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Acute and subchronic oral toxicity studies in rats with nanoscale and pigment grade titanium dioxide particles

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ABSTRACT

Data generated using standardized testing protocols for toxicity studies generally provide reproducible and reliable results for establishing safe levels and formulating risk assessments. The findings of three OECD guideline-type oral toxicity studies of different duration in rats are summarized in this publication; each study evaluated different titanium dioxide (TiO_2) particles of varying sizes and surface coatings. Moreover, each study finding demonstrated an absence of any TiO_2 -related hazards. To briefly summarize the findings: 1) In a subchronic 90-day study (OECD TG 408), groups of young adult male and female rats were dosed with rutile-type, surface-coated pigment-grade TiO_2 test particles (d_{50} = 145 nm – 21% nanoparticles by particle number criteria) by oral gavage for 90 days. The no-adverse-effect level (NOAEL) for both male and female rats in this study was 1000 mg/kg bw/day, the highest dose tested. The NOAEL was determined based on a lack of TiO_2 particle-related adverse effects on any in-life, clinical pathology, or anatomic/microscopic pathology parameters; 2) In a 28-day repeated-dose oral toxicity study (OECD TG 407), groups of young adult male rats were administered daily doses of two rutile-type, uncoated, pigment-grade TiO_2 test particles (d_{50} = 173 nm by number) by daily oral gavage at a dose of 24,000 mg/kg bw/day. There were no adverse effects measured during or following the end of the exposure period; and the NOAEL was determined to be 24,000 mg/kg bw/day; 3) In an acute oral toxicity study (OECD TG 425), female rats were administered a single oral exposure of surface-treated rutile/anatase nanoscale TiO_2 particles (d_{50} = 73 nm by number) with doses up to 5000 mg/kg and evaluated over a 14-day post-exposure period. Under the conditions of this study, the oral LD_{50} for the test substance was >5000 mg/kg bw. In summary, the results from these three toxicity studies – each with different TiO_2 particulate-types, demonstrated an absence of adverse toxicological effects. Apart from reporting the findings of these three studies, this publication also focuses on additional critical issues associated with particle and nanotoxicology studies. First, describing the detailed methodology requirements and rigor upon which the standardized OECD 408 guideline subchronic oral toxicity studies are conducted. Moreover, an attempt is made to reconcile the complex issue of particle size distribution as it relates to measurements of nanoscale and pigment-grade TiO_2 particles. Clearly this has been a confusing issue and often misrepresented in the media and the scientific literature. It is clear that the particle-size distribution for pigment-grade TiO_2, contains a small ("tail") component of nanoscale particles (i.e., 21% by particle number and <1% by weight in the test material used in the 90-day study). However, this robust particle characterization finding should not be confused with mislabeling the test materials as exclusively in the nanoscale range. Moreover, based upon the findings presented herein, there appears to be no significant oral toxicity impact contributed by the nanoscale component of the TiO_2 Test Material sample in the 90-day study. Finally, it seems reasonable to conclude that the study findings should be considered for read-across purposes to food-grade TiO_2 particles (e.g., E171), as the physicochemical characteristics are quite similar.

1. Introduction

Numerous non-guideline studies have been published with the intention of characterizing the mammalian toxicity of titanium

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dioxide particles. While *ad hoc* experimental-type study results may provide some value in investigating a potential mechanism of toxicity, or in postulating a time course of pathological development following exposures, it arguably is the standardized studies following test guidelines (TG) adopted by the Organization for Economic Cooperation and Development (OECD) that provide the most comprehensive findings, transparency and reproducibility of the toxicity results of any tested material. The data generated from these types of well-conducted, standardized studies are commonly used by regulatory agencies and internally by industry to establish safe acceptable exposure limits for their workers.

Another issue which pertains to the relevance of safety studies is the source, synthesis and application of the test material being utilized in the study. For example, in a number of studies, investigators have synthesized forms of nanoscale titanium dioxide in the laboratory which have little relevance, if any, to commercialized forms of TiO_2 to which humans may be exposed (Gao et al., 2012, 2013).

The objective of the studies in this publication were to evaluate the toxicity of characteristic commercial TiO_2 particles in rats following acute to subchronic oral exposures. The results of three independent studies summarized in this paper include a subchronic 90-day oral toxicity study in male and female rats, investigating the hazards of pigment-grade (i.e., fine sized) titanium dioxide particles containing a small tail component (i.e., ~21% by number) of ultrafine or nanoscale particles; contained within the particle size distribution. The second study consisted of a 28-day repeated dose oral toxicity study in male rats with two slightly larger pigment-grade TiO_2 test particles with approximately 11% nanoscale material by number; and the third study represented an acute oral toxicity study in female rats following single exposures to an ultrafine TiO_2 grade that would be considered to be a nanomaterial in the TiO_2 industry (TDMA and TDSC, 2013). Taken together, the findings demonstrate the low toxicity of fine-sized or nanoscale TiO_2 particles following oral exposures.

Regulatory scrutiny and scientific curiosity regarding the potential for different biological effects from nanomaterials has led to a plethora of research in the area of nanotoxicology. Although a substantial portion of this research has led to negative results, these publications are infrequently published due to the lack of a "Wow" factor. The absence of this information has led to the perception that nanoscale size alone is associated with hazard. This coupled with a general lack of appreciation of the difference between number and mass percent content in particulate systems has complicated public interpretations of the potential hazards associated with nanomaterials as well as with materials that only contain trace amounts of nanomaterials. Recently, the Dunkin' Donuts Company was pressured to phase out its whitening agent in the powdered sugar formulation for some of its doughnut products. As You Sow, a Non-Governmental Organization (NGO), had commissioned independent laboratory tests of certain Dunkin' Donuts products and other types of powdered donuts and concluded that they contained a substantial proportion of titanium dioxide nanomaterials. Moreover, this NGO had decided that the nanomaterial component of the formulation posed substantial toxicity for human health when compared to the larger, TiO_2 pigment-grade particle size. Unfortunately, the conclusions of the As You Sow organization were not based upon any reliable hazard data or particle size conclusions. Furthermore, this NGO failed to recognize that the commercial forms of titanium dioxide particles utilized in food applications are composed almost entirely of the pigment-grade variety, (i.e., mean particle sizes greater than >100 nm); as these TiO_2 particle forms have a high refractive index feature resulting in the properties of brightness along with a natural white coloring. These "opacifier" characteristics serve to promote effective (white) pigment qualities and color optimization; properties which are important in applications such as paints, papers, plastics, coatings, along with food items and toothpaste products. The desired light scattering by TiO_2 particulates occurs preferentially in the 200 nm–300 nm particle size range (Braun, 1997). This is the reason why nanoscale TiO_2 particles (i.e., mean particle sizes < 100 nm) are not utilized for these applications. Similar to the TiO_2 particles used in food-grade applications, the pigment-grade titanium dioxide sample evaluated in the 90-day subchronic oral toxicity study was of a similar particle size range (with a d_{50} of 223 nm by mass and 173 nm by number), containing 21% nanoparticles (defined as < 100 nm). However, it should be noted that the 21% represents a particle number value but that the nanoscale component represents <1% of the mass of the particle size distribution. In addition, 90-day exposures to 1000 mg/kg bw/day in rats resulted in no adverse effects at the highest dose tested in this study. Unfortunately, the health-based arguments made by As You Sow to pressure Dunkin Donuts were specious and imprudent Odom (2015).

However, this example illustrates a broader issue of unintended public perception bias which can occur, ironically, as a result of proactive and concerted attempts by policy makers, industry and academia to identify potential hazards in new science areas to preempt unintended human health consequences. The field of nanotoxicology needs to address this issue and further provide comparative context for the relative hazards of nanomaterials versus more traditional chemical and natural materials.

2. Materials and methods

2.1. Identification of materials utilized in the three studies

Four commercial titanium dioxide samples synthesized via the chloride process (http://www.essentialchemicalindustry.org/chemicals/titanium-dioxide.html) were evaluated. Test particle sample No. 1 was assessed in a subchronic 90-oral toxicity study, and consisted of a pigmentary, rutile-type titanium dioxide particulate sample with an alumina surface coating (see Fig. 1). In addition, in a 28-day repeated dose study, the hazards of two nearly identical test samples were evaluated. This study was designed to test whether differences in process variability due to impurities in the ores used to produce TiO_2 particulates resulted in any differences in toxicity. These samples were identified as uncoated, rutile-type pigmentary TiO_2 samples (see Fig. 2). The two samples were

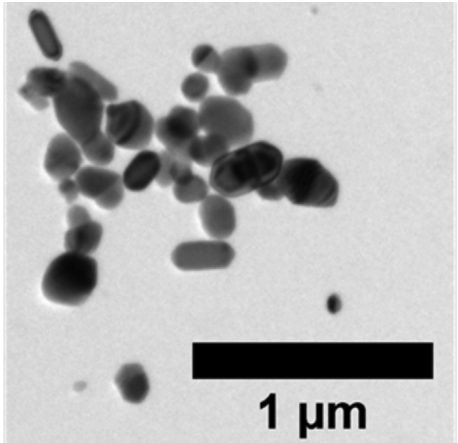

Fig. 1. Low magnification – transmission electron micrograph of Rutile-type, pigment-grade TiO_2 Test Sample No. 1 – surface-coated sample.