

Nanotechnology: Chemical and Toxicological Risk Assessment Issues with Antimicrobials

By

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EXTENDED ABSTRACT

The United States Environmental Protection Agency (U.S. EPA) Office of Pesticide Programs (OPP) is a regulatory office that is responsible for pesticides registration for distribution and sale in the U.S. OPP is mandated by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) to conduct hazard and exposure risk assessments to ensure that agricultural and antimicrobials pesticides and biopesticides, when become commercially available, do not pose unreasonable adverse effects to humans or the environment (air, soil, water, terrestrial and aquatic organisms). OPP reviews enormous amount of scientific and technical data submitted by the registrants as well as has the burden of making regulatory decisions on these pesticides. The new nanoscience and nanotechnology, which may be used in pesticides, present an enormous challenge to the Agency in both domains.

The antimicrobial pesticides are handled in the Antimicrobial Division (AD) in OPP. Uses, application rates, and application scenarios of antimicrobial pesticides are relatively less than those of agricultural pesticides. However, antimicrobials are more used at homes, institutions, hospitals, and other residential-related fields, such as, construction materials, playground and recreational equipments, swimming pool and spa, etc. There are 12 categories of antimicrobial pesticides based on their use patterns. Of these use patterns, eight are categorized as indoor/residential. Some outdoor antimicrobial uses include heavy duty wood preservatives and antifoulants. Each use pattern triggers a set of data requirements.

Manufactured nanomaterials (MN) can be broadly divided into two major types: (1) carbon nanotubes, fullerenes, and functionalized carbon nanotubes or functionalized fullerenes; (2) manufactured nano-metals and nano-metal oxides. Both types can have different physical shapes like cylindrical or spherical etc. From these two types of MNs, a series of nanoproducts can be made. Mike Roco¹ believes that five generations of nanoproducts can be devised from these MNs. These are:

- 1) Passive nanostructures which include: polymers, ceramics, nanostructures of metals, nanoparticles, coatings, and possible applications are in the food industry, consumer products, cosmetics and pharmaceuticals.
- 2) Active nanostructures which include amplifiers, targeted drugs, actuators, adaptive structures and 3D transistors and possible applications are in the fields of

nanobiotechnology, neuroelectronics interfaces, hybrid nanomanufacturing (nanocomposites).

3) Systems of nanosystems which include guided assembling, 3D networking robotics, and possible applications are nanorobotics, regenerative medicine, brain-machine interface, and engineered agriculture products.

4) Molecular nanosystems which include molecular devices ‘by design’, atomic design, emerging functions, and possible applications are neuromorphic engineering, complex systems, human-machine interface

5) Converging Technology which include nano-bio-info from nanoscale, cognitive technologies, large complex systems from nanoscale and applications include hybrid nano-bio-info-medical-cognitive systems. At the present time, research and applications are at the third generation stage.

OPP mandates, through FIFRA, to conduct risk assessment for pesticides. The following data are required and acquired for a) physical/chemical characterization of a pesticide; b) data to show that the intended use of pesticide is efficacious; c) ecological effects, fate and transport of a pesticide in air, water, soils, terrestrial and aquatic organisms, bioaccumulation; d) human exposure risks for workers and post-applicators; e) mammalian toxicity.

OPP requires the submission of physical/chemistry characteristics for all pesticides which are submitted for registration. These are presented Table 1:

Table 1. OPPTS Physical/Chemical Properties Series 830 Test Guidelines

Series#	Title	TGAI ¹	MUP ²	EUP ³
830.1000	Background for prod properties test guidelines	X	X	X
830.1550	Prod. identity and composition	X	X	X
830.1600	Description of materials used to produce the products	X	X	X
830.1620	Description of production process	X		
830.1650	Description of formulation process		X	X
830.1670	Discussion of formation of impurities	X		
830.1700	Preliminary analysis	X		
830.1750	Certified limits	X	X	X
830.1800	Enforcement analytical method	*	*	*
830.1900	Submittal of samples	*	*	*
830.6302	Color	X	X	#
830.6303	Physical state	X	X	X
830.6304	Odor	X	X	#
830.6313	Stability to sunlight at normal and elevated temp.	X		
830.6314	Oxid/reduc chemical incompatibility	X	X	X
830.6315	Flammability		X	X
830.6316	Explodability		X	X
830.6317	Storage stability		X	#
830.6319	Miscibility		X	X
830.6320	Corrosion characteristics		X	X
830.6321	Dielectric breakdown voltage		X	X

Series#	Title	TGAI ¹	MUP ²	EUP ³
830.7000	pH of aqueous solutions and suspensions	X	X	X
830.7050	UV/visible absorption	X		
830.7100	Viscosity		X	X
830.7200	Melting point/melting range	X		
830.7220	Boiling point/boiling range	X		
830.7300	Density/relative density/bulk density	X	X	X
830.7370	Dissociation constant in water	X		
830.7520	Particle size, fiber length, and diameter distribution	X		
830.7550	Partition coefficient (n-octanol/water), shake flask method	X		
830.7560	Partition coefficient	X		
830.7570	Partition coefficient (n-octanol/water) estimation by liquid chromatography	X		
830.7840	Water solubility: column elution method; shake flask method	X		
830.7860	Water solubility, generator column method	X		
830.7950	Vapor pressure	X		

¹ Technical grade active ingredient

² Manufacture use product

³ End use product

Open literature search on nanotechnology and nanoproducts indicates that the determination of the physical/chemical characteristics of nanomaterials is beset with difficulties and uncertainties. This means that the uncertainties will make the data, results and risk assessments less reliable for nanomaterials.

Some of the problems, difficulties and uncertainties discussed here by no means represent a complete and comprehensive list:

1. Metal M or metal oxide MO as a MN is brought for registration. Should OPP consider it a NEW chemical or an old chemical but new use? The second approach will require very little new data. It is becoming quite clear that nanomaterials have some unique and new characteristics not possessed by same material if it is the 'normal' or 'conventional' state of existence. Some of the new and novel characteristics are: surface area, surface activity, size, and size distribution, shape, morphology, enhanced catalytic activity. Therefore treating a nanomaterial formed from a conventional substance as a new chemical appears a better approach.
2. a) Product identification and composition is of critical importance. Evidence abounds that most of the MNs suffer from the presence of impurities, particularly the presence of metals as impurity. How can a nano Technical Grade Active Ingredient (TGAI) be defined? What percent (%) or number of ppm level of impurity will the Agency regard as acceptable or unacceptable?
b) Which analytical techniques would be acceptable for the Agency to be standard enforcement method for the full characterization, identification of nanoproducts? SEM, TEM, AFT is expensive techniques. Moreover, a single technique will not provide a complete characterization of a nanoproduct. It should be pointed out that recent research shows that some traditional

techniques can yield good and reliable data in identification and characterization of nanoparticle, as shown by the recent work by Xu,² Guar-Tzo Wei³, C. Degueldre^{4,5}, and Jorg Bettmer⁶

3. Most of the nanomaterials are insoluble in water. One simple technique used to identify chemicals is the uv-vis spectra. However, because of the solubility issue, should the Agency abandon asking for this data?
4. Similarly using the existing guideline methods as shown in Table 1, some of the characteristics like boiling point, partition coefficients (Kow), vapor pressure, and density can not be determined. Should Agency not require these characteristics? OR should the Agency develop new methods and employ new guidelines for the determination of these and other characteristics?
5. In addition to the data requirements listed in Table 1, should the Agency require new data, and therefore develop new guidelines due to the nanomaterials having new and unique characteristics? Some of the new characteristics that will play pivotal role in the characterization as well as help in the risk assessments are: specific surface area of the MNs, determination of zeta potentials of the MNs, surface charge on MNs, agglomeration/aggregation conditions of the MNs, crystalline phase of metal/metal oxides MNs, grain size, hydrodynamic size, size, length, and shape of MNs etc.

Metal Risk Assessment and Uncertainties:

Scientific community as well as the Agency has recognized that the risk assessment methodology used for conventional pesticides (agricultural as well as antimicrobials) can not be used for the risk assessment for metals. For example, fate and transport studies based on the conventional test guidelines to determine hydrolysis, photolysis, degradation (metabolism) studies under aerobic and anaerobic conditions, bioavailability, bioaccumulation etc. can not be investigated for metals⁷. Some of the reasons are: metals are naturally occurring substances, and are found in nature as mixtures in the environmental media like soils, water, air, plants, humans, animals and microorganisms. Metals do not degrade, biodegrade in the traditional sense, but undergo speciation (exist as different forms of chemical moieties at various PHS, redox conditions, in water, soils sediments, plants, humans and animals. In various organisms all important processes like absorption, distribution, transformation, and excretion of metals depend on the metal, organ's ability to absorb, transform, and excrete or to store the metal. This difficulty gets compounded with metals that are MNs⁸. This gives rise to the following uncertainties and concerns:

- Silver meets the PBT criteria in environmental media like water and soils. The exact hazard associated with nano-silver is not well-defined and hence precise hazard and exposure risk assessments can not be done for nano-silver.
- Despite the claim of nano-silver uses in numerous consumer products (and actual antimicrobial uses have not been figured in), the mass loadings into the environmental media is not known. This is a major barrier to conducting risk assessments. No adverse effects of nano-silver in the environmental media have been substantiated yet.

- Toxicity of silver to bacteria is well established. And some reports indicate that toxicity to bacteria from nano-silver is enhanced. But dose-responses in various environmental media have not been systematically investigated.
- In its bioavailable form, silver is known to be highly toxic to aquatic organisms. A similar database for nanosilver is not yet established.
- Normal silver is generally not a systemic toxic substance to humans. However, nanosilver has shown increased toxicity. Full blown research must be conducted to establish the possibility if nanosilver is a systemic toxic substance.

These issues are for one metal-silver in this case. The trend in the nanoscience and nanotechnology are clear for quite sometime, that the manufacture and applications of new products will include metals and metal oxides. As the recent document has established that a new framework is needed to conduct the risk assessments for metals, perhaps it is true that a brand new framework will be needed to conduct the risk assessment on nano-metals and nano-metal oxides.

Because of their nanometer-range size and high surface area and/or reactivity, nanomaterials possess unique physiochemical properties, and in turn, the toxicity of nanomaterials might be different from conventional materials. Some of the critical issues that EPA is struggling with and may continue to do so for the foreseeable future are:

- How useful are the data generated on conventional size pesticides? Is data bridging between conventional size chemical and nanomaterial possible?
- Would current data requirement be adequate to characterize the hazards and to assess the risks related to the exposure of a nanopesticide?
- Would the Agency need nanopesticide-specific data?
- Would the current test guidelines be suitable and adequate for testing nanopesticide or existing guidelines be modified? Or would new test guideline(s) be needed?
- For toxicity risk assessment would traditional animal testing model be appropriate for testing nanopesticide? What kind of dosimetrics would be appropriate for testing nanomaterial, surface area/activity-based and/or particle size/number-based in addition to mass-based?
- Could the results derived from “non-traditional” *in vivo* method (e.g., intratracheal) be used in hazard characterization and risk assessment?
- Would the data generated on 10 nm nanomaterials be the same as those of 30 nm ones?
- How nanomaterials enter, travel through, and deposit in the body? - PK/PD of nanomaterials in animals
- How would aggregation/agglomeration affect the toxicity of nanomaterials in organisms and environmental media?

The current data requirement for health effects (OPPTS mammalian toxicity series 870 test guidelines) for antimicrobial agents is a “tiered” approach. The Agency requires the Tier I data for all antimicrobial agents unless a waiver is granted based on its use pattern (e.g., in so-called “closed system” use that poses a minimum or negligible concern of exposure) or the physical/chemical properties (e.g., a corrosive agent). The

Tier I data requirement is listed below and all studies are required to test on the technical grade of active ingredient (TGAI). The product-specific acute 6 package may be used for the acute toxicity testing requirement.

Tier I data requirement:

Acute Toxicity Testing:

- 870.1100: Acute oral (one species) (TGAI/MP and EP)
- 870.1200: Acute dermal toxicity (one species) (TGAI/MP and EP)
- 870.1300: Acute inhalation toxicity (one species) (TGAI /MP and EP)
- 870.2400: Primary eye irritation (one species) (TGAI/MP and EP)
- 870.2500: Primary dermal irritation (TGAI/MP and EP)
- 870.2600: Dermal sensitization (TGAI/MP and EP)

Subchronic Toxicity Testing:

- 870.3100: 90-Day oral – rodent (TGAI)

Developmental Toxicity and Reproduction:

- 870.3700: Prenatal developmental toxicity - rodent (TGAI)

Mutagenicity:

- Subdivision F, App. 9, 870.5100: Bacterial reverse mutation assay (TGAI)
- Subdivision F, App. 9, 870.5300: *In vitro* mammalian gene mutation (TGAI)
- Subdivision F, App. 9 870.5380, 870.5385/870.5395: *In vivo* cytogenetics (mutagenicity) (TGAI)

Depending upon the use pattern (e.g., food/feed-contact), the exposure concerns (e.g., long-term/high human exposure), or chemical-specific toxicity (e.g., a neurotoxicity- or immunotoxicity-causing agent), additional testing including route-specific (dermal and/or inhalation), chronic toxicity and/or carcinogenicity studies, and other specific testings may also be required.

The conventional toxicity testing is to determine if there are any health effects in humans and/or animals exposed to a substance via specific exposure route. The health effects and exposure route are defined by the physiochemical properties of the substance as well as the likelihood of human exposure. The commonly used duration of testing includes acute, subchronic, and chronic. The dosage used in conventional toxicity testing is mass-based. Although toxicological endpoints vary among the studies, the lethal concentration level of 50% testing animal die after one exposure in an acute test (LD50/LC50), the maximum tolerated dose (MTD), and the none-observed-adverse-effect level (NOAEL) are defined endpoints for an acceptable level of human exposure. In addition, the histopathological changes of tissues are normally examined by light microscopy techniques.

Nanomaterials characterized by its well-known unique nano size and large surface area as well as surface reactivity would likely manifest a unique toxicity both in humans and

environment. The mass concentration of doses used in the conventional toxicity testing would most likely result in non-representative exposure of nanomaterial particles. In turn, the toxic effects observed in the study are unlikely to be the potential toxic effects of the tested nanomaterial; but rather, the effect of massive blockage, e.g., preventing the ingestion of nutrients due to the amount of foreign material in the stomach in an oral study, or clogging the airways in an inhalation study. In addition, the light microscopic examination used in the conventional testing will not be able to detect any nanomaterials presented in the organs/tissues⁸.

The short-term duration, such as those defined in acute toxicity testing, is unlikely to occur in human exposure to small amount of nanomaterials in reality unless a massive release happens as a result of accident. Therefore, subchronic and/or chronic toxicity testing would be more appropriate to determine the toxic effects of nanomaterials.

The OECD Working Party on Manufactured Nanomaterials report (ENV/CHEM/NANO 2008/7)⁹ indicates that the current OECD testing guidelines are generally appropriate for investigating the human health effects of nanomaterials. However, it is recommended that certain important considerations need to be borne in mind, particularly those related to the physiochemical characteristics, including such characteristics in the actual dosing solution. Considerations should be given as well to what the most appropriate dose metrics would be to the tested nanomaterial. If not known, a series of measurements including mass, particle number and surface area need to be considered. Modification to the testing guideline may also be necessary, particularly via inhalation route, which is considered to be the primary exposure route for nanomaterials. Detailed histopathological examination of the entire respiratory tract is needed.

There are many *in vitro* and “unconventional” *in vivo* (e.g., intratracheal) studies on nanomaterials. It is important to establish a scientifically sounding approach, based on current knowledge and practical solutions, when using these test methods. Nanomaterials have a tendency of translocating to whatever the exposure conditions are, including inside the organism after administration and will likely to show systemic effects. Therefore, it is almost impossible to determine such property of the nanomaterials and their systemic health effects by using single cell *in vitro* testing environment, until more valid screening tests or the relationship between the physiochemical properties of nanomaterials and their toxic effects are established.

The effects of chemical on human health and environment are dependent upon physiochemical and toxicological properties of the substance. To understand how the substance behaves in humans and animals, including persistence, bioavailability, internal distribution and bioaccumulation, are the keys to accurately assess and characterize the toxicological properties of the substance. No exceptions for nanomaterials. The toxicokinetics studies, which are defined to study the absorption, distribution and elimination of nanomaterials in humans and animals, are fundamentally important in assessing their potential health effects. While it is technically challenging, studies

tracking the distribution of nanomaterials *in vivo* at realistic exposure scenarios (dose level and exposure route) seems necessary.

For what have been said, there are knowledge gaps on whether or not or to what extent that extrapolation would be possible from the toxicology of non-nanomaterials and other physical forms (e.g., fiber of the same substance) to the toxicology of nanomaterials and between nanomaterials of different size, range, and shape.

Given the complexity and variety of antimicrobial pesticides (twelve use pattern categories, different use sites and dual jurisdiction with FDA), the data requirement and policy on regulating pesticides containing nanomaterials are a “case-by-case” approach, although there are no registrations of pesticides containing nanomaterials at present time. From the toxicity perspective, the uncertainties of interpretations between *in vitro* and *in vivo* mechanistic studies as well as the limitations of the existing regulatory data requirements are presented.

Research and applications in the field of nanotechnology is fast-paced. Virtually all disciplines of science are or will be impacted by nanotechnology. It has been pointed out¹⁰ recently that from the science and regulatory perspectives, short-term and long policies are needed to have strong grip on the development of the new field.

Some of these short-term policies are:

- Environmental health and safety (EHS) related research funds should be increased, and a peer-reviewed EHS research plan should be instituted.
- A stand-alone Nanotechnology Effects Institute should be established. This Institute should handle all hazards, exposure and safety related issues.
- A similar stand-alone interagency nanotechnology Regulatory Group should form.
- Each Regulatory agency should have its own unique nanotechnology plan

Some of the long-term policies include:

- Existing regulatory laws such as Toxic Substance Control Act (TSCA), Federal Food, Drug, and Cosmetic Act (FFDCA), and Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), should be amended to incorporate nanotechnology related guidelines and make these laws more stringent.
- Introduce new laws concerning the data requirements for nanoproducts
- Enact new regulatory law specific to nanoproducts

REFERENCES

1. **Mike Roco, June 2008, A talk presented at the: International Food Policy Research Institute , Washington, DC**
2. **P.D. Nallathamby, Kerry J. Lee, and Xiao-Hong Nancy Xu, 2008, ^{ACS} NANO, Volume 2(No.7), pp 1371-1380**
3. **Guor-Tzo Wei, and Fu-Ken Liu, 1999, Journal of Chromatography A, 836, pp 253-260**
4. **C. Degueldre et.al, 2004, Analytica Chimica acta, 519, pp137-142**
5. **C. Degueldre et al., 2006, Analytica Chimica Acta, 555, pp 263-268**
6. **Andreas Helfrich, Wolfram Bruchert, and Jorg Bettmer, 2006, Journal of Analytical Atomic Spectrometry, Vol.21, pp 431-434**
7. **Risk Assessment Framework for Metals, 2007/ US EPA, Office of Research and Development.**
8. **Rocks. S, Pollard, S, and Dorey, R., et al. 2008. Comparison of Risk Assessment Approaches for Manufactured Nanomaterials. Final report, Defra Project CB403, Cranfield University. May 30, 2008.**
9. **Working Party on Manufactured Nanomaterials. 2008. Progress Report for Project 4 “Manufactured Nanomaterials and Test Guidelines”. ENV/CHEM/NANO (2008)7. Organization for Economic Co-operation and Development (OECD). May 30, 2008.**
10. **Davies, J. C. 2008. Woodrow Wilson International Center for Scholars, Project on Emerging Nanotechnologies (PEN) # 13.**