NEUROSOME

Exploring The Neurological Exposome

THE SH-SY5Y NEUROBLASTOMA CELL LINE AS A MODEL TO EVALUATE THE EFFECTS OF ENVIRONMENTAL POLLUTANTS ON NEURODEVELOPMENT

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This project has received funding from the European Union's H2020 Framework Programme under grant agreement No - GA - 766251

H2020-MSCA-ITN-2017 GA - 766251

- This project is part of NEUROSOME, an European integrated training network which investigates the causal associations of cumulative exposure to environmental chemicals of children and neurodevelopmental disorders.

Neurodevelopmental disorders and environmental pollutants

H2020-MSCA-ITN-2017 GA - 766251

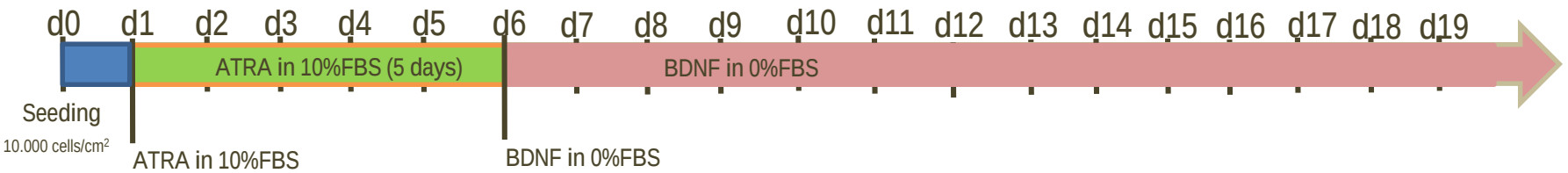
Neurodevelopmental disorders are a group of conditions with onset in the developmental period and that comprise conditions such as attention deficit-hyperactivity disorder (ADHD) and autism spectrum disorder (ASD).
Several environmental pollutants, ranging form heavy metals, pesticides to plastic derivates and other organic compounds, have been associated with neurodevelopmental disorders.
This project aims to understand the effects of cumulative exposure of central nervous system cells models to pollutants.

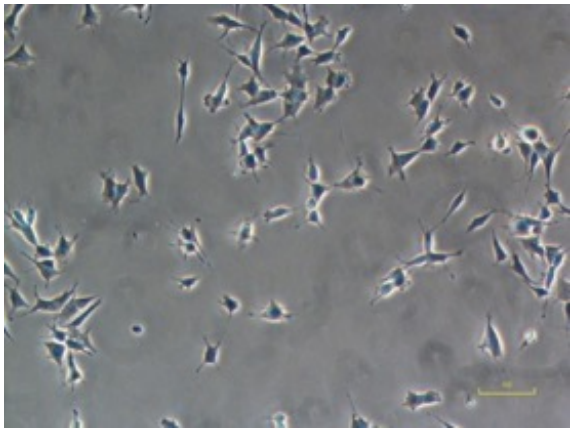
SH-SY5Y neuroblastoma cell line

SH-SY5Y (ATCC® CRL-2266™) is a human cell line that can be indefinitely grown as neuroblast-like cells or can be differentiated into neuron-like cells.

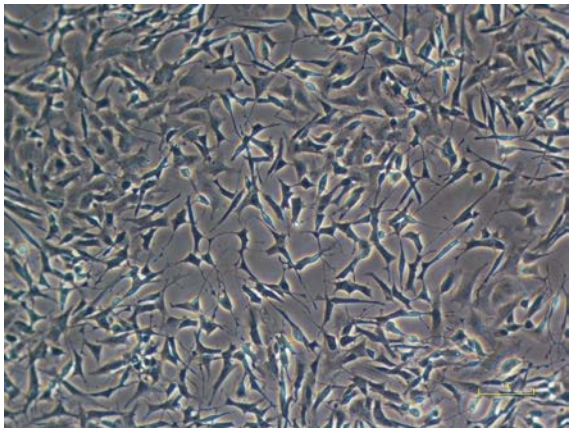
We have developed a differentiation protocol based on Encinas *et al.* (2000) protocol in which, cells are successively treated with **all-trans retinoic acid** (ATRA, 10 µM) and **brain derived neurotrophic factor** (BDNF, 50 ng/mL) to obtain a neuronal morphology resembling that of neuronal primary cell cultures.

SH-SY5Y differentiation protocol

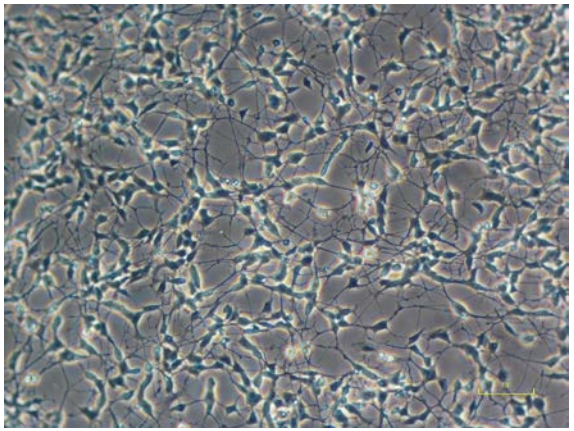




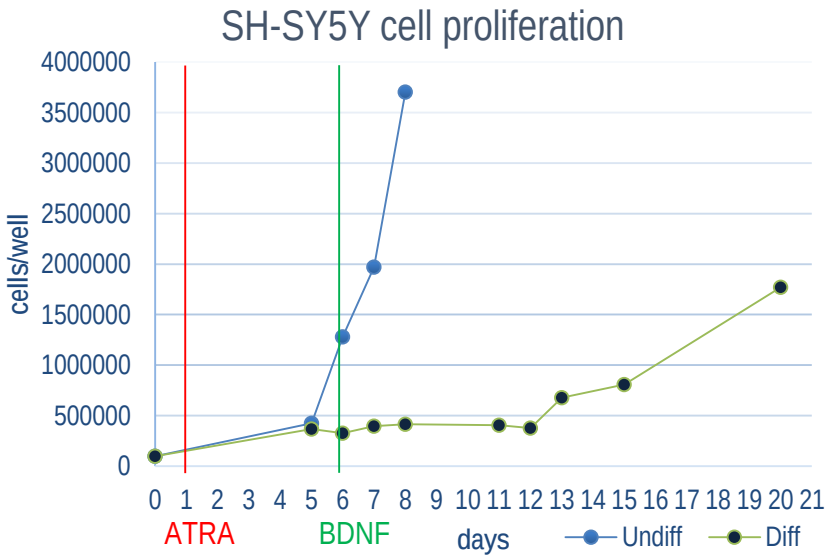
24 h after seeding



7 days in BDNF (12 days of diff.)



5 days in ATRA



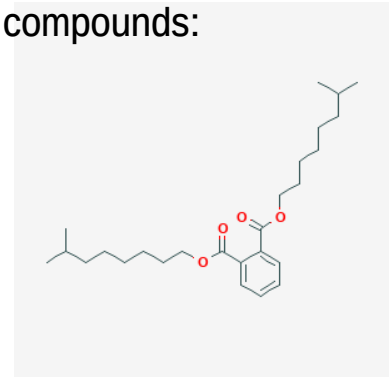
Cell counting performed with a Malassez hematocytometer

Heavy metals:

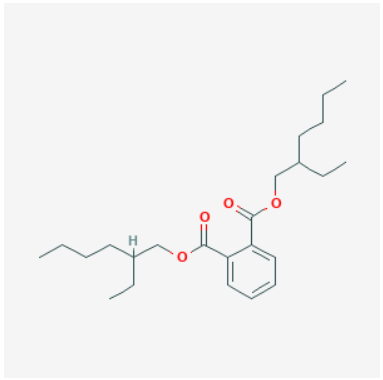
Methylmercury chloride (CH_3HgCl)
Mercury (II) chloride ($HgCl_2$)
Lead dinitrate ($Pb(NO_3)_2$)
Cadmium chloride ($CdCl_2$)
Manganese (Mn)

Prenatal and postnatal exposure to these heavy metals have been adversely associated with neurodevelopment (Tellez-Rojo et al., 2006; Chung et al., 2015; Rothenberg et al., 2016; Wang et al., 2016; Barbone et al., 2018) and increased risk of neurodevelopmental disorders (Lee et al., 2016; Ji et al., 2018)

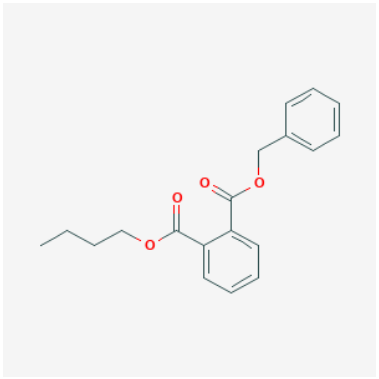
Organic compounds:



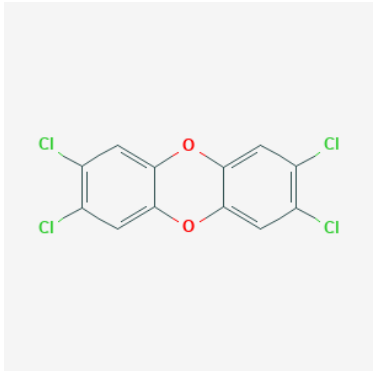
Di(2-ethylhexyl) phthalate (DeHP)



Diisononyl phthalate (DiNP)



Butyl benzyl phthalate (BBzP)



2,3,7,8-thetrachlorodibenzodioxin (TCDD)

Molecular structures from Pubchem

Pollutants

Phthalates can act as endocrine disruptors (Axelsson *et al.,* 2015) and their prenatal and early age exposure has been associated with poorer develpment (Kim et al., 2011; Zhang et al., 2019) and neurodevelopmental disorders (Telsa et al., 2012; Engel et al., 2018).

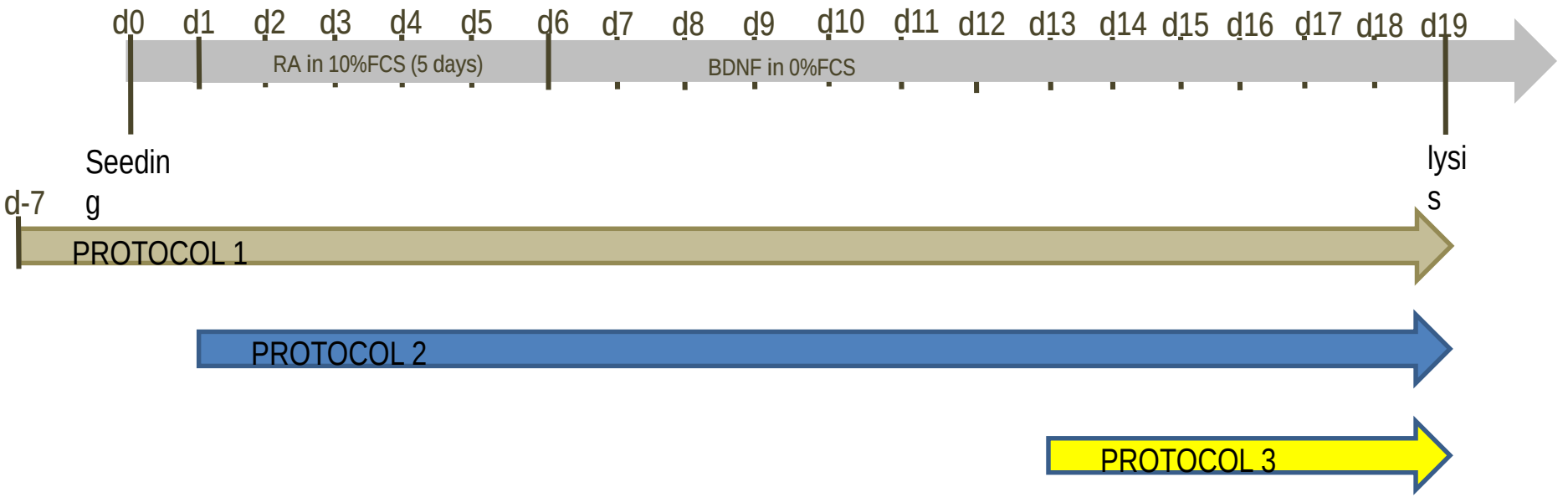
Likewise, TCDD has also been associated with imparied neurodevelopment and several disorders (Nishijo et al., 2014; Tran et al., 2016).

Pollutants Treatment

SH-SY5Y cells will be exposed to pollutants at different stages of differentiation. Pollutants incubation consist of their addition to the growth medium, either alone or in mixture, at concentrations depending on the PBPK modelling for the brain in D. Sariganis' laboratory (S. Karakitsios, AUTH) in the context of HEALS.

		DEHP	DiNP	BBzP	Hg	Pb	Cd
Brain	Max (nM)	13.899	14.882	2.278	89.735	27.053	41.420
	Mean (nM)	1.251	1.488	0.209	23.431	8.213	18.575
	Min (nM)	0.695	0.298	0.038	2.493	4.831	9.181

Pollutants treatment



Analysis

- Genomic expression: qPCR and/or microarrays.
- Protein expression: western blot, immunostaining, ELISA...
- Cell viability: alamar blue, Malassez cell counting, sulforhodamine B.
- Metabolic activity: oxidative stress markers, ATP or NADH levels...

Significance of the results and further research

Genomic expression, protein expression, metabolic activity and cell viability of the cultures will be studied in order to assess the effects of the pollutants (alone or in mixture).

The results of this preliminary model will be indicative for further research in more complex models comprising glial and neuronal cells, as well as representative blood brain barrier epithelial cells.

One of the models that will be used it is **mini-brains** (Pavoni *et al*., 2018), 3D neural microenvironments derived from human iPSCs with neural and glial cells that simulate discrete brain regions.