



Danube River Basin: chemical and *in vitro* bioassays screening of waste water effluents – applying NEREUS guidelines

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NORMAN/AQUALity Workshop on prioritisation
of emerging contaminants in urban wastewater,
Paris, 6 March 2019





WW effluents sampling campaign in the DRB - 2017

Product of:
ICPDR (International
Commission for the
Protection of the
Danube River), Vienna

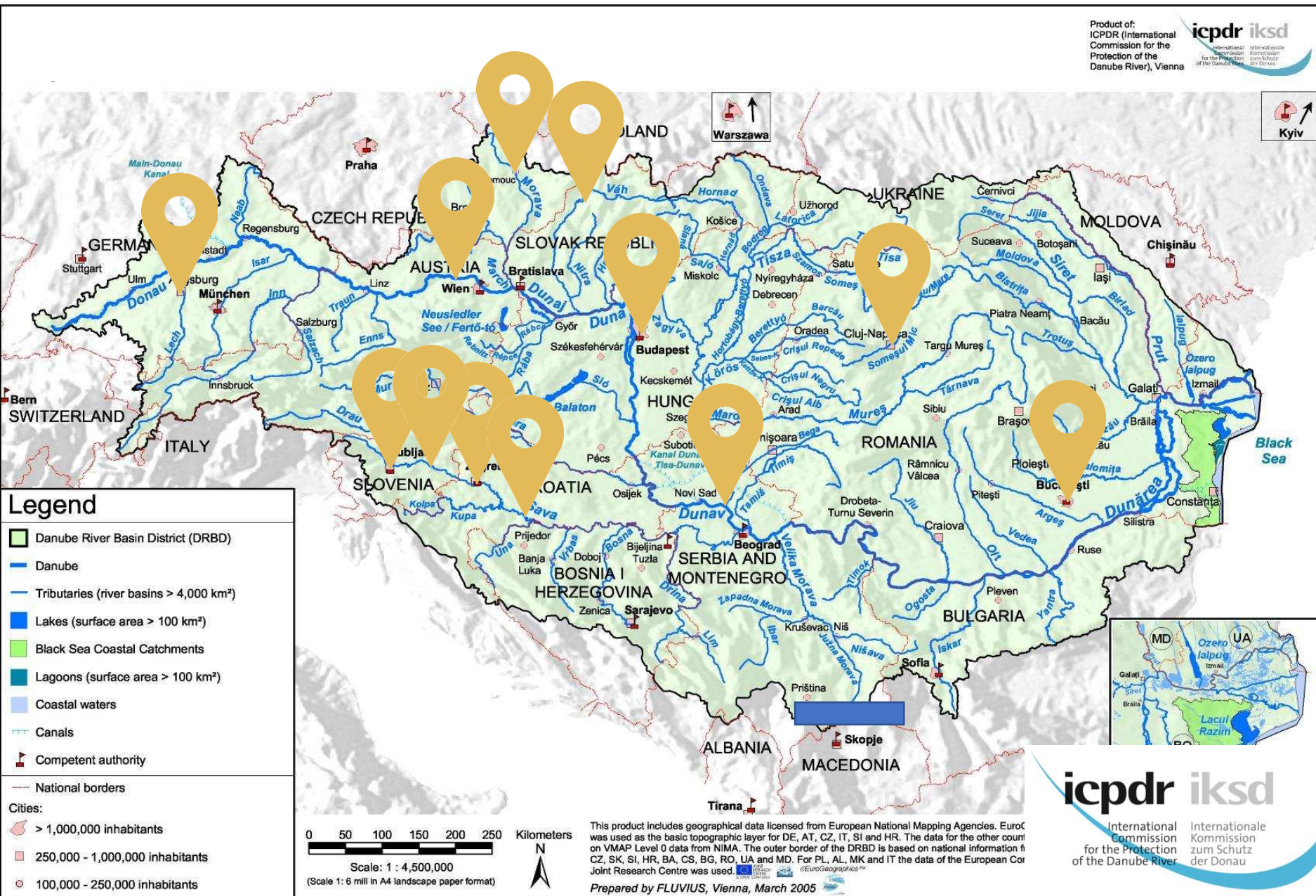
icpdr iksd
International
Commission
for the Protection
of the Danube River
Internationale
Kommission
zum Schutz
der Donau



Kyiv



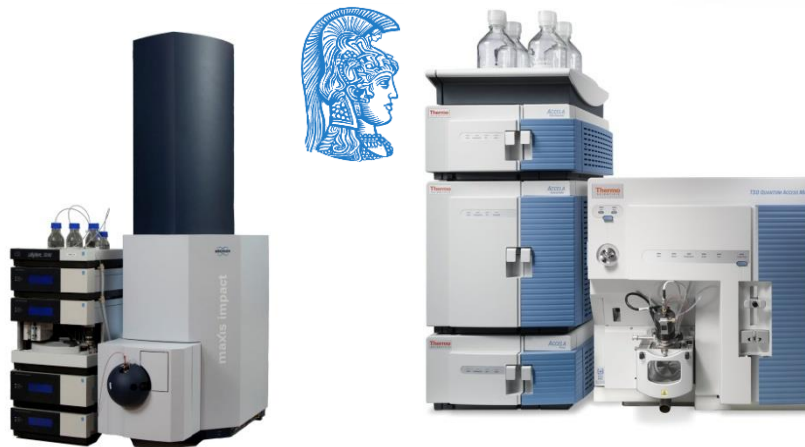
Warszawa



WWTPs in the DRB selected for WW effluent monitoring

Country	Town	PE	Treatment type
Romania	Bucharest	1,327,995	tertiary
Romania	Cluj-Napoca	382,031	tertiary
Serbia	Šabac	84,000	tertiary
Croatia	Varaždin	97,450	secondary
Croatia	Zagreb	842,425	secondary
Slovenia	Ljubljana	537,712	secondary
Slovenia	Vipav	152,487	tertiary
Hungary	Budapest	1,174,643	tertiary
Slovak Republic	Žilina	139,934	tertiary
Czech Republic	Brno-Modřice	397,945	tertiary
Austria	Amstetten	150,000	tertiary
Germany	Augsburg	659,387	tertiary

Sampling campaign in the Danube River Basin



Chemical analysis

Highly sensitive determination of antibiotics and their TPs

Target screening of 2248 compounds
Suspect screening (>40,000 substances)
Non-target screening

Upload of the chromatograms to the DSFP



Bioassays

CALUX ER α CALUX Nrf2
CALUX anti-AR CALUX Cytotox
CALUX anti-PR
CALUX GR
CALUX PPAR α 2
CALUX PPAR γ 2
CALUX PAH
CALUX PXR

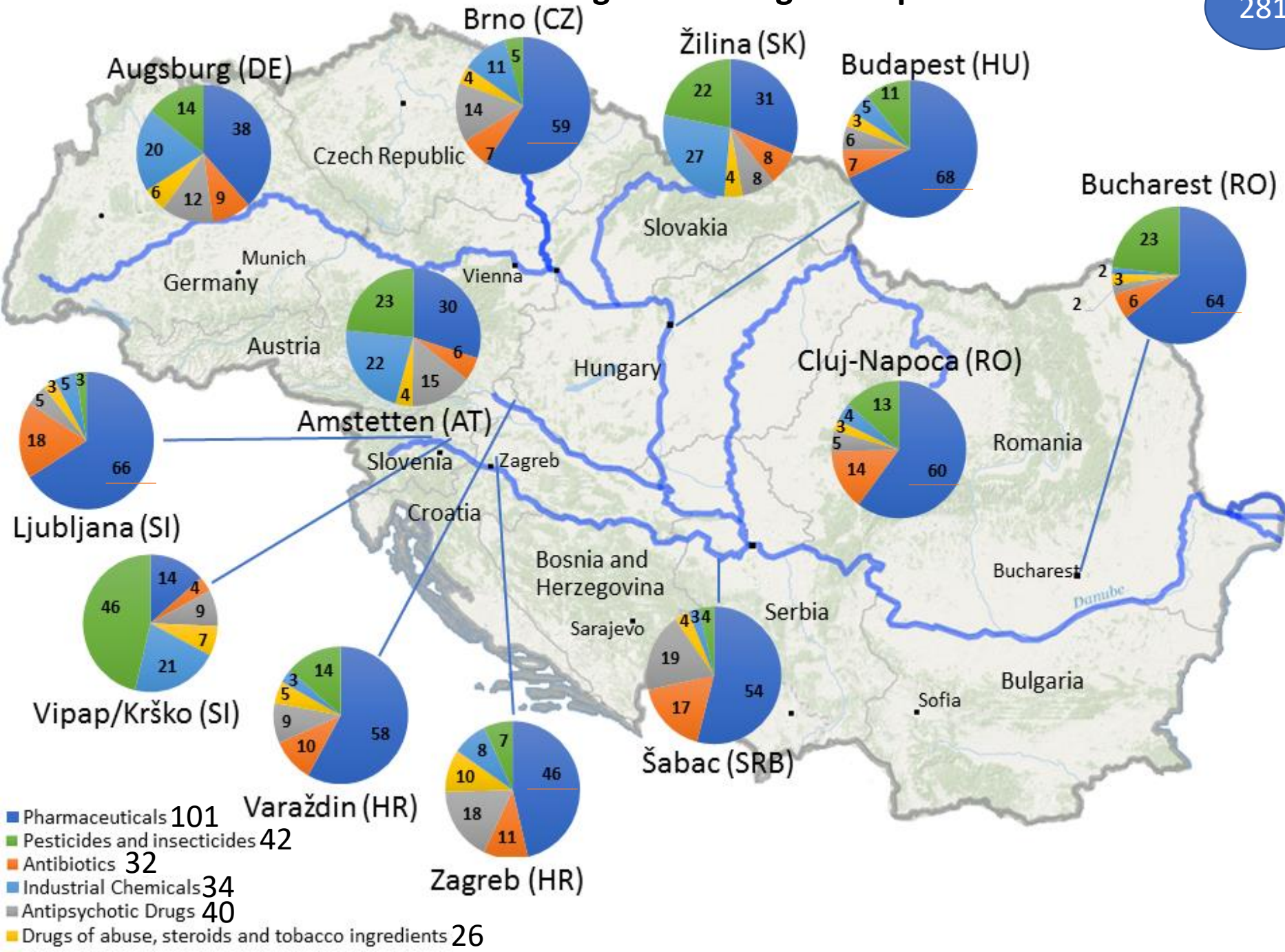


Genomics

Determination of ARGs by multiplex-PCR

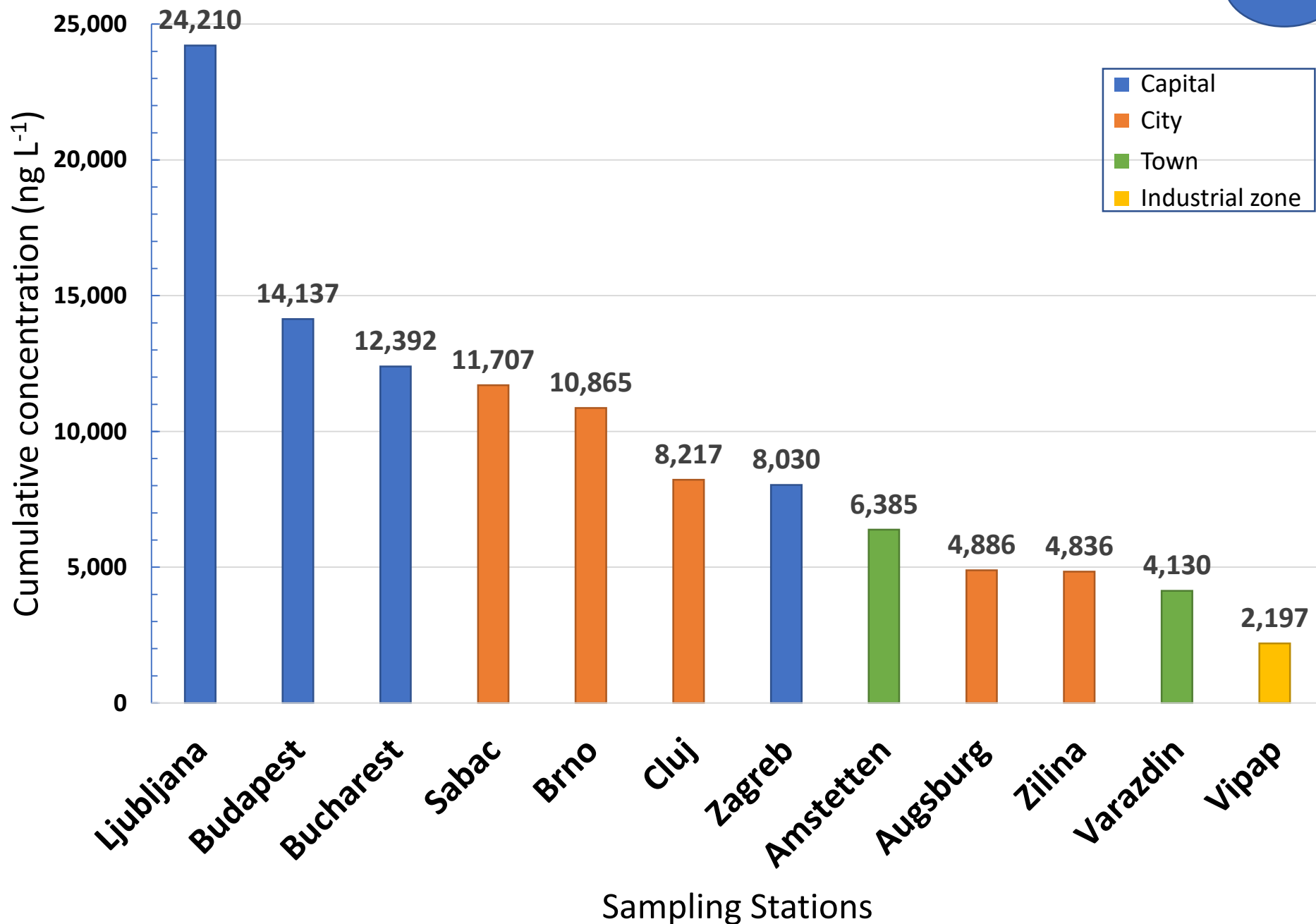
Int1	MecA	blaKaPC
Sul1	qnrS	VanB
TetM	blaSHC	
ermB	Aph3a	
ermF	TetB	
blaOXA	VanA	

% of detected categories of target compounds



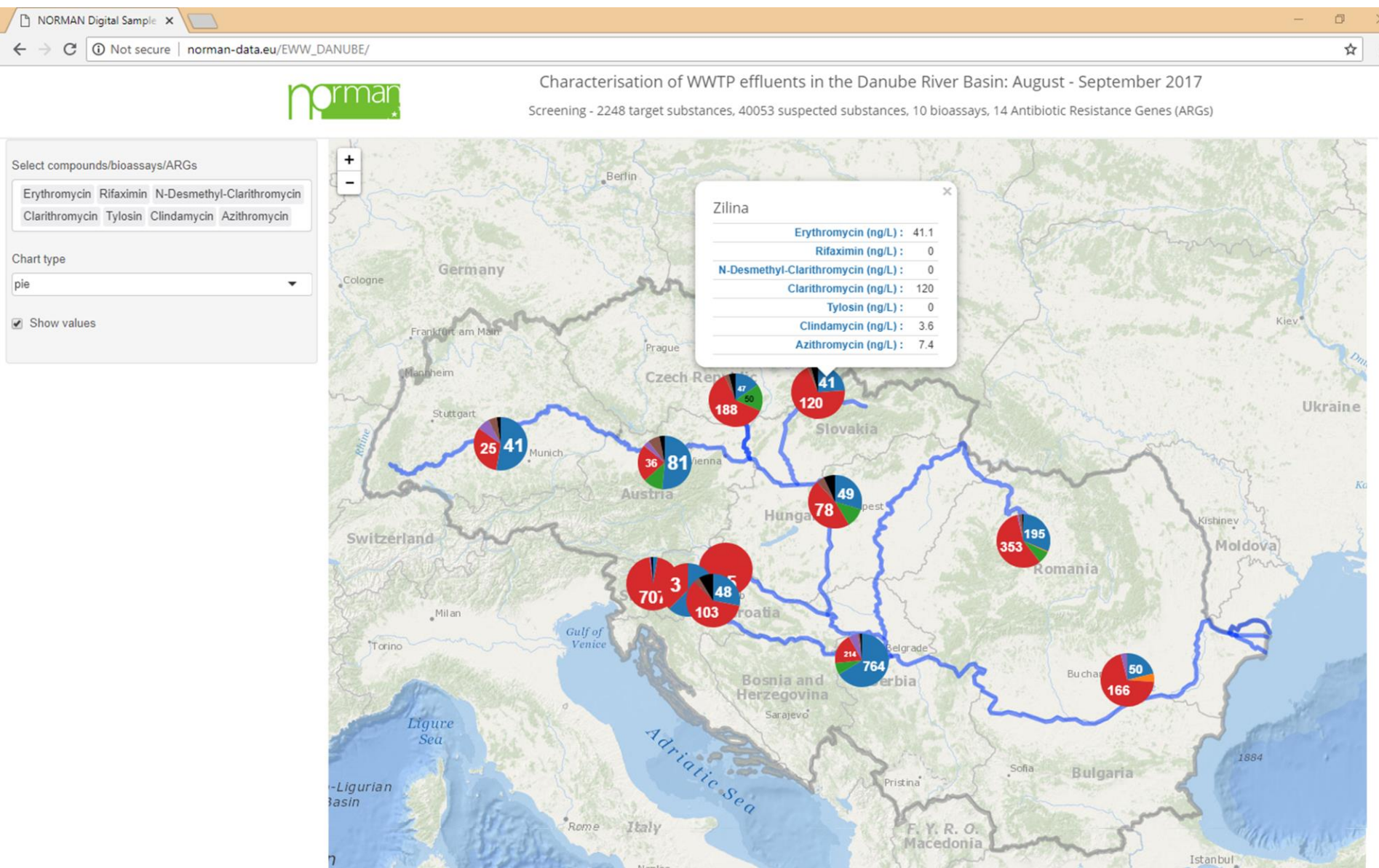
Cumulative concentrations of the detected target compounds

281

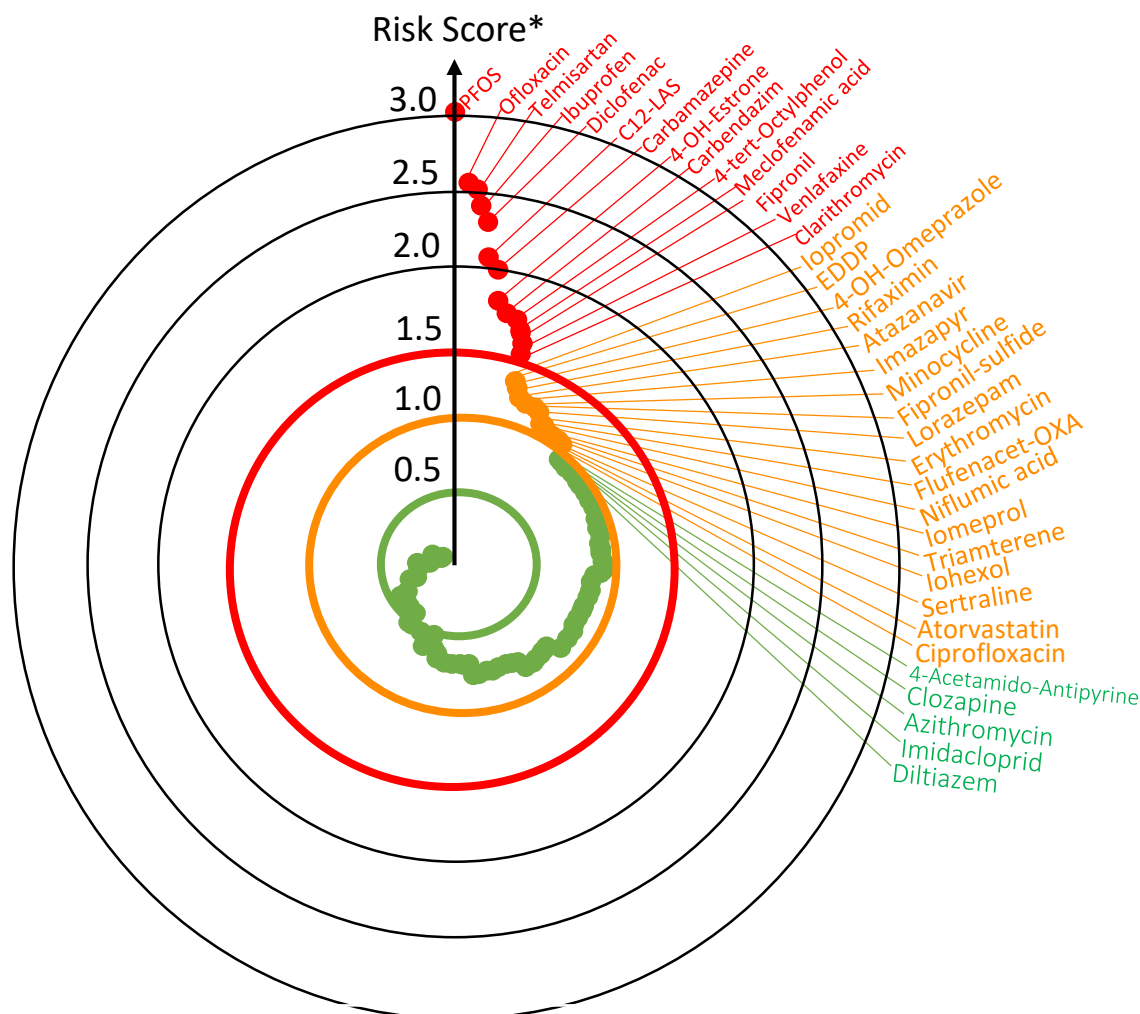


On-line interface – wide-scope target screening data

www.norman-data.eu/EWW_DANUBE/



Chemical risk assessment



Top compounds

PFOS

Ofloxacin

Telmisartan

Ibuprofen

Diclofenac

C12-LAS

Carbamazepine

4-OH-Estrone

Carbendazim

4-tert-Octylphenol

Meclofenamic acid

Fipronil

Venlafaxine

Clarithromycin

* **Risk Score (0 to 3)** = Frequency of Appearance (0 to 1)
 + Frequency of PNEC exceedance (0 to 1)
 + Extent of PNEC exceedance (0 to 1)


Storage of chromatograms for retrospective screening – NORMAN Database System

DSFP - <https://www.norman-network.com/nds/>

NORMAN Digital Sample X

← → ↻ ⓘ Not secure | norman-data.eu/DigitalSFP/

☆



Main Page

Batch mode

Contribute

More tools ▾

Substance name or CAS or StdInChIKey

Compound ▾

OR

Precursor m/z

254.059389

Mass error in Da ☐ Switch to ppm

0.003

0.005 0.01 0.015 0.02 0.03 0.035 0.04 0.045 0.05

0.05

RTI Tolerance (%)

0

20

100

0 10 20 30 40 50 60 70 80 90 100

Submit Job

Contributed Samples

Results

Chromatograms

Interactive Map

Help

Show

10 ▾

 entries

Search:

☐ Select per page

		Country	Matrix	Project
	All <input type="text"/>	All <input type="text"/>	All <input type="text"/>	All <input type="text"/>
543	<input type="checkbox"/> EI_LC-ESI-QTOF_Effluent wastewater Stuttgart Muhlhausen_Stuttgart_Germany_03.05.2018_ITN ANSWER_28543.xlsx	Germany	Water-Waste water-Municipal	ITN ANSWER
541	<input type="checkbox"/> EI_LC-ESI-QTOF_Procedural Blank wastewater from Germany_Gottingen_Germany_03.05.2018_ITN ANSWER_28513.xlsx	Germany	Water-Waste water-Municipal	ITN ANSWER
539	<input type="checkbox"/> EI_LC-ESI-QTOF_Effluent wastewater Bondorf Hallfingen_Herrenberg_Germany_03.05.2018_ITN ANSWER_28545.xlsx	Germany	Water-Waste water-Municipal	ITN ANSWER
535	<input type="checkbox"/> EI_LC-ESI-QTOF_Effluent wastewater Eutin_Neustadt_Germany_03.05.2018_ITN ANSWER_28541.xlsx	Germany	Water-Waste water-Municipal	ITN ANSWER
533	<input type="checkbox"/> EI_LC-ESI-QTOF_Effluent wastewater Wevelinghofen_Grevenbroich_Germany_03.05.2018_ITN ANSWER_28539.xlsx	Germany	Water-Waste water-Municipal	ITN ANSWER
531	<input type="checkbox"/> EI_LC-ESI-QTOF_Effluent wastewater Lauterhofen dry weather_Amberg_Germany_03.05.2018_ITN ANSWER_28537.xlsx	Germany	Water-Waste water-Municipal	ITN ANSWER
529	<input type="checkbox"/> EI_LC-ESI-QTOF_Effluent wastewater Schonerlinde_Berlin_Germany_03.05.2018_ITN ANSWER_28535.xlsx	Germany	Water-Waste water-Municipal	ITN ANSWER
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525	<input type="checkbox"/> EI_LC-ESI-QTOF_Effluent wastewater Hattingen_Hattingen_Germany_03.05.2018_ITN ANSWER_28531.xlsx	Germany	Water-Waste water-Municipal	ITN ANSWER
523	<input type="checkbox"/> EI_LC-ESI-QTOF_Effluent wastewater Lauterhofen_Amberg_Germany_03.05.2018_ITN ANSWER_28529.xlsx	Germany	Water-Waste water-Municipal	ITN ANSWER

Showing 1 to 10 of 306 entries

Previous

1

 2 3 4 5 ... 31 Next

Create Results Visualization

>3 fragments

Yes

330

300

10

10

65

Chemical Class	Number of Structures
4-Acetamidoantipyrine	1000
4-Formylaminoantipyrine	1000
Metoprolol	500
Atenolol	500
TBEP (Tris(2-butoxyethyl) Phosphate)	1000
Lamotrigine	1000
Iorophenoxy)-1-(1H-imidazol-1-yl)-3,3-dimethyl-	500
Bis-(2-ethylhexyl) phthalate	1000
Di-n-octyl phthalate	1000
Tolyltriazole	1000
Ranitidine	1000
Amisulpride	500
Oleic acid	500
Sotalol	500
Carbamazepine	1000
Sulfapyridine	1000
Galaxolidone	1000

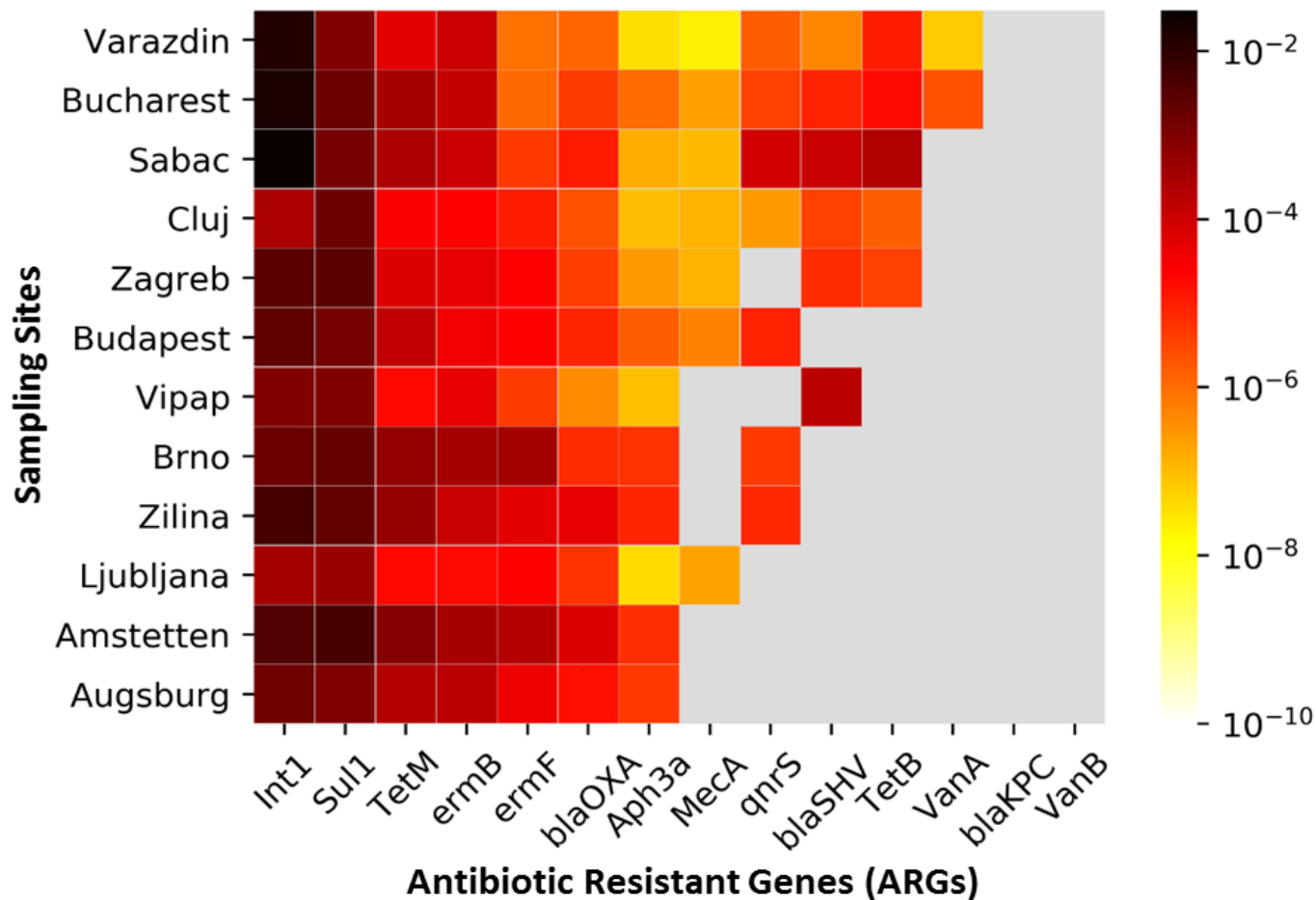
✓ 249 (177+72) compounds with experimental HRMS/MS spectra (>2 experimental fragments)

✓ 95 (72+23) compounds with predicted HRMS/MS spectra (≥ 5 in-silico predicted fragments)

[illegible]

Sabac (SRB)
Zilina (SK)
Krsko (SI)
Ljubljana (SI)
Cluj-Napoca (RO)
Bucharest (RO)
Budapest (HU)
Zagreb (HR)
Varazdin (HR)
Augsburg (DE)
Amstetten (AT)

Concentration of ARGs [Gene copies/16S rRNA]



Int1: Measure of mobile resistant elements present

Sul1: ARG against **sulfonamides**

tetM, tetB: ARG against **tetracycline**

ermB, ermF: ARG against **macrolides and lincosamide**

blaOXA, blaSHV, blaKPC, Meca: ARG against **beta-lactamase**

Aph3a: ARG against **aminoglycosides**

qnrS: ARG against **fluoroquinolones**

VanA, VanB: ARG against **vancomycin**

High correlation factors between A&ARGs in some cases; $r \approx 0.77$ for qnrS and sum of Oxolinic acid, Marbofloxacin, Enrofloxacin, Ofloxacin, Norfloxacin, Ciprofloxacin, Flumequine

Effect-based trigger (EBT) values

Mode of Action	Reference Compound	EBT	1-3 times EBT	3-10 times EBT	1-10 times EBT	> 100 times EBT
Estrogenicity ^a	ng E2 eq./l	0.4	0.4-1.2	1.2-4.0	4.0-40	>40
Anti-androgenicity ^b	µg Flutamide/l	3.3	3.3-9.9	9.9-33	33-330	>330
Glucocorticoid receptor activation	ng Dex./l	100	100-300	300-1000	1000-10000	<10000
Activation of peroxisome proliferator-activated receptor	ng Roz./l	36	36-108	108-360	360-3600	>3600
AhR receptor activation ^c	ng B(a)P/l	6.2	6.2-18.6	18.6-62	62-620	>620
Adaptive Stress (Nrf2)	µg dichlorvos/l	26	26-78	78-260	260-2600	>2600
Early warning chemicals: Activation pregnane x receptor (PXR)	µg DEHP/l	272	272-816	816-2720	2720-27200	>27200

Exceedance of EBTs

	ER? CALUX	anti-AR CALUX	GR CALUX	PPAR ? CALUX	PAH CALUX	Nrf2 CALUX	PXR CALUX
1 - Varazdin	5	5,7	<19	640	72	64	210
2 - Amstetten	1,1	22	<20	<520	122	71	320
3 - Cluj-Napoca	<0,06	31	34	<420	52	LOQ (<79)	210
4 - Augsburg	1	10	72	<410	72	71	330
5 - Vipap	0,65	32	<25	<460	242	120	1200
6 - Budapest	0,56	11	<23	<430	62	73	360
7 - Ljubljana	6,6	8,4	120	<350	62	78	240
8 - Bucharest	7,4	5,7	38	<340	82	200	250
9 - Zilina	2,2	8,9	78	<480	72	94	130
10 - Sabac	1,1	14	<41	<490	72	71	100
11 - Brno	0,54	13	47	<1100	122	130	430
12 - Zagreb	0,8	6	<42	<1100	52	LOQ (<26)	420

Response plan

- If the **measured value/EBT < 1**: no further action required.
- If **1 < measured value/EBT < 3**: quality check data, continue to monitor every three months, until 1 year and until the EBT < 1.
- If **3 < measured value/EBT < 10**: data check, immediate re-sampling and quantify specific target compounds which are known to cause the effects observed in the respective bioassay (toxicity drivers). Continue to monitor every three months, until 1 year and the EBT < 1.
- If **10 < measured value/EBT < 100**: all of the above plus enhance source identification program. Also monitoring of influent waste water to confirm the magnitude of assumed safety factors associated with removal efficiency by the available WWT technology and dilution in the receiving water body.
- If **measured value/EBT > 100**: all of the above plus immediately confer with the local environmental authority to determine the required response action. Confirm WWTP corrective actions through additional monitoring that indicates the measured value/EBT ratio is below at least 100.

Proposed list of performance-based indicator compounds to be included in wastewater effluent monitoring programmes – **treatment technology related, EU list**

Surrogate to bulk parameters e.g. turbidity, electrical conductivity, UV absorbance, TOC...

Indicator chemical	ELV= PNEC*dilution factor (ng/L)	Frequency	LOQ (ng/L)	References - analytical method
Biodegradable¹				
Acesulfam		Every 6 months	tba	Loos et al., 2013
Benzotriazole		Every 6 months	tba	Loos et al., 2013
Diclofenac		Every 6 months	tba	Loos et al., 2013
Gabapentin		Every 6 months	tba	Kasprzyk-Hordern et al., 2008
Trimethoprim		Every 6 months	tba	Kostich et al., 2014
Sulfamethoxazole		Every 6 months	tba	Göbel et al.,
Valsartanic acid		Every 6 months	tba	Schultz et al., 2010
Oxypurinol		Every 6 months	tba	Funke et al., 2015
Not biodegradable, but oxidizable²				
Carbamazepine		Every 6 months	tba	Loos et al., 2013
Difficult to degrade biologically; not amendable to chemical oxidation³				
TCEP (Tris (2-carboxyethyl)phosphine)		Every 6 months	tba	Loos et al., 2013
Sucralose		Every 6 months	tba	Loos et al., 2013

¹ Biodegradable during biofiltration or soil-aquifer treatment.

² Not degradable during conventional activated sludge treatment, biofiltration or soil-aquifer treatment, but amendable to chemical oxidation.

³ Not degradable during conventional activated sludge treatment, biofiltration or soil-aquifer treatment, not amendable to chemical oxidation.

Response plan

- If **$1 < \text{MEC}/\text{ELV} < 10$** : quality check data, continue to monitor every three months, until 1 year and the $\text{MEC}/\text{ELV} < 1$ and preferably is consistently less than 5 times the ratio of MEC/ELV .
- If **$10 < \text{MEC}/\text{ELV} < 100$** : data check, immediate re-sampling and analysis to confirm MEC, also monitor at the point of abstraction. Continue to monitor every three months, until 1 year and the $\text{MEC}/\text{ELV} < 1$ and preferably is consistently less than 5 times the ratio of MEC/ELV .
- If **$100 < \text{MEC}/\text{ELV} < 1,000$** : all of the above plus enhance source identification program. Also monitoring at a point of abstraction and in the distribution system closer to the point of exposure to confirm attenuation of CEC is occurring and to confirm the magnitude of assumed safety factors associated with removal efficiency, dilution and post-treatment.
- **$\text{MEC}/\text{ELV} > 1,000$** : all of the above plus immediately confer with the local environmental authority to determine the required response action. Confirm plant corrective actions through additional monitoring that indicates the CEC levels are below at least an MEC/ELV of 100.

Selection of performance-based indicator compounds

- Target substances chosen to assess treatment performance must **permanently occur** at concentrations significantly above their analytical method detection limit (preferably, the ratio between the measured environmental concentration and the method detection limit should exceed at least 10).
- **Appropriate and commercially available analytical methods** must exist to quantify the target contaminants in treated wastewater.
- Performance-based indicator chemicals used for monitoring should **broadly represent the range of physico-chemical and biological properties** affecting their removals by the various treatment processes within the treatment train.
- Substances with **toxicological relevance**.

Proposed list of chemicals to be included in wastewater effluent monitoring programmes – ecology related (RBSPs), national/RB list

Indicator chemical	ELV= PNEC*dilution factor (ng/L)	Frequency	LOQ (ng/L)	References - analytical method
Biodegradable¹				
RBSP1	tba	Every 6 months	tba	
RBSP2	tba	Every 6 months	tba	
...	tba	Every 6 months	tba	
RBSP6	tba	Every 6 months	tba	
Not biodegradable, but oxidizable²				
RBSP7	500	Every 6 months	tba	
...				
Difficult to degrade biologically; not amendable to chemical oxidation³				
RBSP9	tba	Every 6 months	tba	
RBSP10	tba	Every 6 months	tba	

Dilution factor: large rivers 10, small rivers 2

¹ Biodegradable during biofiltration or soil-aquifer treatment.

² Not degradable during conventional activated sludge treatment, biofiltration or soil-aquifer treatment, but amendable to chemical oxidation.

³ Not degradable during conventional activated sludge treatment, biofiltration or soil-aquifer treatment, not amendable to chemical oxidation.

Conclusions

- Proposal of:
 - EU list of performance-based indicator compounds
 - EU list of *in vitro* bioassays with EBTs to be monitored in WW effluents
 - National/RBS list of RBSPs to be monitored in WW effluents
 - Response plans
- Update of the UWWTD