APPLIED NANOPARTICLES

BioGAS+

Report on Safety and Toxicity of Iron Oxide Nanoparticles

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1. Summary

Superparamagnetic iron oxide nanoparticles are the only clinically approved metal oxide nanoparticles. Given that iron oxides magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) occur naturally as nano-sized crystals in the Earth's crust, it would seem that there is no intrinsic risk associated with these nanoparticles.

Magnetite nanoparticles have attracted much attention not only because of their superparamagnetic properties but also because they have been shown to have low toxicity in the human body. Currently, magnetite nanoparticles are used in a variety of biomedical applications, for example, magnetic resonance imaging, targeted delivery of drugs or genes, targeted destruction of tumor tissue through hyperthermia, magnetic transfections, iron detection, chelation therapy, anemia treatment and tissue engineering.

Several studies have examined the toxicity potential of several different types of magnetite nanoparticles with a range of surface coatings and have generally found low or no toxicity associated with these nanoparticles until high exposure levels (>100 mg/ml). The toxicity was also found to be dependent on various factors such as type of surface coating or its breakdown products, tail length, chemical composition of cell-medium, oxidation state and protein interaction.

In conclusion, many studies have shown that magnetite nanoparticles with a range of surface coatings have low or no toxicity except at very high levels of exposure.

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2. Remarks on nanoparticles safety and toxicity

DOES THE NANOFORM OF A SUBSTANCE ENTAIL AN INCRESED RISK?

How to deal with nanoparticles? This text is intended to describe the basics of nanosafety for the people working in the field.

Introduction. The surprising properties of NPs are fundamentally due to their high surface to volume ratio, finite size effects, collective behavior and interaction with light of any wavelength (for hyperthermia, diagnosis and imaging…). This results in a broad spectrum of chemical, physical, catalyst, optical and magnetic behaviors which can be sized for many uses. Interestingly, their exuberance of degenerated states at the macromolecular level allows to use them as versatile molecular sensors and actuators, as much as make them complicate to master. For similar reasons, nanoparticles are intrinsically unstable and may easily heterogeneously or homogeneously aggregate, chemically transform and corrode and disintegrate. To be exposed to biological systems, for a nanoparticle, it suffices to have few albumin proteins catching it and then they are introduced in physiological environments where they do not interfere much and many are dissolved and metabolized. In principle, it has been observed up to now that cells deal easily with tiny particles and no significant acute toxicity has been found in in vitro and in vivo studies at realistic doses unless toxic components were present in the formulations.

At the origin of nanotoxicity and nanosafety concerns, it was pointed at the well-known fact that cells have problems to deal with *micrometric* unsoluble particles. As asbestos fibers, with dimensions greater than 20 micrometers, up to hundreds, induce frustrated phagocytosis[1], chronic inflammation, asbestosis, and years later, cancer. This is not the case for small, sub-micrometric particles. A concern then was if small NPs could accumulate and aggregate up to such dangerous sizes. In this regard, dose and persistency are key to determine this potential risk. If the nanoparticles do not aggregate, they may dissolve. When they dissolve they yields ions (metal cations) that may be toxic, as in the well-known case

[1] https://diamondenv.wordpress.com/2011/04/15/frustrated-phagocytes-and-the-fibre-paradigm/

of cadmium or silver. In parallel, the corrosion process is a REDOX[2] active process that may stress the cell environment. However this effect has been observed to be transient and only significant at rather high doses. Therefore, regarding nanotoxicity, and the associated risks to work with nanoparticles, Today knowledge indicates that many nanoparticles in their intended uses do not need special care beyond being treated as other chemical substances, even if some particularities may apply (*vide infra*). Nanotoxicity is a young field that can be considered about 10 years old. Despite this youth, many knowledge from metal toxicity, sarcoidosis, asbestosis, silicosis, environmental pollution and other disciplines have contributed significantly to the rapid establishment of the nanosafety discipline. It is also important to be aware that simple nanoparticulate materials have been used in consumer products for long as food additives (E-171 to E-175 nanometric iron oxide, aluminium oxide, titanium dioxide, silver and gold), in cosmetics, as simple as talc, catalysis, paint pigments, coatings and other. Up to now, we have been mainly reproducing, at ease, nanomaterials that are already existing in nature of that somehow it was already produced by man in a more imperfect and unaware manner. Small, of about 20 nm iron oxide nanoparticles have been found in natural unpolluted soils or inside bacterial magnetosomes. When we get next generations of nanoparticles, additionals cares, will be needed.

Before that, and as no acute effects have been observed or identified, more subtle effects will need to be investigated. Also, these results are related to healthy conditions and acute doses. Thus, despite the absence of signs of alarm, it is desirable to perform long term studies at chronic and subtoxic doses and in compromised states (when the body is weakened by disease). Alterations of the immune system and changes in the biodistribution in the case of inflammation might exhacerbate or suppress it and accumulate in organs (if the nanoparticles succeed in entering the body, what is very unusual, even after dermal contact or ingestion). Thus, chronic exposure at subtoxic doses, long term effects, repeated doses, or co-exposure of different types of nanoparticles and other toxins (as LPS, allergens or chemical toxins) or exposure of nanoparticles in the case of disease, e.g.: during cirrhosis NP exposure may be more critical and need to be studied. Focus has to be put also on the immune system, which is responsible to detect, categorize and manage external invasion. The immune system has memory, so repeated exposure to nanoparticles could alter immune response.

Nanoparticles may exist in different forms during their full life cycle, normally: pristine (as synthesized), functionalized (ready to be used and during use), disposed and degraded (after use). The exposure and biological effects depend on the state of the NP at each point of their life. In none of these forms iron oxide nanoparticles have been found toxic unless they were functionalized with toxic moieties.

While it has been observed that nanoparticles do not penetrate the skin and are not up-taken after ingestion, concerns remain with respect to pulmonary exposure. It is the ability of small dry nanoparticles to be aerosolized from dry powders and enter the lungs. Experimental studies in animals have shown that at equivalent mass doses, poorly soluble nanostructured metal oxides in the form of

[2] https://en.wikipedia.org/wiki/Redox

agglomerated or aggregated nanoparticles (e.g., titanium dioxide, aluminum oxide, and manganese dioxide) are more potent in animals than equivalent single particles of similar composition in causing pulmonary inflammation and tissue damage. For these and other poorly soluble particles, a consistent dose-response relationship is observed when dose is expressed as particle surface area. These animal studies suggested that for nanostructured materials and larger particles with similar chemical properties, the toxicity of a given mass dose will increase with decreasing particle size due to the increasing surface area. Therefore, the breathing of solid nanoparticles, specially aggregates made of persistent materials, is highly unadvisable. However, even for poorly soluble particles of relatively low toxicity, some animal studies have identified doses that were not associated with observed adverse responses. For example, a recent animal study reported mass doses of either fine or nanostructured TiO_2 in rats at which the lung responses did not significantly differ from controls, while crystalline silica caused more severe lung responses at the same mass dose. In addition to particle size and surface area, other physical and chemical properties of particles are known to influence biological interactions, including solubility, shape, surface reactive sites, charge, and crystal structure.[3] Note that this is not the case of BioGAS+ which is made of non-persistent nanoparticles, they are not aggregated and they do not carry toxic moieties or toxic additives or toxic excipients.

In the following the main associated causes of nanoparticle induced toxicity are listed. In principle, at realistic doses in a controlled manner, inorganic nanoparticles have basically shown toxicity due to aggregation or dissolution, or because they were carrying toxic moieties.

i.- TOXICITY has been observed in the case of some cationic (positively charged) NPs. This is well known for both biological (antimicrobial peptides) and micrometric (organic) particles where cationic charge at their surface makes them to interact strongly with cell membranes interfering thus with its normal functioning and inducing cell death. See for example ref[4]. The charge is carried by molecules attached to the surface or by the inorganic surface itself if it is at pH lowers than the nanoparticle isoelectric point. Toxicity has only been observed when the cationic charge is maintained in the physiological media. In the case of BioGAS+, it is prepared at basic pH displaying a negatively charged surface which becomes neutral when dispersed in the working environment. At acidic pH, where the BioGAS+ nanoparticles would present positive surface charge, they dissolve.

ii.- TOXICITY has been related to aggregation. Aggregates caused direct acute toxicity when mice were intratracheally instilled with carbon-nanotubes and they suffocated due to tracheal clogging, indicating the poor dispersability of hydrophobic nanostructures in biological systems. Besides, risks have been observed in the case of penetration of non-biodegradable persistent micrometric particles (in principle bigger than 20 micrometers) in the lungs and related with frustrated phagocytosis and the onset of

[3] NANO TC 229 WG 3 072-2007_Revised Draft TR Health and Safety Practices 2007-10-19-1

[4] Chitosan functionalisation of gold nanoparticles encourages particle uptake and induces cytotoxicity and pro-inflammatory conditions in phagocytic cells, as well as enhancing particle interactions with serum components. *Journal of nanobiotechnology* 2015, 13 (1), 84.

chronic inflammation as in the case of silicosis, granulomatosis and asbestosis. When a strange object is detected by the immune system, and not categorized as danger, it is simply phagocytized and removed away from the biological machinery being it denaturalized protein aggregates or cell debries. A typical nanoparticle is 10 nm, a viruses 100 nm, a bacteria 1000 nm and a eukariota cell 10.000 nm. However, when the object is too big (for sizes beyond 10 micrometers), the immune cells cannot engulf it and then starts a chemical defense against the non-biodegradable material. This leads to tissue irritation and in the long run, may cause cancer. Needle-like microparticles -as asbestos 500 x 10 microns- are especially effective to induce this effect.

In determined conditions, nanoparticles could aggregate to micrometric sizes. Besides, as the size increases, the likeliness for exposure and particle penetration also decreases. There are many strategies to avoid aggregation developed for decades in different fields of material science and chemistry. There is two simple ways to avoid aggregation, to avoid high concentrations (if there is few NPs, it is difficult that NPs meet to grow and form an aggregate) and use anti-aggregation agents. Aggregation is a phenomenon driven by the reduction of the high energy surface of the nanoparticles. Absorption of molecules which provide electrostatic charge or steric repulsion to the nanoparticle serve to maintain them isolated even at high concentrations. Therefore, in complex media, it is observed that nanoparticles are rapidly coated by molecules from the environment, their surface energy decreased and their tendency for aggregation cancelled. In the case of BioGAS+ only when the material is prepared there are risks of aggregation, no once they have been dispersed in the working environment. Likely, when inorganic nanoparticles are dispersed in serum they are rapidly coated by proteins (forming the so-called protein corona) what avoids their further aggregation, what would be always subject of concern. Fortunately, nanoparticles they do not cross the skin and do they not get inside the body from the intestinal track (we have been eating soil for ages and naturally small nanoparticles form and dissolve or aggregate constantly). The critical point here are clearly the lungs, even if the mucociliary escalatory system may be effective in removing foreign matter from the lungs (specially small nanoparticles). Therefore, it is not recommended to be exposed to nanoparticle aerosols, and for that, it would be enough to avoid working in the dry phase. Also, aggregation can be programmed, for example as a way of disposal, producing aggregates which are larger than the micrometric critical size and easily operable as bulk materials.

iii.- TOXICITY has been observed when the NP act as a reservoir of toxic ions that are delivered during corrosion. The paradigmatic case are CdSe nanoparticles which *become more toxic* with time, as they corrode and yield Cd ions. Besides, the corrosion process itself is redox active and induces the production of reactive oxygen species (ROS). Indeed, to dissipate surface energy, if the nanoparticles do not aggregate or associate with coating molecules, many of them will disintegrate. This is a common phenomenon in nature and widely studied by geochemistry where a nanoparticle is an intermediate state between the micrometric particle and the dissolved ions. Or like in microbiology, where bacteria synthesize small inorganic nanoparticles of toxic ions to detoxify the environment. Changes in the surroundings when the nanoparticle leaves the synthesis environment lead many nanoparticles to

disintegration, by corrosion and other chemical transformation that dissolves it. In this process the nanoparticle yield ions, and also may yield electrons. Electrons are reactive and generate reactive species which may be toxic if sustained for a long time (if the stress causing the response is mantained). Some metallic cations are often bioactive, as an example, cadium, mercury and lead cations are very toxic to us, nickel is allergenic, cobalt is carcinogenic, silver cations are toxic to bacteria and cupper ions are toxic to fungi (and fungi are toxic to bacteria). Besides, iron is a common ion in biological systems at very high concentrations. Indeed, the slow dissolution of iron oxide nanoparticles into iron ions has make iron nanoparticles (feromuxytol) an active principle to treat ferropenic anemia to control the dosing at the molecular level. Basically, the pattern of exposure, the dosing profile, is different when using directly the ionic species or when those are provided by a dissolving nanoparticle.

iv.- TOXICITY has been related to the capacity of nanoparticles in presenting antigens or allergens. NPs can be good aggregators and orientators of molecules to be presented to the immune system[5]. Indeed, nanoparticles are excellent molecular carriers and a whole scientific field is developing around it. And this could be a cause of major concern if functionalizable nanoparticles were dispersed in the environment and, unfortunately, they will associate with antigens or allergens before homogeneous or heterogeneous aggregation. For example, when the car combustion emission microparticles are coated by pollen grains, they become ultra-allergenic. This is one of the reasons why allergies in the urban areas are more intense than outside in the country side. Therefore, the nanoparticle surface has to be passivated before uncontrolledly dispersed. Fortunately, the concentration of toxins in the environment in comparison with the rest of the inert or tolerable molecules is very low and the promiscuity of the nanoparticle surface very high, so it would be complicated that naked nanoparticles meet toxins, antigen or allergens before their surface is passivated by other molecules.

v.- TOXICITY has been associated also with catalysis, especially in the case of photocatalysis and NPs as TiO_2 that are able to generate toxic free radicals when illuminated. Catalysis is a surface phenomenon and the high surface to volume ratio of small nanoparticles has been exploited for years in the chemical industry. Despite the natural suitability of inorganic nanoparticles for catalysis, it is well known that it is unexpected that nanoparticles will act as powerful catalyst unless they are designed to do so. Indeed, normally, nanoparticle surfaces are rapidly passivated with organic molecules that interface the inorganic core with the environment. Lacking that, a protecting layer, nanoparticles life is extremely brief and they absorb irreversibly or vanish. In this case, the protecting layer dumps the catalytic powers of the inorganic nanoparticle. In any case, iron oxide nanoparticles are not photocatalyst, nor considered affective catalyst with some exceptions for oxidation reactions.

vi.- TOXICITY has been related to hydrophobicity, and since hydrophobic substances hardly disperse in biological environments, attention only has to be paid to amphiphilic or detergent-like molecules that can be transported by nanoparticles. Is the well-known case of gold nanoparticles coated with a cationic

[5] Homogeneous conjugation of peptides onto gold nanoparticles enhances macrophage response *ACS nano* 2009, 3 (6), 1335-1344

detergent like CTAB[6]. This detergent forms a double layer vesicle-like coating on top at the nanoparticle surface and can be dispersed in biological environments and then, in contact with cells, they may expose their hydrophobic core to the cell membrane (which has also a vesicle like structure with an inner hydrophobic core) perturbing it. These are similar effects as those observed with pure detergent molecules; however, it would happen at lower detergent doses in the case of association to nanoparticles. Therefore it seems not advisable the unintended mix of detergents and nanoparticles. Note that detergents are already poorly biocompatibles, fortunately, normally, highly biodegradables.

vii.- TOXICITY has been related to breathing dry (powdered) nanoparticles (and its aggregates). Because of it, it is recommended not to work in the dry phase, but always wet. In a study of chemical contamination in the laboratory by electron microscopy and ICPMS, dispersion of the nanoparticles from the liquid phase was not observed. The conclusions were that once the nanoparticles have been somewhere, tiny rest remains for ever even after washing (similarly happens with ions) but that there were no cross-contamination, even at extreme proximity from the vessels and vials that contained it. The ambient filters and air purifiers where empty of observable nanoparticles (other than the micrometric particles of dust), concluding that the nanoparticles can not leave from the wet phase. At the same time, it has been observed that ultrafine powders of nanoparticles are easily aerosolized and transported long distances.

viii.- TOXICITY can be observed if tissue is irradiated when nanoparticles are present. The only toxicity related to irradiation of a nanoparticle containing body is related to the increased dosing of the received radiation. Therefore one should not be exposed to radiation, magnetic hyperthermia in the case of superparamagnetic nanoparticles or x-ray radiotherapy in the case of heavy metal nanoparticles. Note that for MRI imaging superparamagnetic nanoparticles are used as safe contrast agents.

3. Information, documents and selected works on iron oxides NPs toxicity and safety

Iron oxides nanoparticles have been officially labelled as:

- Not a hazardous substance or mixture according to Regulation **(EC) No. 1272/2008**;
- Not a hazardous substance or mixture according to **EC-directives 67/548/EEC**
- Not a hazardous substance or mixture according to **EC-directives 1999/45/EC**
- Not a dangerous substance or mixture according to the **Globally Harmonised System** (GHS, http://www.unece.org/trans/danger/publi/ghs/ghs_welcome_e.html)
- No Carcinogenic by the International Agency for Research on Cancer (IARC) :

[6] http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2988217/

"No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC."

Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Geneva: World Health Organization, International Agency for Research on Cancer, 1972-PRESENT. (Multivolume work). Available at: http://monographs.iarc.fr/ENG/Classification/index.php p. S7 216 (1987)]

- **Material Safety Data Sheet for Magnetite Nanoparticle (MSDS) by NanoComposix available at** http://nanocomposix.com/pages/magnetite-nanoparticle-safety

- **Safety and toxicity information on Ferraheme® (ferumoxsil), a superparamagnetic iron oxide nanoparticles solution for intravenous injection for the treatment of iron deficiency anemia in adult patients with chronic kidney disease:** http://www.amagpharma.com/products/feraheme/

- **Information on Nanotherm, a concentrated solution of magnetite Nanoparticles for hyperthermia for cancer treatment:** http://www.magforce.de/en/produkte/nanothermr.html

Selected publications on iron oxides NPs toxicity and safety

- **Title: Physical and chemical properties of superparamagnetic iron oxide MR contrast agents: ferumoxides, ferumoxtran, ferumoxsil.**
  Jung CW1, Jacobs P. *Magn Reson Imaging. 1995;13(5):661-74.*
  Abstract:
  The bulk physiochemical properties of the active ingredients in three AMI colloidal, superparamagnetic iron oxide (SPIO), MR contrast agents are described. Ferrous content and X-Ray diffraction (XRD) of the colloids are consistent with nonstoichiometric magnetite phases in all three active ingredients. No separate maghemite (gamma-Fe_2O_3) phases were detected by XRD. XRD line-broadening determinations of representative samples of ferumoxides (dextran coated), Ferumoxtran (dextran covered), and ferumoxsil (siloxane coated) yielded mean crystal diameters (volume weighted distribution) of 4.8-5.6, 5.8-6.2, and 7.9-8.8 nm, respectively. Transmission electron microscopy (TEM) showed that the crystal sizes were lognormally distributed with respective mean crystal diameters (number weighted distribution) of 4.3-4.8, 4.3-4.9, and 8.0-9.5 nm, respectively. Consistent with their small crystal sizes, the three SPIO colloids are superparamagnetic with no remanence after saturation at high applied fields (< 1 T), and showed characteristic relaxed Mössbauer spectra. The Mössbauer spectra of ferumoxides and Ferumoxtran were consistent with the

presence of superparamagnetic relaxation above a blocking temperature of approximately 60 K. Due to the larger crystal sizes of ferumoxsil, its Mössbauer spectra showed the presence of rapid collective magnetic excitations on the Mössbauer time scale (approximately 1-10 ns). All three colloids showed high MR relaxivities. TEM of the SPIO colloids showed that ferumoxides and ferumoxsil are composed of aggregates of nonstoichiometric magnetite crystals, while Ferumoxtran consists of single crystals of nonstoichiometric magnetite. Dynamic light scattering (PCS) measurements showed that Ferumoxtran particles have average hydrodynamic diameters of approximately 21 nm (number weighted distribution) or 30 nm (volume weighted distribution). The data indicate that Ferumoxtran crystals are coated with an 8-12 nm layer of dextran T-10. Ferumoxides aggregates have average particle sizes of approximately 35 nm (number average distribution; TEM and PCS), or approximately 50 nm (volume weighted distribution; PCS). Mean sizes of ferumoxsil aggregates are approximately 300 nm (intensity weighted distribution). A discussion of the various particle size distributions is presented.

- **Title: Ultrasmall cationic superparamagnetic iron oxide nanoparticles as nontoxic and efficient MRI contrast agent and magnetic-targeting tool.**
  Uchiyama MK et al. *Int J Nanomedicine. 2015 Jul 28;10:4731-46*
  Abstract:
  Fully dispersible, cationic ultrasmall (7 nm diameter) superparamagnetic iron oxide nanoparticles, exhibiting high relaxivity (178 mM-1s-1 in 0.47 T) and no acute or subchronic toxicity in Wistar rats, were studied and their suitability as contrast agents for magnetic resonance imaging and material for development of new diagnostic and treatment tools demonstrated. After intravenous injection (10 mg/kg body weight), they circulated throughout the vascular system causing no microhemorrhage or thrombus, neither inflammatory processes at the mesentery vascular bed and hepatic sinusoids (leukocyte rolling, adhesion, or migration as evaluated by intravital microscopy), but having been spontaneously concentrated in the liver, spleen, and kidneys, they caused strong negative contrast. The nanoparticles are cleared from kidneys and bladder in few days, whereas the complete elimination from liver and spleen occurred only after 4 weeks. Ex vivo studies demonstrated that cationic ultrasmall superparamagnetic iron oxide nanoparticles caused no effects on hepatic and renal enzymes dosage as well as on leukocyte count. In addition, they were readily concentrated in rat thigh by a magnet showing its potential as magnetically targeted carriers of therapeutic and diagnostic agents. Summarizing, cationic ultrasmall superparamagnetic iron oxide nanoparticles are nontoxic and efficient magnetic resonance imaging contrast agents useful as platform for the development of new materials for application in theranostics.

- **Title: The suitability of different cellular in vitro immunotoxicity and genotoxicity methods for the analysis of nanoparticle-induced events.**
  Nanotoxicology. 2010 Mar;4(1):52-72.
  Abstract:
  Suitable assays and test strategies are needed to analyze potential genotoxic and immunotoxic health effects caused by nanoparticle exposure. The development and validation of such methods is challenging because nanoparticles may show unexpected behavior, like aggregation or interference with optical measurements, when routine in vitro assays are performed. In our interdisciplinary study, the effects of inorganic gold (4.5 nm) and iron oxide (7.3 nm) nanoparticles with a narrow size distribution were tested on human cells using different assay systems. The results show that cytotoxicity as well as immunotoxicity and genotoxicity induced by these two inorganic nanoparticles was low or absent when using a panel of cell-based tests in different laboratories. However, several technical issues had to be tackled that were specific for working with nanoparticles. The methods used, their suitability for nanotoxicity testing, and the technical problems encountered are carefully described and discussed in this paper.

- **Title: Nanotechnology and in Situ Remediation: A Review of the Benefits and Potential Risks.**
  Barbara Karn, Todd Kuiken and Martha Otto *Environmental Health Perspectives Vol. 117, No. 12 (Dec., 2009), pp. 1823-1831*
  Abstract:
  Although industrial sectors involving semiconductors; memory and storage technologies; display, optical, and photonic technologies; energy; biotechnology; and health care produce the most products that contain nanomaterials, nanotechnology is also used as an environmental technology to protect the environment through pollution prevention, treatment, and cleanup. In this review, we focus on environmental cleanup and provide a background and overview of current practice; research findings; societal issues; potential environment, health, and safety implications; and future directions for nanoremediation. We do not present an exhaustive review of chemistry/engineering methods of the technology but rather an introduction and summary of the applications of nanotechnology in remediation. We also discuss nanoscale zero-valent iron in detail. Data Sources: We searched the Web of Science for research studies and accessed recent publicly available reports from the U.S. Environmental Protection Agency and other agencies and organizations that addressed the applications and implications associated with nanoremediation techniques. We also conducted personal interviews with practitioners about specific site remediations. Data Synthesis: We aggregated information from 45 sites, a representative portion of the total projects under way, to show nanomaterials used, types of pollutants addressed, and organizations responsible for each site. Conclusions: Nanoremediation has the potential not only to reduce the overall costs of cleaning up large-scale contaminated sites but also to reduce cleanup time, eliminate the need for treatment

and disposal of contaminated soil, and reduce some contaminant concentrations to near zero-all in situ. Proper evaluation of nanoremediation, particularly full-scale ecosystem-wide studies, needs to be conducted to prevent any potential adverse environmental impacts.

- **Title: Ecotoxicity of, and remediation with, engineered inorganic nanoparticles in the environment.**
  A. Sanchez , S. Recillas, et al. (2011). *TrAC Trends in Analytical Chemistry 30(3): 507-516.*
  Abstract:
  This article presents recent developments on the use of inorganic nanoparticles (NPs) for environmental remediation in polluted soil, water and gas. The number of publications on these topics has grown exponentially in recent years, especially those focused on wastewater treatment. Among these topics, removal of metals has become the most popular, although some works relate to the use of nanomaterials for the elimination of nutrients (e.g., nitrogen and some persistent organic pollutants). However, this growth has not been accompanied by knowledge about the behavior of NPs once used and released into the environment. In this article, we also comment upon the current situation with respect to NP toxicology (nanotoxicology). A remarkable number of different toxicology tests has been applied to NPs, often making it very difficult to interpret or to generalize the results. We analyze in detail the bioluminescence, Daphnia magna and other tests, and give some preliminary results obtained in our work.

- **Title: Superparamagnetic iron oxide nanoparticles: diagnostic magnetic resonance imaging and potential therapeutic applications in neurooncology and central nervous system inflammatory pathologies, a review**
  J S Weinstein et al. *J Cereb Blood Flow Metab. 2010 Jan; 30(1): 15–35.*
  Abstract:
  Superparamagnetic iron oxide nanoparticles have diverse diagnostic and potential therapeutic applications in the central nervous system (CNS). They are useful as magnetic resonance imaging (MRI) contrast agents to evaluate: areas of blood–brain barrier (BBB) dysfunction related to tumors and other neuroinflammatory pathologies, the cerebrovasculature using perfusion-weighted MRI sequences, and in vivo cellular tracking in CNS disease or injury. Novel, targeted, nanoparticle synthesis strategies will allow for a rapidly expanding range of applications in patients with brain tumors, cerebral ischemia or stroke, carotid atherosclerosis, multiple sclerosis, traumatic brain injury, and epilepsy. These strategies may ultimately improve disease detection, therapeutic monitoring, and treatment efficacy especially in the context of antiangiogenic chemotherapy and antiinflammatory medications. The purpose of this review is to outline the current status of superparamagnetic iron oxide nanoparticles in the context of biomedical nanotechnology as they apply to diagnostic MRI and potential therapeutic applications in neurooncology and other CNS inflammatory conditions.

- **Title: Review: Biocompatibility and Toxicity of Magnetic Nanoparticles in Regenerative Medicine**
  H. Markides, M. Rotherham, and A. J. El Haj *Journal of Nanomaterials, vol. 2012, Article ID 614094*
  Abstract:
  Regenerative medicine is a pioneering field aimed at restoring and regenerating the function of damaged cells, organs and tissues in order to establish normal function. It demands the cross communication of disciplines to develop effective therapeutic stem cell based therapies. Nanotechnology has been instrumental in the development and translation of basic research to the clinically relevant therapies. In particular, magnetic nanoparticles (MNPs) have been applied to tag, track and activate stem cells offering an effective means of monitoring in vitro and in vivo behaviour. MNPs are comprised of an iron oxide core with a biocompatible biological polymer. Safety is an issue of constant concern and emphasises on the importance of investigating the issue of toxicity. Any indication of toxicity can ultimately limit the therapeutic efficiency of the therapy. Toxicity is highly dependent on the physical, chemical and structural properties of the MNP itself as well as dose and intended use. Few in vitro studies have reported adverse effects of MNP on cells at in vitro in therapeutic doses. However, long term in vivo studies have not been studied as extensively. This review aims to summarise current research in this topic highlighting commonly used toxicity assays to investigate this.

- **Title: Review: Potential toxicity of superparamagnetic iron oxide nanoparticles (SPION)**
  N. Singh et al. *Nano Reviews Vol 1 (2010) incl Supplements*
  Abstract:
- Superparamagnetic iron oxide nanoparticles (SPION) are being widely used for various biomedical applications, for example, magnetic resonance imaging, targeted delivery of drugs or genes, and in hyperthermia. Although, the potential benefits of SPION are considerable, there is a distinct need to identify any potential cellular damage associated with these nanoparticles. Besides focussing on cytotoxicity, the most commonly used determinant of toxicity as a result of exposure to SPION, this review also mentions the importance of studying the subtle cellular alterations in the form of DNA damage and oxidative stress. We review current studies and discuss how SPION, with or without different surface coating, may cause cellular perturbations including modulation of actin cytoskeleton, alteration in gene expression profiles, disturbance in iron homeostasis and altered cellular responses such as activation of signalling pathways and impairment of cell cycle regulation. The importance of protein–SPION interaction and various safety considerations relating to SPION exposure are also addressed.

- **Title: Biocompatibility of Fe_3O_4 nanoparticles evaluated by in vitro cytotoxicity assays using normal, glia and breast cancer cells**
  B. Ankamwar et al. *Nanotechnology. 2010 Feb 19;21(7):75102*

Abstract:
In order to reveal the biocompatibility of Fe(3)O(4) nanoparticles and bipolar surfactant tetramethylammonium 11-aminoundecanoate cytotoxicity tests were performed as a function of concentration from low (0.1 microg ml(-1)) to higher concentration (100 microg ml(-1)) using various human glia, human breast cancer and normal cell lines. Cytotoxicity tests for human glia (D54MG, G9T, SF126, U87, U251, U373), human breast cancer (MB157, SKBR3, T47D) and normal (H184B5F5/M10, WI-38, SVGp12) cell lines exhibited almost nontoxicity and reveal biocompatibility of Fe_3O_4 nanoparticles in the concentration range of 0.1-10 microg ml(-1), while accountable cytotoxicity can be seen at 100 microg ml(-1). The results of our studies suggest that Fe_3O_4 nanoparticles coated with bipolar surfactant tetramethylammonium 11-aminoundecanoate are biocompatible and promising for bio-applications such as drug delivery, magnetic resonance imaging and magnetic hyperthermia.

- **Title: A new approach for the in vitro identification of the cytotoxicity of superparamagnetic iron oxide nanoparticles**
  M. Mahmoudi et al. *Colloids Surf B Biointerfaces. 2010 Jan 1;75(1):300-9*
  Abstract:
  Superparamagnetic iron oxide nanoparticles (SPIONs) are increasingly used in medical applications, such as targeting delivery and imaging. In the future, patients are more likely to be exposed to pharmaceutical products containing such particles. The study of toxicity of SPIONs has become of great importance in recent years, although the published data in this arena is limited. The aim of the present work is to investigate the cytotoxicity of SPIONs and the effect of the particles on the cell medium components. For this purpose, uncoated and polyvinyl alcohol (PVA) coated SPIONs with narrow size distribution were synthesized via a well-known coprecipitation method. The mouse fibroblast cell line L929 was exposed to SPIONs to probe the toxicity of magnetic nanoparticles during the bio application. Changes to the cell medium caused by SPIONs were analyzed with zeta potential measurements, ultraviolet visible spectroscopy (UV/vis) and the 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide (MTT) assay. It is observed that gas vesicles are formed in SPION-treated cells. Toxicity is conventionally explained by changes in the DMEM's pH and composition due to the tendency of SPIONs to interact with biomolecules. A new procedure is proposed to examine the in vitro toxicity of nanoparticles in a more rigorous manner, which gives an improvement in the relationship between in vivo and in vitro toxicity studies.

- **Title: Subchronic inhalation toxicity of iron oxide (magnetite, Fe3O4) in rats: pulmonary toxicity is determined by the particle kinetics typical of poorly soluble particles**
  J. Pauluhn *Appl. Toxicol. 2012, 32, 488-504*
  Abstract:
  Wistar rats were nose-only exposed to pigment-sized iron oxide dust (Fe3O4, magnetite) in a subchronic 13-week inhalation study according to the OECD testing guidelines TG#413 and

GD#39. A 4 week pilot study with a 6 month post exposure period served as basis for validating the kinetic modeling approaches utilized to design the subchronic study. Kinetic analyses made during this post exposure period demonstrated that a diminution in particle clearance and lung inflammation occurred at cumulative exposure levels exceeding the lung overload threshold. Animals were exposed 6 h per day, five days per week for 13 consecutive weeks at actual concentrations of 0, 4.7, 16.6 and 52.1 mg m−3 (mass median aerodynamic diameter ≈1.3 µm, geometric standard deviation = 2). The exposure to iron oxide dust was tolerated without mortality, consistent changes in body weights, food and water consumption or systemic toxicity. While general clinical pathology and urinalysis were unobtrusive, hematology revealed changes of unclear toxicological significance (minimally increased differential neutrophil counts in peripheral blood). Elevations of neutrophils in bronchoalveolar lavage (BAL) appeared to be the most sensitive endpoint of study. Histopathology demonstrated responses to particle deposition in the upper respiratory tract (goblet cell hyper- and/or metaplasia, intraepithelial eosinophilic globules in the nasal passages) and the lower respiratory tract (inflammatory changes in the bronchiolo-alveolar region). Consistent changes suggestive of pulmonary inflammation were evidenced by BAL, histopathology, increased lung and lung-associated-lymph node (LALN) weights at 16.6 and 52.1 mg m−3. Increased septal collagenous fibers were observed at 52.1 mg m−3. Particle translocation into LALN occurred at exposure levels causing pulmonary inflammation. In summary, the retention kinetics iron oxide reflected that of poorly soluble particles. The empirical no-observed-adverse-effect level (NOAEL) and the lower bound 95% confidence limit on the benchmark concentration (BMCL) obtained by benchmark analysis was 4.7 and 4.4 mg m−3, respectively, and supports an OEL (time-adjusted chronic occupational exposure level) of 2 mg m−3 (alveolar fraction).

- **Title: Comparison of SPIO and USPIO for in vitro labeling of human monocytes: MR detection and cell function**
  R.D. Oude Engberink et al. *Radiology. 2007 May;243(2):467-74.*
  Abstract:
  In vitro labeling of human monocytes is effective by using SPIOs, not USPIOs. Incubation with SPIOs (1.0 mg Fe/mL) results in efficient labeling detectable on MR images and does not affect cellular viability and activation markers such as cell migration and cytokine production.

- **Title: Clinical toxicities of nanocarrier systems**
  K.R. Vega-Villa, J.K. Takemoto et al. *Adv Drug Deliv Rev. 2008 May 22;60(8):929-38*
  Abstract:

Toxicity of nanocarrier systems involves physiological, physicochemical, and molecular considerations. Nanoparticle exposures through the skin, the respiratory tract, the gastrointestinal tract and the lymphatics have been described. Nanocarrier systems may induce cytotoxicity and/or genotoxicity, whereas their antigenicity is still not well understood. Nanocarrier may alter the physicochemical properties of xenobiotics resulting in pharmaceutical changes in stability, solubility, and pharmacokinetic disposition. In particular, nanocarriers may reduce toxicity of hydrophobic cancer drugs that are solubilized. Nano regulation is still undergoing major changes to encompass environmental, health, and safety issues. The rapid commercialization of nanotechnology requires thoughtful environmental, health and safety research, meaningful, and an open discussion of broader societal impacts, and urgent toxicological oversight action.

- **Title: New Applications of Nanotechnology for Neuroimaging.**
  G. Suffredini, J.E. East and L.M. Levy *AJNR Am J Neuroradiol. 2014 Jul;35(7):1246-53*
  Abstract:
  Advances in nanotechnology have the potential to dramatically enhance the detection of neurologic diseases with targeted contrast agents and to facilitate the delivery of focused therapies to the central nervous system. We present the physicochemical rationale for their use, applications in animal models, and ongoing clinical trials using these approaches. We highlight advances in the use of nanoparticles applied to brain tumor imaging, tumor angiogenesis, neurodegeneration, grafted stem cells, and neuroprogenitor cells

- **Title: Cardioprotective activity of iron oxide nanoparticles.**
  Fei Xiong, Hao Wang et al. *Scientific Reports 5, Article number: 8579 (2015)*
  Abstract:
  Iron oxide nanoparticles (IONPs) are chemically inert materials and have been mainly used for imaging applications and drug deliveries. However, the possibility whether they can be used as therapeutic drugs themselves has not yet been explored. We reported here that Fe2O3 nanoparticles (NPs) can protect hearts from ischemic damage at the animal, tissue and cell level. The cardioprotective activity of Fe2O3 NPs requires the integrity of nanoparticles and is not dependent upon their surface charges and molecules that were integrated into nanoparticles. Also, Fe2O3 NPs showed no significant toxicity towards normal cardiomyocytes, indicative of their potential to treat cardiovascular diseases.

- **Title: Medical application of functionalized magnetic nanoparticles**
  A. Ito, M. Shinkai, H. Honda and T. Kobayashi *J Biosci Bioeng. 2005 Jul;100(1):1-11.*
  Abstract:
  Since magnetic particles have unique features, the development of a variety of medical applications has been possible. The most unique feature of magnetic particles is their reaction to a magnetic force, and this feature has been utilized in applications such as drug

targeting and bioseparation including cell sorting. Recently, magnetic nanoparticles have attracted attention because of their potential as contrast agents for magnetic resonance imaging (MRI) and heating mediators for cancer therapy (hyperthermia). Magnetite cationic liposomes (MCLs), one of the groups of cationic magnetic particles, can be used as carriers to introduce magnetite nanoparticles into target cells since their positively charged surface interacts with the negatively charged cell surface; furthermore, they find applications to hyperthermic treatments. Magnetite nanoparticles conjugated with antibodies (antibody-conjugated magnetoliposomes, AMLs) are also applied to hyperthermia and have enabled tumor-specific contrast enhancement in MRI via systemic administration. Since magnetic nanoparticles are attracted to a high magnetic flux density, it is possible to manipulate cells labeled with magnetic nanoparticles using magnets; this feature has been applied in tissue engineering. Magnetic force and MCLs were used to construct multilayered cell structures and a heterotypic layered 3D coculture system. Thus, the applications of these functionalized magnetic nanoparticles with their unique features will further improve medical techniques.

- **Title: Biodistribution, clearance, and biocompatibility of iron oxide magnetic nanoparticles in rats**

  T. K. Jain et al. *Mol Pharm. 2008 Mar-Apr; 5(2):316-27*

  Abstract:

  It is essential to determine the biodistribution, clearance, and biocompatibility of magnetic nanoparticles (MNPs) for in vivo biomedical applications to ensure their safe clinical use. We have studied these aspects with our novel iron oxide MNP formulation, which can be used as a magnetic resonance imaging (MRI) agent and a drug carrier system. Changes in serum and tissue iron levels were analyzed over 3 weeks after intravenous administration of MNPs to rats. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AKP) levels, and total iron-binding capacity (TIBC) were also measured with time to assess the effect of MNPs on liver function. Selected tissues were also analyzed for oxidative stress and studied histologically to determine biocompatibility of MNPs. Serum iron levels gradually increased for up to 1 week but levels slowly declined thereafter. Biodistribution of iron in various body tissues changed with time but greater fraction of the injected iron localized in the liver and spleen than in the brain, heart, kidney, and lung. Magnetization measurements of the liver and spleen samples showed a steady decrease over 3 weeks, suggesting particle degradation. Serum showed a transient increase in ALT, AST, AKP levels, and TIBC over a period of 6-24 h following MNP injection. The increase in oxidative stress was tissue dependent, reaching a peak at approximately 3 days and then slowly declining thereafter. Histological analyses of liver, spleen, and kidney samples collected at 1 and 7 days showed no apparent abnormal changes. In conclusion, our MNPs did not cause long-term changes in the liver enzyme levels or induce oxidative stress and thus can be safely used for drug delivery and imaging applications.

- **Title: Proton-Promoted Iron Dissolution from Nanoparticles and the Influence by the Local Iron Environment.**

  J. T. N. Knijnenburg et al. *J. Phys. Chem. C, 2014, 118 (41), pp 24072–24080*

  Abstract:

  Nanostructured iron-containing compounds are promising for food fortification and supplementation to alleviate iron deficiency due to their fast dissolution in dilute acid and high dietary iron bioavailability. Furthermore, when such compounds are encapsulated in a nano-CaO matrix, their dissolution rate is increased. Here the relation between that rate and iron structure (amorphous/crystalline Fe_2O_3, crystalline $Ca_2Fe_2O_5$, or monomeric Fe^{3+} inside CaO) is investigated. We used X-ray diffraction (XRD) and electron paramagnetic resonance (EPR) spectroscopy as complementary techniques to study the local iron environment in Ca/Fe oxides as a function of nanoparticle composition. Nanostructured mixed Ca/Fe oxide-containing powders were prepared by flame spray pyrolysis, and their dissolution over time in acidic solutions (pH 1 and 3) was monitored by EPR spectroscopy. Three types of Fe were distinguished in these as-prepared powders: monomeric Fe^{3+} and crystalline $Ca_2Fe_2O_5$ at low Fe content powders ($Ca:Fe \geq 3.6$) and amorphous/crystalline Fe_2O_3 at $Ca:Fe \leq 0.7$. During dissolution, monomeric Fe^{3+} and crystalline $Ca_2Fe_2O_5$ dissolved rapidly (<1 min), while crystalline Fe_2O_3 was more stable and only slowly released Fe^{3+} even at pH 1. The Fe release is discussed within a thermodynamic model based on the nanoparticle lattice energy for each of the nanocrystalline phases, revealing that Fe coordination and lattice dynamics play a more dominant role than particle size. Thus, we demonstrate that control of crystalline structure rather than “nanosizing” may be a prerequisite for rapid dissolution of ferric iron from nanoparticles.