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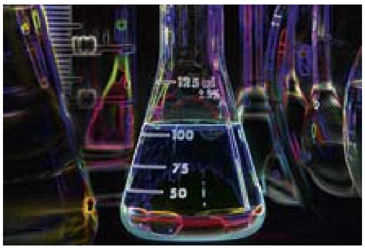
# Chemistry as a tool in nanotoxicology

Safety

*The environmental impact of nanomaterials.*

*This article first gives a rundown of the current state of chemistry and its relation with other sciences. Everything that surrounds us is made up of chemical substances, so chemistry can safely claim to be the science of everyday things. This makes chemistry crucial to human beings, affording many benefits. The image of chemistry, however, is frequently bound up with environmental pollution. This aspect is dealt with in this article, explaining some research projects in which chemistry is trying to mitigate environmental problems. It also looks at the relationship of chemistry with biomedicine and materials science, two multidisciplinary areas that are vital for humankind's well-being. Lastly, a summary is made of some characteristics of the potential environmental and toxicological impact of nanomaterials and the results are given of this research group's project 'Environmental Impact of Nanomaterials'.*



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## Chemistry: some basic concepts

Chemistry is the science that studies the composition, structure, properties and transformations of matter, especially a atomic and molecular level.

The atom and the molecule are chemistry's main subjects. Transformations, or chemical reactions, are the processe whereby one chemical species turns into another.

The atom is the smallest unit of matter that maintains its identity and properties; it cannot be split by chemical processes An atom is made up by a nucleus with a positive charge orbited by electrons, tiny particles with a negative charge.

Chemists' interest in atoms resides in their capacity of bonding to form chemical compounds and matter. In nature there are no isolated atoms (they occur only in extreme conditions or in very controlled laboratory experiments), and they generally combine with each other (an exception to this is a group of chemical elements, the noble gases, which are largely inert).

Chemical substances owe their existence to the fact that they are stable from an energy (thermodynamic) point of view This stability stems from the existence of chemical bonds, which are produced whenever two or more atoms interact by sharing or exchanging electrons. This bonding is basically of three types: ionic, metallic and covalent.

In the ionic bond an atom donates one or more of its electrons to one or more other atoms. The chemical species generated by electron loss or gain, in relation to the electrically neutral atom, is called an ion. If the atom loses electrons it forms a positive ion or cation; if it gains electrons it forms a negative ion or anion. Ionic bonding is formed by the interaction of anions and cations.

Electrons of metallic elements are very loosely united to their nucleus and have a high degree of mobility. Metals are formed by the union of many metal atoms sharing their highly mobile electrons, forming a sort of electronic cloud that is shared by all the metal atoms; this constitutes the metallic bonding. Electrons thus belong to more than one atom and are called delocalised electrons. The electronic mobility is the origin of metals' electrical, magnetic and thermal properties.

The commonest chemical bond is the covalent, which forms when two atoms (identical or distinct) bond by sharing electrons. This union forms the molecule, the basic subject of chemistry. For this reason chemistry has been defined as the molecular science, though this definition in fact falls short of its real range.

The capacity of chemistry to transform matter is based on the chemical reactions suffered by chemical species, fuelled by the creation, breaking and reordering of bonds. This implies movement of electrons within the chemical species.

Everything surrounding us on our planet is made up by chemical species (ionic, metallic or covalent). It can hence safely be claimed that everything is chemistry, and chemistry is the central science.

## Chemistry, the central science

Molecules are useful tools for studying processes and developing theories in other scientific areas, driving the progress of chemistry's sister sciences. On the strength of this characteristic, chemistry has come to be regarded as the central science.

Figure 1 shows chemistry's relation to the other sciences. Toxicology, food science, environmental sciences, materials science, agrarian sciences, medicine, nanotoxicology and physics are only some of the sciences that chemistry constantly interacts with. Note that the arrow bonding chemistry to the rest of the sciences has its source in chemistry. All these sciences tap into concepts and methods of chemistry (based on the use and manipulation of molecules) to study phenomena and/or generate consumer products. The arrow joining physics with chemistry is double headed, reflecting the mutual contribution between both sciences: chemistry contributes molecules for carrying out experiments and verifying theories while physics supplies the conceptual base for chemistry. Finally, chemistry's input to mathematics is modest, with a more important contribution the other way round, maths supplying a theoretical base and numerical methods.

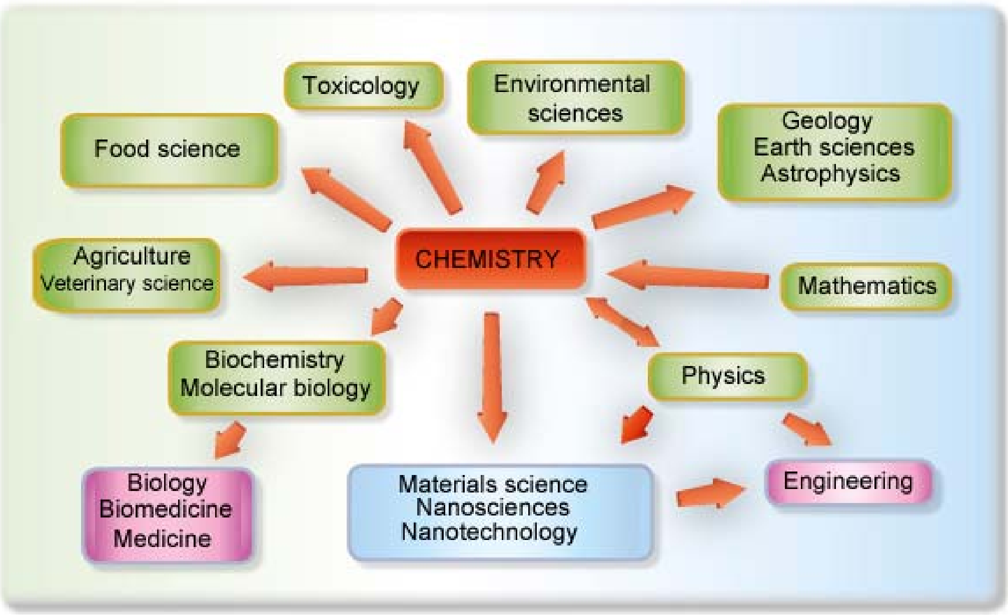


**Figure 1.** Interrelationships of chemistry with other sciences

Whenever two distant areas interact, they have to do so at the lowest common denominator of their respective study areas, which are usually atoms and molecules, and therefore through the domains of chemistry. Chemistry can therefore be considered as the nexus between the two distant scientific areas involved.

Take the example of food. Everything we eat is a mixture of chemical substances (whether natural or artificial); this synergy between chemistry and food science is spawning a brand new scientific discipline, molecular gastronomy. Toxicology, for its part, in its application to environmental sciences, has to analyse the toxic effect of a chemical substance on an organism or ecosystem. To do so it often recurs to an explanation based on the interaction between bio macromolecules and the exogenous substance.

## The science of everyday things

The sheer ubiquity of chemical species, particularly molecules, has led to the commonplace claim that everything is chemistry. This definition is not absolutely correct, however, for certain natural phenomena (especially those bound up with enormous structures like galaxies or with the very high energy levels generated in particle accelerators) stray beyond the realm of chemistry.

For this reason it is perhaps more fitting to define chemistry as the science of everyday things, given that all we human beings interact with thousands of chemical substances every day: the air we breathe, the food or water we eat or drink, the fuel we consume, the clothes we wear, etc.

## Nanomaterials and the environment

Chemistry currently benefits society in all the following ways:

- **Human health.** It provides medicine, diagnosis material and sundry biomaterials.
- **Veterinary science.** Chemical substances help to protect the health of our livestock and pets.
- **Agriculture.** Fertilisers make cropfields more productive. Pesticides, herbicides and other phytosanitary products protect the harvests. Using chemical methods we find out the composition of farmland and optimise the crops to suit the particular characteristics of the land they grow in. Also by chemical methods we analyse the harvested crops to improve their ongoing quality.
- **Food.** The abovementioned improvements to crop- and animal-farming procedures stave off any food production problems. Additives modify and improve food properties.
- **Water purification.** Water impurities are removed by oxidising- and bactericide-agents like ozone or chlorine dioxide. The process is then completed with a series of physical-chemical treatments like flocculation or filtering through membranes, also made from chemicals.
- **Energy.** Our main source of energy is still the burning of fuels deriving from oil and natural gas, which are mixtures of chemical compounds. Combustion is a chemical oxidation reaction. Another source of energy is electrochemical, in which the chemical energy of ions is converted into electricity. This is the working principle of batteries and fuel cells.
- **Everyday materials.** Everything we use on a daily basis is formed by chemical substances.
- **Technological materials.** Chemistry produces molecules with extraordinary electrical, magnetic, optical or mechanical properties, generating materials apt for diverse technological applications such as aeronautics, major engineering works, electronics, computation, etc.
- **Environmental protection.** There is no denying that the overkill use of chemical substances and, above all, excessive energy consumption, impair the environment. This is the toll that has to be paid by a technologically advanced society. Chemical pollution often makes the headlines. Some years ago we were less aware of the environmental hazards of many chemical compounds but these gaps in our knowledge have been progressively filled in by research conducted by chemists.

Many widely used compounds, like fertilisers and drugs, are beneficial in our everyday life, but they should be used in a rational and controlled way; this is not always the case. If the right resources are provided, chemistry can then make a positive contribution to the environment (see later).

## Importance of chemical pollution

One of the biggest problems of contemporary society is pollution and its health and environmental implications. So-called chemical pollution refers to the harmful concentration of chemical substances in a given environment (ranging from an organism up to a complete ecosystem). Given that everything surrounding us is chemical, it comes as no surprise to find that harmful substances are chemical too.

A toxic sludge spill from an aluminium plant in Hungary in October 2010 highlighted the grave damage that can be caused by environmental pollution

Two of the terms used in the above definition call for a closer look: namely «concentration» and «harmful». Concentration is a fundamental concept in chemistry and refers to the amount of a substance present in a given environment (normally expressed in terms of its capacity or volume, which might range from a test tube up to a river, ocean or the whole atmosphere). The social impact of environmental pollution means that news of any significant amount of a chemical substance (drug, consumer product, processing product, etc.) in a given environment makes a big splash in the press. This news is usually couched in terms like «in place x there are y kgs of the substance z». Statements of this kind are meaningless to chemists because the crucial information is missing: in what volume of place x has the substance z been detected, i.e. the concentration.

Secondly, the above description of chemical pollution uses the word «harmful». To ascertain how harmful a chemical substance is, we need information (with hard figures) on its biological activity[1].

One aim of our study is to apply the scientific method to the area of toxicology and chemical environmental sciences, i.e. find out the relation between the structure and toxic effect (a biological activity), in order to come up with an answer to the abovementioned missing information on the «harmful concentration».

Any property of a chemical substance depends on its structure. One aim of this research is to cull dependable information on the toxic effects of chemical substances in relation to the structure. There are three useful consequences of research of this type.

Firstly, if we know the bio-macromolecule structure (enzyme, receptor, nucleic acid) with which a chemical substance interacts, we can then determine its toxic effect at molecular level. Secondly, by building up enough information on the structure and toxicity (Structure-Activity Relationship or SAR studies), we can then forecast the toxicity of a chemical substance before preparing it. Finally, certain toxic substances that are currently in use due to their great utility can be replaced by less toxic alternatives developed on the basis of the accumulated SAR knowledge.

From the environmental point of view a distinction has to be made between two types of chemical pollutants, which not only have different physical properties but also occur in different environments. On one side are the greenhouse gases, CO2 to the fore. This environmental problem is bound up with excessive energy dependence on the combustion of fossil fuels and calls for a global solution.

The other major type of environmental pollutant is made up by consumer and processing substances. This type of pollutant can in turn be broken down into two classes depending on its organic compounds. The high concentration of metals in ecosystems is extremely dangerous (depending on the specific cation involved). Witness the recent sludge spill in Hungary (autumn 2010).

Moreover a very numerous and varied group of chemical pollutants is made up by organic compounds. Worthy of special mention here are chlorinated dibenzodioxins («dioxins»), chlorinated dibenzofurans («furans»), polychlorinated biphenyls (PCBs), polyannulated aromatic hydrocarbons and diverse aromatic and heterocyclic compounds (figure 2). This group of substances are generically called «persistent organic pollutants» (POPs): «persistent» because they persist a long time in the environment or in the organism (they build up in certain organisms, especially in the fatty tissue[2]).

If there is any convincing proof that a chemical compound has a harmful health effect, it should be banned. If the compound involved is technologically useful, a less harmful substitute should be sought. If the compound is generated inadvertently, a method should be sought to avoid its formation or transform it into another less toxic substance. In both possible solutions chemistry plays a key role. Structural studies can draw conclusions on the characteristics responsible for the «good property» of the compound in question and design alternatives with similar benefits but less toxicity. We can safely claim that «the problem of chemical pollution will be solved by chemical methods».

Why has this environmental pollution built up into such a widespread and serious problem? The answer is simple. We have been using a host of chemical substances (conducive to our well-being in some way) without being aware of their side effects («chemistry and chemical substances have these two sides to them»). No information was to hand because there was no regulatory body asking for it and, possibly, human beings were not fully aware of their environmental complications.

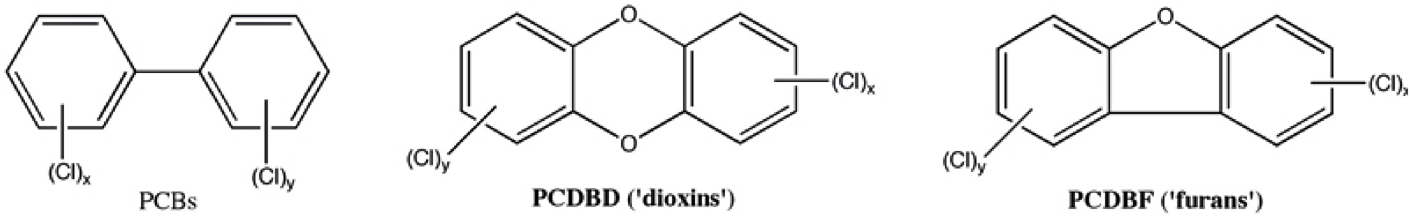

**Figure 2.** Chemical structures of some POPs

## The role of chemistry in environmental protection

Nowadays we are aware of the possible toxicity and environmental impact of chemical substances. Chemists are therefore striving to palliate these effects. In this pursuit the following aspects are now being studied:

- Cuantificación de sustancias químicas en el ambiente.
- Quantification of chemical substances in the environment.
- Determination of the toxicity of chemical compounds and ascertainment of the biological mechanism (in collaboration with biologists).
- Design and synthesis of chemical compounds with a beneficial biological activity (in the right dosage) that might mitigate the effects of other toxic agents.
- Development of more eco-friendly industrial processes (green chemistry and sustainable chemistry).
- Design and implementation of chemical waste-treatment routes.
- Research into physical and chemical-physical processes for selective separation of toxic substances.
- Research into «clean energy» generation processes.

## The chemistry of the future

Science has to tackle the needs posed by society. The challenges to science in the upcoming decades are likely to be in the areas of energy, environment, food, health and technology. This progress also has to be sought with one eye on social fairness, to close the yawning gap between human beings and countries.

All sciences and technologies will need to pull together to meet these needs, with a multidisciplinary approach in which chemistry will continue supplying molecules to prepare materials and will also input methods and concepts to help make sense of the results. Chemistry will therefore continue to be the central science of the twenty first century.

To help tackle these challenges, chemical research is interacting on a multidisciplinary basis with two main areas biomedicine and materials science, which are likely to be the main drivers of scientific advances to benefit society in the future.

## Chemistry and nanotechnology

In the future our instruments will have to be as tiny as possible. One feasible and reachable lower boundary is the size of the molecule, i.e., the scale of tens of angstroms (1 Å = 10-10 m), where assemblies of a small number of molecules will be capable of transforming electrical, luminous or thermal energy into movement (mechanical energy), in so-called molecular machines.

While this objective is being pursued, progress is being made in nanometric scale materials (at the scale of hundreds of nanometres, nm, 1 nm = 10-9 m). This has given rise to a branch of science, nanoscience, and its practical application nanotechnology. The scientific bases are now being laid down (basic research) of this scientific area and the first applications are budding forth. Nowadays there are over 1000 commercial products containing nanoparticles, ranging from suncreams to sporting material. The media has published articles on the spectacular growth of this business area, which is now beginning to be profitable.

## Chemistry, between biomedicine and materials science

Traditionally chemistry has studied analytical aspects (quantification and characterisation of substances), physical aspects (determination of properties) and synthetic aspects (preparation of substances). In recent years chemistry has tended to become much more interdisciplinary in its approach, interacting with other areas, especially biomedicine and materials science.

To contribute towards these emerging areas, chemistry is currently studying more complex aspects such as:

- Reaction mechanisms.
- Intermolecular interactions. n Explanation of natural phenomena at molecular level.
- Reaction mechanisms.
- Intermolecular interactions.
- Explanation of natural phenomena at molecular level
- Design and preparation of materials with given properties.

These new research areas have broadened chemistry's scope, reinforcing its role and status as the central science.

## Chemistry, biomedicine and drugs

For many years now chemistry has been making valuable contributions to biomedicine: drugs, diagnosis material biomaterials (ranging from the «classic» contact lenses to artificial tissue or bones)[3] and compounds for studying biologica processes [4]. These inputs have considerably enhanced our quality of life. Furthermore, the arsenal of available medicaments (prepared by chemists) for treating almost any illness has helped to prolong life expectancy spectacularly[5].

Research into the development and production of drugs (medical chemistry) has progressed notably in recent years, due mainly to two salient factors.

Firstly, awareness is spreading that a rational design beforehand saves time and money afterwards; secondly, knowledge o the action mechanism of active compounds is paramount for understanding a drug's efficacy. These two aspects have beer favoured by the availability of powerful computers and suitable computational methods. Knowledge of these two (wide ranging) aspects of medicinal-chemistry research favours all the following:

- Preparation of compounds with bespoke activity.
- Assessment of pharmacokinetic properties such as absorption and distribution throughout the organism.
- Finding out the drug metabolism and elimination routes.
- Adjustment of the right medicament dose; together with genetic studies (pharmacogenomics) this favours the development of personalised medicine[6].

Although this biomedical research is still a long way from full maturity, undoubted progress has been made in recent year: and is likely to bear fruit in the coming decades.

## Chemistry and toxicology

Although related to medicinal chemistry («the beneficial effect of xenobiotics»), toxicological chemistry («the harmfu effect of xenobiotics») is a more complex research area. The two sometimes even overlap when an excessive drug dosage can turn it toxic.[7]. A drug normally has a single biological target, whereas toxicology is a more complex multidisciplinary science that ranges from molecular to ecological aspects. Chemistry plays a key role in this research area since the prime cause of toxic effects is the structure of the molecules and their interaction.

Research into drug development and production (medicinal chemistry) has developed notably in recent years.

Compared with modern developments of medicinal chemistry, toxicology is still in its infancy and there are several aspect: to bear in mind.

- Firstly, many of the toxicity results (especially in ecosystems) of chemical compounds have been obtained in ar inconsistent manner, and published data often refers to species killed by a given pollutant. Single toxicity scales have been drawn up to palliate this situation (TEF, toxic equivalency factor), but the problem here is that there is very little information to go on and it has been obtained with very diverse experimental procedures. This hinders the drawing o conclusions from any current structural relation study.
- Toxicity results have been published for many compound mixtures (e.g.the Aroclors, which are mixtures o polychlorinated biphenyls, PCBs); the toxicity is taken to be the sum of the individual toxicities without taking intc

account any synergies between them.

- Although it is generally acknowledged that there are pollutants with short-term effects (normally with acute toxicity and long term effects these are not usually brought into relation with chemical aspects. In an earlier study we postulated that the long-term effect of PCBs bears a direct relationship with their dipolar moment and, ipso facto with their interaction capacity with fatty tissue molecules, favouring their retention therein and long term release This aspect has hardly been studied and is worthy of in-depth research. This constitutes one of the objectives of ou research.
- Ignorance of the toxicity mechanism. This is a very complex problem involving many «actors»: different biochemica processes and receptors.
- The metabolism of the toxic agents has hardly been studied. Toxicity is a very complex biochemical process, involving the intervention of diverse cell receptors, (simultaneously or in cascade form), that might unleash varied biochemica responses. It is well known, for example, that there is a close relationship between aromatic hydrocarbon receptor (AhR) and estrogen receptors (ER), two of the main bio-macromolecules responsible for toxic responses[8]. It might also sometimes turn out that the exogenous compound is not actually the cause of the toxic effect but rather undergoes a previous metabolic transformation. Moreover, some of the cell receptors mediating the toxic response might be activated by more than one mechanism. This was recently found to be the case with the aromatic hydrocarbon receptor (AhR), an essential bio-macromolecule in the toxicity processes of aromatic compounds[9].
- Remediation of toxic effects. This is also a very tricky problem. The solution calls for previous knowledge of the abovementioned aspects. Any progress made will be beneficial both to the organism and the ecosystem.

## International initiatives and the role of computational chemistry

Toxicity and its impact on human health and ecosystems is a huge concern of today's society. Growing awareness of this problem has spawned a series of actions at international level, such as the Kyoto Protocol[10], the Stockholm Convention[11 and REACH[12]. Especially important are the latter two, applicable mainly at European level and aiming to eliminate the toxic compounds most harmful to the environment (the persistent organic pollutants, POPs) (the Stockholm Protocol) and analyse the possible toxic effects of chemical substances, adding up to thousands of analyses each year.

All European countries have now ratified the REACH regulation, binding themselves to study all chemical substances produced and marketed in Europe to determine their harmful effects to persons, animals, plants and ecosystems. This regulation also applies to chemical substances already in use. As might well be imagined, implementation and enforcement of the REACH Regulation entails a change of mindset for the chemical industry. There are now thousands of chemica products on the market; obtaining data on all of them therefore entails a huge investment of time and money. In fact, the idea of culling experimental data on all chemical substances currently in use is a pipedream; an alternative to experiments therefore needs to be sought; this is to be found in computational toxicology, to which our group has made outstanding contributions in recent years. This computational research has to be topped up with reliable information on the biologica activity of some compounds, which, once correlated with its structure, allows us to forecast compound toxicity without the need for biological testing.

Chemistry comes into its own here. In the case of the POPs, it can provide solutions for eliminating them; in the case of REACH, we should be able to forecast toxicity by virtual screening, saving much time and money. Reaching this state requires a lot of spadework research.

## Computational methodology in toxicology

Thoroughgoing research into the bioactivity of potentially toxic compounds requires a great deal of physical-chemica information. Although this data may be obtained by experiments, this calls for a great effort in terms of time and money Furthermore, some compounds are not available in pure form (admixture of isomers and congeners is frequent in some environmental pollutants, such as the polyhalogenated compounds). A computational approach is hence an efficient alternative for obtaining this data, also avoiding too much handling of the toxic substances. The tools of computationa chemistry can also help to make sense of results[13].

It is a well known fact that a molecule's form (electronic distribution and geometry) determines its properties. Our research in the toxicology area has shown that numerous data are required for studying and making sense of molecular toxicology including:

- Molecular structure (connectivity of atoms and their geometry).
- Conformational analysis, which provides the energy and geometry of each structure, rounded out by dynamic aspects (mobility among conformers).
- Electrostatic properties, including the molecular electrostatic potential, dipolar moment, quadropole moment, high order electrostatic moments, polarizability (which also impinges on reactivity).
- Reactivity indices, including hardness/softness, polarizability, electrophilicity/nucleophilicity.
- Molecular orbits, especially HOMO and LUMO, which are the reactivity-determining orbits.
- Aromaticity. Many toxic compounds have the property of aromaticity, which has practically never been used for analysing toxicity. Our group has direct experience in this area, having recently established a quantification method[15].
- Modelling of metabolic reactions. Some possible reactions are the generation of radicals, hydroxylation reactions and conjugation with bio-macromolecules. An attempt has been made to model possible metabolic routes of the xenobiotic compounds, which may in turn be more toxic than their precursors.
- Intermolecular interactions. Many of the abovementioned molecular properties are very useful for analysing and predicting non-covalent interactions between organic compounds (homo or hetero-interactions), which can then serve for studying synergistic effects between different pollutants (important in ecotoxicological studies), interaction with certain organs and tissues (e.g. the accumulation in fatty tissue of certain pollutants with long-term harmful effects). Another aspect to study is the possible interactions with the target bio-macromolecule (receptors, enzymes, nucleic acids, etc.).
- Structure-activity relations. Working from the culled data on biological activity and properties, structure-activity relation studies then have to be conducted using various strategies (such as molecular similarity indices or comparison of molecular properties) and methods (statistics, analysis of main components and application of neural nets).
- Docking studies

Where the three-dimensional structure of the target bio-macromolecules is known or can be divined from homology studies computational studies should then be made of their interaction with the xenobiotic compounds, using docking techniques.

## Nanoscience, nanotechnology and the environment

The future well-being of mankind in such areas as consumer goods, electronics, energy and the environment will depend on the availability of suitable materials for carrying out certain functions efficiently. These materials have to meet the requisites of energy efficiency and miniaturisation.

The limit in material design lies at molecular level. Nonetheless, individual manipulation of molecules is still some way off because it involves working at ångström scale. In the meantime we can work with molecule groupings, which generate nanomolecular structures, i.e. about a few hundred nanometres. In recent times nanoscience (the science studying nanomolecular structures) has taken off in a big way, obtaining basic information that is capable of making sense of the particle behaviour of the nanostructures. Applications of this basic research are already on the cards, giving rise to nanotechnology. Among the most frequently studied nanostructures feature the metal nanoparticles, with many applications in areas as diverse as optics[16], catalysis[17], electronic devices[18], chemical detection[19] and biomedicine[20].

Applications of nanoparticles (NPs) can be extended by enhancing their structural and functional versatility. This can be done by modifying the NPs by surface union (through non-covalent interactions) with a variety of organic molecules, generating hybrid organic-inorganic structures studied by our group (see below)[21].

Nonetheless there is little to go on in terms of the potential effect of nanomaterials on living beings and ecosystems. This information needs to be established on a solid scientific basis before it is too late and nanotechnology becomes widely used on an industrial scale, generating chemical substances without due control or proper knowledge (as has been the industrial pattern over the last 200 years, as already pointed out).

The experimental and computational work with these compounds might be complicated (problems of solubility, molecule size, etc.), but progress is now sorely needed in this field[22].

## Summary of the results of the project 'Environmental impact of nanomaterials'

During 2011 we conducted research on the synthesis, characterisation, structure, stability and the biological properties of gold nanoparticles (AuNPs) coated with peptides and derivatives. This is still a work in progress and the results, though promising, are still provisional.

Gold nanoparticles (AuNPs) modified with organic molecules have been widely described in the scientific literature, with some practical applications now bodying forth[23]. There are contradictory results on the toxicity of nanomaterials based on AuNPs[24], so more knowledge is still needed about these compounds. Our findings were that toxicity and the biological activity are highly dependent on the organic part united to the metal core.

AuNPs have been synthesised with different sizes and their application potentialities have been boosted with organic compounds of various structures. The chosen stabilisers for this purpose were peptide-biphenyl hybrids (PBHs), whose structure consists of two peptide chains bonded to the 2 and 2' positions of the biphenyl ring system[25]. This family of compounds has promising features such as dynamic properties in solution, ordered structures in solid phase and biological activity as calpain inhibitors[26].

The structures of some PBHs used are shown in figure 3. The PBH-modified AuNPs were prepared in a phase formed by 2-propanol/water/methanol by reduction of the metal precursor ($HAuCl_4$) with sodium borohydride ($NaBH_4$) as shown in figure 4. The structure of the PBH used in the NP synthesis has been shown to influence the size of the resulting AuNPs and also their stability. An example is shown in figure 5.

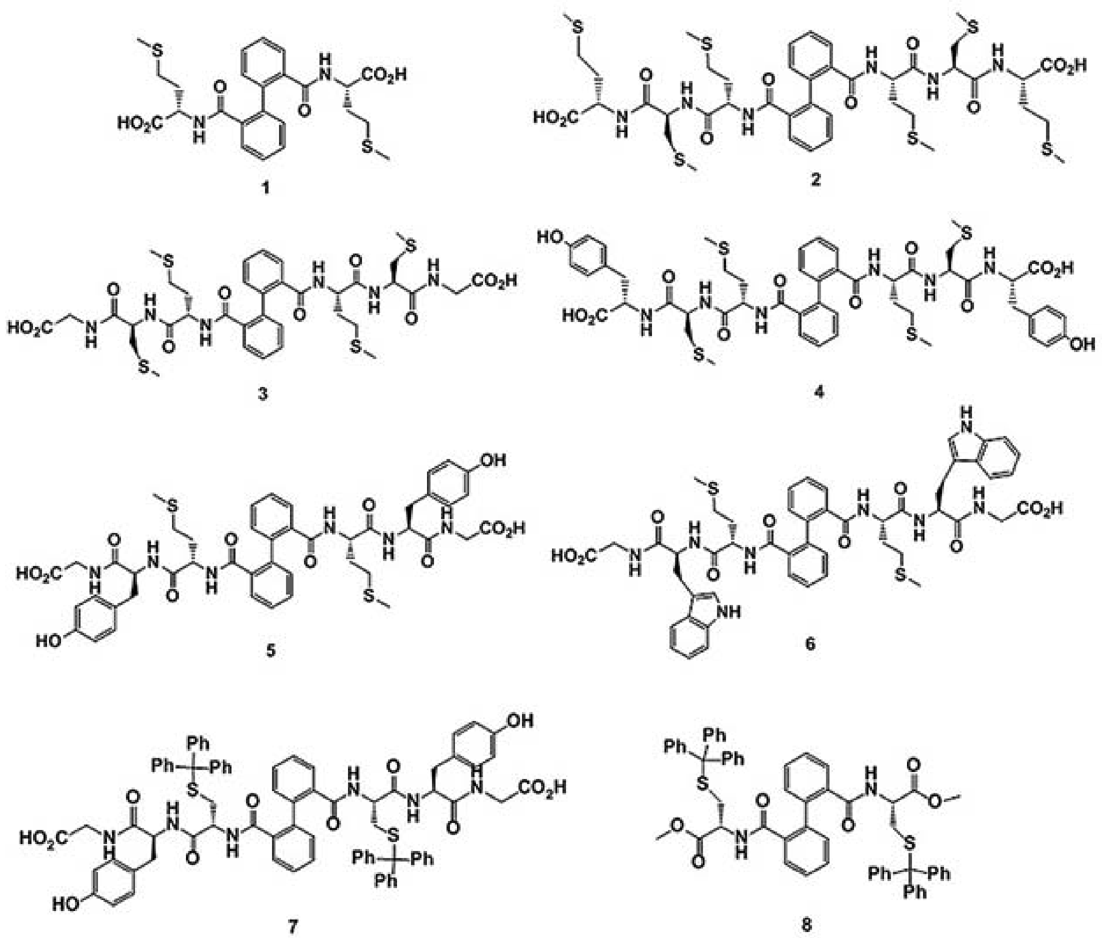

**Figure 3.** Structure of some peptide biphenyl hybrids used to stabilise AuNPs.

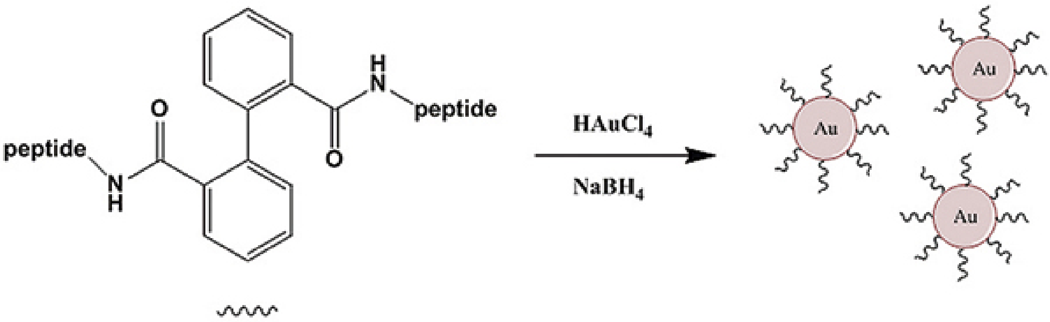


**Figure 4.** AuNP synthesis scheme.

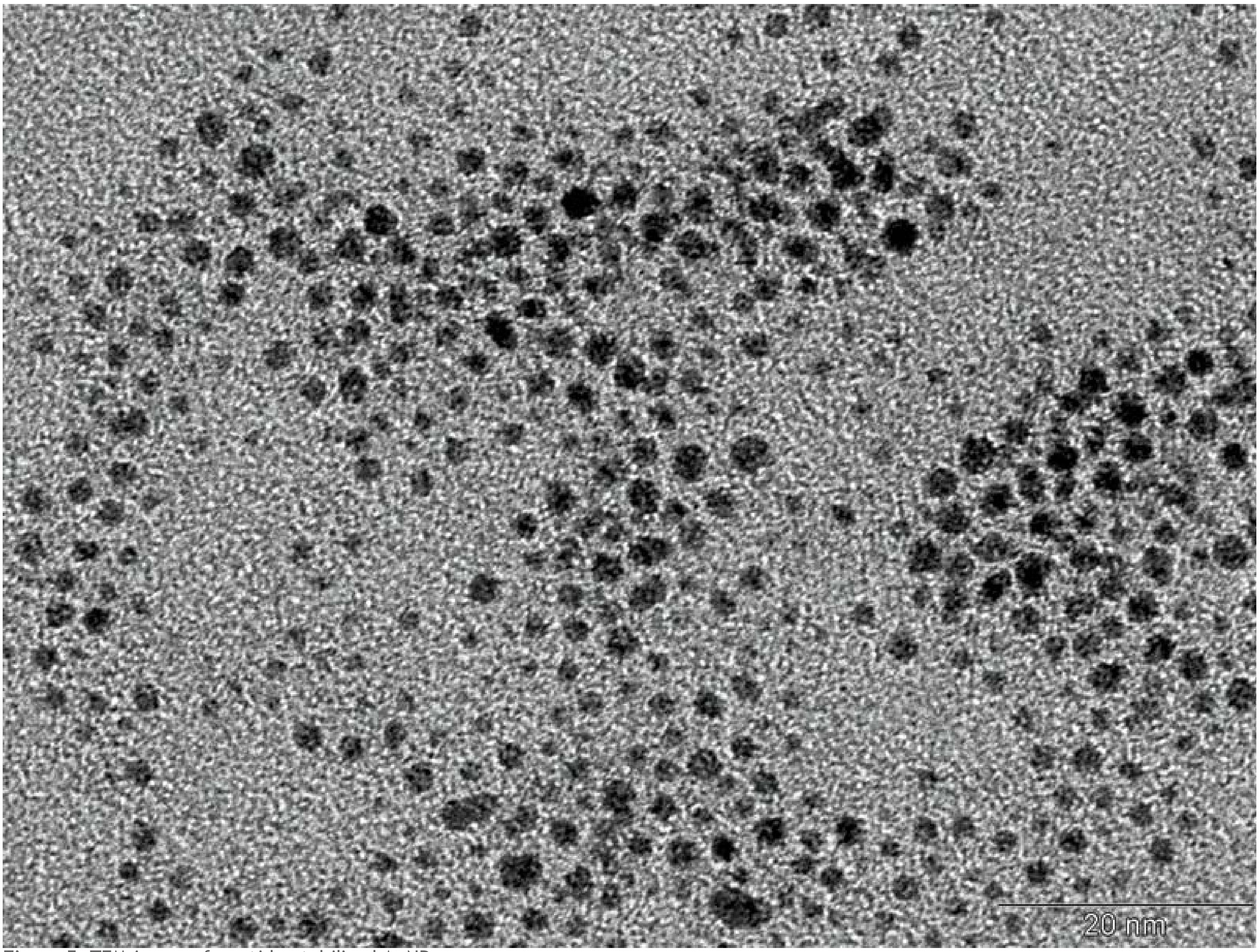


**Figure 5.** TEM image of peptide-stabilised AuNPs.

## AuNP Stability

We found that the organic residues afford the nanoparticle with great stability. The structure of the AuNPs that turned ou to be most stable and also presented the greatest size homogeneity was determined by means of various techniques such a ultraviolet visible spectroscopy (UV-vis), Fourier Transform infrared spectroscopy (FT-IR), transmission electron microscope (TEM-EDX) and elemental analysis. These served to prove their functionality and also afforded information on the functiona groups that might be involved in the PBH bonding with the AuNP.

We also carried out various AuNP stability studies in the culture medium to be used for the cell-level toxicity tests. To do sc we used such techniques as transmission electron microscope (TEM), dynamic light scattering (DLS) and ultraviolet visible spectroscopy (UV-vis).

Thus, by means of the dynamic light scattering (DLS) technique a study was made of the diameter of the AuNPs scattered ir the culture medium. The diameter obtained by this technique is the hydrodynamic diameter; it shows how the NP is spreac through the fluid. We thereby demonstrated that some nanoparticles tend to agglomerate in the culture medium due mainly to the presence of salts. Non-agglomerated AuNPs suspended in solution are preferable in cytotoxicity studies for two reasons: free movement and quicker and easier translocation through the cell membrane[27]. We found non agglomeration tc depend on the structure of the organic compound bound to the nanoparticles, concluding that organic structures afford the AuNPs with greater stability in the culture medium. We have now built up a fair amount of stability data from differen AuNP samples in the culture medium and we intend to carry out anti-malarial activity studies with the most stable of them.

## Biological AuNP studies

Through a collaboration with Professor Bautista (Veterinarian Science School of the UCM), we are testing the anti-malaria activity of the compounds prepared by our group. We supplied some samples of the AuNPs stabilised with peptide-bipheny and found anti-malarial activity. These results are still preliminary and have to be confirmed by the UCM group.

We also conducted some toxicity studies in collaboration with the group of Dr. Navas (INIA). Interesting results are showing up; the biological activity seems to depend on the structure of the organic ligand. We have carried out studies of oxidative stress (in the HEP G2 cell line) and cytotoxicity of the AuNPs in the biological culture medium: EMEM with serum (EMEM/S+ and EMEM without serum (EMEM/S-) (EMEM stands for "Eagle's Minimal Essential Medium", a widely used biological culture medium).

Earlier research had shown that NPs induce oxidative stress as a toxicity mechanism, and this oxidative stress may result in cell death[28]. Cytotoxicity studies were also carried out to determine whether the oxidative stress contributes to cell death in cell lines HEP G2; these studies also showed dependence on exposure time.

In sum, toxicity results showed that this toxicity depends on organic residue of the organic-inorganic hybrid

## Preliminary computational studies of the AuNPs

We conducted some *ab initio* computational studies of gold atom clusters, using HF-DFT and relativistic calculation bases The sheer complexity of the systems makes the calculations long-winded; satisfactory results have not yet beer forthcoming. We are now beginning to use another computational strategy but without results as yet. The important aspec of these structures is the surface, so we are using computational methods that model the surfaces of these materials withou delving into atomic detail. The ADF methodology implemented in Cerius 2 is being used.

## Conclusions

The initial project objectives were limited, due to the short duration thereof. The results described herein show that the approach used is giving satisfactory results. At least from the qualitative point of view, relations between toxicity and AuNF structure can now be determined.

The biological results (both of toxicity and anti-malarial activity) suggest that NPs modified by peptide-biaryl hybrids migh be useful tools in molecular toxicology and in biomedicine.

The medium-long term objectives of this project are to obtain reliable data on the biological and toxicological activity o nanomaterials and the computational modelling thereof. The computational studies will input information on the structure o the materials, enabling relations to be established between this structure and biological activity.

On the basis of these results the toxicity of organic-inorganic hybrid nanoparticles can be determined and analysed tc provide insights in materials science, environmental sciences and chemical toxicity

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## ACKNOWLEDGEMENTS

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