Hazard assessment of nanomaterials in advanced healthy and diseased liver in vitro models

Nanomaterials (NMs) are produced in largely quantities and incorporated in numerous consumer products including cosmetics, textiles, food, sporting goods and medicines. The ever increasing use of these materials is associated with enhanced exposure to humans (through skin, via inhalation or ingestion) and with potential unintentional release to the environment.

The lungs and the gastrointestinal tract are known to be the primary exposure sites for NMs. Research indicates that NMs administered via ingestion, inhalation or intravenous injection rapidly reach the liver in large quantities. In fact, as an exposure site the liver has huge importance, as it has been shown to accumulate NMs in large quantities which could potentially contribute to long-term adverse effects related to oxidative stress and inflammation.

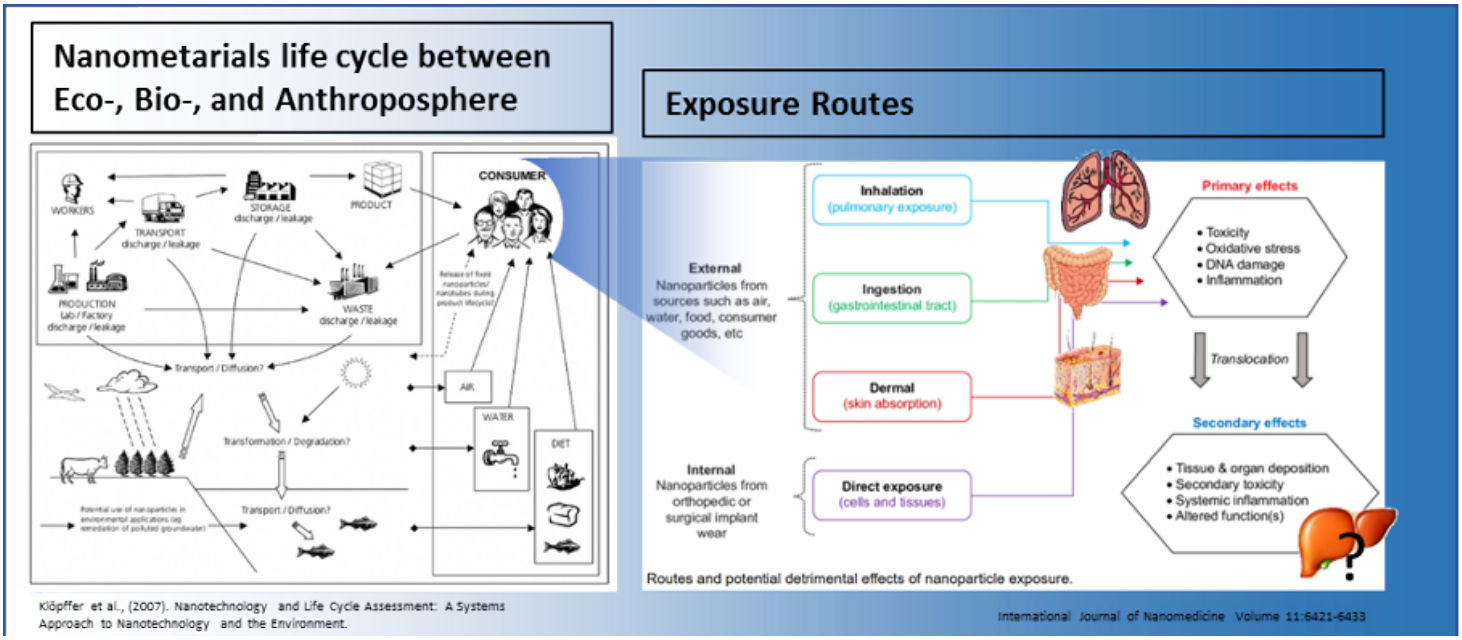

Figure 1: Potential environmental release of NMs during production, transport, use and disposal (left panel). Identified entry routes through which nanomaterials may inflict adverse effects directly at the exposure site or on secondary organs (right panel).

Exposure to NMs could aggravate pre-existing liver conditions, related to western diet and sedentary life-style which eventually can progress from clinically asymptomatic benign fatty liver to life threatening liver diseases such as fibrosis, cirrhosis or liver cancer. According to a recent US cohort study [1] ~95% of suspected non-alcoholic fatty liver disease (NAFLD) patients were not aware of having any liver disease, as in most cases the disease starts without apparent symptoms.

[1] Singh A, et al., Dig Dis Sci. 2020 Apr;65(4):978-986. doi: 10.1007/s10620-019-05700-9

This project has received €12.7M in funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 760813

PATROLS

Advanced Tools for NanoSafety Testing

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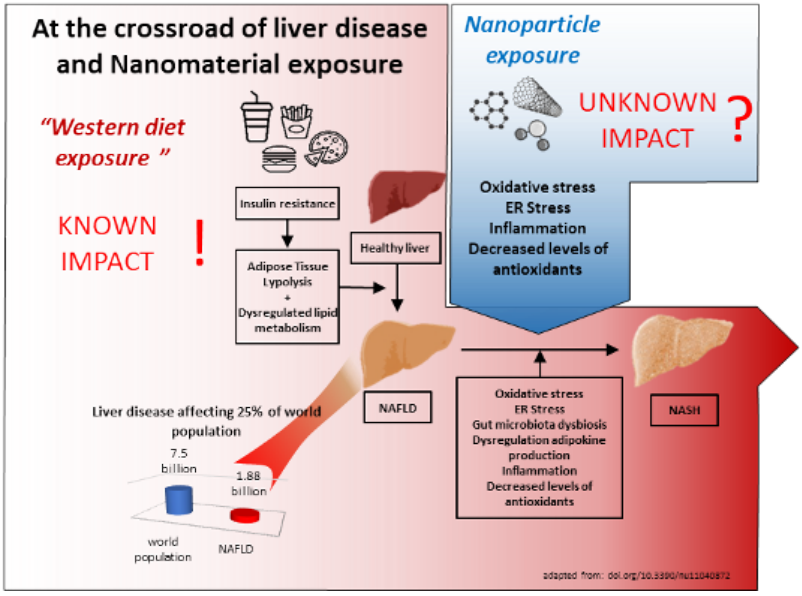
Globally, the prevalence of NAFLD is estimated to be around 25.2%.

Therefore, safety of drugs, chemicals, and NMs should not only be assessed in healthy individuals, but also in individuals representing a sizeable part of the general population, suffering either from obesity, diabetes, or non-alcoholic steatohepatitis (NASH), who might be at higher risk to develop sustained liver injury based on their metabolic predisposition. Metabolic liver diseases and exposure to chemicals or drugs can trigger similar cellular stress response capable to stimulate and accelerate further disease progression.



Here we present an advanced *in vitro* liver test system for investigating potential adverse effects of substances foreign to life, including drugs, chemicals and NMs. The liver models represent three different normo-physiological or diseased states, i.e. healthy, fatty and early, pre-fibrotic NASH. The 3D spheroid models constitute all of the important cell types for driving toxicity, initiation and progression of disease in humans which include liver cells, inflammatory cells, vascular cells and collagen-producing hepatic stellate cells.

Disease induction is formulated as such that respective metabolic states can be achieved within 7 days. The system is ready for compound testing. This dosing regime is extremely flexible and can include repeated dosing up to two weeks. Chronic, repeated treatment more likely reflects realistic exposure situations, be it a medication taken for chronic diseases or a repeated ingestion or inhalation of chemicals or nanomaterials intentionally or accidently released to the environment or into the food chain.

Our data shows that use of very low and physiologically relevant repeated dosing strategies is more than sufficient to represent NM-induced toxicity. Furthermore, we show the importance of incorporation of all cell types for a physiologically relevant toxicological data in the liver. Finally, our results show that inclusion of pre-existing, even mild disease states of the liver is very important for a more comprehensive toxicological profiling for toxins including NMs, chemicals and drugs.

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