

A review of the use of the OECD threshold approach for fish acute studies

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INTRODUCTION

Fish acute toxicity studies (OECD TG 203, Ref 1) are embedded in global regulatory frameworks for chemical risk assessment; however, this study type is ethically controversial as a vertebrate study with a lethality endpoint. Alternative approaches are available (OECD TG 236, OECD TG 249, Refs 2-3) but not yet widely accepted by regulatory authorities. Projects are ongoing to support the use of alternative approaches (CEFIC, SWIFT, Ref 4), however for the meantime LC50 determinations using OECD TG 203 are still required.

Fish acute toxicity studies are routinely conducted at Scymaris Ltd. to support global chemical risk assessments and the OECD threshold approach (Ref 5) is used wherever possible. Not only does this approach negate the need for range finding, but it also reduces animal numbers from 42 to 12 fish in most cases. The threshold approach utilises data generated from algae (OECD TG 201, Ref 6) and *Daphnia magna* (OECD TG 202, Ref 7) studies to set a limit concentration for the fish study. Only if mortality is observed is a full range of 5 test concentrations required. This approach aids the 3R's principles by reducing animal numbers and refinement of the test concentration by utilising data from other test organisms.

METHODS

DESCRIPTION OF THE THRESHOLD APPROACH

4. When acute fish toxicity data need to be generated the threshold approach should be applied. It includes the performance of tests according to OECD guidelines:

- TG 201 – Freshwater Alga and Cyanobacteria, Growth Inhibition Test
- TG 202 – *Daphnia* sp. Acute Immobilisation Test
- TG 203 – Fish, Acute Toxicity Test (Limit test)
- TG 203 – Fish, Acute Toxicity Test.

The following step-wise procedure should be utilized (Figure):

- Derivation of the threshold concentration (Step 1):** The lowest EC50 value of invertebrate (e.g. *daphnia*) or algae is set as threshold concentration (TC). If these data are not available they need to be determined according to TG201 and TG202.
- Assessment of acute fish toxicity (limit test) at the TC (Step 2):** An acute fish test is performed according to the limit test described in TG 203 (paragraph 20) at the TC. If the TC is >100 mg/l, the test substance concentration should be 100mg/l in the limit test. The absence of mortality indicates that the fish is not the most sensitive species and that, with at least 90% of confidence, the LC50 is greater than the threshold concentration. If mortality occurs, a full study according to TG 203 should be conducted.

Figure 1. Description of the threshold approach (Ref 5)

Guideline followed: OECD TG 203: Fish, acute toxicity test

Test species: Rainbow trout (*Oncorhynchus mykiss*), Fathead Minnow (*Pimephales promelas*), Sheepshead Minnow (*Cyprinodon variegatus*), Carp (*Cyprinus carpio*) and Pond Loach (*Misgurnus anguillicaudatus*)

Duration of study: 96 hours

Study temperature: 13 - 25°C (Species dependent)

Photoperiod: 16 hours light and 8 hours dark with 20-minute dawn and dusk



Figure 2. Test species: Pond Loach, Rainbow Trout and Sheepshead Minnow

We conducted a retrospective assessment of the fish acute toxicity studies conducted at our laboratory at the end of our previous Home Office Project Licence (5-year duration). The retrospective assessment evaluated the OECD threshold approach, when and by whom it was used and if we could have reduced fish numbers further if it was utilised more frequently.

We have grouped the fish acute studies conducted over the 5-year project licence period into two categories for this assessment. 1) Standard design, which includes range finding and a definitive study and 2) OECD Threshold design, utilising algae and invertebrate data to refine and reduce animal numbers.

All the studies assessed were reported in compliance with GLP (Good Laboratory Practice) and in accordance with the OECD TG 203.

RESULTS AND KEY FINDINGS

The split of fish acute toxicity studies was 54% standard design with range finding and a definitive test and 46% utilising the OECD Threshold approach (Figure 3). All threshold studies conducted were successful in proving fish were the least sensitive species and a full concentration range was not required.

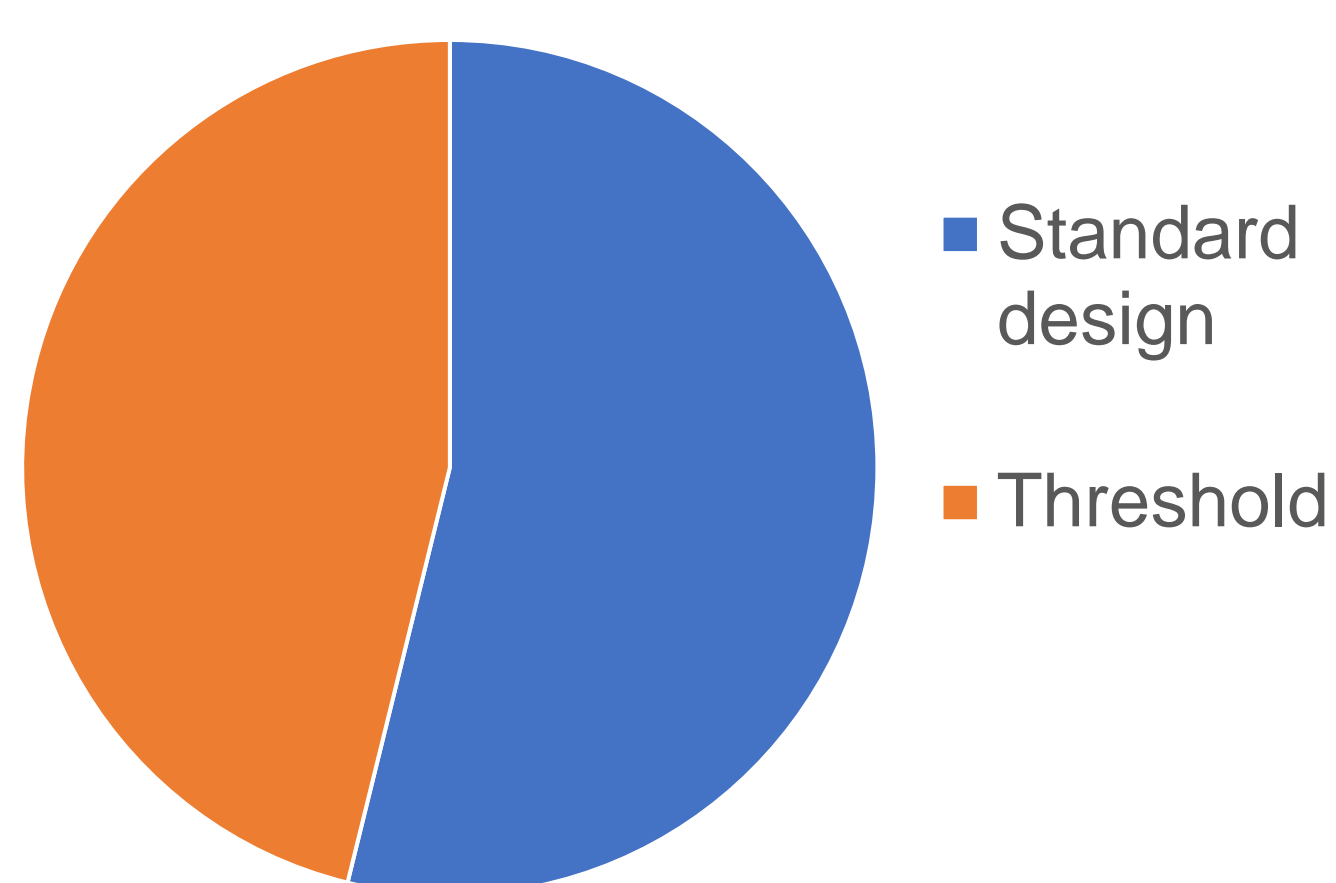


Figure 3. Fish acute toxicity test: Study design type

Of the standard design studies conducted 43% were part of a larger package including algae and *Daphnia* acute studies. The remaining 57% of studies were stand alone fish studies to support global registration of agrochemicals. 83% of the studies conducted as part of a larger package found fish to be the least sensitive species and therefore the threshold test would have been a suitable approach. If the threshold approach had been used it would have reduced animal numbers considerably by removing the requirement of a range finding test and reducing the number of test concentrations to run as a limit study.

When assessing which industry sectors were requesting the standard design versus the threshold approach it can clearly be seen that the study design directly correlates to the industry sector.

The industrial chemicals + others had a much higher uptake of the threshold approach and a heavy weighting towards submission for EU REACH.

Conversely, the agrochemical sector was heavily weighted towards the standard approach and therefore using significantly more animals. The data generated for the agrochemicals had a more diverse use for global regulatory submission.

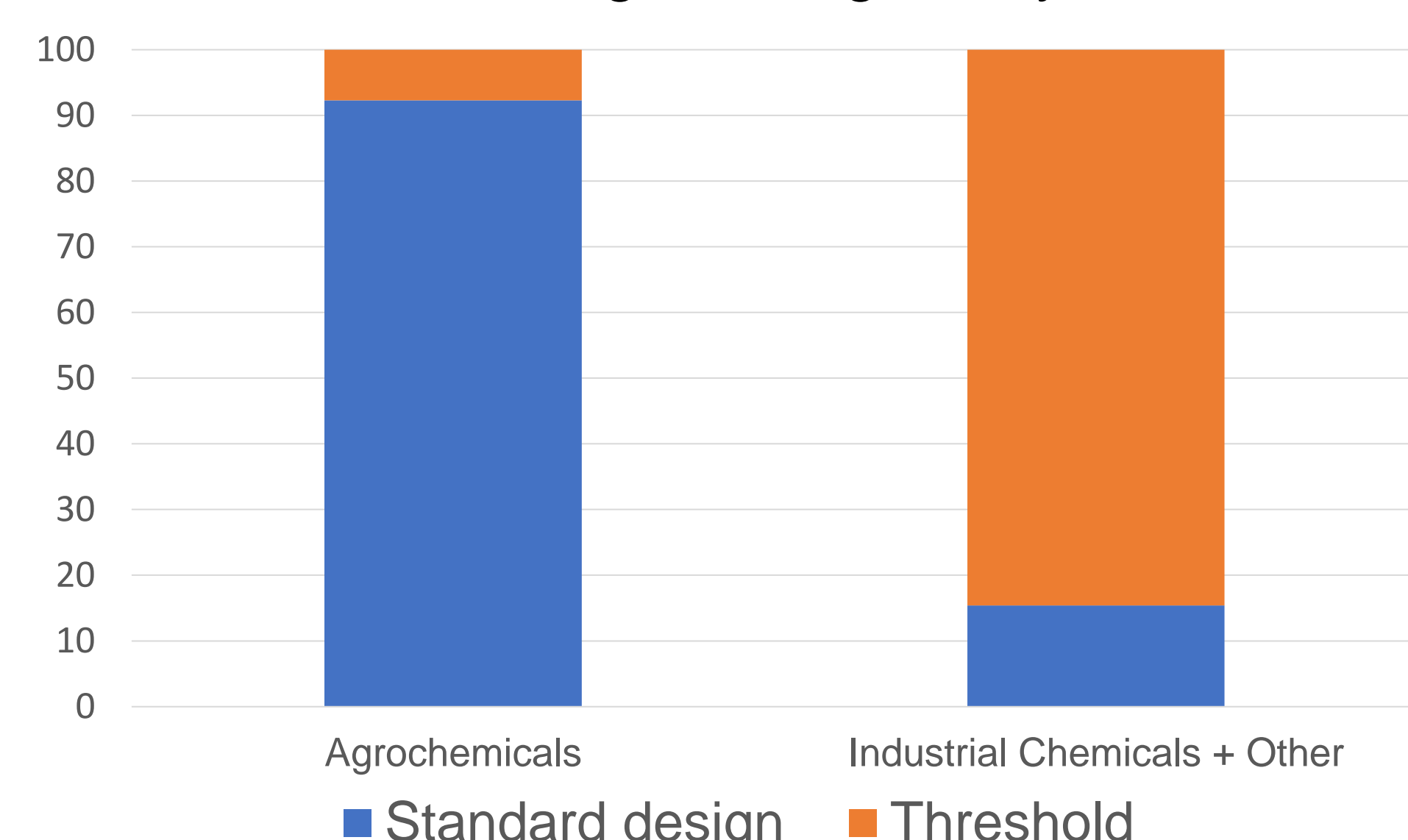


Figure 4. Fish acute toxicity test: Industry sector