RP269-TOXICITY OF CHEMICAL MIXTURES IN STORMWATER: MALATHION AND BENZENE TOXICITY TO *DAPHNIA MAGNA*

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ABSTRACT:

Stormwater is comprised of chemical mixtures from multiple nonpoint runoff sources. The increasing urbanization of land use causes contaminants on the impervious surfaces to be carried away in stormwater and into the nearest waterway. The common landscaping and agricultural practice of over-application of pesticides leaves the excess toxicant to be washed away with the field runoff water. While there is information on individual chemical toxicity, little is known about the toxicity of chemical mixtures. In order to manage risk it is critical to understand how contaminated stormwater can affect the reproduction and survival of non-target organisms. In this 21-day chronic *Daphnia magna* toxicity tests were conducting using >98% reagent grade benzene and analytical grade malathion. The chemicals were tested at the following concentration ranges: malathion 0.035-3.0 µg/L and benzene 450 – 28000 µg/L. A mixture of benzene and malathion was also tested to evaluate the potential additive, synergistic, or antagonistic toxicity: malathion 0.035 – 3.0 µg/L in the presence of 7000 µg/L of benzene. The survival and reproduction rate data collected will be used to construct an age structured population model to determine how the chemical mixture would change the dynamics of a *Daphnia magna* population.

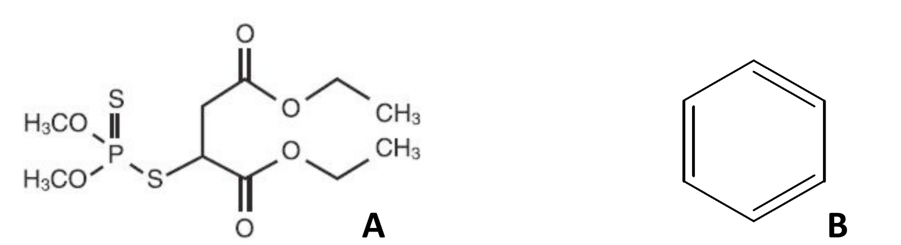

Figure 1. Chemical structure of malathion (A) and benzene (B) (MedcineOnline, 2005; NIST, 2010)

INTRODUCTION:

The urbanization of land use and the increasing amount of impervious surfaces have contributed to the presence of pollutants in stormwater runoff. Runoff from impervious surfaces such as parking lots and roads have detectable concentrations of aromatic hydrocarbons e.g. benzene, toluene, ethylbenzene, and xylene (BTEX) that are constituents of gasoline, along with other oils used in automobiles (Pitt et al., 1995). Semi-pervious surfaces, including, but not limited to lawn and agriculture fields, contribute chemicals to runoff through the used of multiple pesticides often used at high concentrations to ensure effectiveness (Barata et al. 2003). Rainfall, varying from light to intense, essentially washes the ground surface and transports chemicals, organic material, and debris into the nearest waterway (Pitt et al., 1995).

◆ Organophosphate insecticides are among the most commonly used pesticides used on account of their high toxicity and rapid degradation in the environment.

◆ While organophosphates are difficult to measure in the environment due to high biotransformation rates, their presence has been noted to cause severe, long lasting population effects on non-target species arising from a lack of target specificity (Barata et al., 2003).

◆ Non-target organisms, like salmon, are exposed to a mixture of countless chemicals with little known about these chemical mixtures' toxicological properties (Laetz et al. 2009).

◆ In this experiment, the individual and combined toxicity of malathion and benzene on the reproductive capacity of *Daphnia magna* were examined to better understand how the mixtures found in stormwater are affecting non-target organisms' ability to reproduce and sustain a stable population of organisms.

HYPOTHESIS:

It is hypothesized that the addition of benzene will increase the toxicity of malathion to both the survivorship and reproduction of *Daphnia magna* caused by the compounding modes of actions: benzene-induced narcosis and acetylcholinesterase inhibition by malathion.

METHODS:

Three 21-day chronic *Daphnia magna* toxicity tests were conducted using the ASTM protocol E1193-97 (ASTM, 2009) methods as modified by Markiewicz and Sofield, (2010). Each chemical and the mixture were tested at seven concentrations with ten replicates of one daphnia each (less than 24 hours old from Aquatic Bio System Inc.) in 100 mL test solution per beaker. The test solutions were prepared mixing the chemical of interest with reconstituted hard water. No carrier was needed for either chemical, as all of the concentrations were below the maximum water solubility for the chemical (Arnold et al., 1958). The test concentrations were chosen to encompass the geometric mean of the EC_{50}s found in the scientific literature.

Table 1. Prepared treatment dosing concentrations of individual chemical test and chemical mixture tests with ten replicates each.

Chemical	Negative Control	Treatment Concentration						
Malathion (µg/L)	0.00	0.035	0.075	0.150	0.350	0.750	1.50	3.00
Benzene (µg/L)	0.00	450	900	1800	3500	7000	14,000	28,000
Malathion/ Benzene (µg/L)	0.00	0.035/ 7000	0.075/ 7000	0.150/ 7000	0.350/ 7000	0.750/ 7000	1.50/ 7000	3.00/ 7000

◆ Treatments prepared volumetrically using ASTM reconstituted hard water as the dilution water and reagent grade chemicals with no carriers (ASTM, 2009).

◆ Water quality parameters of pH, alkalinity, hardness, and dissolved oxygen were tested and within acceptable range (ASTM, 2009).

◆ Instar daphnia held in a temperature controlled room at 20.0 ± 2.0° C with a photoperiod of 16 hrs light/8 hrs dark and fed *Selenastrum capricornutrum*.

◆ Test solutions were renewed on the 7th and 14th day of the experiment along with the counting and discarding of offspring.

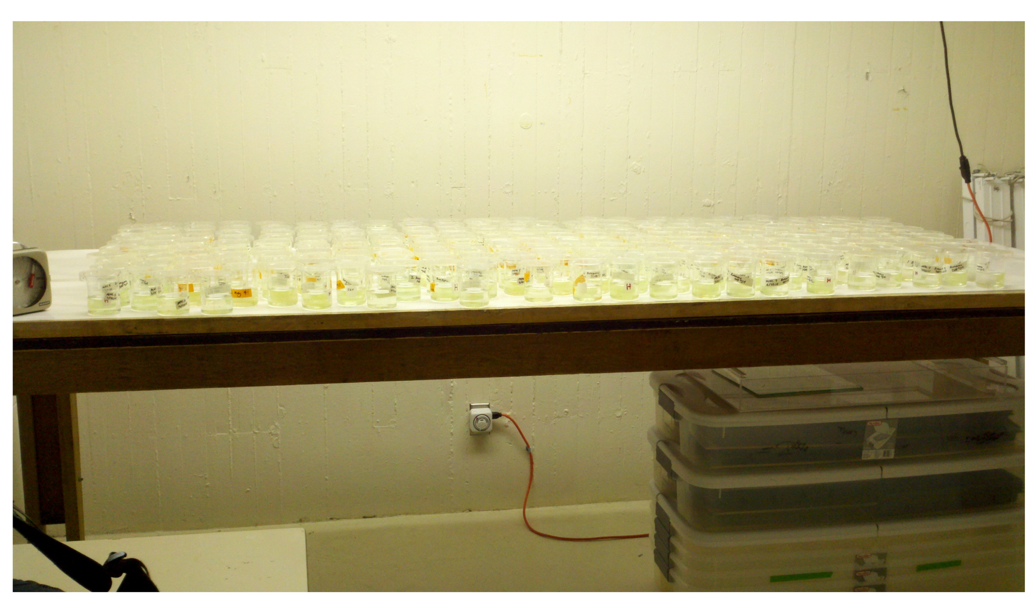

Figure 1. Set up of malathion, benzene, mixture, and negative control test chamber beakers on day one of exposure, February 23rd, 2011.

RESULTS & CONCLUSION:

◆ Probit regression of the survivorship raw data using the statistical software SPSS generated the following dose-concentration response curves for percent mortality (Figures 2A,C).

◆ Survivorship mortality LC_{50} of malathion was 0.057 ug/L (0.0-0.210 ug/L) and the mixture had an LC_{50} of 0.582 (0.0-1.723 ug/L).

◆Comparing the mixture test to the individual chemical test of malathion shows that benzene decreased the lethal toxicity of malathion.

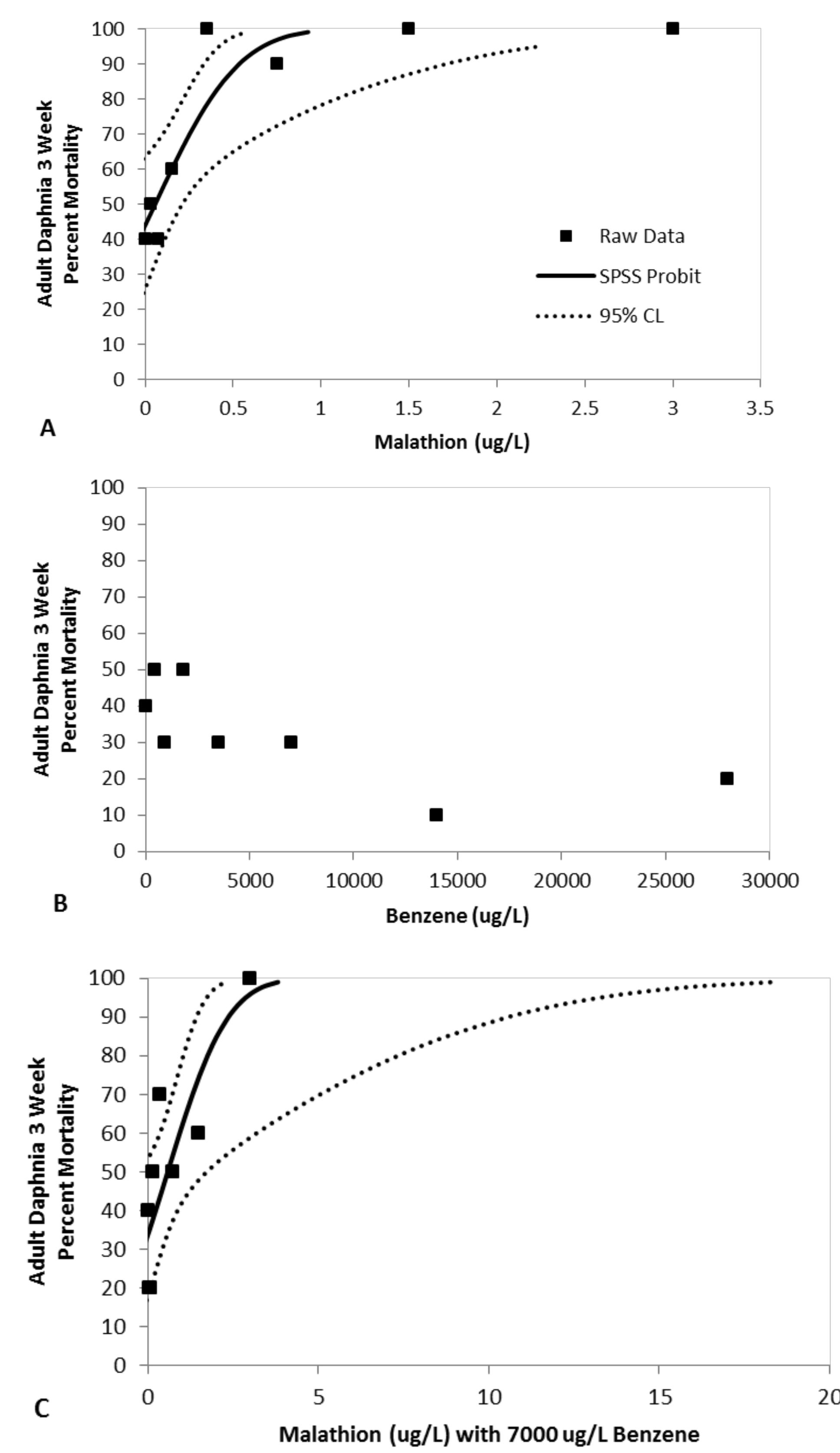

Figure 2. Percent mortality curves for malathion (A) and malathion with benzene (B) with 95% confidence intervals as calculated by using Probit Regression in the statistical software SPSS. SPSS was unable to construct a curve for the benzene mortality data.

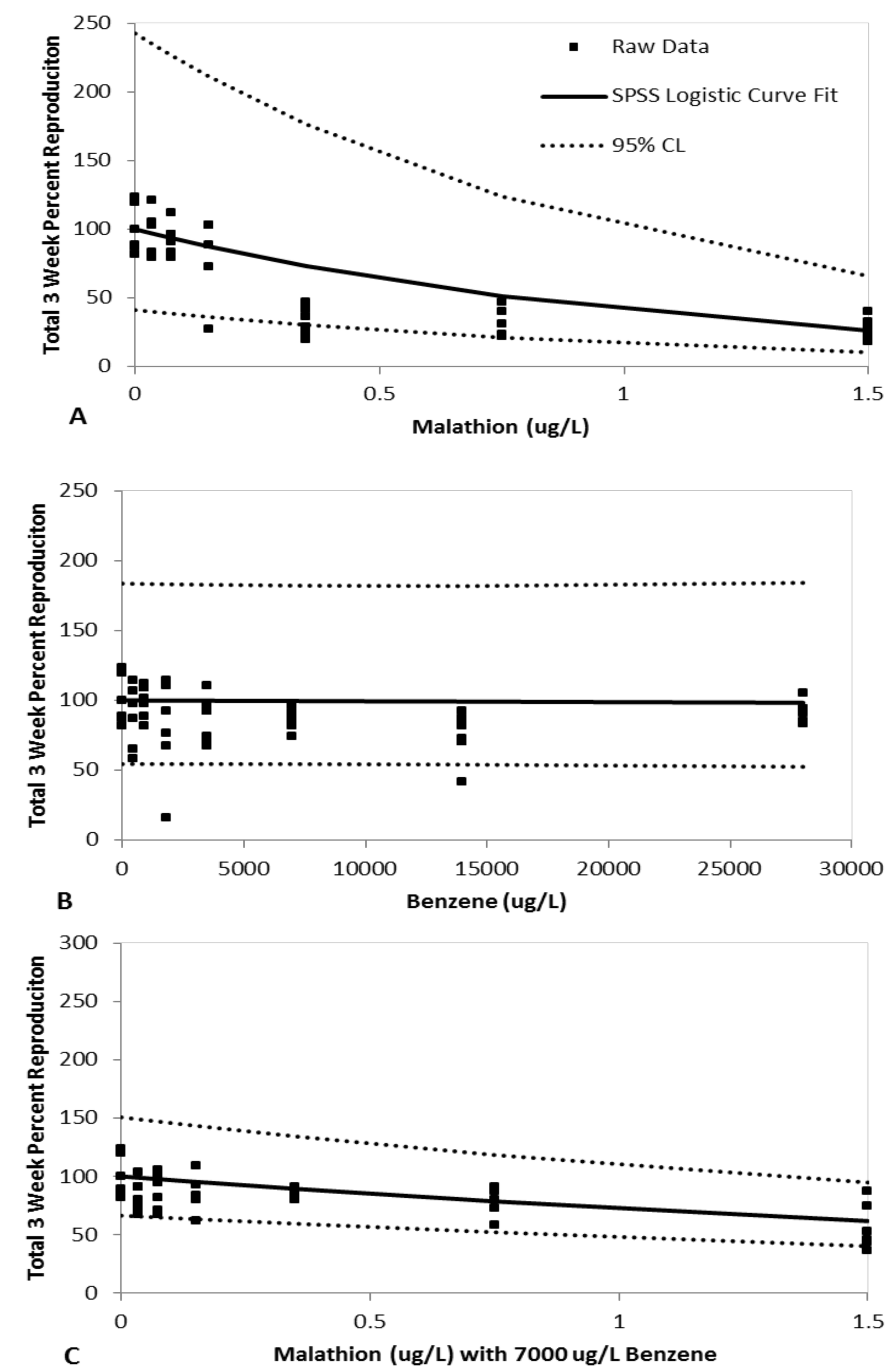

Figure 3. Percent reproduction curves for malathion (A), benzene (B), and malathion and benzene (C) with 95% confidence intervals as calculated by the statistical software SPSS. 100% reproduction was set at the average of the negative control replicates: 55 instars.

◆ Both malathion and the mixture of chemicals saw reduced reproduction with an increase of chemical concentration. No effect was seen in benzene (Figure 3).

◆Malathion reduced reproduction by 50% at 0.75 ug/L while the mixture EC_{50} was greater than 1.5 ug/L.

◆In both survivorship and reproduction, the addition of benzene to malathion decreased the toxicity of malathion.

◆The weekly survivorship and reproduction data will be used to determine how the exposure to the chemical mixture would affect population dynamics compared to a population on exposed to malathion.

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References: Arnold, D., Plank, C., Erickson, E., Pike, F.,1958. Solubility of Benzene in Water. Industrial & Engineering Chemistry Chemical & Engineering Data Series. 3: 253.; ASTM. 2009. Standard guide for conducting *Daphnia magna* life-cycle toxicity tests. E1193-97 (2004), *ASTM Standards on Disc Volume 11.06.* ASTM International, West Conshohocken, PA.; Barata, C., Solayan, A., Porte, C. 2004. Role of B-esterases in assessing toxicity of organophosphorus (chlorpyrifos, malathion) and carbamate (carbofuran) pesticides to Daphnia magna. Aquatic Toxicology 66:125-139.; Laetz, C.A., Baldwin, D.H., Collier, T.K., Herbert, V., Stark, J.D., and Schotz, N.L., 2009. The Synergistic Toxicity of Pesticide Mixtures: Implications for Risk Assessment and the Conservation of Endangered Pacific Salmon. Environmental Health Perspectives. 117(3): 348-353.; Pitt, R., Field, R., Lalor, M., Brown, M. 1995. Urban Stormwater Toxic Pollutants: Assessment, Sources, and Treatability. Water Environment Research 3: 260-275; MedicineOnline, 2005. http://www.medicineonline.com/drugs/O/2236/OVIDE-malathion-LOTION-0-5.html.; NIST Chemistry WebBook, 2010. http://wtt-lite.nist.gov/cgi-bin/openindex.cgi?cid=71432.