E-FET – OECD 236-derived transcriptomic threshold concentrations for chronic toxicity assessment

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REACH regulation currently requires animal-intensive chronic toxicity tests such as the Fish Early Life Stage (FELS) assay (OECD TG 210). To reduce vertebrate use, new approach methods (NAMs) are needed to detect long-term effects, e.g. on growth (GR), survival (SU), and development. The transcriptomic point-of-departure (tPOD) approach offers a promising alternative by identifying early molecular responses through benchmark modeling of concentration-dependent gene expression changes and has shown protective performance relative to apical endpoints. This study investigates zebrafish embryos – a 3R-compliant life stage – as a model to derive tPODs for 16 industrial chemicals with diverse toxicity profiles (NE = no effects, GR and SU). We evaluate whether embryo-derived tPODs are predictive or protective compared to FELS no-effect concentrations (NOECs) and assess potential limitations related to chemical properties and modes of action.

Methodology & Results

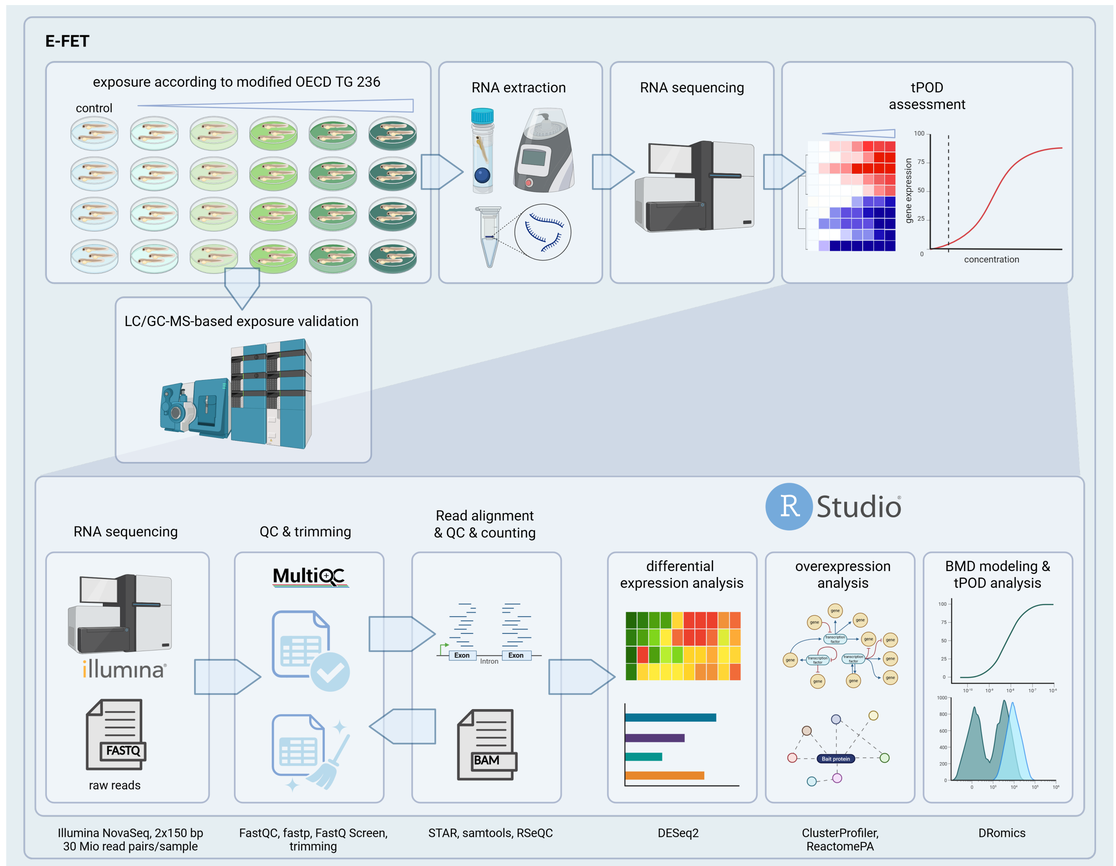

Figure 1: Experimental design and transcriptomic workflow. tPODs were derived from zebrafish embryo testing[1] with five sublethal concentrations (NOEC–LOEC range) with four replicates and verified exposure. After RNA-Seq, Dromics[2] was used to define tPODs. Created with Biorender.com.

Test substances and properties

Test substance	Log Kow	Solubility [µg/L]	Test substance	Log Kow	Solubility [µg/L]
NE1	-3.85	>10,000,000	SU1	-0.38	3,400,000
NE2	3.44	340,000	SU3	3.09	156,000
NE7	6.13	<1.0	SU4	3.39	111,000
GR1	1.80	360	SU5	3.54	14,000
GR4	4.33	547	SU7	4.37	38,000
GR6	4.80	4,380	SU8	6.06	30.0
GR8	8.21	104	SU9	6.34	1,600
GR12	10.77	1,800,000	SU11	7.94	1,190

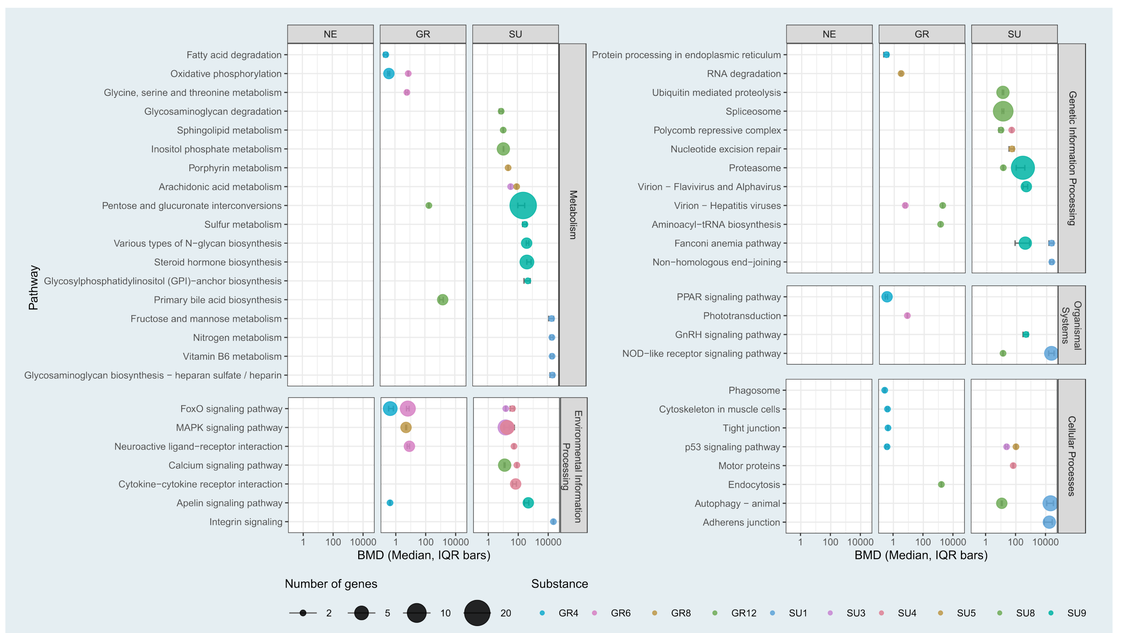

Figure 2: Median pathway-level BMDs (±IQR) for all substances grouped by class (NE, GR, SU). Points represent responsive pathways (size = gene count), highlighting differences in pathway sensitivity and early biological perturbations across substances.

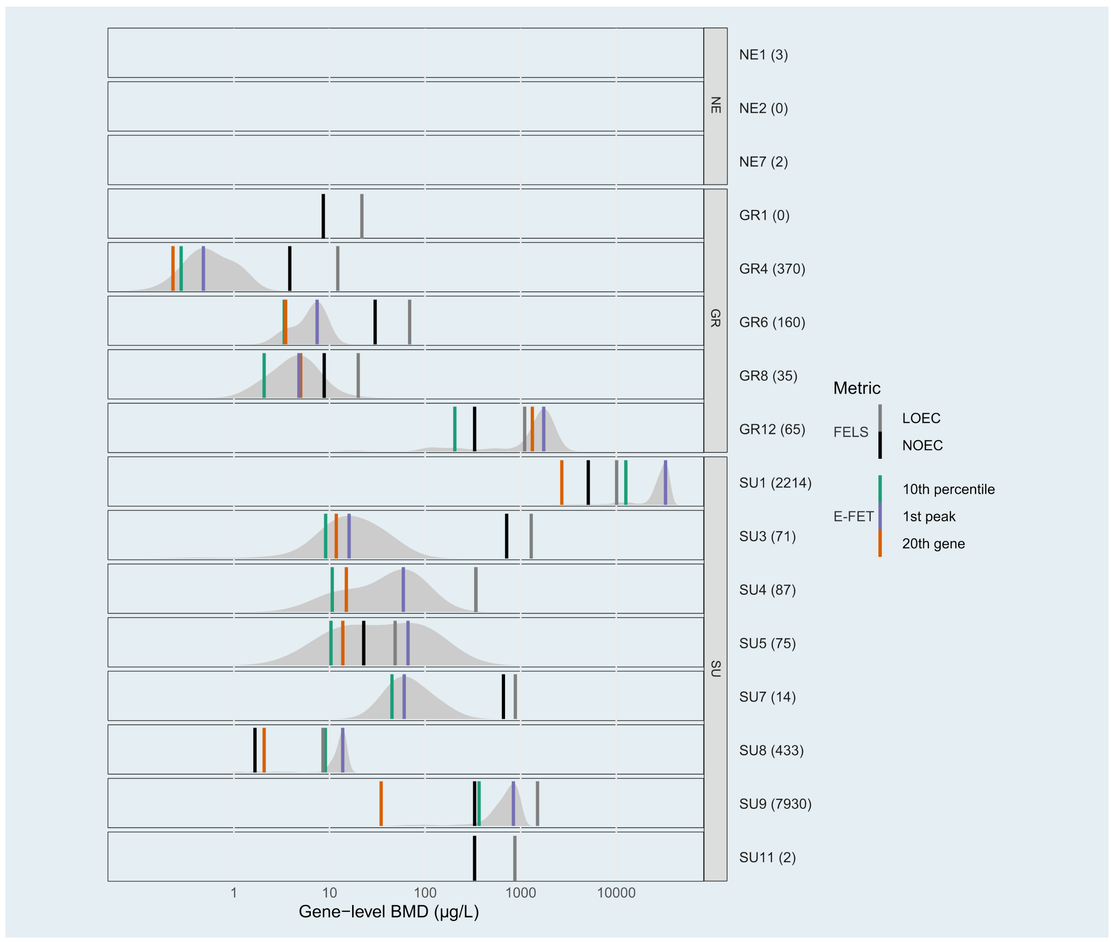

Figure 3: Distribution of gene-level benchmark doses and tPODs for all tested substances. Shown are gene-level BMDs for each substance based on geometric mean measured concentrations, overlaid with tPOD metrics (10th percentile = turquoise line, first-peak = violet line, and 20th-gene BMD values = orange line). Black and grey lines denote experimentally derived NOEC and LOEC values from FELS, respectively. Numbers in brackets represent numbers of concentration-responsive genes.

Conclusion

Zebrafish embryo transcriptomic responses largely mirrored chronic FELS outcomes, with tPODs aligning well with NOECs when exposure was sufficient, supporting their potential as protective indicators. Missing or inconsistent tPODs were mainly linked to exposure limitations (e.g., degradation or solubility in the case of SU1 and SU11), highlighting the need for improved exposure setups. Broader testing is required to confirm these findings.

References

1 OECD (2013) Test No. 236: Fish Embryo Acute Toxicity (FET) Test.
2 Larras, Floriane, et al. "DRomics: a Turnkey Tool to support the use of the dose–response framework for omics data in ecological risk assessment." Environmental science & technology 52.24 (2018): 14461-14468.

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Sponsoring
This study was sponsored by ECHA.

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