



EDC-WFD ILC Final report

FINAL MEETING

EDC 🛞 WFD





Berlin, 22th February 2023

Presentation overview

- Context and aim
- ILC Structure
- Participation
- Results and evaluations
- Conclusion



Context and aim

The interlaboratory comparison (ILC) was carried out within the framework of **the Project 18NRM01 EDC-WFD** "Metrology for monitoring endocrine disrupting compounds under the Water Framework Directive".

OBJECTIVE

to demonstrate the fitness for purpose of optimized and validated methods for estrogens measurements by MS-based and effect-based methods (e.g. $ER\alpha$ -Calux and A-YES), defining **performance characteristics** of the methods in terms of repeatability within laboratories and reproducibility.

Background and bounds

Definition of the ILC structure and timing

Suitable RMs (characteristic, number of units produced, transportation bounds, storage)

Preliminary recoinnaisance (EDC-WFD_ILC Preregistration form - questionnaire)

Available laboratories (analytical capabilities, LOQs, method implementation)

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Schedule

Time	Action
by 30 th September 2022	The ILC Organizer sends the Protocol to the laboratories
by 2 nd November 2022	The test materials sent to each involved laboratory
by 23 rd November 2022 → delayed 30 th November	The involved laboratories provided the results to ilc-empir@isprambiente.it
by 20 th December 2022 → delayed 30 th December 2022	A Preliminary statistical evaluation sent to the involved laboratories
by 14 th January 2023	Comments from the participants
17 th February 2023	The Final Report sent to the involved laboratories.
22 th February 2023	Plenary meeting for the presentation and discussion of the results



Laboratories and RMs

27 Laboratories pre-registered:

- 22 chemical measurements
- 10 EBMs

All the laboratories received the RMs (kit)

Some labs able to carry out both chemical measurements (more instrumental techniques) and EBMs

> Some labs did not returned reporting templates (technical/analytical troubles, units arrived broken, not confident results, method not well implemented, LOQs too high)



Laboratories – CHEM





ARPA SICILIA - Agenzia regionale per la protezione Ambientale (Italy)

ARPA LOMBARDIA - Agenzia regionale per la protezione Ambientale (Italy)

BAM -Bundesanstalt für Materialforschung und -prüfung (Germany)

EAEF - Eurofins analyses pour l'environnement (France)

Ecotox Centre (Switzerland)

Université de Bordeaux, EPOC UMR5805 Equipe LPTC (France)

Eurofins Environment Testing Finland Oy (Finland)

SYKE-Finnish Environment Institute (Finland)

Institut für Energie- und Umwelttechnik e.V. (IUTA) (Germany)

JSI-Jožef Stefan Institute (Slovenia)

Laboratoire de l'Environnement et de l'Alimentation de la Vendée (France)

LANUV NRW-Landesamt für Natur, Umwelt und Verbraucherschutz (Germany)

LDAR 24-Laboratoire Départemental d'Analyses et de Recherche (France)

LNE- Laboratoire National de Métrologie et d'Essais (France)





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Laboratories - EBMs

BioDetection Systems BV (The Netherlands)

Ecotox Centre (Switzerland)

Federal Institute of Hydrology (Germany)

ISPRA-Centro Nazionale per la Rete nazionale dei laboratori-Area Metrologia (Italy

ISSeP - Institut Scientifique de Service Public (Belgium)

IWW Water Center (Germany)

QuoData GmbH (Germany)

Ruhrverband – Kooperationslabor (Gerrmany)

Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft (Germany)



EDC

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VFD







Test materials

- 4 bottles of natural water (2 bottles for each test material, 1L each);
- Ampoules of SPM (suspended particulate matter) and DOC (Dissolved Organic Carbon);
- 2 Vials (EDC-WFD_C1 and EDC-WFD_C2, for chemical measurements; EDC-WFD_B1 and EDC-WFD_B2, for EBMs) containing, at different concentration, 3 mL of the standard of the targeted substances in methanol

QC unit (3 mL target estrogens mixture in methanol) for chemical measurements *Blank unit* for chemical measurement and EBMs

RM reconstitution

The RMs should be **reconstituted**, mixing all the constituents together to the desired whole water sample with a distinct DOC, estrogen content and SPM load (Instruction given into the Protocol).



RMs composition - Chemical methods

EDC-WFD C1	,	SPM mg/L	DOC mg/L	EDC-WFD C2	Medium Concentration Range ng/L	SPM mg/L	DOC mg/L
17βΕ2	0,1-0,5			17βE2	0,5-2,5		
17αEE2	0,02-0,1			17αEE2	0,1-0,5		
E1	0,2-1,0	10	2	E1	0,5-2,5	50	5
17αE2	0,1-0,5			17αE2	0,5-2,5		
E3	0,2-1,0			E3	0,5-2,5		

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RMs composition - EBMs

EDC-WFD B1	Low EEQ Range ng/L βE2 eq	SPM mg/L	DOC mg/L	EDC-WFD B2	Medium EEQ Range ng/L βE2 eq	SPM mg/L	DOC mg/L
Cumulative effect	0,1-1,0	10	2	Cumulative effect	0,5 -5,0	50	5

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Reference estrogens concentration

EDC-WFD C1/B1	Low Concentration ng/L	EDC-WFD C2/B2	Medium Concentration ng/L
17βE2	0,22	17βE2	1,01
17αEE2	0,035	17αEE2	0,210
E1	0.40	E1	1,01
17αE2	0,20	17αE2	1,02
E3	0,41	E3	1,01

Gravimetric determination



Measurements: Chemical methods and EBMs

The RMs reconstituted were analysed in repeatability conditions in **duplicate** (independent measurements).

The overall number of samples for each kit to be analysed was four (4)

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Chemical Measurements (1)

<u>mandatory</u>: Measurements of the proposed three WFD priority substances:17-beta-estradiol ($17\beta E2$), 17-alpha-ethinylestradiol ($17\alpha EE2$), and estrone (E1).

strongly recommended: Measurement of 17-alpha-estradiol (**17**α**E2**) and estriol (**E3**).

Measurements by GC-MS-MS, LC-MS-MS, GC-HRMS and LC-HRMS.



Chemical Measurements (2)

Pre-treatment steps were:

- i) <u>mandatory</u>: a stable isotope dilution method (i.e. isotopically labeled internal standard for each estrogen);
- ii) <u>mandatory</u>: sample preparation technique based on SPE cartridge or SPEdisk (C18, polymeric phase HLB, DVB, polymeric reversed phase);
- iii) <u>strongly recommended</u>: purification step (LC-NH₂, SiOH, Florisil or selective SPE cartridges for estrogens).

Effective Based Methods

<u>The application of ER α -Calux and/or A-YES was preferred</u>, other bioassays were optional.

Pre-treatment steps were:

- i) <u>mandatory</u>: Sample preparation technique based on SPE cartridge or SPE disk (C18, polymeric phase (HLB, DVB, polymeric reversed phase);
- **ii)** <u>strongly recommended</u>: Purification step (LC-NH2, SiOH or selective SPE cartridges for estrogens).

FD

Laboratories partecipating

CHEMICAL METHODS

20 Laboratories 💻

22 templates sent

22nd February 2023

15 labs returned 17 templates



EBMs

10 Laboratories ———— 10 templates sent

9 labs returned 9 templates





Data treatment & Statistical evaluation (1)

Last Reporting template delivered (as revision) on the 7th December

Ad-hoc Data-base were implemented for Chemical methods and EBMs

Verifying of the data (units, gross errors) and < LOQ values not considered.

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EDC

	Measurand	17	β-E2	17α-	EE2		E1	17	α-Ε2	E3	
		1	ig/L	ng	L	r	ıg/L		ng/L	ng/l	-
Lab code	Unit	value	expanded uncertainty	value	expanded uncertainty	value	expanded uncertainty	value	expanded uncertainty	value	expanded uncertainty
	unit 1	<0.3	_	< 0.03	_	<0.3	_	_	_	_	_
1	unit 2	<0.3		< 0.03		<0.3					
	Average value										
	unit 1	0,281		0,029		0,288		0,310			
2	unit 2	0,265		0,031		0,264		0,262			
	Average value	0,273	0,137	0,030	0,015	0,276	0,138	0,286	0,143		
	unit 1	0,252	0,091	0,021	0,007	0,441	0,163				
3	unit 2	0,290	0,104	0,016	0,005	0,442	0,164				
	Average value	0,271	0,098	0,019	0,006	0,442	0,163				
	unit 1	0,198	0,032	0,033	0,009	0,354	0,032	0,196	0,021	0,377	0,038
7-GC	unit 2	0,192	0,032	0,029	0,009	0,331	0,032	0,187	0,021	0,376	0,038
	Average value	0,195	0,032	0,031	0,009	0,343	0,032	0,192	0,021	0,377	0,038
	unit 1	0,210	0,012	0,029	0,007	0,375	0,021	0,197	0,011	0,389	0,019
7-LC	unit 2	0,202	0,012	0,031	0,007	0,355	0,021	0,205	0,011	0,392	0,019
	Average value	0,206	0,012	0,030	0,007	0,365	0,021	0,201	0,011	0,391	0,019
	unit 1	(<loq)< td=""><td>9,0</td><td>(<loq)< td=""><td>9,0</td><td>(<loq)< td=""><td>9,0</td><td></td><td></td><td></td><td></td></loq)<></td></loq)<></td></loq)<>	9,0	(<loq)< td=""><td>9,0</td><td>(<loq)< td=""><td>9,0</td><td></td><td></td><td></td><td></td></loq)<></td></loq)<>	9,0	(<loq)< td=""><td>9,0</td><td></td><td></td><td></td><td></td></loq)<>	9,0				
8	unit 2	(<loq)< td=""><td>9,0</td><td>(<loq)< td=""><td>9,0</td><td>(<loq)< td=""><td>9,0</td><td></td><td></td><td></td><td></td></loq)<></td></loq)<></td></loq)<>	9,0	(<loq)< td=""><td>9,0</td><td>(<loq)< td=""><td>9,0</td><td></td><td></td><td></td><td></td></loq)<></td></loq)<>	9,0	(<loq)< td=""><td>9,0</td><td></td><td></td><td></td><td></td></loq)<>	9,0				
	Average value	(<loq)< td=""><td>9,0</td><td>(<loq)< td=""><td>9,0</td><td>(<loq)< td=""><td>9,0</td><td></td><td></td><td></td><td></td></loq)<></td></loq)<></td></loq)<>	9,0	(<loq)< td=""><td>9,0</td><td>(<loq)< td=""><td>9,0</td><td></td><td></td><td></td><td></td></loq)<></td></loq)<>	9,0	(<loq)< td=""><td>9,0</td><td></td><td></td><td></td><td></td></loq)<>	9,0				
	unit 1	0,23		0,046		0,37				0,37	
9	unit 2	0,25		0,051		0,43				0,36	
	Average value	0,24		0,048		0,40				0,37	
	unit 1	0,28		0,05		0,45		0,25		0,54	
10	unit 2	0,25		0,04		0,39		0,21		0,49	
	Average value	0,26		0,04		0,42		0,23		0,51	
	unit 1	<0,4		0,059		0,467				<2	
11	unit 2	<0,4		0,049		0,445				<2	
	Average value										
	unit 1	0,230		0,030		0,410		0,230		0,410	
13	unit 2	0,230		0,030		0,391		0,210		0,400	
	Average value	0,230		0,030		0,400		0,220		0,405	
	unit 1	0,28	0,03	0,02	0,01	0,34	0,03	0,25	0,02	0,45	0,08
15-GC	unit 2	0,32	0,03	<0,013		0,39	0,04	0,26	0,02	0,57	0,10
	Average value	0,30	0,03	0,02	0,01	0,36	0,04	0,25	0,02	0,51	0,09
	unit 1	0,1	0,02	0,02	0,004	0,3	0,04	0,1	0,01		
15_LC	unit 2	0,1	0,02	0,02	0,004	0,3	0,04	0,1	0,01		
	Average value	0,1	0,02	0,02	0,004	0,3	0,04	0,1	0,01		
	unit 1	0,3	0,2	<loq=1.0< td=""><td></td><td>0,5</td><td>0,2</td><td>0,2</td><td>0,09</td><td></td><td></td></loq=1.0<>		0,5	0,2	0,2	0,09		
19	unit 2	0,3	0,2	< LOQ = 1.0		0,4	0,2	0,2	0,09		
	Average value	0,3				0,4		0,2			
	unit 1	0,22	0,13	<0.1		1,35	0,20	0,18	0,11	0,77	0,29
20	unit 2	0,25	0,15	<0.1		1,40	0,21	0,22	0,13		
	Average value	0,24	0,14	<0.1		1,38	0,21	0,20	0,12	0,77	0,29
	unit 1	0,243		0,046		0,498		0,259		0,507	
21	unit 2	0,255		0,048		0,451		0,259		0,457	
	Average value	0,249		0,047		0,475		0,259		0,482	
	unit 1			< 0.2	0,1	0,4	0,2				
22	unit 2			< 0.2	0,1	0,4	0,2				
	Average value										
	unit 1	0,284	0,041	0,040	0,010	0,415	0,017	0,298	0,040	0,467	0,020
23	unit 2	0,233	0,041	0,036	0,010	0,413	0,017	0,324	0,040	0,465	0,020
	Average value	0,258	0,041	0,038	0,010	0,414	0,017	0,311	0,040	0,466	0,020

EDC-WFD C1

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Lab code unit ingl. <		Measurand	17	7β-Ε2	170	x-EE2		E1	17	α-Ε2		E3
Unit usize expanded uncertainty value uncertainty value value uncertainty value uncertainty <th>Lab code</th> <th></th> <th>1</th> <th>ng/L</th> <th>n</th> <th>g/L</th> <th></th> <th>ng/L</th> <th colspan="2">ng/L</th> <th></th> <th>ng/L</th>	Lab code		1	ng/L	n	g/L		ng/L	ng/L			ng/L
unit 1 1260 oursettaty		Unit	value	expanded	value	expanded	value	expanded	value	expanded	value	expanded
Unit 1 1,260 0,470 0,470 0,470 0,476 0,340 Image for the state of the s			value	uncertainty	Value	uncertainty	vulue	uncertainty	value	uncertainty	Tuluc	uncertainty
1 Unit 2 1,27 0,470 0,680 0,889 0,550 - - - 2 unit 1 0,960 0,210 0,534 1,04 - - 2 unit 1 0,960 0,210 0,534 1,02 - - 4 verage value 1,05 0,53 0,200 0,010 0,594 1,02 - - 3 unit 1 1,161 0,418 0,167 0,052 1,085 0,401 - <		unit 1	1,260	0,470	0,172	0,060	0,786	0,340				
Average value Open Open Open Open Open Open Open 2 unit 2 1,14 0,90 0,534 1,04 0 0 3 unit 2 1,14 0,190 0,591 0,556 1,33 0,52 0 4 unit 1 1,161 0,449 0,177 0,052 1,835 0,401 0 0 4 unit 1 0,851 0,518 0,822 1,835 0,833 0 0 0,929 0,971 0,949 0,987 0,952 0,977 0,949 0,887 0,953 7-CC unit 1 0,997 0,951 0,818 0,952 0,871 0,949 0,887 0,953 7-CC unit 1 0,997 0,431 0,450 0,981 0,431 0,492 0,847 0,843 0,899 0,611 0,973 0,431 0,997 0,431 0,997 0,431 0,997 0,431 0,997 0,431 <td< th=""><th>1</th><th>unit 2</th><th>1,227</th><th>0,470</th><th>0,165</th><th>0,060</th><th>0,829</th><th>0,350</th><th></th><th></th><th></th><th></th></td<>	1	unit 2	1,227	0,470	0,165	0,060	0,829	0,350				
unit1 0.960 0.210 0.534 1.04 2 unit2 1.14 0.190 0.594 1.02 3 unit1 1.161 0.418 0.180 0.924 0.544 4 Verage value 1.246 0.448 0.167 0.052 1.085 0.401 4 verage value 1.246 0.443 0.187 0.051 0.878 0.052 0.877 0.049 0.887 0.053 7-GC unit1 0.887 0.054 0.177 0.051 0.877 0.049 0.887 0.051 7-GC unit1 0.897 0.041 0.192 0.981 0.883 0.952 0.873 0.049 0.887 0.051 0.977 0.047 7-GC unit1 0.410 0.291 0.045 0.973 0.043 1.002 0.051 0.977 0.047 7-GC unit1 0.4100 9.0 </th <th></th> <th>Average value</th> <th></th>		Average value										
2 unit 2 1,1 0,190 0,504 1,102 4 verage value 1,05 0,53 0,520 0,519 0,526 1,33 0,92 3 unit 1 1,161 0,449 0,167 0,052 0,848 0,401 4 verage value 1,246 0,449 0,167 0,052 1,035 0,283 0.401 7-GC unit 1 0,887 0,052 0,873 0,049 0,887 0,052 0,873 0,049 0,887 0,053 74.C unit 1 0,997 0,043 1,002 0,051 0,973 0,043 0,967 0,943 0,973 0,988 0,973 0,988 0,973 0,988 0,971 0,971 0,971 0,971 0,971 0,971 0,971 0,971 0,971 0,971 0,971 0,971 0,971 0,971 0,971 0,971 0,971 0,971 0,971		unit 1	0,960		0,210		0,534		1,04			
Average value 1,65 0,33 0,200 0,100 0,210 0,200 1,03 0,22 I 3 um1 2 1,246 0,449 0,167 0,052 1,085 0,041 I <t< th=""><th>2</th><th>unit 2</th><th>1,14</th><th></th><th>0,190</th><th></th><th>0,504</th><th></th><th>1,02</th><th></th><th></th><th></th></t<>	2	unit 2	1,14		0,190		0,504		1,02			
Junt 1 1,161 0,419 0,167 0,052 0,884 0,401 Average value 1,246 0,433 0,167 0,052 1,835 0,401 T-GC unf 1 0,887 0,054 0,177 0,051 0,872 0,052 0,873 0,049 0,987 0,953 Average value 0,882 0,814 0,173 0,051 0,887 0,052 0,873 0,049 0,987 0,953 Average value 0,882 0,814 0,173 0,045 0,978 0,043 1,002 0,011 0,977 0,047 Average value 0.986 0,411 0,270 0,947 0,943 1,000 0,061 0,957 0,047 Average value 0.980 (<1,00)		Average value	1,05	0,53	0,200	0,100	0,519	0,260	1,03	0,52		
3 unit 2 1,246 0,433 0,167 0,052 1,055 0,401		unit 1	1,161	0,418	0,168	0,052	0,984	0,364				
Average value 1,244 0,433 0,168 0,052 1,035 0,032 0,052 0,073 0,049 0,887 0,053 7-GC unit 1 0,876 0,054 0,177 0,051 0,887 0,052 0,875 0,049 0,887 0,053 Average value 0,887 0,055 0,876 0,044 0,055 0,883 0,892 0,875 0,049 0,887 0,053 unit 1 0.997 0,041 0,155 0,045 0,875 0,043 0,990 0,047 Average value 0.996 0,041 0,201 0,047 0,043 0,997 0,047 Average value (4,00) 9,0 (4,100) 9,0 (4,100) 9,0 (4,100) 9,0 (4,100) 9,0 (4,100) 9,0 (4,100) 9,0 (4,100) 9,0 (4,100) 9,0 (4,101) 1,11 1,14 unit 1 1,35 0,22 1,01 1,13 1,10 1,10	3	unit 2	1,246	0,449	0,167	0,052	1,085	0,401				
unit 1 0.87 0.047 0.047 0.047 0.047 0.047 0.047 0.047 0.047 0.047 0.047 0.049 0.887 0.049 0.887 0.053 Average value 0.882 0.054 0.887 0.051 0.887 0.051 0.877 0.049 0.881 0.053 unit 1 0.997 0.041 0.155 0.045 0.977 0.043 0.989 0.051 0.977 0.047 Average value 0.992 0.041 0.158 0.045 0.973 0.043 0.989 0.051 0.977 0.047 aunit 1 (1.00) 9.0 (<1.00) 9.0 (<1.00) 9.0 0.981 0.973 0.047 aunit 1 1.3 0.22 1.0 0.0 0.0 1.0 1.0 1.0 1.0 aunit 1 1.16 0.23 1.01 1.13 1.07 1.01 1.0 1.01 aunit 2 1.20 0.245		Average value	1,204	0,433	0,168	0,052	1,035	0,383				
7-GC unit 2 0.976 0.054 0.177 0.087 0.087 0.087 0.049 0.887 0.053 unit 1 0.997 0.041 0.182 0.085 0.967 0.043 1.002 0.051 0.887 0.047 rt.C unit 1 0.997 0.041 0.185 0.045 0.977 0.043 1.002 0.051 0.987 0.047 Average value 0.996 0.041 0.219 0.045 0.977 0.043 1.002 0.051 0.987 0.047 unit 1 (1.00) 9.0 (<1.00)		unit 1	0,887	0.054	0,187	0,051	0,879	0,052	0,873	0,049	0,897	0,053
Average value 0.882 0.084 0.045 0.085 0.0875 0.0475 0.047 0.047 r.LC unit 1 0.997 0.041 0.155 0.045 0.977 0.043 0.020 0.051 0.977 0.047 Average value 0.992 0.041 0.201 0.045 0.978 0.043 1.002 0.051 0.977 0.047 Average value 0.992 0.041 0.201 0.045 0.973 0.043 1.002 0.051 0.977 0.047 average value (4LOO) 9.0 (4LOO) 1.0 1.0 1.0 1.0<	7-GC	unit 2	0,876	0.054	0,177	0,051	0,887	0,052	0,877	0,049	0,887	0,053
unit1 0.997 0.041 0.201 0.045 0.977 0.047 0.947 0.047 Average value 0.992 0.041 0.201 0.045 0.978 0.043 0.980 0.051 0.977 0.047 Average value 0.992 0.041 0.198 0.045 0.973 0.043 1.000 0.061 0.973 0.047 Aurage value (4LOQ) 9.0 (1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 <th></th> <th>Average value</th> <th>0,882</th> <th>0.054</th> <th>0,182</th> <th>0,051</th> <th>0,883</th> <th>0,052</th> <th>0,875</th> <th>0,049</th> <th>0,892</th> <th>0,053</th>		Average value	0,882	0.054	0,182	0,051	0,883	0,052	0,875	0,049	0,892	0,053
74.C unit 2 0.986 0.041 0.045 0.978 0.043 0.998 0.051 0.987 0.047 Average value 0.992 0.041 0.198 0.045 0.978 0.043 1.00 0.061 0.973 0.047 B unit 1 (cl.CQ) 9.0 (cl		unit 1	0.997	0.041	0.195	0.045	0.967	0.043	1,002	0,051	0,977	0,047
Average value 0.952 0.041 0.188 0.045 0.973 0.043 1,000 0.061 0.973 0.043 8 unit 1 (ct.OQ) 9,0 (ct.OQ) 1,0 1,0 1,0 1,	7-LC	unit 2	0.986	0.041	0.201	0.045	0.978	0.043	0,998	0,051	0,967	0,047
a unit (cl.OQ) 9,0 (cl.OQ) 1,0 (cl.OQ) 1,0 (cl.OQ) 1,0 (cl.OQ) 1,0 (cl.OQ) 1,0 (cl.OQ) 1,0 1,0 1,0 1,0		Average value	0.992	0.041	0.198	0.045	0.973	0.043	1,000	0,061	0,973	0,047
8 unit 2 (<1.O2)		unit 1	(<loq)< th=""><th>9,0</th><th>(<loq)< th=""><th>9,0</th><th>(<loq)< th=""><th>9,0</th><th></th><th></th><th></th><th></th></loq)<></th></loq)<></th></loq)<>	9,0	(<loq)< th=""><th>9,0</th><th>(<loq)< th=""><th>9,0</th><th></th><th></th><th></th><th></th></loq)<></th></loq)<>	9,0	(<loq)< th=""><th>9,0</th><th></th><th></th><th></th><th></th></loq)<>	9,0				
Average value (4LOQ) 9,0 1,0 1,0 9 unit 1 1,3 0,22 1,0 1,0 1,1 Average value 1,3 0,22 1,03 1,13 1,07 10 unit 1 1,16 0,23 1,01 1,13 1,07 11 unit 2 1,20 0,25 1,07 1,15 1,14 11 unit 2 1,30 0,227 1,096	8	unit 2	(<loq)< th=""><th>9,0</th><th>(<loq)< th=""><th>9,0</th><th>(<loq)< th=""><th>9,0</th><th></th><th></th><th></th><th></th></loq)<></th></loq)<></th></loq)<>	9,0	(<loq)< th=""><th>9,0</th><th>(<loq)< th=""><th>9,0</th><th></th><th></th><th></th><th></th></loq)<></th></loq)<>	9,0	(<loq)< th=""><th>9,0</th><th></th><th></th><th></th><th></th></loq)<>	9,0				
9 unit 1 1,3 0,22 1,0 1,0 1,0 9 unit 2 1,3 0,22 1,0 1,1 1,1 10 unit 1 1,16 0,23 1,01 1,13 1,07 10 unit 2 1,20 0,25 1,07 1,15 1,14 11 unit 2 1,30 0,22 1,04 1,14 1,10 11 unit 2 1,30 0,25 1,07 1,15 1,14 11 unit 2 1,30 0,27 1,096 1 1 11 unit 1 1,20 0,197 1,14 1 1 0 13 unit 1 1,20 0,197 1,10 1,00 0,96 14 1,10 0,193 0,99 1,10 0,99 1,13 0,19 14 1,20 0,12 0,30 0,98 0,95 0,09 1,27 0,99 1,13 0,19 15-GC		Average value	(<loq)< th=""><th>9.0</th><th>(<loq)< th=""><th>9.0</th><th>(<loq)< th=""><th>9.0</th><th></th><th></th><th></th><th></th></loq)<></th></loq)<></th></loq)<>	9.0	(<loq)< th=""><th>9.0</th><th>(<loq)< th=""><th>9.0</th><th></th><th></th><th></th><th></th></loq)<></th></loq)<>	9.0	(<loq)< th=""><th>9.0</th><th></th><th></th><th></th><th></th></loq)<>	9.0				
9 unit 2 1,3 0,22 1,0 1,1 Average value 1,3 0,22 1,03 1,0 1,1 10 unit 1 1,16 0,23 1,01 1,13 1,07 10 unit 2 1,20 0,25 1,07 1,15 1,14 11 unit 1 1,367 0,245 1,144 1 1 11 unit 1 1,367 0,245 1,144 1 1 11 unit 1 1,30 0,277 1,096		unit 1	13		0.22		1.0				1.0	
Average value 1,3 0,22 1,03 1,03 1,03 unit 1 1,16 0,23 1,01 1,13 1,07 10 unit 2 1,20 0,25 1,01 1,13 1,07 Average value 1,18 0,24 1,04 1,14 1,10 unit 1 1,367 0,215 1,144 1 1 unit 1 1,30 0,277 1,096	9	unit 2	13		0.22		10				11	
International and the second		Average value	13		0.22		1.03				1.03	
10 unit 2 1.00 0.02 1.07 1.05 1.07 11 unit 1 1.36 0.25 1.07 1.15 1.14 11 unit 1 1.36 0.24 1.04 1.14 1.10 11 unit 2 1.30 0.245 1.144 1.14 1.16 11 unit 2 1.30 0.227 1.096 - - - 11 unit 2 1.30 0.237 1.096 - - - 13 unit 2 1.10 0.193 0.99 1.10 0.966 Average value 1.15 0.195 1.04 1.10 0.988 - 4 1.10 0.99 0.11 0.99 1.13 0.19 16-CC unit 2 1.00 0.09 26.00 0.961 0.09 1.01 0.988 170-0 Average value 1.16 0.10 0.08 0.11 0.11 0.11 <td< th=""><th></th><th>unit 1</th><th>1 16</th><th></th><th>0.23</th><th></th><th>1.01</th><th></th><th>1 1 3</th><th></th><th>1.07</th><th></th></td<>		unit 1	1 16		0.23		1.01		1 1 3		1.07	
Normal value 1,16 0,27 1,04 1,14 1,16 unit 1 1,367 0,215 1,144 1,14 1,10 unit 1 1,367 0,215 1,144 1,14 1,10 unit 1 1,340 0,227 1,096	10	unit 2	1,10		0.25		1.07		1,15		1.14	
Interference Interference<		Averarie value	1 10		0,23		1.04		1,13		1,14	
Int 1,30 0,213 1,144 Image of the second sec		unit 1	1,10		0,24		1.144		1,14		1,10	
Int Int <thint< th=""> <thint< th=""> <thint< th=""></thint<></thint<></thint<>	11	unit 9	1,307		0,213		1,144					
Unit 1 1,20 0,197 1,10 1,10 1,00 13 unit 2 1,10 0,197 1,10 1,10 0,096 Average value 1,15 0,195 1,04 1,10 0,986 unit 1 1,12 0,12 0,30 0,08 0,08 0,09 1,27 0,09 1,13 0,19 15-GC unit 1 1,12 0,10 0,08 26,00 0,96 0,10 0,93 0,07 <0,2 1,700 Average value 1,16 0,10 0,08 26,00 0,96 0,11 0,08 1,13 0,19 15-LC unit 1 0,1 0,02 0,02 0,004 0,7 0,1 0,1 0,01 0.1 15-LC unit 2 1,0 0,5 0,8 0,3 0,8 0,4 1,0 0,3 10 0,10 0,02 0,27 0,16 3,35 0,50 0,92 0,23 1,42 0,28			1,540		0,221		1,030					
13 unit 1 1,10 1,10 1,10 1,00 13 unit 2 1,10 0,197 1,10 1,10 1,00 Average value 1,15 0,155 1,04 1,10 0,96 unit 1 1,22 0,12 0,30 0,08 0,95 0,09 1,27 0,99 1,13 0,19 15-GC unit 1 1,20 0,12 0,20 0,08 0,95 0,09 1,27 0,99 1,13 0,19 15-GC unit 2 1,00 0,02 0,004 0,85 0,09 1,10 0,08 1,7,00 Average value 1,1 0,10 0,02 0,004 0,8 0,1 0,1 0,01 0.01 15-LC unit 1 1,1 0,1 0,02 0,02 0,004 0,8 0,1 0,01 0,01 0.01 0.01 0.01 0.01 0.01 0.01 0.01 0.01 0.01 0.01 0.03 0.03		Average value	4.00		0.407		4.40		4.40		4.00	
10 umit 1 1,10 0,133 0,193 1,10 0,56 4xerage value 1,15 0,153 1,14 1,10 0,58 0,99 1,10 0,58 unit 1 1,32 0,12 0,30 0,08 0,55 0,09 1,27 0,99 1,13 0,19 15-GC unit 2 1,00 0,09 <0,013 26,00 0,91 0,09 1,00 0,02 1,00 0,09 1,13 0,19 16-GC unit 2 1,16 0,10 0,92 0,004 0,8 0,11 0,11 0,01 -0.2 1,13 0,19 unit 1 0,1 0,02 0,02 0,004 0,7 0,1 0,1 0,01 - - 0,3 0,8 0,4 1,0 0,3 0,3 0,8 0,4 1,0 0,3 0,3 0,8 1,4 0,3 0,3 0,8 1,0 0,3 0,3 0,8 0,4 1,0 0,3 <td< th=""><th>12</th><th>unit 2</th><th>1,20</th><th></th><th>0,197</th><th></th><th>1,10</th><th></th><th>1,10</th><th></th><th>1,00</th><th></th></td<>	12	unit 2	1,20		0,197		1,10		1,10		1,00	
Average value 1,15 0,155 1,04 1,10 0.38 15-GC unit 1 1,22 0,12 0,30 0,85 0,99 1,27 0,99 1,13 0,19 15-GC unit 2 1,00 0,09 <0,013 26,00 0,96 0,10 0,93 0,07 <0.2 17,00 Average value 1,16 0,10 0,08 26,00 0,91 0,09 1,01 0,02 1,13 0,19 15-LC unit 1 0,1 0,02 0,02 0,004 0,7 0,1 0,1 0,01 19 unit 1 1,1 0,5 0,7 0,3 0,8 0,4 1,0 0,3 19 unit 2 1,0 0,5 0,7 0,3 0,8 0,4 1,0 0,3 20 unit 1 1,34 0,27 0,27 0,15 3,55 0,50 0,92 0,23 1,42 0,28 21 <	15	unit 2	1,10		0,193		0,99		1,10		0,96	
Is-GC unit 1 1.32 0.12 0.30 0.08 0.85 0.09 1.72 0.09 1.73 0.09 15-GC unit 2 1,00 0.09 <0.013 26,00 0.95 0.09 1.72 0.09 1.73 0.09 1.73 0.09 1.73 0.09 1.73 0.09 1.73 0.07 <0.2 17,00 Average value 1.16 0.10 0.08 26,00 0.91 0.09 1.10 0.08 1.13 0.19 Unit 1 0.1 0.02 0.02 0.004 0.8 0.1 0.1 0.01 0.1 Unit 2 0.1 0.02 0.02 0.004 0.7 0.1 0.1 0.01 0.3 19 unit 2 1.0 0.5 0.8 0.3 0.8 0.4 1.0 0.3 20 unit 1 1.34 0.27 0.27 0.16 3.35 0.50 0.92 0.23 1.42 0.28		Average value	1,15	0.40	0,195	0.00	1,04	0.00	1,10	0.00	0,98	0.40
19-6C unit 2 1,00 0,09 40,073 26,00 0,95 0,70 0,93 0,70 40,2 17,00 40.2 Average value 1,16 0,00 0,00 0,95 0,70 0,93 0,70 40,2 1,10 0,19 15-LC unit 1 0,1 0,02 0,02 0,004 0,7 0,1 0,1 0,01 . 16-LC unit 2 0,1 0,02 0,02 0,004 0,7 0,1 0,1 0,01 . 19 unit 1 1,1 0,5 0.00 0,7 0,1 0,1 0,01 . 19 unit 1 1,3 0,27 0,27 0,16 3,35 0,50 0,92 0,23 1,0 0,3 20 unit 1 1,34 0,27 0,27 0,16 3,35 0,50 0,92 0,23 1,42 0,28 20 unit 1 1,34 0,27 0,27 0,16 <th< th=""><th>45.00</th><th>unit 1</th><th>1,32</th><th>0,12</th><th>0,30</th><th>0,08</th><th>0,85</th><th>0,09</th><th>1,27</th><th>0,09</th><th>1,13</th><th>0,19</th></th<>	45.00	unit 1	1,32	0,12	0,30	0,08	0,85	0,09	1,27	0,09	1,13	0,19
Average value 1,16 0,10 0,08 25,00 0,91 0,09 1,10 0,08 1,13 0,19 15-LC unit 1 0,1 0,02 0,02 0,004 0,7 0,1 0,1 0,01 0,11	15-60		1,00	0,09	<0,013	26,00	0,96	0,10	0,93	0,07	<0,2	17,00
unit 1 0,1 0,02 0,02 0,04 0,8 0,1 0,01 0.1 0,01 </th <th></th> <th>Average value</th> <th>1,16</th> <th>0,10</th> <th>0,08</th> <th>26,00</th> <th>0,91</th> <th>0,09</th> <th>1,10</th> <th>0,08</th> <th>1,13</th> <th>0,19</th>		Average value	1,16	0,10	0,08	26,00	0,91	0,09	1,10	0,08	1,13	0,19
16-LC unit 2 0,1 0,02 0,04 0,7 0,1 0,1 0,1 0,1 0,1 Average value 0,1 0,02 0,004 0,7 0,1		unit 1	0,1	0,02	0,02	0,004	0,8	0,1	0,1	0,01		
Average value 0,1 0,02 0,02 0,04 0,7 0,1	15-LC	unit 2	0,1	0,02	0,02	0,004	0,7	0,1	0,1	0,01		
unit 1 1,1 0,5 0.8 0,3 0,8 0,4 1,0 0,3 19 unit 2 1,0 0,5 0.7 0,3 0,8 0,4 1,0 0,3 Average value 1,1 0 0,7 0,7 0,3 0,8 0,4 1,0 0,3 unit 1 1,34 0,27 0,27 0,16 3,35 0,50 0,92 0,23 1,42 0,28 unit 1 1,02 0,25 0,25 0,15 3,44 0,52 0,91 0,23 1,42 0,28 unit 1 1,025 0,255 0,255 1,033 0,922 0,91 0,23 1,42 0,28 unit 1 1,025 0,255 1,033 0,922 0,921 0,922 0,921 0,922 Average value 0,956 0,227 0,978 0,984 0,976 0.55 unit 1 1,5 0,8 0,2 0,1 0,9 0,55 0.68 <th></th> <th>Average value</th> <th>0,1</th> <th>0,02</th> <th>0,02</th> <th>0,004</th> <th>0,7</th> <th>0,1</th> <th>0,1</th> <th>0,01</th> <th></th> <th></th>		Average value	0,1	0,02	0,02	0,004	0,7	0,1	0,1	0,01		
19 unit 2 10 0,5 0,7 0,3 0,8 0,4 10 0,3 Average value 1,1 0,7 0,8 0,7 0,8 0,9 1,0 0,3 unit 1 1,34 0,27 0,27 0,16 3,35 0,50 0,92 0,23 1,0 20 unit 2 1,19 0,24 0,22 0,13 3,54 0,53 0,90 0,23 1,42 0,28 Average value 1,27 0,25 0,25 1,033 1,031 1,047 1,029 21 unit 1 1,025 0,255 1,033 1,047 1,029 21 unit 2 0,866 0,219 0,922 0,931 0,932 21 unit 1 1,5 0,8 0,2 0,1 0,9 0,55 0,924 0,936 22 unit 1 1,5 0,8 0,2 0,1 0,9 0,55 0 1 0,976 1		unit 1	1,1	0,5			0,8	0,3	0,8	0,4	1,0	0,3
Average value 1,1 0 0,7 0,8 1,0 unit 1 1,34 0,27 0,27 0,16 3,35 0,50 0,92 0,23 - unit 2 1,19 0,24 0,22 0,13 3,54 0,53 0,90 0,23 1,42 0,28 Average value 1,27 0,25 0,25 0,15 3,45 0,52 0,91 0,23 1,42 0,28 unit 1 1,025 0,25 0,15 3,45 0,52 0,91 0,23 1,42 0,28 unit 2 0,86 0,219 0,922 0,921 0,922 0,921 0,922 unit 2 0,86 0,219 0,922 0,921 0,922 0,922 0,922 0,922 0,922 0,922 0,922 0,922 0,922 0,922 0,922 0,922 0,922 0,922 0,922 0,924 0,922 0,922 0,924 0,922 0,924 0,925 0,924 0,925	19	unit 2	1,0	0,5			0,7	0,3	0,8	0,4	1,0	0,3
unit 1 1,34 0,27 0,27 0,16 3,35 0,50 0,92 0,23		Average value	1,1				0,7		0,8		1,0	
20 unit 2 1,19 0,24 0,22 0,13 3,54 0,53 0,90 0,23 1,42 0,28 Average value 1,27 0,25 0,25 0,15 3,45 0,52 0,91 0,23 1,42 0,28 unit 1 1,025 0,255 1,033 1,047 1,029 0,23 1,42 0,28 21 unit 2 0,886 0,219 0,922 0,921 0,922 0,922 Average value 0,956 0,227 0,978 0,984 0,976 0,976 22 unit 2 0,9 0,5 0,2 0,1 0,9 0,55 0,276 0,976 22 unit 2 0,9 0,5 0,2 0,1 0,9 0,55 0,076 0,076 23 unit 1 1,330 0,072 0,221 0,018 1,047 0,068 1,333 0,072 1,164 0,078 23 unit 1 1,330 0,072 0,		unit 1	1,34	0,27	0,27	0,16	3,35	0,50	0,92	0,23		
Average value 1,27 0,25 0,25 0,15 3,45 0,52 0,91 0,23 1,42 0,28 unit 1 1,025 0,235 1,033 1,047 1,029 1,029 unit 2 0,866 0,279 0,922 0,921 0,922 0,921 0,922 Average value 0,956 0,227 0,978 0,984 0,976 1 unit 1 1,5 0,8 0,2 0,1 0,9 0,5 0 1 0 0,5 0	20	unit 2	1,19	0,24	0,22	0,13	3,54	0,53	0,90	0,23	1,42	0,28
unit 1 1,025 0,235 1,033 1,047 1,029 unit 2 0,886 0,219 0,922 0,921 0,922 Average value 0,956 0,227 0,978 0,984 0,976 unit 1 1,5 0,8 0,2 0,1 0,9 0,5 0 unit 2 0,9 0,5 0,2 0,1 0,9 0,5 0 0 unit 2 0,9 0,5 0,2 0,1 0,9 0,5 0 0 unit 2 0,9 0,5 0,2 0,1 0,9 0,5 0 0 unit 1 1,330 0,072 0,201 0,018 1,047 0,068 1,308 0,072 1,164 0,078 unit 1 1,333 0,072 0,221 0,018 1,079 0,068 1,333 0,072 1,121 0,078		Average value	1,27	0,25	0,25	0,15	3,45	0,52	0,91	0,23	1,42	0,28
21 unit 2 0.866 0.219 0.922 0.921 0.922 Average value 0.956 0.277 0.978 0.984 0.976 0.976 22 unit 1 1,5 0,8 0,2 0,1 0,9 0,5 24 unit 2 0,9 0,5 0,2 0,1 0,9 0,5 4Verage value 0,9 0,5 0,2 0,1 0,9 0,5		unit 1	1,025		0,235		1,033		1,047		1,029	
Average value 0.956 0.277 0.978 0.984 0.976 unt1 1,5 0,8 0,2 0,1 0,9 0,5 - - - unt2 0,9 0,5 0,2 0,1 0,9 0,5 - - - Average value -	21	unit 2	0,886		0,219		0,922		0,921		0,922	
unit 1 1,5 0,8 0,2 0,1 0,9 0,5 22 unit 2 0,9 0,5 0,2 0,1 0,9 0,5 Average value		Average value	0,956		0,227		0,978		0,984		0,976	
unit 2 0,9 0,5 0,2 0,1 0,9 0,5 c c c Average value - <td< th=""><th></th><th>unit 1</th><th>1,5</th><th>0,8</th><th>0,2</th><th>0,1</th><th>0,9</th><th>0,5</th><th></th><th></th><th></th><th></th></td<>		unit 1	1,5	0,8	0,2	0,1	0,9	0,5				
Average value unit 1 1,330 0,072 0,230 0,018 1,047 0,068 1,308 0,072 1,164 0,078 23 unit 2 1,303 0,072 0,221 0,018 1,079 0,068 1,333 0,072 1,121 0,078	22	unit 2	0,9	0,5	0,2	0,1	0,9	0,5				
unit 1 1,330 0,072 0,230 0,018 1,047 0,068 1,308 0,072 1,164 0,078 23 unit 2 1,303 0,072 0,221 0,018 1,079 0,068 1,333 0,072 1,121 0,078 4 1,003 0,072 0,221 0,018 1,079 0,068 1,333 0,072 1,121 0,078		Average value										
23 unit 2 1,303 0,072 0,221 0,018 1,079 0,068 1,333 0,072 1,121 0,078		unit 1	1,330	0,072	0,230	0,018	1,047	0,068	1,308	0,072	1,164	0,078
	23	unit 2	1,303	0,072	0,221	0,018	1,079	0,068	1,333	0,072	1,121	0,078
Average value 1,316 0,072 0,225 0,018 1,063 0,068 1,320 0.072 1,142 0.078		Average value	1,316	0,072	0,225	0,018	1,063	0,068	1,320	0,072	1,142	0,078

EDC-WFD C2

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EDC-WFD – B1

	Measurand	Cumulative effec	
Lab code		nç	g/LβE2eq
	Unit	value	e xpande d unce rtainty
	unit 1	0,56	0,34
5	unit 2	0,51	0,31
	Average value	0,54	0,33
	unit 1	0,28	0,03
9	unit 2	0,34	0,03
	Average value	0,31	0,03
	unit 1	0,47	0,29
12	unit 2	0,61	0,40
	Average value	0,54	0,34
	unit 1	0,12	0,08
16	unit 2	0,15	0,09
	Average value	0,14	0,09
	unit 1	< LOQ	
17	unit 2	< LOQ	
	Average value	< LOQ	
	unit 1	0,006	0,006
18	unit 2	0,006	0,006
	Average value	0,006	0,006
	unit 1	0,129	0,052
24	unit 2	0,195	0,078
	Average value	0,162	
	unit 1	0,18	
25	unit 2	0,31	
	Average value	0,30	
	unit 1	0,206	
26	unit 2	0,226	
	Average value	0,216	

EDC-WFD – B2

	Measurand	Cumu	lative effect
Lab code		nç	J/LβE2eq
	Unit	value	expanded uncertainty
	unit 1	2,5	1,5
5	unit 2	2,4	1,4
	Average value	2,5	1,5
	unit 1	1,57	0,35
9	unit 2	1,70	0,17
	Average value	1,63	0,35
	unit 1	1,78	0,12
12	unit 2	1,48	0,24
	Average value	1,62	0,18
	unit 1	0,88	0,65
16	unit 2	0,70	0,51
	Average value	0,79	0,58
	unit 1	0,683	
17	unit 2	0,634	
	Average value	0,658	
	unit 1	0,233	0,022
18	unit 2	0,329	0,033
	Average value	0,289	0,028
	unit 1	0,629	0,25
24	unit 2	0,784	0,31
	Average value	0,706	
	unit 1	<lod< td=""><td></td></lod<>	
25	unit 2	3,7	
	Average value		
	unit 1	1,06	
26	unit 2	1,32	
	Average value	1,19	

EDC 🕲 WFD

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Data treatment & Statistical evaluation (2)

h and k Statistics (Mandel) according to ISO 5725-2:2019 were used to check the consistency of the data (graphical technique):

- the first (h) evaluates between-laboratory variability
- the second (k) evaluates within-laboratory variability

Examination of the h and k plots can indicate that specific laboratories exibit patterns of results that are markedly different from the others

The critical values of the h statistic depend on the number of laboratories participating in the study while those of the k statistic depend on the number of laboratories and the number of replicates performed by each laboratory

Data treatment & Statistical evaluation (3)

Grubbs's test was applied (iterative procedure) to identify "**straggler**" values (for test statistic > 5% or \leq 1% critical value) "**outliers**" (for test statistic > 1% critical value).

Outliers values were evaluated considering the statistic outcomes. **Single** laboratory results (not duplicates) were not considered

Cochran's test was applied for verifying the homogeneity of variance within laboratories

ED(













Data treatment & Statistical evaluation (4)

Chemical measurements

The performance characteristics of the measurement methods (**repeatability-S**_r and **reproducibility-S**_R) calculated according to ISO 5725-2:2019.

For each RM the mean value is calculated based on the laboratories' measurement results (the number of laboratories n and associated valid data considered for each parameter m (n/m).)

All the measurement results produced were pooled

EDC-WFD C1	S _r %	S _R %	Mean value * ng/L	Reference value ng/L
17-bE2	7,5	13,9	0,25 (12/24)	0,22
17-aEE2	10,5	36,9	0,034 (12/24)	0,035
E1	7,2	16,1	0,39 (14/28)	0,40
17-aE2	7,9	18,6	0,23 (10/20)	0,20
E3	7,7	14,8	0,44 (8/16)	0,41

* Between brackets number of labs and valid data (i.e 12/24)

EDC-WFD C2	S _r %	S _R %	Mean value * ng/L	Reference value ng/L
17-bE2	11,8	14,8	1,16 (15/30)	1,01
17-aEE2	5,9	12,8	0,21 (13/26)	0,21
E1	5,7	18,3	0,92 (15/30)	1,01
17-aE2	7,9	16,0	1,02 (10/20)	1,02
E3	4,7	8,6	1,01 <i>(</i> 8/16)	1,01

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Data treatment & Statistical evaluation (5)

Effective Based Methods

The performance characteristics of the measurement methods (**repeatability-S**_r and **reproducibility-S**_R) calculated according to ISO 5725-2:2019.

Considering the number of valid data for EBMs, not sufficient to give reliable S_r and S_R values, two different evaluation approach were carried out

Measurement results were grouped by the two main different biossays)Era-Calux and A-YES), providing only indicative S_r and S_R values if statistically possible

Measurement results obtained by different bioassays (ISO 19040-1, ISO 19040-2, ISO 19040-3 and p-YES) were pooled



	S _r %	S _R %	mean value ng/L βE2 eq
Cumulative effect	19,4	56,4	0,31 <i>(</i> 7 <i>/</i> 1 <i>4)</i>

* Between brackets number of labs and valid data (i.e 12/24)

EDC-WFD B2

	S _r %	S _R %	mean value ng/L βE2 eq
Cumulative effect	10,8	60,8	1,17 <i>(</i> 8/16)

* Between brackets number of labs and valid data (i.e 12/24)

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	Bioassay		Reproducibility (%)	Repeatability (%)	Mean value (*) ng/LβE2 eq
	<u>ERα-Calux</u>		61,8	11,1	0,33 (3-6)
EDC-MED BI	A-YES	Cumulative effect	29,1	29,0	0,21 (3-6)
EDC-WFD B2	<u>ERα-Calux</u>		74,2	7,3	1,29 (4- <i>8</i>)
	A-YES		37,8 (CV%)	16,0 (CV%)	0,95 (2-4)

* Between brackets number of labs and valid data (i.e 12/24)

Indicative values Insufficient valid data

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Chemical measurements and repeatability



EDC-WFD C1 17-bE2



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EBMs measurements and repeatability



EDC-WFD B1 Cumulative effect





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Lab code	Extraction technique	Extraction sorbent	Purification sorbent	Instrumental analysis technique	NOTE/COMMENTS:
1	SPE offline	Polymeric phase-HLB		LC-HRMS	
2	SPE offline	Polymeric phase-HLB		LC-MS/MS	
3	SPE offline	Polymeric phase-HLB	SPE online su C18	LC-MS/MS	DOC spiking solution added it to the samples without filtering
7-GC	Speedisk	Polymeric phase-HLB	MiSPE	GC-MS/MS	
7-LC	Speedisk	Polymeric phase-HLB	MISPE	LC-MS/MS	
8	SPE offline	Polymeric phase-HLB	LC-NH2	LC-MS/MS	
9	SPE offline	Polymeric reversed phase		LC-MS/MS	Agilent 1290 Infinity II - Agilent G6495A Triple Quadrupole (QQQ)
10	SPE offline	Polymeric phase-HLB	LC-NH2	LC-MS/MS	2 analytical methologies LC/MSMS. First one to analyse 17 E2, 17βE2, E1, E3, 17 E2-d2, 17βE2-13C3, E1-13C3 and E3- d2; organic extract condionned in 100% MeOH with 5 L injected. The second one, more specific, to analyse 17 EE2 and 17 EE2-d4; organic extract condionned in 76/30 MQ water / MeOH/(v/v) with 100 L injected.
11	SPE offline			LC-MS/MS	
13	Speedisk	C18 phase	LC-NH2	LC-MS/MS	
15-GC	Speedisk	C18 phase	SiOH	GC-MS/MS	
15-LC	Speedisk	C18 phase	SiOH	LC-MS/MS	
19	Speedisk	Polymeric phase-DVB		GC-MS/MS	
20	SPE offline	Polymeric phase-HLB		LC-MS/MS	
21	SPE offline	Polymeric phase-HLB	SiOH	LC-MS/MS	Enrichment Factor: 1000
22	Extraction liquide / liquide - acétylation			GC-MS/MS	Standard extracted under the same conditions as the samples
23	Speedisk	C18 phase	LC-NH2	LC-MS/MS	



Lab code	Extraction technique	Extraction sorbent	Specify Other	Purification sorbent	Bloassay	Data Treatment	NOTE/COMMENT\$:
5	SPE offline	Polymeric phase-HLB		no ne	Era-Calux	PCx model. Effectile veliat 5% 3 Parameters Logistic Function	
9	\$PE on¶iine	Polymeric reversed phase	Strata XL (Phenomenex)	no ne	Era-Calux	PCx model. Effectievel at 10%. 4 Parameters Log Logistic Function.	For further information on data treatment see a liso Simon et al (2022) Evaluation of Three I SD Estrogen Receptor Tan mae citivation Assays Applied to 52 Donn etic Effluent Samples. Environmental Toxicology and Chemistry - Volume 41, Number 10 - pp. 2512–252 as a well as international Organization for Standardization. (2018c) I SO 19640-3 Wa Ber quality—Determination of the estrogenic potential of water and wate water—Dart 2: in vitro human cell-based reporter gene assay and international Organization for 58 notacrdization. (2022) I SO 20156 Wa Ber quality—Calcutation of biological e quivalence (BEG) concentrations.
12	SPE offiline	Polymeric phase-HLB		no ne	P-YE\$	Reverse Function Model (for Individual estrogen): 5 Parameters Logistic Function.	p-YE3 allows in a ddition to the effect based analysis the quantification of estrogenic compounds such as E1, E2, bE2 and E3. REPs were determined by quantification of compound signals against the E2-Calibration to express the signal as E2-Equivalence. The REPs are the quotient of the calculated E2-Equivalence divided by the amount of the compound exposed. Quantifications for individual compounds (mean value with n=3 +/- C1 55%) E1, bebE2 and EE2 are reported in ng/l, alpha-E2 is reported in the bat-22 equivalence come intation in ng/l. EDC-WFD-B1:: beta-E2: 0.27 +0.17; EE2: 0.05 +/- 0.05; E1: 0.37 +/- 0.34; alpha-E2: 0.01 +/- 0.02 EDC-WFD-B2:: beta-E2: 0.27 +/- 0.35; EE2: 0.27 +/- 0.03; E1: 0.34; -0.03; alpha-E2: 0.01 +/- 0.06 EDC-WFD-B2:: beta-E2: 0.37 +/- 0.35; EE2: 0.27 +/- 0.05; E1: 0.37 +/- 0.55; alpha-E2: 0.01 +/- 0.06
16	Speedlak	Polymeric phase-HLB		MISPE	Era-Calux	PCx model. Effectievel at 10% 4 Parameters Logistic Function	
17	SPE offline	Polymeric reversed phase	Polystyre ned iviny i benze ne	no ne	YE \$	4 Parameters Logistic Function. Effect le vel not specifie d	For B2 (unit 1 and unit 2) we didn't have enough DOC spiking solution. So instead of adding 4540 µl to the sample we added only 3500 µl.
18	SPE offline	Polymeric phase-HLB		no ne	Era-Calux	According to BDS calculation sheet 4 Parameters Logistic Function.	EDC-WFD B2, unit 2: only 0,72 L Instead of 1 L could be extracted via \$PE due to clogging of the catridge.
24	SPE offine	C18 phase			A-YE\$	BIOVAL© software (new_diagnostics GmbH), calculations according to ISO 19040- 2:2018: 4 Parameters Logistic Function. Effect level at 20%	Enrichment factor for each sample was 250.
25	SPE offine	Polymeric reveræd phase		no ne	A-YES	BioVAL© software (new_diagnostics GmbH), calculations according to ISO 15040- 2:2018. 4 Parameters Logistic Function.	Due to an error in sample preparation, the sample composition to be set for the test of sample B1, unit 2, was diluted with drinking water. Sample B2 unit 1 did not re-discove well after SPE.
26	SPE offine	C18 phase		no ne	A-YE\$	BIOVAL© software (new_dlagnostics GmbH), calculations according to ISO 19040- 2:2018. 4 Parameters Logistic	After SPE with C18 phase, extracts are dried in N2 stream and then dissolved in DMSO. The DMSO extract is diluted with H2O for the A-YES assay to a final DMSO percentage of 5%. Method recenty established; clean-up step for the extracts was not possible.

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EDC-WFD C1/C2

Including labs not considered in the statistics

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EDC-WFD B1/B2











C2

EDC 🕑 WFD





C1

C2

EDC 🕲 WFD



B1

B2

EDC 🛞 WFD

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Some considerations (1)

- Very good agreement between the reference concentration of 5 estrogens in the RMs, gravimetrically determined, and the mean values calculated based on the laboratories chemical measurement results.
- Good application of the measurement procedures by the labs within the ILC context, including also the demanding reconstitution step.



Some considerations (2)

- General good performance characteristics are observed for both level of concentration.
- Repeatability within the laboratories below 12 %, while reproducibility values fall below 19%; the only exception is 17 α EE2 in EDC-WFD C1 (reproducibility around 37%) probably due to the lower and challenging concentration of 17 α EE2 (0,035 ng/L)
- Not possibile to separate performance characteristic by technical instrument

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Some considerations (3)

The evaluation of performance characteristics for EBMs is conditioned by the low number of labs and valid data.

Outputs interesting, considering the characteristic of the RMs, mimicking real samples:

- Concentration significantly lower than those tested in ILC reported in ISO 19040-2 (A-YES) and ISO 19040-3 (ERα-Calux): the lowest concentrations in ISO were respectively 6,4 ng/L βE2 eq and 1,7 ng/L βE2 eq.
- the reduced number of laboratories and valid data for each bioassay may provide only an indicative information on repeatability and reproducibility

Some considerations (4)

Different data treatment used by the $ER\alpha$ -Calux laboratories (e.g. different fitting and effect levels in percentage) might partially justify the higher variability observed in EBMs measurement results.

A-YES laboratories, applying the same data evaluation (BIOVAL), are characterized by lower variability.

EDC-WFD ILC data (S_r and S_R) could also be compared with values observed in ISO 19040-2 (A-YES) and ISO 19040-3 (Calux);

- 20-25 valid data, S_r =7,5-21,4% and S_R =16,6-28,0%.
- 22-25 valid data, $S_r = 13-18\%$, $S_R = 18-33\%$.

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Some considerations (5)

Labs applied in general the indication given by the ILC protocol. SPE-Offline extraction phase was used with higher frequency together with Polymeric-phase HLB as sorbent. Higher percentage of labs used LC-MS/MS.

In EBMs only one laboratory carried out a purification of the extract

Conclusion (1)

Laboratories able to apply the demanding reconstitution step.

RMs tested fit for purpose in EDC-WFD ILC and in future experiences

EDC-WFD ILC show good application and performance characteristics of the tested methods for chemical measurements, facing with low concentration of the selected estrogens, close to the laboratories LOQ, with variable content of the interferents



Conclusion (2)

For EBMs, the low number of laboratories in EDC-WFD ILC suggests the need of new interlaboratory trials characterized by:

- challenging samples with low concentration of estrogenic substances;
- higher number of laboratories (public and private), coming from different European countries



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Thank you for the participation and for your attention



