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Laboratory challenges in meeting the required priority substances EQS levels

K. Clive Thompson
Chief Scientist
ALcontrol Laboratories

Paul Gribble,
Technical Director
ALcontrol Laboratories

DISCLAIMER

The views expressed in this presentation are solely those of the authors and not necessarily those of ALcontrol Laboratories

Directive 2013/39/EU

- Priority Substances Directive
- Sets EQS for Priority Substances and Priority Hazardous Substances.
- 45 chemicals (or groups of chemicals) included in total
- Including 21 Priority Hazardous Substances
- Priority Hazardous Substances
  - Toxic
  - Persistent
  - Bioaccumulative

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Derivation of EQS Values

- How were the relevant EQS values derived?
- The WFD is based upon river catchment areas and maintenance of overall water quality using biota and other measures
- At some of these priority pollutant sub ng/litre level EQS values, the question must be asked were all confounding variables taken into consideration.
- Also how were appropriate precautionary safety factors derived? Are some of these excessive?
- Many of these EQS limits have been grossly exceeded in the past for long periods with some dire effects, but in other instances with very limited effects, such as of DDT, BDEs, PCBs and heptachlor.

Key Issues

- At the very low cited EQS values cited for many substances, will the labs across the 28 countries be able to obtain consistent results
- Unfit for purpose analysis is worse than no analysis when making decisions involving very high expenditure by companies/industries
- The **uncertainty** of both the **sampling** and **analysis needs** to be determined
- Proficiency scheme(s) are needed
- Blind proficiency testing is also needed across the EU

Financial Justification (1)

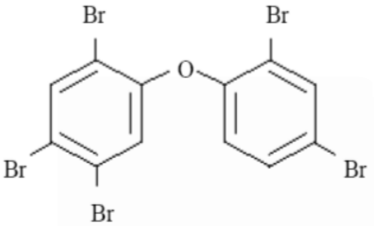
- Do we need to ask: On a risk basis, can the cost of implementing and enforcing some of these ultra low EQS with both fit for purpose sampling and analysis be justified?
- **Money supply is not infinite, especially in the current economic climate.**
- Could some of this money spent to better overall effect to minimise the risks?
- A similar situation arises with Disinfection by Products (DBPs) Over 700 potentially toxic DBPs have been reported in drinking waters. **(Recent 3 day DBP 2014 international conference in Muelheim)**

Financial Justification (2)

- Can consistent fit for purpose samples be taken for sub-ng/litre organic parameter concentrations?
- Have sample stability studies been carried out for real water samples (as per the DWI "Info Letter 12/05" protocol)?
- Can consistent fit for purpose analysis be carried out by routine labs at ng and sub-ng/litre concentrations?
- Can fit for purpose samples be taken for biodegradable **"Free Cyanide"** around the PNEC_{fw} of 52 ng/litre level? This is 1000 times less than the drinking water directive.
- The 71 page EC report by Robert Loos (2012) "**Analytical Methods for the new proposed Priority Substances of the European Water Framework Directive (WFD)"** does not address the sampling aspect other than a mention of passive sampling which does not give required **"total "** results.

Financial Justification (3)

Brominated Diphenyl Ethers (BDEs)

Chemical structure	**AA-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l] (in brackets former values)	**MAC-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]	**EQS biota** [µg/kg]
			
(penta BDE)	Fresh 4.9 10^{-8} µg/l = 49 fg/l (0.00005 ng/l) Salt 2.4 10^{-9} µg/l = 2.4 fg/l (0.0000024 ng/l)	Fresh 0.14 µg/l Salt 0.014 µg/l (n.a.)	0.0085 µg/kg = 8.5 ng/kg

For the group of priority substances covered by brominated diphenylethers (No 5), the **EQS refers to the sum of the concentrations of congener numbers 28, 47, 99, 100, 153 and 154.**

Robert Loos (2012) Analytical Methods for the new proposed Priority Substances of the European Water Framework Directive (WFD)

Financial Justification 4

Sweden gave the following information on the cost of analyses (2012): -

Substances or substance group	Matrix	Cost in EURO	Type of laboratory
Dioxins	Biota	1000	Research laboratory
PFOS	Water or biota	280-330	Research laboratory
15 PFASs	Water or biota	430	Research laboratory
Aclonifen, Cypermethrin, Dicofol (method OMK 51)	Water	225	Research laboratory (accredited)
Method OMK 51 (GC-MS); multi-compound analysis including already prioritised pesticides	Water	395	Research laboratory (accredited)
Bifenox, Cybutryne, Quinoxyfen, and Terbutryn; possibly also Dichlorvos, and Diclofenac	Water	225	Research laboratory (accredited)
Method OMK 57 (LC-MS-MS); multi-compound analysis including already prioritised pesticides	Water	395	Research laboratory (accredited)

Robert Loos (2012) Analytical Methods for the new proposed Priority Substances of the European Water Framework Directive (WFD) **Grand total analysis cost for above parameters ~ €3000/sample**

Financial Justification 5

SI multiples for gram (g)

Submultiples

Value	Symbol	Name	g
10^{0} g	g	gram	1.0
10^{-1} g	dg	decigram	0.1
10^{-2} g	cg	centigram	0.01
10^{-3} g	mg	milligram	0.001
10^{-6} g	µg	microgram (mcg)	0.000001
10^{-9} g	ng	nanogram	0.000000001
10^{-12} g	pg	picogram	0.000000000001
10^{-15} g	fg	femtogram	0.000000000000001
10^{-18} g	ag	attogram	**EQS (ag/l) coming soon?**
10^{-21} g	zg	zeptogram	

Note: - For protein with a MW of 10^{6} a conc of 1 ag/litre is less than 1 molecule per litre!

Specific Compounds (µg/l)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
No	**Name of Substance**	**CAS number**	**AA-EQS Inland surface waters**	**AA-EQS Other surface waters**	**MAC-EQS Inland Surface waters**	**MAC-EQS Other surface waters**	**EQS Biota**
(28)	Benzo(a)pyrene	50-32-8	1,7 x 10^{-4}	1,7 x 10^{-4} **(0.17 ng/l)**	0,27	0.027	5
(30)	Tributyltin compounds (Tributyltincation)	36643-28-4	0,0002	0,0002 **(0.2 ng/l)**	0,0015	0,0015	
(41)	Cypermethrin	52315-07-8	8 x 10^{-5}	8 x 10^{-6} **(0.008 ng/l)**	6 x 10^{-4}	6 x 10^{-5}	
(44)	Heptachlor and heptachlor epoxide	76-44-8/1024-57-3	2 x 10^{-7}	1 x 10^{-8} **(0.00001 ng/l)**	3 x 10^{-4}	3 x 10^{-5}	6.7 x 10^{-3}

Heptachlor/Heptachlor Epoxide (ng/l)

-1	-2	-3	-4	-5	-6	-7	-8
No	**Name of Substance**	**CAS number**	**AA-EQS Inland surface waters**	**AA-EQS Other surface waters**	**MAC-EQS Inland Surface waters**	**MAC-EQS Other surface waters**	**EQS Biota**
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ATSDR 2007 Public Health Statement on Heptachlor and Heptachlor Epoxide "For exposures of up to 10 days, EPA recommends that a child weighing 22 pounds or less not drink water containing more than 0.01 mg heptachlor or heptachlor epoxide per liter of water **0.01 mg/l (10,000 ng/l).** EPA requires that drinking water should not contain more than **0.0004 mg/L (400 ng/l)** heptachlor and 0.0002 mg/L **(200 ng/l)** of heptachlor epoxide."

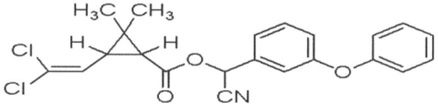
Banned ~ 1988

ATSDR 2007 Public Health Statement on Heptachlor and Heptachlor Epoxide

- **From 1953 to 1974**, heptachlor entered the soil and surface water when farmers used it to kill insects in seed grains and on crops
- In one survey, **the background levels of heptachlor in drinking water and groundwater in the United States ranged from 20 to 800 parts of heptachlor in one trillion parts of water (ppt).** Heptachlor was found in less than 2% of U.S. groundwater samples that are known to be contaminated from pesticide application. The average level of heptachlor in the contaminated groundwater samples was 800 ppt. No information was found for levels of heptachlor epoxide in groundwater or drinking water. **Heptachlor epoxide has been found in surface water (river, lakes) at levels between 0.1 and 10 parts of heptachlor epoxide in one billion parts of water (ppb, 1 ppb is 1 thousand times more than 1 ppt).**

Specific Compounds

Cypermethrin

CAS Number 52315-07-8	**Log Kow** 6.6	**Water Solubility [mg/l]** 0.004
Chemical structure **(MW 416.3)**	**AA-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]	**MAC-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]
	Fresh 8 x 10^{-5} = 0.00008 = 0.08 ng/l = 80 pg/l Salt 8 10^{-6} = 0.000008 = 0.008 ng/l = 8 pg/l	Fresh 6 x10^{-4} = 0.0006 = 0.6 ng/L Salt 6 10^{-5} = 0.00006 = 0.06 ng/l = 60 pg/l

Visual Perspective – Tributyltin EQS

1 drop (20µl) in 20x Olympic sized swimming pools

20x

Visual perspective – Cypermethrin EQS

Need to measure 1 drop (20µl) in 1500m x 500m x 1.33m

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Visual Perspective – Heptachlor EQS

<1 drop in whole course of the Thames 346km x 250m x 2m

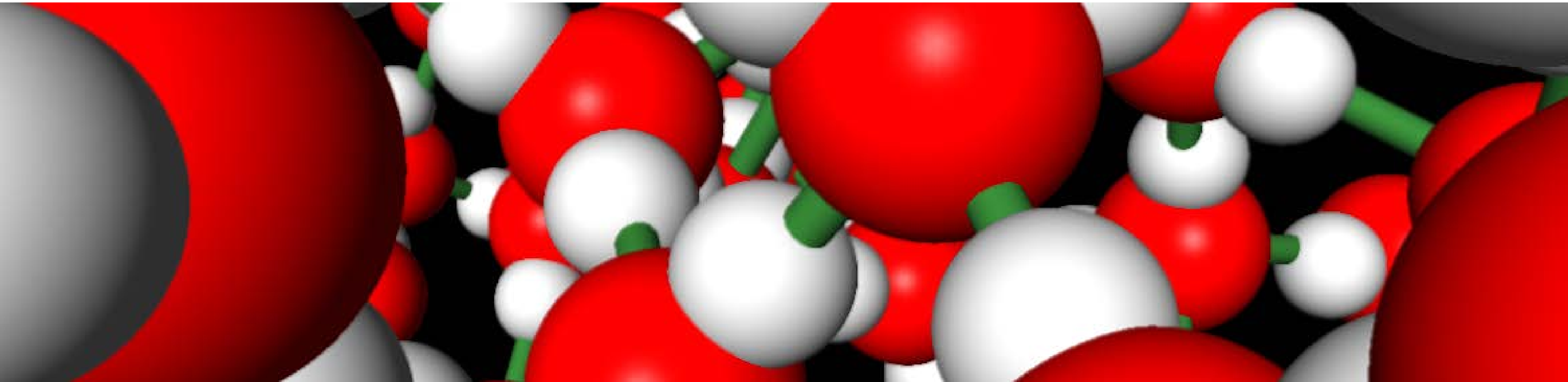
Visual Perspective – Final thought

- How many 20 µl drops were added over the periods during the peak usage of the relevant chemicals?
- Was a clear long-term chronic effect observed in the environment during these periods at levels within one or two orders of magnitude of the extremely low EQS limits?
- It is felt that some of the proposed EQS limits are highly aspirational with respect to routine sampling and analysis monitoring. Especially for Heptachlor and Heptachlor Epoxide
- Can the very high cost of monitoring at these ultra-low concentration levels be cost-justified on a risk-based approach?

Molecules per litre

- Avagadro’s Constant = 6.02×10^{23}
- Number of molecules in a mole

Compound	EQS (µg/litre)	Molecules per litre
Cadmium	0.08	428,432,000,000
Cypermethrin	0.000008 (8 pg/litre)	11,568,664
Heptachlor	0.00000001 (10 fg/litre)	16,126



The sampling challenge

- Large volumes of sample required
- How are large, homogenous samples to be collected for such low levels?
- Surface waters must include solid particulates
- Rules out passive samplers
- Different chemicals need different sampling media
- Stainless Steel or Aluminium for most organics
- LDPE for PFOS and metals

The analytical challenge

- LOD should be 1/7th Annual Average EQS
- For organic analysis, typical sample extracted and concentrated to 1ml
- 1µl injected into GCMS
- Heptachlor current LOD is 0.01 ug/l
- Need to reduce by 7 million!
- Anyone for 7 million litre samples?

Matrix interference

- Typically caused by
  - solids in sample
  - or co-extracted material in much higher concentrations than the target analyte
- Usual way to reduce matrix interference is to dilute it out
- Reduces concentration of target analyte as well
- Increases LOD!

Environmental contamination factors

- Is the extraction analyst a smoker?
- What deodorant is the analyst using?
- Is the analyst on any medication?
- Which soap products are used in the lab toilets?
- How are validation blank samples obtained?
- Were samples taken from next to a busy road?

Background concentrations

- For the compounds with very low EQSs, majority of water courses likely to be above EQS
- This makes matrix specific validation of LOD near impossible
- Leading to lack of robustness in very low results in specific sample matrix
- Also suggests that the majority of river basins will fail good chemical status
- Does this benefit anyone?

Analytical solutions

- Alternative extraction techniques
  - Solid Phase Extraction disks
  - Allow solids and greater sample volume
  - Realistic concentration factor of up 3000x
  - Currently extract concentration step 1000x

Instrumentation

- Higher sensitivity detectors
  - MS/MS
  - ToF

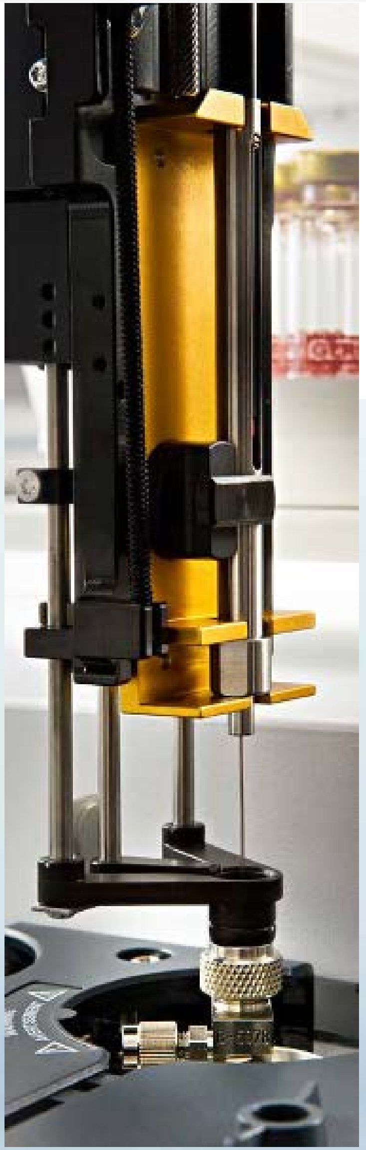
May get 20x more sensitivity

- Use of techniques like Chemical Ionisation can give additional sensitivity gains particularly in heavy matrix samples

Instrumentation

Using PTV injector

- Increase 1µl injection to
  - 100µl injection
  - 100x fold increase



Overall increases

- 3x for extraction (x1000 to x3000)
- 20x for detector change (MS to MS/MS or ToF)
- 100x more into GC
- 3x20x100 = 6000x
- Heptachlor requires 7,000,000x extra sensitivity!
- Still looking at 167 litre sample to achieve EQS
- 1200 litre sample required to achieve Annual Average monitoring LOD

Variable sample types

- These extraction and instrument conditions work for ‘clean’ samples
- Groundwater which may enter surface water could also be subject top the same EQS LOD requirements
- Groundwater on brownfield sites could be heavily impacted
- High potential for carry-over of highly contaminated sample on a method developed for very low LOD’s
- Practical issues with customer education

Summary

- Some EQS detection limits not yet met
- Newest classes of instruments and advanced extraction techniques required
- Homogenous samples nearly impossible
- Sampling and analysis will be expensive
- Some EQS detection limits may never be met
- Proving Compliance with Priority Substances Directive may not be possible
- Samples from different sources will cause issues
- Lots more to be done!

Table 7: Determination of selected polybrominated diphenyl ether (PBDE) congeners in whole water samples with disk-based solid phase extraction

Results of the interlaboratory comparison for validation of EN 16694 – Sample 2

Sample 2: High level sample with SPM

Matrix: Mineral water spiked with 200 mg/L River Scheldt SPM

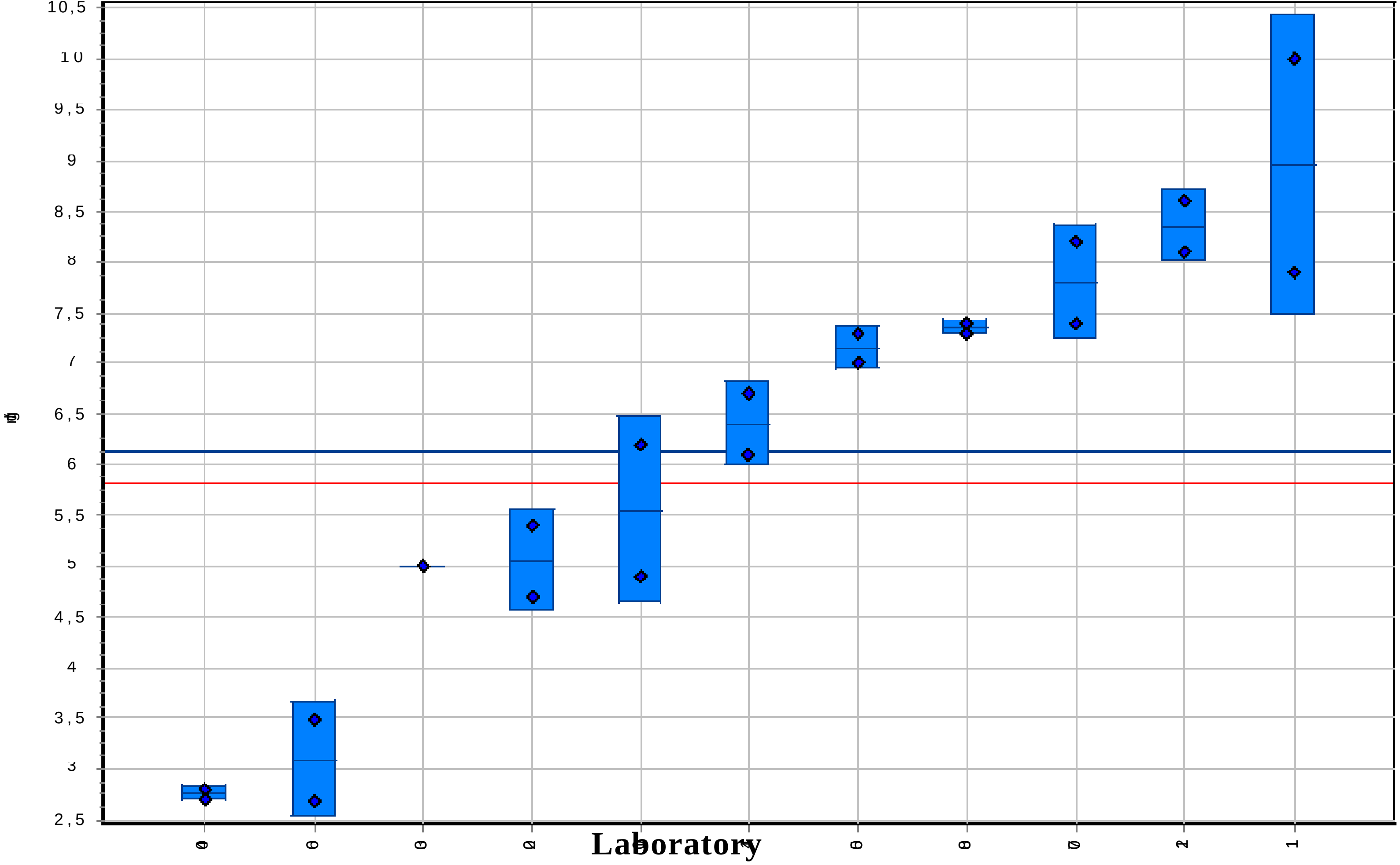
Measurand	l	n	o	X	ẍ	η	s_R	$C_{V,R}$	s_r	$C_{V,r}$
			%	ng/l	ng/l	%	ng/l	%	ng/l	%
BDE-28	8	16	20,0	0,032	0,0296	92,5	0,0076	25,7	0,0032	10,8
BDE-47	11	22	0,0	2,51	2,65	105,7	0,775	29,2	0,273	10,3
BDE-99	11	22	0,0	5,82	6,13	105,3	2,09	34,1	0,625	10,2
BDE-100	11	22	0,0	0,86	0,965	112,2	0,338	35,0	0,068	7,0
BDE-153	11	22	0,0	1,19	1,12	94,2	0,419	37,4	0,156	13,9
BDE-154	9	18	18,2	0,55	0,528	96,0	0,168	31,8	0,021	4,0
Sum 6 BDE	11	22	0,0	10,96	11,74	107,1	3,27	27,8	1,282	10,9

Explanation of symbols:

l	number of laboratories after outlier rejection
n	number of individual test results after outlier rejection
o	percentage of outliers
X	assigned value (estimated concentration)
ẍ	overall mean of results (without outliers)
h	recovery rate
s_R	reproducibility standard deviation
$C_{V,R}$	coefficient of variation of reproducibility
s_r	repeatability standard deviation
$C_{V,r}$	coefficient of variation of repeatability

BDE-99, Sample 2

BDE-47, sample Sample: 2 Mean: 6,13 ng/l Reference value: 5,82 ng/l



Sum 6 BDE, Sample 2

Sample: 2
Measurand: Sum 6 BDE
Reference value: 10,960 ng/l

Mean: 11,740 ng/l
Reproducibility s.d.: 3,267 ng/l
Repeatability s.d.: 1,282 ng/l

Table 3: Results of the interlaboratory comparison for validation of TS 16692 TBT

Sample 1:	Low SPM, Matrix: Mineral water spiked with 20 mg SPM
Sample 2:	High SPM, Matrix: Mineral water spiked with 200 mg SPM
Sample 3:	Spike, Matrix: Mineral Water spiked with TBT
Sample 4:	Blank, Matrix: Mineral Water

Sample	l	fl	o %	X ng/l	ng/l	η %	s_R ng/l	$C_{V,R}$ %	s_r ng/l	$C_{V,r}$ %
1	7	14	0.0	3.8	4.21	110.8	2.25	53.4	1.05	24.9
2	7	14	0.0	3.4	3.51	103.2	1.10	31.3	0.42	12.0
3	6	12	14.3	17	17.4	102.5	2.18	12.5	1.12	6.4
4	7	13	0.0	-	0.32	-	0.20	62.5	0.10	31.3

Explanation of symbols:

l	number of laboratories after outlier rejection
n	number of individual test results after outlier rejection
o	percentage of outliers
X	assigned value (estimated)
	overall mean of results (without outliers)
▱	recovery rate
s_R	reproducibility standard deviation
$C_{V,R}$	coefficient of variation of reproducibility
s_r	repeatability standard deviation
$C_{V,r}$	coefficient of variation of repeatability

6.1 Results of the interlaboratory trial for the validation of “prEN 16691

“Water quality - Determination of polycyclic aromatic hydrocarbons (PAH) in whole water samples using liquid solid extraction combined with gas chromatography mass spectrometry (GC-MS)

Sample 1: Low PAH-level sample containing 20mg/L suspended particulate matter (SPM) Matrix: Mineral water, spiked with PAH containing SPM

Sample 1										
Measurand	l	n	o	X	x	η	s_R	$C_{V,R}$	s_r	$C_{V,r}$
			%	ng/l	ng/l	%	ng/l	%	ng/l	%
Anthracene	10	20	0.0	5.66	3.44	60.89	1.67	48.4	1.15	33
Fluoranthene	13	26	0.0	94.3	51.2	54.26	21.38	41.8	8.82	17
Benzo[b]fluoranthene	11	22	8.3	28.7	33.2	115.69	10.56	31.8	5.18	15
Benzo[k]fluoranthene	11	22	8.3	13.5	17.4	128.65	8.25	47.4	2.77	15
Benzo[a]pyrene	8	16	33.3	14.5	13.5	92.69	2.58	19.1	0.563	4
Benzo[ghi]perylene	10	20	16.7	35.6	34.2	96.10	6.50	19.0	6.50	19
Indeno[1,2,3-cd]pyrene	7	14	36.4	21.6	22.5	104.23	1.67	7.4	0.927	4
Naphtalene	4	8	20.0		7.75		4.01	51.8	1.20	15
Fluorene	6	12	0.0		3.24		1.50	46.3	0.580	17
Acenaphthene	4	7	22.2		2.28		2.49	109.5	0.064	2
Acenaphthylene	8	16	0.0		2.99		2.50	83.6	0.473	15
Phenanthrene	8	16	0.0	45.0	18.6	41.40	5.96	32.0	1.99	10
Pyrene	9	18	0.0	92.7	51.5	55.59	19.48	37.8	10.51	20
Chrysene	8	16	11.1	32.5	30.3	93.15	10.36	34.2	1.45	4
Benzo[a]anthracene	6	12	14.3	18.4	16.6	90.04	2.93	17.7	1.63	9
Dibenzo[a,h]anthracene	7	14	22.2	3.64	4.09	112.25	1.29	31.6	1.09	26

Note: - Proposed Benzo[a]pyrene in fresh and saline waters EQS is 0.17 ng/litre

Conclusions (1)

1. Until **fit for purpose sampling and analysis** can be achieved by all the relevant labs at the concentrations of interest, we need to proceed with caution.
2. There is a need to set up **a group of ISO 17025 accredited labs to work together** to determine what can be routinely achieved on an ongoing basis. As per MCERTS schemes.
3. Need to determine **realistic achievable performance limits** (LOD, precision and bias). As per the MCERTS schemes.

Conclusions (2)

4. A harmonised EU-wide way of **calculating LOD and LOQ values** is a must for ensuring comparability of interpreting a given set of data and assessing whether it meets the directive requirements

5. **Set up an EU-wide proficiency scheme or failing that a UK one.**

6. The EC should set up a **blind proficiency scheme** to cover all 28 EU countries to ensure comparable results

7. Carry out **sample stability testing**. (As per DWI Info Letter 12/05)

Conclusions (3)

8. Need a **risk based approach** in the current financial situation to ensure that the output from this analysis can justify the very high cost.

9. **Regulators should liaise with analytical chemists relating to both sampling and analysis when setting regulatory limits!!!**

Extracts from the Directive (1)

- **The water EQS laid down in this Annex are expressed as total concentrations in the whole water sample**
- **Novel monitoring methods such as passive sampling and other tools show promise for future application, and their development should therefore be pursued.** KCT/PB NOTE: “Passive sampling does **not** give total results”
- By way of derogation from the first subparagraph, in the case of **cadmium, lead, mercury and nickel (hereinafter “metals”), the water EQS refer to the dissolved concentration**, i.e. the dissolved phase of a water sample obtained by filtration through a 0,45 µm filter or any equivalent pre-treatment, or, where specifically indicated, to the bioavailable concentration.
- **Member States may, when assessing the monitoring results against the relevant EQS, take into account: natural background concentrations for metals and their compounds where such concentrations prevent compliance with the relevant EQS;**

Extracts from the Directive (2)

Since the objective of this Directive, namely that of achieving good surface water chemical status by laying down EQS for priority substances and certain other pollutants, cannot be sufficiently achieved by the Member States and can therefore, by reason of the need to maintain the same level of protection of surface water throughout the Union, be better achieved at Union level**, the Union may adopt measures, in accordance with the principle of subsidiarity as set out in Article 5 of the Treaty on European Union**. In accordance with the principle of proportionality, as set out in that Article, **this Directive does not go beyond what is necessary in order to achieve that objective.**

Development of the 1st Watch List under the Environmental Quality Standards Directive JRC 2014 Document

In conclusion, the ten substances recommended for the first Watch List are listed below, subject to the availability of the analytical methodology to monitor them:

Diclofenac
17-Beta-estradiol (E2)
17-Alpha-ethinylestradiol (EE2)
Trichlorfon
Cyclododecane
Aminotriazole
Methiocarb
2-ethylhexyl 4-methoxycinnamate
Erythromycin
Cyanide-free

STOP

HERE

ATSDR 2007 Public Health Statement on Heptachlor and Heptachlor Epoxide

- **From 1953 to 1974**, heptachlor entered the soil and surface water when farmers used it to kill insects in seed grains and on crops
- In one survey, **the background levels of heptachlor in drinking water and groundwater in the United States ranged from 20 to 800 parts of heptachlor in one trillion parts of water (ppt).** Heptachlor was found in less than 2% of U.S. groundwater samples that are known to be contaminated from pesticide application. The average level of heptachlor in the contaminated groundwater samples was 800 ppt. No information was found for levels of heptachlor epoxide in groundwater or drinking water. **Heptachlor epoxide has been found in surface water (river, lakes) at levels between 0.1 and 10 parts of heptachlor epoxide in one billion parts of water (ppb, 1 ppb is 1 thousand times more than 1 ppt).**

Heptachlor/Heptachlor Epoxide (ng/l)

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No	**Name of Substance**	**CAS number**	**AA-EQS Inland surface waters**	**AA-EQS Other surface waters**	**MAC-EQS Inland Surface waters**	**MAC-EQS Other surface waters**	**EQS Biota**
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