Submission re. SCENIHR's preliminary opinion on nanosilver 31/1/14

Friends of the Earth

1. What may be the implications of the widespread use of nanosilver for human health and the environment? Please consider direct, as well as indirect effects occurring via the distribution into the environment (e.g. from use in appliances, discarding dental material, washing out from textiles, etc.). Does this change the existing assessments for silver in general?

Friends of the Earth mostly agrees with SCENIHR's conclusions

- We are concerned that the Opinion refers to nanosilver as if it is a uniform substance. It is important to note that the effects of nanosilver will vary depending on the particle characteristics and surface coatings.
- Given the rate of ion release is generally proportional to the surface area of a particle, nanosilver is more efficient than bulk silver at generating silver ions (Wijnhoven *et al.* 2009).
- Nanosilver also presents new properties, including:
    - the ability to cross many biological barriers, making it highly bioavailable (Loeschner *et al.* 2011)
    - increased production of reactive oxygen species, leading to apoptosis and cell death (Choi *et al.* 2010)
    - capacity to deliver silver ions efficiently to the surface of bacteria (Marambio-Jones & Hoek, 2010)
- We disagree with the statement that "At current levels of use of silver in consumer products, silver concentrations in wastewater sludge are unlikely to pose any risk to soil organisms"(p. 29). A recent European study (Schlich *et al* 2013) suggests that nanosilver in soil has a low mobility and repeated applications of sewage sludge containing nanosilver can cause it to accumulate in soil. Based on previous studies, the authors predicted that the concentration of nanosilver in soil will increase annually by 0.001 mg/kg dry soil, resulting in the predicted no effect concentration being exceeded in approximately 50 years.
- Colman *et al.* (2013) found an adverse impact on plants and microorganisms in a long-term field experiment following the application of sewage biosolids containing a low dose of nanosilver.
- We strongly disagree with the statement that "toxic effects to aquatic organisms are unlikely" (p. 30). Nanosilver is has been found to be toxic to a range of aquatic organisms - including fish, algae and crustacean (Lee *et al.* 2012; Kim *et al.* 2011; Bondarenko *et al* 2013). Bondarenko *et al.* concluded that "the discharge or leaching of biocidal nanomaterials to surface waters may pose threat to aquatic species." Dimkpa *et al.* (2012) have raised concerns regarding the environmental contamination of water streams with nanosilver because of its "extreme toxicity to non-target bacteria and other life forms."
- Browning *et al.* (2013) found that exposure to silver nanoparticles caused zebra fish embryos to develop with head abnormalities and no eyes. Zebra fish have been widely used as a model organism for the study of embryological development in other vertebrates including humans. The researchers found that embryos in earlier developmental stages were much more sensitive to the effects of the nanoparticles than later stage embryos. The results suggest that nanoparticles may disrupt

important developmental processes such cell signaling and gene transcription, creating downstream effects upon embryonic development.

- Abbott Chalew *et al.* (2013) found that conventional treatment resulted in 2–20% of nanosilver or its dissolved ions remaining in finished water. The study concluded that the use of nanoparticles in consumer products is resulting in nanoparticles in drinking water sources and that treatment may not remove them.
- A US Court recently found that exposure to nanosilver in consumer products was 'ubiquitous' and unavoidable because of lack of labelling and that as a result toddlers were being put at risk (*NRDC v EPA*, US Court of Appeals for the Ninth Circuit 2013)
- The German Federal Institute for Risk Assessment (BfR 2010) has recommended manufacturers "*avoid the use of nanoscale silver or nanoscale silver compounds in foods and everyday products until such time that the data are comprehensive enough to allow a conclusive risk assessment which would ensure that products are safe for consumer health."*
- We believe that a precautionary approach should be taken to the regulation of nanosilver. Lack of evidence of harm due to data gaps should not be used as an excuse for regulatory inaction.

2. Could the widespread use of nanosilver, in particular in medical care and in consumer products, increase the risk of selecting silver resistant micro-organisms? Could the widespread use of nanosilver create cross-resistance in micro-organisms?

Friends of the Earth mostly disagrees with SCENIHR's conclusions

- We mostly agree with the relevance of information included in the report, we disagree with the conclusions drawn by the SCENIHR.
- In particular, we disagree with SCENIHR's conclusion that there is a paucity of information on the bacterial resistance mechanisms to silver nanoparticles. In 2001, scientists identified the set of genes primarily responsible for silver resistance in bacteria - the sil operon (Gupta *et al.* 2001). This information provided researchers with the ability to rapidly identify bacterial isolates with levels of resistance to silver.
- Silver resistance in bacteria following the clinical use of silver has been well documented in the literature e.g. Merlino & Kennedy (2010).
- McArthur *et al.* (2012) have warned that "*continued use of [antimicrobial textiles] could result in increased and widespread resistance to specific antimicrobials, especially metals, with an increased resistance to antibiotics. Such increases have the potential to find their way into bacterial populations of human pathogens leading to serious and unintended public health consequences."*
- Stokes and Gillings (2011) explain that "*selection in stressed environments with respect to such compounds as heavy metals are enriched with antibiotic resistance genes*". Thus, the selection of bacteria with silver resistance, also simultaneously selects for other antimicrobial and antibiotic resistance genes. Furthermore, once bacteria have already expressed resistance to these antimicrobials, it is expected that the ongoing usage of these and other antimicrobials will continuously increase levels of resistance to these antimicrobials and antibiotics.
- Resistance genes to silver have been found on a range of plasmids, notorious for containing multiple antibiotic resistance genes (Gupta *et al*. 2001; Silver 2003; Merlino & Kennedy 2010). Baker-Austin *et al.* (2006) have concluded that metal contamination "represents a long-standing, widespread and recalcitrant selection pressure with both environmental and clinical importance that potentially

contributes to the maintenance and spread of antibiotic resistance factors."

- A number of clinically-relevant investigations into the incidence of resistant bacteria and bacterial resistance outbreaks, particularly among Gram negative bacteria, demonstrate the connection between resistance to biocidal metals (including silver) and common antibiotics on identical mobile genetic elements such as plasmids (Sandegren *et al.* 2012; Kremer & Hoffmann 2012; Sutterlin *et al.* 2011; Johnson et al. 2006)
- Qiu et al. (2012) investigated the ability of different nanomaterials to promote the transfer of the multiresistance IncP plasmid RP4. Qui and coauthors found that all tested nanomaterials strongly promoted plasmid transfer, with the authors suggesting an important role in oxidative stress damaging cell membranes, promoting the transfer of genes and nutrients (the "SOS response"). It is believed that nanosilver similarly kills bacteria primarily through this mechanism of oxidative stress damage to cell membranes (Wijnhoven *et al.*, 2009). While the study didn't specifically look at nanosilver, it is reasonable to believe nanosilver will promote the transfer of plasmids in a similar manner.
- Mijnendonckx *et al.* (2013) have raised concerns that "the extensive use of silver-based products will increase the release of silver in the environment, putatively inducing the dissemination of silver resistance (and thereby cross-resistance to antibiotics)".
- A large and growing body of scientific evidence supports the contention that the unrestricted use of nanosilver will drive the further generation and spread of antibiotic resistance in human pathogens (e.g. Reidy *et al.* 2013). Given the high likelihood that nanosilver will further contribute to the pool of bacteria resistant to antimicrobials we believe the only appropriate action is to restrict the use of nanosilver to critical clinical applications and patients.

3. To what extent may the widespread use of nanosilver and the possible increase of resistant micro-organisms reduce the nanosilver's efficacy?

Friends of the Earth mostly disagrees with SCENIHR's conclusions

- While we mostly agree with the relevance of information included in the report, we disagree with the conclusions drawn by the Scientific Committee.
- There is a growing body of evidence that suggests that the widespread use of nanosilver is likely to result in an increase in resistant micro-organisms, thus reducing the efficacy of nanosilver as an antimicrobial (Crocetti & Miller 2011).
- Similar to the well-understood problem of patients not finishing a course of antibiotics, the low level usage of any antimicrobial can stimulate the spread of resistance genes to that antimicrobial (and other antimicrobials – including antibiotics - through co-selection). The induction of bacterial resistance mechanisms following exposure to a low concentration of antimicrobials (biocides) has been reported in a number of studies for a number of antimicrobials (SCENIHR 2009). Similarly, the sub-optimal use of therapeutic antimicrobials for animals, in particular under-dosage, can enhance the development of AMR (European Commission 2011).
- After investigating three biocides, widely used in the food industry, Capita *et al.* (2013) concluded "the use of biocides at sub-inhibitory concentrations could represent a public health risk".
- Experts recognise that to minimise development of resistant bacteria in clinical settings, wound dressings must release high levels of silver ions, in an attempt to kill all bacteria present (Chopra, 2007). Concentrations of silver ions lower than 15 μg/L

have recently been reported to even boost bacterial growth instead of arresting it (Xiu *et al.*, 2012), a response that resembles suboptimal treatment with antibiotics, which creates resistant microbes.

- A recent study (Gunawan *et al.* 2013) reported for the first time that *Bacillus* spp. could develop resistance to nanosilver cytotoxicity upon exposure. The study found that the induced effects of adaptation in the forms of nanosilver and enhanced growth were stable. In other words the effects were still present even upon discontinuation of nanosilver exposure. The authors raised concerns that "this inherent ability of the ubiquitously-occurring *Bacillus sp.* may pose adverse implications to the increasingly wide use of antimicrobial nanosilver, and the environment."
- In hospital settings, nanosilver is used extensively for wound management, particularly for the treatment of burns, ulcers (rheumatoid arthritis-associated leg ulcers, diabetic ulcers, etc.), toxic epidermal necrolysis, healing of donor sites and for meshed skin grafts (Wijnhoven *et al*. 2009).
- Reidy et al. (2013) have raised concerns regarding the overuse of nanosilver and the potential for bacterial resistance to develop. They have called for a risk-benefit analysis for all nanosilver applications and eventually restrictions of the uses where a clear benefitr cannot be demonstrated.
- At a conference held at the BfR in 2012 it was widely agreed that the use of silver may lead to the selection of silver resistant bacteria
- Given the clinical value of nanosilver and the likely increase in resistant micro-organisms that will result from the widespread use of nanosilver we believe it is appropriate to restrict the use of nanosilver to critical clinical applications and patients.
- Overall we strongly disagree with the conclusion of the Scientific Committee that "it is not possible to estimate at this time whether or not resistance will increase and spread in view of a more widespread use of nanosilver in products". Rather, we argue that from the new body of research, it is easy to conclude it is highly likely that the widespread use of nanosilver will increase the incidence of resistant micro-organisms, thus reducing the efficacy of nanosilver.

4. Are there any other safety, health and environmental effects of nanosilver?

Friends of the Earth mostly disagrees with SCENIHR's conclusions

- While we mostly agree with the relevance of information included in the report, we believe that the Scientific Committee has grossly underestimated the potential effect that the widespread use of nano-silver may have on ecosystems.
- Discussion of the potential interaction of nanosilver with other pollutants is also missing from the Opinion.
- The Opinion makes a number of references to silver exposure. Nanomaterials can have markedly different properties to bulk forms of the same material. It is therefore not acceptable to use the results of the hazard assessment of bulk silver to evaluate the eco- and human toxicity of nanosilver. (US EPA SAP report 2010; Sass and Wu 2013).
- Colman *et al.* (2013) found that, in addition to having an adverse impact on plants and microorganisms, the application of sewage biosolids containing a low dose of nanosilver also led to an increase in nitrous oxide (N_2O) fluxes. This is significant – since nitrous oxide is a notorious greenhouse gas, with 296 times the global

warming potential of carbon dioxide. It is also the dominant stratospheric ozone depleting substance.

- Nanosilver is especially toxic to heterotrophic (ammonifying/ nitrogen fixing) and chemolithotrophic bacteria. Chemolithotropic bacteria belong to the lithotropic family of microbes and consume inorganic material. These organisms liberate many crucial nutrients, and are essential in the formation of soil. Ratte (1999) showed that silver ions inhibit enzymes needed by nitrifying bacteria.
- The toxic effect of silver on bacteria also appears to disrupt denitrification processes, with the potential to cause ecosystem-level disruption (Throback et al. 2007).
- Silver can readily bioaccumulate in aquatic organisms (Wijnhoven *et al.* 2009) and accumulates especially strongly in saltwater. Rapid uptake of silver in seawater has been observed in phytoplankton and marine invertebrates, even when few free silver ions are present. Concentrations of silver in phytoplankton have been 10,000 to 70,000 times higher than the concentration of silver in the surrounding water. As many of these organisms are eaten, the silver is then passed up the food chain (Luamo 2008).
- Colman *et al.* (2013) found that several plant species were able to take up silver from nano-silver in soils. This suggests a potential route for nano-silver from sewage waste to enter into the food chain, where it will likely bioaccumulate.
- Preliminary evidence suggests that the incineration of nanomaterials may catalyse the formation of other pollutants (Holder *et al.* 2013). Vejerano *et al.* (2013) reported that the emission of PAHs or chlorinated furans was higher when nanomaterials (including nanosilver) were part of the waste. The incineration of nanosilver could therefore increase the production of by-toxic products.
- The US National Research Council (2013) argues that "inventories are needed that describe what ENMs [Engineered Nanomaterials] are being produced, how they are being used, and what their forms are along the value chain." A mandatory register of nanomaterial use would help regulators determine the quantities and types of nanomaterials currently being produced. This is vital both to characterise the risk associated with nanomaterial pollution, and to develop successful strategies to prevent it.

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