Introduction

Despite an increasing amount of attention, little is understood about how doses of endocrine disrupting chemicals (EDCs) at environmental concentrations affect testes. The majority of studies (studies or mixture) are conducted at doses of estrogen or other EDCs at the NOAEL for the single substance, but not at environmentally relevant dosing or doses that are generally considered to be safe; these include studies showing lower tolerable exposure values like ADK, DNEL, or RfD. To address these concerns, we launched a project to test the endocrine activity of flutamide at environmentally relevant concentrations (EDCs) in a combined pre- and postnatal study design with regulatory testing protocols. Endpoints chosen for the present project have been used to determine the effects of these chemicals in in vivo studies (18), e.g., the effect of randomized lethal and reproductive toxicity testing protocols. Endpoints chosen for the present project have been validated in vitro and in vivo. The design is based on current knowledge on the mechanisms of action of EDCs and is consistent with the endocrine disruption model (19).

Conclusions

Consistent anti-androgenic effects were detected in male offspring at the two top doses of flutamide (Table 3). Salient findings (and not necessarily all endpoints that reached statistical significance) are:

- Decreased anogenital distances
- Increased nipple retention
- Decreased sexual maturation
- Reduced male sex organ weights
- Large subcutaneous fat pads

Female offspring displayed no signs of developmental toxicity at any flutamide dose.

A NOAEL of 0.025 mg/kg body weight/day was determined for pre- and postnatal exposure to flutamide in male offspring (Table 3). Survival of the offspring was not affected by treatment.

Experimental Design

The aim of this study was to determine effects which go beyond adaptive processes to maintain homeostasis in the endocrine system. A pre- and postnatal, two-generation study design was improved by the addition of endpoints measuring prepubertal sex hormone and histopathological examinations (Figure 1).

A statistical significant delay of about 1 day was observed in the 2.5 mg/kg body weight/day group. However, the delay resulting from treatment with 2.5 mg/kg body weight/day was both longer (almost two weeks) and statistically significant (p < 0.01). Furthermore, 4 of 5 male rats of the 2.5 mg/kg body weight/day group had an abnormality of the anterior pituitary gland, which is a pituitary abnormality not seen in controls with the 0.25 mg/kg body weight/day or 0.025 mg/kg body weight/day groups.

One hour after dosing, 5 dams from each group were sacrificed on GD 18 and a caesarian section was performed. The tested doses (Table 1) were selected to mimic a low-effect, the no observed adverse effect level (NOAEL), for endocrine effects, and the Acceptable Daily Intake (ADI), for adverse effects, of flutamide. Although all test animals were exposed to flutamide, only female rats were sacrificed at the end of lactation. Consequently, 0.0025 mg/kg body weight/day was chosen as the lowest dose level. The dose selection was based on the results of previous studies (20) and a calculated NOAEL for flutamide.

The dosing period for each parent group was 14 days. The postnatal period was extended to the age of 120 days. The study design was chosen which is compliant with regulatory testing protocols. The test design was improved by the addition of endpoints measuring prepubertal sex hormone and histopathological examinations (Figure 1).