European Commission

JRC SCIENTIFIC AND POLICY REPORTS

Analytical methods relevant to the European Commission's 2012 proposal on Priority Substances under the Water Framework Directive

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2012

Report EUR 25532 EN

Joint
Research
Centre

European Commission
Joint Research Centre
Institute for Environment and Sustainability

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JRC73257

EUR 25532 EN

ISBN 978-92-79-26642-3 (pdf)
ISBN 978-92-79-26643-0 (print)

ISSN 1831-9424 (online)
ISSN 1018-5593 (print)

doi:10.2788/51497

Luxembourg: Publications Office of the European Union, 2012

Printed in Italy

Analytical methods relevant to the European Commission's 2012 proposal on Priority Substances under the Water Framework Directive

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CONTENTS

ACRONYMS

AA	Annual Average
APCI	Atmospheric Pressure Chemical Ionization
ASE	Accelerated Solvent Extraction
EC	European Commission
ECD	Electron Capture Detector
ECNCI	Electron Capture Negative Chemical Ionization
EQS	Environmental Quality Standard
ESI	Electrospray Ionization
FPD	Flame Photometric Detector
FIMS	Flow Injection Mercury System
GC	Gas Chromatography
GC-MS	Gas Chromatography Mass Spectrometry
GC-MS-MS	Gas Chromatography tandem Mass Spectrometry
HPLC	High Pressure Liquid Chromatography
HRGC/HRMS	High Resolution Gas Chromatography/High Resolution Mass Spectrometry
ISO	International Standard Organization
qTOF	Quadrupole Time-of-Flight
LC	Liquid Chromatography
LC-MS	Liquid Chromatography Mass Spectrometry
LC-MS-MS	Liquid Chromatography tandem Mass Spectrometry (triple quadrupole)
LLE	Liquid-Liquid Extraction
LOD	Limit of Detection
LOQ	Limit of Quantification
MDL	Method Detection Limit
MS	Mass Spectrometry (or Member State)
NPD	Nitrogen-Phosphorus Detector
PFASs	Perfluoroalkyl Substances
PLE	Pressurized Liquid Extraction
SLE	Solid Liquid Extraction
SPE	Solid Phase Extraction
SPM	Suspended Particle Matter
SPME	Solid Phase Micro Extraction
TOF	Time-of-Flight
UHPLC	Ultra High Pressure Liquid Chromatography

ACKNOWLEDGEMENTS

I would like to thank the following people for contributions or comments to this report:

John Batty	Environment Agency	UK
Susanne Boutrup	National Environmental Research Institute, Aarhus University	Denmark
Mario Carere	ISS	Italy
Luba Chepanova	Environment Agency	Bulgaria
Helen Clayton	DG ENV	
Colman Concannon	EPA	Ireland
Marina Coquery	Irstea - Aquaref	France
Karin Deutsch	BMLFUW	Austria
Eric De Wulf	Vlaamse Milieumaatschappij	Belgium
Anja Duffek	UBA	Germany
Bernd Gawlik	DG JRC	
Sabine Geiss	TLUG	Germany
Hannah Green	Environment Agency	UK
Henner Hollert	RWTH Aachen University	Germany
Robert Kase	Oekotoxzentrum / Swiss Centre for Applied Ecotoxicology (Eawag/EPFL)	Switzerland
Jenny Kreuger	SLU	Sweden
Petra Kunz	Oekotoxzentrum / Swiss Centre for Applied Ecotoxicology (Eawag/EPFL)	Switzerland
Maria Linderoth	EPA	Sweden
Luca Lucentini	ISS	Italy
Giulio Mariani	DG JRC	
Anne Morin	Ineris - Aquaref	France
Simon O'Toole	Environmental Protection Agency	Ireland
Alfred Rauchbüchl	BMLFUW	Austria
Ian Rippin	Environment Agency	UK
Craig Robinson	Scottish Government, Marine Laboratory	UK
Jorge Rodriguez Romero	DG ENV	
Sainio Pirjo	Ministry of the Environment	Finland
Nijole Striupkuviene	EPA – Water Division	Lithuania
Simona Tavazzi	DG JRC	
Inge Werner	Oekotoxzentrum / Swiss Centre for Applied Ecotoxicology (Eawag/EPFL)	Switzerland
Caroline Whalley	DEFRA	UK

SUMMARY

This report collects information on chemical analytical methods for the analysis of the new proposed priority substances (PS) of the European Water Framework Directive (WFD) and some existing PS for which the Environmental Quality Standards (EQS) have been changed under the first review of the PS list. First, analytical "standard" methods (ISO, CEN, US EPA) were searched. Then, the EU Member States (MS) were asked via the Chemical Monitoring and Emerging Pollutants (CMEP) expert group to provide validated "in-house methods" used as a national reference and to report their limits of detection (LODs) or quantification (LOQs). Finally, published literature articles were searched to get an overview of today's analytical performance.

Compliance monitoring for the WFD requires the achievement of a LOQ equal or below a value of 30% of the relevant EQS. The achieved method limits of quantification (LOQs) are therefore compared with 30% of the EQS, which is 0.3 × EQS.

Very low annual average AA-EQS values in the picogram-per-liter (pg/l) concentration range have been set for several of the new proposed PS: For Cypermethrin 80 pg/l (8 pg/l for coastal salt waters), for Dichlorvos 60 pg/l in coastal waters, for Dicofol 32 pg/l in coastal waters, for 17-alpha-ethinylestradiol 35 pg/l (7 pg/l in coastal waters), for 17-beta-estradiol 80 pg/l in coastal waters, and for Heptachlor/-Heptachlorepoxide 0.2 pg/l (10 fg/l in coastal waters). Dicofol and Heptachlor/-Heptachlorepoxide, for which biota EQS have been set (biota EQS: 33 µg/kg, and 6.7 ng/kg, respectively), however, are intended to be analysed in biota.

Moreover, a very challenging water EQS has been set for the already existing PS Brominated Diphenylethers (BDEs) (49 femtogram-per-liter (fg/l), and 2.4 fg/l in coastal waters). However, it is intended that BDEs be analysed in biota (EQS: 8.5 ng/kg). In addition, the water EQS for Polyaromatic Hydrocarbons (PAHs) has been lowered to 0.17 ng/l, and a biota EQS of 2-10 µg/kg added, which is more easy to reach.

In general, it is very difficult to reach with currently available analytical instruments LOQs in the low pg/l concentration range. A possibility could be the use of gas chromatography (GC) with high resolution mass spectrometry (HRMS). This technique, however, is not generally available in normal water monitoring laboratories. Also in the field of liquid chromatography mass spectrometry (LC-MS), instruments with improved sensitivity have become available in the last years.

Moreover, lower LOQs can be achieved by extracting higher volumes of water (10-1000 liters). These large-volume techniques, however, are very work and time intensive, and very costly, and are therefore not useful for routine WFD compliance monitoring (analysis of one sample per month).

The most challenging substances proposed as new PS are: Cypermethrin (EQS: 80 pg/l, and 8 pg/l for coastal salt waters), Dichlorvos (EQS: 60 pg/l in coastal waters), 17-alpha-ethinylestradiol (EQS: 35 pg/l, and 7 pg/l in coastal waters), and 17-beta-estradiol (EQS: 0.4 ng/l, and 80 pg/l in coastal waters).

Dicofol, Dioxins and dioxin-like compounds, Heptachlor/Heptachlorepoxide, Hexabromocyclododecane (HBCDD), Perfluorooctane sulfonic acid (PFOS), and the BDEs are intended to be analysed in biota.

Minor analytical problems could be encountered for the following substances: Aclonifen (EQS: 0.12 µg/l, and 12 ng/l for coastal salt waters), Bifenox (EQS: 12 ng/l, and 1.2 ng/l for coastal waters), Cybutryne (=Irgarol) (EQS: 2.5 ng/l), Diclofenac (EQS: 0.10 µg/l, and 10 ng/l for coastal waters), Quinoxyfen (EQS: 0.15 µg/l, and 15 ng/l for coastal waters), and Terbutryn (EQS: 65 ng/l, and 6.5 ng/l for coastal waters).

(All figures are AA-EQS for freshwaters unless otherwise stated)

1. Introduction

The main aim of EU water policy is to ensure that throughout the EU a sufficient quantity of good quality water is available and used more efficiently for the needs of people and businesses, as well as for the protection of the environment. In 2000 the Water Framework Directive (WFD; 2000/60/EC) (European Commission, 2000) established a legal basis to protect and restore clean water across Europe and ensure its long-term, sustainable use. The general objective of the WFD is to get all water – for example, lakes, rivers, streams and groundwater aquifers – into a healthy state by 2015.

Article 16 of the WFD sets out "strategies against pollution of water" outlining the steps to be taken. Article 16(4) of the WFD requires the Commission to review regularly its Annex X which contains the list of priority substances (PS) in the field of water policy, identified among those posing a significant risk to or via the aquatic environment.

The existing list of 33 priority substances was established by Decision No. 2455/2001/EC (European Commission, 2001), and amended by Directive 2008/105/EC (Environmental Quality Standards Directive, EQSD) (European Commission, 2008). The latter also established environmental quality standards (EQS) for all the 33 priority substances and for 8 other pollutants that were already regulated at EU level under existing legislation.

The review of the list as required by the WFD and EQSD has taken more than three years because of the extensive technical analysis involved in identifying possible new PS and setting EQS for them. On 31 January 2012, the European Commission published its proposal for a new Directive amending Directives 2000/60/EC and 2008/105/EC as regards PS in the field of water policy (European Commission, 2012). This proposal (COM(2011)876) includes a revised (second) list of PS, and provisions to improve the functioning of the legislation. The main features of the proposal are:

- 15 additional PS, 6 of them designated as priority hazardous substances;
- stricter EQS for four existing PS and slightly revised EQS for three others;
- the designation of two existing PS as priority hazardous substances;
- the introduction of biota standards for several substances;
- provisions to improve the efficiency of monitoring and the clarity of reporting with regard to certain substances behaving as ubiquitous persistent, bio-accumulative and toxic (PBT) substances;
- a provision for a watch-list mechanism designed to allow targeted EU-wide monitoring of substances of possible concern to support the prioritisation process in future reviews of the priority substances list.

With this proposal, the Commission is proposing to add 15 chemicals to the list of 33 pollutants that are monitored and controlled in EU surface waters. This is another step towards improving the quality of our river, lake and coastal waters. The 15 substances include industrial chemicals as well as substances used in biocides, pharmaceuticals and plant protection products. They have been selected on the basis of scientific evidence that they may pose a significant risk to health.

The newly proposed substances are the outcome of a review that considered the risks posed by some 2000 substances according to their levels in surface waters, and their hazardousness, production and use. For six of the 15 new PS the classification proposed would require their emissions to water to be phased out within 20 years. The proposal also includes stricter standards for four currently controlled substances, and a requirement to phase out the emissions of two others already on the list.

The proposed 15 additional PS are:

- Plant protection product substances: Aclonifen, Bifenox, Cypermethrin, Dicofol, Heptachlor/Heptachlorepoxide, Quinoxyfen
- Substances used in biocidal products: Cybutryne, Dichlorvos, Terbutryn
- Industrial chemicals: Perfluorooctane sulfonic acid (PFOS), Hexabromocyclo-dodecane (HBCDD)
- Combustion by-products: Dioxins and dioxin-like PCBs
- Pharmaceutical substances: 17-alpha-ethinylestradiol, 17-beta-estradiol, Diclofenac

Pharmaceuticals are proposed for the first time. The proposal does not put into question the medicinal value of these substances, but addresses the potential harmful effects of their presence in the aquatic environment. Concentrations above the proposed standards can affect fish health, reducing successful reproduction, for example, and harming other living organisms. Our awareness of the impact of pharmaceuticals in the environment has grown considerably in recent years, and the proposal is based on the latest scientific knowledge.

The Commission also proposes improvements to the monitoring and reporting of chemical pollutants in water, as well as a mechanism to obtain better information on the concentrations of other pollutants that might need to be controlled in the future at EU level. The Commission proposal is accompanied by a report to the European Parliament and Council on the outcome of the review of the existing list of controlled substances.

As a rule, Member States must meet environmental quality standards for new PS by 2021 (the deadline of the 2nd River Basin Management Plan). Longer timelines are possible in specific cases if the conditions for exemptions set out in the WFD are applicable.

Of the additional 15 substances, the following are proposed as Priority Hazardous Substances: Dicofol, Quinoxyfen, PFOS, Heptachlor(epoxide), HBCDD, Dioxin and dioxin-like PCBs. The substances already on the list but which would be subject to stricter standards are: Brominated diphenylethers, Fluoranthene, Nickel, Polyaromatic Hydrocarbons (PAHs). The two existing substances which would become Priority Hazardous Substances are Diethylhexylphthalate and Trifluralin.

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European Commission (EC). **2001**. Decision No 2455/2001/EC of the European Parliament and of the Council of 20 November 2001 establishing the list of priority substances in the field of water policy and amending Directive 2000/60/EC. Official Journal of the European Union L331, 1–5.

European Commission (EC). **2008**. Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy. Official Journal of the European Union L348, 84–97.

European Commission (EC). **2012**. Proposal for a Directive of the European Parliament and of the Council amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. COM(2011) 876 final.

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2. General comments on detection and quantification limits (LODs and LOQs)

It must be noted that there are different definitions for the terms “limit of detection” (LOD) and “limit of quantification” (LOQ), and that laboratories use different methods for the calculation of these method performance characteristics. In general, LODs or LOQs can be estimated from the lowest point of the calibration curve or from blank samples. The LOD corresponds usually to a signal-to-noise ratio (S/N) of 3, and the LOQ to S/N of 10. According to ISO/TS 13530 (ISO, 2009), the LOD is three times the standard deviation of the blank samples, and the LOQ three times the LOD (LOQ = 3 × LOD).

The newer US EPA methods mention that “the detection limits and quantitation levels (of methods) are usually dependent on the level of interferences rather than instrumental limitations”, and that “method(s are usually) performance-based which means that you may modify the method to improve performance (e.g., to overcome interferences or improve the accuracy or precision of the results)”.

To this statement must be added that analytical instruments of the latest generation with higher sensitivity are undoubtedly advantageous to analyse trace amounts of environmental pollutants.

Finally, it should be stressed that LODs or LOQs are not constant values and can change over time. They are dependent on several parameters such as the interferences, blank contaminations, and instrument tunings (daily instrument performance), and hence have to be verified regularly.

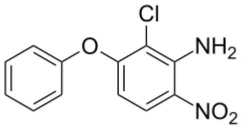
Reference

ISO/TS 13530. **2009**. International Organization for Standardization; Water quality - Guidance on analytical quality control for chemical and physicochemical water analysis.

3. Proposed Priority Substances

3.1. Aclonifen

Aclonifen is an herbicide used in plant protection products.

CAS Number 74070-46-5	**Log K_{OW}** 4.37	**Water Solubility [mg/l]** 1.4
Chemical structure **(MW 264.7)**	**AA-EQS** **Inland (fresh) and other (salt) surface waters**	**MAC-EQS** **Inland (fresh) and other (salt) surface waters**
	Fresh 0.12 µg/l Salt 0.012 µg/l = 12 ng/l	Fresh 0.12 µg/l Salt 0.012 µg/l = 12 ng/l

No analytical “standard” method is available for Aclonifen.

Methods applied by EU Member States and comments received

Germany: Lowest reported LOQ: 0.01 µg/l = 10 ng/l; according to EPA 619; GC-MS (modified); EPA method 619 is an old LLE-GC method using a thermionic bead detector in the nitrogen mode.

Sweden: LOQ: 0.020 µg/l = 20 ng/l; Method: OMK 51 (liquid-liquid extraction with dichloromethane; identification and quantification with GC-MS).

UK-EA: Not been requested but could be added to existing suites.

Northern-Ireland: Has never been requested. A new method of analysis would need to be developed.

Literature methods

Only one article could be found for the analysis of Aclonifen in water. Passeport and co-workers (Passeport et al., 2010) applied SPME-GC-MS for water analysis. The achieved LOQ of 0.10 µg/l of this method, however, is not sufficient for WFD compliance monitoring. In addition, Aclonifen was analysed by Kmellar and co-workers (Kmellar et al., 2008) in vegetables using LC-tandem-MS, by Wang and co-workers (Wang et al., 2010) in berries using LC-MS-MS and UHPLC-qTOF-MS, and by Schummer and co-workers (Schummer et al., 2010) in air samples using GC-MS-MS.

Conclusion

The LOQ of 10 ng/l achieved by one German laboratory (reported in 2011) is **sufficient** for compliance monitoring in inland surface waters (0.3 × EQS = 36 ng/l), but not sufficient (but close) in coastal surface waters (0.3 × EQS = 3.6 ng/l).

References

EPA Method 619. The determination of triazine pesticides in municipal and industrial wastewater. U.S. Environmental Protection Agency, Office of Water, Washington, DC, USA.

Kmellar, B., Fodor, P., Pareja, L., Ferrer, C., Martinez-Uroz, M.A., Valverde, A., Fernandez-Alba, A.R. **2008**. Validation and uncertainty study of a comprehensive list of 160 pesticide residues in multi-class vegetables by liquid chromatography–tandem mass spectrometry. Journal of Chromatography A 1215, 37–50.

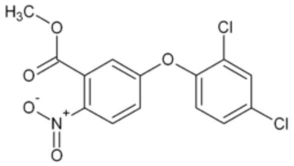
Passeport, E., Guenne, A., Culhaoglu, T., Moreau, S., Bouyé, J.-M., Tournebize, J. **2010**. Design of experiments and detailed uncertainty analysis to develop and validate a solid-phase microextraction/gas chromatography–mass spectrometry method for the simultaneous analysis of 16 pesticides in water. Journal of Chromatography A 1217, 5317–5327.

Schummer, C., Mothiron, E., Appenzeller, B.M.R., Rizet A.-L., Wennig, R., Millet, M. **2010**. Temporal variations of concentrations of currently used pesticides in the atmosphere of Strasbourg, France. Environmental Pollution 158, 576–584.

Wang, J., Leung, D., Chow, W. **2010**. Applications of LC/ESI-MS/MS and UHPLC QqTOF MS for the determination of 148 pesticides in berries. Journal of Agricultural Food and Chemistry 58, 5904–5925.

3.2. Bifenox

Bifenox is an herbicide used as a control of broad leaved weeds in post-emergence applications in winter cereals.

CAS Number 42576-02-3	**Log K_{OW}** 3.64	**Water Solubility [mg/l]** < 0.1
Chemical structure **(MW 342.1)**	**AA-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]	**MAC-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]
	Fresh 0.012 = 12 ng/l Salt 0.0012 = 1.2 ng/l	Fresh 0.04 = 40 ng/l Salt 0.004 = 4 ng/l

No analytical "standard" method is available for Bifenox.

Methods applied by EU Member States and comments received

Germany: Lowest reported LOQ: 0.01 µg/l = 10 ng/l (according to ISO 6468; GC-MS).

Sweden: LOQ: 0.050 µg/l = 50 ng/l (method: OMK 57).

OMK 57 is an on-line SPE-LC-MS-MS method developed by Jansson and Kreuger (Jansson and Kreuger, 2010). Bifenox is not included in this article, but it was confirmed by the author that it has been added afterwards to the method; however, the LOQ of 50 ng/l is high because LC-MS ionization is difficult for Bifenox.

UK-EA: Not been requested but could be added to existing suites.

Northern-Ireland: Has never been requested. A new method of analysis would need to be developed.

Literature methods

Only few articles could be found on the analysis of Bifenox in water samples. Bifenox and Bifenox acid were analysed by Laganà and co-workers (Laganà et al., 2000; 2002) in water using SPE-LC-MS-MS; a LOD of 2-3 ng/l was achieved for Bifenox and Bifenox acid by extracting 0.5-1 l river water. Berenzen and co-workers (Berenzen et al., 2005) achieved only a LOD of 0.05 µg/l using SPE-GC-MS (extraction of 1 liter).

In addition, Bifenox was analysed by Díez and co-workers (Díez et al., 2006) in barley (food) samples using LC–ESI(+)-MS-MS, and in soil samples using GC-MS analysis (Díez et al., 2008), by Nguyen and co-workers (Nguyen et al., 2008) in cabbage (food) using GC-MS, and by Kanrar and co-workers (Kanrar et al., 2010) in tea samples using LC-MS-MS.

Conclusion

The LOQ of 10 ng/l achieved by one German laboratory (reported in 2011) is **not sufficient** for compliance monitoring in inland (0.3 × EQS = 3.6 ng/l) and in coastal surface waters (0.3 × EQS = 0.36 ng/l).

Bifenox can be analysed by GC- or LC-MS methods; the salt water EQS of 1.2 ng/l is not easy to achieve.

References

Berenzen, N., Lentzen-Godding, A., Probst, M., Schulz, H., Schulz, R., Liess, M. **2005**. A comparison of predicted and measured levels of runoff-related pesticide concentrations in small lowland streams on a landscape level. Chemosphere 58, 683–691.

Díez, C., Traag, W.A., Zommer, P., Marinero, P., Atienza, J. **2006**. Comparison of an acetonitrile extraction/partitioning and “dispersive solid-phase extraction” method with classical multi-residue methods for the extraction of herbicide residues in barley samples. Journal of Chromatography A 1131, 11–23.

Díez, C., Barrado, E., Marinero, P., Sanz, M. **2008**. Orthogonal array optimization of a multiresidue method for cereal herbicides in soils. Journal of Chromatography A 1180, 10–23.

EN ISO 6468. **1996**. Water quality - Determination of certain organochlorine insecticides, polychlorinated biphenyls and chlorobenzenes - Gas chromatographic method after liquid-liquid extraction.

Jansson, C.; Kreuger, J. **2010**. Multiresidue analysis of 95 pesticides at low nanogram/liter levels in surface waters using online preconcentration and high performance liquid chromatography/tandem mass spectrometry. Journal of AOAC International 93, 1732-1747.

Kanrar, B., Mandal, S., Bhattacharyya, A. **2010**. Validation and uncertainty analysis of a multiresidue method for 42 pesticides in made tea, tea infusion and spent leaves using ethyl acetate extraction and liquid chromatography–tandem mass spectrometry. Journal of Chromatography A 1217, 1926–1933.

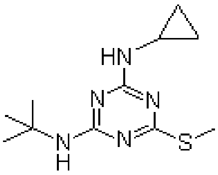
Laganà, A., Fago, G., Fasciani, L., Marino, A., Mosso, M. **2000**. Determination of diphenyl-ether herbicides and metabolites in natural waters using high-performance liquid chromatography with diode array tandem mass spectrometric detection. Analytica Chimica Acta 414, 79–94.

Laganà, A., Bacaloni, A., De Leva, I., Faberi, A., Fago, G., Marino, A. **2002**. Occurrence and determination of herbicides and their major transformation products in environmental waters. Analytica Chimica Acta 462, 187–198.

Nguyen, T.D., Yu, J.E., Lee, D.M., Lee, G.-H. **2008**. A multiresidue method for the determination of 107 pesticides in cabbage and radish using QuEChERS sample preparation method and gas chromatography mass spectrometry. Food Chemistry 110, 207–213.

3.3. Cybutryne = Irgarol

Cybutryne (= Irgarol) is an effective triazine herbicidal biocide (or algicide**)** mainly used as an antifouling agent in paints for boats and vessels. It is applied at marine as well as at inland freshwater sites.

CAS Number 28159-98-0	**Log K_{OW}** 3.95	**Water Solubility [mg/l]** 7
Chemical structure **(MW 253.4)**	**AA-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]	**MAC-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]
	0.0025 = 2.5 ng/l (fresh and salt)	0.016 = 16 ng/l (fresh and salt)

No analytical “standard” method is available for Cybutryne.

Methods applied by EU Member States and comments received

Sweden: LOQ: 0.002 µg/l = 2 ng/l (Method OMK 57). OMK 57 is an on-line SPE-LC-MS-MS method developed by Jansson and Kreuger (Jansson and Kreuger, 2010).

Germany: Lowest reported LOQ: 0.001 µg/l = 1 ng/l; according to EPA 619; GC-MS (modified).

UK-EA: Current minimum reporting value (mrv) = 5 ng/l in freshwater suite. Could meet requirement with some method development.

UK-SEPA: Not currently requested but could possibly be added to existing suites with some method modifications to accommodate lower LOD.

Northern-Ireland: Has never been requested. A new method of analysis would need to be developed.

Literature methods

Many articles on the analysis of Irgarol in water have been published. Some examples are given here:

Extraction (volume)	**Analysis**	**LOD**	**Reference**
SPE	GC-MS	Not reported	Biselli et al., 2000
on-line SPE	HPLC–APCI–MS	5 ng/l	Gimeno et al., 2001
SPE (1 l)	LC–ESI-MS–MS	0.2 ng/l	Lamoree et al., 2002
LLE (2 l)	GC-MS	1 ng/l	Hall Jr. et al., 2005
SPE (2 l)	GC-MS	1 ng/l	Carbery et al., 2006
SPE (0.5 l)	LC-MS	0.2 ng/l	Cai et al., 2006
SPE (2 l)	GC-MS	3.1 ng/l	Gatidou et al., 2007
LLE (1 l)	GC-MS	0.6 ng/l	Hall Jr. et al., 2009

On-line SPE (20 ml)	LC-MS-MS	LOQ: 6 ng/l	Singer et al., 2010
SPE (1 l)	LC-MS-MS	LOD: 0.1 ng/l LOQ: 0.3 ng/l	Sánchez-Rodríguez et al., 2011a

In addition, Irgarol was analysed in harbour sediments using HPLC-APCI-MS (Thomas et al., 2000), LC-MS-MS (Sánchez-Rodríguez et al., 2011b), and GC-NPD (Cassi et al., 2008).

Conclusion

The LOQ of 1 ng/l reported by one German laboratory is **nearly sufficient** for compliance monitoring in inland and coastal surface waters (0.3 × EQS = 0.75 ng/l).

The literature methods show that the analysis of Cybutryne (= Irgarol) by GC- and LC-MS methods is relatively easy.

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Cai, Z., Fun, Y., Ma, W.-T., Lam, M.H.-W., Tsui, J. **2006**. LC–MS analysis of antifouling agent Irgarol 1051 and its decyclopropylated degradation product in seawater from marinas in Hong Kong. Talanta 70, 91–96.

Carbery, K., Owen, R., Frickers, T., Otero, E., Readman, J. **2006**. Contamination of Caribbean coastal waters by the antifouling herbicide Irgarol 1051. Marine Pollution Bulletin 52, 635–644.

Cassi, R., Tolosa, I., De Mora, S. **2008**. A survey of antifoulants in sediments from Ports and Marinas along the French Mediterranean coast. Marine Pollution Bulletin 56, 1943–1948.

EPA Method 619. The determination of triazine pesticides in municipal and industrial wastewater. U.S. Environmental Protection Agency, Office of Water, Washington, DC, USA.

Gatidou, G., Thomaidis, N.S., Zhou, J.L. **2007**. Fate of Irgarol 1051, diuron and their main metabolites in two UK marine systems after restrictions in antifouling paints. Environment International 33, 70–77.

Gimeno, R.A., Aguilar, C., Marcé, R.M., Borrull, F., **2001**, Monitoring of antifouling agents in water samples by on-line solid-phase extraction–liquid chromatography–atmospheric pressure chemical ionization mass spectrometry. Journal of Chromatography A 915, 139–147.

Hall Jr., L.W., Killen, W.D., Anderson, R.D., Gardinali, P.R., Balcomb, R. **2005**. Monitoring of Irgarol 1051 concentrations with concurrent phytoplankton evaluations in East Coast areas of the United States. Marine Pollution Bulletin 50, 668–681.

Hall Jr., L.W., Killen, W.D., Anderson, R.D., Balcomb, R., Gardinali, P.R. **2009**. Ecological risk of Irgarol 1051 and its major metabolite in coastal California marinas and reference areas. Marine Pollution Bulletin 58, 702–710.

Jansson, C.; Kreuger, J. **2010**. Multiresidue analysis of 95 pesticides at low nanogram/liter levels in surface waters using online preconcentration and high performance liquid chromatography/tandem mass spectrometry. Journal of AOAC International 93, 1732-1747.

Lamoree, M.H., Swart, C.P., van der Horst, A., van Hattum, B. **2002**. Determination of diuron and the antifouling paint biocide Irgarol 1051 in Dutch marinas and coastal waters. Journal of Chromatography A 970, 183–190.

Sánchez-Rodríguez, A., Sosa-Ferrera, Z., Santana-del Pino, A., Santana-Rodríguez, J.J. **2011a**. Probabilistic risk assessment of common booster biocides in surface waters of the harbours of Gran Canaria (Spain). Marine Pollution Bulletin 62, 985–991.

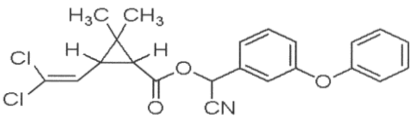
Sánchez-Rodríguez, A., Sosa-Ferrera, Z., Santana-Rodríguez, J.J. **2011b**. Applicability of microwave-assisted extraction combined with LC–MS/MS in the evaluation of booster biocide levels in harbour sediments. Chemosphere 82, 96–102.

Singer, H., Jaus, S., Hanke, I., Lück, A., Hollender, J., Alder, A.C. **2010**. Determination of biocides and pesticides by on-line solid phase extraction coupled with mass spectrometry and their behaviour in wastewater and surface water. Environmental Pollution 158, 3054-3064.

Thomas, K.V., Blake, S.J., Waldock, M.J. **2000**. Antifouling paint booster biocide contamination in UK marine sediments. Marine Pollution Bulletin 40, 739-745.

3.4. Cypermethrin

Cypermethrin is a pyrethroid insecticide.

CAS Number 52315-07-8	**Log K_{OW}** 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 $8\ 10^{-5}$ = 0.00008 = 0.08 ng/l = 80 pg/l Salt $8\ 10^{-6}$ = 0.000008 = 0.008 ng/l = 8 pg/l	Fresh $6\ 10^{-4}$ = 0.0006 = 0.6 ng/L Salt $6\ 10^{-5}$ = 0.00006 = 0.06 ng/l = 60 pg/l

Standard Methods

The US EPA method 1699 reaches a LOQ of $6.6\ 10^{-5}$ µg/l = 0.066 ng/l = 66 pg/l (EPA 1699), and for solid samples 0.0024 µg/kg = 2.4 ng/kg (EPA 1699).

Description of EPA Method 1699: For determination of selected organochlorine, organo-phosphorus, triazine, and pyrethroid pesticides in multi-media environmental samples by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS).

Extraction: Aqueous samples are spiked with stable isotopically labeled analogs of the pesticides into a 1-L sample. The sample is extracted at neutral pH with methylene chloride using separatory funnel extraction (SFE) or continuous liquid/liquid extraction (CLLE).

Clean-up: Extracts of aqueous, solid or mixed phase samples are cleaned up using an aminopropyl SPE column followed by a microsilica column. Extracts may be further cleaned up using gel permeation chromatography (GPC) or solid-phase cartridge techniques. Extracts in which the organo-chlorine pesticides only are to be determined may be further cleaned up using silica gel, Florisil, or alumina chromatography.

Determination by GC/HRMS: The analytes are separated by the GC and detected by a high-resolution (≥8,000) mass spectrometer. Two exact m/z's for each pesticide are monitored throughout a pre-determined retention time window.

Methods applied by EU Member States and comments received

Germany: Lowest reported LOQ: 0.001 µg/l = 1 ng/l (according to ISO 11369; LC-UV).

Bulgaria: Specific substance Draft EQS for Bulgaria 0.1 µg/l; LOQ: 0.001 = 1 ng/l; LLE-GC-ECD (EN ISO 6468). We have no data on biota and sediment.

Sweden: LOQ: 0.010 µg/l = 10 ng/l (method: OMK 51; liquid-liquid extraction with dichloromethane; Identification and quantification with GC-MS).

UK-EA: Will require a new approach using high sensitivity technology and/or increased sample sizes. Major problems expected for marine sediments.

Northern-Ireland: Will require a new method of analysis using more sensitive instrumentation and possibly larger sample volumes.

Literature methods

Only few articles on the analysis of Cypermethrin were found:

Extraction (volume)	Analysis	LOD	Reference
SPE	GC-ECD or FPD	0.05 µg/l	Jergentz et al., 2005
SPE (1 l)	GC-µECD	0.5-15 ng/l	Xue et al., 2005

Conclusion

The LOQ of 1 ng/l reported by one German laboratory (in 2011) is **not sufficient** for compliance monitoring in inland and coastal surface waters (0.3 × EQS = 24 pg/l; 2.4 pg/l for coastal). To reach LOQs in the low pg/l concentration range is **extremely difficult**, if not impossible with current methods. Even the LOQ reported by the EPA method 1699 (66 pg/l) using GC/HRMS is not sufficient. Lower LOQs could be achieved by extracting 10 liters of water (large volume). In addition, a promising strategy might be passive sampling, as indicated by Ireland.

References

EN ISO 6468. **1996**. Water quality - Determination of certain organochlorine insecticides, polychlorinated biphenyls and chlorobenzenes - Gas chromatographic method after liquid-liquid extraction.

EPA Method 1699. December **2007**. Pesticides in water, soil, sediment, biosolids, and tissue by HRGC/HRMS. EPA-821-R-08-001. U.S. Environmental Protection Agency, Office of Water, Washington, DC, USA.

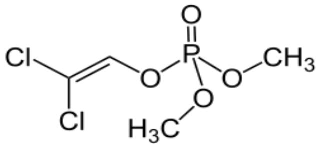
ISO 11369. **1997**. Water quality - Determination of selected plant treatment agents - Method using high performance liquid chromatography with UV detection after solid-liquid extraction.

Jergentz, S., Mugni, H., Bonetto, C., Schulz, R.. **2005**. Assessment of insecticide contamination in runoff and stream water of small agricultural streams in the main soybean area of Argentina. Chemosphere 61, 817–826.

Xue, N., Xu, X., Jin, Z. **2005**. Screening 31 endocrine-disrupting pesticides in water and surface sediment samples from Beijing Guanting reservoir. Chemosphere 61, 1594–1606.

3.5. Dichlorvos

Dichlorvos is an organophosphorous insecticide.

CAS Number 62-73-7	**Log K_{OW}** 1.9	**Water Solubility [g/l]** ~ 8-18
Chemical structure **(MW 221.0)**	**AA-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]	**MAC-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]
	Fresh 6 10^{-4} = 0.0006 = 0.6 ng/l Salt 6 10^{-5} = 0.00006 = 0.06 ng/l = 60 pg/l	Fresh 7 10^{-4} = 0.0007 = 0.7 ng/L Salt 7 10^{-5} = 0.00007 = 0.07 ng/l = 70 pg/l

Standard Methods

The European Standard EN 12918 reaches a LOQ of 0.01 µg/l (EN 12918).

Description: This European Standard specifies the extraction processes and gas chromatographic (GC) methods for determining parathion, parathion-methyl and some other organophosphorus compounds in drinking waters, surface waters and waste waters. This standard may also be suitable for the determination of other organic compounds. The range is dependent on the compound and the source of water and is typically up to 1 µg/l with a reporting limit of 0.01 µg/l for drinking waters involving a 1000 to 1 extraction ratio.

The US EPA Method 622 reaches a LOQ of 0.1 µg/l (EPA 622).

Description of EPA 622: A measured volume of sample, approximately 1 liter, is extracted with 15 % methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to hexane during concentration to a volume of 10 mL or less. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by gas chromatography with a thermionic bead or flame photometric detector in the phosphorus mode.

Methods applied by EU Member States and comments received

Lithuania: LOQ: 0.5 µg/l (EN 12918 and EN ISO 10695).

UK-EA: Will require a new approach using high sensitivity technology and/or increased sample sizes.

Northern-Ireland: Will require a new method of analysis using more sensitive instrumentation and possibly larger sample volumes.

Literature methods

Only few articles on the analysis of Dichlorvos in water were found:

Extraction (volume)	**Analysis**	**LOD**	**Reference**
SPE (1 l)	GC with N,P detector	9 ng/l	Hamers et al., 2003
SPE-disks (1 l)	GC-ion-trap-MS-MS	Not reported	Steen et al., 2001

Apparently, Dichlorvos has been analysed more often in other matrices:

Matrix	Analysis	LOD	Reference
Foodstuff	GC-MS-MS	0.27 ng/g	Arrebola et al., 2003
Sludge	LC-MS-MS	2 ng/g	García-Valcárcel and Tadeo, 2009
Marine sediments	GC-MS-MS	0.770 ng/g = µg/kg	Camino-Sánchez et al., 2011
Air particles	GC-MS-MS	2.63 pg/m^3	Coscollà et al, 2011
Human hair	GC-MS-MS	2 pg/mg = ng/g	Salquèbrea et al., 2012

Conclusion

To reach LOQs in the low pg/l concentration range is **extremely difficult**, if not impossible with current analytical methods. A possibility could be GC high resolution MS. Compliance monitoring requires the achievement of a LOQ equal or below a value of 30% of the relevant environmental quality standards, which is 0.3 × EQS = 0.18 ng/l for fresh waters, and 18 pg/l for coastal waters. Current methods are **not sufficient**.

References

Arrebola, F.J., Martínez Vidal, J.L., Mateu-Sánchez, M., Álvarez-Castellón, F.J. **2003**. Determination of 81 multiclass pesticides in fresh foodstuffs by a single injection analysis using gas chromatography–chemical ionization and electron ionization tandem mass spectrometry. Analytica Chimica Acta 484, 167–180.

Camino-Sánchez, F.J., Zafra-Gómez, A., Pérez-Trujillo, J.P., Conde-González, J.E., Marques, J.C., Vílchez, J.L. **2011**. Validation of a GC–MS/MS method for simultaneous determination of 86 persistent organic pollutants in marine sediments by pressurized liquid extraction followed by stir bar sorptive extraction. Chemosphere 84, 869–881.

Coscollà, C., Castillo, M., Pastorb, A., Yusà, V. **2011**. Determination of 40 currently used pesticides in airborne particulate matter (PM 10) by microwave-assisted extraction and gas chromatography coupled to triple quadrupole mass spectrometry. Analytica Chimica Acta 693, 72–81.

EPA Method 622. The determination of organophosphorus pesticides in municipal and industrial wastewater. U.S. Environmental Protection Agency, Office of Water, Washington, DC, USA.

EN 12918:1999. Water quality - Determination of parathion, parathion-methyl and some other organophosphorus compounds in water by dichloromethane extraction and gas chromatographic analysis. CEN, European Committee for Standardization, Brussels.

EN ISO 10695. **2000**. Water quality - Determination of selected organic nitrogen and phosphorus compounds - Gas chromatographic methods.

García-Valcárcel, A.I., Tadeo, J.L. **2009**. A combination of ultrasonic assisted extraction with LC–MS/MS for the determination of organophosphorus pesticides in sludge. Analytica Chimica Acta 641, 117–123.

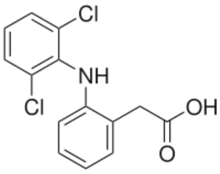
Hamers, T., Van den Brink, P.J., Mos, L., Van der Linden, S.C., Legler, J., Koeman, J.H., Murk, A.J. **2003**. Estrogenic and esterase-inhibiting potency in rainwater in relation to pesticide concentrations, sampling season and location. Environmental Pollution 123, 47–65.

Salquèbrea, G., Schummera, C., Millet, M., Briand, O., Appenzellera, B.M.R. **2012**. Multi-class pesticide analysis in human hair by gas chromatography tandem (triple quadrupole) mass spectrometry with solid phase microextraction and liquid injection. Analytica Chimica Acta 710, 65– 74.

Steen, R.J.C.A., Van der Vaart, J., Hiep, M., Van Hattum, B., Cofino, W.P., Brinkman, U.A.Th. **2001**. Gross fluxes and estuarine behaviour of pesticides in the Scheldt Estuary (1995–1997). Environmental Pollution 115, 65-79.

3.6. Diclofenac

Diclofenac is a non-steroidal anti-inflammatory drug.

CAS Number 15307-86-5	**Log K_{OW}** 4.0-4.5	**Water Solubility [g/l]** 50
Chemical structure **(MW 296.2)**	**AA-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]	**MAC-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]
	Fresh 0.1 µg/l Salt 0.01 µg/l = 10 ng/l	not applicable

Standard Methods

No analytical standard method is available for Diclofenac, but the EPA method 1694 can be applied (EPA 1694).

Description: EPA Method 1694 determines pharmaceuticals and personal care products (PPCPs) in environmental samples by high performance liquid chromatography combined with tandem mass spectrometry (HPLC-MS-MS) using isotope dilution and internal standard quantitation techniques. This method has been developed for use with aqueous, solid, and biosolids matrices.

Methods applied by EU Member States and comments received

Italy: LOQ: 10 ng/l; Internal Method, validated.

France: LOQ: 7 ng/l; Aqua-Ref Method; SPE-MS-MS; 1000 ml water; extraction with 500 mg Oasis HLB.

UK-EA: Not been requested to be developed in water but effluent method exists.

UK-SEPA: Not currently requested but could possibly be added to existing suites.

Northern-Ireland: Has never been requested. A new method of analysis would need to be developed.

Literature methods

Some articles on the analysis of different pharmaceuticals including Diclofenac in water have been selected:

Extraction (volume)	**Analysis**	**LOD**	**Reference**
SPE (0.4 l)	LC-MS-MS	LOQ: 10 ng/l	Hao et al., 2006
SPE (0.2 l); waste water	LC-ion-trap-MS-MS	LOD: 0.4 ng/l LOQ: 1 ng/l	Martínez Bueno et al., 2007
SPE (0.5 l)	LC-ion-trap-MS-MS	LOD: 1 ng/l LOQ: 7 ng/l	Gros et al., 2009
SPE (0.1 l)	LC-ion-trap-MS-MS	LOD: 0.15 ng/l LOQ: 0.49 ng/l	Grujic et al., 2009

Conclusion

The lowest LOQ of 7 ng/l reported by France is **sufficient** for compliance monitoring in inland surface waters (0.3 × EQS = 30 ng/l), but not sufficient in coastal surface waters (0.3 × EQS = 3 ng/l). Literature methods show that LOQs of 1 ng/l are achievable with modern LC-MS-MS instruments.

References

EPA Method 1694. December **2007**. Pharmaceuticals and personal care products in water, soil, sediment, and biosolids by HPLC/MS/MS. EPA-821-R-08-002. U.S. Environmental Protection Agency, Office of Water, Washington, DC, USA.

Gros, M., Petrovic, M., Barceló, D. **2009**. Tracing pharmaceutical residues of different therapeutic classes in environmental waters by using liquid chromatography/quadrupole-linear ion trap mass spectrometry and automated library searching. Analytical Chemistry 81, 898–912.

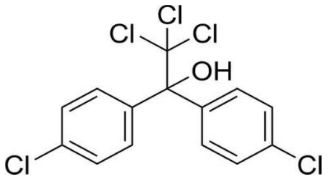
Grujic, S., Vasiljevic, T., Lausevic, M. **2009**. Determination of multiple pharmaceutical classes in surface and ground waters by liquid chromatography–ion trap–tandem mass spectrometry. Journal of Chromatography A, 1216, 4989–5000.

Hao, C., Lissemore, L., Nguyen, B., Kleywegt, S., Yang, P., Solomon, K. **2006**. Determination of pharmaceuticals in environmental waters by liquid chromatography/electrospray ionization/tandem mass spectrometry. Analytical and Bioanalytical Chemistry 384, 505–513.

Martínez Bueno, M.J., Agüera, A., Gómez, M.J., Hernando, M.D., García-Reyes, J.F., Fernández-Alba, A.R. **2007**. Application of liquid chromatography/quadrupole-linear ion trap mass spectrometry and time-of-flight mass spectrometry to the determination of pharmaceuticals and related contaminants in wastewater. Analytical Chemistry 79, 9372-9384.

3.7. Dicofol

Dicofol is an organochlorine pesticide (arcaricide; miticide) that is chemically related to DDT, and used for controlling mites that damage cotton, fruit trees and vegetables.

CAS Number 115-32-2	**Log K_{OW}** 4.08-4.32	**Water Solubility [mg/l]** 0.8	
Chemical structure **(MW 370.5)**	**AA-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]	**MAC-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]	**EQS biota** [µg/kg]
	Fresh $1.3\ 10^{-3}$ = 0.0013 = 1.3 ng/l Salt $3.2\ 10^{-5}$ = 0.000032 = 32 pg/l	not applicable	33 µg/kg

Standard Methods

No analytical standard method is available for Dicofol.

Methods applied by EU Member States and comments received

Germany: Lowest reported LOQ by one German laboratory: 0.005 µg/l = 5 ng/l (according to DIN 38407-2; GC-ECD).

Sweden: LOQ: 0.010 µg/l = 10 ng/l; method OMK 51; liquid-liquid extraction with dichloromethane; Identification and quantification with GC-MS).

UK-EA: Will require a new approach using high sensitivity technology and/or increased sample sizes.

Northern-Ireland: Has never been requested. A new method of analysis would need to be developed.

Literature methods

It was difficult to find articles on the analysis of Dicofol in water or biota.

Recently, Zhong and co-workers (Zhong et al., 2012) presented a large volume analytical method for the analysis of some pesticides (including Dicofol) in seawater. This method consisted in large volume SPE with self-packed glass columns of 1000 l seawater followed by GC-MS determination in electron capture negative chemical ionization mode (EC-NCI). The MDL achieved was 0.2 pg/l.

In addition, another method was found for the analysis of Dicofol in human breast milk using GC-MS after LLE and clean-up, achieving a LOQ of 0.20 ng/g lipid (Fujii et al., 2011).

Conclusion

The lowest LOQ of 5 ng/l reported by one German laboratory (in 2011) is **not sufficient** for compliance monitoring in inland and coastal surface waters (0.3 × EQS = 0.39 ng/l; 9.6 pg/l for coastal). To reach LOQs in the low pg/l concentration range is **extremely difficult**, if not impossible with current methods. Lower LOQs can be achieved by extracting higher volumes of water (large volume: 10-1000 l; see paper by Zhong and co-workers (Zhong et al., 2012)). Such large-volume SPE methods, however, are not very useful for WFD compliance monitoring (one sample per month), as they are very work intensive, and very costly.

Therefore, biota analysis is recommended for Dicofol. The paper by Fujii and co-workers (Fujii et al., 2011) indicates that 1/3 of the EQS (9.9 µg/kg) should be achievable.

References

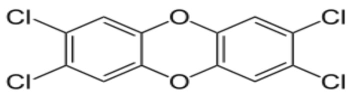
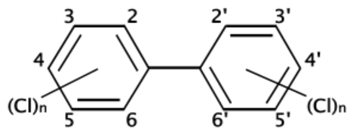
DIN 38407-2. **1993**. German standard methods for the determination of water, waste water and sludge; jointly determinable substances (group F); determination of low volatile halogenated hydrocarbons by gas chromatography.

Fujii, Y., Haraguchi, K., Harada, K.H., Hitomi, T., Inoue, K., Itoh, Y., Watanabe, T., Takenaka, K., Uehara, S., Yang, H.-R., Kim, M.-Y., Moon, C.-S., Kim, H.-S., Wangi, P., Liu, A., Hung, N.N., Koizumi, A. **2011**. Detection of dicofol and related pesticides in human breast milk from China, Korea and Japan. Chemosphere 82, 25–31.

Zhong, G., Xie, Z., Cai, M., Möller, A., Sturm, R., Tang, J., Zhang, G., He, J., Ebinghaus, R. **2012**. Distribution and air - sea exchange of current-use pesticides (CUPs) from East Asia to the high Arctic Ocean. Environmental Science & Technology 46, 259–267.

3.8. Dioxins and dioxin-like PCBs

Dioxins and dioxin-like compounds comprise Polychlorinated dibenzo-*p*-dioxins (PCDDs), Polychlorinated dibenzofurans (PCDFs), Polychlorinated biphenyls (PCBs) (twelve of them have "dioxin-like" properties). There are 75 PCDDs, and seven of them are specifically toxic. There are 135 PCDF congeners, and ten of them have "dioxin-like" properties. Dioxins occur as by-products in the manufacture of some organochlorines, in the incineration of chlorine-containing substances such as PVC (polyvinyl chloride), in the chlorine bleaching of paper, and from natural sources such as volcanoes and forest fires.

	Log K_{OW} > 7	**Water Solubility [mg/l]** 1.77×10^{-4} - 1.35×10^{-6}	
Chemical structure	**AA-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]	**MAC-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]	**EQS biota** [µg/kg]
 			Sum of PCDD+PCDF+PCB-DL 8.0 10^{-3} µg.kg^{-1} TEQ = 0.008 µg.kg^{-1} TEQ = 8 ng/kg TEQ

Standard Methods

LOQ: 1-5 10^{-5} = 10-50 pg/l; 1-5 ng/kg; HRGC-HRMS method (EPA 1613).

Description: EPA Method 1613 was developed for isomer-specific determination of the 2,3,7,8-substituted, tetra through octa-chlorinated, dibenzo-*p*-dioxins and dibenzofurans in aqueous, solid, and tissue matrices by isotope dilution, high resolution capillary column gas chromatography (HRGC)/high resolution mass spectrometry (HRMS). Fish or other solid samples are extracted by Soxhlet extraction.

Methods applied by EU Member States and comments received

Italy: LOQ: PCDD+PCDF 0.2 pgWHO-TE/g lb (biota); DL-PCB: 0.05 pgWHO-TE/g lb (biota) (US EPA method 1613, modified).

Sweden: Biota analysis; the two references by Danielsson and co-workers (Danielsson et al., 2005), and Wiberg and co-workers (Wiberg et al., 2007) were given. No LODs or LOQs, however, are given in these articles.

UK-EA: No problems expected.

Northern-Ireland: Will require a new method of analysis using more sensitive instrumentation and possibly larger sample volumes.

Conclusion

The lowest LOQ of 0.2 pgWHO-TE/g (= ng/kg) lb PCDD+PCDF (biota) (DL-PCB: 0.05 pgWHO-TE/g lb) reported by Italy is **sufficient** for compliance monitoring in biota (0.3 × EQS = 2.4 ng/kg).

References

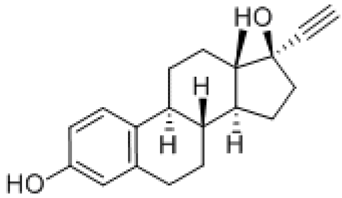
Danielsson, C., Wiberg, K., Korytár, P., Bergek, S., Brinkman, U.A.Th., Haglund, P. **2005**. Trace analysis of polychlorinated dibenzo-p-dioxins, dibenzofurans and WHO polychlorinated biphenyls in food using comprehensive two-dimensional gas chromatography with electron capture detection. Journal of Chromatography A 1086, 61-70.

EPA method 1613. October **1994**. Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS. U.S. Environmental Protection Agency, Office of Water, Washington, DC, USA.

Wiberg, K., Sporring, S., Haglund, P., Björklund, E. **2007**. Selective pressurized liquid extraction of polychlorinated dibenzo-p-dioxins, dibenzofurans and dioxin-like polychlorinated biphenyls from food and feed samples. Journal of Chromatography A 1138, 55-64.

3.9. 17-alpha-Ethinylestradiol

17-alpha-ethinylestradiol is a synthetic estradiol used in contraceptive anti-baby pills and for the treatment of menopausal and post-menopausal symptoms.

CAS Number 57-63-6	**Log K_{OW}** 3.67-4.2	**Water Solubility [mg/l]** 4.7-19
Chemical structure **(MW 296.4)**	**AA-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]	**MAC-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]
	Fresh 3.5 10^{-5} = 0.035 ng/l = 35 pg/l Salt 7 10^{-6} = 7 pg/l	not applicable

Standard Methods

MDL: 1 10^{-4} µg/l = 0.1 ng/l (sample volume: 1 l) (EPA 1698);

LOD: 0.33 ng/l (sample volume: 1 l) (EPA 539).

Description: EPA Method 1698 determines steroids and hormones in environmental samples by isotope dilution and internal standard high resolution gas chromatography combined with high resolution mass spectrometry (HRGC/HRMS). EPA Method 1698 was developed for use with aqueous, solid, and biosolids matrices. This method involves solvent extraction of the sample (LLE for water samples), followed by cleanup with a layered alumina/florisil column, and an option to remove sulfur using copper. Following cleanup, the target analytes are derivatized to their trimethylsilyl-ethers using *N*,*O*-Bis(trimethylsilyl) trifluoroacetamide with trimethylchlorosilane (BSTFA: TMCS) to make them sufficiently volatile for analysis by GC/HRMS. Quantitation is performed by isotope dilution and internal standard techniques, depending on the analyte and the availability of labeled analogs (EPA 1698).

Description of EPA Method 539: Samples are dechlorinated with sodium thiosulfate and protected from microbial degradation using 2-mercaptopyridine-1-oxide sodium salt during sample collection. Samples are fortified with surrogates and passed through solid phase extraction (SPE) disks containing octadecyl (C18) functional groups in order to extract the method analytes and surrogates. The compounds are eluted from the solid phase with a small amount of methanol. The extract is concentrated to dryness with nitrogen in a heated water bath, and then adjusted to a 1-mL volume with 50:50 methanol:water after adding the internal standards. An aliquot of the sample is injected into an LC equipped with a C18 column that is interfaced to a MS/MS. The analytes are separated and identified by comparing the acquired mass spectra and retention times to reference spectra and retention times for calibration standards acquired under identical LC-MS/MS conditions. The concentration of each analyte is determined using the internal standard technique (EPA 593).

Methods applied by EU Member States and comments received

Italy: LOQ: 1.8 ng/l; LC-MS/MS (IT: ISS); Performance data on drinking waters (LOD) in the context of drinking water directive. The methods in some cases are from other MS. The methods are validated with ring-test.

France: LOQ: 1.2 ng/l; Aqua-Ref Method (SPE-MS-MS); 250 ml water; extraction with 200 mg Oasis HLB followed by florisil clean-up (Miège et al., 2009).

LOQ: 5 ng/g for sewage sludge; Aqua-Ref Method (PFE/SPE/LC/MS-MS) (Gabet-Giraud et al., 2010).

UK-EA: Will require a new approach using high sensitivity technology and/or increased sample sizes.

Northern-Ireland: I don't believe that it is possible to reach these LOQs with the instrumentation currently available to the NIEA Trace Organics Team.

Literature methods

Steroid hormones are endocrine-disrupting compounds, which affect the endocrine system at very low concentrations; so interest in the sensitive determination of steroids in the environment has increased in recent years.

Recently a very comprehensive review on the analysis of steroid hormones in environmental samples has been published (Tomsikova et al., 2012); this review cites many other articles including LODs. It is discussed in detail how to enhance the sensitivity of analytical procedures for the determination of female steroid hormones (estrogens and progestogens) in environmental matrices. A number of steps in the analytical procedure, starting with the sample pre-treatment and ending with detection, could significantly contribute to enhancing sensitivity, so they need to be thoroughly optimized. The best results in analysis of estrogens and progestogens have been achieved with liquid chromatography (LC), as separation method, and tandem mass spectrometry (MS-MS), as detection method. Analysis using gas chromatography coupled to MS is discussed as well. Sample preparation depends on the kind of sample. Its optimization is important in reducing matrix interferences and plays a significant role in enhancing sensitivity. Liquid samples were most frequently prepared with off-line solid-phase extraction, while solid samples were also extracted by liquid-liquid, pressurized-liquid, microwave and ultrasound extraction techniques. In several studies, derivatization improved the sensitivity of LC-MS detection (Tomsikova et al., 2012).

Steroid estrogens and phenolic xenoestrogens are weak acids and their ionization on ESI and APCI are not very efficient compared with other more polar chemicals. Chemical derivatization can add on moieties improving ionization and enhance signals. Selective extraction, additional clean-up, efficient LC separation is important for the analysis of estrogens, because matrix effects can cause a loss in sensitivity. Dansyl chloride or pentafluorobenzyl bromide (PFBBr) can react with phenolic groups, significantly improving sensitivity (Lien et al., 2009).

Lien and co-workers (Lien et al., 2012) compared the signal sensitivities and matrix effects of four ionization modes and four reversed phase liquid chromatographic (LC) systems on analyzing Estrone (E1), 17-beta-Estradiol (E2), Estriol (E3), 17-alpha-Ethinylestradiol (EE2), 4-Nonylphenol (NP), 4-*tert*-Octylphenol (OP), Bisphenol A (BPA) and their derivatives of dansyl chloride or pentafluorobenzyl bromide (PFBBr) in water matrixes using a triple-quadrupole mass spectrometer with selected reaction monitoring (SRM). Dansylated compounds with ESI at UHPLC condition had the most intense signals and less matrix effects of the various combinations of ionization and LC systems (Lien et al., 2009).

Grover and co-workers compared GC-MS, GC-MS-MS, and LC-MS-MS for the analysis of steroidal estrogens in environmental water samples (Grover et al., 2009).

Some analytical methods are summarized here:

Extraction (volume)	**Analysis**	**LOD**	**Reference**
On-line SPE derivatization	LC-MS-MS	0.7 ng/l	Salvador et al., 2007
SPE (0.25 l); clean-up with florisil	LC-MS-MS (derivatization)	LOQ: 0.22 ng/l	Matejıcek and Kuban, 2008
SPE (1 l)	LC-MS-MS	MDL: 0.2 ng/l	Vulliet et al., 2008
SPE (2 l)	GC-MS-MS (derivatization) LC-MS-MS	0.3 ng/l 0.4 ng/l	Grover et al., 2009
C18 speedisks	UHPLC-MS-MS (derivatization)	0.91 ng/l	Lien et al., 2009
SPE (0.25 l); clean-up with florisil	LC-MS-MS	LOQ: 1.2 ng/l	Miège et al., 2009
SPE (2 l); clean-up with florisil	UHPLC-MS-MS	0.10 ng/l	Chang et al., 2011

Conclusion

The MDL of 0.1 ng/l of EPA method 1698 is **not sufficient** for compliance monitoring in inland and coastal surface waters (0.3 × EQS = 10.5 pg/l; 2.1 pg/l for coastal). To reach LOQs in the low pg/l concentration range is **extremely difficult**, if not impossible with current analytical methods.

References

Chang, H., Wan, Y., Wu, S., Fan, Z., Hu, J. **2011**. Occurrence of androgens and progestogens in wastewater treatment plants and receiving river waters: Comparison to estrogens. Water Research 45, 732-740.

EPA Method 1698. December **2007**. Steroids and hormones in water, soil, sediment, and biosolids by HRGC/HRMS. EPA-821-R-08-003. U.S. Environmental Protection Agency, Office of Water, Washington, DC, USA.

EPA Method 539. November **2010**. Determination of hormones in drinking water by solid-phase extraction (SPE) and liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS-MS). EPA-815-B-10-001. U.S. Environmental Protection Agency, Office of Water, Washington, DC, USA.

Gabet-Giraud, V., Miège, C., Herbreteau, B., Hernandez-Raquet, G., Coquery, M. **2010**. Development and validation of an analytical method by LC-MS/MS for the quantification of estrogens in sewage sludge. Analytical and Bioanalytical Chemistry 396, 1841–1851.

Grover, D.P., Zhang, Z.L., Readman, J.W., Zhou, J.L. **2009**. A comparison of three analytical techniques for the measurement of steroidal estrogens in environmental water samples. Talanta 78, 1204-1210.

Lien, G.-W., Chen, C.-Y., Wang, G.-S., **2009**. Comparison of electrospray ionization, atmospheric pressure chemical ionization and atmospheric pressure photoionization for determining estrogenic chemicals in water by liquid chromatography tandem mass spectrometry with chemical derivatizations. Journal of Chromatography A, 1216, 956–966.

Matejıcek, D., Kuban, V. **2008**. Enhancing sensitivity of liquid chromatographic/ion-trap tandem mass spectrometric determination of estrogens by on-line pre-column derivatization. Journal of Chromatography A, 1192, 248-253.

Miège, C., Bados, P., Brosse, C., Coquery, M. **2009**. Method validation for the analysis of estrogens (including conjugated compounds) in aqueous matrices. Trends in Analytical Chemistry 28, 237-244.

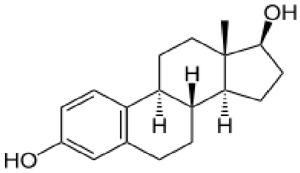
Salvador, A., Moretton, C., Piram, A., Faure, R. **2007**. On-line solid-phase extraction with on-support derivatization for high-sensitivity liquid chromatography tandem mass spectrometry of estrogens in influent/effluent of wastewater treatment plants. Journal of Chromatography A, 1145, 102–109.

Tomsikova, H., Aufartova, J., Solich, P., Sosa-Ferrera, Z., Santana-Rodriguez, J.J., Novakova, L. **2012**. High-sensitivity analysis of female-steroid hormones in environmental samples. Trends in Analytical Chemistry 34, 35-58.

Vulliet, E., Wiest, L., Baudot, R., Grenier-Loustalot, M.-F. **2008**. Multi-residue analysis of steroids at sub-ng/L levels in surface and ground-waters using liquid chromatography coupled to tandem mass spectrometry. Journal of Chromatography A 1210, 84-91.

3.10. 17-beta-Estradiol

17-beta-estradiol is the predominant natural female sex hormone and is the most active of the naturally occurring estrogenic hormones and is also a key intermediate in industrial synthesis of other estrogens and of various hormonal 19-norsteroids.

CAS Number 50-28-2	**Log K_{OW}** 4.0	**Water Solubility [mg/l]** 1.7-3.6
Chemical structure **(MW 272.4)**	**AA-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]	**MAC-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]
	Fresh 4 10^{-4} = 0.4 ng/l Salt 8 10^{-5} = 80 pg/l	not applicable

Standard Methods

MDL: 1 10^{-4} µg/l = 0.1 ng/l (sample volume: 1 l) (EPA 1698);

LOD: 0.39 ng/l (sample volume: 1 l) (EPA 539).

Methods applied by EU Member States and comments received

Italy: 0.9 ng/l; LC-MS/MS (IT: ISS); performance data on drinking waters (LOD) in the context of drinking water directive. The methods in some cases are from other MS. The methods are validated with ring-test.

France: LOQ: 0.5 ng/l; Aqua-Ref Method (SPE-MS-MS); see above.

LOQ: 2-4 ng/g for sewage sludge; Aqua-Ref Method (PFE/SPE/LC/MS-MS).

UK-EA: Will require a new approach using high sensitivity technology and/or increased sample sizes.

Northern-Ireland: I don’t believe that it is possible to reach these LOQs with the instrumentation currently available to the NIEA Trace Organics Team.

Literature methods

Some analytical methods are summarized here:

Extraction (volume)	**Analysis**	**LOD**	**Reference**
On-line SPE; derivatization	LC-MS-MS	0.4 ng/l	Salvador et al., 2007
SPE (0.25 l); clean-up with florisil	LC-MS-MS (derivatization)	LOQ: 0.26 ng/l	Matejıcek and Kuban, 2008
SPE (1 l)	LC-MS-MS	MDL: 0.01 ng/l	Vulliet et al., 2008
SPE (2 l)	GC-MS-MS (derivatization) LC-MS-MS	0.3 ng/l 0.4 ng/l	Grover et al., 2009
C18 speedisks	UHPLC-MS-MS	0.81 ng/l	Lien et al., 2009
SPE (0.25 l); clean-up with florisil	LC-MS-MS	LOQ: 0.6 ng/l	Miège et al., 2009
SPE (2 l)	UHPLC-MS-MS	0.10 ng/l	Chang et al., 2011

Conclusion

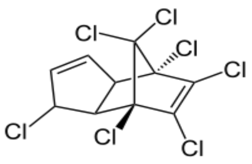
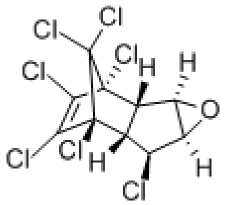
The MDL of 0.1 ng/l of the EPA method 1698 is **sufficient** for compliance monitoring in inland surface waters (0.3 × EQS = 0.12 ng/l), but **not sufficient** in coastal surface waters (0.3 × EQS = 24 pg/l). To reach LOQs in the low pg/l concentration range is **extremely difficult**, if not impossible with current analytical methods.

References

See under 3.9. “17-alpha-ethinylestradiol”.

3.11. Heptachlor and Heptachlorepoxide

Heptachlor is an insecticide which is banned and not used anymore in the EU. Heptachlor-epoxide is its degradation product.

CAS Number 76-44-8; 1024-57-3	**Log K_{OW}** 5.44-6.10	**Water Solubility [mg/l]** 0.06-0.3	
Chemical structure **(MW 373.3; 389.4)**	**AA-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]	**MAC-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]	**EQS biota** [µg/kg]
 	Fresh 2 10^{-7} = 0.2 pg/l Salt 1 10^{-8} = 0.01 pg/l = 10 fg/l	Fresh 3 10^{-4} = 0.3 ng/l Salt 3 10^{-5} = 0.03 ng/l	6.7 10^{-3} µg/kg = 0.0067 µg/kg = 6.7 ng/kg

Standard Methods

Heptachlor: LOQ: 7 pg/l; Heptachlorepoxide: LOQ: 12 pg/l (EPA 1699).

Solid: Heptachlorepoxide: LOQ: 0.3 ng/kg (EPA 1699).

LOD: 1-10 ng/l (EN ISO 6468).

Description of EPA 1699:

LLE-HRGC/HRMS (see under 3.4. “Cypermethrin”).

Description of EN ISO 6468: Describes a method for determining certain organochlorine insecticides, polychlorinated biphenyls (PCBs) and chlorobenzenes (except the mono- and dichlorobenzenes) in drinking water, ground water, surface waters and waste waters. The method is applicable to samples containing up to 0,05 g/l of suspended solids. Principle: LLE followed by GC-ECD.

Methods applied by EU Member States and comments received

Germany: Lowest reported LOQ: 0.001 µg/l = 1 ng/l (according to EN ISO 6468; GC-MS).

Lithuania: 0.005 µg/l = 5 ng/l (EN ISO 6468).

Bulgaria: 0.001 µg/l = 1 ng/l; LLE-GC-ECD (EN ISO 6468).

Draft EQS for Bulgaria 0.01 µg/l; we have no data on biota and sediment.

Sweden: LOQ: 0.010 µg/l = 10 ng/l; Method: OMK 51 (LLE-GC-MS).

UK-EA: Will require a new approach using high sensitivity technology and/or increased sample sizes.

Northern-Ireland: I don’t believe that it is possible to reach these LOQs with the instrumentation currently available to the NIEA Trace Organics Team.

Literature methods

A lot of information is available on the analysis of organochlorine pesticides. Some articles on the analysis of Heptachlor and Heptachlorepoxide in water and biota have been selected:

Extraction (volume)	Analysis	LOD	Reference
SPME	GC-ECD	LOD for Heptachlor: 0.050 µg/l	Ratola et al., 2006
SPE	GC-µECD	MDL for Heptachlorepoxide: 0.11 ng/l	Gao et al., 2008
SPE (1 l)	GC-ECD	LOD for Heptachlor:: 0.15 ng/l LOD for Heptachlorepoxide:: 0.08 ng/l	Zhou et al, 2006

Biota and sediment analysis:

Extraction (species)	**Analysis**	**LOD**	**Reference**
PLE (ASE) (harbor seals)	GC-ion trap MS	MDLs: 5.4-68 pg/g lipid weight (= 5.4-68 ng/kg)	Wang et al., 2007
SLE (birds)	GC-ECD	LOQ for Heptachlor: 0.01 ng/g LOQ for Heptachlorepoxide: 0.015 ng/g	Cid et al., 2007
Soxhlet (fish)	GC-ECD	LOD: 0.10-0.60 ng/g	Zhou et al., 2007
SLE (sediment)	GC-ECD	LOD: 1 ng/g dry weight	Poolpak et al., 2008
Soxhlet (meat)	GC-ECD	LOD: 4 µg/kg	Ahmad et al., 2010
SLE (birds)	GC-ECD	LOD: 0.03-0.54 ng/g	Espin et al., 2010
Soxhlet or cryogenic extraction	GC-ECD	LOQ: 0.1 ng/g (Heptachlor and Heptachlorepoxide)	Thomas et al., 2012

Conclusion

The LOQ of 0.3 ng/kg of EPA method 1699 (LLE-HRGC/HRMS) for Heptachlorepoxide is **sufficient** for compliance monitoring in biota (0.3 × EQS = 2.01 ng/kg). GC-ECD might not be sensitive enough to reach these low LOQs.

In water, the LOQ of 7 pg/l of EPA method 1699 is **not sufficient** for compliance monitoring (0.3 × EQS = 60 fg/l in inland surface waters, and 3 fg/l in coastal waters).

References

Ahmad, R., Salem, N.M., Estaitieh, H. **2010**. Occurrence of organochlorine pesticide residues in eggs, chicken and meat in Jordan. Chemosphere 78, 667–671.

Cid, F.D., Antón, R.I., Caviedes-Vidal, E. **2007**. Organochlorine pesticide contamination in three bird species of the Embalse La Florida water reservoir in the semiarid midwest of Argentina. Science of the Total Environment 385, 86–96.

EN ISO 6468. **1996**. Water quality - Determination of certain organochlorine insecticides, polychlorinated biphenyls and chlorobenzenes - Gas chromatographic method after liquid-liquid extraction.

EPA Method 1699. December **2007**. Pesticides in water, soil, sediment, biosolids, and tissue by HRGC/HRMS. EPA-821-R-08-001. U.S. Environmental Protection Agency, Office of Water, Washington, DC, USA.

Espín, S., Martínez-López, E., Gómez-Ramírez, P., María-Mojica, P., García-Fernández, A.J. **2010**. Assessment of organochlorine pesticide exposure in a wintering population of razorbills (Alca torda) from the southwestern Mediterranean. Chemosphere 80, 1190–1198.

Gao, J., Liu, L., Liu, X., Lu, J., Zhou, H., Huang, S., Wang, Z.., Spear, P.A. **2008**. Occurrence and distribution of organochlorine pesticides – lindane, p,p'-DDT, and heptachlor epoxide – in surface water of China. Environment International 34, 1097–1103.

Poolpak, T., Pokethitiyook, P., Kruatrachue, M., Arjarasirikoon, U., Thanwaniwat, N. **2008**. Residue analysis of organochlorine pesticides in the Mae Klong river of Central Thailand. Journal of Hazardous Materials 156, 230–239.

Ratola, N., Santos, L., Herbert, P., Alves, A. **2006**. Uncertainty associated to the analysis of organochlorine pesticides in water by solid-phase microextraction/gas chromatography–electron capture detection—Evaluation using two different approaches. Analytica Chimica Acta 573–574, 202–208.

Thomas, M., Lazartigues, A., Banas, D., Brun-Bellut, J., Feidt, C. **2012**. Organochlorine pesticides and polychlorinated biphenyls in sediments and fish from fresh water cultured fish ponds in different agricultural contexts in north-eastern France. Ecotoxicology and Environmental Safety 77, 35–44.

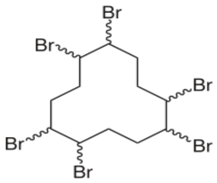
Wang, D., Atkinson, S., Hoover-Miller, A., Lee, S.-E., Li, Q.X. **2007**. Organochlorines in harbor seal (Phoca vitulina) tissues from the northern Gulf of Alaska. Environmental Pollution 146, 268-280.

Zhou, R., Zhu, L., Yang, K., Chen, Y. **2006**. Distribution of organochlorine pesticides in surface water and sediments from Qiantang River, East China. Journal of Hazardous Materials 137, 68–75.

Zhou, R., Zhu, L., Kong, Q. **2007**. Persistent chlorinated pesticides in fish species from Qiantang River in East China. Chemosphere 68, 838–847.

3.12. Hexabromocyclododecane (HBCDD)

Hexabromocyclododecane (HBCDD) is a high production volume chemical used as a flame retardant, mainly within the polymer and textile industry. In theory, HBCDD consists of 16 stereoisomers, with water solubility in the range of 2-50 µg/l.

CAS number 134237-50-6 (α-HBCDD) 134237-51-7 (β-HBCDD) 134237-52-8 (γ-HBCDD)	**Log K_{OW}** 5.07 (α-HBCDD) 5.12 (β-HBCDD) 5.47 (γ-HBCDD)	**Water Solubility [mg/l]** 0.0488 (α-HBCDD) 0.0147 (β-HBCDD) 0.0021 (γ-HBCDD)	
Chemical structure **(MW 641.7)**	**AA-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]	**MAC-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]	**EQS biota** [µg/kg]
	Fresh 0.0016 = 1.6 ng/l Salt 0.0008 = 0.8 ng/l	Fresh 0.5 Salt 0.05	167 µg/kg

No analytical “standard” method is available for HBCDD.

Methods applied by EU Member States and comments received

Sweden: Biota analysis (Sellström et al., 2003).

UK-EA: Not requested in water; Biota and sediment method under development; Water LOQ: challenging.

Northern-Ireland: Has never been requested. A new method of analysis would need to be developed.

Literature methods

HBCDD can be analysed by GC- and LC-MS techniques (Haug et al., 2008). More LC-MS methods are reported in the literature, because LC achieves the separation of α-, β-, and γ-isomers; GC can only report total HBCDD concentrations. Some examples are given here for biota analysis. No analytical methods or data for water or SPM analysis could be found.

Extraction (species)	**Analysis**	**LOD**	**Reference**
Soxhlet (fish)	LC-MS-MS	LOQ: 20-75 pg/g	Janak et al., 2005
Soxhlet (biota)	LC-MS	1.2 µg/kg	Morris et al., 2006
Soxhlet (fish)	GC-MS	LOD: 0.02-0.2 ng/g	Hajslova et al., 2007
SLE (sediment and biota)	LC-MS	LOD: 0.05-0.15 ng/g	Haukas et al., 2009
SLE (seafood)	LC-MS-MS	LODs: 0.02 ng/g for α- and γ-HBCDD, and 0.01 ng/g for β-HBCDD	Nakagawa et al., 2010
ASE (fish)	LC-MS-MS	LOD: 6-21 pg/g	Köppen et al., 2010
Soxhlet (birds)	LC- ion trap-MS	Not reported	Leslie et al., 2011
SLE (fish oil)	LC-MS-MS	LOQs: 0.11 ng/g for α-HBCDD; 0.18 ng/g for γ-HBCDD; 0.20 ng/g for β-HBCDD	Ortiz et al., 2011

Conclusion

No analytical standard methods are available for HBCDD. However, the literature shows that biota analysis at 1/3 of the EQS (0.3 × EQS = 50.1 µg/kg) **should be possible**.

No analytical methods are available for water or SPM analysis.

References

Hajslova, J., Pulkrabova, J., Poustka, J., Cajka, T., Randak, T. **2007**. Brominated flame retardants and related chlorinated persistent organic pollutants in fish from river Elbe and its main tributary Vltava. Chemosphere 69, 1195–1203.

Haug, L.S., Thomsen, C., Liane, V.H., Becher, G. **2008**. Comparison of GC and LC determinations of hexabromocyclododecane in biological samples – Results from two interlaboratory comparison studies. Chemosphere 71, 1087–1092.

Haukås, M., Hylland, K., Berge, J.A., Nygård, T., Mariussen, E. **2009**. Spatial diastereomer patterns of hexabromocyclododecane (HBCD) in a Norwegian fjord. Science of the Total Environment 407, 5907–5913.

Janak, K., Covaci, A., Voorspoels, S., Becher, G. **2005**. Hexabromocyclododecane in marine species from the Western Scheldt Estuary: diastereoisomer- and enantiomer-specific accumulation. Environmental Science & Technology 39, 1987-1994.

Köppen, R., Becker, R., Esslinger, S., Nehls, I. **2010**. Enantiomer-specific analysis of hexabromocyclododecane in fish from Etnefjorden (Norway). Chemosphere 80, 1241–1245.

Leslie, H.A., Leonards, P.E.G., Shore, R.F., Walker, L.A., Bersuder, P.R.C., Morris, S., Allchin, C.R., De Boer, J. **2011**. Decabromodiphenylether and hexabromocyclododecane in wild birds from the United Kingdom, Sweden and The Netherlands: Screening and time trends. Chemosphere 82, 88–95.

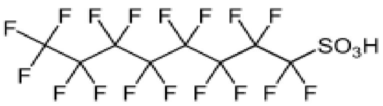
Morris, S., Bersuder, P., Allchin, C.R., Zegers, B. **2006**. Determination of the brominated flame retardant, hexabromocyclodocane, in sediments and biota by liquid chromatography-electrospray ionisation mass spectrometry. Trends in Analytical Chemistry 25, 343-349.

Ortiz, X., Guerra, P., Díaz-Ferrero, J., Eljarrat, E., Barceló, D. **2011**. Diastereoisomer- and enantiomer-specific determination of hexabromocyclododecane in fish oil for food and feed. Chemosphere 82, 739–744.

Sellström, U., Bignert, A., Kirkegaard, A., Häggberg, L., de Wit, C.A., Olsson, M., Jansson, B. **2003**. Temporal trend studies on tetra- and pentabrominated diphenyl ethers and hexabromocyclododecane in guillemot egg from the Baltic Sea. Environmental Science & Technology 37, 5496-5501.

3.13. Perfluorooctansulfonic acid (PFOS)

PFOS (CAS no. 1763-23-1) is a perfluoroalkyl substance which was used (in Europe) in providing grease, oil and water resistance to materials such as textiles, carpets, paper and in general coatings. Other smaller volume uses are in chromium plating, photolithography, photography, and in hydraulic fluids for aviation. PFOS has also been used in fire-fighting foams. Production of PFOS (or its derivatives) has been phased out in Europe.

CAS number 1763-23-1 (acid) 2795-39-3 (potassium salt)	**Log K_{OW}** A reliable measured value is not available.	**Water Solubility [mg/l]** 370-570	
Chemical structure **(MW 500.1)**	**AA-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]	**MAC-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]	**EQS biota** [µg/kg]
	Fresh 6.5 10^{-4} = 0.65 ng/l Salt 1.3 10^{-4} = 0.13 ng/l	Fresh 36 µg/l Salt 7.2 µg/l	9.1 µg/kg

Standard Methods

LOQ: 2.0 ng/l (ISO 25101).

Description: ISO 25101 specifies a method for the determination of the linear isomers of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) in unfiltered samples of drinking water, ground water and surface water (fresh water and sea water) using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Other isomers may be reported separately as non-linear isomers and qualified as such. The method is applicable to a concentration range of 2.0 ng/l to 10 000 ng/l for PFOS and 10 ng/l to 10 000 ng/l for PFOA. Depending on the matrix, the method may also be applicable to higher concentrations ranging from 100 ng/l to 200 000 ng/l after suitable dilution of the sample or reduction in sample size.

Methods applied by EU Member States and comments received

Germany: Lowest reported LOQ: 0.01 µg/l (according to ISO/CD 25101; LC-MS).

Italy: LOD: 1-10 ng/l; LC-MS/MS; Internal Method, validated (IT). Performance data on drinking waters (LOD) in the context of drinking water directive. The methods are in some cases are from other MS. The methods are validated with ring-test.

Biota: 0.1 ng/g fw (laboratory method ISS, validated).

Denmark: NERI analysis PFOS in biota and water with detection limits about 10 times below EQS. The method is according to the ICES guideline for analysis of PFOS. The method is not yet accredited but accreditation of the analysis in serum and maybe later other matrixes is planned.

Sweden: Below is a reference to method for the analysis of PFOS in biota (not a standardised one however) (Holmström et al., 2010).

UK-EA: Will require a new approach using high sensitivity technology and/or increased sample sizes.

Northern-Ireland: Has never been requested. A new method of analysis would need to be developed.

Literature methods

The review article by Jahnke and Berger (Jahnke and Berger, 2009) gives a good overview on the performance of analytical methods for Perfluoroalkyl Substances (PFASs) in different matrices. The results of three international interlaboratory studies on the analysis of PFASs show that analytical methods for PFASs in water and fish have improved considerably (Van Leeuwen et al., 2009; 2011). For the extraction of fish, most laboratories use solid-liquid extraction (SLE) followed by ENVICarb clean-up, first described by Powely and co-workers (Powley et al., 2005). This clean-up has become very popular. Analysis of PFOS is performed by LC-MS-MS.

Selected biota methods:

Extraction (matrix)	**Analysis**	**LOD**	**Reference**
Ion pair extraction (biota)	LC-MS-MS	LOD: 8.5 ng/g	Hansen et al., 2001
SLE; ENVICarb clean-up (soil, sediment, sludge, biota)	LC-MS-MS	LOD: 0.2 ng/g	Powley et al., 2005; 2008
SLE; ENVICarb clean-up (eggs)	LC-MS-MS	MDL: 0.3 ng/g	Holmström et al., 2010

In addition, recently a “matrix effect-free” method for the ultra-trace analysis of PFASs (including PFOS) in dietary food samples (including fish) has been published by Vestergren and co-workers (2012); this method, which employs ion pair extraction and subsequent solid-phase extraction clean-up on Florisil and graphitized carbon followed by LC-MS-MS, achieves for PFOS a MQL of 1.7 pg/g (extraction of 2.5 g fish).

Selected water methods:

PFOS is extracted from water using SPE (Van Leeuwen et al., 2009; 2011); best recoveries are obtained by ion-pair SPE. With modern LC-MS-MS instruments, LOQs in the range of 0.1 ng/l can be achieved in expert laboratories (Labadie & Chevreuil, 2011; Ullah et al., 2011). Yamashita and co-workers (Yamashita et al., 2004) achieved for a 1 liter water extraction a LOD of 0.8 pg/l, by taking extreme care to background contamination. Theobald and co-workers (Theobald et al., 2007) described the determination of various PFASs in seawater samples of 10 up to 30 L using SPE with self-made glass columns followed by LC-MS-MS, and achieved for PFOS a LOQ of 22 pg/l.

Extraction (Volume)	**Analysis**	**LOD**	**Reference**
SPE (1 l)	LC-MS-MS	LOD: 0.8 pg/l	Yamashita et al., 2004
SPE (10-30 l)	LC-MS-MS	LOQ: 22 pg/l	Theobald et al., 2007
SPE ion extraction (1 l)	LC-MS-MS	LOQ: 0.14 ng/l	Labadie & Chevreuil, 2011
SPE (0.5 l)	LC-QTOF-MS	LOQ: 0.08-0.17 ng/l	Ullah et al., 2011

Conclusion

The LOQ of 2 ng/l of the ISO method 25101 is **not sufficient** for compliance monitoring in inland and coastal surface waters (0.3 × EQS = 0.195 ng/l; 39 pg/l for coastal). To reach LOQs in the low pg/l range is difficult, also due to blank problems.

Biota extraction of PFOS by solid-liquid extraction (SLE) followed by ENVICarb clean-up is relatively easy and the LOQs achieved are **sufficient** for biota compliance monitoring (0.3 × EQS = 2.73 µg/kg).

References

ISO 25101. **2009**. Water quality - Determination of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) - Method for unfiltered samples using solid phase extraction and liquid chromatography/mass spectrometry.

Hansen, K.J., Clemen, L.A., Ellefson, M.E., Johnson, H.O. **2001**. Compound-specific, quantitative characterization of organic fluorochemicals in biological matrices. Environmental Science & Technology 35, 766-770.

Holmström, K.E., Johansson, A.K., Bignert, A., Lindberg, P., Berger, U. **2010**. Temporal trends of perfluorinated surfactants in Swedish peregrine falcon eggs (falco peregrinus), 1974-2007. Environmental Science & Technology 44, 4083-4088.

Jahnke, A., Berger, U. **2009**. Trace analysis of per- and polyfluorinated alkyl substances in various matrices - How do current methods perform? Journal of Chromatography A, 1216, 410–421.

Labadie, P., Chevreuil, M. **2011**. Biogeochemical dynamics of perfluorinated alkyl acids and sulfonates in the River Seine (Paris, France) under contrasting hydrological conditions. Environmental Pollution 159, 3634-3639.

Powley, C.R., George, S.W., Ryan, T.W., Buck, R.C. **2005**. Matrix effect-free analytical methods for determination of perfluorinated carboxylic acids in environmental matrixes. Analytical Chemistry 77, 6353-6358.

Powley, C.R., George, S.W., Russell, M.H., Hoke, R.A., Buck, R.C. **2008**. Polyfluorinated chemicals in a spatially and temporally integrated food web in the Western Arctic. Chemosphere 70, 664-672.

Theobald, N., Gerwinski, W., Caliebe, C., Haarich, M. **2007**. Scientific report 202 22 213 of the German Federal Environmental Agency UBA-FB 00 001049, ISSN 1862-4804, 625, 2007, http://www.umweltbundesamt.de.

Ullah, S., Alsberg, T., Berger, U. **2011**. Simultaneous determination of perfluoroalkyl phosphonates, carboxylates, and sulfonates in drinking water. Journal of Chromatography A, 1218, 6388– 6395

Van Leeuwen, S.P.J., Swart, C.P., van der Veen, I., de Boer, J., **2009**. Significant improvements in the analysis of perfluorinated compounds in water and fish: results from an interlaboratory method evaluation study. Journal of Chromatography A 1216, 401–409.

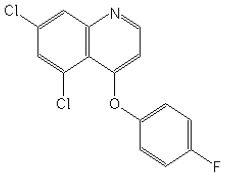
Van Leeuwen et al., IVM, Amsterdam. Third interlaboratory study on perfluorinated compounds in environmental and human matrices, Report R-11/04; 27 May 2011.

Vestergren, R., Ullah, S., Cousins, I.T., Berger, U. **2012**. A matrix effect-free method for reliable quantification of perfluoroalkyl carboxylic acids and perfluoroalkane sulfonic acids at low parts per trillion levels in dietary samples. Journal of Chromatography A 1237, 64-71.

Yamashita, N., Kannan, K., Taniyasu, S., Horii, Y., Okazawa, T., Petrick, G., Gamo, T. **2004**. Analysis of perfluorinated acids at parts-per-quadrillion levels in seawater using liquid chromatography-tandem mass spectrometry. Environmental Science & Technology 38, 5522-5528.

3.14. Quinoxyfen

Quinoxyfen is a fungicide often used to control powdery mildew infections on grapes and hops.

CAS Number 124495-18-7	**Log K_{OW}** 4.66	**Water Solubility [mg/l]** 0.047
Chemical structure **(MW 308.1)**	**AA-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]	**MAC-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]
	Fresh 0.15 µg/l Salt 0.015 µg/l = 15 ng/l	Fresh 2.7 µg/l Salt 0.54 µg/l

No analytical "standard" method is available for Quinoxyfen.

Methods applied by EU Member States and comments received

Germany: Lowest reported LOQ: 0.01 µg/l = 10 ng/l (according to ISO 11369; LC-UV).

Sweden: LOQ: 2 ng/l; Method: OMK 51 (LLE-GC-MS).

UK-EA: Not been requested but could be added to existing suites.

Northern-Ireland: Has never been requested. A new method of analysis would need to be developed.

Literature methods

It was difficult to find analytical methods for the analysis of Quinoxyfen in water.

Pareja and co-workers (Pareja et al., 2011) recently presented a direct water injection LC-MS-MS method (after filtration) for the multi-residue pesticide analysis in paddy field water (injection volume: 5 µl). A new hybrid triple quadrupole-linear ion trap-mass spectrometer (QqLIT) instrument was used in the tandem MS-MS mode. The LOQ achieved for Quinoxyfen was 0.02 µg/l.

Extraction	**Analysis**	**LOD**	**Reference**
Direct injection (5 µl)	LC-MS-MS	0.02 µg/l	Pareja et al., 2011

Several multi-residue LC-MS-MS analytical methods for the analysis of pesticides (including quinoxyfen) in food samples are available (e.g.: Hengel and Miller, 2008; Kmellar et al., 2008; Wang et al., 2010).

Biota analysis:

Extraction (matrix)	Analysis	LOD	Reference
SLE (honeybees)	GC-MS-MS	Not reported	Walorczyk and Gnusowski, 2009
SLE (fish; sediment)	LC-MS	LOD: 0.54 µg/kg	Merli et al., 2010

Conclusion

The LOQ of 2 ng/l reported by Sweden is **sufficient** for compliance monitoring in inland and coastal surface waters (0.3 × EQS = 45 ng/l; 4.5 ng/l for coastal).

References

ISO 11369. **1997**. Water quality - Determination of selected plant treatment agents - Method using high performance liquid chromatography with UV detection after solid-liquid extraction.

Hengel, M.J., Miller, M. **2008**. Analysis of pesticides in dried hops by liquid chromatography−tandem mass spectrometry. Journal of Agricultural Food and Chemistry 56, 6851–6856.

Kmellar, B., Fodor, P., Pareja, L., Ferrer, C., Martinez-Uroz, M.A., Valverde, A., Fernandez-Alba, A.R. **2008**. Validation and uncertainty study of a comprehensive list of 160 pesticide residues in multi-class vegetables by liquid chromatography–tandem mass spectrometry. Journal of Chromatography A 1215, 37–50.

Merli, A., Reeves, G., Meregalli, G., Piccinini, A., Negri, I., Carmignano, P., Balderacchi, M., Capri, E. **2010**. Surface-water exposure to quinoxyfen: Assessment in landscape vineyards. Journal of Hydrology 383, 62–72.

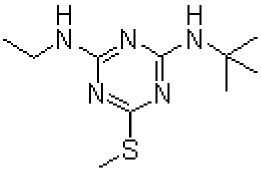
Pareja, L., Martinez-Bueno, M.J., Cesio, V., Heinzen, H., Fernandez-Alba, A.R. **2011**. Trace analysis of pesticides in paddy field water by direct injection using liquid chromatography–quadrupole-linear ion trap-mass spectrometry. Journal of Chromatography A 1218, 4790– 4798.

Walorczyk, S., Gnusowski, B. **2009**. Development and validation of a multi-residue method for the determination of pesticides in honeybees using acetonitrile-based extraction and gas chromatography–tandem quadrupole mass spectrometry. Journal of Chromatography A 1216, 6522-6531.

Wang, J., Leung, D., Chow, W. **2010**. Applications of LC/ESI-MS/MS and UHPLC QqTOF MS for the determination of 148 pesticides in berries. Journal of Agricultural Food and Chemistry 58, 5904–5925.

3.15. Terbutryn

Terbutryn is a triazine herbicide or algicide.

CAS Number 886-50-0	**Log K_{OW}** 3.48	**Water Solubility [mg/l]** ~ 25
Chemical structure **(MW 241.3)**	**AA-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]	**MAC-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]
	Fresh 0.065 µg/l = 65 ng/l Salt 0.0065 µg/l = 6.5 ng/l	Fresh 0.34 µg/l Salt 0.034 µg/l

Standard Methods

LOQ: 0.05 µg/l = 50 ng/l (EPA 619). EPA method 619 is an old LLE-GC method using a thermionic bead detector in the nitrogen mode.

Methods applied by EU Member States and comments received

France: LOQ: 0.08 ng/l = 80 pg/l; SPE-LC-MS; SPE of 250 ml water with 200 mg Oasis HLB (El Mrabet et al., 2006).

Sweden: LOQ: 50 ng/l; Method: OMK 57 (on-line SPE-LC-MS-MS).

UK-EA: Current minimum reporting value (mrv) = 4 ng/l; could meet requirement with some method development.

Northern-Ireland: Has never been requested. A new method of analysis would need to be developed.

Literature methods

Many articles on the analysis of Terbutryn in water have been published. Some examples are given here:

Extraction (volume)	**Analysis**	**LOD**	**Reference**
SPE (1 l)	GC-MS	LOQ: 3 ng/l	Gfrerer et al., 2002
SPE (0.9 l)	GC-MS	LOQ: 30 ng/l	Claver et al., 2006
Stir bar sorptive extraction	GC-MS	LOQ: 1.8 ng/l	León et al., 2006
Direct injection (100 µl)	UHPLC-MS-MS	LOD: 5 ng/l	Diaz et al., 2008
On-line SPE (20 ml)	LC-MS-MS	LOQ: 6 ng/l	Singer et al., 2010
Stir bar sorptive extraction	GC×GC-TOF-MS	MQL: 0.5 ng/l	Gomez et al., 2012

Conclusion

The LOQ of 80 pg/l reported by France is **sufficient** for compliance monitoring in inland and coastal surface waters (0.3 × EQS = 19.5 ng/l; 1.95 ng/l for coastal).

Terbutryn can be analysed by GC- and LC-MS techniques.

References

Claver, A., Pena, O., Rodríguez, L., Ovelleiro, J.L. **2006**. Study of the presence of pesticides in surface waters in the Ebro river basin (Spain). Chemosphere 64, 1437–1443.

Díaz, L., Llorca-Pórcel, J., Valor, I. **2008**. Ultra trace determination of 31 pesticides in water samples by direct injection–rapid resolution liquid chromatography-electrospray tandem mass spectrometry. Analytica Chimica Acta 624, 90-96.

El Mrabet, K., Poitevin, M., Vial, J., Pichon, V., Amarouche, S., Hervouet, G., Lalere, B. **2006**. An interlaboratory study to evaluate potential matrix reference materials for pesticides in water. Journal of Chromatography A 1134, 151-161.

EN ISO 10695. **2000**. Water quality - Determination of selected organic nitrogen and phosphorus compounds - Gas chromatographic methods.

EPA Method 619. The determination of triazine pesticides in municipal and industrial wastewater. U.S. Environmental Protection Agency, Office of Water, Washington, DC, USA.

Gfrerer, M., Martens, D., Gawlik, B.M., Wenzl, T., Zhang, A., Quan, X., Sun, C., Chen, J., Platzer, B., Lankmayr, E., Kettrup, A. **2002**. Triazines in the aquatic systems of the Eastern Chinese Rivers Liao-He and Yangtse. Chemosphere 47, 455–466.

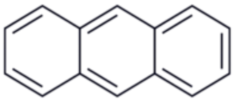
Gómez, M.J., Herrera, S., Solé, D., García-Calvo, E., Fernández-Alba, A.R. **2012**. Spatio-temporal evaluation of organic contaminants and their transformation products along a river basin affected by urban, agricultural and industrial pollution. Science of the Total Environment 420, 134–145.

León, V.M., Llorca-Pórcel, J., Álvarez, B., Cobollo, M.A., Munoz, S., Valor, I. **2006**. Analysis of 35 priority semivolatile compounds in water by stir bar sorptive extraction–thermal desorption–gas chromatography–mass spectrometry. Part II: Method validation. Analytica Chimica Acta 558, 261–266.

Singer, H., Jaus, S., Hanke, I., Lück, A., Hollender, J., Alder, A.C. **2010**. Determination of biocides and pesticides by on-line solid phase extraction coupled with mass spectrometry and their behaviour in wastewater and surface water. Environmental Pollution 158, 3054-3064.

4. Changes relating to existing Priority Substances

4.1. Anthracene

Chemical structure	**AA-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]	**MAC-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l] (in brackets former values)
	Fresh 0.1 Salt 0.1	Fresh 0.1 (0.4) Salt 0.1 (0.4)

Standard Methods

LOQ: 0.01 µg/l; HPLC-Fluorescence (ISO 17993).

Methods applied by EU Member States and comments received

Lithuania: LOQ: 0.001 µg/l (ISO 17993:2004).

Bulgaria: LOQ 0.1 µg/l (EPA 8100); LLE-GC-FID/MS. We have no data on biota and sediment

UK-EA and Northern-Ireland: No problem expected.

Ireland: No problem expected.

Conclusion

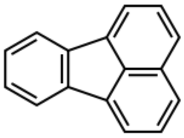
The LOQ of 0.01 µg/l of the ISO method 17993 is **sufficient** for compliance monitoring in inland and coastal surface waters.

References

ISO 17993. **2002**. Water quality - Determination of 15 polycyclic aromatic hydrocarbons (PAH) in water by HPLC with fluorescence detection after liquid-liquid extraction.

EPA method 8100. September **1986**. Polynuclear Aromatic Hydrocarbons.

4.2. Fluoranthene

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] (in brackets former values)	**EQS biota** [µg/kg]
	0.0063 µg/l = 6.3 ng/l (0.1) Fresh and salt	0.12 µg/l (1.0) Fresh and salt	30 µg/kg

Standard Methods

LOQ: 0.01 µg/l; HPLC-Fluorescence (ISO 17993).

LOQ: 0.3 µg/kg (ISO 15753).

Methods applied by EU Member States and comments received

Lithuania: LOQ: 0.005 µg/l (ISO 17993:2004).

Lithuania: 0.4 µg/kg (ISO 13877).

Bulgaria: LOQ: 0.1 µg/l; LLE-GC-FID/MS (EPA 8100); we have no data on biota and sediment.

UK-EA and Northern-Ireland: No problem expected.

Ireland: No problem expected.

Conclusion

To reach the new water EQS of 0.0063 µg/l (= 6.3 ng/l) is challenging. The biota EQS of 30 µg/kg can be achieved (with ISO methods) for compliance monitoring in biota.

References

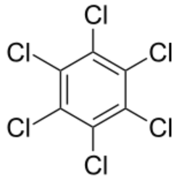
ISO 13877. **1998**. Soil quality -- Determination of polynuclear aromatic hydrocarbons -- Method using high performance liquid chromatography.

ISO 17993. **2002**. Water quality - Determination of 15 polycyclic aromatic hydrocarbons (PAH) in water by HPLC with fluorescence detection after liquid-liquid extraction.

ISO 15753. **2006**. Animal and vegetable fats and oils -- Determination of polycyclic aromatic hydrocarbons.

EPA method 8100. September **1986**. Polynuclear Aromatic Hydrocarbons.

4.3. Hexachlorobenzene (HCB)

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]
	not applicable (0.01 µg/l)	0.05 µg/l Fresh and salt	10 µg/kg

Standard Methods

EN ISO 6468: LLE-GC.

US EPA method 1625: GC-MS; Determination of semivolatile toxic organic pollutants in waters, soils, and municipal sludges; MDL: 51 µg/kg.

Methods applied by EU Member States and comments received

Ireland: Biota analysis; No problem expected.

Literature methods

Two good papers on the exposure assessment of Hexachlorobenzene through food, fish and seafood consumption were found (Falcó et al., 2004; 2008). The highest HCB levels were found in salmon and mackerel with 1.68 and 0.80 ng/g. Moreover, there is an excellent review on HCB in the global environment by Barber and co-workers (2005).

Matrix	**Extraction**	**Analysis**	**LOD**	**Reference**
Fish	SLE	GC-ECD	0.76 µg/kg	Tricklebank et al., 2002
Food samples	Soxhlet; clean-up	GC-HRMS	5 ng/kg	Falcó et al., 2004
Water	LLE	GC-ECD	LOQ: 10 ng/l	Fatta et al., 2007
Fish; seafood	Soxhlet; clean-up	GC-HRMS	5 ng/kg	Falcó et al., 2008
Fish (eel)	ASE	GC-MS-MS	1 µg/kg	Macgregor et al., 2010

Conclusion

Biota monitoring of HCB is not difficult.

References

Barber, J.L., Sweetman, A.J., Van Wijk, D., Jones, K.C. **2005**. Hexachlorobenzene in the global environment: Emissions, levels, distribution, trends and processes. Science of the Total Environment 349, 1-44.

EN ISO 6468. **1997**. Determination of certain organochlorine insecticides, polychlorinated biphenyls and chlorobenzenes – Gas chromatographic method after liquid-liquid extraction.

EPA method 1625. **1989**. Semivolatile Organic Compounds by Isotope Dilution GCMS.

Falcó, G., Bocio, A., Llobet, J.M., Domingo, J.L., Casas, C., Teixido, A. **2004**. Dietary intake of hexachlorobenzene in Catalonia, Spain. Science of the Total Environment 322, 63–70.

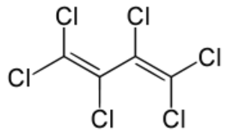
Falcó, G., Llobet, J.M., Bocio, A, Domingo, J.L. **2008**. Exposure to hexachlorobenzene through fish and seafood consumption in Catalonia, Spain. Science of the Total Environment 389, 289-295.

Fatta, D., Michael, C., Canna-Michaelidou, St., Christodoulidou, M., Kythreotoua, N., Vasquez, M. **2007**. Pesticides, volatile and semivolatile organic compounds in the inland surface waters of Cyprus. Desalination 215, 223–236.

Macgregor, K., Oliver, I.W., Harris, L., Ridgway, I.M. **2010**. Persistent organic pollutants (PCB, DDT, HCH, HCB & BDE) in eels (Anguilla anguilla) in Scotland: Current levels and temporal trends. Environmental Pollution 158, 2402-2411.

Tricklebank, K.A., Kingsford, M.J., Rose, H.A. **2002**. Organochlorine pesticides and hexachlorobenzene along the central coast of New South Wales: multi-scale distributions using the territorial damselfish Parma microlepis as an indicator. Environmental Pollution 116, 319–335.

4.4. Hexachlorobutadiene

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]
	not applicable (0.1 µg/l)	0.6 µg/l Fresh and salt	55 µg/kg

Standard Methods

EN ISO 6468; LLE-GC

US EPA method 1625; GC-MS; Determination of semivolatile toxic organic pollutants in waters, soils, and municipal sludges; MDL: 46 µg/kg.

Methods applied by EU Member States and comments received

Ireland: Biota analysis; No problem expected.

Literature methods

Less information on Hexachlorobutadiene is available. Macgregor and co-workers (2010) analysed persistent organic pollutants (PCB, DDT, HCH, HCB & BDE) including Hexachlorobenzene and Hexachlorobutadiene in eel samples.

Matrix	**Extraction**	**Analysis**	**LOD**	**Reference**
Water	LLE	GC-ECD	LOQ: 2 ng/l	Fatta et al., 2007
Fish (eel)	ASE	GC-MS-MS	1 µg/kg	Macgregor et al., 2010

Conclusion

Biota monitoring should not be a problem.

References

Fatta, D., Michael, C., Canna-Michaelidou, St., Christodoulidou, M., Kythreotoua, N., Vasquez, M. **2007**. Pesticides, volatile and semivolatile organic compounds in the inland surface waters of Cyprus. Desalination 215, 223–236.

ISO 6468. **1996**. Water quality -- Determination of certain organochlorine insecticides, polychlorinated biphenyls and chlorobenzenes -- Gas chromatographic method (ECD) after liquid-liquid extraction.

ISO 10301. **1997**. Water quality -- Determination of highly volatile halogenated hydrocarbons -- Gas-chromatographic methods; ECD detection.

ISO 15680. **2003**. Water quality -- Gas-chromatographic determination of a number of monocyclic aromatic hydrocarbons, naphthalene and several chlorinated compounds using purge-and-trap and thermal desorption.

Macgregor, K., Oliver, I.W., Harris, L., Ridgway, I.M. **2010**. Persistent organic pollutants (PCB, DDT, HCH, HCB & BDE) in eels (Anguilla anguilla) in Scotland: Current levels and temporal trends. Environmental Pollution 158, 2402-2411.

4.5. Lead and its compounds

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] (in brackets former values)
	Fresh 1.2 (7.2) Salt 1.3 (7.2)	Fresh 14 (n.a.) Salt 14 (n.a.)

Standard Methods

LOQ: 0.1 µg/l (ICP-MS) (ISO 17294-2).

Methods applied by EU Member States and comments received

Italy: LOD: 0.1 µg/l; ICP/MS (FR, CZ, ES, NL); 0.2; GF/AAS (CZ); Performance data on drinking waters (LOD) in the context of drinking water directive. The methods in some cases are from other MS. The methods are validated with ring-test.

Lithuania: LOQ: 50 µg/l (ISO 11885).

UK-EA and Northern-Ireland: No problem expected.

Ireland: LOQ: 0.5 µg/l; Method development could reduce LOQ to 0.3 of EQS.

Conclusion

The LOQ of 0.1 µg/l of the ISO method 17294-2 is **sufficient** for compliance monitoring in inland and coastal surface waters.

References

ISO 17294-2. **2003**. Water quality -- Application of inductively coupled plasma mass spectrometry (ICP-MS) -- Part 2: Determination of 62 elements.

ISO 11885. **2007**. Water quality - Determination of selected elements by inductively coupled plasma optical emission spectrometry (ICP-OES).

4.6. Mercury and its compounds

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]
	not applicable (0.05)	0.07 µg/l Fresh and salt	20 µg/kg

Standard Methods

EN ISO 17852:2008; Atomic fluorescence spectrometry.

EN 12338:1998; CV-AAS with Amalgamation.

Methods applied by EU Member States and comments received

Ireland: LOQ: 0.05µg/l in water; No problem expected in biota.

Literature methods

Many articles on biota (fish) analysis of mercury are available.

Matrix	**Extraction**	**Analysis**	**LOD**	**Reference**
Fish	Solvent	AAS	10 ng/g	Branco et al., 2007
Fish	Solvent	AAS-FIMS	1 µg/kg	Katner et al., 2010
Fish	Digestion	FIMS mercury analyser	2 ng/g	Burger and Gochfeld, 2011

Conclusion

Biota monitoring of mercury is not a problem.

References

Branco, V., Vale, C., Canario, J., dos Santos, M.N. **2007**. Mercury and selenium in blue shark (Prionace glauca, L. 1758) and swordfish (Xiphias gladius, L. 1758) from two areas of the Atlantic Ocean. Environmental Pollution 150, 373-380.

Burger, J., Gochfeld, M. **2011**. Mercury and selenium levels in 19 species of saltwater fish from New Jersey as a function of species, size, and season. Science of the Total Environment 409, 1418–1429.

ISO 5666. **1999**. Water quality -- Determination of mercury.

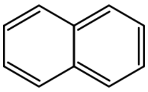
ISO 16590. **2000**. Water quality -- Determination of mercury -- Methods involving enrichment by amalgamation.

ISO 16772. **2004**. Soil quality -- Determination of mercury in aqua regia soil extracts with cold-vapour atomic spectrometry or cold-vapour atomic fluorescence spectrometry.

ISO 17852. **2006**. Water quality -- Determination of mercury -- Method using atomic fluorescence spectrometry.

Katner, A., Sun, M.-H., Suffet, M. **2010**. An evaluation of mercury levels in Louisiana fish: Trends and public health issues. Science of the Total Environment 408, 5707–5714.

4.7. Naphthalene

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] (in brackets former values)
	Fresh 2 (2.4) Salt 2 (1.2)	Fresh 130 (n.a.) Salt 130 (n.a.)

Standard Methods

LOQ: 0.01 µg/l (ISO 17993).

Methods applied by EU Member States and comments received

Lithuania: LOQ: 0.005 µg/l (ISO 17993).

Bulgaria: LOQ 0.1 µg/l; LLE-GS-FID/MS (EPA 8100); we have no data on biota and sediment

UK-EA and Northern-Ireland: No problem expected.

Ireland: LOQ: 0.5µg/l.

Conclusion

The LOQ of 0.01 µg/l of the ISO method 17993 is **sufficient** for compliance monitoring in inland and coastal surface waters.

References

ISO 17993. **2002**. Water quality - Determination of 15 polycyclic aromatic hydrocarbons (PAH) in water by HPLC with fluorescence detection after liquid-liquid extraction.

4.8. Nickel and its compounds

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] (in brackets former values)
	Fresh 4 (20) Salt 8.6 (20)	Fresh 34 (n.a.) Salt 34 (n.a.)

Standard Methods

LOQ: 1 µg/l (ICP-MS) (ISO 17294-2).

Methods applied by EU Member States and comments received

Italy: LOD: 0.1 µg/l; ICP/MS (CZ, ES); 0.1 µg/l; GF/AAS (ES); Performance data on drinking waters (LOD) in the context of drinking water directive. The methods in some cases are from other MS. The methods are validated with ring-test.

Ireland: Ireland: LOQ: 0.5µg/l.

Lithuania: 10 µg/l (ISO 11885); 1000 ug/kg (SVP 1-2-10:2009).

UK-EA: Problems possible

UK-SEPA: No problem expected.

Northern-Ireland: Problems possible for freshwater.

Conclusion

The LOQ of 1 µg/l of the ISO method 17294-2 is **sufficient** for compliance monitoring in inland and coastal surface waters.

References

ISO 17294-2. **2003**. Water quality -- Application of inductively coupled plasma mass spectrometry (ICP-MS) -- Part 2: Determination of 62 elements.

ISO 11885. **2007**. Water quality - Determination of selected elements by inductively coupled plasma optical emission spectrometry (ICP-OES).

4.9. Polyaromatic Hydrocarbons (PAHs)

Chemicals	**AA-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]	**MAC-EQS** **Inland (fresh) and other (salt) surface waters** [µg/l]	**EQS biota** [µg/kg]
Benzo(a)pyrene	Fresh 1.7 10^{-4} = 0.17 ng/l Salt 1.7 10^{-4} = 0.17 ng/l	Fresh 0.27 (0.1) Salt 0.027 (0.1)	SUM [µg/kg] 2 for fish 5 for crustaceans and cephalopods 10 for molluscs
Benzo(b)fluoranthene		Fresh 0.017 (n.a.) Salt 0.017 (n.a.)	
Benzo(k)fluoranthene		Fresh 0.017 (n.a.) Salt 0.017 (n.a.)	
Benzo(g,h,i)perylene		Fresh 8.2 10^{-3} = 8.2 ng/l Salt 8.2 10^{-4} = 0.82 ng/l	
Indeno(1,2,3-cd)pyrene			

The AA-EQS values for PAHs have been changed and lowered. In the EQS Directive from 2008, there was a single AA-EQS of 0.05 µg/l for Benzo(a)pyrene (for fresh and coastal waters), a sum AA-EQS for Benzo(b)fluoranthene, and Benzo(k)fluoranthene of 0.03 µg/l, and a sum AA-EQS for Benzo(g,h,i)perylene and Indeno(1,2,3-cd)pyrene of 0.002 µg/l.

Standard Methods

LOQ: 0.01 µg/l (ISO 17993).

LOQ: 0.2 µg/kg Benzo(a)pyrene, Benzo(b)fluoranthene, Benzo(k)fluoranthene; 1 µg/kg for Benzo(g,h,i)perylene, and 0.3 µg/kg for Indeno(1,2,3-cd)pyrene (ISO 15753:2006).

Methods applied by EU Member States and comments received

Italy: LOQ: 2 ng/l for Benzo(a)pyrene; 6 ng/l for Benzo(b)fluoranthene, Benzo(k)-fluoranthene, Benzo(g,h,i)perylene, and Indeno(1,2,3-CD)pyrene (GC/MS).

Lithuania: LOQ: 0.002 µg/l for Benzo(a)pyrene; 0.005 µg/l for Benzo(b)fluoranthene, Benzo(g,h,i)perylene, and Indeno(1,2,3-CD)pyrene; 0.001 µg/l for Benzo(k)-fluoranthene (ISO 17993:2004); 0.2 µg/kg for Benzo(a)pyrene; 0.4 ug/kg Benzo(b)-fluoranthene, and Indeno(1,2,3-CD)pyrene; 0.1 ug/kg Benzo(k)fluoranthene; 0.3 ug/kg Benzo(g,h,i)perylene (ISO 13877:1998).

UK-EA and Northern-Ireland: No problems expected.

Ireland: No problem expected.

Scotland (Craig Robinson): Using passive sampling, our experience indicates that the required LOQs are achievable. There is an ICES TIMES (Techniques in Marine

Environmental Science) paper that is about to appear on their website (www.ices.dk) that includes details of how to determine water concentrations using passive sampling. We have had close collaboration with Foppe Smedes for a number of years and largely follow his advice in our work (Smedes and Booij, 2012).

As for what are measureable concentrations, we've not yet published concentrations of PAHs in water (except as posters). We have a paper that we are re-submitting to the journal tomorrow, with responses to reviewers, and that measures PAHs in a river catchment using silicone rubber PSDs. But it would be a bit premature to use that.

There was an intercalibration exercise conducted in France by Aquaref, a paper describing the outline of which has been accepted by TrAC (Miége et al., 2012) and a paper on PAHs is in preparation. A presentation on the PAH exercise can be found at the webpage of Aquaref, and shows that labs were able to measure B[*a*]P at <50 pg/l by passive sampling (slides 10 & 12), but does not quote DLs for passive sampling. This is because it is not possible to accurately quote generic DLs for water concentrations using PS techniques. One could derive DLs for PAH absorbed by the sampler (e.g. ng/g), but for concentrations in water, the DLs will vary for every deployment of the sampler, depending upon duration, flow rate, temperature, etc. If a sampler is deployed for a long period of time, then one could get a low DL – so long as the lab is able to get blanks low enough.

However, it must be kept in mind that passive sampling methods extract only the solved fraction in the water phase; therefore, detection of the low-volatile PAHs, which tend to adsorb to suspended solids, could lead to lower findings compared to the total water sample.

Literature methods

Cailleaud and co-workers (Cailleaud et al., 2007) analysed PCBs and PAHs in the water column of the Seine estuary at concentrations in the low ng/l range. Dissolved PAHs were extracted from 2 l water by LLE, and analysed by GC-MS. No LODs are given.

Extraction (volume)	**Analysis**	**LOD**	**Reference**
LLE (2 l)	GC-MS	Not given	Cailleaud et al., 2007
SPE (650 l)	HPLC-fluorescence	LOD: 0.06-0.5 ng/l	Nizzetto et al., 2008
SPE (5 l)	GC-MS	LOD: 18-80 ng/l	Guo et al., 2007

Conclusion

The lowest LOQs between 1-6 ng/l for water analysis achieved are **not sufficient** for compliance monitoring in inland and coastal surface waters (0.3 × EQS = 0.051 ng/l = 51 pg/l). New research shows that lower water LOQs can be achieved by passive sampling. Biota monitoring should be **possible**.

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Nizzetto, L., Lohmann, R., Gioia, R., Jahnke, A., Temme, C., Dachs, J., Herckes, P., Di Guardo, A., Jones, K.C. **2008**. PAHs in air and seawater along a North–South Atlantic transect: Trends, processes and possible sources. Environmental Science & Technology 42, 1580-1585.

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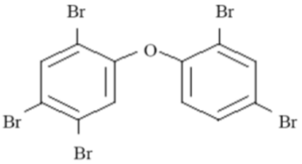
4.10. Brominated Diphenyl Ethers (BDEs)

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] (in brackets former values)	**EQS biota** [µg/kg]
 (penta BDE)	Fresh 4.9 10^{-8} µg/l = 49 fg/l (0.0005 µg/l) Salt 2.4 10^{-9} µg/l = 2.4 fg/l (0.0002 µg/l)	Fresh 0.14 µg/l Salt 0.014 µg/l (n.a.)	0.0085 µg/kg = 8.5 ng/kg

Standard Methods

LOQ: 20-40 pg/l; 2-4 ng/kg; HRGC-HRMS (for penta-heptaBDE; EPA 1614).

Description of ISO 22032:

This International Standard specifies a method for the determination of Polybrominated Diphenylethers in sediment and sludge using gas chromatography/mass spectrometry in the electron impact or electron capture ionisation mode. Extraction of PBDEs from the dried sample by an organic solvent is followed by clean-up of the extract by e.g. multi-layer silica gel column chromatography. For quantification an internal standard calibration is applied.

When applying GC-EI-MS, the method is applicable to samples containing 0.05 µg/kg to 25 µg/kg of tetra- to octabromo congeners and 0.3 to 100 µg/kg of decabromo diphenyl ether (BDE-209), respectively. Approximately ten times lower concentrations can be quantified when using GC-ENCI-MS.

Description of EPA method 1614:

Extraction: Aqueous samples (samples containing less than one percent solids): Stable isotopically labeled analogs of the BDEs are spiked into a 1-L sample. The sample is extracted using solid-phase extraction (SPE), separatory funnel extraction (SFE), or continuous liquid/liquid extraction (CLLE).

Fish and other tissue: A 20-g aliquot of sample is homogenized, and a 10-g aliquot is spiked with the labeled compounds. The sample is mixed with anhydrous sodium sulfate, dried for a minimum of 30 minutes, and extracted for 18-24 hours using methylene chloride in a Soxhlet extractor. The extract is evaporated to dryness, and the lipid content is determined.

Clean-up: Tissue extracts are first cleaned up using an anthropogenic isolation column, and all extracts are cleaned up using back-extraction with sulfuric acid and/or base, and gel permeation, silica gel, and/or Florisil or alumina chromatography, as required.

HRGC-HRMS analysis: After cleanup, the extract is concentrated to 20 µL and labeled injection internal standards are added. An aliquot of the extract is injected into the gas chromatograph (GC). The analytes are separated by the GC and detected by a high-resolution mass spectrometer. Two exact m/z's are monitored at each level of bromination throughout a pre-determined retention time window.

Methods applied by EU Member States and comments received

Italy: LOQ: 0.1 ng/l; Internal Method, validated.

UK-EA: Problems expected in water and possibly in biota.

UK-SEPA: Not currently requested for water. For biota, problems expected to reach required detection limit.

Northern-Ireland: Extremely challenging.

Literature methods

BDEs have been extensively monitored during the last years in biota, sediment, humans, milk, food, dust, etc. Less data on water analysis is available.

Möller and co-workers (Möller et al., 2011) developed a large-volume SPE extraction method followed by GC-ECNCI-MS for the ultra-trace analysis of BDEs in seawater samples. They achieved MDLs in the low fg/l concentration range.

Water analysis:

Extraction (volume)	Analysis	LOD	Reference
SPE (100 l)	GC-MS	MDL: 20-200 pg/l	Oros et al., 2005
LLE (10 l)	GC-MS-MS	LODs: 1.5-8 pg/l (dissolved phase); 2.0-16 pg/l (SPM); BDE-209: 240 pg/l (DP); 410 pg/l (SPM)	Wurl et al., 2006
SPE (1000 l)	GC-ECNCI-MS	MDL: 0.0003-0.014 pg/l (dissolved); 0.24 pg/l for BDE-209; 0.001-0.026 pg/l (particulate); 0.042 pg/l for BDE-209	Möller et al., 2011

Biota analysis:

Extraction (species)	Analysis	LOD	Reference
SLE (fish)	HRGC-HRMS	0.01-0.25 ng/g	Luross et al., 2002
Soxhlet (marine biota)	GC-NCI-MS	0.1 ng/g	Vorkamp et al., 2004
(polar bears)	GC-NCI-MS	0.01-0.05 ng/g	Dietz et al., 2007
Soxhlet (fish)	HRGC-HRMS	0.010-0.127 ng/g	Peng et al., 2007
Soxhlet (fish)	GC-MS	0.02-0.2 ng/g	Hajslova et al., 2007
SLE (liver)	HRGC-HRMS	2.4-14 ng/kg; 85-217 ng/kg for BDE-209	Mariussen et al., 2008
SLE (eegs of sea eagles)	GC-MS	2 ng/g	Nordlöf et al., 2010
SLE (fish)	GC-MS-MS	1-30 ng/kg; 170 ng/kg for BDE-209	Labadie et al., 2010
ASE (fish)	HRGC-HRMS	0.002-0.054 ng/kg (muscle) 0.048-1.1 ng/kg (liver) BDE-209: 0.56-7.8 ng/kg	Munschy et al., 2011
Soxhlet (fish)	GC-NCI-MS	13-16 ng/kg	Montory et al., 2012

Conclusion

The new water AA-EQS for BDEs in the fg/l range is **not achievable** with routine analytical methods. The biota LOQ of 2 ng/kg of the EPA method 1614 (HRGC-HRMS) is **sufficient** for compliance monitoring in biota (0.3 × EQS = 2.55 ng/kg).

References

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5. Annex

5.1. Comment from Germany

Quick inquiry amongst German monitoring laboratories (in 2011)

Early 2011 a quick inquiry amongst German monitoring laboratories which are in charge of WFD monitoring was undertaken in order to assess whether there are analytical methods available to determine the new proposed Priority Substances in water, sediment or biota.

Table 1 summarizes the ranges of reported LOQs from eleven Federal State laboratories and indicates the principle of measurement of the methods with the lowest LOQ for each compound.

In general, the German laboratories in charge of WFD monitoring are used to analyze the matrices water, sediment or suspended matter. The inquiry has shown that methods (standard methods or in-house methods analogue to standard methods) are available for the majority of the new Priority Substances in at least one of the laboratories. But for the majority of substances the reported LOQs were not low enough in respect to the proposed EQS.

The LOQ of the most sensitive method was below 30% of the proposed EQS for: Terbutryn, Lead, Naphthalene, Anthracene, Diclofenac and Nickel.

The LOQ of the most sensitive method was inadequate for the determination of the following Priority Substances in water: Bifenox, Cypermethrin, Dichlorvos, Dicofol, PFOS, 17-beta-estradiol, 17-alpha-estradiol, polyBDEs, Heptachlor/Heptachlorepoxide in water and biota, Aclonifen, Quinoxyfen, Cybutryne (Irgarol), Fluoranthene, Benzo(a)pyrene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(g,h,i)-fluoranthene.

The governmental environmental laboratories in charge of WFD monitoring are not yet prepared to analyze the possible new priority substances in biota. Hence, at the moment there is no information available on which LOQ might be reached when analyzing HBCDD, Dioxins, Fluoranthene, Sum of PAHs and PBDEs in biota.

Table 1: Overview of Limits of Quantification (LOQs) reported by German monitoring laboratories (in 2011).

Priority Substance	Matrix	range of reported LOQ	Unit	Most sensitive method
Aclonifen	WATER	0.01-0.05	µg/l	according to EPA 619 (GC-MS)
Bifenox	WATER	0.01-0.08	µg/l	according to ISO 6468 (GC-MS)
Cybutryne (Irgarol)	WATER	0.001-0.02	µg/l	according to EPA 619 (GC-MS)
Cypermethrin	WATER	0.001-0.01	µg/l	according to ISO 11369 (LC-UV)
Dichlorvos	WATER	0.001-0.1	µg/l	GC-MS
Diclofenac	WATER	0.005-0.15	µg/l	LC-MS/MS
Dicofol	WATER	0.005	µg/l	according to DIN 38407-2(GC-ECD)
HBCDD	WATER	no information		
	BIOTA	no information		
PFOS	WATER	0.01	µg/l	ISO 25101 (LC-MS)
	BIOTA	5	µg/kg	no information
Quinoxifen	WATER	0.01-0.06	µg/l	according to ISO 11369 (LC-UV)
Terbutryn	WATER	0.001-0.03	µg/l	GC-MS
17-ß-estradiol	WATER	0.0005	µg/l	LC-MS/MS
Dioxin	BIOTA	no information		
Heptachlor / Heptachlorepoxide	WATER	0.001-0.01	µg/l	EN 6468 (GC-MS)
	BIOTA	5	µg/kg	no
17-alpha-ethinylestradiol	WATER	0.0005	µg/l	LC-MS/MS
Ibuprofen	WATER	0.005-0.05	µg/l	DIN 38407-F35 (LC-MS/MS)
Anthracene	WATER	0.0025	µg/l	GC-MS
Fluoranthene	WATER	0.0025	µg/l	GC-MS
	BIOTA	no information		
Lead	WATER	0.2	µg/l	no information
Naphthalene	WATER	0.0025	µg/l	GC-MS
Nickel	WATER	0.5	µg/l	no information
PAHs				
Benzo(a)pyrene	WATER	0.0025	µg/l	GC-MS
Benzo(b)fluoranthene	WATER	0.0025	µg/l	GC-MS
Benzo(k)fluoranthene	WATER	0.0025	µg/l	GC-MS
Benzo(ghi)perylene	WATER	0.0025	µg/l	GC-MS
Sum PAHs	BIOTA	no information		
polyBDE	WATER	0.003 – 0.005	µg/l	GC-MS
	BIOTA	no information		

5.2. Comment from Sweden

Substance	Method	Matrix
Aclonifen	OMK 51	surface water LOD 0.008 µg/l LOQ 0.020 µg/l
	OMK 51	rain water LOD 0.005 µg/l LOQ 0.007 µg/l
Bifenox	OMK 57	surface water LOD 0.050 µg/l
Cyanides	-	
Cybutryne (Irgarol)^{B}	Bones et al, 2006	water, solid matrices
	OMK 57	surface water LOD 0.001 µg/l LOQ 0.002 µg/l
Cypermethrin	OMK 51	surface water LOD 0.001 µg/l LOQ 0.010 µg/l
	OMK 51	rain water LOD 0.001 µg/l LOQ 0.010 µg/l
Dichlorvos	-	
Diclofenac	-	
Dicofol^{A}	OMK 51	rain water LOD 0.001 µg/l LOQ 0.010 µg/l
Dioxin	Danielsson 2005, Wiberg 2002, 2007.	biota
17 alpha-ethinylestradiol	-	
17-beta-ethinylestradiol	-	
Heptachlor/heptachlor epoxide	OMK 51	rain water LOD 0.001 µg/l LOQ 0.010 µg/
HBCDD	Sellström, 1996; 2003	biota
PFOS	Bignert et al 2011	biota
Quinoxyfen	OMK 57	surface water LOD 0.010 µg/l LOQ 0.050 µg/l

	OMK 51	rain water
		LOD 0.002 µg/l
		LOQ 0.003 µg/l
Terbutryn	OMK 57	surface water
		LOD 0.010 µg/l
		LOQ 0.050 µg/
	OMK 57	rain water
		LOD 0.005 µg/l
		LOQ 0.005 µg/l
Ibuprofen	-	
Dioxin-like PCBs	Danielsson 2005, Wiberg 2002, 2007.	biota
PCBs (non-dioxin like)		
Zinc	SS-EN ISO 17294-1 and 2 (ICP-MS)	water
		LOD 0.3 µg/l, LOQ 1 µg/l
Zinc	SS-EN 13805 (Digestion in a microwave oven)	biota (fish liver)
	SS-EN ISO 17294-1 and 2 (ICP-MS)	LOD 0.5 µg/g dw
	Borg et al, 1981 (older method)	LOQ 1.5 µg/g dw
Zinc	SS-EN ISO 17294-2:2005	water
	Borg et al, 1981	biota (fish liver)

[A] Not analysed after 2006

[B] Not included in routine monitoring

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5.3. Comment from Italy

Analytical Methods-Italy; B: Biota DW: Drinking waters SW: Surface Waters

Substance		
	Method	**Matrix**
PCDD+PCDF	EPA 1613 modified	0.2 pgWHO-TE/g B (LOD)
PCB DL	EPA 1613 modified	0.05 pgWHO-TE/g B (LOD)
PFOS	(Italy) Internal method validated	0.1 ng/g fw B (LOD)
Diclofenac	Internal Methods-validated (IT)	10 ng/L SW (LLOA)
Ibuprofen	Internal Methods-validated (IT)	1-10 ng/L SW (LLOA)
PFOS	Internal Methods-validated (IT)	1-10 ng/L SW (LLOA)
PBDE	Internal Methods validated (IT)	0,0001 µg/l SW (LLOA)
PFOS	LC-MS/MS (IT)	6.5 x 10^{-4} µg/l DW (LOD)
17alpha-ethinylestradiol	LC-MS/MS (IT)	$1.8x10^{-3}$ µg/l DW (LOD)
17 beta-estradiol	LC-MS/MS (IT)	9 x 10^{-4} µg/l DW (LOD)
PAHs		
	GC/MS (IT)	$1x10^{-4}$ µg/l DW (LOD)
Benzo(a)pyrene	GC/MS (IT)	$2x10^{-3}$ µg/l DW (LOD)
Benzo(b)fluoranthene		$6x10^{-3}$ µg/l DW (LOD)
Benzo(k)fluoranthene		$6x10^{-3}$ µg/l DW (LOD)
Indeno(1,2,3-CD)pyrene		$6x10^{-3}$ µg/l DW (LOD)
Benzo(g,h,i)perylene		$6x10^{-3}$ µg/l DW (LOD)

5.4. Comment from Austria

A. Rauchbüchel, K. Deutsch, 21 March 2011

“The lack of analytical methods for quite a number of parameters in the table and the insufficient sensitivity for some of the available methods confirm our concerns regarding the technical feasibility of compliance checking for the proposed EQS of existing and candidate priority substances.

Furthermore the table displays the situation too optimistic from our point of view insofar as it considers US EPA methods in many cases. These methods usually require highly sophisticated laboratory equipment which presumably is not available in all Austrian (routine) water laboratories.

Additionally the table reflects the urgent need to push forward the development of CEN and /or ISO methods for the listed substances.”

5.5. Cost of analyses (Sweden)

Sweden gave the following information on the cost of analyses:

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)

5.6. Contribution on bioanalytical assays for steroidal oestrogens

by Robert Kase, Petra Kunz, Henner Hollert, and Inge Werner

Bioanalytical receptor binding assay methods for monitoring steroidal oestrogens 17-alpha-ethinylestradiol (EE2) and 17-beta-estradiol (E2) in water bodies

Endocrine disruptive compounds (EDCs) influence the sexual function and differentiation in aquatic organisms, mainly driven by their oestrogenic or androgenic activity. A well-studied *mode of action* is the oestrogenic receptor binding. Within the Water Framework Directive (WFD), the oestrogenic and EDCs 17-alpha-ethinylestradiol (EE2) and the natural hormone 17-beta-estradiol (E2) are listed as candidate priority substances with adopted Environmental Quality Standards (EQS) of 35 pg/l for EE2 and 0.4 ng/l for E2, respectively.

Both EQS are below the analytical limits of quantification (LOQ) of most routine chemical methods (see points 3.9 and 3.10 of this report). In addition, these substances do not accumulate in biota or sediment, thus analysing those compartments will not reduce LOQ problems (as is the case for several of the current priority substances).

To overcome these current detection problems, we therefore propose to use sensitive effect-based tools (i.e. simple *in vitro* estrogen-receptor transactivation assays) for the screening of oestrogenic activity for EQS compliance monitoring. In addition to the monitoring for oestrogenic activities, androgenic activities can be monitored by specific androgen-receptor transactivation assays in parallel.

Three widely used oestrogen receptor transactivation assays are frequently being suggested as suitable tools for monitoring of oestrogenic activity (Kase et al., 2009; 2011, Hecker & Hollert, 2011; Kienle et al., 2011; 2012). These assays have also been compared in several studies (Murk et al., 2002; Leusch et al., 2008; Kase et al., 2009):

1. The **YES (Yeast Estrogen Screen) assay** (Routledge & Sumpter, 1996 adapted to Schultis & Metzger, 2004),
2. the commercial **ER-CALUX®** (Estrogen Receptor-mediated Chemically Activated Luciferace gene expression) (van der Linden et al., 2008), and
3. the non-commercial **T47D-Kbluc assay** (Wilson et al., 2004).

These *in vitro* assays are able to measure the overall receptor binding potential of oestrogens, e.g. 17-alpha-ethinylestradiol (EE2), 17-beta-estradiol (E2) or estrone (E1) and other substances in an environmental sample expressing their combined potency in E2-equivalents (EEQs) and can be compared with the proposed annual average AA-EQS for E2 to determine related risk quotients.

$$\textbf{Risk quotient (RQ)} \quad = \frac{\textbf{MEC or EEQ}}{\textbf{QC}} = ?$$

<1 tolerable risk

>1 intolerable risk

Equation 1: Calculation of risk quotients (RQ), MEC= Measured Environmental Concentration or equivalent concentration, e.g. EEQ; QC= Quality criteria (usually the AA-EQS)

Among the three assays, the **YES assay** was generally found to be the least sensitive with an LOQ for E2 in the low ng/l range (2-3 ng/l E2, personal communication Sebastian Buchinger, Federal Institute of Hydrology, GER) and is in the DIN/ISO standardization program. However, the advantages of the YES are its practicability and robustness also for waste water assessments. A prediction for a potential anthropogenic oestrogenic impact on surface water can be made using the YES by dividing the EEQs by a corresponding dilution factor.

The **ER-CALUX®** and the non-commercial **T47D-Kbluc** are more sensitive than the YES. They reliably detect oestrogenic activity in surface water (LOQs in the range of 0.1 ng/l, reported by Leusch, 2008) and are thus well suited for monitoring of EQS compliance (AA-EQS: 0.4 ng/l E2).

All three in vitro assays can be performed in combination with solid phase extraction (SPE) and passive sampling, so lower LOQs are also possible, depending on the methods used. Different SPE-LOQs of **ER-CALUX®** of 20-40 pg/l are described (e.g. Puijker, 2007). The sensitivity of the **T47D-Kbluc** is expected also here in a similar range.

However, it must be stressed that these methods are integrative receptor binding assays which detect all estrogen like chemicals able to bind (agonistic) to the estrogen receptor. Therefore, they can be applied as screening assays for the whole oestrogenic potential (of a water sample) or single strongly binding substances just as E2.

Samples with positive results that require chemical analytical confirmation for single compounds (RQ >1), can then be further analysed with more sensitive (and costly) chromatographic analytical methods (based on LC- or GC-MS techniques) with LOQs below the recommended AA-EQS for E2 or EE2, as well as analytical screening for other known (and generally weaker) oestrogen receptor binding compounds, such as estrone (E1), nonylphenols, bisphenol A and others.

Reasons for proposing the use of E2-equivalents (EEQ), instead of EE2-equivalents

- E2 (17-beta-estradiol) is a natural steroid hormone and has an *in vitro* and *in vivo* potency between E1 (estrone) and EE2 (17-alpha-ethinylestradiol); therefore it is well suited for assessing mixture effects.
- E2 and E1 are likely the main contributors to oestrogenic activity in surface water, therefore E2 is more representative of oestrogenic substances than EE2.
- E2-equivalents are commonly used in bioanalytics and biomonitoring, thus data are easily comparable with previous studies.
- EE2 has a slightly higher potency *in vitro* than E2, but *in vivo* it is 10-25 times more potent. If EE2 equivalents were to be used, there is a high probability for risk overestimation and obtaining false positive results, due to the possibility of E2 binding at the receptor.

It is known that other environmentally relevant water pollutants, e.g. triclosan, can increase estrogenic activity via an inhibition of the androgen receptor (AR) (Rostowski et al., 2011). Therefore a simultaneous monitoring of ER and AR receptor activation and inhibition is preferred. In addition to the monitoring for oestrogenic activities, androgenic activities can be monitored by commercial **AR-CALUX®** systems, or the non-commercial **MDA-kb2 cell line** with an AR receptor, recommended by the US-EPA (Wilson et al., 2002; Blake et al., 2010; Hecker & Hollert, 2011). Similar to the EEQ approach androgenic hormone equivalents like testosterone, or dihydrotestosterone equivalents can be used as a positive control to calculate the AEQs .

Summary and Recommendation

Using effect-based tools will reduce the high costs of the few currently available analytical “high end” methods for the measurement of E2 and EE2 and provide reliable information on the endocrine disrupting potential of water samples. The bioanalytical cost range is between 60 Euro for a **YES test** and 200 Euro for an **ER-CALUX®** at Bio Detection Systems. Three widely used estrogen receptor transactivation assays, the **YES** (adapted to Schultis & Metzger, 2004), the **ER-CALUX®** (van der Linden, 2008), and the non-commercial **T47D-Kbluc** (Wilson et al., 2004) are recommended as effect-based bioanalytical alternatives to currently available chemical analytical methods.

These bioanalytical methods have proven functionality in environmental samples and can be used for surface water assessment or to assess significant sources of potential endocrine disruptors such as municipial wastewater (Kienle et al., 2011) or sediments (Grund et al., 2011). The knowledge about androgenic and antiandrogenic receptor binding in the aquatic environment is currently limited, which is also the case for other receptor mediated activations (e.g. Kortenkamp et al., 2011). Therefore, additional monitoring with androgen receptor (AR) transactivation assays is proposed in parallel to address both ER- and AR-receptor mediated risks of endocrine disruptors with effect based tools.

Additionally, effect-based tools have also successfully been used for the identification of unknown chemicals and the contribution of single compounds to the overall endocrine effectiveness when combined with the strategy of effect-directed analysis (Hecker & Hollert, 2009; Higley et al., 2012).

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European Commission

EUR 25532 – Joint Research Centre – Institute for Environment and Sustainability

Title: Analytical Methods relevant to the European Commission's 2012 proposal on Priority Substances under the Water Framework Directive

Author: Robert Loos

Luxembourg: Publications Office of the European Union

2012 – 69 pp. – 21.0 x 29.7 cm

EUR – Scientific and Technical Research series – ISSN 1831-9424 (online), ISSN 1018-5593 (print)

ISBN 978-92-79-26642-3 (pdf)

ISBN 978-92-79-26643-0 (print)

doi: 10.2788/51497

Abstract

This report collects information on chemical analytical methods for the analysis of the new proposed priority substances (PS) of the European Water Framework Directive (WFD) and some existing PS for which the Environmental Quality Standards (EQS) have been changed under the first review of the PS list. First, analytical "standard" methods (ISO, CEN, US EPA) were searched. Then, the EU Member States (MS) were asked via the Chemical Monitoring and Emerging Pollutants (CMEP) expert group to provide validated "in-house methods" used as a national reference and to report their limits of detection (LODs) or quantification (LOQs). Finally, published literature articles were searched to get an overview of today's analytical performance.
The achieved method limits of quantification (LOQs) are compared with one third (1/3) of the EQS, mandatory for WFD compliance monitoring.

Very low annual average AA-EQS values in the picogram-per-liter (pg/l) concentration range have been set for several of the new proposed PS: For Cypermethrin 80 pg/l (8 pg/l for coastal salt waters), for Dichlorvos 60 pg/l in coastal waters, for Dicofol 32 pg/l in coastal waters, for 17-alpha-ethinylestradiol 35 pg/l (7 pg/l in coastal waters), for 17-beta-estradiol 80 pg/l in coastal waters, and for Heptachlor/Heptachlorepoxide 0.2 pg/l (10 fg/l in coastal waters). Dicofol and Heptachlor/Heptachlorepoxide, for which biota EQS have been set (biota EQS: 33 µg/kg, and 6.7 ng/kg, respectively), however, are intended to be analysed in biota.

Moreover, a very challenging water EQS has been set for the already existing PS Brominated Diphenylethers (BDEs) (49 femtogram-per-liter (fg/l), and 2.4 fg/l in coastal waters). However, it is intended that BDEs be analysed in biota (EQS: 8.5 ng/kg). In addition, the water EQS for Polyaromatic Hydrocarbons (PAHs) has been lowered to 0.17 ng/l, and a biota EQS of 2-10 µg/kg added, which is more easy to reach.

In general, it is very difficult to reach with currently available analytical instruments LOQs in the low pg/l concentration range. A possibility could be the use of gas chromatography (GC) with high resolution mass spectrometry (HRMS). This technique, however, is not generally available in normal water monitoring laboratories. Also in the field of liquid chromatography mass spectrometry (LC-MS), instruments with improved sensitivity have become available in the last years.

Moreover, lower LOQs can be achieved by extracting higher volumes of water (10-1000 liters). These large-volume techniques, however, are very work and time intensive, and very costly, and are therefore not useful for routine WFD compliance monitoring (analysis of one sample per month).

The most challenging substances proposed as new PS are: Cypermethrin (EQS: 80 pg/l, and 8 pg/l for coastal salt waters), Dichlorvos (EQS: 60 pg/l in coastal waters), 17-alpha-ethinylestradiol (EQS: 35 pg/l, and 7 pg/l in coastal waters), and 17-beta-estradiol (EQS: 0.4 ng/l, and 80 pg/l in coastal waters).

Dicofol, Dioxins and dioxin-like compounds, Heptachlor/Heptachlorepoxide, Hexabromocyclododecane (HBCDD), Perfluorooctane sulfonic acid (PFOS), and the BDEs are intended to be analysed in biota.

Minor analytical problems could be encountered for the following substances: Aclonifen (EQS: 0.12 µg/l, and 12 ng/l for coastal salt waters), Bifenox (EQS: 12 ng/l, and 1.2 ng/l for coastal waters), Cybutryne (=Irgarol) (EQS: 2.5 ng/l), Diclofenac (EQS: 0.10 µg/l, and 10 ng/l for coastal waters), Quinoxyfen (EQS: 0.15 µg/l, and 15 ng/l for coastal waters), and Terbutryn (EQS: 65 ng/l, and 6.5 ng/l for coastal waters).

LB-NA-25532-EN-N

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ISBN 978-92-79-26642-3

Publications Office