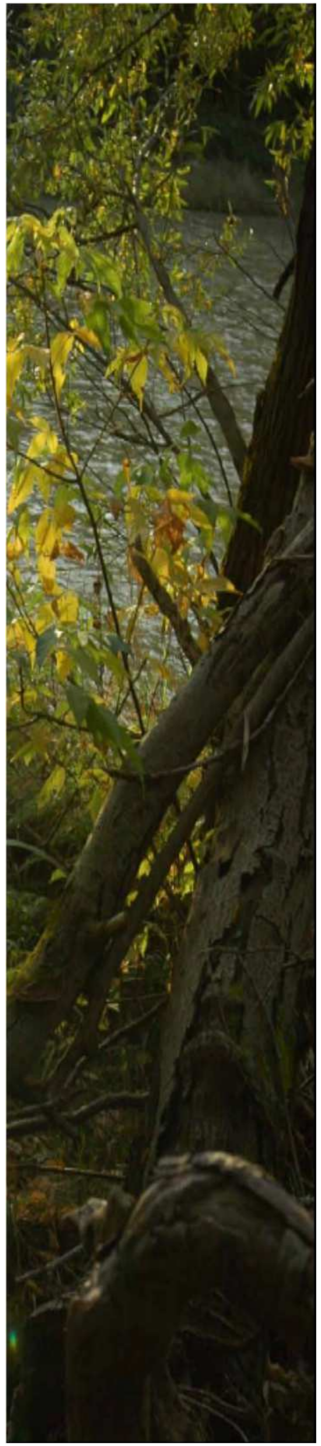
FP7 PROJECT CADASTER

LESSONS FROM COLLECTION OF EXPERIMENTAL DATA AND EXPERIMENTAL TESTING IN CADASTER

Public Health Institute Maribor

Mojca Kos Durjava, PhD



Public Health Institute Maribor

Centre for Risk Assessment of Chemicals with Laboratory

http://ckt.zzv-mb.si/eng/

- **Experimental testing - environmental toxicology and fate & behavior in the environment.**
- Risk assessment of chemicals, PPP, veterinary drugs for the environment.
- Implementation of the Water Framework Directive**.**
- and more, http://ckt.zzv-mb.si/eng/

Participation in national and international projects and project groups.

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REACH

REACH – EC Regulation on chemicals and their safe use (EC 1907/2006)
Registration, **E**valuation, **A**uthorisation and Restriction of **Ch**emical substances.

The aim of REACH: to improve the protection of human health and the environment.

↓

Industry has to assess hazards and risks of the substances.

↓

Information on the hazards that the substances pose to human health and the environment.

↓

Standardised and harmonised toxicological test protocols (OECD Test Guidelines).

ITS approach – (Q)SARs etc.

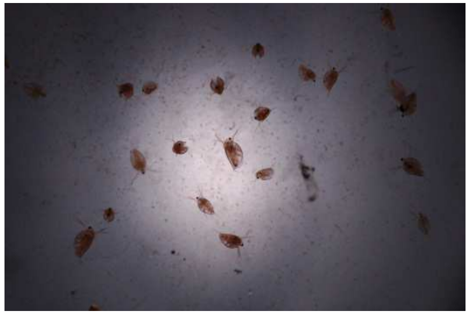
To replace experimental testing.
To strengthen confidence in experimental results.

Manufacturers → Regulatory authority → Registration Approval

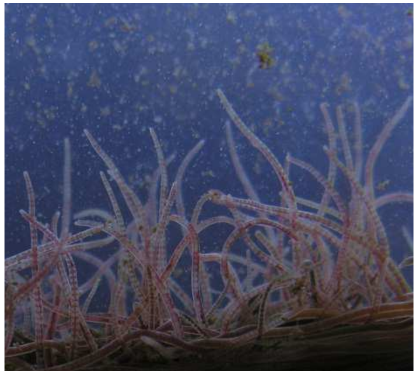
Environmental effects → Ecotoxocity studies

Persistence → Degradability

Bioaccumulation → BCF

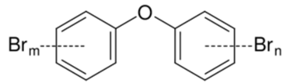


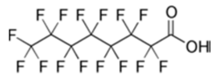
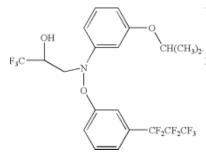
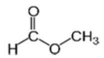
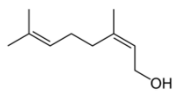
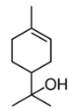
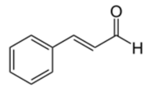
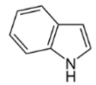
Aquatic toxicity (CLP, REACH)
Fish
Crustaceans (daphnids)
Algae/aquatic plants



LESSONS FROM COLLECTION OF EXPERIMENTAL DATA AND EXPERIMENTAL TESTING IN CADASTER

A data search and experimental testing has been performed on four group of chemicals at RIVM, NL and PHI, SLO on:

1. Polybrominated diphenylethers (PBDE) 

2. Poly- and perfluorinated compounds (PFCs)   
3. Substituted musks/fragrances     
4. Triazoles/benzotriazoles ((B)TAZ)  

LESSONS FROM COLLECTION OF EXPERIMENTAL DATA

A data search on all endpoints of relevance for the environmental risk and hazard assessment of the groups of chemicals included in the case studies.

On	Physico-chemical properties, Environmental fate parameters and Aquatic and terrestrial ecological effect data, other available toxicity data.
From	Literature, EU RAR, Dossiers for Active Substances (PPP), Databases on risk and hazard assessment parameters (IUCLID, AQUIRE,etc.) Industry sources and regulatory agencies: Dupont, RIFM
For	Heterogeneous Brominated Compounds (Flame Retardants) Perfluoroalkylated substances Substituted musks/fragrances Triazoles/benzotriazoles

LESSONS FROM COLLECTION OF EXPERIMENTAL DATA

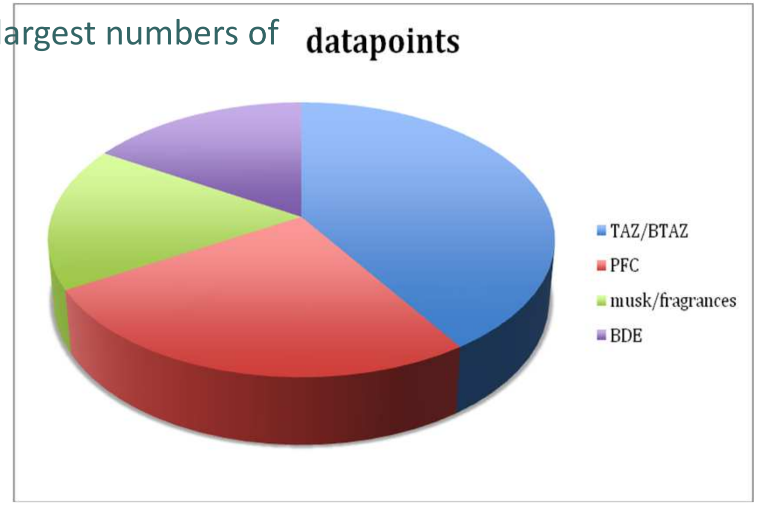
Cadaster Database includes 7823 experimental data within the four classes of chemicaks (1129 chemicals) - primary records without duplicates.

http://www.cadaster.eu/database/

The data available in CADASTER database were collected from numerous articles as well as from public databases. The original source of information is provided for each record.

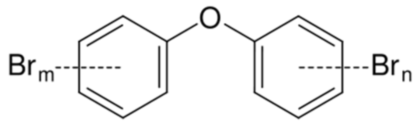
Only limited data are available for the SIDS endpoints.

The distribution of data across different classes indicates that largest numbers of experimental data are available for TAZ/BTAZ.



LESSONS FROM EXPERIMENTAL TESTING IN CADASTER

Polibrominated diphenylethers (PBDEs)



209 congeners ; 10 homologous groups

Used as flame retardants in building materials, textiles and electronic equipment.

Environmental risks and risks to human health.

Penta and octa formulations are banned from EU market since 2004.

Moderate to high hydrophobicity - K_{OW} .
Low solubility in water.
Low volatility.
High bioaccumulation potential.
Toxic effects.

Persistent Organic Chemical - POPs

LESSONS FROM EXPERIMENTAL TESTING IN CADASTER

Bioaccumulation of different PBDEs by *Tubifex tubifex*)

Tubifex tubifex
- A good model organism to replace aquatic vertebrate species such as fish in assessing bioaccumulative properties of substances .
- Sediment dwelling organisms - a worst-case scenario for bioaccumulation effects.

OECD Guidelines for the Testing of Chemicals, No 315
Bioaccumulation in Sediment-dwelling Benthic Oligochaetes, October 2008.

Bioaccumulation tests of PBDE on oligochaete species *Tubifex tubifex* in system water-sediment-tubifex.

LESSONS FROM EXPERIMENTAL TESTING IN CADASTER

Bioaccumulation of different PBDEs by *Tubifex tubifex*

Chemicals

23 PBDEs were tested in four tests:

3 commercial mixtures of PBDEs
(TBDE-71, TBDE-79 and TBDE-83R)
4 individual congeners
(PBDE-077, PBDE-126, PBDE-198 and PBDE-204).

Sediment

Artificial sediment,
according to standard OECD 315.

Static system

Artificial sediment
Tap water

Constituent	Percentage of sediment dry weight	Characteristics
Peat	2 %	Ground sphagnum peat,
Quartz sand	66 %	Grain size: particles 0.05-
	10 %	Grain size: particles 0.180-
Kaolinite clay	22 %	Kaolinite content >30 %
Food source	0.4 %	Folia urticae, powdered leaves of stinging nettle (*Urtica sp.*), finely ground (particle size ≤), in addition to dry sediment

LESSONS FROM EXPERIMENTAL TESTING IN CADASTER

Bioaccumulation of different PBDEs by *Tubifex tubifex*

Test organisms

Oligochaetes *Tubifex tubifex.*
Cultured at 14 ± 2 °C and ~ 250 lx over several years.

Performance of the test

TEST PHASE	PREPARATION FOR UPTAKE PHASE	UPTAKE PHASE (ACCUMULATION)		
			PREPARATION FOR ELIMINATION PHASE	ELIMINATION PHASE
DAY	-6 to 0	0 to 21	22 to 28	28 to 40 (1 to 12)

Analysis of samples

Concentration of PBDEs in water, sediment and tubifex determined by high resolution gas chromatography coupled with high resolution mass spectrometry (HRGC/HRMS).

LESSONS FROM EXPERIMENTAL TESTING IN CADASTER

Bioaccumulation of different PBDEs by *Tubifex tubifex*

METHODS AND RESULTS

Determination of BAF, BAFK, BSAF, BCF

$$C_{org}(t) = C_{org} * e^{-k_{el}} e^{t}$$

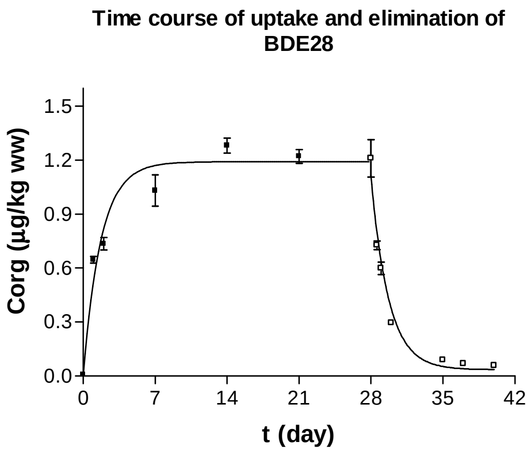
$$BAF = \frac{C_{org}}{C_{sed}}$$

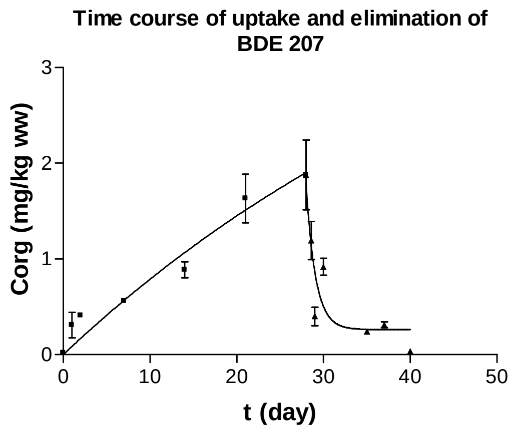
$$BAF_{K} = \frac{k_{up}}{k_{el}}$$

$$BSAF = BAF * \frac{f_{oc}}{f_{lip}}$$

$$BCF = \frac{C_{org}}{C_{w}}$$

$$C_{org} = \frac{k_{up}}{k_{el}} * C_{sed}(1 - e^{-k_{el} * t})$$





LESSONS FROM EXPERIMENTAL TESTING IN CADASTER

Bioaccumulation of different PBDEs by *Tubifex tubifex*

RESULTS AND DISCUSSION

<u>Congeners of PBDEs from commercial mixtures are selectively accumulated.</u>

BAF, BAF_k - higher for the low PBDEs
- lower for the high PBDEs

BAF BCF - in average differ by less than an order of magnitude for low and high PBDEs.

Some congeners did not reach a steady state plateau in the organisms during the timeframe of the test.

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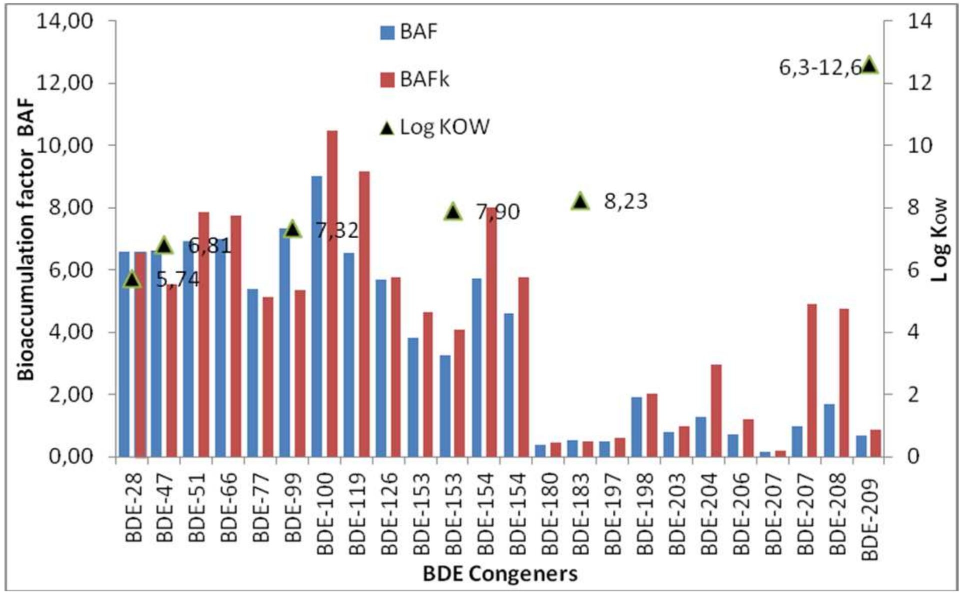
The calculated kinetic BAF and the kinetic BCF are more reliable.



LESSONS FROM EXPERIMENTAL TESTING IN CADASTER

Bioaccumulation of different PBDEs by *Tubifex tubifex*

CONCLUSIONS

- ITS approach - to reduce the number of fish and amphibians tested, the aquatic annelids have become frequently used test species.
- Practicable, good repeatability.
- Possibility to test more congeners at the same time.

The CADASTER dataset generated for PBDE congeners was used to build the QSAR models for predicting the bioaccumulation.

- Mansouri K., Consonni V., Kos Durjava M., Kolar B., Öberg T., Todeschini R., Assessing bioaccumulation of polybrominated diphenyl ethers for aquatic species by QSAR modeling. Chemosphere. 06/2012; 89(4):433-44.
- Kolar B., Mojca Kos Durjava, Lovro Arnus, Willie Peijnenburg, Bioaccumulation of different PBDEs by Tubifex tubifex. Environmental Toxicology and Chemistry. Prepared for submission.

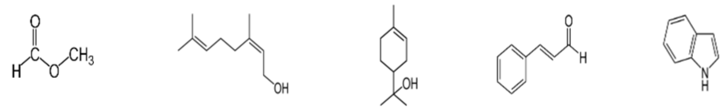
Bioakumulacija polibromiranih difeniletrov v maloščetincih *Tubifex tubifex*

PHOTOS

LESSONS FROM EXPERIMENTAL TESTING IN CADASTER

Substituted musks/fragrances

Esters, terpenes, aromatic, amines…

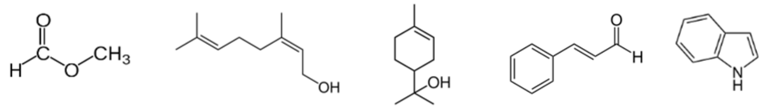


Cosmetics, household products, air fresheners, scented candles…

LESSONS FROM EXPERIMENTAL TESTING IN CADASTER

Substituted musks/fragrances

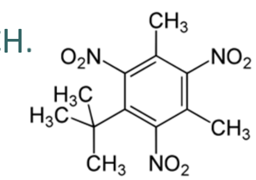
PHYS-CHEM DATA
Substances are volatile.



FATE AND BEHAVIOUR
Some are persistent and bioaccumulative.

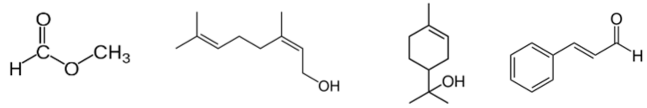
TOXICITY
Some of the musks are quite toxic to fish, aquatic invertebrates and algae.

Musk Xylene
- candidate PBT substance, vPvB;
- substance of very high concern under REACH.



LESSONS FROM EXPERIMENTAL TESTING IN CADASTER

Substituted musks/fragrances



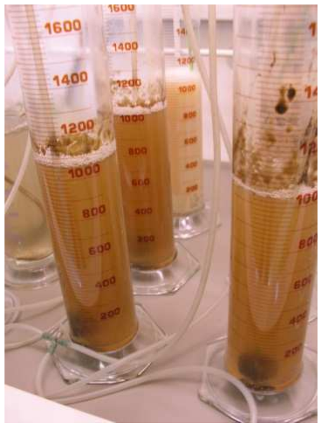
Toxicity testing with:

- Green algae (*Pseudokirchneriella subcapitata*), OECD 201;
- Crustaceans (*Daphnia magna)*, OECD 202.

Ready biodegradability testing, OECD 301D.

List of fragrances relevant for the testing on algae, daphnids and ready biodegradability

ID	Name	Functional Class	CAS Nr
FRA-001	Acethyl cedrene	Terpenes	032388-55-9
FRA-006	Benzyl cinnamate	Cynnamic acid der.	000103-41-3
FRA-022	Hexyl salycilate	Salycilic acid der.	006259-76-3
FRA-023	Hexylcinnamaldeyde	Cynnamic acid der.	000101-86-0
FRA-024	HHCB (Galaxolide)	Musks	001222-05-5
FRA-041	Methyl dihydrojasmonate	Terpenes	024851-98-7
FRA-049	Quinidine	Ref.Comp. (Drug)	000056-54-2
FRA-054	α-amylcinnamyl alcohol	Cynnamic acid der.	000101-85-9
FRA-056	Musk ambrette	Musks	000083-66-9
FRA-065	Cyclopentadecanolide	Musks	000106-02-5
FRA-069	Benzyl Benzoate	other compounds	000120-51-4



Cadaster Workshop, 8-9/10/2012, Munich
Mojca Kos Durjava, PHI

LESSONS FROM EXPERIMENTAL TESTING IN CADASTER

Substituted musks/fragrances

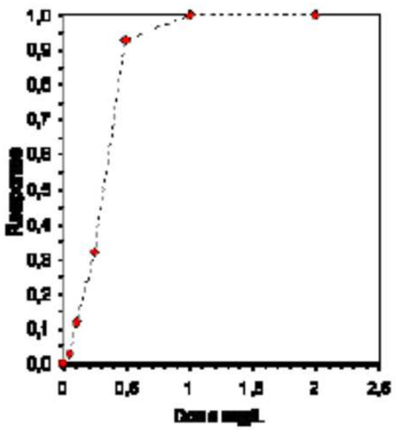
Algae, Growth Inhibition Test, OECD 201, Acute and longterm, ErC50, NOEC, 72 hours test
Toxcalc – Toxicity Data Analysis Software, Version 5.0.32.

Benzyl cinnamate; Cynnamic acid der.
CAS 103-41-3

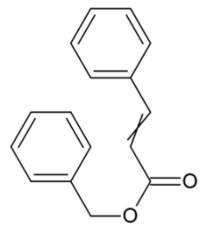
Point	mg/L	SD	95% CL(Exp)		Skew
IC01*	0,0240	0,0217	0,0000	0,1950	0,2787
IC05	0,0766	0,0127	0,0000	0,1765	0,1090
IC10	0,1097	0,0157	0,0000	0,2331	0,2987
IC15	0,1418	0,0169	0,0088	0,2748	-0,1313
IC20	0,1735	0,0153	0,0531	0,2938	-0,1401
IC25	0,2051	0,0164	0,0759	0,3343	0,0355
IC40	0,2826	0,0122	0,1861	0,3791	0,0250
IC50	0,3237	0,0120	0,2293	0,4182	0,1499
IC60	0,3649	0,0130	0,2620	0,4678	0,1758
IC75	0,4266	0,0165	0,2963	0,5569	0,2277
IC80	0,4472	0,0180	0,3053	0,5891	0,2554
IC85	0,4678	0,0196	0,3135	0,6221	0,2842
IC90	0,4883	0,0754	0,0000	1,0827	1,1517
IC95	0,6512	0,1275	0,0000	1,6566	0,1394
IC99	0,9302	0,2073	0,0000	2,5655	-0,8130

* indicates IC estimate less than the lowest concentration

Linear Interpolation (200 Resamples)



Dose response relationship with calculated data for toxicity test with freshwater alga *Pseudokirchneriella subcapitata* for benzyl cinnamate



B-Control

0,06 mg/L

0,12 mg/L

0,25 mg/L

0,5 mg/L

1 mg/L

2 mg/L

LESSONS FROM EXPERIMENTAL TESTING IN CADASTER

Substituted musks/fragrances

Algae, Growth Inhibition Test, OECD 201, Acute and longterm, ErC50, NOEC, 72 hours test
Toxcalc – Toxicity Data Analysis Software, Version 5.0.32.

Toxicity testing with *Pseudokirchneriella subcapitata*, E_rC_{50}-72h (mg/L)

ID	Substance	CAS No	E_rC_{50} – 72h	95 % interval	R^2
FRA-001	Acethyl cedrene	375-22-4	>1.1	/	/
FRA-006	Benzyl cinnamate	355-80-6	0.32	0.23-0.42	0.948
FRA-022	Hexyl salycilate	335-67-1	0.97	0.93-1.02	0.944
FRA-023	Hexylcinnamaldeyde	375-95-1	1.14	0.55-1.73	0.892
FRA-024	HHCB (Galaxolide)	335-76-2	>0.7	/	/
FRA-041	Methyl dihydrojasmonate	2058-94-8	10.3	3.4-17.2	0.954
FRA-049	Quinidine	307-55-1	2.8	2.3-3.3	0.888
FRA-054	α-amylcinnamyl alcohol	101-85-9	3.8	3.4-4.2	0.872
FRA-056	Musk ambrette	83-66-9	0.98	0.71-1.3	0.861
FRA-065	Cyclopentadecanolide	106-02-5	>1.6	/	/
FRA-069	Benzyl Benzoate	120-51-4	0.24	0.17-0.31	0.895

Toxicity results on *Pseudokirchneriella subcapitata*, NOEC-72h (mg/L)

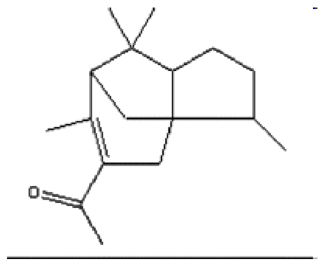
ID	Substance	CAS No	NOEC – 72h
FRA-001	Acethyl cedrene	375-22-4	0.55
FRA-006	Benzyl cinnamate	355-80-6	0.060
FRA-022	Hexyl salycilate	335-67-1	0.060
FRA-023	Hexylcinnamaldeyde	375-95-1	0.40
FRA-024	HHCB (Galaxolide)	335-76-2	0.23
FRA-041	Methyl dihydrojasmonate	2058-94-8	0.21
FRA-049	Quinidine	307-55-1	< 0.1
FRA-054	α-amylcinnamyl alcohol	101-85-9	0.22
FRA-056	Musk ambrette	83-66-9	0.29
FRA-065	Cyclopentadecanolide	106-02-5	0.53
FRA-069	Benzyl Benzoate	120-51-4	< 0.05

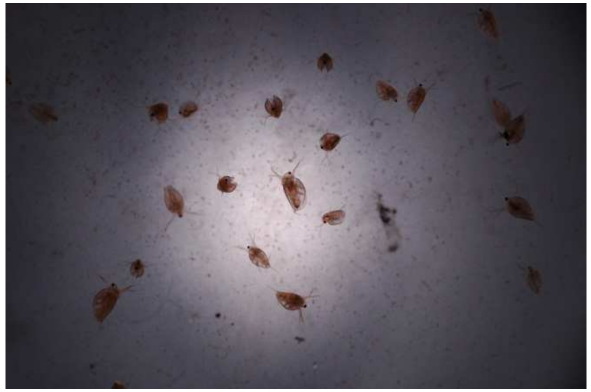
LESSONS FROM EXPERIMENTAL TESTING IN CADASTER

Substituted musks/fragrances

Daphnia sp., Acute Immobilisation Test, OECD 202, Acute, EC50, 48 hours test
EPA Probit Analysis software, Version 1.5
Acetyl cedrene;Terpene
CAS 32388-55-9

Daphnia 48-Hr EC50		
Substance name: Metyl Cedryl Ketone		
CAS Nr.: 32388-55-9		
Test start:		
Test end:		
Freshwatter pH: 7.6		
Parameter	Start	End
Temperature (°C):	20	20
pH (C1)	7,2	7,2
Konc. O_2 (mgO_2/L) (C1)	6,2	6





Results

Test ID	Date (test start)	EC50(mg/L)	Theoretical spike concentration (mg/L)	Measured conc.- test start (mg/L)	Measured conc.- test end (mg/L)
MCK-1	18.8.10	0,53	0,8	0,67	0,64
MCK-2	18.8.10	0,45	0,8	0,67	0,64
MCK-3	18.8.10	0,43	0,8	0,67	0,64

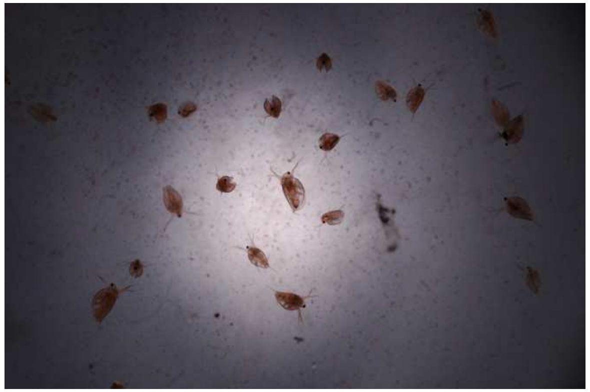
LESSONS FROM EXPERIMENTAL TESTING IN CADASTER

Substituted musks/fragrances

Daphnia sp., Acute Immobilisation Test, OECD 202, Acute, EC50, 48 hours test

Toxicity results on *Daphnia magna*, EC_{50}-48h (mg/L)

ID	Substance	CAS No	EC_{50} – 48h	95 % interval
FRA-001	Acethyl cedrene	375-22-4	0.53	0.42-0.74
FRA-006	Benzyl cinnamate	355-80-6	1.5	1.19-1.92
FRA-022	Hexyl salycilate	335-67-1	0.42	0.34-0.53
FRA-023	Hexylcinnamaldeyde	375-95-1	0.24	0.17-0.29
FRA-024	HHCB (Galaxolide)	335-76-2	0.30	0.24-0.39
FRA-041	Methyl dihydrojasmonate	2058-94-8	20.2	15.1-26.9
FRA-049	Quinidine	307-55-1	5.6	4.0-9.1
FRA-054	α-amylcinnamyl alcohol	101-85-9	1.21	0.94-1.36
FRA-056	Musk ambrette	83-66-9	2.1	1.5-2.5
FRA-065	Cyclopentadecanolide	106-02-5	0.45	0.35-0.59
FRA-069	Benzyl Benzoate	120-51-4	3.8	3.40-4.33



LESSONS FROM EXPERIMENTAL TESTING IN CADASTER

Substituted musks/fragrances

Ready biodegradability test, OECD 301D, 28 days

The persistence of the substance in aerobic aqueous medium; 28 days.
Closed bottle test – substances are volatile.

5-6 concentrations of test substance were:

- inoculated with aquarium water - mixed bacterial population;
- incubated under aerobic conditions in the dark at 20 °C;
- reference compound tested: sodium acetate;
- degradation was followed by chemical analysis at frequent intervals (GC/MS, LC/MS/MS).

Dt, percentage dergadation was calculated.

$$D_t = \left[1 - \frac{C_t - C_{blt}}{C_0 - C_{bl0}}\right] * 100$$

LESSONS FROM EXPERIMENTAL TESTING IN CADASTER

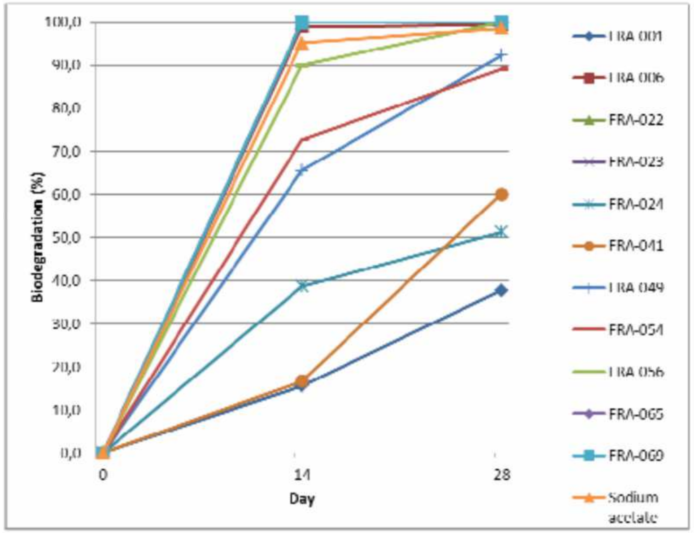
Substituted musks/fragrances

Ready biodegradability test, OECD 301D, 28 days

Mean value of measured concentrations (µg/L) for tested substituted musks/fragrances at day 0, day 14 and day 28

Fragrance ID	C_0	C_{14}	C_{28}	C_{bl0}	C_{bl}[a]
FRA-001	5	4.24	3.15	0.1	0.1
FRA-006	5	0.15	0.13	0.1	0.1
FRA-022	12.5	0.1	0.1	0.1	0.1
FRA-023	5	0.1	0.1	0.1	0.1
FRA-024	0.5	0.35	0.30	0.1	0.1
FRA-041	25	25	12.0	0.1	0.1
FRA-049	100	34.5	7.85	0.1	0.1
FRA-054	5	1.44	0.63	0.1	0.1
FRA-056	0.5	0.14	0.1	0.1	0.1
FRA-065	0.5	0.1	0.1	0.1	0.1
FRA-069	2.5	0.1	0.1	0.1	0.1

[a] C_{bl} represents concentration in blank at day 0, at day 14 and at day 28. All concentrations were below the detection limit of the method (< LOD) and are calculated as LOD/2



The percentage of biodegradation of substituted musks/fragrances and reference substance sodium acetate

LESSONS FROM COLLECTIONS OF EXPERIMENTAL DATA AND EXPERIMENTAL TESTING IN CADASTER

Publicly available report on www.cadaster.eu

Publications

- G. Ding, M. Wouterse, R. Baerselman, W.J.G.M. Peijnenburg. Toxicity of poly- and perfluorinated compounds to lettuce (*Lactuca sativa*) and green algae (*Pseudokirchneriella subcapitata*). Arch. Environ. Contam. Technol., 62, 49-55, 2012.
- G. Ding, E.-J. van den Brandhof, R. Baerselman, W.J.G.M. Peijnenburg. Acute toxicity of poly- and perfluorinated compounds to two cladocerans, *Daphnia magna* and *Chydorus sphaericus*. Environ. Toxicol. Chem., 31(3), 605-10, 2012.- G. Ding and W. Peijnenburg. Physicochemical Properties and Aquatic Toxicity of Poly- and Perfluorinated Compounds – review paper. Rev. Environ. Sci. Technol., accepted for publication, 2012.
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THANK YOU

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