Does Carbon Black Disaggregate in Lung Fluid? A Critical Assessment

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Does Carbon Black Disaggregate in Lung Fluid? A Critical Assessment

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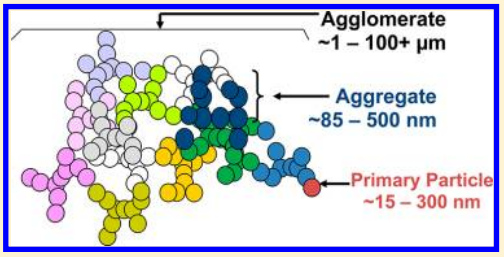
ABSTRACT: Carbon black is an industrially produced particulate form of nearly pure elemental carbon. The basic building blocks of carbon black are (1) primary particles, minute pieces of matter with defined physical boundaries; (2) aggregates, collections of strongly bound or fused particles; and (3) agglomerates, collections of weakly bound aggregates. Industrial carbon black is produced within a closed reactor where the primary particles form aggregates, which become the indivisible entities of carbon black. These aggregates then form agglomerates, which are the typical form of carbon black in commerce. Carbon black is often used in *in vitro* and *in vivo* particle toxicology investigations as a reference nanoparticle. The toxicology studies often report the sizes of the primary particles but rarely the sizes of the aggregates or agglomerates. It appears in many cases that there is a limited understanding of the fact that carbon black typically does not exist as primary particles but instead exists as aggregates and agglomerates. Moreover, many toxicology studies manipulate carbon black particles in order to disperse them so that the form of carbon black used in these toxicology studies may be substantially different from the form that may be encountered in the workplace environment. Since the main exposure route for carbon black is inhalation, the question arose as to whether inhaled carbon black may deagglomerate or disaggregate to either smaller aggregates or primary particles when in contact with lung fluids. This question relates to the concern that there may be additional hazards of smaller particles, such as their ability to translocate to tissues and organs beyond the lung and the ability to pass through the blood−brain barrier. The purpose of this assessment is to review the existing literature for evidence as to whether carbon black deagglomerates or disaggregates into smaller aggregates or primary particles when in contact with lung fluid. On the basis of a review of the physical characteristics of commercial carbon black and various toxicology studies, it appears that commercially produced carbon black in contact with lung fluid is unlikely to deagglomerate or disaggregate into smaller aggregates or primary particles.



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1. INTRODUCTION

Carbon black is a manufactured aciniform (grape-like) particulate form of nearly pure (>95%) elemental carbon. The smallest units of carbon black (the primary particles) typically do not individually exist outside of a closed reactor. Inside the reactor, the near spherical particles coalesce into aggregates that become the basic indivisible entities of commercial carbon black. Strong electrical forces maintain the bond between aggregates and promote the formation of agglomerates, which are the result of hundreds to thousands of strongly adhering aggregates. Carbon black in commerce is encountered as agglomerates.[1] Figure 1 shows the typical sizes and appearance of carbon black primary particles, aggregates, and agglomerates. Figure 2 is an electron microscopy image of a typical industrial aciniform aggregate of carbon black.

The following definitions for particles, aggregates, and agglomerates are provided in a European Union (EU)

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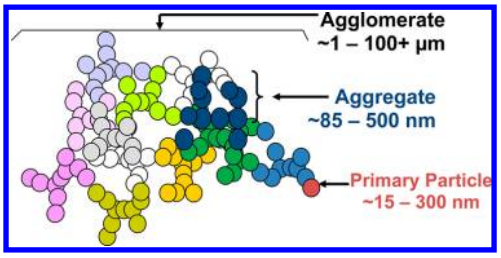

Figure 1. Typical sizes and structures of carbon black primary particles, aggregates, and agglomerates.

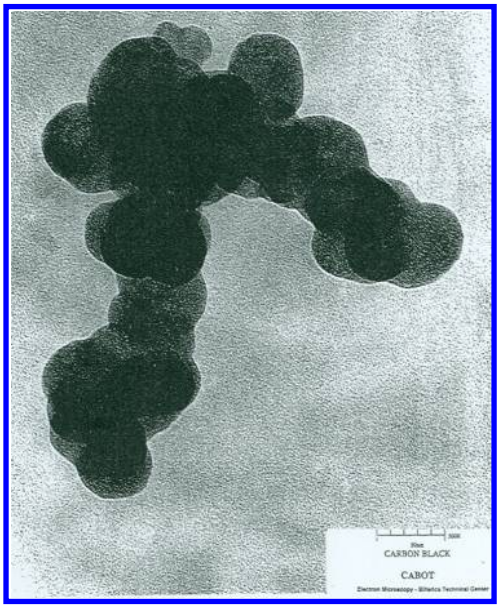

Figure 2. Typical industrial aciniform aggregate of carbon black.

document,[2] namely, (a) particle means a minute piece of matter with defined physical boundaries; (b) agglomerate means a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components; and (c) aggregate means a particle comprising strongly bound or fused particles.

Carbon black is often used for *in vitro* and *in vivo* particle toxicology investigations as a reference nanoparticle (also known as an "ultra-fine" particle). In many cases, however, there seems to be limited understanding of the fact that carbon black typically does not exist as primary particles but instead exists as aggregates and agglomerates. The conditions under which carbon black may disaggregate or deagglomerate are not fully understood. Although carbon black present in workplace air may be mainly in the agglomerated form, a question arose as to whether inhaled carbon black may deagglomerate or disaggregate to either smaller aggregates or primary particles when in contact with lung fluids or other cellular materials. This question relates to the concern that primary particles are typically in the nano size range (<100 nm). Additional hazards may be associated with nanoparticles compared to their bulk counterparts. These hazards may be related to the higher surface area of nanoparticles and other properties, such as their ability to translocate to tissues and organs beyond the lung and the ability to pass through the blood–brain barrier. The purpose of this perspective is to address the existing literature for evidence as to whether carbon black deagglomerates or disaggregates when in contact with lung fluid.

2. RELEVANCE OF THE QUESTION OF CARBON BLACK DISAGGREGATION IN LUNG FLUID

2.1. Translocation of Particles. The main reason to understand potential carbon black disaggregation in the lung is to enhance the understanding of its kinetic behavior once inhaled. If carbon black disaggregates into smaller sizes, there is a greater possibility of translocation to other organs and tissues. Studies using specially prepared carbon particulates in the "ultrafine" or nanoparticle range suggest the possibility of translocation. In a study in 4 week and 18-month-old female Swiss mice,[3] the authors introduced 7 mg of ^{7}Be-labeled carbon black (Elftex 8 furnace black) into the gut by gavage. The isotope distribution in the animals was measured at 4 h, then 1, 2, 4, and 14 days after introduction. There was uptake and distribution from the gut; transit was more rapid in young mice. Peyer's patches (a gut-associated lymphoid tissue) of older mice took up more radiolabeled material than younger mice. However, the International Agency for Research on Cancer (IARC)[4] noted that it was unclear whether the authors verified the stable binding of the isotope to the carbon black particles.

In a study by Oberdorster et al.,[5] carbon black (and other particles) instilled in rat lungs showed that the translocation of particles from the alveolar lumen of the lungs was dependent on particle size. Following intratracheal instillation of 0.5 mg particles of different sizes, smaller particles (12 and 20 nm) penetrated the alveolar epithelial cell barrier and entered the lung interstitium to a greater extent than an equal mass of larger respirable particles (>200 nm) at 24 h. This proportion increased with increasing particle dose as either mass or surface area.

Kreyling et al.[6] conducted translocation studies with iridium or carbon nanoparticles with aggregate sizes between 20 and 80 nm. Following one hour of inhalation of these particles in rats, the particles were measured in liver, spleen, kidneys, heart, and brain. After 24 h, translocation of carbon nanoparticles was found to be lower than iridium nanoparticles. Furthermore, there was significantly less translocation and accumulation with 80-nm than with 20-nm nanoparticle aggregates of iridium. The authors concluded that both nanoparticle characteristics, the material and the size of the chain-type aggregates, determine translocation.

Other studies have shown that ultrafine carbon and other particles can translocate beyond the lungs. Inhaled spark-generated ultrafine ^{13}C-carbon particles of approximately 25 nm in diameter were cleared rapidly from rat lungs and translocated to other organs (e.g., liver and spleen).[7] In this study, significant amounts of particles were found in the livers of rats in the high-exposure group (approximately 5-fold higher amounts in the liver than in the lung at 24 h). It is important to note, however, that this study was not conducted with industrially derived carbon black but instead with singlet carbon particles produced in the laboratory. The results illustrate the need to distinguish results from studies using industrially produced carbon black from other forms of artificially produced, or naturally occurring, carbon particles which do not exist in the form of stable aggregates.

2.2. Lung Toxicity. Exploring the question of potential carbon black disaggregation in the lung can provide a better understanding of the physical/chemical behavior of carbon black in the lung. Researchers have studied possible health effects and toxicity of carbon black in *in vitro*, *in vivo*, and in epidemiological studies.[4] Carbon black toxicity via the

inhalation route has been extensively studied in subchronic studies[8−10] and chronic studies[11,12] in animals. These studies demonstrated a dose-dependent increase in biochemical and cellular markers of lung damage. A critical assessment of the potential for carbon black to deagglomerate or disaggregate in the lung will add to this existing body of knowledge by shedding light on its toxicokinetics, after inhalation.

2.3. Regulatory Definition of Nanomaterials. Under most current definitions of nanomaterials, carbon black is considered a nanomaterial, (dimensions of less than 100 nm (nm)) because the primary particles are in the nanosize range, regardless of the aggregated or agglomerated sizes. A recent EU document[2] makes the following points:

(1) "The definition in this recommendation should be used as a reference for determining whether a material should be considered a "nanomaterial" for legislative and policy purposes in the [European] Union. The definition of "nanomaterial" should be **based solely on the size of the constituent particles** of a material, without regard to hazard or risk. This definition, based only on the size of a material, covers natural, incidental or manufactured materials."

(2) "Agglomerated or aggregated particles may exhibit the same properties as the unbound particles. Moreover, there can be cases during the life cycle of a nanomaterial where the particles are released from the agglomerates or aggregates. The definition in this recommendation should therefore also include particles in agglomerates or aggregates whenever the constituent particles are in the size range 1 nm−100 nm."

Because carbon black generally consists of primary particles of less than 100 nm in size, carbon black will be considered in the EU, and presumably elsewhere worldwide, as a nanomaterial. In addition to the primary particles being nanosized, a significant fraction of aggregates are also less than 100 nm.[4] Therefore, by most definitions, carbon black is considered a nanomaterial, and this consideration would not be affected by additional studies of disaggregation behavior. Regardless of regulatory definitions, however, it is still important to have a complete understanding of the form and structure of carbon black, and the conditions under which carbon black agglomerates and aggregates may break down.

3. RESULTS OF LITERATURE REVIEW

3.1. Physical Characteristics of Commercial Carbon Black. The physical characteristics of commercial black suggest that disaggregation in biological systems is unlikely to occur. It is important to distinguish between the laboratory-derived carbon particulates in the ultrafine range and industrially produced carbon black, which exists as aggregates and agglomerates. In the IARC Monograph on Carbon Black,[4] it is stated that "Carbon black is a generic term for an important family of products that is used principally for the reinforcement of rubber, as a black pigment and because of its electrically conductive properties. It is an extremely fluffy fine powder with a large surface area and is composed essentially of elemental carbon. Carbon black is one of the most stable chemical products. In general, it is the most widely used nanomaterial and its aggregate dimension ranges from tens to a few hundred nanometers (nm); it imparts special properties to composites of which it is a part."

IARC[4] draws a clear distinction between commercially produced carbon black and other environmentally encountered carbonaceous particulates materials such as soot. "In contrast to carbon black, soot is a material of varying and often unknown composition that is an unwanted by-product of the incomplete combustion of all types of material that contain carbon, such as waste oil, coal, paper, rubber, plastic, household waste and also some fuel oils. Soots have a small surface area of available carbon due to their large particle size and low carbon content. They typically contain large quantities of solvent-extractable materials and their ash content can be 50% or more."[4]

Tables 1 and 2, reproduced from IARC,[4] illustrate important size characteristics of some commonly produced carbon black.

Table 1. Particle Range of Rubber-Grade Carbon Black[a]

Group number	Typical average primary particle size (nm)	Average surface area (m^2/g)
0	0–10	>150
1	11–19	121–150
2	20–25	100–120
3	26–30	70–99
4	31–39	50–69
5	40–48	40–49
6	49–60	33–39
7	61–100	21–32
8	101–200	11–20
9	201–500	0–10

[a]Reprinted with permission from ref 4. Copyright 2010 International Agency for Research on Cancer.

Carbon black is typically described as a commercially produced aciniform (grape-like) aggregate particulate material. "These aggregated particles are produced by homogeneous nucleation from a supersaturated fluid initially with the formation of approximately 1-nm scale nuclei that then coagulate to primary particles. These further aggregate, as they collide and stick together, through van der Waal's-type cohesive forces and/or hydrogen bonding and are welded together by the deposition of more material from the fluid phase. The resulting complex particles are aggregates. Once the process is complete, the aggregates are the smallest separable entities; neither nuclei nor "primary particles" exist apart from the aggregate. We use the term "nodule" to describe the welded sphere that once was a primary particle."[13]

Gray and Muranko[13] further state, "From the standpoint of industrial hygiene and the environment, nuclei and primary particles are historic artefacts, aggregates are the permanent entities, and agglomerates are dynamic entities whose diameter represents a balance of the process of agglomeration by second-order collision with sticking and various mechanical dispersion forces, which may exist in their vicinity. For materials with strong particle−particle cohesive forces such as carbon blacks and amorphous silicas, these agglomerates can be very large and very hard to disperse to the single aggregate state."

Gray and Muranko[13] detail the forces required to break down the carbon black aggregates; they note that experimentally, intense mechanical forces have been applied but with little evidence of significant release of nodules (primary particles), although there may be some reduction in aggregate size by internal fracture at weak spots in the necks between nodules. They concluded that the aggregate structure which can withstand these intense mechanical forces is unlikely to be broken down in a biological system in which the forces are very weak by comparison.

Table 2. Summary Information on Particle Size[a]

Carbon black	Surface area (m^2/g)	Approximate diameter of primary particle size (nm)	Diameter of aggregate (nm)	Size of agglomerate
Oil-furnace	12–240	10–400	50–400	Large (<2 mm)
Thermal	6–15	120–500	400–600	Large (<2 mm)
Impingement (channel)		10–30	50–200	Large (<2 mm)
Lampblack	15–25	60–200	300–600	Large (<2 mm)
Acetylene black	15–70	30–50	350–400	Pelletizes poorly

[a]Reprinted with permission from ref 4. Copyright 2010 International Agency for Research on Cancer.

3.2. Studies in biological systems. Although the previous section presented theoretical reasons as to why disaggregation of carbon black aggregates would be extremely unlikely to occur in most environmental situations and biological systems; the evidence to support this supposition needs to be examined. Since few studies have specifically addressed the potential for carbon black to disaggregate in lung fluids, it can be instructive to examine such analyses performed on other similar substances, broadly described as poorly soluble particles.

Maier et al.[14] addressed disaggregation of an aggregated form of titanium dioxide (TiO_2) (P 25, Evonik Industries). TiO_2 is a nanostructured particulate material comparable to carbon black. Like carbon black, commercially produced TiO_2 is highly aggregated and agglomerated. The question of whether lung surfactant may promote the disaggregation of TiO_2 was investigated. Dipalmitoylphosphatidyl-choline (DPPC), the main component of lung surfactant, was used to assess whether it can split the bonds between TiO_2 aggregates and agglomerates. The energy required to split aggregates into primary particles, as well as the energy of the interaction of a TiO_2 surface with a DPPC bilayer, was calculated using a model. Calculated splitting energy was 1 J per square meter (J/m^2) between TiO_2 aggregates and 10 J/m^2 between primary particles. In contrast, the calculated energy generated by the interaction between DPPC and TiO_2 was significantly weaker at 0.05J/m^2. Thus, the energy generated by the interaction of DPCC and TiO_2 is insufficient to break apart aggregates or agglomerates (which are more weakly bound to each other).[14]

To verify these model calculations, the authors measured particle size distributions of TiO_2 suspensions in DPCC. The particle size distribution of TiO_2 did not change demonstrably in the presence of DPCC, indicating that DPCC did not break down TiO_2 aggregates. Therefore, the model calculations supported the measured particle size distribution of TiO_2 suspensions in DPCC. The authors concluded that lung surfactant does not promote the disaggregation of TiO_2 agglomerates and aggregates. Particles such as carbon black and TiO_2 belong to a category of chemically inert, poorly soluble particles as listed in IARC.[4] IARC Monograph 93 grouped carbon black, TiO_2, and talc together because of their similarities, and the main hazard concern which is the effects on the lung as a result of inhaling the particles.[4]

The results of Maier et al.[14] are supported by the findings of the NanoCare project in Germany.[15] The behavior of different nanostructured materials (including carbon black and TiO_2) in the presence and absence of lung fluid were compared. The type of carbon black investigated had a primary particle size of 14 nm (from Evonik Industries). In one study, particle-size distributions were measured for nine nanomaterials, including carbon black. The nanomaterials were dispersed using ultra-centrifugation in serum and porcine broncho-alveolar lavage fluid (pBALF). When dispersed in diluted serum, the fraction of carbon black particles under 100 nm was 0.07% by weight, indicating that the bulk of the material is present in an aggregated or agglomerated form that is larger than 100 nm. Carbon black dispersed in pBALF also showed that the percent of carbon black particles less than 100 nm was 0.05%, indicating that additional disaggregation or deagglomeration did not take place in the presence of lung fluid. TiO_2 showed similar results when dispersed in dilute serum and pBALF. These results support the conclusion that there is no significant disaggregation or deagglomeration of carbon black in lung fluid. Unlike the Maier study[14] that used a component of lung fluid (DPCC), the NanoCare project[15] used complete lung fluid; therefore, these results may be more representative of the actual lung environment. NanoGem is the follow-up project to NanoCare; one of the stated objectives of this project is to study deagglomeration under different conditions.[16]

Porter et al.[17] discuss the development of a synthetic dispersion medium (DM) that is used to disperse agglomerated nanoparticles for toxicity studies. For toxicity studies, agglomerated particles are typically dispersed to smaller agglomerate or aggregate sizes in order to facilitate dosing in both *in vivo* and *in vitro* studies. DM is designed to mimic broncho-alveolar lavage (BAL) fluid and consists of phosphate-buffered saline (PBS), serum albumin, and the lung surfactant, DPCC. The authors studied the dispersion of ultrafine carbon black (UFCB; Degussa Printex 90) in solutions of PBS, BAL fluid, and DM. Dynamic light scattering measurements were conducted to determine particle size. After adding UFCB to the PBS, BAL fluid and DM solutions, the suspensions were first vortexed, and then sonicated with a probe sonicator for 30 min. After vortexing in all three solutions, UFCB formed large agglomerates that could not be measured by dynamic light scattering. However, after sonication in BAL fluid and DM (but not PBS), UFCB particle sizes reduced to approximately 100 nm. Similar results were obtained with ultrafine titanium dioxide ($UFTiO_2$; Degussa Aeroxide P25). The key issue to note in this study is that sonication for approximately 30 min was essential to disaggregate or deagglomerate $UFTiO_2$ and UFCB in both BAL fluid and DM and that disaggregation or deagglomeration did not occur prior to sonication. These results indicate that carbon black is unlikely to disaggregate or deagglomerate if it encounters lung fluid under physiological conditions, which clearly would not involve prolonged sonication.

Jiang et al.[18] studied various types of TiO_2 dispersions and found that agglomerates were dispersed more effectively with probe sonication compared to bath sonication. Probe or bath sonication did not have much effect on dispersing aggregates.

These studies show that high energy levels, such as those dispensed by sonication methods, are needed, even for deagglomeration. This effect of sonication was noted by Maier et al.[14] who stated that sonication of TiO_2 in DPCC leads to an additional dust fraction with aggregate sizes of about 100 nm which is not normally seen without sonication. Because of this finding, test substances were added to lung fluid just with shaking gently by hand.[14] These studies indicate that carbon black is unlikely to disaggregate or deagglomerate in contact with lung fluid under physiological conditions, which would not involve sonication.

Creutzenberg[19] studied whether agglomerates of various materials disintegrated into primary particles following uptake in the respiratory tract. The materials studied included titanium dioxide (TiO_2 P 25 and TiO_2 T 805 from Evonik-Degussa), carbon black (Printex 90 and Printex XE2 from Evonik-Degussa), and several metal oxide particles. One study involved administering the materials to rats through intratracheal instillation, conducting broncho alveolar lavage (BAL) at specified intervals and measuring agglomerate sizes in the BAL fluid. The author noted that the carbon black particles were barely present in BAL fluid and could not be accurately measured. The TiO2 results showed larger agglomerate sizes for TiO_2 P 25 and similar agglomerate sizes for TiO_2 T 805 in the lung lining fluid. It was concluded "Based on the results in various approaches, a tendency of nanoscaled particles to form larger size agglomerates following deposition and interaction with cells (in vitro) or the respiratory tract (in vivo) is predominant. The contrary trend, i.e., the increase of particle number due to a disintegration of agglomerates seems not to be of high relevance."[19]

It is noted that these experiments suspended dry carbon black in lung fluid before measuring dispersion. It appears that agglomeration tends to increase when dry carbon black is placed in biological fluid. If dry carbon black is aerosolized, (as in the case of inhalation experiments), agglomerate sizes are likely to be smaller. However, the possibility of further deagglomeration or disaggregation is still unlikely since the lung environment does not produce the higher levels of energy produced by sonication.

4. DISCUSSION

Commercially produced carbon black is typically encountered as agglomerates, which are composed of hundreds to thousands of aggregates, the basic indivisible entities of carbon black. Aggregates, in turn, are composed of primary particles; however, primary particles typically do not exist outside of a closed reactor. For carbon black, as with other particles, the main exposure route is usually inhalation. Following inhalation, carbon black agglomerates would encounter lung fluid and other cellular materials. If the agglomerates and aggregates break down to release smaller aggregates or primary particles in the nanosize range, there is the concern that the smaller sized carbon black could translocate to other organs and tissues and potentially result in additional toxic effects.

In experimental settings, intense mechanical forces have been applied to aggregates, with little evidence of primary particle release.[13] Primary particles exist only for a short time during the manufacturing process in a closed high-temperature system before they become aggregated. The aggregate structure that can withstand these intense mechanical forces is unlikely to be broken down in a biological system where the forces are weak in comparison.[13] Agglomerates, however, are held together by relatively weak van der Waal's forces and may be able to deagglomerate in biological systems into aggregates.

Maier et al.[14] evaluated whether aggregated TiO_2 could disaggregate in a surfactant present in lung fluid. TiO_2 aggregates did not disaggregate in the presence of a lung fluid surfactant. The authors also conducted modeling calculations that showed that the energy generated by the interaction of lung fluid surfactant and TiO_2 surfactants is much less than the energy required to split apart agglomerates and aggregates. The NanoCare project in Germany[15] measured the particle size distribution of various nanomaterials, including carbon black, before and after dispersion in broncho-alveolar lavage fluid. The particle size distribution of carbon black did not change after the addition of broncho-alveolar lavage fluid. Porter et al.[17] reported that significant breakdown of carbon black agglomerates in lung fluid occurred only after sonication for 30 min and that no breakdown was noted after vortexing carbon black in lung fluid. Jiang et al.[18] also showed that high energy levels, such as those dispensed by sonication methods, are needed even for deagglomeration. Creutzenberg[19] concluded that particles tended to form larger size agglomerates following interaction with the respiratory tract and that the disintegration of agglomerates did not appear to occur.

The dispersion methods used in many toxicology studies often result in a form of carbon black that may be substantially different from the form that is encountered in the workplace environment. The available scientific information supports the perspective that commercially produced carbon black in lung fluid is unlikely to deagglomerate or disaggregate into smaller aggregates or primary particles.

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Notes

The authors are members of the Scientific Advisory Group (SAG) of the International Carbon Black Association (ICBA), whose member companies are engaged in the manufacture of carbon black worldwide. The function of the SAG is to recommend research in all areas of occupational health to the ICBA related to the manufacture and use of carbon black.

ABBREVIATIONS

BAL, broncho-alveolar lavage; DM, dispersion medium; DPCC, dipalmitoylphosphatidyl-choline; EU, European Union; IARC, International Agency for Research on Cancer; J/m^2, joules per square meter; mg, milligrams; nm, nanometers; pBALF, porcine broncho-alveolar lavage fluid; PBS, phosphate-buffered saline; TiO_2, titanium dioxide; UFCB, ultrafine carbon black; $UFTiO_2$, ultrafine titanium dioxide

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