Inhalation toxicology facilities at RIVM for human, animal, and cell exposures to nanomaterials and particulate air pollution.

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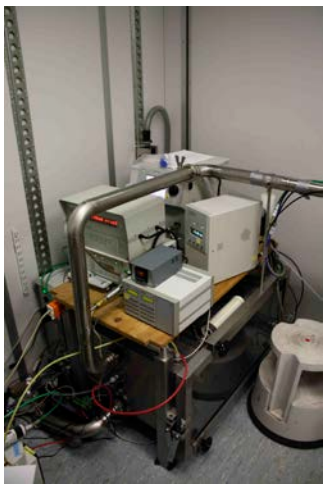
The RIVM history in inhalation toxicology research started in the 1980's. The first years the research focused on the toxicity of air pollution gases as Ozone and Nitrogen-oxides (Rombout *et al* 1989, van Bree *et al* 2001). From approximately 1995 the focus was on the effects of Particulate Matter. Since 1997 we use a mobile laboratory (MAPCEL) to do inhalation studies on locations with high air pollution or close to the (medical) facilities of academic hospitals for collaboration projects. Inside the MAPCEL small laboratory animals, healthy volunteers or patients have been exposed to e.g. outdoor, size fractionated, particulate matter (PM) (Cassee *et al* 2005, Mills *et al* 2008, Gerlofs *et al* 2010), diesel engine exhaust (Mills *et al* 2011) and nanomaterials (Raftis *et al* 2015).



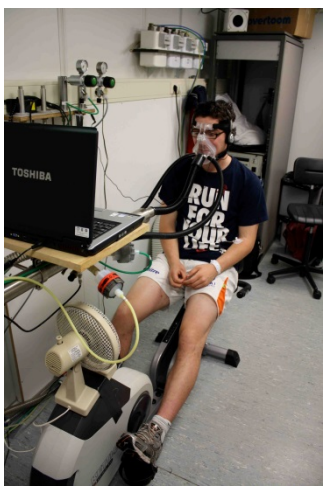


Figure 1A. Generation and analysis setup for NP exposure,
1B. volunteer fit with facemask on exercise bike during NP exposure.

Nanomaterials (NM) studies are performed within our laboratory since 2010. For NM the inhalation route is a major concern for both workplace and indoor/outdoor environment. Exposure characteristics like particle size, shape, number, charge and surface area can have a major impact on the site of deposition inside the respiratory tract. When inside the bloodstream the NM can be distributed to other parts of the body depending on their characteristics.

A good insight in the distribution of the NM over the body in time can be performed with radiolabeled nanoparticles as this has usually very low detection limits and overcomes issues of back ground levels of a given material in de body. The setup of equipment to perform biokinetics studies was described by Kreyling *et al* 2002. It is moved from the Helmholtz Institute of Radiobiology, Neuhergeberg, DE to the RIVM where it will be used in the next years.

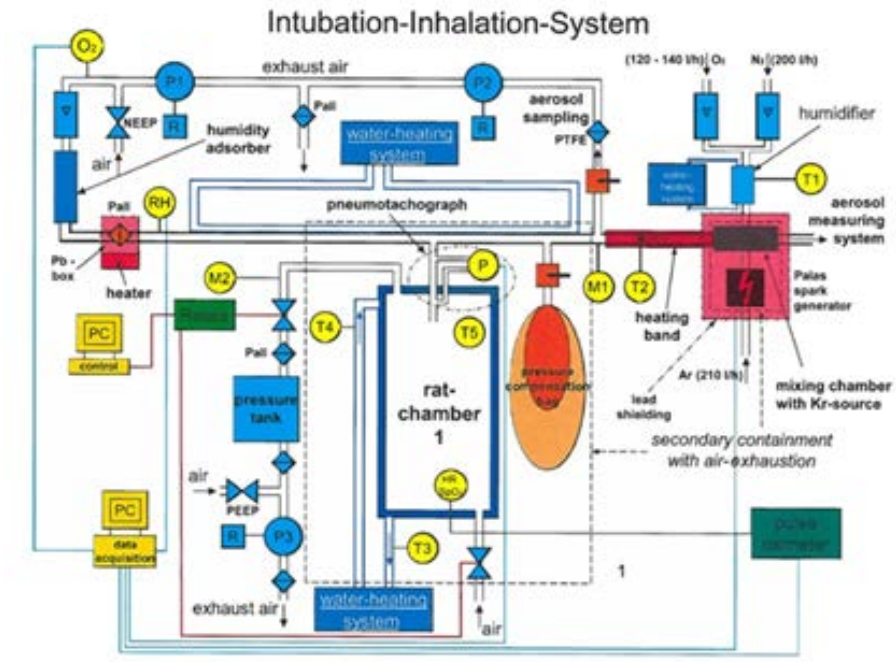


Figure 2 Schematic exposure setup for biokinetics studies. Anaesthetized animals inhale (radiolabeled) NM for 1 or 2 hours in a closed system with controlled ventilation by pressure changes inside the plethysmograph. (from: Kreyling *et al* 2002).

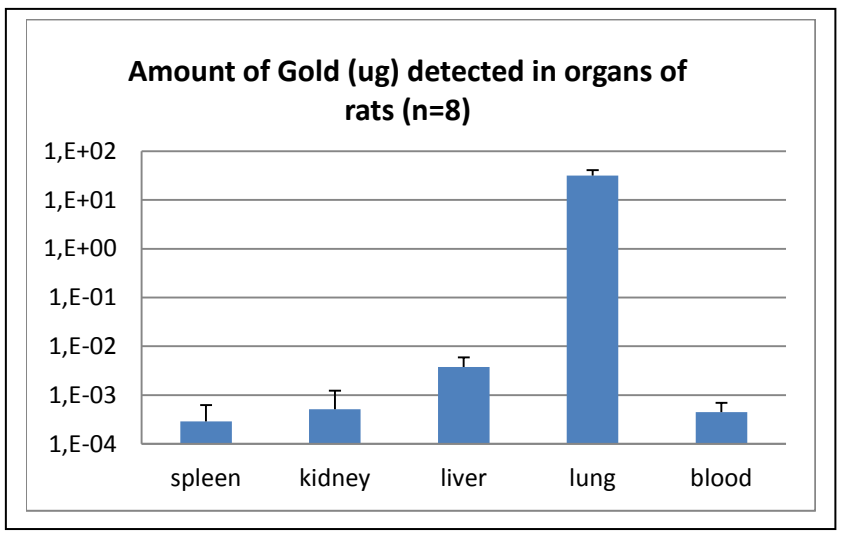


Figure 3. Results of a study in which 8 rats were exposed for 2 hour to (non-radioactive) 20 nm nano-gold particles and in which organs were collected after 24 hours for gold determinations by neutron activation analysis at TU-Delft.

With this setup, four animals (mice, rats) can simultaneously be exposed, while (isoflurane) anaesthetized, to radiolabeled NM of different sizes (by way-length and heat treatment) through a trachea-cannula. The breathing pattern of the rats is harmonized by an alternating negative/ambient pressure change inside the plethysmograph, so the frequency of breathing and the volume per breath is controlled. At different time points after exposure, the uptake/translocation of NM can be measured in the organs of the animals. The radiolabeled NM make it possible to measure over 4 order of magnitude. With some modifications, this system can also be used for future *in vitro* exposures to NM.

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