ID 439 v.05

Results report effect-based analysis

Report number:	Samples received:
Customer: Illustrative report with fictional data	Sampling date:
Contact information:	Report date:

Overview of results

A more detailed summary of the results and limits of detection are presented later in the report.

		Genotoxicity	Acute toxicity	Oxidative stress	Antiandrogenic effects	Androgenic effects	Estrogenic effects	Activation of the Ah receptor
Quality control/accreditation*		**A**	**A**	**I**	**I**	**I**	**I**	**I**
Sample number BioCell Analytica	**Customer description of the sample**							
	WWTP influent – untreated wastewater	Yes	No	Class 5	Class 3	Class 4	Class 5	Class 4
	WWTP effluent – conventional treatment	Yes	No	Class 5	Class 2	Class 5	Class 5	Class 5
	WWTP effluent – after pilot polishing treatment	No	No	Class 1	Class 1	Class 1	Class 2	Class 2
	Receiving water – upstream	No	No	Class 2	Class 1	Class 3	Class 3	Class 1
	Receiving water – downstream	No	No	Class 2	Class 2	Class 4	Class 5	Class 2

*A refers to analyzes performed under accreditation from Swedac, Sweden's national accreditation body. I refers to analyzes performed in accordance with internal quality guidelines.

Information on effect-based analyses

More information on effect-based analyses can be found here.

Classification of results

Genotoxicity and acute toxicity are reported and classified as yes/no. Results from other parameters are classified into five classes based on a comparison either with historical data for the same water type or a guidance values relevant to the water type in question. More information on the classification can be found here.

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Technical description

A technical description of our effect-based analysis can be found here. General information and approval of the report can be found here.

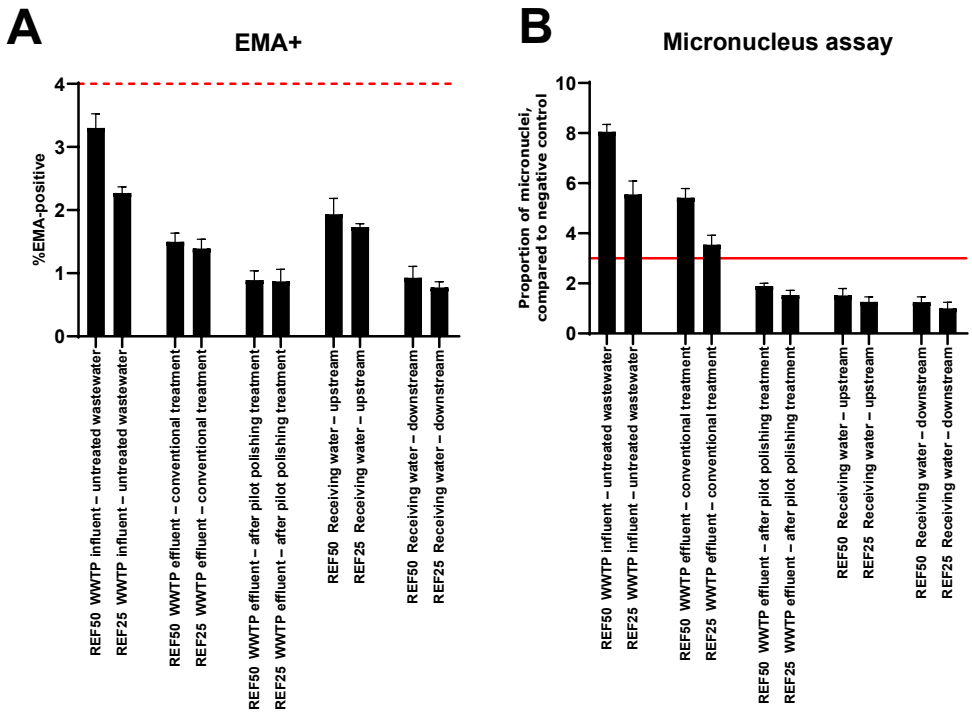
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Genotoxicity

The genotoxicity of the samples has been evaluated with micronucleus test. More information on genotoxicity and micronucleus tests is available here.

This analysis is carried out under accreditation from Swedac.

Sample number BioCell Analytica	Customer description of the sample	Classification genotoxicity
	WWTP influent – untreated wastewater	Yes
	WWTP effluent – conventional treatment	Yes
	WWTP effluent – after pilot polishing treatment	No
	Receiving water – upstream	No
	Receiving water – downstream	No



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Acute toxicity

The acute toxicity in the samples has been evaluated using cultured fish cells. More information on acute toxicity and this analysis is available here.

This analysis is carried out under accreditation from Swedac.

		Acute toxicity single parameters			Classification acute toxicity, summarized assessment
Sample number BioCell Analytica	**Customer description of the sample**	**Metabolic activity**	**Cell membrane integrity**	**Integrity lysosomal membranes**	
	WWTP influent – untreated wastewater	No	No	No	No
	WWTP effluent – conventional treatment	No	No	No	No
	WWTP effluent – after pilot polishing treatment	No	No	No	No
	Receiving water – upstream	No	No	No	No
	Receiving water – downstream	No	No	No	No

* The calculation of EC_{20} values is described here.

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Other parameters

More information on these parameters is available here. Information on the classification of the results is available here.

Sample number BioCell Analytica	Customer description of the sample	Water type*	Oxidative stress (µg/L tBHQ equivalents)		Antiandrogenic effects (ng/L OHF equivalents)		Androgenic effects (ng/L DHT equivalents)		Estrogenic effects (ng/L E2 equivalents)		Activation of the Ah receptor (ng/L TCDD equivalents)	
			Result	LOD[1]	Result	LOD[1]	Result	LOD[1]	Result	LOD[1]	Result	LOD[1]
	WWTP influent – untreated wastewater	Untr	1330	19,9	214	58,2	354	0,208	240	0,0383	18,3	0,0315
	WWTP effluent – conventional treatment	Trea	350	9,97	65,0	29,1	95,0	0,104	60,0	0,00958	5,50	0,0315
	WWTP effluent – after pilot polishing treatment	Trea	<LOD	4,99	<LOD	29,1	<LOD	0,104	0,106	0,00479	0,189	0,0315
	Receiving water – upstream	Rec	7,55	4,99	<LOD	29,1	0,219	0,104	0,176	0,00479	<LOD	0,0315
	Receiving water – downstream	Rec	5,43	4,99	29,1	29,1	0,311	0,104	0,706	0,00958	0,0615	0,0315

* Water type refers to the category of water which the sample has been classified as, for the comparison with historical data or guidance values. [1]LOD refers to limit of detection.

General information and approval

The analyzes have been carried out in our analytical laboratory at the following address: Almas allé 4C, SE-756 51 Uppsala, Sweden. The sampling has been carried out by the customer or by a third party on assignation by the customer. The results relate only to the samples analysed. Further, the results apply to the sample as they were received.

Date(s) when the analyzes were carried out: 202x-xx-xx—202x-xx-xx

Data handling carried out by:

Reviewed and approved by:

The report has been approved:

Effect-based analysis

Effect-based analysis in cultured cells

Effect-based analysis measures the total biological effect of all chemical substances present in a water sample. This means that effect-based methods also detect unknown substances, transformation products, and mixture effects that may arise when several chemical substances interact. The traditional way of monitoring chemical pollutants is targeted chemical analysis of a limited number of selected chemical substances, which does not provide information about unknown substances or mixture effects. BioCell Analytica's effect-based analyses are performed using laboratory-cultured mammalian or fish cells that have been adapted to detect different types of unwanted biological effects, such as endocrine-disruption effects and damage to genetic material (genotoxicity).

How the results are reported

Genotoxicity and acute toxicity are primarily evaluated as yes/no: whether the sample is genotoxic or not, and whether the sample is acutely toxic or not.

For acute toxicity, the sample's effect concentration at 20% (EC_{20}) is calculated. This value describes the concentration level at which the water sample caused a 20% reduction in the cellular functions measured in the test. The sample is tested at 100% (undiluted water) and at different dilutions (e.g., 80% sample + 20% clean water). A low EC_{20} value indicates that the sample has a high acute toxic effect.

For the other parameters, the results are reported as bioequivalent concentrations, for example ng estradiol (E2) equivalents/L. The bioequivalent concentration is the concentration of a reference substance (e.g., estradiol (E2)) that would produce the same biological effect as the sample.

How results from effect-based analysis are used

Results from repeated effect-based analyses can be compared with guidance values and historical data. This provides a comprehensive picture of water quality and early warning signals if the contamination situation changes. By comparing results before and after treatment, the treatment efficiency of different technologies can be evaluated. By analyzing samples upstream in the water system, source tracking can be carried out.

Interpretation and classification of results

The results from effect-based analyses of endocrine-disrupting effects and environmental sensors can be interpreted by comparison with guidance values or with previously analyzed water samples (historical data). This interpretation is presented in the tables in this report through color-coding of the results.

For parameters and water types where guidance values exist, the results are classified into five classes according to the table below. Comparison with historical data shows how the effects observed in the analyzed water samples relate to other comparable samples analyzed in the same way. The comparison with historical data is also presented in five classes, according to the table below.

Class	Historical data	Guidance value
1	<LOD[1]	<LOD
2	>LOD to 25th percentile[2]	>LOD to 25% of guidance value
3	25th to 75th percentile	25-75% of guidance value
4	75th to 90th percentile	75%-100% of guidance value
5	>90th percentile	> guidance value

[1]LOD refers to limit of detection. [2] The 25th percentile means that 25% of the historical dataset for that water type falls below this value. Similarly, the 75th and 90th percentiles indicate that 75% and 90%, respectively, of the historical dataset for that water type fall below this value.

The classification is carried out by comparison with historical data or guideline values relevant to the water type of the current sample, according to the table below.

		Genotoxicity	Acute toxicity	Oxidative stress	Antiandrogenic effects	Androgenic effects	Estrogenic effects	Activation of the Ah receptor
Water type	**Abbr.**	**Classification in relation to**						
Wastewater, untreated	Untr	Yes/No	Yes/No	Hist.	Hist.	Hist.	Hist.	Hist.
Wastewater, treated	Trea	Yes/No	Yes/No	Hist.	Hist.	Guid.	Guid.	Hist.
Wastewater-impacted recipient	Rec	Yes/No	Yes/No	Hist.	Hist.	Guid.	Guid.	Hist.
Raw water for drinking water production	RW	Yes/No	Yes/No	Hist.	Hist.	Guid.	Guid.	Hist.
Drinking water	DW	Yes/No	Yes/No	Hist.	Hist.	Guid.	Guid.	Hist.
Surface water	Surf	Yes/No	Yes/No	Hist.	Hist.	Guid.	Guid.	Hist.

Hist. refers to historical data, *Guid.* refers to guidance values.

Technical description

The analyses are carried out according to the following:

- Genotoxicity is analyzed using the cell line TK-6, in general in accordance with OECD guideline 487 (SOP 056, version 3)
- Acute toxicity in fish cells is analyzed using the cell line RTgill-W1, in general in accordance with ISO standard 21115 (SOP 026, version 3)
- Oxidative stress is analyzed using the cell line AREc32
- Antiandrogenic and androgenic effects are analyzed using the cell line AR-EcoScreen GR KO M1, in general in accordance with OECD guideline 458
- Estrogenic effects are analyzed using the cell line ERα-CALUX, in general in accordance with OECD guideline 455
- Activation of the Ah receptor is analyzed using the cell line DR-EcoScreen

Sample processing
Before analysis, samples are concentrated using solid-phase extraction (SPE) to increase the sensitivity of the analysis. The concentrated water sample is then diluted in the culture medium used for the laboratory-cultured cells. The SPE is performed using an HLB column, which concentrates organic substances in the sample.

For the analysis of genotoxicity, the results are usually reported for two different concentration levels, indicating how many times the sample has been concentrated for the analysis. If either concentration shows a genotoxic effect higher than three times the background level, the sample is classified as genotoxic.

Acute toxicity in fish cells is analyzed in non-concentrated water samples, meaning that all types of contaminants, including inorganic substances, are included in the sample. The results are reported as the impact on three important cell functions. Unaffected cells have a value of 100%. If any of the three cell functions studied decrease below 80%, the sample is classified as acutely toxic.

Control of general effects on the cells
Initially, cell viability (how well the cells are doing) is examined after the cells have been exposed to different concentrations of the water sample. This is carried out to ensure that the results from the specific tests are not influenced by the cells being generally unhealthy. In specific tests, only sample concentrations that do not cause general effects on the cells are used, which means the sample may need to be diluted. This dilution can affect the detection limit of the analysis.

Calculation of bioequivalent concentrations
The bioequivalent concentration is calculated as the ratio between the concentration of the reference substance that produces 20% activation and the concentration factor of the sample that produces 20% activation of the measured biological effect. The bioequivalent concentration is expressed as reference substance equivalents per liter of water, for example x ng estradiol equivalents/L (E2 equivalents/L). This means that the sample has an estrogenic effect equivalent to x ng estradiol/L.

More information on the parameters

Genotoxicity
A genotoxic effect means that the sample contains something that can cause damage to genetic material (DNA). Genotoxicity is a very serious undesirable biological effect that requires extensive testing and investigation before the registration of, for example, pesticides that may contaminate food and the environment. In humans, DNA damage in body cells can lead to cancer and other diseases. In the aquatic environment, DNA damage primarily risks causing reproductive disorders in fish. Unlike many other types of toxicity, there is no safe threshold dose for certain types of genotoxicity, meaning there is no dose below which genotoxic effects can be guaranteed not to occur. It is also unknown which type of genotoxicity causes effects in a complex chemical mixture, such as in a water sample. Because this is such a serious effect, and as a precaution, genotoxic effects in water samples are considered unacceptable. In the evaluation, the response is interpreted simply as yes or no: whether the sample is genotoxic or not.

Acute toxicity in cultured fish cells
Acute toxicity is measured in gill cells from rainbow trout using three different parameters. Acute toxicity observed in these cells has shown good agreement with toxicity in fish. If any of the three analyzed parameters indicate acute toxicity, the sample is classified as acutely toxic.

Oxidative stress
Oxidative stress occurs when reactive oxygen species are produced in excess. Oxidative stress is harmful to cells and can be the starting point for many different damaging effects in humans and animals. It can be caused by many different chemical substances and therefore serves as a useful environmental sensor.

Antiandrogenic effects
Antiandrogenic substances inhibit the function of natural androgens, which have important roles in sexual development and reproduction in both humans and animals. Antiandrogenic effects can be caused by many different chemical substances and therefore serve as a useful environmental sensor.

Androgenic effects
Androgenic substances are endocrine-disrupting, meaning they mimic natural hormones that play important roles in sexual development and reproduction in both humans and animals, or they interfere with the effects of such natural hormones.

Estrogenic effects
Estrogenic substances are endocrine-disrupting compounds, meaning they mimic natural hormones that have important functions in sexual development and reproduction in both humans and animals.

Ah receptor
The Ah receptor (also called the dioxin receptor) has several fundamental functions in cells, but overactivation can lead to various harmful effects in humans and animals. It can be activated by many different chemical substances and therefore serves as a useful environmental sensor.