SNELLER VAN INNOVATIE NAAR MENS

About SLiM

In February 2011 the Ministry of Economic Affairs and both the province and municipality of Utrecht gave a 'Pieken in de Delta' grant to the 'Taskforce Innovation Region Utrecht' (TFI) and its partners, including University of Applied Sciences Utrecht, TNO, University Utrecht, Danone and GlaxoSmithKline. RIVM and NKCA were also project partners, financed by the Ministry of Public Health, Welfare and Sport.

The project SLiM (Sneller van Innovatie naar Mens) aims to realise 'a smarter way from innovation to humans' by stimulating and accelerating the development and acceptance of alternative methods. Together with researchers from research institutes, industry, government and representatives of national and European regulatory authorities, four areas of research were identified as focal points for SLiM: carcinogenicity, allergenicity, reproductive and developmental toxicity, and barrier functions. Apart from the development of methods that will Replace, Reduce and Refine (3Rs) animal experimentation, the activities within SLiM also included the dissemination of good practices, education and the promotion of regulatory implementation.

Final Symposium

"Humans are very conservative beings. Because animal testing is what we have used already for a very long time, we feel a misplaced sense of security when using animal experiments," according to Herman Koëter, Orange House Partnership. Therefore, it will take time and effort to change the attitude of humans towards animal testing. Koëter believes it's time for a different approach. And that was exactly the aim of project SLIM: find a smarter way from innovation to humans by stimulating and accelerating the development and acceptance of alternative methods. On 16 January 2014 the final event of the 'Pieken in de delta project SLIM' took place in Utrecht.

In this final meeting, SLiM, in cooperation with the Netherlands Knowledge Centre on Alternatives to animal use (NKCA), gave an overview of the results obtained and shared the lessons learned on accelerated acceptance of Replacement, Refinement and Reduction (3R) of animals in research with all stakeholders: industry, research institutes, regulatory bodies, legislation authorities and policy makers.

Cyrille Krul kicked off with a short overview of the SLiM project and the conclusions regarding the factors, drivers and barriers that were identified with respect to regulatory acceptance and the use of 3R methods. Furthermore, she presented some actions that could help to align the development process and the lessons learned from the SLiM project, whose results were recently published.

The meeting was chaired by Herman Koëter from Orange House partnerships and by Jan Raaijmakers from GlaxoSmithKline (GSK).

Noble goal

Referring to the goal of SLIM as being to define 'good practices' for the smarter and faster development and acceptance of novel testing approaches for regulatory assessment of the safety or risk of compounds, Herman Koëter concluded that this is a noble goal. All involved – scientists, industry, the legislature, regulatory bodies – should want this. Scientists should want this because it is synonym with more focus on science, innovations and human relevance. Regulators should want this because of the immense workload caused by the amount of chemical substances that must be (re)assessed for safe use in the EU (>30,000) and the time it takes to build a dossier of each compound (2-3 years) and do the evaluation (approx 2 years). And we should all want this because we feel insecure; have we possibly overlooked something or missed something important?

Without really having a full understanding, we keep digging. If we had stopped digging, a new DES or thalidomide disaster might have happened, but it hasn't, so aren't we doing a good job? The industry should support this goal because it take considerable effort to build a dossier. There is freedom to operate, as with the new US FDA Act of January 2011, but data according to the latest scientific insights are required.

But to encourage people to seize the opportunity , rewards for succeeding should be in place, such as the incentives in the food industry to provide quality food to consumers in very low-income countries, the incorporation of good nutrients and avoidance of bad nutrients. Accessibility (affordability & availability) to the Access to Nutrition Index (ATN) based on access to medicine index (ATM). And what about an index for proving safety without animal testing? Is that not another option? According to the SLiM project, it is a real option.

The Science of SLiM

RBL assay
Raymond Pieters of HU and IRAS and Karen Knipping of Nutricia Research BV presented the humanised *in vitro* allergy model. This assay, to test hydrolysates, was successfully validated in three different centres and it clearly gives better results than animal experiments. The animal model (guinea pigs) currently used is neither reliable or predictive. The mouse model may be more useful but why not skip animal models altogether? The RBL assay is both time and cost efficient.

The RBL assay focuses on allergic challenge reaction, NOT on the sensitisation of allergic reaction. However, hydrolysates are meant for children that already suffer from allergy. In addition, the RBL assay can be used both in QA (production chain) and R&D as well as for other allergens (new food products). The assay may have high commercial value. And it is also good for the branding: no animals used for baby food.

It is clear convincing scientific story: a better predictive *in vitro* model. It has improved predictive value with respect to humans. There is internal acceptance by major industrial players (e.g. Nutricia, Friesland Campina) although the current developers of the assay need to help convince other end users and regulators to change the existing guidelines. Only then can all the criteria be met to get new RBL assay fully implemented.

***In vitro* intestinal barrier models**
TNO's Heleen Wortelboer presented a new research strategy using cell and tissue culture methods that has been reviewed for the absorption of substances after oral intake. It involved The collecting relevant literature data, selecting *in vitro* intestinal barrier models and the relevant compounds for detailed study, determining the permeability values of these compounds along with the CYP/UGTs/transporter abundance in the models and developing a software simulation model, called the absorption PBPK model. As a result, the *in vitro* test systems have been characterised and individually evaluated for set of compounds. The PBPK modelling is under development to extrapolate *in vitro* data to predict plasma profiles and bioavailability in humans.

The next step is to test the alternative 3R approach with additional compounds and different chemical characteristics (including peptides, nutrients) to evaluate predictive power for the bioavailability of compounds in humans. For this purpose EU funding has been found to enable us to enhance our understanding of how orally-administered drugs are taken up from the gastrointestinal tract into the body and to apply this knowledge to create new assays and computer models that will better predict the performance of these drugs in patients. This project also involves Unilever, Johnson&Johnson and EMA.

What are the lessons learned from SLiM? It is a big challenge to get industry actively involved in first phase. Secondly, different applications for the Pharma, Food and Chemical industries means different interests in absorption routes (e.g., endogenous transporters, endocytosis, M-cells, chylomicrons, lipid rafts). Therefore, demonstrator data of specific compounds are needed to evaluate the alternative approach within each application and for faster implementation. Differences should be accounted for to achieve this collaboration with partners, also in food and chemical industry with a specific focus on marker compounds for metabolism/transporters, bio-relevant conditions (e.g., after digestion) and regional absorption.

Irrespective of the industrial involvement and willingness to share their compounds and data, the availability of human intestinal test tissue in good condition to measure transporter abundance remains a scientific challenge.

Two species needed?
Can adequate risk assessment be performed for the developmental toxicity testing of pharmaceuticals with only one species? If so, should the rat or rabbit be the default species? If not, what species should be preferred for which compound classes (or is there any other indicator)? Peter Theunissen of HU and RIVM presented a database that has been built for this purpose with data collected from developmental toxicity studies of 440 pharma compounds (from more than 900 studies) in rats and rabbits. The amount of compounds included in the database is higher than expected due to the active involvement of the regulators and industry. Compounds for which registration was applied in the EU between 2004-2012 (CBG, EMA) included failed non-EU registered (US-FDA, US-EPA) compounds via ILSI-HESI-DART members. The preliminary analysis of 164 compounds was performed to compare rats and rabbits. The foetal Lowest Observed Adverse Effect Level (LOAEL) for a rabbit is 10 times lower than that of a rat. Further research will focus on using all the data currently available to perform analyses of kinetics, effect, compound classes/mode of action and human intended exposure. A final analysis is needed to conclude whether the same conclusions can be drawn from testing in only one species compared to two species. The data will be discussed at the Teratology Society in June 2014.

Embryonic Stem Cell models
Marc Teunis, HU
The goal of this project was to identify and confirm biomarkers of early-stage developmental disruption with the use of mouse embryonic stem cells. Research questions that need to be answered are: Can early-stage developmental toxicity be predicted based on a selected number of protein markers? Are there more objective read-outs that can replace beating cardiomyocytes? Could we shorten assay duration to 5 days? Is elimination of the 'hanging droplet' step an option? And does the EST have added value in the replacement of developmental toxicity screening in the 2-species? Two years of research did not produce full answers but changes in the expression of Neuropilin-1 (NRP-1) and FoxC 1 after exposure to developmental toxic compounds were observed and confirmed by PCR. More compounds need to be tested to evaluate whether NRP1 and FoxC are makers with good predictive value. In addition a more feasible single cell culture (instead of the more difficult hanging droplet) looks promising.

2-year rat carcinogenicity study
Ruud Woutersen of WUR and TNO explained the aim of this case study, which was to prove that for non-genotoxic compounds safe exposure levels for consumers can be established without a 2-year carcinogenicity study by:
•Identification of the No-Observed-Adverse-Effect-Level (NOAEL) from a 3- or 6-month repeated-dose toxicity (sub-chronic) study.
•Application of an additional Assessment Factor above the regular Uncertainty Factor (inter- and intraspecies).
A retrospective study was performed by building a database based on three independent databases from the EFSA (13 compounds), Actor Tox Ref from the EPA (192 compounds), and CBG (64 compounds).

Compounds were analysed only when the CAS number, name of compound, strain of rat, dose range and NOAEL/LOAEL of the sub-chronic and the carcinogenicity study were present. The effect on body weight and organ weights, histopathology (hypertrophy, proliferation, hyperplasia, foci of cellular alteration) and the benign and malignant tumours were analysed. The *in vitro* and *in vivo* genotoxic profile of the compounds should be known.

The preliminary conclusion is that the absence of histopathological evidence of putative preneoplastic lesions in a rat sub-chronic (3-6 months) repeated dose study using a whole animal approach is a reliable way to predict a negative tumour outcome in the 2-year carcinogenicity study. The proviso is that 1) the compound is not genotoxic , 2) the compound caused no hormonal perturbation and 3) An Assessment Factor (AF) of 10 is applied to the NOAEL of the sub-chronic study in addition to the generally applied factor of 100 (to determine the Acceptable Daily Intake). The results will be presented by the EPAA in June 2014

Workshops: Boosting innovation in Alternatives for Animal Testing
In the afternoon there were several workshops to look at the possibilities to improve the involvement of the all chain partners in 3R development and to boost commitment for 3R acceptance and implementation. Cyrille Krul of TNO started the afternoon sessions with an overview of progress in the SLiM project with respect to communication to stakeholders and the role of social media. Furthermore, she talked about the role of animal testing and 3Rs in the Corporate Social Responsibility policy. Consumers and patients demand more and more openness and transparency from both public and private organisations. Companies see the interesting possibilities this opens up, including using the 3Rs method for animal testing as part of their Corporate Social Responsibility programme. That's why the SLIM project has looked into the feasibility of setting up an index to benchmark organisations for animal testing and 3Rs methods. This will increase insight into what is already feasible and how other organisations have implemented new strategies. A summary of these results is also given in the reports of the meeting of 18 June and 29 October (see innovativetesting.nl/SLIM) 2013.

A preliminary brainstorm with stakeholders demonstrated that a 3R Index to benchmark organisations would be a good opportunity to create incentives for industry and research organisations. A 3R Index for public and private organisations might be possible. The index will give us more insight in the factors that drive 3R implementation. An index is a "self-regulating system": you need to improve to stay at the forefront. The possibility of a 3R Index should be discussed internationally and will hopefully start at the World Congress on Alternatives in August 2014 in Prague.

Overall the SLiM project team would like to make the following recommendations:

- Communication & Science are inseparable
- Social media will play a role, be prepared!
- Keep the message clear, short and simple
- 3R policy is more than just CSR
- Identify individual stakeholders. You will depend on their motivation and tenacity.
- Reward frontrunners and encourage sharing

After Cyrille Krul's presentation, a 4-minute cartoon entitled 'Boosting innovation in alternatives to Animal testing' was shown to summarise the main non-scientific results thus far as an inspirational message for the workshop session that followed. This cartoon has been uploaded to internet and can be seen via vimeo or www.innovativetesting.nl/slim.

In the workshop the participants were divided into different stakeholder groups. Each group had to answer the following questions:

1. What do you (or your organisation) need to actively use 3R methods?
2. What can you offer others to facilitate 3R implementation?

A summary of the discussions is listed below.

Obtaining Commitment:
To obtain commitment, influence and support are needed at the level of top management, advisory boards and government. But how can we reach them? How can we ensure that scientific advice to implement the 3Rs in daily practice is taken on board? Check out the advisory boards and find out by whom and how they can be (indirectly) influenced. Branch organisations can play a important role in getting the 3Rs implemented.

The regulator is your colleague. Scientists at all levels in the company must collaborate with regulators and understand their 3R perspective. Implementing 3Rs in CSR via a 3R index could be of interest for investors and could enhance the urgency of this topic at top management level.

Politicians influence legislation. So if a change in certain legislation is needed, the industry could lobby and make use of social pressure to make this happen. In addition, regulators have influence on politicians. Daily practice shows that guidelines and support documents should not be confused with legislation. At this moment several guidelines regarding the safety evaluation of new products are an integral part of EU legislation.

However, laws cannot be changed as fast as new scientific insights appear; the law should therefore provide the regulatory framework but practical guidelines with respect to (non-)animal testing must be separated from the law as much as possible to improve the implementation of 3R methods and accelerate innovations.

One of the most important conclusions of the afternoon was that industry, scientists, regulators and NGOs (patient and animal welfare organisations) should work together to make this possible. The Dutch authorities could also play a role in this matter; a lobby among regulatory bodies such as ECHA should be organised.

Only when there is commitment can continuity be guaranteed. Continuity and economic growth can be guaranteed by innovation and improvement of the competitive position. Industry representatives present at the meeting are aware of the need to invest in 3R methods to make this happen.

Continuity of database analyses is also essential for regulators. Database maintenance should be ensured so that scientists have access to the data, input of new data and continuous evaluation of the data. Horizon 2020, CEFIC-LRI, EPAA, ZonMW, NC3R and other (inter)national funds are possibilities that should be explored.

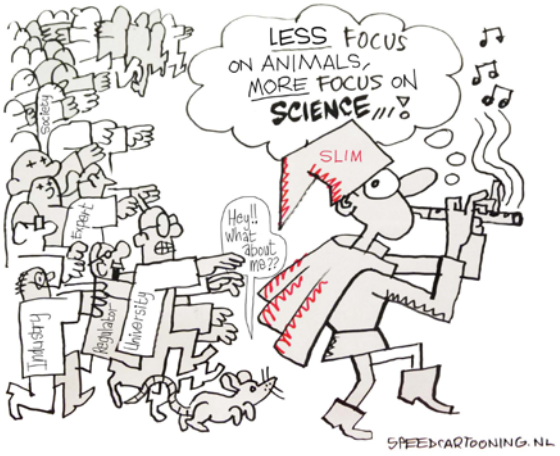
Horizon 2020 as a real possibility for 3R approaches (especially calls in 2015) and must be prioritised in the near future, for instance by looking for other European partners at ESTIV and the World Congress on Alternatives in August 2014.

Communication
How can we make use of public pressure and pressure from investors and shareholders? The general public should be informed by consortia (from industry, research institutes and trade organisations) to put 3Rs on the agenda…….

Within the industry more communication and transparency are needed as well. Data sharing remains important to build enough evidence for new research strategies. Not only reference databases but biological databases (tissue and cells) with demand and supply matching in place could be an option. The regulators added to this topic that they need more data! One of the successes of SLiM is that the data provided by industry of failed compounds could be used for retrospective data analyses. The inclusion of such industry data is a must for a complete evaluation of the data. Regulators support the initiatives of industry to become more open in providing data and sharing positive and negative experiences. Regulators are involved in international forums (for instance ICH and OECD) to facilitate the implementation of new research strategies. The industry should approach these regulators.

Coordination: in science and to improve animal welfare

Science:
There should be more coordination between what industry demands and what research institutes can supply. A good example is the NC3Rs CRACK-it programme in the UK, where scientific proposals presented to a board of experts from industry and academia should make it clear that the 3R goals are realistic, whereby the research focus is not only on the R of replacing but also on the other two Rs



(refinement and reduction). The focus should be on relevance to humans, meaning more focus on science.

Animal welfare:

People in animal welfare ethical committees (*dierexperimenten commissies*, DECs) do have a lot of experience and expertise in animal experiments, statistical methods to reduce the amount of animals, best practices in animal welfare, etc. However, there is no guarantee of knowledge and alternatives to animal experiments on each topic. A culture of openness and honesty about one's own limitations is needed. There should be a national network of experts on alternatives who can be consulted whenever a protocol cannot be judged on the basis of the expertise present in an individual DEC. These experts should be key opinion leaders. It will take effort, time and money to set up such a network. The NKCA mentioned that some effort has been made to start such a network and that it will continue such efforts (or delegate it to the National Committee).