



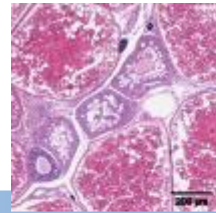


Methods development to detect antibiotic activity in water samples

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Mariel Pikkemaat (RIKILT)

Who is who?

- Grontmij|AquaSense
  - Consultancy and ecotoxicology laboratories
- Waterdienst
  - Directorate-General for Public Works and Water Management (Rijkswaterstaat)
- RIVM
  - National Institute for Public Health and the Environment
- RIKILT
  - Institute of Food Safety
- WUR
  - Wageningen University and Research

Presentation

- Introduction to study on emerging substances
- Method development
- Application (examples)
- Method optimization and outlook

Background…

- NORMAN has identified a list of the currently most frequently discussed emerging substances and emerging pollutants today. Examples of this list are surfactants, pharmaceuticals and personal care products, methyl tert-butyl ether (MTBE) and other related petrol additives and their degradation products, polar pesticides and their degradation products and various proven or suspected endocrine disrupting compounds (EDCs). Another example is nanoparticles, which behave aerodynamically like gas molecules and have a large surface area per unit mass.

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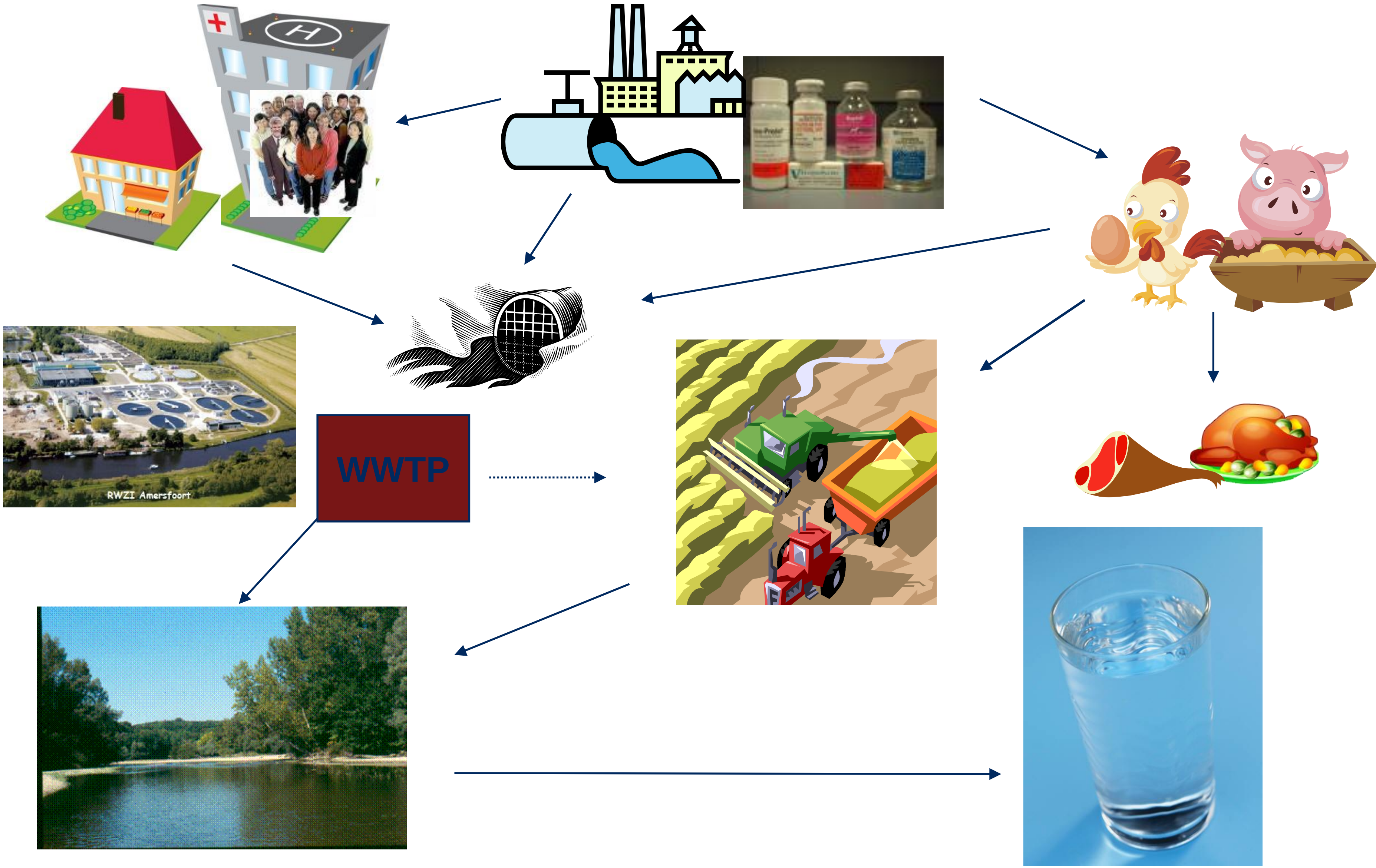
'New substances', ask for 'new methods'

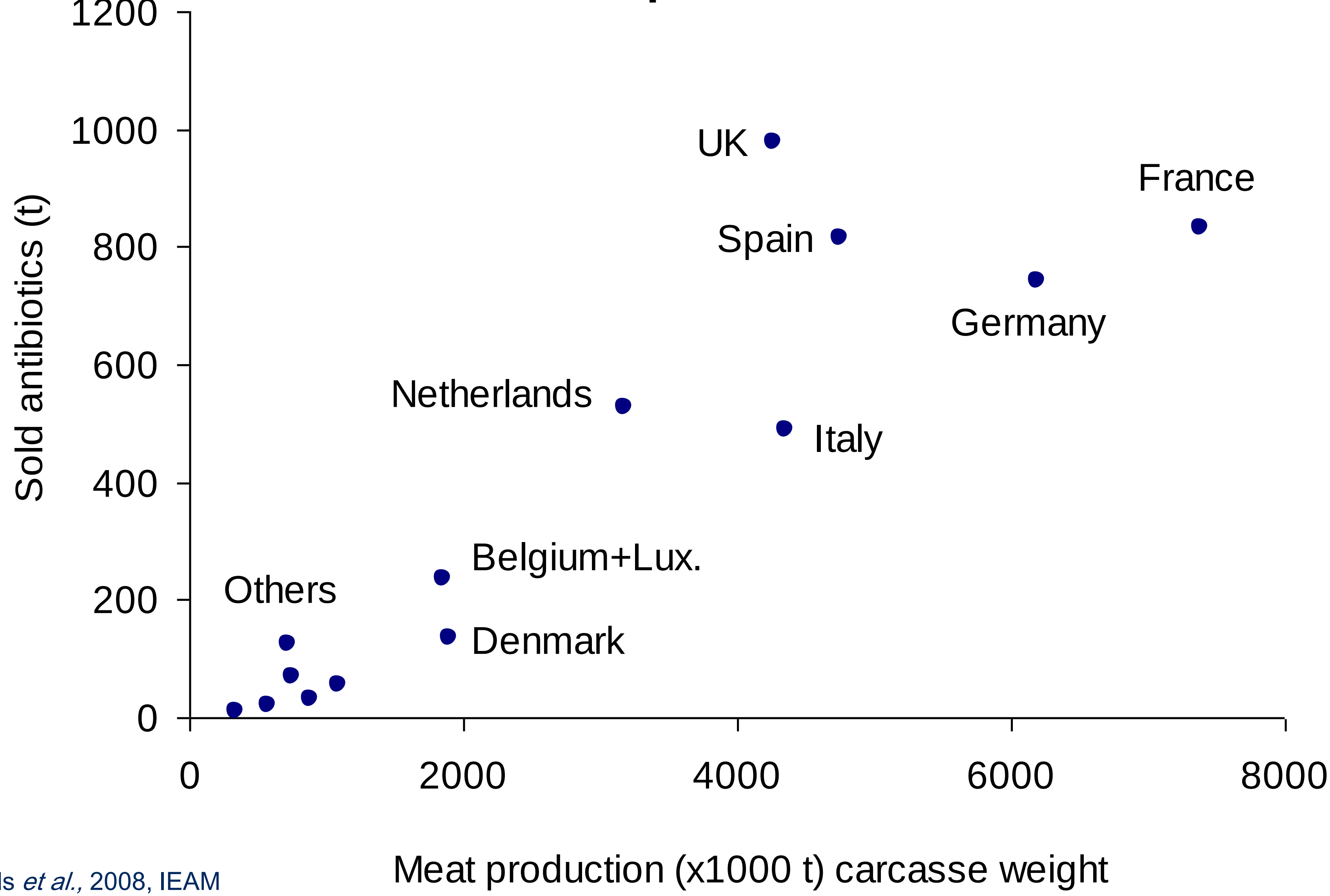
Pharmaceuticals – antibiotics

- Oroginally – natural substances against bacteria, fungi, protozoa:
  - Bactericidal – kill microbes
  - Bacteriostatic – inhibit grotwh
- 1928 – 1st antibiotic → penicillin
- Worldwide use: '*antibiotic era*'
  - Treatment in human and veterinary medicine
  - Threat: antibiotic resistance – prudent use
  - In veterinary medicine for prevention and as growth promoters (2004 stop in EU)

Antibiotics – fate and distribution

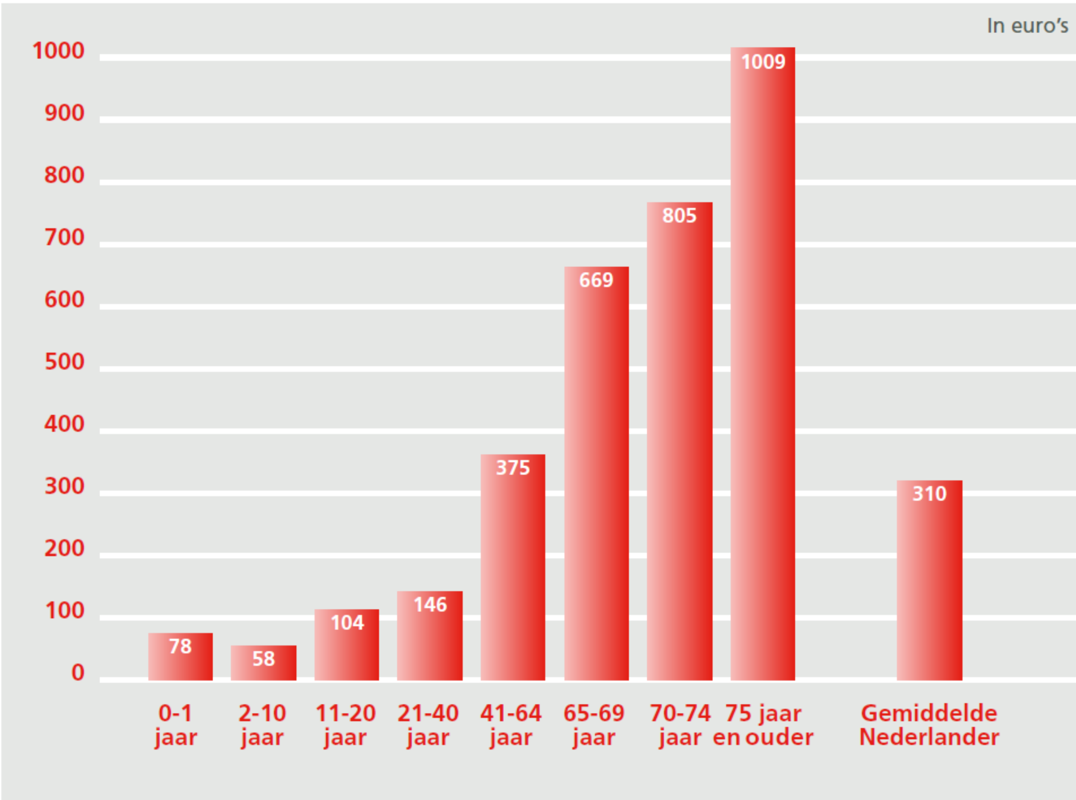


Relation between antibiotics and meat production



Kools *et al.*, 2008, IEAM

Use in households (NL)



Bron: Stichting Farmaceutische Kengetallen

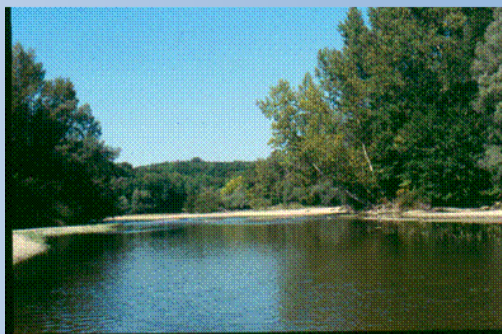
- Older population uses more medicines
- Expected life 2005-2025
  - >65 yr: +60%

Apples and oranges?

Antibiotics groups

- Aminoglycosides (A)
- Macrolides (M)
- Beta – lactams (B)
- Quinolones (Q)
- Sulfonamides (S)
- Tetracyclines (T)




Bioassay measurement

- Chemical analysis:
    - accurate identification? metabolites?

- Bioassays
    - Potential identification of activity
        - Cf. Steroids (Estrogens, Androgens, etc).
        - Cf. Dioxines
    - Including active metabolites

Methods

- Developed at RIKILT (Food Safety)

- **The Nouws antibiotic test (NAT)**
  → **slaughter animals** (residue analysis)

- 5 test plates – group-specific identification
  - T-plate; Q-plate; M&B-plate; A-plate and S-plate

- PRINCIPLE
  - (Specific) BACTERIAL GROWTH INHIBITION IN PRESENCE OF ANTIBIOTICS

Water – Scan Test

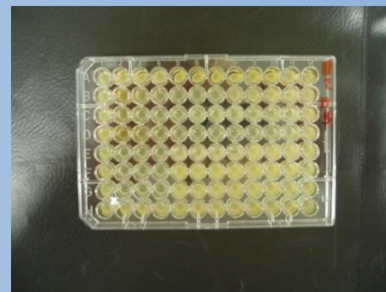
- Antibiotic present → growth inhibition
- 2 – 16 mm → linear correlation between inhibition zone and concentration

The tests

- Different agar compositions were used with different bacteria strains, antibiotic supplements to prepare them
- Applying samples on plates → incubate at 30 C (T, Q, M/B)
  37 C (S,A)

1 concentration

Dilution series



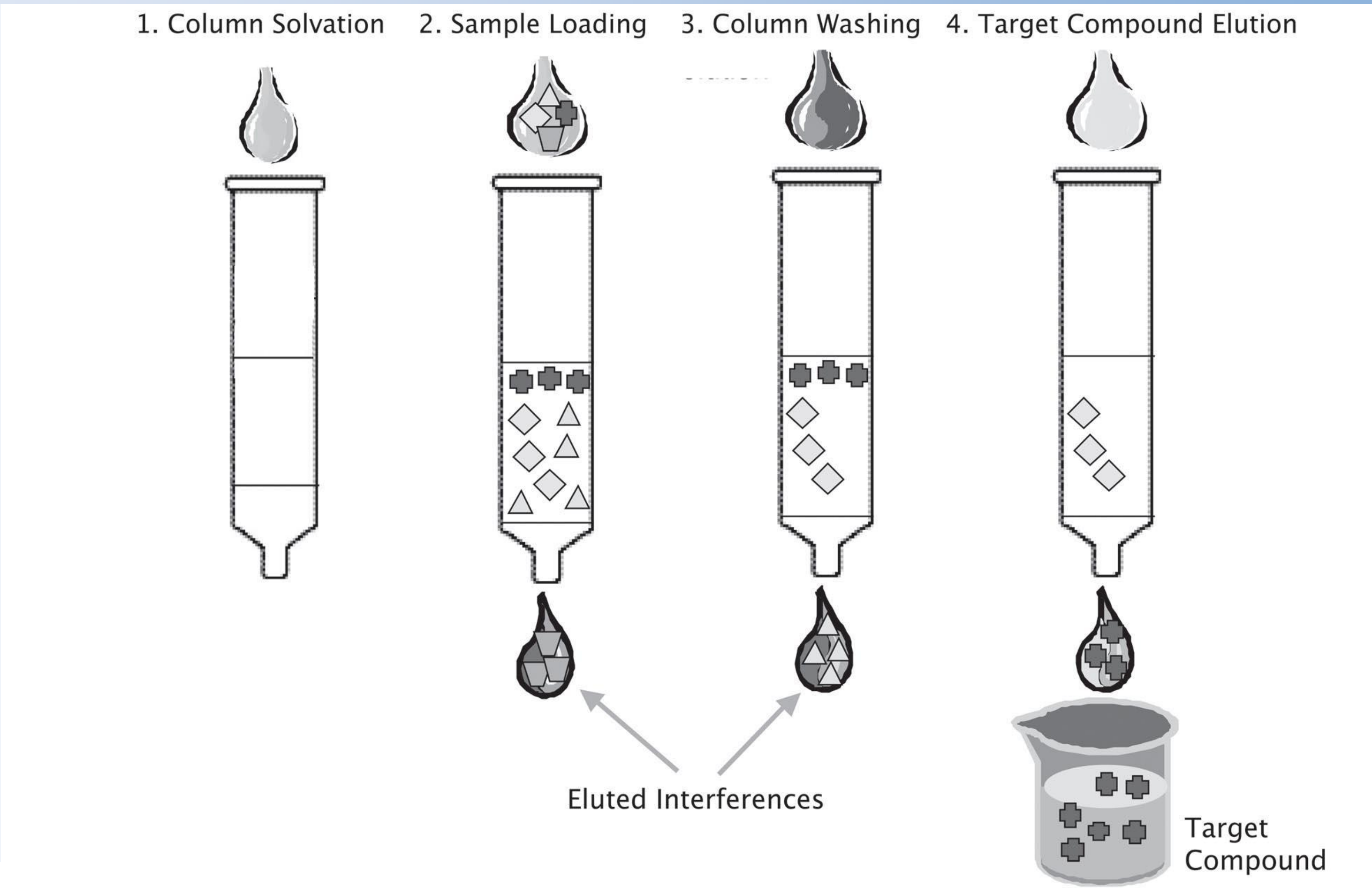
- Measurements after 24 h incubation
  - Water – Scan Test → Inhibition zones were measured
  - 96 well plate test →Optical density (O.D.) (also at T “0”) was measured

Method development

Questions?

- Extraction method needed …
  - SPE, special resins (XAD)?
- Detection limit
- Sensitivity?
- Specificity? Cross-reactivity?

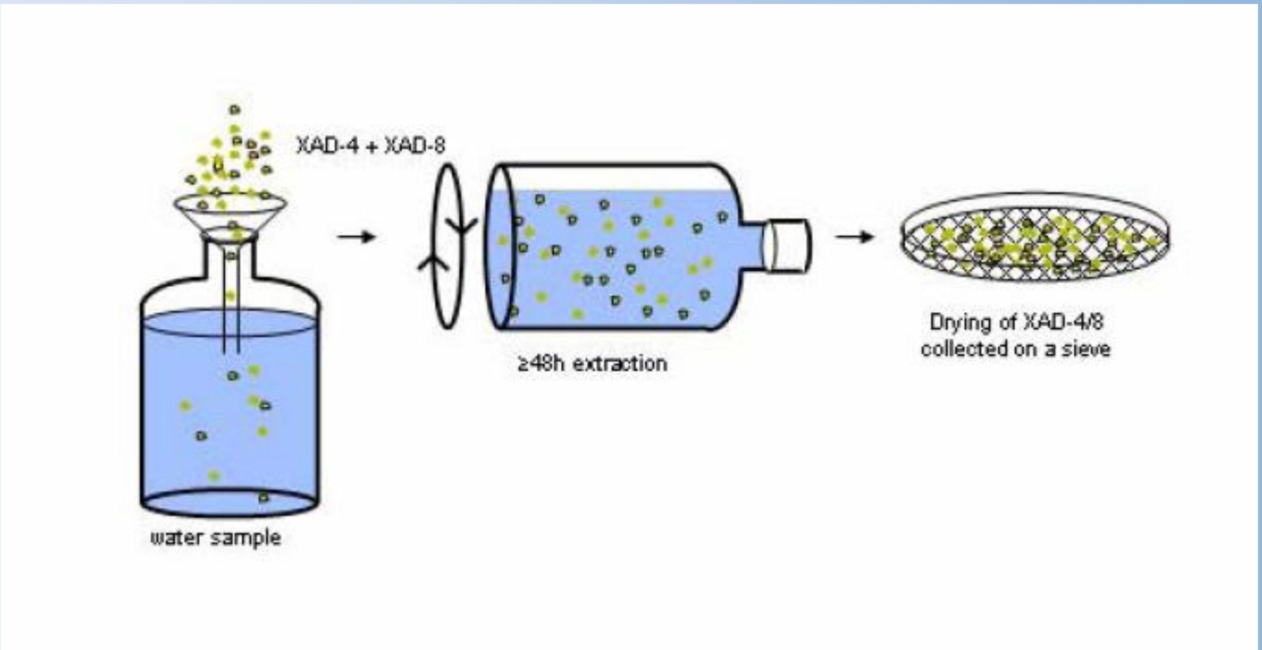
SPE – extraction



http://www.biotage.com/graphics/9223.jpg

XAD 4/8 – extraction

- XAD 4/8 – two synthetic resins used to extract substances



Spike (mix with log Kow range)

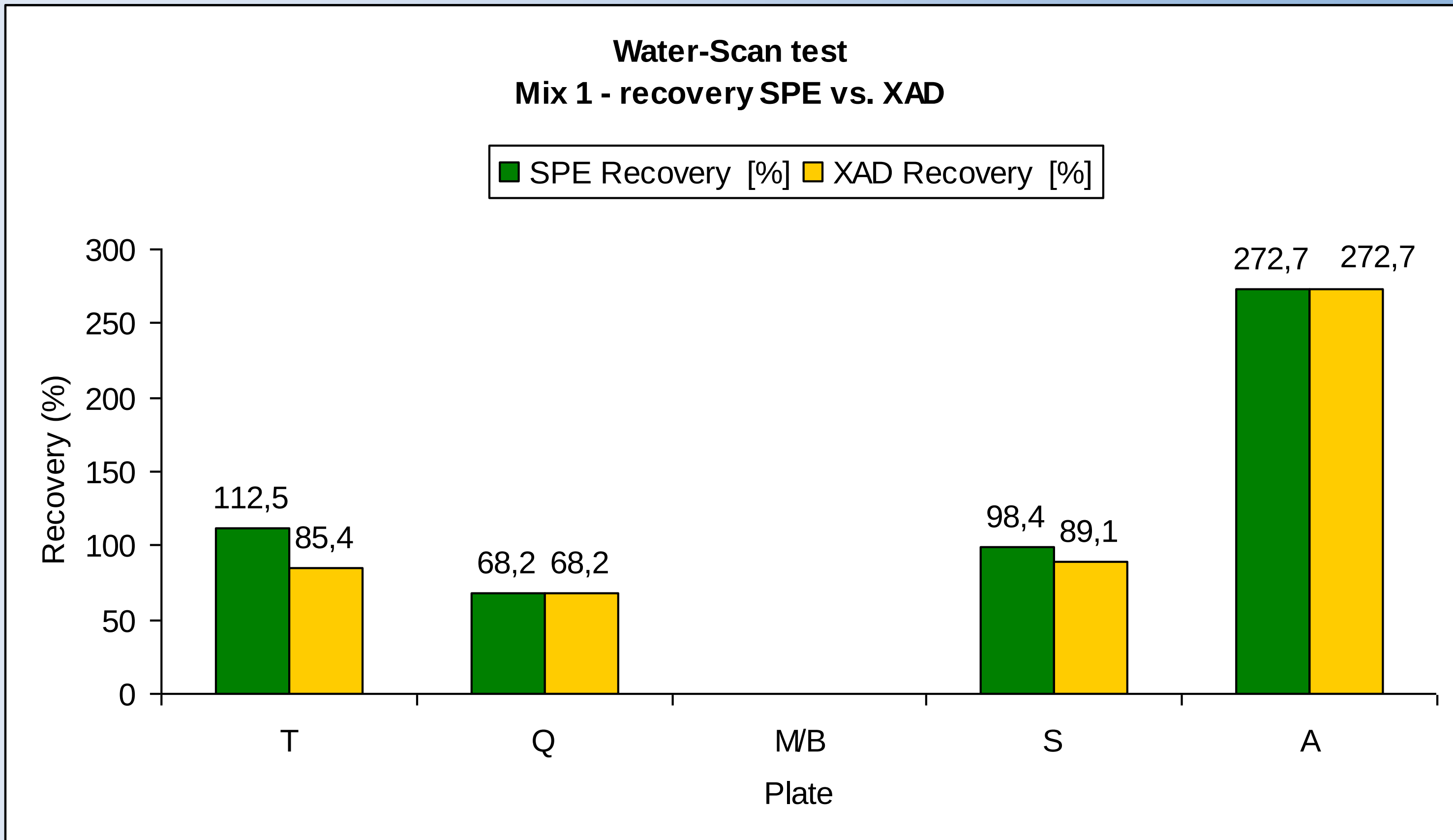
Group	Antibiotic	CAS no	Molecular formula	log Kow Experimental	log Kow Ecosar estim [kowwin]
Aminoglycosides	**Neomycin B**	**144-04-2**	**C23H46N6O13**	**-1.01**	**-9.41**
Aminoglycosides	Streptomycin	57-92-1	C21 H39 N7 O12		-9.07
Aminoglycosides	Apramycin	37321-09-8	C21 H41 N5 O11		-8.12
Aminoglycosides	Kanamycin sulfate		C18H36N4O11	-6.7	-6.7
Tetracyclines	**Oxytetracycline [SPE]**	**79-57-2**	**C22H24N2O9**	**-0.9**	**-2.87**
Cephalosporines	Cefazolin	25953-19-9	C14 H13 N8 O4 S3	-0.58	-2.19
Aminoglycosides	Gentamicin	1403-66-3	C21 H43N5 O7		-1.88
Cephalosporines	Cefalonium	5575-21-3	C20H18N4O5S2		-1.66
Tetracyclines	Doxycycline=vibramycin	564-25-0	C22H24N2O8	-0.02	-1.36
Tetracyclines	Tetracycline [SPE]	60-54-8	C22H24N2O8	-1.3	-1.33
Cephalosporines	Cefacetrile	10206-21-0	C13 H13 N3 O6 S1	-0.45	-1.12
Quinolones	Marbofloxacin	115550-35-1	-		-1.11
Penicillines	Nafcillin	985-16-0	C21 H21 N2 O5 S1 Na1		-1.07
Aminoglycosides	Spectinomycin	1695-77-8	C14 H24 N2 O7		-0.82
Cephalosporines	Cefapirin	21593-23-7	C17H17N3O6S2	-1.15	-0.8
Tetracyclines	**Chlortetracycline [SPE]**	**57-62-5**	**C22H23ClN2O8**	**-0.62**	**-0.68**
Cephalosporines	Cefoperazone	62893-19-0	C25 H27 N9 O8 S2	-0.74	-0.42
Sulfonamides	**Sulfadiazine**	**68-35-9**	**C10 H10 N4 O2 S1**	**-9**	**-0.34**
Sulfonamides	Sulfadoxine	2447-57-6	C12 H14 N4 O4 S1	0.7	-0.24
Quinolones	Ciprofloxacin [SPE]	85721-33-1	C17H18FN3O3	0.28	0
Quinolones	Sarafloxacin	98105-99-8	C20H17F2N3O3		0.12
Macrolides	Lincomycin	154-21-2	C18 H34 N2 O6 S1	0.56	
Cephalosporines	Cefalexin	15686-71-2	C16H17N3O4S	0.65	
Sulfonamides	**Sulfamethoxazole [SPE]**	**723-46-6**	**C10 H11 N3 O3 S1**	**0.89**	**0.48**
Quinolones	Enrofloxacin	93106-60-6	C19H22FN3O3		0.7
Sulfonamides	Trimethoprim	738-70-5	C14H18N4O3	0.91	0.73
Sulfonamides	Sulfamethazine [SPE]	57-68-1	C12 H14 N4 O2 S1	0.89	0.76
Sulfonamides	Dapsone	80-08-0	C12 H12 N2 O2 S1	0.97	0.77
Tetracyclines	Chloramphenicol	56-75-7	C11H12Cl2N2O5	1.14	0.92
Penicillines	Amoxicillin	26787-90-3	C16H19N3O5S	0.87	0.97
Macrolides	Tylosin	1401-69-0	C46 H77 N1 O17	1.63	1.05
Sulfonamides	Sulfadimethoxine [SPE]	122-11-2	C12 H14 N4 O4 S1	1.63	1.17
Quinolones	Danofloxacin	112398-08-0	C19H20FN3O3		1.19
Quinolones	Difloxacin	98106-17-3	C21H19F2N3O3	0.89	1.28
Penicillines	Ampicillin	69-53-4	C16 H19 N3 O4 S1	1.35	1.45
Quinolones	**Oxolinic acid**	**14698-29-4**	**C13H11NO5**		**1.7**
Macrolides	Oleandomycin	3922-90-5	C35 H61 N1 O12	1.69	1.83
Penicillines	Penicillin G	61-33-6	C16 H18 N2 O4 S1	1.83	1.85
Macrolides	**Erythromycine**	**114-07-8**	**C37 H67 N1 O13**	**3.06**	**2.48**
Penicillines	Oxacillin	66-79-5	C19 H19 N3 O5 S1	2.38	2.57
Quinolones	**Flumequine**	**42835-25-6**	**C14H12FNO3**	**1.6**	**2.7**
Sulfonamides	Baquiloprim	102280-35-3	C17 H20 N6		2.84
Penicillines	Cloxacillin	61-72-3	C19 H18 CL1 N3 O5 S1	2.48	3.22
Penicillines	Dicloxacillin	3116-76-5	C19 H17 CL2 N3 O5 S1	2.91	3.86
Macrolides	Tilmicosin	108050-54-0	C46 H80 N2 O13	3.8	4.13
Macrolides	Valnemulin	101312-92-9	C31H52N2O5S	-	4.16

1. Neomycin
2. Oxytetracycline-OTC
3. Chlortetracycline-CTC
4. Sulfadiazine
5. Sulfamethoxazole-SMX
6. Oxolinic acid
7. Erythromycine
8. Flumequine

Log Kow clusters:

?2
1 - 2
0 - 1
0 -"-1"
"-1" - "-2"
? "-2"

Recovery patterns (spiked water)



Conclusions

- Some cross-reactivity noted
  - At plate “A”: aminoglycosides
- Specific?
  - Limited effects (<< cyto toxicity) of others
    - RIVM data
- Overview of ‘indicative’ detection limits for >35 compounds (1-1000 ug/L)
  - Waterdienst data

Application, some examples

- Monitoring
- Screening locations

- Review of treatment techniques
- New treatments, do they remove?

- Prioritization of locations
- Identification of hot-spots

End-of-pipe?

- Antibiotics in river water
- Monitoring/screening
- Hot-spots?

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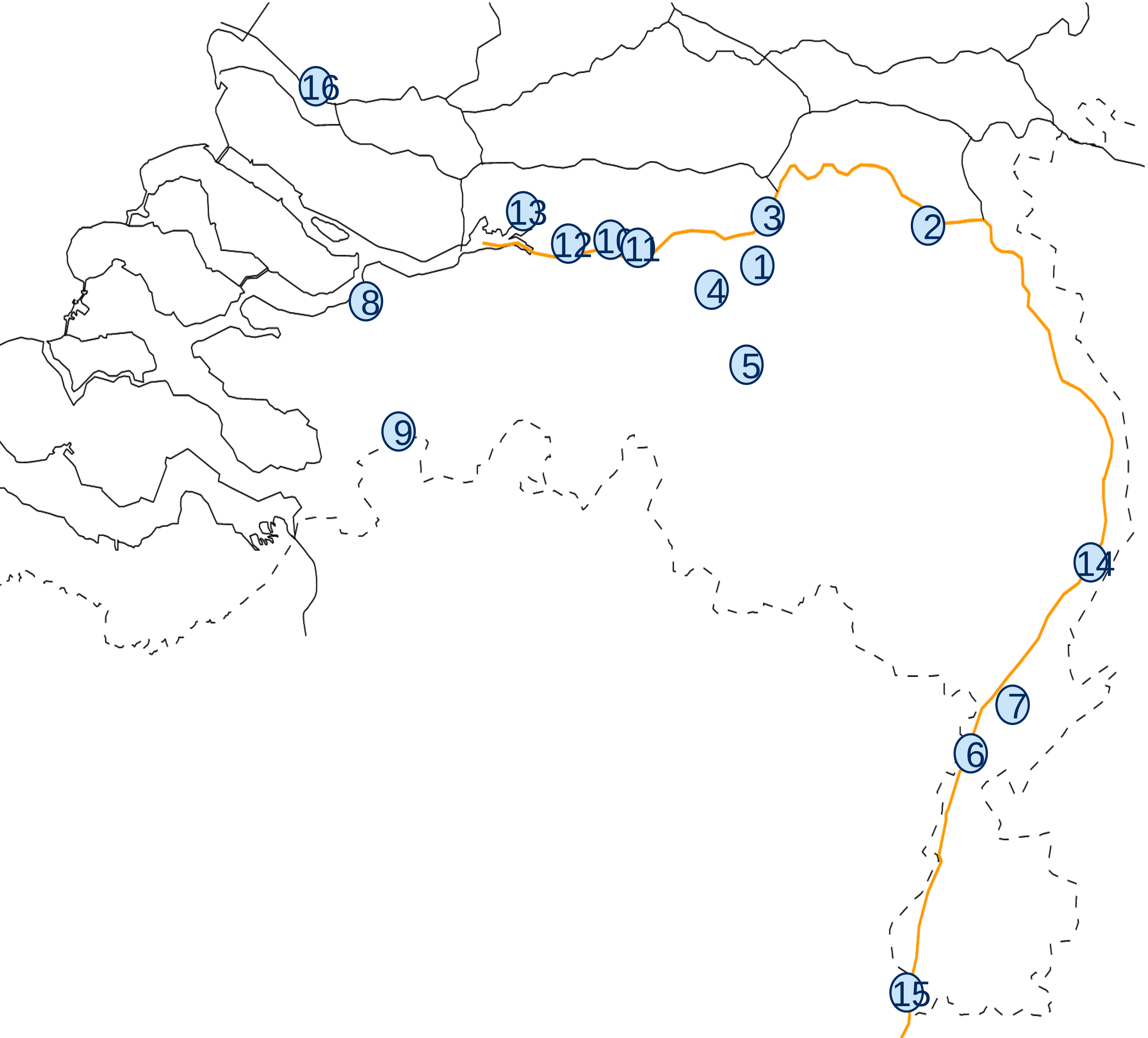
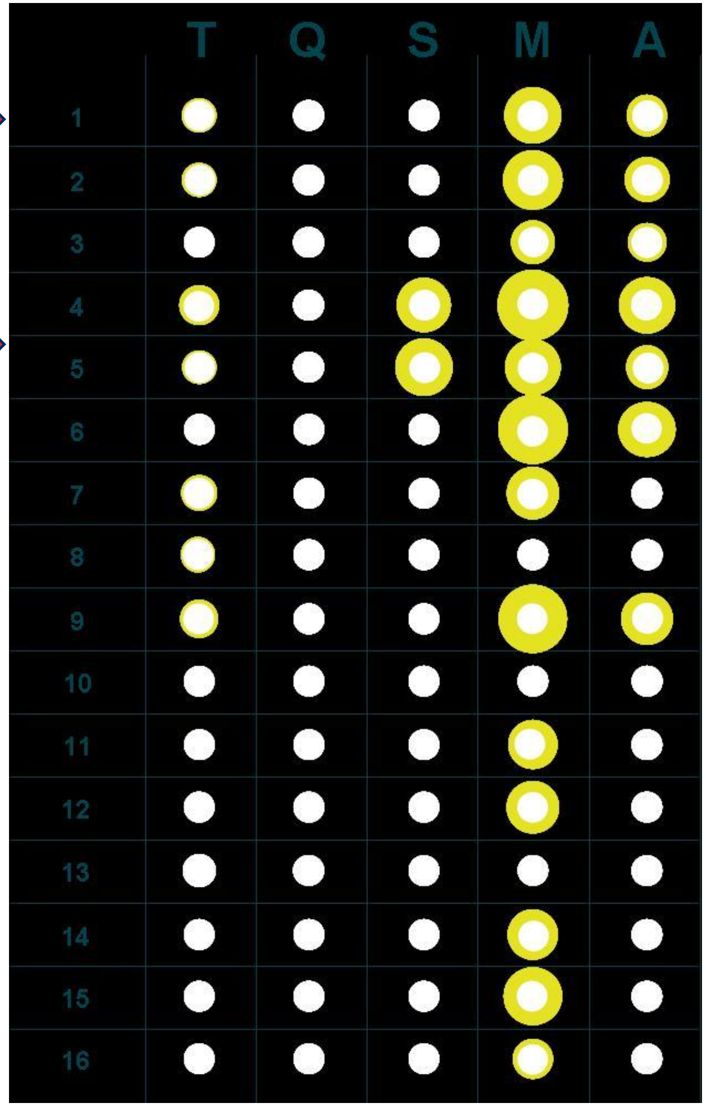
Screening the Dutch Meuse river basin (Waterdienst)



Screening the Dutch Meuse river basin (Waterdienst)

River Aa at 's Hertogenbosch

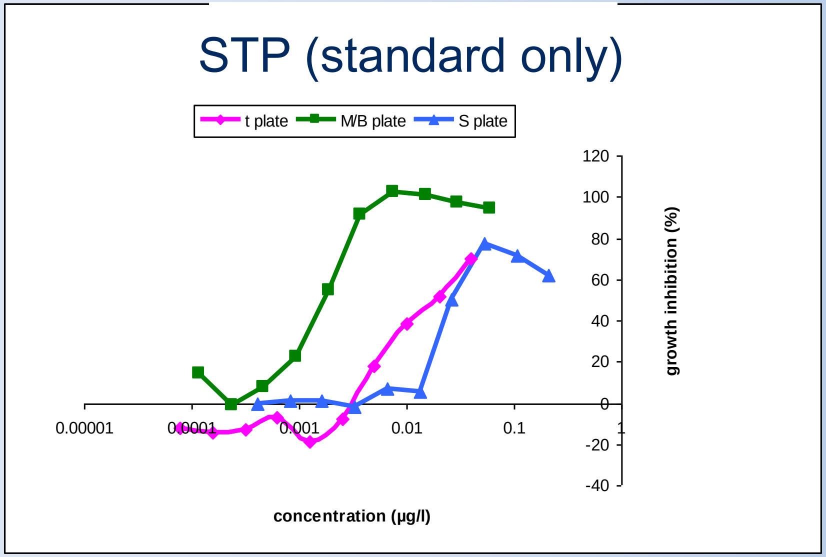


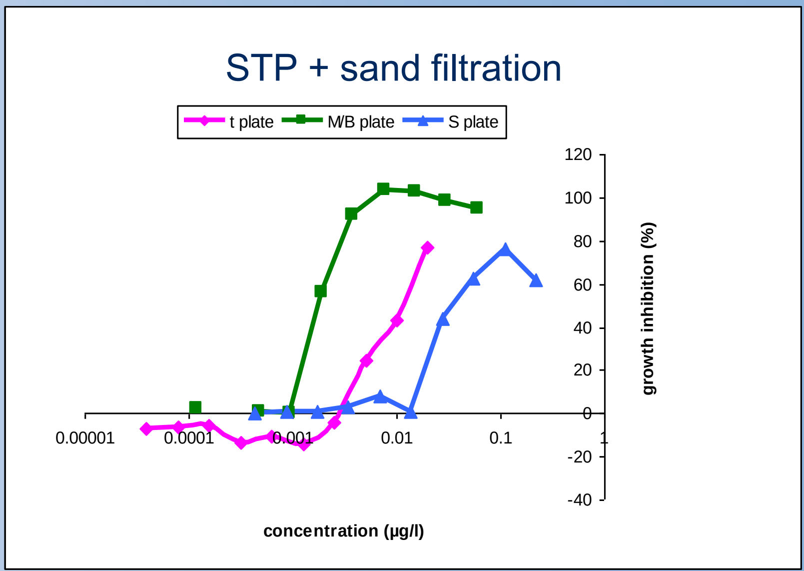
Zandleij near WWTP

Ditch near a pig farm

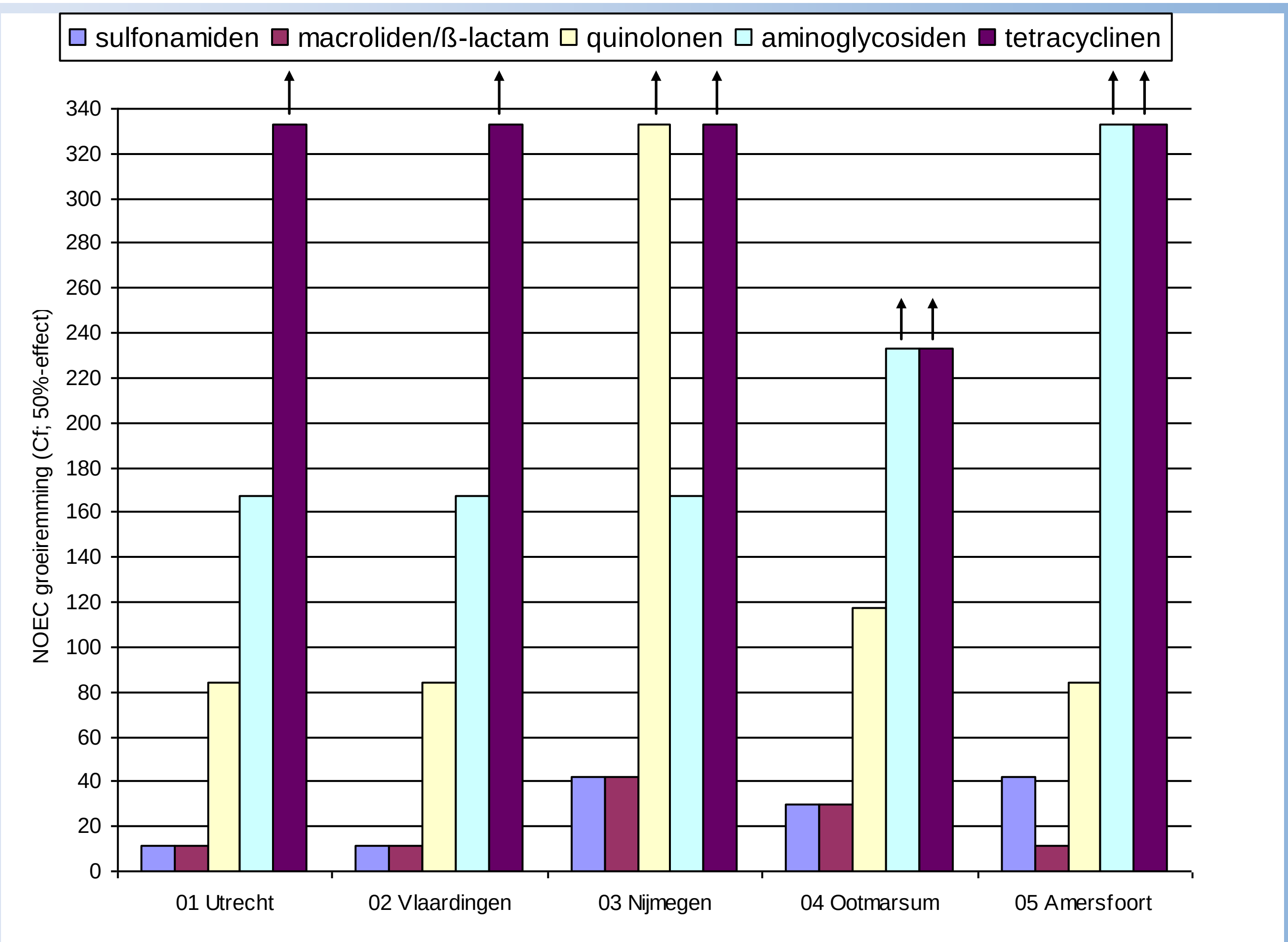
Example: use of antibiotic bioassay

- STP standard sewage treatment plant
- STP + sand filter
  - Response patterns similar in 96-wells test





Antibiotics screening: locations



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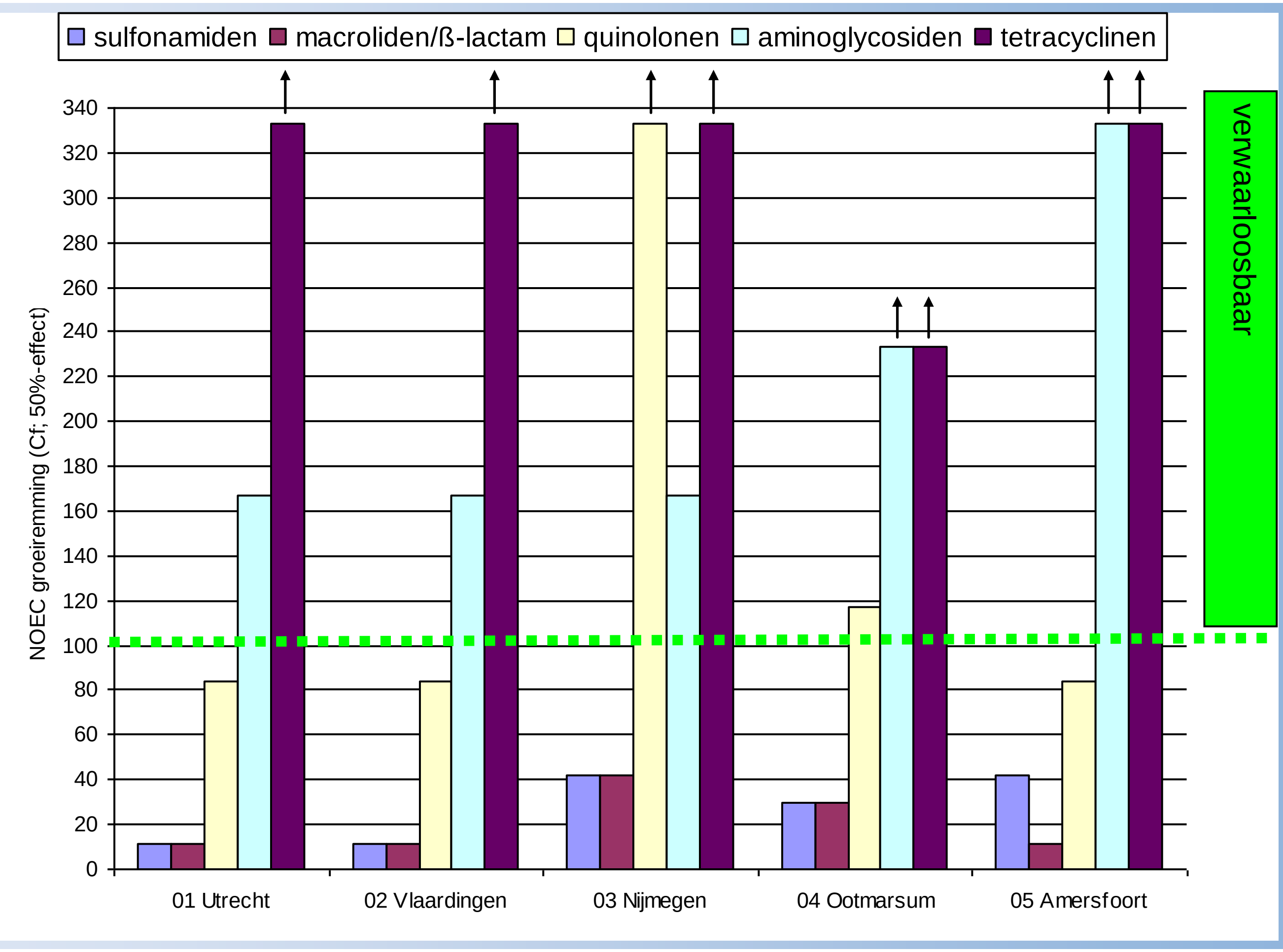
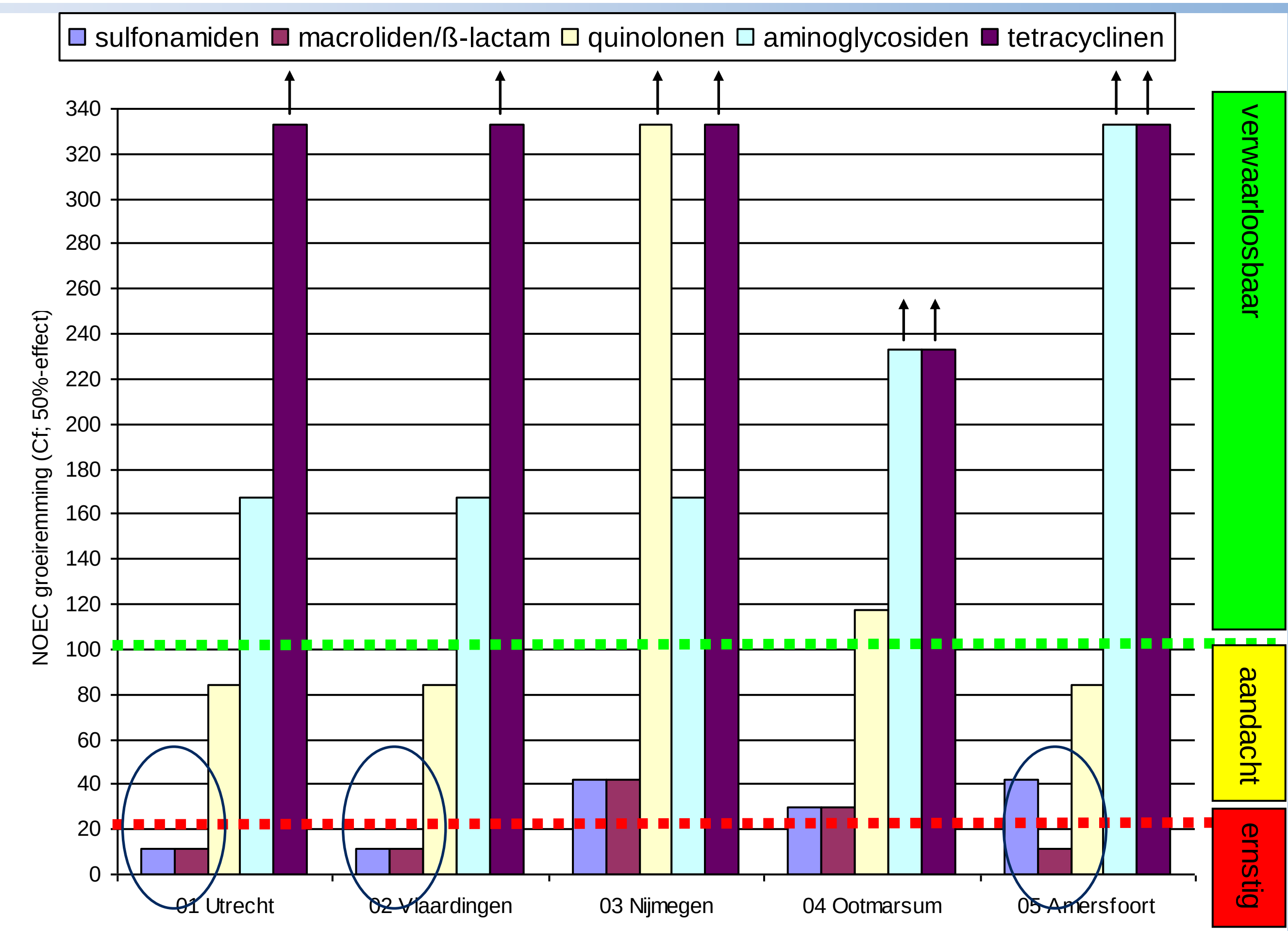
planning connecting
respecting
the future

Antibiotics screening: assessment



Antibiotics screening: assessment



Conclusion

- Method optimization
  - (with interested parties)
- Available for routine screening
  - in combination with other bioassays
  - in combination with chemical analysis
  - (for interested parties)
- Outlook:
  - Possible application in research on the emission of antibiotics from households, hospitals and care-institutions …

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