SOOT AS A SOURCE OF ESTROGEN- AND ANDROGEN RECEPTOR ACTIVATING COMPOUNDS

Klaus Rehmann[*,**], Karl-Werner Schramm[**] and Antonius A. Kettrup[*,**]

[*] Chair of Ecological Chemistry and Environmental Analytics, Technical University Munich, D-85350 Freising-Weihenstephan, Germany
[**] Institute of Ecological Chemistry, GSF-National Research Center for Environment and Health, Ingolstaedter Landstrasse 1, D-85764 Neuherberg, Germany

Introduction

The topic of endocrine disruption has attracted immense attention over the last years e. g. [1, 2]. It became evident, that many man made xenoestrogens enter the environment on the aquatic route via sewage water [3, 4]. However there are other paths as well. For example it has been shown that estrogen-like activities are carried by airborne particles [5]. As aerosols partially derive form incineration processes, we decided to screen soot on its endocrine disrupting properties using a yeast assay on estrogen receptor activating compounds, and since in contrast to environmental estrogens virtually nothing is known about sources of anthropogenic androgens, we included additionally a yeast screen on testosterone receptor activating compounds.
To allow for the identification of relevant compounds and to ensure that possible positive results were not masked by substances disturbing the bioassays the soot extract was submitted to a treatment consisting of a solvent polarity dependent fractionation on a silica gel column followed by a RP-TLC separation of the active fraction(s) from the silica gel column.

Materials and Methods

Chemicals of the highest purity available were purchased from Aldrich, Steinheim, Germany Baker, Griesheim, Germany, Fluka, Neu-Ulm, Germany, Merck, Darmstadt, Germany and Riedel de Hœn, Seelze, Germany. Yeast nitrogen base was obtained from Difco, Augsburg, Germany.
The yeast strains applied were those introduced by Routledge and Sumpter [6] and Gaido et al. [7]. The assays were performed and the data evaluated essentially as described by Rehmann et al. [8, 9] with the composition of the yeast growth media adopted to the strains used, with respect to their specific selection markers. Test cultures were exposed for 2 h (estrogen assay) and overnight (approximately 16 h, androgen assay) with 17β-estradiol and testosterone respectively serving as reference compounds.
The soot investigated was obtained from domestic heating (wood and coal firing). Five grams of soot were subjected to a dichloromethane (DCM):acetone (1:1, 400 mL) soxhlet extraction for 24 h. (An extract prepared with a n-hexane (Hex):acetone (1:1) mixture gave almost identical results). The extract was filtered through anhydrous Na_2SO_4, evaporated to dryness under reduced pressure and resuspended in 10 mL methanol (MeOH). Three millilitre of this suspension were placed on a 40 g silica gel column, sequentially eluted with 500 mL of Hex, DCM, and MeOH, and the solvent volumes were reduced to 3 mL each using a rotary evaporator. A 600 µL subsample of the DCM fraction was separated further by TLC on a RP18 phase applying a

Hex:DCM (3:1) solvent system. TLC fractions were scraped off according to the fluorescence band pattern observed under UV illumination and eluted three times with 5 mL MeOH. All fractions were evaporated to dryness, redissolved in 1 mL MeOH and 100 µL aliquots were withdrawn for chemical analysis. Finally the MeOH was exchanged for a suited volume of DMSO, which was done for all samples to be tested in the yeast assays. Prior to use the DMSO dissolved samples were stored at 4°C in the dark as were the reference compound solutions.

Results and Discussion

The activity of DCM (Hex):acetone soot extracts and subfractions of the extracts in yeast screens for estrogen- and androgen receptor activating compounds respectively was examined to obtain information about the endocrine disrupting potency of incineration by-products.

As can be drawn from Figure 1 the soot raw extract showed a pronounced estrogen-like potency with the activity extracted from 1.35 mg soot corresponding to about 40 % of the response elucidated by a 3 nM solution of 17β-estradiol. Fractionation of the raw extract by polarity-dependent elution from silica gel resulted in a slight increase of the total estrogenic activity recovered which pointed to a finite interference of soot constituents with the assay. The largest fraction of the activity observed was located in the medium polar DCM eluate. Further separation of the DCM eluate by RP-TLC showed that the components responsible for the effects seen resided in the most polar fraction as they possessed very low R_f values. Again, the over all recovery of estrogenic activity from the RP-TLC was slightly higher than 100 % when compared to the DCM eluate.

Our findings indicate that soot and thus airborne soot particles carry a distinct load of estrogen receptor activating compounds. As the chemical analysis of the most potent fractions has not been completed yet one may only speculate about the nature of the active ingredients but one may not be completely wrong when assuming that phenolic compounds will be considerable candidates.

Aromatic compounds which share a relatively planar structure as common feature represent major constituents within all kinds of tar and soot. Thus the less pronounced androgen-like activity observed is not that much surprising since the androgen receptor in contrast to the estrogen receptor does not very strongly interact with planar compounds [6] because its effector molecule testosterone (actually 5α-dihydrotestosterone) is also nonplanar.

To obtain a more convincing picture of the endocrine disrupting capacity of incineration by-products in the near future further investigations are warranted. First of all the active soot components have to be identified. Here initial results will be available soon. Furthermore different kinds of soot have to be compared with respect to their estrogenic and androgenic potency to look for a correlation e. g. between the activities observed and the source or formation conditions of the sample.

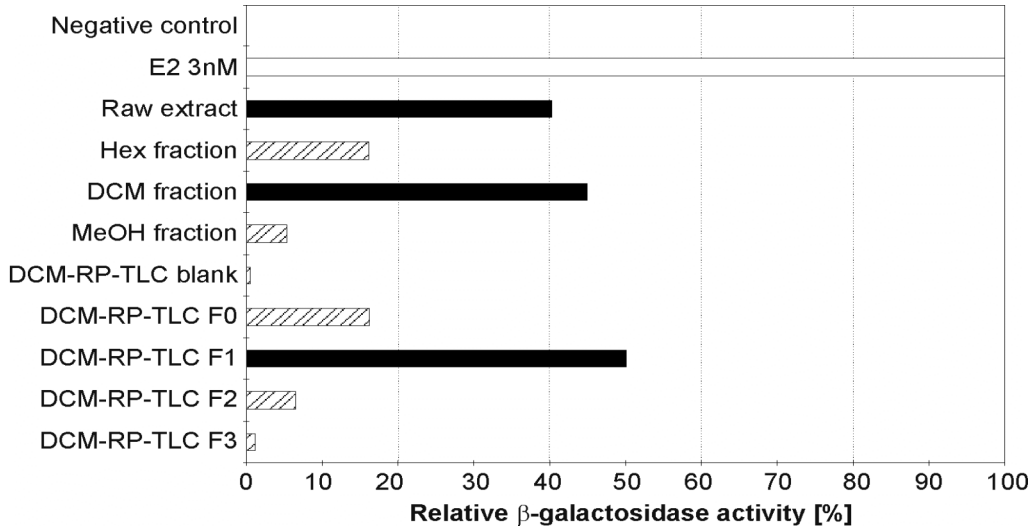

FIGURE 1 Estrogenic activity of soot. Potency of the raw DCM/acetone soot extract, and of the silica gel and RP-TLC fractions as compared to 3 nM 17β-estradiol ($EC_{50,\ 17\beta\text{-estradiol}}$ ≈1 nM). The sample content of the different fractions corresponded to 1.35 mg of soot. R_f-values for the TLC fractions were: Blank 1.14-1.35, F0 -0.02-0.02, F1 0.02-0.09, F2 0.09-0.14, F3 0.14-0.20.The activity of the soot extract and its silica gel fractions in the yeast androgen screen was lower than the estrogenic activity when compared to the respective reference compounds (Figure 2)

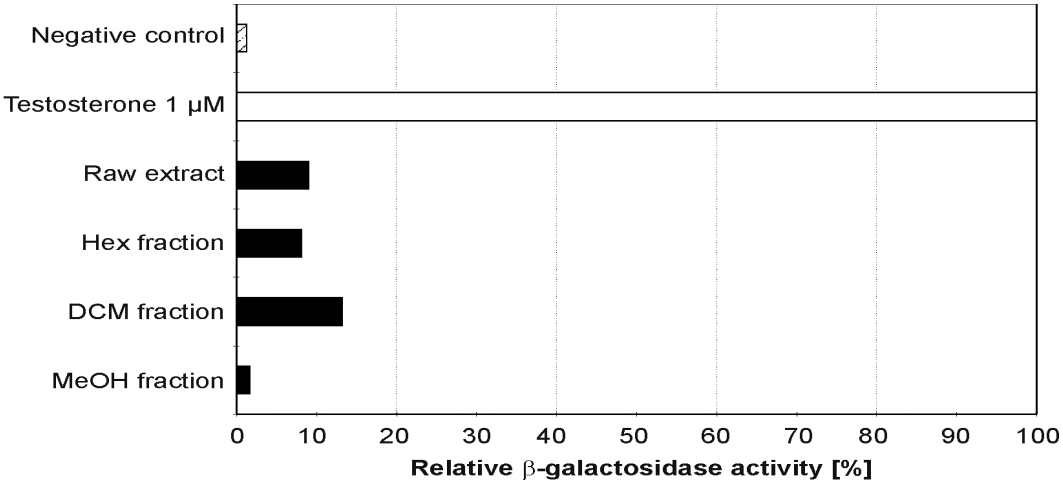

FIGURE 2 Androgenic activity of soot. Potency of the raw DCM/acetone soot extract and of the silica gel column subfractions as compared to 1µM testosterone ($EC_{50,\ testosterone}$ ≈24 nM). The sample content of the different fractions corresponded to 1.35 mg of soot.

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