

## Newsletter 3

### DENAMIC meetings

March 10-11<sup>th</sup> 2014, the DENAMIC midterm workshop with title 'Novel tools and methods for the screening of chemicals for developmental neurotoxicity' was held at the Free University in Amsterdam. DENAMIC partners and international speakers contributed to the workshop by talking about novel tools and methods to screen for (developmental) neurotoxicity; *in vitro* assays, reduction and refinement of animal studies; epidemiological evidence for effects of environmental exposure on child neuropsychological development and the use of biomarkers in developmental neurotoxicity.

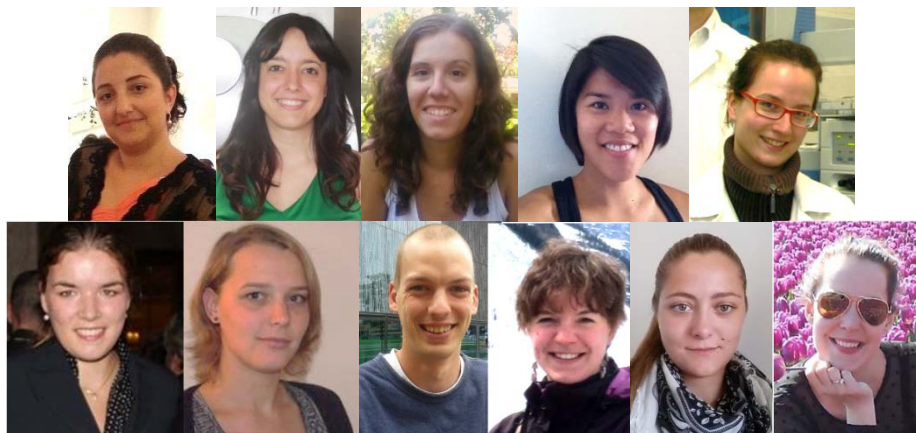
Also our 5<sup>th</sup> progress meeting was held adjacent to the workshop in Amsterdam, during which experimental results were presented and discussed among the partners and member of the Scientific Advisory Board gave their advise about the project.

In October 2014 a progress meeting will be held in Brno (CZ) at the RECETOX Institute with the focus on data integration and risk assessment of mixtures.

DENAMIC results have also recently been presented at several national and international meetings, including the 35th Dutch Society of Toxicology meeting (NL), the DNT4 meeting in Philadelphia (US), the 18th International Congress on *in vitro* Toxicology (ESTIV2014) in Egmond-aan-Zee (NL), SETAC North America Nashville (US), Metabolomic conference (UK).

### Young DENAMIC scientists

A considerable amount of the work in DENAMIC is performed by young scientists, including PhD students and postdocs. A lot of contacts are made during our meetings, and collaborations and visits between the different DENAMIC partners are promoted. On the next pages, our young scientist tell you about their successes and challenges within the DENAMIC project.



Young DENAMIC scientist. From left to right and top to bottom: Iuliana Moldoveanu (INCEMC), Andrea Cabrera Pastor (CIPF), Belén Gómez Gimenez (CIPF), Iwa Lee (UU), Eliska Cechova (MU), Marta Seifertova (MU), Marieke Meijer (IRAS), Max Leenders (IRAS), Marijke de Cock (VU), Sonja Buratovic (UU), Ilona Quak (VU). Not in picture: Jessica Legradi (VU).

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For more information [www.denamic-project.eu](http://www.denamic-project.eu)

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### Young DENAMIC scientists – part 1

PhD student **Iuliana Moldoveanu** (INCEMC): “My main task in the project is to develop new electrochemical sensors for fast screening of neurotransmitters. While finding the best sensors and characterizing them was a very hard work. It is great to see how these new tools can be used for the real samples send by our colleagues from VU Amsterdam and Valencia, and how the behavioural studies will correlate with the changes in neurotransmitters concentrations for zebrafish and rats samples.”

**Andrea Cabrera Pastor** (CIPF): “During the last two years, I have participated in DENAMIC project. I think it is necessary to identify which pesticides could be dangerous for the brain development and to find molecular alterations that are responsible for these effects. Results obtained indicate that some pesticides induce gender-differential effects on spatial learning. We are analyzing *in vivo* the mechanisms affected in learning and memory and motor activity as a consequence of developmental exposure to different types of pesticides. Hence, we expect to better understand how pesticides are altering these processes.”

PhD student **Iwa Lee** (UU): “I think that the DENAMIC-project has a very interesting approach and idea, as it combines different research models and aspects to study the mechanism(s) of action and the connection of early life exposure to neurotoxic agents. Pesticides may induce adult behavioral changes and disrupt neuroprotein levels crucial for normal brain development. We are also investigating exposure to low-dose mixtures of the pesticides, to elucidate which compound contributes most of to the toxicity and the possible mechanism behind it.”

“My name is **Jessica Legradi** (VU) and I work as a PostDoc within Denamic. I develop new assays to assess DNT using zebrafish as a model species. By using an *in vivo* system I hope to be able to better predict risks for human exposures. The main advantage of my assays is that I can test many more substances than with other standard *in vivo* assays in rodents. My biggest challenge is to establish the zebrafish as an expected model for neurotoxicity testing. I enjoy working within the Denamic project, especially with such an international and interdisciplinary team of excellent researchers.”

“My name is **Belén Gómez Giménez** (CIPF). Last year the government of Valencia granted me a fellowship to do my PhD thesis. This year I have performed behavioral studies with the pups of the rats, to study how pesticides affect the brain development. Our next objective is to characterize the molecular mechanisms including microdialysis studies to analyze the neurotransmitters and signal transduction. Furthermore, we have sent samples to PROTEOM SCIENCE, a partner of DENAMIC and we are currently analyzing if the proteins altered are connected to neurotransmission and animal behavior.”

PhD student **Eliska Cechova** (MU): “After my Masters where I determined estrogens in wastewaters I wanted to work with biological samples. I am a second-year PhD student and I am developing analytical methods for determination of organic pollutants in milk, currently working on non-destructive clean-up methods of fat removal. We compare different extraction techniques and instruments in order to obtain the lowest limits of detection. This work brings me interesting experience and the opportunity to meet nice and inspiring people during project meetings.”

### Recent DENAMIC publications (I)

- de Groot MW, Dingemans MM, Rus KH, de Groot A, Westerink RH. Characterization of calcium responses and electrical activity in differentiating mouse neural progenitor cells in vitro. *Toxicol Sci.* 2014; 137(2):428-35.
- 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.
- 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.
- Buratovic S, Viberg H, Fredriksson A, Eriksson P. Developmental exposure to the polybrominated diphenyl ether PBDE 209: Neurobehavioural and neuroprotein analysis in adult male and female mice. *Environ Toxicol Pharmacol.* 2014;38(2):570-585.
- van Staden J.F. and R.I. Stefan-van Staden. Flow-injection analysis systems with different detection devices and other related techniques for the in vivo and in vitro determination of dopamine as neurotransmitter. A review. *Talanta*, 102, 34-43, 2012.

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### Young DENAMIC scientists – part 2

PhD student **Marta Seifertova** (MU): “My first contact with the DENAMIC project was at the beginning of my PhD studies in September 2012. I started developing the analytical procedures for determination of pyrethroids and other neurotoxic compounds and their metabolites in biological matrices. At the moment I am working on the analysis of rat tissues from *in vivo* experiments and with our team we are dealing with mother milk analysis. DENAMIC provides me an interesting opportunity how to get to know the international cooperation of the scientists in the European project and to broaden the horizon of my professional knowledge.”

“I’m **Max Leenders**, a postdoc at the division of Environmental Epidemiology at the Institute for Risk Assessment Sciences (IRAS) of the Utrecht University. Within this function, I’m responsible for performing statistical analyses that are performed in large datasets such as the ones derived from –omics studies. The DENAMIC project offers a great opportunity to perform these types of analyses and, because of the different systems in which the data is collected, to examine whether it’s possible to combine these different datasets with the help of bioinformatics.”

PhD student **Sonja Buratovic** (UU): “Within my PhD project I focus mainly on behavioural disturbances and memory and learning impairments following early life exposure to different classes of pesticides and environmental pollutants. Together with our partners within DENAMIC we are able to integrate different model systems and perform both mechanistic studies as well as studies on the biological impact this exposure may have on the individual. Hopefully the knowledge generated by the DENAMIC project will be used to highlight the importance of studying neurotoxicity of mixtures and provide a firm foundation for risk management.”

“My name is **Marieke Meijer** and I am a PhD candidate at the Neurotoxicology research group, Institute for Risk Assessment Sciences (IRAS). During my study Toxicology and Environmental Health I got interested in *in vitro* neurotoxicology and I was happy to hear that I could work on the DENAMIC project as a PhD candidate. I am involved in developing *in vitro* screening tools for neurotoxicity for which I performed and explored several *in vitro* screening tests. I got some nice results which I disseminated at several international and national congresses and have published in peer-reviewed journals.”

Postdoc **Marijke de Cock** (VU): “Having done my PhD on the OBELIX project, it was very exciting to get offered the opportunity to join DENAMIC as a post-doc researcher. I work on the LINC study, the Dutch cohort. Currently I’m also working on a codebook that we can use for the data-analysis. My OBELIX experience comes in quite handy here, but as I only joined DENAMIC not too long ago, it is also challenging as I am not familiar with all the ins and outs of the project (yet). However, with the great (and very friendly) team of researchers involved, I’m sure this will not take too long, and I’m looking forward to continue on this project.”

PhD student **Ilona Quaak** (VU): “My focus is on early life exposure to neurotoxic compounds and the development of neurodevelopmental disorders during childhood, such as Attention Deficit Hyperactivity Disorder and Autism Spectrum Disorder. I feel this project fits me very well, as I previously studied Clinical and Developmental Psychology at the University of Groningen. For DENAMIC, we are using data from LINC, which is a mother child cohort based on several cities in the Netherlands. I am conducting research at the university on the one hand, but I also maintain and extend the cohort. I hope that with the current study, we will also be able to advise policy makers and put things into practice, so future generations might be protected.”

### Recent DENAMIC publications (II)

- Gugoasa, LA, RI Stefan-van Staden, AA Ciucu, JF van Staden. Influence of the physical immobilization of dsDNA on the carbon based matrices of electrochemical sensors. *Current Pharmaceutical Analysis*, 10(1), 20-29, 2014.
- van Staden, J.F., R. Georgescu, R.I. Stefan-van Staden, I. Calinescu. Graphene based dot microsensors for the assay of adenine, guanine and epinephrine. *J Electrochem Soc.*, 161(2), B3014-B3022, 2014
- Georgescu, R., J. Frederick van Staden, J. Moldoveanu, J. Calinescu, RI Stefan-van Staden. New amperometric microsensors for the analysis of serotonin in urine samples. *J Electrochem Soc.*, 161, 000, 2014