Supplementary material to:

[60]Fullerene is an *in vivo* Powerful Antioxidant With no Acute or Sub-acute Toxicity

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SUPPORTING INFORMATION PARAGRAPH

Preparation of Aqueous suspensions of micronized C_{60}. [60]fullerene (purity: 99.98 %) was purchased from Term USA (Fort Bragg, CA, USA). Its purity was tested by HPLC, UV and MS. No impurity could be observed. It was used without further purification as well as after sublimation. No differences could be observed in the results obtained with both kinds of products. Polyoxyethylene sorbitan monostearate (Tween 60) and 0.9 DS carboxymethyl cellulose (CMC) were purchased from Acros Organics (Noisy-Le-Grand, France). All the other reagents were analytical grade and were purchased from Sigma (St Louis, MO). In the stainless steel milling vessels of a Pulverisette 7 (Fritsch, Idar-Oberstein, Germany), 2 g of fullerene were added to 15 ml of distilled water containing 0.02 % of Tween 60 and 6 stainless steel balls (8 mm of diameter). The mixture was milled during hours (typically 6 days) at 600 revolutions per minute until complete homogenisation of the suspension. Then 0.2 g of CMC and 0.18 g of NaCl were added and the mixture agitated until complete dissolution of CMC. After adjusting the final volume to 20 ml, the resulting homogenous suspension was then sterilized by autoclaving for two hours at 120 °C. The sterilized suspension that is stable for at least 2 months is then ready for use. The C_{60} concentration of each preparation was determined by HPLC (50) before use. The size distribution of the C_{60} particles in these aqueous suspensions (Fig. 1A), as determined by scanning electron microscopy: 7 % of particles with diameter (d) > 1000 nm and < 1650 nm; 43 % (500 nm < d < 1000 nm); 28 % (250 nm < d < 450 nm) and 22 % (d < 200 nm). Particle diameters may be reduced by increasing the milling time.

***In vivo toxicity and biochemical tests*.** Animals received human care and the study protocols complied with our University's guidelines for the care and use of laboratory animals. Male Wistar rats (200 ± 10 g, Charles River, France) were housed by groups of 6 in polypropylene cages at constant temperature (22°C) and humidity (60 %) and with a 12 h light/dark cycle, and fed a standard diet *ad libitum*. The animals used in the kinetic studies were housed in individual metabolic cages enabling urine and excrement collection. All rats were allowed to acclimate to this facility for at least one week before being used in the experiments.

We did not register any spontaneous animal death during our experiments. All animals treated with C_{60} showed no overt clinical signs or behavioural trouble. Moreover, their growth was not different from that of vehicle-treated rats.

At the end of the experiment, body weights were determined and the animals were sacrificed under the same conditions by bleeding through the thoracic aorta after sodium pentobarbital (1.0 ml/kg of body weight) anaesthesia. Small pieces of the right lobe of freshly collected rat livers as well as small pieces of spleens were fixed with pH 7.4 phosphate-buffered 10 % formalin and were processed by embedding in paraffin. Liver histology was evaluated using hematoxylin and eosin or Masson's trichrome staining. Samples for transmission electron microscopy (TEM) examination were processed as described previously (15). Serum alanine aminotransferase (ALT) activities were determined using a Hitashi 911 Analyzer (Roche Diangostics, Meylan, France) according to Bergmeyr's method (51). Vitamin C in sera was determined by HPLC coupled with coulometric detection (52). Circulating levels of vitamins A and E were determined simultaneously by HPLC with diode-array detection (53). Liver content of reduced and oxidised glutathione forms were determined by automated ion-exchange chromatography (54). C_{60} determinations in livers and excrements

were performed by HPLC with diode-array detection as described previously (50). Data are presented as the mean ± standard deviation or as the median and the range. Multiple comparisons were performed by using ANOVA or Kruskall-Wallis tests, according to the homogeneity of variances determined by the Cochran test. All tests were 2-tailed, and $P < 0.05$ was considered statistically significant.