Bio-indicators monitoring of tertiary treatment performance for the removal of micro-pollutants from urban wastewater

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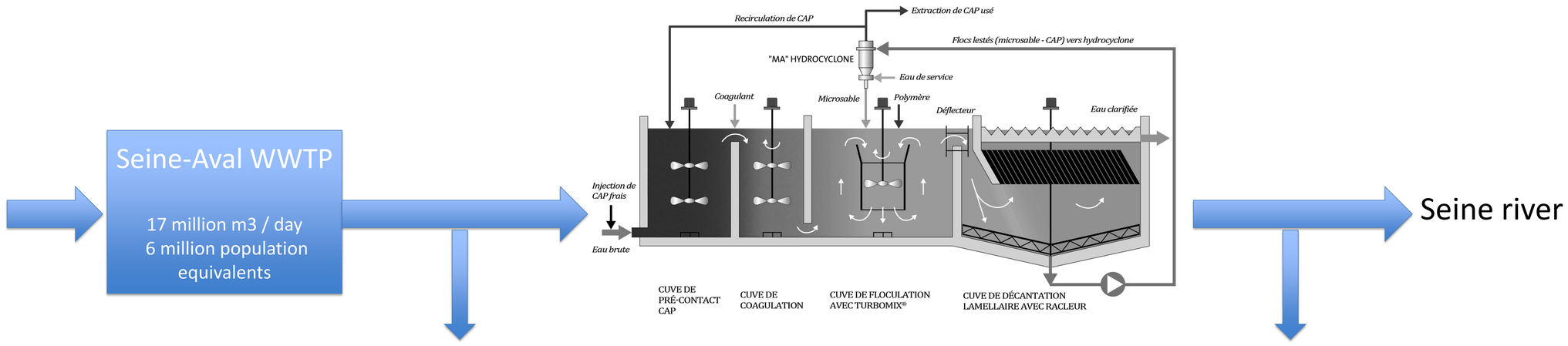
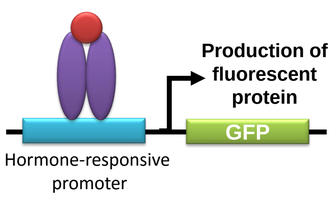
Effluents from wastewater treatment plants (WWTP) contain micropollutants which have endocrine disruption capabilities. Removal of these endocrine disruptive compounds (EDC's) necessitates tertiary or quaternary treatments. Reliable means are required for monitoring of the performance of these processes in order to evaluate their impact and optimize their use. Bio-indicators are currently the tool of choice for this analysis as they provide a global readout of the WWTP effluent, importantly giving information on the net disruptive effect of the overall sample. In this context, SIAAP, Veolia and Watchfrog have jointly investigated the effectiveness of a tertiary treatment process (Actiflo® Carb) coupled with in-line monitoring of the endocrine disruptive effect using bio-indicators (Watchfrog) We used fish fry and tadpoles that generate a fluorescent protein when their endocrine system is impacted by pollutants present in the sample. A specific instrument called the Frogbox, allows continuous, real-time monitoring of the fluorescence of these larvae. We studied the performance of the Actiflo® Carb process over several months using two Frogboxes (inlet and outlet of the process). We demonstrated that the EDC activity varies greatly between different days and weeks of measurement. Chemical analysis of various micropollutants has enabled us to demonstrate that the disruptive effect on the thyroid hormonal axis is well correlated with the concentration of pollutants. These results demonstrate the usefulness of such a tool not only to monitor the performance of the treatment process but also to provide a real-time indication of the activity impact on the environment. The efficiency of the Actiflo® Carb process has also been demonstrated along with possible strategies for optimising activated carbon consumption and their activity impact on wildlife.

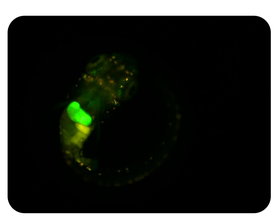


Vertebrate Bio-indicators for endocrine disruption

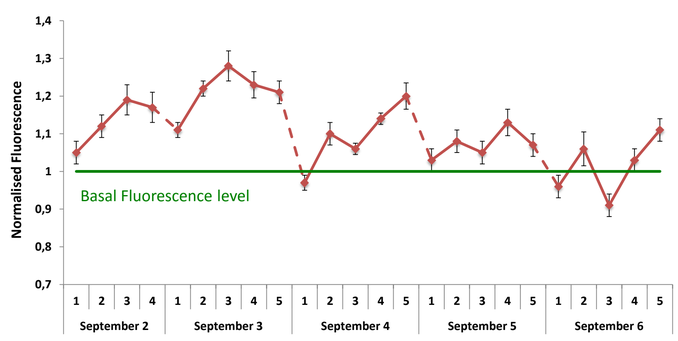


Tadpole from the THbZIP transgenic line carries a genetic construct with GFP expression driven by the THbZIP promoter. The THbZIP gene is directly regulated by thyroid hormones during metamorphosis.

Fish fry from the CHGH-GFP transgenic line carries a genetic construct with GFP expression driven by the choriogenin promoter. The choriogenin gene is involved in the egg production and directly regulated by estrogens hormones

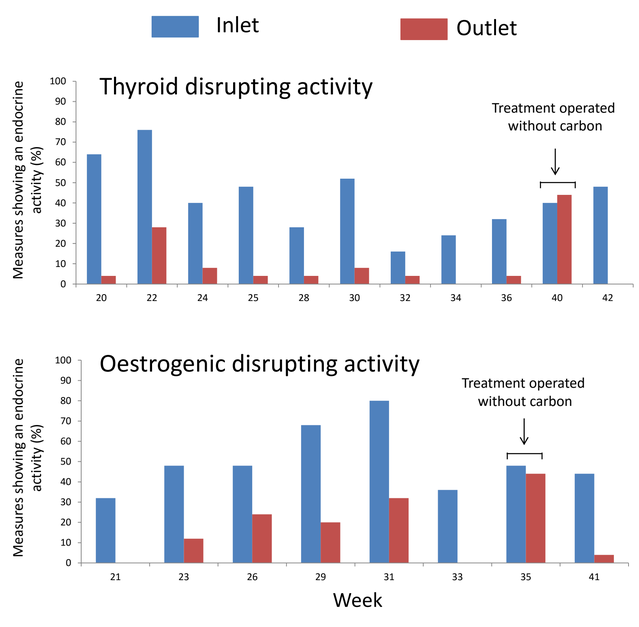


Dynamic profile of endocrine disruption activity in the WWTP effluent



Variations in the endocrine activity detected in line by the FrogBox WWTP effluent. Representative example of the week 36.
Each point indicates the mean of the fluorescence intensity of 50 tadpoles (red line), the results were normalised on the fluorescence intensity of a control group exposed to mineral water (green line). Error bars indicate the standard error of the mean (SEM).

The tertiary treatment reduces the endocrine disrupting activity



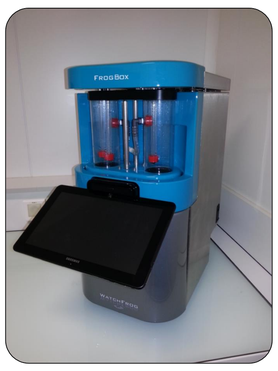
The blue and red bars indicates the percentage of measures showing an endocrine activity respectively in the tertiary treatment inlet or outlet.

In line endocrine disruption assessment

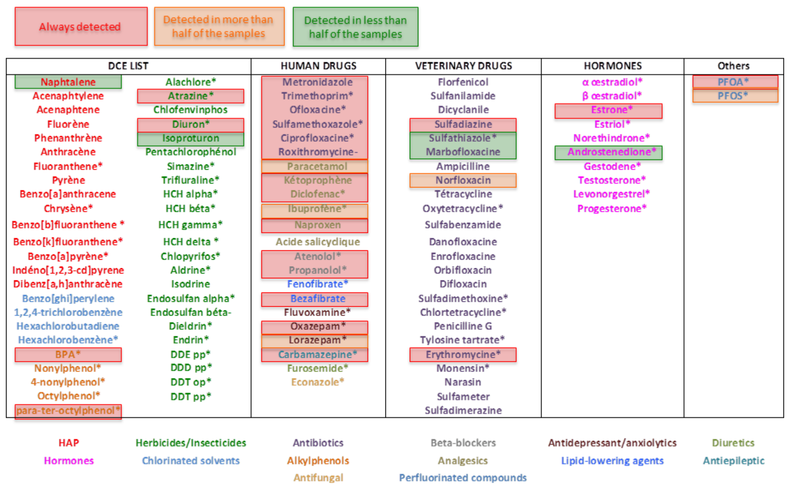
-Until now *in vivo* tests for endocrine disruption were performed laboratory and on specific samples.

-The FrogBox was developed and used for continuously exposing the larvae to sample and quantifying their fluorescence emission at regular intervals

-The FrogBox offers the possibility to be used on site automating both the exposure to the sample and the reading of fluorescence, and therefore the detection of endocrine disrupting activity.



110 Targeted micro-pollutants

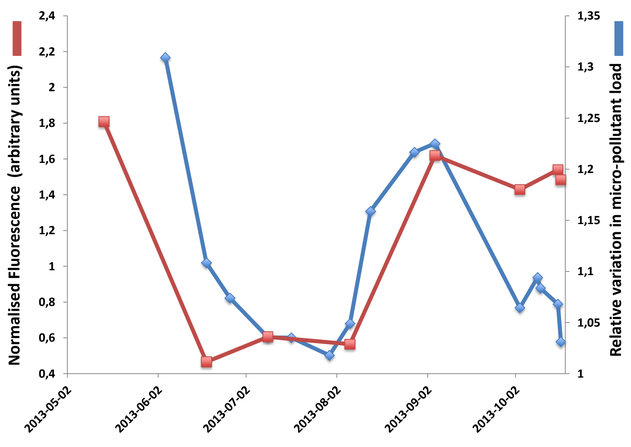


Red, orange and green boxes indicate respectively the molecules identified in all, half or more and less that half of the samples. Molecules without box were never found in the samples. The * indicates the molecules with a supposed or proven endocrine effect.

	Inlet of the tertiary treatment		Outlet of the tertiary treatment	
	Number of measure showing an endocrine effect	Number of measure reaching the endocrine disruption threshold	Number of measure showing an endocrine effect	Number of measure reaching the endocrine disruption threshold
Thyroid disruption : 248 measures	119 (48% of the measures)	1	27 (11% of the measures)	0
Estrogenic disruption : 175 measures	101 (58% of the measures)	1	30 (17% of the measures)	0

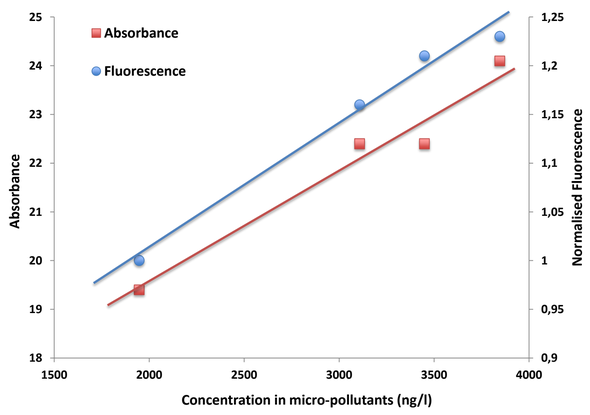
Synthesis of the results obtained on the thyroid and estrogenic activity assessed in the tertiary treatment inlet and outlet.
The threshold for endocrine disruption was reached once for the thyroid tests (week 22) and once for the estrogenic tests (week 26). In both cases this observation was made in inlet samples, all outlet samples induce fluorescence levels below this threshold showing the efficient of the tertiary treatment to eliminate endocrine active pollution.

The thyroid disrupting activity is linked to the micro-pollutant load in the WWTP outlet.



Red line indicates the mean value of the measures of fluorescence made the Tuesday of each week. Blue line indicates the mean relative variation in micro-pollutant load for the 27 molecules detected in more than half of the samples.

The thyroid disrupting activity correlate with the micro-pollutant load in the WWTP outlet.



There is an highly significant correlation between the fluorescence and the concentration of micro-pollutants (Cor=0.99 ; p=0.008) and between the absorbance and the fluorescence (Cor=0.95 ; p=0.04).

Conclusions

The assessment of the endocrine effect using the FrogBox for several consecutives months has highlighted:

-Variations in the endocrine activity in the treated wastewater over the course of days, weeks and months.
-The weakest endocrine effects early august when the population is lowest in the Paris region.
-The thyroid effect to be overall correlated with the changes in concentrations of micro-pollutants targeted by the chemical analysis. Importantly, notable exceptions were observed when the micro pollutants concentration was low but the thyroid activity stayed high showing that the global load in micro-pollutants could not always predict the thyroid activity.
-Bioassays to be only approach integrating the action of all molecules to reveal the physiological effect of the water.

This study also shows the relevance of in-line bioassays for endocrine activity assessment. This technology allowed the characterisation of the efficiency of the tertiary treatment and highlights the efficiency of the activated carbon based tertiary treatment for the removal of the endocrine disruptors.