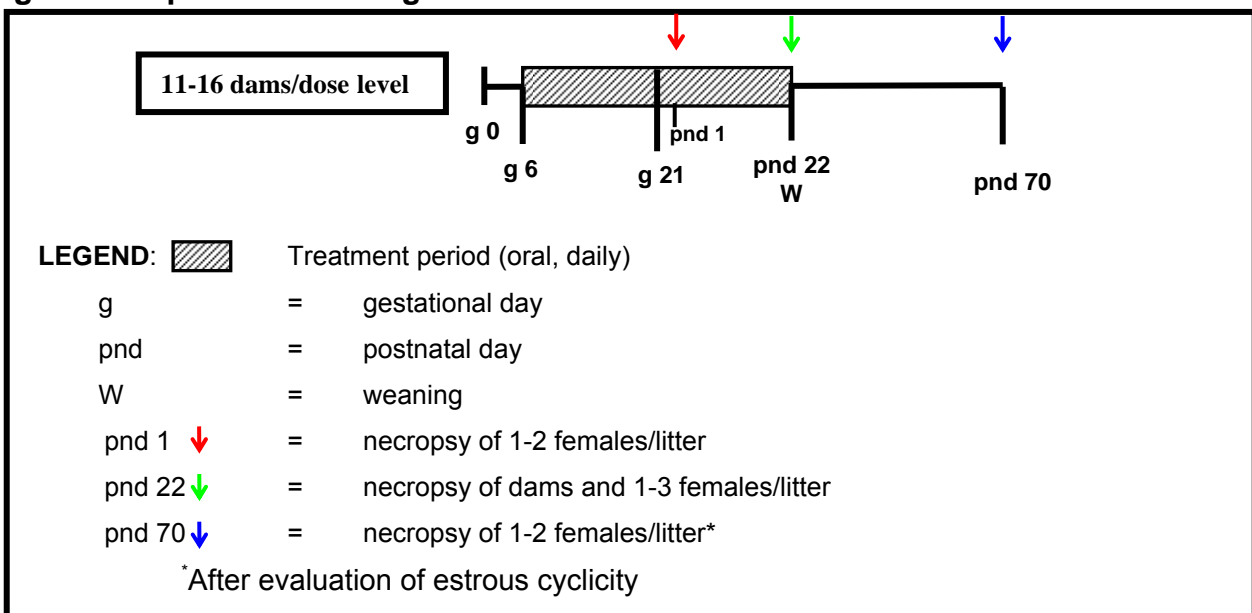


6 Material and methods

6.1 Animals, dose selection and treatment

Wistar rat dams were administered DEHP (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany, lot no. S11126-334) or peanut oil (Bombastus-Werke AG, Freital, Germany) 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. The low and the high doses were respectively 0.015, 0.045, 0.135, 0.405 and 1.215 mg/kg DEHP/day, and 5, 15, 45, 135 and 405 mg/kg DEHP/day. One group of animals received only peanut oil and served as vehicle control. A total number of 11-16 rat dams (litters) per dose were used. The experimental design is shown in Figure 1. 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 the 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). Because the female data in the literature is scarce, the high dose range was based on previous results obtained in males.

Figure 1. Experimental design



6.2 Endpoints of female offspring

The reproductive development of female offspring was investigated in different time points up to adulthood. In addition, landmarks of sexual development were assessed, as follow:

Developmental landmarks

- ✓ Number of nipples - Postnatal day (PND) 13
- ✓ Anogenital distance – PND 22
- ✓ Vaginal opening – starting on PND 33
- ✓ Day of first estrus - starting on PND 33

Postnatal day 1 (PND 1)

- ✓ Body and organ weights (brain and liver)
- ✓ Brain aromatase activity

Postnatal day 22 (PND22)

- ✓ Body and organ weights (brain and liver)
- ✓ Brain aromatase activity

Adult female rats (9 weeks old)

- ✓ Estrous cyclicity
- ✓ Body and organ weights (liver, kidneys, spleen, thymus, thyroid, uterus and ovaries)
- ✓ Serum estradiol and progesterone concentration
- ✓ Ovarian follicle counting
- ✓ Morphometry (uterus and vagina)