



## Conference Reports

# 40<sup>th</sup> international congress on Applied Ethology

Freiburg, Germany, 20 - 22 of November 2008

The congress was organised and hosted by the German Veterinary Association. The presentations focused mainly on livestock, however, investigations of the behaviour of laboratory dogs and horses were also shown.

In his introduction **Hinrich Snell** of the regional council in Bonn gave a short overview over the major current topics, including the national regulations concerning transport of animals.

A number of studies dealt with the effects of living and feeding conditions and social interactions on piglet behaviour. **Milena Buri** presented her study on the quality of different types of nest-building material and its impact on the incidence of dangerous situations for piglets in loose housing farrowing systems. The animals' behaviour was filmed, and it was shown that the sow behaviour hardly differed with straw length but that fewer risky situations occurred with longer straw. **Johannes Baumgartner** investigated three types of free farrowing systems with regard to the use of the creep area by piglets and the incidence of dangerous situations of piglet crushing. The study drew the conclusion that the lying area should be enlarged in the biggest system (7.6 square metres) and the smallest system, with a space of only 4.1 square metres, should be abandoned completely. **Tanja Kutzner** showed that contact between non-littermate piglets reduces social stress and aggressive behaviour. By using a computer-controlled feeding-system and conditioning pigs to acoustic signals, **Manuela Zebunke** showed that the animals were more active than conventionally fed control groups.

A session on fattening bulls, dairy cows and calves dealt with improved housing conditions, introduction of new animals to the herd and sucking behaviour. **Frank Zerbe** compared rubber topped slatted floors with conventional ones with regard to their effect on tail tip injuries of fattening bulls. The major result was that the rubber mats positively affected the animals'

health. Positive handling of the animals was shown by **Johanna Probst** to be of advantage for the meat quality due to reducing the stress for the animals. **Maren Kauke** recorded the extent of stress caused in cows by noise and vibration in the milking parlour. No differences could be proven with respect to udder health. However, it is generally known that noise can negatively affect animals. Dairy herds were investigated by **Lorenz Gygax** as to their social structure and changes therein after introduction of single or pairs of heifers. It proved advantageous to introduce heifers in pairs. **Beatrice Roth** compared the sucking behaviour of mother-bonded calves with that of artificially reared animals with respect to the influence on cross-sucking, health and weight gain. Mother-bonded rearing prevented cross-sucking.

Studies on broilers and laying hens compared different types of litter, studied habituation to a new nest position and asked whether the taste of feathers influences pecking behaviour. The effect of different types of litter on the occurrence of pododermatitis in broilers was studied by **Jutta Berk**. Chopped straw, which is standard for the industry, appeared to be the least suitable and resulted in a significant degradation of footpads. **Tine Lentfer** tried to find out if changing the nest position in aviary systems affects the behaviour of the laying hens. It was shown that the hens can be habituated to a new nest position and the number of mislaid eggs was small. The palatability of feathers and the influence on pecking behaviour was investigated by **Alexandra Harlander-Matauschek**. Hens were offered feathers either soaked in a bitter or sweet substance. The bitter taste resulted in reduced pecking.

The influence of an enriched environment and contact to other animals on the behaviour of goats was investigated in a series of studies. **Janine Aschwanden** studied the influence of structural elements on feeding and lying behaviour of loosely housed goats. It was shown that provision of an enriched situation, by

providing partitions or platforms within the pens, minimised social conflicts and facilitated feeding and lying. The impact of different degrees of separation on the behaviour and vocalisation of goats was investigated by **Katrin Siebert**. Animals in total isolation showed passive and fearful behaviour. Goats which were still in sensory contact with the group were observed to continuously cry as they tried to keep social contact. An approach to evaluating parameters describing the social characteristics of goat groups was presented by **Janine Aschwanden**. The behaviour of the animals was analysed and allowed for defining factors influencing the housing conditions.

**Sylvia Graf** showed that the presence of a buck reduces aggression in group-housed breeding rabbits but also leads to restlessness.

With regard to laboratory animals, **Dorothea Doering** tried to determine which factors are linked to the behaviour of laboratory dogs when encountering an unknown person. It was shown that all animals sought contact and were easy to handle. However, breeding the dogs inside the facility seems to be of advantage compared to acquiring animals from other origins. A comparable study regarding threatening and avoiding behaviour

of horses in stables with feeding stalls and with automatic feeding systems was presented by **Stephanie Streit**. In conclusion, both systems are suitable for horses in run-in sheds, as long as professional management is provided.

As this was the 40th anniversary of the congress, **Josef Troxler, Hans Oester** and **Hans Hinrich Sambraus** gave an overview of the developments in the rearing of pigs, bulls and laying hens in the last decades. A podium session focussed on how livestock ethology may contribute to positive changes in practice. A major goal might be seen in assessing and taking into account the real necessities of the animals instead of creating only a minimum of factors which only slightly reduce animal suffering.

Silke Bitz  
Landesverband Menschen für  
Tierrechte  
Tierversuchsgegner  
Baden-Württemberg e.V.  
Alexanderstrasse 13  
70184 Stuttgart  
Germany

## International meeting on Developmental Neurotoxicity (DNT 2)

Reston, Virginia, USA, 12-14 November 2008

### Background

Chemicals in the environment potentially may have significant consequences for neurodevelopmental disorders and children's health. Concern about health risks for children has increased among policy makers, scientists, representatives of industry, NGOs, the general public and the media and efforts are being undertaken to identify developmental neurotoxicity (DNT)-related hazards via alternative approaches.

Following the first TestSmart meeting, DNT 1 (2006, Reston USA (see Workshop report by Lein et al., 2007)) the second meeting, DNT 2, was also hosted by CAAT. The primary goals of the meeting were to further develop a scientific and policy network to promote the use of *in vitro* and non mammalian methods and concepts to screen for potential developmental neurotoxicity. Furthermore, during the meeting a recommendation document (to be published separately, Crofton et al., in preparation) was shared describing the minimum criteria which test method developers should be concerned about to generate data that can help in predicting and identifying hazards of DNT. Thirdly, the meeting was planned to further develop the concept of DNT 1 as part of the Test Smart programme (an efficient and scientific approach to toxicity data initiated by CAAT). Today, there is only

a limited availability of mechanism-based relevant and reliable test for assessing the potential developmental neurotoxic hazard of chemicals. Presentations and discussion were held on issues including alternative models (various type of cell culture and non-mammalian species), endpoints relevant to DNT testing, the intelligent use of the data produced and their value in different policy frameworks.

### Report on the meeting

On the first day **Kim Boekelheide** (US Brown University) set the stage by discussing the current risk assessment paradigm which is based on high-dose exposure of laboratory animals. In the future, the knowledge on human toxicity pathways and the identification of adaptive responses *versus* toxic responses should lead to alternative approaches reflecting more realistic exposure (see NAS report, Toxicity testing in the 21<sup>st</sup> Century, 2007). Indeed, he emphasised the dosing regime should consist of multiple dosing and endpoints assessed *via* high-throughput means based on human biology. Problems that will need to be solved are related to the validation paradigm which often refers to animals at high dose exposure, the issue of effects by mixtures, metabolism and kinetics, epigenetic factors and other un-



known mechanisms and specific cell-cell and organ interactions. **Cynthia Bearer** a paediatrician from the University of Maryland confronted the audience with the fact that 5 million children are affected by developmental disorders of fetal origin often related to gene and environmental interactions. She was calling on the scientific community to identify biomarkers of exposure of the chemicals humans are exposed to so that interventions can be very focused. **Kevin Crofton** (US EPA) then presented the recommendation document identified above. He stressed the importance on the need for quality data to demonstrate the predictive capacity of new test methods and batteries and identified the need for a sound strategy for data interpretation. **Ellen Fritsche** (University of Duesseldorf, Germany) gave a very detailed overview of different endpoints that she developed using human neural progenitor neurospheres in order to assess critical neurodevelopmental processes such as cell-cell adhesion, migration, proliferation, differentiation and apoptosis. Furthermore, she proposed a test system to tackle the important issue of species differences. **William Mundy** (US EPA) presented a set of endpoints for neural connectivity, neurite outgrowth, synapse formation and function. He mentioned the importance of the use of very specific control endpoints to prove that a test does what it is supposed to do. **Joseph Bressler** (Johns Hopkins University, USA) was the last plenary speaker of the first day giving a detailed overview of endpoints that have a crucial role in neural development and function, the importance of the role of glial cells in DNT and the blood-brain barrier.

During the second day of the DNT 2 meeting different test systems, both *in vitro* cell culture models and non-mammalian systems, were presented. **Lucio Costa** (University of Washington, USA) gave a detailed review on the available *in vitro* cell-based methods to study neurotoxicity and DNT such as cell lines, primary cultures, co-cultures, aggregate cultures, slices, rodent and human stem cells and primary cells from transgenic animals. He stressed the importance whenever possible to use human cells and emphasised once more the critical neuronal-glial cell interactions. **Pamela Lein** (Oregon Health & Science University, USA) detailed non-mammalian alternative models such as nematodes, sea urchin, insects, fish and amphibian test systems. Although species differences are an issue, several genes important for neural developments have been conserved during evolution, enabling the use of non-mammalian systems to predict a set of human-relevant endpoints. On test systems such *C. elegans*, *Drosophila* and zebra fish embryos several data are available indicating their potential for DNT testing. A major advantage of these test systems is that behavioural studies can be performed.

After the scene was set *via* the excellent plenary presentations the concept of concurrent breakout groups was used focusing on two different aspects: discussion on the science available with regard to (1) cell culture systems and (2) models for DNT. Several participants were invited to give a short presentation related to their posters.

The session on data interpretation, integration and policy was introduced by **Robert Kavlock** (US EPA). He gave an overview of the US EPA ToxCast chemical prioritization program where over 300 chemicals are characterised for over 400 endpoints and

emphasised the importance of good logistic support allowing intelligent data generation and interpretation. **James Bus** (Dow Chemicals, USA) mentioned the need for high throughput methods to identify toxicity pathways relevant to identified human DNT effects and to gather information on large set of chemicals. He mentioned the importance of the low end of the dose response curve, the importance of getting insight into the key events of modes of action, to account for species differences and polymorphisms and he brought again the issue of mixtures.

The third day of the meeting was organised as panel discussions on issues related to a framework for policy and for biology. During these sessions a kick-off was presented for a global collaborative research effort to develop agreed guidance criteria for a new generation of DNT test methods based on alternative approaches to be presented and discussed at DNT 3 in 2011, in Europe. A discussion forum as a follow-up on DNT is now hosted at Alttox (<http://www.AltTox.com>) and co-chaired by William Mundy (US EPA) and Sandra Coecke (In Vitro MethodsUnit/ECVAM). AltTox.org is a website dedicated to advancing non-animal methods of toxicity testing including methods for DNT, both to better protect the health of humans, animals and the environment and to reduce the numbers and suffering of animals used in current toxicology assessments. The DNT forum is designed to encourage the exchange of technical and policy information on alternative DNT methods and more importantly to stimulate researchers world-wide to join this global DNT research effort. The summary of DNT 1 and some of the papers from DNT 2 are available on the CAAT website (<http://caat.jhsph.edu/dnt>).

The meeting was closed with a broad and animated discussion of many of the issues raised during the meeting, first by a discussion of a policy framework by **Paul Locke** (Johns Hopkins University, USA) followed by a lively discussion led by **Philip G. Lewis** (Rohm and Haas Company, USA). In his close, he encouraged the audience to continue with the work what has been started in DNT 1 and 2 to provide at DNT 3 the test method data that will allow the integration of data produced by alternative DNT testing into integrated test strategies.

## References

- Coecke, S., Goldberg, A. M., Allen, S. et al. (2007). Incorporating *in vitro* alternative methods for developmental neurotoxicity into international hazard and risk assessment strategies. *Environ. Health Perspect.* 115, 924-931.
- Lein, P., Locke, P. and Goldberg, A. (2007). Meeting report: alternatives for developmental neurotoxicity testing. *Environ. Health Perspect.* 115, 764-768.
- Grandjean, P. and Landrigan, P. J. (2006). Developmental neurotoxicity of industrial chemicals (Review). *Lancet* 368, 2167-2178.
- National Academy of Sciences (2007). *Toxicity Testing in the 21<sup>st</sup> Century*. Washington, D. C: The national academies press.

Sandra Coecke<sup>1</sup>, Anna Bal-Price<sup>1</sup> and Alan Goldberg<sup>2</sup>  
<sup>1</sup>In Vitro MethodsUnit/ECVAM, JRC, Ispra, Italy,  
<sup>2</sup>Johns Hopkins University, CAAT, Baltimore, USA

## Looking in the crystal ball...

The laboratory animal in a changing world  
Utrecht, The Netherlands, 5-7 February 2009

This international symposium was organised by **Frauke Ohl** (Utrecht University, Department of Animals, Science and Society) and **Coenraad Hendriksen** (NVI - Netherlands Vaccine Institute and NCA - Netherlands Centre for Alternatives to Animal Use). The immediate cause for the celebration were several anniversaries: 25 years Division of Laboratory Animal Science, 15 years NCA and 5 years Department of Animals, Science and Society at the veterinary faculty of the University of Utrecht. The goal of the symposium was to give a perspective of the 3R developments within the Netherlands and Europe during the last decades and to outline visions for the future. Many international key players and stakeholders from different areas of 3R research attended the meeting and shared their knowledge with the participants.

**Bert van Zutphen** (Dept. Animals, Science and Society) focussed in his lecture on the developments that have taken place in the field of animal use for research and testing during the past decades in The Netherlands. He compared the situation with developments in other European countries and focussed particularly on the revision of directive 86/609 (animal protection law) currently under discussion in the EU. **Judy MacArthur** (Home Office, UK) spoke about the task of laboratory animal scientists to balance high quality science with animal welfare. She stressed especially the need for a more differentiated and honest dialogue amongst the different stakeholders for the overall benefit of the animals. The hope was expressed that the future would bring better understanding of the environmental and social needs of laboratory animals, based upon scientific studies rather than anthropomorphic judgement. **Coenraad Hendriksen** reminded the first publication of the principle of the 3R by Russell and Burch 50 years ago. Nowadays most of our current laws and regulations in Europe include strict guidance on how to implement the 3Rs. This applies in particular to the proposed revision of the European Council Directive 86/609. **Herman Koeter** (Orange House Partnership, Belgium) gave an overview of the recent conceptual developments in risk assessment of foods. He suggested that current approaches would benefit from fundamental re-thinking and introduction of new concepts. As examples, he explained the use of intelligent hazard prediction and classification tools that make use of existing data. QSARs (quantitative and qualitative structure-activity relationships), QPS (qualified presumptions of safety) and TTC (thresholds of toxicological concern) are already applied to a small extent, but would deserve a more prominent role in risk assessment. He also stressed that the latest generation of *in vitro* pre-screening and hazard identification methods has very much matured. These novel computational and *in vitro* approaches, complemented with recently-developed proteomic, metabolomic and carcinogenic profiling techniques, are expected to provide adequate predictive power to assess the safety (or risk) of substances in

the near future. This would allow a significant reduction of or even complete abandoning of the traditional observational animal studies. **Marcel Leist** (Doerenkamp-Zbinden Chair for alternative *in vitro* methods, University of Konstanz, Germany) gave the audience an insight into new developments in the field of *in vitro* toxicology, such as a movement towards evidence-based toxicology on both sides of the Atlantic, and the US National academy vision of a new toxicology of the 21<sup>st</sup> century. These developments will give a new push for the development of replacement methods. Currently, the major driving forces in Europe are the REACH legislation and the seventh amendment of the cosmetics directive. These efforts are supported by several large-scale integrated projects funded by the EU. A decisive key step for the future of alternative methods in the toxicology area will be the creation of centers that would be responsible for reference compounds and reference compound databases. This will support the development of more robust cell-based and model (invertebrate) organisms-based test systems. The introduction of new endpoints for these systems combined with the use of larger panels of tests may be a way forward to higher predictivity. **Jann Hau** (Department of Experimental Medicine, Veterinary University of Copenhagen, Denmark) analysed a systematic sample of 2800 articles published between 1970 and 2000 to assess the implementation of the 3Rs in biomedical research. In terms of reduction, he documented a 50% decrease in the average number of animals used per published paper. He stressed that Utrecht is internationally recognised as one of the world's most important centres for promoting the 3Rs in biomedical research and teaching. **Paul Flecknell** (Biology Centre, University of Newcastle, UK) reported on progress in "refinement", i.e. the greatest possible reduction of pain and stress experienced by the animals still used in research. This concept can readily be applied to a very wide range of research procedures. Measures such as environmental enrichment, the introduction of humane endpoints and the use of analgesics have been widely accepted as good laboratory praxis, although the detection and quantification of stress and pain behaviour in animals will still require more intensive training of researchers and basic research of veterinarians and behavioural biology scientists. **Frauke Ohl** (Department of Animals, Science and Society, Utrecht University) finally closed the sessions of the first day with "a look into the crystal ball" to identify what will be the future of the 3Rs. The fact that the invitation to Utrecht was accepted readily by such an impressive and interdisciplinary group of people clearly indicates that there is a fundamental willingness and demand to discuss and critically evaluate the future of animal experimentation.

The second day was dedicated to parallel workshops: Housing of laboratory animals: past, present and future; the 3Rs in genetics of animal models; 3Rs and regulatory testing; the 3As



in education: animals, alternatives and attitude; the biotechnical view on the future of the 3Rs; societal aspects of “responsible” use of laboratory animals; better animal models: better therapeutics; fish: issues related to using fish-species for research; translational research: claim and reality; welfare assessment: where do we need to go?; the impact of law and regulations on the implementation of the 3Rs. The most impressive workshop in the eyes of the author was 3Rs and regulatory testing, chaired by **Coenraad Hendriksen**. The speakers **Jean-Marie Schiffelers** (USBO, Utrecht University), **Bas Blaauboer** (Doerenkamp-Zbinden Chair of toxicology, IRAS, Utrecht University), **Herman Koëter** (The Orange House, Belgium), **Ruurd Stolp** (Schering Plough Intervet, The Netherlands) and **Thomas Hartung** (Doerenkamp-Zbinden Chair of evidence based toxicology, Johns Hopkins University and CAAT, Baltimore, USA) gave an excellent overview of the use of laboratory animals in the fields of regulatory safety and quality testing. Many national and international parties, often with divergent interests, are involved in setting the test requirements aimed at efficacy, consumer safety and environmental protection. In addition to the discussion of existing regulatory testing policies, an inventory was made of progress towards and obstacles to the acceptance and implementation of the 3Rs. Surprisingly, nearly 20% of the

workshop participants voted at the end that lack of communication between scientists and regulators is the most hampering obstacle to the implementation of 3R methods. The complicated validation process, “lack of reward” for the 3R scientists and political reasons came next.

The last day was dedicated to a public discussion “Science goes public - listening to society” in the Conference venue “Jaarbeurs” in the historic town center of Utrecht. This part of the meeting was held in Dutch language to optimally involve the local population.

All in all this “looking into the crystal ball” was a very well organised and fruitful event to define the status quo and further developments of the 3Rs. We thank the organisers and the University of Utrecht for the organisation of this event and the high importance it was given. It did not remain unnoticed that the University provided the most noble medieval hall, usually only used for receptions of high ranking guests and University celebrations, for the lectures, and that the dean of the prestigious Faculty of Veterinar Medicine, **Albert Cornelissen**, not only welcomed the guests at the begin of the event but was present during the whole symposium and actively participated in all discussions.

fpg

## Comments

*Franz P. Gruber*

### Opinion of the Scientific Committee on the need for non-human primates

What else could we expect? As already predicted in ALTEX 3/08, the Scientific Committee on Health and Environmental Risks (SCHER) stressed the need for the continued use of non-human primates. With Mark Matfield as the speaker, the “Executive Director of the Research Defence Society“, the result was clear from the start – he had already fought fiercely

against a ban on primate experiments in England in 2004. The EU obviously no longer even strives to maintain the presence of impartiality.

At least the statement contains the open admission that the opinion “does not consider the ethical, economic, cultural and social aspects of NHPs use”. This declaration disqualifies the state-

ment from merit serious consideration. He who openly dismisses ethical aspects should have no chance of succeeding before the European Parliament. Even paying lip service to the 3R Principle will not help here.

Franz P. Gruber  
Editor in chief, ALTEX