# Andreas Kortenkamp on the urgency of new test validation for endocrine disruptors

**Despite tremendous advances in new tests for endocrine disruptors, a slow validation process, low regulatory adoption rates, and a lack of tests for important effects may hamper action under the EU chemicals strategy, Andreas Kortenkamp tells Emma Davies**

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The EU's new chemicals strategy brings a "strong added sense of urgency" to developing and validating new tests for endocrine disruptors (EDs), says Andreas Kortenkamp, professor for human toxicology at the Centre for Pollution Research and Policy, Brunel University London.

The strategy recognises that the exposure of humans and the environment to endocrine-disrupting chemicals requires "specific attention". It includes proposals to create new hazard classes for EDs under the CLP regulation, as well as a category for substances of very high concern under REACH. Under the strategy, the European Commission has also pledged to accelerate the development and uptake of tests for EDs.

"Without these tests, improved regulatory measures for this group of chemicals cannot be fully implemented and will largely remain fantasy," he says. "Bottlenecks" in the system are currently preventing new tests being used for regulatory purposes. In particular, there is a shortage of validated ED tests for adverse effects on the thyroid hormone system, neurodevelopment, metabolic disorders and female reproductive health.

It is almost a decade since Professor Kortenkamp, an expert in endocrine disruptors and chemical mixtures, co-authored a State of the Art Assessment of Endocrine Disruptors for the Commission.

The report continues to be cited in most regulatory science papers on EDs. The field has developed tremendously since it was published, he says. "In many areas we have a better understanding of exposure disease associations, and research on specific chemicals has led to an explosion of new findings, mostly in the direction of increased realisation of new hazards."

There have also been exciting advances in new test methods since the report was published. "What is lagging is getting these new methods validated. The lack of validated tests holds up everything."

## Underinvestment

One problem is that validation of test methods at OECD level is "poorly funded", he says. "Laboratories that organise and conduct the necessary ring trials usually have to find the funds themselves." The entire process is also very time-consuming and slow, he adds.

He identifies two significant bottlenecks in regulatory adoption of new ED tests.

"First, there is the incomplete implementation of validated OECD tests for relatively cheap and fast in vitro tests." Such test methods are available for oestrogen and androgen pathways, as well as for other effects, but they are not prescribed in the various regulations that lay down test methods for REACH, the plant protection products Regulation (PPPR) and the biocidal products Regulation (BPR). The methods currently prescribed in these regulations focus almost exclusively on in vivo tests, he says.

Introducing OECD-validated in vitro test methods would considerably improve the situation, if complemented by quantitative in vitro–in vivo extrapolation strategies, he suggests. "With such a strategy, more substances could be tested, and testing costs could potentially decrease through effective prioritisation of substances predicted

to be active in vivo on the basis of in vitro results and toxicokinetic modelling."

The impact of a second bottleneck is "perhaps more fundamental," he adds. "For a range of ED-relevant effects, such as disruption of the thyroid hormone system, neurodevelopment, metabolic disorders or female reproductive health, we do not have adequate test methods at all."

## Thyroid hormone system

A wide variety of manmade chemicals have the potential to disrupt the thyroid hormone system, yet it is far less represented in testing terms than oestrogen and androgen pathways, partly due to its biological complexity. Thyroid hormones are essential for many biological processes, including brain development.

"Delivery of the right amount of thyroid hormones to the developing brain is absolutely crucial for proper development," explains Professor Kortenkamp. "In early brain development, the foetus relies exclusively on supply from the mother, because the foetal thyroid gland is not yet functional. Too little thyroid hormone to the foetal brain and there are declines in IQ and alterations in other measures of brain function. Too much, and the same happens." He describes a 'Goldilocks' zone, where the thyroid hormone supply is just right.

"This has been shown in large human health studies. Accordingly, chemicals that block the uptake of iodide by the thyroid gland inhibit thyroid hormone synthesis in the thyroid and are likely to disrupt brain development.

"The same applies to substances that inhibit thyroid hormone synthesising enzymes or enzymes that activate thyroid hormones. Chemicals that displace thyroid hormones from transporter proteins in the blood are also a problem.

"In many inland areas of the EU, there is insufficient iodine supply of the population, which the World Health Organization considers worrying." Exposure to chemicals that disrupt the thyroid hormone system will "add to the resulting disease burden", he suggests.

In the EU, the only tests currently listed for information requirements concern changes in the blood levels of thyroid hormones and physical changes in the thyroid gland.

"The assumption behind this strategy is that alterations in blood thyroid hormone levels translate into similar changes at the sites where the hormones act, such as in the developing brain," explains Professor Kortenkamp.

"But we know today that this assumption is not always correct. Alterations of thyroid hormone levels in the blood are important indicators, but they do not reveal the full picture. We know of heritable disorders where gene mutations render certain thyroid hormone transporters or hormone receptors dysfunctional. This means that either the hormone does not reach the brain, or even if it does, it cannot act because the receptor is unable to relay the message. This leads to catastrophic impacts on brain development but the accompanying changes in thyroid hormone blood levels are relatively small and may be overlooked readily."

It is "conceivable" that certain chemicals may significantly disrupt thyroid hormone supplies to the developing brain without altering blood thyroid hormone levels. "Such chemicals would simply be overlooked with the current regulatory tests," he says.

A wide range of new in vitro test methods have been developed over the past 15 years, addressing many entry points for disrupting the thyroid hormone system, including inhibition of iodide uptake and hormone synthesis, says Professor Kortenkamp.

But because they are not yet validated, the tests are not part of the data and testing requirements for EU regulations. "This puts risk assessors and producers alike in an incredibly difficult position. They have to decide on the status of chemicals as endocrine disruptors on the basis of incomplete and insufficient data," he says.

There is still a need for more assays that capture transport processes to the brain, across physiological barriers such as the placenta or the blood-brain barrier. "The biggest difficulty, however, is in measuring downstream effects on the brain."

## ATHENA advances

Professor Kortenkamp coordinates an EU-funded project called ATHENA, which aims to close critical gaps in test methods for chemicals that disrupt the thyroid hormone axis. The project is one of eight forming the European Cluster to Improve Identification of Endocrine Disruptors (EURION), which launched in 2019 and is funded by Horizon 2020.

"Downstream consequences of under- or over-supply of thyroid hormones on brain development is the most serious gap. We are working feverishly to improve this situation in ATHENA," he says. Project partners are also working on improved test methods for capturing the inhibition of thyroid hormone transport across the placenta and the blood-brain barrier.

"Some of the transporters are already well characterised, but there are likely to be additional cell membrane transporters. Partner labs in the ATHENA project are busy plugging this gap," he says.

ATHENA project work also points to "hopeful signs" that misplaced neurons appearing in the white matter of a developing brain could be a suitable endpoint for measuring the consequences of insufficient thyroid hormone supply.

Things are steadily moving in the right direction. As well as highlighting EURION projects, Professor Kortenkamp points to a recent French public-private partnership called PEPPER, which aims to provide the missing link between research and speedier validation of ED test methods.

## Andreas Kortenkamp

Andreas Kortenkamp is professor for human toxicology at Brunel University London, where he directs the Centre for Pollution Research and Policy. He researches the combined effects of chemicals on endocrine diseases. Professor Kortenkamp has served on the US National Research Council Panel on cumulative risk assessment for phthalates, and on the US National Research Council Panel for non-monotonic dose-response relations for endocrine disruptors. He was a member of the US Consumer Health Advisory Panel on the assessment of phthalates and produced the 2009 State of the Art Report on Mixture Toxicology for the European Commission and the 2012 State of the Art Assessment of Endocrine Disruptors. He joined the WHO/Unep panel for evaluating the state of the science of endocrine disruption in 2012. He coordinates the EU-funded ATHENA project on developing new test methods for thyroid hormone system disrupting chemicals.