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REPORT OF THE FINAL RING TEST OF THE DAPHNIA MAGNA REPRODUCTION TEST

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

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Report of the Final Ring Test of the *Daphnia magna* Reproduction Test

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The Environmental Health and Safety Programme co-operates closely with other international organisations. This document was produced within the framework of the Inter-Organization Programme for the Sound Management of Chemicals (IOMC).

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The Inter-Organization Programme for the Sound Management of Chemicals (IOMC) was established in 1995 by UNEP, ILO, FAO, WHO, UNIDO and the OECD (the Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

Foreword

This document is the report of the Ring Test of the February 1994 OECD draft Guideline 202, part II on *Daphnia magna* Reproduction Test, which was conducted in 1994.

It also includes the report of the OECD Workshop on the Final Ring Test of the *Daphnia magna* Reproduction Test, which was held at Sheffield University on 27-28 March 1995 (Appendix E).

The Joint Meeting of the Chemicals Group and the Management Committee of the Special Programme on the Control of Chemicals recommended that this document be derestricted. It is being published on the responsibility of the Secretary-General of the OECD.

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Summary

This report presents the results from the 1994 ring test of a draft of the Organisation for Economic Co-operation and Development's 21-day *Daphnia magna* reproduction test. The aim was to subject the latest revision of OECD Guideline 202, Part II *Daphnia* sp. Reproduction Test, dated February 1994, to international ring testing. The results are compared with those from a previous round of testing in 1985, when the Guideline was less defined, and are also used to investigate variability within and between laboratories. Three substances were used for the 1994 ring test: 3,4-dichloroaniline (DCA), cadmium chloride and phenol.

Forty-eight laboratories in 16 OECD Member countries and the Czech Republic[1] participated. Adherence to the draft Guideline was good, with most laboratories using clone A *Daphnia*, a fully defined medium and the requisite diet, although many appeared not to have supplied the food ration on the basis of organic carbon as recommended. Most laboratories were able to meet the criteria for water quality and for control animal performance stated in the draft Guideline. Unlike the 1985 ring test, no screening and selection of the data according to compliance with the criteria were made. Therefore all the data, with the few exceptions noted in Section 4.1, were included in the statistical analysis.

The results, analysed using the total numbers of juveniles produced, show a clear improvement over those from the 1985 ring test. With DCA the bulk of the data (90%) show that the effect concentrations were within a factor of 8, and that around 50% were within a factor of 2.

The results with cadmium chloride could be divided into two types of response: (1) those where effects were observed, and (2) those which failed to find an effect. This was due to the inclusion or absence of EDTA in the media, the toxicity of cadmium being reduced by the chelating action of this substance. When effect concentrations were identified, 62% of the NOECs and 45% of the EC_{50}s were within a factor of 8. This test substance produced more inter-laboratory variability than did the other two.

The results with phenol, a difficult substance to test due to its biodegradation, show good agreement, with all of the EC_{50}s being within a factor of 10.

Several response variables were examined with the intention of compensating for the loss of reproducing adults due to mortality during the test. Response variables based on juveniles per brood produced higher effect concentrations and larger standard errors than the other response variables examined due to the compensatory effect of fewer broods at higher concentrations, masking the fact that fewer juveniles were also produced at these treatments. For this and other reasons, these response variables are not recommended.

The response variable based on total juveniles per adult less those from adults which died was the most statistically robust variable, producing the most homogeneous variability together with the smallest significant difference, i.e. it produced the most powerful test. This response variable is therefore recommended.

[1] The Czech Republic became an OECD Member country on 21 December 1995.

Clone A was slightly more sensitive to DCA than the other clones used, but the response of all clones was similar for cadmium and phenol. Ration was found to have no influence on toxicity.

The ratio of variability for DCA within and between laboratories was found to be 2. This is an excellent result, comparing favourably with such data from other ring tests. It is indicative of the high degree of development that the draft Guideline has reached.

1. Introduction

Results from a European Union (EU) ring test conducted in 1985, based on a revision of Organisation for Economic Co-operation and Development (OECD) Guideline 202, Part II, *Daphnia* sp. reproduction test (OECD 1984), revealed an unacceptable level of variability in the results between laboratories. These results stimulated a series of investigations, initially within the European Union and later in OECD Member countries, designed to identify the sources of this variation with a view to developing an improved Guideline.

In 1994 a ring test took place, based on a draft Guideline (dated February 1994) which was developed following these investigations. This ring test involved 48 laboratories in 16 OECD countries and the Czech Republic, which became a Member country in 1995. The numbers of laboratories from each country are shown in Table 1.1. The names, addresses and phone and fax numbers of the laboratories are given in Appendix A.

Table 1.1 Number of laboratories taking part by country

Country	No. of laboratories
Germany	12
United Kingdom	7
France	6
United States	5
Netherlands	4
Japan	3
Australia	1
Canada	1
Czech Republic	1
Denmark	1
Finland	1
Italy	1
Norway	1
Portugal	1
Spain	1
Sweden	1
Switzerland	1

The ring test was organised by Professor Peter Calow of Sheffield University, UK, with funding from the Joint Research Centre of the Commission of the European Union and support from the OECD. Analysis of the ring test data was performed by Sheffield University and the Water Research Centre (WRc) (under contract from the Department of the Environment, UK).

1.1 Ring test objectives

The primary objective of the ring test was to evaluate the performance of the February 1994 draft OECD Guideline 202, Part II, by asking laboratories to perform 21-day *Daphnia magna* reproduction studies in accordance with the draft Guideline. Laboratories were requested to carry out reproduction studies using one, two or all three test substances (see section 2.1). Laboratories were also encouraged to perform a repeat study on one test substance (the second study not overlapping with the first) in order that the repeatability of results within laboratories could be assessed in addition to the reproducibility of results between laboratories.

Additional objectives were: (1) to identify how the reproductive output of the *Daphnia* should be expressed (e.g. total number of live offspring per parent over the period of the test, total number of live offspring per parent per reproductive day, etc.); and (2) to determine whether offspring produced by adults which die during the test should be included in the calculations, and if so, how.

1.2 Background

1.2.1 1985 European Union ring test

Until the adoption of OECD Test Guideline 210, fish early-life stage toxicity test, in July 1992, the OECD *Daphnia* sp. reproduction test was the only chronic toxicity test available within Europe for the testing of chemicals and pesticides for notification/registration purposes. However, it was recognised that data from tests performed according to OECD Test Guideline 202, Part II, *Daphnia* sp. reproduction test (adopted in 1984) might be very variable. A European Union ring test, based on OECD Guideline 202, Part II, was therefore initiated in 1985 (Cabridenc 1987) using 3,4-dichloroaniline (DCA) and sodium bromide.

Results from the 1985 ring test showed considerable variation between laboratories. It was thought that this variation was due either to permitted differences in the culture/testing regime (e.g. test species and/or clone, media and food) or to errors in the test protocol regarding feeding. Nevertheless, a revised EU protocol was developed (Draft 4, XI/681/86) following discussions between ring test laboratories and other experts, although it was recognised that further improvements could still be made.

1.2.2 European collaborative research programme

Progress towards improving the Guideline after the 1985 ring test was held back by two factors: (1) an inability to adequately define a method for the long-term culture of *Daphnia magna* which could be employed by everyone, and (2) a poor understanding of the sources of the observed variability in the results. In recognition of the need for improving the EU draft Guideline, a workshop involving interested parties from independent testing laboratories, industry/commerce, regulatory authorities and academia within the European Union was held at Sheffield University in December 1989. Out of this meeting emerged a general consensus that more fully defined culture systems were desirable and that variability in test results within and between laboratories could be reduced through improved standardisation, particularly with respect to the *Daphnia magna* genotype, food and culture medium used. With respect to

culture medium, delegates at the workshop felt the use of a fully defined artificial medium, with no additives or “magic” factors (e.g. seaweed extract, soil extract, "tetramin" etc.), would be desirable for both routine culture and for testing. However, it would have to sustain cultures at desired productivity levels over the long term. A voluntary research programme, designed to identify optimal conditions with regard to genotype, food and culture medium, was agreed. The work on effects of genotype was co-ordinated by Sheffield University, whilst that on effects of culture medium and diet was co-ordinated by WRc.

The results from this research programme were reviewed at a follow-up workshop, again at Sheffield University, in April 1991. Laboratories which had participated in the programme and representatives from all OECD countries were invited to attend. The overall conclusion was that clear recommendations could be made with regard to the standardisation of *Daphnia magna* clone, culture medium and food. Formal updating of the 1984 OECD Guideline 202, Part II, was therefore initiated.

The principal differences between the 1984 Guideline and the draft proposal were that in the latter:

(1) the species to be used was *Daphnia magna* (the 1984 version allows the use of any suitable *Daphnia* species);

(2) the test duration was 21 days (increased from 14 days);

(3) the number of animals to be used in each test and in the controls was reduced from 40, preferably held in four groups of ten, to ten animals held individually;

(4) more specific recommendations were made with regard to genetic clone (i.e Clone A [Baird *et al* 1991], the most commonly used clone in Europe), culture medium (i.e. a fully defined artificial medium [Elendt and Bias 1990]) and feeding conditions (i.e. algal diet provided in terms of mg carbon/*Daphnia*/day [Sims *et al* 1993]).

With regard to recommendations in (4) above, it was recognised that OECD-wide agreement on rigid standardisation would be difficult to achieve. It was therefore suggested that with respect to both genotype and medium, alternatives to those recommended could be used provided that they enabled the validity criterion for juvenile production to be met and resulted in similar susceptibility to a reference substance.

Although considerable progress had been made towards understanding the factors affecting the variability of reproduction in *Daphnia magna*, the 1991 workshop delegates agreed that further work was needed before a definitive Test Guideline could be produced. It was decided that this could be achieved in two stages:

Stage 1: study of the comparative performance of Clone A and the fully defined artificial media against alternatives in order to (a) investigate whether *Daphnia* could be cultured long-term in artificial media, and (b) assess the degree to which standardisation was necessary, i.e. a Pilot Ring Test;

Stage 2: full ring testing of a draft Guideline developed from the results of Stage 1.

In June 1991, a detailed report of the 1991 workshop (including outline proposals for the Pilot Ring Test) and the draft Test Guideline were circulated for comment to National Co-ordinators of the OECD Test Guidelines Programme, workshop participants, and nominated National Experts on aquatic toxicology.

No substantial proposals for amendments were received, with the exception of an impression of concern regarding the effect on the statistical power of changing the test design (see item 3 above). The plans for the Pilot Ring Test were therefore altered to include a comparison of the statistical power of the two test designs. At the same time, there was increasing interest within the scientific community in moving away from the identification of LOECs and NOECs (Lowest Observed Effect Concentration and No Observed Effect Concentration) using analysis of variance (ANOVA) to EC point estimation using regression analysis (i.e. the identification of the concentration causing a particular % effect concentration such as an EC_{50}). It was agreed that the Pilot Ring Test data would be analysed in these two ways.

1.2.3 Pilot Ring Test

The Pilot Ring Test began in April 1992, with a reporting deadline of the end of December 1992. It was co-ordinated by Professor Peter Calow, Sheffield University, with funding from the European Commission, and WRc under contract to the UK Department of the Environment. Thirty-six laboratories from 14 OECD Member countries took part.

There were three components to the Pilot Ring Test: Options 1, 2 and 3. The aim of **Option 1** was to determine the suitability of two fully defined artificial media, Elendt M4 and M7 (Elendt and Bias 1990), to support long-term cultures of *Daphnia magna* and provide (1) an adequate supply of offspring for use in toxicity tests and (2) an adequate quality of offspring, in terms of size and performance in acute tests. Participants were asked to use M4 and/or M7 in their routine culture regimes and to compare performance with their own medium over as long a period of time as possible within the constraints of the timetable of the pilot study. In addition, participants were asked to perform 48h acute toxicity tests on <24 hour old *Daphnia* using DCA at least once during the culture period in order to investigate possible differences in susceptibility of animals produced under different culture regimes.

The aim of **Option 2** was to assess the comparative performance of Clone A (i.e. the recommended clone) in different media and for different test designs (i.e. individually held versus group-held animals) during 21-day reproduction tests. For each regime, laboratories were asked to carry out a 21-day reproduction test using two DCA concentrations, 10 and 25 µg/l, and one control. The selected DCA concentrations were expected to give moderate and high effects on reproduction, respectively. Some laboratories reported results from studies using more than two DCA concentrations.

The aim of **Option 3** was to assess the comparative performance of Clone A and other clones in the two test designs. Laboratories were asked to perform 21-day reproduction tests as described for Option 2.

Throughout Options 2 and 3, laboratories were asked to provide a standard, fixed food ration, in terms of mg carbon/*Daphnia*/day. This was to be algal cells from one of three species (*Chlorella vulgaris, Raphidocellis subcapitata* or *Scenedesmus subspicatus*). Flexibility in ration was not allowed, as feeding was thought to be a key parameter with respect to culture performance.

The effects of clone, medium and test design were analysed using analysis of variance (ANOVA) at the University of Sheffield. The statistical power of the two test designs and the use of regression techniques for data analysis were investigated by WRc under contract to the UK Department of the Environment.

A second workshop to discuss the results, hosted by the UK Department of the Environment and chaired by Professor Calow, was held at Sheffield University on 20-21 March 1993. Following the review and discussion of the results from the Pilot Ring Test, the workshop participants agreed that *Daphnia magna* could be cultured long-term in artificial media and that standardisation did not appear to be as important as had been previously thought. The following general conclusions were made:

(1) Most laboratories were able to achieve satisfactory performance in routine culture using all culture regimes. With few exceptions, all laboratories reported an ability to keep cultures of clone A (and others) in M4 or M7 medium in a satisfactory state over relatively long periods. Nevertheless, some laboratories expressed a preference for retaining their own systems.

(2) Notwithstanding the fact that only two DCA concentrations were used in the reproduction tests, it was very encouraging to find that most laboratories had been able to fulfil both physico-chemical and biological validity criteria, and that LOECs/EC_{50}s fell in a narrow range.

(3) There were no consistent clone or media effects with DCA.

(4) There was little difference between the two test designs in terms of either consistency or statistical power, particularly for detecting a less than 25% reduction in reproduction relative to the control (Sims and Van Dijk in literature). The new test design, involving individually held animals, was generally preferred in terms of ease of management of the test and the biological information that could be derived. This system, however, was considered impractical for use in flow-through tests, for which there was a perception of increasing demand, due to the large numbers of test vessels involved.

(5) Problems associated with statistical analysis of ecotoxicity data using both ANOVA and regression were recognised. For example, the LOEC, derived by ANOVA, is dependent on the test concentrations used, i.e. if two tests are conducted under identical conditions but with different test concentrations, they will yield different LOECs. Determination of a LOEC is also highly dependent upon variability in regard to the mean response value. In addition, no statement of precision (e.g. 95% confidence limits) can be obtained for a LOEC. On the other hand, the regression approach requires selection of an appropriate mathematical model. A decision is also required over what level of “effect”, in this case a reduction in reproduction, to consider as either (a) ecologically significant or (b) sufficiently

robust to form an appropriate basis for decision-making (e.g. classification of a product). There was, however, a recognition that the regression approach might be more appropriate, and delegates looked forward to the review of test design and data analysis that was being prepared for OECD in relation to all aquatic toxicity tests (Pack 1993).

(6) It was felt that a full-scale ring test of the revised draft Guideline (modified in light of results from the Pilot Ring Test, workshop discussions and subsequent comments) was needed before final agreement on a method could be obtained.

2. Ring test methods

2.1 Test substances

Three test substances were used in the Final Ring Test, namely 3,4-dichloroaniline (DCA), cadmium chloride and phenol. These chemicals were chosen because (1) they have well-documented effects on *Daphnia* reproduction, (2) they have different modes of action, (3) stock solutions could be prepared without the use of solubilising agents, and (4) they would be relatively easy and inexpensive to analyse at the concentrations to be used. In addition, DCA was chosen to provide a link with the 1985 EU ring test and the 1992 Pilot Ring Test. Cadmium chloride was chosen to investigate the suitability of M4 and M7 media, which contain a known chelating agent, for testing metals and metal compounds. Phenol was chosen to assess the performance of the draft Guideline when testing a "difficult" substance because it is readily biodegradable in the test system.

2.2 Instructions to participants

Participants were provided with the revised (February 1994) draft of Guideline 202, Part II for use in the ring test. They were also provided with details on the test substances including suppliers, purity, concentrations to be used, and methods for chemical analysis. This documentation is included in Appendix B.

The choice of which substance(s) to use was left to the participants, although the desirability of their conducting repeat tests rather than testing multiple substances was stressed. Repeat tests would be needed to assess within- and between-laboratory variability.

2.3 Spreadsheet

Participants were provided with a 3.5-inch disc containing a spreadsheet for data recording. Excel software on Microsoft Windows was required. As some participants would not have access to this software, paper data sheets were also supplied to standardise data recording. More detail is given in Appendix C.

3. Reported test conditions

This section describes the experimental and environmental conditions reported by the laboratories.

3.1 Clone

The genetic clone is known to influence the sensitivity of *Daphnia magna* to toxicants (Baird *et al* 1991). In order to assess the contribution of clones to the variability of the ring test results, data were requested from the laboratories regarding the clones used.

In total, seven genetically typed clones of *Daphnia magna* were used (Table 3.1), with the majority of laboratories using the European clone, clone A. The data were analysed statistically to examine whether the clone used influenced the results obtained. The results of this analysis are reported in Section 4.5.

Table 3.1: Genetic clones of *Daphnia magna* used by participating laboratories for the three test substances

Clone	No. of laboratories using each clone		
	DCA	Cadmium	Phenol
A	23	10	6
own (unknown)	7	6	2
A and own *	2	0	1
4	1	2	1
2	3	1	0
DM94-8-17.F	0	1	0
DM94-7-22.S	1	0	0
EF	0	1	0
MJ-2	1	1	0
Total	38	22	10

* *These clones were used individually in different experiments by the same laboratory.*

3.2 Medium

3.2.1 Pre-test culture medium

Fourteen laboratories (29%) cultured their *Daphnia* in a different medium to that which they used in the ring test. Of these, only six (43%) reported that they allowed a period of acclimation to the new test medium before starting the ring test. Acclimation periods used varied from 24 hours to three weeks. Eight (17%) of the participants used an organic additive in their pre-test culture media.

3.2.2 Ring test medium

The draft Guideline recommended that a fully defined culture medium, such as Elendt M4 or M7, be used since this avoids the use of undefined additives (e.g. seaweed extract, soil extract, etc.) and improves standardisation between laboratories (Elendt 1990). However, other media could be used during the ring test provided that a full description, including the nature of any additives used, was reported. The types of media used during the ring test are shown in Table 3.2.

Table 3.2: Types of media used for each test substance

Medium	No. of laboratories using each medium		
	DCA	Cadmium	Phenol
Elendt M4	18	6	2
Elendt M7	8	6	4
Elendt M4 and M7	1	2	1
Elendt M4 and own	1	1	1
own	10	7	2
Total	38	22	10

Five laboratories (10%) used media additives during the ring test. The additives used were soil extract (laboratory code L15), seaweed extract (L41), Frippak Booster (L43) and the yeast-based additive YTC (L2 and L45).

<u>Comment</u>: Experience from the Pilot Ring Test showed the desirability of allowing a period of acclimation when changing *Daphnia* cultures from one medium to another. This was highlighted in the documentation supplied for the ring test. The recommended period of acclimation is one generation, i.e. normally about three weeks. However, experience has shown that longer periods may be required.

3.3 Diet and ration

It was recognised from previous work (Groeger *et al* 1991, Naylor *et al* 1992a), that both quantity and quality of food are important for daphnids. The ration provided was also thought to be the most important parameter, with respect to culture performance, from investigations leading to this Final Ring Test (Section 1.2). A diet consisting of one of the following green algae was recommended in the draft Guideline: *Chlorella* sp., *Raphidocellis subcapitata* and *Scenedesmus subspicatus*. Table 3.3 shows the species used.

Table 3.3: Algal species used for each test substance

Algal species	No. of laboratories using each alga		
	DCA	Cadmium	Phenol
Chlorella minutissima	1	1	0
Chlorella vulgaris	8	3	3
Chlorella spp.	3	3	1
Raphidocellis subcapitata	10	8	4
Raphidocellis subcapitata and Ankistrodesmus falcatus	1	1	0
Scenedesmus acutus	1	1	0
Scenedesmus spp.	0	1	0
Scenedesmus subspicatus	12	4	2
not stated	2	0	0
Total	38	22	10

Total organic carbon (TOC) was chosen as a measure of food ration (Naylor *et al* 1992b, Sims 1993). The optimum ration for good reproductive output had been predetermined during earlier phases of this work to be 0.1 to 0.2 mg carbon/daphnid/day (Sims *et al* 1993. The vast majority of laboratories which were successful in analysing carbon reported a daily ration within this range (Table 3.4).

Table 3.4: Rations used for each test substance

Ration (mg C/Daphnid/day)	No. of laboratories using different rations		
	DCA	Cadmium	Phenol
0.1-0.2	19	12	6
> 0.2	1	1	0
unknown	18	9	4
Total	38	22	10

Comment: **Many laboratories did not, or were unable to, provide results for carbon analyses with their test reports. Of those testing DCA, 47% did not report TOC. For cadmium this was 41%, while for phenol it was 40%. As the fecundity of *Daphnia* is directly related to energy input to the system, it is critical that food supplied to the reproducing adults is controlled on the basis of its energy content. In order to achieve this, cell number must be replaced by some measure of energy content as a basis for provisioning the *Daphnia*. Organic carbon provides such a measure, and should be adopted for this purpose.**

3.4 Strategies for neonate removal

The draft Guideline recommended that, for each parent animal, the offspring produced should be removed and counted daily from the appearance of the first brood. The majority of laboratories did in fact do this, as shown in Table 3.5.

Table 3.5: Strategies for neonate removal from test vessels over the 21-day test period for each test substance

Frequency of neonate removal	No. of laboratories		
	DCA	Cadmium	Phenol
daily	28	17	8
weekdays only (5 x weekly)	4	3	0
medium renewals (3 x weekly/ every 48 hours)	6	2	2
Total	38	22	10

Two of the response variables used for analysing the effects of a chemical on the reproductive output of *Daphnia* involved calculating the number of neonates produced per brood (Section 4.3). The number of broods per adult therefore had to be determined. This could only be done accurately if the neonates had been observed on a daily basis, as was the case with the majority of data sets. For laboratories where neonates were counted three times per week, it was only possible to estimate the number of broods. Consequently there was a small chance of double counting, leading to an overestimation of brood numbers in some vessels. However, it was still possible to observe whether the number of broods declined at higher concentrations. These data sets were therefore not excluded from the statistical analysis.

3.5 Presence of males

It is not possible to sex juvenile *Daphnia* accurately when they are the age recommended for initiating a juvenile production test, i.e. less than 24 hours old. This becomes a realistic option only when they are approaching adulthood. Consequently, separation of male animals from the intended all-female parental generation at the outset of a juvenile production study is not possible and this may lead to the occasional inadvertent inclusion of males into the test group.

Data supplied by the laboratories showed that the presence of male *Daphnia* in the parental population at the outset of each experiment was not high (Table 3.6). However, some laboratories did experience problems in this area.

Table 3.6: Occurrence of males by test substance

	DCA	Cadmium	Phenol
No. of laboratories reporting males	6	4	2
No. of tests containing males	6	4	2
No. of males	24	15	3
% males	0.8	0.8	0.3

The presence of males in the parental population presents problems for data analysis. When analysing the results for this ring test, males were discounted from the assessment of effects on reproduction, i.e. one male in a treatment would be deducted from the total so that the mean reproductive output would be calculated as if only nine females were used in that treatment at the outset. However, had the male died, it would have been counted as a mortality and added to any female mortalities in that treatment. In fact, no males died during the ring test.

Comment: At present there is no validity/quality criterion regarding the number of males in a study. This should be addressed. The practice of establishing a few extra vessels for each treatment at the outset, with a view to replacing males with these if required or discarding them if not, may have some merit.

3.6 Test volumes

Information on the test volumes used by each participant in the ring test was provided by 41 participants (85%). Of these, 34 (83%) used 50 ml volumes, as recommended in the draft Guideline (though some expressed concern at this small volume), while five (12%) used 100 ml volumes. The remaining two used 80 ml volumes.

Comment: **It is recommended that the Guideline reflect these findings, i.e. test volumes should be 50 to 100 ml. However, larger volumes may sometimes be necessary to meet requirements of the analytical procedure used for determination of the test substance, although pooling of replicates for chemical analysis is possible.**

3.7 Water quality

The draft OECD Guideline recommended that temperature, concentration of dissolved oxygen, total hardness and pH values should be measured for fresh and old media at least once a week in the control and the highest test concentration.

The strategies used for water quality measurements and the number of laboratories adopting these are given in Table 3.7. All except two of the participating laboratories observed and recorded water quality as required throughout the test period. The laboratory adopting method 5 used the lowest rather than the highest concentration for measurement.

Table 3.7: Frequency of water quality measurements

Assessment strategy (> once a week in fresh and old medium)	No. of laboratories
1) all concentrations	21
2) control and highest concentration	23
3) control, lowest and highest concentration	1
4) control and two highest concentrations	1
5) control and lowest concentration	1

N.B: One laboratory (not shown) conducted water quality measurements once in the control medium.

3.7.1 pH

The draft Guideline recommended that the pH should not vary by more than 1.5 units throughout the 21-day period. No laboratory recorded a pH range which exceeded this recommendation in any of the cadmium or phenol tests. However, five (10%) of the 52 tests with DCA exceeded this range. Maximum and minimum values for each test substance are shown in Table 3.8.

Table 3.8: Ranges of pH for each test substance

	pH (pH unit)		
	DCA	**Cadmium**	**Phenol**
maximum*	9.1	9.2	8.7
minimum*	7.0	6.9	7.0
% of tests exceeding the 1.5 pH variation range	10	0	0

** values from different tests*

Comment: The reported pH values showed that only 5% of the total data sets submitted exceeded a range of 1.5 pH units, i.e. the current Guideline recommendation for this parameter. This suggests that the Guideline pH recommendation is both appropriate and attainable.

3.7.2 Dissolved oxygen

The draft Guideline stated that the concentration of dissolved oxygen should be above 3 mg/l (i.e. around 30% of the air saturation value [ASV] at 20°C), with no upper limit stipulated. Dissolved oxygen was reported as either mg/l or 2% ASV. For comparison, the data have been converted to % ASV (Table 3.9).

Table 3.9: Ranges of dissolved oxygen for each test substance

	Dissolved oxygen (% ASU)		
	DCA	**Cadmium**	**Phenol**
maximum*	141	132	108
minimum*	60	75	41

** values from different tests*

NB: One laboratory reported a single measurement of dissolved oxygen of 25% ASV from one of four tests they conducted.

Comment: As only one laboratory reported concentrations of dissolved oxygen below the current recommended minimum of 3 mg/l (i.e. around 30% ASV at 20°C), this value seems to be appropriate. However, it is worth considering whether an upper value for dissolved oxygen would be desirable.

3.7.3 Temperature

The recommended temperature range for the test was 18-22°C, with any one test not varying by more than 2°C within these limits. Variation outside the recommended range occurred with each test substance (Table 3.10), i.e. in eleven (19%) of the tests with DCA, seven (22%) of those with cadmium, and five (29%) of those with phenol.

Table 3.10: Ranges of temperature for each test substance

	Temperature (°C)		
	DCA	**Cadmium**	**Phenol**
maximum*	25.0	25.3	25.1
minimum*	16.8	16.8	18.2
no. of tests out of 18-22°C range	7 (13%)	6 (20%)	2 (13%)
no. of tests exceeding the ±2°C variation range	11 (19%)	7 (22%)	5 (29%)

** values from different tests*

It was clear from the data submitted that there was confusion in interpreting the draft Guideline as regards temperature measurements. Some were reported as the temperature of the test area (air temperature), and others as the temperature of the test medium. It was not clear to which of these the reported temperatures referred The temperature of the test medium should have been measured, although this was not made explicit in the Guideline.

In total, 20% of the data sets exceeded the current Guideline criterion for variations in temperature. This may indicate that this criterion is currently too stringent, or that the temperature control equipment used by participating laboratories was inadequate. A more likely explanation lies in the confusion over what the draft Guideline required to be measured, air or medium temperature, as outlined above.

The strong influence of temperature on the reproduction of *Daphnia* has been reported (Lewis *et al* 1991, Stuhlbacher *et al* 1993), so a relaxation in the recommended range would seem ill-advised. It would be better to strive to achieve the current criterion.

Comment: From this it is clear that there was some confusion regarding what the draft Guideline required in the way of temperature measurements. It was intended that temperatures of the test solutions be reported, although this was not made explicit in the draft Guideline. This will be rectified in the next version of the Guideline, which will be drafted in the light of these results.

3.7.4 Total hardness

The draft Guideline imposed no limits on the total hardness of the medium, except that it should be monitored for consistency. Any wide fluctuations may reveal an error in medium preparation and indicate that precautionary measures should be taken. In comparing all laboratories, the maximum reported total hardness was 308 mg/l as $CaCO_3$ and the minimum was 178 mg/l as $CaCO_3$. However, individual laboratories reported only marginal fluctuations.

Comment: No criteria for total hardness are needed in the Guideline if synthetic media are recommended. If other media are used, the total hardness should be comparable with that for Elendt media, i.e. around 250 mg/l as $CaCO_3$, although it is acknowledged that this value may be problematic for workers using natural waters of low hardness.

3.8 Validity criteria

In the 1985 ring test several test validity criteria were used to select and reject data. These were that (1) an average of 70 or more juveniles should be produced per adult in the control during the experiment, (2) mortality of control adults should not exceed 10% , and (3) the coefficient of variation for control fecundity should not exceed 20%.

In the draft Guideline used for this ring test, quality criteria were that (1) an average of 60 or more juveniles should be produced per surviving control adult, and (2) mortality of control parents should not exceed 20%. No requirement regarding the coefficient of variation for control fecundity was made.

Table 3.11 shows that the majority of data sets submitted passed these quality criteria.

Table 3.11: Tests which did not conform to control validity criteria

	No. of tests			
	Mean fecundity ≤ 60 juveniles	**Parental mortality ≥ 20%**	**Coefficient of variation ≥ 20%**	**Time to first juveniles ≥ 9 days**
DCA	12	1	17	5
cadmium	3	0	7	1
phenol	1	1	4	2

Note: The total number of tests performed (n) was:

- for DCA: n = 52
- for cadmium: n = 30
- for phenol: n = 16

In order to examine this further, the EC_{50}s for nominal concentrations of the three test substances were plotted against control fecundity, control mortality, and the coefficient of variation for control fecundity. If any of these validity criteria influenced the EC_{50} one would expect a trend in the plotted data such that, for example, as control, mortality increased the EC_{50} became greater. This would indicate a loss of sensitivity as control mortality increased.

Figures 3.1, 3.2 and 3.3 show the results for DCA, cadmium and phenol. The response variable used for these analyses was total juveniles from each parent. The figures show that the data are scattered with no apparent trends, indicating that the EC_{50}s were not influenced by control mortality, fecundity, or coefficient of variation.

It was decided to examine whether total mortalities throughout all test concentrations influenced the EC_{50}, with the aim of investigating whether a validity criterion limit should be set on this. Total mortality was plotted against EC_{50} for the three test substances separately (Figure 3.4). Once again, no relationship was found.

One further validity criterion has been applied to *Daphnia* reproduction tests in the past: that of the time taken for the control animals to produce their first brood. The time to first brood is influenced by temperature and ration, and usually the criterion has been set at nine days. Only eight data sets (8%) failed to meet this criterion (five with DCA, one with cadmium and two with phenol). If ration is supplied at 0.1-0.2 mg C/daphnd/day and the test temperature is maintained at around 20°C, the first brood can therefore be expected in the controls by day 9 (usually day 8).

The inadvertent inclusion of male daphnids in the parental generation at the outset of reproduction studies (Section 3.5) should be addressed, though at this stage no conclusion regarding this as a validity criterion is drawn.

Comment: **Most laboratories were able to comply with the control validity criteria established for the ring test, i.e. adult mortality did not exceed 20% in 98% of the experiments and mean fecundity was at least 60 juveniles per surviving adult. Most laboratories also achieved control performance criteria which have been used as validity criteria in the past (i.e. coefficient of variation for control fecundity and time to first brood). Analysis of the data showed that, within the limits set, there was no relationship between the validity criteria and the outcome of the test in terms of the EC_{50}.**

These findings suggest that the criteria for the performance of the control animals have been set at an appropriate level and that, providing laboratories follow the instructions in the Guideline, they should not have to repeat studies due to not meeting these validity criteria.

3.9 Analysis and maintenance of test substance concentrations

Annex 4 of the draft Guideline recommended a minimum frequency for the chemical analysis of freshly prepared test solutions of once a week in all test concentrations. The same solutions were to be analysed again at the time of renewal in order to assess the stability of the exposure concentrations. Nominal concentrations can be used for data analysis only if the

actual (measured) concentration values fall within 20% of the nominal test concentrations. It is noted that the draft Guideline offers no advice concerning the calculation of actuals from nominals.

Figure 3.5 shows nominal *vs* actual concentrations for each test substance, plotted on log scales. For all three test substances the relationship between log actual and log nominal is linear, with homogeneous variance throughout the ranges used, and the slope of the regression line is virtually 1.0. For DCA the intercept was -0.07, for cadmium 0.02 and for phenol -0.11. This means that the actual concentrations are proportional to the nominal concentrations, and the best estimate of the factor of proportionality is the antilog of the intercept. Thus:

DCA:	actual concentration	=	0.85 x nominal
cadmium:	actual concentration	=	1.05 x nominal
phenol:	actual concentration	=	0.78 x nominal

With respect to the maintenance of test concentrations, Table 3.12 indicates that initial concentrations, i.e. those of the fresh test solutions, the majority of tests were within 20% of the nominal concentrations for all three test substances (90% for DCA, 67% for cadmium, and 85% for phenol).

Table 3.12: Maintenance of exposure concentrations in tests as percentages of nominal and actual concentrations

	No. of tests (%)		
	DCA	**Cadmium**	**Phenol**
fresh medium			
tests in which concentrations were within 20% of nominal	90	67	85
old medium			
tests in which concentrations were within 20% of nominal	31	40	15
tests with total loss	4	0	54

Losses of DCA averaged 23%, with 4% of the data sets showing total loss of the test substance from one or more treatments over the periods between medium renewals.

Losses of cadmium averaged 5%. No data sets showed total loss of this test substance.

With phenol, the biodegradable test substance, the average loss between renewals was 36%, with 54% of the data sets showing total loss of the test substance from one or more treatments over the periods between medium renewals.

Some laboratories reported increases in the measured concentrations of the old solutions as compared to the same solutions when fresh. This occurred most frequently with cadmium (36% of the data sets), though it was seen with the other two test substances to a lesser extent.

A document produced by the UK Ecotoxicity Shadow Group (Stephenson 1992) with the intention of providing cost-effective guidance on chemical analysis recommends the following sampling regime for *Daphnia* chronic toxicity tests:

1) where concentrations are expected to remain within ±20% of nominal:

   analyse the highest and lowest concentration when freshly prepared and again at renewal; this should be conducted once each week for the duration of the test.

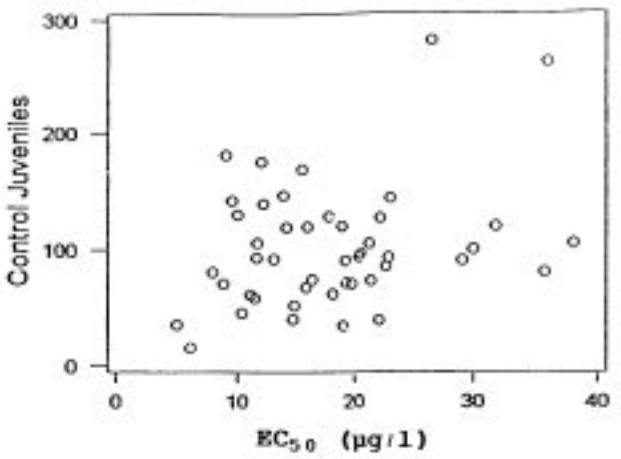
2) where concentrations are not expected to remain within ±20% of nominal:

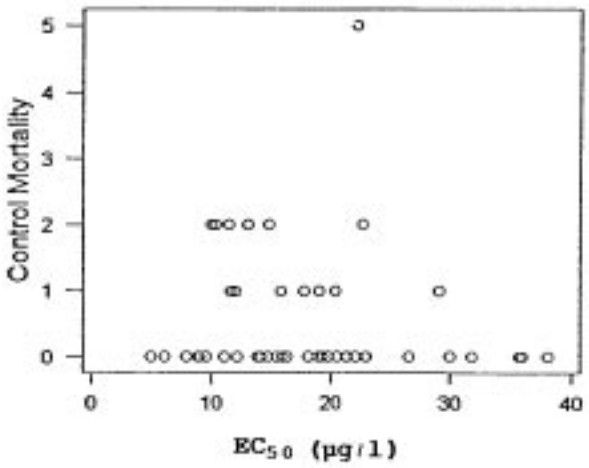
   analyse all concentrations or consider a flow-through test.

Similar conclusions were drawn in an advisory document for the UK Department of the Environment dealing with testing of "difficult" substances (Whitehouse and Mallett 1993). The results concerning the stability of these test substances reinforce the advice given in these documents, that before commencing a toxicity study the test substance should be assessed for stability between planned medium renewal periods. If, as in the case of phenol, the concentration falls by more than 20%, a shorter period between renewals should be investigated or a flow-through system should be considered.

Comment: **With all three test substances the relationship between initial actual and nominal concentrations shows there is consistency across treatments within tests. This conclusion supports the case that chemical analysis need not be performed for every treatment, since a good estimate of the ratio of actual to nominal concentrations can be obtained from analysing one or two treatments.**

The stability of the test substance should be known before undertaking these tests. If concentrations are likely to fall by more than 20% of the initial concentrations, consideration should be given to increasing the frequency of medium renewal or to the use of a flow-through system.





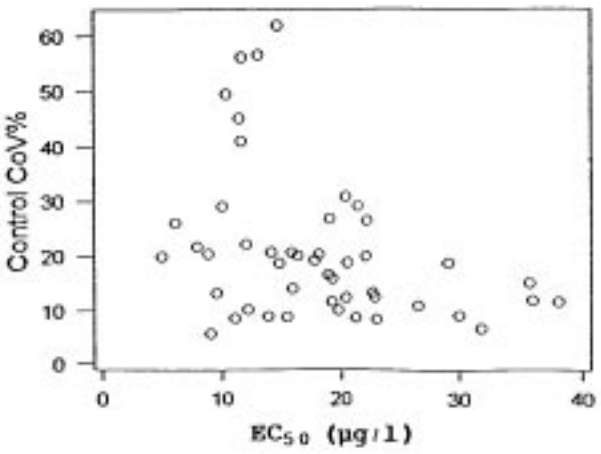
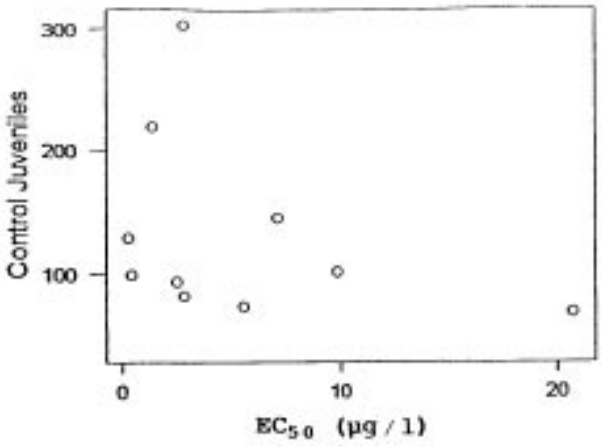
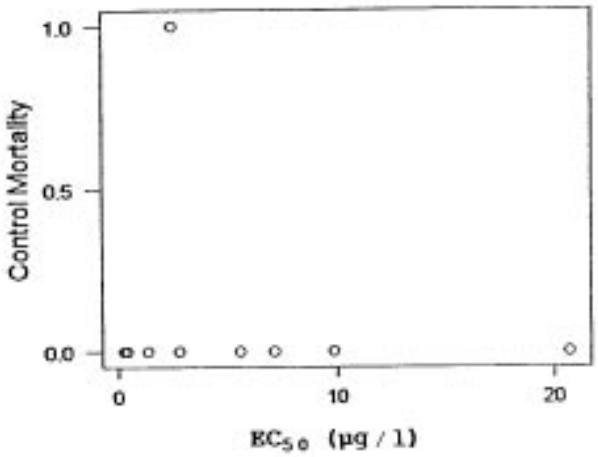


Figure 3.1 Effect of control fecundity, mortality and coefficient of variation on EC_{50} for 3,4 dichloroaniline





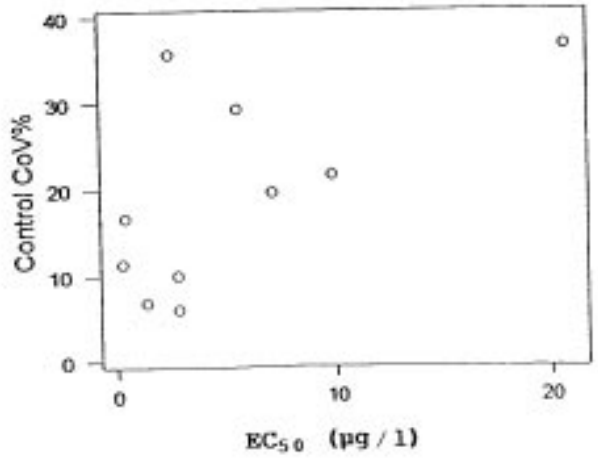
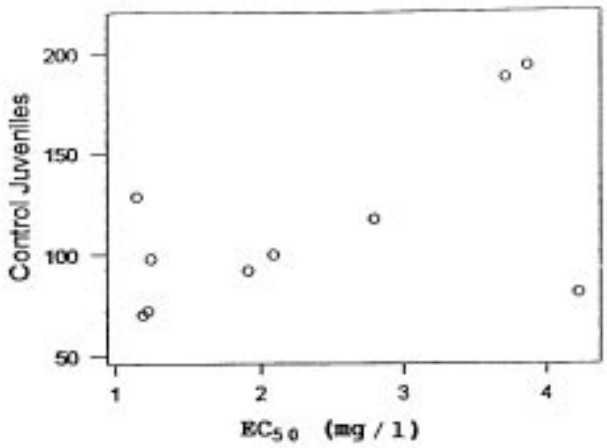
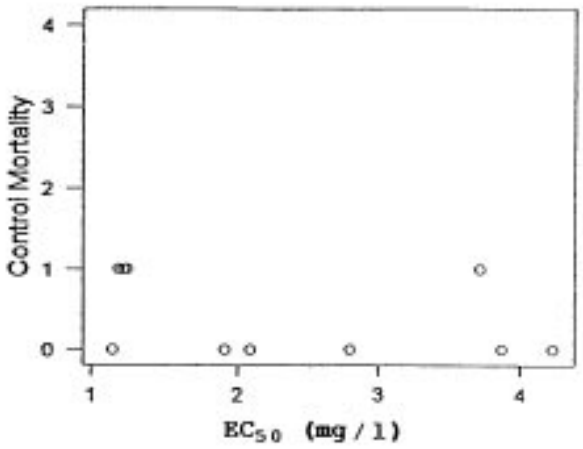


Figure 3.2 Effect of control fecundity, mortality and coefficient of variation on EC_{50} for cadmium chloride





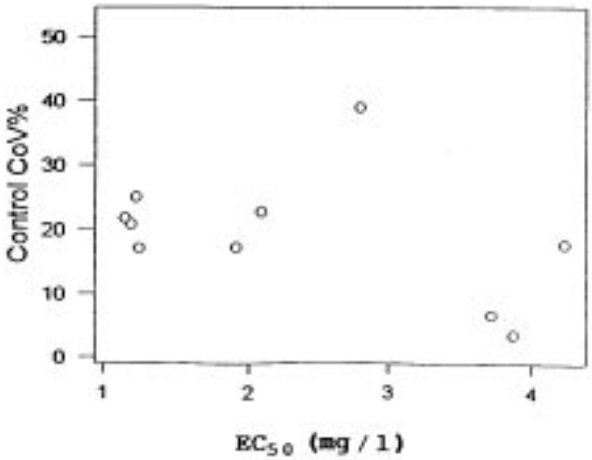
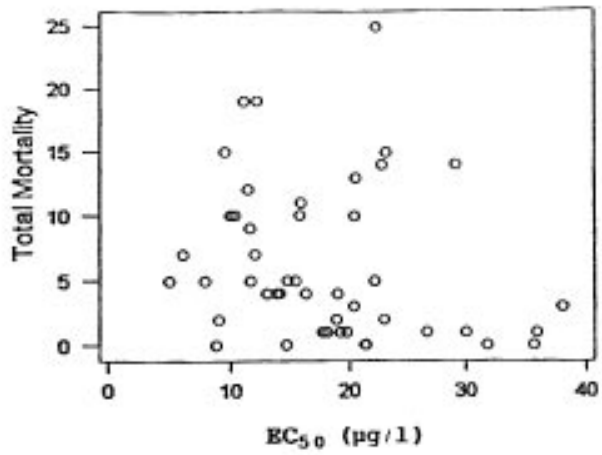
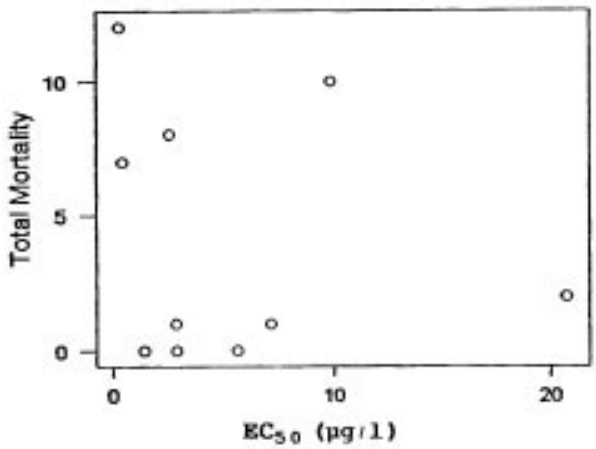


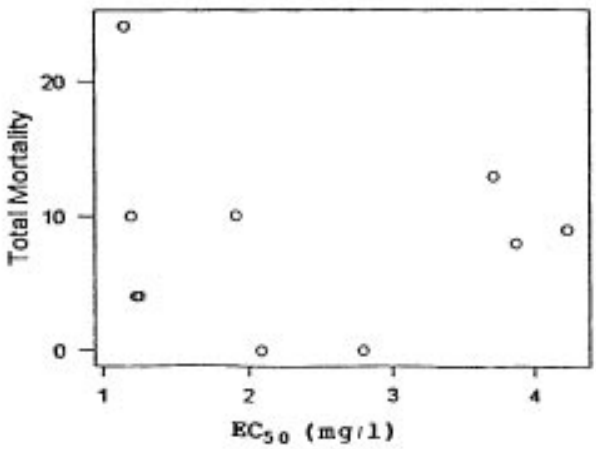
Figure 3.3 Effect of control fecundity, mortality and coefficient of variation on EC_{50} for phenol



DCA



Cadmium chloride



Phenol

Figure 3.4 Effect of total mortality on EC_{50} for the three test substances

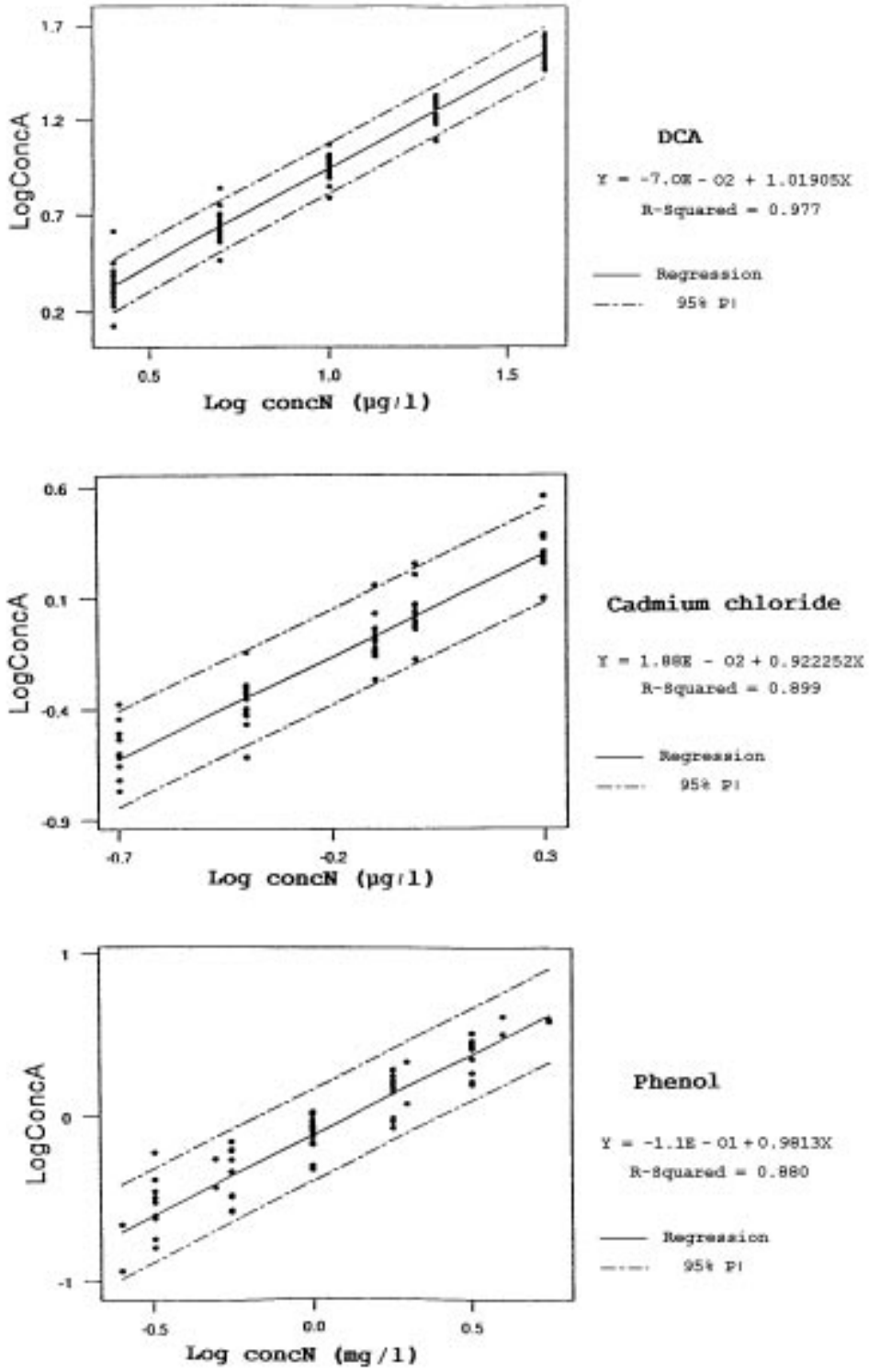


Figure 3.5 Regression plots of logged nominal concentrations (N) on logged actual concentrations (A) for the three test substances

4. Analysis of reproduction data from each parent

This section details the results of statistical analyses of the fecundity data generated during the Final Ring Test.

4.1 Statistical methods

The cut-off date for receipt of the ring test data was 12 September 1994. This was subsequently extended by one month. Only minimal selection of the data according to compliance with validity criteria was carried out. The data from two participants were excluded, one on the basis of exceptionally low fecundity coupled with culturing problems, the other due to insufficient data concerning test conditions being supplied. One data set arrived too late to be included in the statistical analysis, but all the remaining data sets were used. In order to preserve anonymity, each laboratory was assigned a code number and these are used throughout this report.

After completion of the ring test and the data submission stage, an information sheet was sent to each participant requesting details of pre-test culture conditions and any ring test specific data that were not included with the data submission. The information sheet also summarised the fecundity and brood numbers, by treatment, for each data set submitted. Participants were requested to check that the data were correct prior to statistical analysis.

For the purpose of statistical analysis the data were grouped by test substance. Each test substance group was then split into two sets: "nominal concentrations", which included all the data received, and "actual concentrations", which incorporated only those data sets with chemical analysis at every test concentration.

4.1.1 Definition of the NOEC

The NOEC is the “No Observed Effect Concentration”. There is some ambiguity about the NOEC since it can be defined in two different ways:

(1) The NOEC is the test concentration immediately below the lowest significant concentration.

(2) The NOEC is the highest test concentration that is not a significant concentration.

In both cases, a significant concentration is interpreted to mean a concentration exhibiting a statistically significant reduction in fecundity (at $p<0.05$) when compared with the control.

Often the two definitions will lead to the same result because, if one concentration is significant, higher concentrations will generally also be significant. However, it is not unknown for a significant concentration to be followed by a higher concentration that is not significant. If this happens, the two definitions yield different results.

As far as the ring test report is concerned, the NOEC was calculated according to definition (1).

<u>Comment</u>: If all concentrations greater than the NOEC, as defined by (1) above, have a significant effect when compared with the control, the two sets of definitions are equivalent. However, this is not always the case. These points need to be understood.

4.1.2 Calculation of the NOEC

For this ring test the NOEC was calculated according to definition (1) above, using the data grouped as described in Section 4.1. Weighted analysis of variance (ANOVA) was used, followed by a one-sided Dunnett's test using a 5% significance level to obtain the LOEC. The NOEC was taken to be the test concentration immediately below the LOEC. The weighting was necessary in the ANOVA because the variance of the results was not homogeneous, being much smaller where the number of juveniles produced was low. The weights should be proportional to the reciprocal of the variance, but given the fact that, for Poisson distributed data, the mean and variance are equal, the weights used were the reciprocals of the mean number of juveniles observed at each test concentration. For Dunnett's test, the least significant difference (LSD) was based on the standard error for the difference between the control data and the lower test concentration.

4.1.3 Estimation of the 21-day EC_{50}, EC_{20} and EC_{10}

The EC_{50} is the concentration giving rise to a 50% reduction in fecundity compared with the control. Similarly, the EC_{20} and the EC_{10} are the concentrations associated with reductions in fecundity of 20% and 10% respectively. The notation EC_q% will be used to denote a general term, namely the concentration associated with a reduction of q%.

Estimation of an EC_q requires:

(1) the assumption of a model for the relationship between fecundity and concentration - this model will have several unknown parameters;

(2) a method for fitting the data to the model to derive estimates of the parameters of the model.

The model used in analysing the ring test data was a logistic curve. Its formula is given by:

$$Y = \frac{c}{1 + \left(x/x_o\right)^b} \qquad (1)$$

where:

Y = the mean fecundity at concentration X,
c = the mean fecundity at X = 0,
x_o = the EC_{50},
b = a slope parameter.

Note: b is called the slope parameter because, after defining the height of the curve by c and the location of the EC_{50} on the curve by x_0, b is the parameter that determines the steepness of the curve. In fact, the slope of the curve at $x = x_0$ is $-bc/2x_0$.

Although the model is presented in terms of the EC_{50}, it is possible to calculate the EC_q for any q, since substituting EC_q for X and (1-q)c for Y in formula (1) leads to:

$$ECq = x_o \left(\frac{9}{1-9}\right)^{1/b} \qquad (2)$$

Thus:

$EC_{50} = x_0$
$EC_{20} = x_0 (1/4)^{1/b}$
$EC_{10} = x_0 (1/9)^{1/b}$

For example, if $x_0 = 10.0$ and b = 2.0:

$EC_{50} = 10.0$
$EC_{20} = 10.0(1/2) = 5.0$
$EC_{10} = 10.0(1/3) = 3.33$

Note that these values are independent of c. Knowledge of the EC_{50} and the slope parameter are sufficient to enable any EC_q to be calculated.

Because this model is non-linear in parameter b, the curve cannot be fitted by standard linear least-squares techniques. Instead, non-linear iterative methods have to be used. For the ring test, the FITNONLINEAR directive of Genstat (Payne *et al* 1993) was used to perform the curve fitting. After fitting this model, it is possible to estimate the EC_q for any value of q, using formula (2). The standard errors of the EC_q can then be obtained using the approximate method described in Chapter 10 of Kendall and Stuart (1977).

The 21-day EC_{20}s and EC_{10}s provide a valuable contribution to the debate over ANOVA *vs* regression approaches to analysing chronic toxicity data (Section 5). This has been the topic of much recent discussion among ecotoxicologists, regulators and industry within the European Union and the OECD.

4.2. Preliminary analysis using total juveniles from each parent

Initially each data set was analysed statistically using total juveniles per parent produced over the 21-day test period as the response variable. This is the method recommended in OECD Guideline 202, Part II (1984) and was also the response variable used to analyse the 1985 ring test. The NOECs, EC_{50}s, EC_{20}s and EC_{10}s were identified for each test substance.

4.2.1 3,4-dichloroaniline

Thirty-eight laboratories conducted 52 tests with DCA. Of these, 28 data sets had the required analytical data on each exposure concentration used. The results of the NOEC and EC determinations are summarised in Figure 4.1, and individual laboratory results are included in Appendix D. The result for L42 is not considered further, as the statistical programme extrapolated it far beyond the tested concentration range.

NOECs were determined on the basis of nominal exposure concentrations for 48 of the 52 data sets (92%). NOECs could not be determined for three tests (6%) due to significant effects being seen at all test concentrations, and for one test (2%) due to the absence of a significant treatment effect.

The EC_{50}s, EC_{20}s and EC_{10}s derived for nominal and actual DCA, concentrations show peaks of response in the 10 to 20 µg/l range and, as expected, a trend moving from high to lower concentrations as the percentage effect declines. This trend is most striking in the case of actual concentrations, though it is also evident in the data for nominal concentrations. Figure 4.2, a box-whisker plot, shows the median NOEC and EC data (represented by the circled bar in the box), the upper and lower quartiles (represented by the extremes of the box) and the range (represented by the ends of the whiskers). Points plotted as asterisks beyond the ends of the whiskers are potential outliers.

Figure 4.1 shows that, using nominal concentrations, approximately 50% of NOECs, EC_{50}s, EC_{20}s and EC_{10}s lay within a factor of 2, over 75% lay within a factor of 4, and over 90% lay within a factor of 8.

<u>Comment</u>: The results for DCA show very low variability in the derived NOECs, EC_{50}s, EC_{20}s and EC_{10}s. For each of these four summary parameters, approximately 50% of the data sets gave values within a factor of 2, more than 75% within a factor of 4, and over 90% within a factor of 8. The consistency of these results is encouraging. They represent data from 38 laboratories.

4.2.2 Cadmium

A total of 22 laboratories conducted 30 tests with cadmium chloride. Of these, 16 tests had the requisite analytical data. The results of the NOEC and EC determinations are summarised in Figure 4.3 and individual laboratory results are included in Appendix D. The

results for L27 (both tests) and L39 (second test) are not considered further, as non-standard concentration ranges were used.

The NOEC data fall into two groups: (1) those in which a significant effect was found, enabling a NOEC to be identified, and (2) those in which no significant effects were found. This is investigated further in Section 4.4.

The graphs of EC_{50}s, EC_{20}s and EC_{10}s show how the effect concentration moves from higher to lower concentrations as the percentage effect declines. This is evident with both nominal and actual concentrations, though it is clearer with the latter. Figure 4.4 shows median, upper and lower quartiles and the range of the four end points.

Figure 4.3 shows that, using nominal concentrations, 38% of NOECs lay within a factor of 2 and 62% within a factor of 8. The figures for the EC_{50}s and EC_{20}s were 27% and 45%, respectively, and for EC_{10}s, 27% and 55%.

Comment: **The NOEC data for cadmium showed two types of response: those finding an effect and those that did not. This is examined further in Section 4.4. Where effect concentrations were found, 38% of NOECs lay within a factor of 2 and 62% within a factor of 8. The figures for the EC_{50}s and EC_{20}s were 27% and 45%, respectively, and for the EC_{10}s 27% and 55%. There was more inter-laboratory variability with this test substance than with DCA. This degree of variability is not surprising given the small size of the data base and the influence of dilution water composition on the toxicity of this test substance (Section 4.4).**

4.2.3 Phenol

A total of ten laboratories conducted 16 tests with phenol. Fourteen tests had the requisite analytical data. The high proportion of data sets (88%) with full analysis of the test concentrations reflects the simplicity of the analytical method used (ultra-violet spectrophotometry). The results of the NOEC and EC determinations are summarised in Figure 4.5 and individual laboratory results are included in Appendix D.

NOECs showed no instances in which significant effects were found at all concentrations, whether expressed as nominal or actual exposure concentrations. With nominals, five experiments (31% of the total) failed to evoke a significant effect. The data show a broad spread in NOECs, with little evidence of a peak. This is not surprising with so few data points and a biodegradable test substance.

The data for EC_{50}s, EC_{20}s and EC_{10}s, based on both nominals and actuals, show the way in which the effect concentrations move from higher to lower concentrations as the percentage effect declines. Figure 4.6 shows the median, upper and lower quartiles and range of the four end points.

Figure 4.5 shows that, using nominal concentrations, 45% of the NOECs lay within a factor of 3.2 and 82% within a factor of 10. The figures for the EC_{50}s were 70% and 100%, respectively, for the EC_{20}s 50% and 90% and for the EC_{10}s 50% and 80%.

Comment: **Despite the limited number of data sets received, it is clear that inter-laboratory variability with phenol is low, as 82% of NOECs and all the EC_{50}s lay within a factor of 10. However, it is likely that the use of a flow-through dosing system for assessing the effects of phenol on *Daphnia* reproduction would have produced results lower than those found here and with less variability due to better maintenance of exposure concentrations.**

4.3 Analysis to compensate for adult mortality

4.3.1 The problem of adult mortality

One of the issues raised at the *Daphnia* workshops was the problem of adult mortality occurring during the test. Because the test is primarily a reproduction test, with the interest being in fecundity rather than in mortality, deaths among the parent animals are an undesirable complication.

Mortalities may arise by:

- toxic effects - mortality will tend to be greater at higher concentrations;
- accidental damage during manipulations at transfer to fresh medium - these mortalities are independent of treatment;
- other unidentified mechanisms which can be attributed to chance.

At present, the response variable for the *Daphnia* reproduction test is the total number of juveniles produced over 21 days by each parent. The premature death of a parent animal will generally result in the loss of some or all of the juveniles which that adult would have produced had it survived. This has consequences for the subsequent statistical analysis and may affect the apparent toxicity of the substance under test.

Parental mortality tends to produce outliers, particularly where few or no juveniles are produced. These outliers do not satisfy one of the requirements for model fitting, namely that there should be homogeneity of variance. This leads to serious doubts about the suitability of the fitted model.

If this problem is simply ignored and the statistical analysis performed regardless of mortality, the reduction in the response variable leads to a decrease in the sample mean and an increase in the sample variance. The decrease in the sample mean for the treatment may lead to a shift of the NOEC or the EC_{50} to higher or lower concentrations, depending on the treatments at which the mortalities occur. The increase in variability will tend to reduce the power of the analysis to detect real differences between treatments, thus tending to raise the NOEC. It will also lead to wider confidence limits around the EC_{50}.

If mortality is due to a toxic effect, there may be a case for investigating a combined reproduction and mortality model. But this is a complex matter not pursued further here.

4.3.2 Alternative response variables

Because of these problems, it was decided to determine whether some alternative measure of fecundity would be less sensitive to parental mortality than total juveniles over 21 days. If such a variable could be found, it might provide a better fit to the statistical models relating fecundity to treatment concentration, thus overcoming the problem of parental mortality.

Seven different response variables were investigated and compared. Each was based on the same definition of the experimental unit, namely a single vessel housing a single parent. The variables, identified by codes RV1 to RV7, are split into three groups:

Group A: Response variables based on the total number of juveniles

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RV1: total juveniles produced during the 21 days of the test - this is the standard response variable used in the past;

RV2: total juveniles produced during the 21 days of the test, excluding results from any parents that died before the end of the test - this reduces the number of replicates in the statistical analysis.

Compared with RV1, RV2 removes all outliers due to parental mortality.

Group B: Response variables based on juveniles per day

In this group, the total number of juveniles produced during the test is divided by the number of days that the parent survived. In this way, an allowance is made for mortality because the smaller number of juveniles produced where parents die before the end of the test is divided by a smaller number of days.

Three different threshold days are considered:

RV3: juveniles per day, counting days from the start of the test;

RV4: juveniles per day, counting days from an approximation to the “onset of gravidity”; here, day 1 is defined to be three days before the day of the first brood in any control vessel;

RV5: juveniles per day, counting days from the “onset of reproduction”; here, day 1 is the day of the first brood in any control vessel.

RV4 and RV5 provide for different ways of counting “reproductive” days.

Group C: Response variables based on juveniles per brood

In this group, the total number of juveniles produced during the test is divided by the number of broods produced by the parent before it died or before the end of the test. In this way, an adjustment is made for mortality because the smaller number of juveniles produced by

parents that die before the end of the test is divided by the smaller number of broods that they produce.

There are two different response variables in this group:

RV6: juveniles per brood, i.e. total juveniles divided by number of broods for each parent;

RV7: juveniles per brood, as in RV6, but excluding results from any parents that died before the end of the test.

4.3.3 Illustrative example

To help clarify these seven response variables, an illustrative example is given covering a single treatment of ten replicates (see Table 4.1). The vessels have been ranked in order of decreasing number of total juveniles to aid clarity (column 1). The observed data can be summarised thus:

- In vessels 1 to 5, all parents survived to 21 days and produced four broods.
- In vessel 6, the parent survived for 21 days but produced only three broods.
- In vessel 7, the parent survived for only 18 days and produced three broods, giving a total of 64 juveniles.
- In vessel 8, the parent survived for 14 days and produced two broods containing a total of 38 juveniles.
- In vessel 9, the parent survived for eight days and produced 15 juveniles in a single brood.
- In vessel 10, the parent survived to day 6 without producing any juveniles.

The first brood among the control vessels was observed on day 8.

Table 4.1: Example data set

Vessel No.	Days survived (D)	Broods (B)	Response variables (RV)						
			RV1	RV2	RV3	RV4	RV5	RV6	RV7
1	21	4	105.	105.	5.00	6.18	7.50	26.25	26.25
2	21	4	96.	96.	4.57	5.65	6.86	24.00	24.00
3	21	4	89.	89.	4.24	5.24	6.36	22.25	22.25
4	21	4	84.	84.	4.00	4.94	6.00	21.00	21.00
5	21	4	83.	83.	3.95	4.88	5.93	20.75	20.75
6	21	3	77.	77.	3.67	4.53	5.50	25.67	25.67
7	18	3	64.	*	3.56	4.57	5.82	21.33	*
8	14	2	38.	*	2.77	3.80	5.43	19.00	*
9	8	1	15.	*	1.88	3.75	15.00	15.00	*
10	6	0	0.	*	0.00	0.00	*	*	*
		Mean	65.1	89.0	3.36	4.35	7.15	21.69	23.32
		S.D.	35.0	7.9	1.48	1.70	3.00	3.44	2.26
		COV%	54.2	9.0	44.1	39.1	41.9	15.8	9.7

Notes:

RV3 = RV1/D
RV4 = RV1/(D-(F-3-1)) = RV1/(D-4)
RV5 = RV1/(D-(F-1)) = RV1/(D-7)
RV6 = RV1/B
F = 8 is the day of the first brood in control vessels.

This example illustrates a number of important properties of the seven response variables. These are summarised by group:

Group A: Total juveniles

Vessels 1 to 6 show that RV2 is identical to RV1 if the adult survives to term. This property leads to the conclusion that, if no adults die anywhere in the test, RV2 will give a NOEC and an EC_{50} that are identical to those obtained for RV1.

When there are any reductions in fecundity due to mortality, RV2 has a higher mean and a smaller standard deviation, and hence a smaller coefficient of variation, than RV1. This smaller variability will produce a better fit to the statistical model and a more powerful test for finding concentrations that produce a real effect.

Group B: Juveniles per day

Vessels 1 to 6 illustrate that RV3, RV4 and RV5 are proportional to RV1 if the adult survives to term. This property leads to the conclusion that, if no adults die anywhere in the test, these three response variables will each give a NOEC and an EC_{50} that are identical to those obtained using RV1.

Vessels 7, 8 and 9 illustrate that, where adults do die before the end of the test and have produced at least some juveniles, RV3 produces results that are closer to the full 21-day results than does V1. For example, in vessel 9, the RV3 value of 1.88 is nearly one-half of the average of the six full-term results (4.24), but the RV1 result (15) is about one-sixth of the average full-term result (89). Hence, RV3 has made a positive adjustment for mortality. Note the lower coefficient of variation.

A similar argument reveals that RV4 generally makes a better correction than RV3. Note that the coefficient of variation is also lower.

Similarly, RV5 will generally make a better correction than RV4. However, vessel 9 reveals an anomaly that can occur with Response Variable 5. This occurs when an adult produces a brood on the "day of onset of reproduction" (day 8 in this case) and then dies within the next 24 hours. This may produce an excessively high number of juveniles per day (15, in this example compared with an average value of around 6). Thus, RV5 can overcorrect and replace an outlier that was too low by one that is too high.

Vessel 10 illustrates that none of these three variables makes a useful adjustment in vessels where the parent survives beyond the threshold day but produces no juveniles. (For RV5, the data are discarded because the parent did not survive to the onset of reproduction.)

Group C: Juveniles per brood

Vessels 1 to 6 illustrate that RV6 and RV7 are only proportional to RV1 when the adult survives to 21 days if all adults produce the same number of broods. Because adults may produce different numbers of broods, these two response variables may produce different NOECs and EC_{50}s to RV1 even where no adults die in any vessel.

However, as vessel 6 illustrates, these response variables can provide a useful adjustment where fewer broods are produced among adults that survive to full term.

Vessels 7 to 9 illustrate that RV6 makes a useful adjustment for mortality, in the sense of bringing the results for parents that die closer to those for parents that survive.

In vessel 10, no broods were produced so the Response Variables 6 and 7 are not calculable. Both response variables produce a lower coefficient of variation than RV1.

4.3.4 Ring test results

The seven different response variables were investigated and compared using the ring test data. Adult mortality was found to be a fairly common occurrence: 46 of the 52 DCA tests (88%), 21 of the 30 cadmium tests (70%), and 13 of the 16 phenol tests (81%) experienced at least one parental mortality during the test. Figure 4.7 gives an example of the different dose response curves that can be obtained by using the different response variables. It shows how the outliers apparent in Response Variable 1 tend to disappear in Response Variable 2 and to be less extreme in Response Variable 5.

The NOEC, EC_{50}, EC_{20} and EC_{10} were estimated for each response variable in turn for each set of data using nominal concentrations. The results for each test are given in Appendix D. Summaries of the results are given in Tables 4.2 for DCA, 4.3 for cadmium and 4.4 for phenol. The number of results for NOEC, EC_{50}, EC_{20} and EC_{10} lying in each of a set of concentration classes is shown. The class boundaries are based on the sequence of recommended concentrations specified for the ring test. NOEC results which lie on the class boundaries have been placed in the lower class in the tables (e.g. for DCA, a NOEC of 5.0 is placed in the 2.5-5.0 class).

Table 4.2: Summary data for DCA nominal exposure concentrations

4.2.A: Reporting of NOECs

Response variable	No. of experiments reporting NOECs in each concentration range (µg/ l)										
	All sig*	<0.60	0.60 1.20	1.20 2.50	2.50 5.00	5.00 10.0	10.0 20.0	20.0 40.0	40.0 80.0	>80.0	None sig**
1	3			5	12	24	7				1
2	4			6	13	23	3				3
3	3			5	12	25	6				1
4	3			5	12	26	5				1
5	3			4	14	24	6				1
6	3			3	13	23	7				3
7	3			3	13	23	7				3

* *All significant: all treatments produced significant effects compared with the controls*

** *None significant: no effects seen*

4.2.B: Reporting of ECs

Response variable (RV)		No. of experiments reporting ECs in each concentration range (µg/l)									
		<0.60	0.60 1.20	1.20 2.50	2.50 5.00	5.00 10.0	10.0 20.0	20.0 40.0	40.0 80.0	>80.0	No fit*
EC_{50}											
1					1	6	23	16	1	1	4
2						5	24	16	2		5
3					1	6	23	16	1	1	4
4					1	5	24	16	1	1	4
5						6	24	16	1	1	4
6						4	19	19	3		7
7						5	18	20	2		7
EC_{20}											
1				1	2	14	25	6			4
2					3	14	22	8			5
3				1	2	14	24	7			4
4				1	2	15	22	8			4
5				1	1	16	20	10			4
6					2	15	21	7			7
7					2	14	22	7			7
EC_{10}											
1		1			4	14	28	1			4
2				1	5	13	27	1			5
3		1			5	13	28	1			4
4		1			5	13	28	1			4
5				1	5	13	27	2			4
6		1		1	5	15	20	3			7
7		1		1	4	17	19	3			7

** data did not fit model used for analysis*

Table 4.3: Summary data for cadmium nominal exposure concentrations

4.3.A: Reporting of NOECs

Response variable (RV)	No. of experiments reporting NOECs in each concentration range (µg/l)										
	All sig*	<0.05	0.05 0.10	0.10 0.20	0.20 0.40	0.40 0.80	0.80 1.00	1.00 2.00	2.00 4.00	>4.00	None sig**
1	2			5	2	2	6			1	12
2	3			6	2	1	7			1	10
3	2			5	2	2	7			1	11
4	2			5	2	2	6			1	12
5	2			6	2	2	7			1	10
6	3			5	1	2	6			1	12
7	4			5	2	1	5			1	12

* *All significant: all treatments produced significant effects compared with the controls*

** *None significant: no effects seen*

4.3.B: Reporting of ECs

Response variable (RV)	No. of experiments reporting ECs in each concentration range (µg/l)									
	<0.05	0.05 0.10	0.10 0.20	0.20 0.40	0.40 0.80	0.80 1.00	1.00 2.00	2.00 4.00	>4.00	No fit*
EC_{50}										
1				1	2	1	1	3	3	18
2				1	2		3	3	3	17
3				1	2	1	1	3	3	17
4				1	2	1	1	4	3	17
5				1	2	1	1	4	3	17
6					1	1	3	5	2	18
7					1	1	3	4	2	19
EC_{20}										
1			1	3	1	1	3	1	1	18
2			1	3	1	1	4		2	17
3			2	2	1	1	3	1	1	17
4			2	2	1	1	4	1	1	17
5			2	2	1	1	4	1	1	17
6				3	1		6	1	1	18
7				3	1		6		1	19
EC_{10}										
1		2		1	3	1	2		1	18
2		2		1	4		2	1	1	17
3		2		1	4		1	1	1	17
4		2		1	4		2	1	1	17
5		2		1	4		2	1	1	17
6				1	5		2	1	1	18
7				1	4		2	1		19

** data did not fit model used for analysis*

Table 4.4: Summary data for phenol nominal exposure concentrations

4.4.A: Reporting of NOECs

Response variable (RV)	No. of experiments reporting NOECs in each concentration range (µg/l)										
	All sig*	<0.08	0.08 0.16	0.16 0.32	0.32 0.56	0.56 1.00	1.00 1.80	1.80 3.20	3.20 6.40	>6.40	None sig**
1				3	2	1	3	1	1		5
2				3	1	2	2	1	1		6
3				3	2	1	2	2	1		5
4				3	2	1	2	1	1		6
5				3	2	1	2	1	1		6
6	1				2	1	3	1	1		7
7	1				2	1	3	1	1		7

* *All significant: all treatments produced significant effects compared with the controls*

** *None significant: no effects seen*

4.4.B: Reporting of ECs

Response variable (RV)	No. of experiments reporting ECs in each concentration range (µg/l)									
	<0.08	0.08 0.16	0.16 0.32	0.32 0.56	0.56 1.00	1.00 1.80	1.80 3.20	3.20 6.40	>6.40	No fit*
EC_{50}										
1						4	3	3		6
2						4	3	2	1	6
3						4	3	3		6
4						4	3	2	1	6
5						4	3	2	1	6
6						3	2	2	1	8
7						3	2	2	1	8
EC_{20}										
1		1		1	3	1	4			6
2		1			4	1	2	1	1	6
3		1		1	3	1	4			6
4		1			4	1	3		1	6
5		1			4	1	3		1	6
6					3	1	2	1	1	8
7					3	1	1	2	1	8
EC_{10}										
1	1		1		3	2	3			6
2				2	2	1	3	1		7
3	1		1		3	2	3			6
4	1		1		3	1	3		1	6
5	1		1		3	1	3		1	6
6					3	2	2		1	8
7					3	2	2	1		8

** data did not fit model used for analysis*

With Response Variables 6 and 7, there is an increase in the number of experiments producing no significant effects with the NOEC calculations and an increase in the number of data sets which could not fit the logistic model to obtain EC_{50}, $EC_{20}$and EC_{10} estimates.

The tables show that, overall, the distribution of NOEC results is hardly affected by changing the response variable. Reference to Appendix D will reveal that for some response variables the NOEC results are shifted one test concentration higher and others are shifted one lower relative to Response Variable 1. This lack of sensitivity reflects to some extent the non-continuous nature of the NOECs, since the NOEC must always be one of the limited number of test concentrations.

For EC_{50}, EC_{20} and EC_{10} results, the tables show that the different response variables have similar distributions, although there is an indication that there is less spread in the results for RV2, RV6 and RV7 than in the other response variables.

To reveal differences between these response variables, box-whisker plots were constructed for each response variable using the NOEC and EC_{50} data for the three test substances derived using nominal exposure concentrations (Figures 4.8 and 4.9). The median is represented by the circled line in the box, the ends of the box represent the upper and lower quartiles, and the ends of the whiskers represent the range, with asterisks denoting potential outliers.

Figure 4.8 shows that, for DCA and cadmium, the response variable used had no influence on the NOECs. With phenol, Response Variables 6 and 7 (based on juveniles per brood) resulted in higher NOECs than the other five response variables. Figure 4.9 shows that the response variables based on juveniles per brood (Response Variables 6 and 7) produced higher EC_{50} values than the other response variables examined (Response Variables 1 to 5). Reference to Appendix D shows Response Variables 6 and 7 also have larger standard errors in general. Figure 4.9 also shows that the variability of the data increased with the juveniles per brood-based response variables (6 and 7), as evident from the width of the boxes. These effects are investigated further in Figures 4.10 and 4.11.

Figure 4.10 presents an example of the dose-response curves obtained using total juveniles excluding those from adults which died (RV 2) and juveniles per brood excluding those from adults which died (RV 7) for one data set. This shows that Response Variable 7 produced a higher EC_{50} than Response Variable 2. Furthermore, the relative spread of the data points was greater at higher concentrations with Response Variable 7.

Figure 4.11 shows the difference in EC_{50}s between Response Variable 1 (total juveniles) and Response Variables 2, 3 or 4, etc. If the two response variables were identical, they would produce the same EC_{50} and the data would fall on the horizontal zero line. If the response variable produces a lower EC_{50} than that of total juveniles, the data will fall below the zero line; if higher it will plot above the line. Response variables based on numbers of juveniles per brood (RVs 6 and 7) generally have higher EC_{50}s than total juveniles (RV 1).

In order to investigate the increase in EC_{50}s with Response Variables 6 and 7, the number of broods produced at each treatment was examined. The results for all three test substances are shown in Figure 4.12 as box-whisker plots. This shows a reduction in the number of broods produced at the two highest DCA concentrations, the highest concentration

of cadmium, and the highest concentration of phenol. Thus, the increase in EC_{50} and its variability seen with Response Variables 6 and 7 was related to the production of fewer broods by adults exposed to the higher concentrations, masking the effect of lower fecundity at these treatments.

In performing these analyses no distinction has been made between tests with mortalities, and those with no mortalities, because Response Variables 2 to 7 are designed to allow for this. In a test where there are no mortalities, Response Variables 1 to 5 will yield identical NOECs and ECs. Similarly, Response Variable 6 will give the same result as Response Variable 7. Only when mortalities occur will the results differ.

To examine the effect of response variables on the NOEC, the data were grouped, by test substance, according to whether they contained adult mortalities or not. These grouped data were then analysed to identify their least significant differences (LSDs), i.e. the size of effect that would be on the borderline of being declared significant in the Dunnett's test. Any observed effect which is less than the LSD would be declared to be not statistically significant (NB: this does not imply that there is no actual treatment related difference, merely that, if present, it cannot be detected by the statistical test). The higher the LSD, the larger the apparent difference must be before it is declared significant. A small LSD is desirable since this makes for a more discriminating test.

Table 4.5 shows the median LSD, on which the NOECs were based, across all laboratories for each of the three substances and the seven response variables. Table 4.6 gives the largest and smallest LSDs for the same categories.

Table 4.5: Median least significant difference (% of control) for each response variable

Response variable (RV)	Median least significant difference (% of control)			Average* median
	DCA	Cadmium	Phenol	
With parental mortalities				
1	25	25	29	26
2	20	20	22	20
3	25	21	28	24
4	23	21	25	23
5	23	20	24	22
6	20	15	20	18
7	19	14	19	18
No. of cases	46	21	13	
With no parental mortalities				
1	25	20	28	23
2	25	20	28	23
3	25	20	28	23
4	25	20	28	23
5	25	20	28	23
6	24	14	16	18
7	24	14	16	18
No. of cases	6	9	3	

** weighted by number of cases*

Table 4.6: Range of least significant differences for each response variable

Response variable (RV)	Range of least significant differences (% of control)					
	DCA		Cadmium		Phenol	
	min	max	min	max	min	max
With parental mortalities						
1	10	60	12	39	15	45
2	10	53	8	31	5	37
3	10	60	10	75	15	44
4	10	54	9	31	15	36
5	10	62	9	30	7	36
6	9	47	8	24	6	50
7	8	47	8	24	5	49
No. of cases	46		21		13	
With no parental mortalities						
1	12	68	8	31	11	32
2	12	68	8	31	11	32
3	12	68	8	31	11	32
4	12	68	8	31	11	32
5	12	68	8	31	11	32
6	11	29	10	24	6	31
7	11	29	10	24	6	31
No. of cases	6		9		3	

Overall, the median LSDs were around 20%. But note that LSDs as high as 75% and as low as 5% were observed. This means that, generally, observed effects will have to be about 20% to 25% to be declared significant though it is not impossible for considerably larger effects to be declared insignificant. Thus, the true effect at the concentration declared to be the LOEC will typically be about 20% though it may be higher or lower.

The response variables with the lowest LSDs (Table 4.5) are those based on numbers of broods (RVs 6 and 7). However, it has been shown previously that these response variables result in fewer broods, and hence smaller reductions, from the control at the higher treatments. They also tended to have wider confidence limits around the EC_{50}. So, despite their low LSDs, they are not recommended. Of the remaining response variables, RV 2 (juveniles per adult excluding those from adults which died) produced the most homogeneous variability together with the smallest LSD (Table 4.5 and Figure 4.11). This response variable is recommended.

Comment: **From a statistical point of view, the chosen variable must satisfy the assumptions of the model fitting procedure, and this applies to both ANOVA and regression modelling. Statistical theory points to three assumptions: normality, statistical independence, and homogeneity of variance. Normality is not an issue because the methods used to fit the model are robust to deviations from normality. Independence is not an issue because the allocation of treatments to experimental units via randomisation usually guarantees independence. This leaves homogeneity of variance as the only assumption giving rise to concern.**

Five of the response variables, RV1, RV3, RV4, RV5 and RV6, are immediately disqualified because for these variables adult mortality causes outliers. RV7 is also disqualified because variability may increase as total juveniles decrease. All these response variables fail to satisfy the requirement of homogeneity of variance. This leaves RV2 as the only acceptable variable on statistical grounds. RV2 is therefore the recommended response variable.

4.4 Effects of test medium

The presence of ethylene-diamine-tetra-acetic acid (EDTA), a known chelating agent, in Elendt M4 and M7 media is a cause for concern when testing substances amenable to complexation, notably metals. The use of poorly defined media, with the addition of organic additives such as seaweed extract, could also reduce metal toxicity due to the presence of naturally occurring complexing agents. However, the presence of chelating agents in *Daphnia* culture media appears to be an important requirement for long-term maintenance of viable cultures. Delegates at the April 1991 Sheffield workshop agreed that there may be an advantage in using a fully defined medium with a known EDTA content.

Elendt M4 and M7 media have a defined EDTA content, M7 having 25% of the EDTA content of M4. Results from the tests with cadmium carried out in these media were compared with those carried out in other media to determine whether the composition of the test medium had any effect on the result. The effect of the test medium on the toxicity of DCA and phenol was similarly investigated.

The data, by substance, were split into two groups. One group used Elendt media (M4 or M7) with EDTA, the other used "own" media without EDTA (Note: Some laboratories [Section 3.2.2] used organic additives, which could also have caused some complexing.) The response variable of total juveniles per parent (i.e. Response Variable 1) was used for the analysis using nominal concentrations. The results are shown in Table 4.7.

Table 4.7: Effect of test media with and without EDTA

Medium	No. of experiments in which no significant effects were found	No. of experiments in which significant effects were found	Total
DCA			
Elendt (with EDTA)	1	37	38
own (without EDTA)	0	14	14
Total	1	51	52
cadmium			
Elendt (with EDTA)	11	8	19
own (without EDTA)	2	9	11
Total	13	17	30
phenol			
Elendt (with EDTA)	2	10	12
own (without EDTA)	3	1	4
Total	5	11	16

The data for DCA show no significant effects on toxicity due to medium.

The results for cadmium indicate that medium had a large influence on the toxicity of this substance. 58% of the 19 experiments using Elendt media containing EDTA found no significant effects, while only two (18%) out of the eleven experiments without EDTA (but possibly with other complexing agents present) found no significant effects. Fisher's exact test indicated that these proportions were significantly different ($p = 0.04$). Laboratories that used "own" media consistently found effects in the range of test concentrations specified for the ring test, while those using Elendt M4 and M7 media mostly failed to find an effect at these concentrations. Indeed, one participant (L27) using Elendt M4 (four times the EDTA content of M7) increased the concentration range by a factor of 160 to obtain an NOEC of 56 µg/l.

The data for phenol show the opposite effect. There was a higher proportion of cases in which "own" medium found no significant effects, but this was not statistically significant (Fisher's exact test, $p = 0.06$).

Comment: **The demonstrated effect of complexing agents such as EDTA on the toxicity of cadmium has implications for testing other metals, metal-containing effluents, and organo-metallic substances. At present, no fully defined medium free of EDTA exists that is suitable for long-term culturing and testing with *Daphnia*. Adoption of a semi-defined medium may be advantageous when working with test substances that contain metals, compared with the current practice which allows a range of different**

undefined media to be used. This is especially so if improved inter-laboratory reproducibility and better interpretation of test results with metal containing substances is desired. ASTM medium with added seaweed extract has been found in previous work to be satisfactory (Sims *et al* 1993), although the organic additive still exerts a mild complexing action. A consequence of this approach is that, for laboratories keeping stock cultures in Elendt medium for testing organic substances, a separate stock of *Daphnia* may need to be maintained in another medium for testing metal-containing substances.

4.5 Effects of clone

The results of the analyses by clone are shown in Figure 4.13 for nominal concentrations of DCA, cadmium and phenol. Clone A was more susceptible to DCA than the other clones. All clones appeared to be equally susceptible to cadmium and phenol.

4.6 Effects of ration

The results of the analyses by ration are shown in Figure 4.14, for nominal exposure concentrations of DCA, cadmium and phenol. These show that the EC_{50}s of these test substances appeared not to be affected by different rations.

4.7 Variability within and between laboratories

An important objective of the ring test was to obtain information on variability between laboratories, and to assess how this compared with variability within laboratories. Therefore, laboratories were asked, where possible, to repeat the tests under the same conditions and to report the results. The International Organization for Standardization's ISO 5725 method (1986) was used to estimate repeatability (r) and reproducibility (R).

Repeatability (r) is the value below which the absolute difference between two single test results obtained at the same laboratory, under repeatability conditions, may be expected to lie with a probability of 95%.

Reproducibility (R) is the value below which the absolute difference between two single test results obtained at different laboratories, under reproducibility conditions, may be expected to lie with a probability of 95%.

ISO 5725 defines repeatability conditions as conditions in which mutually independent test results are obtained with the same method on identical material in the same laboratory by the same operator, using the same equipment, within short intervals of time. Reproducibility conditions are defined as conditions in which mutually independent test results are obtained with the same method on identical material in different laboratories at different times.

Statistical analysis was performed to estimate r and R for EC_{50} results using nominal concentrations. Only the results for DCA nominals had sufficient data sets to enable this analysis to be carried out. The analysis was limited to EC_{50} values using total juveniles as the

response variable. Furthermore, only laboratories that had performed repeat tests were included.

Twelve laboratories had performed the test twice and one had performed it five times. However, in the laboratory with five data sets only two were performed with the same combination of medium and clone, so the other three data sets were discarded. One other laboratory had used different clones and was therefore excluded. Two laboratories were excluded because only one EC_{50} was calculable.

The remaining ten pairs of results were analysed using ANOVA to distinguish the variation within and between laboratories. The variance within the ten laboratories was estimated to be 12.36, and the estimated variance between them was 38.40. The total variance was thus 50.76. Taking square roots to convert variances to standard deviations yields 3.5 for within laboratories, 6.2 for between laboratories, and a combined standard deviation of 7.1 to measure the variability of single results from different laboratories.

The recommended method for obtaining r and R is to multiply the appropriate standard deviation by the square root of 2 to give the standard error of the difference between two observations and then by 1.96, this being the 95% (two-sided) point of the standard normal distribution. The combined multiplier is 2.8.

Thus, using this multiplier, r = 10 µg/l and R = 20 µg/l, producing a ratio of 2. This compares favourably with r/R data from other ring tests:

- Fathead minnow ring test : 1.3
- *Daphnia* 48h acute toxicity ring test : 2.6
- Chemical analysis, partition coefficient : 1.1 (aniline) to 3.5 (trichloroethyline)

<u>Comment</u>: For DCA, two EC_{50} results from within the same laboratory cannot be considered to be different unless they differ by more than 10 µg/l, and two results from different laboratories cannot be considered different unless they differ by more than 20 µg/l. Another way of looking at this is to say that repeat results between laboratories are twice as variable as repeat results within a laboratory, a ratio of 2. This compares well with such data from other ring tests.

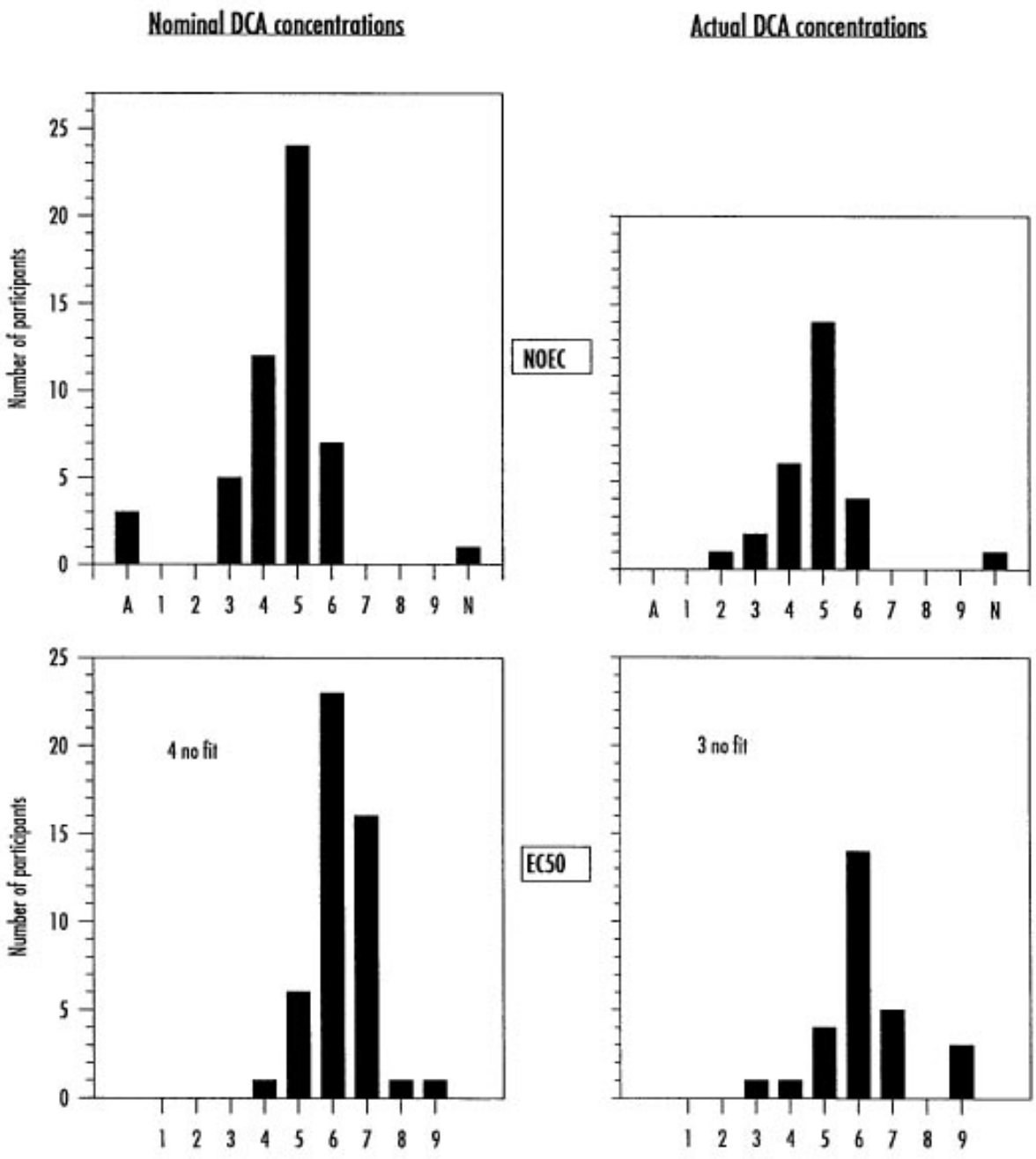


Figure 4.1 Summary of effects across the whole data set grouped by effect category for the test substance DCA.

Effect categories used (values in µg / l): A, all affected (NOECs only) N, none affected (NOECS only)

1	2	3	4	5	6	7	8	9
<0.6	0.6 ↓ 1.2	1.2 ↓ 2.5	2.5 ↓ 5.0	5.0 ↓ 10	10 ↓ 20	20 ↓ 40	40 ↓ 80	>80

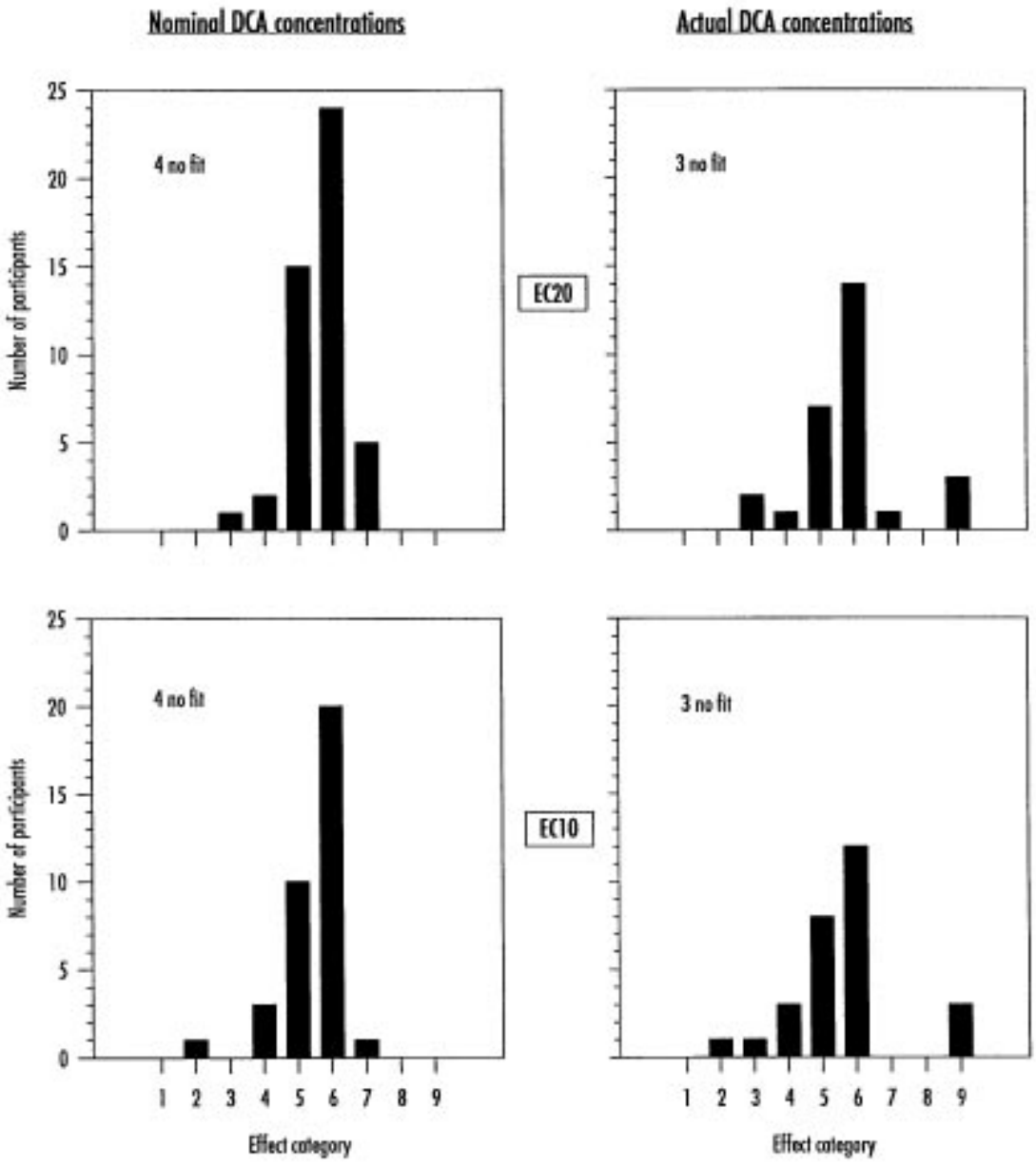


Figure 4.1 (cont.) Summary of effects across the whole data set grouped by effect category for the test substance DCA.

Effect categories used (values in µg / l): A, all affected (NOECs only) N, none affected (NOECS only)

1	2	3	4	5	6	7	8	9
<0.6	0.6	1.2	2.5	5.0	10	20	40	>80
	↓	↓	↓	↓	↓	↓	↓	
	1.2	2.5	5.0	10	20	40	80	

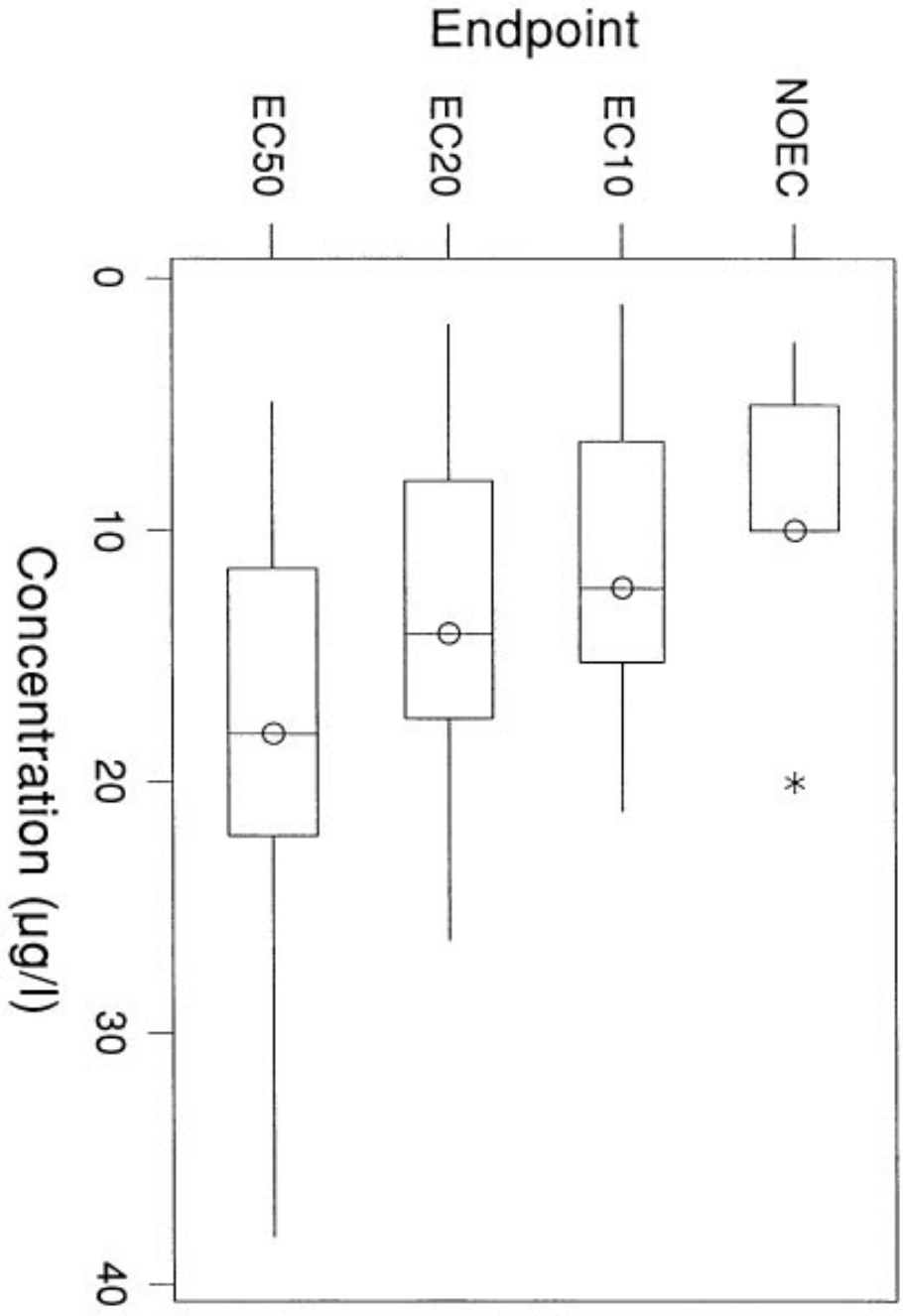


Figure 4.2 Box-whisker plot of four end points for 3,4-dichloroaniline.
Note - circled line = median
sides of box = 25 and 75% quartiles
bars = range
* = outliers

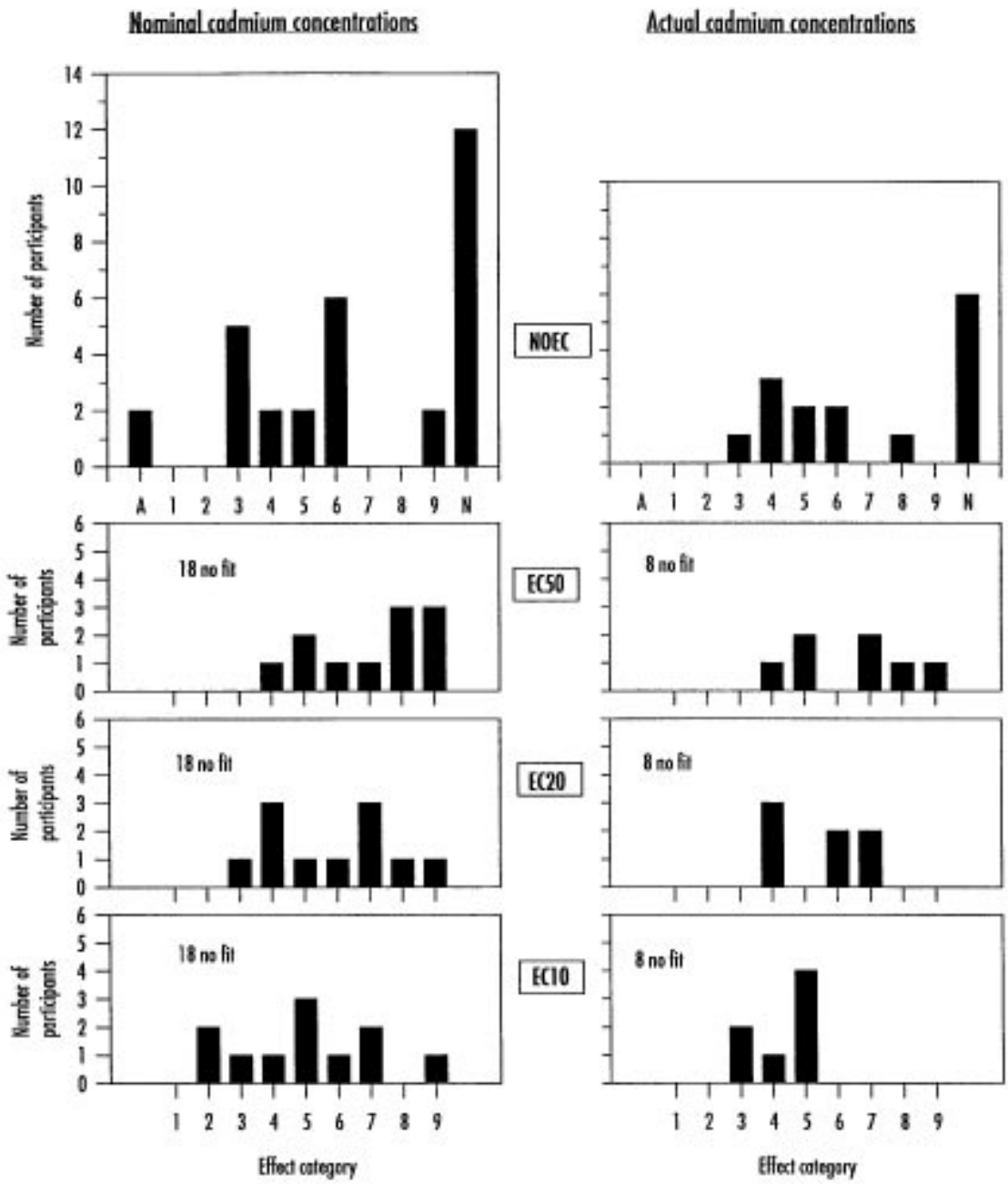


Figure 4.3 Summary of effects across the whole data set grouped by effect category for the test substance cadmium.

Effect categories used (values in µg / l): A, all affected (NOECs only) N, none affected (NOECS only)

1	2	3	4	5	6	7	8	9
<0.05	0.05	0.1	0.2	0.4	0.8	1.0	2.0	>4.0
	↓	↓	↓	↓	↓	↓	↓	
	0.1	0.2	0.4	0.8	1.0	2.0	4.0	

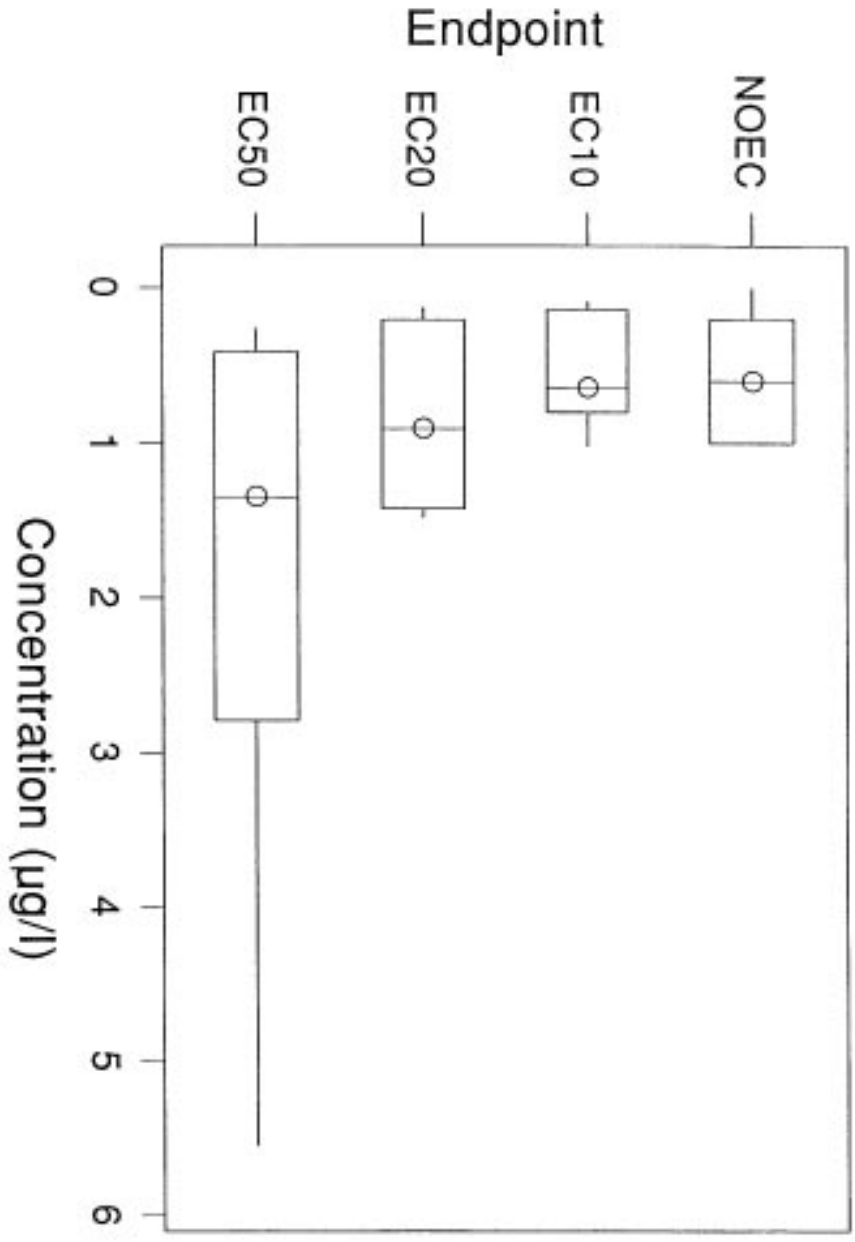


Figure 4.4 Box-whisker plot of four end points for cadmium chloride.
Note - circled line = median
sides of box = 25 and 75% quartiles
bars = range
* = outliers

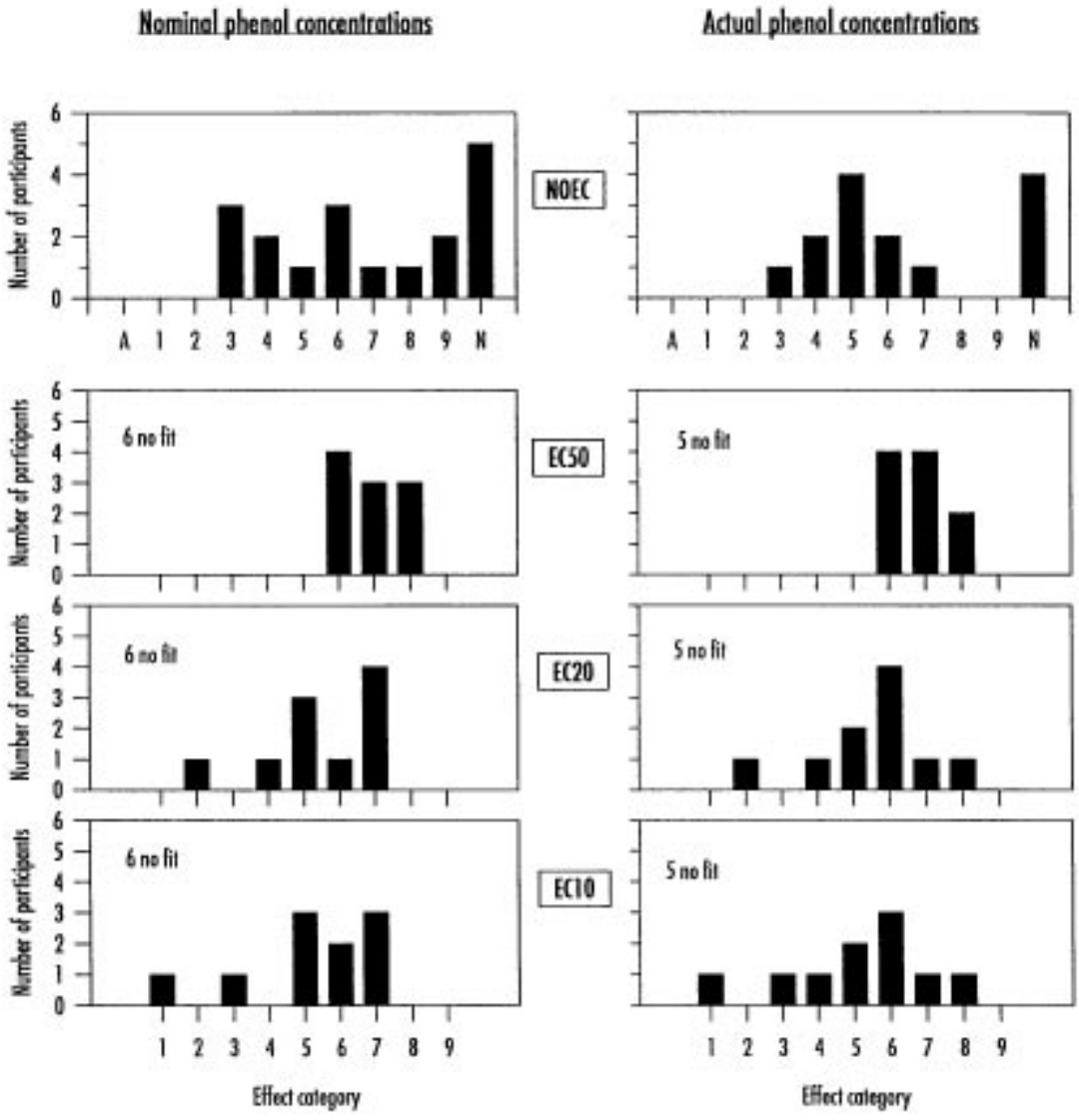


Figure 4.5 Summary of effects across the whole data set grouped by effect category for the test substance phenol.

Effect categories used (values in µg/l): A, all affected (NOECs only) N, none affected (NOECS only)

1	2	3	4	5	6	7	8	9
<0.08	0.08	0.16	0.32	0.56	1.0	1.8	3.2	>6.4
	↓	↓	↓	↓	↓	↓	↓	
	0.16	0.32	0.56	1.0	1.8	3.2	6.4	

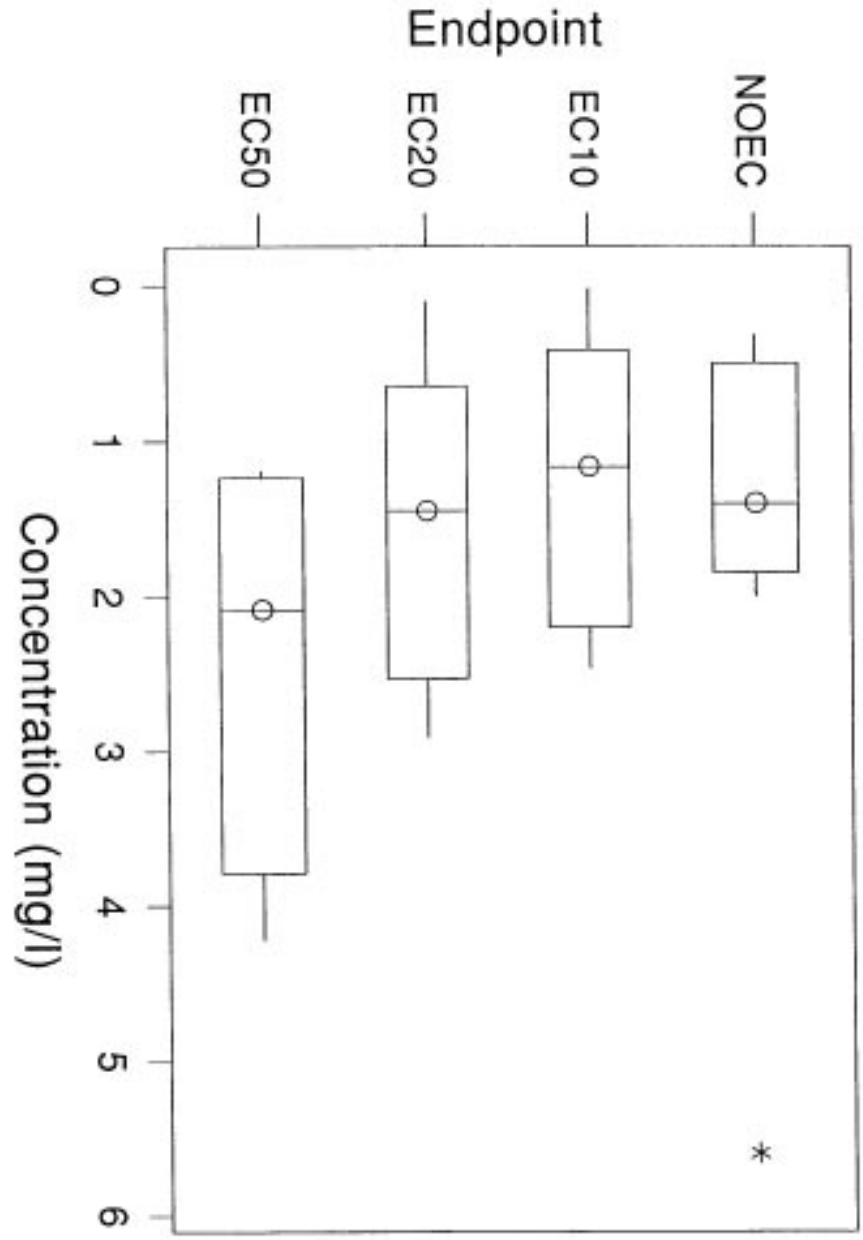


Figure 4.6 Box-whisker plot of four end points for phenol.
Note - circled line = median
sides of box = 25 and 75% quartiles
bars = range
* = outliers

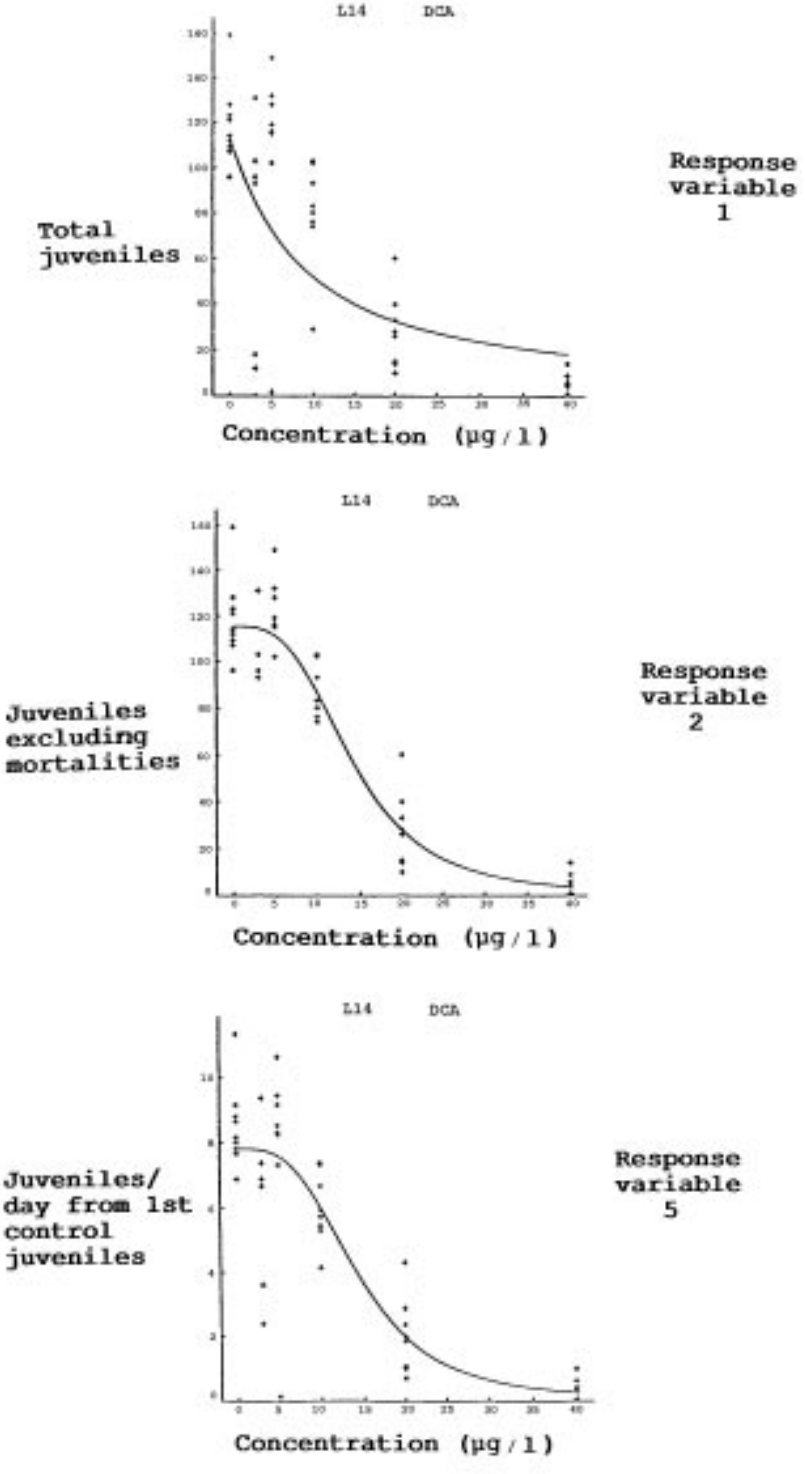
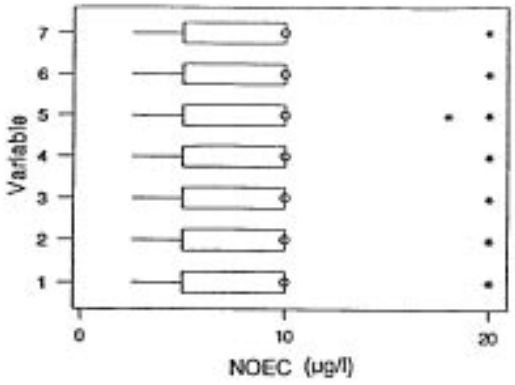
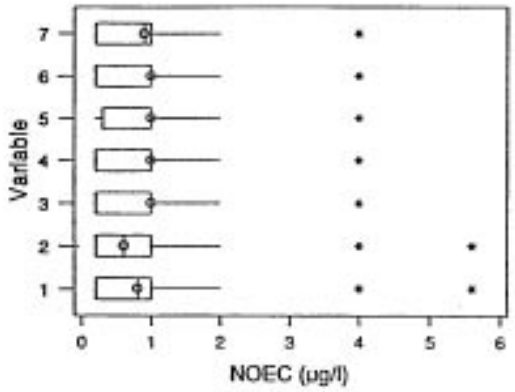


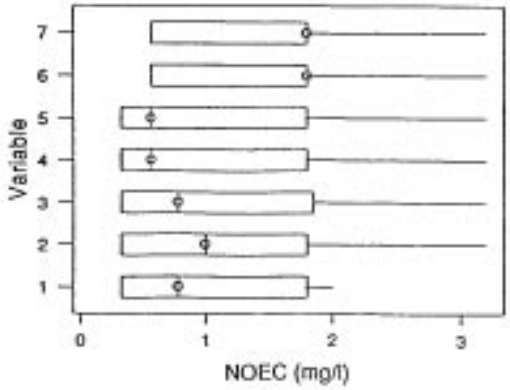
Figure 4.7 Examples of dose-response curves for one data set using:
response variable 1 (total juveniles per adult)
response variable 2 (total juveniles excluding those from adults which died)
response variable 5 (juveniles per day from the day of first control juveniles)



DCA

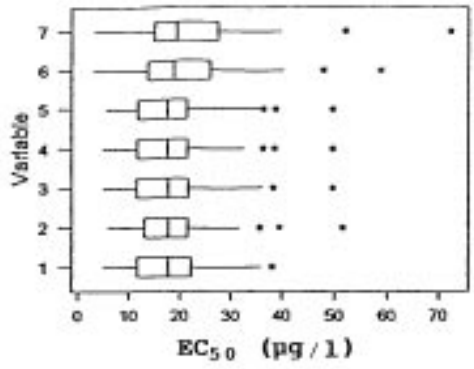


Cadmium chloride

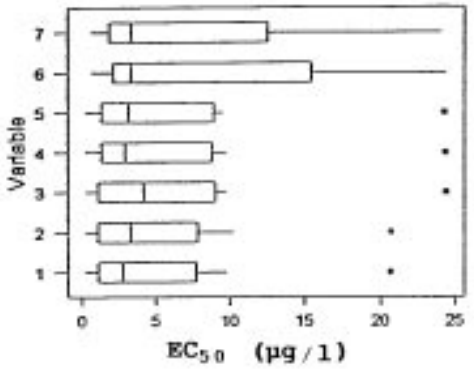


Phenol

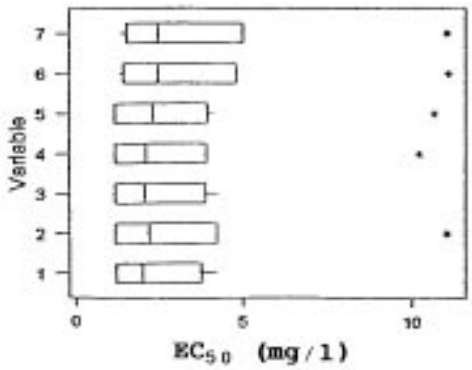
Figure 4.8 Box-whisker plot of the seven endpoints for NOECs of the three test substances.
Note - circled line = median
sides of box = 25 and 75% quartiles
bars = range
* = outliers
response variable 1 = total juveniles per adult
response variable 2 = total juveniles per surviving adult
response variable 3 = juveniles per day from day 1
response variable 4 = juveniles per day from day of first control juveniles - 3 days
response variable 5 = juveniles per day from day of first control juveniles
response variable 6 = juveniles per brood
response variable 7 = juveniles per brood less those from adults which died.



DCA

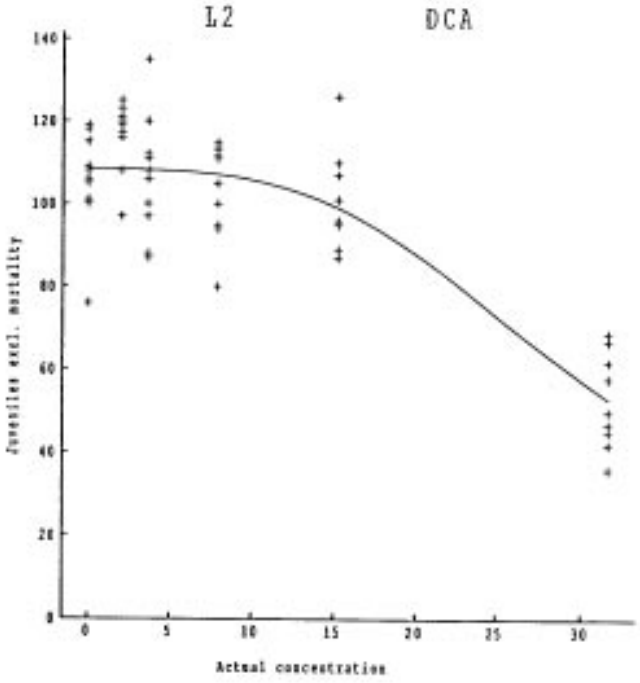


Cadmium chloride



Phenol

Figure 4.9 Box-whisker plot of the seven endpoints for ECs of the three test substances.
Note - line = median
sides of box = 25 and 75% quartiles
bars = range
* = outliers
response variable 1 = total juveniles per adult
response variable 2 = total juveniles per surviving adult
response variable 3 = juveniles per day from day 1
response variable 4 = juveniles per day from day of first control juveniles - 3 days
response variable 5 = juveniles per day from day of first control juveniles
response variable 6 = juveniles per brood
response variable 7 = juveniles per brood less those from adults which died



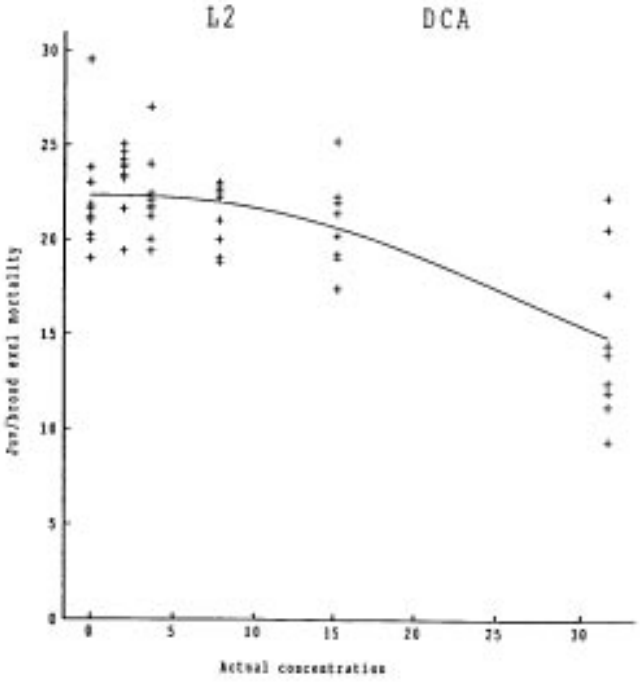


Figure 4.10 Examples of dose-response curves for one data set using:
response variable 2 (total juveniles excluding those from adults which died)
response variable 7 juveniles per brood excluding those from adults which died)

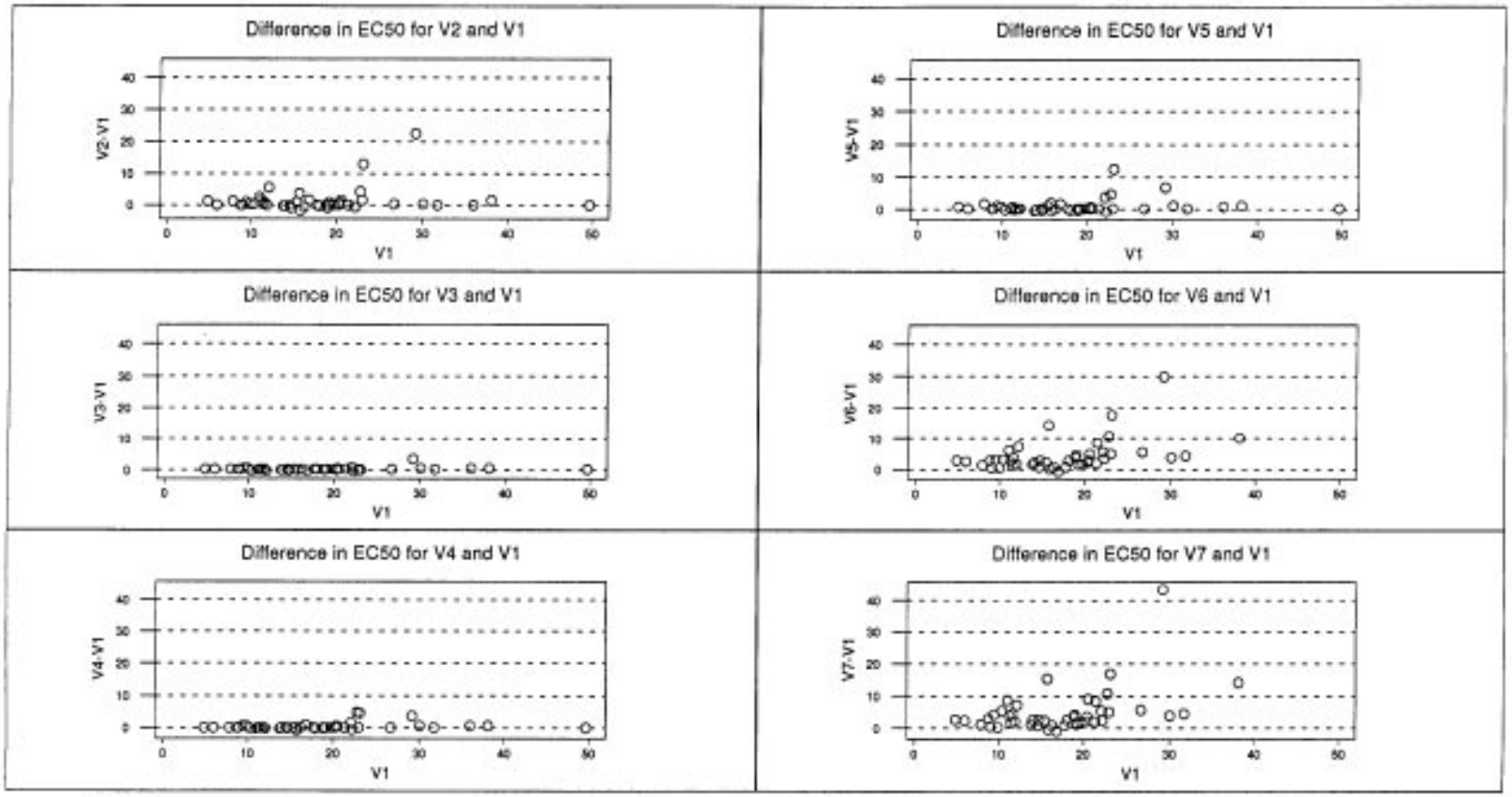
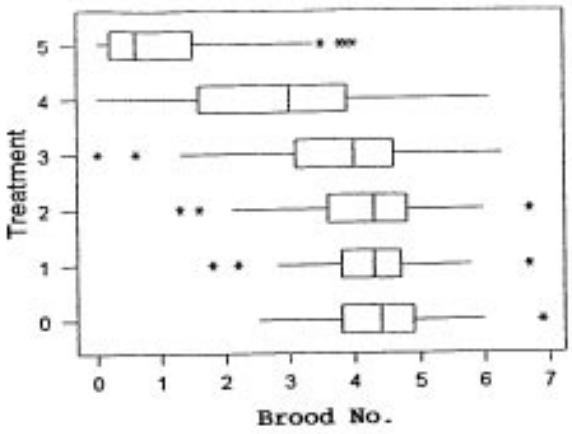
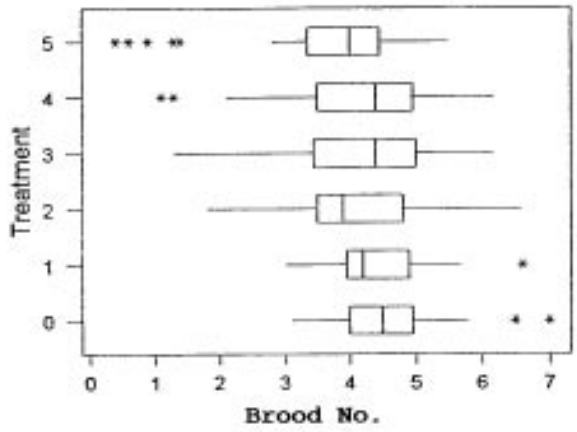


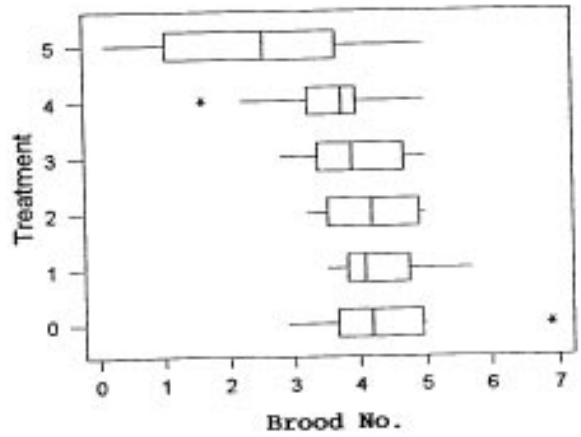
Figure 4.11 Difference between response variable 1 EC_{50} and EC_{50}s of the other response variables.
Note - circled line = median
sides of box = 25 and 75% quartiles
bars = range
* = outliers
response variable 1 = total juveniles per adult
response variable 2 = total juveniles per surviving adult
response variable 3 = juveniles per day from day 1
response variable 4 = juveniles per day from day of first control juveniles - 3 days
response variable 5 = juveniles per day from day of first control juveniles
response variable 6 = juveniles per brood
response variable 6 = juveniles per brood less those from adults which died.



DCA



Cadmium chloride



Phenol

Figure 4.12 Box-whisker plot of numbers of broodes by treatment for the three test substances.
Note - line = median
sides of box = 25 and 75% quartiles
bars = range
* = outliers

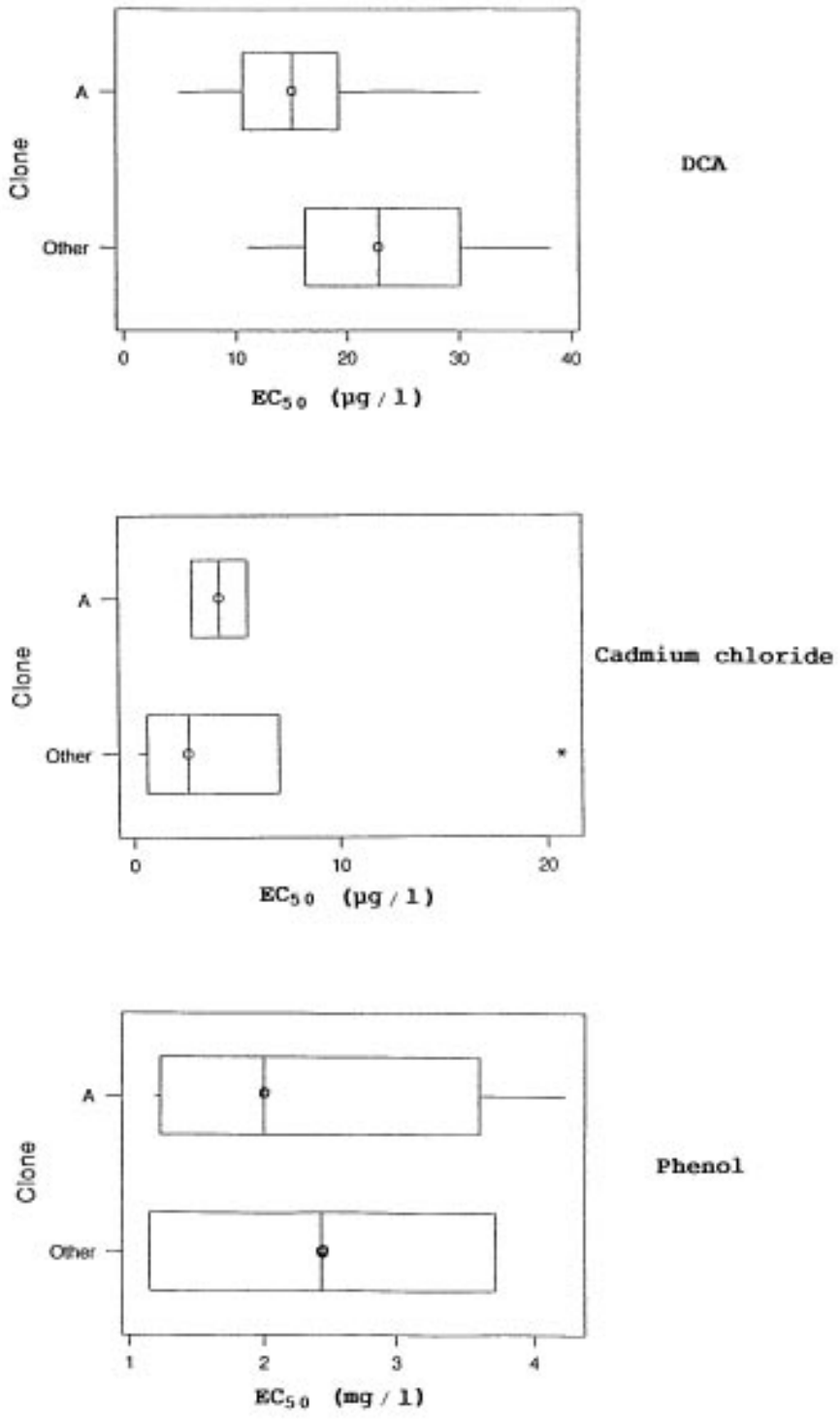


Figure 4.13 Box-whisker plot of EC_{50}s by clone for the three test substances.
Note - circled line = median
sides of box = 25 and 75% quartiles
bars = range
* = outliers

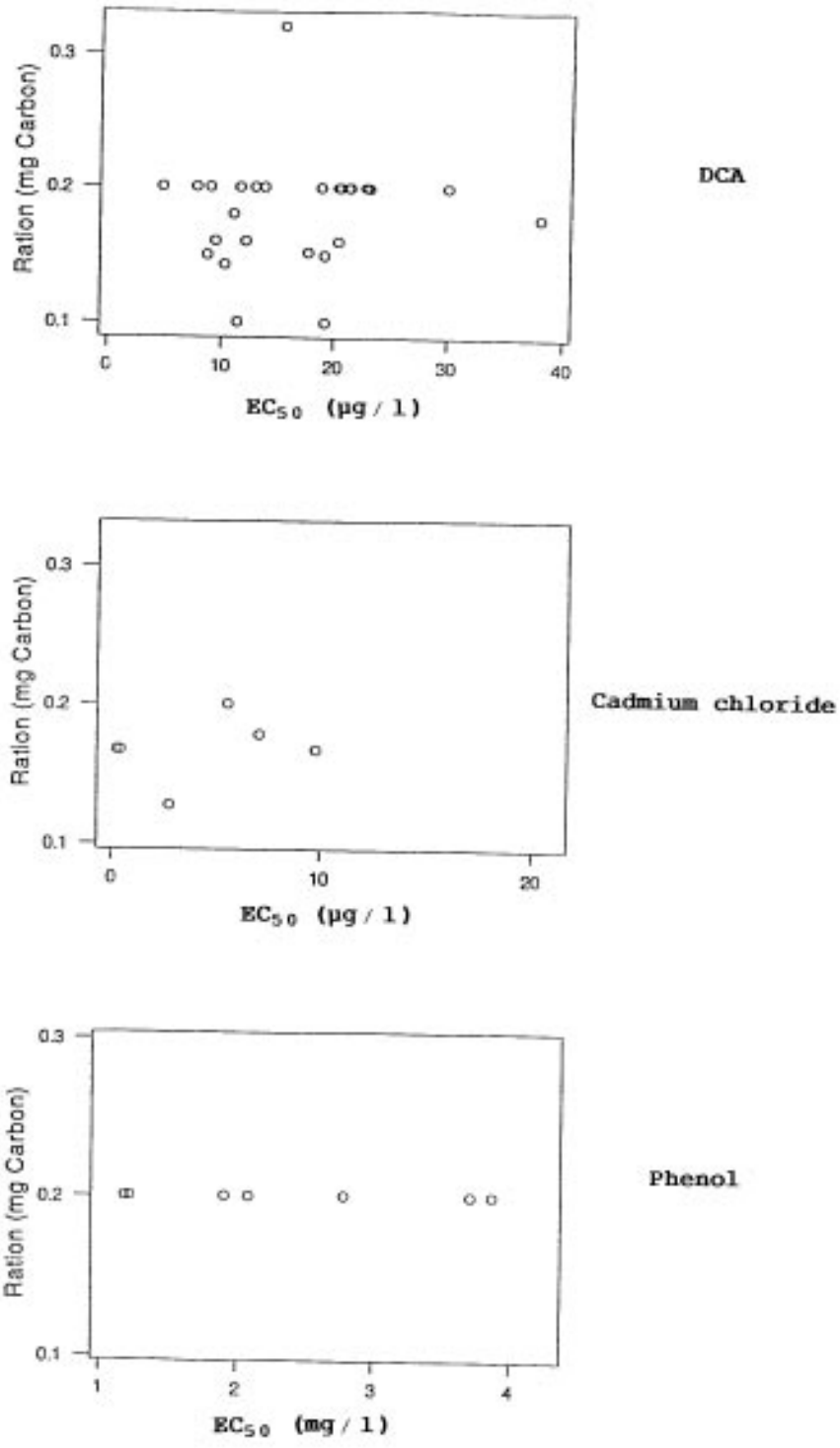


Figure 4.14 Difference between ration and EC_{50} for the three test substances.

5. Comparison of NOEC and EC_q

This section investigates the relationship between different summary parameters of toxicity.

5.1 Background

In 1993, in response to a growing interest within OECD Member countries and the scientific community, a review of guidance given on test design and data analysis for all existing and draft OECD Test Guidelines for aquatic toxicology was prepared (Pack 1993). The "growing interest" really represented an increasing discontent in some circles with the LOEC and NOEC as the principal summary measures to be derived from subchronic and chronic studies, and the desire to move towards the derivation of a concentration-response relationship using statistical modelling (e.g. regression modelling), from which a summary measure such as an EC_{10} or EC_{20} could be reported.

Briefly, the LOEC, and consequently the NOEC, have several disadvantages as a summary measure. For example, the LOEC and NOEC are dependent on the test concentrations used. Therefore, if two tests are conducted under identical conditions but with different concentrations, they are bound to yield different LOECs and NOECs. In addition, no statement of precision (e.g. 95% confidence limits) can be obtained for the LOEC or NOEC. Hence there is no way of reporting their reliability. Although statistical modelling has its drawbacks, such as in the choice of model and the estimation of parameters, it does correct many of the defects inherent in the LOEC and NOEC approach.

Three main recommendations were made in the Pack review:

(1) EC point estimation (e.g. EC_{10}, EC_{20}, EC_{50}) should be considered as the preferred type of analysis.

(2) Member countries should consider extending their networks of experts to include a list of statistical specialists to assist in the development of current and future Guidelines.

(3) The OECD should promote the development of a handbook of statistical methods and computer software to assist in data analysis for aquatic (and potentially other) toxicity Guidelines.

The review was greatly appreciated by Member countries, but it was recognised that the recommendations had a number of important implications for the tests themselves, and also for the subsequent use of the data in risk assessment. For example, with respect to risk characterisation, assessment factors are currently applied to NOECs to estimate Predicted No Effect Concentrations (PNECs). Would it be necessary to derive different assessment factors if EC_qs were reported from tests? Should the same EC_q be derived for all tests? What should be done with the "old" NOEC data?

Subsequent to the proposal to use EC point estimation rather than a LOEC/NOEC approach, a workshop was held in the Netherlands in September 1994 on “How to measure no effect? - Towards a new measure of chronic toxicity in ecotoxicology” (Noppert *et al* 1994). At this workshop, the arguments surrounding EC_q *vs* the NOEC were reviewed and an additional summary measure was proposed for further consideration, the No Effect Concentration (NEC). This assumes that there is a threshold concentration (the NEC) below which the chemical will not affect the organism.

Only two of the three approaches referred to above were used in analysing the ring test data for this report, namely the NOEC and the Ec_q. However, the data files were passed on to Professor Kooijman at the Vrije Universiteit in Amsterdam to allow parNECs to be estimated. This investigation will be reported separately.

5.2 Comparison of NOEC and EC_q in the ring test

The NOEC approach was based on analysis of variance (ANOVA), followed by Dunnett's test to find the LOEC. The second approach is based on fitting a response curve by a non-linear regression procedure and using this to estimate an EC_q, such as the EC_{50}. For further details, see Sections 4.1.2 and 4.1.3.

The least significant difference (LSD) for Dunnett's test indicates the size of the smallest observed effect that would be declared to be statistically significant. This represents the boundary between test concentrations with effects small enough to give rise to a NOEC and those large enough to be declared a LOEC. Table 4.5 shows that, for the ring test data, the median LSD was typically about 20% to 25%, so that a concentration declared to be the No Observed Effect Concentration could easily have an observed effect of up to 20%.

However, the NOEC for individual tests almost always lay below the EC_{20}. This is revealed in Figure 5.1, which shows Box-whisker plots of the difference between the NOEC and the associated EC_{50}, EC_{20} and EC_{10} for each test substance. For the three substances, the EC_{50} was always greater than the NOEC, the EC_{20} was nearly always greater than the NOEC, but the EC_{10} was sometimes greater and sometimes less than the NOEC.

An alternative approach would be to substitute the NOEC into equation (2) in Section 4.1.3 and use this to discover the value of q which gives the best fit of EC_q to NOEC. Table 5.1 summarises the results when this is done.

Table 5: Percentage effect at the NOEC

Substance	mean	median	min	max	standard deviation
DCA	8.8	3.6	0.0	37.1	10.6
cadmium	9.6	9.3	1.4	19.3	5.5
phenol	9.0	4.3	0.1	31.9	10.7

This shows that the median EC at the NOEC was 3.6% for DCA, 9.3% for cadmium and 4.3% for phenol. The mean values for DCA and phenol were considerably higher than their medians because the data were skewed. The large difference between the maxima and minima should also be noted.

5.3 Using EC_{50} and slope

As stated in Section 4.1.3, the logistic curve relating reproduction to concentration has three parameters:

c: the expected reproduction at zero concentration;
x_0: the EC_{50}, and
b: the slope parameter.

The slope of the curve at $X = x_0$ is: $-bc / 4x_0$

The EC_{50} is a more robust estimate than the EC_{20} or EC_{10}, because it is less susceptible to differences in the model formulation. This is important, because it is not possible to define a model suitable for all substances. Therefore, different laboratories testing the same substance may use different models. There will tend to be greater consistency between results from different models if the EC_{50} is used rather than some other Ec_q.

However, the 50% effect expected at the EC_{50} is generally too high for regulatory purposes. The question arises as to whether some factor could be applied to the EC_{50} to make it more acceptable.

Nyholm et al (1994) suggested that the slope of the concentration-response curve at the EC_{50} is an informative parameter that should be reported in addition to the EC_{50} itself. This is because the slope gives an indication of how far the EC_{50} is away from the more extreme EC_qs, for example the EC_{10}. The steeper the slope, the nearer it is to the EC_{10}; the gentler the slope, the greater is the difference between the EC_{50} and the EC_{10}.

One way of using this information on the slope of the concentration response curve is to use the model to calculate the EC_{10} or EC_{20}. But these are sensitive to the form of the model used. An alternative method of incorporating slope which avoids this drawback is to find the concentration at the point where the tangent to the curve at the EC_{50} intersects a horizontal line through the expected response in the control.

This point of intersection occurs at $x_0(1 - b/2)$ and the percentage effect at x_c is given by:

$$\frac{1}{1+\frac{1}{(b-2)^b}}$$

Thus, x_C is not constant but depends only on b.

One disadvantage, which is immediately apparent, is that if the estimate of b is smaller than 2 (and this is not unusual), x_c ill be negative. Therefore, x_c could not be used for a concentration which produces some low toxic effect.

An interesting alternative approach is to work in terms of log-concentrations. The concentration-response curve then takes the form:

$$Y = \frac{c}{1 + \exp\left(bZ - bz_O\right)}$$

where: $Z = \ln(X)$
and $z_0 = \ln(x_0)$ i.e logarithm of the EC_{50}.
b and c take the same meaning as previously.

This curve is symmetrical about the EC_{50}. The slope at $Z = z_0$ is: -bc / 4

The intercept of the tangent at z_0 intersects the horizontal line through the expected response at control (y=c) at:

$z_c = z_0 - 2/b$

The percentage effect at z_c is:

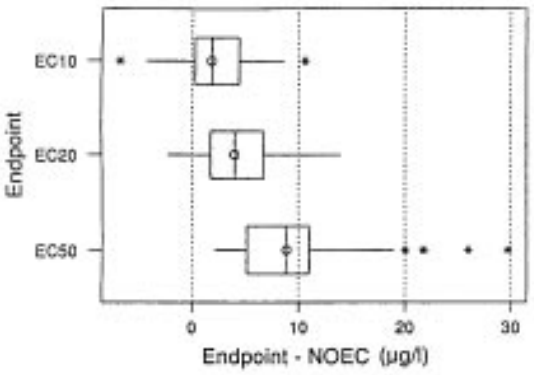
qc= 1-1/(1+exp(-2)) = 0.1192 which is constant for all c, b and z_0

So this method produces the interesting result that the intersection between the tangent at the EC_{50} and the line y = c yields a concentration that is virtually the EC_{12}, and it will give the EC_{12} whatever the value of c, b and EC_{50}.

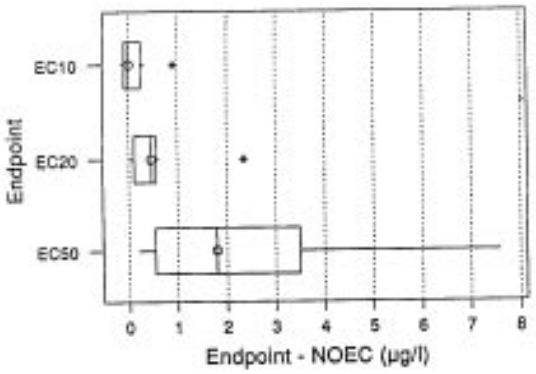
Figure 5.2 provides an illustration of the concept for three cases: b=1, b=2 and b=4; c is fixed at 100 and the EC_{50} is set at 10.

It would be instructive to see whether this concept could be applied to other models.

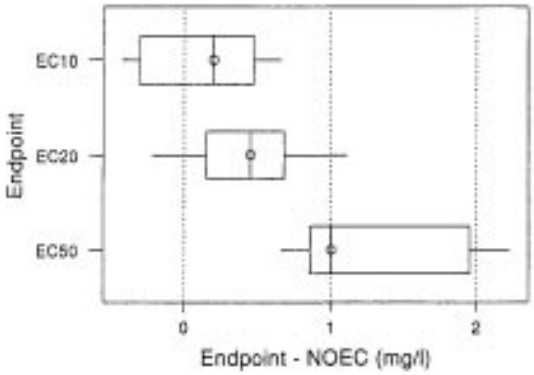
<u>Comment</u>: This idea is of sufficient interest to merit further investigation and consideration.



DCA



Cadmium chloride



Phenol

Figure 5.1 Box-whisker plots of differences between the NOEC and the associated EC_qs for each test substance.

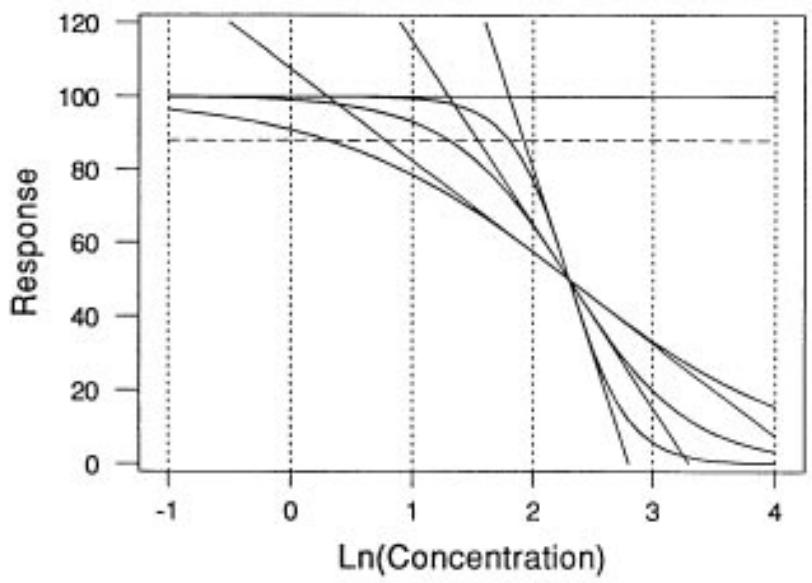


Figure 5.2 Tangents at EC_{50}.

NB - C = expected control value (100 juveniles/adult)
B = slope parameter (1, 2 and 4)
EC_{50} = 10

6. Conclusions and recommendations

The Final Ring Test thoroughly evaluated the performance of the February 1994 draft OECD Guideline 202, Part II, *Daphnia magna* Reproduction Test and demonstrated that variability in the test results within and between laboratories was low. Significant improvements to the Guideline have therefore been made since the first ring test in 1984 (Cabridenc 1987). In addition, it has been possible to investigate various ways of expressing reproductive output of the *Daphnia* and how to treat data from animals which die during the test; proposals for how these issues should be dealt with in future have been made.

From the results presented in the earlier sections, the following conclusions can be made:

(1) Adherence to the instructions in the draft Guideline by the laboratories was generally very good:

- Most used a genetically typed clone (clone A was most frequently used);
- Most used a fully defined test medium (Elendt M4 or M7 were most frequently used);
- Most used test volumes of 50 ml;
- Most used living cells of the preferred algal species, although despite the emphasis in the draft Guideline on the importance of supplying ration on the basis of TOC (or some surrogate), around 50% of the laboratories failed to do so;
- Most removed juveniles either daily or at least five times a week;
- Most made water quality measurements at the required frequency and in the required treatments, although not all participants followed instructions concerning the frequency and extent of test substance determinations.

(2) Most laboratories were able to meet the water quality criteria recommended:

- 95% of experiments reported pH within a range of 1.5 pH units;
- Of the 98 experiments (52 with DCA, 30 with cadmium and 16 with phenol), the dissolved oxygen concentration fell below a minimum of mg/l in only one test; however, the need for an upper limit for dissolved oxygen should be considered;
- 85% of experiments were conducted within the specified temperature range (18-22^{o}C), with 61% reporting temperature fluctuations within 2^{o}C; the need for temperature measurements to be made in the test media rather than in the test area (air temperature) should be made explicit in the next revision of the Guideline and experimenters should make every effort to comply with the temperature criteria.

This suggests that these criteria are appropriate and achievable.

(3) Most laboratories were able to meet the validity control performance criteria in the draft Guideline:

- 98% of experiments reported control mortalities of not greater than 20%;
- 84% of experiments reported mean numbers of live offspring per parent of at least 60.

These validity criteria would therefore appear to be set at appropriate and achievable levels.

(4) The relationships found between nominal and actual concentrations support the case that chemical analysis need not be performed for every treatment if the ratio between nominals and actuals can be obtained. The stability of the test substance should be investigated before a 21-day test is initiated, possibly at the range-finding stage (48h acute toxicity). For very labile substances, consideration should be given to more frequent medium renewal, or to the use of flow-through systems, with the aim of improving the maintenance of exposure concentrations.

(5) The overall variability between laboratories, in terms of the effects on reproduction, was low and much less than for the 1984 ring test. Of all tests that provided estimates of NOEC, EC_{50}, EC_{20} and EC_{10}:

- for DCA, approximately 50% of the NOECs, EC_{50}s, EC_{20}s and EC_{10}s lay within a factor of 2, over 75% lay within a factor of 4, and over 90% lay within a factor of 8;

- for cadmium, 38% of the NOECs lay within a factor of 2 and 62% within a factor of 8; the figures for the EC_{50}s and EC_{20}s were 27% and 45% respectively, and for EC_{10}s 27% and 55%;

- for phenol, 45% of the NOECs lay within a factor of 3.2 and 82% within a factor of 10; the figures for the EC_{50}s were 70% and 100% respectively, for the EC_{20}s 50% and 90%, and for the EC_{10}s 50% and 80%.

Within-laboratory variability (r) and between-laboratory variability (R) for DCA, derived using nominal concentrations, were: r = 10 µg/l and R = 20 µg/l, giving a ratio of 2. This compares favourably with such data reported for other ring tests.

(6) Of the seven response variables examined to compensate for adult mortality, RV1, RV3, RV4, RV5 and RV6 are disqualified because, for these variables, adult mortality causes outliers. RV7 is also disqualified because variability can increase as total juveniles decrease. All these variables fail to satisfy the requirement of homogeneity of variance. This leaves RV2 as the only acceptable variable on statistical grounds. Therefore, the recommended response variable is RV2, i.e. total juveniles per adult excluding results from any adults that die.

(7) The presence of EDTA in the Elendt media caused reductions in apparent cadmium toxicity due to chelation. In order to reduce variability between laboratories when testing metal-containing compounds, media containing EDTA or other known

chelating agents should be avoided, or the EDTA content controlled by using a fully defined medium. Alternatively, the adoption of a semi-defined medium, preferably without strong chelators, for testing such compounds may be the preferred option. ASTM with seaweed extract may prove useful in this respect, but the organic additive may act as a weak chelator.

(8) Clone A appeared to be slightly more sensitive to DCA than the other clones used, but all clones were similarly sensitive to cadmium and phenol.

(9) Until the uncertainty surrounding the preference for NOEC or EC_q is resolved, it is recommended that, for *Daphnia* reproduction studies, the requirements of the existing Guideline 202, Part II of April 1984 are retained with respect to the summary parameters to be reported for effects on reproduction, i.e. both the NOEC and the EC_{50} should be reported. The data reported in this ring test should make a valuable contribution to this debate.

7. Literature

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APPENDIX B

DRAFT OECD TEST GUIDELINE 202, PART II

DAPHNIA MAGNA REPRODUCTION TEST
TO BE USED IN THE FINAL RING TEST

Please note: For purposes of the Final Ring Test, text enclosed in boxes should be ignored.

INTRODUCTION

1. OECD Test Guidelines for Testing of Chemicals are periodically reviewed in the light of scientific progress. With respect to Guideline 202, Part II *Daphnia* sp. Reproduction Test (adopted April 1984), it had generally been acknowledged that data from tests performed according to this Guideline could be very variable. This led, in recent years, to considerable effort being devoted to the identification of the reasons for this variability with the aim of producing a better test method. This updated Guideline is based on the outcome of these research activities and ring tests performed in 1992 (I) and 1994 (2).

2. The main differences between this and the previous version of the Guideline are:

(a) the species to be used is *Daphnia magna*;

(b) the test duration is 21 days;

(c) for semi-static tests, the number of animals to be used at each test concentration has been reduced from at least 40, preferably divided into four groups of 10 animals, to at least 10 animals held individually (although different designs can be used for flow-through tests);

(d) more specific recommendations have been made with regard to test medium and feeding conditions.

3. Definitions used are set out in Annex 1.

PRINCIPLE OF THE TEST

4. Young female *Daphnia* (the parent animals), aged less than 24 hours at the start of the test, are exposed to the test substance added to water at a range of concentrations. The test duration is 21 days. At the end of the test, the effect of the substance on the numbers of offspring produced by the animals is assessed. From this the lowest observed effect concentration (and hence the no observed effect concentrations) and the concentration

estimated to cause a given reduction in reproductive output (i.e. an EC_x where x is a defined % effect) can be derived.

RING TEST PARTICIPANTS SHOULD NOTE THAT, FOR THE PRESENT, PARAGRAPH 4 IS RATHER VAGUE AND DOES NOT GIVE ANY INDICATION OF HOW THE REPRODUCTIVE OUTPUT OF THE *DAPHNIA* IS EXPRESSED. AS MENTIONED IN THE COVERING LETTER TO THIS DOCUMENT, THERE IS STILL SOME CONTROVERSY ON THIS ISSUE. THE RESULTS FROM THE RING TEST WILL BE ANALYSED IN VARIOUS WAYS AND THE ISSUE WILL BE RESOLVED AT THE WORKSHOP WHICH WILL BE HELD TO DISCUSS THE RESULTS OF THE RING TEST.

5. Substance related effects on other parameters, e.g. survival, intrinsic rate of increase and time to production of first brood, may also be examined.

INFORMATION ON THE TEST SUBSTANCE

6. Results of an acute toxicity test (see Guideline 202 Part 1) performed with Daphnia magna should be available. The result may be useful in selecting an appropriate range of test concentrations in the reproduction tests. The water solubility and the vapour pressure of the test substance should be known and a reliable analytical method for the quantification of the substance in the test solutions with known and reported accuracy and limit of detection should be available.

7. Useful information includes the structural formula, purity of the substance, stability in light, pK_a, P_{ow} and results of a test for ready biodegradability (see Guideline 301).

SOME OF THIS INFORMATION FOR THE RING TEST IS PROVIDED BELOW.

VALIDITY OF THE TEST

8. For a test to be valid, the following performance criteria should be met in the control(s):

- the mortality of the parent animals should not exceed 20% at the end of the test;
- the mean number of live offspring produced per parent animal surviving at the end of the test must be $\geq$ 60.

DESCRIPTION OF THE METHOD

Apparatus

9. Test vessels and other apparatus which will come into contact with the test solutions should be made entirely of glass or other chemically inert material. The test vessels will normally be glass beakers.

Other vessels may be appropriate for particular test substances (e.g. vessels with an airtight seal for volatile substances.)

In addition, some or all of the following equipment will be required:

- **oxygen meter (with microlectrode or other suitable equipment for measuring dissolved oxygen in low volume samples);**
- **adequate apparatus for temperature control;**
- **pH meter;**
- **equipment for the determination of the hardness of water;**
- **equipment for the determination of the total organic carbon concentration (TOC) of water or equipment for the determination of the chemical oxygen demand (COD);**
- **adequate apparatus for the control of the lighting regime and measurement of light intensity.**

Test organism

10. The species to be used in the test is *Daphnia magna* Straus.

11. Preferably, the clone should have been identified by genotyping. Research (1) has shown that the reproductive performance of Clone A[1] (which originated from IRCHA in France) (3) consistently meets the validity criterion of a mean of ≥60 offspring per parent animal surviving when cultured under the conditions described in this protocol. However, other clones are acceptable provided that the *Daphnia* culture is shown to meet the validity criteria for a test. Information on the clone used (e.g. source, whether it has been genetically typed) should be included in the test report.

12. At the start of the test, the animals should be less than 24 hours old and must not be first brood progeny. They should be derived from a healthy stock (i.e. showing no signs of stress such as high mortality, presence of males and ephippia, delay in the production of the first brood, discoloured animals etc.). The age of the stock animals should be greater than 14 days. The stock animals must be maintained in culture conditions (light, temperature, medium, feeding and animals per unit volume) similar to those to be used in the test.

Test medium

13. It is recommended that a fully defined medium be used in this test. This avoids the use of additives (e.g. seaweed, soil extract, etc.), which are difficult to characterise, and therefore improves the opportunities for standardisation between laboratories. Elendt M4 and M7 media (see Annex 2) have been found to be suitable for this purpose. However. other media are acceptable providing the *Daphnia* culture is shown to meet the validity criteria for the test.

[1] Clone A is the same clone as Clone 5 which has been used in previous documents and discussions. The clone will now be referred to as Clone A as this is how this genotype is referenced in Baird *et al* (1991) - see reference (3) in Literature section.

14. If media are used which include organic additives, these additives should be specified clearly and information should be provided in the test report on composition, particularly with regard to carbon content as this may contribute to the diet provided. It is recommended that the TOC and/or COD of the stock preparation of the organic additive is determined and an estimate of the resulting contribution to the TOC/COD in the test medium made. It is recommended that TOC levels in the medium (i.e. before addition of the algae) should be below 2 mg/l.[2]

15. When testing substances containing metals, it is important to recognise that the properties of the test medium (e.g. hardness, chelating capacity) may have a bearing on the toxicity of the test substance. For this reason, a fully defined medium is desirable. With respect to the fully defined Elendt media, medium M7 has a lower concentration of EDTA than medium M4.

Test solutions

16. Test solutions of the chosen concentrations are usually prepared by dilution of a stock solution. The chosen concentrations may also be prepared separately by direct addition of the test substance. Stock solutions should preferably be prepared by dissolving the substance in test medium.

17. The use of solvents or dispersants may be required in some cases in order to produce a suitably concentrated stock solution. However, every effort should be made to avoid the use of such materials. Examples of suitable solvents are acetone, ethanol, methanol, dimethyl sulfoxide, dimethylformamide and methylene glycol. Examples of suitable dispersants are Cremophor RH40, Tween 80, methylcellulose 0.01%, HCO-40.

Solvents are used to produce a concentrated stock solution which can be dosed accurately into water. At the recommended solvent concentration (i.e. $\leq$ 0.I ml/l), they will not increase the water solubility of a substance.

Dispersants may assist in accurate dosing and dispersion but at the recommended concentration ($\geq$ 0.01ml/l) they will not increase the water solubility of a substance.

PROCEDURE

Conditions of Exposure

Duration

18. The test duration is 21 days.

[2] Value taken from some chemical characteristics of an acceptable dilution water given in OECD Test Guideline 210 Fish, Early-Life Stage Toxicity Test (adopted 17 July 1992). These values are the same as those recommended in EPA Guidelines intended for use in developing data on the toxicity of substances subject to regulation under the Toxic Substances Control Act (see 40 CFR Part 797-7-1-92 Edition).

Loading

19. Parent animals are maintained individually, one per test vessel, with 50 ml of medium in each vessel.

Larger volumes may sometimes be necessary to meet requirements of the analytical procedure used for determination of the test substance concentration, although pooling of replicates for chemical analysis is also allowable. For flow-through tests, alternative designs may, for technical reasons, be considered (e.g. four groups of 10 animals in a larger test volume), but any changes to the test design should be reported.

Number of animals

20. For semi-static tests, at least 10 animals individually held at each test concentration and at least 10 animals individually held in the control series.

For flow-through tests, 40 animals divided into four groups of 10 animals has been shown to be suitable (1).

Feeding

21. For semi-static tests, feeding should preferably be done daily, but at least five times per week. Deviations from this (e.g. for flow-through tests) should be reported.

22. During the test the diet of the parent animals should preferably be living algal cells of one of the following: *Chlorella* sp, *Raphidocellis subcapitata*[3] and *Scenedesmus subspicatus*. The diet should be supplied based on the amount of carbon provided to each parent animal. Research (1) has shown that, for *Daphnia magna*, ration levels of between 0.1 and 0.2 mg C/*Daphnia*/day are sufficient for achieving the required number of offspring to meet the test validity criteria.

23. If surrogate measures, such as algal cell number or light absorbance, are to be used to feed the required ration level (i.e. for convenience since measurement of carbon content is time consuming), each laboratory must produce its own nomograph relating the surrogate measure to carbon content of the algal culture (see Annex 3 for advice on nomograph production). Nomographs should be checked at least annually and more frequently if algal culture conditions have changed. Light absorbance has been found to be a better surrogate for carbon content than cell number (4).

24. Care should be taken to minimise the volume of algal culture medium transferred to the test vessels by feeding a concentrated algal suspension. This can be achieved by centrifugation followed by resuspension in distilled water, deionised water or *Daphnia* culture medium.

Light

25. 16 hours light at an intensity not exceeding 800 lux.

[3] new name for *Selenastrum capricornutum*

Temperature

26. The test should be conducted at a temperature within the range 18-22°C. However, for any one test, the temperature should not, if possible, vary by more than 2°C within these limits (e.g. 18-20.19-21 or 20-22°C).

Aeration

27. The test vessels must not be aerated during the test.

Test concentrations

28. Normally there should be at least five test concentrations arranged in a geometric series with a separation factor not exceeding 3.2. It is desirable that even at the highest concentration used in the test, mortality should be relatively low. If a NOEC is to be derived, the reproductive output of animals in the lowest test concentration, should not be significantly (p=0.05) lower than that in the control. **ANNEX 4 PROVIDES INFORMATION ON HOW TO PREPARE STOCK SOLUTIONS AND GIVES TEST CONCENTRATIONS WHICH MUST BE USED FOR THE RING TEST SUBSTANCES (NB. IT IS NOT NECESSARY TO USE SOLVENTS OR DISPERSANTS FOR THE RING TEST SUBSTANCES).**

29. Where a solvent or dispersant is used to aid preparation of test solutions, its concentration should not be greater than 0.1 ml/l and should be the same in all test vessels.

Controls

30. One test-medium control series

and also, if relevant, one control series containing the solvent or dispersant should be run in addition to the test series.

31. Generally in a well-run test, the coefficient of variation around the mean number of living offspring produced per parent animal in the control(s) should be ≤25%, and this should be reported for test designs using individually held animals.

Test medium

32. For semi-static tests, the test medium should be renewed at least three times per week. Test vessels are prepared and the parent animals transferred to them by, for example, a glass pipette of suitable diameter. The volume of medium transferred with the *Daphnia* should be minimised.

33. During the test, the dissolved oxygen concentration should be above 3 mg/l and the pH should not vary by more than 1.5 units.

Observations

34. The results of the observations made during the test should be recorded on data sheets. If other measurements are required (see paragraph 5 and 37), additional observations may be required.

0ffspring

35. Preferably, for each parent animal, the offspring produced should be removed and counted daily from the appearance of the first brood to prevent them consuming food intended for the adult. For the purpose of this guideline it is only the number of living offspring that needs to be counted, but the presence of aborted eggs or embryos should be recorded. USE DATA SHEET PROVIDED IN ANNEX 5.

Mortality

36. Any mortality among the parent animals should be recorded daily.

USE DATA SHEET PROVIDED IN ANNEX 5.

Other parameters

37. Although this guideline is designed principally to assess effects on reproduction, it is possible that other effects may also be sufficiently quantified to allow statistical analysis. Other parameters that can be measured or calculated include survival and growth (weight/Iength) of parent animals and time to production of first brood (and subsequent broods), number and size of broods per animal, number of aborted broods, presence of males or ephippia and the intrinsic rate of increase.

Frequency of analytical determinations and measurements

38. Oxygen concentration, temperature, hardness and pH values should be measured at least once a week, in fresh and old media, in the control(s) and in the highest concentration.

USE DATA SHEET PROVIDED IN ANNEX 5.

39. During the test, the concentrations of test substance are determined at regular intervals.

40. In semi-static tests where the concentration of the test substance is expected to remain within ±20% of the nominal, it is recommended that, as a minimum, the highest and lowest test concentrations be analysed when freshly prepared and at the time of renewal on one occasion during the first week of the test (i.e. analyses should be made on the same solution - when freshly prepared and at renewal). These determinations should be repeated at least at weekly intervals thereafter. For tests where the concentration of the test substance is not expected to remain within ±20% of the nominal, it is necessary to analyse all test concentrations but following the same regime as described for more stable substances. Determination of test substance concentrations prior to renewal need only be performed on one replicate vessel at each test concentration.

ANNEX 4 PROVIDES GUIDANCE ON TECHNIQUES FOR DETERMINING THE CONCENTRATIONS OF THE RING TEST SUBSTANCES AND FREQUENCY OF ANALYSIS. USE DATA SHEETS PROVIDED IN ANNEX 6 TO RECORD MEASURED CONCENTRATIONS.

41. If a flow-through test is used, similar sampling regime to that described for semi-static tests is appropriate. However, it may be advisable to increase the number of sampling occasions during the first week (e.g. three sets or measurements) to ensure that the test concentrations are remaining stable. In these types of test, the flow-rate of diluent and test substance should be checked daily.

42. If there is evidence that the concentration of the substance being tested has been satisfactorily maintained within ±20% of the nominal concentration throughout the test, then results can be based on nominal values. If the deviation from the nominal concentration is greater than ±20%, results should be expressed in terms of the mean measured concentration.

DATA AND REPORTING

Treatment of results

43. ANALYSIS OF THE DATA FROM THE RING TEST WILL BE CARRIED OUT BY THE RING TEST ORGANISERS. PARTICIPANTS SHOULD THEREFORE PROVIDE RAW DATA AS SPECIFIED IN PARAGRAPH 44 BELOW.

Test report

44. The test report must include the following:

Test substance:

- physical nature and, where relevant, physiochemical properties;
- chemical identification data, including purity;

Test species:

- the clone, supplier of source (if known) and the culture conditions used.

Test conditions:

- test procedure used (e.g. semi-static or flow-through, volume, loading in number of *Daphnia* per litre);
- photoperiod and light intensity;
- test design (e.g. number of replicates, number of parents per replicate);
- details of culture medium used;
- if used, additions of organic material including the composition, source, method of preparation, TOC/COD of stock preparations, estimation of resulting TOC/COD in test medium; detailed information on feeding, including amount (in mg C/1) and schedule (e.g. type of food(s), including, for algae the specific name (species) and, if known, the strain, the culture conditions);

- method of preparation of stock solutions and frequency of renewal (the solvent or dispersant and its concentration must be given, when used);

Results:

- the nominal test concentrations and the results of all analyses to determine the concentration of the test substance in the test vessels (Use data sheets provided in Annex 6);
- water quality within the test vessels (i.e. pH, temperature and dissolved oxygen concentration, and TOC and/or COD and hardness where applicable) (Use data sheets provided in Annex 5);
- the full record of living offspring by each parent animal (Use data sheets provided in Annex 5); the number of deaths among the parent animals and the day on which they occurred (Use data sheets provided in Annex 5);
- any other parameters such as those described in paragraph 37, if measured;
- explanation for any deviation from the Test Guideline.

LITERATURE

(1) OECD Test Guidelines Programme. Report of the workshop on the *Daphnia magna* Final Ring Test, Sheffield University, UK, 20-21 March 1993.

(2) Final Ring Test report (to be referenced after completion of ring test).

(3) Baird, D.J. *et al* (1991). A comparative study of genotype sensitivity to acute toxic stress using clones of *Daphnia magna Straus.* Ecotoxicology and Environmental Safety, **21**, 257-265.

(4) Sims, I. (1993). Measuring the growth of phytoplankton: the relationship between total organic carbon with three commonly used parameters of algal growth. Arch. Hydrobiol., **128**, 459-466.

ANNEX I

DEFINITIONS AND UNITS

For the purpose of this Guideline the following definitions are used:

Parent animals are those animals present at the start of the test and of which the reproductive output is the object of the study.

Offspring are the young *Daphnia* produced by the parent animals in the course of the test.

TOC is Total Organic Carbon.

COD is Chemical Oxygen Demand.

ANNEX 2

PREPARATION OF FULLY DEFINED ELENDT M7 AND M4 MEDIA

Acclimation to Elendt M4 and M7 media

Some laboratories have experienced difficulty in directly transferring *Daphnia* to M4 and M7 media. However, some success has been achieved with gradual acclimation, i.e. moving from own medium to 30% Elendt, then to 60% Elendt and then to 100% Elendt. The acclimation periods may need to be as long as one month. **IF YOU HAVE ANY PROBLEMS, PLEASE CONTACT PETER CALOW.**

PREPARATION

Trace elements

Separate stock solutions (I) of individual trace elements are first prepared in deionised water. From these different stock solutions (I) a second single stock solution, stock solution II is prepared, which contains all trace elements (combined solution), i.e:

Stock solution(s) I (single substance)	Amount added to deionised water	Concentration (in relation of medium M4)	To prepare the combined stock solution II add the following amount of stock solution I to deionised water	
	mg/l		ml/l	
			M 4	M 7
H_3BO_3	57190	20 000-fold	1.0	0.25
$MnCl_2$ * 4 H_2O	7210	20 000-fold	1.0	0.25
LiCl	6120	20 000-fold	1.0	0.25
RbCl	1420	20 000-fold	1.0	0.25
$SrCl_2$ * 6 H_2O	3040	20 000-fold	1.0	0.25
NaBr	320	20 000-fold	1.0	0.25
Na_2MoO_4 * 2 H_2O	1260	20 000-fold	1.0	0.25
$CuCl_2$	335	20 000-fold	1.0	0.25
$ZnCl_2$	260	20 000-fold	1.0	1.0
$CoCl_2$ * 6 H_2O	200	20 000-fold	1.0	1.0
KI	65	20 000-fold	1.0	1.0
Na_2SeO_3	43.8	20 000-fold	1.0	1.0
NH_4VO_3	11.5	20 000-fold	1.0	1.0
Na_2EDTA * 2 H_2O	5000	2 000-fold	-	-
$FeSO_4$ * 7 H_2O	1991	2 000-fold	-	-
Both Na_2EDTA and $FeSO_4$ solutions are prepared singly, poured together and autoclaved immediately. This gives:				
21 Fe-EDTA solution		1 000-fold	20.0	5.0

M4 and M7 media

M4 and M7 media are prepared using stock solution II, the macro-nutrients and vitamins as follows:

	Amount added to deionised water	**Concentration (related to medium M4)**	**Amount of stock solution added to prepare medium**	
	mg/l		**ml/l**	
			M 4	**M 7**
Stock solution II (combined trace elements)		20-fold	50	50
Macro nutrient stock solutions (single substance)				
C_aCl_2 *2 H_2O	293 800	1 000-fold	1.0	1.0
$MgSO_4$ *7 H_2O	246 600	2 000-fold	0.5	0.5
KCl	58 000	10 000-fold	0.1	0.1
$NaHCO_3$	64 800	1 000-fold	1.0	1.0
Na_2SiO_3 *9 H_2O	50 000	5 000-fold	0.2	0.2
$NaNo_3$	2 740	10 000-fold	0.1	1.0
KH_2PO_4	1 430	10 000-fold	0.1	1.0
K_2HPO_4	1 840	10 000-fold	0.1	1.0
Combined vitamin stock	-	10 000-fold	0.1	0.1
The combined vitamin stock solution is prepared by adding the 3 vitamins to II deionised water, as shown below:				
	mg/l			
Thiamine hydrochloride	750	10 000-fold		
Cyanocobalamine (B12)	10	10 000-fold		
Biotine	7.5	10 000-fold		

The combined vitamin stock is stored frozen in small aliquots. Vitamins are added to the media shortly before use.

N.B. To avoid precipitation of salts when preparing the complete media, add the aliquots of stock solutions to about 500-800 ml deionised water and then fill it up to 1litre.

ANNEX 3

TOC ANALYSIS AND PRODUCTION OF A NOMOGRAPH FOR TOC CONTENT OF ALGAL FEED

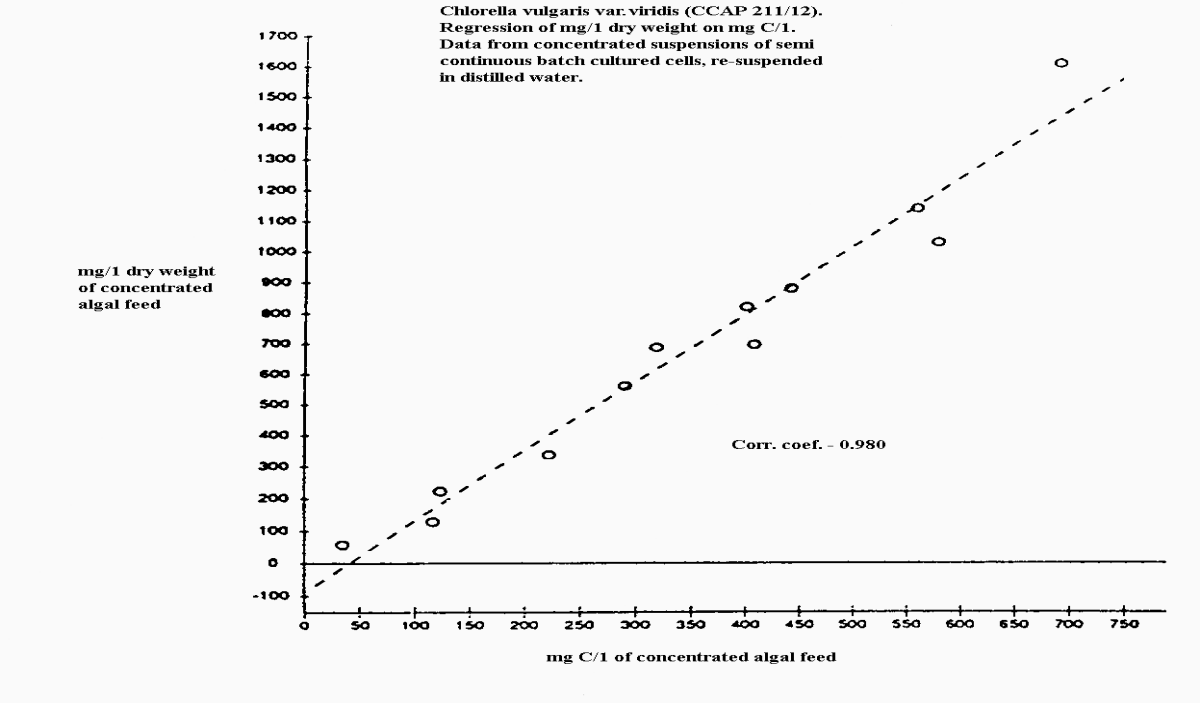
It is recognised that the carbon content of the algal feed will not normally be measured directly but from correlations (i.e. nomographs) with surrogate measures such as algal cell number or light absorbance.

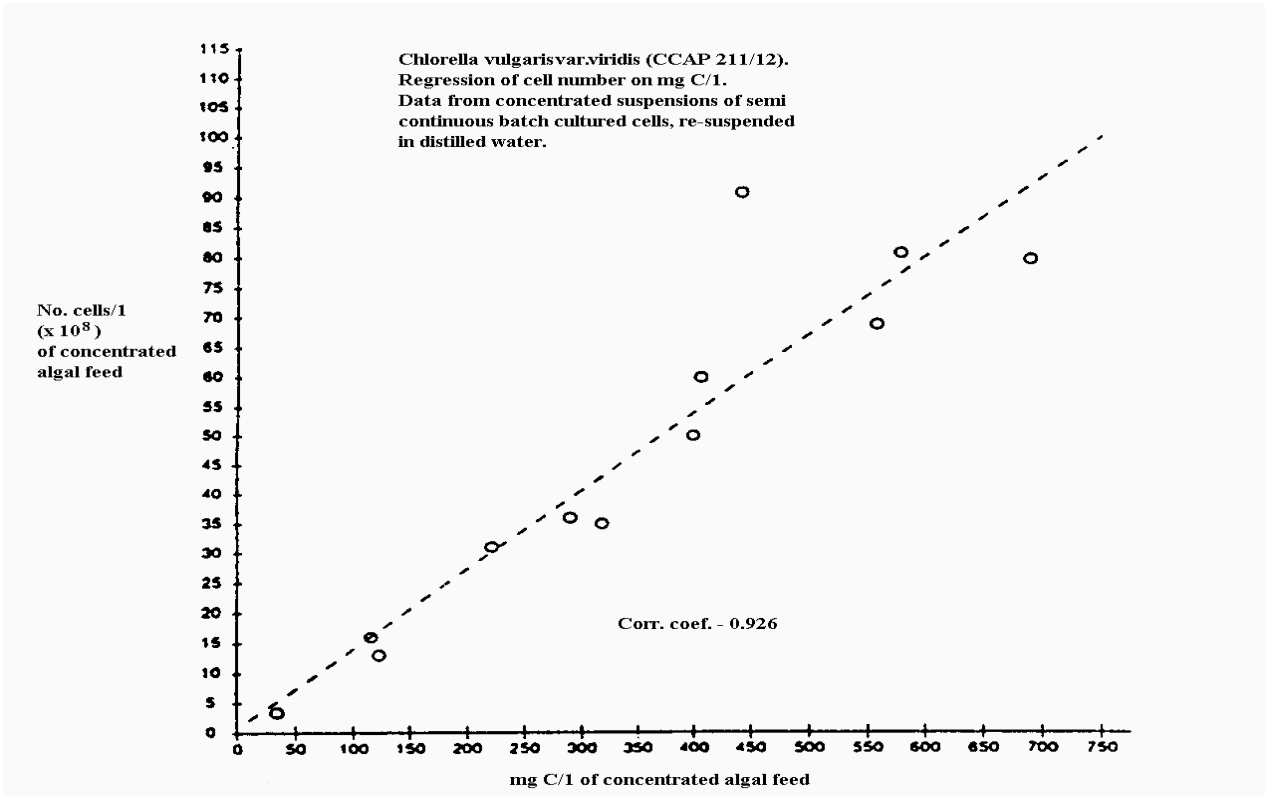
TOC should be measured by high temperature oxidation rather than by UV or persulphate methods. (For advice see: The Instrumental Determination of Total Organic Carbon, Total Oxygen Demand and Related Determinants 1979, HMSO 1980; 49 High Holborn, London WCIV 6HB). **IF YOU NEED FURTHER ADVICE, CONTACT PETER CALOW.**

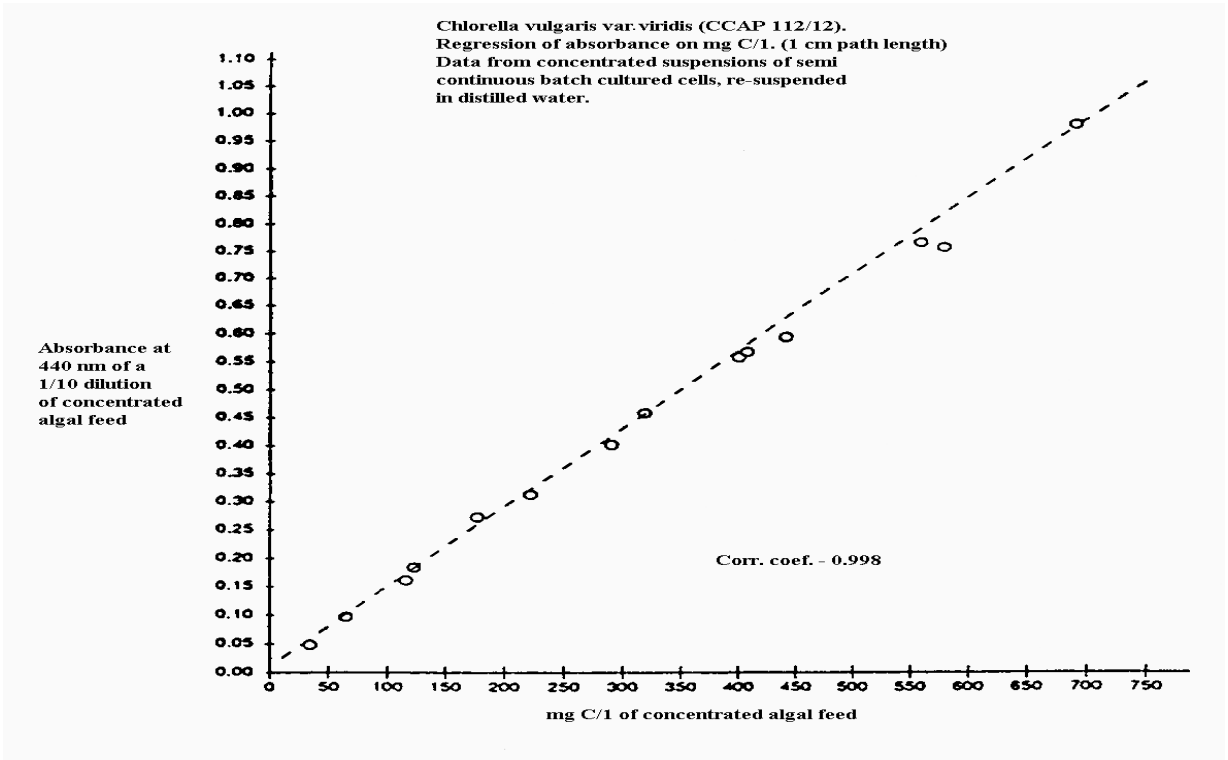
For nomograph production, algae should be separated from the growth medium by centrifugation followed by resuspension in distilled water. Measure the surrogate parameter and TOC concentration in each sample in triplicate. Distilled water blanks should be analysed and the TOC concentration deducted from the algal sample TOC concentration.

Nomographs should be linear over the required range of carbon concentrations. Examples are shown below.

N.B. THESE SHOULD NOT BE USED FOR CONVERSIONS; IT IS ESSENTIAL THAT YOU PREPARE YOUR OWN NOMOGRAPHS.




Chlorella vulgarisvar.viridis (CCAP 211/12).
Regression of cell number on mg C/1.
Data from concentrated suspensions of semi continuous batch cultured cells, re-suspended in distilled water.
No. cells/1 (x 10^8) of concentrated algal feed
Corr. coef. - 0.926
mg C/1 of concentrated algal feed


Chlorella vulgaris var.viridis (CCAP 112/12).
Regression of absorbance on mg C/1. (1 cm path length)
Data from concentrated suspensions of semi continuous batch cultured cells, re-suspended in distilled water.
Absorbance at 440 nm of a 1/10 dilution of concentrated algal feed
Corr. coef. - 0.998
mg C/1 of concentrated algal feed

ANNEX 4

GUIDANCE ON THE PREPARATION AND ANALYSIS OF AQUEOUS TEST SOLUTIONS OF RING TEST SUBSTANCES

I. CADMIUM

1. Test chemical

Name: Cadmium chloride

Chemical formula: $CdCl_2$

Supplier: Aldrich Chemical Company Ltd.
The Old Brickyard
New Road
Gillingham
Dorset
SP8 4Jl
England

Purity: 99.99% pure

2. Test concentrations

The following five nominal concentrations must be tested:

- 0.2 µg/l
- 0.4 µg/l
- 0.8 µg/l
- 1 µg/l
- 2 µg/l

A control will comprise the dilution medium used to prepare the test solutions.

3. Preparation of stock solutions

First dry the solid $CdCl_2$ for 24 hours at a temperature in excess of I00°C. This will reduce the solid test substance to the anhydrous form prior to use.

N.B. If this pre-treatment is not carried out, then water content must be accounted for in all calculations. The following methods for calculating stock and test solutions assumes the anhydrous solid is being used.

Calculate the amount of solid test substance required.

$CdCl_2$ fw / Cd aw = 183.3 / 112.4 = 1.631 (Cd ratio)

fw = formula weight; aw = atomic weight

Solid required (mg) = (1.631)x; where x = required stock concentration (mg/l)

e.g.; 1mg/l = (1.631)l = 1.631 mg

We recommend a stock solution of 1 mg/l. This solution should be kept cool (< 5ºC) and stored for no longer than 72 hours. New stock solutions must be prepared at least every 72 hours.

4. Preparation of test solutions

Test solutions can be prepared by serial dilution of the stock solution.

5. Analysis of the test solutions

AAS*:* Atomic Absorption Spectrometry

Two methods can be used depending on the concentration of cadmium in solution.

5.1 Graphite Furnace AAS

Machine used: Perkin-Elmer 2100 AAS with a HGA 700 and an automatic sampler.

NB: Use of an automatic sampler gives good repeatability.

(The furnace used at Sheffield is a pyroitically coated graphite tube with a platform)

Machine specifications:

Cadmium lamp: current = 4mA

Wavelength: 228.8 nm

Slit width: 0.7 nm (low)

Matrix modifier used: $NH_4H_2PO_4$ conc. $0.2mgl^{-1}$

This apparatus uses a 6 step temperature regime:

STEP	TEMPºC	RUNNING TIME (sec)	RAMP TIME	INTERNAL GAS FLOW
1	90	10	1	300
2	120	20	1	300
3	650	10	1	300
4	20		1	300
5	1600	5 reading taken	0.1	100
6	2700	3 burn off	1	300

Injection temperature: 50°C
Linear to: 50 µg/l
Sensitivity levels: lowest 0.1-0.5µg/l

5.2 Flame AAS

(System used at Sheffield is Perkin-Elmer 2100 AAS with a standard Perkin-Elmer AS-50 automatic sampler)

Machine Specifications:

Cd lamp: current = 4mA
Wavelength: 228.8 nm
Slit width: 0.7 nm (low)
Air-acetylene flame - uses large 10 cm burner (more sensitive than 5 cm)
Fuel flow = 2.6 l/min
Oxidant flow = 7 l/min

Sampling:

Cd flame check -- using 2mg/l standard check baseline absorbance unit is the same as or less than 0.028 (this is the Cd flame characteristic concentration, and is without units).

Integration time 4 secs
2 replicates per sample
Read delay 4 secs

Additional information:

(i) BACKGROUND CORRECTOR: Generally not used with cadmium samples (except digests) because of low contamination problems.

(ii) BURNER POSITION: This will depend largely on the system and varies greatly.

(iii) Impact bead is NOT used but is replaced by a FLOW SPOILER instead.

(iv) LAMP TYPE: Not specific.

6. Schedule for analysis of the test solutions

Freshly prepared test solutions at each exposure concentration should be analysed on one occasion each week through the test. The same solutions should again be analysed at the time of renewal in order to assess the stability of the exposure concentrations. The results of the analysis are to be reported on the appropriate data sheet (Annex 6).

Repeat analyses should be carried out on selected samples in order to assess the repeatability of the procedure. These are to be reported.

II. 3, 4-DICHLOROANILINE

1. Test chemical

Name:	3,4-dichloroaniline (3,4-DCA)
Chemical formula:	$Cl_2C_6H_3NH_2$
Supplier:	Aldrich Chemical Company Ltd. The Old Brickyard New Road Gillingham, Dorset SP8 4JL, England
Purity:	98%

2. Test concentrations

The following five nominal concentrations must be tested:

- 2.5 µg/l
- 5.0 µg/l
- 10.0 µg/l
- 20.0 µg/l
- 40.0 µg/l

A control will comprise the dilution medium used to prepare the test solutions.

3. Preparation of stock solutions

The water solubility of 3,4-DCA at 20°C is approximately 600 mg/l. However, to ensure that true solutions are achieved in this s study it is advised that no stock solutions are prepared at concentrations exceeding 50 mg/l.

A master solution can best be prepared by adding finely crushed (i.e. using a pestle and mortar) 3,4 DCA to the dilution medium (there is no need for a solvent carrier). The solution must be prepared in either a clear glass vessel covered to exclude light or in a brown glass vessel. The mixture should be stirred for 24 hours or sonicated for 1 hour after which it should be checked to ensure that all the chemical has dissolved. Further stirring/sonicating should be carried out if necessary.

A stock solution of suitable concentration (say 1mg/l) to dose the test vessels should be prepared by dilution of the master solution. The concentration of 3,4-DCA in the stock solution should be determined by analysis prior to preparing the test solutions. Once prepared the stock solution must be stored in the dark at 4°C between use. Stored in this way it should

be stable for at least 21 days. However, it is advisable to check this during the course of the study.

4. Preparation of test solutions

Test solutions can be prepared by serial dilution of the stock solution.

5. Analysis of the test solutions

5.1 Material and equipment

(i) Normal laboratory glassware and equipment.

(ii) 3,4-DCA reference standard (purity 98%). Solutions are prepared in the hplc mobile phase to externally calibrate the hplc system. A typical concentration range for standard solutions is 0.01 to 0.1 mg/l.

(iii) Acetonitrile (hplc grade).

(iv) Water (glass distilled).

(v) Bond Elut C_{18} cartridges (3 ml capacity).

(vi) Varian liquid chromatography model 5000 fitted with a variable wavelength UV absorbance detector (model UV. I00) and autosampler.

5.2 Extraction and concentration of water samples

(i) Acetonitrile (10 ml)

(ii) Water (15 ml)

A 100 ml water sample is then passed through a pre-washed cartridge. This volume is normally within the breakthrough volume for 3,4 DCA using a 3 ml capacity cartridge. However each batch of cartridges is to be tested for performance. Water sample flow through the column is maintained at 3 to 5 ml by applying vacuum.

DCA is eluted from the Bond Elut cartridge with acetonitrile (3 ml) and the final volume of the eluate is adjusted to 5 or I0 ml with distilled water. The water extracts are then ready for analysis.

5.3 Water sample analysis by HPLC

The following conditions are used:

Column:	250 x 4.9 mm id stainless steel tube packed with ODS 3-5 µm particle.
Mobile phase:	acetonitrile/water mixture, proportions 70 + 30 by volume.
Flow rate:	1 ml/mn.
Detector:	wavelength 247 nm absorbance range 0.002.

6. Schedule for analysis of the test solutions

Freshly prepared test solutions at each exposure concentration should be analysed on one occasion each week throughout the test. The same, but old, solutions should again be analysed after transferring the daphnids to fresh test medium in order to assess the stability of the exposure concentrations. The old solutions will need to be pooled together in order to obtain a sufficiently large sample for analysis (100 ml).

The results of the analysis are to be reported on the appropriate data sheet (Annex 6).

Repeat analyses should be carried out on selected samples in order to assess the repeatability of the procedure. The repeat analyses are to be reported.

III. PHENOL

1. Test chemical

Name:	phenol
Chemical formula:	C_6H_6O
Supplier:	Aldrich Chemical Company Ltd. The Old Brickyard New Road Gillingham, Dorset SP8 4JL, England
Purity:	99%+

2. Physical properties that may influence test result

Both 3,4 DCA and cadmium chloride are relatively stable under the conditions of the test. Phenol, however, is less so. Its volatility is moderate (Henry's Constant 4.0×10^{-7} bar m^3/mol) but losses, probably due to degradation have been observed at the lower concentrations. Therefore. its inclusion in the ring test will provide data on the use of "difficult substances", as the results will be strongly influenced by the maintenance of concentrations. In order to reduce variability due to this factor, strict adherence to the medium renewal regime must be observed. Also, 150 ml tall-form beakers (c. 5 cm diameter, 9 cm high) are recommended as test vessels, with 100 ml test volumes. (This is a divergence from the Guidelines allowed only for Phenol; 100 ml test volumes are more stable than 50 ml.)

3. Test concentrations

The following five nominal concentrations must be tested:

- 0.32 mg/l
- 0.56mg/l
- 1.0 mg/l
- 1.8 mg/l
- 3.2 mg/l

A control will comprise the dilution medium used to prepare the test solutions.

4. Preparation of stock solutions

The water solubility of phenol at 20°C is approximately 87 g/l. It is advised that stock solutions are prepared at 50 mg/l.

Aqueous stock solutions can best be prepared by adding phenol to the dilution medium. A solvent carrier should not be used. The solutions should be freshly prepared, in volumetric glassware, on each day of use. The mixture should be stirred for at least 30 minutes to ensure dissolution.

5. Preparation of test solutions

Test solutions can be prepared by serial dilution of the stock solution

6. Analysis of the test solutions

6.1 Materials and equipment

Column:	Spherisorb ODS 1,25 cm x 4.6 mm i.d
Flow rate:	1.0 ml/mn
Mobile phase:	80% acetonitrile, 20% water (double distilled)
Temperature:	ambient
Injection volume	10 µl
Detector (UV) wavelength:	220 nm
Instrument:	Hewlett Packard 1050
Sample preparation:	200 µl of sample added to 800 µl acetonitrile

6.2 Spectrophotometric detection of phenol

We used Unicam UV silica spectrophotometer cells (Cat. No. 9423 168 104220) of 1 cm path length.

(Note: this Cat. No. is for a matched pair, the cost being £80.85.)

Supplier:

Unicam Ltd
York Street
Cambridge
CB1 2XP
England

Maximum absorbance was found at 268 nm using a Beckman DU 65 spectrophotometer.

7. Schedule for analysis of the test solutions

Freshly prepared test solutions at each exposure concentration should be analysed on one occasion each week throughout the test. The same solutions should again be analysed at the time of renewal in order to assess their stability. Using UV spectroscopy the concentrations in the vessels may be followed closer if required. The results of the weekly analyses are to be reported on the appropriate data sheet (Annex 6). If more frequent analyses are conducted, these should also be reported.

Repeat analysis should be carried out on selected samples in order to assess the repeatability of the procedure. The repeat analyses are to be reported.

ANNEX 5

EXAMPLE DATA SHEET FOR RECORDING MEDIUM RENEWAL, PHYSICAL/CHEMICAL MONITORING DATA, FEEDING, DAPHNIA REPRODUCTION AND ADULT MORTALITY

Experiment No: Date started: Clone: Medium: Type of food: Test substance: Nominal conc:

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		
Medium renewal (tick)																								
pH♦																							new	
																							old	
O_2 (mg/l)♦																							new	
																							old	
Temp (°C)♦																							new	
																							old	
Food provided (tick)																								
No. live offspring*																								Total
Vessel 1																								
2																								
3																								
4																								
5																								
6																								
7																								
8																								
9																								
10																								
																							Total	
Cumulative adult mortality♠																								

♦ Indicate which vessel was used for the experiment ♠ Record mortality of any adult animals as 'M' in relevant box * Record aborted broods as 'AB' in relevant box

ANNEX 6

DATA SHEET FOR RECORDING RESULTS OF CHEMICAL ANALYSIS

(a) Measured concentrations

Nominal conc.	Week 1 sample		Week 2 sample		Week 3 sample	
	Fresh	Old	Fresh	Old	Fresh	Old

(b) Measured concentrations as a percentage of nominal

Nominal conc.	Week 1 sample		Week 2 sample		Week 3 sample	
	Fresh	Old	Fresh	Old	Fresh	Old

N.B. Space is provided in the tables for any repeat analyses of samples

APPENDIX C

SPREADSHEET

The spreadsheet developed for this ring test had provision for recording initial data. This included the laboratory, test substance, diet, experiment number, clone of *Daphnia*, start date, medium and concentration used. The remainder of the spreadsheet was presented in the form of a work book, one page of the work book for each treatment. Each page included a section for recording water quality, feeding and medium renewals. The remainder of the page comprised a section for recording the numbers of live juveniles produced and numbers of dead adults observed. Distributed with the spreadsheet was a questionnaire on its use. Participants were requested to complete this and return it to the organisers so that an assessment of the spreadsheet's usefulness could be made.

Only 23 (40%) of 58 recipients of the spreadsheet questionnaires returned them (Table A1).

Table A1: Responses to the spreadsheet questionnaire.

Question	Number Responding	
	Yes	No
Did they have the software (Excel 4.0)?	18	4
For those who had the software:		
Were they successful in using the spreadsheet?	13	5
Were the instructions clear and unambiguous?	14	3
Was the option chart easy to use?	15	2
Was the layout suitable for data input?	14	2
Did they have problems entering spreadsheet?	4	14
Did they have problems going from page to page?	2	15
Did they have problems going from cell to cell?	1	15
Did they have problems exiting spread sheet?	6	10

The decision to use Microsoft Excel for the spreadsheet was vindicated by the fact that 82% of those who responded had this software. One participant installed it in order to use the spreadsheet.

Most participants that had Excel and responded (72%) were successful in using the spreadsheet. Those who were unsuccessful said that they found macro errors at various points. This appeared to be due to a problem in compatibility between the English and German versions of Excel. In one case, where Excel 4.0 G was used, file names would not be accepted.

The majority of respondents (82%) said that the instructions supplied were clear and unambiguous, while 88% said that the option chart was easy to use. Most (88%) said that the layout of the spreadsheet was suitable for the purpose of the ring test. However, one participant pointed out that the spreadsheet did not meet Good Laboratory Practice (GLP) data recording standards. A total of 22% said that they had problems accessing the spread sheet, 12% experienced problems moving between pages, 6% (1 respondent) said that movement from cell to cell within a page was problematic and 38% said that they experienced problems exiting the work book. In one case the spreadsheet did not respond to the "end" button.

The comments received showed that some participants thought the fonts used were too small. Others said that they would have liked some space for including results from data analysis and/or a comments section. One respondent indicated that the use of the American notation for the date caused some confusion.

When it came to extracting data from the spreadsheets for data analysis, the use of higher versions of Excel than the one used when compiling the spreadsheet (4.0) caused problems. These were overcome by using a higher version (5.0) to save the affected work books in version 4.0 mode. During the data extraction it was hard to obtain printouts or to get an overview of the data, points also raised by some participants.

These responses show that most participants were happy with the spreadsheet. However, it was clear that some were unsuccessful in using it. The incompatibility between different language versions of Excel had not been foreseen, nor the incompatibility between different versions of Excel. GLP issues of electronic data capture were not addressed while designing the spreadsheet.

Clearly, if this package is to be developed further, as a contribution to the handbook and software package as outlined by Pack (1993) (point 3 in Section 5.1 of this report), it must be written using software which is robust across different nationalities and languages and which is stable over time and not subject to constant updating and revision. These criteria would be fulfilled by using, for example, Visual Basic. Provision of an area on the spreadsheet for comments would be useful, as would improvements to the water quality section. Some further development would be required with a view to improving the method for copying pages in the work book, providing a facility to review the data and improving the method for obtaining hard copy.

Comment: **The problems identified with the current spreadsheet are surmountable. If the OECD wish to pursue the aim of providing tools to standardise data capture and statistical methods, a principle with applications and implications for other Effects on Biotic Systems Guidelines, further work**

would be required. This exercise has been useful in promoting discussion and highlighting the strengths and weaknesses of the spreadsheet approach used.

If the spreadsheet is to be developed further, perhaps with a statistical package for data analysis, this should be done using a "stable" and universal language such as Visual Basic.

APPENDIX D

STATISTICAL ANALYSIS OF FECUNDITY DATA FOR THE THREE TEST SUBSTANCES

NOTE:

RV = response variable, code numbers given in this report
LSD = lowest significant difference
SE = standard error
B = slope parameter
C = expected control response

L01C1 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			58.5	53.8	48.1	49.1	50.6	49.9		
Mortalities:			0	0	1	0	0	0		
Broods/Adult:			4.4	4.0	3.3	2.8	4.6	4.3		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-21.6	*	*	*	*	*	*	*	*
2	*	-14.3	*	*	*	*	*	*	*	*
3	*	-21.3	*	*	*	*	*	*	*	*
4	*	-20.9	*	*	*	*	*	*	*	*
5	*	-20.6	*	*	*	*	*	*	*	*
6	0.40	-16.1	*	*	*	*	*	*	*	*
7	0.40	-16.1	*	*	*	*	*	*	*	*

L02C1 Nominal

Concentrations:			0.00	0.02	0.40	0.80	1.00	2.00		
Juveniles/Adult:			98.7	76.2	53.0	21.7	11.3	5.2		
Mortalities:			0	0	0	1	1	5		
Broods/Adult:			4.9	4.5	3.6	1.6	1.3	0.9		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.20	-25.3	0.41	0.05	0.20	0.04	0.14	*	2.00	97.97
2	0.20	-26.2	0.43	0.05	0.22	0.05	0.16	0.04	2.17	95.94
3	0.20	-25.7	0.41	0.05	0.20	0.04	0.13	*	1.94	4.68
4	0.20	-25.8	0.40	0.05	0.20	0.04	0.13	*	1.93	5.46
5	0.20	-26.0	0.40	0.06	0.20	0.04	0.13	*	1.91	6.56
6	0.20	-23.6	0.95	0.17	0.29	0.11	0.15	0.07	1.18	19.95
7	0.20	-21.7	0.89	0.14	0.31	0.10	0.17	0.08	1.33	19.79

L02C1 Actual

Concentrations:			0.00	0.31	0.46	0.69	1.05	2.01		
Juveniles/Adult:			98.7	76.2	53.0	21.7	11.3	5.2		
Mortalities:			0	0	0	1	1	5		
Broods/Adult:			4.9	4.5	3.6	1.6	1.3	0.9		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.31	-25.3	0.45	0.04	0.26	0.04	0.19	*	2.48	101.25
2	0.31	-26.2	0.47	0.04	0.28	0.04	0.21	*	2.69	99.88
3	0.31	-25.7	0.45	0.05	0.25	0.04	0.18	*	2.43	4.83
4	0.31	-25.8	0.45	0.05	0.25	0.04	0.18	*	2.42	5.64
5	0.31	-26.0	0.45	0.05	0.25	0.04	0.18	*	2.40	6.77
6	0.31	-23.6	0.94	0.15	0.33	0.10	0.18	0.07	1.32	20.34
7	0.31	-21.7	0.88	0.11	0.37	0.09	0.22	0.07	1.59	20.16

L02C2 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			128.5	84.5	33.6	15.8	7.3	1.9		
Mortalities:			0	1	0	0	3	8		
Broods/Adult:			4.9	3.6	1.8	1.3	1.1	0.6		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-15.7	0.26	0.02	0.13	0.02	0.09	*	2.01	129.62
2	*	-16.6	0.26	0.02	0.13	0.02	0.09	*	1.99	130.43
3	*	-15.3	0.26	0.02	0.13	0.02	0.09	*	2.00	6.20
4	*	-15.4	0.26	0.02	0.13	0.02	0.09	*	1.99	7.24
5	*	-15.5	0.26	0.02	0.13	0.02	0.09	*	1.99	8.71
6	0.20	-18.0	0.63	0.06	0.30	0.05	0.19	0.04	1.84	26.35
7	0.20	-19.2	0.64	0.06	0.31	0.06	0.20	0.05	1.86	26.50

L02C2 Actual

Concentrations:			0.00	0.42	0.48	1.07	0.97	2.02		
Juveniles/Adult:			128.5	84.5	33.6	7.3	15.8	1.9		
Mortalities:			0	1	0	0	3	8		
Broods/Adult:			4.9	3.6	1.8	1.3	1.1	0.6		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-15.7	0.39	0.03	0.24	*	0.18	*	2.76	130.40
2	*	-16.6	0.39	0.03	0.23	*	0.17	*	2.63	130.20
3	*	-15.3	0.39	0.03	0.23	*	0.17	*	2.70	6.22
4	*	-15.4	0.39	0.03	0.23	*	0.17	*	2.69	7.25
5	*	-15.5	0.39	0.03	0.23	*	0.17	*	2.67	8.71
6	0.42	-18.0	0.71	0.07	0.37	0.06	0.25	0.06	2.10	27.16
7	0.42	-19.2	0.74	0.08	0.38	0.08	0.26	0.07	2.10	26.99

L05C1 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			70.9	67.1	67.9	67.7	64.6	57.1		
Mortalities:			0	0	0	0	1	1		
Broods/Adult:			5.2	4.8	5.0	4.9	4.7	4.5		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-20.5	*	*	*	*	*	*	*	*
2	1.00	-10.3	*	*	*	*	*	*	*	*
3	1.00	-18.9	*	*	*	*	*	*	*	*
4	*	-12.6	*	*	*	*	*	*	*	*
5	1.00	-10.2	*	*	*	*	*	*	*	*
6	*	-9.6	*	*	*	*	*	*	*	*
7	*	-8.4	*	*	*	*	*	*	*	*

L07C1 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			95.5	86.1	89.3	90.6	92.8	93.3		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			4.3	4.2	4.5	4.6	4.4	4.2		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-17.3	*	*	*	*	*	*	*	*
2	*	-17.3	*	*	*	*	*	*	*	*
3	*	-17.3	*	*	*	*	*	*	*	*
4	*	-17.3	*	*	*	*	*	*	*	*
5	*	-17.3	*	*	*	*	*	*	*	*
6	*	-14.0	*	*	*	*	*	*	*	*
7	*	-14.0	*	*	*	*	*	*	*	*

L09C1 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			58.3	51.4	61.1	41.8	54.4	45.0		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			3.5	3.2	3.5	2.7	3.5	3.2		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-31.1	*	*	*	*	*	*	*	*
2	*	-31.1	*	*	*	*	*	*	*	*
3	*	-31.1	*	*	*	*	*	*	*	*
4	*	-31.1	*	*	*	*	*	*	*	*
5	*	-31.1	*	*	*	*	*	*	*	*
6	*	-19.9	*	*	*	*	*	*	*	*
7	*	-19.9	*	*	*	*	*	*	*	*

L10C1 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			66.5	67.9	78.4	69.8	74.8	75.6		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			3.9	4.1	4.7	4.3	4.5	4.4		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-22.0	*	*	*	*	*	*	*	*
2	*	-22.0	*	*	*	*	*	*	*	*
3	*	-22.0	*	*	*	*	*	*	*	*
4	*	-22.0	*	*	*	*	*	*	*	*
5	*	-22.0	*	*	*	*	*	*	*	*
6	*	-14.1	*	*	*	*	*	*	*	*
7	*	-14.1	*	*	*	*	*	*	*	*

L10C1 Actual

Concentrations:			0.00	0.29	0.51	0.92	1.10	1.91		
Juveniles/Adult:			66.5	67.9	78.4	69.8	74.8	75.6		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			3.9	4.1	4.7	4.3	4.5	4.4		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-22.0	*	*	*	*	*	*	*	*
2	*	-22.0	*	*	*	*	*	*	*	*
3	*	-22.0	*	*	*	*	*	*	*	*
4	*	-22.0	*	*	*	*	*	*	*	*
5	*	-22.0	*	*	*	*	*	*	*	*
6	*	-14.1	*	*	*	*	*	*	*	*
7	*	-14.1	*	*	*	*	*	*	*	*

L11C1 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			159.5	146.1	142.6	131.3	156.9	161.2		
Mortalities:			0	1	0	2	1	0		
Broods/Adult:			4.8	4.5	4.5	4.2	4.7	4.7		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-18.2	*	*	*	*	*	*	*	*
2	0.20	-10.4	*	*	*	*	*	*	*	*
3	*	-17.2	*	*	*	*	*	*	*	*
4	*	-17.0	*	*	*	*	*	*	*	*
5	0.20	-10.6	*	*	*	*	*	*	*	*
6	*	-9.1	*	*	*	*	*	*	*	*
7	*	-8.3	*	*	*	*	*	*	*	*

L11C1 Actual

Concentrations:			0.00	0.25	0.44	0.83	1.16	2.33		
Juveniles/Adult:			159.5	146.1	142.6	131.3	156.9	161.2		
Mortalities:			0	1	0	2	1	0		
Broods/Adult:			4.8	4.5	4.5	4.2	4.7	4.7		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-18.2	*	*	*	*	*	*	*	*
2	0.25	-10.4	*	*	*	*	*	*	*	*
3	*	-17.2	*	*	*	*	*	*	*	*
4	*	-17.0	*	*	*	*	*	*	*	*
5	0.25	-10.6	*	*	*	*	*	*	*	*
6	*	-9.1	*	*	*	*	*	*	*	*
7	*	-8.3	*	*	*	*	*	*	*	*

L11C2 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			134.8	130.5	122.9	132.9	136.4	133.6		
Mortalities:			0	0	1	0	0	0		
Broods/Adult:			4.0	4.0	3.8	4.0	4.0	4.1		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-11.6	*	*	*	*	*	*	*	*
2	*	-8.8	*	*	*	*	*	*	*	*
3	*	-9.6	*	*	*	*	*	*	*	*
4	*	-8.9	*	*	*	*	*	*	*	*
5	*	-8.8	*	*	*	*	*	*	*	*
6	*	-8.4	*	*	*	*	*	*	*	*
7	*	-8.2	*	*	*	*	*	*	*	*

L11C2 Actual

Concentrations:			0.00	0.24	0.44	0.91	1.18	2.42		
Juveniles/Adult:			134.8	130.5	122.9	132.9	136.4	133.6		
Mortalities:			0	0	1	0	0	0		
Broods/Adult:			4.0	4.0	3.8	4.0	4.0	4.1		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-11.6	*	*	*	*	*	*	*	*
2	*	-8.8	*	*	*	*	*	*	*	*
3	*	-9.6	*	*	*	*	*	*	*	*
4	*	-8.9	*	*	*	*	*	*	*	*
5	*	-8.8	*	*	*	*	*	*	*	*
6	*	-8.4	*	*	*	*	*	*	*	*
7	*	-8.2	*	*	*	*	*	*	*	*

L16C1 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			117.8	94.2	94.5	97.6	100.1	96.1		
Mortalities:			1	2	2	2	1	2		
Broods/Adult:			6.5	5.3	4.8	5.4	6.0	5.3		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-39.0	*	*	*	*	*	*	*	*
2	*	-23.9	*	*	*	*	*	*	*	*
3	*	-37.1	*	*	*	*	*	*	*	*
4	*	-31.2	*	*	*	*	*	*	*	*
5	*	-26.5	*	*	*	*	*	*	*	*
6	*	-20.9	*	*	*	*	*	*	*	*
7	*	-19.2	*	*	*	*	*	*	*	*

L18C1 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			81.3	81.4	82.3	75.3	74.8	55.2		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			4.9	4.9	5.0	5.0	4.9	4.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	1.00	-8.3	2.79	0.22	1.48	0.11	1.02	0.14	2.18	81.88
2	1.00	-8.3	2.79	0.22	1.48	0.11	1.02	0.14	2.18	81.88
3	1.00	-8.3	2.79	0.22	1.48	0.11	1.02	0.14	2.18	3.90
4	1.00	-8.3	2.79	0.22	1.48	0.11	1.02	0.14	2.18	4.82
5	1.00	-8.3	2.79	0.22	1.48	0.11	1.02	0.14	2.18	5.85
6	1.00	-10.0	*	*	*	*	*	*	*	*
7	1.00	-10.0	*	*	*	*	*	*	*	*

L18C1 Actual

Concentrations:			0.00	0.22	0.24	0.54	0.67	1.25		
Juveniles/Adult:			81.3	81.4	82.3	75.3	74.8	55.2		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			4.9	4.9	5.0	5.0	4.9	4.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.67	-8.3	1.70	0.12	0.95	0.07	0.67	0.09	2.37	81.93
2	0.67	-8.3	1.70	0.12	0.95	0.07	0.67	0.09	2.37	81.93
3	0.67	-8.3	1.70	0.12	0.95	0.07	0.67	0.09	2.37	3.90
4	0.67	-8.3	1.70	0.12	0.95	0.07	0.67	0.09	2.37	4.82
5	0.67	-8.3	1.70	0.12	0.95	0.07	0.67	0.09	2.37	5.85
6	0.67	-10.0	*	*	*	*	*	*	*	*
7	0.67	-10.0	*	*	*	*	*	*	*	*

L23C1 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			154.4	146.8	121.5	126.4	137.4	108.8		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			5.4	5.3	4.8	5.1	5.2	5.5		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	1.00	-25.8	*	*	*	*	*	*	*	*
2	1.00	-25.8	*	*	*	*	*	*	*	*
3	1.00	-25.8	*	*	*	*	*	*	*	*
4	1.00	-25.8	*	*	*	*	*	*	*	*
5	1.00	-25.8	*	*	*	*	*	*	*	*
6	1.00	-21.9	3.86	1.60	1.17	0.44	0.58	0.39	1.16	28.01
7	1.00	-21.9	3.86	1.60	1.17	0.44	0.58	0.39	1.16	28.01

L23C2 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			104.6	76.1	56.1	63.4	61.4	55.6		
Mortalities:			0	2	1	0	1	1		
Broods/Adult:			4.9	3.8	3.9	4.8	4.4	3.6		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.20	-32.2	*	*	*	*	*	*	*	*
2	0.20	-28.5	*	*	*	*	*	*	*	*
3	0.20	-31.9	*	*	*	*	*	*	*	*
4	0.20	-31.0	*	*	*	*	*	*	*	*
5	0.20	-27.2	*	*	*	*	*	*	*	*
6	0.20	-23.4	*	*	*	*	*	*	*	*
7	0.20	-24.3	*	*	*	*	*	*	*	*

L25C1 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			73.3	74.6	68.7	73.8	56.1	56.6		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			3.5	3.5	3.5	3.8	3.5	3.9		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.80	-24.4	5.55	4.36	1.42	0.64	0.64	0.55	1.02	74.65
2	0.80	-24.4	5.55	4.36	1.42	0.64	0.64	0.55	1.02	74.65
3	0.80	-24.4	5.55	4.36	1.42	0.64	0.64	0.55	1.02	3.56
4	0.80	-24.4	5.55	4.36	1.42	0.64	0.64	0.55	1.02	4.39
5	0.80	-24.4	5.55	4.36	1.42	0.64	0.64	0.55	1.02	5.33
6	1.00	-24.4	3.36	1.18	1.05	0.40	0.53	0.35	1.19	21.87
7	1.00	-24.4	3.36	1.18	1.05	0.40	0.53	0.35	1.19	21.87

L25C1 Actual

Concentrations:			0.00	0.31	0.48	0.86	1.07	2.01		
Juveniles/Adult:			73.3	74.6	68.7	73.8	56.1	56.6		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			3.5	3.5	3.5	3.8	3.5	3.9		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.86	-24.4	4.90	3.27	1.47	0.60	0.73	0.56	1.15	74.74
2	0.86	-24.4	4.90	3.27	1.47	0.60	0.73	0.56	1.15	74.74
3	0.86	-24.4	4.90	3.27	1.47	0.60	0.73	0.56	1.15	3.56
4	0.86	-24.4	4.90	3.27	1.47	0.60	0.73	0.56	1.15	4.40
5	0.86	-24.4	4.90	3.27	1.47	0.60	0.73	0.56	1.15	5.34
6	1.07	-24.4	3.16	0.96	1.12	0.39	0.61	0.36	1.34	21.90
7	1.07	-24.4	3.16	0.96	1.12	0.39	0.61	0.36	1.34	21.90

L26C1 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			100.4	83.4	87.7	65.9	42.5	4.4		
Mortalities:			0	2	0	0	2	6		
Broods/Adult:			4.1	3.8	4.0	3.6	2.6	0.4		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.40	-30.3	0.98	0.06	0.71	0.07	0.58	*	4.23	90.93
2	0.40	-23.7	1.03	0.07	0.64	0.07	0.49	0.07	2.97	97.04
3	0.40	-30.2	0.98	0.06	0.70	0.07	0.58	*	4.22	4.35
4	0.40	-30.2	0.98	0.06	0.70	0.07	0.58	*	4.21	5.72
5	0.40	-28.8	0.95	0.05	0.68	0.06	0.56	*	4.09	7.34
6	0.40	-14.8	1.79	0.25	0.61	0.13	0.32	0.11	1.29	24.18
7	0.40	-14.8	1.86	0.26	0.65	0.14	0.35	0.11	1.31	24.15

L26C2 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			144.3	124.3	97.0	80.5	41.2	17.6		
Mortalities:			0	0	0	0	0	1		
Broods/Adult:			4.5	4.8	3.9	3.7	2.1	1.3		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.20	-28.8	0.71	0.11	0.33	0.09	0.21	0.07	1.83	139.78
2	0.20	-28.8	0.70	0.11	0.32	0.09	0.21	0.07	1.79	140.33
3	0.20	-28.7	0.71	0.11	0.33	0.09	0.21	0.07	1.83	6.66
4	0.20	-28.7	0.71	0.11	0.33	0.09	0.21	0.07	1.83	8.23
5	0.20	-28.7	0.71	0.11	0.33	0.09	0.21	0.07	1.83	9.99
6	0.20	-21.2	1.25	0.20	0.38	0.13	0.19	0.09	1.17	31.00
7	0.20	-21.4	1.26	0.21	0.38	0.13	0.19	0.09	1.16	31.01

L26C2 Actual

Concentrations:			0.00	0.23	0.40	0.71	1.00	1.64		
Juveniles/Adult:			144.3	124.3	97.0	80.5	41.2	17.6		
Mortalities:			0	0	0	0	0	1		
Broods/Adult:			4.5	4.8	3.9	3.7	2.1	1.3		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.23	-28.8	0.70	0.09	0.37	0.08	0.25	0.07	2.15	138.32
2	0.23	-28.8	0.69	0.09	0.36	0.08	0.24	0.07	2.10	138.98
3	0.23	-28.7	0.70	0.09	0.37	0.08	0.25	0.07	2.15	6.59
4	0.23	-28.7	0.70	0.09	0.37	0.08	0.25	0.07	2.15	8.14
5	0.23	-28.7	0.70	0.09	0.37	0.08	0.25	0.07	2.15	9.89
6	0.23	-21.2	1.13	0.16	0.41	0.12	0.22	0.09	1.37	30.88
7	0.23	-21.4	1.13	0.16	0.41	0.12	0.22	0.10	1.36	30.91

L27C1 Nominal

Concentrations:			0.00	32.0	56.0	100.0	180.0	320.0		
Juveniles/Adult:			72.0	63.5	61.9	52.1	55.7	51.0		
Mortalities:			0	0	0	1	0	1		
Broods/Adult:			4.0	3.9	4.0	3.8	4.0	3.5		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	56.00	-16.0	*	*	*	*	*	*	*	*
2	56.00	-14.3	*	*	*	*	*	*	*	*
3	56.00	-15.5	*	*	*	*	*	*	*	*
4	56.00	-15.3	*	*	*	*	*	*	*	*
5	56.00	-15.1	*	*	*	*	*	*	*	*
6	56.00	-14.9	*	*	*	*	*	*	*	*
7	32.00	-13.7	*	*	*	*	*	*	*	*

L27C2 Nominal

Concentrations:			0.00	5.60	10.00	18.00	32.00	56.00		
Juveniles/Adult:			75.9	59.9	50.5	47.9	27.7	8.3		
Mortalities:			0	0	0	0	0	2		
Broods/Adult:			3.6	4.0	3.5	3.3	2.9	1.4		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-16.0	24.69	2.35	12.76	*	8.67	*	2.10	66.35
2	*	-14.7	24.29	2.26	12.18	*	8.13	*	2.01	67.01
3	*	-16.2	24.48	2.40	12.44	*	8.37	1.84	2.05	3.18
4	*	-16.3	24.40	2.42	12.33	*	8.27	1.84	2.03	4.18
5	*	-16.4	24.33	2.44	12.22	*	8.16	1.84	2.01	5.15
6	*	-15.1	24.43	3.17	6.84	1.73	3.26	*	1.09	18.58
7	*	-14.4	24.15	2.91	7.49	1.74	3.78	1.17	1.19	18.39

L31C1 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			302.6	304.1	274.7	272.6	261.2	190.7		
Mortalities:			0	0	0	0	0	1		
Broods/Adult:			5.8	5.7	5.8	5.8	6.0	5.2		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.80	-13.7	2.77	0.35	1.27	0.19	0.80	0.21	1.77	299.45
2	0.20	-8.2	3.79	0.60	1.34	0.16	0.73	0.16	1.33	302.19
3	0.80	-13.0	2.83	0.36	1.27	0.19	0.80	0.20	1.73	14.27
4	0.80	-12.8	2.84	0.36	1.27	0.18	0.79	0.20	1.72	15.77
5	0.80	-12.1	2.90	0.37	1.28	0.18	0.79	0.19	1.69	18.75
6	0.80	-11.3	3.30	0.52	1.18	0.19	0.65	0.18	1.35	52.65
7	0.20	-8.1	4.35	0.84	1.25	0.18	0.61	0.16	1.12	52.88

L31C1 Actual

Concentrations:			0.00	0.17	0.37	0.72	0.94	1.84		
Juveniles/Adult:			302.6	304.1	274.7	272.6	261.2	190.7		
Mortalities:			0	0	0	0	0	1		
Broods/Adult:			5.8	5.7	5.8	5.8	6.0	5.2		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.72	-13.7	2.55	0.33	1.17	0.17	0.74	0.19	1.78	299.27
2	0.17	-8.2	3.52	0.57	1.23	0.15	0.67	0.15	1.32	302.17
3	0.72	-13.0	2.60	0.33	1.17	0.17	0.74	0.18	1.74	14.26
4	0.72	-12.8	2.62	0.33	1.17	0.17	0.73	0.18	1.73	15.77
5	0.72	-12.1	2.67	0.34	1.18	0.16	0.73	0.17	1.69	18.74
6	0.72	-11.3	3.05	0.48	1.09	0.17	0.59	0.17	1.34	52.64
7	0.17	-8.1	4.03	0.78	1.15	0.16	0.55	0.14	1.11	52.88

L31C2 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			218.1	225.6	199.8	174.5	168.0	42.0		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			7.0	6.6	6.6	6.2	6.2	2.8		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.40	-13.0	1.35	0.05	0.91	0.06	0.72	0.06	3.51	214.24
2	0.40	-13.0	1.35	0.05	0.91	0.06	0.72	0.06	3.51	214.24
3	0.40	-13.0	1.35	0.05	0.91	0.06	0.72	0.06	3.51	10.20
4	0.40	-13.0	1.35	0.05	0.91	0.06	0.72	0.06	3.51	11.28
5	0.40	-13.0	1.35	0.05	0.91	0.06	0.72	0.06	3.51	13.39
6	0.80	-10.3	1.89	0.07	1.09	0.09	0.78	0.09	2.49	32.03
7	0.80	-10.3	1.89	0.07	1.09	0.09	0.78	0.09	2.49	32.03

L31C2 Actual

Concentrations:			0.00	0.17	0.34	0.70	0.92	1.80		
Juveniles/Adult:			218.1	225.6	199.8	174.5	168.0	42.0		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			7.0	6.6	6.6	6.2	6.2	2.8		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.34	-13.0	1.23	0.05	0.83	0.05	0.67	0.05	3.59	212.95
2	0.34	-13.0	1.23	0.05	0.83	0.05	0.67	0.05	3.59	212.95
3	0.34	-13.0	1.23	0.05	0.83	0.05	0.67	0.05	3.59	10.14
4	0.34	-13.0	1.23	0.05	0.83	0.05	0.67	0.05	3.59	11.21
5	0.34	-13.0	1.23	0.05	0.83	0.05	0.67	0.05	3.59	13.31
6	0.70	-10.3	1.71	0.06	0.99	0.08	0.72	0.08	2.53	31.93
7	0.70	-10.3	1.71	0.06	0.99	0.08	0.72	0.08	2.53	31.93

L34C1 Nominal

Concentrations:			0.00	0.20	0.50	0.80	1.00	2.00		
Juveniles/Adult:			119.8	108.8	114.2	111.6	107.9	122.6		
Mortalities:			0	1	0	0	0	0		
Broods/Adult:			5.0	4.9	3.5	5.0	5.0	4.8		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-17.9	*	*	*	*	*	*	*	*
2	*	-18.5	*	*	*	*	*	*	*	*
3	*	-17.9	*	*	*	*	*	*	*	*
4	*	-17.9	*	*	*	*	*	*	*	*
5	*	-17.9	*	*	*	*	*	*	*	*
6	*	-9.8	*	*	*	*	*	*	*	*
7	*	-10.1	*	*	*	*	*	*	*	*

L37C1 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			83.0	85.0	81.2	97.4	82.6	105.0		
Mortalities:			1	0	1	0	3	1		
Broods/Adult:			3.9	4.1	3.9	4.4	3.7	4.3		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-29.2	*	*	*	*	*	*	*	*
2	*	-17.0	*	*	*	*	*	*	*	*
3	*	-25.1	*	*	*	*	*	*	*	*
4	*	-23.8	*	*	*	*	*	*	*	*
5	*	-23.3	*	*	*	*	*	*	*	*
6	*	-20.4	*	*	*	*	*	*	*	*
7	*	-12.6	*	*	*	*	*	*	*	*

L37C1 Actual

Concentrations:			0.00	0.36	0.72	1.44	1.80	3.61		
Juveniles/Adult:			83.0	85.0	81.2	97.4	82.6	105.0		
Mortalities:			1	0	1	0	3	1		
Broods/Adult:			3.9	4.1	3.9	4.4	3.7	4.3		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-29.2	*	*	*	*	*	*	*	*
2	*	-17.0	*	*	*	*	*	*	*	*
3	*	-25.1	*	*	*	*	*	*	*	*
4	*	-23.8	*	*	*	*	*	*	*	*
5	*	-23.3	*	*	*	*	*	*	*	*
6	*	-20.4	*	*	*	*	*	*	*	*
7	*	-12.6	*	*	*	*	*	*	*	*

L39C1 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.20	2.40		
Juveniles/Adult:			70.0	61.1	63.9	64.7	70.9	57.1		
Mortalities:			0	0	1	0	0	1		
Broods/Adult:			4.5	4.0	3.9	4.8	4.5	3.8		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-22.2	*	*	*	*	*	*	*	*
2	*	-14.3	*	*	*	*	*	*	*	*
3	*	-21.3	*	*	*	*	*	*	*	*
4	*	-20.7	*	*	*	*	*	*	*	*
5	*	-19.8	*	*	*	*	*	*	*	*
6	*	-13.4	*	*	*	*	*	*	*	*
7	*	-13.0	*	*	*	*	*	*	*	*

L39C2 Nominal

Concentrations:			0.00	80.0	120.0	240.0	480.0	960.0		
Juveniles/Adult:			90.4	80.5	83.4	55.4	0.0	0.0		
Mortalities:			0	1	1	1	10	10		
Broods/Adult:			5.5	4.7	4.8	4.3	0.0	0.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	120.00	-21.5	295.26	42.05	174.13	42.23	126.99	52.43	2.62	88.00
2	120.00	-14.4	308.37	46.49	201.78	29.20	157.20	45.84	3.27	88.76
3	120.00	-20.7	287.44	36.64	182.75	41.11	139.57	54.65	3.05	4.17
4	120.00	-20.5	284.32	35.20	186.72	41.16	144.59	56.06	3.26	5.47
5	120.00	-14.9	332.64	58.51	195.48	32.22	142.49	45.34	2.60	6.78
6	120.00	-14.4	*	*	*	*	*	*	*	*
7	120.00	-14.4	*	*	*	*	*	*	*	*

L39C2 Actual

Concentrations:			0.00	84.4	107.8	186.7	290.0	450.0		
Juveniles/Adult:			90.4	80.5	83.4	55.4	0.0	0.0		
Mortalities:			0	1	1	1	10	10		
Broods/Adult:			5.5	4.7	4.8	4.3	0.0	0.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	107.80	-21.5	220.32	24.44	141.08	27.11	108.50	34.59	3.11	88.88
2	107.80	-14.4	230.46	25.78	160.04	18.72	129.18	29.25	3.80	89.31
3	107.80	-20.7	217.00	21.46	145.38	26.18	114.87	34.82	3.46	4.22
4	107.80	-20.5	215.65	20.51	147.29	25.98	117.40	35.11	3.62	5.53
5	107.80	-14.9	244.94	32.59	154.78	20.82	118.13	29.29	3.02	6.85
6	107.80	-14.4	*	*	*	*	*	*	*	*
7	107.80	-14.4	*	*	*	*	*	*	*	*

L41C1 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			187.4	164.3	187.4	189.7	177.2	145.9		
Mortalities:			1	1	1	0	0	0		
Broods/Adult:			4.9	4.5	4.9	5.0	5.0	5.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	1.00	-19.6	*	*	*	*	*	*	*	*
2	1.00	-13.9	3.18	0.75	1.84	0.19	1.34	0.31	2.54	190.64
3	1.00	-19.4	*	*	*	*	*	*	*	*
4	1.00	-14.9	3.02	0.84	1.92	0.18	1.47	0.35	3.05	11.01
5	1.00	-14.8	3.03	0.83	1.91	0.18	1.47	0.35	3.03	13.38
6	1.00	-13.9	3.10	0.77	1.88	0.18	1.41	0.32	2.79	37.75
7	1.00	-13.9	3.18	0.75	1.84	0.19	1.34	0.31	2.54	38.13

L42C1 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			62.0	54.9	51.4	47.9	47.1	44.9		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			4.1	4.2	3.1	3.3	3.4	4.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.20	-13.9	*	*	*	*	*	*	*	*
2	0.20	-13.9	*	*	*	*	*	*	*	*
3	0.20	-13.9	*	*	*	*	*	*	*	*
4	0.20	-13.9	*	*	*	*	*	*	*	*
5	0.20	-13.9	*	*	*	*	*	*	*	*
6	1.00	-19.3	2.91	0.90	1.81	0.27	1.37	0.47	2.92	15.07
7	1.00	-19.3	2.91	0.90	1.81	0.27	1.37	0.47	2.92	15.07

L42C1 Actual

Concentrations:			0.00	0.19	0.37	0.73	0.91	1.81		
Juveniles/Adult:			62.0	54.9	51.4	47.9	47.1	44.9		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			4.1	4.2	3.1	3.3	3.4	4.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.19	-13.9	*	*	*	*	*	*	*	*
2	0.19	-13.9	*	*	*	*	*	*	*	*
3	0.19	-13.9	*	*	*	*	*	*	*	*
4	0.19	-13.9	*	*	*	*	*	*	*	*
5	0.19	-13.9	*	*	*	*	*	*	*	*
6	0.19	-19.3	2.62	0.80	1.64	0.24	1.25	0.42	2.95	15.07
7	0.19	-19.3	2.62	0.80	1.64	0.24	1.25	0.42	2.95	15.07

L42C2 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			79.9	75.1	72.2	71.2	65.9	64.4		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			4.3	4.8	4.6	4.4	3.7	3.9		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	1.00	-19.9	*	*	*	*	*	*	*	*
2	1.00	-19.9	*	*	*	*	*	*	*	*
3	1.00	-19.9	*	*	*	*	*	*	*	*
4	1.00	-19.9	*	*	*	*	*	*	*	*
5	1.00	-19.9	*	*	*	*	*	*	*	*
6	*	-14.3	*	*	*	*	*	*	*	*
7	*	-14.3	*	*	*	*	*	*	*	*

L42C2 Actual

Concentrations:			0.00	0.19	0.38	0.75	0.94	1.83		
Juveniles/Adult:			79.9	75.1	72.2	71.2	65.9	64.4		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			4.3	4.8	4.6	4.4	3.7	3.9		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.94	-19.9	*	*	*	*	*	*	*	*
2	0.94	-19.9	*	*	*	*	*	*	*	*
3	0.94	-19.9	*	*	*	*	*	*	*	*
4	0.94	-19.9	*	*	*	*	*	*	*	*
5	0.94	-19.9	*	*	*	*	*	*	*	*
6	*	-14.3	*	*	*	*	*	*	*	*
7	*	-14.3	*	*	*	*	*	*	*	*

L49C1 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			100.9	100.5	98.1	98.9	109.9	124.6		
Mortalities:			0	0	0	0	1	0		
Broods/Adult:			3.1	3.0	3.0	3.2	3.6	4.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-16.6	*	*	*	*	*	*	*	*
2	*	-13.3	*	*	*	*	*	*	*	*
3	*	-16.1	*	*	*	*	*	*	*	*
4	*	-15.9	*	*	*	*	*	*	*	*
5	*	-15.6	*	*	*	*	*	*	*	*
6	*	-14.4	*	*	*	*	*	*	*	*
7	*	-11.8	*	*	*	*	*	*	*	*

L49C1 Actual

Concentrations:			0.00	0.24	0.40	0.82	0.99	1.94		
Juveniles/Adult:			100.9	100.5	98.1	98.9	109.9	124.6		
Mortalities:			0	0	0	0	1	0		
Broods/Adult:			3.1	3.0	3.0	3.2	3.6	4.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-16.6	*	*	*	*	*	*	*	*
2	*	-13.3	*	*	*	*	*	*	*	*
3	*	-16.1	*	*	*	*	*	*	*	*
4	*	-15.9	*	*	*	*	*	*	*	*
5	*	-15.6	*	*	*	*	*	*	*	*
6	*	-14.4	*	*	*	*	*	*	*	*
7	*	-11.8	*	*	*	*	*	*	*	*

L50C1 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			64.8	57.5	57.5	58.1	62.1	40.7		
Mortalities:			1	0	0	0	0	1		
Broods/Adult:			5.2	5.6	5.5	5.4	5.6	4.3		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	1.00	-20.7	*	*	*	*	*	*	*	*
2	1.00	-16.5	*	*	*	*	*	*	*	*
3	1.00	-19.9	*	*	*	*	*	*	*	*
4	1.00	-19.7	*	*	*	*	*	*	*	*
5	1.00	-19.5	*	*	*	*	*	*	*	*
6	*	-13.9	*	*	*	*	*	*	*	*
7	*	-14.5	*	*	*	*	*	*	*	*

L50C1 Actual

Concentrations:			0.00	0.50	0.80	1.60	2.40	2.90		
Juveniles/Adult:			64.8	57.5	57.5	58.1	62.1	40.7		
Mortalities:			1	0	0	0	0	1		
Broods/Adult:			5.2	5.6	5.5	5.4	5.6	4.3		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	2.40	-20.7	*	*	*	*	*	*	*	*
2	2.40	-16.5	*	*	*	*	*	*	*	*
3	2.40	-19.9	*	*	*	*	*	*	*	*
4	2.40	-19.7	*	*	*	*	*	*	*	*
5	2.40	-19.5	*	*	*	*	*	*	*	*
6	*	-13.9	*	*	*	*	*	*	*	*
7	*	-14.5	*	*	*	*	*	*	*	*

L52C1 Nominal

Concentrations:			0.00	0.20	0.40	0.80	1.00	2.00		
Juveniles/Adult:			92.6	89. 3	62.2	68.4	57.2	51.6		
Mortalities:			1	0	2	0	3	2		
Broods/Adult:			4.5	5.0	3.9	4.6	4.4	3.8		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.20	-26.0	2.46	1.06	0.29	0.22	0.08	0.10	0.65	93.92
2	*	-12.1	2.00	0.35	0.28	0.09	0.09	0.05	0.71	103.17
3	0.20	-25.4	3.07	1.57	0.31	0.25	0.08	0.10	0.61	4.47
4	0.20	-16.2	2.19	0.60	0.21	0.11	0.05	0.04	0.59	5.76
5	0.20	-16.2	2.36	0.68	0.22	0.12	0.05	0.04	0.58	6.91
6	0.20	-14.1	*	*	*	*	*	*	*	*
7	*	-10.8	*	*	*	*	*	*	*	*

L06P1 Nominal

Concentrations:			0.00	0.32	0.56	1.00	1.80	3.20		
Juveniles/Adult:			53.8	57.4	68.2	62.7	62.8	65.7		
Mortalities:			2	1	0	0	1	1		
Broods/Adult:			3.9	4.2	4.5	4.3	3.7	4.1		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-37.1	*	*	*	*	*	*	*	*
2	*	-23.0	*	*	*	*	*	*	*	*
3	*	-34.6	*	*	*	*	*	*	*	*
4	*	-29.0	*	*	*	*	*	*	*	*
5	*	-25.3	*	*	*	*	*	*	*	*
6	*	-20.3	*	*	*	*	*	*	*	*
7	*	-16.6	*	*	*	*	*	*	*	*

L09P1 Nominal

Concentrations:			0.00	0.32	0.56	1.00	1.80	3.20		
Juveniles/Adult:			72.0	86.7	67.9	44.3	25.8	2.6		
Mortalities:			1	0	1	0	0	2		
Broods/Adult:			3.6	3.9	3.7	2.8	2.5	0.9		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.56	-28.7	1.22	0.11	0.77	0.10	0.59	0.10	3.02	76.69
2	0.56	-22.4	1.18	0.09	0.72	0.08	0.54	0.08	2.79	81.91
3	0.56	-28.4	1.22	0.11	0.77	0.10	0.59	0.10	3.01	3.66
4	0.56	-28.1	1.22	0.11	0.75	0.10	0.57	0.10	2.91	5.15
5	0.56	-27.9	1.21	0.11	0.75	0.10	0.57	0.10	2.91	6.45
6	0.56	-22.8	1.66	0.13	0.98	0.14	0.71	0.14	2.60	20.06
7	0.56	-18.2	1.62	0.10	0.95	0.11	0.69	*	2.59	20.89

L09P1 Actual

Concentrations:			0.00	0.41	0.261	0.76	1.94	2.65		
Juveniles/Adult:			72.0	86.7	67.9	44.3	25.8	2.6		
Mortalities:			1	0	1	0	0	2		
Broods/Adult:			3.6	3.9	3.7	2.8	2.5	0.9		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.26	-27.0	1.02	0.13	0.62	0.12	0.47	*	2.81	74.41
2	0.26	-22.2	0.96	0.11	0.55	0.09	0.40	*	2.51	80.60
3	0.26	-26.6	1.01	0.13	0.62	0.12	0.46	*	2.81	3.55
4	0.26	-26.4	1.03	0.13	0.62	0.12	0.46	*	2.72	4.98
5	0.26	-26.2	1.02	0.13	0.62	0.12	0.46	*	2.72	6.24
6	0.26	-21.4	1.97	0.08	1.55	0.13	1.34	0.15	5.76	18.38
7	0.26	-17.9	1.94	0.07	1.52	0.11	0.31	0.13	5.57	18.95

L09P2 Nominal

Concentrations:			0.00	0.32	0.56	1.00	1.80	3.20		
Juveniles/Adult:			70.3	68.8	51.7	40.1	13.8	0.1		
Mortalities:			1	2	1	1	1	4		
Broods/Adult:			3.9	3.9	3.2	3.2	2.2	0.1		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.32	-26.1	1.19	0.08	0.89	0.08	0.76	0.08	4.95	62.37
2	0.32	-19.0	1.17	0.07	0.86	0.07	0.72	0.06	4.47	68.48
3	0.32	-24.7	1.18	0.08	0.89	0.08	0.75	0.08	4.90	3.01
4	0.32	-24.7	1.18	0.08	0.89	0.08	0.75	0.08	4.90	3.01
5	0.32	-24.4	1.18	0.08	0.89	0.08	0.75	0.08	4.91	3.52
6	0.56	-19.6	1.36	0.09	0.82	0.10	0.61	0.10	2.75	17.90
7	0.56	-18.6	1.34	0.08	0.80	*	0.60	0.09	2.73	18.58

L09P2 Actual

Concentrations:			0.00	0.60	0.70	1.03	1.91	2.80		
Juveniles/Adult:			70.3	68.8	51.7	40.1	13.8	0.1		
Mortalities:			1	2	1	1	1	4		
Broods/Adult:			3.9	3.9	3.2	3.2	2.2	0.1		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.60	-26.1	1.13	0.08	0.88	*	0.76	*	5.44	65.05
2	0.60	-19.0	1.14	0.07	0.86	*	0.73	*	4.95	71.12
3	0.60	-24.7	1.13	0.07	0.87	*	0.75	*	5.41	3.14
4	0.60	-24.7	1.13	0.07	0.87	*	0.75	*	5.41	3.14
5	0.60	-24.4	1.12	0.07	0.87	*	0.75	*	5.41	3.67
6	0.70	-19.6	1.40	0.10	0.87	0.11	0.67	0.11	2.95	18.32
7	0.70	-18.6	1.38	0.09	0.87	0.10	0.66	0.10	2.96	19.00

L13P1 Nominal

Concentrations:			0.00	0.32	0.56	1.00	1.80	3.20		
Juveniles/Adult:			91.9	106.2	75.3	63.7	42.3	44.6		
Mortalities:			0	0	3	2	1	4		
Broods/Adult:			4.2	3.8	3.7	3.7	3.2	2.9		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.56	-28.9	1.91	0.42	0.52	0.24	0.25	0.16	1.07	98.88
2	1.00	-23.5	2.28	0.42	0.70	0.27	0.35	0.20	1.18	99.08
3	0.56	-27.0	2.05	0.43	0.56	0.25	0.25	0.16	1.05	4.74
4	0.56	-26.7	2.11	0.45	0.56	0.25	0.26	0.17	1.05	5.86
5	0.56	-24.5	2.54	0.54	0.65	0.29	0.30	0.19	1.02	7.08
6	*	-49.6	*	*	*	*	*	*	*	*
7	*	-48.5	*	*	*	*	*	*	*	*

L13P1 Actual

Concentrations:			0.00	0.30	0.54	0.88	1.64	3.25		
Juveniles/Adult:			91.9	106.2	75.3	63.7	42.3	44.6		
Mortalities:			0	0	3	2	1	4		
Broods/Adult:			4.2	3.8	3.7	3.7	3.2	2.9		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.54	-28.9	1.82	0.43	0.45	0.22	0.20	0.14	1.00	99.04
2	0.88	-23.5	2.19	0.45	0.62	0.25	0.30	0.18	1.10	99.25
3	0.54	-27.0	1.97	0.46	0.48	0.23	0.21	0.14	0.98	4.74
4	0.54	-26.7	2.03	0.48	0.49	0.24	0.21	0.15	0.98	5.86
5	0.54	-24.5	2.50	0.59	0.57	0.27	0.24	0.17	0.94	7.08
6	*	-49.6	*	*	*	*	*	*	*	*
7	*	-48.5	*	*	*	*	*	*	*	*

L15P1 Nominal

Concentrations:			0.00	0.32	0.56	1.00	1.80	3.20		
Juveniles/Adult:			87.6	114.9	104.4	100.1	105.5	91.6		
Mortalities:			4	0	3	2	2	2		
Broods/Adult:			2.9	3.8	3.4	3.6	3.5	3.3		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-37.3	*	*	*	*	*	*	*	*
2	*	-20.2	*	*	*	*	*	*	*	*
3	*	-29.6	*	*	*	*	*	*	*	*
4	*	-20.7	*	*	*	*	*	*	*	*
5	*	-17.5	*	*	*	*	*	*	*	*
6	*	-19.9	*	*	*	*	*	*	*	*
7	*	-18.5	*	*	*	*	*	*	*	*

L15P1 Actual

Concentrations:			0.00	0.25	0.46	0.81	1.50	2.60		
Juveniles/Adult:			87.6	114.9	104.4	100.1	105.5	91.6		
Mortalities:			4	0	3	2	2	2		
Broods/Adult:			2.9	3.8	3.4	3.6	3.5	3.3		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-37.3	*	*	*	*	*	*	*	*
2	*	-20.2	*	*	*	*	*	*	*	*
3	*	-29.6	*	*	*	*	*	*	*	*
4	*	-20.7	*	*	*	*	*	*	*	*
5	*	-17.5	*	*	*	*	*	*	*	*
6	*	-19.9	*	*	*	*	*	*	*	*
7	*	-18.5	*	*	*	*	*	*	*	*

L16P1 Nominal

Concentrations:			0.00	0.32	0.56	1.00	1.80	3.20		
Juveniles/Adult:			128.3	97.1	68.7	68.8	7.0	0.9		
Mortalities:			0	2	6	2	6	8		
Broods/Adult:			6.9	5.7	4.9	4.6	1.6	0.6		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.32	-36.0	1.14	0.11	0.88	0.13	0.75	0.14	5.23	94.45
2	0.32	-37.0	1.17	0.13	0.86	0.15	0.72	*	4.46	109.51
3	0.32	-36.1	1.12	0.11	0.85	0.12	0.72	0.13	5.03	4.74
4	0.32	-36.1	1.12	0.11	0.85	0.12	0.72	0.13	5.02	4.99
5	0.32	-36.2	1.11	0.11	0.83	0.13	0.69	0.13	4.68	5.99
6	1.00	-36.5	1.32	0.19	0.80	0.19	0.59	0.19	2.77	17.17
7	1.00	-34.8	1.50	0.21	0.97	0.26	0.75	0.28	3.19	16.99

L23P1 Nominal

Concentrations:			0.00	0.32	0.56	1.00	1.80	3.20		
Juveniles/Adult:			97.8	70.3	59.5	46.2	49.0	36.0		
Mortalities:			1	1	0	2	0	0		
Broods/Adult:			4.8	4.5	4.4	3.2	3.7	2.7		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.32	-34.8	1.24	0.63	0.10	*	0.02	*	0.56	98.17
2	0.32	-34.3	1.27	0.62	0.11	0.12	0.03	*	0.57	101.53
3	0.32	-33.7	1.18	0.57	0.10	*	0.02	*	0.56	4.79
4	0.32	-33.3	1.15	0.55	0.10	*	0.02	*	0.57	5.98
5	0.32	-33.0	1.13	0.53	0.10	0.11	0.02	*	0.57	7.35
6	*	-24.2	*	*	*	*	*	*	*	*
7	*	-26.1	*	*	*	*	*	*	*	*

L23P1 Actual

Concentrations:			0.00	0.32	0.61	0.94	1.55	2.71		
Juveniles/Adult:			97.8	70.3	59.5	46.2	49.0	36.0		
Mortalities:			1	1	0	2	0	0		
Broods/Adult:			4.8	4.5	4.4	3.2	3.7	2.7		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.32	-34.8	1.15	0.52	0.13	0.13	0.03	*	0.63	98.14
2	0.32	-34.3	1.18	0.51	0.14	0.14	0.04	*	0.64	101.49
3	0.32	-33.7	1.10	0.47	0.12	0.12	0.03	*	0.63	4.79
4	0.32	-33.3	1.08	0.46	0.12	0.12	0.03	*	0.64	5.97
5	0.32	-33.0	1.05	0.44	0.12	0.12	0.03	*	0.64	7.34
6	*	-24.2	*	*	*	*	*	*	*	*
7	*	-26.1	*	*	*	*	*	*	*	*

L28P1 Nominal

Concentrations:			0.00	0.32	0.56	1.00	1.80	3.20		
Juveniles/Adult:			193.4	202.2	212.3	233.2	248.9	246.1		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			4.5	4.5	4.7	5.0	5.0	5.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-11.1	*	*	*	*	*	*	*	*
2	*	-11.1	*	*	*	*	*	*	*	*
3	*	-11.1	*	*	*	*	*	*	*	*
4	*	-11.1	*	*	*	*	*	*	*	*
5	*	-11.1	*	*	*	*	*	*	*	*
6	*	-5.5	*	*	*	*	*	*	*	*
7	*	-5.5	*	*	*	*	*	*	*	*

L28P1 Actual

Concentrations:			0.00	0.16	0.267	0.505	0.918	1.57		
Juveniles/Adult:			193.4	202.2	212.3	233.2	248.9	246.1		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			4.5	4.5	4.7	5.0	5.0	5.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-11.1	*	*	*	*	*	*	*	*
2	*	-11.1	*	*	*	*	*	*	*	*
3	*	-11.1	*	*	*	*	*	*	*	*
4	*	-11.1	*	*	*	*	*	*	*	*
5	*	-11.1	*	*	*	*	*	*	*	*
6	*	-5.5	*	*	*	*	*	*	*	*
7	*	-5.5	*	*	*	*	*	*	*	*

L28P2 Nominal

Concentrations:			0.00	1.80	3.20	5.60	10.00	18.00		
Juveniles/Adult:			213.3	245.6	241.8	205.0	112.6	0.4		
Mortalities:			0	0	1	0	7	10		
Broods/Adult:			5.0	5.0	4.9	5.0	3.9	0.1		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	5.60	-14.5	*	*	*	*	*	*	*	*
2	5.60	-5.4	11.08	0.61	7.26	0.48	5.67	0.59	3.30	233.64
3	5.60	-14.6	*	*	*	*	*	*	*	*
4	5.60	-14.7	10.24	0.16	8.91	0.22	8.21	0.28	9.93	13.32
5	5.60	-7.0	10.69	0.15	9.08	0.17	8.26	0.22	8.54	16.21
6	5.60	-6.0	11.12	0.25	8.10	0.30	6.72	0.35	4.37	46.00
7	5.60	-5.4	11.08	0.61	7.26	0.48	5.67	0.59	3.29	46.73

L28P2 Actual

Concentrations:			0.00	0.832	1.46	2.74	4.90	11.50		
Juveniles/Adult:			213.3	245.6	241.8	205.0	112.6	0.4		
Mortalities:			0	0	1	0	7	10		
Broods/Adult:			5.0	5.0	4.9	5.0	3.9	0.1		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	2.74	-14.5	4.88	0.10	4.00	0.14	3.57	*	7.00	226.43
2	2.74	-5.4	5.45	0.31	3.53	0.23	2.75	0.29	3.20	233.80
3	2.74	-14.6	5.01	0.10	4.11	0.14	3.65	*	6.93	10.82
4	2.74	-14.7	5.06	0.11	4.10	0.14	3.63	0.17	6.60	13.39
5	2.74	-7.0	5.37	0.11	4.20	0.12	3.64	0.14	5.64	16.32
6	2.74	-6.0	5.66	0.16	3.68	0.16	2.87	0.18	3.23	46.57
7	2.74	-5.4	5.45	0.31	3.53	0.23	2.75	0.29	3.20	46.76

L28P3 Nominal

Concentrations:			0.00	0.56	1.00	1.80	3.20	5.60		
Juveniles/Adult:			194.1	211.3	223.6	207.7	149.6	30.0		
Mortalities:			0	0	0	1	0	7		
Broods/Adult:			5.0	5.0	5.0	4.6	4.6	2.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	1.80	-24.2	3.87	0.19	2.91	0.23	2.46	0.24	4.86	209.80
2	1.80	-11.3	4.23	0.17	2.88	0.18	2.29	0.20	3.59	213.87
3	1.80	-23.2	3.89	0.18	2.90	0.22	2.45	0.24	4.74	10.02
4	1.80	-22.5	3.90	0.18	2.90	0.22	2.44	0.23	4.70	12.40
5	1.80	-20.8	3.90	0.17	2.90	0.20	2.44	0.22	4.67	15.15
6	1.80	-16.2	4.68	0.21	3.15	0.27	2.50	0.30	3.50	42.29
7	1.80	-11.8	5.01	0.27	3.22	0.26	2.49	0.29	3.15	42.67

L28P3 Actual

Concentrations:			0.00	0.264	0.476	0.845	1.64	3.84		
Juveniles/Adult:			194.1	211.3	223.6	207.7	149.6	30.0		
Mortalities:			0	0	0	1	0	7		
Broods/Adult:			5.0	5.0	5.0	4.6	4.6	2.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.85	-24.2	2.20	0.16	1.43	0.17	1.12	0.17	3.25	210.71
2	0.85	-11.3	2.53	0.15	1.45	0.14	1.05	0.14	2.49	214.23
3	0.85	-23.2	2.22	0.16	1.43	0.17	1.11	0.16	3.17	10.06
4	0.85	-22.5	2.22	0.16	1.43	0.16	1.11	0.16	3.15	12.45
5	0.85	-20.8	2.23	0.15	1.44	0.15	1.11	0.15	3.15	15.20
6	0.85	-16.2	2.94	0.20	1.65	0.22	1.18	0.21	2.41	42.38
7	0.85	-11.8	3.24	0.26	1.73	0.21	1.20	0.21	2.21	42.69

L28P4 Nominal

Concentrations:			0.00	0.56	1.00	1.80	3.20	5.60		
Juveniles/Adult:			188.0	203.7	211.9	201.1	135.3	24.1		
Mortalities:			1	1	0	2	5	4		
Broods/Adult:			5.0	4.8	5.0	4.7	4.0	1.5		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	1.80	-26.2	3.71	0.19	2.80	0.22	2.37	0.24	4.89	202.08
2	3.20	-19.3	4.22	0.21	3.30	0.28	2.86	0.31	5.64	206.24
3	3.20	-22.7	3.88	0.17	2.99	0.20	2.56	0.22	5.28	9.72
4	3.20	-22.0	3.95	0.17	3.06	0.20	2.64	0.22	5.43	12.06
5	3.20	-20.4	4.10	0.17	3.18	0.21	2.74	0.23	5.43	14.75
6	3.20	-14.9	4.85	0.19	3.36	0.26	2.71	0.29	3.79	41.16
7	3.20	-12.9	4.99	0.18	3.77	0.33	3.19	0.39	4.92	41.51

L28P4 Actual

Concentrations:			0.00	0.264	0.476	0.971	1.84	3.94		
Juveniles/Adult:			188.0	203.7	211.9	201.1	135.3	24.1		
Mortalities:			1	1	0	2	5	4		
Broods/Adult:			5.0	4.8	5.0	4.7	4.0	1.5		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.97	-26.2	2.26	0.15	1.54	0.16	1.23	0.17	3.63	202.72
2	1.84	-19.3	2.69	0.18	1.94	0.23	1.60	0.24	4.20	206.34
3	1.84	-22.7	2.40	0.14	1.68	0.16	1.37	0.16	3.91	9.74
4	1.84	-22.0	2.46	0.14	1.74	0.16	1.42	0.16	4.02	12.08
5	1.84	-20.4	2.59	0.14	1.83	0.17	1.50	0.17	4.02	14.76
6	1.84	-14.9	3.24	0.17	1.99	0.21	1.50	0.22	2.85	41.19
7	1.84	-12.9	3.38	0.17	2.32	0.28	1.87	0.31	3.69	41.51

L39P1 Nominal

Concentrations:			0.00	0.25	0.50	1.00	2.00	4.00		
Juveniles/Adult:			75.4	67.8	64.2	58.8	68.2	58.0		
Mortalities:			0	1	0	1	0	1		
Broods/Adult:			4.2	4.0	4.0	3.7	3.9	3.8		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-27.9	*	*	*	*	*	*	*	*
2	*	-23.2	*	*	*	*	*	*	*	*
3	*	-26.1	*	*	*	*	*	*	*	*
4	*	-25.0	*	*	*	*	*	*	*	*
5	*	-23.3	*	*	*	*	*	*	*	*
6	*	-22.0	*	*	*	*	*	*	*	*
7	*	-21.3	*	*	*	*	*	*	*	*

L39P1 Actual

Concentrations:			0.00	0.22	0.55	1.07	2.17	4.10		
Juveniles/Adult:			75.4	67.8	64.2	58.8	68.2	58.0		
Mortalities:			0	1	0	1	0	1		
Broods/Adult:			4.2	4.0	4.0	3.7	3.9	3.8		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-27.9	*	*	*	*	*	*	*	*
2	*	-23.2	*	*	*	*	*	*	*	*
3	*	-26.1	*	*	*	*	*	*	*	*
4	*	-25.0	*	*	*	*	*	*	*	*
5	*	-23.3	*	*	*	*	*	*	*	*
6	*	-22.0	*	*	*	*	*	*	*	*
7	*	-21.3	*	*	*	*	*	*	*	*

L39P2 Nominal

Concentrations:			0.00	0.25	0.50	1.00	2.00	4.00		
Juveniles/Adult:			81.2	81.2	81.1	87.8	66.6	44.5		
Mortalities:			0	0	1	1	3	4		
Broods/Adult:			4.6	4.6	4.9	4.9	3.8	2.9		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	2.00	-33.7	4.22	0.63	2.27	0.68	1.58	0.74	2.23	82.95
2	*	-15.9	*	*	*	*	*	*	*	*
3	2.00	-32.8	4.27	0.60	2.36	0.67	1.67	0.75	2.34	3.95
4	*	-24.6	*	*	*	*	*	*	*	*
5	*	-19.1	*	*	*	*	*	*	*	*
6	*	-13.9	*	*	*	*	*	*	*	*
7	*	-12.9	*	*	*	*	*	*	*	*

L39P2 Actual

Concentrations:			0.00	0.115	0.37	0.84	1.19	3.23		
Juveniles/Adult:			81.2	81.2	81.1	87.8	66.6	44.5		
Mortalities:			0	0	1	1	3	4		
Broods/Adult:			4.6	4.6	4.9	4.9	3.8	2.9		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	1.19	-33.7	3.47	0.62	1.66	0.63	1.08	0.63	1.88	82.59
2	*	-15.9	*	*	*	*	*	*	*	*
3	1.19	-32.8	3.52	0.59	1.74	0.64	1.16	0.65	1.98	3.93
4	*	-24.6	*	*	*	*	*	*	*	*
5	*	-19.1	*	*	*	*	*	*	*	*
6	*	-13.9	*	*	*	*	*	*	*	*
7	*	-12.9	*	*	*	*	*	*	*	*

L39P3 Nominal

Concentrations:			0.00	0.32	0.56	1.00	1.80	3.20		
Juveniles/Adult:			55.0	46.8	53.1	58.3	70.2	57.9		
Mortalities:			1	2	2	2	0	0		
Broods/Adult:			3.6	3.6	3.6	3.3	4.1	4.1		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-44.8	*	*	*	*	*	*	*	*
2	*	-29.9	*	*	*	*	*	*	*	*
3	*	-44.4	*	*	*	*	*	*	*	*
4	*	-30.3	*	*	*	*	*	*	*	*
5	*	-29.9	*	*	*	*	*	*	*	*
6	*	-21.1	*	*	*	*	*	*	*	*
7	*	-21.3	*	*	*	*	*	*	*	*

L39P3 Actual

Concentrations:			0.00	0.18	0.324	0.69	1.48	2.82		
Juveniles/Adult:			55.0	46.8	53.1	58.3	70.2	57.9		
Mortalities:			1	2	2	2	0	0		
Broods/Adult:			3.6	3.6	3.6	3.3	4.1	4.1		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-44.8	*	*	*	*	*	*	*	*
2	*	-29.9	*	*	*	*	*	*	*	*
3	*	-44.4	*	*	*	*	*	*	*	*
4	*	-30.3	*	*	*	*	*	*	*	*
5	*	-29.9	*	*	*	*	*	*	*	*
6	*	-21.1	*	*	*	*	*	*	*	*
7	*	-21.3	*	*	*	*	*	*	*	*

L40P1 Nominal

Concentrations:			0.00	0.32	0.56	1.00	1.80	3.20		
Juveniles/Adult:			130.2	133.2	131.0	151.4	130.3	27.0		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			3.2	3.5	3.5	4.0	3.9	2.2		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	1.80	-22.2	2.68	0.20	2.26	0.32	2.04	0.37	7.94	136.03
2	1.80	-22.2	2.68	0.20	2.26	0.32	2.04	0.37	7.94	136.03
3	1.80	-22.2	2.68	0.20	2.26	0.32	2.04	0.37	7.94	6.48
4	1.80	-22.2	2.68	0.20	2.26	0.32	2.04	0.37	7.94	8.50
5	1.80	-22.2	2.68	0.20	2.26	0.32	2.04	0.37	7.94	10.46
6	1.80	-15.8	2.69	0.09	2.05	0.15	1.74	0.17	5.05	37.52
7	1.80	-15.8	2.69	0.09	2.05	0.15	1.74	0.17	5.05	37.52

L40P1 Actual

Concentrations:			0.00	0.35	0.63	1.03	1.76	2.92		
Juveniles/Adult:			130.2	133.2	131.0	151.4	130.3	27.0		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			3.2	3.5	3.5	4.0	3.9	2.2		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	1.76	-22.2	2.50	0.16	2.14	0.27	1.96	0.31	9.01	136.04
2	1.76	-22.2	2.50	0.16	2.14	0.27	1.96	0.31	9.01	136.04
3	1.76	-22.2	2.50	0.16	2.14	0.27	1.96	0.31	9.01	6.48
4	1.76	-22.2	2.50	0.16	2.14	0.27	1.96	0.31	9.01	8.50
5	1.76	-22.2	2.50	0.16	2.14	0.27	1.96	0.31	9.01	10.47
6	1.76	-15.8	2.51	0.08	1.97	0.13	1.71	0.15	5.73	37.52
7	1.76	-15.8	2.51	0.08	1.97	0.13	1.71	0.15	5.73	37.52

L40P2 Nominal

Concentrations:			0.00	0.32	0.56	1.00	1.80	3.20		
Juveniles/Adult:			99.6	115.3	104.0	99.0	67.6	17.5		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			4.0	3.9	3.5	3.8	3.3	2.4		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	1.00	-32.5	2.09	0.16	1.45	0.19	1.17	0.19	3.78	105.98
2	1.00	-32.5	2.09	0.16	1.45	0.19	1.17	0.19	3.78	105.98
3	1.00	-32.5	2.09	0.16	1.45	0.19	1.17	0.19	3.78	5.05
4	1.00	-32.5	2.09	0.16	1.45	0.19	1.17	0.19	3.78	6.62
5	1.00	-32.5	2.09	0.16	1.45	0.19	1.17	0.19	3.78	8.15
6	1.80	-31.1	2.24	0.19	1.41	0.23	1.07	0.23	2.99	27.99
7	1.80	-31.1	2.24	0.19	1.41	0.23	1.07	0.23	2.99	27.99

L40P2 Actual

Concentrations:			0.00	0.24	0.33	0.68	1.40	2.25		
Juveniles/Adult:			99.6	115.3	104.0	99.0	67.6	17.5		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			4.0	3.9	3.5	3.8	3.3	2.4		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	0.68	-32.5	1.59	0.10	1.17	0.13	0.98	0.14	4.58	104.78
2	0.68	-32.5	1.59	0.10	1.17	0.13	0.98	0.14	4.58	104.78
3	0.68	-32.5	1.59	0.10	1.17	0.13	0.98	0.14	4.58	4.99
4	0.68	-32.5	1.59	0.10	1.17	0.13	0.98	0.14	4.58	6.55
5	0.68	-32.5	1.59	0.10	1.17	0.13	0.98	0.14	4.58	8.06
6	1.40	-31.1	1.67	0.12	1.12	0.16	0.88	0.17	3.44	27.71
7	1.40	-31.1	1.67	0.12	1.12	0.16	0.88	0.17	3.44	27.71

L01D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			45.0	45.1	41.0	23.8	4.1	0.0		
Mortalities:			2	1	0	2	2	3		
Broods/Adult:			3.6	4.2	4.3	2.7	1.0	0.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	5.00	-41.4	10.31	1.19	6.91	1.29	5.47	*	3.47	45.15
2	5.00	-27.3	10.60	1.02	6.92	*	5.39	*	3.25	49.66
3	5.00	-35.2	9.91	1.09	6.50	*	5.08	*	3.29	2.26
4	5.00	-34.7	9.90	1.08	6.50	*	5.08	*	3.29	2.80
5	5.00	-34.1	9.91	1.06	6.50	*	5.08	*	3.29	3.40
6	5.00	-30.9	13.28	2.32	6.11	2.18	3.90	*	1.79	11.74
7	10.00	-27.8	15.61	1.78	9.62	2.25	7.25	2.32	2.90	11.24

L02D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			105.7	106.8	106.4	103.9	97.4	48.2		
Mortalities:			0	1	0	0	1	1		
Broods/Adult:			4.8	4.7	4.8	4.9	4.7	3.4		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	20.00	-19.5	38.12	1.80	26.30	3.72	21.17	4.42	3.73	105.93
2	20.00	-13.2	39.56	1.35	26.79	2.71	21.33	3.28	3.56	108.04
3	20.00	-17.2	38.44	1.48	27.38	3.53	22.45	4.30	4.09	5.06
4	20.00	-16.1	38.59	1.36	27.71	3.39	22.82	4.17	4.18	6.27
5	20.00	-14.2	38.90	1.19	28.08	3.11	23.20	3.86	4.25	7.65
6	20.00	-16.8	47.96	4.82	30.78	4.15	23.75	5.82	3.13	21.94
7	20.00	-14.1	52.06	6.28	31.03	3.67	22.91	5.17	2.68	22.27

L02D1 Actual

Concentrations:			0.00	2.01	3.62	7.86	15.22	31.60		
Juveniles/Adult:			105.7	106.8	106.4	103.9	97.4	48.2		
Mortalities:			0	1	0	0	1	1		
Broods/Adult:			4.8	4.7	4.8	4.9	4.7	3.4		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	15.22	-19.5	30.02	1.50	20.28	3.03	16.12	3.55	3.53	105.99
2	15.22	-13.2	31.21	1.13	20.62	2.20	16.17	2.62	3.35	108.14
3	15.22	-17.2	30.29	1.24	21.14	2.87	17.13	3.46	3.86	5.07
4	15.22	-16.1	30.42	1.14	21.40	2.77	17.43	3.36	3.95	6.27
5	15.22	-14.2	30.67	1.00	21.69	2.53	17.71	3.10	4.00	7.66
6	15.22	-16.8	38.33	4.05	23.88	3.41	18.10	4.68	2.93	21.96
7	15.22	-14.1	41.88	5.30	24.03	3.03	17.36	4.14	2.49	22.30

L04D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			89.8	98.1	83.2	82.2	38.6	0.7		
Mortalities:			0	0	1	0	0	0		
Broods/Adult:			4.0	4.0	3.7	4.1	3.2	0.2		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	10.00	-25.0	19.19	0.70	15.48	0.99	13.65	*	6.45	88.30
2	10.00	-21.0	19.02	0.60	15.27	0.84	13.44	*	6.33	90.66
3	10.00	-24.7	19.18	0.69	15.47	0.98	13.64	*	6.44	4.21
4	10.00	-23.9	19.17	0.67	15.44	0.95	13.62	*	6.43	6.82
5	10.00	-21.0	19.02	0.60	15.27	0.84	13.44	*	6.33	9.07
6	10.00	-25.4	20.70	1.82	12.03	2.05	8.76	2.09	2.56	22.93
7	10.00	-22.1	20.31	1.51	11.85	1.72	8.64	1.74	2.57	23.49

L04D1 Actual

Concentrations:			0.00	2.40	5.10	10.20	19.70	40.20		
Juveniles/Adult:			89.8	98.1	83.2	82.2	38.6	0.7		
Mortalities:			0	0	1	0	0	0		
Broods/Adult:			4.0	4.0	3.7	4.1	3.2	0.2		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	10.20	-25.0	18.86	0.71	15.10	0.99	13.25	*	6.23	88.48
2	10.20	-21.0	18.66	0.61	14.87	0.83	13.02	*	6.10	90.90
3	10.20	-24.7	18.85	0.71	15.09	0.98	13.24	*	6.22	4.22
4	10.20	-23.9	18.83	0.68	15.06	0.95	13.22	*	6.21	6.84
5	10.20	-21.0	18.66	0.61	14.87	0.83	13.02	*	6.10	9.09
6	10.20	-25.4	20.48	1.79	11.90	1.99	8.65	2.02	2.55	22.99
7	10.20	-22.1	20.09	1.48	11.74	1.66	5.58	1.69	2.58	23.53

L07D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			81.2	80.8	71.6	69.3	70.9	46.7		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			4.1	4.3	4.3	2.8	3.4	2.4		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	20.00	-27.5	49.73	10.71	25.94	7.54	17.42	8.81	2.12	77.29
2	20.00	-27.5	49.73	10.71	25.94	7.54	17.42	8.81	2.12	77.29
3	20.00	-27.5	49.73	10.71	25.94	7.54	17.42	8.81	2.12	3.68
4	20.00	-27.5	49.73	10.71	25.94	7.54	17.42	8.81	2.12	4.83
5	20.00	-27.5	49.73	10.71	25.94	7.54	17.42	8.81	2.12	5.95
6	20.00	-20.8	*	*	*	*	*	*	*	*
7	20.00	-20.8	*	*	*	*	*	*	*	*

L10D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			66.5	68.2	70.9	64.8	55.9	59.1		
Mortalities:			0	1	0	0	0	0		
Broods/Adult:			3.9	4.3	4.4	4.3	4.0	3.8		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-22.0	*	*	*	*	*	*	*	*
2	*	-22.1	*	*	*	*	*	*	*	*
3	*	-21.4	*	*	*	*	*	*	*	*
4	*	-21.3	*	*	*	*	*	*	*	*
5	*	-21.3	*	*	*	*	*	*	*	*
6	5.00	-12.9	*	*	*	*	*	*	*	*
7	5.00	-13.2	*	*	*	*	*	*	*	*

L10D1 Actual

Concentrations:			0.00	4.15	6.97	11.95	21.65	37.50		
Juveniles/Adult:			66.5	68.2	70.9	64.8	55.9	59.1		
Mortalities:			0	1	0	0	0	0		
Broods/Adult:			3.9	4.3	4.4	4.3	4.0	3.8		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-22.0	*	*	*	*	*	*	*	*
2	*	-22.1	*	*	*	*	*	*	*	*
3	*	-21.4	*	*	*	*	*	*	*	*
4	*	-21.3	*	*	*	*	*	*	*	*
5	*	-21.3	*	*	*	*	*	*	*	*
6	6.97	-12.9	*	*	*	*	*	*	*	*
7	6.97	-13.2	*	*	*	*	*	*	*	*

L12D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			91.9	83.0	80.6	53.6	10.3	0.3		
Mortalities:			1	1	2	2	1	2		
Broods/Adult:			4.2	4.2	3.9	3.1	1.6	0.2		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	5.00	-34.0	11.55	1.04	8.15	1.04	6.64	1.03	3.97	85.84
2	5.00	-27.2	12.29	0.90	8.85	0.94	7.31	0.94	4.23	93.31
3	5.00	-32.7	11.54	0.99	8.15	1.00	6.65	0.98	3.98	4.13
4	5.00	-32.2	11.61	1.01	8.12	1.00	6.60	0.98	3.89	5.45
5	5.00	-31.5	11.59	0.98	8.11	0.98	6.59	0.96	3.89	6.75
6	10.00	-29.3	15.14	1.49	9.62	1.77	7.38	1.80	3.06	20.38
7	10.00	-24.8	15.90	1.23	11.21	1.75	9.14	1.88	3.97	21.30

L12D1 Actual

Concentrations:			0.00	2.83	5.64	10.54	21.17	39.08		
Juveniles/Adult:			91.9	83.0	80.6	53.6	10.3	0.3		
Mortalities:			1	1	2	2	1	2		
Broods/Adult:			4.2	4.2	3.9	3.1	1.6	0.2		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	5.64	-34.0	12.17	1.08	8.70	1.07	7.14	1.06	4.12	85.91
2	5.64	-27.2	13.00	0.95	9.47	0.98	7.87	0.97	4.38	93.22
3	5.64	-32.7	12.16	1.03	8.70	1.03	7.15	1.01	4.13	4.13
4	5.64	-32.2	12.23	1.05	8.68	1.03	7.10	1.01	4.04	5.45
5	5.64	-31.5	12.21	1.02	8.67	1.00	7.09	0.99	4.05	6.76
6	10.54	-29.3	16.03	1.58	10.28	1.86	7.93	*	3.12	20.38
7	10.54	-24.8	16.82	1.31	11.86	1.82	9.67	*	3.97	21.31

L13D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			35.0	27.3	14.0	14.8	3.4	0.0		
Mortalities:			0	0	2	1	0	2		
Broods/Adult:			3.0	3.0	2.1	2.8	1.7	0.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	2.50	-23.0	4.88	0.86	1.81	0.52	1.02	0.37	1.40	35.16
2	*	-18.3	6.05	0.81	2.58	0.56	1.56	0.42	1.62	34.24
3	2.50	-23.0	4.89	0.87	1.82	0.53	1.02	0.37	1.40	1.67
4	2.50	-23.0	4.90	0.87	1.82	0.53	1.02	0.38	1.40	2.07
5	*	-20.8	5.47	0.86	2.16	0.55	1.25	0.40	1.49	2.48
6	2.50	-24.4	7.49	1.27	2.97	0.88	1.73	0.66	1.50	11.70
7	2.50	-24.5	7.27	1.24	2.86	0.85	1.65	0.63	1.49	11.76

L13D1 Actual

Concentrations:			0.00	2.49	4.67	9.22	21.53	45.25		
Juveniles/Adult:			35.0	27.3	14.0	14.8	3.4	0.0		
Mortalities:			0	0	2	1	0	2		
Broods/Adult:			3.0	3.0	2.1	2.8	1.7	0.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	2.49	-23.0	4.68	0.84	1.65	0.48	0.90	*	1.34	35.22
2	*	-18.3	5.77	0.76	2.33	0.50	1.37	0.36	1.53	34.54
3	2.49	-23.0	4.70	0.85	1.66	0.49	0.91	*	1.33	1.68
4	2.49	-23.0	4.71	0.85	1.66	0.49	0.91	*	1.33	2.07
5	*	-20.8	5.26	0.83	1.98	0.50	1.12	0.36	1.42	2.49
6	2.49	-24.4	7.07	1.23	2.60	0.78	1.45	0.56	1.39	11.85
7	2.49	-24.5	6.88	1.21	2.51	0.75	1.39	0.54	1.38	11.90

L13D2 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			80.0	71.7	45.4	33.7	14.1	1.0		
Mortalities:			0	0	0	2	1	2		
Broods/Adult:			3.6	3.8	3.5	3.0	2.1	0.3		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	2.50	-28.8	7.86	1.15	4.08	0.86	2.78	0.70	2.13	75.55
2	2.50	-24.1	8.91	1.27	4.67	0.97	3.13	0.79	2.14	73.57
3	2.50	-28.8	7.86	1.15	4.08	0.86	2.78	0.70	2.13	3.60
4	2.50	-28.8	7.86	1.15	4.08	0.86	2.78	0.70	2.13	4.44
5	2.50	-25.0	9.26	1.28	5.29	1.04	3.54	0.85	2.35	5.13
6	2.50	-21.7	9.00	1.61	2.55	0.82	1.22	0.52	1.10	22.43
7	2.50	-21.7	9.00	1.61	2.55	0.82	1.22	0.52	1.10	22.43

L13D2 Actual

Concentrations:			0.00	2.60	4.90	9.89	21.82	43.13		
Juveniles/Adult:			80.0	71.7	45.4	33.7	14.1	1.0		
Mortalities:			0	0	0	2	1	2		
Broods/Adult:			3.6	3.8	3.5	3.0	2.1	0.3		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	2.60	-28.8	7.69	1.19	3.84	0.85	2.56	0.68	2.02	76.44
2	2.60	-24.1	8.78	1.34	4.39	0.97	2.90	0.77	2.02	74.36
3	2.60	-28.8	7.69	1.19	3.84	0.85	2.56	0.68	2.02	3.64
4	2.60	-28.8	7.69	1.19	3.84	0.85	2.56	0.68	2.02	4.50
5	2.60	-25.0	9.02	1.35	4.88	1.03	3.26	0.83	2.19	5.21
6	2.60	-21.7	9.18	1.72	2.45	0.82	1.14	0.50	1.05	22.47
7	2.60	-21.7	9.18	1.72	2.45	0.82	1.14	0.50	1.05	22.47

L14D1 Nominal

Concentrations:			0.00	3.00	5.00	10.00	20.00	40.00		
Juveniles/Adult:			118.1	99.9	120.7	90.8	23.4	4.6		
Mortalities:			0	2	0	0	1	1		
Broods/Adult:			4.9	4.2	5.0	4.5	2.9	0.7		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	10.00	-25.5	14.08	1.14	9.48	1.27	7.52	1.27	3.50	113.73
2	5.00	-21.7	13.68	0.87	8.85	0.91	6.86	0.89	3.18	121.42
3	10.00	-24.7	14.01	1.10	9.41	1.21	7.45	1.20	3.48	5.45
4	10.00	-24.3	13.98	1.07	9.37	1.18	7.41	1.17	3.47	7.18
5	10.00	-23.9	13.93	1.04	9.33	1.14	7.37	1.14	3.45	8.88
6	10.00	-20.7	16.09	1.44	8.82	1.61	6.20	1.53	2.31	24.54
7	10.00	-20.1	16.60	1.43	8.62	1.48	5.87	1.36	2.12	25.16

L14D2 Nominal

Concentrations:			0.00	3.00	5.00	10.00	20.00	40.00		
Juveniles/Adult:			119.0	58.4	86.3	73.3	24.1	4.6		
Mortalities:			0	5	2	2	1	1		
Broods/Adult:			4.8	2.8	3.6	3.9	3.0	1.7		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-38.0	15.88	2.64	10.46	2.93	8.21	*	3.33	82.62
2	5.00	-28.7	13.78	1.17	8.72	1.20	6.67	1.14	3.03	116.10
3	*	-37.2	15.79	2.52	10.43	2.81	8.19	*	3.34	4.01
4	*	-33.1	14.78	1.87	9.69	2.07	7.57	2.05	3.27	5.45
5	*	-32.0	15.23	1.87	9.93	2.03	7.72	1.99	3.23	6.59
6	5.00	-25.2	15.95	1.72	8.88	1.64	6.30	1.49	2.36	22.94
7	5.00	-27.0	15.31	1.53	8.43	1.44	5.95	1.29	2.32	24.17

L15D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			180.9	187.1	186.3	69.3	4.8	0.1		
Mortalities:			0	0	1	0	1	0		
Broods/Adult:			5.0	5.0	5.0	4.4	1.8	0.1		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	5.00	-10.0	9.01	0.19	6.74	0.24	5.69	0.25	4.79	187.82
2	5.00	-9.5	9.00	0.19	6.72	0.24	5.66	0.25	4.73	188.39
3	5.00	-9.8	9.01	0.19	6.76	0.24	5.71	0.25	4.82	8.98
4	5.00	-9.8	9.02	0.19	6.76	0.24	5.72	0.26	4.83	11.10
5	5.00	-9.8	9.02	0.19	6.77	0.24	5.73	0.26	4.84	13.49
6	5.00	-10.1	9.39	0.27	6.43	0.31	5.15	0.32	3.67	37.94
7	5.00	-10.2	9.37	0.27	6.40	0.31	5.12	0.32	3.63	37.81

L15D1 Actual

Concentrations:			0.00	1.80	3.90	8.10	17.00	30.00		
Juveniles/Adult:			180.9	187.1	186.3	69.3	4.8	0.1		
Mortalities:			0	0	1	0	1	0		
Broods/Adult:			5.0	5.0	5.0	4.4	1.8	0.1		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	3.90	-10.0	7.29	0.16	5.43	0.19	4.57	0.20	4.71	187.43
2	3.90	-9.5	7.29	0.15	5.42	0.19	4.57	0.20	4.69	187.98
3	3.90	-9.8	7.29	0.16	5.44	0.19	4.58	0.20	4.74	8.96
4	3.90	-9.8	7.29	0.16	5.45	0.19	4.59	0.20	4.74	11.08
5	3.90	-9.8	7.29	0.16	5.45	0.19	4.59	0.21	4.75	13.47
6	3.90	-10.1	7.58	0.23	5.06	0.26	4.00	0.26	3.43	37.94
7	3.90	-10.2	7.56	0.23	5.03	0.26	3.96	0.26	3.40	37.83

L16D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			90.4	92.2	82.5	83.8	66.1	25.8		
Mortalities:			1	1	1	1	3	7		
Broods/Adult:			5.8	5.8	6.0	5.7	5.4	2.6		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	20.00	-33.2	29.16	3.13	17.73	3.81	13.25	3.84	2.79	88.45
2	20.00	-19.6	51.62	13.38	21.11	5.65	12.49	5.59	1.55	94.82
3	20.00	-28.5	32.44	2.86	20.16	3.87	15.26	4.05	2.91	4.32
4	20.00	-28.3	32.73	2.86	20.38	3.91	15.45	4.11	2.93	4.55
5	20.00	-26.6	35.69	3.11	21.41	4.28	15.88	4.57	2.71	5.44
6	10.00	-21.8	58.91	17.30	19.81	6.95	10.43	6.25	1.28	15.65
7	10.00	-22.0	72.42	40.36	14.00	7.34	5.35	4.76	0.84	16.50

L19D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			104.9	103.1	104.2	99.0	61.8	1.8		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			4.3	4.0	4.1	4.2	3.8	0.7		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	10.00	-11.5	21.30	0.38	17.13	0.43	15.08	0.48	6.37	103.00
2	10.00	-11.5	21.30	0.38	17.13	0.43	15.08	0.48	6.37	103.00
3	10.00	-11.5	21.30	0.38	17.13	0.43	15.08	0.48	6.37	4.91
4	10.00	-11.5	21.30	0.38	17.13	0.43	15.08	0.48	6.37	6.06
5	10.00	-11.5	21.30	0.38	17.13	0.43	15.08	0.48	6.37	7.36
6	10.00	-10.6	23.21	0.62	16.16	0.68	13.07	0.71	3.83	25.11
7	10.00	-10.6	23.21	0.62	16.16	0.68	13.07	0.71	3.83	25.11

L19D1 Actual

Concentrations:			0.00	2.30	4.50	9.50	21.00	42.00		
Juveniles/Adult:			104.9	103.1	104.2	99.0	61.8	1.8		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			4.3	4.0	4.1	4.2	3.8	0.7		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	9.50	-11.5	22.38	0.40	18.01	0.45	15.86	0.50	6.38	102.88
2	9.50	-11.5	22.38	0.40	18.01	0.45	15.86	0.50	6.38	102.88
3	9.50	-11.5	22.38	0.40	18.01	0.45	15.86	0.50	6.38	4.90
4	9.50	-11.5	22.38	0.40	18.01	0.45	15.86	0.50	6.38	6.05
5	9.50	-11.5	22.38	0.40	18.01	0.45	15.86	0.50	6.38	7.35
6	9.50	-10.6	24.42	0.65	17.00	0.73	13.76	0.75	3.83	25.02
7	9.50	-10.6	24.42	0.65	17.00	0.73	13.76	0.75	3.83	25.02

L20D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			70.1	80.3	52.9	32.2	2.9	0.0		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			4.6	4.7	4.1	3.4	0.8	0.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	2.50	-25.6	8.81	0.72	5.93	*	4.70	*	3.50	70.81
2	2.50	-25.6	8.81	0.72	5.93	*	4.70	*	3.50	70.81
3	2.50	-25.6	8.81	0.72	5.93	*	4.70	*	3.50	3.37
4	2.50	-25.6	8.81	0.72	5.93	*	4.70	*	3.50	4.43
5	2.50	-25.6	8.81	0.72	5.93	*	4.70	*	3.50	5.45
6	5.00	-29.1	11.58	1.54	5.72	1.47	3.81	*	1.98	16.22
7	5.00	-29.1	11.58	1.54	5.72	1.47	3.81	*	1.98	16.22

L22D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			127.0	123.6	143.3	127.3	89.2	1.8		
Mortalities:			5	5	5	5	4	1		
Broods/Adult:			4.1	3.9	4.3	4.3	3.7	0.7		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	20.00	-34.3	22.27	1.21	18.40	1.25	16.46	1.37	7.28	129.99
2	10.00	-21.3	21.44	0.61	17.63	0.67	15.73	0.73	7.08	166.06
3	10.00	-24.7	21.64	0.80	17.82	0.87	15.91	0.98	7.14	7.05
4	10.00	-23.3	21.50	0.75	17.69	0.82	15.78	0.93	7.11	8.48
5	10.00	-22.0	21.27	0.69	17.49	0.78	15.59	0.89	7.07	10.72
6	20.00	-25.4	25.49	1.60	19.43	1.73	16.58	1.82	5.11	31.15
7	20.00	-31.3	24.61	1.57	18.61	1.72	15.80	*	4.96	33.99

L22D2 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			175.0	182.2	166.9	117.0	18.9	0.1		
Mortalities:			1	0	0	1	2	3		
Broods/Adult:			4.7	5.0	5.0	4.4	2.5	0.1		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	5.00	-15.5	11.96	0.45	9.00	0.44	7.62	0.44	4.86	174.10
2	2.50	-10.9	11.95	0.38	8.83	0.38	7.40	0.37	4.59	178.20
3	5.00	-13.3	11.95	0.41	8.98	0.40	7.60	0.40	4.85	8.35
4	5.00	-12.8	11.94	0.40	8.98	0.39	7.59	0.39	4.84	9.77
5	5.00	-10.9	11.98	0.37	8.94	0.36	7.54	0.36	4.74	11.77
6	5.00	-12.4	13.50	0.51	9.10	0.59	7.23	0.60	3.52	36.08
7	5.00	-12.1	13.76	0.53	9.32	0.62	7.41	0.63	3.55	36.13

L25D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			73.3	57.2	52.3	57.2	34.7	3.4		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			3.5	3.8	3.3	3.5	2.8	0.6		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	2.50	-23.5	21.48	1.34	15.77	1.50	13.16	1.57	4.49	59.60
2	2.50	-23.5	21.48	1.34	15.77	1.50	13.16	1.57	4.49	59.60
3	2.50	-23.5	21.48	1.34	15.77	1.50	13.16	1.57	4.49	2.84
4	2.50	-23.5	21.48	1.34	15.77	1.50	13.16	1.57	4.49	3.51
5	2.50	-23.5	21.48	1.34	15.77	1.50	13.16	1.57	4.49	4.26
6	*	-28.2	29.84	4.10	17.11	4.42	12.36	4.44	2.49	17.50
7	*	-28.2	29.84	4.10	17.11	4.42	12.36	4.44	2.49	17.50

L25D1 Actual

Concentrations:			0.00	2.04	3.64	7.08	15.90	34.88		
Juveniles/Adult:			73.3	57.2	52.3	57.2	34.7	3.4		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			3.5	3.8	3.3	3.5	2.8	0.6		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	2.04	-23.5	17.23	1.22	12.13	1.31	9.88	*	3.95	59.59
2	2.04	-23.5	17.23	1.22	12.13	1.31	9.88	*	3.95	59.59
3	2.04	-23.5	17.23	1.22	12.13	1.31	9.88	*	3.95	2.84
4	2.04	-23.5	17.23	1.22	12.13	1.31	9.88	*	3.95	3.51
5	2.04	-23.5	17.23	1.22	12.13	1.31	9.88	*	3.95	4.26
6	*	-28.2	24.82	4.00	12.86	4.00	8.74	3.80	2.11	17.58
7	*	-28.2	24.82	4.00	12.86	4.00	8.74	3.80	2.11	17.58

L27D1 Nominal

Concentrations:			0.00	3.20	5.60	10.00	18.00	32.00		
Juveniles/Adult:			51.5	49.1	47.9	33.4	21.6	2.8		
Mortalities:			2	2	0	0	1	0		
Broods/Adult:			4.7	4.6	5.0	4.5	3.6	1.5		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	5.60	-15.7	14.72	0.81	9.58	0.83	7.43	*	3.21	49.25
2	3.20	-13.4	13.82	0.75	8.71	0.73	6.61	0.69	2.99	51.97
3	5.60	-12.9	14.36	0.73	9.19	0.73	7.08	*	3.11	2.41
4	5.60	-12.3	14.24	0.71	9.08	0.72	6.96	0.68	3.07	3.01
5	3.20	-11.9	14.10	0.71	8.92	0.71	6.82	0.67	3.03	3.70
6	5.60	-12.4	17.56	1.02	9.52	1.02	6.67	0.93	2.27	10.57
7	3.20	-13.9	17.12	1.10	9.12	1.07	6.32	0.96	2.20	10.66

L27D2 Nominal

Concentrations:			0.00	3.20	5.60	10.00	18.00	32.00		
Juveniles/Adult:			74.0	75.1	63.6	53.7	21.8	0.2		
Mortalities:			0	0	0	0	0	4		
Broods/Adult:			3.9	4.0	4.0	3.3	2.8	0.2		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	5.60	-17.7	16.26	0.52	13.56	0.73	12.20	0.83	7.64	66.10
2	5.60	-18.1	15.53	0.65	12.31	0.82	10.84	0.90	6.06	67.25
3	5.60	-17.7	16.26	0.52	13.56	0.73	12.20	0.83	7.64	3.15
4	5.60	-17.7	16.26	0.52	13.56	0.73	12.20	0.83	7.64	4.13
5	5.60	-17.7	16.26	0.52	13.56	0.73	12.20	0.83	7.64	5.08
6	10.00	-16.3	16.84	0.65	12.24	0.97	10.16	1.10	4.35	17.86
7	10.00	-16.3	16.84	0.65	12.24	0.97	10.16	1.10	4.35	17.86

L28D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			93.2	98.1	97.1	92.9	50.5	2.0		
Mortalities:			1	1	0	0	0	1		
Broods/Adult:			4.7	4.9	5.0	5.0	4.3	1.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	10.00	-18.0	20.38	0.58	15.97	0.70	13.85	*	5.69	95.72
2	10.00	-12.7	20.14	0.41	15.77	0.51	13.67	0.56	5.67	98.46
3	10.00	-16.5	20.32	0.54	15.87	0.65	13.74	*	5.62	4.59
4	10.00	-16.1	20.30	0.53	15.84	0.63	13.70	0.70	5.59	5.69
5	10.00	-15.4	20.27	0.51	15.80	0.61	13.65	0.68	5.56	6.93
6	10.00	-17.6	22.52	1.00	15.34	1.11	12.25	1.14	3.61	19.54
7	10.00	-15.5	22.20	0.89	14.85	0.99	11.74	1.01	3.45	19.96

L28D1 Actual

Concentrations:			0.00	2.12	4.31	8.78	12.68	34.80		
Juveniles/Adult:			93.2	98.1	97.1	92.9	50.5	2.0		
Mortalities:			1	1	0	0	0	1		
Broods/Adult:			4.7	4.9	5.0	5.0	4.3	1.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	8.78	-18.0	13.25	0.50	9.65	0.53	8.02	*	4.38	97.97
2	8.78	-12.7	12.95	0.34	9.42	0.38	7.82	*	4.36	101.56
3	8.78	-16.5	13.20	0.46	9.57	0.49	7.93	*	4.31	4.71
4	8.78	-16.1	13.18	0.45	9.54	0.48	7.90	*	4.29	5.83
5	8.78	-15.4	13.15	0.43	9.50	0.46	7.85	*	4.26	7.11
6	8.78	-17.6	15.75	0.95	9.51	0.86	7.08	0.81	2.75	19.95
7	8.78	-15.5	15.36	0.84	9.09	0.75	6.69	0.70	2.64	20.51

L28D2 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			119.3	122.7	119.7	102.5	49.5	1.2		
Mortalities:			0	0	0	0	0	2		
Broods/Adult:			4.5	4.7	4.8	4.6	3.2	0.6		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	10.00	-16.1	18.88	0.53	14.88	0.70	12.94	*	5.82	116.53
2	10.00	-15.9	18.75	0.56	14.52	0.74	12.51	*	5.43	116.85
3	10.00	-16.1	18.88	0.53	14.88	0.70	12.94	*	5.82	5.55
4	10.00	-16.1	18.88	0.53	14.88	0.70	12.94	*	5.82	6.85
5	10.00	-16.1	18.88	0.53	14.88	0.70	12.94	*	5.82	8.32
6	5.00	-14.1	22.42	0.88	15.46	1.00	12.43	1.05	3.73	25.30
7	5.00	-14.1	22.42	0.88	15.46	1.00	12.43	1.05	3.73	25.30

L28D2 Actual

Concentrations:			0.00	2.02	4.14	8.32	16.28	34.50		
Juveniles/Adult:			119.3	122.7	119.7	102.5	49.5	1.2		
Mortalities:			0	0	0	0	0	2		
Broods/Adult:			4.5	4.7	4.8	4.6	3.2	0.6		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	8.32	-16.1	15.21	0.46	11.71	0.59	10.05	*	5.30	117.15
2	8.32	-15.9	15.10	0.49	11.41	0.62	9.69	*	4.95	117.57
3	8.32	-16.1	15.21	0.46	11.71	0.59	10.05	*	5.30	5.58
4	8.32	-16.1	15.21	0.46	11.71	0.59	10.05	*	5.30	6.89
5	8.32	-16.1	15.21	0.46	11.71	0.59	10.05	*	5.30	8.37
6	4.14	-14.1	18.33	0.78	12.22	0.84	9.64	0.87	3.42	25.44
7	4.14	-14.1	18.33	0.78	12.22	0.84	9.64	0.87	3.42	25.44

L28D3 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			85.7	69.9	81.7	69.2	52.0	3.1		
Mortalities:			2	1	0	2	2	7		
Broods/Adult:			4.6	3.9	4.3	4.0	3.6	0.8		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	10.00	-32.1	22.82	1.58	17.81	1.71	15.40	1.80	5.59	76.26
2	10.00	-22.6	26.88	1.82	20.12	1.98	16.98	2.09	4.78	80.34
3	10.00	-31.0	22.67	1.50	17.67	1.63	15.28	1.72	5.57	3.69
4	10.00	-23.8	27.47	2.35	20.80	2.60	17.67	2.75	4.98	4.88
5	10.00	-23.7	27.28	2.29	20.61	2.51	17.49	2.66	4.95	6.08
6	20.00	-25.5	33.40	4.32	18.75	4.06	13.38	4.18	2.40	18.49
7	20.00	-28.0	33.57	4.15	19.72	4.11	14.45	4.32	2.61	18.40

L28D3 Actual

Concentrations:			0.00	2.03	4.19	8.70	17.10	34.30		
Juveniles/Adult:			85.7	69.9	81.7	69.2	52.0	3.1		
Mortalities:			2	1	0	2	2	7		
Broods/Adult:			4.6	3.9	4.3	4.0	3.6	0.8		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	8.70	-32.1	19.51	1.36	15.20	1.47	13.14	1.54	5.56	76.30
2	8.70	-22.6	23.00	1.57	17.18	1.70	14.49	1.79	4.76	80.36
3	8.70	-31.0	19.39	1.29	15.10	1.39	13.04	1.47	5.54	3.69
4	8.70	-23.8	23.51	2.02	17.77	2.23	15.09	2.36	4.96	4.88
5	8.70	-23.7	23.35	1.97	17.62	2.16	14.94	2.28	4.92	6.08
6	17.10	-25.5	28.60	3.70	16.04	3.45	11.44	3.55	2.40	18.50
7	17.10	-28.0	28.76	3.57	16.87	3.51	12.35	3.68	2.60	18.40

L28D4 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			100.1	98.0	98.1	92.9	84.8	20.6		
Mortalities:			0	0	0	0	1	0		
Broods/Adult:			4.4	4.6	4.5	4.2	4.3	3.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	10.00	-12.2	30.04	1.06	22.20	1.42	18.61	1.53	4.59	97.46
2	10.00	-12.2	30.28	1.12	22.57	1.54	19.01	1.67	4.72	97.43
3	10.00	-12.2	30.50	1.10	22.92	1.52	19.39	1.66	4.85	4.64
4	10.00	-12.3	30.65	1.12	23.15	1.57	19.64	1.72	4.94	5.73
5	10.00	-12.4	30.84	1.17	23.44	1.65	19.97	1.81	5.05	6.95
6	20.00	-17.3	33.60	1.64	23.95	2.67	19.65	2.99	4.09	22.28
7	20.00	-17.5	33.70	1.70	24.16	2.85	19.88	3.20	4.16	22.27

L28D4 Actual

Concentrations:			0.00	2.06	4.19	8.56	16.60	33.70		
Juveniles/Adult:			100.1	98.0	98.1	92.9	84.8	20.6		
Mortalities:			0	0	0	0	1	0		
Broods/Adult:			4.4	4.6	4.5	4.2	4.3	3.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	8.56	-12.2	25.12	0.90	18.44	1.21	15.38	1.30	4.48	97.52
2	8.56	-12.2	25.33	0.96	18.75	1.31	15.72	1.41	4.61	97.48
3	8.56	-12.2	25.52	0.94	19.04	1.29	16.04	1.40	4.73	4.64
4	8.56	-12.3	25.65	0.96	19.24	1.33	16.25	1.45	4.82	5.73
5	8.56	-12.4	25.82	1.00	19.49	1.40	16.54	1.53	4.93	6.96
6	16.60	-17.3	28.20	1.41	19.95	2.28	16.30	2.54	4.01	22.28
7	16.60	-17.5	28.29	1.46	20.13	2.43	16.50	2.72	4.08	22.28

L28D5 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			96.7	93.9	103.5	88.2	52.0	2.9		
Mortalities:			0	1	0	1	1	10		
Broods/Adult:			4.4	4.3	4.6	4.2	3.2	0.8		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	10.00	-27.9	20.56	1.06	15.70	1.23	13.41	1.32	5.14	96.04
2	10.00	-25.0	21.90	2.27	12.93	2.84	9.50	3.33	2.63	101.22
3	10.00	-27.1	20.63	1.06	15.60	1.23	13.25	1.31	4.97	4.60
4	10.00	-26.6	20.66	1.05	15.58	1.21	13.21	1.29	4.92	5.70
5	10.00	-25.7	20.69	1.02	15.56	1.17	13.17	1.25	4.86	6.97
6	10.00	-23.8	25.50	1.69	17.29	1.92	13.78	1.96	3.57	21.73
7	10.00	-18.1	29.42	7.45	16.94	2.65	12.26	4.09	2.51	22.22

L28D5 Actual

Concentrations:			0.00	1.98	4.02	8.22	16.13	32.60		
Juveniles/Adult:			96.7	93.9	103.5	88.2	52.0	2.9		
Mortalities:			0	1	0	1	1	10		
Broods/Adult:			4.4	4.3	4.6	4.2	3.2	0.8		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	8.22	-27.9	16.57	0.87	12.60	1.00	10.73	1.08	5.06	96.16
2	8.22	-25.0	17.64	1.80	10.52	2.24	7.77	2.65	2.68	101.21
3	8.22	-27.1	16.63	0.87	12.52	1.00	10.60	1.06	4.89	4.61
4	8.22	-26.6	16.65	0.86	12.51	0.99	10.58	1.05	4.84	5.71
5	8.22	-25.7	16.68	0.84	12.49	0.95	10.54	1.01	4.79	6.98
6	8.22	-23.8	20.64	1.39	13.92	1.56	11.06	1.59	3.52	21.74
7	8.22	-18.1	23.53	5.84	13.72	2.08	10.00	3.24	2.57	22.21

L29D1 Nominal

Concentrations:			0.00	6.00	10.00	18.00	32.00	56.00		
Juveniles/Adult:			39.5	24.4	22.6	22.0	4.0	0.7		
Mortalities:			0	2	2	1	0	0		
Broods/Adult:			3.8	3.0	2.4	2.6	1.2	0.3		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-27.1	22.17	2.10	16.23	2.43	13.52	2.52	4.48	27.96
2	*	-23.7	21.43	1.58	15.71	1.80	13.08	1.86	4.48	31.54
3	*	-26.2	22.78	1.95	17.28	2.34	14.63	2.46	4.99	1.37
4	*	-28.5	23.46	2.08	18.40	2.60	15.93	2.79	5.66	1.84
5	18.00	-61.8	25.77	7.84	22.27	*	20.45	*	9.50	2.75
6	*	-20.0	27.76	1.91	18.60	2.72	14.69	2.94	3.49	9.55
7	*	-20.5	27.39	2.16	17.32	2.86	13.18	2.99	3.02	9.53

L29D2 Nominal

Concentrations:			0.00	6.00	10.00	18.00	32.00	56.00		
Juveniles/Adult:			34.5	31.1	32.0	18.7	3.9	1.1		
Mortalities:			1	1	0	0	1	1		
Broods/Adult:			3.6	3.1	3.2	2.5	1.6	0.6		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	10.00	-25.8	18.99	1.36	12.98	1.47	10.39	1.47	3.64	33.62
2	10.00	-20.2	18.06	1.06	11.82	1.10	9.23	1.07	3.27	36.35
3	10.00	-24.0	18.75	1.30	12.72	1.40	10.14	1.39	3.58	1.63
4	10.00	-23.4	18.61	1.29	12.57	1.38	10.00	1.37	3.54	2.03
5	10.00	-23.0	18.31	1.32	12.26	1.40	9.69	1.38	3.46	2.51
6	10.00	-17.9	23.48	1.51	14.60	1.72	11.06	1.70	2.92	10.27
7	10.00	-17.5	23.14	1.50	14.21	1.67	10.66	1.64	2.84	10.44

L30D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			60.2	59.8	50.3	34.0	8.0	0.5		
Mortalities:			0	0	1	1	7	10		
Broods/Adult:			4.1	4.0	3.2	2.8	1.2	0.1		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	5.00	-32.5	11.04	1.06	7.27	1.03	5.70	0.98	3.32	58.21
2	5.00	-18.9	13.47	1.38	6.53	1.27	4.28	*	1.92	61.46
3	5.00	-34.7	10.85	1.26	6.66	1.19	5.00	1.10	2.84	2.83
4	5.00	-38.6	10.60	1.57	5.99	1.43	4.29	1.28	2.43	3.79
5	5.00	-28.2	11.05	1.25	6.11	1.27	4.31	*	2.34	4.67
6	10.00	-34.4	17.03	2.18	10.03	2.71	7.35	*	2.62	15.57
7	10.00	-31.8	19.60	3.80	11.35	3.24	8.28	3.52	2.55	15.63

L31D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			281.5	248.4	256.4	239.4	199.0	41.3		
Mortalities:			0	0	0	0	0	1		
Broods/Adult:			6.0	5.8	5.6	5.7	5.3	2.8		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	5.00	-14.3	26.71	1.13	19.01	1.33	15.58	1.38	4.08	257.33
2	5.00	-12.9	27.04	1.07	18.91	1.27	15.34	1.32	3.88	257.60
3	5.00	-14.3	26.71	1.13	19.01	1.33	15.58	1.38	4.08	12.25
4	5.00	-14.3	26.71	1.13	19.01	1.33	15.58	1.38	4.08	13.54
5	5.00	-14.3	26.71	1.13	19.01	1.33	15.58	1.38	4.08	16.08
6	10.00	-13.8	32.15	1.38	21.50	1.93	16.98	2.08	3.44	44.80
7	10.00	-13.8	32.15	1.38	21.50	1.93	16.98	2.08	3.44	44.80

L31D1 Actual

Concentrations:			0.00	2.42	4.62	9.33	19.24	38.86		
Juveniles/Adult:			281.5	248.4	256.4	239.4	199.0	41.3		
Mortalities:			0	0	0	0	0	1		
Broods/Adult:			6.0	5.8	5.6	5.7	5.3	2.8		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	4.62	-14.3	25.82	1.10	18.30	1.30	14.96	1.34	4.03	257.19
2	4.62	-12.9	26.14	1.05	18.20	1.24	14.73	1.28	3.83	257.45
3	4.62	-14.3	25.82	1.10	18.30	1.30	14.96	1.34	4.03	12.25
4	4.62	-14.3	25.82	1.10	18.30	1.30	14.96	1.34	4.03	13.54
5	4.62	-14.3	25.82	1.10	18.30	1.30	14.96	1.34	4.03	16.07
6	9.33	-13.8	31.15	1.36	20.73	1.89	16.33	2.03	3.40	44.78
7	9.33	-13.8	31.15	1.36	20.73	1.89	16.33	2.03	3.40	44.78

L31D2 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			262.9	238.8	244.5	241.8	204.6	107.9		
Mortalities:			0	0	0	0	0	1		
Broods/Adult:			6.9	6.7	6.7	6.3	6.1	4.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	10.00	-14.1	36.02	1.80	21.04	2.47	15.36	2.62	2.58	249.37
2	10.00	-14.3	35.83	1.85	21.03	2.47	15.40	2.62	2.60	249.32
3	10.00	-14.3	36.40	1.86	21.05	2.53	15.28	2.68	2.53	11.88
4	10.00	-14.3	36.42	1.87	21.05	2.54	15.28	2.69	2.53	12.47
5	10.00	-14.3	36.51	1.89	21.06	2.56	15.26	2.71	2.52	14.68
6	20.00	-19.1	*	*	*	*	*	*	*	*
7	20.00	-19.0	*	*	*	*	*	*	*	*

L31D2 Actual

Concentrations:			0.00	2.17	4.50	8.99	18.57	32.71		
Juveniles/Adult:			262.9	238.8	244.5	241.8	204.6	107.9		
Mortalities:			0	0	0	0	0	1		
Broods/Adult:			6.9	6.7	6.7	6.3	6.1	4.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	8.99	-14.1	30.11	1.21	19.42	1.85	15.03	2.09	3.16	248.30
2	8.99	-14.3	29.99	1.25	19.42	1.85	15.06	2.09	3.19	248.26
3	8.99	-14.3	30.37	1.26	19.43	1.90	14.96	2.15	3.10	11.83
4	8.99	-14.3	30.39	1.26	19.43	1.90	14.96	2.15	3.10	12.42
5	8.99	-14.3	30.45	1.27	19.43	1.92	14.94	2.17	3.09	14.61
6	18.57	-19.1	*	*	*	*	*	*	*	*
7	18.57	-19.0	*	*	*	*	*	*	*	*

L33D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			61.7	60.7	60.0	41.8	24.2	0.7		
Mortalities:			0	0	0	0	0	1		
Broods/Adult:			4.5	4.1	4.0	3.3	3.2	0.3		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	5.00	-32.1	18.07	1.38	13.57	1.61	11.48	*	4.84	56.23
2	5.00	-32.1	17.62	1.48	12.96	1.67	10.79	*	4.49	56.76
3	5.00	-32.1	18.07	1.38	13.57	1.61	11.48	*	4.84	2.68
4	5.00	-32.1	17.62	1.48	12.96	1.67	10.79	*	4.49	3.34
5	5.00	-32.1	17.62	1.48	12.96	1.67	10.79	*	4.49	4.05
6	10.00	-28.9	20.67	2.09	12.05	2.43	8.79	2.55	2.57	14.93
7	10.00	-28.9	20.67	2.09	12.05	2.43	8.79	2.55	2.57	14.93

L33D1 Actual

Concentrations:			0.00	1.99	3.97	8.30	19.50	46.25		
Juveniles/Adult:			61.7	60.7	60.0	41.8	24.2	0.7		
Mortalities:			0	0	0	0	0	1		
Broods/Adult:			4.5	4.1	4.0	3.3	3.2	0.3		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	3.97	-32.1	17.12	1.65	11.96	*	9.70	*	3.87	56.34
2	3.97	-32.1	16.61	1.75	11.29	*	9.01	*	3.59	56.87
3	3.97	-32.1	17.12	1.65	11.96	*	9.70	*	3.87	2.68
4	3.97	-32.1	16.61	1.75	11.29	*	9.01	*	3.59	3.35
5	3.97	-32.1	16.61	1.75	11.29	*	9.01	*	3.59	4.06
6	8.30	-28.9	20.30	2.54	10.52	2.64	7.15	2.58	2.11	14.96
7	8.30	-28.9	20.30	2.54	10.52	2.64	7.15	2.58	2.11	14.96

L34D1 Nominal

Concentrations:	0.00	2.50	5.00	10.00	20.00	40.00
Juveniles/Adult:	119.8	113.8	114.4	116.6	98.9	34.6
Mortalities:	0	0	0	0	0	0
Broods/Adult:	5.0	4.9	5.0	5.0	4.4	3.9

RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	10.00	-17.6	31.81	1.61	22.00	2.30	17.74	2.49	3.76	116.44
2	10.00	-17.6	31.81	1.61	22.00	2.30	17.74	2.49	3.76	116.44
3	10.00	-17.6	31.81	1.61	22.00	2.30	17.74	2.49	3.76	5.55
4	10.00	-17.6	31.81	1.61	22.00	2.30	17.74	2.49	3.76	6.85
5	10.00	-17.6	31.81	1.61	22.00	2.30	17.74	2.49	3.76	8.32
6	20.00	-19.4	36.09	2.45	28.72	5.73	25.13	6.97	6.07	23.35
7	20.00	-19.4	36.06	2.45	28.72	5.73	25.13	6.97	6.07	23.35

L35D1 Nominal

Concentrations:	0.00	2.50	5.00	10.00	20.00	40.00
Juveniles/Adult:	93.2	89.8	84.7	91.1	60.1	5.1
Mortalities:	0	0	0	0	1	1
Broods/Adult:	5.0	4.7	4.9	4.9	3.8	2.0

RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	10.00	-22.1	23.00	1.06	17.52	1.16	14.94	1.21	5.09	89.86
2	10.00	-18.2	24.36	1.03	18.89	1.13	16.28	1.17	5.45	89.74
3	10.00	-22.1	23.00	1.06	17.52	1.16	14.94	1.21	5.09	4.28
4	10.00	-22.1	23.00	1.06	17.52	1.16	14.94	1.21	5.09	5.29
5	10.00	-22.1	23.00	1.06	17.52	1.16	14.94	1.21	5.09	6.42
6	10.00	-15.8	27.97	1.25	21.28	1.53	18.14	1.63	5.07	18.38
7	10.00	-15.8	27.97	1.25	21.28	1.53	18.14	1.63	5.07	18.38

L35D2 Nominal

Concentrations:	0.00	2.50	5.00	10.00	20.00	40.00
Juveniles/Adult:	71.2	73.3	96.0	72.7	35.3	1.5
Mortalities:	0	0	0	0	1	0
Broods/Adult:	4.8	4.7	4.8	4.7	4.2	0.8

RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	10.00	-24.3	19.26	0.85	14.85	1.08	12.75	1.19	5.32	77.82
2	10.00	-22.0	20.03	0.81	15.65	0.98	13.55	1.08	5.62	77.61
3	10.00	-24.3	19.26	0.85	14.85	1.08	12.75	1.19	5.32	3.71
4	10.00	-24.3	19.26	0.85	14.85	1.08	12.75	1.19	5.32	4.58
5	10.00	-24.3	19.26	0.86	14.85	1.08	12.75	1.19	5.32	5.56
6	10.00	-18.2	20.84	1.17	13.79	1.37	10.83	1.43	3.36	16.50
7	10.00	-18.2	20.84	1.17	13.79	1.37	10.83	1.43	3.36	16.50

L38D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			116.8	110.2	110.4	63.2	20.3	0.7		
Mortalities:			1	0	1	2	1	0		
Broods/Adult:			3.5	4.0	3.4	3.4	1.6	0.3		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	5.00	-40.7	11.21	1.29	7.45	1.22	5.88	1.17	3.40	113.81
2	5.00	-28.3	12.59	1.11	8.78	1.08	7.10	1.05	3.85	119.97
3	5.00	-40.5	11.25	1.29	7.49	1.22	5.91	1.16	3.41	5.42
4	5.00	-40.5	11.27	1.29	7.51	1.22	5.93	1.16	3.42	6.69
5	5.00	-33.9	11.78	1.21	7.99	1.16	6.39	1.11	3.58	8.37
6	5.00	-31.9	13.73	2.11	6.92	*	4.63	*	2.02	29.58
7	5.00	-29.7	15.03	1.98	8.15	1.87	5.69	*	2.26	29.22

L39D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			66.8	55.2	51.2	42.1	20.2	1.2		
Mortalities:			1	0	1	2	3	3		
Broods/Adult:			4.0	3.6	3.8	3.1	1.5	0.6		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	5.00	-31.2	15.77	1.68	10.78	1.74	8.58	*	3.61	56.12
2	*	-20.1	19.34	1.10	14.37	1.31	12.09	*	4.68	58.37
3	5.00	-30.4	15.71	1.65	10.67	1.70	8.52	*	3.59	2.69
4	5.00	-30.1	15.69	1.64	10.64	1.69	8.49	*	3.58	3.54
5	5.00	-26.4	17.50	1.47	12.40	1.66	10.12	*	4.01	4.36
6	2.50	-17.1	29.98	2.35	23.79	3.13	20.78	3.42	5.99	14.62
7	2.50	-15.2	31.02	2.89	25.37	4.09	22.55	4.54	6.89	14.88

L39D2 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			70.5	73.8	70.4	67.2	34.6	1.6		
Mortalities:			0	0	1	0	0	0		
Broods/Adult:			4.7	4.3	4.1	4.1	3.9	0.7		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	10.00	-23.3	19.77	0.79	15.23	0.97	13.07	1.06	5.30	70.95
2	10.00	-18.0	19.55	0.63	14.99	0.77	12.83	0.84	5.22	72.76
3	10.00	-23.3	19.77	0.79	15.23	0.97	13.07	1.06	5.30	3.38
4	10.00	-23.3	19.77	0.79	15.23	0.97	13.07	1.06	5.30	4.43
5	10.00	-23.3	19.77	0.79	15.23	0.97	13.07	1.06	5.30	5.46
6	10.00	-25.0	21.41	1.48	13.63	1.65	10.46	1.67	3.08	16.91
7	10.00	-25.0	21.41	1.48	13.63	1.65	10.46	1.67	3.08	16.91

L40D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			145.9	127.4	125.3	105.3	28.0	4.2		
Mortalities:			0	3	0	0	0	1		
Broods/Adult:			4.0	3.6	4.0	4.0	2.3	1.3		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	5.00	-19.2	13.81	0.86	9.20	0.91	7.26	0.90	3.41	134.80
2	2.50	-15.6	13.23	0.60	8.69	0.62	6.80	0.61	3.30	141.87
3	5.00	-17.8	13.58	0.78	8.95	0.82	7.01	0.80	3.32	6.54
4	5.00	-16.9	13.45	0.73	8.81	0.76	6.88	0.74	3.28	8.67
5	5.00	-14.9	13.20	0.63	8.56	0.65	6.63	0.63	3.19	10.90
6	5.00	-18.6	15.45	1.20	8.37	1.12	5.85	*	2.26	35.00
7	5.00	-17.9	14.93	0.98	8.27	0.92	5.85	*	2.34	35.99

L40D1 Actual

Concentrations:			0.00	1.90	4.20	8.30	17.20	37.50		
Juveniles/Adult:			145.9	127.4	125.3	105.3	28.0	4.2		
Mortalities:			0	3	0	0	0	1		
Broods/Adult:			4.0	3.6	4.0	4.0	2.3	1.3		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	4.20	-19.2	11.57	0.77	7.46	0.80	5.77	0.77	3.16	135.41
2	1.90	-15.6	11.02	0.54	6.99	0.54	5.35	0.51	3.04	142.86
3	4.20	-17.8	11.36	0.70	7.24	0.71	5.55	0.69	3.07	6.57
4	4.20	-16.9	11.24	0.65	7.11	0.66	5.43	0.63	3.03	8.72
5	4.20	-14.9	11.01	0.57	6.88	0.57	5.21	0.54	2.95	10.96
6	4.20	-18.6	13.04	1.09	6.67	*	4.51	*	2.07	35.23
7	4.20	-17.9	12.56	0.88	6.58	*	4.51	*	2.14	36.28

L40D2 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			101.2	121.7	122.6	78.9	35.6	0.4		
Mortalities:			2	0	0	1	1	0		
Broods/Adult:			3.1	3.7	3.5	2.8	2.2	0.1		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	10.00	-43.2	16.81	1.73	12.92	2.00	11.21	*	5.41	106.04
2	10.00	-31.7	18.18	1.10	14.73	1.47	13.01	*	6.57	113.51
3	10.00	-42.2	16.42	1.78	12.60	1.99	10.75	*	5.19	5.11
4	10.00	-38.7	17.53	1.33	14.03	1.76	12.29	*	6.19	6.87
5	10.00	-37.1	18.46	1.19	15.06	1.64	13.37	*	6.81	8.44
6	5.00	-23.7	15.82	1.67	8.24	1.67	5.63	*	2.13	30.00
7	5.00	-24.4	15.57	1.71	7.99	1.68	5.41	1.53	2.08	30.36

L40D2 Actual

Concentrations:			0.00	2.20	4.40	9.10	19.00	37.60		
Juveniles/Adult:			101.2	121.7	122.6	78.9	35.6	0.4		
Mortalities:			2	0	0	1	1	0		
Broods/Adult:			3.1	3.7	3.5	2.8	2.2	0.1		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	9.10	-43.2	16.67	1.49	13.26	1.87	11.58	*	6.02	104.52
2	9.10	-31.7	17.44	1.00	14.26	1.40	12.68	*	6.89	113.10
3	9.10	-42.2	16.59	1.49	13.16	1.86	11.48	*	5.96	5.01
4	9.10	-38.7	16.84	1.22	13.60	1.69	12.00	*	6.48	6.84
5	9.10	-37.1	17.64	1.09	14.51	1.57	12.94	*	7.08	8.42
6	4.40	-23.7	14.78	1.64	7.50	1.60	5.05	*	2.05	29.98
7	4.40	-24.4	14.53	1.67	7.26	1.60	4.84	*	2.00	30.33

L41D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			169.1	195.0	192.2	163.4	37.0	0.8		
Mortalities:			0	0	0	0	2	3		
Broods/Adult:			5.0	5.0	5.0	4.9	2.7	0.3		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	10.00	-16.2	15.42	0.63	11.94	0.79	10.28	0.84	5.41	183.56
2	10.00	-11.8	16.12	0.51	12.37	0.65	10.59	*	5.23	183.04
3	10.00	-16.2	15.42	0.63	11.94	0.79	10.28	0.84	5.41	8.74
4	10.00	-16.3	15.36	0.63	11.84	0.79	10.16	0.85	5.31	10.21
5	10.00	-11.9	16.22	0.51	12.54	0.65	10.79	*	5.39	12.18
6	10.00	-13.3	17.70	0.69	11.86	0.88	9.38	*	3.46	37.23
7	10.00	-13.3	17.70	0.69	11.86	0.88	9.38	*	3.46	37.23

L41D1 Actual

Concentrations:			0.00	2.27	4.91	9.91	19.57	43.91		
Juveniles/Adult:			169.1	195.0	192.2	163.4	37.0	0.8		
Mortalities:			0	0	0	0	2	3		
Broods/Adult:			5.0	5.0	5.0	4.9	2.7	0.3		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	9.91	-16.2	14.88	0.61	11.30	0.77	9.63	0.82	5.05	184.78
2	9.91	-11.8	15.53	0.49	11.62	*	9.81	*	4.78	184.39
3	9.91	-16.2	14.88	0.61	11.30	0.77	9.63	0.82	5.05	8.80
4	9.91	-16.3	14.85	0.62	11.25	0.78	9.57	0.83	5.00	10.27
5	9.91	-11.9	15.58	0.49	11.73	*	9.93	*	4.88	12.28
6	9.91	-13.3	17.32	0.71	11.37	0.89	8.87	*	3.29	37.37
7	9.91	-13.3	17.32	0.71	11.37	0.89	8.87	*	3.29	37.37

L42D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			85.5	85.4	87.8	77.3	73.5	62.3		
Mortalities:			0	0	0	0	0	1		
Broods/Adult:			4.1	3.9	4.1	4.2	4.2	3.9		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	20.00	-23.5	90.36	50.75	26.39	10.34	12.86	9.71	1.13	86.86
2	*	-19.6	*	*	*	*	*	*	*	*
3	20.00	-23.5	90.36	50.75	26.39	10.34	12.86	9.71	1.13	4.14
4	20.00	-23.5	90.36	50.75	26.39	10.34	12.86	9.71	1.13	4.83
5	20.00	-23.5	90.36	50.75	26.39	10.34	12.86	9.71	1.13	5.79
6	*	-26.6	*	*	*	*	*	*	*	*
7	*	-26.6	*	*	*	*	*	*	*	*

L42D2 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			81.3	72.9	70.9	61.5	53.4	52.9		
Mortalities:			0	0	0	1	2	2		
Broods/Adult:			5.0	4.5	4.2	4.0	3.0	3.5		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	10.00	-28.1	*	*	*	*	*	*	*	*
2	5.00	-18.8	*	*	*	*	*	*	*	*
3	10.00	-27.3	*	*	*	*	*	*	*	*
4	10.00	-27.0	*	*	*	*	*	*	*	*
5	5.00	-19.8	*	*	*	*	*	*	*	*
6	*	-27.7	*	*	*	*	*	*	*	*
7	*	-24.1	*	*	*	*	*	*	*	*

L42D2 Actual

Concentrations:			0.00	1.35	2.90	6.15	12.40	29.34		
Juveniles/Adult:			81.3	72.9	70.9	61.5	53.4	52.9		
Mortalities:			0	0	0	1	2	2		
Broods/Adult:			5.0	4.5	4.2	4.0	3.0	3.5		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	6.15	-28.1	*	*	*	*	*	*	*	*
2	2.90	-18.8	*	*	*	*	*	*	*	*
3	6.15	-27.3	*	*	*	*	*	*	*	*
4	6.15	-27.0	*	*	*	*	*	*	*	*
5	2.90	-19.8	*	*	*	*	*	*	*	*
6	*	-27.7	*	*	*	*	*	*	*	*
7	*	-24.1	*	*	*	*	*	*	*	*

L43D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			128.0	132.9	124.5	126.0	42.0	1.0		
Mortalities:			1	0	0	0	0	0		
Broods/Adult:			4.6	5.0	4.7	4.6	3.9	0.7		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	10.00	-17.1	17.74	0.56	14.08	0.84	12.30	*	6.01	128.78
2	10.00	-17.4	17.70	0.57	14.04	0.83	12.26	*	5.98	129.29
3	10.00	-16.7	17.69	0.56	14.03	0.83	12.25	*	5.97	6.16
4	10.00	-16.7	17.68	0.56	14.01	0.83	12.23	*	5.96	8.11
5	10.00	-16.7	17.66	0.57	13.99	0.83	12.20	*	5.95	10.01
6	10.00	-14.7	18.48	0.66	13.12	0.86	10.74	*	4.05	27.55
7	10.00	-15.2	18.47	0.67	13.12	0.87	10.73	*	4.05	27.56

L43D1 Actual

Concentrations:			0.00	1.70	3.80	7.90	17.00	36.00		
Juveniles/Adult:			128.0	132.9	124.5	126.0	42.0	1.0		
Mortalities:			1	0	0	0	0	0		
Broods/Adult:			4.6	5.0	4.7	4.6	3.9	0.7		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	7.90	-17.1	14.92	0.52	11.61	0.75	10.03	*	5.54	128.71
2	7.90	-17.4	14.89	0.52	11.58	0.75	10.00	*	5.51	129.22
3	7.90	-16.7	14.88	0.51	11.57	0.74	9.99	*	5.51	6.16
4	7.90	-16.7	14.87	0.52	11.55	0.74	9.97	*	5.49	8.10
5	7.90	-16.7	14.85	0.52	11.53	0.75	9.95	*	5.48	10.00
6	7.90	-14.7	15.58	0.61	10.73	0.77	8.62	*	3.71	27.55
7	7.90	-15.2	15.58	0.62	10.73	0.78	8.62	*	3.71	27.55

L44D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			15.5	13.5	9.6	3.1	0.6	0.0		
Mortalities:			0	0	0	0	1	6		
Broods/Adult:			2.5	1.8	1.6	1.3	0.3	0.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	2.50	-33.7	6.00	0.80	3.55	0.74	2.62	*	2.65	15.24
2	2.50	-33.5	5.97	0.81	3.49	0.74	2.55	0.68	2.59	15.30
3	2.50	-33.7	6.00	0.80	3.55	0.74	2.62	*	2.65	0.73
4	2.50	-33.7	6.00	0.80	3.55	0.74	2.62	*	2.65	1.17
5	2.50	-33.7	6.00	0.80	3.55	0.74	2.62	*	2.65	1.52
6	5.00	-47.0	8.35	1.27	5.12	1.65	3.85	1.68	2.86	7.16
7	5.00	-47.0	8.35	1.27	5.12	1.65	3.85	1.68	2.86	7.16

L44D2 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			16.0	4.4	1.7	1.2	0.0	0.0		
Mortalities:			0	1	2	4	8	8		
Broods/Adult:			4.3	2.2	1.3	0.6	0.0	0.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	*	-25.0	*	*	*	*	*	*	*	*
2	*	-23.8	*	*	*	*	*	*	*	*
3	*	-25.0	*	*	*	*	*	*	*	*
4	*	-25.0	*	*	*	*	*	*	*	*
5	*	-25.0	*	*	*	*	*	*	*	*
6	*	-24.1	*	*	*	*	*	*	*	*
7	*	-25.1	*	*	*	*	*	*	*	*

L45D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			144.4	151.6	140.1	147.5	101.5	6.4		
Mortalities:			0	1	1	2	2	9		
Broods/Adult:			4.9	4.9	4.6	4.8	3.9	0.4		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	20.00	-36.1	23.16	1.58	18.12	1.70	15.69	1.79	5.65	146.06
2	10.00	-10.0	35.89	2.54	22.67	1.73	17.33	1.89	3.02	149.93
3	20.00	-35.4	22.98	1.51	17.95	1.62	15.53	1.71	5.61	7.07
4	10.00	-20.6	27.62	1.98	18.85	1.93	15.07	2.07	3.63	8.80
5	10.00	-10.1	35.60	2.57	22.77	1.78	17.54	1.95	3.10	10.76
6	10.00	-9.4	40.59	3.99	24.04	2.06	17.70	2.26	2.64	30.47
7	10.00	-7.6	39.99	3.23	23.23	1.64	16.91	1.79	2.55	30.92

L45D1 Actual

Concentrations:			0.00	1.90	4.00	8.10	16.40	36.10		
Juveniles/Adult:			144.4	151.6	140.1	147.5	101.5	6.4		
Mortalities:			0	1	1	2	2	9		
Broods/Adult:			4.9	4.9	4.6	4.8	3.9	0.4		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	16.40	-36.1	19.37	1.51	14.65	1.57	12.44	1.62	4.96	146.21
2	8.10	-10.0	31.85	2.55	18.98	1.65	14.03	1.74	2.68	150.04
3	16.40	-35.4	19.21	1.44	14.51	1.50	12.31	1.55	4.93	7.08
4	8.10	-20.6	23.69	1.93	15.39	1.79	11.96	1.86	3.21	8.81
5	8.10	-10.1	31.58	2.57	19.12	1.71	14.25	1.80	2.76	10.76
6	8.10	-9.4	36.54	4.01	20.32	1.99	14.43	2.08	2.36	30.49
7	8.10	-7.6	35.89	3.24	19.57	1.57	13.72	1.64	2.29	30.94

L46D1 Nominal

Concentrations:			0.00	1.50	3.30	7.30	16.00	35.10		
Juveniles/Adult:			57.7	52.8	50.1	49.5	7.7	0.2		
Mortalities:			2	2	1	2	2	3		
Broods/Adult:			3.6	3.0	3.3	3.3	1.7	0.2		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	7.30	-40.8	11.43	1.66	8.78	2.05	7.52	2.16	5.26	53.55
2	7.30	-37.2	11.59	1.47	8.84	1.85	7.55	1.96	5.12	59.87
3	7.30	-39.1	11.37	1.56	8.71	1.91	7.46	2.01	5.22	2.59
4	7.30	-38.4	11.32	1.50	8.66	1.84	7.41	1.93	5.18	3.43
5	7.30	-36.9	11.11	1.25	8.30	1.45	6.99	1.49	4.75	4.41
6	7.30	-36.1	12.74	1.43	8.71	2.00	6.98	*	3.65	15.78
7	7.30	-32.5	13.16	1.36	9.36	2.11	7.66	*	4.06	16.80

L46D1 Actual

Concentrations:			0.00	0.62	0.80	1.23	3.78	8.80		
Juveniles/Adult:			57.7	52.8	50.1	49.5	7.7	0.2		
Mortalities:			2	2	1	2	2	3		
Broods/Adult:			3.6	3.0	3.3	3.3	1.7	0.2		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	1.23	-40.8	2.41	0.50	1.71	0.55	1.40	0.54	4.03	53.48
2	1.23	-37.2	2.43	0.44	1.70	0.48	1.38	0.48	3.88	59.95
3	1.23	-39.1	2.39	0.48	1.69	0.51	1.38	0.51	4.01	2.59
4	1.23	-38.4	2.38	0.46	1.68	0.50	1.37	0.49	3.99	3.42
5	1.23	-36.9	2.32	0.41	1.60	0.41	1.28	0.40	3.72	4.40
6	1.23	-36.1	2.69	0.47	1.57	0.53	1.14	0.50	2.57	16.04
7	1.23	-32.5	2.78	0.44	1.66	0.51	1.22	0.49	2.68	17.11

L47D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			138.8	117.5	137.2	0.0	20.3	4.0		
Mortalities:			0	1	0	10	6	2		
Broods/Adult:			4.9	4.4	3.7	0.0	1.5	1.6		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	10.00	-28.4	12.12	2.40	7.92	2.55	6.16	*	3.25	132.54
2	10.00	-11.1	17.58	0.78	12.41	0.88	10.12	*	3.98	135.91
3	10.00	-26.1	11.60	1.99	7.39	2.04	5.68	1.92	3.09	6.61
4	10.00	-26.1	11.60	1.99	7.39	2.04	5.68	1.92	3.09	7.71
5	10.00	-24.6	12.03	1.89	7.66	1.94	5.89	*	3.08	9.24
6	10.00	-15.6	19.25	1.69	12.83	1.87	10.14	1.85	3.41	30.66
7	10.00	-15.6	19.25	1.69	12.83	1.87	10.14	1.85	3.41	30.66

L47D1 Actual

Concentrations:			0.00	2.45	4.96	9.98	20.37	39.90		
Juveniles/Adult:			138.8	117.5	137.2	0.0	20.3	4.0		
Mortalities:			0	1	0	10	6	2		
Broods/Adult:			4.9	4.4	3.7	0.0	1.5	1.6		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	9.98	-28.4	12.35	2.47	8.08	2.62	6.29	*	3.26	132.35
2	9.98	-11.1	17.97	0.77	12.81	0.88	10.51	*	4.10	135.83
3	9.98	-26.1	11.80	2.06	7.51	2.11	5.79	*	3.09	6.60
4	9.98	-26.1	11.80	2.06	7.51	2.11	5.79	*	3.09	7.70
5	9.98	-24.6	12.26	1.95	7.81	2.00	6.02	*	3.09	9.22
6	9.98	-15.6	19.54	1.69	13.14	1.88	10.41	1.87	3.48	30.65
7	9.98	-15.6	19.54	1.69	13.14	1.88	10.41	1.87	3.48	30.65

L47D2 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			141.7	127.2	115.4	58.4	28.6	1.6		
Mortalities:			0	1	2	5	1	6		
Broods/Adult:			4.9	4.4	4.5	3.4	3.0	0.6		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	5.00	-24.6	9.51	0.92	5.58	0.80	4.08	0.71	2.60	135.87
2	5.00	-18.8	10.32	0.99	5.34	*	3.63	*	2.10	145.97
3	5.00	-20.7	9.92	0.75	5.89	0.65	4.34	0.59	2.66	6.74
4	5.00	-20.8	10.13	0.75	6.05	0.67	4.48	0.60	2.69	7.85
5	5.00	-21.3	10.47	0.79	6.24	0.70	4.61	0.63	2.68	9.43
6	5.00	-17.9	12.70	1.00	6.40	0.86	4.28	0.74	2.02	29.20
7	5.00	-17.1	13.50	1.25	6.33	1.24	4.08	*	1.84	29.53

L47D2 Actual

Concentrations:			0.00	2.48	5.03	10.11	19.80	39.57		
Juveniles/Adult:			141.7	127.2	115.4	58.4	28.6	1.6		
Mortalities:			0	1	2	5	1	6		
Broods/Adult:			4.9	4.4	4.5	3.4	3.0	0.6		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	5.03	-24.6	9.63	0.92	5.70	0.80	4.19	0.72	2.65	135.47
2	5.03	-18.8	10.37	0.98	5.43	*	3.72	*	2.14	145.71
3	5.03	-20.7	10.03	0.74	6.00	0.65	4.45	0.59	2.70	6.72
4	5.03	-20.8	10.23	0.74	6.16	0.67	4.59	0.60	2.74	7.84
5	5.03	-21.3	10.56	0.77	6.35	0.70	4.72	0.64	2.73	9.41
6	5.03	-17.9	12.74	0.98	6.49	0.85	4.37	0.74	2.06	29.17
7	5.03	-17.1	13.50	1.22	6.42	1.24	4.17	*	1.87	29.50

L48D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			129.2	105.6	121.7	58.4	4.0	0.9		
Mortalities:			2	1	0	5	2	0		
Broods/Adult:			3.8	3.3	4.1	3.4	1.2	0.6		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	5.00	-25.2	9.95	0.56	7.45	0.65	6.29	0.70	4.79	119.19
2	5.00	-23.0	10.25	0.69	7.61	0.76	6.39	0.78	4.64	125.56
3	5.00	-22.7	10.28	0.51	7.80	0.58	6.64	0.62	5.02	5.79
4	5.00	-22.0	10.40	0.48	7.93	0.55	6.78	0.58	5.12	7.78
5	5.00	-23.9	10.70	0.52	8.27	0.58	7.11	0.62	5.37	9.64
6	5.00	-16.7	10.20	0.54	6.65	0.58	5.17	0.57	3.24	32.71
7	5.00	-18.3	10.00	0.71	6.45	0.73	4.99	0.70	3.17	33.59

L48D1 Actual

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			129.2	105.6	121.7	58.4	4.0	0.9		
Mortalities:			2	1	0	5	2	0		
Broods/Adult:			3.8	3.3	4.1	3.4	1.2	0.6		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	5.00	-25.2	9.95	0.56	7.45	0.65	6.29	0.70	4.79	119.19
2	5.00	-23.0	10.25	0.69	7.61	0.76	6.39	0.78	4.64	125.56
3	5.00	-22.7	10.28	0.51	7.80	0.58	6.64	0.62	5.02	5.79
4	5.00	-22.0	10.40	0.48	7.93	0.55	6.78	0.58	5.12	7.78
5	5.00	-23.9	10.70	0.52	8.27	0.58	7.11	0.62	5.37	9.64
6	5.00	-16.7	10.20	0.54	6.65	0.58	5.17	0.57	3.24	32.71
7	5.00	-18.3	10.00	0.71	6.45	0.73	4.99	0.70	3.17	33.59

L50D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			45.3	53.2	49.6	48.0	54.5	5.1		
Mortalities:			1	1	1	1	0	9		
Broods/Adult:			3.8	4.7	4.7	4.3	4.4	0.5		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	20.00	-60.4	*	*	*	*	*	*	*	*
2	*	-24.3	*	*	*	*	*	*	*	*
3	20.00	-60.4	*	*	*	*	*	*	*	*
4	20.00	-54.0	*	*	*	*	*	*	*	*
5	20.00	-47.4	*	*	*	*	*	*	*	*
6	*	-26.1	*	*	*	*	*	*	*	*
7	*	-26.1	*	*	*	*	*	*	*	*

L50D1 Actual

Concentrations:			0.00	1.89	3.84	7.89	15.48	31.40		
Juveniles/Adult:			45.3	53.2	49.6	48.0	54.5	5.1		
Mortalities:			1	1	1	1	0	9		
Broods/Adult:			3.8	4.7	4.7	4.3	4.4	0.5		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	15.48	-60.4	*	*	*	*	*	*	*	*
2	*	-24.3	*	*	*	*	*	*	*	*
3	15.48	-60.4	*	*	*	*	*	*	*	*
4	15.48	-54.0	*	*	*	*	*	*	*	*
5	15.48	-47.4	*	*	*	*	*	*	*	*
6	*	-26.1	*	*	*	*	*	*	*	*
7	*	-26.1	*	*	*	*	*	*	*	*

L51D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			39.7	48.6	23.6	31.8	4.2	0.0		
Mortalities:			0	0	0	0	0	0		
Broods/Adult:			2.7	2.9	1.6	2.3	1.2	0.0		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	10.00	-67.5	14.66	3.64	11.74	4.66	10.35	*	6.31	34.19
2	10.00	-67.5	14.66	3.64	11.74	4.66	10.35	*	6.31	34.19
3	10.00	-67.5	14.66	3.64	11.74	4.66	10.35	*	6.31	1.63
4	10.00	-67.5	14.66	3.64	11.74	4.66	10.35	*	6.31	2.28
5	10.00	-67.5	14.66	3.64	11.74	4.66	10.35	*	6.31	2.85
6	10.00	-28.5	15.21	1.25	11.40	1.64	9.63	1.77	4.80	15.44
7	10.00	-28.5	15.21	1.25	11.40	1.64	9.63	1.77	4.80	15.44

L52D1 Nominal

Concentrations:			0.00	2.50	5.00	10.00	20.00	40.00		
Juveniles/Adult:			93.9	89.0	97.2	89.7	50.3	0.3		
Mortalities:			1	0	1	0	2	6		
Broods/Adult:			5.0	5.0	4.8	5.0	4.1	0.2		
RV	NOEC	LSD%	EC50	SE50	EC20	SE20	EC10	SE10	B	C
1	10.00	-14.4	20.42	0.34	17.34	0.48	15.77	0.61	8.49	92.39
2	10.00	-12.7	20.83	0.40	17.49	0.54	15.79	0.70	7.93	93.47
3	10.00	-12.4	20.72	0.32	17.55	0.41	15.92	0.51	8.35	4.43
4	10.00	-12.1	20.83	0.34	17.51	0.43	15.81	0.53	7.97	5.18
5	10.00	-12.2	20.99	0.36	17.60	0.43	15.88	0.53	7.88	6.24
6	10.00	-13.1	23.25	0.80	17.03	0.85	14.20	0.96	4.46	18.77
7	10.00	-13.5	23.85	1.08	16.93	1.09	13.85	1.27	4.05	18.84

APPENDIX E

CHAIRMAN'S REPORT OF THE OECD WORKSHOP ON THE FINAL RING TEST OF THE *DAPHNIA MAGNA* REPRODUCTION TEST

Sheffield University, UK, 27-28 March 1995

December 1995

INTRODUCTION

Background

1. The programme to improve the OECD Test Guideline 202, Part II, *Daphnia* sp. Reproduction Test (1984), began in 1989 with the first workshop at Sheffield University, UK, on "Sources of Variation in Ecotoxicological Tests with *Daphnia magna*". Laboratory work was subsequently initiated within the EC Member States and the results were reviewed in April 1991 at a follow-up workshop, again in Sheffield, which involved representatives of all OECD Member countries. The workshop participants agreed that further work was needed and decided this could be achieved in two stages of ring testing (i.e. a Pilot Ring Test and a Final Ring Test).

2. The Pilot Ring Test took place during 1992 with the objective of investigating the effect of *Daphnia* clone, culture/test medium and test design on test results. A third workshop to discuss the Pilot Ring Test results was held at Sheffield University in March 1993. The draft guideline was again revised in light of the Pilot Ring Test results (June 1993 draft) and subsequently circulated for comments. This resulted in the February 1994 draft Guideline.

3. The Final Ring Test began in February 1994 with a reporting deadline of 12th September 1994. It was co-ordinated by Professor Peter Calow of Sheffield University with funding from the European Commission.

Objectives and Design of the Final Ring Test

4. The **primary objective** of the Final Ring Test was to evaluate the performance of the February 1994 draft of OECD Guideline 202, Part II, by asking laboratories to perform 21-day *Daphnia magna* reproduction tests in accordance with the draft Guideline. Additional objectives were to (1) identify how the reproductive output of the *Daphnia* should be expressed (e.g. total number of live offspring per parent over the period of the test, total number of live offspring per parent per reproductive day, etc.), and (2) determine whether offspring produced by adults which die during the test should be included in the calculations, and if so, how.

5. There were three ring test substances, 3,4-dichloroaniline (DCA), cadmium chloride and phenol. These substances were chosen because (1) they had well-documented effects on *Daphnia* reproduction, (2) they have different modes of action, (3) stock solutions could be prepared without the use of solubilising agents, and (4) they would be relatively easy and inexpensive to analyse at the concentrations to be used. In addition, DCA was chosen to provide a link with the 1985 EU ring test and the 1992 Pilot Ring Test. Cadmium was chosen to investigate the suitability of Elendt M4 and M7 defined media (which contain a known chelating agent) for the testing of metals and metal compounds. Phenol was chosen to assess the performance of the draft Guideline when testing a “difficult” substance because it is readily biodegradable in the test system.

6. Participants were requested to carry out reproduction tests using one, two or all three substances. They were also encouraged to perform a repeat test on one substance in order that the repeatability of results within laboratories could be assessed in addition to the reproducibility of results between laboratories. Forty-eight laboratories from 15 OECD Member countries and the Czech Republic took part in the Final Ring Test.

WORKSHOP TO DISCUSS FINAL RING TEST RESULTS

7. A workshop to discuss the Final Ring Test results, hosted by the UK Department of the Environment and chaired by Professor Calow, was held at Sheffield University on 27-28 March 1995. The workshop Agenda is given in Annex 1. There were 50 participants from 13 Member countries, the Czech Republic and the European Commission (see Annex 2), and included representatives from laboratories that took part in the Final Ring Test and additional experts nominated by Member countries.

Objectives

8. The workshop objectives were to:

(i) discuss the results of the Final Ring Test;
(ii) revise the draft Test Guideline in light of the Final Ring Test results.

Documents Available

9. The following documents were mailed to participants prior to the meeting.

(i) Draft Report of the Final Ring Test of the *Daphnia magna* Reproduction Study [March 1995].
(ii) Draft OECD Test Guideline 202 Part II - *Daphnia Magna* Reproduction Test [March 1995] (i.e. the February 1994 version revised in light of the ring-test results).

10. The draft report of the Final Ring Test was developed by WRc Medmenham (under contract from the UK Department of the Environment), with input from Sheffield University. In this report, the reproduction data were analysed using both analysis of variance to derive No Effect Concentrations (NOEC) and regression analysis to estimate EC_{50}s, EC_{20}s and EC_{10}s.

11. In addition, and in relation to the current debate on alternatives to the NOEC, the following documents, prepared by Professor Kooijman and Dr. Bedeaux of the Free University of Amsterdam, were made available at the workshop.

(i) Analysis of OECD Ring Test Data on *Daphnia* Reproduction.
(ii) Analysis of Toxicity Tests on *Daphnia* Survival and Reproduction.
(iii) Statistical Properties of No Effects Levels.
(iv) Statistical Analysis of Bioassays, Based on Hazard Modelling.

12. These documents concern the use of the parametric No Effect Concentration (NEC) as an alternative to both the NOEC and EC_x and, in the first document, the results of the analysis of the ring test data using the NEC approach are presented.

Summary of Final Ring Test Results

13. The meeting heard that adherence to the draft Guideline used in the ring test was good, with most participants being able to meet the water quality and validity control performance criteria.

14. With respect to effects of the ring test substances on reproduction, the results showed a clear improvement over those from a previous EC ring test performed in 1985, i.e. variability between laboratories was considerably reduced. For DCA, 38 laboratories conducted 52 reproduction studies. Ninety per cent of the effect concentrations were within a factor of 8, and 50% were within a factor of 2. Ten laboratories conducted 16 tests with phenol; all EC_{50}s and 82% of the NOECs lay within a factor of ten. For cadmium, 22 laboratories conducted 30 tests. The results with cadmium showed two types of response split between (1) those where effects were observed and (2) those which failed to find an effect. This was due to the inclusion of EDTA, a metal chelating agent, in some media (e.g. Elendt M4 and M7) -- the toxicity of cadmium being reduced by the EDTA. When effects concentrations were identified, 50 to 100% were within a factor of 8, depending on which measure of toxicity (i.e. NOEC, EC_{50} etc.) was used.

15. Several response variables were examined in an attempt to compensate for adult mortality occurring during the reproductive period. The response variable recommended in the draft report was juveniles per parent per day from the day juveniles first appeared in the control. (Note: there was considerable discussion on this issue at the workshop. See paragraph 18.)

Discussion of Final Ring Test Results

16. During the discussion of the ring test results, a number of issues for which further debate and decision-making were needed were identified. The main discussion items and the subsequent decisions and proposals for the revision of Guideline 202 are described below.

Principle of the Test

17. Two issues were discussed:

(i) how should the reproductive output of the *Daphnia magna* be expressed (i.e. which response variable should be used?)?;
(ii) which summary statistic should be reported (i.e. NOEC, EC_{50}, NEC, etc.)?

18. The participants agreed that one single response variable should be used. The participants rejected the proposal made in the draft ring test report (March 1995) for the use of "juveniles per parent per day from the day juveniles first appeared in the control" and agreed that the use of the "total number of living offspring per parent" should be used instead. There was some enthusiasm for allowing other response variables to be reported, but these would have to be added in addition to "total living offspring per parent".[1]

[1] Immediately following the workshop, the two statisticians who had been involved in the Final Ring Test planning and in the data analysis (i.e. Peter Chapman, Zeneca Agrochemicals, UK and Peter van Dijk, WRc, UK) met to consider, in more depth, which response variable would be the most suitable from the statistical point of view (there had been insufficient time for discussion of this issue at the workshop). As a result of their discussions, they now recommend that reproductive output be expressed as total juveniles

19. Until the issue of alternatives to the NOEC is resolved,[2] it was agreed that the requirements in the existing Guideline 202, Part II (1984) should be retained, i.e. the NOEC and the EC_{50} with the dose-response curve should be reported.

Validity criteria

20. The workshop agreed with the recommendations for validity criteria given in the March 1995 draft Guideline 202, i.e.:

- control mortality $\leq$20%;
- control fecundity $\geq$60 juveniles per parent.

21. It was recognised that other performance criteria, such as the coefficient of variation, the time to first brood in control could be mentioned in the Guideline but that they should not be used as validity criteria (i.e. a test would not be automatically invalid if these performance criteria are not reached). The general opinion was that the coefficient of variation around the mean number of living offspring produced per parent animal should be $\leq$25%.

Frequency of medium renewal

22. It was agreed that the frequency of medium renewal will depend on the stability of the test substance, but that it should be at least three times a week. If from preliminary stability tests the test substance is not stable between the maximum renewal period (i.e. 3 days), then consideration should be given to more frequent medium renewal, or to the use of a flow-through test.

Frequency of analytical determinations

23. There was general agreement that if there was sufficient evidence to show that the test substance is stable and would remain within ±20% of the nominal between renewal periods, then as a minimum, determinations would only need to be done on the highest and lowest test concentrations (when freshly prepared and at the time of renewal) on a weekly basis. However, if the test substance is not expected to remain within ±20% of nominal, then it would be necessary to analyse all test concentrations (on a weekly basis).

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per parent alive at the end of the test (i.e. juveniles produced from adults that die during the test are excluded from the calculations). Full justification for this is given in the revised Final Ring Test Report (June 1995). However, note that for tests where animals are held in groups (e.g. in flow-through tests) it will not be possible to express the reproductive output in this way. For these tests, it is proposed that the total number of living offspring per parent should be used.

[2] Issues surrounding test design and data analysis for aquatic toxicity testing are being addressed within the OECD Test Guidelines and Hazard Assessment Programmes.

Nominal vs measured concentrations

24. There was considerable discussion with respect to whether effect concentrations should be expressed using nominal or measured concentrations. The workshop reached consensus on the following recommendations:

- when the test substance is stable within ±20% of the nominal or measured initial concentration between the renewal period (i.e. maximum 3 days), results should be based on nominal or measured initial concentrations;

- if, from preliminary stability studies, the test substance concentration is not maintained within ±20% of the nominal or measured initial concentration, results should be expressed in terms of the time-weighted average.

Proposed Changes to the Draft Guideline

25. Following the discussion of the Final Ring Test results and the remaining issues, the revised version of Guideline 202, Part II (i.e. March 1995) was reviewed paragraph by paragraph. The recommendations have been incorporated into a revised draft [see Draft OECD Test Guideline 202 Part II -- *Daphnia magna* Reproduction Test (August 1995)].

Future action

26. The draft Chairman's report of the workshop and the draft Guideline (updated based on workshop discussions of the Final Ring Test results) will be circulated to workshop participants for comments.

27. The final Chairman's Report of the workshop (revised in light of comments of workshop participants) and draft Guideline (updated based on workshop discussions) will be circulated to National Co-ordinators of the Test Guideline Programme and nominated National Experts for comments.

ANNEX 1

THE OECD WORKSHOP ON THE FINAL RING TEST OF THE *DAPHNIA MAGNA* REPRODUCTION TEST

27-28 March 1995, University of Sheffield, UK

WORKSHOP AGENDA

Monday 27 March

14.00	Welcome Domestic arrangements Aims of the workshop	Peter Calow (Univ. Sheffield)
14.10	Background Participating laboratories	Nicky Grandy (OECD)
14.20	Resumé of exercise Use of clone, media, food Maintenance of water quality criteria	Jane Gamble (Univ. Sheffield)
	Tea	
15.00	Ring test results	Ian Sims (WRc)
16.45	Statistical techniques Peter Chapman (Zeneca) S.A.L.M. Kooijman (Free Univ. Amsterdam)	Peter van Dijk (WRc)

Tuesday 28 March

09.00	Summary of previous day's discussions	Peter Calow (Univ. Sheffield)
09.30	Implications for the OECD Test Guideline 202, *Daphnia magna* Reproduction Test	Nicky Grandy/ Marie-Chantal Huet
	Coffee	
12.30	Lunch	
14.00	Continued discussions on draft Guideline	
16.30	Summary	Peter Calow (Univ. Sheffield)
17.00	Close	

ANNEX 2

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Daphnia Ring Test Workshop
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