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# Development of linear and threshold no significant risk levels for inhalation exposure to titanium dioxide using systematic review and mode of action considerations

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ABSTRACT

Titanium dioxide ($TiO_2$) has been characterized as a poorly soluble particulate (PSP) with low toxicity. It is well accepted that low toxicity PSPs such as $TiO_2$ induce lung tumors in rats when deposition overwhelms particle clearance mechanisms. Despite the sensitivity of rats to PSPs and questionable relevance of PSP-induced tumors to humans, $TiO_2$ is listed as a possible human carcinogen by some agencies and regulators. Thus, environmental toxicity criteria for $TiO_2$ are needed for stakeholders to evaluate potential risks from environmental exposure and regulatory compliance. A systematic review of the literature was conducted to characterize the available data and identify candidate datasets upon which toxicity values could be derived. Key to this assessment, a survey of mechanistic data relevant for lung cancer was used to support quantitative inhalation risk assessment approaches. A total of 473 human studies were identified, 7 of which were epidemiological studies that met inclusion criteria to quantitatively characterize carcinogenic endpoints in humans. None of these studies supported derivation of toxicity criteria; therefore, animal data were used to derived safety values for $TiO_2$ using different dosemetrics (regional deposited dose ratios, $TiO_2$ particle surface area lung burden, and volumetric overload of alveolar macrophages), benchmark dose modeling, and different low-dose extrapolation approaches. Based on empirical evidence and mechanistic support for nonlinear mode of action involving particle overload, chronic inflammation and cell proliferation, a no significant risk level (NSRL) of 300 μg/day was derived. By comparison, low-dose linear extrapolation from tumor incidence in the rat lung resulted in an NSRL value of 44 μg/day. These toxicity values should be useful for stakeholders interested in assessing risks from environmental exposure to respirable $TiO_2$.



## 1. Introduction

Titanium (Ti) is an abundant metal in the earth crust; however, it is not found in its free, pure metal form in nature but exists primarily as titanium dioxide ($TiO_2$) or as ilmenite ($FeTiO_3$) (Leyens and Peters, 2006). $TiO_2$ exists as one of three mineral crystal structures: anatase, rutile, and brookite (the least common) (Warheit, 2013). The rutile crystal structure is often associated with fine particle sizes (typically in the μm size range), whereas the anatase crystal structure is generally associated with smaller ultrafine particles that are often more chemically reactive than rutile crystals (Shi et al., 2013; Warheit and Donner, 2015). There are nanoparticles many definitions of ultrafine or nanoparticles, but are generally defined as having at least one dimension that is 1–100 nm (Nazarenko et al., 2015).

Titanium enriched metal alloys are used in the construction of aircraft parts, electronics, military equipment, and medical devices (Leyens and Peters, 2006). During the production process, metal dusts including $TiO_2$ may be generated and released to ambient air. $TiO_2$ is also used in pigment production; such pigments are in

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numerous consumer products including paints, cosmetics, plastic, and food as whitening agents (NIOSH, 2011). Pigment-grade $TiO_2$ is usually found as fine particles (<2.5 μm), often ranging from 200 to 300 nm in diameter, and commonly as aggregates and agglomerates rather than discrete particles (Baan, 2007; IARC, 2010; Warheit and Donner, 2015). Primary ultrafine (nanoscale) $TiO_2$ particles typically range from 10 and 50 nm, and agglomerate to particles <150 nm in diameter (Baan, 2007; IARC, 2010). Nanoscale $TiO_2$ particles are not typically used in whitening applications because they lack the light scattering properties of larger sized particles; however, nanoscale $TiO_2$ particles are more reactive and serve as catalysts and scavengers of UV light (Warheit and Donner, 2015; Warheit et al., 2007).

Because various consumer products contain $TiO_2$, environmental exposure among the general population is expected, albeit at lower levels compared to worker populations. Oral ingestion of pigment-grade $TiO_2$ can occur from additives in foods and toothpaste, whereas exposure to fine and ultrafine $TiO_2$ could come from inhalation exposures as a result of swallowing and mucocilliary clearance from lungs (Nazarenko et al., 2015; Shi et al., 2013). As fine and ultrafine $TiO_2$ can be found in many topical skin products, there is also potential for dermal exposure. However, most studies suggest that $TiO_2$ does not readily penetrate beyond the epidermis, and there is relatively little absorption from the gastrointestinal tract (Shi et al., 2013; Warheit and Donner, 2015), and thus these exposure routes have not proven to be of toxicological concern (IARC, 2010). As such, inhalation is the primary route of $TiO_2$ exposure.

Inhalation exposure to $TiO_2$ results in lung tumors in rats (Heinrich et al., 1995; Lee et al., 1985; Muhle et al., 1991). In 2010, citing sufficient evidence in experimental animals and inadequate evidence from epidemiologic studies, the International Agency for Research on Cancer (IARC) characterized $TiO_2$ as "possibly carcinogenic to humans (Group 2B)" (IARC, 2010). As a result, the Office of Environmental Health Hazard Assessment (OEHHA) within the California Environmental Protection Agency added airborne, unbound $TiO_2$ particles of respirable size to the list of chemicals known to the State of California to cause cancer under Proposition 65; however, no safe harbor level or No Significant Risk Level (NSRL) has been set by OEHHA (OEHHA, 2011). Compliance with Proposition 65 in the case of $TiO_2$ exposures necessitates the development of a NSRL, even if risk to humans is low. The National Institute for Occupational Safety and Health (NIOSH) has developed occupational standards for $TiO_2$ based on the occurrence of $TiO_2$-induced lung tumors in rats. However, no environmental standards have been proposed for which stakeholders can assess the potential impact of non-occupational exposure.

Of particular interest to the development of toxicity values (e.g. NSRL) for $TiO_2$ is the availability of human epidemiological data and mechanistic information regarding carcinogenesis in rats. Currently, there are no data available to characterize environmental exposures to $TiO_2$, and information on occupational $TiO_2$ exposures are mostly related to the production of pigment-grade (fine) $TiO_2$. Between 1970 and 2000, occupational exposures to fine $TiO_2$ in United States and Europe were reported to be highest among manufacturing workers in jobs of packing, milling, site cleaning, and maintenance (<1 to 5 $mg/m^3$) (Sleeuwenhoek, 2005). Workers in pigment $TiO_2$ manufacturing may also be exposed to ore, other dusts, and asbestos (IARC, 2010). No data are currently available for characterizing worker exposures to ultrafine $TiO_2$ particles.

With regard to mechanistic information, it is well-recognized that $TiO_2$ is a poorly soluble particle (PSP), and that PSPs are known to induce pulmonary toxicity and lung cancer in rats by particle overload leading to chronic inflammation and cell proliferation (ECETOC, 2013; IARC, 2010; ILSI, 2000). The European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC, 2013) has published an adverse outcome pathway (AOP) for PSPs, where the molecular initiating event (MIE) is described as impairment of pulmonary particle clearance. The relevance of this AOP for humans is uncertain, as rats are known to be more sensitive to the effects of PSPs compared to other species (ECETOC, 2013; ILSI, 2000). Moreover, $TiO_2$ has not shown clear evidence of increased lung cancer among workers (IARC, 2010; NIOSH, 2011).

While particle overload is strongly implicated in fine $TiO_2$-induced lung tumors in rats, the toxicity of nanoparticles, including $TiO_2$, is more uncertain. Although nanoparticles are generally considered more potent toxicants, there are several studies that indicate otherwise (Grassian et al., 2007; Warheit et al., 2006, 2007). Moreover, many toxicity studies employ relatively pure nanoparticles, whereas consumers are typically exposed to products (i.e. mixtures) that may contain some fraction of nanomaterial along with many other ingredients (Nazarenko et al., 2014, 2012a, 2012b). It is known, however, that nanoparticles tend to agglomerate and aggregate to decrease the surface to volume ratio and decrease the free energy of the system (Simakov et al., 2007). Similarly, ultrafine $TiO_2$ also has a tendency to aggregate or agglomerate into particles that exceed nanoscale (EPA, 2010; Warheit and Donner, 2015). For example, one study with three different ultrafine $TiO_2$ samples reported that the median diameters were >100 nm in water and >2.5 μm in phosphate buffered saline (Warheit et al., 2007). Recent studies with marketed cosmetic and spray products containing nanomaterials (some including $TiO_2$), found that nanoparticles within the products tend to agglomerate, thereby lessening exposure to ultrafine material (Nazarenko et al., 2014, 2012a, 2012b). Moreover, these studies also predicted that the vast majority of product deposition occurred in the head airways as opposed to alveolar regions of the lung (Nazarenko et al., 2012a, 2014). It is also notable that some products marketed as containing nanoparticles were not found to have any nanoscale particles, whereas some products not marketed as containing nanomaterials clearly contained nanoscale materials. These authors have concluded that toxicity findings in studies of pure nanoscale materials likely differ from potential toxicity in real world consumer products (i.e. environmental exposure) where nanoscale products agglomerate and deposit differently within airways.

The objective of this study was to derive NSRL values for $TiO_2$ that stakeholders can use to evaluate compliance. A systematic review of the literature was conducted in order to characterize available human and animal data, and to support the selection of a dataset(s) to be utilized in the development of NSRL values. Key to this assessment, known mechanistic data and mode of action (MOA) and AOP information were used to support approaches for the development of inhalation toxicity criteria for environmental exposure to $TiO_2$. As such, both nonlinear and linear (default) low-dose extrapolation approaches were investigated. The toxicity criteria developed herein were informed by United States Environmental Protection Agency (EPA) risk assessment practices and modeling approaches implemented by Dankovic et al. (2007) and NIOSH (2011). These toxicity criteria should be useful in the quantitative risk assessment of potential carcinogenic risk from exposure to $TiO_2$.

## 2. Methods

### 2.1. Systematic literature review and identification of candidate datasets

A literature search was conducted to identify datasets that could serve as the basis of toxicity values associated with carcinogenicity