In vitro assays for assessment of androgenic and estrogenic activity of defined mixtures and complex environmental samples.

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Point sources of endocrine active compounds to aquatic environments such as waste water treatment plants, pulp and paper mills, and animal feeding operations invariably contain complex mixtures of chemicals. The current study investigates the use of targeted *in vitro* assays designed to detect specific hormonal activities. Two stable cell line assays are being evaluated as tools fro screening environmental effluents. MDA-kb2 is being used as a screen for androgenic activity and T47D-KBluc for estrogenic activity. In characterizing the utility of these assays for screening such complex environmental samples, it is important to first understand how mixtures of compounds with different mechanisms of action would affect assay results. To this end, several defined mixture studies have been conducted. EC_{50} and Hillslope data from individual compounds were incorporated into a dose addition model to predict mixture responses. Modeled predictions were then tested experimentally to determine if the mixtures interacted. The MDA-kb2 assay was evaluated using a seven androgen mixture and binary mixtures of androgen-estrogen, androgen-trichlocarban and androgen-antiandrogen. In the estrogen assay, binary mixtures of an endogenous estrogen (estradiol-17β, E_2) with exogenous estrogens (bisphenol A (BPA) or its analogues bisphenol AF and tetrabromo-bisphenol A) were tested and their interaction evaluated across a broad range of concentrations to test if environmental estrogens function in a dose-additive manner with endogenous estrogens. Six E_2 concentrations were each tested with six BPA concentrations in order to develop a surface response plot and to evaluate interactions at low doses. Overall, these studies indicate that mixtures of androgens or estrogens act in a dose additive manner in their respective assays. Additional androgen assay data suggests that binary mixtures of an androgen and an estrogen most reliably reflect activity of the androgen, some environmental chemicals like trichlocarban, could enhance androgenicity of a mixture, and, for the environmental anti-androgens tested, comparatively high concentrations were required to inhibit detection of androgens with varying potencies. Our results with defined mixtures support the use of these assays as reliable screens for androgenic or estrogenic activity in complex environmental samples. *This abstract does not necessarily reflect USEPA policy.*