Project Completion Report (R/UW-BEP-001)

Effect of Clean and Polluted Groundwater on Reproduction and Development of Daphnia

Period of contract: **July 1, 2001 – June 30, 2002**

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Project Summary

Title:
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Project I.D.:
R/UW-BEP-001

Investigator:
Principal Investigator - Stanley Dodson, Professor
Department of Zoology
University of Wisconsin-Madison

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Background/Need:

There is a need for an efficient and effective whole-animal screen for ecological effects of pollutants (see Table 1). Previous work in our lab has focused on herbicides and vertebrate hormones. Several of these chemicals changed normal *Daphnia* development and sex determination, at concentrations found in groundwater. The current proposed research focuses on laboratory assays of low-level concentrations of common-use insecticides that have been reported to be contaminants of groundwater. Information on insecticide effects will contribute toward our evaluation of the *Daphnia* reproduction assay. This assay has been developed and used successfully in the lab for a number of chemical contaminants. Preliminary results suggest that it is crucial to survey insecticides. Our assays provide information on "contamination," (whether due to parent compounds, breakdown products, and chemical mixtures) because we look at the whole-animal response. This is an important feature, because there are so few cost-effective assays that can detect sublethal effects on whole organisms.

Our ultimate goal is to establish the *Daphnia* development and reproduction assays as a surrogate, cost-effective bioassay system for risk assessment. Our results suggest these assays do have value, because *Daphnia* are sensitive to ambient concentrations of contaminant herbicides, and because *Daphnia* respond in characteristic ways to some vertebrate hormones such as thyroxine and some steroid hormones.

Objectives:

The project objective was to characterize effects of common-use insecticides classified as endocrine disruptors on development and sex determination of *Daphnia* magna, using well-established short- (six day) and long-term (30 day) life-table type assays. The endpoints include:

growth in length, molting frequency, and population growth rate
fecundity and sex ratio
deviations from normal morphology in neonates and adults.

Methods:

We exposed *Daphnia* to common-use insecticides in two kinds of assays. These assays have been developed and polished in our lab over the last few years. In the first assay, adult female *Daphnia* are grown under environmental conditions that naturally induce about 50 percent males. After six days (the equivalent of two instars or molting periods), and a renewal of culture medium, the offspring are scored as to gender, survival, and morphology, and the adult females are scored as to

survival, fecundity, morphology, and size. Animals grown in uncontaminated artificial lake water, or in water contaminated with low levels (100 ppb or lower) of a common-use insecticide that occurs in groundwater. In the second assay, neonates are followed with daily observations throughout their lifetime. Animals are measured each day, and we record details of reproduction and development.

Results and Discussion:

Effects of estrogen modulating compounds

Toxaphene (polychlorinated camphenes), an insecticidal mixture of over 670 congeners and widely classified as estrogenic, was the only chemical tested that affected sexual differentiation in *D. magna* (Fig. 1). *Daphnia magna* exposed to 50 and 100 μg/L toxaphene produced 17-44 percent more male clutches compared to the control *D. magna* ($p<0.01$, Fig.1). In addition to increasing male production, toxaphene exposure (50 μg/L) decreased the average clutch size from 17 to 13 individuals ($p=0.02$). At the higher concentration of 100 μg/L toxaphene had no effect on fecundity. Toxaphene exposure concentrations below 50 μg/L had no effects on reproduction or growth (Table 2).

Another putative estrogenic insecticide o'p'-DDT, did not alter the natural sex ratio at any of the concentrations tested. However, at 100 μg/L o'p'-DDT decreased survivorship, killing the majority of the *D. magna* by day three of the assay. Di-*n*-butyl phthalate had no observable effects on the developmental or reproductive endpoints examined in *D. magna* (Table 2).

Effects of thyroid modulating pesticides

Three herbicides reported as disrupting normal thyroid function in vertebrates; acetochlor, metribuzin and alachlor, did not affect sexual differentiation, survivorship, resting egg production, or morphology in *D. magna* (Table 2). The only thyroid modulating herbicide that appeared to have any effect on *D. magna* was acetochlor, and effects were seen below the listed EC_{50} (48 hours) for *Daphnia* (16 mg/L; Tomlin 1994). An EC_{50} value represents the concentration at which 50 percent of the organisms show any toxic effect (Effective Concentration). Adult *D. magna* with a six-day exposure to 100 μg/L acetochlor were significantly smaller (4.16 mm) than their respective control (4.28 mm; $p=0.04$). However, the clutch size of the acetochlor-exposed *D. magna* was not affected by the smaller adult size, hence a smaller brood chamber.

Effects of pesticides with LH, androgenic, or insulin activity

Pesticides with reported activity in vertebrate androgen systems had no effects on *Daphnia*. The o'p'-DDT metabolite p'p-DDE, which has been shown to function as a hormone (androgen) antagonist in vertebrates, did not impair reproductive or developmental processes in *Daphnia* at sublethal concentrations; however, p'p'-DDE was toxic to *D. magna* at 100 μg/L (Table 1 and 2). Likewise, the androgenic herbicide, linuron, had no toxic effects on *D. magna* at the concentrations tested.

Amitraz an insecticide that has been shown to inhibit insulin secretion in rats (Abu-Basha *et al*. 1999) did not exert toxicity on the daphnid developmental and reproductive processes monitored in this study. The herbicide 2,4-D that has been correlated with elevated LH levels in humans (Garry *et al.* 2001) had no observable effects on the developmental or reproductive endpoints examined in *D. magna*.

Effects of pesticides with no known endocrine activity

Five pesticides were examined that currently have no known impacts on vertebrate endocrine systems: cyanazine, diflubenzuron, chlorsulfuran, diquat and metolachlor. Exposure to 100 μg/L cyanazine significantly reduced the number of *D. magna* that reproduced to 23 percent ($p=0.01$), while only four percent of the control daphnids failed to produce a clutch in the 12 days monitored. In addition, the average clutch size of the reproducing adult *D. magna* exposed to 100 μg/L cyanazine was significantly smaller ($p=0.04$) with an average clutch size of 14 individuals vs. 11 individuals in the control. Cyanazine had no effect on sex determination.

Diflubenzuron was highly toxic to *D. magna,* significantly decreasing survivorship at 0.01 μg/L ($p=0.005$; Fig. 2). The LC_{50} value for diflubenzuron in this six-day assay fell between 0.10 and 0.01μg/L. Lower diflubenzuron concentrations elicited no adverse effects on growth/molting or reproduction of the daphnids (Table 2).

The remaining pesticides with no known endocrine activity, chlorsulfuran, diquat and metolachlor, did not affect *D. magna* at the concentrations tested (Tables 1 and 2). The herbicide chlorsulfuran has been reported in surface waters at very low concentrations (Table 1). This study indicates that these environmentally relevant concentrations appear to have no apparent effects on *Daphnia* (Tables 1 and 2). Diquat did not affect any of the reproductive or development endpoints monitored in *D. magna* at the concentrations tested. Metolachlor, an herbicide that has been found at concentrations as high as 143 μg/L in Midwestern U.S. streams and rivers, had no effects on *Daphnia* at similar concentrations (Battaglin *et al*. 2000, Table 2).

Conclusions/Implications/Recommendations:

Applicability of Daphnia *as a screen for endocrine modulating compounds*

Several pesticides affected reproductive and developmental process in *Daphnia;* however, there does not appear to be a pattern between pesticides with particular endocrine classification (reported from vertebrate systems) and effects on specific reproductive and developmental processes in *Daphnia*. Toxaphene a common groundwater contaminant was the only estrogenic compound that affected sexual differentiation in *Daphnia.* Toxaphene exposure (50 and 100 μg/L) increased male production in *Daphnia,* and yet several known estrogenic chemicals (o'p'-DDT, and Di-*n*-butyl Phthalate) had no effect on sexual differentiation in *Daphnia* (Fig. 1, Table 2). This suggests that estrogens may not play a direct role in *Daphnia* sexual differentiation. However, data in other studies imply that weakly estrogenic compounds such as dieldren and atrazine do affect sex ratio in *Daphnia*. Dodson and colleagues found a decreased proportion of males among young produced by *Daphnia* exposed to dieldrin (1999 a), and an increased proportion of males among young produced by *Daphnia* exposed to atrazine (1999 b).

The question of whether or not pesticides elicit estrogenic activity in *D. magna* is further complicated because there is no universal "gold standard" of estrogen action among vertebrate bioassays (Coldham *et al.* 1997). Toxaphene, although commonly referred to as having estrogenic properties, has also been classified as having thyroid and antiandrogen properties (Waritzet *et al.* 1998, Arcaro *et al.* 2000). Toxaphene has also been reported as not having estrogenic properties (Table 1; Palmer *et al.* 1998). Classification of various chemicals as estrogenic or nonestrogenic is still debated in the scientific community. Therefore, to draw generalizations about all estrogenic compounds is premature.

Developmental and reproductive impairments in *D. magna* by the thyroid modulating compounds (TMC) were inconsistent. Acetochlor was the only chemical with known thyroid activity in vertebrates to have any observable effects on *D. magna*. Acetochlor reduced adult size in the six-day exposure (Table 2). Acetochlor did not decrease daphnid fecundity in this study. Therefore, it is possible that the reduction in growth rate was an endocrine related response; however, the remaining TMC's tested did not have similar affects on growth rates. Although this study did not find consistent evidence of TMC on *D. magna*, it is conceivable that *D. magna* would be affected by TMC. It is unknown if *D. magna* have a thyroid system similar to vertebrates, but other invertebrates do respond to thyroxine. Chino *et al.* (1994) isolated thyroid hormones in the sea urchin (*Hemicentrotus pulcherrimus*) and determined that thyroid hormones function in the formation of the adult rudiment. Thyroxine has also been found to accelerate larval development in the Crown of Thorns Starfish (*Acanthaster planci*; Johnson and Cartwright 1996). Based on results from this study, using *D. magna* to screen for chemicals with thyroid activity may not be effective.

Other vertebrate hormones that have affected invertebrates, such as vertebrate-type steroidal androgens,have disrupted crustacean growth and reproduction. Olmstead and LeBlanc (1998) found that exposure of female daphnids to testosterone significantly inhibited the rate of development of their abdominal process. However, *D. magna* fecundity was not reduced when exposed to the androgenic compounds linuron and DDE at concentrations tested.

Due to the lack of chemicals cited as having effects on insulin or LH activity, only one pesticide was tested from each of these categories. Lutenizing hormone is known to stimulate the crustacean Sand Shrimp (*Crangon crangon)* ovaries resulting in an increase in the number of the generative oocytes, and the number of oogonia (Zukowska-Arendarczyk 1981). Therefore, an herbicide such as 2,4-D, which elevates LH levels in humans, may have a measurable effect on *D. magna* even though the presence of LH in *D. magna* is still unknown (Garry *et al*. 2001); however, based on the reproductive and developmental endpoints examined in this study, I found no effects of 2,4-D on *D. magna*. An insulin-like immunoreactive material was found in the mussel *Mytilus edulis*; therefore, if a chemical can disrupt normal insulin function in vertebrates then it may also be possible to disrupt similar pathways in invertebrates (Fritsch *et al.* 1976). However, amitraz did not disrupt any of the reproductive or development processes examined in *D. magna*.

Many pesticides have never been tested for endocrine effects, and no chemicals have been tested against all hormone systems (Table 1). Therefore, several chemicals with no known endocrine activity were assayed. These chemicals did not elucidate any patterns regarding effects on reproductive and developmental endpoints, therefore, no conclusions can be drawn regarding their effects on the daphnid endocrine system.

Although generalization regarding endocrine classification cannot be drawn from this study, several pesticides did elicit toxic effects on developmental and reproductive processes in *D. magna*. Cyanazine is a triazine herbicide in the same family as atrazine, an herbicide which Dodson *et al.* (1999) found decreased *Daphnia* sex ratio. Although cyanazine had no effect on sex determination in this study, as atrazine did in the Dodson *et al.* (1999) study, cyanazine did lower fecundity and the number of adults that produced offspring. It is possible that *D. magna* reproduction is mediated by endocrine functions, and that 100 μg/L cyanazine disrupts normal endocrine function involved in *D. magna* reproduction.

Based on results from this study, it appears unlikely that *D. magna* would make a good screen for vertebrate endocrine modulating compounds because there was no apparent pattern between pesticides reported with estrogenic, androgenic, thyroid, insulin or LH activity in vertebrates and effects on *D. magna*. However, this *D. magna* assay which monitored sublethal effects related to endocrine-regulated processes such as growth, fecundity and sex determination consistently detected ecologically relevant effects of these pesticides on *D. magna* at environmentally relevant concentrations.

Ecological Implications (daphnid sensitivity)

Any chemical that affects an organism's fitness (i.e. survival, growth rate, fecundity, and/or sexual determination) is likely to have effects that transcend individual responses and affect the entire ecosystem. Several of the pesticides tested in this study appear to disrupt individual developmental and reproductive processes at environmentally relevant concentrations. Toxaphene is a persistent insecticide heavily used in the United States until its use was restricted in 1982. Toxaphene accumulates in ecosystems due to its lipopohilic, persistent, volatile nature and appears in regions where it has never been used (DeGeus 1999). Toxaphene has been detected in groundwater at concentrations ranging from 0.1 to 1 mg/L (Bell *et al.* 1996). These concentrations are higher than the concentrations in this study that impaired reproductive and developmental processes in *Daphnia*. Therefore, the effective levels reported in this study are realistic exposures that suggest that *Daphnia* exposed to toxaphene in the wild could be at risk of impaired reproductive development.

Toxaphene not only affected sexual differentiation, but it also decreased the average clutch size (Table 2). Sanders (1980) came to similar conclusions, finding that 0.12 μg/L toxaphene significantly reduced the production of young over a 21-day period in *D. magna*. Toxaphene may affect *Daphnia* population growth rates, since a reduction in mean clutch size (fecundity) is likely to result in a decrease in *Daphnia* population growth rate. This decrease in population growth rate may be further amplified by a reduction in asexual females, which reproduce faster than their sexual counterparts do.

Cyanazine and acetochlor had similar negative impacts on *D. magna*. This study shows reproductive impairment of *D. magna* at a concentration (100 μg/L) substantially lower than the current EC_{50} listing (42-106 mg/L; Tomlin 1994). Acetochlor disrupted the normal growth patterns of *D. magna*. Acetochlor exposed (100 μg/L) *D. magna* were, on average, 0.12 mm smaller than their respective controls. The acetochlor EC_{50} (48 hours) for *Daphnia* is currently listed as 16 mg/L (Tomlin 1994) an order of magnitude higher than the concentration that decreased *D. magna* growth in this study.

Diflubenzuron has been regarded as one of the least hazardous insecticides (to vertebrates), primarily due to its specificity to selectively affect chitin synthesis inhibitors (Marx 1977). Chitin, a polysaccharide, is a major component of insect cuticles. Chitin synthesis inhibitors inhibit molting, killing the organism before maturation and preventing reproduction. Nontarget organisms, like crustaceans, also produce chitin. Currently, the listed *Daphnia* EC_{50} for diflubenzuron (48 hours) is 7.1 μg/L, while this six-day *D. magna* assay found diflubenzuron toxic to *D. magna* at 0.01μg/L (Table 2). Similarly, Savitz and Wright (1994) found that substantially lower concentrations (0.78 μg/L) than the reported EC_{50} affected naupliar survival and development in the copepod, *Eurytemora affinis.* Decreased survivorship will have larger ecological ramifications than decreases in fecundity and growth rate.

Daphnia play a key ecological role in lakes and ponds as the dominant herbivores that aid in the transfer of energy from autotrophs to the top of the food web. Determining the vulnerability of *D. magna* to sublethal but environmentally relevant pesticide concentrations is important for the establishment of environmental health standards that will maintain ecological integrity. Pesticides have been widely broadcast and are routinely found in surface and groundwaters at concentrations ranging from 0.001 to 100 μg/l (Table 1). Based on results from this study, *D. magna* are vulnerable to many pesticides found within this range in nature. *Daphnia magna* may be particularly vulnerable to chitin synthesis inhibitors such as diflubenzuron, which reduce survivorship at very low concentrations. Reproductive and developmental processes in *D. magna* are affected by acetochlor, cyanazine, and toxaphene at concentrations found in surface waters. The *D. magna* bioassay appears to be an invaluable tool in determining sublethal but environmentally relevant toxicity of pesticides on aquatic communities and *D. magna* may serve as a useful indicator of water quality.

Related Publications:

Kashian, D.R. and S.I. Dodson. 2002. Evaluation of the use of *Daphnia* for toxicity testing of endocrine disruptors: Effects of vertebrate hormones on development and sex determination in *Daphnia magna.* In Review: submitted to *Journal of Aquatic Ecosystem stress and recovery.*

Kashian D.R., 2002. Reproduction and development in *Daphnia:* The role of hormones, pesticides and detoxification. PhD dissertation. University of Wisconsin-Madison, Wisconsin.

Kashian, D.R. 2002. An investigation of xenobiotic detoxification through P-450 induction in *Daphnia magna.* To be submitted to *Environ. Toxicol. Chem*.

Kashian, D.R. and S.I . Dodson. 2002. Disruption of developmental and sexual determination processes in *Daphnia magna*: A survey of 10 agricultural chemicals. In Review: submitted to *Environ. Toxicol. Chem.*

Key Words:
toxicity, toxaphene, Acetochlor o'p'-DDT, Di-n-butyl phthalate, p'p-DDE, linuron, alachlor, metribuzin, amitraz, 2,4-D chlorosulfuran, cyanazine, diflubenzuron, metolachlor and diquat

PROPOSAL NARRATIVE

INTRODUCTION:

Pesticide-contaminated groundwater is of concern, because much of the population depends on groundwater for drinking. Extensive herbicide use in agricultural areas has resulted in widespread occurrence of herbicides in agricultural streams and shallow groundwater (Nowell et al. 2000, Sullivan & Richards 1996).

Currently, there is considerable uncertainty in estimating pesticide exposure risks. For example, most contamination occurs as pesticide mixtures, whereas most toxicity and exposure assessments are based on controlled experiments with a single contaminant. In addition, some breakdown products, for which there are no established standards or guidelines, may have effects similar to their parent pesticides (Barbash and Resek 1996). A whole-animal assay, using *Daphnia* (the water flea), can be used to test for the presence of chemicals (i.e., individual, mixtures, or breakdown products) that interfere with normal *Daphnia* growth and reproduction.

Pesticides can be transported to groundwater by water that percolates below the crop root zone. Bason and Colborn (1998) estimated that nationally 9,850 community and 445,000 rural drinking water wells are contaminated. The USEPA conducted a survey examining the pesticide content of drinking water wells, and found that 10 percent of community wells and 4 percent of rural wells were contaminated with at least one pesticide at or above minimum reporting level (USEPA 1999). Other recent surveys (US-EPA 1998, USGS 1999) found approximately half of the wells tested contained detectable amounts of two or more pesticides. These pesticides are typically present at low concentrations (1 mμ g/L) exceeding USEPA safe drinking water levels in less than one percent of sampled wells (USGS 1999, Kolpin *et al.* 1998). Regardless of these supposed low or "safe" levels of contamination, based on current toxicity assessments, the question remains as to the long-term safety of exposure to these chemicals and their effects on aquatic life and human health (Snell and Carmona 1995, Shurin and Dodson 1997).

Daphnia (the water flea) can be used to screen for the presence of groundwater contaminants that interfere with normal *Daphnia* reproduction. The use of *Daphnia* (an inhabitant of surface waters) in a groundwater study can be justified in several ways:

- Ecologically important *Daphnia* species live in surface waters (ponds and lakes) that are often fed by groundwater springs.
- Results from assays can be used to generate "uncertainty factors" in drinking water policy.
- The *Daphnia* reproductive bioassay routinely gives information relevant to endocrine and developmental disruption for two generations of an invertebrate whole animal.
- Results from *Daphnia* assays can indicate the need for further (expensive and time-consuming) assays using mammals.

Research in our lab and others (Baldwin et al. 1997, LeBlanc and McLachlan 1999) is exploring links between (1) nonlethal effects of pesticides on *Daphnia* development and sex determination, (2) effects of vertebrate and arthropod hormones on *Daphnia*, and (3) similarities between *Daphnia* and vertebrate endocrine systems. Contaminants that affect *Daphnia* sexual development will certainly have important ecological effects, because of the key ecological position of *Daphnia* as a dominant grazer on algae and as a major food for fish. These contaminants may also be of concern for health issues in vertebrates; an effect on *Daphnia* would be justification for designing further testing with vertebrates, such as rodents. There is currently a strong interest in sublethal effects of environmental chemicals on development of organisms, including humans. Low dose effects are subtle but apparently real, and they do not always follow a simple linear dose-response relationship (Kaiser 2000).

We have developed two biological assays sensitive to nonlethal but ecologically important effects on *Daphnia* (Dodson et al. 1999a; Kashian & Dodson 2000). Preliminary results suggest that short-term and long-term assays can give different results, as if *Daphnia* have a detoxification system (such as the P450 enzymes) that takes about six days to be activated. For this reason, we assay chemicals with both the short and long protocols. Some subtle effects are not evident if short protocols are used, and some short-term effects are not seen in long-term exposures.

- The short-term assay has the advantages of speed (six days), suitability (development in whole animals), and trans generational integration (2 generations: adults & offspring).
- The long-term assay (30 days) has the additional advantages of increased sensitivity and relevance to *Daphnia* population ecology. This life-table assay produces data on age-specific and accumulative effects of chemicals, and allows calculation of population growth rates.

Several common groundwater contaminants have been shown (in the laboratory) to modify *Daphnia* sexual determination or morphological development, including nonylphenol (Shurin and Dodson 1997, Baldwin et al. 1997), dieldrin (Dodson et al. 1999a), atrazine (Dodson et al. 1999b), and methoprene (Peterson et al., 2001). The insecticide endosulfan had a nearly significant tendency to inhibit male production (Dodson et al. 1999a). The insecticide carbaryl affects normal carapace development (Hanazato and Dodson 1993).

In this proposed research, we will expose *Daphnia* to a variety of common-use chemicals that have a variety of modes of action (Table 1). These chemicals are insecticides typical of Wisconsin groundwater (Battaglia and Tauchen 1995).

The project objective was to characterize effects of ambient concentration levels of common-use insecticides, found in groundwater, on the development and sex determination of *Daphnia magna*, using short (six day) and long-term (30 day) life-table type assays. The endpoints included: growth in length, population growth rate, molting frequency, fecundity, sex ratio, and deviations from normal morphology in neonates and adults. The results of this research provide to augment our development of a surrogate, cost-effective bioassay system for risk assessment.

PROCEDURES AND METHODS:

The research used both short- and long-term assays (six days and ca. 21 days). A single short-term assay (40 control jars and 40 treatment jars) can be completed in six days, and one graduate student (50 percent RA) performed at least two assays per week, with the assistance of an hourly helper (to assist in maintaining cultures, setting up and transferring assays, and scoring). It was necessary to replicate each assay. Data was recorded and preliminary analyses were performed at the end of each assay. It was necessary to do assays at a minimum of three dilutions to estimate exposure-response relationships.

A single long-term assay (ca 12 control jars and 12 treatment jars) can be completed in 30 days, and one person (50 percent) RA can do one assay at a time, if a short-term assay is also in progress. Most long-term assays were duplicated.

The overall control water for these exposure treatments was an artificial high-hardness lake water (**Combo medium**, Kilham et al. 1998). The short-term *Daphnia* Reproductive Assay were performed as described in Dodson et al. (1999b), with current modifications (including observing clutches of individual adults, and feeding *Ankistrodesmus*). Each assay was made up of 40 replicates per treatment, and typically two treatments: a control and an exposure treatment.

The long-term *Daphnia* assay was comprised of a control and treatment series of replicates. One *Daphnia* was used per 30 ml, and individuals are introduced as neonates. Individuals were measured every day (or three days for later adults), and the number of male and female offspring are recorded. As in the short-term assay, any morphological variation is recorded. This assay lasts for 30 days, which is most of the life span of an average individual *D. magna*. The algae was grown in

Combo, settled and then resuspended in the test water. This allowed the *Daphnia* to be exposed to 100 percent test water.

Test animals are transferred to new medium after three days, and neonates produced from day six to eight were scored for the following endpoints: sex ratio of neonates, survivorship of adults and neonates, fecundity, adult instar duration, body length, resting egg production, neonate morphology (body form, length of swimming setae, neck teeth), and neonate lipid metabolism.

The raw data were analyzed with appropriate statistics: Chi-square test for goodness of fit for sex ratio and adult survivorship (where the control is the expected distribution) or one-way anova for the other parameters.

RESULTS AND DISCUSSION: *I put this information in the first Results and discussion… Not sure why there are two sections for this and the methods.*

CONCLUSIONS AND RECOMMENDATIONS: The use of sublethal endpoints in toxicity testing allows for a better understanding of toxicity than solely using mortality; mortality being the most frequently used endpoint by many agencies (ASTM 2001; EPA 1989). Based on results from this study the six-day *Daphnia* bioassay allows for the detection of ecologically relevant toxicity that other acute assays may miss. The use of *Daphnia* does not appear to be a good predictor of vertebrate endocrine disruption; however, daphnid growth and reproduction impairments may reflect endocrine-related impairments occurring in other zooplankton. Further research needs to be conducted to determine if *Daphnia* can be a model system for other zooplankton endocrine disruption. The *Daphnia* bioassay is useful in detecting the presence of toxic substances in groundwater through monitoring of reproductive and developmental endpoints.

REFERENCES

ASTM. 2001. Standard guide for conducting *Daphnia magna* life-cycle toxicity tests. In Annual book of ASTM standards, E 1193-97. *American Society for Testing and Materials*, Philadelphia, Pa., pp 418-433.

Baldwin W.S., S.E. Graham, D. Shea, and G.A. LeBlanc. 1997. Metabolic androgenization of female *Daphnia magna* by the xenoestrogen 4-nonylphenol. *Environm Tox Chem* 16:1905-1911.

Barbash, J.E., and E. A. Reske, 1996. Pesticides in groundwater distribution, trends, and governing factors, v. 2 of Gilliom, R. J., ed., Pesticides in the hydrologic system: Chelsea, Mich., Ann Arbor Press, 588 p.

Bason C.W. and T. Colborn. 1998. US application and distribution of pesticides and industrial chemicals capable of disrupting endocrine and immune systems. *Journal of Clean Technology, Environmental Toxicology, and Occupational Medicine* 7: 147-156.

Battaglia, R.J. and R.T. Tauchen. 1995. Wisconsin Agricultural Statistics Service. Wisconsin DATCAP, Madison, Wis.

Dodson, S.I., C.M. Merritt, L. Torrentera, K.M. Winter, C.K. Tornehl, and K. Girvin. 1999a. Dieldrin reduces male production and sex ratio in *Daphnia galeata mendotae*. *Toxicol Ind Health* 15:192-199.

Dodson, S.I., C.M. Merritt, J. P. Shannahan, and C.M. Schults. 1999b. Low doses of Atrazine increase male production in *Daphnia pulicaria*. *Environmental Toxicology and Chemistry*. 18:1568-1573.

Kaiser, J. 2000. Panel cautiously confirms low-dose effects. *Science* 290:695-697.

Kashian, D.R. and S.I. Dodson. 2000. The effects of xenobiotics on sex determination and reproduction in *Daphnia*. Poster. SETAC annual meeting.

Kilham, S.S., D.A. Kreeger, S.G. Lynn, C.E. Goulden, L. Herrera. 1998. COMBO: A defined freshwater culture medium for algae and zooplankton. Hydrobiologia *in press*.

Kratzer, C.R. 1999. Transport of diazinon in the San Joaquin River Basin. *California Journal of the American Water Resources Association* 35:(2)379-395.

LeBlanc, G.A. and J.B. McLachlan. 1999. Molt-independent growth inhibition of *Daphnia magna* by a vertebrate antiandrogen. *Environmental Toxicology and Chemistry* 18:1450-1455.

Nowell, L.H., P.D.Capel, and P.D. Dileanis. 2000. Pesticides in stream sediment and aquatic biota-Distribution, trends, and governing factors, v. 4 of Gilliom, R.J., ed., Pesticides in the hydrologic system: Chelsea, Mich., Ann Arbor Press. *in press*.

Peterson, J.K., D.R. Kashian, and S.I. Dodson. 2001. Methoprene and 20-OH-ecdysone affect male production in *Daphnia pulex*. *Environmental Toxicology and Chemistry in press*.

Shurin, J.B. & S.I. Dodson. 1997. Sublethal toxic effects of cyanobacteria and nonylphenol on environmental sex determination and development in *Daphnia*. *SETAC Journal* 16:1269-1276.

Snell, T. W. and M.J. Carmona. 1995. Comparative toxicant sensitivity of sexual and asexual reproduction in the rotifer Branchionus calycilflorus. *Environmental Toxicology and Chemistry* 14: 415-420.

Sullivan, D. J. and K. D. Richards. 1996. Pesticides in streams of western Lake Michigan drainages, Wisconsin and Michigan, 1993-95: U.S. Geol. Fact Sheet FS-107-96, 4p.

US EPA. 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA 600/4-89/001. U.S. Environmental Protection Agency, Cincinnati, Ohio.

US EPA. 1998. Wisconsin Migrant Worker Camp Drinking Water Quality Study. USEPA Region V, Safe Drinking Water Branch. Released July, 1998. (Joe Janczy, Life Scientist).

USGS. 1999. The quality of our nation's waters – nutrients and pesticides. US Geological Survey circular 1225, available at http://water.usgs.gov/pubs/circ/circ1225/

Table 1. Summary of pesticides and their reported known status as endocrine disrupters, used in a bioassay examining pesticide affects on *Daphnia*. The carrier is reported for each pesticide tested.

Pesticide	**Carrier**[a]	**Highest & lowest concentration tested:** μg/L	**Range of pesticide concentrations in surface or ground water (Reference):** μg/L	**Hormone system affected in vertebrate systems (Reference)**
Acetochlor	Acetone	100	>0.01-25.1 (Battaglin *et al*. 2000)	Thyroid (Hurley *et al*. 1998)
Alachlor	Water	100-10	0.05-50 (Scribner *et al.* 2000)	Thyroid (Wilson *et al*. 1996)
Amitraz	Acetone	10	N/A	Insulin (Abu-Basha *et al.* 1999)
Chlorsulfuran	Acetone	1-0.001	0.013 (Battaglin *et al.* 2000)	Unknown
Cyanazine	Acetone	100-10	0.1-80 (Scribner *et al.* 2000)	Unknown
p'p'-DDE	Acetone	100-10	0.07 -300 (Abbassy *et al.* 1999; LBDA 1985)	Androgen (Kelce *et al.* 1995)
o'p'-DDT	Acetone	100-1	0.001- 300 (LBDA 1985)	Estrogen (Lascombe *et al*. 2000)
Diflubenzuron	Water	100-0.001	0-16 (Savitz and Wright 1994)	Unknown
Diquat	Water	100-10	3 (Fernandez *et al.* 1998)	Unknown
Linuron	Water	100-10	2800 (Caux *et al.* 1998)	Androgen (Lambright *et al.* 2000)
2, 4-D	Acetone	100	0.26 (Donald *et al.* 2001)	Luteinizing hormone (Garry *et al.* 2001)
Metolachlor	Water	100-1	0-143 (Battaglin *et al.* 2000)	Unknown
Metribuzin	Water	100	0.1-10 (Scribner *et al.* 2000)	Thyroid (Porter *et al.* 1993)
Di-n-butyl Phthalate	Acetone	100	177 (Cincinelli *et al.* 2001)	Estrogen (Jobling *et al*. 1995) Anti-androgen (Ashby and Lefevre 2000)
Toxaphene	Acetone	100-0.0001	0.001- 6000 (Bell *et al.* 1996)	Estrogen (Palmer *et al.* 1998) Thyroid (Waritz *et al.* 1998) Antiestrogen (Arcaro *et al.* 2000)

[a] Acetone concentrations were <0.06mg/L in culture medium

Table 2. Pesticides that significantly (α=0.05) affected various response variables in *Daphnia magna* acute assays. The number represents the pesticide concentration where the effect was observed in μg/L. Dashes represent no effect. EC_{50} values in μg/L, taken from Tomlin (1994). Tomlin (1994) did not report EC_{50} concentrations for all the pesticides tested, therefore they are reported as N/A.

Pesticide tested	***Increased male production***	***Decreased fecundity***	***Decreased growth***	***Decreased survivorship***	**EC_{50} (48 hours) for *Daphnia)***
acetochlor	--	--	100	--	1600
alachlor	--	--	--	--	1000
amitraz	--	--	--	--	0.035
chlorsulfuran	--	--	--	--	37,000
cyanazine	--	100	--	--	4200-10,600
o'p'-DDT	--	--	--	100	N/A
p'p-DDE		--	--	100	N/A
diflubenzuron	--	--	--	.01	7.1
diquat	--	--	--	--	N/A
linuron	--	--	--	--	75
metolachlor	--	--	--	--	2500
metribuzin	--	--	--	--	450-3500
di-*n*-butyl phthalate	--	--	--	--	N/A
toxaphene	100, 50	50	--	--	N/A
2,4-D	--	--	--	--	23,500

Principal Investigator (15 percent time spent on the project**)**
STANLEY I. DODSON **Curriculum Vitae**

Formal Education

- **Ph.D.** Department of Zoology. University of Washington, Seattle
- **B.A.** Yale University

Professional Activities and Awards:

US Patent: *Daphnia* Reproductive Bioassay for Testing Toxicity of Aqueous Samples and Presence of an Endocrine Disruptor. P96080US (Awarded 1999)

- **Editorial Board: *Ciencia* Ergo Sum** (1995-present)
- **Univ. Library Committee,** UW-Madison (1995-1999)
- **Editorial Board: *Hydrobiologia*** (1994-present)
- **Editorial board: Ecology** (1999-present)

Professional Experience:

- **Founder of *BioAssay,* Inc.,** *Madison, Wisconsin.* *(1997)*
- **Professor** Department of Zoology. UW-Madison (1982-present)
- **Chair** Department of Zoology. UW-Madison (1991-1993)
- **Associate Chair** Dept. of Zoology. UW-Madison (1989-1991)
- **Associate Professor** Dept. of Zoology. UW-Madison (1975-1982)
- **Assistant Professor** Dept. of Zoology. UW-Madison (1970-1975)

Books:

S.I. Dodson (ED.) **Ecology**. Oxford University Press. 1998.
S.I. Dodson et al. **Ecology Readings**. Oxford University Press. 1999.
M.B. Balcer, N.L. Korda, and S.I. Dodson. **Zooplankton of the Great Lakes**. UW Press, 1984

Selected Recent Publications:

Peterson, J.K., D.R. Kashian, and S.I. Dodson. 2001. Methoprene and 20-OH-ecdysone affect male production in *Daphnia pulex*. *Environm. Toxicology & Chemistry in press*.

Dodson, S.I., S.E. Arnott, and C.L. Cottingham. 2000. The Relationship in Lake Communities between Primary Productivity and Species Richness. *Ecology*. *in press*.

Dodson, S.I., C.M. Merritt, J.P. Shannahan, and C.M. Schults. 1999. Low doses of Atrazine increase male production in *Daphnia pulicaria*. *Journal of the Society for Environmental Toxicology and Chemistry*. 18:1568-1573.

Dodson, S.I., C.M. Merritt, L. Torrentera, K.M. Winter, C.K. Tornehl. 1997. An *In Vivo Daphnia* Reproduction Bioassay to Screen to Endocrine Disruption Activity in Freshwater. *Environmental Health Perspectives*. 15:192-199.

Weigel, B., J. Lyons, S.I. Dodson, L.K. Paine, and D.J. Undersander. 1999. Using stream arthropods to compare riparian land-use practices on cattle farms in southwestern Wisconsin. *J. North American Benthol. Soc.* in review.

Shurin, J. and S.I. Dodson. 1996. Sublethal Toxic Effects of Cyanobacteria and Nonyl phenol on Environmental Sex Determination and Development in *Daphnia*. *Environmental Toxicology & Chemistry* 16:1269-1276.

Dodson, S.I., T. Hanazato and P. Gorski. 1995. Behavioral Responses of *Daphnia pulex* exposed to Carbaryl & *Chaoborus* Kairomone. *Environ. Toxicol. and Chem.* 14:43-50.

Hanazato, T. and S.I. Dodson. 1995. Synergistic Effects of Low Oxygen Concentration, Predator Kairomone, and a Pesticide on the Cladoceran *Daphnia pulex*. *Limnology and Oceanography*. 40: 700-709.

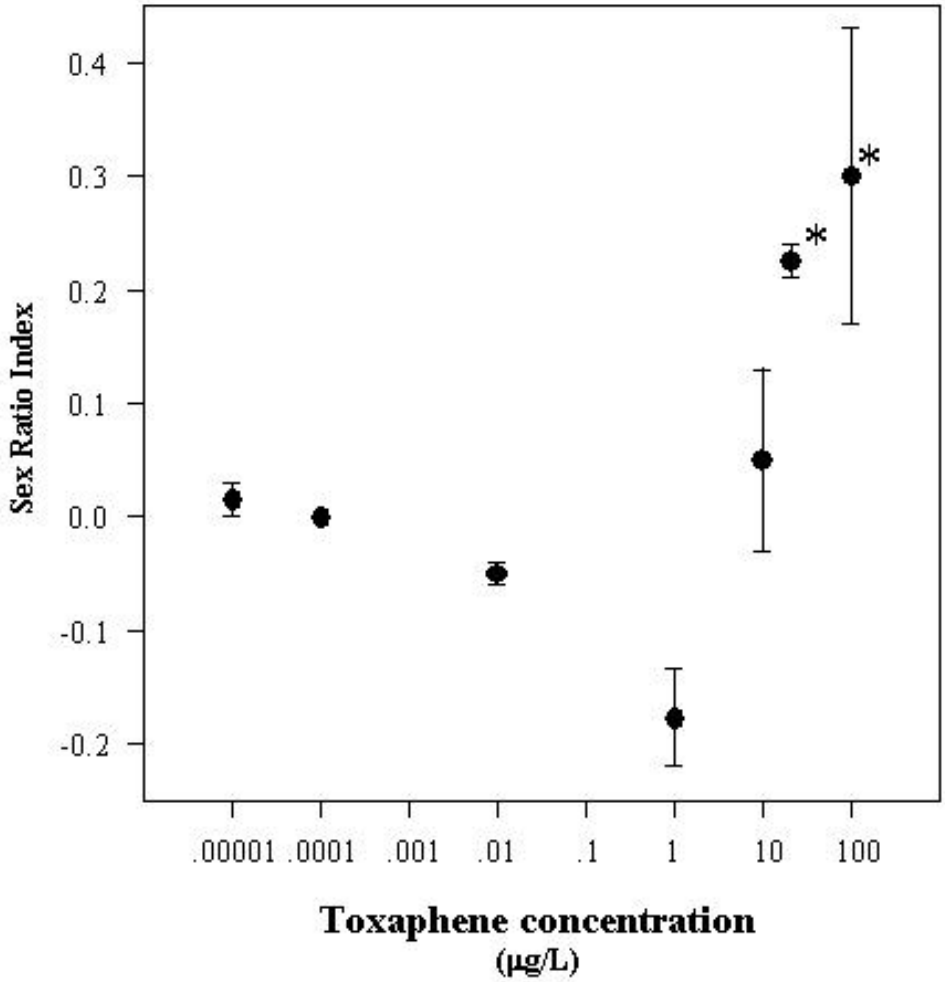

Fig. 1. Effects of toxaphene on offspring sex of *D. magna*. Data is presented as the mean sex ratio index ± standard error of two assays (n=2) that independently compared the frequency of male and female broods of *D. magna* with and without toxaphene exposure at several concentrations. The sex ratio index is the difference between the treatment and control sex-ratio (male/total offspring) in each assay. Significance was determined for individual assays (G-Goodness of Fit test) with the p-value combined using the method given in Sokal and Rohlf (1995). * indicates significance at α=0.05.

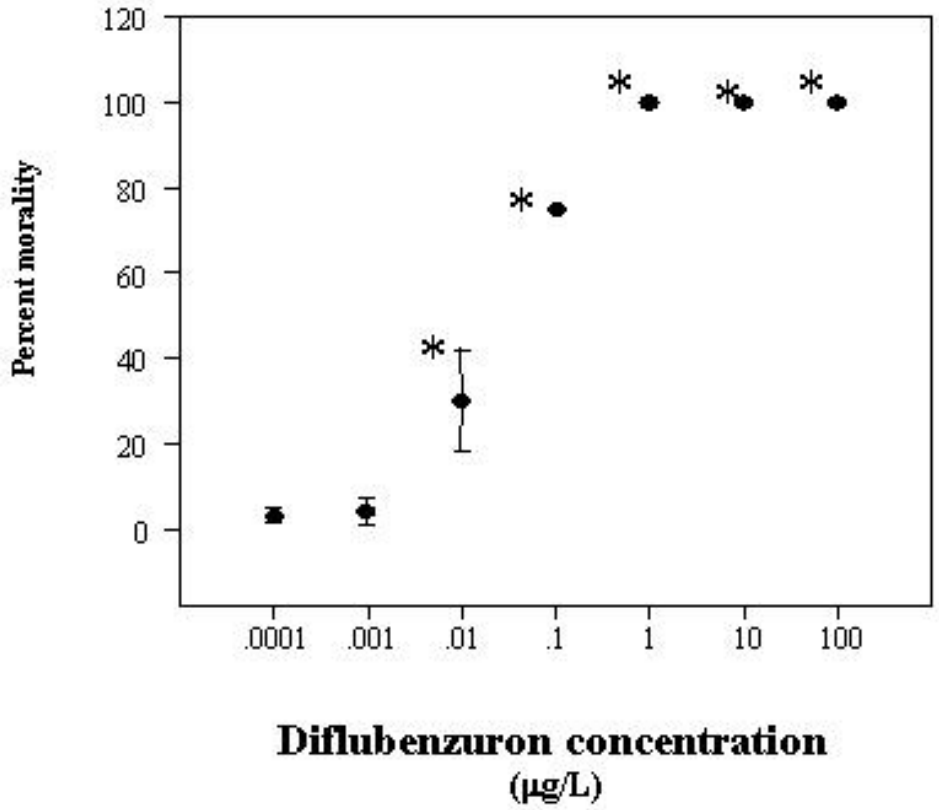

Fig. 2. Effects of diflubenzuron on short-term (6-days) adult *Daphnia* survival. indicates significance at α=0.05; G-Goodness of Fit test. Error bars represent the standard error between assays.