THE TOXICITY OF LIQUID EDGER HERBICIDE ON THE BRINE SHRIMP *ARTEMIA FRANCISCANA*

by

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ABSTRACT

The effect of 0.0001 and 0.00001 mg/L concentrations of Liquid Edger (LE) herbicide was evaluated on the parental generation, F_1 and F_2 of *Artemia franciscana*. The criteria evaluated were parental life span, survival to adulthood, fecundity, fertility and sex ratio. Only the parental generation was directly exposed to the herbicide. Only the F_2 generation was evaluated for survival to adulthood and sex ratio. The 0.00001mg/L treatment of LE reduced the life span of females from the parental generation ($P<0.005$). The longevity of F_1 females whose parents were exposed to the 0.00001mg/L treatment was significantly reduced ($P<0.005$). Parental females that were exposed to either of the treatments released significantly fewer broods than those from the controls ($P<0.005$). F_1 females did not have significantly fewer broods ($P>0.005$). Parental females that were exposed to either of the treatments had significantly lower fecundity and fertility than did control females ($P<0.005$). F_1 females from treated parents did not have significantly lower fecundity or fertility than did control F_1 females ($P>0.005$). Liquid Edger did not affect survival to adulthood or sex ratio of the F_1 or F_2. Liquid Edger caused female debility and the F_1 generation showed effects probably caused by residues of pesticide that were passed from the mother to the offspring.

RESUMEN

En la presente investigación se evaluó el efecto de las concentraciones 0.0001 y 0.00001mg/L del herbicida “Liquid Edger” sobre la generación parental, filial 1 y 2 de *Artemia franciscana*. Los criterios evaluados fueron longevidad parental, sobrevivencia a adulto, fecundidad, fertilidad y proporción de sexos. El tratamiento 0.00001 mg/L de “Liquid Edger” redujo la longevidad de las hembras de la generación parental ($P<0.005$). La longevidad de las hembras de la filial 1 cuyos padres fueron expuestos al tratamiento 0.00001 mg/L se redujo significativamente ($P<0.005$). Las hembras expuestas a los dos tratamientos de LE produjeron significativamente menos camadas que las hembras del control ($P<0.005$). Las hembras de la filial 1 no redujeron el número de camadas ($P>0.005$). En la generación parental, la fecundidad y fertilidad de las hembras expuestas a los dos tratamientos fue significativamente menor que las del control ($P<0.005$). No hubo reducción significativa en fecundidad y fertilidad en las hembras de la filial 1 ($P>0.005$). No se afectó la sobrevivencia a adulto y relación de sexos en las filiales 1 y 2. “Liquid Edger” causó debilidad de las hembras y los efectos vistos en la filial 1 posiblemente fueron causados por residuos de pesticida que la madre le transmitió a su descendencia.

To God, my family and friends.

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1 INTRODUCTION

Pesticides are physical, chemical or biological agents that are used to control, repel and combat agricultural pests that devastate crops and to eradicate insect-borne diseases (Sailaja, 2006; Preston, 2001). Pesticides are widely used throughout the world in agriculture to protect crops and in public health programs to control diseases transmitted by vectors or intermediate hosts. They have contributed to the enhancement of economic, health, and food conditions; however they have caused environmental contamination and generated an ecological impact. Pesticides can eliminate the useful species and result in proliferation of resistant plagues. Also they generate potential risks for the health of people exposed to them (Bull, 2006; Madrigal, 2002; Hoyos, et al. 1996).

When pesticides are released, they have an impact on every component and phase of the environment (Bhalli, 2006; Pastor, 2001; Gots, 1993). First, they enter the air, soil and water; throughout these phases they affect all life forms, because they are not specific and they affect many non-targeted organisms (Sailaja, 2006; Lee et al., 2002; Maroni et al., 2000). Humans are the organisms that receive the highest concentrations of contaminants, because they occupy the top of the food chain (Vuyyuri, 2006, Madrigal, 2002; Vallejo, 1996).

It is important to emphasize that in addition to acute intoxication, pesticides are responsible for several adverse effects on human and animal health. Occupational exposure or exposure

to contaminated water, air, or food may cause chronic diseases, genetic mutations, cancer, congenital malformations (teratogenesis), endocrine or hormonal alterations, reproductive problems, and immunological system problems (Costa et al., 2006; Bul et al., 2006; Paz et al., 2002; Nivia, 2000).

Pesticide use (including herbicides) is widespread and misuse of them has generated health problems for persons who are exposed directly or indirectly, and wide contamination of natural resources (Bull, 2006; Lucero et al., 2000; Hoyos et al, 1996).

The short-term toxic effects of acute exposure to pesticides are well known, but the long-term genotoxic effects (mutagenic, carcinogenic, and teratogenic) are not so well known. These last effects are often produced by chronic exposure to low doses (Paz et al., 2002; Ramirez et al., 2002; Hoyos, 1996; Bolognesi et al., 1993).

Liquid Edger herbicide has a wide domestic use and its genotoxicity studies are rare. Its principal ingredient is arsenic, which has genotoxic effects in different biological models (Basu, 2001). Arsenic is a naturally occurring metal in aquatic ecosystems, but its levels are increasing due to pollution. In ocean water the average residues are (2-3ug/L of arsenic) (Moore et al.1984 and Blanck et al, 1989 in Tisler, 2002). It was found that in rivers that crossed industrial areas and received runoff from agricultural areas treated with herbicides contained residues of 1-20µg/L and 10-33 µg/L arsenic respectively (Moore et al., 1984 in Tisler, 2002).

Artemia is an invertebrate component of the fauna of hypersaline aquatic ecosystems. It plays an important role in the energy flow of the food chain and it is used as a laboratory bioassay organism (Kanwar, 2007). The brine shrimp *Artemia franciscana* is an organism frequently used to detect many mutagen effects (Grosch, D. S. 1967, 1970, 1973, 1976; Martinez 1987; Squire, 1970; Squire and Grosch, 1970; Squire, 1973), using survival to adulthood and reproductive ability as evaluation criteria.

Invertebrate species are used primarily as true sentinel organisms on the basis of abundance, sampling facility, and their wide spectrum of ecological characteristics and sensitivity to chemicals. The use of invertebrates as bioindicators or bioaccumulators is therefore implemented before vertebrates, but their applications are marginal and primarily involve developmental toxicity and genotoxicity testing (Kanwar, 2007).

In this work, the effect of concentrations of 0.0001 and 0.00001 mg/L of Liquid Edger was evaluated on the parental generation, F_1 and F_2 generations of *Artemia franciscana*. The criteria evaluated were fecundity, fertility, number of broods, survival to adulthood and sex ratio. The F_2 was only evaluated in terms of survival and sex ratio.

The present work contributes to knowledge related to the chronic effect of Liquid Edger herbicide on *Artemia franciscana,* which is very important because it is an organism that is well adapted to high salinity and plays an important role in its ecosystem. Brine shrimp feed on microorganisms such algae and bacteria and help rid the lake water of contaminants such

phosphorus and nitrogen (Kanwar, 2007). It serves as food for many shore species of birds and is a major food source for fish and invertebrates in both fresh and salt water aquaculture.

The objective of this study was to determine the effects of 0.0001 and 0.00001 mg/L concentrations of Liquid Edger on *Artemia franciscana*. The criteria for evaluation included survival to adulthood, longevity of brine shrimp, average number of broods per female, average fecundity per female, average fertility per female and sex ratios of progeny.

2 Literature Review

2.1 *Artemia* sp.

Artemia is an anostracan crustacean that inhabits inland salt lakes, coastal lagoons and solar saltworks (Persoone, 1987). They are distributed in all continents except Antartica (Triantaphyllidis et al.1998). The abiotic factors that determine their presence and distribution are high salinity, temperature, ionic composition and biotic interactions. These biological communities vary in environment attributes such as permanence, seasonality, and predictability (Lenz, 1987). The brine shrimp is able to tolerate extreme environment conditions because it has various important adaptations like the interchangeable (diapausing cysts versus nauplii) life cycle.

The genus *Artemia* (Crustacea, Anostraca) is a complex of eight bisexual species and many parthenogenetic types. In the Old World both bisexual and parthenogenetic populations exist, and only two bisexual species have been described for the New World, these are *Artemia persimilis* (Piccinelli and Prosdocimi) and *Artemia franciscana* (Kellogg). *Artemia franciscana* is widely distributed in North, Central, South America and the Caribbean and it has been highly studied, whereas *A. persimilis* is distributed only just in Argentina and Chile (Gajardo et al., 2004; Gajardo et al., 2001; Cohen et al., 1999). Although *Artemia franciscana* is endemic to the New World both permanent and temporary populations also

exist worldwide, due to introductions of different strains (Browne, 1991).

Its survival and reproduction are deeply dependent on the interaction between salinity and temperature (Kappas et al, 2004). *Artemia* can tolerate brief exposures to temperatures as extreme as -18 to 40 ° C although most strains do not survive <6ºC or >35ºC. The tolerance to different temperatures is strain specific (Browne et al. 1988). The optimal temperature for cyst hatching and adult grow out is 24-29°C, but there are differences between strains, for the San Francisco bay strain it is 22 degrees °C as compared to 30 °C for the Great Salt lake strain (Browne, 2000). They can live in fresh water for about 5 hours before they die. According to Hammer and Hurlbert (1992) *Artemia franciscana* prefers salinity greater than 38 ppt (approx. 1.027 specific gravity). However, Squire (personal communication, 2008) stated that while *A. franciscana* from San Francisco kept at a specific gravity of 1.023 survived to maturity at levels equal to those kept at 75 ppt. (1.054 specific gravity), adult life span and reproductive ability were reduced by more than 90%. This emphasizes that simple survival to adulthood may not be a valid criterion for judging optimal salinities. With relation to pH, *Artemia* grows in neutral and alkaline environments (8-9 pH), and cyst hatchability ability decreases when pH is low (Browne, 2000). When the salinity increases, the dissolved oxygen content of brine decreases. Thus *Artemia* has compensated for low O_2 levels by being able of synthesize the respiratory pigment haemoglobin. The haemoglobin enables *Artemia* to live longer and to obtain more oxygen from the external medium (Gilchrist, 1956). It has been reported that *Artemia* lives in environmental with less than 1.0mg/L oxygen, and also

above saturation point, when the algae flora increase oxygen level (Hontoria 1993, Cisneros, 2002).

"They are well adapted to high salinity and play an important role in its ecosystem. Brine shrimp feed on microorganisms such algae (*Chlamydomonas, Tetrahedron* and *Dunaliella*) and bacteria and help rid the lake water of contaminants such phosphorus and nitrogen. Under optimal conditions, brine shrimp can live up to three months or more. However, due to changes such as temperature and food supply in the lake, the average life cycle is closer to 1½ months. Other variables that can affect a brine shrimp's growth and survival include light, pH, oxygen, and salinity of the water" (Kanwar, 2007). Light plays a role in brine shrimp hatching" (Vanhaecke et al.,1981).

2.2 Brine Shrimp *Artemia* sp.

The "brine shrimp", *Artemia*, belongs to the phylum Arthropoda, subphylum Crustacea, class Branchiopoda, order Anostracha, family Artemidae and genus *Artemia* (Leach, 1819). The genus *Artemia* is a complex of sibling species and superspecies, defined by the criterion of reproductive isolation. Early taxonomists assigned species names to populations with different morphologies, collected at different temperatures and salinities. Afterward, all brine shrimp was referred to as *Artemia salina* Linnaeus 1758. Unfortunately, some authors continue this practice today in spite of the different species and even racial characteristics. Reproductively isolated populations or clusters of populations have different names:

- *A. salina* Linnaeus 1758: Lymington, England (now extinct), Mediterranean area;
- *A. tunisiana* Bowen and Sterling 1978 synonym of *A. salina*;
- *A. parthenogenetica* Barigozzi 1974, Bowen and Sterling 1978: Europe, Africa, Asia, Australia;
- *A. urmiana* Gunther 1900: Iran;
- *A. sinica* Yaneng 1989: Central and Eastern Asia;
- *A. persimilis* Piccinelli and Prosdocimi 1968: Argentina and Chile;
- *A. franciscana* superspecies: Americas, Caribbean and Pacific islands, including populations reproductively isolated in nature like *A.*(*franciscana*) *franciscana* Kellogg 1906 and *A.(franciscana) monica* Verrill 1869 (Mono Lake, California) (Stappen. 1996).

2.3 Artemia franciscana

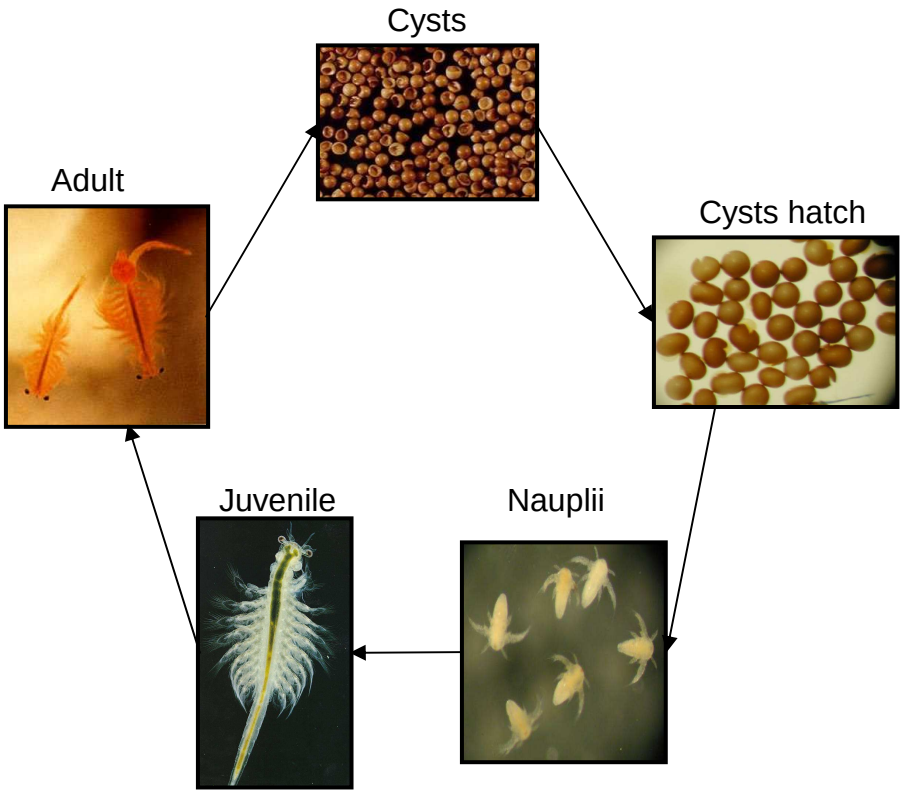

Figure 2-1 Life cycle of *Artemia*

2.3.1 Reproduction

Artemia adults are sexually dimorphic (Figure 2-1). Female is larger than male and it has a brood pouch or uterus behind the 11th pair of thoracopods. Male has large claspers (modified second antennae, also called “graspers”) that easily distinguish them from the females and it has a paired penis in the posterior part of their trunk. In some species and populations of

Artemia (for example, Europe), males may be rare (or absent) and females reproduce by parthenogenesis. Female *Artemia* apparently contain a limited number of undifferentiated oogonial stem-cells which undergo mitoses, producing an undifferentiated oogonium and an oogonium that differentiates into a primary oocyte and undergoes meioses (Squire, 1970; Iwasaki, 1973), just as continuing spermatogenesis depends upon a stem cell population of spermatogonia in the male. Any damage to this undifferentiated oogonial stem-cell population may permanently reduce fecundity and fertility (Squire, 1970) just as gonial damage in males is well known to produce such damage.

Artemia franciscana reproduce both oviparous (cysts) and ovoviviparous (nauplii) manner. The type of reproduction depends of the environmental conditions, for example if there are high salinity and low oxygen levels it will reproduce oviparously more frequently. During mating, males deposit sperm in the female ovisac where eggs are fertilized. In oviparous reproduction, the embryo is covered with a cyst wall by the shell gland. Once fertilized, eggs quickly undergo cleavage and development through the gastrula stage; they enter a state of metabolic dormancy (diapause) and after one to a few days, cysts are released by the female (oviposition). Multiple batches of cysts may be released at intervals of every few days by the same female (Persoone et al., 1980).

Artemia female can produce two types of eggs; rarely thin-shelled “summer eggs” and thick-shelled, brown “winter eggs”. The former continue developing and hatch quickly after being released and in the latter their development is arrested at about early gastrula stage. When

they have dried in the encysted form, they survive in a metabolically inactive state (termed cryptobiosis) for up to 10 or more years while still retaining the ability to survive extreme environmental conditions (Persoone, 1980). In fact, Squire (personal communication) has kept a jar of commercially obtained cysts from San Francisco *Artemia* for 30 years in the freezer at -32°C, and although the hatchability has declined somewhat, they are still quite good and produce healthy adults.

Alternatively, females may give birth to ovoviviparous broods in which the naupliius can be clearly seen inside of the ovisac, is not encysted with a shell, and lacks the embryonic chorion (structure secreted by shell glands of the maternal reproductive tract), although they are surrounded by a fertilization membrane and a thin fibrous cuticle (Criel and Macrae, 2002).

2.3.2 Embryology

Cleavage of the developing egg is total and yolk is equally distributed among blastomeres. While within the female brood sac, egg development continues rapidly though cleavage and blastula stages. Eggs are then deposited in the environment where they may remain encysted, with embryonic development arrested at about early gastrula stage. At this time, there are about 4,000 cells in the embryo and these are highly organized, but no organs are discernible. When encysted eggs are exposed to favorable conditions (rehydration), the eggs swell and rapid development of the embryo resumes, resulting in completion of the nauplial stage.

Hatching occurs in about 1-2 days, depending on temperature. For the first few hours, the nauplius stays within a hatching membrane that hangs beneath the cyst shell. This is also called the “umbrella stage” in which development of the nauplius is completed (Stappen, 1996).

2.3.3 Larval Stages and Growth

The larval stage of *Artemia* has 14 instars, at each instar they molt. They reach their sexual maturity when about 12 molts are completed and their terminal size when 14 molts are completed. Instar 1 begins at the moment the brownish-orange (color given by stored yolk material) nauplius emerges. The body consists mainly of a head and its length is approximately 0.4-0.5 mm. They are able to swim freely after about 12-20 hrs. Their swimming speed depends on temperature. Its “head” has three pairs of appendages and a ventral mouth covering by a labrum. The appendages are: a pair of small first antennae (antennules), a pair of well-developed second antennae, and a pair of mandibles. The antennules have sensorial function, the second antennae have locomotory and filter-feeding function and the mandibles have food uptake function. There is a small median naupliar eye that senses light. The posterior end of the nauplius there is a short, undifferentiated, and unsegmented structure which will became in a trunk. The nauplius has incomplete digestive tract, thus they does not immediately feed. Their energy source is the stored yolk (Browne, 1991).

The instar 2 begins when the nauplius molts and it becomes the metanauplius. The metanauplius is about 0.6 mm in length and translucent in color. Their trunk region enlarged and continues to lengthen and differentiate through the next series of molts. Different to nauplius, the metanauplius swim vigorously because their second antennae are more developed and help them to better move. Its digestive tract is developed and starts filter-feeding. They feed on microalgae, bacteria, and detritus. There are three molts into this stage. When organisms are in later metanaupliar stage, they have developed mouthpart appendages (maxillules and maxillae) and a longer thoracic region, with some definition of thoracic segments. After that, there are seven postnaupliar (larval) stages. At these stages the antennae get smaller and paired thoracic appendages start forming. These appendages become more numerous, long and functional at each stage. The compound eyes become more fully developed, the labrum is reduced in size, and abdominal segments become defined (Persoone, 1980). Subsequently, there are five postlarval stages. In these stages, the antennae continuing reducing size, there is multiplication of ommatidial facets in the compound eyes, lengthening of the eyestalks, and sexual organs are formed. Completion of the 17th molt marks the end of post-embryonic development and the beginning of the final adult stage. At the 17th molt occur, the post-embryonic development end and beginning of the final adult stage. Brine shrimp grow extremely rapidly. The adult stage is reached about three weeks after hatching. At adult size, biomass is about 500 times more than the nauplius biomass. Adults may live up to about 3 months (Abatzopoulos, 2002).

2.3.4 Adult

Artemia has an elongated body, its body size is about 8 mm in length but it can vary. It is formed by head, thorax and abdomen. The head has a pair of antennae (antennules), a pair of mandibules, a pair of maxillules and maxillae and two stalked complex eyes. In both sexes, the antennules are short and the maxillules and maxillae are greatly reduced in size. The mandibles are enlarged and modified as claspers in males, but they are short and thickened in females. The anterior part of the body is not covered by a shield or carapace. It has 20 trunk segments. The first 11 trunk segments are classified as thoracic segments and bare paired, paddle-like appendages called phyllopods. The phyllopods are used for swimming (ventral side up), feeding, and respiration. Posterior to the thorax segments, there are seven abdominal segments that don't bear appendages. The last body segment bears a pair of long tail filaments and there is the anus. "During swimming, appendages move in a rhythmic and wave-like pattern, at a frequency of about 5-10 waves per second. Each wave of movement starts out in posterior segments and then, rapidly and sequentially, progresses into more anterior segments. During the "power stroke" of each cycle of movement, the paddle-like appendages push water in a rearward direction, thus smoothly propelling the animal forward. Importantly, such water currents also function in food gathering, as well as in respiration, since thoracic appendages also have gills" (Persoone, 1980).

2.3.5 Evaluation of the Effect of Pesticides on Artemia

Varo et al. (2006) evaluated the efficacy of the *Artemia* cyst's chorion as a barrier to the organophosphorus pesticide chlorpyrifos, in whole and decapsulated cysts. They exposed the cysts to 10 mg/ L chlorpyrifos in sea water separately during hydration and hatching phase. Their results demonstrated the ability of the cyst's chorion to obstruct the passage of chlorpyrifos molecules through this protective structure. They found that the concentration of chlorpyrifos in exposed decapsulated cysts was higher than in exposed whole cysts. After removing the chorion of exposed cysts, the concentration of chlorpyrifos in the embryo was lower than that of cysts exposed, so this demonstrated the retention of chlorpyrifos molecules by the shell. Hatching was not severely affected by exposure to the insecticide whereas survival at 44 h of the nauplius exposed to chlorpyrifos was significantly different from the controls. Survival of naupli hatched from exposed decapsulated cysts was higher than that from those hatched from exposed whole cysts. They suggested that it was because of the lower vitality of the latter, due to depletion of energy reserves during hatching.

Koutsaftis et al. (2007) evaluated the toxicity of binary (in several proportions), ternary and quaternary mixtures of biocides: Zinc pyrithione (ZPT), Copper pyrihione (CPT), Chlorothalonil and Diuron using the brine shrimp *Artemia salina* as the test organism. The ZPT-CPT combination had a strictly synergistic effect. The binary mixtures of Diuron with the metal pyrithiones exhibited various interactive effects (synergistic, antagonistic or additive) depending on concentration ratios, whereas all binary mixtures that contained

Chlorothalonil exhibited antagonistic effects. They showed that the binary mixtures of different chemicals and different amounts of them can produce different effects. The four ternary mixtures tested also exhibited various interactive effects, and the quaternary mixture exhibited synergism.

Grosch (1973) investigated the reproductive performance of brine shrimp *Artemia salina* (actually *A. franciscana* from California) after exposure to sublethal doses of nine types of organic compounds occurring early in the degradation of non-persistent pesticides. Three of these failed to reduce adult life span or depress fecundity at 10 parts per million (ppm): benzazimide and 3-hydroxy-methylbenzazimide from azinphosmethyl and 3,5,6-tricholoro-2-pyridinol from Dursban. He found that all treated *Artemia* adults had a decreased life span. The average total number of broods per pair was decreased by every agent. In addition, 1, 5-dihydroxynaphthalene caused a delay in the appearance of the first brood for more than a week. A decrease in the number of broods was accompanied by a decrease of the average total number of zygotes produced.

Brix et al. (2003) determined the chronic toxicity of arsenic (sodium arsenate) on *Artemia franciscana*. They determined the chronic toxicity by measuring the adverse effects of arsenic on brine shrimp growth, survival, and reproduction. The test endpoints that they evaluated in this study were: survival of the parental generation prior to reproductive pairing on day 12 and survival on days 21 and 28; growth of the parental generation prior to reproductive pairing (day 12) and at day 28; reproduction in the parental generation on days

21 and 28; survival of the F_1 generation through day 12; and growth of the F_1 generation through day 12. The parental and F_1 generation were directly exposed to the chemical. They began their study with <24-h-old nauplii, continued through reproduction of the parental generation, and ended after 28 days of exposure. They tested concentrations of 4, 8, 15, 31, and 56mg/L dissolved arsenic. Their result showed that adult survival was the most sensitive biological endpoint, with growth and reproduction somewhat less sensitive than survival. The "no observed effect concentration" (NOEC) for survival was 8mg/L, and the lowest observed effect concentration (LOEC) was 15mg/L dissolved arsenic. The LOEC for growth and reproduction was greater than the highest concentration tested 56mg /L. Based on survival, the final chronic value (geometric mean of the NOEC and LOEC) was 11mg/L dissolved arsenic. The F_1 generation appeared to acclimate to the prior arsenic exposure of the parental generation and was significantly less sensitive than the parental generation.

Brix et al. (2006) estimated median effective concentrations (EC50s) of 7, 5, and 28 µg/L for Cd, Cu, and Zn on *Artemia franciscana*. Their research results suggested that brine shrimp hatching success is not particularly sensitive to Cd and Zn, but it is sensitive to Cu. They suggested that brine shrimp are at significant risk from Cu and Zn in Great Salt Lake (GSL), UT, where ambient concentrations as high as 10 and 14 µg/L, respectively, have been measured. However, site-specific water-quality conditions ensure that brine shrimp cyst hatching success is not significantly affected by any of these metals at the normal background concentrations that occur in GSL (<15 µg/L).

Hadjispyrou et al. (2001) tested the effects of three organotin compounds —trimethyltin chloride, dimethyltin dichloride, and dibutyltin diacetate — and two heavy metals—cadmium and hexavalent chromium — on *Artemia franciscana* mortality. They found that the toxicity order was trimethyltin chloride > potassium dichromate > dimethyltin dichloride > dibutyltin diacetate > cadmium chloride. The big difference in toxicity between dialkyltin and trialkyltin was not accompanied by an equally big difference in bioaccumulation. At a Sn concentration in water of 10 mg/L, the bioconcentration factor was 50 for dimethyltin dichloride and 75 for trimethyltin chloride. At a Sn concentration in water of 100 mg/L, the bioconcentration factor was 6 for dimethyltin dichloride and 9 for trimethyltin chloride. The interactive effect of trimethyltin chloride and cadmium, as well as that of trimethyltin chloride and chromium, was found to be synergistic. Also found to be synergistic was the interactive effect of trimethyltin chloride with cadmium and chromium applied together.

Sanchez et al. (1997) evaluated the toxicity of chlorpirifos, methylclorphirifos, parathion and methylparathion to three age classes of *Artemia salina*. They found that 24-h old *A. salina* was less sensitive to the pesticides than 48 h old *A. salina* and 48 h old *A. salina* was significantly more tolerant than 72 h old *A. salina*. There were some differences among the three age classes of *A. salina* in relative order of toxicity of OPI tested. The rank order of toxicity to 48 h old *A. salina* was methylparathion < parathion < methylclorphirifos < chlorpirifos, while to 24 and 72h h old *A. salina* it was methylparathion = parathion < methyl-chlorpiryfos < chlorpyrifos.

Barahona et al. (1996) evaluated the acute toxicity of some phenolic compounds (pentachlorophenol (PCP), 2,6 dichloroindophenol (2,6-DCIP), 2,4-dinitrophenol (2,4-DNP), o-nitophenol (o-NP), p-nitrophenol (p-NP), diaminophenol and 2,6 dymethylphenol (2,6-DMP)) on 24-48 and 168 hr old *Artemia salina*. They found that *Artemia salina* larvae aged 168 hr were 4.76 and 24.30 times more sensitive for 2,6 DMP than *Artemia salina* larvae 24 and 48 hr old, respectively. Their analysis of 24–hr LC50 values revealed that the sensitivity of three age classes of *Artemia salina* to PCP, 2,4-DNP,o-NP,p-NP,diaminophenol and diamidophenol is in the order of 168-hr = 48hr > 24hr old. In the case of 2,6-DCIP it was 168 hr=48hr>24 hr old.

2.3.6 Liquid Edger®

Liquid Edger® (LE) is an arsenical herbicide distributed by the Gordon Corporation. It is recommended for the control of a wide variety of unwanted vegetation around the buildings, along sidewalks, driveways, trees and ornamentals. It is deactivated on contact with the soil. This product is ready to use. One gallon is able to cover up to 250 square feet or 1200 linear feet (with a band 2.5 inches wide).

The Oral Lethal Dose 50 on rat is >1500 mg/kg and the Dermal Lethal Dose 50 on rabbit is >2000 mg/kg (MSDS, 2001).

LE is a liquid mixture. The active ingredients are dimethylarsinic (cacodylic acid) 0.09% (Figure 2-2), sodium cacodylate 0.53 % (Figure 2-3) and inert ingredients (99.38%). The

hazard category of this herbicide is acute and chronic. Its routes of entry in humans and animals are contact, inhalation, and ingestion. If there is a contact with the eyes, this may cause mild irritation, if the contact is with skin, it may cause irritation, if there is inhalation of this herbicide, it may cause irritation to the respiratory tract by prolonged or repeated overexposure. If Liquid Edger is ingested, it may irritate the gastrointestinal tract (MSDS, 2001).

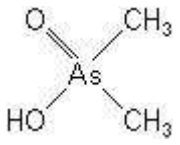

Figure 2-2 Molecular structure of dimethylarsinic acid (cacodylic acid) C2H7AsO2

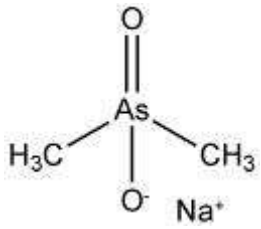

Figure 2-3 Molecular structure of sodium cacodylate (CH3)2AsNaO2 3H2O

According the Liquid Edger® MSDS 2001, its physical and chemical properties are: boiling point 82ºC, vapor pressure (mm Hg) <17 @ 28F, vapor density (Air= 1) : >1, specific gravity (H_20=1):1.0000, solubility in water: infinite. Appearance and odor: Liquid; very slight garlic odor. pH 6.0-7.0. Density 8.37 pounds/gallon, freezing point <35˚F. It is stable, it has no incompatibility with any material, may produce arsenic fumes and it has no hazardous polymerization.

2.3.7 Acute and Chronic Toxicity of Arsenic to Some Aquatic Organisms

The effect of arsenic was evaluated on the bacterium *Vibrio fischeri*. The luminescence was measured after 30 min for acute toxicity assessment and after 24 h to measure chronic effects. The acute toxicity increased slowly with increasing concentrations, 50% reduction of luminescence (30min EC50) occurred at 72.4 mg/L. Table 2.1 shows the values of acute toxicity and chronic toxicity on *Vibrio ficheri*.

Table 2.1 Arsenic toxicity to luminescent bacteria (Tisler, 2002)

Luminescent bacteria	
Acute toxicity	**Chronic toxicity**
30min EC20 = 13.4 mg/L	24h EC20 = 3.7 mg/L
30min EC50 = 72.4mg/L	24h EC50 = 20.4mg/L
30min EC80 = 395 mg/L	24h EC80 = 115 mg/L

The effect of arsenic was evaluated on the green alga *Scenedesmus subspicatus*. Algal growth was determined by measuring the cell density to evaluate the toxicity test. Arsenic was toxic to algae; the EC50 values for algal biomass and the specific growth rate were 34.7mg/L and 60.3mg/L, respectively. The biomass determination was more sensitive endpoint than the growth rate. Table 2.2 shows the toxicity values for green alga *Scenedesmus subspicatus*.

Table 2.2 Toxicity of arsenic to algae. (Tisler, 2002)

	Cell growth (biomass)	Specific growth rate
72h EC10 (mg/L)	9.4	34.7
72h EC50 (mg/L)	34.7	60.3
72h EC90 (mg/L)	128.8	104.7

Acute toxicity and chronic toxicity of arsenic was evaluated on *Daphnia magna*. Acute toxicity was evaluated by counting the immobile daphmids after a 24 hr and 48 hr exposure period. The endpoints of chronic toxicity test were mortality of daphmids, appearance of the first offspring, and total young per female after 21 d. The results of acute and chronic toxicity tests with daphmids indicated great sensitivity of daphmids to arsenic in comparison to other organisms. The EC50 values were 2.7 mg/L after 24 h and 2.5 mg/L after 48 h. Table 2.3 shows the values of acute toxicity and chronic toxicity on *Daphnia magna*.

Table 2.3 Acute and chronic arsenic toxicity to *Daphnia magna* (Tisler, 2002)

Acute toxicity		Chronic toxicity
24h EC10 =2.0mg/L	48h EC10 =1.9 mg/L	21d IC10=1.3 mg/L
24h EC50 =2.7mg/L	48h EC50 =2.5mg/L	21d IC25=1.9 mg/L
24h EC90 =3.8mg/L	48h EC90 =3.4mg/L	21d LOC=3.70 mg/L

Acute arsenic toxicity was tested on juvenile rainbow trout *Oncorhynchus mykiss* and zebrafish *Brachydanio rerio*. The endpoint of acute toxicity test was survival of fish during 96 hr of exposure, every 24 hr dead fish were counted and removed. Table 2.4 shows the

values of acute arsenic toxicity in rainbow trout and zebrafish. Arsenic was more toxic to rainbow trout than to zebrafish.

Table 2.4 Acute arsenic toxicity in rainbow trout and zebrafish (Tisler, 2002)

	All values are in mg/L		
Zebra fish	LC10	LC50	LC90
24h	27.3	34.9	44.8
48h	24.5	32.7	43.6
72h	22.2	28.5	36.7
96h	21.9	28.1	36.0
Rainbow trout			
24h	-	-	-
48h	15.5	23.2	34.7
72h	13.4	17.7	23.4
96h	12.1	15.3	19.4

2.3.8 Genetic Toxicology of Arsenic

According EPA 2003, Poma et al. (1981) evaluated the clastogenic effect (capacity to induce chromosomal aberrations) of arsenic on mouse bone marrow cells and on spermatogonia *in vivo* following the co-administration of ethyl methane sulfonate (EMS). They did not observe synergic effects in chromosomal aberrations. However they observed that sodium arsenite increased the clastogenic effect in both bone marrow cells and spermatogonia at a concentration of 0.77µM in drinking water.

Rogers et al. (1981) evaluated the teratogenic potential of cacodylic acid in the rat and mouse. He administered cacodylic acid to time-pregnant albino CD rats and CD-1 mice on days 7-16 of gestation. Rats received 0, 7.5, 15, 30, 40, 50, and 60 mg/kg/day in 0.2 ml/day intubation volume; mice received 0, 200, 400, or 600 mg/kg/day in 0.1 ml/day. They observed fetal and maternal toxicity in both species. In the mouse, maternal toxicity was evident at the lowest dose, while the teratogenic response was confined to cleft palate at 400 and 600 mg/kg/day. The effective maternal toxic dose in the rat was 40 mg/kg/day. In this species, incidence of irregular palatine rugae, was significantly dose-related ($p < 0.001$). Their results suggested an apparent no effect level for this anomaly below 30 mg/kg/day.

Yamanaka et al. (1989) evaluated DNA damage induced by administration of dimethylarsinic acid (DMAA) to rats and mice. He found that at 12 h after administration of DMAA, DNA single-strand breaks were markedly induced in lungs. The majority of dimethylarsine (a further metabolite of DMAA) in the expired air was excreted within 6-18 h after administration of DMAA to rats. *In vitro* experiments using nuclei isolated from lungs of mice indicated that DNA strand breaks were caused by dimethylarsine. Their results suggested that the strand breaks were induced not by dimethylarsine itself, but by active oxygen produced both by dimethylarsine and molecular oxygen. When DNA was exposed to dimethylarsine, thiobarbituric acid (TBA)-reactive intermediates and cis-thymine glycols were produced.

Yamanaka et al. (1994) administered dimethylarsinic acid (DMAA) orally to mice. They found lung-specific DNA damage, i.e., DNA single-strand breaks and the clumping of heterochromatin. The lung-specific strand breaks were not caused by DMAA itself, but by a further metabolite of DMAA called dimethylarsine.

Kato et al. (1995) administered dimethylarsinic acid (DMAA) to an embryonic cell line of alveolar epithelial (L-132) cells .They observed that alkali-labile sites in DNA were produced prior to DNA single-strand breaks (SSB) and DNA-protein crosslinks (PC) in L-132 cells by exposure to 10mM DMAA. Their findings suggested that SSB and PC induced by exposure of L-132 cells to DMAA occurred via the formation of AP sites (Apurinic and Apyrimidinic sites) in DNA. SSB were produced by a beta-elimination reaction on AP sites in the DNA and PC by a Schiff-base reaction between amino groups of nuclear proteins and aldehyde groups of AP sites.

Rin et al. (1995) reported that dimethylarsinic acid (DMAA) induced DNA single-strand breaks (SSB) both *in vivo* and in cultured alveolar type II (L-132) cells *in vitro*, possibly via the production of dimethylarsenic peroxyl radicals. Also, he investigated the interaction of superoxide anion radicals ($O2^-$) in the induction of SSB in L-132 cells using paraquat, an (O_2^-)-producing agent. He observed a significant enhancement of SSB formation in the DMAA-exposed cells when coexposed to paraquat. This study suggested that the DMAA exposure caused some modification of DNA such as DNA-adducts, which was recognized by active oxygen to form SSB.

Ochi et al. (1996) investigated the inducibility of apoptosis in cultured human HL-60 cells by arsenic compounds, such as arsenite, arsenate, methylarsonic acid (MAA), and dimethylarsinic acid (DMAA), together with the role of glutathione (GSH) in the induction. They found that among the arsenic compounds DMAA was the most potent in terms of the ability to cause the morphological changes (formation of nuclear fragmentation and apoptotic bodies). Fragmentation of inter-nucleosomal DNA was induced by DMAA. The results suggested that the death of cells caused by DMAA was due to apoptosis and that GSH is involved in the induction of apoptosis by the arsenic compound.

Zhao et al. (1997) demonstrated an association between arsenic and malignant transformation. They observed hypomethylation of DNA and aberrant gene expression in a rat liver epithelial cell line. The tested concentrations of sodium arsenite were 0.125, 0.25 y 0.5 mM. They suggested that there was a DNA hypomethylation induced as a mechanism of carcinogenesis due chronic exposition to arsenic.

Yamanaka et al. (1997) observed DNA single-strand breaks due to the inhibition of repair polymerization in cultured human pulmonary epithelial (L-132) cells after exposure to dimethylarsinic acid (DMAA). After exposure of the L-132 cells to 10mM DMAA, the breaks occurred in a time-dependent manner during incubation for 1-6 h under the inhibition of aph-sensitive polymerases with 50 µg /ml aph plus 10 mM hydroxyurea (HU) for the last 1 h of the DMAA exposure. Also, when DNA polymerase beta was inhibited with 10 mM

DDT plus 1 µM methotrexate (MTX), the exposure of L-132 cells to 10mM DMAA for 6 h significantly induced DNA single-strand breaks.

Kashiwada et al. (1998) investigated the cytogenetic effects of dimethylarsinic acid (DMAA), on mouse bone marrow cells after a single intraperitoneal injection to mice. They observed that DMAA significantly increased mitotic indices at 16, 24 and 48 h after injection, and prolonged the average generation time 1.5 h at the 24 h. Their results suggested that DMAA may cause mitotic arrest *in vivo* as well as *in vitro* and that aneuploidy induced by DMAA might be associated with carcinogenicity of arsenic.

Hamilton et al. (1998) used a 14 day old chick embryo liver *in vivo* model to study the effect of chromium and arsenic on the expression of a model-inducible gene. They observed that Phosphoenolpyruvate carboxykinase (PEPCK) arsenite significantly altered both basal and hormone inducible expression at nontoxic doses in the chick embryo *in vivo* (100µmol/Kg) and in hepatoma rat H411E cells culture (0.33-1.0µM). They suggested that arsenate has direct and indirect effects on the specific transcription factors and other signaling pathways rather than on DNA *per se*.

Schaumloffel et al. (1998) observed a suppression of arsenic-induced micronuclei in V79 cells for by trivalent antimony. They observed that significantly elevated frequencies of micronuclei (MN) were obtained with 0.25 µM arsenic trioxide.

Ochi et al. (1998) investigated changes in cytoskeletal organization of cultured V79 cells exposed to arsenite and dimethylarsinic acid (DMAA) and related changes, such as mitotic arrest and induction of multinucleated cells. They found that DMAA caused mitotic arrest and induction of multinucleated cells with a delay of 12 h relative to the mitotic arrest. By contrast, he observed that arsenite at equitoxic concentrations to DMAA was less effective than DMAA in causing mitotic arrest and in inducing multinucleated cells.

Ochi et al. (1999) investigated the localization of alpha-tubulin, because of interest in the mechanism of the induction of aberrant mitotic spindles in Chinese hamster V79 cells exposed to dimethylarsinic acid (DMAA). Their results reported that DMAA-induced multiple signals of alpha-tubulin were integrated into one signal at the center of multinucleated cells, surrounded by multiple nuclei as the cell cycle progressed to the next interphase, suggesting the presence of a self-integration mechanism of centrosomal MTOCs during the cell cycle.

According EPA 2003, Lynn et al. (2000) demonstrated that arsenic induced oxidative damage in DNA in human vascular smooth muscle cells in vitro. Human aorta cells (VSMCs) were treated four hr at one to 10µL arsenite and DNA strand breaks were detected by single-cell alkaline electrophoresis.

Ochi et al. (2000) evaluated the effects of dimethylarsinic acid (DMAA) on microtubule disruption and reorganization. They observed that DMAA induced aberrant cytokinesis, such

as tripolar and quadripolar division, in a concentration-dependent manner. Their results, suggested that the centrosome is the primary target for the induction of multipolar spindles by DMAA and the resultant induction of multinucleation and multipolar division.

Mass et al. (2001) evaluated the genotoxic potential of trivalent arsenic species in a DNA nicking assay. Their results indicated that the species can damage the naked DNA without exogenous enzymatic or chemical activation.

Ochi et al. (2002) investigated the effect of acid (DMAA) on microtubule-based motors in the induction of abnormal centrosome integrity by dimethylarsinic. Their results suggested that abnormal centrosome integrity caused by DMAA was not simply due to dispersion of fragments of microtubule-organizing centers, but was dependent on the action of kinesin.

3 Materials and Methods

In this research was used Gordon's Liquid Edger® (Dimethylarsicinic CAS No 75605 and Sodium cacodylate CAS No 124652). This chemical was used in pure form, adding it with a micropipette to the brine solution to achieve the respective concentrations. *Artemia franciscana* used for this research were reared from commercial cysts from San Francisco, California. Cysts were hatched in seawater (specific gravity 1.023, salinity approx. 32ppt) at approximately 25ºC ± 2ºC. Forty eight hours old were used to initiate the study. Saline water was collected from the salt flats at the U.S Fish and Wildlife Service's Cabo Rojo National Wildlife Refuge, one of the nine components of the Caribbean Islands National Wildlife Refuge Complex. A Special Use Permit for the collection of brine (and PR *Artemia* cysts) in the Salinas was kindly granted by Mr. Joseph J. Schwagerl, Deputy Project Leader, to Drs. Richard Squire and Robert Mayer. The saline water was stored in several 5-gallon Jerry cans until used for testing. Distilled water was used to dilute the initial saline to the experimental solution (specific gravity 1.050 = 70ppt) and pH 8.0. Salinity was measured using either a refractometer or a hydrometer.

The lighting for the test experimental setup consisted of fluorescent lights set on a 24 h light photoperiod. The 25ºC ± 2ºC temperature was maintained constant. *Artemia* were observed and fed with 0.5 ml of commercial yeast diluted in distilled water daily. Evaporated water from the brine was replaced periodically by addition of distilled water.

3.1 Analysis of Biological Data

Toxicity was measured using the following criteria: Survival to adulthood, longevity, average number of broods per female, average fecundity per female, average fertility per female, sex ratio of progeny of the *Artemia franciscana*.

3.2 Toxicity Test

Commercial dry cysts of *Artemia franciscana* were hatched in pure sea water (specific gravity 1.023, salinity approx. 32ppt) during 48 hours. The test solutions were prepared by adding pure Liquid Edger® to the 70ppt brine solution to achieve the following concentrations: 0.01 mg/L, 0.001 mg/L, 0.0001 mg/L, 0.00001 mg/L, 0.000001 mg/L, 0.0000001 mg/L, 0.00000001 mg/L. Each brine solution with its respective Liquid Edger concentration was transferred to five quart jars which represented the replicates. Twenty five nauplii were randomly transferred into each jar with a Pasteur pipette. The jars were incubated under constant temperature and light. When organisms achieved their maturity (20 days) they were counted. The 50% Lethal Concentration was obtained and 0.0001 and 0.00001 mg/L concentrations were selected for the following experiments.

3.3 Effects of test solutions on the Parental Generation

Dry cysts of *Artemia franciscana* were hydrated and hatched in a glass dish which contained sea water at 1.023 specific gravity. They remained at 25 °C during 24 hours. Brine solution at 70 ppt. (1.050 specific gravity) was transferred into three-liter mass culture jars. Approximately 100 nauplii were randomly transferred at each jar using a Pasteur pipette. These jars were maintained under 24 h fluorescent light illumination at 25ºC ±2ºC until pairs were observed (approximately 22 days old). At that time, 0.0001 and 0.00001 mg/L concentrations of Liquid Edger were prepared. The stock solution of Liquid Edger was prepared by dissolving 0.35 and 3.5 mL LE in 3500 mL in brine solution at 1.050 specific gravity to produce 0.0001 and 0.00001 mg/L concentrations respectively. Each concentration was transferred into fifteen quart jars (700mL), although data was analyzed based on thirteen pairs for the control, fourteen pairs for 0.0001 mg/L treatment and fifteen replicates for 0.00001 mg/L treatment. The other pairs were excluded because the death of the mating pairs females was not attributed to Liquid Edger. Brine solution (1.050 sp. gr.) without pesticide was used as control. A single *Artemia* pair was randomly transferred to each pint jar with a meat baster. They were incubated at constant temperature and illumination until the female died. The length of time that females lived gave us information about their longevity. The mating pairs were maintained in the test because a male died, his female was supplied with a male from the same test group that had recently lost his mate.

Fecundity is the potential reproductive capacity of an organism or population, measured by the number of gametes. Female fecundity was defined as the total sum of all recoverable gametes produced by a single female throughout her reproductive history (Squire, 1979). Likewise, female **fertility** was defined as the total sum of recovered nauplii from ovoviviparous and oviparous broods; it combined the factors included in fecundity and hatchability (Squire, 1979).

To evaluate fecundity and fertility, the number of cysts or nauplii released by all females was recorded daily. These data yielded information about the number of broods and number of gametes released (fecundity) by each female. When nauplii were observed in the brine solution, the parents were removed to a Petri dish and the nauplii were counted under a dissection microscope using a Pasteur pipette. Then, the parents were returned to their original jar. When cysts were observed in the brine solution, the parents were removed from their culture solution to a Petri dish. Their brine solution was filtered with a Whatman #4 filter paper. Subsequently, the parents were returned to original jar. The filter paper was dried at environmental temperature and placed in the freezer at 5 ºC. Afterwards, these cysts were counted using a paintbrush and hydrated in brine solution (specific gravity 1.023). The number of nauplii that hatched was registered every day during seven days. This data gave us information about percentage hatchability.

3.4 Effect of the tests solutions on the F_1 Generation

To evaluate survival to adulthood and sex ratio, each time when the parental females produced viviparous broods, 25 nauplii were randomly transferred to quart jars which contained brine solution without pesticide. When they were 22 days old, they were counted and sexual classification was made. A chi-square contingency test was used to analyze these results.

To evaluate female longevity, fecundity and fertility on the F_1, fifteen single pair matings per treatment from the previous survival experiment were each transferred to a pint jar. Pint jars contained brine solution without pesticide. The organisms were maintained at conditions similar to the parental generation and the same factors were evaluated. This tested the delayed effects of the pesticide.

3.5 Effect on the F_2 Generation

To evaluate survival and sex ratio, each time when an F_1 female produced oviparous broods, 25 nauplii were randomly transferred to quart jars which contained brine solution without pesticide. When they were 22 days old, they were counted to test for survival to adulthood and alterations in sex ratio using the same set up as the original experiment.

3.6 Data

Data were analyzed using a commercial software package Infostat (version 3). Data for the various test endpoints were first evaluated for normality and homogeneity of variance. The assumption of normality was tested by calculating the ANOVA model residuals and testing these residuals for normality by the normal probability plot method. The assumption of homogeneity of variances was evaluated by Q-plot test. The non-parametric Student t-test and Chi squared test were used to test for differences among levels. Each treatment was represented by fifteen pairs but the data was analyzed based on thirteen pairs for the control, fourteen pairs for 0.0001 mg/L treatment and fifteen pairs for 0.00001 mg/L treatment. The other pairs were excluded because the death of the mating pair's females was not attributed to Liquid Edger. The mating pairs were maintained in the test because when a male died, his female was supplied with a male from the same test group that had recently lost his mate.

4 RESULTS

4.1 Effect of Liquid Edger on the Parental generation

4.1.1 Female Life Span

The results suggest that females exposed to pesticide treatments had shorter longevity than those from the control, but only females' longevity from the 0.00001mg/L treatment was significantly different ($P < 0.05$) (Figure 4-1). In the control, the average longevity of the parental generation was 38 and 37 days for females and males respectively. In the 0.0001mg/L treatment the average longevity was 29 and 32 days for females and males respectively and in the 0.00001mg/L treatment it was 29 and 40 days for females and males respectively (Table 4-1). Longevity of males from treatments was not shorter than from the control.

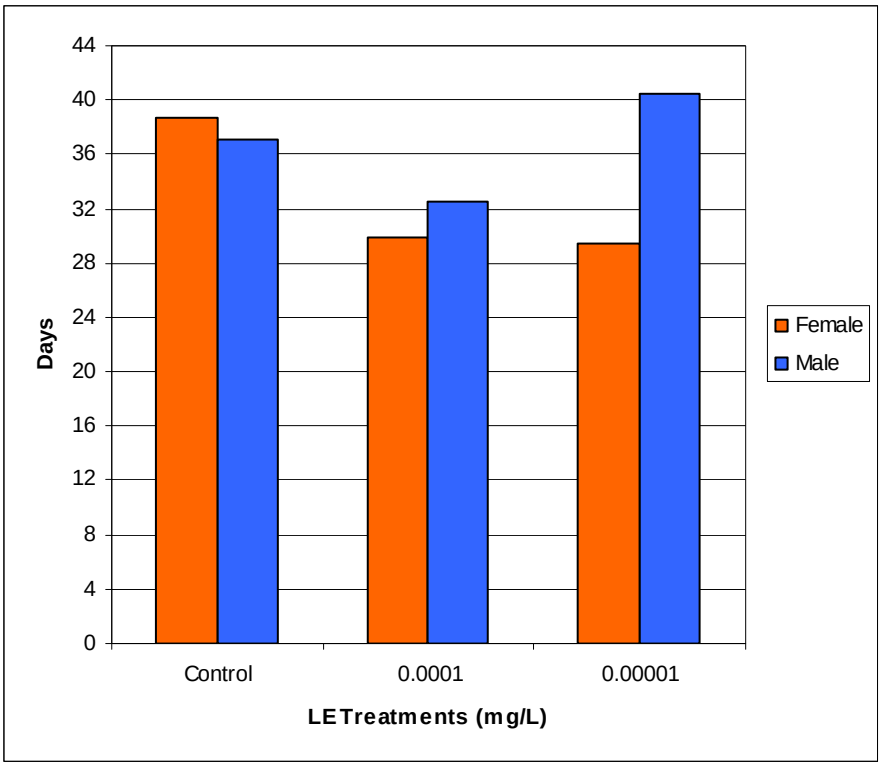

Figure 4-1 Parental generation average adult life span in *Artemia franciscana* after treatment with Liquid Edger

Table 4-1 Parental generation average adult life span in *Artemia franciscana* after treatment with Liquid Edger

Dosage level (mg/L)		n	X(Days)	t-test value	P
Control	Female	13	38.67		
Control	Male	13	37.07		
0.0001	Female	14	29.93	1.644	P>0.05
0.0001	Male	14	32.53		
0.00001	Female	15	29.4	1.8623	P<0.05
0.00001	Male	15	40.53		

n - Number of pairs at each concentration level
X - Average adult life span at each concentration level

4.1.2 Reproductive Performance

4.1.2.1 Number of Broods

Females exposed to treatments and those from the control released their broods approximately every four days. The average number of broods released by females exposed to Liquid Edger was significantly lower than that from the control ($P<0.05$). In the control, the average number of released broods by females was 4.41, in the 0.0001 and 0.00001 mg/L treatments it was 3.38 and 3.1 respectively (Table 4-2).

Table 4-2 Average of broods produced by adult *Artemia* females in the P_1 generation after treatment with Liquid Edger

Dosage level (mg/L)	n	X	t-test value	P
Control	13	4.41		
0.0001	14	3.38	6.12	P<0.05
0.00001	15	3.1	8.71	P<0.05

n - Number of pairs at each concentration level
X - Average number of broods at each concentration level

4.1.2.2 Hatchability

The cysts hatchability was a measure of dominant lethal events. Hatchability tests were conducted for all oviparous broods. The percentage of hatched cysts was calculated for each brood. The arcsine transformation was then obtained for each value. Untransformed treatment means and t-test comparisons are given in table 4-3.

The amount of cysts released by females exposed to treatments was lower than from the control females, but there were no significant differences (P>0.05). The control females released a cysts average of 114.40, and females exposed to 0.0001 and 0.00001 mg/L released cyst averages of 78.50 and 75.67, respectively. The hatchability in the control was 51.37, and in 0.0001 and 0.00001 mg/L treatments it was 20.12 and 33.78, respectively (Table 4-3).

Table 4-3 Average of hatchability of cysts produced by adult *Artemia* females in the P_1 generation after treatment with Liquid Edger

Dosage level (mg/L)		X	t-test value	P
Control	Cyst	114.40		
Control	Hatchability	51.37		
0.0001	Cyst	78.507		
0.0001	Hatchability	20.12	1.2	P>0.05
0.00001	Cyst	75.67		
0.00001	Hatchability	33.78	1.44	P>0.05

X – Average of cysts and percentage of hatchability at each concentration level

4.1.2.3 Fecundity and Fertility

Female **fecundity** was defined as the sum total of all recoverable gametes produced by a single female throughout her reproductive history (Squire, 1979).

Females exposed to Liquid Edger produced significantly less gametes than those from controls P<0.05 (Figure 4-2). The average of gametes produced by females from the control

in each brood was 100.4643, and in 0.0001 and 0.00001 mg/L treatments it was 46.07 and 49.89 respectively (Table 4-4).

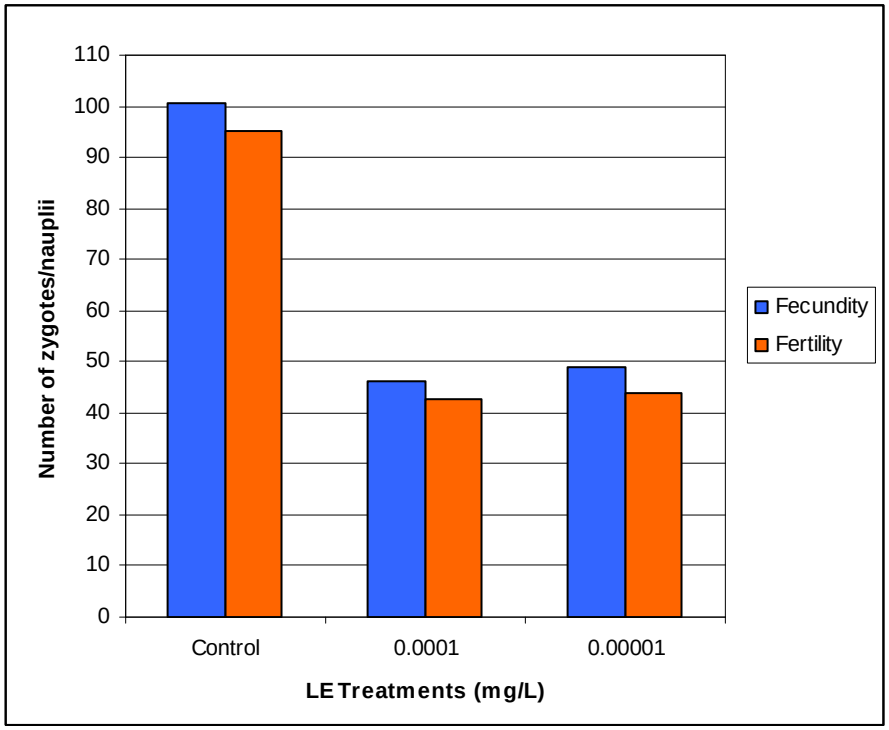

Figure 4-2 Average fecundity and fertility of *Artemia* females in the P_1 generation after treatment with Liquid Edger

Table 4-4 Average fecundity of *Artemia* females in the P_1 generation after treatment with Liquid Edger

Dosage level (mg/L)	n	X	t-test value	P
Control	13	100.4643		
0.0001	14	46.07	4.35	P<0.05
0.00001	15	49.89	4.43	P<0.05

X - Average female fecundity at each concentration level
n - Number of *Artemia* pairs at each concentration level

Female **fertility** was defined as the sum total of recovered nauplii from ovoviviparous and oviparous broods, and combines the factors included in fecundity and hatchability (Squire, 1979). The number of nauplii released by females exposed to Liquid Edger treatments was significantly lower than from control females P<0.005 (Figure 4-2). In the control, the females released an average of 95.2 nauplii in each brood, and in 0.0001 and 0.00001 mg/L treatments it was 51.0 and 52.9 respectively (Table 4-5).

Table 4-5 Average fertility of *Artemia* females in the P_1 generation after treatment with Liquid Edger

Dosage level (mg/L)	n	X	t-test value	P
Control	13	95.2		
0.0001	14	51.0	3.784	P<0.05
0.00001	15	52.9	3.673	P<0.05

n - Number of *Artemia* pairs at each concentration level
X - Average female fertility at each concentration level

4.2 Effect of Liquid Edger on the F_1 generation

4.2.1 Survival and Sex Ratio

The F_1 *Artemia* were not exposure directly to Liquid Edger after they were removed from their parent's jar. Nauplii were placed in brine solution without pesticide. When they were 20 days old they were counted and the sex ratio was calculated.

Parental exposure to Liquid edger did not affect the survival to adulthood. The average number of organisms that survived (out of 25 initial nauplii) in the control and the treatments is presented in the Table 4-6.

Table 4-6 Contingency chi square values for average survival to adulthood of the F_1 generation

Dosage level mg/L	n	X	Contingency X^2 values treated versus control	P
Control	13	18.95	----------	-------
0.0001	14	18.05	0.000342	P>0.05
0.00001	15	19.34	0.008119	P>0.05

n - Number of jars at each concentration level. Each jar had 25 *Artemia* individuals
X - Average survival to adulthood at each concentration level

In the controls, the observed number of males was slightly higher than the number of females. On the contrary, in the treatments the observed number of males was lower than the number of females. There was no significant difference in the sex ratio in control vs. treatments (P >0.05) (Table 4-7). In the control the male average was 9.17 and female average was 9.77. In

0.0001mg/L treatment the average was 9.37 for males and 8.67 for female, in 0.00001mg/L treatment it was 9.78 for female and 9.56 for male (Figure 4-3).

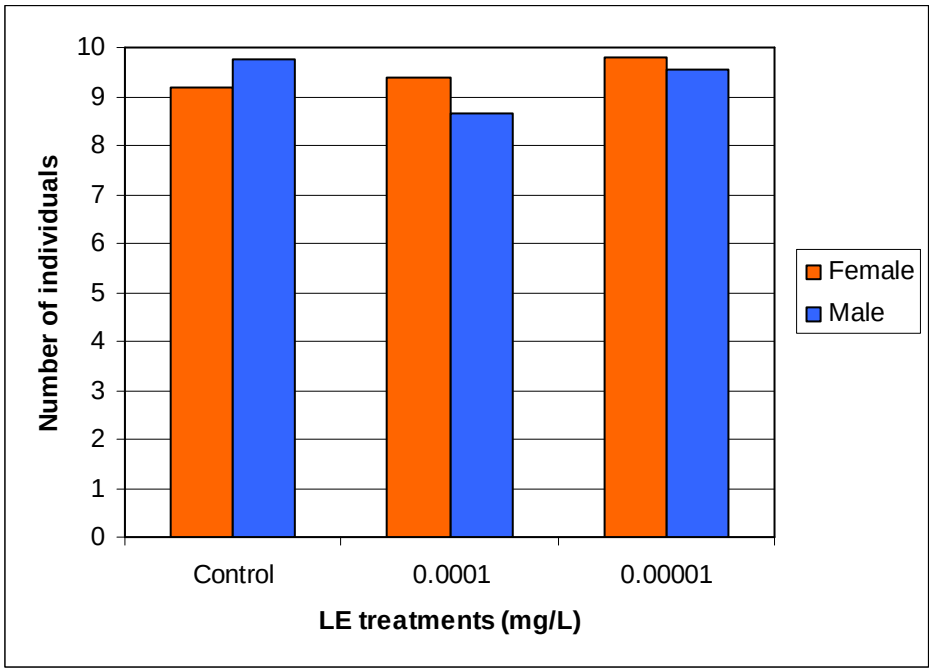

Figure 4-3 Female and male survival of F_1 *Artemia*

Table 4-7 Contingency chi square values for sex ratio (males/total animals) in the F_1 generation

Dosage level mg/L	n	X	Contingency X^2 values treated versus control	P
Control	13	0.5134	----------	--------
0.0001	14	0.5268	0.0001728	P>0.05
0.00001	15	0.4980	0.0002205	P>0.05

n - Number of jars at each concentration level. Each jar had 25 *Artemia* individuals
X - Average sex ratio at each concentration level

4.2.2 F_1 Adult Life Span

Females whose parents were exposed to pesticide treatments had shorter longevity than those from control parents, but only females' longevity from the 0.00001mg/L treatment was significantly different ($P < 0.05$) (Figure 4-4). In the controls, the average longevity of the parental was 35.5 and 38.3 days at females and males, respectively. In the 0.0001mg/L treatment the average longevity was 31.6 and 36.7 days for females and males, respectively and in 0.00001mg/L treatment it was 26.6 and 36.9 days for females and males, respectively (Table 4-8). Longevity of males from treatments was not shorter than from those in the controls.

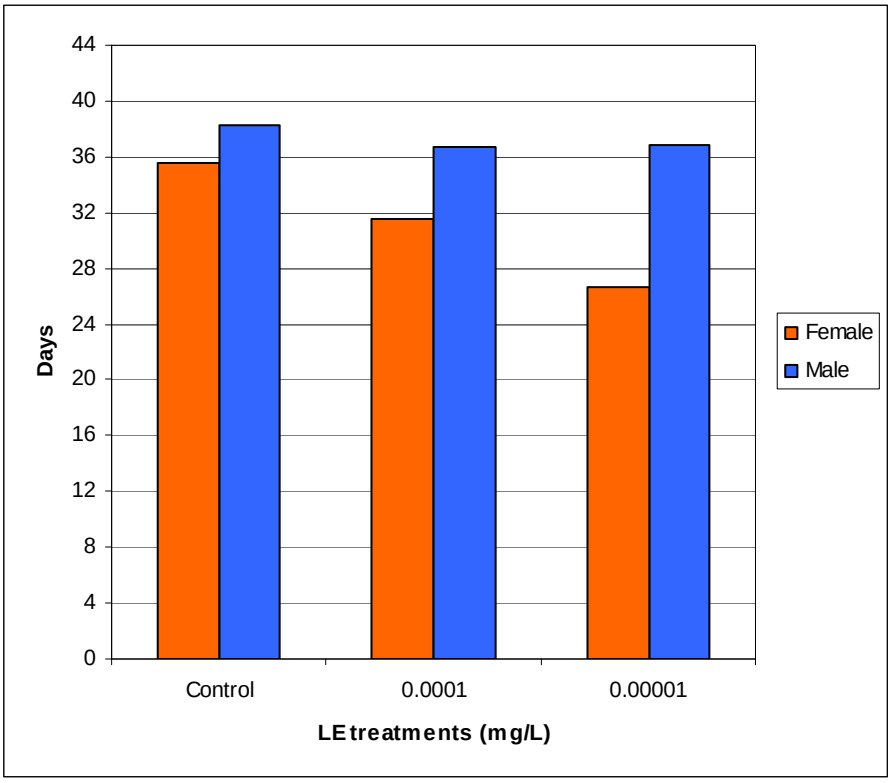

Figure 4-4 F_1 generation average adult life span of *Artemia franciscana*

Table 4-8 F_1 generation average adult life span of *Artemia franciscana*

Dosage level (mg/L)		n	X(Days)	t-test value	P
Control	Female	13	35.5		
Control	Male	13	38.3		
0.0001	Female	14	31.6	0.1689	P>0.05
0.0001	Male	14	36.7		
0.00001	Female	15	26.6	2.0804	P<0.05
0.00001	Male	15	36.9		

n - Number of pairs at each concentration level
X - Average adult life span at each concentration level

4.2.3 Reproductive Performance

4.2.3.1 Number of Broods

Females whose parents were exposed to treatments and those from control released their broods approximately every four days. The average number of broods released by these experimental F_1 females was slightly lower than that of the controls, but there was no significant difference between them (P>0.05). In the control, the average of released broods by females was 3.47, in the 0.0001 and 0.00001 mg/L treatments it was 2.92 and 3.07, respectively (Table 4-9).

Table 4-9 Average number of broods produced by adult *Artemia* females in the F_1 generation

Dosage level (mg/L)	n	X	t-test value	P
Control	13	3.47		
0.0001	14	2.92	0.7306	P>0.05
0.00001	15	3.07	0.7242	P>0.05

n - Number of pairs at each concentration level
X - Average number of broods at each concentration level

4.2.3.2 Hatchability

The amount of cysts released by daughters of exposed parents was lower than that those from the control, but there was no significant difference (P>0.05). The control F_1 females released an average of 50 cysts and daughters from females exposed to 0.0001 and 0.00001 mg/L released averages of 48 and 40 respectively. The hatchability in the control was 41.82, and in 0.0001 and 0.00001 mg/L treatments it was 37.50 and 42.50 respectively (Table 4-10).

Table 4-10 Average hatchability of cysts produced by adult *Artemia* females in the F_1 generation

Dosage level (mg/L)		X	t-test value	P
Control	Cyst	50.00		
Control	Hatchability	41.82		
0.0001	Cyst	48.00		
0.0001	Hatchability	37.50	0.013	P>0.05
0.00001	Cyst	40.00		
0.00001	Hatchability	42.50	1.933	P>0.05

X – Average of cysts and percentage of hatchability at each concentration level

4.2.3.3 Fecundity and Fertility

Females whose parents were exposed to Liquid Edger produced slightly fewer gametes than those ones from the control but there was no significant difference P>0.05 (Figure 4-5). The average number of gametes produced by females from the control in each brood was 100.5, and in 0.0001 and 0.00001 mg/L treatments it was 103.5 and 85.3 respectively (Table 4-11).

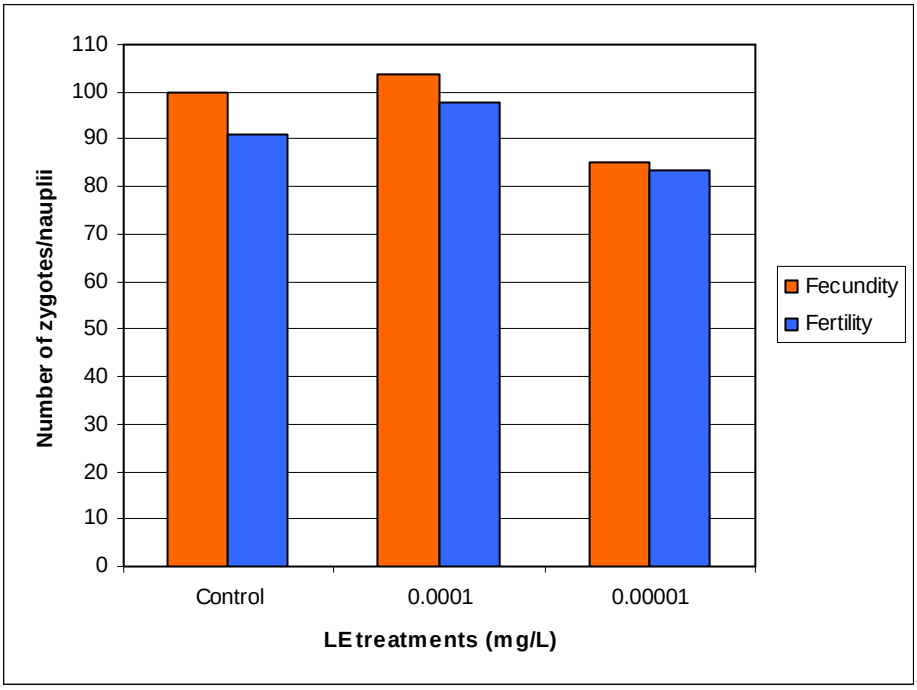

Figure 4-5 Average fecundity and fertility of *Artemia* females in the F_1 generation

Table 4-11 Average fecundity of *Artemia* females in the F_1 generation

Dosage level (mg/L)	n	X	t-test value	P
Control	13	100.5		
0.0001	14	103.5	3.783	P>0.05
0.00001	15	85.3	3.650	P>0.05

X - Average female fecundity at each concentration level
n - Number of *Artemia* pairs at each concentration level

The average number of nauplii released by females whose parents were exposed to Liquid Edger treatments was lower than that those from control F_1 females but there was no significant difference P>0.05 (Figure 4-5). In the control, the females released an average of 99.62 nauplii in each brood, and in 0.0001 and 0.00001 mg/L treatments it was 94.71 and 83.30 respectively (Table 4-12).

Table 4-12 Average fertility of *Artemia* females in the F_1 generation

Dosage level (mg/L)	n	X	t-test value	P
Control	13	99.62		
0.0001	14	94.71	0.251	P>0.05
0.00001	15	83.30	0.30	P>0.05

n - Number of *Artemia* pairs at each concentration level
X - Average female fertility at each concentration level

4.3 Effect of Liquid Edger on Survival and Sex Ratio of the F_2 generation

4.3.1 Survival and Sex Ratio

Liquid edger did not significantly affect the survival to adulthood of F_2 *Artemia*. The average number of *Artemia* that survived in the control and the treatment groups is presented in Table 4-13.

Table 4-13 Contingency chi square values for average survival to adulthood of the F_2 generation

Dosage level mg/L	n	X	Contingency X^2 values treated versus control	P
Control	13	14.4		
0.0001	14	15.2	0.0003418	P>0.05
0.00001	15	14.6	0.008119	P>0.05

n - Number of jars at each concentration level. Each jar had 25 *Artemia* individuals
X - Average survival to adulthood at each concentration level

In the control F_2, the number of males was slightly higher than the number of females. On the contrary, in the treated F_2 the number of males was lower than the number of females. There was no significant difference in the sex ratio in control vs. treatments P >0.05 (Table 4-14). In the control the male average was 7.0 and female average was 7.4. In 0.0001mg/L treatment the average was 7.9 for males and 7.3 for females, in 0.00001mg/L treatment it was 7.26 for female and 7.34 for males (Figure 4-6).

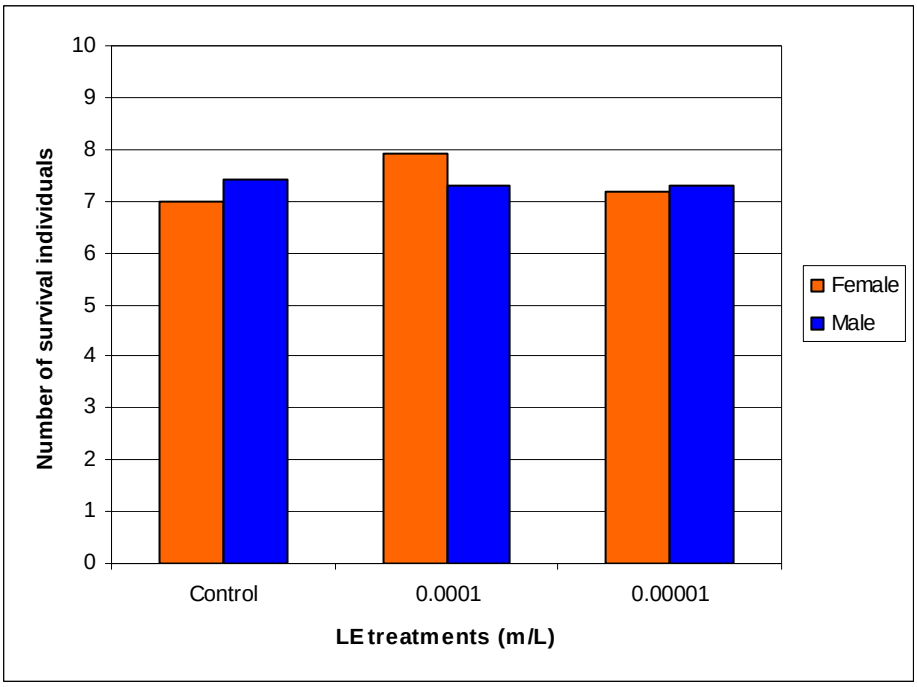

Figure 4-6 Female and male survival of F_2 *Artemia*

Table 4-14 Contingency chi square values for sex ratio (males/total animals) in the F_2 generation

Dosage level mg/L	n	X	Contingency X^2 values treated versus control	P
Control	13	0.53	--------	-------
0.0001	14	0.51	0.0003419	P>0.05
0.00001	15	0.51	0.001079	P>0.05

n - Number of jars at each concentration level. Each jar had 25 *Artemia* individuals
X - Average sex ratio at each concentration level

5 DISCUSSION

In this research, Liquid Edger herbicide was used because it is a common domestic pesticide which is used to kill unwanted plants and its chronic impact is unknown. Its principal component is arsenic, which generates a potential risk to any organism exposed to it. It is known that some times ingredients that are used in agricultural formulations are significantly more toxic to fish, amphibians and aquatic invertebrates in the pure state than when they are mixed (Lajmanovich, 2003). However, in other cases when the compounds are mixed, the potential effect is the highest because of the synergic effect (Rico 2000), that is, the effect of a component is greater when it is mixed with other components (Lajmanovich 2003).

Artemia franciscana was used because is an important biological model to test toxicology of many chemicals; plays an important role in its ecosystem, and is a "necessary" food source in aquaculture. *Artemia* is the main food supply for the millions of shorebirds that use their habitat as a migratory stopover or breeding ground (Brix, 2002). Also, brine shrimp feed on microorganisms such algae and bacteria and help rid the water of contaminants such phosphorus and nitrogen (Kanwar, 2007). *Artemia* are a major food source in marine and fresh water aquaculture (Bengtson el., 1991), and exposure of *Artemia* populations to environmental contamination may result in population decline and the production of contaminated cysts which are potentially harmful if used in aquaculture.

I evaluated the effect of Liquid Edger herbicide on the parental generation (P_1), the F_1 and the F_2. The parental generation was exposed to Liquid Edger concentrations, as were the ovovivarously produced F_1 nauplii for a short time before they were transferred to herbicide-free media. Thus, any effect on the F_1 can be viewed as a chronic effect or a delayed effect. Chronic effects are produced following a prolonged exposure (months, years, and decades) and they persist after the exposition has been stopped. Also, the effects could be attributed to genetic damage because the highest numbers of mutations that are produced in the organisms usually delay a generation to express themselves.

The 0.0001 and 0.00001 mg/L treatments were chosen after the toxicity test of Liquid Edger on *Artemia franciscana*. Each treatment was represented by fifteen pairs but the data was analyzed based on thirteen pairs for the control, fourteen pairs for 0.0001 mg/L treatment and fifteen pairs for 0.00001 mg/L treatment. The other pairs were excluded because the death of the mating pair's females was not attributed to Liquid Edger. The mating pairs were maintained in the test because when a male died, his female was supplied with a male from the same test group that had recently lost his mate.

In *Artemia*, reproductive performance is influenced by some intrinsic factors; the reproductive lifespan of the adults, the number of broods produced per female (Cunningham, 1978) and the number of offspring per brood. The longer they live the larger they grow, and

the more zygotes per brood a female can produce. However some females produce fewer offspring per brood but may produce more broods over their lifespan.

The longevity of parental females (P_1) treated with Liquid Edger was lower than the one from control, but only the longevity of females from the 0.00001 mg/L treatment was significantly lower than the one from control. This also occurred in the F_1; the females whose parents were exposed to 0.00001 mg/L treatment had significantly lower longevity that those from control. The males did not have a reduced longevity. This could suggest that the females were more sensitive to pesticide than the males.

These results are similar to those obtained by Grosch (1976), who reported that male *Artemia* lived longer than their respective females when they were exposed to different concentrations of antifouling paints, algaecides and an aquatic herbicide. Also, Varo et al. (1998) reported that females were more vulnerable when they tested the acute lethal toxicity of chlorpyrifos pesticide on different species and strains of *Artemia*. Moreover, Cunningham (1978) reported that female parents were more physiologically stressed than males after exposure of mercuric chloride and methyl mercury chloride.

The shorter longevity of females can be explained because in this species the female is the heterogametic sex, which is usually less resistant to environmental stress and often shorter lived in general (Squire, 1970). Likewise, females and males may respond differently to

chemical substances (e.g., females produce eggs full of yolk); there are variations in the susceptibility of an individual during its life. These differences are caused by factors that can affect the absorption speed, the distribution in the body, the biotransformation speed and the excretion of a determined substance as well as hereditary factors (Silbergeld, 2001).

The longevity of F_1 females was affected although they were not directly exposed to the Liquid Edger over most of their lifespan. The F_1 females and males were exposed *in utero* while they were unfertilized eggs and embryos prior to being released by their mother. It is known that small molecules which are hydrosoluble and lyposoluble easily go trough the layers that protect the embryo. Therefore, the offspring could get a secondary exposure through the mother while they were born. Some times those lowest doses, which do not have an immediate visible effect, could affect the organism after the first expos

ure. That would mean that they were exposed during the first life stages but the chemical was not lethal and did not have immediately obvious effect.

The females whose parents were exposed to Liquid Edger died sooner than the ones from control. When they were 24 h old (nauplius stage) they were placed into a brine solution without pesticide. This means that their shorter longevity resulted because the chemical had an effect on their first development stages or even parental gametogenic cells. If their mother had ovoviparous reproduction, the nauplii were indirectly exposed through their mother and they were directly exposed during a few hours when they were released to brine solution with

Liquid Edger but they were resistant to it. Some researches have demonstrated that the naupliar stage of *Artemia*, is the most resistant stage to chemicals. Sánchez et al. (1997) evaluated the toxicity of chlorpirifos, methylclorphirifos, parathion and methylparathion to three age classes of *Artemia salina*. They found that 24-h old *A. salina* was less sensitive to the pesticides than 48 h old *A. salina* and 48 h old *A. salina* was significantly more tolerant than 72 h old *A. salina.* Likewise, Barahona et al. (1996) evaluated the acute toxicity of some phenolic compounds (pentachlorophenol (PCP), 2,6 dichloroindophenol (2,6-DCIP), 2,4-dinitrophenol (2,4-DNP), o-nitophenol (o-NP), p-nitrophenol (p-NP), diaminophenol and 2,6 dymethylphenol (2,6-DMP)) 24-48- and 168 hr old on *A. salina*. They found that *A. salina* larva aged 168 hr were 4.76 and 24.30 times more sensitive for 2,6 DMP than *A. salina* larvae 24- and 48 hr old, respectively.

On another hand, if a parental female had oviparous reproduction, the cysts from which the offspring hatched were exposed directly and indirectly for several hours, but the probability of the pesticide went through the chorion seems low. Varo et al. (2006) demonstrated the ability of the cyst chorion to obstruct the passage of the organic pesticide chlorpyrifos molecules through this protective structure. It was possible that the cyst chorion obstructed the passage LE molecules as it did with chlorpyrifos.

It could be that LE concentrated in nauplii, without causing death to them. Wan et al. in 1996 demonstrated that persistent chlorinated compounds (DDTs) could be concentrated in high

levels in *Artemia* nauplii from water, after 24 h pesticide contamination. These researchers reported that concentration in nauplii exposed to 1µg/L was about twice in organisms exposed to 0.5µg/L of pesticide. In general, the decrement induced by LE on P_1 life span can be seen as a summation of innumerable deleterious effects on the animal's physiological processes and cannot be ascribed to genetic damage alone.

The females exposed to pesticide released their broods sooner than those ones from the control. Furthermore, proportionately more of the zygotes from treated adults were deposited as cysts. Presumably this was a characteristic response to environmental stress (Grosch, 1967). In general the first broods had a number of offspring lower that the later offspring; this behavior is typical of *Artemia* females and may also be seen when parents are faced with stress in the environment.

Both pesticide treatments significantly decreased the number of broods released by P_1 females. A decrease in number of broods was accompanied by a decrease of the average total number of zygotes produced. Any change in the general health of female *Artemia* may temporarily or permanently alter the number of gametes recovered per brood (Squire, 1967). The number of broods released by F_1 females was not decreased significantly. The deficit of number of broods in the parental experimental groups could be a consequence of the decrease in life span. However, the 0.0001mg/L treatment did not significantly decrease the life span

of females exposed to it. Thus, it indicates that this treatment even though it did not cause female death, it affected a reproductive factor.

The frequency of cyst production generally tended to increase, possibly this was a response to stress caused by Liquid Edger. However normal females also produced oviparous as well as ovoviviparous broods. It could be that various environmental factors such as diet, temperature, and salinity affected the cyst production at one time or another. The proportion of zygotes encysted may be the most important index to strain survival, provided some of the embryos are viable (Grosch, 1973). Since cyst production is a prompt response important for surviving an environmental change, it was not surprising to observe a significant shedding of cysts within a day or two after a chemical contamination of a jar population.

The control and treatment cysts collected from the filter papers were re-hydrated for the hatchability test. In general, the hatchability in treatments was lower than in the control, but there was no significant difference. However, it is important to consider that, ovoviviparous nauplii are thin-membraned zygotes that develop within the female's brood sac, while cysts are the resistant zygotes encapsulated within a thick shell, which are voided directly into the environment (Nakanishi 1962 in Cunningham 1978). Shell gland dysfunction may result in reduction in viability. The tertiary envelope (outer cell layer) of the cysts is secreted by the shell gland and chemical or structural modifications in the cell wall might reduce the resistance of the cysts to desiccation, temperature changes, and other environmental stresses,

thus reducing hatchability (Lochhead in Cunningham, 1978). Squire (1979), explained that other factors which may conceivably affect cyst hatchability include: failure of fertilization, nutritional inadequacies of oocyte resulting from damage to the oocyte, or nutritive cells of the complex, or general physiological changes of the female, improper shell deposition, and genetic lethality.

Liquid Edger significantly reduced the fecundity and fertility of the P_1 generation. Fecundity and fertility of F_1 individuals, whose parents were exposed to the pesticide, were lower than those from controls; however the difference was not significant. It is possible that the values of fecundity and fertility were not affected because they produced their first broods earlier than those from the control. Several females from treatments released their offspring 3 days before females from control.

It was observed that the fecundity and fertility values of P_1 individuals from both treatments were similar; while in the F_1 the values of 0.0001mg/L treatment were slightly higher than those from 0.00001 mg/L treatment. This was a characteristic response to environmental stress. A similar behavior was found by Grosch (1967) when he evaluated the effect of DDT on reproductive performance of *Artemia*. He found that the frequency of brood deposit was unaltered and more broods were deposited by animals given the higher dose of DDT. Furthermore, proportionately more of the zygotes from treated adults were deposited as cysts. Shunting offspring into a dormant encysted state is the usual *Artemia* response to unfavorable

external factors. By interfering with this response both Liquid Edger concentrations added to jar cultures had the effect of increasing the vulnerability of shrimp to their environment.

Fitness is usually defined as the relative capacity for leaving offspring that attain reproductive maturity. The maximum number of offspring is limited by the number of zygotes. Along with many other organisms, *Artemia* has adopted the reproductive strategy of investing maximum energy in gamete production. The quantity and the quality or type of zygote is significant for the survival of shrimp populations (Grosh, 1980). The loss of a considerable proportion of potential offspring can be serious. A reduction of the number of zygotes to 1/3 or less than the control average, as caused by both Liquid Edger treatments, implies impending population collapse if the population continued to be exposed to LE. In long-term studies of irradiated populations, more than half of the normal reproductive capacity constituted a reserve necessary for buffering environmental changes (Grosch, 1969 in Grosch, 1973).

The organisms that were used to evaluate survival and sex ratio were not permanently exposed to the pesticide. When they were 24 h old, they were placed into brine solution without pesticide. Many toxic substances have different responses depending on the sex of the exposed organism. Some differences can be explained because there are metabolic differences that can be determined by physiological state, the genetic structure of the exposed individual (Peña et al., 2001) or the kind of genetic alteration. Genetic alterations are able to

produce, for example, a sex-linked recessive lethal mutation. In many species, the heterogametic XY male expresses any lethal mutation that was induced in the X chromosome of their mother (Winchester, 1996). This has led to the traditional explanation for the increased susceptibility of male humans, mice, rats and *Drosophila* to chemical and radiation exposure. In *Artemia*, the female is the heterogametic ZW sex, but it is not clear to what extent the Z and W chromosomes have differentiated from each other or whether the W and Z chromosomes remain essentially similar. The increased susceptibility of female *Artemia* to environmental insult may be due to physiological differences, genetic differences or both.

The offspring from control and experimental groups in both the F_1 and F_2 generations showed an excellent proportion of larvae surviving to maturity. There was neither a predominance of females (heterogametic sex) nor males (homogametic sex) as the sex ratios were close to 1.0. This showed that there was no action of induced recessive sex-linked lethal mutations in a hemizygous condition.

In general, Liquid Edger had a harmful effect on *Artemia franciscana*. The P_1 generation exposed to Liquid Edger had significantly fewer offspring than that from control. This can be associated with the shorter longevity of treated females, the low number of broods released by females, the low production of gametes and the low hatchability. The F_1 generation appeared to recover from the prior Liquid Edger exposure of the parental generation although longevity of females was reduced.

Grosch (1972) found that males and females of *Artemia* exposed to naphthalene compounds had short adult life spans. He explained that the compound caused the effect because it is able to inhibit mitoses. Female and male brine shrimp have several molts during their life and consequently there are epidermal mitoses. Thus, he associated short adult life spans with molt periods. In this study, it was found that only females had shorter adult life spans, thus it is possible that Liquid Edger did not strongly affect the epidermal mitoses, but it is possible that it caused damage in the mitoses necessary to form the germinal cells or later meiotic products. In fact, many agricultural and other pesticides (aimed at insect, fungal, and plant pests), which end up in the marine environment, were designed primarily to inhibit the process of cell division, through effects on the mitotic spindle apparatus (Dixon, 2002).

Moreover, Kashiwada et al. (1998) who worked on mouse bone marrow cells suggested that dimethylarsinous acid (DMA) caused mitotic arrest in vivo as well as *in vitro*. Also, Ochi et al. in 1998 investigated changes in cytoskeletal organization of cultured V79 cells exposed to arsenite and dimethylarsinic acid. They found that dimethylarsinic acid caused mitotic arrest and induction of multinucleated cells with a delay of 12 h relative to the mitotic arrest.

It is known that there are toxic agents that merely cause maternal debility and others that attack upon reproduction. These have the vulnerability of dividing cells. On this basis, damage is expected in the steam cell component of *Artemia* gonads and in the cells of the

cleaving embryo. Embryos attain the blastula stage within the female before a choice between encystment or ovoviviparity is made for them (Lochhead, 1941 in Grosch, 1973).

The influence of sub-lethal exposures on population dynamics must not be underestimated. Although sub-lethal effects are initially less visible than those induced by acute exposures, a serious population decline may result if reproductive performance of the exposed adults and survivorship of the resulting offspring are severely impaired (Cunningham, 1978).

Considering some toxic studies with arsenic on different species, it is seen that there is great variability in both toxicity of different pesticides and sensitivity of different species. The concentrations that were evaluated in those studies were higher than the ones evaluated in this experiment. Arsenic affected survival, reproduction, and growth, of the exposed organisms. Tisler et al. (2002) gave results from their acute and chronic toxicity tests on daphnids, fishes, algae, and bacteria. They found that arsenic induced toxic effects in all tested organisms. The most sensitive species was *Daphnia magna*; the 48 hr EC50 was determined to be 2.5 mg/L. Daphmids were followed by rainbow trout *Oncorhynchus mykiss* with 96 hr LC50 at 15.3 mg/L. In chronic exposures daphmids were more sensitive than bacteria or algae; the low effect level (IC25) was at 1.9 mg/L of arsenic.

Brix et al. (2003) determined the chronic toxicity of arsenic (sodium arsenate) on the *Artemia franciscana*, using concentrations of 4, 8, 15, 31, and 56 mg/L. They found that adult

survival was the most sensitive biological endpoint, with growth and reproduction less sensitive than survival. The no observed effect concentration (NOEC) for survival was 8 mg/L, and the lowest observed effect concentration (LOEC) was 15 mg/L dissolved arsenic. The LOEC for growth and reproduction was greater than the highest concentration tested 56 mg /L. Based on survival, the final chronic value was 11 mg/L dissolved arsenic. The F_1 generation appeared to acclimate to the prior arsenic exposure of the parental generation and was significantly less sensitive than the parental generation.

The concentrations used in this research are lower than those used in other arsenic toxicity tests. Synergism refers to the phenomenon in which two or more agents acting together create an effect greater than that predicted by knowing only the separate effects of the individual agents. It is possible that this phenomenon occurred in this study. Arsenic is the principal component of Liquid Edger (Dimethylarsinic acid (0.09%) and sodium cacodylate (0.53%). It is possible that when the 2 different chemical forms of arsenic acted together or with other Liquid Edger inert ingredients (99.38%), an effect greater than the one produced by the arsenic alone was produced.

The mechanisms by which arsenic affects *Artemia* are unknown. Sometimes the chemical itself does not affect the organism, but its metabolites formed when they are in the organism cause the damage. It is possible that any metabolite produced when the arsenic was in the organism reacted with any biological molecule. Yamanaka et al. (1994) administered

dimethylarsinic acid (DMAA) orally to mice. The lung-specific strand breaks were not caused by DMAA itself, but by dimethylarsine, a further metabolite of DMAA. Some evidences indicates that these trivalent, methylated, and relatively less ionizable arsenic metabolites may be unusually capable of interacting with cellular targets such as proteins and even DNA.

Arsenic is a naturally occurring metal in aquatic ecosystems, but its levels are increasing due to pollution. The background levels of most unpolluted freshwaters are below 1µg/L of arsenic. In ocean water the average residues are slightly higher (2-3µg/L of arsenic) (Moore et al., 1984 and Blanck et al. 1989 in Tisler, 2002). Rivers crossing industrial areas that contain residues of 1-20µg/L were detected in the streams receiving runoff from agricultural areas treated with herbicides containing arsenic compounds (Moore and Ramomoothy, 1984 in Tisler, 2002).

According arsenic in drinking water report, a limit of 0.01mg/L was set for drinking water by the US EPA (2001) and the WHO (2001). The concentrations used in this research were 0.0001mg/L and 0.00001mg/L.These concentrations are lower than the limits established by EPA and yet they had harmful effects on *Artemia*, an important organism into its ecosystem. This suggests that the EPA limits may need reconsideration.

The marine environment provides a sink for many natural and anthropogenic derived chemicals. For example, in 1994 the Organization for Economic Co-operation and Development (OECD) estimated that 1500 new chemicals are being added annually to the 100,000 already present in the natural environment. It has been argued that contaminant exposure could lead to detrimental effects at the population level, such as the loss of genetic diversity, which could have serious implications for species survival and ecosystem functioning (Bickham et al., 2000 in Dixon 2002).

Ecotoxicants released into the environment, including arsenic, often accumulate most rapidly in aquatic habitats where they enter the biota and are subsequently transferred to higher trophic levels and, in many cases, eventually to humans. Extremely high levels of arsenic have been observed in many fish taxa (Juresa, 2003) and have been shown to be toxic (Tisler, 2002). Some species possess specific arsenic-binding proteins (Oladimeji, 1985 in Tisler, 2002) that may increase bioaccumulation.

It is recognized that at the DNA and chromosome levels, marine invertebrates express qualitatively similar types of induced damage to those found in higher organisms (e.g. point mutations, strand breaks and chromosomal aberrations). Many of these species are linked directly or indirectly to the human food chain. Because of this, it is important that we are aware of their exposure to environmental mutagens and carcinogens (Dixon 2002). Many of these organisms have the capacity to transform these agents to biologically active metabolites

and accumulate toxicants in their cells and tissues at concentrations several orders of magnitude above that found in the environment.

6 CONCLUSIONS

Artemia franciscana was exposed to 0.0001 and 0.00001 mg/L treatments of Liquid Edger herbicide. The 0.00001mg/L treatment reduced the longevity of females from parental and F_1 generations. The F_1 was affected although it was not exposed directly to herbicide except prior to fertilization, while still in the ovisac and shortly after "birth". Liquid Edger caused female debility.

Both treatments of Liquid Edger decreased the fecundity and fertility of females from the parental generation. This was associated with the short lifespan of females, the reduced number of broods and fewer released gametes. The F_1 generation appeared to partially recover from the prior arsenic exposure.

The offspring from control and experimental groups in both the F_1 and F_2 showed an excellent proportion of larvae surviving to maturity. There was no change in sex ratio.

In general, the decrement induced by LE on P_1 life span can be seen as a summation of innumerable deleterious effects on the animal's physiological processes and cannot be ascribed to genetic damage alone.

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