NORMAN Non-Target Screening Trial

Non-target screening of organic substances in river water samples

WRAP UP

NORMAN SOLUTIONS Workshop on Non-Target Screening
Towards the harmonisation of methods for
non-target screening of environmental samples

16–17 September 2014, Eawag, Dübendorf

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NORMAN Collaborative Trial on non target screening: *Background*

- Part of NORMAN JPA for 2013
  - Follow-up to the NORMAN-JRC workshop in Stresa (2010); need for comparison and harmonisation of non-target screening methods in Europe

- Organization, scientific and technical preparation, collection and evaluation of the results, preparation of the evaluation report, organisation of the related discussion workshop and dissemination of the results
  - EI, Eawag, UFZ, UMEA and LfU

- Synergy with the international Joint Danube Survey 3 organised by the International Commission for the Protection of the Danube River (ICPDR; August/September 2013)
  - Test material sampled within the survey

NORMAN CT – non target screening

Objectives

- Main objective:

  To draft recommendations by the NORMAN Association on the use of non-target and suspect screening for the identification of WFD river basin specific pollutants

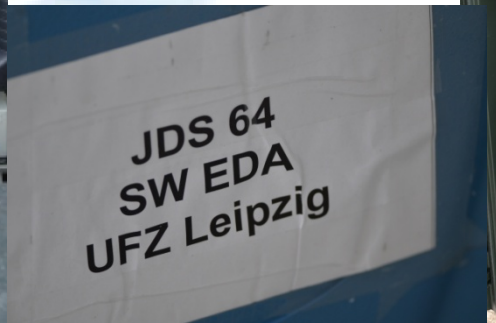
- Specific objectives
  - The analysis of samples using MS techniques established in each of the participating laboratories and declaration of
    - *How many substances are present in the sample, and*
    - *How many of them can be provisionally identified by suspect and non-target screening*
- NEW!!!
  - Training dataset for storage and re-processing of raw mass spectral data
  - Scientific publication(s)

NORMAN CT – non target screening

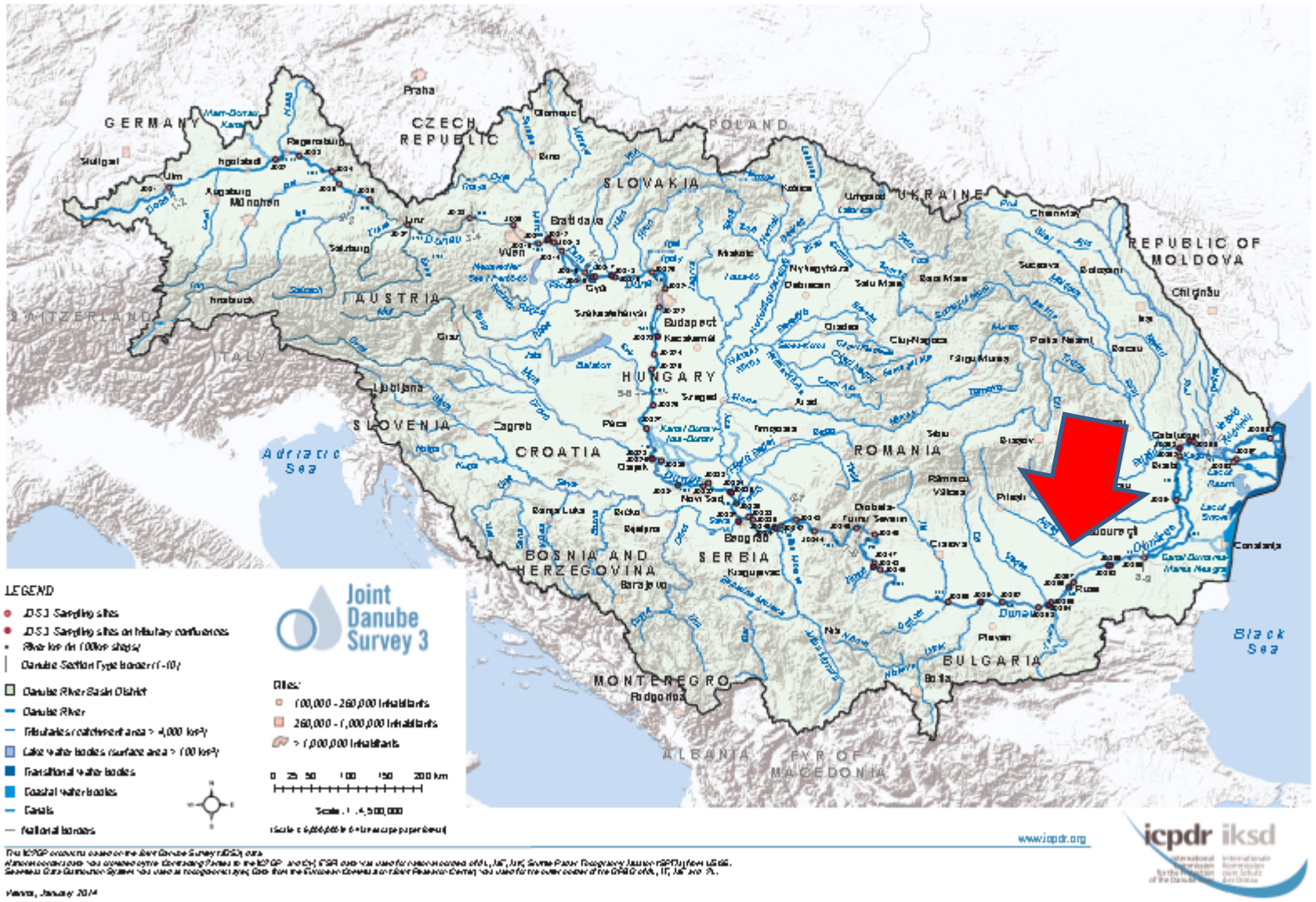
Setup

- *Sampling*
  - JDS57 - Downstream Ruse/Giurgiu (RO/BG; rkm 488), 18/09/2013
- *Sample preparation*
  - Large volume solid-phase extraction of 1000 liters of water sample
  - Freeze dried aliquot of 1.5 l water to each participant
  - Fabrication blank
- *Sample dispatch – UFZ to EI, EI to participants*
  - *Retention index mixtures: LC-MS UNI Munich; GC-MS retention indices EI*
- *Sample reconstitution*
  - *According to instructions, but based on the needs of the analytical method*
- *Analysis*
  - *LC-HR-MS(MS) / GC-MS*
  - *Within two days from sample arrival*
  - *Result submission – two month from dispatch of results*
- *Reporting – evaluation of results – final discussion meeting*

Large Volume Sampling



Joint Danube Survey 3 - Overview map



NORMAN CT – non target screening

Timetable of the exercise

Application deadline	15 October 2013
Sample distribution	December 2014
Result submission	mid March 2014
Final discussion meeting	mid/end June 2014 (September 2014)

Preparation of recommendation report	November 2014

Stability and homogeneity studies by EI and UFZ –
December 2013/January 2014

Expectations

- Agreement on:
  - Harmonised reporting formats
  - Workflows
  - Terminology
- Two?? publications
  - General
  - Mass spectrometry
- Follow up?

NORMAN CT – non target screening

Received datasets

	Name of organization / institute	Name of laboratory	Total DCTs	LC-MS	GC-MS
1	IAREN- Water Institute of the Northern Region	Laboratory of Chromatography			
2	NIVA	NIVA Oslo	1	1	
3	SUEZ Environment	CIRSEE	1	1	1
4	T. G. Masaryk Water Research Institue	Reference Laboratory for Environment Components and Waste			
5	University of Antwerp	Toxicological Centre	1	1	
6	Technische Universitaet Muenchen	Chair of Urban Systems Engineering	1	1	
7	University Jaume I (UJI)	Research Institute for Pesticides and Water	1		1
8	EAWAG	Environmental Chemistry	1	1	
9	Rijkswaterstaat	Monitoring en Laboratorium	1	1	1
10	National and Kapodistrian University of Athens / Department of Chemistry	Laboratory of Analytical Chemistry	1	1	1
11	NILU-Norwegian Institute for Air Research	Department of Environmental Chemistry			
12	University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental protection	Laboratory for Environmental Chemical Analysis			
13	Veolia Environnement Recherche & Innovation	Pole Analyse Innovation Chimie	1	1	1
14	Ministry of the Environment of Canada	Laboratory Services Branch			
15	BRGM	Laboratory Division			
16	IRSTEA	LAMA	1	1	
17	Environmental Institute (EI), SK	Analytical Laboratory	1		1
18	Helmholtz-Centre for Environmental Research - UFZ	Effect-directed analysis	1	1	
19	University of Padua / Department of Chemistry	Group of Analytical Chemistry	1	1	
20	University of Bordeaux	team LPTC, laboratory EPOC (UMR 5805 CNRS)			
21	Masaryk University / Faculty of Science	RECETOX			
22	Bundesanstalt für Gewässerkunde	Gewässerchemie	1	1	
23	Zweckverband Landeswasserversorgung	Betriebs- und Forschungslaboratorium	1	1	
24	Croatian Waters	Central Water Management Laboratory	1	1	
25	University of Tuebingen	Environmental Chemistry	1	1	
26	University of Umea	Department of Chemistry	1		1
			18	**16**	**7**

LC-MS – Re-categorization of results

	Methods_original	Methods_re
NIVA	4	4
SUEZ-CIRSEE	8	8
UniAntw_TC	4	4
TUM	2	2
EAWAG	1	1
RWS	36	1
UoA	9	3
VEOLIA	1	1
IRSTEA	1	1
UFZ	4	4
UniPad	9	7
BFG	1	1
LW	2	2
CW	240	1
UniTueb	1	1
UJI	48	2
	371 →	**43**

Target + Suspect	Non-target + Unknown
34	3174
92	274
11	8
74	0
161	8536
0	36
169	1661
17	0
27	3
72	52
8	92
3	4057
126	2028
229	10
18	1432
31	17
1072	**21380**

Summary – methods used

Institute		Liquid chromatograph								Mass spectrometer						
name	no	column				type	solvent	flow	temp	inj vol	type	MS1				MS2	
		type	L [mm]	ID [mm]	PS [µm]			[ml/min]	[°C]	[µl]		scan range [m/z]	Resolution	acc [ppm]	Ionz	Mode	Type
BFG	1	**RP-C18**	150	2	3	**HPLC**	H2O/ACN (FA)	0.2	30	10	**q-ToF**	100-1200	30,000	5	ESI±	CID	
Eawag	2	**RP-C18**	50	2	3.5	**HPLC**	H2O/MeOH (FA)	0.2	30	20	**Orbitrap**	100-1000	140,000	5	ESI±	HCD	DD/DIA
TUM	3	**RP+ HILIC**	150	2	5/2.7	**LC + LC**	H2O/ACN (NH4ac)		25	10	**ToF**	100-1700		5	ESI±	-	
Croatian Water	4	**RP-C18**	150/100	2	1.8	**U-HPLC**	H2O/MeOH (NH4ac)	0.4	50	100	**q-ToF**	100-1000	40,000	<5	ESI±	CID	DD/DIA
IUPA Spain	5	**RP-C18**	100	2	1.7	**U-HPLC**	H2O/MeOH (FA)	0.3	40	50	**q-ToF**	50-1000	20,000	5	ESI±	CID	
Langenau	6	**RP-C18**	150	2	3.5	**HPLC**	H2O/ACN (FA)	0.3	40	5	**q-ToF**	100-1200			ESI±	CID	
NIVA	7	**RP-C18**	100	2	1.7	**U-HPLC**	H2O/MeOH (NH4ac)	0.45	50	5	**q-ToF**	50-1200	22,500	5	ESI±	CID	
RWS	8	**RP-C18**	150	2	3.5	**HPLC**	H2O/MeOH (FA)	0.2	35	10	**Orbitrap**	50-1000	30,000	5	ESI±		
UFZ	9	**RP-C18**	100	3	2.6	**HPLC**	H2O/MeOH (FA)	0.2	22	10	**Orbitrap**	100-1000	100,000	3	ESI±	HCD	
Veolia	10	**RP-C18**	100	2	3	**HPLC**	H2O/MeOH (FA)	0.2	35	20	**Orbitrap**	80-1500	30,000	5	ESI±	CID	
UniAntwerp	11	**RP-C8**	150	2	3.5	**HPLC**	H2O/ACN (NH4ac)	0.25	50	10	**q-ToF**	50-1300		5	ESI±	CID	
UniPadua	12	**RP-C18**	100	2	1.7/2.6	**U-/HPLC**	H2O/ACN (FA)	0.3	30	40	**q-ToF**	50-2000	18,000		ESI±	CID	
UniTübingen	13	**RP-C18**	50	2	1.8	**U-HPLC**	H2O/ACN (FA)	0.5	40	2	**q-ToF**	50-1200	20,000	1	ESI±	CID	
UniAthen	14	**RP-C18**	100	2	3	**HPLC**	H2O/ACN (FA)	0.2		10	**QqQ**		unit		ESI±	CID	
	14	**RP-C18**	100	2	2.2	**HPLC**	H2O/MeOH (NH4fa)	0.2-0.5	30	10	**q-ToF**	50-1000	40,000	<2	ESI±	CID	
SUEZ	15	**RP-C18**	50	2	1.9	**HPLC**	H2O/MeOH	0.3	30	5	**Orbitrap**	70-1000	70,000	5	ESI±	HCD	

GC-MS – Re-categorization of results

Participant	Methods Original	Methods	Target	Suspect	'Non-target - Unknown'	Target + Suspect	'Non-target + Unknown'
SUEZ-CIRSEE	1	1			15		15
UJI	28	5	9	9	10	18	10
RWS	40	3		40		40	
UoA	1	3	8	10		18	
VEOLIA	3	1		31		31	
EI	10	2	10	5	91	15	91
UmU	1	1	58	168		226	
	84 →	**16**	**85**	**263**	**116**	**348**	**116**

WRAP UP

- CT - good experience for many labs
- GC-MS and LC-MS **complementary**
- LC-MS provides much more information but not yet fully used
- HRMS and MS/MS needed for real non-target analysis
- **Target** and **Suspects screening** dominates, almost no non-targets due to time limitation and missing automatic workflows
- Clear terminology important: definitions agreed on -linked to **confidence levels**
- **Harmonisation of reporting format and workflow** needed so that automatic upload is possible
- **Storage of the data for retrospective analysis?**

WRAP UP GC-MS

- **Soft ionization** (APCI, PCI, soft EI) and HRMS would be useful for suspect/NT screening
- Need for more specific **EI libraries, APCI libraries, exact mass libraries**
- Increased confidence
  - **RT prediction** for both GC and GCxGC useful
  - **Retention Index (RI)** to be included in the workflow
- Selection of **common quantification internal standard** for semi-quantification

- Different workflow for low resolution GC and LC??
- Consider sample reconstitution – loss of compounds especially for GC analysis

WRAP UP LC-MS

- **Workflow –**
  - **C18 columns** –first choice
  - Consider special columns for **complementary range of polar compounds** in a separate run (HILIC or mixed mode phases)
- Micro and nano flow LC to be considered (not routinely used yet; advantage - matrix suppression)
- Use of **normal HPLC or long run UHPLC** preferred to short run UHPLC in NT screening (to get all info in one run; matrix effects enhanced in UHPLC)
- Selection of **solvent** (AcN, MeOH) – probably MeOH preferred
  - Better for NI depending on the source
  - Good to use one solvent only for building RTI libraries
  - Purity of the solvent essential (LC-MS grade)

WRAP UP LC-MS

- **Injection volume** - as much as possible
- **Resolution** of MS – what is good enough for enviro analysis
  - Go for the highest resolution on your instrument affordable by your chromatography (40000 - 60000 a good compromise)
  - Mass accurracy to be considered
- **QqQ not to be used for NT screening**
- **ESI in PI/NI** preferred
  - SW needed to bring the results together
- **MS/MS in NT screening** – data independent MS/MS preferred (quantitation of fragments)??; data dependent – reinjection of samples – state of the art
- **Mass range** – 50 – 2000 optimal (MS/MS range to be defined)

WRAP UP - **Target screening**

- **Quantitative target analysis** preferred vs. target screening – terms to be clearly distinguished
- **MS/MS needed** (in some cases extracted ion chromatogram and RT sufficient), identification points required
- The wider **target list** the better

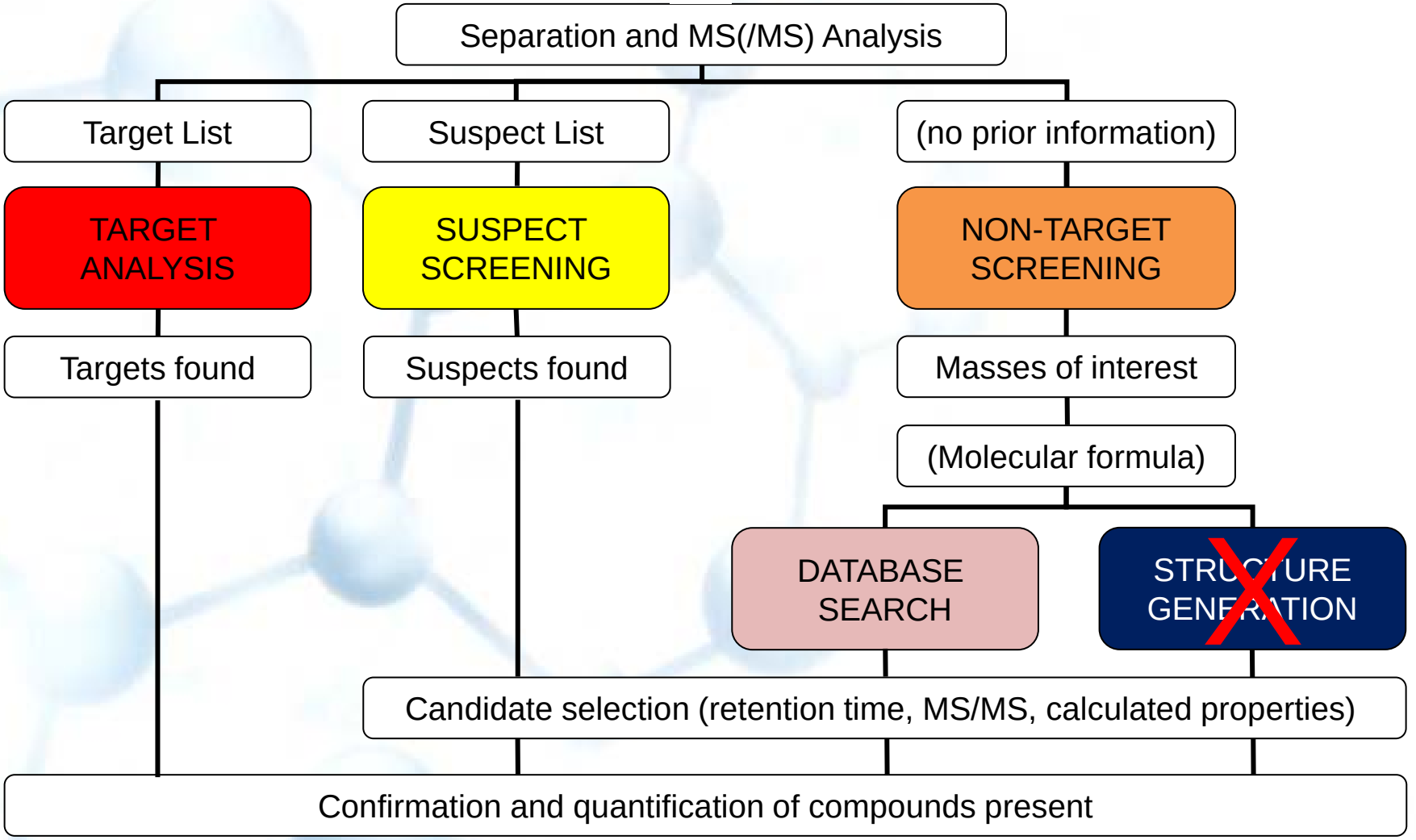
WRAP UP - **Suspect screening**

- Match exact mass and isotope pattern
- Follow by MS/MS and RT characteristic
- How to compile suspect list? **Screen 'big' vs. 'smart'**
  - Add specific info to each compound (pharmaceuticals, ionisation type of expected pollution, usage, found in real world samples...)
  - Create platform for all existing databases (NORMAN, StoffIdent, **sharing in-house databases**...)

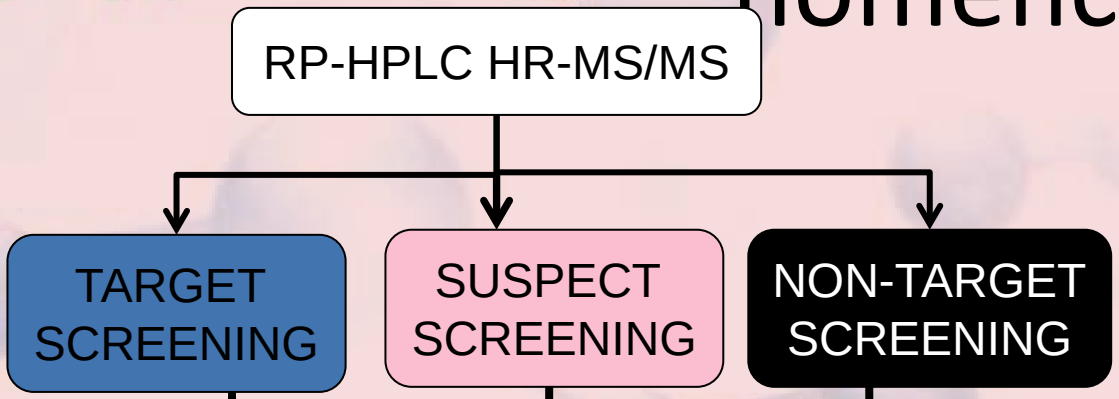
WRAP UP

- **NON-TARGET ANALYSIS/SCREENING**
  - No difference between unknown and non-target
  - Confidence levels 1-5 (Schymanski et al.) a good start for categorisation of substances (by all labs)
- Retention time information (for LC-MS)
  - Not widely used – too much uncertainty
  - Still useful for selection of candidates (eliminating false positives)
  - More work needed
  - Correlation done by each lab independently by its own standard set, comparison through logD values or normalised RTI values

Status quo of identification approaches for LC-MS



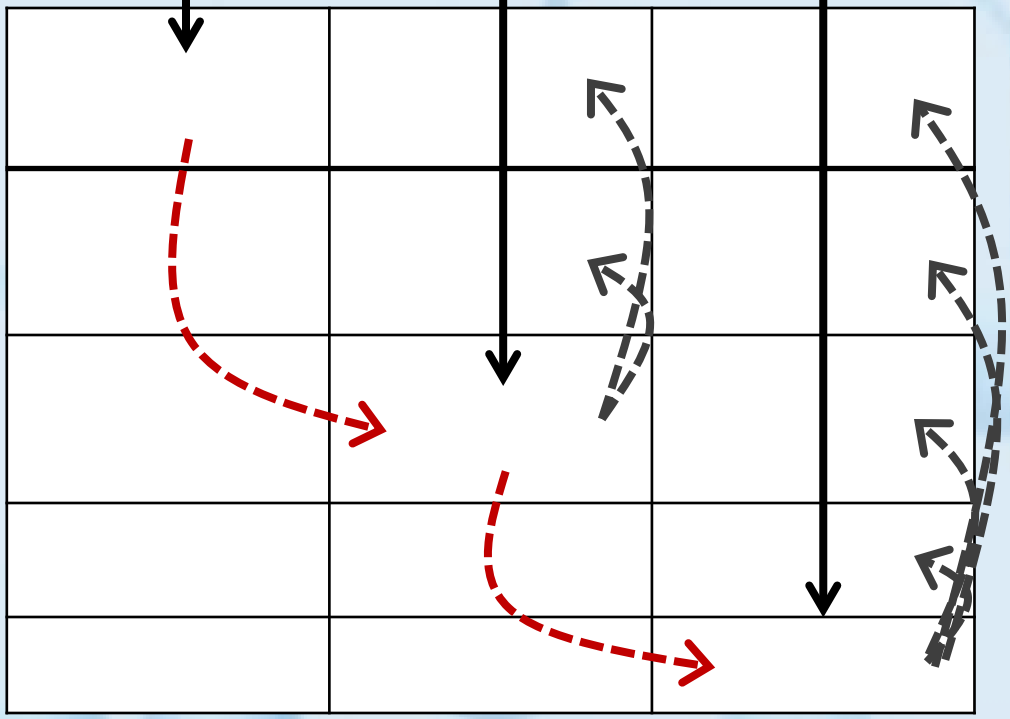
Workflow nomenclature



Available data before analysis:

- List of targets
- List of suspect

Available data after analysis:



Identification confidence

Level 1: Confirmed structure by reference standard

Level 2: Probable structure by library/*diagnostic evidence*

Level 3: Tentative candidate(s) structure, substituent, class

Level 4: Unequivocal molecular formula

Level 5: Exact mass of interest

WRAP UP

- Next CT
  - **Goal NT screening – FAILED**
  - Invest even more effort into harmonisation of target/suspect screening before going into NT
  - Guidance document – Core Team drafting sending around for confirmation
  - Next time sample also specifically for GC-MS (spiked with IS for RT comparison)
  - PROPOSAL - **Use current data for NT screening in 2015**

Lessons learned

- **First** non-target trial ever!!!
  - "**Collaborative trial**", not a competition => no winners, no losers *BUT* concrete aims and comparisons are needed to learn from the experiences of all participants
- Very ambitious trial with a **huge scope**
- **Timing**: much more work as expected… resulted in late arrival of samples and incomplete data evaluation, time buffers would help
- Simplify data reporting: ***Make life as easy as possible for the participants!***

NORMAN - NEXT STEPS

- Report
- Publication in special issue on HRMS in Anal. Bioanal. Chem. – Deadline January 2015
- Guidance document - harmonisation of methods for non-target screening techniques
- Who wants to join the NORMAN Expert Group on non-target screening
  - Harmonisation, prioritisation

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