# Determination of the estrogenic activity in effluents of a landfill leachate treatment plant analyzed by the E-screen

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**Objective**

The aim of this study was to establish the E-screen assay for analyzing the total estrogenic activity of different clean-up steps (biodegradation, sedimentation and charcoal treatment) of a landfill leachate treatment plant in Japan.

**Introduction**

The rapid screening of a large number of chemicals and samples for their estrogenic potencies requires simple, sensitive, and specific *in vitro* bioassays. In the last 10 years several *in vitro* bioassays have been established and used for analyzing endocrine disrupting chemicals (EDC).

In a recent report of Anderson et al.[1)] 19 endocrine disrupting chemicals (EDC) were analyzed by three laboratories with different bioassays. In this study the E-screen data of the three different laboratories showed comparable results.

Koerner et al.[6,7)] has already successfully established the E-screen to analyze effluents of a municipal sewage sludge plant. In this study we tried for the first time to apply the E-screen for analyzing the total endocrine activity in effluents of a landfill leachate treatment plant.

First we validated the E-screen with some already known EDC like bisphenol A, butylbenzylphtalate (BBP), butylhydroxyanisole (BHA) and ethinylestradiol (Anderson et al.[1)], Soto et al.[2)], Villalobos et al.[3)], Sonnenschein et al.[4)], Schuller[5)] and Koerner et al.[6,7)]).

We analyzed by E-screen the estrogenic activity of treated water samples taken in each step of the biodegradation/sedimentation and charcoal treatment of a landfill leachate treatment plant in comparison to a nearby river water.

Finally, we discuss some data of PCDDs/PCDFs, PCBs, Co-PCBs, bisphenol A, nonylphenol, octylphenol and phtalates analyzed by GC/MS and 17β-estradiol analyzed by ELISA of this landfill leachates.

## Materials and Methods

### 1. E-screen

Cultivation of the MCF-7 cells and performance of the proliferation experiment was carried out according to the principle method described by Koerner et al.[6,7].
Briefly, the cells were cultivated in DME medium with 5 % fetal calf serum (FCS) in a humidified atmosphere with 5 % $CO_2$. For starting the proliferation experiment the cells were seeded in 24 well plates with a density of 5000 cells per well. After 24 hours the medium was changed to the experimental medium, phenolred-free DME medium containing 5 or 10 % CD-FCS (FCS treated with charcoal-dextrane to remove all steroids). Four wells per assay were the negative control without hormones. The other wells additionally contained 17ß-estradiol (E2) in concentrations between $10^{-12}$ M and $10^{-9}$ M as positive control or the validation standards in final concentrations between $10^{-9}$ M and $10^{-4}$ M, respectively. Each concentration of a compound was tested in four wells. The stock solutions of the compounds were prepared in DMSO or ethanol (0.1 %). Five days later the cells in each well were counted by using the sulforhodamine B (SRB) assay.

### 2. Procedure for handling the water samples

The water treatment was done according to the method descripted published by Koerner et al.[6,7]. For this study we analyzed four different kind of water samples from a municipal landfill: a) the leachate from this landfill, b.) the water after the biodegradation/sedimentation, c.) the water after the additional treatment with charcoal (which is comparable with the final effluent of the landfill into a nearby river) and d.) a water from this river. All samples were stored in brown glass bottles until extracted. All samples were extracted within a few days after sampling. For all samples solid phase extraction with a 200 mg polystyrene copolymer resin ENV+ (6 ml) from ICT (Bad Homburg, Germany) was applied with 1 L water.

### 3. GC/MS analysis

The GC/MS analysis of the PCDD/PCDFs, PCBs, Co-PCBs, bisphenol A, nonylphenol, octylphenol and different phtalates were done according to the Analytical Manual published by the Japan Environmental Agency.

## Results and Discussion

### 1. Testing of different charcoal-dextrane stripped Fetal calf serum (CD-FCS)

We have tested different FCS of different manufacturer (Gibco and Wako), which were treated with charcoal/dextrane to make them steroid-free. We found comparable results with the FCS of Koerner et al.[6,7] used Gibco Mycoclone Super plus and our favorite FCS from Gibco named Certified 16000. Also the best two FCS of Wako Life Science (Whittaker Bio, USA and Trace Bioscience, Australien) showed good results.
Our prefered CD-FCS Gibco C 16000 showed a $EC_{50}$ value for estradiol of 9 x $10^{-12}$ M. This results are closer to that of Koerner et al.[6,7] used Mycoclone Super (Gibco; $EC_{50}$ value of estradiol of 8 x $10^{-12}$ M).

## 2. Validation of the optimized E-screen

Five known EDC were tested in this study by E-screen: 17β -Estradiol (Sigma, Germany), bisphenol-A 97 % (Fluka, Japan), ethinylestradiol (Sigma, Germany) and 3-tert.-butyl-4-hydroxyanisole 98 % (BHA, Wako, Japan) and benzylbutylphthalate 98 % (BBP, Wako, Japan).
In Table 1 the relative potencies ($EC_{50}$) we obtained in this study are compared with the data recently published in the literature [1-7].

**Table 1:** $EC_{50}$ values (mol) of the validation standards in the E-screen of this study and 4 other laboratories (Andersen et al.[1], Schuller[5] and Koerner et al.[6,7])

| Compound | Andersen [1] | Soto [1] | Olea [1] | Koerner/ Schuller [5,6,7] | Our study |
|---|---|---|---|---|---|
| 17β -Estradiol | $6x10^{-13}$ | $7x10^{-12}$ | $1x10^{-13}$ | $1.1x10^{-11}$ (n=44) | $6.2x10^{-12}$ (n=34) |
| Ethinyl-E2 | NE | $8x10^{-12}$ | $3x10^{-12}$ | $8.1x10^{-12}$ | $6.4x10^{-12}$ (n=2) |
| Bisphenol A | $2x10^{-7}$ | $7x10^{-7}$ | $2x10^{-7}$ | $2.6x10^{-7}$ | $1.1x10^{-8}$ (n=11) |
| BBP | R<50 | $5x10^{-6}$ | $1x10^{-6}$ | $3.1x10^{-6}$ | $5.1x10^{-7}$ (n=9) |
| BHA | NA | NA | NA | $5.3x10^{-6}$ | $7.7x10^{-7}$ (n=1) |

NE, value could not be estimated from the response curve, R<50, maximal response observed for the test chemical at the concentration tested was below 50% of the maximal 17β-estradiol.
NA, not analyzed in the study.

As shown in Table 1, the results of this study calculated as a $EC_{50}$ values and the resulting estradiol equivalent (EE) for 17β-estradiol and ethinyl-estradiol (EE=1.1) were comparable to those published by Anderson et al.[1], Soto et al.[2], Villalobos et al.[3], Sonnenschein et al.[4], Schuller[5] and Koerner et al.[6,7].
The EE of this study show a one magnitude higher value for BBP ($1.2x10^{-5}$, n=9) and bisphenol A ($5.6x10^{-4}$, n=11) and a similar data for BHA ($9.1x10^{-6}$, n=1) compared with the values of Andersen et al.[1] and Koerner et al.[6,7].
The deviations for some of the other validation standards may mainly be due to the different quality of this compounds, as we used chemicals from other distributors or different charges.

## 3. The total estrogenic activity of landfill leachates determined by E-screen

In the first results of the estrogenic activity in landfill leachates, the final effluent didn't show a higher MCF-7 cell proliferation compared with the water of a nearby river.
The resulting EE values of the final effluent and a river water sample are in the same range as observed with effluents from a sewage sludge treatment plant (2-7 ng EE/L) reported by Koerner et al.[6,7].
However, the untreated leachates and the leachates only treated by biodegradation/sedimentation didn't show a clear dose-response in the proliferation assay with MCF-7 cells which differ from what we expected. This may be caused by various substances in the uncleaned leachates, which influence the cell growth of the MCF-7 cells. Currently, we are studying the possible interferences in the E-screen.

**4. GC/MS and ELISA data of endocrine disruptors in landfill leachates**

In Table 2 the PCDDs/PCDFs, PCBs, Co-PCBs, nonylphenol, octylphenol and bisphenol A concentrations in the different water samples determined by GC/MS are given.

The three leachate treatment steps of the landfill show in case of the PCDDs/PCDFs and Co-PCBs/PCBs a reduction (80%) of the resulting PCDDs/PCDFs/PCB-TEQ (WHO/IPCS 1993). Also the amounts of bisphenol A, nonylphenol and octylphenol decreased by each treatment. The 17β-estradiol data analyzed by ELISA suggests that the major reduction of its amount in the leachate came from the last step, the charcoal treatment.

**Table 2:** PCDDs/PCDFs, PCBs, Co-PCBs, bisphenol A, nonylphenol, octylphenol and estradiol concentrations in the landfill leachates

| Compound | Conc | I. Leachate | II. Biodegradation/ sedimentation | III. Charcoal/ effluent | River water | Detection limit |
|---|---|---|---|---|---|---|
| PCDDs (TEQ) | pg/l | 21 (0.037) | 5.8 (0.012) | 5.2 (0.0093) | 26 (0.74) | |
| PCDFs (TEQ) | pg/l | 8.7 (0.029) | 7.9 (0.018) | 3.3 (0.015) | 15 (0.24) | |
| Co-PCB (TEQ) | pg/l | 300 (0.058) | 100 (0.016) | 9.5 (0.0024) | 170 (0.032) | |
| Sum of TEQ | pg/l | 0.124 | 0.046 | 0.027 | 1.012 | |
| PCB | ng/l | 46 | 6.8 | 1.2 | 3.9 | |
| Bisphenol A | µg/l | 0.13 | 0.013 | n.d. | 0.012 | n.d.<0.005 |
| Nonylphenol | µg/l | 2.8 | n.d. | n.d. | n.d. | n.d.<0.05 |
| Octylphenol | µg/l | n.d. | n.d. | n.d. | n.d. | n.d.<0.03 |
| Estradiol (ELISA) | µg/l | 0.005 | 0.009 | n.d. | n.d. | n.d.<0.001 |

Phtalates (e.g. BBP, DBP, DEHP, dicycohexylphtalate, diethyl-, dipropyl-, dipentyl- and dihexyl-phtalates) were under the detection limit ( 0.1-0.3 µg/l).

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