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Report on the Validation of the Lymnaea Stagnalis Reproduction Toxicity Test

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REPORT ON THE VALIDATION OF THE LYMNAEA STAGNALIS REPRODUCTION TOXICITY TEST

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FOREWORD

The project to develop mollusc reproduction tests was initiated by Denmark, Germany, France and the United Kingdom and included in the work plan of the Test Guidelines Programme in 2011.

Originally the project envisaged the development of a single Test Guideline (TG) on mollusc reproduction test, including both the mollusc species *Lymnaea stagnalis* and *Potamopyrgus antipodarum*. However, the Validation Management Group on Ecotoxicity (VMG-Eco) supported the development of two separate TGs for these two mollusc species after discussion at the 10th VMG-Eco meeting in December 2014.

The separate draft validation report for the Test Guideline using *Lymnaea stagnalis* was finalised in June 2015 and subsequently circulated to the Working Group of the National Coordinators of the Test Guidelines Programme (WNT) for review and commenting in July 2015 and again in December 2015.

No comments were received for the validation report in support of the Test Guideline using *Lymnaea stagnalis*, which was subsequently approved by the WNT at its 28th meeting in April 2016. The Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology agreed to declassification of the validation report on 8 July 2016.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology.

REPORT ON THE VALIDATION OF THE LYMNAEA STAGNALIS REPRODUCTION TOXICITY TEST

Report prepared by Virginie Ducrot[1] and Sandrine Charles[2].

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BACKGROUND

1. 1n 2010 the OECD issued a Detailed Review Paper (DRP) on the published literature relating to mollusc life-cycle toxicity testing of chemicals. 1t recommended the development of protocols for tests with the freshwater gastropods *Potamopyrgus antipodarum* and *Lymnaea stagnalis*. Only results for *L. stagnalis* are discussed in this report.

2. Denmark, France, Germany and the United Kingdom took the initiative of developing such tests and therefore submitted a Standard Project Submission Form (SPSF) to the OECD in February 2011. Based upon this SPSF, the OECD test guideline program has been extended to establish a reproduction (partial life-cycle, static renewal) protocol for assessing the toxicity of chemicals to these two mollusc species.

3. Since 2011, a 56d reproductive static-renewal test method has been evaluated in a first ring-test using *L. stagnalis* and involving seven laboratories in Europe. The results of this first ring-test were presented in a detailed report, which was discussed during the 9th meeting of the Validation Management Group for Ecotoxicity Testing (VMG-eco 9) in 2013, and during the meeting of the OECD Ad hoc Expert Group on 1nvertebrate Testing in 2013.

4. Based on above-mentioned discussions and the valuable experience gained in the first ring-tests, a consolidated draft standard operating procedures (SOPs) has been produced, which presents both rearing and toxicity test procedures and their application to evaluate reproductive toxicants.

5. The validation studies have started in 2013 based upon the consolidated SOPs. This validation study has been conducted according to the 2013-2015 workplan that was submitted to VMG-eco in September 2013 and discussed in the 9th meeting of the VMG-eco in 2013.

6. The aim of the validation ring-tests were three fold: (i) assessing the reproducibility of test results among a large number of laboratories with different levels of experience in mollusc testing (from inexperienced to expert ones), (ii) assessing consistency and reproducibility of toxicity values (i.e. range of NOEC and ECx values found by all partners) between the ring-tests (i.e. first ring-test/pre- validation vs. validation) and (iii) assessing responses of the snails to a larger number of chemicals than studied in the first ring-tests.

7. The preliminary results of these validation studies for both species were presented in a detailed report, which was discussed during the 10th meeting of the Validation Management Group for Ecotoxicity Testing (VMG-eco 10) in 2014.

8. This report presents an update of the progress report presented in December 2014 and discussed during VMG-Eco 10. In the present report, results are discussed in the light of measured chemical concentrations and appropriate statistical tests for NOEC and LOEC determination for *Lymnaea stagnalis*. The results of test design optimization studies with *L. stagnalis* are also presented, with conclusions regarding the optimal test duration and core test endpoints to be used in the forthcoming test guideline for reproductive toxicity tests with *Lymnaea stagnalis*.

EXECUTIVE SUMMARY

9. 1n tests with *L. stagnalis*, the production of clutches (egg-laying behaviour) and eggs (fecundity) were analysed as two independent endpoints. Endpoint values were then compared. The choice of the reproduction endpoint did not influence the values of the NOEC, LOEC or ECx, so that both endpoints can be used. Results for both endpoints are presented in this report.

10. Seven laboratories participated in the cadmium ring-test with *L. stagnalis.* Mean measured concentrations were in the range between 19 to 300 µg/L. Cadmium exposure induced a concentration-dependant decrease in fecundity in all laboratories. NOEC values were less than the lowest tested concentration in many instances and could thus not be determined. 1n laboratories where NOEC could be determined, it varied between 33 and 79 µg/L. LOEC values varied between 19 and 154 µg/L. EC_X values were estimated for six out of seven laboratories and were similar, except for Lab 8. 1n Lab 8, lower $EC_{50\text{-}56d}$ values were found for both endpoints. The softness of test water used in this laboratory (<50 mg $CaCo_3.L^{-1}$) probably explains these results, since water softness is known to increase the toxicity of cadmium. The mean values (and standard deviation) for $EC_{50\text{-}56d}$ were 139 µg/L (± 77) and 103 µg/L (± 40), based on the number of clutches or eggs per individual-day, respectively. A 6-fold or 4-fold difference was obtained between the lowest and the highest estimated $EC_{50\text{-}56d,}$ based on the number of clutches or eggs per individual- day, respectively. The corresponding coefficients of variation of the $EC_{50\text{-}56d}$ among all laboratories were 55 and 39%, respectively (42 and 28% when data from Lab. 8 are excluded). This inter-laboratory variability is comprised in the range of acceptable variation defined for reference chemicals in OECD acute toxicity tests guidelines for invertebrates. Results were consistent with results from the previous (pre-validation) ring-test with cadmium and *L. stagnalis*.

11. Seven laboratories participated in the TBT ring-test with *L. stagnalis.* Mean measured concentrations were in the range between 39 and 435 ng TBT/L. Results of these reproduction tests again showed a good agreement among participating laboratories. A concentration- dependant decrease in fecundity was observed in all laboratories. NOEC and LOEC values were quite similar among laboratories where significant effects could be detected. 1ndeed, NOEC values varied between 118 and 251 ng TBT/L and LOEC values varied between 251 and 435 ng TBT/L. EC_X values were estimated for all laboratories and endpoints, and were similar except for Lab. 8 where lower $EC_{50\text{-}56d}$ values were found for both endpoints. The mean values (and standard deviation) for $EC_{50\text{-}56d}$ were 382 ng TBT/L (± 194) and 326 ng TBT/L (± 154), based on the number of clutches or eggs per individual-day, respectively. A 4-fold and 3-fold difference was obtained between the lowest and the highest estimated $EC_{50\text{-}56d}$, based on the number of clutches or eggs per individual-day, respectively. The corresponding coefficients of variation of the $EC_{50\text{-}56d}$ among all laboratories were 51 and 47%, which is again an acceptable inter-laboratory variability. Results were also consistent with the results from the previous (pre-validation) ring-test with TBT and *L. stagnalis*.

12. Four laboratories participated in the prochloraz ring-test with *L. stagnalis.* Mean measured concentrations were in the range between 8 and 378 µg/L. Exposure to prochloraz inhibited snail reproduction in all laboratories. NOEC values varied in the range between 75 and 412 µg/L (which generally corresponded to the fourth tested concentration), while LOEC values were in the range between 496 and 873 µg/L, which generally corresponded to the highest tested concentration.

The mean values (and standard deviation) for $EC_{50\text{-}56d}$ were 382 µg/L (±193) and 326 µg/L (±154),), based on the number of clutches or eggs per individual-day, respectively. A 4-fold and 3-fold difference was obtained between the lowest and the highest estimated $EC_{50\text{-}56d}$, based on the number of clutches or eggs per individual-day, respectively. The corresponding coefficients of variation of the $EC_{50\text{-}56d}$ among all laboratories were 30 and 42%, which is again an acceptable inter-laboratory variability.

13. Two laboratories participated in the trenbolone ring-test with *L. stagnalis.* Mean measured concentrations were in the range between 9 and 394 ng/L. No decrease in snail fecundity was observed in this concentration range. Since trenbolone did not affect the reproduction of *L. stagnalis*, it was decided to stop the testing with this test substance.

14. Finally, data from the pre-validation and validation studies with *L. stagnalis* were used for the optimisation of the test duration and for the choice of the core test endpoint (i.e. number of eggs vs. number of clutches produced per individual-day) to be used. Statistical evaluation of data resulting in the protocol optimisation are presented in this report, which shows that monitoring the number of clutches produced per individual-day over 28 ensures both adequate sensitivity and cost-effectiveness of the proposed toxicity test, on the basis of both EC_{50} values and NOECs.

15. The robustness and the reproducibility of the reproduction test with *Lymnaea stagnalis* have been demonstrated based upon measured concentrations in two validation exercises with four test compounds. 1n total 16 partners from 9 countries participated in these ring-tests coming from industries, government and academia. Within these validation studies, 38 reproduction tests have been performed, thereof seven tests did not achieve the given validity criteria. Two laboratories had technical issues to satisfy the temperature of 20 °C, one laboratory had issues in maintaining the appropriate concentration of dissolved oxygen in test water and three other laboratories did not meet the biological criteria (maximum control mortality or minimum clutch number in control groups). This suggests that the given criteria are appropriate and achievable. For all tested chemicals the inter-laboratory reproducibility of the test has been shown as most of the laboratories detected comparable NOEC, LOEC and $EC_{50\text{-}56d}$ values with overlapping 95% credible intervals for the latter. Furthermore the repeatability could be demonstrated between the validation and pre-validation ring-tests.

OECD MOLLUSC TESTS - VALIDATION PHASE CADMIUM RESULTS OVERVIEW FOR ALL PARTNER LABS - MEASURED CONCENTRATIONS

Overview of measured concentrations

16. On a general point of view, Cadmium concentrations were measured at three different dates during the experiment. Each measurement consisted in two Cd concentration values: the first one (”conc.on”) is obtained at water renewal time; the second one (”conc.off”) is obtained after an interval of three days.

17. As for the data analysis of the pre-validation phase, measured Cd concentrations were calculated for each lab and each nominal concentration as the arithmetic mean of all measured values, $n = 2$ to $n = 12$ depending on the lab and on the measurement date.

18. Figure 1 shows the relationship between nominal concentrations and measured ones for all partner labs separately. Table 1 gives calculated arithmetic means and the associated standard deviations for all partner labs. Figure 2 shows the relationship between the arithmetic mean Cd concentrations measured over the test period and the nominal concentrations for all partner laboratories.

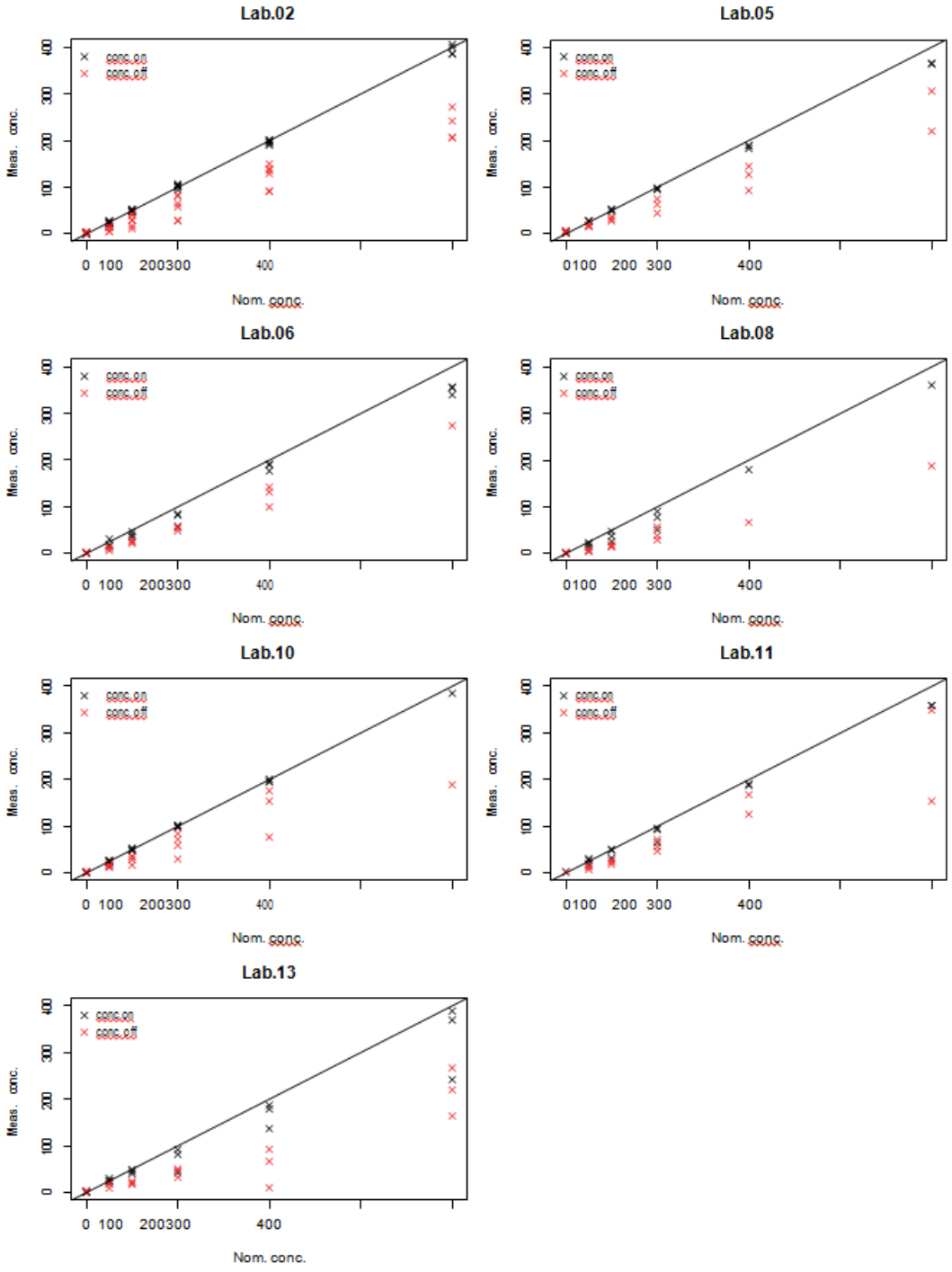

Figure 1: Relationship between nominal and measured concentrations (in µg.L−1): in black, ”conc.on” measurements at water renewal time; in red, ”conc.off” measurements after an interval of three days. The plain line corresponds to the first bisector.

Lab.num	conc.nom	conc.mean	conc.sd
Lab.02	0	1.333	1.723
Lab.02	25	18.33	7.691
Lab.02	50	39.25	14.26
Lab.02	100	79.58	28.58
Lab.02	200	159.9	42.27
Lab.02	400	313.1	89.67
Lab.05	0	4.342	2.164
Lab.05	25	22.47	5.796
Lab.05	50	41.6	10.91
Lab.05	100	78.62	21.87
Lab.05	200	154.3	39.61
Lab.05	400	314	68.57
Lab.06	0	1.21	0.8861
Lab.06	25	16.18	8.368
Lab.06	50	32.72	9.668
Lab.06	100	64.33	15.34
Lab.06	200	155.4	36.97
Lab.06	400	331.5	39.08
Lab.08	0	1.015	1.419
Lab.08	25	12.39	7.84
Lab.08	50	26.6	13.51
Lab.08	100	57.22	23.27
Lab.08	200	123.4	79.97
Lab.08	400	274.5	122.3
Lab.10	0	1.229	1.139
Lab.10	25	20.08	5.692
Lab.10	50	39.45	12.91
Lab.10	100	80.74	25.88
Lab.10	200	166.7	47.31
Lab.10	400	287	138.6
Lab.11	0	2.165	1.252
Lab.11	25	18.86	8.972
Lab.11	50	33.6	13.27
Lab.11	100	71.48	19.5
Lab.11	200	168.2	29.96
Lab.11	400	305.2	100.9
Lab.13	0	1.817	1.238
Lab.13	25	21.22	7.254
Lab.13	50	32.78	13.92
Lab.13	100	57.67	23.65
Lab.13	200	112.2	68.52
Lab.13	400	275.3	87.77

Table 1: Arithmetic means (”conc.mean”) and associated standard deviations (”conc.sd”) of concentration measurements corresponding to each nominal Cd concentrations for all partner labs. All concentrations are expressed in µg.L−1.

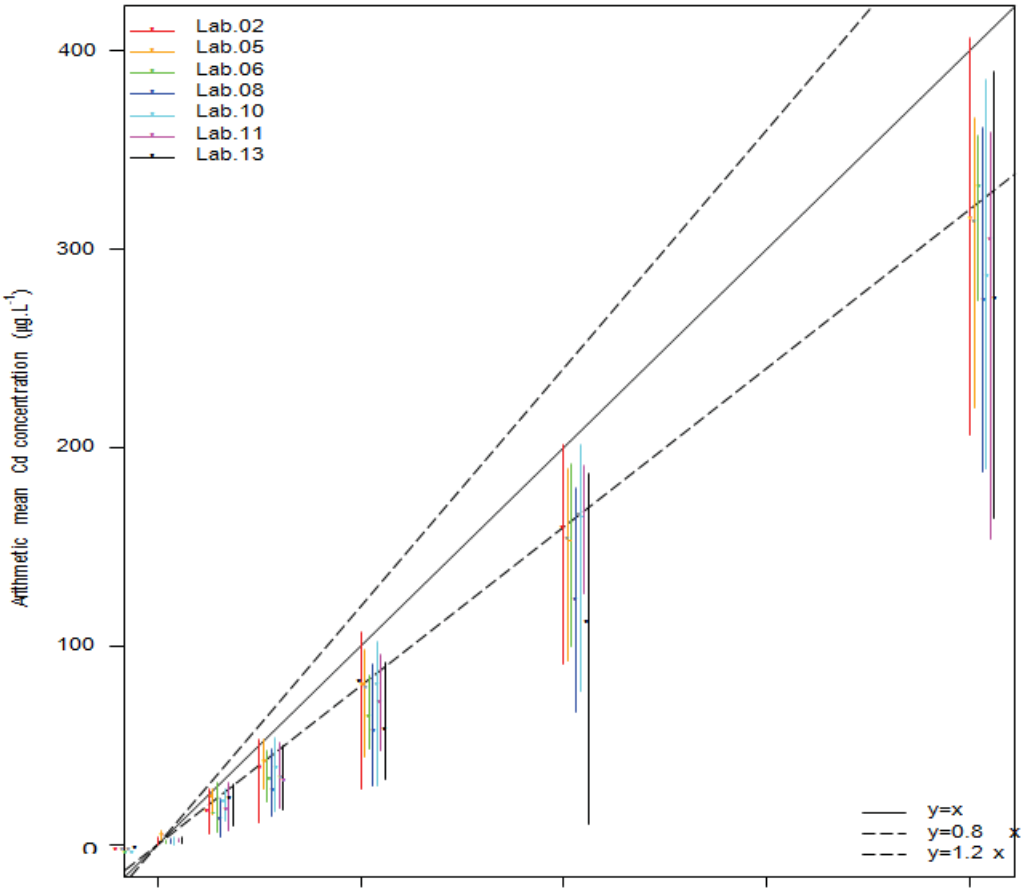

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Figure 2: Relationship between the arithmetic mean Cd concentrations measured over the test period and the nominal concentration for all each partner laboratories. Reported values correspond to the means (circles) and their spread (segments; i.e., ranges from minimal to maximal measured values). The solid line represents the theoretical case where actual concentrations are similar to the nominal concentrations (first bisector), while dashed lines represent theoretical cases where actual concentrations reach 80% and 120% of the nominal concentrations, respectively.

EC_X estimates from Cd measured concentrations

Figure 3 shows EC_{50} for all labs, estimated from clutch or egg data.

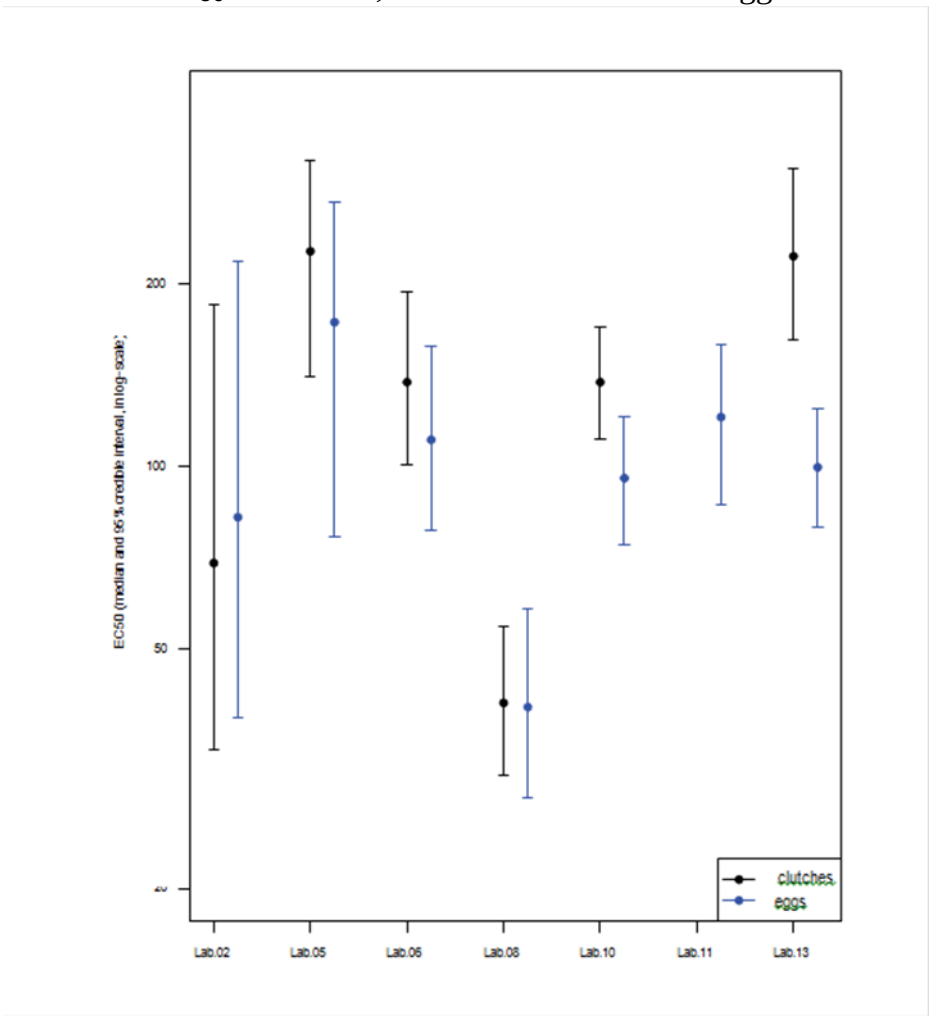

Figure 3: $EC50$ estimates (medians and 95% credible intervals for all partner labs): in black, $EC50$ estimated from clutch data; in blue, $EC50$ estimated from egg data.

19. Tables 2 and 3 give EC_X estimates with their 95% credible intervals for $x = 5, 10, 20, 50$, when it was possible to estimate such toxicity values. Tables show results with different orders of lines:

Table 2 Results are ordered per lab and per endpoint;

Table 3 Results are ordered per endpoint and per lab.

Lab	Tox	Endpoint	Model	med.EC5	Q2.5.EC5	Q97.5.EC5	med.EC10	Q2.5.EC10	Q97.5.EC10	med.EC20	Q2.5.EC20	Q97.5.EC20	med.EC50	Q2.5.EC50	Q97.5.EC50
Lab.02	Cadmium	clutches	P	4.69e-02	2.14e-46	1.43e+00	2.90e-01	2.92e-34	3.95e+00	2.11e+00	3.59e-21	1.20e+01	6.93e+01	3.41e+01	1.85e+02
Lab.02	Cadmium	eggs	GP	7.73e-03	2.52e-68	1.30e+00	8.09e-02	1.05e-50	3.79e+00	1.01e+00	1.43e-31	1.25e+01	8.25e+01	3.85e+01	2.18e+02
Lab.05	Cadmium	clutches	GP	5.80e+01	1.44e+00	1.96e+02	8.18e+01	4.84e+00	2.16e+02	1.19e+02	1.79e+01	2.42e+02	2.27e+02	1.41e+02	3.20e+02
Lab.05	Cadmium	eggs	GP	2.28e+01	7.78e-02	1.14e+02	3.81e+01	4.88e-01	1.39e+02	6.62e+01	3.48e+00	1.73e+02	1.73e+02	7.65e+01	2.73e+02
Lab.06	Cadmium	clutches	GP	8.64e+00	1.68e+00	2.60e+01	1.74e+01	5.07e+00	4.12e+01	3.72e+01	1.66e+01	6.90e+01	1.38e+02	1.01e+02	1.94e+02
Lab.06	Cadmium	eggs	GP	4.27e+00	7.99e-01	1.34e+01	9.76e+00	2.65e+00	2.43e+01	2.39e+01	9.71e+00	4.64e+01	1.11e+02	7.85e+01	1.58e+02
Lab.08	Cadmium	clutches	P	4.47e+00	1.64e+00	9.31e+00	7.81e+00	3.58e+00	1.41e+01	1.43e+01	8.31e+00	2.25e+01	4.07e+01	3.09e+01	5.45e+01
Lab.08	Cadmium	eggs	GP	9.63e+00	3.96e+00	1.89e+01	1.38e+01	6.66e+00	2.47e+01	2.04e+01	1.16e+01	3.33e+01	4.01e+01	2.84e+01	5.80e+01
Lab.10	Cadmium	clutches	GP	3.78e+01	1.81e+01	6.48e+01	5.25e+01	2.91e+01	8.14e+01	7.49e+01	4.87e+01	1.05e+02	1.38e+02	1.11e+02	1.69e+02
Lab.10	Cadmium	eggs	GP	2.39e+01	1.15e+01	4.13e+01	3.40e+01	1.87e+01	5.35e+01	4.98e+01	3.14e+01	7.17e+01	9.57e+01	7.41e+01	1.21e+02
Lab.11	Cadmium	eggs	GP	1.60e+01	5.66e+00	3.67e+01	2.67e+01	1.15e+01	5.27e+01	4.66e+01	2.46e+01	7.86e+01	1.21e+02	8.62e+01	1.59e+02
ab.13	Cadmium	clutches	GP	1.96e+01	4.60e+00	5.80e+01	3.64e+01	1.21e+01	8.49e+01	7.10e+01	3.38e+01	1.29e+02	2.23e+02	1.62e+02	3.10e+02
Lab.13	Cadmium	eggs	GP	1.62e+01	8.45e+00	2.84e+01	2.58e+01	1.50e+01	4.09e+01	4.25e+01	2.80e+01	6.12e+01	9.97e+01	7.92e+01	1.25e+02

Table 2: ECx ordered per lab and per endpoint.

Lab	Tox	Endpoint	Model	med.EC5	Q2.5.EC5	Q97.5.EC5	med.EC10	Q2.5.EC10	Q97.5.EC10	med.EC20	Q2.5.EC20	Q97.5.EC20	med.EC50	Q2.5.EC50	Q97.5.EC50
Lab.02	Cadmium	clutches	P	4.69e-02	2.14e-46	1.43e+00	2.90e-01	2.92e-34	3.95e+00	2.11e+00	3.59e-21	1.20e+01	6.93e+01	3.41e+01	1.85e+02
Lab.05	Cadmium	clutches	GP	5.80e+01	1.44e+00	1.96e+02	8.18e+01	4.84e+00	2.16e+02	1.19e+02	1.79e+01	2.42e+02	2.27e+02	1.41e+02	3.20e+02
Lab.06	Cadmium	clutches	GP	8.64e+00	1.68e+00	2.60e+01	1.74e+01	5.07e+00	4.12e+01	3.72e+01	1.66e+01	6.90e+01	1.38e+02	1.01e+02	1.94e+02
Lab.08	Cadmium	clutches	P	4.47e+00	1.64e+00	9.31e+00	7.81e+00	3.58e+00	1.41e+01	1.43e+01	8.31e+00	2.25e+01	4.07e+01	3.09e+01	5.45e+01
Lab.10	Cadmium	clutches	GP	3.78e+01	1.81e+01	6.48e+01	5.25e+01	2.91e+01	8.14e+01	7.49e+01	4.87e+01	1.05e+02	1.38e+02	1.11e+02	1.69e+02
Lab.13	Cadmium	clutches	GP	1.96e+01	4.60e+00	5.80e+01	3.64e+01	1.21e+01	8.49e+01	7.10e+01	3.38e+01	1.29e+02	2.23e+02	1.62e+02	3.10e+02
Lab.02	Cadmium	eggs	GP	7.73e-03	2.52e-68	1.30e+00	8.09e-02	1.05e-50	3.79e+00	1.01e+00	1.43e-31	1.25e+01	8.25e+01	3.85e+01	2.18e+02
Lab.05	Cadmium	eggs	GP	2.28e+01	7.78e-02	1.14e+02	3.81e+01	4.88e-01	1.39e+02	6.62e+01	3.48e+00	1.73e+02	1.73e+02	7.65e+01	2.73e+02
Lab.06	Cadmium	eggs	GP	4.27e+00	7.99e-01	1.34e+01	9.76e+00	2.65e+00	2.43e+01	2.39e+01	9.71e+00	4.64e+01	1.11e+02	7.85e+01	1.58e+02
Lab.08	Cadmium	eggs	GP	9.63e+00	3.96e+00	1.89e+01	1.38e+01	6.66e+00	2.47e+01	2.04e+01	1.16e+01	3.33e+01	4.01e+01	2.84e+01	5.80e+01
Lab.10	Cadmium	eggs	GP	2.39e+01	1.15e+01	4.13e+01	3.40e+01	1.87e+01	5.35e+01	4.98e+01	3.14e+01	7.17e+01	9.57e+01	7.41e+01	1.21e+02
Lab.11	Cadmium	eggs	GP	1.60e+01	5.66e+00	3.67e+01	2.67e+01	1.15e+01	5.27e+01	4.66e+01	2.46e+01	7.86e+01	1.21e+02	8.62e+01	1.59e+02
Lab.13	Cadmium	eggs	GP	1.62e+01	8.45e+00	2.84e+01	2.58e+01	1.50e+01	4.09e+01	4.25e+01	2.80e+01	6.12e+01	9.97e+01	7.92e+01	1.25e+02

Table 3: ECx ordered per endpoint and per lab.

Between-lab variability

20. Coefficients of variation (CV) were calculated from median values of EC_{50} estimates for all labs and both endpoints[1].

- → From EC_{50} medians estimated on clutch data, we got a CV equal to 55%.
- → From EC_{50} medians estimated on egg data, we got a CV equal to 39%.

21. The between-lab variability appeared high mainly due to results from Lab.08 with EC_{50} estimates much lower than for the other labs (see Figure 3). This lab will be excluded from further analyses based on experimental conditions not in accordance with validity criteria.

22. Table 4 allows us to compare both prevalidation and validation phases on the basis of the coefficients of variation for both endpoints, clutches end eggs.

Endpoint	CV-prevalidation	CV-validation (without Lab.08)
Clutches	31% (n = 5)	42% (n = 5)
Eggs	22% (n = 5)	28% (n = 6)

Table 4: Coefficients of variation for clutch and egg endpoints and both phases, calculated from EC_{50} median values. Results from Lab.08 have been excluded.

23. The between-lab variability was slightly higher within the validation phase. Notice that extreme results from Lab.08 were excluded in order not to bias the CV calculation. Notice also that the protocol changed between the two phases and that all labs were different between the two phases.

24. In addition to Table 4, Figure 4 shows the comparison between EC_{50} estimates of the pre- and the validation phases, as well as the comparison between EC_{50} estimates from clutch and egg data.

[1] As a reminder, CV = $\frac{\mu}{\sigma}$, where μ and σ are mean and standard deviation of the EC_{50} medians.

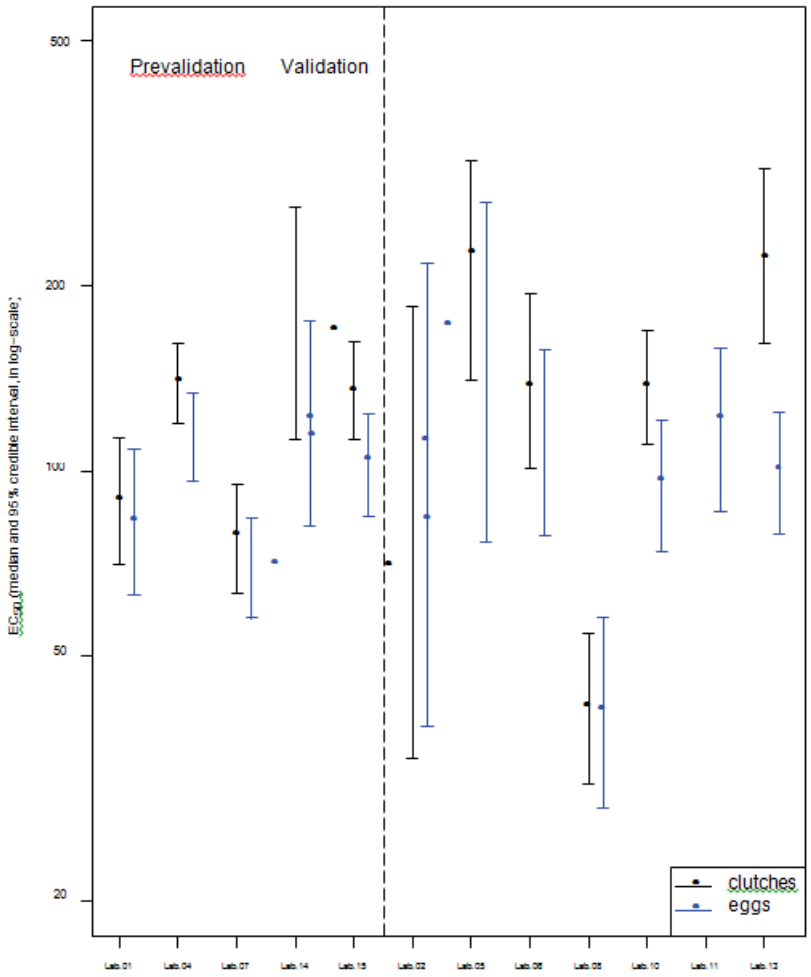

Figure 4: Comparison between $EC50$ estimates of the prevalidation phase (left to the dotted vertical black line) and the validation phase (right to the dotted vertical black line). Circles stand for EC_{50} median values and flat arrows for their 95% credible intervals: in black, when clutch data were used; in blue when egg data were used.

EXPERIMENTAL DESIGN OPTIMISATION

Experiment duration

25. The possible reduction in the experiment duration has been studied by comparing the EC_{50} estimates (median ± 95% credible interval) at each target time (from 21 to 56 days) to the interval $EC_{50-56d} \pm sd$, where EC_{50-56d} is the median estimates at day 56 and *sd* is the standard deviation of the EC_{50-56d} medians between labs of a given phase.

26. Because protocols were different between the two phases, two different analyses were performed: (i) within the pre-validation phase, including five labs; two different sd_{preval} values were calculated for EC_{50-56d} medians from clutch and egg data (Figure 5). (ii) within the validation phase, including six labs (Lab.08 was excluded); two different sd_{val} values were calculated for EC_{50-56d} medians from clutch and egg data (Figure 6).

27. From these analyses, it was possible to extract the first target time at which the EC_{50} median was outside the interval $EC_{50-56d} \pm sd$:

(i) Table 5 for the prevalidation phase;

(ii) Table 6 for the validation phase.

28. Because the between-lab variability was less important in the prevalidation phase than in the validation phase, target times are much greater (Table 5 versus Table 6). Based on these two tables, we could conclude differently according to the phase and the endpoint under consideration (see red and green values in Tables 5 and 6). Nevertheless, consider- ing that the experimental protocol was almost in its final version in the validation phase, we should refer to the corresponding results to conclude. Hence, based on Table 6 and Figure 6, we could reasonably suggest 28 days of experiment for clutches and 32 days for eggs. Such results need to be further confirmed from experiments with other toxicants (see TBT analyses, in particular).

Number of replicates

29. Our first idea was to explore the following items related to the number of replicates:

- reduce the number of replicates and keep the number of tested concentrations?
- reduce the number of replicates and increase the number of tested concentrations for the same experimental effort?
- reduce the number of replicate concomitantly with the reduction in the experiment duration?

30. After the OECD mollusc meeting in Odense (Danemark, 18-20 February 2015), it was decided not to consider these issues yet, but only to focus on the reduction in the experiment duration.

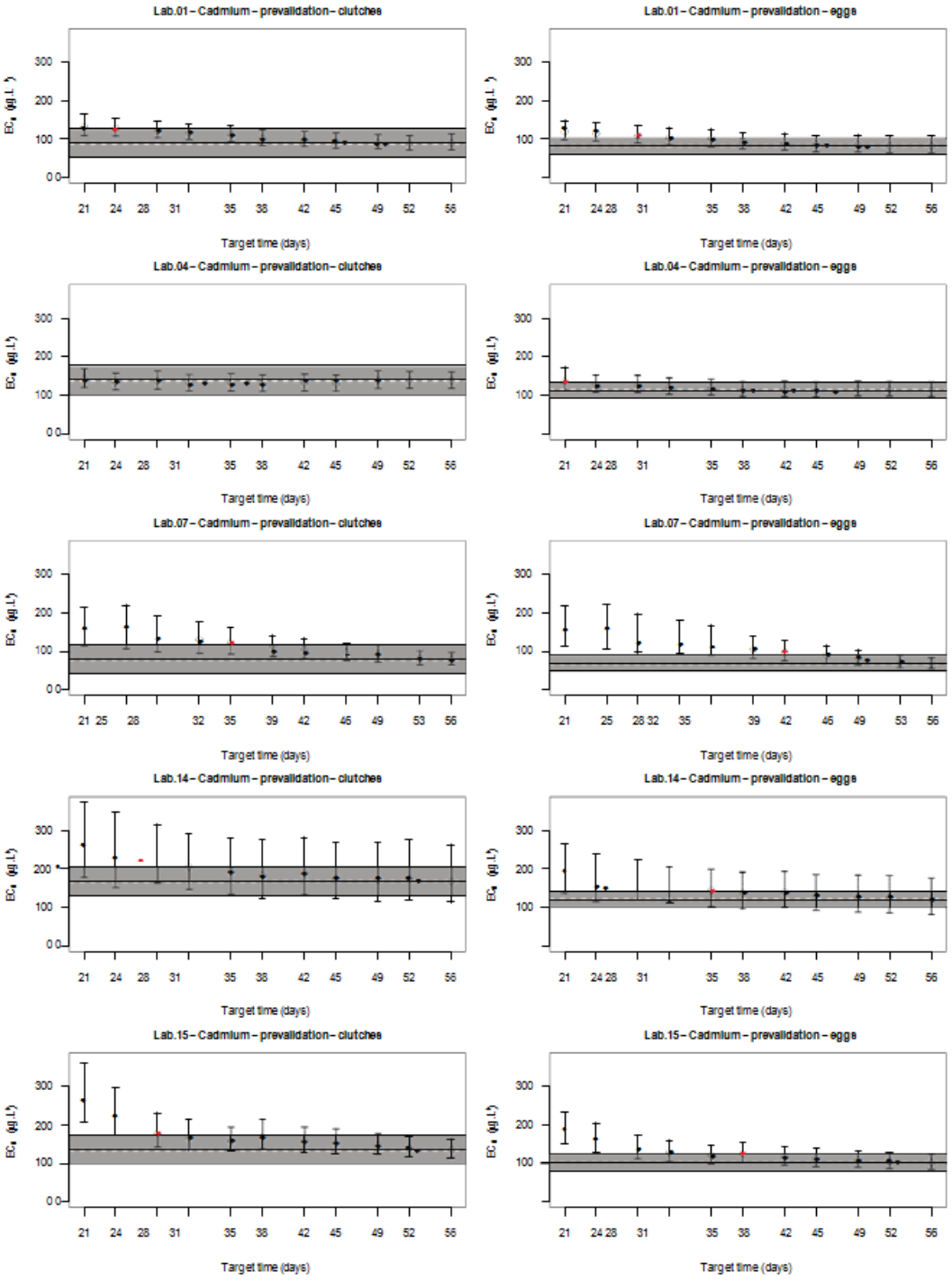

Figure 6: EC_{50} estimates (medians and 95% credible intervals) according to the target time (in days) for all labs and both endpoints of the validation phase. The red circle points out the first target time at which the EC_{50} median is outside the interval $EC_{50-56d} \pm sd_{val}$ (grey band).

Lab	Tox	Phase	Endpoint	Target time
Lab.01	Cadmium	prevalidation	clutches	24
Lab.01	Cadmium	prevalidation	eggs	28
Lab.04	Cadmium	prevalidation	clutches	< 21
Lab.04	Cadmium	prevalidation	eggs	21
Lab.07	Cadmium	prevalidation	clutches	35
Lab.07	Cadmium	prevalidation	eggs	42
Lab.14	Cadmium	prevalidation	clutches	28
Lab.14	Cadmium	prevalidation	eggs	35
Lab.15	Cadmium	prevalidation	clutches	28
Lab.15	Cadmium	prevalidation	eggs	38

Table 5: First target time at which the EC_{50} median is outside the interval $EC_{50-56d} \pm sd_{preval}$ for the prevalidation phase. The red value stands for the highest target time among labs for clutches; the green value stands for the highest target time among labs for eggs.

labnumbers	toxnames	phasenames	endpointnames	target.choice.val
Lab.02	Cadmium	validation	clutches	21
Lab.02	Cadmium	validation	eggs	21
Lab.05	Cadmium	validation	clutches	<21
Lab.05	Cadmium	validation	eggs	21
Lab.06	Cadmium	validation	clutches	<21
Lab.06	Cadmium	validation	eggs	24
Lab.10	Cadmium	validation	clutches	<21
Lab.10	Cadmium	validation	eggs	<21
Lab.11	Cadmium	validation	clutches	ND
Lab.11	Cadmium	validation	eggs	31
Lab.13	Cadmium	validation	clutches	21
Lab.13	Cadmium	validation	eggs	32

Table 6: First target time at which the EC_{50} median is outside the interval $EC_{50-56d} \pm sd_{val}$ for the validation phase. The red value stands for the highest target time among labs for clutches; the green value stands for the highest target time among labs for eggs.

OECD MOLLUSK TESTS - VALIDATION PHASE TBT RESULTS OVERVIEW FOR ALL PARTNER LABS - MEASURED CONCENTRATIONS

OVERVIEW OF MEASURED CONCENTRATIONS

31. Two labs were omitted from the TBT analyses because data were not complete.

32. As for cadmium analyses, TBT concentrations were measured at three different dates during the experiment. Each measurement consisted in two TBT concentration values: the first one (”conc.on”) is obtained at water renewal time; the second one (”conc.off”) is obtained after an interval of three days.

33. As for the Cd analysis, measured TBT concentrations were calculated for each lab and each nominal concentration as the arithmetic mean of all measured values, $n = 4$ or $n = 12$ depending on the lab and on the measurement date.

34. Figure 1 shows the relationship between nominal concentrations and measured ones for all partner labs separately. Table 1 gives calculated arithmetic means and the associated standard deviations for all partner labs. Figure 2 shows the relationship between the arithmetic mean Cd concentrations measured over the test period and the nominal concentrations for all partner laboratories simultaneously.

35. We mainly notice that measured concentrations for Lab.03 are very very low compared to nominal ones, what makes suspicious further analyses with this lab. Should it be maintained in the analysis?

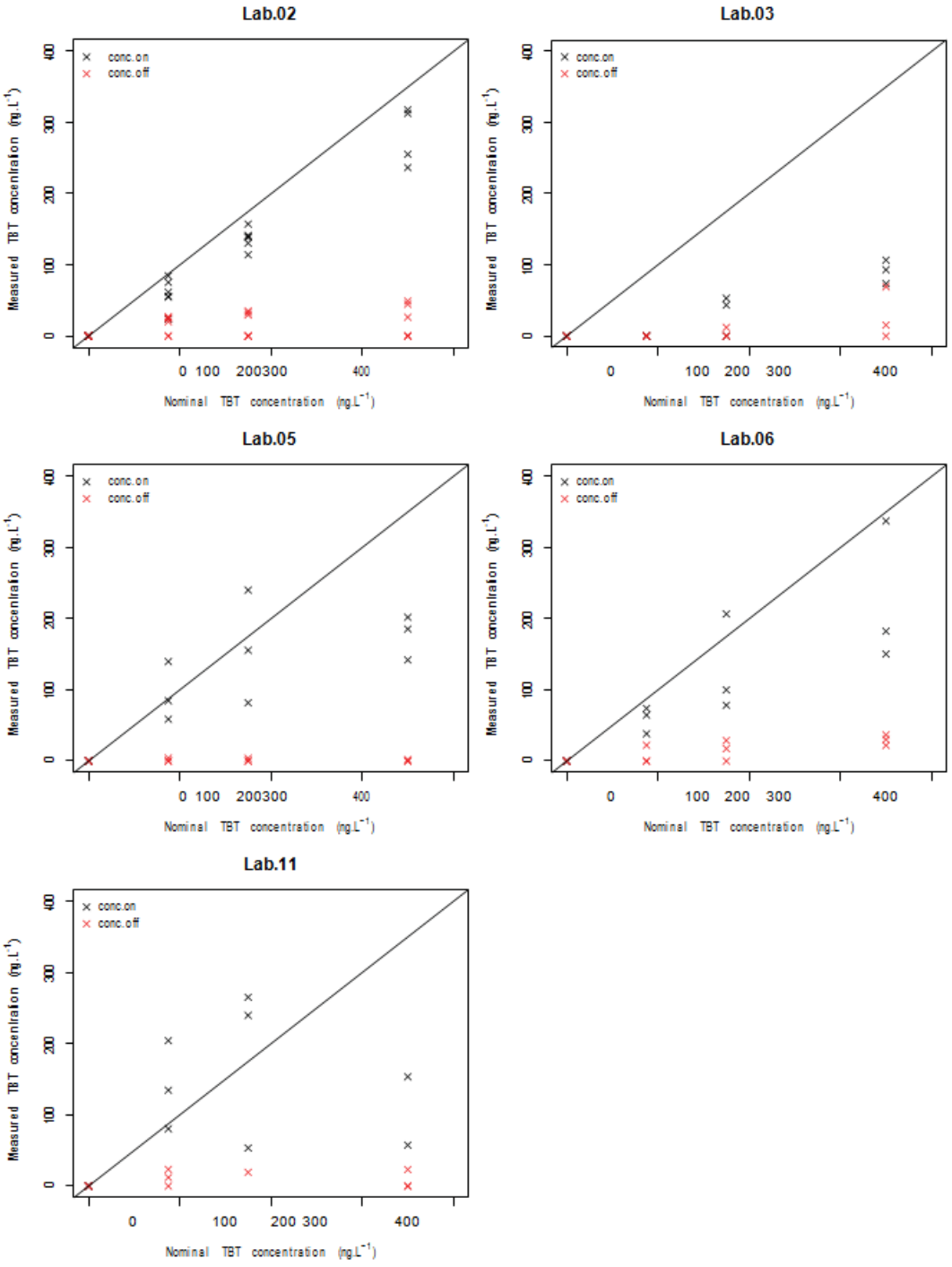

Figure 1: Relationship between nominal and measured concentrations (in ng.L^{-1}): in black, ''conc.on'' measurements at water renewal time; in red, ''conc.off'' measurements after an interval of three days. The plain line corresponds to the first bisector.

lab.num	conc.nom	conc.mean	conc.sd
Lab.02	0	0	0
Lab.02	87.5	37.83	27.99
Lab.02	175	76.67	64.92
Lab.02	350	189.7	199.4
Lab.02	700	273.5	267.2
Lab.02	1400	808.5	776.9
Lab.03	0	0	0
Lab.03	87.5	0	0
Lab.03	175	18.14	24.06
Lab.03	350	59.65	42.77
Lab.03	700	95.34	78.68
Lab.03	1400	167.9	116.1
Lab.05	0	0	0
Lab.05	87.5	47.92	57.3
Lab.05	175	80.24	99.99
Lab.05	350	88.58	98.32
Lab.05	700	184.8	193.6
Lab.05	1400	291.2	310.8
Lab.06	0	0	0
Lab.06	87.5	33.22	31.66
Lab.06	175	71.93	76.17
Lab.06	350	126.6	123.6
Lab.06	700	345.8	238.3
Lab.06	1400	295.1	274.5
Lab.11	0	0	0
Lab.11	87.5	75.86	80.75
Lab.11	175	144.6	126.1
Lab.11	350	125.9	201.9
Lab.11	700	355.1	477.7
Lab.11	1400	610.9	759.5

Table 1: Arithmetic means (”conc.mean”) and associated standard deviations (”conc.sd”) of con- centration measurements corresponding to each nominal TBT concentrations for all partner labs. All concentrations are expressed in $ng.L^{-1}$.

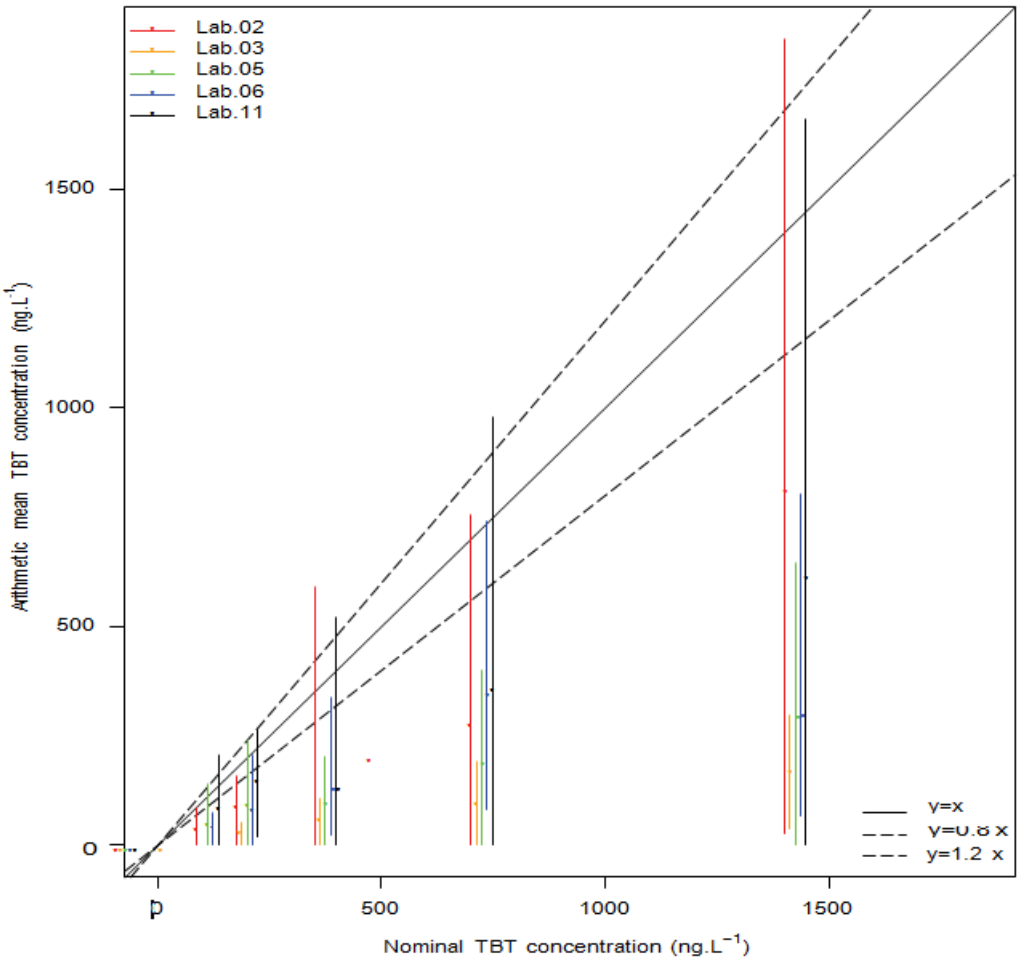

Figure 2: Relationship between the arithmetic mean TBT concentrations measured over the test period and the nominal concentration for all each partner laboratories. Reported values correspond to the means (circles) and their spread (segments; i.e., ranges from minimal to maximal measured values). The solid line represents the theoretical case where actual concentrations are similar to the nominal concentrations (first bisector), while dashed lines represent theoretical cases where actual concentrations reach 80% and 120% of the nominal concentrations, respectively.

EC_X ESTIMATES FROM TBT MEASURED CONCENTRATIONS

Figure 3 shows EC_{50} for all labs, estimated from clutch or egg data.

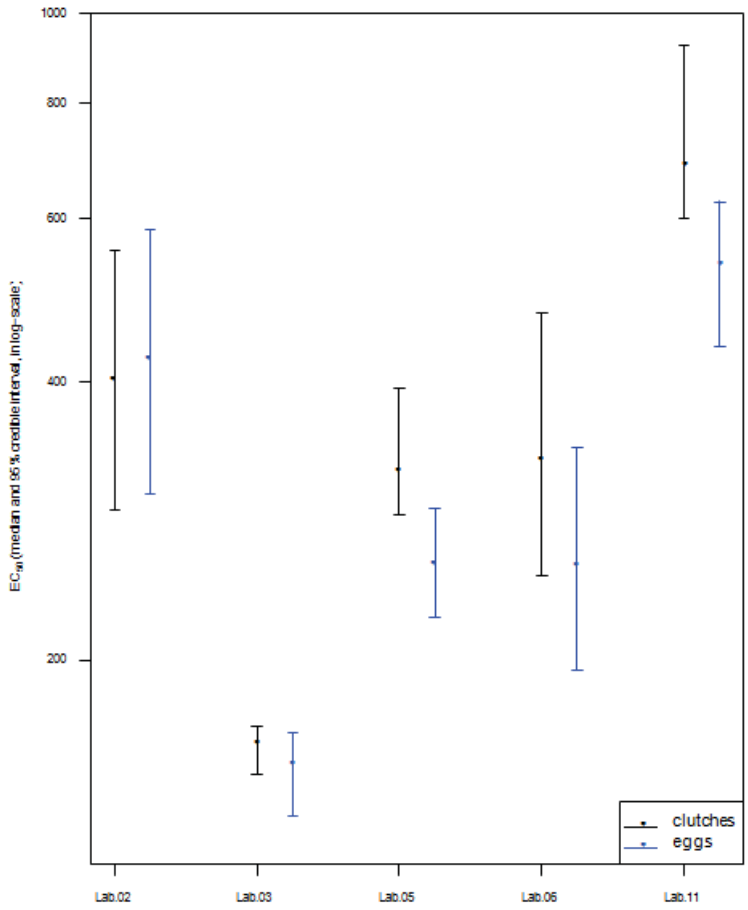

Figure 3: EC_{50} estimates (medians and 95% credible intervals for all partner labs): in black, EC_{50} estimated from clutch data; in blue, EC_{50} estimated from egg data.

36. Tables 2 and 3 give EC_x estimates with their 95% credible intervals for $x = 5, 10, 20, 50$, when it was possible to estimate such toxicity values. Tables show results with different orders of lines:

Table 2 Results are ordered per lab and per endpoint;

Table 3 Results are ordered per endpoint and per lab.

Lab	Tox	Endpoint	Model	med.EC5	Q2.5.EC5	Q97.5.EC5	med.EC10	Q2.5.EC10	Q97.5.EC1	med.EC20	Q2.5.EC20	Q97.5.EC2	med.EC50	Q2.5.EC50	Q97.5.EC50
Lab.02	TBT	clutches	GP	2.10e+01	4.31e+00	6.12e+01	4.43e+01	1.31e+01	1.04e+02	1.00e+02	4.35e+01	1.85e+02	4.03e+02	2.90e+02	5.55e+02
Lab.02	TBT	eggs	GP	3.18e+01	6.75e+00	8.73e+01	6.14e+01	1.85e+01	1.38e+02	1.25e+02	5.41e+01	2.26e+02	4.26e+02	3.02e+02	5.83e+02
Lab.03	TBT	clutches	GP	1.16e+02	7.08e+01	1.60e+02	1.26e+02	8.63e+01	1.62e+02	1.39e+02	1.07e+02	1.64e+02	1.63e+02	1.50e+02	1.69e+02
Lab.03	TBT	eggs	GP	1.03e+02	5.75e+01	1.58e+02	1.14e+02	7.23e+01	1.60e+02	1.28e+02	9.20e+01	1.62e+02	1.55e+02	1.36e+02	1.67e+02
Lab.05	TBT	clutches	GP	1.22e+02	5.79e+01	2.51e+02	1.57e+02	9.03e+01	2.64e+02	2.05e+02	1.44e+02	2.79e+02	3.23e+02	2.86e+02	3.93e+02
Lab.05	TBT	eggs	GP	1.14e+02	6.09e+01	1.88e+02	1.40e+02	8.63e+01	2.08e+02	1.75e+02	1.24e+02	2.32e+02	2.56e+02	2.22e+02	2.92e+02
Lab.06	TBT	clutches	GP	6.38e+01	1.29e+01	1.63e+02	9.66e+01	2.91e+01	2.00e+02	1.52e+02	6.95e+01	2.53e+02	3.30e+02	2.47e+02	4.74e+02
Lab.06	TBT	eggs	GP	5.72e+01	1.43e+01	1.28e+02	8.34e+01	2.92e+01	1.57e+02	1.26e+02	6.28e+01	1.98e+02	2.55e+02	1.95e+02	3.39e+02
Lab.11	TBT	clutches	P	1.73e+02	3.14e+01	5.18e+02	2.46e+02	7.00e+01	5.45e+02	3.64e+02	1.67e+02	5.75e+02	6.91e+02	6.00e+02	9.24e+02
Lab.11	TBT	eggs	GP	1.27e+02	3.20e+01	2.80e+02	1.83e+02	6.35e+01	3.35e+02	2.73e+02	1.34e+02	4.10e+02	5.39e+02	4.37e+02	6.26e+02

Table 2: ECx ordered per lab and per endpoint.

Lab	Tox	Endpoint	Model	med.EC5	Q2.5.EC5	Q97.5.EC5	med.EC10	Q2.5.EC10	Q97.5.EC1	med.EC20	Q2.5.EC20	Q97.5.EC2	med.EC50	Q2.5.EC50	Q97.5.EC50
Lab.02	TBT	clutches	GP	2.10e+01	4.31e+00	6.12e+01	4.43e+01	1.31e+01	1.04e+02	1.00e+02	4.35e+01	1.85e+02	4.03e+02	2.90e+02	5.55e+02
Lab.03	TBT	clutches	GP	1.16e+02	7.08e+01	1.60e+02	1.26e+02	8.63e+01	1.62e+02	1.39e+02	1.07e+02	1.64e+02	1.63e+02	1.50e+02	1.69e+02
Lab.05	TBT	clutches	GP	1.22e+02	5.79e+01	2.51e+02	1.57e+02	9.03e+01	2.64e+02	2.05e+02	1.44e+02	2.79e+02	3.23e+02	2.86e+02	3.93e+02
Lab.06	TBT	clutches	GP	6.38e+01	1.29e+01	1.63e+02	9.66e+01	2.91e+01	2.00e+02	1.52e+02	6.95e+01	2.53e+02	3.30e+02	2.47e+02	4.74e+02
Lab.11	TBT	clutches	P	1.73e+02	3.14e+01	5.18e+02	2.46e+02	7.00e+01	5.45e+02	3.64e+02	1.67e+02	5.75e+02	6.91e+02	6.00e+02	9.24e+02
Lab.02	TBT	eggs	GP	3.18e+01	6.75e+00	8.73e+01	6.14e+01	1.85e+01	1.38e+02	1.25e+02	5.41e+01	2.26e+02	4.26e+02	3.02e+02	5.83e+02
Lab.03	TBT	eggs	GP	1.03e+02	5.75e+01	1.58e+02	1.14e+02	7.23e+01	1.60e+02	1.28e+02	9.20e+01	1.62e+02	1.55e+02	1.36e+02	1.67e+02
Lab.05	TBT	eggs	GP	1.14e+02	6.09e+01	1.88e+02	1.40e+02	8.63e+01	2.08e+02	1.75e+02	1.24e+02	2.32e+02	2.56e+02	2.22e+02	2.92e+02
Lab.06	TBT	eggs	GP	5.72e+01	1.43e+01	1.28e+02	8.34e+01	2.92e+01	1.57e+02	1.26e+02	6.28e+01	1.98e+02	2.55e+02	1.95e+02	3.39e+02
Lab.11	TBT	eggs	GP	1.27e+02	3.20e+01	2.80e+02	1.83e+02	6.35e+01	3.35e+02	2.73e+02	1.34e+02	4.10e+02	5.39e+02	4.37e+02	6.26e+02

Table 3: ECx ordered per endpoint and per lab.

BETWEEN-LAB VARIABILITY

37. Coefficients of variation (CV) were calculated from median values of EC_{50} estimates for all labs and both endpoints[2].

→ From EC_{50} medians estimated on clutch data, we got a CV equal to 51%.

→ From EC_{50} medians estimated on egg data, we got a CV equal to 47%.

38. The between-lab variability appears high due to low estimates from Lab.03 data, for which measured concentrations are particularly low compared to nominal ones (see Table 1).

39. Table 4 shows coefficients of variation for both phases, pre-validation and validation, and for both endpoints, clutches and eggs. In addition to Table 4, Figure 4 shows the comparison between EC_{50} estimates of the pre- and the validation phases, as well as the comparison between EC_{50} estimates from clutch and egg data.

Endpoint	CV-prevalidation	CV-validation
Clutches	25% (n = 3)	51% (n = 5)
Eggs	42% (n = 3)	47% (n = 5)

Table 4: Coefficients of variation for clutch and egg endpoints and boht pre- and validation phases, calculated from EC_{50} medians values.

[2] As a reminder, CV = $\frac{\mu}{\sigma}$, where μ and σ are mean and standard deviation of the EC_{50} medians.

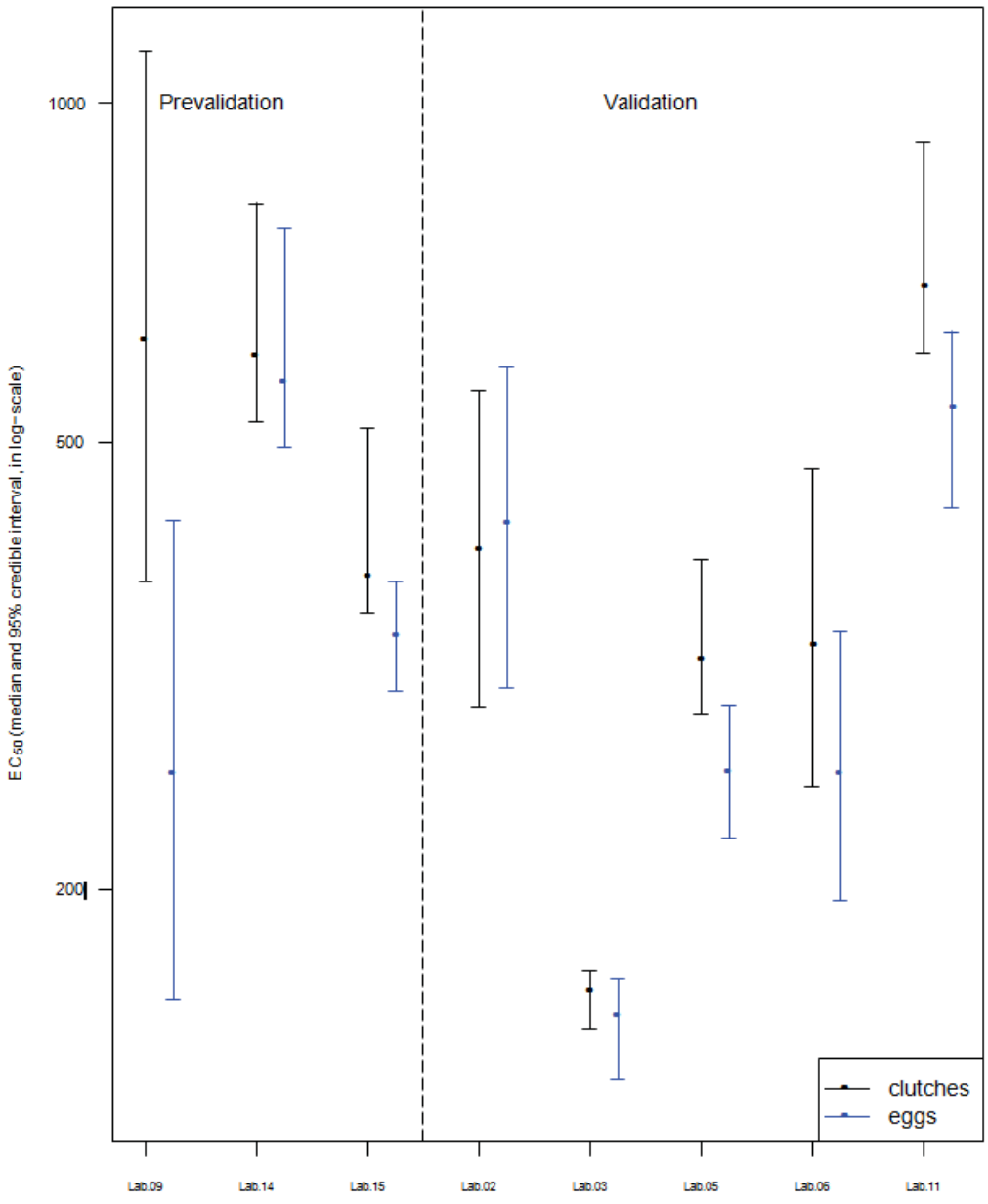

Figure 4: Comparison between EC_{50} estimates of the prevalidation phase (left to the dotted vertical black line) and the validation phase (right to the dotted vertical black line). Circles stand for EC_{50} median values and flat arrows for their 95% credible intervals: in black, when clutch data were used; in blue when egg data were used.

EXPERIMENTAL DESIGN OPTIMIZATION

Experiment duration

40. The possible reduction in the experiment duration has been studied by comparing the EC_{50} estimates (median ± 95% credible interval) at each target time (from 21 to 56 days) to the interval EC_{50-56d} ± sd, where EC_{50-56d} is the median estimates at day 56 and *sd* is the standard deviation of the EC_{50-56d} medians between labs of a given phase. This work was performed using the computing facilities of the CC LBBE/PRABI.

41. Because protocols were different between the two phases, two different analyses were per- formed: (i) within the pre-validation phase, including five labs; two different sd_{preval} values were calculated for EC_{50-56d} medians from clutch and egg data (Figure 5). (ii) within the validation phase, including six labs (Lab.08 was excluded); two different sd_{val} values were calculated for EC_{50-56d} medians from clutch and egg data (Figure 6).

42. From these analyses, it was possible to extract the first target time at which the $EC50$ median was outside the interval $EC50{-}56d \pm sd$: (i) Table 5 for the prevalidation phase; (ii) Table 6 for the validation phase.

43. Based on these results, we could reasonably suggest 35 days of experiment for both clutches and eggs. Such results would need to be further confirmed from experiments with other types of toxicants. In particular, these results should be compared to those obtained with Cd and prochloraz.

Number of replicates

44. As for Cd, it was finally decided not to explore issues related to the number of replicates.

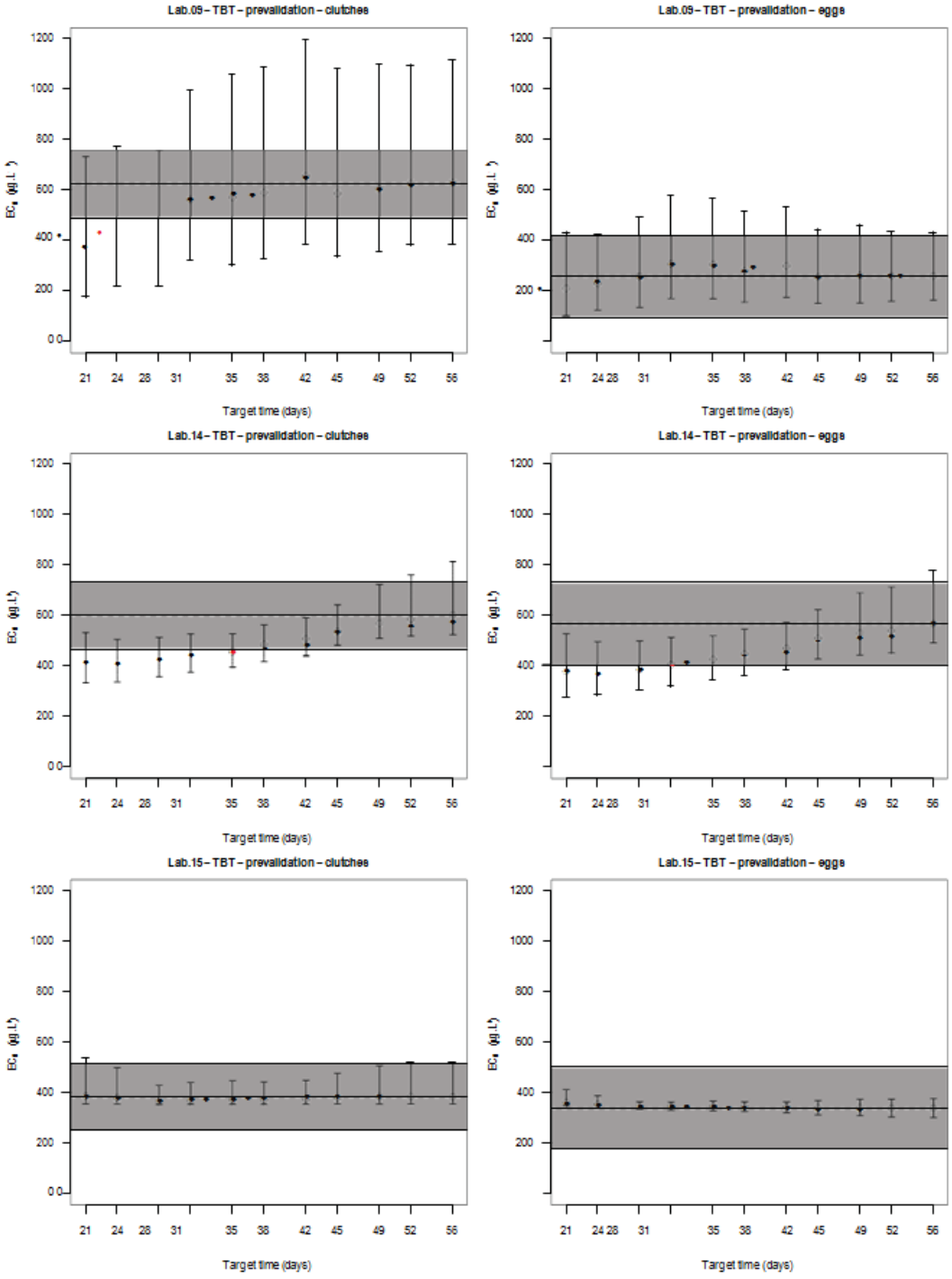

Figure 5: EC_{50} estimates (medians and 95% credible intervals) according to the target time (in days) for all labs and both endpoints of the prevalidation phase. The red circle points out the first target time at which the EC_{50} median is outside the interval $EC_{50-56d} \pm sd_{preval}$ (grey band).

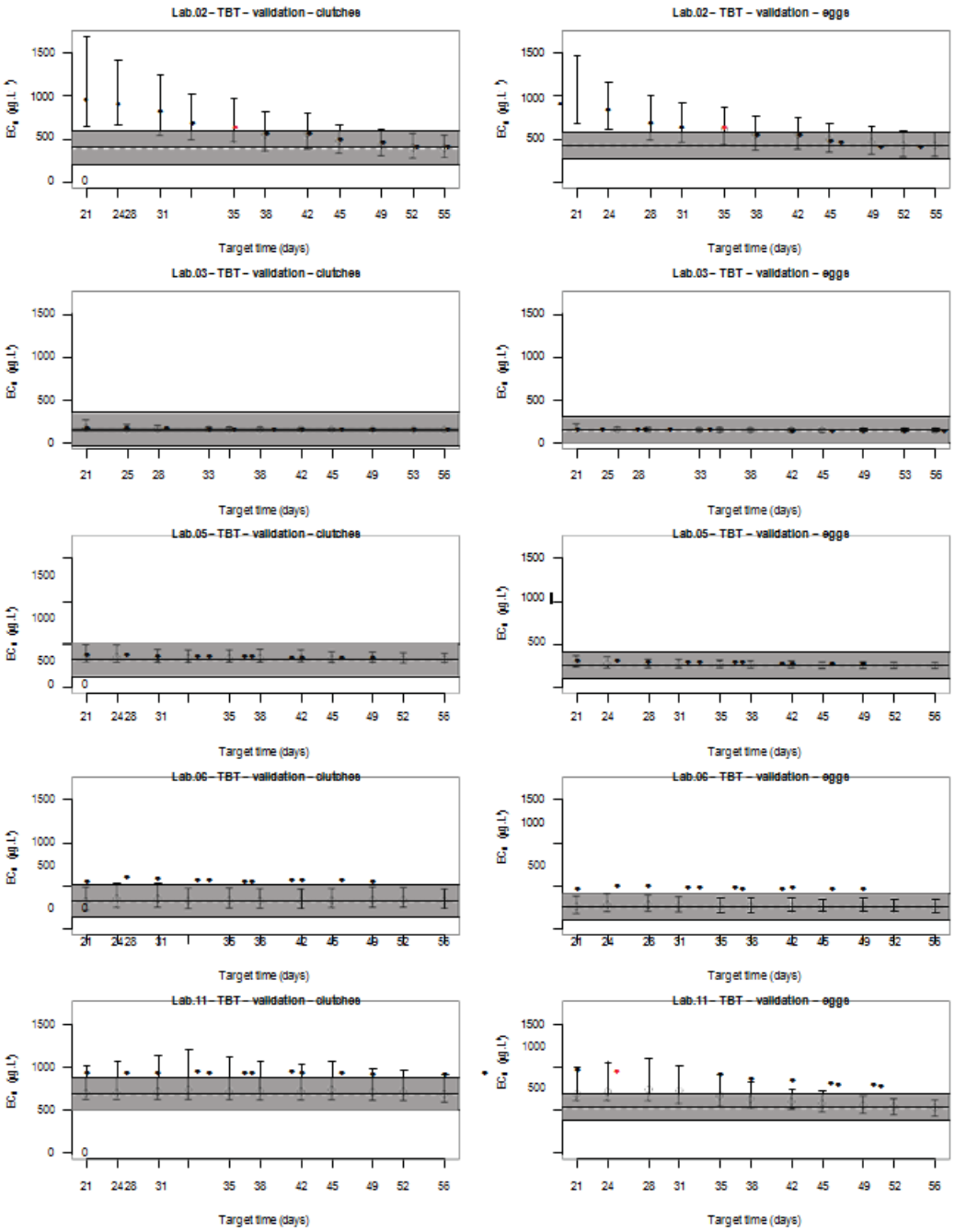

Figure 6: EC_{50} estimates (medians and 95% credible intervals) according to the target time (in days) for all labs and both endpoints of the validation phase. The red circle points out the first target time at which the EC_{50} median is outside the interval $EC_{50-56d} \pm sd_{val}$ (grey band).

Lab	Tox	Phase	Endpoint	Target time
Lab.09	TBT	prevalidation	clutches	28
Lab.09	TBT	prevalidation	eggs	<21
Lab.14	TBT	prevalidation	clutches	35
Lab.14	TBT	prevalidation	eggs	31
Lab.15	TBT	prevalidation	clutches	<21
Lab.15	TBT	prevalidation	eggs	<21

Table 5: First target time at which the EC_{50} median is outside the interval $EC_{50-56d} \pm sd_{preval}$ for the prevalidation phase. The red value stands for the highest target time among labs for clutches; the green value stands for the highest target time among labs for eggs.

Lab	Tox	Phase	Endpoint	Target time
Lab.02	TBT	validation	clutches	35
Lab.02	TBT	validation	eggs	35
Lab.03	TBT	validation	clutches	<21
Lab.03	TBT	validation	eggs	<21
Lab.05	TBT	validation	clutches	<21
Lab.05	TBT	validation	eggs	<21
Lab.06	TBT	validation	clutches	<21
Lab.06	TBT	validation	eggs	<21
Lab.11	TBT	validation	clutches	<21
Lab.11	TBT	validation	eggs	31

Table 6: First target time at which the EC_{50} median is outside the interval $EC_{50-56d} \pm sd_{val}$ for the validation phase. The red value stands for the highest target time among labs for clutches; the green value stands for the highest target time among labs for eggs.

OECD MOLLUSC TESTS - VALIDATION PHASE PROCHLORAZ RESULTS OVERVIEW FOR ALL PARTNER LABS - MEASURED CONCENTRATIONS

OVERVIEW OF MEASURED CONCENTRATIONS

45. Four labs tested prochloraz as a toxicant.

46. As for cadmium and TBT analyses, prochloraz concentrations were measured at different dates during the experiment. Each measurement consisted in two prochloraz concentration values: the first one (”conc.on”) is obtained at water renewal time; the second one (”conc.off”) is obtained after an interval of three days. In Lab.07, there are four replicates of conc.on and conc.off at each measurement dates.

47. As for the Cd and TBT analysis, measured prochloraz concentrations were calculated for each lab and each nominal concentration as the arithmetic mean of all measured values (from n = 6 to n = 24 depending on the lab).

48. Figure 1 shows the relationship between nominal concentrations and measured ones for all partner labs separately. Table 1 gives calculated arithmetic means and the associated standard deviations for all partner labs. Figure 2 shows the relationship between the arithmetic mean Cd concentrations measured over the test period and the nominal concentrations for all partner laboratories simultaneously.

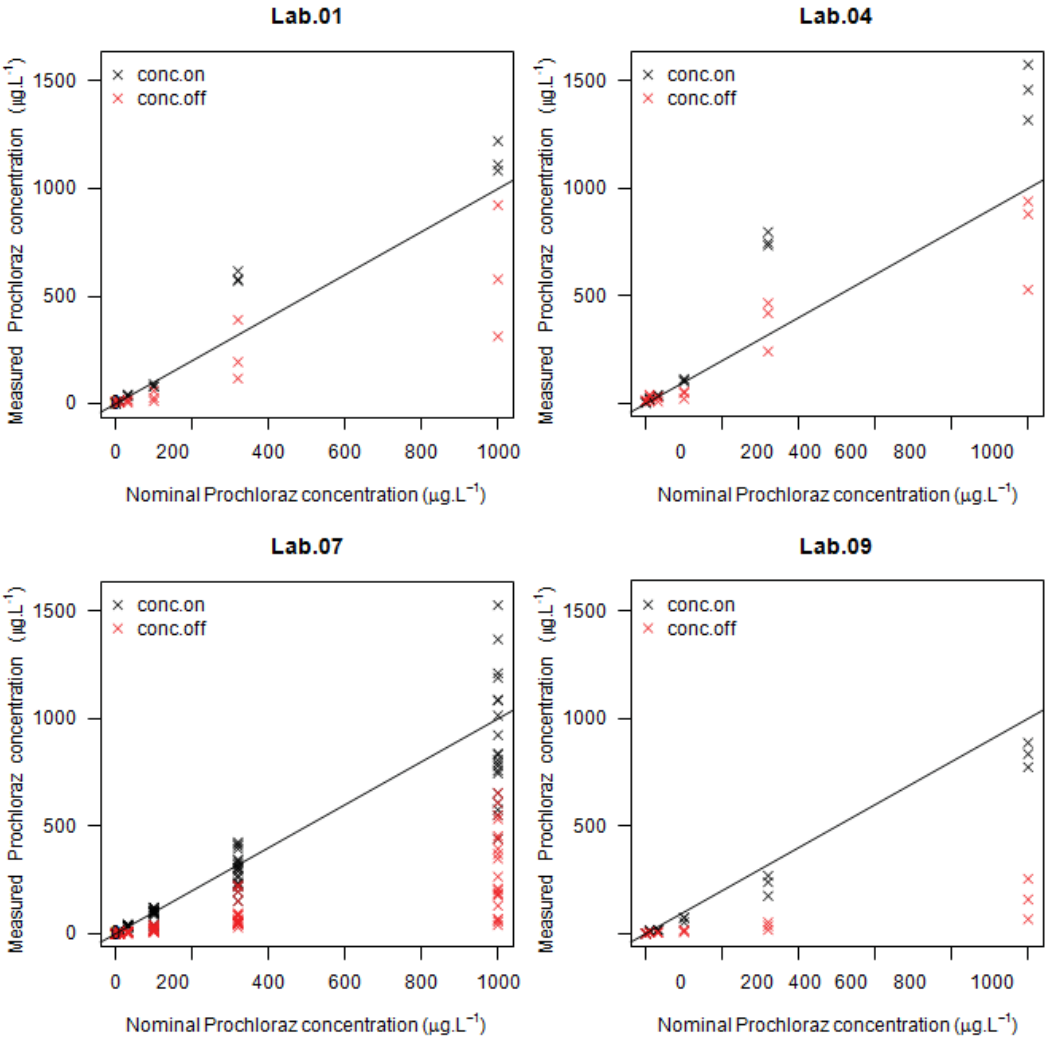

Figure 1: Relationship between nominal and measured concentrations (in μg.L^{-1}): in black, "conc.on" measurements at water renewal time; in red, "conc.off" measurements after an interval of three days. The plain line corresponds to the first bisector.

lab.num	conc.nom	conc.mean	conc.sd
Lab.01	0	5.63	4.76
Lab.01	10	10.95	5.413
Lab.01	32	25.98	15.74
Lab.01	100	58.96	32.09
Lab.01	320	411.6	214.2
Lab.01	1000	872.6	353.9
Lab.04	0	9.478	4.827
Lab.04	10	25.05	11.53
Lab.04	32	29.74	10.88
Lab.04	100	75.13	37.92
Lab.04	320	568.7	224.1
Lab.04	1000	1117	400.1
Lab.07	0	0.6284	1.271
Lab.07	10	6.63	5.608
Lab.07	32	18.76	15.6
Lab.07	100	54.92	42.4
Lab.07	320	193.6	126.8
Lab.07	1000	594	385
Lab.09	0	0	0
Lab.09	10	8.087	5.086
Lab.09	32	10.58	6.434
Lab.09	100	39.25	31.13
Lab.09	320	131.3	110.2
Lab.09	1000	496.1	374.6

Table 1: Arithmetic means (”conc.mean”) and associated standard deviations (”conc.sd”) of concen- tration measurements corresponding to each nominal Prochloraz concentrations for all partner labs. All concentrations are expressed in $\mu g.L^{-1}$.

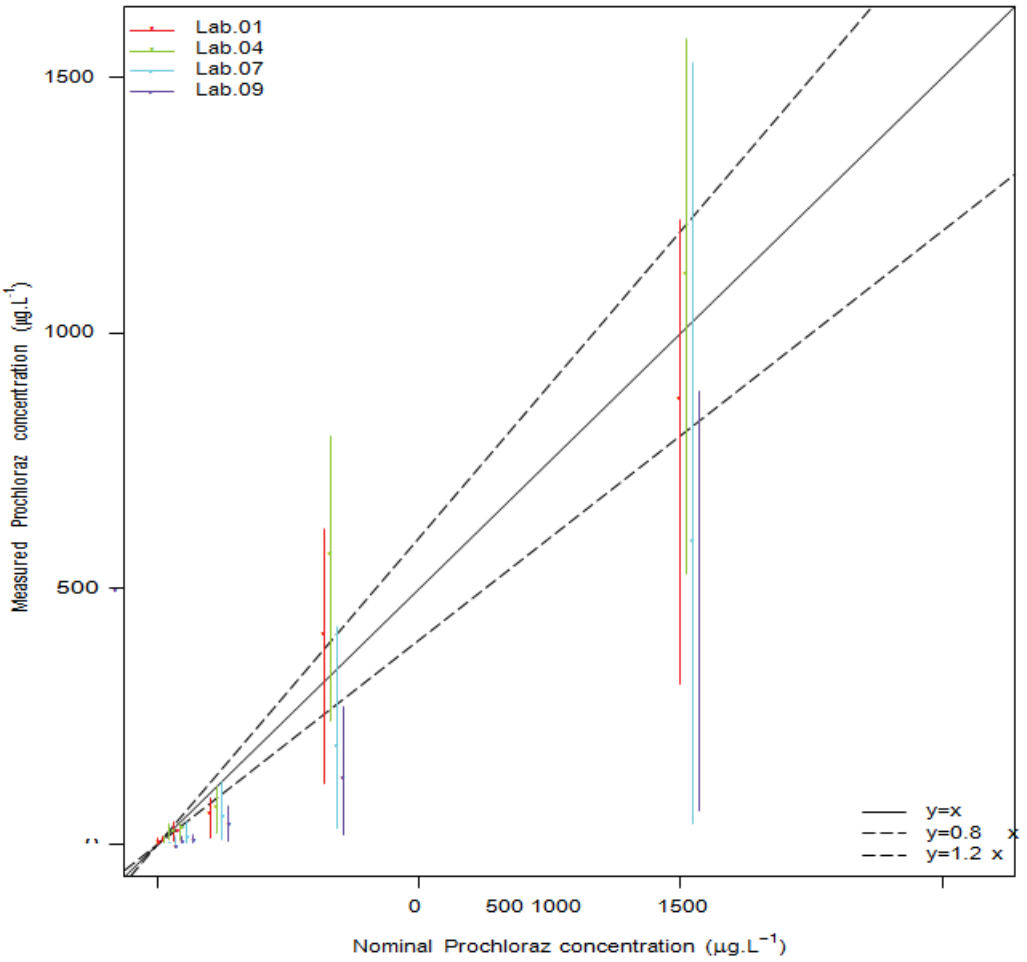

Figure 2: Relationship between the arithmetic mean Prochloraz concentrations measured over the test period and the nominal concentration for all each partner laboratories. Reported values correspond to the means (circles) and their spread (segments; i.e., ranges from minimal to maximal measured values). The solid line represents the theoretical case where actual concentrations are similar to the nominal concentrations (first bisector), while dashed lines represent theoretical cases where actual concentrations reach 80% and 120% of the nominal concentrations, respectively.

EC_X ESTIMATED FROM PROCHLORAZ MEASURED CONC.

49. Figure 3 shows EC50 for both labs, estimated from clutch or egg data. On a general point of view, although the Jonckheere-Terpstra test is significant for all datasets (what has justified to fit a concentration-effect model), fitting results must be considered with a lot of caution. Indeed, EC_{50} medians are often larger than the highest tested concentration, and the very large credible interval around the EC_{50} median makes questionable the whole estimation process (see details in the corresponding sheets).

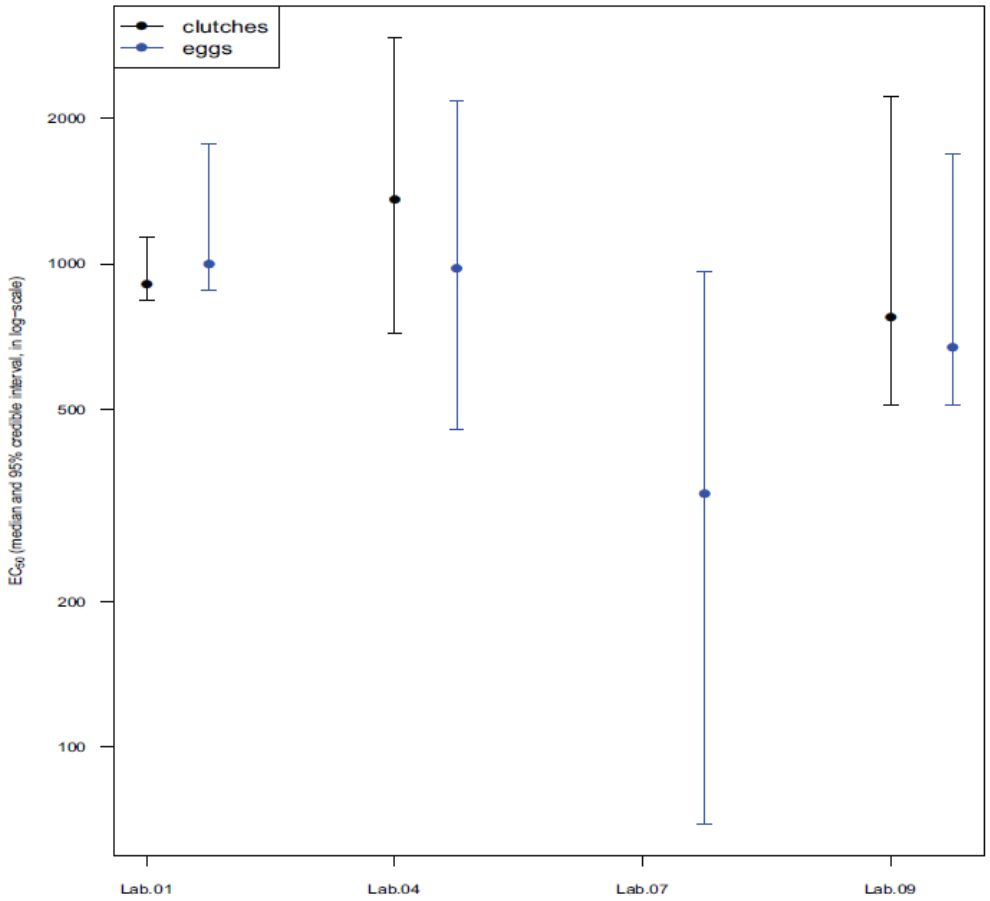

Figure 3: EC_{50} estimates (medians and 95% credible intervals for all partner labs): in black, EC_{50} estimated from clutch data; in blue, EC_{50} estimated from egg data.

50. Tables 2 (ordered per lab and per endpoint) and 3 (ordered per endpoint and per lab) give ECx estimates with their 95% credible intervals for x = 5, 10, 20, 50, when it was possible to estimate such toxicity values.

BETWEEN-LAB VARIABILITY

51. Coefficients of variation (CV) were calculated from median values of EC50 estimates for all labs and both endpoints[3].

→ From EC_{50} medians estimated on clutch data, we got a CV equal to 30% (n = 3).

→ From EC_{50} medians estimated on egg data, we got a CV equal to 42% (n = 4).

[3] As a reminder, CV = $\frac{\mu}{\sigma}$, where μ and σ are mean and standard deviation of the EC_{50} medians.

Lab	Tox	Endpoint	Model	med.EC5	Q2.5.EC5	Q97.5.EC5	med.EC10	Q2.5.EC10	Q97.5.EC10	med.EC20	Q2.5.EC20	Q97.5.EC20	med.EC50	Q2.5.EC50	Q97.5.EC50
Lab.01	Prochloraz	clutches	P	4.83e+02	1.86e+02	8.28e+02	5.70e+02	2.87e+02	8.40e+02	6.84e+02	4.48e+02	8.55e+02	9.09e+02	8.43e+02	1.13e+03
Lab.01	Prochloraz	eggs	GP	5.30e+02	9.39e+01	8.40e+02	6.31e+02	1.90e+02	8.52e+02	7.60e+02	3.96e+02	8.81e+02	9.97e+02	8.80e+02	1.77e+03
Lab.04	Prochloraz	clutches	P	7.85e-01	1.49e-02	1.76e+01	5.20e+00	2.77e-01	5.61e+01	4.08e+01	6.21e+00	1.98e+02	1.36e+03	7.18e+02	2.94e+03
Lab.04	Prochloraz	eggs	GP	3.92e-01	2.75e-03	2.41e+01	2.85e+00	6.81e-02	6.70e+01	2.43e+01	2.09e+00	2.10e+02	9.75e+02	4.53e+02	2.18e+03
Lab.07	Prochloraz	eggs	GP	1.22e+00	1.06e-19	4.82e+02	4.92e+00	2.60e-14	5.18e+02	2.26e+01	1.54e-08	5.60e+02	3.34e+02	6.93e+01	9.62e+02
Lab.09	Prochloraz	clutches	GP	2.52e+02	1.50e+00	5.51e+02	3.40e+02	8.03e+00	5.89e+02	4.59e+02	4.96e+01	6.86e+02	7.73e+02	5.11e+02	2.22e+03
Lab.09	Prochloraz	eggs	GP	3.88e+02	6.39e+01	8.11e+02	4.43e+02	1.34e+02	8.44e+02	4.98e+02	2.82e+02	9.22e+02	6.71e+02	5.09e+02	1.68e+03

Table 2: ECx ordered per lab and per endpoint. The empty line for Lab.01 and eggs is due to a non significant Jonckheere-Terpstra test in this case, thus preventing to fit any concentration-effect model.

Lab	Tox	Endpoint	Model	med.EC5	Q2.5.EC5	Q97.5.EC5	med.EC10	Q2.5.EC10	Q97.5.EC10	med.EC20	Q2.5.EC20	Q97.5.EC20	med.EC50	Q2.5.EC50	Q97.5.EC50
Lab.01	Prochloraz	clutches	P	4.83e+02	1.86e+02	8.28e+02	5.70e+02	2.87e+02	8.40e+02	6.84e+02	4.48e+02	8.55e+02	9.09e+02	8.43e+02	1.13e+03
Lab.04	Prochloraz	clutches	P	7.85e-01	1.49e-02	1.76e+01	5.20e+00	2.77e-01	5.61e+01	4.08e+01	6.21e+00	1.98e+02	1.36e+03	7.18e+02	2.94e+03
Lab.09	Prochloraz	clutches	GP	2.52e+02	1.50e+00	5.51e+02	3.40e+02	8.03e+00	5.89e+02	4.59e+02	4.96e+01	6.86e+02	7.73e+02	5.11e+02	2.22e+03
Lab.01	Prochloraz	eggs	GP	5.30e+02	9.39e+01	8.40e+02	6.31e+02	1.90e+02	8.52e+02	7.60e+02	3.96e+02	8.81e+02	9.97e+02	8.80e+02	1.77e+03
Lab.04	Prochloraz	eggs	GP	3.92e-01	2.75e-03	2.41e+01	2.85e+00	6.81e-02	6.70e+01	2.43e+01	2.09e+00	2.10e+02	9.75e+02	4.53e+02	2.18e+03
Lab.07	Prochloraz	eggs	GP	1.22e+00	1.06e-19	4.82e+02	4.92e+00	2.60e-14	5.18e+02	2.26e+01	1.54e-08	5.60e+02	3.34e+02	6.93e+01	9.62e+02
Lab.09	Prochloraz	eggs	GP	3.88e+02	6.39e+01	8.11e+02	4.43e+02	1.34e+02	8.44e+02	4.98e+02	2.82e+02	9.22e+02	6.71e+02	5.09e+02	1.68e+03

Table 3: ECx ordered per endpoint and per lab. The empty line for Lab.01 and eggs is due to a non significant Jonckheere-Terpstra test in this case, thus preventing to fit any concentration-effect model.

EXPERIMENTAL DESIGN OPTIMIZATION

52. The possible reduction in the experiment duration is studied by comparing the EC_{50} estimates (median ± 95% credible interval) at each target time (from 21 to 56 days) to the interval EC_{50-56d} ± sd, where EC_{50-56d} is the median estimates at day 56 and *sd* is the standard deviation of the EC_{50-56d} medians between labs. Results are shown on Figure 4.

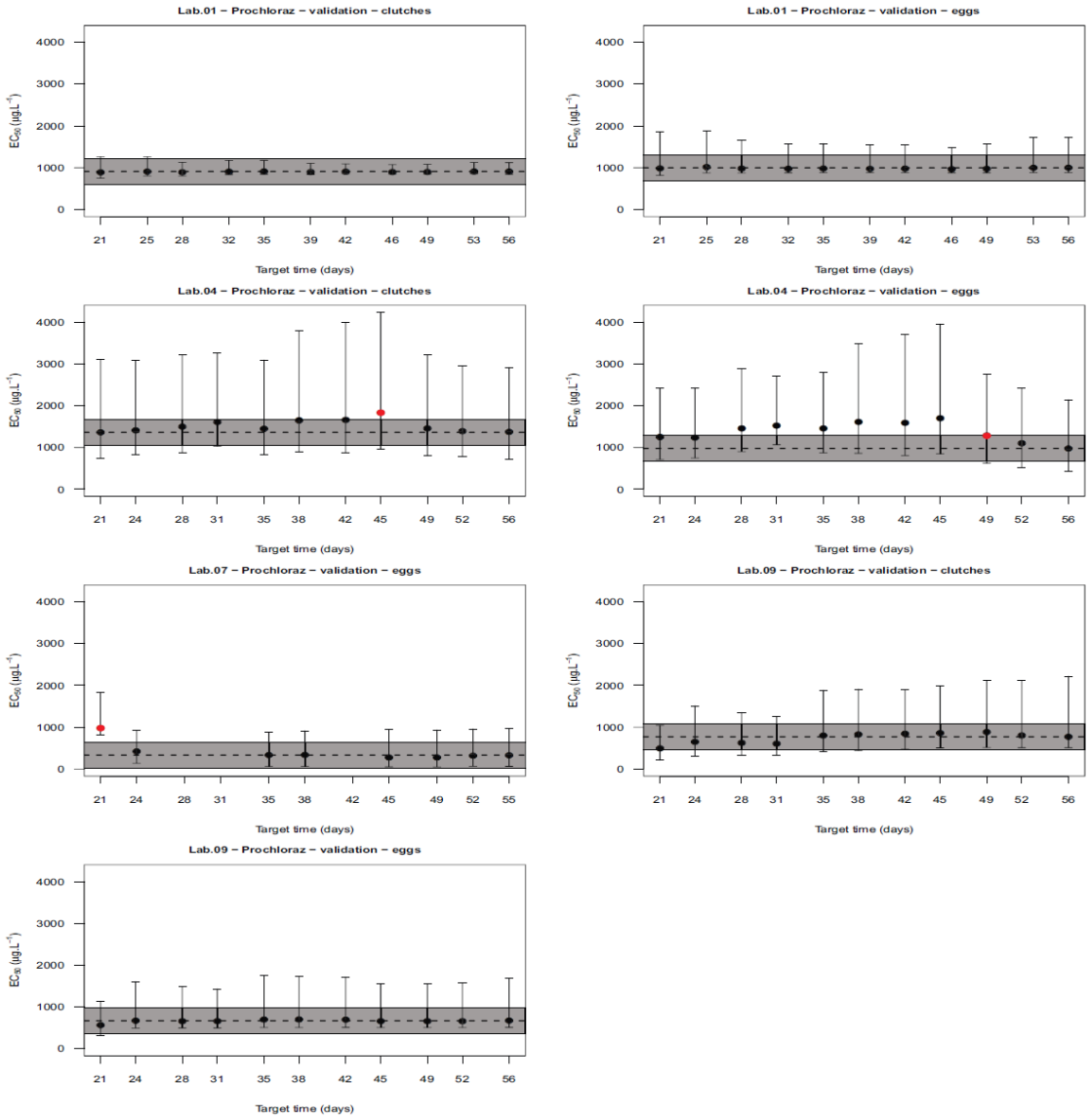

Figure 4: EC_{50} estimates (medians and 95% credible intervals) according to the target time (in days) for both labs and clutches of the validation phase. The red circle points out the first target time at which the EC_{50} median is outside the interval $EC_{50-56d} \pm sd_{val}$ (grey band). Missing EC_{50} values

for Lab.07 correspond to cases where the Jonckheere-Terpstra test is non significant, so that no concentration-effect model was fitted.

53. Based on these results and those from Cd and TBT, any experiment duration more than 28 days would be sufficient, even if for Lab.04, the first target time at which the EC_{50} median is outside the interval $EC_{50-56d} \pm sd_{val}$ is more than 45 days.

OECD MOLLUSC TESTS - RESULTS AT DAY 28 AND COMPARISON WITH RESULTS AT DAY 56

KRUSKALL-WALLIS VS JONCKHEERE-TERPSTRA

54. As recommended by J. Green, we evaluated the use of the Jonckheere-Terpstra (JT) hypothesis test in order to discriminate datasets for which the contaminant effect was not significant, so datasets for which it was not appropriate to fit a concentration-effect model. Results shown in Table 1 allows us to compare p-values obtained either with a Kruskall-Wallis (KW) hypothesis test (that we used at first) or with a JT hypothesis test, at day 28 and at day 56.

55. Notice that it was not possible to run an exact JT hypothesis test due to ties in some samples. We thus used the normal approximation for which a number of iterations has to be arbitrary chosen; J. Green recommend to increase the number of iterations until the confidence interval (CI) for the p-value is entirely on one side of 0.05. Unfortunately, when using the R software to perform JT hypothesis test with package 'clinfun' and function 'jonckheere.test', the CI is not provided. So we used a fixed number of 10^6 iterations.

56. At day 56, there are four discrepancies between KW and JT p-values for Lab.11, Cadmium, vali- dation, clutches, Lab.07, Prochloraz, validation, eggs and Lab.09, Prochloraz, validation clutches and eggs (Table 1, blue cells). Regarding JT p-value, the contaminant effect is significant, thus leading to fit a concentration-effect model. Nevertheless, the fitting provided rather bad EC_x estimates (see Appendices).

57. At day 28, there are three cases for which the JT hypothesis test is significant while the KW one is not (Table 1, green cells): Lab.13, Cadmium, validation, clutches, Lab.09, Prochloraz, validation, eggs and Lab.11, TBT, validation, clutches. In the two latter case, EC_x estimates are not reliable, with 2.5% quantiles greater than the highest tested concentration (see Appendices).

58. Finally, the JT hypothesis test, compared to the KW one, allowed us to conveniently include Lab.13, Cadmium, validation, clutches in our pool of datasets to fit at day 28. However, it compelled us to include questionable EC_{50} estimates for Lab.09, Prochloraz, validation, eggs and Lab.11, validation, clutches, Cadmium and TBT. At the end, it remained only two cases for which the JT hypothesis test was not significant: Lab.07, Prochloraz, validation, eggs and Lab.11, Cadmium, validation, clutches at day 28 (Table 1, red cells).

Tox	Endpoint	Phase	Lab	kw28.pval	jt28.pval	kw56.pval	jt56.pval
Cadmium	clutches	prevalidation	Lab.01	2.270e-05	1.000e-05	9.850e-05	1.000e-05
Cadmium	clutches	prevalidation	Lab.04	1.050e-05	1.000e-05	9.060e-06	1.000e-05
Cadmium	clutches	prevalidation	Lab.07	3.280e-04	1.000e-05	1.160e-05	1.000e-05
Cadmium	clutches	prevalidation	Lab.14	1.300e-04	1.000e-05	2.530e-05	1.000e-05
Cadmium	clutches	prevalidation	Lab.15	1.920e-06	1.000e-05	1.650e-06	1.000e-05
Cadmium	clutches	validation	Lab.02	3.140e-03	5.000e-05	3.530e-04	1.000e-05
Cadmium	clutches	validation	Lab.05	3.180e-03	2.400e-03	1.250e-03	2.500e-04
Cadmium	clutches	validation	Lab.06	1.900e-04	1.000e-05	1.300e-04	1.000e-05
Cadmium	clutches	validation	Lab.10	3.220e-04	1.000e-05	1.770e-04	1.000e-05
Cadmium	clutches	validation	Lab.11	8.570e-01	6.200e-01	6.750e-02	9.280e-03
Cadmium	clutches	validation	Lab.13	5.360e-02	4.680e-03	5.410e-03	1.000e-05
Cadmium	eggs	prevalidation	Lab.01	4.850e-05	1.000e-05	2.080e-04	1.000e-05
Cadmium	eggs	prevalidation	Lab.04	1.400e-05	1.000e-05	8.940e-06	1.000e-05
Cadmium	eggs	prevalidation	Lab.07	5.260e-04	2.000e-05	9.270e-06	1.000e-05
Cadmium	eggs	prevalidation	Lab.14	5.620e-05	1.000e-05	1.490e-05	1.000e-05
Cadmium	eggs	prevalidation	Lab.15	5.470e-07	1.000e-05	3.140e-07	1.000e-05
Cadmium	eggs	validation	Lab.02	8.330e-04	1.000e-05	4.290e-04	1.000e-05
Cadmium	eggs	validation	Lab.05	1.960e-03	1.700e-04	1.040e-03	4.000e-05
Cadmium	eggs	validation	Lab.06	1.080e-04	1.000e-05	3.930e-05	1.000e-05
Cadmium	eggs	validation	Lab.10	1.440e-04	1.000e-05	7.800e-05	1.000e-05
Cadmium	eggs	validation	Lab.11	1.560e-03	1.000e-05	2.530e-05	1.000e-05
Cadmium	eggs	validation	Lab.13	7.140e-03	2.000e-05	1.200e-03	1.000e-05
Prochloraz	clutches	validation	Lab.01	1.120e-02	9.310e-03	5.800e-03	3.490e-03
Prochloraz	clutches	validation	Lab.04	5.360e-04	1.000e-05	1.230e-04	1.000e-05
Prochloraz	clutches	validation	Lab.09	9.230e-03	7.000e-05	1.300e-01	1.810e-03
Prochloraz	eggs	validation	Lab.01	4.100e-02	2.190e-02	2.800e-02	2.280e-03
Prochloraz	eggs	validation	Lab.04	3.210e-04	1.000e-05	1.280e-04	1.000e-05
Prochloraz	eggs	validation	Lab.07	1.930e-01	8.000e-02	3.030e-01	1.360e-02
Prochloraz	eggs	validation	Lab.09	9.330e-02	4.460e-03	3.820e-01	3.810e-02
TBT	clutches	prevalidation	Lab.09	4.380e-04	1.000e-05	6.760e-04	1.000e-05
TBT	clutches	prevalidation	Lab.14	1.040e-03	2.200e-04	2.990e-03	3.270e-03
TBT	clutches	prevalidation	Lab.15	4.510e-03	2.890e-02	2.520e-02	2.460e-02
TBT	clutches	validation	Lab.02	3.920e-03	2.000e-05	8.500e-05	1.000e-05
TBT	clutches	validation	Lab.03	4.880e-03	4.170e-03	1.650e-03	3.730e-03
TBT	clutches	validation	Lab.05	7.520e-03	1.130e-02	1.190e-03	4.820e-03
TBT	clutches	validation	Lab.06	2.640e-03	6.600e-04	4.800e-03	4.110e-03
TBT	clutches	validation	Lab.11	1.290e-01	2.340e-03	1.340e-03	1.000e-05
TBT	eggs	prevalidation	Lab.09	1.040e-04	1.000e-05	5.880e-05	1.000e-05
TBT	eggs	prevalidation	Lab.14	2.540e-03	1.100e-04	1.520e-02	1.110e-02
TBT	eggs	prevalidation	Lab.15	7.770e-03	7.880e-03	8.620e-03	2.420e-03
TBT	eggs	validation	Lab.02	2.700e-03	4.000e-05	1.100e-04	1.000e-05
TBT	eggs	validation	Lab.03	1.150e-02	1.600e-03	4.930e-03	5.060e-03
TBT	eggs	validation	Lab.05	5.410e-04	8.810e-03	3.000e-04	5.250e-03
TBT	eggs	validation	Lab.06	3.430e-04	9.000e-05	1.510e-04	2.000e-05
TBT	eggs	validation	Lab.11	2.540e-03	1.000e-05	5.820e-04	1.000e-05

Table 1: P-values of Kruskall-Wallis (KW) and Jonckheere-Terpstra (JT) hypothesis tests performed for all toxicants, endpoints, phases and labs: 'kw28.pval' stands for KW p-value of the test performed on the cumulated number of reproduction outputs (clutches or eggs) per individual-day at day 28; 'kw56.pval' stands for KW p-value of the test performed on the cumulated number of reproduction outputs (clutches or eggs) per individual-day at day 56; 'jt28.pval' stands for JT p-value of the test performed on the cumulated number of reproduction outputs (clutches or eggs) per individual-day at day 28; 'jt56.pval' stands for JT p-value of the test performed on the cumulated number of reproduction outputs (clutches or eggs) per individual-day at day 56. Blue and green cells highlight discrepancies between KW and JT hypothesis test significance. Red cells highlight non significant hypothesis tests; green cells stand for differences between KW and JT tests at day 28, while blue cells stand for differences between KW and JT tests at day 56.

RESULTS AT 28 DAYS OF EXPERIMENT

59. During the OECD mollusc meeting in Odense (Denmark, 18-20 February 2015), it was decided to reduce the experiment duration at 28 days whatever the contaminant for both clutch and egg data. This decision was based on results giving EC_{50} estimates according to the experiment duration for the three contaminants studied during the prevalidation and the validation phases: cadmium, TBT and prochloraz.

60. It is first interesting to compare the between-lab variability at day 28 and at day 56 based on coefficients of variation (Table 2). Three other tables give EC_{10} and EC_{50} estimates (medians ± 95% credibility intervals) both at day 28 and 56, as well as the p-value of the JT hypothesis test and the fitted model type for the three toxicants: cadmium (Table 3), TBT (Table 4) and prochloraz (Table 5). In addition to these tables, Figures 1, 2 and 3 allow us to visually compare results at day 28 and 58 both for clutch and egg data.

61. In order to make easier this comparison, two kinds of ratios were calculated: in Table 6, the ratio between EC_{50} medians at 28 and 56 days; in Table 7, the ratio between EC_{50} medians at 28 days from clutches and EC_{50} medians at 56 days from eggs. In these two tables, only three ratios are slightly over 2 (red cells), what gives us other arguments in favour of 28-days experiments.

62. For cadmium, there is less variability at day 28 than at day 56 (smaller CVs); the high CV value for clutches, validation at day 56 (52.5%) is due to the high estimate of EC_{50} for Lab.11 (see previous section). For TBT, the variability is reduced between day 28 and 56 but only for the prevalidation phase; the high CV values for clutches (57.4%) and eggs (63.4%) of the validation phase at day 28 are due to highest estimates of EC_{50} for Lab.02 compared to those obtained at day 56 (see Figure 2). For TBT, low EC_{50} estimates from Lab.03 (which used very young snails compared to the other labs) probably also bias CV calculations (Figure 2). At last, for prochloraz, CV values are similar between day 28 and day 56, as well as between clutches and eggs.

Tox	Endpoint	Phase	Duration	CV	n
Cadmium	clutches	prevalidation	28d	26.8	5
Cadmium	clutches	prevalidation	56d	31.0	5
Cadmium	clutches	validation	28d	33.5	5
Cadmium	clutches	validation	56d	52.5	6
Cadmium	eggs	prevalidation	28d	15.5	5
Cadmium	eggs	prevalidation	56d	21.8	5
Cadmium	eggs	validation	28d	27.2	6
Cadmium	eggs	validation	56d	28.0	6
Prochloraz	clutches	validation	28d	44.5	3
Prochloraz	clutches	validation	56d	30.4	3
Prochloraz	eggs	validation	28d	38.1	3
Prochloraz	eggs	validation	56d	41.8	4
TBT	clutches	prevalidation	28d	8.4	3
TBT	clutches	prevalidation	56d	24.8	3
TBT	clutches	validation	28d	57.3	5
TBT	clutches	validation	56d	50.7	5
TBT	eggs	prevalidation	28d	21.2	3
TBT	eggs	prevalidation	56d	42.0	3
TBT	eggs	validation	28d	63.4	5
TBT	eggs	validation	56d	47.2	5

Table 2: Coefficients of variation per tox, endpoint and phase, for both duration, 28 or 56 days. *n* stands for the number of concerned labs. (*)Excluding Lab.11 would provide $CV_{Cd-clutches-56d} = 42.0\%$.

Tox	Endpoint	Phase	Lab	Duration	Model	JT.pval	med.EC10	Q2.5.EC10	Q97.5.EC10	med.EC50	Q2.5.EC50	Q97.5.EC50
Cadmium	clutches	prevalidation	Lab.01	28d	P	0.0000	4.0122e+01	2.3822e+01	5.9110e+01	1.2178e+02	1.0251e+02	1.4587e+02
Cadmium	clutches	prevalidation	Lab.01	56d	P	0.0000	1.6754e+01	7.0698e+00	2.9368e+01	8.8692e+01	7.0680e+01	1.1294e+02
Cadmium	clutches	prevalidation	Lab.04	28d	P	0.0000	5.1152e+01	3.3682e+01	7.3290e+01	1.3899e+02	1.1763e+02	1.6458e+02
Cadmium	clutches	prevalidation	Lab.04	56d	P	0.0000	5.2968e+01	3.5860e+01	7.4389e+01	1.3966e+02	1.1953e+02	1.6168e+02
Cadmium	clutches	prevalidation	Lab.07	28d	P	0.0000	4.8186e+01	1.3982e+01	1.2930e+02	1.3498e+02	9.5612e+01	1.8943e+02
Cadmium	clutches	prevalidation	Lab.07	56d	P	0.0000	1.8801e+01	9.5130e+00	3.0485e+01	7.8146e+01	6.3353e+01	9.5359e+01
Cadmium	clutches	prevalidation	Lab.14	28d	P	0.0000	3.7457e+01	8.7951e+00	9.0309e+01	2.2689e+02	1.6331e+02	3.1689e+02
Cadmium	clutches	prevalidation	Lab.14	56d	P	0.0000	1.1500e+01	2.3320e+00	3.3565e+01	1.6907e+02	1.1261e+02	2.6889e+02
Cadmium	clutches	prevalidation	Lab.15	28d	P	0.0000	2.1474e+01	9.6889e+00	3.8912e+01	1.7942e+02	1.4113e+02	2.3212e+02
Cadmium	clutches	prevalidation	Lab.15	56d	P	0.0000	1.4710e+01	7.6732e+00	2.3418e+01	1.3466e+02	1.1257e+02	1.6246e+02
Cadmium	clutches	validation	Lab.02	28d	P	0.0001	2.5074e+00	3.4972e-03	2.0279e+01	1.2654e+02	6.2680e+01	2.5930e+02
Cadmium	clutches	validation	Lab.02	56d	P	0.0000	2.8951e-01	2.9175e-34	3.9482e+00	6.9285e+01	3.4119e+01	1.8511e+02
Cadmium	clutches	validation	Lab.05	28d	GP	0.0024	1.0935e+02	3.5149e+01	2.6447e+02	2.4120e+02	1.7642e+02	3.1813e+02
Cadmium	clutches	validation	Lab.05	56d	GP	0.0003	8.1767e+01	4.8413e+00	2.1630e+02	2.2677e+02	1.4076e+02	3.2042e+02
Cadmium	clutches	validation	Lab.06	28d	P	0.0000	3.3275e+01	1.1456e+01	6.8815e+01	1.6088e+02	1.1711e+02	2.1513e+02
Cadmium	clutches	validation	Lab.06	56d	GP	0.0000	1.7382e+01	5.0708e+00	4.1245e+01	1.3783e+02	1.0087e+02	1.9401e+02
Cadmium	clutches	validation	Lab.10	28d	GP	0.0000	5.6872e+01	2.9950e+01	9.0062e+01	1.4525e+02	1.1600e+02	1.7714e+02
admium	clutches	validation	Lab.10	56d	GP	0.0000	5.2500e+01	2.9074e+01	8.1396e+01	1.3786e+02	1.1085e+02	1.6942e+02
Cadmium	clutches	validation	Lab.11	28d		0.6200						
Cadmium	clutches	validation	Lab.11	56d	GP	0.0093	1.5246e+02	2.5309e+01	3.9353e+02	3.5908e+02	2.6890e+02	6.0419e+02
Cadmium	clutches	validation	Lab.13	28d	P	0.0047	1.0534e+02	3.9597e+01	2.5200e+02	2.7115e+02	2.1519e+02	3.3504e+02
Cadmium	clutches	validation	Lab.13	56d	GP	0.0000	3.6385e+01	1.2084e+01	8.4908e+01	2.2256e+02	1.6178e+02	3.0964e+02
Cadmium	eggs	prevalidation	Lab.01	28d	GP	0.0000	4.2228e+01	2.4819e+01	6.3650e+01	1.0935e+02	8.8883e+01	1.3588e+02
Cadmium	eggs	prevalidation	Lab.01	56d	GP	0.0000	2.3858e+01	1.1841e+01	4.0924e+01	8.2274e+01	6.2837e+01	1.0835e+02
Cadmium	eggs	prevalidation	Lab.04	28d	GP	0.0000	6.8921e+01	4.9866e+01	9.5935e+01	1.2636e+02	1.0670e+02	1.5393e+02
Cadmium	eggs	prevalidation	Lab.04	56d	GP	0.0000	5.8783e+01	4.2849e+01	7.8121e+01	1.1303e+02	9.6070e+01	1.3365e+02
Cadmium	eggs	prevalidation	Lab.07	28d	GP	0.0000	5.8128e+01	2.0659e+01	1.5730e+02	1.3762e+02	9.8765e+01	2.0018e+02
Cadmium	eggs	prevalidation	Lab.07	56d	GP	0.0000	2.0572e+01	1.2537e+01	3.0585e+01	6.9728e+01	5.7689e+01	8.4143e+01
Cadmium	eggs	prevalidation	Lab.14	28d	GP	0.0000	3.6780e+01	1.2864e+01	7.3882e+01	1.6714e+02	1.2096e+02	2.2169e+02
Cadmium	eggs	prevalidation	Lab.14	56d	GP	0.0000	1.5424e+01	4.6963e+00	3.5710e+01	1.2076e+02	8.1556e+01	1.7545e+02
Cadmium	eggs	prevalidation	Lab.15	28d	GP	0.0000	2.6463e+01	1.4737e+01	4.3116e+01	1.3958e+02	1.1167e+02	1.7398e+02
Cadmium	eggs	prevalidation	Lab.15	56d	GP	0.0000	1.8885e+01	1.1235e+01	2.8480e+01	1.0245e+02	8.4188e+01	1.2383e+02
Cadmium	eggs	validation	Lab.02	28d	GP	0.0000	3.4666e+00	2.5868e-02	1.8730e+01	1.0965e+02	6.0401e+01	2.1133e+02
Cadmium	eggs	validation	Lab.02	56d	GP	0.0000	8.0907e-02	1.0516e-50	3.7896e+00	8.2502e+01	3.8455e+01	2.1753e+02
Cadmium	eggs	validation	Lab.05	28d	GP	0.0002	8.1002e+01	1.2576e+01	1.9913e+02	2.0130e+02	1.2487e+02	2.8812e+02
Cadmium	eggs	validation	Lab.05	56d	GP	0.0000	3.8062e+01	4.8804e-01	1.3906e+02	1.7313e+02	7.6519e+01	2.7272e+02
Cadmium	eggs	validation	Lab.06	28d	GP	0.0000	1.5958e+01	4.3153e+00	4.0864e+01	1.2857e+02	8.4515e+01	1.8736e+02
Cadmium	eggs	validation	Lab.06	56d	GP	0.0000	9.7565e+00	2.6541e+00	2.4299e+01	1.1072e+02	7.8539e+01	1.5778e+02
Cadmium	eggs	validation	Lab.10	28d	GP	0.0000	4.3487e+01	2.4976e+01	6.7539e+01	1.0861e+02	8.5303e+01	1.3540e+02
Cadmium	eggs	validation	Lab.10	56d	GP	0.0000	3.3994e+01	1.8658e+01	5.3539e+01	9.5690e+01	7.4083e+01	1.2075e+02
Cadmium	eggs	validation	Lab.11	28d	GP	0.0000	6.3637e+01	2.1915e+01	1.1307e+02	1.8711e+02	1.3325e+02	2.3347e+02
Cadmium	eggs	validation	Lab.11	56d	GP	0.0000	2.6705e+01	1.1478e+01	5.2701e+01	1.2068e+02	8.6203e+01	1.5880e+02
Cadmium	eggs	validation	Lab.13	28d	GP	0.0000	4.8042e+01	2.9889e+01	7.0845e+01	1.3790e+02	1.1326e+02	1.6558e+02
Cadmium	eggs	validation	Lab.13	56d	GP	0.0000	2.5757e+01	1.4992e+01	4.0925e+01	9.9733e+01	7.9202e+01	1.2468e+02

Table 3: Cadmium results: EC_{10} and EC_{50} estimates (medians with 2.5% and 97.5% quantiles) for all labs, both endpoints (clutches and eggs), both phases (pre- and validation) and both durations (28 and 56 days): gray background lines stand for results at day 28. JT.pval stands for the p-value of the Jonckheere-Terpstra hypothesis test, while Model stands for the concentration-effect fitted model: Poisson (P) or Gamma-Poisson (GP). The empty line stands for no results from the concentration-effect model due to no significant effect of the contaminant.

Tox	Endpoint	Phase	Lab	Duration	Model	JT.pval	med.EC10	Q2.5.EC10	Q97.5.EC10	med.EC50	Q2.5.EC50	Q97.5.EC50
TBT	clutches	prevalidation	Lab.09	28d	P	0.0000	9.2590e+00	1.0491e-01	2.3456e+02	4.2127e+02	2.1709e+02	7.5014e+02
TBT	clutches	prevalidation	Lab.09	56d	P	0.0000	8.7130e+00	7.2170e-01	4.6102e+01	6.2055e+02	3.7539e+02	1.1114e+03
TBT	clutches	prevalidation	Lab.14	28d	P	0.0002	1.6997e+02	8.3447e+01	2.9969e+02	4.2682e+02	3.5186e+02	5.0744e+02
TBT	clutches	prevalidation	Lab.14	56d	P	0.0033	3.1425e+02	1.5327e+02	4.9779e+02	5.9918e+02	5.2095e+02	8.1407e+02
TBT	clutches	prevalidation	Lab.15	28d	P	0.0289	2.9630e+02	2.1302e+02	3.4127e+02	3.6506e+02	3.5039e+02	4.2598e+02
TBT	clutches	prevalidation	Lab.15	56d	GP	0.0246	2.8342e+02	1.7291e+02	3.4201e+02	3.8163e+02	3.5187e+02	5.1341e+02
TBT	clutches	validation	Lab.02	28d	P	0.0000	1.2048e+02	1.3961e+01	6.0540e+02	8.2670e+02	5.4297e+02	1.2425e+03
TBT	clutches	validation	Lab.02	56d	GP	0.0000	4.4301e+01	1.3129e+01	1.0386e+02	4.0276e+02	2.9050e+02	5.5506e+02
TBT	clutches	validation	Lab.03	28d	P	0.0042	1.3016e+02	7.0331e+01	1.6346e+02	1.7374e+02	1.6438e+02	2.1369e+02
TBT	clutches	validation	Lab.03	56d	GP	0.0037	1.2601e+02	8.6331e+01	1.6170e+02	1.6335e+02	1.5036e+02	1.6938e+02
TBT	clutches	validation	Lab.05	28d	P	0.0113	1.4584e+02	6.4356e+01	2.8004e+02	3.3895e+02	2.8687e+02	4.3706e+02
TBT	clutches	validation	Lab.05	56d	GP	0.0048	1.5651e+02	9.0252e+01	2.6361e+02	3.2293e+02	2.8642e+02	3.9299e+02
TBT	clutches	validation	Lab.06	28d	GP	0.0007	7.5004e+01	1.9314e+01	2.0075e+02	3.5618e+02	2.5626e+02	5.1666e+02
TBT	clutches	validation	Lab.06	56d	GP	0.0041	9.6589e+01	2.9148e+01	2.0002e+02	3.3005e+02	2.4661e+02	4.7370e+02
TBT	clutches	validation	Lab.11	28d	P	0.0023	5.5352e+02	1.3766e+02	6.5486e+02	7.1936e+02	6.2257e+02	1.1443e+03
TBT	clutches	validation	Lab.11	56d	P	0.0000	2.4642e+02	7.0014e+01	5.4467e+02	6.9075e+02	5.9954e+02	9.2369e+02
TBT	eggs	prevalidation	Lab.09	28d	GP	0.0000	1.3306e+00	5.6842e-03	1.7755e+01	2.5168e+02	1.2842e+02	4.9264e+02
TBT	eggs	prevalidation	Lab.09	56d	GP	0.0000	2.7721e+00	1.2651e-01	1.5474e+01	2.5494e+02	1.5943e+02	4.2606e+02
TBT	eggs	prevalidation	Lab.14	28d	GP	0.0001	1.4914e+02	6.8100e+01	2.9120e+02	3.8727e+02	3.0408e+02	4.9870e+02
TBT	eggs	prevalidation	Lab.14	56d	GP	0.0111	2.8138e+02	1.2039e+02	4.9595e+02	5.6816e+02	4.9474e+02	7.7397e+02
TBT	eggs	prevalidation	Lab.15	28d	GP	0.0079	2.7936e+02	2.0909e+02	3.3814e+02	3.4825e+02	3.3329e+02	3.6435e+02
TBT	eggs	prevalidation	Lab.15	56d	GP	0.0024	2.2074e+02	1.2434e+02	3.3151e+02	3.3754e+02	3.0024e+02	3.7497e+02
TBT	eggs	validation	Lab.02	28d	GP	0.0000	1.1918e+02	1.9213e+01	4.1212e+02	7.3812e+02	4.9008e+02	1.0293e+03
TBT	eggs	validation	Lab.02	56d	GP	0.0000	6.1381e+01	1.8547e+01	1.3782e+02	4.2571e+02	3.0177e+02	5.8253e+02
TBT	eggs	validation	Lab.03	28d	GP	0.0016	1.1758e+02	6.1348e+01	1.6168e+02	1.6667e+02	1.4765e+02	1.9038e+02
TBT	eggs	validation	Lab.03	56d	GP	0.0051	1.1418e+02	7.2282e+01	1.5990e+02	1.5527e+02	1.3551e+02	1.6669e+02
TBT	eggs	validation	Lab.05	28d	GP	0.0088	1.3132e+02	7.0751e+01	2.2180e+02	2.7182e+02	2.2822e+02	3.2630e+02
TBT	eggs	validation	Lab.05	56d	GP	0.0053	1.3980e+02	8.6321e+01	2.0754e+02	2.5560e+02	2.2225e+02	2.9162e+02
TBT	eggs	validation	Lab.06	28d	GP	0.0001	6.6433e+01	1.8356e+01	1.5136e+02	2.8043e+02	2.0618e+02	3.9462e+02
TBT	eggs	validation	Lab.06	56d	GP	0.0000	8.3392e+01	2.9218e+01	1.5686e+02	2.5468e+02	1.9513e+02	3.3886e+02
TBT	eggs	validation	Lab.11	28d	GP	0.0000	2.5848e+02	4.0695e+01	5.8397e+02	7.4935e+02	6.1735e+02	1.0849e+03
TBT	eggs	validation	Lab.11	56d	GP	0.0000	1.8260e+02	6.3503e+01	3.3530e+02	5.3919e+02	4.3690e+02	6.2599e+02

Table 4: TBT results: EC_{10} and EC_{50} estimates (medians with 2.5% and 97.5% quantiles) for all labs, both endpoints (clutches and eggs), both phases (pre- and validation) and both durations (28 and 56 days): gray background lines stand for results at day 28. JT.pvalstands for the p-value of the Jonckheere-Terpstra hypothesis test, while Modelstands for the concentration-effect fitted model: Poisson (P) or Gamma-Poisson (GP).

Tox	Endpoint	Phase	Lab	Duration	Model	JT.pval	med.EC10	Q2.5.EC10	Q97.5.EC10	med.EC50	Q2.5.EC50	Q97.5.EC50
Prochlora	clutches	validation	Lab.01	28d	P	0.0093	5.4973e+02	2.3793e+02	8.3993e+02	8.9094e+02	8.0581e+02	1.1355e+03
Prochloraz	clutches	validation	Lab.01	56d	P	0.0035	5.7008e+02	2.8667e+02	8.4034e+02	9.0888e+02	8.4333e+02	1.1328e+03
Prochlora	clutches	validation	Lab.04	28d	P	0.0000	4.2273e+01	4.2877e+00	2.5567e+02	1.4941e+03	8.7669e+02	3.1805e+03
Prochloraz	clutches	validation	Lab.04	56d	P	0.0000	5.2029e+00	2.7715e-01	5.6050e+01	1.3620e+03	7.1758e+02	2.9425e+03
Prochlora	clutches	validation	Lab.09	28d	P	0.0001	3.1601e+01	1.0081e+00	4.2149e+02	6.2227e+02	3.3382e+02	1.3299e+03
Prochloraz	clutches	validation	Lab.09	56d	GP	0.0018	3.3956e+02	8.0312e+00	5.8885e+02	7.7275e+02	5.1066e+02	2.2194e+03
Prochlora	eggs	validation	Lab.01	28d	GP	0.0219	6.4238e+02	2.2490e+02	8.5503e+02	9.7627e+02	8.7573e+02	1.6256e+03
Prochloraz	eggs	validation	Lab.01	56d	GP	0.0023	6.3131e+02	1.9049e+02	8.5246e+02	9.9737e+02	8.7959e+02	1.7710e+03
Prochlora	eggs	validation	Lab.04	28d	GP	0.0000	3.0023e+01	5.4754e+00	1.1989e+02	1.4484e+03	9.0916e+02	2.8517e+03
Prochloraz	eggs	validation	Lab.04	56d	GP	0.0000	2.8494e+00	6.8066e-02	6.7005e+01	9.7536e+02	4.5305e+02	2.1784e+03
Prochlora	eggs	validation	Lab.07	28d		0.0800						
Prochloraz	eggs	validation	Lab.07	56d	GP	0.0136	4.9204e+00	2.6016e-14	5.1828e+02	3.3429e+02	6.9266e+01	9.6200e+02
Prochlora	eggs	validation	Lab.09	28d	GP	0.0045	1.8487e+02	1.0477e+01	4.8356e+02	6.6780e+02	4.9940e+02	1.4911e+03
Prochloraz	eggs	validation	Lab.09	56d	GP	0.0381	4.4270e+02	1.3434e+02	8.4449e+02	6.7095e+02	5.0889e+02	1.6847e+03

Table 5: Prochloraz results: *EC*10 and *EC*50 estimates (medians with 2.5% and 97.5% quantiles) for all labs, both endpoints (clutches and eggs), both phases (pre- and validation) and both durations (28 and 56 days): gray background lines stand for results at day 28. JT.pval stands for the p-value of the Jonckheere-Terpstra hypothesis test, while Model stands for the concentration-effect fitted model: Poisson (P) or Gamma-Poisson (GP). Empty lines stand for no results from the concentration-effect model due to no convergence of the algorithm.

Tox	Endpoint	Phase	Lab	Ratio.EC50
Cadmium	clutches	prevalidation	Lab.01	1.40
Cadmium	clutches	prevalidation	Lab.04	1.00
Cadmium	clutches	prevalidation	Lab.07	1.70
Cadmium	clutches	prevalidation	Lab.14	1.30
Cadmium	clutches	prevalidation	Lab.15	1.30
Cadmium	clutches	validation	Lab.02	1.80
Cadmium	clutches	validation	Lab.05	1.10
Cadmium	clutches	validation	Lab.06	1.20
Cadmium	clutches	validation	Lab.10	1.10
Cadmium	clutches	validation	Lab.11	
Cadmium	clutches	validation	Lab.13	1.20
Cadmium	eggs	prevalidation	Lab.01	1.30
Cadmium	eggs	prevalidation	Lab.04	1.10
Cadmium	eggs	prevalidation	Lab.07	2.00
Cadmium	eggs	prevalidation	Lab.14	1.40
Cadmium	eggs	prevalidation	Lab.15	1.40
Cadmium	eggs	validation	Lab.02	1.30
Cadmium	eggs	validation	Lab.05	1.20
Cadmium	eggs	validation	Lab.06	1.20
Cadmium	eggs	validation	Lab.10	1.10
Cadmium	eggs	validation	Lab.11	1.60
Cadmium	eggs	validation	Lab.13	1.40
Prochloraz	clutches	validation	Lab.01	0.98
Prochloraz	clutches	validation	Lab.04	1.10
Prochloraz	clutches	validation	Lab.09	0.81
Prochloraz	eggs	validation	Lab.01	0.98
Prochloraz	eggs	validation	Lab.04	1.50
Prochloraz	eggs	validation	Lab.07	
Prochloraz	eggs	validation	Lab.09	1.00
TBT	clutches	prevalidation	Lab.09	0.68
TBT	clutches	prevalidation	Lab.14	0.71
TBT	clutches	prevalidation	Lab.15	0.96
TBT	clutches	validation	Lab.02	2.10
TBT	clutches	validation	Lab.03	1.10
TBT	clutches	validation	Lab.05	1.00
TBT	clutches	validation	Lab.06	1.10
TBT	clutches	validation	Lab.11	1.00
TBT	eggs	prevalidation	Lab.09	0.99
TBT	eggs	prevalidation	Lab.14	0.68
TBT	eggs	prevalidation	Lab.15	1.00
TBT	eggs	validation	Lab.02	1.70
TBT	eggs	validation	Lab.03	1.10
TBT	eggs	validation	Lab.05	1.10
TBT	eggs	validation	Lab.06	1.10
TBT	eggs	validation	Lab.11	1.40

Table 6: Ratios between EC_{50} medians at 28 and 56 days for all tox, both end points, both phases and all labs. Red cells highlight ratios over 2.

Tox	Phase	Lab	Ratio.EC50
Cadmium	prevalidation	Lab.01	1.50
Cadmium	prevalidation	Lab.04	1.20
Cadmium	prevalidation	Lab.07	1.90
Cadmium	prevalidation	Lab.14	1.90
Cadmium	prevalidation	Lab.15	1.80
Cadmium	validation	Lab.02	1.50
Cadmium	validation	Lab.05	1.40
Cadmium	validation	Lab.06	1.50
Cadmium	validation	Lab.10	1.50
Cadmium	validation	Lab.11	
Cadmium	validation	Lab.13	2.70
Prochloraz	validation	Lab.01	0.89
Prochloraz	validation	Lab.04	1.50
Prochloraz	validation	Lab.07	
Prochloraz	validation	Lab.09	0.93
TBT	prevalidation	Lab.09	1.70
TBT	prevalidation	Lab.14	0.75
TBT	prevalidation	Lab.15	1.10
TBT	validation	Lab.02	1.90
TBT	validation	Lab.03	1.10
TBT	validation	Lab.05	1.30
TBT	validation	Lab.06	1.40
TBT	validation	Lab.11	1.30

Table 7: Ratios between EC_{50} medians at 28 days from clutches and EC_{50} medians at 56 days from eggs, for all tox, both end points, both phases and all labs. The red cell highlights the ratio over 2.

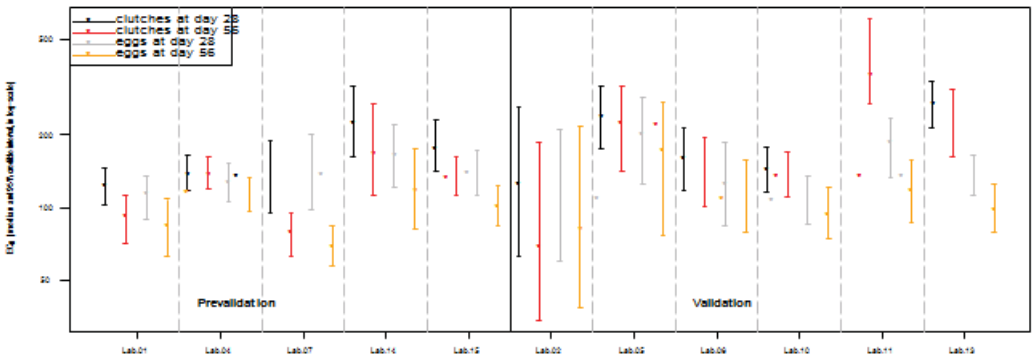

Figure 1: Cadmium $EC50$ estimates (medians as circles, 95% credibility intervals as flat arrows): from clutch data at day 28 (in black), from clutch data at day 56 (in red), from egg data at day 28 (in gray), from egg data at day 56 (in orange).

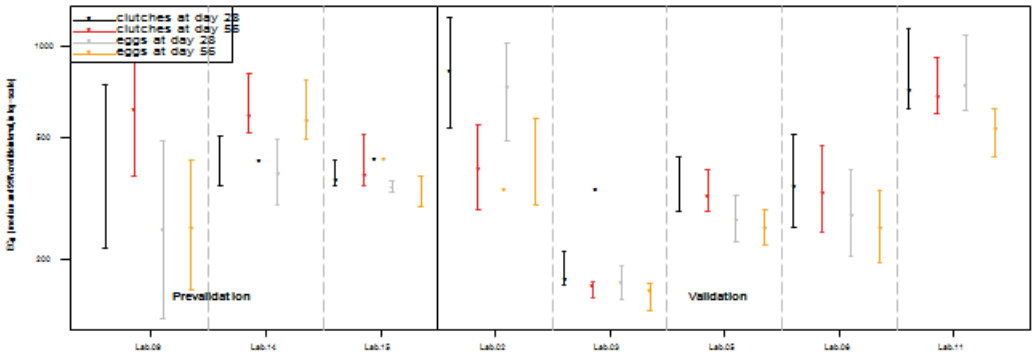

Figure 2: TBT $EC50$ estimates (medians as circles, 95% credibility intervals as flat arrows): from clutch data at day 28 (in black), from clutch data at day 56 (in red), from egg data at day 28 (in gray), from egg data at day 56 (in orange).

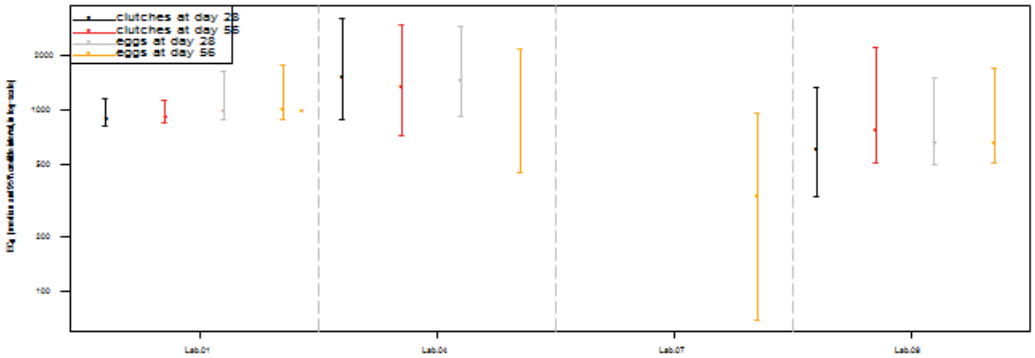

Figure 3: Prochloraz $EC50$ estimates (medians as circles, 95% credibility intervals as flat arrows): from clutch data at day 28 (in black), from clutch data at day 56 (in red), from egg data at day 28 (in gray), from egg data at day 56 (in orange).

CLUTCH VERSUS EGG EC_{50} ESTIMATES

63. The objective of this section is to help in deciding if only clutches can be counted or if eggs must also be counted. The study was performed at day 28 only. As a first help decision tool, we can check if EC_{50} estimates from clutch or egg data are similar, that is with similar modes of their posterior dis- tribution but also with similar uncertainties. Recall that EC_{50} were estimated by Bayesian inference as recommended by Delignette-Muller et al. (2014)[1].

64. We already have an idea of this issue from Figures 1, 2 and 3 were posterior distributions of EC_{50} estimates were simply summarized with medians, 2/5% and 97.5% quantiles of EC_{50} estimates. In order to complete previous results, we can compare whole posterior probability distributions obtained for EC_{50} estimates from both clutch and egg data. As shown on Figure 4, posteriors on EC_{50} from clutch or egg data are close from each other for all labs: distributions have similar positive skewness and similar kurtosis; pics of the distributions are also closely located. Even if EC_{50} medians from clutches are most often larger than EC_{50} medians from eggs, values remain pretty similar according to ratios between EC_{50} medians that are close to 1, except for Lab.13, Cadmium, validation (see Table 9). Notice also that posteriors for prochloraz are far from priors thus indicating that estimates are far from what it was expected before the experiment. This fact is also due to rather bad estimates (very large uncertainty and high EC_{50} medians) for all prochloraz datasets.

65. Based on the fact that priors on EC_{50} were log-normally distributed, we tried to fit a log-normal distribution on posteriors in order to estimate a standard deviation. This estimated standard de- viation could be used as a quantitative proxy of the uncertainty on EC_{50} estimates; nevertheless, such an assertion must be used with caution because the estimation is based on the hypothesis that posteriors also follow a log-normal distribution. Log-normal distributions were thus fitted on EC_{50} posterior MCMC samples with R package fitdistrplus (Delignette-Muller and Dutang, 2015[2]). The last appendix shows posterior fittings with log-normal distributions. For all labs, toxicants, phases and endpoints, there are two graphs: on the left, the Cullen and Frey graph which is a skewness-kurtosis plot to compare the observed value from posteriors to different values from common distributions as a tools to help in choosing the distribution to fit; on the right, the histogram of posteriors against the fitted density log-normal distribution.

66. All these results are summarized in Table 8, from which we notice that $Median_{EC_{50}}$ and Q_{extend} are very good proxies of $\mu_{EC_{50}}$ and $\sigma_{EC_{50}}$ with $\mu_{EC_{50}} \simeq Median_{EC_{50}}$ and $\sigma_{EC_{50}} \simeq Q_{extend}/4$. Based on $CV_{EC_{50}}$ values we can say that EC_{50} estimates from clutch or egg data have very similar uncertainties: $CV_{EC_{50}}$ values are always equal from clutches and eggs, except in two cases (blue cells in Table 8). $Median_{EC_{50}}$ from clutches are almost always larger than $Median_{EC_{50}}$ from eggs, except in few cases (see green cells in Table 8). The ratio between $Median_{EC_{50}}$ from clutches and $Median_{EC_{50}}$ from eggs is rarely greater than 30% except in few cases (red cells in penultimate column of Table 9).

67. In the perspective of being the more protective as possible, it seems rather difficult to consider only *medians* of EC_{50} from clutch data; the *uncertainty* around the median must also be taken into account. An alternative, when using only clutch data, could be to consider the 2.5% quantile of the EC_{50} estimate, which is pretty close to the EC_{50} median from eggs, except in three cases; see red

cells in Table 8 or the last column in Table 9 (red cells highlight ratios over 1.3). Anyway, the ratio between $Median_{EC_{50}}$ from clutches and $Median_{EC_{50}}$ from eggs remains small enough to cause no issue from the risk assessment point of view, where factors 10 are arbitrary and systematically applied on EC_{50} values.

[1]Delignette-Muller, M.L., Lopes, C., Veber, P., Charles, S., 2014. Statistical handling of reproduction data for exposure-response modeling. Environ. Sci. Technol. 48, 7544–51.

[2]Delignette-Muller, M.L., Dutang, C., 2015. `fitdistrplus` : An R Package for Fitting Distributions. *J. Stat. Softw.* 64(4). 1-34

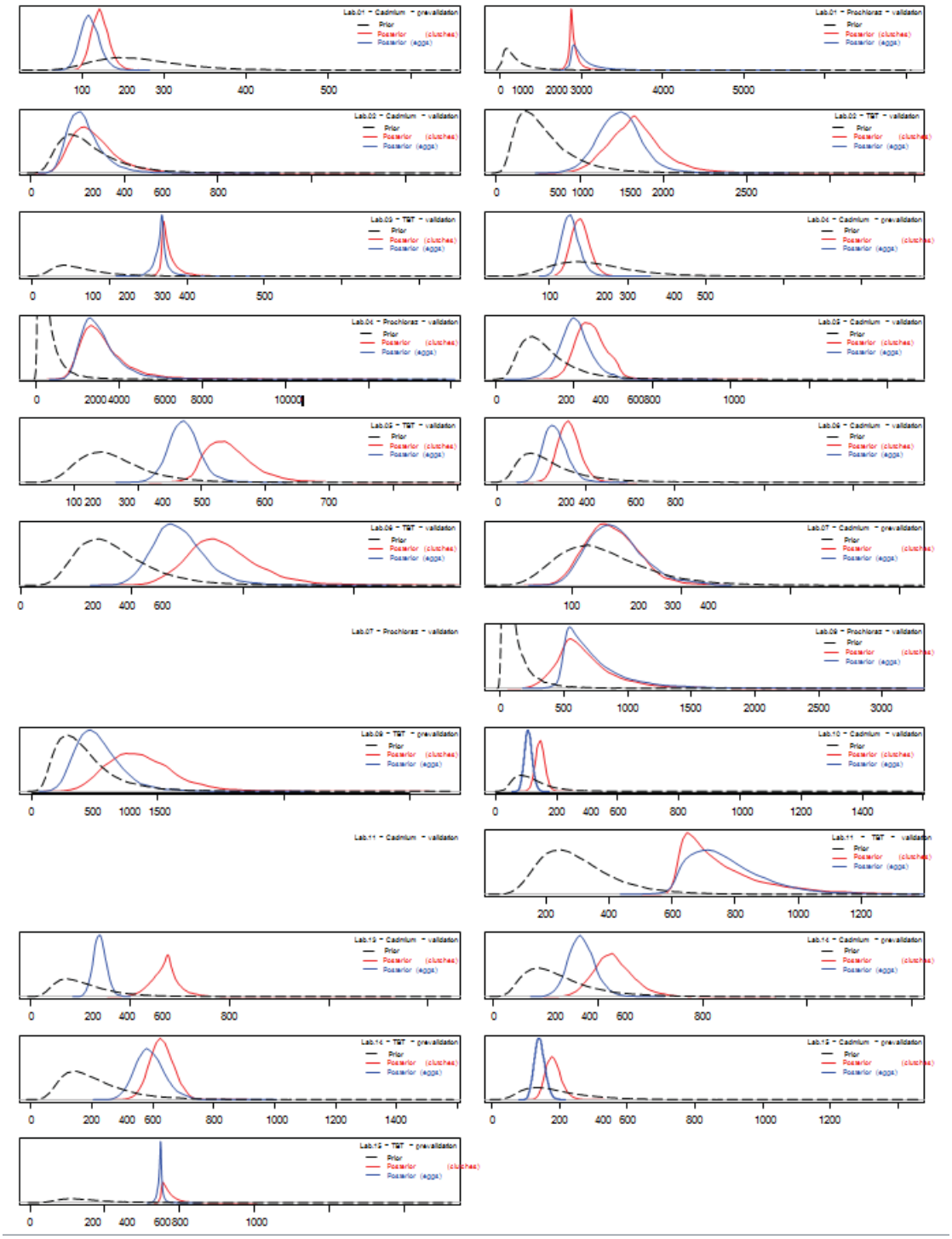

Figure 4: $EC50$ posterior distributions from both clutch (in red) and egg (in blue) data superimposed to prior distributions (dotted line in black) for all labs, all toxicant and both pre- and validation phases.

Tox	Phase	Lab	Endpoint	Median$_{EC50}$	Q2.5$_{EC50}$	Q97.5$_{EC50}$	μ_{EC50}	σ_{EC50}	CV$_{EC50}$	Q_{extend}	$4\times\sigma_{EC50}$
Cadmium	prevalidation	Lab.01	clutches	121.8	102.5	145.9	122.4	10.9	0.1	43.4	43.7
Cadmium	prevalidation	Lab.01	eggs	109.3	88.9	135.9	110.1	11.9	0.1	47.0	47.6
Cadmium	prevalidation	Lab.04	clutches	139.0	117.6	164.6	139.4	11.8	0.1	47.0	47.4
Cadmium	prevalidation	Lab.04	eggs	126.4	106.7	153.9	127.3	12.0	0.1	47.2	48.1
Cadmium	prevalidation	Lab.07	clutches	135.0	95.6	189.4	137.0	24.1	0.2	93.8	96.4
Cadmium	prevalidation	Lab.07	eggs	137.6	98.8	200.2	140.2	25.1	0.2	101.4	100.3
Cadmium	prevalidation	Lab.14	clutches	226.9	163.3	316.9	230.0	38.8	0.2	153.6	155.2
Cadmium	prevalidation	Lab.14	eggs	167.1	121.0	221.7	168.4	25.8	0.2	100.7	103.4
Cadmium	prevalidation	Lab.15	clutches	179.4	141.1	232.1	181.2	23.0	0.1	91.0	92.0
Cadmium	prevalidation	Lab.15	eggs	139.6	111.7	174.0	140.4	15.9	0.1	62.3	63.7
Cadmium	validation	Lab.02	clutches	126.5	62.7	259.3	135.5	50.3	0.4	196.6	201.3
Cadmium	validation	Lab.02	eggs	109.7	60.4	211.3	116.1	37.7	0.3	150.9	150.7
Cadmium	validation	Lab.05	clutches	241.2	176.4	318.1	243.8	38.6	0.2	141.7	154.5
Cadmium	validation	Lab.05	eggs	201.3	124.9	288.1	202.8	41.8	0.2	163.2	167.2
Cadmium	validation	Lab.06	clutches	160.9	117.1	215.1	162.1	25.3	0.2	98.0	101.3
Cadmium	validation	Lab.06	eggs	128.6	84.5	187.4	130.5	26.8	0.2	102.8	107.4
Cadmium	validation	Lab.10	clutches	145.2	116.0	177.1	145.6	15.7	0.1	61.1	63.0
Cadmium	validation	Lab.10	eggs	108.6	85.3	135.4	109.1	12.8	0.1	50.1	51.3
Cadmium	validation	Lab.11	clutches								
Cadmium	validation	Lab.11	eggs	187.1	133.2	233.5	186.2	26.6	0.1	100.2	106.5
Cadmium	validation	Lab.13	clutches	271.1	215.2	335.0	270.4	29.0	0.1	119.8	115.9
Cadmium	validation	Lab.13	eggs	137.9	113.3	165.6	138.3	13.3	0.1	52.3	53.0
Prochloraz	validation	Lab.01	clutches	890.9	805.8	1135.5	912.6	78.1	0.1	329.7	312.5
Prochloraz	validation	Lab.01	eggs	976.3	875.7	1625.6	1043.8	178.4	0.2	749.8	713.7
Prochloraz	validation	Lab.04	clutches	1494.1	876.7	3180.5	1625.1	544.6	0.3	2303.8	2178.6
Prochloraz	validation	Lab.04	eggs	1448.4	909.2	2851.7	1547.1	457.7	0.3	1942.5	1831.0
Prochloraz	validation	Lab.07	eggs								
Prochloraz	validation	Lab.09	clutches	622.3	333.8	1329.9	674.9	235.7	0.3	996.1	942.9
Prochloraz	validation	Lab.09	eggs	667.8	499.4	1491.1	747.0	233.1	0.3	991.7	932.5
TBT	prevalidation	Lab.09	clutches	421.3	217.1	750.1	436.9	141.2	0.3	533.1	564.6
TBT	prevalidation	Lab.09	eggs	251.7	128.4	492.6	266.2	93.1	0.3	364.2	372.5
TBT	prevalidation	Lab.14	clutches	426.8	351.9	507.4	428.2	40.3	0.1	155.6	161.3
TBT	prevalidation	Lab.14	eggs	387.3	304.1	498.7	390.8	49.0	0.1	194.6	196.2
TBT	prevalidation	Lab.15	clutches	365.1	350.4	426.0	371.0	19.7	0.1	75.6	78.7
TBT	prevalidation	Lab.15	eggs	348.2	333.3	364.4	348.1	7.3	0.0	31.1	29.1
TBT	validation	Lab.02	clutches	826.7	543.0	1242.5	840.2	173.0	0.2	699.5	692.1
TBT	validation	Lab.02	eggs	738.1	490.1	1029.3	741.6	138.6	0.2	539.2	554.3
TBT	validation	Lab.03	clutches	173.7	164.4	213.7	177.9	12.6	0.1	49.3	50.5
TBT	validation	Lab.03	eggs	166.7	147.6	190.4	166.6	9.8	0.1	42.7	39.2
TBT	validation	Lab.05	clutches	339.0	286.9	437.1	344.7	37.9	0.1	150.2	151.8
TBT	validation	Lab.05	eggs	271.8	228.2	326.3	272.8	24.4	0.1	98.1	97.5
TBT	validation	Lab.06	clutches	356.2	256.7	516.7	363.6	65.0	0.2	260.4	260.1
TBT	validation	Lab.11	clutches	719.4	622.6	1144.3	782.8	128.2	0.2	521.8	512.7
TBT	validation	Lab.11	eggs	749.4	617.3	1084.9	774.1	117.3	0.2	467.6	469.0

Table 8: Uncertainty on EC50 estimates from clutch and egg data, for the three toxicants, both phases and all labs. MedianEC50 , Q2.5EC50 and Q97.5EC50 stand for 50%, 2.5% and 97.5% quantiles of the EC50 posterior distribution, respectively. μEC50 and σEC50 are the estimated mean and standard deviation from fitting of the log-normal distribution on posteriors.CV = σEC50 and Q = Q97.5 EC50 − Q2.5 EC50 .

Tox	Phase	Lab	$\frac{EC_{50,clutches}}{EC_{50,eggs}}$	$\frac{EC_{50,eggs}}{Q_{2.5\%,clutches}}$
Cadmium	prevalidation	Lab.01	1.10	1.10
Cadmium	prevalidation	Lab.04	1.10	1.10
Cadmium	prevalidation	Lab.07	0.98	1.40
Cadmium	prevalidation	Lab.14	1.40	1.00
Cadmium	prevalidation	Lab.15	1.30	0.99
Cadmium	validation	Lab.02	1.20	1.70
Cadmium	validation	Lab.05	1.20	1.10
Cadmium	validation	Lab.06	1.30	1.10
Cadmium	validation	Lab.10	1.30	0.94
Cadmium	validation	Lab.11		
Cadmium	validation	Lab.13	2.00	0.64
Prochloraz	validation	Lab.01	0.91	1.20
Prochloraz	validation	Lab.04	1.00	1.70
Prochloraz	validation	Lab.07		
Prochloraz	validation	Lab.09	0.93	2.00
TBT	prevalidation	Lab.09	1.70	1.20
TBT	prevalidation	Lab.14	1.10	1.10
TBT	prevalidation	Lab.15	1.00	0.99
TBT	validation	Lab.02	1.10	1.40
TBT	validation	Lab.03	1.00	1.00
TBT	validation	Lab.05	1.20	0.95
TBT	validation	Lab.06	1.30	1.10
TBT	validation	Lab.11	0.96	1.20

Table 9: Two ratios are given for the three toxicants, the both phases and all labs: the ratio between $EC50$ medians from clutch and egg estimates; the ratio between $EC50$ medians from egg estimates and $Q2.5\%$ from clutch estimates.

CONCLUSION

68. Based on our results, it seems reasonable to conduct 28-days experiments by counting only clutches at each time-point. Our goal is indeed to make the test both sensitive and cost effective. As clearly illustrated in this report, the gain in exposing during 56 days (resp. counting eggs) is negligible compared to the gain in exposing 28 days (resp. counting only clutches). This gain is too small to justify the investment in terms of human resources and experimental costs that occur when doubling the experiment duration and significantly increase the workload when counting eggs, which is the most time-consuming part of the experiment.

APPENDICES

Discrepancies between KW and JT tests at day 56

3 Fit of the concentration-response model

Data concern: Lab.11, Cadmium, validation, clutches

Type of concentration:
concmeas

The model with a Gamma Poisson stochastic part was used to analyse the data. Additional parameter ω stands for overdispersion.

3.1 Fitted curve

Model parameters are given in Table 1.

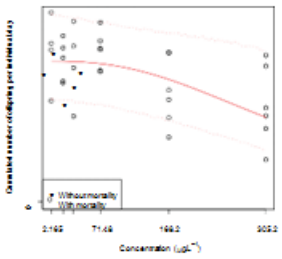

Figure 3: Plain red line: fitted curve superimposed to observed data expressed in cumulated number of offspring per individual-day; Parameters are equal to their median value. Dotted red lines: 95% credible band.

	median	Q2.5	Q97.5
d	0.23	0.21	0.265
b	2.51	0.804	65.7
e	359	269	604
omega	0.0038	0.000249	0.0126

Table 1: Estimated parameters with 95% credible interval. Q2.5 (resp. Q97.5) is the 2.5% quantile (resp. 97.5%) of the posterior distribution. *d* stands for the expected number of offspring per individual-day in the control, *e* is the 50% effective concentration (EC_{50}) and *b* is a slope parameter.

3.2 Estimated EC_x

Estimated values of some effective concentrations are given in Table 2.

	median	Q2.5	Q97.5	Min	Max
EC5	112.92	10.198	381.71	0.029912	925.29
EC10	152.46	25.309	393.53	0.28578	932.35
EC20	212.21	67.564	415.24	3.3098	940.06
EC50	359.08	268.9	604.19	188.79	1141

Table 2: Estimated values with 95% credible interval as well as minimal and maximal values. Q2.5 (resp. Q97.5) is the 2.5% quantile (resp. 97.5%) of the posterior distribution.

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Figure 5: Results from the fit of the concentration-effect model on data concerning Lab.11, Cadmium, validation and clutches at day 56.

3 Fit of the concentration-response model

Data concern: Lab.07, Prochloraz, validation, eggs Type

of concentration:
concmeas

The model with a Gamma Poisson stochastic part was used to analyse the data. Additional parameter ω stands for overdispersion.

Jonckheere-Terpstra trend test: p-value = 0.014 .

3.1 Fitted curve

Model parameters are given in Table 1.

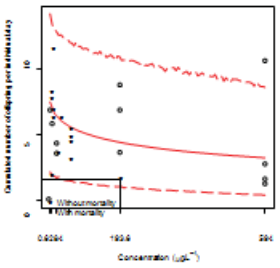

Figure 3: Plain red line: fitted curve superimposed to observed data expressed in cumulated number of offspring per individual-day; Parameters are equal to their median value. Dotted red lines: 95% credible band.

	median	Q2.5	Q97.5
d	7.74	5.66	9.93
b	0.538	0.0605	10.1
e	334	69.3	962
omega	1.14	0.63	2.33

Table 1: Estimated parameters with 95% credible interval. Q2.5 (resp. Q97.5) is the 2.5% quantile (resp. 97.5%) of the posterior distribution. *d* stands for the expected number of offspring per individual-day in the control, *e* is the 50% effective concentration (EC_{50}) and *b* is a slope parameter.

3.2 Estimated EC_x

Estimated values of some effective concentrations are given in Table 2.

	median	Q2.5	Q97.5	Min	Max
EC5	1.2207	1.0563e-19	481.95	1.0016e-126	2063.9
EC10	4.9204	2.6016e-14	518.28	2.4345e-94	2084.7
EC20	22.585	1.5381e-08	559.5	3.4172e-59	2130.8
EC50	334.29	69.266	962	12.581	3433.1

Table 2: Estimated values with 95% credible interval as well as minimal and maximal values. Q2.5 (resp. Q97.5) is the 2.5% quantile (resp. 97.5%) of the posterior distribution.

Figure 6: Results from the fit of the concentration-effect model on data concerning Lab.07, Prochloraz, validation and clutches at day 56.

3 Fit of the concentration-response model

Data concern: Lab.09, Prochloraz, validation, clutches Type of

concentration:
concmeas

The model with a Gamma Poisson stochastic part was used to analyse the data. Additional parameter ω stands for overdispersion.

Jonckheere-Terpstra trend test: p-value = 0.0015 .

3.1 Fitted curve

Model parameters are given in Table 1.

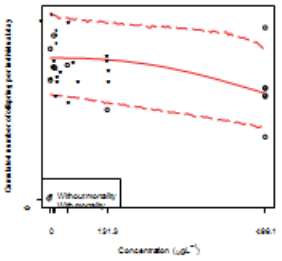

Figure 3: Plain red line: fitted curve superimposed to observed data expressed in cumulated number of offspring per individual-day; Parameters are equal to their median value. Dotted red lines: 95% credible band.

	median	Q2.5	Q97.5
d	0.214	0.216	0.273
e	773	511	2.22e+03
omega	0.00358	0.000463	0.01

Table 1: Estimated parameters with 95% credible interval. Q2.5 (resp. Q97.5) is the 2.5% quantile (resp. 97.5%) of the posterior distribution. *d* stands for the expected number of offspring per individual-day in the control, *e* is the 50% effective concentration (EC_{50}) and *b* is a slope parameter.

3.2 Estimated EC_x

Estimated values of some effective concentrations are given in Table 2.

	median	Q2.5	Q97.5	Min	Max
EC5	252.07	1.5	550.72	0.00097142	3801.5
EC10	339.56	8.0312	588.85	0.032187	3850.4
EC20	458.56	49.583	686.38	1.4375	3904.2
EC50	772.75	510.66	2219.4	293.91	8226

Table 2: Estimated values with 95% credible interval as well as minimal and maximal values. Q2.5 (resp. Q97.5) is the 2.5% quantile (resp. 97.5%) of the posterior distribution.

Figure 7: Results from the fit of the concentration-effect model on data concerning Lab.09, Prochloraz, validation and clutches at day 56.

3 Fit of the concentration-response model

Data concern: Lab.09, Prochloraz, validation, eggs Type

of concentration:
concmeas

The model with a Gamma Poisson stochastic part was used to analyse the data. Additional parameter ω stands for overdispersion.

Jonckheere-Terpstra trend test: p-value = 0.039 .

3.1 Fitted curve

Model parameters are given in Table 1.

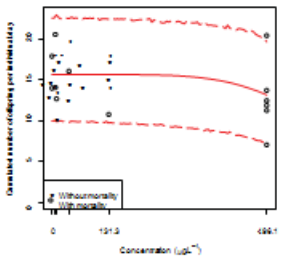

Figure 3: Plain red line: fitted curve superimposed to observed data expressed in cumulated number of offspring per individual-day; Parameters are equal to their median value. Dotted red lines: 95% credible band.

	median	Q2.5	Q97.5
d	15.7	14.7	16.8
e	671	509	1.68e+03
omega	0.623	0.385	1.1

Table 1: Estimated parameters with 95% credible interval. Q2.5 (resp. Q97.5) is the 2.5% quantile (resp. 97.5%) of the posterior distribution. *d* stands for the expected number of offspring per individual-day in the control, *e* is the 50% effective concentration (EC_{50}) and *b* is a slope parameter.

3.2 Estimated EC_x

Estimated values of some effective concentrations are given in Table 2.

	median	Q2.5	Q97.5	Min	Max
EC5	387.77	63.919	811.24	1.3702	4074
EC10	442.7	134.34	844.49	8.1098	4151
EC20	497.84	282	921.92	55.86	4236.3
EC50	670.95	508.89	1684.7	498.38	5145.4

Table 2: Estimated values with 95% credible interval as well as minimal and maximal values. Q2.5 (resp. Q97.5) is the 2.5% quantile (resp. 97.5%) of the posterior distribution.

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Figure 8: Results from the fit of the concentration-effect model on data concerning Lab.09, Prochloraz, validation and eggs at day 56.

Discrepancies between KW and JT tests at day 28

Laboratory: Lab.09 Toxicant: Prochloraz
Endpoint: eggs (called offspring afterwards) Phase: validation
→ Use of actual concentrations (arithmetic means of measured values).
Jonckheere-Terpstra trend test: p-value = 0.0045 .

Fit of the concentration-response model

The model with a Gamma Poisson stochastic part was used to analyse the data. Additional parameter ω stands for overdispersion.

Model parameters are given in Table 1.

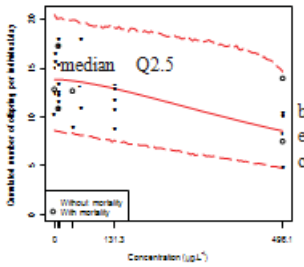


Figure 1: Plain red line: fitted curve superimposed to observed data expressed in cumulated number of offspring per individual-day; Parameters are equal to their median value. Dotted red lines: 95% credible band.

			Q97.5			
			d	13.9	12.7	15.7
b	1.6	0.486	35.8			
e	668		499	1.49e+03		
omega			0.591	0.359		1.06

Table 1: Estimated parameters with 95% credible

97.5%) of the posterior distribution. *d* stands for the expected number of offspring per individual-day in the control, *e* is the 50% effective concentration (EC_{50}) and *b* is a slope parameter.

Estimated values of some effective concentrations are given in Table 2.

	median	Q2.5	Q97.5	Min	Max
EC5	114.42	2.3293	472.11	0.00042166	1529.5
EC10	184.87	10.477	483.56	0.021916	1555.3
EC20	311.94	52.155	508.7	1.5954	1583.8
EC50	667.8	499.4	1491.1	259.4	5471.4

Table 2: Estimated values with 95% credible interval as well as minimal and maximal values. Q2.5 (resp. Q97.5) is the 2.5% quantile (resp. 97.5%) of the posterior distribution.

Figure 9: Results from the fit of the concentration-effect model on data concerning Lab.09, Prochloraz, validation and eggs at day 28.

Laboratory: Lab.11 Toxicant: TBT
Endpoint: clutches (called offspring afterwards) Phase: validation
→ Use of actual concentrations (arithmetic means of measured values).
Jonckheere-Terpstra trend test: p-value = 0.0023 .

Fit of the concentration-response model

The model with a Poisson stochastic part was used to analyse the data.
Model parameters are given in Table 1.

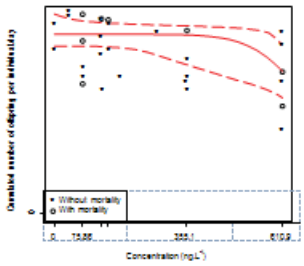


Figure 1: Plain red line: fitted curve superimposed to observed data expressed in cumulated number of offspring per individual-day; Parameters are equal to their median value. Dotted red lines: 95% credible band.

	median	Q2.5	Q97.5
d0.301	0.281		0.335
b8.21	1.1	75.7	
e	719	623	1.14e+03

Table 1: Estimated parameters with 95% credible interval. Q2.5 (resp. Q97.5) is the 2.5% quantile (resp. 97.5%) of the posterior distribution. d is the expected number of offspring per individual-day in the control, e is the 50% effective concentration (EC_{50}) and b is a slope parameter.

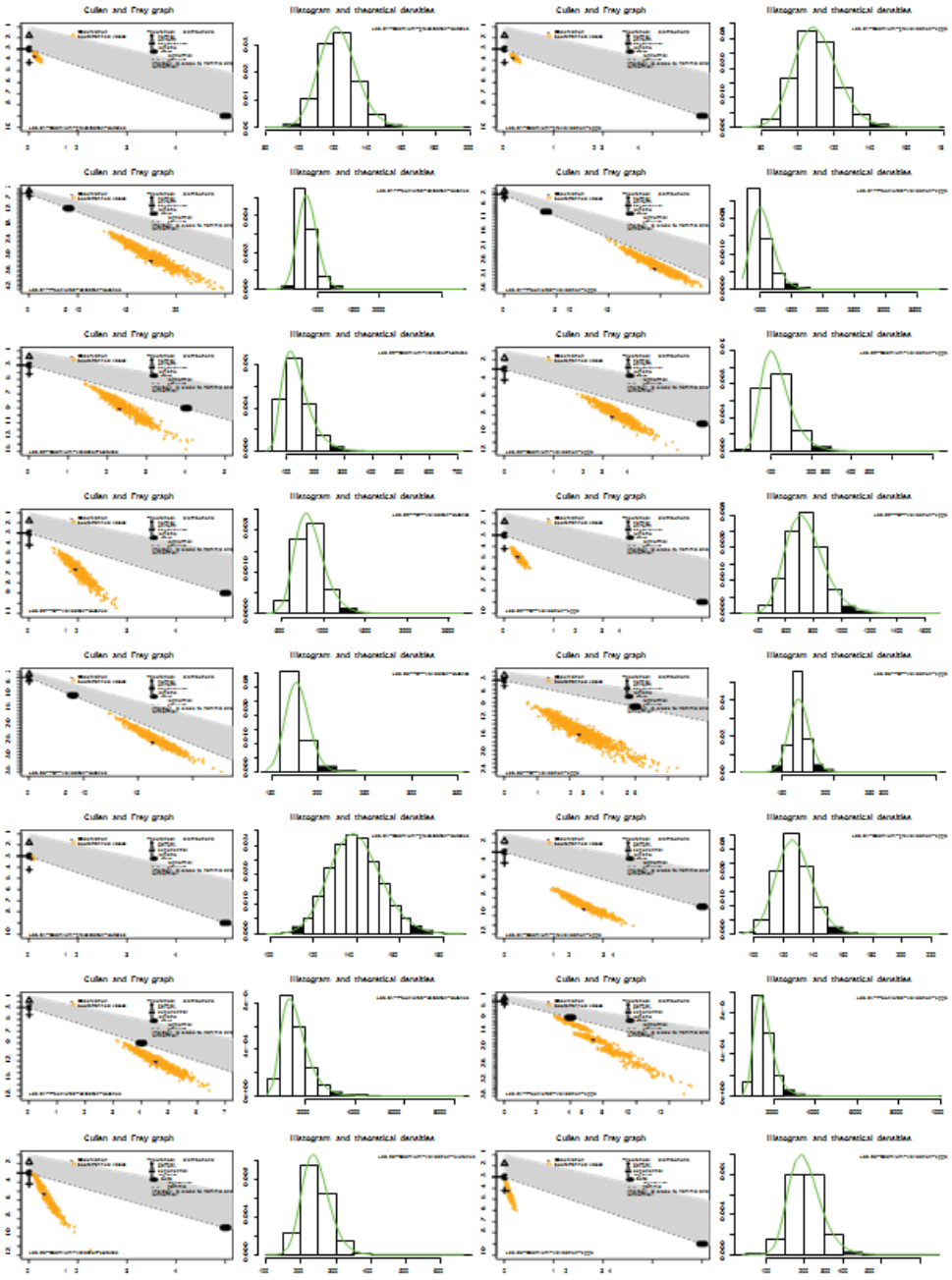
Estimated values of some effective concentrations are given in Table 2.

	median	Q2.5	Q97.5	Min	Max
EC5	506.32	68.777	626.62	2.7944	1065.8
EC10	553.52	137.66	654.86	12.332	1079.1
EC20	604.4	282.65	704.11	61.767	1093.8
EC50	719.36	622.57	1144.3	553.63	1930

Table 2: Estimated values with 95% credible interval as well as minimal and maximal values. Q2.5 (resp. Q97.5) is the 2.5% quantile (resp. 97.5%) of the posterior distribution.

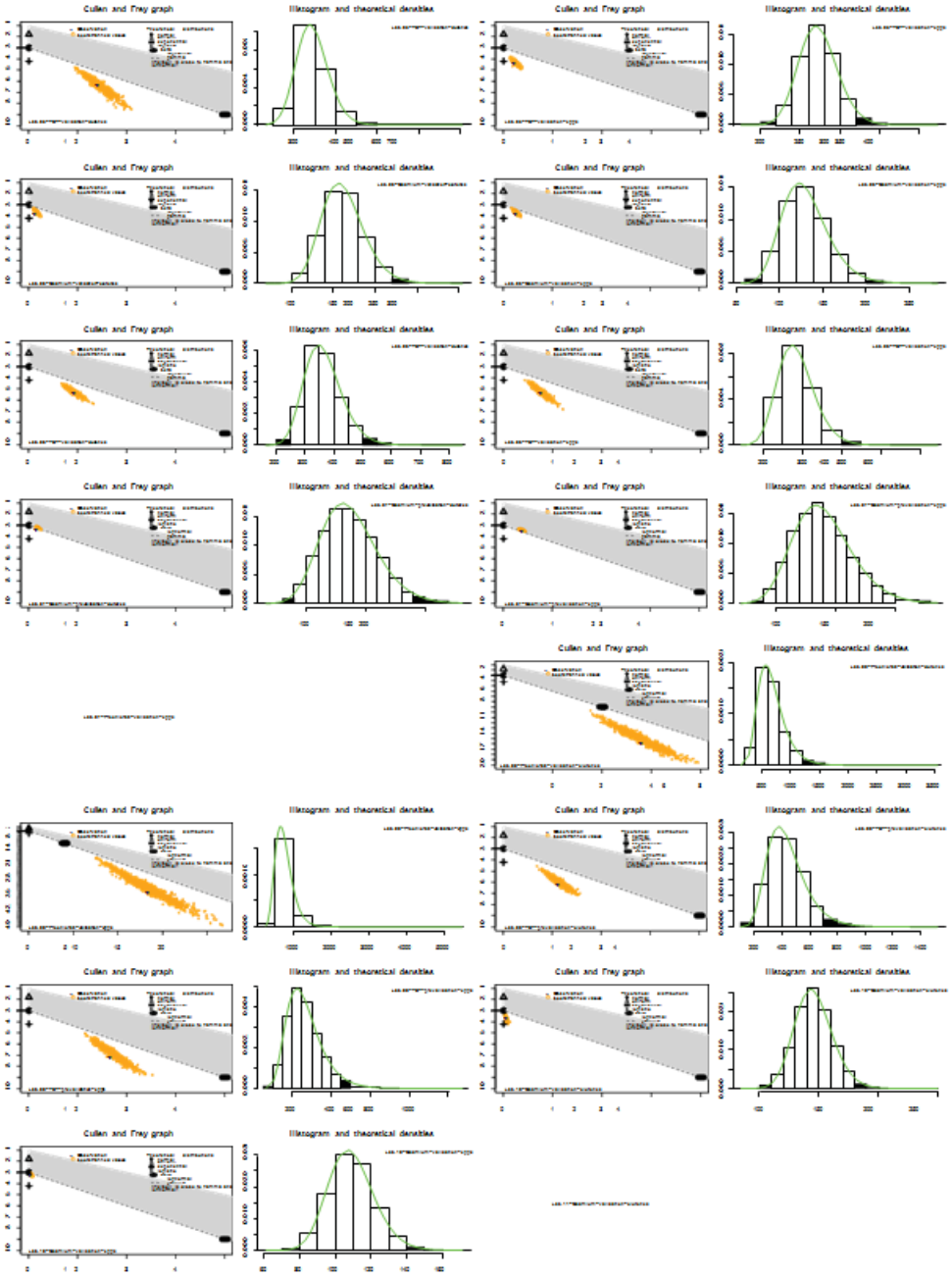
Figure 10: Results from the fit of the concentration-effect model on data concerning Lab.11, TBT, validation and clutches at day 28.

5.3 Posterior fittings



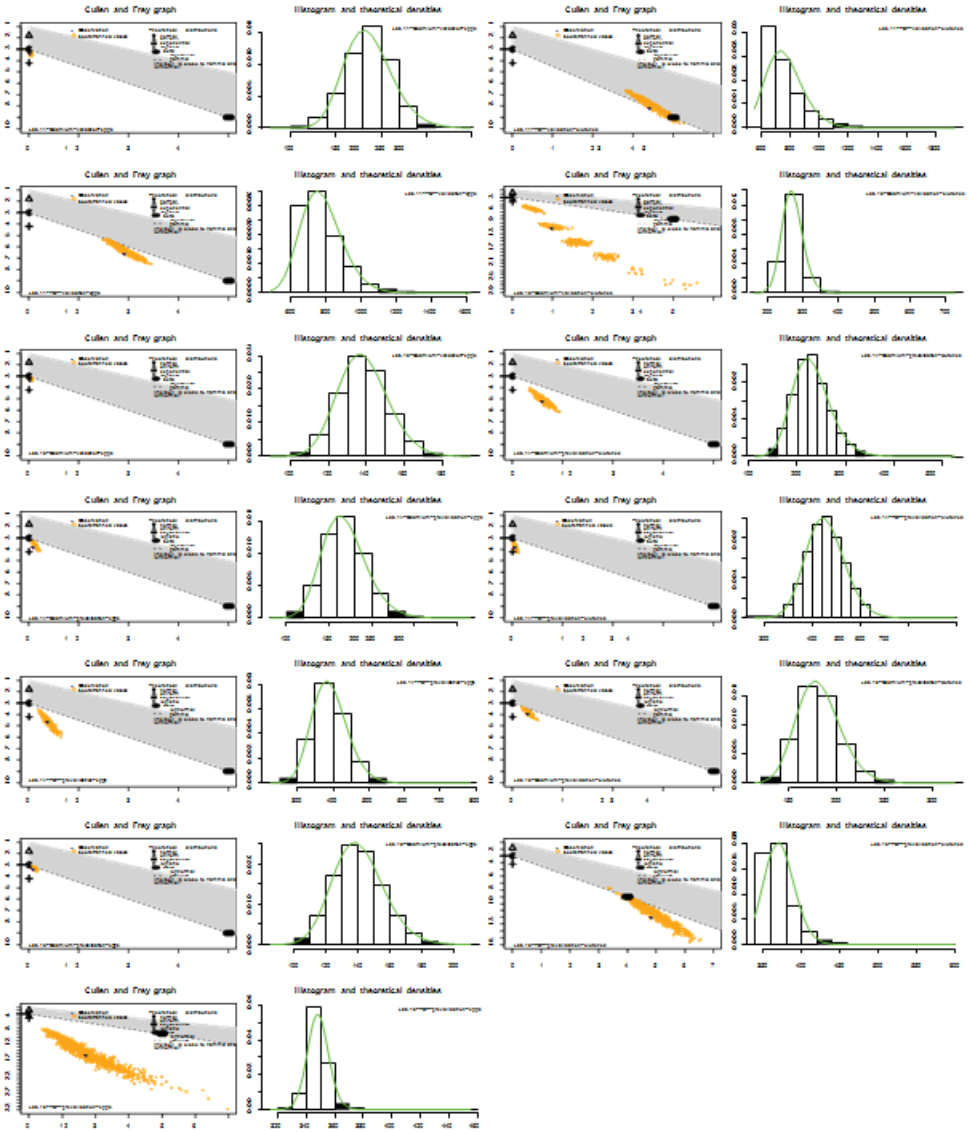
(a) From Lab.01 to lab.05

Figure 11: Posterior fittings with log-normal distributions. For all labs, toxicants, phases and endpoints, there are two graphs: on the left, the Cullen and Frey graph which is a skewness-kurtosis plot to compare the observed value from posteriors to different values from common distributions as a tools to help in choosing the distribution to fit; on the right, the histogram of posteriors against the fitted density log-normal distribution.



(b) From Lab.05 to Lab.11

Figure 11: Posterior fittings with log-normal distributions. For all labs, toxicants, phases and endpoints, there are two graphs: on the left, the Cullen and Frey graph which is a skewness-kurtosis plot to compare the observed value from posteriors to different values from common distributions as a tools to help in choosing the distribution to fit; on the right, the histogram of posteriors against the fitted density log-normal distribution.



(c) From Lab.11 to Lab.15

Figure 11: Posterior fittings with log-normal distributions. For all labs, toxicants, phases and endpoints, there are two graphs: on the left, the Cullen and Frey graph which is a skewness-kurtosis plot to compare the observed value from posteriors to different values from common distributions as a tools to help in choosing the distribution to fit; on the right, the histogram of posteriors against the fitted density log-normal distribution.