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Environmental Protection Agency
Office of Pesticide Programs
Regulatory Public Docket EPA-HQ-OPP-2012-0594
Environmental Protection Agency
1200 Pennsylvania Ave., N.W.
Washington, DC 20460–0001

To Whom It May Concern:

RE: Registration Review docket for Antimicrobial pesticide product Nanosilva; Docket ID EPA-HQ-OPP-2012-0594.

These comments are submitted on behalf of People for the Ethical Treatment of Animals (PETA) and the Physicians Committee for Responsible Medicine (PCRM), which together represent more than three million members and supporters who are concerned with EPA's continuing decision to regulate nanosilver as a traditional pesticide, rather than as a nanomaterial. The parties to this submission are national animal protection and scientific advocacy organizations that share the common goal of promoting reliable and relevant regulatory testing methods and strategies that protect human health and the environment while reducing, and ultimately eliminating, the use of animals.

We appreciate the opportunity to comment on the Environmental Protection Agency's (EPA's) proposed decision to register a nanosilver-containing antimicrobial pesticide product named "Nanosilva", and specifically on the Agency's proposal to require the conduct of new animal studies as listed below:

- 90-day inhalation toxicity in rats (OCSPP 870.3465) modified to include *in vivo* bone marrow assay and functional observation battery, motor activity and detailed neuropathology;
- Reproduction and developmental toxicity screening tests in rats (Modified OCSPP 870.3550/OECD TG 421)

Based on the information provided below, we believe that the requested studies would be duplicative, and that the EPA needs to assess nanosilver-containing products more strategically and comprehensively, rather than requiring animal tests on individual products. Finally, and most importantly, the EPA must facilitate the development and regulatory use of QSARs and human-relevant *in vitro* methods without delay.

Background

Nanosilva is a silver-based product and is used as a non-food-contact preservative to protect plastics and textiles from odor and stain causing microorganisms. It is a liquid suspension containing 1% nanosilver by weight with average diameters between 6.9 and 10.6 nm (minimum diameter of 3 nm and maximum diameter of 18 nm), where the nanosilver surface is coated with sulfur and polyvinylpyrrolidone (PVP), and is attached to silica. Nanosilva is formulated into a polymeric intermediate known as a master batch and eventually incorporated into polymer and polymer based products to suppress the growth of bacteria, algae, mold, etc. It works as a broad spectrum antimicrobial by releasing ionic silver.

Several health effects tests have been performed on the Nanosilva suspension including acute toxicity, oral, dermal, and inhalation; acute eye and dermal irritation; and skin sensitization. A plastic leaching study was also performed. The company producing Nanosilva requested waivers for a number of *in vivo* tests including: Fish Acute Toxicity Test, Freshwater and Marine (OPPTS 850.1075), Avian Acute Oral Toxicity Test (OPPTS 850.2100), 90-Day Dermal Toxicity (OPPTS 870.3250), Prenatal Developmental Toxicity Study (OPPTS 870.3700), Mammalian Bone Marrow Chromosome Aberration Test (OPPTS 870.5385), and Immunotoxicity (OPPTS 870.7800). Waivers for the studies were requested because of the lack of acute toxicity noted in the six acute toxicity studies performed, the fact that Nanosilva has a specific gravity greater than water and a low solubility in water, and the low likelihood of leaching as indicated by a lack of detection of silver at concentrations greater than the analytical detection limit in leaching studies conducted with a plastic incorporating Nanosilva.

Difficulties with Testing Nanosilver as a Pesticide

EPA's State of the Science report on Nanosilver[1] indicates that tests that have already been conducted on nanosilver using animals display a considerable lack of reproducibility in experimental results. Variations can be traced to the vast differences in morphological shape of nanosilver (from rods, to spheres, particles, cubes, truncated triangles, wires, and films) and even, more importantly, to the differing animal species used in experiments. As the available data also demonstrate, nanosilver properties change depending on the exact size/surface area[2] of the nanosilver particles, the carrier used[3], any coatings[4], and whether the nanosilver has surface modifications[5], which in turn can have effects on its biological activity. These variable properties also can contribute to nanosilver behaving differently from bulk silver.

The bioavailability, fate and transport of nanosilver are dependent upon numerous factors. These include particle size, ease of aggregation, surface characteristics, and colloidal stability, which in turn is a function of the capping agent, pH, ionic strength, and presence of ligands, to name a few. Nanosilver toxicity has been shown to vary with route of exposure, oxidation state, and most importantly, physicochemical characteristics. The State of the Science report[6] declares "The physicochemical features of the nanoparticles must be characterized under the experimental setting so that definitive associations between these parameters and any biological responses observed may be identified." It is also noted in the State of the Science report that "…a good dispersion of the silver nanoparticles is required for effective toxicological and/or antibacterial activities, and might influence its subsequent toxicity[7,8]. Toxicity determination of nanosilver

particles may be dependent on the size distribution of the particles[9,10]." Thus, for an animal test to have any validity, characteristics such as the concentration, diameter and chemical composition of nanosilver in the exposure scenario must be identified and mimicked in the experimental setting. Yet, the report goes on to say that there are difficulties in accurately measuring and delivering doses as well as monitoring nanomaterial behavior, particularly in solution (and *in vivo*). With a potential biological response dependent on numerous factors – and many of these factors lacking reliable means of measurement – it is impossible to generate meaningful information by conducting the above-mentioned animal tests. For example, it will undoubtedly prove technically challenging to deliver representative and appropriate doses of nanosilver to rats in the inhalation toxicity test being proposed for this antimicrobial pesticide registration review. Therefore, due to the high potential for generating the type of equivocal results seen in many other animal studies with nanosilver, it is likely that these tests may need to be repeated and so would be a waste of animal lives and resources.

Uncertainties Associated with Extrapolation from Animal Studies to Humans

In addition to the considerable uncertainty in toxic potential associated with the physicochemical variability of nanomaterials, there are many documented uncertainties in extrapolating from laboratory animal studies to the human situation including: differences in meaningful dosages, variations in responses to chemicals and in target organs and tissue effects with different species and strains of animals, as well as different toxic thresholds between species including humans[11]. Importantly, biological relevance is unlikely with the currently-used rat tests – issues relating to breathing mode, physiology, relative sizes of nerve bulbs[12] and the different rate of particle clearance of rats[13] compared to humans all point to important anatomical and physiological differences that preclude clear data extrapolation between species. Certainly, some of these differences will make interpretation of the proposed inhalation study results challenging and given the likely unrealistic oral dosages and exposure times involved, it seems impossible that the rat reproductive/developmental toxicity test being called for in this pesticide registration review could provide meaningful data.

Risk of longer-term exposure to Nanosilva would seem to be greatest at the factory where workers handle, mix, and load the product. Yet, EPA has issued strict mitigation measures to ensure very low exposure potential. These include: 1) closed system loading of Nanosilva suspensions; 2) NIOSH certified full-face respirators immediately available for use in emergency; 3) use of gloves that are chemically resistant to all components of Nanosilva liquid suspension; 4) use of long-sleeve shirts, long pants, and shoes plus socks. It would appear extremely unlikely that any exposures under these conditions would result in long-term reproductive and developmental effects in workers, so that results of rat reproductive/developmental screening under relatively high dosages are unlikely to provide any useful, human-relevant information.

The Required Studies Would be Duplicative

Furthermore, there is no clear rationale given in the Draft Decision Document for Conditional Registration of Nanosilva[14] for conducting a 90-day inhalation study. In 2012, EPA was reviewing a similar silica-nanosilver composite product, HeiQ AGS-20, which contains

nanosilver particle sizes in the 1-10 nm range, similar to Nanosilva. At that time, EPA required a 90-day inhalation study in rats be conducted to confirm findings of cited non-guideline studies[15,16] submitted for HeiQ AGS-20. These same studies (i.e., Sung et al. 2008, 2009) were cited for Nanosilva as well as a newer inhalation study (Song et al. 2012[17]). Sung et al. (2009) conducted a 90-day inhalation study using nanosilver particles that EPA notes were of similar size to the pesticide product under review, and a NOAEL of 133 ug/m^3 was determined. Song et al. (2012) followed up with a lung-recovery study that indicated lung pathology findings similar to Sung et al. (2009) and showed gradual clearing in females, but persistent inflammation in high-dose males. A NOAEL of 49 ug/m^3 was derived from this study. In addition, two 28-day inhalation studies are available[18, 19]. EPA states in the above document[20] that the Song et al. (2012) study is the most appropriate study for inhalation exposure and is using the lower NOAEL of that study as the point of departure (POD) for short- and intermediate-term inhalation exposures to the nanosilver in Nanosilva. EPA goes on to state that the inhalation NOAEL of 49 ug/m^3 is expected to be protective of developmental and reproductive effects of nanosilver, as well as neurotoxic effects.

However, a supplemental DER was issued on 8/1/2013 for the 90-day inhalation toxicity test for nanosilver, and indicated that while the Song et al. (2012) study was acceptable and both a NOAEL and a LOAEL could be determined, the study still did not satisfy the guideline requirements for OCSPP 870.3465. EPA should provide further details on what necessary information is lacking and how this information, if gathered, would be used to modify labeling and further mitigate the already stringent Nanosilva handling procedures. EPA should also provide evidence that the two products (Nanosilva and HeiQ AGS-20) are substantially different in toxicologically-relevant ways that justify performing two separate 90-day inhalation studies. Otherwise, the results of the pending HeiQ AGS-20 study should be obtained before requiring inhalation studies on Nanosilva.

Similarly, requiring a reproduction/developmental toxicity screen for both materials may be duplicative. EPA should wait for the results of the testing on HeiQ AGS-20 before requiring that hundreds more animals be killed

Uncertainties Associated with Exposure

Further, when considering the hazard assessment of nanosilver, its potential ubiquity in the environment cannot be ignored. Of the more than 250 consumer products claiming to contain nanosilver[21], only a relatively few are being registered as pesticide products. Nanosilver is found in food packaging materials, textiles, electronics, cosmetics, medical devices, water disinfectants, and room sprays. However, there are very little data quantifying its presence or characterizing its forms in the environment or describing the amounts to which humans are cumulatively exposed. There are many questions regarding the degree to which nanosilver leaches from products, and what forms it may take, whether it remains as nanoparticles or aggregates, whether it dissolves or converts to ionic silver, and under what conditions these transformations may take place. Lacking information on the characterization and presence of nanosilver in the environment, data on amounts and forms being released from all types of products, and a clear understanding of the possible interactions with the surrounding environment, it is difficult to see

how a realistic hazard assessment and exposure scenario can be determined for the product subject to this pesticide registration review.

Assessment Must Be Approached in a Comprehensive Manner

Due to the physicochemical complexity of nanosilver in the environment, EPA must approach its assessment in a comprehensive manner, rather than requiring animal tests on individual products. We urge the EPA to first gain an understanding of nanosilver's forms, fate and transport in the environment, characterize the scenarios under which it is most bio-active, and determine the variables that influence its toxicity. Much of the preliminary toxicity evaluation could be done using *in vitro* or other non-animal methods. There are already a number of promising nanomaterial-specific *in vitro* techniques available and in development that are likely to provide high-throughput and reliable data on the cellular toxicity of nanosilver. These techniques will also be of use when looking at mechanisms and interactions of nanosilver within human systems, which will allow for better predictivity as well as allow assessment of cumulative exposures. Once this knowledge is gained, a screening model and other *in silico* methods could be developed to predict toxicity based on characteristics of the particular nanosilver product, its use and length of exposure. At that point, specific nanosilver products that show the highest potential for toxicity could be identified for further testing. Using such an integrated evaluation and testing approach is more strategic, and more efficient in the long run, than testing on animals on a product-by-product basis. Not only will the latter approach kill thousands of animals, but the results will be difficult to interpret, particularly when so many more non-pesticide-regulated products containing nanosilver go uncharacterized and may represent a greater threat. We understand that not all of these products are under the Office of Pesticide Programs regulatory purview; even so, a more holistic, cooperative approach is essential.

The EPA's Nanomaterial Research Strategy[22] focuses on a tiered approach based on *in vitro* toxicity testing methods and recognizes the critical differences in assessing nanomaterials and bulk chemicals. We recommend that instead of applying unproven and unvalidated animal-based methods used to test bulk chemicals, EPA consider nanosilver as a nanomaterial first and foremost and allow for the safety testing of nanosilver using available non-animal testing methods.

Because nanomaterials differ from traditional chemicals and have proven difficult to test using some of the animal-based methods used for traditional chemicals, it makes more sense to apply an Integrated Testing Strategy (ITS) that takes into account the data available for the nanomaterial of interest (in this case, for nanosilver) and provides a rational testing strategy to satisfy regulatory needs while minimizing animal testing. Methods that have proven most efficient and sensitive to nanomaterials include *in vitro* methods using bacterial, fungal, and algal toxicity tests as well as human cell- and tissue-based toxicity tests to assess human safety for these nanoparticles[23,24,2526,27,28,29,30,31,32]. Moreover, many of EPA's newer testing designs[33,34,35,36] could be modified slightly to accommodate the robust testing of nanomaterials.

Another promising approach is described in a report[37] focused on elucidating the impact of ingesting silver nanoparticles using a novel *in vitro* digestion model that has been accepted to the journal of Nanotoxicology (publication date is to be determined). This study assessed

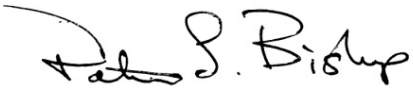
nanoparticle effect after coming into contact with artificial saliva, gastric, and intestinal solutions. Because of the model's specificity to nanosilver and its ability to assess the nanoparticles as they traverse the digestive system, we request that EPA consider this method for further use in risk assessment as well.

In order to most efficiently regulate nanomaterial-containing pesticides, EPA must require manufacturers to further develop and use *in vitro* and *in silico* human and environmentally-relevant methods that are high throughput, efficient and flexible enough to handle the infinite variations that are possible with a nanomaterial-based product. If the agency decides to require additional registration and testing of products containing true nanosilver according to antimicrobial pesticide regulations, then manufacturers should be encouraged to assess these risks using a human-relevant intelligent testing scheme that relies on high-throughput, reliable, non-animal methods rather than resorting to the problematic, expensive and time-consuming animal-based methods used for traditional pesticides.

It makes little sense to require animal testing on a product-by-product basis. EPA should delay decisions on toxicity testing requirements until nanosilver products can be characterized and compared for similarities and differences, and until realistic exposure levels can be determined. We disagree with Scientific Advisory Panel recommendations that bridging opportunities may be limited among nanosilver products and request that a full examination of the physicochemical properties be completed before requiring any animal tests. A recent review of the applicability of QSARs to nanotoxicology[38] highlights the promise of computational toxicology methods in evaluating nanomaterials, although the article does point out that some major gaps in research required to accelerate the use of QSAR methods for regulatory purposes do exist.

We urge EPA to support human-relevant toxicity test methods for assessing nanosilver. *In vivo* methods are not validated for testing nanomaterials and given the myriad of conflicting data from animal studies with nanomaterials, testing nanomaterials *in vivo* is not only subject to the same problems and constraints that plague all animal-based testing, but is further confounded by even more practical difficulties than experienced with traditional chemicals and pharmaceuticals. EPA must employ a more robust and reliable system to assess nanomaterial safety and we urge EPA to facilitate the development, implementation, and use of relevant and reliable non-animal-based methods.

Sincerely,



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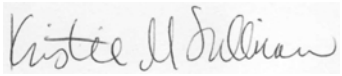

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