

ASAT

Starting the Programme

's Gravenhage, 1 March 2008

Index

Samenvatting	3
1. The ASAT-Initiative in its various challenging aspects	5
2. The Government of the Netherlands and the ASAT-Initiative	6
3. The Four Workshops	7
3. 1. General	7
3. 2. General Outline of the ASAT Research Plan	8
3. 3. Four Comprehensive Case Studies	13
3. 4. Animal Testing: societal and political context	18
4. Conclusions	18
5. Programme - Organisation, Network, Communication and Funding	19
5. 1. Start Up after 1 March 2008	19
5. 2. Programme organisation 2009 – 2014	20
5. 3. The Scientific Programme	21
5. 4. Road Map on ASAT	21
5. 5. Network	21
5. 6. Communication	22
5. 7. Funding	23
Programme and key Note Speeches	24
Participants Workshops	26
Appendix 1 ASAT, Assuring Safety without Animal Testing. Working towards Sustainable Protection of Human Health and the Environment	
Appendix 2 'Starting ASAT in the Netherlands' 22 August 2007	
Appendix 3 Chronology	
Appendix 4. CVs	

Samenvatting

ASAT staat voor een nieuwe benadering van veiligheid (van voedsel, geneesmiddelen, cosmetica, chemische stoffen) met als doel een duurzame en transparante bescherming van de gezondheid van mens en milieu. Uitgangspunt van ASAT is het aanvaardbare risico voor mens of milieu; deze risico's worden van tevoren geïdentificeerd en vervolgens beoordeeld in nieuw ontwikkelde, transparante en betrouwbaardere methoden van risico beoordeling (risk assessment), die het mogelijk maken de ernst te beoordelen naar aard, omvang en preventie. Door een geïntegreerde toepassing van genomics technologieën, geavanceerde informatie technologie en systeembioïologie worden nieuwe experimentele modellen mogelijk die de gegevens genereren voor deze nieuwe methoden van risico beoordeling in een perspectief waarbij enerzijds de humane veiligheid beter kan worden gegarandeerd dan op dit moment het geval is en waarbij anderzijds het niet meer nodig zal zijn gegevens te genereren met behulp van proefdieren.

De afgelopen drie jaren is intensief gewerkt aan het ASAT concept waarin 'in vitro testing' het stap voor stap moet gaan overnemen van 'in vivo [proefdier] testing'. Sluitstuk van deze periode vormde een viertal internationale werk conferenties die in januari en februari 2008 hebben plaats vonden over ASAT op uitnodiging van de Directeur Generaal van de Volksgezondheid. Elk van de conferenties had een ASAT-bouwsteen als onderwerp en had als doel om een bijdrage te leveren aan een voorlopig advies aan de Directeur Generaal met betrekking tot de haalbaarheid van het concept en de vorming van een netwerk in Nederland om ASAT te implementeren. Uitgangspunt bij het inrichten van het Netwerk was dat het:

- een aantal buitenlandse groepen zou kunnen omvatten
- gemakkelijk zou zijn voor toekomstige groepen uit de publiek en private sector te participeren
- een nadere invulling zou kunnen geven van de bouwstenen voor ASAT en voor de voorziene verantwoordelijkheidsverdeling tussen ASAT en de overheid.

Het verzoek was om op 1 maart 2008 te rapporteren.

Op grond van de resultaten van de vier werk conferenties begin dit jaar kan nu -conform verzoek- aan de DG van de Volksgezondheid advies worden uitgebracht.

Voorgesteld wordt om de periode april-december 2008 te gebruiken om het ASAT Programma verder voor te bereiden en uit te werken voor de periode 2009 tot en met 2014.

In 2008 wordt dan gewerkt aan:

- Het ontwikkelen van een Road Map voor een geïntegreerd ASAT Programma 2009-2014
- De voorbereiding van een viertal 'case studies' zoals voorgesteld tijdens de werkconferenties; dit om op alle vier geselecteerde "demo gebieden" een "Proof of Principle" te krijgen, geschikt om als algemene benadering toe te passen ook op andere gebieden van de toxicologie
- Het verder uitbreiden en onderhouden van het netwerk dat vorm heeft gekregen door de vier werkconferenties en door eerdere bijeenkomsten
- Het inzetten van dit netwerk om andere (buitenlandse) groepen en overheden te laten inhaken en verantwoordelijkheid te laten nemen voor de uitvoering van delen van het ASAT Programma
- Het uitwerken van een communicatie strategie niet alleen ter ondersteuning van het netwerk maar vooral ook om de publieke acceptatie van in vitro testing verder te faciliteren.

Teneinde deze getrapte aanpak mogelijk te maken wordt aanbevolen om de huidige ondersteuning van het ASAT-Initiatief in Nederland verder te verbreden en te professionaliseren. Overheidssteun voor 2008 maar ook voor de periode daarna, kan daarbij niet worden gemist. Voor 2009-2014 wordt gestreefd naar verdere internationalisering, niet alleen om de basis van het concept wetenschappelijk en ook richting Europese regelgevers te verbreden maar ook om de kosten ervan te delen met internationale partners en 'matching' van nationale budgetten te verkrijgen vanuit de EU programma's. Geleidelijk aan zal ook de betrokkenheid van de industrie eerst in kind en later ook in cash worden vergroot.

Voor de periode 2009-2014 wordt de volgende programma structuur voorgesteld:

Een 'Programme Board' is eindverantwoordelijk voor het ASAT Programma.

Leden van deze [eerst nationale en later internationale] Programme Board vertegenwoordigen in eerste instantie Overheidsorganisaties die verantwoordelijk zijn voor de beoordeling van gezondheids- risico's en voor risk management, de private sector en de Wetenschap; zodra mogelijk en opportuun, zal ook de industrie haar vertegenwoordiging hebben.

De Programme Board is onafhankelijk in haar vaststelling en financiering van onderzoeksprojecten.

Een eerst nationale, later internationale 'Government Group' zal -zo spoedig als mogelijk is- optreden als coördinatiepunt en zal zorg dragen voor een adequate financiering van het programma; zij zal de leden van de Programme Board benoemen. De leden van de groep zelf worden benoemd door de deelnemende landen cq ministers.

Ook wordt voorgesteld indien mogelijk al in maart 2008, een besluit te nemen over het instellen en financieren van een klein, hoog gekwalificeerd en goed geëquipeerd Project Bureau dat:

- De uitwerking en implementatie van de Road Map ter hand kan nemen
- Het programma voor 2009-2014 kan uitwerken, voor wat betreft inhoud zowel als structuur en organisatie
- De fondsen kan genereren voor de uitvoering van het Programma 2009-2014
- Verdere internationalisering kan voorbereiden via COST, EUREKA en Programma's van de Europese Commissie.

Een exacte inschatting van de voor ASAT benodigde budgetten is op dit moment nog niet te maken; de recente werkconferenties hebben geen verandering gebracht in eerder weergegeven aannames voor wat betreft orde grootte. Het jaar 2008 zal ook op dit aspect verder duidelijkheid moeten brengen en begrotingen moeten opleveren voor zowel organisatie als inhoud van het ASAT Programma. Voor 2008 worden de kosten geschat op of € 1,25 m.

1. The ASAT-Initiative in its various challenging aspects

Society expects the air they breathe, the food they eat, the water they drink and the products they use to be safe. At the same time large parts of society dislike animal testing. Unfortunately, today's reality is that chemical safety of air, water, food, medicines, cosmetics, products for personal hygiene, cleaning agents, fertilisers and any other products can only be guaranteed when animal testing has been involved one way or another.

The ASAT-Initiative involves an innovative approach in assessing risks posed by human¹ exposure to chemicals, boosting scientific efforts in this area and making use of the most modern technologies. Since it starts from human health risks it is innovative and adds value by providing a more transparent and better public health protection. In a step by step approach ASAT aims at replacing animal testing for generating the hazard and kinetics data required for risk assessments; as such it will first limit and later on abolish the use of animal testing in validating new concepts.

The ASAT concept takes advantage of rapid advances in science and technology. It is based on 'a full reversing of toxicological paradigms' and it encompasses no less than a step change in the way we think about risk.

In getting there it will require a fundamental change in the way risk managers and risk assessors assess the public health risks of a particular chemical compound.

ASAT builds on the results from various research programmes aiming at the improvement, harmonisation and validation of tests and procedures within the EU -at National and European levels- and in North America, Japan and various other countries.

In assessing safety, ASAT is fully risk based; it considers the nature of a compound, its use and therefore the exposure to the general public or categories of the general public such as consumers, patients or workers e.g. drug related liver damage, the risk of breast cancer and the use of deodorants, the risk of infertility for males in specific industrial settings. This will result in the establishment of such a health risk either for the general public or for one or several categories depending on how that particular chemical is used and disposed of.

Once a public health risk has been assessed, a decision can be made as to whether that risk can be considered small enough to be accepted or not. In the former case the use of that particular chemical will be deemed safe. In the latter case measures will be sought to reduce the risk to an acceptable level. Alternatively one will have to refrain from the use of that chemical for that application.

ASAT is Science based since it requires a thorough understanding of the biological mechanisms, networks and processes in man responsible for the health risks of concern. This is where Biological Science, Clinical Medicine and Experimental Toxicology will have to line up and co-operate.

Old and new technologies such as Gene Expression Analysis (Transcriptomics), Proteomics, Metabolomics and other 'Omics' technologies, Systems Biology, Bioinformatics as well as advanced statistics and data management will have to be applied in conjunction. This will allow for the definition of relevant indicators, biomarkers, for the risks to be scrutinised.

Exposure to chemicals in experimental models -that include the biological processes in man relating to the risks to be assessed- will allow measuring the very biomarkers that allow assessment of specific relevant risks. The objective is to develop an approach in safety decision making that is based on health risks for humans using experimental data derived from a thorough understanding of human biology.

¹ ASAT includes the protection of human health as well as that of domestic and agricultural animals and the biotic environment. This report is limited to the protection of human health, in view of the limited time that was available for preparation.

ASAT is different from the many existing research efforts in the area of chemical safety because it focuses on risk assessment and public health protection, its key drivers being:

1. the development of models, databases and tools for integrating large amounts and different types of data (existing & new; clinical, animal and non-animal); and
2. the targeted generation of new non-animal data critical for the risk-based safety decision (involving the development of more-predictive non-animal models and the application of new technologies).

It derives its data and information from experimental test systems that reflect the very biological systems and networks that are involved in the public health risks under scrutiny. It is therefore strictly building on the science of human biology. It will contribute substantially to the creation of alternatives to animal testing by 'reverse engineering' of the current safety paradigms. This makes ASAT ambitious and daring.

2. The Government of the Netherlands and the ASAT-Initiative

The Dutch Government has been informed about the concept by Netherlands Organization for Health Research and Development, ZonMw, in 2004. In January 2007 the Government has been approached to support the Initiative (appendix 1). In August 2007 the report Starting ASAT in the Netherlands (2) made several recommendations on how to continue and implement the ASAT concept. Major recommendation was to organise four workshops to ask national and international experts for comments on the ASAT-concept and to share views on opportunities and limitations of today's as well as tomorrow's science and technology developments to this context.

Meanwhile the workshops have been organised, early January and late February 2008. They were hosted by the Director General for Public Health, Ir. Hans de Goeij. This report² reflects the Workshops' outcome and will be used as a basis for further decision making.

It was initially expected to address the following issues:

- Develop a programme proposal:
 - Establish a Dutch national network as a tool for implementation of the ASAT Concept
 - Include partners from abroad whenever necessary
- Make recommendations regarding the organisational set up and modus operandi within the network (defining roles and responsibilities of the various groups).

Later on the following aspects were added:

- The development of this 'ASAT committed' network but in such manner that it can be linked to other networks and that it allows for participation by the private sector at a later stage
- The development of concrete research oriented project proposals for the four themes within ASAT 1) Risk Assessment, Exposure Modelling and (Toxico)kinetics and Biotransformation, 2) Experimental Models, 3) Measurement technology and 4) Bio-informatics and Systems-Biology
- The development of a proposal on how a distribution of responsibilities and a repartition of tasks between ASAT and the Government should look like.

Meanwhile the Government Policy regarding Alternatives to Animal Testing is under review.

² The workshops and this report have been prepared by Ada Knaap, Gerard Mulder, Bart Sangster, Marjanne Slot and Pol van den Bergen. See appendix 4 for their CVs. Unfortunately Gerard Mulder was not able to contribute to the report due to an engagement abroad.

The objective of this report is to clarify the added value of ASAT to innovative scientific developments and to risk assessment in the context of public health policies. ASAT will be judged on the basis of the newly developed inter-ministerial policies on alternative testing. Thus it will support the Government in decision making on ASAT within the context of this 'policy to be re-defined'.

3. The Four Workshops

3.1. General

Four workshops have been organised since; they focused on the four building blocks of ASAT:

1. Risk Assessment, Exposure modelling and (Toxico)kinetics & Bio-transformation
2. Experimental Models
3. Measurement Technology
4. Bioinformatics and Systems Biology

Each workshop had a mix of experts from science areas to be discussed and from other Science & Technology domains, to be connected possibly with ASAT. Participants (see list of participants) were informed that the objective of the workshops was to make recommendations to the Dutch Government on the establishment of a network that has the capability to develop and deliver ASAT. This network should consist of science groups from academia, institutions and industry in the Netherlands and –if and when appropriate- from abroad.

Even though people had been invited at a very short notice, many reacted almost immediately and showed their interest in the subject and their willingness to participate. The total number of participants was around 50 from the Netherlands and abroad.

'A good starting point would be, based on inventory, to identify a key area in biology that is linked to a key clinical effect and to take that as an opportunity for the Netherlands to start working on. It could become a key economic driver.'

Notwithstanding the fact that many participants were not involved on a day to day basis in risk assessment or in safety testing, the discussion in the workshops showed a quick and firm support for the ASAT Concept. It was emphasised that ASAT could only add value if it could keep up its daring character focusing on the science of biological mechanisms and networks which operate in a direct relation to assessment of human health risks. It was also underlined that much is already going on in particular in the areas of developing new tests and gathering data; duplication should by all means be avoided, collaboration, be it national or international, should be sought.

'Don't limit the plan to the Netherlands or Europe. Find out what's going on and be pragmatic as to where you put your funds.'

Added value of ASAT was considered present and clear in the sense that the concept was:

- Science and Risk driven
- Public Health focused
- Aiming at the combination of novel and existing technologies
- Multidisciplinary and relatively top down

Many participants from abroad stated explicitly that the reason they came over for the workshop was because the invitation was from the Government which they considered an essential prerequisite for success. Top down and Government driven would be the best way to make a programme like this work.

Although it may be anticipated that ASAT is particularly appealing to representatives from chemical and cosmetics industries, participants with a pharma-background were interested as well because of the central position of human biology in ASAT. The pharmaceutical industry is interested in safe products not so much because of regulations, but because of corporate responsibility. For several reasons pharma-industry would prefer to avoid expensive use of animals in testing and to use human cellular systems and human biology based in-silico data instead of animal testing.

The current approach and the uncertainties of today's animal models were briefly discussed as well. Participants agreed that since current practice has certain limitations the overall objective should be to reduce the uncertainties inherent to the animal models. The proposed new systems/models are aimed at the development of methods that are more closely related to the human situation than the present models. This was considered one of the appealing characteristics of the approach. Whether the health based risk models will be better predicting disease/health outcome can only be assessed once they have been developed. However, the prospect was considered to be of ample potential.

3.2. General Outline of the ASAT Research Plan

Many scientific and technological issues were discussed that need to be resolved. This confirmed the size and the complexity of ASAT. There was agreement that the final 'product to be delivered' had to be validated in in-vitro test models that can be exposed to chemicals to generate targeted data critical for the risk-based safety decision for specific public health risks.

There was a general agreement that this concept could only be proven viable and feasible if developed and validated within well-specified and representative cases. Most participants confirmed that using four building blocks would be very helpful to developing ASAT and adjust the various scientific activities to enable the delivery of ASAT.

'Be realistic; one project cannot provide the solution, but starting something may make the difference and demonstrate proof of principle for the approach.'

As time progressed and more discussions had been held a consensus was reached that the advancement of the highly innovative ASAT approach of non-animal safety testing and risk assessment will gain momentum from a transparent demonstration of feasibility. Specific cases within current toxicology have to be selected which can provide a proof of principle within a reasonable time frame. In the longer term, the principle can subsequently be used as a general approach applicable to other areas of toxicology. Such a specific case study ideally complies with a number of criteria:

1. The study should address an organ or a pathological process which is related to significant public health issues, in order to be able to demonstrate that ASAT can indeed have a significant public health impact.
2. The fundamental biology underpinning toxic modes-of-action should be well known, so that the required data bases can be effectively loaded relatively quick and simple, and research can focus on the toxicology of the system and relatively rapid progress can be made.
3. For developing these case studies within the national ASAT research programme, it is imperative that a large body of expertise is already available in the Netherlands: in that sense, the existence of the Netherlands Toxicogenomics Centre and other Genomics Centres is considered very helpful.
4. Human samples and data should ideally be available for comparative study, as the human is the target for risk assessment.

5. Priority should be given to an area where current animal use for safety assessment is relatively high, so that the gain in terms of reduction of animal use is likewise high.
6. In vitro alternatives, such as cell cultures, which mimic the in life physiology of the model, should be available for study.
7. The model of choice should represent a niche that has not been already studied in great detail with ASAT-type approaches by other major initiatives. This will allow ASAT to develop its own scientific face. Furthermore, this will facilitate the identification of ASAT as an independent major player in the area of animal-free alternatives for safety testing, and it will position ASAT as a partner of interest for other major players in the field.
8. Finally, the model of choice should be a low-hanging fruit that can relatively easily be harvested. An area should be selected in which in a short time significant progress can be made to the extent that in 4-6 years' time a proof of principle for the ASAT approach can be provided.

These ASAT case studies should focus on developing specific stepping stones, in order to reach the all-over important deliverable of a combination of validated in-vitro tests usable for the purpose of human risk assessment.

The following subjects were also discussed:

1. ASAT and Risk Assessment

The need to work with risk assessors and risk managers upfront was emphasized. This will lead to an unambiguous selection of combinations of risks that will have to be assessed for different compounds and different applications, agreement on what information is needed for carrying out the assessments and what indicators are suitable for generating that information. It will help risk assessors and risk managers to appreciate the relation between the risk underpinning human biology and the newly to be developed in-vitro tests.

2. ASAT and biological response to realistic exposure

Within the ASAT concept, in the transition from hazard identification to risk assessment, it is of crucial importance to be able to generate relevant data on toxic responses at "daily life" exposure levels, e.g. at rather low dose. Few toxic dose-range finding studies, however, have evaluated 'omics technologies as to whether they are more sensitive in comparison to 'classical' toxicological methods. First results indicate that 'omics technologies do harvest relevant data on toxic responses at lower dose than classical methods can do. However, to what extent toxicogenomics technologies are capable of reliably performing at the really low dose range has not been fully explored yet, in in-vitro models, in laboratory animals nor in humans.

In addition, humans are never exposed to single compounds, but always to mixtures. This has been appreciated by toxicologists for a long time now, but classic toxicology has not really delivered scientific models relevant for evaluating effects of mixtures. Toxicogenomics approaches have provided first proof-of-principle (within the Netherlands Toxicogenomics Centre by TNO) that applying 'omics technologies to test hypotheses on mixture toxicity yields relevant insights. This should be further explored.

3. ASAT and 'omics technologies

It is evident that 'omics technologies are very powerful in generating complex, multilayered data from cells responding to toxicants, thereby having the potential of tremendously increasing our understanding of toxic modes-of-action. Omics technologies are already demonstrating their potential to identify chemical hazard and the associated risk assessment. For ASAT, it is critical to maintain the current state of technological quality, and to be able to integrate innovations where/whenever of relevance, by upgrading existent 'omics technologies and absorbing emerging novel applications.

4. ASAT, alternatives to animal testing and new technologies

All current in-vitro models generate hazards. In-vitro models that are highly predictive for human safety are still generally lacking. It is essential that models are set up to transform these hazards into risks for humans. Obviously, generating in vitro alternatives to current rodent toxicity tests requires an emphasis on cellular technologies leading to in vitro assays which reliably represent the (rodent and/or human) in vivo situation. Currently, within the Netherlands Toxicogenomics Centre as well as internationally, focus is on developing cellular assays for chemical carcinogenesis, reproduction toxicity, endocrine disruption, immunotoxicity including sensitization, and organ toxicity (liver, lung, kidney, skin, blood). The ASAT case studies should deepen this, thereby focusing on the extrapolation of data generated by such in vitro assays to humans in vivo, by taking the systems biology approach.

In-vitro systems should ideally predict acute as well as chronic health risks that could be expected in humans under realistic exposure conditions. Disturbances in cellular homeostasis are the basis of toxicity-related pathology at the functional/organ level. These are diverse but would typically be manifested by (subtle) changes in, for instance, cellular organelle functions, as well as at the transcriptome and proteome level. Toxic effects are currently analyzed in cells from different organs that are often dedifferentiated and displaying oncogenic transformations; translation of the effects to the *in vivo* situation is limited. 3D-organization and the micro-environment are generally not taken into account and reliable omics-based biomarkers for specific in-vitro toxicity screening are not yet available. There are high expectations of (live)-cell imaging-based technology using low resolution fluorescence microscopy, but it needs further development: there is still a lack of pathology-relevant cell biology-based mid- or end-points that can be visualized and quantified (in real-time) using high-content high resolution imaging screening methods. Focus should be on establishing:

- 1) human (conditionally) immortalized cell lines with differentiation characteristics, representing the key organ target cells;
- 2) key cell biology-based markers representing human (chronic) toxicity-related pathological conditions in crucial target organs to be used for imaging-based analysis;
- 3) further omics-based biomarkers for organ pathologies which can be translated into cell-based assays;
- 4) automated high content and high resolution fluorescence imaging approaches and image analysis methodologies for cell-based predictive (chronic) toxicity profiling.

Development of a high throughput system of (human) cell lines using imaging techniques in order to detect the major toxicities to be expected in humans in vivo after realistic exposure, thus seems promising. In particular mechanistic information should be gained from these cell lines. Promising results on the prediction of human liver toxicity using imaging techniques with the HepG2 cell line underscore the feasibility to predict human toxicity based on high throughput imaging screening of living cells. It is anticipated that within the next 5 years a dedicated effort along those lines will identify several major human toxicities, based on a mechanism-based approach.

It was noted that most pharmaceutical companies have many -but confidential- data. Therefore, ASAT should get the pharmaceutical industry involved such that their confidential data base can be used to generate reliable and predictive publicly available assays. The assays should be applicable to all kind of molecules, independent of intended use e.g. medicines, pesticides, industrial chemicals etc and enable the analysis of the major relevant (in terms of use) types of health risks of chemicals: liver damage, organ-specific cancer etc, etc...

Stem cell technology was extensively discussed. The general conclusion was that stem cell technology might be of limited value in developing ASAT. However, it was likely to be able to add value when assessing the risk of health effects with a functional nature such as rhythm disturbances in the heart or disturbances in nerve conduction. Alternatively, the potential of embryonic (rodent and human) stem cells in developing in vitro assays seems exciting, predominantly because of the opportunities for developing robust, non-transformed cell lines for organ toxicity. Such stem cell assays however should not be developed by ASAT, but by building on experience gathered by the Netherlands Toxicogenomics Centre and the Cancer Genomics Centre, ASAT should explore to what extent stem cell lines developed elsewhere, can be used as in vitro assays representing target organs and processes for toxicity.

5. ASAT and PK-PD modelling

Once developed, to be used in an ASAT context, these essays should be complemented with toxicokinetic interfaces, in order to reliably extrapolate the hazards determined in (cellular) in vitro systems to a risk assessment for humans in vivo under realistic exposure conditions and to predict dose-dependent responses from in vitro models to humans in vivo. There was consensus that predicting kinetics and biotransformation are an essential prerequisite for risk assessment. When assessing the risk of organ related health effects due to exposure to a chemical understanding the level of the parent molecule and its metabolites in that particular organ is required. There is sufficient evidence that this could be made possible in the forthcoming years.

The degree of toxicity of a chemical depends, in the first place, on the exposure of the target tissue(s) to the compound itself or to its toxic metabolite(s). Thus, toxicokinetics is an essential aspect of risk assessment, not only to relate effect size with plasma or blood levels, but also to predict tissue exposure to metabolites. For the purpose of ASAT models to predict human toxicokinetics should be based on (human) in-vitro data exclusively combined with proper physiologically-based toxicokinetic (PBTK) modelling.

Currently, increasingly successful models are explored for medicinal drug development (Physiologically-Based Pharmacokinetic models, PBPK). They are mainly available for drug-like molecules and focus on prediction of plasma levels, primarily based on intestinal absorption, hepatic metabolic clearance and renal excretion. More biological, physiological, and genetic information is needed to extend these models to exposure of the major toxicologically relevant target organs, including e.g. liver, kidney, intestine, brain, lung, heart, blood cells, placenta and foetus. Increasingly PBTK models are available also for (environmental) chemicals, based, however, on animal in vivo data.

Recently the relevance of interplay between transporters and drug metabolising enzymes is increasingly appreciated. To assess this, cell lines/systems with expressed combinations of transporters and metabolic enzymes are needed; these are currently available only to a limited extent. The availability of such cell lines/systems with the most relevant combinations of transporters and enzymes is essential to allow proper assessment of risks after exposure. Highly predictive human models ideally are based on human tissue, having both drug metabolism and transporters in their proper physiological abundance. Such models need to be developed. A tissue bank to ensure the efficient and ethically controlled availability of human tissue for research is needed.

Once the toxicokinetic parameters of a chemical in humans have been derived and the potential hazards have been identified, the next essential step is to assess the risks for humans in vivo. This requires that the in vitro effect size in relation to the dose-effect response has to be converted to an in vivo risk. In order to achieve this, the pharmacokinetics-pharmacodynamics (PK-PD) approach is very promising. This method has been validated extensively for medicinal drugs, in particular to extrapolate, in a strictly quantitative manner animal data to humans as well as in vitro receptor-drug interactions to the in vivo situation. For (non-selective) toxic effects this approach has much less been

applied so far. In combination with PBPK modelling this is a powerful approach to risk assessment based exclusively on in vitro data.

In order to validate the approach it will be highly profitable if extensive data bases available in the pharmaceutical industry can be used. A major requirement, therefore, is to involve (several) companies in this endeavour, which can be done in a non-confidential way.

Mechanism based PK-PD modelling was seen as a very powerful tool that could be applied. However, developed in a pharma context and considering its cost it was questioned to what it would be affordable outside pharmaceutical R&D. Elements should be applied across ASAT such as the need to distinguish between chemical related properties and system specific properties.

At the Leiden/Amsterdam Center for Drug Research, at the Radboud University in Nijmegen, and at the University of Groningen, there is ample expertise available for constructing the PK-PD modelling as described.

6. ASAT and systems biology.

The systems toxicology approach refers to the ‘-omics-based evaluation of biological systems upon perturbation by chemical stressors, by monitoring molecular networks and toxicological endpoints and by iteratively integrating these response data to model the toxicological system. A key strength of applied systems toxicology is its emphasis on the integration of data from different studies and analytical platforms to produce a richer and biologically more refined understanding of the toxicological response of a cell, organ, or organism. It is expected that such integration of data will yield a more complete picture of the biology behind the pathway modulations that are associated with a particular toxicant treatment, shedding light not only on what the cell is planning (transcriptomics), but also on what occurs in the proteome and metabolome. Therefore, it needs to be facilitated that transcriptomics, proteomics, metabolomics, and high-throughput techniques are collectively used to collect quantitative data for the iterative construction and validation of systems models for toxicity.

ASAT should prioritize delivering proof-of-principle that such a systems toxicology approach actually can be applied to the demands of chemical risk assessment. Therefore, ASAT will focus on a limited number of cases which clearly represent important health care issues and of which considerable scientific knowledge and (genomics-based) research data are already available including first concepts of mechanistic models. High-level expertise is available, within the Centres of the Netherlands Genomics Initiative.

7. ASAT, bioinformatics and databases

The development of novel animal-free approaches or approaches using significantly less animals that are risk-based require intensive evaluation of such approaches against existing knowledge on toxicity profiles of chemicals and drugs in animals and man.

In building integrated models for hazard identification and risk assessment, there is a need for a computational bioinformatics infrastructure to manage the increasingly expanding information from functional genomics and proteomics gained from experiments in animals, cells or tissues, or in humans, in addition to information on the chemical properties (physico-chemical properties), exposure (toxicant, dose, route, intervention), kinetics (absorption, distribution, metabolism, excretion), biological sample (species, tissue, stage) and disease outcome (time, risk, phenotype). Such infrastructures, i.e. databases, will help delineating pathways and biological regulatory networks for specific diseases as well as for chemicals or classes of toxicants, developing novel biomarkers indicative of exposure and/or predictive of adverse effects, by integrating modern computing and information technology with toxicological, physiological, and pathological data, both at the “omics” level and the phenotypic level.

A number of databases are currently being developed by different institutions across the world. If the Netherlands desires to play a central role in the development of ASAT, the added value of creating another database in the Netherlands must lay in the possibility to structure the database in such a fashion that it allows generating and coordinating information that is specifically applicable to ASAT. Such a database would enable to store data available at different sites and stored in different ways, perhaps for different reasons. For this reason the database should have interactions and be able to communicate with other such databases. A crucial addition to data already present will be human data.

A crucial factor supporting the development of ASAT is the quality and quantity of the data that constitute the information stored. Chemical and pharmaceutical industries produce a vast amount of data on different chemicals and chemical classes in preclinical and clinical settings, often using high-throughput methodologies. In fact most information (especially pertaining to information in humans) required to fill databases may actually be predominantly present within the industrial setting. Unless provisions are made to include such data without breaching confidentiality and maintaining high ethical standards, adequately functioning databases may be difficult to establish. Studies submitted for evaluation by regulatory authorities in the Netherlands may serve as an avenue to retrieve such information, provided specific provisions for confidentiality are in place. An organization structure not directly related to industries, for instance government-based, would best be fit to ensure quality of the database, access, confidentiality and the highest ethical standards.

To integrate toxicogenomic approaches into risk assessment practice, management of a large, public database for storing and integrating the results of toxicogenomic analyses with conventional toxicity-testing and exposure data should be developed as part of an international network of databases. Since this should allow access to experimental data as well as human clinical data, historical data as well as future data and data from the open literature as well as of a confidential nature great effort has to be put in the infrastructure of this data base and in the more complex international infrastructure of which it has to be a part. It seems unlikely this will be feasible without Government taking a lead in its establishment. Similarly careful preparation is needed before deciding on its structure and set up to achieve an attractive cost benefit ratio.

Utilizing the data and information requires advanced bio computational tools and are as such a precondition for deciding on any database. To further enhance systems biology/toxicology approaches, which will depend to a considerable extent on such databases, bioinformatics tools, such as software, analysis, and statistical tools need to be further developed. Algorithms that facilitate accurate identification of orthologous genes and proteins in species used in toxicological research need to be defined. Tools to integrate data across multiple analytical platforms (for example, gene sequences, transcriptomics, proteomics, and metabolomics) need to be developed. This will provide computational models to enable the study of network responses and systems-level analyses of toxic responses.

3.3. Four Comprehensive Case studies

As a first result from the Workshops, each of the four ASAT building blocks has been elaborated in some further detail. In this task, participants focused onto those areas where the work could be started at a rather short notice; in addition in each of the areas a research group could be identified to take responsibility for the implementation of the project. The selection of these case studies obviously complies to the criteria set in section 3.2.

There was general support for starting two case studies:

Case 1: The risk of male infertility

Fertility is a public health issue of major concern. Sub fertility and infertility nowadays lead more than 15% of couples with child wish in the Netherlands to consult a fertility clinic. Sperm abnormalities such as oligozoospermia and azoospermia occur in higher frequency in humans than in any other mammal studied. Time-related reductions in human sperm quality in the developed world have been an issue of extensive scientific debate. Sperm quality issues have tentatively been related to environmental endocrine disrupter exposures. The still ongoing increase in testicular cancer incidence observed especially in western societies has given rise to worries about life style-related chemical exposures.

Current knowledge about safety testing of chemicals indicates that the testis is probably the reproductive organ that is most sensitive to chemical exposure. The development of the oocyte is hardly ever seen to be affected. This is probably related to the fact that spermatogenesis is a continuous process throughout adult life including all phases from the spermatogonium through meiosis into fully differentiated sperm. Oocyte development partly occurs in utero, and in the adult woman oocytes remain silent until ovulation. These notions indicate that spermatogenesis is most likely the most vulnerable process in the area of fertility.

The fundamental biology of spermatogenesis in the testis is well described. Gonocyte differentiation occurs under the influence of trophic and sex hormones produced and regulated by distinct cell types in the testis. Leydig cells and Sertoli cells, as well as gonocytes can be cultured in cell culture systems and their function can be studied in isolation. The regulation of sex hormone homeostasis through the aromatase enzymes is also well known, and various in vitro systems allow the study of effects on sex hormone homeostasis in e.g. testicular and adrenal cell lines. Such cell lines are also available to some extent from human material, allowing interspecies extrapolation. In addition, human samples from clinical resections can be used for comparative study. Clinically, sperm function parameters are routinely assessed with fully standardized methodology, on the basis of global WHO standards for sperm quality assessment.

Animal use for testing possible effects of chemicals on fertility is particularly high. Within the EU REACH programme for chemical safety assessment it has been estimated that 70% of animals will be used in reproductive toxicity testing, the major part of which is employed in 1- and 2-generation studies for fertility effect assessment. This testing will be required for 10.000 out of the 30.000 chemicals under REACH in the coming semi decade.

Although basic knowledge of the biology of the testis is relatively comprehensive, the system has so far not been used as a major model for applying the omics approaches. Some preliminary and relatively small studies have been performed, which suggest that the testicular system allows analysis with an omics approach. Thus, the area of testicular toxicity testing fulfils all above-mentioned criteria for providing a relatively unique niche for the advancement of the ASAT principles.

The Netherlands Toxicogenomics Centre runs a programme on reproduction toxicology, thereby focusing on fetal toxicity and fertilization, but not on male fertility. This case study thus will be complementary to the NTC programme while simultaneously building on its experience on omics-based approaches to reproduction toxicology, thus creating synergy.

Case 2: Respiratory sensitivity: the risk of respiratory inflammation and irritation

The prevalence of respiratory sensitization has considerably increased over the last decades, and this is due to a large extent to environmental factors, among which exposure

to chemicals. Respiratory sensitization may lead to chronic inflammatory diseases such as rhinitis and asthma. Also non-immunologic inflammatory reactions in the respiratory tract may occur, and symptoms due to non-specific irritants and sensitizers may have similar characteristics, such as reduction of lung function, airway hyperreactivity, bronchitis, non-infectious pneumonitis, bronchial obstruction, etc.

Indirect immunotoxicity in the respiratory tract occurs when a chemical, often after covalent binding to proteins, is recognized by the immune system as an antigen, leading to sensitization (allergy). Larger molecules, for instance detergents, can often be recognized by the immune system as such.

The cellular response of the respiratory tract to contact with irritating and sensitizing compounds occurs along the following cascade of processes: compounds entering the respiratory tract are able to disrupt membranes of viable cells lining the lumen of the tract, resulting in the release of cytoplasm into extra cellular spaces. The cytoplasm of such lung lining epithelial cells contain pro-inflammatory cytokines, such as for instance IL-1 α , which induce an inflammatory cascade and secondary molecular responses, followed by morphological alterations and finally the onset of symptoms such as influx of inflammatory cells, production of mucus, and increased reactivity of the airways to other non-specific stimuli that may result in bronchoconstriction. Irritation in the respiratory tract may also be induced by oxidative stress. Reactive oxidative species (ROS) cause cellular damage by oxidising nucleic acids, proteins and membrane lipids, which may lead to altered gene expression or direct cytotoxicity. Another mechanism by which external insults may affect the barrier function in the respiratory tract is modulation of the lipid bilayers of cell membranes. At sub-cytotoxic concentrations, the interaction of compounds with cellular membranes may change membrane fluidity, which may consequently have an impact on receptor-mediated signal transduction and thus induce responses leading to irritation.

The vast majority of lung lining cells are epithelial cells, and as such bronchus-associated epithelial cells are often the first cells to encounter sensitizers and irritants. Alveolar epithelium and alveolar macrophages reside deeper in the lungs, and for those exposures that reach these areas, such cells are obviously target cells. In response to contact with foreign antigens, these cells release a range of cytokines and growth factors that mediate uptake of the protein-hapten complex or proteins by antigen-presenting cells and their maturation and migration to draining lymph nodes, where T cells are primed to elicit an immediate immune response upon subsequent encounter with the same allergen. Lung epithelium and macrophages are therefore considered to be suitable targets to examine the (differential) responses to sensitizers and irritants, and to be crucial for the typical response of dendritic cells (DC) to sensitizers.

There are no validated animal inhalation tests to predict respiratory sensitizing activity of chemicals. Sensitization testing has been developed *in vivo* in animals (predictive testing) and in humans (diagnostic testing). The Local Lymph Node Assay (LLNA) in mice is currently used to identify sensitizing properties and potency of chemicals. The test is designed for predicting skin sensitizers, but to date all respiratory sensitizers tested are also positive in the LLNA, and cytokine profiles within the context of the LLNA are suitable for discrimination of respiratory from skin sensitizers.

As the LLNA is an animal test, there is a need for validated in-vitro methods for identification of sensitizers. Alternative test methods available include QSAR models, although they have not been sufficiently developed and validated. In addition, bronchus-associated epithelial cell and dendritic cell cultures are available and will be used for priority setting. The key steps in the sensitization process (allergen detection, uptake and processing, cytokine signalling, migration/maturation of antigen-presenting cells (DC's), activation and proliferation of T cells) have been used as read-out parameters, but tests based on these are still far from being perfect and further work is needed before in-vitro systems for respiratory sensitization can be used for regulatory purposes (i.e. classification and labelling, and dose response assessment). Better understanding of the complicated processes of respiratory sensitization through broad examination of induction of specific pathways and mediators is of crucial importance for the development of adequate immunotoxicological tests.

For the prediction and risk assessment of respiratory sensitizers and irritants, the ASAT programme should aim at

- Creating the required data base on relevant respiratory functional biology and toxicology, both by exploiting existing rodent and human data, and adding novel experimental results coming from functional genomics approaches, that include toxicogenomics
- Applying complex data analyses to understand the multi-dimensional sensitization response and how this predisposes to respiratory inflammation and irritation
- To build a systems model out of that, adding PK-PD modelling to that for the purpose of predicting respiratory responses to application of low levels of immunotoxicants.

The Netherlands Toxicogenomics Centre runs a small programme on skin sensitization, establishing toxicological pathways induced in epithelial cells by sensitizers. Both skin and respiratory sensitizers are positive in the LLNA, indicating that initial responses in the LLNA should be comparable for both types of sensitizers. The outcome of the work done by NTC will be incorporated with the work done under ASAT, in order to get the full view on sensitization.

These two case studies would be able to develop a framework in the first year and collect existing data. In year two collection of existing data will be continued (bio-informatics) enabling thorough understanding of the relevant biological networks (systems biology) and identifying existing knowledge gaps. In year three and four gaps can be closed by focussed projects. Priority chemicals and their exposure scenarios can be developed. In parallel test models can be developed and omics testing started and the results processed in the biological network model. Year four and five can be used for further refining the in-vitro test models from qualitative to quantitative predictive biomarkers to generate input in the risk assessment paradigms. Year five and six can be used for validation.

This would enable delivering a comprehensive tool for two specific public health risks:

- 1) A thorough understanding of the risk and the underpinning human biology
- 2) A risk paradigm for the relevant risk or risks when more mechanisms require to be assessed
- 3) An in-vitro test model or in-vitro test models that can generate data for the risk assessment paradigm(s)
- 4) Validation of the tool.

It was concluded that there would be a need for two additional case studies based on the following considerations:

First, the cases should be relevant from an international scientific co-operation perspective. They should therefore be less of a niche and in areas where other groups are active as well. Second, the model should be relevant across different application areas (food to pharma). Third, it should in addition be comprehensive enough to deliver a tangible end product that will allow risk assessment for that particular risk. The following case studies were supported:

Case 3: The risk of liver damage due to inflammation and/or degeneration

In the liver, often organ-specific toxic effects are observed in long-term rodent assays. Furthermore, in the liver, unexpected toxicity is regularly observed in humans, during clinical trials or once a product has been put on the market. These idiosyncratic effects are a major reason not to further develop lead compounds or even, to withdraw drugs from the market, both having huge financial consequences.

This high attrition rate of compounds in in-vivo toxicity testing following a positive evaluation in in-vitro tests demonstrates the need for improved model systems for liver toxicity.

In the area of hepatotoxicity most focus so far is on short term animal studies in order to predict long term effects. Translational research to the human situation, such as in clinical settings and phase 1-2-3 studies for drug development, thereby focusing on biomarkers in blood / plasma, are lacking. An integrated approach on the various omics technologies in target and surrogate target tissues from humans and animals is needed. Furthermore, in-vitro liver models should be developed or improved, such as by incorporating stem cell technologies, co-cultures of various cell types, and 3D-organ cultures, aiming at pertaining liver-specific functions essential for pharmacology/toxicology and liver-specific mechanisms of toxicity (e.g. hepatitis, inflammation, cholestasis, etc.). These in-vitro models will enable a better understanding and investigation of the mechanisms of action for liver toxicants, thereby returning mechanism-based testing systems.

Within the Netherlands Toxicogenomics Centre, several of these aspects are addressed, but for a limited number of compounds, a few mechanisms, and a focus on in vitro (mostly mouse primary hepatocytes and HepG2 cell line). The ASAT case study will create synergy with this by focusing on in vivo issues of hepatotoxicity, by studying low dose effects, and by expanding the NTC approach to applications for human risk assessment, through PK-PD modelling.

Case 4: The risk of developing adenocarcinoma of the female breast

Cancer, the second leading cause of death in the Western world, is primarily caused by genome instability. Many chemicals have the potential to adversely affect the DNA of our body cells, thereby causing (epi)genetic alterations that might eventually lead to cancer development. Since the pharmaceutical, chemical, cosmetic and food industries are synthesizing increasing numbers of chemicals it is of crucial importance to test these substances, using highly sensitive methodologies that can reliably predict human carcinogen risk before these new chemicals reach the market. Discrimination between genotoxic and non-genotoxic carcinogens is an important component as well. The overall objective is to look at the pathways and mechanisms leading to the initiation, growth and metastasis of malignant cells, especially how the application of toxicogenomics approaches can be utilized to define these changes and help in hazard identification.

More specifically, breast cancer is a leading cause of death for women aged from their late 30s to their early 50s, with more than 270,000 women being diagnosed with breast cancer each year in the EU. While factors related to reproductive history and hormonal status increase risk, the large geographic variation in risk indicates that environmental factors also make an important etiological contribution. Existing evidence suggests that, in addition to nutrition and lifestyle, environmental carcinogens may also have an important role. Recent reviews of the epidemiological literature concluded that the evidence to date provides support for an association between breast cancer and exposure to Cadmium, PCBs and PAHs. This provides the rationale for focusing on breast cancer risk within ASAT.

Within the Netherlands Toxicogenomics Centre, a systems toxicology approach to liver carcinogenesis is being undertaken. In a complementary study, the ASAT programme will address carcinogenesis of the female breast, thereby targeting on non-genotoxic events, analyzing the role of epigenetic and hormonal factors in carcinogenesis, and thereby building on experiences within NTC but also, and most importantly, within the Netherlands Cancer Institute/Cancer Genomics Centre. Furthermore, the ASAT case study will contribute to the relevance of system modelling for human cancer risks by adding PK-PD modules.

3. 4. Animal testing: societal and political context

Most participants in the Workshops proved conscious of the fact that -depending on statistics used- testing for safety purposes, comprised about 10% only of all animals used for scientific purposes within the EU³.

National and EU research efforts in the area of 'Alternatives' are aiming at reducing, refining and replacing animal testing in general but they do not single out safety testing as a separate area of interest.

All participants were very much aware of the complex societal and political context of ASAT. Society, in particular in Europe, has become more and more critical on the justification of the use of animals for scientific or safety purposes.

Concern about information available on the many chemicals that in large volumes are being used, made in/ imported into the European Union, has urged a need for more comprehensive safety data relating to volume, use and properties.

This need has resulted in a Chemicals Directive and in "REACH", an acronym for Registration, Evaluation, Authorisation and Restriction of Chemical substances.

REACH in itself will cause a huge increase in animal testing for an initial period that is estimated at about 10 years; this because of the fact that it will ask for complete dossiers on chemicals, imported, used or manufactured within the EU. Unfortunately -by now- it is clear that those data can only be generated via animal testing.

Concern about Animal Testing triggered an intense public focus on testing cosmetics and their ingredients. In Europe this resulted in an adoption of the 7th Amendment of the EU-Cosmetics Directive by the European Parliament. Marketing within the EU will be banned for all cosmetics when ingredients have been tested in animals after 2009. Only a few tests are exempted and a general ban will be effective for ingredients tested after 2013. It does not matter whether the testing has been carried out within the EU or elsewhere.

Also all products used for personal hygiene such as soap, shampoo and tooth paste will be struck by this ban since they are within the remit of the Cosmetics Directive. In a breathtaking contradiction to this European regime the USA and China request by law animal data for cosmetics in order to be approved and allowed on the market.

Within the EU this situation has all the characteristics of Catch 22. On the one hand animal testing will be prohibited whereas on the other hand, animal testing is being promoted albeit in an indirect manner.

Globally there is an impossible situation where in cosmetics in one part testing of ingredients will pre-empt marketing whereas in other parts it is a precondition to marketing such products. ASAT looks like an attractive way out of the stalemate.

Medicines however are different from other chemicals because they are designed to interfere with human biological processes in order to cure disease or for prevention. In these products animal testing is involved in research and development as well as in assessing safety. Moreover in many countries animal testing in two species -one being a non-rodent- is mandatory.

4. Conclusions

Many scientists from a variety of disciplines have been 'exposed' to ASAT and discussed its feasibility and relevance for public health protection. They were from the Netherlands, other

³ Average per annum in the EU is about one million animals, mainly rodents.

European countries and the USA. They are involved in pharma, food and general product safety as well as environmental protection. Generally they have expressed an interest in ASAT, supported its feasibility and most have expressed the need to take action because time seems to be ready for a risk based approach to health protection that builds on the assessment of specific public health risks. They expressed support for a slightly unorthodox top down approach to align the many simultaneous activities that are required for actual delivery of test models and risk assessment paradigms that can be used in everyday health protection. Moreover during the workshops several scientists showed an interest in participating in the ASAT Programme.

A strong need for involving regulators upfront as well interest groups was strongly recommended. Last but not least validation was confirmed as a requirement for acceptance once completed.

Based on this it is recommended to start in 2008 and use this year to:

1. Prepare the execution of the four case studies
2. Develop a road map to deliver a comprehensive ASAT programme; 2009 -2014
3. Maintain and expand a network that has been created through the workshops
4. Use the networks to invite other (foreign) groups and governments to join the programme and take responsibility for the execution parts of it
5. Set up a communication tool supporting the network.

5. Programme - Organisation, Network, Communication and Funding

5.1. Start Up after 1 March 2008

Right after 1 March 2008 – when the Report on ASAT has been delivered to the Minister-programme preparations could be started depending on a decision by the Minister for Health, Welfare and Sports. Given the complexity of the issue on the one hand and the need to get to an international positioning of the Initiative on the other, in the event of a positive decision by the Minister, these preparations are expected to last till the end of 2008.

From a content point of view focus will be on the further elaboration of the four case studies which have been identified in the four Workshops in January & February 2008 to deliver a proof of principle. The intention will be to per 'Building Block' define a project based approach involving the best experts from the Netherlands and from abroad. This to enable the already participating research groups to build a high quality infrastructure, with high quality people and equipment, to eventually deliver top quality science.

From an organisational point of view efforts have to be undertaken to upgrade and expand the team presently working on ASAT to eventually realise and implement the ASAT concept in The Netherlands and abroad.

The Government will have to play a major role here in a strong commitment especially for the running costs of the programme not only for the rest of 2008 but also for the seven years to come; be it that for the period 2009-2014 collaboration will be sought with international partners to spread the burden and match the funding.

For the year 2008 this strong commitment should involve urgent decision making on:

- 1) The establishment of a 'lean and mean' Project Office:
 - To pave the way for a more definite programme & project infrastructure for the period 2009-2014
 - To prepare for the implementation of the Road Map mentioned above
 - To raise funds for project implementation on a national as well as an international level.

- 2) The establishment of a – for the time being - national Programme Board.
- To support, steer and guide the Programme and the Office.
 - To prepare for further internationalisation via COST, EUREKA and the European Commission programmes.

This year should be used to actively pursue support and commitment from industry primarily in kind to facilitate the programme by providing data and actively participating in research projects. Alignment with initiatives by the Commission and European Trade Associations such as EPAA will sought.

This year the programme board will only be at reduced strength and have a national character; given the work to be done members this first period will mainly be representatives from academia, government and governmental bodies, like regulatory authorities. The period after, the number of Members will go up and industry representatives will be involved. The Programme by then will be internationalized. The number should be limited between five and maximal ten members.

Chapter 5. 2 hereafter will provide the definite and more specific set up of the organization envisaged and the approach to funding. Main difference for the period 2009-2014 is in the international character envisaged.

A budget of € 1,25 m is expected to cover the 2008 cost for preparing the four case studies and the road map including the assessment of Industry support, setting up the network and communication and the Project Office staff running cost.

5. 2. Programme and Organisation 2009-2014

The **Programme Board** will be the highest Authority in the Programme implementation; it will be responsible for the implementation of the Road Map and for the generation of the projects on the four building Blocks mentioned.

Members of the [international] Programme Board will be representatives from Governmental Bodies and Institutions involved in health risk assessment and risk management, Industry and Academia.

As a decision maker the Programme Board will have an independent position even to the context of funding the projects.

A specific international **Government Group** will coordinate and provide resources to implement the programme. This international Government Group is also charged with the task to appoint the members of the Programme Board. Members of the Government Group will be appointed by the participating Ministries from the Netherlands and/or abroad.

The Government Group will also invite a well renowned public figure to act as an ASAT-Ambassador. A Figurehead could help to raise interest in ASAT nationally and internationally, with government, Industry and Academia.

A **Project Office**, lean and mean as defined above, will be tasked with the support of activities of the Programme Board and the Government Group.

They will generate, secure finance, monitor and evaluate the ASAT projects.

They will act on behalf of the Programme Board in executing the Scientific Programme and in the interaction with the research groups and institutions, responsible for carrying out the projects.

They will manage the Network, the workshops and the Communication.

They will -in close consultation with the Programme Board- establish and stop, if necessary Specific Steering Committees on technical matters (science and applied technology).

At this stage the cost of the Programme as a whole is estimated of the order of magnitude as indicated in appendix 1.

5.3. The Scientific Programme

The scientific ASAT programme basically should consist out of four building blocks to be developed in parallel and in conjunction. This approach will lead to one integrated system to assess safety to be used for decision making in any specific situation (chemical and its application) on which risks should be assessed and on which data and information should be generated for such risk assessments. This explains why the scientific programme will probably ask for only a light top down type of co-ordination. It also explains why communication between the different research groups and the individual scientists, working on specific projects, is of paramount importance.

Within each building block, several elements of related scientific questions/ uncertainties will have to be identified that require resolving. Right after, experts' agreement on the exact nature of these questions and on the "knowledge gaps" to be closed, will lead to the definition of specific scientific projects and the research groups to implement them.

Over the four building blocks, problem analysis, project development and project execution will be done in parallel but different 'problem areas' might be in different phases because of:

- The inherent complexity differs
- The right experts have not been identified yet or are not yet available
- Resources are limited.

A step by step approach might be advantageous here:

- It will facilitate different elements to benefit from knowledge and experiences gained in other elements
- Projects will be executed at a higher pace
- The Dutch programme might function as a crystallization point for other EU Member states [and beyond] for the Private Sector and for the European Commission.

In such an approach the Dutch scientific programme will have the potential to expand almost in a natural way into an European or at the longer term even a Global programme.

5.4 Road Map on ASAT

In order to orchestrate the process described above, a Road Map will be developed. This Road Map will facilitate executing the 2009 -2014 programme and should at least include:

- An outline of the main aims and goals of the ASAT program
- A description of the Network to be established in relation to:
 - Regulating Authorities & Governments
 - Science
 - Industry
- A reflection on the programme set up in
 - The generation of projects
 - The financing of projects
 - The process of appraising, monitoring & evaluating of results
 - Picture the National & International Fund raising approaches
 - Define the organisational set up of the Programme
 - Schedule a time path.

5.5. Network

The implementation of the ASAT-Programme will benefit [even depend on] of the possibility to consult and involve the best experts in a wide range of science domains. The added value has already been proven in the international workshops, organised in January and February in The Hague.

Communication has been identified as key for the ASAT Programme.

As a consequence a network should be developed, maintained and managed as an integral part of ASAT.

This network should be primary focussed on:

- Scientists to be involved in Programme implementation
- Scientists interested in ASAT
- Officials involved in risk management
- Government officials responsible for policy on public health and chemical safety matters from food to pesticides and medicines.

It should also be accessible for:

- NGOs with an interest in chemical safety and its assessment
- Politicians.

The Network might function as a Platform where scientists involved in the Scientific Programme can be informed about progress and exchange information. The Platform will contribute to openness and transparency by informing a much larger audience who can ask questions or make suggestions. This will help to identify scientists, able to contribute to the Programme by participating in workshops on specific issues to be discussed or analysed. It will enable Research Groups to identify areas where they can participate and join the Scientific Programme.

The network will create a 'Platform facility' for consultation, debate and confrontation. Input from risk managers, policy makers and NGOs no doubt will be very helpful in giving further shape to the programme by anticipating questions and concerns by other people than those intimately involved in the science. It will contribute to the creation of a virtual ASAT-Community consisting of those directly involved and other stakeholders such as future users and people who represent the interests of the general public or with a mission in public health, safety and animal testing.

The Network with its Platform will facilitate meetings, discussions and other encounters. Occasionally it can even be the starting point for creating and providing technical support for small teams that will be sent out on fact finding missions for specific subjects or to address specific questions.

5.6. Communication

Transparency and openness is a prerequisite for the eventual (public) acceptance of the work that is being carried out, to raise awareness and to generate input into the process.

Communication in ASAT should provide:

- Interaction within the network and its participating scientists
- Information sharing within the network about the programme, its progress, difficulties and deliverables
- Access to information about ASAT, the actual work and deliverables
- Information about discussions meetings and their outcomes in the Platform.

It is foreseen that communication will depend on web based technology. Mail and printed information should be minimised. Information for a wider audience -than the scientists involved in the programme- should be scrutinised for accessibility, factual correctness and appeal. It is proposed to use English as the primary language of communication.

5.7. Funding

Basic idea in establishing the ASAT Programme is the mixed national/international/European character in its origin and its implementation.

As far as the funding for the Programme and -later on- for the ASAT Projects is concerned, this basic philosophy implies the following considerations:

The Netherlands' Government triggered developments in ASAT by enabling initiators to check the feasibility of the Programme in a series of four international Workshops; these workshops clearly have proven the viability of the ASAT Concept but lot's has to be done still to get things on track and this can only be realised with a continued strong support and commitment from this same Government.

Based on this continued support commitment will have to be sought from the European Commission, COST and the EUREKA Programme; COST (in a COST Action) for the establishment of a strong scientific Network on the longer term, EUREKA more specifically to get support for Industry led research projects.

The Programme as well as its projects can only be funded from these European sources when adequate and reliable financial support is secured on a National basis.

At a later stage when setting up the Programme Board and the Government Group mentioned above international support has to be acquired to establish a virtual Common Pot for the funding of international ASAT Projects.

Programme and key note presentations

Format of the workshops

All four workshops have been organised in the same format, starting with 2 or 3 keynote presentations, followed by breakout sessions, in which several aspects of the topic of the workshop were discussed.

Day 1

13.00 – 14.00	Registration and lunch
14.00 – 14.05	Welcome by chairman
14.05 – 14.20	Introduction of ASAT
14.20 – 16.20	Keynote presentations
16.20 – 16.50	Coffee & Tea Break
16.50 – 18.35	Break out session 1
18.35 – 19.30	Drinks
19.30	Dinner

Day 2

08.30 – 09.15	Continuation of break out session 1
09.15 – 10.00	Report to plenary
10.00 – 10.30	Coffee break
10.30 – 11.30	Break out session 2
11.30 – 12.00	Report to plenary
12.00	End of workshop, lunch

Keynote presentations

Workshop 1: Risk assessment and (toxico)kinetics & modeling

Risk based safety assurance; rationale and science

Dr. Julia Fentem, Head of Safety & Environmental Assurance Centre (SEAC), Unilever UK

Prediction of kinetics and biotransformation of chemicals in humans based on exclusively in vitro data: which data are required and how reliable is the prediction?

Dr Amin Rostami, Professor of Clinical Pharmacokinetics and Drug Metabolism, University of Sheffield, UK

Workshop 2: Experimental models

Opportunities of stem cell research and technology for the future of experimental models to predict health risks from chemicals. How close can we get to the human biology behind the risks we want to assess?

Dr Helen Rippon, Research Fellow, Division Investigative Science, Imperial College of London, UK

New cell systems for function analysis of toxic effects

Dr Christine Mummery, ICIN Professor of Developmental Biology, Hubrecht Institute, NL

Workshop 3: Measurement Technologies

Omic technologies in advancing biology: data generation

Dr Jos Kleinjans, Professor and Head of Department of Health Risk Analysis and Toxicology, Faculty of Health, Medicine and Life Sciences, Maastricht University

In vivo modelling of (toxic) effects of chemicals based on in vitro findings. PK/PD from in vitro to in vivo: which data are required for health protection without animal experiments?

Dr Meindert Danhof, Professor and Head of Division of Pharmacology, Leiden-Amsterdam Center for Drug Research, Leiden University

The report from the NAS National Research Council (NRC) *“Applications of Toxicogenomic Technologies to Predictive Toxicology and Risk Assessment”*

Dr John Quackenbush, Professor of Computational Biology and Bioinformatics, Dana-Farber Cancer Institute, Harvard School of Public Health, USA
Member of the committee at the US National Academy of Sciences that prepared the report

Workshop 4: Bioinformatics and Systems Biology

Bio-informatics matchmaker between toxicology and public health protection?

Dr Peter van der Spek, Professor and Head of Bioinformatics Department, Erasmus Medical Center, Rotterdam

Learning biological networks; pathway analysis, identifying biomarkers and their potential for risk assessment of health risk relating to the use of chemicals

Dr Bart Hendriks, Senior Scientist Systems Biology, Pfizer Research Technology Center, USA

Participants

Workshops

Barlow, Susan Independent consultant in toxicology UK	3, 4
Beers, Saskia, Policy Officer Animal Welfare, Department of Knowledge, Ministry of Agriculture, Nature and Food Quality	2
Bessems, Jos Senior project manager, Centre for Substances and Integrated Risk Assessment, National Institute for Public Health and the Environment (RIVM)	2
Biegstraaten-Meeuws, Marianne Programme Secretary, the Netherlands Organisation for Health Research and Development (ZonMw)	3
Brouwer, Bram Director BioDetectionSystems, Director Ecogenomics Consortium	2, 3, 4
Callant Cransveld, Conchita Advisor Innovations Life Sciences & Health, Senter Novem / Ministry of Economic Affairs	3, 4
Cook, Alison Programme Manager 3Rs and Regulatory Use of Animals, NC3Rs, UK	1
Crommelin, Daan Scientific Director, Top Institute Pharma	3, 4
Danhof, Meindert Professor and Head of Division Pharmacology, Leiden-Amsterdam Center for Drug Research, Leiden University	3
Delft, Joost van Associate Professor Genetic Toxicology and Toxicogenomics, Department of Health Risk Analyses and Toxicology, Faculty of Health, Medicine and Life Sciences, Maastricht University	4
Driel, Roel van Professor of Biochemistry, University of Amsterdam, Founding director Netherlands Institute for Systems Biology (NISB)	4
Fentem, Julia Head of Safety & Environmental Assurance Centre (SEAC), Unilever UK	All
Greim, Helmut Director of the Institute of Toxicology and Environmental Hygiene, Technical University Munich, Germany	1, 2
Groothuis, Geny Professor Pharmacokinetics and Drug Delivery, Department of Pharmacy, University of Groningen	1
Groten, John Executive Director Toxicology & Drug Disposition, NV Organon, a part of Schering Plough Corporation	1
Hakkert, Betty Head of REACH Bureau, Expert Centre for Substances, National Institute for Public Health and the Environment (RIVM)	All

Hartung, Thomas Head of European Centre for the Validation of Alternative Methods (ECVAM)	1, 2
Hendriks, Bart Senior Scientist Systems Biology, Pfizer Research Technology Center, USA.	4
Hensing, Janna Ministry of Education, Culture and Science	4
Hof, Paulion Van Department for Research and Science Policy, Ministry of Education, Culture and Science	4
Keijer, Jaap Head of Food Bioactives, Institute of Food Safety (RIKILT)	1, 2
Kleinjans, Jos Professor and Head of Department of Health Risk Analysis and Toxicology, Faculty of Health, Medicine and Life Sciences, Maastricht University	2, 3, 4
Koëter, Herman Deputy Executive Director and Director of Science, European Food Safety Authority (EFSA)	1, 2
Loizou, George Head of Computational Toxicology Team, Mathematical Sciences Unit, Health Improvement Group, Health and Safety Lab, UK	1
Loveren, Henk van Head of Section Immunotoxicology and Infection, National Institute for Public Health and the Environment (RIVM), Professor Immunotoxicology, University of Maastricht	All
Mullenders, Leon Professor and head of Department of Toxicogenetics, Leiden University Medical Centre	3
Mummery, Christine ICIN Professor of Developmental Biology, Hubrecht Institute	2
Peijnenburg, Ad Leader of Toxicology and Effect monitoring Group, Institute of Food Safety (RIKILT), Wageningen University	3, 4
Piersma, Aldert Professor of Reproductive and Developmental Toxicology Laboratory for Health Protection Research, National Institute for Public Health and the Environment (RIVM)	3, 4
Quackenbush, John Professor of Computational Biology and Bioinformatics, Dana-Farber Cancer Institute, Harvard School of Public Health, USA	3, 4
Rippon, Hellen Research Fellow, Division Investigative Science, Imperial College of London, UK	2
Roggen, Erwin Manager Pharma Protein Development, R&D Molecular Biotechnology, NovoZymes	3, 4
Rostami, Amin Professor of Clinical Pharmacokinetics and Drug Metabolism, University of Sheffield, UK	1
Sandt, Han van de Manager Research Chemical Safety, TNO Quality of Life	3

Schoonen, Willem Group Leader In Vitro Toxicology and Toxicogenomics, Section DMPK & Safety, Department Pharmacology, NV Organon, a part of Schering Plough Corporation	2
Schothorst, Evert van Researcher/Projectleader Transcriptomics and related Bioinformatics, Institute of Food Safety (RIKILT), Wageningen University	3, 4
Spek, Wouter Director EuroBioFund, European Science Foundation	4
Spek, Peter van der Professor and Head of Bioinformatics Department, Erasmus Medical Center	4
Stegg, Harry van Laboratory of Health Effects Research, National Institute of Public Health and the Environment (RIVM)	3
Stierum, Rob Head Toxicogenomics, Business unit Biosciences, TNO Quality of Life	4
Tritscher, Angelika Joint Secretary to JECFA and JMPR, International Program on chemical safety, WHO	1
Vermeulen, Nico Professor Molecular Toxicology, Department of Chemistry and Pharmacology, Vrije Universiteit of Amsterdam	1
Water, Bob van de Professor Drug Safety Sciences, Centre for Drug Research, Leiden University	2, 3
Woutersen, Ruud Manager, Toxicology and Applied Pharmacology Department, TNO	4
Xu, Jim Head of Predictive Toxicology, Systems Biology Group, Pfizer Research Technology Centre, USA	1, 2
Organisation	
Alting von Geusau, Marie José Project Manager ASAT, Ministry of Health, Welfare and Sports	All
Bergen, Pol van den Trencavel Technology Management	All
Knaap, Ada Toxicologist, formerly senior scientific advisor at the National Institute for Public Health and the Environment (RIVM)	All
Mulder, Gerard Emeritus Professor Toxicology at the University of Leiden	All
Roos, Jacqueline Liaison officer, Directorate International Affairs, Ministry of Health, Welfare and Sports	All
Sangster, Bart Retired medical doctor and toxicologist	All
Slot, Marjanne Project Manager ASAT-Initiative	All
Thole, Esther Science Writer	All

Appendix 1

ASAT

Assuring Safety without Animal Testing

Working towards

**Sustainable Protection of Human Health and the
Environment**

ASAT Programme - summary

Objective	Sustainable and transparent protection of human health and the environment; quicker, cheaper and without animal testing.
Means	1) Protection on the basis of clearly-defined health risks, 2) Transparent, improved risk assessment methods, 3) On the basis of data from experimental chemical safety models, based on the new technological possibilities offered by the application of genomics technologies, advanced information technology and systems biology.
Organisation	An independent, cooperative, accountable team of three with a background in business, health and/or environmental regulations and science. This team of three is operated by and accountable to the government, and is supported by an executive office.
Task	1. Formulating research targets, forming the various research groups and (commissioning) the realisation of large scale research programmes. 2. Monitoring the progress of and the cohesion between the various aspects of the programme. 3. Facilitating a European network from the Netherlands by linking up the various national networks starting with the UK, Germany and Belgium. 4. Involving the European Commission in the initiative, so that it will eventually assume (co-)leadership.
Period	A total of 7 years (2007 through 2014), consisting of 1 launch year + 6 years.
Budget	A total of € 110 million:
NL	€ 65 million (launch year 5M, 6 years x 10M) The take-off year is intended to set up the organisation, start up research projects and organise the field; to get all the involved parties 'pulling in the same direction'; the next step will be the realisation of large-scale, coherent projects with a duration of 2-4 years.
EU:	€ 15 million (6 x 2.5M) The objective is to gain access to foreign research groups with knowledge or expertise essential to the development of ASAT. As a spin off, the involvement of these groups in the work will facilitate the participation of foreign governments.
Global:	€ 17.5 million (7 x 2.5M) An active think-tank and facility for the generation and exchange of ideas will help attract the most creative and innovative minds to take part in the development process. This will increase the chances of success and speed up both the process and international acceptance.
Contingencies:	€ 12.5 million

Introduction

Dutch citizens expect optimal protection of their health and the environment. This relates to safe foods, safe and clean drinking water and clean air to breathe. It also includes safe products, varying from personal care items to medicines or DIY products.

Determining whether something is safe takes a great deal of time and money. This applies to both the formulation of standards and the testing of products. Testing programmes take several years, after which the data they yield must be processed and interpreted. Only then can the experts draw solid conclusions. The current approach to safety was developed during the nineteen fifties. This approach is based on assessing the extent to which a substance is hazardous. Animal testing is an integral part of the process. This practice is internationally accepted by the responsible authorities. Nevertheless, there is a significant degree of dissatisfaction with the current approach. In addition to resistance against animal testing, the approach has not always proved to be optimal. New, recently introduced technologies now allow for other, adequate and more effective risk assessment methods with far less need for animal testing. The ASAT-Initiative offers this perspective.

Rational ASAT

ASAT stands for a new approach to safety (of food, medicines, cosmetics, chemical substances) aimed at realising sustainable and transparent protection of human health and the environment. The starting point is an acceptable level of risk to human health or the environment; these risks are identified in advance and subsequently assessed by means of newly developed, transparent and reliable risk assessment methods. These risk assessment methods allow for the risks to be assessed in terms of their nature, scale and frequency. The integrated application of genomics technologies, advanced information technology and systems biology yields new experimental models that can be used to generate data for these new risk assessment methods.

Good risk assessment methods increase the sustainability of society and the economy: new products with added value can be assessed more effectively and efficiently in terms of the risks they pose to human health, the natural environment and the living environment of the general public. ASAT thus contributes to a (more) sustainable allocation of private and public sector R&D funds. This will engender a high level of societal acceptance, in combination with the gradual reduction and eventual phasing out of animal safety testing due to ASAT.

Why now

The ASAT-Initiative is both possible and likely to succeed thanks to a number of simultaneous developments:

- Over the past few years, the realisation has set in that the traditional method of protecting human health and the environment is in need of change. Hazard assessment alone will no longer suffice. Products and technologies must be assessed on the basis of their quantified risk to human health and the environment. Only then can measures designed to reduce risks to an acceptable level be assessed in terms of their effectiveness.
- The exploration of the Human Genome and the genomics technologies that have made this possible have developed in leaps and bounds. These genomics technologies are now being applied on a large scale in various areas, including the development of new medicines and concepts for prevention, diagnostics and food production. This has yielded entirely new, novel insights and knowledge. The recent boom in bioinformatics is generating new insights and potentials for risk assessment. As a result, the ASAT-Initiative can now be realised, a development that would have been impossible five years ago.

Urgency

The scientific world and industry have both expressed a great deal of interest in the ASAT-Initiative. The same applies to the Dutch government. This resulted in a letter from the Minister of Health, Welfare and Sport, on behalf of the Ministers of Agriculture, Nature Management and Fisheries, Housing, Spatial Planning and that of the Environment and Education, Culture and Science to the Lower House of Dutch Parliament in December of 2005, endorsing the potential of the ASAT-Initiative.

The time is right: in a few years time, the 7th Amendment to the EU Cosmetics Guideline will block any innovation in the European cosmetics industry (including personal care products such as shampoo and toothpaste) involving animal testing. The procedures for safety testing on medicines are in need of improvement. The European Policy on Chemicals (REACH) is expected to result in a vast increase in the use of animal testing. Application of the same genomics technologies that are to make ASAT possible will make today's models for safety assessments based on animal testing look increasingly questionable. Increasingly, society regards animal testing as undesirable and unacceptable.

An opportunity for the Netherlands

The ASAT-Initiative has a European/international scope. After all, risk assessment does not stop at the Netherlands' borders. The Netherlands is in an excellent position to play a leading role in this area within Europe. The knowledge and scientific disciplines required for ASAT are well-organised within our universities and research institutions. In one example, the Netherlands' Toxicogenomics Centre, NTC, oversees collaboration between all the relevant academic groups and research institutions. The Netherlands is also well-represented in the numerous European bodies charged with the implementation of European legislation.

The FDA and EPA are already working with the business sector to improve risk assessment in the US. If Europe lags behind, we will find ourselves in the undesirable situation of having diverging acceptance policies. This would be bad for both citizens and international business: businesses would then be forced to invest even more in the acceptance of products, while consumers would have to foot the bill in the form of more expensive products and delayed market introduction.

Here lies an opportunity and vital interest for the Netherlands. There are many companies in our country that would benefit greatly from more effective risk assessment methods. A leading and pioneering role in this area will undoubtedly result in new industrial activity. This activity will centre around the development and manufacturing of tests and testing methods, the development and management of databases, the testing of substances and the conducting of risk assessments for the business sector or government bodies.

Collaboration between government & commercial sector

The development and implementation of new methods in the area of risk assessment are one of the government's primary responsibilities. After all, the government determines the legal frameworks for the acceptance of new products. Nevertheless, good cooperation with the commercial sector is vital in developing and implementing new methods: new legislation directly affects a company's market position. This is especially relevant when it comes to products with added societal value, such as food products that prevent the development of diabetes.

Broad political support

Broad political support will be essential in ensuring successful realisation of the ASAT-Initiative and its subsequent spin-off effects on our economy. ASAT is based around cooperation between people from a broad array of disciplines: broad political support will make it possible and easier to actively bring together these disciplines – not used to working together – and stimulate them to work towards a common goal. If these groups commit to the initiative and dedicate their efforts to the government's objective rather than their own individual interests, a great deal can be achieved. This approach will also further realisation of the ASAT-Initiative's essential international dimension.

Broad political support for the ASAT programme will also stimulate alignment of the project's realisation. It will prevent any unnecessary discussions with regard to competencies. This will make it all the more visible that the initiative transcends individual policy areas. After all, the project affects public health and the environment, and has direct consequences in the area of work and health: the policy remit of the Ministries of Transport, Public Works and Water Management, Agriculture, Nature Management and Fisheries, Housing, Spatial Planning and the Environment and Social Affairs and Employment. The issues affect the scientific community and the position of Dutch universities and knowledge institutions; the Ministry of Education, Culture and Science. Finally, ASAT affects the competitive position of Dutch business and new commercial activities: the Ministry of Economic Affairs.

The ASAT Programme

The ASAT programme approaches multiple issues on a simultaneous and coherent basis. Multidisciplinary research groups, consisting of academics, regulators and commercial researchers will be set up in order to identify new models and tests that can yield the data needed to assess the predefined health risks.

The usual bottom-up approach to scientific programmes is not suited to this Programme. If the initiative is to succeed, a result-oriented approach will be required whereby top down groups and institutions are convinced of the advantages of teamwork. Within this context, it is vital to allow as much freedom as possible for scientific creativity. This is why a decision was made to assign executive responsibility to a team of three with a background in the commercial sector, health and/or environmental regulations and science. This team of three will be accountable to the government. This activity will require a significant investment in terms of time by the members of the team. The team of three is charged with active process leadership and is responsible for bringing together the various groups. Coordination and alignment on the basis of a single common approach is essential. The groups are responsible for the joint realisation of various projects that serve as building blocks for the new approach to environmental and human health protection. The team of three will be supported by a small-scale office.

The size of the budget for activities in the Netherlands is based on the estimated maximum absorption capacity of the Dutch university groups, knowledge institutions and various services involved in assessing risks to human health and the environment. The business sector will participate by providing capacity; the companies involved are responsible for financing this capacity. As is the case for other groups, they are responsible for the scientific quality of the projects. They will benefit equally from the insights gained through Programme participation. In view of the ASAT-Initiative's important role in developing new and improved regulations on market access for new products, it is extremely important that policy staff at the various relevant national ministries and the European Commission participate in the ASAT programme. This will ensure that the results of the ASAT programme can be translated into new policies and legislation as soon as possible.

In addition to activities and budgets for research in the Netherlands, funds will also be needed in order to involve – initially European – research groups in the initiative. The objective here is twofold: 1. Gaining access to expertise and technology developed elsewhere. Synergy could be achieved between Dutch expertise in the area of observational technology, Toxicogenomics, and experience in the UK with regards to the development of experimental biological models. 2. Involving foreign groups in the ASAT Programme will generate attention and possibly even pressure on foreign governments to align with the Dutch approach and network.

One aspect of the Programme is the realisation of collaborations with – initially – the UK, Germany and Belgium in an effort to set up a programme and network of activities that supplement the Dutch programme, working on the basis of the Dutch approach. Ideally, the combined network should be under joint management. This would serve as a stepping stone towards a European programme managed (as is the case in the Netherlands) on behalf of the Committee on a top-down basis, with a clearly defined objective. As is the case in the Dutch Programme, the business sector can participate at both national and European level by providing capacity.

Finally, a think-tank will be set up. This think-tank will allow experts from within and outside the EU to voice critical and constructive views on scientific aspects, solutions to technical problems, the interpretation of data and the implementation of results in policy and legislation. A think-tank creates an environment that invites the analysis, debate and discussion of complex scientific problems. Specific issues can be addressed by bringing together the best scientists in an environment that stimulates the development of creative and original solutions. New developments in the world of science and technology can then be assessed in an early stage in terms of their potential for application in the realisation of the Programme and implementation of the results in policy and legislation.

Appendix 2

Starting ASAT in the Netherlands

Ada Knaap, Gerard Mulder and Bart Sangster

and
Marjanne Slot

The Hague, 22 August 2007

Introduction

The Assuring Safety without Animal Testing (ASAT) programme, subtitled “Towards Sustainable Protection of Health and the Environment” is a proposal towards a work plan that was presented to the Ministries of Health, Welfare and Sport, Agriculture, Nature Management and Fisheries, Housing, Spatial Planning and the Environment and Education, Culture and Science on 4 January. The objective is to realise: “Sustainable and transparent protection of human health and the environment; quicker, cheaper and without animal testing”. It is the outcome of a process that started in 2004 with a memorandum from ZonMw (Netherlands Organisation for Health Research and Development) to the Minister of Health, Welfare and Sport. This was followed up by a recommendation from the KNAW (Royal Netherlands Academy of Arts and Sciences) and ZonMw, based on an invitational conference of experts aimed at assessing the scientific feasibility of ASAT. The Minister of Health, Welfare and Sport informed the Lower House of Netherlands’ Parliament of the outcome on 20 December 2005. The January 2007 proposal was the result of consultations with staff from the Ministries involved during the second half of 2006.

At the behest of the Ministry of Health, Welfare and Sport, three persons have been made available for a ‘preparatory Quadrant’ to facilitate ASAT. The Quadrant will be complemented with a ‘government representative’ invited by the Ministry of Health, Welfare and Sport.

The past few weeks saw meetings by the preparatory team held on 13 and 21 August. The following issues were discussed:

- Contents, form and organisational structure of a future ASAT Programme in the Netherlands.
- The potential added value of Quadrant playing a central role in such a programme.
- The current and future relationship with the Ministry of Health, Welfare and Sport in its capacity as the Ministry responsible for Animal Testing Policy and the other Ministries in view of their responsibility for a broad range of issues relating to ASAT such as health and environmental protection, scientific development and economic activity.

This document provides a summary of the preparatory team’s initial conclusions and insights as regards the internal debate on ASAT at the Ministry of Health, Welfare and Sport and the ongoing inter-ministerial consultations. On 16 August of this year, the Ministry of Health, Welfare and Sport commissioned the formulation of a concrete programme by 1 March 2008, in order to answer the following questions:

- Which groups in the Netherlands:
 - have sufficient expertise and experience in order to generate building blocks for ASAT?
 - would be prepared to generate the required capacity if funding were made available?
 - would be prepared to participate in a scientific network that facilitates coordination and alignment, aimed at the realisation of ASAT?
- Which foreign groups are suitable for participation in the ASAT network?
- Which expectations and minimal criteria are applied by risk assessors and risk managers in the Netherlands, the UK, Germany, Belgium and at EU-level with regards to Risk Based Safety Assurance?

A. Brief outline of the preparatory team’s background.

See appendix 1 for a brief summary of the preparatory team.

The preparatory team’s availability is determined to a large extent by the political and administrative support for this process, and the resulting perspective on an operational ASAT Programme for the Netherlands. The nature and scale of operational support are also relevant in this regard.

B1a. ASAT objectives

- Protection of human health
- Protection of the 'animal environment'
- Without the use of animal testing
- Via risk based safety assessment (from hazard to risk)
- Through an approach that can be applied to all chemicals (food, pesticides, medicine, etc.)
- Based on risk underpinned science (as opposed to test driven science)

B1b. ASAT starting points⁴

- The process involves collaboration between various scientific institutes:
 - Top down management on the basis of a common objective.
 - Institutions retain their individual responsibility.
 - Scientists are stimulated to exercise their academic creativity.
- Optimal use of existing knowledge and technology at:
 - Dutch research institutions and universities.
 - Pharmaceutical companies and umbrella organisations.
 - Companies producing consumer products for personal care (cosmetics), home care and food, and their umbrella organisations.
 - Chemical companies and their umbrella organisations.
- Optimal cooperation in order to prevent duplication.
- All deliverables of the programme such as new tests, methods and risk assessment procedures will only become relevant and applicable after validation. Validation is thus an integral part of the programme.
- A convincing case must be made for regulators, policy makers and society in order to achieve acceptance and application.

B2. Further detailing of ASAT

For each of the substances to be introduced for a specific use, (from medicines to cleaning agents) data on (concentration-dependant) potential toxic effects ("hazards") will be collected in a quick and relatively inexpensive manner by means of automated high throughput in vitro screening ("hazard profile"). This process also takes into account the substance's potential metabolites through the use of adequate testing systems.

Based on the expected application(s) (pesticide, colouring agent, etc) an estimate will be made of the exposure (via inhalation, skin or swallowing).

Biotransformation and (toxico)kinetic data on the substance will be generated on the basis of the estimated external exposure using – amongst other methods - PB/PK models. This will generate the internal dose to the compound and its metabolites.

Working on this basis, the compound and its metabolites will then be tested in risk based model/technology combinations (yet to be developed tests). The outcome of these tests (data) will form the input for the assessment of the identified general risks in terms of general health protection. Additional data from other model/technology combinations may be necessary to assess the risks associated with specific applications such as medicines or pesticides or on the basis of structure alerts (QSAR) or hazard data.

This will allow for the identification of potential health risks related to application of the compound.

These health risks, their nature, scale, probability and degree of (un)certainty will allow the risk manager to make a decision (also taking into account other relevant considerations) on the approval of and limitations to application.

⁴ ASAT includes the protection of human health as well as that of domestic and agricultural animals and the biotic environment. This report is limited to the protection of human health, in view of the limited time that was available for preparation.

C. Proposed ASAT organisational structure

Organisation An independent, cooperative Quadrant that takes responsibility for results and has its roots in business, health and/or environmental regulations, science and the Dutch government. This Quadrant is operated by the government to which it is held accountable.

The Quadrant is supported by an executive office.

Task 1. Formulating research targets, forming the various research groups and (commissioning) the realisation of large scale research programmes.

2. Monitoring the progress of and the cohesion between the various aspects of the programme.

3. Facilitating a European network from the Netherlands by linking up the various national networks starting with the UK, Germany and Belgium.

4. Involving the European Commission in the initiative, so that it will eventually assume (co) leadership.

Period A total of 7 years (2007 through 2014), consisting of 1 launch year + 6 years.

D. Programme for preparatory phase until March 2008 and budget⁵.

Inventory

The preparatory team has considered the knowledge domains necessary in order to realise ASAT. Examples of Dutch institutions with expertise and experience have been listed for each domain. This list is in no way limitative, but does serve an illustrative purpose for this phase.

The proposal is to invite experts from four domain clusters. The experts from each cluster will be supplemented by experts from other domains. A limited number of foreign experts⁶ with crucial expertise will also be invited in order to gain optimal insight of the various possibilities. The common language will be English. A proposal has been made to the effect that participants should receive a fee in order to increase their commitment.

Information will be sent to the participants prior to the meeting. After a brief introduction by the preparatory team, one or more keynote lectures will be held focusing on the application of innovative technology or approach to risk assessment. The experts will then be invited to respond to the concept and share their knowledge on ASAT's current and future scientific/technological possibilities. Whilst these workshops are intended as brainstorming sessions on the one hand, they will also serve as a basis for decision-making as to whether and how this area is to be fleshed out in ASAT: the preparatory Q will have to make decisions on the basis of these workshops.

In order to benefit optimally from these experts and entice them to make time in their schedules, each of the four meetings will run from 14:00 on the first day to 12:00 the next day. This will allow for (informal) contact in the evenings and time for reflection after dinner. In view of the ambition to involve some of these experts in more than one meeting, the possibility of organising the meetings in tandem is currently being considered.

The following four cluster meetings are currently under consideration:

1. Risk Assessment, Exposure Models and (Toxico-)Kinetic & Biotransformation

⁵ This is part of the ASAT programme's launch year as proposed on 4 January 2007. The lack of a broader perspective could affect the commitment of discussion partners and groups interested in participating. Due to the fact that the think-tank has not yet been made operational, there is no input from leading international scientists from the relevant sub-disciplines on the one hand or regulators from the EU Members States, EU and other regulatory blocks such as the US, Japan and China. Interaction with NGOs and other stakeholders depends on further development of the communication with regard to ASAT.

⁶ In order to attract foreign experts with the appropriate qualifications, it will be necessary in certain cases to pay a full fee in addition to travel and accommodation costs.

2. Bioinformatics and Systems Biology
3. Experimental Models: cells in vitro
4. Measurement Technology: how can effects be measured in vitro

A non-limitative overview of institutions offering relevant expertise:

Risk Assessment

IRAS (Utrecht University), RIVM, RIKILT, VWA, TNO

Exposure Models

RIVM, RIKILT

(Toxico-)Kinetic & Biotransformation

LACDR (Leiden University), Groningen University Pharmacology Department

Bioinformatics

Via NGI who have bundled various institutions' expertise in this area.

Systems biology

Via NGI which has bundled various institutions' expertise in this area.

Experimental Models

These models can be based on cell lines, tissue culture, biopsy material and material from slaughterhouses

NKI, Amsterdam and Cell Biology research units at various universities

Measurement Technology

This includes 'omics', cytological profiling, biomarkers and QSAR

NTC/NGI for 'omics' and various institutions and (technology) universities.

And experts from the various domains employed by companies such as AKZO-Nobel, DSM, Organon, Unilever.

Brokerage

Based on the findings from the four meetings, the preparatory team will draw up a proposal towards the establishment of a network in the Netherlands in order to realise ASAT. In all likelihood, a number of foreign research groups will be included. Recommendations will also be made with regard to the working methods adopted within the network.

The preparatory team is aware that this does not address all elements in the request of 16 August. An initial elaboration of the second and third point in that request found that this would result in:

- Complex negotiations with potential partners, due to ambiguities as to which area the partner in question was to research.
- Resulting in limited cooperation from institutions due to uncertainty as to the perspective from which they are to offer capacity in order to provide the preparatory team with information.
- A disproportionate strain on the preparatory team.
- Risks with regard to quality⁷ due to the lack of structured professional office support.
- Legal and political risks due to the interests of the potential participants in the network.

⁷ In addition to the amount of work, this also relates to issues such as minutes of discussions and monitoring of the quality of the collection and archiving of data. The ex ante recording of procedures and evaluation criteria is also a part of this process, and contributes to the level of transparency. This is important with a view to subsequent auditing by the Court of Auditors and any legal proceedings in the unlikely event that an institution is not satisfied with the outcome of the process.

Appendix 3.

Chronology

2004

28 July; Letter by ZonMw to Minister VWS; 'Notice on alternatives to animal testing'; 2004/16424/ZONMW

The Feasibility of Replacing Animal Testing for Assessing Consumer Safety: A Suggested Future Direction, Julia Fentem, Mark Chamberlain and Bart Sangster; ATLA 32, 617–623, 2004

2005

3 October Invitational Conference hosted by ZonMw and KNAW on feasibility of ASAT at the request of Ministers of VWS, OCW and LNV

2 November; Letter by ZonMw en KNAW to Minister VWS about invitational conference; 2005/21254/ZONMW

7 November; Brussels; 'Europe goes alternative. Conference Organised by EU Commissioners for Enterprise and Research, MEPs and Industry

http://ec.europa.eu/enterprise/events/animal_tests/index_en.htm

Start of EPAA; European Partnership for Alternative Approaches to Animal Testing

<http://ec.europa.eu/enterprise/epaa/brochure.htm>

November 8-10 Brussels; Toxicology Forum European meeting session III
Safety Without Animal Testing: Reality or Fantasy?

http://www.toxforum.org/ToxForumContent/Templates/ToxForumSub.aspx?NRMODE=Published&NRNODEGUID=%7b86fb30a8-3b56-4200-8508-7b6374e27908%7d&NRORIGINALURL=%2fContent%2fArchived%2bMeetings%2fEuropean_Meetings%2f2005_European_Meetings%2f&NRCACHEHINT=NoModifyGuest#Session3

20 December; Letter from Minister of VWS to Parliament

<http://www.minvws.nl/kamerstukken/vgp/2005/dierproeven.asp>,
http://www.minvws.nl/images/dierproeven_tcm19-114535.pdf p. 8/9

2006

21 June, Vught; Toxicogenomics Workshop, organised by the Netherlands Genomics Initiative

9 November: Genomics Momentum, organised by the Netherlands Genomics Initiative

<http://www.genomicsmomentum2006.org/GM2006/Programme/Workshop%202.aspx>

2007

4 January; ASAT; Naar een Duurzame Bescherming van Gezondheid en Milieu; Appendix 1

April, Business Plan Netherlands Genomics Initiative 2008-2012

www.genomics.nl/GetFile.aspx?aliaspath=%2Fresources%2FPublications%2FCorporate%2FNGI_SP_NL_pdf

12 June; Letter by Minister of OCW

<http://ikregeer.nl/static/pdf/KST107956.pdf>

19 June; Global Environment Facility Proposal for Global ASAT-Initiative

22 augustus; "Start ASAT in Nederland". Rapportage van de kwartiermakers Quadrant. Appendix 2

December 5-7 Lisbon: EuroBioForum, organised by Euro Bio Fund of the European Science Foundation

<http://www.esf.org/activities/eurobiofund/eurobioforum-2007/asat-a-future-with-reduced-animal-testing.html>

Appendix 4

Ada Knaap

Until recently, Ada Knaap was senior scientific advisor at the Centre for Substances and Integrated Risk Assessment of the RIVM (Dutch National Institute for Public Health and the Environment). She studied biology at Leiden University. She is a toxicologist, specialised in genetic toxicology, carcinogenesis, exposure and risk assessment.

Her current field of expertise comprises toxicological evaluations and risk assessments of chemicals in food, consumer products and the environment. She is a member of various national and international Advisory and Expert Committees including EU, WHO and OECD and is vice-chairman is vice-chairman of the EFSA scientific committee.

Gerard Mulder

Gerard Mulder has been professor *emeritus* in Toxicology at Leiden University since 2006. Having completed his studies in Biochemistry and obtained a doctorate in Biochemical Toxicology, he worked at Groningen University until 1984. In 1984, he became a professor in Toxicology at the Leiden University Leiden/Amsterdam Centre for Drug Research (LACDR). As a post-doc and later visiting scientist, he had tenures at the National Institutes of Health (NIH) in Bethesda, MD and the National Centre for Toxicological Research (NCTR) at the FDA in Jefferson, Arizona (USA). He was dean at the faculty of Mathematics and Natural Sciences at Leiden University (1993 and 1994) and scientific director of the LACDR from 2001 to 2005. He has over 300 publications to his name. His current positions are:

- Chairman of the Health and Occupational Exposure to Substances Committee (formerly WGD) of the Health Council, which formulates exposure limits for the working environment (since 1999)
- Member of the Health Council (since 1997)
- Member of the Committee for the Safety of Medicines (since 1997)
- Chairman of the Novel Foods Safety Committee (since 2006)
- Chairman of the Central Animal Testing Committee (CCD; since 1998)
- Member of the Supervisory Committee of the RIVM (Bilthoven; since 2000)

Bart Sangster

Dr Bart Sangster is a physician, specialised in Internal Medicine and Toxicology. Up until his retirement in June of 2007, he was Senior Vice President Safety and Environmental Assurance at Unilever.

Amongst other positions, he was Professor of Clinical Toxicology at Utrecht University, head of the RIVM's National Poison Control Centre and head of Intensive Care (reanimation and toxicology department) at the Academic Hospital in Utrecht. Before becoming responsible for the safety of consumers, employees and the environment at Unilever, he was Director of Public Health at RIVM and later Director General for Health at the Ministry for health, Welfare and Sport.

He is the author of over 150 publications in scientific and medical periodicals and books.

- Member of the EFSA Management Board
- Member of the Board at InnovatieNetwerk
- Member of the Strategic Focus Group at VWA
- Member of the advisory board at LOI

Marjanne Slot

Project Manager

After obtaining a law degree from Leiden University, Marjanne Slot started working as coordinator of projects and organiser of events at the crossroads of science, technology and industry. She joined forces with communication agency Publismarket in organising innovative events for clients such as the Ministry of Agriculture, Nature and Food Quality, NOVEM and the Netherlands Genomics Initiative (NGI). For NGI she has been involved in many different projects in the Life Sciences, e.g. the start up of the Netherlands Toxicogenomics Center, and the organisation of an international workshop on genomics & alternatives to animal testing. Currently she is working on preparing the ASAT-Initiative.

Pol van den Bergen

Mr. Pol van den Bergen educated as a lawyer , is specialized in the conversion of knowledge into business. It is his main task as a partner in the Trencavel Technology Management consultancy company.

After an engagement of several years at the Ministry of Economic Affairs in industry policy making, he was one of the founders of the SENTER Organization in The Hague, later on he was the Director International Affairs of that same organization.

He chaired the National Coordinators during the Dutch Chairmanships in the EUREKA Program in 1991-1992 and 2004-2005.

From 1995-2000 as a member of the Corps Diplomatique, he served as the Secretary General of the EUREKA Program in Brussels.

From 2000-2004 he headed the Government Foundation "Dreamstart", tasked to advise the Minister of Economic Affairs on a new policy to generate and promote high tech start ups in the Netherlands .

Since 2000 he is a Member of the Board of Commissioners of Parnassia/Bavo Group, Mental Hospitals and Clinics in The Hague, The Netherlands.