Stable Isotope Tracing - a way forward for Nanotechnology?

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ABSTRACT

Numerous publications and reports have expressed health and safety concerns about the production and use of nanoparticles, especially in areas of exposure monitoring, personal use, and environmental fate and transport. We suggest that stable isotopic tracers, which have been used widely in the earth sciences and in metabolic and other health-related studies for several decades, could be used to address many of these issues.

Key words: isotope tracers, nanoparticles, sunscreen, zinc oxide, titanium dioxide.

BACKGROUND

Because of their small size, much concern has been expressed about the potential for adverse health effects arising from the ability of nanoparticles to penetrate cell walls and the blood–brain barrier. These concerns include possible detrimental health effects during manufacture and transport as well as their fate and transport in the environment. Currently, there are no occupational health and safety guidelines for the production, use and disposal of nano-products or process by-products. The concern over lack of guidelines has resulted in a coordinated collaborative effort between several U.S. agencies—including the National Institutes of Health, the National Toxicology Program headquartered at the National Institute of Environmental Health Sciences, the U.S. EPA, the Centers for Disease Control and Prevention, the National Institute for Occupational Safety and Health, and the Occupational Safety and Health Administration —and comprehensive reports by The Royal Society and the Royal Academy of Engineering of the United Kingdom (2004), a European Commission report (SCENIHR 2005), and a White paper from the U.S. EPA (2005). More recently Maynard's Nature article [1] proposed five "grand challenges" to stimulate imaginative and innovative research relevant to the safety of nanotechnology. Perhaps the two most relevant of these "grand challenges" relevant to this paper are (i) *Develop models for predicting the potentia impact of engineered nanomaterials on the environment and human health, within the next 10 years and (ii) Develop and validate methods to evaluate the toxicity of engineered nanomaterials, within the next 5–15 years.*

By design, many of the nanotechnology products in development or in use contain a metal (or metalloid in the case of arsenic) (see Table 1). We suggest that many of the concerns outlined above can be addressed with the approach of isotopic tracing, whereby a stable isotope of the element of interest is incorporated into the product allowing any transfer to be easily detected leveraging the recent advances in mass spectrometry using techniques such as inductively coupled mass spectrometry (ICP-MS), high resolution ICP-MS, multi-collector ICP-MS or thermal ionization mass spectrometry (TIMS). This approach differs from tracing methods using radio labelled metals, such as copper-64, which generally have a short half-life.

STABLE ISOTOPE TRACING

Stable isotopes are chemical isotopes that are not radioactive. The technique of stable isotope tracing has been widely used in the earth sciences to understand the origin of rocks and in ecological and biological studies. It has also been applied in nutritional and metabolic balance studies and health investigations [2].

We use the term "stable" for metal isotopes rather than its common application to the light stable isotopes of hydrogen, carbon, nitrogen and sulfur. There are two main approaches in the use of isotope tracing, one based on naturally occurring differences between stable isotopes and the other that uses the addition of a tracer of the separated isotope.

In the first approach, use is made of the variations in isotopic abundance of the stable end products arising from radioactive decay. For example, the stable end products of lead-206 (206Pb), 207Pb, and 208Pb are derived by long-term radioactive decay of parent's uranium-238 (238U), 235U, and thorium-232, respectively. The fourth lead isotope 204Pb has no known radioactive parent. Hence, over geological time, lead is formed into mineral deposits that have major differences in isotopic composition that can accumulate in the bones of inhabitants. Such naturally occurring isotopic differences have been used in recent investigations of the mobilization of lead from the maternal skeleton during pregnancy and lactation [3].

In this Australian study, investigators examined differences in the lead isotopic signature of long-term Australian residents and the environment exposed to geologically old mine lead (formed > 1,700 million years ago) and compared this with the signature in migrants to Australia, who were generally exposed to geologically young lead (formed ~ 300–400 million years ago). By monitoring blood and urine lead isotopic values during pregnancy and lactation and comparing these isotopic values with those in the environment (diet, air, water, soil, dust), it was possible to estimate the extra amount of lead coming from the maternal skeleton. Similarly, variations in the four isotopes of strontium (84Sr, 86Sr, 87Sr, 88Sr) have been used to investigate migration paths and dietary habits of humans and animals.

The second approach applies to elements that do not have a radioactive parent. In this case, there are negligible or very small variations in natural abundance between the different isotopes. To trace such elements, a stable non-radioactive isotope or tracer whose abundance is different from that occurring naturally is incorporated into the product. Many tracers are available from commercial suppliers and their price usually depends on the natural abundance, with the lower the natural abundance the higher the cost. This technique can be applied easily for some of the elements and compounds currently under being designed or in used as nanoparticles (see Table 1).

Where possible, if not cost-prohibitive, the tracer can be added to enhance the isotopic abundance to show a difference from that occurring naturally, thus making it easier to detect in a study. The ability to detect differences is dependent on a number of factors, including the purity of the separated tracer, the total amount of the element under investigation, and the sensitivity of the instruments for measuring the isotopic abundances. Thermal ionization mass spectrometry (TIMS) is considered to be the "gold standard" for isotopic measurements although multi-collector ICP-MS or high-resolution ICP-MS may also be suitable, depending on conditions.

POSSIBLE USES FOR ISOTOPIC TRACING IN NANOTECHNOLOGY

The latter of the above approaches offers the greatest potential for use in nanotechnology. For example, zinc oxide and titanium dioxide nanoparticles are being used in sunscreens and also other personal care products. Zinc has five isotopes whose naturally occurring abundances are listed in Table 1. 68Zn with a natural abundance of 18.6% is the isotope of choice for studies of zinc oxide because of cost considerations. We are in the process of conducting investigations using 68Zn and 46Ti as tracers in sunscreen products containing zinc oxide and titanium dioxide to determine *in vivo* their dermal absorption and excretion in humans.

Use of zinc isotopes is not just limited to sunscreen nanoparticles, as zinc is found in several other nanoparticles such as quantum dots which are being researched as potential artificial fluorophores for detection of tumors using fluorescence spectroscopy. In one product under development, the metals giving rise to the fluorescence of CdSe or cadmium telluride (CdTe), have a coating of ZnS to minimize exposure to the highly toxic Cd and potentially toxic Se or Te. There is some concern that the coating may degrade before the quantum dots are excreted from the body, but apart from mice and cellular studies, there are no human data [4].

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Table 1.
Some products used in nanotechnology and their natural relative isotopic abundances (%)

Product	Symbol	Isotope and natural relative abundances in brackets parentheses
Cadmium sulfide	CdS	Cd: 106 (1.2), 108 (0.9), 110 (12.4), 111 (12.8), 112 (24.0), 113 (12.3), 114 (28.8), 116 (7.6)
	S	S: 32 (95.0), 33 (0.75), 34 (4.2), 36 (0.02)
Cadmium selenide	CdSe	Se: 74 (0.9), 76 (9.0), 77 (7.5), 78 (23.5), 80 (50), 82 (9)
Cadmium telluride	CdTe	Te: 120 (0.09), 122 (2.4), 123 (0.87), 124 (4.6), 125 (7.0), 126 (18.7), 128 (31.8), 130 (34.5)
Calcium	Ca	Ca: 40 (96.9), 42 (0.65), 43 (0.14), 44 (2.08), 46 (0.003), 48 (0.19)
Chromium	Cr	Cr: 50 (4.35), 52 (83.8), 53 (9.5), 54 (2.36)
Iron	Fe	Fe: 54 (5.8), 56 (91.7), 57 (2.14), 58 (0.31)
Gallium phosphide/arsenide	GaP/GaAs	Ga: 69 (60), 71 (40)
Gallium antimonide/selenide/telluride	GaSb/Se/Te	Sb:121 (57.3), 123 (42.7)
Indium phosphide/arsenide/antimonide	InP/As/Sb	In: 113 (4.3), 115 (95.7)
Lead sulfide/selenide/telluride	PbS/Se/Te	Pb: 204 (1.4), 206 (24.1), 207 (22.1), 208 (52.4)
Magnesium	Mg	Mg: 24 (79), 25 (10), 26 (11)
Molydenum	Mo	Mo: 92 (14.8), 94 (9.1), 95 (15.9), 96 (16.7), 97 (9.5), 98 (24.4)
Silicon germanium/carbide	SiGe/C	Si: 28 (92.2), 29 (4.7), 30 (3.1);Ge: 70 (20.7), 72 (27.5), 73 (7.7), 74 (36.4), 76 (7.7)
Tantalum	Ta	Ta: 180 (0.01), 181 (99.9)
Silver	Ag	Ag: 107 (51.4), 109 (48.7)
Titanium dioxide	TiO2	Ti: 46 (8.0), 47 (7.5), 48 (73.7), 49 (5.5), 50 (5.3)
Tungsten	W	W: 180 (0.13), 182 (26.3), 183 (14.3), 184 (30.7), 186 (28.6)
Vanadium	V	V: 50 (0.25), 51 (99.7)
Zinc oxide/sulfide/selenide/telluride	ZnO/S/Se/Te	Zn: 64 (48.9), 66 (27.8), 67 (4.1), 68 (18.6), 70 (0.62)
Zirconium	Zr	Zr: 90 (51.4), 91 (11.2), 92 (17.1), 94 (17.5), 96 (2.8)

The integrity of the coating could be monitored by using either a single isotopic tracer such as 68Zn incorporated into the coating or an "overkill" that could employ a multiple isotopic approach, as Cd has eight naturally occurring isotopes, Se has six isotopes, Te has eight, isotopes (and Zn has five isotopes) (Table 1).

Stable isotope tracing for monitoring exposures to nanoproducts has many potential uses including the incorporation of a tracer in the production process to monitor worker exposure from isotopic measurements of wipes from areas such as protective clothing, hands, and face, or collection of biomarkers such as blood and urine. Furthermore, simple surface wipes and/or dust accumulation methods using petri dishes could be used for air monitoring exposures, followed by isotopic analysis.

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In addition to the occupational aspects there are concerns over the fate and transport of nanoparticles in the environment and/or cytotoxicity studies such as those undertaken by the National Toxicology Program could also be addressed using the stable isotope tracing approach.

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CONCLUSION

The stable isotope tracing approach has many advantages for use for monitoring purposes in the nanotechnology field including (a) many of the metals/metalloids currently used in nanotechnology have more than one isotope and this approach can be readily implemented (b) the stable, usually high-purity, isotopes used in such studies are non-radioactive (c) the stable isotopes do not have the disadvantage of radioactive tracers which commonly have very short half-lives and may give low radiation doses and thus allow long-term monitoring and (d) the methods for isotopic measurements of ICP-MS (and TIMS) are applicable to most metals and are routinely employed in environmental and health investigations.

While the initial investment to obtain the isotopic tracers may be high, the dividends of providing reliable scientific evaluations for monitoring these nanoparticles in environmental and biological systems will more than offset the initial cost.

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