

Evaluation of the endocrine disrupting potential of the binary exposure to trilostane and prochloraz in H295R cells and Medaka fish

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Introduction

Although living organisms are exposed to mixtures of environmental chemicals, most of previous studies on endocrine disrupting chemical (EDCs) have only evaluated individual chemical -induced effects. The objective of this study was to evaluate the mixture effect of two model EDCs, trilostane and prochloraz, on the reproduction axis, especially the steroidogenesis. Prochloraz is a commonly used imidazole fungicide, which has been reported to affect reproduction and development in fish and wildlife by inhibiting *CYP* genes, including steroidogenic cytochrome P450 c 17 α hydroxylase, 17,20-lyase (*CYP17*) and aromatase (*CYP19*). Trilostane is an inhibitor of 3 β -hydroxysteroid dehydrogenase (3 β HSD) that is upstream of *CYP17* and *CYP19* on the steroidogenesis pathway. The testable hypothesis is that co-exposure of prochloraz and trilostane could synergistically affect the production steroids, and thereby the reproduction of fish. Single and binary exposure of prochloraz and trilostane were conducted in both the H295R cells and small fish model Medaka. Trilostane (0.01-0.3 μ M) and prochloraz (0.001-0.03 μ M) synergistically inhibited the production of several steroid hormones by H295R cells after 12 h exposure. In medaka, co-exposure with trilostane (60 and 600 μ g/L) caused no further inhibition on fish egg production by prochloraz (30 μ g/L).

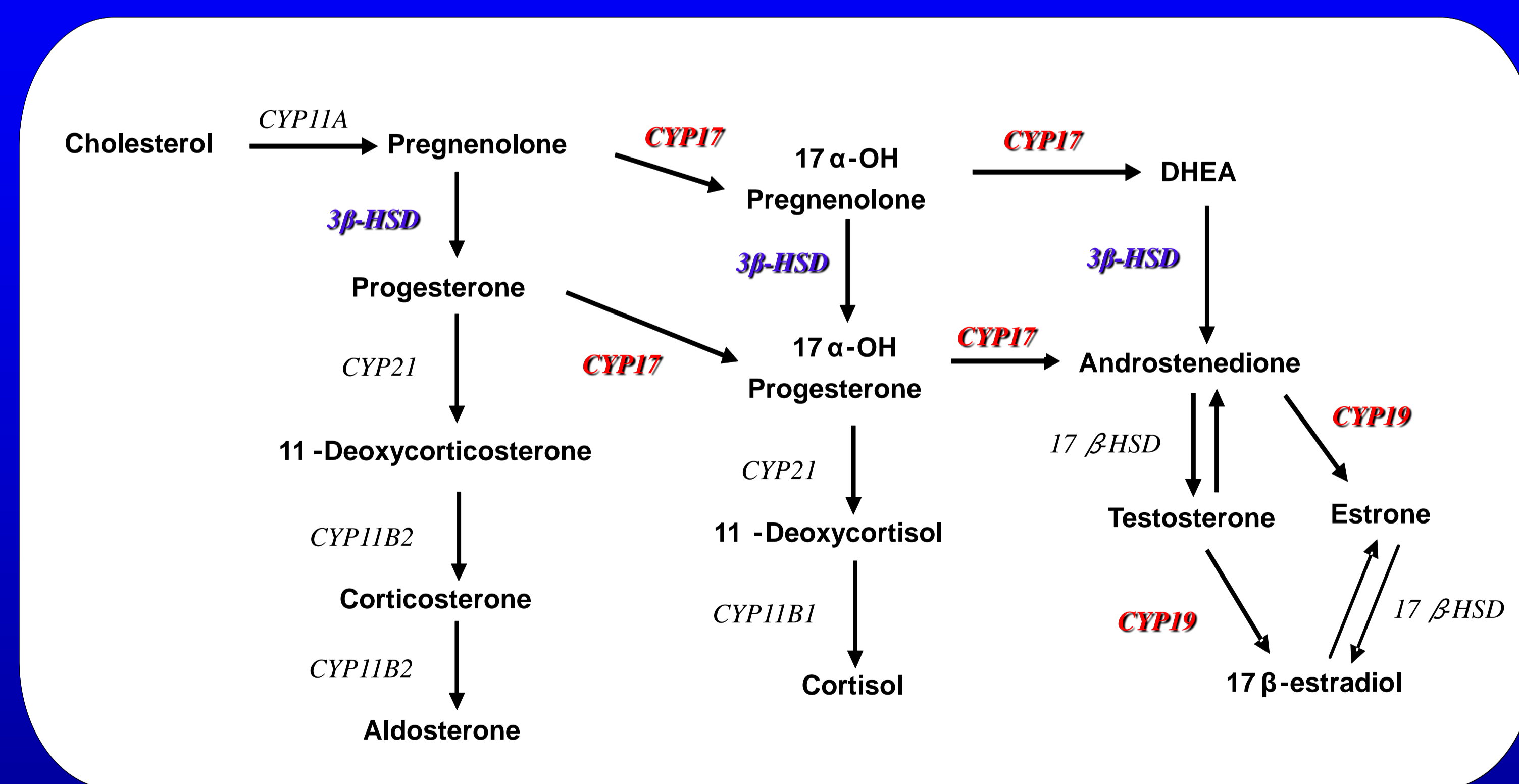
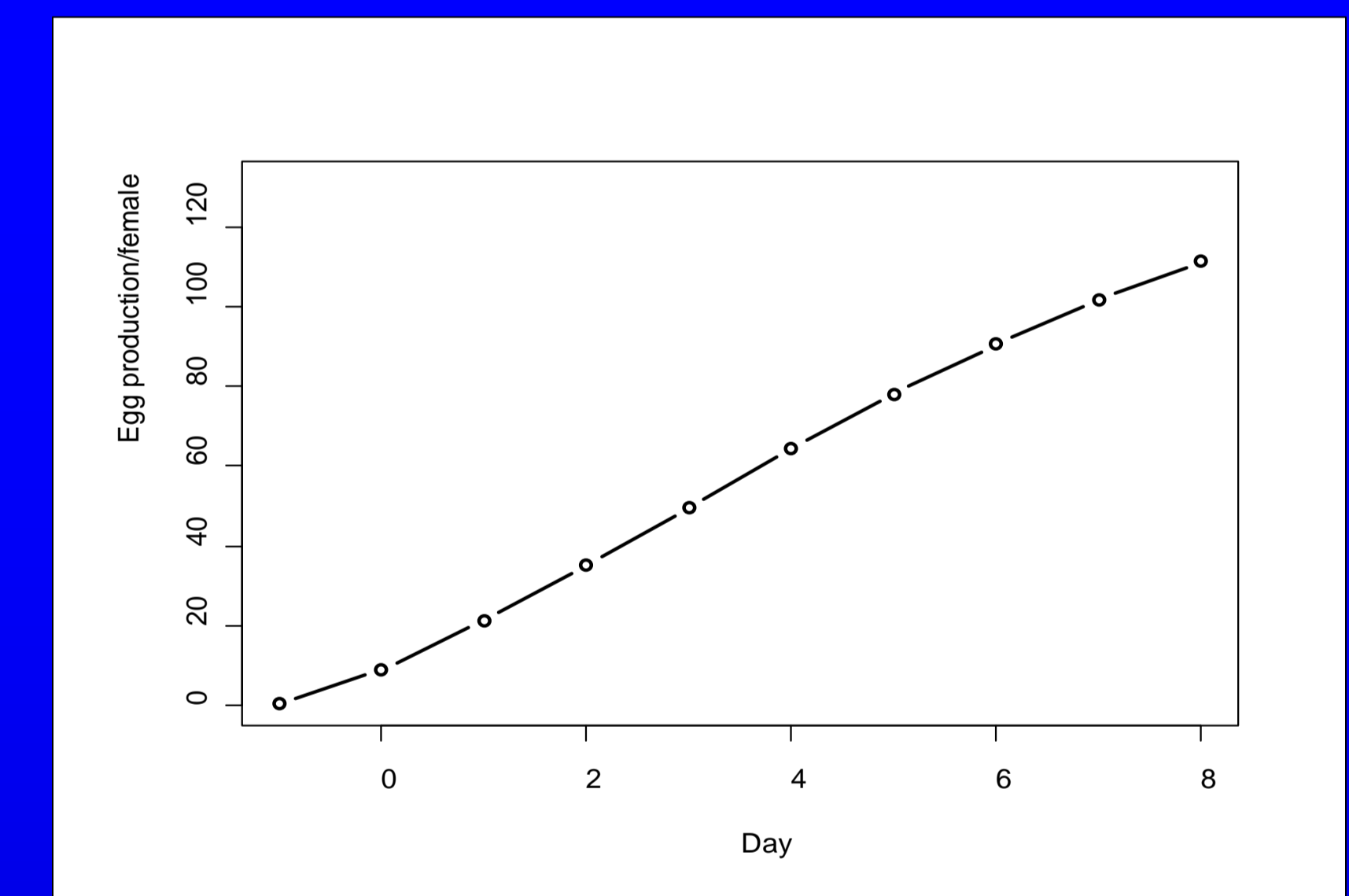
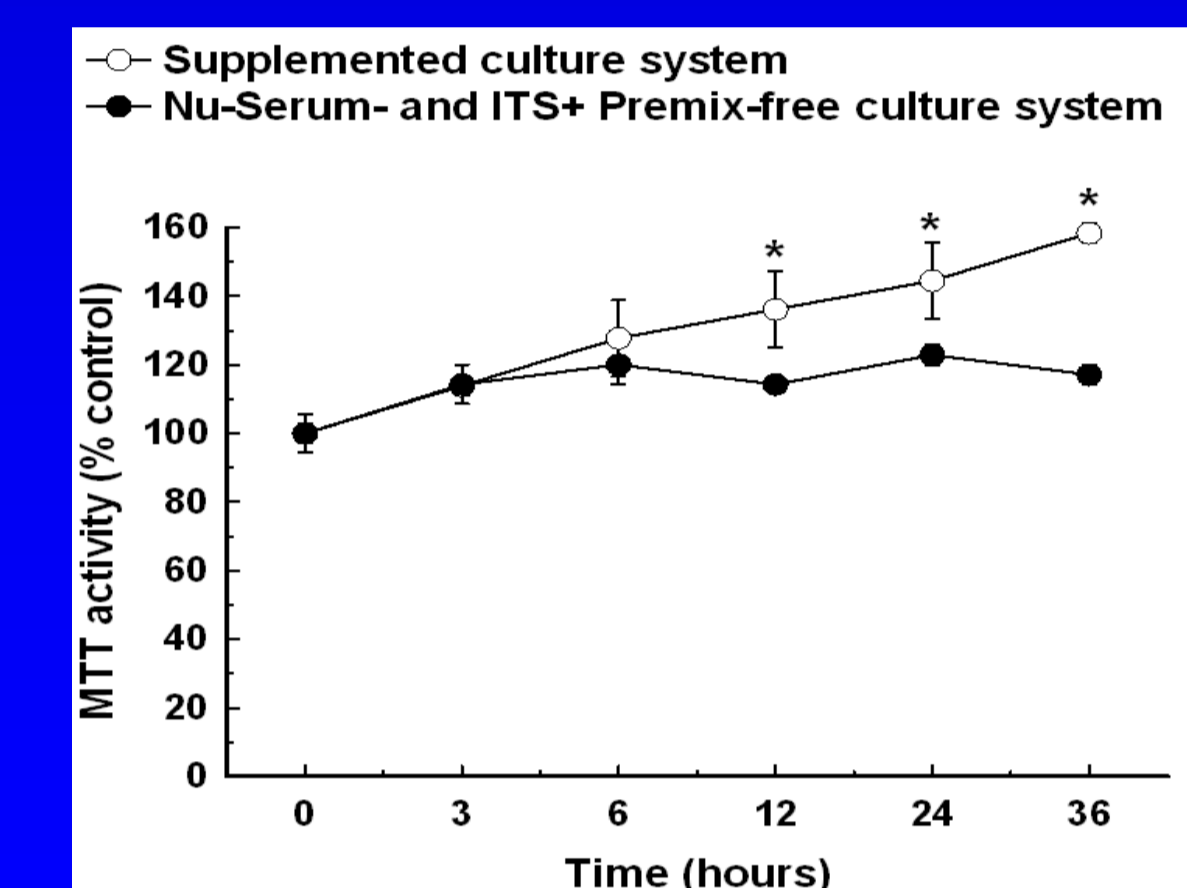


Figure 1, Steroidogenesis pathway affected by endocrine disrupting chemicals. Prochloraz is an inhibitor of CYP17 and CYP19 as indicated by the red bold font; Trilostane is an inhibitor of 3 β HSD as indicated by the blue bold font.

Results

Figure 2. Comparison of cell numbers (determined as MTT activity) to supplemented and Nu-Serum- and ITS+ Premix-free H295R culture system after changing medium. Values represent mean \pm S.E.M. of four replicate samples. Significant differences between 0 h and other time points is indicated by * P <0.05.



Discussions

1. Advantage of Nu-Serum- free H295R cell culture system in chemical testing.

H295R cells was more stable in the unsupplemented culture system, including (1) comparatively unchanged cell number, (2) steroidogenic gene expression.

2. Prochloraz had higher inhibitory potency in Serum-free H295R culture system .

1) Deoxycorticosterone, 2) 17 α OH Progesterone, 3) Androstanedione.

3. Prochloraz synergistically enhanced the inhibitory effect of trilostane on steroidogenesis.

1) Corticosterone, 2) Androstanedione.

4. Synergistic effect of co-exposure of trilostane and prochloraz was not observed at the reproduction tract of Medaka fish. Future works should investigate the effects of binary of exposure on the hypothalamus pituitary adrenal (HPA) axis in animals.

Reference

Zhang et al., *Environ. Sci. Technol.* **2008**, 42 (22), 8614.
Villeneuve et al., *Toxicol. Sci.* **2008**, 104 (1), 113-23.