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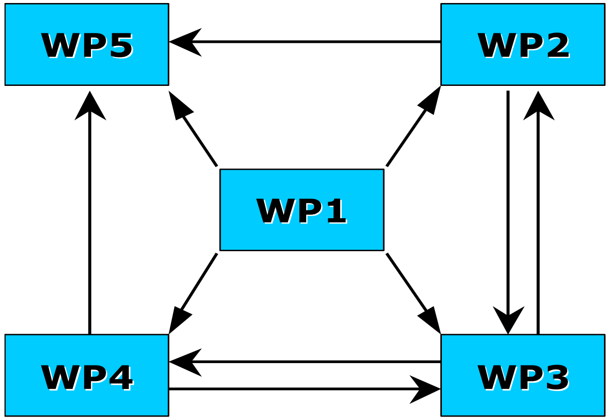

Willie Peijnenburg*, Mojca Durjava, Paola Gramatica, Tomas Öberg, Magnus Rahmberg, Igor Tetko, Nina Jeliaskova, Mark Huijbregts, Mike Comber

Rijksinstituut voor Volksgezondheid en Milieu
Ministerie van Volksgezondheid, Welzijn en Sport

Exemplification of the integration of tools within REACH: the CADASTER project

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CADASTER

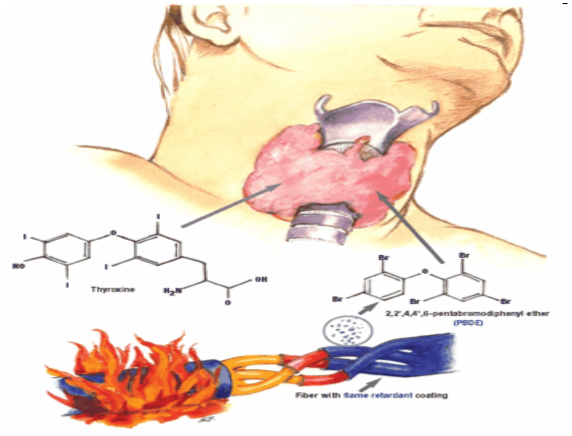
CAse studies on the Development and Application of in-Silico Techniques for Environmental hazard and Risk assessment

Diapozitiv 1

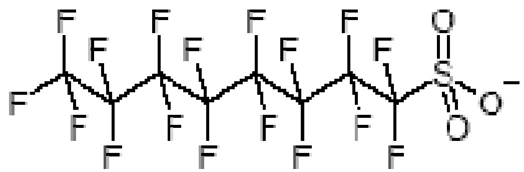
a1 anonymous; 26.11.2010

CADASTER: Exemplification of tools within REACH

CADASTER: CAse studies on the Development and Application of *in-Silico* Techniques for Environmental hazard and Risk assessment



PBDE: "The PCB's of the future"



Classification of PFOS-compounds in 22 categories according to OECD

CADASTER

Beneficiary Number *	Beneficiary name	Beneficiary short name	Country
1 (coordinator)	Rijksinstituut voor Volksgezondheid en Milieu (RIVM)	RIVM	Nl
2	Public Health Institute Maribor	PHI	Si
3	University of Insubria (Varese)	UI	Italy
4	IVL Swedish Environmental Research Institute	IVL	S
5	University of Kalmar	HIK	S
6	Helmholtz Zentrum München - German Research Center for Environmental Health (GmbH)	HMGU	Ge
7	Ideaconsult Ltd.	IDEA	Bu
8	Radboud University Nijmegen	RUN	Nl
9	Mike Comber Consulting	MCC	Be

REACH

Registration, Evaluation, Authorisation and Restriction of Chemicals

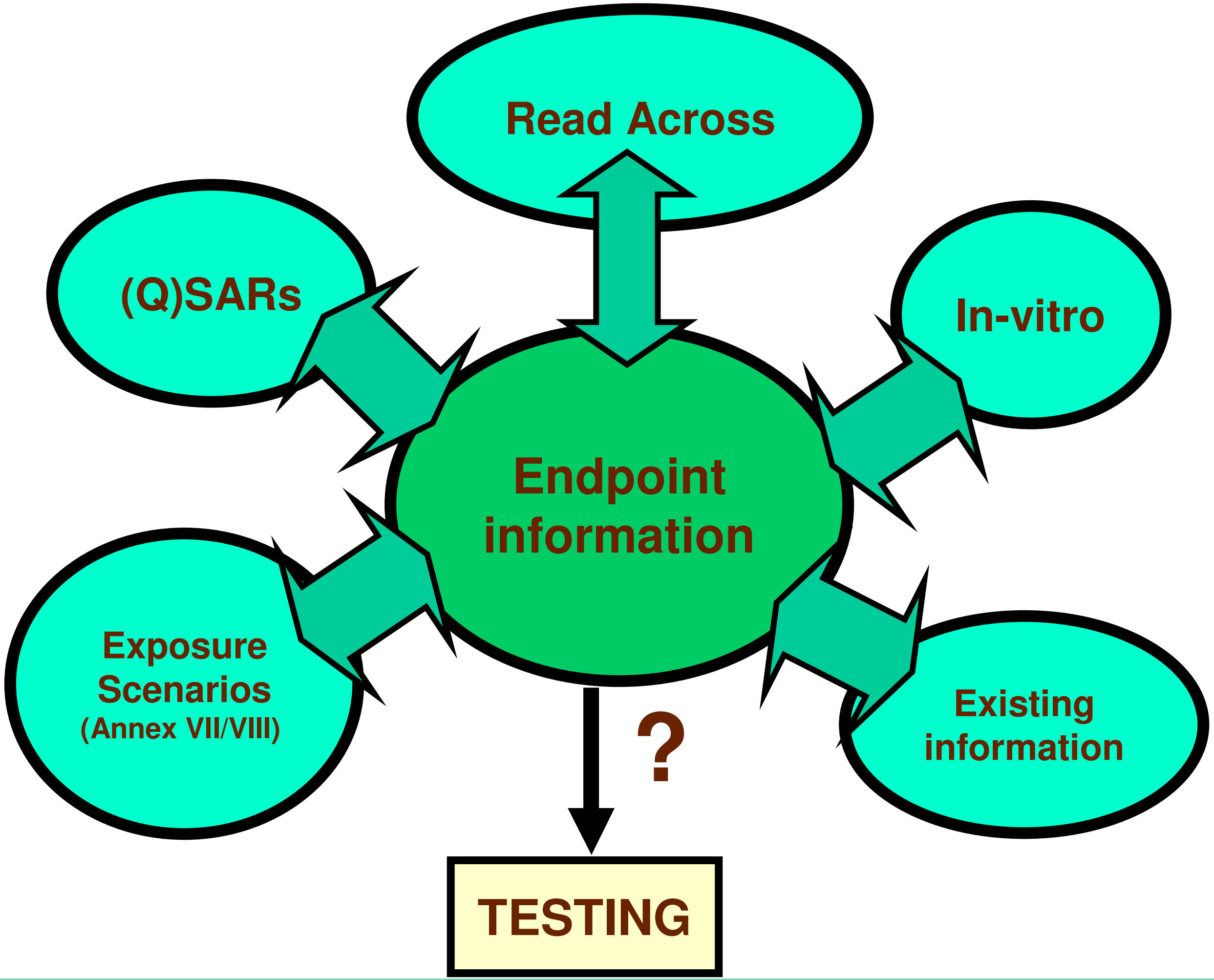
REACH requires demonstration of safe manufacture and use of chemicals

REACH based on precautionary principle, aims at achieving proper balance between social, economic and environmental objectives

REACH aims to optimise the use of scarce and scattered info on substances

REACH aims to minimise animal testing by optimal use of info on “related” compounds

Intelligent Testing Strategies (ITS)



Goals:

- Exemplify the integration of information, models, strategies for safety-, hazard-, risk assessment for large numbers of substances
- Carry out “real” risk assessment for large numbers of substances according to the basic philosophy of REACH: < costs, animal testing, time
- Exemplify how to increase non-testing information whilst quantifying and reducing uncertainty

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Aim:

Provide full environmental hazard and risk assessment according to the REACH philosophy for chemicals belonging to 4 classes of emerging chemicals:

- 1 – Polybrominated diphenylethers (PBDE), hydrophobic chemicals that pose a threat to man and the environment.
- 2 - Perfluoroalkylated substances and their transformation products, like perfluoroalkylated sulfonamides, alkanoic acids, sulfonates. Persistent hydrophilic compounds that may be toxic for man and environment.
- 3 – Substituted musks/fragrances; a heterogenic group of chemicals of varying composition like substituted benzophenones, polycyclic musks, terpene derivatives. Common emission pattern in the environment.
- 4 - Triazoles/benzotriazoles: increasingly used as pesticides and anti-corrosives.

Outcome:

DSS – regularly updated for new compound classes:

-New testing strategies

-New testing data

-New models

-Actual integrated evaluations, including uncertainty and variability

-On-line and stand-alone tool

Activities

1: **Collection of data and models**

- Experimental data intrinsic hazards – Screening Initial Data Set Dossier (SIDS)
- Models – Screening Initial Data Set Dossier (SIDS)

- Generation new data essential for validation and proper hazard/risk assessment
- Database data/models: dissemination purposes

Activities

2: **Development/validation QSAR models**

- Evaluate performance
- Similarity analysis and multivariate ranking methods for identification of priority chemicals to orient the experimental testing
- Develop new QSARs where gaps are identified due to lack of existing models or due to models of insufficient quality.
- Documentation of the performance of the (final) models selected and developed.

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Activities

3: **Integration of QSARs within hazard and risk assessment**

- Integration in probabilistic risk assessment framework: characterize variability/ uncertainty, sensitivity analyses, modeling of variability with regard to application in SSDs
- Evaluate ECETOC TRA screening RA tool
- Evaluate methods and decision points for establishing scientific validity and applicability domains for QSAR models
- Explore possibilities for economic valuation of substitution of chemicals from within chemical classes

Activities

4: **Outreach:** website, newsletters/ workshops, stand-alone tools for dissemination of project results

- Development of on-line, stand-alone DSS: develop, publish, use QSAR/QSPR models for REACH
- Integration of the developed models with the QSAR Application Toolbox developed by OECD: establish the com-patibility of the models with the (Q)SAR Model Reporting Format (QMRF) format
- Provision of a sustainable dissemination of project results by the WWW and as stand-alone tools
- Communication including newsletters and workshop(s).

Some findings

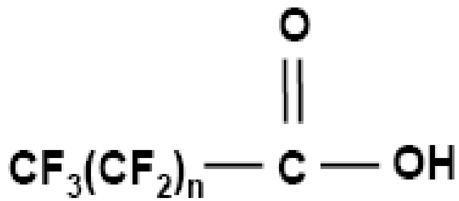
- Lack of sufficient data for relevant endpoints
- Lack of models for relevant endpoints and relevant chemical classes
- Difficult to obtain data from industry

- > 7500 data entries relevant for RA – 4 classes
- Overview of suited (Q)SAR models available
- Identified: need for new/improved models

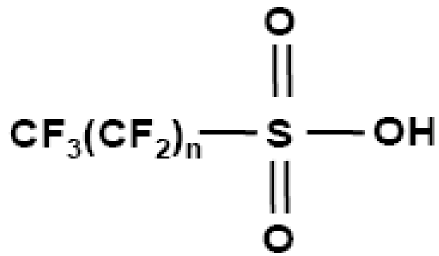
CASE STUDY

Toxicity testing of PerFluorinated Compounds

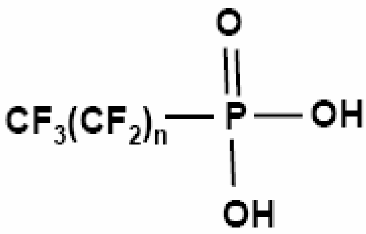
Poly- and Perfluorinated Chemicals (PFC)



Perfluorocarboxylic acids
(e.g., PFCAs, n = >7 - 14)



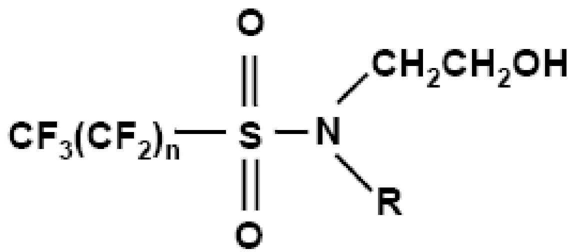
Perfluorosulfonic acids
(e.g., PFSAs, n = >5 - 9)



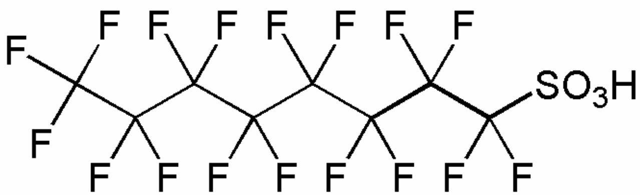
Perfluorinated phosphonic acids
(PFPAs, n = 5,7 and 9)

$CF_3(CF_2)_nCF_2CH_2CH_2OH$

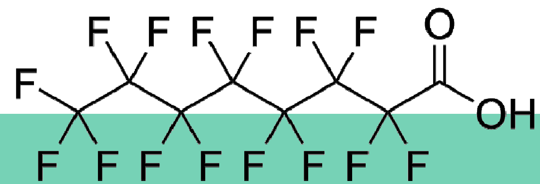
Fluorotelomer alcohols
(e.g., FTOH, n=4, 6 and 8)



Perfluorosulfonamidoethanols
(PFOSE, R=CH_3, CH_2CH_3; e.g., n=7)



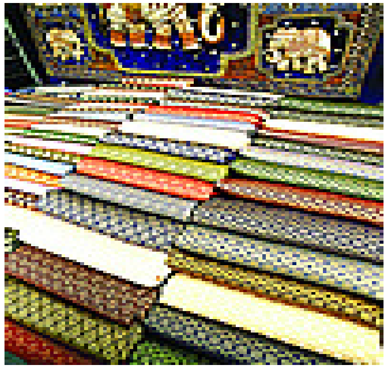
PFOS



PFOA

PFC Applications

Insecticide formulations



Water/Oil repellent textile

Treated paint and carpet

Food packaging products

Non-stick cookware

Fire-fighting foams

How many PFCs are there?

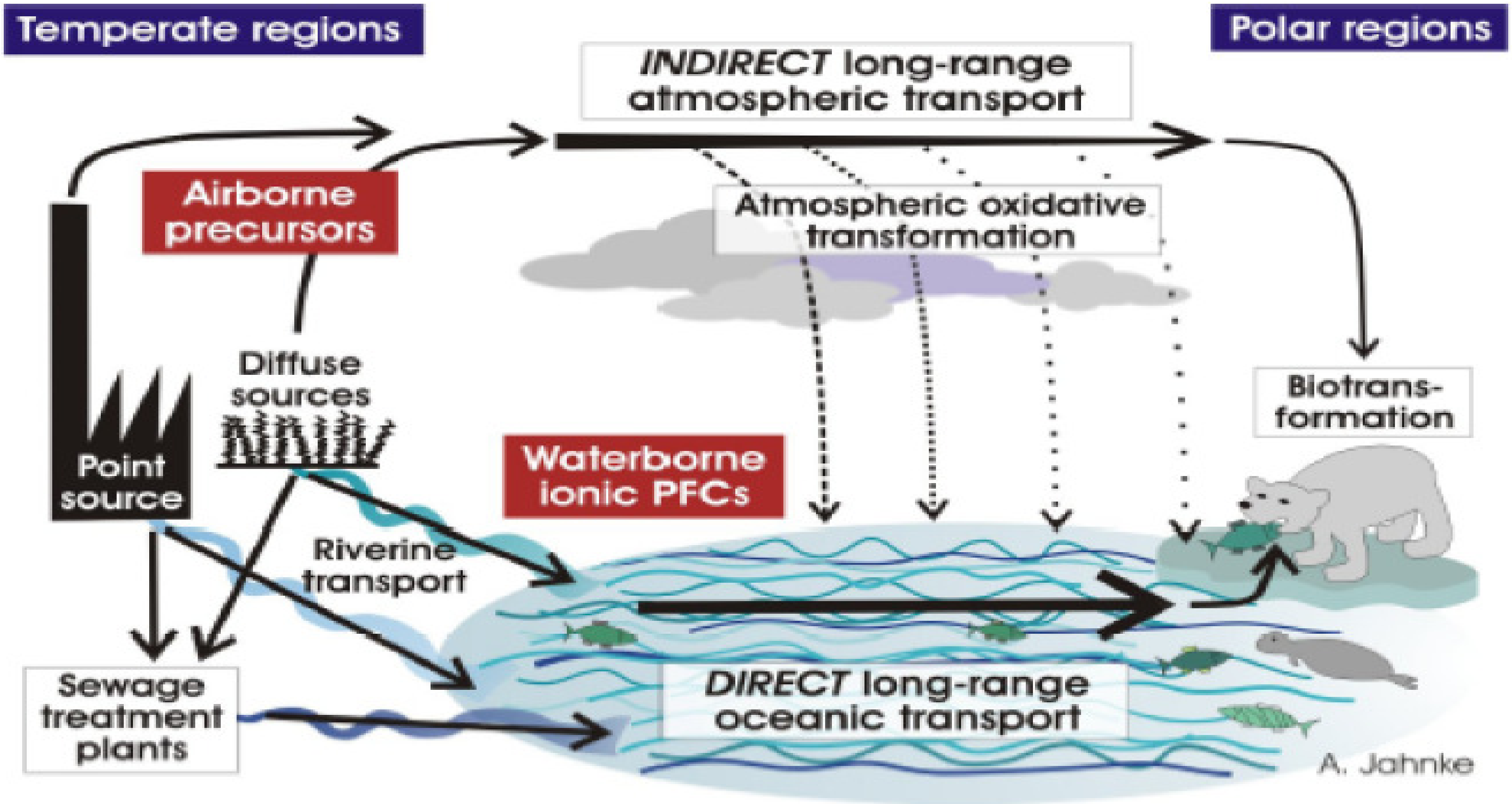
$924 < N(PFCs) < 1070$

- **165 PFOS and related substances**
- **260 Perfluoroalkyl Sulfonate (PFAS) and Related Compounds**
- **30 PFOA and related substances**
- **615 Fluorinated Chemicals that Potentially Degrade to PFCA**
  - 146 chemicals in part one (perfluoro chemicals)
  - 469 chemicals in part two (polyfluoro chemicals that have fully fluorinated carbon moieties plus a number of CH2- groups.)

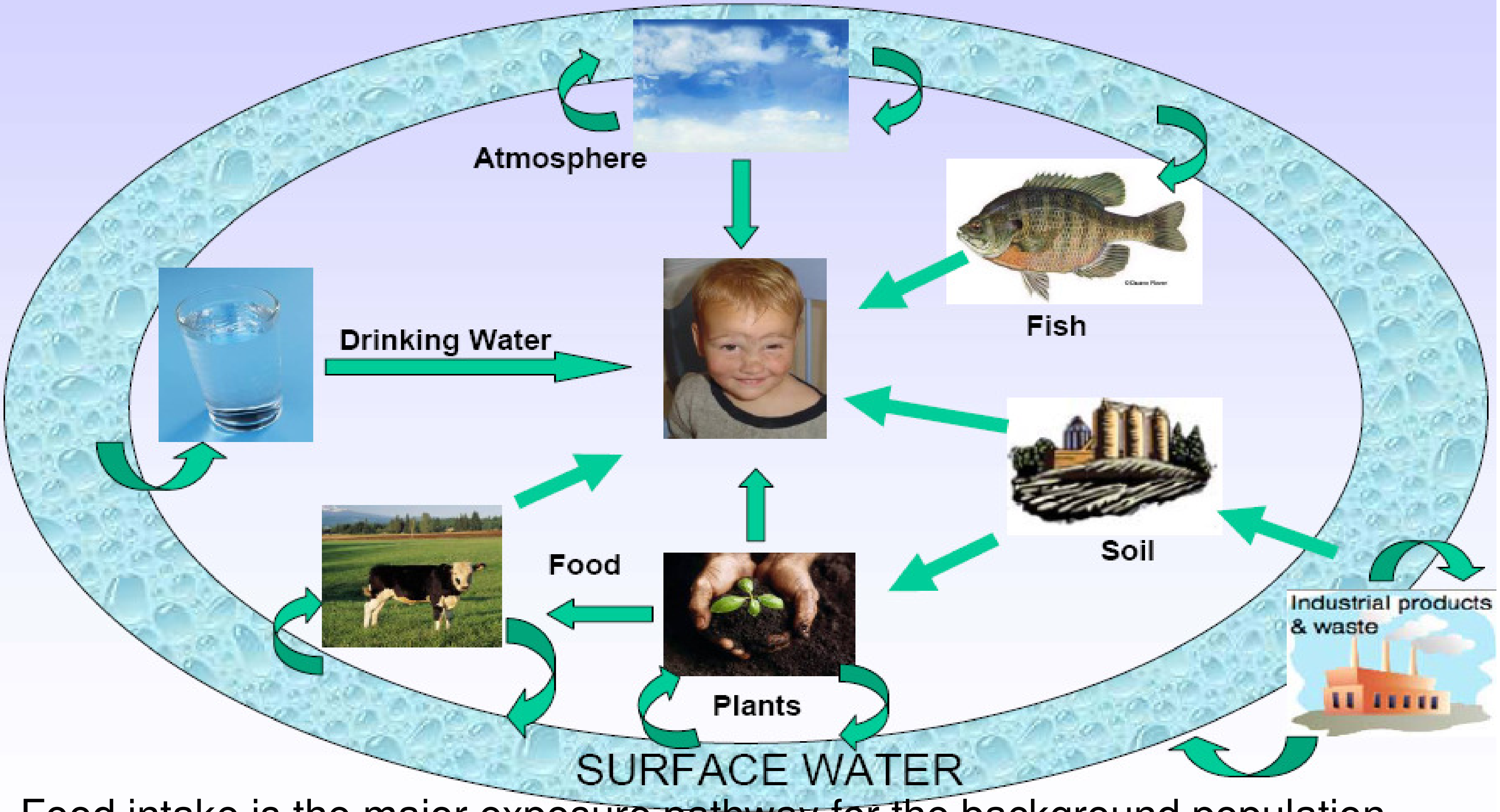
OECD. Lists of PFOS, PFAS, PFOA, PFCA, related compounds and chemicals that may degrade to PFCA. ENV/JM/MONO(2006)15.

PFCs and Sources to the Environment

(Butt *et al.*, 2009 *Sci Total Environ.* in review)



Human Exposure Pathways



Food intake is the major exposure pathway for the background population, whereas drinking water exposure is dominant for populations near sources of contaminated drinking water. *Vestergren and Cousins, Environ. Sci. Technol.,* ***2009****,* ***43*** *(15), 5565–5575.*

What about their aquatic toxicity?

	Algae			Aquatic Plant					Water flea		Planarians		Green neon shrimps	Midge	Fish				Bacteria	Amphibian
	Green algae	Diatom algae	blue-green algae	*Lemna gibba*	*Myriophyllum sibiricum*	Lettuce	cucumber	pakchoi	*Daphnia magna*	*Daphnia pulicaria*	*Dugesia japonica*	*Physa acuta*	*Neocaridina denticulate*	*Chironomus tentans*	Rainbow trout	Tilapia	Fathead minnow	Bluegill sunfish	Vibrio fischeri	African clawed frog
PFBS																				
PFHxS																				
PFHpS																				
PFOS	6			2	6	2	2	2	5	2	4	4	4	4	1	2	1	1		1
PFBA																				
PFHxA	1	1	1																1	
PFHpA	1	1	1																1	
PFOA	1	1	1	1	6	2	2	2	2		4	4	4			2			1	
APFO									3						1					
PFNA	1	1	1																1	
PFDA																				
PFUnA																				
PFDoA	1																			
PFTeA	1																			
PFHxDA																				
PFOcDA																				
4:2 FTOH																				
6:2 FTOH																2				
8:2 FTOH																1				
10:2 FTOH																				

Insufficient data for environmental hazard and risk assessment, and insufficient data for modelling to predict other data.

↓

It is necessary to get some toxicity data.

↓

Which chemicals should be tested?

Strategy:

1 – Experimental design: PCA + read across toxicity data other (rodent) species

2 – Selection of ideal set of test compounds

3 – Acquiring test compounds

4 – Design non-ideal set of test compounds

5 – Toxicity assessment

6 - Modelling

Work of University of Insubria

- Inhalation study: 4 descriptors based MLR model
  - Hydrophobicity (M*log*P) → negative
  - Electronegativity (Jhetv, X3v and MATS1e) → positive
- Oral study: 4 descriptors based MLR model
  - Fingerprint descriptors representing frequency of atom pairs like C-C, C-F and C-O are prominent
- 376 extra PFCs predicted including PFCs listed in ECHA
- Prediction and prioritization of toxic PFCs based on rodents toxicity

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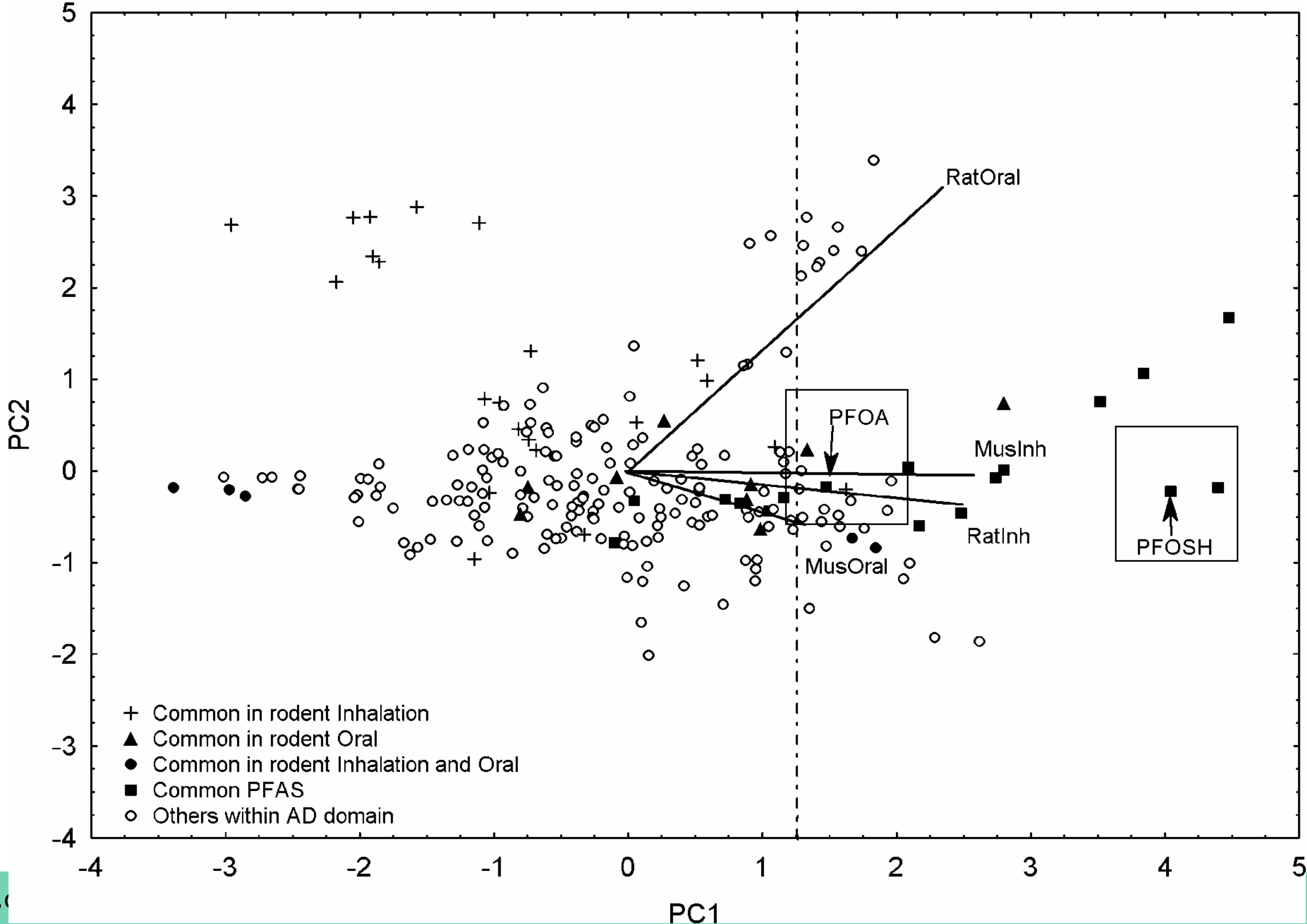

RatOral
MusInh
RatInh
MusOral
PFOA
PFOSH
PC1
PC2
Common in rodent Inhalation
Common in rodent Oral
Common in rodent Inhalation and Oral
Common PFAS
Others within AD domain

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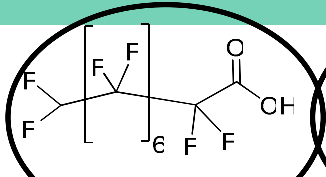
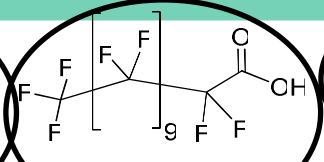
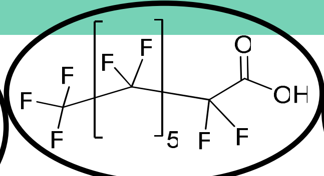


76-21-1

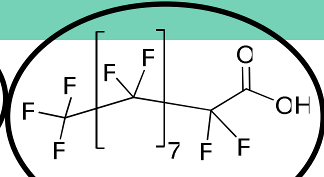


307-55-1



335-67-1

PFOA



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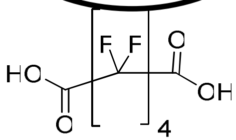
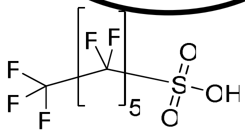


336-08-3



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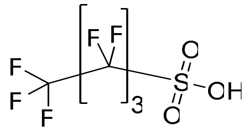

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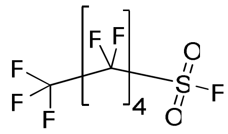

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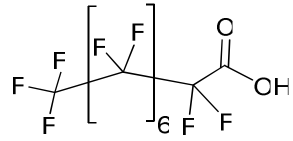

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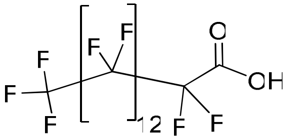

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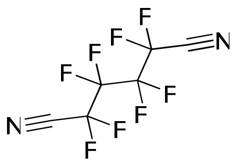

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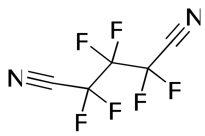

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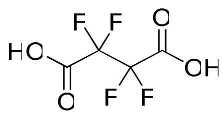

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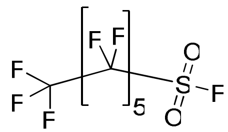

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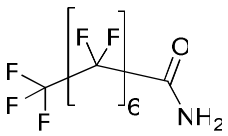

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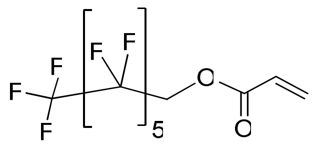

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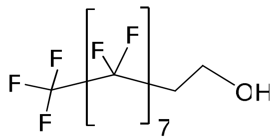

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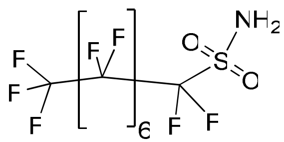

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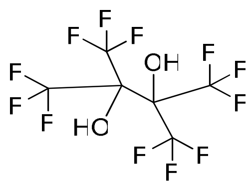
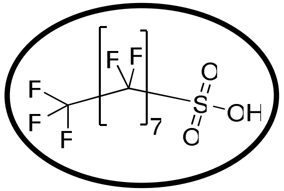


918-21-8

PFOSH



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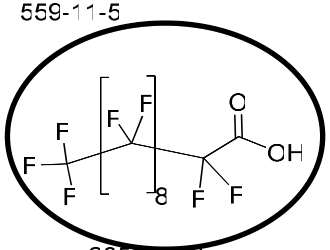

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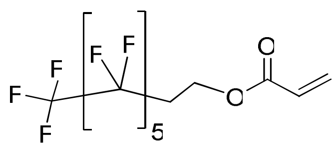

17527-29-6

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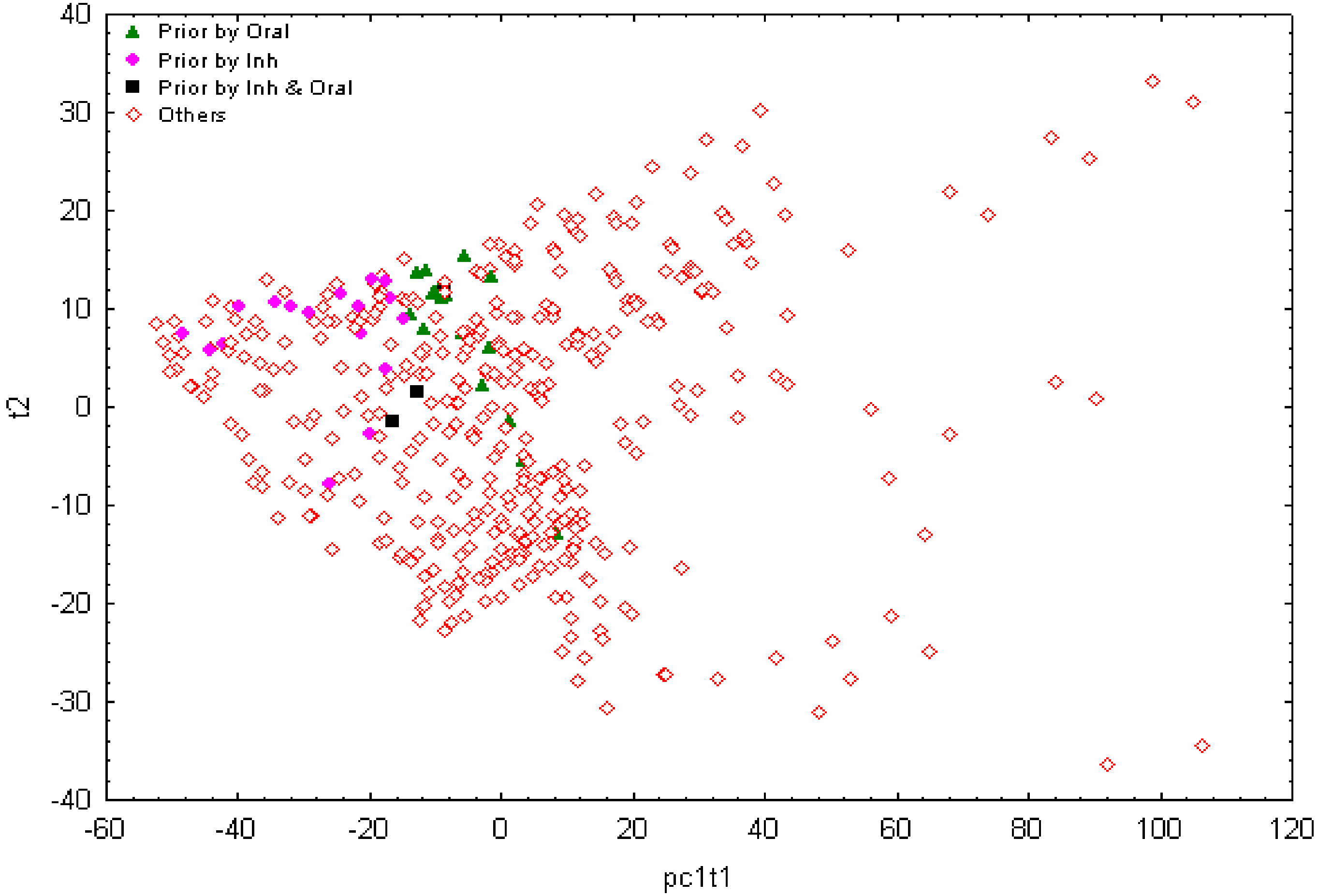
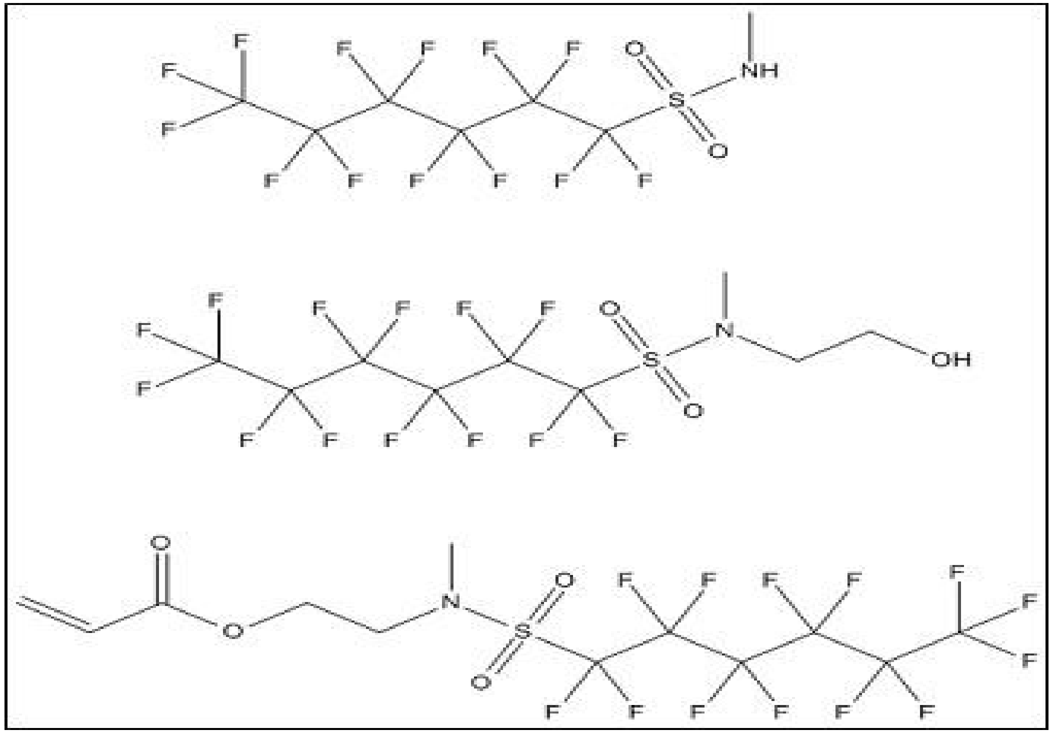
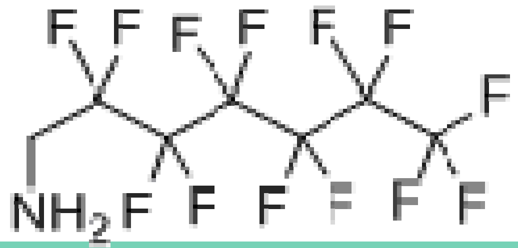
PCA DRAGON descriptors



Hotelling T2 ellipses of PCA analyses for 434 PFCs
using 1436 molecular structure descriptors

Extended set of compounds





Butanoic acid, heptafluoro-, ethyl ester

Methacrylic acid, 2,2,3,3,4,4,4-heptafluorobutyl ester

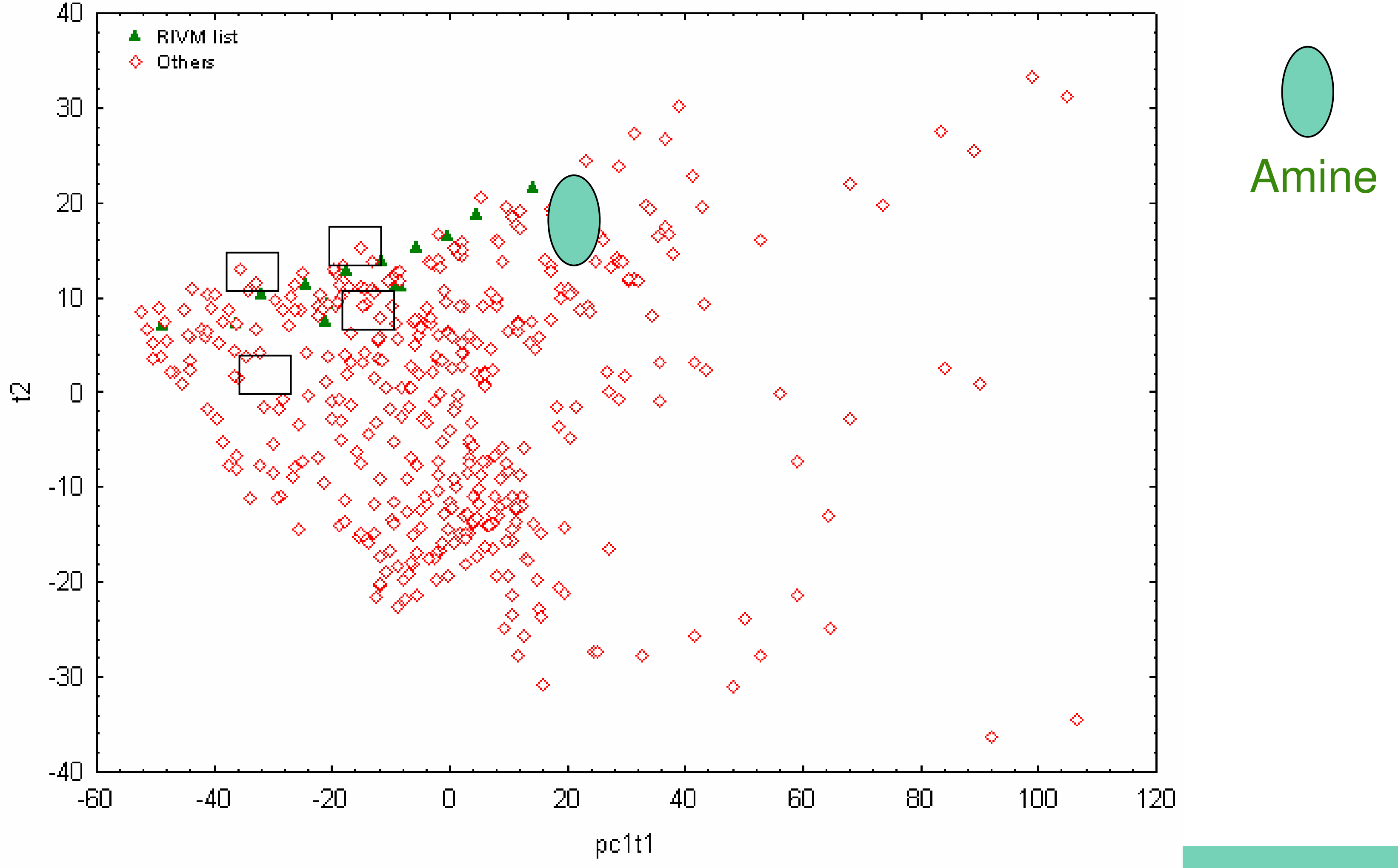
3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluoro-1-octanethiol

1H,1H,2H,3H,3H-Perfluorononane-1,2-diol; 97%

1H,1H,2H,2H-Perfluorooctyl isobutyrate

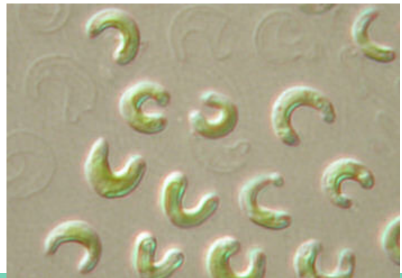
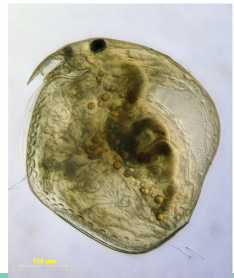
2,2,3,3,4,4,5,5,6,6,7,7-Dodecafluoro-1,8-octanediol

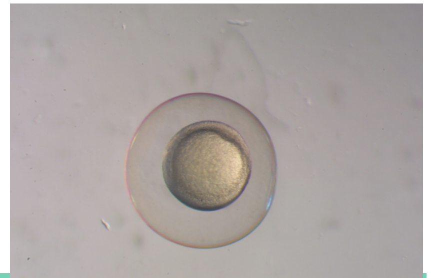
Extended set of compounds

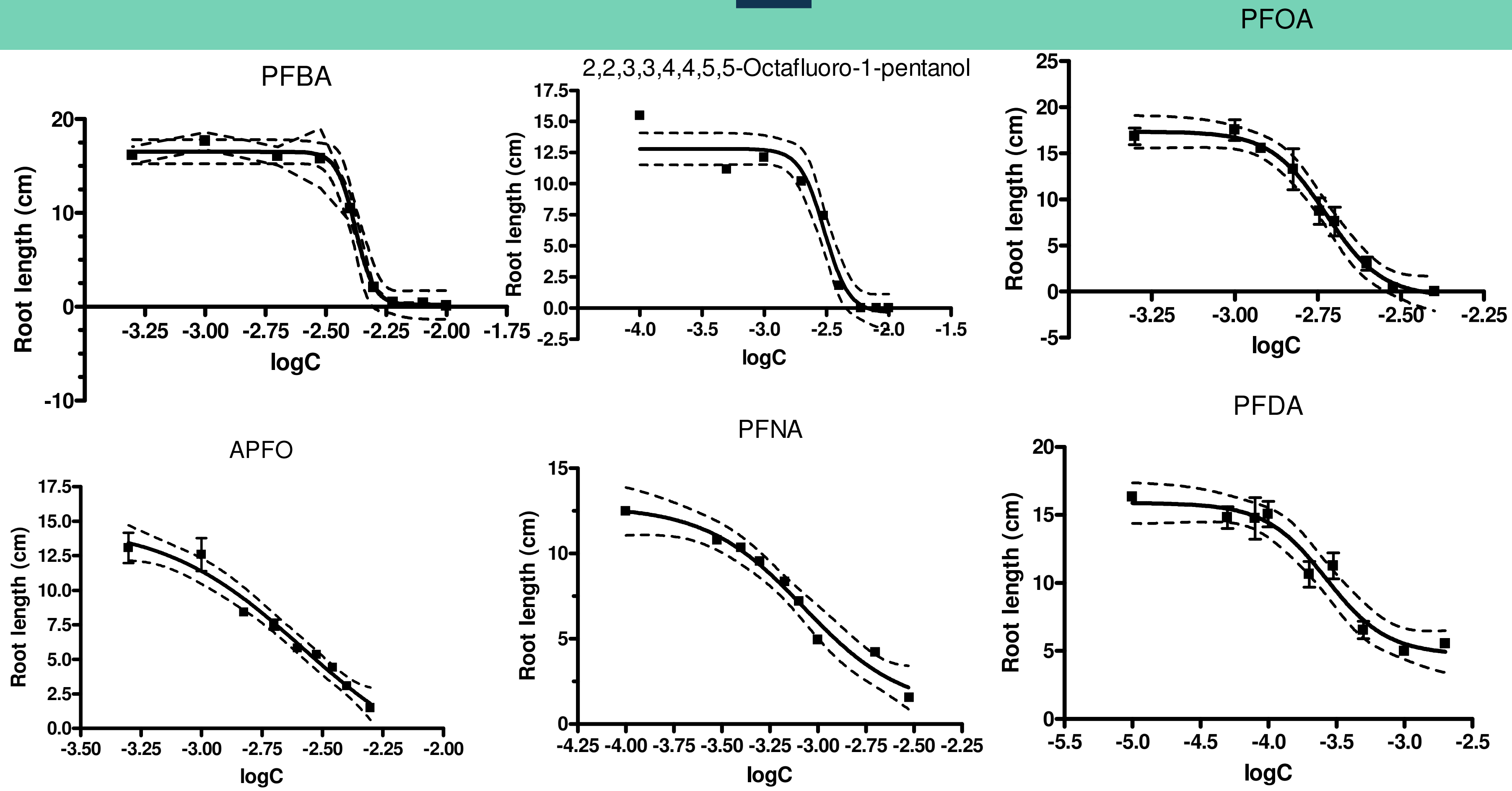


Aquatic toxicity

- Lettuce (*Lactuca sativa*) – Seed germination/root elongation test
- Green algae (*Pseudokirchneriella subcapitata*) – PAM test
- Water flea (*Chydorus sphaericus* and *Daphnia magna*) – Acute immobilisation test□
- Zebrafish (*Danio rerio*) – Fish embryo toxicity test□

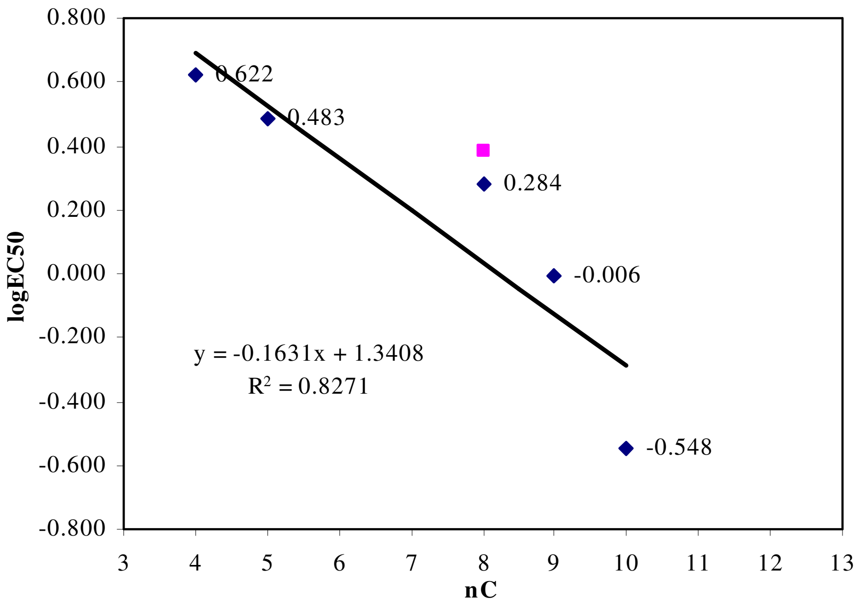






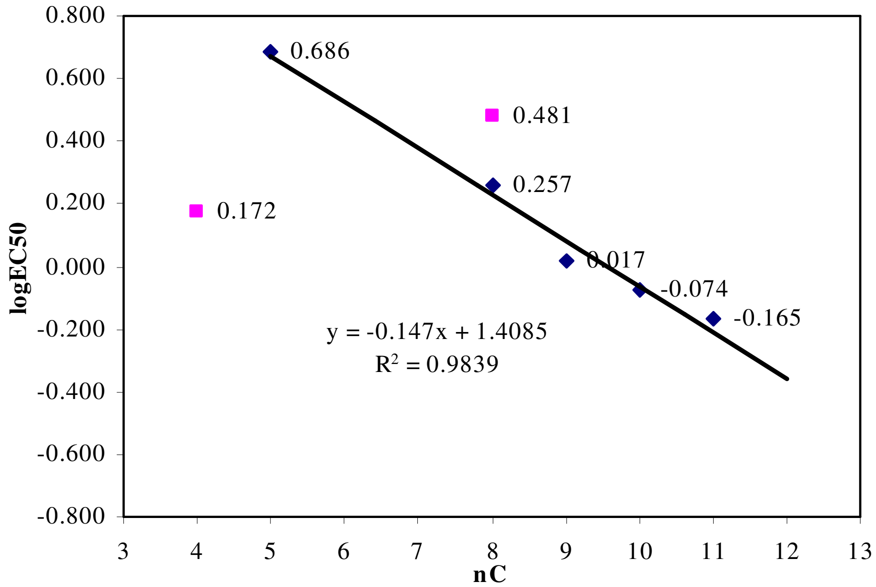


The highest concentrations of **PFUnA** and **PFDoA** have no effect.

Lettuce seed



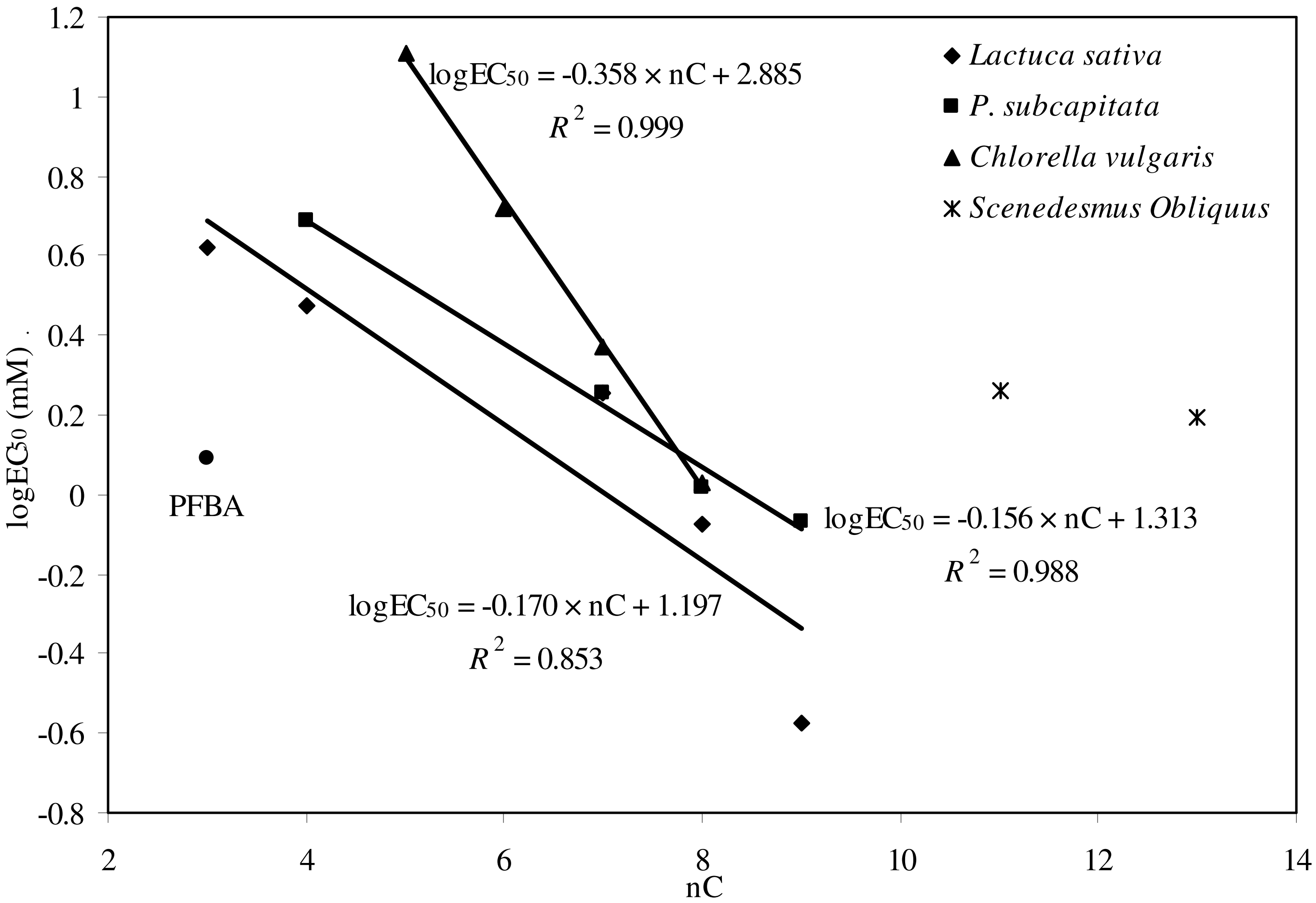
Green algae *(Pseudokirchneriella subcapitata)*



Results - acids

Log EC50, lettuce = – 0.170 × nC + 1.197
***n* = 5, *R*² = 0.853, *p* = 0.0252**

Log EC50, algae = – 0.156 × nC + 1.313
n = 4, R² = 0.988, p = 0.006



CADASTER

Interspecies extrapolation read across daphnids

Daphnia magna

Log EC50, 24h = – 0.127 × nC + 0.646
$n = 5$, $R^2 = 0.986$, $p = 7.090 \times 10$-4

Log EC50, 48h = – 0.131 × nC + 0.615
$n = 6$, $R^2 = 0.971$, $p = 3.265 \times 10$-4

Chydorus sphaericus

Log EC50, 24h = – 0.214 × nC + 1.013
$n = 7$, $R^2 = 0.972$, $p < 0.0001$

Log EC50, 48h = – 0.221 × nC + 0.876
$n = 7$, $R^2 = 0.925$, $p = 5.394 \times 10$-4

24h toxicity:

Log EC50, C. sphaericus = 1.560 × log EC50, D. magna – 0.113
$n = 5$, $R^2 = 0.888$, $p = 0.016$

For 48-h toxicity:

Log EC50, C. sphaericus = 1.494 × log EC50, D. magna – 0.277
$n = 6$, $R^2 = 0.846$, $p = 0.009$

Thanks for your attention!

谢谢！