APPLIED NANOPARTICLES: STASTING UP UNDER RESPONSIBLE RESEARCH AND INNOVATION (RRI) PRINCIPLES

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Abstract. APPLIED NANOPARTICLES S.L. (AppNPs) is a spin-off of the Catalan Institute of Nanoscience and Nanotechnology (ICN2), the University Autònoma of Barcelona (UAB) and the Institut Català de Recerca i Estudis Avançats (ICREA). Among its co-founders are scientists from these institutions, and experts on Responsible Research and Innovation (RRI), e-communication and business development and technology transfer. AppNPs has the office address in Barcelona. The main current objective of AppNPs is the commercial exploitation of the patent application "biogas production" (BioGAS+), in the U.S. and Europe, consisting on the use of iron oxide nanoparticles as additives to optimise the production of biogas by feeding with essential iron the involved bacterial consortia responsible for that. AppNPs also develops projects on the production, characterisation and commercialization of model nanoparticles, as well as consulting work related to other possible industrial uses of inorganic nanoparticles. AppNPs business is based on the principles of Responsible Innovation, focusing on the process design of nanoparticles, low energy consumption, low toxicity, waste minimization and reduction of emissions.

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1.- Methanisation and Iron Oxide Nanoparticles

The majority of the energy around us comes directly or indirectly from the sun. The small remaining extra portion comes from heat at the centre of the planet that is slowly cooling, from the gravitational pendulum of the moon and the seas, and from splitting heavy atoms in nuclear reactors. The rest, like the wind, is produced by the sun, which heats air masses that expand and become lighter displacing cooler ones. The wind also moves the waves. There is also the sun energy stored in chemical bonds by biology. This is why wood burns. And coal is fossilized wood. Photosynthesis is a very interesting way to store energy. Trees are made of condensed air by converting CO_2 into organic molecules and carbon based materials. Only a part of the water and little amounts of essential minerals are taken by the roots and come up to the leaves by capillarity. The roots are also made of air, from the CO_2 in air that is reduced by the sun via the photosynthesis. Thus, when the tree and the trunk are burned, the heat generated is a portion of the energy they took from the sun to build themselves. A portion, because there is always a loss of energy in any transformation, dissipated in form of entropy.

A chemical reduction reaction that begins with CO_2 in the atmosphere, accumulates it in the form of hydrocarbons and, progressively, in a multistep manner, evolves towards carbon, petroleum and shale gas. CO_2 is taken from the atmosphere, digested, transformed into more complex molecules and oxygen released. Ironically, the end of life in the planet will occur when no more CO_2 is available, when all the Carbon has been buried in solid and liquid forms into the rocks and the ocean depths. "Ironically", because today the massive extraction of fossil fuels that is taking place is subtly altering the chemical composition of the atmosphere by releasing vast amounts of CO_2 that was previously reduced by photosynthesis, thus going back on geochemical time, with very apparent consequences for climate change[1].

All organic matter, the biosphere, in its natural reduced state, is immersed in an atmosphere rich in oxygen, and has stored energy. As such, organic waste, pig manure and excrements also store energy. When something stops living, it decomposes. Being alive prevents us from decaying in a few hours. This decomposition ends up returning humidity in the form of water vapour, degraded organic matter, ultimately in the form of CO_2, small parts of other gases, and ashes with nitrates, phosphates and small amounts of other inorganic matter. Interestingly, if this process occurs in conditions where there is a low oxygen concentration, such as the naturally occurring underwater, underground or in man-made closed recipients; the organic matter is degraded into methane, CH_4. This is because a fraction of this organic matter, in the form of *archaea* bacteria, can breathe the oxygen bound in organic molecules and release methane. This molecule can easily be stored and transported for its posterior burning into CO_2, releasing thus all the energy contained in its four chemical bonds.

[1] At the beginning, when the Earth was very young, the atmospheric concentration of CO_2 was 98%. Now it is only 0.03%. Of course the temperature before was 240-340 ºC on the surface of the planet while it is 13ºC now. Similarly, the concentration of free oxygen was almost non-existent and today it is at 21%.

This process of transformation of organic matter into methane, or methanization, is not a spontaneous chemical process.[2] It is produced by consortiums of specialized archaic bacteria. These bacteria were among the first inhabitants of our planet. It is said that in that free-oxygen-less world then, life forms incorporated carbon into their organic matter by capturing CO_2 through photosynthesis. In the process they released 2 atoms of oxygen that progressively accumulated as a toxic waste in the atmosphere. Oxygen is of course very reactive, and it was very toxic back then. This is why we can still use hydrogen peroxide, H_2O_2, as a disinfectant. It burns things. And it was this waste from life, from initial metabolism, that caused the first massive extinction 2.400 million years ago in the Precambrian era[3]. Not all Precambrian forms of life disappeared. Some stayed alive in places without a good oxygen supply, at the bottom of wells, between rocks, inside of living things (the concentration of free oxygen inside the body is very low; it's all transported by haemoglobin), and they produce methane. This is why sewers explode when there is an accidental spark. These bacteria are everywhere, proliferating whenever they have the opportunity to access organic matter in the absence of oxygen – excrement, under skin or corpse. When exposed to oxygen, many die while some form spores to wait for more optimal conditions for their biochemical living.

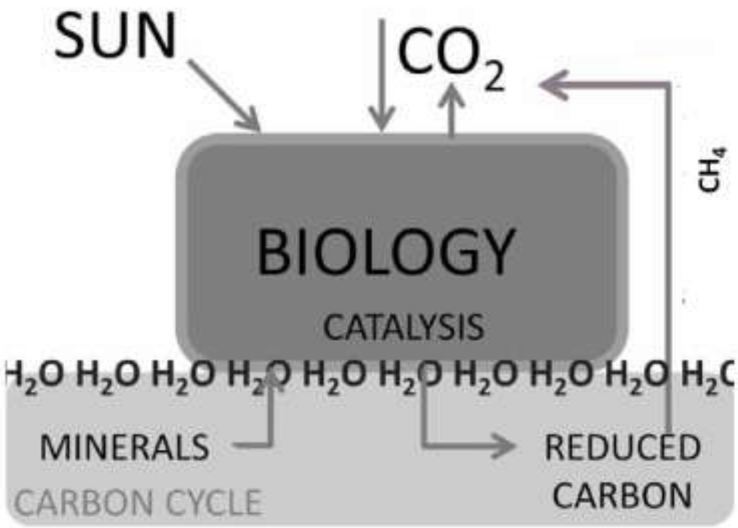

Fig.1 Carbon Cycle. Note that part of the reduced carbon is transformed into CH_4 which is constantly emitted to the atmosphere where it will be slowly oxydized to CO_2. Before that, in the form of CH_4, it produces about 20 times more green house effect than CO_2.

Both anaerobic and aerobic bacteria need iron for their functioning, like animals, plants and fungi. In fact, all life forms base part of their metabolism on the oxidative reduction of iron ions between valence states +2 and +3. In physiological conditions, iron can easily afford to donate or take an electron. Taking and giving away electrons is the essence of (bio) chemistry. Normally, bacteria does not store iron, as mammals do with ferritin, therefore, they need to take it from the environment. In the environment, there is a great abundance of iron in its

[2] A steak lost in space will be around forever. Putrefaction is not a physicochemical degradation like the one that molded cliffs and valleys, but rather a biological process.
[3] Apparently, we needed four more to get ready to produce the sixth.

inorganic form. The planet's core is made of iron and it is the fourth most abundant element in the crust. But it takes an important biochemical effort to transform the iron found in rocks into biologically available, like in blood. For us, eating screws or red soil will never cure anaemia, but microbes can do it, even if they also prefer to take iron already inserted in the biological units. Thus, when bacteria infect an organism, the largest and most immediate genetic expression alteration they experiment has to do with the finding, trapping and use of iron for their proliferation. And it is for this reason that when bacteria are detected by the immune system, one of the first defence actions is to remove iron and sugar available in blood[4].

Interestingly, in this context, in conditions of anaerobic breakdown, in the absence of oxygen, small doses of mixed iron oxide nanoparticles (NPs) serve as a *catalyst* that stimulates bacteria metabolism and accelerates the production of biogas (a mixture of different gases produced by the breakdown of organic matter in the absence of oxygen, mainly CO_2 and CH_4) up to three times with cellulose as feedstock in laboratory conditions (DIN-38414)[5]. Thus, the process that converts organic waste into raw matter for energy production is optimized by simply adding a small dose of iron NPs either to a large waste treatment reactor, a septic tank or a homemade biodigester.

This is based on the effects of the presence of essential trace elements in the methanogenesis process, and the optimized dosing when using small unstable NPs that corrode and dissolve as ions provider. In fact, a challenging area of anaerobic digestion research remains largely unchartered with respect to understanding the role of trace metals in enabling biogas production. This major knowledge gap and scientific challenge is a multifaceted problem involving metal chemistry, physical interactions of metal and solids, microbiology and technology optimisation.

2.- Climate Change and transition to renewable energy systems

Due to the fact that every molecule of CH_4 ends up being oxidized to CO_2 and that a molecule of CH_4 causes up to 20 times more greenhouse effect than a molecule of CO_2, it is the responsibility of everyone, in order to create a cleaner planet with a more stable atmosphere, to prevent CH_4 from entering the atmosphere and rather to introduce it into our stoves, vehicles and heaters. When this CH_4 comes from CO_2 in the atmosphere that was trapped by recent photosynthesis, returning it to the atmosphere will assure the maintenance of a constant concentration of gases in the atmosphere, while combustion of fossil fuels is altering it.

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[4] This is a classic example of burning a city and its reserves before it falls in the hands of the enemy. The infected person is thus weakened so as not to provide nutrients to the bacteria that have invaded the organism. Ironically, many years ago, someone thought it was a good idea to combat the symptoms of an infection, like apathy, tiredness and anemia with iron supplements, which exacerbated the virulence of the infection at once.

[5] 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

In this sense, the urgency in tackling climate change and promoting renewable sources of energy has been universally agreed and it is the responsibility of all citizens, including the scientific and business communities[6]. The UN General Assembly adopted unanimously on 25 September 2015 the New Sustainable Development Agenda (*Transforming our world: the 2030 Agenda for Sustainable Development*) with 17 global goals at its core, being Goal 7 "*Ensure Access to affordable, reliable, sustainable and modern energy for all* and Goal 13 *Take urgent action to combat climate change and its impacts"*. Interestingly, unlike their predecessor (the Millennium Development Goals) the Sustainable Development Goals (SGSs) explicitly call on all business and scientific communities to apply their creativity and innovation to solve sustainable development challenges: *"We the peoples"* are the celebrated opening words of the Charter of the United Nations. It is *"we the peoples"* who are embarking today on the road to 2030: *The journey should involve Governments as well as parliaments, the United Nations system and other international institutions, local authorities, indigenous peoples, civil society, business and the private sector, the scientific and academic community – and all people. Millions have already engaged with, and will own, this Agenda. It is an Agenda of the people, by the people and for the people – and this, we believe, will ensure its success.*

Aligned with this broad framework, the EU is building a regulatory framework favouring the development of energy from renewable sources that, ideally, should be closely linked to increased energy efficiency and decentralized energy production. In this sense, one of the most promising renewable energy sources is the Biogas produced during anaerobic digestion of organic substrates. Biogas production represents a non-polluting, carbon neutral energy source from local raw materials. Also, methane is rising as an alternative to store energy from other renewable sources. Furthermore, biogas production is aligned with the EU strategy and current regulation on waste management.

There is not a biogas sectorial regulation, but the biogas sector is being directly affected by divers EU directives in a wide variety of fields (among others): Directive 2003/55/EC (Injection of biogas into the natural gas network. Aims to open the existing gas network from other inputs than natural gas); Directive 1999/31/EC (Gradual reduction of the filling of biodegradable municipal waste on landfills, by 2016 to 35% of the level of 1995); Directive 2009/28/EC (Promotion of the use of energy from renewable sources); Directive 91/676/EEC (The Nitrate Directive setting limits to the quantity of nitrates that can be spread on agricultural land); Directive 86/178/EEC (The sewage sludge directive); Directive 96/61/EEC (IPPC directive); Directive 98/70/CE; (Quality of petrol and diesel fuels with specific rules for biofuels).

Recently, after Germany, transition to renewable energy was designed by the France's government in 2015, and -in line with the experiences in north of Italy, Sweden and Denmark- a new ideal is being created for the development of biogas and renewable energy. At the same time, untreated waste from organic matter continues to pollute the planet and sends uncontrolled amounts of CH_4 into the atmosphere.

[6] www.un.org/ga/search/view_doc.asp?symbol=A/RES/70/1&Lang=E

3.- Creation and development of Applied Nanoparticles S.L.

i) Basic data

Applied Nanoparticles SL 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 funded on October 17th, 2013. The company is made up by 13 shareholders with no single shareholder having more than a 10% of shares, a very particular feature that has clearly defined the direction taken by our company[7]. As per today, 10 out of those 13 shareholders are or have been directly or indirectly involved in the day to day work. This structure makes it very diverse and robust against modifications and unexpected transformation of the initial aims, needing a democratic majority of shareholders for fundamental decision making. Up to now, the company is structured without explicit CEO. Decisions and strategies are agreed between the legal representative, the scientific director, workers and active shareholder. The implementation of the resolutions adopted is delegated to the most suitable and most available person in a knowledge based democratic system.

The core project and main reason for setting up Applied Nanoparticles SL is the commercial exploitation of a patent based on the use of engineered iron oxide NPs for enhanced biogas production, named BioGAS+. In this sense, our company forms part of an innovation model, as explained by Jones[8], focused on the direct translation of research from Universities into direct spin-off companies (often supported by venture capital and foundations), and that *systematically underestimates the value of tacit knowledge and know-how in manufacturing processes, and overestimates the importance of protectable IP*. We consider that our company has departed from this model thanks in part to the incorporation of the Responsible Research and Innovation principles to the organization, and the consequences that this incorporation has meant.

ii) Company developments

The work on the use of iron oxide nanoparticles to enhance biogas production started in 2006 with a project called NANOCLEAN founded by the Spanish Environmental Ministry, where it was observed the enhanced production of Biogas in the presence of iron oxide nanoparticles (up to a 200% increase). This was observed when studying the potential toxic effects of

[7] Among them there are Researchers in nanoscience and nanotechnology and/or Environmental Science –and inventors of the Biogas+ Patent- and the remaining are from other disciplines like, lawyers, marketing experts, e-communication experts, graphic designers and alike. This unusual composition has been of paramount importance on the direction that our company has decided to take.

[8] Jones, R.A.L. (2011) What has nanotechnology taught us about contemporary technoscience? http://eprints.whiterose.ac.uk/43375/

different common NPs in bacterial consortia intended for waste water treatment. To our surprise, instead of toxic, some iron oxide nanoparticles were beneficial. Note that more generation of Biogas from the same residue implies an increased degradation of it, this is why we couple the generation of renewable energy with the treatment of waste. After repeating the experiments and performing the corresponding control experiments, the phenomenon consistently reproduced and we decided to start filling a patent[9] to protect it. At this point, the institutions –patent owners- carried out a series of non-fructiferous technology transfer promotion actions in specialized forums and conferences. We had some interested companies on our product, normally technology brokers, but it never went any far as the technology was considered still too immature, so not yet interesting for (nanotechnology) business angels. Roughly, in the Technology Readiness Levels (TRL, 1 to 9, from the Eureka! to the placement in the market), one hurries up to protect his discoveries at levels 2-3 and it is not minimally commercializable until levels 6-7.

Therefore, we decided to overcome this TRL gap by looking for altruist funding from private foundations or public bodies through technology transfer national and European support programs. In 2011 we obtained funding from the Bill & Melinda Gates Foundation Grand Challenges Explorations Grants (proposal OPP1044410). In 2012 we present our project to the Fundación Repsol[10] Entrepreneurs Fund[11]. At that time we were selected as pre-awarded only, because our technology was too immature and lacked the infrastructure behind to develop it. The pre-award consisted in a reduced funding and coaching for one year, with the objective to create the commercial tool to develop our own patent and precisely define our business model, project and product. In 2013 we also obtained the Ibero-American Secretariat General Innovation and Entrepreneurship Award[12]. In 2014 we submitted a new proposal to the Fundación Repsol and we got awarded their incubation prize which consisted on mentoring, education in entrepreneurship (business-school like), and basic funding for two years (finishing in September 2016).

As a mean to obtain and explore different funding venues we diversified activities in services analysis and characterization, sales of model nanoparticles and technical reports (as on the effectiveness of photocatalytic NPs of titanium oxide in the urban pavement for the oxidation

[9] Using magnetite NPs in the production of biogas through anaerobic digestion of cellulose has been patented by the Universitat Autònma de Barcelona (UAB), the Institut Català de Recerca i Estudis Avançats (ICREA) and the Catalan Institute of Nanotechnology (ICN2) (WO 2012/123331 A1, published 20/09/2012 and PCT/EP2012/054022,11/09/2013). In July 2015 Applied Nanoparticles SL signed with the Patent owners an exclusive licensing agreement for BioGAS+.

[10] Fundación Repsol is one of the means by which Repsol fulfills its commitment to social responsibility, acting as a cannel for the group'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.

[11] 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 posible. www.fondoemprendedores.fundacionrepsol.com/en/entrepreneurs-fund

[12] The Ibero-American Secretariat General (SEGIB) is an international support organisation of the Ibero-American Summit of Heads of State and Government, implement its mandates and drive Ibero-American Cooperation in the areas of Education, social cohesion and culture. Sgib.org/en/who-we-are.

of NO to NO_3, and its potential toxicity). We have also obtained funding from the EU cooperation programs (H2020) and support for SMEs 1st stage. In October 2015 the H2020 NanoFASE collaborative project started, dealing with the potential ecotoxicity and environmental fate of industrial nanoparticles-based products, in which BioGAS+ is one of the project's case studies and AppNPs a partner of the research consortium.

In a spin-off company, human resources are critical: with no financial strength, neither infrastructures, only knowledge and human resources can be our competitive tools. Therefore, highly motivated and skilled employees are needed, and one may not be able to retain them in small companies for a long period (whether they will pursue their academic and investigation career or because you will not be able to compete financially). In our case, the way to retain human capital has been a combination of 2 concepts, namely: "company in the making" and "company with purpose".

When talking of "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 without formal CEO in the company's structure and personal responsibility of follow up actions). This gives plenty of room for personal development possibilities, depending on the real level of involvement that each one is able to provide. The company thus serves as a platform for project development and opportunities generation. Ideas, in our field of action, nanoparticles engineering and production, are welcome and considered.

By "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 at these early times of nanotechnology development driven by principles of Responsible Research and Innovation in order to generate wealth responsibly, minimizing deleterious size effects, with special care on sustainability (understanding that our survival instinct expands to our sons and the sons of our sons and so on). All our activities, resources and funds are directed to this objective. The vision is present on the day to day of the laboratory work. Principles and action go together and can be directly experienced.

iii) BioGAS+ project development

With regard to product development we have been working in the following: i) Reformulating the synthesis process, optimizing costs, raw materials and processes, ii) Preservation and aging of samples[13], iii) Scaling up and study of industrial production[14], iv) Synthesis products with recycled iron precursors and industrial grade NaOH, v) Testing similar products from other producers, vi) Developing a small simple in-house system to test biogas production to be

[13] Wherever observed rapid surface oxidation followed by a progressive slower oxidation of the whole NP who starts by being pure magnetite (mixed Fe^{3+}/Fe^{2+}) and ends up being pure maghemite (pure Fe^{3+}). The more oxidized is the particle, the more inert it is. Nitrogen preservation and deoxygenated water allows the product to remain unchanged for months.

[14] We have produced the NPs on scales of 100 and 500 liters. The product obtained is not identical to the one obtained in the lab, but is more reproducible.

independent from other to test the power of our produced NPs and vii) Developing the capacity of producing 1 Kg of NPs per day with a set of three 10 L reactors. viii) Increasing our knowledge and know-how in manufacturing processes: For that we have searched for alliances with industrial partners (from providers to end users) with which we share part of our know-how and exclusive rights on part of our product and patent in exchange of know-how and resources.- In this sense, it has been important the decision of selecting only industrial partners ready to share resources and knowledge, and conscious of the time needed for getting the right, safe and sustainable products, and discarding any negotiation with venture capitalists that may imposes requirements for unrealistically fast timescales to bring products to market, or transform the project into some financial derivative to rise funds.

During this time we tested the ability of our product to increase the production of biogas from different types of feedstock in collaboration with several industrial partners (sewage sludge, meat-processing waste, etc.), where clear enhancement of the biogas production was observed when employing different doses of BioGAS+ in the different types of residues. The initial variability of observed results depend on the intrinsic biological variability, exacerbated when working in batch reactors, changing often residue and inoculum; the variability in the oxidation state of the NPs, and the change in buoyancy of NPs, what ultimately determines the dose of iron offered to bacteria. Also, we have made progress in understanding the kinetics of the process which must allow us in the future to reformulate and optimize the process and adapt it to different requirements imposed by the different types of waste, different type of reactor/digesters and different types of work regimes, and customize our solution.

We have also committed a considerable amount of time and resources to study both the toxicity and exposure of our BioGAS+ during the whole LCA of the product, thus assessing its potential risks. Those studies are going to be continued within the NanoFASE[15] project, part of the EU H2020 cooperation program, which, besides further studies on toxicity and risk assessment, will add studies on the modelling of the environmental fate of the product. As per today (the project its only on its 6th month), the first preliminary results regarding toxicity of BioGAS+ as a product are consistent with the very low toxicity and high biocompatibility of iron oxides nanoparticles already reported in the literature[16].

4.- Responsible Research and Innovation at Applied Nanoparticles S.L.

i) Why RRI?

Our previous experience in the fields of nanosafety & nanosustainability -mainly through collaborative projects under EU FP6 and FP7 and the participation of some of us in the Centre for NanoBioSafety and Sustainaility (CNBSS[17]) development-, the need to design our legal

[15] Detailed information on the project avilable at: www.nanofase.eu

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

[17] http://www.cnbss.eu/

strategy and our interest in nanotechnology education and dissemination can be seen as the main reasons that motivate our interest in the concept of RRI and its practical expression in the daily work of our company.

Let's briefly comment on these three reasons;

Our work on nanosafety & nanosustainability makes us familiars with concepts as green chemistry, safety by design, LCA, workers H&S with nanomaterials, etc. Indeed it was while studying potential toxicity of NPs to the bacterial consortium responsible for the transformation of organic waste into biogas that we discovered the benefits of using iron oxide NPs.

Regarding the design of our legal strategy and as it is well known, 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[18]. As the current regulatory regime applicable to Nanotechnology and Nanomaterials (N&N) was not designed for nanomaterials, it is (up to some point) inadequate[19]. The consequence of this inadequacy to private economic operators regarding their legal obligations (their legal compliance) is legal uncertainty. Consequently, where explicit nano regulation do not exist and the economic operator is faced with open legal concepts[20], it is of upmost importance to draw on all viable regulatory options given by the regulator, either in hard or soft law[21]. It is clear that a comprehensive legal strategy[22] is out of

[18] The lack of a nano-specific legislation measures does not mean that nanotechnologies are an entirely unregulated enterprise. Their regulation falls to existing provisions designed to manage risks associated with conventional, bulk-sized materials. Van Calster, G. (2006) *Regulating Nanotechnology in the European Union*. *Nanotechnology Law & Business*. 3 (2006). 359 374. At 359. Ronit, J-H., Tmar, D. *Europan risk governance of nanotechnology: Explaining the emerging regulatory policy* Research Policy 44 (2015) 1527 – 1536. We can refer to horizontal legislation (General Product Safety, Product Liability legislation, Chemical legislation (REACH,CLP), Worker Protection legislation, sectorial regulation –air, water, soil, waste, IPPC…-).

[19] Those inadequacies are basically, knowledge, regulatory design and information gaps. They are being filled, basically, 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 (like the one under consideration regarding REACH); c) promoting anticipatory legal governance to private operators, based on the use of soft law mechanisms.

[20] For instance, the Chemical Agents Directive 98/24/EC shows the minimum requirements for protecting workers from the adverse effects of chemical agents that are present at the workplace. Employees are required to assess the risks and control them while Ocuppational Exposure Limits (OELs) does not exist for nanomaterials. For individual companies, a derivation of an OEL requires large amounts of toxicity data which is complicated and expensive to obtain. In most cases, a provisional benchmarks levels alternative to OELs may be developed by public institutions (or in other fields private organizations like the ISO). The competent authority may recommend its adoption as a soft law instrument. Private operators may positively react to its adoption as offers temporary certainty with regard to their legal obligation to take preventive action. Van Broekhuuizen, P., Dorbeck-Jung, B. <*Exposure limits Values for Nanomaterials – Capacity and willingness of users to Apply a Precautionary Approach.* Journal of Acuppational and Environmental Hygiene. Vol. 10, Issue 1. January 2013. 46-53.

[21] Whether Enforced self-regulation (mandatory reporting scheme), Voluntary self-regulation (Codes of conduct, risk management systems, reporting schemes) or Guidelines and Standards (ISO, OECD, government authorities).

reach of any start up, whether in human or economic resources. What then? Confronted with these limitations, the most sensible course of action is to focus on risk avoidance that 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, to develop safe and sustainable nanomaterials, thus applying RRI principles. But it also has to be noted that any comprehensive legal strategy, to be successful, also has to start by the same rationale: developing safe and sustainable products is the cornerstone of a successful (legal) strategy.

Some of our shareholders have been for a long time working in the field of multidisciplinary education & communication convinced that for a smooth introduction of nanotechnology, openness and education are fundamental factors. Among undertaken projects we can mention the information service Nanowiki[23], exhibitions based on electron microscope images[24], NanoColoringBook[25], GoldLight Quantum Jewellery[26] and several general audience books related with Nanoscience and Nanotechnology[27]. What is the meaning of these activities? We consider that to the scientist, art, understood as a communication device, forces him to condensate and depurate his ideas about the observed subject. Aesthetical experience, similar to conceptual understanding, has neurochemical effects, as increased secretion of endorphins. When both are couple together, the discovery experience is well more pleasant. Therefore, the practice of art improves us as scientists. Regarding communication, art is a wonderful media to reach a broad part of the population which is not sensitive to direct scientific communication. Emotions related to the artistic experience help recording the memories and deepen the learning. We believe that probably, instead of finding wonderful new solutions for the challenges we face -something that only really happens from time to time- our better contribution to society should be to improve its educational level ("there is no technology that will save us from ourselves"[28]). We also believe that research is needed in order to be able to teach.

ii) Our approach to RRI.

We were starting to work with RRI principles when the focus was on “emerging technologies” (nanotechnology, synthetic biology, geo-engineering, stem cell science, etc.), so potential

[22] We understand that a comprehensive legal strategy have to tackle the following areas: audit insurance portfolio, audit and inventory potential hazards, audit and monitor regulation, assess compliance with standards and best practices, audit and monitor workplace safety, assess production chain contracts, identify and address post manufacturing issues, tackle risk communication and information accessibility and set up a nanotechnology risk expert team.

[23] Nanowiki information service has been considered among the 10 most influential nanotechnology accounts: ”Nanotech: 10 Twitter accounts to follow to keep up to date” by Hope Reese, TechRepublic http://www.techrepublic.com/article/nanotech-10-twitter-accounts-to-follow-to-keep-up-to-date/

[24] Paisatges artificials, un viatge aéri a nanolandia (2002) http://straddle3.net/openfridays/#2; Promenade in nanoland (2004). http://straddle3.net/context/03/en/2004_01_27.html https://archive.org/details/promenade_in_nanoland; http://metode.cat/Revistes/Metodart/Nano

[25] Coloring book. https://archive.org/details/NanoColoreja; https://archive.org/details/NanoColorea

[26] http://www.theguardian.com/what-is-nano/precious-particles; https://www.facebook.com/goldlightjewels/

[27] https://archive.org/search.php?query=creator%3A%22v%C3%AD%C2%ADctor%20puntes%22

[28] The tragedy of commons http://science.sciencemag.org/content/162/3859/1243.full

controversial areas of research and innovation at an early stage of development, and where the day to day work on the lab was of paramount importance on the whole conceptual architecture. We have (dishearteningly) witnessed the evolution of the concept to a set of “keys” of RRI[29] that as stated by De Saille “are not necessarily specific to RRI or even to R&D policy, but are broadly the result of legal changes to the governance of the EU, and to an evolution in the understanding of the rights of citizens to have a say in how they are governed”[30]. Especially considering that the majority of innovation in nanotechnology today is based on spin-off companies generated in the academic environment that are developing the new frontiers of nano-related scientific development before larger companies buy the developed technology. Indeed, it seems to be lacking more concrete activities (“soft” interventions) focused on the day to day laboratory work of spin-off companies[31] and about effective policies for helping them to balance societal and economic considerations (because it is clear that as per today, promotion clearly outweighs precaution).[32]

Following the definition given by Von Schomberg[33] when developing responsible innovation, we consider the *Product and Process dimensions*. As within the Product Dimension, the focal points are Ethical Acceptability, Safety and Sustainability while on the Process Dimension we have Stakeholder Involvement, Transparency and Responsibility Awareness.

Within ethical acceptability, one finds *Constitutional Values* and *Grand Challenges*:
Constitutional Values. It is commonly understood that RRI is aimed at the pursuit of particular values (normative targets or ends) that, in the EU context, can be found on the Treaty of the European Union and the Charter of Fundamental Rights of the EU (also named “constitutional values”)[34]. These normative targets have been democratically agreed and provide the

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[29] Namely: inclusive engagement; gender equality; more science education; ethics; open access to data; new models of governance. Responsible Research and Innovation: Europe’s ability to respond to societal challenges. KI-31-12-921-EN-C). http://bookshop.europa.eu.

[30] De Saille, ”Innovating Innovation Policy: The emergence of “Responsible Reseach and Innovation”. Journal of Responsible Innovation 2:2.
http://www.tandfonline.com/doi/abs/10.1080/23299460.2015.1045280

[31] Van Greenhuizenn, M., Ye, Q. “Responsible Innovators: an Exploration of Networks Conditions in View of Transition”. Triple Helix Conference 2013; Scholten, V.E., Van der Duin, P.A. “Responsible innovation among academic spin-offs: how responsable practices help developing absorptive capacity” Journal on Chain and Network Science 2015; 15(2): 165-179.

[32] Phelps, R., Fisher, E. “Legislating the laboratory? Promotion and Precaution in a Nanmaterials Company”. In Sarah J. Hurst (ed.). Biomediacal Nanoatechnology: Methods and Protocols. Methods in Molecular Biology. Vol 762. Springer 2011. 339 – 359.

[33] ”A transparent, interactive process by which societal actors and innovators become mutually responsive to each other with a view to the (ethical) acceptability, sustainability and societal desirability of thee innovation process and its marketable products (in order to allow a proper embedding of scientific and technological advances in our society)”. Von Schomberg, R. Prospects for technology assessment in a framework of responsable research and innovation. In: Dusseldorp, M., Beecroft, R. (Eds.), Technikfolgen Abschätzen Lehren: Bildungspotenziale Transdisziplinarer, Vs Verlag. Methoden, Wiesbaden.

[34] Namely: Promotion of scientific and Technological Advance; Promotion of social justice, gender equality, solidarity, Fundamental Rights; Quality of Life, High level of protection (human health and Environment); Sustainable Development; Competitive Social Market Economy. Ozolina, Z., Mitcham C., Schroeder, D., Mordini, E., McCarthy, P. & Crowley J. (2012) Ethical and Regulatory Challenges to Science

legitimate basis for defining the type of impacts that research and innovation should pursue[35]. It is clear that such high level normative anchor points provide a legitimate starting point concerning the goals, purposes and motivations, and that tensions and even conflict may exist in particular situations among them; but we consider extremely important to discuss, understand and integrate such constitutional values. It is precisely in these moments were it has to be clearly differentiated between common and democratic agreed constitutional values and particular policy actions. Only this differentiation will permit to advance in democratic terms. At Applied Nanoparticles SL those constitutional values has been discussed among shareholders and workers and finally entered in our *Code of Conduct*. We do 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 bases.

As we will see in more detail on Section 5 of this Chapter, the values that we have agreed upon are those that we all recognize as universal (human dignity, solidarity, sustainability, social justice, transparency and democratic participation) and 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)[36] and will carry out actions for internal application, knowledge and dissemination. As it will be seen from our Code of Conduct, we do consider that RRI is best understood as a normative-political orientation that seeks to alter the present sociotechnical order, so steeped in the philosophy of deliberative democracy and in social constructivist approaches of science. In this sense, we consider that presenting RRI to policy-makers as a politically neutral tool risks trivializing and undermining the very policy changes RRI advocates seek to instigate: “Adopting a more politically laden language of agendas, interests, impacts, and power may be an unconventional and provocative choice of policy strategy, but may ultimately be more fruitful”.[37]

In this same line of thought it can be said that the RRI governance cannot be credible if an equal access to the marketplace is not guaranteed: as per today, to develop products under RRI require more time and economic resources (at least in the short term). This situation makes developments under RRI a voluntary and moral decision (the same can be said about Corporate Social Responsibility –CSR-) that will never have a real impact in changing current technoscience trajectories and, in the end, in global economic dynamics. In any case, it can be easily acknowledge that the less corrupted societies are, the safer and wealthier.

Grand Challenges. As we have seen in Section 2 of this Chapter, the urgency in tackling climate change and promoting renewable sources of energy has been universally agreed as one of the

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and Research Policy at the Global Level. Expert Group report, Directorate General for Research and Innovation of the European Commission. Luxemburg: Publication Office of the EU.

[35] Von Schomberg, R. (2013) “*A vision of responsible innovation*”. In: R. Owen, M. Heintz and Bessant, J. (Eds.). responsible Innovation. London: John Wiley.2013.

[36] https://www.ecogood.orgg/en

[37] Michiel van Oudheusden (2014) *Where are the politics in responsible innovation? European governance, technology assessment, and beyond*, Journal of Responsible Innovation, 1:1, 67-86, http://dx.doi.org/10.1080/23299460.2014.882097.

Grand Challenges of our time. We also have seen the 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 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. Many existing technologies are approaching this problem (i.e. pre-treatment of the biomass, thermalization of the waste, combination of feedstock and inoculums) and 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 BioGAS+ project proposes a disruptive, effective and adaptable solution to enhance existing biogas production technologies in biogas plants. BioGAS+ is the first ready to use nano-additive that transforms the actual process of methanisation into an economically viable process. Consequently, empowering more transformation of organic waste into biogas. In this sense, our project could thus help in combating climate change and promoting renewable energy systems.

Regarding *Safety*, within Applied Nanoparticle SL, we take advantage of the previous experience of our personnel working with nanoparticles and nanomaterials (such as the avoidance of using dry powders at any time, working always in the wet phase in order to strongly reduce exposure to NMs, and thus associated risk, etc.) combined with following broader Nanosafety Guidances and Frameworks published by some European institutions focused on nanosafety, basically:

i) *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 a 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 a EU H2020 Project in which our product is one of the chosen case studies.

ii) 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). Even if it is very general on dealing with all sorts of nanomaterials in a safe way, and that we consider that some amendments should be done to become more nanoparticles focused to completely suit our circumstances, 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.

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Regarding *Sustainability*, Applied Nanoparticles is very committed to work in the greenest and environmentally friendly conditions possible. After some research on existing literature on Green Chemistry and additionally to our endorsement to broader and aforementioned Millennium Goals, we observed that the most accepted and extended guidance on the topic is the list of 12 Principles of Green Chemistry developed by Paul Anastas and John Warner on 1998[38], a list of requirements that an ideal "green" or environmentally friendly chemical, process or product would follow or accomplish. These 12 principles would be as follows[39]:

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

We are confident that the production process of our main product, based on magnetite (Fe_3O_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[40], being iron a life-essential *oligoelement* and iron oxides, even in the nanometric form, very natural abundant materials[41]. Our raw materials (iron I and II chlorides) derive from these natural abundant oxides and 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

[38] Anastas, P. T.; Warner, J. C. Green Chemistry: Theory and Practice, Oxford University Press: New York, 1998, p.30

[39] List and description taken from: http://www.acs.org/content/acs/en/greenchemistry/what-is-green-chemistry/principles/12-principles-of-green-chemistry.html

[40] 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. Ankamwar 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*

[41] http://www.lulu.com/shop/v%C3%ADctor-puntes-and-josep-salda%C3%B1a-cavall%C3%A9/nanoparticles-before-nanotechnology/ebook/product-20635604.html

temperature, the production process has a very low energetic demand (except from the generation of the required stirring power). In terms of waste management and degradation, the size and dose of the nanoparticles are purposely designed to completely dissolve during the tens of days of a standard anaerobic digestion, and by-products of the synthesis generated are basically basic waters from purification steps, which are recycled and reused as starting basic solution for further synthesis (completing it with the corresponding amount of new base).

Regarding *Process Dimension*, it is key the involvement of Stakeholders, Transparency and Responsible Awareness. Although acknowledging that the continuous and consistent involvement of society in the research and innovation process is one key aspect of RRI[42], it has to be recognised that from the perspective of a start-up the task has to be approached with caution and respect. It is not only the economic constrains but the inherent difficulty in setting up public engagement practices (methods of participation, the purposes, the evaluation criteria, etc.). 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 (http://www.appliednanoparticles.eu/news/newsletter/). These tools goes well beyond current information services as our aim is to filter, curate and contextualize information in the fields of biogas and nanotechnology.[43]

Our final aim is to generate debate (inside and outside our company) on fundamental topics. From an RRI perspective one of such topics is the ethical debates around biogas[44]. It has to be underlined that such debates are influencing our approach to market (and our business plan) in a fundamental way[45].

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[42] Sutcliff, H. "A Report on Responsible Research and Innovation for the European Commission". MATTER. 2011.

[43] 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 do this work by filtering and curating in the field of nanotechnology. There was a unique process and 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 sought to provide an extra value: the context. This is achieved by information itself and promoting debate / controversy and / or contrast of opposing paradigms in the same capsule (by means of the context and follow up). The compilation of the capsules over time became an online database with their own search tools and labelling information, and is updated and contextualized in book format (electronic and paper) with a unique signature graphic.

[44] 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

[45] The German market represents approximately the 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 confront us with the ethical "food vs. fuel" debate (bioenergy production may compete, directly or indirectly, with food production, and as a consequence the food security may be adversely affected). Based on ethical ground we have decided to stop any possible commercialization of BioGAS+ to any

Regarding Transparency we would like to point out to the increasing problem to transparency posed by the increased generalization of Non Disclosure Agreements (NDA) that have to be signed with every possible client and supplier and at a very early stage of any negotiation.

Finally, we turn to the Responsibility Awareness: Following Schuurbiers[46] we consider that researchers have a moral responsibility to critically reflect on the wider socio-ethical context of their work:

- The "value-based socio-ethical premises" of research;
- Epistemological and ontological assumptions;
- Methodological norms of scientific culture;
- The socio-ethical consequences of research

As we are working in and from the laboratory, we are especially interested in the "midstream"[47] phase as an opportunity for addressing social and ethical concerns. While preparing the ground for the effective application of modulation strategies in our company we have been closely following the "midstream modulation"[48] framework and the STIR project[49] methodologies.

Having in mind our economic and human constrains we have structured the modulation around periodical informal review meetings. In those meetings we analyse the technology and business parts of our business and a review of the most interesting information published by our newsletter is discussed. Overall, a wide range of topics are reviewed (environmental health and safety, sustainability, patenting, long-term research and business strategies, ethical issues and the responsibility of scientists to communicate with society).

Having in mind that our scientists have been working under RRI related principles for a long time, we considered appropriate to point to potential social and ethical aspects (accepting the "tracker-dog" role) and simultaneously to use the SocioTechnical Integration Research (STIR)

Anaerobic digestion plant using crop as a feedstock. Having in mind the market figures given, no need to say the far reaching consequences of our decision.

[46] Schuurbiers, 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.

[47] '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.

[48] Midstream modulation refers to research and innovation decisions that implement, or enact, strategic visions and objectives. Midstream modulation plays out in three iterative phases: De facto modulation, Reflexive modulation and Deliberate modulation. Delivered modulation involves putting the increased reflexive awareness generated in the previous step to work during research or strategic decision-making. This may be done to improve either the products or the processes of decisions. It typically results from the practitioner perceiving a broader variety of either decision alternatives or decision values to be at play in her actions. Fisher, E., Rip, A., "Responsible Innovation: Multi-Level Dynamics and Soft Intervention Practices". R. Owen, M. Heintz and Bessant, J. (Eds.). Responsible Innovation. London: John Wiley.2013.

[49] SocioTechnical Integration Research (STIR) led by E. Fisher (http://cns.asu.edu/ stir). The STIR project is a form of collaborative inquires between natural scientists and engineers on one hand, and social scientists and humanities scholars on the other, with the objective of building multi-level capacities for anticipatory and reflexive governance. Fisher, E. "Ethnographic Invention: Probing the Capacity of Laboratory Decisions". NanoEthics (2007) 1: 155-165; Schuurbiers, D., Fisher, E., "Lab-scale intervention". EMRO reports. Vol. 10. No. 5. 2009. 424 – 427.

decision protocol. In this sense, our experience could point 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.).

5.- Code of Conduct

The first draft of our Code of Conduct reads a 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 the 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 carry out actions for internal application, and knowledge dissemination.
These principles and objectives emanate standards of ethical behavior listed below:

4.- ETHICAL STANDARDS OF CONDUCT

RELATIONSHIP WITH WORKERS
4.1.- HEALTH AND SAFETY AT 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 steaming from the nontechnology academic laboratories and environments as the nanosafety cluster (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 in 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 the responsible that increased value. Employees authorized personal use of

resources 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 developed of work, following general visions and interest and not restricted to the strictly planed and commanded, what would be a poor method for progress allowing for more exploration and broadening of the impact of our activities,
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 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 the 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 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 the company should share in the profits it generates. This is in line to prioritize 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. Besides, up to now, we have been working exclusively with ethical banking and/or cooperative. Basically because in our short experience, we get a better service at a better price in these institutions.

4.5.- RELATIONSHIP WTH 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 Assembles 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.

6.- Potential Nanoparticles Toxicity

Today, the majority of innovation in nanotechnology is restricted to spin-off generated in the academic environment where funding agencies set research efforts towards common goods, in medicine, energy and environment. Besides, 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, 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 works.

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 using 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 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[50].

At the origin of nanotoxicity and nanosafety concerns, it was pointed at the well-known fact that cells have problems to deal with *micrometric* insoluble particles. As asbestos fibers, with dimensions greater than 20 micrometers, up to hundreds, induce frustrated phagocytosis[51], 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, Today knowledge indicates that many NPs in their intended uses do not need special care beyond being treated as other chemical substances, even if some particularities may apply. Nanotoxicity is a young field that can be considered about 10 years old. Despite this youth,

[50] Harald F. Krug Angewandte Chimie 2014, 53, 12304-1231

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

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 long, 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 other. Up to now, we have been mainly reproducing, at ease, nanomaterials that are already existing in nature or that somehow they are already produced by man in a more imperfect and unaware manner. Small, of about 20 nm, iron oxide NPs have been found in natural unpolluted soils or inside bacterial magnetosomes, and nanometric TiO_2 has been used by the tone in the cosmetic industry as sun screens and others for decades now. When we get next generations of nanoparticles, additional cares 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 the biodistribution in the case of inflammation might exhacerbate or suppress it and accumulate in organs (if the NPs 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 NPs and other toxins (as LPS, allergens or chemical toxins) or exposure of NPs 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 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. 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 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 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[52]. Therefore, the breathing of solid nanoparticles, especially aggregates made of persistent materials, is highly unadvisable. However, even for poorly soluble particles of relatively low toxicity, animal studies have identified doses that were not associated with observed adverse responses[53]. 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[54] . 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.[55] Note that this is not the case of 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 to interact strongly with cell membranes interfering thus with its normal functioning and inducing cell death. See for example Chitosan functionalization of gold nanoparticles[56]. 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[57]. 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 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

[52] 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.

[53] Warheit, D. B., Webb, T. R., Sayes, C. M., Colvin, V. L., and Reed, K. L., Toxicol. Sci. 91(1), 227-236, 2006

[54] Warheit, D. B., Webb, T. R., Colvin, V. L., Reed, K. L., and Sayes, C. M., Toxicol. Sci. 95(1), 270-280, 2006.

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

[56] 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.

[57] Warheit DB1, Laurence BR, Reed KL, Roach DH, Reynolds GA, Webb TR. Toxicol Sci. 2004 Jan;77(1):117-25

biological machinery being it denaturalized protein aggregates or cell debris. This applies for nanoparticles (3 to 100) nm, a viruses (20-400 nm, a bacteria ≈1000 nm and a eukariota cell ≈10.000 nm. However, when the object is too big (for sizes beyond 10 micrometers)[58], 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 -as asbestos 10 x 500 microns- are especially effective to induce this effect.

In determined conditions, NPs 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 are two simple ways to avoid aggregation, to avoid high concentrations (if there are few NPs, it is difficult that NPs 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 them isolated 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 there is risk of aggregation, no 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) what avoids their further aggregation, what would be always 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 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 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 (specially small NPs). 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. 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 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, 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 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.

[58] Kostas Kostarelos Nature Biotechnology 2008, 26, 774 – 776.

iv.- Toxicity has been observed when the NP act as a reservoir of toxic ions that are delivered during corrosion. A 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, as an example, cadmium, 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 NPs into iron ions has make 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 directly the ionic species or when those 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[59]. 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 associated with antigens or allergens before homogeneous or heterogeneous aggregation. For example, when the car combustion emission microparticles are coated by pollen grains, they become more allergenic. This is one of the reasons why allergies in the urban areas are more intense than outside in the country side. Therefore, the NP surface has to be passivated before 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 complicated that naked nanoparticles meet toxins, antigen 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_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 NPs for catalysis, it is well known that it is unexpected that NPs will act as powerful catalyst unless they are designed to do so. Indeed, normally, nanoparticle surfaces are rapidly passivated with

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

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. This is the case of TiO_2 coated with a thin transparent layer of Al_2O_3 in sun screens. The TiO_2 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_2 induced toxicity. In any case, iron oxide nanoparticles are not photocatalyst, nor considered affective 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. Is the well-known case of gold NPs coated with a cationic detergent like CTAB[60]. This detergent forms a double layer vesicle-like coating on top at 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 as those observed with pure detergent molecules; however, it would happen at lower detergent doses in the case of association to NPs. Therefore it seems not advisable the unintended mix of detergents and NPs. Note that detergents are already poorly biocompatibles, fortunately, normally, highly biodegradables.

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 working 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 then result to be unsafe but they are already developed. Otherwise, we are going to suffer it until it is forbidden once problems have already created, and regarding 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 hazards are found, NPs have to be re-designed so that detrimental specific NP characteristics are decreased while maintaining the desired unique intended parental

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

properties. These strategies should not only focus on reducing NP hazard, but on reducing NP release from matrices or promoting its degradability after release.

To reduce toxicity of the NPs.
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
To minimize release of NPs from their matrices.
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).
To reduce persistence of NPs
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)

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 to be fully evaluated yet, but in principle, protection against chemical substances does work for protection against nanoparticulate matter. When necessary, technological solutions has 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 available technologies, technological improvements in water and air filtering (e.g., foam technology) and treatment (destruction of NPs) may need to be developed.

7.- Conclusion

In their basal article “Developing a framework for responsible innovation”, Owen and colleagues[48] presents four dimensions of responsible innovation, namely; Anticipation, Reflexivity, Inclusion and Responsiveness. The 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. It is said that you should never attribute to malice what can be attributed to stupidity. In an over simplified manner, who contaminates the river or slave the workers, he/she does not do so because enjoys the river death or making others suffer. When the businessman says that he cannot compete without pressing on workers, polluting the environment and cheating on consumers (see recent "clean diesel"[61] case) is due to a lack of intellectual capacities (inability to find a better -smarter- way of solving technological problems have not been found). As Winston Churchill said, "gentlemen, we have run out of money, now we have to think". We cannot afford being stupid, it is too expensive. Responsibility requires complex thinking, wide angle analysis and forecasting consequences. Even more in a world getting more and more sophisticated thanks to technological development. And now that we should start to develop more efficient technologies, and truly understand sustainability, we can witness with dismay that a new illiteracy is rising and signs of post-enlightenment are starting to be visible[62], probably as a consequence of the increased complexity. Early in our anthropocene era, our technology is still primitive. In the future we will design wise technology. Today, our societies may not be ready to develop intelligent technologies. This may be why we pollute so much and waste so much energy. This is why the final aim of this company, of this project, 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 to sustainable progress.

[61] https://en.wikipedia.org/wiki/Volkswagen_emissions_scandal
[62] https://longsworde.wordpress.com/2010/08/22/our-post-enlightenment-era-the-sleep-of-reason-breeds-monsters/