NeuroToxicology 44 (2014) 366

Contents lists available at ScienceDirect

NeuroToxicology

Reply to Letter to the Editor

## Reply on 'Prerequisites for a reliable introduction of *in vitro* neurotoxicity testing within the REACH framework'

*To the Editor,*

In his letter to the editor, Dr. Zarros (2014) underlines the need for *in vitro* and *in silico* neurotoxicity testing. I obviously agree, but in my view, current *in silico* neurotoxicity testing is still hampered by an overall lack of functional *in vitro* data. As outlined earlier (Westerink, 2013), reliable modeling approaches are available for just a small subset of the thousand of chemicals. In his letter, Dr. Zarros (2014) advocates the implementation and further development of the use of neuropathological and cytopathological parameters. Even though neuronal function may be disturbed before neuropathological and cytopathological damage is visible, the use of pathological parameters can have an important advantage; neuropathological damage is an obvious sign of neurotoxicity.

Nonetheless, as also holds for *in vitro* parameters for neuronal function, it is less clear exactly how much damage is required before (sub) clinical symptoms occur. Solving this challenge will require translational research to bridge the gap between *in vitro* and *in vivo* (Llorens et al., 2012; van Thriel et al., 2012), but will be a big step from a legislative perspective. To do so, we may need to know the biological relevance of the hundreds of endpoints studied in *in vitro* assays, including pathological endpoints.

While doing this, we need to keep in mind that the biological relevance of a particular endpoint and the degree of effect on that endpoint is not fixed. For example, even when looking at cell viability, it can be challenging to determine the biological relevance of a particular effect as the degree of biological variations and the sensitivity of the *in vitro* assay may not be compatible with the biological relevance. For *in vitro* cell viability data, standard deviations of ~5% are not uncommon. In practice this means that it will be difficult to reliably establish effects of 1–2%. One could argue that effects <5% lack biological relevance and that increasing the concentration of the test compound will result in a larger effect (thereby allowing for the use of a benchmark concentration). However, depending on the *in vitro* model used, this can result in false negatives. As an example, heterogeneous neuronal cultures can consist of a wide diversity of cell types. If a particular cell type represents only 2% of the total cell population, a specific but lethal effect on just this single cell type will not show up in cell viability assays, also not at higher concentrations of the test compounds. Nevertheless, this 2% of the cells may be essential for, *e.g.*, neuronal network function. This may be even more challenging for developmental neurotoxicity, where (small) effects may be deleterious within a specific developmental window, but (relatively) harmless at other developmental stages. Nonetheless, these difficulties can be tackled by using a battery of *in vitro* tests covering multiple endpoints.

For sure, this does not mean that *in vitro* research is useless, but we may need to focus on characterization of *in vitro* models, identification of key processes for neuronal function and survival in the models, and defining minimal relevant effect sizes for these endpoints, also at particular developmental windows. Doing so will aid in implementing the use of *in vitro* data for REACH and other legislative frameworks (Bal-Price et al., 2008, 2010; Llorens et al., 2012; van Thriel et al., 2012). Combined with validation of the (biological) relevance of the *in vitro* observed effects in alternative species (Levin et al., 2009; Peterson et al., 2008), the increase in *in vitro* data will ultimately pave the way for further implementation of *in silico* data in legislative frameworks as suggested by Dr. Zarros.

### Conflicts of interest

The author declares that no conflicts of interest exist.

### References

Bal-Price AK, Hogberg HT, Buzanska L, Lenas P, van Vliet E, Hartung T. *In vitro* developmental neurotoxicity (DNT) testing: relevant models and endpoints. Neurotoxicology 2010;31:545–54.

Bal-Price AK, Suñol C, Weiss DG, van Vliet E, Westerink RH, Costa LG. Application of *in vitro* neurotoxicity testing for regulatory purposes: symposium III summary and research needs. Neurotoxicology 2008;29:520–31.

Levin ED, Aschner M, Heberlein U, Ruden D, Welsh-Bohmer KA, Bartlett S, et al. Genetic aspects of behavioral neurotoxicology. Neurotoxicology 2009;30:741–53.

Llorens J, Li AA, Ceccatelli S, Sunõl C. Strategies and tools for preventing neurotoxicity: to test, to predict and how to do it. Neurotoxicology 2012;33:796–804.

Peterson RT, Nass R, Boyd WA, Freedman JH, Dong K, Narahashi T. Use of non-mammalian alternative models for neurotoxicological study. Neurotoxicology 2008;29:546–55.

van Thriel C, Westerink RH, Beste C, Bale AS, Lein PJ, Leist M. Translating neurobehavioural endpoints of developmental neurotoxicity tests into *in vitro* assays and readouts. Neurotoxicology 2012;33:911–24.

Westerink RH. Do we really want to REACH out to *in vitro*? Neurotoxicology 2013;39:169–72.

Zarros A. Prerequisites for a reliable introduction of *in vitro* neurotoxicity testing within the REACH framework. Neurotoxicology 2014;44:365.

Remco H.S. Westerink*
*Neurotoxicology Research Group, Toxicology Division, Institute for Risk Assessment Sciences (IRAS), Faculty of Veterinary Medicine, Utrecht University, The Netherlands*

*Correspondence to: P.O. Box 80.177, NL-3508 TD Utrecht, The Netherlands. Tel.: +31 30 2533487; fax: +31 30 2535077
*E-mail address:* r.westerink@uu.nl (Remco H.S. Westerink).

Received 6 June 2014
Available online 13 June 2014

http://dx.doi.org/10.1016/j.neuro.2014.06.004
0161-813X/