APPENDIX 1: NanoRiskCat●●●|●● Template

Literature methodology/sources of information

The following sources of information were used to fill out the NanoRiskCat●●●|●● for nanoTiO2:

1. Stone V, Hankin S, Aitken R, Aschberger K, Baun A, Christensen F, Fernandes T, Hansen SF, Hartmann NB, Hutchinson G, Johnston H, Micheletti G, Peters S, Ross B, Sokull-Kluettgen B, Stark D, Tran L. 2009. Engineered Nanoparticles: Review of Health and Environmental Safety (ENRHES). Available at: http://ihcp.jrc.ec.europa.eu/whats-new/enhres-final-report (Accessed July 15, 2010)

2. Shinohara, N., Nakamishi, J., Gamo, M. 2009. Risk Assessment of Manufactured Nanomaterials –TiO2. Available: http://www.aist-riss.jp/main/modules/product/nano_rad.html?ml_lang=en (Accessed July 15, 2010)

3. NIOSH, 2011, *Current Intelligence Bulletin 63 Occupational Exposure To Titanium Dioxide*, Department Of Health And Human Services Centers For Disease Control And Prevention National Institute For Occupational Safety And Health, Washington, D.C. (Accessed July 15, 2010)

4. Regulation (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 (available: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:353:0001:1355:EN:PDF (Accessed March 25, 2012)

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Human hazard profile

1. **HARN: Does the nanomaterial fulfill the HARN paradigm?**

   Answer: No

   Arguments and explanation: Although various forms and shapes exist of nanoTiO2-particles these are not normally associated with HARN

2. **Bulk – "Level A CLP": Is the bulk form of the nanomaterial known to cause or may cause serious damaging effects?**

   Answer: No

   Arguments and explanation: TiO2 is not classified in the Annex VI of Regulation (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006

3. **Bulk – "Level B CLP": Is the bulk form of the nanomaterial classified for other less adverse effects according to the CLP?**

   Answer: No

   Arguments and explanation: TiO2 is not classified in the Annex VI of Regulation (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006

4. **Nano – Acute toxicity: Is the specific nanomaterial known to be acute toxic?**

   Answer: No

   Arguments and explanation: According to Stone *et al.* (2009) no in vivo studies have

been identified in regard oral and dermal acute toxicity. In regard to inhalation toxicity, several authors have shown that TiO2 nanoparticles (with a size in the range of about 20-30 nm) is considerably more toxic than its micro- TiO2 (> 100nm) counterpart (see e.g. Ferin *et al.* 1992; Renwick *et al.* 2004; Chen *et al.* 2006; Inooue *et al.* 2008 cited in Stone et al. (2009)). After having exposed 2 times 10 mice to nanoTiO2 via intraperitional injection, Chen *et al.* (2006) reported observing that a total of five mice died after exposure to 1944 and 2592 mg/kg, respectively. From this can be derived that the acute toxicity estimates are > 5 mg/l.

5. **Are there indications that the nanomaterial causes genotoxic-, mutagenic-, carcinogenic-, respiratory-, cardiovascular, neurotoxic, reproductive effects or carcinogenicity in humans and/or laboratory animals or has organ-specific accumulation been documented?**

   Answer: Yes

   Arguments and explanation:

   a. **Genotoxicity and mutagenicity:** According to Stone *et al.* (2009) "TiO2 *nanoparticles are not expected to cause direct mutagenicity/genotoxicity (although further testing may be needed to fully confirm this), but may trigger genotoxicity via an indirect threshold driven mechanism involving oxidative stress.*"

   b. **Respiratory tract toxicity:** According to Stone *et al.* (2009) several authors have shown that TiO2 nanoparticles (with a size in the range of about 20-30 nm) is considerably more toxic than its micro- TiO2 (> 100nm) counterpart (see e.g. Ferin *et al.* 1992; Renwick *et al.* 2004; Chen *et al.* 2006; Inooue *et al.* 2008). Most studies identified used a single dose of particles, administered via intratracheal instillation and toxicity observed included: pulmonary inflammatory response (characterised by neutrophil and macrophage infiltration) (Ferin *et al.* 1992; Chen *et al.* 2006; Warheit *et al.* 2007; Inoue *et al.* 2008; Renwick *et al.* 2004; Grassian *et al.* 2007); epithelial damage, increased permeability of the lung epithelium, and cytotoxicity, which were measured within the bronchoalveolar lavage fluid (BALF) (Renwick *et al.* 2004); and morphological alteration within the lung (Chen *et al.* 2006). Finally, Ahn *et al.* (2005) using a high dose (4 mg kg-1) investigated what processes were responsible for particulate mediated stimulation of excessive mucus secretion within humans. TiO2 exposure stimulated an increase in goblet cell hyperplasia, which is, in part, attributed to an increase in muc5 gene expression and IL-13

production. Therefore, it could be speculated that particle mediated increases in mucus secretion contributed to the aggravation of chronic airway disease symptoms within humans, and therefore warrants further investigation. Grassian *et al.* (2007) investigated the toxicity of TiO2 nanoparticles (5 and 21 nm) within mice, subsequent to inhalation (0.7 or 7 mg m-3, for 4 hours) or nasal instillation (up to 150 µg per 50 µl). An elevated macrophage population was associated with the inhalation of particles (4 and 24 hours post exposure), and were observed to internalise particles. An infiltration of neutrophils was associated with the nasal instillation of TiO2. Several authors suggested that the response subsequent to TiO2 exposure was dose driven (e.g. Chen *et al.* 2006; Renwick *et al.* 2004). In the Renwick *et al.* (2004) study, no toxicity was seen at 125 µg per rat (corresponding to 0.5 µg kg-1 assuming a rat weight of 250 g), whereas toxicity was seen at the high dose of 500 µg per rat (particle size 29nm). Chen *et al.* (2006) exposed mice and found toxicity (inflammation and histological changes in the lung) at the lowest dose of 100 µg per mouse (corresponding to 33 µg kg-1 assuming a mouse weight of 30 g) (particle size 19-21 nm). Although the Chen *et al.* (2006) study does not indicate a no effect level, it seems justified (assuming the rat is more sensitive) to estimate, a No Observed Adverse Effect Level (NOAEL) of 125 µg per rat (corresponding to 0.5 µg kg-1). The crystallinity of TiO2 nanoparticles is thought to influence the toxicity with the anatase form expected to be more toxic that the rutile form (Warheit *et al.* 2007).

c. **Cardiovascular toxicity:** According to Stone *et al.* (2009) "*Helfenstein et al. (2008) showed that* TiO2 *nanoparticles were able to affect cardiomyocyte electrophysiology, enhance ROS production, and reduce myofibril organisation, whereas Peters et al. (2004) found* TiO2 *relatively low-toxic to HDMEC endothelial microvascular cells (with minimal IL-8 release).*"

d. **Neurotoxicity:** Long *et al.* (2006, 2007) indicates that TiO2 nanoparticles caused a ROS driven toxicity to some types of cells of the CNS in vitro. According to Stone *et al.* (2009) *"Wang et al. (2008a) investigated the distribution of rutile (80 nm) and anatase (155 nm) TiO2 particles within the mouse brain, following nasal instillation exposure (500 µg per mouse, every other day for a total of 30 days) and determined if any neurotoxicity associated with exposure. Both forms of TiO2 were able to access the brain, with accumulation within the cerebral cortex, thalamus and hippocampus evident, and was postulated to occur via the olfactory bulb. This route of uptake however, was unlikely to be mediated via penetration into the cardiovascular system and via the blood. Instead, TiO2*

delivery to the brain occurred via neuronal transport, with preferential localisation evident within the hippocampus and olfactory bulb. Accumulation of TiO2 resulted in morphological alterations and loss of neurones in the hippocampus, which was accounted for by the higher distribution of TiO2 within this brain region. In addition it was suggested that TiO2 elicited oxidative stress within the brain due to the elevation of superoxide dismutase (SOD), and catalase activity, and evidence of increased lipid peroxidation and protein oxidation. Therefore neuronal mediated translocation of TiO2 to the brain, following nasal instillation, was observed, with the hippocampus illustrated as being the main target of accumulation and toxicity. Wang et al. (2008b) expanded upon these findings and found that the phenomenon was time dependent (was maximal at 30 days), and that an inflammatory response (indicated by IL-1β, and TNFα) within the brain was also stimulated by TiO2 exposure. The response was measured at day 2, 10, 20, and 30. It was apparent that repeated exposures, over a period of 30 days, were required to enable the accumulation of TiO2 within the brain. It is therefore of interest that the neuronal transport of nanoparticle containing substances between the nose and CNS could be exploited, in order to bypass the blood brain barrier".

e. **Reproductive damage:** Komatsu *et al.* (2008) has shown that TiO2 nanoparticles are taken up by and affect viability, proliferation and gene expression of Leydig cells (testosterone producing cells of the testis) in vitro, whereas one in vitro study suggests that TiO2 nanoparticles may be toxic towards Leydig cells. However, given the toxico-kinetics, it can be questioned whether TiO2 can indeed reach these cells. No studies investigating female fertility were identified. Overall, no conclusion can be drawn (Stone *et al.* 2009). No information has been identified on developmental toxicity and hence and no conclusion can be drawn.

f. **Carcinogenicity:** One study has described finding tumour following chronic inhalation after repeated exposure (Heinrich *et al.* 1995). The study used very high doses and had a long duration (high death in the control group). NIOSH (2005) concluded, based on those data that TiO2 is carcinogenic in rats and that it cannot be excluded to be carcinogenic in humans. It is expected that carcinogenicity occurs following pulmonary overload and thus has a threshold (Stone *et al.* 2009). It should be noted that also the International Agency for Research on Cancer have assessed TiO2 (even the microform – if exposure is high enough) to be a Class 2B carcinogen (Possibly carcinogenic to humans) (IARC 2006).

g. Does the nanomaterial accumulate in tissue and/or organs: As noted by Stone *et al.* (2009) there is limited evidence in regard to whether TiO2 accumulate in tissue and/or organs. According to Stone *et al.* (2009) *"Fabian et al. (2008) determined the tissue distribution of TiO2 nanoparticles (20-30 nm) within rats, at 1, 14 and 28 days post exposure, via intravenous injection (5 mg kg-1). TiO2 was cleared from the blood and primarily accumulated within the liver, but was also apparent within the spleen, lungs and kidneys. The level of TiO2 was retained over the observation time within the liver, however levels decreased with time within the other organs. No serum cytokine or enzyme changes, which insinuated that no toxicity was associated with TiO2 exposure, however further investigations, including histopathological analysis would be necessary to confirm this. Wang et al. (2008a) investigated the distribution of rutile (80 nm) and anatase (155 nm) TiO2 particles within the mouse brain, following nasal instillation exposure (500 µg per mouse, every other day for a total of 30 days) and determined if any neurotoxicity associated with exposure. Both forms of TiO2 were able to access the brain, with accumulation within the cerebral cortex, thalamus and hippocampus evident, and was postulated to occur via the olfactory bulb."*

6. **Overall evaluation of human hazard**

We conclude that the color-code that best reflects the human hazard profile of nanoTiO2 is ● based on the following considerations:

1. The widely reported respiratory damage caused by nanoTiO2
2. NanoTiO2 has been associated with carcinogenic-, cardiovascular and neurotoxic and reproductive damage.

Environment hazard profile

1. **Bulk – "Level A CLP": Is the bulk form of the nanomaterial classified as CLP Acute 1 or Chronic 1 or Chronic 2?**

Answer: No

Arguments and explanation: TiO2 is not classified in the Annex VI of Regulation (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006

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2. **Nano – LC_{50}<10 mg/l: Is the nanomaterial in question reported to be hazardous to environmental species i.e. LC50 or EC 50 <10 mg/l?**

   Answer: Yes

   Arguments and explanation: Following U.S. Environmental Protection Agency (2002) standard protocol, Zhu *et al.* (2008) reported deriving an LC50,72h of 2.02 mgl-1 for nanoTiO2 on the crustacean Daphnia magna.

3. **Overall evaluation of environmental hazard**

   We conclude that the color-code that best reflects the environmental hazard profile of nanoTiO2 is ● based on the fact that nanoTiO2 has been reported to be hazardous to environmental species i.e. LC50 or EC50 <10 mg/l.