6 Materials and methods

6.1 Animals, dose selection and treatment

Wistar rat dams were administered DEHP or peanut oil by daily gavage from day 6 of gestation (mating=day 0) to day 21 of lactation. Two wide ranges of doses, low and high, were used:

- Low-dose range: 0.015, 0.045, 0.135, 0.405 and 1.215 mg DEHP/kg/day
- High-dose range: 5, 15, 45, 135 and 405 mg DEHP/kg/day

One group of animals received only peanut oil and served as vehicle control. A total number of 11-16 rat dams (litters) per group were used. The low-dose range was selected starting from a dose (0.015 mg/kg/day) similar to the estimated median daily intake of the general German population (0.0138 mg/kg/day) reported by Koch *et al*. (2003). Four additional doses were calculated by applying a space factor of 3 between doses. The high-dose range was chosen starting from 5 mg/kg/day and with a space factor of 3, so that the highest level (405 mg/kg/day) would be a dose known to induce reproductive adverse effects in male offspring rats without causing overt maternal toxicity (Moore *et al*., 2001).

6.2 Evaluation of male offspring

Male offspring was investigated in different stages of life to detect possible reproductive tract abnormalities and induction of effects on reproductive and endocrine functions. A list of the endpoints evaluated is shown in table 1.

Table 1. Endpoints investigated in male offspring exposed *in utero* and during lactation to DEHP

Age	Number of animals	Endpoints
PND 1	1-2 males per litter [a]	Organ weights (brain, liver); Intratesticular testosterone; Histopathology of the testis; Brain hypothalamic pre-optic area (HPOA) aromatase activity.
PND 13	All males	Number of nipples.
From PND 15	All males	Testis descent.
PND 22	1-3 males per litter [a]	Organ weights (brain, liver, testis, epididymis); Anogenital distance; Histopathology of the testis; Diameter of seminiferous tubule; Brain hypothalamic pre-optic area (HPOA) aromatase activity.
From PND 33	All males	Preputial separation.
PND 110	1-2 males per litter	Sexual Behaviour.
PND 130	1-2 males per litter	Mating (fertility) study.
PND 140	1-2 males per litter	Organ weights (brain, liver, kidney, spleen, thymus, testis, epididymis, prostate, seminal vesicle); Daily sperm production; Sperm morphology; Testicular morphometry; Sertoli cell number; Ratio of leptotene spermatocytes to Sertoli cells; Serum testosterone concentration.

PND = postnatal day

[a] litters with less then 3 male pups were not used at this age