Introduction
The Göttingen minipig is a promising non-rodent animal model for juvenile toxicity studies, because of favourable characteristics such as a large mean litter size (3-4 newborns per sow), high rate of growth and rapid achievement of sexual maturity. We have conducted a study to evaluate the husbandry and handling issues involved in performing juvenile studies in this animal model, and to evaluate the response to reference toxicants. In this study, Göttingen piglets were treated during the neonatal period with enalapril (an angiotensin converting enzyme (ACE) inhibitor which induces persistent renal injuries in juvenile rats and in farm pigs) and sotalol (an anti-arrhythmic drug which results in increased QT intervals in a range of laboratory animals, including minipigs). This study permitted us to evaluate the practical aspects of working with Göttingen minipig sows and piglets, the feasibility of different examinations in unweaned piglets and the impact of litter size, lactation and husbandry needs on the study design.

Materials and Methods
Animals
Three pregnant Göttingen sows in the last third of the gestation period were obtained from Ellegaard Göttingen Minipigs A/S, Daløse, Denmark. On arrival at CIT the sows were housed in dedicated units with a separate heated space for piglets. The sows were acclimated to the study conditions, weighed every week and remained with their litters for the entire duration of the study. In the days following birth, the piglets were individually identified by an implantable intramuscular microchip, and received an iron injection to prevent porcine iron-deficiency anaemia. In addition, the animals were submitted to a general veterinary examination and a neuro-developmental evaluation (data not shown). All the piglets were acclimated to the study conditions.

Study design
Piglets were allocated to groups one or two days after birth. The first two litters of 7 and 4 newborns were randomized by cross-fostering on study day 1 and the piglets were allocated to groups 1 and 2. The third litter of 9 piglets was divided into groups 3 and 4. Vehicle, enalapril or sotalol was administered by oral gavage at a dosage volume of 1 mL/kg/day. Group 4 received no treatment.

Evaluations
Body weight and crown-rump length were measured twice a week throughout the study. On study days 18 and 19, urine samples were manually taken from some piglets for urinalysis. Blood was sampled from all piglets on study day 21 to collect specific data in hematology and blood biochemistry. Electrocardiograms (ECG) were recorded on all group 3 piglets on study day 26 (predose) and study day 27, one hour after treatment with sotalol. ECG were also recorded in some piglets from the other groups on study day 27. The piglets were tranquilized prior to ECG recordings, which were performed with standard bipolar and unipolar limb leads DI, DII, DIII, measuring aVr, aVL, aVF with automated analysis. Ophthalmology was assessed on study day 28 on those piglets not submitted to ECG. All the piglets were sacrificed on study day 34 and submitted to a full macroscopic examination, fourteen organs were weighed and microscopic examination was performed on eleven tissues.

Results and discussion
Husbandry
The randomization by cross-fostering was successful and no maternal aggressiveness was observed between piglets, suggesting appropriate management and environment of the animals. The oral treatment by gavage was easily achieved in unweaned piglets from postnatal day 7. Blood and urine samples, electrocardiography and ophthalmological examinations were successfully performed in unweaned piglets and thus enabled the collection of background data for juvenile Göttingen minipigs.

Study results
No mortality was observed during the study. Body weight increased by 500% in the month from birth to weaning and length increased proportionally by 100%. Group 2 piglets treated with enalapril had the same physical development rate as for group 1 animals until study day 32 (see graph 1). In the enalapril treated animals, increased creatinine and urea blood levels were measured, suggesting abnormalities in the renal function. The average weight of the kidneys was increased in both sexes and renal macroscopic lesions (not seen in other study animals) were observed during the necropsy (see table 2). At microscopy, the renal lesions consisted in multifocal increased amount of fibrous tissue in interstitium, tubular vacuolization and tubular dilatation in cortex. These dilated tubules were often basophilic, lined by a flattened epithelium and occasionally contained sloughed degenerated cells in their lumens. These interstitial and tubular renal lesions (photo C) correlated with the blood biochemistry results (seen at study day 21), and were similar to those seen in previous published studies with enalapril in rats and farm piglets (12). At electrocardiography one hour after treatment with sotalol, group 3 piglets presented a bradycardia associated with increased PQ and QT intervals (see table 3). No other abnormalities in clinical, laboratory or histopathology investigations were observed for these piglets.

Conclusion
In this evaluation study we were able to demonstrate that:
- cross-fostering is a feasible method to standardize study treatment groups,
- juvenile minipigs treated with reference drugs enalapril and sotalol show the expected renal or cardiovascular toxicity,
- repeated investigations (oral gavage treatment, blood samplings, electrocardiograms…) can be easily achieved in Göttingen piglets and can allow the characterization of lesions induced by drugs.

The findings of our study support the use of Göttingen minipigs in juvenile animal studies for the toxicity assessment of pediatric medicines.

References