

Unclassified

ENV/JM/MONO(2008)20

Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

24-Jul-2008

English - Or. English

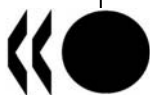
**ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

**SERIES ON TESTING AND ASSESSMENT
Number 93**

**REPORT OF THE VALIDATION OF AN ENHANCEMENT OF OECD TG 211: DAPHNIA MAGNA
REPRODUCTION TEST**

JT03249178

Document complet disponible sur OLIS dans son format d'origine
Complete document available on OLIS in its original format



**ENV/JM/MONO(2008)20
Unclassified**

English - Or. English

ENV/JM/MONO(2008)20

OECD Environment, Health and Safety Publications

Series on Testing and Assessment

No. 93

**REPORT OF THE VALIDATION OF AN ENHANCEMENT OF OECD TG 211:
DAPHNIA MAGNA REPRODUCTION TEST**

IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among UNEP, ILO, FAO, WHO, UNIDO, UNITAR and OECD

Environment Directorate

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

Paris 2008

Also published in the Series on Testing and Assessment:

- No. 1, *Guidance Document for the Development of OECD Guidelines for Testing of Chemicals (1993; reformatted 1995, revised 2006)*
- No. 2, *Detailed Review Paper on Biodegradability Testing (1995)*
- No. 3, *Guidance Document for Aquatic Effects Assessment (1995)*
- No. 4, *Report of the OECD Workshop on Environmental Hazard/Risk Assessment (1995)*
- No. 5, *Report of the SETAC/OECD Workshop on Avian Toxicity Testing (1996)*
- No. 6, *Report of the Final Ring-test of the Daphnia magna Reproduction Test (1997)*
- No. 7, *Guidance Document on Direct Phototransformation of Chemicals in Water (1997)*
- No. 8, *Report of the OECD Workshop on Sharing Information about New Industrial Chemicals Assessment (1997)*
- No. 9, *Guidance Document for the Conduct of Studies of Occupational Exposure to Pesticides during Agricultural Application (1997)*
- No. 10, *Report of the OECD Workshop on Statistical Analysis of Aquatic Toxicity Data (1998)*
- No. 11, *Detailed Review Paper on Aquatic Testing Methods for Pesticides and industrial Chemicals (1998)*
- No. 12, *Detailed Review Document on Classification Systems for Germ Cell Mutagenicity in OECD Member Countries (1998)*
- No. 13, *Detailed Review Document on Classification Systems for Sensitising Substances in OECD Member Countries (1998)*
- No. 14, *Detailed Review Document on Classification Systems for Eye Irritation/Corrosion in OECD Member Countries (1998)*
- No. 15, *Detailed Review Document on Classification Systems for Reproductive Toxicity in OECD Member Countries (1998)*
- No. 16, *Detailed Review Document on Classification Systems for Skin Irritation/Corrosion in OECD Member Countries (1998)*

- No. 17, *Environmental Exposure Assessment Strategies for Existing Industrial Chemicals in OECD Member Countries (1999)*
- No. 18, *Report of the OECD Workshop on Improving the Use of Monitoring Data in the Exposure Assessment of Industrial Chemicals (2000)*
- No. 19, *Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals used in Safety Evaluation (1999)*
- No. 20, *Revised Draft Guidance Document for Neurotoxicity Testing (2004)*
- No. 21, *Detailed Review Paper: Appraisal of Test Methods for Sex Hormone Disrupting Chemicals (2000)*
- No. 22, *Guidance Document for the Performance of Out-door Monolith Lysimeter Studies (2000)*
- No. 23, *Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (2000)*
- No. 24, *Guidance Document on Acute Oral Toxicity Testing (2001)*
- No. 25, *Detailed Review Document on Hazard Classification Systems for Specifics Target Organ Systemic Toxicity Repeated Exposure in OECD Member Countries (2001)*
- No. 26, *Revised Analysis of Responses Received from Member Countries to the Questionnaire on Regulatory Acute Toxicity Data Needs (2001)*
- No. 27, *Guidance Document on the Use of the Harmonised System for the Classification of Chemicals Which are Hazardous for the Aquatic Environment (2001)*
- No. 28, *Guidance Document for the Conduct of Skin Absorption Studies (2004)*
- No. 29, *Guidance Document on Transformation/Dissolution of Metals and Metal Compounds in Aqueous Media (2001)*
- No. 30, *Detailed Review Document on Hazard Classification Systems for Mixtures (2001)*
- No. 31, *Detailed Review Paper on Non-Genotoxic Carcinogens Detection: The Performance of In-Vitro Cell Transformation Assays (2007)*

- No. 32, *Guidance Notes for Analysis and Evaluation of Repeat-Dose Toxicity Studies (2000)*
- No. 33, *Harmonised Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures (2001)*
- No. 34, *Guidance Document on the Development, Validation and Regulatory Acceptance of New and Updated Internationally Acceptable Test Methods in Hazard Assessment (2005)*
- No. 35, *Guidance notes for analysis and evaluation of chronic toxicity and carcinogenicity studies (2002)*
- No. 36, *Report of the OECD/UNEP Workshop on the use of Multimedia Models for estimating overall Environmental Persistence and long range Transport in the context of PBTS/POPS Assessment (2002)*
- No. 37, *Detailed Review Document on Classification Systems for Substances Which Pose an Aspiration Hazard (2002)*
- No. 38, *Detailed Background Review of the Uterotrophic Assay Summary of the Available Literature in Support of the Project of the OECD Task Force on Endocrine Disrupters Testing and Assessment (EDTA) to Standardise and Validate the Uterotrophic Assay (2003)*
- No. 39, *Guidance Document on Acute Inhalation Toxicity Testing (in preparation)*
- No. 40, *Detailed Review Document on Classification in OECD Member Countries of Substances and Mixtures Which Cause Respiratory Tract Irritation and Corrosion (2003)*
- No. 41, *Detailed Review Document on Classification in OECD Member Countries of Substances and Mixtures which in Contact with Water Release Toxic Gases (2003)*
- No. 42, *Guidance Document on Reporting Summary Information on Environmental, Occupational and Consumer Exposure (2003)*
- No. 43, *Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment (2008)*
- No. 44, *Description of Selected Key Generic Terms Used in Chemical Hazard/Risk Assessment (2003)*
- No. 45, *Guidance Document on the Use of Multimedia Models for Estimating Overall Environmental Persistence and Long-range Transport (2004)*

- No. 46, *Detailed Review Paper on Amphibian Metamorphosis Assay for the Detection of Thyroid Active Substances (2004)*
- No. 47, *Detailed Review Paper on Fish Screening Assays for the Detection of Endocrine Active Substances (2004)*
- No. 48, *New Chemical Assessment Comparisons and Implications for Work Sharing (2004)*
- No. 49, *Report from the Expert Group on (Quantitative) Structure-Activity Relationships [(Q)SARs] on the Principles for the Validation of (Q)SARs (2004)*
- No. 50, *Report of the OECD/IPCS Workshop on Toxicogenomics (2005)*
- No. 51, *Approaches to Exposure Assessment in OECD Member Countries: Report from the Policy Dialogue on Exposure Assessment in June 2005 (2006)*
- No. 52, *Comparison of emission estimation methods used in Pollutant Release and Transfer Registers (PRTRs) and Emission Scenario Documents (ESDs): Case study of pulp and paper and textile sectors (2006)*
- No. 53, *Guidance Document on Simulated Freshwater Lentic Field Tests (Outdoor Microcosms and Mesocosms) (2006)*
- No. 54, *Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application (2006)*
- No. 55, *Detailed Review Paper on Aquatic Arthropods in Life Cycle Toxicity Tests with an Emphasis on Developmental, Reproductive and Endocrine Disruptive Effects (2006)*
- No. 56, *Guidance Document on the Breakdown of Organic Matter in Litter Bags (2006)*
- No. 57, *Detailed Review Paper on Thyroid Hormone Disruption Assays (2006)*
- No. 58, *Report on the Regulatory Uses and Applications in OECD Member Countries of (Quantitative) Structure-Activity Relationship [(Q)SAR] Models in the Assessment of New and Existing Chemicals (2006)*
- No. 59, *Report of the Validation of the Updated Test Guideline 407: Repeat Dose 28-Day Oral Toxicity Study in Laboratory Rats (2006)*

- No. 60, *Report of the Initial Work Towards the Validation of the 21-Day Fish Screening Assay for the Detection of Endocrine Active Substances (Phase 1A) (2006)*
- No. 61, *Report of the Validation of the 21-Day Fish Screening Assay for the Detection of Endocrine Active Substances (Phase 1B) (2006)*
- No. 62, *Final OECD Report of the Initial Work Towards the Validation of the Rat Hershberger Assay: Phase-1, Androgenic Response to Testosterone Propionate, and Anti-Androgenic Effects of Flutamide (2006)*
- No. 63, *Guidance Document on the Definition of Residue (2006)*
- No. 64, *Guidance Document on Overview of Residue Chemistry Studies (2006)*
- No. 65, *OECD Report of the Initial Work Towards the Validation of the Rodent Uterotrophic Assay - Phase 1 (2006)*
- No. 66, *OECD Report of the Validation of the Rodent Uterotrophic Bioassay: Phase 2. Testing of Potent and Weak Oestrogen Agonists by Multiple Laboratories (2006)*
- No. 67, *Additional data supporting the Test Guideline on the Uterotrophic Bioassay in rodents (2007)*
- No. 68, *Summary Report of the Uterotrophic Bioassay Peer Review Panel, including Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the follow up of this report (2006)*
- No. 69, *Guidance Document on the Validation of (Quantitative) Structure-Activity Relationship [(Q)SAR] Models (2007)*
- No. 70, *Report on the Preparation of GHS Implementation by the OECD Countries (2007)*
- No. 71, *Guidance Document on the Uterotrophic Bioassay - Procedure to Test for Antioestrogenicity (2007)*
- No. 72, *Guidance Document on Pesticide Residue Analytical Methods (2007)*
- No. 73, *Report of the Validation of the Rat Hershberger Assay: Phase 3: Coded Testing of Androgen Agonists, Androgen Antagonists and Negative Reference Chemicals by Multiple Laboratories. Surgical Castrate Model Protocol (2007)*
- No. 74, *Detailed Review Paper for Avian Two-generation Toxicity Testing (2007)*

- No. 75, *Guidance Document on the Honey Bee (Apis Mellifera L.) Brood test Under Semi-field Conditions (2007)*
- No. 76, *Final Report of the Validation of the Amphibian Metamorphosis Assay for the Detection of Thyroid Active Substances: Phase 1 - Optimisation of the Test Protocol (2007)*
- No. 77, *Final Report of the Validation of the Amphibian Metamorphosis Assay: Phase 2 - Multi-chemical Interlaboratory Study (2007)*
- No. 78, *Final report of the Validation of the 21-day Fish Screening Assay for the Detection of Endocrine Active Substances. Phase 2: Testing Negative Substances (2007)*
- No. 79, *Validation Report of the Full Life-cycle Test with the Harpacticoid Copepods Nitocra Spinipes and Amphiascus Tenuiremis and the Calanoid Copepod Acartia Tonsa - Phase 1 (2007)*
- No. 80, *Guidance on Grouping of Chemicals (2007)*
- No. 81, *Summary Report of the Validation Peer Review for the Updated Test Guideline 407, and Agreement of the Working Group of National Coordinators of the Test Guidelines Programme on the follow-up of this report (2007)*
- No. 82, *Guidance Document on Amphibian Thyroid Histology (2007)*
- No. 83, *Summary Report of the Peer Review Panel on the Stably Transfected Transcriptional Activation Assay for Detecting Estrogenic Activity of Chemicals, and Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-up of this Report (2007)*
- No. 84, *Report on the Workshop on the Application of the GHS Classification Criteria to HPV Chemicals, 5-6 July Bern Switzerland (2007)*
- No. 85, *Report of the Validation Peer Review for the Hershberger Bioassay, and Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-up of this Report (2007)*
- No. 86, *Report of the OECD Validation of the Rodent Hershberger Bioassay: Phase 2: Testing of Androgen Agonists, Androgen Antagonists and a 5 α -Reductase Inhibitor in Dose Response Studies by Multiple Laboratories (2008)*
- No. 87, *Report of the Ring Test and Statistical Analysis of Performance of the Guidance on Transformation/Dissolution of*

Metals and Metal Compounds in Aqueous Media (Transformation/Dissolution Protocol) (2008)

No.88 *Workshop on Integrated Approaches to Testing and Assessment (2008)*

No.89 *Retrospective Performance Assessment of the Test Guideline 426 on Developmental Neurotoxicity (2008)*

No.90 *Background Review Document on the Rodent Hershberger Bioassay (2008)*

No.91 *Report of the Validation of the Amphibian Metamorphosis Assay (Phase 3) (2008)*

No.92 *Report of the Validation Peer Review for the Amphibian Metamorphosis Assay and Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-Up of this Report (2008)*

No.93 *Report of the Validation of an Enhancement of OECD TG 211: Daphnia Magna Reproduction Test (2008)*

© OECD 2008

Applications for permission to reproduce or translate all or part of this material should be made to: Head of Publications Service, OECD, 2 rue André-Pascal, 75775 Paris Cedex 16, France

About the OECD

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 30 industrialised countries in North America, Europe and the Asia and Pacific region, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialised committees and working groups composed of member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD's workshops and other meetings. Committees and working groups are served by the OECD Secretariat, located in Paris, France, which is organised into directorates and divisions.

The Environment, Health and Safety Division publishes free-of-charge documents in ten different series: **Testing and Assessment; Good Laboratory Practice and Compliance Monitoring; Pesticides and Biocides; Risk Management; Harmonisation of Regulatory Oversight in Biotechnology; Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents; and the Safety of Manufactured Nanomaterials.** More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (<http://www.oecd.org/ehs/>).

This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organizations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 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 participating organisations are FAO, ILO, OECD, UNEP, UNIDO, UNITAR and WHO. The World Bank and UNDP are observers. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

This publication is available electronically, at no charge.

**For this and many other Environment,
Health and Safety publications, consult the OECD's
World Wide Web site (www.oecd.org/ehs/)**

or contact:

**OECD Environment Directorate,
Environment, Health and Safety Division**

**2 rue André-Pascal
75775 Paris Cedex 16
France**

Fax: (33-1) 44 30 61 80

E-mail: ehscont@oecd.org

FOREWORD

This document presents the validation report of the neonate sex ratio endpoint in the 21-day Daphnia Reproduction Test. In 2004, the 16th Meeting of the Working Group of National Coordinators of the Test Guidelines Programme (WNT) agreed on the inclusion of a project on the revision of the existing Test Guideline 211 on the Daphnia reproduction test in the Test Guidelines work plan. The objective of the project was to validate the new endpoint, sex ratio of the neonates, as a responsive endpoint to juvenile hormone agonists.

The validation study described in this report was conducted in 2006-2007. Twelve laboratories participated in the ring-test. The draft validation report was prepared by Japan and circulated to the Validation Management Group for ecotoxicity testing (VMG-eco) for comments in October 2007. Comments received were addressed with responses to comments, and in January 2008, a second draft report was available. Following the 6th Meeting of the VMG-eco in January 2008, all pending issues were addressed, with further changes made to the validation report, mostly in relation to statistics. The Task Force on Endocrine Disrupters Testing and Assessment and the WNT respectively endorsed and approved the validation report at their meetings in April 2008.

Authors:

Taisen Iguchi

Okazaki Institute for Integrative Bioscience
National Institutes of Natural Science
5-1 Higashiyama, Myodaiji, Okazaki 444-8787,
Japan

Norihisa Tatarazako

Research Center for Environmental Risk
National Institute for Environmental Studies
16-2 Onogawa, Tsukuba, Ibaraki 305-8506,
Japan

Shigeto Oda

Research Center for Environmental Risk
National Institute for Environmental Studies
16-2 Onogawa, Tsukuba, Ibaraki 305-8506,
Japan

John Green

DuPont Applied Statistics Group, USA

Jukka Ahtiainen

Consultant
Environment, Health and Safety Division
OECD
2, rue André-Pascal
F- 75775 Paris Cédex 16

Anne Gourmelon

Environment, Health and Safety Division
OECD
2, rue André-Pascal
F- 75775 Paris Cédex 16

TABLE OF CONTENTS

| | |
|---|----|
| SUMMARY | 16 |
| ACKNOWLEDGMENT | 17 |
| 1 INTRODUCTION | 18 |
| 2 ORGANISATION OF THE VALIDATION | 19 |
| 2.1 Daphnia strain comparison study..... | 19 |
| 2.2 Participants of the validation exercise | 20 |
| 2.3 Distribution of material..... | 20 |
| 3 TEST PERFORMANCE AND CONDITIONS..... | 21 |
| 3.1 Test substances | 21 |
| 3.2 Test organism and culturing | 21 |
| 3.3 Test media..... | 21 |
| 3.4 Water quality parameters | 22 |
| 3.5 Test protocol | 22 |
| 3.6 Test design and concentrations | 22 |
| 3.7 Observations and measurement variables..... | 22 |
| 3.8 Supporting chemical analysis | 22 |
| 3.9 Data assessment and statistical analysis | 23 |
| 4 RESULTS..... | 27 |
| 4.1 Adult mortality..... | 27 |
| 4.1.1 Performance in the controls | 27 |
| 4.1.2 Effects of the test substances | 27 |
| 4.2 Reproduction..... | 27 |
| 4.2.1 Performance in the controls | 27 |
| 4.2.2 Effects of the test substances | 27 |
| 4.3 Sex ratio of offspring | 27 |
| 4.3.1 Controls..... | 27 |
| 4.3.2 Effects of the test substances | 28 |
| 4.4 Overall data evaluation | 29 |
| 5 DISCUSSION..... | 34 |
| 5.1 Test design | 34 |
| 5.2 Biological observations and practicability..... | 34 |
| 5.3 Sources of variability | 34 |
| 5.4 Statistical power analysis..... | 34 |

| | | |
|-----|--|----|
| 5.5 | Validity criteria | 34 |
| 6 | CONCLUSIONS AND RECOMMENDATIONS FOR TG ENHANCEMENT | 35 |
| 7 | LITERATURE | 36 |

Annexes

| | | |
|---------|--|-----|
| Annex 1 | Statistical Analysis of <i>Daphnia Magna</i> sex ratio experiments | 37 |
| Annex 2 | Power properties of the sex ratio endpoint | 95 |
| Annex 3 | Proposal for enhanced TG 211 | 135 |

SUMMARY

Several studies have demonstrated recently that juvenile hormones and their analogs are involved in sex determination and thus exposure to juvenile hormones or pesticides mimicking juvenile hormones induce *Daphnia magna* to produce male neonates. The existing OECD Test Guideline 211 on the *Daphnia* reproduction test was used with small modifications in an inter-laboratory study to investigate the effect of juvenile hormone mimics on the induction of male neonates. Two substances were tested: one as a juvenile hormone analog and another as a known reproductive toxicant. Results show that the *Daphnia* strain used responded as expected: induction of male neonates in the controls was minimal (<5%) and pyriproxyfen (JH agonist) induced all male population in 8 out of 11 laboratories, while 3,5-dichlorophenol (toxicant) did not induce male. The test design used (5 concentrations, 10 replicates) yielded acceptable power, as demonstrated with the power curve. This report will be used as a reference to support the limited modifications proposed in the existing OECD Test Guideline 211.

ACKNOWLEDGMENT

We would like to thank the following laboratories for participating in this validation study:

- Finnish Environment Institute, Finland
- French National Institute for Industrial Environment and Risks, France
- Laboratoire Ecotoxicité et Santé Environnementale Equipe CNRS UMR, France
- Aachen University, Germany
- Bayer CropScience AG, Germany
- Institute for Biological Analysis and Consulting, Germany
- UMWELTBUNDESAMT, Germany
- Laboratory of Hydrobiology, Hungary
- National Institute of Health, Italy
- Agricultural Chemicals Inspection Station, Japan
- Kureha Special Laboratory, Co., Ltd., Japan.

Thanks are also due to all who made constructive discussion at the Invertebrate Expert Meeting at the OECD.

Statistical analysis was performed by Japan NUS Co., Ltd.

This work was supported partly by a grant from the Ministry of the Environment, Japan, and by a grant from the European Commission, DG Research.

1 INTRODUCTION

1. The cladoceran crustacean *Daphnia magna* is a cyclical parthenogen: females, genetically identical to their mothers, are produced asexually as long as environmental conditions are favorable. Males, also genetically identical to mothers, appear under changing environmental conditions, such as shortening photoperiod, decreasing food concentration, and increasing population density (Hobaek and Larson, 1990; Kleiven et al., 1992). Because of its relatively short life cycle and ease handling in the laboratory, *D. magna* is the recommended test animal for OECD TG 202 and TG 211 (OECD, 1998, 2004).

2. Several studies have demonstrated recently that juvenile hormones and their analogs are involved in sex determination (Olmstead and LeBlanc, 2002, 2003; Tatarazako et al., 2003; Oda et al. 2005a) and thus exposure to juvenile hormones, or to pesticides engineered as juvenile hormone mimics, induce *D. magna* to produce male neonates. This phenomenon is of potential concern as an all-male population cannot reproduce without a female. The same phenomenon occurs in other cladoceran taxonomic groups, including the genera *Moina* and *Ceriodaphnia* (Oda et al., 2005b).

3. OECD Test Guidelines for Testing of Chemicals are periodically reviewed and revised in the light of scientific progress. This is of particular interest when scientific progress enables the refinement of an existing test guideline, e.g., the collection of additional hazard information. On the basis of an increased understanding of the endocrinology of cladocerans in recent years, a proposal was made by Japan to update the existing Test Guideline 211 which covers various endpoints related to growth and reproduction. Options were open in the existing TG 211 to measure the sex ratio of offspring but no mode of action had yet been associated with the endpoint. In the present inter-laboratory study, the impact of a chemical interfering with juvenile hormone-regulated sex determination is investigated. Sensitivity, statistical significance and power, and reproducibility of findings are assessed. Changes to the existing OECD Test Guideline 211 on *Daphnia magna* reproduction will be proposed to enable collection of additional data without major modification to the test design.

4. The main difference between this enhanced version and the previous version of the Guideline is an inclusion of new endpoint (offspring sex ratio) for the detection of possible endocrine disruption in *Daphnia*.

5. Rationale to include these new measurement variables and observations is supported by recent knowledge on endocrinology of *Daphnia* as already stated above (Olmstead and LeBlanc, 2002, 2003; Tatarazako et al., 2003; Baldwin et al. 2001).

6. Enhancement of the OECD Test Guideline 211 *Daphnia magna* reproduction test as a new screening method for possible endocrine disrupting chemicals with juvenile hormone activity was proposed by Japan in May 2004. The enhancement, measurement of offspring sex ratio, was first tested for its validity in the pre-validation tests at the National Institute for Environmental Studies (NIES) in Japan from February to June 2005. The tests were conducted using seven genetically distinct strains of *D. magna* from six countries to study the genetic difference in sensitivity to test chemicals and its influence on the interpretation of test results (Table 1).

7. Based on the results of the pre-validation tests (Oda et al., 2006), a ring-test was then planned and conducted in 2007 with 12 laboratories from six countries under the initiative of NIES (Table 2). The strain that had been maintained for more than 20 years at NIES was distributed as a test strain to the participating laboratories. Pyriproxyfen, one of the juvenoid Insect Growth Regulators (IGRs), was chosen as a positive control. 3,5-Dichlorophenol (3,5-DCP) was also tested because this has been already tested with copepod and mysid in other ring-tests. These test chemicals were provided by NIES.

2 ORGANISATION OF THE VALIDATION

2.1 *Daphnia* strain comparison study

8. First, as a pre-validation step, acute and reproductive toxicity tests were conducted on seven strains of *Daphnia magna* from six laboratories in five countries. 3,4- dichloroaniline (DCA) and fenoxycarb were used as test chemicals, the former as a known reproductive toxicant in aquatic invertebrates and the latter as a known juvenile hormone agonist.

9. Seven strains of *D. magna*, six of which were provided by external laboratories (Table 1), were used for the strain comparison study. According to the laboratory data sheets provided, strains from US Environmental Protection Agency and Japanese National Institute for Environmental Studies had a common “ancestor”, as did AstraZeneca and UK Environment Agency. However, this feature does not necessarily mean that the strains are genetically identical.

Table 1. Laboratories that provided the *D. magna* strains

| Institution | Country |
|---|---------|
| US Environmental Protection Agency | USA |
| Bayer CropScience AG | Germany |
| Technical University of Denmark | Denmark |
| Environment Agency | UK |
| AstraZeneca UK Limited | UK |
| Finnish Environment Institute | Finland |
| National Institute of Environmental Studies | Japan |

10. Concentrations of test chemicals in acute toxicity tests were determined for all concentrations at the start and the end of each test. For 21-day reproduction tests, concentrations of test chemicals were determined once a week for all concentrations when freshly prepared and at renewal of the media.

11. Acute toxicity tests revealed that estimated EC₅₀ (50% effective concentration) values for DCA varied by a factor of 2.1 among strains (310 - 640 µg/L), whereas the EC₅₀ values for fenoxycarb varied by a factor of 4 (210 – 860 µg/L).

12. EC₅₀ values for reproductive toxicity tests with DCA ranged from 5.9 to 38 µg/L among strains.

13. Fenoxycarb exposure induced the production of male neonates in all the strains, including the strain used in the present study. Estimated EC₅₀ values for the induction of male offspring were highly variable among strains: sensitivity to fenoxycarb differed by a factor of approximately 23 (0.45–10µg/L).

14. This pre-validation exercise (see Oda et al, 2007) suggested that induction of male offspring by a juvenile hormone analog is universal among genetically different strains. Decreased total numbers of offspring at increased concentrations of fenoxycarb as well as other juvenoids may, however, obscure the incidence of male neonates production in the 21-day reproduction tests due to a reduced statistical power.

2.2 Participants of the validation exercise

15. Based on the results of the above presented pre-validation tests for strain comparison (Oda et al., 2007), a validation exercise (ring-test) was conducted with 12 laboratories from six countries under the initiative of NIES ([Table 2](#)).

Table 2. Participating laboratories in the ring-test

| Laboratory | Country |
|---|---------|
| Finnish Environment Institute | Finland |
| French National Institute for Industrial Environment and Risks | France |
| Laboratoire Ecotoxicité et Santé Environnementale Equipe CNRS UMR | France |
| Aachen University | Germany |
| Bayer CropScience AG | Germany |
| Institute for Biological Analysis and Consulting | Germany |
| UNWELTBUNDESAMT | Germany |
| Laboratory of Hydrology | Hungary |
| National Institute of Health | Italy |
| Agricultural Chemicals Inspection Station | Japan |
| Kureha Special Laboratory, Co., Ltd. | Japan |
| National Institute for Environmental Studies | Japan |

2.3 Distribution of material

16. The *D. magna* strain was distributed by mail by NIES as a unique test strain to all participating laboratories.

17. The test chemicals pyriproxyfen (CAS 95737-68-1), and 3,5-dichlorophenol (CAS 591-35-5) were sent to the participants from the same chemical repository at NIES.

3 TEST PERFORMANCE AND CONDITIONS

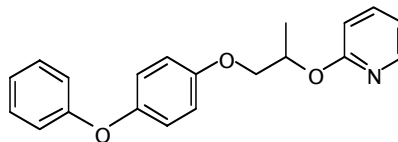
3.1 Test substances

18. The test chemical pyriproxyfen (CAS 95737-68-1), a juvenoid Insect Growth Regulators (IGR), was chosen as a positive control.

19. 3,5-dichlorophenol (CAS 591-35-5) was chosen as it had been used in several other studies in copepod and mysid, and it had been used quite widely as a reference compound in other international ring tests.

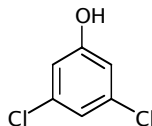
Pyriproxyfen

Molecular formula $C_{20}H_{19}NO_3$
 Molecular weight 321.4
 CAS no. 95737-68-1
 Purity (%) 99.6%
 Supplier Wako Pure Chemical Industries Ltd. (Osaka, Japan)



3,5-Dichlorophenol

Molecular formula $Cl_2C_6H_3OH$
 Molecular weight 163.0
 CAS no. 591-35-5
 Purity (%) 99.2%
 Supplier Wako Pure Chemical Industries Ltd. (Osaka, Japan)



3.2 Test organism and culturing

20. The species used in the test was *Daphnia magna* Straus. The strain that had been maintained for more than 20 years at NIES was distributed as a test strain to the participating laboratories.

21. To acclimate the animals, they were reared under test conditions for at least four generations before the start of testing. Elendt M4 medium, as described in OECD TG 211 (OECD, 1998), was used as the culture medium.

3.3 Test media

22. Elendt M4 (OECD, 1998) was used as the culture medium.

23. The dissolved oxygen concentration was kept above 3 mg/L at the beginning and during the test. The pH was within the range 6 - 9, and it was recommended that it should not vary by more than 1.5 units in any test, as in the guideline TG 211. Hardness above 140 mg/l (as $CaCO_3$) was recommended.

3.4 Water quality parameters

24. Water quality parameters fulfilled the recommendations of the protocol (TG 211).

3.5 Test protocol

25. The test protocol to be followed was the existing TG 211, plus the new observation on neonates for sex identification. Details of the procedure for the sex determination of neonates can be found in [Annex 3](#).

26. The two validity criteria for the performance of the test are presented in [Table 3](#).

Table 3. Validity of the test in OECD Test Guideline 211

| For a test to be valid, the following performance criteria should be met in the control(s). | |
|---|--|
| 1 | The mortality of the parent animals (female <i>Daphnia</i>) does not exceed 20% at the end of the test. |
| 2 | The mean number of live offspring produced per parent animal surviving at the end of the test is >60. |

3.6 Test design and concentrations

27. There were five test concentrations (25, 74, 220, 670, and 2000 ng/L) of pyriproxyfen together with solvent control (DMF, $\leq 0.01\%$ v/v) and control and five test concentrations (12, 37, 110, 330, 1000 $\mu\text{g/L}$) of 3,5-DCP together with control. There were ten replicates (i.e., adult females held individually) per test concentration and the numbers of male and female offspring per replicate were recorded at regular intervals.

3.7 Observations and measurement variables

28. The observations and measurement variables included:

- Mortality of the parent animals,
- Total number of alive male and female neonates (offspring) produced daily over the 21-day period,
- Total number of alive male neonates per replicate over the entire 21-day period.

29. The sex of neonates was identified under a stereomicroscope using the length and morphology of the first antenna as defining characteristics. The offspring sex ratio was defined as the ratio of males to the total number of neonates to a mother or mothers during experimental period (21 days) observed at each concentration.

3.8 Supporting chemical analysis

30. Originally, all participating laboratories were expected to do the chemical analysis of the test media in order to verify the concentrations during the 21 day test. Unfortunately, not all laboratories could perform this analytical verification and hence the results were presented as nominal concentrations. The analytical results available for 3,5-CDP and pyriproxyfen are presented in [Table 4](#) and [Table 5](#), respectively.

Table 4 Measured concentrations of 3,5-DCP. Concentrations were time-weighted.

| Laboratory | Control | 12 µg/L | 37 µg/L | 110 µg/L | 330 µg/L | 1000 µg/L |
|------------|---------|---------|---------|----------|----------|-----------|
| LAB B | N.D. | 9.7 | 23.8 | 72.0 | 240.1 | 838.9 |
| LAB F | N.D. | 17.2 | 22.2 | 44.5 | 184.6 | 601.0 |
| LAB G | N.D. | 9.9 | 32.6 | 88.8 | 300.1 | 930.7 |
| LAB H | N.D. | 12.9 | 26.4 | 78.4 | 262.5 | 922.9 |

Table 5 Measured concentrations of pyriproxyfen. Concentrations were time-weighted.

| Laboratory | Control | Solvent Control | 25 ng/L | 74 ng/L | 220 ng/L | 670 ng/L | 2000 ng/L |
|------------|---------|-----------------|---------|---------|----------|----------|-----------|
| LAB B | N.D. | N.D. | 37.8 | 54.0 | 123.2 | 438.1 | 1228.2 |
| LAB G | N.D. | N.D. | 17.1 | 49.4 | 166.1 | 526.8 | 1497.9 |
| LAB H (1) | N.D. | N.D. | 20.9 | 44.9 | 157.2 | 495.3 | 1695.8 |
| LAB H (2) | N.D. | N.D. | 16.0 | 39.9 | 117.4 | 365.0 | 1356.3 |
| LAB I | N.D. | N.D. | 13.2 | 25.8 | 69.1 | 264.2 | 1187.2 |
| LAB J | N.D. | N.D. | 9.2 | 19.8 | 46.5 | 155.2 | 657.3 |

3.9 Data assessment and statistical analysis

31. As mentioned above, the test results were based by nominal concentrations.

Data quality assessment

32. There were nine laboratories reporting identically designed experiments with dilution water and solvent controls, and five test concentrations of pyriproxyfen and 3,5-dichlorophenol. There were ten replicates (adult females held individually) per test concentration and the numbers of male and female offspring per replicate were recorded at regular intervals. Some laboratories recorded offspring numbers on a daily basis, while others recorded offspring every other day or in some other uniform fashion. The total number of males per replicate and total number of females per replicate over the entire 21-day period was used in the analysis.

33. There was some inconsistency in the information recorded on offspring sex ratios when an adult died before the end of the test. Some laboratories reported no offspring data at all, while others reported offspring production prior to the adult death but not after. For consistency, none of the offspring information was included in the analysis of sex ratios when the corresponding adults died before the end of the experiment at day 21.

Statistical analysis

34. A detailed schematic diagram of the statistical decision procedure is provided in Figures 1a, 1b, and 1b. Details of how these procedures were applied are discussed here for clarity.
35. When the data did not satisfy homoscedasticity, they were log-transformed before analysis. Nonparametric analysis was performed if no normalizing transform is found.
36. Proportional data (offspring sex ratio) were arc-sin square-root transformed to normalize the response and to stabilize the variance prior to analysis.
37. At first, solvent control was compared with dilution water control for its statistical significance in the tests with 3,5-DCP, as dimethylformamide (DMF) was used as a solvent. For comparisons with other concentrations of the test chemical, solvent control was used regardless of significance of difference between solvent control and water control.
38. LOEC and NOEC were calculated by Williams's test after ANOVA or Kruskal-Wallis test followed by multiple Mann-Whitney tests with a Bonferroni adjustment to the significance levels for total number of neonates. Ratios in each vessel were used to obtain LOEC and NOEC for offspring sex ratio by Williams's test.
39. EC₅₀ values for total number of neonates and offspring sex ratio were estimated by logistic regression by using values (either number or ratio) of each vessel.
40. All the statistical analyses were conducted with JMP (ver. 5.1, SAS Institute, Inc., Cary, NC, USA) except for Williams test, which was performed by Stat Light (Yukms Co., Ltd., Tokyo, Japan).

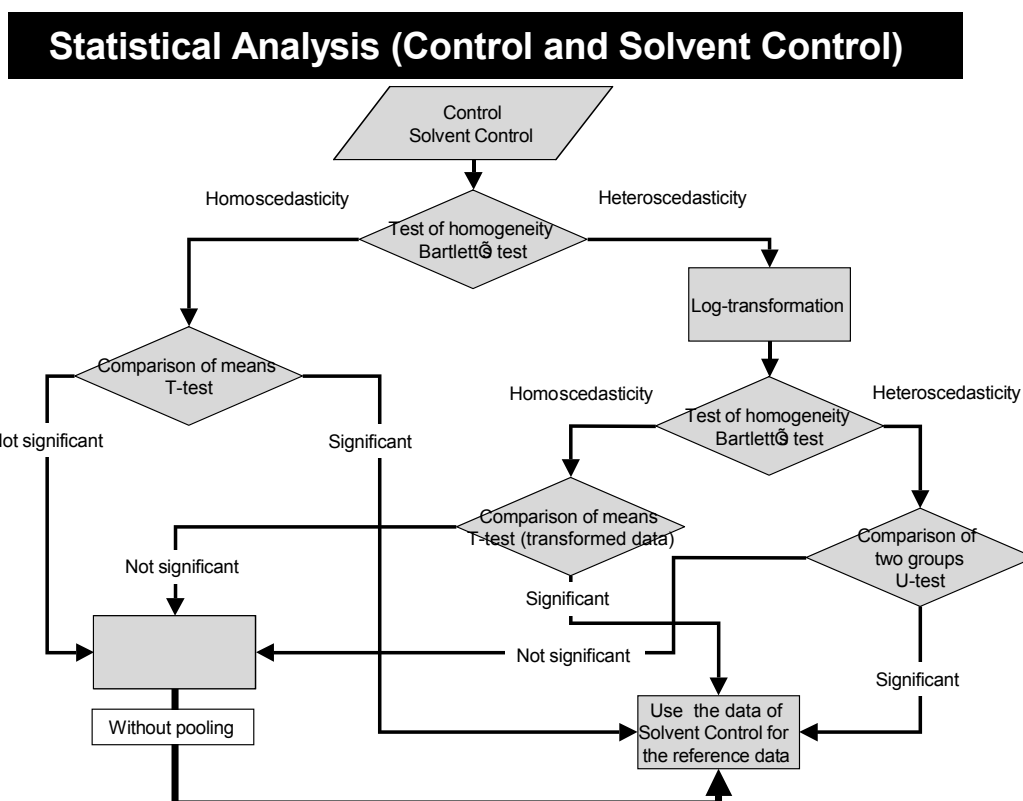


Figure 1a. The schematic diagram of the statistical decision procedure

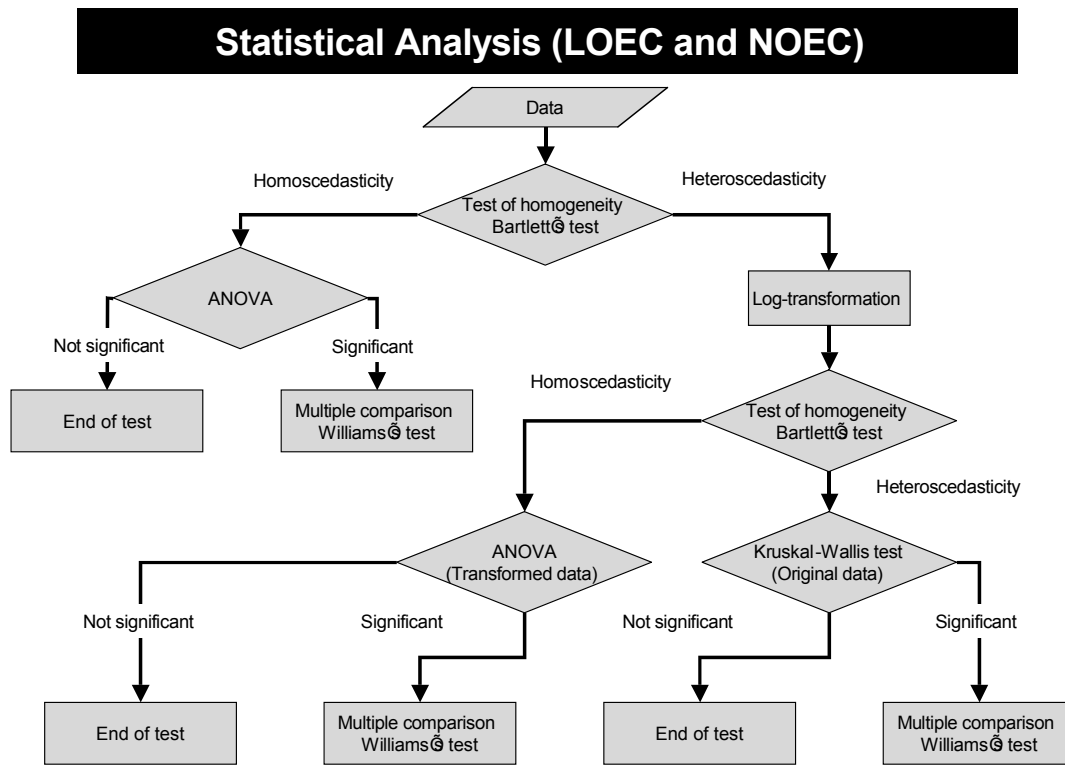


Figure 1b. The schematic diagram of the statistical decision procedure.
Note: Bartlett's test can be replaced by Levene's test.

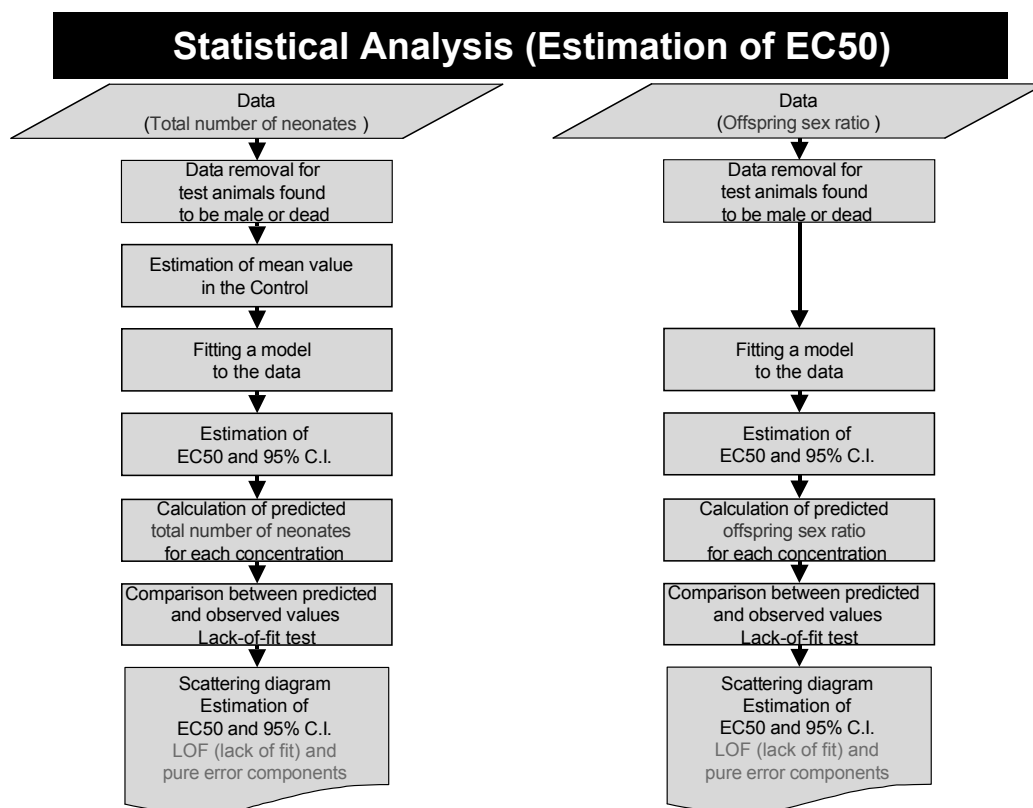


Figure 1c. The schematic diagram of the statistical decision procedure continued from the previous page

41. Annex 2 provides a detailed discussion of the multiple comparisons tests that can be used for the analysis of the sex ratio, in addition to the Williams test proposed in Figure 1b. Additionally, the impacts of the loss of replicates and of reduced replicate size (due to decreased fecundity) on the power properties of the various test proposed are investigated in Annex 2.

4 RESULTS

4.1 Adult mortality

4.1.1 Performance in the controls

42. In most controls (see exception below), the adult female mortality was under 20 % during the test, thus the validity criterion was met in those laboratories.

4.1.2 Effects of the test substances

43. The adult female mortality was under 20 % during the tests except for Laboratory A with 3,5-DCP and Laboratories I and J with pyriproxyfen. These laboratories did not satisfy one of the validity criterion; mortality greater than 20% was observed. Therefore, data from Laboratories A and J was not included in the analysis of sex ratios when the parent died before the end of the experiment. Lab I in Fig. 2 (with pyriproxyfen) was, however, regarded as “valid” because the mortality of the test animals in the solvent control did not exceed 20% at the end of the test (although the mortality was greater than 20% in the water control).

4.2 Reproduction

4.2.1 Performance in the controls

44. Some of the laboratories had difficulties to fulfill the validity criterion of adequate neonate production (Labs A, C, E, and J in [Figure 2](#) and Lab A in [Figure 3](#)). The mean number of live offspring produced per parent animal surviving in the control at the end of the test should have been greater than 60.

4.2.2 Effects of the test substances

45. The effect of the five concentrations of pyriproxyfen on the reproduction is presented in [Figure 2](#) and the effect of 3,5-DCP concentrations in [Figure 3](#).

46. The total number of neonates decreased in a concentration-related manner after exposure to pyriproxyfen in all the laboratories ([Figure 2](#)).

47. Exposure to 3,5-DCP decreased total number of neonates in *D. magna* in a concentration dependent manner in all the laboratory except for those that did not fulfill the validity criteria ([Figure 3](#)).

4.3 Sex ratio of offspring

4.3.1 Controls

48. A small number of male neonates also appeared in the controls, solvent controls and 3,5-DCP exposure treatments in some laboratories (Labs E, F, and in [Figure 3](#), and Labs B, D, and E in [Figure 5](#)).

49. There was one laboratory, in which more than 50% of neonates in the solvent control were males in the test of pyriproxyfen (Lab B in [Figure 2](#)). The results of an additional test with a solvent (Dimethylformamide, DMF) by the laboratory B, however, did not show the induction of male neonates production by DMF.

50. It is thus considered reasonable and appropriate to exclude the data for the laboratory B with pyriproxyfen from further discussions. A validity criterion for the acceptable rate or number of male neonate in controls is needed and proposed further in this report, based on experience from this study and historical data.

4.3.2 *Effects of the test substances*

51. The effects of pyriproxyfen and 3,5-dichlorophenol on the neonate sex ratio are presented in [Figure 4](#) and [Figure 5](#), respectively.

52. The production of male neonates was induced in response to pyriproxyfen exposure in most of the laboratories ([Figure 4](#)).

53. In Lab A and Lab J concentration-dependent male induction was not observed following exposure to pyriproxyfen ([Figure 4](#)). These studies did not satisfy either or both of the validity criteria ([Table 3](#)). Besides, in one of these laboratories, the measured concentration of pyriproxyfen was about 30% of nominal concentration at the highest concentration (Lab J in [Figure 4](#)).

4.4 Overall data evaluation

54. Production of male neonates in the controls or solvent controls, as well as in the treatment groups of 3,5-DCP exposure, was observed in some laboratories. In most of the cases, only a small number of male neonates appeared as background noise (up to 5%). This background rate could be used as a basis to develop a validity criterion on the acceptable level of male neonates in the controls. More than 50% of neonates were male in the solvent control in the test with Pyriproxyfen by one laboratory (Lab B). An additional test, however, showed that no male neonate was produced after exposure to the solvent dimethylformamide (DMF). Production of male neonates in small numbers should be accepted as background noise.

55. The results in which concentration-dependent production of male neonates after exposure to pyriproxyfen had not been observed were excluded from the further analysis because the data did not satisfy the criteria (“validity of the test” in OECD TG 211) in the present ring-test. It is not clear why male neonates were not produced in a concentration-dependent manner in response to pyriproxyfen exposure in these laboratories (Labs A and J in [Figure 4](#)) and if this has something to do with satisfying the criteria “validity of the test”.

56. From the results obtained in the pre-validation tests using several genetically distinct strains of *D. magna*, it seems to be desirable to use specific strains so that enough neonates are produced for sex identification of neonates. Thus, the use of specific strains and adoption of additional criteria for the validity of the test (e.g., “the number of male neonates should not exceed ‘5%’ percent of total number of neonates in control(s)”) for an enhanced version of TG 211 are highly recommended.

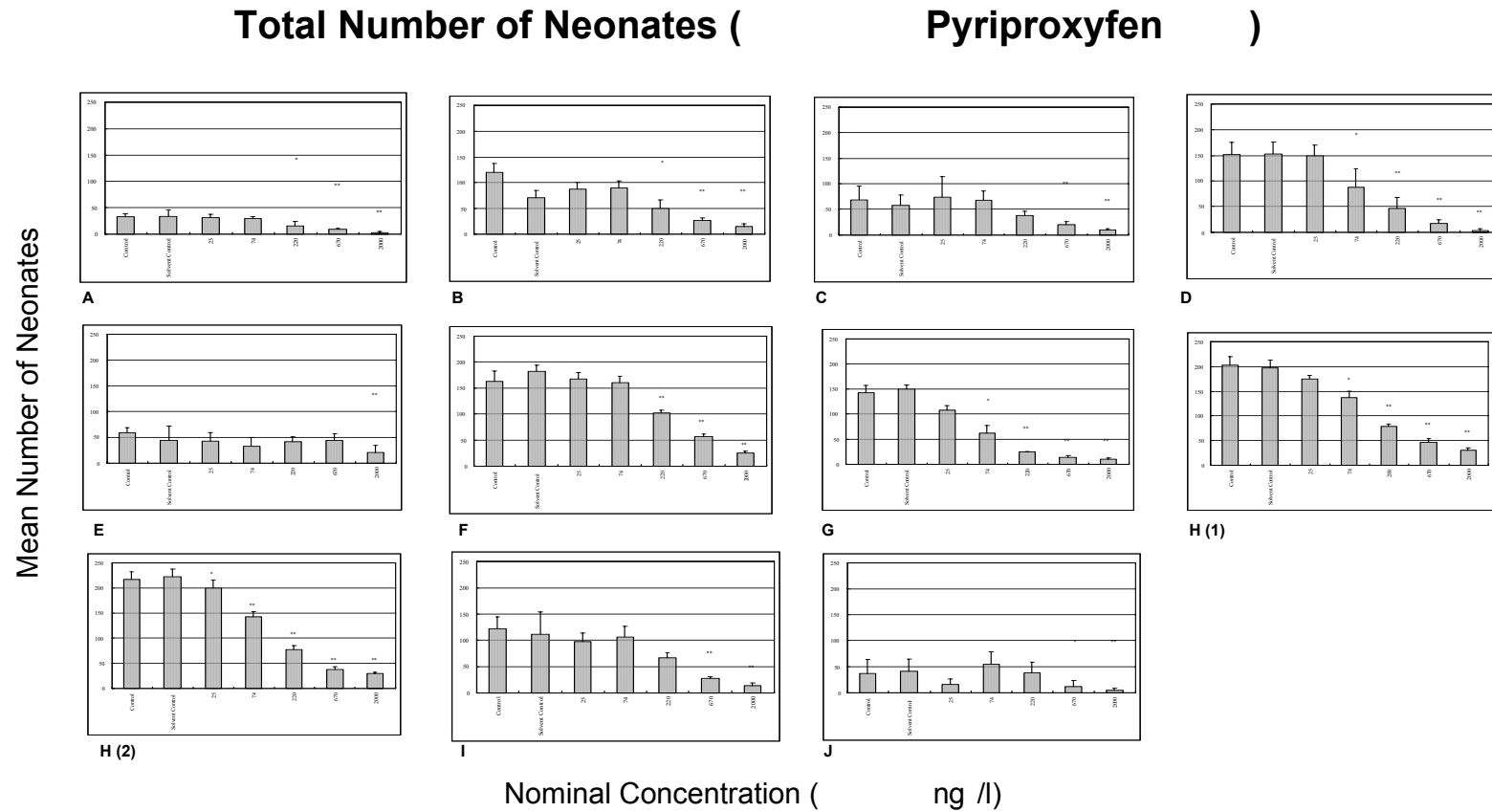


Figure 2. Total number of neonates is presented for the results of pyriproxyfen by ten laboratories. Laboratories were named randomly with alphabet letters, which correspond to those in other figures. For the laboratory H, results of two tests are shown. Error bars indicate standard deviations. Asterisks mean statistically significant difference from solvent control (* $p < 0.05$, ** $p < 0.01$).

Total Number of Neonates (3,5-DCP)

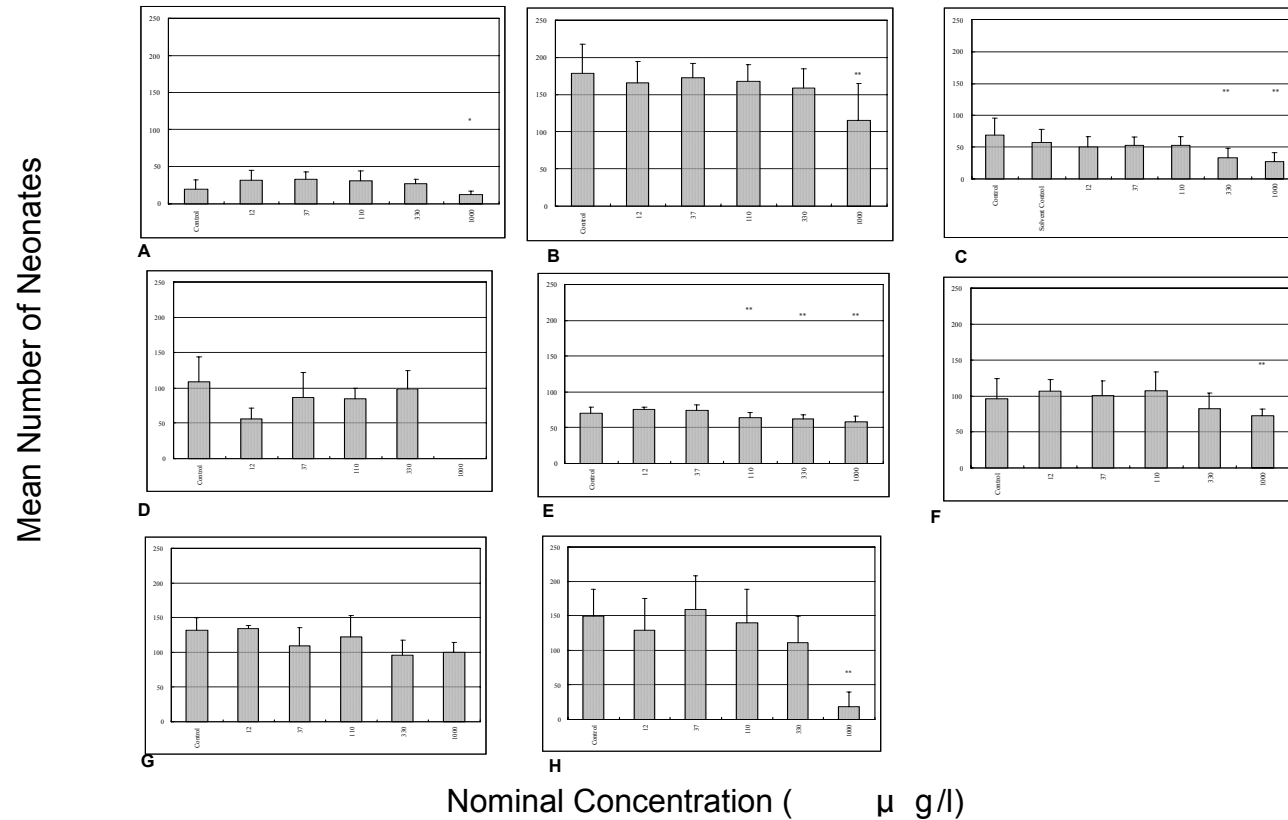


Figure 3. Total number of neonates is presented for the results of 3,5-DCP by eight laboratories. Laboratories were named randomly with alphabet letters, which correspond to those in other figures. Error bars indicate standard deviations. Asterisks mean statistically significant difference from solvent control (* $p < 0.05$, ** $p < 0.01$).

Offspring Sex ratio (Pyriproxyfen)

Offspring Sex Ratio (% male/total)

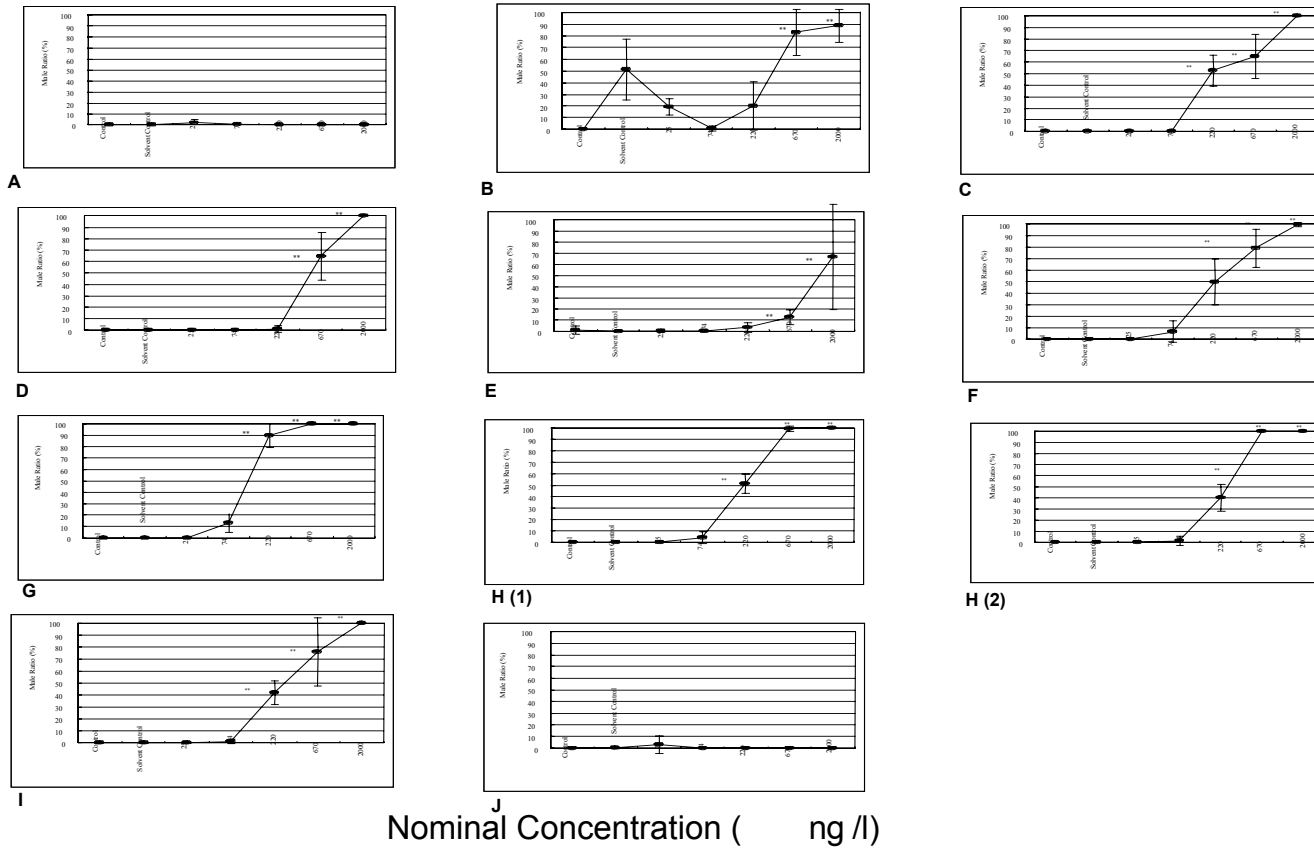


Figure 4. Offspring sex ratio is presented for the results of pyriproxyfen by ten laboratories. Laboratories were named randomly with alphabet letters, which correspond to those in other figures. For the laboratory H, results of two tests are shown. Error bars indicate standard deviations. Asterisks mean statistically significant difference from solvent control (* $p < 0.05$, ** $p < 0.01$).

Offspring Sex Ratio (3,5-DCP)

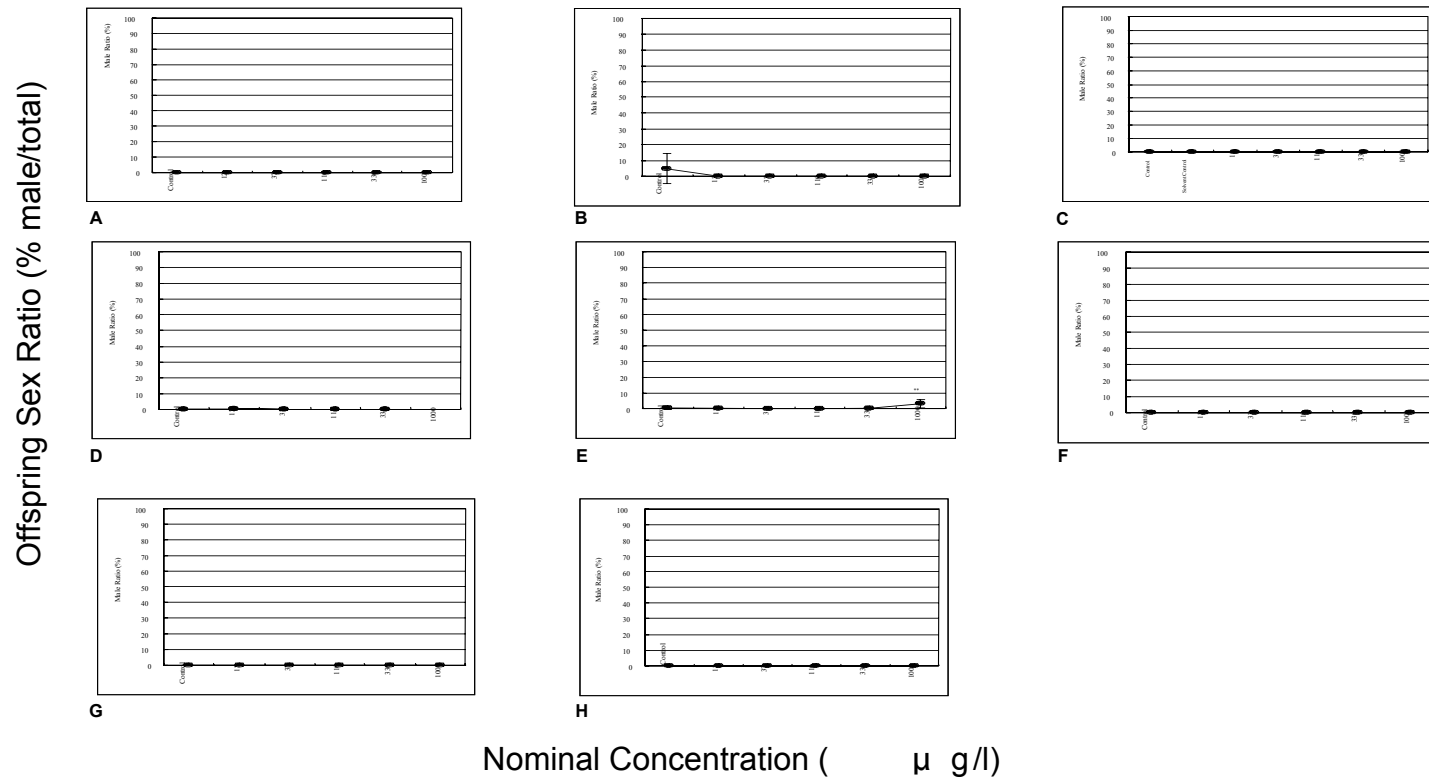


Figure 5. Offspring sex ratio is presented for the results of 3,5-DCP by eight laboratories. Laboratories were named randomly with alphabet letters, which correspond to those in other figures. Error bars indicate standard deviations. Asterisks mean statistically significant difference from solvent control (* $p < 0.05$, ** $p < 0.01$).

5 DISCUSSION

5.1 Test design

57. A test design with 10 replicate adults per control and test concentration is adequate to detect the effects causing a change in the sex ratio (i.e. production of male neonates).

5.2 Biological observations and practicability

58. Most laboratories which participated in this validation exercise found the sexing of the neonates feasible. However, it would also be desirable to find other ways to collect data for the analysis of offspring sex ratio because it is much more laborious to identify the sex of all the neonates in the test compared to the existing TG 211.

5.3 Sources of variability

59. In any biological test, the condition of the organisms and their acclimatization is a potentially large source of variability. At least one laboratory had difficulty attaining adequate reproduction of neonates even though all laboratories used the same strain.

5.4 Statistical power analysis

60. Power studies on the detection of shifts in sex ratio demonstrate that adequate power is achieved with experimental designs having 10 replicates per treatment level. Power is increased when replicates are doubled in the control group, while having five treatment groups plus controls (see [Annex 2](#) and [Annex 3](#) for further details).

5.5 Validity criteria

61. The validity criteria from the existing TG 211 were met in 5 out of 10 laboratories for pyriproxyfen and 7 out of 8 laboratories for 3,5-DCP, and they should be maintained in the updated version of the TG 211.

62. An additional validity criterion is needed for the acceptable level of male neonates in controls, as crowding and test conditions may cause additional stress, possibly inducing male neonates in the controls. The experiments described in this validation report indicate that 5% of male neonate in control replicate is common and can represent the background noise.

6 CONCLUSIONS AND RECOMMENDATIONS FOR TG ENHANCEMENT

63. Using a genetically identical strain, concentration-dependent production of male neonates following exposure to a juvenoid mimic, pyriproxyfen, was observed in all the tests meeting the validity criteria.

64. On the basis of available information, it is not possible to predict which of the sex ratio or of the reproduction endpoint will be more sensitive; however, there are indications this increase in the number of males might be less sensitive than the decrease in offspring.

65. After the discussions in the OECD Invertebrate Expert Group meeting in June 2007 in Columbia (United States) it was decided that only minor modifications are needed to the existing Test Guideline 211. The existing TG 211 already mentions in paragraph 44, that the production of male neonates could be an optional endpoint. An additional *Annex 7* will be included to provide guidance on the identification of the sex of the neonates. The resultant changes and the additional wording used in the revised TG 211 are provided in Annex 3.

7 LITERATURE

Baldwin, W.S., Bailey, R., Long, K.E., and Klaine, S. (2001). Incomplete ecdysis is an indicator of ecysteroid exposure in *Daphnia magna*. *Environmental Toxicology and Chemistry* 20, 1564-1569.

Hobaek, A., Larsson, P., 1990. Sex determination in *Daphnia magna*. *Ecology* 71, 2255–2268.

Kleiven, O.T., Larsson, P., Hobaek, A., 1992. Sexual reproduction in *Daphnia magna* requires three stimuli. *Oikos* 65,197–206.

Oda, S., Tatarazako, N., Watanabe, H., Morita, M., Iguchi, T., 2005a. Production of male neonates in *Daphnia magna* (Cladocera, Crustacea) exposed to juvenile hormones and their analogs. *Chemosphere* 61, 1168–1174.

Oda, S., Tatarazako, N., Watanabe, H., Morita, M., Iguchi, T., 2005b. Production of male neonates in 4 cladoceran species exposed to a juvenile hormone analog, fenoxycarb. *Chemosphere* 60, 74–78.

Oda, S., Tatarazako, N., Dorgerloh, M., Johnson, R., Kusk, K.O., Leverett, D., Marchini, S., Nakari, T., Williams, T., Iguchi, T. (2007) Strain difference in sensitivity to 3,4-dichloroaniline and insect growth regulator, fenoxycarb, in *Daphnia magna*. *Ecotoxicology and Environmental Safety*, 67, 399-405.

OECD, 1998. *Daphnia magna* reproduction test (21pp.). In: OECD Guidelines for Testing of Chemicals. Organization for Economic Cooperation and Development, Paris.

OECD, 2004. *Daphnia sp.*, acute immobilization test (12pp.). In: OECD Guidelines for Testing of Chemicals. Organization for Economic Cooperation and Development, Paris.

Olmstead, A.W. and LeBlanc, G.A. (2002). Juvenoid hormone methyl farnesoate is a sex determinant in the crustacean *Daphnia magna*. *J. Exp. Zool.*, 293, 736–739.

Olmstead, A.W. and LeBlanc, G.A. (2003). Insecticidal juvenile hormone analogs stimulate the production of male offspring in the crustacean *Daphnia magna*. *Environ. Health Perspect.*, 111, 919-924.

Tatarazako, N., Oda, S., Watanabe, H., Morita, M., and Iguchi, T. (2003). Juvenile hormone agonists affect the occurrence of male *Daphnia*. *Chemosphere*, 53, 827-833.

ANNEX 1**Statistical Analysis of *Daphnia Magna* Sex ratio Experiments**

John W. Green, Ph.D.

Senior Consultant: Biostatistics, DuPont Applied Statistics Group

1. A detailed schematic diagram of the statistical decision procedure is provided below in Figures 1a and 1b. Details of that decision procedure are discussed here for clarity.
2. The sex ratios of the water and solvent controls were compared by the Mann-Whitney test in the two cases where different male proportions were observed in the two controls. In neither case was the difference in the controls statistically significant. Consequently, the two controls were pooled for further analysis.
3. Sex ratios in offspring differ from sex ratios in earlier experiments because the number of offspring is not fixed at the beginning of the experiment. This means the Cochran-Armitage and Fisher exact tests are not appropriate. The viable tests for determining the LOEC and NOEC are (1) Dunnett's test or Williams test on arc-sin square-root transformed proportion males or proportion females if this (or another transform) normalizes the response and stabilizes the variance, (2) the Jonckheere-Terpstra test applied in step-down fashion, or (3) Dunn's test or multiple Mann-Whitney tests with a Bonferroni-Holm adjustment to the significance levels if no normalizing transform is found. In the case of the Williams or Jonckheere test, an assessment of dose-response monotonicity is also made and serious deviation from monotonicity would preclude either of these tests.
4. The tests done were 1-sided for an increasing response on the proportion males and 1-sided for a decreasing response on the proportion females. The NOECs and LOECs are the same for both proportions and only the results for males are summarized in this report.

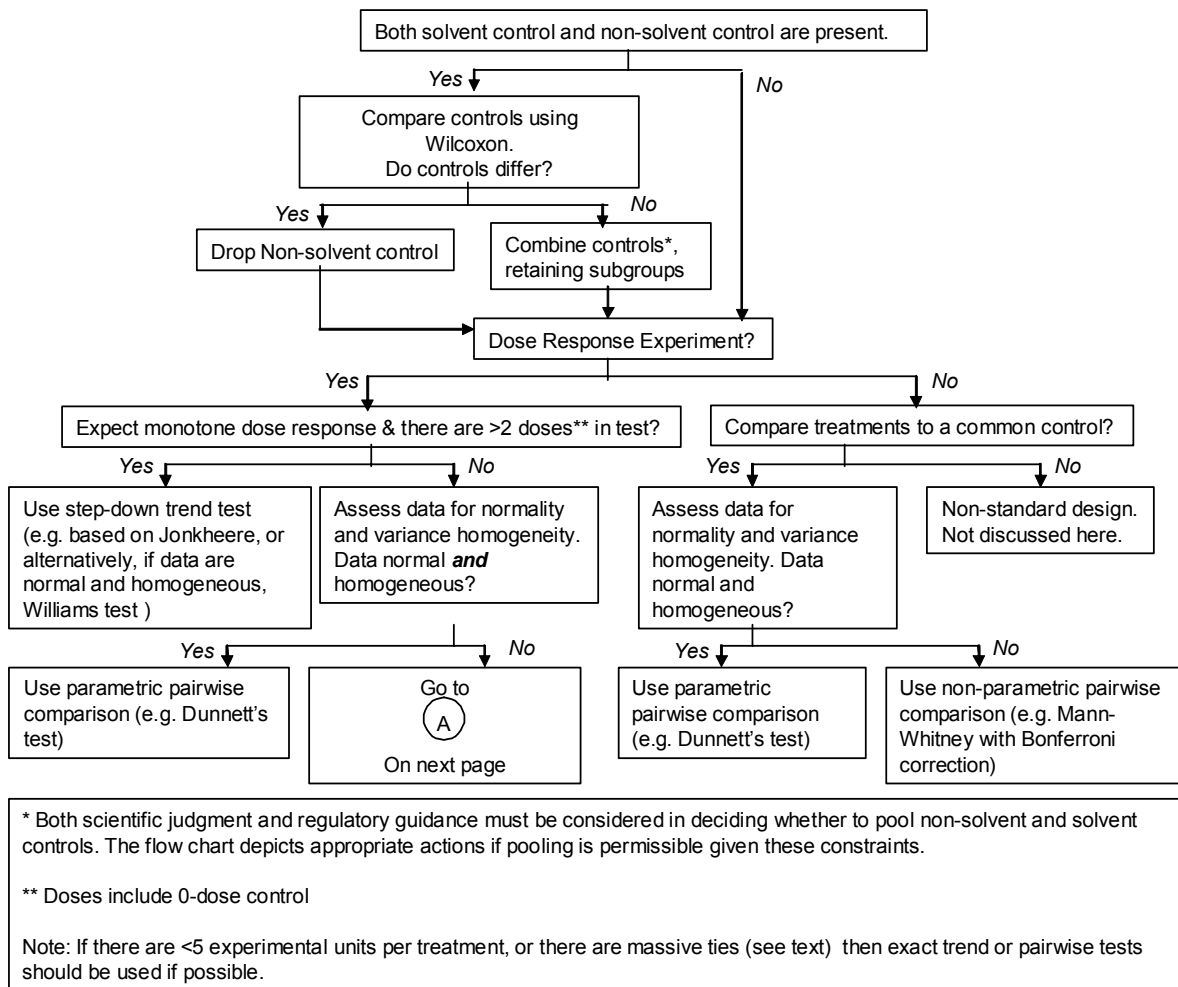
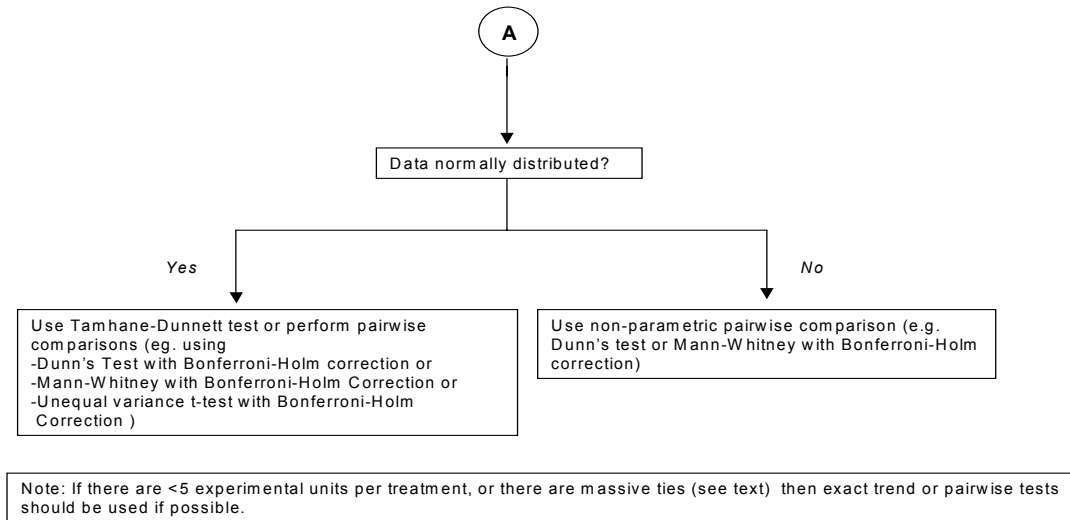


Figure 1a. The schematic diagram of the statistical decision procedure



POWER PROPERTIES OF THE SEX RATIO ENDPOINT

Power Properties of Jonckheere Test

5. A complete discussion of the power properties of the Jonckheere-Terpstra test applied to sex-ratio data will be deferred. However, the following plot in [Figure 6](#) captures the essential power properties for experiments such as analyzed in this report, where there are ten replicates per test concentration and 5 test concentrations plus a control. The power plot assumes the same number of replicates in control and treatments, whereas the present data have double the number of replicates in the control due to the two controls. Thus, the power properties for the current experiments will be greater than shown in this plot. Nevertheless, this will serve as a baseline.

6. What [Table 7](#) below indicates is that the Jonckheere test found significant effects of about the same size as the standard deviation. The horizontal axis in the power curve below in [Figure 6](#) is in standard deviation units, so that, for example, 1 indicates an effect of 1 standard deviation; 2 indicates an effect of 2 standard deviations in magnitude, etc. The power curve above the horizontal axis shows that the power to detect an effect of magnitude 1 std is around 70%. The results from the current experiments are thus consistent with these general power properties.

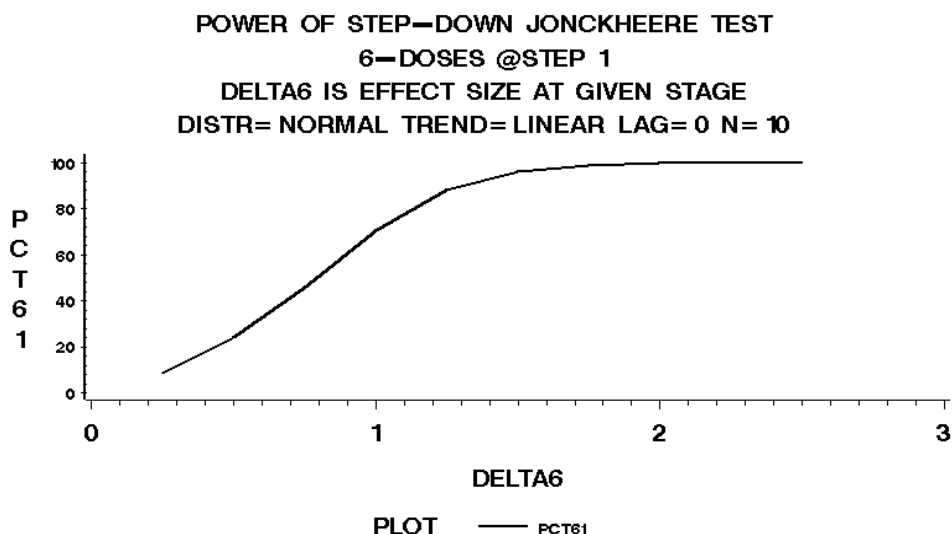


Figure 6. The power curve of the step-down Jonckheere test

Table 7. Summary of Results with Statistical Significance

SYMBOL KEY:

- * Parametric comparison to control (Dunnett/Tamhane-Dunnett) significant
- @ Nonparametric comparison to control (Dunn's) significant
- # Trend test (Jonckheere-Terpstra) significant
- ~ next to control mean indicates no analyses were performed
- All symbols use alpha=0.05 level
- Tests were for an increasing dose-response
- Results are reported for the proportion males as

| | | LAB I DAPHNID | | | |
|----------------------|------------|---------------|-----------|-----------|------------|
| | | 1 | 2 | 3 | 4 |
| Group: | 5 | | | | |
| Concentration (ug/L) | 6 | | | | |
| | 670 | 0 | 25 | 74 | 220 |
| | 2000 | | | | |
| PRPMALE | | 0.000 | 0.000 | 0.012# | 0.418@# |
| 0.759@# | 1.000@# | 0.000 (15) | 0.000 (8) | 0.019 (9) | 0.102 (10) |
| 0.287 (8) | 0.000 (10) | | | | |
| | | LAB A DAPHNID | | | |
| | | 1 | 2 | 3 | 4 |
| Group: | 5 | | | | |
| Concentration (ug/L) | 6 | | | | |
| | 670 | 0 | 25 | 74 | 220 |
| | 2000 | | | | |
| PRPMALE | | 0.000 | 0.019@ | 0.005 | 0.000 |
| 0.000 | 0.000 | 0.000 (18) | 0.029 (7) | 0.013 (7) | 0.000 (6) |
| 0.000 (8) | 0.000 (7) | | | | |

| | | LAB D DAPHNID | | | |
|----------------------|-----------|---------------|-----------|-----------|-----------|
| Group: | | 1 | 2 | 3 | 4 |
| 5 | 6 | | | | |
| Concentration (ug/L) | | 0 | 25 | 74 | 220 |
| 670 | 2000 | | | | |
| PRPMALE | | 0.000 | 0.000 | 0.000 | 0.012# |
| 0.647@# | 1.000@# | 0.000 (19) | 0.000 (8) | 0.000 (8) | 0.028 (8) |
| 0.209 (9) | 0.000 (7) | | | | |

ENV/JM/MONO(2008)20

LAB B DAPHNID

| Group: | | 1 | 2 | 3 | 4 |
|----------------------|-----------|------------|------------|------------|------------|
| 5 | 6 | | | | |
| Concentration (ug/L) | | | | | |
| 670 | 2000 | 0 | 25 | 74 | 220 |
| PRPMALE | | 0.000 | 0.193@ | 0.007 | 0.201# |
| 0.829@# | 0.887@# | | | | |
| | | 0.000 (16) | 0.071 (10) | 0.021 (10) | 0.208 (10) |
| 0.198 (10) | 0.139 (9) | | | | |

LAB H DAPHNID

| Group: | | 1 | 2 | 3 | 4 |
|----------------------|------------|------------|------------|------------|------------|
| 5 | 6 | | | | |
| Concentration (ug/L) | | | | | |
| 670 | 2000 | 0 | 25 | 74 | 220 |
| PRPMALE | | 0.000 | 0.000 | 0.013 | 0.403@# |
| 1.000@# | 1.000@# | | | | |
| | | 0.000 (20) | 0.000 (10) | 0.042 (10) | 0.121 (10) |
| 0.000 (10) | 0.000 (10) | | | | |

LAB G DAPHID

| Group: | | 1 | 2 | 3 | 4 |
|----------------------|------------|------------|------------|-----------|------------|
| 5 | 6 | | | | |
| Concentration (ug/L) | | | | | |
| 670 | 2000 | 0 | 25 | 74 | 220 |
| PRPMALE | | 0.000 | 0.000 | 0.146@# | 0.897@# |
| 1.000@# | 1.000@# | | | | |
| | | 0.000 (20) | 0.000 (10) | 0.068 (9) | 0.107 (10) |
| 0.000 (10) | 0.000 (10) | | | | |

LAB E DAPHNID

| Group: | | 1 | 2 | 3 | 4 |
|----------------------|------------|------------|------------|-----------|------------|
| 5 | 6 | | | | |
| Concentration (ug/L) | | | | | |
| 670 | 2000 | 0 | 25 | 74 | 220 |
| PRPMALE | | 0.006 | 0.005 | 0.002 | 0.035# |
| 0.128@# | 0.667@# | | | | |
| | | 0.026 (19) | 0.016 (10) | 0.007 (9) | 0.043 (10) |
| 0.064 (10) | 0.471 (10) | | | | |

ENV/JM/MONO(2008)20

LAB F DAPHNID

| Group: | | 1 | 2 | 3 | 4 |
|----------------------|------------|------------|------------|------------|------------|
| 5 | 6 | | | | |
| Concentration (ug/L) | | 0 | 25 | 74 | 220 |
| 670 | 2000 | | | | |
| PRPMALE | | 0.000 | 0.001 | 0.065# | 0.448@# |
| 0.791@# | 0.995@# | | | | |
| | | 0.001 (20) | 0.004 (10) | 0.094 (10) | 0.244 (10) |
| 0.165 (10) | 0.015 (10) | | | | |

LAB C DAPHNID

| Group: | | 1 | 2 | 3 | 4 |
|----------------------|------------|------------|-----------|-----------|------------|
| 5 | 6 | | | | |
| Concentration (ug/L) | | 0 | 25 | 74 | 220 |
| 670 | 2000 | | | | |
| PRPMALE | | 0.000 | 0.000 | 0.000 | 0.527@# |
| 0.651@# | 1.000@# | | | | |
| | | 0.000 (18) | 0.000 (9) | 0.000 (9) | 0.134 (10) |
| 0.189 (10) | 0.000 (10) | | | | |

7. In all cases, there was no significant difference between the solvent and water control for proportion males, so the two controls could be combined for further analysis to determine the NOEC and LOEC. A formal analysis between controls was conducted in Lab E and Lab F, where the difference was small but not statistically significant, thus allowing combination of controls to increase the statistical power of the tests.

Table 6: LOEC Summary

| | LOEC by Dunn | LOEC by JT |
|-------|--------------|------------|
| Lab I | 220 | 74 |
| Lab A | 25* | >2000 |
| Lab D | 670 | 220 |
| Lab B | 25** | 220 |
| Lab H | 220 | 220 |
| Lab G | 74 | 74 |
| Lab E | 670 | 220 |
| Lab F | 220 | 74 |
| Lab C | 220 | 220 |

* Only the 25 ug/L conc effect was statistically significant

** Only the 25, 670, and 2000 ug/L conc effects were statistically significant

The statistics on male neonates induction in the 3,5-DCP experiments are not shown here as there was no significant findings in these tests.

8. No normalizing transformation for the proportion male or proportion female was found. Consequently, only non-parametric tests were done. No significant non-monotonicity was found, so trend-based tests were appropriate. Both a pair-wise test, Dunn's test, and a trend-based test, the Jonckheere-Terpstra, were done. [Table 6](#) summarizes the results of the two tests. [Table 7](#) provides greater detail. The complete statistical output is provided in [Annex 2](#).

9. Results from Lab A were noticeably different from the other laboratories. There was no significant effect at any test concentration found using the Jonckheere-Terpstra test and only the effect at the 25 ug/L concentration were found significant by Dunn's test. Apart from this laboratory, all labs found significant effects at the three highest test concentrations by the Jonckheere-Terpstra test and three labs also found significant effects at 74ug/L by that test. Results from Dunn's test were less consistent.

Details of Statistical Analyses

Comparison of Controls in Lab E

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 12:47 WEDNESDAY 27JUN07
 DAPHNID MEASUREMENTS FROM DATASET CTRLLLHH
 GROUP STATISTICS FOR PRPMALE BY DOSE, Unweighted

| dose | doseval | COUNT | MEAN | MEDIAN | STD_DEV | STD_ERR |
|------|---------|-------|----------|--------|----------|----------|
| 1 | 0 | 10 | 0.011290 | 0 | 0.035703 | 0.011290 |
| 2 | 0 | 9 | 0.000000 | 0 | 0.000000 | 0.000000 |

NOTE
 Kruskal-Wallis analysis specifically requested.
 Normality and equality of variance have not been checked.

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 12:47 WEDNESDAY 27JUN07
 Kruskal-Wallis Test on PRPMALE
 DAPHNID MEASUREMENTS FOR FULL DATA

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable RESPONSE
 Classified by Variable dose

| dose | N | Sum of Scores | Expected Under H0 | Std Dev Under H0 | Mean Score |
|------|----|---------------|-------------------|------------------|------------|
| 1 | 10 | 104.50 | 100.0 | 4.743416 | 10.450 |
| 2 | 9 | 85.50 | 90.0 | 4.743416 | 9.500 |

Average scores were used for ties.
 Wilcoxon Two-Sample Test

Statistic 85.5000

Normal Approximation

Z -0.8433
 One-Sided Pr < Z 0.1995
 Two-Sided Pr > |Z| 0.3991

t Approximation

One-Sided Pr < Z 0.2051
 Two-Sided Pr > |Z| 0.4101

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square 0.9000
 DF 1
 Pr > Chi-Square 0.3428

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 12:47 WEDNESDAY 27JUN07
 Modified Dunn's Multiple Comparisons (Two-sided) on PRPMALE
 DAPHNID MEASUREMENTS FOR FULL DATA USING ALPHA=0.05

| dose | doseval | COUNT | N0 | MRANK | ABS_DIFF | CRIT_05 | CRIT_01 | SIGNIF | p_val |
|------|---------|-------|----|-------|----------|---------|---------|---------|--------|
| 1 | | 0 | 10 | 10 | 10.45 | 0.00 | 1.91034 | 2.51061 | . |
| 2 | | 0 | 9 | 10 | 9.50 | 0.95 | 1.96268 | 2.57940 | 0.1714 |

Thus, there is no statistically significant difference between the two controls. These controls will be combined to determine the NOEC for Lab E.

Comparison of Controls in Lab F

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 12:51 WEDNESDAY 27JUN07
 DAPHNID MEASUREMENTS FROM DATASET CTRLNYK
 GROUP STATISTICS FOR PRPMALE BY DOSE, Unweighted

| dose | doseval | COUNT | MEAN | MEDIAN | STD_DEV | STD_ERR |
|------|---------|-------|------|------------|---------|------------|
| 1 | | 0 | 10 | .000558659 | 0 | .001766636 |
| 2 | | 0 | 10 | 0 | 0 | 0 |

NOTE
 Kruskal-Wallis analysis specifically requested.
 Normality and equality of variance have not been checked.

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 12:51 WEDNESDAY 27JUN07
 Kruskal-Wallis Test on PRPMALE
 DAPHNID MEASUREMENTS FOR FULL DATA

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable RESPONSE
 Classified by Variable dose

| dose | N | Sum of Scores | Expected Under H0 | Std Dev Under H0 | Mean Score |
|------|----|---------------|-------------------|------------------|------------|
| 1 | 10 | 110.0 | 105.0 | 5.0 | 11.0 |
| 2 | 10 | 100.0 | 105.0 | 5.0 | 10.0 |

Average scores were used for ties.
 Wilcoxon Two-Sample Test

Statistic 110.0000

Normal Approximation
 Z 0.9000
 One-Sided Pr > Z 0.1841
 Two-Sided Pr > |Z| 0.3681

t Approximation
 One-Sided Pr > Z 0.1897

Two-Sided Pr > |Z| 0.3794

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square 1.0000
 DF 1
 Pr > Chi-Square 0.3173

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 12:51 WEDNESDAY 27JUN07
 Modified Dunn's Multiple Comparisons (Two-sided) on PRPMALE
 DAPHNID MEASUREMENTS FOR FULL DATA USING ALPHA=0.05

| dose | doseval | COUNT | N0 | MRANK | ABS_DIFF | CRIT_05 | CRIT_01 | SIGNIF | p_val |
|------|---------|-------|----|-------|----------|---------|---------|--------|--------|
| 1 | 0 | 10 | 10 | 11 | 0 | 1.95996 | 2.57583 | . | |
| 2 | 0 | 10 | 10 | 10 | 1 | 1.95996 | 2.57583 | | 0.1587 |

Thus, there is no statistically significant difference between the two controls. These controls will be combined to determine the NOEC for Lab F.

Comparison of Controls in Other Labs

There was no difference in proportion males in the two controls for any other lab, so no formal test is needed to conclude that no statistically significant difference exists. These controls will be combined in each lab to determine the NOEC for that lab.

NOEC for LAB I

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:07 WEDNESDAY 27JUN07
 LAB I DAPHNID MEASUREMENTS FROM DATASET SEXRATIOS
 GROUP STATISTICS FOR PRPMALE BY DOSE, Unweighted

| dose | doseval | COUNT | MEAN | MEDIAN | STD_DEV | STD_ERR |
|------|---------|-------|---------|---------|---------|---------|
| 1 | 0 | 15 | 0.00000 | 0.00000 | 0.00000 | 0.00000 |
| 2 | 25 | 8 | 0.00000 | 0.00000 | 0.00000 | 0.00000 |
| 3 | 74 | 9 | 0.01199 | 0.00000 | 0.01928 | 0.00643 |
| 4 | 220 | 10 | 0.41768 | 0.41311 | 0.10213 | 0.03229 |
| 5 | 670 | 8 | 0.75950 | 0.88462 | 0.28695 | 0.10145 |
| 6 | 2000 | 10 | 1.00000 | 1.00000 | 0.00000 | 0.00000 |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:07 WEDNESDAY 27JUN07
 SHAPIRO-WILK TEST OF NORMALITY OF PRPMALE
 AQUATIC DAPHNID: FULL DATA

| OBS | STD | SKEW | KURT | SW_STAT | P_VALUE | SIGNIF |
|-----|---------|----------|---------|---------|---------|--------|
| 60 | 0.10682 | -1.01631 | 6.20207 | 0.66691 | 0.0001 | ** |

Outliers & Influential Observations
 PRPMALE FROM FULL DATA Unweighted
 AQUATIC DAPHNID: Concentrations in ug/L

| Obs | DAPHNID | dose | doseval | GROUP | OBSER | Pred | SE_PRED |
|-----|---------|------|---------|-------|---------|---------|----------|
| 1 | 1 | 3 | 74 | 3 | 0.00000 | 0.01199 | 0.037219 |
| 2 | 2 | 3 | 74 | 3 | 0.00000 | 0.01199 | 0.037219 |
| 3 | 3 | 3 | 74 | 3 | 0.00000 | 0.01199 | 0.037219 |
| 4 | 4 | 3 | 74 | 3 | 0.00000 | 0.01199 | 0.037219 |
| 5 | 5 | 3 | 74 | 3 | 0.00806 | 0.01199 | 0.037219 |
| 6 | 7 | 3 | 74 | 3 | 0.03540 | 0.01199 | 0.037219 |
| 7 | 8 | 3 | 74 | 3 | 0.05310 | 0.01199 | 0.037219 |
| 8 | 9 | 3 | 74 | 3 | 0.00000 | 0.01199 | 0.037219 |
| 9 | 1 | 4 | 220 | 4 | 0.42623 | 0.41768 | 0.035309 |
| 10 | 2 | 4 | 220 | 4 | 0.31746 | 0.41768 | 0.035309 |
| 11 | 3 | 4 | 220 | 4 | 0.48276 | 0.41768 | 0.035309 |
| 12 | 4 | 4 | 220 | 4 | 0.39437 | 0.41768 | 0.035309 |
| 13 | 5 | 4 | 220 | 4 | 0.55319 | 0.41768 | 0.035309 |
| 14 | 6 | 4 | 220 | 4 | 0.40000 | 0.41768 | 0.035309 |
| 15 | 7 | 4 | 220 | 4 | 0.58824 | 0.41768 | 0.035309 |
| 16 | 8 | 4 | 220 | 4 | 0.29268 | 0.41768 | 0.035309 |
| 17 | 9 | 4 | 220 | 4 | 0.42857 | 0.41768 | 0.035309 |
| 18 | 10 | 4 | 220 | 4 | 0.29333 | 0.41768 | 0.035309 |
| 19 | 2 | 5 | 670 | 5 | 1.00000 | 0.75950 | 0.039476 |
| 20 | 3 | 5 | 670 | 5 | 1.00000 | 0.75950 | 0.039476 |
| 21 | 4 | 5 | 670 | 5 | 0.76923 | 0.75950 | 0.039476 |
| 22 | 5 | 5 | 670 | 5 | 1.00000 | 0.75950 | 0.039476 |
| 23 | 6 | 5 | 670 | 5 | 0.37500 | 0.75950 | 0.039476 |
| 24 | 7 | 5 | 670 | 5 | 1.00000 | 0.75950 | 0.039476 |
| 25 | 9 | 5 | 670 | 5 | 0.35484 | 0.75950 | 0.039476 |

| Obs | L95M | U95M | Resid | LB | UB |
|-----|----------|---------|----------|-------------|------------|
| 1 | -0.06263 | 0.08661 | -0.01199 | -.000784861 | .000470917 |
| 2 | -0.06263 | 0.08661 | -0.01199 | -.000784861 | .000470917 |
| 3 | -0.06263 | 0.08661 | -0.01199 | -.000784861 | .000470917 |
| 4 | -0.06263 | 0.08661 | -0.01199 | -.000784861 | .000470917 |
| 5 | -0.06263 | 0.08661 | -0.00393 | -.000784861 | .000470917 |
| 6 | -0.06263 | 0.08661 | 0.02341 | -.000784861 | .000470917 |
| 7 | -0.06263 | 0.08661 | 0.04111 | -.000784861 | .000470917 |
| 8 | -0.06263 | 0.08661 | -0.01199 | -.000784861 | .000470917 |
| 9 | 0.34689 | 0.48847 | 0.00855 | -.000784861 | .000470917 |
| 10 | 0.34689 | 0.48847 | -0.10022 | -.000784861 | .000470917 |
| 11 | 0.34689 | 0.48847 | 0.06508 | -.000784861 | .000470917 |
| 12 | 0.34689 | 0.48847 | -0.02332 | -.000784861 | .000470917 |

| | | | | | |
|----|---------|---------|----------|-------------|------------|
| 13 | 0.34689 | 0.48847 | 0.13551 | -.000784861 | .000470917 |
| 14 | 0.34689 | 0.48847 | -0.01768 | -.000784861 | .000470917 |
| 15 | 0.34689 | 0.48847 | 0.17055 | -.000784861 | .000470917 |
| 16 | 0.34689 | 0.48847 | -0.12500 | -.000784861 | .000470917 |
| 17 | 0.34689 | 0.48847 | 0.01089 | -.000784861 | .000470917 |
| 18 | 0.34689 | 0.48847 | -0.12435 | -.000784861 | .000470917 |
| 19 | 0.68035 | 0.83864 | 0.24050 | -.000784861 | .000470917 |
| 20 | 0.68035 | 0.83864 | 0.24050 | -.000784861 | .000470917 |
| 21 | 0.68035 | 0.83864 | 0.00973 | -.000784861 | .000470917 |
| 22 | 0.68035 | 0.83864 | 0.24050 | -.000784861 | .000470917 |
| 23 | 0.68035 | 0.83864 | -0.38450 | -.000784861 | .000470917 |
| 24 | 0.68035 | 0.83864 | 0.24050 | -.000784861 | .000470917 |
| 25 | 0.68035 | 0.83864 | -0.40466 | -.000784861 | .000470917 |

Outliers & Influential Observations
 PRPMALE FROM FULL DATA Unweighted
 AQUATIC DAPHNID: Concentrations in ug/L

| Obs | DAPHNID | dose | doseval | GROUP | OBSER | Pred | SE_PRED |
|-----|---------|------|---------|-------|---------|---------|----------|
| 26 | 10 | 5 | 670 | 5 | 0.57692 | 0.75950 | 0.039476 |

| Obs | L95M | U95M | Resid | LB | UB |
|-----|---------|---------|----------|-------------|------------|
| 26 | 0.68035 | 0.83864 | -0.18258 | -.000784861 | .000470917 |

NOTE

This set of outliers will be excluded from all outlier omitted analyses for this response. In order for the outliers from the transformed analysis to be used for outlier omitted analysis instead, run the analysis again and either specify the transform (instead of allowing the system to decide) or manually omit these observations using the exclusion functionality.

NOTE

The data was NOT normally distributed.
 A nonparametric analysis will be performed.

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:07 WEDNESDAY 27JUN07
 Kruskal-Wallis Test on PRPMALE
 LAB I DAPHNID MEASUREMENTS FOR FULL DATA

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable RESPONSE
 Classified by Variable dose

| dose | N | Sum of Scores | Expected Under H0 | Std Dev Under H0 | Mean Score |
|------|----|---------------|-------------------|------------------|------------|
| 1 | 15 | 217.50 | 457.50 | 55.131680 | 14.500000 |
| 2 | 8 | 116.00 | 244.00 | 43.280872 | 14.500000 |
| 3 | 9 | 194.50 | 274.50 | 45.462748 | 21.611111 |
| 4 | 10 | 390.00 | 305.00 | 47.449795 | 39.000000 |
| 5 | 8 | 377.00 | 244.00 | 43.280872 | 47.125000 |
| 6 | 10 | 535.00 | 305.00 | 47.449795 | 53.500000 |

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square 54.8629
 DF 5
 Pr > Chi-Square <.0001

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:07 WEDNESDAY 27JUN07
 Modified Dunn's Multiple Comparisons (Increasing) on PRPMALE
 LAB I DAPHNID MEASUREMENTS FOR FULL DATA USING ALPHA=0.05

| dose | doseval | COUNT | N0 | MRANK | ABS_DIFF | CRIT_05 | CRIT_01 | SIGNIF | p_val |
|------|---------|-------|----|---------|----------|---------|---------|--------|--------|
| 1 | 0 | 15 | 15 | 14.5000 | 0.0000 | 13.9627 | 17.2747 | . | |
| 2 | 25 | 8 | 15 | 14.5000 | 0.0000 | 16.7407 | 20.7116 | | 1.0000 |
| 3 | 74 | 9 | 15 | 21.6111 | 7.1111 | 16.1227 | 19.9471 | | 0.7622 |
| 4 | 220 | 10 | 15 | 39.0000 | 24.5000 | 15.6108 | 19.3137 | ** | 0.0007 |
| 5 | 670 | 8 | 15 | 47.1250 | 32.6250 | 16.7407 | 20.7116 | ** | 0.0001 |
| 6 | 2000 | 10 | 15 | 53.5000 | 39.0000 | 15.6108 | 19.3137 | ** | 0.0001 |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:07 WEDNESDAY 27JUN07
 MONOTONICITY CHECK OF PRPMALE - FULL DATA
 DOSES 0, 25, 74, 220, 670, 2000 ug/L
 LAB I DAPHNID OF AQUATIC DAPHNID

PARAM p_t SIGNIF
 DOSE TREND 0.0001 **
 DOSE QUAD 0.0005 **

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:07 WEDNESDAY 27JUN07
 ANALYSIS OF PRPMALE USING ALPHA=0.05
 FULL DATA

----- JONCKHEERE-TERPSTRA TEST KEY -----
 JT IS JONCKHEERE STATISTIC
 Z_JT IS STANDARDIZED JONCKHEERE STATISTIC
 PR_JT IS P-VALUE FOR UPWARD TREND
 PL_JT IS P-VALUE FOR DOWNWARD TREND
 P-VALUES ARE FOR TIE-CORRECTED TEST WITH CONTINUITY CORRECTION FACTOR
 SIGNIF RESULTS ARE FOR Increasing ALTERNATIVE HYPOTHESIS

Check for ties in PRPMALE
 Percent of all data tied at 3 most frequently observed values
 Since 47 percent of observations have the same value
 Exact methods (StatXact) are recommended

| COUNT | SUMWTS | NMISS | NOBS | RESPONSE | TIES | TIEPCT |
|-------|--------|-------|------|----------|------|--------|
| 28 | 60 | 2 | 62 | 0.00000 | 28 | 47 |
| 14 | 60 | 2 | 62 | 1.00000 | 42 | 70 |
| 1 | 60 | 2 | 62 | 0.00806 | 43 | 72 |

Check for Number of Reps in PRPMALE Thru 2000 ug/L
 Maximum Number of Reps in Any Treatment Group or Control is 15
 Total Number of Reps in All Treatment Groups is 60
 Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 5 Doses thru 2000 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 1239.5 | 8.1394639 | . | 0.0001 | ** | 6 |

Check for Number of Reps in PRPMALE Thru 670 ug/L
 Maximum Number of Reps in Any Treatment Group or Control is 15
 Total Number of Reps in All Treatment Groups is 50
 Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 4 Doses thru 670 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|----------|--------|--------|--------|------|
| 779.5 | 6.806719 | . | 0.0001 | ** | 5 |

Check for Number of Reps in PRPMALE Thru 220 ug/L
 Maximum Number of Reps in Any Treatment Group or Control is 15
 Total Number of Reps in All Treatment Groups is 42

ENV/JM/MONO(2008)20

Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 3 Doses thru 220 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 447.5 | 5.5596326 | . | 0.0001 | ** | 4 |

Check for Number of Reps in PRPMALE Thru 74 ug/L
 Maximum Number of Reps in Any Treatment Group or Control is 15
 Total Number of Reps in All Treatment Groups is 32
 Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 2 Doses thru 74 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 105 | 2.8180764 | . | 0.0046 | ** | 3 |

NOTE
 Remaining data is constant.
 No further tests are possible.

NOTE
 Jonckheere test results are included in the summary table.
 Group means should be examined to check for lack-of-fit

=====

AUTO TRANSFORM FULL DATA

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:07 WEDNESDAY 27JUN07
 SHAPIRO-WILK TEST OF NORMALITY OF SQRT(PRPMALE)
 AQUATIC DAPHNID: FULL DATA

| OBS | STD | SKEW | KURT | SW_STAT | P_VALUE | SIGNIF |
|-----|----------|----------|---------|---------|---------|--------|
| 60 | 0.076079 | -0.62463 | 3.28114 | 0.80355 | 0.0001 | ** |

NOTE
 No auto-transform fixes data non-normality.
 Returning to untransformed response.

NOEC Determination for Lab A

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:07 WEDNESDAY 27JUN07
 A DAPHNID MEASUREMENTS FROM DATASET SEXRATIOS
 GROUP STATISTICS FOR PRPMALE BY DOSE, Unweighted

| dose | doseval | COUNT | MEAN | MEDIAN | STD_DEV | STD_ERR |
|------|---------|-------|----------|--------|----------|----------|
| 1 | 0 | 18 | 0.000000 | 0 | 0.000000 | 0.000000 |
| 2 | 25 | 7 | 0.019490 | 0 | 0.028673 | 0.010837 |
| 3 | 74 | 7 | 0.004926 | 0 | 0.013033 | 0.004926 |
| 4 | 220 | 6 | 0.000000 | 0 | 0.000000 | 0.000000 |
| 5 | 670 | 8 | 0.000000 | 0 | 0.000000 | 0.000000 |
| 6 | 2000 | 7 | 0.000000 | 0 | 0.000000 | 0.000000 |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:07 WEDNESDAY 27JUN07
 SHAPIRO-WILK TEST OF NORMALITY OF PRPMALE
 AQUATIC DAPHNID: FULL DATA

| OBS | STD | SKEW | KURT | SW_STAT | P_VALUE | SIGNIF |
|-----|----------|---------|---------|---------|---------|--------|
| 53 | 0.010699 | 2.80807 | 14.8328 | 0.53929 | 0.0001 | ** |

Outliers & Influential Observations
 PRPMALE FROM FULL DATA Unweighted
 AQUATIC DAPHNID: Concentrations in ug/L

| Obs | DAPHNID | dose | doseval | GROUP | OBSER | Pred | SE_PRED |
|-----|---------|------|---------|-------|----------|-----------|------------|
| 1 | 2 | 2 | 25 | 2 | 0.000000 | 0.019490 | .004253340 |
| 2 | 4 | 2 | 25 | 2 | 0.024390 | 0.019490 | .004253340 |
| 3 | 5 | 2 | 25 | 2 | 0.000000 | 0.019490 | .004253340 |
| 4 | 6 | 2 | 25 | 2 | 0.037037 | 0.019490 | .004253340 |
| 5 | 8 | 2 | 25 | 2 | 0.075000 | 0.019490 | .004253340 |
| 6 | 9 | 2 | 25 | 2 | 0.000000 | 0.019490 | .004253340 |
| 7 | 10 | 2 | 25 | 2 | 0.000000 | 0.019490 | .004253340 |
| 8 | 2 | 3 | 74 | 3 | 0.034483 | 0.004926 | .004253340 |
| 9 | 3 | 3 | 74 | 3 | 0.000000 | 0.004926 | .004253340 |
| 10 | 4 | 3 | 74 | 3 | 0.000000 | 0.004926 | .004253340 |
| 11 | 5 | 3 | 74 | 3 | 0.000000 | 0.004926 | .004253340 |
| 12 | 6 | 3 | 74 | 3 | 0.000000 | 0.004926 | .004253340 |
| 13 | 7 | 3 | 74 | 3 | 0.000000 | 0.004926 | .004253340 |
| 14 | 9 | 3 | 74 | 3 | 0.000000 | 0.004926 | .004253340 |
| 15 | 1 | 6 | 2000 | 6 | 0.000000 | -0.000000 | .004253340 |
| 16 | 2 | 6 | 2000 | 6 | 0.000000 | -0.000000 | .004253340 |
| 17 | 3 | 6 | 2000 | 6 | 0.000000 | -0.000000 | .004253340 |
| 18 | 5 | 6 | 2000 | 6 | 0.000000 | -0.000000 | .004253340 |
| 19 | 6 | 6 | 2000 | 6 | 0.000000 | -0.000000 | .004253340 |
| 20 | 8 | 6 | 2000 | 6 | 0.000000 | -0.000000 | .004253340 |
| 21 | 9 | 6 | 2000 | 6 | 0.000000 | -0.000000 | .004253340 |

ENV/JM/MONO(2008)20

| Obs | L95M | U95M | Resid | LB | UB |
|-----|-----------|----------|-----------|-------------|------------|
| 1 | 0.010933 | 0.028046 | -0.019490 | -1.1806E-20 | 1.9676E-20 |
| 2 | 0.010933 | 0.028046 | 0.004901 | -1.1806E-20 | 1.9676E-20 |
| 3 | 0.010933 | 0.028046 | -0.019490 | -1.1806E-20 | 1.9676E-20 |
| 4 | 0.010933 | 0.028046 | 0.017547 | -1.1806E-20 | 1.9676E-20 |
| 5 | 0.010933 | 0.028046 | 0.055510 | -1.1806E-20 | 1.9676E-20 |
| 6 | 0.010933 | 0.028046 | -0.019490 | -1.1806E-20 | 1.9676E-20 |
| 7 | 0.010933 | 0.028046 | -0.019490 | -1.1806E-20 | 1.9676E-20 |
| 8 | -0.003631 | 0.013483 | 0.029557 | -1.1806E-20 | 1.9676E-20 |
| 9 | -0.003631 | 0.013483 | -0.004926 | -1.1806E-20 | 1.9676E-20 |
| 10 | -0.003631 | 0.013483 | -0.004926 | -1.1806E-20 | 1.9676E-20 |
| 11 | -0.003631 | 0.013483 | -0.004926 | -1.1806E-20 | 1.9676E-20 |
| 12 | -0.003631 | 0.013483 | -0.004926 | -1.1806E-20 | 1.9676E-20 |
| 13 | -0.003631 | 0.013483 | -0.004926 | -1.1806E-20 | 1.9676E-20 |
| 14 | -0.003631 | 0.013483 | -0.004926 | -1.1806E-20 | 1.9676E-20 |
| 15 | -0.008557 | 0.008557 | 0.000000 | -1.1806E-20 | 1.9676E-20 |
| 16 | -0.008557 | 0.008557 | 0.000000 | -1.1806E-20 | 1.9676E-20 |
| 17 | -0.008557 | 0.008557 | 0.000000 | -1.1806E-20 | 1.9676E-20 |
| 18 | -0.008557 | 0.008557 | 0.000000 | -1.1806E-20 | 1.9676E-20 |
| 19 | -0.008557 | 0.008557 | 0.000000 | -1.1806E-20 | 1.9676E-20 |
| 20 | -0.008557 | 0.008557 | 0.000000 | -1.1806E-20 | 1.9676E-20 |
| 21 | -0.008557 | 0.008557 | 0.000000 | -1.1806E-20 | 1.9676E-20 |

NOTE

This set of outliers will be excluded from all outlier omitted analyses for this response. In order for the outliers from the transformed analysis to be used for outlier omitted analysis instead, run the analysis again and either specify the transform (instead of allowing the system to decide) or manually omit these observations using the exclusion functionality.

NOTE

The data was NOT normally distributed.
A nonparametric analysis will be performed.

STATISTICAL ANALYSIS LIST FILE
REPORT CREATED ON 19:07 WEDNESDAY 27JUN07
Kruskal-Wallis Test on PRPMALE
A DAPHNID MEASUREMENTS FOR FULL DATA

The NPAR1WAY Procedure
Wilcoxon Scores (Rank Sums) for Variable RESPONSE
Classified by Variable dose

| dose | N | Sum of Scores | Expected Under H0 | Std Dev Under H0 | Mean Score |
|------|----|---------------|-------------------|------------------|------------|
| 1 | 18 | 450.0 | 486.0 | 24.388456 | 25.000000 |
| 2 | 7 | 255.0 | 189.0 | 17.435804 | 36.428571 |
| 3 | 7 | 201.0 | 189.0 | 17.435804 | 28.714286 |
| 4 | 6 | 150.0 | 162.0 | 16.316935 | 25.000000 |
| 5 | 8 | 200.0 | 216.0 | 18.435940 | 25.000000 |
| 6 | 7 | 175.0 | 189.0 | 17.435804 | 25.000000 |

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square 15.9649
 DF 5
 Pr > Chi-Square 0.0069

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:07 WEDNESDAY 27JUN07
 Modified Dunn's Multiple Comparisons (Increasing) on PRPMALE
 A DAPHNID MEASUREMENTS FOR FULL DATA USING ALPHA=0.05

| | C | | M | | A | C | | C | | S | |
|---|------|----|----|---------|---------|---------|---------|----|---|---|--------|
| d | O | | R | | S | R | R | I | I | I | p |
| o | U | | A | | D | T | T | I | I | G | |
| s | N | N | N | N | I | | | 0 | 0 | N | v |
| d | T | 0 | K | K | F | 5 | 1 | F | F | F | a |
| e | | | | | | | | | | | l |
| l | | | | | | | | | | | |
| 1 | 0 | 18 | 18 | 25.0000 | 0.0000 | 5.48536 | 6.78650 | | | | . |
| 2 | 25 | 7 | 18 | 36.4286 | 11.4286 | 7.33012 | 9.06884 | ** | | | 0.0007 |
| 3 | 74 | 7 | 18 | 28.7143 | 3.7143 | 7.33012 | 9.06884 | | | | 0.5962 |
| 4 | 220 | 6 | 18 | 25.0000 | 0.0000 | 7.75747 | 9.59756 | | | | 1.0000 |
| 5 | 670 | 8 | 18 | 25.0000 | 0.0000 | 6.99249 | 8.65113 | | | | 1.0000 |
| 6 | 2000 | 7 | 18 | 25.0000 | 0.0000 | 7.33012 | 9.06884 | | | | 1.0000 |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:07 WEDNESDAY 27JUN07
 MONOTONICITY CHECK OF PRPMALE - FULL DATA
 DOSES 0, 25, 74, 220, 670, 2000 ug/L
 A DAPHNID OF AQUATIC DAPHNID

| PARAM | p_t | SIGNIF |
|------------|--------|--------|
| DOSE TREND | 0.0309 | * |
| DOSE QUAD | 0.2062 | |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:07 WEDNESDAY 27JUN07
 ANALYSIS OF PRPMALE USING ALPHA=0.05
 FULL DATA

----- JONCKHEERE-TERPSTRA TEST KEY -----
 JT IS JONCKHEERE STATISTIC
 Z_JT IS STANDARDIZED JONCKHEERE STATISTIC
 PR_JT IS P-VALUE FOR UPWARD TREND
 PL_JT IS P-VALUE FOR DOWNWARD TREND
 P-VALUES ARE FOR TIE-CORRECTED TEST WITH CONTINUITY CORRECTION FACTOR
 SIGNIF RESULTS ARE FOR Increasing ALTERNATIVE HYPOTHESIS

ENV/JM/MONO(2008)20

Check for ties in PRPMALE

Percent of all data tied at 3 most frequently observed values

Since 92 percent of observations have the same value

Exact methods (StatXact) are recommended

| COUNT | SUMWTS | NMISS | NOBS | RESPONSE | TIES | TIEPCT |
|-------|--------|-------|------|----------|------|--------|
| 49 | 53 | 2 | 55 | 0.000000 | 49 | 92 |
| 1 | 53 | 2 | 55 | 0.024390 | 50 | 94 |
| 1 | 53 | 2 | 55 | 0.034483 | 51 | 96 |

Check for Number of Reps in PRPMALE Thru 2000 ug/L

Maximum Number of Reps in Any Treatment Group or Control is 18

Total Number of Reps in All Treatment Groups is 53

Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 5 Doses thru 2000 ug/L

| JT | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|------|-----------|--------|--------|--------|------|
| 87.5 | -0.466018 | 0.3251 | . | | 6 |

NOTE

Jonckheere test results are included in the summary table.

Group means should be examined to check for lack-of-fit

AUTO TRANSFORM FULL DATA

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:07 WEDNESDAY 27JUN07
 SHAPIRO-WILK TEST OF NORMALITY OF SQRT(PRPMALE)
 AQUATIC DAPHNID: FULL DATA

| OBS | STD | SKEW | KURT | SW_STAT | P_VALUE | SIGNIF |
|-----|----------|---------|---------|---------|---------|--------|
| 53 | 0.046125 | 1.80158 | 7.30967 | 0.60983 | 0.0001 | ** |

NOTE

No auto-transform fixes data non-normality.

Returning to untransformed response.

NOEC Determination for Lab D

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:07 WEDNESDAY 27JUN07
 LAB D DAPHNID MEASUREMENTS FROM DATASET SEXRATIOS
 GROUP STATISTICS FOR PRPMALE BY DOSE, Unweighted

| dose | doseval | COUNT | MEAN | MEDIAN | STD_DEV | STD_ERR |
|------|---------|-------|---------|---------|---------|----------|
| 1 | 0 | 19 | 0.00000 | 0.00000 | 0.00000 | 0.000000 |
| 2 | 25 | 8 | 0.00000 | 0.00000 | 0.00000 | 0.000000 |
| 3 | 74 | 8 | 0.00000 | 0.00000 | 0.00000 | 0.000000 |
| 4 | 220 | 8 | 0.01213 | 0.00000 | 0.02827 | 0.009996 |
| 5 | 670 | 9 | 0.64653 | 0.61538 | 0.20926 | 0.069752 |
| 6 | 2000 | 7 | 1.00000 | 1.00000 | 0.00000 | 0.000000 |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:07 WEDNESDAY 27JUN07
 SHAPIRO-WILK TEST OF NORMALITY OF PRPMALE
 AQUATIC DAPHNID: FULL DATA

| OBS | STD | SKEW | KURT | SW_STAT | P_VALUE | SIGNIF |
|-----|----------|---------|---------|---------|---------|--------|
| 59 | 0.078334 | 0.32254 | 10.5416 | 0.52253 | 0.0001 | ** |

 Outliers & Influential Observations
 PRPMALE FROM FULL DATA Unweighted
 AQUATIC DAPHNID: Concentrations in ug/L

| Obs | DAPHNID | dose | doseval | GROUP | OBSER | Pred | SE_PRED |
|-----|---------|------|---------|-------|---------|---------|----------|
| 1 | 1 | 4 | | 220 4 | 0.00000 | 0.01213 | 0.028972 |
| 2 | 2 | 4 | | 220 4 | 0.00000 | 0.01213 | 0.028972 |
| 3 | 3 | 4 | | 220 4 | 0.00000 | 0.01213 | 0.028972 |
| 4 | 4 | 4 | | 220 4 | 0.01639 | 0.01213 | 0.028972 |
| 5 | 5 | 4 | | 220 4 | 0.00000 | 0.01213 | 0.028972 |
| 6 | 6 | 4 | | 220 4 | 0.00000 | 0.01213 | 0.028972 |
| 7 | 7 | 4 | | 220 4 | 0.00000 | 0.01213 | 0.028972 |
| 8 | 8 | 4 | | 220 4 | 0.08065 | 0.01213 | 0.028972 |
| 9 | 1 | 5 | | 670 5 | 0.52000 | 0.64653 | 0.027315 |
| 10 | 2 | 5 | | 670 5 | 0.61538 | 0.64653 | 0.027315 |
| 11 | 3 | 5 | | 670 5 | 0.93333 | 0.64653 | 0.027315 |
| 12 | 5 | 5 | | 670 5 | 0.68182 | 0.64653 | 0.027315 |
| 13 | 6 | 5 | | 670 5 | 0.94444 | 0.64653 | 0.027315 |
| 14 | 7 | 5 | | 670 5 | 0.76190 | 0.64653 | 0.027315 |
| 15 | 8 | 5 | | 670 5 | 0.60000 | 0.64653 | 0.027315 |
| 16 | 9 | 5 | | 670 5 | 0.42857 | 0.64653 | 0.027315 |
| 17 | 10 | 5 | | 670 5 | 0.33333 | 0.64653 | 0.027315 |

| Obs | L95M | U95M | Resid | LB | UB |
|-----|----------|---------|----------|-------------|------------|
| 1 | -0.04598 | 0.07024 | -0.01213 | -2.7756E-16 | 1.6653E-16 |
| 2 | -0.04598 | 0.07024 | -0.01213 | -2.7756E-16 | 1.6653E-16 |
| 3 | -0.04598 | 0.07024 | -0.01213 | -2.7756E-16 | 1.6653E-16 |
| 4 | -0.04598 | 0.07024 | 0.00426 | -2.7756E-16 | 1.6653E-16 |
| 5 | -0.04598 | 0.07024 | -0.01213 | -2.7756E-16 | 1.6653E-16 |
| 6 | -0.04598 | 0.07024 | -0.01213 | -2.7756E-16 | 1.6653E-16 |
| 7 | -0.04598 | 0.07024 | -0.01213 | -2.7756E-16 | 1.6653E-16 |
| 8 | -0.04598 | 0.07024 | 0.06852 | -2.7756E-16 | 1.6653E-16 |
| 9 | 0.59174 | 0.70132 | -0.12653 | -2.7756E-16 | 1.6653E-16 |
| 10 | 0.59174 | 0.70132 | -0.03115 | -2.7756E-16 | 1.6653E-16 |
| 11 | 0.59174 | 0.70132 | 0.28680 | -2.7756E-16 | 1.6653E-16 |
| 12 | 0.59174 | 0.70132 | 0.03529 | -2.7756E-16 | 1.6653E-16 |
| 13 | 0.59174 | 0.70132 | 0.29791 | -2.7756E-16 | 1.6653E-16 |
| 14 | 0.59174 | 0.70132 | 0.11537 | -2.7756E-16 | 1.6653E-16 |
| 15 | 0.59174 | 0.70132 | -0.04653 | -2.7756E-16 | 1.6653E-16 |
| 16 | 0.59174 | 0.70132 | -0.21796 | -2.7756E-16 | 1.6653E-16 |
| 17 | 0.59174 | 0.70132 | -0.31320 | -2.7756E-16 | 1.6653E-16 |

NOTE

This set of outliers will be excluded from all outlier omitted analyses for this response. In order for the outliers from the transformed analysis to be used for outlier omitted analysis instead, run the analysis again and either specify the transform (instead of allowing the system to decide) or manually omit these observations using the exclusion functionality.

NOTE

The data was NOT normally distributed.
A nonparametric analysis will be performed.

STATISTICAL ANALYSIS LIST FILE
REPORT CREATED ON 19:07 WEDNESDAY 27JUN07
Kruskal-Wallis Test on PRPMALE
LAB D DAPHNID MEASUREMENTS FOR FULL DATA

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable RESPONSE
Classified by Variable dose

| dose | N | Sum of Scores | Expected Under H0 | Std Dev Under H0 | Mean Score |
|------|----|---------------|-------------------|------------------|------------|
| 1 | 19 | 399.0 | 570.0 | 50.189355 | 21.0000 |
| 2 | 8 | 168.0 | 240.0 | 36.773494 | 21.0000 |
| 3 | 8 | 168.0 | 240.0 | 36.773494 | 21.0000 |
| 4 | 8 | 211.0 | 240.0 | 36.773494 | 26.3750 |
| 5 | 9 | 432.0 | 270.0 | 38.619894 | 48.0000 |
| 6 | 7 | 392.0 | 210.0 | 34.734057 | 56.0000 |

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square 54.1449
 DF 5
 Pr > Chi-Square <.0001

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:07 WEDNESDAY 27JUN07
 Modified Dunn's Multiple Comparisons (Increasing) on PRPMALE
 LAB D DAPHNID MEASUREMENTS FOR FULL DATA USING ALPHA=0.05

| dose | doseval | COUNT | N0 | MRANK | ABS_DIFF | CRIT_05 | CRIT_01 | SIGNIF | p_val |
|------|---------|-------|----|--------|----------|---------|---------|--------|--------|
| 1 | 0 | 19 | 19 | 21.000 | 0.000 | 10.5546 | 13.0582 | . | |
| 2 | 25 | 8 | 19 | 21.000 | 0.000 | 13.7109 | 16.9631 | | 1.0000 |
| 3 | 74 | 8 | 19 | 21.000 | 0.000 | 13.7109 | 16.9631 | | 1.0000 |
| 4 | 220 | 8 | 19 | 26.375 | 5.375 | 13.7109 | 16.9631 | | 0.9044 |
| 5 | 670 | 9 | 19 | 48.000 | 27.000 | 13.1640 | 16.2865 | ** | 0.0001 |
| 6 | 2000 | 7 | 19 | 56.000 | 35.000 | 14.3836 | 17.7954 | ** | 0.0001 |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:07 WEDNESDAY 27JUN07
 MONOTONICITY CHECK OF PRPMALE - FULL DATA
 DOSES 0, 25, 74, 220, 670, 2000 ug/L
 LAB D DAPHNID OF AQUATIC DAPHNID

| PARAM | p_t | SIGNIF |
|------------|--------|--------|
| DOSE TREND | 0.0001 | ** |
| DOSE QUAD | 0.0001 | ** |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:07 WEDNESDAY 27JUN07
 ANALYSIS OF PRPMALE USING ALPHA=0.05
 FULL DATA

----- JONCKHEERE-TERPSTRA TEST KEY -----
 JT IS JONCKHEERE STATISTIC
 Z_JT IS STANDARDIZED JONCKHEERE STATISTIC
 PR_JT IS P-VALUE FOR UPWARD TREND
 PL_JT IS P-VALUE FOR DOWNWARD TREND
 P-VALUES ARE FOR TIE-CORRECTED TEST WITH CONTINUITY CORRECTION FACTOR
 SIGNIF RESULTS ARE FOR Increasing ALTERNATIVE HYPOTHESIS

ENV/JM/MONO(2008)20

Check for ties in PRPMALE

Percent of all data tied at 3 most frequently observed values

Since 69 percent of observations have the same value

Exact methods (StatXact) are recommended

| COUNT | SUMWTS | NMISS | NOBS | RESPONSE | TIES | TIEPCT |
|-------|--------|-------|------|----------|------|--------|
| 41 | 59 | 2 | 61 | 0.00000 | 41 | 69 |
| 7 | 59 | 2 | 61 | 1.00000 | 48 | 81 |
| 1 | 59 | 2 | 61 | 0.01639 | 49 | 83 |

Check for Number of Reps in PRPMALE Thru 2000 ug/L

Maximum Number of Reps in Any Treatment Group or Control is 19

Total Number of Reps in All Treatment Groups is 59

Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 5 Doses thru 2000 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 845.5 | 6.7733003 | . | 0.0001 | ** | 6 |

Check for Number of Reps in PRPMALE Thru 670 ug/L

Maximum Number of Reps in Any Treatment Group or Control is 19

Total Number of Reps in All Treatment Groups is 52

Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 4 Doses thru 670 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|----------|--------|--------|--------|------|
| 481.5 | 5.242061 | . | 0.0001 | ** | 5 |

Check for Number of Reps in PRPMALE Thru 220 ug/L

Maximum Number of Reps in Any Treatment Group or Control is 19

Total Number of Reps in All Treatment Groups is 43

Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 3 Doses thru 220 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 76.5 | 2.1330176 | . | 0.0300 | ** | 4 |

NOTE

Remaining data is constant.

No further tests are possible.

NOTE

Jonckheere test results are included in the summary table.

Group means should be examined to check for lack-of-fit

=====

AUTO TRANSFORM FULL DATA

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:07 WEDNESDAY 27JUN07
 SHAPIRO-WILK TEST OF NORMALITY OF SQRT(PRPMAL)
 AQUATIC DAPHNID: FULL DATA

| OBS | STD | SKEW | KURT | SW_STAT | P_VALUE | SIGNIF |
|-----|----------|---------|---------|---------|---------|--------|
| 59 | 0.061085 | 0.78608 | 7.17325 | 0.64910 | 0.0001 | ** |

NOTE
 No auto-transform fixes data non-normality.
 Returning to untransformed response.

NOEC Determination for Lab B

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:08 WEDNESDAY 27JUN07
 LAB B DAPHNID MEASUREMENTS FROM DATASET SEXRATIOS
 GROUP STATISTICS FOR PRPMALE BY DOSE, Unweighted

| dose | doseval | COUNT | MEAN | MEDIAN | STD_DEV | STD_ERR |
|------|---------|-------|---------|---------|---------|----------|
| 1 | 0 | 16 | 0.00000 | 0.00000 | 0.00000 | 0.000000 |
| 2 | 25 | 10 | 0.19280 | 0.21834 | 0.07102 | 0.022458 |
| 3 | 74 | 10 | 0.00659 | 0.00000 | 0.02085 | 0.006593 |
| 4 | 220 | 10 | 0.20070 | 0.19830 | 0.20778 | 0.065706 |
| 5 | 670 | 10 | 0.82875 | 0.86607 | 0.19806 | 0.062631 |
| 6 | 2000 | 9 | 0.88721 | 1.00000 | 0.13904 | 0.046348 |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:08 WEDNESDAY 27JUN07
 SHAPIRO-WILK TEST OF NORMALITY OF PRPMALE
 AQUATIC DAPHNID: FULL DATA

| OBS | STD | SKEW | KURT | SW_STAT | P_VALUE | SIGNIF |
|-----|---------|----------|---------|---------|---------|--------|
| 65 | 0.12155 | -0.97329 | 2.91655 | 0.89220 | 0.0001 | ** |

Outliers & Influential Observations
 PRPMALE FROM FULL DATA Unweighted
 AQUATIC DAPHNID: Concentrations in ug/L

| Obs | DAPHNID | dose | doseval | GROUP | OBSER | Pred |
|-----|---------|------|---------|-------|---------|---------|
| 1 | 10 | 2 | 25 | 2 | 0.00000 | 0.19280 |
| 2 | 1 | 4 | 220 | 4 | 0.00000 | 0.20070 |
| 3 | 2 | 4 | 220 | 4 | 0.37097 | 0.20070 |
| 4 | 3 | 4 | 220 | 4 | 0.00000 | 0.20070 |
| 5 | 4 | 4 | 220 | 4 | 0.37879 | 0.20070 |
| 6 | 5 | 4 | 220 | 4 | 0.00000 | 0.20070 |
| 7 | 6 | 4 | 220 | 4 | 0.46154 | 0.20070 |
| 8 | 7 | 4 | 220 | 4 | 0.37879 | 0.20070 |
| 9 | 8 | 4 | 220 | 4 | 0.39130 | 0.20070 |
| 10 | 9 | 4 | 220 | 4 | 0.00000 | 0.20070 |
| 11 | 10 | 4 | 220 | 4 | 0.02564 | 0.20070 |
| 12 | 1 | 5 | 670 | 5 | 1.00000 | 0.82875 |
| 13 | 3 | 5 | 670 | 5 | 1.00000 | 0.82875 |
| 14 | 5 | 5 | 670 | 5 | 0.35000 | 0.82875 |
| 15 | 7 | 5 | 670 | 5 | 1.00000 | 0.82875 |
| 16 | 8 | 5 | 670 | 5 | 0.69565 | 0.82875 |
| 17 | 5 | 6 | 2000 | 6 | 0.75000 | 0.88721 |
| 18 | 8 | 6 | 2000 | 6 | 0.75000 | 0.88721 |
| 19 | 10 | 6 | 2000 | 6 | 0.66667 | 0.88721 |

| Obs | SE_PRED | L95M | U95M | Resid | LB | UB |
|-----|----------|---------|---------|----------|-----------|---------|
| 1 | 0.040033 | 0.11270 | 0.27291 | -0.19280 | -0.084421 | 0.12312 |
| 2 | 0.040033 | 0.12060 | 0.28081 | -0.20070 | -0.084421 | 0.12312 |
| 3 | 0.040033 | 0.12060 | 0.28081 | 0.17027 | -0.084421 | 0.12312 |
| 4 | 0.040033 | 0.12060 | 0.28081 | -0.20070 | -0.084421 | 0.12312 |
| 5 | 0.040033 | 0.12060 | 0.28081 | 0.17809 | -0.084421 | 0.12312 |
| 6 | 0.040033 | 0.12060 | 0.28081 | -0.20070 | -0.084421 | 0.12312 |
| 7 | 0.040033 | 0.12060 | 0.28081 | 0.26084 | -0.084421 | 0.12312 |
| 8 | 0.040033 | 0.12060 | 0.28081 | 0.17809 | -0.084421 | 0.12312 |
| 9 | 0.040033 | 0.12060 | 0.28081 | 0.19060 | -0.084421 | 0.12312 |
| 10 | 0.040033 | 0.12060 | 0.28081 | -0.20070 | -0.084421 | 0.12312 |
| 11 | 0.040033 | 0.12060 | 0.28081 | -0.17506 | -0.084421 | 0.12312 |
| 12 | 0.040033 | 0.74864 | 0.90885 | 0.17125 | -0.084421 | 0.12312 |
| 13 | 0.040033 | 0.74864 | 0.90885 | 0.17125 | -0.084421 | 0.12312 |
| 14 | 0.040033 | 0.74864 | 0.90885 | -0.47875 | -0.084421 | 0.12312 |
| 15 | 0.040033 | 0.74864 | 0.90885 | 0.17125 | -0.084421 | 0.12312 |
| 16 | 0.040033 | 0.74864 | 0.90885 | -0.13310 | -0.084421 | 0.12312 |
| 17 | 0.042199 | 0.80277 | 0.97164 | -0.13721 | -0.084421 | 0.12312 |
| 18 | 0.042199 | 0.80277 | 0.97164 | -0.13721 | -0.084421 | 0.12312 |
| 19 | 0.042199 | 0.80277 | 0.97164 | -0.22054 | -0.084421 | 0.12312 |

NOTE

This set of outliers will be excluded from all outlier omitted analyses for this response. In order for the outliers from the transformed analysis to be used for outlier omitted analysis instead, run the analysis again and either specify the transform (instead of allowing the system to decide) or manually omit these observations using the exclusion functionality.

NOTE

The data was NOT normally distributed.
A nonparametric analysis will be performed.

STATISTICAL ANALYSIS LIST FILE
REPORT CREATED ON 19:08 WEDNESDAY 27JUN07
Kruskal-Wallis Test on PRPMALE
LAB B DAPHNID MEASUREMENTS FOR FULL DATA

The NPAR1WAY Procedure
Wilcoxon Scores (Rank Sums) for Variable RESPONSE
Classified by Variable dose

| dose | N | Sum of Scores | Expected Under H0 | Std Dev Under H0 | Mean Score |
|------|----|---------------|-------------------|------------------|------------|
| 1 | 16 | 248.00 | 528.0 | 62.292286 | 15.500000 |
| 2 | 10 | 348.50 | 330.0 | 52.174414 | 34.850000 |
| 3 | 10 | 171.50 | 330.0 | 52.174414 | 17.150000 |
| 4 | 10 | 318.00 | 330.0 | 52.174414 | 31.800000 |
| 5 | 10 | 548.50 | 330.0 | 52.174414 | 54.850000 |
| 6 | 9 | 510.50 | 297.0 | 49.944941 | 56.722222 |

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square 53.7743
 DF 5
 Pr > Chi-Square <.0001

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:08 WEDNESDAY 27JUN07
 Modified Dunn's Multiple Comparisons (Increasing) on PRPMALE
 LAB B DAPHNID MEASUREMENTS FOR FULL DATA USING ALPHA=0.05

| | C | | M | A | B | C | C | S | |
|---|------|----|----|---------|---------|---------|---------|----|--------|
| d | O | | R | — | S | R | R | I | p |
| e | U | | A | D | I | I | I | G | — |
| o | N | N | N | I | F | — | — | N | v |
| v | T | 0 | K | F | — | 0 | 0 | I | a |
| s | | | | | 5 | 1 | 1 | F | l |
| a | | | | | | | | | |
| e | | | | | | | | | |
| l | | | | | | | | | |
| 1 | 0 | 16 | 16 | 15.5000 | 0.0000 | 14.7524 | 18.2517 | . | |
| 2 | 25 | 10 | 16 | 34.8500 | 19.3500 | 16.8203 | 20.8101 | * | 0.0186 |
| 3 | 74 | 10 | 16 | 17.1500 | 1.6500 | 16.8203 | 20.8101 | | 1.0000 |
| 4 | 220 | 10 | 16 | 31.8000 | 16.3000 | 16.8203 | 20.8101 | | 0.0604 |
| 5 | 670 | 10 | 16 | 54.8500 | 39.3500 | 16.8203 | 20.8101 | ** | 0.0001 |
| 6 | 2000 | 9 | 16 | 56.7222 | 41.2222 | 17.3859 | 21.5098 | ** | 0.0001 |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:08 WEDNESDAY 27JUN07
 MONOTONICITY CHECK OF PRPMALE - FULL DATA
 DOSES 0, 25, 74, 220, 670, 2000 ug/L
 LAB B DAPHNID OF AQUATIC DAPHNID

| PARAM | p_t | SIGNIF |
|------------|--------|--------|
| DOSE TREND | 0.0001 | ** |
| DOSE QUAD | 0.0002 | ** |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:08 WEDNESDAY 27JUN07
 ANALYSIS OF PRPMALE USING ALPHA=0.05
 FULL DATA

----- JONCKHEERE-TERPSTRA TEST KEY -----
 JT IS JONCKHEERE STATISTIC
 Z_JT IS STANDARDIZED JONCKHEERE STATISTIC
 PR_JT IS P-VALUE FOR UPWARD TREND
 PL_JT IS P-VALUE FOR DOWNWARD TREND
 P-VALUES ARE FOR TIE-CORRECTED TEST WITH CONTINUITY CORRECTION FACTOR
 SIGNIF RESULTS ARE FOR Increasing ALTERNATIVE HYPOTHESIS

Check for ties in PRPMALE

Percent of all data tied at 3 most frequently observed values
 Since 46 percent of observations have the same value
 Exact methods (StatXact) are recommended

| COUNT | SUMWTS | NMISS | NOBS | RESPONSE | TIES | TIEPCT |
|-------|--------|-------|------|----------|------|--------|
| 30 | 65 | 2 | 67 | 0.00000 | 30 | 46 |
| 8 | 65 | 2 | 67 | 1.00000 | 38 | 58 |
| 2 | 65 | 2 | 67 | 0.37879 | 40 | 62 |

Check for Number of Reps in PRPMALE Thru 2000 ug/L
 Maximum Number of Reps in Any Treatment Group or Control is 16
 Total Number of Reps in All Treatment Groups is 65
 Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 5 Doses thru 2000 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 1359.5 | 6.7123449 | . | 0.0001 | ** | 6 |

Check for Number of Reps in PRPMALE Thru 670 ug/L
 Maximum Number of Reps in Any Treatment Group or Control is 16
 Total Number of Reps in All Treatment Groups is 56
 Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 4 Doses thru 670 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|----------|--------|--------|--------|------|
| 889 | 5.340913 | . | 0.0001 | ** | 5 |

Check for Number of Reps in PRPMALE Thru 220 ug/L
 Maximum Number of Reps in Any Treatment Group or Control is 16
 Total Number of Reps in All Treatment Groups is 46
 Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 3 Doses thru 220 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 413 | 2.6360691 | . | 0.0051 | ** | 4 |

Check for Number of Reps in PRPMALE Thru 74 ug/L
 Maximum Number of Reps in Any Treatment Group or Control is 16
 Total Number of Reps in All Treatment Groups is 36
 Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 2 Doses thru 74 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 188 | 1.3205155 | . | 0.0956 | | 3 |

NOTE

Jonckheere test results are included in the summary table.
 Group means should be examined to check for lack-of-fit

=====

AUTO TRANSFORM FULL DATA

STATISTICAL ANALYSIS LIST FILE
REPORT CREATED ON 19:08 WEDNESDAY 27JUN07
SHAPIRO-WILK TEST OF NORMALITY OF SQRT(PRPMAL)

AQUATIC DAPHNID: FULL DATA

| OBS | STD | SKEW | KURT | SW_STAT | P_VALUE | SIGNIF |
|-----|---------|----------|---------|---------|---------|--------|
| 65 | 0.14540 | -0.52778 | 1.96429 | 0.85467 | 0.0001 | ** |

NOTE
No auto-transform fixes data non-normality.
Returning to untransformed response.

NOEC Determination for Lab H

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:08 WEDNESDAY 27JUN07
 LAB H DAPHNID MEASUREMENTS FROM DATASET SEXRATIOS
 GROUP STATISTICS FOR PRPMALE BY DOSE, Unweighted

| dose | doseval | COUNT | MEAN | MEDIAN | STD_DEV | STD_ERR |
|------|---------|-------|---------|---------|---------|----------|
| 1 | 0 | 20 | 0.00000 | 0.00000 | 0.00000 | 0.000000 |
| 2 | 25 | 10 | 0.00000 | 0.00000 | 0.00000 | 0.000000 |
| 3 | 74 | 10 | 0.01338 | 0.00000 | 0.04231 | 0.013380 |
| 4 | 220 | 10 | 0.40338 | 0.42449 | 0.12057 | 0.038129 |
| 5 | 670 | 10 | 1.00000 | 1.00000 | 0.00000 | 0.000000 |
| 6 | 2000 | 10 | 1.00000 | 1.00000 | 0.00000 | 0.000000 |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:08 WEDNESDAY 27JUN07
 SHAPIRO-WILK TEST OF NORMALITY OF PRPMALE
 AQUATIC DAPHNID: FULL DATA

| OBS | STD | SKEW | KURT | SW_STAT | P_VALUE | SIGNIF |
|-----|----------|---------|---------|---------|---------|--------|
| 70 | 0.046150 | 0.12127 | 6.76992 | 0.58802 | 0.0001 | ** |

Outliers & Influential Observations
 PRPMALE FROM FULL DATA Unweighted
 AQUATIC DAPHNID: Concentrations in ug/L

| Obs | DAPHNID | dose | doseval | GROUP | OBSER | Pred |
|-----|---------|------|---------|-------|---------|---------|
| 1 | 1 | 3 | 74 | 3 | 0.00000 | 0.01338 |
| 2 | 2 | 3 | 74 | 3 | 0.00000 | 0.01338 |
| 3 | 3 | 3 | 74 | 3 | 0.00000 | 0.01338 |
| 4 | 4 | 3 | 74 | 3 | 0.00000 | 0.01338 |
| 5 | 5 | 3 | 74 | 3 | 0.00000 | 0.01338 |
| 6 | 6 | 3 | 74 | 3 | 0.13380 | 0.01338 |
| 7 | 7 | 3 | 74 | 3 | 0.00000 | 0.01338 |
| 8 | 8 | 3 | 74 | 3 | 0.00000 | 0.01338 |
| 9 | 9 | 3 | 74 | 3 | 0.00000 | 0.01338 |
| 10 | 10 | 3 | 74 | 3 | 0.00000 | 0.01338 |
| 11 | 1 | 4 | 220 | 4 | 0.53247 | 0.40338 |
| 12 | 2 | 4 | 220 | 4 | 0.41860 | 0.40338 |
| 13 | 3 | 4 | 220 | 4 | 0.43038 | 0.40338 |
| 14 | 4 | 4 | 220 | 4 | 0.51163 | 0.40338 |
| 15 | 5 | 4 | 220 | 4 | 0.57333 | 0.40338 |
| 16 | 6 | 4 | 220 | 4 | 0.24691 | 0.40338 |
| 17 | 7 | 4 | 220 | 4 | 0.30000 | 0.40338 |
| 18 | 8 | 4 | 220 | 4 | 0.46667 | 0.40338 |
| 19 | 9 | 4 | 220 | 4 | 0.30380 | 0.40338 |
| 20 | 10 | 4 | 220 | 4 | 0.25000 | 0.40338 |
| 21 | 1 | 6 | 2000 | 6 | 1.00000 | 1.00000 |
| 22 | 2 | 6 | 2000 | 6 | 1.00000 | 1.00000 |
| 23 | 3 | 6 | 2000 | 6 | 1.00000 | 1.00000 |
| 24 | 4 | 6 | 2000 | 6 | 1.00000 | 1.00000 |
| 25 | 5 | 6 | 2000 | 6 | 1.00000 | 1.00000 |

ENV/JM/MONO(2008)20

| Obs | SE_PRED | L95M | U95M | Resid | LB | UB |
|-----|----------|----------|---------|----------|----|----|
| 1 | 0.015153 | -0.01689 | 0.04365 | -0.01338 | 0 | 0 |
| 2 | 0.015153 | -0.01689 | 0.04365 | -0.01338 | 0 | 0 |
| 3 | 0.015153 | -0.01689 | 0.04365 | -0.01338 | 0 | 0 |
| 4 | 0.015153 | -0.01689 | 0.04365 | -0.01338 | 0 | 0 |
| 5 | 0.015153 | -0.01689 | 0.04365 | -0.01338 | 0 | 0 |
| 6 | 0.015153 | -0.01689 | 0.04365 | 0.12042 | 0 | 0 |
| 7 | 0.015153 | -0.01689 | 0.04365 | -0.01338 | 0 | 0 |
| 8 | 0.015153 | -0.01689 | 0.04365 | -0.01338 | 0 | 0 |
| 9 | 0.015153 | -0.01689 | 0.04365 | -0.01338 | 0 | 0 |
| 10 | 0.015153 | -0.01689 | 0.04365 | -0.01338 | 0 | 0 |
| 11 | 0.015153 | 0.37311 | 0.43365 | 0.12909 | 0 | 0 |
| 12 | 0.015153 | 0.37311 | 0.43365 | 0.01523 | 0 | 0 |
| 13 | 0.015153 | 0.37311 | 0.43365 | 0.02700 | 0 | 0 |
| 14 | 0.015153 | 0.37311 | 0.43365 | 0.10825 | 0 | 0 |
| 15 | 0.015153 | 0.37311 | 0.43365 | 0.16995 | 0 | 0 |
| 16 | 0.015153 | 0.37311 | 0.43365 | -0.15647 | 0 | 0 |
| 17 | 0.015153 | 0.37311 | 0.43365 | -0.10338 | 0 | 0 |
| 18 | 0.015153 | 0.37311 | 0.43365 | 0.06329 | 0 | 0 |
| 19 | 0.015153 | 0.37311 | 0.43365 | -0.09958 | 0 | 0 |
| 20 | 0.015153 | 0.37311 | 0.43365 | -0.15338 | 0 | 0 |
| 21 | 0.015153 | 0.96973 | 1.03027 | 0.00000 | 0 | 0 |
| 22 | 0.015153 | 0.96973 | 1.03027 | 0.00000 | 0 | 0 |
| 23 | 0.015153 | 0.96973 | 1.03027 | 0.00000 | 0 | 0 |
| 24 | 0.015153 | 0.96973 | 1.03027 | 0.00000 | 0 | 0 |
| 25 | 0.015153 | 0.96973 | 1.03027 | 0.00000 | 0 | 0 |

 Outliers & Influential Observations
 PRPMALE FROM FULL DATA Unweighted
 AQUATIC DAPHNID: Concentrations in ug/L

| Obs | DAPHNID | dose | doseval | GROUP | OBSER | Pred |
|-----|---------|------|---------|-------|---------|---------|
| 26 | 6 | 6 | 2000 | 6 | 1.00000 | 1.00000 |
| 27 | 7 | 6 | 2000 | 6 | 1.00000 | 1.00000 |
| 28 | 8 | 6 | 2000 | 6 | 1.00000 | 1.00000 |
| 29 | 9 | 6 | 2000 | 6 | 1.00000 | 1.00000 |
| 30 | 10 | 6 | 2000 | 6 | 1.00000 | 1.00000 |

| Obs | SE_PRED | L95M | U95M | Resid | LB | UB |
|-----|----------|---------|---------|---------|----|----|
| 26 | 0.015153 | 0.96973 | 1.03027 | 0.00000 | 0 | 0 |
| 27 | 0.015153 | 0.96973 | 1.03027 | 0.00000 | 0 | 0 |
| 28 | 0.015153 | 0.96973 | 1.03027 | 0.00000 | 0 | 0 |
| 29 | 0.015153 | 0.96973 | 1.03027 | 0.00000 | 0 | 0 |
| 30 | 0.015153 | 0.96973 | 1.03027 | 0.00000 | 0 | 0 |

NOTE

This set of outliers will be excluded from all outlier omitted analyses for this response. In order for the outliers from the transformed analysis to be used for outlier omitted analysis instead, run the analysis again and either specify the transform (instead of allowing the system to decide) or manually omit these observations using the exclusion functionality.

NOTE

The data was NOT normally distributed.
A nonparametric analysis will be performed.

STATISTICAL ANALYSIS LIST FILE
REPORT CREATED ON 19:08 WEDNESDAY 27JUN07
Kruskal-Wallis Test on PRPMALE
LAB H DAPHNID MEASUREMENTS FOR FULL DATA

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable RESPONSE
Classified by Variable dose

| dose | N | Sum of Scores | Expected Under H0 | Std Dev Under H0 | Mean Score |
|------|----|---------------|-------------------|------------------|------------|
| 1 | 20 | 400.0 | 710.0 | 68.965290 | 20.00 |
| 2 | 10 | 200.0 | 355.0 | 53.420284 | 20.00 |
| 3 | 10 | 220.0 | 355.0 | 53.420284 | 22.00 |
| 4 | 10 | 455.0 | 355.0 | 53.420284 | 45.50 |
| 5 | 10 | 605.0 | 355.0 | 53.420284 | 60.50 |
| 6 | 10 | 605.0 | 355.0 | 53.420284 | 60.50 |

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square 67.6709
DF 5
Pr > Chi-Square <.0001

ENV/JM/MONO(2008)20

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:08 WEDNESDAY 27JUN07
 Modified Dunn's Multiple Comparisons (Increasing) on PRPMALE
 LAB H DAPHNID MEASUREMENTS FOR FULL DATA USING ALPHA=0.05

| dose | doseval | COUNT | N0 | MRANK | ABS_DIFF | CRIT_05 | CRIT_01 | SIGNIF | p_val |
|------|---------|-------|----|-------|----------|---------|---------|--------|--------|
| 1 | 0 | 20 | 20 | 20.0 | 0.0 | 13.4231 | 16.6071 | . | |
| 2 | 25 | 10 | 20 | 20.0 | 0.0 | 16.4399 | 20.3395 | | 1.0000 |
| 3 | 74 | 10 | 20 | 22.0 | 2.0 | 16.4399 | 20.3395 | | 1.0000 |
| 4 | 220 | 10 | 20 | 45.5 | 25.5 | 16.4399 | 20.3395 | ** | 0.0008 |
| 5 | 670 | 10 | 20 | 60.5 | 40.5 | 16.4399 | 20.3395 | ** | 0.0001 |
| 6 | 2000 | 10 | 20 | 60.5 | 40.5 | 16.4399 | 20.3395 | ** | 0.0001 |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:08 WEDNESDAY 27JUN07
 MONOTONICITY CHECK OF PRPMALE - FULL DATA
 DOSES 0, 25, 74, 220, 670, 2000 ug/L
 LAB H DAPHNID OF AQUATIC DAPHNID

| PARAM | p_t | SIGNIF |
|------------|--------|--------|
| DOSE TREND | 0.0001 | ** |
| DOSE QUAD | 0.0001 | ** |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:08 WEDNESDAY 27JUN07
 ANALYSIS OF PRPMALE USING ALPHA=0.05
 FULL DATA

----- JONCKHEERE-TERPSTRA TEST KEY -----
 JT IS JONCKHEERE STATISTIC
 Z_JT_ IS STANDARDIZED JONCKHEERE STATISTIC
 PR_JT_ IS P-VALUE FOR UPWARD TREND
 PL_JT_ IS P-VALUE FOR DOWNWARD TREND
 P-VALUES ARE FOR TIE-CORRECTED TEST WITH CONTINUITY CORRECTION FACTOR
 SIGNIF RESULTS ARE FOR Increasing ALTERNATIVE HYPOTHESIS

Check for ties in PRPMALE
 Percent of all data tied at 3 most frequently observed values
 Since 56 percent of observations have the same value
 Exact methods (StatXact) are recommended

| COUNT | SUMWTS | NMISS | NOBS | RESPONSE | TIES | TIEPCT |
|-------|--------|-------|------|----------|------|--------|
| 39 | 70 | 2 | 72 | 0.00000 | 39 | 56 |
| 20 | 70 | 2 | 72 | 1.00000 | 59 | 84 |
| 1 | 70 | 2 | 72 | 0.13380 | 60 | 86 |

Check for Number of Reps in PRPMALE Thru 2000 ug/L
 Maximum Number of Reps in Any Treatment Group or Control is 20
 Total Number of Reps in All Treatment Groups is 70
 Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 5 Doses thru 2000 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 1457 | 8.2661065 | . | 0.0001 | ** | 6 |

Check for Number of Reps in PRPMALE Thru 670 ug/L
 Maximum Number of Reps in Any Treatment Group or Control is 20
 Total Number of Reps in All Treatment Groups is 60
 Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 4 Doses thru 670 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 957 | 7.2114282 | . | 0.0001 | ** | 5 |

Check for Number of Reps in PRPMALE Thru 220 ug/L
 Maximum Number of Reps in Any Treatment Group or Control is 20
 Total Number of Reps in All Treatment Groups is 50
 Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 3 Doses thru 220 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 457 | 5.2161012 | . | 0.0001 | ** | 4 |

Check for Number of Reps in PRPMALE Thru 74 ug/L
 Maximum Number of Reps in Any Treatment Group or Control is 20
 Total Number of Reps in All Treatment Groups is 40
 Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 2 Doses thru 74 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 34.5 | 1.4142136 | . | 0.2457 | | 3 |

NOTE

Jonckheere test results are included in the summary table.
 Group means should be examined to check for lack-of-fit
 to a linear trend before trend test results are accepted.

=====

AUTO TRANSFORM FULL DATA

STATISTICAL ANALYSIS LIST FILE
REPORT CREATED ON 19:08 WEDNESDAY 27JUN07
SHAPIRO-WILK TEST OF NORMALITY OF SQRT(PRPMAL)

AQUATIC DAPHNID: FULL DATA

| OBS | STD | SKEW | KURT | SW_STAT | P_VALUE | SIGNIF |
|-----|----------|---------|---------|---------|---------|--------|
| 70 | 0.054530 | 3.11752 | 19.8560 | 0.56609 | 0.0001 | ** |

NOTE

No auto-transform fixes data non-normality.
Returning to untransformed response.

NOEC Determination for Lab G

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:08 WEDNESDAY 27JUN07
 LAB G DAPHID MEASUREMENTS FROM DATASET SEXRATIOS
 GROUP STATISTICS FOR PRPMALE BY DOSE, Unweighted

| dose | doseval | COUNT | MEAN | MEDIAN | STD_DEV | STD_ERR |
|------|---------|-------|---------|---------|---------|----------|
| 1 | 0 | 20 | 0.00000 | 0.00000 | 0.00000 | 0.000000 |
| 2 | 25 | 10 | 0.00000 | 0.00000 | 0.00000 | 0.000000 |
| 3 | 74 | 9 | 0.14608 | 0.13750 | 0.06840 | 0.022800 |
| 4 | 220 | 10 | 0.89718 | 0.94071 | 0.10696 | 0.033824 |
| 5 | 670 | 10 | 1.00000 | 1.00000 | 0.00000 | 0.000000 |
| 6 | 2000 | 10 | 1.00000 | 1.00000 | 0.00000 | 0.000000 |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:08 WEDNESDAY 27JUN07
 SHAPIRO-WILK TEST OF NORMALITY OF PRPMALE
 AQUATIC DAPHID: FULL DATA

| OBS | STD | SKEW | KURT | SW_STAT | P_VALUE | SIGNIF |
|-----|----------|----------|---------|---------|---------|--------|
| 69 | 0.045438 | -1.68151 | 9.14962 | 0.62540 | 0.0001 | ** |

Outliers & Influential Observations
 PRPMALE FROM FULL DATA Unweighted
 AQUATIC DAPHID: Concentrations in ug/L

| Obs | dose | doseval | GROUP | OBSER | Pred | SE_PRED |
|-----|------|---------|-------|---------|---------|----------|
| 1 | 3 | 74 | 3 | 0.13750 | 0.14608 | 0.015736 |
| 2 | 3 | 74 | 3 | 0.07143 | 0.14608 | 0.015736 |
| 3 | 3 | 74 | 3 | 0.02899 | 0.14608 | 0.015736 |
| 4 | 3 | 74 | 3 | 0.18605 | 0.14608 | 0.015736 |
| 5 | 3 | 74 | 3 | 0.19118 | 0.14608 | 0.015736 |
| 6 | 3 | 74 | 3 | 0.12857 | 0.14608 | 0.015736 |
| 7 | 3 | 74 | 3 | 0.17500 | 0.14608 | 0.015736 |
| 8 | 3 | 74 | 3 | 0.13514 | 0.14608 | 0.015736 |
| 9 | 3 | 74 | 3 | 0.26087 | 0.14608 | 0.015736 |
| 10 | 4 | 220 | 4 | 1.00000 | 0.89718 | 0.014928 |
| 11 | 4 | 220 | 4 | 0.84000 | 0.89718 | 0.014928 |
| 12 | 4 | 220 | 4 | 0.92308 | 0.89718 | 0.014928 |
| 13 | 4 | 220 | 4 | 0.96154 | 0.89718 | 0.014928 |
| 14 | 4 | 220 | 4 | 0.76000 | 0.89718 | 0.014928 |
| 15 | 4 | 220 | 4 | 0.88889 | 0.89718 | 0.014928 |
| 16 | 4 | 220 | 4 | 0.68000 | 0.89718 | 0.014928 |
| 17 | 4 | 220 | 4 | 1.00000 | 0.89718 | 0.014928 |
| 18 | 4 | 220 | 4 | 0.95833 | 0.89718 | 0.014928 |
| 19 | 4 | 220 | 4 | 0.96000 | 0.89718 | 0.014928 |

ENV/JM/MONO(2008)20

| Obs | L95M | U95M | Resid | LB | UB |
|-----|---------|---------|----------|-------------|------------|
| 1 | 0.11463 | 0.17752 | -0.00858 | -1.1102E-15 | 6.6613E-16 |
| 2 | 0.11463 | 0.17752 | -0.07465 | -1.1102E-15 | 6.6613E-16 |
| 3 | 0.11463 | 0.17752 | -0.11709 | -1.1102E-15 | 6.6613E-16 |
| 4 | 0.11463 | 0.17752 | 0.03997 | -1.1102E-15 | 6.6613E-16 |
| 5 | 0.11463 | 0.17752 | 0.04510 | -1.1102E-15 | 6.6613E-16 |
| 6 | 0.11463 | 0.17752 | -0.01751 | -1.1102E-15 | 6.6613E-16 |
| 7 | 0.11463 | 0.17752 | 0.02892 | -1.1102E-15 | 6.6613E-16 |
| 8 | 0.11463 | 0.17752 | -0.01094 | -1.1102E-15 | 6.6613E-16 |
| 9 | 0.11463 | 0.17752 | 0.11479 | -1.1102E-15 | 6.6613E-16 |
| 10 | 0.86735 | 0.92702 | 0.10282 | -1.1102E-15 | 6.6613E-16 |
| 11 | 0.86735 | 0.92702 | -0.05718 | -1.1102E-15 | 6.6613E-16 |
| 12 | 0.86735 | 0.92702 | 0.02589 | -1.1102E-15 | 6.6613E-16 |
| 13 | 0.86735 | 0.92702 | 0.06435 | -1.1102E-15 | 6.6613E-16 |
| 14 | 0.86735 | 0.92702 | -0.13718 | -1.1102E-15 | 6.6613E-16 |
| 15 | 0.86735 | 0.92702 | -0.00829 | -1.1102E-15 | 6.6613E-16 |
| 16 | 0.86735 | 0.92702 | -0.21718 | -1.1102E-15 | 6.6613E-16 |
| 17 | 0.86735 | 0.92702 | 0.10282 | -1.1102E-15 | 6.6613E-16 |
| 18 | 0.86735 | 0.92702 | 0.06115 | -1.1102E-15 | 6.6613E-16 |
| 19 | 0.86735 | 0.92702 | 0.06282 | -1.1102E-15 | 6.6613E-16 |

NOTE

This set of outliers will be excluded from all outlier omitted analyses for this response. In order for the outliers from the transformed analysis to be used for outlier omitted analysis instead, run the analysis again and either specify the transform (instead of allowing the system to decide) or manually omit these observations using the exclusion functionality.

NOTE

The data was NOT normally distributed.
A nonparametric analysis will be performed.

STATISTICAL ANALYSIS LIST FILE
REPORT CREATED ON 19:08 WEDNESDAY 27JUN07
Kruskal-Wallis Test on PRPMALE
LAB G DAPHID MEASUREMENTS FOR FULL DATA

The NPAR1WAY Procedure
Wilcoxon Scores (Rank Sums) for Variable RESPONSE
Classified by Variable dose

| dose | N | Sum of Scores | Expected Under H0 | Std Dev Under H0 | Mean Score |
|------|----|---------------|-------------------|------------------|------------|
| 1 | 20 | 310.0 | 700.0 | 71.149765 | 15.50 |
| 2 | 10 | 155.0 | 350.0 | 55.206020 | 15.50 |
| 3 | 9 | 315.0 | 315.0 | 52.815003 | 35.00 |
| 4 | 10 | 465.0 | 350.0 | 55.206020 | 46.50 |
| 5 | 10 | 585.0 | 350.0 | 55.206020 | 58.50 |
| 6 | 10 | 585.0 | 350.0 | 55.206020 | 58.50 |

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square 66.7038
 DF 5
 Pr > Chi-Square <.0001

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:08 WEDNESDAY 27JUN07
 Modified Dunn's Multiple Comparisons (Increasing) on PRPMALE
 LAB G DAPHID MEASUREMENTS FOR FULL DATA USING ALPHA=0.05

| dose | doseval | COUNT | N0 | MRANK | ABS_DIFF | CRIT_05 | CRIT_01 | SIGNIF | p_val |
|------|---------|-------|----|-------|----------|---------|---------|--------|--------|
| 1 | 0 | 20 | 20 | 15.5 | 0.0 | 13.8886 | 17.1830 | . | |
| 2 | 25 | 10 | 20 | 15.5 | 0.0 | 17.0100 | 21.0448 | | 1.0000 |
| 3 | 74 | 9 | 20 | 35.0 | 19.5 | 17.6288 | 21.8104 | * | 0.0252 |
| 4 | 220 | 10 | 20 | 46.5 | 31.0 | 17.0100 | 21.0448 | ** | 0.0001 |
| 5 | 670 | 10 | 20 | 58.5 | 43.0 | 17.0100 | 21.0448 | ** | 0.0001 |
| 6 | 2000 | 10 | 20 | 58.5 | 43.0 | 17.0100 | 21.0448 | ** | 0.0001 |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:08 WEDNESDAY 27JUN07
 MONOTONICITY CHECK OF PRPMALE - FULL DATA
 DOSES 0, 25, 74, 220, 670, 2000 ug/L
 LAB G DAPHID OF AQUATIC DAPHID

| PARM | p_t | SIGNIF |
|------------|--------|--------|
| DOSE TREND | 0.0001 | ** |
| DOSE QUAD | 0.0056 | ** |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:08 WEDNESDAY 27JUN07
 ANALYSIS OF PRPMALE USING ALPHA=0.05
 FULL DATA

----- JONCKHEERE-TERPSTRA TEST KEY -----
 JT IS JONCKHEERE STATISTIC
 Z_JT IS STANDARDIZED JONCKHEERE STATISTIC
 PR_JT IS P-VALUE FOR UPWARD TREND
 PL_JT IS P-VALUE FOR DOWNWARD TREND
 P-VALUES ARE FOR TIE-CORRECTED TEST WITH CONTINUITY CORRECTION FACTOR
 SIGNIF RESULTS ARE FOR Increasing ALTERNATIVE HYPOTHESIS

ENV/JM/MONO(2008)20

Check for ties in PRPMALE

Percent of all data tied at 3 most frequently observed values

Since 43 percent of observations have the same value

Exact methods (StatXact) are recommended

| COUNT | SUMWTS | NMISS | NOBS | RESPONSE | TIES | TIEPCT |
|-------|--------|-------|------|----------|------|--------|
| 30 | 69 | 2 | 71 | 0.00000 | 30 | 43 |
| 22 | 69 | 2 | 71 | 1.00000 | 52 | 75 |
| 1 | 69 | 2 | 71 | 0.02899 | 53 | 77 |

Check for Number of Reps in PRPMALE Thru 2000 ug/L

Maximum Number of Reps in Any Treatment Group or Control is 20

Total Number of Reps in All Treatment Groups is 69

Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 5 Doses thru 2000 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 1640 | 9.0048822 | . | 0.0001 | ** | 6 |

Check for Number of Reps in PRPMALE Thru 670 ug/L

Maximum Number of Reps in Any Treatment Group or Control is 20

Total Number of Reps in All Treatment Groups is 59

Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 4 Doses thru 670 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 1170 | 8.2288922 | . | 0.0001 | ** | 5 |

Check for Number of Reps in PRPMALE Thru 220 ug/L

Maximum Number of Reps in Any Treatment Group or Control is 20

Total Number of Reps in All Treatment Groups is 49

Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 3 Doses thru 220 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 700 | 6.8232838 | . | 0.0001 | ** | 4 |

Check for Number of Reps in PRPMALE Thru 74 ug/L

Maximum Number of Reps in Any Treatment Group or Control is 20

Total Number of Reps in All Treatment Groups is 39

Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 2 Doses thru 74 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 288 | 4.8633754 | . | 0.0001 | ** | 3 |

NOTE

Remaining data is constant.
No further tests are possible.

NOTE

Jonckheere test results are included in the summary table.
Group means should be examined to check for lack-of-fit

NOEC Determination for Lab E

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:09 WEDNESDAY 27JUN07
 LAB E DAPHNID MEASUREMENTS FROM DATASET SEXRATIOS
 GROUP STATISTICS FOR PRPMALE BY DOSE, Unweighted

| dose | doseval | COUNT | MEAN | MEDIAN | STD_DEV | STD_ERR |
|------|---------|-------|---------|---------|---------|---------|
| 1 | 0 | 19 | 0.00594 | 0.00000 | 0.02590 | 0.00594 |
| 2 | 25 | 10 | 0.00513 | 0.00000 | 0.01622 | 0.00513 |
| 3 | 74 | 9 | 0.00247 | 0.00000 | 0.00741 | 0.00247 |
| 4 | 220 | 10 | 0.03454 | 0.01923 | 0.04303 | 0.01361 |
| 5 | 670 | 10 | 0.12828 | 0.12533 | 0.06441 | 0.02037 |
| 6 | 2000 | 10 | 0.66667 | 1.00000 | 0.47140 | 0.14907 |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:09 WEDNESDAY 27JUN07
 SHAPIRO-WILK TEST OF NORMALITY OF PRPMALE
 AQUATIC DAPHNID: FULL DATA

| OBS | STD | SKEW | KURT | SW_STAT | P_VALUE | SIGNIF |
|-----|---------|----------|---------|---------|---------|--------|
| 68 | 0.17572 | -1.88840 | 8.28370 | 0.59209 | 0.0001 | ** |

Outliers & Influential Observations
 PRPMALE FROM FULL DATA Unweighted
 AQUATIC DAPHNID: Concentrations in ug/L

| Obs | DAPHNID | dose | doseval | GROUP | OBSER | Pred | SE_PRED |
|-----|---------|------|---------|-------|---------|---------|----------|
| 1 | 8 | 1 | 0 | 1 | 0.11290 | 0.00594 | 0.041908 |
| 2 | 7 | 2 | 25 | 2 | 0.05128 | 0.00513 | 0.057766 |
| 3 | 3 | 3 | 74 | 3 | 0.02222 | 0.00247 | 0.060891 |
| 4 | 1 | 4 | 220 | 4 | 0.00000 | 0.03454 | 0.057766 |
| 5 | 3 | 4 | 220 | 4 | 0.00000 | 0.03454 | 0.057766 |
| 6 | 4 | 4 | 220 | 4 | 0.06250 | 0.03454 | 0.057766 |
| 7 | 5 | 4 | 220 | 4 | 0.08696 | 0.03454 | 0.057766 |
| 8 | 7 | 4 | 220 | 4 | 0.00000 | 0.03454 | 0.057766 |
| 9 | 8 | 4 | 220 | 4 | 0.00000 | 0.03454 | 0.057766 |
| 10 | 9 | 4 | 220 | 4 | 0.11905 | 0.03454 | 0.057766 |
| 11 | 10 | 4 | 220 | 4 | 0.00000 | 0.03454 | 0.057766 |
| 12 | 2 | 5 | 670 | 5 | 0.19697 | 0.12828 | 0.057766 |
| 13 | 3 | 5 | 670 | 5 | 0.19444 | 0.12828 | 0.057766 |
| 14 | 4 | 5 | 670 | 5 | 0.00000 | 0.12828 | 0.057766 |
| 15 | 5 | 5 | 670 | 5 | 0.10417 | 0.12828 | 0.057766 |
| 16 | 8 | 5 | 670 | 5 | 0.18605 | 0.12828 | 0.057766 |
| 17 | 9 | 5 | 670 | 5 | 0.05660 | 0.12828 | 0.057766 |
| 18 | 10 | 5 | 670 | 5 | 0.17857 | 0.12828 | 0.057766 |
| 19 | 1 | 6 | 2000 | 6 | 1.00000 | 0.66667 | 0.057766 |
| 20 | 2 | 6 | 2000 | 6 | 1.00000 | 0.66667 | 0.057766 |
| 21 | 4 | 6 | 2000 | 6 | 0.00000 | 0.66667 | 0.057766 |
| 22 | 5 | 6 | 2000 | 6 | 1.00000 | 0.66667 | 0.057766 |
| 23 | 6 | 6 | 2000 | 6 | 1.00000 | 0.66667 | 0.057766 |
| 24 | 7 | 6 | 2000 | 6 | 1.00000 | 0.66667 | 0.057766 |
| 25 | 8 | 6 | 2000 | 6 | 0.00000 | 0.66667 | 0.057766 |

| Obs | L95M | U95M | Resid | LB | UB |
|-----|----------|---------|----------|-----------|----------|
| 1 | -0.07783 | 0.08971 | 0.10696 | -0.020734 | 0.018710 |
| 2 | -0.11034 | 0.12060 | 0.04615 | -0.020734 | 0.018710 |
| 3 | -0.11925 | 0.12419 | 0.01975 | -0.020734 | 0.018710 |
| 4 | -0.08093 | 0.15002 | -0.03454 | -0.020734 | 0.018710 |
| 5 | -0.08093 | 0.15002 | -0.03454 | -0.020734 | 0.018710 |
| 6 | -0.08093 | 0.15002 | 0.02796 | -0.020734 | 0.018710 |
| 7 | -0.08093 | 0.15002 | 0.05241 | -0.020734 | 0.018710 |
| 8 | -0.08093 | 0.15002 | -0.03454 | -0.020734 | 0.018710 |
| 9 | -0.08093 | 0.15002 | -0.03454 | -0.020734 | 0.018710 |
| 10 | -0.08093 | 0.15002 | 0.08450 | -0.020734 | 0.018710 |
| 11 | -0.08093 | 0.15002 | -0.03454 | -0.020734 | 0.018710 |
| 12 | 0.01281 | 0.24376 | 0.06869 | -0.020734 | 0.018710 |
| 13 | 0.01281 | 0.24376 | 0.06616 | -0.020734 | 0.018710 |
| 14 | 0.01281 | 0.24376 | -0.12828 | -0.020734 | 0.018710 |
| 15 | 0.01281 | 0.24376 | -0.02412 | -0.020734 | 0.018710 |
| 16 | 0.01281 | 0.24376 | 0.05776 | -0.020734 | 0.018710 |
| 17 | 0.01281 | 0.24376 | -0.07168 | -0.020734 | 0.018710 |
| 18 | 0.01281 | 0.24376 | 0.05029 | -0.020734 | 0.018710 |
| 19 | 0.55119 | 0.78214 | 0.33333 | -0.020734 | 0.018710 |
| 20 | 0.55119 | 0.78214 | 0.33333 | -0.020734 | 0.018710 |
| 21 | 0.55119 | 0.78214 | -0.66667 | -0.020734 | 0.018710 |
| 22 | 0.55119 | 0.78214 | 0.33333 | -0.020734 | 0.018710 |
| 23 | 0.55119 | 0.78214 | 0.33333 | -0.020734 | 0.018710 |
| 24 | 0.55119 | 0.78214 | 0.33333 | -0.020734 | 0.018710 |
| 25 | 0.55119 | 0.78214 | -0.66667 | -0.020734 | 0.018710 |

Outliers & Influential Observations
 PRPMALE FROM FULL DATA Unweighted
 AQUATIC DAPHNID: Concentrations in ug/L

| Obs | DAPHNID | dose | doseval | GROUP | OBSER | Pred | SE_PRED |
|-----|---------|------|---------|--------|---------|---------|----------|
| 26 | 9 | 6 | | 2000 6 | 1.00000 | 0.66667 | 0.057766 |
| 27 | 10 | 6 | | 2000 6 | 0.00000 | 0.66667 | 0.057766 |

| Obs | L95M | U95M | Resid | LB | UB |
|-----|---------|---------|----------|-----------|----------|
| 26 | 0.55119 | 0.78214 | 0.33333 | -0.020734 | 0.018710 |
| 27 | 0.55119 | 0.78214 | -0.66667 | -0.020734 | 0.018710 |

NOTE

This set of outliers will be excluded from all outlier omitted analyses for this response. In order for the outliers from the transformed analysis to be used for outlier omitted analysis instead, run the analysis again and either specify the transform (instead of allowing the system to decide) or manually omit these observations using the exclusion functionality.

NOTE

The data was NOT normally distributed.
 A nonparametric analysis will be performed.

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:09 WEDNESDAY 27JUN07
 Kruskal-Wallis Test on PRPMALE
 LAB E DAPHNID MEASUREMENTS FOR FULL DATA

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable RESPONSE
 Classified by Variable dose

| dose | N | Sum of Scores | Expected Under H0 | Std Dev Under H0 | Mean Score |
|------|----|---------------|-------------------|------------------|------------|
| 1 | 19 | 458.00 | 655.50 | 62.447878 | 24.105263 |
| 2 | 10 | 250.50 | 345.00 | 49.289776 | 25.050000 |
| 3 | 9 | 225.00 | 310.50 | 47.161771 | 25.000000 |
| 4 | 10 | 361.50 | 345.00 | 49.289776 | 36.150000 |
| 5 | 10 | 528.50 | 345.00 | 49.289776 | 52.850000 |
| 6 | 10 | 522.50 | 345.00 | 49.289776 | 52.250000 |

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square 36.1728
 DF 5
 Pr > Chi-Square <.0001

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:09 WEDNESDAY 27JUN07
 Modified Dunn's Multiple Comparisons (Increasing) on PRPMALE
 LAB E DAPHNID MEASUREMENTS FOR FULL DATA USING ALPHA=0.05

| dose | N | Mean | Lower | Upper | Lower | Upper | Lower | Upper | Significance |
|------|----|---------|---------|---------|---------|-------|-------|-------|--------------|
| 1 | 19 | 24.1053 | 0.0000 | 12.7383 | 15.7598 | | | | . |
| 2 | 10 | 25.0500 | 0.9447 | 15.3389 | 18.9773 | | | | 1.0000 |
| 3 | 9 | 25.0000 | 0.8947 | 15.8874 | 19.6559 | | | | 1.0000 |
| 4 | 10 | 36.1500 | 12.0447 | 15.3389 | 18.9773 | | | | 0.1693 |
| 5 | 10 | 52.8500 | 28.7447 | 15.3389 | 18.9773 | ** | | | 0.0001 |
| 6 | 10 | 52.2500 | 28.1447 | 15.3389 | 18.9773 | ** | | | 0.0001 |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:09 WEDNESDAY 27JUN07
 MONOTONICITY CHECK OF PRPMALE - FULL DATA
 DOSES 0, 25, 74, 220, 670, 2000 ug/L
 LAB E DAPHNID OF AQUATIC DAPHNID

| PARAM | p_t | SIGNIF |
|------------|--------|--------|
| DOSE TREND | 0.0001 | ** |
| DOSE QUAD | 0.0114 | * |

 STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:09 WEDNESDAY 27JUN07
 ANALYSIS OF PRPMALE USING ALPHA=0.05
 FULL DATA

----- JONCKHEERE-TERPSTRA TEST KEY -----
 JT IS JONCKHEERE STATISTIC
 Z_JT IS STANDARDIZED JONCKHEERE STATISTIC
 PR_JT IS P-VALUE FOR UPWARD TREND
 PL_JT IS P-VALUE FOR DOWNWARD TREND
 P-VALUES ARE FOR TIE-CORRECTED TEST WITH CONTINUITY CORRECTION FACTOR
 SIGNIF RESULTS ARE FOR Increasing ALTERNATIVE HYPOTHESIS

Check for ties in PRPMALE
 Percent of all data tied at 3 most frequently observed values
 Since 65 percent of observations have the same value
 Exact methods (StatXact) are recommended

| COUNT | SUMWTS | NMISS | NOBS | RESPONSE | TIES | TIEPCT |
|-------|--------|-------|------|----------|------|--------|
| 44 | 68 | 2 | 70 | 0.00000 | 44 | 65 |
| 6 | 68 | 2 | 70 | 1.00000 | 50 | 74 |
| 2 | 68 | 2 | 70 | 0.03846 | 52 | 76 |

Check for Number of Reps in PRPMALE Thru 2000 ug/L
 Maximum Number of Reps in Any Treatment Group or Control is 19
 Total Number of Reps in All Treatment Groups is 68
 Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 5 Doses thru 2000 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 1130.5 | 5.9876219 | . | 0.0001 | ** | 6 |

ENV/JM/MONO(2008)20

Check for Number of Reps in PRPMALE Thru 670 ug/L
Maximum Number of Reps in Any Treatment Group or Control is 19
Total Number of Reps in All Treatment Groups is 58
Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 4 Doses thru 670 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 711 | 5.0765391 | . | 0.0001 | ** | 5 |

Check for Number of Reps in PRPMALE Thru 220 ug/L
Maximum Number of Reps in Any Treatment Group or Control is 19
Total Number of Reps in All Treatment Groups is 48
Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 3 Doses thru 220 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 263.5 | 2.5899854 | . | 0.0040 | ** | 4 |

Check for Number of Reps in PRPMALE Thru 74 ug/L
Maximum Number of Reps in Any Treatment Group or Control is 19
Total Number of Reps in All Treatment Groups is 38
Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 2 Doses thru 74 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 62.5 | 0.5002899 | . | 0.4017 | | 3 |

NOTE

Jonckheere test results are included in the summary table.
Group means should be examined to check for lack-of-fit

=====

AUTO TRANSFORM FULL DATA

STATISTICAL ANALYSIS LIST FILE
REPORT CREATED ON 19:09 WEDNESDAY 27JUN07
SHAPIRO-WILK TEST OF NORMALITY OF SQRT(PRPMAL)
AQUATIC DAPHNID: FULL DATA

| OBS | STD | SKEW | KURT | SW_STAT | P_VALUE | SIGNIF |
|-----|---------|----------|---------|---------|---------|--------|
| 68 | 0.19458 | -1.54241 | 5.28322 | 0.75235 | 0.0001 | ** |

NOTE

No auto-transform fixes data non-normality.
Returning to untransformed response.

NOEC Determination for Lab F

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:09 WEDNESDAY 27JUN07
 LAB F DAPHNID MEASUREMENTS FROM DATASET SEXRATIOS
 GROUP STATISTICS FOR PRPMALE BY DOSE, Unweighted

| dose | doseval | COUNT | MEAN | MEDIAN | STD_DEV | STD_ERR |
|------|---------|-------|---------|---------|---------|----------|
| 1 | 0 | 20 | 0.00028 | 0.00000 | 0.00125 | 0.000279 |
| 2 | 25 | 10 | 0.00125 | 0.00000 | 0.00395 | 0.001250 |
| 3 | 74 | 10 | 0.06482 | 0.00662 | 0.09370 | 0.029631 |
| 4 | 220 | 10 | 0.44785 | 0.42206 | 0.24403 | 0.077169 |
| 5 | 670 | 10 | 0.79126 | 0.84211 | 0.16522 | 0.052248 |
| 6 | 2000 | 10 | 0.99524 | 1.00000 | 0.01506 | 0.004762 |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:09 WEDNESDAY 27JUN07
 SHAPIRO-WILK TEST OF NORMALITY OF PRPMALE
 AQUATIC DAPHNID: FULL DATA

| OBS | STD | SKEW | KURT | SW_STAT | P_VALUE | SIGNIF |
|-----|---------|----------|---------|---------|---------|--------|
| 70 | 0.11183 | -0.70067 | 5.03896 | 0.79545 | 0.0001 | ** |

Outliers & Influential Observations
 PRPMALE FROM FULL DATA Unweighted
 AQUATIC DAPHNID: Concentrations in ug/L

| Obs | DAPHNID | dose | doseval | GROUP | OBSER | Pred | SE_PRED |
|-----|---------|------|---------|-------|---------|---------|----------|
| 1 | 1 | 3 | 74 | 3 | 0.00000 | 0.06482 | 0.036718 |
| 2 | 3 | 3 | 74 | 3 | 0.14583 | 0.06482 | 0.036718 |
| 3 | 4 | 3 | 74 | 3 | 0.27211 | 0.06482 | 0.036718 |
| 4 | 5 | 3 | 74 | 3 | 0.14013 | 0.06482 | 0.036718 |
| 5 | 6 | 3 | 74 | 3 | 0.00000 | 0.06482 | 0.036718 |
| 6 | 7 | 3 | 74 | 3 | 0.01325 | 0.06482 | 0.036718 |
| 7 | 8 | 3 | 74 | 3 | 0.00000 | 0.06482 | 0.036718 |
| 8 | 9 | 3 | 74 | 3 | 0.00000 | 0.06482 | 0.036718 |
| 9 | 10 | 3 | 74 | 3 | 0.00000 | 0.06482 | 0.036718 |
| 10 | 1 | 4 | 220 | 4 | 0.42105 | 0.44785 | 0.036718 |
| 11 | 2 | 4 | 220 | 4 | 0.70526 | 0.44785 | 0.036718 |
| 12 | 3 | 4 | 220 | 4 | 0.26000 | 0.44785 | 0.036718 |
| 13 | 4 | 4 | 220 | 4 | 0.64220 | 0.44785 | 0.036718 |
| 14 | 5 | 4 | 220 | 4 | 0.77064 | 0.44785 | 0.036718 |
| 15 | 6 | 4 | 220 | 4 | 0.42308 | 0.44785 | 0.036718 |
| 16 | 7 | 4 | 220 | 4 | 0.00000 | 0.44785 | 0.036718 |
| 17 | 8 | 4 | 220 | 4 | 0.63462 | 0.44785 | 0.036718 |
| 18 | 9 | 4 | 220 | 4 | 0.40594 | 0.44785 | 0.036718 |
| 19 | 10 | 4 | 220 | 4 | 0.21569 | 0.44785 | 0.036718 |
| 20 | 1 | 5 | 670 | 5 | 0.94737 | 0.79126 | 0.036718 |
| 21 | 2 | 5 | 670 | 5 | 0.94545 | 0.79126 | 0.036718 |
| 22 | 3 | 5 | 670 | 5 | 0.69355 | 0.79126 | 0.036718 |
| 23 | 4 | 5 | 670 | 5 | 0.45098 | 0.79126 | 0.036718 |
| 24 | 5 | 5 | 670 | 5 | 0.72549 | 0.79126 | 0.036718 |
| 25 | 6 | 5 | 670 | 5 | 0.98148 | 0.79126 | 0.036718 |

| Obs | L95M | U95M | Resid | LB | UB |
|-----|----------|---------|----------|-----------|----------|
| 1 | -0.00853 | 0.13818 | -0.06482 | -0.010268 | 0.013780 |
| 2 | -0.00853 | 0.13818 | 0.08101 | -0.010268 | 0.013780 |
| 3 | -0.00853 | 0.13818 | 0.20729 | -0.010268 | 0.013780 |
| 4 | -0.00853 | 0.13818 | 0.07530 | -0.010268 | 0.013780 |
| 5 | -0.00853 | 0.13818 | -0.06482 | -0.010268 | 0.013780 |
| 6 | -0.00853 | 0.13818 | -0.05158 | -0.010268 | 0.013780 |
| 7 | -0.00853 | 0.13818 | -0.06482 | -0.010268 | 0.013780 |
| 8 | -0.00853 | 0.13818 | -0.06482 | -0.010268 | 0.013780 |
| 9 | -0.00853 | 0.13818 | -0.06482 | -0.010268 | 0.013780 |
| 10 | 0.37449 | 0.52120 | -0.02680 | -0.010268 | 0.013780 |
| 11 | 0.37449 | 0.52120 | 0.25742 | -0.010268 | 0.013780 |
| 12 | 0.37449 | 0.52120 | -0.18785 | -0.010268 | 0.013780 |
| 13 | 0.37449 | 0.52120 | 0.19435 | -0.010268 | 0.013780 |
| 14 | 0.37449 | 0.52120 | 0.32279 | -0.010268 | 0.013780 |
| 15 | 0.37449 | 0.52120 | -0.02477 | -0.010268 | 0.013780 |
| 16 | 0.37449 | 0.52120 | -0.44785 | -0.010268 | 0.013780 |
| 17 | 0.37449 | 0.52120 | 0.18677 | -0.010268 | 0.013780 |
| 18 | 0.37449 | 0.52120 | -0.04191 | -0.010268 | 0.013780 |
| 19 | 0.37449 | 0.52120 | -0.23216 | -0.010268 | 0.013780 |
| 20 | 0.71791 | 0.86462 | 0.15611 | -0.010268 | 0.013780 |
| 21 | 0.71791 | 0.86462 | 0.15419 | -0.010268 | 0.013780 |
| 22 | 0.71791 | 0.86462 | -0.09771 | -0.010268 | 0.013780 |
| 23 | 0.71791 | 0.86462 | -0.34028 | -0.010268 | 0.013780 |
| 24 | 0.71791 | 0.86462 | -0.06577 | -0.010268 | 0.013780 |
| 25 | 0.71791 | 0.86462 | 0.19022 | -0.010268 | 0.013780 |

Outliers & Influential Observations
 PRPMALE FROM FULL DATA Unweighted
 AQUATIC DAPHNID: Concentrations in ug/L

| Obs | DAPHNID | dose | doseval | GROUP | OBSER | Pred | SE_PRED |
|-----|---------|------|---------|--------|---------|---------|----------|
| 26 | 7 | 5 | | 670 5 | 0.84211 | 0.79126 | 0.036718 |
| 27 | 8 | 5 | | 670 5 | 0.84211 | 0.79126 | 0.036718 |
| 28 | 9 | 5 | | 670 5 | 0.84615 | 0.79126 | 0.036718 |
| 29 | 10 | 5 | | 670 5 | 0.63793 | 0.79126 | 0.036718 |
| 30 | 2 | 6 | | 2000 6 | 0.95238 | 0.99524 | 0.036718 |

| Obs | L95M | U95M | Resid | LB | UB |
|-----|---------|---------|----------|-----------|----------|
| 26 | 0.71791 | 0.86462 | 0.05084 | -0.010268 | 0.013780 |
| 27 | 0.71791 | 0.86462 | 0.05084 | -0.010268 | 0.013780 |
| 28 | 0.71791 | 0.86462 | 0.05489 | -0.010268 | 0.013780 |
| 29 | 0.71791 | 0.86462 | -0.15333 | -0.010268 | 0.013780 |
| 30 | 0.92188 | 1.06859 | -0.04286 | -0.010268 | 0.013780 |

NOTE

This set of outliers will be excluded from all outlier omitted analyses for this response. In order for the outliers from the transformed analysis to be used for outlier omitted analysis instead, run the analysis again and either specify the transform (instead of allowing the system to decide) or manually omit these observations using the exclusion functionality.

NOTE

The data was NOT normally distributed.
A nonparametric analysis will be performed.

STATISTICAL ANALYSIS LIST FILE
REPORT CREATED ON 19:09 WEDNESDAY 27JUN07
Kruskal-Wallis Test on PRPMALE
LAB F DAPHNID MEASUREMENTS FOR FULL DATA

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable RESPONSE
Classified by Variable dose

| dose | N | Sum of Scores | Expected Under H0 | Std Dev Under H0 | Mean Score |
|------|----|---------------|-------------------|------------------|------------|
| 1 | 20 | 367.50 | 710.0 | 72.295260 | 18.3750 |
| 2 | 10 | 193.50 | 355.0 | 55.999667 | 19.3500 |
| 3 | 10 | 284.50 | 355.0 | 55.999667 | 28.4500 |
| 4 | 10 | 439.50 | 355.0 | 55.999667 | 43.9500 |
| 5 | 10 | 546.00 | 355.0 | 55.999667 | 54.6000 |
| 6 | 10 | 654.00 | 355.0 | 55.999667 | 65.4000 |

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square 60.8775
DF 5
Pr > Chi-Square <.0001

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:09 WEDNESDAY 27JUN07
 Modified Dunn's Multiple Comparisons (Increasing) on PRPMALE
 LAB F DAPHNID MEASUREMENTS FOR FULL DATA USING ALPHA=0.05

| dose | doseval | COUNT | N0 | MRANK | ABS_DIFF | CRIT_05 | CRIT_01 | SIGNIF | p_val |
|------|---------|-------|----|--------|----------|---------|---------|--------|--------|
| 1 | 0 | 20 | 20 | 18.375 | 0.000 | 14.0713 | 17.4090 | . | |
| 2 | 25 | 10 | 20 | 19.350 | 0.975 | 17.2337 | 21.3216 | | 1.0000 |
| 3 | 74 | 10 | 20 | 28.450 | 10.075 | 17.2337 | 21.3216 | | 0.4346 |
| 4 | 220 | 10 | 20 | 43.950 | 25.575 | 17.2337 | 21.3216 | ** | 0.0014 |
| 5 | 670 | 10 | 20 | 54.600 | 36.225 | 17.2337 | 21.3216 | ** | 0.0001 |
| 6 | 2000 | 10 | 20 | 65.400 | 47.025 | 17.2337 | 21.3216 | ** | 0.0001 |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:09 WEDNESDAY 27JUN07
 MONOTONICITY CHECK OF PRPMALE - FULL DATA
 DOSES 0, 25, 74, 220, 670, 2000 ug/L
 LAB F DAPHNID OF AQUATIC DAPHNID

| PARAM | p_t | SIGNIF |
|------------|--------|--------|
| DOSE TREND | 0.0001 | ** |
| DOSE QUAD | 0.0001 | ** |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:09 WEDNESDAY 27JUN07
 ANALYSIS OF PRPMALE USING ALPHA=0.05
 FULL DATA

----- JONCKHEERE-TERPSTRA TEST KEY -----
 JT IS JONCKHEERE STATISTIC
 Z_JT_ IS STANDARDIZED JONCKHEERE STATISTIC
 PR_JT IS P-VALUE FOR UPWARD TREND
 PL_JT IS P-VALUE FOR DOWNWARD TREND
 P-VALUES ARE FOR TIE-CORRECTED TEST WITH CONTINUITY CORRECTION FACTOR
 SIGNIF RESULTS ARE FOR Increasing ALTERNATIVE HYPOTHESIS

Check for ties in PRPMALE
 Percent of all data tied at 3 most frequently observed values
 Since 49 percent of observations have the same value
 Exact methods (StatXact) are recommended

| COUNT | SUMWTS | NMISS | NOBS | RESPONSE | TIES | TIEPCT |
|-------|--------|-------|------|----------|------|--------|
| 34 | 70 | 2 | 72 | 0.00000 | 34 | 49 |
| 9 | 70 | 2 | 72 | 1.00000 | 43 | 61 |
| 2 | 70 | 2 | 72 | 0.84211 | 45 | 64 |

ENV/JM/MONO(2008)20

Check for Number of Reps in PRPMALE Thru 2000 ug/L
 Maximum Number of Reps in Any Treatment Group or Control is 20
 Total Number of Reps in All Treatment Groups is 70
 Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 5 Doses thru 2000 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 1697.5 | 8.6969743 | . | 0.0001 | ** | 6 |

Check for Number of Reps in PRPMALE Thru 670 ug/L
 Maximum Number of Reps in Any Treatment Group or Control is 20
 Total Number of Reps in All Treatment Groups is 60
 Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 4 Doses thru 670 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 1094 | 7.1306513 | . | 0.0001 | ** | 5 |

Check for Number of Reps in PRPMALE Thru 220 ug/L
 Maximum Number of Reps in Any Treatment Group or Control is 20
 Total Number of Reps in All Treatment Groups is 50
 Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 3 Doses thru 220 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 582 | 5.3088021 | . | 0.0001 | ** | 4 |

Check for Number of Reps in PRPMALE Thru 74 ug/L
 Maximum Number of Reps in Any Treatment Group or Control is 20
 Total Number of Reps in All Treatment Groups is 40
 Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 2 Doses thru 74 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 201.5 | 2.9065775 | . | 0.0010 | ** | 3 |

Check for Number of Reps in PRPMALE Thru 25 ug/L
 Maximum Number of Reps in Any Treatment Group or Control is 20
 Total Number of Reps in All Treatment Groups is 30
 Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 1 Doses thru 25 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 34 | 0.5594024 | . | 0.3333 | | 2 |

NOTE

Jonckheere test results are included in the summary table.
 Group means should be examined to check for lack-of-fit
 to a linear trend before trend test results are accepted.

=====

AUTO TRANSFORM FULL DATA

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:09 WEDNESDAY 27JUN07
 SHAPIRO-WILK TEST OF NORMALITY OF SQRT(PRPMAL)
 AQUATIC DAPHNID: FULL DATA

| OBS | STD | SKEW | KURT | SW_STAT | P_VALUE | SIGNIF |
|-----|---------|----------|---------|---------|---------|--------|
| 70 | 0.12480 | -1.33565 | 8.97528 | 0.78948 | 0.0001 | ** |

NOTE

No auto-transform fixes data non-normality.
 Returning to untransformed response.

NOEC Determination for Lab C

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:09 WEDNESDAY 27JUN07
 LAB C DAPHNID MEASUREMENTS FROM DATASET SEXRATIOS
 GROUP STATISTICS FOR PRPMALE BY DOSE, Unweighted

| dose | doseval | COUNT | MEAN | MEDIAN | STD_DEV | STD_ERR |
|------|---------|-------|---------|---------|---------|----------|
| 1 | 0 | 18 | 0.00000 | 0.00000 | 0.00000 | 0.000000 |
| 2 | 25 | 9 | 0.00000 | 0.00000 | 0.00000 | 0.000000 |
| 3 | 74 | 9 | 0.00000 | 0.00000 | 0.00000 | 0.000000 |
| 4 | 220 | 10 | 0.52699 | 0.52558 | 0.13363 | 0.042257 |
| 5 | 670 | 10 | 0.65092 | 0.68993 | 0.18944 | 0.059905 |
| 6 | 2000 | 10 | 1.00000 | 1.00000 | 0.00000 | 0.000000 |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:09 WEDNESDAY 27JUN07
 SHAPIRO-WILK TEST OF NORMALITY OF PRPMALE
 AQUATIC DAPHNID: FULL DATA

| OBS | STD | SKEW | KURT | SW_STAT | P_VALUE | SIGNIF |
|-----|----------|---------|---------|---------|---------|--------|
| 66 | 0.086263 | 0.54123 | 5.85067 | 0.68785 | 0.0001 | ** |

 Outliers & Influential Observations
 PRPMALE FROM FULL DATA Unweighted
 AQUATIC DAPHNID: Concentrations in ug/L

| Obs | DAPHNID | dose | doseval | GROUP | OBSER | Pred | SE_PRED |
|-----|---------|------|---------|-------|---------|---------|----------|
| 1 | 1 | 4 | 220 | 4 | 0.58333 | 0.52699 | 0.028393 |
| 2 | 2 | 4 | 220 | 4 | 0.47368 | 0.52699 | 0.028393 |
| 3 | 3 | 4 | 220 | 4 | 0.52174 | 0.52699 | 0.028393 |
| 4 | 4 | 4 | 220 | 4 | 0.38776 | 0.52699 | 0.028393 |
| 5 | 5 | 4 | 220 | 4 | 0.52941 | 0.52699 | 0.028393 |
| 6 | 6 | 4 | 220 | 4 | 0.36000 | 0.52699 | 0.028393 |
| 7 | 7 | 4 | 220 | 4 | 0.58140 | 0.52699 | 0.028393 |
| 8 | 8 | 4 | 220 | 4 | 0.78125 | 0.52699 | 0.028393 |
| 9 | 9 | 4 | 220 | 4 | 0.66667 | 0.52699 | 0.028393 |
| 10 | 10 | 4 | 220 | 4 | 0.38462 | 0.52699 | 0.028393 |
| 11 | 1 | 5 | 670 | 5 | 0.80000 | 0.65092 | 0.028393 |
| 12 | 2 | 5 | 670 | 5 | 1.00000 | 0.65092 | 0.028393 |
| 13 | 3 | 5 | 670 | 5 | 0.40000 | 0.65092 | 0.028393 |
| 14 | 4 | 5 | 670 | 5 | 0.55556 | 0.65092 | 0.028393 |
| 15 | 5 | 5 | 670 | 5 | 0.73684 | 0.65092 | 0.028393 |
| 16 | 6 | 5 | 670 | 5 | 0.75000 | 0.65092 | 0.028393 |
| 17 | 7 | 5 | 670 | 5 | 0.69565 | 0.65092 | 0.028393 |
| 18 | 8 | 5 | 670 | 5 | 0.68421 | 0.65092 | 0.028393 |
| 19 | 9 | 5 | 670 | 5 | 0.42857 | 0.65092 | 0.028393 |
| 20 | 10 | 5 | 670 | 5 | 0.45833 | 0.65092 | 0.028393 |

| Obs | L95M | U95M | Resid | LB | UB |
|-----|---------|---------|----------|-------------|------------|
| 1 | 0.47019 | 0.58378 | 0.05635 | -1.6653E-16 | 2.7756E-16 |
| 2 | 0.47019 | 0.58378 | -0.05330 | -1.6653E-16 | 2.7756E-16 |
| 3 | 0.47019 | 0.58378 | -0.00525 | -1.6653E-16 | 2.7756E-16 |
| 4 | 0.47019 | 0.58378 | -0.13923 | -1.6653E-16 | 2.7756E-16 |
| 5 | 0.47019 | 0.58378 | 0.00243 | -1.6653E-16 | 2.7756E-16 |
| 6 | 0.47019 | 0.58378 | -0.16699 | -1.6653E-16 | 2.7756E-16 |
| 7 | 0.47019 | 0.58378 | 0.05441 | -1.6653E-16 | 2.7756E-16 |
| 8 | 0.47019 | 0.58378 | 0.25426 | -1.6653E-16 | 2.7756E-16 |
| 9 | 0.47019 | 0.58378 | 0.13968 | -1.6653E-16 | 2.7756E-16 |
| 10 | 0.47019 | 0.58378 | -0.14237 | -1.6653E-16 | 2.7756E-16 |
| 11 | 0.59412 | 0.70771 | 0.14908 | -1.6653E-16 | 2.7756E-16 |
| 12 | 0.59412 | 0.70771 | 0.34908 | -1.6653E-16 | 2.7756E-16 |
| 13 | 0.59412 | 0.70771 | -0.25092 | -1.6653E-16 | 2.7756E-16 |
| 14 | 0.59412 | 0.70771 | -0.09536 | -1.6653E-16 | 2.7756E-16 |
| 15 | 0.59412 | 0.70771 | 0.08593 | -1.6653E-16 | 2.7756E-16 |
| 16 | 0.59412 | 0.70771 | 0.09908 | -1.6653E-16 | 2.7756E-16 |
| 17 | 0.59412 | 0.70771 | 0.04474 | -1.6653E-16 | 2.7756E-16 |
| 18 | 0.59412 | 0.70771 | 0.03329 | -1.6653E-16 | 2.7756E-16 |
| 19 | 0.59412 | 0.70771 | -0.22235 | -1.6653E-16 | 2.7756E-16 |
| 20 | 0.59412 | 0.70771 | -0.19258 | -1.6653E-16 | 2.7756E-16 |

NOTE

This set of outliers will be excluded from all outlier omitted analyses for this response. In order for the outliers from the transformed analysis to be used for outlier omitted analysis instead, run the analysis again and either specify the transform (instead of allowing the system to decide) or manually omit these observations using the exclusion functionality.

NOTE

The data was NOT normally distributed.
A nonparametric analysis will be performed.

STATISTICAL ANALYSIS LIST FILE
REPORT CREATED ON 19:09 WEDNESDAY 27JUN07
Kruskal-Wallis Test on PRPMALE
LAB C DAPHNID MEASUREMENTS FOR FULL DATA

The NPAR1WAY Procedure
Wilcoxon Scores (Rank Sums) for Variable RESPONSE
Classified by Variable dose

| dose | N | Sum of Scores | Expected Under H0 | Std Dev Under H0 | Mean Score |
|------|----|---------------|-------------------|------------------|------------|
| 1 | 18 | 333.00 | 603.00 | 63.398837 | 18.50 |
| 2 | 9 | 166.50 | 301.50 | 48.852085 | 18.50 |
| 3 | 9 | 166.50 | 301.50 | 48.852085 | 18.50 |
| 4 | 10 | 444.00 | 335.00 | 51.040913 | 44.40 |
| 5 | 10 | 491.00 | 335.00 | 51.040913 | 49.10 |
| 6 | 10 | 610.00 | 335.00 | 51.040913 | 61.00 |

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square 62.8071
 DF 5
 Pr > Chi-Square <.0001

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:09 WEDNESDAY 27JUN07
 Modified Dunn's Multiple Comparisons (Increasing) on PRPMALE
 LAB C DAPHNID MEASUREMENTS FOR FULL DATA USING ALPHA=0.05

| dose | doseval | COUNT | N0 | MRANK | ABS_DIFF | CRIT_05 | CRIT_01 | SIGNIF | p_val |
|------|---------|-------|----|-------|----------|---------|---------|--------|--------|
| 1 | 0 | 18 | 18 | 18.5 | 0.0 | 13.5878 | 16.8109 | . | |
| 2 | 25 | 9 | 18 | 18.5 | 0.0 | 16.6416 | 20.5890 | | 1.0000 |
| 3 | 74 | 9 | 18 | 18.5 | 0.0 | 16.6416 | 20.5890 | | 1.0000 |
| 4 | 220 | 10 | 18 | 44.4 | 25.9 | 16.0773 | 19.8909 | ** | 0.0004 |
| 5 | 670 | 10 | 18 | 49.1 | 30.6 | 16.0773 | 19.8909 | ** | 0.0001 |
| 6 | 2000 | 10 | 18 | 61.0 | 42.5 | 16.0773 | 19.8909 | ** | 0.0001 |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:09 WEDNESDAY 27JUN07
 MONOTONICITY CHECK OF PRPMALE - FULL DATA
 DOSES 0, 25, 74, 220, 670, 2000 ug/L
 LAB C DAPHNID OF AQUATIC DAPHNID

| PARM | p_t | SIGNIF |
|------------|--------|--------|
| DOSE TREND | 0.0001 | ** |
| DOSE QUAD | 0.0001 | ** |

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:09 WEDNESDAY 27JUN07
 ANALYSIS OF PRPMALE USING ALPHA=0.05
 FULL DATA

----- JONCKHEERE-TERPSTRA TEST KEY -----
JT IS JONCKHEERE STATISTIC
ZJT IS STANDARDIZED JONCKHEERE STATISTIC
PRJT IS P-VALUE FOR UPWARD TREND
PLJT IS P-VALUE FOR DOWNWARD TREND
 P-VALUES ARE FOR TIE-CORRECTED TEST WITH CONTINUITY CORRECTION FACTOR
 SIGNIF RESULTS ARE FOR Increasing ALTERNATIVE HYPOTHESIS

Check for ties in PRPMALE

Percent of all data tied at 3 most frequently observed values
 Since 55 percent of observations have the same value
 Exact methods (StatXact) are recommended

| COUNT | SUMWTS | NMISS | NOBS | RESPONSE | TIES | TIEPCT |
|-------|--------|-------|------|----------|------|--------|
| 36 | 66 | 2 | 68 | 0.00 | 36 | 55 |
| 11 | 66 | 2 | 68 | 1.00 | 47 | 71 |
| 1 | 66 | 2 | 68 | 0.36 | 48 | 73 |

Check for Number of Reps in PRPMALE Thru 2000 ug/L

Maximum Number of Reps in Any Treatment Group or Control is 18
 Total Number of Reps in All Treatment Groups is 66
 Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 5 Doses thru 2000 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 1386 | 8.1212836 | . | 0.0001 | ** | 6 |

Check for Number of Reps in PRPMALE Thru 670 ug/L

Maximum Number of Reps in Any Treatment Group or Control is 18
 Total Number of Reps in All Treatment Groups is 56
 Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 4 Doses thru 670 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 836 | 6.4776517 | . | 0.0001 | ** | 5 |

Check for Number of Reps in PRPMALE Thru 220 ug/L

Maximum Number of Reps in Any Treatment Group or Control is 18
 Total Number of Reps in All Treatment Groups is 46
 Monte Carlo Exact Permutation Methods Used

Jonckheere Trend Test on Dose 0 + Lowest 3 Doses thru 220 ug/L

| ___JT___ | Z_JT | XPL_JT | XPR_JT | SIGNIF | DOSE |
|----------|-----------|--------|--------|--------|------|
| 382.5 | 4.9592565 | . | 0.0001 | ** | 4 |

NOTE

Remaining data is constant.
 No further tests are possible.

NOTE

Jonckheere test results are included in the summary table.
 Group means should be examined to check for lack-of-fit

=====

AUTO TRANSFORM FULL DATA

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:09 WEDNESDAY 27JUN07
 SHAPIRO-WILK TEST OF NORMALITY OF SQRT(PRPMAL)

AQUATIC DAPHNID: FULL DATA

| OBS | STD | SKEW | KURT | SW_STAT | P_VALUE | SIGNIF |
|-----|----------|---------|---------|---------|---------|--------|
| 66 | 0.055563 | 0.14785 | 4.62832 | 0.70374 | 0.0001 | ** |

NOTE

No auto-transform fixes data non-normality.
 Returning to untransformed response.

=====

AUTO TRANSFORM FULL DATA

STATISTICAL ANALYSIS LIST FILE
 REPORT CREATED ON 19:08 WEDNESDAY 27JUN07
 SHAPIRO-WILK TEST OF NORMALITY OF SQRT(PRPMAL)

AQUATIC DAPHID: FULL DATA

| OBS | STD | SKEW | KURT | SW_STAT | P_VALUE | SIGNIF |
|-----|----------|----------|---------|---------|---------|--------|
| 69 | 0.040514 | -1.69177 | 11.2168 | 0.58680 | 0.0001 | ** |

NOTE

No auto-transform fixes data non-normality.
 Returning to untransformed response.

ANNEX 2**Statistical Issues in Daphnia Reproduction Experiments****John W. Green, Ph.D., Ph.D., DuPont****Timothy A. Springer, Ph.D., Wildlife International**

This paper addresses the following topics: (1) The use of a significant ANOVA F-test or non-parametric Kruskal-Wallis test as a “gatekeeper” requirement before doing multiple comparisons of treatment groups to control in a toxicity experiment; (2) the appropriateness of analysis of proportion responses (fecundity, sex ratio, parent survival) by parametric tests (Dunnnett, Williams), non-parametric tests (Jonckheere-Terpstra, Mann-Whitney, Dunn), quantal models (Cochran-Armitage and Rao-Scott Cochran-Armitage tests, and logistic regression); (3) the effect of loss of replication (e.g., through parent mortality or reduced fecundity) on the above named tests; (4) the power properties of the above named tests; (5) the utility of regression analysis (EC_x) for sex ratio; (6) a proposed statistical protocol for reproductive endpoints.

If an adequate database is made available, then a follow-up paper will deal with (1) repeated measures analysis of fecundity and sex ratio to take time into account; (2) joint probabilistic assessment of sex ratio and fecundity.

Previously reported investigations have demonstrated that it is advantageous to express sex ratio as the percentage of males in the offspring population. Thus, this definition of sex ratio is used throughout the following discussion.

Summary of Findings

The initially proposed experimental design consists of five concentrations with 10 replicates (chambers containing a single parthenogenetically reproducing female) at each concentration. A smaller experimental design with fewer replicates per test concentration would be sufficient for NOEC determination. If the initially proposed simulated design is retained, then regression analysis to estimate EC₅₀, EC₂₅, or EC₁₀ values for daphnia sex ratio would provide more useful information than would the NOEC determination

Power comparisons were made of seven different statistical tests that could be used for NOEC determination. The rarity of male offspring in controls causes the statistical tests to be very sensitive to extremely small changes in percent males, so much so as to make interpretation of the results problematic. The quality of EC₁₀, EC₂₅ and EC₅₀ estimates from probit analysis was examined in detail and found satisfactory. It is a biologically based decision which value of x in EC_x is most appropriate for this screening experiment.

It would be important to validate the simulations against real laboratory data. The dose-response shapes used in the simulations are based on previous ecotoxicological responses and the plots given in Tatarazako *et al* (2003). Laboratory data from the labs that conducted the daphnia experiments for the VMG-eco might have different shapes that would affect the conclusions in this report.

Effect of multiple observation of the same sex ratio

The expected control incidence of percent males among daphnia neonates is zero, and in the three dose-response scenarios investigated, the same zero incidence is expected one or more test concentrations. This means that with 10 replicates per concentration, there will be numerous replicate percent male values of 0. This has three major effects on statistical analysis. First, the assumption of normality required by the Dunnett and Williams tests cannot be satisfied regardless of transformation used. One implication is that these tests are not valid for Daphnia sex ratio. A second major effect is that the estimated variance from these data will be very small by virtue of pooling zero between-rep variance from the control and some low test concentrations. An implication of this is that all of the tests will be very sensitive to small changes in percent males, so much so as to make interpretation of the results problematic. The simulations discussed below verify the very high power properties of these tests. It should be observed that the same problems arise if there are numerous replicate proportions male tied at some other value, such as 0.5. It is the ties, not the zeros, which contribute most to the problem.

A third major effect is that much smaller experimental designs can be used to determine a NOEC for Daphnia sex ratio. Since the present design is used to allow analysis of other biological endpoints, such as fecundity, a protocol that randomly selects a smaller number of replicates in each concentration for sex ratio NOEC determination may prove adequate. Such a subsample would still have the problem of many zero replicate proportions. It may be that calculation of a useful NOEC is not viable for Daphnia sex ratio.

The expected rarity of males among control offspring simplifies calculations in the regression analysis, as the control percentage of males in this group essentially can be ignored. Thus, in the daphnia reproduction study, the EC50 for sex ratio is the concentration that results in 50 percent males, not a 50 percent *change* in the percentage of males relative to the control. The above considerations suggest that for when sex ratio is expressed as the percentage of male offspring, it is advantageous to fit responses to a regression model from which an ECx is estimated. Numerous ties (zeros and other low counts) among the replicates in treatment groups can also cause problems with regression models, but the effects need not be as severe as they are for NOEC determinations. It is likely that because of the high frequency of ties, the confidence intervals for ECx estimates will be unrealistically tight. The simulations are based on known values of ECx, x=10, 25, 50, so the quality of the regression estimates is readily obtained. Green (2008) reported on such estimates in the context of emergence and survival of non-target plants. The results from that work do not carry over entirely to Daphnia sex ratio because of the multiple zero replicate proportions discussed above, which is not as severe a problem for the stated non-target plant responses.

Use of “Gatekeeper” Tests Preceding Multiple Comparisons in Toxicology

In many basic statistics courses on analysis of variance (ANOVA), one performs an overall assessment of differences among treatment levels using the F-test. If that test is not significant, then no further exploration of treatments is done. If the F-test is significant, then one moves on to the question of which treatments differ. In this second stage of analysis, there are distinct schools of thought on how to proceed as well as numerous different statistical tests that could be used. One school of thought is to use so-called protected t-tests. The idea is that the F-test provides protection against false positives at the 5% level, so given that differences were found to exist, additional pairwise comparisons of treatment effects can be done without adjustment for the

number of comparisons made. As Hsu (1996) (among many references) indicates, this procedure increases the false positive rate among the pairwise comparisons. Another school of thought is to use some sort of adjustment for multiple comparisons, such as the simple Tukey honest significant difference (HSD). There are many tests that could be used.

One reason that a multiplicity adjustment for pairwise differences should be considered comes from an understanding of the F-test. The F-test is equivalent to testing simultaneously the hypothesis that no contrast of treatment means is different from zero (Scheffe (1953)). A contrast has the form

$$c_1\mu_1 + c_2\mu_2 + c_3\mu_3 + \dots + c_k\mu_k$$

where

μ_i , $i=1, 2, 3, \dots, k$ are the true treatment means and
 $c_1+c_2+c_3+\dots+c_k=0$.

Thus, a significant F-test may arise, for example, because of a significant difference between a low treatment group and the average of the two high treatment groups and yet it is possible that no pairwise difference ($\mu_i - \mu_j$) will be significant. It therefore makes sense to make some adjustment in the p-values for the number of pairwise comparisons being made after a significant F-test is observed.

However, it is equally important to consider the multiple comparison issue from the opposite perspective in the context of toxicology. The F-test provides simultaneous protection against any false positive among all contrasts tested (a very large number), not just the one in question. What this indicates is that if only a select few comparisons of treatment means is of interest, then using a significant F-test as a “gatekeeper” before making the comparisons of interest can lead to a higher false negative rate. Because the F-test protects against false positives among all possible contrasts, it is overly protective when only a few contrasts are of interest.

In toxicology, the only comparisons of interest are comparisons of treatments to the control, i.e., contrasts of the form $\mu_i - \mu_1$, $i=2, 3, 4, \dots, k$. So, rather than begin with the overly protective F-test, one uses a procedure focused on the comparisons of interest. Dunnett’s and Williams’ tests are designed for this situation and make all adjustments needed. Their use is independent of the significance of the F-test. It is possible for the F-test not to be significant and Dunnett or Williams to find significant effects and it is possible for the F-test to be significant and neither Dunnett nor Williams find any significant effect. These are possibilities because the F-test and the Dunnett and Williams tests are not testing the same hypotheses.

Hsu (1996) discusses some of the above on pp 177ff. Dunnett and Tamhane (1992) discuss step-up tests and include general discussion of the issues described above and related matters. It is evident after careful reading of these papers that no gatekeeper test is required. Indeed, many of the papers on multiple comparisons assume the reader is familiar with this non-requirement. For example, there is no explicit comment on the F-test in Tamhane, *et. al* (2001), but as a co-author of the paper, I am certain that no protective F-test was assumed, and it is clear from the paper that no previous F-test (or any other test) for overall treatment effect is made. The references in the previous two citations are also of interest. In particular, the papers on step-down procedures are important. These are the basis for the step-down Jonckheere-Terpstra and Cochran-Armitage tests. Dunnett and Tamhane (1991) give a version of Dunnett’s test for the heterogeneous

variance problem. This is what I call the Tamhane-Dunnett test. Dunnett (1955) gives the original Dunnett test and no mention is made of using a previous F-test. Again, it may require a careful reading to be clear that no such gatekeeper test is involved.

Westfall *et al* (1999) contains extensive discussion of multiple comparisons. Section 3.4, p53, discusses comparisons with a common control. As with most articles, it does not explicitly say no previous F-test is required, but it does talk about the increase in power from using a procedure focused on comparison to a control rather than a general procedure for all comparisons.

Selwyn (1988) discusses step-down trend tests used in toxicology. He explicitly discusses the step-down Cochran-Armitage test and gives a detailed flow chart that does not involve a previous significant chi-square test or any other omnibus test.

Salsburg (1986) discusses on pp85ff the power advantage of using a focused trend test instead of a general ANOVA test (i.e., F-test). He advocates Bartholomew's test for trend and Dunnett's test when a trend test is not appropriate. He does explicitly state the inappropriateness of using the F-test as a gatekeeper (though he does not use that term). I do not recommend Bartholomew's test because of the great complexity of the mathematics, some unresolved issues regarding its application to studies with more than four treatment groups, and the lack of readily available software.

An excellent reference on multiple comparisons is Hochberg and Tamhane (1987). The first short chapter talks about the short comings of the F-test. It may not be clear to a non-statistician that all subsequent development is done without regard to any gatekeeper test such as the F-test, but such is the case. It is implied in this chapter, but, as stated, may not be obvious on casual reading.

It should be emphasized that all of these sources advocate a need to adjust the significance levels for the number of comparisons. There are numerous ways to do that. There is no justification in toxicology for simply doing numerous single comparisons of treatments, all at the 5% false positive rate, ignoring the other comparisons. The references provided are among many on this subject, but perhaps these will suffice to make the point that no F-test, Kruskal-Wallis test, chi-squared test, or any other gatekeeper test is needed before doing the recommended multiple comparisons in toxicology experiments. This comment should not be taken to mean that tests for normality, variance homogeneity, and dose-response monotonicity are not needed. Those are entirely different issues to help decide which multiple test is appropriate.

Unit of analysis in aquatic toxicology experiments

Previous reports have addressed the issue of what is the appropriate unit of analysis in an aquatic toxicology experiment. It was argued that the replicate test vessel is the appropriate unit of analysis, not the individual subject. In daphnia experiments, each test vessel corresponds to a single, parthenogenetically reproducing, female. The neonates for that vessel thus all come from the same parent. In addition to the usual concern that the parent and offspring in the same test vessel are correlated in a different way from parents and offspring in a different test vessel, there is no way that offspring from the same parent can be considered independent experimental units. It has been suggested that this lack of independence can be accommodated by using logistic regression. The idea behind the logistic regression approach is to adjust for varying mean proportion males among reps in the same test concentration by including a rep "effect" in the

model. This is an interesting idea, but it is ultimately based on the individual neonates being independent experimental units within reps. This is biologically objectionable and exaggerates the degree of freedom. The test vessel is the unit of analysis and it is appropriate to analyze the proportion male or the number of neonates from that vessel.

Certainly it can be argued that a repeated measures analysis should be done to take into account different patterns of reproduction or presence of males over time. A simple approximation to this would be to analyze these endpoints at different time points over the course of the experiment. That is, analyze cumulative reproduction and number of males within each of several time intervals. The remainder of this paper does not consider the time element. It is assumed that the cumulative response is analyzed within an appropriate time interval.

Multiple comparison tests for sex ratio (expressed as percent males) and fecundity

Analysis of fecundity is based on comparisons of the number of neonates per replicate. Experience suggests that these numbers will be sufficiently variable to allow use of the Dunnett and Williams tests after a suitable transform. A square-root transform on such count data is the standard normalizing transform to investigate.

In *Daphnia* studies, the proportion of males in the control and one or more low concentrations is usually zero or close to zero in every rep. This means there will be little or no variation among rep means, so that regardless of transform used, the normality requirement of the Dunnett and Williams' tests will not be satisfied. This would rule out those tests for analysis of sex ratio data. Thus alternative tests must be used to determine a NOEC for sex ratio. Appropriate alternative tests considered here are the Mann-Whitney and Dunn tests with Bonferroni-Holm adjusted p-values, the Jonckheere-Terpstra test, and the Cochran-Armitage tests (with or without the Rao-Scott adjustments). All of these tests are applied to replicate proportions, not individual neonates. Each of these tests is explored below.

A closely related question is whether all neonates should be sexed or only a random fixed-size sample from each replicate. The labor-intensive nature of sexing neonates may suggest the latter action. In that event, consideration must be given to how large a sample is required. Simulations were done for 10, 25, and 100 neonates per replicate vessel. This would yield 100, 250, and 1000 neonates per concentration to be sexed and analyzed statistically. Table 1 below shows the 95% confidence bounds for the sample proportion males possible from each of the three indicated sampling schemes. The width of the confidence interval varies as the true proportion varies, with the maximum width occurring when males constitute 50% of the population in a test concentration.

Table 1: Half-width of 95% confidence interval for percent males

| Repsize | True % | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 |
|---------|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 10 | | 6 | 8 | 9.2 | 9.8 | 10 | 9.8 | 9.2 | 8 | 6 |
| 25 | | 3.8 | 5.1 | 5.8 | 6.2 | 6.3 | 6.2 | 5.8 | 5.1 | 3.8 |
| 100 | | 1.9 | 2.5 | 2.9 | 3.1 | 3.2 | 3.1 | 2.9 | 2.5 | 1.9 |

For example, with 10 neonates sexed per rep, if the true percent males is 30%, then the 95% confidence bounds for the sample percent is 30 +/- 9.2.

Trend tests such as Williams, Jonckheere-Terpstra, and Cochran-Armitage gain additional accuracy because they use the entire dose-response shape in determining the NOEC. The Dunnett, Dunn, and Mann-Whitney tests use only the percents in each test concentration, so Table 1 is more indicative of the variability in sample percent males.

Of the tests considered, only the Cochran-Armitage test takes the number of neonates per rep directly into account and only with the Rao-Scott adjustment is between-rep variance taken into account. The Rao-Scott adjusted Cochran-Armitage test is thus biologically satisfying on this matter. It should be recognized that the usual justification for this test is based on the binomial distribution, which in turn assumes independence of subjects within each rep. This is the same objection voiced above regarding the logistic regression approach. However, in fact both versions of the Cochran-Armitage test rely only on the proportion of males in each replicate and require only independence of the reps, which is not in dispute. The advantage of the Rao-Scott adjusted version of the Cochran-Armitage test is the way it captures between-rep variance as well as varying sample sizes. This is a good test, but other tests should be not ruled out without further consideration. Such consideration is provided below

As indicated in the above discussion, each test has some theoretical difficulties. One of the uses of the power simulations will be to indicate how the tests perform.

Given that the rep is the unit of analysis, the fact that replicate sex ratios may not all be based on the same number of neonates does not mean those replicate sex ratios are not comparable. The effect of varying numbers of neonates per replicate is primarily on the among-rep variance. The power analyses presented below considered the effect of this extra-binomial variance. As will be evident from the power analyses, the Jonckheere test is a powerful option even when the number of neonates per rep varies.

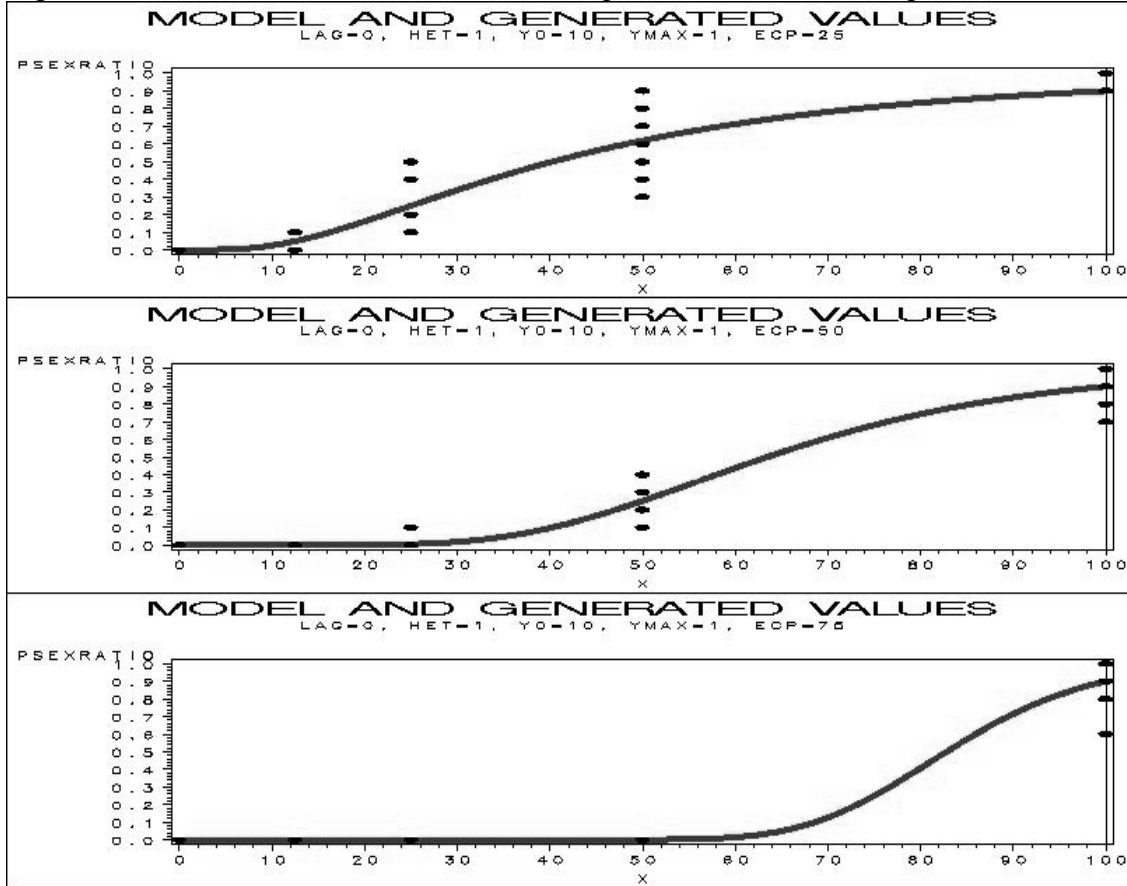
Power Simulations

The power characteristics of these tests depend on the shape of the dose-response curve, the number and spacing of test concentrations, the number of neonates sexed per rep, the amount, if any, of extra-binomial variance observed among the rep proportion males. Three sigmoid dose-response shapes were simulated, shown in figure 1, corresponding to a rapid rise in the percent males, a more gradual rise, or a steep rise following a near zero incidence at low concentrations.

These curves are templates for the simulations. What is shown are three base shapes following the commonly observed Bruce-Versteeg “probit-type” dose-response with a 99% effect (i.e., 99% male population) in the high test concentration and 0% males in the control. By sliding the high test concentration to the left and rescaling, the same shapes can be simulated with the effect at the high concentration varying between 99% and 1%. It can be shown that the Bruce-Versteeg curve is completely determined by specifying EC25, the effect at the high concentration, and the maximum concentration. In these simulations, the high concentration is standardized at 100, so specifying EC25 and the maximum effect suffices. ECPB specifies EC25 in the base curves as 25, 50, or 75.

The power plots following show the likelihood (i.e., probability) of finding a statistically significant effect at the highest test concentration or at each lower concentration. The horizontal axis is the percent effect (i.e., % males) in the indicated given test concentration

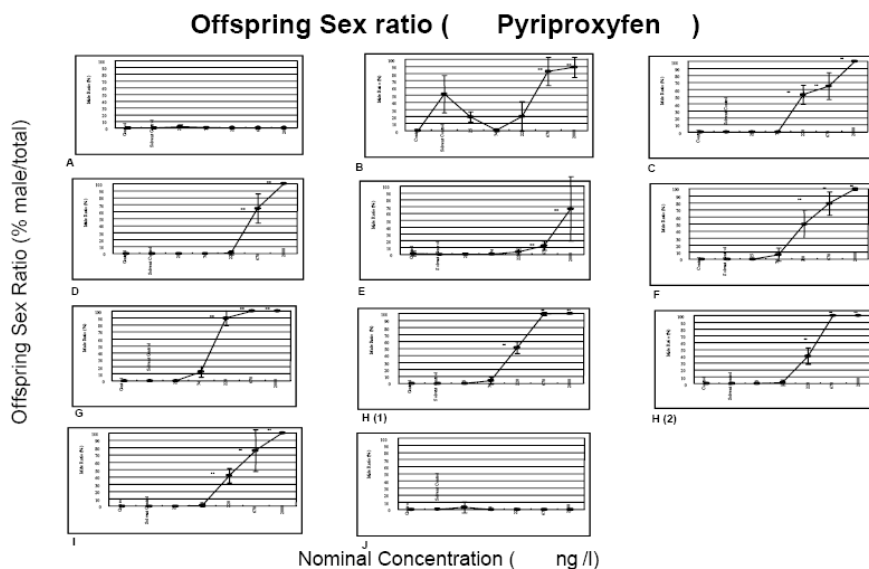
Figure 1: Dose-Response Shapes Simulated.



The red curve is the underlying dose-response shape. The black dots, some of which will be invisible in a black and white printout, are sample replicate proportions in one simulation. There are 10 replicates per concentration. It will be observed that there are not 10 dots at any concentration. This is because in addition to multiple ties at zero at low concentrations, there are also multiple ties at non-zero values in higher concentrations. All such ties contribute to the low sample variance and consequent high power of the tests.

The following figure, taken from Tatarazako *et al* (2003), is consistent with the bottom of the three simulated shapes in Figure 1 (labeled ECPB=75) or with the middle shape (labeled ECPB=50).

Figure 2: Offspring Sex Ratio from Tatarazako *et al* (2003)



1
2
3
4
5
6

Figure 4. Offspring sex ratio is presented for the results of pyriproxyfen by ten laboratories. Laboratories were named randomly with alphabet letters, which correspond to those in other figures. For the laboratory H, results of two tests are shown. Error bars indicate standard deviations. Asterisks mean statistically significant difference from solvent control (* $p < 0.05$, ** $p < 0.01$).

It has been suggested that the Jonckheere-Terpstra test is not sufficiently powerful for the analysis of sex ratio and that this test ignores the possibility of varying numbers of neonates across replicates, especially at higher test concentrations. As the power curves will demonstrate, the first concern is not correct. It is certainly true that the Jonckheere test does not take varying sample sizes into account. Since the replicate is the unit of analysis, the main impact of varying sample sizes is to increase the among-replicate variance. The impact on the Jonckheere test, among others, has been addressed in the power simulations as indicated in the following paragraph.

Simulations were done assuming alternatively 0% and 30% extra-binomial variance. The term “extra-binomial variance” is defined as follows. The variance of replicates drawn from a binomial distributed population has a fixed value depending on the probability of an effect (in this case, change of sex to male) and the number of animals exposed. If the variance exceeds this, factors other than random variability from sampling are contributing to the variance of the replicates. This additional variance is thus called extra-binomial variability. Extra binomial variance might arise from varying numbers of neonates in different reps or from other factors. Secondly, an average 30% random mortality among parents and an additional average 30% reduction among neonate production was simulated in the two highest test concentrations. Thus, the simulations included situations with (1) only binomial variance and no mortality, (2) 30% extra-binomial variance and no mortality, (3) only binomial variance but 30% parent mortality and 30% reduced fecundity in the highest two test concentrations, and (4) 30% extra-binomial variance and 30% parent mortality and 30% reduced fecundity in the highest two test concentrations. These power simulations were carried out for all seven of the tests explored in this report. Because of the

concerns raised above and time constraints, the power simulations did not include the logistic regression approach.

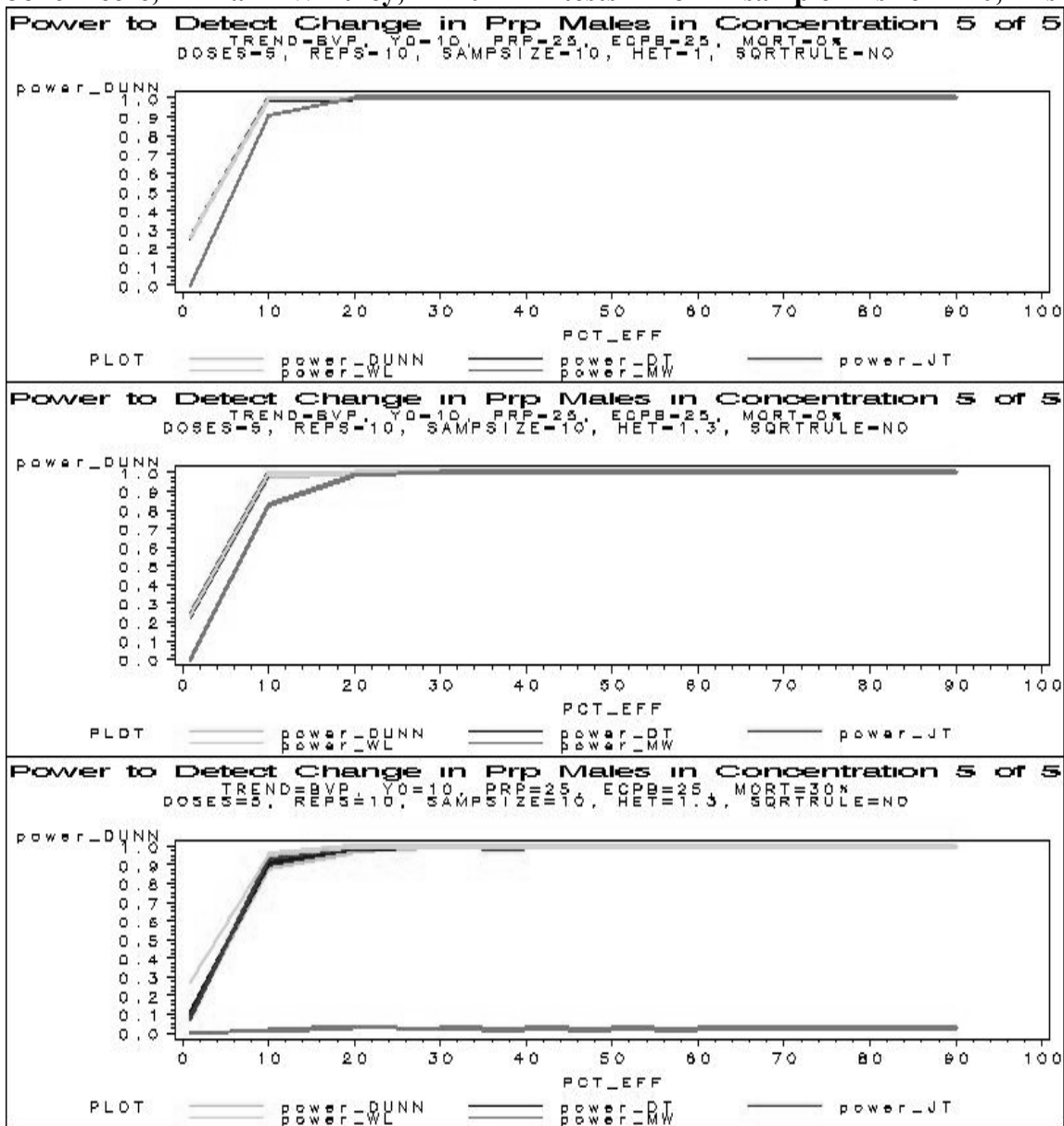
In the following figures, HET refers to the variance heterogeneity simulated, with HET=1 corresponding to no variance heterogeneity (i.e., 1 times the usual standard deviation) and HET=1.3 referring to a 30% increase (1.3 times the usual standard deviation). PRP=25 and ECPB are reference points used in constructing the dose-response curves and can be ignored. Y0 refers to the assumed control female count, so Y0=10 when the sample size is 10 means the controls are simulated with all females. The reader can ignore these terms other than HET and ECPB, with no loss of understanding.

Furthermore, simulations were restricted to five concentrations, 4 or 10 replicates per concentration, 10, 25, or 100 neonates per replicate. In those simulations where parental mortality or reduced neonate productivity were included, a 30% mortality and 30% reduction in neonate production were modeled. It should be understood that these 30% figures are averages. A random number generator was used so that in a given high test concentration, the actual parental mortality rate might be higher or lower. The same applies to simulated neonate production. Similarly, when 30% extra-binomial variance is simulated with or without varying the parental survival or neonate production rates, this 30% is an average and the effect on a given test concentration will vary. These approaches add reality to the simulations.

Power Properties for Dose-Response Shape 1 (ECPB=25)

Figure 3 shows the power for the Williams, Dunnett, Jonckheere, Mann-Whitney, and Dunn tests for the case of 10 neonates per rep, showing the results for the first dose-response shape described above. The top figure assumes no extra-binomial variance, the second assumes 30% extra-binomial variance, and the bottom figure assumes 30% extra-binomial variance, and both 30% parental mortality and 30% reduced neonate fecundity in the two highest test concentrations.

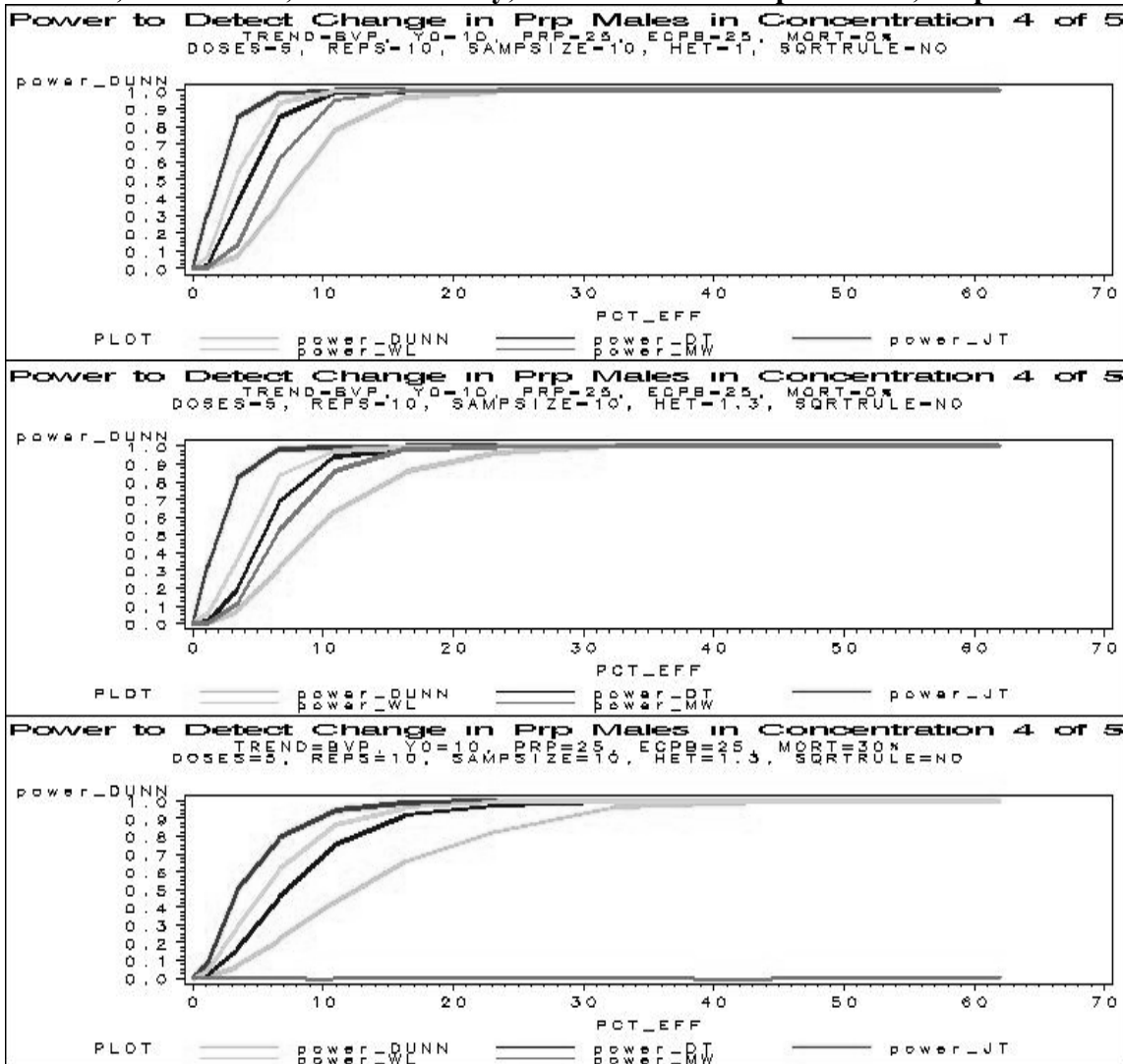
Figure 3: Power to detect an effect in the high test concentration from Williams, Dunnett, Jonckheere, Mann-Whitney, Dunn tests for sample size 10, shape 1



The graphs show the power to detect the indicated percentage of males. The simulations illustrated in the top panel assume no extra-binomial variance, the middle panel assumed 30% extra-binomial variance, and the bottom panel assumed 30% extra-binomial variance, and both 30% parental mortality and 30% reduced neonate fecundity in the two highest test concentrations. The vertical axis gives power as proportion.

Some plots coincide with the Williams plot and are thus not visible. All tests except Mann-Whitney have 80% power to detect an 8% increase in males at the high test concentration. The loss of power of the Mann-Whitney when there is parent mortality or reduced fecundity in the high test concentrations is notable.

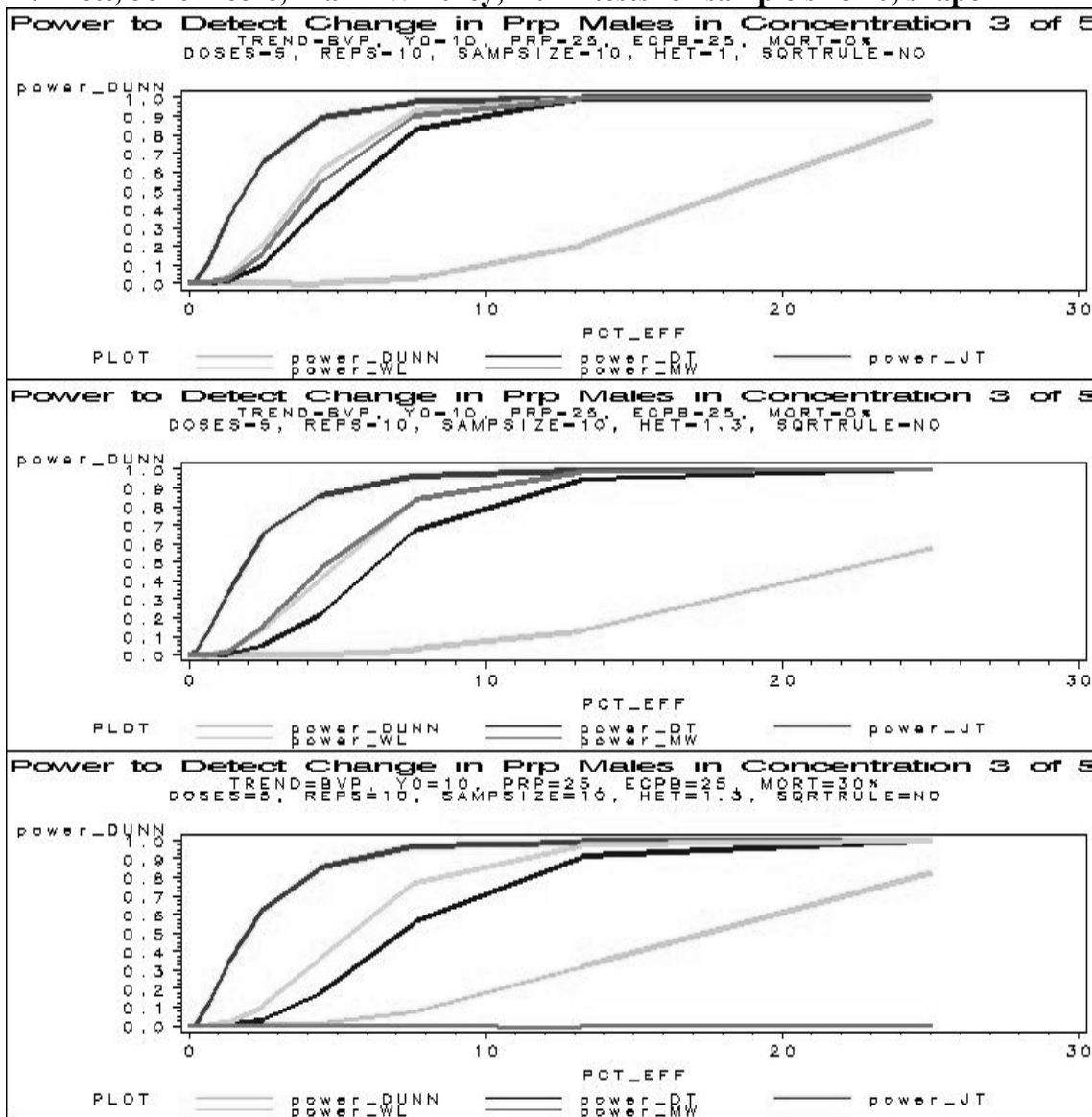
Figure 4: Power to detect an effect in the second highest test concentration from Williams, Dunnett, Jonckheere, Mann-Whitney, Dunn tests for sample size 10, shape 1



The power curves begin to separate at step 2 in the step-down process. (Of course, Dunnett, Dunn, and Mann-Whitney are not affected by the step-down process.) It will be seen that the Jonckheere test has 80% power to detect an increase to 4-7% in males, whereas Dunnett's test has 80% power to detect an increase of 8-12% in males and Dunn's has power 80% to detect an increase of 11-24%. Mann-Whitney power is slightly lower than Dunnett power except in the presence of parental mortality or reduced fecundity in the high test concentrations, where the power is nearly zero.

It will be observed that the power plots for step 1 are rather crude due to simulated increments. It is not possible for the power to detect an effect in step 2 to be higher than the power to detect the same size effect in step 1

Figure 5: Power to detect an effect in the third highest test concentration from Williams, Dunnnett, Jonckheere, Mann-Whitney, Dunn tests for sample size 10, shape 1



The differences among the power curves is sharper still in step 3 of the step-down process, with the Dunn test power markedly less powerful than Jonckheere, Williams, Dunnnett, and in the top two variance scenarios, the Mann-Whitney. Note that the range of the horizontal axis is not the same as in the previous plots.

There is little power to detect an effect in step 4, as the effect simulated there is quite small. Consequently, plots for steps 4 and beyond are not shown.

Figures 6-8 shows the power of the two Cochran-Armitage tests to detect an effect at the high test concentration for the first simulated shape (ECPB=25) and same three variance scenarios described for Figures 3-5. Figure 6 shows the power to detect an effect in the high concentration, while Figures 7 and 8 deal with effects in the next two highest concentrations. Here Power_RS (green) and Power_CA (magenta) refer to the power for the Rao-Scott and standard versions, respectively, of the Cochran-Armitage test.

These power properties are consistent with those for the Jonckheere test given in Figures 3-5. As before, it will be observed that there is little difference between a general 30% extra-binomial variance and that plus 30% parental mortality and reduced neonate fecundity in the two highest test concentrations. In all three scenarios, there is 80% power to detect an increase of 4-6% in proportion males using the Rao-Scott adjusted Cochran-Armitage test.

Figure 6: Power to detect an effect in the high concentration with the standard and Rao-Scott adjusted Cochran-Armitage tests for sample size 10, shape 1

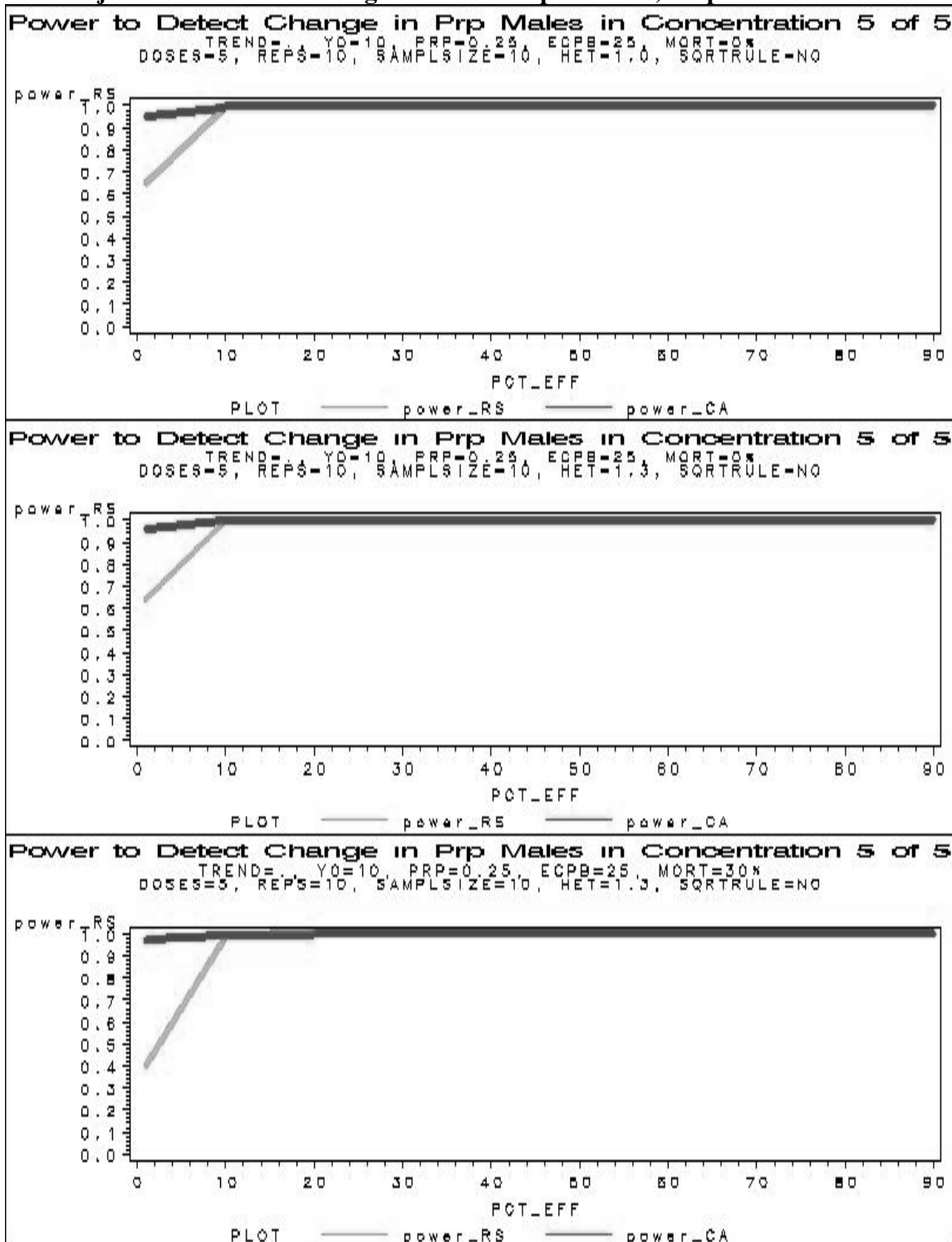


Figure 7: Power to detect an effect in the second highest concentration with the standard & Rao-Scott adjusted Cochran-Armitage tests for sample size 10, shape 1

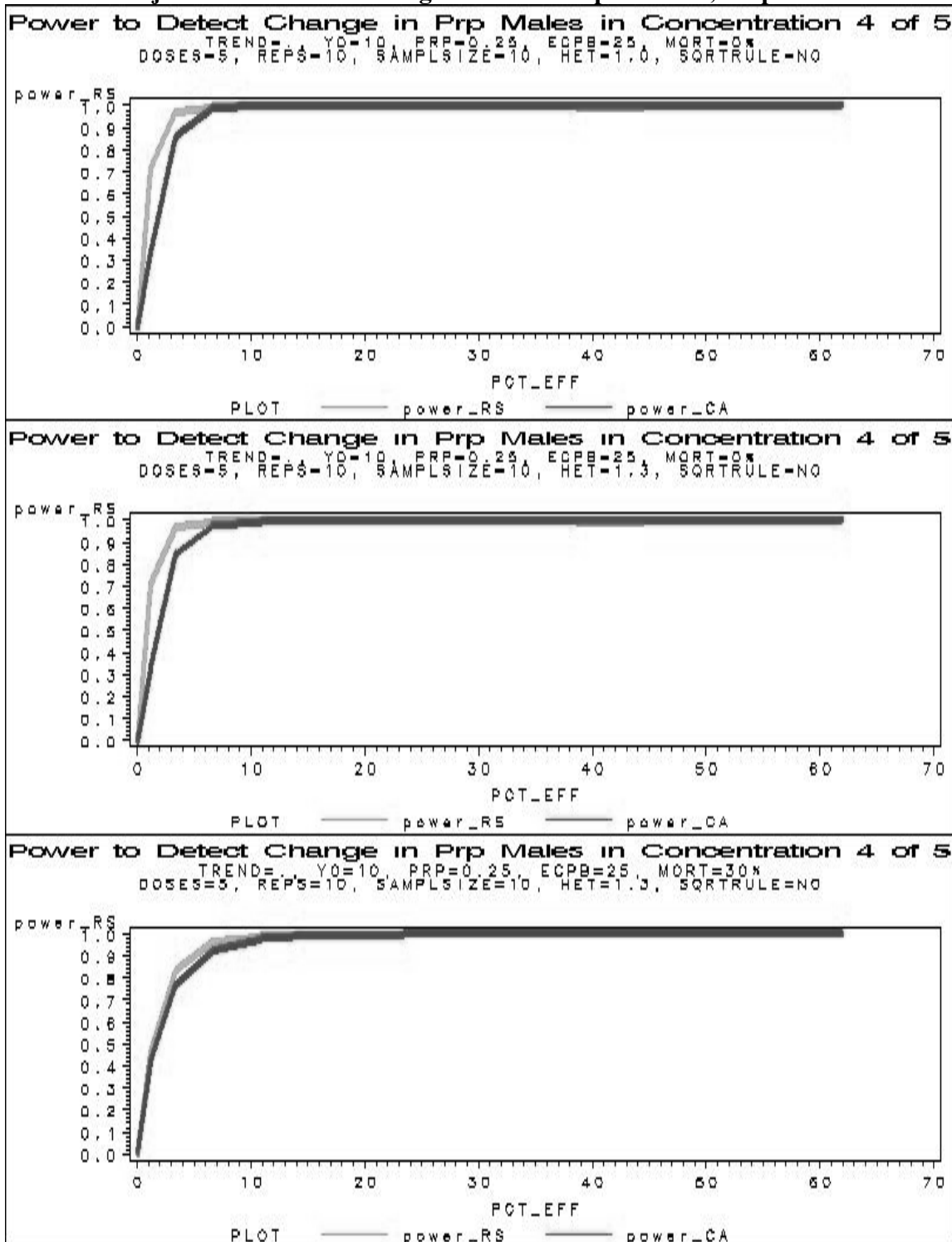


Figure 7: Power to detect an effect in the third highest concentration with the standard & Rao-Scott adjusted Cochran-Armitage tests for sample size 10, shape 1

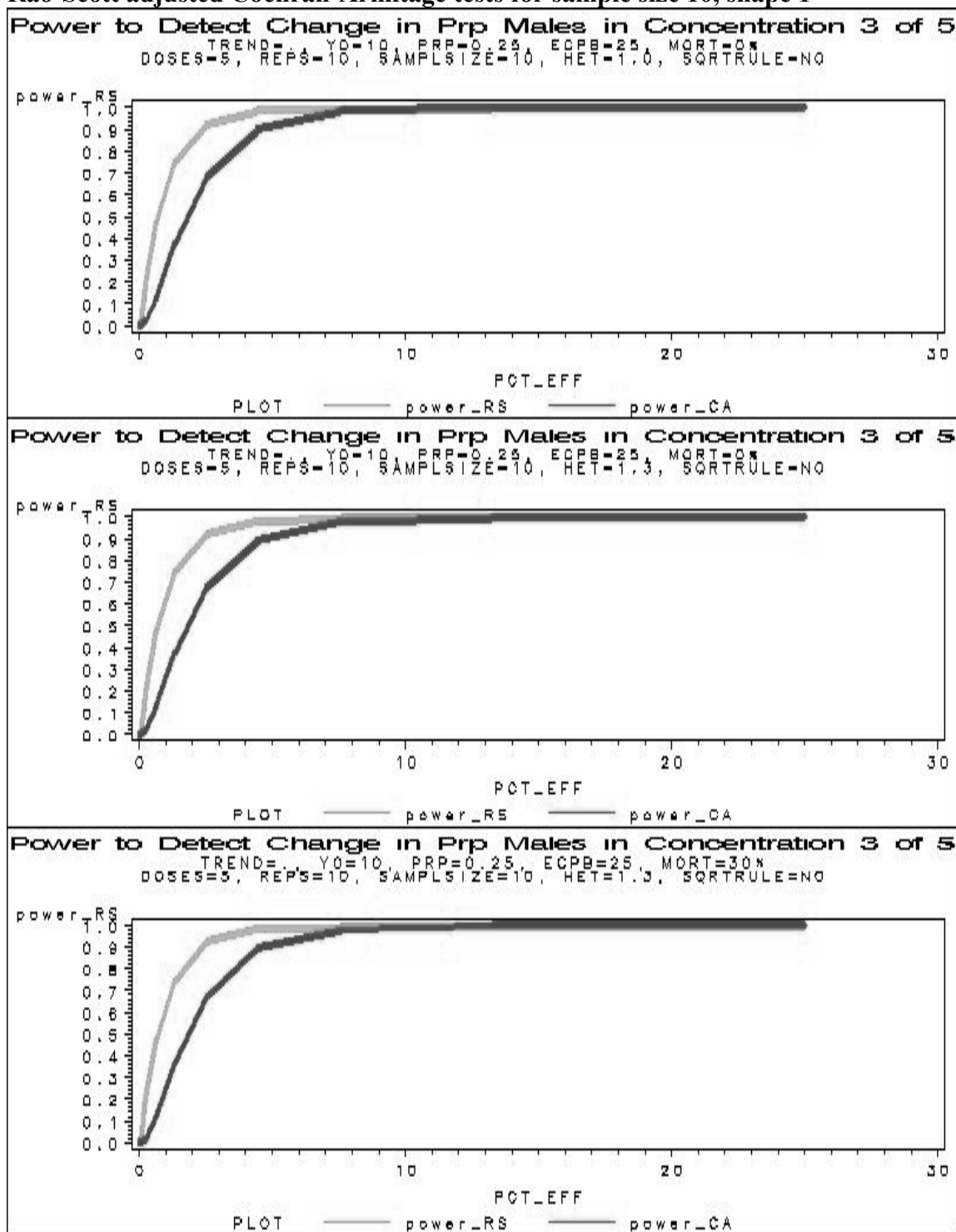
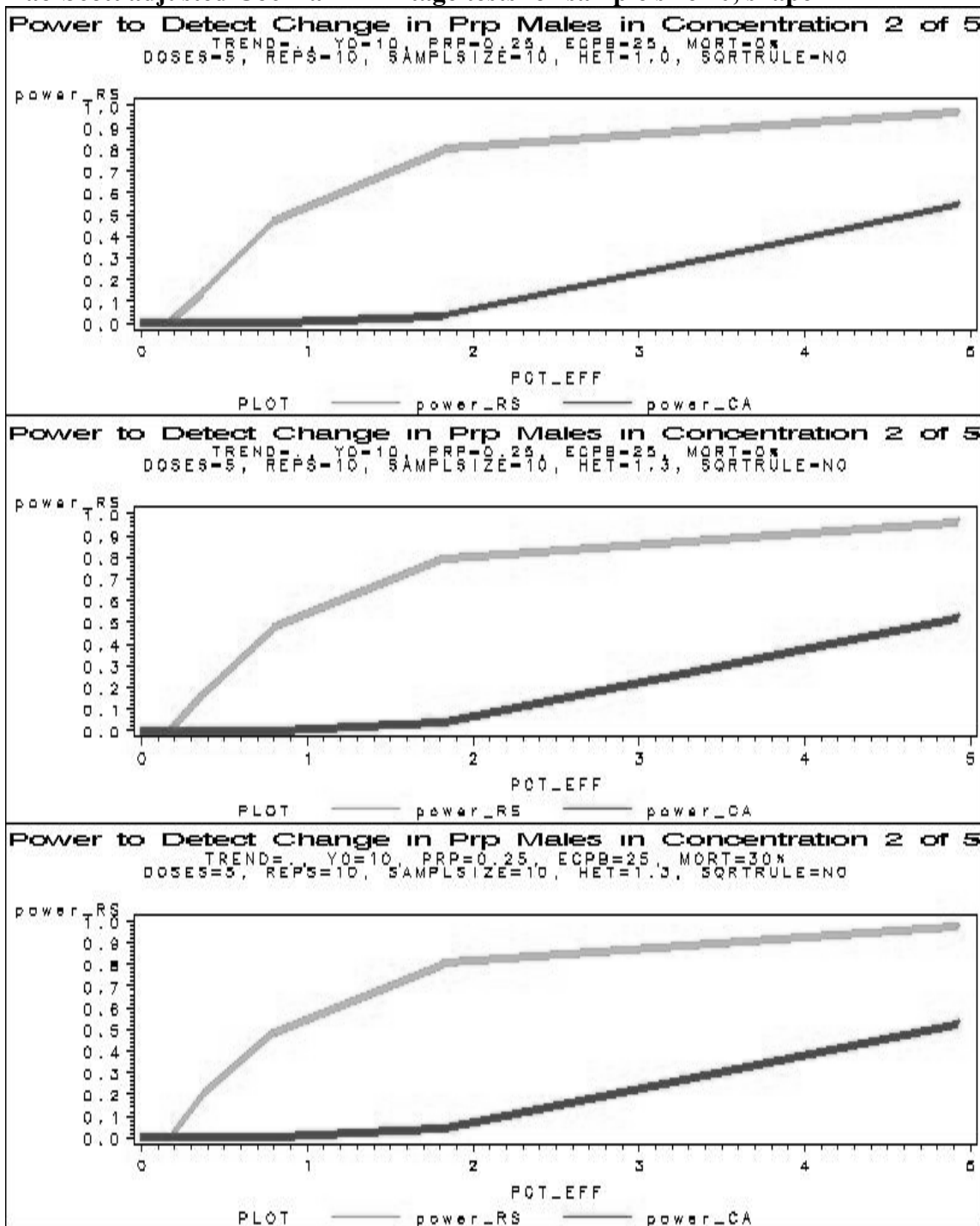


Figure 8: Power to detect an effect in the fourth highest concentration with the standard & Rao-Scott adjusted Cochran-Armitage tests for sample size 10, shape 1



The Rao-Scott adjusted Cochran-Armitage test is so sensitive that interpreting the statistical tests could be problematic for the dose response shape 1. However, shape 1 (ECPB=25) appears unlikely based on the results in Tatarazako *et al* (2003).

Power Properties for Dose-Response Shape 2 (ECPB=50)

Figures 9 and 10 show the power to find significant an effect in the highest two test concentrations, respectively, for the Williams, Dunnett, Jonckheere, Mann-Whitney, and Dunn tests for the case of 10 neonates per rep, showing the results for the second dose-response shape described above (ECPB=50). In each figure, the top plot is for the case of no extra-binomial variance, the second plot assumes 30% extra-binomial variance, and the bottom plot assumes 30% parental mortality and 30% reduced neonate fecundity in the two highest test concentrations.

It will be observed that in all three plots, the Jonckheere, Williams, Dunnett, and Dunn tests have essentially the same power properties (some curves are hidden behind the Williams curve). In the first two variance scenarios (top and middle plots), the power for the Mann-Whitney test is lower, but not markedly so. However, when there is parental mortality or reduced fecundity in the two highest test concentrations, the Mann-Whitney loses almost all power to detect an effect, no matter how large.

Figure 9: Power to find an effect in the high test concentration with Williams, Dunnett, Jonckheere, Mann-Whitney, Dunn tests for sample size 10, shape 2

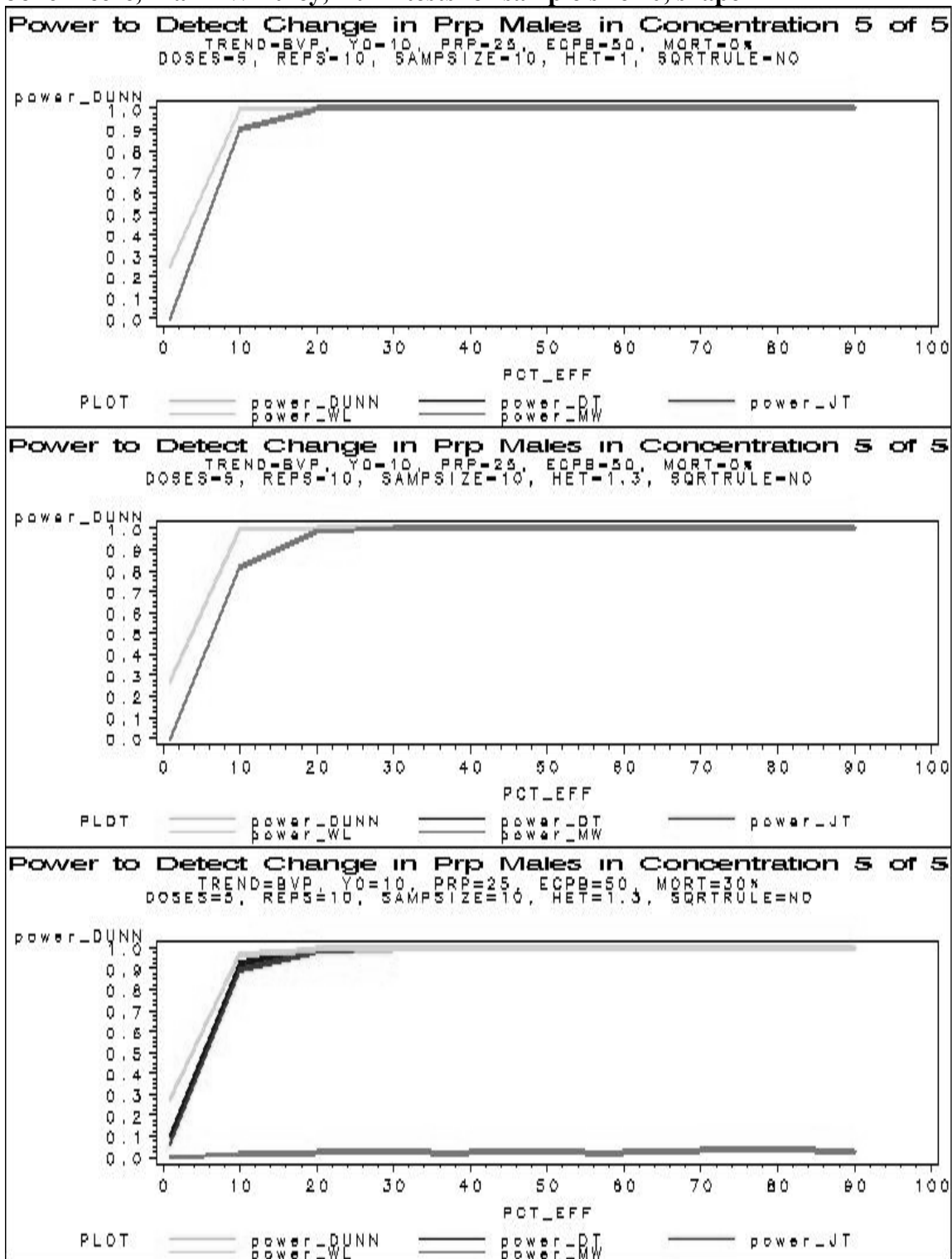
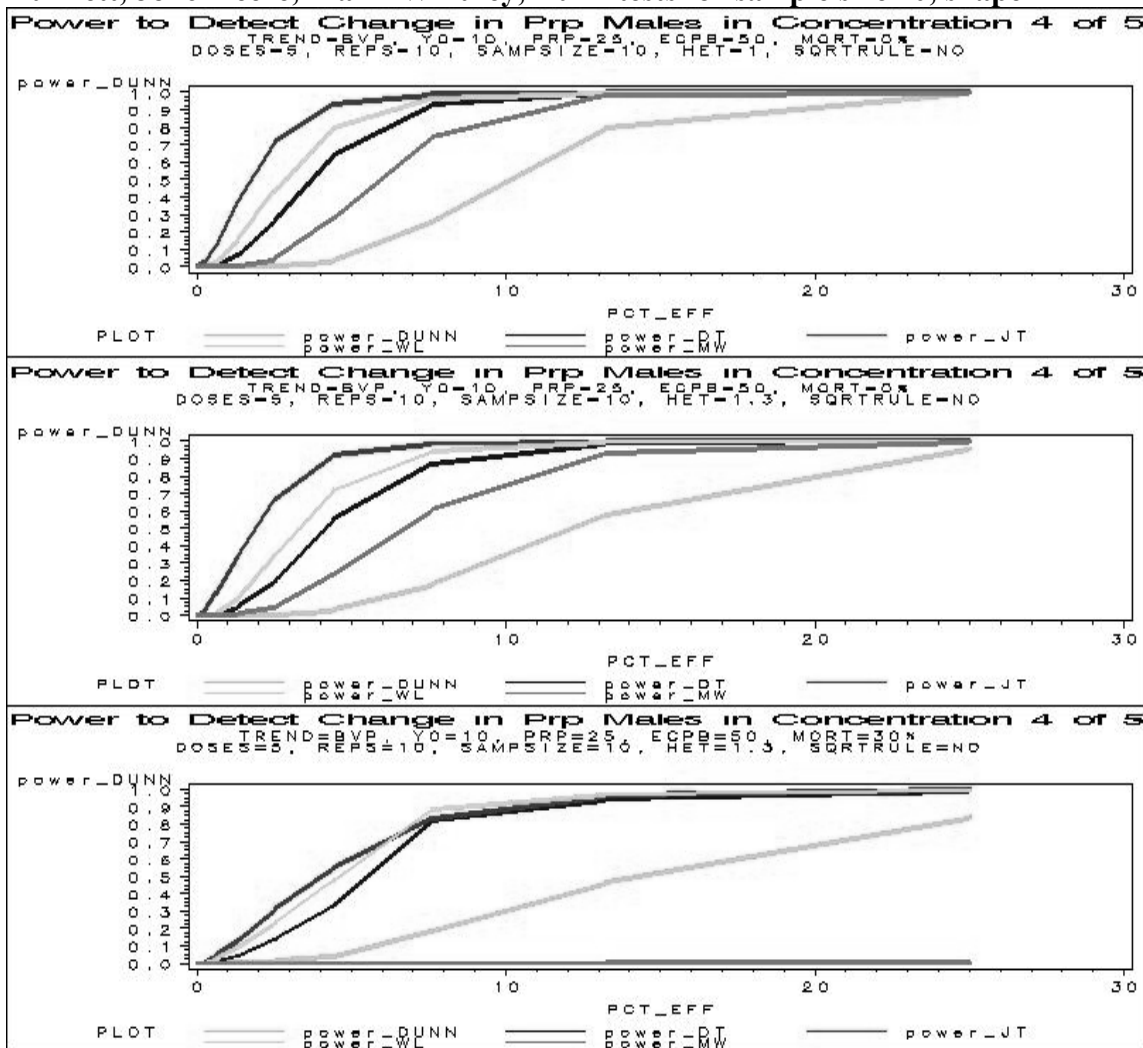


Figure 10: Power to find an effect in the second highest test concentration with Williams, Dunnnett, Jonckheere, Mann-Whitney, Dunn tests for sample size 10, shape 2



In the top 2 variance scenarios, the Jonckheere test has 80% power to detect an effect of about 4%, while Dunnnett’s test has 80% power to detect an effect of about 7%, with the Williams’ power curve in between, Mann-Whitney noticeably lower and Dunn considerably lower in power. In the third variance scenario, where 30% parental mortality and 30% reduction in fecundity among surviving parents, Mann-Whitney power drops to essentially zero, and the Jonckheere, Williams, and Dunnnett tests all have 80% power to detect an effect of about 7-8%. The Dunn test achieves 80% power to detect an effect of about 23%.

Under dose-response shape 2, the effect in the third highest test concentration is so small that none of the tests has appreciable power to detect an effect. These plots are not shown.

Figures 11 and 12 show the power to detect an effect at the high test concentration using the two Cochran-Armitage tests for the second simulated shape (ECPB=50) and the same three variance scenarios as before. It will be observed that there is little difference between a general 30% extra-binomial variance and that plus 30% parental mortality and reduced neonate fecundity in the two highest test concentrations. In all three scenarios, there is 80% power to detect an increase of 16-22% in proportion males using the Rao-Scott adjusted Cochran-Armitage test, with only minor reduction in power using the standard Cochran-Armitage test.

Figure 11: Power to detect an effect in the highest concentration with the standard & Rao-Scott adjusted Cochran-Armitage tests for sample size 10, shape 2

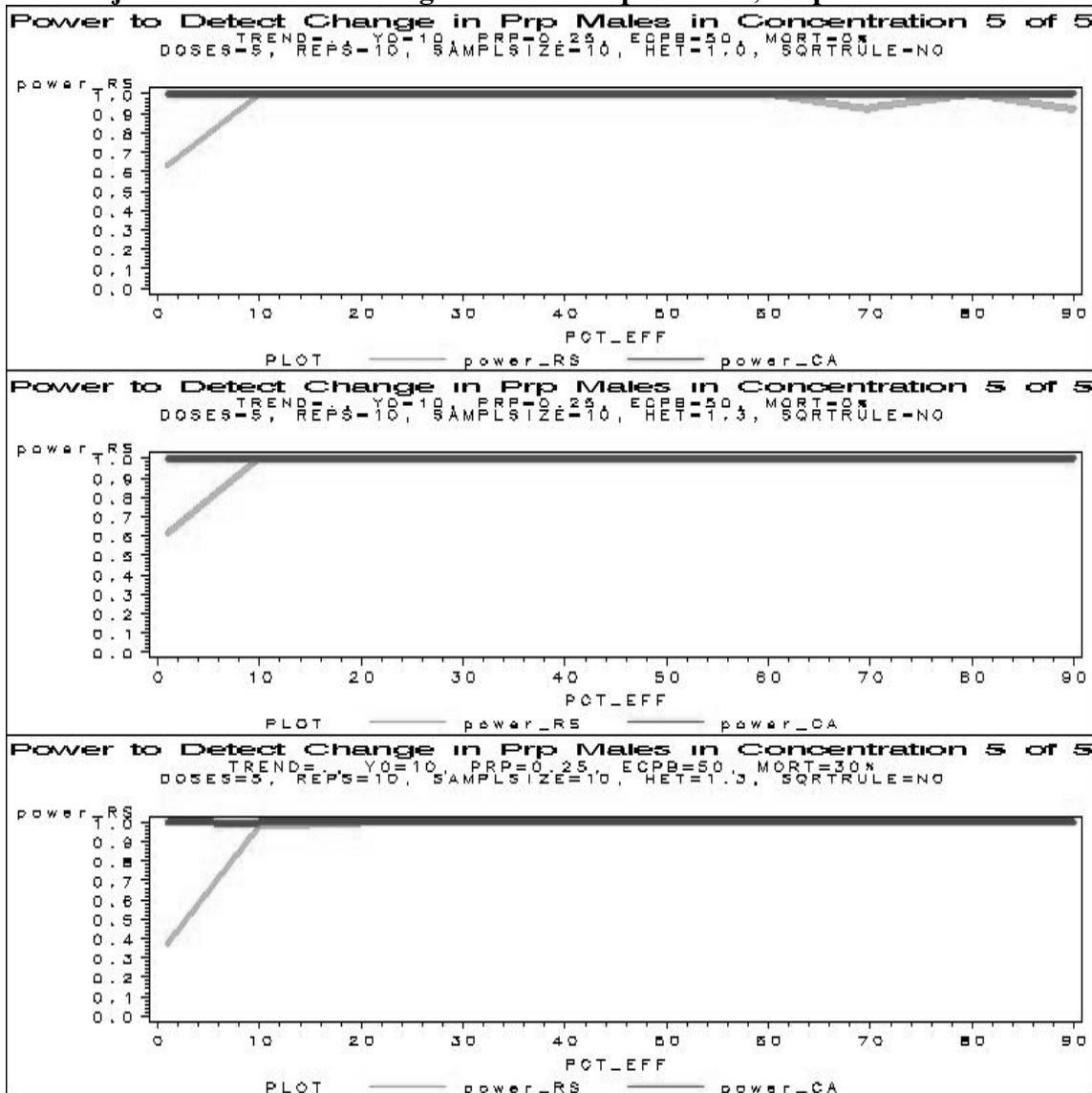
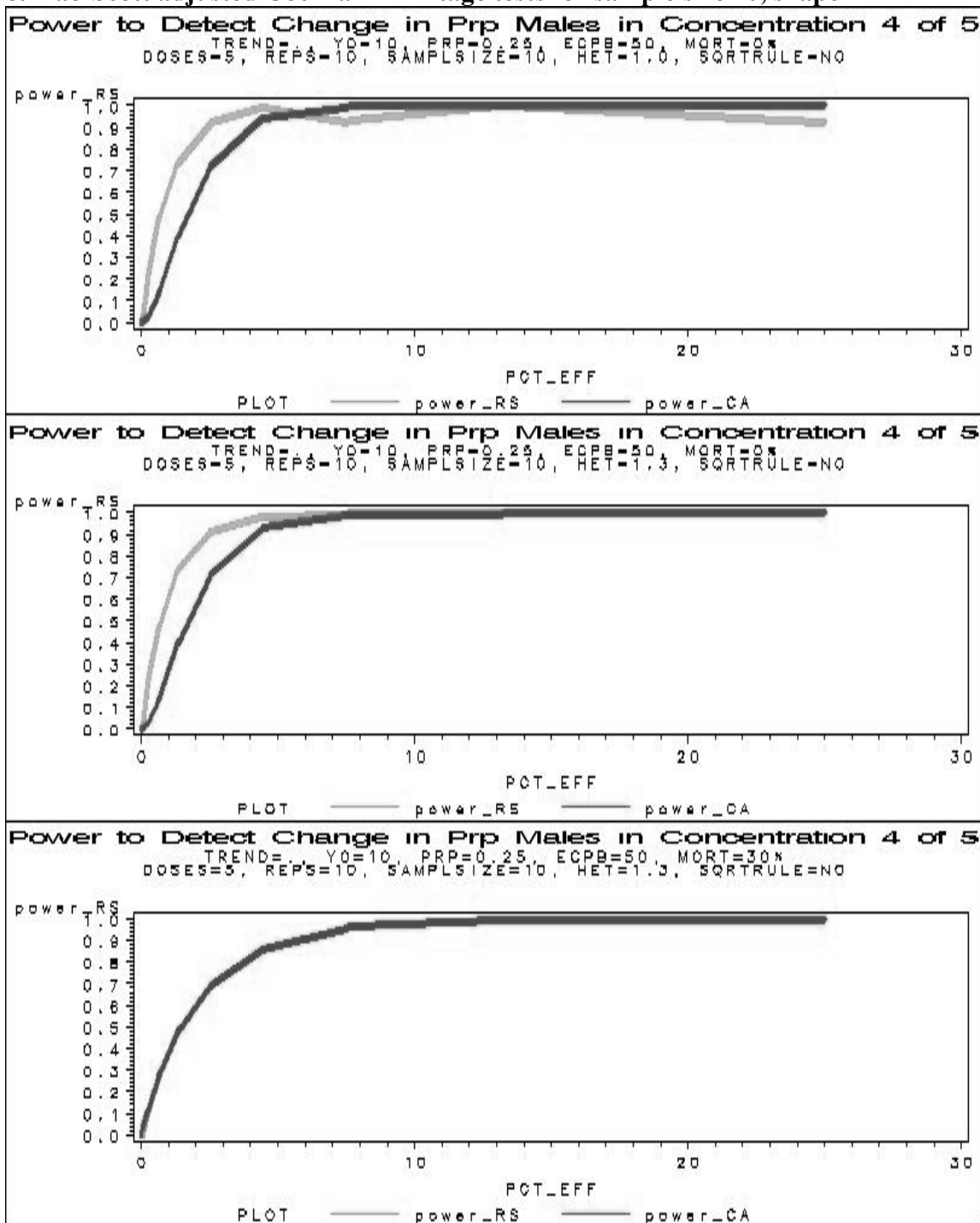


Figure 12: Power to detect an effect in the second highest concentration with the standard & Rao-Scott adjusted Cochran-Armitage tests for sample size 10, shape 2



As with dose-response shape 1, the power to detect an effect in the highest test concentration is so high that interpreting the results will be problematic.

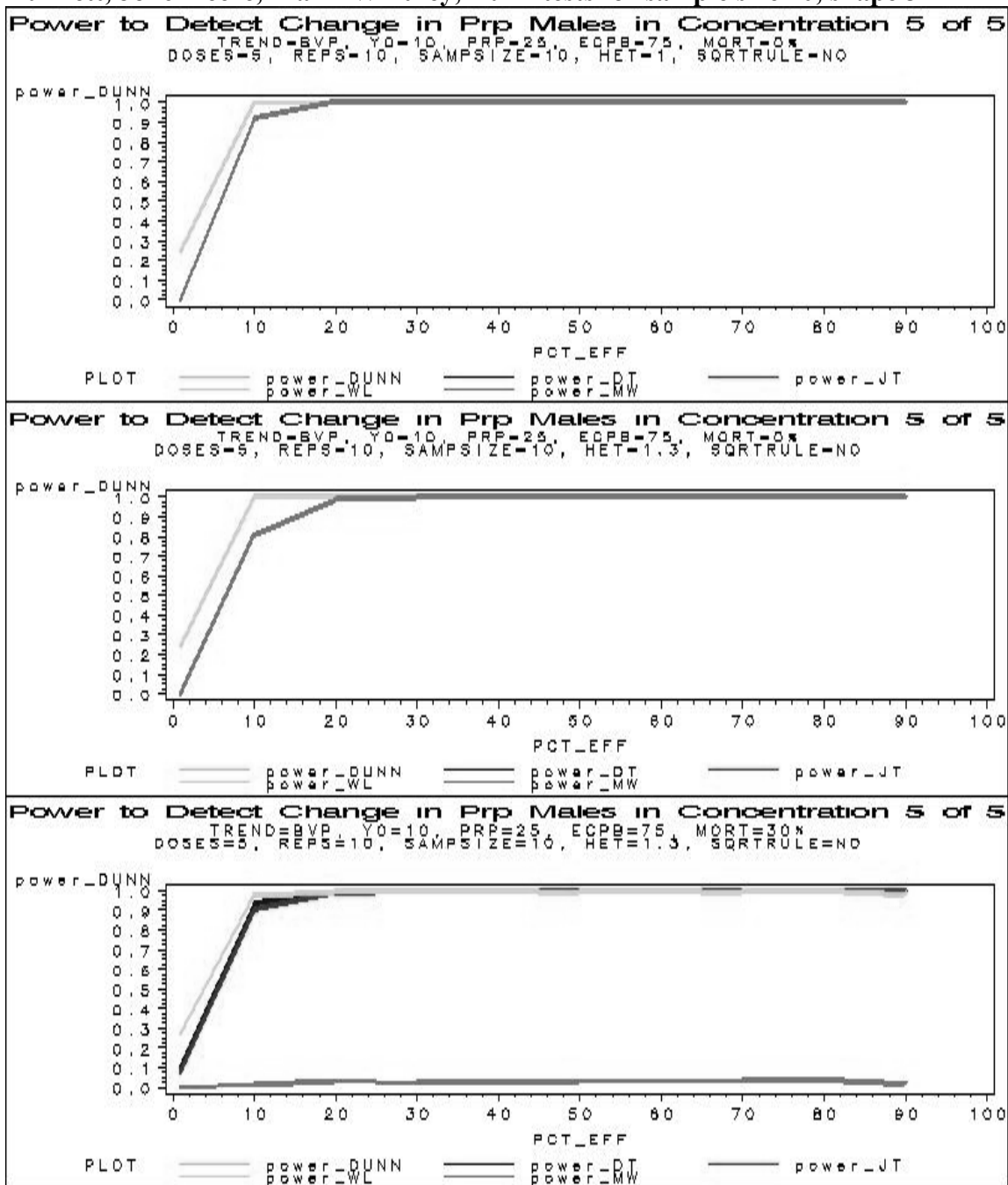
Dose-Response Shape 3 (ECPB=75)

Figure 13 shows the power to detect an effect in the highest test concentration using the Williams, Dunnett, Jonckheere, Mann-Whitney, and Dunn tests for the case of 10 neonates per rep, showing the results for the third dose-response shape (ECPB=75). The top plot assumes no extra-binomial variance, the second assumes 30% extra-binomial variance, and the bottom plot assumes 30% parental mortality and 30% reduced neonate fecundity in the two highest test concentrations.

It will be observed that in all three plots, the Williams, Jonckheere, and Dunnett tests have essentially the same power properties, so only the Williams is seen, and the Dunn power properties are very little different. The Mann-Whitney power properties are about the same for the top two variance scenarios, but again the power is almost zero power for the bottom variance scenario. It can be seen that these tests have 80% to detect an increase of about 8% in proportion males. The work of Tatarazako *et al* (2003) suggests this dose-response shape does occur. There is little difference found with experimental designs with more test concentrations or more neonates sexed per rep.

It should be understood that for this dose-response shape, there is essentially zero percent males in the lower concentrations, so that the associated powers are essentially zero. These plots are not shown. The NOEC in almost every case is the second highest test concentration.

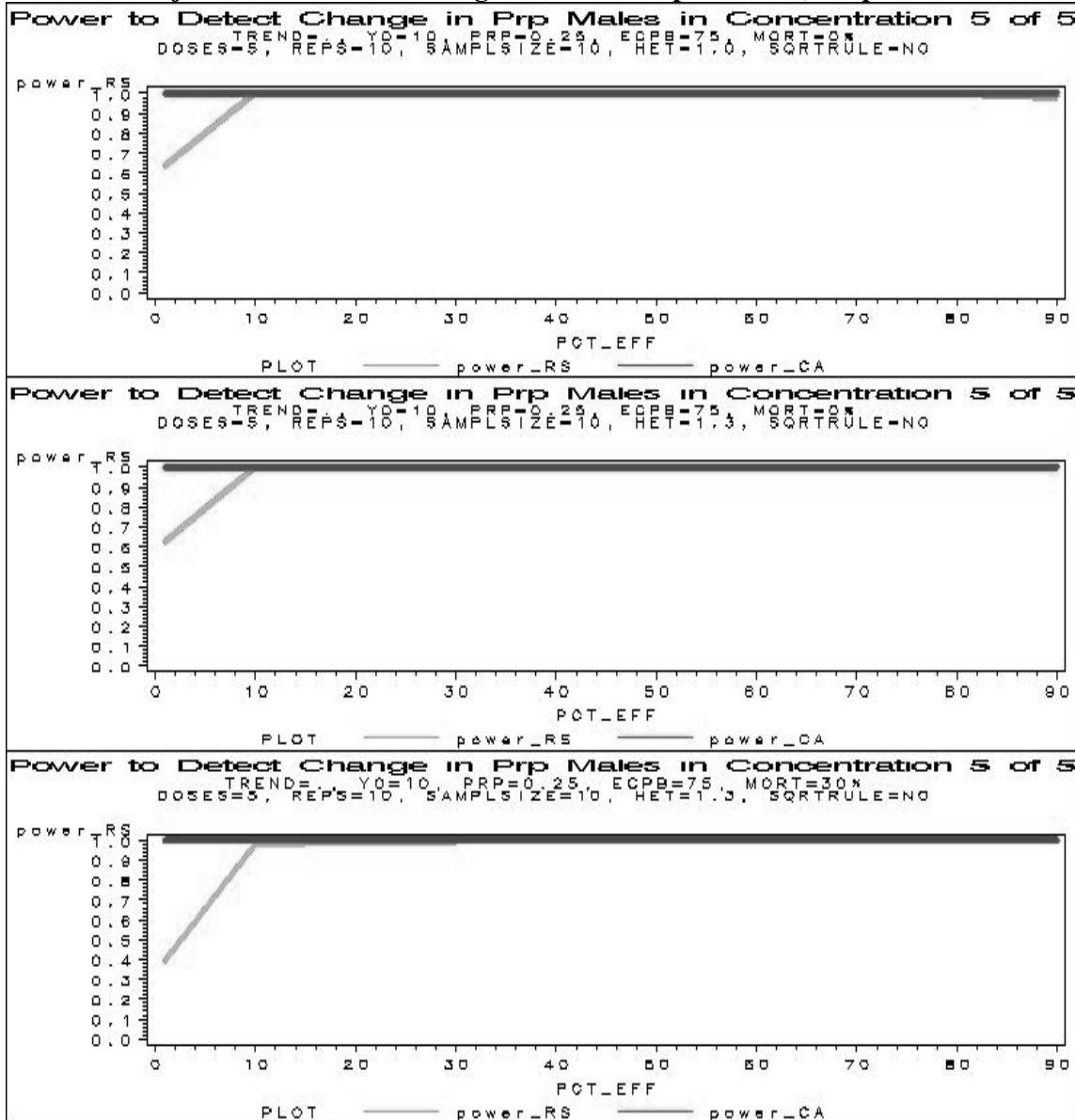
Figure 13: Power to detect an effect in the highest test concentration with the Williams, Dunnett, Jonckheere, Mann-Whitney, Dunn tests for sample size 10, shape 3



[Note that JT and others hidden beneath Williams]

Figure 14 shows the results for the two Cochran-Armitage tests for the third simulated shape (ECPB=75) and three variance scenarios. It will be observed that there is essentially no difference between the standard and Rao-Scott adjusted Cochran-Armitage tests and little between among the three variance scenarios. In all three scenarios, there is 80% power to detect an increase of about 75% in proportion males. This supports the conclusion reached above that one or two additional test concentrations are needed for this dose-response shape.

Figure 14: Power to detect an effect in the highest test concentration with the standard & Rao-Scott adjusted Cochran-Armitage tests for sample size 10, shape 3



[Note that curves overlap and appear to be one.]

Regression Results

The same set of simulation conditions that were used in the evaluation of the NOEC approach were incorporated into simulations of the use of probit regression to estimate EC_x , $x=10, 25, 50$. Two experimental designs were studied, with the first design having 4 parental replicates each providing 25 neonates for sex ratio determination, and the second design having 10 parental replicates each providing 10 neonates for sex ratio determination. It should be understood that probit analysis ignores reps, so that 4 reps of 25 is treated the same as 10 reps of 10 or one rep of size 100 since all involve 100 neonates. We have argued that reps should not be ignored, and we continue to recommend that replicates not be ignored. However, experience with non-target plant emergence and survival studies documented in Green (2008) suggest probit analysis might provide good estimates of EC_x , although confidence interval estimates are perhaps unrealistically tight. With some qualifications, the following simulations verify this. Therefore, for sake of simplicity we have performed simulations using probit analysis to explore the utility of the EC_x approach.

As stated, in probit analysis, the division of each treatment group into replicates is ignored. However, this does not mean that the number of replicates in a study (or in the simulations) can be ignored in evaluating experimental designs, or in the interpretation of EC_x estimates. Consider the consequence if a study is designed to have four replicate parental females per treatment, and toxicity results in 30% parent mortality and 30% reduction in fecundity. With only four replicates, random mortality with a mean of 30% could result in 1, 2, or 3 reps being missing, leading to a 25-75% reduction in neonates. The 30% reduction of fecundity within the remaining replicates would be additive with the initial reductions, so apparent variability could be quite high and the offspring used to assess sex ratio could be from a very small number of parents. This suggests that careful consideration should be given to how reps are randomly selected for analysis from a larger experiment. If only reps with parents surviving to the end of the experiment are sampled, this is a restriction on randomization. It would seem nevertheless to be a prudent restriction.

In the simulations, the true EC_x is known, so the quality of the EC_x estimates can be determined. For each value of x , the true EC_x is compared to the median estimated EC_x (EC_xEST) and to the minimum (EC_xMIN), maximum (EC_xMAX), 10th and 90th percentiles of the point estimates (EC_xQ10 and EC_xQ90), and to the median upper and lower 95% confidence bounds (EC_xLB and EC_xUB) on EC_x . The maximum percent effect (PCTEFF) is the percent effect in the highest treatment group. This is done for each of the three dose-response shapes and the same range of values for the maximum percent effect at the highest test concentration. In addition, the number of successful fits (NFIT) of the probit model is compared to the number of simulations.

It is important that regression estimates provide confidence bounds. Failure to provide such bounds suggests problems with the fit of the regression model to the data, since probit analysis usually provides such bounds. This complicates interpretation of the results. While the simulations given below indicate the point estimates of EC_x are reliable under most simulated conditions, in a particular experiment, there is no way of knowing what the true EC_x values are, and the inability to obtain confidence bounds casts doubt on the value of the EC_x estimates.

It may be of interest to determine the quality of ECx estimates from other regression models, such as the Bruce-Versteeg, which are based on the replicate mean proportions. Such an approach has the theoretical advantage of using the replicate information, such the between-replicate variance. Such simulations are being done and will be reported later.

Simulation results for two experimental designs are provided in the following tables. Tables 2 and 3 give simulation results for a study having 4 parental replicates with each providing 25 neonates for sex ratio determination. Tables 4 to 12 give simulation results for a study having 10 parental replicates with each providing 10 neonates for sex ratio determination. This is unimportant, since probit analysis ignores reps and analyzes both designs in the same way. It is only relevant for the simulations with regard to how parent mortality and reduced fecundity are simulated. Only results for dose-response shapes 2 (ECPB=50) and 3 (ECPB=75) are reported.

Table 2: EC10 estimates from shape 2 (ECPB=50), 4 reps of 25, HET=1, Mort=0. See text for definition of column headings.

| NFIT | iters | EC10MAX | EC10Q90 | EC10Q75 | EC10EST | EC10LB | EC10UB | EC10Q25 | EC10Q10 | EC10MIN | PCTEFF | ECPB | EC10 |
|------|-------|---------|---------|---------|---------|--------|--------|---------|---------|---------|--------|------|-------|
| 985 | 1000 | 191.8 | 110.8 | 108.0 | 106.1 | | | 104.6 | 102.4 | 98.8 | 4 | 50 | 114.3 |
| 999 | 1000 | 136.8 | 104.6 | 103.4 | 101.5 | 81.5 | 152.7 | 100.0 | 98.8 | 91.5 | 8 | 50 | 103.6 |
| 1000 | 1000 | 104.6 | 98.8 | 97.7 | 96.8 | 72.0 | 103.8 | 95.6 | 94.9 | 79.0 | 16 | 50 | 92.1 |
| 1000 | 1000 | 101.5 | 97.2 | 96.4 | 95.3 | 66.5 | 92.0 | 94.3 | 93.1 | 73.8 | 20 | 50 | 88.2 |
| 1000 | 1000 | 99.4 | 95.6 | 94.9 | 94.0 | 63.8 | 87.4 | 92.8 | 91.4 | 73.0 | 24 | 50 | 84.8 |
| 1000 | 1000 | 96.8 | 94.6 | 93.7 | 92.6 | 61.9 | 84.2 | 91.6 | 78.2 | 68.0 | 28 | 50 | 81.9 |
| 1000 | 1000 | 95.3 | 92.1 | 91.4 | 90.5 | 59.6 | 80.0 | 89.0 | 71.9 | 65.0 | 36 | 50 | 76.8 |
| 1000 | 1000 | 93.4 | 91.4 | 90.5 | 89.5 | 58.6 | 78.3 | 72.3 | 70.0 | 63.4 | 40 | 50 | 74.5 |
| 1000 | 1000 | 92.3 | 90.3 | 89.5 | 88.4 | 57.8 | 76.5 | 70.0 | 67.6 | 62.7 | 44 | 50 | 72.4 |
| 1000 | 1000 | 91.9 | 89.5 | 88.8 | 86.7 | 57.1 | 75.4 | 68.3 | 65.6 | 60.0 | 48 | 50 | 70.3 |
| 1000 | 1000 | 89.3 | 87.5 | 86.5 | 67.0 | 55.9 | 72.5 | 64.1 | 62.2 | 57.2 | 56 | 50 | 66.4 |
| 1000 | 1000 | 88.8 | 86.7 | 67.8 | 65.5 | 54.6 | 70.5 | 62.6 | 60.5 | 55.3 | 60 | 50 | 64.5 |
| 1000 | 1000 | 87.9 | 85.2 | 65.8 | 63.2 | 54.1 | 68.8 | 60.8 | 58.9 | 53.6 | 64 | 50 | 62.6 |
| 1000 | 1000 | 86.7 | 65.7 | 63.6 | 61.4 | 53.3 | 66.7 | 58.9 | 57.1 | 52.2 | 68 | 50 | 60.7 |
| 1000 | 1000 | 84.7 | 61.1 | 59.1 | 57.1 | 50.4 | 62.4 | 55.0 | 53.2 | 48.4 | 76 | 50 | 56.7 |
| 1000 | 1000 | 83.3 | 58.5 | 56.6 | 54.9 | 49.1 | 59.8 | 52.9 | 51.1 | 46.9 | 80 | 50 | 54.5 |
| 1000 | 1000 | 62.7 | 55.6 | 54.2 | 52.6 | 47.0 | 57.1 | 50.9 | 49.2 | 44.7 | 84 | 50 | 52.2 |
| 1000 | 1000 | 59.6 | 52.7 | 51.6 | 49.3 | 44.3 | 53.9 | 48.1 | 46.8 | 42.4 | 88 | 50 | 49.6 |
| 1000 | 1000 | 48.5 | 45.0 | 43.7 | 42.2 | 37.4 | 45.9 | 40.7 | 39.5 | 36.1 | 96 | 50 | 42.0 |
| 1000 | 1000 | 48.9 | 45.0 | 43.5 | 42.1 | 37.5 | 45.9 | 40.9 | 39.7 | 35.6 | 96 | 50 | 42.0 |
| 1000 | 1000 | 50.0 | 44.7 | 43.7 | 42.2 | 37.7 | 45.9 | 40.9 | 39.8 | 35.9 | 96 | 50 | 42.0 |
| 1000 | 1000 | 50.0 | 45.0 | 43.6 | 42.2 | 37.5 | 45.9 | 41.0 | 39.7 | 36.1 | 96 | 50 | 42.0 |
| 1000 | 1000 | 49.4 | 44.7 | 43.4 | 42.1 | 37.4 | 45.9 | 40.7 | 39.5 | 36.1 | 96 | 50 | 42.0 |

It will be observed that when the maximum percent effect, PCTEFF, is 16% or over, the quality of the estimates is good. It would be poor practice to use probit analysis to estimate EC10 if less than 10% males was observed in any test concentration. Indeed, Table 1 suggests the EC10 estimate may be unreliable if the maximum observed percent effect is less than 20. However, Tatarazako *et al* (2003) suggests a much higher incidence of males may be observed in high test concentrations.

Table 3: EC10 estimates from shape 2 (ECPB=50), 4 reps of 25, HET=1.3, Mort=30

| NFIT | iters | EC10MAX | EC10Q90 | EC10Q75 | EC10EST | EC10 | EC10LB | EC10UB | EC10Q25 | EC10Q10 | EC10MIN | PCTEFF |
|------|-------|---------|---------|---------|---------|-------|--------|--------|---------|---------|---------|--------|
| 369 | 1000 | 21246.0 | 114.7 | 113.9 | 112.9 | 144.8 | | | 110.2 | 107.4 | 104.5 | 1 |
| 988 | 1000 | 3029.6 | 108.5 | 105.6 | 102.7 | 100.0 | 80.6 | 407.5 | 100.9 | 99.1 | 87.3 | 10 |
| 985 | 1000 | 1872.5 | 108.2 | 105.4 | 102.7 | 100.0 | 82.6 | 640.2 | 100.8 | 99.0 | 84.9 | 10 |
| 999 | 1000 | 228.1 | 102.2 | 100.0 | 97.8 | 85.6 | 70.4 | 126.2 | 95.7 | 93.6 | 70.8 | 20 |
| 1000 | 1000 | 154.5 | 102.3 | 100.0 | 97.7 | 85.6 | 70.9 | 124.0 | 95.7 | 93.8 | 72.1 | 20 |
| 1000 | 1000 | 124.2 | 97.4 | 95.8 | 94.1 | 76.5 | 60.5 | 91.9 | 91.0 | 77.1 | 66.6 | 30 |
| 1000 | 1000 | 135.0 | 97.5 | 95.9 | 93.9 | 76.5 | 60.7 | 91.7 | 88.6 | 76.7 | 63.8 | 30 |
| 1000 | 1000 | 98.8 | 94.3 | 92.9 | 90.3 | 69.5 | 56.9 | 83.7 | 73.5 | 69.7 | 60.5 | 40 |
| 1000 | 1000 | 118.3 | 94.6 | 93.0 | 90.1 | 69.5 | 56.9 | 83.4 | 73.1 | 69.7 | 57.1 | 40 |
| 1000 | 1000 | 95.3 | 91.9 | 90.3 | 70.9 | 63.5 | 53.9 | 78.0 | 67.3 | 63.8 | 56.0 | 50 |
| 1000 | 1000 | 95.8 | 91.8 | 90.2 | 71.1 | 63.5 | 54.0 | 78.1 | 67.5 | 63.8 | 53.3 | 50 |
| 1000 | 1000 | 94.7 | 88.9 | 69.1 | 65.0 | 58.0 | 51.1 | 72.9 | 61.7 | 58.6 | 50.7 | 60 |
| 1000 | 1000 | 94.1 | 89.3 | 69.3 | 64.9 | 58.0 | 50.9 | 72.9 | 61.5 | 58.7 | 52.2 | 60 |
| 1000 | 1000 | 90.6 | 66.5 | 63.0 | 58.7 | 52.7 | 46.7 | 66.8 | 55.4 | 52.7 | 44.4 | 70 |
| 1000 | 1000 | 92.3 | 66.3 | 62.6 | 58.9 | 52.7 | 46.8 | 66.7 | 55.6 | 53.2 | 46.3 | 70 |
| 1000 | 1000 | 87.4 | 58.3 | 55.0 | 52.0 | 47.1 | 42.1 | 59.3 | 49.0 | 46.6 | 40.3 | 80 |
| 1000 | 1000 | 88.5 | 58.8 | 55.4 | 52.2 | 47.1 | 42.3 | 59.6 | 48.8 | 46.2 | 41.4 | 80 |
| 1000 | 1000 | 60.5 | 49.5 | 46.8 | 44.0 | 40.3 | 36.0 | 50.5 | 41.3 | 39.5 | 32.6 | 90 |

In this more variable scenario, the EC10 estimate is observed to be unreliable if the maximum percent effect in the high concentration is 20% or less. Otherwise, the estimates appear to be good.

Table 4: EC10 estimates from shape 3 (ECPB=75), 10 reps of 10, HET=1, Mort=0

| NFIT | iters | EC10MAX | EC10Q90 | EC10Q75 | EC10EST | EC10 | EC10LB | EC10UB | EC10Q25 | EC10Q10 | EC10MIN | PCTEFF |
|------|-------|---------|---------|---------|---------|-------|--------|--------|---------|---------|---------|--------|
| 382 | 1000 | 115.7 | 114.7 | 113.9 | 112.2 | 116.6 | | | 110.2 | 108.2 | 101.9 | 1 |
| 988 | 1000 | 115.6 | 108.2 | 104.7 | 102.5 | 100.0 | | | 100.8 | 99.0 | 94.3 | 10 |
| 982 | 1000 | 114.8 | 107.4 | 104.6 | 102.7 | 100.0 | | | 100.0 | 99.0 | 94.7 | 10 |
| 997 | 1000 | 113.1 | 101.9 | 100.0 | 97.8 | 93.7 | | | 96.2 | 95.2 | 91.6 | 20 |
| 996 | 1000 | 112.9 | 101.7 | 100.0 | 97.8 | 93.7 | | | 96.3 | 95.2 | 92.3 | 20 |
| 1000 | 1000 | 105.6 | 97.8 | 96.3 | 95.2 | 89.5 | | | 93.8 | 92.7 | 90.1 | 30 |
| 999 | 1000 | 102.7 | 98.1 | 96.5 | 95.2 | 89.5 | | | 93.8 | 92.7 | 90.0 | 30 |
| 999 | 1000 | 100.0 | 95.2 | 94.0 | 92.8 | 86.0 | | | 91.8 | 91.0 | 87.8 | 40 |
| 1000 | 1000 | 98.8 | 95.2 | 93.9 | 92.9 | 86.0 | | | 91.9 | 90.9 | 88.5 | 40 |
| 1000 | 1000 | 98.1 | 93.0 | 92.0 | 91.1 | 82.8 | | | 90.1 | 89.4 | 87.3 | 50 |
| 1000 | 1000 | 98.5 | 93.2 | 92.2 | 91.1 | 82.8 | | | 90.3 | 89.6 | 86.3 | 50 |
| 1000 | 1000 | 95.3 | 91.3 | 90.5 | 89.7 | 79.8 | | | 88.8 | 88.1 | 85.8 | 60 |
| 1000 | 1000 | 94.3 | 91.3 | 90.5 | 89.7 | 79.8 | | | 88.9 | 88.1 | 86.4 | 60 |
| 1000 | 1000 | 93.3 | 89.8 | 89.1 | 88.2 | 76.7 | 54.9 | 74.3 | 87.5 | 86.9 | 66.8 | 70 |
| 1000 | 1000 | 92.2 | 89.8 | 89.2 | 88.4 | 76.7 | 53.9 | 75.9 | 87.6 | 87.0 | 67.4 | 70 |
| 1000 | 1000 | 92.4 | 88.5 | 87.9 | 87.1 | 73.2 | 46.5 | 72.8 | 86.5 | 85.8 | 61.6 | 80 |
| 1000 | 1000 | 93.8 | 88.5 | 87.9 | 87.1 | 73.2 | 53.9 | 73.4 | 86.4 | 85.7 | 64.4 | 80 |
| 1000 | 1000 | 89.3 | 87.2 | 86.6 | 85.9 | 68.6 | 53.9 | 71.3 | 85.2 | 84.6 | 61.3 | 90 |
| 1000 | 1000 | 89.8 | 87.2 | 86.6 | 85.9 | 68.6 | 53.5 | 71.2 | 85.2 | 84.6 | 61.6 | 90 |
| 1000 | 1000 | 90.4 | 87.4 | 86.7 | 86.0 | 68.6 | 53.8 | 72.6 | 85.3 | 84.6 | 62.8 | 90 |
| 1000 | 1000 | 90.9 | 87.2 | 86.5 | 85.9 | 68.6 | 53.0 | 72.0 | 85.2 | 84.5 | 62.0 | 90 |
| 1000 | 1000 | 90.9 | 87.2 | 86.7 | 85.9 | 68.6 | 53.8 | 72.0 | 85.2 | 84.6 | 62.9 | 90 |
| 1000 | 1000 | 90.9 | 87.2 | 86.6 | 85.9 | 68.6 | 54.2 | 71.9 | 85.3 | 84.6 | 63.3 | 90 |

The point estimates are very good, but the inability to obtain confidence intervals for the EC10 estimates when the maximum percent effect at the high test concentration is less than 70 indicates problems with the fit of the model and complicates interpretation. This is especially disturbing since ideal conditions are simulated, that is, no extra-binomial variance and no parent mortality or reduced fecundity at high concentrations.

Table 5: EC10 estimates from shape 3 (ECPB=75), 10 reps of 10, HET=1.3, Mort=30

| NFIT | iters | EC10MAX | EC10Q90 | EC10Q75 | EC10EST | EC10 | EC10LB | EC10UB | EC10Q25 | EC10Q10 | EC10MIN | PCTEFF |
|------|-------|---------|---------|---------|---------|-------|--------|--------|---------|---------|---------|--------|
| 382 | 1000 | 115.7 | 114.7 | 113.9 | 112.2 | 116.6 | | | 110.2 | 108.2 | 101.9 | 1 |
| 988 | 1000 | 115.6 | 108.2 | 104.7 | 102.5 | 100.0 | | | 100.8 | 99.0 | 94.3 | 10 |
| 982 | 1000 | 114.8 | 107.4 | 104.6 | 102.7 | 100.0 | | | 100.0 | 99.0 | 94.7 | 10 |
| 997 | 1000 | 113.1 | 101.9 | 100.0 | 97.8 | 93.7 | | | 96.2 | 95.2 | 91.6 | 20 |
| 996 | 1000 | 112.9 | 101.7 | 100.0 | 97.8 | 93.7 | | | 96.3 | 95.2 | 92.3 | 20 |
| 1000 | 1000 | 105.6 | 97.8 | 96.3 | 95.2 | 89.5 | | | 93.8 | 92.7 | 90.1 | 30 |
| 999 | 1000 | 102.7 | 98.1 | 96.5 | 95.2 | 89.5 | | | 93.8 | 92.7 | 90.0 | 30 |
| 999 | 1000 | 100.0 | 95.2 | 94.0 | 92.8 | 86.0 | | | 91.8 | 91.0 | 87.8 | 40 |
| 1000 | 1000 | 98.8 | 95.2 | 93.9 | 92.9 | 86.0 | | | 91.9 | 90.9 | 88.5 | 40 |
| 1000 | 1000 | 98.1 | 93.0 | 92.0 | 91.1 | 82.8 | | | 90.1 | 89.4 | 87.3 | 50 |
| 1000 | 1000 | 98.5 | 93.2 | 92.2 | 91.1 | 82.8 | | | 90.3 | 89.6 | 86.3 | 50 |
| 1000 | 1000 | 95.3 | 91.3 | 90.5 | 89.7 | 79.8 | | | 88.8 | 88.1 | 85.8 | 60 |
| 1000 | 1000 | 94.3 | 91.3 | 90.5 | 89.7 | 79.8 | | | 88.9 | 88.1 | 86.4 | 60 |
| 1000 | 1000 | 93.3 | 89.8 | 89.1 | 88.2 | 76.7 | 54.9 | 74.3 | 87.5 | 86.9 | 66.8 | 70 |
| 1000 | 1000 | 92.2 | 89.8 | 89.2 | 88.4 | 76.7 | 53.9 | 75.9 | 87.6 | 87.0 | 67.4 | 70 |
| 1000 | 1000 | 92.4 | 88.5 | 87.9 | 87.1 | 73.2 | 46.5 | 72.8 | 86.5 | 85.8 | 61.6 | 80 |
| 1000 | 1000 | 93.8 | 88.5 | 87.9 | 87.1 | 73.2 | 53.9 | 73.4 | 86.4 | 85.7 | 64.4 | 80 |
| 1000 | 1000 | 89.3 | 87.2 | 86.6 | 85.9 | 68.6 | 53.9 | 71.3 | 85.2 | 84.6 | 61.3 | 90 |
| 1000 | 1000 | 89.8 | 87.2 | 86.6 | 85.9 | 68.6 | 53.5 | 71.2 | 85.2 | 84.6 | 61.6 | 90 |
| 1000 | 1000 | 90.4 | 87.4 | 86.7 | 86.0 | 68.6 | 53.8 | 72.6 | 85.3 | 84.6 | 62.8 | 90 |
| 1000 | 1000 | 90.9 | 87.2 | 86.5 | 85.9 | 68.6 | 53.0 | 72.0 | 85.2 | 84.5 | 62.0 | 90 |
| 1000 | 1000 | 90.9 | 87.2 | 86.7 | 85.9 | 68.6 | 53.8 | 72.0 | 85.2 | 84.6 | 62.9 | 90 |
| 1000 | 1000 | 90.9 | 87.2 | 86.6 | 85.9 | 68.6 | 54.2 | 71.9 | 85.3 | 84.6 | 63.3 | 90 |

The point estimates are good, but the inability to obtain confidence intervals for the EC10 estimates when the maximum percent effect at the high test concentration is less than 70 indicates problems with the fit of the model and complicates interpretation.

Table 6: EC25 Estimates from shape 2 (ECPB=50), HET=1, Mort=0

| NFIT | iters | EC25MAX | EC25Q90 | EC25est | EC25 | EC25LB | EC25UB | EC25Q25 | EC25Q10 | EC25MIN | PCTEFF |
|------|-------|---------|---------|---------|-------|--------|--------|---------|---------|---------|--------|
| 985 | 1000 | 383.3 | 120.2 | 114.5 | 136.0 | | | 112.7 | 110.1 | 106.0 | 4 |
| 999 | 1000 | 235.3 | 112.7 | 109.3 | 123.2 | 113.5 | 481.2 | 107.5 | 106.0 | 103.2 | 8 |
| 1000 | 1000 | 140.1 | 106.7 | 103.7 | 109.6 | 102.7 | 192.9 | 102.7 | 101.5 | 97.1 | 16 |
| 1000 | 1000 | 129.1 | 104.8 | 101.9 | 104.9 | 95.5 | 137.8 | 101.1 | 100.0 | 92.6 | 20 |
| 1000 | 1000 | 121.1 | 102.7 | 100.3 | 100.9 | 91.0 | 119.4 | 99.3 | 98.4 | 91.0 | 24 |
| 1000 | 1000 | 121.1 | 101.5 | 99.0 | 97.4 | 87.6 | 111.1 | 97.9 | 96.8 | 85.7 | 28 |
| 1000 | 1000 | 104.4 | 98.1 | 96.3 | 91.4 | 81.0 | 97.5 | 94.7 | 88.8 | 82.2 | 36 |
| 1000 | 1000 | 99.7 | 97.3 | 95.1 | 88.7 | 78.5 | 94.0 | 89.5 | 85.7 | 77.1 | 40 |
| 1000 | 1000 | 98.4 | 96.1 | 93.8 | 86.1 | 76.3 | 91.2 | 85.2 | 82.7 | 78.3 | 44 |
| 1000 | 1000 | 97.9 | 95.1 | 91.9 | 83.7 | 74.1 | 88.4 | 82.2 | 80.1 | 74.0 | 48 |
| 1000 | 1000 | 94.9 | 92.8 | 79.8 | 79.0 | 70.6 | 84.0 | 77.4 | 75.6 | 70.9 | 56 |
| 1000 | 1000 | 94.3 | 91.8 | 77.3 | 76.7 | 68.9 | 82.2 | 75.3 | 73.4 | 68.9 | 60 |
| 1000 | 1000 | 93.2 | 90.1 | 75.0 | 74.4 | 67.4 | 80.2 | 73.0 | 71.1 | 66.9 | 64 |
| 1000 | 1000 | 91.8 | 77.1 | 72.4 | 72.1 | 65.6 | 77.6 | 70.5 | 68.6 | 63.9 | 68 |
| 1000 | 1000 | 89.5 | 71.6 | 67.7 | 67.4 | 61.8 | 72.7 | 65.9 | 64.3 | 60.5 | 76 |
| 1000 | 1000 | 87.8 | 68.5 | 65.0 | 64.8 | 59.5 | 69.8 | 63.2 | 61.5 | 57.2 | 80 |
| 1000 | 1000 | 71.5 | 65.3 | 62.1 | 62.1 | 57.1 | 66.6 | 60.5 | 59.2 | 56.1 | 84 |
| 1000 | 1000 | 68.4 | 62.0 | 58.9 | 58.9 | 54.2 | 63.2 | 57.5 | 56.4 | 52.3 | 88 |
| 1000 | 1000 | 56.2 | 52.3 | 50.1 | 50.0 | 46.0 | 53.7 | 48.9 | 47.8 | 45.0 | 96 |
| 1000 | 1000 | 56.8 | 52.4 | 50.1 | 50.0 | 46.1 | 53.7 | 48.8 | 47.8 | 43.4 | 96 |
| 1000 | 1000 | 56.6 | 52.3 | 50.1 | 50.0 | 46.1 | 53.7 | 48.9 | 47.8 | 44.4 | 96 |
| 1000 | 1000 | 57.1 | 52.3 | 50.1 | 50.0 | 46.1 | 53.7 | 48.9 | 47.8 | 44.8 | 96 |
| 1000 | 1000 | 56.9 | 52.2 | 50.0 | 50.0 | 45.9 | 53.6 | 48.7 | 47.8 | 44.7 | 96 |

So long as the maximum effect at the high concentration is 20% or higher, the quality of the EC25 estimates is good.

Table 7: EC25 Estimates from shape 2 (ECPB=50), HET=1.3, Mort=30

| NFIT | iters | EC25MAX | EC25Q90 | EC25est | EC25 | EC25LB | EC25UB | EC25Q25 | EC25Q10 | EC25MIN | PCTEFF |
|------|-------|----------|---------|---------|-------|--------|--------|---------|---------|---------|--------|
| 369 | 1000 | 270944.3 | 124.6 | 122.5 | 179.6 | | | 119.5 | 116.1 | 112.5 | 1 |
| 988 | 1000 | 19106.5 | 118.1 | 110.6 | 124.0 | 118.8 | 3712.3 | 108.5 | 106.3 | 99.5 | 10 |
| 985 | 1000 | 9687.1 | 117.6 | 110.6 | 124.0 | 118.8 | 8772.1 | 108.4 | 106.2 | 100.3 | 10 |
| 999 | 1000 | 516.4 | 119.7 | 105.6 | 106.1 | 102.9 | 342.1 | 103.4 | 101.9 | 94.3 | 20 |
| 1000 | 1000 | 337.3 | 115.7 | 105.5 | 106.1 | 103.9 | 310.6 | 103.4 | 101.9 | 92.0 | 20 |
| 1000 | 1000 | 223.7 | 111.7 | 101.9 | 94.8 | 91.3 | 148.0 | 100.0 | 98.4 | 85.1 | 30 |
| 1000 | 1000 | 259.9 | 111.0 | 101.9 | 94.8 | 91.5 | 149.9 | 99.9 | 98.0 | 85.1 | 30 |
| 1000 | 1000 | 130.2 | 103.2 | 98.8 | 86.1 | 84.0 | 115.8 | 95.9 | 90.5 | 79.9 | 40 |
| 1000 | 1000 | 197.6 | 103.5 | 98.4 | 86.1 | 83.8 | 113.8 | 95.6 | 90.5 | 77.8 | 40 |
| 1000 | 1000 | 125.6 | 98.5 | 91.6 | 78.7 | 77.5 | 99.7 | 86.7 | 83.2 | 74.8 | 50 |
| 1000 | 1000 | 117.3 | 98.4 | 92.0 | 78.7 | 77.5 | 99.6 | 86.6 | 83.2 | 73.8 | 50 |
| 1000 | 1000 | 101.1 | 94.7 | 83.2 | 72.0 | 72.3 | 91.9 | 80.0 | 77.2 | 68.4 | 60 |
| 1000 | 1000 | 102.6 | 95.2 | 83.5 | 72.0 | 72.3 | 92.4 | 80.1 | 77.5 | 69.9 | 60 |
| 1000 | 1000 | 96.5 | 83.5 | 76.1 | 65.4 | 66.6 | 84.9 | 73.1 | 70.5 | 62.1 | 70 |
| 1000 | 1000 | 105.6 | 83.5 | 76.3 | 65.4 | 66.7 | 85.0 | 73.2 | 70.8 | 62.9 | 70 |
| 1000 | 1000 | 92.8 | 74.3 | 68.5 | 58.4 | 60.0 | 76.5 | 65.5 | 63.1 | 56.3 | 80 |
| 1000 | 1000 | 93.9 | 74.6 | 68.7 | 58.4 | 60.1 | 76.7 | 65.7 | 63.2 | 56.0 | 80 |
| 1000 | 1000 | 74.6 | 65.3 | 59.8 | 50.0 | 52.2 | 66.9 | 56.9 | 54.6 | 48.5 | 90 |

The table is for 10 reps of size 10 rather than 4 reps of size 25. The latter has the same pattern of missing confidence intervals as seen for EC10 estimates with MORT=30 and HET=1.3. This appears to be a function of the algorithm used to simulate the indicated parent mortality and reduced fecundity. With only 4 reps, a 30% parent mortality rate would often eliminate 1 or 2 reps (and occasionally 3) for a reduction of 25-50% and sometimes 75% in the number of neonates that are sexed. This suggests care in the random selection of reps to analyze. It might be appropriate to randomly select reps from among those where the parents survive to the end of the experiment. This is a restriction on randomization to be sure, but the desirable properties of the fit and quality of estimated ECx values would seem to outweigh the disadvantage of the restricted randomization.

Table 8: EC25 Estimates from shape 3 (ECPB=75), HET=1, Mort=0

| NFIT | iters | EC25MAX | EC25Q90 | EC25est | EC25 | EC25LB | EC25UB | EC25Q25 | EC25Q10 | EC25MIN | PCTEFF |
|------|-------|---------|---------|---------|-------|--------|--------|---------|---------|---------|--------|
| 382 | 1000 | 125.9 | 124.6 | 121.8 | 127.5 | | | 119.6 | 116.9 | 109.7 | 1 |
| 988 | 1000 | 125.7 | 116.9 | 110.4 | 109.3 | | | 108.4 | 106.1 | 100.8 | 10 |
| 982 | 1000 | 124.8 | 116.2 | 110.5 | 109.3 | | | 107.5 | 106.1 | 101.1 | 10 |
| 997 | 1000 | 122.8 | 109.6 | 105.0 | 102.5 | | | 103.0 | 101.8 | 97.6 | 20 |
| 996 | 1000 | 122.4 | 109.3 | 104.9 | 102.5 | | | 103.0 | 101.8 | 98.4 | 20 |
| 1000 | 1000 | 114.0 | 104.9 | 101.8 | 97.8 | | | 100.2 | 98.9 | 95.8 | 30 |
| 999 | 1000 | 110.5 | 105.1 | 101.8 | 97.8 | | | 100.0 | 98.9 | 95.8 | 30 |
| 999 | 1000 | 107.4 | 101.9 | 99.1 | 94.0 | | | 97.9 | 96.9 | 93.0 | 40 |
| 1000 | 1000 | 105.9 | 101.9 | 99.1 | 94.0 | | | 97.9 | 96.8 | 94.0 | 40 |
| 1000 | 1000 | 105.1 | 99.3 | 97.0 | 90.6 | | | 95.8 | 95.1 | 92.6 | 50 |
| 1000 | 1000 | 105.6 | 99.4 | 97.0 | 90.6 | | | 96.0 | 95.3 | 91.2 | 50 |
| 1000 | 1000 | 101.8 | 97.2 | 95.4 | 87.2 | | | 94.4 | 93.6 | 90.8 | 60 |
| 1000 | 1000 | 100.8 | 97.3 | 95.3 | 87.2 | | | 94.4 | 93.6 | 91.6 | 60 |
| 1000 | 1000 | 99.5 | 95.5 | 93.6 | 83.8 | 71.5 | 86.6 | 92.8 | 92.1 | 80.3 | 70 |
| 1000 | 1000 | 98.4 | 95.5 | 93.9 | 83.8 | 72.3 | 90.7 | 92.9 | 92.2 | 82.3 | 70 |
| 1000 | 1000 | 98.4 | 94.0 | 92.4 | 80.0 | 65.6 | 86.4 | 91.6 | 90.8 | 74.3 | 80 |
| 1000 | 1000 | 100.0 | 94.0 | 92.3 | 80.0 | 69.6 | 85.2 | 91.5 | 90.7 | 76.3 | 80 |
| 1000 | 1000 | 95.0 | 92.5 | 90.9 | 75.0 | 66.8 | 81.5 | 90.1 | 89.3 | 73.4 | 90 |
| 1000 | 1000 | 95.3 | 92.4 | 90.9 | 75.0 | 66.5 | 82.8 | 90.2 | 89.4 | 71.3 | 90 |
| 1000 | 1000 | 96.0 | 92.6 | 91.0 | 75.0 | 68.1 | 83.7 | 90.2 | 89.4 | 73.2 | 90 |
| 1000 | 1000 | 96.8 | 92.4 | 90.9 | 75.0 | 67.1 | 82.5 | 90.1 | 89.3 | 71.1 | 90 |
| 1000 | 1000 | 96.7 | 92.5 | 90.9 | 75.0 | 67.7 | 82.7 | 90.1 | 89.4 | 74.1 | 90 |
| 1000 | 1000 | 96.7 | 92.4 | 91.0 | 75.0 | 68.0 | 83.2 | 90.2 | 89.5 | 74.7 | 90 |

The point estimates are good, but the inability to provide confidence intervals when the maximum effect at the high concentration is less than 70% suggests problems with the fit and complicates interpretation.

Table 9: EC50 Estimates from shape 2 (ECPB=50), HET=1, Mort=0

| NFIT | iters | EC50MAX | EC50Q90 | EC50Q75 | EC50EST | EC50 | EC50LB | EC50UB | PCTEFF |
|------|-------|---------|---------|---------|---------|-------|--------|--------|--------|
| 632 | 1000 | 137.5 | 137.5 | 137.5 | 137.5 | 228.0 | | | 1 |
| 1000 | 1000 | 559.7 | 122.5 | 119.4 | 116.5 | 157.5 | 155.2 | 3522.4 | 10 |
| 1000 | 1000 | 2860.9 | 122.5 | 118.6 | 116.5 | 157.5 | 150.1 | 1881.2 | 10 |
| 1000 | 1000 | 246.4 | 151.8 | 113.9 | 110.8 | 134.7 | 122.0 | 252.4 | 20 |
| 1000 | 1000 | 218.0 | 151.8 | 113.9 | 110.8 | 134.7 | 122.4 | 252.4 | 20 |
| 1000 | 1000 | 185.8 | 131.2 | 122.3 | 107.9 | 120.4 | 108.9 | 155.6 | 30 |
| 1000 | 1000 | 171.1 | 131.2 | 124.1 | 108.4 | 120.4 | 109.2 | 160.6 | 30 |
| 1000 | 1000 | 130.2 | 117.3 | 112.2 | 107.4 | 109.4 | 100.1 | 126.5 | 40 |
| 1000 | 1000 | 141.5 | 117.3 | 112.2 | 107.8 | 109.4 | 100.2 | 128.0 | 40 |
| 1000 | 1000 | 123.1 | 106.7 | 103.1 | 100.0 | 100.0 | 92.6 | 111.0 | 50 |
| 1000 | 1000 | 117.6 | 106.6 | 103.1 | 100.0 | 100.0 | 92.6 | 111.3 | 50 |
| 1000 | 1000 | 102.1 | 96.9 | 94.2 | 91.5 | 91.4 | 84.8 | 99.8 | 60 |
| 1000 | 1000 | 108.3 | 96.6 | 94.0 | 91.2 | 91.4 | 84.4 | 99.0 | 60 |
| 1000 | 1000 | 93.3 | 87.3 | 85.5 | 83.1 | 83.0 | 77.1 | 90.0 | 70 |
| 1000 | 1000 | 97.5 | 87.1 | 85.7 | 83.4 | 83.0 | 77.4 | 90.6 | 70 |
| 1000 | 1000 | 86.0 | 78.2 | 76.2 | 74.2 | 74.2 | 68.7 | 80.3 | 80 |
| 1000 | 1000 | 85.6 | 78.1 | 76.1 | 74.1 | 74.2 | 68.7 | 80.2 | 80 |
| 1000 | 1000 | 70.4 | 66.7 | 65.2 | 63.6 | 63.5 | 59.0 | 68.6 | 90 |
| 1000 | 1000 | 70.2 | 66.7 | 65.1 | 63.5 | 63.5 | 58.9 | 68.4 | 90 |
| 1000 | 1000 | 71.8 | 66.7 | 65.1 | 63.6 | 63.5 | 58.9 | 68.6 | 90 |
| 1000 | 1000 | 72.0 | 66.7 | 65.1 | 63.5 | 63.5 | 58.9 | 68.5 | 90 |
| 1000 | 1000 | 71.8 | 66.7 | 65.1 | 63.5 | 63.5 | 58.9 | 68.5 | 90 |
| 1000 | 1000 | 71.9 | 66.6 | 65.1 | 63.5 | 63.5 | 58.9 | 68.5 | 90 |

Table 10: EC50 Estimates from shape 2 (ECPB=50), HET=1.3, Mort=30

| NFIT | iters | EC50MAX | EC50Q90 | EC50Q75 | EC50EST | EC50 | EC50LB | EC50UB | EC50Q25 | EC50Q10 | EC50MIN | PCTEFF |
|------|-------|-----------|---------|---------|---------|-------|--------|----------|---------|---------|---------|--------|
| 369 | 1000 | 4584439.4 | 136.6 | 135.6 | 134.1 | 228.0 | | | 130.7 | 126.7 | 122.2 | 1 |
| 988 | 1000 | 147845.1 | 128.9 | 124.2 | 120.0 | 157.5 | 162.6 | 46233.8 | 117.6 | 115.0 | 106.8 | 10 |
| 985 | 1000 | 60151.8 | 128.5 | 124.1 | 120.0 | 157.5 | 162.6 | 171438.2 | 117.5 | 114.8 | 108.0 | 10 |
| 999 | 1000 | 1280.2 | 173.6 | 117.9 | 114.4 | 134.7 | 136.8 | 1256.5 | 111.7 | 109.8 | 104.4 | 20 |
| 1000 | 1000 | 803.0 | 162.0 | 117.7 | 114.2 | 134.7 | 137.1 | 1011.1 | 111.7 | 109.8 | 104.8 | 20 |
| 1000 | 1000 | 430.1 | 158.1 | 125.2 | 110.6 | 120.4 | 120.0 | 301.5 | 108.3 | 106.6 | 102.6 | 30 |
| 1000 | 1000 | 542.7 | 161.5 | 125.9 | 111.0 | 120.4 | 119.6 | 312.0 | 108.3 | 106.5 | 101.7 | 30 |
| 1000 | 1000 | 211.8 | 143.2 | 129.7 | 109.9 | 109.4 | 110.4 | 199.1 | 106.3 | 104.6 | 100.6 | 40 |
| 1000 | 1000 | 349.5 | 144.3 | 129.2 | 110.5 | 109.4 | 109.8 | 192.9 | 106.5 | 104.8 | 101.1 | 40 |
| 1000 | 1000 | 207.1 | 128.8 | 120.5 | 111.5 | 100.0 | 102.5 | 153.3 | 104.8 | 103.0 | 95.5 | 50 |
| 1000 | 1000 | 181.3 | 130.7 | 120.7 | 110.9 | 100.0 | 102.4 | 150.8 | 104.9 | 102.6 | 93.2 | 50 |
| 1000 | 1000 | 151.2 | 120.2 | 113.1 | 107.4 | 91.4 | 96.6 | 131.2 | 102.8 | 100.0 | 82.1 | 60 |
| 1000 | 1000 | 150.4 | 121.0 | 113.8 | 107.3 | 91.4 | 97.0 | 133.3 | 102.6 | 100.0 | 90.0 | 60 |
| 1000 | 1000 | 158.0 | 110.5 | 105.6 | 101.0 | 83.0 | 90.2 | 119.1 | 97.4 | 93.6 | 85.1 | 70 |
| 1000 | 1000 | 167.0 | 111.1 | 106.1 | 101.2 | 83.0 | 90.5 | 118.7 | 97.2 | 93.7 | 81.6 | 70 |
| 1000 | 1000 | 125.8 | 101.0 | 96.8 | 92.6 | 74.2 | 82.9 | 106.8 | 89.0 | 85.5 | 77.3 | 80 |
| 1000 | 1000 | 122.8 | 101.0 | 96.9 | 93.0 | 74.2 | 83.2 | 107.4 | 89.5 | 86.5 | 75.3 | 80 |
| 1000 | 1000 | 105.5 | 90.9 | 87.3 | 83.5 | 63.5 | 74.5 | 95.5 | 79.7 | 76.7 | 65.2 | 90 |
| 1000 | 1000 | 105.5 | 91.2 | 87.2 | 83.4 | 63.5 | 74.4 | 95.8 | 79.6 | 76.4 | 64.6 | 90 |
| 1000 | 1000 | 110.2 | 91.1 | 87.1 | 83.4 | 63.5 | 74.3 | 95.6 | 79.9 | 77.1 | 64.4 | 90 |
| 1000 | 1000 | 140.9 | 91.6 | 87.3 | 83.5 | 63.5 | 74.4 | 96.2 | 80.1 | 77.0 | 68.9 | 90 |
| 1000 | 1000 | 104.8 | 90.4 | 87.0 | 83.1 | 63.5 | 74.1 | 95.4 | 79.7 | 76.7 | 64.3 | 90 |
| 1000 | 1000 | 105.9 | 90.8 | 87.1 | 83.7 | 63.5 | 74.7 | 95.7 | 80.1 | 76.6 | 68.3 | 90 |

If the maximum effect at the high concentration is at least 40%, the estimates are good. It would not be advisable to estimate EC50 when there is much less than a 50% effect observed in any test concentration.

Table 11: EC50 Estimates from shape 3 (ECPB=75), HET=1, Mort=0

| NFIT | iters | EC50MAX | EC50Q90 | EC50Q75 | EC50EST | EC50 | EC50LB | EC50UB | EC50Q25 | EC50Q10 | PCTEFF |
|------|-------|---------|---------|---------|---------|-------|--------|--------|---------|---------|--------|
| 986 | 1000 | 137.5 | 131.5 | 127.5 | 124.7 | 123.1 | | | 122.5 | 119.4 | 4 |
| 1000 | 1000 | 137.5 | 124.7 | 120.8 | 118.6 | 118.1 | | | 116.5 | 114.7 | 8 |
| 1000 | 1000 | 122.5 | 115.5 | 113.2 | 111.9 | 112.5 | | | 110.8 | 109.3 | 16 |
| 1000 | 1000 | 117.5 | 112.6 | 111.3 | 109.8 | 110.5 | | | 108.4 | 107.5 | 20 |
| 1000 | 1000 | 114.7 | 110.3 | 109.3 | 107.9 | 108.7 | | | 106.8 | 105.7 | 24 |
| 1000 | 1000 | 111.9 | 108.4 | 107.5 | 106.4 | 107.2 | | | 105.3 | 104.4 | 28 |
| 1000 | 1000 | 108.4 | 105.7 | 104.7 | 103.8 | 104.3 | | | 102.9 | 102.0 | 36 |
| 1000 | 1000 | 107.5 | 104.7 | 103.5 | 102.6 | 103.1 | | | 101.8 | 100.7 | 40 |
| 1000 | 1000 | 106.8 | 103.2 | 102.3 | 101.5 | 101.8 | | | 100.7 | 100.0 | 44 |
| 1000 | 1000 | 105.0 | 102.0 | 101.3 | 100.5 | 100.6 | | | 99.8 | 99.0 | 48 |
| 1000 | 1000 | 102.3 | 100.0 | 99.3 | 98.6 | 98.2 | | | 97.6 | 97.2 | 56 |
| 1000 | 1000 | 101.5 | 99.0 | 98.5 | 97.6 | 97.0 | | | 97.0 | 96.3 | 60 |
| 1000 | 1000 | 101.3 | 98.1 | 97.4 | 96.7 | 95.8 | | | 96.0 | 95.3 | 64 |
| 1000 | 1000 | 99.5 | 97.2 | 96.5 | 95.8 | 94.6 | | | 95.1 | 94.4 | 68 |
| 1000 | 1000 | 96.7 | 95.1 | 94.6 | 93.9 | 92.0 | | | 93.2 | 92.7 | 76 |
| 1000 | 1000 | 96.7 | 94.4 | 93.7 | 92.9 | 90.5 | | | 92.1 | 91.5 | 80 |
| 1000 | 1000 | 95.1 | 93.2 | 92.4 | 91.8 | 88.9 | | | 90.9 | 90.3 | 84 |
| 1000 | 1000 | 93.4 | 91.8 | 91.2 | 90.6 | 87.0 | | | 89.9 | 89.3 | 88 |
| 1000 | 1000 | 90.3 | 88.9 | 87.9 | 87.4 | 81.2 | 68.9 | 79.3 | 86.7 | 86.0 | 96 |
| 1000 | 1000 | 90.6 | 88.9 | 87.9 | 87.4 | 81.2 | 68.4 | 78.9 | 86.7 | 86.0 | 96 |
| 1000 | 1000 | 90.3 | 88.9 | 87.9 | 87.4 | 81.2 | | | 86.7 | 86.0 | 96 |
| 1000 | 1000 | 90.9 | 88.9 | 87.9 | 87.4 | 81.2 | 69.6 | 80.0 | 86.0 | 84.7 | 96 |
| 1000 | 1000 | 89.9 | 88.5 | 87.9 | 87.4 | 81.2 | 66.9 | 77.6 | 86.7 | 85.3 | 96 |

The point estimates are all good, but the inability to provide confidence intervals is disturbing and suggests problems with the model. This will complicate interpretation.

Table 12: EC50 Estimates from shape 3 (ECPB=75), HET=1.3, Mort=30

| NFIT | iters | EC50MAX | EC50Q90 | EC50Q75 | EC50EST | EC50 | EC50LB | EC50UB | EC50Q25 | EC50Q10 | EC50MIN | PCTEFF |
|------|-------|---------|---------|---------|---------|-------|--------|--------|---------|---------|---------|--------|
| 382 | 1000 | 138.2 | 136.6 | 135.4 | 133.5 | 140.8 | | | 130.7 | 127.4 | 119.0 | 1 |
| 988 | 1000 | 138.0 | 127.3 | 123.1 | 119.7 | 120.7 | | | 117.4 | 114.7 | 108.5 | 10 |
| 982 | 1000 | 136.9 | 126.8 | 122.8 | 119.9 | 120.7 | | | 116.5 | 114.7 | 108.6 | 10 |
| 997 | 1000 | 134.5 | 118.9 | 116.1 | 113.5 | 113.2 | | | 111.1 | 109.6 | 104.8 | 20 |
| 996 | 1000 | 134.0 | 118.6 | 116.1 | 113.4 | 113.2 | | | 111.2 | 109.6 | 105.6 | 20 |
| 1000 | 1000 | 124.1 | 113.4 | 111.3 | 109.6 | 108.0 | | | 107.9 | 106.3 | 102.6 | 30 |
| 999 | 1000 | 119.9 | 113.5 | 111.6 | 109.6 | 108.0 | | | 107.7 | 106.3 | 102.6 | 30 |
| 999 | 1000 | 116.2 | 109.8 | 108.1 | 106.5 | 103.8 | | | 105.1 | 103.9 | 99.2 | 40 |
| 1000 | 1000 | 114.5 | 109.8 | 108.0 | 106.5 | 103.8 | | | 105.1 | 103.9 | 100.5 | 40 |
| 1000 | 1000 | 113.5 | 106.7 | 105.3 | 104.1 | 100.0 | | | 102.6 | 101.7 | 98.8 | 50 |
| 1000 | 1000 | 114.2 | 107.0 | 105.6 | 104.1 | 100.0 | | | 102.9 | 101.9 | 97.1 | 50 |
| 1000 | 1000 | 109.6 | 104.3 | 103.3 | 102.2 | 96.3 | | | 100.9 | 100.0 | 96.5 | 60 |
| 1000 | 1000 | 108.5 | 104.4 | 103.2 | 102.1 | 96.3 | | | 100.9 | 100.0 | 97.6 | 60 |
| 1000 | 1000 | 106.8 | 102.3 | 101.3 | 100.0 | 92.6 | 91.7 | 107.2 | 99.1 | 98.3 | 94.8 | 70 |
| 1000 | 1000 | 105.8 | 102.3 | 101.4 | 100.3 | 92.6 | 93.3 | 118.7 | 99.2 | 98.4 | 94.8 | 70 |
| 1000 | 1000 | 105.7 | 100.4 | 99.6 | 98.6 | 88.4 | 89.0 | 113.3 | 97.6 | 96.8 | 91.4 | 80 |
| 1000 | 1000 | 107.4 | 100.5 | 99.6 | 98.5 | 88.4 | 88.8 | 104.1 | 97.6 | 96.6 | 92.1 | 80 |
| 1000 | 1000 | 101.7 | 98.8 | 97.9 | 96.9 | 82.8 | 83.3 | 97.0 | 95.9 | 95.0 | 87.5 | 90 |
| 1000 | 1000 | 101.9 | 98.6 | 97.9 | 96.9 | 82.8 | 84.4 | 100.9 | 96.0 | 95.0 | 83.5 | 90 |
| 1000 | 1000 | 102.5 | 98.8 | 97.9 | 97.0 | 82.8 | 85.2 | 100.8 | 96.1 | 95.3 | 86.7 | 90 |
| 1000 | 1000 | 103.8 | 98.6 | 97.7 | 96.9 | 82.8 | 84.0 | 99.5 | 95.9 | 95.0 | 82.8 | 90 |
| 1000 | 1000 | 103.5 | 98.8 | 97.9 | 96.9 | 82.8 | 85.0 | 99.8 | 96.0 | 95.2 | 88.5 | 90 |
| 1000 | 1000 | 103.5 | 98.6 | 97.9 | 96.9 | 82.8 | 85.9 | 99.4 | 96.0 | 95.3 | 88.7 | 90 |

The point estimates are good, but the inability to provide confidence intervals when the maximum effect at the high concentration is less than 70% suggests problems with the fit and complicates interpretation.

Conclusions

Results for experiments with 10 replicates per treatment each providing 25 and 100 neonates have not been presented in order to keep this presentation to a reasonable size. Furthermore, the power properties of statistical tests do not differ dramatically when the number of offspring from each rep is greater than 10. This is because there are still only ten reps per concentration even though each rep mean proportion is determined from a larger sample size. There is some reduction in variability (hence increase in power) from the larger sample sizes if no extra-binomial variance is assumed.

None of these tests have desirable power properties for any of the dose-response shapes. They are all so sensitive as to make interpretation of results problematic. Adding more test concentrations would not alter this conclusion. Given that the results of Tatarazako *et al* (2003) suggests dose-response shapes 2 and 3 do occur, some modification of the originally proposed experimental design and statistical evaluation tools should be considered.

These results show that smaller experiments using fewer replicates per test concentration, thereby reducing the total number of test subjects needed, would retain adequate power to detect effects of biological importance. Such smaller designs should be considered. Additional power simulations with fewer reps per test concentration will be presented at a later date.

If this experimental design is to be retained in order to accommodate other biological endpoints besides sex ratio, then it is more appropriate to analyze daphnia sex ratio, which has a 0% background incidence rate, using a suitable regression model to estimate EC50 or perhaps EC25. This is quite different from the situation with fish sex ratio, where the background incidence rate is around 50% and probit analysis (and ECx in general) is not reliable. There are various regression models that can be applied to replicate percent males, such as the Bruce-Versteeg model. In extensive computer simulations for non-target plants [Green (2008)], the probit model was found to perform very well at estimating ECx values even though rep effects are ignored. The same is shown to be true for sex ratio, although confidence intervals remain problematic

Based on earlier regression simulations done by Green (2008) it is expected that fewer reps per concentration will be sufficient for good EC25 or EC50 estimation as well as for NOEC determination for all three dose-response shapes. However, how much reduction in replication can be justified is not yet known. Such work is under way and will be reported later.

References

- Hsu, J.C. (1996); Multiple Comparisons Theory and Methods, Chapman and Hall, Boca Raton.
- Dunnett, C. W. (1955); A Multiple Comparison Procedure for Comparing Several Treatments with a Control, Journal of the American Statistical Association, 50, 1096-1121.
- Dunnett, C. W., and Tamhane, A. C. (1992); A Step-Up Multiple Test Procedure, Journal of the American Statistical Association, 87, 162-170.
- Dunnett, C. W., and Tamhane, A. C. (1991); Step-Down Multiple Tests for Comparing Treatments With a Control in Unbalanced One-Way Layouts, Statistics in Medicine, 10, 939-947.
- Green, J. W. (2008); Smaller experiments through improved experimental design and statistical methodology, in preparation. [Accepted for presentation at SETAC Warsaw, 2008]
- Hochberg, Y., and Tamhane, A.C. (1987); Multiple Comparison Procedures, Wiley, New York.
- Salsburg, D. S. (1986); Statistics for Toxicologists, Marcel Dekker, New York.
- Scheffe, H. (1953); A Method of Judging All Contrasts in the Analysis of Variance, Biometrika, 40, 87-104.
- Selwyn, M. (1988); Preclinical Safety Assessment, pp 231-272, in K. E. Peace, editor, Biopharmaceutical Statistics for Drug Development, Marcel Dekker, New York.
- Tamhane, A.C., Dunnett, C. W., Green, J. W., Wetherington, J D. (2001); Multiple Test Procedures for Identifying the Maximum Safe Dose, Journal of the American Statistical Association, 96, 835-843.
- Tatarazako, N., Oda, S., Watanabe, H., Morita, M., Iguchi, T. (2003); Juvenile hormone agonists affect the occurrence of male *Daphnia*, Chemosphere, 2003 (Vol. 53, No. 8) 827-833

ANNEX 3**Proposal for the enhanced TG 211****PROPOSAL FOR UPDATED GUIDELINE 211****Daphnia magna Reproduction Test**

(proposed changes are in bold in the text below)

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 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 (1) and 1994 (2).

2. The main differences between this and the **initial previous**-version of the Guideline (**dated April 1984**) 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.
- (e) **Annex 7 has been added to describe procedures for the identification of neonate sex if required. In line with previous versions of this guideline sex ratio is an optional endpoint.**

(...)

VALIDITY OF THE TEST

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

- the mortality of the parent animals (female *Daphnia*) does 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 is ≥ 60 .

(...)

Other parameters

44. 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. Growth measurements are

highly desirable since they provide information on possible sublethal effects which may be more useful than reproduction measures alone; the measurement of the length of the parent animals (i.e. body length excluding the anal spine) at the end of the test is recommended. Other parameters that can be measured or calculated include time to production of first brood (and subsequent broods), number and size of broods per animal, number of aborted broods, presence of male neonates (**reference to the validation report**) or ephippia and possibly the intrinsic rate of population increase (see Annex 1 for definition and **Annex 7 for the identification of the sex of neonates**).

(...)

ANNEX 7 of the enhanced TG 211 (all new text)

Guidance for the identification of the neonate sex

Production of male neonates can occur under changing environmental conditions, such as shortening photoperiod, temperature, decreasing food concentration, and increasing population density (Hobaek and Larson, 1990; Kleiven et al., 1992). Male production is also a known response to certain insect growth regulators (Oda et al., 2005). Under conditions where chemical stressors are inducing a decrease in reproductive offspring from the parthenogenic females, an increased number of males would be expected (“Validation report” reference). On the basis of available information, it is not possible to predict which of the sex ratio or of the reproduction endpoint will be more sensitive; however, there are indications (reference “validation report”, part 1) this increase in the number of males might be less sensitive than the decrease in offspring. Since the primary purpose of the Test Guideline is to assess the number of offspring produced, the appearance of males is an optional observation. If this optional endpoint is evaluated in a study, then an additional test validity criterion of no more than 5% males in the controls should be employed.

The most practical and easy way to differentiate sex of *Daphnia* is to use their phenotypic characteristics, as males and females are genetically identical and their sex is environmentally determined. Males and females are different in the length and morphology of the first antennae, which are longer in males than females (Fig. 1). This difference is recognizable right after birth, although other secondary sex characteristics develop as they grow up (e.g., see Fig. 2 in Olmstead and LeBlanc, 2000).

To observe the morphological sex, neonates produced by each test animal should be transferred by pipet and placed into a petri dish with test medium. The medium is kept to a minimum to restrain movement of the animals. Observation of the first antennae can be conducted under a stereomicroscope ($\times 10-60$).

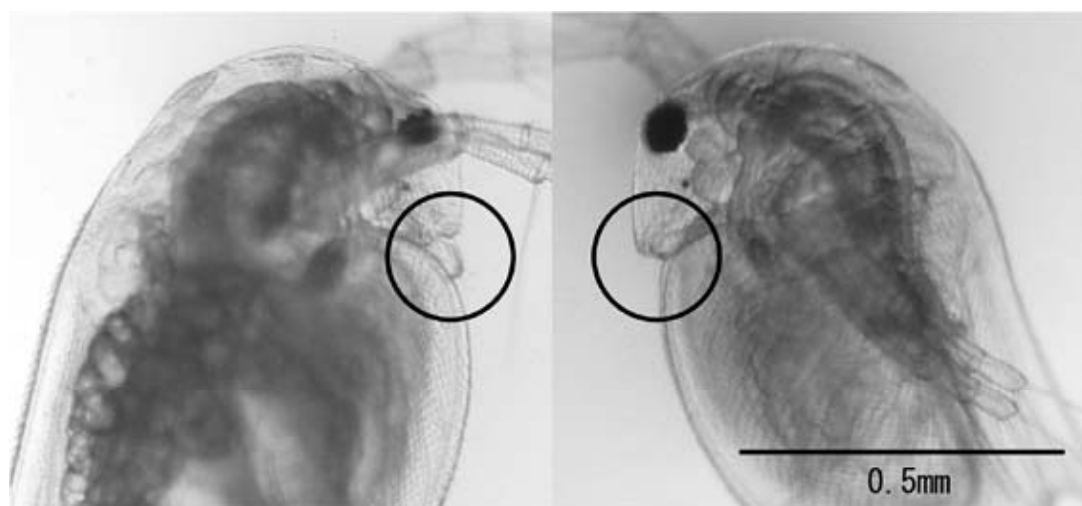


Fig. 1 24-hour-old male (left) and female (right) of *D. magna*. The male was from a mother exposed to 2 000ng/L of fenoxycarb (juvenoid IGR). Males can be distinguished from females by the length and morphology of the first antennae as shown in the circles.

References

- Olmstead, A.W., LeBlanc, G.A., 2000. Effects of endocrine-active chemicals on the development characteristics of *Daphnia magna*. *Environmental Toxicology and Chemistry* 19, 2107-2113.
- Hobaek A and Larson P. 1990. Sex determination in *Daphnia magna*. *Ecology* 71: 2255-2268.
- Kleiven O.T., Larsson P., Hobaek A. 1992. Sexual reproduction in *Daphnia magna* requires three stimuli. *Oikos* 65, 197-206.
- Oda S., Tatarazako N, Watanabe H., Morita M., and Iguchi T. 2005. Production of male neonates in *Daphnia magna* (Cladocera, Crustacea) exposed to juvenile hormones and their analogs. *Chemosphere* 61:1168-1174.
- Tatarazako, N., Oda, S., Abe, R., Morita M. and Iguchi T., 2004. Development of a screening method for endocrine disruptors in crustaceans using *Daphnia magna* (Cladocera, Crustacea). *Environmental Science* 17, 439-449.