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PROJECT FINAL REPORT

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Project acronym: RADAR

Project title: Rationally Designed Aquatic Receptors integrated in label-free biosensor platforms for remote surveillance of toxins and pollutants

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4.1 Final publishable summary report

4.1.1 Executive summary

RADAR is a 7-member consortium that has developed in the past four years a robust, sensitive, and versatile biochemical sensor platform for spot measurements and on-line monitoring of toxins and pollutants, with a focus on Endocrine Disrupting Compounds (EDCs) and Polycyclic Aromatic Hydrocarbons (PAHs), in food production processes and in the aquatic environment.

One originality of the project was the design and production of aquatic organisms derived receptors i.e. proteins, derived from aquatic organisms, that recognize and respond to a specific class of pollutants and toxins. These receptors were designed on the basis of the in-vivo occurring receptors affected by the presence of the class of pollutants targeted. The targeted receptors, namely the Estrogen Receptor (ER) and the Aryl Hydrocarbon Receptor (AhR), are sensitive not only to a single toxic molecule, but to an entire class of potentially hazardous molecules. To the best of our knowledge, RADAR has produced the first

- Aryl Hydrocarbon Receptor from aquatic organisms with highest affinity towards Polycyclic Aromatic Hydrocarbons (PAHs), e.g. epigallocatechin gallate, indole 3,2 b-carbazole (I32bC), or 6-formylindole-3,2 b-carbazole (FICZ).
- Estrogen Receptor and genetically engineered mutants from aquatic organisms with an increase of their specificity (from uM to nM IC50), sensitivity (more than a factor of 5x) and robustness (shelf life longer than 9 months at -20C).

Another originality of the project was the unique combination of an automated filtration/ separation/ pre-concentration module with a novel detection module. The resulting platform has allowed attaining unsurpassed sensitivity in a continuous label-free monitoring platform towards toxins and pollutants, allowing for early detection of class-specific compounds thanks to

- The pre-concentration factor of up to 1000x as achieved for E2 (17-β estradiol) in the automated filtration/separation/pre-concentration module in fresh and marine water samples.
- The integrated compact detection module based on a combination of WaveGuide Grating (WGG) biochips and the ARGOS reader. This module has reached comparable performance to best-in-class commercial label-free platform at a fraction of the cost.
- The novel assay format based on peptides specific to ER yielding conformational change upon ligand binding. Amplification of the signal lowered the limit of quantification of ER-ligand complexes down to the low ppb level (working range between1-10 ppb). For 17β-estradiol (E2), the LOD is 5 ppt with 200 times pre-concentration.

Finally, to the best of our knowledge, when combined with its wireless communication module, the RADAR platform is the first automated compact platform designed

- to perform label-free, robust, specific and sensitive detection of toxins and pollutants, and
- to send an alarm signal to a remote control station.

Several dozens of samples ranging from fresh to marine water originating from various locations such as sludge tank, input and output filters of fish tanks, sewage, were successfully processed and measured (with positive samples for E2 found in fish farm at the filter output and in the sludge tank), proving the versatility of the module for different real-life applications and proving the validity of the method and the platform for the detection of EDCs in food production processes and in the aquatic environment.

4.1.2 Summary description of project context and objectives

RADAR stands for Rationally Designed Aquatic Receptors integrated in label-free biosensor platforms for remote surveillance of toxins and pollutants.

Project context

Environmental monitoring is becoming increasingly important as the number of compounds in use continues to increase. Within the last ten years, some of the 85,000 chemicals just in the United States have been found to coincidentally have hormonal effects, where the most common effect is to mimic estrogen (e.g. alkylphenols). Although the European Community has achieved major strides in environmental performance during the last two decades, threats of environmental damage and depletion still exist. It is estimated that more than 150,000 contaminated sites exist in the EU.

Furthermore, increased intensity of water use, discharge of untreated domestic and industrial wastes, excessive application of fertilisers, pesticides and insecticides in agriculture, and accidental spills of harmful substances have led to increased pollution of water-bodies throughout Europe. The importance of monitoring activities at polluted sites has gained attention from national authorities due to increased evidence of contamination from past improper waste management, industrial, and agricultural practices. Today, both, monitoring and characterization activities require water and food samples to be taken on-site and then analyzed at a laboratory using conventional analytical methods such as LC-MS, which is costly and time consuming.

Developing timely and cost-effective monitoring and characterisation technologies is critical, both to reduce costs to the European economy in addressing contamination problems, and to promote European competitiveness in global environmental and food markets. RADAR novel technology can contribute to the health and safety of the European population by reducing risks associated with contaminants in the environment and food.

Project objectives

The RADAR project aimed at developing biosensor platforms for the monitoring of toxins and pollutants in the environment and for the surveillance of production processes using aquatic organisms' derived biological recognition elements. To achieve the goals, the RADAR project integrated various state-of-the-art technologies that advanced biosensors beyond the current state-of-the-art. It implemented sensitive, specific, and versatile biological recognition elements based on recombinant receptors derived from aquatic organisms on robust, label-free, multiplexed, remotely-controlled, and portable biosensor platforms with an integrated automated in-line sample preparation unit.

In this context, the RADAR consortium defined the project's main objectives as:

Objective 1: To increase the sensitivity, specificity and versatility of biosensors using nanostructured surfaces and genetically engineered recombinant bio-receptors derived from aquatic organisms.

Objective 2: To provide a robust, label-free, remotely-controlled, and portable biosensor platform for cost-effective spot measurements and on-line monitoring with integrated fully automated sample preparation for non-experts.

Objective 3: To validate the RADAR biosensor and demonstrate its application for cost-effective spot measurements and on-line monitoring of toxins and pollutants in food processes and in the aquatic environment.

Objective 1: Increase the sensitivity, specificity and versatility of biosensors

Aquatic organisms have proteins capable of binding endocrine disruptive compounds (EDCs) such as estrogenic substances, polychlorinated compounds, polycylic aromatic hydrocarbons, steroids, pesticides, etc. Such proteins are ideal candidates as biological recognition elements for biosensors, since they will bind any EDC present in the analyzed sample. Rather than selecting a single protein and use it as is as biosensor, RADAR identified the particular structural and functional features required to bind EDCs through the use of genetic, structural and functional information, complemented by computational modelling tools and mutational experiments.

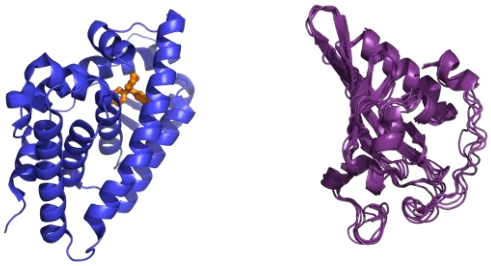

Figure 1: Simulation of the receptors. Three-dimensional structures of the estrogen receptor (blue, left) and aryl hydrocarbon receptor (purple, right). A chemical pollutant is shown in orange inside the estrogen receptor. Only the region of the protein responsible for binding of hormones and pollutants is shown.

RADAR succeeded in the design, production, purification, and testing of the Estrogen Receptor (ER) and the Aryl Hydrocarbon Receptor (AhR), (Figure 1). Both receptors have been successfully produced in E.Coli, a low cost bacterial system with minimal requirements in terms of safety and containment of manufacturing processes. RADAR also succeeded in the production of rationally designed mutated receptors for the Estrogen receptor. Protein mutants have been designed by structural and genetic analysis with the intent of increasing the specificity and selectivity of the receptors so that they could be used to recognize and differentiate different classes of pollutants (Table 1).

	IC_{50}		
COMPOUNDS	ER_wt	ER_M421F	ER_M421I
EE2	12 nM ± 6	2 nM ± 1	90 nM ± 21
E2	16 nM ± 4	7 nM ± 2	191 nM ± 47
BPA	12 µM ± 2	2 µM ± 1	100 µM ± 9
4NP	8 µM ± 1	4 µM ± 2	60 µM ± 29
4TOP	11 µM ± 1	10 µM ± 4	200 µM ± 61
TAM	47 nM ± 14	55 nM ± 40	835 nM ± 300

Table 1: IC50 values of the mutants ER_M421F, ER_M421I compared to the wild type estrogen receptors (ER_wt) IC50 values.

The characterization of expressed proteins was performed mainly in terms of i) purity, thorough SDS-PAGE; ii) structure stability, by means of Circular Dichroism Spectroscopy; and iii) ligand binding, using a fluorescence competitive binding assay. The in vitro testing and selection of best receptors by affinity binding measurements to selected toxins and pollutants was achieved, for ER, towards six selected compounds thanks to the commercially available PolarScreen™ ER Alpha Competitor Assay, Green.

In summary, RADAR has achieved the following results towards objective 1:

- Receptors for both Estrogen (ER) and Aryl Hydrocarbon (AhR) were successfully derived from aquatic organisms and genetically engineered to increase their specificity (from uM to

nM IC50), sensitivity (more than a factor of 5x) and robustness (shelf life longer than 9 months at -20C). Moreover, they were expressed and purified in quantities compatible for application testing during the last 12 months of the project.

- Nanostructured surface were implemented with enhanced sensitivity through higher activity of receptors at the surface. Detection of 17β-estradiol (E2) binding to ER and EPGC binding to AhR was detected with the WGG sensors by direct detection for EPGC binding and indirect detection by peptide enhancement for E2 binding.
- Generic binding of the receptors to the surface was achieved via amine coupling as well as Ni(II)-NTA surface chemistries on several 2D and 3D surfaces (Optodex C, XC200, Surfix, CMD50) showing the versatility of the biochips.

Objective 2: Provide a robust, label-free, remotely-controlled, and portable biosensor platform

This objective combines multiple objectives providing a best-in-class biosensor platform for the versatile, specific and sensitive detection of toxins and pollutants:

1. *Optimized Label-Free detection with nanostructured, chemically modified biochip*

   For the label-free biosensor, RADAR used an evanescent field based sensors (waveguide grating: WGG), transparent and non-metallic (tantalum pentoxide) tuned for a penetration depth of the evanescent wave matching surface chemistry and assay. The optical biochip is read with a novel Angle Interrogating Optical Sensor (ARGOS) that provides best-in-class sensitivity while keeping the expected versatility and cost effectiveness of a platform instrument (Figure 2).

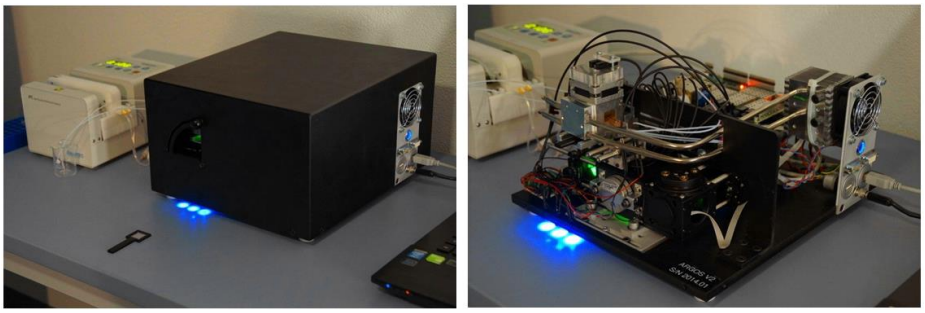

Figure 2: Actual pictures of one of the three ARGOS V2 reader platforms. Left with the chip holder (on the table), ready to be placed in the system for measurements.

   WGG sensors were nanostructured in order to achieve best-in-class specificity and sensitivity to EDCs and therefore to improve the performance of the platform compared to state of the art ones. The physico-chemical properties of the surface were engineered at the nanoscale to control the spatial distribution, density and conformation of immobilized bio-receptors. The surface nanostructuring had two effects: Bio-receptors activity improvement thanks to proteins confinement on the nanostructure & Detection sensitivity improvement by creating nano-grating enhancing the evanescent wave coupling.

2. *Easy to use sample preparation with integrated sample preparation unit (SPU)*

   The samples were collected and automatically prepared on a microfluidic device to increase the accuracy of the biosensors. The integrated sample preparation unit (SPU) had three functions, filtration/separation/pre-concentration integrated in an automated module for delivery to the ARGOS detection unit (Figure 3).

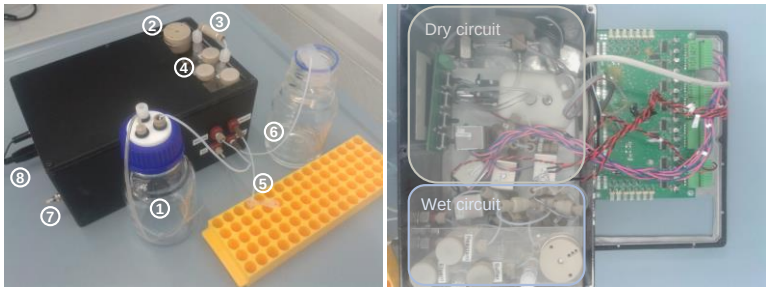

Figure 3: Overview of the sample preparation prototype: 1)Sample, 2) Degasser, 3) SPE column, 4) Reservoirs for eluent, dilution buffer and reactant, 5) Output to sensor, 6) Output to waste container, 7) Pressure regulator, 8) Connections to power supply and computer

3. Remotely controlled capability via Wireless Connectivity

Our biosensor was equipped with a wireless connectivity module to inform users as quickly as possible about any contamination. Mobile telephony (GSM) challenge was to allow operation on batteries for very long period of time while being able to send alarms within minutes. New protocols with wake-up functions were implemented to achieve ultra-low power function.

In summary, RADAR has achieved the following results towards objective 2:

- Thanks to an automated filtration/separation/pre-concentration module, a pre-concentration factor of up to 1000x was achieved for E2 (17β estradiol). The module was tested with fresh and marine water samples showing the versatility of the module next to its sensitivity.
- An integrated version of the detector was successfully designed, constructed and tested that, by performing angle scanning, can be applied as SPR and WGG readers. The sensor module showed superior performance than best-in-class commercial WGG systems. Label-free was achieved by design of the WaveGuide Grating (WGG) based sensor biochip and the ARGOS reader. Cost-effectiveness was achieved both at the biochip level (by developing and testing biological regeneration procedures) and at the instrument level (by developing a compact platform with COGs at a fraction of the cost of the competitive products).
- Several dozens of samples ranging from fresh to marine water originating from various locations such as sludge tank, input and output filters of fish tanks, sewage, were successfully filtered, separated, and pre-concentrated, proving the versatility of the module for different real-life applications. Sensitivity and specificity to E2 were achieved down to 5ppt proving the validity of the platform for monitoring food production processes and in the aquatic environment.

Objective 3: Validate the RADAR biosensor and demonstrate its application

To demonstrate the full potential and applicability of the developed RADAR platform, fresh and marine water, fish feeds, urine, were collected and measured for EDCs.

Sample No.	Material	SPR receptor test		GCMS		ELISA	
		Resp (RU)	ppt E2 (% recovery)	Ratio b-E2/E2-d3	ppt E2	EE2 ppt	E2 ppt
1 NL	Hydrolysed MilliQ	12	<5	0.01	<5	0.17	0.75
2	" + 50 ppt E2	424	41 (82)	0.77	33	0.28	37
3 NL	MilliQ	12	<5	0.01	<5	0.15	0.2
4	" + 50 ppt E2	347	35 (70)	0.83	36	0.38	50
5 SI	0035-9 Monitoring Station Sept	12	<5	0.01	<5	0.18	0.75
6	" + 50 ppt E2	384	38 (76)	0.85	37	0.37	40
7 SI	0035-11 Monitoring Station Nov	12	<5	0.00	<5	0.14	0.6
8	" + 50 ppt E2	378	37 (74)	0.82	35	0.31	38
9 SI	K1-B9 Sewage outlet (bottom) Sept	12	<5	0.00	<5	0.17	0.7
10	" + 50 ppt E2	294	30 (60)	0.98	42	0.36	32
11 SI	K1-O11 Sewage outlet (surface) Nov	15	<5	0.02	<5	0.23	1.4
12	" + 50 ppt E2	416	41 (82)	0.88	38	0.36	37
133 SI	K1-B11 Sewage outlet (bottom) Nov	14	<5	0.02	<5	0.15	0.8
14	" + 50 ppt E2	408	40 (80)	0.92	40	0.30	40

15*NL	MilliQ	15	<1	-	-	-	0.09
16*	" + 10 ppt E2	469	6.6 (66)	-	-	-	8.4
17* SI	0035, 20.11.14 Fish farm	11	<1	-	-	-	0.26
18* SI	0014 Estuary of the Rizana river	2	<1	-	-	-	0.32
19* SI	BOJ Buoy in the central part of the Gulf of Trieste	17	<1	-	-	-	0.32
20* SI	KCN River Rizana, below the outflow from the sewage treath plant	11	<1	-	-	-	0.34

*500 ml sample purified and concentrated by SPE in 100 ul methanol and 50 ul (equivalent to 250 ml samples) was used for analysis.
- not analysed.

Table 2. Results obtained with water samples taken in Slovenia. Of most of the samples, 200 ml were purified and concentrated by SPE in duplicate (blank and spiked with 50 ppt E2) and the residue dissolved in 200 µl methanol which was divided in 4 portions of 50 µl (50 ml of sample per portion). In the Biacore, 200 µl was added to the 50 µl methanol and this was mixed with receptor (1:1; v/v) of which 90 µl was injected.

In summary, RADAR has achieved the following results towards objective 3:

- Selected visits were organized with potential end-users and testing sites were identified for the project. Sample harvesting was accomplished by application partners to build a collection of samples that were subsequently analyzed.
- Several matrices (fresh and marine water, fish feeds, sewage) were successfully processed and measured, proving the versatility of the module for different real-life matrices.
- Most importantly, the platform was tested for several matrices for E2 (17β estradiol) allowing the identification of positive and negative samples confirmed by standard methods, validating the overall method and platform for monitoring of toxins and pollutants in food processes and in the aquatic environment.

4.1.3 Main S&T results/foregrounds

RADAR aimed at developing a biosensor platform for the monitoring of complex biomolecules such as toxins and pollutants in the environment and for the surveillance of production processes by integrating the following advances:

- Specific and sensitive biological recognition elements based on recombinant receptors derived from aquatic organisms,
- Organized nanostructured, chemically modified substrates,
- Efficient sample preparation and pre-concentration modules,
- Compact and cost-effective label-free detection modules,
- Deployable remotely-controlled robust biosensor platforms

The complete scientific and technological development was designed around four poles of activity, corresponding to WP1-4 () which are centered to the system testing WP5 (Figure 4). The pole WPs can operate simultaneously, and are strongly interlinked and iterative. Each pole WP is in strong connection and collaboration with the other three pole WPs, and once the two prototypes based on WGG and SPR have been realized, they will finally be tested in WP5.

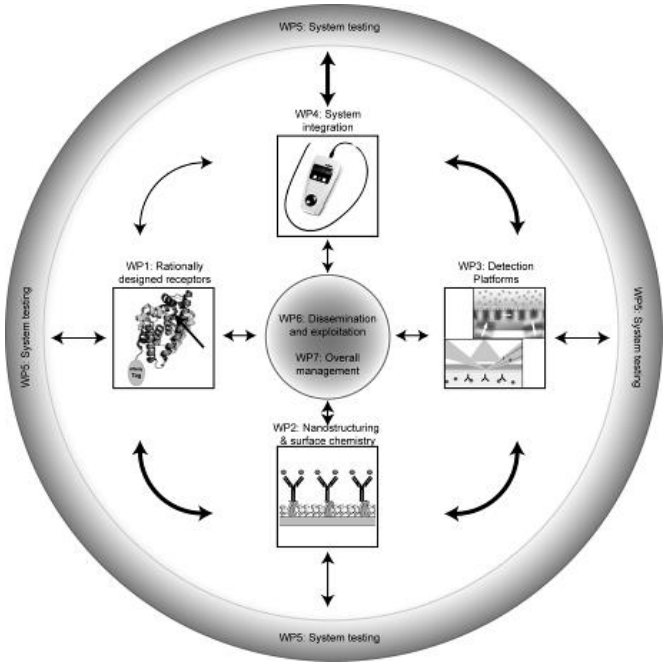

Figure 4. The four poles of the RADAR project and the iterative nature of the project rationale.

Hereafter the main S&T results and foregrounds are reported with a brief description of main activities. Further details are reported in the referenced deliverable documents. Deliverables and milestone description are also reported.

Work Package 1

Rationally designed recombinant receptors derived from aquatic organisms

WP Leader: JRC-IES

Executive Summary

WP1 was the key-starting step of the project which objectives were focused on

1. Production of two recombinant receptors namely the Estrogen Receptor (ER) and the Aryl Hydrocarbon Receptor (AhR) for the detection of chemical pollutants including toxin in complex matrices e.g. water, and food;
2. Rational design of modified ER and AhR receptors (mutants) in order to increase specificity and sensitivity towards class of pollutants.

The final goal of the WP1 was to provide rationally designed battery of recombinant ERs (wild type + mutants) and wild type AhR to be immobilized on surfaces (WP2), to be tested for their binding, sensitivity and specificity (WP4, WP5).

Production of Estrogen Receptor (ER) and Aryl Hydrocarbon Receptor (AhR) as recombinant receptors

This objective has been successfully achieved. RADAR could express, purify and produce high yield of the two recombinant receptors in Escherichia coli, a very cheap heterologous system (see D1.1, D1.2 and D1.2, D1.4 for ER and AhR, respectively). Characterization of the two recombinant receptors was performed to test the ligand binding activity and stability, two key parameters required for the immobilization on surface.

Estrogen receptor characterization has been quite easy since an in vitro binding assay is commercially available which confirmed the ability of binding, first to the natural ligand binding (17β-estradiol) and then to several class of compounds (see D1.5). The recombinant ER also has been also tested for complex matrices such as chemical mixture showing its binding ability.

For the AhR, a setup of the binding assay has been required and therefore took more time, the first approach has been the development of an assay similar to the ER assay based on fluorescence ligand molecule as described in D1.6. Since among the four designed molecules, no positive results could be seen, an assay was setup based on radio-ligand [3H] dioxin (TCDD), which has a strong binding affinity for the AhR to verify the activity of AhR. The results, shown in the amended D1.7, confirmed the ability of the recombinant AhR to bind to the ligands.

Rational design of modified ER and AhR (mutants)

To generate rationally modified recombinant receptors, computational analysis was carried out to identify the amino acids to be mutated in order to modify the affinity and therefore the specificity toward class of pollutants.

As predicted in the project, this approach has been considered as high risk for the AhR since no crystal structure is available, comparing to ER foreseen as low risk due to well-known crystal structure which provides which amino acids interact with the ligand. The outcome of this objective has confirmed the prediction, indeed RADAR could succeed for ER and only partially for AhR.

Based on computational analysis three mutants for ER have been identified which were experimentally expressed and purified (D1.4). Their ability to actively bind to the ligands have been tested as described in deliverable D1.5 and in the peer-reviewed publication (Ferrero et al., PloS One 2014). Particularly one ER mutant showed an increased affinity for some class of pollutants designated as the best mutant receptor.

A more complex analysis has been performed for AhR, due to the lack of crystal structure, models have been considered to predict potential amino acids involved into the ligand binding (D1.7). Some potential mutations were identified and the mutants generated; however the expression did not work, probably an expression protocol needs to be set up.

Under the WP1, Milestone 1.1, 1.2, 1.3 (partially) and M6.1 have been achieved.

Main Tasks

Identification of ligand binding domain for the EDCs through genetic, structural and functional comparison: Identification of ligand bind domain was achieved for both, estrogen receptor (ER) and Aryl Hydrocarbon receptors (AhR). They are the recombinant receptors in RADAR (D1.1).

Selection of amino acids to be mutated based on structural analysis and cloning in suitable expressing vector: Expression and purification of ER and AhR2 as well as identification of amino acids to be mutated in ER were completed. Regarding the mutant construct generation for ER, three mutants were generated. Identification of AhR2 amino acids to be mutated was not possible due to a lack of crystal structure (D1.2/ D1.3/D1.4).

Expression and purification of wild type and mutant constructs in suitable organism: The expression and purification of ER and AhR2 wild type recombinant receptor was completed. Purification of mutants constructs for ER were achieved (D1.2/D1.4).

Characterization of expressed protein: The expressed recombinant receptors were characterized in term of stability, storage and binding activity. It was already demonstrated their binding affinity towards natural ligands end EDCs: 17β-Estradiol, 17α-EthynylEstradiol, Tamoxifen, BisPhenol-A, 4-NonylPhenol and 4-tert-OctylPhenol (D1.6).

In vitro testing and selection of best receptors by affinity binding measurements to selected toxins and pollutants: The affinity binding measurements allowed the selection of wild type ER, mutants ER_M421F, ER_M421L, -ER_ M421I, wild type AHR2. AHR2 mutants were designed and a mutant containing only the ligand binding domain (AHR2LBD, generated by deletion of 515 aminoacids) was successfully expressed in E.coli. D1.7 was focused on AhR activity analysis. (D 1.7)

Deliverable Number	Due Date	Actual Date	Deliverable Description
D 1.1	5	5	Progress report on first sequence alignments identifying the common motif for ER and AhR receptors in aquatic organisms: Identification of the conserved domain among the aquatic organism and synthesis of the ligand binding domain (LBD)

			encoding gene for ER and DNA binding and ligand bind domains for AhR.
D 1.2	9	9	Progress report on expression and purification of wild type ER and AhR: The encoding genes for ER and AhR were expressed and purified. The purification method was based on Ni-affinity system.
D 1.3	13	13	Progress report on expression and purification of a representative, rationally designed mutant: Structural conformational analysis allowed identifying a set of residues to be mutated. One mutant was successfully tested for the expression and purification.
D 1.4	17	17	Progress report on cloning and generation of mutants: Other mutants (a set of three) have been subsequently cloned and expressed.
D 1.5	29	29	Data report on expression and purification protocols of recombinant receptors: High levels of pure wild type and four mutants of ER were obtained. We also expressed and purified the wild type Aryl Hydrocarbon Receptor. The structure stability was confirmed through circular dichroism spectroscopy.
D 1.6	32	32	Data report on characterization of recombinant receptors: Recombinant receptors of ER were characterized through fluorescence competitive ligand binding assay. The binding activities of wild type and mutant ERs were demonstrated towards six compounds: 17α-EthynylEstradiol (EE2), 17β-Estradiol (E2), BisPhenol-A (BPA), 4-NonylPhenol (4NP), 4-tert-OctylPhenol (4TOP) and Tamoxifen (TAM). A ligand binding assay for AhR is under development.
D 1.7	34	40	Data report on in vitro test for binding activities of wild type and mutant receptors: A mutant of AhR containing only the ligand binding domain (AHR2LBD) was successfully expressed in E.coli and activity verified by a radiolabeled assay using [3H]TCDD as marker. Mutants in single amino acid positions of AhR were designed and expressed but not active.

Milestone N.	Due Date	Actual Date	Milestone Description
MS3	12	12	First set of pilot recombinant receptors, as starting point for the testing of engineered biosensor substrates: Wild type recombinant receptors have been successfully produced. The recombinant receptors were tested for their stability and be further used as substrate for the immobilization on biosensor.
MS5	18	18	Second set of recombinant receptors, as validation of our approach: Identification of the first set of estrogen receptor mutants has been achieved. These receptors have been expressed and purified. Characterization of one of these mutants e.g. in vitro binding assay has been performed.
MS8	34	40	Final set of recombinant receptors, as best-in-class receptors: ER-wt, AhR-wt and four mutants were produced with different affinities for toxicant classes

Work Package 2

WP 2: Engineered biosensor substrate with optimized receptor immobilization

WP Leader: JRC-IHCP

Executive Summary

The main objective of work package 2 was to engineer the biosensor chip surfaces for optimizing the immobilization of the Estrogen and Aryl hydrocarbon Receptors developed in the frame of work package 1. The final goal was to maximize the performances of the biosensor in particular to enable the detection of estrogenic compounds.

The main difficulty of this task was related to the small size of the compounds to be detected which have size lower than 500 Daltons representing the limit of detection of most label free biosensors in particular of surface plasmon resonance (SPR).

In order to control their orientations, Estrogen (ER) and Aryl receptors (AhR) with poly-histidine tags were immobilized via specific metal (Ni)-protein interactions. Two sensing platforms were used i.e. surface plasmon resonance (SPR) and wavelength interrogated optical sensors (WIOS). Several surface chemistries were tested in parallel i.e. Nitrilotriacetic acid derivatised carboxymethyldextran hydrogel (NTA-CMD) on SPR and WIOS and NTA derivatized Mercapto-hexadecanoic acid Self Assembled Monolayer (MHDA-SAMs) on SPR. Above mentioned functionalized surfaces were nanostructured for evaluating further potential detection signal enhancement. The chosen protein model to test the performance of the surfaces was the His tagged-Transthyretin (TTR) and Tyroxine hormone (T4, size 770 Da). The surface nano-structuring methods were optimized, fully characterized and tested with the TTR/T4 model.

The best results were obtained with the NTA-MHDA nanostructured surfaces using the TTR/T4 model. We observed an increase of the signal of about 20% even if the active area of nanostructured surface is only 15% of the uniform surface. The results obtained with nanostructured NTA-CMD chemistry did not give any significant enhancement. Next to the nanostructuration, the buffer composition was optimized for ERs immobilization. The goal was to find the conditions giving the highest immobilization level of receptors. HBS buffer containing 10mM Hepes, 150mM NaCl, 0.003% Tween 20 has been selected giving a good compromise between the binding capacity and compatibility with circular dichroism (Triton free). The ERα-wt and mutants ER M421L, ER M421F and ER L346M were successfully immobilized on both platforms (SPR and WIOS) functionalized with NTA-MHDA and NTA-CMD chemistries nanostructured or uniforms. The immobilization experiments gave similar results on both nanostructured and uniform chemistries (> 1000 pg/mm2). β-Estradiol detection was monitored at different concentrations. Only a slight detectable signal has been observed on the nanostructured surfaces (see Figure 3 of deliverable 2.4). Nevertheless, the obtained signal was found too low to develop a robust assay for real samples.

WIOS sensor has been used to determine the best running buffer enabling AhRs receptors immobilization. Two buffers have been tested namely NaP and NaP/NaCl buffers. The presence of NaCl in the buffer decreases the immobilization efficiency of the receptors. Receptor surface coverage is respectively 1400 and 770 pg/mm2 for NaP and Nap/NaCl buffer. Experiments of Epigallocatechin (EPGC) detection have been performed. Noticeably, whereas no EPGC binding is detected on AhRs immobilized in NaP buffer, binding was slightly detected for concentration from 1 to 10 μM with AhRs immobilized in NaP/NaCl buffer. NaCl seems to have a positive effect

of AhRs folding that allows a better exposition of binding site of immobilized receptors. Nevertheless the reproducibility of the results was very poor.

Complementary experiments have been performed using Biacore which is a one of the most sensitive SPR biosensor instrument. Several methods of receptors immobilization have been tested i.e. random immobilization with amine coupling or oriented immobilizations using NTA chips and chips coated with an antibody against the His-tag. However, the interaction of the immobilized receptors with high concentrations of the estrogenic compounds in solution resulted in low or no response. Similar results were obtained with an alternative reversed system in which Estrogen derivatives were immobilized on the surface and the interaction with the ER in solution was investigated.

As conclusion, Due to the inherent limit of detection of the used sensors and the small size of the analyte, detection of chemical compounds such as estradiol and tamoxifen by using direct assay has not been possible. A New assay format has been successfully developed. Peptides specifics to ER conformational change upon ligand binding have been used as amplification agents. By immobilizing these peptides on the sensor surface, limit of quantification of ER-ligand complex was found lower than 10 nM. The optimization of the assays is described in WP4.

Main Tasks

Optimization of the chemical composition and geometry of the nanostructures: Two different nanostructured surfaces have been tested: Carboxy-methyldextran hydrogel (CMD) and Mercapto-hexadecanoic acid Self Assembled Monolayer (MHDA-SAMs). NI(II)-NTA derivatization protocol has been optimized. Nano-structuring protocols have been optimized for both chemistries. (D2.1 and D2.3)

Physical-chemical characterization: Developed surfaces have characterized at each step of fabrication by XPS, AFM, SEM. (D2.2)

Immobilization and activity test of nanostructured surfaces: TTR/T4 model tested on SPR with and without nano-structures. Signal enhancement observed with SPR detection method.

Array patterning and immobilization of the novel receptors: Binding of wild type ER on nanostructured surfaces SPR. Binding of wild type AhR on non-nanostructured surfaces SPR. Binding of wild type AhR on nanostructured surfaces SPR. SPR detection with wild type ER. Binding of wild type ER on non -nanostructured surfaces WGG. WGG detection of pollutants with wild type AhR. Satisfying receptor loading on both SPR and WGG platforms.

Initial assay development / performance testing: Bioaffinity Mass Spectrometry (BioMS) competitive inhibition assay was developed with LC-QqQ-MS. Direct assay with ER immobilized on the sensor surface. Direct assay with ER immobilized on the sensor surface. Due to the inherent limit of detection of the used sensors and the small size of the analyte to detect, detection of chemical compound such as estradiol and tamoxifen by using direct assay has not been possible.

Deliverable Number	Due Date	Actual Date	Deliverable Description
D 2.1	5	5	Preliminary description of nano-engineered surfaces and preparation protocols: Protocols for carboxy-methyldextran hydrogel (CMD) and Mercapto-hexadecanoic acid Self Assembled Monolayer (MHDA-SAMs). NI(II)-NTA derivatization protocol has been optimized. Nano-structuring protocols have been optimized for both chemistries.
D 2.2	9	9	Data report on the extended characterization of the nanostructure surfaces: AFM, XPS and SEM analysis have been performed. Chemical characterisation by XPS is limited due to the limit of detection of this method.
D 2.3	13	13	Data report on performances of the model test on the developed nanostructure surfaces: Immobilized His-tag TTR density close to those obtained with the CMD surface. NTA derivatized carboxymethyldextran hydrogel surfaces give promising results for both SPR and WGG platforms.SPR signal is 5 times higher than for uniform Dextran for normalized area.
D 2.4	17	17	Sensor surfaces with immobilized AhR, ER and mutant receptors against toxin/ pathogens in array format: All the receors developed in the frame of WP1 have been immobilized successfully with the desired surface density.
D 2.5	29	29	Data report on initial assay development and biophysical properties of AhR and ER protein constructs against toxins/pathogens: Due to the inherent limit of detection of the used sensors and the small size of the analyte to detect, detection of chemical compound such as estradiol and tamoxifen by using direct assay has not been possible. Another strategy has been implemented.

Milestone N.	Due Date	Actual Date	Milestone Description
MS4	12	12	Nanostructured and chemically treated surfaces available with array-like patterned AhR and ER receptors for SPR and WGG: The sensitivity enhancement by using structured surfaces has been observed with SPR and Model proteins(TTR/T4). No enhancement has been observed with the WIOS and ERα / estradiol.

Work Package 3

WP 3: Detection platform design, fabrication and validation

WP Leader: Optics Balzers

Executive Summary

WP3 aimed at the development of a robust and highly sensitive label-free detection platform. These criteria were the main drivers during the development of all system components involved, meaning the sensor chip itself, the cartridge and its microfluidics, the optical read-out system and the related electronics. During the entire development, the future integration with the subsystem developed in WP4 and additional factors like production compatibility at reasonable costs for sensor and consumables were considered.

In Task 3.1, the sensor cartridge was developed. The cartridge is used to apply the concentrated analyte dissolved in aqueous solution on the sensitive part of the optical transducer chip. In addition to the transport of the fluid onto the chip, the cartridge needs to satisfy other requirements like leak-tightness, low dead-volume, easy replacement and it should preferably be mass producible at a wafer level. After the development of some computer assisted design (CAD) models and finite element calculations of the flow, several cartridges have been produced and tested. The current cartridge supports eight measurement channels in parallel, whereas six are used as measurement and two as reference channels. In contrast to the reference channel, the measurement channel is split into two channels, just before the actual sensor part of the chip. This T-junction allows guiding the liquid either over the sensor or to a waste channel. This approach allows switching from one liquid to the next within a few seconds, without mixing of the two liquids as in the previous systems: the two liquids are being separated by an air bubble, hence the two liquids are unable to mix.

Since air bubbles cannot be flown over the sensor part due to loss of a stable baseline, the bubble needs to be by-passed. To do so, an internally developed, low-cost, non-invasive bubble detector at the inlet of the measurement channel detects an incoming bubble, which will trigger a valve at the outlet to close the measurement channel but open the waste channel and due to the hydrostatic back-pressure the bubble will exit via the latter. A second bubble detector at the outlet inverts the process and toggles the valve and the second liquid will directly be applied on the sensor surface. As this switching directly takes place on the sensor chip itself, the dead- as well as the mixing volume is marginal and leads therefore to very fast switching times. Additionally, together with an automated selector valve, this switching principle allows “walk-away-operation-mode”, since the new liquid is detected automatically and so is the switching of the valve. An entire assay can be preprogrammed and autonomously been run on the system.

As the system’s sensitive element, the optical transducer chip and its performance is of utmost importance to achieve a stable and sensitive measurement. The sensor optimizations were part of Task 3.2. Extensive computational simulations on various parameters have been performed to gain sensitivity and stability. Various test structures have been produced and tested. Simulations and experiments indicate a rather high potential to optimize the previous sensor design regarding both, sensitivity and stability. One of the benefits of the current system with the MEMS micro-mirror (as described) is its independency of the selected laser wavelength. As the simulations suggest, a shorter wavelength leads to a higher sensitivity regarding surface related binding processes but lower sensitivity to bulk effects of the cover liquid. The current system is equipped with a 532nm green DPSS laser, compared to 762nm in the previous wavelength scanning system. These simulations have independently been confirmed experimentally in T3.6 at DLO

and CSEM by comparing the sensor sensitivities towards refractive index changes and the adsorption of molecules to the sensor surface. Whereas the measurements at DLO have been performed directly with the ARGOS sensor and compared to the corresponding sensitivities of a commercial SPR system (Biacore 3000), the measurements at CSEM have previously been collected ex-situ with different light sources.

The transduced signal, generated by the sensor chip, is being read by the portable sensor module developed in Tasks 3.3 and relies on an angular interrogation scheme to measure the change of the effective refractive index induced by the adsorption of the analyte (here EDC) onto the waveguide grating transducer surface. The heart of the optical module consists of so-called opto-mechanical cage structure, housing the laser source, diaphragm, the MEMS mirror and plano-convex lenses. This sturdy structure can be mounted in the reader platform as a whole at its predefined position and can also easily be removed for maintenance if needed, as well. It is decoupled from the related electronics needed to drive the system and acquires, processes and stores the sensor data. The (mechanical) decoupling of the optical module from the rest of the reader has an additional advantage, since it's less prone to external effects like vibrations, thermal drift etc. The system design again aims at highest performance regarding both sensitivity and stability, with additional constraints like size, manufacturability, cost and power-consumption. The newly developed optical reader system performs at a superior performance level compared to commercial WGG sensor systems on the market (BR8, Dynetix) and comparable to expensive and bulky lab systems (e.g. Biacore 3000). The performance has been analyzed by refractometric (glycerol) measurements as well as biosensing via IgG immunoassays before the systems have been shipped to the partners.

Main Tasks

Cartridge design and manufacturing for on-line EDC detection for SPR and WGG: Standardized cartridge based on simple plastic design (suitable for molding) including 6 measurement and 2 reference channels was designed and manufactured. Working cartridge. 100 times faster switching times.

WGG Sensor substrate optimization: Extensive in-silico simulations finalized. New structures manufactured tested in newly developed reader module. Better sensor design (stability and sensitivity) New theoretical models.

Design and adaption of portable sensor module for WGG and SPR: Transferred to WP4 (system integration).Running prototype.

Multiplex assay development on portable SPR and WGG biosensors: Testing of integrated system done with glycerol and IgG demonstrating state of the art sensitivity level of the final ARGOS V2 version. k-casein anti-k-casein interaction assay performed on many surface chemistries

Comparison of performance of the developed assays in commercial SPR and WGG machines and the portable SPR and WGG instruments: Transferred to WP5. ARGOS about 5x less sensitive compared to SPR Biacore but portable and low cost

Deliverable Number	Due Date	Actual Date	Deliverable Description
D 3.1	11	11	Report on the cartridge design for on-line detection compatible with SPR and WGG: After computer assisted design (CAD) models and finite element calculations of the flow, several

			cartridges have been produced and tested. The final design (mainly outer shape) highly depends on the integration of the subsystems, which is an upcoming task in WP4 and can easily be adapted if necessary. UPDATE: Final cartridge/holder delivered in RP3.
D 3.2	13	13	Report on the optimized WGG sensor substrate: Extensive computational simulations on various parameters have been performed to gain sensitivity and stability. Various test structures have been produced and tested. Simulations and experiments indicate a rather high potential to optimize the previous sensor design regarding both, sensitivity and stability.
D 3.3	21	21	Report on the portable sensor module for WGG: Working prototype with high sensitivity and stability (electronics, optics, MEMS actuator, software) delivered for WP4.
D 3.4	21	21	Report on the portable sensor module for SPR: Working prototype with high sensitivity and stability (electronics, optics, MEMS actuator, software) delivered for WP4.

Milestone N.	Due Date	Actual Date	Milestone Description
MS2	21	21	Portable SPR and WGG Detection Platform ready: Portable WGG Detection Platform delivered and transferred to WP4, overall sensor integration. Compact, low-cost but robust system proves best in class performance.
MS6	36	44	Portable SPR and WGG Detection Platform and specific assays for EDC detection ready: Three ARGOS V2 systems available for use at partners.

Work Package 4

WP 4: System integration and validation with sample preparation module

WP Leader: CSEM

Executive Summary

The main goal of Work Package 4 was the integration of the biosensor platform, including development of the sample preparation unit and of the wireless communication module.

Prior to any measurement, sample preparation is the key step for a sensitive and selective detection of target analytes. If the selectivity of the analysis is provided by the use of biological receptors, immunoassays used for the detection are sensitive to matrix effects and pre-treatment is crucial to prevent denaturation of the biomolecules. Moreover EDCs are present at the ng/L level in the environment, when limit of detections of optical biosensors reach the 1 µg/L level. A pre-concentration is therefore needed before analysis. In this work package, solid phase extraction (SPE) was chosen. For the development of the sample preparation unit, the natural hormone 17β-estradiol (E2) was chosen as the model compound due to its affinity with the estrogen receptor use for the biosensor assay. Prototypes were built that enable to perform fully automated SPE, with 500 fold pre-concentration and full recovery of E2, starting with 1 to 100 ng/L in only 100 ml sample volume. The output of the sample preparation unit is a 20-200 µl volume of methanol 5% or 10% v/v, totally compatible with analysis by immunoassay. This unit was successfully applied to the extraction and pre-concentration of E2 from real sea water samples with analysis by enzyme-linked immuno-sorbent assay (ELISA) on a well-plate format.

A prototype for wireless communication was developed and further integrated. This device enables transfer of data from the system to a database accessible by the user, and for remote control of the biosensor platform. The mobile telecommunication technology, global system for mobile telecommunication (GSM), was chosen as it provides a world-wide network coverage and high bandwidth requirements. The communication unit is a gateway between the biosensor platform and the GSM network, capable of transporting the information collected on the sensor to a central location for controlling, monitoring and data storage. The gateway is using GSM as radio interface and establishes a point of connection to Internet. An on-field monitoring application implies limited power resources for the overall platform. Therefore a high concern was the power consumption of the communication module. In order to spare energy, the wireless module is kept in sleep mode most of the time. It then periodically wakes up to establish a link to the controlling server. Remote monitoring on the field is then possible for the user.

Finally, the optical biosensor that was developed in Work Package 3 was further integrated to provide a compact, robust, user friendly and performant sensor for the analysis of EDCs by receptor immunoassay. The electronics was developed with a modular approach. Both sample preparation and biosensor units have independent electronic controls that enable to work with two stand-alone instruments. On the other hand, there were made compatible so that the two system units can be used in-line for full assay, from sample preparation to immunoassay and data analysis. A receptor immunoassay was transferred from gold standard surface plasmon resonance biosensor to the optical biosensor developed in the project.

In conclusion, the collaboration of the partners within WP4 lead to the development and the release of a modular biosensor platform allowing for remote monitoring of EDCs in the environment. The device includes sample preparation, with extraction and pre-concentration of analytes, sample analysis by immunoassay on an optical biosensor, and the possibility for

remote control. The biggest challenge in monitoring programs is to obtain a result as close as possible as the sample upon collection. The overall platform offers an alternative to standard analytical methods with this regard. The different units allow to reduce the costs associated to monitoring programs, by proposing high quality low-cost instruments for either in-laboratory or on-field analysis. The complete automation of the process steps helps reducing human-related errors, and provides a high sensitivity throughout the process.

Main Tasks

Specification gathering of overall biosensor platform based on WGG and SPR: List of potential compounds for the receptors defined. Two model ligands for ER and AhR: respectively E2 and BaP. First priority is given to water samples.

Elaboration of extraction procedure and module: Solid phase extraction has been chosen instead of isotachophoresis. A transfer of knowledge from DLO to CSN has been done, and CSN is developing a new method for the extraction, separation and preconcentration of the EDCs from water samples. 500 to 1000 fold preconcentration of 17β-estradiol from di-water demonstrated.

Platform design and fabrication of module for bringing EDCs from solid into liquid phase: Replaced by extended T4.2 / As priority is given to liquid samples, this module might be developed in a later phase of the project.

Sieving element to remove solid particles and gas bubbles: Working prototype for macro-filtration ready and successfully tested. Proof of concept for micro-filtration done. Device available for the removal of gas bubbles.

Isotachophoresis for EDC pre-concentration, separation and extraction: Isotachophoresis replaced by Solid Phase extraction. 10-fold and 500- fold concentrations on water samples

Development of global wireless connectivity module: Prototype of the wireless module ready & tested. Characterization of its power consumption done. Integrated on the electronic boards' stack.

Biosensor platform design, construction and assembly including microcontroller and electronic instrument control unit: A first set of prototypes was tested with real samples and ER assay. A second set of improved systems were delivered.

System control firmware and software: **-**

Integration of all subsystems in biosensor: Two separate prototypes compatible for use in line.

Optimization and Validation of system with final receptors: Optimized assay developed for ER, and transferred to SPR platform. AhR-based assay not optimized (not quantitative)

Deliverable Number	Due Date	Actual Date	Deliverable Description
D 4.1	5	5	Report on the four-month specifications gathering for the instrument from the final users: List of potential compounds for the receptors defined, as well as two model ligands for ER and AhR. First priority is given to water samples. The frequency of analysis will depend on the application.

D 4.2	14	14	Report on wireless connectivity module performance: Prototype of the wireless module ready and tested. Characterization of its power consumption done.
D 4.3	26	26	Report on design and performances of sieving, preconcentration, separation and extraction modules of sample preparation platform for food and water: Development and validation of separate elements of the sample preparation module: sample collection, filtration, gas removal and pre-concentration by solid phase extraction.
D 4.4	27	27	Report on biosensor platform design: Description of the opto-electronic and fluidic components of the WGG biosensor and characterization of the first prototype by injections of glycerol.
D 4.5	29	29 (+48)	Report on integrated WGG and SPR biosensor platform prototypes: Optimization of the solid phase extraction process and system. Update on the opto-electronics for the biosensor. Choice of materials for the wetted parts of the system.
D 4.6	36	36 (+48)	Data report on WGG and SPR biosensor platforms performances with final receptors: Tests done with the WGG biosensor prototype, with antibodies, on different sensor chips (different surface chemistries). No tests with the receptors yet.

Milestone N.	Due Date	Actual Date	Milestone Description
MS1	5	5	Specifications of overall biosensor platform based on WGG and SPR: List of potential compounds for the receptors defined, as well as two model ligands for ER and AhR. First priority is given to water samples. The frequency of analysis will depend on the application.
MS7	26	26	Sample-preparation platform for food and water ready: Two automated prototypes for pre-concentration of filtered water samples have been developed and validated. 10-fold and 500-fold concentrations on water samples with E2 were demonstrated.
MS10	36	40	THREE portable biosensor prototypes based on WGG and SPR ready: Transfer of the prototypes to the application partners

Work Package 5

WP 5: Biosensor platform system testing

WP Leader: RIKILT

Executive Summary

The main objectives of the Work Package 5 can be stated as follows:

1. To demonstrate the application of the label-free WGG and SPR biosensors, to detect EDCs with high precision and specificity in the following testing sites: sea water, fresh water, fish farming, dairies, fish lines and fruit juice processes.
2. To demonstrate the use of WGG and SPR biosensors as an alternative low cost monitoring strategy that may document the impact of pollution.
3. To carry out a series of field sampling campaigns that will generate a database of information that will enable evaluation of the biosensors and guidelines for the extension of the results to other targets of the same family.

The biosensor estrogen receptor (ER) assay works very well in the SPR-based biosensor. The peptide-coated CM5 chips coated with biotinylated peptide (via streptevidin) or with amine-peptide, are very stable for months and for hundreds of injections. The diluted ER is also usable during a working week. The assay is fast (ca. 10 min) and works in 10 % methanol which is good for the combination with the SPU. The sensitivity for E2 and EE2 is similar and in the low ppb level (working range between1-10 ppb) and can be improved to the low ppt level by SPE extraction and concentration.

However, the test does not work properly in the WGG (ARGOS) system. Successful tests performed with Surfix chips could not be reproduced consistently. We see binding of the receptor to the peptide-coated surfaces but non reproducible influence of E2. This needs to be investigated to get this application working in the ARGOS. The overall conclusion is that it is too early to switch to the ARGOS to get good results with the receptor test and additional research is necessary.

The Biacore SPR assay was tested with blank MilliQ water and drinking water and with "off-line" SPE and resulted in a limit of detection of 5 ppt after a 200 times preconcentration and with an average recovery of 75% of E2 spiked at 50 ppt and these data were confirmed by GCMS and ELISA. All samples obtained from fish farms from the UK and Slovenia and from sea and river water samples near and in Slovenia were found negative for E2 equivalents with the biosensor assay (< 5 ppt in 200 times concentrated samples and < 1 ppt in 1000 times concentrated samples). This was confirmed by the E2 and EE2 ELISAs. In two fish farm water samples from the UK, low levels of EE2 of 0.27 and 0.44 ppt were found with ELISA and one of these sample contained E2 (2.7 ppt). One sewage sample from Slovenia showed low levels of E2 (1.4 ppt) and 0.23 ppt EE2.The spiked samples at 50 ppt gave recoveries of 60 to 82 %. The SPU gave varying recoveries (6-100%) with an average of 53 ± 31% when samples were spiked at 50 ppt and concentrated (87 to 147 times) and an improvement of the system is required to provide better results.

For the application in food, the EU inspection programs for meat control focus on sample materials that are more suitable for testing for banned substances, especially if the animals are still on the farm, such as urine and faeces or hair. To test the assay with positive samples, urine

samples from horses (n=20) were used and they gave varying concentrations of E2 in the biosensor ranging from 0.3 to >10 ng/ml (ppb) and this was confirmed by ELISA.

Main Tasks

Technical support from all RADAR partners: Support was given in the supply of reagents, instruments, samples and know-how.

SPU tested with fish farming and fresh water: EPL and MBS sampled both salt and freshwater from fish farms and sea and river water samples.

SPU tested with sea water and fish farming water: See above

WGG tested Fish, fruit juices, milk testing: Drinking water, beer and urine samples were taken.

Fish, fruit juices, milk testing: Samples were tested

Fish farming and fresh water testing: Testing was done on the benchtop SPR biosensor in RIKILT

Sea water and fish farming testing: See above

Deliverable Number	Due Date	Actual Date	Deliverable Description
D 5.1	48	48	Report on the performance of the WGG sensor for laboratory use and remote-controlled in situ monitoring of several pollutants and toxins in fish farm and fresh water: The SPU was tested with fish farm water samples and gave varying recoveries (6-100%) with an average of 53 ± 31% when samples were spiked at 50 ppt and concentrated (87 to 147 times).
D 5.2	48	48	Report on the performance of the WGG sensor for remote-controlled in situ monitoring of several pollutants and toxins in sea water and fish farms: The SPU was tested with water samples from sea water, a fish farm and river water and gave varying recoveries (10 to 118%) and an improvement of the system is required to provide better results.
D 5.3	48	48	Report on the performance of the WGG sensor for laboratory use and monitoring of several pollutants and toxins in food processing, specifically in fish, fruit juices and milk: The overall conclusion is that it is too early to switch to the ARGOS to get results with the receptor test for food.
D 5.4	44	48	Report on the performance of the SPR based and/or other benchmarking: sensor for laboratory use and monitoring of several pollutants and toxins in food processing, specifically in fish, fruit juices and milk: The biosensor estrogen receptor assay works well in the Biacore SPR-based biosensor with a working range between1-10 ppb of E2.
D 5.5	44	48	Report on the performance of the SPR based and/or other benchmarking sensor for laboratory use and remote-controlled in situ monitoring of several pollutants and toxins in fish farm and fresh water: Levels of E2 were found to be below 5 ng/L (ppt)

			and all spiked samples at 50 ppt resulted in levels between 35 and 41 ppt of E2 (70 to 82% recovery). Trace levels of other hormones (testosterone (approx. 17ppt) and progesterone (<5ppt) were found in one of the samples.
D 5.6	44	48	Report on the performance of the SPR based and/or other benchmarking sensor for remote-controlled in situ monitoring of several pollutants and toxins in sea water and fish farms: All samples obtained from a fish farm from Slovenia and from sea and river water samples near and in Slovenia were found negative for E2 equivalents with the biosensor assay (< 5 ppt in 200 times concentrated samples and < 1 ppt in 1000 times concentrated samples).

Milestone N.	Due Date	Actual Date	Milestone Description
MS12	48	48	WGG and SPR based biosensor platforms testing completed in water and food processes: The ER assay in the SPR based platform was applied during the End user week.

4.1.4 The potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and exploitation of results

1. Policy impact

We expect the scientific evidence gathered using the new platforms and technologies resulted from the project to be translated into guidelines and recommendations for control of endocrine disrupting compounds in food and water. Our dissemination strategy views the policy impact as a key factor in the subsequent success of raising public awareness and commercial development of the outputs from the project. We engaged with the policy makers, for example via the Symposium “Small solutions for big water-related problems”, held in Rome, through the project partner JRC and through our external advisor Prof. I. Werner, of EAWAG, Switzerland, to inform governmental environment, food and health agencies at an EU-wide and national level, and NGOs in these areas.

The feasibility of developing a technically sound, cost-effective control strategy is an important part of developing effective guidelines for controlling pollutants such as endocrine disruptors and RADAR has been and will continue to be active in promoting its outputs for policy consideration.

2. Societal impact

Outside the pure scientific and technical impact, this is the first area where the RADAR project is expected to have an impact. We have used the outputs of the consortium work to raise awareness of the issues around endocrine disrupting compounds to the general public and also within the context of trade and its impact on the businesses producing food and water. This has been achieved through use of the website which has pointed interested parties to the science base behind the news stories on these compounds, for example referring to the EU roadmap on EDCs and topical news from reliable source such as Food Manufacture; through consortium members’ open days for schools and through face to face discussions with individual contacts at meetings. This first area of societal influence will be continued over the coming year as the outputs from the programme are collected and publicised in trade journals and magazines.

Socio-economic impacts as well as wider societal implications of the project include a better monitoring of water supply world-wide, monitoring from the source to the waste throughout all distribution channels. Water is a scarce supply, so preventing pollution of water supply by monitoring toxin and pollutants is critical and the RADAR platform just allows for this to happen.

3. Commercial impact

The commercial area is the second main area of impact benefitting the consortium members and creating added value from the support provided by the EU FP7 programme. This impact arises from the solution to the problem of how to monitor the endocrine disrupting compounds in non-laboratory conditions making it feasible to consider implement a monitoring programme at an industrial scale. The individual components of the RADAR instrument is designed to perform this monitoring function are valuable intellectual assets for exploitation in their own right and may be used for detecting compounds in the case of the receptor assays in different market areas such as within the pharmaceutical, veterinary or medical sectors and the instrumentation is likely to be used for monitoring of a range of other chemical compounds of environmental interest by pairing with different assay types. The RADAR consortium has now entered active commercialisation phase post-project with a number of confidential discussions underway for the partners with a view to progressing the development of commercial outputs from the research.

4. Scientific and technical impact

The expected final scientific impact of the project is manifold. At the end of the project, i.e. Month 48, we can proudly cite three technical results of the project with high impact much beyond the project itself:

To the best of our knowledge, RADAR has produced the first Aryl Hydrocarbon Receptor from aquatic organisms with highest affinity towards Polycyclic Aromatic Hydrocarbons (PAHs), e.g. epigallocatechin gallate.

For the first time to the best of our knowledge, an Estrogen Receptor and several mutants were produced from aquatic organisms with a successful increase of the specificity and the selectivity of the receptors to recognize and differentiate different classes of pollutants. This technical progress beyond the state-of-the-art is opening new routes towards class specific robust capture biomolecules for EDCs compound detection.

To the best of our knowledge, the ARGOS compact label-free platform with the ER coated biochips linked to the automated filtration/separation/pre-concentration module is the first combined label-free system successfully able to detect EDCs at relevant concentration levels in the aquatic environment and in food processes.

The potential use of the ARGOS compact label-free platform with the ER coated biochips linked to the automated filtration/separation/pre-concentration module is the field of monitoring EDCs in the aquatic environment and in food processes. Knowing that the number of EDCs is increasing, such a platform approach is without doubt promised to be a success as we have now proved that its sensitivity and specificity is matching the regulatory values as shown for the specific case of E2. Moreover, its potential use is greatly extended as, thanks to the use of ER or the AhR receptors, it can detect all molecules that interact with the receptors and not only specific toxins and pollutants (class specific and not molecule specific).

The ARGOS compact label-free platform with the ER coated biochips linked to the automated filtration/separation/pre-concentration module can have a significant impact on the way water treatment and monitoring is performed today and will be performed tomorrow. Even without regulatory incentives, the platform, due to its limited cost of ownership, should be able to penetrate the market by providing a direct feedback (monitoring) of level of toxins and pollutants, thus allowing the significant reduction of chemicals (costly !) used in the water treatment cycle. Further applications will target the field of monitoring: With the integration of the system with a wireless module for on-line detection of toxins and pollutants in diverse matrices, the final system has shown to have a huge potential impact for monitoring of class specific toxins and pollutants in the aquatic environment.

5. Main dissemination activities

The website was then used as the primary dissemination vehicle for information on our general activities. It has been regularly updated throughout the course of the project with information on scientific progress, consortium meetings and as a way of advertising future events of general and technical interest. Links were provided to relevant websites for both general and specialist resources and news of consortium members speaking activities at conferences etc. highlighted as well as news articles of interest.

An important part of the dissemination process has been reporting scientific progress via publication of learned articles in scientific journals and presentation of work in progress at international and national conferences, technical meetings and congresses. To date the consortium have published 5 original articles, 8 oral presentations at conferences across the EU and USA, 9 poster presentations including the best poster prize winner at the Conference on Bio-Sensing Technology in Sitges, Spain in 2013.

The RADAR consortium joined forces with another EU FP7-funded project µAQUA to organise a joint scientific symposium entitled “Small solutions for big water-related problems” on sensors for water quality and food security in Rome in October 2014. The meeting had 145 delegates, 30 speakers and a similar number of poster presentations. A short film showing a tree planting ceremony during the conference and clips from the meeting is available on YouTube RADAR members provided oral presentations for the meeting provided a scientific overview of progress made in the project to date and we have guest edited a special edition of the International Journal of Environmental Research and Public Health associated with the consortium as well as providing articles due for publication in 2015.

The educational aspects of the dissemination process have also been considered by the consortium with posters presented at open days for the general public and school children, at least three open days and general meetings including the Food Matters Live event in London in November 2014. The distribution list for general dissemination of the final summary factsheet, now in production, includes all the major water companies or authorities within the EU, a wide range of food industry companies including the retail, primary production and manufacturing sectors.

The technological advances made by the RADAR consortium have generated opportunities for commercialisation of knowledge gained. The options for further development the components of the RADAR instrument for on-line monitoring of endocrine disrupting compounds in environmental samples which is the focus of the project, including specific types of proteins, biosensor assays, sample preparation units and the detection and reporting hardware and software modules have been considered within the work package by all the consortium members. The potential for commercial development of the RADAR outputs is indicated by the filing of a patent by CSEM and Optics Balzers with another patent filing under consideration for the receptor work at JIC Ispra. Technology offering factsheets have been prepared or are in preparation to aid the dissemination of knowledge and provide an easy to understand description of the innovation or product prototype for future development.

The commercial background and likely areas of interest and concern for technology developers have been considered within the consortium's commercialisation plan and will provide a starting point for future commercial exploitation. RADAR partners will continue to work on the commercial and dissemination activities to maximise the social and financial impact of the EU FP7 funding throughout 2015.

Project Logo in jpeg format:

Link to a short video from Valentina Turk on RADAR
https://docs.google.com/a/ktn-uk.org/file/d/0B7t4FeM5_YfOazkzUTcyT3FQWTQ/edit

4.1.5 The address of the project public website, if applicable as well as relevant contact details.

Project website: www.fp7-radar.eu

RADAR project main contact persons

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WP3	**Detection platform design, fabrication and validation**	Florian Kehl Optics Balzers AG Neugrüt 35 LI-9496 Balzers florian.kehl@opticsbalzers.com T +423 388 9200 F +423 388 9390
WP4	**System integration and validation with sample preparation module**	Sarah Heub CSEM AG Bahnhofstrasse 1 CH-7302 Landquart sarah.heub@csem.ch T +41 81 307 8137 F +41 81 307 8100
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WP6	**Dissemination and exploitation**	Mr. John Bostock Deiniol Road Unit 2, Mentec

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WP7	**Overall management and consortium coordination**	Silvia Generelli CSEM AG Bahnhofstrasse 1 CH-7302 Landquart silvia.generelli@csem.ch T +41 81 307 8139 F +41 81 307 8100

4.2 Use and dissemination of foreground

Section A (public)

The dissemination achieved to date and the plan for dissemination of the completed outputs of the RADAR project has been addressed in the RADAR Dissemination Report (see public version below). This also includes the general dissemination activities to the general public, the technical communities and the commercial stakeholders in the food, water and environmental communities. Examples are pasted below of the summary factsheets, one of the technical offering documents (factsheets for commercialisation purposes).

A summary factsheet outlining the achievements of the RADAR project is in draft form (see Figure below) and will be finalised once the last set of testing data is available for distribution (Jan/Feb 2015). This will be disseminated widely (distribution list in Appendix 1 of Dissemination Document) to water companies and food companies directly and via third party websites.

-radar-

Project Details

Duration: 2011–2014

DRAFT FINAL FACTSHEET FOR RADAR

Background

There has been a growth in opportunities for monitoring of pollutants and toxins driven by policy commitments designed to reduce exposure to health-endangering carcinogens, mutagens and reproductive toxicants. RADAR is an EU-funded programme that has developed an integrated platform system for on-site monitoring of pollutants without 3rd party sample collection and lab-based detection requirements.

The large number of different chemicals that have to be measured for their potential ecological toxicity (for example, as required by the REACH legislation) puts a huge pressure and at the same time, creates opportunities for the development of analytical tools such as the RADAR biosensor platform.

of different chemicals (or chemical between different biosensors. An ideal platform would be able to simultaneously detect different chemicals in a mixture.

OPTICS BALZERS elysium csem NIB

The Technology Offering Documents outlining the technological outputs of the RADAR project and seeking to discuss partnering or licence opportunities serve mainly as a commercialisation tool. However, judicial use with the overall broad factsheet provides an opportunity for a slightly more in depth dissemination opportunity. These extra sheets will be made available to interested parties on the list in Appendix A of the Dissemination Document.

Technology Offering Documents

An electronic document provided in PDF will be developed for the various technology offerings as they are finalised:

1. Sample Preparation System
2. ARGOS Instrument
3. Biosensor receptor assay for the detection of oestrogens in water
4. RADAR integrated instrument for the detection of oestrogens

These documents provide an easy reference material for the technology under consideration and will be used to initiate discussions with prospective partners for commercialisation.

The RADAR consortium members have all taken part in dissemination activities throughout the project and this report will provide an indication of the types of interactions made. The nature of dissemination is that the activities tend to occur later in the project cycle as interesting data and real-world opportunities become available due to progress in the science and also closer working of the consortium members, hence the activities will extend into 2015 beyond the scope of the project to ensure maximum coverage of the outputs from the EU funding.

1. RADAR Website (http://www.fp7-radar.eu)

The website was launched with the project in 2011 and was given a substantial remodelling in 2012. The content has been actively managed throughout the project as shown in the examples and screen shots below.

The website is aimed at both a general audience (see the screenshot of the home page, Figure 1 as an example) and also as a resource for scientists to find out more about the aims, news and progress of the RADAR programme.

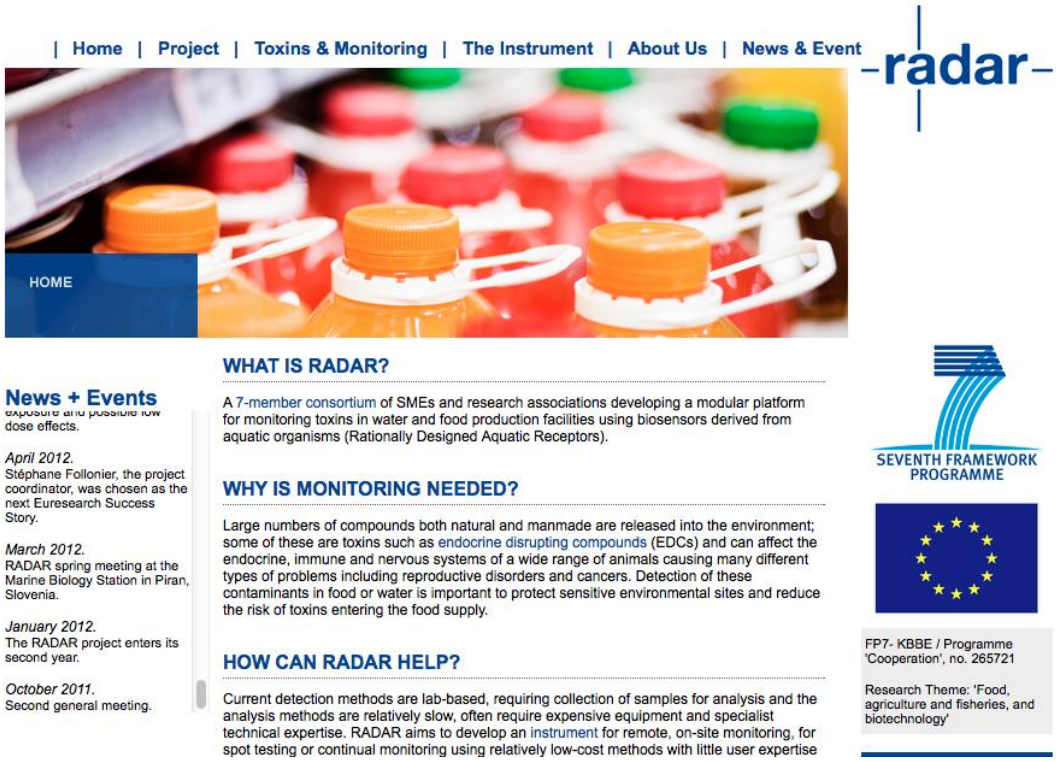


Figure 1 Home page of website

The website news function serves to highlight pieces in various media print, audio and video that are topical or of general interest. This includes following the developing story of the potential regulation of endocrine disruptors in the EU, US and other countries (see Figure 2 below)

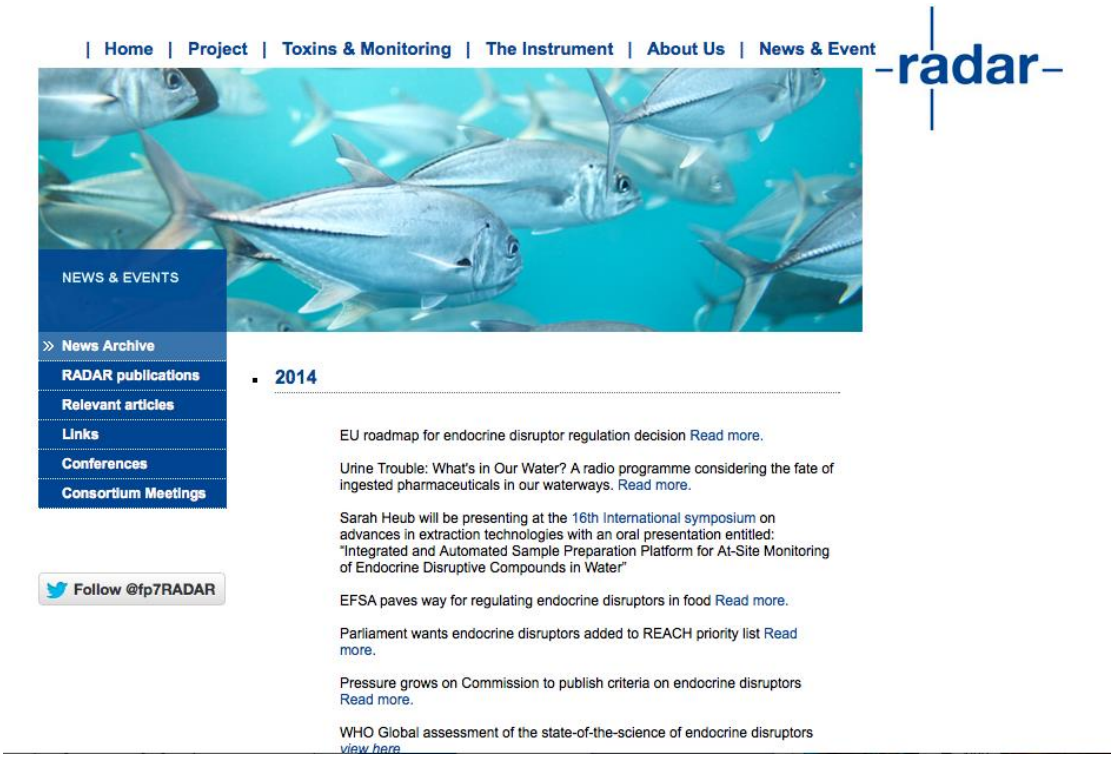


Figure 2 News & Events Page of RADAR website

Figure 2 also shows the tabs for publications from RADAR work and other relevant publications that may be of interest to the community. Consortium members taking part in dissemination activities such as the symposium on advances in extraction technologies are also illustrated in this figure.

The website also provides an opportunity to put faces to the names of consortium members through updates on consortium meetings (see Figure 3 a report on the Spring 2014 meeting at RIKILT in the Netherlands). The text in these reports is deliberately informal to democratise the work of EU-funded scientists and help the general public understand better that the issues being studied are real problems of relevance to all citizens.

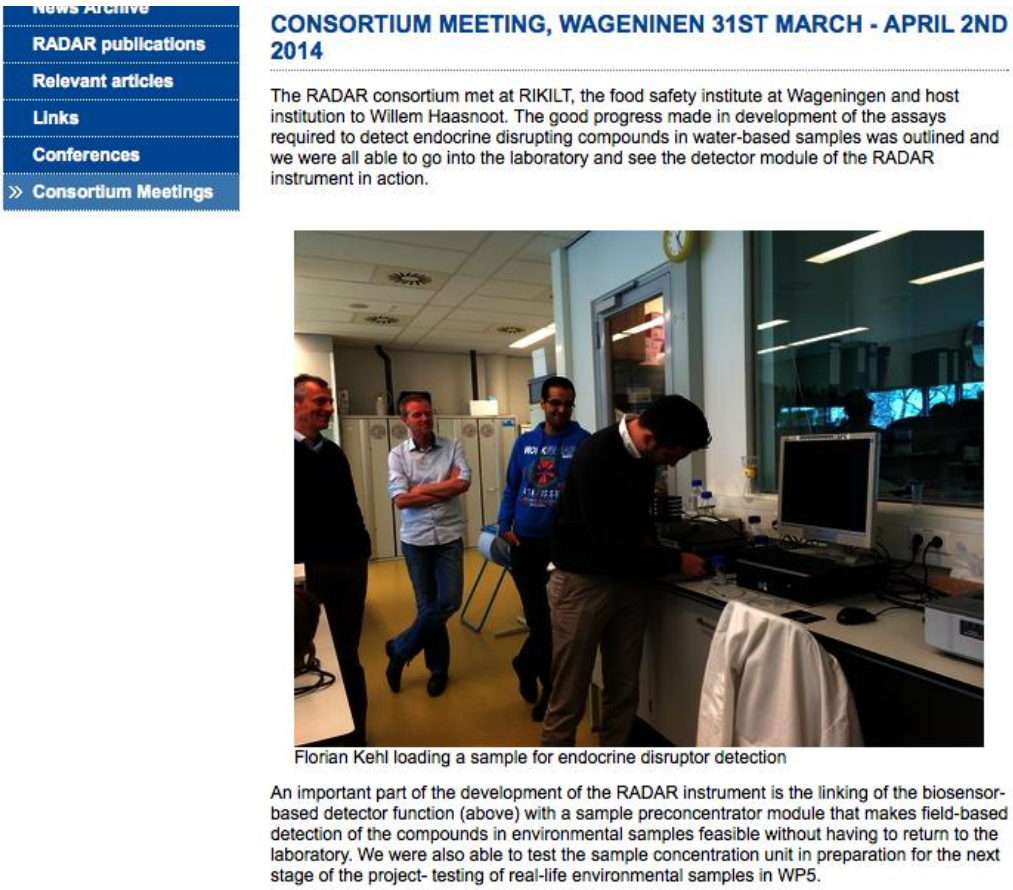


Figure 3 Consortium meeting report on website

The website provides links to other relevant websites for both general and specialist knowledge (see Figure 4).

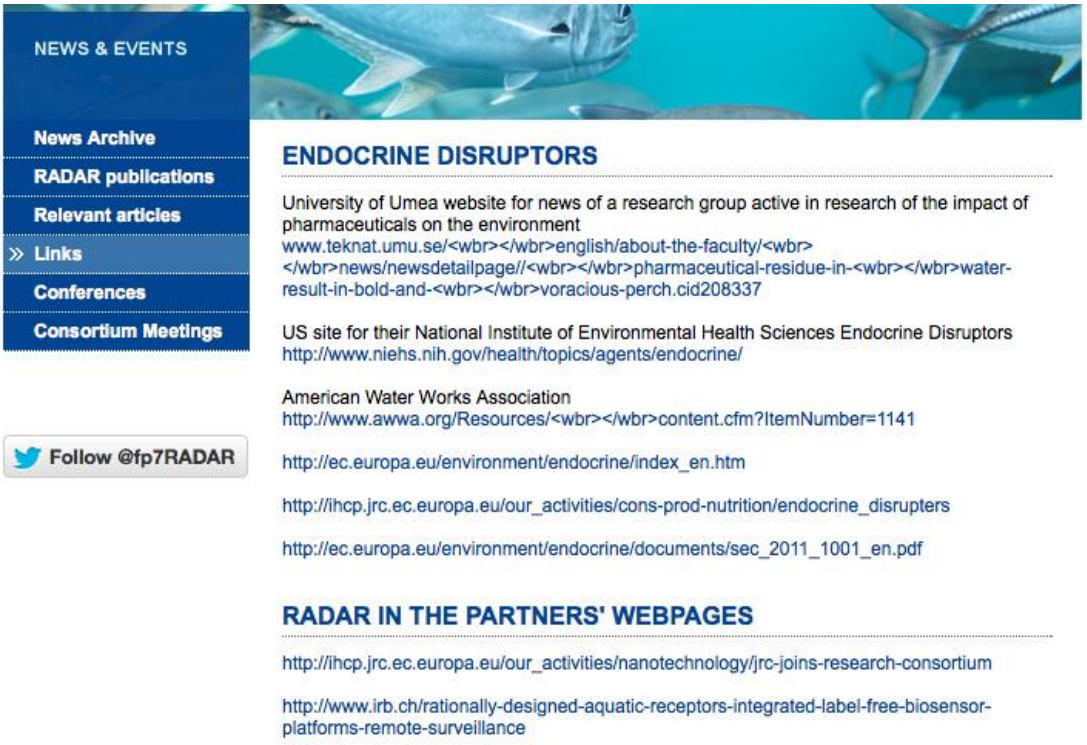


Figure 4 Links page on RADAR website

RADAR members also have posted information on other websites to encourage further dissemination of RADAR activities through our network of networks. The example shown in Figure 5 is a posting to the UK's Knowledge Transfer website with over 6000 registered subscribers. Postings were made on at least quarterly basis as the programme developed with a minimum in excess of 120 viewings for each post.

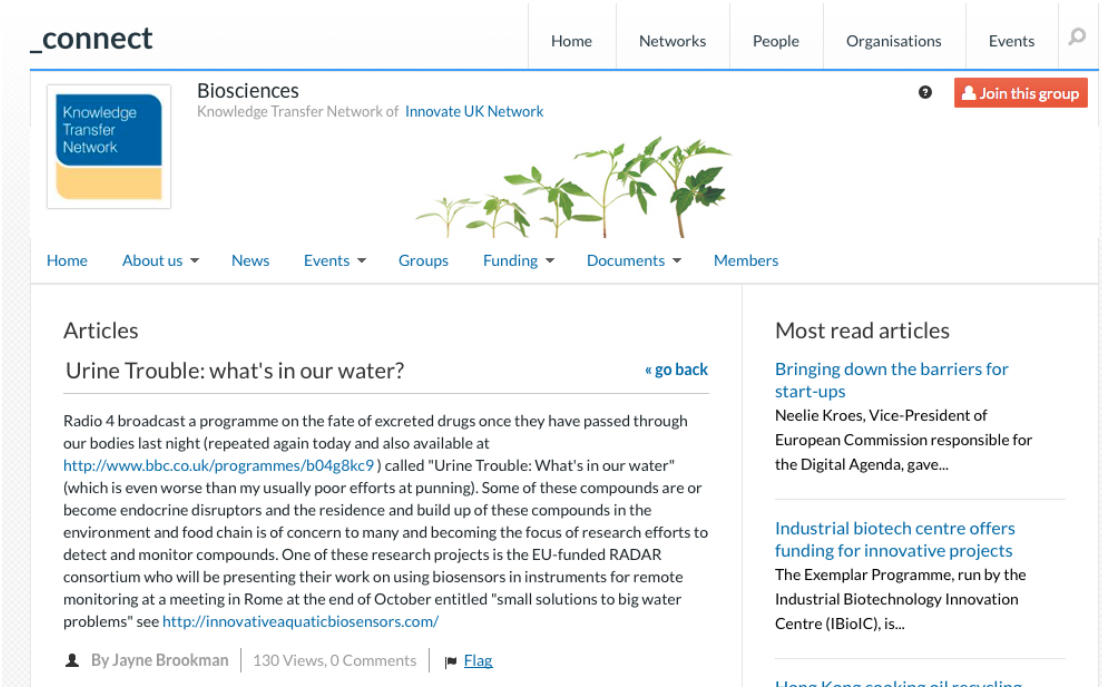


Figure 5 Post of RADAR activity on KTN website

2. Publications and Promotional Materials: Scientific and Learned Articles:

The RADAR consortium has made a series of posters for scientific dissemination at learned meetings etc. and these have been catalogued in the periodical reports for RADAR (Reports on Deliverables RADAR 6-2, 6-3 and 6-4).

The most recent success for the team is that the article submitted by Sarah Heub and colleagues to the scientific publication, Journal of Chromatography A, has been accepted for publication and is available on-line from 8th January 2015 see screen shot of article below in Figure 5.

The RADAR website will be updated as further scientific and popular publications are accepted from the RADAR project outputs through 2015.

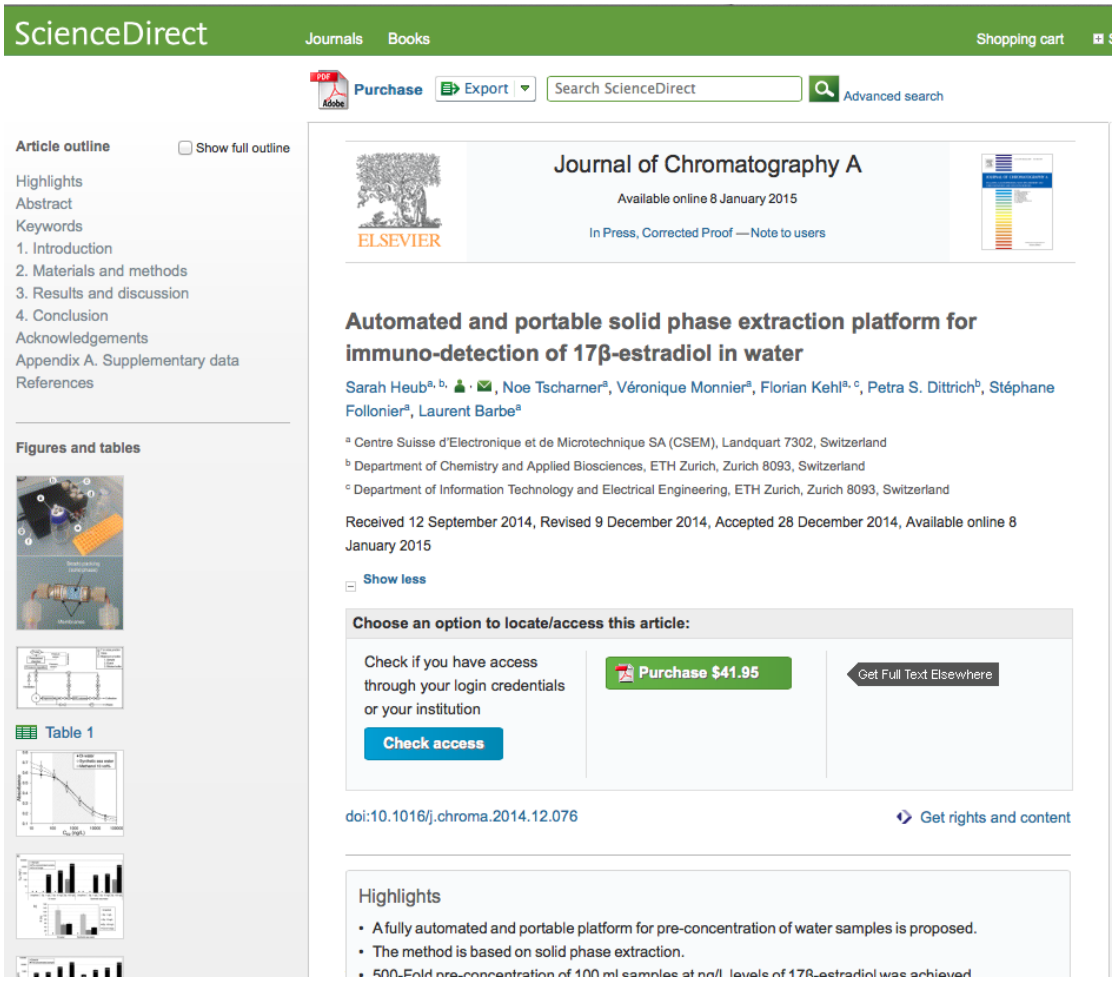


Figure 6. Screen Shot Showing Heub et al. in press

3. Publications and Promotional Materials: RADAR Conference

The RADAR consortium organised a joint symposium with the FP7 project µAQUA to bring together two communities working on different but overlapping areas of concern for the water industry.

The event was called Small Solutions for Big Water-Related Problems (see homepage of website pasted below) and was held at the Italian National Institute for Health, in Rome on the 28-30th October 2014. The symposium had 145 delegates, 30 speakers and a similar number of poster presentations. A short film showing a tree planting ceremony during the conference and clips from the meeting is available on YouTube .

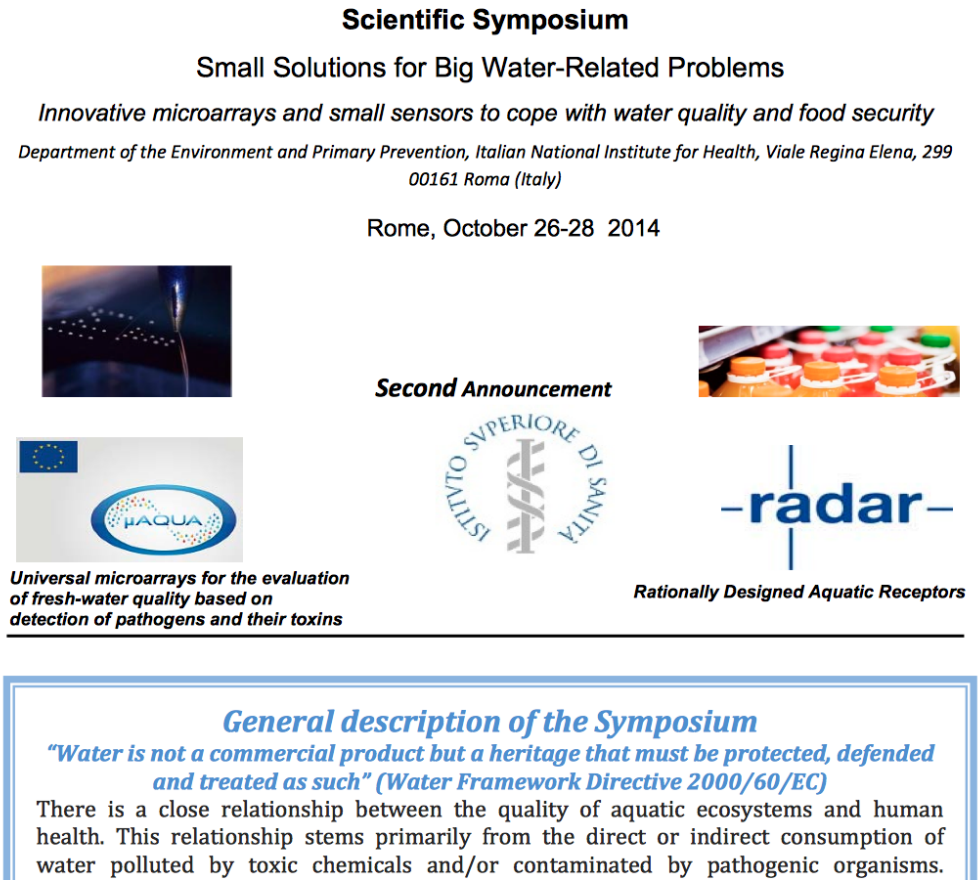
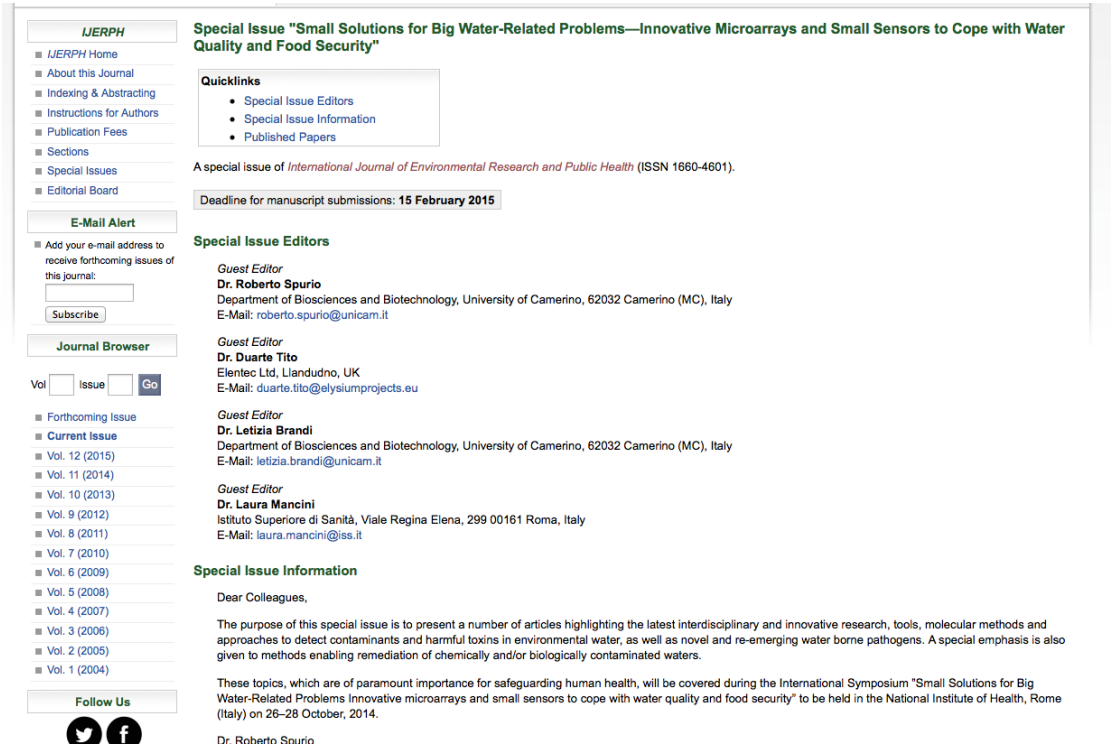


Figure 7. Screenshot of Small Solutions for Big Water-Related Problems website

The presentations from the meeting, including 5 RADAR contributions (outlined in RADAR deliverables reports 6-2 and 6-4) will contribute to a special edition of the scientific journal International Journal of Environmental Research and Public Health, see link and screenshot below.



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- Vol. 6 (2009)
- Vol. 5 (2008)
- Vol. 4 (2007)
- Vol. 3 (2006)
- Vol. 2 (2005)
- Vol. 1 (2004)

Follow Us

Special Issue "Small Solutions for Big Water-Related Problems—Innovative Microarrays and Small Sensors to Cope with Water Quality and Food Security"

Quicklinks

- Special Issue Editors
- Special Issue Information
- Published Papers

A special issue of *International Journal of Environmental Research and Public Health* (ISSN 1660-4601).

Deadline for manuscript submissions: **15 February 2015**

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Special Issue Information

Dear Colleagues,

The purpose of this special issue is to present a number of articles highlighting the latest interdisciplinary and innovative research, tools, molecular methods and approaches to detect contaminants and harmful toxins in environmental water, as well as novel and re-emerging water borne pathogens. A special emphasis is also given to methods enabling remediation of chemically and/or biologically contaminated waters.

These topics, which are of paramount importance for safeguarding human health, will be covered during the International Symposium "Small Solutions for Big Water-Related Problems Innovative microarrays and small sensors to cope with water quality and food security" to be held in the National Institute of Health, Rome (Italy) on 26–28 October, 2014.

Dr. Roberto Spurio

Figure 8 Screenshot showing details of special edition of International Journal of Environmental Research and Public Health resulting from our conference.

4. Publications and Promotional Materials: Summary Factsheets

A summary factsheet outlining the achievements of the RADAR project is in draft form (see Figure 8 below) and will be finalised once the last set of testing data is available for distribution (Jan/Feb 2015). This will be disseminated widely and will be downloadable from the RADAR website together with many other of the presentations and publications of a more technical nature generated through the course of the RADAR programme.

The Technology Offering Documents (see RADAR Commercialisation Plan and fragment replicated below) outlining the technological outputs of the RADAR project and seeking to discuss partnering or licence opportunities serve mainly as a way to provide information for people considering working with RADAR to help develop the research outputs commercially. These will also be available on the website for download for anyone interested in finding out a little more about the outputs from our research.

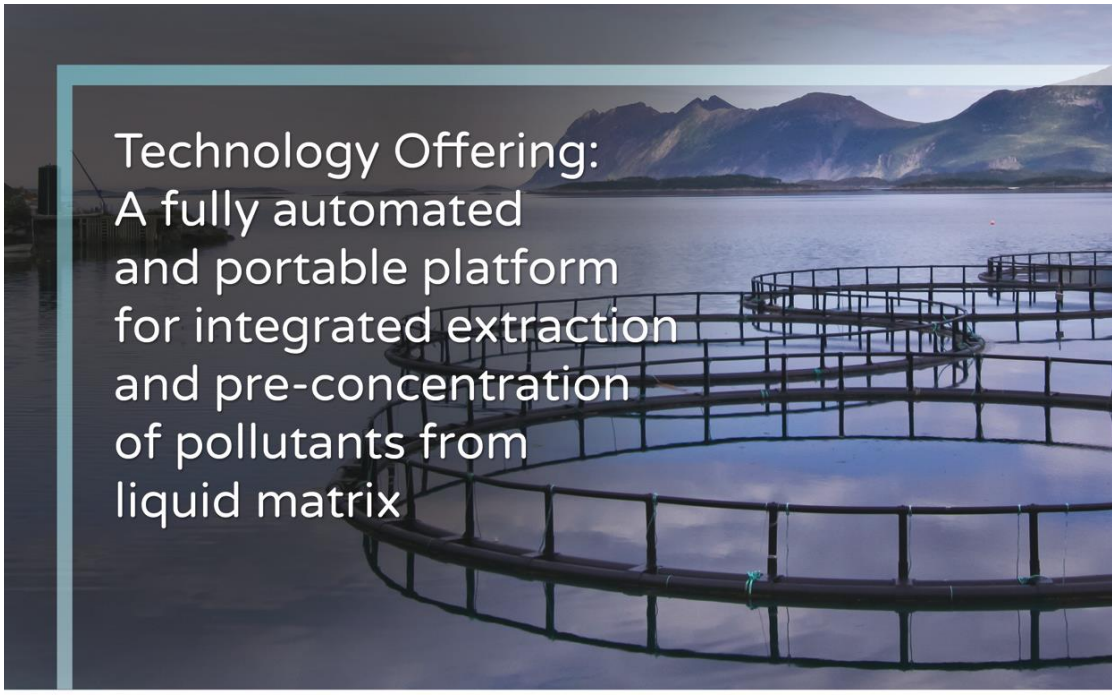
Technology Offering Documents

An electronic document provided in PDF will be developed for the various technology offerings as they are finalised:

1. Sample Preparation System
2. ARGOS Instrument
3. Biosensor receptor assay for the detection of oestrogens in water
4. RADAR integrated instrument for the detection of oestrogens

These documents provide an easy reference material for the technology under consideration and will be used to initiate discussions with prospective partners for commercialisation.

The first of these factsheets is pasted below.



Project Details

A 7-member consortium of SMEs and research associations developing a modular platform for monitoring toxins in water and food production facilities using biosensors derived from aquatic organisms (Rationally Designed Aquatic Receptors). Funded under the FP7-KBBE programme 2011-2014.

This innovative platform for liquid sample preparation has been developed as part of an EU project (RADAR) developing a real-time, label-free, biosensor-based monitoring system for toxins and pollutants using the endocrine-disrupting hormone 17 α-ethinylestradiol (EE2, found in many oral contraceptives) and the natural hormone 17β-estradiol (E2) as test compounds. The extraction system is automated, portable and modular in nature, so it can be used independently or in-line with a biosensor (see Figure 1 for prototype in use).

The system incorporates a micro- in Figure 2, and fabricated to support the channels and hold the miniaturized solid phase extraction (SPE) column for sample pre-concentration before target chemical detection. A pre-concentration step is necessary as the target chemical is present at very low levels in environmental formed by an elastomer gasket pressed between two plastic layers made from polyether-ether-ketone (PEEK) chosen for its chemical resistance and as it was free in composition from contaminating endocrine disrupting compounds. The sample preparation system is modular and contained within a single 25x25x10cm unit with ports loading of the solutions required for immunoassay, see Figure 1, and it also holds the solid phase extraction removable chip (Figure 2, 2 x 1.5 x 0.6cm)

The sample preparation system has been tested in its full prototype form using model and environmental samples including seawater from the Adriatic Sea and from an on-shore β-estradiol (E2) at 1, 10 or 100ngl-1 with less than 100ml solution were successfully concentrated with recovery rates ranging between 24 and 100% depending on the sample matrix. The concentration achieved is in the range of 10 to 1000-fold. Furthermore, the concentrated sample is directly compatible with ELISA or biosensor based assay systems.

Benefits of Sample Preparation System Offered

The extraction platform is particularly suitable for applications where the target compound is present at low levels, such as in environmental monitoring of watercourses or food production lines or where there is a desire for continuous or frequent monitoring.

It has several advantages over traditional solid phase extraction methods including:

- an automated system designed for minimal user requirements
- portable
- substantially faster concentration cycle < 2h
- very low solvent use
- ELISA/biosensor-friendly output

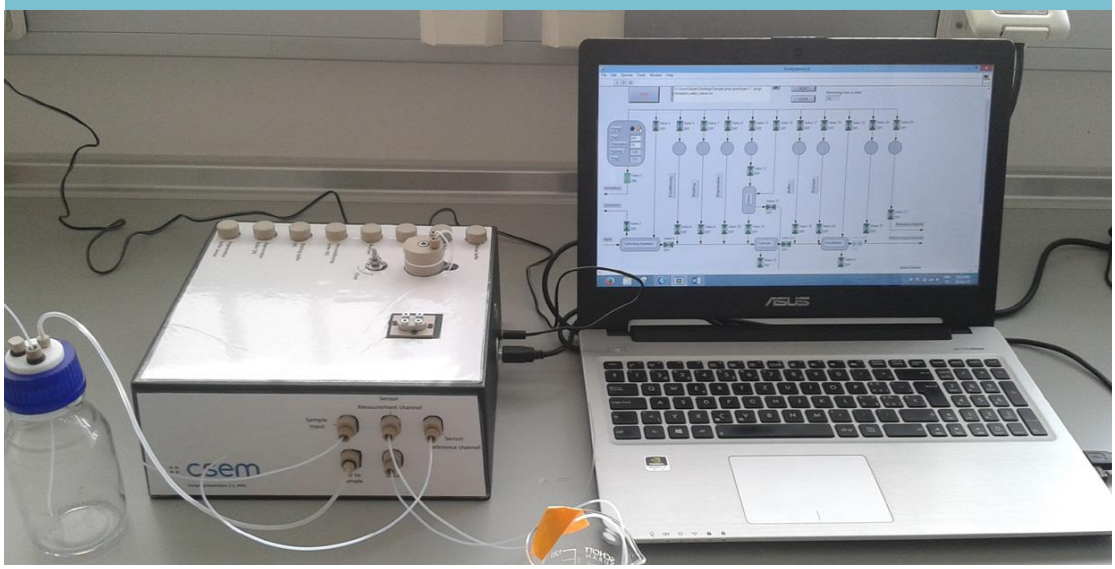


Figure 1: complete system as used on a bench.

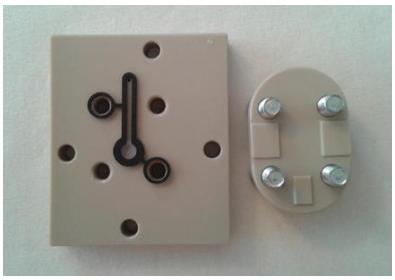


Figure 2: exchangeable micro-SPE chip.

customising the unit for your particular sample preparation needs or if you are interested in discussing commercialisation routes please contact:

Dr Silvia Generelli, CSEM, email: silvia.generelli@csem.ch

5. Popular Articles and Future Opportunities

Raising a broader awareness of the RADAR outputs and the context of the work performed is important and the RADAR consortium are keen to engage and inform as wide an audience as possible. We have approached two learned societies with broad technical membership to offer contributions to their member magazines to spread the message. We will continue to seek opportunities to publish popular and summary type articles throughout the course of 2015.

Elysium and all other consortium members will also use their networks to disseminate the RADAR outputs further to relevant academic, industrial and policy recipients whenever the opportunity arises.

For example, Elysium has been asked to address the Sensors for Water Interest Group swig at their Summer meeting and will discuss the RADAR project. Similarly they will disseminate orally at a number of UK water interest groups through 2015. Materials will be shared with UK industry sector groups through the Knowledge Transfer Network in the UK.

The dissemination of RADAR outputs will continue throughout 2015 and the website will be maintained by Elysium and updated regularly. Furthermore full support will be given to consortium members wishing to develop the commercialisation of the RADAR opportunities further.

The dissemination and exploitation work package (WP6) is led by Elysium Projects Ltd. with all consortium members taking an active role throughout the four years of the RADAR project. The work package aims to ensure that the work done within the project to develop a robust and portable instrument for the monitoring and detection of endocrine disrupting compounds in environmental samples is well known within the scientific community and awareness of the surrounding issues and opportunities raised for the general public. The exploitation component of the work aims to ensure that wherever possible the scientific and technological advances made during the course of the project are developed for both commercial and social benefit.

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES

NO.	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers[2] (if available)	Is/Will open access[3] provided?
1	Receptor-based high-throughput screening and identification of estrogens in dietary supplements using bioaffinity liquid-chromatography ion mobility mass spectrometry	P. Aqai, L. Varani, V. E. F. Ferrero, W. Haasnoot et al.	Analytical and Bioanalytical Chemistry		Springer Verlag		01/10/2013	epub	10.1007/s00216-013-7384-1	yes
2	Rational Modification of Estrogen Receptor by Combination of Computational and Experimental Analysis	V. Ferrero, T.Lettieri et al.	PLoS ONE	Volume 9 July 2014 |			2014	pp 1-10	doi:10.1371/journal.pone.0102658	yes

[2] A permanent identifier should be a persistent link to the published version full text if open access or abstract if article is pay per view) or to the final manuscript accepted for publication (link to article in repository).
[3] Open Access is defined as free of charge access for anyone via Internet. Please answer "yes" if the open access to the publication is already established and also if the embargo period for open access is not yet over but you intend to establish open access afterwards.

3	Mixtures of Chemical Pollutants at European Legislation Safety Concentrations: How Safe are They?	R.Carvalho, T. Lettieri et al.	Toxicological Science	13 August 2013	Oxford University Press on behalf of Toxicological Sciences		2014	pp 1-16	doi: 10.1093/toxsci/kfu118	yes
4	FEM-Based Method for the Simulation of Dielectric Waveguide Grating Biosensors,	T. Guillod, F. Kehl, and C. V. Hafner	European Economy	Vol. 137	Progress In Electromagnetics Research		2013	565-583	doi:10.2528/PIER13020502	yes
5	Automated and portable solid phase extraction platform for immuno-detection of 17β-estradiol in water	S. Heub , N. Tscharner , V. Monnier , F. Kehl , S. Follonier et al.	Journal of Chromatography A	Vol. 1381	Elsevier	Netherlands	01/02/2015	22-28	10.1016/j.chroma.2014.12.076	no

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES								
NO.	Type of activities[4]	Main leader	Title	Date	Place	Type of audience[5]	Size of audience	Countries addressed
1	Web sites/Applications	Elysium Projects Limited	RADAR project website: www.fp7-radar.eu	30/09/2011	-	Civil society	0	-
2	Posters	OPTICS BALZERS AG	3rd International Conference on Bio-Sensing Technology	12/05/2013	Sitges, Spain	Scientific community (higher education, Research)		Worldwide
3	Posters	JRC -JOINT RESEARCH CENTRE-EUROPEAN COMMISSION	3rd International Conference on Bio-Sensing Technology	12/05/2013	Sitges, Spain	Scientific community (higher education, Research)		Worldwide

[4] A drop down list allows choosing the dissemination activity: publications, conferences, workshops, web, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters, Other.
[5] A drop down list allows choosing the type of public: Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias ('multiple choices' is possible.

4	Posters	JRC -JOINT RESEARCH CENTRE-EUROPEAN COMMISSION	3rd International Conference on Bio-Sensing Technology	12/05/2013	Sitges, Spain	Scientific community (higher education, Research)		Worldwide
5	Posters	CSEM CENTRE SUISSE D'ELECTRONIQUE ET DE MICROTECHNIQUE SA - RECHERCHE ET DEVELOPPEMENT	Label-free Technologies	01/11/2012	Amsterdam	Scientific community (higher education, Research) - Industry		Worldwide
6	Posters	CSEM CENTRE SUISSE D'ELECTRONIQUE ET DE MICROTECHNIQUE SA - RECHERCHE ET DEVELOPPEMENT	Integrated and automated sample preparation platform for at-site monitoring of endocrine disruptive compounds in water	09/05/2013	San Diego, CA, USA	Scientific community (higher education, Research) - Industry		Worldwide
7	Posters	CSEM CENTRE SUISSE D'ELECTRONIQUE ET DE MICROTECHNIQUE SA - RECHERCHE ET DEVELOPPEMENT	Integrated and automated sample preparation platform for at-site monitoring of endocrine disruptive compounds in water	18/06/2012	Varese, Italy	Scientific community (higher education, Research)		Worldwide
8	Posters	JRC -JOINT RESEARCH CENTRE-EUROPEAN COMMISSION	Design of recognition biological elements for label-free biosensor platforms for detection of endocrine disruptor pollutants in water	18/06/2012	Varese, Italy	Scientific community (higher education, Research)		Worldwide

9	Oral presentation to a scientific event	JRC -JOINT RESEARCH CENTRE-EUROPEAN COMMISSION	Environmental Microbiology and Biotechnology in the frame of the knowledge-based bio and green economy	10/04/2014	Bologna (Italy)	Scientific community (higher education, Research) - Industry	100	all
10	Posters	JRC -JOINT RESEARCH CENTRE-EUROPEAN COMMISSION	SETAC Society for Environmental Toxicology and Chemistry	10/05/2014	Basel (Switzerland)	Scientific community (higher education, Research)	1500	all
11	Posters	JRC -JOINT RESEARCH CENTRE-EUROPEAN COMMISSION	SETAC Society for Environmental Toxicology and Chemistry	03/05/2014	Barcelona (Spain)	Scientific community (higher education, Research)	200	all
12	Oral presentation to a scientific event	CSEM CENTRE SUISSE D'ELECTRONIQUE ET DE MICROTECHNIQUE SA - RECHERCHE ET DEVELOPPEMENT	RADAR: an innovative platform for remote surveillance of chemical pollutants and toxins	26/10/2014	Rome (Italy)	Scientific community (higher education, Research) - Policy makers	80	Europe
13	Oral presentation to a scientific event	FONDAZIONE PER L'ISTITUTO DI RICERC A IN BIOMEDICINA	Rational design of estrogen receptors for biosensor development	26/10/2014	Rome (Italy)	Scientific community (higher education, Research) - Policy makers	80	Europe

14	Oral presentation to a scientific event	JRC -JOINT RESEARCH CENTRE-EUROPEAN COMMISSION	Label-free biosensor platforms for detection of endocrine disruptor pollutants	26/10/2014	Rome (Italy)	Scientific community (higher education, Research) - Policy makers	80	Europe
15	Oral presentation to a scientific event	CSEM CENTRE SUISSE D'ELECTRONIQUE ET DE MICROTECHNIQUE SA - RECHERCHE ET DEVELOPPEMENT	Combined FEM and analytical method for the simulation and optimization of planar sielectric waveguide grating biosensors	02/07/2012	Zurich (Switzerland)	Scientific community (higher education, Research)		all
16	Oral presentation to a scientific event	CSEM CENTRE SUISSE D'ELECTRONIQUE ET DE MICROTECHNIQUE SA - RECHERCHE ET DEVELOPPEMENT	Rationally designed aquatic receptors for on-site monitoring of endocrine disruptive compounds in waters and food processes	10/09/2014	Davos (Switzerland)	Scientific community (higher education, Research)		Switzerland
17	Oral presentation to a scientific event	CSEM CENTRE SUISSE D'ELECTRONIQUE ET DE MICROTECHNIQUE SA - RECHERCHE ET DEVELOPPEMENT	Integrated and automated sample preparation platform for at-site monitoring of endocrine disruptive compounds in water	25/05/2014	Chania (Crete)	Scientific community (higher education, Research)		Europe
18	Oral presentation to a scientific event	CSEM CENTRE SUISSE D'ELECTRONIQUE ET DE MICROTECHNIQUE SA - RECHERCHE ET	Fully automated and portable platform for integrated extraction and pre-concentration of toxins and pollutants from liquid samples	26/10/2014	Houston (USA)	Scientific community (higher education, Research)		all

		DEVELOPPEMENT						
19	Oral presentation to a scientific event	CSEM CENTRE SUISSE D'ELECTRONIQUE ET DE MICROTECHNIQUE SA - RECHERCHE ET DEVELOPPEMENT	Development of an integrated, label-free, waveguide grating based biosensor platform for on-the-spot measurements and online monitoring of toxins and pollutants in food production processes and in the aquatic environment	26/10/2014	Rome (Italy)	Scientific community (higher education, Research) - Policy makers	80	Europe
20	Posters	CSEM CENTRE SUISSE D'ELECTRONIQUE ET DE MICROTECHNIQUE SA - RECHERCHE ET DEVELOPPEMENT	Angle interrogating optical sensor ARGOS: scanning MEMS mirror for higher performance label-free optical biosensing	16/05/2012	Cancun (Mexico)	Scientific community (higher education, Research)		all
21	Posters	CSEM CENTRE SUISSE D'ELECTRONIQUE ET DE MICROTECHNIQUE SA - RECHERCHE ET DEVELOPPEMENT	Angle interrogating optical sensor ARGOS: scanning MEMS mirror for higher performance label-free optical biosensing	12/09/2012	Davos (Switzerland)	Scientific community (higher education, Research)		Switzerland
22	Posters	CSEM CENTRE SUISSE D'ELECTRONIQUE ET DE MICROTECHNIQUE SA - RECHERCHE ET DEVELOPPEMENT	Automated and portable sample preparation platform for immuno-detection of toxins and pollutants in liquid samples	23/07/2014	Heidelberg (Germany)	Scientific community (higher education, Research) - Industry		all

23	Posters	CSEM CENTRE SUISSE D'ELECTRONIQUE ET DE MICROTECHNIQUE SA - RECHERCHE ET DEVELOPPEMENT	Fabrication, simulation and chaacterization of waveguide grating based biosensors	22/09/2014	Lausanne (Switzerland)	Scientific community (higher education, Research)		all
24	Oral presentation to a scientific event	Elysium Projects Limited	Small solutions of rbig water-related problems	27/10/2014	London (UK)	Scientific community (higher education, Research) - Industry	75	Europe

Section B (Confidential[6] or public: confidential information to be marked clearly)

Part B1

TEMPLATE B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.					
Type of IP Rights[7]:	**Confidential Click on YES/NO**	**Foreseen embargo date dd/mm/yyyy**	**Application reference(s) (e.g. EP123456)**	**Subject or title of application**	**Applicant (s) (as on the application)**
Patent	yes		WO2014EP61740	MEASUREMENT METHOD BASED ON AN OPTICAL WAVEGUIDE SENSOR SYSTEM	CSEM Centre Suisse d'Electronique et de Microtechnique SA
Provisional patent	yes		EP14169774.8	Portable system for automated preparation of liquid and/or solid samples	CSEM Centre Suisse d'Electronique et de Microtechnique SA

[6] Note to be confused with the "EU CONFIDENTIAL" classification for some security research projects.

[7] A drop down list allows choosing the type of IP rights: Patents, Trademarks, Registered designs, Utility models, Others.

Part B2

Type of Exploitable Foreground[8]	Description of exploitable foreground	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application[9]	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
Commercial exploitation of R&D results	Measurement method based on waverlength interrogation on the output pad of a waveguide sensor system	Yes		The "ARGOS V2" measurement instrument	A3.2. Aquaculture; C10.5. Manufacturing of dairy products, C11.0. Manufacture of beverages, E. Water	2015	WO2014EP61740	CSEM Centre Suisse d'Electronique et de Microtechnique SA
Commercial exploitation of R&D results	Automated sample preconcentration and preparation module "SPU"	Yes		Automated prototype of the Sample Preparation Unit	A3.2. Aquaculture; C10.5. Manufacturing of dairy products, C11.0. Manufacture of beverages, E. Water	2015	EP14169774.8 (provisional)	CSEM Centre Suisse d'Electronique et de Microtechnique SA
Commercial exploitation of R&D results	Aryl Hydrocarbon Receptor ligand binding domain AhR2LBD	Yes		The AhR2LBD as part of a kit for the detection of potential toxicants	A3.2. Aquaculture; C10.5. Manufacturing of dairy products, C11.0. Manufacture of beverages, E. Water	2016	In preparation	JRC - Joint Research Centre - European Commission; Fondazione per l'Istituto di Ricerca in Biomedicina
Commercial exploitation of R&D results	Estrogen receptor (ER) mutants	Yes		The ER mutants as part of a kit for the detection of potential endocrine disruptors	A3.2. Aquaculture; C10.5. Manufacturing of dairy products, C11.0. Manufacture of beverages, E. Water	2016	In preparation	JRC - Joint Research Centre - European Commission; Fondazione per l'Istituto di Ricerca in Biomedicina,
Commercial	RADAR instrument	Yes		Monitoring instrument	A3.2. Aquaculture;	2016		Consortium

[19] A drop down list allows choosing the type of foreground: General advancement of knowledge, Commercial exploitation of R&D results, Exploitation of R&D results via standards, exploitation of results through EU policies, exploitation of results through (social) innovation.
[9] A drop down list allows choosing the type sector (NACE nomenclature) : http://ec.europa.eu/competition/mergers/cases/index/nace_all.html

Type of Exploitable Foreground[8]	Description of exploitable foreground	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application[9]	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
exploitation of R&D results				for at-site monitoring of endocrine disruptive compounds	C10.5. Manufacturing of dairy products, C11.0. Manufacture of beverages, E. Water			
Commercial exploitation of R&D results	Biosensor receptor assay	Yes		Assay kit for endocrine disruptive compounds	A3.2. Aquaculture; C10.5. Manufacturing of dairy products, C11.0. Manufacture of beverages, E. Water	2014	Interest being evaluated	JRC - Joint Research Centre - European Commission; Fondazione per l'Istituto di Ricerca in Biomedicina, RIKILT

Measurement method based on wavelength interrogation on the output pad of a waveguide sensor system

The measurement method using waelenght interrogation of the output pad of a waveguide sensor system has been protected by patent. Thanks to this new measurement method, in concomitance of the optimization of the waveguide geometry, the wavelenght of the light used for the interrogation and other technical parameters, it has been possible to reach a quality of the signal unreached so far with this type of technology, at a price very competitive with the system that are at this day on the market with the same signal quality characteristics.

In addition Optics Balzers AG will exploit the know-how on waveguide-based biochips to expand the portfolio of his offerings. OBL will use the on-line monitoring platform as a basis for a field deployable biosensor and present it to its extended network of potential partners and users. One person has been hired in the company to continuously monitor the ongoing biosensors market and other relevant market segments.

In addition Optics Balzers AG will exploit the know-how on waveguide-based biochips to expand the portfolio of his offerings. OBL will use the on-line monitoring platform as a basis for a field deployable biosensor and present it to its extended network of potential partners and users. One person has been hired in the company to continuously monitor the ongoing biosensors market and other relevant market segments.

Automated sample pre-concentration and preparation module "SPU"

CSEM will benefit from the project results with the demonstration of its know-how in the field of sample preparation. The compact, automated sample preparation setup raised the interest of the analytical laboratories offering water analysis services. The use of the device will shorten the time of sample preparation from 1-2 days, typically, to 1 to several hours, without the need of intervention by an operator during the process. The system has been protected by a provisional patent, while the full patent is pending. Even though the actual prototype is not suitable yet for direct industrialization, the commercial potential of the device pushes CSEM to further invest in the active research on industrial partners for commercialization of the SPU.

Aryl Hydrocarbon Receptor ligand binding domain AhR2LBD

The IRB is a world leading institute in the rational modification and production of human antibodies; with RADAR it demonstrated its ability to also design and produce recombinant proteins with high quality control. It will thus become a recognized player in the field of protein design, which is expected to have an expanding importance in years to come.

The AhR is a particularly interesting receptor from the commercial viewpoint, as there is at this stage no toxicity assessment assay based on this protein. Work at JRC is in progress - outside the framework of RADAR - to develop a fluorescent assay kit based on the AhR2LBD developed in RADAR. As the commercial potential of this application is high, patentability and commercialization of both AhR2LBD molecule and the future kit are being evaluated.

Estrogen receptor (ER) mutants

The IRB is a world leading institute in the rational modification and production of human antibodies; with RADAR it demonstrated its ability to also design and produce recombinant proteins with high quality control. It will thus become a recognized player in the field of protein design, which is expected to have an expanding importance in years to come.

As for the AhR, the mutants of ER are newly synthetized molecules, ans, as it has been shown in WP1, they show different affinities for different classes of toxicants.

RADAR instrument

CSEM will benefit from the project results with the demonstration of its know-how in the field of sample preparation and system integration for toxin and pollutant sensor-based applications. CSEM will be able to exploit IP developed within this project as well as background information in future industrial projects. It is commercially very interesting, since it streamlines on the same knowledge axis created within previous detection systems, thus providing additional value to commercial exploitation effort in this field. Potential applications for this technology extend beyond the domains of environmental monitoring and surveillance to, for example, safety, health, automotive and personal applications.

Biosensor receptor assay

Based on the ER-wt the RIKILT developed a SPR-based toxicity assay to detect and quantify estrogen disruptive compounds. The assay proved very robust during the tests with E2, and is thus of great commercial interest, as well as of immediate interest for RIKILT, that can now add this offer to their analytical services portfolio.

Moreover RIKILT, JRC and MBS will exploit the new knowledge and expertise gained within the project for industrial and governmental projects and will, where applicable, use the newly developed assays for their legislative tasks. RIKILT will focus on the field and agriculture market. JRC will mostly exploit the foreground applications in the field of environmental monitoring of European waters (Institute for Environment and Sustainability) and food products monitoring (Institute for Health and Consumers Protection). MBS will apply the new knowledge in marine monitoring, especially in offshore fish farms and in wastewater.

Elysium Projects Ltd. will present the RADAR project foreground as products and services to potential commercial partners and will work actively during 2014 with the academic members and other SMEs to ensure that all opportunities are exploited. Effort will be done to give the project maximum visibility (see dissemination).

The commercial partner search strategy will be a mixture of approaching known contacts within individual consortium member's networks and a more generic approach based on use of technology offering factsheets (see below). These factsheets will be sent to a range of potentially interested parties as detailed in the dissemination report, to the companies identified in this report, to personal contacts and also to a number of service websites/fora, including LinkedIn groups publicising the technology offerings available.

Individual consortium members will use the technology factsheets to start discussions with their network of interested companies. Elysium has already initiated a discussion with technology intermediaries such as Isle Utilities (http://www.isleutilities.com) who have EU-wide links and scope.

Technology Offering Documents

An electronic document provided in PDF will be developed for the various technology offerings as they are finalised:

1. Sample Preparation System
2. ARGOS Instrument
3. Biosensor receptor assay for the detection of oestrogens in water
4. RADAR integrated instrument for the detection of oestrogens

These documents provide an easy reference material for the technology under consideration and will be used to initiate discussions with prospective partners for commercialisation.

The financial aspects of prototype and product development can be supplied by a partnership with a larger partner and/or attracting new funds for the project. These can be through the usual financial channels such as debt, angel or venture capital funds. An alternative approach is to use crowdfunding either for the project or to fund a company through equity funding. The RADAR outputs would have a reasonable chance of success in this type of funding activity as they could be easily communicated to potential funders.

There are several relevant crowdfunders within the EU, the Netherlands in particular has relatively relaxed legislation on this type of funding. One Planet Crowd offers subordinated convertible loans to finance projects which are converted to equity at a later funding stage (oneplanetcrowd) . Indiegogo has a technology section (indiegogo) and is a well established platform as is Crowdcube (crowdcube)

Purchasing decisions for on-line water monitoring solutions were found to be technically driven with individuals responsible within companies performing their own practical evaluations of the suitability of individual monitoring systems for the role required (see van den Broeke et al. (2014) Compendium of Sensors and Monitors and Their Use in the Global Water Industry, published by WERF) This indicates that a working prototype/basic model system with bespoke modifications plus a maintenance contract may be a sensible market model to follow for commercialisation of the RADAR instrument.

- Assistance with the development of commercial offering through into a product is available from a number of EU-funded schemes including:
- the EIP Water on-line marketplace where there are groups that may be of assistance including a SME group in collaboration with the National Water Partnerships (sme support ag131)
- an eco-innovations gateway for information and publicity (ecoweb),
- the AugMent - Water Monitoring for Decision Support (AG124) group (augment) for information exchange and a report on funding for cleantech SMEs and researchers (financing).

The SME instrument is another potential route to develop the prototype further into the commercialisation pathway (sme-instrument). The consortium is considering one or more Eurostars application (eurostars) for the March deadline as part of the future effort to develop the standalone assays and the RADAR instrument.

In addition to forming part of the RADAR instrument the assay developed using the engineered receptor could be exploited as a stand-alone assay. Consortium discussions around the possible commercialisation options are outlined below together with some information on possible partners in addition to RIKILT's current commercialisation partners.

The ER Receptor alone can be offered for commercialisation in a partner's preferred format or the receptor can be developed as part of an "Assay in a box" offering where the reagents required are packaged together with the receptor and protocols for a complete service. Examples of this type of all-inclusive assay format are provided by INDIGO biosciences (see below) for their nuclear receptor cell line based assays. The assay would need to be developed in a format that was compatible with use in 96- and 384-well format to complement the equipment used by most research laboratories and possibly 1024-well format for high throughput screening.

An analysis of the freedom to act in a commercial environment with all parts of the proposed development in particular the use of receptor peptide combination in the assay would need to be performed as part of any commercialisation activity.

The advantage of the assay developed by RADAR consortium is that is more sensitive than the current ELISA method for the hormone. Very importantly it is effect driven rather than structural so can determine the presence of both known and unknown molecules binding to the receptor.

The market opportunity is likely to be for exploitation by diagnostic companies for applications in food environment and health. NGOs could be seen as a major potential market user as customers for monitoring activities in response to current concerns over the presence of EDCs in the food supply chain.

The receptor is an engineered protein expressed by transgenic cells and so is scalable in production and should provide a relatively easy source of material which is cheap to deliver at a commercial scale.

Future ideas for development of the assay include the possibility of developing it as part of a multiplex assay format lab on a chip for a range of pollutants rather than as a single assay. In terms of future

commercialisation negotiating a licence for 3rd party diagnostic companies to exploit is a simple route to market. RIKILT have used this approach with antibodies for a range of applications.

The consortium is considering developing this further via a EUROSTARS application.

Furthermore full support will be given to consortium members wishing to develop the commercialisation of the RADAR opportunities further. This will include Elysium providing support with applications such as EUROSTARS and in connecting with potential partner companies.

It is not possible yet to be precise on the financial impact of these commercialisation opportunities due to the development stages of the opportunities but the market sizes of the initial selected areas are in excess of €100m for many if not all of the applications so are likely to be worthy of consideration for further development. A full analysis of the markets open to the RADAR instrument and its components is provided in the commercialisation plan.

The initial stages of the commercialisation process have begun with discussions starting with potential investors and potential technology partners on routes to commercialising the RADAR outputs.

4.3 Report on societal implications

A General Information *(completed automatically when **Grant Agreement number** is entered.*

Grant Agreement Number:	265721
Title of Project:	Rationally designed aquatic receptors integrated in label-free biosensor platforms for remote surveillance of toxins and pollutants
Name and Title of Coordinator:	Dr. Stéphane Follonier, Vice President CSEM AG

B Ethics

1. Did your project undergo an Ethics Review (and/or Screening)? • If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements'*	***No***
2. Please indicate whether your project involved any of the following issues (tick box) :	***NO***
RESEARCH ON HUMANS	
• Did the project involve children?	No
• Did the project involve patients?	No
• Did the project involve persons not able to give consent?	No
• Did the project involve adult healthy volunteers?	No
• Did the project involve Human genetic material?	No
Did the project involve Human biological samples?	No
Did the project involve Human data collection?	No
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	No
• Did the project involve Human Foetal Tissue / Cells?	No
• Did the project involve Human Embryonic Stem Cells (hESCs)?	No
• Did the project on human Embryonic Stem Cells involve cells in culture?	No
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	No
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	No
• Did the project involve tracking the location or observation of people?	No
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	No
• Were those animals transgenic small laboratory animals?	No
• Were those animals transgenic farm animals?	No

• Were those animals cloned farm animals?	No
• Were those animals non-human primates?	No
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	No
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	No
DUAL USE	
• Research having direct military use	No
• Research having the potential for terrorist abuse	No

C Workforce Statistics

Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator		1
Work package leaders	3	4
Experienced researchers (i.e. PhD holders)	8	11
PhD Students	1	1
Other	3	4

How many additional researchers (in companies and universities) were recruited specifically for this project?	**5**
Of which, indicate the number of men:	1

D Gender Aspects

5. Did you carry out specific Gender Equality Actions under the project?

○ Yes
■ No

6. Which of the following actions did you carry out and how effective were they?

		Not at all effective				Very effective
❑	Design and implement an equal opportunity policy	○	○	○	○	○
❑	Set targets to achieve a gender balance in the workforce	○	○	○	○	○
❑	Organise conferences and workshops on gender	○	○	○	○	○
❑	Actions to improve work-life balance	○	○	○	○	○
○	Other:					

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

○ Yes- please specify

■ No

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

■ Yes- please specify

Master students and PhDs were formed during the project. Presentation for schools were organized

○ No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

■ Yes- please specify

Posters

○ No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

Main discipline[10]: 4.1
Associated discipline[10]: 2.2 ○ Associated discipline[10]:1.5

G Engaging with Civil society and policy makers

11a Did your project engage with societal actors beyond the research community? *(if 'No', go to Question 14)*

■ Yes
○ No

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

○ No
○ Yes- in determining what research should be performed
○ Yes - in implementing the research

[10] Insert number from list below (Frascati Manual).

■ Yes, in communicating /disseminating / using the results of the project

11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	○	Yes
	■	No

12. Did you engage with government / public bodies or policy makers (including international organisations)

○ No
○ Yes- in framing the research agenda
○ Yes - in implementing the research agenda
■ Yes, in communicating /disseminating / using the results of the project

13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

○ Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
■ Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
○ No

3b If Yes, in which fields?

Agriculture		Energy		Human rights	
Audiovisual and Media		Enlargement		Information Society	
Budget		Enterprise		Institutional affairs	
Competition		**Environment**		Internal Market	
Consumers		External Relations		Justice, freedom and security	
Culture		External Trade		**Public Health**	
Customs		**Fisheries and Maritime Affairs**		Regional Policy	
Development Economic and Monetary Affairs		**Food Safety**		Research and Innovation	
Education, Training, Youth		Foreign and Security Policy		Space	
Employment and Social Affairs		Fraud		Taxation	
		Humanitarian aid		Transport	

13c If Yes, at which level?

- ■ Local / regional levels
- ■ National level
- ■ European level
- ■ International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?	**5**
To how many of these is open access[11] provided?	**4**
How many of these are published in open access journals?	**4**
How many of these are published in open repositories?	**4**
To how many of these is open access not provided?	**1**
Please check all applicable reasons for not providing open access:	
❑ publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available ■ no suitable open access journal available ❑ no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other[12]: ……	

15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*		**2**
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	**0**
	Registered design	**0**
	Other	**0**
17. How many spin-off companies were created / are planned as a direct result of the project?		**0**
Indicate the approximate number of additional jobs in these companies:		

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

❑	Increase in employment, or	❑	In small & medium-sized enterprises
❑	Safeguard employment, or	❑	In large companies
❑	Decrease in employment,	❑	None of the above / not relevant to the project
■	Difficult to estimate / not possible to quantify		

[11] Open Access is defined as free of charge access for anyone via Internet.
[12] For instance: classification for security project.

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs:	*Indicate figure:*
Difficult to estimate / not possible to quantify	■

I Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

○ Yes ■ No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

■ Yes ○ No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

■	Press Release	■	Coverage in specialist press
❑	Media briefing	■	Coverage in general (non-specialist) press
❑	TV coverage / report	■	Coverage in national press
❑	Radio coverage / report	❑	Coverage in international press
■	Brochures /posters / flyers	■	Website for the general public / internet
❑	DVD /Film /Multimedia	■	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

Language of the coordinator	■	English
Other language(s)		

***Question F-10*:** Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]
1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)
1.3 Chemical sciences (chemistry, other allied subjects)
1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)
1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2 ENGINEERING AND TECHNOLOGY

2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)

2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]

2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)

3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)

3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)

4.2 Veterinary medicine

5. SOCIAL SCIENCES

5.1 Psychology

5.2 Economics

5.3 Educational sciences (education and training and other allied subjects)

5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary , methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)

6.2 Languages and literature (ancient and modern)

6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group]