



# RRI Case Study

APPLIED NANOPARTICLES SL: SPINNING OFF  
UNDER RESPONSIBLE RESEARCH AND  
INNOVATION (RRI) PRINCIPLES

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## Executive Summary

Applied Nanoparticles SL (AppNP) is a science-based spin off company founded on 17<sup>th</sup> October 2013 whose purpose is to research, study and develop nanoparticles and their applications based on Responsible Research and Innovation principles.

We understand RRI as a normative-political orientation that seeks to alter the present sociotechnical order; we are convinced that taking a Responsible Research and Innovation approach to research is the only way of solving the problems we have in the world. From this perspective, a radical reformulation of traditional company values, structures and innovation procedures has been implemented and is explained in the Case Study.

Our company is structured without an explicit CEO, and leadership is taken per project and expertise. Shareholders are actively involved in the day-to-day work and decision making processes of the company. Similarly, employees (scientists) are encouraged to creatively develop and shape their work, and are requested to participate in company governance to help them to enhance their awareness of consequences and their responsibilities. This RRI alignment also affects our legal strategy, and the projects and

*AppNP's ultimate aspiration is to transform waste into appealing raw materials in an efficient and sustainable way while contributing to key European policy objectives.*

tools developed for knowledge sharing, generation of debate, and foresight (multidisciplinary education and communication projects, newsletter and twitter account).



AppNP's core project is the commercial exploitation of a patent named BioGAS+. BioGAS+ is the first ready to use additive based on safe and sustainable engineered iron nanoparticles directed to the optimization of anaerobic digestion processes, which increases the production of biogas from organic waste. BioGAS+ is a disruptive

technology because it obtains the highest ever-reported improvement of biogas production, triples the biogas yield with cellulose as feedstock in laboratory conditions, and obtains over a 30% methane ratio increase in real industrial settings, among other additional advantages. AppNP's ultimate aspiration when developing BioGAS+ is to transform waste into appealing raw materials in an efficient and sustainable way while contributing to key European policy objectives, such as climate change targets, energy and food security, resource efficiency, improved air quality, the development of bioeconomy and circular economy, bioenergy, and the prevention of contamination.

Experience taken from our daily work in the laboratory suggests that we have to focus on an overarching dimension of RRI: Intelligence (which implies education, study, formation, verification, scepticism, criticism, historical perspective etc.). Responsibility requires complex thinking, wide angle analysis and forecasting of consequences. *This is why the ultimate aim of this company, of this project, and of all these efforts, is education in all its forms:* proper education is the only way we know to become more intelligent than we are, and is central to achieving sustainable progress. And we can confirm with optimism that young nanotechnology scientists are eager to adopt a responsible approach to research and innovation, realize that technology is never value neutral, and accept their moral responsibilities; they only need the proper research and innovation environment.

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## The Company - Applied Nanoparticles SL

Applied Nanoparticles SL (AppNPs) is a technology-based spin off company derived from the Universitat Autònoma de Barcelona (UAB), the Institut Català de Recerca i Estudis Avançats (ICREA), and the Catalan Institute of Nanoscience and Nanotechnology (ICN2). The company was founded on October 17th 2013.

The company's purpose is to research, study and develop nanoparticles and their applications. This includes design, production and characterization of nanoparticles, consultancy activities and dissemination, as well as training and education in nanoscience, nanotechnology, and related areas.



## The Product - BioGAS+ by AppNP

AppNP's core project and the main reason behind setting up the company is the commercial exploitation of a patent based on the use of engineered iron oxide nanoparticles for enhanced biogas production, named BioGAS+.

### The Concept

BioGAS+ is the first ready to use additive based on safe and sustainable engineered iron nanoparticles directed to the optimization of anaerobic digestion processes which increases the production of biogas (a mixture of different gases produced by the breakdown of organic matter in the absence of oxygen, mainly CO<sub>2</sub> and CH<sub>4</sub>). Thus, the process that converts organic waste into raw matter for energy production is optimized by simply adding a small dose of iron (Fe) based nanoparticles (NPs) to either a large waste treatment reactor, a septic tank, or a homemade biodigester.

### Technical Description

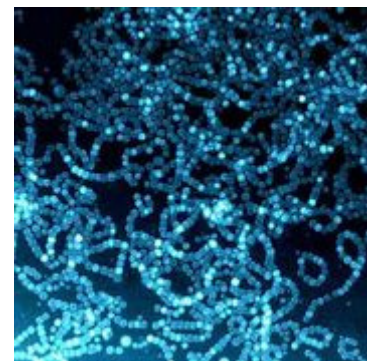
It is known that the addition of Fe ions to an anaerobic bacterial reactor can increase methane production, however introducing such ions can give rise to toxicity and excess reactivity. These problems are solved with BioGAS+ iron based nanoparticles: NPs can be designed to corrode and dissolve in a controlled manner, thereby providing a Fe optimized dosing source because of their denseness, chemical composition, crystal structure, nanometric size, and high reactivity. In conditions of anaerobic digestion small doses of mixed iron oxide NPs serve as a *catalyst* that stimulates bacteria metabolism and accelerates the production of biogas.

## Product Development

Work on the use of iron oxide NPs to enhance biogas production started in 2008 with a project called NANOCLEAN, funded by the Spanish Environmental Ministry. The initial objective was the study of the potential toxic effects of different common NPs in bacterial consortia intended for waste water treatment plants (WWTPs). If NPs became common in consumer products, they will inevitably end up in WWTPs, our environmental gate keepers. To our surprise, instead of proving toxic, some iron oxide nanoparticles were beneficial. After repeating the experiments and performing the corresponding controls, the phenomenon was consistently reproduced, and so we decided to start filing a patent to protect it. After that, the institutions involved in the project – the patent owners - carried out a series of unfruitful technology transfer promotion activities in specialized forums and conferences. Technology brokers, mainly, showed some interest in our product at international and local science-business fairs, but this did not develop far as the technology was considered too immature, and not yet of interest to (nanotechnology) business angels. Indeed, Today, regarding the Technology Readiness Levels (TRLs, 1 to 9, from the Eureka! to placement in the market),<sup>2</sup> inventors hurry to protect their discoveries at levels 2-3 but they are usually not minimally commercializable until TRL 6-7. Therefore, we decided to overcome what we called “TRL gap” by looking for alternative funding, namely, altruist funding, industrial partners, EU / national support programs and activity diversification:



1. Altruist Funding: In 2011 we obtained funding from the Bill & Melinda Gates Foundation Grand Challenges Explorations Grants (proposal OPP1044410). In 2012 we presented our project to the Fundación Repsol<sup>3</sup> Entrepreneurs Fund<sup>4</sup> and were selected for a one year pre-incubation award (as our technology was immature). In 2013 we obtained the Ibero-American Secretariat General Innovation and Entrepreneurship Award.<sup>5</sup> In 2014 we submitted a new proposal to the Fundación Repsol and were awarded their incubation prize, which consisted of mentoring, education in entrepreneurship, and basic funding for two years (finishing in September 2016).



<sup>2</sup> TRL is a measurement system of the maturity level of particular technologies. This measurement system provides a common understanding of technology status and addresses the entire innovation chain. [https://ec.europa.eu/research/participants/data/ref/h2020/wp/2014\\_2015/annexes/h2020-wp1415-annex-g-trl\\_en.pdf](https://ec.europa.eu/research/participants/data/ref/h2020/wp/2014_2015/annexes/h2020-wp1415-annex-g-trl_en.pdf)

<sup>3</sup> Fundación Repsol is one of the means by which Repsol fulfills its commitment to social responsibility, acting as a channel for the company's social and cultural work. Its objective is to contribute to social wellbeing, improve people's quality of life, and achieve greater social, educational, environmental and cultural development [www.fundacionrepsol.com/en/the-foundation](http://www.fundacionrepsol.com/en/the-foundation)

<sup>4</sup> The Fundación Repsol Entrepreneurs Fund is aimed at entrepreneurs with innovative technological projects in the field of energy and energy efficiency who have set up or intend to set up a company. The Fund's objective is to support them in taking their solutions to the market as quickly as possible. [www.fondoemprendedores.fundacionrepsol.com/en/entrepreneurs-fund](http://www.fondoemprendedores.fundacionrepsol.com/en/entrepreneurs-fund)

<sup>5</sup> The Ibero-American Secretariat General (SEGIB) is an international support organisation of the Ibero-American Summit of Heads of State and Government, which implements its mandates and drives Ibero-American Cooperation in the areas of education, social cohesion and culture <http://segib.org/en/who-we-are/>

2. Industrial Partners: We have searched for alliances with industrial partners with the strategic objective of sharing part of our know-how and exclusive rights to BioGAS+ in exchange for their know-how and resources. We have seen that Industrial Partners are conscious of the time needed for getting the right, safe, sustainable products. On the other hand, since BioGAS+ reached TRL 6/7 we have received several proposals from venture capitalists / business angels that we have rejected as they impose requirements for unrealistically fast timescales to bring products to market, or transform the project into some financial derivative in order to raise funds.
3. EU / national Support Programs: We have obtained funding from EU cooperation programs (H2020), and support from the SMEs Instrument (1<sup>st</sup> stage).
4. Activity Diversification: Services analysis and characterization, sales of model nanoparticles and consultancy.

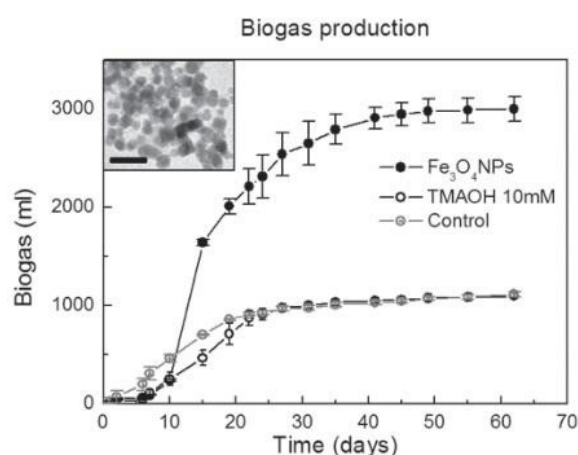
## Technology Readiness Level

As explained, BioGAS+ is the result of intensive research that started in 2008. Since then we have travelled the long road from laboratory to market. The first tests were carried out in laboratory settings with discontinuous digesters. Subsequently these results were confirmed with different feedstock (urban waste, slaughterhouse waste, manure, and weed) directly provided by different end-users (companies) and tested in continuous pilot laboratory digesters.

At present, BioGAS+ is being tested at the industrial premises of a global sewage sludge biogas plant operator (starting with test digesters of 1.5m<sup>3</sup> -TRL7- and then in digesters of 500m<sup>3</sup> or above -TRL8). A specific plan for testing BioGAS+ in real industrial digesters for other selected sectors and feedstock (agricultural residues, farm waste, food and beverage, and organic fraction of municipal solid waste) has also been set up, and will be implemented in 2017. BioGAS+ has already been produced in (pre) bulk quantities which prove the industrial scalability of our product (60 kg in 500 L reactors). During 2017 we will be producing hundreds of kgs and afterwards tonnes (t) of BioGAS+. For these reasons, we consider that TRL9 will be achieved in 2017.

## Differential advantages

Increase biogas and biomethane production: BioGAS+ is a disruptive technology because it obtains the highest ever-reported improvement of biogas production: triples the biogas yield with cellulose as feedstock in laboratory conditions -DIN-38414-<sup>6</sup> and obtains over a 30% methane ratio increase in real industrial settings, with real feedstock and with optimal concentrations below the 1% (with respect to the Volatile Solids). Such a methane production increase is far above any known technology aimed at increasing biogas



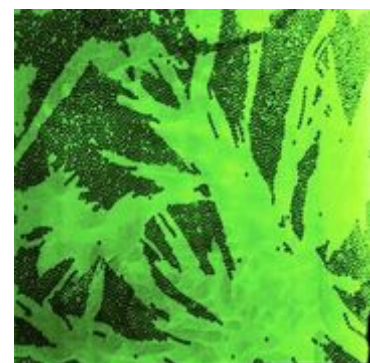
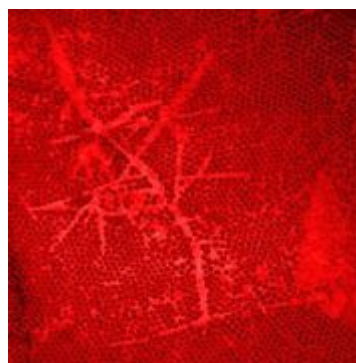
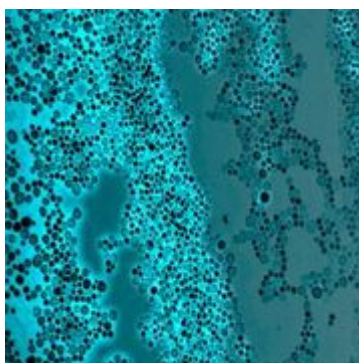
<sup>6</sup> E Casals, R Barrena, A García, E González, L Delgado, M Busquets-Fité et al. Programmed iron oxide nanoparticles disintegration in anaerobic digesters boosts biogas production. *Small* 10, 2801-2808, 2014.

production: many existing technologies approach this problem (i.e. pre-treatment of the biomass, thermalization of the waste, combination of feedstock and inoculums) but only obtain modest production increases. Moreover, many tend to be costly to implement since they usually require structural changes in the biogas production process. The unprecedented methane ratio increase is the most appealing advantage of BioGAS+, but it also offers additional differential advantages, including:

- Improved biomass to biomethane conversion efficiency.
- Better biogas composition (higher methane share).
- Higher waste degradation. An improvement of anaerobic digestion of biomass will lead to a less bioactive end-of-waste digestate, which is more appealing for composting and re-use.
- Acceleration of the digestion process. Reduction in retention / residential time<sup>7</sup> and in the digestate fraction.
- Enrichment of the residual material (digestate) with iron ions to obtain by-products with increased economic value such as high quality fertilizers.
- Additive (it does not require any change in the biogas plant industrial process).
- As proved suitable for “difficult to digest” feedstock it enlarge available biomass feedstock previously discarded or recalcitrant organic matter. .
- Reduced AD plant energy consumption.
- Minimization of undesirable side effects in biogas plants such as the odours associated with HS and NH<sub>3</sub>, thus reducing the cost of associated conditioning measures.

## Patent

The Private Foundation Catalan Institute of Nanoscience and Nanotechnologies (ICN2), the Catalan Institute for Research and Advanced Studies (ICREA), and the Autonomous University of Barcelona (UAB) are the owners of <<*a method for increasing the production of biogas in anaerobic digestion processes of biodegradable material by adding nanoparticles iron oxide*>>. The Patent owners and Applied Nanoparticles SL have signed an Exclusive Licencing Agreement dated 21/07/2015. This method is protected in Europe by patent application 12707361.7.1352 Ref. P1923EP01/EP2683662 and in the USA under Patent US 9,416,373 B2 dated August 16<sup>th</sup>, 2016 BIOGAS PRODUCTION



<sup>7</sup> Reducing the residential time of the feedstock inside the digester allows rationalization of a biogas plant's investment strategies (plant dimensions / new plants).

## AppNP, BioGAS+ and RRI

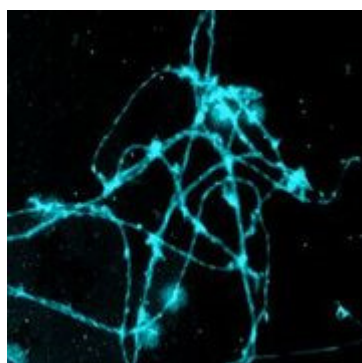
We understand RRI as a normative-political orientation that seeks to alter the present sociotechnical order. From this perspective a radical reformulation of traditional company values, structures and procedures (including decision making and innovation procedures) has to take place. This is what we are trying to achieve at AppNPs with BioGAS+. Otherwise, we will be facing what can be named “responsible-washing” (following the well-known “green-washing”<sup>8</sup> in the Corporate Social Responsibility framework).

In this Section we present our work on RRI. In doing so, we follow the RRI concept as developed by the RRI Tools Project<sup>9</sup> that specifies **outcomes** and **process requirements**.<sup>10</sup> RRI outcomes can be separated into **learning outcomes** (engaged publics, responsible actors, responsible institutions), **research and innovation outcomes** (ethically acceptable, sustainable, socially desirable), and **societal outcomes** (solutions to societal challenges - Grand Challenges). In order to achieve these outcomes, the RRI process has to meet certain process requirements that have been divided into four clusters, namely, **diversity and inclusion, anticipation and reflection, openness and transparency, responsiveness and adaptive change**. Following this framework, we present a set of AppNPs outcomes linked to the corresponding process requirements.

### RRI outcomes

#### Learning outcomes

##### *Shareholding and decision-making process (Diversity & Inclusion)*



The company is made up of 13 shareholders, with no single shareholder having more than 10% of shares. Among them are researchers in Nanoscience and Nanotechnology or Environmental Science (inventors of Biogas+) and representatives of other disciplines; law, marketing, e-communication and graphic design. 10 out of the 13 shareholders are or have been directly or indirectly involved in the day to day work of the company.

This structure makes the company diverse, and robust against modifications and unexpected transformation of the initial aims, as a democratic majority of shareholders is required for fundamental decision making. The company is structured without an explicit CEO. The implementation of the resolutions adopted is delegated to the most suitable and most available person, in a knowledge-based functional (expertise and facts driven) system where efficiency is prioritized over efficacy, and efficacy is not at all neglected. In general, decisions and strategies are agreed between the legal representative, the scientific director, workers and active shareholders.

<sup>8</sup> Spends more time and money claiming to be “green” (e.g. in advertising) than implementing business practices that minimize environmental impact.

<sup>9</sup> Funded under the Framework Programme FP7 (2007 – 2013), RRI Tools has been set up to develop a set of digital resources to advocate, train, disseminate and implement RRI under Horizon 2020. <https://www.rri-tools.eu/>

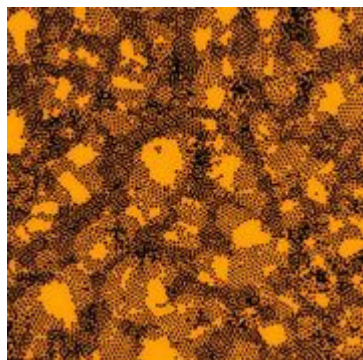
<sup>10</sup> “Responsible Research and Innovation is a dynamic, iterative process by which all stakeholders involved in the R&I practice become mutually responsive and share responsibility for both the outcomes and the processes involved”. [https://www.rri-tools.eu/documents/10184/107098/RRITools\\_D1.1-RRIPolicyBrief.pdf/c246dc97-802f-4fe7-a230-2501330ba29b](https://www.rri-tools.eu/documents/10184/107098/RRITools_D1.1-RRIPolicyBrief.pdf/c246dc97-802f-4fe7-a230-2501330ba29b)



*Retaining talented employees (Diversity & Inclusion / Responsiveness & adaptive change)*

In a spin-off company, human resources are critical: with no financial strength or infrastructures, knowledge and human resources are our only competitive tools. Therefore, highly motivated and skilled employees are needed, but one may not be able to retain them in small companies for a long period (they may pursue their academic and investigation career, or you may not be able to compete financially). In our case, the way to retain human capital has been a combination of 2 concepts, namely: “a company in the making” and “a company with purpose”.

When talking of “a company in the making” we refer to the possibility of directly influencing the direction the company is heading based on the decision taking procedure explained above (collaborative decision making procedure and personal responsibility for follow up actions). This gives plenty of room for personal development possibilities, depending on the level of involvement that each person is able and willing to provide. The company thus serves as a platform for project development and opportunities generation.



By “a company with purpose” we refer to our vision as a company and how we want to achieve it. We want to become a reference for nanotechnology applications in these early days of nanotechnology development, driven by principles of Responsible Research and Innovation in order to generate wealth responsibly, minimize deleterious side effects, and pay special care to sustainability. All of our activities, resources and funds are directed to this objective. The vision is present in the day-to-day laboratory work; principles and action go together and can be directly experienced.

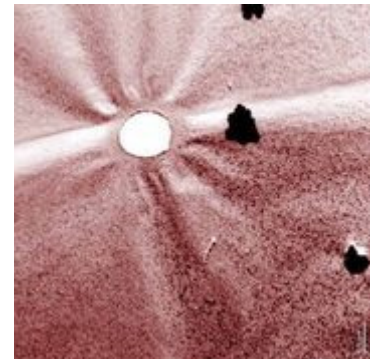
*Innovating responsibly as the cornerstone of the legal strategy (Responsiveness & adaptive change)*

It is well known that there are no specific regulations for nanotechnologies or nanomaterials at EU level. Instead, the manufacture, use and disposal of nanomaterials are covered, at least in principle, by a complex set of existing regulatory regimes. As the current regulatory regime applicable to Nanotechnology and Nanomaterials (N&N) was not designed for nanomaterials, it is (in some ways) inadequate.<sup>11</sup> The consequence of this inadequacy to private economic operators regarding their legal obligations (compliance) is legal uncertainty. Consequently, where explicit nano-regulations do not exist, and the economic operator is faced with open legal concepts, the most sensible course of action is to focus on risk avoidance, which implies low impact production techniques - green chemistry - as well as the way in which nanomaterials are embedded into products - safe by design / design for safety. In other words, in the current legal framework and social context, companies need to develop safe and sustainable nanomaterials, and applying RRI principles is the best way we found to achieve it.

<sup>11</sup> Those inadequacies are essentially: knowledge, regulatory design and information gaps. They are filled by 3 different regulatory methods: a) by entering explicit “nano” references to existing regulatory regimes for chemicals (Regulation (EC) No. 987/2008 amending REACH as regards Annexes IV and V, OJ L268/14 by which Carbon and Graphite were removed from exemption list Annex IV; b) by proposing regulatory reviews on nanomaterials (such as the one under consideration regarding REACH); and by c) promoting anticipatory legal governance to private operators, based on the use of soft law mechanisms.

*Newsletter and twitter account (Anticipation & reflection / Openness & transparency)*

Whilst acknowledging that the continuous and consistent involvement of society in the research and innovation process is one key aspect of RRI, it has to be recognised that from the perspective of a start-up, this task has to be approached with caution and respect. It is not only economic constraints, but the inherent difficulty in setting up public engagement practices (methods of participation, purposes, evaluation criteria, etc.) that make this a challenge. For these reasons we have chosen to involve stakeholders and society at large by promoting a permanent dialogue through our twitter account (<https://twitter.com/biogasplu>), and our open source monthly newsletter <https://www.biogasplus.info/biogas-newsletter/>. These tools go well beyond current information services, as our aim is to filter, curate and contextualize information in the fields of biogas and nanotechnology.<sup>12</sup>



Our final aim is to generate debate and foresight (inside and outside our company) on fundamental topics. From an RRI perspective, one such topic is the ethical debate around biogas<sup>13</sup> which, as we will see when discussing our Code of Conduct, below, has influenced our approach to market (and our business plan) in a fundamental way.

*Researchers' Responsibility Awareness (Anticipation & reflection)*

Following Schuurbijs<sup>14</sup> we consider that researchers have a moral responsibility to pay attention to and critically reflect on the wider socio-ethical context of their work, specifically:

- i. The “value-based socio-ethical premises” of research;
- ii. Epistemological and ontological assumptions;
- iii. Methodological norms of scientific culture; and
- iv. The socio-ethical consequences of research.

As we are working in and from the laboratory, we are especially interested in the “midstream”<sup>15</sup> phase as an opportunity for addressing social and ethical concerns.

<sup>12</sup> BioGAS+ newsletter and twitter concept: Today we have an unlimited amount of content; what is valuable is filtering and curating; making sense of the information. BioGAS+ Newsletter does this work by filtering and curating in the field of nanotechnology, biogas and responsible innovation. There is a unique process of on-going monitoring, selection and presentation of information from the original source, with correspondence, and in some cases even collaboration with, the authors of the research. The capsules (news) resulting from the monitoring and filtering of information seek to provide extra value: the context. This is achieved by the information itself and also by promoting debate / controversy and / or a contrast of opposing paradigms in the same capsule (by means of the context and follow up). The compilation of the capsules over time has become an online database with its own search tools and labelling information, which is updated and contextualized in book format (electronic and paper) with a unique signature graphic.

<sup>13</sup> Just a few examples of different news published and contextualized in our newsletter regarding ethical concerns: May 2015. [“If global food waste were a country, it would rank third in terms of greenhouse emissions”](#); April 2015. [Restricted Expansion of Food for Fuel: Waste-Biogas, Next Generation Biofuel](#); March 2015. [Waste-biogas or crop-biogas?](#); January 2015. [“We need to move from the food versus fuel debate to a food and fuel debate”](#); December 2014. [Biogas and Food Security Debate](#); December 2014. UK: [Milk in biodigesters](#)

<sup>14</sup> Schuurbijs, D., “Social Responsibility in Research Practice. Engaging applied scientists with the socio-ethical context of their work”. Ed. Brey, P., Kroes, P., Meijers, A. 2010.

<sup>15</sup> ‘Midstream’ denotes the phase of the research and development before scientific results are translated into products or services, but after authorization and funding decisions have been taken. Fisher, E., Mahajan, R.L., Mitcham, C. “Midstream Modulation of Technology: Governance From Within”. *Bulletin of Science, Technology & Society*. Vol. 26, No. 6. December 2006, 485-496.

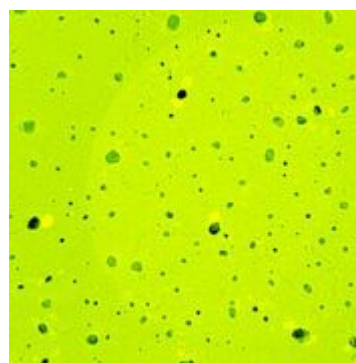
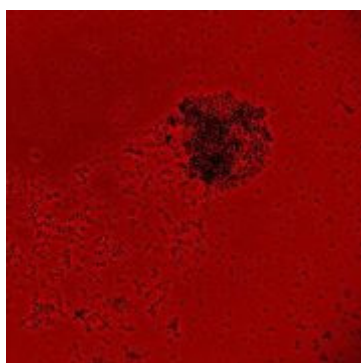
Bearing in mind our economic and human constraints we have structured the midstream modulation around periodical informal review meetings. In those meetings we analyse the technology and business aspects of our business and a review of the most interesting information published by our newsletter (and feedback received) is discussed. Overall, a wide range of topics are reviewed (environment, health and safety, sustainability, patenting, long-term research and business strategies, ethical issues and the responsibility of scientists to communicate with society). Our experience points to a “two way embedding” between social and natural scientists. All members of the organization, whatever his/her background, become involved and aware of the social and institutional constraints in which we have to operate (balancing scientific and commercial interests, coping with the demands of clients, the complexities of research practices etc).

*Responsible Nanosafety Experts (Anticipation & reflection / Responsiveness & adaptive change)*

Our vision is that every (nano)scientist should become a “responsible (nano)safety expert”. This is, all workers involved in our project have basic skills on nanosafety and nanosustainability. This concept came to us from the “expert patient” definition, and it is putting in practice the idea that all responsible citizens should know first aid techniques. We are currently developing (within the framework of a collaborative H2020 project) a training course for nano-doctoral students that will combine “hands on RRI in the lab” with “tutorials on broader RRI concepts”. The final objective is that the doctoral students re-analyse their doctoral thesis in the light of the learned RRI concepts and practices.

*Multidisciplinary education and communication projects (Openness & transparency)*

We are convinced that for a smooth introduction of nanotechnology in society, openness and education are fundamental factors. For this reason, AppNPs and / or our shareholders are very active in undertaking projects that bring Nanoscience and Nanotechnology (N&N) to society. Among others, we can mention the information service Nanowiki,<sup>16</sup> NanoColoringBook,<sup>17</sup> GoldLight Quantum Jewellery,<sup>18</sup> production and / or sponsoring of general and technical audience books related to N&N,<sup>19</sup> and collaboration with the Barcelona University (UB) Nanodivulga Project,<sup>20</sup> among other.



<sup>16</sup>Nanowiki information service has been considered among the 10 most influential nanotechnology accounts by Hope Reese from TechRepublic: <http://www.techrepublic.com/article/nanotech-10-twitter-accounts-to-follow-to-keep-up-to-date/>

<sup>17</sup> Coloring book. <https://archive.org/details/NanoColoreja>; <https://archive.org/details/NanoColorea>

<sup>18</sup> <http://www.theguardian.com/what-is-nano/precious-particles>; <https://www.facebook.com/goldlightjewels/>

<sup>19</sup> <https://archive.org/search.php?query=creator%3A%22v%C3%AD%C2%ADctor%20punter%22>

<https://archive.org/details/NanoOncologyTheTurningPoint>

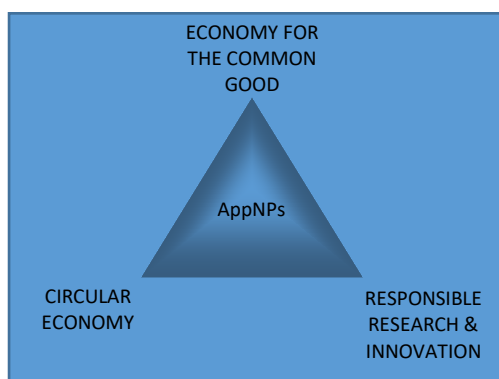
<sup>20</sup> <http://www.ub.edu/laubdivulga/nanodivulga/>. A set of activities aimed at bringing the world of nanotechnology to the public.

## R&I outcomes

### *Ethical acceptability (Diversity & Inclusion / Openness & transparency)*

Within a company, its core values (or constitutional values) are expressed within its Code of Conduct (CoC).<sup>21</sup> The normative targets set in the CoC “have been democratically agreed and provide the legitimate basis for defining the type of impacts that research and innovation should pursue”<sup>22</sup> within AppNPs. At AppNPs those “anchor points”<sup>23</sup> have been discussed among shareholders and workers. We believe that this exercise helps to understand the higher values that have to be taken into consideration when laboratory decisions on safety and sustainability are taken on a daily basis.

The values that we have agreed upon for our CoC are those that we all recognize as universal (human dignity, solidarity, sustainability, social justice, transparency and democratic participation) and they imply that all actions and decisions should be directed to seek social, economic and environmental sustainability, and not to seek individual or only economic benefits. In this sense, our company understands that these values should also substantiate economic relations and, in this regard, shares the principles of the Economy for the Common Good (ECG).<sup>24</sup>



We understand that the CoC has to be a “living framework” from which concrete ethical discussions and lines of action have to be derived. In this sense, and from an RRI perspective, one such topic is the ethical debates around biogas that are influencing our approach to market (and our business plan) in fundamental ways. For example, the German market represents approximately 50% of the total biogas market in the EU. From it, 48% (by weight) or 77% (by energy output) of biogas is produced with “crop feedstock”. This reality confronts us with the ethical

“food vs. fuel” debate (bioenergy production may compete, directly or indirectly, with food production, and as a consequence food security may be adversely affected). Based on ethical grounds we have decided to develop products for anaerobic digestion plants using waste as feedstock. Having in mind the market figures given, there is no need to expand further on the far reaching consequences of our decision.

### *Safe and Sustainable outcomes (Anticipation & reflection)*

Our previous experience in the fields of nanosafety & nanosustainability<sup>25</sup> - mainly through collaborative projects under EU FP6 and FP7 and the participation in the Centre for NanoBioSafety and Sustainability (CNBSS<sup>26</sup>) - have made us familiar with how to follow and implement concepts and procedures such as “safety by design”, “green chemistry” and Life Cycle Assessment (LCA). We provide our product BioGAS+ to other research laboratories under the H2020 program to openly study the potential (eco)toxicity of our product.

<sup>21</sup> <https://www.biogasplus.info/2017/01/25/applied-nanoparticles-code-of-conduct/>

<sup>22</sup> Von Schomberg, R. (2013) “A vision of responsible innovation”. In: R. Owen, M. Heintz and Bessant, J. (Eds.). Responsible Innovation. London: John Wiley.2013.

<sup>23</sup> Ibid.

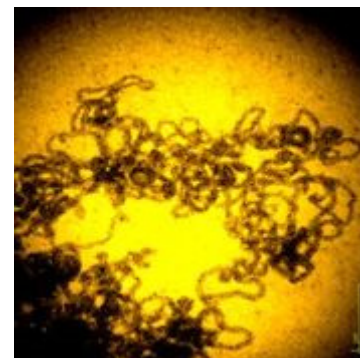
<sup>24</sup> <https://www.ecogood.org/en>

<sup>25</sup> <https://www.biogasplus.info/2017/01/25/potential-nanoparticles-toxicity/>

<sup>26</sup> <http://www.cnbss.eu/>

Regarding *Safety*, we follow broader Nanosafety Guidance and Frameworks published by some European institutions focused on nanosafety, as *NanoRiskCat – A Conceptual Decision Support Tool for Nanomaterials* (from the Environmental Protection Agency of the Danish Ministry of the Environment)<sup>27</sup>, and *Working Safely with Nanomaterials in Research & Development* (developed by The UK NanoSafety Partnership Group and the Institution of Occupational Safety and Health (IOSH) within the Health and Safety Executive (HSE) of the UK Government).<sup>28</sup>

Regarding *Sustainability*, AppNP is committed to work in the greenest and most environmentally friendly conditions possible, by following the 12 Principles of Green Chemistry developed by Paul Anastas and John Warner in 1998; a list of requirements that an ideal "green" or environmentally friendly chemical, process or product would follow or accomplish.<sup>29</sup>



We are confident that the production process of our main product, based on magnetite ( $\text{Fe}_3\text{O}_4$ ) nanoparticles follows all of the aforementioned principles to some degree, starting with the low inherent hazard of the product itself. There is plenty of literature about the innocuous or very low toxic nature of magnetite nanoparticles,<sup>30</sup> iron being a life-essential *oligoelement*, and iron oxides, even in the nanometric form, are natural abundant materials.<sup>31</sup> Our raw materials cannot be considered scarce or non-renewable feedstock. Moreover, in our production process the nanoparticles are synthesized *in situ* in aqueous media at room temperature and are always processed as a colloid, never as a dry powder, thus avoiding airborne exposure. Being carried out at room temperature, the production process has a very low energetic demand (except for the generation of the required stirring power). The washing waters of the NP production are recovered and directly used as base for further synthesis. Regarding the

<sup>27</sup> *NanoRiskCat – A Conceptual Decision Support Tool for Nanomaterials* (from the Environmental Protection Agency of the Danish Ministry of the Environment). We follow it as our benchmark framework on Risk Assessment of Nanomaterials. It provides clear and detailed guidance on mapping and assessing risk that yields into a simple and visual final report for each given nanomaterial based on five-colour coded dots; three of them covering areas on exposure potential (for professional end-users, for consumers and for the environment), and the remaining two covering hazard evaluation (for humans and for the environment). We chose it for the simplicity of the approach and the visual clarity of the final report obtained as output. We are confident that we can apply it to our BioGAS+ product, as most of the required input data is already available, and we will obtain the missing one from an EU H2020 Project in which our product is one of the chosen case studies.

<sup>28</sup> *Working Safely with Nanomaterials in Research & Development* (developed by The UK NanoSafety Partnership Group and the Institution of Occupational Safety and Health (IOSH) within the Health and Safety Executive (HSE) of the UK Government). This is very general on dealing with all sorts of nanomaterials in a safe way, and we consider that some amendments should be made to include more nanoparticles focused to completely suit our circumstances, but we follow it for its concise and useful guidance on some areas, especially on Engineered Exposure Control Measures, Personal Protection Equipment, Disposal of Nanomaterials and Labelling and Signs.

<sup>29</sup> Anastas, P. T.; Warner, J. C. *Green Chemistry: Theory and Practice*, Oxford University Press: New York, 1998, p.30. These 12 principles are as follows: 1. Prevention; 2. Atom (matter) Economy; 3. Less Hazardous Chemical Syntheses; 4. Designing Safer Chemicals; 5. Safer Solvents and Auxiliaries; 6. Design for Energy Efficiency; 7. Use of Renewable Feedstocks; 8. Reduce Derivatives; 9. Catalysis; 10. Design for Degradation; 11. Real-time analysis for Pollution Prevention; 12. Inherently Safer Chemistry for Accident Prevention. List and description taken from: <http://www.acs.org/content/acs/en/greenchemistry/what-is-green-chemistry/principles/12-principles-of-green-chemistry.html>

<sup>30</sup> i) J S Weinstein et al. *J Cereb Blood Flow Metab.* 2010 Jan; 30(1): 15–35. ii) N. Singh et al. *Nano Reviews Vol 1 (2010) incl Supplements* iii) B. Ankanwar et al. *Nanotechnology.* 2010 Feb 19;21(7):75102 iv) M. Mahmoudi et al. *Colloids Surf B Biointerfaces.* 2010 Jan 1;75(1):300-9 v) A. Sanchez, S. Recillas, et al. (2011). *TrAC Trends in Analytical Chemistry* 30(3): 507-516

<sup>31</sup> <http://www.lulu.com/shop/v%C3%ADctor-puntes-and-josep-salda%C3%B1a-cavall%C3%A9/nanoparticles-before-nanotechnology/ebook/product-20635604.html>

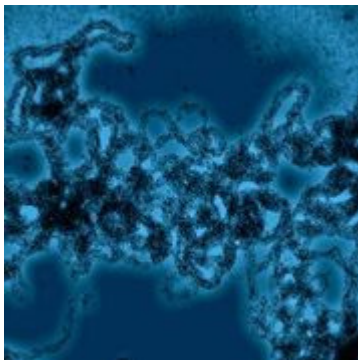
application, the size and dose of the nanoparticles are purposely designed to completely dissolve during the tens of days of a standard anaerobic digestion retention times.

## Societal outcomes

### *Solution to societal challenges – Grand Challenges - (Responsiveness & adaptive change)*

AppNP's final aspiration when developing BioGAS+ is to transform waste into appealing raw materials in an efficient and sustainable way while contributing to key European policies, such as **climate change targets, energy and food security, resource efficiency, improved air quality, the bioeconomy and circular economy, bioenergy, prevention of contamination.**

Specifically, the urgency in tackling climate change and promoting renewable sources of energy has been universally agreed as one of the Grand Challenges of our time. We have also seen EU policy and regulatory alignment with this global challenge. But as per today, biogas production represents a small share of the total renewable energy sector (10.9 Mtoe<sup>32</sup> of biogas in 2010 and an estimated production of 39.5 Mtoe in 2020, i.e. approximately 10% of EU natural gas consumption). The main cause of this underuse is the difficult optimization of the complex processes occurring inside anaerobic digesters and, as a consequence, the low conversion rates of biomass (waste) to energy (gas/methane), leading to economic inefficiency. As we have seen, BioGAS+ can transform actual biogas production into a profitable business, thus helping to combat climate change and promote renewable energy systems.



## Lessons learned

In their foundational article “Developing a framework for responsible innovation”, Owen and colleagues<sup>33</sup> present four dimensions of responsible innovation, namely; Anticipation, Reflexivity, Inclusion and Responsiveness. Experience taken from our daily work in the laboratory suggests that we have to add a fifth dimension, or perhaps an overarching *sine qua non*: Intelligence (which implies education, study, formation, verification, scepticism, criticism, historical perspective etc.).

In an over simplified manner, those who contaminate the river or abuse the workers do not do so because they enjoy river death or making others suffer. When the businessman says that he cannot compete without pressurising workers, polluting the environment and cheating on consumers (see the recent “clean diesel”<sup>34</sup> case) this is due to a lack of intellectual capacities or low knowledge and reasoning capabilities. As Winston Churchill said, “gentlemen, we have run out of money, now we have to think”. Now we are not running out of money, we are running out of a clean planet. Therefore, we cannot afford foolishness, it is too expensive. Responsibility requires complex thinking, wide angle analysis and forecasting of

<sup>32</sup> Million Tonnes of Oil Equivalent.

<sup>33</sup> Owen et al., “A Framework for Responsible Innovation”. In: R. Owen, M. Heintz and Bessant, J. (Eds.). Responsible Innovation. London: John Wiley. 2013.; Stilgoe, J., Owen, R., Macnaghten, P., “Developing a framework for responsible innovation”. Research Policy 42 (2013) 1568 – 1580.

<sup>34</sup> [https://en.wikipedia.org/wiki/Volkswagen\\_emissions\\_scandal](https://en.wikipedia.org/wiki/Volkswagen_emissions_scandal)

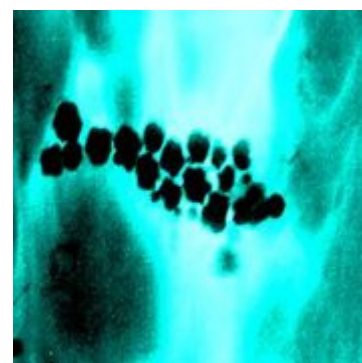
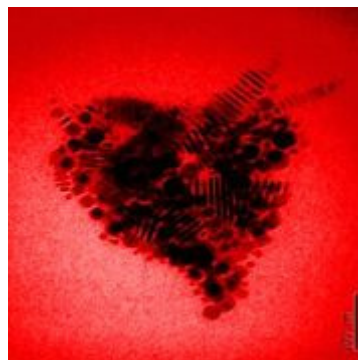
consequences. This is increasingly the case in a world becoming more and more sophisticated thanks to technological development. Now that we need to start to develop more efficient technologies, and truly understand sustainability, we witness with dismay the new illiteracy that is rising, where signs of post-enlightenment are starting to be visible,<sup>35</sup> probably as a consequence of the increased complexity. This is why the final aim of this company, of this project, and of all these efforts, is education in all its forms: proper education is the only way we know to become more intelligent than we are, and the only way towards sustainable progress.

From our experience (through conferences, EU nanosafety cluster, twitter account and so on) we have seen that mature and established professionals often, probably influenced for an interest in the actual status quo, think that they are already “responsible”, and look at this movement with sympathy and condescendence, while young nanotechnology scientists, who have not yet established professional lives are more eager to adopt a responsible approach and realize that technology is never value neutral, but always value-laden.<sup>36</sup> They accept their moral responsibility (to critically reflect on the wider socio-ethical context of their work), and are thus ready to understand RRI as a political tool. They only need the proper innovation environment.

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*This is why the ultimate aim of this company, of this project, and of all these efforts, is education in all its forms.*

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(\* All pictures have been taken by Dr Victor Puentes using a Transmission Electron Microscopy – TEM)

<sup>35</sup> <https://longsworde.wordpress.com/2010/08/22/our-post-enlightenment-era-the-sleep-of-reason-breeds-monsters/>

<sup>36</sup> “Science for the Twenty- First Century. A new Commitment”. Declaration on Science and the use of Scientific Knowledge. World Conference on Science. UNESCO. Budapest. Hungary. 1999.

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## Appendix 1: AppNPs Code of Conduct

Our Code of Conduct reads as follows:

### 1.- OBJECT

The object of this Code of Conduct is to define the principles and standards of ethical conduct that should govern the actions of the related persons in the exercise of their professional activities in their relationship with the Company.

### 2.- SCOPE

The Code shall apply to Applied Nanoparticle SL and the following people:

- i) Managers, directors and any person employed by Applied Nanoparticles SL,
- ii) Any natural or legal person hired by Applied Nanoparticles SL for the provision of services and for the Company and
- iii) Any other natural or legal person determined by the directors.

### 3.- ETHICAL PRINCIPLES

The values that should govern the actions of all persons subject to it are those that we all recognize as universal: human dignity, solidarity, sustainability, social justice, transparency and democratic participation (in line with the guidelines established at the European level by art. 3 ap. 1,2, and 3 of the Maastricht Treaty and the Charter of Fundamental Rights of the European Union. OJ C83 of 30.03.2010).

In all areas of our company these universal values imply that all actions and decisions should be directed to seek social, economic and environmental sustainability and not to seek individual or only economic benefits. In this sense, our company understands that the values embodied should also substantiate economic relations and, in this regard, shares the principles of the Economy for the Common Good and will carry out actions for internal application, and knowledge dissemination.

These principles and objectives create standards of ethical behaviour listed below:

### 4.- ETHICAL STANDARDS OF CONDUCT

#### RELATIONSHIP WITH WORKERS

4.1.- HEALTH AND SAFETY IN THE WORKPLACE. Aware of the limitations of applicable law, AppNPs considers that it has to go beyond compliance with regulations on Safety and Health at Work. Therefore internally we apply good practices stemming from the nontechnology academic laboratories and environments of the nanosafety cluster ([www.nanosafetycluster.eu](http://www.nanosafetycluster.eu)). We are also educating our workers on basics of NP toxicity and safety from a broad and fundamental point of view to transform our workers into nanosafety experts.

#### 4.2.- WORKERS RIGHTS & OBLIGATIONS

We seek the active participation of workers in all aspects of the organization and recognize their contribution to the increased value of the company by applying incentives and bonuses to share with those responsible for that increased value. Employees have authorized personal use of the resources of AppNPs, but this should not be excessive, nor for personal gain or illegal purposes and should not be abusive in any other way. Employees should not use their position to obtain direct or indirect personal benefit.

We promote the smooth development of work, following general visions and interests, rather than being restricted to the strictly planned and commanded agenda (which would be a poor method for progress).

We advocate equal opportunities; we maintain an environment free of harassment and other primitive behaviour, and we ensure privacy of personal data.

#### 4.3.- RELATIONSHIP WITH THE PRODUCTS OF INNOVATION

We develop all our products under the principles of Responsible Innovation, both from the point of view of the product (it has to be useful, sustainable and safe) and process (it has to be collaborative and inclusive). As a result: Innovation is addressed to social benefit; ethical considerations of impacts at social and environmental levels are considered; the security of the product is studied through its full life cycle, from its production to its disposal or reuse, addressing the health and safety of workers and consumers.







#### 4.4.- RELATIONSHIP WITH INTEREST GROUPS

We need collaboration with suppliers and customers throughout the supply and value chain. In this context, transparency and disclosure of information is mandatory to open a sincere 2-way dialogue with all stakeholders and society. Our priority will be to interact with customers and suppliers who share the values, principles and standards reflected in the Code of Conduct. We understand a market economy based on cooperation between operators reflecting the finding that scientific innovation is a historic cooperation of countless thinkers and researchers. We also understand that only people working in and / or for the company should share in the profits it generates. This is in line with prioritizing productive economy over financial economy. We do not contemplate the possibility of sharing the profits of the enterprise among people who have not added value to the organization. Any investment in the Company will be addressed (internally) to strengthen the capacities of Responsible Innovation and (in the external environment) to those activities oriented to the common good and to improve the quality of life. We are working exclusively with ethical banking and / or cooperatives. Our experience is that in these institutions we get a better service at a better price.

#### 4.5.- RELATIONSHIP WITH SOCIETY

In our relation with society we are committed to sustainable development. We understand sustainable development in its three dimensions: environmental, economic and social. In this sense: i) the product of our innovation should be aligned with the 2030 Agenda for Sustainable Development of the UN (Resolution adopted by the General Assemblies of September 25, 2015. A / 70 / L1), and we are committed to integrating the ten principles of the Covenant UN World in our business strategy and operations by implementing its Management Model, and ii) the product of our innovation will have as design requirements: environmental protection, collective security and public health.

## Appendix 2: Potential Nanoparticles Toxicity

Today, the majority of innovation in nanotechnology is restricted to spin-offs generated in the academic environment where funding agencies set research efforts towards common goods, in medicine, energy and environment. From the societal point of view, and for similar reasons (small companies cannot financially support worldwide patents), patents are only presented in *rich* countries where benefits are more secured, allowing its free implementation in the rest of the world. Therefore, in these cases, the most critical point regarding responsible development is safety, and safety comprises human and environmental safety. Therefore, the question we need all to pose is: *does the nanoform of a substance entail an increased risk?* This question is fundamental to us. The potential negative impact of nanotechnology in health and the environment has worried society, and even if this is often overlooked by scientists, technologies need to get introduced into social environments and therefore the applications that develop are shaped by a mix of social and technological forces. If society embraces and finds uses for a technology, then it survives. Otherwise, not matter how good the technology is, it will die. Thus, effective communication is mandatory, which should include, in addition to state-of-the-art performance, safety studies, and a nanosafety-by-design approach contributing to full life cycle assessments and viability studies. Because of this, we take the advantage to describe our position regarding potential nanoparticles risk in the frame of our work.

The surprising properties of NPs are fundamentally due to their high surface to volume ratio, finite size effects, collective behaviour and interaction with light of any wavelength (for hyperthermia, diagnosis and imaging purposes) This results in a broad spectrum of chemical, physical, catalyst, optical and magnetic behaviours which can be sized for many uses. Interestingly, their exuberance of degenerated states at the macromolecular level allows their use as versatile molecular sensors and actuators, as much as it makes 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 absorbed onto it and then they can be introduced in physiological environments where 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.<sup>37</sup>

At the origin of nanotoxicity and nanosafety concerns, it was pointed at the well-known fact that cells have problems dealing with *micrometric* insoluble particles. Asbestos fibers, with dimensions greater than 20 micrometers, up to hundreds, induce frustrated phagocytosis<sup>38</sup>, 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, NP dose and persistency are key to determine this potential risk. If the NPs do not aggregate, they may dissolve. When they dissolve they yield ions (metal cations) that may be toxic, as in the well-known case of cadmium or silver NPs. In parallel, the corrosion process is a redox 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 NPs, current knowledge indicates that many NPs in their intended uses do not need special care beyond being treated like other chemical substances, even if some particularities may apply. Nanotoxicity is a young field that can be considered to be about 10 years old. Despite this youth, much knowledge from metal toxicity, microparticle 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 a long time, as food additives (E-171 to E-175 have a nanometric portion of iron oxide, aluminium oxide, titanium dioxide, silver and gold, respectively), in cosmetics, as simple as talc, catalysts, paint pigments, coatings and others. Up to now, we have been mainly reproducing nanomaterials that already exist in nature or that somehow are already produced by man in a more imperfect and unaware manner. Small, (about 20 nm) iron oxide NPs have been found in natural unpolluted soils or inside bacterial magnetosomes, and nanometric TiO<sub>2</sub> has been used by the tonne in the cosmetic industry as sun screens and other formulations for decades now. When we get the next generations of nanoparticles, additional care will need to be considered. 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 biodistribution in the case of inflammation might exacerbate or suppress acute effects and accumulate in organs (if the NPs succeed in entering the body, which 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 NPs and other toxins (such as LPS, allergens or chemical

<sup>37</sup> Harald F. Krug *Angewandte Chemie* 2014, 53, 12304-1231

<sup>38</sup> <https://diamondenv.wordpress.com/2011/04/15/frustrated-phagocytes-and-the-fibre-paradigm/>

toxins), or exposure to NPs in the case of disease, e.g.: during cirrhosis may be more critical and need to be studied. Focus has to be put also on the immune system, which is responsible for detecting, categorizing and managing external invasion. The immune system has memory, so repeated exposure to NPs 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. Iron oxide nanoparticles have not been found toxic in any of these forms unless they were functionalized with toxic moieties.

While it has been observed that NPs 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 NPs 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 agglomerated or aggregated nanoparticles (e.g., titanium dioxide, aluminium oxide, and manganese dioxide) are more potent in animals than equivalent single well dispersed 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 suggest 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.<sup>39</sup> Therefore, the breathing of solid nanoparticles, especially aggregates made of persistent materials, is highly inadvisable. However, even for poorly soluble particles of relatively low toxicity, animal studies have identified doses that were not associated with observed adverse responses.<sup>40</sup> For example, a recent animal study reported mass doses of either fine or nanostructured TiO<sub>2</sub> 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.<sup>41</sup> 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.<sup>42</sup> Note that this is not the case for BioGAS+ which is made of non-persistent NPs; they are not aggregated and they do not carry toxic moieties or toxic additives or toxic excipients.

In the following the main causes associated to NP induced toxicity are listed. In principle, at realistic doses in a controlled manner, inorganic NPs 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 interact strongly with cell membranes, thus interfering with its normal functioning and inducing cell death. See for example Chitosan functionalization of gold nanoparticles.<sup>43</sup> This charge is carried by molecules attached to the surface or by the inorganic surface itself if it is at pHs lower than the NP isoelectric point, although 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+ NPs 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.<sup>44</sup> Risks have also 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 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 whether denaturalized protein aggregates or cell debris. This applies for nanoparticles (3 to 100 nm), viruses (20-400 nm), bacteria (≈1000 nm),

<sup>39</sup> Oberdörster, G., Ferin, J., Lehnert, B. E., Correlation between particle-size, in-vivo particle persistence, and lung injury, *Environ. Health Perspect.* 102 (S5), 173–179, 1994.

<sup>40</sup> Warheit, D. B., Webb, T. R., Sayes, C. M., Colvin, V. L., and Reed, K. L., *Toxicol. Sci.* 91(1), 227-236, 2006

<sup>41</sup> Warheit, D. B., Webb, T. R., Colvin, V. L., Reed, K. L., and Sayes, C. M., *Toxicol. Sci.* 95(1), 270-280, 2006.

<sup>42</sup> NANO TC 229 WG 3 072-2007\_ Revised Draft TR Health and Safety Practices 2007-10-19-1

<sup>43</sup> 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.

<sup>44</sup> Warheit DB1, Laurence BR, Reed KL, Roach DH, Reynolds GA, Webb TR. *Toxicol Sci.* 2004 Jan;77(1):117-25

and eukariota cells ( $\approx 10.000$  nm). However, when the object is too big (beyond 10 micrometers),<sup>45</sup> the immune cells cannot engulf it and then triggers a chemical defence against the non-biodegradable material. This leads to tissue irritation and in the long run, may cause cancer. Needle-like microparticles such as asbestos 10 x 500 microns, are especially effective to induce this effect.

In determined conditions, NPs could aggregate to micrometric sizes. But 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 are two simple ways to avoid aggregation; to avoid high concentrations (if there are few NPs, it is difficult for NPs to meet to grow and form an aggregate), and to use anti-aggregation agents. Aggregation is a phenomenon thermodynamically favored, 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 their isolation even at high concentrations. 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 is there is risk of aggregation, and none once they have been dispersed in the working environment. Likely, when inorganic NPs are dispersed in serum they are rapidly coated by proteins (forming the so-called protein corona) which avoids their further aggregation, which would always be a subject of concern. 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 related to breathing dry (powdered) nanoparticles (and its aggregates).** Fortunately, NPs do not cross the skin and do not get inside the body from the intestinal tract (humans have been eating soil for millenia and naturally small NPs 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 (especially small NPs). Therefore, it is not recommended to be exposed to nanoparticle aerosols, and for that reason, it would be enough to avoid working in the dry phase. Wet NPs do not leave the solution, they stay in the liquid body and are not transferred to the atmosphere; if the drop is dried they aggregate and stick to the substrate. In a study of chemical contamination in the laboratory by electron microscopy and ICPMS, dispersion of the NPs from the liquid phase was not observed. The conclusions were that once the NPs have been somewhere, a tiny residue remains for ever even after washing (similarly with ions) but that there was no cross-contamination, even at extreme proximity from the vessels and vials that contained the solution. The ambient filters and air purifiers were empty of observable NPs (other than the micrometric particles of dust), concluding that the NPs cannot 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.

**iv.- Toxicity has been observed when the NP act as a reservoir of toxic ions that are delivered during corrosion.** The paradigmatic case is CdSe nanoparticles which *become more toxic* with time, as they corrode and yield Cd ions. 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 NPs to disintegration, by corrosion and other chemical transformation that dissolves it. In this process the NP yield ions, and also may yield electrons. Electrons are reactive and generate reactive oxygen species (ROS) which may be toxic if sustained for a long time (if the stress causing the response is maintained). Metallic cations are often bioactive, for example, cadmium, mercury and lead cations are very toxic to us, nickel is allergenic, cobalt is carcinogenic, silver cations are toxic to bacteria, and copper 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 NPs into iron ions has made iron NPs an active principle (feromuxytol) to treat ferropenic anaemia, controlling the dosing at the molecular level. Basically, the pattern of exposure, the dosing profile, is different when using the ionic species directly, or when these are provided by a dissolving NP (acute vs sustained dosing).

**v.- Toxicity has been related to the capacity of NPs in presenting antigens or allergens.** NPs can be good aggregators and orientators of molecules to be presented to the immune system.<sup>46</sup> Indeed, nanoparticles are excellent molecular carriers and a whole scientific field is developing around it, which could be a cause of major concern if functionalizable nanoparticles were dispersed in the environment and unfortunately associated with antigens or allergens before homogeneous or heterogeneous aggregation. For example, when car combustion

<sup>45</sup> Kostas Kostarelos Nature Biotechnology 2008, 26, 774 – 776.

<sup>46</sup> Homogeneous conjugation of peptides onto gold nanoparticles enhances macrophage response ACS nano 2009, 3 (6), 1335-1344

emission microparticles are coated by pollen grains, they become more allergenic. This is one of the reasons why allergies in urban areas are more intense than in the countryside. Therefore, the NP surface has to be passivated before being uncontrolledly dispersed. Fortunately, the concentration of toxins and allergens 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 unlikely that naked nanoparticles meet toxins, antigens or allergens before their surface is passivated by other molecules.

**vi.- Toxicity has been associated also with catalysis**, especially in the case of photocatalysis with NPs as TiO<sub>2</sub> 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 NPs for catalysis, it is well known that it is unexpected that NPs will act as powerful catalysts 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 protecting layer, nanoparticle life is extremely brief and they absorb irreversibly or vanish. In this case, the protecting layer dumps the catalytic powers of the inorganic nanoparticle. This is the case of TiO<sub>2</sub> coated with a thin transparent layer of Al<sub>2</sub>O<sub>3</sub> in sun screens. The TiO<sub>2</sub> is still able to absorb the high energy photon, but the electron thus generated is buried at the interlayer and rapidly recombines without generating the free radicals responsible for photocatalytic TiO<sub>2</sub> induced toxicity. In any case, iron oxide nanoparticles are not photocatalyst, nor considered effective catalyst with some exceptions for oxidation reactions.

**vii.- Toxicity has been related to hydrophobicity**, and since hydrophobic substances hardly disperse in biological environments, attention has to be paid to amphiphilic or detergent-like molecules that can be transported by NPs, such as in the well-known case of gold NPs coated with a cationic detergent like CTAB<sup>47</sup>. This detergent forms a double layer vesicle-like coating on the NP 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 to those observed with pure detergent molecules; however, it would happen at lower detergent doses in the case of association to NPs. Therefore the unintended mix of detergents and NPs it seems inadvisable. Note that detergents are already poorly biocompatible, although fortunately they are normally highly biodegradable.

**viii.- Toxicity can be observed if tissue is irradiated when nanoparticles are present.** The only toxicity related to irradiation of a NP 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.

All this knowledge allows us to work under nanosafety by design paradigms. Safety by design, from its definition, is a concept and movement that encourages construction or product designers to "design out" health and safety risks during design development. The concept supports the view that along with quality, program and cost; safety should be determined during the design stage in order to avoid development of technologies that result in being unsafe once they are already developed. Otherwise, we will suffer until the technology is forbidden, after problems have already been created, and the environment, polluted for decades.

Thus, risk mitigation methodologies can be developed and implemented taking into consideration the whole life cycle of a nano-enabled product. Innovative safer-by-design approaches beyond surface modifications can be designed, taking into account all the existing information on structural features that determine NPs' toxicity, release and degradation. In the case where hazards are found, NPs have to be re-designed so that detrimental specific NP characteristics are decreased while maintaining the desired unique intended parental properties. These strategies should not only focus on reducing NP hazards, but on reducing NP release from matrices or promoting their degradability after release.

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<sup>47</sup> <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2988217/>

<b>To reduce toxicity of the NPs.</b>
Shape and size modifications.
Increase in hydrophilicity to decrease the potential to cross biological membranes.
Increase in lipophilicity to promote aggregation and precipitation.
Modification of the intrinsic bulk composition of NPs or changing oxidation state to mitigate reactivity.
Design NPs that lose their catalytic activity when released from their embedding matrices
<b>To minimize release of NPs from their matrices.</b>
Induction of strong van der Waals and covalent bonding between the NPs and their matrices.
Development of barriers by multilayer approaches involving development of multilayer films, or multicoating approaches.
Self-healing pairs of NP / matrix by developing a suitable ionic approach to improve the formation of ionic bonding inside the host.
Induction of self-assembly of NMs in aqueous media or at high temperatures by introducing labile or different functionalities to increase the coalescent character of the NPs (sintering).
<b>To reduce persistence of NPs</b>
Development of new high biodegradable NPs under certain temperature or oxidative conditions.
Modify oxidation state of NPs.
Introduce impurities to increase the instability and degradability of NPs.

Table 1. Safety by design approximation extracted from GuideNANO ([www.guidenano.eu](http://www.guidenano.eu))

In addition to safer-by-design approaches, best practices for handling / packaging, different levels of confinement, and use of general exposure control measures and personal protective equipment (PPE) have to be included. The protection factors towards NPs for existing PPEs have yet to be fully evaluated, but in principle, protection against chemical substances does work for protection against nanoparticulate matter. When necessary, technological solutions have to be developed for exposure reduction and PPE (e.g., selecting less permeable materials, introducing double layers, use of nonwoven fabrics, and ventilated/pressurized systems). Depending on the efficiency of different exposure reduction technologies available, technological improvements in water and air filtering (e.g., foam technology) and treatment (destruction of NPs) may need to be developed.



# RESPONSIBLE INNOVATION COMPASS

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