

Methylmercury was proposed as Poison of the Month for April by the Working Group 3R Practice/ Alternative Methods

...because methylmercury exemplifies why developmental neurotoxicity requires new testing strategies. Human-based in vitro methods can more specifically reflect sensitive developmental processes of the nervous system than classical animal studies and thereby contribute to a more mechanistically informed and human-relevant risk assessment.

Mercury in aquatic ecosystems

Inorganic mercury enters aquatic ecosystems mainly via the atmosphere. Important sources include coal combustion in power plants, metal smelting, cement production, waste incineration, and artisanal small-scale gold mining. Natural processes such as volcanic eruptions, weathering of mercury-containing rocks, and wildfires also contribute to its release.

In aquatic systems, mercury is usually present as inorganic Hg(II), bound to particles, sulfide, chloride, or organic matter. In sediments and oxygen-poor zones, microorganisms can convert this Hg(II) into methylmercury. This organic form is particularly bioavailable and accumulates along aquatic food chains.



Methylmercury

Methylmercury: Why developmental neurotoxicity requires alternative testing methods

Methylmercury (CH_3Hg^+) is a highly toxic organic mercury compound. It is formed predominantly by microbial methylation of inorganic mercury in aquatic ecosystems and accumulates along the food chain. Particularly high concentrations are found in long-lived predatory fish such as tuna, swordfish, shark, or pike. For humans, the consumption of fish and seafood is therefore the main source of exposure.

From a toxicological perspective, the primary concern is not acute poisoning, but chronic exposure to low doses, particularly during pregnancy and early childhood. Methylmercury is efficiently absorbed in the gastrointestinal tract and forms cysteine complexes that structurally resemble neutral amino acids. Corresponding amino acid transporters facilitate transfer across the placenta into the fetal circulation, as well as transport across the blood–brain barrier into the central nervous system.

The vulnerability of the fetus results from the high temporal and spatial precision of prenatal neurodevelopment. Processes such as neuronal proliferation, migration, differentiation, synaptogenesis, and network maturation must occur in a coordinated manner within defined developmental windows. Methylmercury can interfere with several of these processes. Disturbances of calcium homeostasis, mitochondrial dysfunction, oxidative stress, cytoskeletal alterations, impaired neuronal migration, and effects on glia–neuron interactions have all been described. As a result, the architecture and function of neuronal circuits may be permanently altered without necessarily inducing gross morphological malformations. Functional consequences often become apparent only later, for example as subtle cognitive, motor, or sensory impairments. Accordingly, the tolerable weekly intake (TWI) of 1.3 $\mu\text{g}/\text{kg}$ body weight derived by EFSA is not based on acute maternal toxicity, but on epidemiological data on prenatal exposure, particularly associations between maternal mercury exposure and neuropsychological developmental parameters in children.

Developmental neurotoxicity is particularly challenging from a regulatory



DNT in vitro battery

Developmental neurotoxicity is still difficult to address in regulatory assessment, because classical studies are resource-intensive and species-specific differences limit their transferability to humans. International efforts therefore focus on new testing strategies. A central role is played by the OECD, which harmonizes methods for chemical safety testing. With contributions from EFSA, national authorities, and scientific working groups, the so-called DNT in vitro battery was established and is currently being further developed within the European research partnership PARC.

The DNT in vitro battery is not a single test, but a combination of different cell culture assays. These assays are designed to reflect key processes of brain development, including cell proliferation, neuronal differentiation, migration, neurite outgrowth, synapse formation, and network activity. The aim is to identify substances that can interfere with these processes and to prioritize them for further assessment.

From a regulatory perspective, the DNT in vitro battery is currently used primarily for screening, prioritization, and mechanistic interpretation within weight-of-evidence assessments. Methylmercury is particularly suitable as a reference substance in this context, because its developmental neurotoxicity is well documented and it can be used to test whether new systems reliably detect sensitive neurodevelopmentally relevant effects.

perspective because it is not defined by a single endpoint, but by disturbances of temporally coordinated processes such as proliferation, migration, differentiation, neurite outgrowth, synaptogenesis, and network maturation. This is precisely where classical animal studies reach their limits: developmental timing, maturation rates, brain organization, and toxicokinetic processes differ between species, meaning that sensitive exposure windows and subtle functional changes can only be transferred to humans to a limited extent. The added value of modern alternative methods lies in their ability to specifically assess key processes of human neurodevelopment and to distinguish specific developmental disturbances from unspecific cytotoxicity.

For methylmercury, this approach has already been applied in human-based model systems. These include neuronal progenitor cells, which can give rise to neurons and glial cells. Such models can be used to examine whether methylmercury interferes with early developmental steps such as cell proliferation, maturation, migration, or initial network formation. They show that methylmercury can impair developmental processes at concentrations that do not primarily cause cell death. Thus, methylmercury is not only a well-characterized example of developmental neurotoxicity, but also a reference substance for evaluating the performance of human-based in vitro methods, including in the context of the DNT in vitro battery.

For regulatory assessment, methylmercury remains challenging, as the main source of exposure, fish, also provides valuable nutrients such as omega-3 fatty acids, iodine, and selenium. The aim must therefore be to protect vulnerable groups without losing sight of the health benefits of occasional fish consumption.

By Ute Haßmann

Literature and links:

- [Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food](#)
- [Methylquecksilber in Fisch und Meeresfrüchten – gesundheitliche Bewertung neuer Daten aus der BfR-MEAL-Studie](#)
- [Methylquecksilber: Warum Schwangere und Stillende manche Arten von Fisch meiden sollten - BfR](#)
- Tal, T. et al. 2024. New approach methods to assess developmental and adult neurotoxicity for regulatory use: a PARC work package 5 project DOI: <https://doi.org/10.3389/ftox.2024.1359507>
- [Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity \(DNT\) In-Vitro Testing Battery \(EN\)](#)
- Foto of [Big Dodzy](#) on [Unsplash](#)

