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Comparison of an array of in vitro assays for the assessment of the estrogenic potential of natural and synthetic estrogens, phytoestrogens and xenoestrogens

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Abstract

Many chemicals in surface waters and sediments have recently been discovered to have estrogenic/antiestrogenic activity. Among these compounds, known as 'endocrine disrupters', are natural and synthetic hormones, phytoestrogenes and a variety of industrial chemicals, such as certain detergents and pesticides. These substances are supposed to affect the development and reproduction in wildlife and humans and may also be involved in the induction of cancer. In order to assess the estrogenic/antiestrogenic potential of pure compounds and complex environmental samples we compared an array of in vitro test systems, (i) two luciferase reporter gene assays using transgenic human MVLN cells (derived from MCF-7 cells) and HGELN cells (derived from HeLa cells); (ii) a competitive binding assay with recombinant human estrogen receptors (ER) α and β; and (iii) a proliferation assay with MCF7-cells (E-Screen). The sensitivity of the assays for 17-β-estradiol decreased in the order: MVLN-cells = E-Screen > HGELN-cells > binding to ER-α > binding to ER-β. A good correlation was obtained between the estrogenic potencies of 11 compounds (17-β-estradiol (E_2), estrone (E_1), estriol (E_3), ethinylestradiol (EE_2), diethylstilbestrol (DES), coumestrol, β-sitosterol, genistein, 4-nonylphenol, 4-octylphenol, bisphenol A) in the three tissue culture assays. The relative potencies of the compounds obtained by the cell free binding assays were one to two orders of magnitude higher compared with the cell culture assays. The phytoestrogens showed a preference to bind to ER-β, but only genistein showed a much lower activity in the E-Screen (growth induction in breast cancer cells) compared with the luciferase induction in MVLN and HGELN-cells. © 2001 Published by Elsevier Science Ireland Ltd.

Keywords: Endocrine disrupter; Water; Bioassay; In vitro assay; Xenoestrogen; Phytoestrogen

Abbreviations: E_2, 17-β-estradiol; EE_2, ethinylestradiol; E_3, estriol; EEq, estradiol equivalent factor; H4IIE.Luc, transgenic rat hepatoma cell line; HGELN, (HeLa Gal-ER-Luc-Neo) transgenic human cell line; MVLN, (MCF-7-p-Vit-tk-Luc-Neo) transgenic human cell line.

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1. Introduction

During the last years, there is growing concern about environmental chemicals, which interact with the endocrine system, because of their possible adverse effects to the health of humans and wildlife (Colburn et al., 1996). The risk assessment of environmental hormones in surface waters and sediments is currently under debate (Safe, 1995; Daston et al., 1997; Juberg, 2000). Biological effects of these endocrine modulators were detected in wildlife including abnormal reproductive development in alligators from Lake Apopka, FL (Guillette et al., 1994), vitellogenin induction in fish living near sewage outfalls with relatively high concentrations of ethinylestradiol (Purdom et al., 1994), changes in sex steroids in the circulating serum and sperm quality of fish near craft-mill outfalls (Munkuttrick et al., 1992), disturbance in sexual phenotype of fish (Bortone and Davis, 1994) and nearly complete mortality of trout in Lake Ontario, Canada, in the sac-fry stage, presumably resulting from exposure to dioxin-like compounds. So far, about 50 different compounds were identified as possible 'endocrine disrupting chemicals' (EDC) (Matthiesen et al., 1996). These compounds are able to act as estrogens, like natural (17-β-estradiol (E_2); estriol (E_3); estrone (E_1)) and synthetic hormones (ethinylestradiol (EE_2), diethylstiblestrol (DES)), phyto- and mycoestrogens, pharmaceutical or therapeutic agents and industrial chemicals (Matthiesen et al., 1996). Other EDC could act as antiestrogens like chlorinated dioxins and -furanes, PCB-congeners and PAH; as androgens like tributyltin, or as antiandrogens, like p.p′-DDE or the fungicide vinclozolin. These substances have been identified in chemical analysis (Stumpf et al., 1996; Desbrow et al., 1998) or in bioassays (Zacharewski et al., 1995; Murk et al., 1996; Guelden et al., 1997; Gutendorf et al., 2000). Many approaches were made to identify endocrine disrupting chemicals, using a variety of biological screening assays. However, no testing guidelines for the in vitro assays have been evaluated so far (EPA, 1998). In most cases only a single assay is used by one laboratory and the comparability of the different assays have not been investigated. Therefore, it is difficult to compare the data about the estrogenic potential of environmental samples evaluated by different laboratories with different methods. In the present investigation we compared the estrogenic potential of a variety of chemicals known to act as estrogens or antiestrogens in an array of in vitro assays with different endpoints.

2. Materials and methods

2.1. Chemicals

2,4,6,7,8 [^{3}H]-17-β-estradiol, (85 Ci/mmol, > 98% pure), was purchased from Amersham International (Uppsala, Sweden). DCC-fetal bovine serum (FBS) was from Hyclone (Erembodegem/Aalst, Belgium). Acetic acid, acetonitrile (HPLC grade) (CH_3CN), bisphenol A, dichloromethane (DCM), disodium hydrogen phosphate, dimethylsulfoxide (DMSO), ethanol (HPLC grade) (EtOH), ethylenediaminetetraacetate (EDTA), magnesium chloride, methanol (HPLC grade) (MeOH), 4-octylphenol, sodium dodecyl sulfate (SDS) were from Merck (Darmstadt, Germany). FBS, Dulbecco's Modified Eagle Medium without phenol red and trypsin-EDTA were purchased from Gibco BRL (Grand Island, NY). Bovine insulin and bovine serum albumine (BSA) were from ICN Biochem. (Costea Mesa, CA). Estradiol (E_2), estriol (E_3), estrone (E_2), ethinylestradiol (EE_2), DES, dithiotreitol (DTT), DMSO, Dulbecco's Modified Eagle Medium with Ham's F12 Nutrient Mixture (DME), genistein, glutamin, glycerol, hydroxylapatite (HAP), hydroxytamoxifen, lactate dehydrogenase (LDH), MTT, neomycin, penicillin, RPMI-1640 (phenolred-free), β-sitosterol, sodium acetate, sodium pyruvate, streptomycin, tamoxifen, Tris (all of molecular biological grade) and Triton-X 100 were obtained from Sigma Chemical (St. Louis, MO). 4-Nonylphenol was purchased from Dr Ehrensdorfer, Germany. Coumestrol was from Fluka (Deisenhofen, Germany). The human estrogen receptors (ER) α and β were purchased from PanVera (Madison, USA). ICI 182 780 was obtained from Zeneca (Macclesfield, UK). The luciferase reporter gene kit was obtained from

Promega (Mannheim, Germany), the LDH kit was purchased from Boehringer Mannheim (Mannheim, Germany) and the protein determination kit was from Bio-Rad (Muenchen, Germany).

2.2. Luciferase-reporter-gene assay for the detection of the hormone activity

The assay was conducted according to the procedure of De Wet et al. (1987), Pons et al. (1990) with minor modifications. Two transgenic human cell lines were applied for the detection of the hormone-activity. The first one, (MVLN-cells, MCF-7-p-Vit-tk-Luc-Neo) were derived from MCF-7 cells containing an estrogen regulated luciferase gene driven by an estrogen-receptor-responsive-element (ERE) in front of the vitellogenin-tyrosin-kinase-promoter (Gagne et al., 1992; Pons et al., 1992). The MVLN cells were a gift of J. Giesy and A. Blankenship, Michigan State University, USA, and were constructed in the laboratory of M. Pons (Institute National de la Santé et de la Recherche Medicale, Montpellier, France). The second cell line (HGELN cells) was stably cotransfected with a plasmid conferring resistance to neomycin, p17m5-βGlob-Luc and the chimeric receptor expression plasmid GAL4-ER (Chen et al., 1995). HGELN cells were a gift of H. Gronemeyer and P. Chambon, (INSERM, Strasbourg, France). Both cell lines expressed ER-α but not ER-β.

The MVLN and the HGELN cells were maintained in Dulbecco's Modified Eagle's Medium, MVLN cells additional with Ham's 12 nutrient mixture, both without phenol red. The mediums were supplemented with 10% fetal calf serum (FCS), 1% penicillin–streptomycin and 1% glutamine. The medium used for MVLN cells additionally contained 1-mM sodiumpyruvate and 1 μg/ml bovine insulin. The medium used for HGELN cells was supplemented with 0.8 mg/ml neomycine. Two days before induction, 10 000 cells per well were seeded in 96-well plates in medium with 10% FCS. After twenty-four hours the medium was changed against medium containing 5% hormone reduced FBS (DCC-FBS). At the day of induction the medium was changed against medium without FCS and the cells were treated for 48 h with the test compounds. The detection of luciferase was performed using a commercial detection kit (Promega). The luciferase activity was calculated in relative light units (RLU) per mg protein.

2.3. Protein determination

As an indirect measurement of the cell density the protein concentration was determined in triplicate according to the method of Bradford (1976). Crystalline BSA was used as protein standard.

2.4. Cell viability

In order to control the conditions of the cell culture of the reporter-gene assays, we performed a colorimetric assay, based on the measurement of extracellular LDH activity (LDH determination kit from Boehringer Mannheim) (Mosman, 1983). The cell lysing agent Triton-X 100 was used as a positive control.

2.5. Competitive binding assay

The competitive binding assay was carried out with modifications according to the hydroxylapatite method as described by Murdoch et al. (1990) using human estrogen receptors α and β. The estrogen receptors (1 pmol) were diluted in TEGM buffer (10 mM Tris–HCl, 1.5 mM Na_2–EDTA, 10% glycerol, 1 mM DTT, pH 7.7). Aliquots of the receptors were incubated with 250 nM [^{3}H]-E_2 and increasing concentrations of the unlabeled competitors in presence and absence of a 200-fold excess of 17-β-estradiol at 4 °C for 18 h. Receptor–ligand complexes were bind to hydroxylapatite (250 μl of 0.16% (w:v)) by incubation at 4 °C for 15 min. After centrifugation at $2000 \times g$ for 5 min, unbound steroid was removed by several washing steps. The supernatants were removed, pellets were suspended in 1-ml ethanol, added to 5.0 ml scintillation fluid and counted in a Beckmann scintillation counter (Beckmann, Fullerton, CA). Triplicate probes were run at each dose level.

2.6. E-Screen

The E-Screen was performed according to the method of Soto et al. (1992) with modifications. The day before treatment 3.000 MCF-7 cells per ml (HTB-22, ATCC) were seeded in 96-well-plates in RPMI-1640 medium containing 10% FCS. At days 2 and 5 the medium was changed against medium containing 5% DCC-FBS and treated with the test substances. At day 8, the MTT-test (Denizot and Lang, 1986) was performed. These assays based on the cleavage of the yellow tetrazolium salt MTT to purple formazan crystals by metabolic active cells. For the test the cells were labeled with 10 µl of the labeling reagent (5mg/ml MTT diluted in phosphate buffered saline (PBS)). After the incubation period of 4 h (incubator, humified atmosphere), the formed formazan crystals were solubilized by adding 100 µl of the solubilization solution (DMSO 9.94 ml:acetic acid 60 µl:SDS 1 g). After shaking the plate and allowing to stand for 5 min, the spectrophotometrical absorbance of the formazan product was measured using a microtiter plate reader. The wavelength of measurement was 540 nm, the reference wavelength was 620 nm. A correlation between the MTT-readings and the number of cells was established before.

3. Results

The aim of this study was to evaluate the applicability of several in vitro assays for the determination of the estrogenic/antiestrogenic potential of environmental occurring compounds. The test systems consisted of two reporter-gene assays in transgenic human cell lines (HGELN-cells and MVLN-cells), a growth induction test in a human breast cancer cell line (MCF-7, E-Screen) and a binding assay with human estrogen receptors α and β.

The sensitivity of the different assays represented by the EC_{50}-values for the standard estrogen β-estradiol (E_2) is shown in Table 1. According to these values the sensitivity of the test systems decrease for 17-β-estradiol in the order, MVLN-cells = E-Screen > HGELN-cells > binding to ER-α > binding to ER-β. All the assays were also applied to an array of compounds with known estrogenic or antiestrogenic activity. These substances belong to natural estrogens (E_1, E_2, E_3), synthetic estrogens (EE_2, DES), phytoestrogens (β-sitosterol, genistein, coumestrol), xenoestrogens (4-nonylphenol, 4-octylphenol, bisphenol A) and ER-receptor antagonists (ICI-164 384, tamoxifen, hydroxytamoxifen). As shown

Table 1
EC_{50} of standard compounds obtained with the test systems

	Luciferase reporter gene assay with MVLN cells	Luciferase reporter gene assay with MVLN cells	E-Screen	Competitive binding with the ER α	Competitive binding with the ER β
17-β-Estradiol	5 pM	40 pM	5 pM	3.5 nM	65 nM
Estriol	60 pM	100 pM	70 pM	50 nM	250 nM
Estrone	480 pM	720 pM	500 pM	500 nM	1 µM
Ethinylestradiol	4 pM	7 pM	4 pM	3 nM	45 nM
Diethylstilbestrol	4 pM	5 pM	2 pM	2 nM	50 nM
4-Nonylphenol	400 nM	500 nM	400 nM	20 µM	30 µM
5-Octylphenol	60 nM	50 nM	50 nM	5 µM	10 µM
Bisphenol A	200 nM	210 nM	200 nM	15 µM	25 µM
Genestin	38 nM	50 nM	40 nM	35 µM	2 µM
β-Sitosterol	50 nM	55 nM	52 nM	4 µM	4 µM
Coumestrol	40 nM	40 nM	45 nM	3 µM	3 µM
Tamoxifen	600 nM	1 µM	700 nM	1.5 nM	40 nM
OH-Tamoxifen				150 nM	1.2 µM
ICI 182 384				1 nm	20 µM

The EC_{50} were calculated by probit analysis utilizing the Graph Pad Prism Sofware 3.0, San Diego, USA.

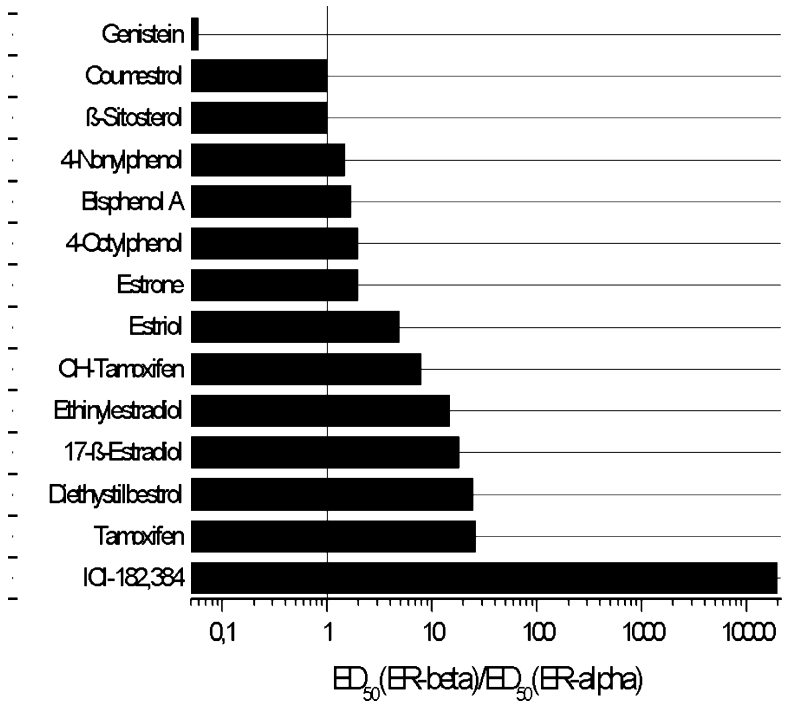

Fig. 1. Relative potencies of the test compounds to bind to ER α and -β determined by the competitive binding assay. For further information concerning the test procedure see Section 2.

in Table 1, the rank of the sensitivity of the different assays for the test compounds does not always correlate with that of 17-β-estradiol. An interesting observation is the activity of the antagonist tamoxifen in the cell culture assays, confirming earlier observations about the partial agonistic/antagonistic properties of this compound.

The relative binding of the test compounds to both estrogen receptors is shown in Table 1 and Fig. 1. Similar binding to the ER-receptors occurs for the phytoestrogens coumestrol and β-sitosterol, the xenoestrogens 4-nonylphenol, bisphenol A, 4-octylphenol and the estradiol metabolite estriol. Some higher affinity to ER-α was observed for estriol, hydroxytamoxifen, ethinylestradiol, diethystilbestrol and tamoxifen. The antagonist ICI-182 384 binds almost exclusively to ER-α, whereas the affinity of the phytoestrogen genistein for ER-β is much higher than that for ER-α.

The relative potencies ($E_2 = 1$) of ER-agonistic compounds determined as ED_{50}-values from the dose response curves are shown in Table 2. The synthetic estrogens EE_2 and DES were the most potent in all assays with relative potencies of 1.25–2.5. The metabolites of E_2 were less potent than the parent compound ranging between 10^{-1} and 10^{-2}, followed by the phytoestrogens (10^{-3}–10^{-4}) and the xenoestrogens (10^{-4}–10^{-5}). The relative potencies determined by the cell culture assays were all very similar, whereas the values obtained from the binding assays with ER-α and -β were higher by one to two orders of magnitude.

An interesting question was the comparability of the potencies derived from the different assays. Taking MVLN-cells as the standard assay, we plotted the relative potencies obtained by these cells against that derived from the other assays in a double logarithmic scale and calculated the correlation by a linear curve fitting. The results are shown in Fig. 2a–d. There was a relatively good comparison of the potencies derived by HGELN and MVLN cells, with a slope of 0.957 and a R-value of 0.99938 (Fig. 2a). Similar results were

obtained if MVLN cells and the E-Screen were compared (slope = 1.049, $R = 0.9891$; Fig. 2b). Only the phytoestrogen genistein did not fit well to this curve. The potency of genistein in the E-Screen was lower by one order of magnitude compared with that obtained with MVLN-cells. The correlation between the potencies obtained by the tissue culture assay with MVLN cells and the cell free binding assays with isolated human ER-α and -β was much lower. The slope of the curves were only 0.7431 (ER-α) and 0.5075 (ER-β), indicating an overestimation of the relative potencies derived from these assays (Fig. 2c and d).

4. Discussion

The terms 'endocrine disrupter' or 'endocrine modulator' describe environmentally occurring compounds exerting effects on the hormonal regulation of various organisms. Especially compounds mimicking the action of 17-β-estradiol have been extensively investigated during the last decade and the possible significance for humans and wildlife has been discussed (Juberg, 2000). Among such compounds are naturally or synthetic estrogens (17-β-estradiol, ethinylestradiol), phytoestrogens (genistein, β-sitosterol, coumestrol) and many industrial chemicals (alkylphenols, DDT and metabolites, PCB's). Although the impact of such compounds on the endocrine system of humans is a controversial issue, there is general agreement that these compounds may influence wildlife (Juberg, 2000). To determine the estrogenic potential of environmental substrates, such as water, sediment or soil, a variety of in vitro and in vivo assays have been introduced. Among these are receptor-reporter-gene assays using transgenic human cell lines (Pons et al., 1990) or yeast (Routledge and Sumpter, 1997), a growth induction test with a human breast cancer cell line, the E-Screen (Soto et al., 1992) and cell free binding assays using estrogen receptors α and β (Kuiper et al., 1997). In vivo assays monitoring changes in the weight of rodent uteri (Odum et al., 1997) and the vitellogenin blood level of fish (Purdom et al., 1994) are also available. Most laboratories use only one of these test systems to describe the estrogenic potential of pure chemicals or complex mixtures. However, because of the different endpoints used, it cannot be expected that the correlation of the results obtained by these assays is always good. In the present investigation some important in vitro assays for the determination of estrogenicity were used to compare the response of 11 pure compounds. The substances used in this study belong to different chemical classes, all of which occur in the environment, either from natural or anthropogenic sources.

Table 2
Relative potencies ($E_2 = 1$) of ER-agonistic compounds determined as ED_{50}-values from the dose response curves

Compound	Chemical group	MVLN cells	HGELN cells	E-Screen	Binding to ER-α	Binding to ER-β
17-β-Estradiol	Natural estrogen	1	1	1	1	1
Estriol	Natural estrogen	0.083	0.4	0.071	0.07	0.26
Estrone	Natural estrogen	0.01	0.056	0.01	0.007	0.065
Ethinylestradiol	Synthetic estrogen	1.25	5.71	1.25	1.16	1.44
Diethylstilbestrol	Synthetic estrogen	1.25	8.0	2.5	1.75	1.3
4-Nonylphenol	Xeno-estrogen	1.25×10^{5}	8.0×10^{-5}	1.3×10^{-5}	1.75×10^{-4}	2.3×10^{-3}
4-Octylphenol	Xeno-estrogen	8.33×10^{-5}	8.0×10^{-4}	1.0×10^{-4}	7.0×10^{-4}	6.5×10^{-3}
Bisphenol A	Xeno-estrogen	2.5×10^{-5}	1.9×10^{-4}	2.5×10^{-5}	2.3×10^{-4}	2.6×10^{-3}
Genistein	Phyto-estrogen	1.32×10^{-4}	8.0×10^{-4}	1.3×10^{-5}	1.0×10^{-4}	0.032
β-Sitosterol	Phyto-estrogen	1.0×10^{-4}	7.3×10^{-4}	9.6×10^{-5}	8.75×10^{-4}	0.016
Coumestrol	Phyto-estrogen	1.25×10^{-4}	1.0×10^{-3}	1.1×10^{-4}	1.17×10^{-3}	0.022
Tamoxifen	Antagonist	8.33×10^{-6}	7.1×10^{-7}	4.0×10^{-5}	0.023	0.054
OH-Tamoxifen	Antagonist			2.33	1.25	
ICI 182 384	Antagonist			3.5	3.25	

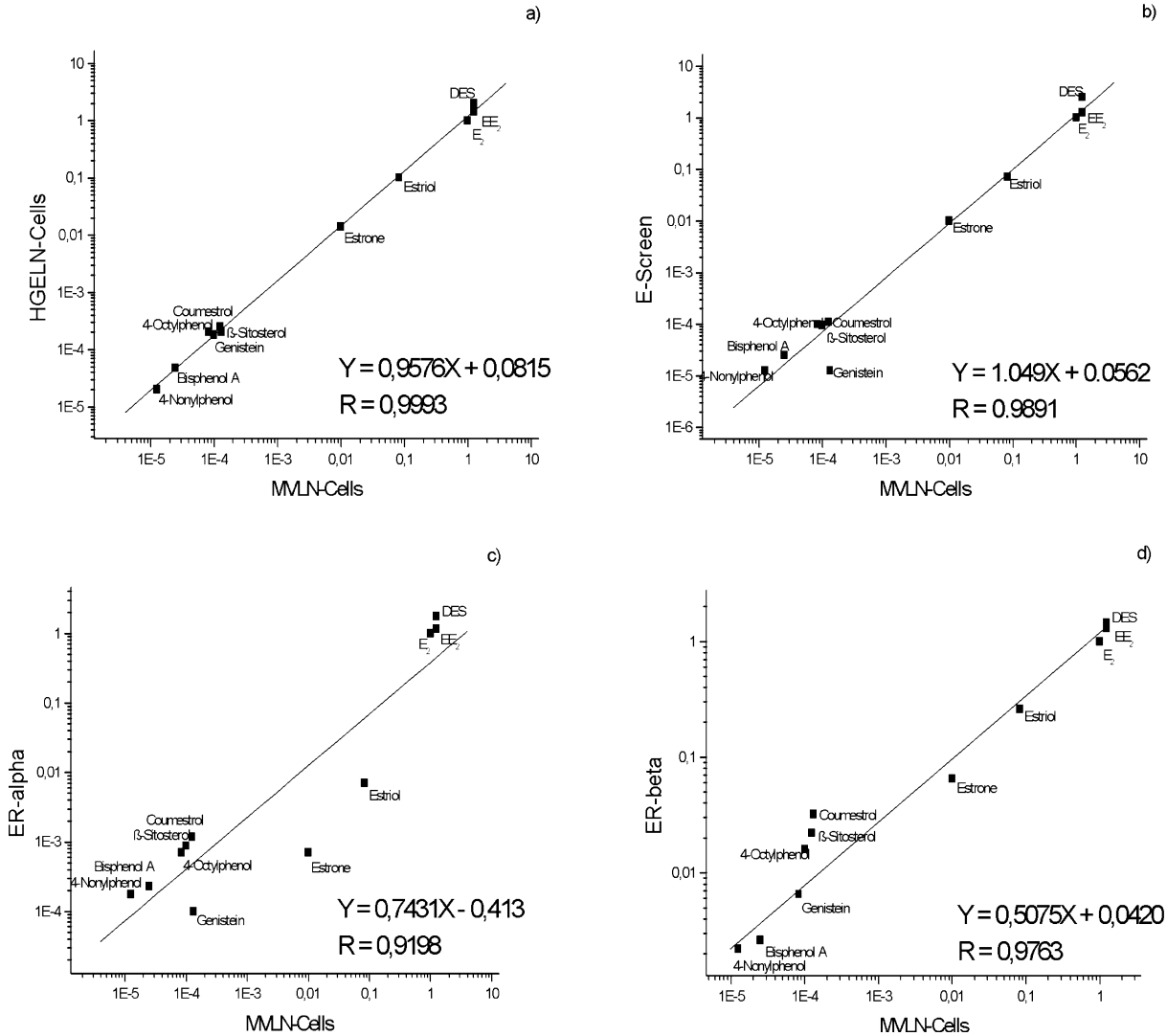

Fig. 2. (a–d) Correlation between the relative potencies ($E_2 = 1$) obtained by the luciferase reporter gene assay with MVLN cells and (a) the luciferase-reporter-gene-assay with the HGELN cells; (b) E-Screen; (c) competitive binding assay with ER α; (d) competitive binding assay with ER β. The luciferase activity was calculated in estradiol equivalents (EEQ) per mg protein. Experiments were performed in duplicate. The error bars represent S.E. values of four parallels per experiment. For further information concerning the test procedure see Section 2. DES, diethylstilbestrol, E_2, 17-β-estradiol; EE_2, ethinylestradiol.

The potency of a certain compound is best characterized by the ED_{50}-value. A comparison of these values obtained with the test compounds in an array of in vitro assays shows considerable differences. Taken E_2 as an example, the ED_{50} varies between 5 (MVLN cells, E-Screen) and 65 nM (binding to ER-β). In HGELN cells, E_2 is eight times less potent compared with MVLN cells, although both assays use luciferase-transfected human cell lines, whereas the synthetic hormone ethinylestradiol is almost equally potent in both luciferase assays.

The test compounds used in this investigation represent different classes of chemicals contributing to the estrogenic potential of environmental samples such as surface water or aquatic sedi-

ments. As shown in Table 1 the relative potencies of the compounds vary within five orders of magnitude. Most potent are E_2 and the synthetic hormones DES and EE_2 followed by the E_2-metabolites estrone and estriol. The phyto- and xenoestrogens are 10^4–10^5 times less potent compared with E_2. Similar results for the potency of the phyto- and xenoestrogens used in this study were also reported by other authors (Soto et al., 1992; Earl Gray et al., 1997; Kuiper et al., 1997, 1998). A comparison of all assays, which was performed in this study, was not carried out in the same laboratory.

Analog to the calculation of TEQ-values for Ah-receptor active compounds (Van-den-Berg et al., 1998), the estrogenic potential of complex mixtures, such as environmental extracts, should be expressed in estradiol-equivalents (EEq). The contribution of the different compounds present in such mixtures is expressed by the equation

$$\mathrm{EEq} = \sum x_i c_i$$

where x_i represents the relative potency and c_i the concentration of a certain compound in the mixture. Because the EEq is used for environmental risk assessment calculations, it is important to know, whether the results obtained by the use of different assays are in accordance. We, therefore, performed a calculation of the EEq of a hypothetical mixture of estrogenic active compounds in an array of different in vitro assays. The results are shown in Table 3. The EEq of this mixture is calculated according to the equation above using the relative potencies obtained by the different assays. The data for a luciferase reporter gene assay in yeast are taken from the literature (Gaido et al., 1997). The EEq-values calculated vary between 26 (yeast) and 302 pM (ER-β). The values obtained by MVLN cells, E-Screen and yeast are similar, whereas HGELN cells revealed a much higher value, mostly due to the high relative potency of DES in this assay. The example shows that the relative estrogenic potential of complex mixtures may vary greatly if different assays are used for the determination of such values.

The comparability of the different assays used in this study was also demonstrated by plotting the relative potencies of the test compounds achieved in one assay against that obtained in another assay (Fig. 2a–d). A double logarithmic scale was used because of the wide range of these potencies. Comparison of the relative potencies of the test compounds obtained by the different tissue culture assays revealed slope values in the range between 0.957 (HGELN/MVLN-cells) and 1.049 (E-Screen/MVLN-cells), whereas comparison of the potencies obtained by the luciferase assay using MVLN-cells with that obtained by the estradiol replacement test using human ER-α and -β is much lower (0.749 for ER-α and 0.5075 for ER-β).

At least two different estradiol receptors exist in humans and other higher organisms. The response of different organs on estradiol can be modified by variation of the expression of these two receptors. Exogenous compounds with estrogenic activity may exert a spectrum of activity different from that caused by endogenous hormones because of different affinities of these compounds to ER-α and -β. This was demonstrated by an array of eleven estrogenic active compounds used in this study. As shown in Fig. 1, E_2 itself binds preferentially to ER-α. Similar results were obtained with the synthetic hormones EE_2 and DES. The xenoestrogens and phytoestrogens, except genistein, showed an almost equal binding affinity for both receptor subtypes. Compared with E_2 this should result in a stronger induction of genes related to ER-β. Genistein binds preferentially to ER-β and should, therefore, stimulate the activity of osteoblasts, which is thought to be related to ER-β (Windahl et al., 2000), without inducing cell proliferation in breast or uterine tissue. This is confirmed by the lack of activity of genistein in the E-Screen (proliferation of breast cancer cells) in the present investigation. These data support the hypothesis that food plants rich in genistein, such as soybeans, have a beneficial effect on osteoporosis in postmenopausal woman without the negative side effects of a hormone substitution on the incidence of breast and endometrium cancer (Wisemann, 2000).

In summary, this investigation has shown the suitability of an array of in vitro tests, including reporter gene assays and receptor binding assays

Table 3
Comparison of the EEQ-values of a hypothetical mixture of estrogenic active compounds in different test systems

	Concentration (pM)	Luciferase reporter-gen-assay MVLN cells	Luciferase-reporter-gen-a E-Screen ssay HGELN cells		Luciferase-reporter-ge n-assay yeast[a]	Competitive binding assay ER α	Competitive binding assay ER β
17-β-Estradiol	10	10	10	10	10	10	10
DES	10	12.5	80	25	6.4	17.5	13
4-Nonyiphenol	30.000	0.35	2.4	6.38	6.00	5.25	69
Bisphenol A	50.000	1.25	9.5	1.25	3.33	11.50	130
β-Sitosterol	50.000	5.00	36.2	4.80	0.23	43.75	80
Sum of EEQ		29.13	138.10	41.43	25.96	88.00	302

The EEQ values of the different compounds in the mixture were calculated by multiplication of the relative potency of the test compounds (estradiol = 1) with the concentration of the compounds in the mixture (pM).

[a] The data of the luciferase reporter gene assay with yeast are by Gaido et al. (1997).

for the analysis of the estrogenic capacity of compounds belonging to different chemical classes. It was also demonstrated that the relative estrogenic potency of complex environmental mixtures determined with different assays might vary greatly. Standardized assays should be used, therefore, for the comparison of the estrogenic capacity of environmental mixtures from different locations.

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