NATIONAL WATER RESEARCH INSTITUTE

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PROCEEDINGS OF THE WORKSHOP ON ORGANOTIN COMPOUNDS IN THE CANADIAN AQUATIC ENVIRONMENT SIDNEY, BRITISH COLUMBIA FEBRUARY 19-20, 1996

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Proceedings of the Workshop on Organotin Compounds in the Canadian Aquatic Environment Sidney, British Columbia February 19-20, 1996

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Management Perspective

These workshop proceedings indicate that the 1989 regulation of antifouling uses of tributyltin under the Pest Control Products Act has not been effective in reducing TBT levels at many locations to below toxicity endpoints or Canadian Water Quality Guidelines for the protection of aquatic life. These proceedings make recommendations for further action in the areas of regulation, research and monitoring.

Sommaire à l'intention de la direction

Ce compte rendu d'atelier révèle qu'en de nombreux endroits, la réglementation de 1989 relative aux applications antisalissure du tributylétain, en vertu de la Loi sur les produits antiparasitaires, n'a pas atteint son objectif de faire passer la concentration du TBE sous les seuils de toxicité ou sous la concentration prévue dans les Recommandations pour la qualité des eaux au Canada pour assurer la protection des organismes aquatiques. On recommande également dans ce compte rendu l'adoption de nouvelles mesures dans les champs de la réglementation, de la recherche et de la surveillance du milieu.

Abstract

The Workshop on Organotin Compounds in the Canadian Aquatic Environment was held at the Institute of Ocean Sciences, Sidney, British Columbia, on February 19-20, 1996. This was the first Canadian workshop on organotin compounds. Its purpose was to bring together all researchers, managers of fisheries resources, regulators, and other interested parties to discuss, primarily, the Canadian situation with respect to the extremely toxic antifouling agent tributyltin (TBT), and to a lesser extent, other pesticidal and non-pesticidal organotin compounds. These proceedings summarize formal presentations and discussions on (i) the history of TBT research and monitoring in Canada, (ii) what effect the regulation of antifouling uses of TBT under the Pest Control Products Act in 1989 has had on concentrations of TBT in water, sediment and aquatic biota in Canada, (iii) the present status of non-pesticidal organotin compounds in Canada under the Canadian Environmental Protection Act, (iii) recent advances in analytical methods, biological chemistry and environmental pathways of organotin compounds, (iv) the leaching of organotin compounds from chlorinated poly(vinyl chloride) pipe into drinking water, and (v) bioaccumulation, biomonitoring and the toxicity of organotin compounds to aquatic biota. It was concluded that the 1989 TBT regulation has not been effective in reducing TBT levels at many locations to below toxicity endpoints or Canadian Water Quality Guidelines for the protection of aquatic life. These proceedings make recommendations for further action in the areas of regulation, research and monitoring. These proceedings also include the workshop program, abstracts of the presentations, addresses of the participants, their current and planned activities on organotin compounds, a list of their publications, and the addresses of those few other organotin researchers (and managers) in Canada who did not attend the workshop.

Résumé

L'atelier sur les organo-étains dans l'environnement aquatique du Canada s'est déroulé les 19 et 20 février 1996 à l'Institut des sciences de la mer de Sidney, Colombie-Britannique. Il s'agissait du premier atelier canadien à porter sur ce type de composés. L'objectif cherché était de réunir tous les chercheurs, tous les gestionnaires de ressources halieutiques et tous les responsables de la réglementation, ainsi que d'autres intéressés, pour étudier notamment la situation canadienne au regard du tributylétain (TBE), un agent antisalissure très toxique, et, secondairement, pour étudier d'autres organo-étains utilisés comme pesticides ou non. Le compte rendu fournit un résumé des présentations formelles et des échanges qui portaient sur 1) l'historique de la recherche sur le TBE et sur sa surveillance au Canada, 2) l'impact que la réglementation de 1989 sur les applications antisalissure du TBE aux termes de la *Loi sur les produits antiparasitaires* a pu exercer sur la concentration du TBE dans l'eau, les sédiments et les biotes aquatiques au Canada, 3) la situation au regard des organo-étains non utilisés comme pesticides au Canada dans le cadre de la *Loi sur la protection de l'environnement*, 4) les derniers progrès dans les domaines des méthodes d'analyse, de la chimie biologique et de l'étude des voies de pénétration des organo-étains dans l'environnement, 5) l'entraînement dans l'eau potable des organo-étains contenus dans les tuyaux fabriqués en polychlorure de vinyle, et 6) la bioaccumulation, la surveillance biologique et la toxicité des organo-étains dans les biotes aquatiques. Les participants se sont entendus pour dire qu'en de nombreux endroits, la réglementation de 1989 relative au TBE n'a pas atteint son objectif de faire passer la concentration du TBE sous les seuils de toxicité ou sous la concentration prévue dans les Recommandations pour la qualité des eaux au Canada pour assurer la protection des organismes aquatiques. On recommande également, dans ce compte rendu, l'adoption de nouvelles mesures dans les champs de la réglementation, de la recherche et de la surveillance du milieu. Ce compte rendu comprend également le programme de l'atelier, les résumés des présentations, l'adresse des participants, leurs travaux en cours ou prévus qui portent sur les organo-étains, une liste de leurs publications ainsi que l'adresse des quelques autres chercheurs (et gestionnaires) qui s'occupent des organo-étains au Canada et qui n'ont pu participer à l'atelier.

Summary of Proceedings

Introduction

The Workshop on Organotin Compounds in the Canadian Aquatic Environment was organized by J.A.J. Thompson of the Institute of Ocean Sciences, Department of Fisheries and Oceans, Sidney, British Columbia, and Y.K. Chau of the National Water Research Institute, Department of the Environment, Burlington, Ontario. This was the first Canadian workshop on organotin compounds. Its purpose was to bring together all researchers, managers of fisheries resources, regulators, and other interested parties to discuss, primarily, the Canadian situation with respect to the extremely toxic antifouling agent tributyltin (TBT), and to a lesser extent, other pesticidal and non-pesticidal organotin compounds. The workshop was held at the Institute of Ocean Sciences, Sidney, British Columbia, on February 19-20, 1996. Approximately 35 people attended the workshop, from government, academia and the private sector. Formal presentations were made, and wide-ranging general discussions ensued, on (i) the history of TBT research and monitoring in Canada, (ii) what effect the regulation of antifouling uses of TBT under the Pest Control Products Act in 1989 has had on concentrations of TBT in water, sediment and aquatic biota in Canada, (iii) the present status of non-pesticidal organotin compounds in Canada under the Canadian Environmental Protection Act, (iii) recent advances in analytical methods, biological chemistry and environmental pathways of organotin compounds, (iv) the leaching of organotin compounds from chlorinated poly(vinyl chloride) pipe into drinking water, and (v) bioaccumulation, biomonitoring and the toxicity of organotin compounds to aquatic biota. These proceedings summarize the conclusions of the workshop, and make recommendations for further action in the areas of monitoring, research and regulation. These proceedings also include the workshop program, abstracts of the presentations, addresses of the participants, their current and planned activities on organotin compounds, a list of their publications, and the addresses of those few other organotin researchers (and managers) in Canada who did not attend the workshop.

Summary

Session 1: Environmental Concentrations

Although in many small harbours and marinas the concentration of TBT has decreased after the regulation of antifouling uses of TBT in 1989 under the Pest Control Products Act, concentrations in water in some locations exceed Canadian Water Quality Guidelines for the protection of aquatic life, and concentrations in sediments at many locations are still high enough to cause toxicity to benthic organisms. TBT concentrations in some

aquatic organisms (notably bivalves) in some locations continue to be high. TBT is fairly persistent in sediments, and it is possible that the TBT found in water results from disturbance of the sediments by storms, dredging, boat activity and bioturbation. However, in some locations, the ratio of concentrations of TBT to its degradation products suggests a fresh source of TBT. At locations not frequented by vessels longer than 25 m, fresh TBT may arise from illegal use, or the use of existing stocks of TBT-containing antifouling paint. (It appears that the 1989 regulation did not recall TBT-containing antifouling paint stocks to the distributor/manufacturer, nor stipulate that TBT-containing paints only be applied by licenced applicators.) There is also the possibility that boats may be painted with TBT in other jurisdictions, and then returned to Canadian waters. A 1995 survey of shipyards in the B.C. Lower Mainland and Vancouver Island found that 9 of 23 shipyards allowed boats to be painted with paints supplied by the boat owners. These paints were not scrutinized by facility managers and were of unknown source and registration. The shipyard managers must be made fully aware of their responsibility to monitor these activities and to insist on the use of registered antifouling paints.

It appears that the regulation of antifouling uses of TBT in 1989 under the Pest Control Products Act has had less impact on TBT concentrations (especially in sediments and shellfish) in larger marine harbours (in particular Vancouver Harbour and Halifax Harbour) where there are many vessels > 25 m. Tributyltin concentrations in water in some locations exceed Canadian Water Quality Guidelines for the protection of aquatic life, and concentrations in sediments at many locations are still high enough to cause toxicity to benthic organisms. Tributyltin concentrations in some aquatic organisms (notably bivalves) in some locations continue to be high. TBT is fairly persistent in sediments, and it is possible that the TBT that continues to be found in water results from disturbance of the sediments by storms, dredging, boat activity and bioturbation. However, there is continued leaching of TBT from vessels > 25 m. Other organotin compounds have also been found in harbours and in sewage sludges (*e.g.*, methyltin, phenyltin, octyltin, propyltin and cyclohexyltin species, as well as dibutyltin and monobutyltin).

Session 2: Biological Effects

This session focused on recent research on (i) the accumulation and toxicity of TBT to the starfish *Leptasterias polaris* (that indicated the importance of considering both thermodynamic and kinetic approaches in considerations of uptake), (ii) early imposex studies on the West coast that indicated the widespread occurrence of imposex in whelks (with recovery of some populations, but not all) and some local extinctions, (iii) a recent imposex survey in Eastern Canada that indicated extensive imposex

occurrence in harbour areas, (iv) a survey of blue mussels (*Mytilus edulis*) in the southern Gulf of St. Lawrence that indicated substantial accumulation of TBT and its degradation products (this research will also examine immunotoxicological effects on the mussels), and (v) the relationship between bioaccumulation and toxicity of TBT in *Hyalella azteca* (illustrating the importance of relating toxicity to an organism to the internal dose or concentration of the toxic chemical, not just to external concentrations in the medium used).

Session 3: Chemical Considerations

This section focused on recent research on (i) the effects of organotin compounds on the permeability of model biological membranes, (ii) the production of volatile organotin compounds in municipal landfills (*e.g.*, tetramethyltin, *n*-butyltin hydride, *n*-butylmethyltin hydride), (iii) the demonstration of the leaching of monobutyltin and dibutyltin from new chlorinated poly(vinyl chloride) pipe, (iv) the demonstration of the extreme sensitivity of electrospray tandem mass spectrometry (positive ion mode - Multiple Reaction Monitoring) for TBT analysis, and (v) the demonstration of the utility of the *in situ* ethylation procedure for the preparation of ethyl derivatives of TBT and its degradation products for analysis by gas chromatography with detection by mass spectrometry or inductively-coupled plasma mass spectrometry.

Recommendations

Because TBT is the organotin compound of most environmental significance, most recommendations given below are for TBT. However, it should be borne in mind that some other organotin compounds used and/or found in Canada are fairly toxic (*e.g.*, dibutyltin), and some of the recommendations may also apply to those other organotin compounds. Because of the declining resources available from governments for research in general, it is becoming increasingly important to tie research and monitoring more closely to solving problems. For example, from a fisheries perspective we need to ensure that organotin compounds are not affecting fisheries resources that are used for commercial, recreational and cultural purposes. From an ecological viewpoint it is important, for example, that local extinctions of whelks caused by TBT be prevented, or remedied.

Regulation and Management

Pesticidal uses of TBT are currently regulated under the Pest Control Products Act, which is administered by the Pest Management Regulatory Agency of the Department of Health. We need to determine if current regulations are working. In the seven years since the 1989 TBT regulation, it

appears that there has been some reduction in TBT concentrations in some ecosystems, but not in others. We need to determine if further regulations or other control options will lead to reduced levels in the environment and reduced adverse effects. Some of the recommendations given below are those that could be addressed under the Pest Control Products Act. An alternative would be to address TBT hazards under the Government of Canada's Toxic Substances Management Policy. This possibility is also discussed below.

- It should be determined when, or if, the last stocks of TBT-containing paint were used up in Canada (*i.e.*, those stocks originally destined for use on boats < 25 m).

- The possibility of joint provincial-state agreements should be explored with a view to harmonizing TBT-containing antifouling paint regulations, restriction of painting activities to licenced applicators, and increased public education.

- Public information strategies should be continued to address illegal use of TBT-based antifoulants on recreational boats, especially where there is the possibility of painting small Canadian boats in other jurisdictions. The recently-produced fact sheets on organotin compounds by both the Institute of Ocean Sciences and the Environmental Protection Service - Pacific and Yukon Region are good examples of public education initiatives.

- The status of current international TBT regulations should be determined. Efforts should be made to encourage countries that do not have TBT regulations to develop regulations similar to those in place in Canada, U.S.A., United Kingdom and France, for example. Efforts should also be made to strengthen current regulations where it appears they are not effective, and to promote international harmonization of the regulations.

- Where it is feasible, the scale and economic cost to date of TBT contamination in Canada on the oyster industry (and other shellfish industries) should be documented.

- An alternative to dealing with the continuing environmental hazards of TBT in Canada under the Pest Control Products Act may be through the Federal Government's Toxic Substances Management Policy (TSMP), especially if there were a case for designating TBT as a Track 1 substance (a substance that is persistent, bioaccumulative and primarily the result of human activity, and which should be virtually eliminated from the environment). This would require an assessment

of the toxicity of TBT at least as rigourous as that used to qualify substances as "CEPA-toxic" under the Department of the Environment's Priority Substances Assessment Program. It should be noted, however, that even if TBT were banned from use on all vessels under TSMP, initiatives would still have to be undertaken to ban TBT internationally in order to prevent its release in Canada from vessels > 25 m in length that had been legally painted in other jurisdictions.

Research

(i) Analytical Method Development

- Intercalibration exercises should be held among Canadian laboratories to ensure the quality of chemical measurements for organotin compounds in water, sediment and biota.

(ii) Occurrence, Persistence, Fate and Environmental Pathways

- More information is required on the occurrence and persistence of TBT and its degradation products in sediment cores from different ecosystems (marine and freshwater) in order to assess more fully the persistence of tributyltin in sediments in Canada. More information is also required on the chemical forms of TBT in sediments as they affect its persistence, fate and bioavailability. In addition, the fate of TBT in dredged harbour sediments that are removed to ocean disposal sites requires further attention.

- The importance of the production of volatile organotin species in some environments should be investigated more fully. Although rates and yields of such volatile species are extremely small in laboratory experiments, it is possible that on much longer time scales the production of volatile tin species may be important in the global cycling of tin. It is important to determine the lifetime of volatile tin compounds in the atmosphere and the possibility of their long-range transport (for example, by sampling Arctic ice cores).

- More work is required to assess the importance of the occurrence of organotin compounds in landfill leachates, and the occurrence of organotin compounds in potable water piping (especially piping that has been in service for some time).

- More work is required to develop suitable biomonitors for TBT in freshwater. At this stage, likely candidates are the amphipod *Hyalella azteca* and bivalves (*e.g., Dreissena polymorpha*).

(iii) Effects

In general, more information is required on the mechanisms of toxic action of organotins at the cellular level (*e.g.,* on DNA). The study of quantitative structure-activity relationships at the molecular and cellular level may provide useful information on what other chemicals may be potent reproductive toxins. Tributyltin (and DBT) are immunosuppressive agents in mammals. Information is urgently needed on their possible immunosuppressive effects in aquatic organisms. The standardization of toxicity tests is also desirable in order to ensure consistency between laboratories.

(a) Sea Water

- With regard to imposex in whelks there are a number of areas that should be explored: (i) the role played by whelks in coastal ecosystems, and the impact on ecosystem structure of widespread imposex in whelks; (ii) what factors other than TBT may also contribute to imposex (other toxic chemicals or ecosystem-specific properties such as availability of nutrients, temperature, *etc.*).

- Determination of possible sublethal effects of chronic exposures of organisms other than whelks to TBT in contaminated areas (*e.g.*, oysters, lobsters, Manila clams, blue mussels, Dungeness crabs).

- Determination of the significance of high concentrations of TBT in marine surface microlayers for intertidal species.

(b) Fresh Water

- Although it is likely that freshwater aquatic biota in Canada have been harmed by TBT in some locations, there has been no actual demonstration of such harmful effects. Research is therefore required on effects of TBT on freshwater organisms in contaminated areas. A possible sentinel organism at this stage, both for biomonitoring as described above, and reproductive toxicity, is *Hyalella azteca*. Other possible sentinel organisms are freshwater snails. However, compared to their marine counterparts, freshwater snails are much less important to secondary production and as food for fish than are crustacea.

Monitoring

Continued monitoring of water, sediment and aquatic biota is necessary to determine the status and trends of organotin contamination of aquatic ecosystems in Canada, and to determine the effectiveness of existing

and future regulations and other control measures. More emphasis should be placed on indicator organisms.

- Monitoring should continue at commercial harbours, marinas, and aquaculture sites close to areas of boating and shipping activity.

- Modest baseline surveys should be done at remote sites, including the Arctic, where TBT degradation rates in water are expected to be much slower than in southern Canada.

- Imposex incidence in whelks and dogwhelks in seawater appears to be a useful indicator of TBT contamination, and should be used more in monitoring programs. (Care must be taken to differentiate between local extinctions due to TBT and those due simply to the absence or loss of suitable habitat.) Imposex determination is also a good tool for screening samples for the more costly chemical analyses. (Another way to mitigate the high cost of chemical analyses is to screen samples first by determining total hexane-extractable tin by a spectrophotometric or emission technique before the more costly chromatographic speciation analyses are done.) It should be noted that, unlike dogwhelks on the East coast of Canada which have a life-span of about 2 years, some whelk species on the West Coast have life-spans of up to 5 years. Consequently, it may have been too early even in 1994 to determine if whelk populations in some locations on the West Coast were recovering after the 1989 TBT regulation.

- Limited monitoring of aquatic organisms other than whelks and dogwhelks is recommended. If such monitoring indicates areas of high TBT contamination, the monitoring program should be extended to birds and other wildlife that eat such aquatic organisms.

Human Health-Related Research and Information Needs

- More work is required on the occurrence and toxicological significance of organotin residues in fish and shellfish destined for human consumption.

- More work is required on the occurrence and toxicological significance of organotin residues in Canadian drinking water.

Workshop Program

Session 1: Environmental Concentrations
(Chair: W.R. Cullen)

(1) Canadian Environmental Protection Act assessment of non-pesticidal organotin compounds (R.J. Maguire)
(2) Butyltin compounds in water, sediment and biological samples in Canada (Y.K. Chau, R.J. Maguire, M. Brown, F. Yang and S.P. Batchelor)
(3) Butyltin compounds in the aquatic environment of British Columbia (C.L. Garrett)
(4) Changes in butyltin residue concentrations in Atlantic Canada between 1984 and 1994 (W.R. Ernst, G. Julien, P. Hennigar and J. Hanson)
(5) An overview of butyltin studies in B.C. coastal waters (J.A.J. Thompson)

Session 2: Biological Effects
(Chair: J.A.J. Thompson)

(6) The use of starfish in toxicological studies of organotin contamination in coastal environments (É. Pelletier, A. Mercier, C. Normandeau and C. Rouleau)
(7) Early imposex studies in Canada (D.V. Ellis)
(8) TBT contamination and imposex in the dogwhelk *Nucella lapillus* at sites in eastern Canada (N. Prouse)
(9) Cellular toxicology of TBT in the blue mussel *Mytilus edulis* (S.D. St-Jean, É. Pelletier and S.C. Courtenay)
(10) Tributyltin accumulation and toxicity to *Hyalella azteca* (U. Borgmann, Y.K. Chau, P.T.S. Wong, M. Brown and J. Yaromich)

Session 3: Chemical Considerations
(Chair: Y.K. Chau)

(11) Tin compounds and membrane permeability (W.R. Cullen, F.G. Herring and B.U. Nwata)
(12) Volatilization of tin compounds in the environment (J. Feldmann, A.V. Hirner and W.R. Cullen)
(13) Organotin leachates in drinking water from chlorinated poly(vinyl chloride) (CPVC) pipe (D.S. Forsyth and B. Jay)
(14) Measurement of tributyltin in sediments using electrospray tandem MS (E. Gentil and J.H. Banoub)
(15) A simple ethylation procedure for the speciation of metals (C.F. Harrington, J. Feldmann, G.K. Eigendorf and W.R. Cullen)

Rapporteur: R.J. Maguire

Abstracts of Presentations

Canadian Environmental Protection Act Assessment of Non-Pesticidal Organotin Compounds

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Abstract

In the past thirty years organotin compounds have developed into important industrial commodities. Tin probably has more organometallic applications than any other metal. These include uses as poly(vinyl chloride) stabilizers, industrial catalysts, industrial and agricultural biocides, and wood preserving and antifouling applications. The organotin compound of most environmental concern is tributyltin, because of its extremely high toxicity to aquatic organisms. A review is given of organotin use in Canada, occurrence, fate and effects in the environment, the 1989 regulation of tributyltin antifoulants under the Pest Control Products Act, and the 1993 Canadian Environmental Protection Act assessment of non-pesticidal organotin compounds. Non-pesticidal organotin compounds were judged not to be toxic to the environment.

Butyltin Compounds in the Canadian Environment - A Review of Their Occurrence Since Regulation of Use in 1989

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Abstract

National surveys of the occurrence of butyltin compounds in water and sediment in Canadian harbours and rivers and several sewage treatment plants were carried out in 1993 and 1994 to assess the environmental pollution status of butyltins after the regulation of antifouling uses of tributyltin in 1989. Apart from the butyltin and methyltin species, other organotin species such as propyltin, phenyltin, octyltin and dicyclohexyltin were also found in some sediments. Both the monooctyltin and dioctyltin species were frequently found in significant concentrations in sewage sludge. The results suggest that there has been a general decrease in concentrations of butyltin compounds in the environment as a result of the 1989 regulation.

Butyltin Compounds in the Aquatic Environment of British Columbia

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Abstract

Between 1984 and 1995, Environment Canada - Pacific and Yukon Region conducted sampling programs to determine the presence of butyltin compounds in the coastal environment of British Columbia. Surveys focused on suspected sources of release including harbours (primarily shipyards and commercial docks), marinas, recreational boating areas, and salmon farms.

Especially high concentrations of butyltin compounds were detected in marinas located in confined and poorly flushed areas and in harbours in the vicinity of shipyards and commercial docks. Prior to the introduction of Canadian regulations on the use of TBT-based antifoulants in 1989, TBT concentrations in the hundreds to thousands of ng Sn/L range were detected in surface waters from south coastal marinas. Mussels and sediments contained butyltin compounds at concentrations of up to several thousand ng Sn/g (wet weight in mussels, dry weight in sediments). In harbours, TBT concentrations of up to several thousand ng Sn/g were detected in sediment samples (up to 42,000 ng Sn/g). Concentrations of butyltin compounds in crabs, fish, clams, mussels and shrimp from harbours were also elevated in comparison to reference areas. Elevated concentrations of butyltin compounds were also detected in the water, sediment, and farmed salmon collected from coastal salmon farms using TBT-containing antifoulants for treating net pens, and in shellfish from some recreational boating areas during the peak summer boating season.

Environment Canada surveys conducted subsequent to the introduction of regulations in 1989 indicate that TBT concentrations in mussels, and possibly surface waters, at recreational marinas have declined since the 1980s. In contrast, TBT concentrations in the surface water and mussels at Vancouver Harbour shipyards and commercial docks have not declined. The concentrations of butyltin compounds in bottom sediments from both marina and harbour areas remain high. In addition, despite the lower concentrations of butyltin compounds detected in water samples from marinas in the 1990s, TBT concentrations in all surface water samples

collected from both marinas and harbours still exceed the current Canadian Water Quality Guideline for the protection of aquatic life in marine and estuarine areas. Recent information on concentrations of butyltin compounds at salmon farms and recreational boating areas is lacking. However, it is likely that concentrations have declined at these sites since regulations have prohibited the use of TBT-containing antifoulants at aquaculture facilities and on most recreational boats since 1989.

The continued presence of high concentrations of butyltin compounds in the environment indicates the need for further evaluation of current regulations on these compounds. Environment Canada surveys indicate that existing regulations have been effective in reducing contamination originating from recreational boats, but may not be as effective at reducing contamination from commercial vessels in harbours and shipyards.

Changes in Butyltin Residue Concentrations in Atlantic Canada between 1984 and 1994

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Abstract

Due to effects of butyltins on marine organisms which have been documented throughout the world, Canada implemented control on the use of TBT as aquatic antifoulants in 1989. Samples of marine sediments obtained from large and small vessel harbours of Atlantic Canada in 1988 were compared with sediments obtained from the same areas in 1994. The results of the analysis of samples for tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT) indicate that residue concentrations generally decreased in the pleasure craft areas while they generally increased in the large vessel harbours. A determination of the percentage of TBT in those samples suggests probable fresh inputs in large vessel harbours while in pleasure craft marinas the butyltin content was likely the result of older inputs. The risk to benthic organisms in large vessel harbours still remains high.

An Overview of TBT Studies in B.C. Coastal Waters

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Abstract

The Institute of Ocean Sciences has been actively engaged in TBT-related studies since 1992 when a program funded by the Toxic Chemicals Green Plan was initiated. In-house studies have concentrated on surface microlayer and sediment chemistry and the depositional history of TBT and its degradation products. We have also provided funding and field support for imposex studies at the University of Victoria. Results of the water and sediment studies will be described in some detail and discussed in the context of temporal and spatial changes since the imposition of controls on TBT-based paints in 1989. Imposex studies will be discussed with respect to butyltin residue concentrations found in tissues collected in 1994.

The Use of Starfish in Toxicological Studies of Organotin Contamination in Coastal Environments

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Abstract

This presentation summarizes our efforts in the last five years to study various chemical and toxicological aspects of the contamination of echinoderms, namely starfish, *Leptasterias polaris*, by organotin compounds. *L. polaris*, a six-armed sea star widely distributed in the St. Lawrence Gulf and Estuary, was first used as a biological model in long-term trophic transfer studies of methylmercury about 10 years ago. The simplified food chain model: suspended particles → blue mussel → starfish, was used in many instances to study the chemical behaviour and the toxicological effects of marine contaminants under near natural conditions.

For example, *L. polaris* was used to assess the degradation potential and toxicological implications of tributyltin (4.8 µg TBT/g) ingested with food for a 53-day period. Sequential debutylation occurred within 2 weeks, first yielding dibutyltin (DBT) which seemed to accumulate more readily in the caeca (up to 1.1 µg/g) and then monobutyltin (MBT) which appeared only in few individuals at low concentrations. All chemical species remained barely detectable in the gonads. Nevertheless, a histological study revealed smaller mature oocytes and thinner epithelium in the gonads of contaminated individuals compared to control organisms.

In another study, whole-body autoradiography (WBARG) and a multicompartmental model were used in conjunction to describe, quantify and compare the distribution kinetics of trophic single doses of methylmercury, tributyltin, and their corresponding ions, Hg(II) and Sn(IV). WBARG indicated that transfer of labeled compounds from the stomach to pyloric caeca, and from pyloric caeca to the rest of the starfish, proceeded mainly by transport *via* the pyloric ducts and by diffusion in the coelomic fluid, respectively, with a negligible contribution from the haemal system. Although organ distribution of methylmercury and TBT at steady-state were rather similar, their kinetics were radically different, TBT distribution kinetics being the slowest of all studied chemicals. This finding enhances the

importance of considering both thermodynamic and kinetic approaches in our studies of organometal uptake. In a similar study conducted with starfish exposed to contaminated seawater (and not to food), WBARG revealed that uptake of the labeled TBT was trapped into the external epidermis of the body wall. This direct observation of organometal deposition confirmed that translocation of TBT is a more difficult and slower process compared to its corresponding inorganic ion. High steric hindrance and low solubility in seawater may explain this behaviour of TBT.

Finally, our first study involving *L. polaris* was undertaken last fall (1995) in an attempt to assess the presence of organotin compounds in coastal waters of the St. Lawrence Estuary. Preliminary results indicate the presence of TBT and DBT in almost all starfish tissues from all sites sampled from Bic (near Rimouski) to Gaspé. Concentrations of TBT and DBT ranged from 5 to 70 ng/g (wet weight) with no apparent geographical trend. Sediment, mussels and starfish samples near a dry dock at Les Méchins indicated the presence of a point source in that vicinity possibly originating from sandblasting operations in the dry dock.

Early TBT-Imposex Studies in Canada

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Abstract

Investigations of the TBT bioindicator, imposex in neogastropod molluscs, were started in Canada in 1986, with preliminary observations on intertidal whelks around Victoria. These were formalized into targeted surveys through to 1991 by various graduate students, undergraduate students, and research assistants, in British Columbia, Southeast Asia and Fiji. The assessment technique makes an excellent teaching tool to illustrate the concept of bioindicators, and has been taught in a variety of marine and pollution courses at the University of Victoria. A 1972 Honours student thesis on antifouling paints had compared TBT-based and other paints.

On the Pacific coast of Canada, 4-5 species of intertidal whelks are readily collectible intertidally. On a multi-species coast, the anatomy, life cycles, pathological responses and taxonomy of each species needs to be determined, because these features affect suitability as bioindicators. We opted to concentrate on simple measures of frequency and female penis length in all species thus facilitating geographical scale surveys.

Results of the surveys through 1988 showed that imposex occurred to that time in all female whelks throughout the Strait of Georgia, Strait of Juan de Fuca and Puget Sound. The only sites in southern British Columbia with some species free of imposex were on remote parts of the outer west coast of Vancouver Island. In 1993 after the 1989 TBT controls, investigations were started to measure recovery rates from prior contamination. By 1993 there were some signs of lesser imposex levels, but most Pacific coast species live longer than the 4 years since controls were implemented, so old affected specimens still dominated the populations. The recovery investigations were expanded in 1994 by a joint investigation with the Institute of Ocean Sciences. Some recovery from TBT contamination has now appeared, particularly on the west coast of Vancouver Island, but there is little sign of recovery in this TBT-induced whelk pathology near Victoria and Vancouver.

The generalized conclusion from the Canadian Pacific coast investigations is that imposex is a useful geographical scale bioindicator, and can pinpoint sites for sampling sediments for chemical measures of TBT contamination. We do not use imposex as an indicator of levels of TBT contamination, although such correlations have been developed elsewhere.

From 1986 to 1993 the investigations were supported by seed and instructional funds from the University of Victoria. Government support was started in 1994 through the Green Plan Toxic Chemicals Program, Department of Fisheries and Oceans, through a collaborative agreement with the University of Victoria.

My laboratory at the University of Victoria is now being disbanded as I approach retirement. My collection of about 200 reprints on TBT and imposex is being catalogued for the University archive. The catalogue will be available initially in hard copy and disk, and eventually electronically. Reprints will be available through interlibrary loan.

Survey of Eastern Canada for Imposex, a Bioindicator of Tributyltin (TBT) Contamination, in the Dogwhelk *Nucella lapillus*

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Abstract

In the presence of tributyltin (TBT) male characteristics, *i.e.*, a penis and *vas deferens*, are imposed on female neogastropods (marine snails) leading to reproductive failure. This abnormal phenomenon, termed imposex, is well documented in the dogwhelk *Nucella lapillus* and other neogastropods and is used as a bioindicator of TBT contamination. *N. lapillus* is common on rocky intertidal shores in eastern North America from southern Labrador to New York.

Sites were surveyed during 1995 in eastern Canada to determine the occurrence of imposex in populations of *N. lapillus*. Imposex was observed at 13/34 sites near shipyards, marinas, and boating activities and the frequency of imposex ranged from 29 to 100%. Halifax Harbour had imposex frequencies ranging from 65 to 100% at 6 sites examined. Sources of TBT are likely from shipyards and continued use of TBT on vessels over 25 m. Other harbours where imposex was observed included Sydney Harbour (100%) and Chester Harbour (88%) in Nova Scotia, Les Méchins Harbour (95%) in Québec, Saint John Harbour (66%) in New Brunswick, and Arnold's Cove Harbour (56%) and Come-by-Chance Harbour (29%) in Newfoundland. Specimens collected near a shipyard at Les Méchins had the highest relative penis size (RPS) index (28.5%) in the survey, and were severely imposexed.

Imposex was not found in 7 sites with little or no boating activity. Another 14 harbour sites, with seemingly ideal habitat, had no *N. lapillus* and their presence in the past is unknown. Possibly TBT contamination caused the disappearance of dogwhelks at these sites.

There have been no previous surveys for imposex in eastern Canada to determine if conditions are improving since TBT was regulated in 1989. Sites will be resurveyed and new sites examined in 1996.

Cellular Toxicology of TBT in the Blue Mussel (*Mytilus edulis*)

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Abstract

Despite a partial ban on the usage of tributyltin (TBT) in most industrialized countries, studies from around the world continue to show high levels of butyltins in surface waters, sediments and biota. Data collected by Environment Canada (Atlantic Region) showed that in many cases levels in sediments of the southern Gulf of St. Lawrence were actually higher 5 years after the 1989 restriction in TBT usage than they had been before. In response to these studies, and to a paucity of related monitoring and research on TBT effects in Maritime biota that was identified at the 1994 mid-program review of the Department of Fisheries and Ocean's Toxic Chemicals Program, blue mussels from 5 sites in the southern Gulf of St. Lawrence were sampled for organotins. Whole-body, wet weight concentrations of TBT ranged from low (10 ng/g) in Cardigan Bay, Prince Edward Island, to medium (245 ng/g) in Summerside, Prince Edward Island. Sampling of surficial sediments in these sites is under way. In the laboratory, a study of the effects of chronic exposure to environmentally relevant concentrations has begun. In this presentation we will discuss the toxicological response in the blue mussel *Mytilus edulis* to TBT at the cellular level. Specifically, we will look at the effect of this compound on the immune system through the level of apoptosis, the number of hemocytes and the phagocytosis capability of natural killer cells' equivalent in the mussel.

Tributyltin Accumulation and Toxicity to *Hyalella azteca*

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Abstract

The relationship between bioaccumulation and toxicity of tributyltin in *Hyalella azteca* was determined for future use in identifying sites of TBT induced toxicity in the field. *Hyalella* accumulated waterborne TBT rapidly, reaching equilibrium within 1 week. Short exposure times are, therefore, sufficient to measure TBT availability in laboratory or field exposures to contaminated water or sediments. Accumulation was not affected significantly by body size for animals between 0.1 and 0.6 mg dry weight, eliminating the need for a body size correction factor. Young amphipods were more sensitive to TBT than adults when both were exposed for 1 week. The 4-wk LC_{50} initiated with 0-1 week old young was 4.8 nM. Tributyltin accumulation was approximately proportional to TBT in water, and the 4-week LC_{50} expressed on a body concentration basis was 110 nmole/g dry weight. Accumulation of TBT by adult *Hyalella* to concentrations of about 100 nmole/g or more in short term (1-wk) exposures to environmental samples would be predicted to result in chronic TBT-induced toxicity.

The Effect of Organotin Compounds on the Permeability of Model Biological Membranes

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Abstract

The efflux of dimethylarsinic acid (DMA) from liposomes formed from egg phosphatidylcholine (EPC) is increased when tributyltin chloride (TBT) is added to the extraliposomal compartment; however the addition of butyltin trichloride (MBT) slows down the efflux. When the liposomes are prepared from EPC and organotin compound different mechanisms for DMA efflux seem to operate: TBT-EPC liposomes show a mixture of facilitated and passive diffusion; MBT-EPC liposomes show only passive diffusion. The facilitated diffusion of DMA^- seems to be stopped by the addition of TBT to the extraliposomal compartment.

Volatilization of Organotin Compounds in the Environment

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Abstract

It is well known that microorganisms are able to methylate tin compounds. Besides environmental methylation of inorganic tin compounds, anthropogenic compounds, such as TBT, also enter the environment. Most of these compounds do not appear to enter the environment, because of their high boiling points. However, after methylation or hydride generation of these compounds to fully uncharged species their boiling points decrease (*e.g.*, tin tetrahydride, -52 °C; tetramethyltin, 78 °C, *n*-butyltin trihydride, 100 °C) and they can enter the atmosphere.

For instance, methylation takes place on municipal landfill sites and in harbours, as well as during the putrefaction of sewage sludge in fermentation tanks. The methane/carbon dioxide gases from landfills and fermentation tanks were sampled. After cryogenic sampling on-site, the compounds in the gas samples were separated without any cleanup by using a non-polar column (SP-2100 on Supelcoport) and a low temperature gas chromatograph. ICP-MS was used as an element-specific, sensitive detector. A rhodium solution, which was introduced simultaneously into the plasma after nebulization, was used as a continuous internal standard. Volatile tin standards were produced by using hydride generation methodology. The separation showed a linear relationship between the boiling points of standards and their retention times ($r^2 = 0.999$). Therefore this relationship was used to determine unknown volatile tin compounds.

Besides tetramethyltin as a major compound, higher alkylated tin compounds were found (*e.g.*, *n*-butyltin hydride, *n*-butylmethyltin hydride). The concentrations of total volatile tin species in different landfill gases from

municipal landfills were determined to be between 0.12 and 99.7 $\mu g/m^3$. In sewage gases the concentrations were much lower (0.005-0.17 $\mu g/m^3$).

Organotin Leachates in Drinking Water from Chlorinated Poly(vinyl chloride) (CPVC) Pipe

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Abstract

A solid phase extraction method, using bonded silica sorbent, was developed for the isolation of mono- and di-methyltin, -butyltin and -octyltin from drinking water. Recoveries averaged 92% over two tested sample weights and spiking levels. Ethyl derivatives were made by Grignard reaction for determination by gas chromatography - atomic absorption spectrometry. Static and repetitive extraction studies were conducted at 24 and 65 °C. Butyltins were rapidly leached into drinking water kept in CPVC pipe samples. Monobutyltin and dibutyltin levels reached 17.6 and 169.5 ng/g, respectively, in water collected from CPVC pipe heated to 65 °C. Butyltins were still leached from CPVC pipe after 20 repetitive extracts, suggesting that new CPVC water systems contaminate the supplied water with organotins for some time after installation.

Measurement of Tributyltin in Sediments Using Electrospray Tandem Mass Spectrometry

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Abstract

The tributyltin (TBT) concentration in a sediment reference material (PACS-1) and in other sediments was determined using electrospray tandem mass spectrometry in the positive ion mode. Tributyltin was extracted using two different protocols: extraction with 1-butanol followed by evaporation and dissolution in ethanol or by ultrasonication in 10% HCl followed by extraction with hexane, evaporation and dissolution in ethanol. Samples were introduced into the electrospray source *via* a continuous flow of acetonitrile:water (70:30) at a flow rate of 20 µL/min using a Shimadzu LC-10AD pump. Quantification of TBT was achieved by ES MS/MS through Multiple Reaction Monitoring (MRM) (using four different channels) of the product ion/precursor ion pairs m/z 235/291, 179/291. 179/235 and 123/179.

Determination of Organotin Speciation Using an *in-situ* Ethylation Procedure and Detection by Gas Chromatography - Mass Spectrometry (GC-MS) and Inductively Coupled Plasma MS (GC-ICP-MS)

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Abstract

It is widely accepted that the determination of the total concentration of an element in the environment reveals little about its toxicity, biogeochemistry or environmental persistence. This is particularly true for elements such as tin, lead and mercury which undergo biological methylation in sediments, which considerably changes their environmental impact. For these reasons a great deal of research has been carried out into developing analytical techniques that are able to quantitatively determine the form in which elements such as these exist in the environment.

Capillary column gas chromatography coupled to mass spectrometry (electron impact mode) allows the structural determination of the exact organotin compound present in the environment. However, the use of GC necessitates conversion of the organotin compound to a more volatile form, such as hydride (*via* sodium borohydride) or alkyl (*via* Grignard reagent) derivative. Both of these suffer from significant disadvantages when analyzing environmental samples. A recent development involves the aqueous phase ethylation of the organotin compound with sodium tetraethylborate to facilitate the necessary increase in volatility.

This paper will outline work being carried out to study the butyl- and cyclohexyltin compounds in coastal marine waters using GC-MS. Recent results using gas chromatography coupled to ICP-MS for the determination of TBT in marine surface waters and microlayer samples will be presented. Preliminary results showing the application of this technique to the simultaneous determination of organolead, organotin and organomercury compounds in water samples will also be presented.

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Current and Planned Activities of Workshop Participants on Organotin Compounds (Where Identified)

J.H. Banoub

- occurrence of organotin compounds in lobster tissues (with J. Uthe, C.L. Chou and N. Prouse)
- study of organotin-tropolone complexes by electrospray tandem mass spectrometry

U. Borgmann

- uptake and toxicity of TBT to *Hyalella azteca* and use of TBT accumulation in *Hyalella* to identify sites of biological impact (with Y.K. Chau).

Y.K. Chau

- national survey on the occurrence of organotins in harbours, rivers, aquatic biota and sewage treatment plants (with R.J. Maguire); a study of toxicity of TBT to *Hyalella azteca vs.* body burden (with U. Borgmann); investigation of the persistence and degradation of butyltins in the environment using sediment cores in locations of organotin pollution (with R.J. Maguire); assessment of the feasibility of monitoring *Hyalella azteca* as an indicator of bioavailability and effects at TBT-contaminated sites (with U. Borgmann).

S.C. Courtenay

- cellular toxicology of TBT in the blue mussel *M. edulis* (Ph.D. project of S. St-Jean, co-directed by É. Pelletier).

W.R. Ernst

- inspections of ship repair facilities in Atlantic Region to determine whether their activities can be improved to reduce the quantities of organotins reaching the aquatic environment

J. Feldmann (with W.R. Cullen)

- identification of the higher alkylated tin species, which have been found in landfill gas and in gases from sewage sludge formation, by using GC-ICP-MS.
- determination of the stability of volatile organotin compounds in the environment. Sampling of ambient air close to possible sources of volatile organotin species. These sites are polluted sites like municipal waste deposits and water treatment plants, and unpolluted sites such as hot springs and swamps.
- microbiological experiments with cultures of polluted and unpolluted sites will be carried out to determine the biomethylation of tin. We hope we can shed some light on the question: what kind of tin compounds are necessary to form volatile tin compounds?
- improvement of the detection limits of organotin compounds by using ethylation derivatization methodology and GC-ICP-MS.

D.S. Forsyth

- current activities have centered on organotins leached from CPVC pipe used for potable water delivery. Planned activities will include a seafood survey from supermarkets, and an examination of seafood consumed locally on the East and West coasts, particularly from regions near or in areas where imposex has been noted.

C.L. Garrett

- completing the publications listed in the List of Publications. These documents contain the findings of all of our work conducted on organotin compounds between 1984 and 1995. Although an interest in organotin issues remains, no new work on these compounds has been planned for the next fiscal year.

C.F. Harrington (with W.R. Cullen)

- currently developing a method for the determination of butyltin speciation in aqueous samples which involves the use of sodium tetraethylborate as the derivatizing agent. The advantages of this method over the current methods in use are its simplicity and lack of interferences. We are also developing the coupling of gas chromatography and inductively-coupled mass spectrometry (GC-ICP-MS) for the multielement speciation of metals such as tin, mercury and lead in environmental samples. Future work is planned to investigate the rates and pathways of the degradation of butyltin compounds in sediments, and to study the methylation of these breakdown compounds in the environment, to determine how this affects their cycling and toxicity.

R.J. Maguire

- occurrence of tributyltin in freshwater harbours and marinas (water, surface microlayer, sediment, suspended solids, caged and native biota) (with Y.K. Chau)
- development of biomonitors for tributyltin in freshwater
- determination of occurrence/persistence of tributyltin, dibutyltin and monobutyltin in sediment cores in freshwater, and comparisons with cores from seawater (with Y.K. Chau)
- toxicity of tributyltin and dibutyltin to freshwater invertebrates (snails, bivalves)
- assessing the feasibility of dealing with tributyltin as a Track 1 chemical under the Toxic Chemicals Management Policy

É. Pelletier

- uptake, degradation and excretion rate of organotin in starfish (lab and field work) (M.Sc. project of C. Normandeau)
- uptake, degradation and toxic effects of TBT *via* water and food in symbiotic sea anemones (Ph.D. project of A. Mercier)
- cellular toxicology of TBT in the blue mussel *M. edulis* (Ph.D. project of S. St-Jean, co-directed by S.C. Courtenay)
- effects of TBT and other trace metals on the phagocytosis capability of blue mussel haemocytes (*in vitro* exposure) (M.Sc. project of N. Bouchard, co-directed by M. Fournier, Université du Québec à Montréal
- development of an analytical method for organotin species in micro-samples (≤ 100 mg of biological tissues) (under contract with Pavlova Océanologie Inc.)

R.C. Pierce (see also R. Waters, J.A.J. Thompson and N. Prouse)

- funding Department of Fisheries and Oceans studies to determine the geographic extent and trends in organotin contamination in freshwater and marine environments and to determine the extent of biological change (*i.e.*, imposex) in selected organisms
- for the next fiscal year, we plan to extend Department of Fisheries and Oceans work to other recreational and commercial harbours on both coasts and to try to more fully assess the causal link between organotins (specifically tributyltin) and biological effects.

N. Prouse (also J. Uthe)

- measure concentrations of TBT in edible lobster tissues, focusing on Halifax Harbour
- attempt, by contract, to determine potential biological effects of TBT on Halifax Harbour lobster
- use the dogwhelk imposex bioindicator of TBT contamination to estimate potential problem areas for the lobster fishery
- continue the imposex study of *Nucella lapillus* (dogwhelks) in Halifax Harbour, including a re-survey of sites and transplant experiments at selected sites
- examine how far imposex extends outward from Halifax Harbour
- study the relationship between imposex and TBT concentrations in dogwhelks and their food supply, supporting sediments, and seawater
- study imposex in the edible whelk *Buccinum undatum* from Halifax Harbour
- based on the results of the Halifax Harbour studies, the following investigations are contemplated: (i) concentrations of TBT in lobster from other TBT-impacted areas, (ii) re-survey sites outside Halifax Harbour for imposex in dogwhelks, (iii) survey new sites for imposex, (iv) determine the occurrence of imposex in the edible whelk from fishing areas in the Gulf of St. Lawrence

S.D. St-Jean

- cellular toxicology of TBT in the blue mussel *M. edulis* (Ph.D. project - directed by É. Pelletier and S.C. Courtenay)

J.A.J. Thompson

- during the last year of funding under the current DFO Toxic Chemicals Green Plan, complete analyses of sediments taken in 1995 from Prince Rupert Harbour and the Strait of Georgia. The latter were collected as two approx. 60 cm cores which will be sectioned and dated.

- contingent upon funding, further imposex surveys, examination of the use of semi-permeable membrane devices (SPMDs) in monitoring, and research applications of electrospray mass spectrometry to TBT problems.

R. Waters (see also R.C. Pierce)

- gathering information for DFO on TBT as one of many contaminants in the Pacific region. This is in connection with a national generic regional assessment of toxic chemicals.

I. Wilkin

- surveys of marinas and shipyards to monitor the use of antifoulants and particularly tin. A survey of commercial ship yards done last year found that the average yard is bottom painting 100 boats a year and tin is used on 8% of vessels painted. Storage sheds were inspected and very few problems were found. Previous distributors were visited and stock inspected for registered product. Marinas in Washington State have been contacted and sent posters for Canadian customers to educate both them and the American storefront distributors about the regulation of antifouling paints. Ship yards will not be visited now for another two years. This coming season small marinas will be visited to educate in terms of regulations, and to inspect storage sheds.

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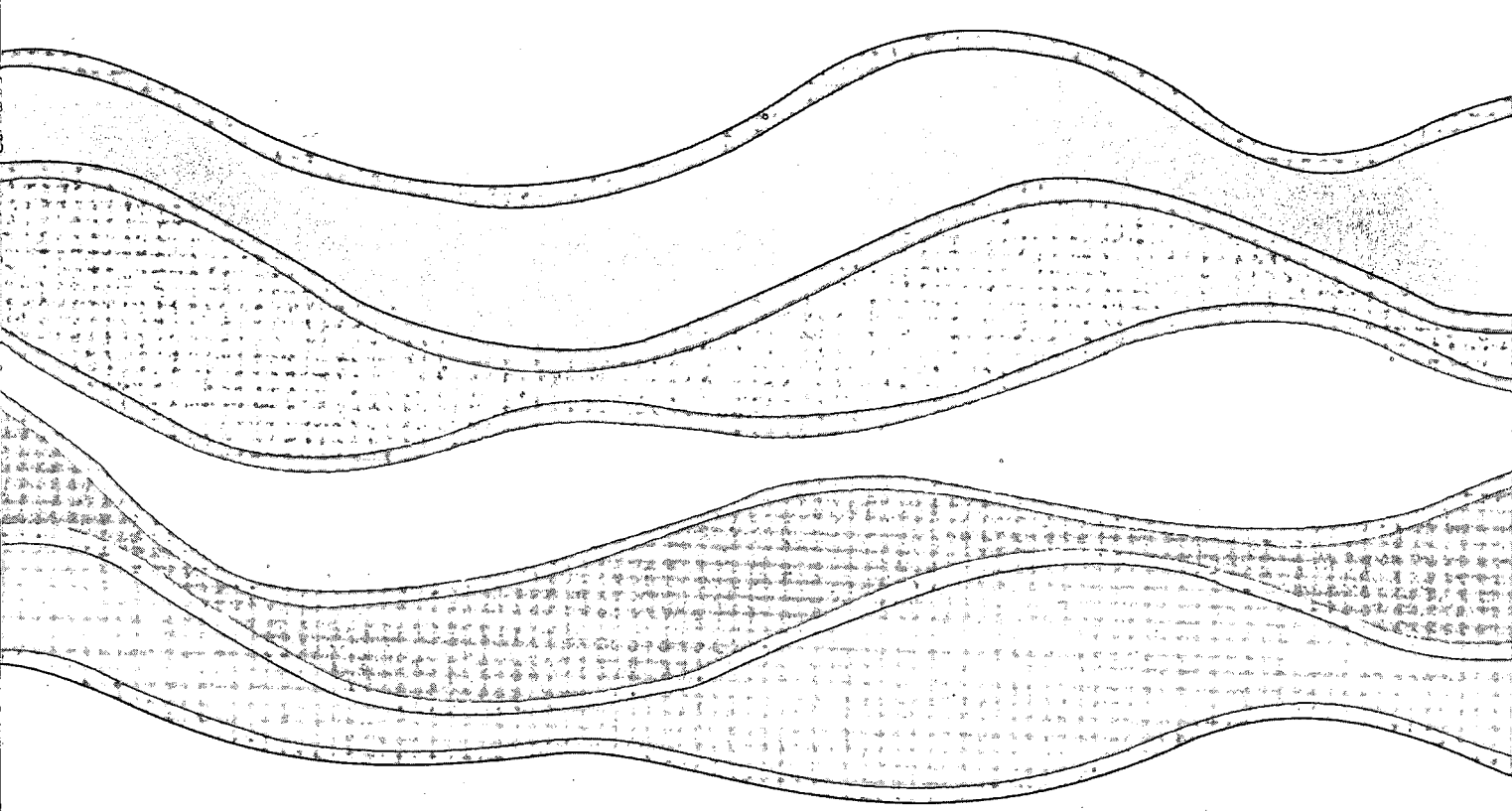

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