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Deliverable of WG3

Deliverable 13

White Paper on the existing knowledge with regard to wastewater and biological hazards

November 2018

COST EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

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This article is based upon work from the COST Action ES1403: New and emerging challenges and opportunities in wastewater reuse (NEREUS), supported by COST (European Cooperation in Science and Technology).

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Executive summary

Deliverable 13 "*White Paper on the existing knowledge with regard to wastewater and biological hazards*" is compiled by members of WG3 "*Effect-based bioassays required for wastewater reuse schemes*" within the framework of the NEREUS COST Action ES1403 "*New and emerging challenges and opportunities in wastewater reuse*".

The intention of this Deliverable was to summarize published scientific knowledge from the application of *in vitro* biotests on the effluents of wastewater treatment plants (WWTPs) and to correlate determined effects with treatment technologies as a base for technical risk management measures. This approach is known for organic trace pollutants and should have been transferred to the application of *in vitro* biotests suggested in Deliverable 14.

It is known, that low loaded WWTPs based on conventional activated sludge system (CAS) implementing full nitrification show higher removal performance for organic trace pollutants compared to high loaded plants for carbon removal only. It is hypothesized, that the further removal of organic trace pollutants correlates to a higher efficiency in the removal of biological effects addressed by various *in vitro* tests too. This is of special interest, as WWTPs with nitrification link up to requirements for eutrophication sensitive areas according to the UWWD Directive.

The literature review revealed, that only one paper (Välitalo et al., 2017) provides sufficient metadata describing technology and operation of the WWTPs investigated with effect-based *in vitro* biotests. In order to demonstrate the work done for Deliverable 13, results from literature review are given for estrogenic activities in the inflow and effluents of CAS plants without a correlation on technologies applied. Results show an average removal of about 90-95% of the estrogenic response.

In order to overcome the shortage in metadata for assessing the performance of CAS plants, the following minimum information on metadata should be provided:

- Design capacity (Population equivalents)
- Effective utilization (Population equivalents)

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- Share of industrial wastewater (%) and most significant type of industry (and physicochemical characteristics)
- Average flow (m^3/d)
- Type of treatment and solid separation
- Location of sampling and type of sample
- SRT (d) and / or F/M ratio (kg BOD/m^3/d)
- Nitrification rate (%)

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1. Aim of the Deliverable

Wastewater treatment plants (WWTPs) are of central importance for the quality of receiving water bodies and compliance with environmental quality standards for achieving a good ecological and chemical status as e.g. defined in the WFD (EC, 2000). Besides the assessment of biological quality criteria, the basic approach in the WFD follows a concentration-oriented approach for the status of a water bodies. Despite the fact that environmental quality standards are based on toxicological assessments the application of biotests for quality assessment of water bodies is not wide spread. Only when new substances and substance classes as endocrine disrupting chemicals and corresponding adverse effects in surface waters were detected and identified as such, it became obvious, that approaches with chemical analysis alone or the application of traditional acute and chronical ecotox-tests are not sufficient to assess certain adverse effects in receiving waters, as they are not sensitive enough in doing so. As a consequence, toxicological tests already applied in REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) for the assessment of chemicals were applied to environmental water samples too and new test systems were developed.

With increasing pressures on the water resources available globally and an increasing demand to reuse the wastewater for various purposes, human health related aspects (hygiene and human toxicology) additionally received increased importance. Biotests to address specific endpoints as estrogenicity, mutagenicity, cytotoxicity also are applied to the effluents of wastewater treatment plants in order to show their contribution to adverse effects in the receiving waters or for reuse. Both, environmental and human health aspects resulted in the core question about the removal of human and ecotoxicity by wastewater treatment plants.

Within the framework of WG3 ″Effect-based bioassays required for wastewater reuse schemes″, the basic intention of the Deliverable 13 was to summarize published scientific knowledge from the application of *in vitro* biotests on the effluents of wastewater treatment plants and try to correlate effects with treatment technologies as a base for technical risk management measures. Whereas such information at least partially is available for the chemical side, no compilation of such information for *in vitro* biotests is available. A correlation between toxicological assessments and technologies requires a certain amount of metadata describing the WWTP technology and operational mode applied.

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Dynamic fluctuations in wastewater itself and in operation additionally can hamper the approach.

2. Status of topic related

WWTPs are the core step in the urban water cycle to remove anthropogenic pollution introduced into the water by human activities or industrial processes. Historically, organic substances measured as COD, BOD or D/TOC in the mg/L range were responsible for severe pollutions of water bodies and high loaded WWTPs with short sludge retention times were built in order to degrade the (usually) readily degradable organic substances by aerobic biological processes. The removal of organic substances in those wastewater treatment plants resulted in a significant decrease of acute toxicological effects compared to the raw sewage too. Subsequent increase in the requirements for effluent water qualities towards the removal of ammonia toxicity and eutrophication resulted in the design of a next generation of low loaded wastewater treatment plants, where nitrification and denitrification were implemented. Additionally, local requirements as disinfection or further removal of effluent suspended matter led to the implementation of additional technical steps. For that status of technologies traditional parameters as COD, nutrient concentrations and suspended effluent matter (still) are appropriate quality criteria and therefore implemented in permits and legislation worldwide (see e.g. EC 1998).

The "success" behind biological wastewater treatment based on activated sludge processes is the decoupling of the hydraulic retention time of wastewater and the growth of the bacteria performing the biological removal. Whereas the hydraulic retention time is in the range of hours, the sludge (or solid) retention time (SRT) is in the range of days. The above mentioned high loaded WWTPs have a sludge retention time of few days only, whereas low loaded plants designed for nitrification have SRTs of >1 week and for denitrification even higher. Following Monod kinetics, the growth rate of a microorganism is related to the substrate available. The less substrate (starvation conditions) available the less the growth rate of bacteria. In order to gain energy under starvation conditions, previously recalcitrant substances are degraded under those low loaded (low food / microorganism – F/M ratio) conditions. This effect can be seen when correlating SRT as a synonym for organic loading (F/M ratio) and removal of organic trace pollutants as e.g. described by Clara et al. (2005) and recently by Achermann et al. (2018).

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Schaar et al. (2010) describe the situation of a wastewater treatment plant that is upgraded from a high loaded C-removal only plant to a low loaded plant implementing nitrification and denitrification. Investigations on the removal of organic trace pollutants for the same city was done prior and after that upgrade showing the difference in the removal for several organic trace compounds (Figure 1). Further on, the effluent from the new, low loaded plant was further treated by an ozonation step, resulting in even higher removal of parent compounds.

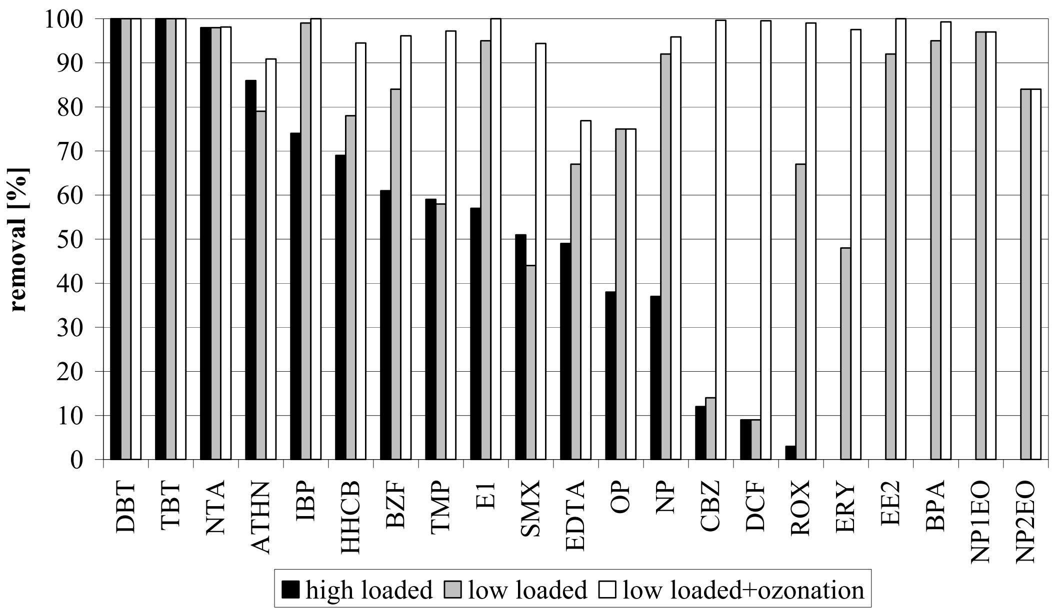

Figure 1 - Mean values for the removal of selected micropollutants under high and low loading and under low loading conditions with a subsequent ozonation at 0.6 g O_3 g DOC_0^{-1} (Schaar et al. 2010).

17α-ethinylestradiol: EE2; Estrone: E1; Bisphenol-A: BPA; Nonylphenol: NP; Nonylphenol monoethoxylate: NP1EO; Nonylphenoldiethoxylate: NP2EO; Octylphenol: OP; Bezafibrate: BZF; Carbamazepine: CBZ; Diazepam: DZP; Diclofenac: DCF; Erythromycin: ERY; Ibuprofen: IBP; Iopromide: IPM; Roxithromycin: ROX; Sulfamethoxazole: SMX; Trimethoprim: TMP; Galaxolide: HHCB; Tonalide: AHTN; Ethylenediaminetetra-acetic acid: EDTA; Nitrilotriacetic acid: NTA; Diethylenetriamine-pentaacetic acid: DTPA; 1,3-propylenediamine-tetraacetic acid: 1,3-PDTA; Diuron: DIU; Di-n-butyltin: DBT; Tri-n-butyltin: TBT; Tetra-n-butyltin: n.a.; Di-n-phenyltin: DPT; Tri-n-phenyltin: TPT.

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From the publications mentioned it could be concluded, that in parallel with the increasing reduction of organic trace compounds, a removal of substance that triggers adverse effects in traditional acute and chronic *in vivo* tests as well as in *in vitro* tests could be expected too. It was the goal of the Deliverable 13 to investigate on that particulate aspect.

In order to do so, basic engineering data of the WWTPs have to be provided by the researchers presenting results from biotests. The assessment of a WWTP without giving substantial metadata for that plant is not really helpful as WWTPs implementing e.g. the activated sludge process due to different design and operation may show differences in all effluent parameters by more than one order of magnitude. With regard to the correlation of WWTP technologies and resulting responses to *in vitro* biotests a single (!) publication could be found that a) provided at least basic operational and design parameters and b) discussed that very correlation directly in the paper. All other publications describe the investigated WWTPs only in an insufficient way and e.g. only give basic information as size, hydraulic retention time, few effluent concentrations and only a very rough description of the system applied (e.g. conventional activated sludge plant or MBR)

Only Välitalo et al. (2017) provide a detailed description of 7 WWTPs investigated in their study for toxicological tests on cytotoxicity, androgenic activity, estrogenic activity (x2), thyroid disruption, genotoxicity (x2) and zebrafish embryo test, most of the tests being based on *in vitro* bioassays. They conclude that within the investigated endpoints the key effects were estrogenic activity, thyroid disruption and fish embryo toxicity. Further they concluded, that toxicity removal efficiency of the WWTPs did not show dependency between the operational parameters or WWTP characteristics, but rather showed similar patterns for each toxicological endpoints. This conclusion is not surprising, as all of the plants have SRTs > 9 days with full nitrification implemented and major changes in the removal of organic trace pollutants is reported to be around 7 days; being the minimum required SRT for nitrification. This is important for the reason that WWTPs for eutrophication sensitive areas according to the UWWD (EC, 1998) require nitrification and therefore SRTs > 7 days, whereas other WWTPs only require secondary treatment that translates to C removal only in high loaded WWTPs.

As a consequence of the literature review done within WG3, the intended goal of Deliverable 13 cannot be achieved, as except from Välitalo et al. (2017) nowhere sufficient

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metadata on the WWTP is provided! A citation derived from that paper supports that fact: "*To our knowledge, there are no previous studies on the correlation between sludge age or sludge retention time (SRT) and general toxicity removal. Previous studies have been focused on individual chemical compounds, such as pharmaceuticals or hormones.*"

In order to demonstrate the way, work in D13 was initiated and performed, results for the most abundant information in literature, the application of estrogenic and androgenic endpoints for *in vitro* assay is summarized below. Literature on removal of estrogenic effects by CAS (conventional activated sludge) plants as well as advanced technologies (ozonation and activated carbon adsorption) was reviewed and information transferred to a database, summarizing effect targeted, the test system applied, the equivalent substance providing the unit stated and data for inflow as well as effluent noted. If data for the removal efficiency is given, that information was summarized too. Accessed literature is provided in the literature chapter at the end of the Deliverable.

Table 1 provides an overview on the papers assessed for information on inflow and effluent concentrations from conventional WWTPs. In quite a lot of cases more than one WWTP was investigated, so in a second sheet, all individual results were collected. Results on reported inflow and effluent concentrations (ng/L Estradiol equivalents) are depicted in Figure 2. Results in Figure 2 show an average inflow concentration of about 100 ng EEQ/L compared to an average of 5 ng EEQ/L (not considering 2 outliers with about 170 ng EEQ/L). This translates to an average removal of the estrogenic signal of about 90-95% in conventional activated sludge plants. Compared with the suggested trigger value of 0.5 ng EEQ/L (please refer to Deliverable 14) this still is one order of magnitude above and subsequently causing estrogenic effects in the effluent water itself. Considering a 1:10 dilution of wastewater effluents in receiving waters, the environmental trigger value already is close. It has to be stressed, that it cannot be concluded, if the WWTPs analysed are representative. But combining the results with the results provided by Clara et al. (2005) in Figure 1, it could be concluded, that SRT of the assessed WWTPs is expected to exceed 7 days and therefore fostering nitrification.

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Table 1 - Summary of literature referring to the removal of estrogenic effects in conventional wastewater treatment plants.

Target	Test system	Unit	WWTPs			Reference
			Influent	Effluent	Removal (%)	
Anti-Androgenic	Anti-AR-Calux	µg F-EQ/L	< LOD – 170	< LOD – 810	-	Gehrmann et al. (2016)
Anti - Androgenic	Anti-AR-Calux	ng F-EQ/L	N.D	500 – 2500	-	Houtman et al. (2018)
Anti - Androgenic	Anti-AR-Calux	ng F-EQ/L	-	< 22000	-	F. D. L. Leusch et al. (2018)
Anti - Androgenic	Anti-AR-Calux	ng F-EQ/L	-	14000 - 36000	-	Frederic D. L. Leusch, Neale, Hebert, Scheurer, and Schriks (2017)
Anti-Androgenic	YAAS	µg F-EQ/L	< LOD – <460	< LOD	-	Gehrmann et al. (2016)
Anti-Androgenic	YAAS	µg F-EQ/L	-	82000 - 1400000		Frederic D. L. Leusch et al. (2017)
Anti-Estrogenic	Anti-ER-Calux	µg TMX-EQ/L	62 – 1700	< LOD – 680	-	Gehrmann et al. (2016)
Anti-Estrogenic	YAES	µg OHT-EQ/L	7860 – > 31000	< LOD – 4830	-	Gehrmann et al. (2016)
Anti-Estrogenic	Anti-ER-Calux	µg TMX-EQ/L	-	< 4200	-	F. D. L. Leusch et al. (2018)
Anti-Estrogenic	Anti-ER-Calux	ng TMX-EQ/L	-	< 500	-	Frederic D. L. Leusch et al. (2017)
Anti-Estrogenic	YAES	ng TMX-EQ/L	-	13000 - 97000	-	Frederic D. L. Leusch et al. (2017)
Androgenic	AR-Calux	ng DHT-EQ/L	< LOD – 41	< LOD – 36	-	Gehrmann et al. (2016)
Androgenic	AR-Calux	ng DHT-EQ//L	LOD – 68		-	Valitalo et al. (2017)
Androgenic	AR-Calux	ng DHT-EQ//L	-	< 4	-	F. D. L. Leusch et al. (2018)
Androgenic	AR-Calux	ng DHT-EQ//L	-	< 0.1 – 2	-	Frederic D. L. Leusch et al. (2017)
Androgenic	YAS	ng T-EQ/L	650 – 800	10 – 250	62.1 – 98.4	Liu et al. (2009)
Androgenic	YAS	ng T-EQ/L	237 – 654	< LOD	-	Gehrmann et al. (2016)
Androgenic	YAS	ng DHT-EQ//L		< 0.5 – 635	-	Frederic D. L. Leusch et al. (2017)
Estrogenic	ER-Calux	ng E-EQ/L	580 – 620	160 – 170	73	Z.-h. Liu, Ito, Kanjo, and Yamamoto (2009)
Estrogenic	ER-Calux	ng E-EQ/L	29 - 185	< LOD – 6.4	90 – 99	Frédéric D. L. Leusch et al. (2006)
Estrogenic	ER-Calux	ng E-EQ/L	1.5 – 152.64	0.05 – 2.5	> 99	Vethaak et al. (2005)
Estrogenic	ER-Calux	ng E-EQ/L	0.65 – 74.93	N.D – 0.599	> 99	Vethaak et al. (2005)
Estrogenic	ER-Calux	ng E-EQ/L	9.8 – 50.5	< LOD – 43.5	13.8 – 99	Avbersek, Zegura, Filipic, Uranjek-Zevart, and Heath (2013)
Estrogenic	ER-Calux	ng E-EQ/L	20.4 – 57.7	< LOQ – 57.7	> 95	Avbersek et al. (2013)
Estrogenic	ER-Calux	ng E-EQ/L	-	0.925 – 23.8	-	Valitalo et al. (2016)
Estrogenic	ER-Calux	ng E-EQ/L	0.45 – 42	1 – 2	> 95	Valitalo et al. (2017)
Estrogenic	ER-Calux	ng E-EQ/L	35 – 90	< 1	> 99	Houtman, Ten Broek, and Brouwer (2018)
Estrogenic	ER-Calux	ng E-EQ/L	0.4 – 0.8	< LOD – 0.4	-	Gehrmann et al. (2016)
Estrogenic	ER-Calux	ng E-EQ/L	-	0.03 – 22.93	-	Kase et al. (2018); Könemann et al. (2018)
Estrogenic	ER-Calux	ng E-EQ/L	-	< 0.6 – 0.78	-	F. D. L. Leusch et al. (2018)
Estrogenic	ER-Calux	ng E-EQ/L	-	< 0.05 – 30	-	Frederic D. L. Leusch et al. (2017)
Estrogenic	E-Screen	ng E-EQ/L	108 – 356	<1 – 14.8	> 95	Tan (2006)
Estrogenic	E-Screen	ng E-EQ/L	14 – 56	N.D – 5.6	91	Körner et al. (2000)
Estrogenic	E-Screen	ng E-EQ/L	229.55 – 233.63	1.74 – 3.13	83-93	Shappell et al. (2007)
Estrogenic	MELN bioassay	ng E-EQ/L	46 – 63	2 – 24	62 – 97	Cargouet, Perdiz, Mouatassim-Souali, Tamisier-Karolak, and Levi (2004)
Estrogenic	MELN bioassay	ng E-EQ/L	-	1.2 – 81.2	-	Jarošová, Bláha, Giesy, and Hilscherová (2014)
Estrogenic	MELN bioassay	ng E-EQ/L	-	0.037 – 19.72	-	Kase et al. (2018); Könemann et al. (2018)
Estrogenic	MELN bioassay	ng E-EQ/L	-	< 0.03 – 24	-	Frederic D. L. Leusch et al. (2017)
Estrogenic	YES	ng E-EQ/L	590 – 600	180 – 195	73	Liu, Ito, Kanjo, and Yamamoto (2009)
Estrogenic	YES	ng E-EQ/L	20.26 – 18.30	6.75 – 7.40	17.7 – 66.68	Balsiger, de la Torre, Lee, and Cox (2010)
Estrogenic	YES	ng E-EQ/L	22.09 – 18.11	ND – 7.42	17.7 - > 99	Balsiger et al. (2010)
Estrogenic	YES	ng E-EQ/L	< LOD – < 2.8	< LOD – 8.0	-	Gehrmann et al. (2016)
Estrogenic	YES	ng E-EQ/L	-	0.01 – 12	-	Kase et al. (2018); Könemann et al. (2018)
Estrogenic	YES	ng E-EQ/L	-	< 0.02 – 98	-	Frederic D. L. Leusch et al. (2017)

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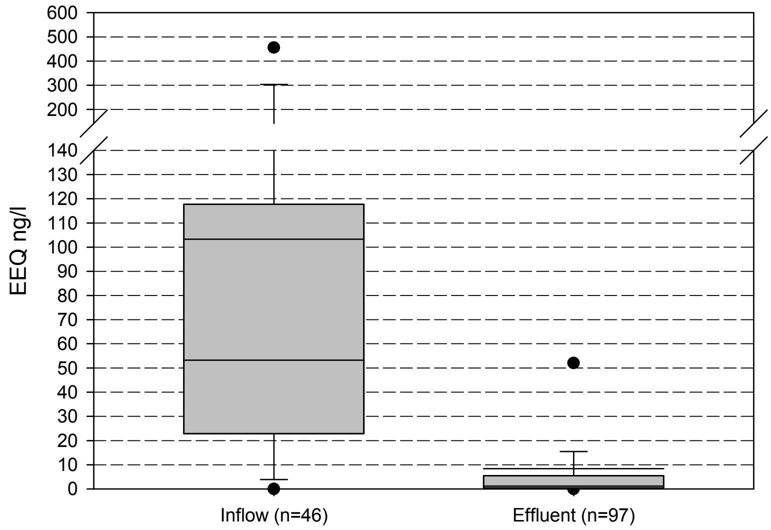

Figure 2 - Box plot of all inflow and effluent EEQ concentrations from CAS plants (The values were derived from Table 1).

3. Summary and suggested approach

Engineers have to figure out technologies and operation conditions for wastewater treatment that comply with evaluation quality standards (EQS) or trigger values defined by toxicologists as treatment targets. As a first step, proven and commonly applied technologies have to be assessed, in order to obtain a baseline of what can be expected from readily available technologies according to best available technology. Especially in the case of new test systems that access new endpoints – as effect-based *in vitro* biotests can be considered – metadata for applied technologies and operation conditions have to be supplied. That essential information is missing in most cases, resulting in the unfavourable situation, that no immediate suggestions for technologies necessary to meet required quality criteria can be given.

In that regard the following minimum information on metadata to describe the WWTPs and treatment technologies applied should be provided for Conventional activated sludge plants (CAS):

- Design capacity (Population equivalents)
- Effective utilization (Population equivalents)

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- Share of industrial wastewater (%) and most significant type of industry (and physicochemical characteristics)
- Average flow (m^3/d)
- Type of treatment and solid separation
- Location of sampling and type of sample
- SRT (d) and / or F/M ratio (kg $BOD/m^3/d$)
- Nitrification rate (%)

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