7 Results and discussion

7.1 Effects on female rat reproductive development (Grande *et al*., 2006)

In the present study, maternal and reproductive outcome data were not adversely affected. DEHP had no significant effect on maternal weight gain, litter size, sex ratio and number of viable pups at any dose tested. On PND 1, we observed a significant increase in liver weight in female offspring exposed to 135 and 405 mg/kg/day. In addition, an increase in liver and kidney weight was detected in dams (PND 22) at the highest dose (405 mg/kg/day). Data from previous studies (DeAngelo *et al*., 1986; Poon *et al*., 1997) consistently show that liver and kidney are primary targets for DEHP. On PND 22, however, no effects were seen in body, liver and brain weight of female offspring.

In contrast to males, sexual differentiation of females is largely hormone-independent, yet still susceptible to hormonal disruption, e.g., masculinisation of the female fetus by exposure to androgens (Hughes, 2001; Sharpe, 2001). Inhibition of nipple development and increased anogenital distance in female offspring has been used as sensitive indicators of masculinisation (Wolf *et al*., 2002). In the present study, no significant changes were detected in nipple development and anogenital distance of female offspring at any dose level. These results confirm and extend the observations made by Moore *et al*. (2001) showing that both endpoints (nipple development and anogenital distance) are not affected in female offspring.

The age at vaginal opening is a sensitive marker of the onset of puberty in rats. In our results, a significant delay in this variable (approximately 2 days) was detected in animals exposed to 15, 45, 135 and 405mg DEHP/kg/day. In addition, a similar delay in the age at first estrus was also observed at 135 and 405mg DEHP/kg/day, although not significantly so. Body weight was unaffected or even higher in treated animals when compared to the control group. The age at vaginal opening and first estrus are mainly dependent on increasing levels of estradiol during puberty and a delay in these processes may suggest antiestrogenic or androgenic activity of test compounds. However, other factors not specifically controlled by estrogen/androgen status can also influence the time of pubertal onset. Interestingly, in our results a delay in the age at preputial separation was also observed in males (Andrade *et al*., 2006a), and the dose levels that affected this

endpoint were the same that resulted in delayed vaginal opening in females. Taken together, these results suggest that developmental exposure to DEHP may interfere with a common mechanism controlling the puberty onset in both sexes.

7.2 Effects on brain aromatase activity (Andrade *et al*., 2006b)

The aromatase enzyme is responsible for the conversion of androgens to estrogens. During perinatal development, locally formed estrogens (mediated by aromatase) are thought to influence sexual differentiation, acting as permanent organizers of specific neural structures (George and Ojeda, 1982; Lephart, 1996; Roselli and Klosterman, 1998). In addition, there is evidence that estrogens are also involved in more general aspects of brain development in both males and females (George and Ojeda, 1982). In the present study, the results obtained with control rats confirmed the developmental profile of brain aromatase activity described in the literature (George and Ojeda, 1982; Lephart *et al.,* 1992; Lephart, 1996) showing that: (1) specific brain areas (e.g., hypothalamic pre-optic area [HPOA]) display higher levels of aromatase activity than the whole brain; (2) HPOA aromatase activity is higher in males than in females during the perinatal period and (3) HPOA aromatase activity is higher during the perinatal period than during postnatal development (e.g., PND 22), in both males and females.

The effects of DEHP on brain HPOA aromatase activity were investigated in male and female rats on postnatal days (PNDs) 1 and 22. In males on PND 1, the plot of the relationship between dose and HPOA aromatase activity showed a nonmonotonic dose response, with low dose inhibition and high dose stimulation, resembling a J-shaped curve. No significant changes in HPOA aromatase activity were detected in females for any dose at this age. In contrast to the findings on PND 1, aromatase activity at weaning (PND 22) was more affected in females than in males. An increase in aromatase activity was observed at only one dose in males (0.405 mg/kg/day) while an increase in activity was observed at all doses in the females except for 0.045 and 5 mg DEHP/kg/day.

These results suggest that there are temporal differences between males and females in aromatase susceptibility to DEHP exposure. Such temporal differences may reflect different regulatory mechanisms of aromatase expression and activity operating in males and females, including differences in gonadal steroid hormone profiles. However,

the exact mechanisms underlying DEHP effects on aromatase are difficult to determine, as the endogenous factors controlling the developmental expression of this enzyme in the rat are not fully understood (Lauber *et al.,* 1997; Lephart, 1996). The observed changes in brain aromatase activity of female offspring were not associated with reproductive abnormalities during adulthood (Grande *et al*., 2007).

7.3 Reproductive effects on adult female offspring (Grande *et al*., 2007)

The endpoints investigated during adulthood were largely unaffected by DEHP treatment. The only adverse response observed in adult female offspring was a significant increase in the incidence of ovarian tertiary atretic follicles at the highest dose (405 mg/kg/day). Females at all doses tested presented a normal pattern of estrous cyclicity. Mean cycle length (days) and the presence of prolonged estrus or diestrus were similar in control and DEHP-treated animals. No significant changes were detected in hormone concentrations (serum estradiol and progesterone), body/organ weights and vaginal and uterine epithelial cell height at any dose tested.

A statistically significant increase in tertiary atretic follicles was observed in the 405 mg/kg/day dose when compared to control group. The mechanism underlying this effect is unknown, but could be related to activation of peroxisome proliferator-activated receptors (PPARs). Lovekamp-Swan and Davis (2003) hypothesized that DEHP (through its main metabolite, mono-(2-ethylhexyl) phthalate, MEHP) may activate PPAR subtype δ, disrupting the timing of growth and differentiation of the ovarian follicles. In another study, Lovekamp-Swan *et al*. (2003) showed a molecular mechanism by which MEHP, through activation of PPAR α and PPAR δ alters the expression of genes critical in granulosa cell estradiol production and metabolism. The hypothesis that DEHP is involved in the degenerative process of tertiary follicles is consistent with the effects of DEHP on male reproductive tract. Previous studies reported that postnatal exposure to high DEHP or MEHP doses (400 – 2000 mg/kg/day) increase germ cell apoptosis in rat testis (Richburg *et al*., 2000, Dalgaard *et al*., 2001, Park *et al*., 2002).