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Deliverable 4.3. - Report on the review of bioassays and biosensors and (Q)SAR models as candidate for the intended use

Deliverable of WP4

D.4.3: Report on the review of bioassays and biosensors and (Q)SAR models as candidate for the intended use

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1 Introduction on the deliverable

A previous deliverable, Delvierable 4.1, introduced the concepts of alternative methods, and the two main catergories of in vitro and in siclio methods. We described the basic theory and the possible applications opened by using these advanced approaches. We also discussed the validation status and initiatives relative to some of these methods, as coordinated by ECVAM. These activities are mainly addressing bioassays, and we reported a table with a series of endpoints.

Here we cover a more practical analysis of the existing methods and tools, which can be already adopted and applied by stakeholders.

Thus, this document is dedicated to the possible solutions present on the market or freely available, and on this basis the stakeholders may find useful solutions to the issue to obtain information on the properties of interest for the characterisation of the waste material and contaminants it contains.

Some important general considerations should be done, to clarify the context of the use of these alternative tools.

As we clarified within Deliverable 4.1 some regulatory initiatives exist, dedicated to the validation of the alternative methods. However, the REACH legislation clarly refers to "valid" QSAR methods, and not to "validated" methods. Explaining this, Annex XI of REACH states the "scientific validity" should be considered. Thus, for QSAR methods, which represent a major group of the in silico methods (other in silico methods are the docking methods, which are important for pharmaceutical industry and drug discovery, and will not be addressed here), it is important to underline that, according to legislation, the scientific validity is the key factor, and not a formal process of validation.

Thus, we will adhere to this position.

Similarly, we remember that also for the practical application of bioassays, such as biosensors and tools which can be easily implemented to produce high number of values for many compounds, the recent position of the US is to take into consideration the scientific validity of the tool, and not necessaliry the validation process. This is the philosophy adopted within the Tox21 and ToxCast initiatives. The reason for this is that the validation process takes ten years or more before a method is officially approved, and this period would seriously limit the possibility to rapidly pro-

duce the necessary data needed within the next few years. Furthermore, the techniques are rapidly changing.

Thus, also in case of the biosensors and bioassays, what we describe in this deliverable is not necessarily the result of a formal validation process (for this refer to Deliverable 4.1 and the information provided there), but conversely we preferred to provide a list which is scientifically updated and may offer advanced, modern tools useful to stakeholders.

A second important consideration refers to the applicability of these two different categories of approaches. QSAR methods have to be applied to individual chemicals. The QSAR approach can be profitably used coupled to a chemical analysis, exploting the information on the contaminant occurrence. However, if the user wants to address a mixture of chemicals, they have to be addressed separately and individually. In case of unknown compounds, QSAR cannot be used.

Conversely, bioassays and biosensors can be used on the whole waste, and provide replies which refer also to the mixture effects.

2 *Biosensors and bioassays*

A series of practical tools can be used. Table 1 lists them while Table 2 more specifically addresses some advanced devises using nanomaterials.

From this overview it is clear that tools exist for ecotoxicological endpoints, such as acute toxicity. In addition, some tools can be applied for human toxicity endpoints, like genotoxicity. Other tools refer to the particular situation of the endocrine disruptors, where tools are listed too.

These tools can be applied both the individual contaminant and to the waste.

As expected, tools related to other properties are lacking, such as physico-chemical properties.

Table 1: Bioluminescence and fluorescence bacterial biosensors and some estrogenic receptor based (ER) bioassays reported in the literature.

Effect	Microorganism	Transduction		References
		Luminescent or fluorescent	Natural or induced	
Acute toxicity	*Pseudomonas fluorescens*	Fluorescence	10568	(Bhattacharyya et al. 2005)
	Vibrio fischeri	Bioluminescence	Natural	(Bhattacharyya et al. 2005) (Lee and Gu 2005)
	Photobacterium phosphoreum			(Pooley et al. 2004) (Yoo et al. 2007)
	Escherichia coli	Luminescence	DH5α	(Bhattacharyya et al. 2005)
			HB101	(Bhattacharyya et al. 2005)
	Pseudomonas putida		TVA8	(Bhattacharyya et al. 2005)
Genotoxicity	*Photobacterium phosphoreum*	Bioluminescence	Dark variant	(Troegl et al. 2005)
	Escherechia coli	Luminescence	-	(Premkumar et al. 2002)
			DPD1718 recA'::lux	(Polyak et al. 2000)
			GC2	(Gu and Choi 2001)
	Pseudomonas fluorescens		HK44	(Lee et al. 2007)
	Escherichia coli		recA'::lux V. fischeri	(Rosen et al. 2000)
	Salmonella typhmurium TA1535		TL210 and TL210ctl	(Taguchi et al. 2004)
Toxicity and genotoxicity	*Escherichia coli*	Luminescence	DPD2511, DPD2540, DPD2794 and TV1061	(Kim and Gu 2003)
Effect	**Analite**	**Transduction**		**References**
Androgenicity	17-β-testosterone	Fluorescence		(Bovee et al. 2008)
Estrogenicity	diethylstilbestrol, 17-β-estradiol, 17-α-estradiol, 2-OH-estrone, bisphenol A, p,p′-dichlorodiphenyldichloroethylene	Nanomechanical		(Dutta et al. 2007)
	17-β-estradiol	Electrophoretic mobility shift assay		(Andres et al. 2008)
	17-β-estradiol	Fluorescence		(Wozei et al. 2006)
	Estrogens	SPR		(Ramakrishnan et al. 2005)
	Estrogens	SPR		(Butala and Sadana 2003)
	17β-Estradiol	Cyclic voltametry		(Murata et al. 2001)
	Estrogens, progestogens, bisphenol A, 4-nonylphenol and tamoxifen.	SPR		(Usami et al. 2002)
	17β-Estradiol, synthetic estrogens and xenostrogens	SPR		(Hock et al. 2002)
	Estrogens and xenoestrogens	SPR		(Seifert et al. 1999)
Androgenicity and estrogenicity	Ligans of nuclear hormone receptors	Fluorescence		(Muddana and Peterson 2003)

Table 2: Nanomaterials based biosensors for Pathogens and natural toxins detection. Currently the application of nanomaterials to improve the biosensors activity is growing; these new biosensors are becoming a new interdisciplinary frontier between biological detection and materials science.

Microorganism	Nanomaterial	Recognition	Detection	Reference
Campylobacter jejuni	SAMs	Antigen-Antibody	SPR	(Wei, Oyarzabal et al. 2007)
			QCM	(Safina, van Lier et al. 2008)
E. coli O157:H7	SAMs of cys-teamine	Antigen-Antibody	QCM	(Poitras and Tufenkji 2009)
	SAMs		SPR	(Subramanian, Irudayaraj et al. 2006)
			LRSP-FS)	(Huang, Dostalek et al. 2011)
	Magnetic NPs		IMS+Plating	(Varshney, Yang et al. 2005)
	-	Phage with luxI gene insert	Bioluminescence	(Ripp, Jegier et al. 2008)
Escherichia coli	Gold nanowire ar-ray	Antigen-Antibody	Electrochemical imped-ance spectroscopy	(Basu, Seggerson et al. 2004)
	Cu@Au NPs		Anodic stripping voltam-metry	(Zhang, Geng et al. 2009)
	Polymeric NPs	Adhesin-receptor	TEM	(Edgar, McKinstry et al. 2006)
	-	Enzyme estearase 2	Electrochemical	(Pöhlmann, Wang et al. 2009)
	-	Antigen-Antibody	Amperometric	(Abu-Rabeah, Ashkenazi et al. 2009)
	-	Antigen-Antibody	Magnetostrictive micro-cantilever	(L.Fu et al 2010)
	-	Lambda phage with luxI gene insert	Bioluminescence	(Birmele, Ripp et al. 2008)
	-	Lytic phage. Amine coupling of phages with carboxylic groups at a carbon surface	Impedimetric	(Shabani, Zourob et al. 2008)
	-	Lambda phage with a luxI based acyl homoserine lactone	Bioluminescence	(Ripp, Jegier et al. 2006)
Listeria monocy-togenes	SAMs	Antigen-Antibody	QCM	(MINUNNI, #160 et al. 1996)
	-	cFv phages with affinity for ActA (= a virulence factor that is expressed on the cell surface of L.monocytogenes)	SPR	(Nanduri, Sorokulova et al. 2007)

Table 2 (continuation)

Salmonella enteriditis	-	Antigen-Antibody	SPR with wavelength modulation	(Koubová, Brynda et al. 2001)
	-		Impedometric sensor	(Kim and et al. 2007)
Salmonella typhimurium	SAMs	Antigen-Antibody	SPR	(Oh, Kim et al. 2004)
	Colloidal Au-NPs			(Ko, Park et al. 2009)
	-	Peptide displaying phage	QCM	(Olsen, Sorokulova et al. 2006)
Salmonella typhimurium and *Bacillus anthracis*	-	Peptide displaying phage	Magnetoelastic	(Huang, Yang et al. 2009)

Self Assembling monolayers (SAMs); Nanoparticles (NPs); Surface plasmon resonance (SPR); Quartz crystal microbalance (QCM); Long-range surface plasmon-enhanced fluorescence spectroscopy (LRSP-FS); Immunomagnetic separation (IMS)

Regarding bioassays used for toxicological purpose, a list of about 290 suggested methods is included in the ECVAM database service on alternative methods to animal experimentation (DB-ALM) focusing on these endpoints:

1. Acute Systemic Toxicity;
2. Basal Cytotoxicity;
3. Carcinogenicity;
4. Tumour promotion;
5. Cardiotoxicity;
6. Digestive System Toxicity;
7. Effects on Reproduction;
8. Developmental toxicity;
9. Ecotoxicity: Air, Aqueous and Soil contamination;
10. Endocrine Organs Toxicity;
11. Genotoxicity and Mutagenicity;
12. Haematotoxicity;
13. Hepatotoxicity and Metabolism-mediated Toxicity;
14. Immunotoxicity;
15. Eye and Skin Irritations;
16. Phototoxicity;
17. Myotoxicity;
18. Nephrotoxicity;
19. Neurotoxicity;
20. Respiratory Tract Toxicity;
21. Photoallergenicity;
22. Percutaneous Absorption;
23. Biotransformation;
24. Drug Discovery and Activity Testing;
25. Biocompatibility & Safety Testing.

The full list includes both validated and under validation methods.

A survey of the regulatory status is also provided, indicating those procedures that are accepted by EU, OECD and / or the US.Moreover it’s important to underline that new techniques are worldwide developed day by day by cell and molecular biologists in order to face the need of in vitro tools for toxicological investigation.

According to those reviewed and proposed by ECVAM, we suggest to use one or more of these in vitro methods as a first choice to investigate the toxicological potential of chemicals and additives. In particular, validated methods have to be preferred if compared with under validation or not validated assays in order to obtain high quality and consistent data.

3 *In silico*

3.1 General tools

Below we list general tools which can be used for several endpoints. We describe below some tools which gather collections of models, or of values, and some useful links which cover series of endpoints, providing several QSAR models.
In the following section we list individual models which can be used for specific end-points.

A. QSAR models databases

Joint Research Center QSAR Model Database

In the regulatory assessment of chemicals (e.g. under REACH), (Q)SAR models are playing an increasingly important role in predicting properties for hazard and risk assessment. This implies both a need to be able to identify relevant (Q)SARs and to use them to derive estimates and/or have access to their pre-calculated estimates. The Joint Research Center (JRC) is developing an inventory of (Q)SAR models which are made available. This inventory includes a collection of robust summaries of (Q)SAR models compiled by using a standard (Q)SAR Model Reporting Format (QMRF). The QMRF template is available for download.The JRC QSAR Model Database is freely accessible from this website.
The QSAR Model Reporting Format (QMRF) is a harmonised template for summarising and reporting key information on (Q)SAR models, including the results of any validation studies. The information is structured according to the OECD (Q)SAR validation principles. The QSAR Prediction Reporting Format (QPRF) is a harmonised template for summarising and reporting substance-specific predictions generated by (Q)SAR models.

- Developers and users of (Q)SAR models can submit to the JRC information on (Q)SARs by using the (Q)SAR Model Reporting Format (QMRF).
- The JRC will perform a quality control (i.e. adequacy and completeness of the documentation) of the QMRFs submitted.
- Properly documented summaries of (Q)SARs (i.e. robust summaries) will be included in the JRC QSAR Model Database.

- The QSAR Model Database will help to identify valid (Q)SARs. e.g. for the purposes of REACH.
- The QMRF is expected to be a communication tool between industry and the authorities under REACH.
- Inclusion of the model in the QSAR Model Database does not imply acceptance or endorsement by the JRC or the European Commission.
- Responsibility for use of the models lies with the end-users.

Danish (Q)SAR Database

To support the regulatory assessment of chemicals, the Danish Environmental Protection Agency (EPA) constructed a (Q)SAR database comprising predictions made by some 70 models for about 166,000 organic chemicals for a wide range of different endpoints. In 2004, a collaborative project was set up between the Danish EPA and the JRC to develop an internet-accessible version of this database. The internet version of the Danish (Q)SAR Database was constructed to enable different types of searching, including structure (substructure/exact match) searching, ID (CAS number, name) searching and parameter (endpoint) searching.

Key features of the program:

- Internet platform requiring Java functionality.
- Enables structure searches by drawing of 2D fragments / structures.
- Enables searches on CAS, chemical name and any of the parameter fields (endpoint, inventory).
- Displays (Q)SAR predictions and 2D structure image in a html format for individual records.

B. QSAR models.

TOXTREE

The JRC commissioned the development of an open source computer program capable of estimating different types of toxic hazardby applying decision tree approaches. Toxtree is suitable for use on a standalone PC, and has been designed with flexible capabilities for future extensions. Currently, plug-ins are available for applying the following rulebases:

- the Cramer classification scheme for TTC (Threshold of Toxicological Concern) estimation;
- an extended Cramer scheme;
- the Verhaar scheme for predicting the mode of toxic action in aquatic species;
- decision trees for estimating skin and eye irritation and corrosion potential, based on the BfR rules,
- the Benigni-Bossa rulebase for mutagenicity and carcinogenicity;
- the ToxMic rulebase for the in vivo micronucleus assay;
- structural alerts for the identification of Michael acceptors;
- the START rulebase for persistance / biodegradation potential.

Toxtree is a flexible and user-friendly open-source application that places chemicals into categories and predicts various kinds of toxic effect by applying decision tree approaches.

Toxtree was developed by Ideaconsult Ltd (Sofia, Bulgaria) under the terms of a JRC contract. The software is made freely available as a service to scientific researchers and anyone with an interest in the application of computer-based estimation methods in the assessment of chemical toxicity.

[More info: http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree]

TOXMATCH

The JRC commissioned the development of an open source computer program that encodes several chemical similarity indices in order to to facilitate the grouping of chemicals, thereby supporting the development of chemicals categories and the application of read-across between analogues. Toxmatch is a flexible and user-friendly open-source software application that encodes several chemical similarity indices to facilitate the grouping of chemicals into categories and read-across.

The core functionalities include the ability to compare datasets based on various structural and descriptor-based similarity indices as well as the means to calculate pair wise similarity between compounds or aggregated similarity of a compound to a set.

Toxmatch was developed by Ideaconsult Ltd (Sofia, Bulgaria) under the terms of a

JRC contract. The software is made freely available as a service to scientific researchers and anyone with an interest in the application of computer-based estimation methods in the assessment of chemical toxicity.

[More info: http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxmatch]

DART

DART (Decision Analysis by Ranking Techniques) is a powerful and user-friendly software tool designed for the ranking of chemicals according to their environmental and toxicological concern based on the most recent ranking theories. Different kinds of order ranking methods, roughly classified as total (also called even-scoring) and partial-order ranking methods (Hasse diagram technique), are implemented in DART. These methods can be used to rank chemicals on the basis of more than one variable. The JRC commissioned the development of an open-source computer program to implement a variety of ranking methods. These are decision support techniques used for the ranking of various alternatives on the basis of more than one variable.

DART was developed by Talete srl (Milan, Italy) under the terms of a JRC contract. The software is made freely available as a service to scientific researchers and anyone with an interest in the application of computer-based ranking methods.

[More info:http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/DART]

T.E.S.T.

The Toxicity Estimation Software Tool (T.E.S.T.) has been developed by US EPA program to allow users to easily estimate toxicity using a variety of QSAR methodologies. T.E.S.T allows a user to estimate toxicity without requiring any external programs. Users can input a chemical to be evaluated by drawing it in an included chemical sketcher window, entering a structure text file, or importing it from an included database of structures. Once a chemical has been entered, its toxicity can be estimated using one of several advanced QSAR methodologies. The program does not require molecular descriptors from external software packages (the required descriptors are calculated within T.E.S.T.).

T.E.S.T allows you to estimate the value for several toxicity end points:

- 96 hour fathead minnow LC50 (concentration of the test chemical in water in mg/L that causes 50% of fathead minnow to die after 96 hours)
- 48 hour Daphnia magna LC50 (concentration of the test chemical in water in mg/L that causes 50% of Daphnia magna to die after 48 hours)
- 48 hour Tetrahymena pyriformis IGC50 (concentration of the test chemical in water in mg/L that causes 50% growth inhibition to Tetrahymena pyriformis after 48 hours)
- Oral rat LD50 (amount of chemical in mg/kg body weight that causes 50% of rats to die after oral ingestion)
- Bioaccumulation factor (ratio of the chemical concentration in fish as a result of absorption via the respiratory surface to that in water at steady state)
- Developmental toxicity (whether or not a chemical causes developmental toxicity effects to humans or animals)
- Ames mutagenicity (a compound is positive for mutagenicity if it induces reverTant colony growth in any strain of Salmonella typhimurium)

T.E.S.T. also allows you estimate several physical properties:

- Normal boiling point (the temperature in °C at which a chemical boils at atmospheric pressure.
- Density (the density in g/cm^3)
- Flash point (the lowest temperature in °C at which it can vaporize to form an ignitable mixture in air)
- Thermal conductivity (the property of a material in units of mW/mK reflecting its ability to conduct heat)
- Viscosity (a measure of the resistance of a fluid to flow in cP defined as the proportionality constant between shear rate and shear stress)
- Surface tension (a property of the surface in dyn/cm of a liquid that allows it to resist an external force)
- Water solubility (the amount of a chemical in mg/L that will dissolve in liquid water to form a homogeneous solution)

[More info: http://www.epa.gov/nrmrl/std/cppb/qsar/#TEST]

CAESAR Models

CAESAR was an EC funded project, which was specifically dedicated to develop QSAR models for the REACH legislation.

Five endpoints with high relevance for REACH have been addressed within CAESAR:

- bioconcentration factor
- skin sensitization
- carcinogenicity
- mutagenicity
- developmental toxicity

CAESAR models have been assessed according to the OECD principles for the validation of QSAR. For the model validity the developers used a wide series of statistical checks. They also used external tests, to verify that the models performs correctly on new compounds.

CAESAR models are now freely available in the SOFTWARE section of the website: [http://www.caesar-project.eu/software/index.php].

3.2 Specific QSAR models

Here we list a series of QSAR models which can be used for specific endpoints of interest within RISKCYCLE. Some models are freely available, others not. We give the preference of free, public models, because they are freely available through the internet, the are more transparent than commercial programs (which for commercial reasons keep several components of the model not available) and in our experience they do provide similar results compared to commercial programs.

The fact that a model is listed here it does not necessarly means that the model can be used for a certain compound, since appropriate chack should be done.

A. Physico-chemical properties.

VAPOUR PRESSURE

- SPARC (University of Georgia) - http://archemcalc.com/sparc.

WATER SOLUBILITY

- EPISUITE (U.S. EPA) http://www.epa.gov/oppt/exposure/pubs/episuite.htm/;
- T.E.S.T.(US EPA) http://www.epa.gov/nrmrl/std/cppb/qsar/

PARTITION COEFFICIENT n-Octanol/Water

- EPISUITE http://www.epa.gov/opptintr/exposure/pubs/episuite.htm

DISSOCIATION CONSTANT

- EPISuite http://www.epa.gov/opptintr/exposure/pubs/episuite.htm

B. Environmental behaviour properties

BIODEGRADABILITY

- EPISuite http://www.epa.gov/oppt/exposure/pubs/episuite.htm

ABIOTIC DEGRADATION

- EPI Suite http://www.epa.gov/oppt/exposure/pubs/episuite.htm

HYDROLYSIS

- HYDROWIN www.epa.gov/oppt/exposure/pubs/episuitedl.htm

ABSORPTION/DESORPTION

- KOCWIN EPI Suite www.epa.gov/oppt/exposure/pubs/episuitedl.htm

BIOCONCENTRATION FACTOR

- BCFWIN http://www.epa.gov/oppt/exposure/docs/episuitedl.htm
- CAESAR http://www.caesar-project.eu
- T.E.S.T. http://www.epa.gov/nrmrl/std/cppb/qsar/index.html#TEST

C. Ecotoxicological properties.

ACUTE TOXICITY ON INVERTEBRATES (DAPHNIA)

- ECOSAR http://www.epa.gov/oppt/newchems/tools/21ecosar.htm
- T.E.S.T. http://www.epa.gov/nrmrl/std/cppb/qsar/index.html#TEST

GROWTH INHIBITION ON ALGAE

- ECOSAR http://www.epa.gov/oppt/newchems/tools/21ecosar.htm

ACUTE TOXICITY ON FISH

- Demetra http://www.demetra-tox.net/
- ECOSAR http://www.epa.gov/oppt/newchems/tools/21ecosar.htm
- Lazar http://lazar.in-silico.de/
- T.E.S.T. http://www.epa.gov/nrmrl/std/cppb/qsar/index.html#TEST

ACTIVATED SLUDGE RESPIRATION INHIBITION TESTING

- EPI Suite http://www.epa.gov/oppt/exposure/pubs/episuite.htm

ACUTE TOXICITY TO INVERTEBRATES

- EPI Suite v.4.1 www.epa.gov/oppt/exposure/pubs/episuitedl.htm

CHRONIC TOXICITY ON DAPHNIA

- ECOSAR http://www.epa.gov/oppt/newchems/tools/21ecosar.htm

CHRONIC TOXICITY ON FISH

- ECOSAR http://www.epa.gov/oppt/exposure/docs/episuitedl.htm

D. Toxicological properties.

GENE MUTATION IN BACTERIA

- CAESAR project models (CAESAR consortium) http://www.caesar-project.eu/#;
- T.E.S.T. http://www.epa.gov/nrmrl/std/cppb/qsar/, EPA;
- Toxtree http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree.

ACUTE TOXICITY

- T.E.S.T. http://www.epa.gov/nrmrl/std/cppb/qsar/, EPA

REPEATED DOSE TOXICITY

- Lazar (human MRTD) http://lazar.in-silico.de

REPRODUCTIVE TOXICITY

- OSIRIS property explorer http://www.organic-chemistry.org/prog/peo/.
- CAESAR http://www.caesar-project.eu

CARCINOGENICITY STUDY

- CAESAR project models (CAESAR consortium) http://www.caesar-project.eu/
- Toxtree http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree

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