Special attention to the neurotoxic potential of 91 compounds from NTP, assessment using zebrafish

Celia Quevedo, Arantza Muriana and Ainhoa Alzualde

Biobide; Pº Mikeletegi 56, San Sebastian, Spain *alzualde@biobide.es*

OBJECTIVE

Focus on the neurotoxic potential of 91 compounds from NTP program

Introduction and methodology in brief

Developmental toxicity and neurotoxicity of 91 compounds sent by National Toxicology Program (NTP) were assessed **blinded** using zebrafish embryos. After evaluating the Maximum Tolerated Concentration, developmental toxicity assay was performed testing chemicals at 5–8 concentration points and assessing morphological alterations and mortality at 2 and 4 dpf (days-post-fertilization). Then, teratogenic index (TI) was calculated as the ratio between LC50 and EC50. **For neurotoxicity evaluation**, the lowest concentration where morphological effects appeared was selected as the highest concentration for behavioral assay. Embryos were treated at 3 dpf with 5 concentrations of each chemicals. After 48 hours of exposure, locomotor activity was analyzed as indicative of behavior.

Larvae from developmental toxicity assay treated at the highest concentration without effect and at the first toxic concentration were used for **internal dosing analysis** to determine the real concentration at which toxic effects were induced. Moreover, concentration of chemicals in the medium was also evaluated in the same experimental groups.

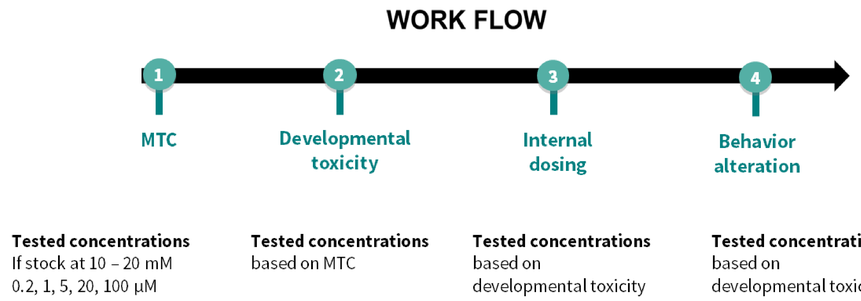

Figure 1: Workflow of the battery of assays performed to evaluate potential toxicity of 91 tested compounds

1 Compounds classified as neurotoxic / neuroactive

Behavioral assay effects:

Neuroactive is considered any compound that increased locomotor activity or altered behavioral profile (reaction to light – dark).

Neurotoxic is considered any compound that induced hypoactivity in concentrations where larvae were morphologically non altered.

Test item identification			Behavioral assay		Developmental toxicity	
			Effect	LOAEL (µM)	NOAEL (µM) at 4	Classification
1174	PE-2086	Deltamethrin	neuroactive	0.005	0.05/ND	1
1445	PE-2044	Deltamethrin	neuroactive	0.025	0.02/ND	1
1092	PE-2050	Dieldrin	neuroactive	0.05	0.2/288.4	1
1215	PE-2077	Tetraethylthiuram disulfide	neurotoxic	0.3	0.1/ND	1
1814	PE-2067	Permethrin	neuroactive	0.5	1/5.02	1
1937	PE-2028	Benzo(k)fluoranthene	neuroactive	0.5	0.05/1.95	1
1453	PE-2055	Heptachlor	neuroactive	0.5	4/337.2	1
1324	PE-2061	Lindane	neuroactive	0.5	2/282.9	1
1401	PE-2049	Dichlorodiphenyltrichloroethane (DDT)	neuroactive	0.5	1/443.5	1
1918	PE-2040	Chlorpyrifos (Dursban)	neuroactive	1	2/882.1	1
1367	PE-2006	2,2',4,4'-Tetrabromodiphenyl ether	neuroactive	2	15/559.0	4
1196	PE-2001	2-Ethylhexyl diphenyl phosphate (EHDP)	neuroactive	3	6/363.6	2
1881	PE-2054	Fluorene	neuroactive	4	15/1876	2
1232	PE-2025	Benzo(a)pyrene	neuroactive	5	>5/2.03	3
1994	PE-2066	Parathion	neuroactive	8	4/696.7	1
1262	PE-2075	Tebuconazole	neurotoxic	10	15/408.0	1
1109	PE-2014	Acenaphthene	neuroactive	15	20/25.52	1
1756	PE-2021	Amoxicillin	neuroactive	15	>100/ND	3
1705	PE-2046	Diazepam	neurotoxic	15	4/18.05	1
1123	PE-2000	1-Methyl-4-phenylpyridinium iodide	neuroactive	30	>100/16.16	3
1965	PE-2084	Valproic acid sodium salt	neuroactive	50	50/130.0	4
1786	PE-2042	Colchicine	neurotoxic	50	75/0.503	2
1884	PE-2030	Berberine chloride	neuroactive	60	>100/12.01	3
1681	PE-2012	6-Hydroxydopamine hydrochloride	neurotoxic	100	>5/ND	3
1327	PE-2069	Phenobarbital	neurotoxic	100	>100/22.52	3

x 2

DNT/NT
Flame Retardant
PAH
Other/Drug

Figure 2: List of neuroactive and neurotoxic compounds detected amongst 91 compounds sent by NTP program. Compounds are sorted by LOAEL from the most toxic ones. NOAEL values obtained in the developmental toxicity assay are also shown linked to real concentration measured in the embryos obtained by internal dosing analysis (red). Developmental classification: likely teratogenic (1), toxic but not teratogenic (2), not toxic for zebrafish (3), inconclusive (4).

2 Importance of the internal dosing analysis

Percentage of concentration of the compounds in the embryos was calculated dividing **measured concentration** (obtained in the internal dosing analysis) by **treatment concentration** at NOAEL.

Considering this percentage, concentrations in embryos at LOAEL of behavioral assay was estimated.

In this way, neurotoxic compounds can be sorted by estimated embryo exposure.

Test item identification			Behavioral assay		
			Effect	LOAEL (µM) treatment concentration	LOAEL (µM) estimated in embryos
1174	PE-2086	Deltamethrin	neuroactive	0.005	< 0.05
1445	PE-2044	Deltamethrin	neuroactive	0.025	< 0.05
1215	PE-2077	Tetraethylthiuram disulfide	neurotoxic	0.3	< 0.5
1756	PE-2021	Amoxicillin	neuroactive	15	< 0.25
1681	PE-2012	6-Hydroxydopamine hydrochloride	neurotoxic	100	< 0.25
1786	PE-2042	Colchicine	neurotoxic	50	0.34
1232	PE-2025	Benzo(a)pyrene	neuroactive	5	2.03
1814	PE-2067	Permethrin	neuroactive	0.5	2.51
1123	PE-2000	1-Methyl-4-phenylpyridinium iodide	neuroactive	30	4.85
1884	PE-2030	Berberine chloride	neuroactive	60	7.21
1109	PE-2014	Acenaphthene	neuroactive	15	19.14
1937	PE-2028	Benzo(k)fluoranthene	neuroactive	0.5	19.50
1327	PE-2069	Phenobarbital	neurotoxic	100	22.52
1453	PE-2055	Heptachlor	neuroactive	0.5	42.15
1705	PE-2046	Diazepam	neurotoxic	15	67.69
1324	PE-2061	Lindane	neuroactive	0.5	70.73
1092	PE-2050	Dieldrin	neuroactive	0.05	72.10
1367	PE-2006	2,2',4,4'-Tetrabromodiphenyl ether	neuroactive	2	74.53
1965	PE-2084	Valproic acid sodium salt	neuroactive	50	130.00
1196	PE-2001	2-Ethylhexyl diphenyl phosphate (EHDP)	neuroactive	3	181.80
1401	PE-2049	Dichlorodiphenyltrichloroethane (DDT)	neuroactive	0.5	221.75
1262	PE-2075	Tebuconazole	neurotoxic	10	272.00
1918	PE-2040	Chlorpyrifos (Dursban)	neuroactive	1	441.05
1881	PE-2054	Fluorene	neuroactive	4	500.27
1994	PE-2066	Parathion	neuroactive	8	1393.40

x 2

Figure 3: List of neuroactive and neurotoxic compounds sorted by the estimated embryo concentration. In the first 5 cases this value could not be calculated because of the limit of quantification.

3 Robustness of the battery of assays proposed

NTP classification	n	# duplicated	Percentage
Suspected developmental neurotox / neurotoxic	**39**	**2**	**%**
Detected as neuroactive	16	1	41.02
Detected as developmentally toxic	6	1	15.4
Detected as toxic (unspecific effect)	4	-	10.2
TOTAL DETECTED	**26**	**2**	66.62
discarding cpds with limited uptake	20/21		**95.2**
Not detected	**13**	-	33.3
discarding cpds with limited uptake	1/21		**4.7**
Limited uptake* in 12 out of 13 not detected compounds (92.3%) and 6 out of 26 active cpds (23%)			

*concentration in the embryo < 20 µM

Figure 4: Summary of the compounds suspected to be developmental neurotoxic / neurotoxic that were detected / not detected in Biobide's assay. Amongst the not detected compounds **Manganese diacetate** is the only one with a concentration in the embryos higher than 20 µM (127 µM).

CONCLUSIONS

- Out of the 91 tested compounds **twenty-four were classified as neuroactive / neurotoxic**. Six corresponded to DNT/NT group, four were PAH, two flame retardants and two were drugs classified as others.
- There was a **limitation** of the maximum tested concentration (100 µM) **due to the stock solution**. In some cases, where compounds were classified as inactive at the tested concentration, they would be detected as active at higher concentrations (for instance caffeine).
- One limitation of zebrafish embryo toxicity assays is the low uptake of **hydrophilic compounds** (logP < 1) and this highlights the **importance of conducting internal dosing assays** for proper test item classification.

Conflict of interest: The authors declare no conflict of interest.
*This poster does not necessarily reflect views of NTP/NIEHS