Appendix to ISO/OECD report on the analysis of tox data

Examples of data analysis using the DEBtox method

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The following four examples were all calculated with software package DEBtox.

1 Bioassay on acute mortality of *Daphnia magna*

Data

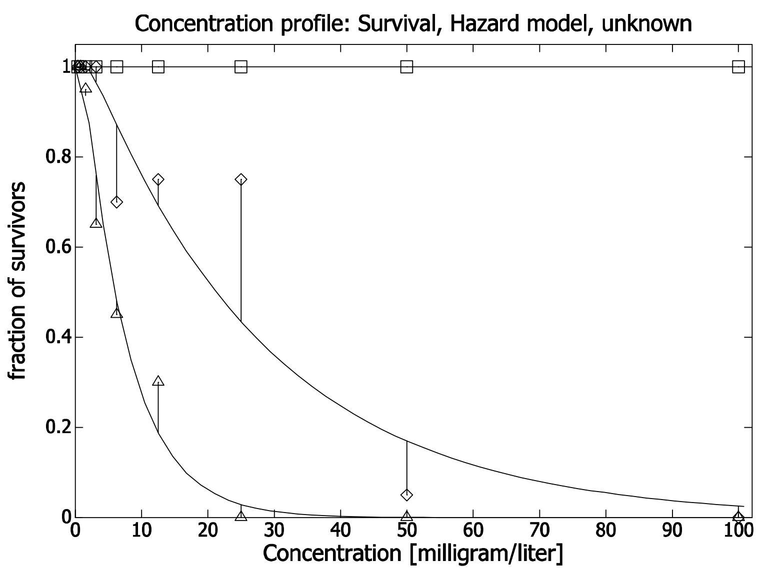
Time: day, Conc: milligram/liter, Resp: Number of survivors

	0.39	0.78	1.56	3.13	6.25	12.50	25.00	50.00	100.00
0	20	20	20	20	20	20	20	20	20
1	20	20	20	20	14	15	15	1	0
2	20	20	19	13	9	6	0	0	0

Parameters and asymptotic standard deviations (ASD)

Survival, Hazard model			ASD	Correlation coefficients	
Blank mortality rate	1e-010	d^{-1}	0.000		
No-effect concentration-time	1.473	$mg\ l^{-1}\ d$	0.344	0.000	
Killing acceleration	0.07524	$l\ mg^{-1}\ d^{-2}$	0.010	0.000	0.359
Deviance	23.29				

Graphical test of model predictions against data



LCx values (derived from parameter values) in mg/l.

Day	LC0	ASD	LC50	ASD
1	1.48	0.344	21.1	2.26
2	0.738	0.172	5.94	0.574

Comments

Slow kinetics appeared to fit the data best, which means that the elimination rate was too small to be estimated reliably. This means that the model loses this parameter. The consequence is that only the killing acceleration (which is the product of the killing rate and the elimination rate) can be estimated, not the killing rate itself. Similarly the ratio of the NEC and the elimination rate, called the no-effect concentration time, could be estimated, rather than the NEC itself; the bioassay did not last long enough for this compound. The 95% confidence interval for the NEC can still be estimated, however, and was found to be (0, 0.95) mg/l, on the basis of the profile likelihood function. The package DEBtool has been used to obtain the profile likelihood function.

The background morality rate was found to be nil. Notice that, excluding this parameter, a total of two parameters have been fitted on 18 data points.

2 Bioassay on algal growth

Data for effects of Atrazine in μg/l on the growth of *Selenastrum capricornutum* in cells/ml.

Time: hour, Conc: microgram/liter, Resp: cells ml^{-1}

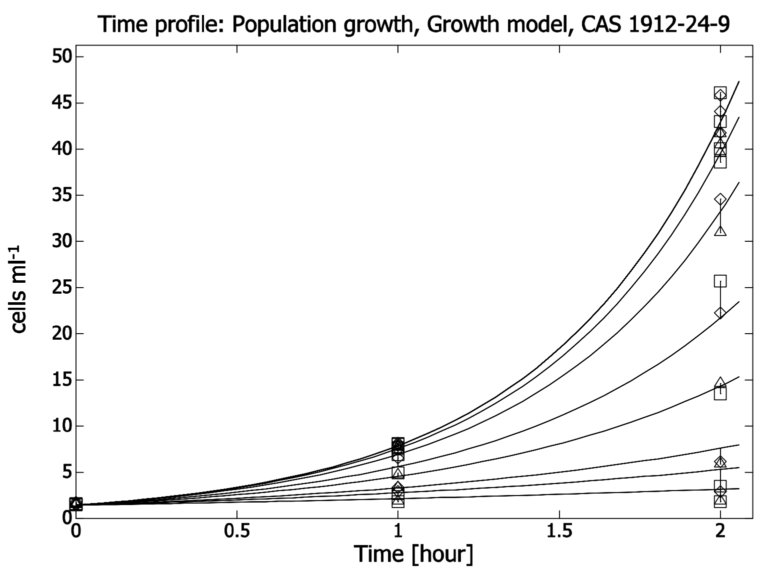
	0	0	0	0	0	0	10	10	20	20	30
0	1.5403	1.5239	1.5279	1.5586	1.5553	1.6126	1.5093	1.7349	1.6586	1.5529	1.5446
1	7.6709	7.9489	7.7606	8.0956	8.1069	7.7463	8.0036	8.1433	7.4276	7.5383	7.5066
2	46.1206	44.0773	41.6406	42.9973	45.8606	40.4706	40.0639	41.7773	39.5539	38.5706	34.5839

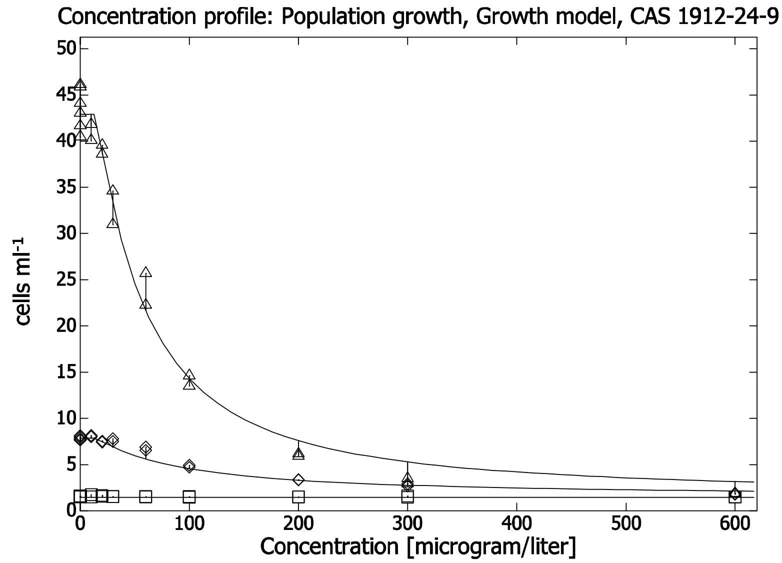
	30	60	60	100	100	200	200	300	300	600	600
0	1.5273	1.5426	1.5223	1.5406	1.4833	1.5099	1.5049	1.5929	1.4529	1.4879	1.5003
1	7.8069	6.8963	6.5459	4.7089	4.9329	3.3593	3.3193	2.6889	2.9189	1.8833	1.8033
2	30.9639	25.6973	22.2406	14.6173	13.4873	6.1586	5.8823	3.4849	2.8926	1.8826	1.8259

Parameter estimates and Asymptotic Standard Deviations (ASD)

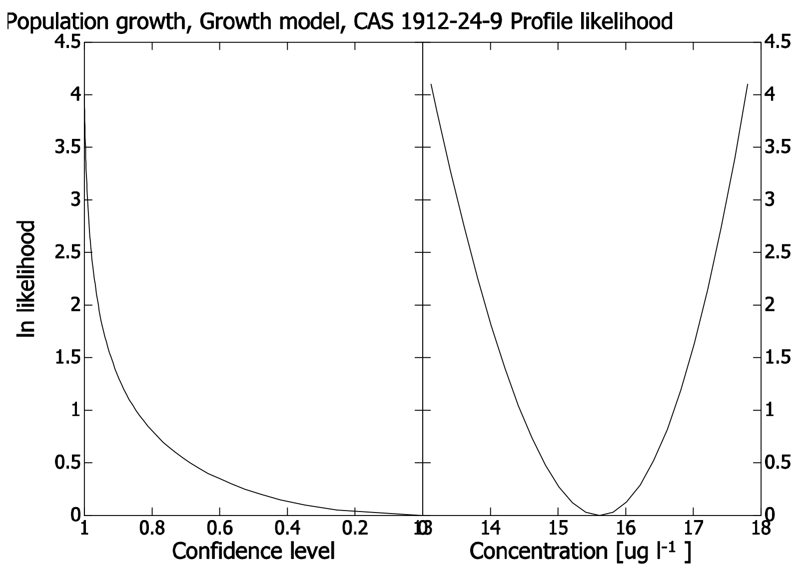
Population growth, Growth model			ASD	Correlation coefficients		
Inoculum size	1.446	·cells ml^{-1}	0.099			
Population growth rate	1.695	h^{-1}	0.035	-0.991		
No-effect concentration	15.61	ug l^{-1}	1.160	0.106	-0.161	
Tolerance concentration	176.6	ug l^{-1}	10.834	-0.570	0.559	-0.489
Mean deviation	1.13	·cells ml^{-1}				

Graphical test of model predictions against data





Profile likelihood for NEC estimate



ECx values

hour	EC0	ASD	EC50	ASD
1	15.6	1.16	139	5.75
2	15.6	1.16	61	2.03

Comments

The model for effects on the growth rate fits quite acceptably, but those for effects on adaptation and hazard fitted slightly better with similar NEC values (see table). The effects on growth have been selected here to improve the comparability with the concentration-response method. The 99% confidence intervals for the NEC values in μg/l for the three models are:

Hazard	5.89	14.4
Adaptation	4.46	9.59
Growth	13.2	17.4

3 Bioassay on *Daphnia* reproduction

Data for the cumulative number of offspring per female as affected by an unkown compound. The data were weighted in the estimation of parameters by the number of surviving females (data not given here).

Time: day, Conc: milligram/liter, Resp: Number of offspring

	0.000	0.015	0.053	0.190	0.670	2.350	8.230
0	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
7	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
10	7.14286	8.28571	6.28571	7.28571	4.57143	3.28571	0.00000
12	9.42857	8.85714	9.14286	13.95240	14.71430	3.95238	0.00000
14	24.00000	26.28570	21.42860	24.11900	21.28570	19.45240	0.00000
17	52.00000	53.85710	42.42860	47.45240	44.85710	42.28570	0.00000
19	84.57140	86.57140	64.14290	72.95240	60.42860	53.61900	0.00000
21	84.57140	86.57140	72.28570	76.78570	71.42860	63.45240	0.00000

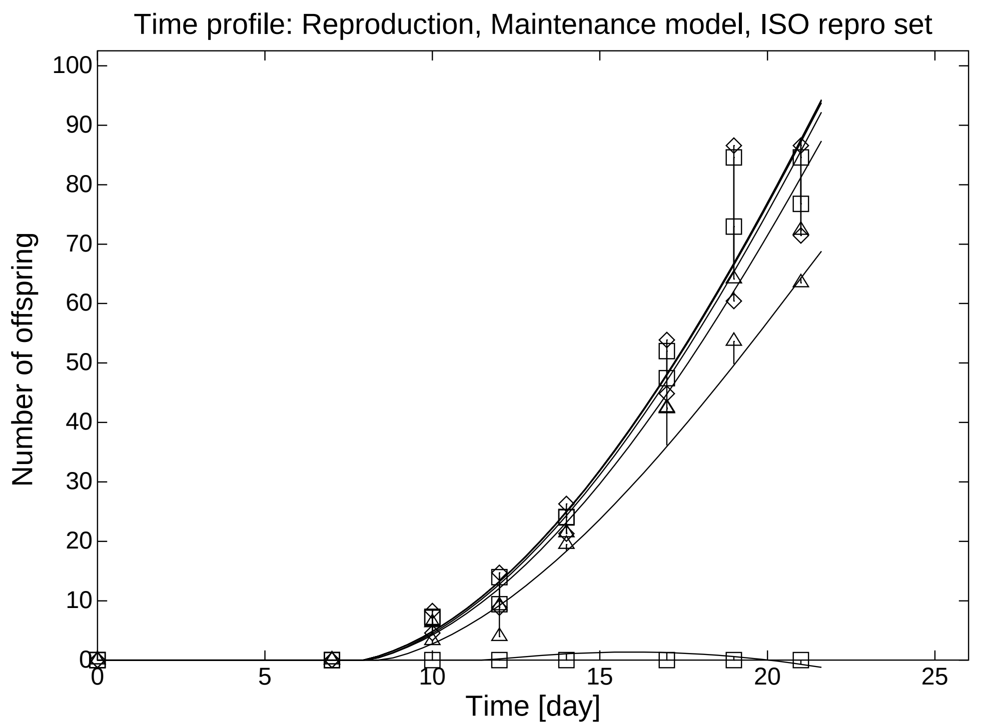
Parameter estimates and Asymptotic Standard Deviations (ASD)

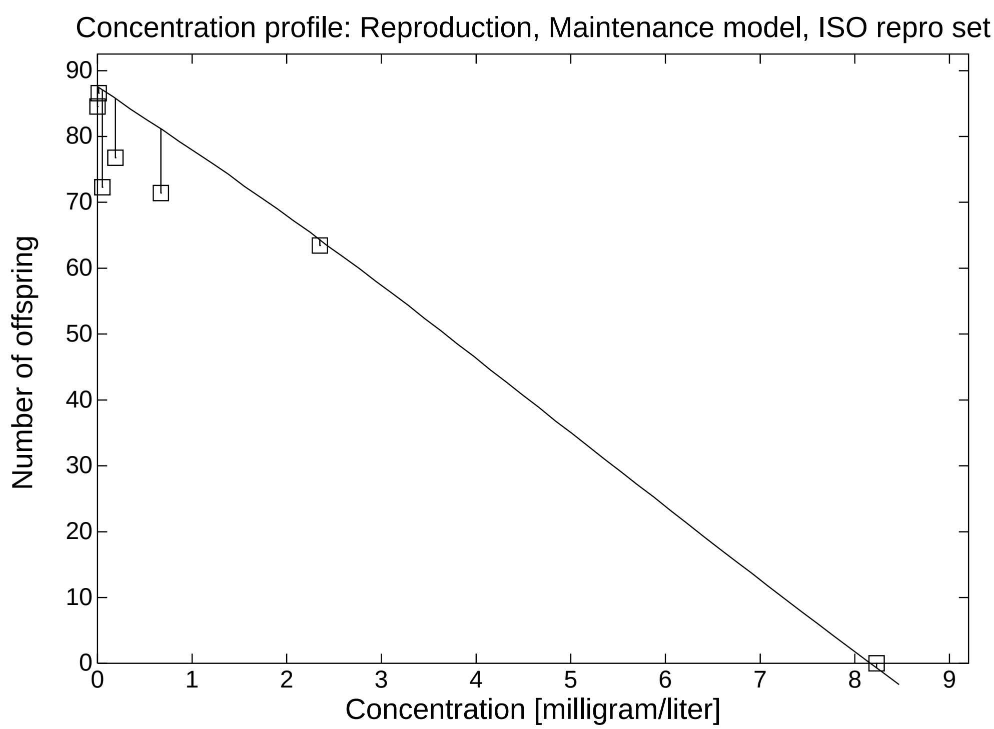
Reproduction, Maintenance model			ASD	Correlation coefficients		
No effect concentration	3.895e-009	mg l^{-1}	0.004			
Tolerance concentration	0.2265	mg l^{-1}	35.175	0.233		
Maximal reproduction rate	15.9	No d^{-1}	0.646	-0.872	-0.030	
Elimination rate	0.001268	d^{-1}	0.199	0.233	1.000	-0.031
Von Bertalanffy growth rate	0.1	d^{-1}				
Scaled length at birth	0.13					
Scaled length at puberty	0.61					
Energy investment ratio	1					
Mean deviation	5.207					

ECx values (derived from parameter values) in mg/l.

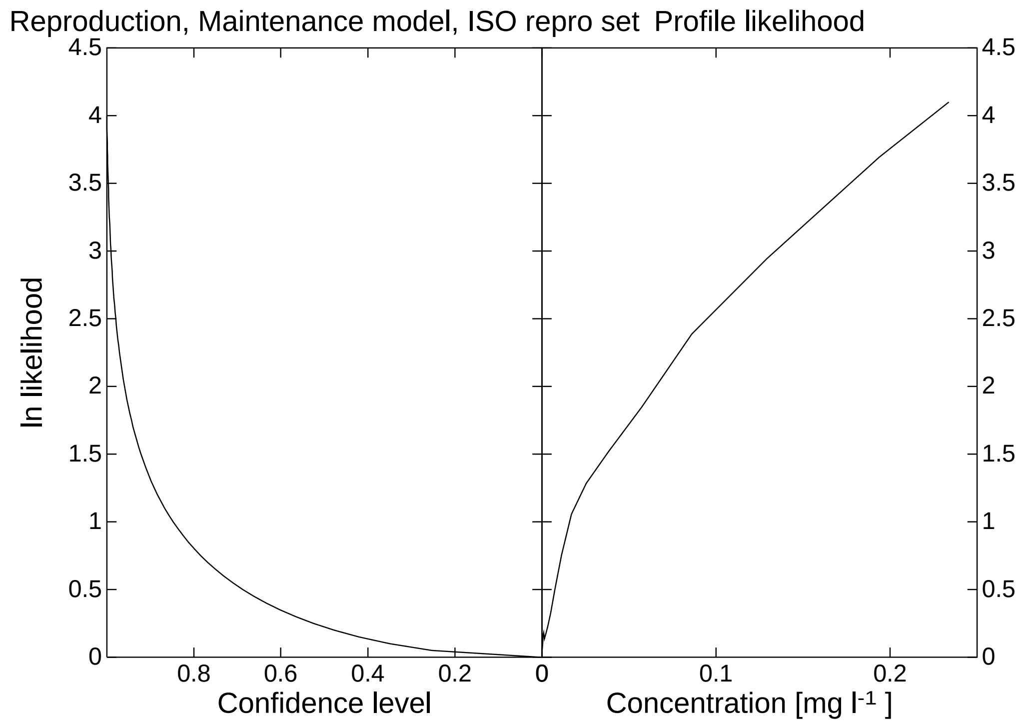
Day	EC0	ASD	EC50	ASD
21	1.10^{-5}	0.44	4.22	6.59

Graphical test of model predictions against data

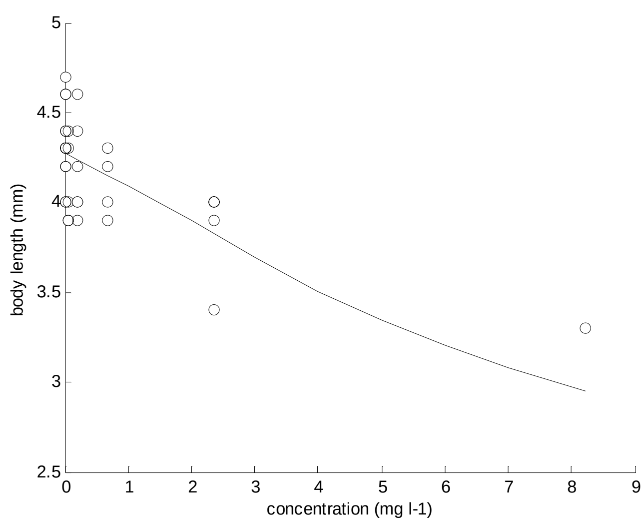

Time profile: Reproduction, Maintenance model, ISO repro set
Number of offspring
Time [day]
0
5
10
15
20
25
0
10
20
30
40
50
60
70
80
90
100


Concentration profile: Reproduction, Maintenance model, ISO repro set
Number of offspring
Concentration [milligram/liter]
0
1
2
3
4
5
6
7
8
9
0
10
20
30
40
50
60
70
80
90

Profile likelihood for NEC estimate



Body length at 21 days



Comments

This dataset is special in several respects. We have counts of offspring at relatively few points in time, not for each day as the guideline recommends. This reduces the effectiveness of the biology-based method; the fact that these data do not give detailed information about the start of the reproduction is especially troublesome. Reproduction starts here later than expected on the basis of the default value of 0.42 for the scaled length at puberty. Therefore, this scaled length has been set at 0.61 to mimic this late start. The model for effects on maintenance appeared to fit the data best; an increase in maintenance costs reduces the ultimate length of the daphnids. This is confirmed by the length data, shown in the last plot; the fitted length at 21 d are calculated with DEBtool; the plotted curve involves the estimation of a single parameter: the ultimate length in the blank. All other parameters are already fixed by the reproduction data, and determine the toxicokinetics, including the dilution by growth, and the effects on growth during exposure. The good fit of the length data, while no effect parameters were estimated for these data, confirms the effects on growth as expected from the observed effects on reproduction. Direct effects on reproduction would not affect body size; direct effects on growth would affect the growth rate, but not the ultimate size. The data not give information about growth, but it is likely that growth almost ceased before 21 d for daphnids, even in the stressed situation. The NEC was found to be not significantly different from zero, with a 95% confidence interval of (0, 0.082) mg/l.

4 Bioassay on fish growth

Data: Mean initial volumetric length of *Oncorhynchus mykiss* is 1.222 g $^{1/3}$

Time: d;	Conc: mg/l; Response: mean volumetric length, g $^{1/3}$						
	0	1	2.2	4.6	10	22	46
21	1.403	1.389	1.418	1.398	1.365	1.355	1.152

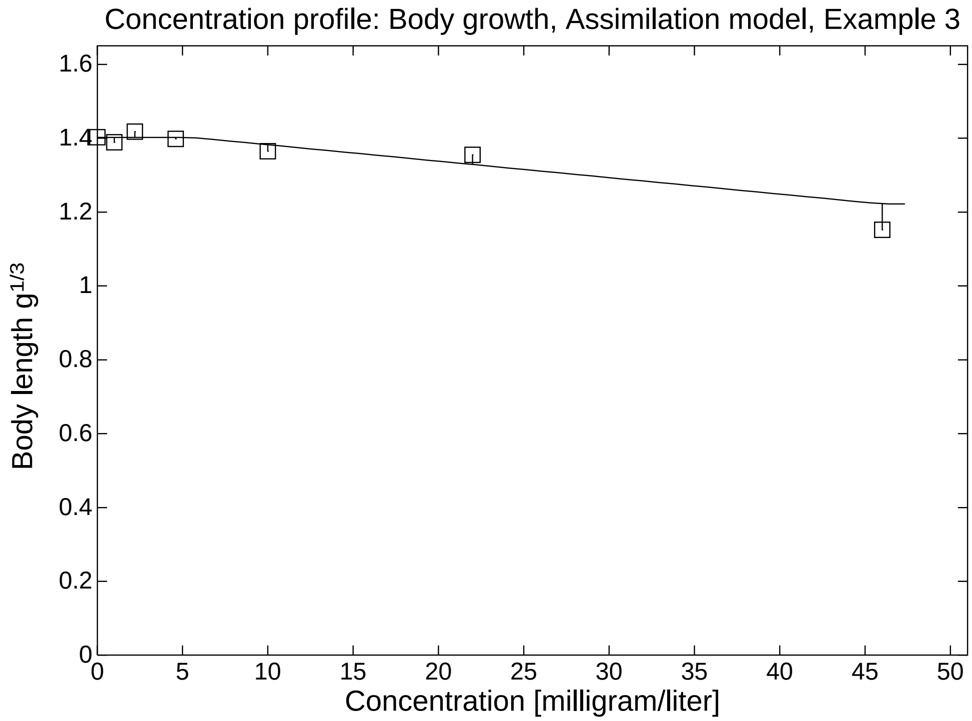
Parameter estimates and Asymptotic Standard Deviations (ASD)

Body growth, Assimilation model			ASD	Correlation coefficients	
No effect concentration	5.597	mg l^{-1}	7.399		
Blank ultimate length	15.84	$g^{1/3}$	1.221	-0.456	
Tolerance concentration	43.78	mg l^{-1}	11.884	-0.818	0.284
Elimination rate	Infinity	d^{-1}			
Initial length	1.222	$g^{1/3}$			
Von Bertalanffy growth rate	0.00059	d^{-1}			
Energy investment ratio	1				
Mean deviation	0.03006	$g^{1/3}$			

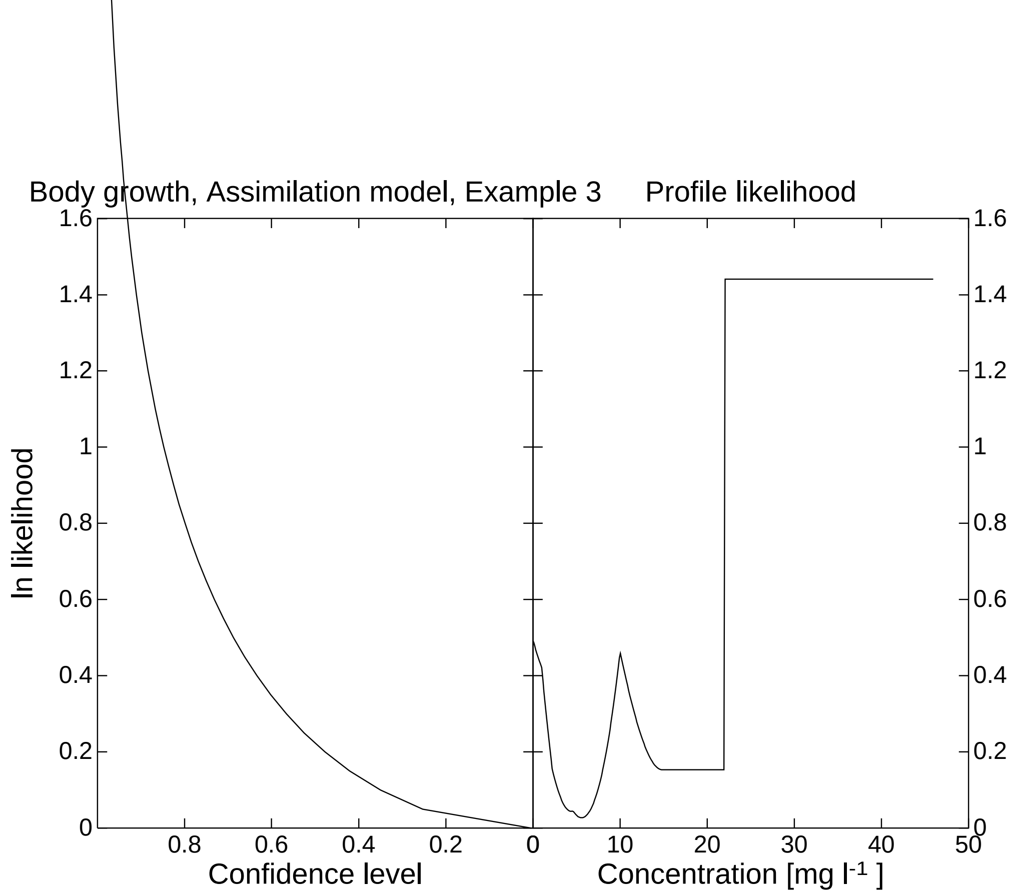
ECx values (derived from parameter values) in mg/l.

Day	EC0	ASD	EC10	ASD
21	5.6	7.4	37.4	5.71

Graphical test of model predictions against data



Profile likelihood for NEC estimate



Comments

The mean body size at the highest concentration was lower than the initial one; the increase in weight during 21 d in the blank was very small. The model for effects on assimilation fitted slightly better than for effects on maintenance or growth costs. The von Bertalanffy growth rate was fixed at 5.9 10^{-4} d (from Kooijman, 2000), and the initial size at 1.222 $g^{1/3}$ (measured mean value). The EC50 is on body length, which is not meaningful in this case since 50% of the blank length is far below the initial length.