SETTING UP THE PROCESS FOR TESTING DEVELOPMENTAL TOXICITY IN ZEBRAFISH FOR PETROCHEMICAL PRODUCTS

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Abstract

There is an increasing need to provide reassurance that products and discharges are safe to both man and the environment. For example, under the EU REACH regulations, chemicals produced over 1000 t/year must be fully assessed for reproductive and developmental toxicity to mammals following the standard OECD 414 and 416 guidelines. However, these standard procedures use a lot of animals and are not suited to be used as screening tests for product development and assessing effluent quality. Although OECD-422 tests are typically performed during product development, because these are useful for dose selection and prioritizations, they are time consuming.

One alternative which is becoming established as a product screening tool is the zebrafish model [1,2]. This is a medium to high throughput method which offers high genetic homology with humans (over 85%) and possesses comparable developmental processes throughout embryogenesis. The size of the zebrafish larvae enable assays to be performed in a multi-well plate. This test provides both a relevant environmental assessment and a predictive capacity for many of the vital endpoints normally measured during a classic developmental toxicity rodent study.

In this pilot-study we investigated how the assay could be applied to assess 5 petrochemical substances and compared results obtained by direct injection, as used in the standard zebrafish Teratox Assay, with those obtained when embryos were exposed to Water Accommodated Fractions (WAF). The substances were tested at six concentrations on four different developmental stages between 30 hours and 4 days post fertilization.

The results indicate that the zebrafish embryos could provide an alternative screening model to assess developmental toxicity of petrochemicals, potentially decreasing animal use in both fish and rodent studies. However, it appears that the dosing method influences the effects seen and both the dose method and doses at which apparent developmental toxicity is observed need to be put into context when interpreting data from these tests.

Material and Methods

Material: 2-4 hpf (hours post fertilization) 1050 zebrafish embryos from wild type AB strain.**Compounds:** petrochemical compounds (see Table 1).

Treatment: Embryos were distributed in 24-well plates and treated with 5 different concentrations in a half log (3.3) starting at 10 or 100, testing 15 embryos per condition, with 5 embryos per well up until 1% of DMSO.

Method: the test items will be tested at six concentrations (including 0) on four different developmental stages (30 hpf, 2 days post fertilization (dpf), 3 dpf and 4 dpf).

Analysis:

Analysis:

	30 hpf	2 dpf	3 dpf	4 dpf
Viability	x	x	x	x
Body shape	x	x	x	x
Heart morphology		x		
Somite morphology		x		
Notocord morphology		x		
Tail morphology		x	x	
Fin morphology				x
Facial structure morphology				x
Brain morphology				x
Hatching			x	
Edema			x	x
Severely affected *		x	x	x

Table 2.
List of end points tested for the teratogenciity assay and theconcrete moment of development where they are analyzed.

List of compounds

Test items
PE-0535
PE-0536
PE-0537
PE-0538
PE-0539
PE-0540 *
PE-0541**

Table 1.
List of petrochemicals tested for the teratogenciity assay

*Control -= Sucrose 300 µM

**Control + = Retinoic Acid 100nM

Results

Test items	Toxicity in zebrafish	Lowest toxic concentration/loading rate tested (mg/L)
PE-0535	Yes	3
PE-0535_WAF	No	_
PE-0536	No	1
PE-0536_WAF	No	10
PE-0537	No	1
PE-0537_WAF	No	10
PE-0538	No	_
PE-0538_WAF	No	_
PE-0581	No	_
PE-0581_WAF	No	_
PR-0231 *	No	_
PR-0052 **	Yes	0.1 µM

- PE-0535 resulted lethal prepared in DMSO at 3mg/L but no effect was detected used after WAF preparation at the loading rates tested.
- PE-0536 and PE-0537 caused developmental defects in both cases (regular and WAF preparations).
- PE-0538 and PE-0581 did not caused any effect at the concentrations / loading rates tested in the embryos from 30hpf to 4 dpf.

Table 3.
List of petrochemicals tested for the teratogenciity assay and the results obtained in zebrafish teratogenicity assay with the lowest toxic concentration/loading rate tested

Conclusions

- The zebrafish is an ideal vertebrate model to evaluate petrochemicals induced toxicities, as an interesting alternative screening model to assess developmental toxicity of petrochemicals.
- The zebrafish model allow potentially decreasing animal use in both fish and rodent studies.
- Dosing methods seems to influence the developmental toxicity effects and should be put into context, developing in some cases bioavailability test (HPLC-Ms-Ms) to interpretate the data properly.
- Results obtained by direct injection, as used in the standard zebrafish Teratox Assay from BBD BioPhenix, gave similar results to those obtained when embryos were exposed to Water Accommodated Fractions (WAF).

Bibliography

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