

Research at the National Food Institute has demonstrated harmful effects of endocrine disruptors, and how even small doses of chemicals can have a significant adverse effect if they occur in a chemical cocktail.

📷 Testicular tissue from rat. National Food Institute

The unborn child must be better protected from chemical cocktails

Even small doses of a chemical substance can be harmful when it occurs in combination with other substances. Knowledge about the cocktail effect is pivotal when legislators set the allowable limits for substances that are allowed in industrial products. At its core, the National Food Institute's research and advice is a matter of protecting the unborn child.

"The most important aim of our research is to protect the unborn fetus against the harmful effects of endocrine disruptors," Senior Researcher and Head of Research Group Terje Svengen says.

The Institute's research focuses on how chemicals disrupt the body's hormones in relation to reproduction. Many chemicals have turned out to be able to imitate or disrupt sex hormones. If there are chemicals present in a pregnant woman's body from food, cosmetics, etc., such chemicals can disrupt the hormonal development of the fetus. Phthalates block, for example, the synthesis of testosterone.

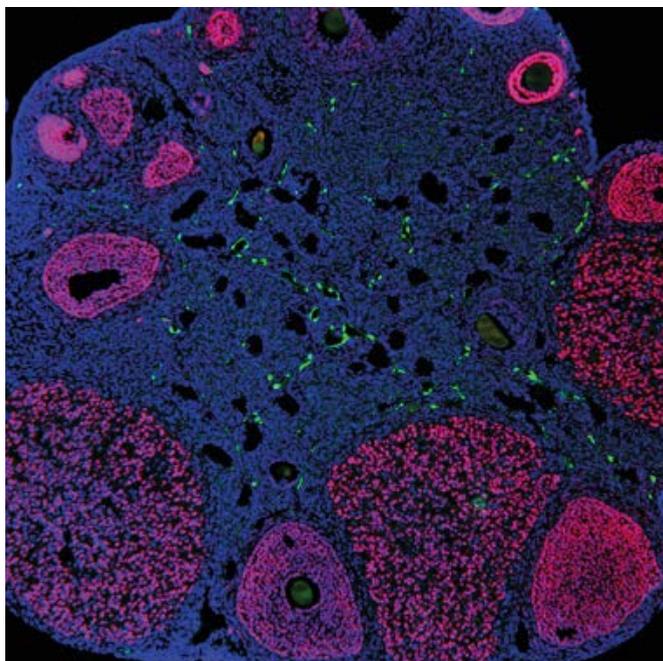
Because of the chemical impact on the pregnant woman, an increasing number of boys are born with deformed genitals. An example of deformation could be that the urethra does not open from its usual location on the head of the penis, also called hypospadias. This condition is associated with an increased risk of having or developing other issues related to the reproductive system.

Girls are underdiagnosed

"The number of girls with congenital genital malformations is probably underdiagnosed. For example, the location of the urethral opening is more difficult to detect in girls than in boys. However, some researchers assume that this is also a problem for an increasing number of girls. If such malformations are associated with other reproductive problems - such as lower fertility - this is a problem we should look into," Terje Svengen says.

Another example of endocrine disruptors' impact on girls is that they can enter puberty earlier, and correspondingly hit menopause earlier as grown women. Furthermore, PCOS (polycystic ovary syndrome) and ovarian cancer may be associated with endocrine disruptors.

The National Food Institute's extensive knowledge about the harmful effects of endocrine disruptors is brought into play in two projects supported by the EU's special programme for research and innovation, Horizon 2020. The purpose of both



Research from the National Food Institute has provided knowledge of how endocrine disruptors affect the female reproductive system.

Adult rat ovarie. National Food Institute

projects is to develop new and better methods to test the harmful effects of different chemical substances.

Better protection of women's fertility

One project, FREIA, is named after the goddess of fertility in Norse mythology. The purpose is precisely to explore how chemicals can disrupt women's ability to have children, and how better testing of chemicals can protect women's reproductive health.

By collecting new data on how the chemicals affect ovaries and puberty in animals, the project will provide more knowledge of the harmful effects chemicals have on women's fertility. Moreover, the researchers are going to develop so-called QSAR computer models that can predict the chemicals' possible harmful effects.

Researchers from 11 partners from seven EU countries participate in this project.

Thyroid hormones and brain development

The other project, ATHENA, shares its name with the Greek goddess of, amongst other things, wisdom and is focused on the hormonal impact on the brain. The project aims to develop better methods to screen for chemical substances that affect the thyroid's production of hormones, which in turn regulate the body's metabolism. These hormones play a crucial role in a fetus' brain development, and it is very important that mother and child have normal hormone levels during pregnancy. If pregnant women are exposed to certain chemicals, the child may be at risk of mental development disruptions, which affect both the ability to learn and intelligence.

We are not protected well enough

A significant part of the problem with the harmful effect of these substances on human reproductive health is the so-called cocktail effect. When chemicals occur together - in a chemical cocktail - even small doses of each substance can cause harm. Therefore, the Institute has in recent years worked to calculate their cocktail effect and to limit the number of substances we are exposed to. In projects commissioned by the national authorities, the researchers have examined how chemicals typically work together. The experiments and mapping clearly indicate that if many chemicals are present even in small quantities, it may have a considerable harmful effect. In other words, 'little strokes fell great oaks.'

Knowledge from the research projects shows that the current method for assessing risk does not sufficiently protect people because the harmful effect of a chemical is only considered in isolation, not in combination. What's more, the substances typically occur in combination when we come across them in foods, cosmetics, and other products in our daily lives.

New tools for risk assessment

Therefore, in two national research projects, the researchers at the National Food Institute have developed a toolkit that takes cocktail effects into account when assessing the risk of being exposed to chemical substances.

A so-called Hazard Index can predict the overall risk of chemicals when they occur in a specific mixture. The calculations can be made at several levels depending on the amount of data that is available for each chemical in the mixture. At the lowest level, all chemicals are put into one group regardless of the effect, and most data will be rough estimates as there is only very little data on the chemicals. At the highest level, the calculation of the Hazard Index is based on actual, measured values, and the chemicals are grouped according to their effects. Here the calculation will result in a more precise estimate of the risk of cocktail effects. The more available data, the more accurate the calculations.

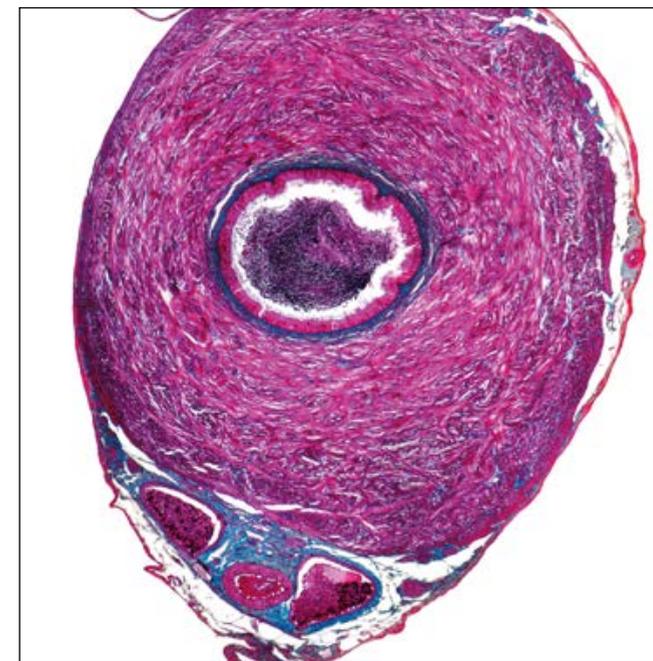
The Institute's software - Cocktail Effect Calculator - can provide information on each chemical in a given mixture. The software can also calculate predicted harmful effects.

"We are in the middle of a paradigm shift. We know we need to get away from looking at only one substance at a time. Now the big question which we are focused on at the moment is what tools are most useful when we assess the risks. In order to improve and expand the toolbox, we need more basic knowledge of how chemicals interact with molecules and cells in the human fetus, and how they harm the fetus," Terje Svingen says.

Research helps companies and authorities

Knowledge of cocktail effects helps authorities to determine allowable limits for the chemical content in the products that companies manufacture. This means that we as consumers can look forward to safer products as the researchers map the cocktail effect and make it possible to measure it even more precisely.

"In Denmark, the National Food Institute works closely with the Danish Veterinary and Food Administration and the Danish Environmental Protection Agency. This has made Denmark a frontrunner in the area of chemicals for many years. Denmark is a country that encourages other countries within this area," Terje Svingen says.



The National Food Institute uses a number of methods to understand how chemicals affect living organisms, including QSAR models, cell experiments and animal studies.

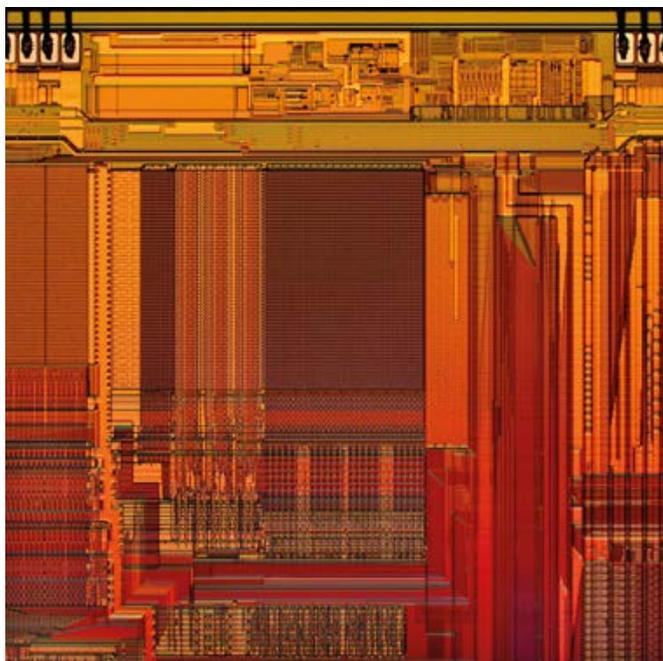
Cross section of a ductus deferens from a rat. Colourbox

Less animal testing

3R stands for the concepts of reduction, refinement, and replacement. The Institute wants to reduce the number of animal experiments, reduce the discomfort related to necessary animal experiments as much as possible (refinement) - and, in some cases, completely replace animal experiences with other approaches.

An understanding of the mechanisms behind the effects of possible toxic chemicals based on cell experiments and computer models may help replace or reduce the number of animal experiments.

In 2016, researchers from the Institute received the 3R Centre's award for their work on predicting harmful effects of chemicals by developing and using computer models, so-called QSAR models, as a contribution to avoiding animal experiments.



DTU develops so-called QSAR models to predict the harmful effects of chemical substances.
Microprocessor component. Antonio Romero/Science Photo Library/Scanpix

Computer models predict harmful effects

The National Food Institute has developed advanced QSAR computer models that are able to predict the harmful effects on health of chemical substances. QSAR stands for Quantitative Structure-Activity Relationship.

The models can predict the harmful effects of the substances, based on whether the chemical structure of the substances resembles other substances that have already been tested.

The Institute's researchers have built a QSAR database, which contains predictions from more than 200 QSAR models and for more than 650,000 organic substances.

The database is publicly available and is continuously developed.

In 2018, the EU decided to recognize four phthalates as having an endocrine disrupting effect on humans and to acknowledge the cocktail effect. The National Food Institute has contributed a significant amount of the documentation for this proposal. The decision is the first step towards stricter regulation relating to the use of these substances in consumer products.

Important test of OECD's requirements to the industry

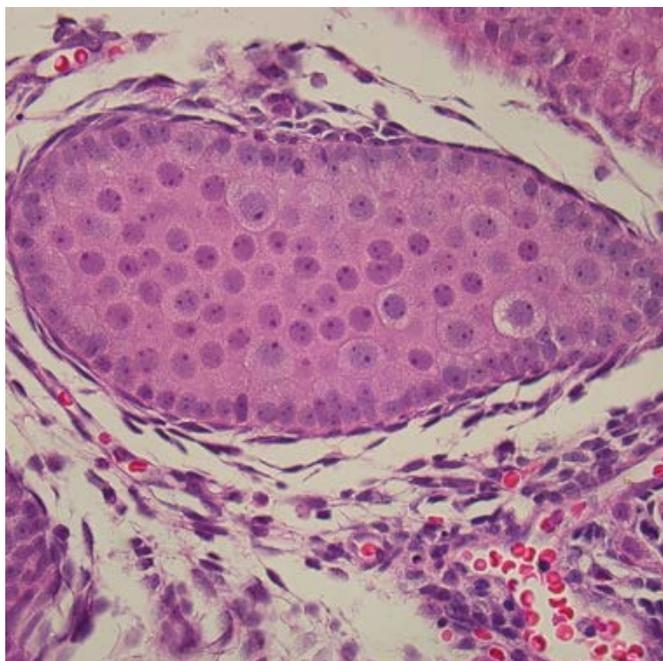
The OECD's Test Guideline Programme develops globally recognized standard test methods that are used to assess the safety of chemicals. The National Food Institute has the important job of filling one of Denmark's two national coordinator positions in relation to the OECD test guidelines. Thus, the Institute is able to influence the statutory tests, which the industry must carry out before they can bring e.g. endocrine disrupting chemicals on the market.

"In 2016 and 2018 we have been able to ensure that the industry has to measure the AGD - the anogenital distance - in order to have a substance approved. When the industry conducts experiments in which rats are exposed to relevant substances, the experiments must include a measurement of the distance between the anus and the genitals. This distance is a biomarker and can be applied to determine the sex of e.g. kittens. In male rats, the distance is normally twice as long compared with females. The distance will be relatively shorter if the level or the effect of testosterone is lower than normal. This reveals whether the substances cause an endocrine disrupting effect in the form of a lower level or function of testosterone, which is often associated with poor sperm quality, testicular cancer, and other disorders. We are proud to have had the AGD test included on the list, as it is an important marker for endocrine disrupting effects," Terje Svingen says.

“The ambition is to build enough knowledge and develop good tools that can be used to quickly and efficiently analyze the potential harmful effects of all new chemical substances on a child's development. The dream scenario is to develop an advanced computer programme which can tell that this specific substance has an effect at given levels of exposure if three other given substances are also included in the cocktail.”

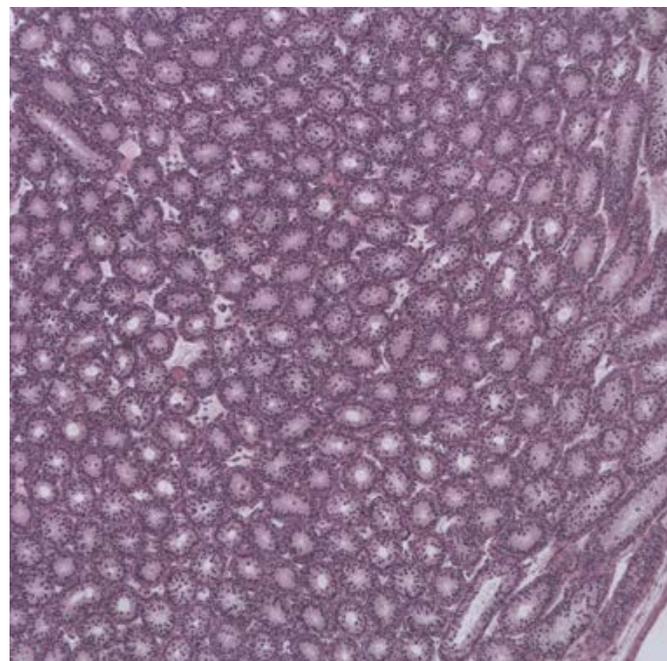
Terje Svingen

Senior Researcher and Head of Research Group



Studies from the National Food Institute show that if rats are exposed to phthalates during early development, it can lead to altered testicular development and lower sperm count.

☐ Fetal rat testis. The National Food Institute



The National Food Institute studies the degree of human exposure to chemical substances through food and consumer goods, and the harmful effects of these substances on laboratory animals' reproductive systems.

☐ Young rat testis. The National Food Institute

Animal testing facilities help produce important knowledge

When computer calculations and cell-based test methods on their own do not provide the knowledge needed, the National Food Institute has animal testing facilities at its disposal. Here, the researchers collect information on harmful or beneficial effects of dietary factors, chemical substances, and microorganisms, including genetically modified microorganisms.

The studies support the Institute's work within the area of food safety and the health-related effects of chemical substances which we are exposed to in our daily lives.

The animal testing facilities include a number of ordinary animal rooms with the opportunity to house smaller test animals

such as rats and mice. In insulators, the researchers can handle germ-free mice (mice born without any gut bacteria) or work with dangerous substances. Moreover, the Institute has room for behavioural testing, dissection rooms, and various auxiliary rooms for feed manufacturing, cage washing, etc.

The animal testing facilities also conduct animal experiments for other departments at DTU, other research institutions, and companies.

On 1 September 2019, the animal testing facilities at DTU will be gathered in DTU's new joint centre, Bio Facility, which will be anchored under the National Food Institute.

THE NATIONAL FOOD INSTITUTE IS IMPORTANT FOR DANISH CONSUMERS

"The National Food Institute is very important for Danish consumers. The Institute's research in respect of e.g. food safety, antimicrobial resistance, pesticides, and endocrine disruptors helps make Danish foods and their packaging safer. Moreover, research in nutrition and eating habits forms the basis of dietary advice and other initiatives which make it easier for consumers to make healthy choices.

It is very important to have research that does not have a one-sided commercial goal, but which can strengthen our knowledge of risks and consumer protection, and which can form a scientific basis for the legislation in Denmark and in the rest of the EU," says Senior Food Adviser Camilla Udsen from the Danish Consumer Council, who is also a member of the Advisory Board at the National Food Institute.



☐ The Danish Consumer Council

Camilla Udsen
The Danish Consumer Council