UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF **C**HEMICAL **S**AFETY
AND **P**OLLUTION **P**REVENTION

October 13, 2017

MEMORANDUM

SUBJECT: *Daphnia magna* acute protocol post-meeting (9/14/2017) action item follow-up

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The agency had a teleconference call with Poly-Technical Solutions and its representatives on 9/14/2017 to discuss their questions on the agency's review of a *Daphnia magna* acute toxicity test protocol with

NSPW-L30SS[1], which contains silver nanoparticles, and their follow-up revision[2]. This memorandum addresses the three follow-up questions the agency agreed to answer for Poly-Technical Solutions.

1) Guidance on how to conduct a kinetic/dissolution study with a metallic nanomaterial

The agency currently has no OCSPP 835 guideline for a kinetic dissolution study with a metallic nanomaterial dispersion. The primary goal of the study should be to provide basic information on the dissolution rate of silver from the amorphous silica-silver complex. Tests should be run using the product used to form batch material under environmentally relevant conditions as represented in aquatic toxicity tests required under 40 CFR Part 158W, and quantify and identify resulting changes to free ionic silver, other silver forms or species, and precipitation of secondary silver species over time. The agency recommends consulting the references provided below to develop a protocol for the amorphous silica-silver complex product (NSPW-L30SS). The agency also recommends including a paired silver nitrate reference and silver nanomaterial reference as part of the test.

- Miller, L. and Chappell, M. 2016. Environmental Consequences of Nanotechnologies: Nanomaterial Dispersion/Dissolution Characterization. Scientific Operating Procedure SOP-F-1. US Army Corps of Engineers Research and Development Center, ERCD/EL SR-16-1. 37 pp http://acwc.sdp.sirsi.net/client/en_US/search/asset/1049986

- Paydary, P. and Larese-Casanova, P. 2015 Separation and quantification of quantum dots and dissolved metal cations by size exclusion chromatography-ICP-MS. *International Journal of Environmental Analytical Chemistry* 95(15):1450-1470.

- Adam, N., Schmitt, C., Galceran, J., Companys, E., Vakurov, A., Wallace, R., Knapen, D. and Blust, R. 2014. The chronic toxicity of ZnO nanoparticles and $ZnCl_2$ to Daphnia magna and the use of different methods to assess nanoparticle aggregation and dissolution. *Nanotoxicology* 8(7):709-717.

- Harmon, A., Kennedy, A., Poda, A., Bednar, A., Chappell, M., and Steevens, J. 2014. Determination of nanosilver dissolution kinetics and toxicity in an environmentally relevant aqueous medium. *Environmental Toxicology & Chemistry* 33(8):1783-1791.

- Levard, C., Reinsch, B., Michel, F., Oumahi, C., Lowry, G. and Brown, Jr., G. 2011. Sulfidation processes of PVP-coated silver nanoparticles in aqueous solution: impact on dissolution rate. *Environmental Science & Technology* 45:5260-5266.

While the primary goal of the Miller and Chappell (2016) standard operating procedure provides a standardized methodology, the agency recommends combining elements of the standard approach with some elements needed for preliminary testing discussed in OCSPP 850.1000 (USEPA, 2016). Added elements should be designed to aid in determining the type of *D. magna* exposure study that needs to be conducted (static, static renewal, flow-through) and to determine what if any modifications or alterations to the test vessel exposure system may be needed to aid in maintaining a constant aqueous dispersion of NSPW/NSPW-L30SS (*e.g.*, Boyle *et al*., 2015; Capper, 2006; Schmidt-Dallmier *et al*., 1992) throughout the exposure duration of the test. For example, the Miller and Chappell (2016) protocol calls for placing flasks in an orbital shaker, and shaking at

[1] DP Barcode 44138[6]: July 27, 2017 Memorandum from D. Randall and A. Byro USEPA/OCSPP/OPP/AD/RASSB to J. Herbert, S. Murasaki, Z. Bain USEPA/OCSPP/OPP/AD/RMB I, Re: Protocol Review of an acute *Daphnia magna* toxicity test with NSPW-L30SS, 6 pp.
[2] DP Barcode 44[1386]: September 7, 2017 Memorandum from D. Randall and A. Byro USEPA/OCSPP/OPP/AD/RASSB to J. Herbert, S. Murasaki, Z. Bain USEPA/OCSPP/OPP/AD/RMB I, Re: Review of revised test protocol for an acute *Daphnia magna* toxicity test with NSPW-L30SS, 4 pp.

approximately 60 revolutions per minute for a 48-hour period but the *D. magna* acute toxicity testing protocol submitted for testing NSPW-L30SS identified it would be conducted as a static or static renewal test. Harmon *et al*. (2014) examined dissolution kinetics for silver nanoparticles in a static versus dynamic systems, and they differ. To inform whether or not the static test proposed for the *D. magna* acute toxicity test will result in stable amorphous silica-nanosilver concentrations and dispersions inclusion of a static test system in the kinetic dissolution study is recommended. Similarly, if a dynamic test system other than placement on an orbital shaker is to be used to maintain a homogeneous dispersion of amorphous silica-nanosilver complex (*e.g*., exposure chamber system in Boyle *et al*., 2015) in the aquatic toxicity tests, recommend including the same dynamic test systems in the kinetic dissolution study. The Miller and Chappell (2016) SOP calls for covering the test system flasks with aluminum to prevent dissolution from photooxidation. Toxicity tests are typically conducted with light/dark photoperiod, so including a paired test system without foil for one or more of the test systems is important for informing the acute *D. magna* toxicity test design. A test period longer than the 48 hours identified in Miller and Chappell (2016) may be more appropriate if the amorphous silica-nanosilver complex is stable over 48 hours, and longer static or static renewal exposure periods (*e.g*., 72 hours) are contemplated for the fish ELS and/or chronic *D. magna* toxicity tests.

Acute effects on *D. magna* (*i.e.,* immobilization and mortality) may potentially occur below the analytical detection limits for silver, silver ions, and particle counts. In such a case, to obtain estimates of an EC_{50} on a free silver or dissolved silver basis or on the number of nanoparticles requires making some assumptions about the behavior of the amorphous silica-nanosilver complex below detection limits. To develop appropriate assumptions, the Agency needs information on the behavior of the amorphous silica-nanosilver complex (e.g., agglomeration, dispersion, formation rate and amount of free silver and silver complexes) in a dilution series across detectable levels. The study should include at least five concentrations: one concentration above but close to the detection limit, one at the expected highest test concentration, and three concentrations in between. The kinetic dissolution study should at a minimum use the aqueous test media to be used in the *D. magna* toxicity test. The Agency also recommends strategic inclusion of other environmentally representative aquatic test laboratory conditions (*e.g*., higher hardness, lower pH, saltwater media) to aid in design of other required aquatic toxicity tests, and to inform the agency of the fate and behavior under a select number of environmentally relevant conditions.

2) Request modification of collection of dead daphnia for histological examination

The laboratory expressed concern over the requirement to collect daphnia that had died, due to the potential for deterioration of the organisms before they could be collected. The agency recognizes there is real potential for this to be an issue. The agency still recommends collecting organisms that have died or at 48 hours those that are alive but immobilized. If an organism has deteriorated to where it cannot be collected, note that the remains of the organism could not be located. If the organism is partially deteriorated, the histologist should be able to note which examinations are not appropriate due to the condition of the organism.

3) Provide example of type of language agency is looking for regarding clean laboratory techniques for inclusion in protocol

"Trace metal clean techniques will be used throughout the experiment as described in detail in [*citation*]. [*Summarize a couple of examples, list type of clean facility to be used in preparing exposure media, identify any ongoing monitoring programs/performance based procedures*] The analytical facility conducting the trace metal analysis is responsible for providing information on trace metal clean techniques to be incorporated during that phase." Clean technique language in Miao, Wang, and Juneau (2005) is also an example. The aquatic and analytical testing facility's

(facilities') contamination control plan(s) can be cited for details (IAEA, 2003; Mindak and Capar (2014)).

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