APPENDIX 1: NanoRiskCat●●●|●● Template

Literature methodology/sources of information

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

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. Dufour, E. K., Kumaravel, T., Nohynek, G. J., Kirkland, D. & Toutain, H. 2006. Clastogenicity, photo-clastogenicity or pseudo-photo-clastogenicity: Genotoxic effects of zinc oxide in the dark, in pre-irradiated or simultaneously irradiated Chinese hamster ovary cells. Mutation Research 607 (2): 215-224.
3. Sayes, C. M., Reed, K. L. & Warheit, D. B. 2007. Assessing toxicity of fine and nanoparticles: comparing in vitro measurements to *in vivo* pulmonary toxicity profiles. Toxicological Sciences 97(1): 163-180.
4. Gojova, A., Guo, B., Kota, R. S., Rutledge, J. C., Kennedy, I. M. & Barakat, A. I. 2007. Induction of inflammation in vascular endothelial cells by metal oxide nanoparticles: effect of particle composition. Environmental Health Perspectives 115(3): 403-409.

Human hazard profile

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

   Answer: No

   Arguments and explanation: To the best of our knowledge, nanoZnO particles do not fulfill 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: ZnO 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: ZnO 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: Unknown

   Arguments and explanation: Not known as the nanomaterial used is not reported

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

   Answer: Yes

   Arguments and explanation:

   a. Genotoxicity and mutagenicity: Dufour *et al.* (2006) has investigated the genotoxicity of ZnO in concentrations up to 500 µg ml^{-1} within CHO cells and it was found that ZnO nanoparticles elicited a dose dependent decrease in cell viability, with prior irradiation being observed to enhance ZnO mediated cytotoxicity. ZnO caused an increase in

chromosome structure aberrations, which was most pronounced within irradiated cells (Stone et al. 2010). Karlsson *et al.* (2008) furthermore found that ZnO decreased cell viability and induced oxidative stress and DNA damage within A549 lung epithelial cells, at concentrations of up to 80 µg ml^{-1} for a period of up to 18 hours.

b. Respiratory tract toxicity: Sayes *et al*. (2007) exposed rats to nanoparticulate (50-70nm) or microparticulate (<1000nm) via a single intratracheal instillation (1 or 5mg/kg), and from 24 hours to 3 months post exposure BALF was collected. A short term, potent pulmonary inflammatory response characterised by a neutrophil infiltration (Sayes et al. 2007; Stone et al. 2010).

c. Cardiovascular toxicity: Gojova *et al.* (2007) exposed HAEC endothelial cells to ZnO (20-70nm) nanoparticles *in vitro* in concentrations up to 50 µg ml^{-1} for 1 to 8 hours and found ZnO stimulated a pronounced pro-inflammatory response as well as cytotoxicity.

d. Neurotoxicity: No information available

Reproductive damage: No information available

e. Carcinogenicity: No information available

f. Does the nanomaterial accumulate in tissue and/or organs?:

No information available

6. Overall evaluation of human hazard

We conclude that the color-code that best reflects the human hazard profile of the nanomaterial used is ● based on *in vivo* and *in vivo* evidence of a combination of hazards from testing of the nanomaterial

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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: Yes

   Arguments and explanation: ZnO is classified as Aquatic Acute 1 as well as Aquatic Chronic 1 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

2. **Overall evaluation of environmental hazard**

   We conclude that the color-code that best reflects the environmental hazard profile of nanoZnO is ● based on bulk CLP classification of Acute 1 or Chronic 1 or Chronic 2