INVESTIGATION OF THE BEHAVIOURAL RESPONSE OF THE AMPHIPOD *COROPHIUM VOLUTATOR* TO THE PESTICIDE ENDOSULFAN

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ABSTRACT

Pesticides sprayed on farmlands can go into rivers and estuaries by transportation through water runoff; and are sufficiently persistent in the environment to be toxic to aquatic communities. During the summer of 2004, a series of experiments were conducted on the behaviour of the amphipod *Corophium volutator* exposed to six pesticides namely, Endosulfan I + II, Azinphos-methyl, Carbofuran, Chlorothalonil, Atrazine and Hexazinone over a wide range of concentrations. Endosulfan was the only pesticide out of the six tested showing properties as an attractant at lower concentrations and as a repellent at higher concentrations. The behavioural response of the amphipod *Corophium volutator* to the pesticide endosulfan was further investigated. The experiments were repeated to observe: scale of movement, effect of weathered pesticides and behaviour over three concentrations of contaminated sediments with changes in time. Lipid percent relative to dry animal mass was also examined as a potential biological effect representing changes in lipid metabolism. The pesticide partitioning for 'sediment organic carbon-water' was calculated based on the published octanol-water partitioning coefficient. Based on these results, possible concentrations of endosulfan entering the Wilmot River estuary were estimated. Behavioural experiments on *Corophium sp.* changed with time and survival rate was low. The animals were less resistant to manipulation in the winter and consequently, sampling time should be recorded and small animals as well as pregnant females should not be used. Lipid extractions revealed a higher lipid percent for exposed animals and varying with time. The partition coefficient calculation for organic carbon in comparison with water showed a higher affinity for organic enriched particles. The concentrations used for behavioural experiments were realistic compared to the exposure levels expected in an estuarine environment. Hence, a long term research goal would be to determine if commonly used pesticides in the conventional potato industry have an effect on the marine community of an estuary.

Keywords: Behavioural ecotoxicology--pesticides--endosulfan--*Corophium Volutator.*

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INTRODUCTION

1.1 Research Summary

Pesticides sprayed on farmlands can go into rivers and estuaries in the form of runoff and can be toxic to aquatic communities. The primary focus of this thesis is to determine the behavioural response of the amphipod *Corophium volutator* to the pesticide endosulfan. The long-term goal would be to determine if commonly used pesticides in the conventional potato industry have an effect on the marine community of an estuary.

Many fish kills have occurred in Prince Edward Island (PEI) as toxic pesticides entered rivers and streams, as a result of runoff caused by erosion from heavy rainfalls (Langer, 2002). In summer 2000, thousands of fish were found dead in the Wilmot River of Prince Edward Island (Freeze 2002). Further investigation showed that the fish kill was caused by the pesticide Azinphos-methyl, used to control bugs and blight on potato farms. (CBC News Online staff 2002). Some pesticides used in the potato industry are among the most toxic products registered in Canada: azinphos-methyl, endosulfan, carbofuran, and chlorothalonil (Langer, 2002). Fish as well as other species such as frogs, turtles, mussels and water birds are likely to be affected by toxic runoff and contaminated sediment (Langer, 2002).

Pesticides released in the environment can be transported and can accumulate in soil, water and air (Kamrin 1997). Chemical and physical properties of a compound such as volatility, solubility in water, resistance to degradation within the atmosphere and hydrosphere and chemical reaction or metabolism within an organism determine their tendency to pollute. If a compound degrades rapidly there is less possibility of a toxic concentration to build up (Reeve 2002). Pesticide persistence in soil is defined as a half-life. A pesticide with a short half-life has less chance to move far in the environment. The six pesticides chosen have a half-life between 30 and 100 days at 20-25°C and greater at lower

temperature. Consequently, these pesticides have a moderate chance to be persistent in the environment. On the other hand, pesticides can be more or less readily degraded by bacteria in soil and by photolysis. In addition, the solubility of a pesticide in water is also a factor determining its mobility into the environment (Kamrin, 1997).

Pesticides are soluble in water to varying degrees, can bind to particles and deposit in sediments. This tendency is evaluated with the log K_{ow} (octanol-water partition coefficient) where the affinity of a chemical to octanol, which represents a replacement to the lipids of animals or to the organic carbon of sediments, is compared to the affinity of a chemical to partition in water. In the literature, the values for log K_{ow} indicate a potential marginal pesticide's affinity for the sediments. Since amphipods ingest sediments or respire interstitial water, if pesticides are present within them, it could affect the behaviour and the survival rate of the amphipods in the test tanks. Pesticides tend to bind with neutral lipids and the percentage of lipid content in *Corophium volutator* will vary with age and over time. The exposure of amphipods to contaminants, including pesticides, can also disturb the lipid metabolism of animals. This is usually observed as an increase in lipid content relative to reference or unexposed animals. This biochemical toxicity response was further investigated by determining the lipids percent (per dry mass of animals) of exposed and non-exposed animals.

Pesticides could be sufficiently persistent in the environment to cause damage to aquatic organisms. Consequently, the investigation of behavioural ecotoxicology is very important. "Observation of behaviour reveals both direct and indirect effects of pollutants and understanding sub-lethal effects is critical for assessing environmental effects and for limiting environmental problems "(Dell'Omo, G., 2002, p.xvi). Pesticides have a neurological effect on organisms, changing their behaviour by blocking important enzymes needed for the proper functioning of the nervous system (Kamrin 1997). Behavioural toxicity decreases an organism's ability to adapt and survive in the environment (Reeve 2002). Since pesticides

are neurotoxins, behavioural response was chosen to determine toxicity. Endosulfan is a chlorinated hydrocarbon referred to as an organochlorine. Organochlorines are powerful pesticides and can stimulate the nervous system by affecting nerve fibres, along the length of the fibre and by disturbing the transmission of the nerve pulse. The result is that the organism will be sent a transmission continuously rather than in response to stimuli affecting the central nervous system, as a symptom of poisoning.

Endosulfan

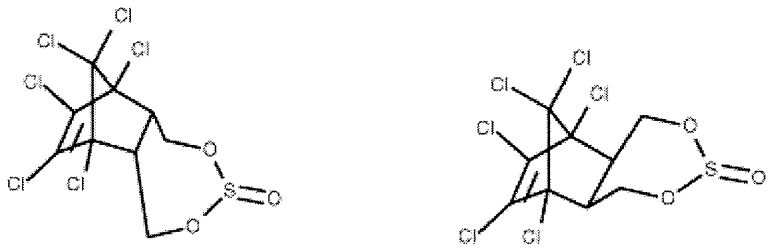

Figure 1.1.0: Endosulfan Alpha and Beta. (Sigma-Aldrich Canada 2004)

Corophium volutator was selected as a desirable test organism for our experiments since many species are found in abundance in benthic and pelagic communities and play an important role in disturbance events. Conlan (1994) describes a disturbance as a biological, chemical, or physical event which alters or destroys food and space resources or the physical environment affecting an organism, population, community or ecosystem. *Corophium volutator* is a deposit feeder and is more susceptible to particle bound contaminants due to the ingestion of sediments, bacteria and diatoms (Chapman 1998). Moreover, *Corophium volutator* is an important species in the food chain; it represents a prey for many fish, crustaceans, mammals and birds. This invertebrate is easily cultured in captivity, broadly distributed, and is found in large numbers in the Bay of Fundy; density can go up to 52,280/m^2 (Boates and Smith 1979), and more importantly it has a short life-cycle and a high reproductive potential (Chapman 1998).

Experiments were repeated to see changes in time and to further explore the behavioural pattern of *Corophium sp.* exposed to lower concentrations of endosulfan. In a run off situation the concentration of pesticides will be reduced in estuaries, with much lower levels than in streams and rivers. Instead of further investigating the lethal doses, the study focuses on the real long-term effects of pesticides on the aquatic niche. Our objective is to discover if low concentrations of pesticides have a chronic toxic effect on amphipods. For this reason, the sub-lethal effect of avoidance/preference behaviour was refined with endosulfan. Experiment tested: change in time, scale of movement, weathered pesticides and preference of amphipods exposed to 3 choices of sediment. As well as investigating behavioural effect, the lipid percent relative to dry animal mass and pesticide partitioning in sediment and water were evaluated using an environmental model (partitioning was not determined experimentally in the laboratory since the analytical equipment required was under repair). The estimation of the sediment organic carbon partitioning was possible based on results of octanol-water coefficient found in the literature and by knowing the concentration of pesticides added to the sediments. The concentrations of pesticides used to conduct the experiments were compared with estimated concentrations likely to end up in the Wilmot River estuary calculated based on watershed and agricultural land use data.

This research project is a continuation of previous work done as a Co-op term in summer 2004. Some of the results and data obtained during summer 2004 were used in this report.

MATERIALS AND METHODS

During summer 2004, a series of experiments were done on the behavioural change of the amphipod *Corophium volutator* exposed to each of six selected pesticides: Endosulfan I + II, Azinphos-methyl, Carbofuran, Chlorothalonil, Atrazine and Hexazinone. The behavioural patterns of *Corophium volutator* including avoidance/preference, swimming, crawling or burrowing preference were investigated. For each set of experiments, 7 tanks separated in the middle by a removable divider were used. In each tank, 50g of Hantsport sediment was placed on each side of the divider. Six test tanks were spiked on the left side with a selected concentration of pesticide and each concentration was done in duplicates. One reference tank containing clean sediments on both sides was used for each set of experiments to measure the effect of handling animals when no pesticides are present. Twenty amphipods were added in each tank; on the spiked sediment for one tank in a duplicate and on the reference sediment for the second tank in a duplicate (Fig 2.0.1).

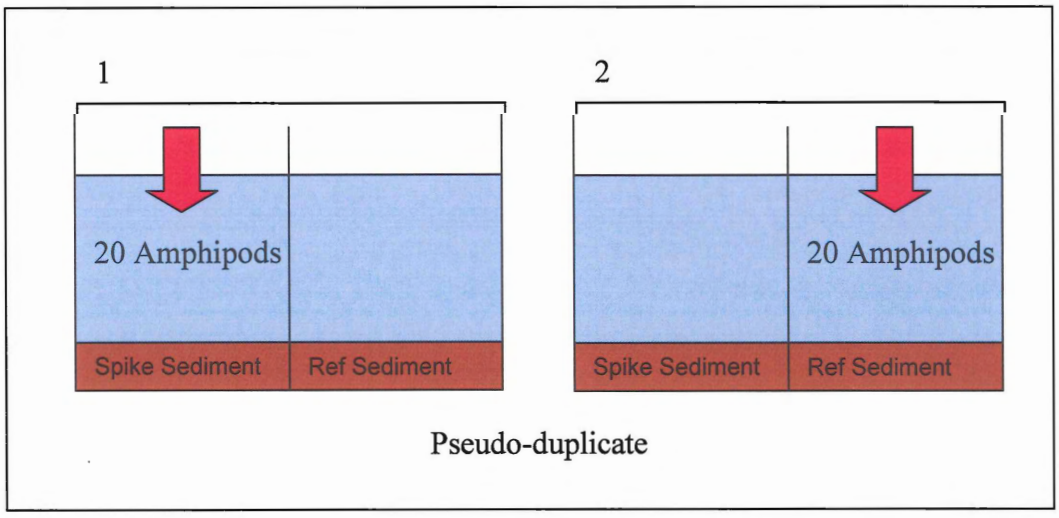

Figure 2.0.1: Experimental tanks for testing Avoidance/Preference; illustrating pseudo-duplicate (same concentration of pesticides in on the left side of both tanks). After 48 hrs, the dividers are put back in place and animals are counted on each side of the tank.

For each test (Fig: 2.0.2; Hellou et al. 2005), the percent mortality, number of animals found alive after 48hrs divided by the initial number of animal multiplied by 100, and percent preference for the reference sediments, number of animals found alive after 48hrs in the reference sediment divided by the number of animal found alive in the tank after 48hrs multiplied by 100. Interpretation consisted in evaluating: if survival rate was higher than 80%; if the percent difference between duplicate tests was minimal; and if there was a trend in the preference. The lethal toxic dose, LC_{50}, was also determined for each of the pesticides tested.

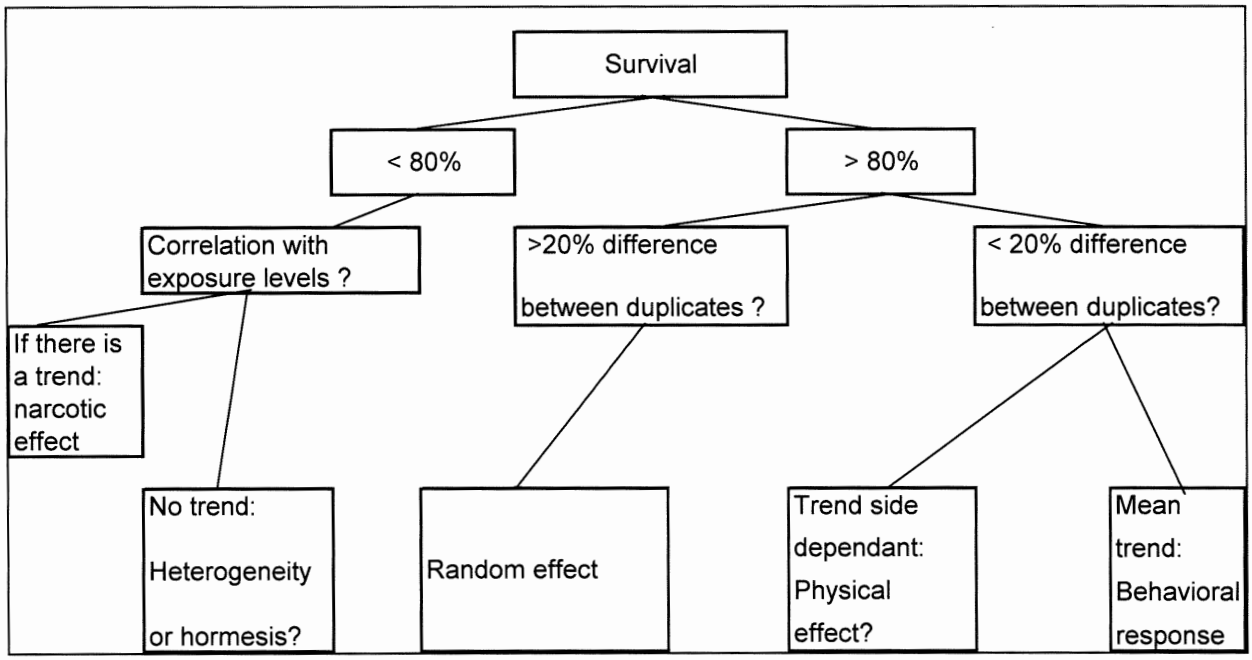

Figure 2.0.2: Interpretative flow chart (Hellou et al. 2005): Approach used for interpreting the results of the behavioural experiments.

Results were plotted in a histogram showing the preference percent in the Y axis and the pesticide concentrations in the X axis. Each pesticide concentration tested was done in duplicate and was illustrated in four columns in the graph. The first column represented for a concentration, "Start S", indicates that the amphipods were placed on the spiked side of the tank at the beginning of the experiment and the height of the bar on the Y axis for this category represents the percentage of surviving animals that preferred the reference sediment after 48hrs. The second column, "Dead S", indicates the percentage of animals found

dead after 48 hrs when initially placed on the spiked side of the tank. The third column, "Start H", indicates that the animals were placed on the reference side of the tank where sediments are clean and the height of the bar on the Y axis for this category represents the percentage of animals that were found in the reference sediment at the end of the 48hrs experiment. The fourth and last column for a pesticide concentration tested, "Dead H", indicates the percentage of animal found dead when initially placed on the reference side (Hantsport sediments).

Endosulfan was the only pesticide out of the six tested for avoidance/preference during summer 2004 showing a clear trend both as an attractant and repellent. Amphipods showed a preference for contaminated sediments at low concentrations and avoidance for higher concentrations of endosulfan.

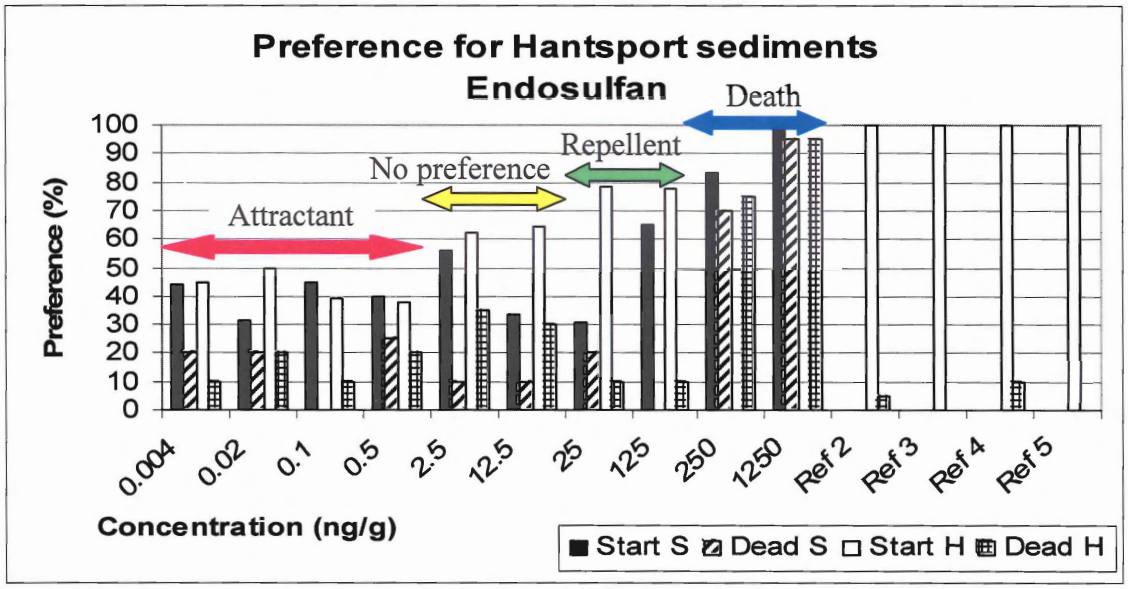

Figure 2.0.3: Avoidance/Preference behavioural response of amphipods exposed to sediments spiked with the pesticide Endosulfan over a wide range of concentrations. "Start S" indicates that the amphipods were placed on the spiked side of the tank at the beginning of the experiment and the height of the bar on the Y axis for this category represents the percentage of surviving animals that preferred the reference sediment after 48hrs. "Dead S" indicates the percentage of animals found dead after 48 hrs when initially placed on the spiked side of the tank. "Start H" indicates that the animals were placed on the reference side of the

tank where sediments are clean and the height of the bar on the Y axis for this category represents the percentage of animals that were found in the reference sediment at the end of the 48hrs experiment. "Dead H" indicates the percentage of animal found dead when initially placed on the reference side (Hantsport sediments).

Based on the results obtained for the behavioral response of amphipods to endosulfan in the summer of 2004, it was deduced that experiments should be repeated with target concentrations of 0.1, 2.5, and 62.5ng/g to see if the results were going to be consistent with time and to further investigate the behavior of *Corophium volutator.*

2.1 Sampling *Corophium volutator* and sediments

Amphipods and sediments were collected at low tide at Hantsport and Cheverie mudflats (See Appendix A: Maps and geology of the Sampling areas). Amphipods and sediments were collected at the surface of the mud flats with gardening shuffles and put in buckets. Only brown sediments from the upper layer of the beach were collected. The buckets, closed with lids, were kept in big coolers for transportation. At destination, the amphipods and their mud were sieved through 1mm sieves, counted and gently handled with twisters and disposed in a beaker containing salt water. The sediments were sieved through a 1mm sieve to discard any organic material and other biological life and frozen for later use.

2.2 Maintaining *Corophium volutator* in captivity

Amphipods were maintained in 20 litre tanks, with about 2 cm of sieved sediments in the bottom and about 12 litres of filtered salt water of PH 7.88 from Bedford basin in an incubator at 15°C with 12 hrs of light and 12hrs of dark. Around 2000 amphipods were placed in each tank. Three air stones were installed at the water surface of each tank; sediment was stirred every day to prevent sediment from becoming anaerobic and amphipods were fed every

Friday. Food consisted of 10 ml of fresh water added to 1g of TetraMin fish food crushed and stirred. The yellow solution formed on top of the solid food was collected and fed to the amphipods. Every Friday the water from the reference tanks was siphoned out, drained through a sieve and collected in a bucket. Afterwards, new salt water was added, sediment was stirred and once sediment settled in the bottom of the tank, the TetraMin solution was added to the water and after a few minutes the air tubing and stones were placed back in the tank.

2.3 Avoidance/Preference Experiments

1. For each set of experiments, 7 tanks separated in the middle by a removable divider were used. In each tank, 50g of Hantsport sediment was placed on each side of the divider. From the main reference tank, 140 amphipods were collected in 7 beakers containing 10ml of salt water and 20 amphipods were placed in each. A 500µl sieve was used to collect the animals. Animals of nearly the same size were selected. Pregnant females with rounder bodies and a transparent but visible pouch visible, as well as smaller animals were put back into the reference tank. Dead and weak animals were removed and replaced by healthier ones. Each test tank was spiked on the left side with a selected concentration of pesticide, each concentration was done in duplicate and a reference tank was also used (unspiked sediment on both sides) for comparison. After spiking the sediment, time was allowed for the acetone to evaporate, 15 minutes for 125µl, 45 minutes for 1mL. Afterwards, the sediment was stirred to insure a homogenous distribution of the contaminant. Amphipods were then added on a different side of the test tanks for each of the duplicate; on the spiked side for one of the tank and on the reference side for the other one, and on the left side for the reference tank. Around 800mL of filtered salt water was added to the reference side of the test tanks and air tubing was installed in each tank. To that effect a tube was attached to an air pump with a needle at the end, so as not to cause major turbulence in the tanks. Finally, the dividers were removed from the test tanks and time and temperature were recorded. The experiments were conducted at room temperature 20 -25°C. Every few hours the animals in the

tanks were monitored. Observation consisted of noting the number of amphipods that were swimming, crawling, burrowing or stayed immobile on the sediments. Also temperature was monitored and time marked during observation. The objective was to see if there were any changes in amphipod behaviour over time. After 48hrs, dividers were put back into place in each tank and air tubing was removed. Water from each of the tanks was drained from the reference side using a tube and a siphon. Water representing the highest concentration of a set of exposures to one pesticide was kept in a bottle, 20ml of dichloromethane was added and bottles were stored for pesticides analysis later. Spiked sediments from the highest concentration of the set was also kept on a glass dish, dried and frozen. Subsequently, amphipods were collected with tweezers, counted and put in beakers for drying. Amphipods were counted separately for each side of the tank, dead or missing animals were also recorded.

2. The same experiment was repeated with animals collected in the fall and winter to see if the experiments were repeatable in time.

3. The same experiment was repeated; however, the tanks with spiked sediment and water were prepared a week in advance, prior to adding the animals, to see if behavioural changes would be observed due to the weathering of the pesticide.

4. The same experiment was repeated in 80 x 80 mm watch glass, 20g of sediment on each side and with only one animal at a time in order to observe individual behaviour and scale of movement.

5. The same experiment was repeated, using 3 divisions, giving the animals three different choices of sediments and placing animals in the lowest and highest concentration of spiked sediment present, in duplicate attempts. Also for two of the experiments, reference sediment was placed at the centre between two contaminated sediments, and the animals were added to the water.

2.4 Determination of neutral lipid percent per dry mass in *Corophium sp.*

Once avoidance/preference experiments were completed, amphipods were collected with tweezers, counted and put in beakers for drying. Amphipods were counted separately for each side of the tank and preserved under R if the animals were collected on the reference side of the tank and as S for the animals found on the spiked side of the tank. The exposure concentration was also recorded and pseudo-duplicates were combined. Lipids were extracted from R and S exposed to a specific concentration. All Glassware was cleaned with acetone, hexane and dichloromethane. Amphipods were dried from 24 to 72 hours. Amphipods were counted and weighed on a small piece of aluminium fold. Animals were transferred to Teflon tubes and crushed with a glass rod. Five mL of hexane: dichloromethane (1:1) mixture and a scoop of sodium sulphate were added. The solution was sonicated at power 60 for 3 minutes and centrifuged for 8 minutes at ¾ speed. Extraction with hexane: dichloromethane, sonication and centrifugation were performed 3 times. After each extraction, the resulting solution was transferred to a 100ml round bottom flask through filtration over a paper filter inserted in a funnel. Each solution was evaporated and 1ml of solution was left in the container. The solution was transferred into 4ml pre-massed small brown vials with a Pasteur pipette and the round bottom flask were once again rinsed three times and transferred to the vials. The samples were then placed under a stream of nitrogen to evaporate the solvent. Samples were massed as soon as they were dried. Percentage of the animals lipid content was calculated for each sample using the formula % Lipids = (lipids mass/mass dry animal)*100.

2.5 Determination of partitioning coefficients for endosulfan

Published values for the log K_{ow} of endosulfan ranges between 2.2 and 3.6. Therefore, the octanol-water partition coefficient ranges between 158 and 3981. The calculations for the sediment organic carbon partitioning are described by Mackay (1991).

Calculation for the partition of pesticide in water

- **Csed/Cw = 0.4 K_{ow} x organic carbon content x sediment density**
- Cseds = Concentration of pesticides in sediment in ng/g (known)
- Cw = Concentration of pesticide in water in ng/ml (unknown)
- K_{ow} = 158 or 3981
- Organic carbon % is 0.66% for Hantsport sediments (Sediments in the Wilmot River estuary contained up to 7% organic carbon)
- Sediment density is 2.11g/ml for Hantsport sediment (Tremblay 2004)
- 1ml of water=1g

2.6 Determination of possible concentrations of endosulfan existing in runoff and in the estuary of the Wilmot River watershed

The concentrations of endosulfan calculated are based on only one spraying event. A GIS map was used to calculate the total potato farm surface area in the Wilmot river watershed as well as the total surface area of the watershed. Runoff defined as the total discharge of surface water was expressed as an equivalent depth of water in mm and along with the total surface area of the watershed were used to calculate the total volume of surface water present in the watershed. The total concentration of endosulfan present in the surface water was determined based on the partitioning coefficient calculations identical to section 2.5. Many assumptions were made and some important factors usually included in hydrological calculations were not considered. Calculations described by Schnoor (1996).

Assumptions:

--Every potato farm area received the same initial quantity of pesticide for 1 spray

--Total quantity of endosulfan present in treated soil is transferred to runoff by an amount calculated based on partitioning coefficient defined in section 2.5

--Total pesticide mass dissolved and transported by runoff was discharged in the Wilmot River

--Total quantity of pesticides discharged into the Wilmot River was dissolved in the river but entirely transported to the estuary.

Factors that are not considered including:

–Quantity for contaminated soils particles transported within runoff as a result of erosion

--Fresh water tributary streams entering Wilmot River

--Whether Wilmot River flow is turbulent or laminar

--pH values, total dissolved oxygen, biomass content in runoff and streams

--Meteorological influences, transport processes, diffusion of the chemical, rate of flow, degree of mixing and thermal effect

--Partition coefficient transport across water air interface in the distribution process

--Transformation process such as biological conversion and degradation processes

Calculation for concentration of pesticides in surface runoff:

$(Cs/0.056 \times K_{ow})/ (\text{Runoff} \times \text{total area in } m^3)$

Calculation of endosulfan discharge in the Wilmot River estuary

C x Q= concentration entering Wilmot river estuary in Kg/s per 1000L of water

C= concentration of pesticide present in the water in Kg/m^3

Q= Wilmot river discharge in m^3/s

RESULTS

3.1 Sampling *Corophium volutator* and sediments

Amphipods and sediments were sampled from Hantsport mud flats (08/07/2004, 15/01/2005) and Cheverie Beach (06/10/2004, 15/01/2005) (see sampling map in Appendix A). In the fall and winter, the density was much lower and the animals were smaller.

3.2 Maintaining *Corophium volutator* in captivity

Animals left from the previous collection were kept in two separate reference tanks and counted on January 14th and February 4th 2005. Animals in one of the tanks were collected in the summer, 8th of July 2004, and the others in the fall, 6th of October 2004. After the first count they were combined and at the end the decrease in population was evaluated to be 4%/day.

Table 3.2.1: Population decline rate in of Corophium in captivity

# of animals	Summer	Fall	Total
1/14/2005	357	130	487
2/4/2005	No data available	No data available	161
Population decline rate	No data available	No data available	**4%/day**

3.3 Behavioural response of amphipods to endosulfan

Behavioural experiments with the pesticide endosulfan were repeated in time to see if concentrations of 0.1ng/g, 2.5ng/g and 62.5ng/g would be comparable with the results obtained in the summer of 2004.

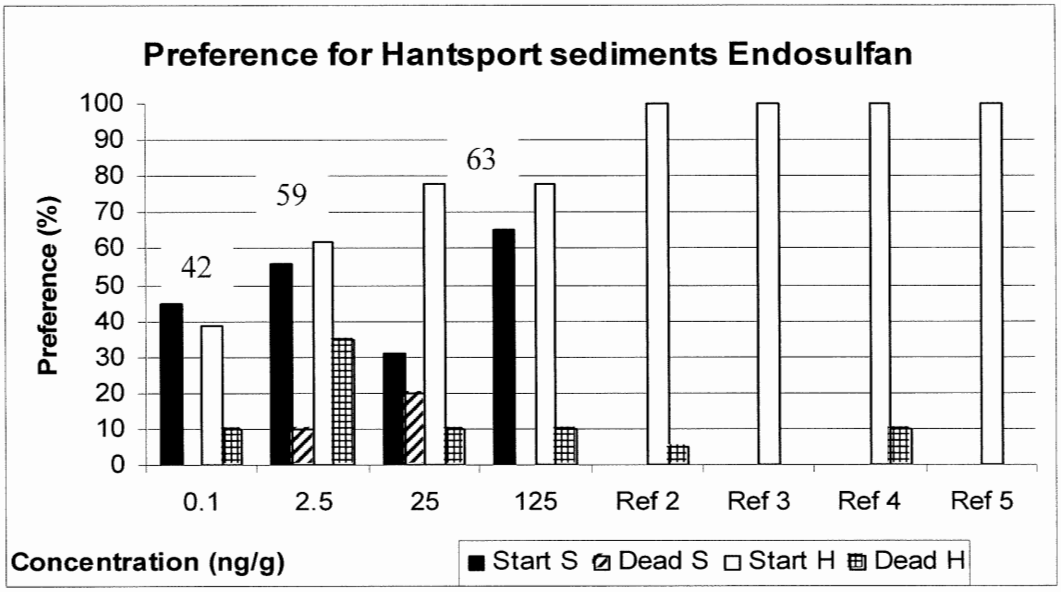

Figure 3.3.1: Preference/Avoidance results for sediments spiked with Endosulfan in summer 2004. "Start S" indicates that the amphipods were placed on the spiked side of the tank at the beginning of the experiment and the height of the bar on the Y axis for this category represents the percentage of surviving animals that preferred the reference sediment after 48hrs. "Dead S" indicates the percentage of animals found dead after 48 hrs when initially placed on the spiked side of the tank. "Start H" indicates that the animals were placed on the reference side of the tank where sediments are clean and the height of the bar on the Y axis for this category represents the percentage of animals that were found in the reference sediment at the end of the 48hrs experiment. "Dead H" indicates the percentage of animal found dead when initially placed on the reference side (Hantsport sediments). The numbers at the top of each level of exposure refer to mean preference between the pseudo-duplicates.

The experiment was repeated on: 22nd, 29th of October 2004, 14th of December 2004, 29, 31 of January 2005 and the 9th of February 2005.

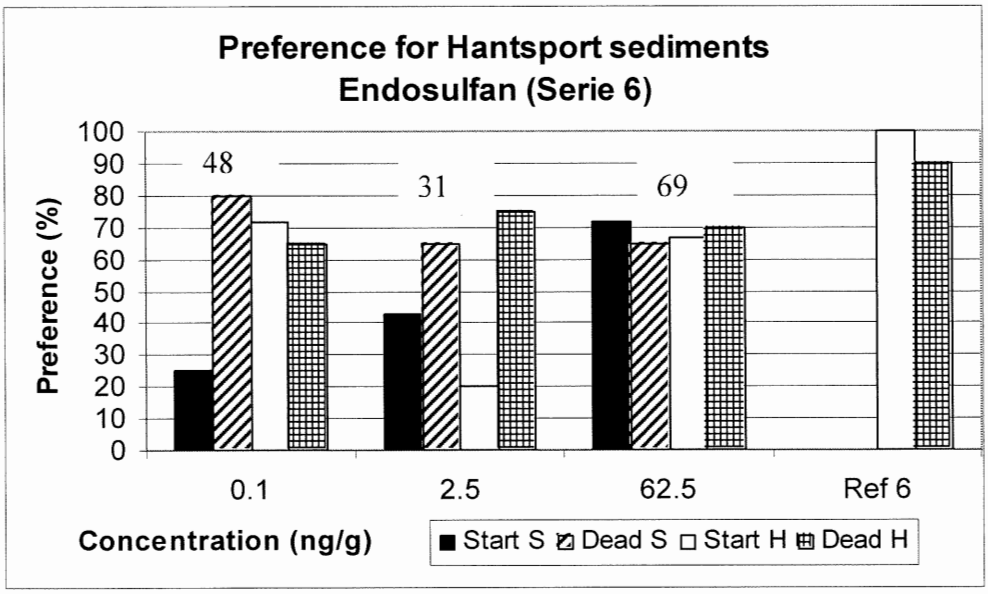

Figure 3.3.2: Preference/Avoidance results for sediment spiked with Endosulfan the 20th of October 2004. The mean death rate for exposed animals to endosulfan was 72% compared to 90% for the reference concentration. "Start S" indicates that the amphipods were placed on the spiked side of the tank at the beginning of the experiment and the height of the bar on the Y axis for this category represents the percentage of surviving animals that preferred the reference sediment after 48hrs. "Dead S" indicates the percentage of animals found dead after 48 hrs when initially placed on the spiked side of the tank. "Start H" indicates that the animals were placed on the reference side of the tank where sediments are clean and the height of the bar on the Y axis for this category represents the percentage of animals that were found in the reference sediment at the end of the 48hrs experiment. "Dead H" indicates the percentage of animal found dead when initially placed on the reference side (Hantsport sediments). The numbers at the top of each level of exposure refer to mean preference between the pseudo-duplicates.

The next experiment was performed using spiked sediment with added water that were prepared a week before adding the animals (Fig 3.2.3). The animals used for the experiment were collected on the 6th of October 2004 at a new location, Cheverie Beach, and they were smaller in comparison to the summer animals.

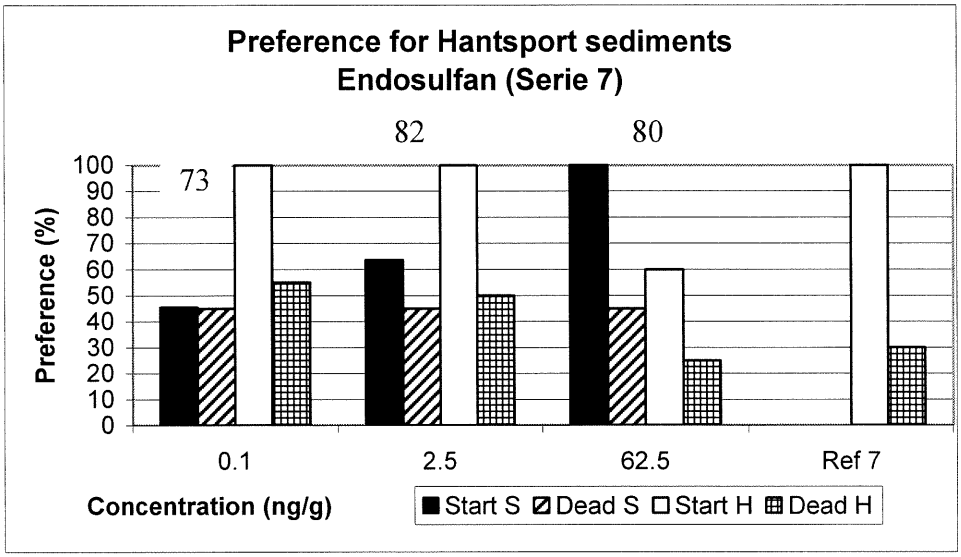

Figure 3.3.3: Preference/Avoidance results for sediment spiked with Endosulfan the 27th of October 2004. The sediment and the water were prepared a week in advance. "Start S" indicates that the amphipods were placed on the spiked side of the tank at the beginning of the experiment and the height of the bar on the Y axis for this category represents the percentage of surviving animals that preferred the reference sediment after 48hrs. "Dead S" indicates the percentage of animals found dead after 48 hrs when initially placed on the spiked side of the tank. "Start H" indicates that the animals were placed on the reference side of the tank where sediments are clean and the height of the bar on the Y axis for this category represents the percentage of animals that were found in the reference sediment at the end of the 48hrs experiment. "Dead H" indicates the percentage of animal found dead when initially placed on the reference side (Hantsport sediments). The numbers at the top of each level of exposure refer to mean preference between the pseudo-duplicates.

This experiment was conducted for a period of 24 hrs instead of 48 hrs (Fig 3.3.4).

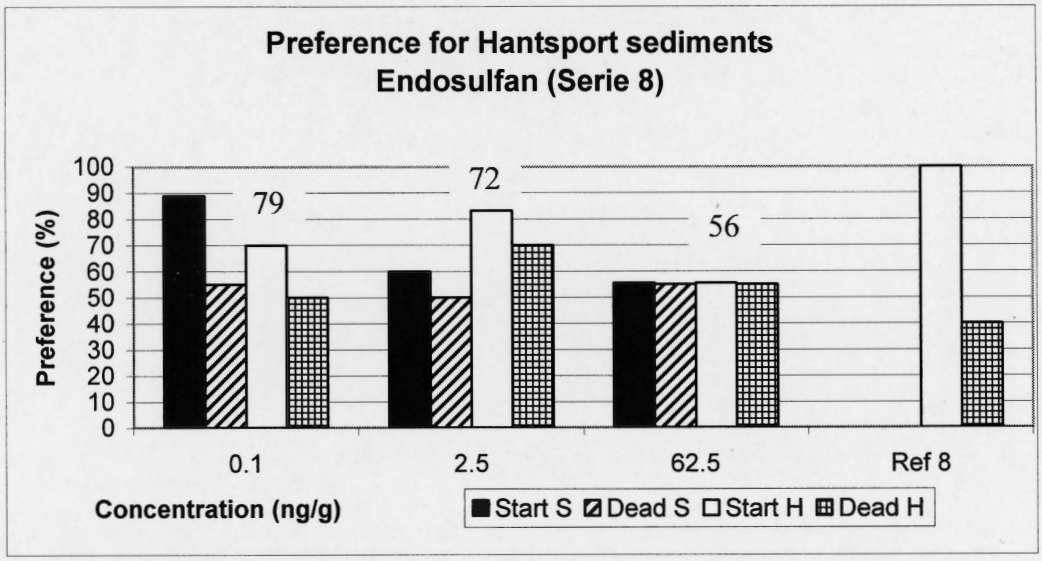

Figure 3.3.4: Preference/Avoidance results for sediment spiked with Endosulfan the 10th of December 2004. (24hrs experiment) The mean death rate for exposed animals was 52% compared with 40% for the reference. "Start S" indicates that the amphipods were placed on the spiked side of the tank at the beginning of the experiment and the height of the bar on the Y axis for this category represents the percentage of surviving animals that preferred the reference sediment after 48hrs. "Dead S" indicates the percentage of animals found dead after 48 hrs when initially placed on the spiked side of the tank. "Start H" indicates that the animals were placed on the reference side of the tank where sediments are clean and the height of the bar on the Y axis for this category represents the percentage of animals that were found in the reference sediment at the end of the 48hrs experiment. "Dead H" indicates the percentage of animal found dead when initially placed on the reference side (Hantsport sediments). The numbers at the top of each level of exposure refer to mean preference between the pseudo-duplicates.

The following experiment was performed in larger tanks with only one replicate using 60 animals that were exposed for a period of 72 hrs and the animals were added to the water instead of being placed in the sediments. The animals used for this experiment were collected in the winter on the 15th of January 2005.

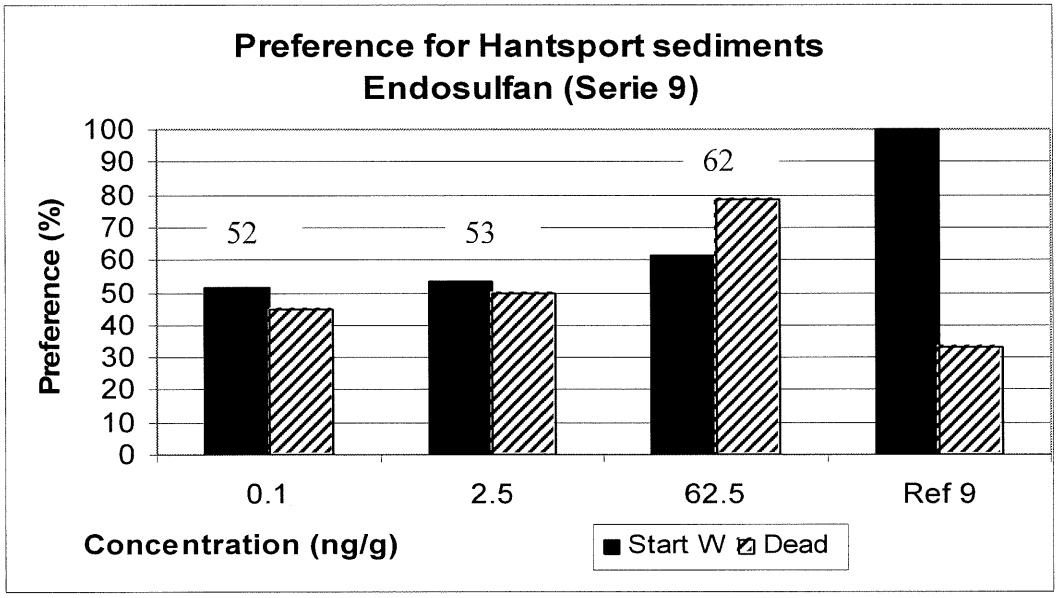

Figure 3.3.5: Preference/Avoidance results for sediment spiked with Endosulfan the 26th of January 2005. 3L tanks were used with only one replicate per concentration for 72 hrs exposure. 60 animals were added to water in each tank. Mean death rate for exposed animals is 58% compared with 30% for non-exposed animals. "Start S" indicates that the amphipods were placed on the spiked side of the tank at the beginning of the experiment and the height of the bar on the Y axis for this category represents the percentage of surviving animals that preferred the reference sediment after 48hrs. "Dead S" indicates the percentage of animals found dead after 48 hrs when initially placed on the spiked side of the tank. "Start H" indicates that the animals were placed on the reference side of the tank where sediments are clean and the height of the bar on the Y axis for this category represents the percentage of animals that were found in the reference sediment at the end of the 48hrs experiment. "Dead H" indicates the percentage of animal found dead when initially placed on the reference side (Hantsport sediments). The numbers at the top of each level of exposure refer to mean preference between the pseudo-duplicates.

The following experiments (Fig 3.3.6, 3.3.7) were performed at 15°C for a period of 48 hrs.

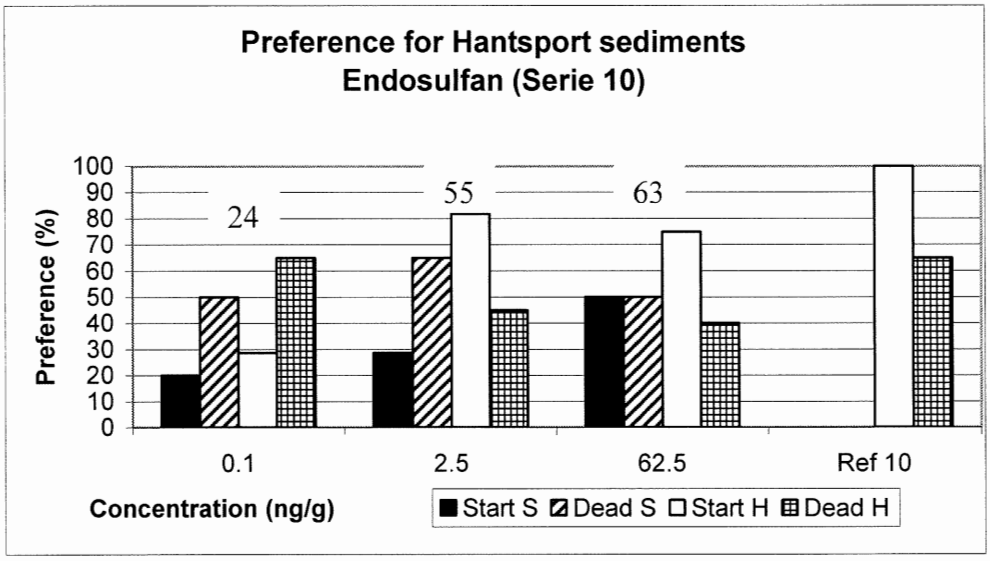

Figure 3.3.6: Preference/Avoidance results for sediment spiked with Endosulfan the 29th of January 2005. Animals were exposed for 48 hrs. "Start S" indicates that the amphipods were placed on the spiked side of the tank at the beginning of the experiment and the height of the bar on the Y axis for this category represents the percentage of surviving animals that preferred the reference sediment after 48hrs. "Dead S" indicates the percentage of animals found dead after 48 hrs when initially placed on the spiked side of the tank. "Start H" indicates that the animals were placed on the reference side of the tank where sediments are clean and the height of the bar on the Y axis for this category represents the percentage of animals that were found in the reference sediment at the end of the 48hrs experiment. "Dead H" indicates the percentage of animal found dead when initially placed on the reference side (Hantsport sediments). The numbers at the top of each level of exposure refer to mean preference between the pseudo-duplicates.

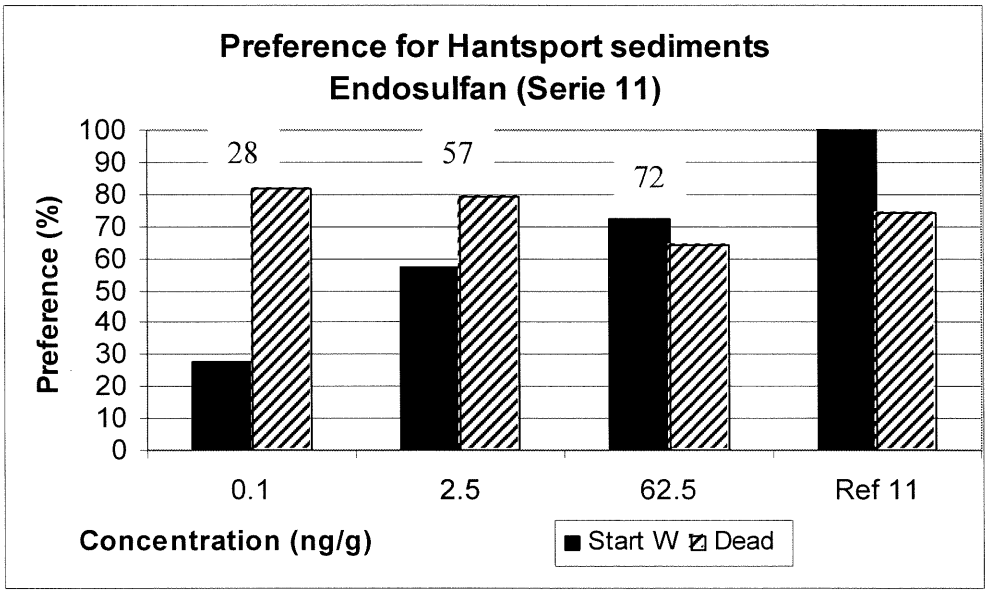

Figure 3.3.7: Preference/Avoidance results for sediment spiked with Endosulfan the 7th of February 2005. 3L tanks were used with only one replicate per concentration for 48 hrs exposure. Mean death rate is 75% for exposed animals and 75% for non-exposed. "Start S" indicates that the amphipods were placed on the spiked side of the tank at the beginning of the experiment and the height of the bar on the Y axis for this category represents the percentage of surviving animals that preferred the reference sediment after 48hrs. "Dead S" indicates the percentage of animals found dead after 48 hrs when initially placed on the spiked side of the tank. "Start H" indicates that the animals were placed on the reference side of the tank where sediments are clean and the height of the bar on the Y axis for this category represents the percentage of animals that were found in the reference sediment at the end of the 48hrs experiment. "Dead H" indicates the percentage of animal found dead when initially placed on the reference side (Hantsport sediments). The numbers at the top of each level of exposure refer to mean preference between the pseudo-duplicates.

3.3.1. Avoidance/Preference in Beakers

Behavioural experiments were also conducted with the same concentrations of 0.1, 2.5, 62.5 ng/g of spiked sediment, using only one animal at a time to observe behavioural response and migration of the animal exposed to contaminated sediment. (Results presented in Appendix D)

3.3.2. Avoidance/Preference Three Choices

Preference/Avoidance experiments were performed in tanks containing two dividers, giving the animals three different choices of sediment (Fig 3.3.8). Tank 1 and 2 divisions contained sediments with endosulfan concentrations of 0.1, 2.5, 62.5ng/g. Animals were initially placed on sediment with endosulfan concentrations of 0.1ng/g for Tank 1 and on sediment with endosulfan concentrations of 62.5 for Tank 2. Tank 3, 4, 5 and 6 divisions contained concentration of 0.1, 62.5ng/g of endosulfan and reference sediment in the middle and the animals were initially placed in the water.

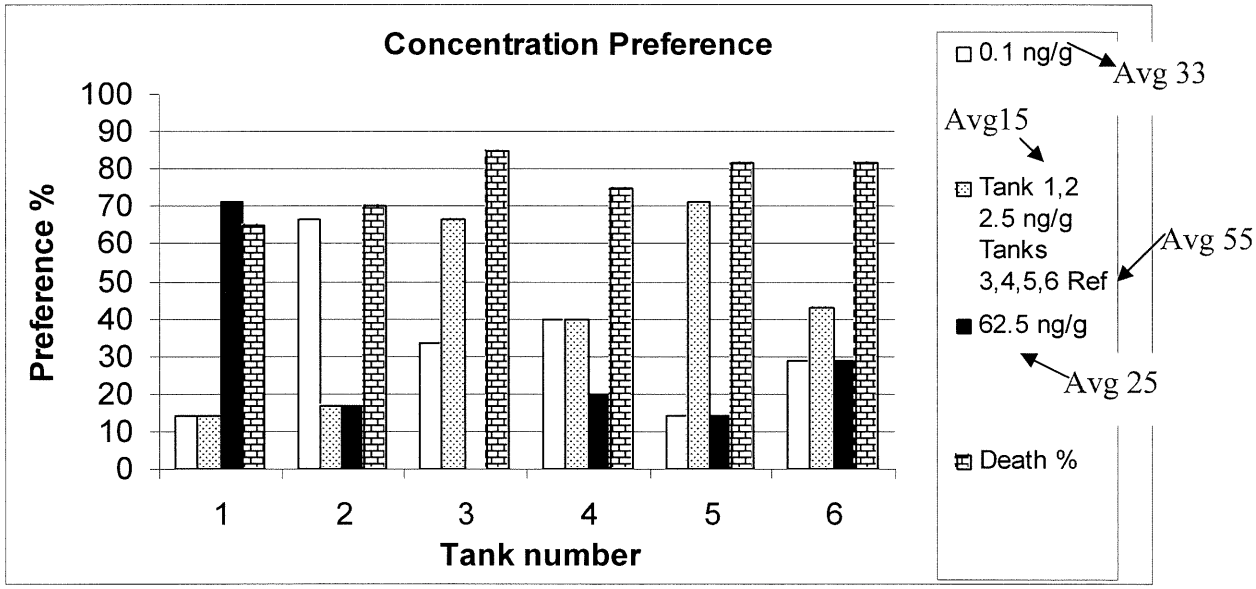

Figure 3.3.8: Preference/Avoidance results for sediment spiked with Endosulfan. 3L tanks were used in duplicate giving three choices of sediments for 48 hrs exposure.

3.4 Lipid percent per dry mass of amphipods

Lipids for the six pesticides tested over summer 2004 were extracted in the fall using different procedures to improve the quality and precision of the data. Lipids per dry animal mass were extracted to determine if animals exposed to pesticides store different amounts of lipids than animals that were not-exposed.

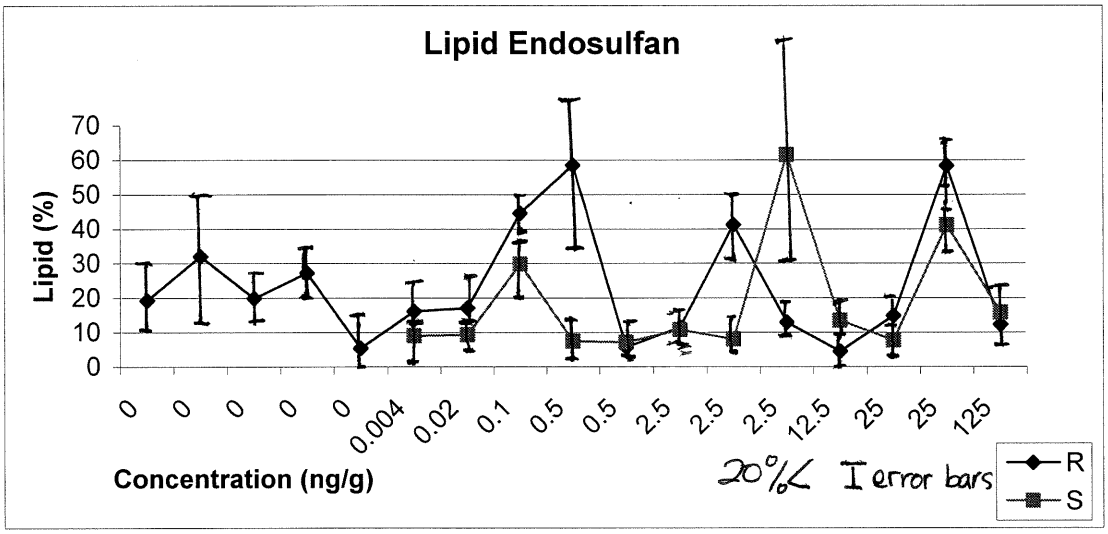

Figure 3.4.1 Lipids % per dry mass for amphipods exposed to endosulfan in summer 2004. "R" represents the results for the animals collected on the reference side of the tank and "S" represents lipid for the animals collected on the spiked side of the tank for each concentration.

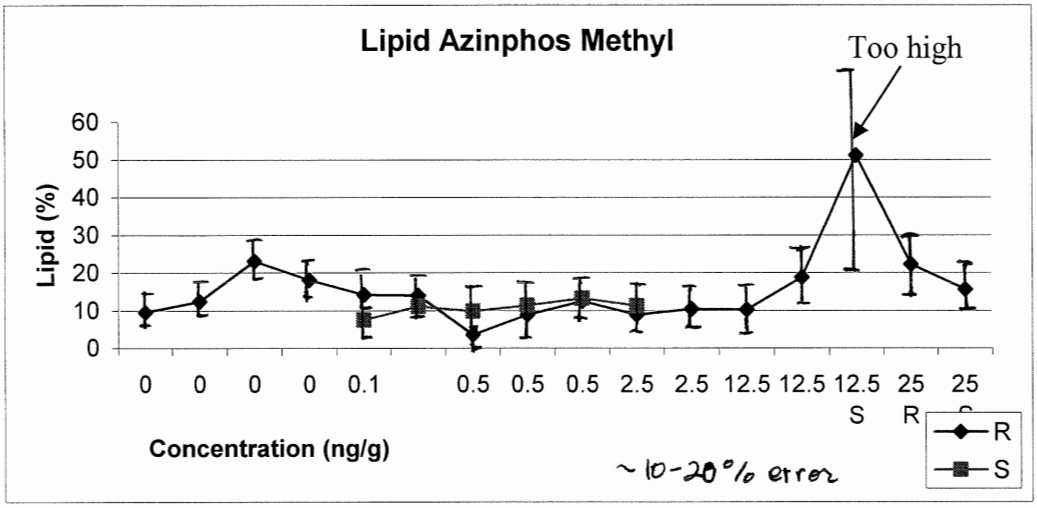

Figure 3.4.2: Lipids % per dry mass for amphipods exposed to Azinphos Methyl in summer 2004. "R" represents the results for the animals collected on the reference side of the tank and "S" represents lipid for the animals collected on the spiked side of the tank for each concentration.

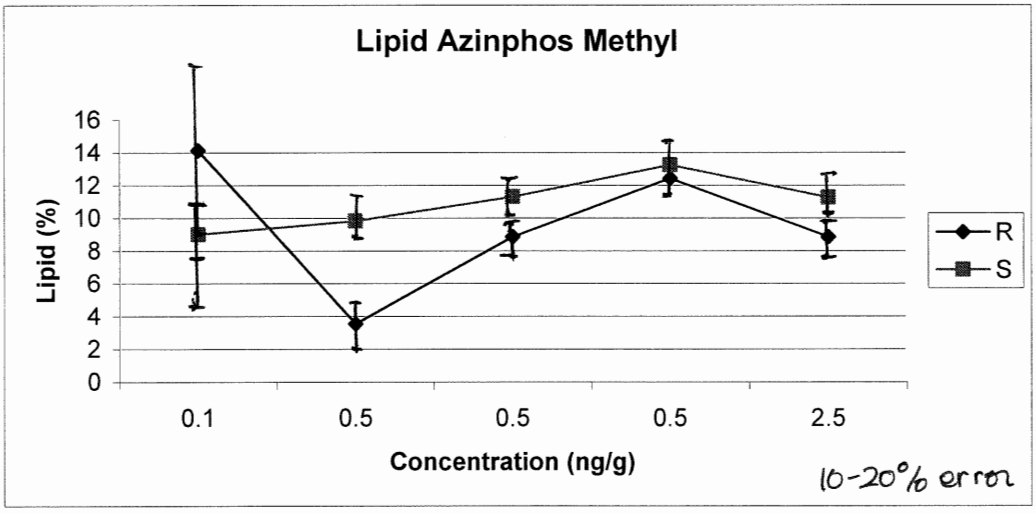

Figure 3.4.3: Zoom on Lipids % per dry mass for concentrations (0.1-2.5 ng/g) for amphipods exposed to Azinphos Methyl in summer 2004. "R" represents the results for the animals collected on the reference side of the tank and "S" represents lipid for the animals collected on the spiked side of the tank for each concentration.

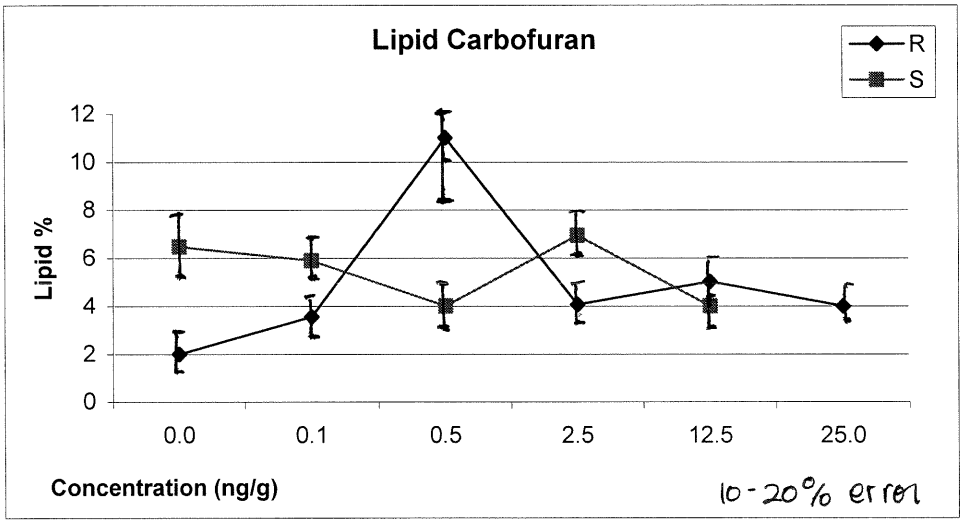

Figure 3.4.4 Lipids % per dry mass for amphipods exposed to Carbofufan in summer 2004. "R" represents the results for the animals collected on the reference side of the tank and "S" represents lipid for the animals collected on the spiked side of the tank for each concentration.

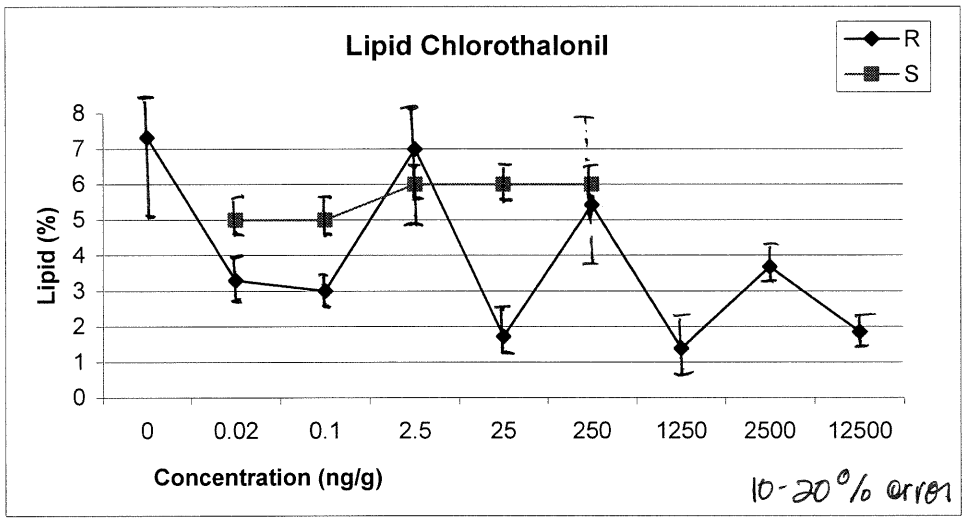

Figure 3.4.5: Lipids % per dry mass for amphipods exposed to Chlorothalonil in summer 2004. "R" represents the results for the animals collected on the reference side of the tank and "S" represents lipid for the animals collected on the spiked side of the tank for each concentration.

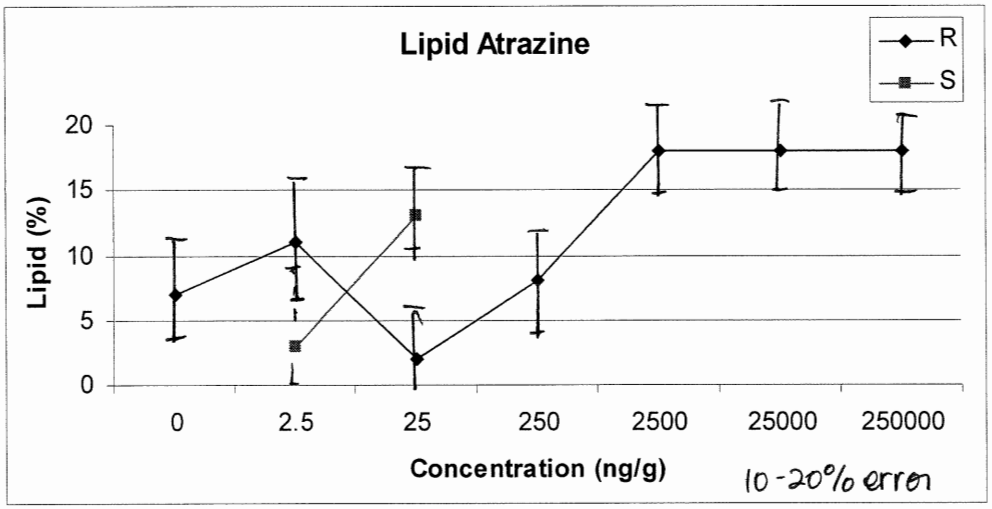

Figure 3.4.6 Lipids % per dry mass for amphipods exposed to Atrazine in summer 2004. "R" represents the results for the animals collected on the reference side of the tank and "S" represents lipid for the animals collected on the spiked side of the tank for each concentration.

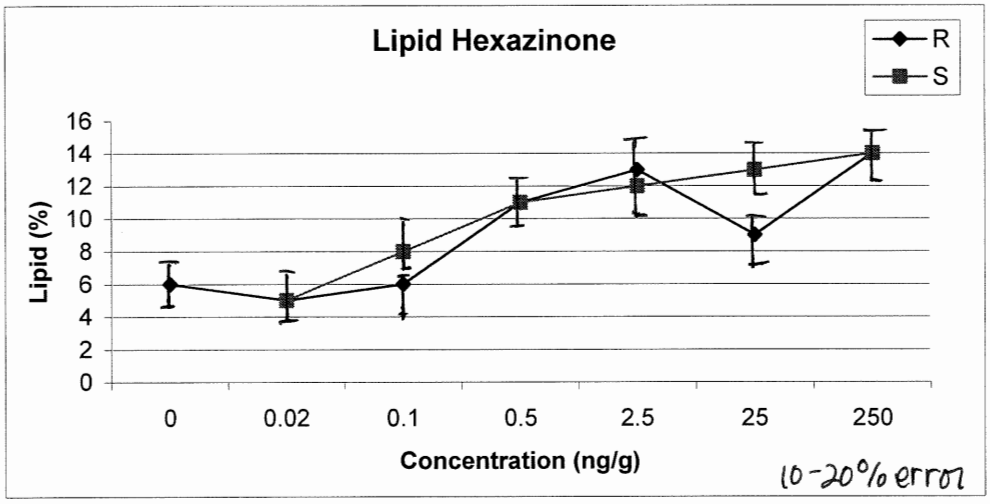

Figure 3.4.7: Lipids % per dry mass for amphipods exposed to Hexazinone in summer 2004. "R" represents the results for the animals collected on the reference side of the tank and "S" represents lipid for the animals collected on the spiked side of the tank for a given concentration.

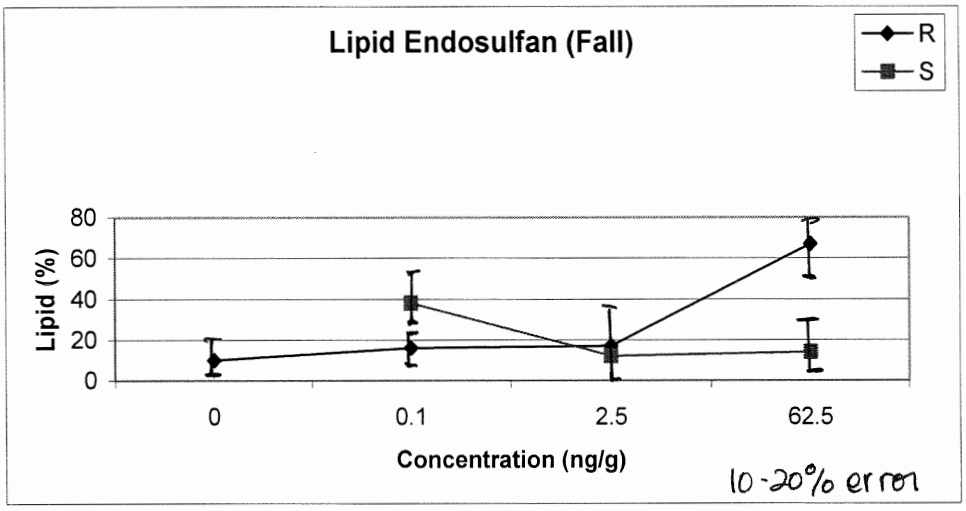

Figure 3.4.8 Lipids % per dry mass for amphipods exposed to endosulfan in fall 2004 for 24hrs. R" represents the results for the animals collected on the reference side of the tank and "S" represents lipid for the animals collected on the spiked side of the tank for a given concentration.

In the following figures, the animals found on the spiked side were combined with the ones found on the reference side in each tank to increase the mass of each sample extracted, as well as the accuracy of the results. These samples were compared with the reference tank of the set where sediments were 100% cleaned on both sides.

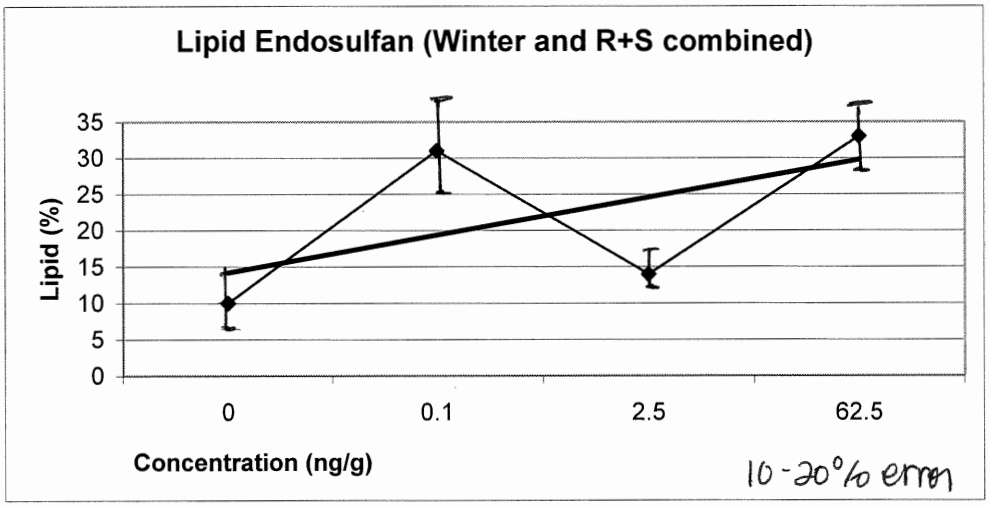

Figure 3.4.9: Lipid % per dry mass for amphipods exposed to endosulfan in winter 2004.

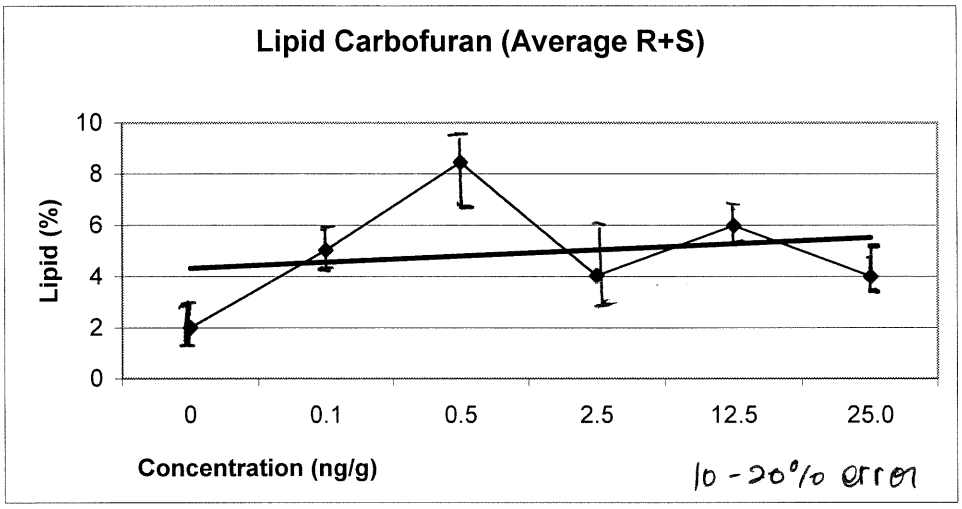

Figure 3.4.10: Lipid % per dry mass for amphipods exposed to carbofuran in summer 2004.

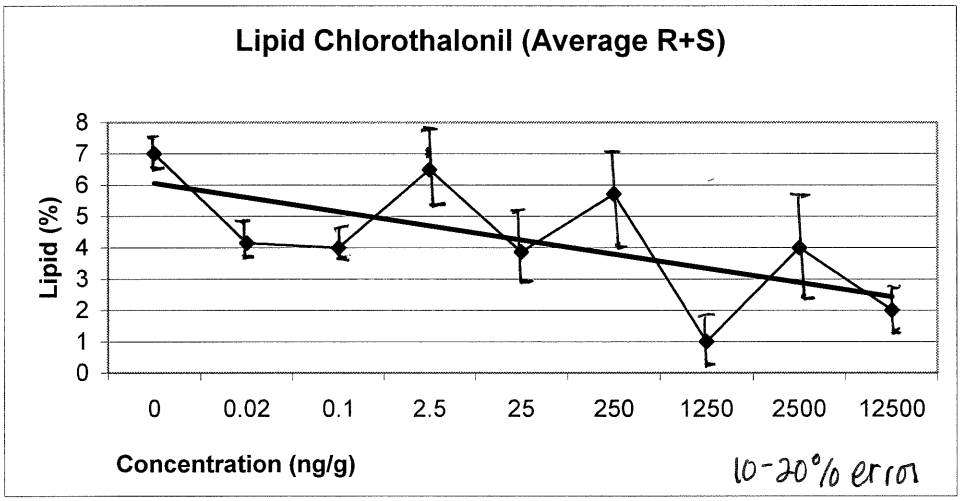

Figure 3.4.11: Lipid % per dry mass for amphipods exposed to Chlorothalonil in summer 2004. Chlorothalonil, as opposed to the other pesticides, is showing a decrease in lipids content as concentration increased. Chlorothalonil is recognized as a stimulant (appendix B). When animals were collected from the tank at the end of the avoidance/preference experiments, they were very agitated and vigorous compared with the other pesticides (Tremblay 2004). Animals are using all of their lipids by being active under the neurological effects of chlorothalonil.

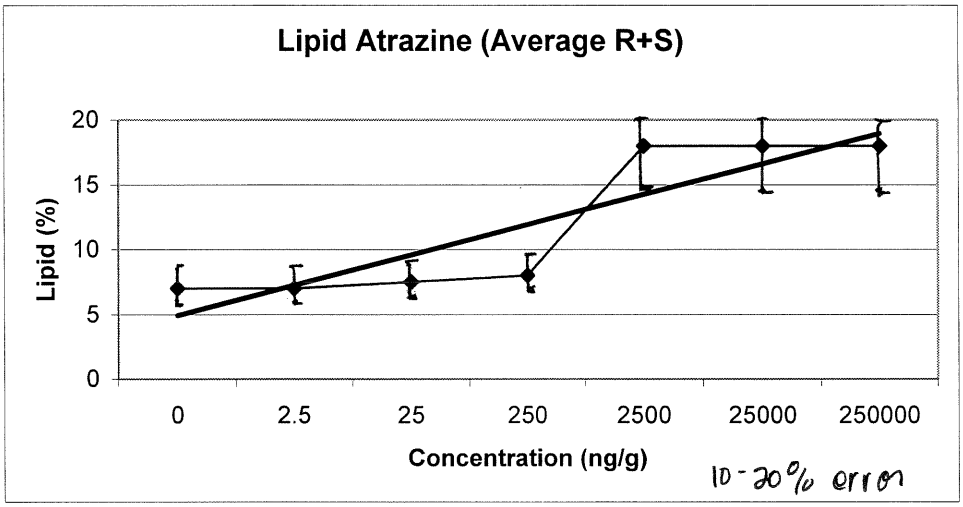

Figure 3.4.12: Lipid % per dry mass for amphipods exposed to atrazine in summer 2004.

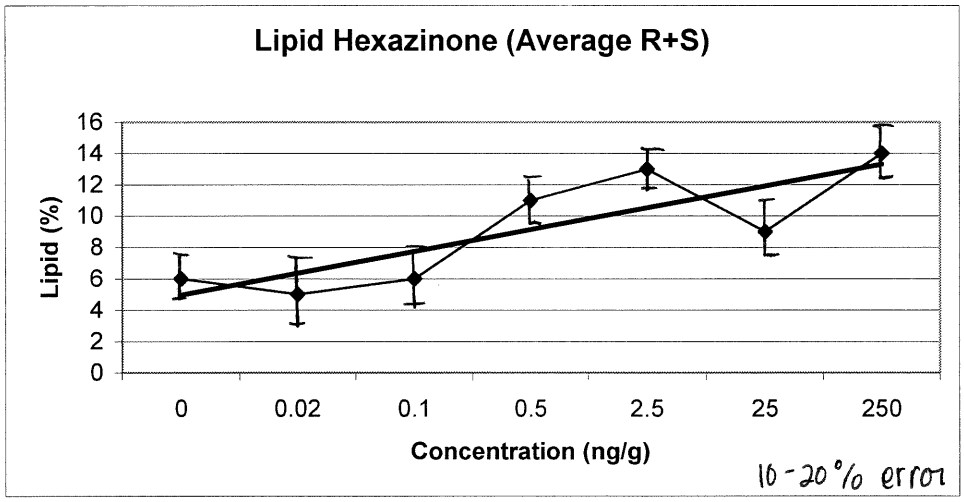

Figure 3.4.13: Lipid % per dry mass for amphipods exposed to Hexazinone in summer 2004.

a.

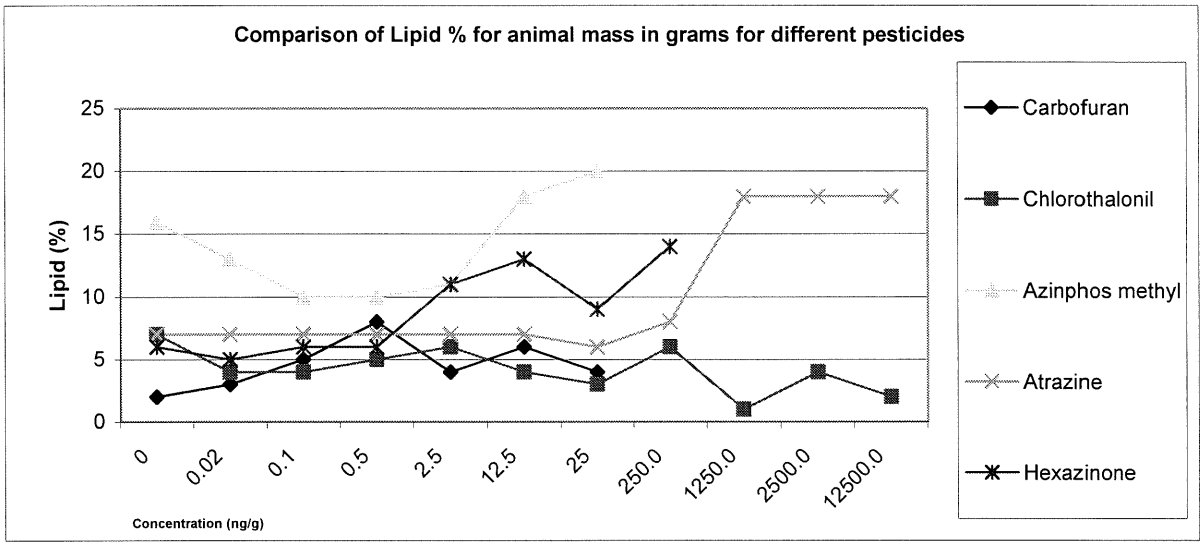

Figure 3.4.14 a: Lipids % for mass in grams for amphipods exposed to 5 different pesticides in summer 2004. The figure shows the variability in lipid percent for the different pesticides.

b.

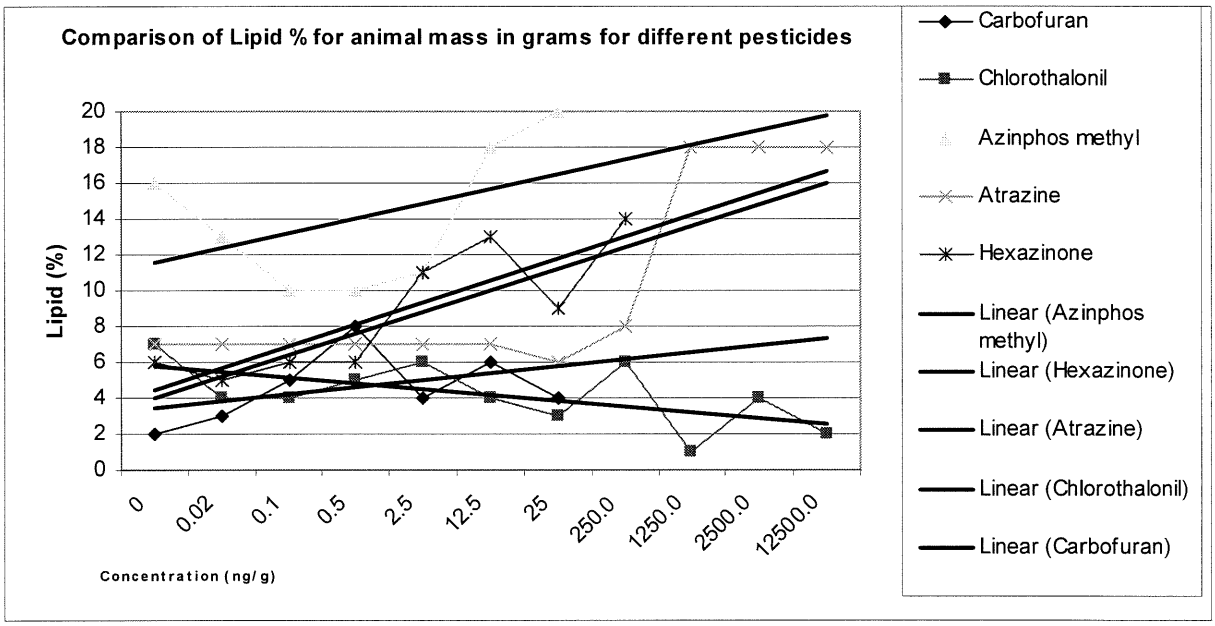

Figure 3.4.14 b: Lipids % for mass in grams for amphipods exposed to 5 different pesticides in summer 2004. The figure shows trend lines demonstrating that for all pesticides but chlorothalonil, the amount of lipid per animal mass increased as the animals are exposed to increasing concentrations of pesticides (although regressions are not significant).

3.5 Partitioning coefficient calculations

1. Calculation for the partition of pesticide in water

Csed/ Cw = 0.4 K_{ow} x organic carbon x sediment density

- Cseds = Concentration of pesticides in sediment in ng (known)
- Cw = Concentration of pesticide in water in ng/ml (unknown)
- K_{ow} = 158 or 3981
- Organic carbon % is 0.66% for Hantsport sediments (Higher for sediments in PEI; Wilmot River estuary sediment up to 7%)
- Sediment density is 2.11g/ml for Hantsport sediment (Laurie 2004)
- 50g of sediment and 500ml of water are used

Let use K_{ow} = 158, and 0.1ng/g in sediments, than;

50g x 0.1ng/g/0.0056 x158 = 500ml x ? = **0.011 ng/ml in water**

If use K_{ow} = 3981, and 0.1 ng/g in sediments, than;

50g x 0.1ng/g/0.0056 x3981 = 500ml x ? = **0.00004ng/ml**

3.6 Concentrations of Endosulfan entering Wilmot River estuary

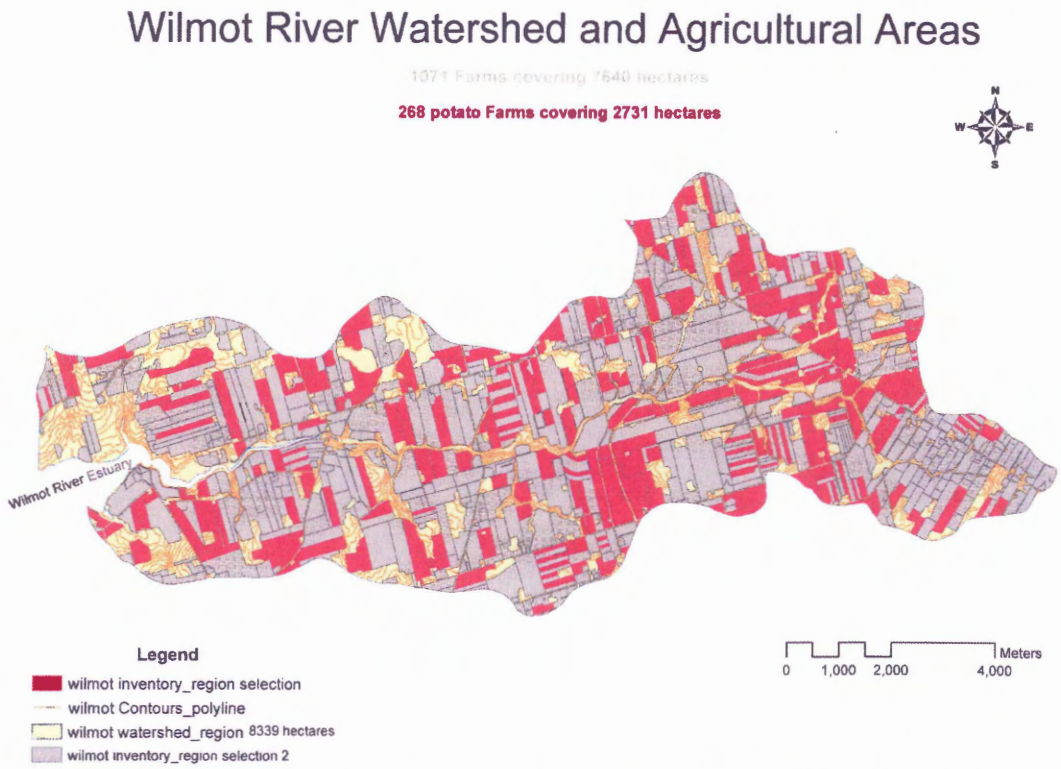

Figure 3.5.1: GIS map of the Wilmot River watershed. The red zones represent the surface area of the potato farms and the grey zones represent other types of agricultural area. Wilmot River watershed has an area of 8339 hectares and the potato farms cover an area of 2731 hectares.

Potato farm surface area: 2731 hectares

Amount of Endosulfan applied: 0.6-1.1 Kg a.i/ha

Total mass of endosulfan (minimum): 1639 Kg

Total mass of endosulfan (maximum): 3004 Kg

Wilmot River (April to September)

Mean annual flow = 0.33 to 0.99 m^3/s

Drainage area of the Wilmot River: 8339 ha

Runoff: 640mm (discharge per unit area)

Calculation for surface runoff:

1. Calculation for the partition of pesticide in water

Csed/ Cw = 0.4 K_{ow} x organic carbon x sediment density

- Cseds = Concentration of pesticides in sediment in Kg (known)
- Cw = Concentration of pesticide in water in ng/ml (unknown)
- K_{ow} = 158 or 3981
- Organic carbon % is up to 7% for the Wilmot River estuary sediment
- Sediment density is about 2.00 g/ml
- Runoff x Total area = Total water volume
- 1 hectare [ha] = 10 000m^2
- 1m^3=1000 dm^3=1000 litre

[1639Kg/(0.056 x 3981)]/(0.640m x 27 310 000m^2)= **4.2E-07 Kg/m^3 or 0.42ng/ml**

[(3004Kg/(0.056x158)]/(0.640m x 27 310 000m^2)=**1.94E-05 Kg/m^3 or 19.4ng/ml**

Calculation of endosulfan discharge in the Wilmot River estuary

C x Q= mixed concentration

4.02E-07 Kg/m^3 dissolved into 0.33 m^3/s = **133 ng/s (min)** per ml of water

1.94E-05 Kg/m^3 dissolved into 0.99 m^3/s =**19 230 ng/s (max)** per ml of water

DISCUSSION

4.1 Sampling *Corophium volutator* and sediments

The density of the *Corophium sp.* on the mudflats of the Bay of Fundy is higher in June -July and declines during the following months. Sampling is more difficult in the late fall-winter month since the animals are found in much lower density and they are not as visible because they are not moving around on the mudflat but hide in their burrow under the surface of the beach.

4.2 Maintaining *Corophium volutator* in captivity

The decline of amphipods in reference tanks at 15°C is of about 4% per day for animals that were kept in captivity over two months (Table 3.2.1). It was found that the longest the animals are kept in captivity, the faster they decline. Also the animals collected late in the fall and during the winter were more sensitive to the laboratory conditions. Some of the factors influencing the survival of amphipods in the laboratory are: sediments size, sediment type, moisture content, sediment thickness and temperature (Meadows 1963, 1964, 1981).

4.3 Behavioural response of amphipods to endosulfan

Behavioural experiments with the pesticide endosulfan were repeated in time to see if concentrations of 0.1ng/g, 2.5ng/g and 62.5ng/g would be comparable with the results obtained in the summer of 2004. In summer 2004, it was concluded that the concentration of endosulfan 0.1ng/g acted as an attractant and that the animals preferred sediments containing 0.1 ng/g and lower concentration of contaminant when compared to clean reference sediment. Therefore the experiment was repeated on: 22nd, 29th of October 2004, 14th of December 2004, 29, 31 of January 2005 and the 9th of February 2005. The first experiment conducted (Fig 3.3.2), showed that animals preferred concentrations 0.1ng/g and 2.5ng/g and avoided the sediment containing 62.5ng/g. However, percent death for this experiment was very high, from 68% to 73%, and up to 90% for the reference tank. The animals used for this experiment were collected in the

summer on the 8th of July 2004. When animals are kept in captivity for a long time, they become discoloured, weary, and are less resistant to manipulation. Survival was much lower than during the summer (25% death in the summer vs >80% in the fall and winter) and this high death rate makes the interpretation of the behavioural results difficult since they are not as meaningful. As outlined in Fig 2.0.2, our criteria have as a first step in assessing the results that a low death and variability 20-30% respectively be observed within a pseudo-duplicate. This was not the case in the fall.

The next experiment was performed using spiked sediment and water that were prepared a week before adding the animals (Fig 3.3.3). The animals used for the experiment were collected on the 6th of October 2004 at a new location, Cheverie Beach, and they were smaller in comparison to the summer animals. Overall, the animals preferred the clean sediment and the death rate was lower but not optimum (<20%). The lower rate of death was explained by the use of freshly collected animals that were vigorous and healthy (only 30% death in the reference tank) and also possibly due to the weathered pesticide. However, a high variability (35-55%) between pseudo-replicates is still observed, while death was more similar between the pseudo-replicates and various levels of exposure. The preference for the reference sediment could be due to the size of the animals; smaller animals might be more sensitive to contaminants. Although survival rate was better (60-25%) than for the experiment conducted on the 20th of October (Figure 3.3.2), it is still much higher than the death rate observed in the summer and the behaviour differs since the amphipods showed preference for Hantsport sediments for all tested concentrations. The death percent for animals exposed to endosulfan was higher than for the animals in the reference tank; however it did not increase according to exposure levels (48-30%).

Because of the high death percentage in the two last experiments, the next one was conducted for a period of 24 hrs instead of 48 hrs (Fig 3.4.4). This time, in comparison to the previous results, the preference decreased with increasing

concentration, but the death rate remained higher (52-40%) making the observation less significant. The difference between pseudo-duplicates was acceptable (0-25% between each exposure level), although survival rate was still too low (need>80%). Once again death in experimental tanks was higher in the reference tanks (55%-40%). The results can be omitted for preference since the animals were exposed to the concentration for a different amount of time.

The following experiment was performed in larger tanks with only one replicate using 60 animals that were exposed for a period of 72 hrs and the animals were added to the water instead of being placed in the sediments. The animals used for this experiment were collected in the winter on the 15th of January 2005. Preferences were consistent with the ones obtained in the summer 2004, however the death rate was still high. Because of their short life-cycle (1yr), the animals hibernating in the winter might have a harder time surviving in captivity at a temperature reaching up to 20-25°C; therefore the following experiments were all conducted at a temperature of 15°C. Mean death was of 51% after 72hrs (Fig 3.3.5) compared to 52% after 48 hrs (Fig 3.3.4), while the reference displayed 40% and 35% death, respectively. Therefore, in both cases, survival in experimental tanks was <50% demonstrating that animals are less resistant when collected from an environment with much lower temperature than the temperature used for their maintenance. Animals from this experiment were also used to determine the neutral lipid percent per dry mass for the winter animals.

The following experiments (Fig 3.3.6, 3.3.7) were performed at 15°C for a period of 48 hrs. The results for preference are consistent with the ones obtained in the summer but with a greater death rate, and more variation between pseudo-duplicates.

Based on the obtained results, it can be concluded that the smaller animals collected during the fall and the winter period were more vulnerable to the pesticides. They showed a higher death rate than in the reference tanks in 3

sets of experiments; a similar death rate in 1 and slightly lower death in 2 series of experiments. The general trend shows an overall preference for the low concentrations of contaminants and a clear avoidance of higher concentration. Some of the experiments were conducted at a temperature of 20ºC -25°C which seemed to be a factor contributing to the higher death rate of the animals during the fall, while temperature was not an issue in the summer experiments (Tremblay 2004). Although the experiments were conducted at the same temperature over the summer time and that the species *Corophium volutator* is known to be very adaptable over a wide range of temperatures, it appeared that the smaller animals collected in the fall were not adapting to this temperature, when kept in the incubator at 15ºC. Therefore the result for avoidance/preference could be consistent with time however the death rate was much higher in the fall and winter than in the summer. Also, due to their short life-cycle (1 year), *Corophium volutator* were more sensitive to manipulation as they were collected during the fall and winter as they hibernate; they were transferred from a cold fall environment to a temperature of up to 25°C. Further experiments should be conducted in the summer only, the period of the year where they would also be exposed to pesticides in the field. Our experiments indicate that if pesticides would be present in the natural environment, animals would be more vulnerable to effects, i.e. higher death in the fall and winter enhanced by a natural decline in population during those seasons.

4.3.1. Avoidance/Preference in Beakers

Behavioural experiments were also conducted with the same concentrations of 0.1, 2.5, 62.5 ng/g of spiked sediment, using only one animal at a time to observe behavioural response and migration of the animal exposed to contaminated sediment. During the first six hours of the experiment it was observed that the animals migrated from the contaminated side to the clean side back and forth showing a preference for the clean sediment. Although the experiments were set to last 48hrs, the animals were found dead after 24hrs. Consequently, no correlation or trend could be concluded or compared with previous experiments.

It is possible that once again the animals were more vulnerable to pesticide and temperature variation due to their size and their life cycles. (Results presented in Appendix D)

4.3.2. Avoidance/Preference Three Choices

Preference/Avoidance experiments were performed in tanks containing two dividers, giving the animals three different choices of sediment. Overall, they preferred the clean reference sediment at 55%; however this was following an increase in preference for the clean sediment as concentration increased.

Avoidance/preference experiments did not replicate in time, the survival rate of *Corophium sp.* was low and animals were less resistant to manipulation in the winter. Consequently, sampling time should be reported and animals as well as pregnant females should not be used. Experiments should be performed exclusively in the summer when animals are more resistant and found in larger densities. Drastic temperature changes could have affected the population kept in captivity and a rapid decline can be caused by disease of animals. It would be interesting to reuse animals, once they have been exposed to a pesticide, to conduct the same experiments and further investigate the adaptation and behaviour of *Corophium sp.* over repetitive exposures. Studying the effects of combined pesticides as being synergistic or antagonistic would also be interesting since many different pesticides are used within an agricultural season.

4.4 Lipid percent per dry mass of amphipods

Lipids for the six pesticides tested over summer 2004 were extracted in the fall using different procedures to improve the quality and precision of the data. Lipids were extracted to determine if animals exposed to pesticides store different amounts of lipids than not-exposed animals. As it was explained earlier, pesticides bind to neutral lipids, therefore if an animal accumulated more fat it will also increase its ability to accumulate contaminants. The lipids of animals exposed to endosulfan in summer 2004 were extracted with different procedures

and errors were encountered. Manipulation errors occurred as very small masses were weighed in heavy Teflon tubes. Errors were also caused by the inaccuracy of the analytical scale for the small amount weighed (five decimal place). Percent lipids were too high when compared to the literature and there was too much variation between samples. The results for lipids percent per dry mass for animals exposed to the pesticides endosulfan and azinphos-methyl had large variations and errors (Fig 4.3.1 and 4.3.2).

Errors and large variations in lipid results occurred when not enough animals were available for the extraction and as a result the mass of lipids extracted as well as the mass of animal were too small for the precision of the scale (See Tables in APPENDIX E). For the following extractions, the animals were combined to obtained numbers larger than 20, to insure a higher animal and lipid mass.

Results for pesticides Endosulfan, Azinphos Methyl, Carbofuran, Atrazine and Hexazinone show that in general the % of lipids per dry animal mass is higher for animals exposed to pesticides. The same results were obtained for animals exposed to Halifax harbour sediment. Often when the lipid percent peaked above average it was due to error in mass because fewer animals were available for the extraction and the scale available was not precise enough. For Atrazine and Hexazinone there was a high death rate and only animals from the reference sediment were available for extraction. However it was found that the lipids fit in the normal range between 3 and 13% per dry animal weight. Lipids percent per dry mass were extracted for animals exposed to endosulfan in the fall with a 24 hrs exposure instead of 48hrs to prevent a higher death rate (Fig 4.3.8). Animals were extracted in small numbers because death rate was high in the experimental tanks.

Whether the animals collected in the tank were only exposed to the side they were collected in the tank or whether they moved and were exposed to both

spike and reference side during the 48hrs experiment is unknown and it make the interpretation of the results difficult. Consequently, the animals found on the spiked side were combined with the ones found on the reference side in each tank to increase the mass of each sample extracted, as well as the accuracy of the results. These samples were compared with the reference tank of the set where sediments were 100% cleaned on both sides. The percent lipids were compared for 5 different pesticides and correlated with the time at which animals were exposed (Fig 4.4.14). It was concluded that the lipid percent increased with larger concentrations of pesticides, except for chlorothalonil where it was varying in time. Chlorothalonil is a stimulant affecting the nervous system. The animals exposed become very agitated and stop storing fat in the process.

Corophium volutator feed on food hiding in sediment, take oxygen from water and, if pesticide is present in these environments, the contaminant might bind to their neutral lipids. Since for all pesticides tested, except for chlorothalonil, lipid percent per dry animal mass increased as the animals were exposed to a higher concentration of pesticides, the effect is doubled in the sense that accumulating more lipids also increased the animals' ability to store pesticides. *Corophium sp.* is an important organism in the food change and biomagnifications effects will be greater.

4.5 Partitioning coefficient calculations

The log K_{ow} for endosulfan is not well defined therefore a range of published values were used to make prediction. The partition coefficient calculation for organic carbon and lipid in comparison with water shows a higher affinity for organic enriched particles as the results indicate that there are more pesticides per g of sediments than in ml of water. Sediments analysis revealed a higher carbon content in PEI sediments and consequently, increasing the likelihood for pesticides to bind to particle and affect the benthic fauna.

4.6 Concentrations of Endosulfan entering Wilmot River estuary

Many assumptions were made and many important factors in the distribution of contaminants in the environment were omitted. However it gives an estimate of

the quantity of endosulfan that could be discharged in Wilmot River estuary. The concentration discharged in the water per ml are greater than the ones calculated for our test tanks and since pesticides have a greater affinity for sediments and that the organic carbon content is up to 7% in PEI sediments, even larger concentrations of pesticides can be expected in the sediment of the estuary. Endosulfan is applied up to 8 times over a period of 4 months on potato farms. The fact that it takes 4 week to degrade the pesticides from surface water at pH 7 to concentration that are not detectable. Endosulfan applied up to 8 times in a summer season; therefore it is constantly discharged in the Wilmot river estuary for that period of time. However, seawater and sediments were sampled from the Wilmot River estuary in July-Oct after rain events and pesticides were analysed by Dr. Hellou's group at DFO. Water sampled showed non-detectable concentration (<10ng/L), while level of endosulfan were ~1ng/g in the field sediments. It should also be considered that Wilmot River watershed has 7640 hectares of agricultural land and many of them using endosulfan and that many other pesticides and fertilisers are use during the periods that possibly magnified the amount of contaminant discharged in this estuary. However, the calculations were very rough and didn't consider many important aspects of contaminants' distribution and transport into the environment. It would be interesting to use environmental modelling software such as Swat or Basin to calculate more precisely the concentrations of pesticides likely to end up in the Wilmot river estuary.

The lethal dose (LC50) for fish exposed to endosulfan is 1µg/L. In the summer 2004, the lethal dose was found to be 250ng/g for the *Corophium sp.* and the animals were indicating preference at 0.004-0.5ng/g and avoidance at 25-125ng/g before death. In the winter, they indicated preference at 0.1ng/g, avoidance at 2.5-12.5ng/g and death at 62.5ng/g showing that the animals are much more sensitive in the winter months. Consequently, a long-term goal would be to determine if commonly used pesticides in the conventional potato industry have an effect on the marine community of an estuary.

CONCLUSIONS

- Avoidance/preference experiments did not replicate in time, the survival rate of *Corophium sp*. was low and animals were less resistant to manipulation in the winter.
- Lipid percent relative to dry animal mass increased as the animals were exposed to higher concentrations of pesticides, except for the pesticide chlorothalonil.
- The effect is doubled in the sense that accumulating more lipids also increased the animals' ability to store pesticides. Since *Corophium sp.* is an important organism in the food change, the biomagnification effects would also be greater.
- The partition coefficient calculation for organic carbon and lipid in comparison with water shows a higher affinity for organic enriched particles. Sediments analysis revealed a higher carbon content in PEI sediments and consequently, increasing the likelihood for pesticides to bind to particle and affect the benthic fauna.
- The concentrations used to perform the behavioural experiments were realistic when compared to the exposure levels expected in an estuarine environment.
- Long term research goal would be to determine if commonly used pesticides in the conventional potato industry have an effect on the marine community of an estuary.

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APPENDIX A

Sampling location and mudflat geology

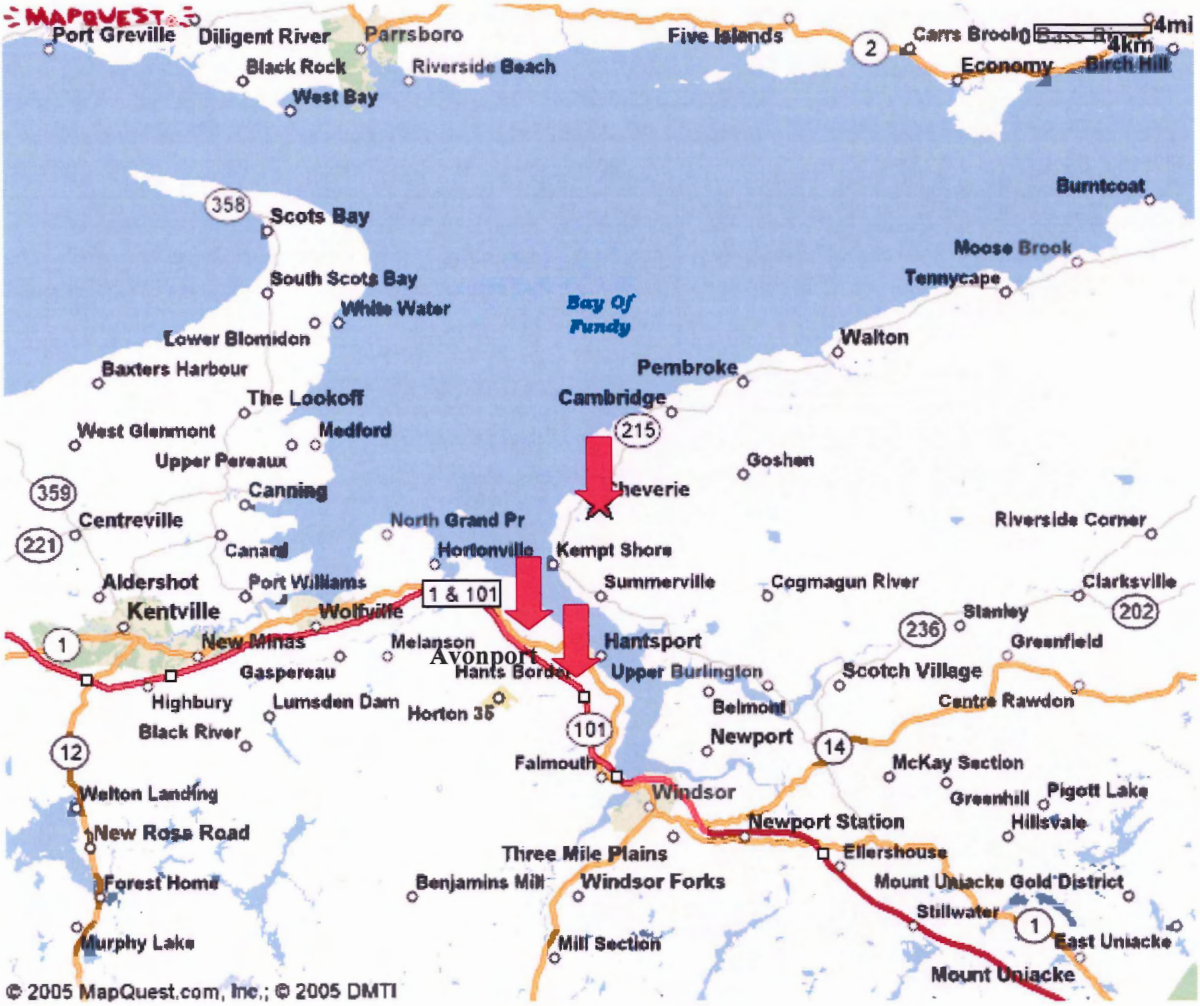

Figure A1: Sampling locations; Avontport, Hantsport and Cheverie.

Figure A2 : Hantsport sampling location

Figure A3 : Cheverie sampling location

The mudflats are located along the Avon River estuary. The Avon River drains into the Minas Basin of the Bay of Fundy. The mudflats were formed and vary as a global result of the geology, glacial history, sea level rising, high tides of the Bay of Fundy, coastal erosion, sediments brought by Avon River, and human activity such as agriculture and forestry. The mudflats are the habitat of many invertebrates including species of polychaete worms, soft-shell clams, intertidal snails and crustaceans, as well as the tube dwelling amphipod *Corophium volutator.* High sedimentation rates, destruction of marsh land and pollution can affect the productivity of the marine ecosystem of the mudflats in the upper Bay of Fundy (Percy 1996).

TABLES A1: Sediment Size, A2: Sediment Density, A3: Moisture Content

A1

Avonport (30/06/04)					Hantsport (06/07/04)				
Sieve size (µm)	Boat mass (g)	Boat and dry sediment (g)	Mass of sediment	%	Sieve size (µm)	Boat mass (g)	Boat and dry	Mass of sediment	%
>63	1.322	7.129	5.807	8.0	>63	2.623	12.629	10.006	14.3
>212	1.318	1.978	0.66	0.9	>212	1.315	1.339	0.024	0.0
>500	1.312	1.466	0.154	0.2	>500	1.322	1.323	0.001	0.0
		Total sand	6.621	9.1			Total sand	10.031	14.3

A2

Avonport (26/05/04)

1 mL Sample	Mass (g)
1	1.889
2	1.885
3	1.827
4	2.095
5	1.952

AVERAGE (g/mL)	1.930
STDDEV	0.102
CV (%)	5.312

Hantsport (31/05/04)

1 mL Sample	Mass (g)
1	2.000
2	2.212
3	2.169
4	1.968
5	2.184

AVERAGE (g/mL)	2.107
STDDEV	0.114
CV (%)	5.390

Hantsport (22/06/04)

1 mL Sample	Mass (g)
1	2.637
2	2.512
3	2.555
4	2.611
5	2.500

AVERAGE (g/mL)	2.563
STDDEV	0.060
CV (%)	2.342

A3

Avonport (26/05/04)

Sample	Mass Watch Glass Empty (g)	Watch Glass wet sediment (g)	Mass of wet sediments (g)	Mass of Glass with dry sediment (g)	Dry Sediment (g)	% of Moisture
1	48.575	49.754	1.179	49.118	0.636	46.056
2	34.940	35.772	0.832	35.319	0.453	45.553
3	54.027	55.150	1.123	54.536	0.614	45.325
					Average % moisture	45.645
					STDEV	0.305
					CV (%)	0.669

Avonport (30/06/04) Sediment was previously frozen

Sample	Mass Watch Glass Empty (g)	Watch Glass wet sediment (g)	Mass of wet sediments (g)	Mass of Glass with dry sediment (g)	Dry Sediment (g)	% of Moisture
1	1.297	9.191	7.894	6.588	2.603	67.026
2	1.317	9.607	8.29	7.449	2.158	73.969
3	1.319	13.435	12.116	9.974	3.461	71.434
					Average % moisture	70.810
					STDEV	2.869
					CV (%)	4.051

Hantsport (31/05/04)

Sample	Mass Watch Glass Empty (g)	Watch Glass wet sediment (g)	Mass of wet sediments (g)	Mass of Glass with dry sediment (g)	Dry Sediment (g)	% of Moisture
1	48.582	49.607	1.025	49.194	0.413	59.707
2	54.098	55.16	1.062	54.753	0.407	61.676
3	35.034	36.087	1.053	35.65	0.437	58.500
					Average % moisture	59.961
					STDEV	1.309
					CV (%)	2.183

APPENDIX B

Pesticides

Azinphos-Methyl

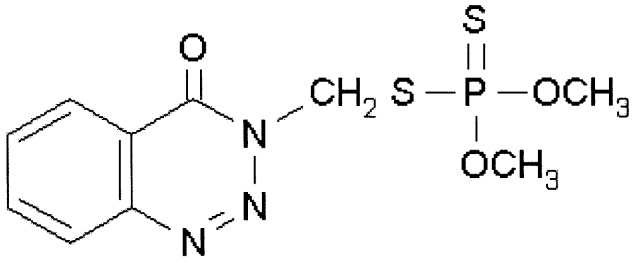

Figure B-1: Azinphos Methyl (Sigma-Aldrich Canada 2004)

Azinphos Methyl is an organophosphate, a highly persistent and broad spectrum insecticide. It is one of the most toxic of all organophosphates and its use is now banned in Canada (Environment Canada 2004). This pesticide affects the nervous system by disrupting the enzyme that regulates acetylcholine, a neurotransmitter. The OP compound inactivates the enzyme acetylchlolinesterase in the nerve cells causing the nerve transmission to continue indefinitely. It damages the normal functioning of cholinesterase, an enzyme essential in proper nervous system function. Azinphos-methyl has a low mobility in soil because it adsorbs strongly to soil particles, has low water solubility and degrades rapidly under UV radiation. Azinphos-methyl is highly toxic to aquatic invertebrates (Kamrin 1997).

Carbofuran

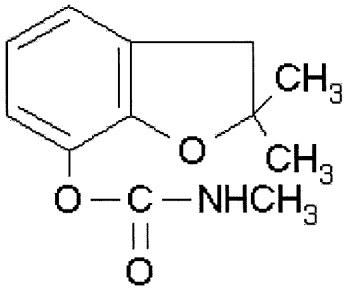

Figure B-2: Carbofuran (Sigma-Aldrich Canada 2004)

Carbofuran is a carbamate compound. This pesticide is used to kill insects, mites or nematodes on contact or after ingestion. Carbamate pesticides affect the nervous system by disrupting an enzyme that regulates acetylcholine, a neurotransmitter. Carbofuran degraded in soil by chemical hydrolysis, bacterial process, and sunlight (Kamrin 1997).

Chlorothalonil

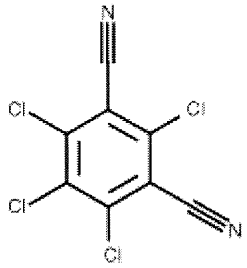

Figure B-3: Chlorothalonil (Sigma-Aldrich Canada 2004)

Chlorothalonil is an organochlorine fungicide classified as a chlorinated hydrocarbon. The chlorinated hydrocarbons are stimulants to the nervous system. Chlorothalonil is highly toxic to aquatic invertebrates. It does not store in fatty tissues and is rapidly excreted from the body. Its half life varies from 1-3 months and it is not degraded by sunlight. It has high binding and low mobility in silky clay and has a solubility of 0.6 mg/L @ 25C° in water (Kamrin 1997).

Atrazine

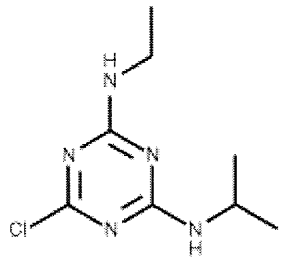

Figure B-4: Atrazine (Sigma-Aldrich Canada 2004)

Atrazine is a triazine, a selective herbicide used to control broadleaf and grassy weeds. It is absorbed by the plants and acts by interfering with photosynthesis. As a result the plant doesn't have enough energy to grow. It is slightly toxic to fish and other aquatic life. It is absorbed dermally, orally and by inhalation. It is highly persistent in soil and has a half life of 60 to 100days. Its water solubility is 28mg/L @ 20C° (Kamrin 1997).

Hexazinone

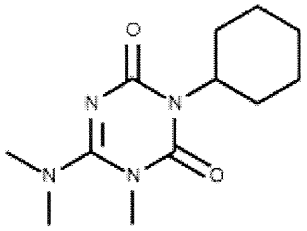

Figure B-5: Hexazinone (Sigma-Aldrich Canada 2004)

Hexazinone is a triazine herbicide used against many weeds. Rain fall or irrigation water is required in order to activate Hexazinone. It acts the same way as Atrazine and is also slightly toxic to fish and other aquatic life. Hexazinone is poorly absorbed to soil particles, very soluble in water and degrades slowly. Consequently, Hexazinone is highly mobile in soil and can contaminate ground water (Kamrin 1997).

APPENDIX C

C: *Corophium volutator* (Pallas)

Corophium volulator is an abundant organism in estuarine mudflats of the Minas Basin in the Bay of Fundy. The species occur in density up to 52,280/m^2 (Boates and Smith 1979). Another species of amphipods (*Gammarus Lawrencianus*) is present on the mudflats of Minas Basin. However, Gammarus *Lawrencianus* are lesser in number and their different anatomy makes the two species easily differentiated.

Figure C1: *Corophium volutator* (Percy 1999)

Corophium volutator has a chitinous shell and its entire body is divided into a series of short tubular segments. Strong, flexible joints between segments allow the animal to straighten or bend into a ball. Its body size range between 1 and

11 mm. The maxilla, behind the antenna, on the underside of the body, is a grouping of small limbs used for capture, handling and ingestion of food (Percy 1999). *Corophium volutator* ingests particles between 4 -63 µm in diameter. Food consists of bacteria, diatoms and particulate organic matter (Neal and Avant 2004). They can feed on particles lying on the seafloor (deposit feeding) as well as suspended in the water (filter feeding). The periopods are several long flexible walking legs used to crawl on the sea floor and pleopods are miniature paddles allowing Corophium to swim. Corophium volutator has been observed to swim, crawl and burrow. Their shell is similar to lobster and molt as they grow. Copulation occurs soon after a female has molted. Females lay on average 38 eggs and the breeding season in the upper Bay of Fundy extends from early May until early August. (Percy 1999). The lipid content of *Corophium volutator* is of 1.2% wet weight, 6% dry weight, and is made up 55.3% phospholipids, 31.4% triglyceride and minor amount of other lipids (Ackman1979).

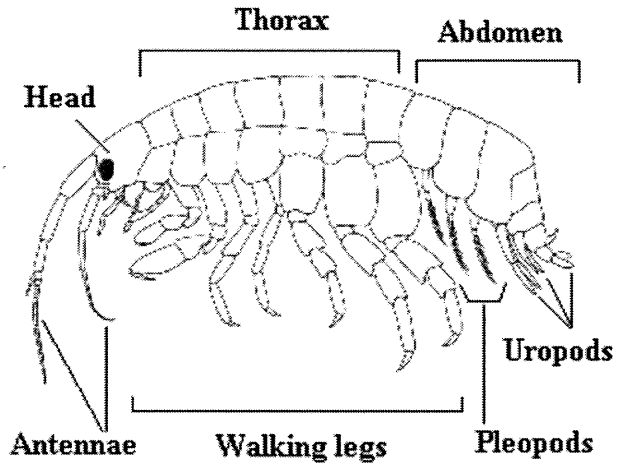

Figure C2: Anatomy of *Corophium volutator* (Museum of Victoria Australia 1996)

Corophium volutator are very adaptable and can support a range of temperatures and salinities. They can live in water saltier than seawater, ~ 34ppt of salt, as

well as in water only slightly saltier than fresh water, ~5ppt of salt, and tolerate everything in between. *Corophium volutator* has a very specific preference for muddy sand or mud as a suitable substratum (Neal, K.J. & Avant, P., 2004). If the substrate is too fine or has too much water, it makes it difficult for the animal to burrow since the tube shape hole would collapse. If the mud is too coarse, it will lack in organic content and will be unattractive for the animals (Cybernat). A constant augmentation of the *Corophium* population is noticeable where salt marsh invasion is greatest. *Corophium* have an influence on the ability of mudflats to withstand the eroding forces of currents and waves. Diatoms, bacteria and other microorganisms in the surface layers of mud secrete sticky organism substance that makes sediment particles stick together and reduce erosion by moving water. *Corophium* consume large amount of these organisms and as a result there is a reduction in the sticky stuff produced.

Various environmental factors represent a threat to the population of *Corophium.* Heavy ice overlying the mudflats during harsh ice conditions crush burrowing holes where animals are living. Also changes in sediment dynamics and mudflat composition such as heavy sedimentation rate can lead to a decrease and even an extermination of the species (Percy 1999). In addition, amphipods have been shown to be very sensitive to contaminated sediment and are among the first taxa to disappear from benthic communities impacted by pollution. (ASTM1993).

APPENDIX D

Table D-1: Small scale Avoidance/Preference 20th of October 2004

ENDOSULFAN (Beakers and 1 animal put on spike side) Vol. 2 p.15 20/10/04					
Beaker #	Conc (ng/g)	Observation			
		Immediately	20min	40min	60min
1	0.1	Animals crawled around the glass, are agitated and move from reference side to spike without showing preference	Crawl reference side	immobile Spike side	immobile Spike side
2	2.5		Crawl reference side	immobile Clean side	immobile Clean side
3	62.5		animal was replaced since the first one died on clean side	immobile Clean side	immobile Clean side
4	R		Immobile	Immobile	Immobile

Animals were put in 1L beakers with 100g of sediment in the bottom, one side of the beaker spiked and just enough water for the sediment to not dry. Experiment was conducted at room temperature 20-25°C. On the 22/10/04 All animals were dead because the s

Table D-2: Small scale Avoidance/Preference 22nd of October 2004

ENDOSULFAN (Beakers and 1 animal put on Ref side) Vol. 2 p.16 22/10/04					
Beaker #	Conc (ng/g)	Observation			
		Immediately	20min	40min	60min
1	0.1	Animals crawled around the glass, are agitated and move from reference side to spike without showing preference			
2	2.5				
3	62.5				
4	R				

Animals were put in 500ml beakers with 50g of sediment in the bottom, one side of the beaker spiked and 200ml water dry. Experiment was conducted at room temperature 20-25°C. On the 27/10/04 All animals were dead.

Table D-3: Small scale Avoidance/Preference 27th of October 2004

ENDOSULFAN (Tanks and 5 animals added to water) Vol. 2 p.27 10/12/04					
Beaker #	Conc (ng/g)	Observation			
		6HRS	24HRS	48HRS	72HRS
1	0.1	1 in Ref immobile	1 crawling in ref, 1 c in spike		3 immobile, 1 crawling in ref
2	2.5	3 swimming in Ref, 1 in spike	1 crawling in ref, 1 immobile in spike		2 swimming
3	62.5	2 swimming in Ref, 1 in spike	1 swimming in ref, 1burrowing in spike		2 dead in spike, 1 immobile in ref
4	R	2 swimming , 1 crawling	1 crawling, 1 immobile in ref, 1 immobile in spike		1 burrowing, 2 dead

5 animals were added to the 500ml of water in each tanks, temperature was 21.5°C. On the 13/12/2004 all animals were found dead except for 1 in the reference side of 0.1ng/g and 1 in the reference tank.Temperature was 22°C.

Table D-4: Small scale Avoidance/Preference 13th of December 2004

ENDOSULFAN (Glass Watch and 5 animals) Vol. 2 p.29 13/12/04				
Beaker #	Conc (ng/g)	Observation		
		6HRS	24HRS	48HRS
1	0.1 S	1 dead in Spike side, 1 crawling in Ref	1 dead R, 1 dead S	All DEAD
2	0.1 S	1 on its back moving its leg in Ref	1 dead S	
3	0.1 S	1 on its back moving its leg in Ref	1 dead S	
4	0.1 S	1 crawling in the middle	1 dead R, 1 dead S	
5	0.1 R	1 immobile in Ref	?	
6	0.1 R	1 dead in reference	?	
7	0.1 R	1 immobile in Ref	1 dead R	
8	0.1 R	1 dead in Spike side	1 dead S	
9	R	1 crawling	1 crawling	

5 animals in each glass watch where one side was spiked, in 4 of the glass watch animals were put on the spike side and in 4 other animals were put on the clean side, temperature was 22°C. After 48hrs all animals were found dead.

Table D-5: Small scale Avoidance/Preference 3rd of November 2004

Time	Observation (Concentration 0.1 ng/g)				
	1	2	3	4	R
9:05	Crawling in Spike sediment	Immobile in Reference sediment	Crawling in Spike sediment	Crawling in Spike sediment	Immobile
9:10	Burrowing in Spike sediments	Crawling in Reference sediment	Crawling in Reference sediment	Crawling in Middle	Immobile
9:15					
9:20	Burrowing in Spike sediments	Burrowing in Reference sediments	Immobile in Reference sediment	Crawling in Spike sediment	Immobile
9:25	Burrowing in Spike sediments	Immobile in Reference sediment	Crawling in Reference sediment	Crawling in Spike sediment	Not visible
9:30	Burrowing in Spike sediments	Immobile in Reference sediment	Immobile in Reference sediment	Crawling in Spike sediment	Immobile
9:35	Not visible	Immobile in Reference sediment	Immobile in Reference sediment	Immobile in Spike sediment	Not visible
9:40	Animal dead added new one	Crawling in Reference sediment	Crawling in Reference sediment	Burrowing in Reference sediments	Crawling
9:45	Immobile in Reference sediment	Immobile in Reference sediment	Immobile in Reference sediment	Animal dead added new one	Immobile
9:50	Immobile in Reference sediment	Crawling in Spike sediment	Immobile in Reference sediment	Burrowing in Reference sediments	Crawling
9:55	Immobile in Reference sediment	Immobile in Reference sediment	Immobile in Reference sediment	Burrowing in Reference sediments	Immobile
10:00	Immobile in Reference sediment	Immobile in Reference sediment	Immobile in Reference sediment	Burrowing in Reference sediments	Immobile
10:05	Immobile in Reference sediment	Immobile in Reference sediment	Immobile in Reference sediment	Burrowing in Reference sediments	Crawling
Found dead after 48 hrs	R	S	S	S	Dead

Animals were put in larger 15x15cm Glass Watch with 100g of sediment in the bottom, one side of the beaker spiked just enough water so the sediment wouldn't dry. Experiment was conducted at room temperature 20-25°C. Three out of 4 died in the spiked sed

Table D-6: Small scale Avoidance/Preference

ENDOSULFAN (Beakers and 1 animal put on spike side) **Vol. 2 p.19 03/11/2004**					
Time	**Observation (Concentration 0.1 ng/g)**				
	1	**2**	**3**	**4**	**R**
8:40	Burrowing in Spike sediments	Crawling in from Spike to reference sediment	Crawling in from Spike to reference sediment	Burrowing in Spike sediments	Burrowing
8:45	Crawling in Spike sediment	Crawling in from Spike to reference sediment	Immobile in Spike sediment	Crawling in Spike sediment	Crawling
8:50	Immobile in Spike sediment	Crawling in Reference sediment	Immobile in Spike sediment	Burrowing in Spike sediments	Immobile
8:55	Crawling in Spike sediment	Immobile in Reference sediment	Burrowing in Spike sediments	Not visible	Immobile
9:00	Burrowing in Spike sediments	Immobile in Reference sediment	Crawling in Spike sediment	Not visible	Crawling
9:05	Immobile in spike sediment	Crawling in Reference sediment	Immobile in Spike sediment	Burrowing out Spike sediments	Immobile
9:10	Burrowing out Spike sediments	Immobile in Reference sediment	Crawling in Spike sediment	Immobile in the Middle	Immobile
9:15	Immobile in Spike sediment	Immobile in Reference sediment	Immobile in Reference sediment	Immobile in the Middle	Immobile
9:20	Crawling in Spike sediment	Crawling in Reference sediment	Immobile in Spike sediment	Crawling in Spike sediment	Immobile
9:25	Immobile in Spike sediment	Immobile in Reference sediment	Immobile in Spike sediment	Immobile in Spike sediment	Immobile
9:30	Burrowing in Spike sediments	Immobile in Reference sediment	Immobile in Spike sediment	Immobile in Spike sediment	Immobile
9:35	Immobile in Spike sediment	Immobile in Reference sediment	Immobile in Spike sediment	Immobile in Spike sediment	Immobile
9:40	Immobile in Spike sediment	Immobile in Reference sediment	Immobile in Spike sediment	Immobile in Spike sediment	Immobile

Animals were put in 80x40 mini beakers with 40g of sediment in the bottom, one side of the beaker spiked and 50ml water. Experiment was conducted at room temperature 20-25°C. Animals were disposed after th experiment. Three out of 4 chosed the spiked sed

Table D-7: Small scale Avoidance/Preference ENDOSULFAN (Beakers and 1 animal put on spike side)

Time	Observation (Concentration 0.1 ng/g)				
	1	2	3	4	R
8:40	Burrowing in Spike sediments	Crawling in from Spike to reference sediment	Crawling in from Spike to reference sediment	Burrowing in Spike sediments	Burrowing
8:45	Crawling in Spike sediment	Crawling in from Spike to reference sediment	Immobile in Spike sediment	Crawling in Spike sediment	Crawling
8:50	Immobile in Spike sediment	Crawling in Reference sediment	Immobile in Spike sediment	Burrowing in Spike sediments	Immobile
8:55	Crawling in Spike sediment	Immobile in Reference sediment	Burrowing in Spike sediments	Not visible	Immobile
9:00	Burrowing in Spike sediments	Immobile in Reference sediment	Crawling in Spike sediment	Not visible	Crawling
9:05	Immobile in spike sediment	Crawling in Reference sediment	Immobile in Spike sediment	Burrowing out Spike sediments	Immobile
9:10	Burrowing out Spike sediments	Immobile in Reference sediment	Crawling in Spike sediment	Immobile in the Middle	Immobile
9:15	Immobile in Spike sediment	Immobile in Reference sediment	Immobile in Reference sediment	Immobile in the Middle	Immobile
9:20	Crawling in Spike sediment	Crawling in Reference sediment	Immobile in Spike sediment	Crawling in Spike sediment	Immobile
9:25	Immobile in Spike sediment	Immobile in Reference sediment	Immobile in Spike sediment	Immobile in Spike sediment	Immobile
9:30	Burrowing in Spike sediments	Immobile in Reference sediment	Immobile in Spike sediment	Immobile in Spike sediment	Immobile
9:35	Immobile in Spike sediment	Immobile in Reference sediment	Immobile in Spike sediment	Immobile in Spike sediment	Immobile
9:40	Immobile in Spike sediment	Immobile in Reference sediment	Immobile in Spike sediment	Immobile in Spike sediment	Immobile

Animals were put in 80x40 mini beakers with 40g of sediment in the bottom, one side of the beaker spiked and 50ml water. Experiment was conducted at room temperature 20-25°C. Animals were disposed after th experiment. Three out of 4 chosed the spiked sed

APPENDIX E

E1: Lipid Table Amphipods exposed to **Endosulfan** (except for 7: 1E, 1L, 1S, 1X, 1AC)

Sample Name	Sample #	Spike (ng/g)	Tubes Empty (g)	Tubes + dry amphipods (g)	Mass amphipods (g)	# of Animals	Mass/am-phipods (mg)	Empty vial (g)	Vial + Lipids (g)	Lipids mass (g)	Lipid %	Lipid/ani-mal (mg)
1E	7C + 7C	0	54.9530	54.996	0.0430	15	2.9	5.1098	5.1181	0.0083	19	0.55
1L	7C + 7C	0	-	-	0.0312	19	1.6	6.5394	6.5494	0.01	32	0.53
1S	7C + 7C	0	27.5325	27.5693	0.0368	20	1.8	5.1509	5.1582	0.0073	20	0.36
1X	7C + 7C	0	57.3987	57.4372	0.0385	18	2.1	5.0466	5.0571	0.0105	27	0.58
1AC	7C + 7C	0	-	-	0.0412	20	2.1	5.1080	5.1102	0.0022	5	0.11
1Z	1S + 2S	0.004 R	-	-	0.0322	19	1.7	5.1221	5.1273	0.0052	16	0.27
1Y	1C + 2C	0.004 S	-	-	0.0290	15	1.9	5.1038	5.1064	0.0026	9	0.17
1M	1C + 2C	0.02 R	29.2347	29.2581	0.0234	13	1.8	5.1824	5.1864	0.004	17	0.31
1N	1S + 2S	0.02 S	28.7490	28.7856	0.0366	19	1.9	5.0627	5.0661	0.0034	9	0.18
1F	1C + 2C	0.1 R	58.0800	58.1152	0.0352	16	2.2	5.0692	5.0849	0.0157	45	0.98
1G	1S + 2S	0.1 S	56.4468	56.4951	0.0483	22	2.2	5.0520	5.0664	0.0144	30	0.65
1H	3C + 4C	0.5 R	56.5836	56.6031	0.0195	12	1.6	5.1082	5.1196	0.0114	58	0.95
1J	5C + 6C	0.5 R	-	-	0.0362	17	2.1	5.1582	5.1609	0.0027	7	0.16
1I	3S + 4S	0.5 S	-	-	0.0281	19	1.5	5.0804	5.0820	0.0016	6	0.08
1K	5S + 6S	0.5 S	-	-	0.0310	17	1.8	5.1850	5.1872	0.0022	7	0.13
1A	1C + 2C	2.5 R	57.7209	57.7478	0.0269	17	1.6	5.1011	5.1042	0.0031	12	0.18
1O	3C + 4C	2.5 R	28.3485	28.3765	0.0280	13	2.2	5.0816	5.0846	0.003	11	0.23
1T	1C + 2C	2.5 R	56.1580	56.1849	0.0269	18	1.5	5.0394	5.0505	0.0111	41	0.62
1B	1S + 2S	2.5 S	58.3971	58.4246	0.0275	14	2.0	5.0642	5.0664	0.0022	8	0.16
1P	3S + 4S	2.5 S	29.5875	29.6295	0.0420	21	2.0	5.1191	5.1245	0.0054	13	0.26
1U	1S + 2S	2.5 S	58.9285	58.9506	0.0221	13	1.7	5.1199	5.1335	0.0136	62	1.05
1Q	5C + 6C	12.5 R	29.2803	29.3097	0.0294	15	2.0	5.1900	5.1913	0.0013	4	0.09
1R	5S + 6S	12.5 S	29.3278	29.3642	0.0364	17	2.1	5.1965	5.2014	0.0049	13	0.29
1C	3C + 4C	25 R	58.1816	58.2066	0.0250	11	2.3	5.1304	5.1341	0.0037	15	0.34
1V	3C + 4C	25 R	58.0389	58.0725	0.0336	19	1.8	5.2003	5.2199	0.0196	58	1.03
1D	3S + 4S	25 S	57.6929	57.7241	0.0312	16	1.9	5.1711	5.1735	0.0024	8	0.15
1W	3S + 4S	25 S	57.6922	57.7243	0.0321	15	2.1	5.0771	5.0903	0.0132	41	0.88
1AB	3S + 4S	125 R	-	-	0.0204	11	1.9	5.1991	5.2016	0.0025	12	0.23
1AA	3C + 4C	125 S	-	-	0.0482	27	1.8	5.2136	5.2212	0.0076	16	0.28

E2: Lipid Table Amphipods exposed to **Azinphos Methyl** (except for 7:2E, 2J, 2Q, 2U)

Sample Name	Sample #	Spike (ng/g)	Tube mass (g)	Tube + dry amphipods (g)	Mass amphipods (g)	# of Amphipods	Mass per amphipods (mg)	Empty vial (g)	Vial + Lipids (g)	Lipids mass (g)	Lipid %	Lipid/animal (mg)
2A	1C + 2C	2.5 R	-	-	0.0192	11	1.75	5.1161	5.1178	0.0017	9	0.15
2B	1S + 2S	2.5 S	-	-	0.0577	26	2.22	5.1567	5.1632	0.0065	11	0.25
2C	3C + 4C	25 R	58.7240	58.7623	0.0383	21	1.82	5.1036	5.1121	0.0085	22	0.40
2D	3S + 4S	25 S	58.4210	58.4434	0.0224	14	1.60	5.1004	5.1039	0.0035	16	0.25
2E	7C + 7C	0	58.4828	58.5227	0.0399	19	2.10	5.1772	5.1810	0.0038	10	0.20
2F	1S + 2S	0.1 S	56.6300	56.67	0.0400	18	2.22	5.1275	5.1305	0.0030	7	0.17
2G	3C + 4C	0.5 R	56.0700	56.1068	0.0368	18	2.04	5.0970	5.0983	0.0013	4	0.07
2H	3S + 4S	0.5 S	56.3630	56.3976	0.0346	16	2.16	5.1620	5.1654	0.0034	10	0.21
2I	5C + 6C	12.5 R	53.1983	53.2432	0.0449	25	1.80	5.1720	5.1766	0.0046	10	0.18
2J	7C + 7C	0	54.3449	54.3838	0.0389	19	2.05	5.1649	5.1697	0.0048	12	0.25
2K	1C + 2C	0.1 R	-	-	0.0269	14	1.92	6.5994	6.6032	0.0038	14	0.27
2L	1S + 2S	0.1 S	-	-	0.0435	19	2.29	6.6870	6.6918	0.0048	11	0.25
2M	3C + 4C	0.5 R	-	-	0.0644	20	3.22	6.6535	6.6592	0.0057	9	0.29
2N	3S + 4S	0.5 S	-	-	0.0239	10	2.39	6.6267	6.6294	0.0027	11	0.27
2O	5C + 6C	12.5 R	-	-	0.0563	26	2.17	6.6363	6.6469	0.0106	19	0.41
2P	5S + 6S	12.5 S	-	-	0.0219	10	2.19	6.6879	6.6991	0.0112	51	1.12
2Q	7C + 7C	0	-	-	0.0480	18	2.67	6.6426	6.6537	0.0111	23	0.62
2R	1C + 2C	0.5 R	-	-	0.0314	19	1.65	5.2046	5.2085	0.0039	12	0.21
2S	1S + 2S	0.5 S	-	-	0.0370	15	2.47	5.1284	5.1333	0.0049	13	0.33
2T	3C + 4C	2.5 R	-	-	0.0443	19	2.33	5.1428	5.1474	0.0046	10	0.24
2U	7C + 7C	0	-	-	0.0320	18	1.78	5.1621	5.1679	0.0058	18	0.32

E3: Amphipods exposed to **Carbofuran** (except for 7:3E, 3J, 3Q)

Sample Name	Spike (ng/g)	Mass amphipods (g)	# of Amphipods	Mass per amphipods (mg)	Empty vial (g)	Vial + Lipids (g)	Lipids mass (g)	Lipid %	Lipid/animal (mg)	Date
3A	2.5 R	0.0418	20	2.09	5.0846	5.0863	0.0017	4	0.08	09/07/2004
3B+3C+3D	2.5S,25SR	0.0678	40	1.69	5.0437	5.0461	0.0024	4	0.06	16/03/2005
3F+3G	0.5RS	0.0305	20	1.53	5.0376	5.0391	0.0015	5	0.08	16/03/2005
3H+3O	12.5 R	0.0498	23	2.17	5.1575	5.1600	0.0025	5	0.11	15/07/2004
3I+3P	12.5 S	0.0504	27	1.87	5.1654	5.1689	0.0035	7	0.13	15/07/2004
3K	0.1 R	0.0337	20	1.69	5.1434	5.1446	0.0012	4	0.06	22/07/2004
3L	0.1 S	0.0401	20	2.01	5.0797	5.0823	0.0026	6	0.13	22/07/2004
3M	0.5 R	0.0236	15	1.57	5.0920	5.0946	0.0026	11	0.17	22/07/2004
3N	0.5 S	0.0339	22	1.54	5.0974	5.0994	0.0020	6	0.09	22/07/2004
3E+3J+3Q	R	0.0870	52	1.67	5.0217	5.0233	0.0016	2	0.03	16/03/2005

E4: Lipid Table Amphipods exposed to **Chorothalonil** (except for 7:4G, 4M, 4R, 4V)

Sample Name	Sample #	Spike (ng/g)	Mass amphipods (g)	# of Amphipods	Mass per amphipods (mg)	Empty vial (g)	Vial + Lipids (g)	Lipids mass (g)	Lipid %	Lipid/animal (mg)	Date
4A+4B	(1C + 2C)+(1S + 2S)	2.5 R+2.5 S	0.0498	30	1.66	5.0686	5.0716	0.0030	6	0.10	16/07/2004
4C+4T	(3C + 4C)+(3C + 4C)	25 R+25R	0.0990	26	3.81	5.0350	5.0367	0.0017	2	0.07	16/07/2004
4D	3S + 4S	25 S	0.0577	23	2.51	4.9182	4.9219	0.0037	6	0.16	16/07/2004
4E+4U	(5C + 6C)(5C+6C)	250 R+250R	0.0517	30	1.72	5.0190	5.0218	0.0028	5	0.09	16/07/2004
4F	5S + 6S	250 S	0.0580	23	2.52	4.9275	4.9307	0.0032	6	0.14	16/07/2004
4G,4M,4R,4V	7	0	0.1080	71	1.52	5.0093	5.0172	0.0079	7	0.11	16/07/2004
4J+4K	(3C + 4C)(3S + 4S)	0.5 R+0.5S	0.0545	24	2.27	5.0557	5.0575	0.0018	3	0.08	26/07/2004
4L	5C + 6C	2500 R	0.0381	26	1.47	5.0241	5.0255	0.0014	4	0.05	26/07/2004
4N+4H	(1C + 2C)(1C + 2C)	0.02 R+0.1R	0.0608	43	1.41	4.9995	5.0015	0.0020	3	0.05	03/08/2004
4O+4I	(1S + 2S)+(1S + 2S)	0.02 S+0.1S	0.0323	22	1.47	5.0785	5.0802	0.0017	5	0.08	03/08/2004
4P	3C + 4C	1250 R	0.0217	21	1.03	5.0596	5.0599	0.0003	1	0.01	03/08/2004
4Q	5C + 6C	12500 R	0.0217	21	1.03	5.0415	5.0419	0.0004	2	0.02	03/08/2004
4S	1C + 2C	2.5 R	0.0164	17	0.96	5.0401	5.0412	0.0011	7	0.06	16/03/2005

E-4

E5: Amphipods exposed to Atrazine (except for 7:5F,5J)

Sample Name	Sample #	Spike (ng/g)	Mass amphipods (g)	# of Amphipods	Mass per amphipods (mg)	Empty vial (g)	Vial + Lipids (g)	Lipids mass (g)	Lipid %	Lipid/animal (mg)	Date
5A	1C + 2C	2.5 R	0.02598	16	1.62375	5.04129	5.04414	0.00285	11	0.178125	16/03/2005
5B	1S + 2S	2.5 S	0.02086	13	1.604615385	4.93335	4.93406	0.00071	3	0.054615	16/03/2005
5C	3C + 4C	25 R	0.03147	17	1.851176471	5.0677	5.06846	0.00076	2	0.044706	16/03/2005
5D	3S + 4S	25 S	0.02039	15	1.359333333	4.95062	4.95334	0.00272	13	0.181333	16/03/2005
5E	5C + 6C	250 R	0.047	25	1.88	4.9842	4.9879	0.0037	8	0.148	27/09/2004
5G+5H+5I	1C + 2C	2500 R, 25000R, 250000R	0.07318	50	1.4636	5.03779	5.05062	0.01283	18	0.2566	16/03/2005
5F+5J	7	0	0.0671	35	1.917142857	5.0093	5.0141	0.0048	7	0.137143	27/09/2004

E6: Amphipods exposed to **Hexazinone** (except for 7:6E, 6K)

Sample Name	Sample #	Spike (ng/g)	Mass amphipods (g)	# of Amphipods	Mass per amphipods (mg)	Empty vial (g)	Vial + Lipids (g)	Lipids mass (g)	Lipid %	Lipid/animal (mg)	Date
6A	**1C + 2C**	**2.5 R**	0.0309	20	1.55	4.9315	4.9355	0.0040	13	0.20	05/08/2004
6B	**3C + 4C**	**25 R**	0.0318	20	1.59	5.0162	5.0190	0.0028	9	0.14	05/08/2004
6C+6D	**5C + 6C**	**250 RS**	0.0422	29	1.46	5.0639	5.0700	0.0061	14	0.21	16/03/2005
6E+6K	**7**	**0**	0.0388	27	1.44	4.9710	4.9735	0.0025	6	0.09	05/08/2004
6F+6G	**1C + 2C**	**0.02 RS**	0.0460	32	1.44	5.0260	5.0283	0.0023	5	0.07	16/03/2005
6H	**3C + 4C**	**0.1 R**	0.0397	23	1.73	4.9691	4.9715	0.0024	6	0.10	11/08/2004
6I+6J	**5C + 6C**	**0.5 RS**	0.0421	27	1.56	4.9336	4.9381	0.0046	11	0.17	16/03/2005

E7: Amphipods exposed to Endosulfan (except for 7,11, 15)

Sample Name	Spike (ng/g)	Mass amphipods (g)	# of Amphipods	Mass per amphipods (mg)	Empty vial (g)	Vial + Lipids (g)	Lipids mass (g)	Lipid %	Lipid/animal (mg)
1	**0.1S**	0.0112	11	1.02	4.9281	4.93628	0.0081	73	0.74
2	**0.1R**	0.0244	15	1.63	5.0687	5.07259	0.0039	16	0.26
3	**2.5S**	0.0246	15	1.64	5.0436	5.04646	0.0029	12	0.19
4	**2.5R**	0.0114	11	1.03	5.0575	5.05939	0.0019	17	0.17
5	**62.5S**	0.0301	18	1.67	5.0187	5.02420	0.0055	18	0.30
6	**62.5R**	0.0109	10	1.09	5.0282	5.03550	0.0073	67	0.73
7	**R**	0.0231	14	1.65	4.9435	4.94572	0.0022	10	0.16
8	**0.1 S**	0.0521	33	1.58	4.9203	4.92263	0.0023	4	0.07
9	**2.5S**	0.0609	30	2.03	5.0539	5.06144	0.0076	12	0.25
10	**62.5S**	0.0146	13	1.12	4.9134	4.91880	0.0054	37	0.42
11	**R**	0.0670	40	1.67	5.0049	5.01011	0.0053	8	0.13
12	**0.1 S**	0.0106	18	0.59	5.0384	5.04000	0.0016	15	0.09
13	**2.5S**	0.0172	21	0.82	5.0046	5.00680	0.0022	13	0.10
14	**62.5S**	0.0243	36	0.68	5.0273	5.02940	0.0021	9	0.06
15	**R**	0.0251	26	0.97	5.0080	5.01100	0.0030	12	0.12