Oxidatively Damaged DNA in Rats Exposed by Oral Gavage to C_{60} Fullerenes and Single-Walled Carbon Nanotubes

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**BACKGROUND:** C_{60} fullerenes and single-walled carbon nanotubes (SWCNT) are projected to be used in medicine and consumer products with potential human exposure. The hazardous effects of these particles are expected to involve oxidative stress with generation of oxidatively damaged DNA that might be the initiating event in the development of cancer.

**OBJECTIVE:** In this study we investigated the effect of a single oral administration of C_{60} fullerenes and SWCNT.

**METHODS:** We measured the level of oxidative damage to DNA as the premutagenic 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) in the colon mucosa, liver, and lung of rats after intragastric administration of pristine C_{60} fullerenes or SWCNT (0.064 or 0.64 mg/kg body weight) suspended in saline solution or corn oil. We investigated the regulation of DNA repair systems toward 8-oxodG in liver and lung tissue.

**RESULTS:** Both doses of SWCNT increased the levels of 8-oxodG in liver and lung. Administration of C_{60} fullerenes increased the hepatic level of 8-oxodG, whereas only the high dose generated 8-oxodG in the lung. We detected no effects on 8-oxodG in colon mucosa. Suspension of particles in saline solution or corn oil yielded a similar extent of genotoxicity, whereas corn oil per se generated more genotoxicity than the particles. Although there was increased mRNA expression of 8-oxoguanine DNA glycosylase in the liver of C_{60} fullerene-treated rats, we found no significant increase in repair activity.

**CONCLUSIONS:** Oral exposure to low doses of C_{60} fullerenes and SWCNT is associated with elevated levels of 8-oxodG in the liver and lung, which is likely to be caused by a direct genotoxicability rather than an inhibition of the DNA repair system.

**KEY WORDS:** cancer, DNA damage, DNA repair, nanoparticle, oxidative stress. *Environ Health Perspect* 117:703–708 (2009). doi:10.1289/ehp.11922 available via [http://dx.doi.org/](http://dx.doi.org/) [Online 12 November 2008]

Humans have been exposed to particulate matter during much of evolution, but technological achievements such as combustion engines and nanotechnology have yielded unique types of particles. Engineered nanotechnological materials are projected to be used, for instance, in electronics, cosmetics, cleaning materials, coatings, food packaging, and medicines, with increasing human exposure. In addition, consumer products containing nanomaterials will inevitably end up as waste, and subsequent processing or deposition may liberate particles to the environment, where they might accumulate along the food chain because some are highly persistent (Helland et al. 2007). Reinforced attention to the hazardous properties of engineered nanoparticles has been evoked by a report showing that a single intraperitoneal application of carbon nanotubes generated mesotheliomas in p53−/− mice (Takagi et al. 2008). These data are supported by another recent report showing that a single injection of carbon nanotubes into the peritoneal cavity of mice elicited an inflammatory reaction and granulomas at the peritoneal surface of the diaphragm (Poland et al. 2008). Similarly, pulmonary exposure to single-walled carbon nanotubes (SWCNT) produced granulomas in the lung of rodents (Lam et al. 2004; Shvedova et al. 2005; Warheit et al. 2004). It is possible that such nanotubes possess the same hazardous effects as other fibrous materials such as asbestos. On the other hand, particles such as C_{60} fullerenes might be less hazardous (Nielsen et al. 2008). For example, in a recent inhalation experiment, Baker et al. (2008) found that exposure to C_{60} fullerenes resulted in little pulmonary toxicity.

Generation of reactive oxygen species (ROS) and oxidative stress is generally accepted to be an important mechanism of action of nanoparticles (Ayres et al. 2008; Nel et al. 2006; Oberdörster et al. 2005). Exposure to combustion particles in urban air or diesel exhaust particles (DEP) is associated with oxidative stress, generated by particles themselves or by cell-mediated inflammatory responses and increased formation of oxidatively damaged DNA (Knaapen et al. 2004; Risom et al. 2005). 8-Oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) is considered to be an important oxidatively generated DNA lesion in carcinogenesis because a) it is mutagenic; b) mammalian cells have a highly versatile repair system for its removal; and c) the level of 8-oxodG is elevated in several types of tumor tissue (Evans et al. 2004). In addition, high urinary excretion of 8-oxodG is associated with increased risk of lung cancer among nonsmokers (Loft et al. 2006; Loft and Møller 2006). The relevance of 8-oxodG is further strengthened by observations that this lesion is elevated in animal tissues and human blood cells upon exposure to urban air pollution, diesel exhaust, or DEP (Møller et al. 2008). In particular, a single oral dose of DEP was associated with increased levels of 8-oxodG in liver, lung, and colon tissue (Danielsen et al. 2008b). Effect modification by the DNA repair system is likely to occur by continuous exposure. For example, ingestion of DEP in the diet for 3 weeks led to an up-regulation of the DNA repair system and unaltered levels of 8-oxodG in the liver and colon mucosa cells (Dybdahl et al. 2003), whereas measurements of lung tissue indicated unaltered regulation of the DNA repair system and elevated levels of oxidatively damaged DNA (Møller et al. 2004).

The aim of the present study was to investigate the effect of a single oral administration of C_{60} fullerenes and SWCNT. C_{60} fullerenes consist of 60 carbon atoms arranged in an aromatic soccer-ball structure with a nanosized diameter. SWCNT consists of two dimensions < 100 nm, whereas the axial dimension is much larger. Both C_{60} fullerenes and SWCNT are hydrophobic and hence difficult to suspend in saline solution, but they suspend more easily in oils. Consequently, rats received the particles in either saline solution or corn oil by oral gavage. We then assessed the level of 8-oxodG, a highly validated biomarker [European Standards Committee on Oxidative DNA Damage (ESCODD) et al. 2005]. We investigated alterations in the regulation of DNA repair by mRNA levels of 8-oxoguanine DNA glycosylase (OGG1), mutY homolog (E. coli) (MUTYH), and nudix (nucleoside...
Materials and Methods

Particle exposure of animals. We obtained 84 female Fisher 344 rats from Taconic (Ry, Denmark). Animals were acclimatized for at least 1 week before entering the experiments. The rats were housed in a temperature-controlled (22–24°C) and moisture-controlled (40–70%) room with a 12-hr light/12-hr dark cycle. All animals had free access to tap water and Standard Atrinom no. 1314 rat chow (Atrinom, Lage, Germany) during the acclimation and housing/treatment periods. Rats were sacrificed at 9 weeks of age. Animals were treated humanely and with regard for alleviation of suffering. All animal procedures followed the guidelines for the care and handling of laboratory animals established by the Danish government, and the Animal Experiment Inspectorate, Ministry of Justice, approved the study (no. 2006/561-1161).

The dry powder of C₆₀ fullerenes was described by the manufacturer to be a 99.9% pure preparation with a primary particle size of 0.7 nm (Sigma–Aldrich, Brondby, Denmark). The dry powder of SWCNT was described by the manufacturer to have a primary particle size of 0.9–1.7 nm and a fiber length < 1 µm (Thomas Swan and Co Ltd, Consett, UK). We suspended the particles in either saline or corn oil (Sigma–Aldrich) by sonication at 70 W and 42 kHz (Branson 1510, VWR–Bie & Bernten A/S, Herlev, Denmark) in a 5-day period for 10 hr each day and again 30 min before administration. For C₆₀ fullerenes and SWCNT, the gas exchange surface areas were < 20 m²/g and 731 ± 2 m²/g and the average pore sizes were 0 and 15 nm respectively (Jacobsen et al. 2008b). We used dynamic light scattering, as described by Jacobsen et al. (2008b), to measure the particle size in suspensions at the same concentration as administered to the rats by oral gavage. In general, it was difficult to determine the presence of nanoparticles in both types of solutions by dynamic light scattering because the solutions contain agglomerates with larger particle sizes. The particle sizes of C₆₀ fullerenes in the saline solution were 407 nm in the low dose and 621 and 5,117 nm in the high dose. In saline solution, the particle sizes of SWCNT were 195, 797, and 5,457 nm (low dose); the particle size in the highest dose could not be determined by dynamic light scattering. The particles were easier to suspend in corn oil than in saline. We obtained size modes of the C₆₀ fullerenes in corn oil by dynamic light scattering as follows: 234 nm (low dose); 40, 713, and 3,124 nm (high dose). The SWCNT had size modes as follows: 34 and 178 nm (low dose) and 1,015 nm (high dose). The SWCNT contained transition metals (2% iron and traces of cobalt, nickel, and manganese) and polycyclic aromatic hydrocarbons (PAHs; 417 ng/g of the U.S. Environmental Protection Agency (EPA) priority PAH compounds (U.S. EPA 1991)), whereas neither transition metals nor PAH could be detected in C₆₀ fullerenes.

The rats received a single intragastric dose of the particle preparations by oral gavage (0.064 and 0.64 mg/kg body weight suspended in saline or corn oil; n = 8), saline solution (control; n = 10), or corn oil (control; n = 10). Each rat received 200 µL fluid. The rats were sacrificed by cervical dislocation 24 hr after intragastric administration. The liver, lung, and colon tissues were snap-frozen in liquid nitrogen and stored at −80°C. We investigated effects in these organs because they are along the likely local and systemic exposure route and we have data from similar experiments on DEP as Standard Reference Material 2975 (SRM2975; National Institute of Standards and Technology, Gaithersburg, MD, USA), which was previously reported to be associated with increased levels of DNA damage 24 hr after a single intragastric application (Danielsen et al. 2008b). For all experiments, rats from each group (0, 0.064, and 0.64 mg particles/kg body weight) were treated the same day.

ROS-generating ability. The ability of the C₆₀ fullerenes, SWCNT, SRM2975, and Printex 90 carbon black (Degussa-Hüls, Frankfurt, Germany) to generate ROS in aqueous solution was determined by oxidation of 2′,7′-dichlorodihydrofluorescin (Molecular Probes, Portland, OR, USA). The particle suspensions were prepared in Hank’s balanced saline solution and sonicated immediately before incubation, as described previously by Jacobsen et al. (2008b). The oxidation product (2′,7′-dichlorofluorescein) was determined by fluorescence spectrometry with excitation at 490 nm and emission at 520 nm on a fluorescence spectrophotometer (VICTOR Wallac-2 1420; PerkinElmer, Hvidovre, Denmark).

Oxidatively damaged DNA. We obtained suspensions of colon epithelial cells by scraping off the cells on the luminal side of the colon with a glass slide in ice-cold Merchant buffer, as described previously by Dybdahl et al. (2003). The DNA was extracted from colon mucosa cells, liver, and lung tissue according to the procedure recommended by ESOCOD (et al. 2005). We isolated nuclei in buffer containing deoxyribozyme mesylate (Sigma–Aldrich) to prevent spurious oxidation; this was followed by lysis, RNase, and proteinase treatment (Sigma–Aldrich). We extracted DNA in buffer containing 40 mM Tris, 20 mM Na₂EDTA, 7.6 M NaI, and 0.3 mM deoxyribozyme mesylate, pH 8.0 (Sigma–Aldrich), 2-propanol, and ethanol. We digested DNA extracts to nucleosides using nuclease P1 and alkaline phosphatase (Merck, Darmstadt, Germany) and measured 8-oxodG and dG by HPLC with electrochemical and ultraviolet detection, respectively.

mRNA expression of HO1, MUTYH, NEIL1, NUDT1, and OGG1. We purified total RNA from liver and lung tissue using TRizol Reagent (Invitrogen, San Diego, CA, USA) and DNase treatment according to the manufacturer’s protocol (SV Total RNA isolation kit; Promega, Madison, WI, USA). For CDNA synthesis, 100–200 ng RNA was used in a reaction volume of 20 µL using GeneAmp RNA PCR (polymerase chain reaction) Kit (Applied Biosystems, Naram, Denmark) as recommended by the manufacturer.

We analyzed mRNA levels of the following genes: HO1 (GeneID 24451 (National Center for Biotechnology Information 2008)), MUTYH (GeneID 170841), NEIL1 (GeneID 3567090), NUDT1 (GeneID 117260), OGG1 (GeneID 81528) on a Taqman AB17900 (Applied Biosystems) as described by Risom et al. (2003b). We used Taqman probes and primers MUTYH (RN00591196), NEIL1 (RN01422330), NUDT1(RN00589097), and Tagman 185 probe (Euk 185 rRNA FAM/MGB probe mix) from Applied Biosystems. All probes and primers span exon junctions and are cDNA specific. We used oligonucleotides of HO1 and OGG1 as follows (final concentrations are shown in parentheses):

- RnHO1 forward primer (900 nM), 10F: 5′–CCA CAG CTC GAC AGC ATG T-3′;
- RnHO1 reverse primer (900 nM), 10R: 5′–GGC AAT ATG GGC GAG ATG AC-3′;
- RnOGG1 forward primer (900 nM), 10F: 5′–GAT TGC TAC TGC TCC TCA-3′;
- RnOGG1 reverse primer (900 nM), 10R: 5′–TGC AGT TAT CAG TCC TCA-3′.
reverse primer (900 nM), 5'-GGA GGC CAT CAT CAG CTT AAA-3'; Taqman probe (250 nM), 135F: 5'-6-FAM- TCT AGA AAT TCC AAG GTG T-3'; reverse primer (900 nM) 609R, 5'-TAC TTC TGG ACC AGC CAG GG-3'; Taqman probe (250 nM), 468T: 5'-6-FAM- CTG TTT TTC CAA CAA CAA CAT TG CATGC C-TAMRA-3'.

For the PCR reactions, we mixed 4 µL of the cDNA preparation with mastermix and water to a final volume of 228 µL. Aliquots were mixed with probes and primers to a final volume of 36 µL; and then transferred to three wells at 10 µL/well. For the PCR reaction, we used the following protocol: activation of the polymerase, 95°C for 20 sec; followed by 45 cycles of 95°C for 1 sec; and finally 60°C for 20 sec. We measured the expressions of reference (18S) and target genes in separate tubes. The expression levels are reported as target mRNA/5S rRNA. The statistical analysis was carried out using Statistica for Windows Version 5.5 (StatSoft, Inc., Tulsa, OK, USA).

Results

ROS-generating ability. Figure 1 shows ROS generation of particles in aqueous solution. The ROS generation was 3.3-fold (95% CI, 2.1–4.5) and 5.5-fold (95% CI, 4.2–6.6) in incubations containing SWCNT at 1 and 10 µg/mL, respectively. The C60 fullerene increased ROS production at the highest dose by 1.6-fold (95% CI, 1.5–1.9), SRM2975 and carbon black increased the level of ROS production by 4.4-fold (95% CI, 4.3–4.5) and 7.6-fold (95% CI, 7.4–7.9), respectively, at 10 µg/mL (Figure 1).

Oxidatively damaged DNA. Figure 2 shows the level of 8-oxodG in colon, liver, and lung tissue of rats administered C60 fullerene or SWCNT dispersed in either saline or corn oil. We observed significant single-factor effects of corn oil, which was associated with 25% (95% CI, 12–40), 30% (95% CI, 20–40), and 38% (95% CI, 29–47) higher level of 8-oxodG in the colon, liver, and lung, respectively.

We found no interactions between type of particle and vehicle exposure, indicating that particles in corn oil generated the same level of DNA damage as particles in saline. The generation of 8-oxodG is based on data from both types of vehicles (corresponding to estimates of single-factor effects). In the liver, the exposure to C60 fullerene was associated with 17% (95% CI, 4–34) and 25% (95% CI, 11–41) increases of 8-oxodG in groups treated with the low dose and high dose of particles, respectively. Exposure to SWCNT increased the level of 8-oxodG in the liver by 22% (95% CI, 8–38) and 20% (95% CI, 7–36) in the rats given the low dose and high dose, respectively. There were increased levels of 8-oxodG in the lung after exposure to SWCNT at both the low (21% [95% CI, 9–33]) and high doses
[23% (95% CI, 11–35)], whereas only the high dose of C60 fullerences was associated with significantly elevated levels of 8-oxodG in the lung (18% (95% CI, 4–31)). Oral exposure to particles was not associated with increased levels of 8-oxodG in colon mucosa tissue.

**mRNA expression of HO1, MUTYH, NEIL1, NUDT1, and OGG1.** Table 1 outlines the gene expression of OGG1, HO1, NEIL1, MUTYH, and NUDT1 mRNA in liver and lung tissue. The type of vehicle did not affect the level of gene transcription (p > 0.05, single-factor effect in ANOVA). The low and high doses of C60 fullerences increased the gene expression of OGG1 by 1.30-fold (95% CI, 0.9–1.9) and 1.80-fold (95% CI, 1.2–2.6), respectively, in the liver, whereas a similar effect of SWCNT did not reach statistical significance. In contrast, we found no significant effects in the expression of HO1, NEIL1, MUTYH, and NUDT1 mRNA in liver and lung tissue. Colon tissue was not analyzed because there was unaltered level of 8-oxodG and limited amounts of material were available.

**OGG1 repair activity.** Based on the observation that the gene expression of OGG1 was increased in the liver after oral exposure to particles, we also measured the OGG1 repair activity of liver extracts (Figure 3). Neither the exposure to particles nor the vehicle had an effect on the OGG1 repair activity, although a dose-related effect of C60 fullerences is suggested (p = 0.20, ANOVA).

**Table 1. mRNAs expression levels of genes involved in the removal of oxidized DNA base lesions in the liver and lung 24 hr after oral administration of C60 fullerences and SWCNT in saline or corn oil.**

| Tissue | Particle dose in saline (mg/kg body weight) | Particle dose in corn oil (mg/kg body weight) |
|--------|-------------------------------------------|---------------------------------------------|
| C60 fullerences | | |
| Lung | 10.4 ± 1.70 | 6.20 ± 0.76 | 6.28 ± 0.96 | 5.78 ± 0.58 | 8.63 ± 2.41 | 7.66 ± 1.50 |
| | 6.26 ± 1.23 | 2.29 ± 0.56 | 4.75 ± 0.96 | 6.65 ± 1.50 | 7.71 ± 1.67 | 8.94 ± 3.46 |
| | 3.16 ± 0.49 | 2.28 ± 0.51 | 2.01 ± 0.33 | 2.94 ± 0.86 | 2.97 ± 0.87 | 3.53 ± 1.01 |
| | 0.34 ± 0.04 | 0.27 ± 0.02 | 0.40 ± 0.09 | 1.26 ± 0.75 | 1.37 ± 0.49 | 1.29 ± 0.97 |
| | 0.55 ± 0.07 | 0.34 ± 0.06 | 0.38 ± 0.05 | 0.49 ± 0.07 | 0.57 ± 0.11 | 0.57 ± 0.10 |
| Liver | 1.05 ± 0.12 | 1.55 ± 0.28 | 1.32 ± 0.18 | 1.47 ± 0.28 | 1.49 ± 0.37 | 1.27 ± 0.22 |
| | 0.36 ± 0.07 | 0.45 ± 0.09 | 0.69 ± 0.30 | 0.35 ± 0.04 | 0.34 ± 0.05 | 0.40 ± 0.08 |
| | 0.56 ± 0.16 | 0.18 ± 0.25 | 1.57 ± 0.74 | 0.72 ± 0.10 | 0.58 ± 0.13 | 0.70 ± 0.08 |
| | 0.25 ± 0.07 | 0.35 ± 0.1 | 0.47 ± 0.18 | 0.26 ± 0.09 | 0.18 ± 0.03 | 0.21 ± 0.07 |
| | 0.12 ± 0.02 | 0.14 ± 0.02 | 0.25 ± 0.07* | 0.10 ± 0.02 | 0.14 ± 0.03 | 0.16 ± 0.03* |
| SWCNT | | | |
| Lung | 10.4 ± 1.70 | 5.03 ± 1.04 | 14.1 ± 7.81 | 5.78 ± 0.58 | 7.15 ± 1.73 | 8.14 ± 2.79 |
| | 6.26 ± 1.23 | 3.48 ± 1.07 | 9.76 ± 3.54 | 6.65 ± 1.50 | 8.65 ± 2.33 | 6.33 ± 0.68 |
| | 3.16 ± 0.49 | 2.11 ± 0.56 | 2.78 ± 0.75 | 2.94 ± 0.86 | 4.88 ± 1.45 | 2.24 ± 0.53 |
| | 0.34 ± 0.04 | 0.82 ± 0.08 | 1.78 ± 0.81 | 1.26 ± 0.75 | 2.29 ± 1.15 | 0.67 ± 0.12 |
| | 0.55 ± 0.07 | 0.54 ± 0.03 | 1.17 ± 0.59 | 0.49 ± 0.07 | 0.54 ± 0.10 | 0.45 ± 0.06 |
| Liver | 1.05 ± 0.12 | 1.56 ± 0.23 | 1.65 ± 0.19 | 1.47 ± 0.28 | 1.05 ± 0.14 | 1.30 ± 0.22 |
| | 0.36 ± 0.07 | 0.36 ± 0.05 | 0.51 ± 0.09 | 0.35 ± 0.04 | 0.37 ± 0.05 | 0.30 ± 0.06 |
| | 0.56 ± 0.16 | 0.88 ± 0.19 | 0.83 ± 0.17 | 0.72 ± 0.10 | 0.68 ± 0.11 | 0.57 ± 0.08 |
| | 0.25 ± 0.07 | 0.22 ± 0.03 | 0.48 ± 0.20 | 0.26 ± 0.09 | 0.37 ± 0.12 | 0.26 ± 0.05 |
| | 0.12 ± 0.02 | 0.15 ± 0.03 | 0.16 ± 0.04 | 0.10 ± 0.02 | 0.15 ± 0.04 | 0.17 ± 0.04 |

The mRNA expression is relative to the expression of 18S per 10^6. Values are mean ± SE. *p < 0.05 (single-factor effect of the particle exposure, ANOVA).

**Discussion**

In this study we found increased levels of oxidatively damaged DNA in liver and lung tissue 24 hr after oral administration of C60 fullerences and SWCNT in saline or corn oil. There were virtually no alterations of the repair activity of oxidized base lesions in the same tissues, indicating that the level of DNA damage is not underestimated as a consequence of increased repair.

The levels of 8-oxodG were approximately 20% increased in the liver and lung tissue after the exposure to C60 fullerences and SWCNT, whereas the particle did not affect the particle-generated genotoxicity. The effect of C60 fullerences on OGG1 mRNA expression in the liver and 8-oxodG in the liver showed clear dose–response relationships, whereas other dose–response relationships were more flat. Possible agglomeration of particles affecting uptake and effects might have influenced the dose relationships. This could also be relevant for the lack of genotoxicity in colon mucosa cells. These cells have high turnover, which could dilute any possibly transient DNA damage, whereas liver and lung cells have a low proliferation rate, allowing damage to accumulate. We have previously shown that rats given the same oral dose of DEP as SRM2975 had approximately 50% elevated level of 8-oxodG in colon mucosa cells, liver, and lung (Danielsen et al. 2008b). Thus, our results indicate that C60 fullerences and SWCNT are genotoxic in rats, but the effect is lower than that observed for SRM2975. The C60 fullerences and SWCNT were administered in rather low doses because we wanted to use the SRM2975 preparation as benchmark particles. In comparison, a recent study with intravenous administration of 150 mg pégylated SWCNT per mouse argued that concentrations between 10- and 100-fold lower were sufficient in biomedical applications (Schipper et al. 2008). Other recent studies on pulmonary toxicity of SWCNT have used doses in the range of 20–40 mg/kg body weight (corresponding to about 1–2 mg/kg body weight) by pharyngeal aspiration (Han et al. 2008; Shvedova et al. 2008). The doses used in the present study can thus be considered as being in the lower range of exposures.

To the best of our knowledge, our data are the first to demonstrate that C60 fullerences and SWCNT generate oxidatively damaged DNA in rodent organs. Other reports have shown that C60 fullerences and SWCNT exposure of cells in culture is associated with elevated levels of DNA damage measured by the comet assay (Dhawan et al. 2006; Kisin et al. 2007; Pacurari et al. 2008). These types of lesions represent general genotoxicity rather than oxidatively damaged DNA, which can be assessed by a modified version of the comet assay including digestion with DNA glycosylase or endonuclease enzymes (Moller 2006). We used this approach in a cell culture experiment and demonstrated that both C60 fullerences and SWCNT generated oxidatively damaged DNA (Jacobsen et al. 2008b). The oxidizing effect of C60 fullerences on biomolecules in cell culture settings could be due to generation of singlet oxygen induced by photosensitization (Kamat et al. 2000; Yamakoshi et al. 2003). Oxidations of DNA in internal organs probably arise from mixed ROS generation, as supported by increased mortality after C60 fullerence exposure in zebrafish in the dark and by enhanced mortality resulting
from coexposure of C60 fullerenes with hydro-
gen peroxide or glutathione depletion (Usenko et al. 2008). Hydroxyl radicals appear to be the
important type of ROS generated by SWCNT in
cell cultures (Manna et al. 2005; Pacurari et al. 2008). The level of transition metals is
not likely to explain the differences in geno-
toxicity between the particles used in the pres-
ent study and SRM2975, because the latter has
very low levels, whereas the SWCNT used
here contained 2% iron, as well as traces of
other metals, and the C60 fullerenes had unde-
tectable levels. Moreover, SWCNT at low
concentrations produced higher levels of ROS in
cell-free systems than did SRM2975 and C60
fullerenes, whereas SRM2975 appeared to
induce more guanine oxidation than the
engineered particles in cell culture (Danielsen et al. 2008a; Jacobsen et al. 2008b).
The SRM2975 preparation contains substan-
tial amounts of PAHs, which show low lev-
els in SWCNT and are undetectable in C60
fullerenes. The content of PAHs could explain
the higher DEP-induced level of 8-oxodG in
blood, the increased expression of
OGG1 mRNA in leukocytes and to the engi-
neered particles in cell culture (Danielsen et al. 2008a; Jacobsen et al. 2008b).
The OGG1 (Ser326Cys) genotype, high expres-
sion levels of OGG1 mRNA in leukocytes is
associated with increased risk of lung cancer
(Hatt et al. 2008).

Although our results strongly indicate that
oral exposure to C60 fullerenes and SWCNT
are associated with increased generation of oxi-
dized DNA, it is still unresolved how appli-
cation of particulate matter in the gut can
oxidize biomolecules in internal organs. A
somewhat naïve notion suggests that the epi-
thelial lining of the gut is designed to absorb
substances and thus may allow passage of
particulate matter. In fact, we suspended the
particles in either corn oil or saline solution
because of the notion that their hydropho-
bic nature would let them follow the regular
passage of lipids in the gut. Because there
was no difference in genotoxicity between
the particles suspended in corn oil and saline
solution, we believe that orally adminis-
tered particles will distribute in the chyle
of the intestinal juice, despite the fact that
they may reach the gastrointestinal tract as
agglomerates, as indicated by aqueous par-
ticle suspension characterization. The results
suggest that the particles are absorbed from
the gastrointestinal tract to blood circulation
and secondary organs. However, at pres-
ent the extent of translocation of particulate
matter across epithelial barriers is a highly
controversial issue. Studies of the pulmonary
translocation of model particles to systemic
circulation and secondary organs indicate
only minute passage (Kreyling et al. 2002;
Mills et al. 2006; Möller et al. 2008; Wiebert et al. 2006). On the other hand, whole-body
inhalation exposure to ultrafine carbon par-
ticles have suggested some deposition in the
liver, which has been speculated to originate
from gastrointestinal exposure and uptake
from the gut (Oberdörster et al. 2002). In
addition, the uptake of C60 fullerenes and
poly styrene latex microspheres from the
gastrointestinal tract into blood circulation
has been estimated in the range of 1% of the
applied dose (Carr et al. 1996; Yamago et al.
1995). However, it is interesting to note that
in drug delivery research, there is acceptance
that particulate matter can be absorbed from
the gastrointestinal tract, and this feature of
nanomaterials is being used as an approach
of altering the pharmaco kinetic behavior of
drugs (Florence 2004).

We observed an effect of corn oil in all
three organs. This was not part of our a priori
hypothesis. We chose the volume of corn oil
to be identical to that of the saline solution
(200 µL), which would allow a reasonable
volume for the suspension in the aqueous
solution. It should be emphasized that corn
oil is rich in polyunsaturated fatty acids
and was sonicated, which might produce geno-
toxic compounds. In addition, rat studies
indicated that a diet rich in corn oil increased
8-oxoG excretion in urine (Loft et al. 1998)
as well as the levels of 5-hydroxymethyl-2´-
deoxyuridine, another marker of oxidatively
DNA from blood and mammary gland
(Follmann et al. 2007).

In conclusion, the data obtained from the
present study indicate that C60 fullerenes and
SWCNT generated oxidatively damaged DNA
in liver and lung cells by a gastrointestinal
route. The genotoxic effect resulting from
exposure to C60 fullerenes and SWCNT was
smaller than that from DEP exposure; how-
ever, it may be a cause for concern in humans.

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DNA oxidation and mutagenicity (Jacobsen et al. 2007), indicating that particles as such
have the ability to induce oxidative stress.

Accordingly, ROS generation in an acellular
system does not appear to predict the
ability of particles to oxidatively damage DNA
cell culture or in vivo.

The notion that C60 fullerenes and
SWCNT induce less oxidative stress compar-
ated with DEP is supported by the unal-
terated regulation of HO1, which in our
previous study was up-regulated by exposure
to SRM2975 (Danielsen et al. 2008b).
In the present experiments, we observed unaltered
expression levels of NEIL1, MUTYH, and
NUT1, whereas the increased expression of
OGG1 mRNA in the liver of rats exposed
to C60 fullerenes was not associated with higher
repair activity, which might be due to the
fact that longer exposure times are required
to observe increased repair activity. Indirect
evidence of this effect modification comes from
studies of inhalation exposure to DEP where
repeat exposures on 4 consecutive
days were associated with up-regulation of
OGG1 and unaltered 8-oxodG in pulmonary
tissue (Risom et al. 2003a), whereas an identi-
cal exposure scenario yielded higher levels of
8-oxodG in OGG1 knockout mice (Risom et al. 2007).
Although increased expression levels of OGG1 might be considered benefi-
cial to the cells, it should be emphasized that in
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