DENAMIC

Developmental neurotoxicity assessment of mixtures in children

Newsletter 4

DENAMIC update

Since last newsletter, the DENAMIC consortium has come together at the RECETOX Institute in Brno for a progress meeting (October 2014). The DENAMIC project has now entered its final phase, in which all generated exposure and experimental data will be combined and integrated for the final assessments.

The consortium is currently also working on the second periodic review. At the end of the DENAMIC project a Final Workshop will be organized in Amsterdam (7-8 December). DENAMIC partners and guest speakers will discuss 1) tools and methods for (developmental) neurotoxicity testing; 2) experimental and epidemiological neurodevelopmental studies; 3) socio-economic impact of neurodevelopmental disorders. Moreover, the DENAMIC documentary will have its premiere.

The topic of this newsletter will be the DENAMIC data that is already published in the peer-reviewed scientific literature. A list is included on the right (continued on the next pages). Furthermore, DENAMIC researchers have contributed commentaries.

Coordinator:
Pim Leonards
Institute for Environmental Studies
VU University Amsterdam
+31 20 5989 509
pim.leonards@ivm.vu.nl

Subcoordinator:
Milou ML Dingemans
Institute for Risk Assessment Sciences
Utrecht University
+31 30 2534387
m.dingemans@uu.nl

This project is carried out with financial support from the European Community's Seventh Framework Programme [FP7/2007-2013], grant agreement n° 282957.

For more information **www.denamic-project.eu**

DENAMIC project publications

2012

van Staden JF, van Staden RI. Flow-injection analysis systems with different detection devices and other related techniques for the in vitro and in vivo determination of dopamine as neurotransmitter. A review. Talanta. 2012, 15;102:34-43.

2013

de Groot MW, Westerink RH, Dingemans MM. Don't judge a neuron only by its cover: neuronal function in in vitro developmental neurotoxicity testing. Toxicol Sci. 2013, 132(1):1-7.

Lee I, Viberg H. A single neonatal exposure to perfluorohexane sulfonate (PFHxS) affects the levels of important neuroproteins in the developing mouse brain. Neurotoxicology. 2013, 37:190-6.

Llop S, Julvez J, Fernandez-Somoano A, Santa Marina L, Vizcaino E, Iñiguez C, Lertxundi N, Gascón M, Rebagliato M, Ballester F. Prenatal and postnatal insecticide use and infant neuropsychological development in a multicenter birth cohort study. Environ Int. 2013, 59:175-82.

Llop S, Lopez-Espinosa MJ, Rebagliato M, Ballester F. Gender differences in the neurotoxicity of metals in children. Toxicology. 2013, 311(1-2):3-12.

Quaak I, Brouns MR, Van de Bor M. The dynamics of autism spectrum disorders: how neurotoxic compounds and neurotransmitters interact. Int J Environ Res Public Health. 2013, 10(8):3384-408.

DENAMIC

Developmental neurotoxicity assessment of mixtures in children

SEVENTH FRAMEWORK PROGRAMME

Newsletter 4

DENAMIC project publications (continued)

2014

Gugoasa LA, Stefan-van Staden RI, Ciucu AA, van Staden JF. Influence of the physical immobilization of dsDNA on the carbon based matrices of electrochemical sensors. Current Pharmaceutical Analysis 2014; 10(1), 20-29.

Legradi J, El Abdellaoui N, van Pomeren M, Legler J. Comparability of behavioural assays using zebrafish larvae to assess neurotoxicity. Environ Sci Pollut Res Int. 2014.

Meijer M, Dingemans MM, van den Berg M, Westerink RH. Inhibition of voltage-gated calcium channels as common mode of action for (mixtures of) distinct classes of insecticides. Toxicol Sci. 2014, 141(1):103-11.

Meijer M, Hamers T, Westerink RH. Acute disturbance of calcium homeostasis in PC12 cells as a novel mechanism of action for (sub)micromolar concentrations of organophosphate insecticides. Neurotoxicology. 2014, 43:110-6.

Meijer M, Hendriks HS, Heusinkveld HJ, Langeveld WT, Westerink RH. Comparison of plate reader-based methods with fluorescence microscopy for measurements of intracellular calcium levels for the assessment of *in vitro* neurotoxicity. Neurotoxicology. 2014, 45:31-7.

Roca M, Leon N, Pastor A, Yusà V. Comprehensive analytical strategy for biomonitoring of pesticides in urine by liquid chromatography-orbitrap high resolution mass spectrometry. J Chromatogr A. 2014.

Roca M, Miralles-Marco A, Ferré J, Pérez R, Yusà V. Biomonitoring exposure assessment to contemporary pesticides in a school children population of Spain. Environ Res. 2014, 131:77-85.

Stefan-van Staden RI, Moldoveanu I, van Staden JF. Pattern recognition of neurotransmitters using multimode sensing. J Neurosci Methods. 2014, 229:1-7.

Legradi et al. Environ Sci Pollut Res Int 2014: Comparability of behavioural assays using zebrafish larvae to assess neurotoxicity.

"The zebrafish (*Danio rerio*) is a well-established model species in developmental biology and an emerging model in behavioural and neurological studies. Zebrafish larvae display numerous behavioural patterns highly similar to rodents and humans. Behavioural tests performed with zebrafish embryos and larvae offer a great opportunity to improve neurotoxicity testing. No other system is capable of detecting such complex and diverse mechanisms in a living organism in such a high throughput manner and short time. In the last years, the number of behavioural studies conducted with zebrafish larvae has increased notably. The goal of this review was to provide an critical overview of behavioural assays commonly used to test substances for developmental neurotoxicity. Based on our findings, we provide recommendations which could help improve future behavioural studies performed with zebrafish larvae. To be fully accepted as a robust model for neurotoxicity testing, the observed behavioural effects need to be better characterized, understood and linked to neurotoxic mechanisms. The applied tests require a robust and reproducible design in order to maximize the knowledge gained from the tests. Standard procedures need to be defined and applied. Ideally, a standard for reporting and performance of these tests should be established." **- Jessica Legradi, PhD (Institute for Environmental Studies, VU University Amsterdam, the Netherlands)**

DENAMIC

Developmental neurotoxicity assessment of mixtures in children

SEVENTH FRAMEWORK PROGRAMME

Newsletter 4

Meijer et al. Neurotoxicology 2014: Acute disturbance of calcium homeostasis in PC12 cells as a novel mechanism of action for (sub)micromolar concentrations of organophosphate insecticides.

Meijer et al. Toxicol Sci 2014: Inhibition of voltage-gated calcium channels as common mode of action for (mixtures of) distinct classes of insecticides.

"We have published 2 papers on the acute inhibition of voltage-gated calcium channels (VGCCS) by (mixtures of) insecticides. In these papers we have demonstrated that insecticides from different classes potently inhibit VGCCs in a concentration-dependent manner. We thus argue that the inhibition of VGCCs is a common mode of action of insecticides related to calcium homeostasis and cell function and as such should be included in risk assessment. For binary mixtures of insecticides, mainly additive effects were found which illustrates the need to include mixture effects in human neurotoxicity risk assessment." **- Marieke Meijer, MSc (Institute for Risk Assessment Sciences, Utrecht University, Utrecht, the Netherlands)**

Roca et al. J Chromatogr 2014: Comprehensive analytical strategy for biomonitoring of pesticides in urine by liquid chromatography-orbitrap high resolution mass spectrometry.

"In this study we propose an analytical strategy that combines a target approach for the quantitative analysis of contemporary pesticide metabolites with a comprehensive post-target screening for the identification of biomarkers of exposure to environmental contaminants in urine using liquid chromatography coupled to high-resolution mass spectrometry (LC–HRMS). The quantitative method for the target analysis of 29 urinary metabolites of organophosphate (OP) insecticides, synthetic pyrethroids, herbicides and fungicides was validated after a previous statistical optimization of the main factors governing the ion source ionization and a fragmentation study using the high energy collision dissociation (HCD) cell. The method – LOQ was lower than 3.2 g L^{-1} for the majority of the analytes. For post-target screening a customized theoretical database was built, for the identification of 60 metabolites including pesticides, PAHs, phenols, and other metabolites of environmental pollutants. The results obtained showed that the proposed strategy is suitable for the determination of target pesticide biomarkers in urine in the framework of biomonitoring studies, and appropriate for the identification of other non-target metabolites." **- Marta Roca, PhD (Centre of Public Health Research, Valencia, Spain)**

Lee and Viberg Neurotoxicology 2013: A single neonatal exposure to perfluorohexane sulfonate (PFHxS) affects the levels of important neuroproteins in the developing mouse brain.

Perfluorinated compounds (PFCs) are commonly used in products such as surfactant and protective coatings and are globally found in the environment as well as in human cord blood, serum and breast milk. This study shows that neonatal exposure to perfluorohexane sulfonate (PFHxS) can alter neuroprotein levels, e.g. CaMKII, GAP-43, synaptophysin and tau, which are essential for normal brain development in mice.

DENAMIC
Developmental neurotoxicity assessment of mixtures in children

SEVENTH FRAMEWORK PROGRAMME

Newsletter 4

Gugoasa et al. Current Pharmaceutical Analysis 2014: Influence of the physical immobilization of dsDNA on the carbon based matrices of electrochemical sensors

"The influence of the physical immobilization of dsDNA in diamond paste, fullerene paste and carbon nanotubes on the behavior of biosensors was shown using three neurotransmitters: dopamine, epinephrine and norepinephrine." **– Prof Dr Raluca-Ioana Stefan-van Staden (National Institute of Research for Electrochemistry and Condensed Matter, Bucharest, Romania)**

Roca et al. Environ Res 2014: Biomonitoring exposure assessment to contemporary pesticides in a school children population of Spain.

"The exposure to pesticides amongst school-aged children (6 11 years old) was assessed in this study. One hundred twenty-five volunteer children were selected from two public schools located in an agricultural and in an urban area of Valencia Region, Spain. Twenty pesticide metabolites were analyzed in children's urine as biomarkers of exposure to organophosphate (OP) insecticides, synthetic pyrethroid insecticides, and herbicides. These data were combined with a survey to evaluate the main predictors of pesticide exposure in the children's population. A total of 15 metabolites were present in the urine samples with detection frequencies (DF) ranging from 5% to 86%. Statistical significant differences were found between exposure subgroups. In conclusion, to our knowledge this is the first HBM study developed in Spain reporting the exposure to non-persistent pesticides in children population. Therefore, the result of this pilot study have allowed us to determine the feasibility of the methodology employed as well as an evaluation of the limitations and "lessons learned" to take them into consideration in future larger scale biomonitoring studies" **- Marta Roca, PhD (Centre of Public Health Research, Valencia, Spain)**

Stefan-van Staden et al. J Neurosci Methods 2014: Pattern recognition of neurotransmitters using multimode sensing.

"The multimode microsensors based on diamond paste modified with 5,10,15,20-tetraphenyl-21H,23H-porphyrine, hemin and protoporphyrin IX, proved to be reliable screening tools for pattern recognition of neurotransmitters such as dopamine, epinephrine and norepinephrine in whole blood and urine samples. Accordingly, they can be used as tools for assessment of neurotoxicity induced by hazardous substances (e.g., pesticides) and also to assess the health state of a population. A multimode sensing system was proposed for pattern recognition, based on stochastic mode and differential pulse voltammetry mode. Accordingly with the statistic evaluation of the results at 99.00% confidence level, both modes can be used for pattern recognition and quantification of neurotransmitters with high reliability. The best multimode microsensor was the one based on diamond paste modified with protoporphyrin IX." **- Prof Dr Raluca-Ioana Stefan-van Staden (National Institute of Research for Electrochemistry and Condensed Matter, Bucharest, Romania)**

DENAMIC

Developmental neurotoxicity assessment of mixtures in children

SEVENTH FRAMEWORK PROGRAMME

Newsletter 4

DENAMIC partners and guest speakers will discuss:

- Tools and methods for (developmental) neurotoxicity testing
- Experimental and epidemiological studies of developmental neurotoxicity
- Socio-economic impact of neurodevelopmental disorders