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Organotins in zebra mussels (*Dreissena polymorpha*) and sediments from the Saint-Lawrence River.

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Abstract

Toxic antifouling agents such as tributyltin (TBT) and triphenyltin (TPT) have been released in aquatic ecosystems through the use of antifouling paint applied to ship hulls, pleasure crafts and fish nets. The purpose of this study was to assess the use of zebra mussels (*Dreissena polymorpha*) as a biomonitor for investigating the geographical variations of organotin bioavailability along the St. Lawrence River. Organotins TBT and TPT and their degradation products were first measured in the soft tissues of zebra mussels collected from along the River. High concentrations of TBT were found in mussels from Bassin Louise, a marina in Quebec City (1442 ng/g wet weight). TPT concentrations were elevated at 2 sites near Quebec City (252 and 530 ng/g wet weight). A follow-up study assessed the extent of the distribution of organotins from the contaminated marina to the River system by measuring organotin concentrations in zebra mussels and in sediments collected River near Quebec City. The highest concentration of TBT was found in Bassin Louise (1078 ng/g wet weight) and elevated concentrations were found in two other marinas. The concentrations decreased sharply to background levels just outside the marinas. All butyltins were detected in all sediments analysed. There was a significant correlation between TBT in sediments and mussels. These studies suggest that organotin contamination may remain a problem in localized freshwaters in the St. Lawrence River.

Résumé

Des agents toxiques comme le tributylétain (TBT) et le triphenylétain (TPT) sont relâchés dans les systèmes aquatiques à cause de leur usage dans des peintures protectrices appliquée sur les coques de bateaux et les filets de pêche. L'objectif de ce projet est d'évaluer l'utilisation de la moule zébrée (*Dreissena polymorpha*) comme biomoniteur pour déterminer les variations géographiques de la bioviabilité des étains organiques le long du fleuve Saint-Laurent. TBT et TPT et leurs produits dérivés ont été mesurés dans les tissus des moules zébrées échantillonnées le long du fleuve. La concentration de TBT la plus élevée (1442 ng/g) se trouva dans les moules du Bassin Louise, la marina de la ville de Québec. Les concentrations de TPT étaient élevées dans deux stations proches de Québec (252 et 530 ng/g). Une deuxième étude porta sur la distribution des étains organiques à partir de la marina contaminée vers le fleuve, en mesurant les concentrations dans les moules zébrées et dans des sediments échantillonnés le long du fleuve à proximité de la ville de Québec, la concentration de TBT la plus élevée étant dans les moules du Bassin Louise (1078 ng/g). Les moules échantillonnées de deux autres marinas avaient des concentrations élevées en TBT. Les concentrations étaient peu élevées en dehors des marinas. TBT et ses dérivés étaient présents dans tous les sediments analysés. Les concentrations de TBT dans les moules étaient directement correlées avec les concentrations dans les sediments. Ces études suggèrent que la contamination des étains organiques demeure un problème dans certains endroits du Fleuve Saint Laurent.

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I thank my supervisor, Dr. Laurie Chan, for giving me the opportunity to work with him, for his guidance and support and for introducing me to such an interesting field of study. His enthusiasm, encouragement and motivation have been driving forces behind this thesis.

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Preface

The following is included in accordance with the regulations of the McGill University Faculty of Graduate Studies:

"The student has the option, subject to the approval of the Department, of including as part of the thesis the text, or duplicated published text, of an original paper, or papers. In this case the thesis must still conform to all other requirements explained in Guidelines Concerning Thesis Preparation. Additional material (procedural and design data as well as descriptions of equipment) must be provided in sufficient detail (e.g. appendices) to allow a clear and precise judgement to be made of the importance and originality of the research reported. The thesis should be made more than a mere collection of manuscripts published or to be published. It must include a general abstract, a full introduction and literature review and a final overall conclusion. Connecting texts, which provide logical bridges between different manuscripts, are usually desirable in the interests of cohesion.

The inclusion of manuscripts co-authored by the candidate and others is acceptable but the candidate is required to make an explicit statement on who contributed to such work and to what extent, and supervisors must attest to the accuracy of the claims, e.g. before the Oral Committee. Since the task of the Examiners is made more difficult in these cases, it is in the candidate's interest to make responsibilities of the authors perfectly clear. Candidates following this option must inform the Department before it submits the thesis for review."

Contribution of Authors:

The overall purpose of this study was to assess the bioavailability of organotins in the St. Lawrence River using zebra mussels as a biomonitor. The study is divided into two sections to be published as separate papers, one of which has been accepted with revision by the Journal of Great Lakes Research, and the other is to be submitted to Hydrobiologia for publication. The co-authors of both thesis sections are my supervisor, Dr. Laurie Chan, and Yves de Lafontaine from the St. Lawrence Center at Environment Canada. Dr. Laurie Chan contributed to the formulation of the thesis objectives and methodology and revision of drafts. Dr. Yves De Lafontaine was responsible for coordinating the field sampling and the revision of drafts. I was responsible for literature review, all chemical and data analysis, sample collection in the 2nd paper, data interpretation and preparation of the manuscripts.

In the first paper I survey the concentrations of organotins from sites along the St. Lawrence River between Cornwall and Ile d'Orléans, and determine point sources of organotin contamination. In the second paper, I investigate the distribution of organotins from possible contaminated sites.

CHAPTER 1

INTRODUCTION

1. 1: History of organotin use

Organotin compounds are generally of anthropogenic origin (apart from methyltins). The first commercially registered organotin compound was marketed in 1936 for use as a stabilizer for synthetic polymers (Guard et al., 1981). Since the biocidal properties of trialkylated organotins were recognized in the 1950's, the variety of applications, products, and consumption increased considerably. To date, organotins are the most widely used organometals. They are mainly employed as pesticides, but also find important non-pesticidal application such as polyvinyl chloride (PVC) stabilizers and catalysts for polyurethane and silicone elastomers (Blunden et al., 1986). About 23% of the total organotin production is used as agrochemicals and as general biocides. The agricultural and biocidal use of organotins probably gives rise to the largest proportion of free organotins in the environment, due to the direct introduction into the soil, air, and water. Growing consumption of a particular form of organotin, tributyltin (TBT), lies in its use as a preservative for timber and wood, textiles, paper and leather. This compound is also used, in a small percentage, in dispersion paints as protection against microbial or fungal attack.

TBT gained widespread application as an effective antifouling paint biocide on pleasure boats, large ships, and docks in the 1970's and 1980's (Maguire, 1998). In the late 1970's, antifouling paints were found to cause detrimental environmental impacts. As TBT leaches directly from paints into water, harbours for pleasure boats and commercial boats and coastal areas can be highly contaminated. Triphenyltin (TPT) has also been employed as a co-toxicant with TBT in some antifouling paints.

However, the major employment of TPT compounds lies in agriculture, where they are used as fungicides in crop protection (potato, celery, sugar beet, coffee, rice, Stäb et al., 1995).

Organic derivatives of tin are far more toxic than inorganic tin, and the toxicity of organotins increases with progressive introduction of organic groups at the tin atom, with maximum toxicity for trialkylated compounds. Toxicity decreases with increasing length of the organic moiety (WHO, 1990).

Environmental degradation of dissolved TBT proceeds by successive dealkylation reaction to produce dibutyltin (DBT) and monobutyltin (MBT) (Figure 1.1). Diphenyltin (DPT) and monophenyltin (MPT) are degradation products of TPT (Figure 1.2).

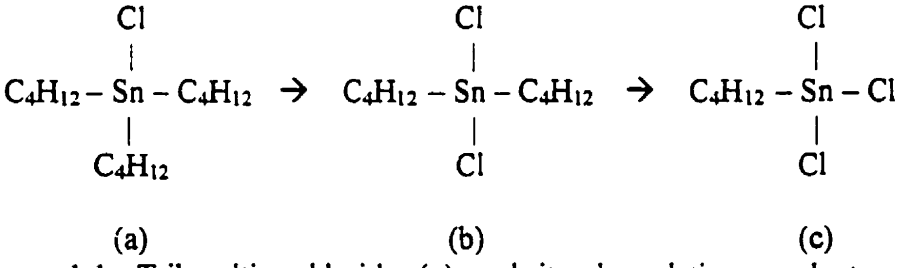


Figure 1.1: Tributyltin chloride (a) and its degradation products: dibutyltin dichloride (b) and monobutyltin trichloride (c).

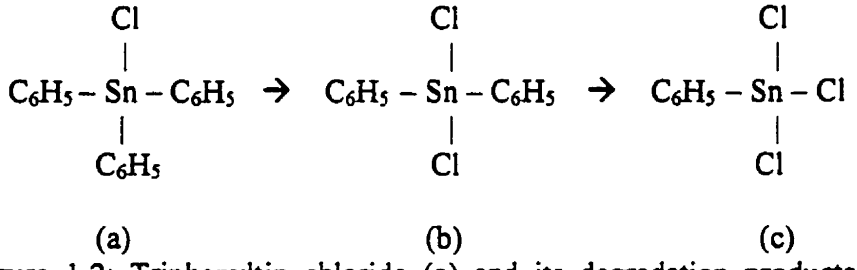


Figure 1.2: Triphenyltin chloride (a) and its degradation products: diphenyltin dichloride (b) and monophenyltin trichloride (c).

TBT and TPT derivatives have high fungicidal, bactericidal, algicidal, and acaricidal properties (Stäb et al., 1995) and, therefore, are particularly harmful to the environment.

1. 2: Organotin toxicity in aquatic life

The ecotoxicological hazards of organotin compounds have been recognized after deleterious effects occurred in the late 1970s on oyster populations in Arcachon Bay in France (Alzieu et al., 1982). The shells of pacific oysters (*Cassostrea gigas*) began to exhibit morphological abnormalities and the spatfall also declined dramatically. Shell abnormalities including chambering and the formation of protein-containing jelly, were observed. Between 1977 to 1983, oyster production in Arcachon was severely affected, resulting in estimated economical losses of $150 million (Alzieu, 1991). Shell thickening was also observed in *C. gigas* in the U.K (Waldock and Thain, 1983). Oyster malformations were later detected along the coasts of Spain (Morcillo et al., 1997) and North America (Uhler et al., 1993).

Negative effects on reproduction were found in marine neogastropods. Imposition of male sex organs, including a penis and vas deferens, on female mud snails (*Nassarius obsoletus*) was linked to TBT contamination in England (Smith, 1981). This phenomenon was termed 'imposex' or 'pseudohermaphroditism'. The degree of penis development and the frequency of imposex was related to TBT levels, and increased near harbors and marinas. Bryan et al. (1986) further documented the imposex phenomenon in other marine neogastropods. Their studies on dogwhelks (*Nucella lapillus*) showed a clear relation between TBT contamination and the

occurrence of imposex. Imposex was described in *N. lapillus* in England, the Netherlands, and the coastline of the North Sea. Imposex also occurs in other whelk species such as *Cronia*, *Morula* and *Thais* in Canada, West Africa, New Zealand, Australia, Malaysia, Singapore, and Indonesia, indicating a global occurrence (Ellis and Pattisina, 1990). Marine molluscs are the most susceptible organisms to TBT. TBT causes adverse effects on the population structure of dogwhelks, with changes in the percentage of adult females. Chronic effects may also occur in freshwater snails, as reduced egg laying in *Biomphalaria glabrata* has been observed (Ritchie et al, 1974). Imposex has been reported in a subtropical freshwater gastropod (*Marisa cornuarietis*) (Bettin et al., 1996).

1. 3: Restrictions on organotin use in water

Because of its potential impact in the environment, the use of TBT-containing antifouling paints is now controlled and banned in many countries. France was the first country to ban the use of such paints in 1982 on boats less then 25 m in length. Similarily, in 1987, a total ban was implemented in the U.K. on the use of TBT paints on such small boats (<25m) and fishing nets. An environmental quality standard of 20 ng/L for fresh water, and 2 ng/L for seawater was also set. The US restricted the use of antifouling paints nationwide in 1988. Canada regulated TBT in 1989 for large ships and prohibited the use on vessels less than 25 m in length. The use of TBT in fishnets is banned in Japan. Switzerland and Germany implemented a sales ban on organotin antifouling paints in 1990, and Germany also prohibited its use. A number of countries including Australia, the Baltic States, Norway, Ireland, etc. implemented

similar restrictions and bans. Regardless of the regulations, antifouling paints remain an important source of organotins to the aquatic environment due to their use in large vessels and applications in countries without regulations. It should be noted that restrictions of TBT use did not necessarily diminish its consumption, as a change in application from antifouling paints to wood preservation occurs at present (Fent, 1996).

1. 4: Zebra mussels as a biomonitor for organotins in freshwater

The pollution of freshwaters by organotins can be studied by the analysis of concentrations in water, sediments or in some members of the indigenous biota common to a region under study. Although the analysis of pollutant levels in water is the most direct way of studying organotin levels, it is frequently at levels below detection limits and gives variable measurements. Because of the filter-feeding behavior and the generally high potential for accumulation of contaminants, including organotins, bivalves have been widely used as sentinel organisms for monitoring the contamination of aquatic ecosystems. Common molluscs, including *Littorina littorina* and *Mya arenaria*, and oysters may serve as bioindicator organisms of organotin pollution in marine ecosystems, as they have a limited ability for metabolism and elimination of this compound (Fent, 1996). In freshwaters, zebra mussels (*Dreissena polymorpha*) are of particular interest. Zebra mussels are among those species showing high bioaccumulation. As a consequence, they have been used as a biomonitor in Europe where they are widely distributed geographically, sedentary, and easy to collect (Stäb et al., 1995; Fent and Hunn, 1991; Fent and

Looser, 1995; Becker et al., 1992, Carlier-Pinasseau et al., 1996). Following their accidental introduction from Europe into North America during the 1980's, zebra mussels rapidly dispersed throughout the Great Lakes (Hébert et al., 1991; Nalepa and Schloesser, 1993). They were first noted in the fresh waters of the St. Lawrence River in 1989 (Lapierre et al., 1994) and are now integrated into the Great Lakes and St. Lawrence River systems.

1. 5: Objectives

There is no information on the sources and availability of organotins in the St. Lawrence River.

The two objectives of this project are: 1) to survey the concentrations of organotins in zebra mussels along the freshwaters of the Saint-Lawrence River in order to provide a measure of their bioavailability, 2) to identify point sources and determine the degree of dispersion. For the first objective, zebra mussels were collected from 11 sites along the St. Lawrence River between Cornwall and Île d'Orléans and their body burden of organotins analysed. This study provided a measure of organotin bioavailability in zebra mussels in the river system, and identified potential point sources. The identified hot spots near Quebec City were studied in greater detail by a follow-up sample collection. The potential of organotins dispersing from the contaminated area to the river system was investigated.

CHAPTER 2

LITERATURE REVIEW

2. 1: Introduction

An extensive amount of literature has been published concerning the aquatic toxicity and the environmental fate of TBT compounds, and to a lesser extent TPT compounds, released into the water by antifouling paints in boat hulls (Becker and Tarradellas, 1994; Chau et al., 1997a, Cardwell and Sheldon, 1986; Fent and Hunn, 1991). Most data from various localities describe concentrations of TBT and TPT in water, sediments and biota, mainly from places with high boating activities. The environmental fate, toxicity and monitoring data of TBT are summarized here and evaluated in respect to environmental safety.

2. 2: Environmental fate of organotins (TBT and TPT)

Two processes may affect the persistence of TBT and TPT in the aquatic environment; photolysis and microbial degradation.

2. 2.1: Abiotic degradation

The UV part of sunlight reaching the earth's surface (>290 nm) induces the cleavage of the C-Sn bond. This results in a stepwise debutylation of TBT, and a dephenylation of TPT (Länge, 1987). There is only limited information on the efficiency of this process in the natural environment. Maguire et al. (1983) found that photolysis is not very effective under natural conditions. Investigations on the light attenuation in oceans and lakes revealed that in moderate latitudes the UV part of the light penetrating the natural waters is very quickly absorbed and attenuated by scattering. Photolysis therefore may not play a major role in reducing environmental organotin contamination in the water column. Maguire et al. (1983) showed that the degradation rate was slow with the half-life of TBT being more than 89 days,

when the UV part of the light is excluded. On the other hand, considerably higher concentrations of TBT degradation products (DBT and MBT) were found in the surface microlayer, indicating a rather high photolytic activity (Maguire and Tkacz, 1987). The photolytic degradation may, therefore, reduce only the surface concentrations of TBT.

2. 2.2: Biological degradation

Bacterial and algal communities contribute to the decomposition process, which results in a stepwise debutylation of the tributyltin cation (Maguire and Tkacz, 1985). Half-lives of environmental TBT concentrations were found to be between 20 to 30 weeks when incubated in the dark at 20°C. Seligman et al. (1986) studied the microbial degradation of TBT in water samples from San Diego Bay and Skidaway estuary (Georgia) and found the half-lives to be 5 to 15 days. The temperature varied between 12 and 29°C. Olson and Brinckman (1986) reported that TBT concentrations in water samples from harbours and marinas in Chesapeake Bay spiked with 1 μg/L TBT were not significantly reduced in a two week period at 5°C in the dark. In contrast, microbial degradation took place in samples spiked with 0.3 μg/L TBT to a rate of 25 to 35% in 7 days when incubated in the dark at 28°C, and to 50% when incubated under light conditions. Waldock et al. (1987) suggested that temperature had a large influence on the degradation rate; at 5°C half-lives were in the order of 60 days, but at 20°C half-lives were reduced to 6 days under dark conditions. Degradations in sediments have been investigated to a lesser degree. Estimates for the half-life of TBT in sediments range from 4 months up to over 10 years, depending on the nature of the sediment and the form of the TBT present. For example, if TBT was present as TBT containing paint particles, it would be very resistant to degradation (Chau et al., 1997b).

2. 3: Ecological Effects

2. 3.1:Toxicity in short term exposure

The toxicity of TBT compounds in the aquatic environment has been intensively investigated and numerous data for freshwater organisms have been reported. The toxicity data are summarized in Table 2.1.

The most sensitive freshwater organism to acute toxicity of TBT is a coelenterate (*hydra spora*) at levels of 400 ng/L, followed by toxic concentrations of 1600 ng/L TBT for rainbow trout (*Oncorhynchus mykiss*, Wong et al., 1982). Other test species are less sensitive to short-term exposure.

There are less toxicity data for the degraded compounds such as DBT and MBT. Alga (*Scenedesmus obliquus*) is the most sensitive freshwater organism to acute DBT exposure; levels of 6500 ng/L reduces photosynthesis, and chlorophyll-a production (Chau et al., 1997a). Acute toxicity of MBT occurs first in the freshwater bacterium *Pseudonomas putida* at levels of 53000 ng/L (Chau et al., 1997a).

Table 2.1: Acute Toxicity of TBT to Aquatic Organisms (laboratory studies).

Test Species	TBT Conc. ng/L	Toxic Effects	Exposure Duration	Reference
Freshwater Algae *Scenedesmus obliquus*	16000-20000	EC_{50}, growth effects	96hr	Wong et al., 1982
Coelenterate *hydra spora*	400	EC_{50}, response to prodding	48hr	Brooke et al., 1986
Water flea *Daphnia magna*	1700-14000	LC_{50}	48hr	Cardwell and Sheldon, 1986
Fathead minnow larvae *Phoxinus phoxinus*	7100	LC_{50}	96hr	Fent and Hunn, 1991
Rainbow trout *Onchorhynchus mykiss*	6900-21000 1600	LC_{50} LC_{50}	72hr 96hr	Cardwell and Sheldon, 1986 Wong et al., 1982

EC_{50}: effective dose to 50% of observations
LC_{50}: lethal dose to 50% of observations

2. 3.2: Toxicity of long term exposure.

Toxic effects from long term exposure to freshwater organisms are summarized in Table 2.2.

Table 2.2: Chronic Toxicity of TBT to Aquatic Organisms (laboratory studies)

Test Species	TBT Conc. ng/L	Toxic Effect	Exposure Duration	Reference
Freshwater Algae *Scenedesmus obliquus*	5000-8000	EC_{50}, growth defects	8 days	Wong et al., 1982
Bleak *Alburnus alburnus*	6000-8000	LC_{50}	60 days	Fent and Hunn, 1991
Rainbow trout	1400	LC_{50}	90 days	Fent and Hunn, 1991
Oncorhynchus mykiss	200-1000	EC_{50}, reduced growth	55 days	Seinen et al., 1981
(fish fry)	5000	100% mortality	12 days	Seinen et al., 1981
(yolk sac fry)	290	LC_{50}	32 days	Seinen et al., 1981
Fathead minnow *Pimephales promelas*	33	EC_{50}, growth effects	33 days	Brooke et al., 1986
Fathead minnow larvae *Phoxinus phoxinus*	230	EC_{50}, reduced growth	30 days	Fent and Hunn, 1991
Mussel larvae *Dreissena polymorpha*	26000	EC_{50}, growth defects	105 days	Becker and Tarradellas, 1994
Clam *Metilus edulis*	310	EC_{50}, reduced growth	66 days	Becker and Tarradellas, 1994

Hatch fathead minnows (*Pimephales promelas*) show growth defects with chronic exposure to TBT at levels of 33 ng/L. Results of this experiment were used for setting the Canadian water quality guideline. A safety factor of 10 was applied and the guideline level was set at 3.3 ng/L TBT for the protection of freshwater life (Moore et al., 1992). Seinen et al. (1981) studied long term effects on rainbow trout fry and found 100% mortality at 5000

ng/L TBT after 12 days, while growth was reduced at 1000 and 200 ng/L after 55 and 103 days respectively. The histopathological effects postulated to occur at these levels are difficult to evaluate with respect to their ecological significance.

Rainbow trout is also most sensitive to chronic exposures of DBT, with mortalities at concentrations of 95000 ng/L. Chronic toxicity of MBT is not known.

TBT in sediment at levels of 41 ng/g dry weight caused population declines in polychaetes (*Scoloplos armiger*) and amphipods (*Urothoe poseidonis*, Matthiessen and Thain, 1989). Chronic toxic effects in the clam *Scrobicularia plana* occur at TBT sediment concentrations of 300 ng/g dry weight (Langston and Burt, 1991). A study by Day et al, 1998, determined chronic toxicity expressed as reduced growth for 3 freshwater benthic species, *Chironomus riparius* at 4300 ng/g dry weight, *Hyalella azteca* at 1400 ng/g dry weight, *Hexagenia* spp. at 600 ng/g dry weight. *Tubifex tubifex* showed reduced reproduction capacity at 1600 ng/g dry weight.

In comparison to TBT, the ecotoxicity of phenyltin compounds is not well known. Data on the acute toxicity of TPT were gathered in the 1970s, but effects at environmentally relevant concentrations with appropriate chemical analysis are only beginning to be investigated recently. The results indicate that the effects of TPT seem to occur at the same order of magnitude as those of TBT (Bruschweiler et al., 1995).

2. 3.3: Toxic body burdens for marine and freshwater species

Table 2.3 shows the toxic effects to aquatic species with respect to their body burdens. Marine organisms *Metilus edulis* and *Ruditapes decussata* are the most sensitive species with toxic effects starting at 70 and 53 ng/g wet weight body burdens.

Table 2.3: Tissue concentrations of TBT and toxicological effects to aquatic organisms

Test Species	TBT Conc. ng/g (w.w)	Toxic Effect	Reference
Freshwater species			
Zebra mussel larvae *Dreissena polymorpha*	6200	Reduced growth	Becker et al., 1992
Water flea *Daphnia magna*	1700	Reduced growth	Fent and Hunn, 1991
Marine species			
Blue mussel *Metilus edulis*	1500 70	Reduced growth Shell thickening	Salazar and Salazar, 1991 Page et al., 1996a
Marine clam *Scrobicularia plana*	1700	Reduced growth	Langston and Burt, 1991
Marine clam *Ruditapes decussata*	53	reduced reproduction	Morcillo et al., 1998

2. 4: Monitoring data from freshwater ecosystems

In order to assess the potential risks of TBT to the aquatic environment, the toxicity data have to be analysed in the context of environmental relevant concentrations. An extensive study was conducted by the National Water Research Institute of Canada on the organotin levels in water and sediments of a large sample of rivers, lakes, harbours and coastal waters. Water and sediment samples were collected in 1994 at 80 freshwater locations. Results of the monitoring surveys are summarized in Table 2.4.

Table 2.4: Organotin levels in water and sediment for freshwaters of Canada.

Compound	Water		Sediment	
	% locations with organotin detected	Highest concentration Detected (ng/L)	% locations with organotin detected	Highest concentration detected (ng/g)
TBT	13	17.8	53	975
DBT	6	9.3	54	391
MBT	2	14.8	41	327
TPT	N/D	N/D	4	19.6
DPT	N/D	N/D	1	4.3
MPT	N/D	N/D	5	10

Adopted from Chau et al., 1997a.

The concentration of TBT was found to be exceeding the interim Canadian water quality guideline of 3.3 ng/L in 12 of 89 freshwater samples collected, with peak levels of up to 17.8 ng/L TBT. Similarly TBT was found in concentrations exceeding the detection limit (0.02 ng/g dry weight) in 42 of 80 sediment samples, with peak levels of up to 975 ng/g. TBT by-products (DBT, MBT) were also detected. In freshwater sediments DBT was found at all sites where TBT was found but at lower concentrations. MBT was also found at most locations where TBT was found.

TPT was not detected at any water samples in freshwater, although 3 out of the 80 sediment samples had TPT concentrations above the detection limit. MPT was found where TPT was found but at lower concentrations. DPT concentration was detected only at one site. Fent and Hunn (1991) analysed phenyltins in sediments and found considerable TPT concentrations at the top of sediment cores. The breakdown products of TPT showed greater variability than TBT, suggesting that biotic and abiotic factors in sediments may have a greater influence on the degradation of TPT than of TBT.

TBT in water and sediment were reported in other countries, such as Switzerland, the

Netherlands, France, England and Germany. Peak levels were in the range of 55.6 to 1337 ng/L TBT, generally found at or very close to marinas, and dock locations for small boats (Table 2.5). Other areas, sometimes even adjacent to harbours and marinas, had values close or below the detection limit of 5 ng/L TBT.

Freshwater concentrations have been reported by Becker and Tarradellas (1994) from 2 lakes in Switzerland, with TBT concentrations of 3.01 to 55.61 ng/L in water, and 27 and 4764 ng/g in sediment for natural and marina sites respectively. Fent and Hunn (1991) compared TBT concentrations in spring, when TBT concentrations peeked with winter concentrations in Lake Lucerne. Becker et al. (1992) examined 5 contaminated locations near marinas in Lake Geneva in Switzerland and France, and compared the data to a clean reference site. Schebek et al. (1991) analysed TBT levels along the Rhine River in Germany.

Waite et al. (1989) determined TBT concentrations in water of Bure and Yare Rivers in England. Dowson et al. (1993) determined sediment TBT concentrations in 6 rivers on the South-East coast of England.

Table 2.5: TBT- monitoring results of freshwater ecosystems

Location	**water: ng/L**		**sediment: ng/g dry wt**		**Reference**
	Most contaminated site	*Natural site*	*Most contaminated* site	*Natural site*	
Swiss freshwaters	55.61	3:01	476	2.7	Becker and Tarradellas, 1994
Lake Lucerne, Switzerland	752 (summer)	77-125 (winter)	1085	N/A	Fent and Hunn, 1991
France and Switzerland	353	13	4764	40	Becker et al., 1992
Germany	73	ND	182	13	Schebek et al., 1991
England	1337	ND	N/A	N/A	Waite et al., 1989
England	ND	ND	3500	ND	Dowson et al., 1993

2. 5: Risk Assessment

2. 5.1: Comparison of toxicity and monitoring data

Evaluation of the risk of TBT in aquatic ecosystems has to be based on the comparison of the environmental concentrations to the toxicity data. TBT levels in most freshwaters of Canada were well below 20 ng/L. On the other hand, areas with high TBT input (marinas, dry dock facilities) showed TBT concentrations close to 20 ng/L. These concentrations are well below the concentrations found to cause acute toxicity in the most sensitive organisms determined in the laboratory (400 to 17000 ng/L, Table 2.1). However, they are approaching the values for chronic toxicity (30 to 8000 ng/L, Table 2.2). Some aquatic organisms, especially mollusc larvae and some macroalgae species, may suffer inhibiting effects on growth or reproductivity in these locations.

Concentrations of TBT in the water column have decreased since the banning of the use of TBT containing antifoulant paints to pleasure craft in Canada (Chau et al., 1997a). The mean TBT/total butyltin ratio for samples collected in 1994 was 78% for freshwater, 51% for sediment in freshwater locations (Chau et al., 1997a). This high TBT/total butyltin ratio indicates little degradation of TBT and/ or recent input of TBT to the ecosystem. High TBT percentages in freshwater small craft marinas may indicate recent contamination by TBT leaching from small boats. However, the resuspension of TBT from the contaminated sediments in those locations cannot be dismissed.

2. 5.2: Fate and pathway of TBT and TPT

A generalized scheme of the distribution, transfer pathways and fate of TBT was derived by Fent (1996) based on studies on concentrations in harbours in Switzerland. Based on the contamination pattern and physiochemical properties of TPT, its fate is assumed to be

similar. Concentrations of TBT sorbed to particulates were about 3 orders of magnitude higher than in water. The sedimentation of these particulates result in a significant accumulation in the sediment where a 1000 times enhancement compared with the water column was observed. Bioaccumulation is another transfer pathway. In mussels, enhancement factors of four orders of magnitude relative to the water column were observed. Contaminant residues in biota are often given as bioconcentration factors (BCF), which are usually calculated by the ratio between the concentration in the organism relative to that in the ambient water. Zebra mussels show high bioaccumulation of organotins and may have BCF of over 12,000 for TBT. Even higher factors are found for TPT (26,000), because of its more lipophilic character (Fent and Hunn, 1991). Morcillo et al. (1997) studied TBT/DBT ratios in molluscs and found that they are species dependent. TBT and TPT in harbour sediments are persistant and stored there for years to decades. Therefore, sediments may act as important reservoirs from which these compounds may be mobilized.

2. 6: Monitoring organotin concentrations in biota

A fundamental prerequisite of a useful biomonitor is that the contaminant accumulated in the organism must reflect spatial and temporal variations of the ambient concentration of the contaminant in a definable manner (Phillips and Rainbow, 1993). A whole range of physiological and environmental factors can influence metal bioaccumulation; and these intrinsic and extrinsic factors vary spatially and temporally in a complex manner. Two very important considerations are the effects of growth of the biomonitor and the seasonal effect on its physiology.

2. 6.1: Effects of growth and size

Contaminants will increase with age and size when growth is slow relative to the rate of contaminant accumulation (Phillips, 1976). The opposite is true when growth rate in biomass exceeds the accumulation rate, then it will have a dilution effect in concentration. In a fully grown organism, whether contaminant accumulation in the soft tissues would increase with time would depend on its corresponding rate of absorption and excretion. Size effect on organotin accumulation has been demonstrated in a number of studies (Stäb et al., 1995; Fent and Hunn, 1991; Becker et al., 1992).

2. 6.2: Effects of season

Seasonal changes in physiology of the biomonitor with respect to growth, reproductive status, feeding habit and energy reserve have been shown to have a decisive influence on tissue contaminant contents in a number of marine bivalves (Phillips and Rainbow, 1993; Becker et al., 1992). The seasonal pattern is further complicated by other biotic factors such as temporal fluctuations in input of organotins, or in hydrological processes which may vary seasonally to affect both dissolved organotins and their bioavailabilities. Many studies recommend that samples should be collected at the same time each year in long term biomonitoring studies. Interpretation of results without taking seasonal effects into considerations will lead to gross errors.

2. 6.3: Studies on organotin concentrations in zebra mussels

Zebra mussels are communly used as a sentinel for heavy metal and organic contaminants in freshwater systems (Chau et al., 1997, Kwan et al., in press; Stäb et al., 1995; Fent and Hunn, 1991; Fent and Looser, 1995). Their extensive use in Europe and in North

America as a biomonitor allows for a means of comparison of organotin concentrations between different freshwater systems (Table 2.6).

Table 2.6: Comparison of butyltin and triphenyltin concentrations in zebra mussels (*Dreissena polymorpha*) from different studies. For comparison, data presented in dry weights was divided by a factor of 10 assuming an average water content of 90%.

Location	Number of sites	Concentration (ng/g wet wt)				Shell Length (mm)	References
		TBT	DBT	MBT	TPT		
Ontario, Canada	13	3-880	n.d-133	3.2-122	N/A	10-15	Chau et al., 1997
Lake Geneva, France	5	1466-9337	119-1390	N/A	N/A-2796	13-34	Becker et al., 1992
Switzerland (reference)	4	N/A-54	N/A	N/A	N/A	N/A	Becker and Tarradellas, 1995
Switzerland (marinas)	4	138-2022	N/A-217	N/A	N/A	N/A	Becker and Tarradellas, 1995
Netherlands	56	1-1550	N/A-205	n.d-120	7-800	20-35	Stäb et al., 1995
Lake Zurich, Switzerland	3	976	96	144	N/A	N/A	Carlier-Pinasseau et al., 1996
Lake Westeinder, Netherlands	4	18-250	<2-16	2-12	14-92	N/A	Stäb et al., 1996
Lake Lucerne, Switzerland	1	2800	470	100	640	18-25	Fent and Hunn, 1991

2. 7: Mechanism of toxicity

The toxic manifestation of the exposure of an organism to environmental chemicals is largely influenced by the bioavailability of the toxicant, the route of exposure, and the level and time course of exposure. The first reactions of organisms to toxic compounds take place at the molecular and cellular levels of target organs and tissues, before effects become visible at a higher level of biological organization. Sublethal compensatory and repair responses may prevent injury during prolonged exposure to toxicants, until the regulatory and adaptation mechanisms of the cell fail.

Organotin compounds exert a number of important cellular, biochemical, and molecular effects. The basic modes of action are similar in aquatic organisms and mammals, but specific actions are also evident in aquatic organisms. In this respect, the biochemical and molecular basis of the high susceptibility of marine organisms, including oysters and neogastropods, deserves particular attention.

After the toxicants enter marine animals via food, water or sediment, they can be stored or eliminated. The kinetics of both storage and elimination for toxic compounds are affected by the metabolism or lack of it by an animal. Many toxic compounds are highly hydrophobic, leading to accumulation in lipid-rich parts of tissues and cells. The elimination of such hydrophobic compounds are facilitated by their biotransformation to water-soluble polar compounds. Thus, metabolism of a compound generally reduces persistence, increases elimination and results in a reduction of toxicity. However, for some compounds, the metabolites are more toxic than the parent compounds (Lee, 1991). Toxic pollutants and their metabolites can be destroyed by certain detoxification systems (e.g. epoxide hydrase, or by binding covalently to cellular proteins and nucleic acids). The cytotoxic effects of some toxicants are due to covalent binding of their reactive metabolites to cellular proteins. In crabs

exposed to TBT in the food, up to 35% of the TBT metabolites were covalently bound to cellular proteins. A number of decapod crustaceans including blue crabs (*Callinectes sapidus*) and spider crabs (*Libinia emarginata*) can metabolize TBT which enters via food and water (De Mora, 1996). In contrast, molluscs in general show a very limited ability to metabolize TBT. Oysters exposed to TBT in water for up to 6 days resulted in only a small amount of the TBT being metabolized, primarily to DBT, and the clam (*Mya Arenaria*) has only a limited ability to metabolize TBT (De Mora, 1996).

Some of the basic and important modes of action of organotins are: 1) Organotin compounds are cytotoxic and potent inhibitors of energy production in cells, by binding to cell receptors, or destroying plasma membranes. They act as inhibitors of mitochondrial oxidative phosphorylation in a variety of cells and as uncouplers of photophosphorylation in chloroplasts. 2) They are potent inhibitors of ion transport proteins such as ATPase in the cell membrane. They can act as potent cell membrane toxicants leading to perturbations of plasma membrane bound enzymes involved in the cellular transport of nutrients. One possible ultimate basis of these reactions seems to be the alteration of calcium homeostasis. Increased intracellular calcium can activate cytotoxic mechanisms that result in perturbations of cellular structure (disruption of cytoskeletal organization, development of membrane abnormalities) and function (Orrenius et al., 1989). This mechanism is also responsible for the apoptosis of thymocytes, which results in immunotoxicity. 3) Organotins interact not only with membrane proteins, but also with intracellular proteins. These interactions are based on the coordination of organotin molecules with amino acids, such as cysteine and histidine (Fent, 1996).

The inhibition of the hepatic michrosomal cytochrome P450 systems are found to occur in various fish. Various components of this crucial detoxification system are affected: both

P450 protein and enzyme activity, and the reductases. Cytochrome P4501A, which is induced by important environmental organic chemicals, seems to be selectively affected in fish by TBT and TPT in vitro and in vivo (Brushweiler et al., 1995), but other P450 forms, including those with testosterone hydroxylase activity, are also affected at high concentrations.

Imposex development in gastropods, the most sensitive sign of the ecotoxicity of TBT, is mediated by steroid hormones. Recent studies indicated that dogwhelk females exposed to TBT showed an increase in testosterone titers, and injection of testosterone in the absence of TBT promoted penis development (Spooner et al., 1991). Imposex development may not be a direct effect of TBT exposure. It may be mediated by steroid hormones; TBT led to an increase in testosterone, possibly by inhibiting the cytochrome P450 systems that control the conversion of cholesterol into a variety of hormones. TBT may destroy or inhibit aromatase and lead to disturbances in the aromatization of androgens to estrogens (Stroben et al., 1991). Among invertebrate groups, molluscs appear to be the most affected by TBT. Molluscs are characterized by very low cytochrome P-450 content and mixed function oxygenase activity in the digestive gland. This low detoxifying activity may explain their susceptibility to TBT.

Effects of TBT on calcification mechanisms resulting in wafer-like chambering of the shell with formation of an interlamellar jelly were first observed in 1974 in Arcachon Bay (Alzieu, 1981). They progressively spread to all of the oyster culture areas along the Atlantic coast. Héral et al. (1981) described the successive phases of the phenomenon as follows: (1) hyper-secretion of a jelly appearing suddenly at the beginning of July and synchronously across populations of various ages; (2) deposition of a fine calcic layer completely enclosing the jelly within 15 days after its appearance, thus forming a gelatinous pocket; (3) disappearance of the jelly around the end of October or beginning of November, leaving a cavity called a chamber. The gelatinous substance contained in the 'chambers' was a protein which differed from the

calcification protein (conchyolin) in that its threonine content was higher and it showed a lower proportion of those amino acids providing the link with calcium: aspartic acid, glycine and serine (Alzieu, 1991). In vertebrates, the cytochrome P-450 system is involved in the synthesis of vitamin D which in turn regulates calcium metabolism. Thus, the abnormal shell growth in oysters after TBT exposure may be related to inactivation of cytochrome P-450 (Lee, 1991).

CHAPTER 3

METHODOLOGY

3. 1: Organotin Analysis by GC/MS

This chapter describes in detail the methodology employed for organotin analysis. There are many methods to analyse organotins in environmental samples. The two most common methods involve the use of Gas Chromatography and Liquid Chromatography. Because of the availability of the Gas Chromatogrophy/ Mass Spectrometer (GC/MS) in our laboratory, we use the GC/MS for all samples analysed. The first step in analysing environmental organotins using GC/MS is to derivatize the tin compounds to volatile compounds. Standards of pentylated derivatives were used to calculate derivatization efficiencies, to evaluate detector performance and finally, to accurately determine concentrations in samples. Pentylation is often used because it yields derivatives which are not too volatile. As a result, losses due to evaporation during work up, are prevented, while the derivatives can still be determined easily by GC/MS. Such pentylated standards are not commercially available and must therefore be synthesized. The 6 organotin compounds investigated were tributyltin (TBT), dibutyltin (DBT), monobutyltin (MBT), triphenyltin (TPT), diphenyltin (DPT) and monophenyltin (MPT). Methylated tricyclohexyltin (TCT) was used as a surrogate for checking recovery of tin compounds in zebra mussel analysis as TCT is not usually found in biota. The procedure used in this study was a protocol modified from Stäb et al., 1992.

Preparation of organotin standards

About 100 mg of organotin compound were weighed and dissolved in 10 mL hexane/toluene (50/50) in a 50 mL centrifuge tube and cooled in an ice bath. Grignard reagent (0.7 mL) was added and the tube closed. After the initial, slightly violent reaction, the tube was removed from the ice bath, and transferred to a water bath of 30°C and shaken occasionally. After 40 min, 5 mL of 2 M sulphuric acid were added to destroy the excess Grignard reagent. The organic layer was separated and dried over anhydrous sodium sulphate. The solvent was evaporated under a mild flow of nitrogen on a 60°C waterbath until an oily residue remained and no further decrease in weight was observed. The extract was dissolved in 1 mL hexane and applied to the top of a glass column containing 6 g of Alumina B. Elution was done with 12 mL of n-hexane/diethylether (9/1). Fractions (2 mL) were collected and the presence of the pentylated organotins was checked by GC/MS.

Table 3.1 demonstrates the yield for each compound analysed.

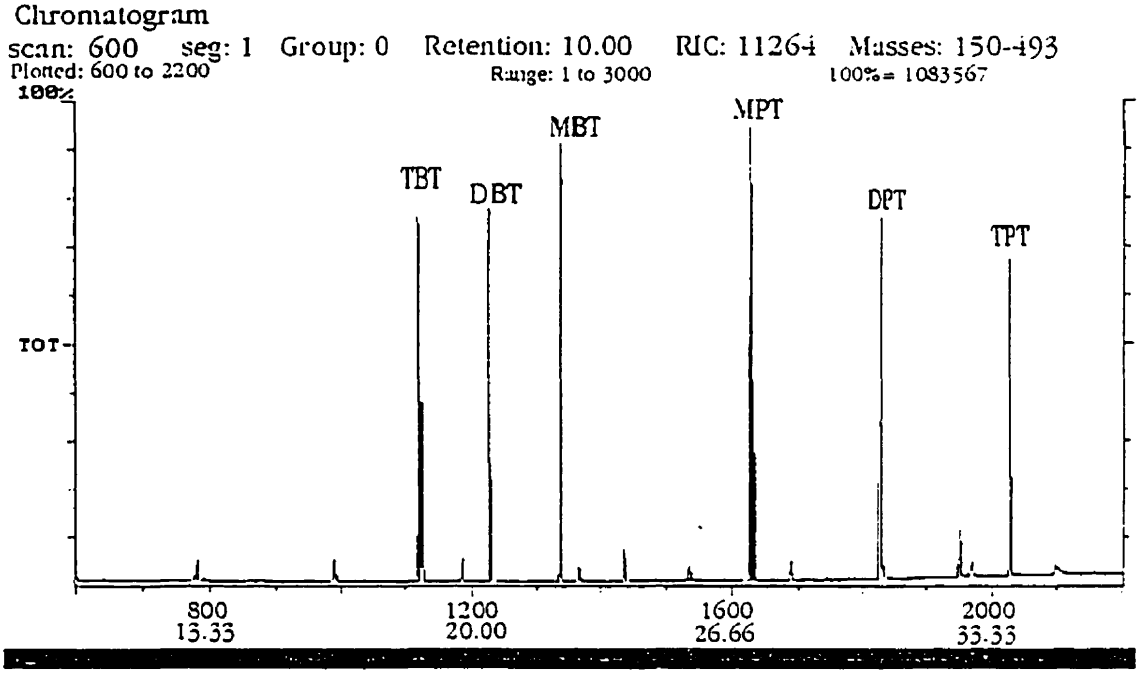
Table 3. 1: Yields of organotin standard fractions

Compound	Fraction (mL)	Yield %
$BuSnPe_3$	0.0-6.0	87%
Bu_2SnPe_2	2.0-6.0	87%
Bu_3SnPe	4.0-8.0	84%
$PhSnPe_3$	4.0-10.0	56%
Ph_2SnPe_2	4.0-14.0	65%
Ph_3SnPe	16.0-36.0	82%
Cy_3SnPe	2.0-10.0	78%

The final products were dissolved in hexane to obtain stock solutions of about 10 mg/mL, which were kept in the dark at –20°C.

A GC/MS chromatogram of organotin standards is shown in Figure 3.1.

Figure 3.1: A typical GC/MS chromatogram of organotin standards.



3. 2: Quality control and quality assurance

Quality control was applied routinely to all stages of sample preparation and organotin analysis to ensure acceptable accuracy and precision of the results obtained. The assessment of the analytical accuracy and precision must be an integral part of organotin analysis in this project. The quality control adopted in this study includes the following measures:

1) Monitoring organotin recovery in sample preparation procedure using certified reference materials (CRM): dry fish tissue (*Letealobrax japonicus*) from the National Institute for Environmental Sciences, Japan for zebra mussel analysis, and sediment PACS-II from the National Research Council, Canada for sediment analysis. Duplicates of the appropriate CRM were included in each batch of samples prepared. Samples and CRM were handled and processed in an identical fashion.

2) An analytical blank (hexane) was included in each batch of samples extracted and derivatized to monitor contamination during procedure.

3) Relative standard deviations of organotin determination in duplicate must be less than 10%.

4) Certified reference material must yield a recovery of within 10% of their certified value.

5) Absorbance of the analytical blanks must not be higher than the absorbance corresponding to the detection limit of the element being analysed.

CHAPTER 4

ORGANOTINS IN ZEBRA MUSSELS (*DREISSENA POLYMORPHA*) ALONG THE ST. LAWRENCE RIVER

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4.1: Abstract

Toxic antifouling compounds such as organotins have been released in aquatic ecosystems throughout the world and can be bioaccumulating in biota. The purpose of this study was to assess the use of zebra mussels (*Dreissena polymorpha*) as a biomonitor for organotins by investigating the geographical variations of organotin bioavailability along the St. Lawrence River system. The presence of organotins tributyltin (TBT) and triphenyltin (TPT) and their degradation products were measured in the soft tissues of zebra mussels collected from 11 sites along the Saint Lawrence River (between Cornwall and Ile d'Orléans) in June 1996. TBT and TPT were generally low (<1 to 30 ng/g wet weight) and were similar to those reported in Ontario and in the Netherlands. The highest concentration of TBT (1442 ng/g wet weight) was found in mussels from Bassin Louise, a marina in Quebec City. This suggests that residual levels in the sediment, or illegal use of antifouling paints still remain problematic in localized sectors of the river. A more detailed study on the availability of TBT in marinas and harbours along the freshwater part of the St. Lawrence River is recommended.

4.2: Introduction

Biocidal properties of trialkylated organotins were recognized in the 1950's and have since been used in a variety of applications and products (Blunden et al., 1986). In Canada, organotins are commonly used as a stabilizer for products containing polyvinyl chloride. The greatest potential for input to the aquatic environment is from organotin usage in antifouling paints used on boats.. Several organotin compounds (tributyltin (TBT), triphenyltin (TPT)) are routinely used in paints as preservatives against biological fouling on exposed underwater surfaces. The widespread use of organotin-based antifouling paints on boat hulls, lobster traps, and fishing nets has resulted in elevated concentrations of these compounds in freshwater, estuarine and marine environments (Moore, 1992). Due to degradation and photodegradation processes, the half-life of organotins in the environment is expected to be between 60 to 240 days but residence times for TPT and TBT of over 10 years in sediments have also been reported (Fent and Hunn, 1991). TBT is particularly toxic to molluscs (Ritsema et al., 1991). Chronic toxic effects on oysters (abnormal shell thickening) and marine gastropods (sterilization of females) occur at aqueous concentrations of a few nanograms per liter (Fent and Hunn, 1991; Ritsema et al., 1991). Important declines in dog whelk populations have been attributed to TBT exposure. The phenomenon of imposex (male genitalia imposed on females) in shoreline whelks has been reported in South East Asia (Indonesia, Singapore and Malaysia), as well as along the coasts of North Atlantic (Ellis and Pattisina, 1990). Ecotoxicological studies on organotins have mainly been focused on TBT, there is little information about the toxicity of TPT (Fargasova, 1998). It was suggested that

adverse effects occur at the same order of magnitude as those of TBT (Fent and Hunn, 1991).

The high potential environmental impact of TBT have led to restrictions on the use of antifouling paints in France, the U.K., Australia, the Netherlands, Switzerland, Japan, Denmark, and Hong Kong. In 1989, under the Pest Control Product Act, restrictions on using antifoulants on boats less than 25m and the banning of their use on nets and on lobster traps was implemented in Canada (Chau et al., 1997).

Despite the regulation, a number of studies have found that concentrations of TBT in the Canadian aquatic environment remained a continuing concern. Pelletier et al. (1996) found TBT and dibutyltin (DBT) in almost all starfish (*Leptasterias polaris)* tissues collected in coastal waters of the St. Lawrence estuary between Rimouski to Gaspe. The levels of TBT and DBT ranged from 5 to 70 ng/g (wet weight) with no apparent geographical trend. Prouse (1996) conducted an imposex survey on gastropods (*Nucella lapillus*) in Eastern Canada. Imposex was observed in 13 of the 34 sites, and ranged from 29% to 100%. In the harbours of Halifax, Sidney, Chester (Nova Scotia), and Les Mechins (Quebec) imposex frequencies were between 88% to 100%. Chau et al. (1997) reported TBT concentrations of 879.9 ng/g TBT (wet weight) in zebra mussels (*Dreissena polymorpha)* at Kingston harbour in Ontario and 289.1 ng/g in mussels at Port Stanley in Ontario.

Following their accidental introduction from Europe into North America during the 1980's, zebra mussels rapidly dispersed throughout the Great Lakes

(Hébert et al., 1991; Nalepa and Schloesser, 1993). Zebra mussels were first noted in the fresh waters of the St. Lawrence River in 1989 (Lapierre et al., 1994). Attaching on hard substrates such as found on dock walls and harbours, zebra mussels are generally regarded as a nuisance, as they block flows in water intakes and waterlines, and have some adverse impact on local fauna. Being able to bioaccumulate many contaminants due to their high filtration capacity and limited ability to metabolize many chemicals, zebra mussels have often been used as biomonitors for trace heavy metals and organic chemicals in Europe and more recently, in North America (Stäb et al., 1995; Fent et Hunn, 1991; Fent et Looser, 1995; Chau et al., 1997; Kwan et al. In preparation). Zebra mussels are ideal biomonitors for high energy river systems not only because of their ability to bioconcentrate contaminants they can also integrate contaminant uptake from the water, sediment (Bruner et al., 1994), and suspended particulates by virtue of their benthic, filter-feeding life-style (Hébert, 1991). Zebra mussels were used for assessing the bioavailability of organotins in the Detroit and St. Clair rivers in Canada (Chau et al., 1997), and Leidse Rijn in the Netherlands (Stäb et al., 1995).

The purpose of this study was to survey the concentrations of organotins in zebra mussels along the St-Lawrence River in order to provide a measure of their bioavailability. Results can be used to infer hot spot point sources and will serve as a baseline for temporal monitoring of organotins in the river system.

4.3: Materials and Methods

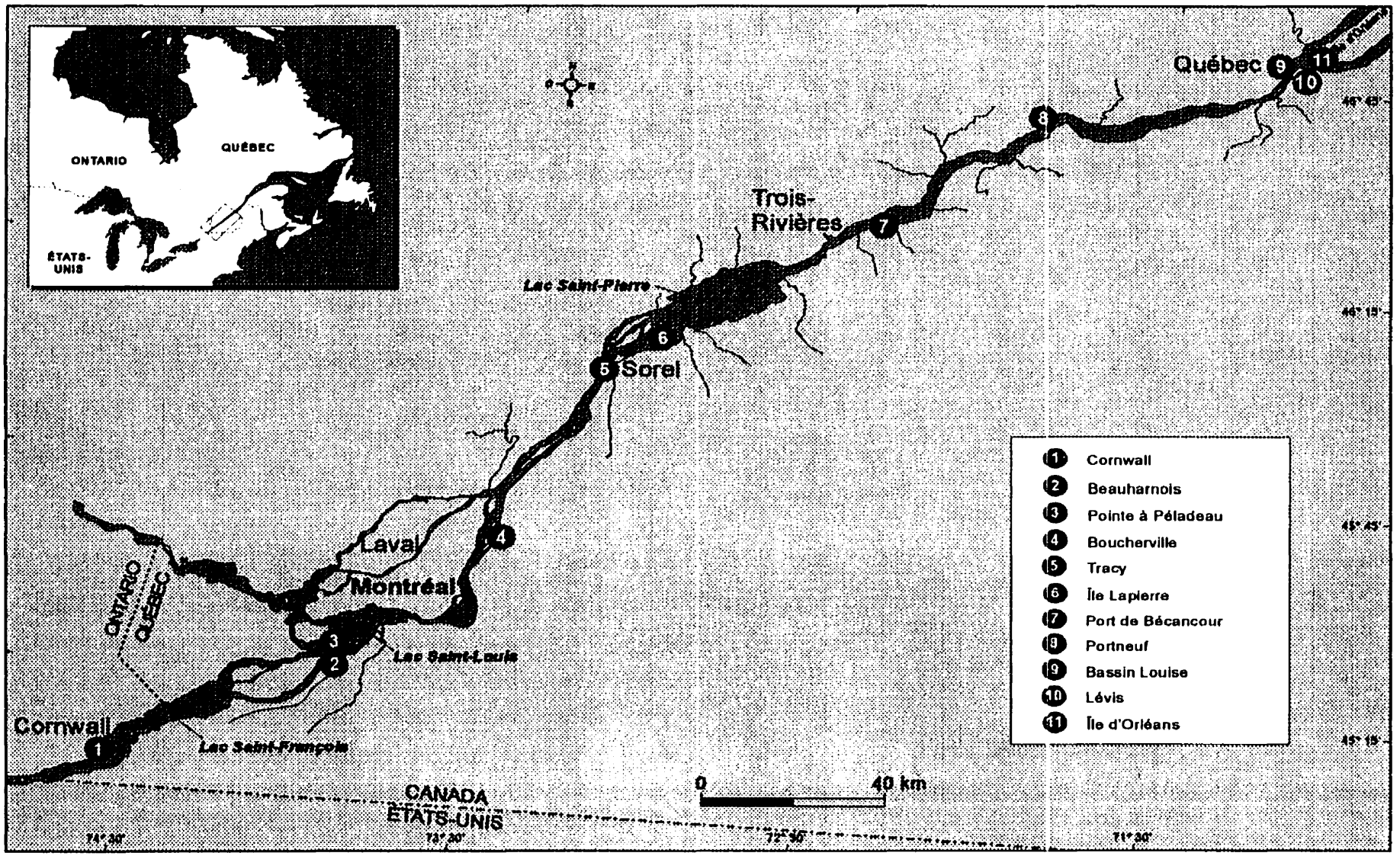
4.3.1: Chemicals and Standards

GC/MS grade solvents were obtained from Fisher Scientific (Montreal, QC). Organotin standards of butyltin (tributyltin, dibutyltin, monobutyltin), phenyltins (triphenyltin, diphenyltin, monophenyltin), and tricyclohexyltin were purchased from Aldrich Chemical Company, Inc. (WI, USA). Derivatizing agents including tropolone, pentyl magnesium bromide, and methyl magnesium iodide were also purchased from Aldrich Chemical Company, Inc. (WI, USA). Alumina type WB-2: basic was purchased from Sigma Company Co. (MO, USA). Chrysene (internal standard) was purchased from Ultra Scientific (RI, USA).

4.3.2: Mussel Sampling and Preparation

Zebra mussels were collected from 11 sampling stations at 3 to 5 meters in depth along the St. Lawrence River (Figure 4.1), in June 1996 by scuba divers. Mussels were brought to the surface and maintained in a container filled with river water for 24 hours to allow gut purging of ingested chemicals still sorbed to sediment particles. All samples were put in glass jars and stored in a -20°C freezer. Zebra mussels with shell length between 20-25 mm were used for this study. The small size range helps reduce variability in the data.

Figure 4.1: Sampling sites along the St. Lawrence River where zebra mussels were collected in June 1996.



Mussel samples were thawed at room temperature. The mussel tissue was removed from the shell, and byssus threads removed. Soft tissues of 15 mussels from each site were pooled (giving approximately 2 g wet weight mussel tissue) and homogenized using a polytron homogenizer (Brinkmann Instruments, N.Y., USA).

A modified procedure from Stäb et al. (1995) was used for the extraction of organotin compounds. Briefly, the procedure consisted of three steps: 1) acid digestion of the sample, 2) extraction, 3) derivatization (pentylation by a Grignard reagent) and clean-up.

Extraction and Derivatization:

Mussel homogenate (2g) was weighed in a 50ml centrifuge tube. Two replicates from each mussel homogenate were analysed. The homogenate was acidified to pH 1.5-2.0 with approximately 0.3ml of 1mol/L hydrochloric acid, 2 mL of freshly prepared concentrated sodium chloride solution and 6 mL of diethyl ether containing 0.3% tropolone added.

For recovery determinations, the sample was spiked with 5 μL of 20 ng/uL solution of methylated tricyclohexyltin (TCT) dissolved in hexane. The mixture was vortexed for 15 s, sonicated for 5 min, and vortexed again. Phase separation was performed by centrifugation at 2,200 rpm for 3 min. The organic layer was separated, and the extraction was repeated. The two extracts were combined and dried on anhydrous sodium sulfate. The extract was concentrated to 1 mL under a gentle stream of nitrogen. Diethyl ether (1 mL) and 0.7 mL of

Grignard reagent pentyl-magnesium-bromide were added. The reaction tube was shaken for 5 s and incubated at 30°C for 40 min. The reaction mixture was cooled in an ice bath and 3 mL hexane was added. Excess Grignard reagent was destroyed by adding 5 mL of 2 M sulphuric acid. The reaction tube was then shaken and centrifuged at 2,000 rpm for 5 min to obtain phase separation. The organic layer was dried on sodium sulfate and its volume reduced to 1 to 2 mL. Cleanup was performed within 2 hours using alumina column chromatography. A glass column (1 cm id X 30 cm) was wet-packed with 5g of pre-cleaned alumina in hexane. Samples were applied to the column and eluted with a 30 mL diethyl ether in hexane (1:9) mixture. Fractions collected were concentrated on a rotary evaporator to about 2 mL, transferred in 15 mL conical centrifuge tubes and evaporated to approximately 100 µL under a gentle stream of nitrogen. The concentrated fractions were spiked with 5 µL chrysene dissolved in iso-octaone (20 ng/µL) as an internal standard.

4.3.3: Conditions for GC/MS

Separation and quantitation of the organotin compounds were performed using a Gas Chromatography/Mass Spectrometer. The conditions were similar to those described by Stäb et al, (1992). GC analyses were carried out using a Varian 3400CX gas chromatograph coupled with a Varian Saturn 4D MS/MS ion trap mass spectrometer (Varian, Walnut Creek, CA). Samples were introduced through a Varian 8200 autosampler equipped with a programmable septum injector (SPI). Separation was performed on a DB-5 MS-ITD column (30m x 0.25mm id x 0.25*u*m film thickness, J&W Scientific Folson, CA). The mass

spectrometer was calibrated daily with perfluorotributylamine. 1 μL of sample solution was injected with the following temperature program: initial SPI injector temperature of 135°C was immediately raised to 300°C for 45 min. Initial GC oven temperature of 80°C was held for 1 min, and raised to 120°C within 3 min, then raised to 260°C within 28 min, and finally raised to 300°C within 20 min, and left at this temperature for an additional 6 min to clean out the column.

Solutions of organotin mixtures at 3 different concentrations were used as external standards. Peak areas, relative to the internal standard (chrysene) which was monitored at m/z 240, were used for plotting calibration curves. Organotins were quantified using the selected ion mode. The ions used for quantitation for each organotin compound are shown in Table 4.1.

100 ng of each organotin compound was pentylated and spiked to 2 g wet weight mussel homogenate. 10 replicates were performed, and all analytical results corrected for with the recovery values shown in Table 4.1.

Table 4.1: Recovery rate (%) and detection limits for organotins in zebra mussels during this study.

Organotin Compound	Quantitation mass m/z	Recovery (%) n=10	Detection Limit (ng/g wet wt.)
MBT	191	54	1
DBT	191	64	1
TBT	177	88	1
MPT	197	46	1
DPT	273	50	1
TPT	348	94	1
TCT	203	89	5

4.3.4: Quality Control

Our laboratory participated in a Quasimème laboratory performance study for Organotins in Biota and standard solution (Aberdeen, Quasimème Round 12, 1998).

A biological reference material (dry fish tissue) from the National Institute for Environmental Studies, Japan Environment Agency (Ibaraki, Japan) and a blank sample were measured with each batch of mussel analysis. TBT concentrations measured were always within 8% of the certified value. No organotin compounds were detected in the blank samples.

4.4: Results

On their respective GC-MS ion traces, all peaks of the organotins were well separated from each other and from interfering compounds (refer to the appendix p. 88).

The presence of an organotin species was tentatively confirmed if it occurred within the appropriate chromatographic window, and the concentrations were above the limit of quantitation (LOQ) for the particular sample (a signal three times the noise level). In this study, the LOQ was 0.02 ng/μL or 1 ng/g for all butyltin and phenyltin compounds, and 0.1 ng/μL or 5 ng/g for TCT. Every organotin peak was identified by its mass spectrum by comparing with standard spectra (Table 4.1).

TBT was detected (>1 ng/g) at 9 out of the 11 sites, where concentrations ranged from 6 to 1442 ng/g wet weight (Table 4.2).

TPT was detected at 5 sites only with a maximum concentration of 530 ng/g wet weight measured at the most downstream site (Ile d'Orléans). No TPT degradation products (DPT and MPT) were detected in any of the samples. The highest concentrations of all butyltin compounds were found in Bassin Louise and values appeared generally higher at all harbour sites relative to other sites (Table 4.2). Except for the Bassin Louise site, neither DBT nor MBT, which were probably breakdown products of TBT, was detected at other sites. No organotin was detected at Tracy and Ile Lapierre, two relatively close sites located at the upstream end of Lake St. Pierre.

Table 4.2: Concentrations of organotins in zebra mussels (ng/g wet weight) at different locations in St. Lawrence River.

Site	Area and substrate	Water content (%)	TBT	DBT	MBT	TPT
1- Cornwall	Harbour	90.5	24	<1	<1	<1
2- Beauharnois	natural bottom	87	6	<1	<1	<1
3- Pointe-a-Peladeau	natural bottom	87	17	<1	<1	<1
4- Boucherville	natural bottom	87	10	<1	<1	2
5- Tracy	Dock wall	87	<1	<1	<1	<1
6- Ile Lapierre	natural bottom	84	<1	<1	<1	<1
7- Port de Becancour	Harbour	85	44	<1	<1	4
8- Portneuf	Harbour	85	16	<1	<1	<1
9- Bassin Louise	Harbour	88	1442	351	322	16
10- Quai Levis	Harbour	87	42	<1	<1	252
11- Ile d'Orleans	natural bottom	86	67	<1	<1	530

4.5: Discussion

This is the first investigation on the availability of organotin compounds in freshwater biota of the St. Lawrence River. The large range in response of over 3 orders of magnitude in TBT levels detected among sites reinforces the suggestion that zebra mussels offer a good potential as a biomonitor or sentinel species for organotins in aquatic ecosystems. Organotin concentrations measured in zebra mussels in Bassin Louise was comparable to those found in Switzerland, the Netherlands, and areas of the Great Lakes system within Canada (Table 4.3).

Figure 4.3: Locations with high organotin concentrations (ng/g wet weight).

Country	Site	Shell Length (mm)	Organotin compounds (ng/g wet weight)			Source
			TBT	DBT	TPT	
Netherlands	Brielse meer	20-35	1353	203	141	Stab et al, 1995
	Vinkeveen	20-35	1550	1975	800	Stab et al, 1995
Switzerland	Marina: Lake Lucerne	18-25	2800	470	640	Fent and Hunn, 1991
Canada (marinas and harbours)	Lakeview Marina, Windsor	10-15	189	24	no data	Chau et al., 1997b
	Kingston Harbour	10-15	880	133	no data	Chau et al., 1997b
	Kettle Creek Marina	10-15	289	31	no data	Chau et al., 1997b
	Bassin Louise Marina	20-25	1442	351	16	This Study

TBT concentrations measured in mussels from Bassin Louise (1442 ng/g) were the highest reported for zebra mussels in freshwaters of Canada. The difference in organotin concentrations may, however, partly be due to the difference in the size of mussels. Mussels analysed in this study were larger (20-25 mm), and possibly older (2 to 3 years old) than those collected in the previous study by Chau et al., 1997 (10-15 mm) (1 year old). There is no systemic study on the relationship between mussel size and organotin concentrations, so size adjustment is not possible. However, organotin concentrations are reported to be higher in bigger mussels (Stäb et al., 1995).

Fent and Hunn (1991) reported high quantities of TBT (1200-3700 ng/g wet weight) in zebra mussels from marinas in a Swiss lake. In our study, high concentrations were also found in a marina located in a confined and poorly flushed area and in harbours in the vicinity of shipyards and commercial docks. Bassin Louise is the largest marina near Quebec City and its access is controlled by a lock limiting water exchange with the St. Lawrence River. It contains, on average, 300 pleasure craft of ranging sizes between 7 to 17 meters.

There are no data on LD_{50} of TBT on zebra mussels. Becker and Tarradellas (1994) reported that neither growth nor mortality was affected in a field experiment where TBT body concentrations reached up to 26000 ng/g dry weight, or 2600 ng/g wet weight, assuming 90% water content in the organisms. Therefore, the TBT concentrations (1442 ng/g wet weight) found in mussels of the Bassin Louise Marina may not have a significant effect on growth or mortality. However, chronic toxicity effects on zebra mussel larvae and/or

juvenile stages have been observed, resulting in reduced growth rates, at TBT burdens of 6200 ng/g wet weight (Becker et al., 1992). Reduced juvenile growth rates were observed at TBT concentrations of 1500 ng/g wet weight in tissue of the marine blue mussel, *Metilus edulis* (Salazar and Salazar, 1991). Growth defects occurred at 1700 ng/g wet weight TBT for other aquatic species such as water fleas (*Daphnia magna)* (Fent and Hunn, 1991) and 90 ng/g wet weight in the marine clam *Scrobicularia plana* (Langston and Burt, 1991). Moreover, more subtle effects such as physiological changes may occur at much lower TBT concentrations. For example, Morcillo et al. (1998) showed that testosterone concentrations were significantly increased in the clam (*Ruditapes decussata*) when they were exposed to 91 ng/L TBT for 7 days, resulting in body residue levels of 52.8 ng/g wet weight. The ecological significance of these physiological changes, however, is not clear.

It has been reported that organotins caused shell thickening in oysters (*Cassostrea gigas*) by disrupting the mechanism of calcium secretion (Alzieu, 1991). We observed a considerable thinning in the shells of mussels collected from Bassin Louise. Whether it was due to organotin accumulation was however not clear.

Because TPT was used in small amounts together with TBT in organotin containing antifouling paints, it is expected that TPT would be present when high TBT concentrations are found as in Bassin Louise. Mussels from Quai Lévis and Ile d'Orléans sites had relatively low TBT concentrations but relatively high TPT concentrations suggesting that the source of organotin in these sites may be

different than those in Bassin Louise. The main use of TPT is as a fungicide for crop protection (potato, celery and sugar beet, Stäb et al., 1995). None of the degraded products of TPT (DPT and MPT) were detected in the mussel samples. It is possible that TBT can be more readily degraded than TPT (Kannan and Lee, 1996). Alternatively, TPT may be subject to a lower rate of metabolization in zebra mussels, than TBT (Stäb, et al., 1996).

In summary, organotin concentrations in zebra mussels at most stations in the St. Lawrence River were generally low and sometimes undetected. Marinas and harbours in the St. Lawrence River appear to be the main potential source of TBT exposure to organisms in the river. The high levels in the mussels from the Bassin Louise marina suggest that organotin residuals in the sediment or illegal use of the antifouling paint may remain a problem locally. Further studies on the concentrations and fate of organotins in commercial harbours, shipyards, and marinas, and close to areas of high boating and shipping activity need to be conducted to assess where the levels of organotin may be a potential danger of organotins to freshwater biota in the St. Lawrence River and elsewhere. In this regard, the use of zebra mussels, now a commonly distributed species in North America, is advocated to allow comparison among studies.

CONNECTING STATEMENT

The bioavailability of organotins along the St. Lawrence River were generally found to be low. However zebra mussels from sampling sites close to Quebec City, more specifically, Bassin Louise, Quai Lévis and Île d'Orléans, had elevated organotin concentrations. A follow-up study was conducted in the Quebec City Harbour area to determine the distribution of organotin contamination from these sites to the St. Lawrence River.

CHAPTER 5

Organotins in zebra mussels (*Dreissena polymorpha*) and sediments in the Quebec City Harbour area of the St. Lawrence River.

To be Submitted to Hydrobiologia

5.1: Abstract

Toxic antifouling agents such as tributyltin (TBT) and triphenyltin (TPT) have been released in aquatic ecosystems through the use of antifouling paint applied to ship hulls, pleasure crafts and fish nets. The purpose of this study was to assess the extent of the distribution of organotins from a contaminated marina to the St. Lawrence River system by measuring organotin concentrations in zebra mussels (*Dreissena polymorpha*) and in sediments collected from 9 sites along the St. Lawrence River near Quebec City in July 1998. TBT concentrations in zebra mussels were between 37 and 1078 ng/g wet weight, with the highest value found in the marina Bassin Louise and elevated concentrations were found in two other marinas. The concentrations decreased sharply to background levels just outside the marinas. All butyltins were detected in all sediments analysed, with highest values found in the Bassin Louise Marina. Phenyltins were detected in 3 of the 9 sites in low concentrations (<55 ng/g) in zebra mussels. There was a significant correlation between TBT in sediments and mussels. This study suggests that TBT contamination may remain a problem in localized freshwaters.

5.2: Introduction

Organotins gained widespread application as an effective antifouling paint biocide on pleasure boats, large ships, and docks in the 1970's and 1980's. As tributyltin (TBT) leaches directly from paints into water, high contamination of pleasure boat and commercial boat harbours and coastal areas resulted. This is the major pathway of entry of TBT into the aquatic environment. Triphenyltin (TPT) compounds have also been employed as a co-toxicant with TBT in some antifouling paints. However, the major employment of TPT compounds lies in agriculture, where they are used as fungicides in crop protection (potato, celery, sugar beet, coffee, rice) (Stäb et al., 1995).

The environmental degradation of dissolved TBT proceeds by successive dealkylation reaction to produce dibutyltin (DBT) and monobutyltin (MBT). Diphenyltin (DPT) and monophenyltin (MPT) are degradation products of TPT.

The ecotoxicological hazards of organotin compounds have been recognized after deleterious effects occurred in the late 1970s on oyster populations in Arcachon Bay in France (Alzieu et al., 1982). The shells of pacific oysters *Cassostrea gigas* began to exhibit morphological abnormalities and the spatfall also declined dramatically. Shell abnormalities including chambering and the formation of protein-containing jelly, were observed. Oyster malformations were also detected along the coast of England (Waite et al., 1991), Spain (Morcillo et al., 1997) and North America (Uhler et al., 1993). Important negative effects on reproduction were found in neogastropods in England. The imposition of male sexual organs, including a penis

and vas deferens, on female mud snails *Nassarius obsoletus* was linked to TBT contamination (Smith, 1981).

Therefore, the use of TBT-containing antifouling paints is now controlled and banned in many countries, resulting in a decrease of TBT contamination of coastal and harbor waters. Canada regulated TBT in 1989 for large ships and prohibited the use on vessels <25 m in length (Chau et al., 1997a). A water quality value of 3.3 ng/L was set for freshwaters and 0.41 ng/L for marine waters. However, antifouling paints remain an important source of organotins to the aquatic environment due to their use in large vessels, illegal use on pleasure craft and applications in countries without regulations.

The pollution of freshwaters by organotins can be studied by the analysis of concentrations in water, sediments or in some members of the indigenous biota common to a region under study. Although the analysis of pollutant levels in water is the most direct way of studying organotin levels, it is frequently costly, gives variable measures, and is often at levels under detection limits. Because of the filter-feeding behavior and the generally high potential for bioaccumulation of contaminants, including organotins, bivalves have been widely used as sentinel organisms for monitoring the contamination of aquatic ecosystems. In freshwaters, zebra mussels (*Dreissena polymorpha*) are of particular interest. Zebra mussels have been used as a biomonitor in Europe where they are widely distributed geographically, sedentary, and easy to collect (Stäb et al., 1995; Fent and Hunn, 1991; Fent and Looser, 1995; Becker et al., 1992, Carlier-Pinasseau et al., 1996). Following their accidental introduction from Europe into North America during the 1980's, zebra mussels rapidly

dispersed throughout the Great Lakes (Hébert et al., 1991; Nalepa and Schloesser, 1993). They were first noted in the fresh waters of the St. Lawrence River in 1989 (Lapierre et al., 1994) and are now integrated into the Great Lakes and St. Lawrence River systems.

Our previous study (Chapter 4) surveyed organotin concentrations in zebra mussels collected from 12 sites along the St. Lawrence River in 1996 and found that there may be point sources of TBT in marinas, particularly in the Quebec City area. Quebec City harbour is the deepwater connection to the agricultural and industrial heartlands of Quebec, Canada. With 15 meters of water depth at low tide, vessels carrying up to 150,000 tons can be accommodated. Just after Quebec City, the river not only becomes narrower, but also more shallow, and therefore, restricts the passage upstream to fewer and smaller ships. This makes the Quebec City area one of the few freshwaters exposed to such large ships.

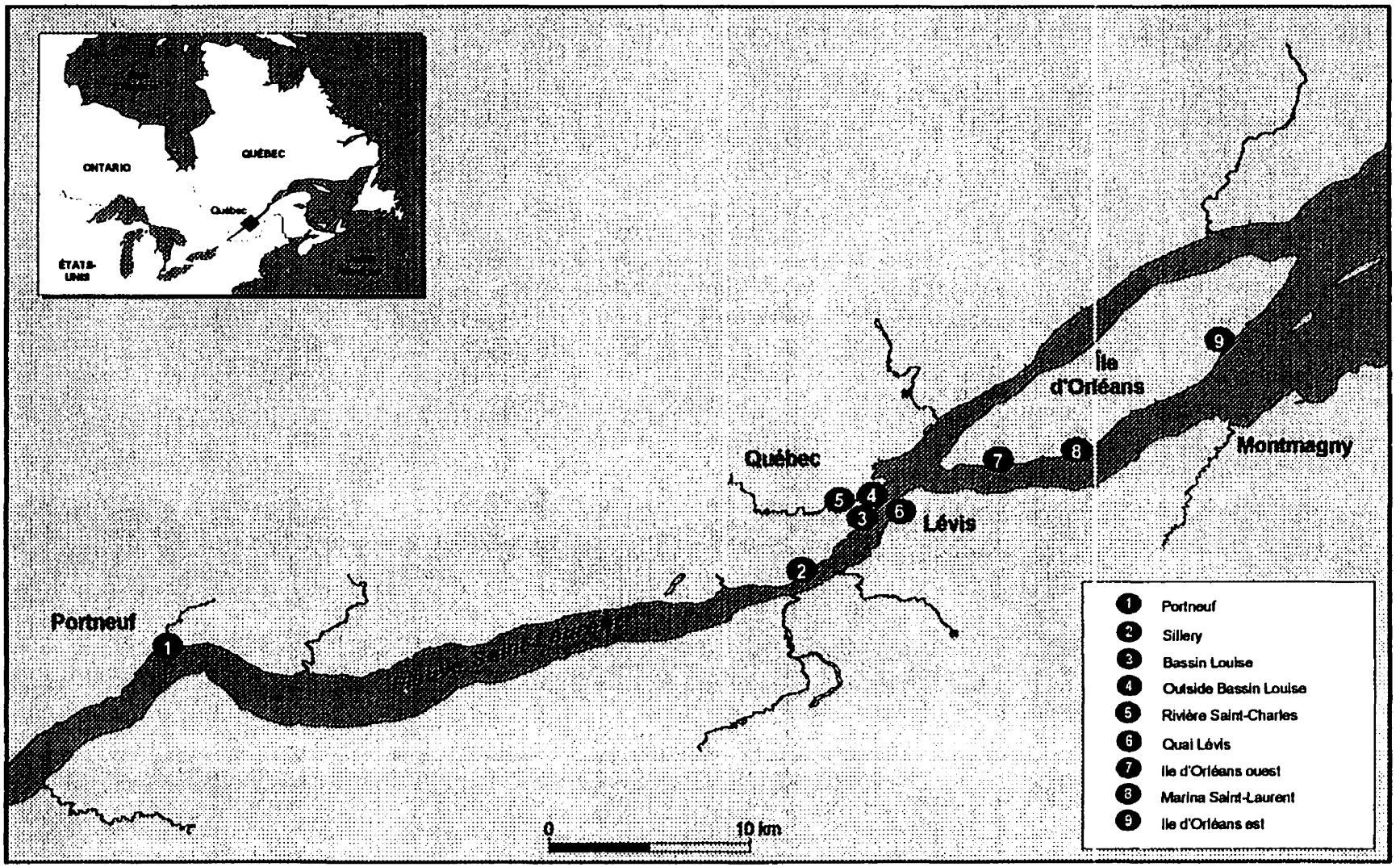
The objective of this study is to investigate the extent of distribution of TBT from the marinas to the river system using zebra mussels as a biomonitor. Sediment samples were also analysed to investigate the bioavailability of organotins in the system.

5.3: Materials and Methods

5.3.1: Sampling

Zebra mussels and the surface layer of sediments were collected from 9 sites along the Saint-Lawrence River (Figure 5.1a)

Figure 5.1a: Sampling sites near Quebec City where zebra mussels and sediments were collected in July 1998.



Samples were collected at 3 stations from each of the 9 sites. Mussels were collected at 3 to 10 meters deep along dock walls. Only site 7, Ile d'Orleans West, zebra mussels were collected from the natural bottom. Zebra mussels were brought to the surface and maintained in a container filled with river water for 24 hours to allow gastric purgation. Sediments samples were taken by grabbing 3 handfuls of the top layer of sandy bottom just below the site where zebra mussels were collected. The sampling period was made as short as possible to allow meaningful comparison between all locations. The period was set between the 13th and the 16th of July, 1998, which is a time of peak yachting and shipping activity.

5.3.2: Sample preparation for GC/MS analysis

Zebra mussel analysis for samples collected in 1998 was performed using a protocol similar to Stäb et al. (1995) and described previously (Chapter 4). Two replicates per station were analysed. Approximately 20 mussels of 20-25 mm shell lengths were used per replicate.

Organotin compounds in sediment were measured using a modified protocol of Cédric et al. (1998).

Sediment samples were first sieved using a 1mm mesh, freeze dried and then crushed in a mortar, and sieved again using a 100 μm mesh. A fraction of 2 g was used to determine water content, 2 g for determining organic content by ashing and 1 g for organotin analysis.

Samples of 1 g of sediment were put in a 50 mL centrifuge tube with a cap.

Samples were then filled with 25 mL of 1 M sodium acetate, 1M acetic acid in methanol solvent. Samples were heated within 5 minutes to 85°C. The solvent was extracted 5 times, and between each extraction, 4 mL of new solvent was added. Samples were rinsed with 4 mL of solvent and purged in nitrogen. Combined extracts were transferred into a 250 mL volumetric flask containing 7 g NaCl. The pH was adjusted to 5.0 with 1 M NaOH. Flasks were filled to 250 mL with nanopure water, and 2 mL hexane and shaken for 12 hours at room temperature.

Grignard reagent (0.5 mL) was added to 0.4 mL of the extract in a 15 mL centrifuge tube and put in a 30°C water bath for 40 min. Five mL of H_2SO_4 was added to destroy the excess Grignard reagent and the samples were centrifuged at 1,800 rpm for 5 min. The organic layer was put in a 15 mL centrifuge tube containing Na_2SO_4 and dried to 2 mL under nitrogen.

Samples were further cleaned within 2 hours using alumina column chromatography. A glass column (1 cm id X 30 cm) was wet packed with 1 g of Na_2SO_4 and 5 g of alumina in hexane. Two mL of samples were applied to the column and eluted with a 30 mL diethyl ether in hexane (1:9) mixture. The eluents were concentrated on a rotary evaporator to about 2 mL, and transferred in 15 mL conical centrifuge tubes, and evaporated to 100 μL under a gentle stream of nitrogen. The concentrated samples are spiked with 5 μL chrysene dissolved in iso-octane (20 ng/μL) as an internal standard.

Analysis

The presence of an organotin species was tentatively confirmed if it occurred within the appropriate chromatographic window, and the concentrations were above the limit of detection (LOD) (a signal three times the noise level) found to be 0.02 ng/μL for all butyltin and phenyltin compounds, and 0.1 ng/μL for TCT. The limit of quantitation (LOQ) for the particular sample was 4 ng/g for all butyltin and phenyltin compounds in 0.4 g of dry sediment.

Conditions for GC/MS

Separation and quantitation of the organotin compounds were performed using a Gas Chromatography/Mass Spectrometer. The conditions used were similar to those described by Stab et al, (1992). GC analysis were carried out using a Varian 3400CX gas chromatograph coupled with a Varian Saturn 4D GC/MS ion trap mass spectrometer (Varian, Walnut Creek, CA). Samples were introduced through a Varian 8200 autosampler equipped with a programmable septum injector (SPI). Separation was performed on a DB-5 MS-ITD column (30m x 0.25mm id x 0.25*u*m film thickness, J&W Scientific Folson, CA). The mass spectrometer was calibrated daily with perfluorotributylamine. 1 uL of sample solution was injected with the following temperature program: initial SPI injector temperature of 135°C was immediately raised to 300°C for 45 min. Initial GC oven temperature of 80°C was held for 1 min, and raised to 120°C within 3 min, then raised to 260°C within 28 min, and finally raised to 300°C within 20 min, and left at this temperature for an additional 6 min to clean out the column. Solutions of organotin mixtures at 3 different concentrations were used as

external standards. Peak areas, relative to the internal standard (chrysene) which was monitored at m/z 240, were used for plotting calibration curves.

5.3.3: Quality Control

Methods of quality control were applied to the monitoring of organotin recoveries in sample preparation procedures in this study. Certified reference materials (CRM) were used: dry fish tissue from the National Institute for Environmental Sciences (NIES), Japan, for zebra mussel analysis, and sediment PACS-II from the National Research Council (NRC) of Canada, for sediment analysis. Recoveries were within 8% of the referred value for fish tissue and within 10% for sediment material.

5.4: Results

The variation between 2 replicates from each station was within 10%. Concentrations of organotins in zebra mussels are summarized in Table 5.1. The data are a mean value ± SD of the samples for the 3 stations. TBT was detected at all 9 sites analysed, and concentrations were greater than 25 ng/g at all sites.

Table 5.1: Concentrations of organotins in zebra mussels (ng/g wet weight) collected in 1998 in the St. Lawrence River near Quebec City.

Site	Substrate/ Area	Water Content (%)	TBT (ng/g) n=3 ±SD	DBT (ng/g) n=3 ±SD	MBT (ng/g) n=3 ±SD	TPT (ng/g) n=3 ±SD
1-Port Neuf	Harbour	89	37 ±9	<1	<1	7 ±3
2-Sillery	Marina	89	327 ±73	61 ±15	23 ±11	<1
3-Bassin Louise	Marina	91	1078 ±222	158 ±54	134 ±60	<1
4-Outside Bassin Louise	Harbour	89	895 ±170	111 ±16	88 ±12	<1
5-St. Charles River	Harbour	91	480 ±218	106 ±60	34 ±12	<1
6-Quai Lévis	Harbour	90	71 ±14	<1	10 ±0	28 ±17
7-Île d'Orleans West	Natural bottom	90	77 ±14	<1	<1	<1
8-St. Lawrence Marina	Marina	91	225 ±61	71 ±19	27 ±8	52 ±2
9-Île d'Orléans East	Peer	91	66 ±16	<1	12 ±4	<1

Highest butyltin concentrations occurred in Bassin Louise. By-product DBT was detected at 5 of the 9 sites, where TBT concentrations were highest (>200 ng/g). MBT was detected at 7 of the 9 sites, at the same sites where DBT was detected and in 2 more locations where TBT was lower than 200 ng/g (site 3 and 9). TPT was detected at 3 of the 9 sites at levels ranging from 7 to 52 ng/g. No by-products of TPT (DPT and MPT) were found in any of the mussel samples. Organotin levels in sediments are presented in Table 5.2.

Table 5.2: Organotin concentrations in sediments (ng/g dry weight) collected in July 1998, from the St. Lawrence River in the Quebec City area.

Site	Organic carbon Content (%)	Water Content (%)	TBT Conc. (ng/g) N=3 ±SD	DBT Conc. (ng/g) n=3 ±SD	MBT Conc. (ng/g) n=3 ±SD	DPT Conc. (ng/g) n=3 ±SD
1- Port Neuf	5.63	45.7	97 ±25	286 ±137	989 ±85	<1
2- Sillery	7.11	50.5	146 ±15	165 ±98	87 ±57	<1
3- Bassin Louise	8.69	52.0	888 ±203	997 ±524	203 ±51	<1
4- Outside Bassin Louise	6.17	46.0	807 ±282	634 ±54	185 ±60	<1
5- St. Charles River	8.21	50.0	330 ±79	579 ±279	165 ±63	<1
6- Quai Lévis	2.65	32.8	173 ±102	496 ±158	123 ±46	15 ±30
8- St. Lawrence Marina	3.05	33.6	209 ±73	389 ±112	4 ±8	101 ±90
9- Île d'Orléans East	6.13	57.2	211 ±36	45 ±12	6 ±12	<1

Organotins were detected in all sediments analysed except for site 7, Île d'Orléans West where sediment was too rocky and no samples could be analysed. All 3 butyltins compounds were detected in all sites. TBT concentrations ranged from 97 to 888 ng/g dry weight, and again the highest value at the Bassin Louise site. DBT concentrations ranged from 45 to 997 ng/g dry weight, with again the highest concentration in the Bassin Louise site. The range of concentrations of MBT was from 4 to 989 ng/g dry weight, and the highest value was found at the Port Neuf site. Phenyltins were detected in 2 sites, in the form of DPT, at concentrations of 15 and 101 ng/g dry weight at Quai Lévis and St. Lawrence marina respectively.

5.6: Discussion

Organotin contamination in sediments in this study were more comparable to Canadian marine harbour sites than to freshwater harbour sites (Chau et al., 1997a, Table 5.3).

Table 5.3: Total organotin concentrations in sediments in Canadian Harbours.

Canadian Harbours	Total Butyltin ng/g dry wt	References
Freshwater harbours		
Hamilton Harbour	62.6- 218.3	Chau et al.. 1997a
Toronto Harbour	92.2- 563.7	Chau et al., 1997a
Kingston Harbour	59.8- 1493.0	Chau et al., 1997a
Montreal Harbour	62.6- 332.4	Chau et al., 1997a
Marine Harbours		
Saint John Harbour	157.2- 3666.6	Chau et al., 1997a
Halifax Harbour	796.6- 2520.4	Chau et al., 1997a
Burard Inlet (Vancouver)	80.9- 3250.7	Chau et al., 1997a
Quebec City Harbour area	262.0- 2088.0	This study

Results of this study have confirmed our previous finding that Bassin Louise is a hot spot of organotin contamination. A primary concern was whether the Bassin Louise Marina acted as a point source of contamination outside of the marina. Zebra mussels and sediments collected just outside the marina (site 3) were high in butyltins, with just slightly lower values than in the marina (site 4). The marina therefore acts as a source of contamination to the river system. The next closest site analysed is Quai Lévis (site 6), across the St. Lawrence River, where TBT concentrations in zebra mussels are relatively low. The contamination gradient declined sharply within this 1 Km distance, probably due to dilution in the high current of the river and removal of TBT to sediments (Figure 5.1b).

Figure 5.1b: Sites 3,4,5 where zebra mussels and sediments were collected in July 1998.

The physical aspects of the river around the Quebec City area are unique in that east of Quebec City, the St. Lawrence River becomes a funnel-shaped estuary. Because the river suddenly widens, this area is tidal, and its currents are strong. The alternate rise and fall of the surface of the water occurs twice a day with tidal ranges of up to 7 meters along the shores of the river. For this reason there is constant mixing of water in the Quebec City area (approximately between Sillery, and mid-way through Île d'Orléans) (Gingras et al., 1997).

TBT concentrations in zebra mussels collected from the Saint-Charles River (site 5), another site in proximity to Bassin Louise, were also elevated. The St. Charles River flows out to the St. Lawrence River. These elevated levels could possibly be due to a gradient effect from the contamination at Bassin Louise, or may be a result from the presence of large cargo ships docking on either side of the river. Therefore, St. Charles River docks may also act as a source of TBT contamination to the St. Lawrence River. Quai Lévis (site 6), Ile d'Orléans West (site 7), and Ile d'Orléans East (site 9) are sites within the St. Lawrence River exposed to diluted contamination gradients from several point sources, and its own exposure to the ships' traffic. TBT concentrations in zebra mussels from these sites demonstrate a homogenous level throughout this area of the river (average concentration: 71 ng/g) indicating minimal contamination.

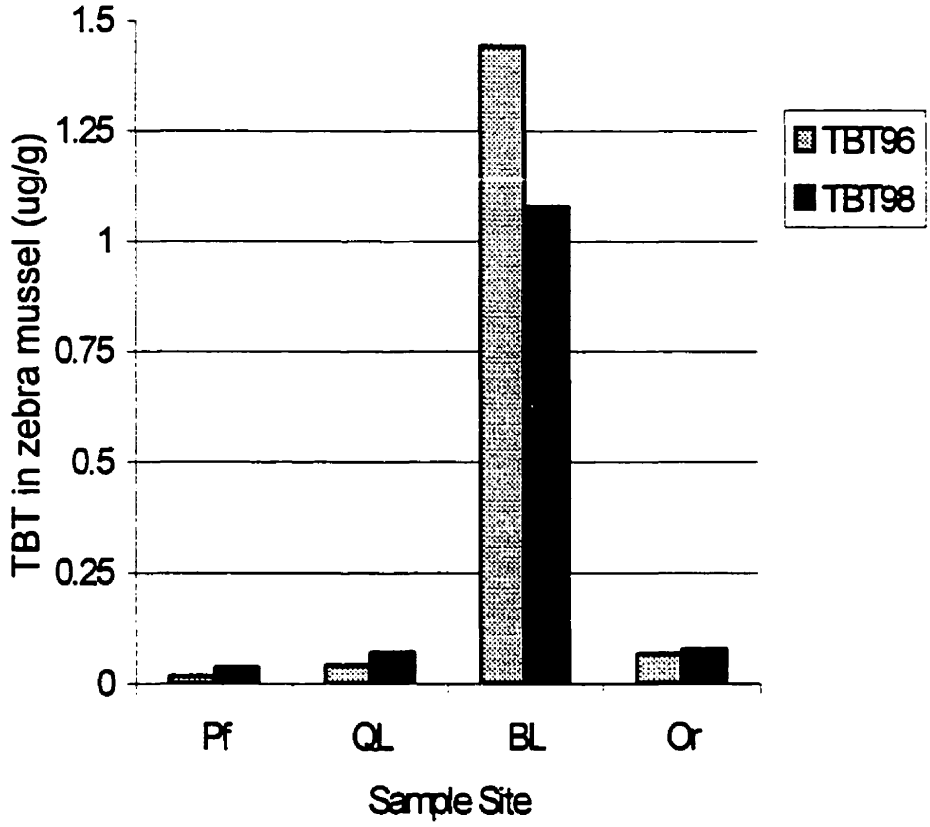
Sillery (site 2) and St. Lawrence Marina (site 8) sites are isolated marinas along the river. Sillery is a relatively large and old marina, with a capacity for 150 pleasure crafts. St. Lawrence Marina is a smaller and more recent marina with approximately 70 pleasure craft. Both marinas showed elevated levels of TBT in zebra mussels (Table 5.1).

Butyltin concentrations in sediments were within the levels found in the rest of the river (see Table 5.2).

Butyltin concentrations were low in zebra mussels of the Port Neuf site (site 1). In contrast, MBT concentrations were high in sediments. A marina was constructed in 1997 at this site. Contamination may occur from antifouling paint particulates off new dock walls, or extensive resuspension of sediments have exposed underlying degraded organotins.

A temporal trend was assessed using four sites where zebra mussels had been collected in 1996 (Chapter 4) (Figure 5.2). These sites are Port Neuf (site 1), Bassin Louise Marina (site 3), Quai Lévis (site 6), and Île d'Orléans West (site 7).

Figure 5.2: Comparison of TBT in zebra mussels collected at 4 sites in 1996 vs 1998



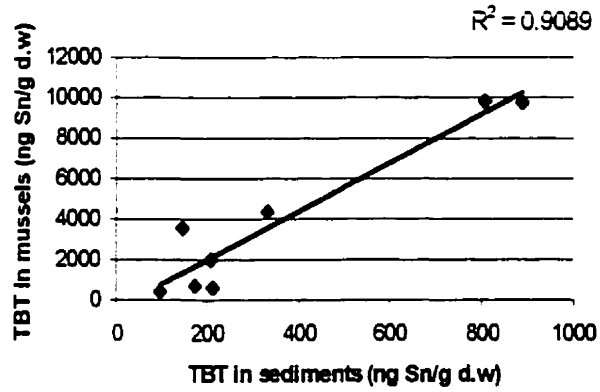
In 3 of the 4 sites, TBT concentrations have increased (from 16 to 37, 42 to 71, and 67 to 77 ng/g wet weight respectively). This may indicate higher boat trafficking in the area. But, there is the possibility that an increase is due to a 3 week difference in sampling times (late June 1996, and mid-July in 1998). TBT concentrations declined at the Bassin Louise site (1442 to 1078 ng/g wet weight), from the concentration in zebra mussel tissue collected in 1996. This is also true for DBT and MBT at this site. This may indicate a decrease in anti-fouling paint use in the marina. In addition, the water

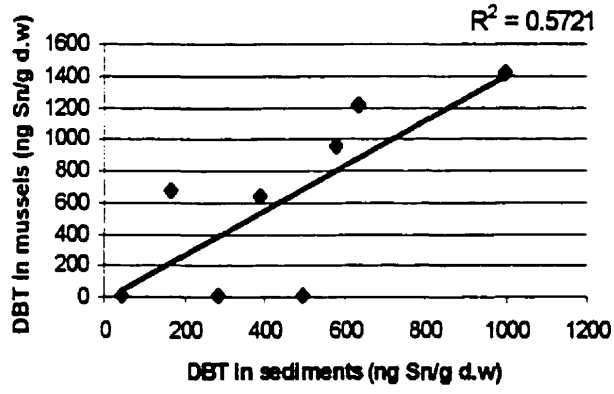
from the marina had been flushed out in the spring, and this may account for the lower values of butyltins.

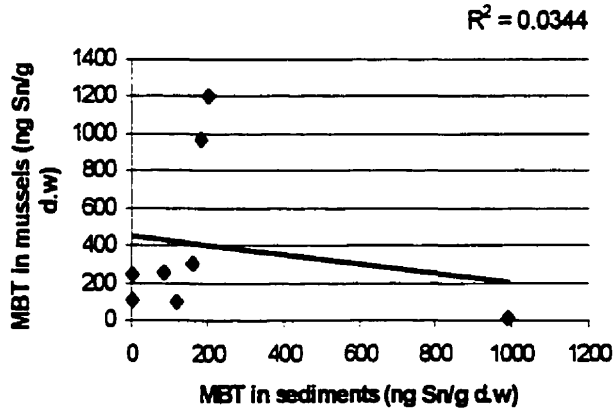
TPT levels detected in Quai Lévis were low and not detected in Île d'Orléans West in 1998, although relatively high concentrations were found at these sites 2 years before. TPT is mostly used as biocides for agriculture, but these are illegal in Canada. Stäb et al. (1995) detected wide fluctuations in TPT levels in zebra mussels within months.

There was a significant correlation between TBT in sediment and in zebra mussels (Figure 5.3a) meaning that TBT in the physical environment was available to the zebra mussels. Becker and Tarradellas (1994) determined accumulation factors for TBT between zebra mussels and sediment in the range 21-254 for 4 Swiss marinas. Chau et al., 1997a, found similar results in the range of 2-222 for 9 locations. The ranges found for TBT in this study, obtained from 8 sites, were between 3-25. The highest concentrations of the butyltin species in zebra mussels were usually found in areas of high sediment contamination.

Figure 5.3: Butyltin concentrations in zebra mussels and sediments; a)TBT, b)DBT, c)MBT (a; P<0.001, y=11.958x - 376.34, b; P=0.06, y=1.427x - 23.575, c; P=0.916, y=-0.2551x - 455.56).







The correlations for DBT and MBT in mussels and sediments were not significant. This suggests that either these organotin compounds are more easily eliminated by zebra mussels, or that they are not as bioavailable as TBT.

Both Langston and Burt (1991) and Becker and Tarradellas (1994) argue that sediment bound organotins are not important sources of zebra mussel contamination because organotins bind to sediment particulates, and are made biologically unavailable to fauna and biota, both in terms of bioaccumulation and ultimately toxicity. Assuming this is true, zebra mussels then represent the organotin availability in the water column. However, others such as Fent and Hunn (1991), Quevauviller et al. (1994), Uhler et al. (1993) argue that sediments not only act as important environmental sinks for TBT in the freshwater environment, but they may also be important sources of TBT to biota. Storm events, dredging, or seasonal changes in the water column influence the resuspension and bioavailability of sediment to freshwater organisms such as molluscs and may diffuse the contaminants farther away from its source. The frequency and magnitudes of many of these events are transient and unpredictable on a year-to-year basis, making it difficult to predict accurately the overall input of organotins from sediments to a local aquatic environment.

All mussels collected in 1998 in this study were taken from dock walls (at the Île d'Orléans West site, mussels were collected from the natural bottom, but analysis was not done on sediments) several meters above the sediments, therefore mussels most likely accumulate organotin particles from the water rather than from sediments, especially in closed systems, where water movement is low. Also, because most sampling sites were along dock walls, there is protection against strong currents and

therefore from sediment resuspension. These sites may not represent the mixing occurring across the river.

The sediment was not fully characterized, however, organic carbon content was less than 10% and water content was around 50% (Table 5.2), showing that the sediment samples were sandy. Total butyltins in sediments ranged from 262 (Ile d'Orleans East) to 2088 (Bassin Louise Marina) ng/g. The rate at which organotins build up (or are lost) in sediments is a function of input rates and subsequent removal through degradation. The ratio of concentrations of TBT to those of its degradation products is often used as an indication of degradability in different media, or organisms. TBT proportions in this study were on average 43%. Sarradin et al. (1994) found similar proportions of 49%. Chau et al. (1997b) found proportion of 51% in Canadian freshwaters. The consistently significant proportions of DBT and MBT to total butyltin in this study indicates that the degradation of TBT in sediments is rather quick, at least at the sediment surface. Breakdown of TBT in estuarine waters are rapid at 28 °C (half-life of one week) and is due to the high metabolic activity of microrganisms at summer temperatures (Olson and Brinkmann, 1986). Water temperatures in the St. Lawrence River, at the time of sampling in 1998, were in the range of 21- 24°C.

Total butyltin concentrations ranged from 37 (Port Neuf) to 1370 (Bassin Louise) ng/g in wet weight zebra mussel tissue. The average TBT/total butyltin ratio for 7 sites (only zebra mussel tissues from sites containing degradation products) was 0.799 (±0.10). Similarly, Chau et al. (1997b) determined as TBT /total butyltin ratio with an

average of 0.71 (±0.10). Slow metabolism of TBT in mussels may account for the high levels of TBT.

The study by Becker et al. (1992) on zebra mussel accumulation and depuration rates indicates bioconcentration factors varying between 6,700 and 260,000 for TBT. The variations are great, and makes it difficult to compare concentrations of organotin in water, which are punctual indications and highly variable measurements, with concentrations measured in an organism, which integrates the amounts of pollutants in the water. It is therefore difficult to extrapolate the toxicological implications to more sensitive organisms based on the TBT residues in mussel tissues of this study.

Zebra mussels in the St. Lawrence River have only inhabited this ecosystem for the past ten years, and consequently trophic food webs are in a state of flux. It is possible that high concentrations of TBT (and other lipophilic chemicals) may be passed more easily to higher organisms via primary consumers of zebra mussels such as diving ducks, and recently introduced round goby (in the Great Lakes) than what might have been the case in the absence of zebra mussels (Chau et al., 1997b).

5.7: Conclusions

Our study has found that sediments in the Quebec City harbour are high in butyltins. However, if resuspended into the water column, butyltin concentrations are diluted by the tidal currents typical of the area, and the availability to the biota, in this case to zebra mussels, remain low.

In marinas, zebra mussels have elevated TBT levels that are similar to concentrations found in coastal harbours, where detrimental effects to sensitive organisms have been observed.

CHAPTER 6

CONCLUSIONS &

RECOMMENDATIONS

Zebra mussels *Dreissena polymorpha* are shown to be suitable biomonitors to assess the bioavailability of butyl- and phenyltin compounds in freshwater estuaries.

The use of *D. polymorpha*, now a commonly distributed species in North America, is advocated to allow comparison between sites and between different studies. Analysing the bioavailability of organotins in zebra mussels, however, does not allow you to interpret what the concentrations represent in terms of toxicity to more sensitive freshwater organisms. Some work is needed to better understand the variability in accumulation rates in this organism to determine a reliable bioconcentration factor (BCF) to allow extrapolations on the availability and risks to susceptible organisms.

Organotin concentrations in zebra mussels along the St. Lawrence River were generally low and sometimes undetected. Marinas and harbours in the St. Lawrence River appeared to be the main potential source of TBT exposure to organisms in the river. The high levels found in the mussels from Bassin Louise suggest that organotin residues in the sediment or illegal use of the antifouling paint may be a potential danger to local freshwater biota. The positive correlations of TBT in sediments and zebra mussels indicate that sediments can be a source. The limited data permitting comparison between the profiles of organotin bioavailability in 1996 and 1998, suggests that environmental levels of TBT were not decreasing.

Ecotoxicological effects on susceptible organisms are known to occur in the concentration ranges presently observed in many parts of the world, mostly marine systems, but also in freshwater: coastal England (Waite et al., 1991), lakes in Switzerland (Fent, 1996; Becker and Tarradellas, 1995), lakes in the Netherlands (Stäb et al., 1995), coastal North-America (Uhler et al., 1993; Page et al., 1996), Japan (Guruge et al., 1996), and the Mediterranean sea (Morcillo et al., 1997; Axiak et al., 1995, Quevauviller et al., 1994). In Canada, Chau et al., (1997a) found that 12 of 89 sites (freshwater marinas and harbours) across Canada exceeded the Canadian water quality guideline of 3.3 ng/L for TBT. TBT levels found in sediments of the Quebec City area were comparable to those found in coastal harbours known to cause detrimental effects to susceptible marine organisms such as oysters and gastropods. The most susceptible organisms in freshwater are fish larvae and coelenterates (Brooke et al., 1986; Seinen et al., 1981). The contamination of freshwater ecosystems by TBT is of concern.

Chronic toxic effects are generally more important in ecological terms than acute effects. The detrimental effects caused by TBT on the reproduction of marine molluscs have lead to reproduction failures and decreasing populations. Pollutants causing such detrimental ecological impacts must have the highest priority in ecotoxicology. Several research recommendations are:

1) Continued monitoring of butyltin residues in fresh water, sediment and biota to determine the effects of the regulation of tributyltin-containing paints in

various countries. Triphenyltin and copper which are other antifoulants and potential replacements for tributyltin, should also be monitored.

2) More information is required on the freshwater toxicity based on body burdens.
3) Development of suitable freshwater biological indicators of butyltin contamination.
4) More data on the persistence of butyltin species in freshwater sediments, and verification of common assumptions made in persistence determinations by sediment core research (knowledge of sedimentation rates, accounting for mixing effects).

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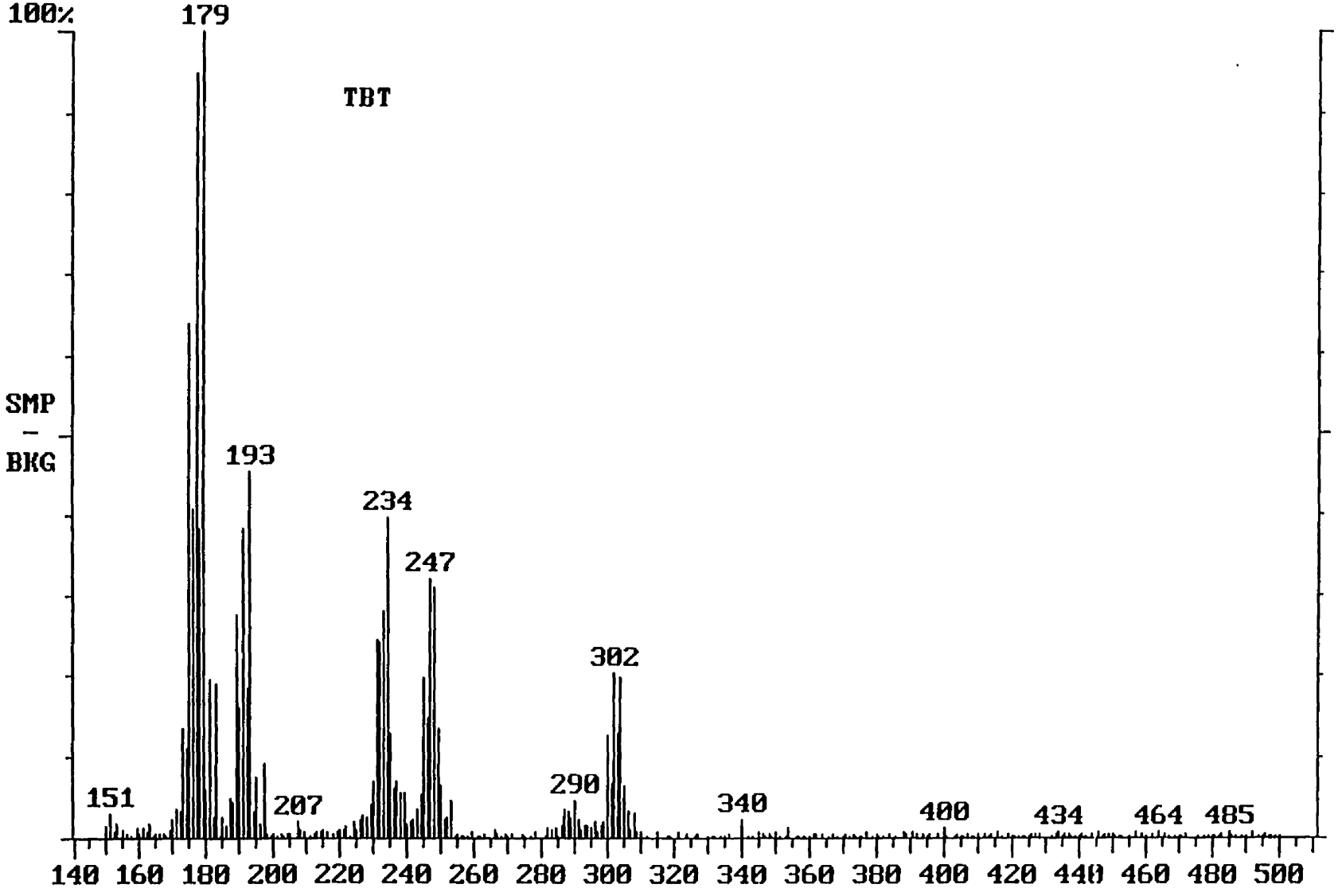
APPENDIX

A chromatogram for each organotin compound studied, TCT and chrysene is shown with their respective ion traces.

Background Subtract D:\SEPTEMBE\MS\98090402 Date: 09/04/98 16:26:43
Comment: 2PPM
Average of: 932 to 936 Minus: 953 to 957 100% = 5811



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Comment: 2PPM
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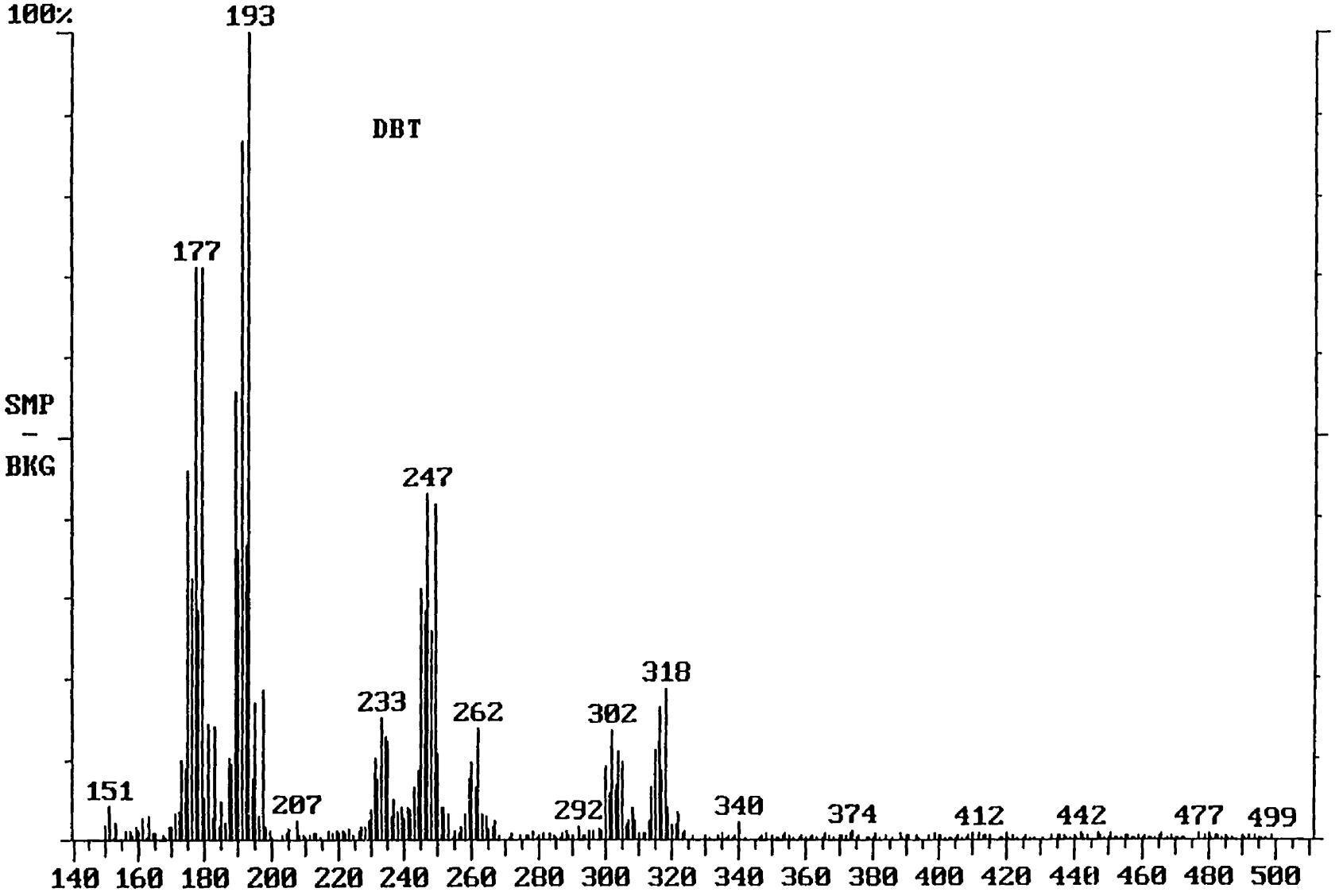

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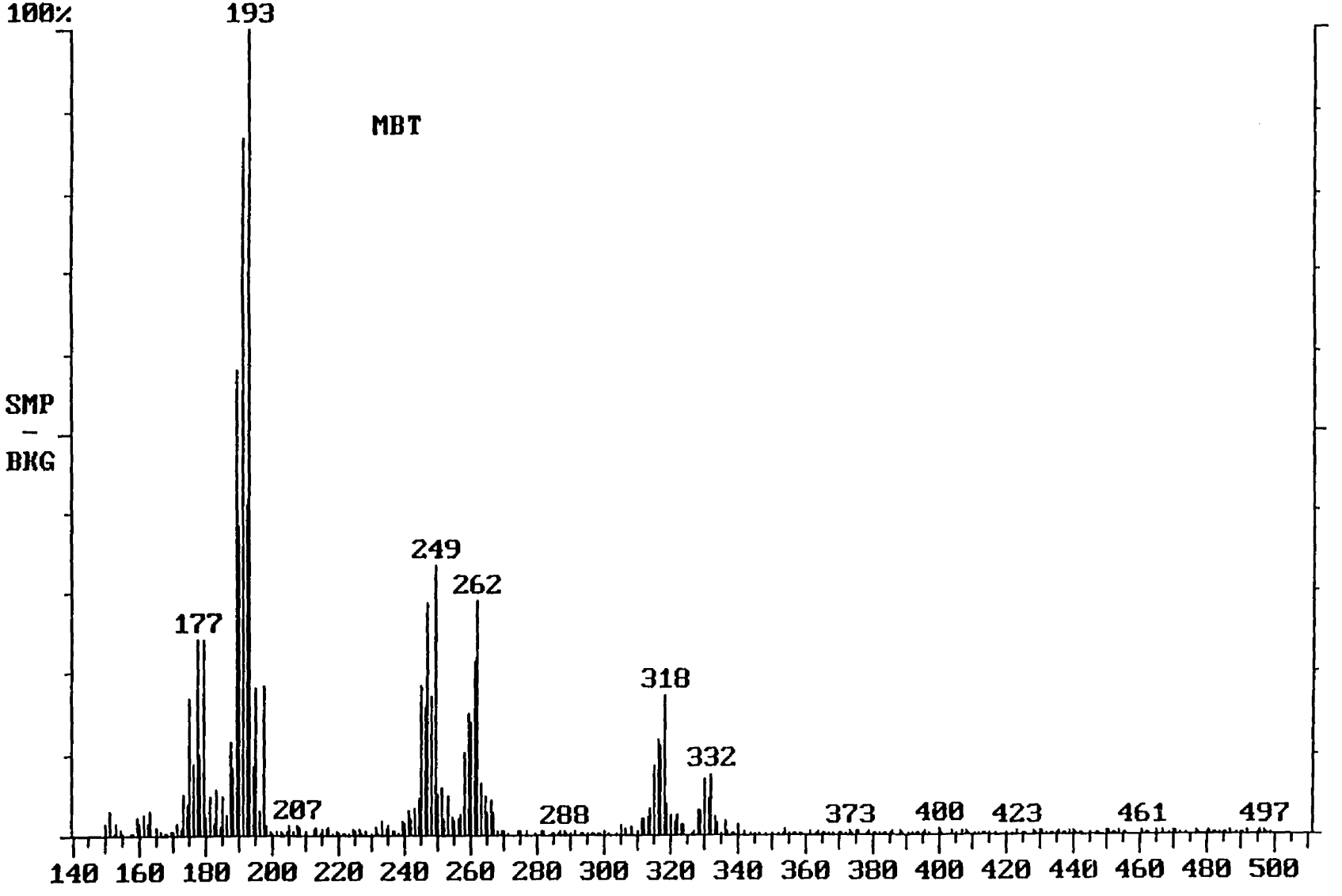

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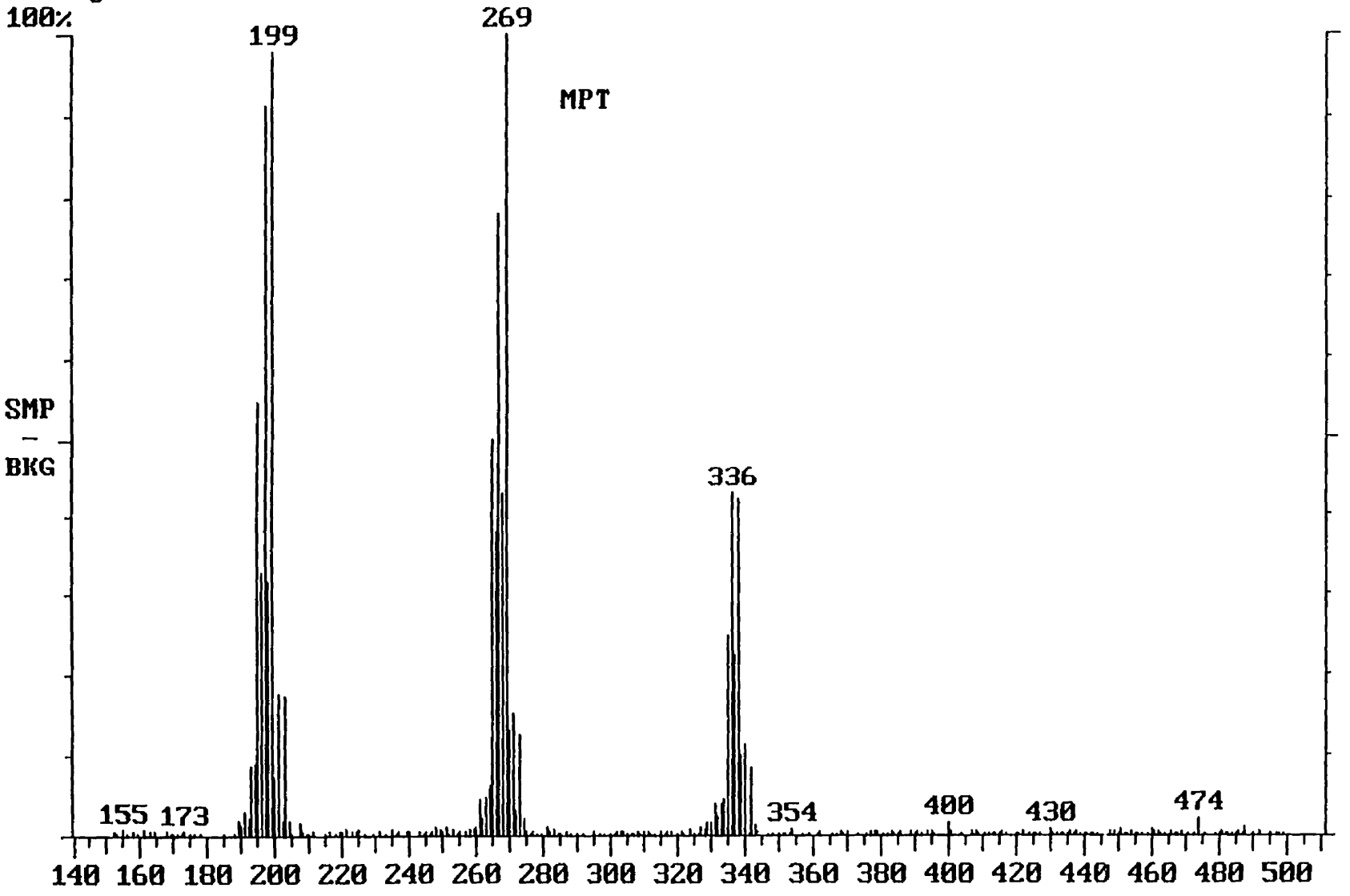

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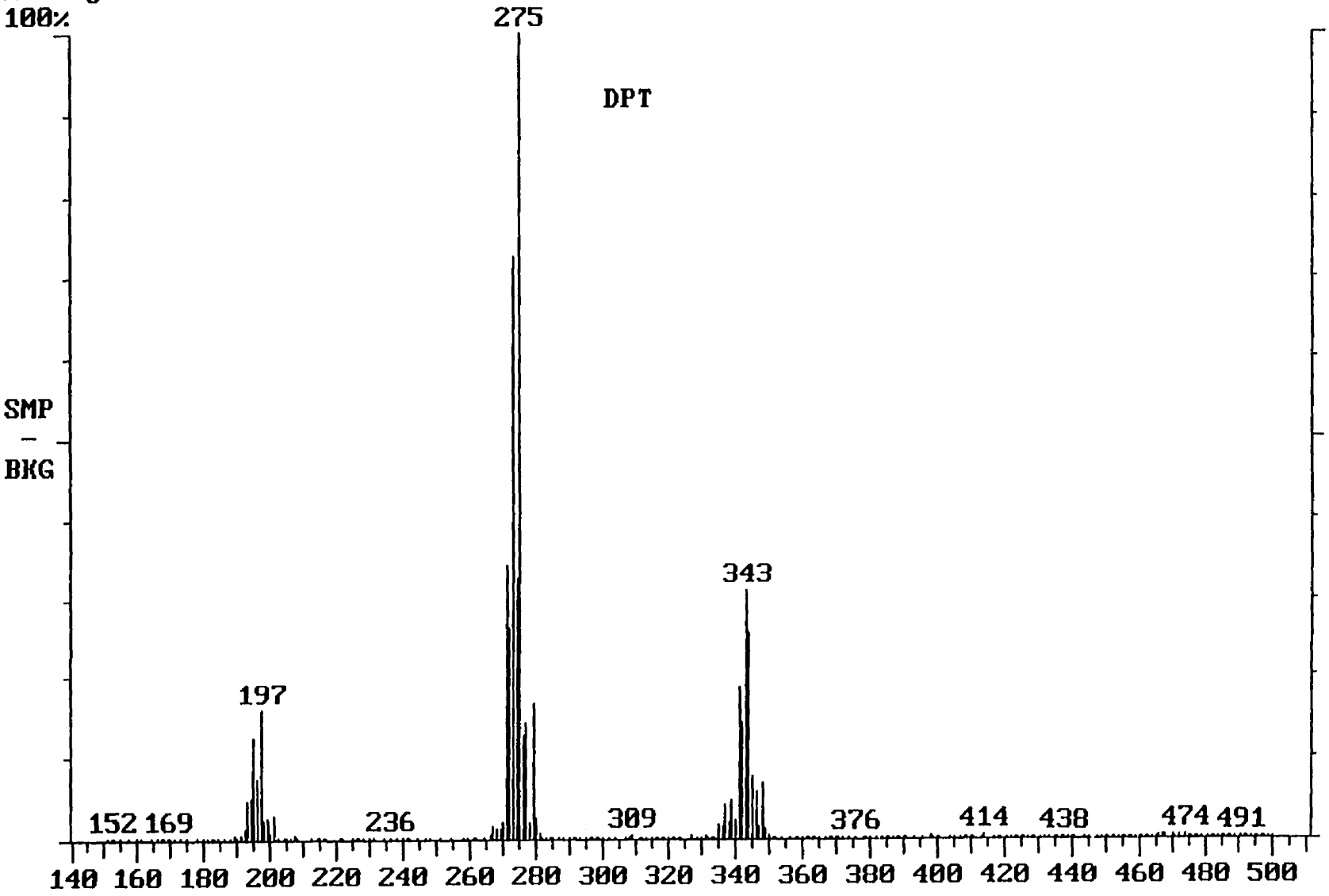

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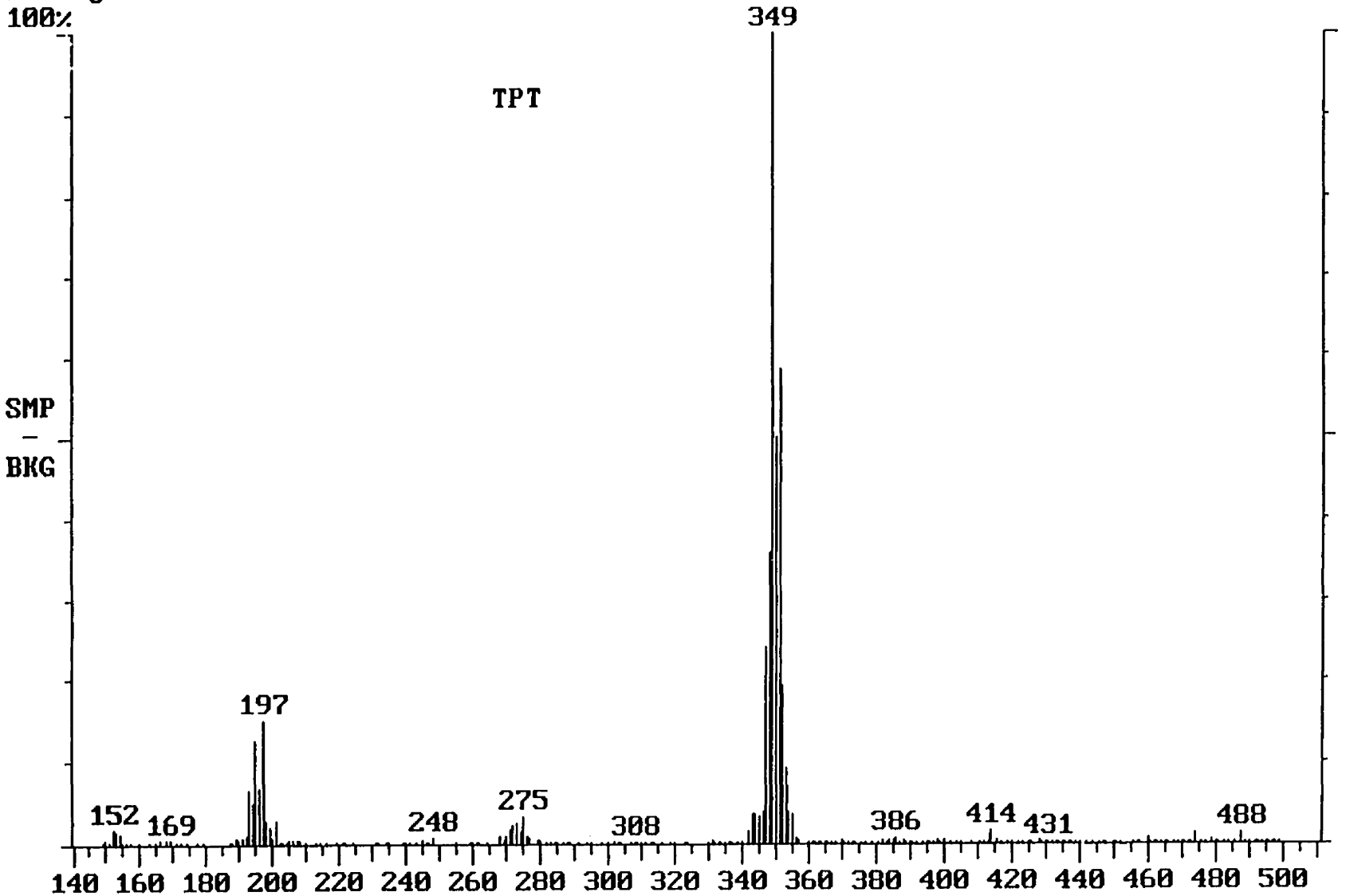
Comment: 2PPM
Average of: 1835 to 1839 Minus: 1809 to 1813 100% = 10764



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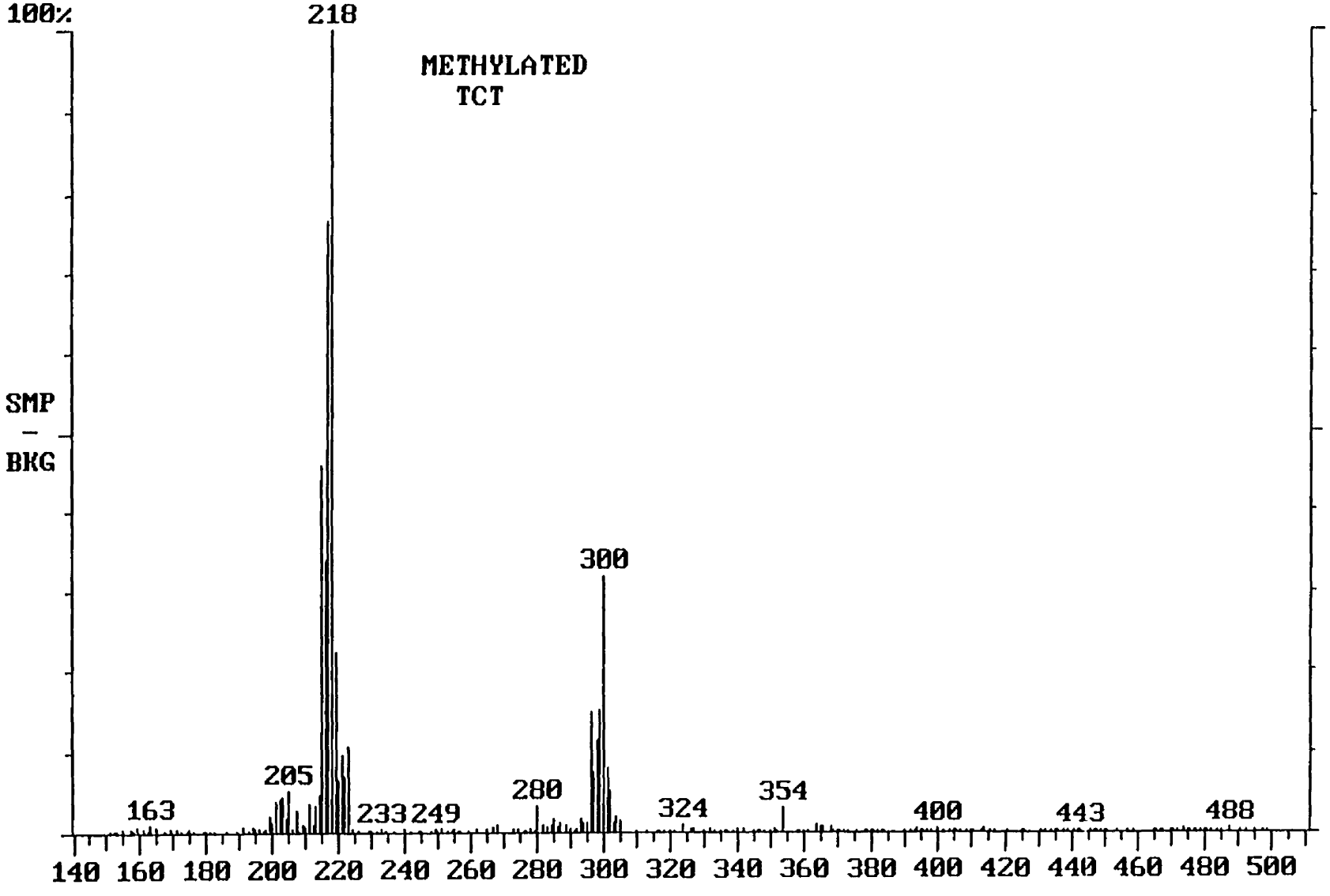

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Average of: 1755 to 1759 Minus: 1727 to 1731 100% = 9804