

Brussels, February 5, 2018

### **Study design: effects of soluble silver on the gut bacterial microbiota**

Four week-old male (40) and female (40) Wistar rats will be purchased from Janvier Labs (St Berthevier, France), and kept at the local animal facility (Animalerie Centrale, Université catholique de Louvain, Brussels, Belgium) under SPF-like conditions in a controlled environment (22°C, 55% relative humidity, 16-h light/8-h dark cycle, with acidified water and food ad libitum) in autoclaved air-filtered polycarbonate cages with conventional sawdust (Carfil, Oud-Turnhout, Belgium).

Upon receipt, all animals will be identified by individual ear tags.

During two weeks before exposure, litters will be mixed every day to homogenize the baseline gut microbiota across the cages [1].

Mice will then be randomly assigned to experimental groups (maximum 3 rats/cage, 4 cages/dose group) and fed with control or AgAc-supplemented pellets during 28 d.

Target treatment doses are 0, 0.4, 4 and 40 mg AgAc/kg bw/d based on [2].

AgAc will be incorporated in food pellets by Carfil (Turnhout, Belgium). Considering an average weight of 200 or 300 g, and based on a daily average consumption of 20 or 30 g food/d for M or F, respectively, concentrations of AgAc in pellets will be 4, 40 and 400 mg AgAc/kg.

Animals will be weighted at the beginning of the exposure and 3 x/week along the exposure and just before sacrifice.

Food consumption will also be recorded 3 x/week per cage to calculate actual AgAc daily intake.

After 7 or 14 d (tbd), blood will be collected from the tail vein and 48 h-feces will be collected in cages.

After 28 d exposure, rats will be euthanized by an intra-muscular injection of pentobarbital and intra-cardiac blood will be collected.

The 3 distal centimeters of the ileum, the caecum, and the 3 proximal centimeters of the colon will be excised and their content will be gently collected by scraping. Feces samples will be pooled per rat and stored at -20°C.

Ileum and colon samples will be collected and immediately stored in Trizol for subsequent analysis if appropriate.

Feces DNA will be extracted with a QIAamp DNA Stool Mini kit (Qiagen, Hilden, Germany) and stored at -20°C until next generation sequencing (NGS) analysis at MR DNA lab (Shallowater, USA). The gut microbial populations will be identified and quantified on an Illumina MiSeq platform with specific primers of the bacterial 16S rRNA gene (2x300bp PE Illumina 20,000 sequence diversity assay). Raw sequences will be processed using USEARCH v8.0.1623 (Tiburon, USA) to generate operational taxonomic units (OTU). The taxonomic assignment of each OTU will be carried out using a local BLAST against the Greengenes database version 13.5. Analyses of the  $\alpha$ - (richness and evenness within a microbial community) and  $\beta$ - (differences in composition among communities) diversities and statistical analyses will then be performed using the R software (version 3.1.2., Vienna, Austria) as described in [3].

Ag, Cu and Se contents will be measured by inductively coupled plasma mass spectrometry (ICP-MS) in serum and feces (Ag only) collected after 7 or 14 d and intestinal feces collected after 28 d. Fecal materials will probably have to be mineralized before analysis. Serum ceruloplasmin (ferroxidase) activity will be assessed at 28 d by a colorimetric kit (Sigma, St Louis, USA).

## References

1. Ma BW, Bokulich NA, Castillo PA, Kananurak A, Underwood MA, Mills DA *et al.*: Routine habitat change: a source of unrecognized transient alteration of intestinal microbiota in laboratory mice. *PLoS One* 2012, 7: e47416.
2. Sprando RL, Black T, Keltner Z, Olejnik N, Ferguson M: Silver acetate exposure: Effects on reproduction and post natal development. *Food Chem Toxicol* 2017, 106: 547-557.
3. van den Brule S, Ambroise J, Lecloux H, Levard C, Soulas R, De Temmerman PJ *et al.*: Dietary silver nanoparticles can disturb the gut microbiota in mice. *Part Fibre Toxicol* 2016, 13: 38.