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BIOACCUMULATION OF BUTYLTIN COMPOUNDS BY MUSSELS IN HARBOURS

by

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RÉSUMÉ

La bioaccumulation de composés de butylétain par les moules d'eau douce (Elliptio complanata) provenant des sédiments contaminés a été étudiée en laboratoire et sur place dans les ports d'Oshawa et de Whitby en Ontario. En laboratoire, l'accumulation de TBT (exprimé sous forme de Sn) par les moules a atteint un maximum de 24 ug/kg à partir des sédiments du port de Whitby qui contenaient 66-110 ug/kg de tributylétain. La concentration de tributylétain dans les sédiments du port d'Oshawa était faible (32-38 ug/kg) et l'accumulation par les moules n'était pas significative. La vitesse et le degré d'accumulation par les moules sont directement proportionnels à la concentration de TBT dans les sédiments.

L'accumulation de tributylétain par les moules était plus élevée dans les deux emplacements du port de Whitby que dans le port d'Oshawa. La vitesse d'accumulation était de 2,1 et 0,7 ng/g/jour respectivement pour les ports intérieurs de Whitby et d'Oshawa pour une période d'exposition de 140 jours. Les facteurs de concentration chez les moules par rapport à l'eau variaient de $4,8 \times 10^3$ à $18,5 \times 10^3$. Les facteurs de concentration par rapport aux sédiments étaient de 0,1 à 10,0 pour le port d'Oshawa et de 2,5 à 18,1 pour le port de Whitby.

Le TBT accumulé dans les sédiments portuaires est disponible pour le biote.

EXECUTIVE SUMMARY

Bioaccumulation of Butyltin Compounds by Mussels in Harbours

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The bioaccumulation of butyltin compounds by freshwater mussels (Elliptio complanata) from contaminated sediments has been investigated in the laboratory and in the field in Oshawa and Whitby Harbours in Ontario. Under laboratory conditions, accumulation of TBT (expressed as Sn) by mussels reached a maximum of 24 ug Kg^{-1} from Whitby Harbour sediment, which contained 66-110 ug Kg^{-1} of tributyltin. Concentration of tributyltin in Oshawa Harbour sediment was low (32-38 ug Kg^{-1}), accumulation by mussels was not significant. Accumulation rate and level of accumulation by mussels is positively related to the concentration of TBT in sediment.

Accumulation of tributyltin by mussels was higher in both locations in Whitby Harbour than in Oshawa Harbour. Accumulation rates were 2.1 and 0.7 ng $g^{-1}day^{-1}$ respectively for the inner harbours of Whitby and Oshawa over a 140-day exposure period. Concentration factors of mussels over water ranged from $4.8x10^3$ to $18.5x10^3$. Concentration factors over sediment were 0.1 to 10.0 for Oshawa Harbour, and 2.5 to 18.1 for Whitby Harbour.

TBT accumulated in harbours sediment is available to biota.

RÉSUMÉ

La bioaccumulation de composés de butylétain par les moules d'eau douce (Elliptio complanata) provenant des sédiments contaminés a été étudiée en laboratoire et sur le terrain dans les ports d'Oshawa et de Whitby en Ontario. Les concentrations de tributylétain (TBT) chez les moules étaient plus élevées dans le port de Whitby que dans le port d'Oshawa. Les concentrations de TBT chez les moules et la vitesse de bioconcentration étaient proportionnelles à la concentration de TBT dans les sédiments.

Mots-clés : bioaccumulation, tributylétain, moules, sédiments.

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Abstract

The bioaccumulation of butyltin compounds by freshwater mussels (Elliptio complanata) from contaminated sediments has been investigated in the laboratory and in the field in Oshawa and Whitby Harbours in Ontario. Tributyltin (TBT) was concentrated by mussels to higher concentrations in Whitby Harbour than in Oshawa Harbour. Concentrations of TBT in mussels and rates of bioconcentration related positively to the concentration of TBT in sediment.

Keywords: Bioaccumulation, tributyltin, mussels, sediment.

Introduction

Tributyltin has been found in many harbour locations as a result of its use as an antifouling agent in ship paint formulations (Maguire et al., 1986; Seligman et al., 1989). Tributyltin degrades under environmental conditions to dibutyltin and monobutyltin, and eventually to tin(IV) species, with tributyltin being the most toxic form to aquatic organisms. Apart from its degradation, little is known about its pathways and impact on the food chain. Organometallic compounds are notoriously known for their lipophilic characteristics which facilitate their bioaccumulation. In our previous studies on bioaccumulation of alkyllead compounds by mussels from the St. Clair River sediments, the rates of

accumulation for triethyllead and diethyllead were 1.96 and 0.26 ng g^{-1} day^{-1} respectively over a 52-day study period (Chau et al., 1988).

Tributyltin was found to accumulate in oysters and mussels with accumulation factors of 30,000 and 10,000 respectively relative to water (Dooley et al., 1987). It was also accumulated in the muscle tissue of salmon reared in sea pens treated with a biocide tri-n-butyltin. Accumulation of 0.3-0.9 ug g^{-1} was observed in Chinook salmon reared for 3-19 months in sea pens in Alaska (Short and Thrower, 1986) and 0.5-1.0 ug g^{-1} for Atlantic salmon in Scotland (Davies and McKie, 1987). Tributyltin and its degradation product dibutyltin were also found in fish and aquatic organisms in harbour locations. For example, as high as 1653 ng g^{-1} (wet wt.) tributyltin was found in a carp in Whitby Harbour, Ontario, in May 1988 (Wong et al., unpublished data).

The use of caged mussels for the investigation of the bioavailability of chemicals has been widely applied. The objectives of the present study are: (1) to investigate the direct bioaccumulation of butyltin compounds in contaminated areas, and (2) to investigate the possibility of <u>in vivo</u> transformation of butyltin compounds.

Experimental

Indigenous freshwater mussels (<u>Elliptio complanata</u>), 6.0-7.0 cm in length, were collected from Balsam Lake, Ontario. These mussels were analyzed and found to be free of contamination by butyltin compounds.

Glass aquaria (40x20x25 cm LWH), each filled with ca. 3 kg (wet weight) of sediment collected from Oshawa Harbour (site 1) and Whitby Harbour (site 1), were used for laboratory exposure experiments. Sediment from

Balsam Lake was free from contamination of butyltin compounds and was used as a control. Dechlorinated tap water (10 dm^3), which has the same composition as Lake Ontario water, was put in each aquarium and allowed to equilibrate with the sediment for 7 days before the test mussels were put in. The aquarium experiments were conducted under static conditions with no continuous water circulation except with air bubbling at mid-water level through air stones to supply oxygen to the system without disturbing the sediment layer. The test mussels were fed with Chlorella vulgaris cultured in CHU-10 medium three times a week. Four mussels were sampled from each tank at 4-day intervals at the beginning of the experiment and at weekly intervals thereafter. The mussels were mechanically opened, blot-dried with filter papers, weighed and analyzed individually on a whole mussel, wet-weight basis for butyltin compounds according to the procedures given below.

The cages (25x25x11 cm, LWH) were fabricated from 1.27 cm open mesh galvanized wire gauze with an opening from one side. Clay bricks were used at both ends to weigh down the cage to contact the sediment. The cages were anchored to shore, using 1/4" polypropylene ropes, fastened to the bottom with bricks at every 3 metres. Six cages, each containing 20 mussels, were placed at 3 locations in Whitby Harbour and 3 locations in Oshawa Harbour, Ontario. At each harbour, 2 cages were placed in the inner locations while one cage was placed outside the harbour in the lake, free from the circulation of the harbour water, for control (Fig. 1A, 1B).

The cages were installed in May 1988. Five mussels were collected from each site in June, August and October for the determination of butyltin species. At each sampling, associated water and sediment samples were also collected for the determination of butyltin species and other

chemical parameters such as dissolved and particulate organic carbon, heavy metals, etc. to provide more information on the two study sites.

Speciation of butyltin compounds in mussels.

The following is a modified procedure from our published GC-AAS technique for the speciation of methyltin and alkyllead compounds (Chau et al., 1982; Chau et al., 1984).

The whole mussel was digested in a large test tube in 10 cm^3 of 20% TMAH solution (Tetramethyl ammonium hydroxide) in a water bath at 60°C for 1-2 hr until the solution turned pale-yellow in colour. After cooling and removal of undissolved debris, the solution was neutralized with 50% HCl to pH 8 + 0.2, and extracted with 3 cm^3 of 0.5% tropolone in hexane solution for 1 hr in a mechanical shaker. The mixture was centrifuged and 2 cm^3 of the hexane phase was transferred to a glass-stoppered, graduated centrifuge tube for ethylation with 0.2 cm^3 of ethylmagnesium bromide. The excess ethylmagnesium bromide was destroyed with 2 cm^3 of 0.5 mol dm^{-3} H_2SO_4. The hexane layer was transferred to a small vial containing a pinch of anhydrous sodium sulfate for storage.

Freeze-dried sediment samples (1-2 g) were extracted for 1 hr with 5 cm^3 of 0.5% tropolone hexane solution after addition of 20 cm^3 of water, 6 g NaCl, 2 g sodium benzoate and 1 g KI. One cm^3 of the tropolone extract was removed for ethylation with 0.2 cm^3 of ethylmagnesium bromide in a small graduated centrifuge tube. Excess ethylmagnesium bromide was destroyed with 2 cm^3 of 0.5 mol dm^{-3} H_2SO_4. The hexane phase was stored in a small vial containing anhydrous sodium sulfate for analysis in the GC-AAS system.

Water samples (1 dm^3, preserved with 1.1 cm^3 conc. HCl), after addition of 40 g of NaCl, were extracted for 1 hr with 10 cm^3 of 0.5% tropolone in hexane solution. The hexane phase was collected in a 50 cm^3 beaker containing a few g of anhy. sodium sulfate. After addition of 0.5 cm^3 iso-octane as a keeper, the hexane was evaporated to 1 cm^3 first in a rotary evaporator and then in a vortex evaporator. The hexane extract was ethylated with 0.2 cm^3 of ethylmagnesium bromide and similar procedures were followed as for sediment analysis. There was no need for sample clean-up for water and sediment samples.

Clean-up of mussel samples.

Sample clean-up was carried out in a glass column (L 15 cm x dia 1.5 cm) packed with 8 cm in height of kiesel-gel 60 (3% water) suspended in hexane. The column was sealed with a 1 cm layer of anhydrous sodium sulfate and a layer of glass wool on top to prevent disturbance of the column bed during elution and to remove any water in the sample. Exactly 1 cm^3 of the ethylated sample was loaded into the column. When the sample had almost passed through the sodium sulfate layer, and the interior walls of the column were rinsed with a few drops of hexane, elution was initiated with 30 cm^3 of hexane at a rate of 1 $cm^3 min^{-1}$. The eluate was collected in a 100 cm^3 round bottom flask. After 0.5 cm^3 of iso-octane was added to the hexane eluate as a keeper, the eluate was evaporated first in a rotary evaporator, and then in a vortex evaporator at room temperature to a final volume of 0.5 cm^3. The finished sample was transferred to a small vial which was tightly sealed before injection into the GC-AAS system.

The gas chromatography-atomic absorption spectrometry (GC-AAS) system.

The GC-AAS system has been described in a previous publication (Chau et al., 1982) except that a J & W fused silica megabore column (DB-1, 30 m, 1.5 um film thickness) was used instead of the 2 m glass column with 3% OV-1 on Chromosorb previously used. Sensitivity, resolution, and analytical time were all improved. Temperatures of the injection port and transfer line were both 150°C. The N_2 carrier gas flow rate was at 10.5 $cm^3 min^{-1}$; the temperature program was 90-200°C at 20°C min^{-1}. The AAS furnace gases were H_2, 84 $cm^3 min^{-1}$ at 1.38×10^6 dyne cm^{-2}; air, 21 $cm^3 min^{-1}$ at 2.41×10^6 dyne cm^{-2}. The 224.6 nm Sn line was generated by an electrodeless discharge lamp operated at 8 W. Deuterium background correction was used. Peak areas were measured with a HP3392A integrator. Tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT) were determined with detection limits for water, sediment (dry wt.) and mussels (wet wt.) of 5 ng dm^{-3}, 5 ng g^{-1}, and 3 ng g^{-1} (expressed as Sn) respectively. Overall percentages of recovery for the three butyltin species for water (spiked at 1 ug dm^{-3}), sediment (spiked at 100 ng g^{-1}) and mussels (spiked at 100 ng g^{-1}) ranged from 84 to 95±6%.

Results and Discussion

Bioaccumulation of butyltin compounds.

Mussels exposed to sediments from Balsam lake (control), Oshawa Harbour and Whitby Harbour in the laboratory were analyzed for all butyltin species. Concentrations of TBT and other butyltin species in Balsam Lake sediments were less than detection limit (5 ng g^{-1}), while the levels of

TBT in Oshawa Harbour sediments were quite low (32-38 ng g^{-1}). Mussels exposed to these sediments contained very low levels of TBT (3 ng g^{-1}) even after 72 days of exposure. Concentrations of TBT in sediments from Whitby Harbour were much higher (66-110 ng g^{-1}). Mussels concentrated TBT slowly from the Whitby Harbour sediment and reached a maximum of 24 ng g^{-1} after 24 days of incubation, with an average accumulation rate of 1 ng of TBT per gram of mussel tissue per day. After the 24th day, the TBT level started to decline (Fig. 2). It was not fully understood why such a decline tobk place. Similar phenomena have been observed in other laboratory bioaccumulation studies with alkyllead by mussels (Chau et al., 1988), and with tributyltin by eelgrass (Francois et al., 1989). In both cases, the decline after the accumulation maximum was attributed to the in vivo degradation of the accumulated compounds. Because no other butyltin species were found in mussels in this study, the in vivo degradation of TBT in mussels was not likely the reason for the decline of TBT concentration.

Caged mussels in harbour locations showed definite patterns of accumulation over the 5-month exposure period (Table 1A & 1B). Accumulations of tributyltin were distinctly higher in both locations in Whitby Harbour than in Oshawa Harbour. From the regression lines of accumulation, it was estimated that the rates of accumulation were 0.7 and 2.1 ng/g/day respectively for the inner harbours (Site 1) of Oshawa and Whitby over a 140-day exposure period. While tributyltin concentrations in sediment could have positive effects on the rates of bioaccumulation, other parameters such as organic content and redox conditions of the sediment, and circulation patterns of the system could have a strong influence on bioaccumulation. The inner harbour at Whitby

is not directly exposed to the lake circulation, and the tributyltin accumulated in the sediment is not readily washed away by circulation. This is one of the reasons that sediment at inner harbour of Whitby contained 1.5 to 7.8 times more butyltin species than that of the inner dOshawa harbour. Although other water quality parameters (e.g. dissolved and particulate organic carbon, particulate nitrogen, etc.) were determined at these sites, the values did not show any significant deviations that could be used to account for the differences in accumulation rates.

The dibutyltin (DBT) and monobutyltin (MBT) were also present in sediments of both harbours, but in lower concentrations than TBT. DBT and MBT were not frequently found in the exposed mussels, except in one or two occations when they were found in low concentrations.

The control sites were outside the breakwaters of both harbours in Lake Ontario in order to avoid as much as possible the influence of harbour water circulation. There was, however, TBT accumulated in the mussels at both control sites, but at very low levels. Such observations indicated that TBT contamination already extended outward to the lake.

Since the sink for tributyltin leached out from biocides in harbour areas would probably be in the sediment, the concentration of butyltin in water is therefore transient. Unlike laboratory experiments where the test animal is exposed to an uniform level of a chemical in solution, the field test biota is exposed to a non-homogeneous medium. Therefore, the concentration factors of tributyltin between mussels and water obtained in the field can only be estimated in certain locations where its water concentration is detectable, and the values obtained could vary

considerably depending on the variation in the concentration of tributyltin in water. Concentration factors ranging from 4.8×10^3 to 18.5×10^3 were obtained which are within the literature values reported for mussels and bivalves (Laughlin, Jr., 1986; Dooley et al., 1987).

The tributyltin concentrations in sediment are less variable. It is possible to calculate the concentration factors of TBT between mussel and sediment. Since mussels and clams are bottom dwellers, and filter the water close to the sediment-water interface, sediment concentration of a chemical may be a more significant parameter than that in the overlying water phase in the estimation of their bioconcentration factors. For Oshawa Harbour, concentration factors between mussels and sediment varied from 0.1 to 10.0 over the experimental period from June to October. For Whitby, higher concentration factors, ranging from 2.5 to 18.1 were obtained. It is evident that concentration factors do depend on the tributyltin concentrations and its bioavailability in the medium as previously discussed for alkyllead compounds (Chau et al., 1988).

Transformation of butyltin compounds.

Although it has been well documented that tributyltin compounds are degraded under aerobic and anaerobic conditions through a debutylation process in harbours and under laboratory controlled conditions (Maguire and Tkacz, 1985; Seligman et al., 1986; Tian et al., 1989), in this study both the DBT and MBT species were only occasionally found in sediment samples of Site #1 of both harbours in May to June, but not in later months. The DBT species was present only in two occasions in mussel in Whitby Harbour at both site 1 and 2 in June (4.2-4.5 ng g^{-1}) reflecting its presence in sediment of the same locations (12.6 ng g^{-1}). The MBT

species was not found in water or mussels in either harbour location. Therefore there are not enough data to discuss whether the dibutyltin in mussels was a result of direct accumulation or *in vivo* transformation as was observed in mussels in the alkyllead study (Chau et al., 1988). There are not sufficient data in the present study to follow the *in vivo* degradation of TBT under environmental conditions.

Conclusions

Mussels accumulate tributyltin from contaminated sediments in harbours. While other chemical and physical properties may play an important role in affecting the bioconcentration of TBT by mussels from sediment, the rates of bioaccumulation and concentrations of tributyltin in mussels relate positively to the concentrations of TBT in sediment.

TBT was the major species found in harbours. Its degradation products, DBT and MBT were found less frequently in sediment and in mussels. Limited data did not provide sufficient grounds for discussion on whether degradation of TBT occurred in the sediment or in the mussels after its was accumulated.

TBT in sediment of contaminated areas is available to biota.

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Table 1A. Concentrations of butyltin compounds in caged mussels and in sediment in Oshawa Harbour, Ontario.

Exposure Days	Control site			Site #1			Site #2		
	TBT	DBT	MBT	TBT	DBT	MBT	TBT	DBT	MBT
Caged Mussels (ng g^{-1})									
0	-	-	-						
34	-	-	-	21	-	-	5	-	-
95	-	-	-	70	-	-	12	-	-
140	-	-	-	91	-	-	73	-	-
Sediment (ng g^{-1})									
0	-	-	-	31	13	-	34	10	-
34	-	-	-	27	-	29	36	-	-
95	-	-	-	7	-	-	36	11	-
140	-	-	-	19	-	-	45	18	-

Table 1B. Concentrations of butyltin compounds in caged mussels and in sediment in Whitby Harbour, Ontario.

Exposure Days	Control site TBT	DBT	MBT	Site #1 TBT	DBT	MBT	Site #2 TBT	DBT	MBT
Caged Mussels (ng g^{-1})									
0	-	-	-						
34	-	-	-	98	4	-	73	5	-
95	16	-	-	682	4	-	397	-	-
140	-	-	-	121	-	-	130	-	-
Sediment (ng g^{-1})									
0	-	-	-	55	21	35	-	-	-
34	23	-	-	39	13	25	-	-	-
95	-	-	-	55	19	18	22	-	-
140	-	-	-	34	-	-	17	-	-

- Not detected: Detection Limits (as Sn): sediment, 5 ng g^{-1}; mussel, 3 ng g^{-1}.

Concentration values are geometric means of replicate analysis: mussels (n=5), sediment (n=3).

TBT- Tributyltin; DBT- Dibutyltin; MBT- Monobutyltin.

Figure Legends

Figure 1A. Caged mussels exposure sites in Oshawa Harbour, Ontario.

-- Cage site; C -- control site.

Figure 1B. Caged mussels exposure sites in Whitby Harbour, Ontario.

-- Cage sites; C -- control site.

Figure 2. Concentrations of TBT in mussels exposed to Whitby Harbour sediment (site #1). Data for mussel are geometric means of replicate (n=4) analyses; Concentration of TBT in Whitby sediment, 66-110 ng/g (dry weight).

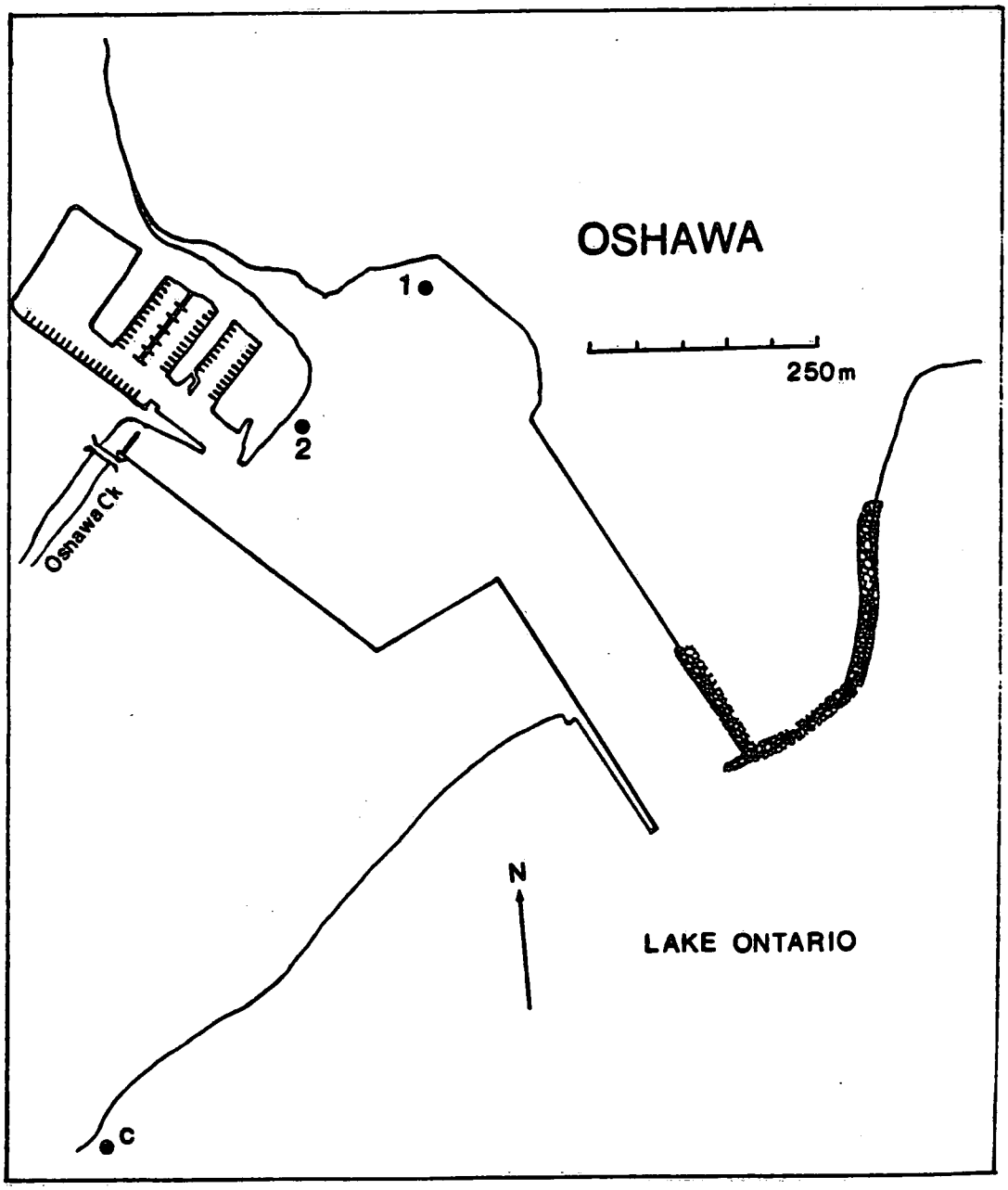
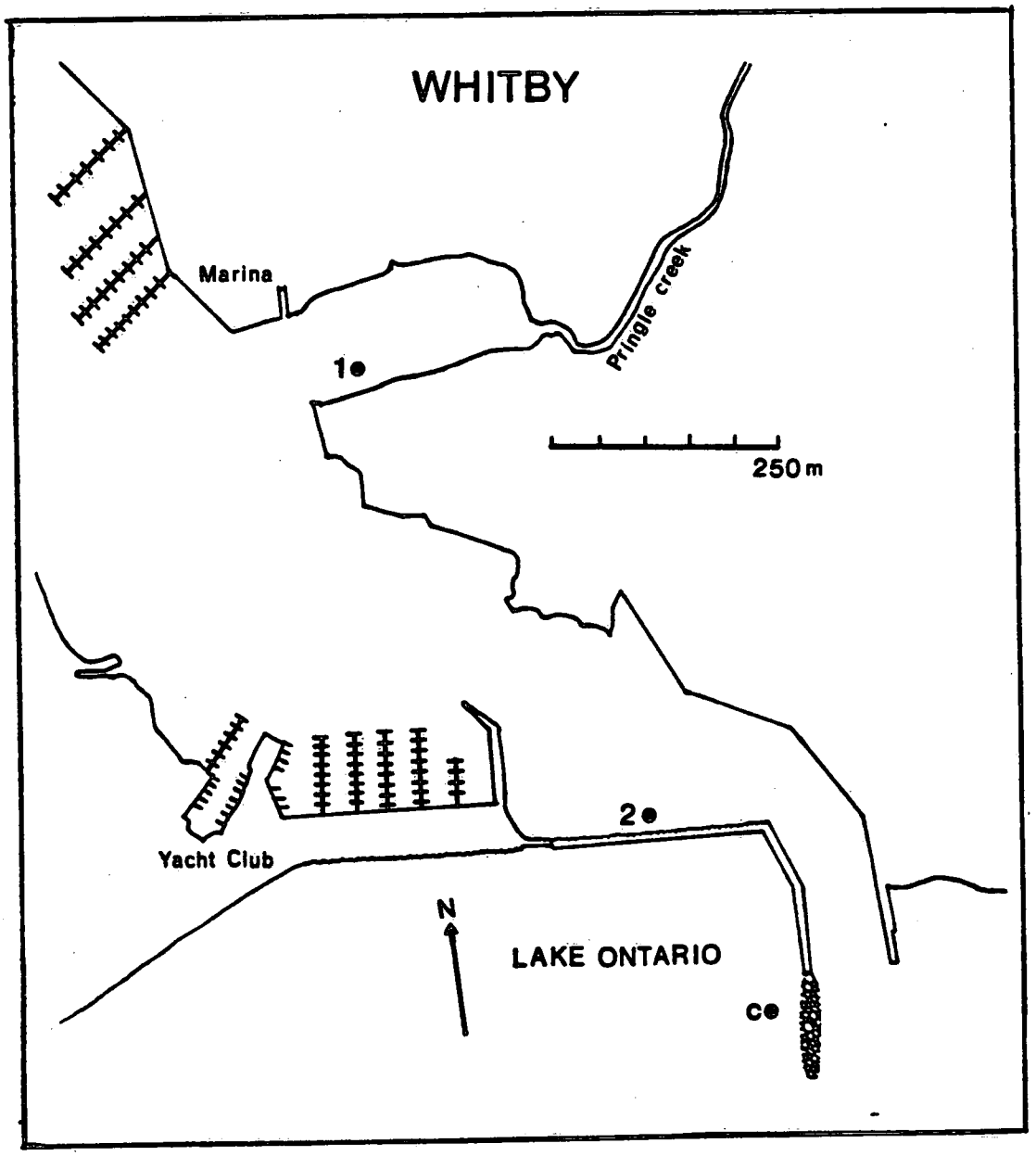


Fig. 1A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Marina
Pringle creek
1
250 m
Yacht Club
2
N
LAKE ONTARIO
C

Fig. 1B

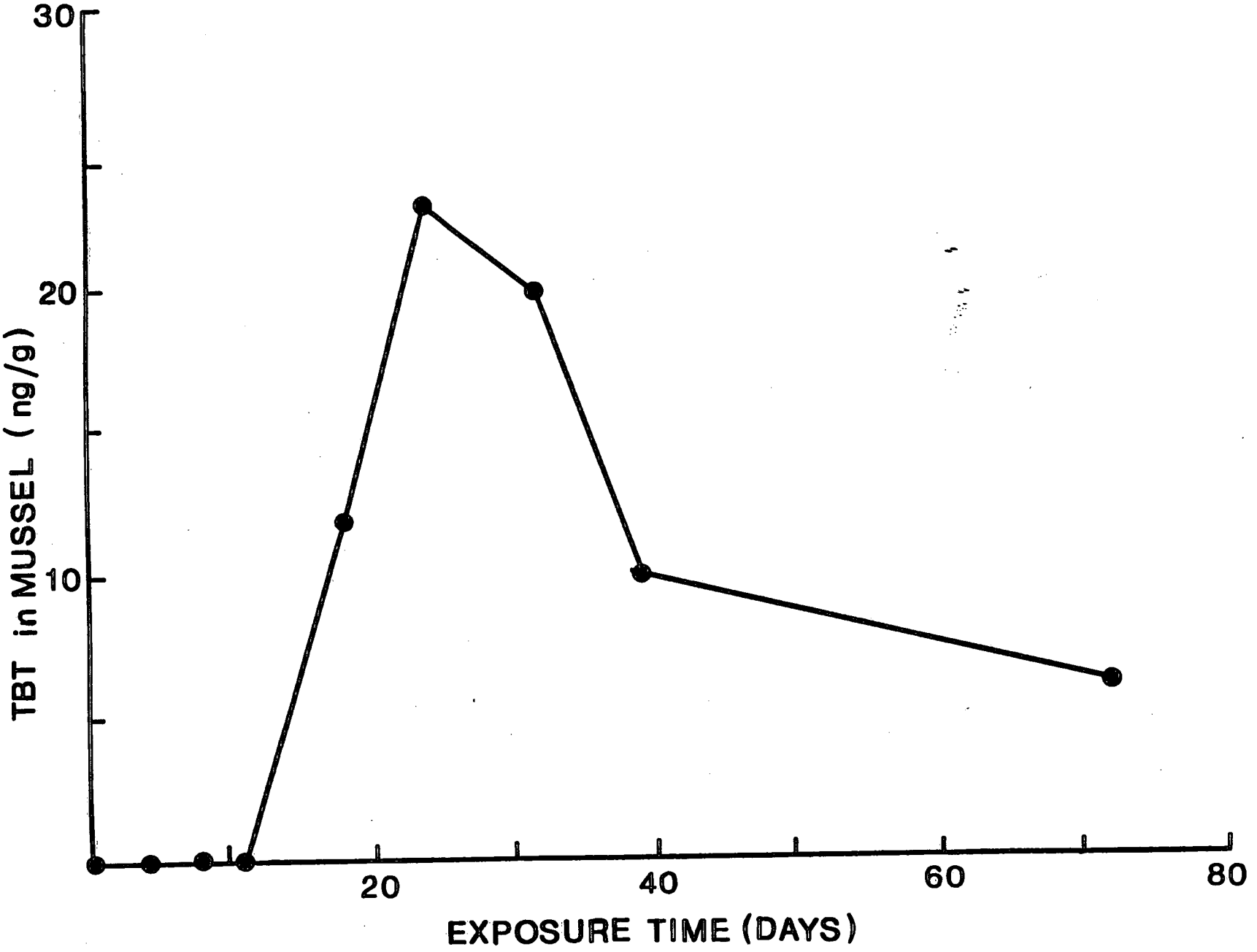

Fig. 2