Supplemental Material

Negative controls. Since we specifically examined the effect of gastrointestinal digestion processes on the production of estrogenic PAH metabolites, we corrected for background signals coming from the digestion matrix as such. Therefore we tested blank digest suspensions, which are typically the stomach, small intestinal or colon suspension from the SHIME reactor to which no PAHs had been added. No positive responses were expected from the blank stomach or small intestinal digests, but the colon suspension from the SHIME reactor is a complex mixture of microbiota and hundreds of different metabolites among which some compounds may posses pseudo-estrogenic properties. The latter may be capable of binding the human estrogen receptor in the bioassay test and hence lead to a small, yet positive response. Blank stomach and small intestine digests indeed did not induce a significant response in the yeast estrogen bioassay (Figure S1a), hence no corrections for these digests were performed in later experiments. We noted however an inhibition in yeast growth when the bioassay was performed on blank small intestine digests. This inhibtion was previously explained by the presence of bile salts (De Boever et al. 2001). These compounds are present in the small intestine digests at 0.2 mmol/L and may disrupt the membrane integrity of the yeast cell wall. In contrast to the stomach and small intestine digests, the blank colon digest elicited a positive response of 28% EE2 equivalence at the highest concentration (Figure S1a), thus making corrections on experimental data necessary. This value may seem high, but mainly originated from low absorbance values at 630 nm, which increases the final response, calculated as $(A540/A\ 630)_{net}$. All dose-response curves of colon digests were hence corrected for this matrix background signal.

PAH contaminated soil samples were also included in this research and they too were subjected to the digests from the SHIME model. Besides PAHs and their possible metabolites, these soils may also contain pseudo-estrogenic compounds, phytoestrogens for instance. We

examined whether the presence of phytoestrogens in a soil sample could be of any concern in the yeast estrogen bioassay. We therefore incubated a clover (Family Leguminosae) soil, putatively rich in phytoestrogens to a stomach, duodenal and colon digest and analyzed these samples in the bioassay. Very low estrogenic signals of 5.6% EE2 equivalence were observed for stomach or duodenal digests on this soil (Figure S1b). A colon digestion on this leguminosae soil did lead to a slightly positive response in the lower 2^1 to 2^4 dilution range, however not significantly different from the blank colon suspension. After correction for this colon background signal, a maximal estrogen response of 4.5% was obtained (Figure S1b). We therefore concluded that no corrections for possible phytoestrogens in the PAH contaminated soil were needed, since on the latter soil there was primarily growth of Poaceae, which produce phytoestrogens at much lower concentrations than Leguminosae (Grippo et al. 1999).

Supplemental references.

De Boever P, Demare W, Vanderperren E, Cooreman K, Bossier P, Verstraete W. 2001. Optimization of a yeast estrogen screen and its applicability to study the release of estrogenic isoflavones from a soygerm powder. Environ Health Persp 109: 691-697.

Grippo AA, Xie Y, Rougeau BL, Wyatt WV. 1999. Analysis of phytoestrogens by high performance liquid chromatography. Journal of the Arkansas Academy of Science 53: 61-66.

Supplemental figures: captions

Figure S1. Dose-response curves in the estrogen yeast assay for negative control samples. Values are averages of 4 replicates. Error bars represent standard deviation and may disappear in the datapoint if too small. **a.** blank stomach, small intestine and colon digests to which no PAHs had been dosed, were screened for estrogenic effects in the bioassay. **b.** stomach, small intestine and colon digests on a clover soil, rich in fytoestrogens were tested for their estrogenic response in the yeast estrogen bioassay. The estrogen response for the colon digest has been corrected for the matrix background response coming from colon suspension (Figure S1a).

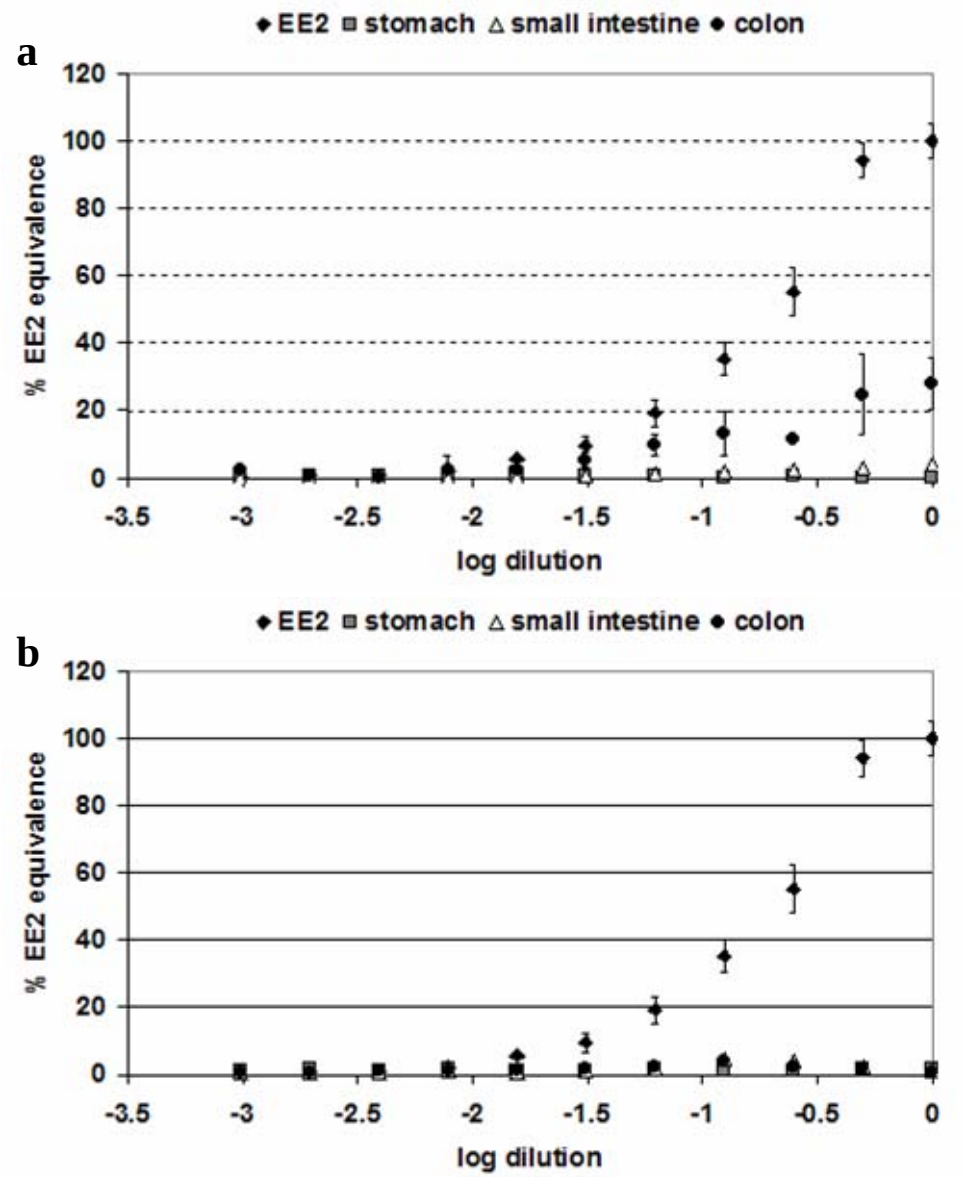
a
◆ EE2 ▫ stomach △ small intestine ● colon
% EE2 equivalence
120
100
80
60
40
20
0
-3.5
-3
-2.5
-2
-1.5
-1
-0.5
0
log dilution
b
◆ EE2 ▫ stomach △ small intestine ● colon
% EE2 equivalence
120
100
80
60
40
20
0
-3.5
-3
-2.5
-2
-1.5
-1
-0.5
0
log dilution

Figure S1.