Environmental sex determination occurs in a species when the sex of offspring is dictated by prevailing environmental conditions (Korpelainen 1990). Species exhibiting this form of sex determination can be found among rotifers, nematodes, polychaetes, crustaceans, insects, fish, and reptiles (Korpelainen 1990). In all cases of environmental sex determination, animals interpret cues from the environment that indicate whether male or female progeny would maximize population sustainability. Hormonal or metabolic pathways are altered in receptive individuals in response to the environmental stimuli that lead to the production of offspring of the desired sex. Human activity, including the introduction of xenobiotics into the environment, can disrupt this process. For example, exposure of turtle eggs to some polychlorinated biphenyls can skew sex ratios of offspring in favor of males. Juvenile hormone analogs (JHAs) mimic the action of methyl farnesoate, resulting in the inappropriate production of male offspring. The occurrence of an effect in the environment could have dire consequences on susceptible crustacean populations (Bliss 1939). This model assumes that the two chemicals elicit a common effect (altered sex ratios) by acting at different sites along the signaling pathway leading to sex determination.

Materials and Methods

Daphnid culture. Daphnids (Daphnia magna) were cultured in incubators at a density of 40 adults in 1 L of medium at a temperature and photoperiod of 20°C and 16 hr light. Algae (Selenastrum capricornutum), cultured in Bold’s basal medium, was used as a food source for daphnids during culturing and feeding. Juvenile hormone analogs (JHAs) represent a class of insecticides that were designed specifically to disrupt endocrine-regulated processes relatively unique to insects. Recently we demonstrated that the crustacean juvenile hormone methyl farnesoate programs oocytes of the crustacean Daphnia magna to develop into males. We hypothesized that insecticidal JHAs might mimic the action of methyl farnesoate, producing altered sex ratios of offspring. Daphnids were exposed chronically (3 weeks) to sublethal concentrations of methyl farnesoate, the JHA pyriproxyfen, and several nonjuvenile chemicals to discern whether excess male offspring production is a generic response to stress or a specific response to juvenile hormones. Only methyl farnesoate and pyriproxyfen increased the percentage of males produced by exposed maternal organisms. As previously reported with methyl farnesoate, acute exposure (24 hr) to either pyriproxyfen or the JHA methoprene caused oocytes maturing in the ovary to develop into males. We performed experiments to determine whether combined effects of a JHA and methyl farnesoate conformed better to a model of concentration addition (indicative of same mechanism of action) or independent joint action (indicative of different mechanisms of action). Combined effects conformed better to the concentration-addition model, although some synergy, of unknown etiology, was evident between the insecticides and the hormone. These experiments demonstrate that insecticidal JHAs mimic the action of the crustacean juvenile hormone methyl farnesoate, resulting in the inappropriate production of male offspring. The occurrence of such an effect in the environment could have dire consequences on susceptible crustacean populations. Key words: crustacea, Daphnia, endocrine disruption, invertebrate, methoprene, methyl farnesoate, mixtures, pyriproxyfen. Environ Health Perspect 111:919–924 (2003). doi:10.1289/ehp.5982 available via http://dx.doi.org/(Online 5 February 2003)
experimentation. Algae (1.4 × 10^6 cells) were provided to each 1-L culture twice daily, and offspring were removed from the cultures at least three times weekly. Cultures were nutritionally supplemented with a fish food homogenate, prepared as described previously (Baldwin and LeBlanc 1994) and provided to the cultures at 4 mg (dry weight) twice daily. Cultured daphnids reproduce asexually under these conditions, with virtually all progeny (> 95%) being female.

**Male progeny production during chronic exposure.** We determined previously that the strain of daphnids used in our laboratory produces male progeny in response to high population density and reduced food availability (Olmstead and LeBlanc 2001). We also demonstrated that culturing of daphnids under environmental conditions that permitted a basal level of male progeny production allowed for increased male production upon exposure to the JHA methoprene (Olmstead and LeBlanc 2001). Therefore, daphnids were exposed to various chemicals under conditions of high population density (15 daphnids in 200 mL of media) and low food level (2.1 × 10^6 cells provided twice daily), and the stimulation of male progeny production was evaluated.

Experiments were initiated with neonatal daphnids (< 24 hr old) and proceeded through approximately four brood cycles (21 days). Four treatment levels were evaluated for each chemical, and each treatment was replicated nine times. Test solutions were maintained at 20°C under a 16-hr light photoperiod. Solutions were changed and offspring removed every 3 days. Sex of individual offspring was determined microscopically (10× magnification), with males being discerned from females by the longer primary antennae (Olmstead and LeBlanc 2000).

Several chemicals were evaluated for their ability to stimulate male progeny production. Exposure concentrations for each chemical were within the range of concentrations that affected parthenogenetic reproduction in standard life cycle tests. The pesticidal JHA pyriproxyfen (Chem Service, West Chester, PA) was evaluated to further test our hypothesis that this class of compounds specifically stimulates male progeny production through its action as a methyl farnesoate agonist. Methyl farnesoate (synthesized by M. Feldlaufer, U.S. Department of Agriculture, Beltsville, MD; provided by H.H. Rees and G. Wainwright, University of Liverpool, Liverpool, UK) was used as the positive control (Olmstead and LeBlanc 2002). The herbicide atrazine (Chem Service) was evaluated because this chemical was reported previously to stimulate male progeny production (Dodson et al. 1999). Fenarimol (Chem Service) was selected because this fungicide functions as an antecdysteroid (Mu and LeBlanc 2002), and ecdysteroids have been implicated in male progeny production (Peterson et al. 2001). Pentachlorophenol (Chem Service), a polar narcotic and an uncoupler of oxidative phosphorylation (Schuurmann et al. 1997), was used to determine whether male progeny production occurs in response to general metabolic stress. Atrazine, fenarimol, and pentachlorophenol were selected for use in these experiments because they all had the potential to stimulate male offspring production although they are nonjuvenile in structure and function. Ethanol (Aaper, Shelbyville, KY) was assessed because this alcohol was used as a carrier solvent for the other chemicals and its potential effect on male production required evaluation. All chemicals (except ethanol) were dissolved in ethanol as a carrier solvent.

![Figure 1](image-url)  
Figure 1. Chemical structures of endogenous and synthetic terpenoid hormones. Juvenile hormone III and methyl farnesoate are endogenous to insects and crustaceans, respectively. Methoprene and pyriproxyfen are pesticides that function as juvenile hormone III mimics.

![Figure 2](image-url)  
Figure 2. Effects of various chemicals on male progeny production in daphnid populations. Bars represent the average and standard deviation (error bars) of nine individually evaluated daphnid populations. *Significantly different from the control populations (ANOVA, Dunnett’s t-test, p ≤ 0.05).
The concentration of carrier present in any given test solution never exceeded 0.005% vol/vol. Control solutions contained the same concentration of ethanol as was present in the respective chemical treatments. Significant differences (p < 0.05) among treatments were evaluated using analysis of variance (ANOVA) and Dunnett’s t-test (JMP software; SAS Institute, Cary, NC).

**Exposure to JHAs during oocyte development.** Methyl farnesoate was previously shown to program maturing oocytes in the ovary to develop into males (Olimestone and LeBlanc 2002). After ovarian maturation, the oocytes are transferred to the brood chamber of the maternal organism, where the embryos develop. Free-swimming neonates are released from the brood chamber upon completion of embryo development. The transfer of oocytes from the ovaries to the brood chamber coincides with the molting of the maternal organism’s exoskeleton, and release of neonates from the brood chamber coincides with the next molt. Maternal daphnids were exposed to concentrations of pyriproxyfen, methoprene, or methyl farnesoate during oocyte maturation, and sex of the resulting progeny exposed in the ovary was determined. Should pyriproxyfen and methoprene program sex of daphnids via the same mechanism as methyl farnesoate, then sex determination should occur during the same window of susceptibility.

Adult female daphnids carrying embryos in their brood chambers were selected from the cultures and placed individually in 50-mL beakers containing 40 mL of media. Beakers were examined every 12 hr for the presence of a molted exoskeleton. Forty-eight hours after detecting a molted exoskeleton, we transferred the daphnid to test media containing the appropriate concentration of the test chemical. The daphnid was maintained in this solution for 24 hr, which encompassed the sex-determining period of ovarian oocyte maturation. Daphnids then were transferred to juvenoid-free medium and maintained until the brood of offspring exposed to the juvenoid in the ovary was released. Food (*S. capricornutum*, 7 × 10^6 cells, and fish food homogenate, 0.2 mg dry weight) was provided to each beaker twice daily. Daphnids typically produce only female offspring under these nonstressed culture conditions. Sex of individual offspring was determined as described above. Results from these experiments were fitted to concentration–response curves with Origin software (MicroCal Software Inc., Northampton, MA) using the following concentration–response equation:

\[
R = \frac{100}{1 + 10^{\log(EC_{50}) - \log(C)}} \quad [1]
\]

where \( R \) is the combined response of chemicals \( x \) and \( y \) and \( C \) and \( C \) are the concentrations of chemicals \( x \) and \( y \). The power of this curve, \( p \), is the average of the slopes from the individual concentration–response curves of the two chemicals. The independent joint-action model (Bliss 1939) was generated with the following equation, which is derived from probability theory:

\[
R = R_x + R_y - R_x R_y \quad [3]
\]

where \( R_x \) and \( R_y \) are the responses for the individual chemicals \( x \) and \( y \), respectively.

Various combinations of pyriproxyfen and methyl farnesoate or methoprene and methyl farnesoate were then experimentally evaluated for the stimulation of male progeny production using the same methods as used with the individual chemicals described above. Model predictions of male offspring production were then generated for each chemical combination using the concentration-additivity model and the independent joint-action model. Model predictions were compared with actual results by calculation of coefficients of determination \( (r^2) \) for each model (Zar 1996). The model producing the highest coefficient of determination best represented the experimental results.

**Results**

**Increased male progeny production from chemical exposure.** Six chemicals were evaluated for their ability to stimulate male progeny production among daphnids. Only the juvenoid hormone methyl farnesoate and the JHA pyriproxyfen altered sex ratios of offspring in favor of males (Figure 2). Under these exposure conditions, pyriproxyfen was 2–3 orders of magnitude more potent at stimulating male progeny production than was methyl farnesoate. Altered sex ratios were not caused by differential embryo mortality because the total number of offspring produced among daphnids exposed to either methyl farnesoate or pyriproxyfen was not significantly different from the controls (data not shown). These results demonstrate that increased male production is not a generalized response of daphnids to chemical stress, but appears to be specific to juvenoid hormones.

**Male-sex determination during oocyte exposure.** We performed experiments to determine whether, like methyl farnesoate, JHAs determined the sex of daphnids during ovarian oocyte maturation. Maternal daphnids were exposed to the juvenoids under conditions that promoted the production of only female offspring. The sex of offspring that were present, as oocytes, in the ovaries of the maternal daphnids during juvenile exposure was determined. Exposure of oocytes to methyl farnesoate during ovarian development programmed the oocytes to develop into male offspring in a
concentration-dependent manner (Figure 3A) with an EC$_{50}$ of 87 nM. Pyriproxyfen stimulated male progeny production among oocytes during ovarian development (Figure 3B) with an EC$_{50}$ of 0.31 nM. Methoprene also stimulated oocytes to develop into males, but only at much higher exposure concentrations (EC$_{50}$ of 1,140 nM). The EC$_{50}$ value for male production by methoprene was approximately the concentration that is lethal for male production by methoprene was approximately the concentration that is lethal for methoprene–methyl farnesoate or methoprene and methyl farnesoate. (Figure 4). These models are presented as contour plots (Figure 4) to illustrate differences between the concentration-addition and independent joint-action models. The greatest difference between model predictions was in the shape of the contour lines across the surface. Contour lines were straight along the entire response surface when using the concentration-addition model (Figure 4A) and were concave using the independent joint-action model (Figure 4B). The concave character of the contour plot generated with the independent joint-action model indicates that combined effects predicted with this model are less than those predicted by simple concentration additivity.

Similar contrasts between the shape of the response surface were evident with the methoprene–methyl farnesoate combinations (Figure 4C,D). In addition, the independent joint-action model predicted a response surface that was less steep relative to the concentration-addition model, with greater differences predicted between the two models (Figure 4C,D). Contour lines generated from the independent joint-action model had virtually no slope at the lower methoprene concentrations. This implies that the lower methoprene concentrations, within the range evaluated, would have a minimal effect on male sex determination.

The incidence of male progeny production from actual binary combinations of the chemicals was then experimentally determined and compared with the two models of concentration addition and independent joint action. The expected (model) and measured (experimental) responses are presented in Tables 1 and 2. For both binary mixtures, the experimental results correlated better to the concentration additive model. Coefficients of determination ($r^2$) between observed and modeled results according to concentration additivity were 0.69 (pyriproxyfen–methyl farnesoate) and 0.60 (methoprene–methyl farnesoate). Lower $r^2$ values were derived between observed and modeled results when using the independent joint-action model (0.09, pyriproxyfen–methyl farnesoate; 0.14, methoprene–methyl farnesoate). Residuals, the measured minus the expected responses, were consistently lower when using the concentration additive model for all binary combinations used. Residuals also were typically greater than zero in both experiments. These results are consistent with the hypothesis that the JHAs alter sex ratios of offspring by the same mechanism as methyl farnesoate; however, some synergy exists between the JHAs and methyl farnesoate.

**Discussion**

Having previously established that the juvenoid hormone methyl farnesoate is a male sex determinant in daphnids (Olmstead and LeBlanc 2002), we hypothesized that insectidal JHAs also would influence the sex of offspring through a mechanism of methyl farnesoate agonism. We reported previously that exposure of maternal daphnids to the JHA methoprene altered sex ratios of offspring in favor of males (Olmstead and LeBlanc 2001). Those results are consistent with our current hypothesis. However, methoprene also may have elicted general stress upon the organisms, which may stimulate male sex determination among offspring. To test this possibility, we exposed maternal daphnids to several diverse chemicals, and determined the effects on offspring sex ratios. Only the juvenoid hormone methyl farnesoate and the JHA pyriproxyfen increased the percentage of male offspring born among exposed maternal daphnids. These experiments confirmed that the increased production of male progeny is not a generalized stress response of the daphnids and suggest that
insecticidal JHAs function as methyl farnesoate agonists. Consistent with previous observations with methyl farnesoate, both insecticidal JHAs caused male sex determination during ovarian oocyte maturation. In aphids, the absence of juvenile hormone causes loss of one of the sex chromosomes during oocyte maturation (Hales and Mirtler 1987). The resulting X0 embryos develop into males. We suggest that a similar mechanism of sex determination is operative in daphnids, where exposure to methyl farnesoate or JHA insecticides causes sex chromosome diminution to the male genotype. This unique period of susceptibility (oocyte maturation) that is common to both the juvenile hormone methyl farnesoate and the insecticidal JHAs further supports the hypothesis that the JHAs function as methyl farnesoate agonists in this crustacean species.

Peterson et al. (2001) reported that methoprene reduced the production of male progeny in Daphnia pulex after a 6-day exposure of adults. The reason for the discrepancy between this study and our results is not known. Perhaps experimental conditions favored the action of methoprene as a methyl farnesoate agonist in our studies but favored its action as an antagonist in Peterson et al.’s (2001) study. The herbicide atrazine was previously reported to stimulate male production by Daphnia pulicaria (Dodson et al. 1999). We were unable to demonstrate the stimulation of male progeny production by atrazine in the present study. Differences in toxicity of this herbicide to the different algal species used as daphnids food in the two studies could have caused differential food deprivation and resulting differences in male offspring production.

Binary combinations of either JHA with methyl farnesoate stimulated male progeny production in a manner that better correlated to the model for concentration additivity than with that for independent joint action. However, both models were deficient in defining the interactions because a synergistic response was evident between the JHAs and the juvenile hormone. The mechanism responsible for this synergy is not known. A likely scenario involves the ability of the JHAs to interfere with metabolism or clearance of the hormone by competitively binding to enzymes or active transporters that modulate activity or levels of the hormone. Similar synergistic interactions have been reported (Bigley and Vinson 1979; El-Guindy et al. 1980; Pratt 1975), and further research is required to illuminate the mechanisms behind this combined response. Although neither model precisely defined the combined action of the JHAs with methyl farnesoate, the greater concordance with the model for concentration additivity contributes further support that the JHAs stimulate male sex determination by acting as methyl farnesoate agonists.

Traylor and Davis (1996) reported a 48-hr LC50 for pyriproxyfen and Daphnia carinata of 250 nM. They also noted that exposure of daphnids to 31 nM pyriproxyfen for 14 days significantly reduced fecundity and stimulated resting egg production. Resting, or diapause, eggs are haploid eggs that require fertilization and typically are produced after the production of males by females who have entered the sexual reproductive cycle. Thus, it is likely that pyriproxyfen stimulated increased male offspring production in this experiment; however, sex of the offspring was not evaluated. Reduced fecundity (i.e., reduced parthenogenic production of offspring) was likely a consequence of entry of the organisms into the sexual reproductive phase. Schaefer and Miura (1990) similarly reported a reduction in fecundity of mixed populations of cladocerans and ostracods at pyriproxyfen exposure levels of 31 nM (Schaefer and Miura 1990).

Methoprene’s ability to alter sex ratios in some crustacean populations would be of limited toxicologic concern under recommended usage conditions, as discussed previously (Olmstead and LeBlanc 2001). Male sex determination occurred during acute exposure only at methoprene concentrations that were lethal to some portion of the population. These exposure levels are not likely to be of environmental relevance. However, methoprene also was demonstrated to stimulate male production under experimental conditions and exposure levels that were not lethal to the organisms (Table 2; Olmstead and LeBlanc 2002). Therefore, the sex-determining effect of methoprene was not an artifact of differential toxicity to male and female offspring.

The potency with which pyriproxyfen stimulates the production of male offspring may be of concern. Pyriproxyfen has been used historically in the United States for flea and tick control in veterinarian applications, and in fire ant bait (Center of Integrated Pest Management 2002). However, this insecticide is increasingly recommended for agricultural uses such as the control of white fly on cotton and scale insects on fruit trees (Center of Integrated Pest Management 2002). Although pyriproxyfen is not recommended for direct application to the aquatic environment, the possibility of runoff and leaching into aquatic systems exists, and there the biologic activity of this compound can remain up to 2 months depending upon the amount of organic material in the water (Schaefer et al. 1988). The extreme potency (100X to 1,000X that of methyl farnesoate) of pyriproxyfen and its ability to elicit effects after acute exposure warrants concern in its ability to alter sex ratios in some crustacean populations.

Alterations in sex determination could have dire consequences to populations of cyclic parthenogens such as D. magna. The production of male offspring is an early event in the transition from parthenogenic to sexual reproduction by these organisms. Sexual reproduction is critical to the survival of populations during periods of environmental adversity (i.e., winter), whereas parthenogenic reproduction allows for high fecundity and rapid population growth during periods of

---

**Table 1. Stimulation of the production of male progeny by binary combinations of pyriproxyfen and methyl farnesoate and model predictions of the combined exposures.**

| Pyriproxyfen (nM) | OBS | CA | IJA | OBS | CA | IJA | OBS | CA | IJA | OBS | CA | IJA |
|------------------|-----|----|-----|-----|----|-----|-----|----|-----|-----|----|-----|
| 0.11             | 15  | 15 | 5   | 12  | 30 | 12  | 86  | 58 | 37  | 89  | 86 | 77  |
| 0.19             | 49  | 35 | 18  | 71  | 50 | 24  | 98  | 71 | 46  | 99  | 90 | 80  |
| 0.33             | 100 | 66 | 56  | 100 | 75 | 58  | 100 | 85 | 70  | 100 | 94 | 89  |
| 0.59             | 100 | 80 | 88  | 100 | 91 | 89  | 100 | 94 | 92  | 100 | 97 | 97  |
| 1.04             | 100 | 97 | 98  | 100 | 98 | 98  | 100 | 98 | 98  | 100 | 99 | 99  |

**Table 2. Stimulation of the production of male progeny by binary combinations of methoprene and methyl farnesoate and model predictions of the combined exposures.**

| Methoprene (nM) | OBS | CA | IJA | OBS | CA | IJA | OBS | CA | IJA | OBS | CA | IJA |
|-----------------|-----|----|-----|-----|----|-----|-----|----|-----|-----|----|-----|
| 64              | 0   | 3  | 5   | 28  | 17 | 18  | 97  | 56 | 51  | 100 | 90 | 84  |
| 116             | 0   | 5  | 5   | 13  | 21 | 19  | 92  | 59 | 51  | 100 | 91 | 84  |
| 200             | 16  | 8  | 5   | 33  | 27 | 19  | 97  | 64 | 51  | 100 | 92 | 84  |
| 360             | 17  | 17 | 5   | 100 | 39 | 19  | 90  | 72 | 51  | 100 | 93 | 84  |
| 640             | 91  | 38 | 14  | 100 | 59 | 26  | 100 | 82 | 56  | 100 | 95 | 86  |

Observed results (OBS) are presented along with model predictions for concentration addition (CA) and independent joint action (IJIA) Data are presented as percentage males per brood. Each experimental data point (OBS) represents the average of five individual daphnids. Model predictions were derived from the concentration–response surface models depicted in Figure 4.
high resource availability. The aberrant production of males by insecticide exposure could interfere with sexual population growth, threatening sustenance of the population as well as populations of consumers that rely upon the daphnids as an energy source.

References

Baldwin WS, LeBlanc GA. 1994. Identification of multiple steroid hydroxylases in *Daphnia magna* and their modulation by xenobiotics. *Environ Toxicol Chem* 13:1013–1021.

Bergeron JM, Crews D, McLachlan JA. 1994. PCBs as environmental estrogens: turtle sex determination as a biomarker of environmental contamination. *Environ Health Perspect* 102:760–761.

Bigley W, Vinson S. 1979. Effects of piperonyl butoxide and DEF on the metabolism of methoprene by the imported fire ant, *Solenopsis invicta* Buren. *Pestic Biochem Physiol* 10:14–22.

Bliss C. 1939. The toxicity of poisons applied jointly. *Ann Appl Biol* 26:585–615.

Borst D, Tsukimura B, Laufer H, Couch E. 1994. Regional differences in methyl farnesoate production by the lobster mandibular organ. *Biol Bull* 186:9–16.

Center of Integrated Pest Management. 2002. North Carolina Agricultural Chemicals Manual. Raleigh, NC: North Carolina State University. Available: http://ipmwww.ncsu.edu/agchem/agchem.html [accessed 16 August 2002].

Chung ACK, Durica DS, Clifton SW, Roe BA, Hopkins PM. 1998. Cloning of crustacean ecdysteroid receptor and retinoid-X receptor gene homologs and elevation of retinoid-X receptor mRNA by retinoic acid. *Mol Cell Endocrinol* 139:209–227.

Dodson S, Merritt C, Shannahan J, Shults C. 1999. Low dose concentrations of atrazine increase male production in *Daphnia pulex*. *Environ Toxicol Chem* 18:1568–1573.

El-Guindy M, El-Sattar M, Madi S. 1980. Synergists as poten-tiatiors to juvenile hormone analogues in susceptible and fenitrothion-resistant strains of the cotton leafworm *Spodoptera littoralis* (Boisd). *Z Ang Ent* 90:520–525.

Gesser P. 1995. Isotopographic analysis of interactions: an update on applications and utility. *Toxicology* 105:161–179.

Hebert PDN. 1998. The population biology of *Daphnia* (Crustacea, Daphniidae). *Biol Rev* 53:387–426.

Horrocks S, Merritt C, Shannahan J, Shults C. 1999. Low dose concentrations of fenitrothion increase male production in *Daphnia pulex*. *Environ Toxicol Chem* 18:1568–1573.

Jones G, Sharp PA. 1997. Ultraspiracle: an invertebrate nuclear receptor for juvenile hormones. *Proc Natl Acad Sci USA* 94:13499–13503.

Korpelainen H. 1990. Sex ratios and conditions required for environmental sex determination in animals. *Biol Rev* 65:147–184.

Lauber H, Ahl JSB, Sagli A. 1993. The role of juvenile hormones in crustacean reproduction. *Am Zool* 33:365–374.

Laufer H, Biggers W. 2001. Unifying concepts learned from methyl farnesoate for invertebrate reproduction and postembryonic development. *Am Zool* 41:442–457.

MERRITT C, SHANNAHAN J, SHULTS C. 1999. Low dose concentrations of atrazine increase male production in *Daphnia pulex*. *Environ Toxicol Chem* 18:1568–1573.

Olmstead AW, LeBlanc GA. 2000. Effects of endocrine-active chemicals on the development of sex characteristics of *Daphnia magna*. *Environ Toxicol Chem* 19:2107–2113.

Schaefer C, Miura T. 1999. Chemical persistence and effects of S-31183, 2-(1-methyl-2-(4-phenoxyphenoxy)ethoxy)pyridine, on aquatic organisms in field tests. *J Econ Entomol* 83:1768–1776.

Schaefer C, Miura T, Dupras E, Mulligan F, Wilder W. 1988. Efficacy, nontarget effects, and chemical persistence of S-31183, a promising mosquito (Diptera: Culicidae) control agent. *J Econ Entomol* 81:1648–1655.

Schuurmann G, Segner H, Jung K. 1997. Multivariate mode-of-action analysis of acute toxicity of phenols. *Aquat Toxicol* 38:277–296.

Traylor K, Davis J. 1996. Sensitivity of *Daphnia carinata* sensu lato to the insect growth regulator, pyriproxyfen. *Ecotoxicol Environ Safety* 33:154–156.

Zer JH. 1996. Biostatistical Analysis. Upper Saddle River, NJ: Prentice-Hall.