An inter-laboratory case study to determine the added value of the Zebrafish Light-dark transition test to predict developmental neurotoxicity

Ainhoa Alzualde[1], Jui-Hua Hsieh[2], Lee Ellis[3], Valentina Schiavone[4], Lisa Truong[5], Jessica Legradi[6], Davide Rubbini[4], Cindy Woodland[7], Nils Kluver[8], Kristen Ryan[2], Mamta Behl[2], Javier Terriente[4], Arantza Muriana[1], Robyn Tanguay[5], Magdalini Sachana[9], Anna Price[10], Bridgett Hill[11, 12], Stephanie Padilla[12], Tim Shafer[12], Ellen Hessel[13]

[1] Biobide, Paseo Mikeletegi 56, 20009 San Sebastian, Spain. alzualde@biobide.es; [2] Division of the National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA.;[3] National Research Council of Canada, 1411 Oxford Street, Halifax, Nova Scotia, B3H 3Z1.; [4] ZeClinics SL, IGTP Edifici Muntanya (c/de Can Ruti, Camí de les Escoles s/n; 08916 BDN, Lab P0-8), 08916 Barcelona, Spain.; [5] Department of Environmental and Molecular Toxicology, Sinnhuber Aquatic Research Laboratory, Oregon State University, Corvallis, OR ; [6] Environment & Health, VU University Amsterdam, 1081 HV, Amsterdam, the Netherlands. ; [7] Innovation and Science Integration Unit, New Substances Assessment and Control Bureau, Healthy Environments and Consumer Safety Branch, Health Canada / Government of Canada.; [8] Department of Bioanalytical Ecotoxicology, Helmholtz Centre for Environmental Research GmbH - UFZ Leipzig, Germany; [9] Organisation for Economic Co-operation and Development (OECD), Paris, France.; [10] European Commission -DG Joint Research Centre (JRC), Ispra, Italy.; [11] Oak Ridge Institute for Acience and Education, Oak Ridge, TN, USA; [12] Center for Computational Toxicology and Exposure,Biomolecular and Computational Toxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA; [13] Centre for Health Protection, National Institute for Public Health and the Environment (RIVM), P.O. Box 1, 3720BA Bilthoven

INTRODUCTION

Developmental neurotoxicity (DNT) entails one of the most complex areas in toxicology.

OECD test guidelines for DNT (TG 426 and 443) are only occasionally carried out and the predictivity of these *in vivo* animal tests for human health effects may be limited. There is a high need for human-relevant *in vitro* models to assess DNT potential of chemicals.

OECD is, therefore, building a guidance document containing a testing strategy to predict DNT. This testing strategy consists of a combination of *in vitro* tests encompassing the critical processes in brain development.

The aim of this study is to investigate the added value of the zebrafish DNT behavioral model in this testing strategy.

MATERIAL AND METHODS

Testing system: zebrafish larvae up to 120 hours post fertilization (hpf), not considered experimental animal under the current European Directive 2010/63/EU.

Treatment: 6 - 120 hpf in 96 well plates, at 28.5 °C. 7 concentrations per chemical + vehicle control.

Assay: light-dark transition test: acclimation (5' light + 5' dark) + tracking (10' light + 10' dark + 10' light + 20' dark). Additional 40' light + 40' dark for some labs.

Testing chemicals: 28 chemicals will be tested **blinded** (Table 1)

Statistic analysis: Benchmark dose analysis (BMC)[1].

- focus on dose-response trend and onset of the response;
- used in quantitative risk assessment.

Institutions involved: 12 (see poster authors).

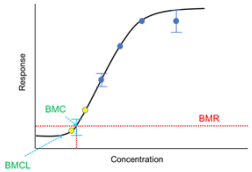

Table 1: Testing chemicals

Testing compounds	CAS N	Testing compounds	CAS N
2-Ethylhexyl diphenyl phosphate	1241-94-7	Kepone	143-50-0
3,3',5,5'-Tetrabromobisphenol A	79-94-7	Methyl parathion	298-00-0
Acetamiprid	135410-20-7	Nicotine	54-11-5
Aldicarb	116-06-3	Parathion	56-38-2
Allethrin	584-79-2	Permethrin	52645-53-1
Benomyl	17804-35-2	tert-Butylphenyl diphenyl phosphate	56803-37-3
Chloramben	133-90-4	Thiacloprid	111988-49-9
Chlorpyrifos	2921-88-2	Tri-o-cresyl phosphate	78-30-8
Cypermethrin	52315-07-8	Trichlorfon	52-68-6
Deltamethrin	52918-63-5	Triphenyl phosphates isopropylated	68937-41-7
Diazinon	333-41-5	Tris(1,3-dichloro-2-propyl) phosphate	13674-87-8
Dieldrin	60-57-1	Tris(2-chloroethyl) phosphate	115-96-8
Dimethoate	60-51-5	Tris(2-chloroisopropyl)phosphate	13674-84-5
Heptachlor	76-44-8	Tris(methylphenyl) phosphate	1330-78-5

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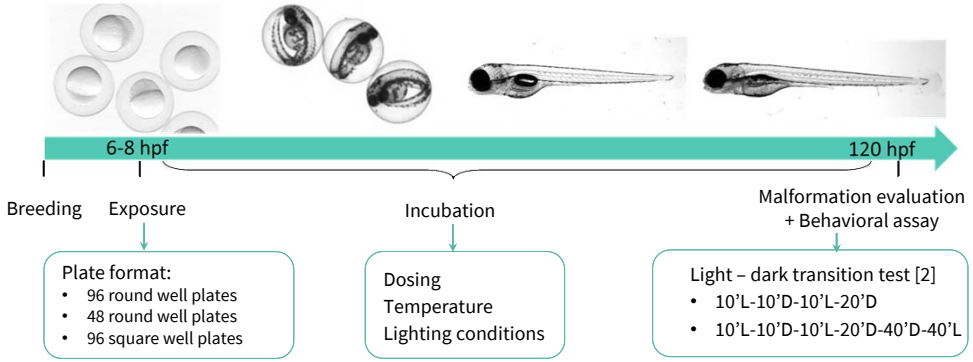
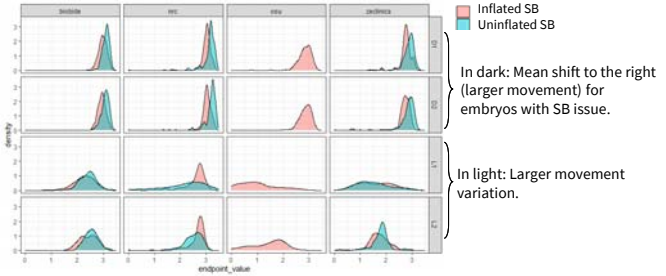
Experimental workflow



What we learnt so far

- Any malformed larvae should be discard from the Behavioral analysis.
- Swim bladder formation is essential for a normal performance of the larvae on the light-dark transition test.
- Well volume is critical for proper embryo development and specificaly for swim bladder formation (Figure 1).

Figure 1: effect of swim bladder (SB) inflation on light-dark transition test



CONCLUSIONS

- ✓ Zebrafish behavior models can have an added value to the OECD guidance document for DNT.
- ✓ Harmonization of the Protocol is essential. Key players in the field are working together.
- ✓ Inter-laboratory replication is a challenge.
- ✓ Well volume is critical for proper embryo development and behavioral performance.

REFERENCE
[1] Hsieh et al., 2019. Toxicological Sciences167 (1): 92–104.
[2] Quevedo et al., 2019. Toxicological Sciences 168(1): 225-240.