Reproductive effects of lead acetate administered in drinking water to Wistar rats in an extended one generation toxicity study

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Introduction
The Extended One-Generation Toxicity Study (EOGRT) is a study design intended to replace the two generation (OECD 415) or providing additional information with the object of the study design in a mid-size contract research organisation. We present here data obtained on reproductive effects of treatment with lead acetate.

Materials and methods
Lead acetate was provided in drinking water to FO adult Wistar rats at 0 (NaAc control), 100, 800 and 1700 ppm Pb, from 2 to 4 weeks pre-mating, through gestation and lactation, to F1 rats from birth to adulthood.

FO adults: observations
The FO adults were mated and evaluated for standard in-life parameters, reproductive function and histopathology. Parameters monitored in the FO/Parental Generation included clinical observations, body weight, food/water consumption, mating and reproductive performance, vaginal smears, distance, organ weight measurements. Sperm analysis, macroscopic evaluation and organ weight measurements were performed.

FD generation: observations
For FD Generation, each litter was examined on PND0 to determine the number and viability, the sex and anogenital distance of pups and any gross abnormalities. The pups were weighed at birth (PND0) and on PND 4, 7, 14 and 21. On PND4, litters were culled to 5 males and 5 females/litter as possible. All culled pups were subjected to necropsy with detailed macroscopic examination. On PND12, males were examined for retention of nipples/areolae. On PND17, caudal brain across the cranial colliculi and cerebellum with cerebellar peduncles and pons. In the pups selected for continuation, mortality and clinical signs, body weights, food and water intake were recorded. In addition, as of PND21, vaginal patency was evaluated daily for each FD female; the body weight of each female was recorded on the day of vaginal patency, then the oestrus cycles were monitored for 2 weeks thereafter. As of PND35, all FD1 males were evaluated for epididymis and testis weight and the body weight recorded upon separation. One male and one female from each resulting litter were randomly assigned to one of five subgroups, for clinical pathology/Thyroid function/Neurotoxicity (subset 1, groups A and B), Immunotoxicity (subset 2) and reproductive effects (subset 3). Subset pups were evaluated for developmental milestones and growth, neurobehavioural assessment (FOB, grip strength, landing foot splay, motor activity and automated image analysis via a SMAFIT® System), TDAR Immunotoxicity (IgM response to sheep-RBC challenge) and thyroid hormones (T3, T4 and TSH). Neuro-histopathology with histopathology and serial brain sections were performed. Subset 3 was euthanized shortly after PND70, as no additional assessment of reproductive performance was considered required. Vaginal smears were examined prior to necropsy; sperm evaluation, macroscopic evaluation and organ weight measurements were performed, and blood was collected for measurements of Pb levels (n=20 rats/sexdose group).

Discussion and conclusion
There were no effects on the FO reproductive performance at any dose level, despite the (~10%) reduction in the sperm count and motility and presence of the abnormal sperm morphology. The FO offspring, the vaginal opening, organ weight and functional anogenital distance showed treatment-related effects mostly at the high dose, with a similar trend noted in the mid dose group. The findings were consistent with literature data on lead. The study design was therefore considered to be suitable for identification of reproductive effects.

Results
There were no body weight effects, clinical signs or neurotoxicity signs in the FO animals. Water intake was reduced in the mid and high groups, affecting the test item intake. There were no effects on reproductive performance; mating, fertility and gestation indices were unaffected in FO animals. The only reproductive parameters found to be affected at FO were significant increases in abnormal sperm morphology. In the F1 generation, the vaginal opening, preputial separation and female reproductive parameters were considered required. Vaginal smears were examined prior to necropsy.

Figure 1: study design FO males

Figure 2: FO males - Water consumption (Mean mL/animal/day)

Figure 3: FO females - Water consumption (Mean mL/animal/day)

Figure 4: F1 males - sperm parameters

Figure 5: F1 mean pup body weight

Figure 6: pup developmental parameters

Figure 7: vaginal opening evolution

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