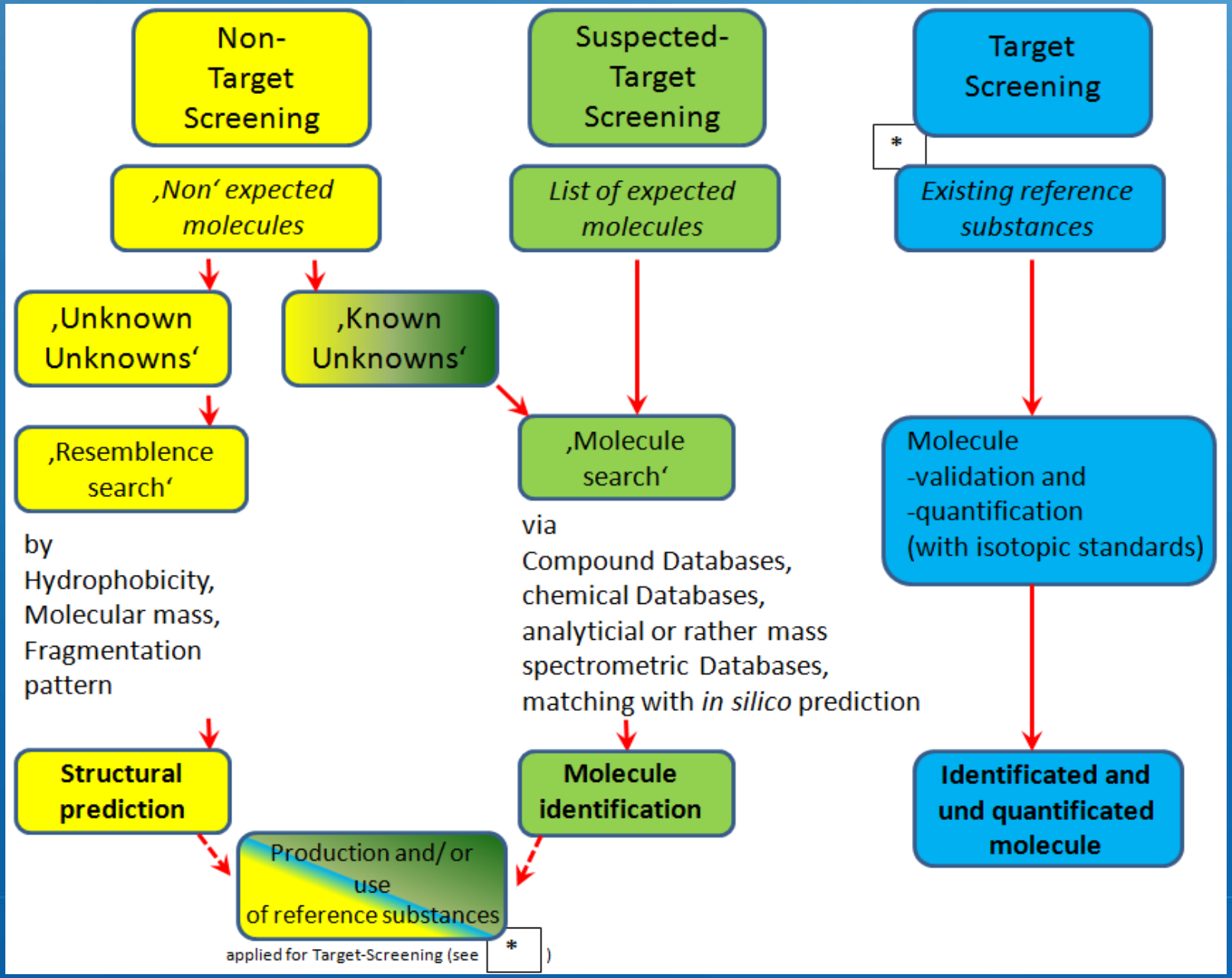
Retention time index (RTI)

in the field of LC:

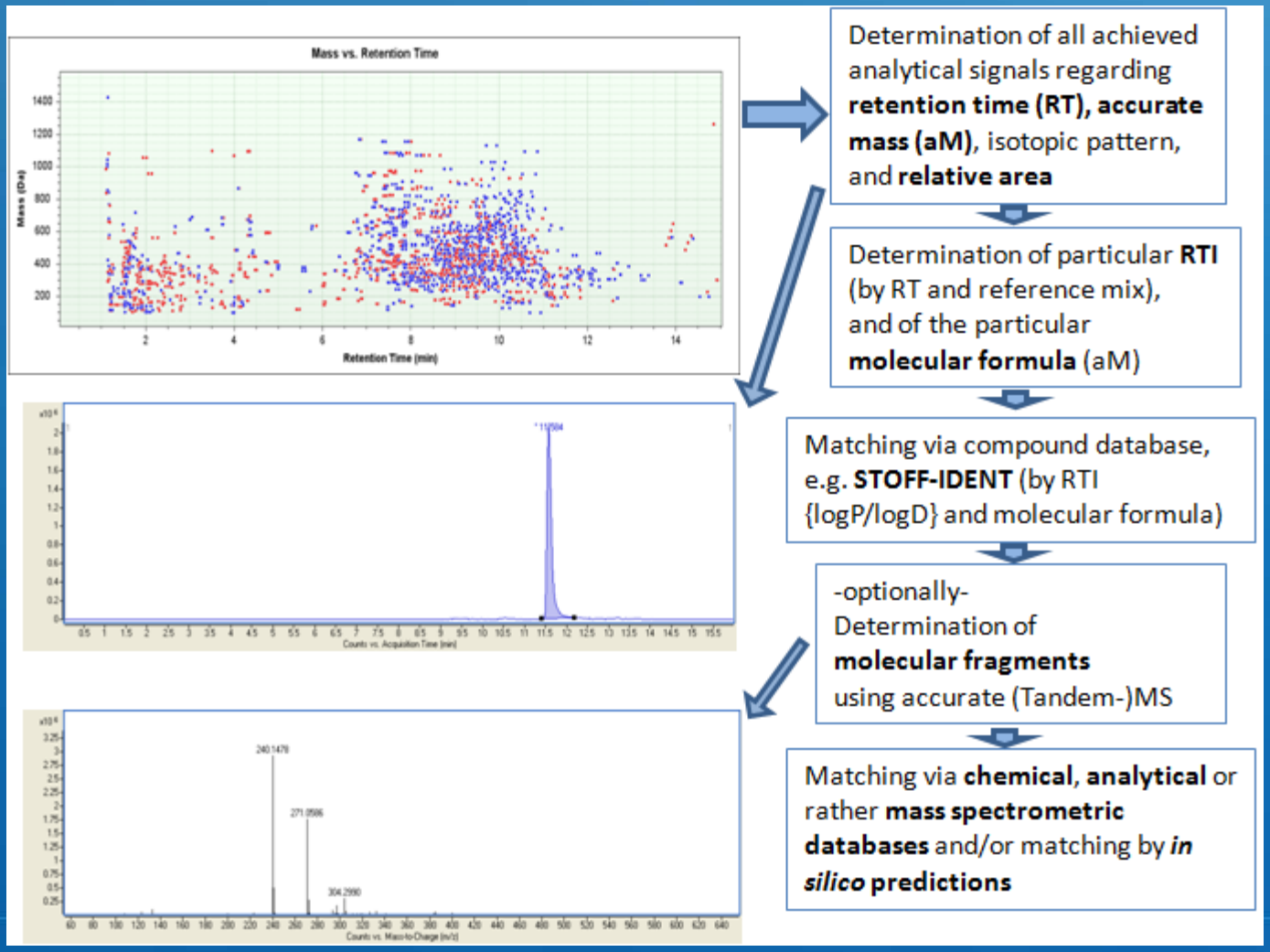
Status Quo

openMASP Workshop;
Freising, den18./19.04.13

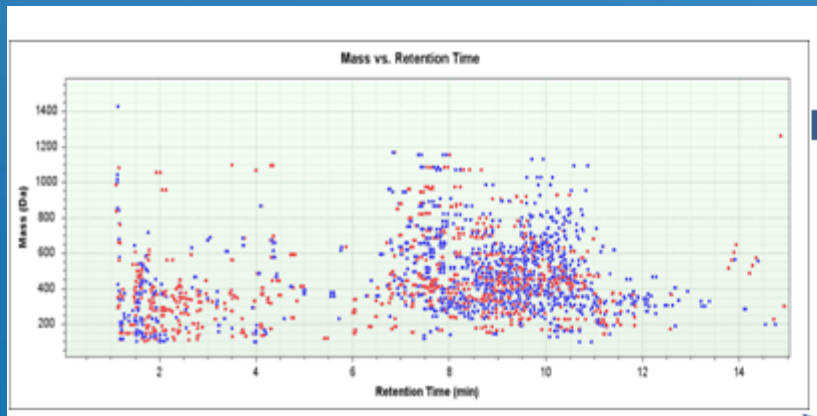
PD Dr. Thomas Letzel

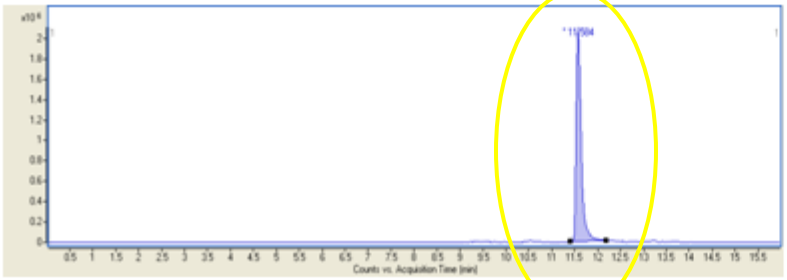

Non-Target Screening
Suspected-Target Screening
Target Screening
*
'Non' expected molecules
List of expected molecules
Existing reference substances
'Unknown Unknowns'
'Known Unknowns'
'Resemblence search'
'Molecule search'
Molecule -validation and -quantification (with isotopic standards)
by Hydrophobicity, Molecular mass, Fragmentation pattern
via Compound Databases, chemical Databases, analyticial or rather mass spectrometric Databases, matching with *in silico* prediction
Structural prediction
Molecule identification
Identificated and und quantificated molecule
Production and/ or use of reference substances
applied for Target-Screening (see *)

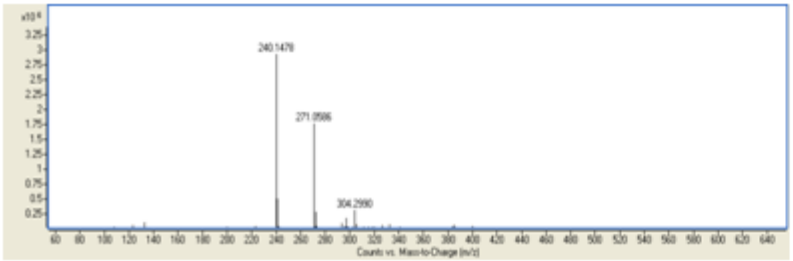
‚Known Unknowns' (über Suspected-Target Screening Pathway)



‚Known Unknowns' (über Suspected-Target Screening Pathway)







Determination of all achieved analytical signals regarding **retention time (RT), accurate mass (aM)**, isotopic pattern, and **relative area**

Determination of particular **RTI** (by RT and reference mix), and of the particular **molecular formula** (aM)

Matching via compound database, e.g. **STOFF-IDENT** (by RTI {logP/logD} and molecular formula)

-optionally-
Determination of **molecular fragments** using accurate (Tandem-)MS

Matching via **chemical**, **analytical** or rather **mass spectrometric databases** and/or matching by ***in silico* predictions**

Overview

Interlaboratory test:

a) Intraday and Interday validation in one laboratory
b) comparison between laboratories
c) RTI calculation (i.e. here logP) of unknown molecules
d) simple prediction tool for retention time

	total	In acidic condition	In neutral condition	Different column type	C18 endcapped	C18 non-endcapped	C18 polar endcapped	C18 polar embedded
No. Labs	26							
No. Methods (total)	38	31	7	22	36	0	2	2
No. Methods (with MeOH)	18	14	4	11	17	0	1	0
No. Methods (with ACN)	20	17	3	11	19	0	1	2

Overview - Map

Participants from

Germany

Austria

Switzerland

Sweden

Greece

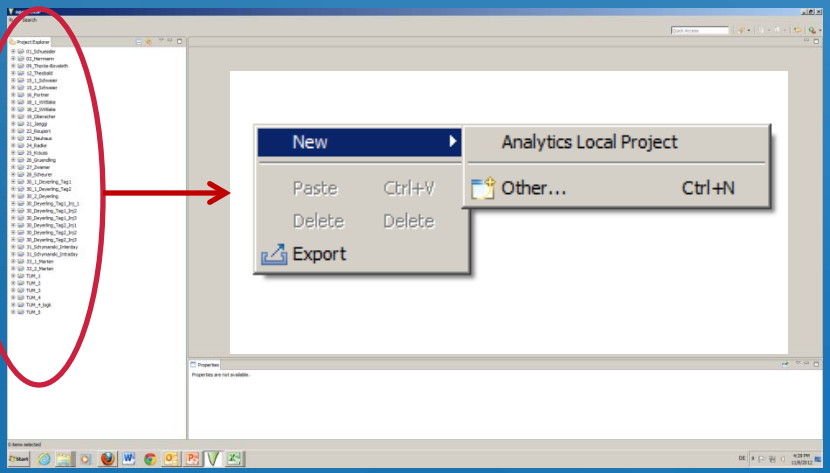
Interlaboratory test (Chemical Sets)

RTI standards and ‚Unknown solution‘

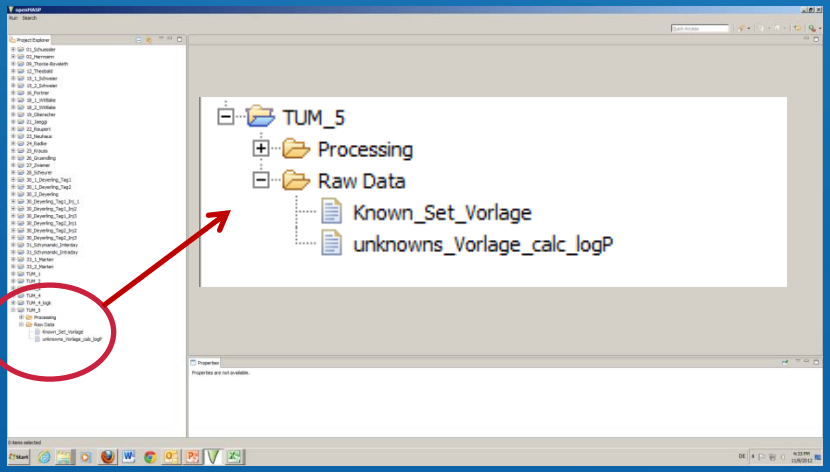
name	Retention time [min]	logP	RTI
Metformin	0.62	-1.36	50.0
Chloridazon	2.23	1.11	87.2
Carbetamide	2.62	1.65	95.3
Monuron	2.74	1.93	99.5
Metobromuron	3.22	2.24	104.2
Chlorbromuron	3.57	2.85	113.4
Metconazole	3.87	3.59	124.5
Diazinon	4.22	4.19	133.6
Quinoxyfen	4.40	4.98	145.5
Fenofibrate	4.47	5.28	150.0

name	Retention time [min]	RTI calc	logP calc	logP theor [1]
Unknown 1	2.09	84.0	0.90	1.27
Unknown 2	3.50	111.6	2.73	2.68
Unknown 3	3.99	127.6	3.80	4.31

RTI calculation - Softwaretool (openMASP)



1. step: create a new analytical project

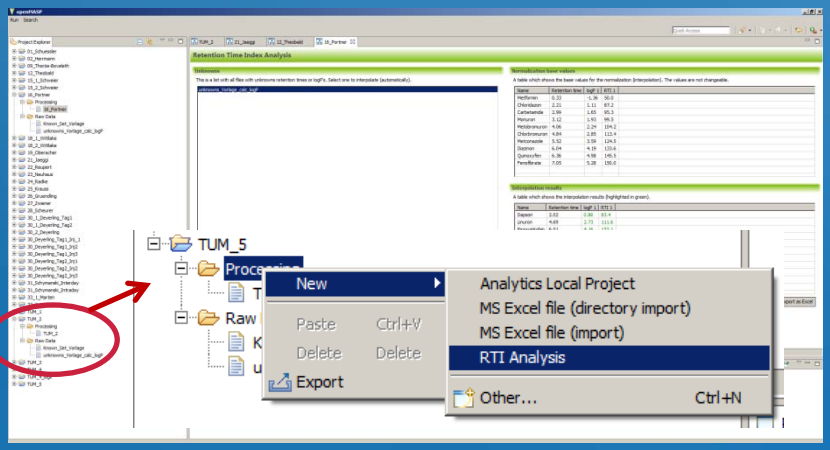


2. step: import Excel-Files

Known_Set_Vorlage for the reference substances

Unknowns_Vorlage_calc_logP for the unknowns

RTI calculation - Softwaretool (openMASP)



3. step: create a RTI Analysis

Normalization base values

A table which shows the base values for the normaliza

Name	Retention time	logP 1	RTI 1
Metformin	0.33	-1.36	50.0
Chloridazon	2.21	1.11	87.2
Carbetamide	2.99	1.65	95.3
Monuron	3.12	1.93	99.5
Metobromuron	4.06	2.24	104.2
Chlorbromuron	4.84	2.85	113.4
Metconazole	5.52	3.59	124.5
Diazinon	6.04	4.19	133.6
Quinoxyfen	6.36	4.98	145.5
Fenofibrate	7.05	5.28	150.0

Table of reference substances with retention time, logP value and normalized RTI

Interpolation results

A table which shows the interpolation results (highligh

Name	Retention time	logP 1	RTI 1
Dapson	2.02	0.86	83.4
Linuron	4.69	2.73	111.6
Picoxystrobin	6.01	4.16	133.1

Table of unknown substances with the calculated RTI and logP value

Interlaboratory test

a) Intraday- and Interday validation in one laboratory (Helmholtz Zentrum München, Hr. Dominik Deyerling)

	RTI	RTI	RTI	RTI	RTI	RTI	Intraday s [%]	Intraday s [%]	Interday s [%]
	day 1 Inj 1	day 1 Inj 2	day 1 Inj 3	day 2 Inj 1	day 2 Inj 2	day 2 Inj 3	day 1	day 2	day 1 + 2
Dapson	87.0	87.0	86.3	86.3	86.6	86.9	0.4	0.3	0.3
Linuron	111.7	112.0	111.7	111.5	111.3	111.7	0.1	0.1	0.2
Picoxy-strobin	130.4	131.3	130.3	130.6	130.6	131.0	0.3	0.1	0.3

Interlaboratory test

b) comparison between laboratories

total no. of injections: **63** in 26 labs

	total (all methods)	**methods with MeOH**	**methods with ACN**
	s [%]	s [%]	s [%]
Dapson	5.7	4.0	2.2
Linuron	1.6	1.4	1.7
Picoxystrobin	4.4	1.2	2.1

Interlaboratory test

c) RTI/ logP calculation of unknown molecules

An example of a method using **Acetonitril** as solvents in acidic mode

name	Retention time [min]	logP	RTI
Metformin	0.62	-1.36	50.0
Chloridazon	2.23	1.11	87.2
Carbetamide	2.62	1.65	95.3
Monuron	2.74	1.93	99.5
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name	Retention time [min]	RTI calc	logP calc	logP theor [1]
Unknown 1	2.09	84.0	0.90	1.27
Unknown 2	3.50	111.6	2.73	2.68
Unknown 3	3.99	127.6	3.80	4.31

Data from Stockholm University, Hr. Michael Radke
[1] www.chemicalize.org

Interlaboratory test

c) RTI/ logP calculation of unknown molecules

An example of a method using **Methanol** as solvents in acidic mode

name	Retention time [min]	logP	RTI
Metformin	2.60	-1.36	50.0
Chloridazon	9.60	1.11	87.2
Carbetamide	10.80	1.65	95.3
Monuron	11.20	1.93	99.5
Metobromuron	12.10	2.24	104.2
Chlorbromuron	13.10	2.85	113.4
Metconazole	14.20	3.59	124.5
Diazinon	14.10	4.19	133.6
Quinoxyfen	16.40	4.98	145.5
Fenofibrate	15.40	5.28	150.0

name	Retention time [min]	RTI calc	logP calc	logP theor [1]
Unknown 1	7.80	77.6	0.47	1.27
Unknown 2	12.90	111.6	2.73	2.68
Unknown 3	13.50	117.5	3.12	4.31

Data from TUM, Hr. Thomas Letzel

[1] www.chemicalize.org

Retention Time Index (RTI via „logP“)

	method 1	method 2	Sigma	pubchem	Tomlin	chemicalize.org	ACD	pH 7.4	Marvin pH 7.4			
molecular formula	RT	RT	logP	logP	logP	logP	logP	logD	logD	**average logP**	s log P	s^2 logP
$C_6H_{12}N_2O_3$				-3,1	-1,5	-1,34	-2,16	-4,52	-3,63	**-2,03**	0,69	-34,2
$C_5H_4N_4O_2$	1.07			-0,9		-1,67	-1,2	-1,55	-2,08	**-1,26**	0,32	-25,2
$C_2H_8NO_2PS$			-0,93	-0,9	-0,8	-0,32	-0,78	-0,78	-0,32	**-0,75**	0,22	-29,6
$C_7H_{12}N_4O_3S_2$	5.7	13,45		-0.2		-0,54	-0,5	-0,5	-0,54	**-0,52**	0,02	-3,8
$C_9H_{20}N_2O_2$		5,53	1,38	1,20	-2,6	0,77	1,1	-0,72	-1,12	**0,37**	1,50	404,9
$C_6H_3Cl_2NO_2$		6,31		2,3	-2,63	2,23	1,59	-1,56	-1,22	**0,87**	2,04	233,9
$C_{10}H_8ClN_3O$	6.3	13,07	1,14	0,8	1,19	1,11	0,73	0,73	1,11	**0,99**	0,19	19,1
$C_9H_{11}ClN_2O$	9.1		k.A.	1,9		1,93	1,89	1,89	1,93	**1,91**	0,02	0,9
$C_9H_{10}Cl_2N_2O_2$	13	18,05	3	3,2	3	2,68	3,2	3,2	2,68	**3,02**	0,19	6,3
$C_{12}H_9ClN_2O_3$		21,23	k.A.	3,8	4,37	3,84	4,37	4,37	3,84	**4,10**	0,28	6,7
$C_9H_{11}Cl_3NO_3PS$		22,59	5,27	5,3	4,7	4,78	4,77	4,77	4,78	**4,96**	0,26	5,3
$C_{13}H_{19}N_3O_4$		22,94	k.A.	5,2	5,18	4,82	5,56	5,56	4,82	**5,19**	0,26	5,0

Outlook – charged molecules (logP/logD)

Standard	Ladung pH 3	Ladung pH 5	Ladung pH7	Theoretischer logP [1]	Calc. logP MeOH, pH 3	Calc. logP MeOH, pH 7
Diclofenac	neutral	negativ	negativ	4.26	3.44	2.45

Standard	logD [2] pH 3	logD [2] pH 5	logD [2] pH7
Diclofenac	4.22	3.21	1.37

[1] www.chemicalize.org
[2] http://www.chemaxon.com/marvin/sketch/index.jsp

Projektteam