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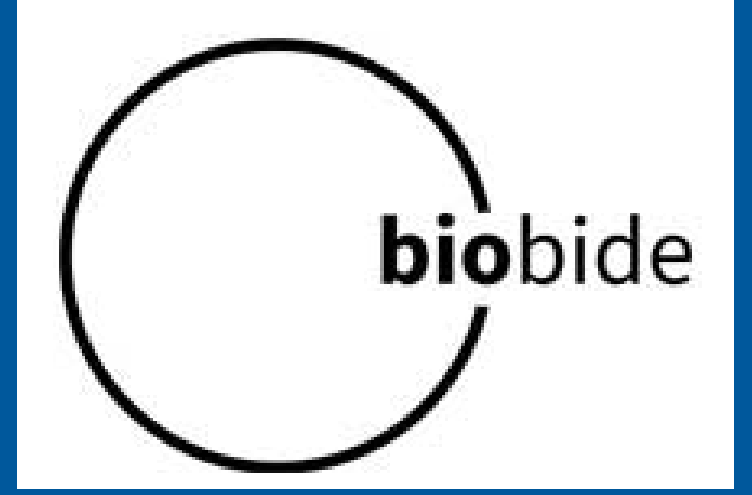
An inter-laboratory validation of the zebrafish embryo assay for the detection of developmental toxicity

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Overview

Zebrafish embryos are being used by many laboratories for de-risking developmental toxicity of chemical compounds helping to reduce the number of in vivo embryo-fetal toxicity studies. The overall concordance to mammalian developmental toxicity has been reported between 60-87% (e.g. [1]). However, it was indicated that different laboratories did not classify the same set of compounds correctly. Therefore, further research is needed to better understand the need for harmonization of used protocols and to identify factors causing variability.

Objectives

- Evaluate the performance of the zebrafish embryo assay in three different laboratories using a limited number of compounds to challenge the use of different protocols
- Optimize the protocols with the intent to make the assay conducive to high content and automatic screening

Conclusions

- Overall the results and effect concentrations between the three different laboratories were very similar
- In many cases the quantitative endpoints were more sensitive than scoring of morphological features by an experienced observer
- Swim bladder inflation was the most sensitive endpoint. Although interference with swim bladder inflation cannot yet be associated to any structural mammalian alteration, potential links to adverse developmental effects may be established in the future

Results & Discussion

Comparison of effect concentrations between the three different laboratories

Substance	Laboratory	NOAEL (µM)		EC ₅₀ (µM)		LC ₅₀ (µM)		TI #		Highest tested concentration
		2 dpf	4 dpf	2 dpf	4 dpf	2 dpf	4 dpf	2 dpf	4 dpf	
Loratadine	Biobide	5	1	10.78	1.64	>30	11.51	>2.8	7.1	30 µM
	Sanofi	8	2	9.31	7.1	13.9	9.25	1.5	1.3	30 µM
	BIOTOX-UFZ	9.8	4	10.34	0.65	19.14	12.82	1.8	19.7	26 µM
Methotrexate	Biobide	250	100	337.3	216.1	>1000	351.2	>3	1.6	1000 µM
	Sanofi	150	50	260	75.4	321	101	1.2	1.3	500 µM
	BIOTOX-UFZ	220	110	244.48	184.4	357.8	304.8	1.5	1.6	550 µM
Dexamethasone*	Biobide	300	10	>600	>600	>600	>600	-	-	600 µM
	Sanofi	>500	250	>500	>500	>500	>500	-	-	500 µM
	BIOTOX-UFZ	>255	>255	>255	>255	>255	>255	-	-	255 µM
Topiramate	Biobide	400	100	863.5	198.6	>1500	671.7	>1.7	3.4	1500 µM
	Sanofi	1000	100	767	325	1279	678	1.7	2.1	1000 µM
	BIOTOX-UFZ	187.5	250	551.2	291.6	1224.1	937.9	2.2	3.2	1500 µM
Glybenclamide	Biobide	>500	>500	>500	>500	>500	>500	-	-	500 µM
	Sanofi	200	200	>200	>200	>200	>200	-	-	200 µM
	BIOTOX-UFZ	>101.2	>101.2	>101.2	>101.2	>101.2	>101.2	-	-	101.2 µM

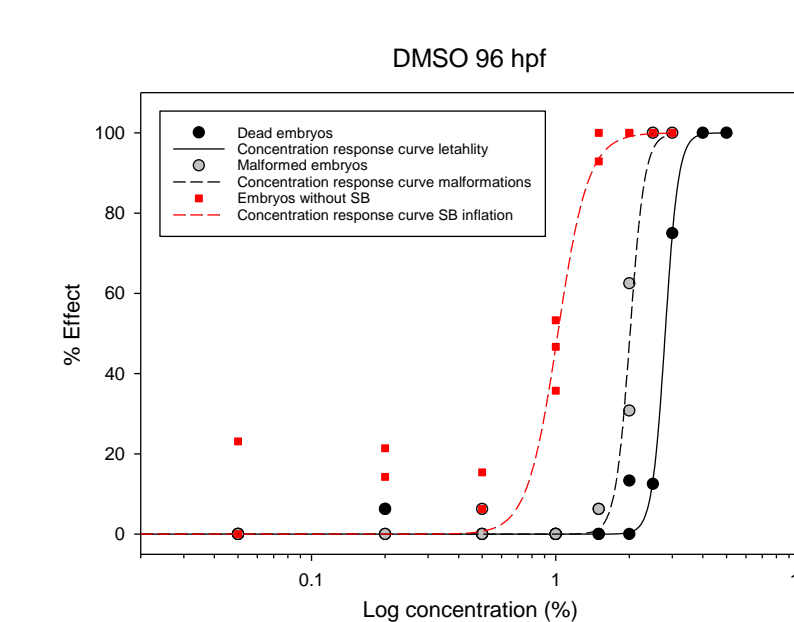
* Precipitation was noted
Teratogenic Index (TI = LC₅₀/EC₅₀)

Zebrafish classification among the laboratories

	Laboratory	Zebrafish negative		Zebrafish positive	
		True negative	False positive	True positive	False negative
Non-teratogen	Biobide				
	Sanofi	Glybenclamide	Loratadine		
	UFZ				
Teratogen	Biobide			Methotrexate	Topiramate
	Sanofi			Dexamethasone*	
	UFZ	Dexamethasone*		Methotrexate	Topiramate

* Additional endpoints implemented at UFZ showed effects and provide future support to determine the potential teratogenicity of a compound
50% < embryotoxicity > 20% resulted in positive outcome without calculated Teratogenic Index (TI = LC₅₀/EC₅₀).

Solvent toxicity



Exposure duration	Endpoint	% DMSO
96h	LC ₅₀	2.8
	Malformations EC ₅₀	2.0
	Eye size EC ₁₀	1.6
	Body length EC ₁₀	2.0
	Swim bladder EC ₅₀	1.1
	LMR OA EC ₅₀	1.3
	Heart Rate EC ₁₀	1.9
48h	LC ₅₀	3.23
	Malformations EC ₅₀	2.27
	Eye size EC ₁₀	2.4
	Body length EC ₁₀	1.7
	Heart Rate EC ₁₀	1.9

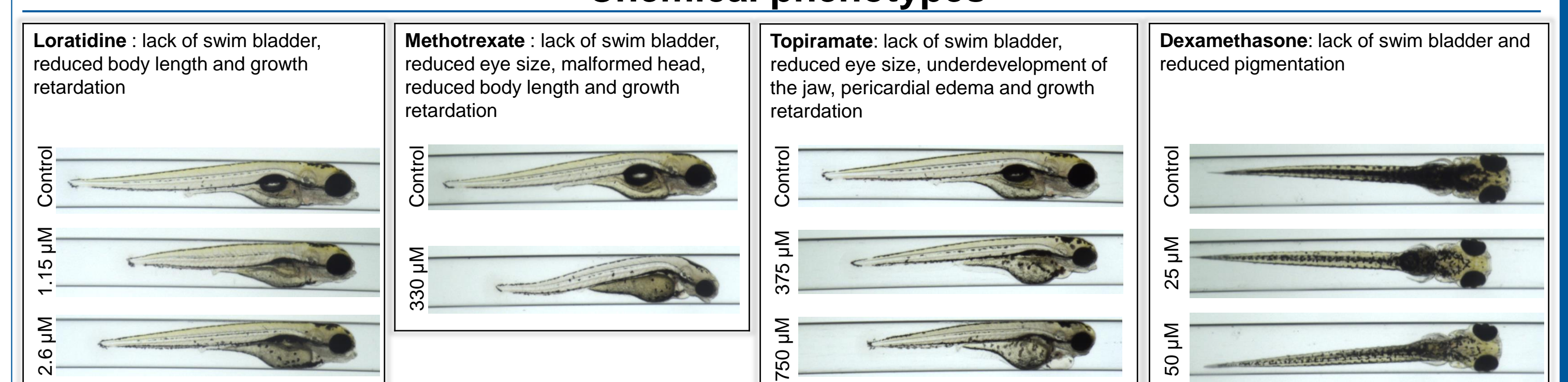
DMSO solvent concentration of testing solution could influence some endpoints, but it is not changing the overall classification of compounds

Additional endpoints measured by UFZ (automatic feature detection)

Substance	48hpf EC ₁₀ (µM)			96 hpf EC ₁₀ (µM)			96 hpf LMR EC ₅₀	96 hpf Swim bladder EC ₅₀
	Eye size	Body length	Heart rate	Eye size	Body length	Heart rate		
Loratadine	10.1	9.9	2.3	2.5	3.5	1.1	0.4	0.3
Methotrexate	185.7	>330	>330	66.5	183.0	>440	326.6	96.7
Dexamethasone	n.d.	n.d.	n.d.	>255	>255	>255	>255	17.33
Topiramate	900.8	>1252	699.3	118.0	335.4	65.2	570.1	46.3
Glybenclamide	n.d.	n.d.	n.d.	>101.2	>101.2	>101.2	>101.2	>101.2

n.d.: not determined

Chemical phenotypes

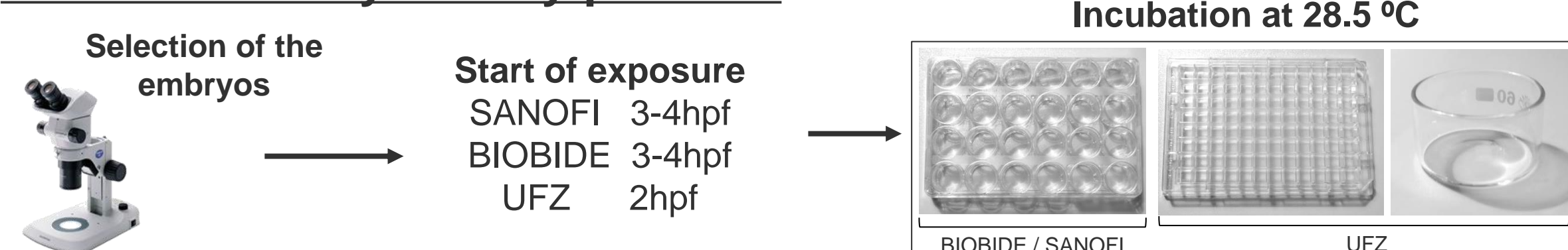


Materials & Methods

Substances selected for the validation study

Substance	Cas no.	Class	Teratogenic classification
Loratadine	79794-75-5	Antihistaminic	Non-teratogen
Methotrexate	59-05-2	Antineoplastic	Teratogen
Dexamethasone	50-02-2	Glucocorticoid	Teratogen
Topiramate	97240-79-4	Antiepileptic	Teratogen
Glybenclamide	10238-21-8	Antidiabetic	Non-teratogen

Zebrafish embryo assay protocol



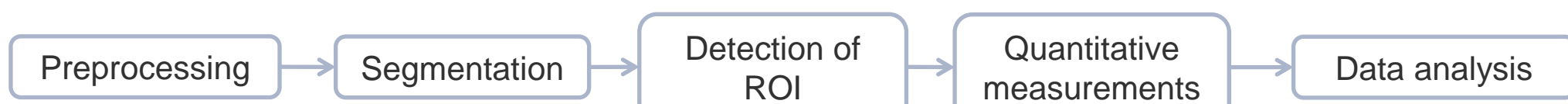
Endpoints

	48 hpf	96 hpf
SANOFI	Morphological assessment: Relative abundance (%) based on yes or no classification (0, 1)	Morphological assessment: Relative abundance (%) based on yes or no classification (0, 1)
BIOBIDE	Morphological assessment: Relative abundance (%) based on yes or no classification (0, 1)	Morphological assessment: Relative abundance (%) based on yes or no classification (0, 1)
UFZ	Morphological features - VAST Eye size Body length Heart rate	Morphological features - VAST Eye size Body length Heart rate SB inflation
		Behavior-LMR

VAST Biomag platform (Vertebrate Automated Screening Technology)

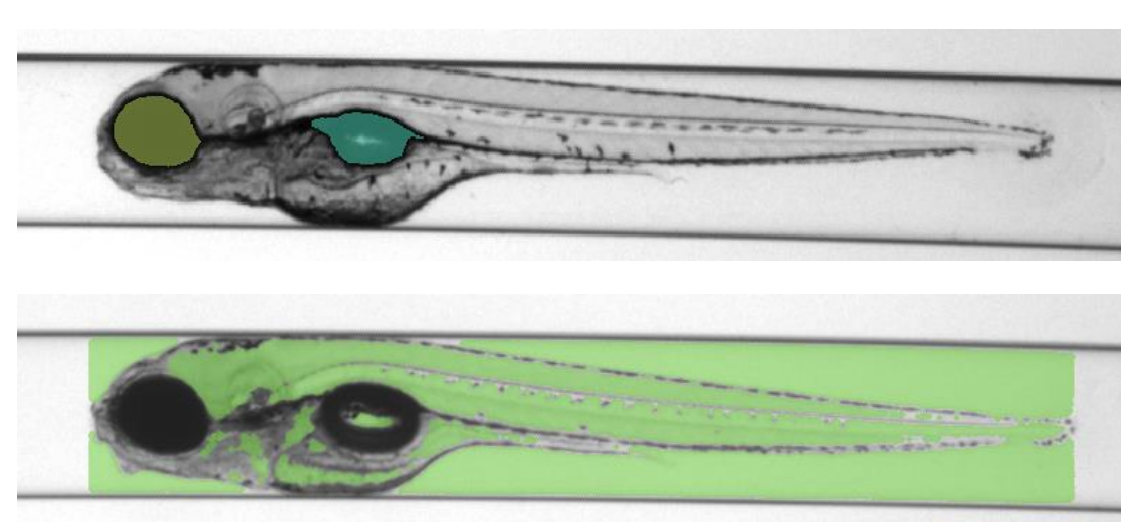


Automated feature detection

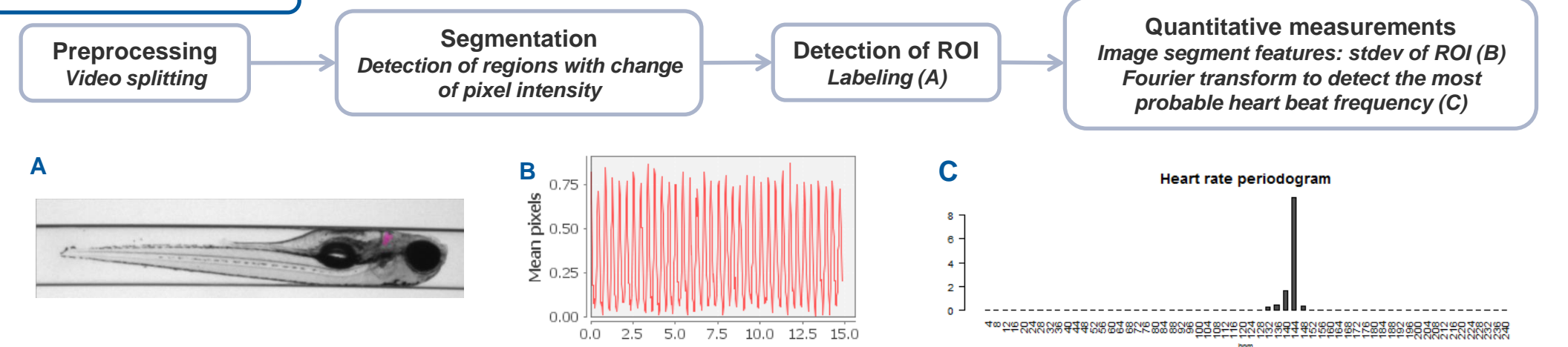


Eye size
SB inflation

Body length



Heart rate



References

[1] A.-L. Gustafson, D.B. Stedman, J. Ball, J.M. Hilleagass, A. Flood, C.X. Zhang, et al., Inter-laboratory assessment of a harmonized zebrafish developmental toxicology assay - progress report on phase I., *Reprod. Toxicol.* 33 (2012) 155–64.

Acknowledgements

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