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Wedebye, Eva Bay; Dybdahl, Marianne; Christiansen, Sofie; Svingen, Terje

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3R ACTIVITIES IN THE RESEARCH GROUP FOR MOLECULAR AND REPRODUCTIVE TOXICOLOGY AT THE NATIONAL FOOD INSTITUTE

Over the past two years, we have asked Aarhus University and the 3R department at Novo Nordisk respectively to describe their 3R efforts for our annual reports. This year, we asked the research group from Molecular and Reproductive Toxicology at the National Food Institute to describe their efforts to integrate the 3Rs into the use of computer, cellular and animal models. We would be happy to hear from other institutions that would like to describe their approach to making a difference in the 3R area.

**By Eva Bay Wedebye, Marianne Dybdahl,
Sofie Christiansen and Terje Svingen**

Traditionally, animal experimentation has been crucial in chemical assessments, and some questions can still only be answered by means of animal experimentation. In our group at the National Food Institute, we conduct research into, among other things, the development of animal-free methods to help achieve a paradigm change in how risk assessments of chemicals can be done in the future.

Molecular and Reproductive Technology is a large multidisciplinary research group at the National Food Institute. Our overarching aim is to protect humans against possible harmful effects that can occur when they are exposed to environmental chemicals. We have a long-standing tradition of examining especially potential hazardous effects that arise when a foetus is exposed to chemicals with endocrine-disruptive effects.

In order to chart the specific effects of various substances and advise authorities and others how best to protect people against these same effects, we apply different strategies which jointly help us to achieve these goals in the best possible way. We take a holistic approach and use computer modelling, cell-based methods and animal experimentation to illustrate the problem. However, we only carry out animal experimentation where this is necessary, and we continuously endeavour to base our research and advise on the 3R principles. Thus, the group works to find alternatives to animal experimentation (Replacement), to develop methods which use as few animals as possible and ensure that we get the most out of the laboratory animals that still have to be used (Reduction) and to ensure that the animals are provided with the best possible conditions (Refinement).

In silico approach

Over two decades, our research group has developed many Quantitative Structure-

Activity Relationship (QSAR) computer models to predict the harmful impact of chemicals based on the chemical structure. We made our database of QSAR predictions freely available on the internet (qsar.food.dtu.dk) in 2015. The models build on previous results from human, animal and cell models.

We use QSAR predictions to assess chemicals, usually together with information from other alternative methods or with historical data from animal experimentation whose quality is not sufficient to stand alone. The model predictions can be used to set priorities to bring focus to bear on the most problematic chemicals, and they can help in the design of safer chemicals that have a lower risk of subsequently turning out to be problematic and prompt comprehensive animal experimentation.

In addition, the research group develops and uses other *in silico* methods, such as physiologically-based kinetic (PBK) modelling. PBK models enable us to predict the fate of chemical substances in the body, i.e. absorption, distribution, conversion and excretion. These items are integrated into the animal model and animal-experimentation procedures, and they are also important to identify in an alternative approach based on cell-based procedures and so-called non-test methods.

In vitro approach

Another important area of the group's research is conducted using cell-based (*in vitro*) testing methods. These are primarily based on human cells, but in some instances on animal cells as well. These cell-based testing methods are often particularly well-suited for determining how chemical substances affect the body at molecular level and thus can give rise to undesirable effects. As chemical substances can affect humans in

many different ways, the group has focused on building up a wide spectrum of in vitro testing methods which jointly cover many different mechanisms.

Seeing that procedures based on human cells are expected to be best suited for predicting effects notably in humans, much of the group's research involves the development of additional new in-vitro methods based on such cells, e.g. human stem cells. We use in vitro models particularly in our research into endocrine-disruptive effects and, in this context, we have established a panel of methods for studying the efficacy on sex steroids, thyroid hormones, etc. We are continuously enlarging the panel of methods with a view to identifying additional relevant mechanisms. Other approaches we use in the group to illuminate underlying mechanisms are so-called omic and high-content technologies.

In vivo approach

When computer calculations and in vitro testing in themselves do not provide enough knowledge to be able to protect the population against possible harmful effects from environmental chemicals, we at the National Food Institute have animal experimentation facilities that are set up to be able to conduct animal experimentation. These procedures can provide information on hazardous or beneficial effects of dietary factors, chemical substances and products, as well as micro-organisms, including genetically modified micro-organisms. Our group conducts reproductive procedures in gestating animals and examines the impact of different chemicals on offspring. The advantage of in vivo experiments is that they make it possible to measure the effect on the entire organism or the developing foetus.

Many different animal-experimentation methods are used to study whether chemical substances can affect foetuses and offspring. Many of these methods are standardized and, for example, the OECD has developed



guidelines (recommended methodological precepts) describing how to use the methods. The methods are used for regulatory testing of chemical substances.

Overarching perspective

Developments in the area of molecular biology and toxicology will in future pave the way for developing better alternative methods for assessing chemicals. The use of traditional animal experimentation for assessing the hazard of chemicals will gradually be replaced or supplemented by the use of alternative methods such as in vitro examinations and computer-based predictions. It is also expected that more targeted animal experiments will be eventually be used in instances where they are indispensable. This transformation is already under way, and we actively contribute to these efforts, including together with the Danish EPA under the auspices of the OECD and EU.

Contributing to international work

Our research group actively participates in OECD efforts to develop Adverse Outcome Pathways (AOP) and Integrated Approaches to Testing and Assessment (IATA). For each harmful effect, an AOP systematically describes the available knowledge of the biological mechanisms causing the effect. Charting correlations and basic mechanisms makes it possible to use relevant mechanical knowledge from things like cell-based methods and QSAR to predict harmful effects in humans. AOPs covering a wide range of different effects in areas such as cancer and damage to the reproductive and nervous systems are freely available at the AOP wiki website (aopwiki.org/).

IATA combines all available information from in silico, in vitro and in vivo studies relating to a chemical assessment. The combined use of these methods can reinforce the interpretation of individual data, which is why IATA could prompt authorities to increasingly use data from alternative methods. IATA can also guide a test strategy where new experimental investigations are required. Together with the Danish EPA, we contribute to the OECD's efforts, including in relation to the development of guidelines on the use of IATA aimed at ensuring that the authorities use mechanistic knowledge in a uniform manner.

The OECD Test Guideline Programme (TGP) develops internationally recognized standard testing methods that are used pursuant to the Danish Chemicals Act. Since 2010, our group has been one of two national (Danish) coordinators of the OECD Test Guideline Programme. When a test is carried out using

an OECD Test Method, the Member State mutually accepts the data generated in the test. This means that companies do not need to test their substances for the same effect in different procedural designs, depending on where in the world the substances are used. A number of in vitro methods have been developed as OECD Test Guidelines, and our research group actively participates in these efforts. It often takes several years to validate, standardize, comment on and adopt these methods.

Our research group has also been very active in efforts to develop new and improve existing in vivo reproduction guidelines with different endocrine-relevant end points. By enlarging the test methods, they can now be used to a much greater extent than previously to assess the possible endocrine-disruptive effects of chemicals. This improvement (Reduction) will be carried out without using more animals than those already in the procedure.

QSAR TOOLBOX + DATABASE

For many years, we have been working to increase the use of QSAR and other animal-free methods in international assessments of chemicals in the EU and OECD. We do this together with the Danish EPA by demonstrating how the methods can be used when we, for example, contribute to assessments of specific substances or to prioritization exercises, and we also

contribute to international guidelines in the area. At the OECD, we have been an active team player in terms of authorities giving higher priority to the use of animal-free methods, and we contribute to efforts to develop the OECD's and EU's QSAR Toolbox which everyone is free to use for their alternative assessments of chemicals.