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Evaluation of dermal and eye irritation and skin sensitization due to carbon nanotubes

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ABSTRACT

The present paper summarizes the results of our studies on dermal and eye irritation and skin sensitization due to carbon nanotubes (CNTs), whose potential applications and uses are wide and varied, including CNT-enhanced plastics, electromagnetic interference/radio-frequency (EMI/RFI) shielding, antistatic material, flexible fibers and advanced polymers, medical and health applications, and scanning probe microscopy. Skin and eyes have the highest risk of exposure to nanomaterials, because deposition of nanomaterials to the surficial organs has the potential to be a major route of exposure during the manufacturing, use, and disposal of nanomaterials. Two products composed of single-walled carbon nanotubes (SWCNTs) and two products composed of multi-walled carbon nanotubes (MWCNTs) were tested regarding acute dermal and acute eye irritation using rabbits, and skin sensitization using guinea pigs. The concentrations of the CNTs in the substances were the maximum allowable for administration. The two products of SWCNTs and one of the products of MWCNTs were not irritants to the skin or eyes. The other product of MWCNTs caused very slight erythema at 24 h, but not at 72 h, after patch removal in the dermal irritation experiments and conjunctival redness and blood vessel hyperemia at 1 h, but not at 24 h, in eye irritation experiments. These findings showed that one product of MWCNTs was a very weak acute irritant to the skin and eyes. No products of SWCNTs and MWCNTs exhibited skin-sensitization effects. Our knowledge of the toxicological effects of CNTs is still limited. Further information is needed to clarify the potential for irritation and sensitization given the complex nature of CNTs.

1. Introduction

Nanomaterials are defined as small-scale substances that are less than 100 nm in at least one dimension (ISO, 2008), which exhibit physical, chemical and/or biological characteristics associated with a nanostructure (Oberdörster et al., 2005a). Humans have been exposed to airborne nanoparticles throughout evolution, but exposure has increased dramatically because of anthropogenic factors including combustion engines, power plants, and other sources of thermodegradation (Oberdörster et al., 2005b). The rapidly developing field of nanotechnology, which is creating materials with size-dependent properties, is likely to become another source of exposure to nanosubstances. These nanosubstances have an increased surface area:mass ratio thereby greatly enhancing their chemical/catalytic reactivity compared to normal-sized forms of the same substance. Nanomaterials are used in a variety of areas including advanced materials, electronics, magnetics and optoelectronics, biomedicine, pharmaceuticals, cosmetics, energy, and catalytic and environmental detection and monitoring (Penn et al., 2003; Liu, 2006).

Carbon nanotubes (CNTs) are an important new class of technological materials that have numerous novel and useful applications. CNTs are fiber-shaped substances that consist of graphite hexagonal-mesh planes (graphene sheet) present as a single-layer or as multi-layers with nest accumulation, and include single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). CNTs are regarded as nanomaterials because of their nanoscale diameter. CNTs have received much attention and are widely used in cutting-edge technologies due to their excellent physical–chemical properties. The potential applications and uses are broad and varied, including CNT-enhanced plastics, electromagnetic interference/radio-frequency (EMI/RFI) shielding, antistatic material, flexible fibers and advanced polymers, medical and health applications, and scanning probe microscopy (ENRHES, 2009). Widespread production and use have caused the release of increasing amounts of nanomaterials into the environment. The introduction of these novel materials into industry requires safety evaluations as well as an understanding of the potential impact on human health, because the unique properties and size of nanomaterials may also result in new health risks, which cannot be

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predicted from the toxicological effects of larger substances of the same composition (Murray et al., 2009). Despite growing concern over the potential risk that nanomaterials pose, there is a lack of information on their potential toxicity. There is a gap in knowledge between the increasing development and use of nanomaterials and the prediction of possible health risks. At present, information on the toxicological effects of CNTs is limited.

Surficial organs such as the skin and eyes have the highest risk of exposure to nanomaterials, because deposition of nanomaterials to surficial organs has the potential to be a major route of exposure during the manufacturing, use, and disposal of nanomaterials. Information on skin and eye irritation and sensitization is a fundamental part of the identification of hazardous chemicals. Studies on skin and eye irritation and skin sensitization are essential components of the minimum set of toxicity screening which provides a fundamental characterization of the potential hazards of nanomaterials (Warheit et al., 2007). Only a few reports are available on irritation regarding CNTs. Soot with a high content of SWCNTs (Huczko and Lange, 2001) and MWCNTs (Kishore et al., 2009) were not irritating. Information on the dermal and eye irritation and sensitization caused by CNTs is still lacking. In the present study, we carried out dermal and eye irritation experiments in rabbits and skin sensitization experiments in guinea pigs using two different products of SWCNTs and MWCNTs in compliance with OECD guidelines.

2. Materials and methods

The experiments were conducted in 2010 at Ina Research Inc. (Ina, Japan). The study design complied with OECD guidelines, but not in accordance with the principles for Good Laboratory Practice. All procedures involving the care and use of animals adhered to animal welfare regulations, "Partial Amendments to the Law for the Humane Treatment and Management of Animals (Law No. 68, June 22, 2005, Japan)", and the "Guidance for Animal Care and Use (revised on November 7, 2007)" of Ina Research Inc. and were in accordance with the protocol reviewed by the Institutional Animal Care and Use Committee (IACUC) of Ina Research Inc., which is fully accredited by AAALAC International (Accredited Unit No. 001107).

2.1. Test materials

Two different products of SWCNTs and MWCNTs were tested for dermal and eye irritation and skin sensitization. Physicochemical properties of Nikkiso-SWCNTs (N-SWCNTs), Super-growth SWCNTs (SG-SWCNTs), Nikkiso-MWCNTs (N-MWCNTs), and Mitsui product of MWCNTs (MWNT-7) were previously reported by Nakanishi (2011), Kobayashi et al. (in press) and Naya et al. (in press), Morimoto et al. (2011), and Kobayashi et al. (2010), respectively. In brief, N-SWCNTs were purchased from Nikkiso Co. Ltd. (Tokyo, Japan). N-SWCNTs, whose mean diameter was 1.8 nm and BET surface area was 878 m^2/g, contained 43,700 ppm iron, 56 ppm rubidium, 22 ppm zinc, 12 ppm gallium, 10 ppm copper, 9 ppm nickel, and 6 ppm lead. SG-SWCNTs were synthesized by a water-assisted chemical vapor method with iron as the catalyst at the National Institute of Advanced Industrial Science and Technology, Japan (AIST). SG-SWCNTs, with a mean diameter of 3.0 nm and BET surface area of 1064 m^2/g, contained 145 ppm iron, 103 ppm nickel, 34 ppm chromium, 15 ppm manganese, 12 ppm aluminum. N-MWCNTs were purchased from Nikkiso Co. Ltd. N-MWCNTs, whose mean diameter was 44 nm and BET surface area was 69 m^2/g, contained 176 ppm gallium, 80 ppm aluminum, 53 ppm iron, 16 ppm cadmium, and 0.5 ppm lithium10. MWNT-7 was purchased from Mitsui & Co. Ltd. (Tokyo, Japan). MWNT-7, with a mean diameter of 60 nm and BET surface area of 23 m^2/g, contained 3600 ppm iron, 14 ppm chromium, 6 ppm bismuth, and 4 ppm nickel.

Olive oil was used to prepare a paste for dermal application in irritation and sensitization experiments and liquid form for ocular application in eye irritation experiment, because the olive oil was highly refined (neutral, denatured, and free of antioxidants) (Said et al., 2007) and widely used as the negative control in these experiments (Stevens, 1967; Said et al., 2007).

2.2. Animals

Male Kbl:NZW rabbits and male Slc:Hartley guinea pigs were purchased from Kitayama Labes Co. Ltd. (Ina, Japan) and Japan SLC Inc. (Hamamatsu, Japan), respectively. Animals were housed individually in standard aluminum cages and acclimated to the laboratory for 6 days prior to the start of the experiment. Only animals found to be in good health were selected for use. The animals were reared on a diet (LRC4; Oriental Yeast Co. Ltd., Tokyo, Japan) and tap water *ad libitum*, and maintained in an air-conditioned room at 19.0–25.0 °C, with a relative humidity of 40–70%, a 12-h light (7:00–19:00)/dark (19:00–7:00) cycle, and ventilation of 15–21 air changes/h.

2.3. Dermal irritation experiment

CNTs were moistened with a minimum amount of olive oil to prepare a paste for dermal application. The CNT-paste contained 1% N-SWCNTs, SG-SWCNTs or N-MWCNTs, or 2% MWNT-7. This amount of CNT was the maximum not leading to overflow when patched and the sample fully coated the skin.

The dermal irritation experiment was performed according to OECD Guideline 404 "Acute Dermal Irritation/Corrosion" (OECD, 2002a). Only rabbits with healthy, intact skin were used (17 weeks of age, in the range of 2.7–3.6 kg in body weight). An area of about 10 × 15 cm on the back of each rabbit was made free of fur using electric clippers and an electric shaver 24 h prior to testing. The CNT-paste (0.5 g) was evenly spread on a lint cloth (2.5 × 2.5 cm), applied to the skin, and covered with a gauze patch, which was held in place with non-irritating elastic bandage (Silkytex, Alcare Co. Ltd., Tokyo Japan). The animals were fitted with Elizabethan collars during application of the patches. The patches were removed after a 4-h exposure period, and the remaining test articles on the application sites were wiped off using gauze soaked in lukewarm water (Water for Injection, JP, Otsuka Pharmaceutical Factory Inc., Tokyo, Japan). No dermal reactions were observed at 3 min and 1 and 4 h after patch removal. The test was repeated with two additional rabbits to confirm the initial findings, because the rabbit in the initial test did not exhibit any dermal reaction. This strategy was used for the test CNTs. At 1, 24, 48 and 72 h after removal of the patches, dermal responses were determined in accordance with OCED Guideline (2002a). For each animal, dermal response scores (sum of the scores for erythema and eschar formation and edema formation) at 1, 24, 48 and 72 h after removal of the patches were summed and then divided by three to obtain a mean irritation score per time point. The mean scores at 24, 48, and 72 h were summed and averaged to obtain the primary irritation index (PII).

2.4. Eye irritation experiment

CNTs were mixed with a minimum amount of olive oil to prepare a test material available in liquid form for ocular instillation. The test material contained 0.1% N-SWCNTs, 0.5% SG-SWCNTs, 0.25% N-MWCNTs, or 1% MWNT-7. The amount of CNT was the maximum resulting in good contact and uniform distribution to the eye, but no overflow, when instilled into the conjunctival sac.

The eye irritation experiment was conducted in accordance to the OECD Guideline 405 "Acute Eye Irritation/Corrosion" (OECD, 2002b). Only rabbits with no abnormalities in the anterior eye