In vitro toxicity of hydrocarbon pollutants

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An investigation of the potential of polar oil related pollutants to disrupt or modulate steroidogenesis *in vitro*, using a human adrenocortical carcinoma cell line, the H295R assay.

INTRODUCTION

To date, knowledge of how oil pollutants could impair fish reproduction, via their ability to disrupt steroidogenesis, the essential biochemical pathway responsible for the production of the sex steroid hormones, is not well studied. For this reason, we have investigated the potential of polar oil related pollutants to disrupt or modulate steroidogenesis *in vitro*, using a human adrenocortical carcinoma cell line, the H295R assay. In the first instance, this *in vitro* study focused on evaluating the deleterious effects of naphthenic acids (NA), alkyl phenols (AP) and the polar fraction of produced water (PW) on steroid hormone production. The results of this *in vitro* study will indicate the potential of oil pollutants to disrupt endocrine function in wildlife, specifically the vitally important process of steroidogenesis.

Materials and methods

The human adrenocortical carcinoma cell line, H295R, expresses all the key enzymes for steroidogenesis, and thus is an ideal model to investigate effects of chemicals on steroid hormone production (Fig. 1).

Cells were exposed to short chained AP (C2 and C3) with a similar composition as North Sea produced water (station 3 data, Harman et al., 2009), a commercial naphthenic acid mixture, and the polar fraction extracted from a produced water sample. The exact compositions of the exposure compounds were determined by gas chromatography. See Fig. 2 for AP composition of PW.

High = 10mg/L (PW=4mg/L)

Med = 1mg/L (PW=0,4mg/L)

Low = 0,1mg/L (PW=0,04mg/L)

After 48 hours exposure medium was collected and analyzed for hormone production (estradiol, testosterone and progesterone). Hormone concentrations were obtained by solid- phase radioimmunoassay kits. None of the exposure concentrations were cytotoxic according to Alamar Blue assays.

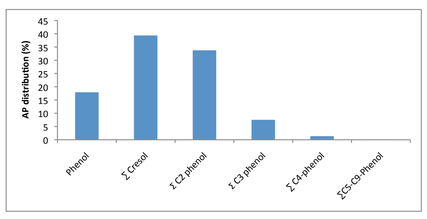

Figure 2. *Distribution in % of alkylphenols present in the extracted sample of PW. Total amount of AP in the extracted polar fraction of PW is 64%.*

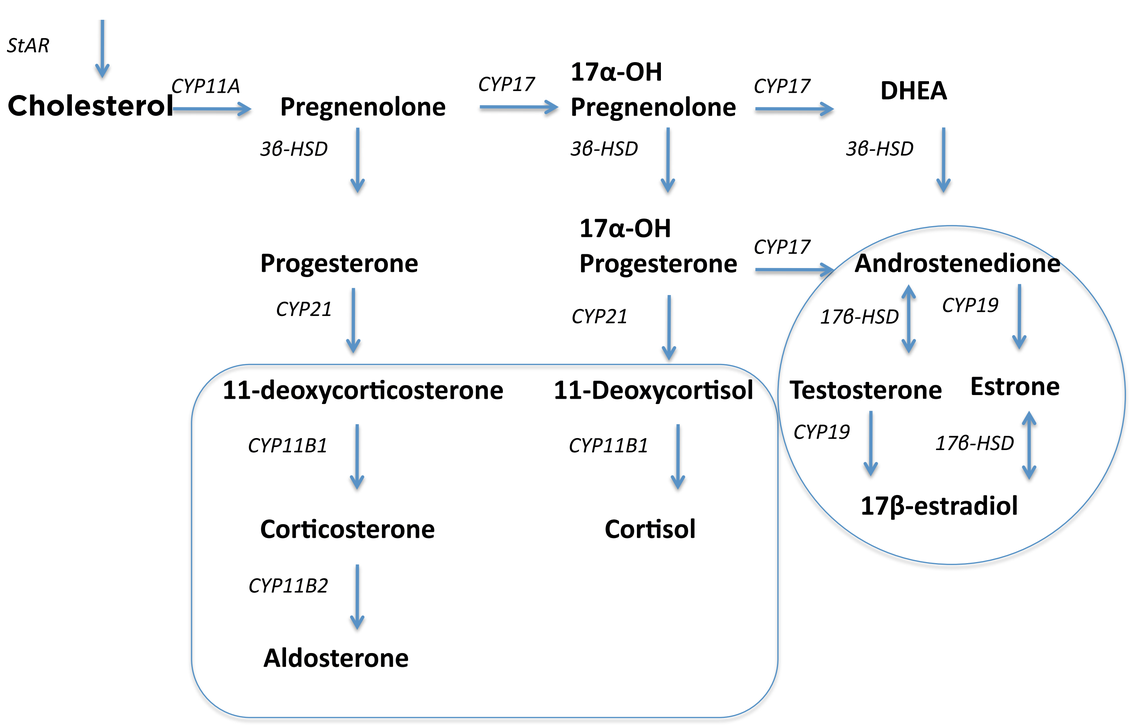

Figure 1. Steroidogenic pathway in H295R cells, adapted from Hecker & Giesy, 2008

Enzymes. **Hormones.** Corticosteroid pathways/products. Sex steroid pathways/products.

Results

ESTRADIOL

A significant increase in estradiol production was detected in the cells exposed to the highest dose of oil pollutants. See figure 3: Comparisons with medium collected from unexposed cells (MB) using the Tukey test, *= p<0.06, **=p<0,03

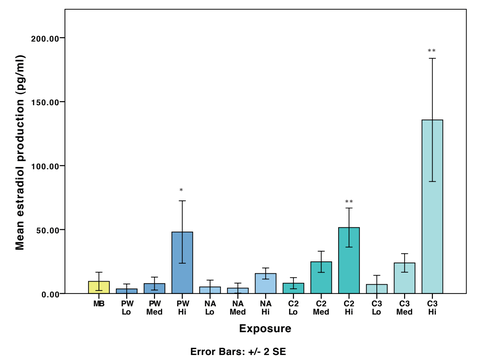

Figure 3. *Estradiol production in cells exposed to hydrocarbon pollutants.*

PROGESTERONE

A significant increase in progesterone production was detected in the cells exposed to the highest dose of oil pollutants. See figure 4: Comparisons with medium collected from unexposed cells (MB) using the Tukey test, ***= p<0.003

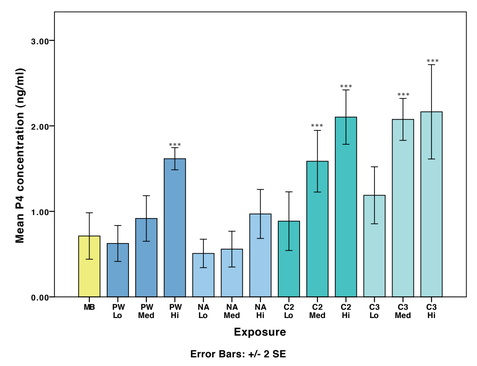

Figure 4. *Progesterone production in cells exposed to hydrocarbon pollutants.*

TESTOSTERONE

No significant alterations in testosterone production in the cells exposed to oil pollutants (using the Kruskall-Wallis test), see figure 5.

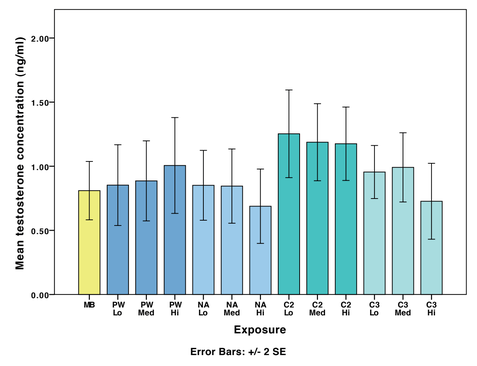

Figure 5. *Testosterone production in cells exposed to hydrocarbon pollutants.*

Conclusions

Naphthenic acids, C2 and C3 alkylphenols and the polar fraction of produced water induce the production of both estradiol and progesterone in exposed H295R cells. This indicates that polar hydrocarbons have the ability to modulate the steroidogenesis.

REFERENCES

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