Optimising concentration setting for aquatic vertebrate endocrine tests

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Introduction

There is a growing demand for fish and amphibian *in vivo* tests for the identification of chemical-induced endocrine activity and disruption. A key challenge is setting appropriate test concentrations [1]:

- How to distinguish between primary endocrine interactions and secondary (non endocrine-mediated)/confounding effects (e.g. mortality).
- Effects may depend on life-stage and species.
- The maximum tolerated concentration (MTC) is inconsistently defined in the available Guidelines.

There is a need for a data-driven rationale to improve concentration setting. This will increase confidence in test results and maximise the utility of the information generated. It will also help avoid additional (including higher-tier) vertebrate testing, and unnecessary repeat testing/suffering in test animals.

This cross-sector, multi-stakeholder initiative builds on previous work to define a strategy for MTC setting in fish *in vivo* endocrine studies (Figure 1).

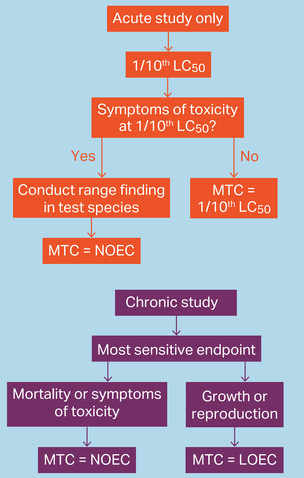

Figure 1. *Simplified approach to setting the MTC depending on the availability of acute (A) and chronic (B) fish data. Adapted from Figure 2 in [2].*

Aim: through retrospective data analysis, conduct an evidence-based activity to define MTCs for fish and amphibian endocrine screening and definitive tests.

Methods and results

Establishing a database

Data were collated from publicly available regulatory studies on pesticide active ingredients:

- 32 Fish short term reproduction assays (FSTRA, OECD TG 229; see [3])
- 31 Amphibian metamorphosis assays (AMA, OECD TG 231)
- Corresponding fish acute toxicity tests (OECD TG 203/OPPTS 850.1075) and fish early life- stage (FELS) toxicity tests (OECD TG 210; fathead minnow (FHM) and rainbow trout (RT)) and fish full life cycle tests (OPPTS 850.1500, any species)

Data analysis step 1: Compare MTCs derived using 1/3rd or 1/10th of the fish 96 h LC_{50} to acute study outcomes

These potential MTCs were compared to the no observed effect concentrations (NOECs) and lowest observed effect concentrations (LOECs) to determine whether (sub)lethal symptoms of toxicity were observed in proximity to the selected MTCs (n = 25 for RT and n = 2 for FHM). Examples can be seen in Table 1.

Fish acute toxicity, Rainbow trout											
Substance	96 h LC_{50} (mg/L)	NOEC (mg/L)	LOEC (mg/L)	NOEC mortality (mg/L)	LOEC mortality (mg/L)	MTC based on 1/3rd LC_{50} (mg/L)	MTC based on 1/10th LC_{50} (mg/L)	Highest concentration used in AMA (mg/L)	AMA conc/ LC_{50}	Highest concentration used in FSTRA (mg/L)	FSTRA conc/ LC_{50}
2,4-Dichlorophen-oxyacetic acid	240	100	180	180	320	80	24	113	0.47	96.5	0.4
Abamectin	0.0036	0.00078	0.0013	0.0022	0.0036	0.0012	0.0004	0.0096	2.67	0.0024	0.67
Benfluralin	0.081	0.017	0.04	0.04	0.052	0.027	0.008	0.0744	0.92	0.0365	0.45
Carbofuran	0.82	<0.1	0.1	0.56	1	0.273	0.082	0.467	0.57	0.435	0.53
Chlorothalonil	0.039	0.022	0.046	0.022	0.046	0.013	0.004	0.005	0.13	0.01	0.26
Diazinon	3.1	0.7	1.7	1.7	3.6	1.03	0.31	0.82	0.26	0.82	0.26
Dimethoate	24	<1.2	1.2	1.2	2.3	8	2.4	100	4.17	96	4

Table 1. *Examples of RT fish acute toxicity data, including calculation of MTCs based on 96 h LC_{50} values and comparisons with highest concentrations tested in AMA and FSTRA studies. The highest concentrations selected ranged from 0.04 (1/25th) or 4.17 times the respective 96 h fish acute LC_{50} for AMAs and from 0.004 (1/250th) to 4.56 times the LC_{50} for FSTRAs. Key: green = MTC or highest concentration ≤ sublethal NOEC; yellow = MTC or highest concentration > sublethal NOEC but < LOEC; red = MTC or highest concentration ≥ sublethal NOEC and/or NOEC mortality; purple = cannot conclude as sublethal NOEC is undetermined.*

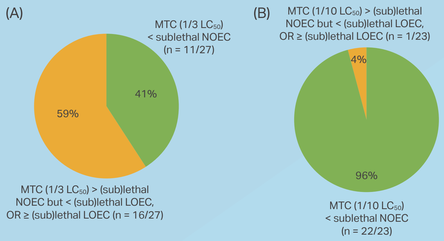

Figure 2. *Comparison of MTCs derived using 1/3rd 96 h LC_{50} (A) or 1/10th 96 h LC_{50} (B) to fish acute study outcomes.*

Green = where significant (sub)lethal symptoms of toxicity were not observed at or below the potential MTC, it is less likely that the highest concentration would elicit lethality/ significant sublethal effects – i.e., the MTC would be unlikely to be compromised by the onset of systemic toxicity.

Yellow = Where (sub)lethal symptoms of toxicity were observed at or below the potential MTC, or when it is not clear from the data (i.e., the potential MTC falls between the NOEC and the LOEC for (sub)lethal acute effects), it is possible that the concentration selected would be too high, and over a longer-term exposure these effects might lead to mortality - i.e. the MTC could be compromised by the onset of systemic toxicity.

In 4/27 cases the MTC of 1/10th LC_{50} was lower than the sublethal LOEC, but the sublethal NOEC was undetermined; these data are not shown.

Data analysis step 2: Assess how MTCs derived from outcomes of standard acute and chronic studies relate to the highest concentrations tested in endocrine screening studies

a) The highest concentrations used in the AMAs and FSTRAs were compared to the NOECs and LOECs from the fish acute toxicity tests to determine whether (sub)lethal symptoms of toxicity may potentially be observed in proximity to the selected highest concentrations. The analysis of RT acute toxicity data (n = 25) is presented here (Table 2 and Figure 3).

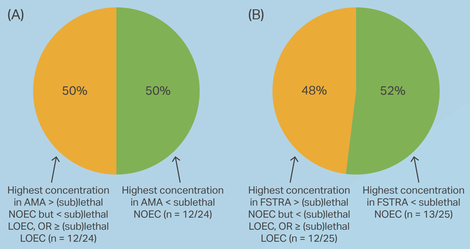

Figure 3. *Comparison of highest concentrations used in AMAs (A) and FSTRAs (B) to fish acute toxicity test outcomes.*

b) The highest concentrations used in the AMAs and FSTRAs were compared to the fish chronic toxicity test NOECs (where the fish chronic LOEC was driven by survival/mortality) or LOECs (where the fish chronic LOEC was driven by effects other than survival*; see Figure 1). The analysis of RT ELS data is presented here (n = 14; Table 2 and Figure 4).

*Further consideration will be given to the types and severity of non-survival effects in Next Steps.

Fish early lifestage study data, Rainbow trout											
Substance	MTC = ELS NOEC (mg/L)	MTC = ELS LOEC (mg/L)	LOEC driven by	Highest concentration used in AMA (mg/L)	AMA conc/ ELS LOEC	AMA conc vs. ELS NOEC	AMA conc vs. ELS LOEC	Highest concentration used in FSTRA (mg/L)	FSTRA conc/ ELS LOEC	FSTRA conc vs. ELS NOEC	FSTRA conc vs. ELS LOEC
Abamectin	0.00052	0.00096	growth	0.0096	10	> NOEC	> LOEC	0.0024	2.5	> NOEC	> LOEC
Carbofuran	0.0248	0.0567	survival and growth	0.467	8.24	> NOEC	> LOEC	0.435	7.67	> NOEC	> LOEC
Fenbutatin Oxide	0.0002	0.0005	survival and growth	0.000307	0.61	> NOEC	< LOEC	0.00166	3.32	> NOEC	> LOEC
Imidacloprid	9.02	26.9	time to hatch and swim-up	18.5	0.69	> NOEC	< LOEC	9.02	0.34	< NOEC	< LOEC
Myclobutanil	0.98	2.2	growth	2.04	0.93	> NOEC	< LOEC	3.3	1.5	> NOEC	> LOEC
Trifluralin	0.00114	0.00218	growth	0.232	106.42	> NOEC	> LOEC	0.0215	9.86	> NOEC	< LOEC

Table 2. *Examples of RT ELS data, including determination of MTCs based on the FELS NOECs and LOECs and comparisons with highest concentrations tested in AMA and FSTRA studies. The highest concentrations selected for the AMAs ranged from between 0.08 (1/13th) and 166.67 times the LOEC reported in the FELS test, and for FSTRAs from between 0.34 (1/3rd) and 38.55 times the LOEC.*

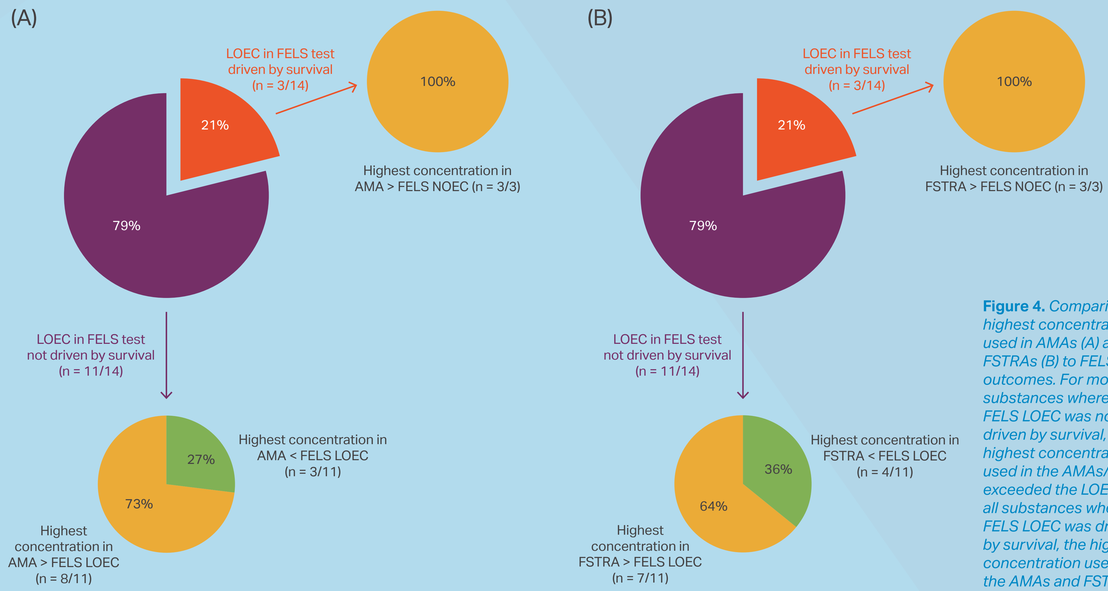

Figure 4. *Comparisons of highest concentrations used in AMAs (A) and FSTRAs (B) to FELS test outcomes. For most substances where the FELS LOEC was not driven by survival, the highest concentration used in the AMAs/FSTRAs exceeded the LOEC. For all substances where the FELS LOEC was driven by survival, the highest concentration used in the AMAs and FSTRAs exceeded the FELS NOEC.*

Conclusions

In agreement with previous findings, when considering the use of fish acute toxicity data to derive an MTC based on 1/3rd or 1/10th of the LC_{50} value, 1/10th of the LC_{50} is less likely to result in potential non-endocrine related toxicities – relevant where only acute data are available. Relative to acute and chronic toxicity study outcomes, the highest concentrations selected in the AMA and FSTRA studies were often higher than 1/10th LC_{50} or the LOEC from a relevant chronic study (where LOEC not driven by survival) or the NOEC (where LOEC is driven by survival).

Use of these as highest test concentrations may compromise findings through induction of significant systemic toxicities and/or mortality. These analyses support the assertion that all available data should be considered when setting the MTC.

Next steps

- Examine how the acute and chronic toxicity outcomes relate to the findings in the endocrine activity screening studies.
- Consider how to incorporate a weight of evidence approach to setting the MTC for fish and amphibian endocrine screening studies.
- Include recommendations to avoid setting of concentrations that are potentially too low to detect endocrine-mediated effects.
- Explore MTC-setting for higher tier endocrine studies, e.g. medaka extended one generation tests (MEOGRT, OECD TG 240) and larval amphibian growth and development assays (LAGDA, OECD TG 241).

References

[1] Burden *et al.* (2021) *Integr Environ Assess Manag* 18(2): 442–458.

[2] Wheeler *et al.* (2013). *Chemosphere* 92(9): 1067–76.

[3] Wheeler *et al.* (2019). *Reg Toxicol Pharmacol* 108: e104424.