Minutes of the 6th Annual *ecopa* Workshop on Nanotech, Biotech and other new technologies: safety testing and research requirements. Sheraton Airport Hotel, Brussels, December 17-18, 2005.

DAY 1

The welcome lecture: New technology and competitiveness by Mr. Stéphane Hogan,

Mr. Hogan's starting point for discussion was first of all an overview of the EU research policy & the reminder why competitiveness is at the centre of research policy under Framework program 7 and secondly, the alternative methods with regard to the public perception & competitiveness.

The objectives of the EU research policy & competitiveness are to increase competitiveness of EU industries and improve quality of life especially, when dealing with issues such as health, environment and transport. 4, 4% of the EU budget for 2005 was dedicated for research in overall spending, which is quite undersized.
The main motivations for doing research lies in the pooling and leveraging of resources, fostering human capacity and excellence in S&T and a better integration of European R&D.

The main elements and change of EC proposal in FP7 compared to FP6 was the ambitious goal to double the annual budget from €5 billion to €10 billion … Final figures were not known yet, a significant increase of budget would be more likely than a doubling. There was also asked for an increase of the budget for collaborative research for Health from €600m to €1 billion. A continuity in the themes, the types of funding instruments and an ongoing objective to simplify procedures, and have the duration of the programme extended to 7 years instead of 4.
The real novelty lies in the European Research Council with a substantial annual budget of €1.5 billion per year in the proposal for Basic Research and not necessarily Collaborative Research, and the Joint Technology Initiatives in particular for Novelty medicine.
This very ambitious proposal on EU level was explained by the fact that the EU currently invests about 1.9% of GDP in research, which is a comparatively low overall investment figure. The EU is just behind Japan and US and will soon be caught up by China by 2008, in terms of proportional spending.

Mr. Hogan explained that there is a lot of continuity of the current program in FP 7, with regard to the 9 thematic priorities .The only major novelty was "Security and space" that never got much attention until now.

Mr. Hogan highlighted especially 'Health' and 'Environment' in his presentation.
Health, being divided in 3 main areas, with a focus on Biotechnology, generic tools and technologies for human health, were among other things following points are addressed; predicting the suitability, safety and efficacy of therapies incl. alternatives to animal testing (for pharmaceutical uses) and for chemical uses in the Environment programme .

The aim of Innovative Medicines Initiative is to remove major R&D bottlenecks, identified under the leadership of industry (EFPIA), in drug development and acting where research is the key.
The long term objective is to increase competitiveness of European pharmaceutical industry and foster Europe as the most attractive place for pharmaceutical R&D, thereby enhancing access to innovative medicines for patients.

The second part of the lecture was dedicated to alternative methods. The main objectives of the alternative methods are to increase competitiveness by better, quicker and more reliable results, and improve quality of life for humans and animals.
The EU support for Alternative methods is motivated by the competitiveness in Framework programs. The development of novel alternative *in vitro* tests has been a priority in successive EC research programmes since the 80s. There has been an evolution to fewer but larger projects; In FP5 (1999-2002), the EC awarded €65 million to 43 projects. In FP6 (2003-2006) research funding on non-animal testing methods is mostly within Thematic Priority 1 (health).In FP6 €55.5 million awarded to 12 projects in first 3 years and a further ~€20-30 million is foreseen in 4th year.
However, still oversubscribed: i.e. some very good projects will not be funded due to limited funds: alternatives for alternative? Can industry provide some money?

Mr. Hogan gave an overview of the several EU funded projects in FP6 in Food and in Health. He also illustrated how public perception can have a considerable impact on the competitiveness for the worse or the better , e.g. cases such as Bovine growth hormone (BSt), the US import was stopped by the EC, with trade sanctions as a consequence and a privation of the farmers to this technology .
With regard to the alternative methods (pharmaceuticals & chemicals), there is a positive impact by the public perception. A delicate balance needs to be achieved, In respect to the impact on competitiveness for chemicals, there is still a debate going on, and the concern was expressed that due to ”*animal welfare*” lobby, one could be pushing for a (too) fast transition.

Dialogue and communication have to be encouraged in creating a common understanding. Therefore fora for constructive dialogue are needed such as *ecopa* and the initiative of *“Europe goes Alternative”* conference (Brussels, 7th Nov. 2005) was also a positive sign and should be followed up in action.
Mr. Hogan ended with the message to communicate success stories and explain short-term costs v long-term gains.
Finally, contact info on his team was provided and a referral to the website www.cordis.lu/lifescihealth for more info.

SESSION I: New Technologies require different thinking.

New Technologies: Threat or Chance for Alternative Method Development by Dr. Bernward Garthoff.

New technologies or trends often develop without being fully understood or recognized by the scientific, regulatory communities and the public. The same remark goes for the alternative methods scene, e.g -omics was left out in the first ECVAM status report, which created a gap into the discussion area. New technologies might be regarded as a threat, but they also offer opportunities for alternative methods development as well as to improve EU competitiveness. One does not exclude the other.
Dr. Garthoff stated with respect to the domain of the regulatory background that the problem with directives in the regulatory areas is, that they are only reactive.
No alternatives are mentioned in the list of existing Directives regulating pharmaceuticals, Cosmetics, plant protection products, Biotech Products, GMOs.,…For example, with regard to REACH out, testing was required and it was thanks to the *ecopa*'s sign-in action that the level of understanding at the EC changed and led to an intervention at Commission level, resulting in the inclusion of alternative methods in 6th FP.

"New"technologies should /can assist in finding new alternatives: An „old"example is the one of radioimmunoassay saving more lives of animal labs, as the hormone detection was done with mice (in the past).Dr. Garthoff pointed out that OECD should proactively develop new methods and he commented on the OECD quote „*At present, we do not have work underway for test guidelines in these areas. "…" However, the topics of Nanotech and Biotech are very interesting to the OECD and we have work programs in those areas*" that this is too reactively. .It also illustrates the fact that directives are not pushing forward.

Dr. Garthoff explained the difference between hazard and risk assessment, as there is still a lot confusion between these terms. RA is an objective quantification of probabilities and consequences of adverse effects. There is no such thing as a" safe dose" and it is more appropriate to call it an estimation according to a set of rules. Risk perception is the perception of how much risk of what sort is acceptable to the consumer, which is a subjective factor as nor the public neither the media discriminate between the different degrees of hazard and risk.

The EC uses "the precautionary principle" as an easy way of handling with the new methodology. It has the advantage to inhibit new technologies being introduced into EU, also those for alternative method development to some extent .It leaves the risk taking to other parts of the world. It prevents untested novel techs to reach and impact the consumer. There is a maximal risk reduction for the EU public and it may decrease animal testing as certain new technologies do not even reach the EU.
The disadvantage is that it does not need, and use sound science as a base and rather thrives on public fear. No responsibilities are taken nor liabilities accepted of agencies and registration authorities as well and it has to some extent a downturn effect on innovation.

The phenomenon, of the pipeline for innovation and alternatives drying up, is going on for some time and can only be helped by new technologies filling the gap.
It has to be accepted that they need to be further researched and developed, also for the use in alternative methods. New technologies need novel proactive approaches, these might help foster the introduction of new proactively thinking at regulatory agencies. On the question if it is up to the EC and its Framework Programs to take the lead, Dr. Garthoff replied that *ecopa i*s pushing for the uptake in the FP. and cited OECD "*that a coordinated and target aimed cooperation is needed between the four stakeholders government, industry, animal welfare and academia in order to provide the breeding ground for new ideas and approaches in hazard assessment as well as in validation, taking into account both animal and non-animal approaches and integration of both.*"

With regard to biotechnology, he raised the question if new technology helped in testing its own products, in terms of developing alternatives? Unfortunately, there is no proactive approach. Dr. Garthoff illustrated that a lot of EU companies, although situated in the EU have to consider requirements from outside the EU/US, which impact EU and gave an example with regard to Safety assessment of GMO's *in vivo*. For proteins *in vivo,* he mentioned that in India they require a specific Brown Norway rat for animal models to detect potential allergens, and that these global requirements also impact the EU.

Dr. Garthoff also mentioned that the mentality of first thinking of *in vivo* methods should change and stressed that is important to consider the advantages of new technologies instead of focusing on the risk, as the public and the European authority are bound to do.

When new technologies are viewed as a threat, following arguments can be used.
New technologies require more testing of the whole organism *in vivo* .It may take too long to be used for alternatives. There will always be the excuse not to pursue other development. New technologies are not easy to regulate, especially GMO's
However, when regarded as an opportunity; it allows following and pursuing new developments. There is a need for basic research with applications in alternative method development. The impact of the "European**"** competitiveness on others organizations such as ICH, OECD… Awareness is being fostered and 'early on' alternatives are available.

Dr. Garthoff concluded that as the source is drying up, early signals can not risk to be overlooked. Therefore there is still a huge need for initiatives as eSI (the *ecopa* science initiative). Secondly, he considered it detrimental for the EU being competitive, to only develop and simply validate „the same old stuff" and urged to make use of the current existing biotech SME industry know how. Finally, he said that alternative method development has to go hand in hand with the evolution of new technologies, otherwise *in vivo*-testing will remain.

Current testing of nanotechnology products, and requirements for regulatory purposes by Dr.Puolamaa

Mrs. Puolomaa gave a lecture from the point of view of the EU Scientific Committee ENIHR opinion on how to assess potential risks of nanotechnology products.
The Scientific Committee on Emerging and Newly Identified Health Risks consists of a group of 13 European independent experts, selected by the EC and is one of the three independent Scientific Committees providing advice in the fields of consumer safety, public health and the environment, specialised in new technologies, medical devices, physical hazards and methodologies of risk assessment.

The European approach to nanotechnologies is one of a safe, integrated and responsible development of new technologies. The regulatory aspects are still under development. An 'Action Plan for Europe 2005-2009' has been adopted by the E.C. in June 2005, wherein in Chapter 6 about Health and Environment two co issues are addressed, namely the improvement of the knowledge base, which is mainly based on info supplied by Framework Programme 4-7, ETPs & SCENIHR and the development of an inventory of the existing legislation. This is done in order to improve the regulatory basis.

Nanotechnology offers huge opportunities, especially in the health area, however as there are a lot of unpredictable potential risks, SCENIHR was asked how to assess the potential risks of nanotechnology products.

The SCENIHR mandate was to examine the appropriateness of existing methodologies to assess risks associated with nanotechnology, secondly in case of inaptness, how to adapt and/or complete the existing methodologies. And thirdly to define the major knowledge gaps necessary to underpin risk assessment in the areas of concern.
The SCENIHR opinion was that at present there are insufficient data to identify systematic rules for nanotoxicology, as nanoparticles are not behaving as bulk which makes them unpredictable. Small size particles often exert greater toxic effects, as they are more likely to interact with biomolecules such as DNA, RNA or proteins. There is lack of data on biological behavior, and more importantly on human and environmental exposure.
The outcome on the first question was that risk assessment methods require modification. Classical toxicological and ecotoxicological methods may not be sufficient. In addition, modified tests are necessary with specific attention to exposure routes to reflect relevant exposure scenarios. Mass characterization is no longer adequate for dose evaluation and should be completed with surface area and N of particles. Existing methods for environmental exposure assessment are not necessarily appropriate.

With regard to the adaptation, completion, new methodologies for routine and physico-chemical characterization as well as routine measurements are needed. In addition, new methods and tests for hazard and new methodological principles for systemic distribution of nanoparticles will have to be developed, dealing with in *vitro /in vivo.*

Mrs Puoloamma summed up that there are quite comprehensive knowledge gaps such as the mechanisms of nanoparticle toxicity, toxicokinetics (target organ identification and doses for hazard assessment), the possibility of extrapolation, human and environmental

exposure levels, health effects and environmental impacts and fate, distribution, persistence and bioaccumulation.
She concluded that existing risk assessment procedures require modification for nanoparticles. There are no rules for nanotoxicology yet. Therefore, a need exists for case by case risk assessment and remarkable knowledge gaps have to be filled.

Mrs. Puolamaa was quite briefly about regulation, given this is still under development. She mentioned though that there will be materialization, and not only tonnage will be a determinative factor, relating to regulatory legislation like REACH
There is a need for identification of novel physico-chemical properties classification and labeling for human and environmental health.

Dr.G. Nohynek: Nanoparticles in cosmetic preparations. Is there a health risk?

Dr. Nohynek summarized what nanoparticles (NPs) are. He mentioned the different sources (biological, natural and man-made) and properties (huge surface, quantum effects) and stressed that typical toxicological properties of NPs do not exist. It was also said that individual molecules in solution usually are smaller than NPs and that it is difficult to keep NPs airborne because they have the tendency to stick together. NPs have different ways of entering the body by inhalation and by the oral and dermal route.
The major concern of nanotoxicology is inhalation toxicity. Dr. Nohynek explained that when NPs are inhaled, their deposition in the lungs depends on the particle size. Their toxicity depends on the total surface area. Particles smaller than 1 µm are immediately exhaled, as for particles bigger than 1µm, they diffused into the alveolar region and deposit there. The lung has a defense mechanism, macrophages, to eliminate these particles. Macrophages, however, cannot remove nanotubes because they are made from fibers. The toxicity of nanotubes is similar to that of asbestos. When the macrophages ingest them they try to destroy the fiber but as a consequence the macrophages die due to the fibertoxicity (not nanotoxicity), which results into inflammation.

Dr.Nohynek mentioned that there is a theory out that NPs could penetrate via the nose into the brain through the olfactory bulb, but this needs to be better documented.

The second route of entering into the body is by oral exposure. It is not very clear what the target organs are. Dr. Nohynek stressed the fact that all the studies done show possible exposure but not toxicity. There is no evidence yet for adverse effects or storage in the human body.

The third way NPs may enter the body is via dermal exposure, in particular via the use of cosmetics based on NPs. Dr. Nohynek showed *in vivo* and *in vitro* penetration studies and concluded that there is no significant penetration into the deeper layers of the skin but only in the stratum corneum. When small molecules are in solution, they penetrate easily through the skin. But insoluble NPs do not move by diffusion but need a mechanical force to penetrate into the skin.

Dr. Nohynek mentioned there was a request from the SCCP (former SCCNFP) to perform photo- and genotoxicity tests for ZnO and TiO_2 NPs. These tests were

performed and the conclusion was that fine particle TiO_2 was not expected to present a genotoxic or photogenotoxic risk for humans under normal conditions of use. For ZnO the data are less conclusive, but point to no geno- and photo-genotoxicity.

The speaker added that there are a lot of hypes about nanotoxicity, but that the facts have to be taken into account. Many studies suggest nanotoxicity but without a valid confirmation. It was also stated that nanotechnology is of great importance for the pharmaceutical industry as there is a need for drugs with a good bioavailability when inhaled/after inhalation or by dermal exposure.

With respect to alternative methods, Dr. Nohynek stated that hazard studies (*in vivo* and *in vitro*) should be performed only if penetration into the living tissues has been shown. These should include standard substances in order to distinguish substance-related from particle-size related aspects. For inhalation toxicity, no alternatives are available yet. Although some alternatives are available or under study for dermal toxicity, Dr. Nohynek expressed his doubts about their usefulness today.

SESSION II: Biotechnology and Nanotechnology: Is biotechnology and small particle research and testing different?

Prof. B. Wynne: Risk perception and assessment of new technologies and the public understanding of science: can alternatives make a difference?

Prof. Wynne started by explaining the audience that there is a difference between risk perception as seen by scientists and in the eyes of the general public. He explained that it is not easy to know the opinion of the public because questionnaires usually contain closed questions, so participants are forced to answer in a certain way.

According to Prof. Wynne, it is more appropriate to make use of qualitative questions as they allow us to learn more about the public opinion. People will bring in issues that scientists have neglected. Scientists are assuming that the issue is risk whereas the public reactions are based upon risk perception. The public is more concerned about the driving force behind innovation. That is not a risk question but a question about the assumption of upstream processes in R&D.

Prof. Wynne also emphasized the distinction between *production-oriented* and *protection-oriented* science. Risk perception is protection-oriented. The primary reason, however, to invest in framework research programs is global competitiveness, which has nothing to do with risk perception. So the disconnection is quite obvious.

Scientific authorities base their decisions on RA, but they do not really take into account the opinion of the general public. The latter, however, does not understand the scientific background and worries about unpredictive effects of new technologies, including genetically modified organisms (GMO).

A study conducted by Prof. Wynne and his team clearly showed the distressing lack of scientific knowledge among the general public. In an open question asking for the

people's opinion about GM crops, the majority indicate CFCs and phenyl amide as the most worrying features of this new technology. It is clear that the public needs sound scientific information and reassuring facts.

In analogy, the suggestion to declare a compound as being safe when no exposure is expected implies a huge confidence in the scientific estimation of the expected exposure. It is not self-evident that this belief is present in the general public. Therefore, Prof. Wynne advised that scientists would take the public concerns into account while defining the exact questions they want to answer, and, more importantly, for what purpose.

It would be wise to include some societal aspects in the scientific risk perception.

Prof. Hartung: Validated methods and methodology currently validated by ECVAM in these areas.

Prof. Hartung gave an overview of the work of ECVAM on biologicals. He explained that more than 50% of new drugs are biologicals. As the classical safety toxicology fails, a predictive toxicology is highly needed. Then he showed how ECVAM research activities are nowadays structures, and that this fits better into the actual needs.

The European Commission adopted a new Action Plan on the 7th of June 2005: "*Nanosciences and nanotechnologies: an action plan for Europe 2005-2009*". The main goal of this plan is to ensure the safety of nanotechnology. ECVAM is actively involved, since 2003, in *in vitro* nanotoxicology studies on manufactured NPs. Prof. Hartung explained that there are different types of exposure to metallic nanoparticles (NPs): environmental, occupational and biomedical. They are mainly working on metallic NPs, e.g. heavy metals, and apply existing cellular models that are also used for chemicals. Initial interest goes to the toxicokinetics of NPs because it is important to know which particles can be taken up by the cells. Then basic parameters such as cytotoxicity and genotoxicity are addressed. To know whether man-made particles are involved in the same pathologies as particles from the environment is one of the goals.

Prof. Hartung explained further that ECVAM is working on prevalidation (since November 2004) of a study in which the EU, USA and Japan are involved. The study is an *in vitro* Cell Transformation Assay. Particles are radiolabelled and it was observed that they were taken up by fibroblasts more easily than was the case for soluble molecules. It was also seen that the majority of the particles were located in the nucleus which is of importance for geno- and mutatoxicity. It can be concluded that the particles are **actively** taken up by the fibroblasts, but NPs seem to be more toxic than their soluble counterparts although toxicity was not as elevated as the uptake level increased. The fibroblasts used were derived from cell lines of human origin because these are easy to get. But netter options would be using primary human cells or adult stem cells, however, both are difficult to get. In particular, the latter are very expensive and labor-intensive. Prof. Hartung, however, is convinced that they will become cheaper when more frequently used.

In the future, full integration of alternative methods into a R&D European Strategy for Nanotechnology will become a reality. At this moment, however, there is not a single

alternative test that can be proposed to be validated according to the ECVAM strategy. A workshop, however, will be held how to validate genomic tests in the context of NPs.

Prof. Hoet: Nanotech and toxicity studies

Prof. Hoet started with defining "nanotechnology" and in general particles smaller than 100 nm are considered to be nanoparticles (NPs). He explained how there could be a link between exposure to NPs and toxicity, with particular emphasis on inhalation. He posed the question whether nanotech has a specific toxicology problem and explained that both chronic and acute exposure may occur and can have a different outcome. Most data available today are derived from acute exposure. Acute exposure is not easy to diagnose, but chronic exposure is even more difficult to diagnose. It is certainly not easy to link an illness, such as cancer, to NPs. At this moment, it is not yet possible to state there is a specific nano-problem at all. The message is that no universal NP exists and that the various properties of the NPs play a decisive role in the final outcome. The key point is to characterize the various NPs. A good characterization and the use of correct test conditions are key factors.

The second question was about the possibility whether NPs can penetrate into the systemic circulation through the skin. He gave a literature overview and mentioned that it has been shown that NPs can enter the brain via the nose, but the question remains if this is toxicologically relevant. Then he spoke about inhalation: after inhalation, it is clear that particles from all sizes can induce an inflammation in the lungs and the mediators can enter into the systemic circulation. Ultrafine particles (Ø <0,1 µm), when inhaled, pass into the circulation and can exert direct effects on cardiovascular endpoints. This is seen also in humans by inhalation of radiolabelled Tc-carbon particles. Only long-term studies will be able to provide sufficient information.

After oral uptake, it is clear that systemic exposure happens but the target organs are not yet known.

At this moment, not enough data and long term studies are available to make a good risk characterization. There is definite need for a strategy in order to obtain more data.
Two options are possible. One possibility is more focused on fundamental – mechanistic research where we have to link the toxicokinetics to the physico-chemical properties of the material. The second possibility is to do preliminary testing, with high throughput, during the development of new materials like *ex vivo* and *in vitro* testing. The problem is that for these tests rats are mainly used, but the physiology of the lungs of rats is not similar to that of humans. Better would be to make a balance between the use of human and animal cells.

SCIENCE FAIR: Basic research on novel alternative approaches.

A young researcher's forum was, as established during the previous Annual *ecopa* Workshops, also present. The different aspects of the NP's research was highlighted by Prof. Tamara Vanhaecke, who introduced the three young doctoral researchers involved in this session, namely An Ranquin (VUB, Brussel, Belgium), Rodger Duffin (Edinburgh University, Scotland) and Jorina Geys (KUL, Leuven, Belgium).

Drs. An. Ranquin: Self organizing nanostructures: an alternative strategy for directed enzyme-prodrug therapies

Drs. Ranquin stressed the fact that at this moment there are different problems encountered in chemotherapy. The drug concentration in the tumor is insufficient; systemic toxicity of drugs occurs, there is a lack of selectivity of the drug tumor cells and drug resistant tumor cells occur. There are different ways to overcome these problems. On one of them, is their new strategy.

Namely, they try a new strategy to specifically deliver prodrug activating enzymes to tumor tissues using a nanosome. This is composed of a synthetic triblock copolymer. It is possible to introduce different functional head groups to copolymers allowing different coupling chemistries. These copolymers form, in aqueous solution, spontaneously nanosomes. The prodrug activating enzyme nucleoside hydrolase is encapsulated in these nanosomes. The nanosomes are permeabelised by incorporation of the porine OmpF to allow diffusion of prodrugs to the interior of the nanosome, hereby creating a nanoreactor. To specifically target the nanoreactors to tumor tissue, they are covalently linked to camel antibodies (nanobodies) directed against tumor associated antigens. Compared with the classical antibody-directed enzyme-prodrug therapy this new system has some advantages: the non-human prodrug activating enzyme is shielded from the environment to prevent immunogenicity and a large number of enzymes molecules are delivered per bound antibody instead of just one.

Drs. Ranquin explained that at this moment these nanoreactors are optimized and characterized. The fact that different functional headgroups can be introduced to the copolymers opens up a wide range of applications including drug carrier function, inductor of apoptosis, a diagnostic tool, delivery vectors for genes and RNAi, imaging tool and a role in immunotherapy.

Dr. Duffin: The toxicology of nanoparticles.

Dr. Duffin showed the knowledge gap between *'accidental'* versus *'engineered'* nanoparticles (NPs) and the effects of NPs when they are inhaled. One of the target organs is definitely the lungs. NPs can lead to inflammation and affect also the cardiovascular system by penetrating through the alveolar space into the blood vessels. Of course the macrophages do their work but at a certain time they will get saturated and no longer function well. Another target organ is the brain. The nose-inhaled NPs penetrate into the brain passing by the olfactory bulb. There also it can have a systemic effect because it can be transported into the blood vessels.

Dr. Duffin showed a case study where the relationship between the mass of NPs and inflammation had been examined. The study led to the conclusion that there is no relationship between the mass and the inflammation but that there is a relationship between surface area and inflammation. When looking to the hypothetical mechanism of inflammation, it can be derived that different NPs cause oxidative stress because of different properties.

Dr. Duffin also drew attention to the fact that there may be a new kind of asbestos: the nanotubes. There are 'single wall' and 'multi wall' nanotubes. They have the same characteristics: thin (between 5 and 200 nm), long (up to 1 mm) and insoluble in the lungs. These are exactly properties that make asbestos pathogenic.

There is no general rule to let us think that learning about one type of NPs tells us about another type of NPs. The audience wanted to know whether there already exist alternative methods for studying the toxicology of NPs, but Dr. Duffin replied that there are not yet *in vitro* alternatives. The Regulatory Authorities want to see one *in vitro* test for all types of NPs, but that seems impossible viewing the fact that all NPs are different.
There has to be a good characterization before a good *in vitro* test can be performed. Dr. Duffin and his team are first doing a lot of preliminary tests and then performing an animal experiment, as it is important to have a well-planned animal experiment in order to obtain plenty of information that can be used to develop a suitable *in vitro* test.

Drs. J. Geys: *In vitro* study of the pulmonary translocation of nanoparticles. A preliminary study.

Drs. Geys explained the mechanism of translocation of particles from the lungs into the systemic circulation *in vitro*. To perform the *in vitro* study, 3 types of epithelial cells were examined: A549 human alveolar epithelial cells (cell line), Calu-3 human bronchial epithelial cells (cell line) and primary rat type II pneumocytes. She showed the cell culture model that was used for the *in vitro* study. The cells were seeded on permeable membranes of different pore size. The integrity of the cell monolayer was verified by measuring the transepithelial electrical resistance (TEER) and passage of sodium fluorescein. The cells which gave the tightest monolayer on both pore sizes were the Calu-3 cells.

Drs. Geys performed translocation studies with amine – or carboxyl-modified, fluorescent polystyrene particles. When these particles were incubated on membranes

without a cellular monolayer, significant translocation was observed on 3 µm pore size. After incubation of 25µg/ml particles for 14-16h on a Calu-3 monolayer there was no significant translocation observed for both carboxyl and amine-modified particles.

She concluded that there is passage of particles through a "tight" cell layer but that the translocation model needs to be refined.

Round table: Hazard and risk assessment: how much is needed for new technologies? And how much of animal testing seems necessary?

During the following podium discussion, moderated by Dr. O.de Silva, the discussants R. Kolar, A. Van Iersel, W. Pfaller, P. Van Parys, G. Klotz and B. Wynne addressed the main question which parties should collaborate , since with new technologies there is more to it than pure science.
With regard to new technologies, such as bio- or nanotechnology, it was said that not only scientists but also regulatory authorities should be more proactively involved in the discussion about, and "the making of" new technologies.
From Animal Welfare's point of view, the society as a whole has to decide whether or not animal testing is necessary and it should not be restricted to a decision of just the stakeholders.
At this moment, there are too few studies to make a meaningful decision on how much risk assessment and safety assessment is needed. The knowledge basis on nanotechnology has to be built up as there are too many aspects of nanoparticles(NPs) yet unknown. Therefore it is too early in the development of nanotechnology to make a conclusion how much animal testing is necessary. Scientists need to use an integrated approach and go back to basic research.
However, the quality of the basic scientific knowledge should be questioned before initiating the development of an adequate risk assessment.
From the industrial point of view though, some *in vitro* models are available to test some aspects of NPs.
A fundamental question that needs to be addressed is the one of risk management. Indeed, although difficult to measure, it is important to quantify nano exposure, as it can be questioned whether we do need animal testing when there is actually no exposure. In addition," accidental" exposure has to be taken into account as well.
Recognizing risk assessment as a part of the process is a great step forward in realizing the situation. Risk assessment and risk perception are important on the one hand, on the other hand there is the aspect of risk communication. Risk communication needs to be adapted and refined with regard to new technologies as the general public has problems with risk communication, in general. The perception of the public is different, as in reality it is very much driven by industry and global markets, and there is a lack of knowledge and appreciation in that respect.
As a conclusion, the need for education was stressed. Indeed, expert judgements are based on current available knowledge. An open dialogue must be encouraged regarding the current knowledge and new developments between all partners involved, namely regulators, politicians and scientists.

***ecopa* linked EU Integrated Project**

ReProTect, presented by Prof. Horst Spielmann.

The publishable executive summary of ReProTect can be found on the *ecopa* website.
Through the combination and application of *in vitro*, tissue and sensor technologies, the ReProTect project aims to develop a novel approach in hazard and risk assessment of reproductive toxicity.
In the framework of REACH, reproductive and developmental toxicity testing is estimated to cost between 700 and 1100 million euro. In addition, reproductive toxicity testing consumes the largest amount in experimental animals when compound to other toxicity endpoints.
So far, an agreement has been obtained on the list of chemicals to be tested and a list of substances for initiating the studies is made available. Effort has been put into rodent metabolic activation systems and a metabolic system for robust CALUX® cell lines has been initiated.
One of the partners (15) organized a workshop on Metabolic Activation in November 2004. Array technologies revealed a list of oestrogen receptor-alpha-, and androgen – receptor-related genes, allowed to select a number of genes associated with endocrine related pathways.
The future perspectives are the validation work on toxicological signatures, which is planned to start in 2006.

PREDICTOMICS, presented by Prof. Jose Castell

http://www.predictomics.com/

The publishable executive summary can be found on the ecopa website.
The major objectives of PREDICTOMICS include (i) the development of advanced cell culture systems which accurately represent the human liver and kidney in vivo, (ii) the identification of early mechanistic markers of toxin-induced cell alterations and (iii) the establishment and pre-validation of a screening platform.
The liver group will focus on the improvement of the phenotypic stability in organotypic cultures. In addition, besides exploring the possibility of generating stable highly differentiated human hepatocellular cell lines, the potential of creating functional human hepatocytes from adult stem cells will also be challenged.
In the renal group, efforts have been put in the development of (co-)culture systems for renal cells. In a next phase, the focus will be on the optimization of tools and assays (e.g. development of cytomics assays). Finally, mechanisms of nephrotoxicity will be investigated and toxicity markers identified. The renal group also announced the expansion to gene profiling. This basically means that all the gene profiles identified in the advanced cell culture models used in PREDICTOMICS will be compared to gene profiles that have been established earlier for renal tissue from live graft donors and renal biopsies. This information will allow the establishment a “gold standard” for human renal proximal tubular gene profiles and will allow the identification of genes relevant for risk assessment in nephrotoxicity.

ACuteTox, presented by Prof. Michael Ryan
http://www.acutetox.org/

ACuteTox' major objective is to develop and pre-validate a simple and robust *in vitro* testing strategy for predicting human acute toxicity in order to replace animal experiments for regulatory testing.
Although acute toxicity can be relatively well assessed in the current available *in vitro* methods (prediction +/-70%), some compounds are misclassified.
ACuteTox will deal with these specific outliers and will try to identify additional parameters in order to improve the *in vitro, in vivo* correlations.
Chemicals and solvents are selected, standard operating on procedure are defined within each package systems.

CONAM, presented by Prof. Vera Rogiers
http://ecopa.vub.ac.be/

CONAM is a SSA, and is about consensus networking on 3R alternative methods within Europe. The main objectives are building a solid network and exchanging scientific, societal, technological information on development and validation of 3R alternatives.
This is basically translated in the emphasis on to formation of new NCPs, the creation of a newsletter on 3R alternatives, the website expansion, and the organization of workshops, meetings and the stimulation of the international cooperation.
The work has been split up in four working groups, translated to the work packages. Prof. Rogiers gave an overview of the tasks of the four work packages, which were all on scheme. WP1 overachieved with the newsletter; there was an active participation in FP6 projects on 3R. New calls, Carcinogenomics and Lintop were entered. New and future NCP's were induced such as Ireland, and Denmark and Hungary are ready to join as full members. WP2 performed their task with the info collection on chemicals and the organization of the chemical workshop on 01.02.06.WP3 created their info pages "education", "legislation" and "database" on the website and held lectures in different EU countries. WP4 was formed and an ethics workshop was organised in Ljubljana on 10-12/6/2005 with the theme "assessing pain & suffering against research aims" and "assessing relevance of species difference".
CONAM is a part of ecopa and the new topics will be discussed on the Sunday morning session.

Introduction of the new EU projects, wherein *ecopa* is involved. .
The abstract is to be found at the ecopa website.

Sens-it-iv, presented by Dr. Erwin Roggen
http://www.sens-it-iv.com

Sens-It-iv will deal with the development of novel testing strategies for the *in vitro* assessment of allergens.
Ultimately, these *in vitro* tests and testing strategies will allow testing the sensitizing potency of both new and existing chemicals produced by European industries.
In addition, they will find their application in the classification and labeling as imposed by the new EU legislation, and in risk assessment as required by the 7th Amendment to the Cosmetic Directive.

LINTOP (Liver, INTestine OPtimisation), presented by Dr. Flavia Zucco

Lintop has as major goal the optimisation and provision of established protocols & experimental *in vitro* models for testing intestinal & liver absorption, metabolism and toxicity of pharmaceutical compounds.
In order to achieve this purpose a combination of genomic /proteomic methods, biotransformation & metabolic assays and analytical techniques will be employed.

CARCINOGENOMICS, presented by Prof. Joost Van Delft.

CARCINOGENOMICS project aimed at developing a high throughput genomics-based test for assessing genotoxic and carcinogenic properties of chemical compounds *in vitro*, as an alternative to current rodent bioassays.
The reason behind this project is that there is a lack in accurate *in vitro* or short-term *in vivo* testing systems for genotoxic carcinogens.In addition, many rodent non -genotoxic carcinogens are likely not of any relevance for humans.
The project is divided into 12 work packages. A relevant selection of chemicals will be studied in liver, kidney and lung models.
The ultimate goal of the CARCINOGENOMICS project is to establish an optimised battery of organ-specific, genomics-based *in vitro* assays, and to submit this battery to ECVAM for further formal validation.

General Annual Assembly:
Session I A: *ecopa* Management Board

This was followed by the General Annual Assembly wherein the elections for the *ecopa* board took place .The following new members were elected, Roman Kolar (Akademie für Tierschutz, DE), Jan van der Valk (NCA, NL) and Odile de Silva (L'Oréal, F).

Asked by Prof. Maier all 11 ecopa members unanimously confirmed the board with the representatives of the National Consensus Platforms being: Prof. Walter Pfaller, (Austria), Dr. JP Beaufays (Belgium), Dr. Miroslaw Cervinka (Czech Republic), Mrs. Riitta Salmi (Finland), Dr. Bernward Garthoff (Germany), Dr. Annalaura Stammati (Italy), Dr. Janne Kuil (The Netherlands), Prof. José Castell (Spain), Mrs. Karin Gabrielson (Sweden), Prof. Peter Maier (Switzerland) and Prof. Duncan Banks (United Kingdom).

The newly elected and confirmed ecopa board thus comprised the following persons:
- Prof. Vera Rogiers (President)
- Prof. José Castell (Vice-President)
- Dr. Bernward Garthoff (Treasurer)

- Prof. Peter Maier (Delegate)
- Mr. Roman Kolar (Delegate)
- Prof. Walter Pfaller (Delegate)
- Dr. Jan Van Der Valk (Delegate)

- Dr. Flavia Zucco (3-R-Expert)
- Dr. Odile De Silva (3-R-Expert)
- Prof. Horst Spielmann (3-R-Expert)

A discussion on the internal regulation about publication rules took place.
The text was adapted and voted upon and accepted on Sunday morning.

1. For statements from Working Groups, statements from *ecopa* and similar documents deemed to be official *ecopa* papers in particular with the *ecopa* logo, the following procedure is mandatory:

- the draft must be circulated by email to all NCP representatives in the *ecopa* board at least 7 working days before the publication date of the document,
- the NCP representatives must maintain active email addresses and communicate these addresses to *ecopa*;
- NCP representatives in the *ecopa* board disagreeing with the content of such a document must issue an immediate “veto” message to the board members and the Secretary General, preferably with a suggestion for acceptable text.
- Documents are to be declared acceptable by clear email messages, by all NCP representatives in the board, to all board members and the Secretary General within the 7 working days.
- This procedure does not apply for statements and documents which are clearly signed as personal contribution.

2. For publications, presentations (including poster presentations), round-table contributions… forum comments and similar documents, the proposed document/speech/slide presentation or comment must be sent to the three members of the Management Board (Chairperson, Deputy Chairperson and Treasurer) 7 working days before the event, with a request for the document to be checked. The check must be performed quickly and consent not withheld unreasonably.

3. Working group representatives must, in agreement with the group they represent, provide a name with the email address and telephone/telefax number of another member of the group they represent. This person will be contacted should the official representative be unavailable. *ecopa* cannot be held responsible if adequate contact details are not provided.

If the official *ecopa* logo is used, it has to be written in small letters and italics.

DAY 2
SESSION I B: *ecopa* Management Board Presentations:

Activities report by Prof. Vera Rogiers

Prof. Rogiers gave an overview of the activities of the four Work Packages:
*WP1: The website has been considerably expanded. Prof. Rogiers mentioned the involvement of *ecopa* in EU projects within FP6, by taking care of the dissemination of info through the website and newsletter. With regard to the newsletter, Prof. Rogiers asked the attendants for input, as it is a good tool to spread information and to access a large network.
Contact building new platforms and relevant bodies; Ireland was a candidate for an associate membership. Further contact building was needed with respect to France, Luxembourg, Portugal, and Greece. Denmark and Hungary wanted to be admitted as a full member. Prof.Rogiers said that help could be offered with an eventual start up process of a platform, e.g. with the preparation of the statutes but stressed at the same time that *ecopa* never would interfere with the country in question.
Reviews, board and workgroup meetings were going on at a regular basis. Prof. Rogiers pleaded to have more board members involved in EU projects and have them attend meetings of general interest for *ecopa.* The produced reports are published in the newsletter and website and accordingly archived. Finally, the date of 25-26/11/2007 for the next 7th Annual Meeting was communicated.
*WP2: The REACH workshop is planned for 01.02.06 and will accordingly result in a consensus paper.
*WP3: The set up of an educational program has been done; Spain took up a course and wants to transfer it to other countries. Italy was interested to collaborate with Spain on this matter.
* WP4: A draft of the consensus paper has been made, an extra meeting will be organized by the WP leader in order to get the discussion finalized. The ethics database on legislation, experimental animals in EU member state is established and needs an update every 2 years.

Prof. Rogiers welcomed everyone to brainstorm for additional topics.

Further activities of *ecopa* in 2006; next to the CONAM obligations, Prof Rogiers mentioned that the eSI Workshop will be repeated in September 2006, bringing young scientists in contact with more senior researchers. The involvement in SusChem (Sustainable Chemistry Platform) and FP7 EU research projects was brought up and she invited everyone to provide input regarding the EU projects. The difference between the advisory role of an *ecopa* board member and the individual contribution as a scientific partner in an EU Project was thoroughly explained, when some attendants questioned the role of the *ecopa* board in EU projects.
Another idea was to prepare similar *ecopa* brochures, in order to distribute news on EU projects on alternatives.

Scientific projects by Prof. Jose Castell

Prof Castell gave an overview of the tentative roadmap of the EU 7th FP- specific program 2007-2013.
He estimated that the first contracts will be signed by mid 2007 and that practical research will start by the end of 2007, beginning 2008.
After having searched the nine different themes on the specific *"in vitro" or" alternative"* wording, it was basically only in the health activity area where these wording could be retrieved. *"Predicting suitability, safety and efficacy of therapies to develop and validate biological markers, in vivo and in vitro methods including simulation, pharmaceutical…. to animal testing.*" The precise priorities need to be clarified. Also in "climate change, pollution and risk", alternatives are briefly mentioned.
Prof. Castell suggested the follow up by the board on the implementation, the description of priorities and to make sure alternatives are taken up in FP7.
On the question if the text was also screened for "animal use", without mentioning alternatives, Prof Castell answered that this was a second level of analysis that had to be done.

Financials by Dr. Bernward Garthoff.

Budget Overview (Status Nov.2005)

EU Projects :	
Predictomics	6.248€
CONAM	49.736€
General	
Miscellaneous expenses	31.867€
Sponsoring eSI	24.709€
Sponsoring Reach Workshop, 6th Annual Workshop, a.o. activities	45.896€
Total	**102.472€**

Estimated expenses in 2005-2006

6th Annual Workshop	27.544€
Reach Workshop	25.000€
eSI workshop	40.000€
Total Expenses	**92.544€**

Dr. Garthoff gave the budget overview of the EU projects, where *ecopa* has predominantly has the role of disseminating the news. .He precised that the sum mentioned next to CONAM, could be misinterpreted as this also includes the wages of the general secretary. This amount still needed to be deducted. With regards to Predictomics, there was no salary involved for *ecopa*. He explained the different accounts related to CONAM vs *ecopa* and explained which account is being used for reimbursing travel expenses of CONAM board members and WP leaders…Dr. Garthoff also precised, referring to previous discussions, that the abstracts quoted in the newsletter are the initial abstracts submitted to the EU. In the final contract, there may be changes compared to the initial abstract. After the contract has changed, the Gantt Chart is adopted to the new version.

Dr. Garthoff explained the auditor Mrs. Stammati that the huge amount of the remainder was due to the fact that the sponsorship funds in most cases are transferred to the next year. He also assured that there was no extra surcharge related to the different VUB accounts.

The budget was approved by unanimous voting.
Mr.Sten Velshow (Denmark) and Mrs. Annalaura Stammati (Italy) were appointed as financial auditors for next year.

***ecopa* business Matters; National Consensus Platform.**

DACOPA-Danish Consensus Platform for alternatives by Prof. Ove Svendsen.

Prof. Svendsen gave an update on the status of the Danish platform. Contrary to other platforms, the Danish platform is a governmental platform, which might explain why it took nearly six years before being fully established. He mentioned that *dacopa* participated in every annual meeting from of ecopa 2000 including 2005. Numerous initiatives were taken from 1999 on, which resulted in a Parliamentary Hearing of 4 June 2003 on alternatives to animal experimentation.
Following, he explained that the organization of the licensing authority, that is licensing animal experimentation, is a bit similar to the concept of *ecopa*, dealing with four stakeholders. This simplified the mutual understanding at the moment of negotiating.
He also added that a new law about cloning of animals was decided by the Parliament October 2005 and that the regulatory authority for cloning of animals is identical with the licensing authority.
After the parliamentary hearing, initiatives were taken by the Ministry. A thorough investigation took place and meetings/hearings were organized with the different stakeholders, academia, industry, animal welfare organizations, other EU member states

with established platforms, *ecopa* regarding legal matters, etc…Those hearings were finalized in 2004, but as the government had quite a lot of different priorities, (note: each year 300 million of animals are killed, of which 350.000 in animal experimentation.) the official founding could not be finalized in 2004.
Bylaws and statutes are finalized by now .The organization of *dacopa* as established in the statutes consists of 9 members. Eight ordinary members representing the four stakeholders, who are identified and suggested by the organization they represent and one chairman, who is appointed by the ministry. The Danish ministry confirms the acceptance of members and a rejection of an ordinary member is only possible in case of legal obstacles linked to this nomination. The chairman and the ordinary members are elected for a 4 year period. To make it complete, the Danish ESAC member for ECVAM will participate in the meetings and activities.
The current timeline is that *dacopa* is to be established in January 2006. The only thing that is missing, are the names of the nominations of the members by the ministries and organizations.
With regard to the funding, he said that the ministry will provide a secretariat and reimburse relevant traveling and accommodation costs for members of the platform, such as participation in the *ecopa* annual meeting. Sponsors have been identified within the four legs of the NCP.
Prof. Svendsen was happy to conclude that 6 years of intensive work finally resulted in the establishment of a Danish Consensus Platform for Alternatives: *dacopa*.

HUCOPA by Dr. Lajos Balogh

Dr. Balogh admitted that Hungary started quite late, namely in 2004. After attending the *ecopa*/ECVAM JRC Enlargement Action IHCP W3 – Stakeholder Workshop, 4-6 June 2004 in Prague, it was decided to start up a Hungarian platform.
The ECVAM / *ecopa* Stakeholder workshop"On the formation of National Consensus Platform for Alternatives to Animal Testing in the new EU Member States and Candidate Countries" had as a result *Hucopa* first founding conference on the 4th of December, 2OO4, NRIRR, Budapest.
Hucopa counts 44 participants, of which 36 are founding members. The president is Prof Peter Rudas(*note : whose activities are now momentary being handled by Mrs. Nagy, due to the decease of Prof. Rudas in february 2006*). The Board consists of the 4 stakeholders, with (1)Dr Zsuzsa Somfai representing industry, with12 founding members, (2)Mariann Molnar, stands for animal welfare, 4 founding members, (3)Dr Laszlo Pallos, represents the government with 3 founding members,(4) and Dr Lajos Balogh, acting for the academia, with17 founding members.
As for now, no more meetings were organized as *Hucopa* had trouble finding long term supporters and consequently no sponsors have been involved. There are still 36 members of the platform since no additional members joined *Hucopa* since the founding.
The activities take place on a rather smaller scale such as the work in the laboratories.
Dr.Balogh gave information on the experimental use in his laboratory. From 2002 on, there is significant decrease in animal numbers. Much less kinetic examinations are performed with normal animals. They started with pathological models and to involve veterinary patients.

The basics of the decrease in animal numbers used lies in the following:
Instead of using manual immuno suppression in mice, inherited athymic mice are used.
The *in vivo* tumor passage has been stopped. *In vitro* tumor cell-radiopharmaceutical binding assay, before *in vivo* biodistribution studies. They make use of the well developed imaging modalities instead of large scale standard bio assays. They included veterinary patients into efficacy studies, instead of inducing pathological models.

Prof. Rogiers offered assistance to help *Hucopa* by addressing companies who have subsidiaries in Hungary for sponsoring the following meetings which are planned end of May and in October. Hungary could apply for help, when needed.

POLCOPA by Dr. Maciej Stepnik

Dr. Stepnik gave an overview at the 6th Annual Meeting of the activities of *Polcopa*. The *Polcopa* Founding Committee has been established after some time. There is a good equilibrium between the four stakeholders.
A draft of the *Polcopa Statute* has been sent out to the Founding Committee members for consultation. The draft is prepared according to the Polish Act on Associations (1989), which assumes that *Polcopa* is a non-profit organization.
He gave an overview of the aims of *Polcopa* as mentioned in §8 of the statutes, such as (1) organizing and supporting any activities aiming at development and promotion of scientific research in the field of alternative methods in toxicity testing on animals. (2) The gathering of people working in this field. (3) The dissemination of the scientific achievements to different target groups and (4) representing the Polish Association on a national and international level.

The Association realizes its aims through (1) organizing scientific meetings, seminars, training courses, conferences, public lectures etc. (2) The cooperation with National and Local Ethics Committees, Ministry of Health, Ministry of Education and Science, Ministry of the Environment, as well as other departments, universities, institutions in Poland and abroad. (3) The cooperation with related associations in Poland and abroad. (4) Publishing activity. (5) Giving an opinion on current status and needs in the field of alternatives in Poland. (6) Scientific advising and consultations concerning animal experimentation.

Polcopa plans in the first trimester of 2006 a meeting of the Founding Committee, which will result in the preparation of a Founding Document that will be submitted to the National Court Register.
They also prepared the *Polcopa* questionnaire on alternative methods, which is available in Poland. Dr. Stepnik gave a preview of the *Polcopa* website (polish/English version: http://www.imp.lodz.pl/polcopa), that is fully operational.
He also added that he will urge his colleagues to fill in the membership questionnaire in order to obtain full membership at ecopa by next year.

Ireland by Prof Michael Ryan

With regard to the foundation of an Irish NCP, Prof. Michael Ryan stated that it is still in an embryonic stage.

He gave an overview of a possible composition of the four stakeholders in Ireland and mentioned organizations and institutions that could be eligible.

With regard to the government, Prof. Ryan said that several departments are involved and can be approached, as the competences are quite spread between them. Prof .Ryan considered it most likely to approach first of all the Department of Health as this department issues licences for animal experiments, nominates Irish representative to ESAC-ECVAM, sponsors National Bioethics Committee, the Irish Medicines Board regulatory agency for human & animal health, the Food Safety Authority of Ireland and finally, the Health Research Board which funds research in the health area.
Other Departments that can be approached are the Department of Enterprise, Trade and Employment, that is one of the Health and Safety Authority, which looks after occupational health & safety and chemicals. He mentioned the Chemicals Science Foundation Ireland, which is the major science funding agency in Ireland. (2) The Department of Agriculture, (3) the Department of the Environment, (4) the Department of Marine, involved in Marine Biotoxin Monitoring, (Mouse Bioassay and Research Funding)

With regard to academia, he was quite confident, as there is a well extended organization of academia, that it would not be a problem finding academia willing to participate.

He thought that industry would be easy to involve in the foundation as 15 of top 20 pharmaceutical companies in the world had subsidiaries in Ireland. There is also a significant biotechnology industry, quite a number of SME's and industry associations.

As for animal welfare, Prof. Ryan pointed out the need to research in this area. He asked the Eurogroup for Animal Welfare for help identifying the Irish animal welfare section. There was no immediate indication of such a representative group.
He mentioned the Dr Hadwen Trust, U.K. that co-funded a research project with Health Research Board, which was a very successful project and got a lot of publicity in the National press.

Finally, with regard to the future, Prof. Ryan planned to organise meetings probably through the Department of Health as they carry responsibility for the health licenses. He will make use of his personal contacts to get the momentum going. Additionally, he would urge young investigators to get involved and seek funding from the department of Health for the start-up process. He required the assistance of *ecopa* to draw on experience and to establish a draft constitution.

NORWAY by Dr.Live Kleveland.

Mrs Live Kleveland representing the Norwegian AW Alliance and the Norwegian School of Veterinary Science, talked about the current situation of the founding of a consensus platform in Norway.

Norway is in the process of establishing an official National Platform, together with a Fund for Alternatives to animal research. The Norwegian Parliament, 2003'...requested the government to continue the initiative to establish a national Platform with a fund for research and development, based on the Swedish model.'
The Food Safety Authority asked the Norwegian School of Veterinary Science in 2004 to start the work to establish a Platform for alternatives, and provides financial support for the work. The mandate given was to identify areas of concern for the 3 Rs in Norway, gather information about existing Platforms, gather and publish guidelines for animal research, contribute to the harmonisation and increased quality of the training in animal experimentation, stimulate to an increased knowledge about fish as experimental animals, (more than 91% of the experimental animals are fish), assist the group working with a new Animal Welfare Act and publish all the information gathered.

Until now, the following has been realised by the associated Norwegian platform
The organisation of a conference on the care and use of fish in research, (http://oslovet.veths.no/fish).
The gathering and publishing guidelines on the care and use of mammals & fish in research, (http://oslovet.veths.no).Approximately 14 publications have been made, including updated information for animal researchers.An organisational stucture for an *ecopa*-style National Platform in Norway is proposed.

The establishment of the platform has to fulfill certain criteria such as:
-The Food Safety Authority stated that the platform should manage an independent state fund for alternatives.
-*ecopa*: include representatives from the "four parties",to work by the consensus principle and to be open and democratic.
-compliance with the Norwegian national legislation

The proposed organisational structure for a Norwegian Platform should be a foundation, governed by a Board. The 12 board members should represent the general interest of a party, not particular bodies. A Council, consisting of representatives for the "four parties", which may advise and control the Board and nominate Board members. Observers are welcomed, and may participate in discussions etc.(private persone, state council,....) And finally, a panel of experts to advise the Board and a secretariat to assist the Board.

With regard to the future , The Food Safety Authority has indicated unofficially that a Platform and a Fund will be established, probably in 2006. If the necessary financial basis is presented, a Norwegian Platform can be formally established within a few weeks as the structure is ready. When established, the Board of the Platform will decide which areas to prioritize.

A final remark was made that funding is needed in order to be able to start, the parliament has foreseen it without precising the exact amount of the funds.The acceptance of the Norwegian associated platform as a full *ecopa* member is therefore foreseen for the 7th Annual Meeting.

Ireland was accepted as an associated membership, *Hucopa* and d*acopa* were admitted as an official member by unanimous voting of the present eleven *ecopa* member state representatives.

SESSION II: Proposals for further Mid Range Activities of the ecopa Workung Groups, composition and comunication.

WP 1 represented by Prof. Rogiers, came with the proposal of an international study on the availability of funding for alternative methods with the Member States. The aim of a questionnaire is to gather as much information as possible, from each member state, on the available budget and pattern of allocation with respect to alternative methods funding. The aim is to present the results, after compilation and analysis, at the 7th Annual Meeting and to post them on the *ecopa* website. A financial contribution for this study would be made available for each NCP that returned a completed questionnaire with usable results. This study is also to be seen as a test case on the ability to cooperate on gathering information of general concern for alternative methods.

WP2 Mrs. Karin Gabrielson mentioned that the Chemical Workshop, postponed due to the EC delay in decision on REACH, will finally take place on 01.02.06, on which the Animal Calculator tool will be presented.
She suggested following future activities:
1. To look at the implementation and the different levels of interpretation of the legislation and flexibility on testing. The use of good alternatives should be maximized.
2. Developing further involvement and keeping up-to-date with the European partnership task force that is formed by EC and Industry.
3. Continuing the work on the globally harmonized system for classification and labeling, (GHS).
4. Try to network harder and interact with environmental groups.

WP3 education, Prof. Cervinka first mentioned the *ecopa* representation at the "Skin in vitro 2005 conference in Cologne", and at the JRC Enlargement action – IHCP W11: ALTERNATIVES 2005 – International Workshop on Promotion of the Three Rs in September 2005, Ljubljana, Slovenia Inv.

Discussion on the future activities: Standard is taking care of parts of the *ecopa* website, done on regular basis by Prof.Jan Van Der Valk. The relevant pages of the *ecopa* website have been checked, updated and completed during the year with regard to the section Legislation, Databases and Courses. In 2006 similar activities will take place.

With regard to refinement and reduction, the activities of Spain and Italy to organize a course for proper animal use should be supported. For members of ethical committees for example, complementary courses under the name of *ecopa* could be organized.
As for replacement, he suggested animal free teaching at selected faculties, which could be done in cooperation with Eurca.

Mr. Cervinka proposed to discuss whether some workshops should be dedicated to education at the Alicante meeting, as the validation of alternative methods in teaching is a missing spot in this area. Another comment was to keep in mind all kind of 3R activities: Refinement, reduction, courses of animal science, proper use of lab animals.

WP 4 Prof. Tjard De cock Buning confirmed that all the aspects listed, were performed. He explained the different meanings of "ethics".
e*copa* subscribed the proposal of the working group on Ethics at the annual meeting in November 2003, the actual stakeholder members of the National Platforms (instead of the national representatives who meet each other in the annual *ecopa* meeting) to unite in a workshop and to initiate among them a process of an open and constructive dialogue that crosses the cultural differences between the diverse stakeholders and between countries. The organization of this workshop was executed in spring 2005. The meeting took place in Ljubljana, Slovenia from the 10th till the 12th of June 2005 and 27 participants from 11 different NCPs attended the meeting.
On the first and second day the dialogue training workshops took place. The workshops were designed according to the focus group method and are further discussed below. The national delegates were mixed in three dialogue-groups of 8-11 participants from the various countries, and in such a setting that the stakeholders were equally balanced. Two issues were discussed under supervision of three skilled moderators. Shared values and areas of consensus and dissensus were explored in an inspiring context. The third day was dedicated to a discussion on the various ways NCPs choose their policy goals and how they organized their activities and the way different stakeholders managed to contribute to the three Rs. Practical experiences were exchanged between older and newer NCPs. This resulted in an overview of the different NCPs and their achievements in managing the platforms over the years.
The focus for 2006 will be on the guidance to human data and material derived from human material.