APPENDIX 1: NanoRiskCat●●●|●● Template

Literature methodology/sources of information

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

1. 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)

2. 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)

3. Luoma, S. N. (2008): Silver Nanotechnologies and the Environment Old Problems or New Challenges? PEN 15. Washington, DC: Project on Emerging Nanotechnologies, Woodrow Wilson International Center for Scholars.

4. Pronk, M. E. J.; Wijnhoven, S. W. P.; Bleeker, E. A. J.; Heugens, E. H. W.; Peijnenburg, W. J. G. M.; Luttik, R.; Hakkert, B. C. (2009): Nanomaterials under REACH: Nanosilver as a case study. National Institute for Public Health and the Environment, Bilthoven, the Netherlands

5. Nanowerk (2010): Nanomaterial Database Search. http://www.nanowerk.com/phpscripts/n_dbsearch.php (Accessed 5 November, 2010).

6. Sanford, J., Venkatapathy, R., El-Badawy, A., Feldhake, D. 2010. State of the Science Literature Review: Everything Nanosilver and More. Washington, DC: U.S. Environmental Protection Agency Office of Research and Development Available: http://www.epa.gov/nanoscience/files/NanoPaper1.pdf (Accessed 08-04-2012)

7. Mikkelsen, S.H., Hansen, E., Christensen, T.B., Baun, A., Hansen, S.F., Binderup, M-L. 2011. Survey on basic knowledge about exposure and potential environmental and health risks for selected nanomaterials. Environmental Project No. 1370 2011. Copenhagen: Danish Ministry of the Environment. Danish Environmental Protection Agency

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Human hazard profile

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

   Answer: No

   Arguments and explanation: Specific information about the size and shape of the nanosilver particles used in this product is not available however it is assumed that the nanosilver particles used in this products do not fulfils HARN. Nanosilver is characterized by being spherical particles of a size ranging from 1-250 nm and is commercialized as powder, flakes, grains, ingots, etc., and is sold in suspension (in water, alcohol or surfactant) and as a dry powder. Nanosilver is available in different sizes and shape spherical, rod-shaped, truncated triangular nanoplates (Luoma *et al.,* 2007; Nanowerk, 2010; Pronk *et al.,* 2009, Mikkelsen et al. 2011), but according to the US EPA (2010): "The most produced shape is spherical with a size of less than 20 nm." and hence most produced nanosilver particles do have a aspect ratio higher than 10:1 and do not fulfil 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: Silver 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: Silver 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 tox: Is the specific nanomaterial known to be acute toxic?**

Answer: No

Arguments and explanation:

In a substantial review of the literature, Stone *et al.* (2009) did not find indications of significant acute toxicity.

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: Kim *et al*. (2008) found no statistically significant effects after having investigated the *in vivo* genotoxicity using a bone marrow micronucleus test after oral administration of 60 nm silver nanoparticles for 28 days at various doses (US EPA 2010). According to Stone et al. (2010) and Mikkelsen et al. (2011) no mutagenicity or genotoxicity studies classically used in chemical regulatory setting have been identified. According to Stone et al. (2010) genotoxicity cannot be ruled out as nanosilver particles have been found to initiate a ROS drive process that might eventually lead to cytotoxicity and genotoxicity.

b. Respiratory tract toxicity: Hyun et al**. (**2008**)** observed no significant toxicological effect in rats in concentrations of silver nanoparticles (13 –15 nm) up to 61 µg/m3 in a 28 days inhalation study rats were rats were exposed in an inhalation chamber 5 days a week and 6 h per day to 3 different doses. A slight effect was observed on the neutral mucins in the respiratory mucosa (Hyun *et al.,* 2008). Under the same exposure condition Ji et al. (2007) did observe some toxicity (cytoplasmic vacuolation and heptatic necrosis) within the liver, but histopathological analysis did not reveal any distinct toxicity within other organs. Following OECD guidelines 413, Sung et al. (2008, 2009) exposed rats to various doses of 18-19 nm silver nanoparticles for 6 h/day and 5

days/week in a 90 days whole body inhalation study. Lungs and liver were found to be main target organs for accumulation of silver and toxicity and a inflammatory response was observed within the lung after prolonged exposure duration along with induced alterations in lung function, at all par ticle concentrations (Sung *et al.* 2008).

c. Cardiovascular toxicity:

No info available (Mikkelsen et al. 2010)

d. Neurotoxicity:

No information available (Mikkelsen et al. 2010)

e. Reproductive damage: Using LDH and MTT assays, Bradyich-Stolle *et al.* (2005) invetstigated the cytotoxicity of different nanoparticles (Silver (15 nm), molybdenum trioxide (MoO3, 30 nm), and aluminum (Al, 30 nm)) in an *in vitro* study on mouse spermatogonial stem cell. A concentration-dependent toxicity was observed for all types of particles tested and silver nanoparticles were found to be the most toxic.
Corresponding soluble salts had no significant effect. Using the same cell line, Bradyich-Stolle *et al.* (2010) investigated the cytoxicity and cell proliferation of 10, 25-30 and 80 nm nanosilver particles that had been coated with either polysaccharide (Ag-PS) or hydrocarbon (Ag-HC). A size and concentration dependant reduction was observed in the viability and cell proliferation of mouse spermatogonial stem cell and coatings were found not to provided any form of protection. Some developmental toxicity in the form of malformation and death has been observed in two embryo fish studies (Bar-Ilan *et al.*, 2009).

f. Carcinogenicity: According to Stone et al. (2010) and Mikkelsen et al. (2011) no studies investigating the carcinogenicity of silver nanoparticles have been identified. As nanosilver may cause genotoxocity, carcinogenicity cannot be ruled out (Stone et al. 2010).

g. Organ-specific accumulation: A dose-related increase of silver deposition has been observed in testes, liver, kidneys, brain, lungs and blood of treated rats (Mikkelsen et al. 2011).

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6. Overall evaluation of human hazard

We conclude that the color-code that best reflects the human hazard profile of nanosilver is ● based on *in vivo* evidence of a combination of hazards from testing of the nanomaterial. Nanosilver has been associated with respiratory tract toxicity in a number of scientific studies and genotoxicity and mutagenicty cannot be ruled out at this point in time. Nanosilver has furthermore been associated with reproductive effects *in vitro* and some organ-specific accumulation has been documented.

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Environment hazard profile

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

   Answer: No

   Arguments and explanation: Silver 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

   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

   Argument and explanation:

   According to Mikkelsen et al. (2011) a number of studies have reported nanosilver to be hazardous to environmental species i.e. LC50 or EC 50 <10 mg/l. In a 48 hour static toxicity tests on adult zebrafish (*Danio rerio*) Griffitt *et al.*, (2008) and Smith et al. (2007) derived LC50-values of 7.07 (6.04-8.28) mg/L and 7.20 (5.9-8.6) mg/L, respectively. A dose-dependent decrease has also been observed in the hatching rates, weak heart beats, edema and abnormal notochords in zebra fish embryos after 48 hours exposure of 0.01 mg/L and 0.02 mg/L 10-20 nm Ag nanoparticles suspended in tap water (Yeo and Kang 2008). Short-term toxicity testing on adult *Daphnia pulex* and *Ceriodaphnia dubia* neonates reported LC50, 48 h, to be 0.040 (0.030-0.050) mg/L and 0.067 mg/L (Griffitt *et al.,* 2008), respectively. In the same study an EC50 of 0.19 mg/L after 96 hours was found for green algae (*P. subcapitata*). For another alga species (*Chlamydomonas reinhardtii*) EC50 ranged from 0.355 mg/L ± 0.062 mg/L after 1 hour, to around 0.092 ± 0.011 mg/L after 3-5 hours. Expressed as a function of free Ag+, EC50 was estimated to range from 3.6 ± 0.5 ug/L after 1 hour, to 0.9 ± 0.08 ug/L after 5 hours (Navarro *et al.,* 2008). This study is important because it was found that the toxicity of AgNP cannot solely be explained by the free ion (Ag+) (Navarro *et al.,* 2008, Mikkelsen et al. 2011).

10. **Overall evaluation of environmental hazard**

    We conclude that the color-code that best reflects the environmental hazard profile of

APPENDIX 1: NanoRiskCat●●●|●● Template

nanosilver is ● based on nanospecific LC50 or EC50 < 10 mg/l.