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Combined effects of estrogenic chemicals with the same mode of action using an estrogen receptor binding bioassay

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ABSTRACT

The increasing amounts of various estrogenic chemicals coexisting in the aquatic environment may pose environmental risks. While the concept of estradiol equivalent (EEQ) has been frequently applied in studying estrogenic mixtures, few experiments have been done to prove its reliability. In this study, the reliability of EEQ and the related model concentration addition (CA) was verified based on the two-hybrid recombinant yeast bioassay when all mixture components had the same mode of action and target of action. Our results showed that the measured estrogenic effects could be well predicted by CA and EEQ for all laboratory-made mixtures using two designs, despite the varying estrogenic activity, concentration levels and ratios of the test chemicals. This suggests that when an appropriate endpoint and its relevant bioassay are chosen, CA should be valid and the application of EEQ in predicting the effect of non-equi-effect mixtures is feasible.

1. Introduction

The development of analytical measurement and the constant pollutant discharges lead to the increasing detection rates and concentrations of endocrine disrupting chemicals (EDCs) (Petrovic et al., 2004). The reproductive and developmental toxicities of EDCs to the aquatic organisms evoke worries about the impacts on human health, and their adverse effects after the long-term exposure to environmental observed concentrations (ng/L) aggravate these worries (Hotchkiss et al., 2008; Vandenberg et al., 2012; Zha et al., 2007). Particularly, steroid estrogens (both natural and synthetic) and phenolic xenoestrogens receive more attention because of their non-ignorable estrogenicities and widespread applications (Peng et al., 2006). These substances have high or moderate affinity to the estrogen receptor (ER), mimicking the normal function of natural estrogen and disrupting the endocrine

Abbreviations: 4-NP, 4-nonylphenol; 4-t-OP, 4-*tert*-octylphenol; BPA, bisphenol A; CA, concentration addition; DES, diethylstibestrol; E1, estrone; E2, 17β-estradiol; E3, estriol; EC50, half maximal effective concentration; EDCs, endocrine disrupting chemicals; EE2, 17a-ethinylestradiol; EED, equivalent effect design; EEQ, estradiol equivalent; ER, estrogen receptor; EV, estradiol valerate; MoA, mode of action; NOEC, no observable effect concentration; OD, optical density; RA, response addition; REP, relative potency; RMD, randomly mixed design; ToA, target of action; YES, yeast estrogen screen.

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Table 1 – Information, properties, EC_{50}s and REPs of the nine test chemicals.

Abbr.	Name	CAS no.	MW	EC_{50}	REP
E2	17-β-estradiol	50-28-2	272.39	18.3	1.00
E1	Estrone	53-16-7	270.37	2.52E+2	7.28E−2
EE2	17a-Ethinylestradiol	57-63-6	296.44	13.1	1.40
E3	Estriol	50-27-1	288.39	6.06E+3	3.03E−3
DES	Diethylstilbestrol	56-53-1	268.38	5.69E+2	3.22E−2
EV	Estradiol valerate	979-32-8	356.50	4.53E+4	4.04E−4
4-t-OP	4-*Tert*-octylphenol	140-66-9	206.36	1.33E+5	1.38E−4
4-NP	4-Nonylphenol	104-40-5	220.39	1.14E+5	1.60E−4
BPA	Bisphenol A	80-05-7	228.31	1.87E+6	9.82E−6

Abbr. = abbreviation; CAS = chemical abstract services registry number; MW = molecular weight (g/mol); EC_{50} = half maximal effective concentration (ng/L); REP = relative potency, REP(i) = EC_{50}(E2)/EC_{50}(i).

and reproductive systems. They are emitted from a variety of sources and seldom exist individually in any environmental compartment, leading to the combined adverse effects on organisms. There have been a number of surveys measuring either the estrogenic activity by use of bioassay, or concentration levels of the estrogenic substances by chemical analysis in water samples (Beck et al., 2006; Lavado et al., 2009; Mahomed et al., 2008; Matthiessen et al., 2006). To bridge the two measurements, concentrations could be transformed into biological activity using the estrogenic relative potency (REP).

In mixture toxicity research, concentration addition (CA) and response addition (RA) are two best-known and widely used models (USEPA, 2000). When taking no interaction into account, their differences mainly depend on whether all components of the mixture are toxicologically similar (Bliss, 1939; Loewe, 1928). Estradiol equivalent (EEQ) is a derived approach of CA, which just simplifies the calculation process by summing the component concentrations after adjusting them for each component's potency. In risk assessment, by comparing EEQs obtained from both analytical determination and bioassay, the major and minor contributors of a complex sample could be identified. When this was applied to environmental waters, some work reported that the predicted overall EEQs were similar to observed ones (Beck et al., 2006; Jiang et al., 2012; Liscio et al., 2009), but some found that the predictions were higher or lower (Cargouët et al., 2004; Furuichi et al., 2004). These contradictory conclusions may result from the contribution of non-targets chemicals such as unknown estrogen agonist, antagonist and humics, or the multiple involved modes of action (MoAs) and the resulting interaction of mixture components (Villeneuve et al., 2000). However, few studies have focused on the adaptations of EEQ and REP which are essential in the causality analysis. Thus, there is a need to confirm the validity of CA and EEQ for the most common ER-binding effects and EDCs with high detection rates by conducting a certain bioassay and eliminating the disturbance of other chemicals.

In the present study, we aimed to test the validity of CA and EEQ in calculating the binding capability to ER when the MoA and target of action (ToA) of all components are the same. To achieve the goal, we adopted a two-hybrid recombinant yeast bioassay to study the combined effects: the dimerization of ERs with estrogens leads to the conformational change of the ligand–receptor complex; then, the recruitment of co-activator and the transcription and translation of downstream reporter gene occur. Like the one-hybrid yeast estrogen screen (YES), this assay also offers single MoA (binding to ER) and ToA (ER). However, it is consistent with the in vivo tests better through introducing the co-activator (Li et al., 2008b; Sheeler et al., 1999). During each experiment, the high purity of yeast strain and setting of control groups should be guaranteed for the validity of test results.

2. Materials and methods

2.1. Chemicals

Estrone (E1, 99%), 17β-estradiol (E2, 97%), estriol (E3, 97%), bisphenol A (BPA, 99%), and dimethyl sulfoxide (DMSO, 99.5%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). 17a-ethinylestradiol (EE2, 99%), diethylstilbestrol (DES, 98%), and estradiol valerate (EV, 99.5%) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). 4-*Tert*-octylphenol (4-t-OP, 97%) was obtained from Sigma–Aldrich and 4-nonylphenol (4-NP, a mixture of branched chain isomers) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Information on the test chemicals is listed in Table 1. All the test chemicals were dissolved in DMSO and stored below −20 °C. They solved well and existed stably in the standard solution and diluted gradients, and the short exposure time would not change their concentrations in culture medium (Knox et al., 2011; Tetko et al., 2005). Therefore, the actual and nominal concentrations were considered in good agreement with each other.

2.2. Experimental design for the mixtures

Nine representative estrogenic chemicals were selected for mixture preparation, which have been frequently detected in aquatic environment and demonstrated to play a role in estrogenic pollution. They included three natural estrogens (E1, E2, and E3), three synthetic estrogens (EE2, DES, and EV) and three phenolic compounds (4-t-OP, 4-NP, and BPA). Two experimental designs were applied to create the laboratory produced mixtures: some were made to cause equal estrogenic effects, and some were arranged according to the related environmental concentrations.

Initially, twelve mixtures (M1–M12), including two mixtures with three components, six with four components, three with five components, and one with nine components, were