

# *In vivo* behavior of full length and ultra-short single-walled carbon nanotubes



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## Introduction

Carbon nanotube (CNT) materials are of special interest as potential tools for biomedical applications. However, available toxicological data concerning single-walled carbon nanotubes (SWNTs) remain contradictory. Here, we compared the effects of SWNTs as a function of dose, length and surface chemistry in Swiss mice.

## Methods

### Carbon nanotube materials:

- HiPco produced, raw, full-length, SWNTs (R-SWNTs) average lengths of *ca.* 1-2  $\mu\text{m}$ , *ca.* 25% residual iron catalyst by weight.
- Purified SWNTs (P-SWNTs); purification of R-SWNTs achieved through non-acidic (liquid Br<sub>2</sub>) treatment, < 4% residual iron catalyst by weight.
- Ultra short SWNTs (US-tubes); obtained from SWNTs after fluorination and pyrolysis, sonicated in conc. HCl and water washed; average lengths of 20-80 nm, < 1.5% residual iron catalyst by weight.

### *In vivo* tests:

#### Oral administration:

The study was conducted according to the European Community Directive. Male Swiss mice were divided into groups of 10 mice and received an oral dose of:

- R-SWNTs,
- P-SWNTs or
- US-tube suspensions,
- vehicle (water) [control group].

The suspensions were administered at a dose level of 1000 mg/kg of body weight (b.w.). The animals were observed daily. At day 14 they were sacrificed for blood and organ collection.

#### Intraperitoneal administration:

Mice (divided into groups of 6 mice each) received an i.p. injection of a 1 mL single bolus dose of:

- R-SWNTs or P-SWNTs at increasing doses (50, 300 and 500 mg/kg b.w.);
- US-tubes (50, 300, and 1000 mg/kg b.w.); or
- vehicle (aqueous Tween solution) [control group].

The animals were observed daily for 14 days (animals treated with all doses) or 150 days (animals treated with US-tubes or P-SWNTs, 300 mg/kg b.w.). At the end of the experiment, the animals were sacrificed for blood and organ collection.

## Results

In an acute oral toxicity test, SWNTs administered at a dose level up to 1000 mg/kg b.w. were not lethal and did not cause any growth or behavioral troubles in Swiss mice.

Intraperitoneally administered SWNTs, irrespective of their length or dose (50-1000 mg/kg b.w.), can coalesce inside the body to form fiber-like structures. When structure lengths exceeded 10  $\mu\text{m}$ , they irremediably induced granuloma formation [Fig. 1]. Smaller aggregates did not induce granuloma formation, but they persisted inside cells for up to 5 months after administration [Fig. 2]. Short (<300 nm) well-individualized SWNTs can escape the reticuloendothelial system and are excreted through the kidneys and bile ducts [Fig. 3].

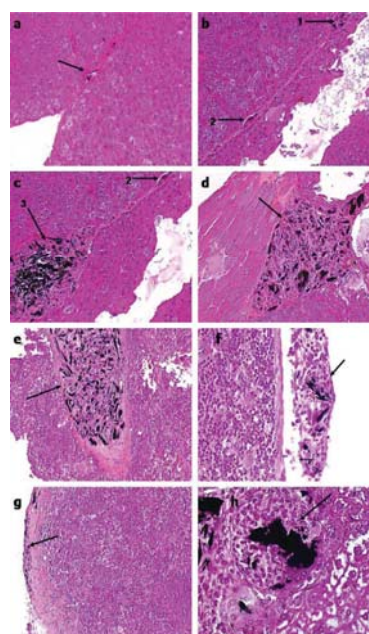


Figure 1. Light micrographs after hematoxylin-eosin staining of spleen and liver sections from mice i.p. injected with a single dose of P-SWNTs or US-tubes, 14 days after administration. The arrows indicate (a) small SWNT aggregates inside hepatic sinusoids; (b, c) granulomas of US-tube aggregates inside lymphatic vessels of the liver; (d) a granuloma loaded with US-tube aggregates inducing adherence between the liver (right) and the surrounding connective tissue (left); (e) a granuloma loaded with US-tube aggregates inside the spleen; (f) a granuloma with SWNT aggregates sticking out from the spleen surface; (g) a granuloma with US-tube aggregates strongly adherent to the serosal surface of the spleen; and (h) high magnification of an US-tube-laden foreign-body-giant cell inside a granuloma. (Magnification: 40x for panels a, b, c, and f; 10x for panels d, e and g; and 100x for panel h.)

Figure 2. Transmission electron microscopy (TEM) micrographs of P-SWNTs found in Kupffer cells in the liver sections of a treated mouse 5 months post administration. The letter N denotes the cell nucleus and the fleshes point P-SWNTs.

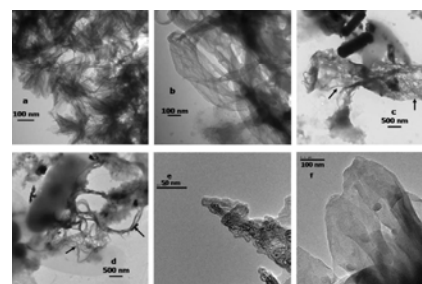
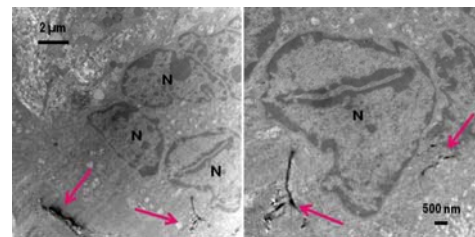


Figure 3. (a-d) TEM and (e, f) high resolution TEM (HRTEM) micrographs showing CNTs in mouse urine and fecal samples 3 days after administration. (a) Urine from P-SWNT treated mice (500 mg/kg b.w.); (b) urine and (c, d) fecal samples from the US-tube treated groups (1000 mg/kg b.w.); (e, f) HRTEM of urine samples (a, b).

## Conclusions

These findings suggest that if the potential of SWNTs for medical applications is to be realized, they should be engineered into discrete, individual particles.

## Published paper

Kolosnjaj-Tabi J, Hartman KB, Boudjemaa S, Ananta JS, Morgant G, Szwarc H, Wilson LJ, Moussa F. *In vivo* behavior of large doses of ultrashort and full-length single-walled carbon nanotubes after oral and intraperitoneal administration to Swiss mice. *ACS Nano*. 2010 Mar 23;4(3):1481-92.