MODELLING ECOTOXICITY OF POLYBROMINATED DIPHENYL ETHERS IN AQUATIC ECOSYSTEMS

Masekoameng Kolobe Elizabeth
BEnvSc (honours) . University of Venda, South Africa

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ABSTRACT

Modeling ecotoxicity of polybrominated diphenyl ethers in aquatic ecosystems

Masekoameng Kolobe Elizabeth
Environmental Applied Science and Management
Ryerson University

Polybrominated diphenyl ethers (PBDEs) are commercially-produced substances that are used as flame retardants in a wide variety of consumer products. They are among chemicals of emerging environmental concern and are found to be ubiquitous in the environment – they were detected in sediments, water, fish, and wildlife and in human adipose tissues. Environmental concentrations are lower than those of other persistent organic pollutants (POPs). However, present data show that while levels of POPs such as PCBs and DDT are decreasing, PBDE levels are definitely on the rise. The two most prevalent PBDEs in the environment are BDE47 and BDE99.

This research studied the toxicity of PBDEs using *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*), *Daphnia magna,* and *Hyalella azteca* in laboratory bioassays, by exposing each species to 5 different concentrations (0, 12.5, 25, 50, 100, and 200 µg/l) of BDE47 and BDE99 congeners. PBDEs showed toxicity to *D. magna* and *P. subcapitata* and growth was inhibited at the lowest concentration tested, (12.5 µg/l). Neither of the two congeners had measurable effects (in particular, mortality) on *H. azteca* at the concentrations tested (up to 200 µg/l).

A model was developed in order to understand effects of PBDEs on grazing (or predator-prey) relationships using *P. subcapitata* as a prey species and *D. magna* as a grazer or predator species. In general, PBDEs have demonstrated the ability to have significant impact on population dynamics of species in a grazing relationship, even at concentrations that caused minimal effects in growth parameters of isolated species. While single species bioassays showed a decrease in biomass of both species with increasing concentrations of PBDEs, our model predicts an increase in algal population, and a disproportionate and significant decline in *Daphnia*. The research suggests that PBDEs in the natural environment therefore, will not only cause toxic effects on individual sensitive species but also on populations of other organisms with which they interact.

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Dinah T and Johannes M, Masekoameng

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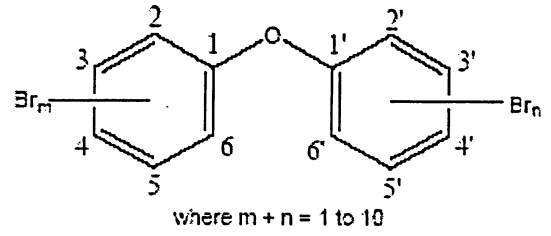
1. INTRODUCTION

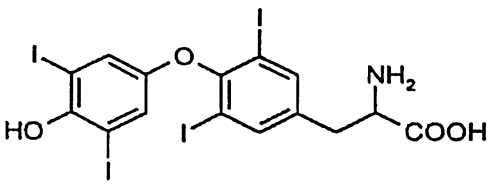
1.1 PBDEs AS EMERGING CONTAMINANTS

Polybrominated diphenyl ethers (PBDEs) are commercially-produced substances that are used as flame retardants in a wide variety of consumer products. They are added to polyurethane foam and various plastics, which in turn may be used in many consumer products including furniture stuffing, carpet backings, non-clothing textiles, electrical insulation, and computer and television casings (Health Canada, 2004). It has been estimated that over 90% of PBDEs (in particular, pentabrominated diphenyl ethers) produced globally are used in polyurethane foams in office and residential furniture, automotive upholstery, sound insulation, and wood imitation products (Environment Canada, 2004).

The interest in these contaminants lay in the fact that they may be of environmental concern for many reasons. They have the potential to be toxic to a variety of aquatic organisms and thus, their ecotoxicological status must be investigated. PBDEs are made of two phenyl rings with an ether linkage between the rings (Figure 1). The linkage makes PBDEs structurally similar to the thyroid hormone thyroxine and, accordingly, these compounds may interfere with the normal functioning of the endocrine system (Meerts *et al.*, 2000).



PBDEs



3,3',5,5'-Tetraiodo-L-thyronine (T_4)

Figure 1: General chemical structure for PBDEs relative to the thyroid hormone thyroxine (T4)(Meerts et al., 2000)

The problem with endocrine-disrupting chemicals (EDC) is that they can cause disruptive effects at exposure levels that are several orders of magnitude lower than carcinogenic exposure levels of concern. EDCs usually have detrimental effects on the reproductive, nervous, and immune systems, and the thyroid function.

There are 209 PBDE congeners (Appendix 1) but only a few exist in commercial products/mixtures. Commercial products occur as a mixture of PBDE congeners and the name is based on the major congener present. For example, decaBDE products contain mainly the decaBDE (BDE209) congener with a small percentage of other congeners (Table 1).

Table1: Congeners found in commercial PBDE mixtures (Hale et al., 2003)

Commercial PBDE mixture	**Components (Congeners) in the PBDE**
PentaBDE	BDE 47, 99, 100, 153, 154, 85
OctaBDE	BDE 183, 153, unknown octa and nona-
DecaBDE	BDE 209, unknown nonaBDE

BDE47 and BDE99 (the main components of a pentaBDE mixture), and BDE209 (the main component of decaBDE) are the most commonly found congeners in the environment. Components of octaBDE also occur in the environment but they are normally detected at very low ratios compared to those of penta mixtures. This corresponds to the low global use of octaBDE mixtures.

1.2. GLOBAL PBDE CONSUMPTION

In general, there has been an increase in global PBDE consumption over the past few decades. In 2001, the total worldwide market demand for PBDEs was about 67,400 tonnes, including 56,100 tonnes of decaBDE mixture, 7,500 tonnes of pentaBDE mixture and about 3,800 tonnes of octaBDE mixture (Lindberg *et al.*, 2004). This is a substantial increase relative to the estimated 1992 annual global consumption of 40,000 metric tonnes, consisting of 30,000 metric tonnes of decaBDE, 6,000 metric tonnes of octaBDE, and 4,000 metric tonnes of pentaBDE (Renner, 2000). Such an increase in PBDE consumption was despite a reported decrease in PBDE consumption in European markets (Renner, 2000).

1.3. RELEASE OF PBDEs INTO THE ENVIRONMENT

It is important to note that PBDEs are used as 'additive' fire retardants. Unlike reactive fire retardants such as tetrabromobisphenol-A (TBBP-A), which are covalently bonded to the plastic itself, additive fire retardants such as PBDEs, are only dissolved in the material; hence, they are believed to be able to leach out of the material into the environment (Kolic *et al.*, 2004). Environmental release of PBDEs may therefore occur 1) as part of dust formed during their initial synthesis, 2) when incorporated into polymers or related finished products, 3) during the use of products or, 4) as a result of disposal or recycling (Renner, 2000). For

example, Julander *et al.* (2005) found PBDE concentrations of up to 208.6 ng/m^3 in the dust from an electronic recycling facility in Sweden. Another pilot study by Schecter *et al.* (2005) analyzed 4 computer wipe samples and 9 domestic sweeping samples for 13 PBDE congeners and all samples tested positive for PBDEs. Logically therefore, in the presence of a carrier such as water or wind, PBDEs released from product use will make its way into systems such as sewage treatment plants (STPs) and eventually into the environment. The release into the environment via STP, for example, occurs because most STPs are not designed to fully eliminate emerging compounds from sludge and effluents (Petrovic, 2003). A study by Hale *et al.* (2006) also suggests that discharge from wastewater treatment effluents and associated particulates to surface waters may be important sources of PBDEs to the environment.

In North America, initial PBDE synthesis is not likely to be responsible for continent-wide PBDE dispersal. This is because production of PBDEs in North America is currently dominated by only two US companies, both with major manufacturing facilities in Arkansas (Hale *et al.*, 2003). In contrast, facilities incorporating PBDEs into polymers and subsequent use in electronics, automobile padding, furniture, and textiles are more widespread. It is these widespread facilities that could be responsible for environmental contamination, considering that polymer-based products may contain up to 30% PBDE by weight (Hale *et al.*, 2003).

1.4. FATE AND PARTITIONING OF PBDEs IN THE ENVIRONMENT

Several studies (Palm *et al.*, 2002; Gouin and Harner, 2003; Buckley *et al.*, 2004; Shoeib, 2004) have been undertaken to model the fate of PBDEs in the environment. In general,

PBDEs will partition in different environmental media depending on the physicochemical properties of specific congeners, as shown in Table 2.

Table 2: Physicochemical properties of PBDE congeners commonly found in the environment (Wania and Dugani, 2003; Environment Canada, 2004).

Property	**TetraBDE**	**PentaBDE**	**DecaBDE**
Molecular weight	485.8	564.7	959,2
Water solubility (25°C; µg/L)	10.9	2.4	<0.1
Log K_{ow}	6.39	6.76	~8.5
Log K_{OA}	10.44	11.26	~15

These physicochemical properties of PBDEs are extremely important when determining partitioning and subsequent fate of these contaminants in the environment.

1.4.1 Water and Sediment Partitioning

PBDEs have high log octanol-water coefficients (K_{ow}; as shown in Table 2), which means that they are more likely to partition into solid medium than into water. Thus, in aquatic environments, PBDEs will accumulate mainly in sediments, suspended organic matter, and biota, with a smaller portion partioning into water. In the water column, solubility of PBDEs will depend on the congener, decreasing with an increase in the number of bromines.

1.4.2 Soil and Air Partitioning

Modeling results by Gouin and Harner (2003) show that in a terrestrial environment, most of the PBDEs will partition in the soil with lighter congeners (*i.e.* those with relatively fewer bromines) partitioning slightly into the air. The reported log octanol-air partitioning co-efficient (K_{OA}) for BDE47 and BDE99 are 10.44 and 11.26 respectively (Wania and Dugani, 2003). This means that generally, PBDEs have greater affinity for soil than air.

The fate of PBDEs in soil-air interfaces may often be affected by seasonal emergence of foliage and snow events. A study by Gouin and Harner (2003) reports that there is a pulse of atmospheric PBDEs in the early spring, followed by a dramatic decrease in atmospheric concentrations coincident with the emergence of foliage. Air samples collected from January to June in 2002 in southern Ontario showed a rise in gas-phase concentration of PBDEs from below detection limits in winter to 19 pg/m3 in early spring, only to decrease again following bud burst (Gouin *et al.,* 2005). The authors hypothesized that in winter, snow scavenging and other deposition processes increase levels of PBDEs in the snow pack. When the snow melts, a fraction of the PBDE burden is released back into the atmosphere (with the remainder partitioning between soil particles and melted snow runoff), resulting in a short period of elevated air concentrations. When foliage emerges, PBDEs could be effectively scavenged from the atmosphere by plant leaves (Gouin and Harner, 2003).

1.4.3 Gas-Aerosol Partitioning in the Atmosphere

PBDEs in the atmosphere exist in the gas phase and in association with atmospheric particles such that their fate (deposition, transport, and residence time) is affected by gas-aerosol (atmospheric particles) partitioning (Shoeib, 2004) i.e. the amount that attaches to particles vs.

the amount in gas phase. Atmospheric particles are important potential sorbents for various organic contaminants, including PBDEs (Buckley *et al.*, 2004). A study by Buckley *et al.* (2004) predicts that PBDEs are more likely to partition in atmospheric particles such as soot than in the gas phase, compared to other organohalogenated compounds such as organochlorine pesticides. Hence, atmospheric residence time and subsequent deposition of atmospheric PBDEs would be affected by the fate of the particle itself. The extent of particle partitioning of individual PBDE congeners depend on the K_{OA} of each congener (the higher the K_{OA} of a congener, the higher the percentage in aerosol phase) (Buckley *et al.*, 2004).

1.4.4 Persistence

As shown earlier, most of the PBDEs will primarily partition in soil and sediments for terrestrial and aquatic environments, respectively. The residence time (persistence) will depend on the media and congener type (relative half-lives are shown in Table 3).

Table 3: Half lives (hours) of different PBDE congeners in various environmental media (Palm et al., 2002)

Half life	Unspecified di brominated phenyl ether	BDE47	BDE99	BDE209
In air	36.7	256	467	7620
In water	360	3600	3600	3600
In soil	360	3600	3600	3600
In sediments	1440	14400	14400	14400

Generally, there is an increase in persistence of PBDEs in air with an increase in degree of bromination (Table 3), with residence time being relatively high in sediments compared to water and air. The actual mechanism of degradation of PBDEs in all these media is not well-documented nor understood. However, a study by Sanchez-Prado *et al.* (2005) suggests that photodegradation is a likely degradation path for PBDEs, where degree of bromination is low enough (ie. BDE209, which is a decaBDE, has great persistence in air). Reductive debromination by successive losses of bromine atoms was observed after UV-irradiation of PBDEs. On the other hand, Gouin and Harner (2003) hypothesised that there is a possibility for PBDEs to be removed by the reaction with hydroxyl radicals in air. The hydroxyl radical is produced by the reaction of excited atomic oxygen and water vapour. It is considered an atmospheric "detergent" because it is very reactive and therefore able to remove hydrogen from volatile organic compounds. They also suggest that microbial action may be important degradation pathways in soil and sediments. However, Boom *et al.* (2002) insist that PBDEs are resistant to degradation.

1.4.5 Long-Range Transportation of PBDEs

There is also an indication that PBDEs may travel to remote locations *i.e.* they are subject to significant long-range transport processes (Wania and Dugani, 2003). The presence of PBDEs in air samples from remote rural areas of England and the west coast of Ireland (concentrations up to 37 pg/cm^3 (Lee *et al.*, 2004)) and the arctic regions of the north Pacific Ocean (concentrations up to 198.9 pg/m^3 (Wang *et al.*, 2005)) confirms that, despite their relatively low volatility, long-range atmospheric transport of these compounds occurs. Biotic samples also confirm long-range transport of PBDEs. Congeners were detected in adipose tissue of

female polar bears from subpopulations in arctic Canada, eastern Greenland, Svelbard, and northwestern Alaska. Concentrations ranged from 7.6 ng/g in the Canadian arctic to 70 ng/g lipid weight in Greenland (Muir *et al.*, 2006). Similarly, PBDEs were found in tissues of fulmars (*Fulmarus glacialis*) (marine birds feeding mainly on crustaceans and fish) of Farve Islands, far away from known point sources (Fangstrom *et al.*, 2005).

The actual mechanisms that induce long distance travel of PBDEs in the environment are currently being studied. Gouin and Harner (2003) explained the possible mechanism for transport of tetraBDE in Canada. They suggested that BDE47 in the atmosphere is likely to be scavenged by snow during winter and become concentrated in the snow pack. When the snow melts, a fraction of this will be released back into the atmosphere. During transport, most of the PBDEs are re-immobilized by partitioning into other media (soil, sediment, and water) or scavenging by plants. It is therefore possible that this congener may be transported to remote locations through a series of deposition and volatization processes (Gouin and Harner, 2003).

1.5. BIOACCUMULATION AND BIOCONCENTRATION OF PBDES IN WILD LIFE

A number of studies showed the ability of PBDEs to increase in concentration from the media of exposure to the organism, and from one organism to another in the foodchain. For example, a study in the Scheldt estuary of the Netherlands showed that PBDE concentrations were 100 times higher in lipids of near-sediment dwelling organism (mysids) than in the sediments themselves (Verslycke *at al.*, 2004). Experiments by Kierkegaard *et al.* (1999) showed that when rainbow trout were fed with food spiked with decaBDE, concentrations in muscle increased from less than 0.6 ng/g (fresh weight) to 38 (±14) ng/g after 120 days. Corresponding

liver concentrations went from 5 to 870 (±219) ng/g (Kiergaard *et al.*, 1999). Hardy (2004) reported a bioconcentration factor (BCF) of ~14,350 for pentaBDE commercial mixtures based on an 8-week laboratory bioconcentration study using Japanese carp. In the same study, differences in bioconcentration of the various components of the product were observed. The major constituent of the product, BDE99, showed no significant accumulation (BCF~73), but the BCF of BDE47 was ~35,000 (Hardy, 2004).

Due to the ability of PBDEs to bioconcentrate, these compounds are found in biological samples at higher concentrations than those generally found in the environment (especially BDE47 and BDE99). In Switzerland, PBDE levels were found in white fish from eight Swiss lakes and in rainbow trout from Swiss fish farms with total PBDE concentrations ranging between 36 and 165 ng/g lipid weight in both species (Zennegg *et al.*, 2002). The main congeners of concern were BDE47 and BDE99 (Zennegg *et al.*, 2002).

PBDEs have also been detected in species representing different trophic levels of the North Sea foodweb. These include invertebrate species such as whelk (*Buccinum undatum*), seastars (*Asterias rubens*), and hermit crab (*Pagurus bernhardus*), and the fish species whiting (*Merlangius merlangus*) and cod (*Gadus morhua*), and the marine mammal species harbour seals (*Phoca vitulina*) and harbour porpoises (*Phocoena phocoena*) (Boon *et al.*, 2002). A study by Hale *et al* (2001) showed PBDE concentrations in Virginia freshwater fish fillets ranging from >5 to 47,900 μg/kg (lipid) with BDE47 contributing 40-70% of the total PBDEs observed.

PBDE concentrations in biota samples from the west coast and Northwest Territories of Canada were also reported and the highest concentration of total PBDE residues (2,269 μg/kg lipid) was found in the blubber of a harbour porpoise from the Vancouver area. A tetraBDE congener accounted for slightly more than half of the total PBDE in the sample (Environment Canada, 2004).

Recently, PBDEs, ranging in concentrations from 16 and 500 μg/kg lipid, were detected in the eggs of herring gulls (*Larus argentatus*) from Big Sister Island in Lake Michigan and 1,830 μg/kg from Port Colbourne in Lake Erie (Law *et al.*, 2003). The herring gull is a year-round resident species in the Great Lakes region with a diet consisting of alewife and rainbow smelt, which are abundant prey fish in the Great Lakes. Thus, the herring gull is ideal for biomonitoring persistent organic pollutants.

Only a few studies have been conducted to examine the accumulation or presence of PBDEs in terrestrial organisms. Lindberg *et al.* (2004) observed that terrestrial organisms from lower trophic levels tend to have generally lower PBDE concentrations compared to aquatic food webs, with the exception of the higher trophic level organisms such as the peregrine falcon, a marine predatory bird (*Falco peregrinus*) (Lindberg *et al.*, 2004). A recent study by D'Have *et al.* (2005) showed PBDE concentrations, from 1 to 1178 ng/g, in the carcasses of the European hedgehog (*Erinaceus europaeus*), a terrestrial omnivorous species.

1.6. HUMAN EXPOSURE

Trends show that levels of PBDEs in mothers' milk are increasing. In Sweden, PBDEs in human milk have increased markedly from 1972 to 1997, with a doubling period of 5 years,

whereas levels of other organohalogen contaminants such as PCBs decreased during the same period (Noren and Meironyte, 2000). This was consistent with observations of Japanese adipose tissues where samples collected in 1970 and 2000 revealed significant increases in adipose PBDE levels, with a concomittant decrease in other organochlorine contaminants (Watanabe and Sakai, 2003).

While data from 1998 to 2000 indicate that levels of PBDEs in human adipose tissues in European countries (*e.g.* Sweden) are decreasing (Watanabe and Sakai, 2003; Betts, 2001), observations are not the same in North America. The assessment of PBDEs in the breast milk of North American women indicates that the body burden of Americans and Canadians is the highest in the world (40 times greater than the highest levels reported for women in Sweden) (Betts, 2001). The levels of PBDEs in North Americans appear to be doubling every two to five years.

Dietary intake of contaminated food seems to be the primary route of human exposure to PBDEs (Sjodin *et al.*, 2003). Although not much has been researched about the concentrations of PBDEs in food samples, preliminary data from Japan showed the presence of PBDEs in edible tissue of four species of fish (yellowtail, salmon, mackerel, and yellow tuna) and one species of shellfish (clam) purchased from two food markets (Domingo, 2004). Concentrations ranged between 17.7 and 1720 ng/kg wet weight (Domingo, 2004). For vegetables and tubers, the same study found PBDE concentration averages to be 38.4 ng/kg for carrots and 134 ng/kg for spinach. Generally, the highest levels were found in food with fats and oils and lowest

levels corresponded to fruits and vegetables, as PBDEs tend to accumulate in fatty tissues and may biomagnify along food chains (Sjodin *et al.*, 2003).

Sjodin *et al.* (2003) further attributed detectable concentrations in farm products (vegetables in particular) to application of biosolids in agricultural areas. They reported that high levels of PBDEs have been found in sewage sludge destined for land applications in North America. Eleven samples of biosolids had levels of 1,100-2,290 ng/kg of tetra to hexa-BDE and <75 to 9,160 ng/kg of BDE209. They suggest that application of PBDE-contaminated biosolids in agricultural areas may be a potential route of human exposure, though this requires further investigation (Sjodin *et al.*, 2003).

1.7 TRENDS IN ENVIRONMENTAL CONCENTRATIONS OF PBDEs

There is evidence that levels of PBDEs in the environment are increasing over time (Renner 2000). Sediment cores from Lake Ontario show a steady increase in the flux of PBDEs into the lake over the past thirty years (Alaee, 2001). Ikonomou *et al.* (2002) documented that polybrominated flame retardants are now increasing exponentially in the North American arctic, so rapidly that they will overtake PCBs as the most prevalent organohalogen contaminant in that region by 2050 if production and use volumes are not reduced.

The increase in environmental concentration of PBDEs is even more prominent in biota. Temporal trends of polybrominated diphenyl ethers (PBDEs) in lake trout from Lakes Superior, Michigan, Huron, and Ontario, and walleye from Lake Erie, collected during the period of 1980-2000, were analyzed by Zhu and Hites (2004). Their data showed that the sum PBDE

concentrations in fishes from the five lakes increased exponentially with time, doubling every 3-4 years (Figure 2).

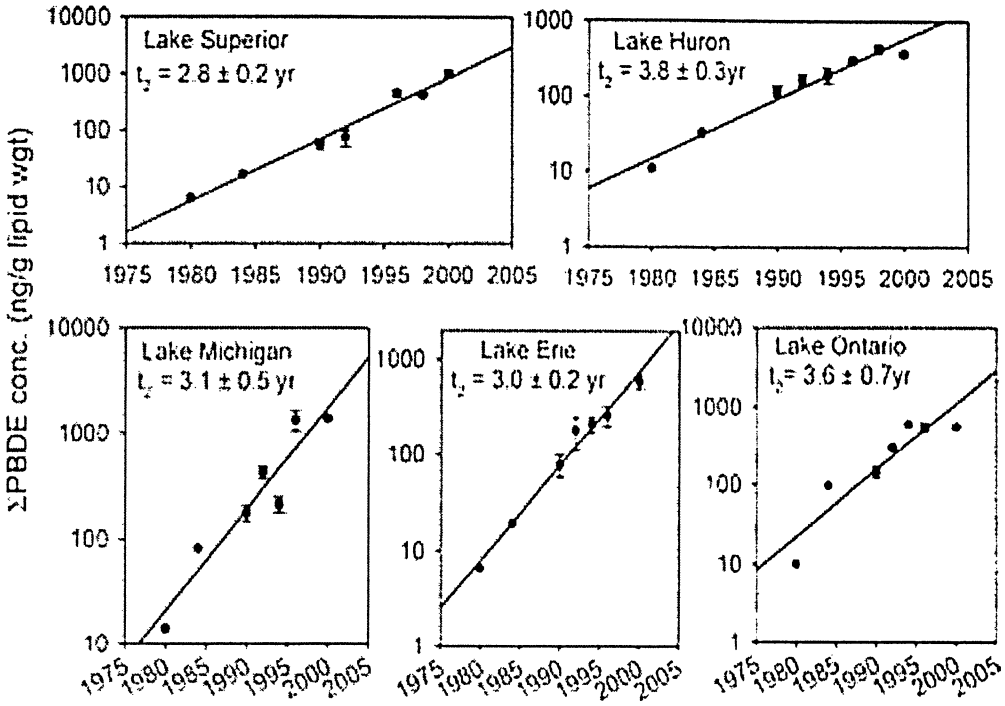


Figure 2. Temporal trend of the sum (Σ) PBDE concentrations in fishes from the Great Lakes (Zhu and Hites, 2004)

The same study suggests that the relative proportion of BDEs-47, -99, and -100, compared to other congeners, increased significantly as a function of time .

1.8 OBJECTIVES

The objectives of this research were:

- To assess the toxicity of tetraBDE (BDE47) and pentaBDE (BDE99) on three aquatic organisms

- To model potential effects of PBDEs on ecological relationships, in particular algae-grazer population dynamics (based on predator-prey models)

The two congeners, tetraBDE and pentaBDE, were studied for the purpose of this study because previous research showed that although deca-BDE accounts for most PBDE consumption, it is the less brominated congeners, tetra-BDE and penta-BDE, and in particular 2,4,2′,4′-tetra-BDE (BDE47) and 2,4,5,2′,4′-penta-BDE (BDE99) that are most commonly found in the environment (Renner, 2000). This prevalence may possibly be due to debromination of decaBDE (Kierkegaard *et al.*, 1999; Renner, 2000). TetraBDE and pentaBDE were also recognized as contributing more than 90% to the total PBDE levels in Lake Ontario (Watanabe and Sakai, 2003). The two are also likely to pose greater environmental concern (Palm *et al.*, 2002) because they have more bioaccumulation potential than the decaBDE congeners (Boon *et al.*, 2002). Assessment of PBDEs in biota samples shows that BDE47 and BDE99 constitute a greater percentage in biological samples than other congeners (Palm *et al.* 2002), with more than 82% reported in polar bears (Muir *et al.*, 2006).

1.9 RESEARCH JUSTIFICATION

PBDEs are clearly increasing in the environment and in biota, including humans. Although environmental concentrations are still lower than those of PCBs, it is the rate at which they are increasing that raises concern about their ecotoxicity.

1.9.1 Deficiency of PBDE Ecotoxicity Information

Despite the availability of data on the ubiquity of PBDEs in environmental media and biota, ecotoxicity is still an unresolved issue. There is a consensus amongst scientists that there is a lack of data that characterize toxicity of these compounds, especially in the environment (Renner, 2000; Gouin and Harner, 2003; Environment Canada, 2004).

Correlations have been found between concentrations of PBDEs and neonate development. Viberk (2003) indicated that exposure of neonatal mice to PBDEs can induce developmental neurotoxicity effects such as changes in spontaneous behaviour (locomotion, rearing, and total activity), and impairments in learning and memory. This was based on laboratory observations where male mice were orally exposed to different concentrations of BDE153 (0.45, 0.9, or 9.0 mg/kg) (Viberk, 2003). These concentrations are, however, higher than those normally found in water and biotic samples from the natural environment, but they are in parallel with reported sediment concentrations.

In other experiments, the larvae of the harpacticoid copepod (*Nitocra spinipes*), a shrimp-like crustacean, were exposed to PBDEs and significant delays in larval development and increases in mortality were observed at 0.04 - 0.4 mg/L tetraBDE (Beitholtz and Wollenberger, 2003).

The inhibition of algal growth using the green algae *Pseudokirchneriella subcapitata* and acute toxicity to the zooplankton *Daphnia magna* by BDE99 was examined by Evandri *et al* (2003). The compound showed a toxicity to *D. magna* with 48-h EC50 value of 25 μ/L. In contrast, BDE99 was nontoxic to *P. subcapitata* at the highest concentration tested (56,000 μg/L) (Evandri *et al.*, 2003).

Due to paucity of information available on toxicity of PBDEs, decision-makers around the world are faced with the challenge of deciding whether PBDEs warrant more strict regulatory measures. This is evident because while European countries have placed a ban on PBDEs, North American regulations are still flexible on the use of these compounds. Thus, it is very necessary to provide further toxicity information that can aid in the decision-making process, leading to potential regulation of these compounds.

1.9.2 The Need for Modeling Effects on Ecological Relationships

Unlike classical toxicology, where a single species (usually mice or rats) is used to represent human physiology, ecotoxicology has to consider effects of toxicants on more than one species. Most importantly, ecotoxicolgy has to consider effects on interactions among species, in order to improve representativeness of complex and dynamic ecological systems. The latter (effects on species interactions) is, however, not well-considered in most ecotoxicological studies. More often than not, individual species are tested in isolation from other species with which they interact, in order to clearly understand correlation between chemicals and effects per species. Mathematical models then become useful in conceptualizing, based on known interaction patterns, the effects of chemicals on populations of not isolated, but interacting species.

Mathematical models have been instrumental in advancing the quantitative description and understanding of ecological systems (Bartell *et al.*, 2003). For example, in the late 1700s, Malthus developed a model to describe the dynamics of populations. By the early 1900s, Lotka and Volterra developed simple models of predator-prey systems (Bartell *et al.*, 2003). The

Lotka-Volterra models are of particular interest to this research because while they have been extensively used in the management of renewable natural resources, little work has been done to incorporate them into ecotoxicological data. The significance of predator-prey models to ecotoxicology lies in the fact that an adverse effect on ecological relationships such as predator-prey interactions can lead to local extinction of a species population, even if toxicant concentrations are below those causing diminished growth, reproduction, or survival of individuals (Newman and Unger, 2003).

Current toxicological models concerning environmental chemicals are inclined towards understanding fate and transport, and bioaccumulations and biomagnification along foodchains. For example, Carrer *et al.* (2000) modeled the fate of dioxins in an aquatic food web by coupling ecotoxicological data and the trophic network model. A trophic network model describes trophic interactions (which are determined by feeding, reproduction, growth, respiration, excretion, and natural mortality) among components of the ecosystem. They used knowledge of such interactions, combined with energy transfer equations, K_{ow} of chemicals, and levels of organic carbon in the sediments to model/estimate the amount of toxicants that is transferred to an organism in a trophic network. On the other hand, Campfens and Mackay (1997) developed a fugacity-based (tendency of a substance to prefer one phase (liquid, solid, gas) over another) model to understand bioaccumulation of PCBs in aquatic foodwebs. They used physicochemical properties of compounds, and the equations that define organisms' metabolic rates in order to estimate concentrations of PCBs at different trophic levels.

Attempts have been made to incorporate toxicological data into population dynamics models. Preston and Snell (2001) used models to simulate the population dynamics of the predatory rotifer *Asplanchna girodi* and six prey species of herbivorous rotifers in response to sublethal concentrations (110, 190, or 330 mg/L) of pentachlorophenol (PCP). Their study showed that 190 mg/L PCP (the concentration that caused only reproductive impairment and no mortality in rotifers) resulted in a 20% decrease in prey division rate while the average prey density at these concentrations increased by 1.5–2-fold due to the higher sensitivity of *A. girodi* to PCP than the prey. This disproportionately lowered *A. girodi* density relative to the prey, causing a decrease in predation pressure (Preston and Snell, 2001).

Following the Preston and Snell (2001) example, the current study will couple toxicological data with predator-prey models to understand effects of PBDEs on algae-grazer population dynamics. Preston and Snell's model assumed a condition where a given toxicant concentration had similar effects on the predator species as on prey species. Our model, on the other hand, accounts for the differential toxicity of compounds on individual predator and prey species. The model owes its existence primarily to the mathematics of Lotka-Volterra predator-prey population dynamics, which assumes that a population will grow exponentially until reaching its carrying capacity such that dN/dT (change in population over time) is a function of division rate "r", population size"N" and carrying capacity "K", as shown in Equation A below (Deaton and Winebrake, 2000). In the presence of a predator, the logistical growth of a population may be affected. Introduction of a predator may tend to place a downward pressure on the prey population; hence the introduction of a constant predation-efficiency term, (Pe) shown in Equation B. The downward pressure may, however, be autoregulating *i.e.* if the prey

population gets too low, the predator population will likely die back as well, offering the opportunity for the prey species to increase in size (Deaton and Winebrake, 2000).

dN/dT = Nr (K- Nr)/K .....[eq. A]

dN/dT = Nr (K-Nr)/K – PeN.....[eq. B]

1.10. TEST ORGANISMS

1.10.1 *Pseudokirchneriella subcapitata* (formerly known as *Selenatrum capricornutum*)

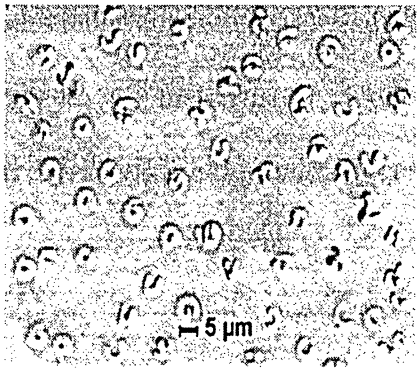


Figure 3: Pseudokirchnerialla subcapitata cells as seen through the microscope

Pseudokirchneriella subcapitata is a non-motile, unicellular crescent-shaped green alga (Class: Chlorophyceae), which is ubiquitous in most fresh waters across North America (Bostan *et al.*, 2005), where it obtains its nutrients from the surrounding water. As a primary producer and an important source of food for zooplankton (Wong *et al.*, 2001), *P. subcapitata* plays a crucial role in aquatic ecosystems and is therefore considered essential in toxicity testing (Bostan *et al.*, 2005). Changes in *P. subcapitata* biomass can affect animals that use it as a source of food. In addition, algal populations can affect dissolved oxygen concentrations, pH, alkalinity, and taste of surface water (Wong *et al.*, 2001; Shehata *et a.l*, 1997). Green algae are widely used in phytotoxicity testing and several agencies, including the USEPA (2003) and Environment

Canada (1992), have standardized test methods for this organism. The algal growth inhibition toxicity test using the freshwater algae in this research was one of the several aquatic toxicity tests selected to be standardized sufficiently to help meet Environment Canada's testing requirements (Environment Canada, 1992).

The advantage of using *P. subcapitata* is that it can be easily cultured in the laboratory and is readily available from reliable suppliers. Its uniform and distinctive morphology makes it ideal for enumeration either with an electric particle counter or a haemocytometer under the microscope. (Environment Canada, 1992). Tests using *P. subcapitata* are relatively short compared to those of many aquatic floras (usually 96 hours is sufficient for algal growth inhibition test (USEPA, 2003)). Hence, it provides more rapid and economically-viable results.

1.10.2 *Daphnia magna*

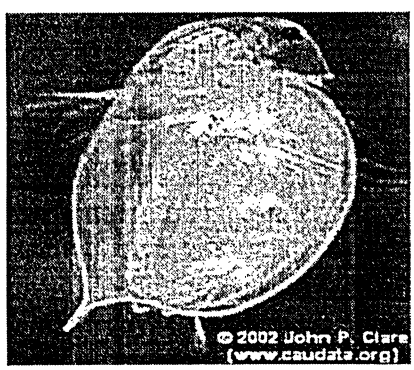


Figure 4: Daphnia magna

Daphnia is a filter-feeding zooplankton in fresh water ecosystems and is widely distributed in North America. It occurs largely in ephemeral habitats such as small ponds and rockpools where vertebrate predators are rare (Koivisto, 1995). In large lakes, they are a major food source for many kinds of fish such as sticklebacks, minnows, the fry of larger fish, and also larval amphibians (Clare, 2002).

Daphnia feed on particles floating in the water-column and on attached vegetation or decaying organic material, but the predominant foods are free-living algae, bacteria, and fungi. In the summer months, they can often be seen "blooming" in ponds and lakes as the concentration of algae builds up (Clare, 2002). In terms of physical conditions, *Daphnia* are generally tolerant of a wide range of oxygen levels, ranging from low to super-saturated concentrations. However, *Daphnia* do appreciate a good oxygen supply (USEPA (2000) suggest dissolved oxygen concentrations of >4 mg/L). pH between 6.5 and 9.5 is acceptable and ammonia should be considered toxic to this organism (Clare, 2002) at concentrations ≥5 mg/L (USEPA, 2000).

Over the years, *Daphnia* have been used in regulatory testing as well as in basic ecotoxicological research (Koivisto, 1995). Tests with *Daphnia magna* are formally endorsed by major international organizations, such as Organization for Economic Cooperation and Development (OECD, 1998) and the USEPA (USEPA, 2002). However, *Daphnia pulex* and *Ceriodaphnia dubia* are also used (Mark and Solbe, 1998). The choice of *Daphnia* over other crustaceans used in bioassays was strongly influenced by the fact that, under favourable conditions (temperature and enough food, etc.), *Daphnia* reproduces asexually (by cyclic parthenogenesis), which circumvent problems associated with identification of opposite sexes for assessing reproductive toxicity. Of all species of *Daphnia*, *D. magna* is the largest and the easiest to handle (Adema, 1978) and this makes it ideal for experimental work.

1.10.3 *Hyalella azteca*

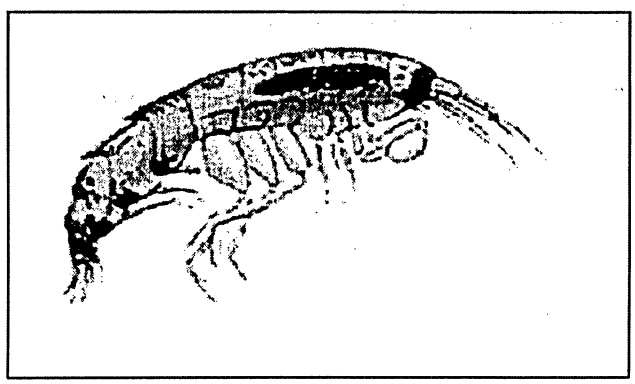


Figure 5: Hyalella azteca

Hyalella azteca is an epibenthic, detritus-feeding, sediment-burrowing freshwater amphipod. It resides in temperate lakes, ponds, and slow-flowing streams, in close association with 1-2cm of sediments (Environment Canada, 1997). The species is distributed across the North American continent's fresh water. It is reported to selectively feed on bacteria and alga that adhere to sediment particles. Fresh water amphipods like *H. azteca* are an important source of food for fish and aquatic birds (Kokkotis and McLaughlin, 2002).

H. azteca has been the subject of numerous ecological studies and it is used extensively in toxicological testing (Hirsch, 1998; Kokkotis and McLaughlin, 2002; Wilcoxen *et al.,* 2003; Trimble and Lydy, 2006). Although *Hyalella* is commonly used for testing toxicity of freshwater sediments, *water-only* acute and chronic tests have been used on this organism (Wilcoxen *et al.,* 2003; Trimble and Lydy, 2006) with various contaminants including organic chemicals (Trimble and Lydy, 2006).

Reviews of sensitivity of *Hyalella* to chemicals indicate that this species is one of the most sensitive of 24 organisms tested in a study of Great Lakes sediment (USEPA, 2000). Using

acute lethality as an endpoint, *water-only* tests, with a number of industrial effluents, indicated that the sensitivity of *Hyalella* was similar to that of the rainbow trout, *Oncorhynchus mykiss* (Environment Canada, 1997). Similarly, acute tolerance of *Hyalella* to potassium chloride was similar to that of *Ceriodaphnia dubia* (Environment Canada, 1997)

The relevance of a benthic organism such as *Hyalella* in studying toxicity of PDBEs relates to the fact that most PBDEs in aquatic environments are likely to partition into sediments (Gouin and Harner, 2003) and therefore, benthic organisms are at the highest risk of exposure.

2. METHODOLOGY

2.1 CHEMICALS

Ninety-eight percent pure BDE47 (2,2'; 4,4' tetrabromodiphenyl ether) and BDE99 (2,2'; 4,4'5 pentabromodiphenyl ether) analytical standards, dissolved in toluene and nonane respectively, were purchased from Wellington Laboratories Inc. (Guelph, Ontario) at concentrations of 50 μg PBDE/mL. Toluene is toxic and is not soluble in water (water solubility = 515 mg/L at 23°C) (Nahar *et al.*, 2000). Similarly, nonane is highly toxic to aquatic organisms at less than ppb levels (Aboul-kassim and Simoneit, 2001). Due to the inherent toxicity of the two solvents (toluene and nonane), a solvent transfer was required for using PBDEs in the toxicity experiments. The analytical standards were evaporated using gentle nitrogen gas-stripping in a fume hood (Evandri et al.,2003). The remaining PBDEs were then re-dissolved in DMSO to prepare 100 μg/mL stock solutions for each congener. DMSO was chosen because it was found to be the least toxic of four commonly used solvents (dimethyl sulfoxide (DMSO), glycerol, ethane, and formamide) (Dresser *et al.,* 1992). The stock solutions were stored in the refrigerator at 5°C for less than 24 hours prior to use.

Solvent exchange procedure testing

In order to ensure that the evaporation process did not remove PBDEs, the presence of PBDEs in a new stock solution (in DMSO) was analyzed using gas chromatography. The equipment used a capillary column, helium gas as a carrier and flame ionization detector. The chromatograms are shown in appendix 2.

2.2 ALGAL TOXICITY TEST

2.2.1 Division rate and Maximum Standing Population

Algal toxicity tests were performed according to USEPA guidelines (USEPA, 2003). The AAP algal growth medium was prepared in 200 mL flasks using equal proportions of nutrient solutions (Appendix 3)

Five different concentrations (200, 100, 50, 25 and 12.5 μg/L) of each PBDE congener were prepared by adding appropriate volume of the stock solutions to the growth medium. Additional DMSO was added to the dilution series such that all flasks had the same final DMSO concentration (0.2% vol/vol). The PBDE concentration range was based on reported 10% *Pseudokirchneriella subcapitata* growth reduction at sediment PBDE concentrations of 3.3μg/L (Hellstroom 2000) (congener not specified). Four mL of the growth media with PBDEs were transferred to glass cuvettes (5 replicates for each concentration of each congener). In addition, there were five replicates of each of two controls: one contained the AAP medium only, and the other one contained AAP medium plus DMSO (0.2% vol/vol) to ensure that any potential toxicity was not due to the carrier.

All cuvettes were then inoculated with 100 μl algae culture (less than 2 weeks old, 5×10^6 cells/mL) grown in AAP medium, resulting in an initial cell density of 1.25×10^5 cells/mL. The cuvettes were partially covered with parafilm to minimize evaporation and were placed in a Sanyo MIR153 incubator under continuous illumination (4000 lux) and constant temperature (24°C).

In order to determine division rates, the absorbance of each test and control cuvette was recorded at different time intervals: 0, 6, 18, 24, 44, 51, 67, 73, 96 and 144 hours. Preliminary

experiments showed that *P. subcapitata,* under these growth conditions, reached maximum standing stock after 144 hours and stabilized thereafter. From the 144th -hour absorbance reading therefore, the maximum standing population at different PBDE concentrations was also calculated. The maximum standing population is simply the number of cells per mL after 144 hrs. Effects on algal division were measured by considering effects of PBDEs on cell division rate given by: log N_t –logN_0 / log2 (t) (Prescott *et a.l*, 2005). Where, N_t was the population size after 144 hours, N_0 is the initial population size, t is 144 hours.

The absorbance was determined using a spectrophotometer (Spectronic 20$^+$) set at 750nm (USEPA 2003).

2.2.2 Calibration

Because absorbance is a function of the volume, size, and pigmentation of the algae (USEPA 2003), a calibration curve was constructed to establish the relationship between absorbance and cell density. This was achieved by preparing five stocks of known algal densities (determined using a haemocytometer), and measuring their corresponding absorbance values (Appendix 4), using the spectrophotometer (Spectronic 20$^+$) set at 750nm. From the equation in the calibration curve, cell densities were determined based on the sample's absorbance.

2.3 *DAPHNIA MAGNA* ACUTE TOXICITY TEST

Five different concentrations of each PBDE congener (200, 100, 50, 25 and 12.5 µg/L) were prepared by adding appropriate volumes of the stock solutions to the bottled spring water (mineral contents shown in Appendix 5). Spring water has been previously used with success in bioassays. Pagnout *et al.* (2006) used spring water for toxicity testing of PAHs to

Ceriodaphnia dubia. Ferrari and Ferard (1996) also recommended spring water as part of *D. magna* growth medium. DMSO was added to the dilution series such that all flasks had the same final DMSO concentration (0.2% vol/vol). The dilution series was prepared in 50 mL centrifuge tubes, with a final volume of 35 mL in each tube (USEPA (2002) recommends a minimum 30-mL volume test chamber). A total of 5 replicates were made for each concentration of each congener. The control treatment contained only spring water and the carrier control contained spring water and DMSO without PBDE (0.2% vol/vol). Bioassay organisms were obtained from a 10 L culture tank containing continuously-aerated dechlorinated water. Three young daphnids (1 day old) were placed in each vessel (USEPA, (2002) recommends less than 5 organism per test chamber), and were each fed one time with fish food (Tetramin® tropical flake) immediately after placement. The tubes were then covered with plastic lids (not air-tight) to minimize evaporation. Mortality was determined after 48 hours of exposure to PBDEs.

The LC_{50} was determined by first converting percent *Daphnia* mortality into logit values in order to linearize the sigmoid dose response curve. Due to the difficulty of accurate determination of the LC_{50} from the sigmoid curve, methods have been developed to linearize sigmoid curves for accurate computation of LC_{50} for normally distributed quantal responses (Newman and Unger (2003). This is done by firstly converting the units of mortality to logit units.

$$\text{Logit} = \ln [\, P(1-P) \,]$$

Where, P is the proportion of the population dying. A linear relationship was established between concentration and logit values. Unlike with the sigmoid curve, the linear curve has one point where the 0-probit value (which is equivalent to 50% mortality) meets the concentration value- LC_{50} value.

2.4 *DAPHNIA MAGNA* CHRONIC AND REPRODUCTIVE TOXICITY TESTS

The *Daphnia magna* bioassay was prepared according to OECD (1998) guidelines with some modifications. For 21-day chronic toxicity tests, four different concentrations of PBDEs (100, 50, 25 and 12.5 μg/L) were prepared as above. Three young daphnids (1 day old) were obtained from culture tanks, placed in each vessel, and fed every other day with fish food (Tetramin® tropical flakes). The bioassay was monitored daily and new offspring were counted and removed to prevent them from consuming food intended for the adult (OECD, 1998). The number of live adults in each vessel was also counted for every reproductive event in order to determine the average number of offspring per adult (OECD, 1998). To compensate for evaporation, dilution water (spring water) was added to maintain the 35 mL volume. After 21 days, the test was terminated and the number of offspring and live adults was tabulated.

2.5 *HYALELLA AZTECA* TOXICITY TEST

Five different concentrations of each PBDE congener (200, 100, 50, 25 and 12.5 μg l^{-1}) were prepared as for *D. magna* experiments. The PBDE solutions were added to fill 10 mL microplate wells. Although USEPA (2000) suggests 100 mL volume for biological tests with *Hyalella*, preliminary experiments showed successful survival of this organism in 10 mL microplates. A total of 6 replicates were made for each PBDE concentration and for each

control. A 1 cm^2 piece of gauze was placed in each well to provide a substrate for attachment by the amphipods (Environment Canada, 1997).

Hyalella azteca used for bioassays were obtained from a 10 L culture tank containing continuously-aerated, dechlorinated water. Three young amphipods (< 1 week) (Environment Canada, 1997) were placed in each vessel and were each fed every three days with fish food (Tetramin® tropical flake). The microplates were covered to minimize evaporation. The number of live individuals was determined after 48 hours, 7 days, and 21 days of exposure. Reproductive test were not undertaken for this organism due to the difficulty of identifying different sexes of this species, especially at neonatal stage.

2.6 STATISTICAL ANALYSIS

Analysis of variance (ANOVA) was performed for all bioassay data and post-hoc pair-wise comparisons (Fisher's least-significant difference tests) were performed using SYSTAT 6.0.1 (student version) in order to evaluate the difference between the treatment and the control. Analysis of variance (ANOVA) is considered the most efficient parametric method available for the analysis of data from experiments (Armstrong *et al.*, 2002). It allows the user to test the differences between several different groups of treatments, thus circumventing the problem of making multiple comparisons between the group means using t-tests (Armstrong *et al.*, 2002). A linear regression analysis was performed in order to test if the slope of the dose response curves was non-zero *i.e.* if there was a linear relationship between PBDE concentration and observed effects

2.7 MODELING

2.7.1 Coupling Predator-Prey Models with Toxicological Data

In order to better understand potential effects of PBDEs on ecological relationships, a model was developed based on the mathematics of Lotka-Volterra predator-prey population dynamics explained in section 1.7.3. The model was to give an understanding of how the pattern of the two co-existing populations might be affected in the presence of PBDEs. This was achieved by running a predator-prey relationship model to simulate the predator-prey fluctuation pattern (over time), both in the absence and in the presence of a toxicant (in this case PBDE).

The following assumptions were made in developing a predator-prey toxicological model using algae (*P. subcapitata*) as prey and *Daphnia magna* as a predator:

- A fixed-area ecosystem such that population growth of the two species occurs under carrying capacity constraints.
- The predator's (in this case *Daphnia's*) only source of food is the prey population (*P. subcapitata*) that exist in the same ecosystem such that the introduction of a *Daphnia* modifies the logistic growth of *P. subcapitata* population
- No immigration or emigration of predator or prey
- PBDEs are the only chemicals/stressor in the system

Data from toxicity experiments (in section 2.2 to 2.4) were used in the modeling process in order to understand how the effects of PBDEs on parameters such as division rates, carrying

capacity, birth rates, and death rates, might eventually influence the dynamics of *D. magna- P. subcapitata* populations.

2.7.2 Modeling Environment

The research used Stella® v8.1.4 (Isee Systems Inc) as a tool for modeling this system. Stella is an icon-based software package, specifically designed for building dynamic system models. The software has a broad range of applications, including population dynamics, nutrient cycling, atmospheric pollution, economics, politics, etc. (Richmond *et al*, 1987). It uses four building blocks/components (shown in Appendix 6). The software also allows the user to define functional relationships between system components (Costanza and Vionov, 2001). Such relationships can be mathematical/algebraic, logical, graphical, or numerical constants.

Our conceptual model design (Stella version) was based on the example by Deaton and Winebrake (2000) with some modification. They used Stella® to develop a conceptual model for wolf-deer population dynamics in a park and we used a relatively similar approach to model algae-daphnid dynamics. However, since their model was a teaching material, some parameters such as carrying capacity, predation efficiency, births, and death rates were chosen arbitrarily for students to understand Stella®. Our model, on the other hand, was based on some ecological facts and experimental data.

2.7.3 Determinimg Carrying Capacity of *Daphnia* for Model Input

In order to determine the carrying capacity of *Daphnia*, it was assumed that the average biomass of each algal cell is approximately 100 pg (dry weight). This was based on the highest mass of *P. subcapitata* carbon content grown under sufficient light and nutrients (Hessen *et al.*,

2002). Thus 5 x 10^{10} cells (maximum carrying capacity of algae in this research) will contain 5 x 10^{12}pg or 5 g of biomass. The mass of *Daphnia* was considered to be 500 µg based on dry weight of *Daphnia* in control vessels in the experiment by Hosmer *et al.* (1998).

Assuming that only 10% of biomass in the primary producers is transferred to primary consumers in a lake ecosystem (Odum and Barrett, 2005) :

- 1g algae will be transferred and support 0.1g to *Daphnia*
- 5g (maximum population) will then support 0.5g *Daphnia*

  0.5 g / 500µg(mass of each *Daphnia*)

  = 1000 = number of *Daphnia* supported by 5 g algae = carrying capacity for *Daphnia*

3. RESULTS AND DISCUSSIONS

The aim of this research was to assess toxicity of PBDEs on *Pseudokirschneriella subcapitata*, *Daphnia magna,* and *Hylella azteca* in laboratory bioassays and to understand potential effects on algae-grazer population dynamics (based on predator-prey models).

3.1 EFFECTS OF PBDES ON *PSEUDOKIRSCHNERIELLA SUBCAPITATA* DIVISION RATE

Both BDE47 and BDE99 induced a dose-dependent decrease in algal division rates relative to the control. BDE47 was, however, inhibitory at lower concentrations than BDE99 (Figures 6).

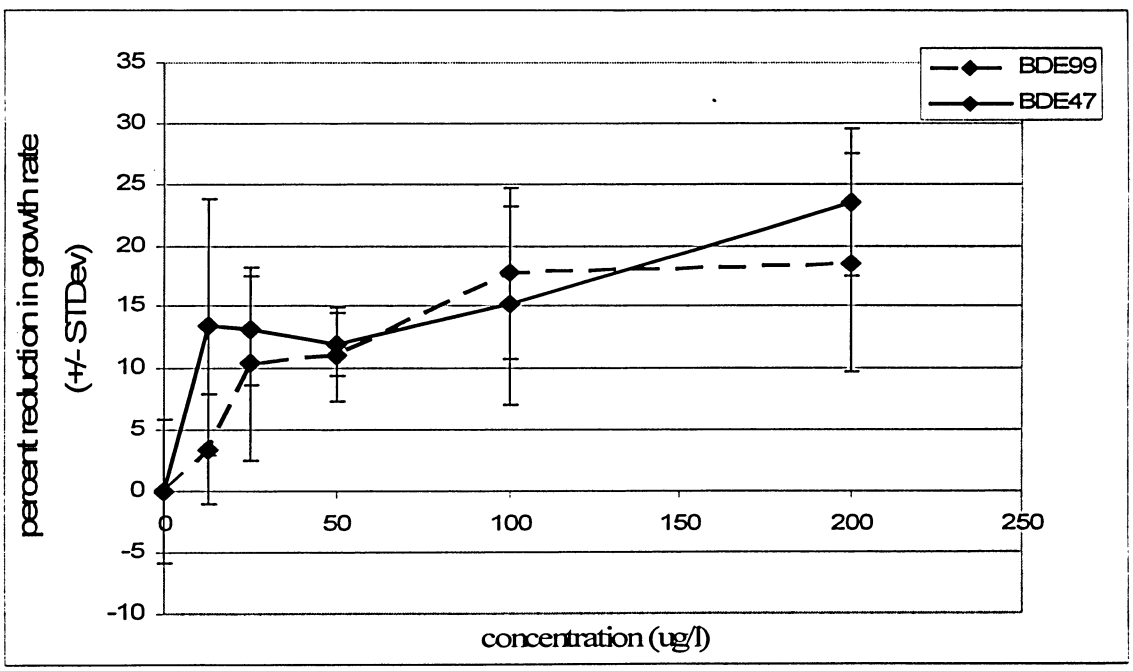


Figure 6: Percent reduction (relative to control) in Pseudokirschneriella subcapitata division rate with increasing concentrations of BDE47 and BDE99

Analysis of variance (ANOVA), performed with Fisher's least-significant difference tests, gave a pairwise comparison of the difference between means of multiple groups. The analysis showed that there was a significant statistical difference between the treatments and control for BDE47 ($F_{6,28} = 8.94$, $p < 0.001$), with significant treatment effects even at the lowest concentration (Fisher's Least Significant Difference (LSD) post-hoc test, $p = 0.014$). There was also a significant statistical difference between treatments and control for BDE99 ($F_{6,28} = 6.54$, $p < 0.001$) but this was only observed at concentrations above 100 μg/L (Fisher's LSD, $p = 0.006$ and $p = 0.014$ for 100 μg/L and 200 μg/L, respectively).

3.2 EFFECTS OF PBDES ON MAXIMUM STANDING POPULATION OF *PSEUDOKIRCHNERIELLA SUBCAPITATA*

The maximum standing population, which is the maximum density of *Pseudokirchneriella subcapitata* after 144 hours, was also measured at different concentrations of PBDEs. This parameter was measured separate from division rate because although a chemical might not slow division rate (which accounts only for the exponential phase of population growth curve), it might have an effect on the maximum number of individual algae at the stationary/equilibrium phase.

In general, the maximum standing population of algae decreased with an increase in concentrations of PBDEs (Figures 7).

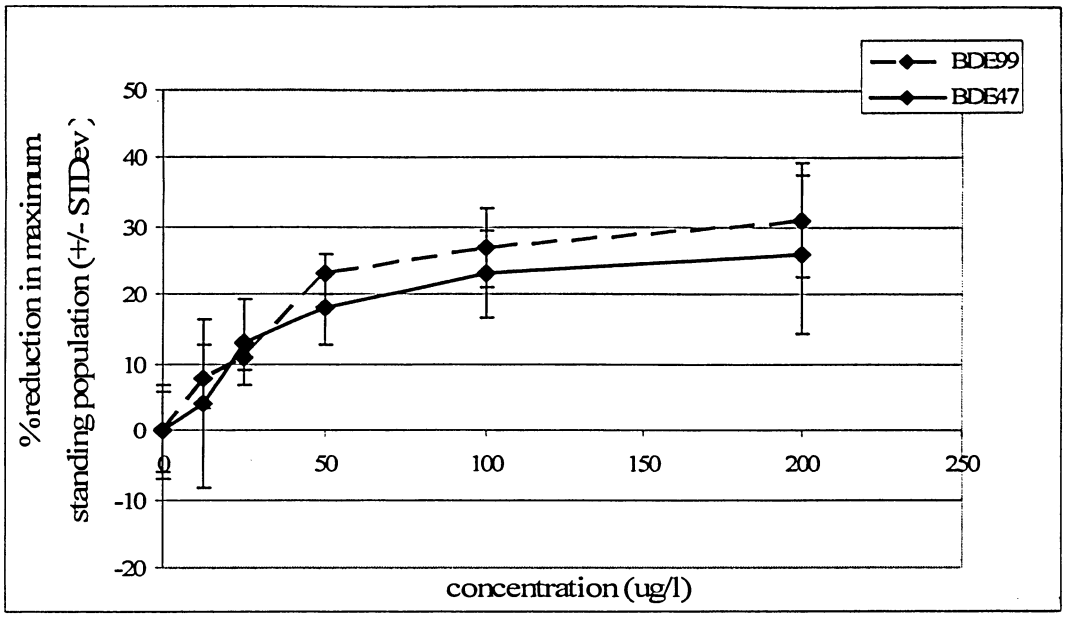


Figure 7: Percent reduction in maximum standing population (relative to control) of P. subcapitata with increasing concentrations of BDE47 and BDE99

ANOVA results showed a significant statistical difference between treatments and controls for both congeners ($F_{6,28}$=8.5, $p < 0.001$), ($F_{6,28} = 26.30$, $p < 0.001$) for BDE47 and BDE99 respectively. For BDE47 however, a significant difference was observed only at concentrations above 25μg/L. For all experiments using *P. subcapitata*, linear regression analysis showed that there was a linear relationship between PBDE concentration and observed effects (i.e. algal cell division rate, maximum standing population; $p < 0.001$ for each) (appendix 7).

In the literature, there seems to be a great inter-laboratory variation among PBDE toxicity results using *P. subcapitata*. Hellstroom (2000) reported a 10% reduction in algal division rate at a concentration of 3.3 μg/L of an unspecified congener. Evandri *et al.* (2003), on the other hand, found that BDE99 had no effect on growth of *P. subcapitata* at concentrations up to

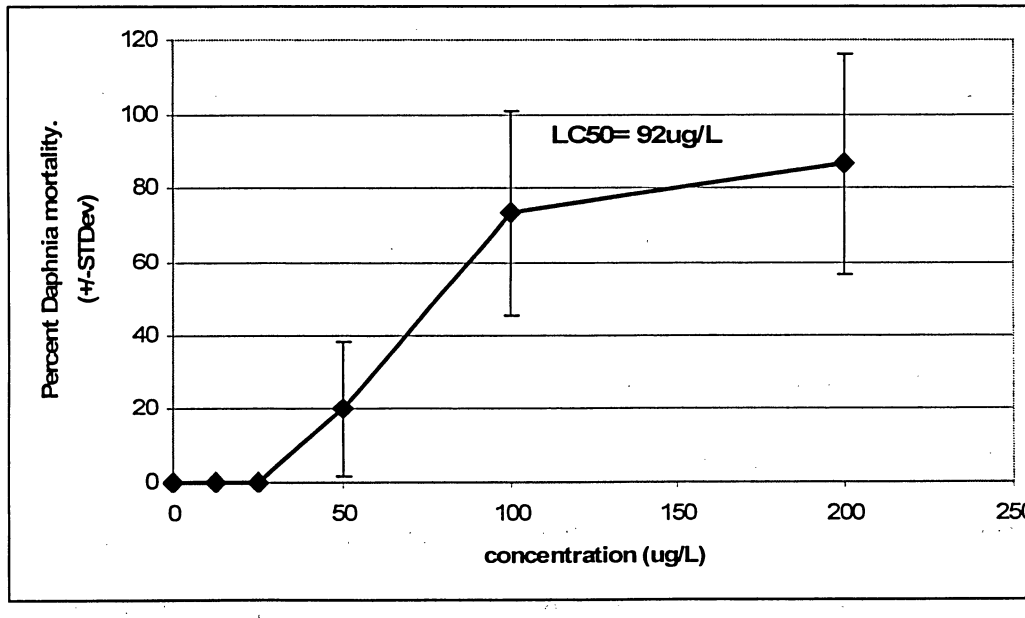
56,000 µg/L. This was the concentration at which more than 20% reduction in maximum standing crop was observed in our experiments with BDE99 (Figure 7). Test conditions for this research and Evandri *et al.* (2003) were very similar and any discrepancies observed in experimental outcomes are due to the intrinsic variabilities in biological experiments at any given time.

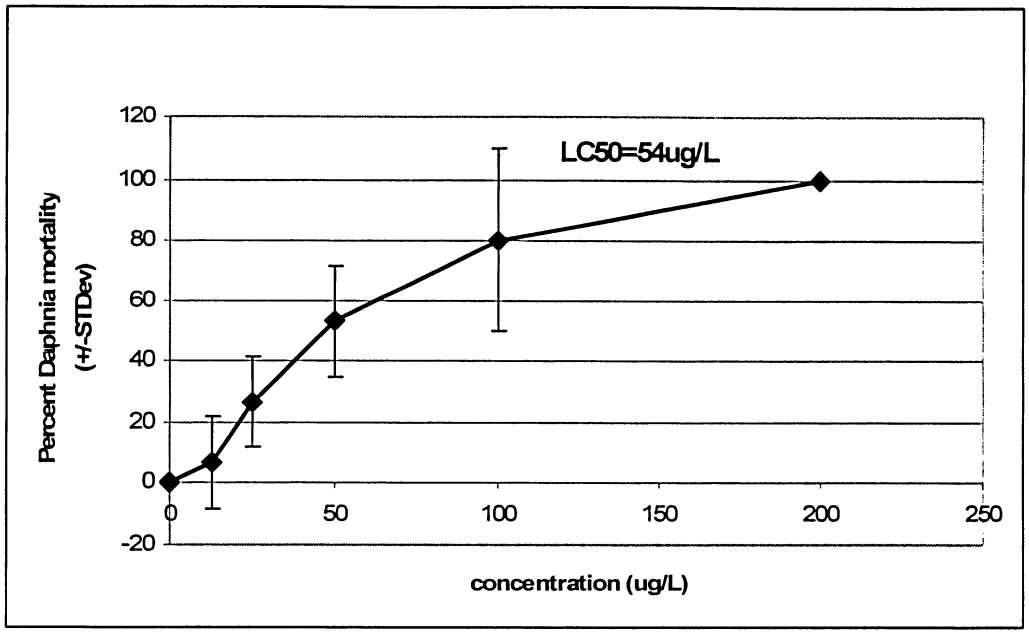
Photodegradation was not taken into account in our algal experiments given the relatively short length of the bioassay and relatively long half-lives of PBDEs in water (table 3). However, future bioassays under light conditions should consider the possibility of PBDEs to undergo photodegradation under UV radiation as suggested by Sanchez-Prado et al (2005).

3.3 ACUTE EFFECTS OF PBDES ON *DAPHNIA MAGNA*

Both PBDE congeners caused a concentration-dependent increase in *Daphnia magna* mortality (Figures 8a, b).

a)





b)

Figure 8. Dose response curve showing 48hr LC50 for BDE47(a) and BDE99(b)

ANOVA tests indicated a treatment effect for each congener ($F_{6,28} = 24.98$, $p < 0.001$ and $F_{6,28} = 30.24$, $p < 0.001$ for PBE47 and BDE99, respectively). Significant effects on mortality were observed at concentrations of 100 µg/L (Fisher's LSD, $p < 0.001$) BDE47 and as low as 25 µg/L (Fisher's LSD, $p = 0.004$) for BDE99.

The observed BDE47 LC_{50} (76 µg/L) was higher than that reported by Barman *et al.* (2004) which was 4.60 µg/L BDE47 for *Ceriodapnia dubia,* a closely related species to *Daphnia magna.* Evandri *et al.* (2003) reported a 48-hour EC_{50} of 25 µg/L for BDE99, considering number of non-motile individuals (excluding mortal individuals). While our results indicated

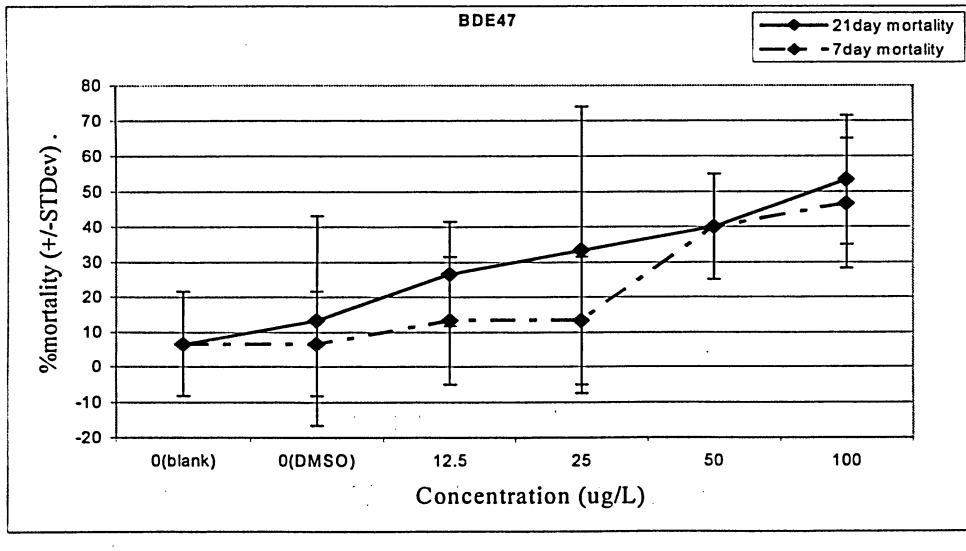
that at 25 µg/L BDE99, only 30% mortality occurred (Figure 8b), a greater percentage of individuals might have suffered other chemical-induced physiological stresses. We thus feel our results are very comparable.

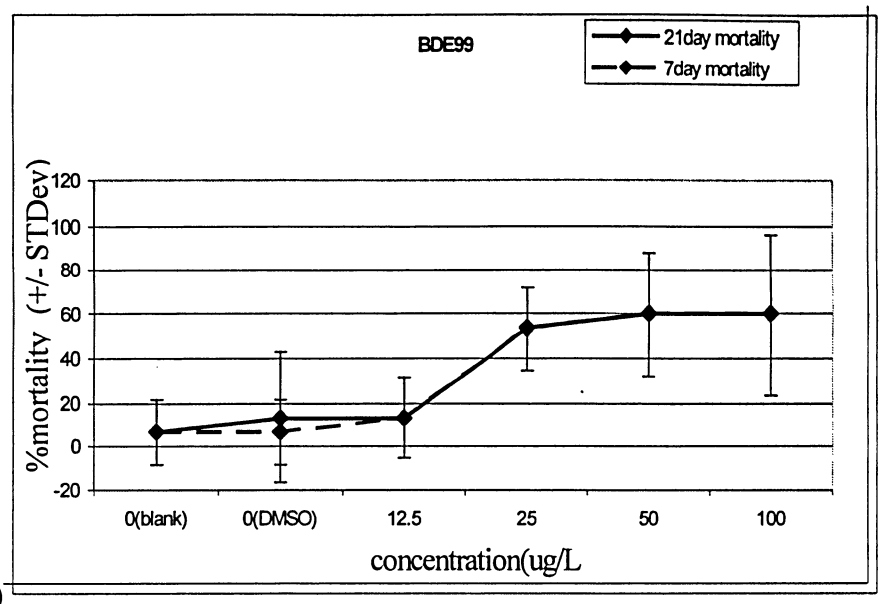
Hardy (2002), conversely, reported a 48-hour EC_{50} of 14 µg/L of a commercial pentaBDE mixture (based on slight reduction in mean body length). Lethal endpoints were not determined in their study due to limits of solubility of the product (solubility = 13.3 µg/L) and no carrier solvent was used (Hardy, 2002). The problem associated with the use of commercial mixtures such as those used by Hardy (2002) is the lack of knowledge on the actual congener that are interfering with the system or causing toxicity (Per-Ola, 2003).

3.4 CHRONIC EFFECTS OF PBDES ON *DAPHNIA MAGNA*

Chronic exposure tests showed a general increase in *Daphnia* mortality with increasing PBDE concentrations, including a marginally significant treatment effect ($F_{5,24} = 2.50$, $p = 0.059$) for BDE47 and a significant effect ($F_{5,24} = 5.13$, $p = 0.002$) for BDE99 (Figures 9a, b).

a)





b)

Figure 9: Chronic mortality of Daphnia at different concentrations of BDE47(a) and BDE99(b)

Fisher's LSD post-hoc tests showed statistically significant treatment effects at concentrations above 25 μg/L and 50 μg/L ($p<0.05$) for BDE99 and BDE47, respectively. For both acute and chronic tests and for both congeners, linear regression analysis showed that there was a linear relationship between PBDE concentration and daphnia mortality (appendix7).

Average chronic-mortality rates in the 21-day experiment were consistent with those from acute tests (Figures 9a,b). This suggests that mortality in the chronic tests was likely to happen within the first week of exposure (especially for BDE99), with survivors being resistant to further PBDE-induced mortality.

For both acute and chronic *Daphnia* mortality experiments, BDE99 showed higher toxicity to *D. magna* than BDE47. This could be attributed to the higher number of bromine atoms in BDE99 (5 bromine atoms versus 4 in BDE47), supporting the hypothesis that the toxicity of

PBDE increases with the number of bromines (Palm *et al.*, 2002). However, relative sensitivity to different congeners could be organism-specific. For example, while this research concludes that BDE99 was more toxic than BDE47 to *D. magna*, experiments by Breitholtz and Wollenberger (2003) showed that BDE47 was more inhibiting than BDE99 to larval development of the harpacticoid copepod, *Nitocra spinipes*. This shows the significance of using multiple species in assessment of environmental chemicals and that no single species is the sole universal indicator of toxicity.

3.5 REPRODUCTIVE EFFECTS OF PBDES ON *DAPHNIA MAGNA*

There was a general trend towards decrease in the number of neonates per individual adult in the presence of PBDEs at concentrations below 50 µg/L. However, neither congener had a statistically significant effect on *Daphnia* reproduction at the tested concentrations ($F_{5,24} = 1.51$, $p = 0.225$ for BDE47; $F_{5,24} = 1.22$, $p = 0.329$ for BDE99) (Figure 10).

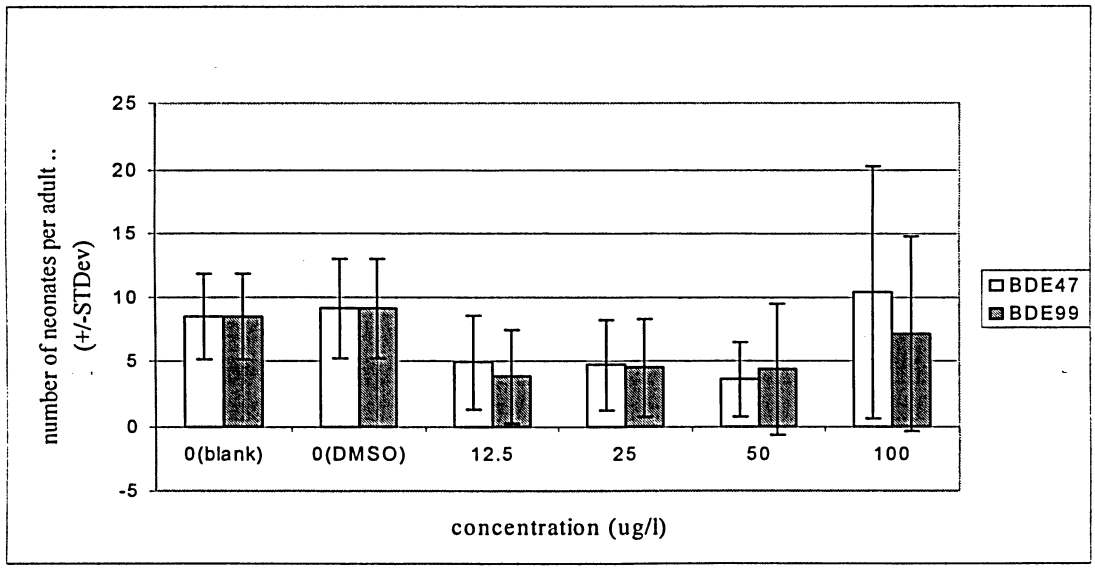


Figure 10: Total number of neonates per adult Daphnia at different BDE47and BDE99 concentrations (cumulated during 21 days).

A high number of neonates per adult was observed in the control vessels and in the highest concentration of BDE47 (100 µg/L). The latter (100ug/L treatments) was also characterised by the highest variability among replicates. All 100 µg/L replicates (both congeners) with 2 or 3 adults surviving throughout the duration of the experiment had no neonates produced. Reproduction occurred only in those vessels with 1 adult. On the other hand, controls with 2 or 3 adults were able to reproduce with success. Thus, it is suggested that future research should consider 1 adult per vessel in an attempt to obtain a clearer correlation between PBDE concentrations and reproduction. While the use of a number of adults per vessel for reproductive tests is in line with conventional protocols (OECD, 1998), the use of 1 adult per vessel will increase uniformity of both treatments and replicates throughout the duration of the experiment.

For all experiments with daphnia, observed toxicity of PBDEs might have been affected by partitioning of these compounds into food. Given the K_{ow} values of PBDEs (Table2), these compounds are likely to partition into flake food; and given a relatively slower consumption rate by daphnia, not all of the PBDEs might have been available for daphnia.

3.6 CHRONIC EFFECTS OF PBDES ON *HYALLELA AZTECA*

Neither congener had effects on *Hyallela azteca* mortality even at the highest concentration tested (200 µg/L). These results were comparable to a reported sublethal 28-day EC_{50} of > 50 mg/kg dry sediments for a commercial pentaBDE mixture (Hardy, 2003). Both studies suggest that *Hyalella* is less sensitive to tested PBDEs when mortality is a considered endpoint.

Although Environment Canada (1997) finds sensitivity of *H. azteca* to toxicants to be comparable to *D. magna*, there are some cases where *H. azteca* is actually less sensitive to toxicants than *D. magna*. For example, the evaluation of aquatic toxicity of fluoxetine (pharmaceutical antidepressant) in single species laboratory toxicity tests resulted in average LC_{50} values for *D. magna* being 820 µg/L while *H. azteca* mortality was not affected by much higher levels found in the sediment (Brooks *et al.*, 2003).

It is however acknowledged that, although *Hyalella* may exhibit less sensitivity than *Daphnia* to some PBDEs in terms of lethality, the organism might have experienced sublethal effects at concentrations lower than those required to cause death. Future research should attempt to monitor sublethal endpoints such as growth and reproductive fecundity and develop EC_{50s} using pure PBDE compounds.

In general, our results of toxicity correlate well with literature values (Hardy, 2002; Evandri et al., 2003). However, since the PBDE congeners used for this study are shipped in a mixture of toluene and nonane, and that, for technical reasons (peak overlapping), our experiments did not test for nonane and toluene in DMSO solution prior to bioassays, the possibility of potential contamination with the initial carrier was suspected. Thus a test was performed by evaporating nonane and toluene using nitrogen gas stripping as in section 2.1 and re-dissolving the sample in hexane for proper separation and analysis with gas chromatography. Redissolution with hexane was due to the forth-mentioned difficulties in chromatographic separation of nonane from DMSO, hence hexane was used as a solvent for nonane and toluene analysis. The results showed the presence of nonane and toluene in the hexane solution. Therefore, absolute values of toxicity in this research should be taken with caution. However, this observation does not

affect the significance of predator-prey system modeling. Modeling of predator-prey system with species of different sensitivity to chemicals proved to be reliable, interesting and necessary. This research also recommend alternative sources of PBDEs for toxicity research *i.e.* custom-build (synthesized and prepared) PBDE standards in DMSO.

3.7 ENVIRONMENTAL CONCENTRATIONS VERSUS TOXICITY

Currently reported concentrations of PBDEs in the aquatic environment (in particular surface water) are usually lower than those that were found to cause toxic effects to some of the most sensitive species (such as those used in this research). PBDE levels in the water columns of most aquatic systems are usually in pica grams per liter (Environment Canada, 2004), while reported EC_{50s} are in the order of micrograms per liter. However, sediment concentrations have already reached hundreds of micrograms per kg (Hale *et a.l*, 2003). This might pose a higher potential for exposure to benthic organisms. Although water-column concentrations are still relatively low, it is their increasing trend that is of concern. PBDE concentrations are increasing at such a rate that, with no intervention, toxic levels will soon be reached.

It is also important to note that environmental concentrations from one medium may not serve as a true surrogate measure of the actual amount of chemicals that enters the body of living organisms. Although concentrations in water, for example, may be relatively low, significant exposures may also occur during feeding and may cause adverse effects independent of environmental concentrations. For example, when *Daphnia* were fed with algae that were treated with different BDE99 concentrations (5 and 100 μM), there was a dose-dependent decrease in survival and impairment of reproduction (Evandri *et al.*, 2003). Effects of PBDE-

contaminated algae, when fed to *Daphnia,* were observed at concentrations that were found not to be toxic to algae themselves (Evandri *et a.l*, 2003). This shows that although PBDEs may not be as toxic to algae as they are to *Daphnia*, surviving algae pose a source of exposure to other organisms through feeding. Food chain exposures are even more relevant for PBDEs because they are able to bio-concentrate. Bio-concentration factors of 35,000x have been reported for BDE47 in laboratory-based studies using Japanese carp (Hardy, 2003).

Suspended particulate matter also constitute yet another source of PBDE exposures in the water column, adding to water concentrations. De Boer *et al* (2002) suggests that suspended particulate matter (SPM) is an important carrier for brominated diphenyl ethers in the aquatic environment. PBDEs in SPM samples from various locations in the Netherlands were detected at concentrations up to 4,600 µg/kg dry weight (De Boer *et al,* 2002).

3.7 EFFECTS OF PBDES ON PREDATOR-PREY RELATIONSHIPS

3.7.1 Conceptualization and Model Input

Stella® (Isee Systems Inc.) was used as a tool to model *P. subcapitata* and *D. magna* population dynamics when organisms were exposed to varying levels of BDE99. PBDE 99 was chosen for modeling impacts because it is the congener that is the main constituent of the PentaBDE mixture; a mixture whose components are most common in the environment. Model output included the standing populations of *P. subcapitata* (PN, cells/L) and *D. magna* (DN, *Daphnia*/L) at a given time.Model inputs were based on laboratory experiments to determine PBDE effects on *P. subcapitata* cell division rate and maximum standing population and *D.*

magna mortality and reproduction (described above). Time steps for the model were 1 day. The model is diagrammatically presented in Figure 11.

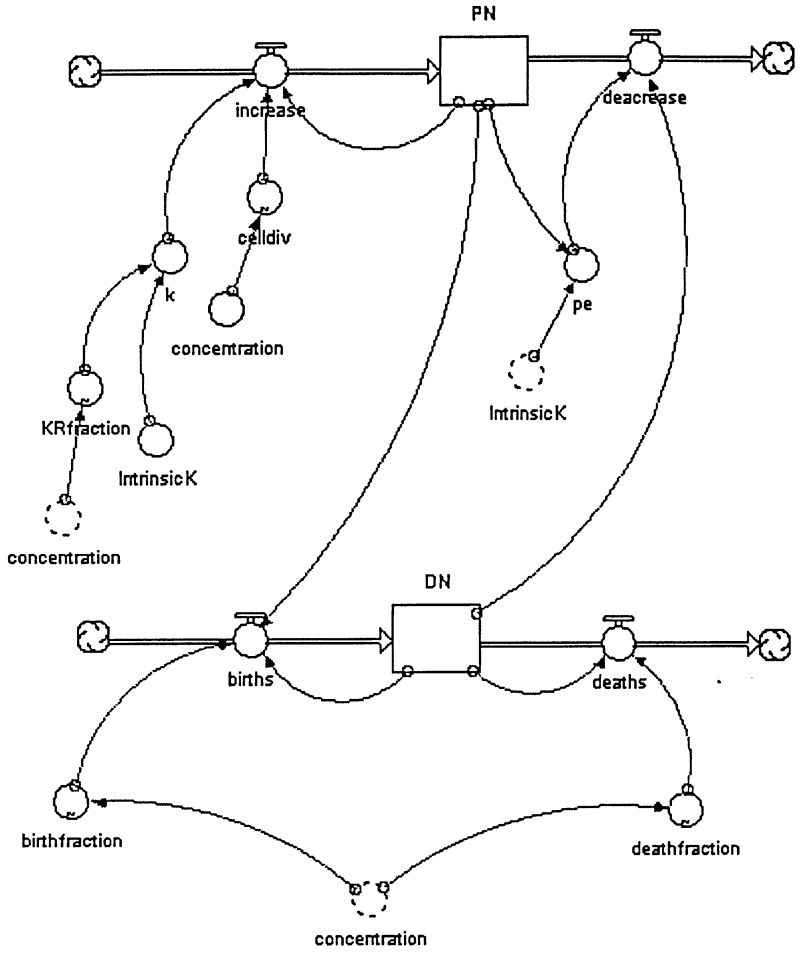


Figure 11. Conceptual model for a grazing (predator-prey) relationship between P. subcapitata and D. magna.

The model follows the general Lotka-Voltera equations for the population of the two species given by the equation A and B for the prey and predator respectively.

dN/dT = Nr (K- Nr)/K .....[eq. A]

dN/dT = Nr (K-Nr)/K – PeN.....[eq. B]

BDE99 data was used as an example contaminant (output data for BDE47 will be shown in appendix 8). Variables for this model were defined as follows in units of individuals of the two species:

***A) Pseudokirchneriella subcapitata* (the prey)**

The prey population size (PN) at any given time is the difference between the number of individuals algal cells produced (increase) and number of cells consumed by *Daphnia* (decrease). It was defined as:

$$PN = Increase - Decrease \quad \textbf{[eq.1]}$$

Where,

"**Increase**" in *P. subcapitata* population each day (cells/L per day) is given by the equation:

$$Increase = PN \times celldiv \times (K-PN)/K \quad \textbf{[eq. 2]}$$

Celldiv = the rate of division (day^{-1}) as a function of PBDE concentration, determined by division rate experiments (Figure 6b).

K denotes carrying capacity or maximum standing population of *P. subcapitata* population at a given concentration of a chemical. It is a product of intrinsic carrying capacity (IntrinsicK) and the fraction by which the intrinsicK is reduced (KRfraction)

$$K = Krfraction \times IntrinsicK \quad \textbf{[eq. 3]}$$

KRfraction is the fraction by which the carrying capacity (IntrinsicK) is reduced by a given concentration of PBDE relative to the control. It was therefore computed as the maximum standing population in each treatment in the standing population experiments, divided by the maximum standing population in the control. As such, KRfraction became a graphical function of concentration as shown in figure 12 below.

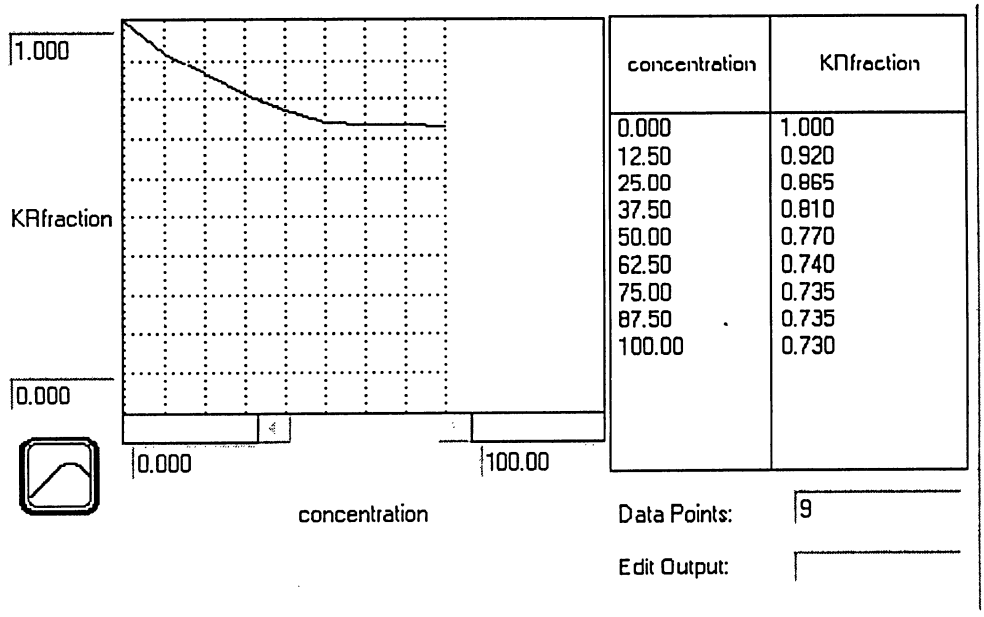


Figure 12: KR fraction as a function of PBDE concentration

IntrinsicK is a constant (5 x 10^{10}cells/L) which denotes carrying capacity for *P. subcapitata* based on the maximum standing population in control vessels of bioassays. The fraction at which IntrinsicK is affected by the chemical is defined by KRfraction.

'**Decrease**' in *P. subcapitata* population each day (cells/L per day) was only due to *Daphnia* grazing.

$$Decrease = DN \times Ge \qquad \textbf{[eq. 4]}$$

Ge (grazing efficiency) is the efficiency of *D. magna* to prey on *P. subcapitata.* According to section 2.7.3., maximum number of *P. subcapitata* in the system (5×10^{10}) would support 1000 *Daphnia.* This means that 1 daphnid will prey and be supported by (5×10^{10} / 1000) or 5×10^{7} units of *P. subcapitata* at a given time. Thus, the intrinsic Ge is 5×10^{7} cells per *Daphnia.* However, Ge is a function of algal population size in the sense that as population size of *P. subcapitata* is at its maximum intrinsic carrying capacity (enough food), *Daphnia* become effective in grazing and their efficiency will decrease as the population of *P. subcapitata* approaches zero. Hence, GE is defined according to the equation:

$$Ge = 5 \times 10^{7} (PN/IntrinsicK). \qquad \textbf{[eq. 5]}$$

B) *Daphnia magna* (the predator)

The predator/grazer population size (DN) at any given time is the difference between the number of births and number of deaths of *Daphnia* in the system. It was defined as

$$DN = births - deaths \qquad \textbf{[eq. 6]}$$

Where,

births (individuals/day) is given by the equation:

$$births = (birthfraction/21) \times DN \times (PN/5x10^{9}) \times (1000-DN)/1000 \qquad \textbf{[eq. 7]}$$

Birth fraction (defined below) is divided by 21 to obtain births per *Daphnia* per day (duration of the reproductive toxicity experiment was 21 days). The factor DN (1000-DN)/1000 is a logistic growth term for *Daphnia* (see section 2.7.3). SN/5x10^9 denotes the dependence of *Daphnia* growth on *P. subcapitata* population size based on carbon transfer between trophic levels. A population of *P. subcapitata* of 5 x 10^{10} cells, assuming a trophic transfer efficiency of 10%, can support the production of 1,000 *Daphnia* (see section 2.7.3). The fraction transferred decrease as the algal population approaches zero because in the absence of algae, there would be no growth of *Daphnia.*

birthfraction denotes the number of births per *Daphnia* recorded during 21-day reproductive toxicity experiments (Figure 10).

deaths (individuals/day) denotes the number of Daphnids dying per day.

$$deaths = deathfraction \times DN \qquad \textbf{[eq. 8]}$$

deathfraction is the percentage of deaths recorded during the 21-days chronic toxicity experiment. It is a function of concentration as shown figure 9.

3.7.2 Model Output

When the model was run at different concentrations of PBDEs (0, 12.5, 25, 50 and 100µg/L), the following observations were made.

Pseudokirchneriella subcapitata

In the absence of a contaminant, algae population (PN) oscillates between ~ 1 x 10^9 and 1 x 10^{10} cells/L, never approaching intrinsic carrying capacity (~ 5 x 10^{10}). At a PBDE concentration of 12.5µg/L, the magnitude of the oscillations was initially greater (oscillations between ~ 1 x 10^{10} and 2.5 x 10^{10}) with shorter periodicity and greater damping in the oscillations through time. At PBDE concentrations above 25µg/L, periodicity of oscillations was much shorter still and damping of oscillations was stronger, with populations reaching fairly stable levels 1.5 - 2.0 x 10^{10} cells (Figure 13a).

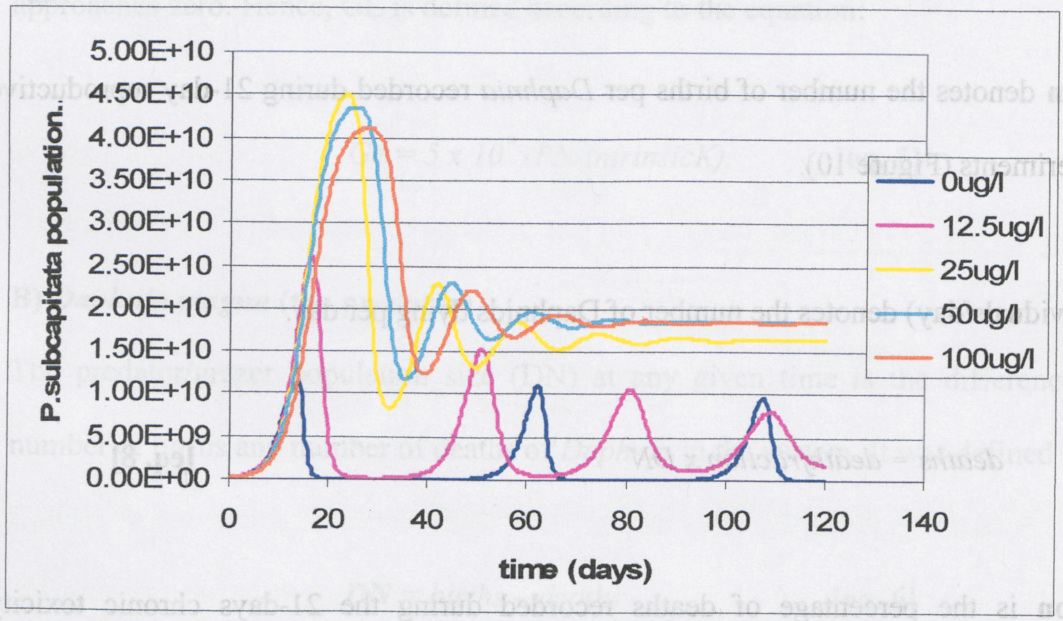


Figure 13a : Population dynamics of P. subcapitata in a predator- prey system at different BDE99 concentrations

The increase in algal standing population with increasing PBDE concentration might have some implications in a lake ecosystem. High algal densities are known to induce the probability of a lake to become more productive and eutrophic/ nutrient-rich; and according to

Odum and Barrett (2005), high-nutrient natural environments increase productivity and decrease species diversity due to increased dominance. Nutrient-rich lakes are also less preferable from the stand point of water quality for domestic use and recreation.

Daphnia magna

In the absence of a contaminant, daphnid populations oscillate between 5 and 180 individuals, never approaching intrinsic carrying capacity (1000). At PBDE concentrations above 25µg/L, the *Daphnia* population oscillates between lower population densities (20-50 individuals), with populations reaching fairly stable levels at around 30 individuals (Figure 13b).

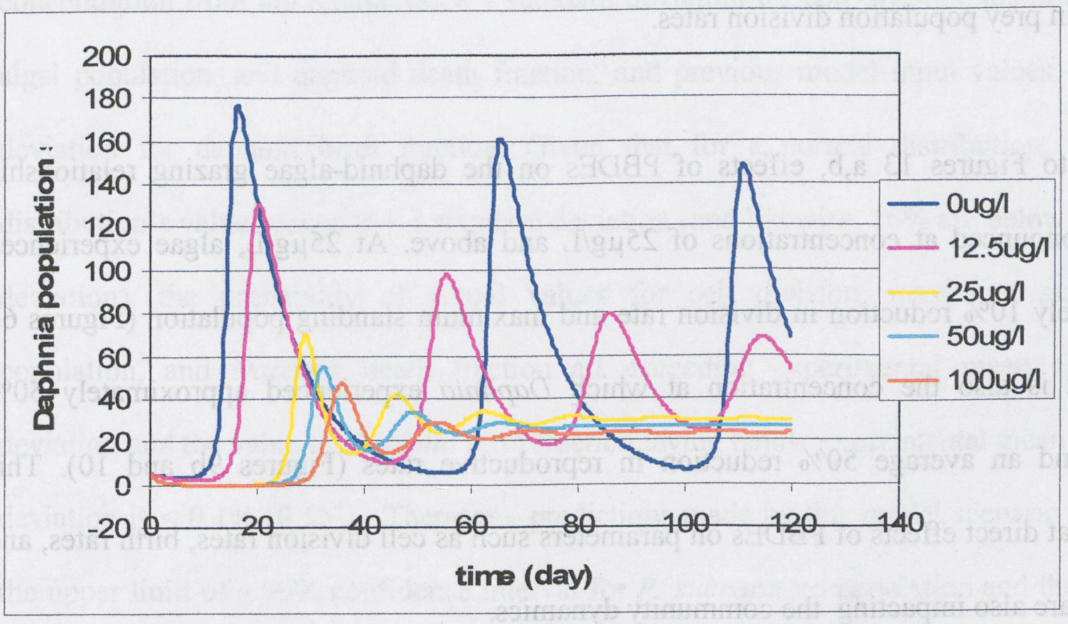


Figure 13b : *Population dynamics of Daphnia magna in a predator-prey system at different BDE99 concentrations*

In the natural environment, *Daphnia magna* is not only an algal predator, but it is a source of food for fish and other zooplankton. Thus, a decrease in the *Daphnia* population will not only

increase phytoplankton productivity but will also affect the populations of those species that feed on it.

In general, contrary to the bioassay observations, there was a general trend towards an increase in the algal population with an increase in PBDE concentrations (Figure 13a), corresponding to a disproportional decrease in the daphnid population (Figure 13b). This suggests that the *Daphnia* population suffered more from contaminant impact than did the algal population. Thus, there were few predators, allowing the algae population to grow. Differential reproductive toxicity between *Daphnia* and algae resulted in the release of prey populations from predation pressure, leading to higher prey population densities, despite toxicant-induced reductions in prey population division rates.

According to Figures 13 a,b, effects of PBDEs on the daphnid-algae grazing relationship become pronounced at concentrations of 25µg/L and above. At 25µg/L, algae experienced approximately 10% reduction in division rate and maximum standing population (Figures 6b and 7b). It is also the concentration at which *Daphnia* experienced approximately 50% mortality and an average 50% reduction in reproductive rates (Figures 9b and 10). This confirms that direct effects of PBDEs on parameters such as cell division rates, birth rates, and death rates are also impacting the community dynamics.

Looking at the algal population; while our single-species tests predicted that PBDEs would decrease algal biomass, when effects on *Daphnia* are also considered, it appears more likely that PBDEs will increase algal biomass as they will have a disproportionate effect on *Daphnia*.

This indicates that, while conventional single-species toxicity tests are the fulcra to which ecotoxicology is pivoted, the environmental significance of those tests might be difficult to glean, as organisms exist and interact with other organisms. Only when studied in an ecological context can we begin to predict the consequences of environmental pollutants such as PBDEs.

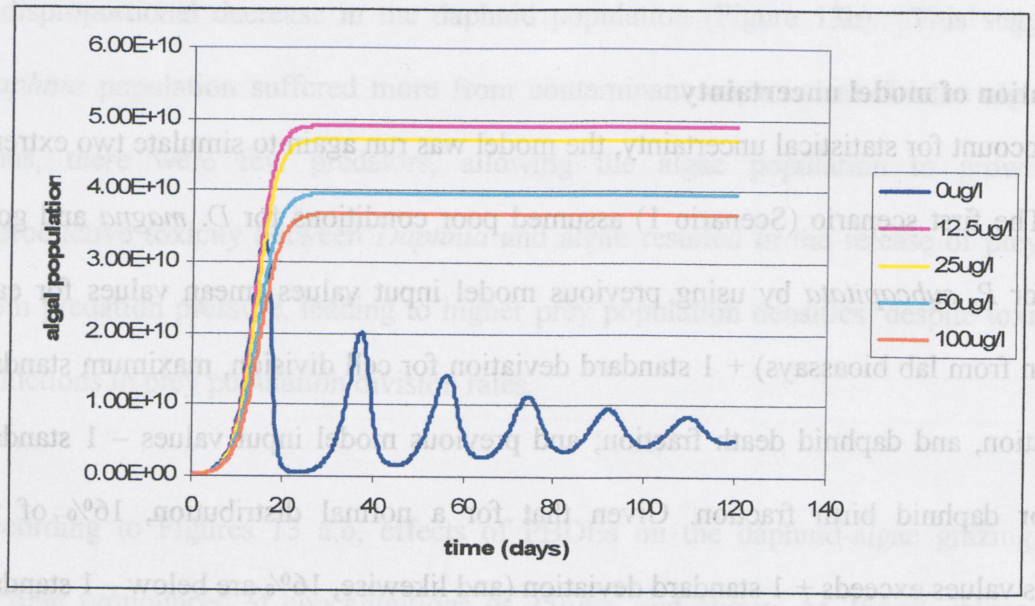
3.7.3 Estimation of model uncertainty

In order to account for statistical uncertainty, the model was run again to simulate two extreme scenarios. The first scenario (Scenario 1) assumed poor conditions for *D. magna* and good conditions for *P. subcapitata* by using previous model input values (mean values for each concentration from lab bioassays) + 1 standard deviation for cell division, maximum standing algal population, and daphnid death fraction; and previous model input values – 1 standard deviation for daphnid birth fraction. Given that for a normal distribution, 16% of the distribution's values exceeds + 1 standard deviation (and likewise, 16% are below – 1 standard deviation), the probability of actual values for cell division, maximum standing algal population, and *Daphnia* death fraction all exceeding experimental mean + 1 standard deviation and the value of *Daphnia* birth fraction laying below experimental mean – 1 standard deviation is < 0.1% (0.16^4). Therefore, predictions made by this model scenario approximate the upper limit of a 99% confidence interval for *P. subcapitata* population and the lower limit at a 99% confidence interval for *D. magna.* The second scenario (Scenario 2) was the direct opposite of Scenario 1 with one exception - death rate. It assumed poor conditions for *P. subcapitata* and good conditions for *D. magna* by using mean values - 1 standard deviation for cell division, maximum standing algal population; + 1 standard deviation for *Daphnia* birth fraction; and the mean value for daphnid death fraction. The mean value for death fraction was

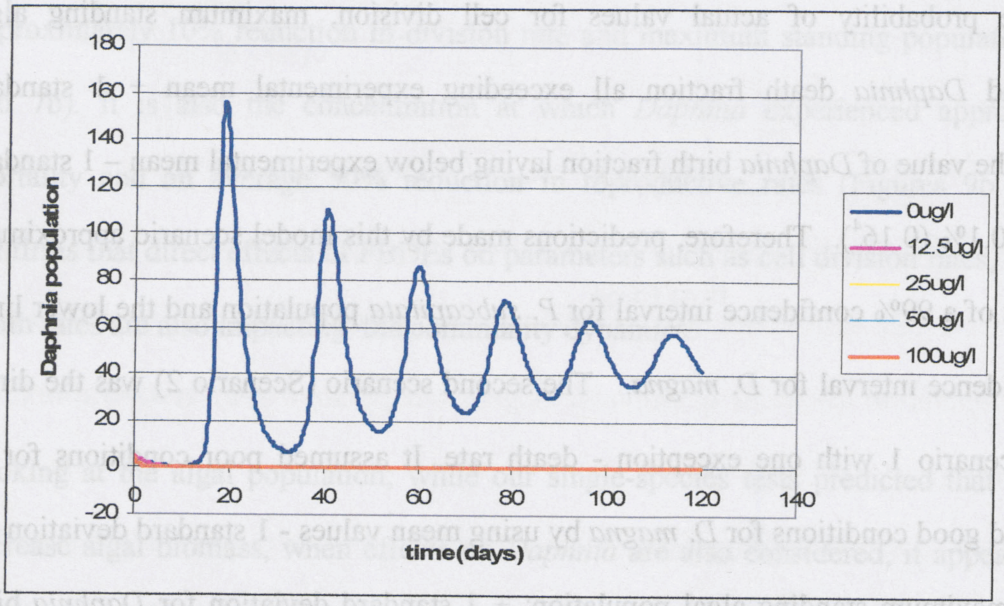
considered (instead of mean -1 standard deviation) for Scenario 2 because mean -1 standard deviation for death fraction is zero at 0µg/L, which is not an environmentally relevant assumption.

The following results were obtained:

Scenario 1



a)



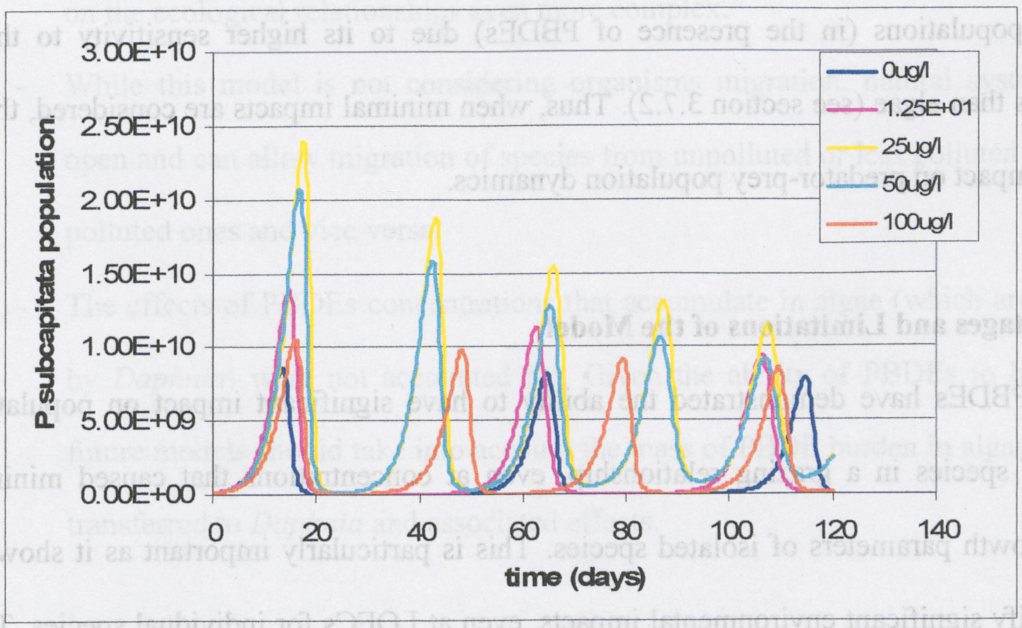
b)

Figure 14: Scenario 1 growth pattern for algae and Daphnia in a grazing relationship

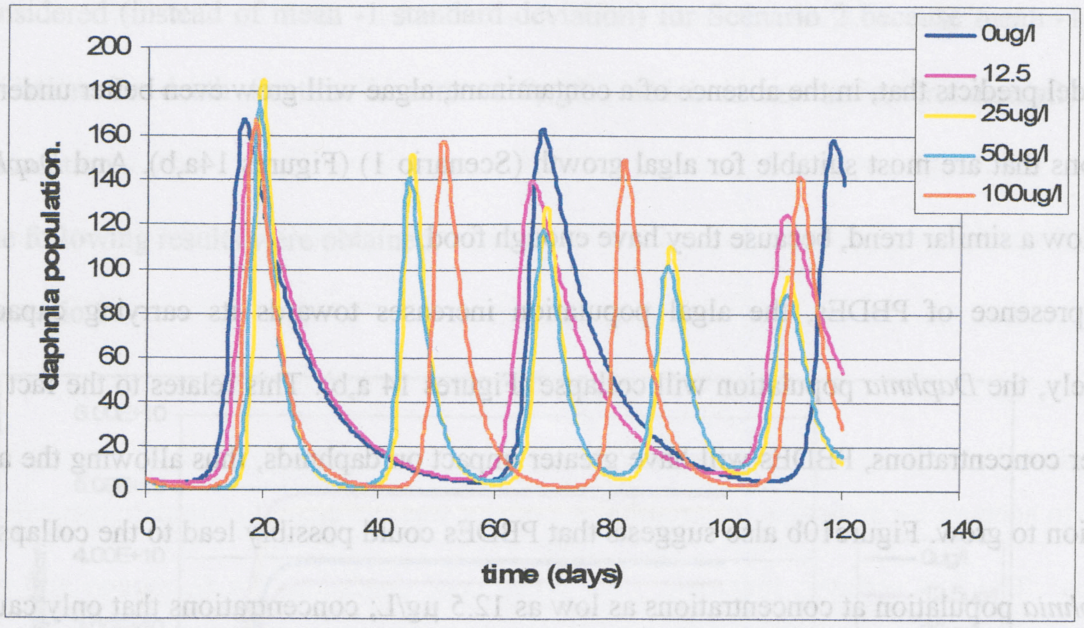
Our model predicts that, in the absence of a contaminant, algae will grow even better under the conditions that are most suitable for algal growth (Scenario 1) (Figures 14a,b). And *Daphnia* will follow a similar trend, because they have enough food.

In the presence of PBDEs, the algal population increases towards its carrying capacity; conversely, the *Daphnia* population will collapse (Figures 14 a,b). This relates to the fact that at higher concentrations, PBDEs will have greater impact on daphnids, thus allowing the algal population to grow. Figure10b also suggests that PBDEs could possibly lead to the collapse in the *Daphnia* population at concentrations as low as 12.5 µg/L; concentrations that only caused reproductive effects and only 10% mortality. It is therefore possible that adverse effect on ecological relationships such as predator-prey interactions can lead to local extinction of a species population at concentrations lower than conventional EC50s.

Scenario 2



a)



b)

Figure 15: Scenario 2 growth pattern for algae and Daphnia in a grazing relationship

The second scenario (Scenario 2) shows no significant PBDE impact on population dynamics of the two species at any concentration. This is because Scenario 2 assumed the most minimal impact on *Daphnia* by PBDEs. Accordingly, *Daphnia* have greater influence on the dynamics of the two populations (in the presence of PBDEs) due to its higher sensitivity to these contaminants than algae (see section 3.7.2). Thus, when minimal impacts are considered, there is minimal impact on predator-prey population dynamics.

3.7.3 Advantages and Limitations of the Model

In general, PBDEs have demonstrated the ability to have significant impact on population dynamics of species in a grazing relationship, even at concentrations that caused minimal effects in growth parameters of isolated species. This is particularly important as it shows a way to identify significant environmental impacts, even at LOECs for individual species. This

is very relevant for today's type of diffuse pollution caused by a plethora of emerging contaminants. This research therefore finds it very appropriate to consider effects on ecological relationships as toxicity endpoints in assessment of risks associated with various chemicals found in the environment.

The research provided a basic model for use in understanding potential effects of PBDEs on predator-prey dynamics. The model might also be used to asses other chemicals based on their inherent toxicological characteristics. Like any mathematical modeling of biological processes however, the interpretation of associated results should be tempered with the understanding that it is a simplified image of reality. Natural systems are too complex to allow complete simulations.

The following facts about natural systems might affect the representativeness of our model.

- The model assumed constant concentrations over time. In nature, levels of chemicals are subject to long- and short-term temporal and spatial variations, making the effects on the ecological relationships even more complex.
- While this model is not considering organisms migration, natural systems are more open and can allow migration of species from unpolluted or less polluted areas to more polluted ones and vice versa.
- The effects of PBDEs concentrations that accumulate in algae (which are preyed upon by *Daphnia*) were not accounted for. Given the ability of PBDEs to bioaccumulate, future models should take into account the mass of PBDE burden in algae that could be transferred to *Daphnia* and associated effects.

- Our model used two species. Natural systems on the other hand are characterized by more complex foodwebs, where the impact of contaminants might have effects on not only one, but multiple predator-multiple prey and on competition relationships.

4. CONCLUSIONS AND RECOMMENDATIONS

This study was able to give an understanding of toxicity of PBDEs on individual species and on grazing relationships. There is an indication that PBDEs are potentially toxic to aquatic species at ppb levels. However, observed toxicity might be due to a combination of effects of PBDE themselves and solvents in which they were initially present. Although current environmental concentrations are significantly lower than those that have been seen to cause toxicity, organisms may be exposed to high levels through feeding because of the ability of PBDEs to bioconcentrate. Also, with environmental concentrations of these compounds increasing exponentially, action against their release might be a necessity.

This study also indicated that effects of PBDEs on individual species are likely to affect the dynamics of two populations in a grazing relationship. Thus suggesting that conventional single-species toxicity tests alone might not give a broad indication of chemical impact, but only when studied in an ecological context can we begin to predict the consequences of environmental pollutants such as PBDEs.

Future modeling of PBDE effect on predator-prey relationships may use *Hyalella* as a predator to see how the system-response will change in cases where the prey is suffering more from chemical effect than the predator. Future models can also include multiple (more than just two) species, forming a foodweb, in order to enhance the ecological relevance of the model.

Europen countries have already placed a ban on the use of PBDEs and their actions are already paying off- PBDE levels in the European environment and humans are decreasing. North America, on the other hand, is relying largely on voluntary phase-out by industries, which is a

relatively passive measure. This research therefore suggests that North American countries should follow the European lead and take active action against PBDE release.

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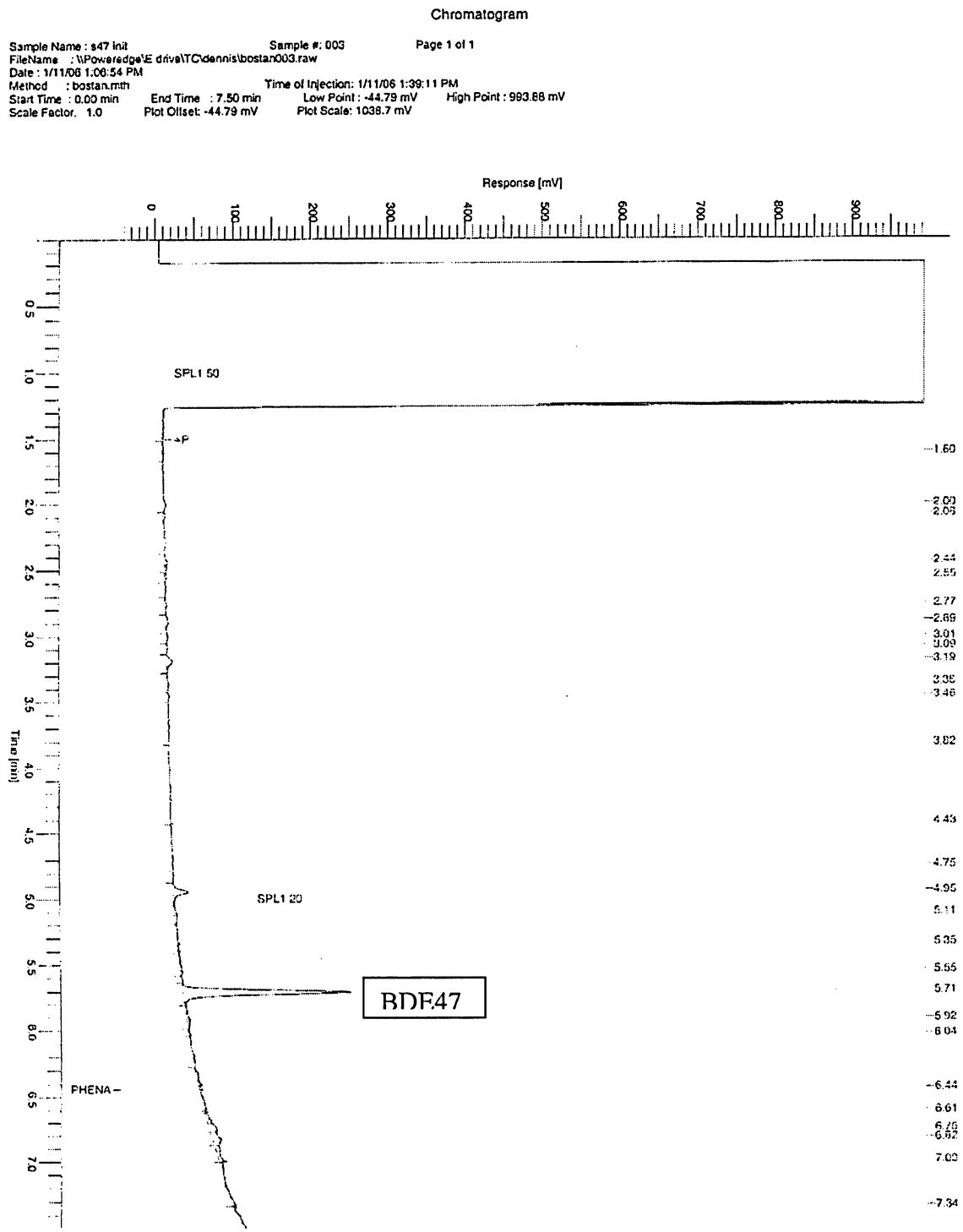
6. APPENDICES

Appendix 1: PBDE numenclature

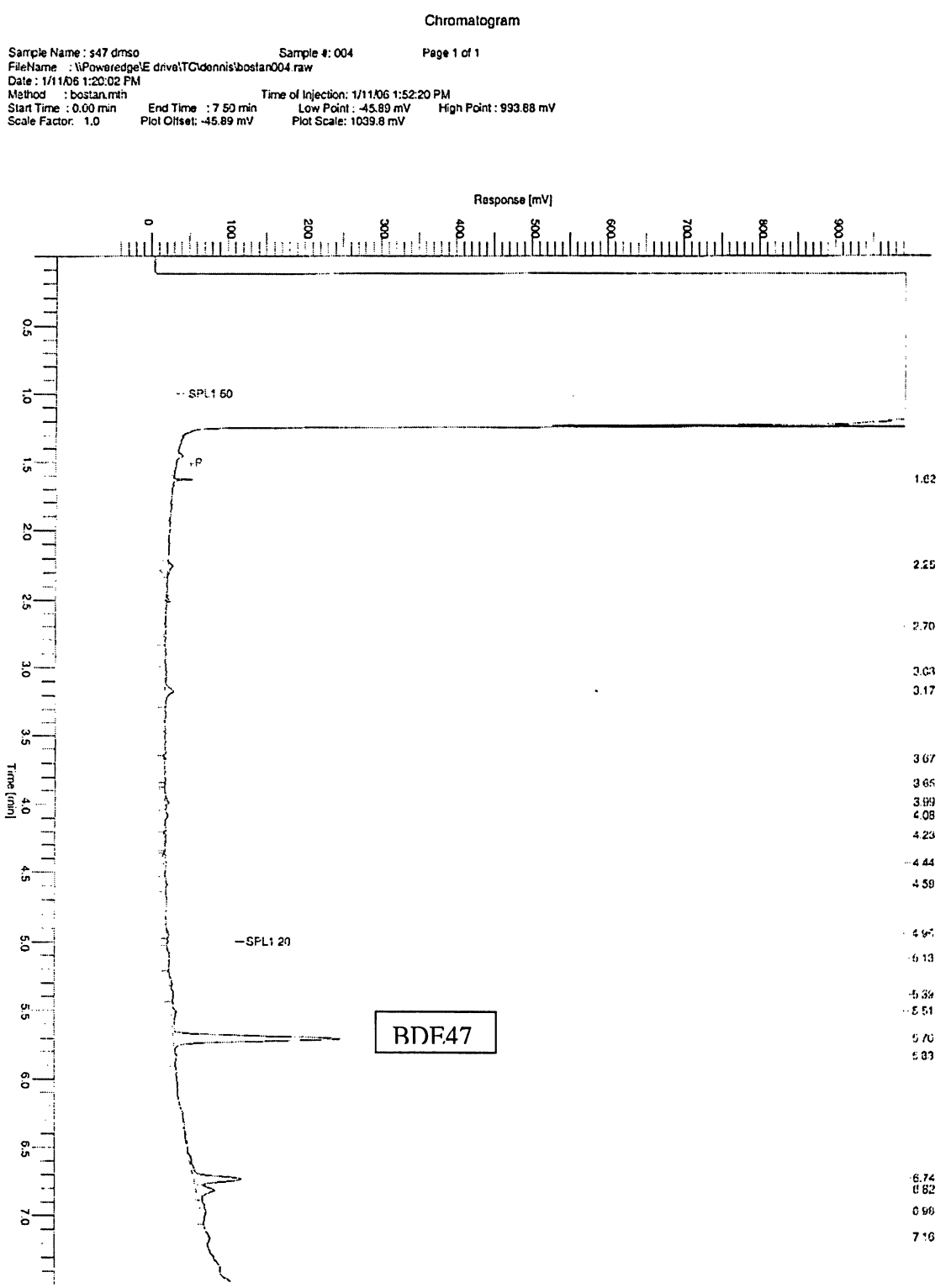
The 209 possible PBDE congeners are identified in the International Union of Pure and Applied Chemistry (IUPAC) numbering system, which arranges them in ascending numerical order of the degree of bromination. For example, BDE-99 is 2,2',4,4',5-Penta-BDE. Example is in the table below.

IUPAC NUMBER	PBDE Congener
BDE-15	4,4'-Di-BDE
BDE-17	2,2',4-Tri-BDE
BDE-25	2,3',4-Tri-BDE
BDE-28	2,4,4-Tri-BDE
BDE-30	2,4,6-Tri-BDE
BDE-33	2',3,4'-Tri-BDE
BDE-47	2,2',4,4'-Tetra-BDE
BDE-49	2,2',4,5'-Tetra-BDE
BDE-51	2,2',4,6'-Tetra-BDE
BDE-66	2,3',4,4'-Tetra-BDE
BDE-71	2,3',4',6-Tetra-BDE
BDE-75	2,4,4',6-Tetra-BDE
BDE-77	3,3',4,4'-Tetra-BDE
BDE-79	3,3',4,5'-Tetra-BDE
BDE-99	2,2',4,4',5-Penta-BDE
BDE-100	2,2',4',4',6-Penta-BDE
BDE-119	2,3',4,4',6-Penta-BDE
BDE-138	2,2',3,4,4',5'-Hexa-BDE
BDE-140	2,2',3,4,4',6-Hexa-BDE
BDE-153	2,2',4,4',5,5'-Hexa-BDE
BDE-154	2,2',4,4',5,6'-Hexa-BDE
BDE-155	2,2',4,4',6,6'-Hexa-BDE
BDE-166	2,3,4,4',5,6'-Hexa-BDE
BDE-180	2,2',3,4,4',5,5'-Hepta-BDE
BDE-183	2,2',3,4,4',5',6-Hepta-BDE
BDE-190	2,3,3',4,4',5,6-Hepta-BDE
BDE-203	2,2',3,4,4',5,5',6-Octa-BDE
BDE-209	2,2',3,3',4,4',5,5',6,6'-Deca-BDE

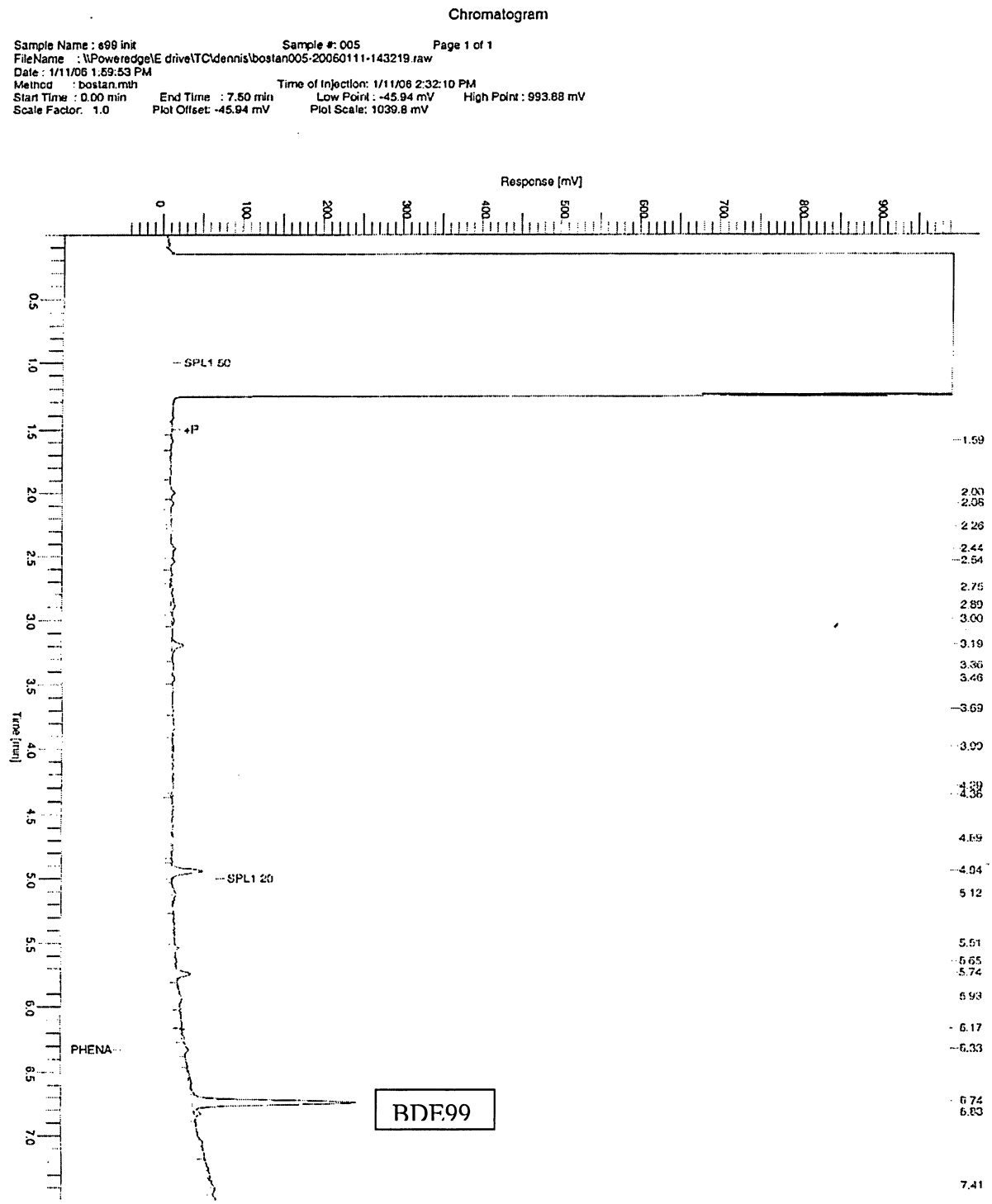
***Appendix 2**: chemical analysis: Presence of PBDEs before and after re-dissolving the stock solution*



PBDE47 in the original solvent (nonane)



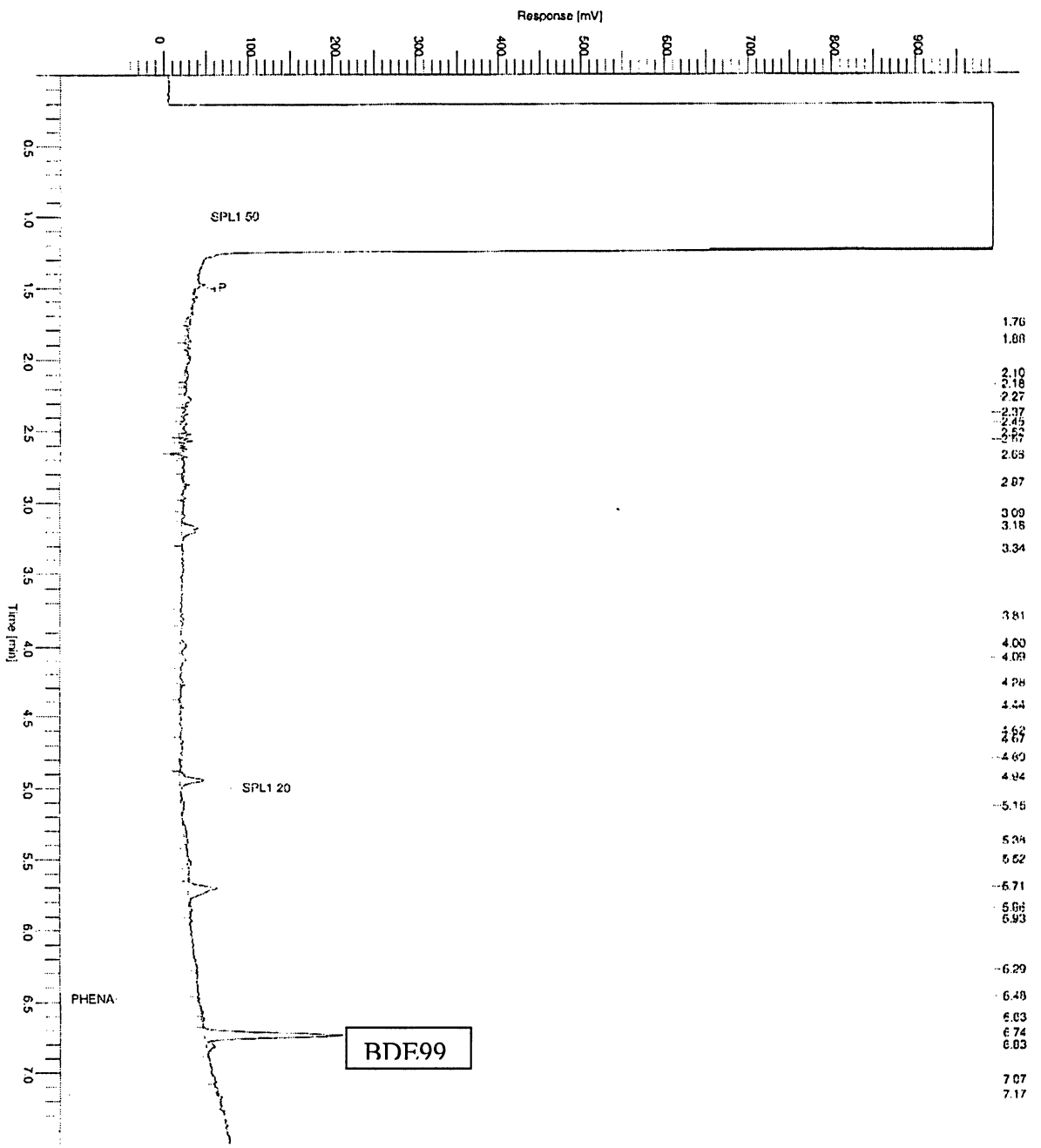
BDE47 in DMSO



BDE 99 in nonane and toluene

Chromatogram

Sample Name : s99 dmso Sample #: 006 Page 1 of 1
FileName : \\Poweredge\E drive\TC\dennis\bostan006.raw
Date : 1/11/06 1:46:09 PM
Method . bostan.mth Time of Injection: 1/11/06 2:18:27 PM
Start Time : 0.00 min End Time : 7.50 min Low Point : -44.57 mV High Point : 993.88 mV
Scale Factor: 1.0 Plot Offset: -44.57 mV Plot Scale: 1038.4 mV



BDE99 in DMSO

Appendix 3: Pseudokirchneriella subcapitata growth media

TABLE 1. NUTRIENT STOCK SOLUTIONS FOR MAINTAINING ALGAL STOCK CULTURES AND TEST CONTROL CULTURES

STOCK SOLUTION	COMPOUND	AMOUNT DISSOLVED IN 500 mL MILLI-Q[k] WATER	
1.	MACRONUTRIENTS		
A.	$MgCl_2 \cdot 6H_2O$	6.08	g
	$CaCl_2 \cdot 2H_2O$	2.20	g
	$NaNO_3$	12.75	g
B.	$MgSO_4 \cdot 7H_2O$	7.35	g
C	K_2HPO_4	0.522	g
D.	$NaHCO_3$	7.50	g
2.	MICRONUTRIENTS		
	H_3BO_3	92.8	mg
	$MnCl_2 \cdot 4H_2O$	208.0	mg
	$ZnCl_2$	1.64	mg[1]
	$FeCl_3 \cdot 6H_2O$	79.9	mg
	$CoCl_2 \cdot 6H_2O$	0.714	mg[2]
	$Na_2MoO_4 \cdot 2H_2O$	3.63	mg[3]
	$CuCl_2 \cdot 2H_2O$	0.006	mg[4]
	$Na_2EDTA \cdot 2H_2O$	150.0	mg
	Na_2SeO_4	1.196	mg[5]

[1] $ZnCl_2$ - Weigh out 164 mg and dilute to 100 mL. Add 1 mL of this solution to Stock 2, micronutrients.

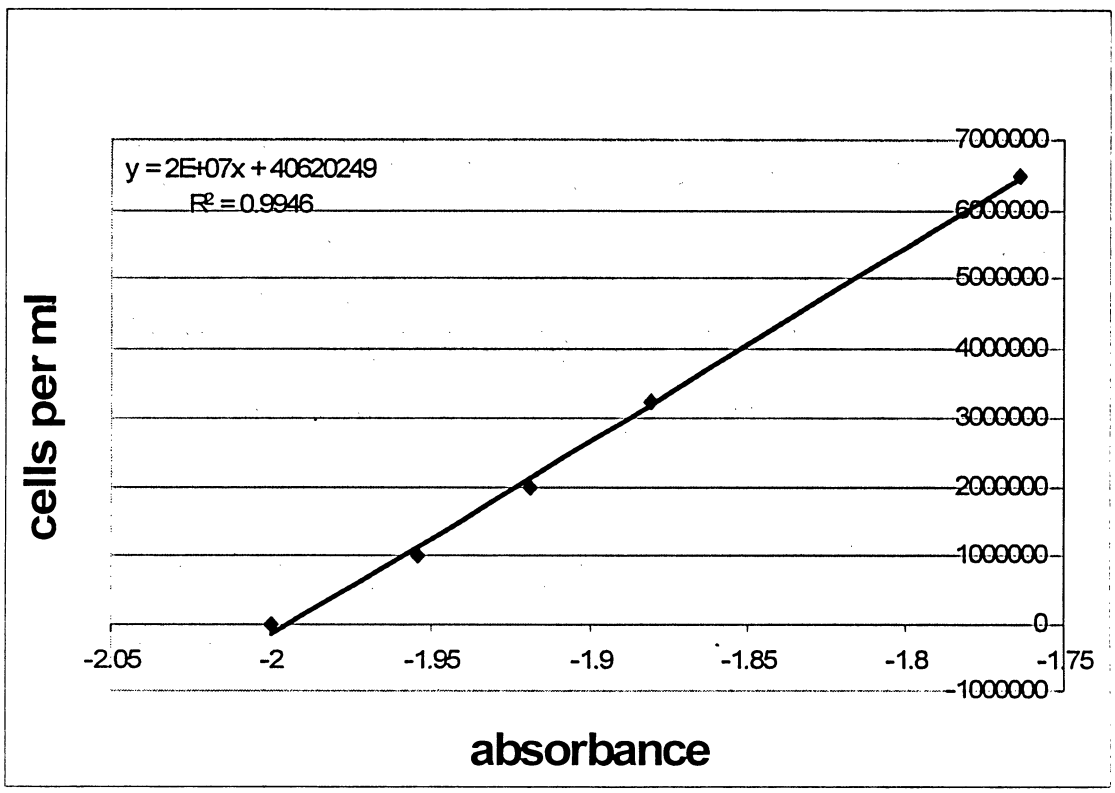
[2] $CoCl_2 \cdot 6H_2O$ - Weigh out 71.4 mg and dilute to 100 mL. Add 1 mL of this solution to Stock 2, micronutrients.

[3] $Na_2MoO_4 \cdot 2H_2O$ - Weigh out 36.6 mg and dilute to 10 mL. Add 1 mL of this solution to Stock 2, micronutrients.

[4] $CuCl_2 \cdot 2H_2O$ - Weigh out 60.0 mg and dilute to 1000 mL. Take 1 mL of this solution and dilute to 10 mL. Take 1 mL of the second dilution and add to Stock 2, micronutrients.

[5] Na_2SeO_4 - Weigh out 119.6 mg and dilute to 100 mL. Add 1 mL of this solution to Stock 2, micronutrients.

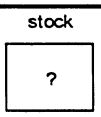
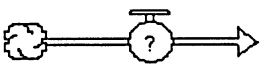
Appendix 4: Calibration curve: Pseudokirchneriella subcapitata cell density Vs absorbance



Appendix 5: Spring water characteristics

Mineral	Concentration (mg/L)
As	<0.001
Ca^{++}	48.2
Cu	<0.1
Mg^{++}	29.4
Pb	<0.001
Na^{+}	6.9
Zn	<0.1
HCO_3^{-}	306
Cl^{-}	1.9
F^{-}	<0.1
NO_3^{-}	8.2
K^{+}	1.0
SO_4^{--}	3.8

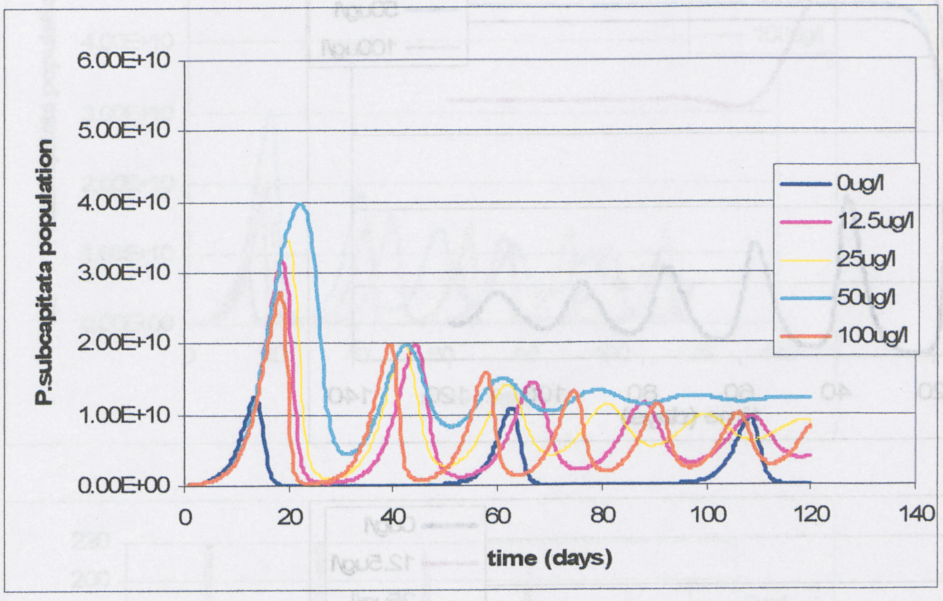
Appendix 6: Four building blocks used in Stella® and their modeling symbols (Richmond et al, 1987; Deaton and Winebrake, 2000)

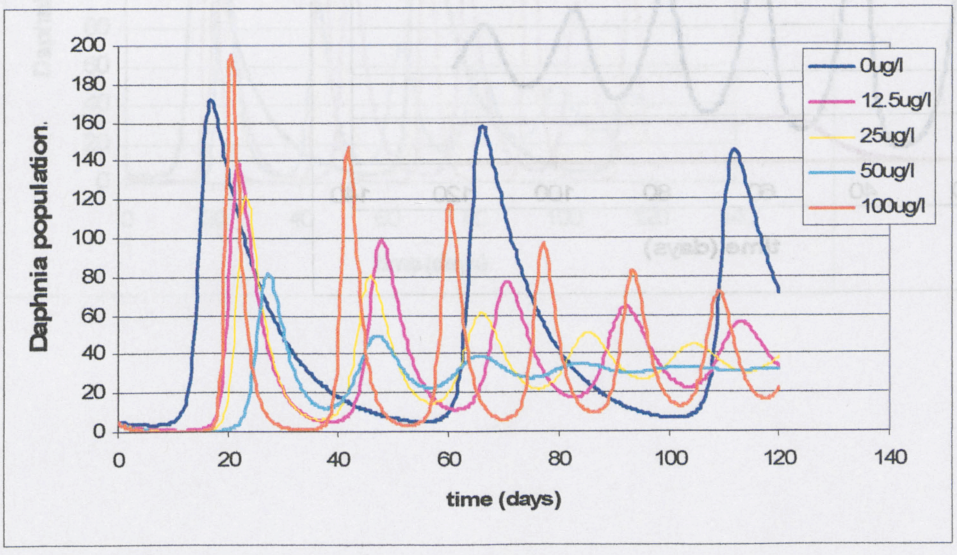
Name	Description	Symbol
Stock/Reservoir	A component of the system where something is accumulated. The components of a reservoir may go up and down over time	
Flows	Activities that determine the values of reservoir over time. Flows feed and drain stocks. The direction of a positive flow is indicated by an arrow ahead.	 the flow
Converters	System quantities that dictate the rates at which processes operate and reservoirs change	 the converter
Interrelation-ships	Define the cause-effects relationships between system elements	 Connector

***Appendix7**: Linear regression data for bioassays. Test of Hypothesis that slope = 0.*

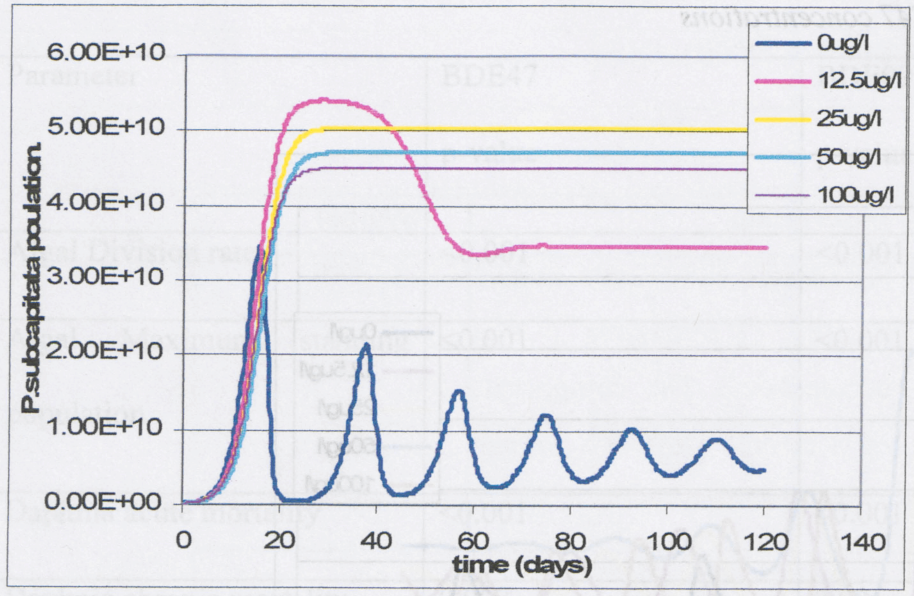
Parameter	BDE47 p-value	BDE99 p-value
Algal Division rate	<0.001	<0.001
Algal Maximum standing population	<0.001	<0.001
Daphnia acute mortality	<0.001	<0.001
Daphnia chronic mortality	0.002	0.001
Daphnia reproduction	0.597	0.698

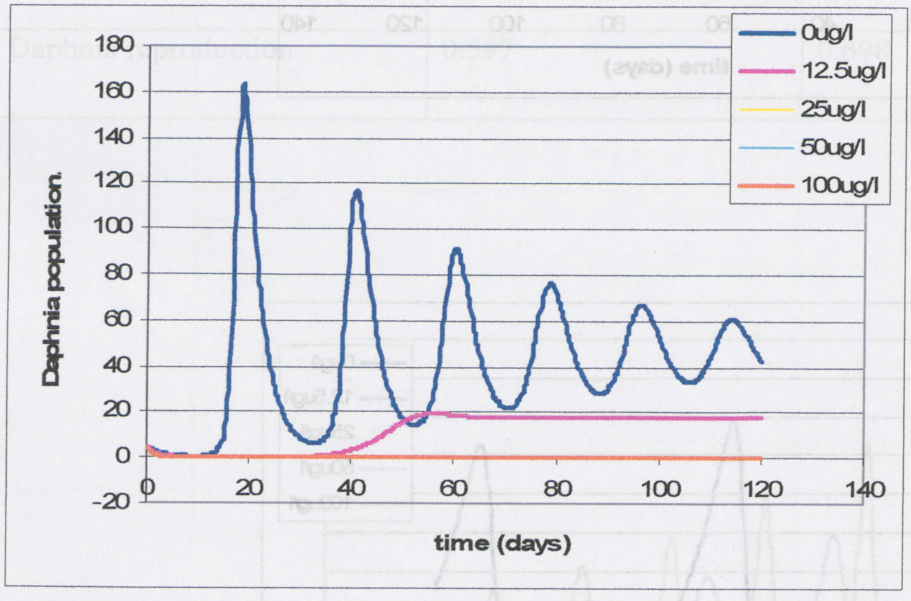
Appendix 8: *Population dynamics of P. subcapitata and Daphnia magna in a predator- prey system at different BDE47 concentrations*



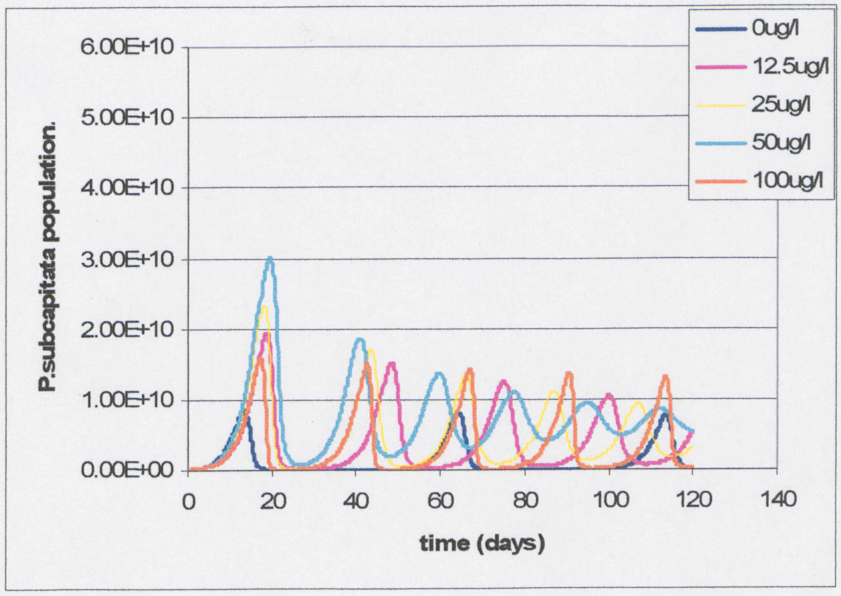


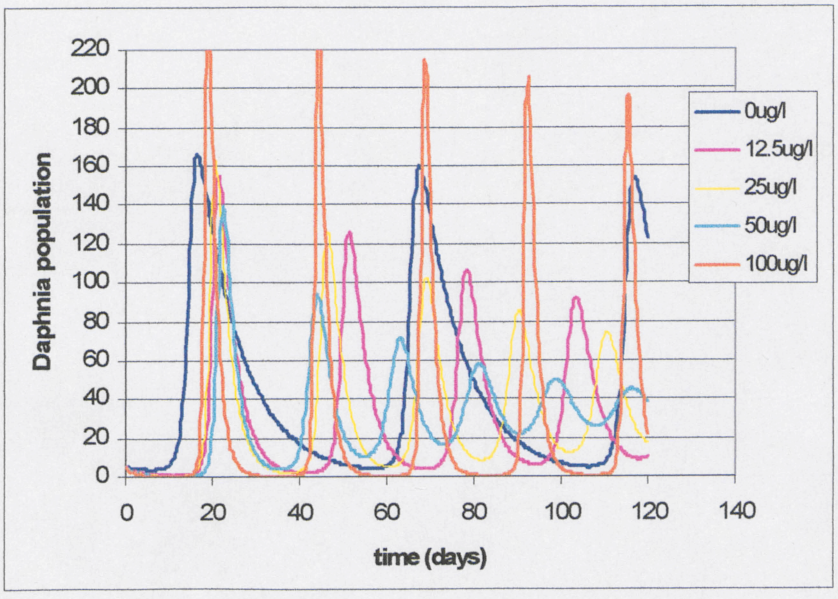
Scenario1


6.00E+10
5.00E+10
4.00E+10
3.00E+10
2.00E+10
1.00E+10
0.00E+00
P.subcapitata poulation.
0
20
40
60
80
100
120
140
time (days)
0ug/l
12.5ug/l
25ug/l
50ug/l
100ug/l


180
160
140
120
100
80
60
40
20
0
-20
Daphnia population.
0
20
40
60
80
100
120
140
time (days)
0ug/l
12.5ug/l
25ug/l
50ug/l
100ug/l

Scenario2


0ug/l
12.5ug/l
25ug/l
50ug/l
100ug/l
P.subcapitata population.
6.00E+10
5.00E+10
4.00E+10
3.00E+10
2.00E+10
1.00E+10
0.00E+00
0
20
40
60
80
100
120
140
time (days)


0ug/l
12.5ug/l
25ug/l
50ug/l
100ug/l
Daphnia population
220
200
180
160
140
120
100
80
60
40
20
0
0
20
40
60
80
100
120
140
time (days)