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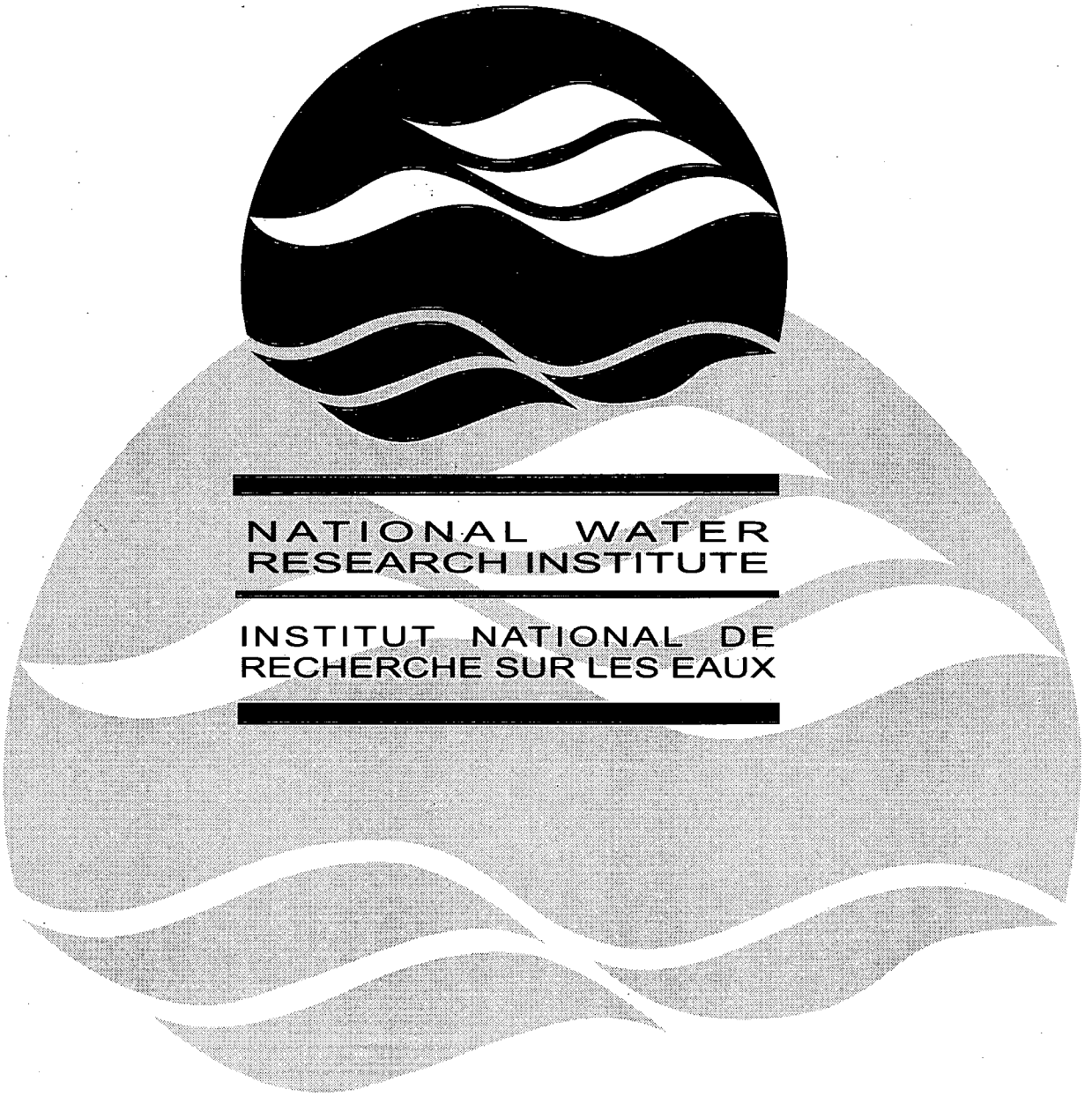

1ST ANNUAL WORKSHOP ON POLYBROMINATED DIPHENYL ETHERS IN THE ENVIRONMENT

M. Alaee, J.M. Luross, and D.B. Sergeant

NWRI Contribution No. 00-153

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AUGUST 19 - 20, 1999
Canada Centre for Inland Waters

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MANAGEMENT PERSPECTIVE

Polybrominated diphenyl ethers (PBDEs) are additive flame-retardants embedded in polymers and plastics to reduce their ability to catch fire. The annual global worldwide production of PBDEs in 1992 was 40 000 tons (EHC: 162, 1994).

The presence of PBDEs in environmental matrices was first detected in the early 1980's. Since then there have been several reports on the levels of PBDEs in various environmental compartments.

Based on preliminary results, in February 1999, a proposal to study *The Impact of PBDEs on Canadian Environment and Health of Canadians* was submitted to the Toxic Substances Research Initiative (TSRI). The project was funded for 3 years. The first step in this study was to inform all of the participants of the latest developments in this field and to exchange information between the participants. The 1st annual workshop on PBDEs in the environment was held at CCIW on August 19-20, 1999. Due to extraordinary interest by other scientists the workshop was opened to the scientific community. As a result more than 50 participants from the Departments of Environment, Fisheries and Oceans, Health Canada, Ontario Ministry of Environment, USDA, California Department of Toxic Substances Control, Carlton University, University of Guelph and University of Indiana participated in the two-day workshop.

The first day was dedicated to presentations. Professor Åke Bergman from Stockholm University delivered the keynote lecture which was followed by 13 presentations that ranged from the sources of analytical standards to risk assessment. The second day of the workshop was dedicated to open discussion between participants on fine-tuning of the research program. Results from the discussions along with the summary of the presentations are presented in these workshop proceedings.

SOMMAIRE À L'INTENTION DE LA DIRECTION

Les éthers de diphényle polybromés (EDPB) sont des additifs incorporés aux polymères et aux plastiques comme ignifugeants. La production mondiale annuelle d'EDPB en 1992 atteignait 40 000 tonnes (EHC: 162, 1994).

La présence d'EDPB dans des matrices de l'environnement a été décelée pour la première fois au début des années 1980. Depuis, plusieurs rapports ont été publiés sur les concentrations d'EDPB dans divers compartiments de l'environnement.

À partir de résultats préliminaires obtenus en février 1999, un projet d'étude des répercussions des EDPB sur l'environnement canadien et la santé des Canadiens a été présenté à l'Initiative de recherche sur les substances toxiques (IRST). Le projet a été financé pendant 3 ans. Dans la première étape de cette étude, on a informé tous les participants des derniers développements dans le domaine, et ces derniers ont échangé de l'information entre eux. Le premier atelier annuel sur les EDPB dans l'environnement a eu lieu au CCEI, les 19 et 20 août 1999. Étant donné l'intérêt extraordinaire démontré par d'autres chercheurs, l'atelier a été ouvert à la communauté scientifique. C'est ainsi que plus de 50 participants d'Environnement Canada, de Pêches et Océans Canada, de Santé Canada, du ministère de l'Environnement de l'Ontario, de l'USDA, du California Department of Toxic Substances Control, de l'Université Carleton, de l'Université de Guelph et de la University of Indiana ont pris part à l'atelier de deux jours.

Le premier jour a été consacré à des exposés. Le professeur Åke Bergman de l'Université de Stockholm a présenté le discours-programme, lequel a été suivi de 13 exposés qui ont traité des sources des étalons d'analyse jusqu'à l'évaluation du risque. La deuxième journée de l'atelier a été consacrée à une discussion ouverte entre les participants pour mettre la dernière main au programme de recherche. Les résultats des discussions ainsi que le résumé des exposés sont présentés dans le compte rendu de l'atelier.

ABSTRACT

The first annual workshop on polybrominated diphenyl ethers (PBDEs) in the Environment was held in Burlington, at the Canada Centre for Island Waters in Burlington, Ontario on August 19-20, 1999. Even though the main focus of the workshop was on PBDEs, the occurrence and environmental relevance of other brominated compounds such as tetrabromobisphenol A (TBBPA), hexabromocyclododecane (HBCDD), and halogenated dimethy bipyrroles were discussed.

The first day of the workshop consisted of fourteen presentations covering a variety of topics that ranged from sources of commercial standards and environmental distribution, to toxicity of brominated flame-retardants. The lectures included an overview of TSRI process, followed by the keynote lecture on the environmental issues of brominated flame-retardants. The presentations continued with an update on the current status and availability of commercial PBDEs, and was followed by a presentation on a method for the simultaneous determination of polybromianted and polychlorinated diphenyl ethers. The talks on the levels of PBDEs in the Canadian environment included the levels in the biota from coastal British Colombia, lake trout from the Great Lakes, marine mammals from the Arctic and selected wildlife tissue. The contributions on the proposed studies included the study of the distribution of the PBDEs in the sediment and biota from St. Lawrence Estuary, estimation of human exposure, toxicity assays for PBDEs, toxicity of PBDEs to aquatic organisms, and probabilistic risk assessment of PBDEs. Also there were two additional talks on chemical disruption and measurement of thyroidal status in fish, and on identification, distribution, and toxicological activity halogenated dimethyl bipyrroles.

The second day of the workshop was dedicated to open discussions. The first group discussed the determination, distribution and fate of PBDEs in the environment. The second group discussed the toxicity and risk assessment. The

workshop discussions from each workshop are summarized in the workshop report section, and the presentation materials used during the workshop are included in this document.

Key Words: brominated flame-retardants (BFRs), polybrominated diphenyl ethers (PBDEs), environmental distribution, toxicity

RÉSUMÉ

Le premier atelier annuel sur les éthers de diphényle polybromés (EDPB) dans l'environnement a eu lieu au Centre canadien des eaux intérieures (CCEI), à Burlington (Ontario), les 19 et 20 août 1999. L'atelier portait principalement sur les EDPB, mais on a également discuté de la présence et des effets d'autres composés bromés dans l'environnement, comme le tétrabromobisphénol A (TBBPA), l'hexabromocyclododécane (HBCDD) et les diméthylbipyrroles halogénés.

La première journée de l'atelier a consisté en quatorze exposés traitant de divers sujets allant des sources des étalons commerciaux et de la distribution des ignifugeants bromés dans l'environnement à la toxicité de ces substances. Les exposés comprenaient un aperçu des activités de l'IRST, suivi du discours-programme sur les problèmes entourant les effets des ignifugeants bromés sur l'environnement. Les exposés se sont poursuivis avec une mise à jour de l'état actuel et de la disponibilité des EDPB commerciaux, suivie d'un exposé sur une méthode de détermination simultanée des éthers de diphényle polybromés et polychlorés. Les exposés sur les concentrations d'EDPB dans l'environnement au Canada portaient sur les concentrations chez le biote sur les côtes de la Colombie-Britannique, chez le touladi dans les Grands Lacs, chez les mammifères marins de l'Arctique et dans les tissus de certains animaux sauvages. Les contributions dans les études proposées comprenaient l'étude de

la distribution des EDPB dans les sédiments et le biote de l'estuaire du Saint-Laurent, l'estimation de l'exposition humaine, les essais sur la toxicité des EDPB, la toxicité des EDPB pour les organismes aquatiques et l'évaluation probabiliste des risques des EDPB. En outre, deux autres exposés ont porté sur la perturbation chimique et la mesure de la fonction thyroïdienne chez les poissons, ainsi que sur l'identification, la distribution et l'activité toxicologique des diméthylbipyrroles halogénés.

La deuxième journée de l'atelier a été consacrée à des discussions ouvertes. Le premier groupe a discuté de la détermination, de la distribution et du devenir des EDPB dans l'environnement. Le deuxième groupe a discuté de la toxicité et de l'évaluation du risque. Les discussions de chaque atelier sont résumées dans la section de l'atelier consacrée au rapport, et le matériel utilisé dans le cadre des exposés de l'atelier est inclus dans ce document.

Mots clés : ignifugeants bromés, éthers de diphényle polybromés (EDPB), distribution dans l'environnement, toxicité

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Introduction:

Long before mankind learned to harness fire as a source of energy, he was horrified by the devastation caused by it. Fire is still a major source of damage to properties and loss of life. In fact, in the US, every year over 3,000,000 fires are reported, which result in 29,000 injuries, 4,500 deaths, and direct losses of over $8 billion (EHC: 192, 1997). Recent advances in technology have resulted in an increase in use of synthetic polymers, electronic equipment, and other ignitable materials loads in our commercial and residential habitat. This has drastically contributed to fire hazard. In order to reduce the chances of ignition and burning of these materials the application of flame-retardants has increased.

The idea of flame retardant materials dates back to about 450 BC, when the Egyptians used alum to reduce the flammability of wood. Romans (about 200 BC) used a mixture of alum and vinegar to reduce the combustibility of wood. Today, there are more than 175 chemicals classified as flame-retardants. Flame-retardants are divided into four major groups: inorganic, halogenated organic, organophosporus and nitrogen based flame-retardants which account for 50%, 25%, 20% and >5% of the annual production, respectively (EHC: 192, 1997).

Halogenated organic flame-retardants are divided into chlorinated and brominated flame-retardants (BFRs). BFRs account for the majority of the halogenated flame-retardants currently in use. BFRs are divided into two subgroups: reactive and additive. Reactive flame-retardants, which are a group of compounds such as tetrabromobisphenol A (TBBPA), are chemically bonded into the plastics. On the other hand, additive flame-retardant compounds such as polybrominated diphenyl ethers (PBDEs) and hexabromocylododecane (HBDD) are only mixed together with the other components of the polymers. As a result, additive flame retardant compounds can be easily released from discarded components and find their way into the environment (Hutzinger et. al 1976, Hutzinger and Thoma 1987). PBDEs were the first group of BFRs to be detected in environmental compartments. In 1979 the presence of BDE-209 (deca-BDE) in soil and sludge (DeCarlo 1979), was detected in the areas surrounding plants where PBDEs were manufactured in the US. Two years later, Anderson and Blomkvist (1981) reported the presence of PBDEs in samples collected along Visken River in Sweden. Jansson et. al. (1987) first indicated that PBDEs are global contaminants by demonstrating their presence in fish eating birds and marine mammals in samples collected from Baltic Sea, North Sea and Arctic Ocean. PBDE congeners were also observed in marine fish, shellfish, and sediment (Watanabe et al. 1987); and were also found in air particulate from Japan and Taiwan (Watanabe et al. 1992). PBDEs were also reported in cod liver and herring from the North Sea (de Boer1989), and in eels from fresh water systems in the Netherlands (de Boer 1990). Stafford (1983) reported the presence of PBDEs in eggs and tissues of fish-eating birds from 6 states in the US and from Ontario, Canada.

PBDEs are lipophilic compounds and were shown to bioaccumulate through the food web (Sellström et al. 1993). PBDEs have also been detected in human adipose tissue (Stanley et al. 1991). Norén and Meironyté (1998) showed that the concentration of PBDEs in breast milk doubled every 5 years over the past 25 years (Figure 1).

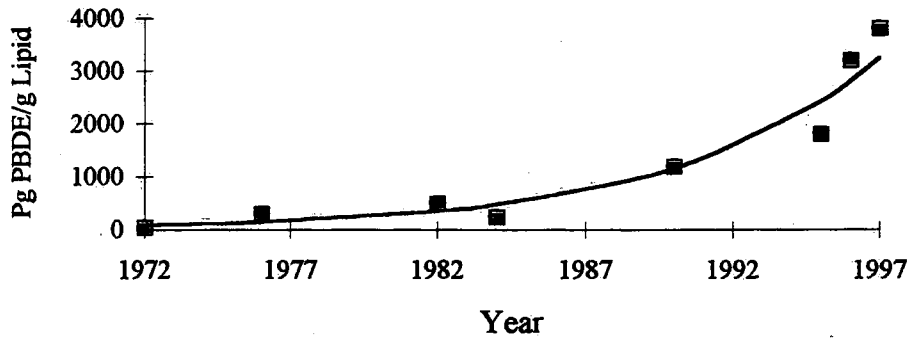

Figure 1. PBDE concentration in breast milk in Sweden, between 1972 and 1997. Results show that the concentrations of PBDEs have doubled every 5 years in humans.

In a recent study Sjödin et al. (1999) reported detectable levels of PBDEs and other BFRs in human plasma collected from hospital workers, data entry clerks, and workers in a electronic dismantling plant. Results from this study showed elevated levels of PBDEs in the plasma of workers in an electronic dismantling plant compared to the other groups.

In 1997, a proposal to study the levels of PBDEs in the aquatic environment was funded by the DFO under the Toxic Chemicals Program. As the result of this project, method based on HRGC/HRMS for the determination of PBDEs in environmental samples were developed at Canada Centre for Inland Waters (CCIW) and Institute of Ocean Sciences (IOS). These methods are based on isotope dilution techniques and provide the most accurate results to-date. These methods were tested for accuracy and precision through an inter-laboratory study between CCIW and IOS. Results from this study showed detectable levels of PBDEs CRMs which consisted of lake trout from Lake Ontario, salmon and herring from Pacific Ocean (Sergeant et al. 1998). PBDEs have subsequently been found in lake trout from the Great Lakes (Luross et.al. 1998), dungeness crab and harbor seals from the strait of Georgia BC, and sturgeon from the Fraser River BC (Ikonomou et al. 1998).

In another investigation funded under the Northern Contaminants Program (NCP), PBDEs were detected in air samples from Alert, Northwest Territories (Alaee et al. 1998) and marine mammals from the Arctic (Muir et al. 1998).

The Wildlife Toxicology Program of the Canadian Wildlife Service (CWS) has been actively seeking to identify organic chemical compounds in wildlife tissues since the early 1970's. The Laboratory Services Section of the Program has provided chemical determinations on tissues since 1986 to support various research projects. During 1997-98, this investigation determined that brominated diphenyl ethers were present in a number of different wildlife tissues taken from various locations in Canada. The extent of the contamination of wildlife with these compounds and the temporal trends are unknown.

Information on PBDEs in various environmental compartments is still lacking. With the current state of information and knowledge on distribution, movement, and toxicity of PBDEs in the Canadian environment adequate risk assessment cannot be done. To address these gaps a proposal to determine the levels of PBDEs in various environmental compartments; to study their toxicity; and to investigate their sources and fate of PBDEs in the Canadian environment was developed. This proposal was submitted for funding to Toxic Substance Research Initiative (TSRI), and was funded in April 1999. The initial step for this project was to hold a workshop to bring all participants up to date with the recent determinations and to exchange information. During the workshop it was suggested to put together the presentations materials for future reference, as the result this proceedings was prepared.

As the result of the enthusiastic support of the participants it was decided to hold this workshop on annual basis at CCIW for the duration of this project. The next workshop is scheduled for summer 2000.

Acknowledgements:
We would like to acknowledge the hard work and dedication of X. Wang, C. Spencer, J. Cooper, L. Brown, J. Martin, P. Hoekstra, and R. Wilkinson who contributed effectively to the success of this workshop. We thank Lianne Schouls for her dedication and support during the preparation of this publication.

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Workshop on Polybrominated Diphenyl Ethers (PBDEs) in the Environment
August 19-20,1999

Workshop A - Determination, Distribution, Fate
9:00-12:00 August 20, 1999

Session leader : Dr. J. J. Ryan, Health Canada
Rapporteur: Bryan Wakeford, Environment Canada

Dr. Ryan suggested that the discussion take place on the following topics:

1. Reference Standards and samples and their availability
2. Methods to be used and determination parameters
3. The necessity for inter-laboratory comparison data on common reference samples
4. Reporting of data - requirements and in what format
5. Basic QA/QC protocols for trace organic analyses

Discussion Topics

Topic #1 Reference Standards and samples and their availability

The total number of potential congeners is the same as that for PCBs - 209. The PCB numbering convention will be used for convenience. Of this number, there are twenty-three ^{12}C congeners which are available in a cocktail mixture. One ^{13}C congener is also included in this mix. The congeners span the homologous series out to the hepta BDE range. There is also one spiking solution available containing five chlorinated ^{13}C labeled diphenyl ethers and a performance standard with one ^{13}C labeled BDE congener and one ^{13}C-CDE congener.

A group of 14 new ^{12}C congeners up to the hepta BDE range have been prepared and are now available in mixtures. Three ^{13}C labeled BDE can be made available through CIL pending an order. There will be a Br_4, a Br_5, and a Br_6.

Concerns were raised:

Are we going to be getting the most appropriate congeners?

Pending toxicology testing there is no way to know which congeners are the most significant. However, from the work available to us now, we will be getting the majority of the congeners that have been present at the highest levels. Congeners of interest which accumulate in biotic samples are numbers: 47 (tetra), 99, 100 (both pentas), 153, 154 (hexas), 183 (hepta), and 209 (deca). These would be of most interest for analytical purposes.

Who else in the world is going to use these standards? Can we share expenses? Should we also encourage secondary suppliers?

Some participants had a perception that this TSRI project team will be the major users of the synthesized standards and therefore would pay the majority of the development costs. D. Sergeant assured the group that the costs were very reasonable - this was backed up by J. Huwe (USDA) who stated that similar syntheses contracted from her lab had been more expensive. M. Simon indicated that some congeners may be available from a second source - no consensus developed around purchasing from two companies at this time although in principle it is a good idea. In particular sometime it would be necessary to check the primary ^{12}C standards against a second independent source. For this purpose a second supplier would be needed.

Should bring up the topic at Dioxin 99?

There was strong support for the idea that the topic should be brought up at the Dioxin 99 in Venice.

Should the new congeners be blended with the old at CIL or should each lab get the two mixtures?

Opinions were split on whether the 23 congeners should be mixed with the 14 newly available congeners. If CIL is contracted to do this, it may be very expensive. If each lab makes the mixtures, there may be lab to lab variation. If the standards are kept separate then two injections of standards are needed. Spiking may have to be done with two solutions that may induce errors

How should the distribution of standards to TSRI labs be done? Directly from CIL or via CCIW?

The administration and handling would be easiest if CIL does it but the extra packaging might increase costs and the importation paper burden would increase. This question is left to be decided later. The octa, nona and deca Br congeners are not available at this time.

When they become available they should not be mixed with other congeners because the time of elution from regular length chromatography columns will be too long. T. Grim (CIL) indicated that they will soon have a deca std available- in approx. 2-3 months. However he could not estimate when nona and octa congeners might be available.

Sediments will likely contain the higher BDE congeners - how will they be dealt with?

This question is unresolved for the present. A lab may use technical material -bromkal mixtures that have been cleaned up. Check with Bergman or DeBoer (interlab study being proposed check letter)

Consensus Proposal-

The TSRI project should order the three new ^{13}C BDE congeners. The mixtures of the 14 new native BDE congeners should also be purchased.

Topic #2 Methods to be used

There was a general consensus that no detailed analytical protocol would be imposed on the collaborating labs. The main criteria would be based on the labs performing satisfactorily in making appropriate determinations of BDEs in reference materials.

Sample preparation

General DFO procedure described by Ikonomou - extraction followed by GPC then silica and alumina columns. Some participants suggested replacement of GPC with acidic silica columns - concern was raised that some congeners may breakdown in acid.

*Huwe (USDA) reported no problem with one penta congener which was put through acid silica cleanup.

*Some participants are concerned that the GPC may not be effective because the collection window may have to be too wide. Luross reported needing multiple passes through GPC column to get adequate cleanup but this may be related to large amounts of lipid present.

*Alumina column is probably necessary to cleanup matrices such as blubber with lots of co-extractants. Ikonomou routinely uses silica and alumina Sergeant stated that he needs alumina as well.

*The carbon column used in PCDD analysis is not recommended as part of any cleanup regime since PBDPE are not adsorbed to this material.

GC/MS

*High resolution MS is available to all participants and may be necessary - a lot of published work was reported using Br ions (79, 81) in NCI. MS operation at 10000 may not be needed in all cases. A resolution that is needed to eliminate likely interferences should be determined.

*The use of ECD for the determinative step is not advisable. This would negate the use of surrogate congeners for spiking.

*If deca BDE is to be determined there are a number of practical concerns. Bergman recommends on column injection and short columns with thin coatings. Also the cleanup column conditions would need special attention because of a tendency for the compound to stick to glassware.

Topic #3 The necessity for inter-laboratory comparison data on common reference samples

There is a strong consensus that labs will participate in analysis of an interlab calibration sample. It is suggested that the Lake Trout reference material which is available from CIL (EDF-2525) be used initially by all labs. There is a reasonable supply of the material but Grim (CIL) stated that there would not be enough for it to be used indefinitely as a regular QC material. The levels of PBDEs in this reference fish sample are high. Further work may necessitate a reference material with lower concentrations of PBDEs.

*There is an intercalibration study being organized by De Boer in Europe. It was proposed that the TSRI participants join that study. The data generated by our TSRI group could be reported internally to Alaee for our own project QA. Copies of a letter from De Boer, inviting participation, were distributed. There will be an opportunity at Dioxin 99 for some informal discussions on how we might join the study.

*Regular QC sample - no consensus on what should be analyzed routinely for labs. The CRM of NRC (carp-1 or carp-2) should be tested.

Topic #4 Reporting of data requirements and in what format.

*Bergman suggests reporting be done on a molar basis i.e. mmole PBDE per weight of samples. Toxicity testing is done on a molar basis and with the large range in weight among the bromine congeners the potential for mis-interpretation is greater if weight reporting is used. The molecular weights of the compounds would follow the IUPAC conventions for multiple isotopes. He also reminded the group that deca Br production is very high. The determination of this congener should not be ignored.

*There is a lot inertia behind reporting by weight in the regulatory system. The final TSRI report may contain the information in both formats i.e. congener data on a gram basis and on a molar basis.

*We will report results for all the individual congeners for which we have standards and report other homologues calculated with an average response factor. The retention times of the first and last member of each homologous group can be predicted to enable the setup of SIM windows.

*It was suggested that homologue totals be reported, even though it is accepted that there are variable relative response factors among them.

Topic #5 Basic QA/QC protocols for trace organic analyses

Other items mentioned on QA but not mentioned above.

Blanks - are to be analyzed along with samples.

What should be looked for if blanks are contaminated?

Solvents are a potential source of contamination and should be verified.

Because of the ubiquitous nature of the compounds there may be contamination through the air supply to a lab if it is under negative pressure compared to the other areas in a building (usual case). This could also produce variations of air contamination throughout a day.

*The use of rotary evaporators may be suspect due to the seals used.

*Instrumentation containing printed circuit boards (nearly all of them) probably contain fire retardants and may be sources of blank responses.

Workshop on Polybrominated Diphenyl Ethers
(August 19-20, 1999)

Toxicology Workgroup

Workgroup Participants:

Nigel Bunce (Chair)
Mehran Alaee
Scott Brown
Paul Sibley (Rapporteur)
Chris Metcalfe (Absent, but left copy of presentation)

Background

The Toxicology Workgroup was charged with identifying information gaps related to the toxicology and chemistry of polybrominated diphenyl ethers (PBDE's) and to provide recommendations that may serve to address these gaps as part of future research programs.

Research regarding toxicological aspects of PBDE compounds is scant, particularly from an environmental perspective. Consequently, there is little information regarding the potential risk posed by these compounds to both humans and the environment. While the workgroup felt that it was important to evaluate potential risks of PBDE's in terms of both human and environmental health, primary consideration was directed toward assessing risk in an environmental context. Using the general question "Do PBDE's pose a risk to Canadians and the Canadian environment?", the toxicology group identified several important aspects that must be addressed in order to advance our current understanding of the toxicity and environmental activity of PBDE's.

1) What congeners should be evaluated?

It is neither practical nor possible to evaluate the toxicity of each of the 209 congeners of PBDE's. Perhaps the most important consideration limiting the number of congeners that can be assessed is the availability and cost of sufficient quantities of each; at present the cost of producing large quantities of pure or radio-labeled PBDE's may prohibit long-term, extensive toxicological evaluations. Based on previous work and existing information, the following congeners were identified as appropriate and relevant candidates for detailed toxicological investigation:

Congener 47 (2,2,4,4)
Congener 99 (2,2,4,4,5)
Congener 100 (2,2,4,4,6)
Congener 153 (2,2,4,4,5,5)
Congener 154 (2,2,4,4,5,6)
Congener 183 (2,2,4,4,5,5,6)
Congener 209 (Br_{10})

2) What assays are available to assess toxicity of PBDE's

A number of cellular and organismal assays were proposed for use in assessing the toxicity of PBDE's. Some of these assays have already been applied in relation to polychlorinated diphenyl ethers (PCDE's). These include:

Cellular

1) AhR ligand binding assays

2) Post-binding assays, including:

- ✸ DRE activation
- ✸ EROD/Acetanilide hydroxylase (ACOH) assays (the group suggested adding ACOH for CYP 1A2 activity, and performing EROD in rat/chick/trout)
- ✸ Kinase assay

Organismal

- *Medaka* embryotoxicity assay (includes developmental endpoints)
- Thyroid histology, including a deiodination assay (to be conducted in fish such as *Medaka* or trout). The group suggested adding a reproductive endpoint for the fish that are to be injected with PBDEs for the thyroid assays: see later)
- Amphibian metamorphosis (using *Xenopus*)

The organismal assays have been applied primarily to fish and amphibians and these organisms could serve as primary models for future research. In fact, since many of the target systems (e.g., thyroid gland) for these compounds are conserved across species, it may not be necessary to conduct tests on a large number of organisms. Rather, it may be possible to make predictions/inferences about the likelihood of effects across species based on toxicological research conducted using selected organisms from a given group (e.g., fish, amphibians). The cellular-level assays would be conducted using liver hepatocytes or cell lines (if available).

In contrast to fish and amphibians, there has been virtually no work conducted with invertebrates, possibly because invertebrates lack many of the target systems affected by PBDE's which are present in these higher organisms (e.g., Ah Receptor, thyroid gland). A possible target system in invertebrates is the nervous system which could be manifest sublethally as changes in behavior. An assay that was proposed that could be used to evaluate such effects is a "righting assay" that has been used in snails (the endpoint is the time required for a snail to right itself following perturbation).

The above assays will provide information to determine the nature and magnitude of the toxicity of PBDE's. Since these compounds exist in the environment at concentrations that will

most likely be manifested at sublethal levels, most effort should be directed toward evaluation of sublethal endpoints of toxicity. Indeed, the above assays are largely directed toward assessment of potential sublethal effects and indicators of exposure, including growth, embryotoxicity, enzyme induction, and ligand binding.

One endpoint not considered in the above suite of assays is reproduction. This may be an important endpoint to include in the assessment of PBDE's for two reasons. First, assessment of reproduction facilitates establishment of quantitative links between lower levels of biological organization (e.g., cellular and organismal) and higher levels of complexity (e.g., populations and communities). This is an important link in terms of ecological risk assessment since approaches such as probabilistic risk assessment (PRA) are based on the premise of protecting populations and communities rather than individuals, yet are largely based on information derived from assays conducted at the organismal and sub-organismal levels. Second, some PBDE's have been implicated as endocrine disrupting compounds whose effects are often manifest at the level of reproduction. Consistent with the endocrine disruption hypothesis, it will be important to focus on effects that occur at environmentally relevant exposure levels.

From a practical standpoint, assessment of reproduction could be achieved via modification of the *Medaka* bioassay. Reproductive evaluation using the fathead minnow (*Pimephales promelas*) could also be conducted, although this would require larger exposure systems and possibly greater quantities of chemical. One way to overcome the amount of chemical that would be required to conduct a reproductive assessment in fish and amphibians would be to inject fish intraperitoneally prior to, or during, the reproductive period.

While sublethal endpoints may be most important in the assessment of PBDE's, there is also a significant lack of information on acute toxicity (e.g., lethality) which may be useful in the application of risk assessment procedures. Some of these data gaps may be addressed at the upcoming Dioxin 99 meeting.

In evaluating the toxicity of PBDE's, one of the first issues to address is the nature of the activity associated with commercially available mixtures relative to the activity of individual congeners. This must be conducted to establish the nature and magnitude of effects (if present) that might be rendered by impurities in the mixture resulting from commercial preparation. It was proposed that as far as possible, all toxicity assays should be conducted so as to compare the activity of the pure congeners (e.g., those listed above) with that of the commercial mixtures containing the same congeners. Should a discrepancy be detected, the commercial mixtures would have to be examined for the compound(s) which may be causing the discrepancy. This would have to be a follow up project; the standards would not be available for inclusion of such work in the present project.

The workgroup also identified the need to understand the extent to which these compounds are metabolized in organisms (for example, PBDE compounds must be hydroxylated before they can interact with the thyroid system). However, in view of the relatively poor understanding of the basic toxicity of PBDE's in aquatic organisms, and the fact that this type of research would require radio-labeled compounds which are presently unavailable, it was felt that this type of research would also not be part of the present project.

3) Application of toxicity and chemistry information in risk assessment

Conducting both human and ecological risk assessment (ERA) requires sufficient information on exposure and toxicity, particularly if probabilistic methods are employed. From an environmental perspective, there appears to an growing, if not adequate, data-base of information regarding exposure (e.g., measured concentrations of various PBDE's in various environmental matrices, including tissues) that could be applied in ERA. In contrast, there appears to be limited information on environmental effects; indeed, much of the existing effects data has been generated in tests using mammalian species (e.g., rats) in the context of human health risk assessment.

While there appears to be a lack of toxicity information, the workgroup proposed that it would be beneficial to ascertain exactly what information is currently available by conducting a literature review. As indicated, some data is available for mammals (rats), most recently from a review issue in the journal *Environmental Health Perspectives*. Some recent reviews on PBDE's indicate that there are probably less than 100-200 papers that directly address toxicity of PBDE compounds. The vast majority of these are directed toward chemistry and environmental concentrations. It was proposed that these papers should be acquired and made available to those working on PBDE's. Mehran Alaee agreed to organize a central repository of literature when he returns from the Dioxin 99 meeting.

The Toxic Substances Research Initiative: A Targeted Approach to Toxic Substances Research in Canada

David M. Kane

Toxic Substances Research Initiative Secretariat

Environmental Health Directorate

Health Canada

www.hc-sc.gc.ca/ehp/ehd/tsri

Health Canada Santé Canada

Background

- Perceived need to strengthen research
- Health & environmental problems
- High levels of exposure
- Impacts of pollution are expensive

Key Objectives

- Strengthen the knowledge base on toxic substances
- Reduce adverse effects of toxic substances
- Protect and preserve ecosystem and human health
- Provide sound science on which to base public policy decisions

Guiding Principles

- Multidisciplinary research approaches
- Partnerships & leveraged resources
- Public understanding and involvement
- Target based approach

Priority Areas

- Persistent Organic Pollutants (POPs)
- Metals
- Endocrine Disrupting Chemicals (EDCs)
- Airborne Pollutants
- Cumulative Ecosystem and Human Health Effects

Funding

- $40 Million research investment
- Partnerships encouraged
- Leveraged funding anticipated
- Incremental research
- Policy driven

Administration

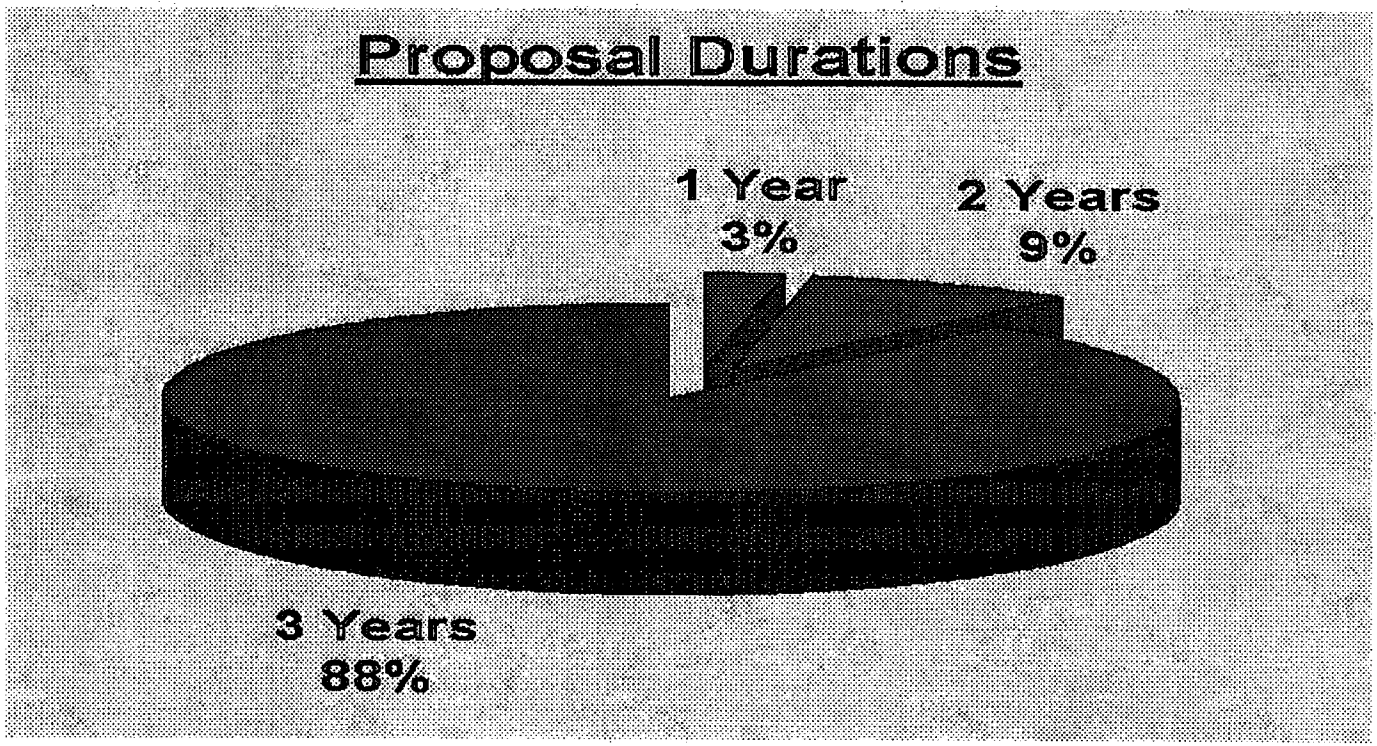
- Science Management Committee
  - Strategic Direction
  - Final Funding Approvals
- Technical Review Committees
  - Review and recommendations for funding
- Secretariat
  - Initiative administration

Funding/Review Cycle - Year 1

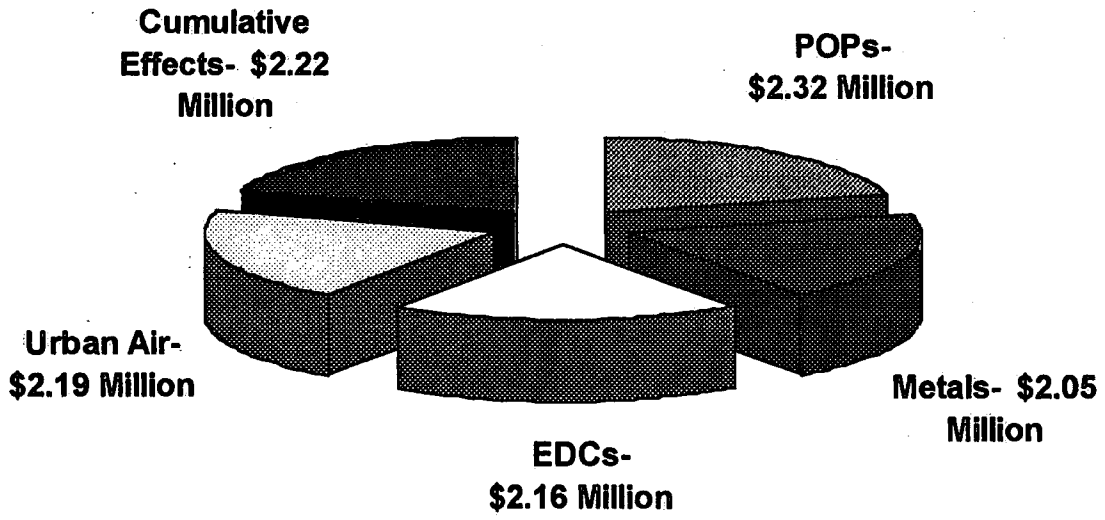
- Application deadline
  - February 26, 1999
- Review and funding decisions
  - April - May 1999
- Release of funds
  - Starting June 1999

Number of Funded Projects

● POPs	17
● Metals	15
● Endocrine Disrupting Chemicals	17
● Urban Air	14
● Cumulative Effects	18


Proposal Durations
1 Year
3%
2 Years
9%
3 Years
88%

Funding Distribution Between Priority Areas



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Distribution of Researchers

DISTRIBUTION OF RESEARCHERS BETWEEN PRIORITY AREAS	
PRIORITY AREA	NUMBER OF RESEARCHERS*
Persistent Organic Pollutants (POPs)	77
Metals	66
Endocrine Disrupting Chemicals (EDCs)	108
Urban Air	47
Cumulative Effects	73

* The number of researchers is calculated as the sum of researchers per approved proposal. Where a researcher is involved in more than one approved proposal, repetition may occur.

Examples of Projects

- POPs
  - **An examination for banned POPs in agricultural soils in Canada, the US and Central America to determine effects of LRT on levels in Canada**
  - **Effects of POPs in northern fish and wildlife and their dietary effects on native populations**
  - **Effects on amphibian populations**
  - **Emerging POPs - Polybrominated diphenyl ethers, fluorinated surfactants, phthalate esters**

Examples of Projects - 2

- Metals
  - **Cycling of mercury in the environment**
  - **Toxic effects of mercury - effect of forest fires on mercury cycling to the aquatic environment and effects on sports fishermen**
  - **Transport of metals from mine tailings - one study of effects on freshwater ecosystems and one on effects of marine disposal**
  - **Bioavailability of metals from sediments and soils**
  - **Lead toxicity in wildlife and subsequent transfer to consumers**

Examples of Projects - 3

- Endocrine Disruptors
  - **Several studies of effects on reproduction in fish (field and laboratory) and wildlife (amphibians, birds, polar bears)**
  - **Studies of reproductive toxicity effects in humans; effects of neonatal exposure, effects of organotin**
  - **Effects of human activity - agricultural practices, municipal sewage effluents, pulp and paper mill effluents**
  - **Effect of adding a synthetic estrogen to a lake system**

Examples of Projects - 4

- Urban Air Quality
  - **Epidemiological studies - comparisons of air quality data with mortality and hospital admission data**
  - **Inhalation toxicity studies - interaction of ozone and particulate matter and modulating effects of CO and NO; study of exposure to particulate matter and effects on asthmatics**
  - **Study of cardiac patients - effects of air pollution levels on cardiac response to exercise**
  - **Development of new particulate matter characterization techniques**

Examples of Projects - 5

Cumulative Effects

- **Studies in Sydney, NS - environmental risk factors for cancer (breast, colon, lung); environmental effects and remediation of PAH mixtures**
- **Effects of exposure to disinfection by-product mixtures**
- **Effects of Po-210 on human and animal systems**
- **Study of relationship between traditional Innu harvesting practices, environmental contaminants and wildlife and human exposure/health effects**
- **Effects of exposure to aromatic amines in human milk**

Conclusions

- 81 projects funded in five priority areas
- Projects cover a wide variety of different topics, addressing identified knowledge needs in each area
- Projects are multidisciplinary, with multiple partnerships
- Most projects bring in significant amounts of leveraged funding

Next Steps

- Second call for proposals for 2000/2001
  - Deadline December 22, 1999
  - Funding in place by April, 2000
- Renewal of the Initiative beyond March, 2002
  - Examination of current state of knowledge in priority areas
  - Identification of emerging issues in toxic substances research
  - Demonstrate effectiveness of current Initiative

Toxic Substances Research Initiative Secretariat

Web site:

www.hc-sc.gc.ca/ehp/ehd/tsri

Health Canada Santé Canada

BROMINATED FLAME RETARDANTS – AN ENVIRONMENTAL ISSUE OF PARTICULAR CONCERN?

Åke Bergman

Brominated flame retardants (BFRs) consist of a variety of organic substances whose cumulative world production is approximately 150 000 tons. More specifically, BFRs make up ¼ of all flame retardants produced annually (1). Major BFRs are tetrabromobisphenol A (TBBPA) and derivatives thereof (2) and polybrominated diphenyl ethers (PBDEs) (3). Environmentally relevant characteristics of BFRs may be hypothesized from their 3D structures, potential chemical reactivities and physico-chemical parameters. Taken together with existing information on the areas where they are applied and the volumes consumed, these factors may be used to identify potentially hazardous chemicals for humans and wildlife, and for the selection of BFR classes for in depth scientific assessments.

So far the major data set of any class of BFRs, has been produced on PBDEs even though some data on TBBPA and hexabromocyclododecane (HBCDD) have also been published. Ever since the identification of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47 (4)) in the environment in 1981 (5), PBDE congeners substituted with 4-6 bromine atoms have been analyzed for in the environment (6 and references cited therein). Some recent data indicate levels of BDE-47, being the dominating PBDE in the environment, range from 500-1700 ng/g lipid weight (l.w.) in pilot whale blubber sampled at the Faeroe islands (7), and approximately 180-200 ng/g l.w. in salmon from the Baltic (8). In comparison to Baltic salmon, 1700 ng/g l.w. of BDE-47 has been measured in steelhead trout from Lake Michigan (9). Presently available data indicate that the background concentrations of BDE-47 and 2,2',3,4,4',5,6-heptabromodiphenyl ether (BDE-183) in Swedish human blood are 1-2 ng/g l.w. and 0.1-0.2 ng/g l.w., respectively, while their concentrations in humans exposed during dismantling of electronics are 2.9 ng/g l.w. and 7.8 ng/g l.w., respectively (10). In the latter study, decabromodiphenyl ether (BDE-209) was quantified in humans with background exposures, and in individuals exposed at work. In contradiction to this result, BDE-209 has previously been claimed not to be bioavailable (3). Further, it is intriguing to see the increasing trend of PBDE concentrations in Swedish mothers milk (11). Environmental levels of TBBPA and HBCDD are much more uncertain.

The presence of BFRs in the environment is highly dependent on their abiotic and biological transformation rates. PBDE congeners and TBBPA are affected by UV light (12,13). The metabolism rates of BDE-47 and 2,2',4,4',5-pentabromodiphenyl ether (BDE-99) are low (14,15). These two PBDE congeners have been shown to be transformed to hydroxylated metabolites. In contrast, TBBPA is readily excreted as TBBPA after formation of glucuronic acid conjugates, and enterohepatic circulation under which conditions deconjugation occurs (16). Only 2% of the dose remained in the rats after 72 h confirming the rapid elimination of TBBPA from the body.

Both TBBPA and hydroxylated PBDE metabolites have structural resemblance to the thyroid hormones T4 and T3. These compounds have thus been studied for binding potencies to the thyroxin transporting protein, transthyretin (TTR), and shown to compete with the natural ligand (17,18). PBDEs have been shown to act both as inhibitors and inducers of the Ah receptor (19). These congeners may significantly alter the learning abilities of mice exposed neonatally (20), and similar to several other organohalogen compounds HBCDD was recently shown to be a potential carcinogen by intragenic recombination in mammalian cells (21). This is some recent toxicological data that needs to be added to information reported previously (2,3).

Methoxylated PBDEs (MeO-PBDEs) have been determined in lipids of fish and mammals (22, 8), and hydroxylated PBDEs (OH-PBDEs) have been determined

in fish blood (8). Recently some of these compounds have been structurally identified by comparison to authentic reference standards (23). Several sources of the OH- and MeO-PBDEs have been proposed while other data are extremely limited.

In conclusion, there are both environmental presence and toxicological concerns that have to be addressed regarding the use of BFRs. In particular, substances with hormone-like structures need to be further investigated to determine any potential endocrinological effects. However, reproductive, neurological, immunological and genotoxic effects should also not be neglected.

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Brominated Flame Retardants - An Environmental Issue of Particular Concern?

Background
Hypothesis of BFRs in the environment
Some recent facts on BFRs
Related compounds
Conclusions and Needs

Å. Bergman, Workshop on PBDEs in the Environment,
Burlington (Canada), August 19-20, 1999

Flame Retardants of Commercial Use

Brominated flame retardants

Decaabromobiphenyl
Decabromodiphenyl ethane
Decabromodiphenyl ether
Octabromodiphenyl ether
Pentabromodiphenyl ether
Tetrabromobis phenol A
Tetrabromobisphenol A bis-(2,3-dibromopropyl ether)
Tetrabromobisphenol A bis-(2-hydroxyethyl ether)
Tetrabromobisphenol A bis-(allyl ether)
Tetrabromobisphenol A dimethyl ether
Tetrabromobispenol S
Ethylene-bis-(tetrabromophthal imide)
Dibromoneopentylglycol (1,3-propanediol, 2,2-bis(bromomethyl))
Tribromoneopentyl alcohol
Vinylbromide
Tribromophenyl allyl ether
(Poly) pentabromobenzyl acrylate
Pentabromotoluene
2,3-Dibromo-2-butene-1,4-diol
(Poly)bromophenols: 2,4-Dibromophenol, 2,4,6-Tribromophenol, Pentabomophenol
1,2-Bis(2,4,6-tribromophenoxy) ethane 1,1-(1,2-ethanediyl bis(oxy), bis 2,4,6-tribromo-benzene
Di-(2-ethylhexyl)tetrabromophthalic ester
Tetrabromophthalic acid Na salt
Tetrabromophthalic acid diol [2-Hyroxypropyl-oxy-2-(2-hydroxyethyl)-ethyl-tetrabromophthalate]
Tetrabromophthalic anhydride
N,N'-Ethylene-bis-(tetrabromophthal imide)
Bromo-chlorinated paraffins
1,3-Butadiene homopolymer brominated
Bis(tribromophenoxy)-ethane
Tetradecabromodiphenoxybenzene
Poly(2,6-dibromo-phenylene oxide)
Poly-tribromostyrene
Brominated polystyrene
Polydibromostyrene
Hexabromocyclododecane
1,2-Dibromo-4(1,2 dibromomethyl) cyclohexane
Ethylene-bis(5,6-dibromo-norbornane-2,3-dicarboximide
Dibromostyrene grafted PP
1,3,5-tris(2,3-dibromo-propoxy)-2,4,6-triazine

Chlorinated flame retardants

Chlorinated paraffins
Chlorendic acid
Chlorendic anhydrid
Dodecachlorodimethan o-dibenzocyclo-octane
Hexachlorocyclopentadiene
Tetrachlorophthalic anhydrid-TCPA
Bromo-chlorinated paraffins
2,2',6,6-Tetrachlorobisphenol A
Tetrachlorophthalic anhydride

Halogenated organophosphorus flame retardants

Tris(1,3-dichloro-2-propyl)phosphate
Tris(2-chloroethyl) phosphate
Tris(2-chlororethyl) phosphate
Tris(2-chlororethyl) phosphate polymer
Tris(2-chloro-1-propyl) phosphate
Tris(1-chloro-2-propyl) phosphate
Bis(2-chloroethyl) vinyl phosphate
Mixture of monomeric chloroethyl phosphonates and high boiling phosphonates
2,4-Dibromophenyl phosphate
Tris(tribromoneopentyl) phosphate
Chlorinated brominated phosphate ester
Bromine-, chlorine and phosphorus containing polyol

Organophosphorus flame retardants

Dimethylphosphono-N-hydroxymethyl-3-propionamide
Tris (2-butoxyethyl) phosphate
Isopropylphenyl diphenyl phosphate
Tricresyl phosphate
Triphenylphosphate
Dimethyl-methyl-phosphonate (DMMP)
Resorcinol dipheny-Iphosphate
Diethyl-ethyl-phosphonate (DEEP)
Cyclic phosphonate ester
Isodecyldiphenyl phosphate
O,O-Diethyl-N,N-bis(2-hydroxyethyl) aminomethyl phosphonate
Dimethyl-3(hydroxymethylamino)-3-oxopropyl phosohonate
Dimethyl phosphonate
Cresyl diphenyl phosphate
Octyl diphenyl phosphate
Tris(2-ethyl hexyl) phosphate
Trioctyl phosphate
Triethyl phosphate
2-Ethylhexyldiphenyl phosphate
Tetrakis (hydroxymethyl)-phosphonium salts (THP salts)
Phosphonic acid derivative
Bis(5,5-dimethyl-2-thiono-1,3,2-dioxaphosphorinamyl) oxide
Tris(hydroxymethyl) phosphine oxide
Trixylenyl phosphate
Tris(isopropy-Iphenyl)phosphate

Nitrogen-based and miscellaneous flame retardants

Melamine
Melamine phosphate
Melamine cyanurate
Ferrocene

Inorganic flame retardants

Potassium fluorotitanate
Potassium fluorozirconate
Aluminium hydroxide
Antimony pentoxide
Antimony trioxide
Zinc oxide
Boric acid
Sodium borate (borax)
Zinc borate
Ammonium sulfamate
Ammonium orthophosphate
Ammonium carbarnate phosphate
Di-ammonium phosphate
Ammonium polyphosphate
Huntite-hyromagnesite
Ammonium octamolybdate
Magnesium hyroxide
Ammonium bromide
Barium metaborate
Molybdenum trioxide
Ammonium sulfate
Ammonium chloride
Zinc hydroxystannate
Red phosphorus
Sodium tungstate
Sodium antimonate

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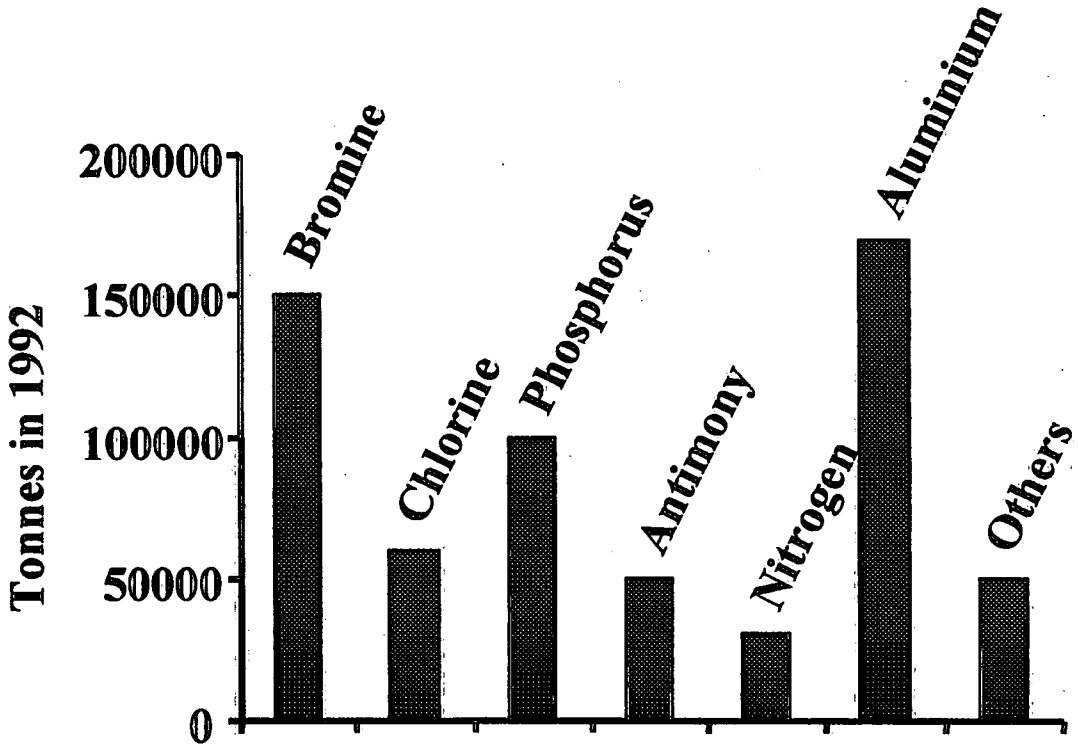
World Wide Demand of Flame Retardants, Grouped According to Chemical Class



Environmental Health Criteria 192,
World Health Organization, 1997

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ADDITIVE BFRs

Decaabromobiphenyl
Decabromodiphenyl ethane
Decabromodiphenyl ether
Octabromodiphenyl ether
Pentabromodiphenyl ether
Tetrabromobisphenol A Derivatives
bis-(2,3-dibromopropyl ether)
bis-(2-hydroxyethyl ether)
bis-(allyl ether)
dimethyl ether
Hexabromocyclododecane
Bis(tribromophenoxy)-ethane
Pentabromotoluene
Bromo-chlorinated paraffins
Di-(2-ethylhexyl)tetrabromophthalic ester
Ethylene-bis-(tetrabromophthal imide)
Tetradecabromodi phenoxybenzene
1,2-Dibromo-4(1,2 dibromomethyl) cyclohexane
Ethylene-bis(5,6-dibromo-norbornane-2,3-dicarbox imide
1,3,5-tris(2,3-dibromo-propoxy)-2,4,6-triazine

REACTIVE BFRs

Tetrabromobis phenol A
Tetrabromobispenol S
2,4-Di-, 2,4,6-Tri- and pentabomophenol
Tribromoneopentyl alcohol
Vinylbromide
Tribromophenyl allyl ether
2,3-Dibromo-2-butene-1,4-diol
Tetrabromophthalic acid Na salt
Tetrabromophthalic anhydride
N,N´-Ethylene-bis-(tetrabromophthal imide)

BROMINATED POLYMERS

Brominated polystyrene
1,3-Butadiene homopolymer brominated
Poly(2,6-dibromo-phenylene oxide)
Poly-tribromostyrene
Dibromostyrene grafted PP
Polydibromostyrene
(Poly) pentabromobenzyl acrylate

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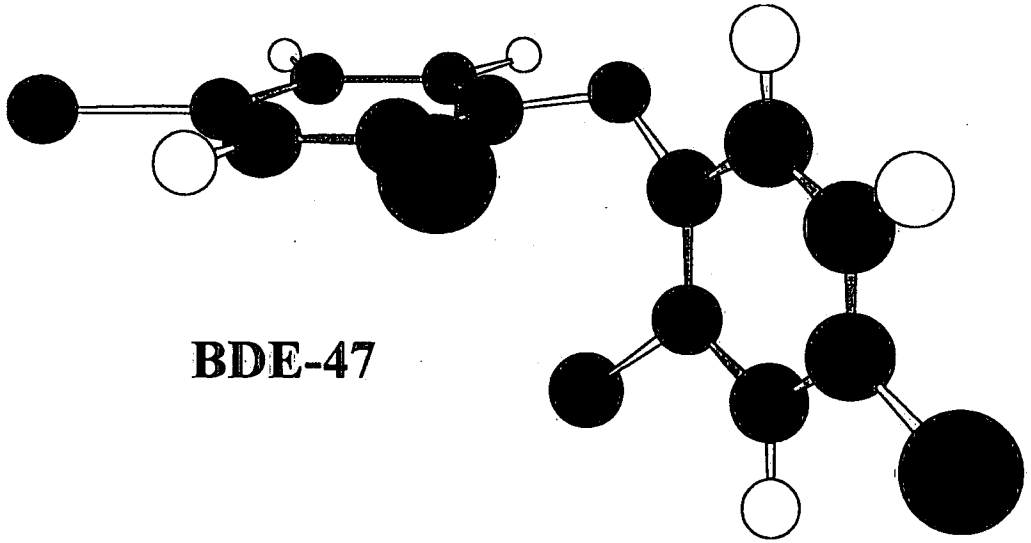
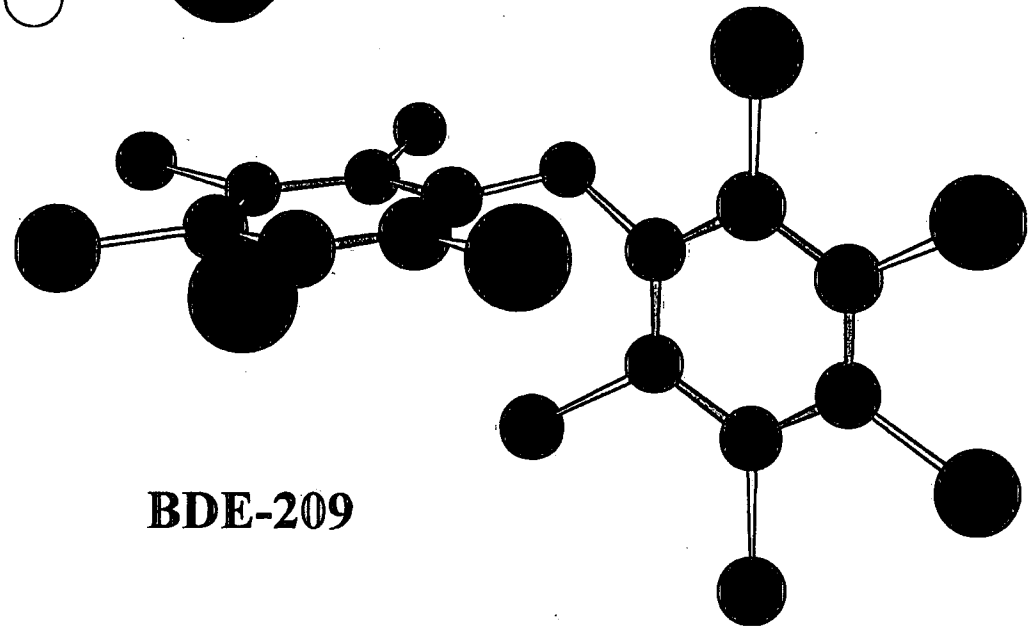
3D Structures of two PBDEs



BDE-47



BDE-209

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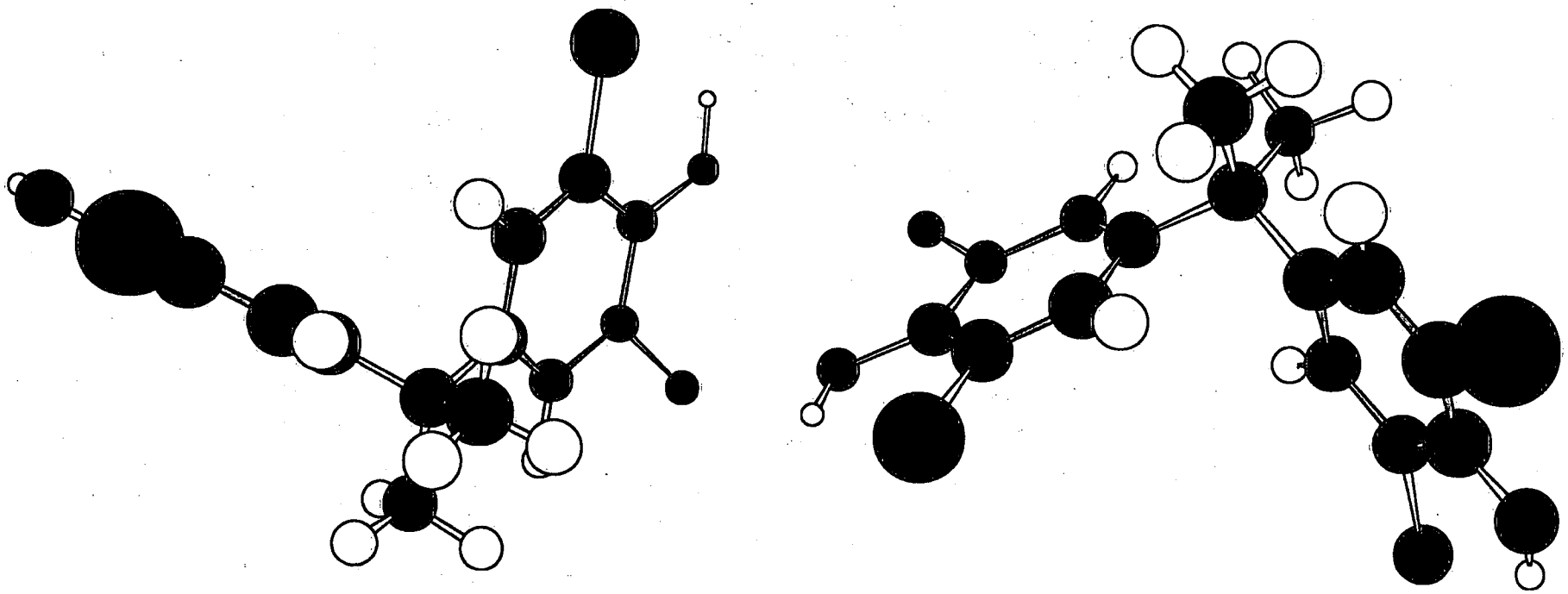
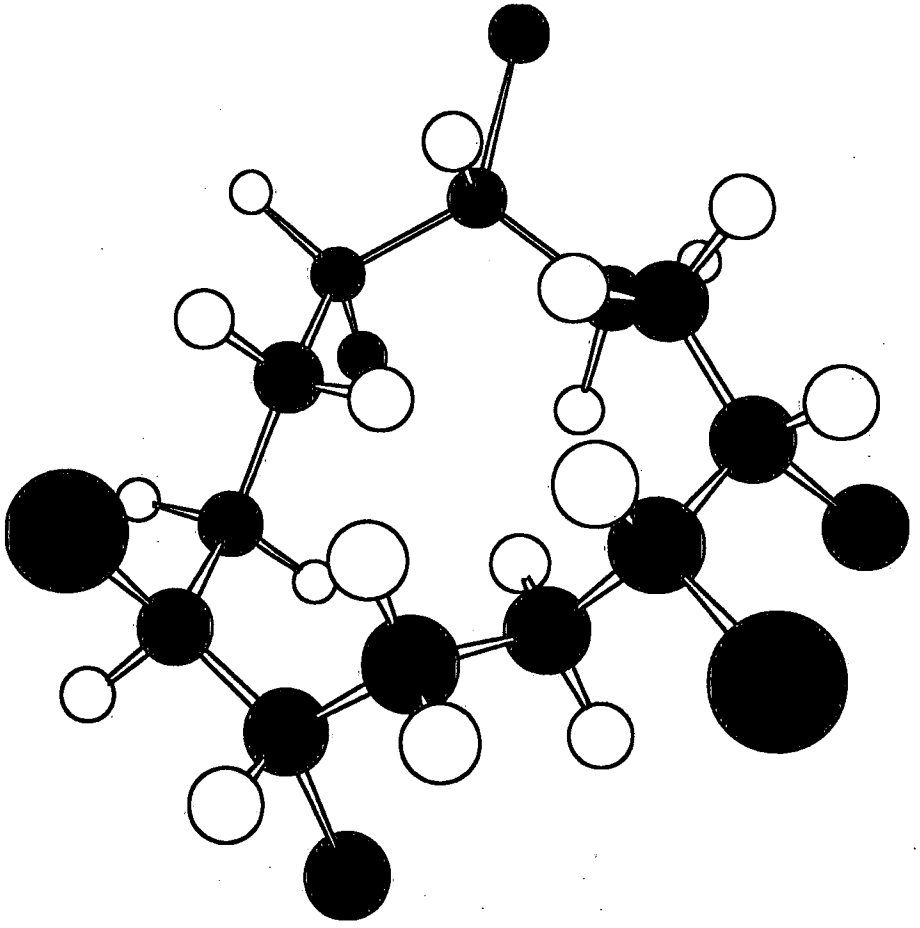
Burlington (Canada), August 19-20, 1999

Two 3D Structures for TBBPA



Hexabromocyclododecane - HBCDD



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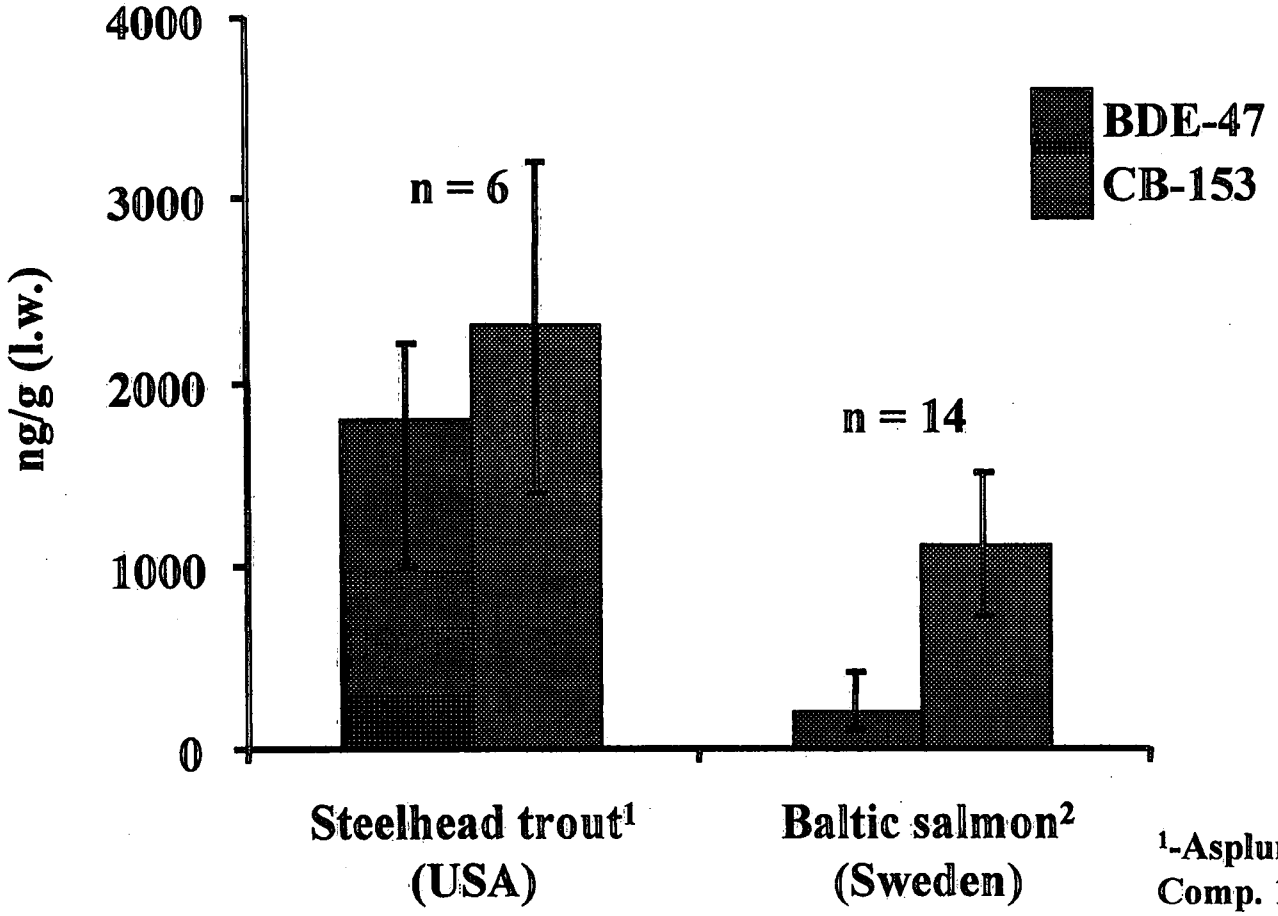
PBDEs in Biota
(in ng/g lipid weight)

Species	n	BDE-47	BDE-99	BDE-100	Literature Reference
Pike (muscle)[1]	4	2400	-	-	Andersson & Blomkvist, 1981
Harbour seal, blubber	3	2200	300	190	de Boer, *et al.* 1998
Pilot whale, blubber	5[2]	1100	370	-	Lindström, *et al.* 1999
Sperm whale, blubber	3	190	44	26	de Boer, *et al* 1998
Steelhead trout, muscle	6	1800	-	-	Asplund *et al* 1999
Baltic Salmon, muscle	14	200	54	47	Asplund, *et al.* 1999
Guillemot, egg	10	480	36	61	Sellström, Thesis, 1996

[1] Contaminated river (Viskan) in Sweden
[2] Average of 5 pooled samples

Concentrations of BDE-47 and CB-153 in Muscle Tissue from Lake Michigan Steelhead Trout and Baltic Salmon

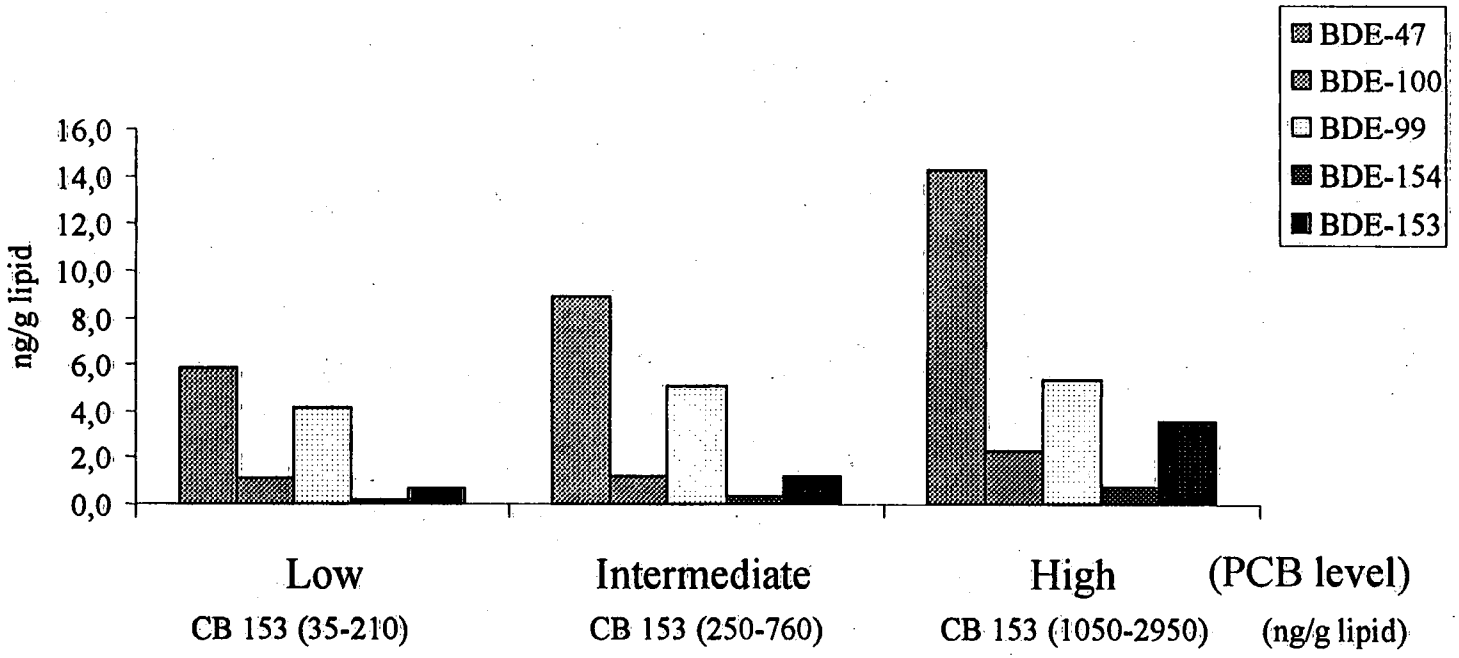
(Error bars indicating range)



[1]-Asplund *et al.* Organohalogen Comp. 1999, In press

[2]-Asplund *et al.* Ambio 1999

PBDEs in women from the Faeroe islands



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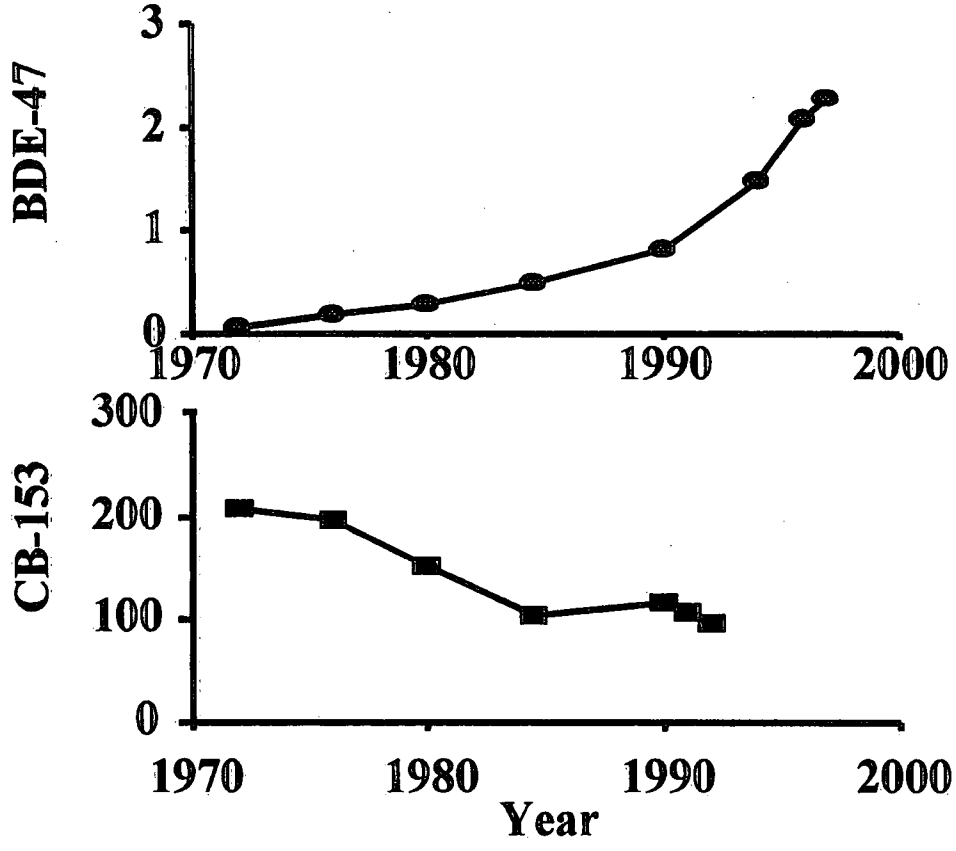
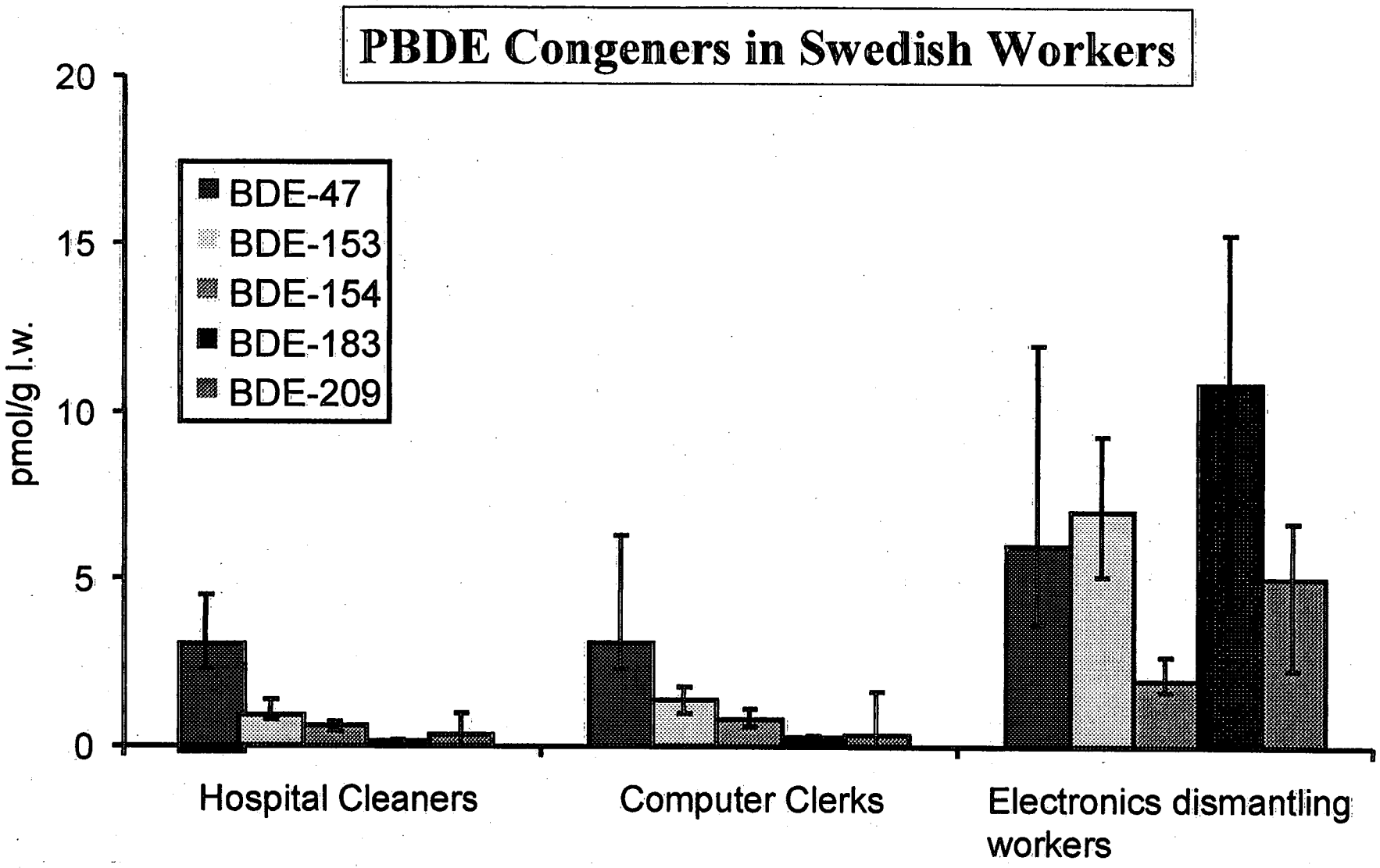
Time Trends of BDE-47 and CB-153 in Human Mothers Milk

(Concentrations given in ng/g lipid weight)

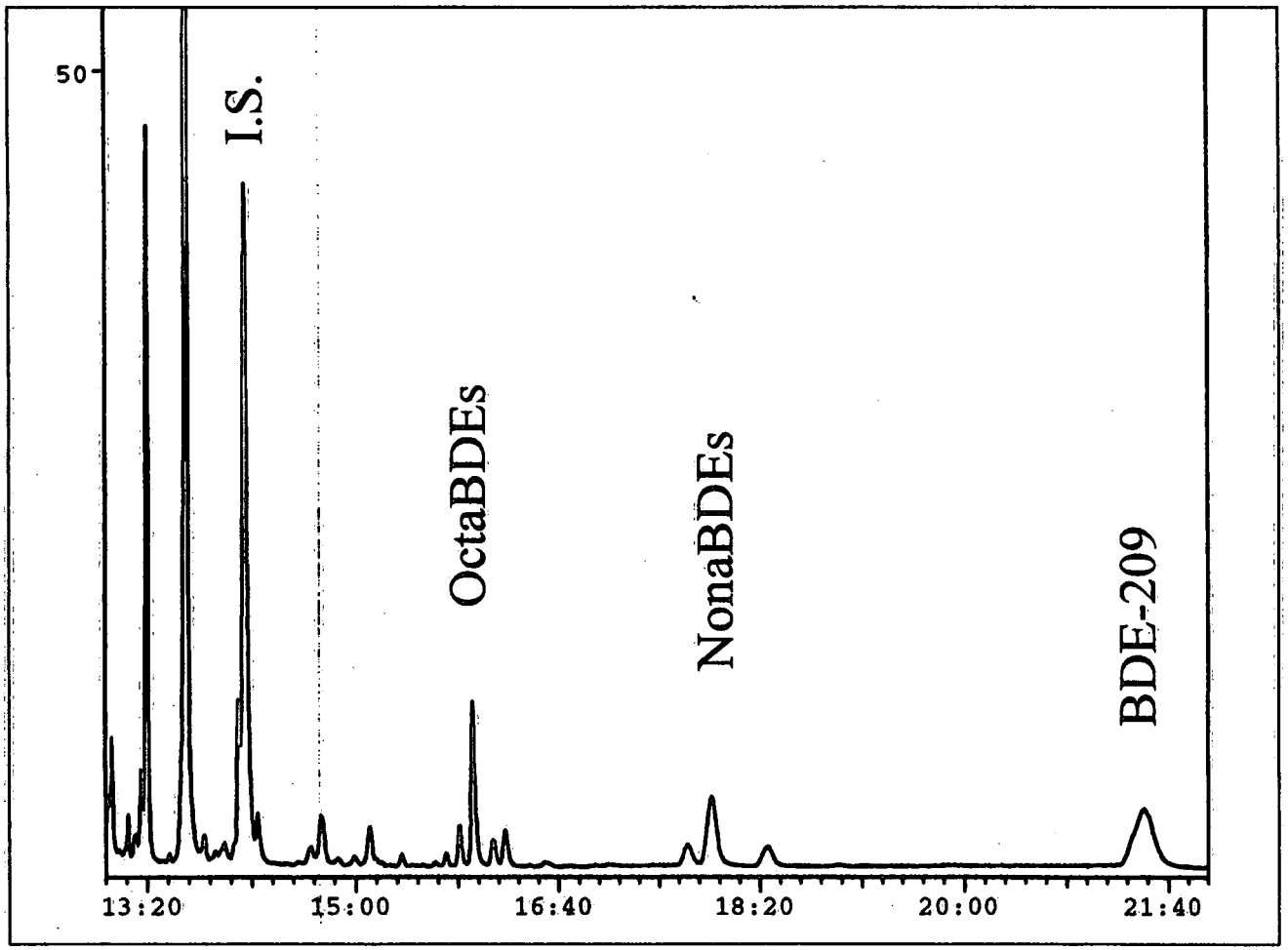


BDE-47 according to Meironyté *et al.* Organohalogen compounds 1998
CB-153 according to Norén *et al.* Environ Health Perspec, 1996



From Sjödin et al, EHP 107 (1999) 643

Higher brominated PBDEs in the Swede in front of you



BDE-209 concentration:
14 pmol/g

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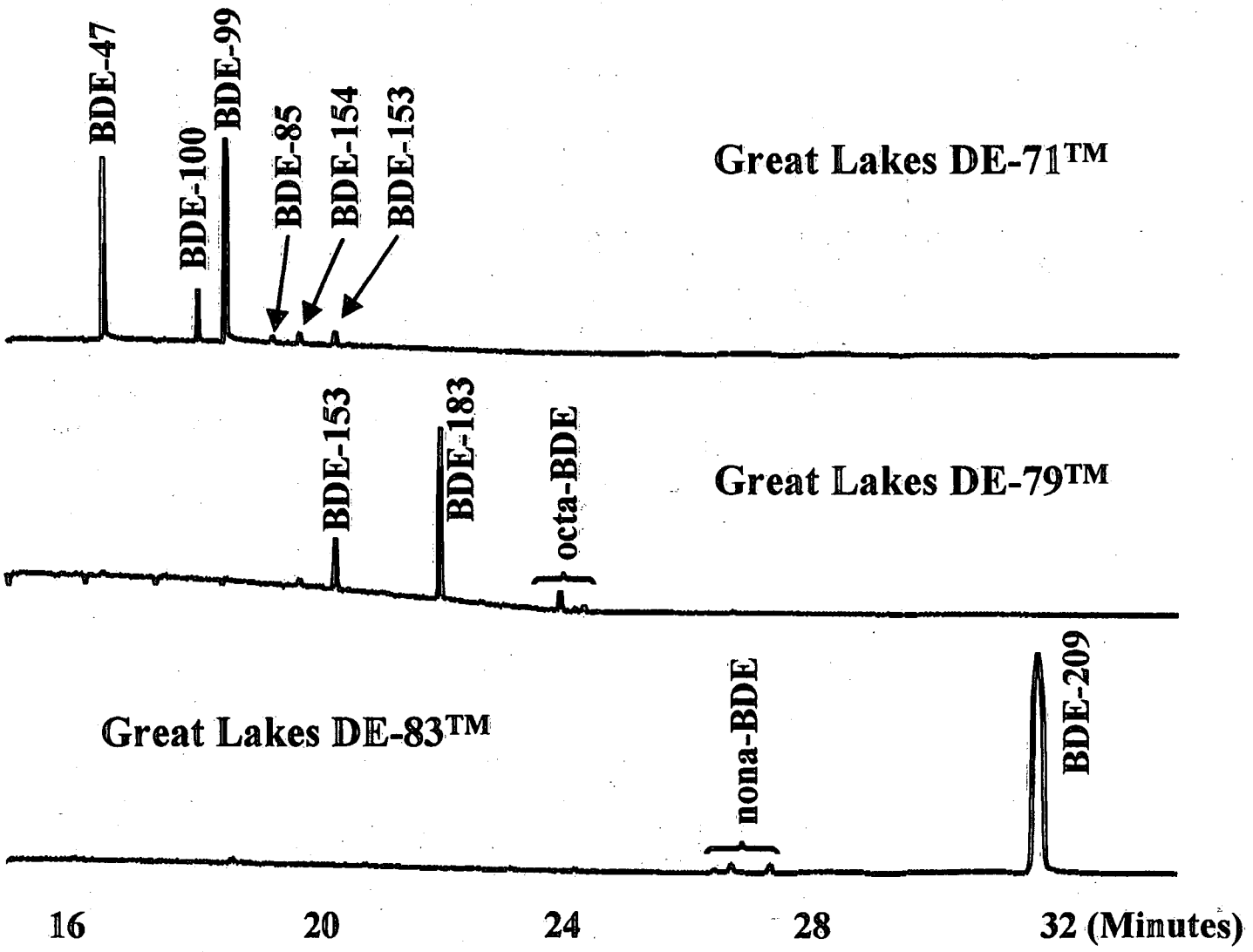
Burlington (Canada), August 19-20, 1999

Three Commercial PBDE Products



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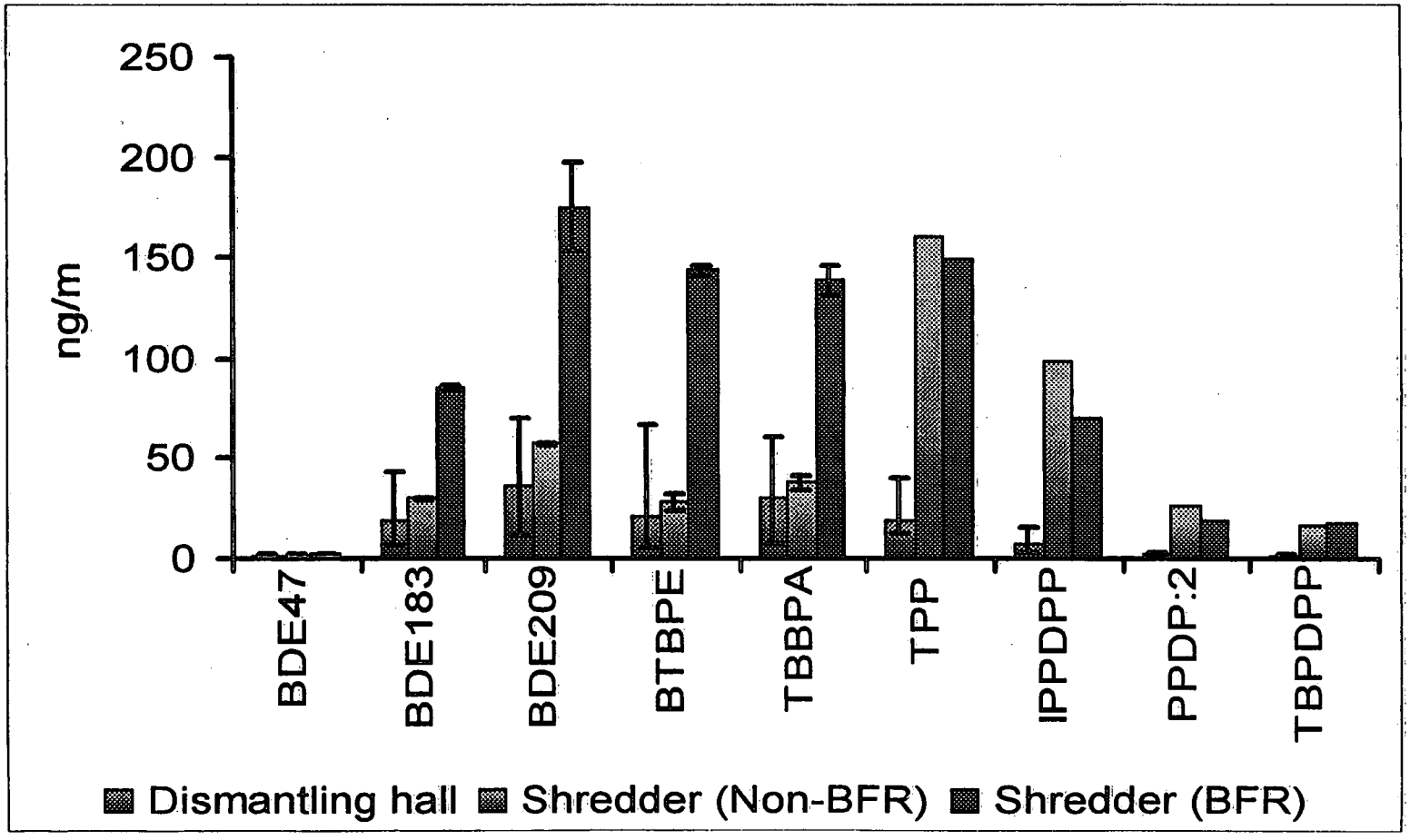
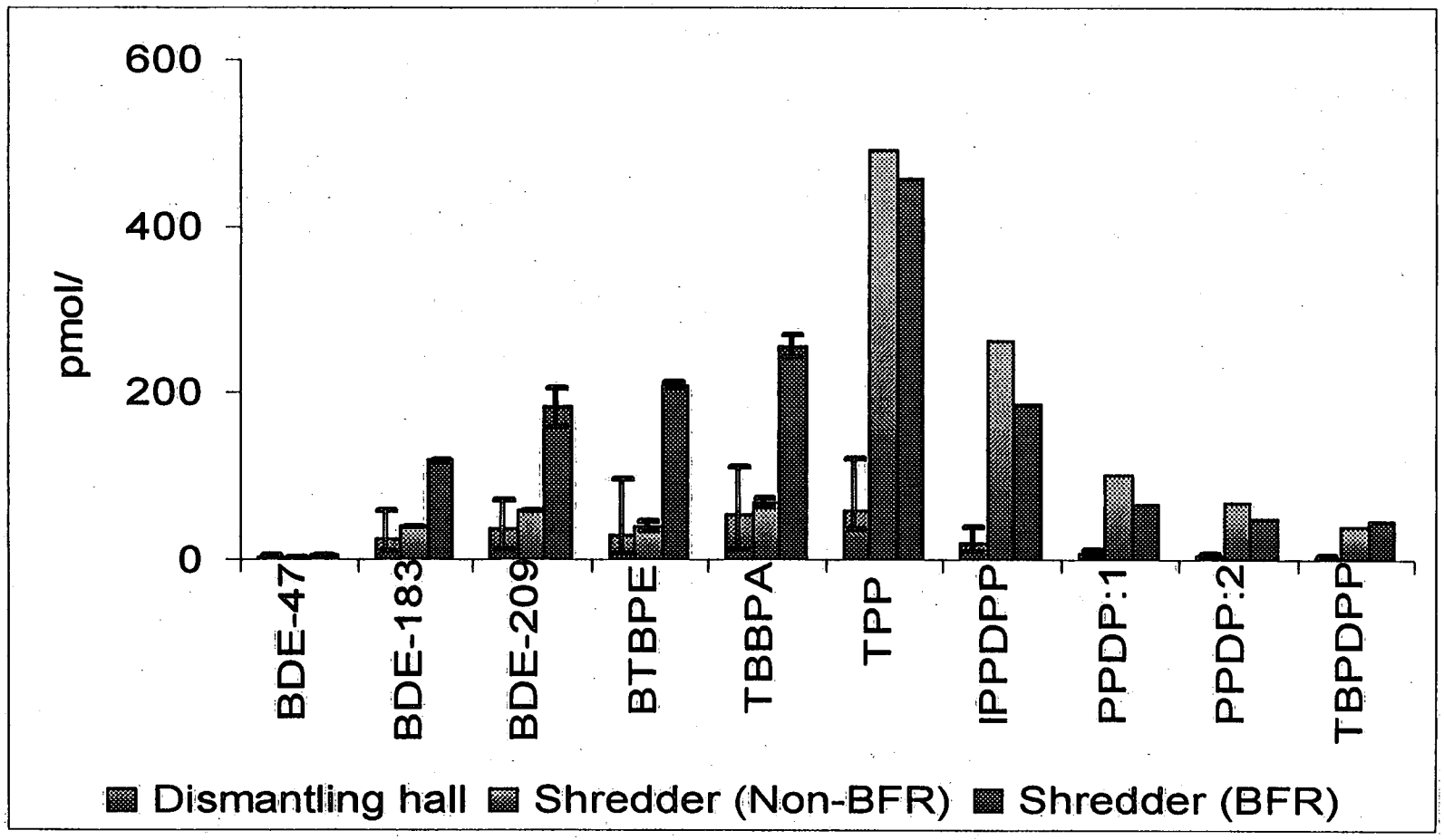
Air Concentrations (ng/m^3) of Flame Retardants at a Plant for Dismanteling of Electronics



Å. Bergman, Workshop on PBDEs in the Environment,
Burlington (Canada), August 19-20, 1999

Air Concentrations (pmol/m^3) of Flame Retardants at a Plant for Dismanteling of Electronics



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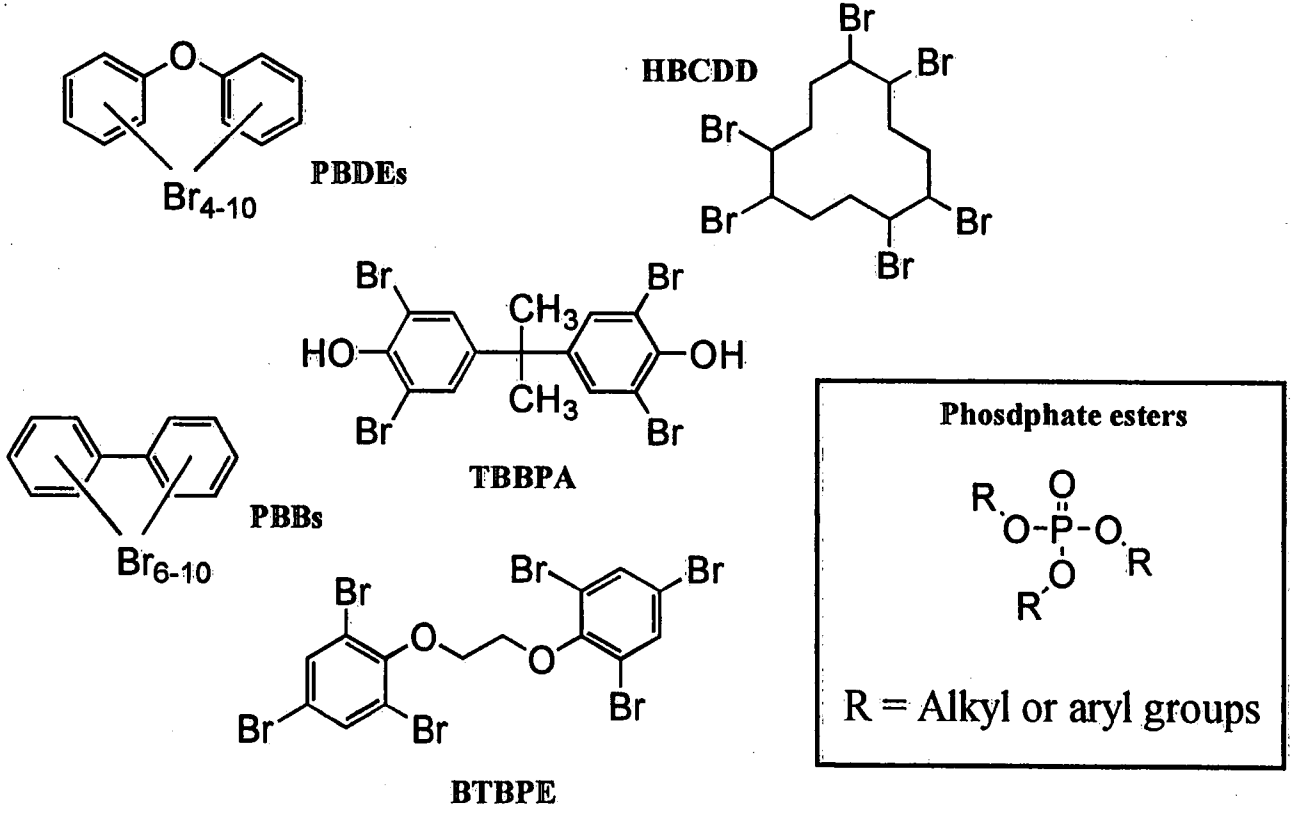
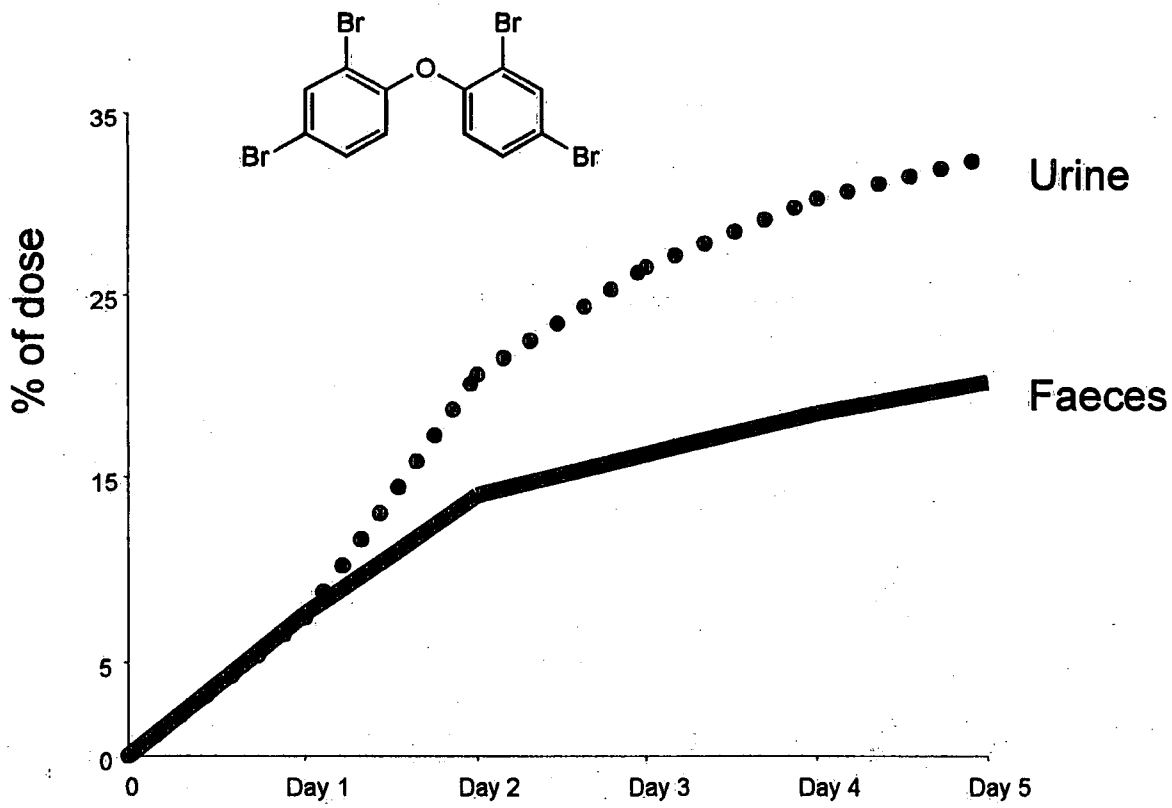
Burlington (Canada), August 19-20, 1999

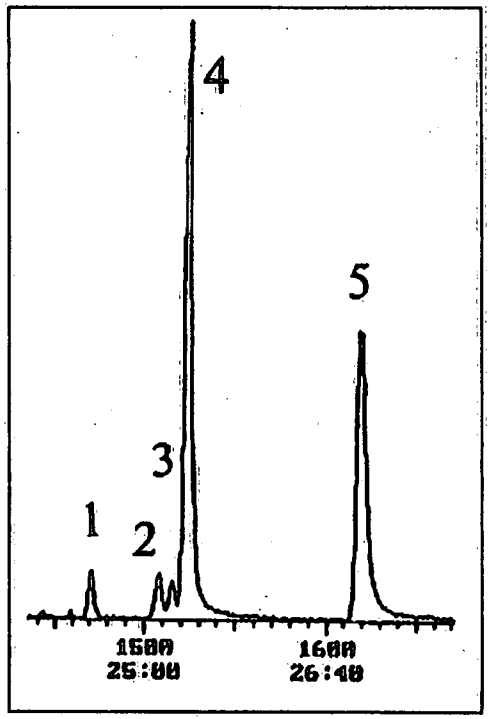
Structures of Some Flame Retardants Identified in Air at an Electronics Recycling Plant



. Bergman, Workshop on PBDEs in the Environment,
Burlington (Canada), August 19-20, 1999

Metabolism of 2,2',4,4'-Tetrabromodiphenyl Ether (BDE-47)

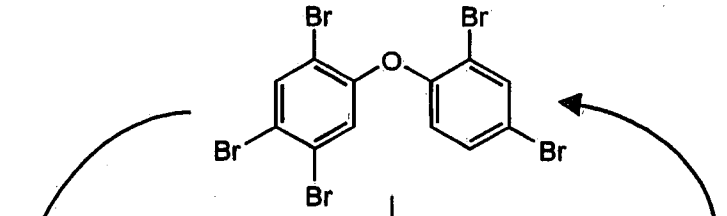




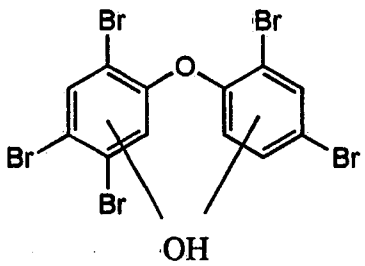
From Orn and Klasson Wehler, Xenobiotica, 28 (1998) 199

Å. Bergman, Workshop on PBDEs in the Environment, Burlington (Canada), August 19-20, 1999

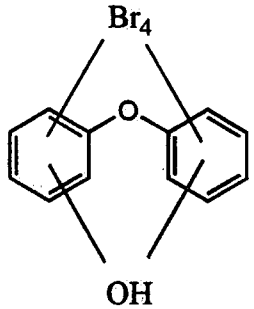
Fecal metabolites of 2,2',4,4',5-Pentabromodiphenyl Ether



INTERMEDIATE



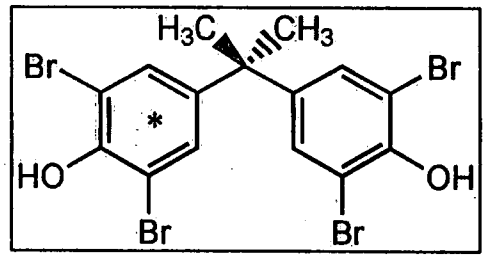
Two congeners



Two congeners

Haak et al, Organohaogen Compounds 1999, In press

^{14}C-TBBPA Metabolism in Bile-duct Cannulated Rats



Cumulative excretion after 72h

Bile:	71%
Faces:	21%
Tissue residues	2.1%
Total:	94%

Identified metabolites in the bile

Diglucuronide conjugate

Sulfate-glucuronide conjugate

TBBPA undergoes enterohepatic ciculation

From Larsen *et al*, Organohalogen Comp. 1998

Potential Endocrine Effects

Theoretically: Structural resemblance between OH-PBDEs and T4/T3

In vitro: OH-PBDE congener competition with T4 for TTR
High affinity of certain OH-PBDE for THR-β and THR-α
TBBPA binds to TTR

In vivo: Reduced T4 levels (BDE-47)

More data available

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Other Effects

Cancer: HBCDD induces intragenic recombination in mammalian cells
Tris(2,3-dibromopropyl)phosphate is carcinogenic

Neurotoxic effects: Behavioural disability in mice induced by BDE-99

Enzyme induction/inhibition: Ah-receptor and PBDE congeners (17 compounds tested)

Limited data on Reproductive and Immunological effects

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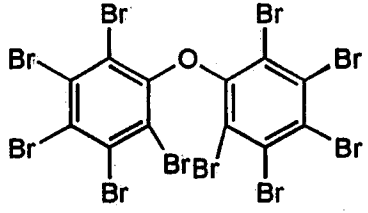
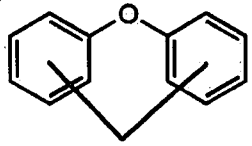
Photochemical transformations of DecaBDE



BDE-209

UV-light

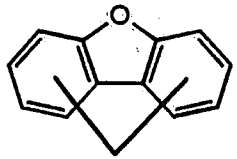
PBDEs



Br_3 - Br_8

10 - 20 Congeners

PBDFs

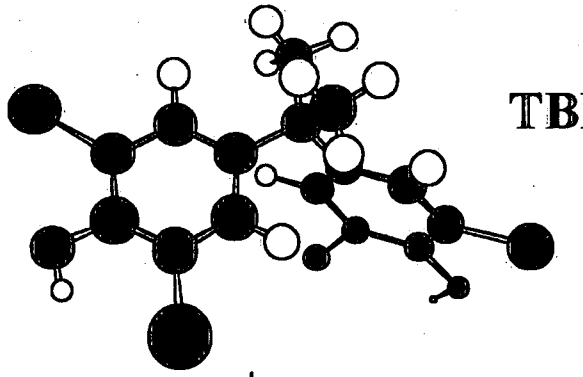


Br_1 - Br_6

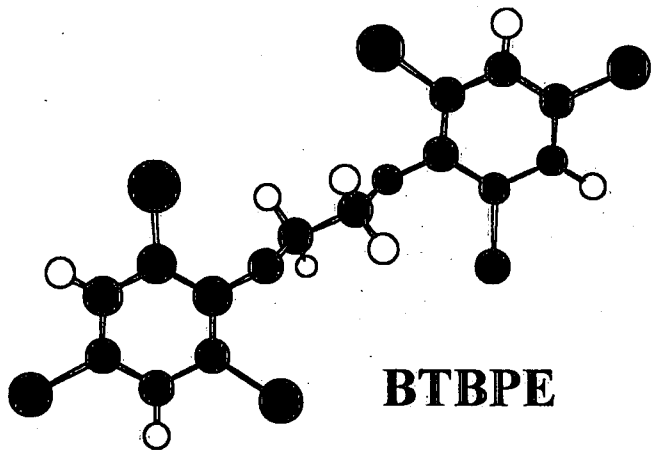
10 - 20 Congeners

Watanabe and Tatsukawa in Bull. Environ. Contam. Toxicol 39 (1987) 953

Photochemical Transformations



TBBPA



BTBPE

UV-light

?

2,4,6-Tribromophenol

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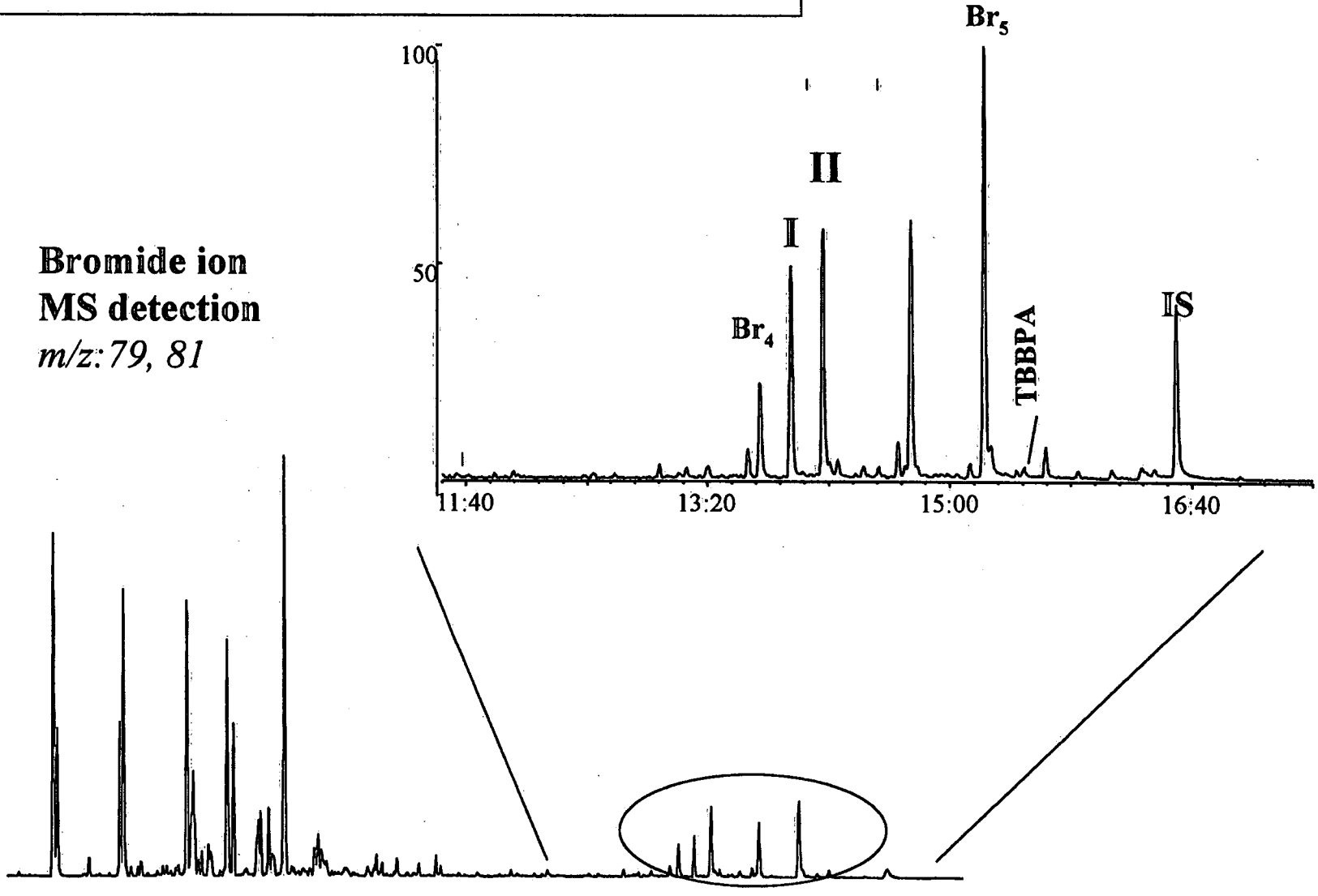
Burlington (Canada), August 19-20, 1999

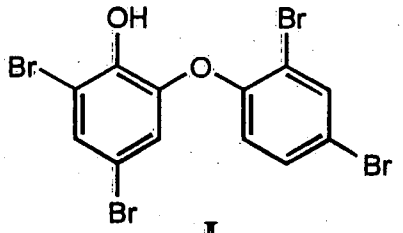
Phenol fraction in Baltic Salmon blood

Bromide ion
MS detection
m/z: 79, 81

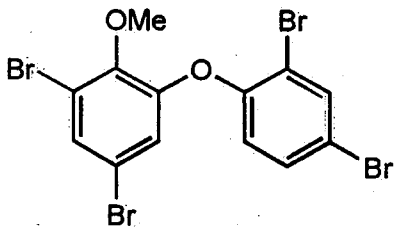


From Asplund *et al*, Ambio 28 (1999) 67

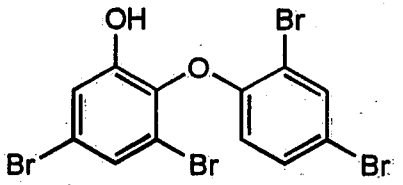
Hydroxylated and methoxylated PBDEs in Wildlife



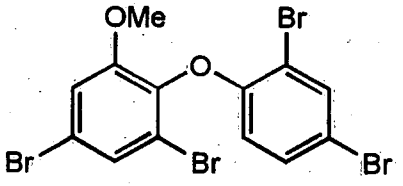
I



I methyl ether



II



II methyl ether

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Burlington (Canada), August 19-20, 1999

CONCLUSIONS

Concern: BFR Production Volumes
Number of BFRs
Additive BFRs are potentially more Environmentally Hazardous than Reactive BFRs
Persistency and Reactivity of BFRs
Bioavailability and Bioaccumulation
Potential Endocrine Effects
Neurotoxic effects and others

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Burlington (Canada), August 19-20, 1999

NEEDS

Needs: Pure Standards for Analysis and Toxicity Testing
Improved (New) Analytical Methods
Comparative Exposure Data (Internal and External)
Relevant Toxicological Studies (*in vitro* and *in vivo*)
Studies on a Larger Number of BFRs (and FRs)

THE ANSWER

YES

YES

YES

YES

New Question: Didn't we learn anything from the DDT and PCB issues?

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Burlington (Canada), August 19-20, 1999

POLYBROMINATED DIPHENYL ETHER STANDARDS

Terry Grim

Cambridge Isotope Laboratories (CIL) has been producing Polybrominated Diphenyl Ether Standards for approximately three years. In 1997, a standard containing 23 native analytes and one ^{13}C-labeled standard (3,3',4,4'-TeBrDPE) was produced for breakthrough investigations by Canada's Departments of Environment and Fisheries & Oceans. Two other labeled mixes were prepared. Because only one ^{13}C-labeled BrDPE standard was available, ^{13}C-labeled ClDPE standards were used.

In 1999, 14 additional analytes were added to the product line, and ^{13}C-3,3',4,4'5-PeBrDPE was produced. Discussions with interested parties are underway to produce cocktails that will complement the 1997 mixes, using additional ^{13}C-labeled BrDPE standards.

Polybrominated Diphenyl Ether Standards

1st Annual Workshop on Polybrominated Diphenyl Ethers in the Environment

August 20, 1999

Terry Grim

Cambridge Isotope Laboratories, Inc.
50 Frontage Road
Andover, MA, 01810, USA

Phone: 978-749-8000
Fax: 978-749-8000
email: terryg@isotope.com

Brominated Diphenyl Ether Standards Now Available from CIL (11/96)

All Solutions are 50 ± 5.0 µg/ml (ppm) in n-Nonane

Unlabeled:

Catalog#	*Description:*	*Amount:*
EO-4098	2-Bromodiphenyl Ether (BDE# 1)	1.2 ml
EO-4099	3-Bromodiphenyl Ether (BDE# 2)	1.2 ml
EO-4100	2,4-Dibromodiphenyl Ether (BDE# 7)	1.2 ml
EO-4101	2,4'-Dibromodiphenyl Ether (BDE# 8)	1.2 ml
EO-4102	2,6-Dibromodiphenyl Ether (BDE# 10)	1.2 ml
EO-4103	3,4-Dibromodiphenyl Ether (BDE# 12)	1.2 ml
EO-4104	3,4'-Dibromodiphenyl Ether (BDE# 13)	1.2 ml
EO-4105	4,4'-Dibromodiphenyl Ether (BDE# 15)	1.2 ml
EO-4106	2,4,6-Tribromodiphenyl Ether (BDE# 30)	1.2 ml
EO-4107	2,4',6-Tribromodiphenyl Ether (BDE# 32)	1.2 ml
EO-4108	2',3,4-Tribromodiphenyl Ether (BDE# 33)	1.2 ml
EO-4109	3,3',4-Tribromodiphenyl Ether (BDE# 35)	1.2 ml
EO-4110	3,4,4'-Tribromodiphenyl Ether (BDE# 37)	1.2 ml
EO-4111	2,2',4,4'-Tetrabromodiphenyl Ether (BDE# 47)	1.2 ml
EO-4112	2,3',4,4'-Tetrabromodiphenyl Ether (BDE# 66)	1.2 ml
EO-4113	2,3',4',6-Tetrabromodiphenyl Ether (BDE# 71)	1.2 ml
EO-4114	2,4,4',6-Tetrabromodiphenyl Ether (BDE# 75)	1.2 ml
EO-4115	3,3',4,4'-Tetrabromodiphenyl Ether (BDE# 77)	1.2 ml
EO-4092	2,2',3,4,4'-Pentabromodiphenyl Ether (BDE# 85)	1.2 ml
EO-4091	2,2',4,4',5-Pentabromodiphenyl Ether (BDE# 99)	1.2 ml
EO-4116	2,3',4,4',6-Pentabromodiphenyl Ether (BDE# 119)	1.2 ml
EO-4093	2,2',4,4',5,5'-Hexabromodiphenyl Ether (BDE# 153)	1.2 ml
EO-4117	2,3,3',4,4',5,6-Heptabromodiphenyl Ether (BDE# 190)	1.2 ml

Carbon-13 Labeled:

EO-1439	3,3',4,4'-Tetrabromodiphenyl Ether (BDE# 77, U-$^{13}C_{12}$, 99%)	1.2 ml

Cambridge Isotope Laboratories, Inc.

50 Frontage Road, Andover, MA 01810-5413 USA
PH: 800.322.1174 (USA) PH: 978.749.8000
PH: 800.643.7239 (Canada) fax: 978.749.2768
e-mail: cilsales@isotope.com URL: http://www.isotope.com

New Flame Retardants (July 1999)

All solutions are 50±5 µg/ml (ppm) in nonane.

Catalog#	*Description:*	*Amount:*
CLM-4694	Tetrabromobisphenol A (ring-$^{13}C_{12}$, 99%)	1.2ml
EO-4930	3,3', 4,4',5-Pentabromodiphenyl ether (BDE# 126, ring $^{13}C_{12}$, 99%)	1.2ml
EO-4915	4-Monobromodiphenyl ether (BDE# 3, unlabeled)	1.2ml
EO-4916	3,3'-Dibromodiphenyl ether (BDE# 11, unlabeled)	1.2ml
EO-4919	2,2',4-Tribromodiphenyl ether (BDE# 17, unlabeled)	1.2ml
EO-4917	2,3',4-Tribromodiphenyl ether (BDE# 25, unlabeled)	1.2ml
EO-4920	2,4,4'-Tribromodiphenyl ether (BDE# 28, unlabeled)	1.2ml
EO-4918	2,2',4,5'-Tetrabromodiphenyl ether (BDE# 49, unlabeled)	1.2ml
EO-4194	2,2',4,4',6-Pentabromodiphenyl ether (BDE# 100, unlabeled)	1.2ml
EO-4921	2,3,4,5,6-Pentabromodiphenyl ether (BDE# 116, unlabeled)	1.2ml
EO-4922	2,2',3,4,4',5'-Hexabromodiphenyl ether (BDE# 138, unlabeled)	1.2ml
EO-4925	2,2',4,4',5,6'-Hexabromodiphenyl ether (BDE# 154, unlabeled)	1.2ml
EO-4923	2,2',3,4,4',6'-Hexabromodiphenyl ether (BDE# 140, unlabeled)	1.2ml
EO-4926	2,2',4,4',6,6'-Hexabromodiphenyl ether (BDE# 155, unlabeled)	1.2ml
EO-4924	2,3,4,4',5,6-Hexabromodiphenyl ether (BDE# 166, unlabeled)	1.2ml
EO-4927	2,2',3,4,4',5,6-Heptabromodiphenyl ether (BDE# 181, unlabeled)	1.2ml

Cambridge Isotope Laboratories, Inc.

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e-mail: cilsales@isotope.com URL: http://www.isotope.com

Technical Data Sheet

Compound: Polybrominated Diphenyl Ether Analytical Standard Solution

Catalog Number: EO-4149

Consists of each of the following compounds in the indicated concentration:

Compound	Concentration
2 - Bromodiphenyl Ether (BDE# 1)	100 ng/ml (ppb)
3 - Bromodiphenyl Ether (BDE# 2)	100 ng/ml (ppb)
2,4 - Dibromodiphenyl Ether (BDE# 7)	100 ng/ml (ppb)
2,4' - Dibromodiphenyl Ether (BDE# 8)	100 ng/ml (ppb)
2,6 - Dibromodiphenyl Ether (BDE# 10)	100 ng/ml (ppb)
3,4 - Dibromodiphenyl Ether (BDE# 12)	100 ng/ml (ppb)
3,4' - Dibromodiphenyl Ether (BDE# 13)	100 ng/ml (ppb)
4,4' - Dibromodiphenyl Ether (BDE# 15)	100 ng/ml (ppb)
2,4,6 - Tribromodiphenyl Ether (BDE# 30)	100 ng/ml (ppb)
2,4',6 - Tribromodiphenyl Ether (BDE# 32)	100 ng/ml (ppb)
2',3,4 - Tribromodiphenyl Ether (BDE# 33)	100 ng/ml (ppb)
3,3',4 - Tribromodiphenyl Ether (BDE# 35)	100 ng/ml (ppb)
3,4,4' - Tribromodiphenyl Ether (BDE# 37)	100 ng/ml (ppb)
2,2',4,4' - Tetrabromodiphenyl Ether (BDE# 47)	100 ng/ml (ppb)
2,3',4,4' - Tetrabromodiphenyl Ether (BDE# 66)	100 ng/ml (ppb)
2,3',4',6 - Tetrabromodiphenyl Ether (BDE# 71)	100 ng/ml (ppb)
2,4,4',6 - Tetrabromodiphenyl Ether (BDE# 75)	100 ng/ml (ppb)
3,3',4,4' - Tetrabromodiphenyl Ether (BDE# 77)	100 ng/ml (ppb)
2,2',3,4,4' - Pentabromodiphenyl Ether (BDE# 85)	150 ng/ml (ppb)
2,2',4,4',5 - Pentabromodiphenyl Ether (BDE# 99)	150 ng/ml (ppb)
2,3',4,4',6 - Pentabromodiphenyl Ether (BDE# 119)	150 ng/ml (ppb)
2,2',4,4',5,5' - Hexabromodiphenyl Ether (BDE# 153)	200 ng/ml (ppb)
2,3,3',4,4',5,6 - Heptabromodiphenyl Ether (BDE# 190)	250 ng/ml (ppb)
$^{13}C_{12}$ 3,3',4,4'- Tetrachlorodiphenyl Ether (CDE# 77)	100 ng/ml (ppb)
$^{13}C_{12}$ 2,3,3',4,4'- Pentachlorodiphenyl Ether (CDE# 105)	100 ng/ml (ppb)
$^{13}C_{12}$ 2,2',3,3',4,4'- Hexachlorodiphenyl Ether (CDE# 128)	200 ng/ml (ppb)
$^{13}C_{12}$ 2,3,3',4,4',5- Hexachlorodiphenyl Ether (CDE# 156)	200 ng/ml (ppb)
$^{13}C_{12}$ 2,2',3,3',4,4',5- Heptachlorodiphenyl Ether (CDE# 170)	200 ng/ml (ppb)
$^{13}C_{12}$ 2,2',3,3',4,4',5,5'- Octachlorodiphenyl Ether (CDE# 194)	300 ng/ml (ppb)
$^{13}C_{12}$ 3,3',4,4'- Tetrabromodiphenyl Ether (BDE# 77)	100 ng/ml (ppb)

Lot Number:	HJ-386
Concentration:	See above
Amount:	1.0 milliliter
Solvent:	n-Nonane
Date Prepared:	10/98

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Technical Data Sheet

Compound: Bromo / Chloro Diphenyl Ether
Surrogate Spiking Solution

Catalog Number: EO-4150

Consists of each of the following compounds in the indicated concentration:

Compound	Concentration
$^{13}C_{12}$ 3,3',4,4'- Tetrachlorodiphenyl Ether (CDE# 77)	20 ng/ml (ppb)
$^{13}C_{12}$ 2,3,3',4,4'- Pentachlorodiphenyl Ether (CDE# 105)	20 ng/ml (ppb)
$^{13}C_{12}$ 2,3,3',4,4',5- Hexachlorodiphenyl Ether (CDE# 156)	40 ng/ml (ppb)
$^{13}C_{12}$ 2,2',3,3',4,4',5- Heptachlorodiphenyl Ether (CDE# 170)	40 ng/ml (ppb)
$^{13}C_{12}$ 2,2',3,3',4,4',5,5'- Octachlorodiphenyl Ether (CDE# 194)	60 ng/ml (ppb)

Lot Number:	HJ-387
Concentration:	See above
Amount:	1.0 milliliter
Solvent:	n-Nonane
Date Prepared:	04/99

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Technical Data Sheet

Compound: Bromo / Chloro Diphenyl Ether
Performance Standard Mixture

Catalog Number: EO-4151

Consists of the following compounds in the indicated concentrations:

Compound	Concentration
$^{13}C_{12}$ 2,2',3,3',4,4'- Hexachlorodiphenyl Ether (CDE# 128)	100 ng/ml (ppb)
$^{13}C_{12}$ 3,3',4,4'- Tetrabromodiphenyl Ether (BDE# 77)	100 ng/ml (ppb)

Lot Number:	HJ-388
Concentration:	See above
Amount:	1.0 milliliter
Solvent:	n-Nonane
Date Prepared:	04/99

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New Standards- Winter 1999?

EO-????
Bromodiphenyl Ether Analytical Standard Solution:

Compound	Concentration
4 - Bromodiphenyl Ether (BDE# 3)	100 ng/ml
3,3' - Dibromodiphenyl Ether (BDE# 11)	100 ng/ml
4,4' - Dibromodiphenyl Ether (BDE# 15)	100 ng/ml
2,2',4 - Tribromodiphenyl Ether (BDE# 17)	100 ng/ml
2,4',6 - Tribromodiphenyl Ether (BDE# 32)	100 ng/ml
2,4,4' - Tribromodiphenyl Ether (BDE# 28)	100 ng/ml
2,2',4,5' - Tetrabromodiphenyl Ether (BDE# 49)	100 ng/ml
2,2',4,4',6 - Pentabromodiphenyl Ether (BDE# 100)	150 ng/ml
2,3,4,5,6 - Pentabromodiphenyl Ether (BDE# 116)	150 ng/ml
3,3',4,4',5 - Pentabromodiphenyl Ether (BDE# 126)	150 ng/ml
2,2',3,3',4,4' - Hexabromodiphenyl Ether (BDE# 128)	200 ng/ml
2,2',3,4,4',5' - Hexabromodiphenyl Ether (BDE# 138)	200 ng/ml
2,2',3,4,4',6' - Hexabromodiphenyl Ether (BDE# 140)	200 ng/ml
2,2',4,4',5,6' - Hexabromodiphenyl Ether (BDE# 154)	200 ng/ml
2,2',4,4',6,6' - Hexabromodiphenyl Ether (BDE# 155)	200 ng/ml
2,3,4,4',5,6 - Hexabromodiphenyl Ether (BDE# 166)	200 ng/ml
2,2',3,4,4',5,6 - Heptabromodiphenyl Ether (BDE# 181)	250 ng/ml
2,2',3,4,4',5',6 - Heptabromodiphenyl Ether (BDE# 183)	250 ng/ml
Decabromodiphenyl Ether (BDE# 209)	400 ng/ml
$^{13}C_{12}$ 3,3',4,4',5- Pentabromodiphenyl Ether (BDE# 126)	150 ng/ml

New Standards- Winter 1999?

EO-????
Higher Bromodiphenyl Ether Performance Standard Mix

Compound	Concentration
$^{13}C_{12}$ 3,3',4,4'5- Pentabromodiphenyl Ether (BDE# 126)	150 ng/ml

Additional ^{13}C PBrDPEs may be added...

Other concerns:

- PCDFs are a natural byproduct during PCB formation- can be on the order of 1/10th of 1%

  Are PBrDDs and PBrDFs a significant byproduct of PBrDPEs?

- PCDDs and PCDFs are produced during PCB incineration

  Are PBrDDs and PBrDFs produced during PBrDPE incineration?

DETERMINATION OF POLYBROMINATED AND POLYCHLORINATED DIPHENYL ETHERS IN BIOTA

D.B. Sergeant, M. Alaee, J. Luross, and M.G. Ikonomou

Polybrominated diphenyl ethers (PBDEs) are rapidly becoming chemicals of concern. Like polychlorinated biphenyls they are capable of forming 209 congeners. Due to stringent fire regulations in many countries the consumption of fire retardants such as PBDEs continues to grow. The extensive use of products containing PBDEs has resulted in the release of these compounds into the environment. PBDEs are lipophilic compounds have been shown to bio-accumulate through the food chain, and have been detected in both freshwater and marine organisms. PBDEs have also been found in human adipose tissue, mother's milk and in the blood of exposed workers. In the case of breast milk it has been shown that the concentration of PBDEs in breast milk doubled every 5 years over the past 25 years. A recently published study reported measureable levels of PBDEs in human plasma collected from hospital workers, data entry clerks, and workers in an electronics dismantling plant. Results from this study showed elevated levels of PBDEs in the plasma of workers in the electronics dismantling plant compared to the other groups. A literature review indicated that the majority of the information on PBDEs originated from Europe, particularly Sweden. A high priority for the study of PBDEs exists since there is very little information available on the levels of PBDEs in the Canadian environment and recent data indicated that PBDEs are more toxic than previously suggested.

Polychlorinated diphenyl ethers (PCDEs) are another group of halogenated contaminants. PCDEs have been used as heat exchangers, flame retardants, plasticisers, lubricants, and hydraulic fluids. They are widely used as intermediates in chemical reactions and as such they have been reported as an impurity in chlorophenols, and phenoxy acids, and not surprising they are known to be present in chemical effluents. PCDEs are widespread contaminants, and have been shown to bio-accumulate in fish, marine mammals, birds, and humans. In the past PCDEs were considered as interferences in the determination of PCDFs, and the majority of the literature on PCDEs deals with separation and elimination of this interference. PCDEs are classified as toxic pollutants by the US-EPA however there is limited information on the toxicity of PCDEs.

In order to evaluate the global distribution, movement and fate of PBDEs and PCDEs in the environment, a sensitive, comprehensive, and interference free analytical method is required for their determination in complex environmental matrices. A number of procedures for the determination of PBDEs in the environment have been reported. However, during our initial investigation of the mass spectrometry of PBDEs it was determined that low resolution quadrupole instruments did not have high mass to detect PBDEs. Since all 209 congeners are not available for PCDEs and PBDEs we have to add the areas under the peak to determine homologue distribution. A high resolution GCMS method and its selectivity would be necessary for comprehensive analyses of both the PBDEs and PCDEs.

A dioxin-like HRGC/HRMS based analytical method was developed for the determination of congener specific PDPEs compounds in biota samples and has been extended to include PCDEs. The recoveries for the internal standards ranged between 65% and 120% for PBDE analyses and 77 to 107% for the PCDE analyses. The method was validated by comparison of GLLFAS's data on biota CRMs with that of IOS. The coefficients of variance for lake trout, herring, and Sockeye salmon were 15%, 22%, and

37% respectively. Significant levels of BDPEs were found in both Lake Ontario lake trout and pacific herring; and sufficient quantities of these materials are commercially available for a round-robin study. Our method is also capable of determining PCDEs in biota samples.

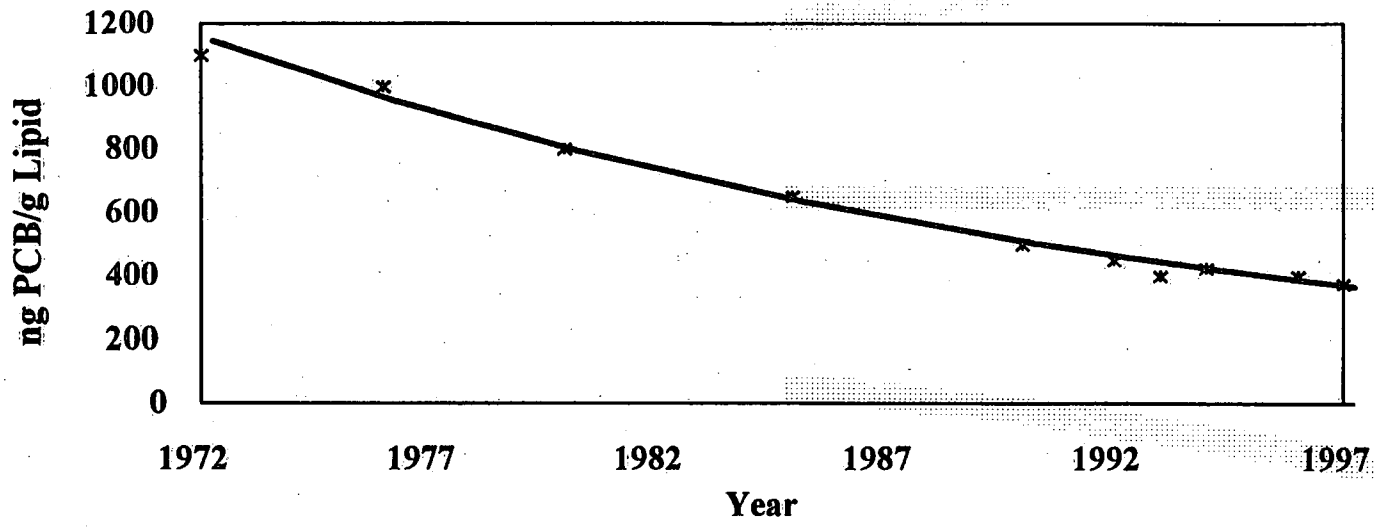
Determination of Polybrominated and Polychlorinated Diphenyl Ethers in Biota

Dave Sergeant, Mehran Alaee,
Jennifer Luross, and Michael Ikonomou

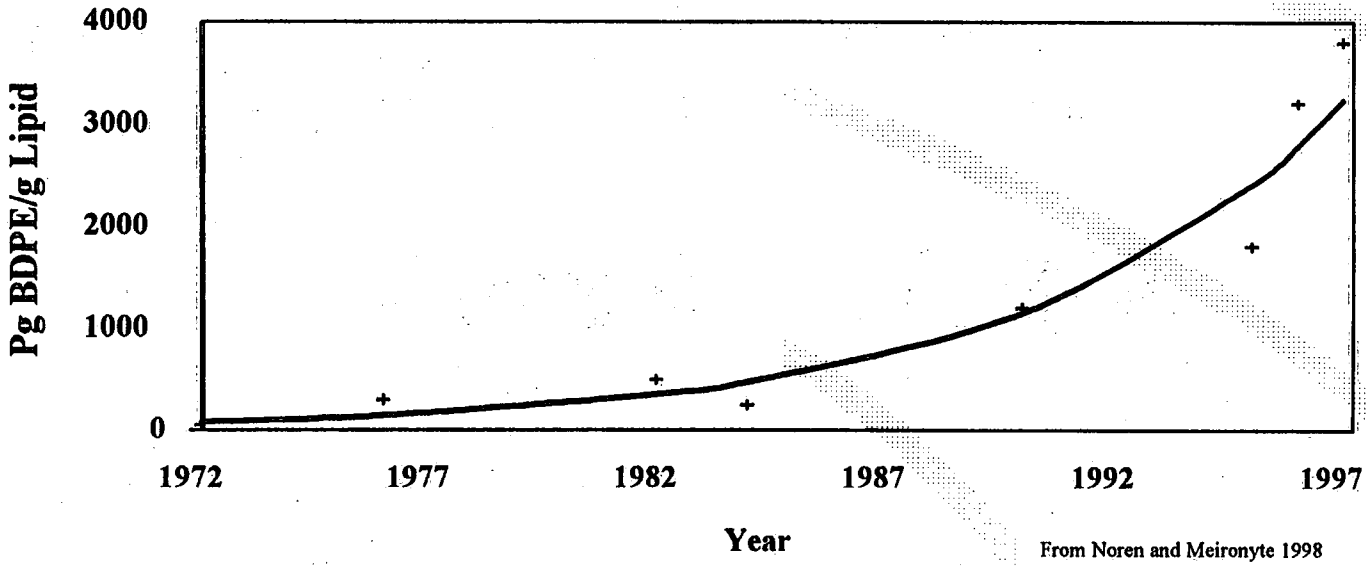
OVERVIEW

- Objective
- Analytical Method
- Results
- Conclusion

Concentration of PCBs in Human Milk Sampled During Different Time Periods



Concentration of PBDE in Human Milk Sampled During Different Time Periods



From Noren and Meironyte 1998

OBJECTIVES

- To develop analytical methods for PBDEs and PCDEs in environmental matrices
- To validate the methods using CRMs
- To apply the methods to environmental samples to generate data

WHY NEED FOR CRMs?

- PBDEs found in the environment
- Various laboratories are generating data
- The methods and standards used in analysis are different
- To compare data from different laboratories a "ruler" is needed to ensure measurements are similar
- CRMs are necessary

- Objective
- Analytical Method
- Results
- Conclusion

Extraction and cleanup

- 10 g fish tissue homogenates
- ground with 130 g sodium sulfate
- packed in glass column
- spiked with 20 uL C13-PCDE surrogates
- eluted with 300 mL dichloromethane
- GPC cleanup for lipid removal
- silica gel and alumina(if needed) cleanups

Instrumental Analysis

- GC run with 1 ul on-column injections
- 60 m RT_X5 column
- 110°C for 1 min. then 15°C to 180°C and then at 2°C to 280°C and hold for 35 min. (90 min. total run time)
- AutoSpec-Q , EI mode, 10 000 resolution
- 8 SIM descriptors for mono- to hepta-PBDE

PBDEs ANALYZED

- 2 mono-, 6 di-, 5 -tri, 5 tetra-, 3 penta-, 1 hexa--, and 1 hepta-PBDE
- 5 C13-PCDE surrogates
- 1 hexa-PCDE AND 1 tetra-PBDE as performance standards (IOS used these as surrogates as well)

PCDEs ANALYZED

- 2 mono-, 3 di-,3-tri, 3 tetra-, 3 penta-, 1 hexa-, 1 hepta-, 1 octa-, and deca PCDE
- 5 C13-PCDE surrogates
- 1 hexa-PCDE AND 1 tetra-PBDE as performance standards (IOS used these as surrogates as well)

- Objective
- Analytical Method
- Results
- Conclusion

Results

- Individual PBDE congeners were run using full scan GCMS at 10 000 resolution. A 70 amu mass window around the M^+ and $M\text{-}2Br^+$ ion clusters was scanned
- Well resolved peaks were observed and the retention times of all congeners was determined. We had only 23 of the 209 congeners

Results

- Limited PBDE congeners are available
- Therefore, with no window mixture it was necessary to interpolate between the congeners available to determine the 8 SIM windows. Overlaps between the congener groups might exist.
- The M^+ ion was monitored for the mono- through tri PBDEs and the $M\text{-}2Br^+$ ion for the tetras onwards

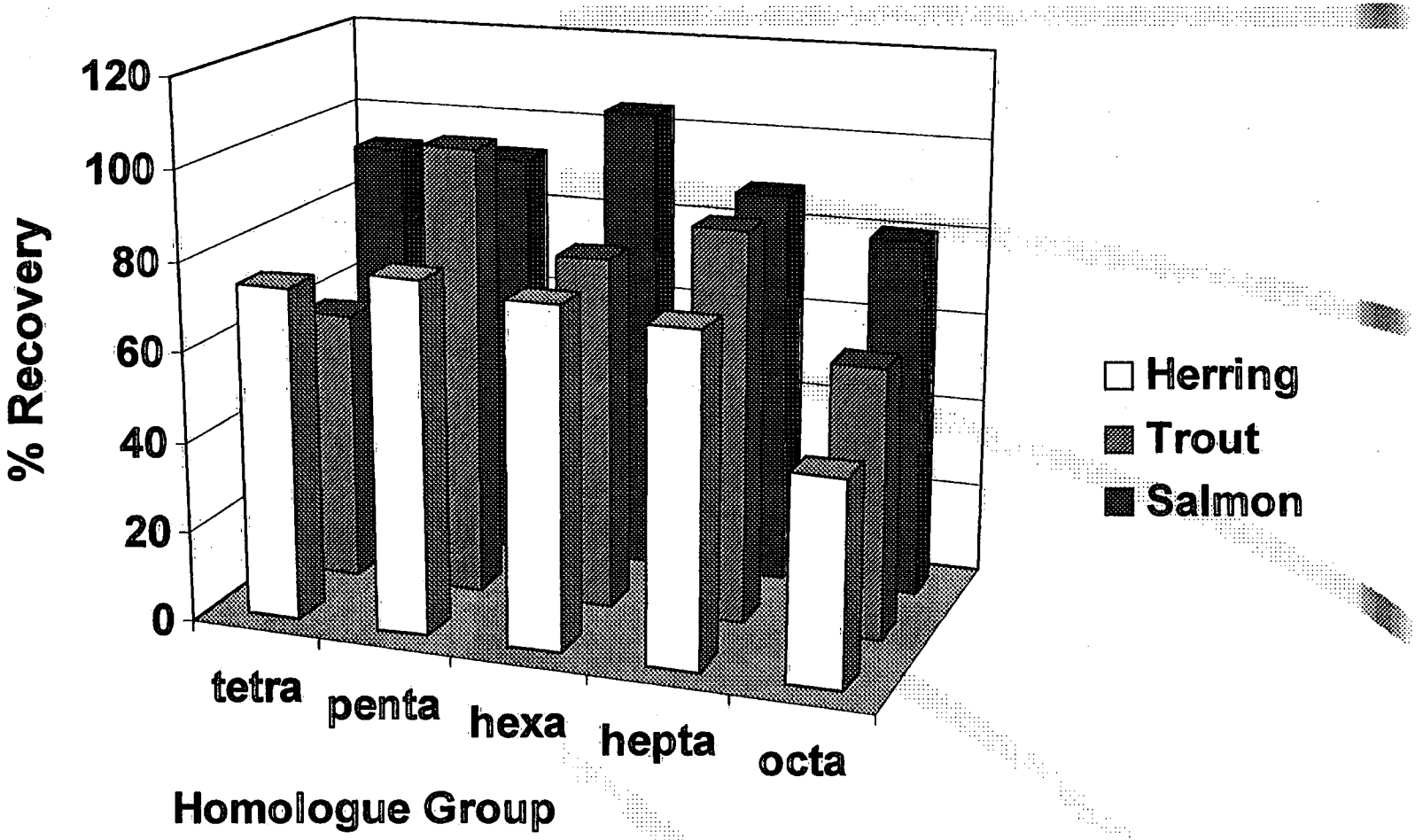
Results

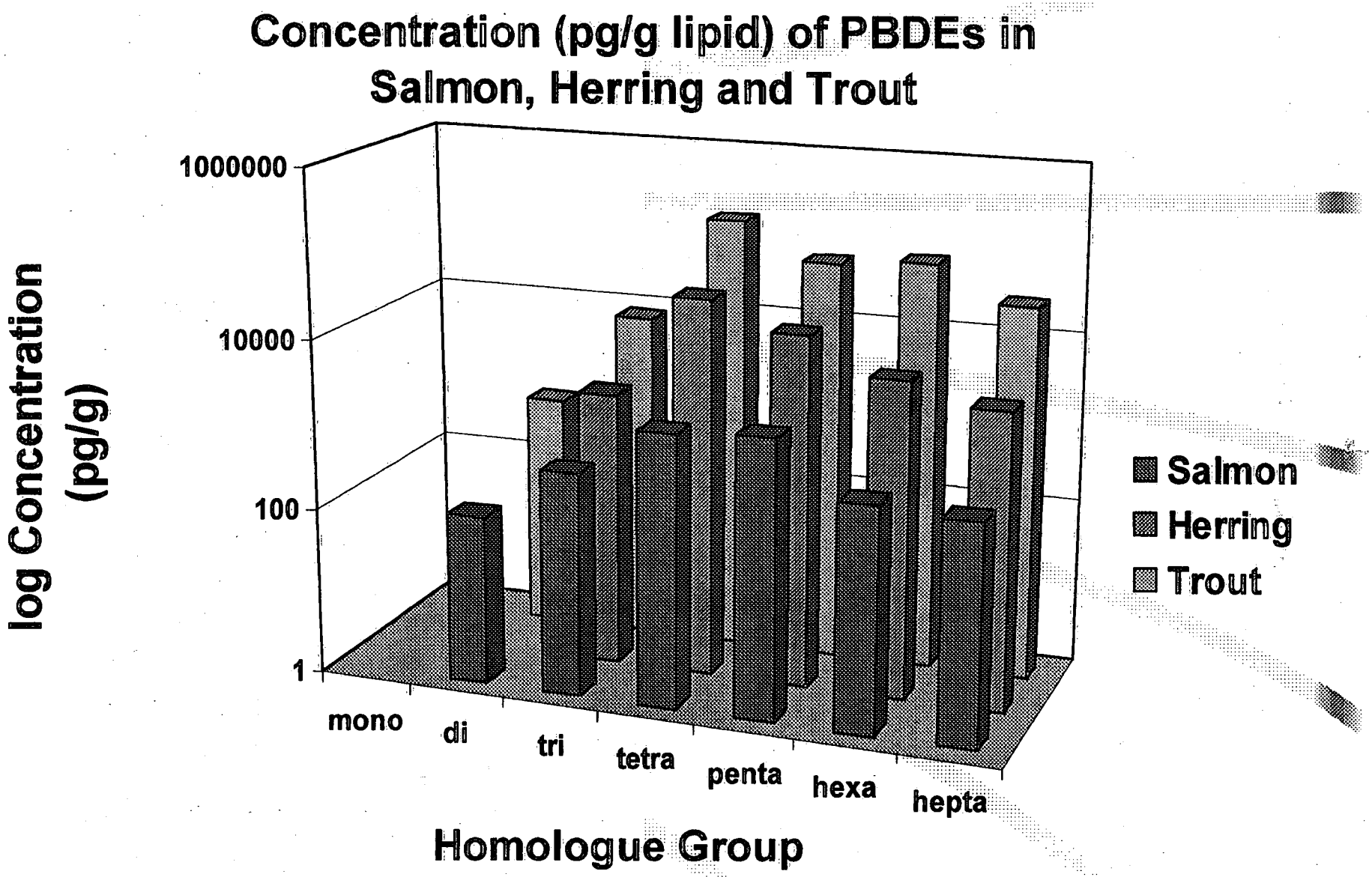
- For 3,3',4,4'-tetra BDE the M^+ ion was more intense than the M-2Br^+ ion, a separate window was created using the M^+ ion
- Windows were verified by analysis of Bromkal 70-5DE (tetra to hepta-BDEs and Bromkal 79-8-DE (penta to deca-BDEs)

Results

- We have detected PBDEs in fish, air, water, and sediments. This method is adequate for analysis of environmental matrices.
- The method is reproducible as shown by RSDs of samples ranging from 10 to 25 %
- Surrogate % recoveries are acceptable based on a 40- 120 % range as QA/QC protocol for data acceptance.

% Recovery of PCDE surrogates in Salmon, Herring and Trout




Concentration (pg/g lipid) of PBDEs in Salmon, Herring and Trout
log Concentration (pg/g)
1000000
10000
100
1
mono
di
tri
tetra
penta
hexa
hepta
Homologue Group
Salmon
Herring
Trout

Lake Trout (n=7)
(pg/g wet weight)

BDPE	Mean	Stdev	Median	Min	Max
Mono	nd	-	-	-	-
Di	60	40	60	nd	80
Tri	660	120	620	520	840
Tetra	12100	2420	11900	9760	16820
Penta	4600	540	4520	4080	5500
Hexa	5600	980	5240	4220	7080
Hepta	2180	4580	540	40	12540

Comparison of Lake Trout Means (pg/g wet weight)

(A: GLLFAS, B: IOS)

BDPE	A (n=7)	B (n=3)
Mono	0	0
Di	60	30
Tri	660	0
Tetra	12100	10931
Penta	4600	5792
Hexa	5600	8755
Hepta	2180	571

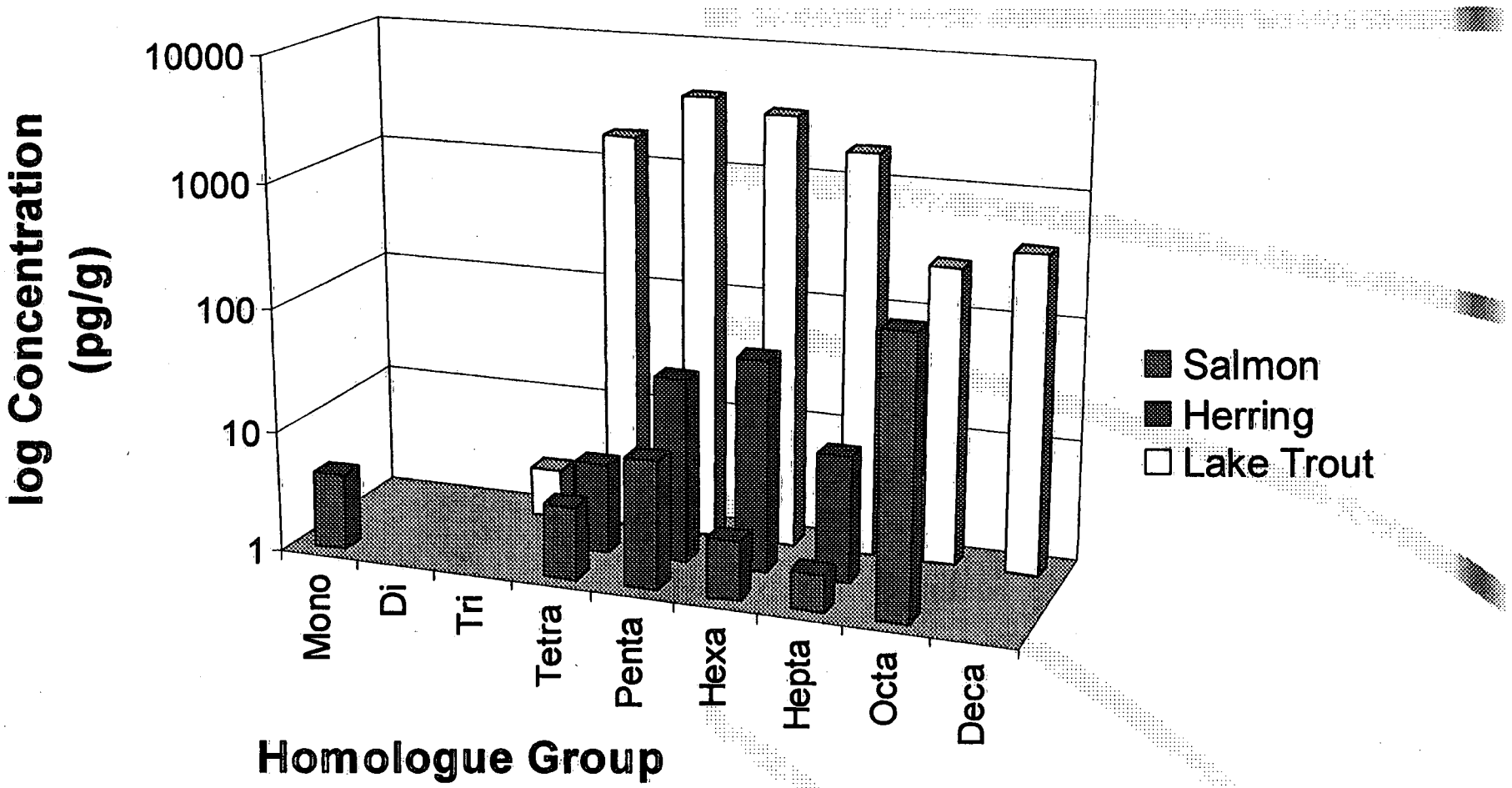
Average MDLs (pg/g)

BDPE	Salmon	Herring	Trout
Mono	1	8	17
Di	1	8	18
Tri	1	9	14
Tetra	2	10	69
Penta	0	3	9
Hexa	0	3	9
Hepta	1	9	28

Interlab. Data Comparison

- GLLFAS and IOS data for the 3 CRMs were compared and a favorable data intercomparison was observed.
- Interlaboratory differences of less than 25% on average were found.

Concentration (pg/g wet weight) of PCDEs in Salmon, Herring and Trout



CONCLUSIONS

- Our methods were successfully applied to these analyses of environmental samples
- PBDEs were identified and quantified in commercially available fish CRMs (dioxin)
- Similar results were obtained in an interlaboratory comparison between GLLFAS and IOS using the CRMs

DISTRIBUTION OF POLYBROMINATED DIPHENYL ETHERS IN THE CANADIAN ENVIRONMENT

Jennifer Luross, Mehran Alaee, Dave Sergeant, Mike Whittle, Derek Muir, and Keith Solomon

Polybrominated diphenyl ethers (PBDEs) are flame retardant compounds that are added to paints, plastics, textiles, and electrical devices. The annual production of PBDEs in 1992 was 40,000 tons, and it continues to increase with the extensive use and manufacturing of fire resistant products. PBDEs are lipophilic compounds that have been shown to bio-accumulate through the food web yet the majority of information pertaining to PBDE levels is from European environments and therefore a method to determine the levels in North America is needed. The initial step was to develop an analytical method for the determination of PBDE in environmental samples which was presented last year at Dioxin 98. This analytical procedure was then applied to various environmental samples, such as air, water, sediment and biota. Data obtained from lake trout from the Great Lakes and marine mammals from Arctic are presented. Average concentrations of PBDEs in lipid from the Great Lakes lake trout were 545 ng/g for Lake Ontario, 237 ng/g for Lake Huron and 135 ng/g for Lake Superior. The main congener in the tetra homologue group was 2, 2', 4, 4'-tetra-BDE, which was observed in all lake trout samples from each of the three Great Lakes, followed by 2,2',4,4',5-penta-BDE. Average concentrations for female ringed seals were 10.0 ng/g, male ringed seals 19.4 ng/g; and average concentration for PBDEs were 28.8 ng/g and 56.8 ng/g for female and male belugas respectively. Similar to the results obtained from Great Lakes lake trout, 2,2',4,4' -tetra-BDE was the main congener found in the samples followed by 2,2',4,4',5-penta-BDE. These results suggest that PBDEs are ubiquitous pollutants in the Canadian environment; and there is a need to further investigate the sources and fate of PBDEs in North America.

Distribution Of Polybrominated Diphenyl Ethers In The Canadian Environment

Jennifer Luross

M. Alaee, D. Sergeant, M. Whittle,

D. Muir, K. Solomon

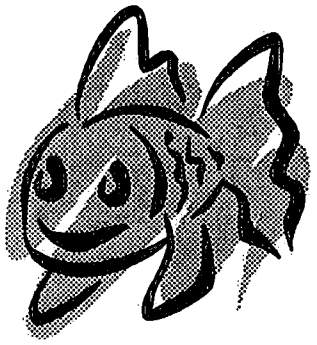
UNIVERSITY of GUELPH

Fisheries and Oceans Canada | Pêches et Océans Canada

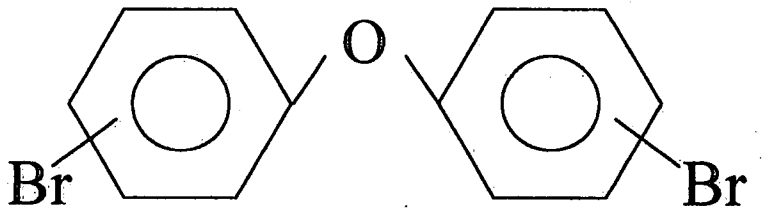
Environment Canada | Environnement Canada

Objectives

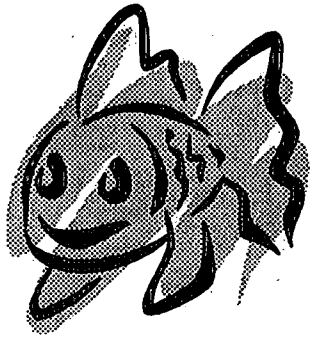
- ✓Background
- ✓Method
- ✓Results and Discussion
- ✓Summary
- ✓Future Directions
- ✓Acknowledgements



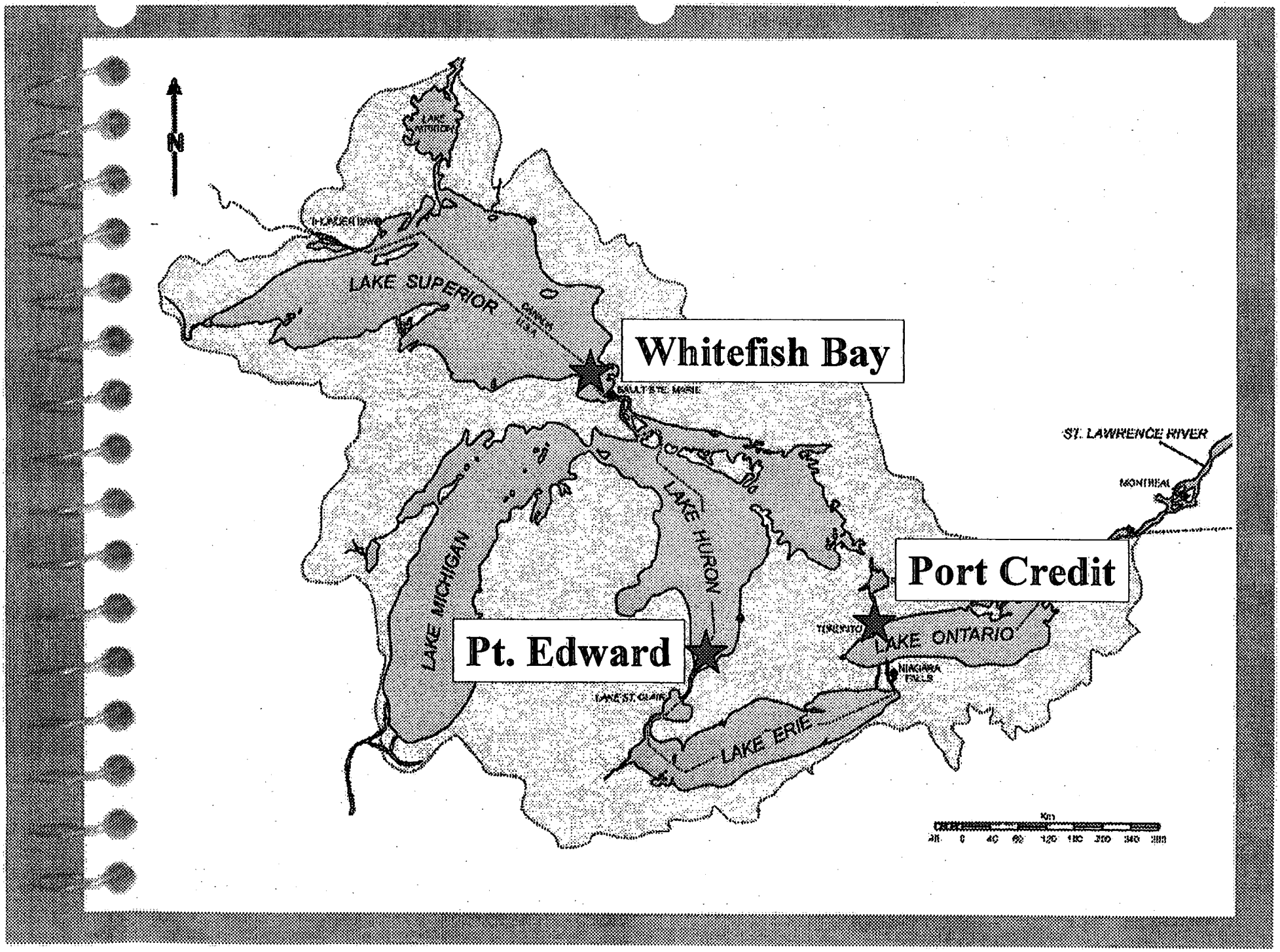
Background



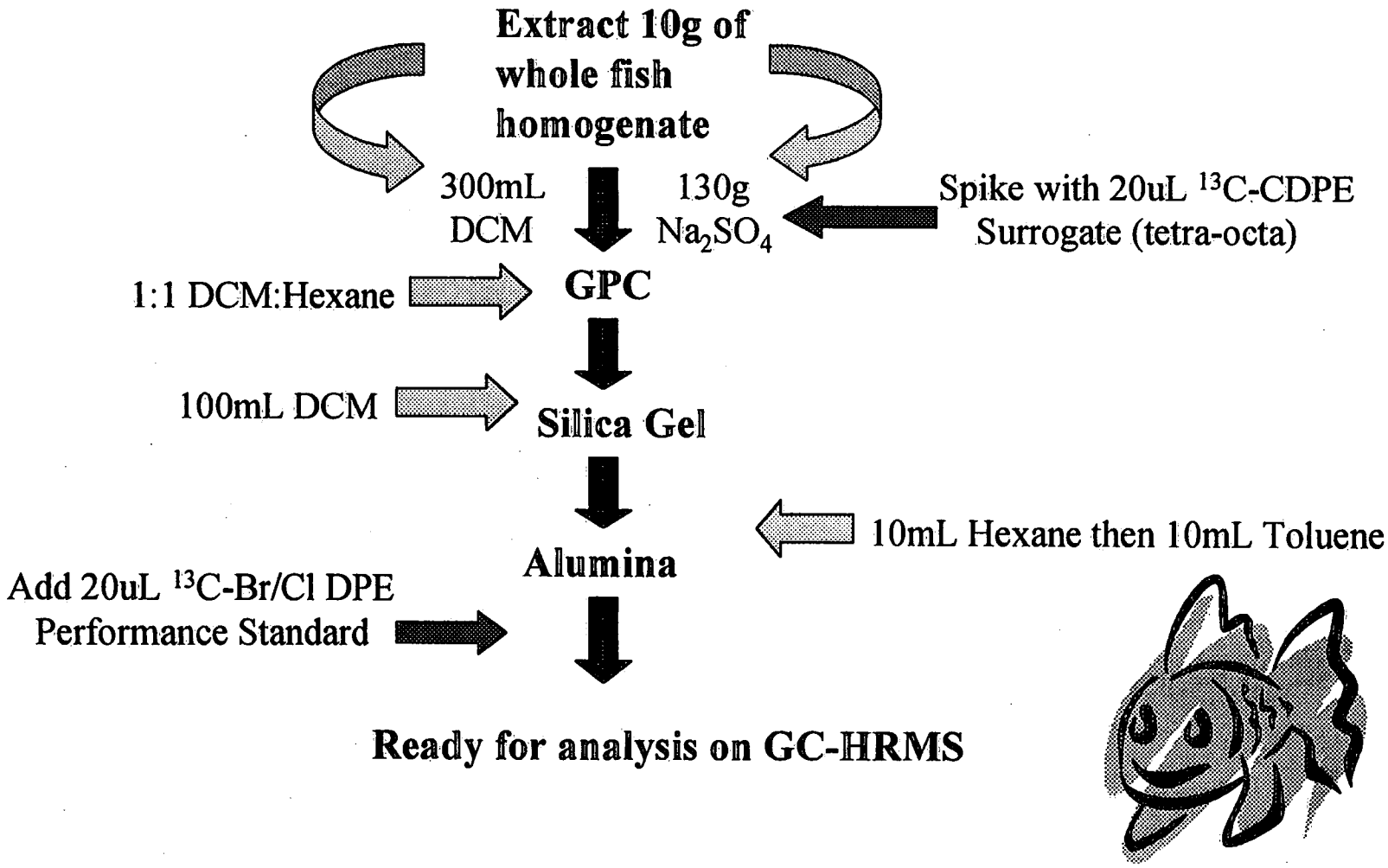
- ✓lipophilic compounds
- ✓flame retardants
- ✓paints, plastics, textiles
- ✓209 congeners
- ✓same substitution as PCBs, possibility of similar toxicity
- ✓bioaccumulate



Why Lake Trout ?


N
LAKE SUPERIOR
Whitefish Bay
SAULT STE. MARIE
ST. LAWRENCE RIVER
MONTREAL
LAKE HURON
LAKE MICHIGAN
Port Credit
Pt. Edward
TORONTO
LAKE ONTARIO
NIAGARA FALLS
LAKE ST. CLAIR
LAKE ERIE

Method


Extract 10g of whole fish homogenate
300mL DCM
130g Na_2SO_4
Spike with 20uL ^{13}C-CDPE Surrogate (tetra-octa)
1:1 DCM:Hexane
GPC
100mL DCM
Silica Gel
10mL Hexane then 10mL Toluene
Alumina
Add 20uL ^{13}C-Br/Cl DPE Performance Standard
Ready for analysis on GC-HRMS

Results and Discussion

- ✓ similar homologue patterns as CRMs
- ✓ ^{13}C-CDPE recoveries between 65-120 %
- ✓ 2, 2', 4, 4'-tetra BDPE had high levels in all samples analyzed followed by 2, 2', 4, 4', 5-penta BDPE

  (main ingredients of Bromkal 70-5DE)

Figure 1: Concentrations of PBDEs (ng/g lipid) in Lake Trout from Lake Superior, Lake Huron, and Lake Ontario

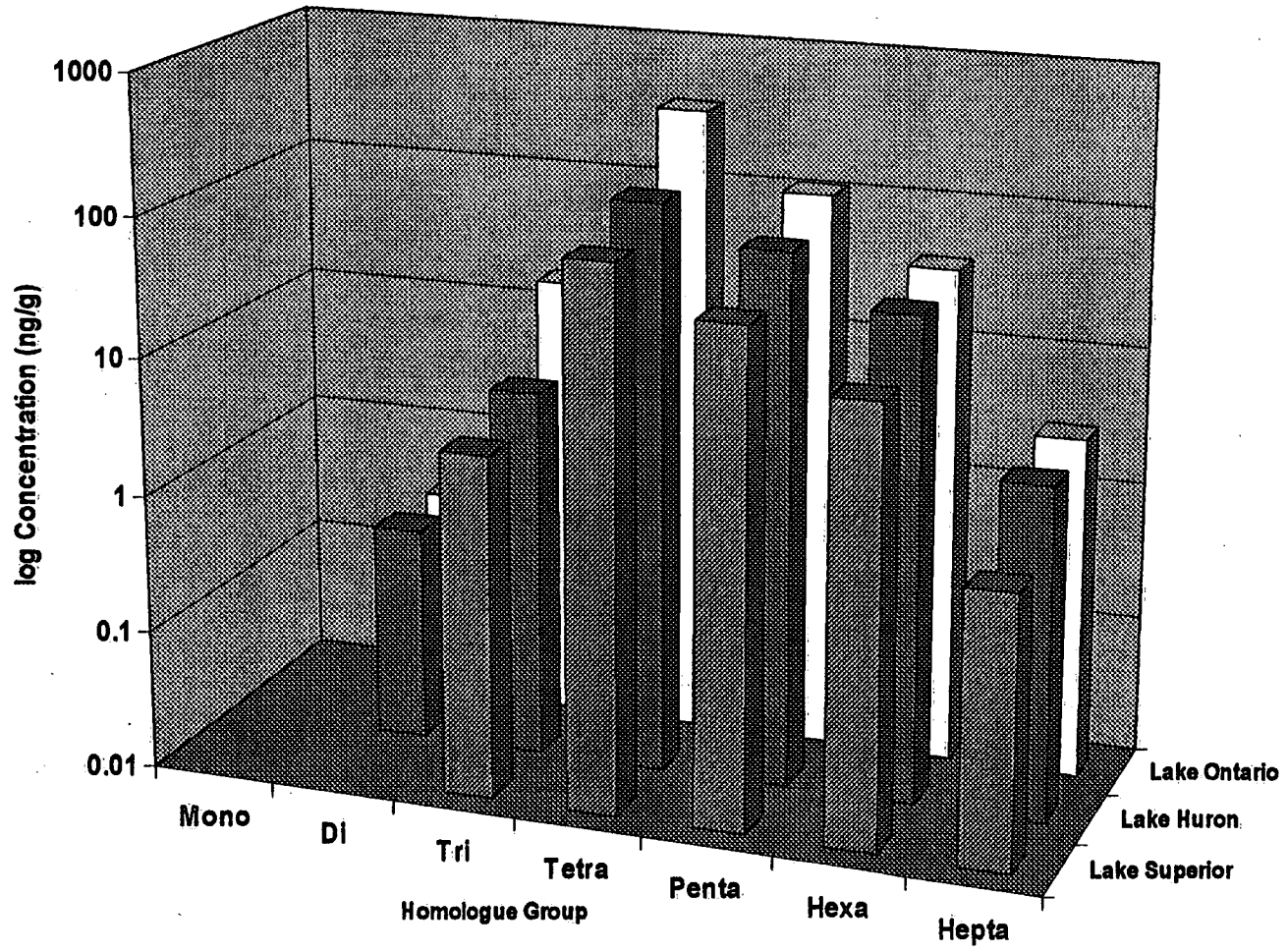
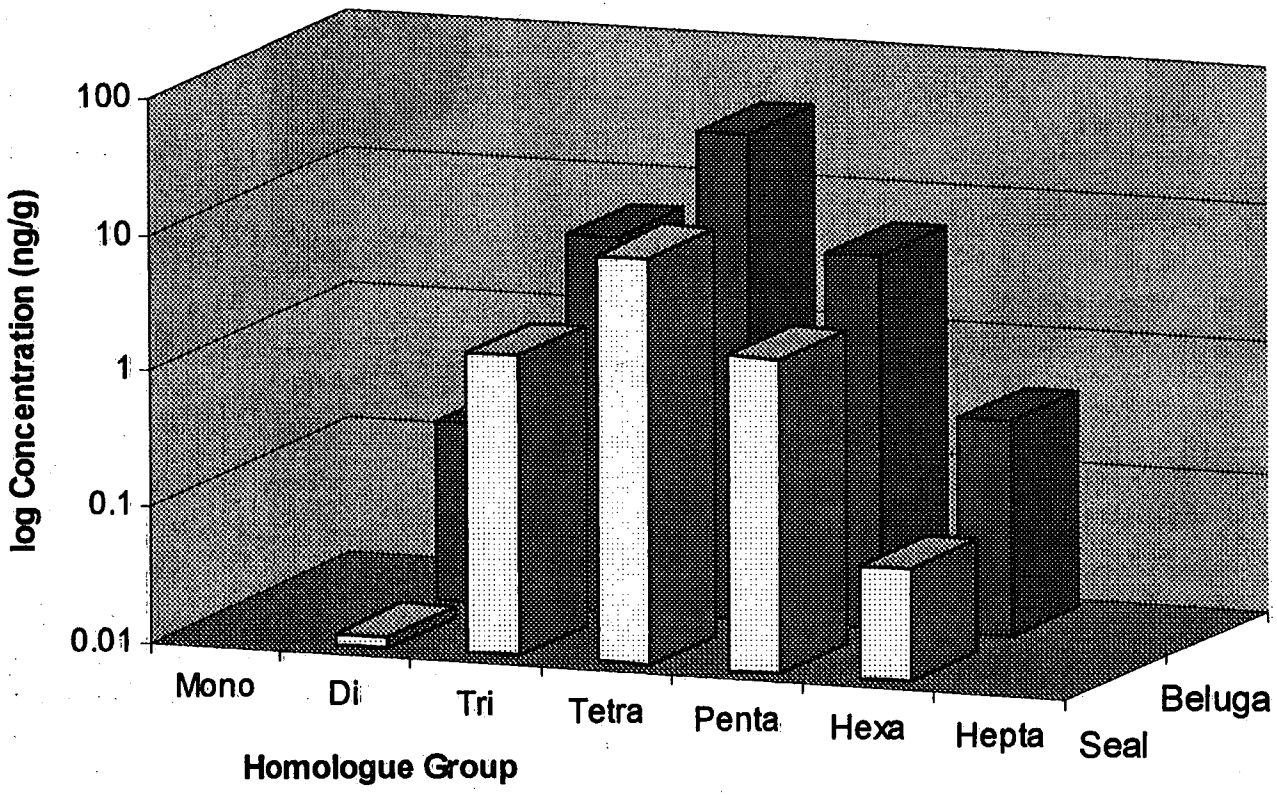
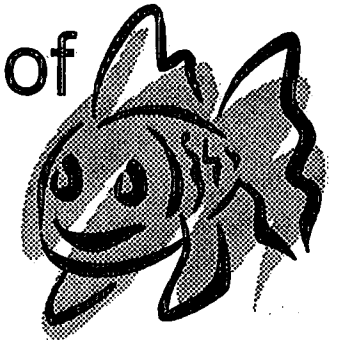


Figure 2: Concentration of PBDEs (ng/g lipid) in Marine Mammals



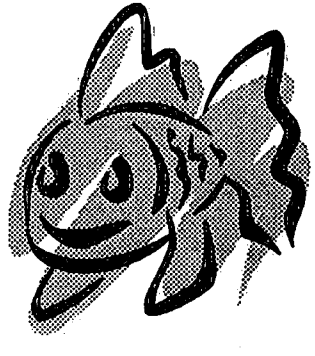
Summary

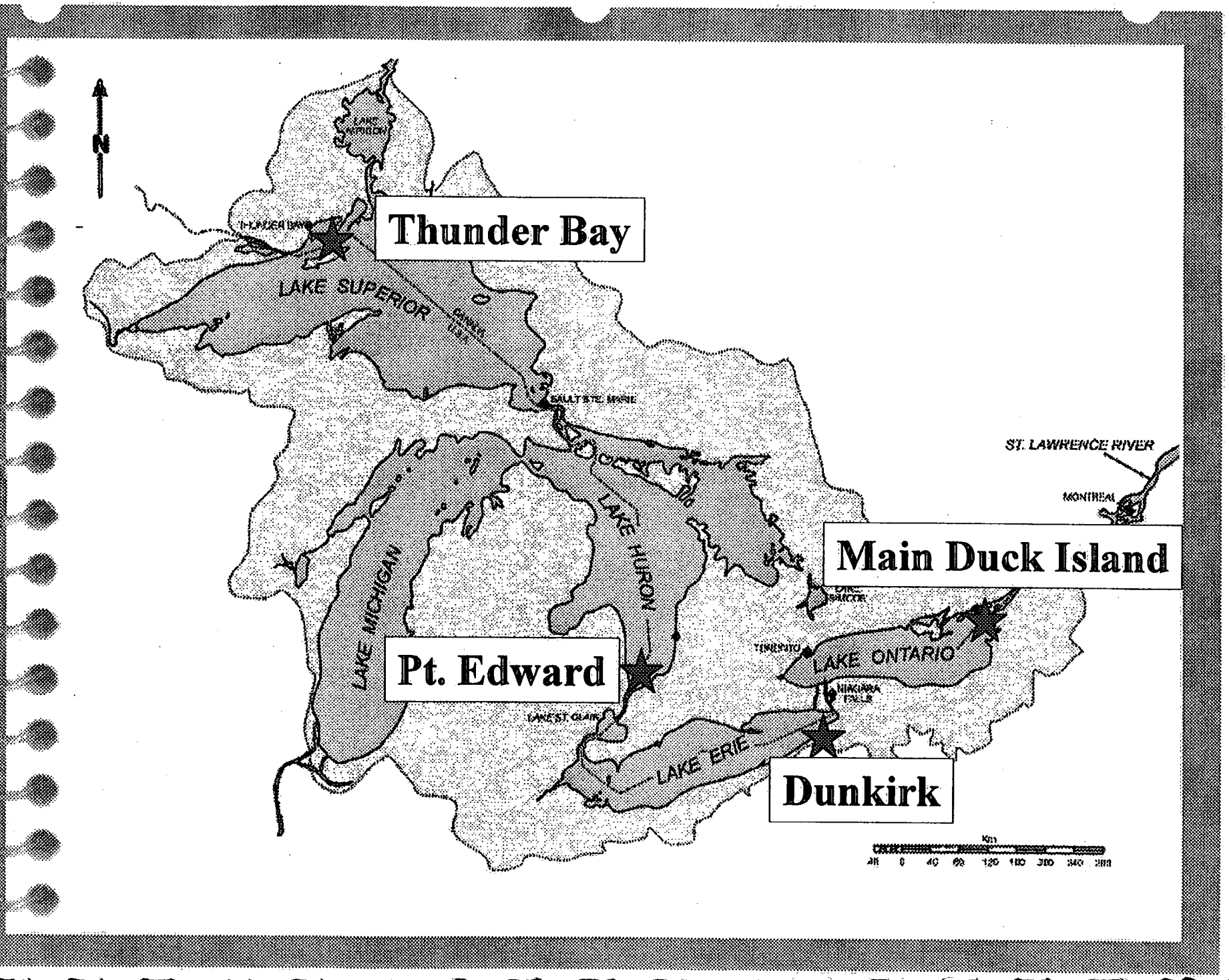
- ✓ concentrations in Lake Ontario were the highest at 545ng/g followed by Lake Huron with 237ng/g, and 135ng/g in Lake Superior
- ✓ female ringed seals had an average of 10ng/g, males 19.4ng/g
- ✓ female belugas had an average of 28.8ng/g, males 56.8ng/g



Future Directions

- ✓ determine spatial and temporal distribution of PBDEs in lake trout from the Great Lakes
- ✓ determine biomagnification of PBDEs in aquatic ecosystems
- ✓ ecotoxicological research



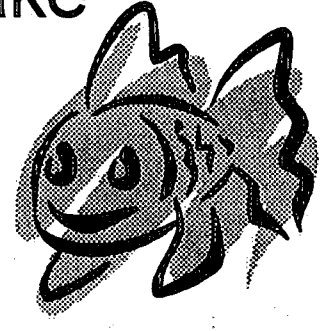

N
Thunder Bay
LAKE SUPERIOR
SAULT STE. MARIE
ST. LAWRENCE RIVER
MONTREAL
Main Duck Island
LAKE MICHIGAN
LAKE HURON
Pt. Edward
LAKE ONTARIO
LAKE ST. CLAIR
LAKE ERIE
Dunkirk
Km

Temporal

- ✓ Lake Ontario
- ✓ 1978, 1983, 1988, 1993, 1998
- ✓ age = 4+, n=12
- ✓ site of capture: Eastern Basin

Spatial

- ✓ Lake Ontario, Lake Huron, Lake Erie, Lake Superior
- ✓ 1997
- ✓ age = 6+, n = 12
- ✓ same site of capture for each lake



Acknowledgements

- ✓ Drs. M. Alaee, D. Muir, and K. Solomon
- ✓ D. Sergeant, B. Wilkinson, and M.Whittle
- ✓ staff from the Ultra-Trace Laboratory at CCIW

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POLYBROMINATED-DIPHENYL-ETHERS IN BIOTA SAMPLES FROM COASTAL BRITISH COLUMBIA.

Michael G. Ikonomou, Norman Crewe, Tim He, and Maike Fischer

Polybrominated diphenyl ethers (PBDEs) are used as additive flame retardants in polymeric materials such as polystyrenes. They are added to plastics, paints, textiles, machines and electronic devices and many thousand tons are produced annually. The penta-, octa- and decabromo substituted diphenyl ethers are the major components. These additives are only dissolved within the material and, therefore, can leach out into the environment. Bioaccumulation of PBDEs is possible since they are highly lipophilic and resistant to degradation. They have been shown to be more persistant than polychorinated biphenyls (PCBs) during *in vitro* biotransformation tests with hepatic microsomes. The efficiency of PBDE uptake is thought to decrease with the degree of bromination due to an increased difficulty to pass through the membrane. Since the structure of these ethers resemble that of PCBs and dioxins, they may display similar toxicity. Although, in contrast to PCBs, the mono-ortho chloro-substituted PCDE are the most immunotoxic congeners. PCDEs have been shown to effect, for example, the mixed-function oxidase enzymes and ultra structure of rat and trout liver. Differing only by the type of halogen, PBDEs are also suspected to be toxic. PBDEs have been found in samples from remote areas which suggests a world-wide distribution. Therefore, more data on the levels and effects of PBDE contamination are required.

Over the past few years our interests have been: a) to develop comprehensive ultra-trace analytical methods for the determination of PBDEs in environmental samples; and b) to monitor the environmental levels and congener distribution of PBDEs in the aquatic environment. The limited number of authentic standards available (natives and surrogates), the lack of certified and/or standard reference materials, and the difficulties associated with the GC/MS analysis of these compounds makes quantitative determination of these compounds challenging. Some of the difficulties associated with the GC/MS analysis are: a) high boiling point of the highly Br substituted congeners; b) limited information on their elution order; c) large variation in instrument response for congeners from the same homologue group and d) long term stability of instrument response since the fragmentation characteristics of these compounds depends on a number of hard to control experimental parameters (condition of the GC injector, oxygen in the ion source and ion source conditioning). Calculating totals of homologue series is also difficult since there is limited information on the elution order of PBDEs and the fragmentation patterns of all congeners. In this presentation we will discuss the measures taken to deal with the instrumental analysis difficulties outlined above and the type of samples and procedures used to assess the accuracy and precision of the overall analytical method.

In this presentation we will also discuss the results obtained from the analysis of a number of representative samples collected from the coastal waters of British Columbia. The samples examined were tissue such as Dungeness crab hepatopancreas, muscle and liver of sturgeon and blubber of porpoises, seals and killerwhales. Pacific Sockeye salmon, Pacific herring and lake trout CRMs were also analyzed. In all samples examined no mono-BDE were detected and the di-, tri-, hexa- and hepta-BDE were ca. <10% of the total PBDE measured. The relative contributions of the individual PBDEs measured were similar for all samples examined with 2,2',4,4'-TeBDE and 2,2',4,4',5-PeBDE being the most abundant.

In all our data the following trends were observed among the congeners measured: 2,2',4,4'-TeBDE followed by 2,2',4,4',5-PeBDE were present at significantly higher levels (ca. 100 fold) than 2,3',4,4'-TeBDE and 2,3',4,4',6-PeBDE, and (ca. 1000 fold) than 2,3',4',6-TeBDE, 2,4,4',6-TeBDE and 3,3',4,4'-TeBDE. The previous studies generally observed more 2,2',4,4'-TeBDE than 2,2',4,4',5-PeBDE which is consistent with our findings. The congener 2,2',3,4,4'-

PeBDE was not detected in all samples, except in the blubber of the porpoise from Tsawassan. Generally, higher concentrations of the 4 major congeners were found in the blubber samples than in the tissue samples. The Dungeness crab showed higher levels of 2,3',4',6-TeBDE and 2,4,4',6-TeBDE than the blubber samples. Except for the trout CRM, the fish samples (sturgeon, salmon and herring) contained very low amounts of TeBDE and PeBDE. Similarly, low levels were detected in tissues (liver, red and white muscle) of other sturgeon species captured in the Fraser river. The differences in PBDE levels among the biota sampled suggest that uptake and excretion of PBDE vary among species.

The congener distribution observed in biota samples is substantially different from what is observed in commercial products that are known to contain PBDEs. Also, as discussed above, similar congener patterns are detected among similar biota samples, i.e. marine samples collected in the West Coast of Canada versus European samples.

Polybrominated-diphenyl-ethers in Biota Samples from Coastal British Columbia

Michael G. Ikonomou, Norman Crewe, Tim He and Maike Fischer

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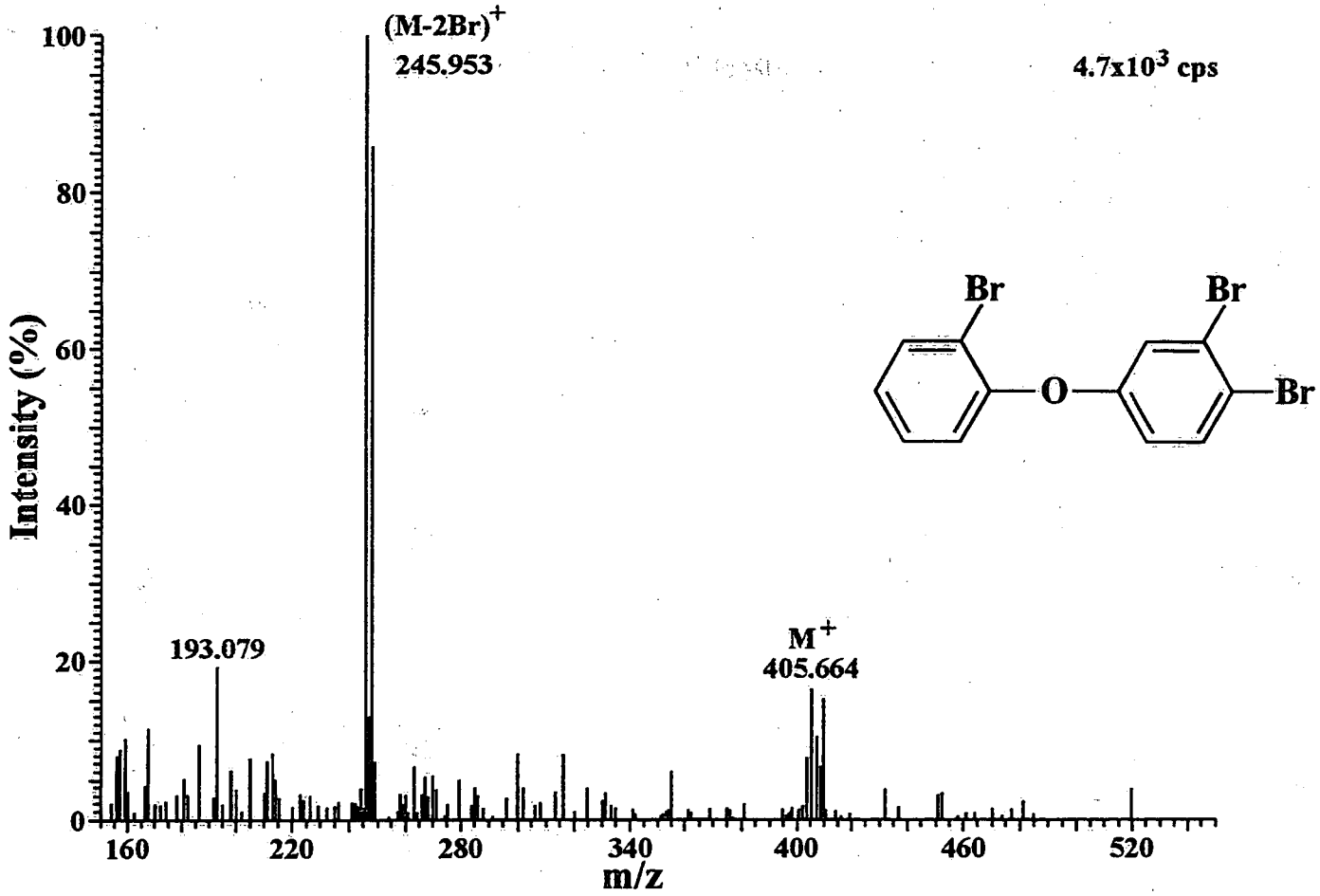
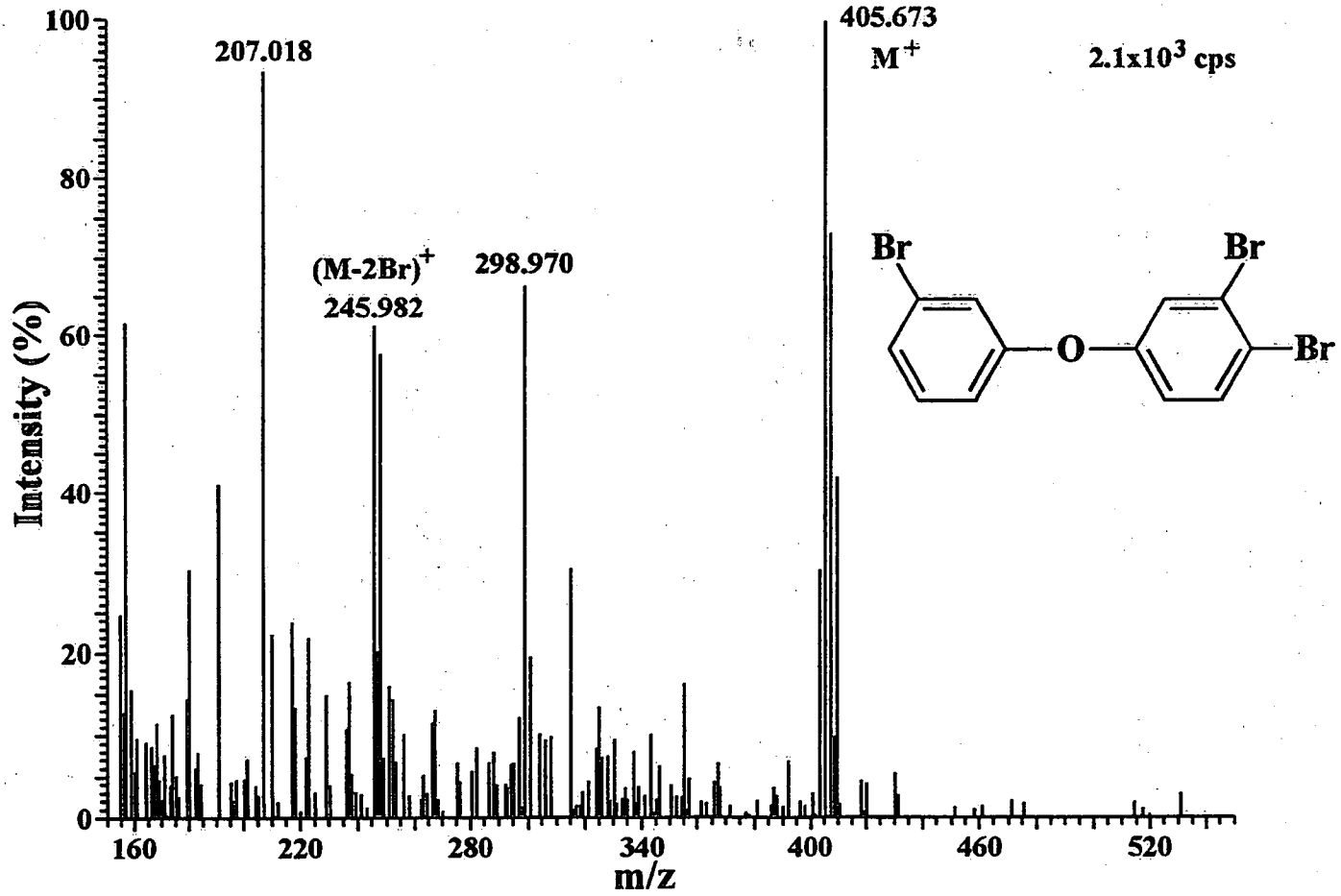
Sidney, BC, Canada, V8L 4B2

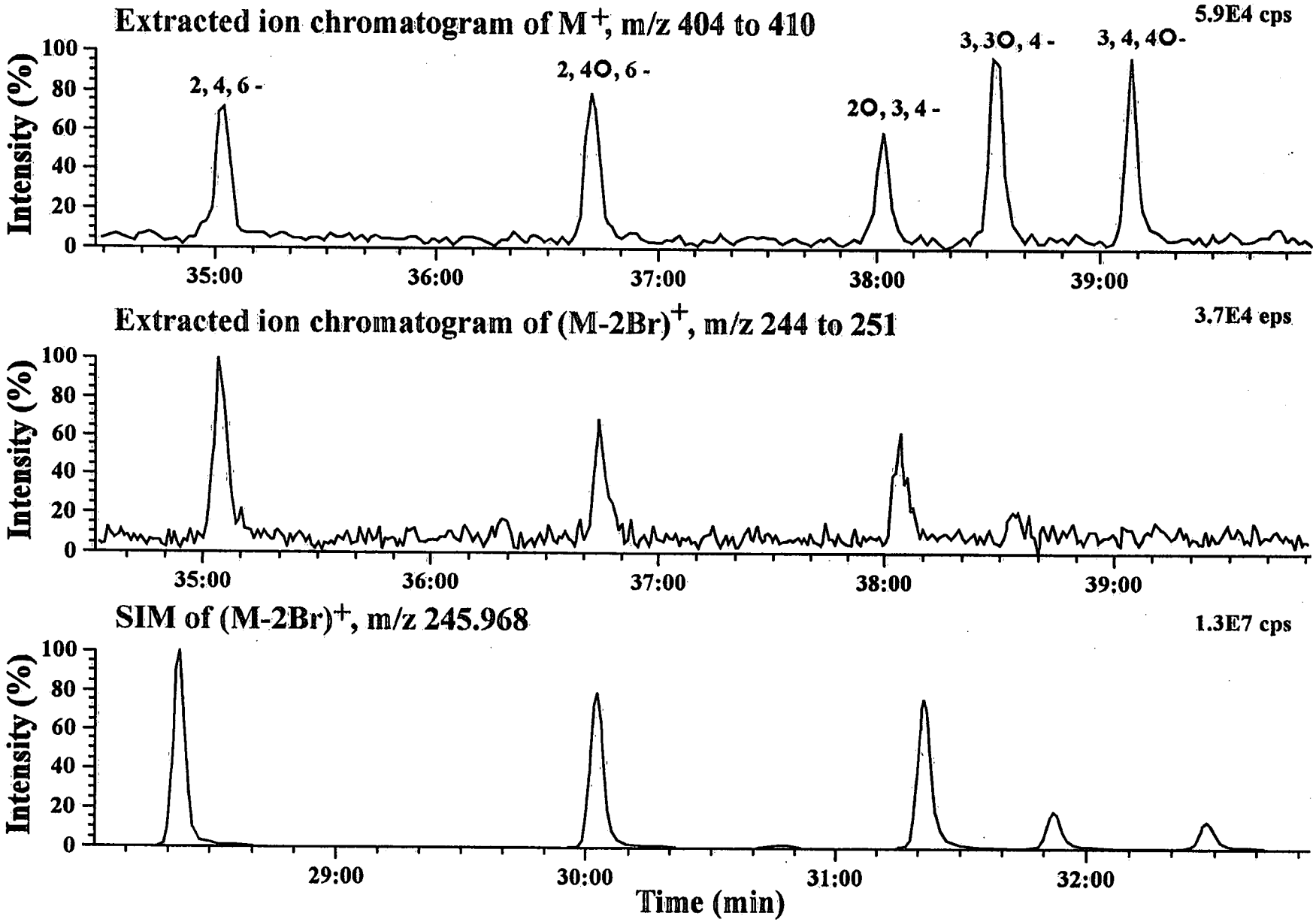
Bromodiphenylethers, GC/HRMS Parameters

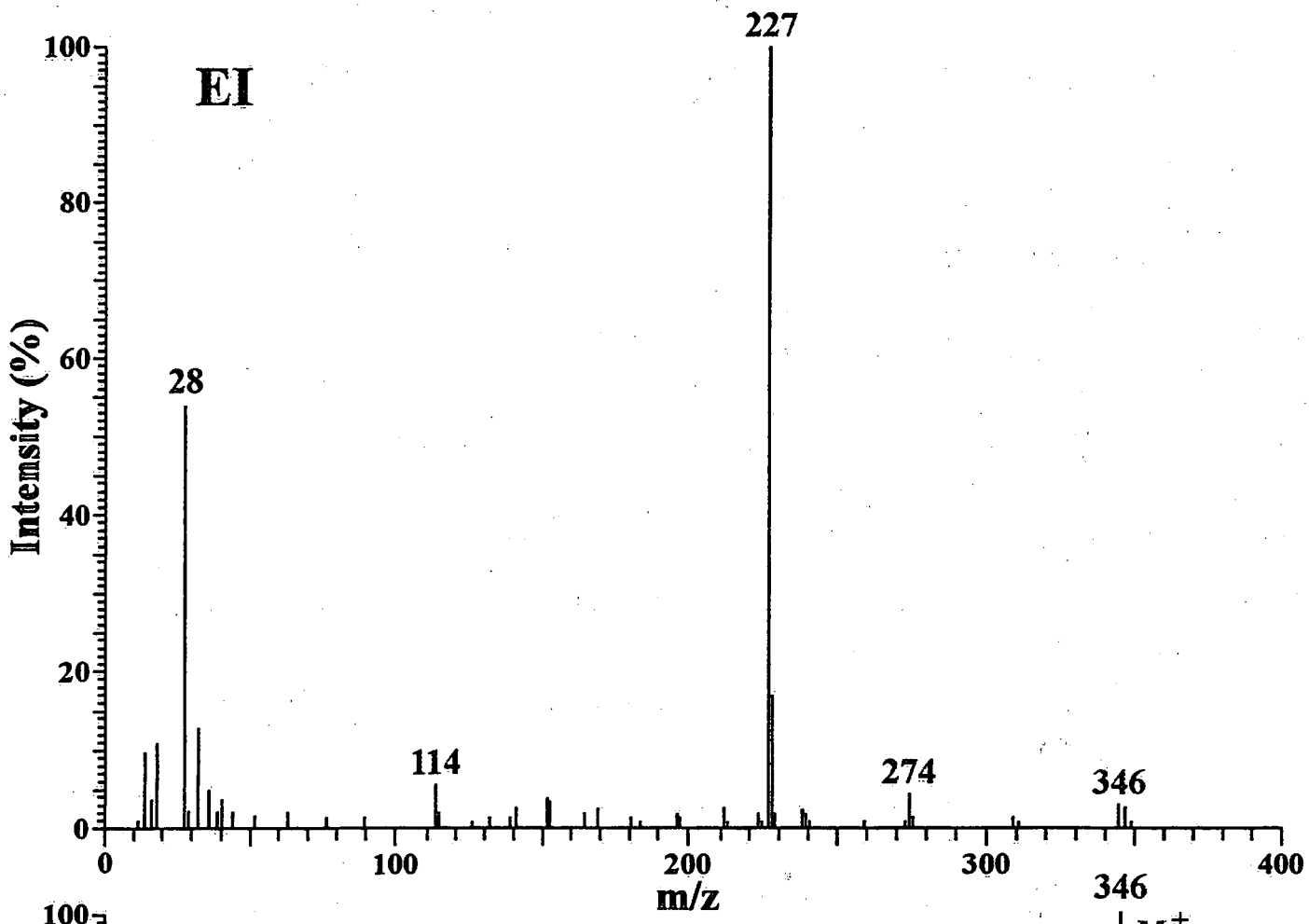
Congeners		RT min:sec	Concentration pg/uL	m/z monitored	ion of m/z	RRF
13C-3,3',4,4'-TeBDPE(77)	RS	38:55	100	496/498	M^+	
13C-2,3,3',4,4',5-HxCDPE(156)	IS	36:40	200	388/390	M^+	1.09
2-MBDPE(1)		17:07	100	248/250	M^+	1.32
3-MBDPE(2)		17:30	100	248/250	M^+	1.93
2,6-DiBDPE(10)		22:43	100	328/330	M^+	0.59
2,4-DiBDPE(7)		24:12	100	328/330	M^+	0.50
2,4'-DiBDPE(8)		24:55	100	328/330	M^+	0.55
3,4-DiBDPE(12)		25:17	100	328/330	M^+	0.84
3,4'-DiBDPE(13)		25:21	100	328/330	M^+	0.86
4,4'-DiBDPE(15)		25:53	100	328/330	M^+	0.92
,4,6-TrBDPE(30)		28:38	100	246/248	$(M-2X)^+$	2.51
2,4',6-TrBDPE(32)		30:18	100	246/248	$(M-2X)^+$	2.20
2',3,4-TrBDPE(33)		31:37	100	246/248	$(M-2X)^+$	2.24
3,3',4-TrBDPE(35)		32:07	100	246/248	$(M-2X)^+$	0.60
3,4,4'-TrBDPE(37)		32:43	100	246/248	$(M-2X)^+$	0.45
13C-2,2',3,3',4,4',5-HpCDPE(170)	IS	40:26	200	422/424	M^+	0.42
2,4,4',6-TeBDPE(75)		35:45	100	484/486	M^+	1.01
2,3',4',6-TeBDPE(71)		36:24	100	484/486	M^+	0.82
2,2',4,4'-TeBDPE(47)		37:04	100	484/486	M^+	1.35
2,3',4,4'-TeBDPE(66)		37:47	100	484/486	M^+	0.77
3,3',4,4'-TeBDPE(77)		38:56	100	484/486	M^+	1.55
13C-2,2',3,3',4,4',5,5'-OcCDPE(194)	IS	42:35	300	456/458	M^+	0.37
2,3',4,4',6-PeBDPE(119)		41:21	150	404/406	$(M-2X)^+$	4.29
2,2',4,4'5-PeBDPE(99)		42:05	150	404/406	$(M-2X)^+$	3.37
2,2',3,4,4'-PeBDPE(85)		44:02	150	404/406	$(M-2X)^+$	1.99
,2',4,4',5,5'-HxBDPE(153)		46:35	200	482/484	$(M-2X)^+$	1.54
2,3,3',4,4',5,5'-BDPE(189)		53:24	250	562/564	$(M-2X)^+$	0.68

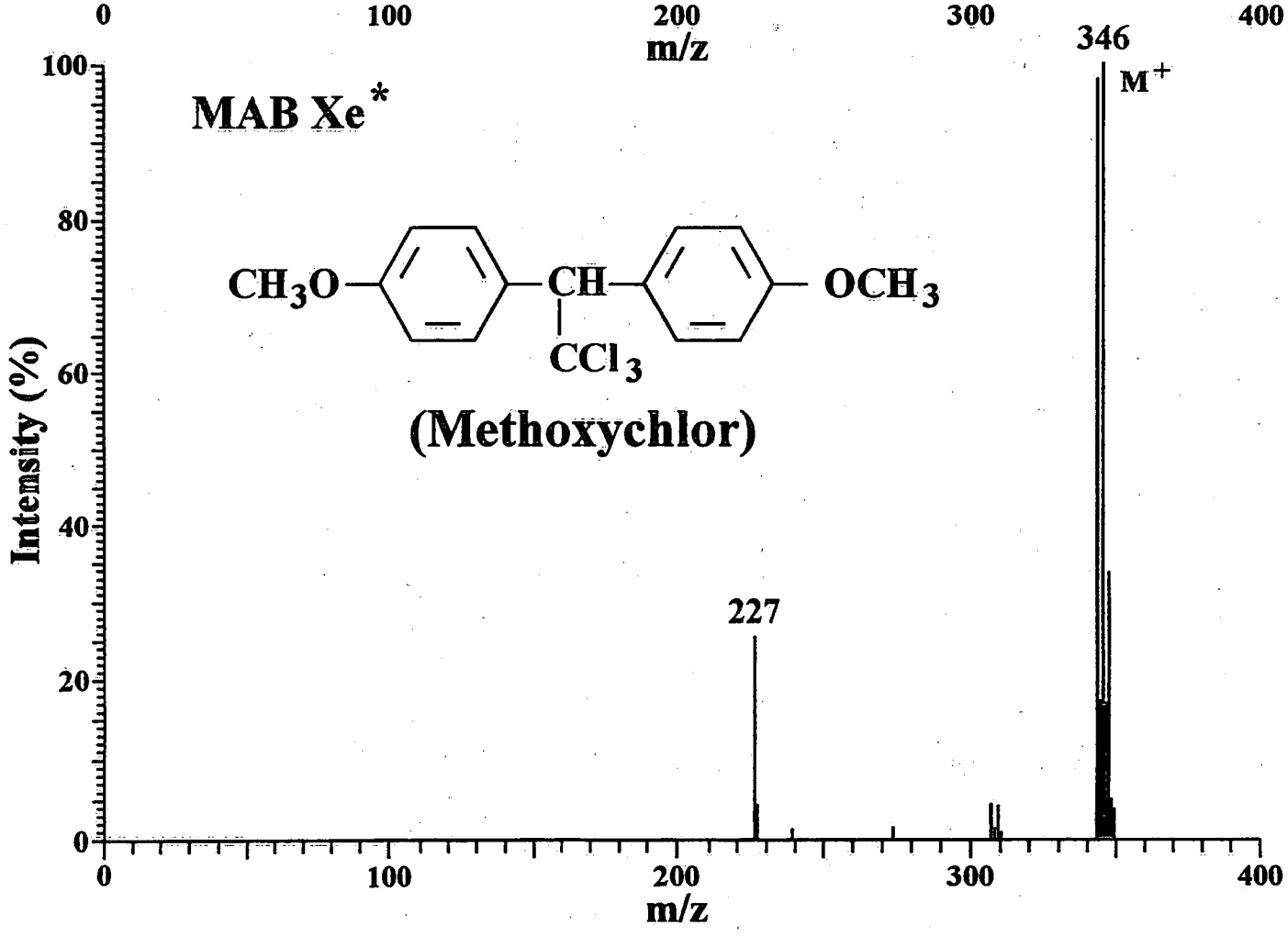

(M-2Br)+
245.953
4.7x10^3 cps
Br
Br
O
Br
193.079
M+
405.664
Intensity (%)
100
80
60
40
20
0
160
220
280
340
400
460
520
m/z


405.673
M+
2.1x10^3 cps
207.018
(M-2Br)+
245.982
298.970
Br
Br
O
Br
Intensity (%)
100
80
60
40
20
0
160
220
280
340
400
460
520
m/z

TrBDEs in Calibration Standard, +ve EI Experiments

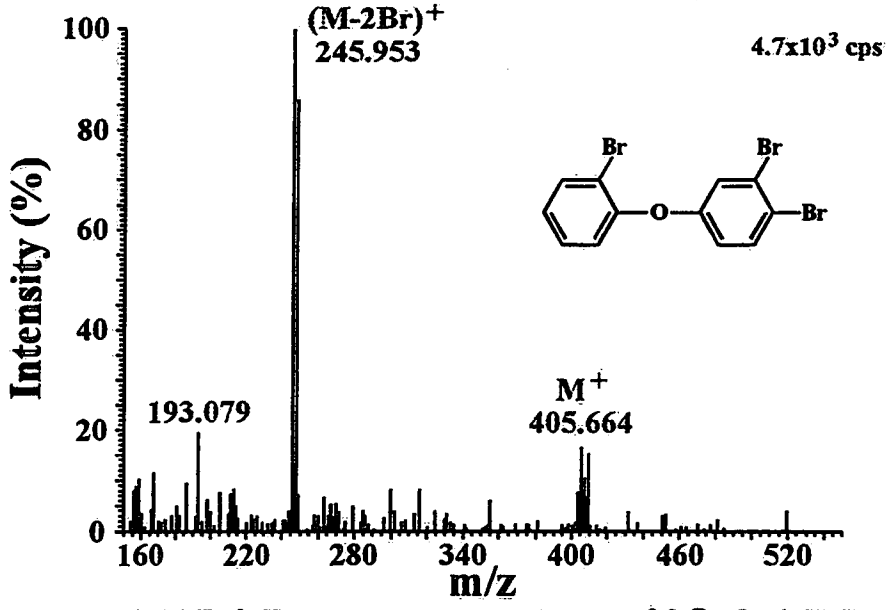




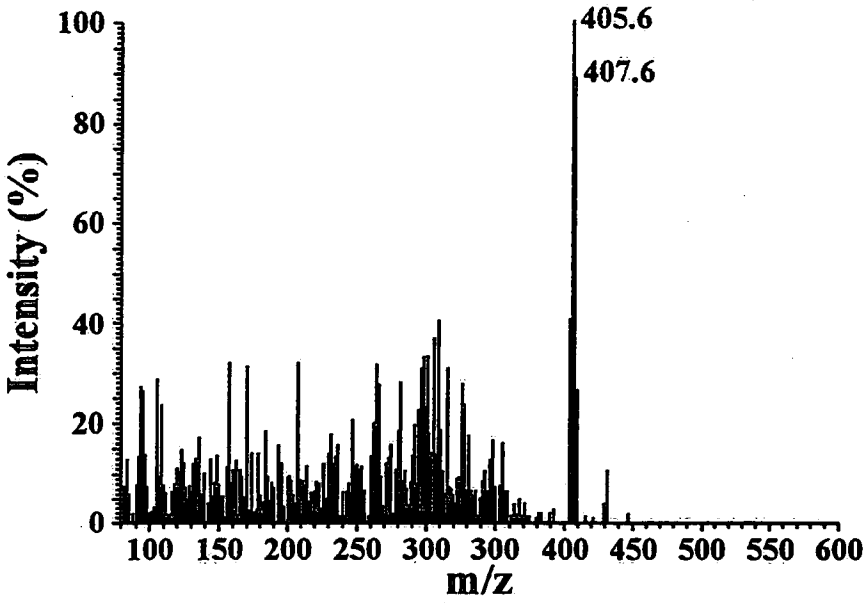


(Methoxychlor)

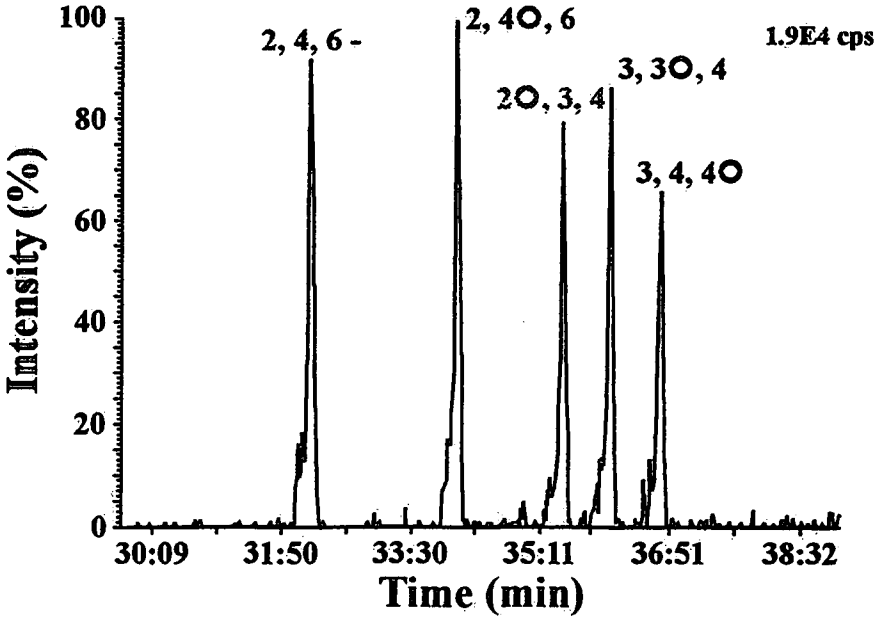
+ve EI full scan mass spectrum of 2O, 3, 4-TrBDE



+ve MAB full scan mass spectrum of 2O, 3, 4-TrBDE



Extracted ion chromatogram of M^+, m/z 404 to 410, +ve MAB experiments



Calculation of PCDE 1/2 rrts

(Nevalainen et al. Environ. Sci. Technol. 1994, 28, 1341-1347)

1. Synthesize 54 PCDE congeners and determine experimental rrts

2. Use the 54 experimental rrts to calculate 1/2 rrts using multiple linear regression:

$$y = m_1 x_1 + m_2 x_2 + m_3 x_3 + ... + m_{20} x_{20} + b$$

where:

y = experimental relative retention time for a given congener

b = constant -- set to zero

x_1, x_2, x_3 ... = substitution variables with values of 0, 1 or 2 depending on whether a particular substitution pattern doesn't occur, occurs once or occurs twice in a given congener

m_1, m_2, m_3 ... = 1/2 RRT coefficients for each phenyl substitution pattern

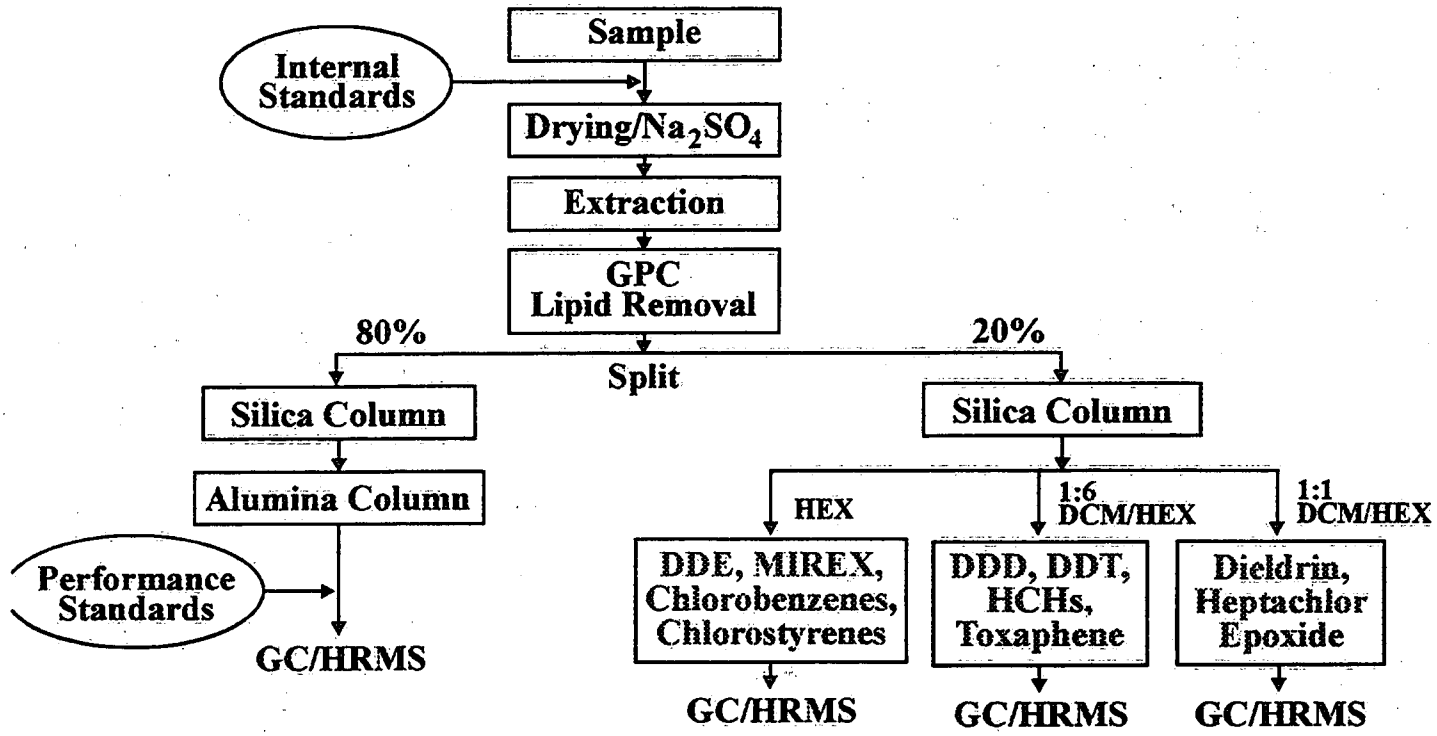
3. Use sum of two 1/2 rrts (out of 20 possible) to estimate the rrt of an unknown congener

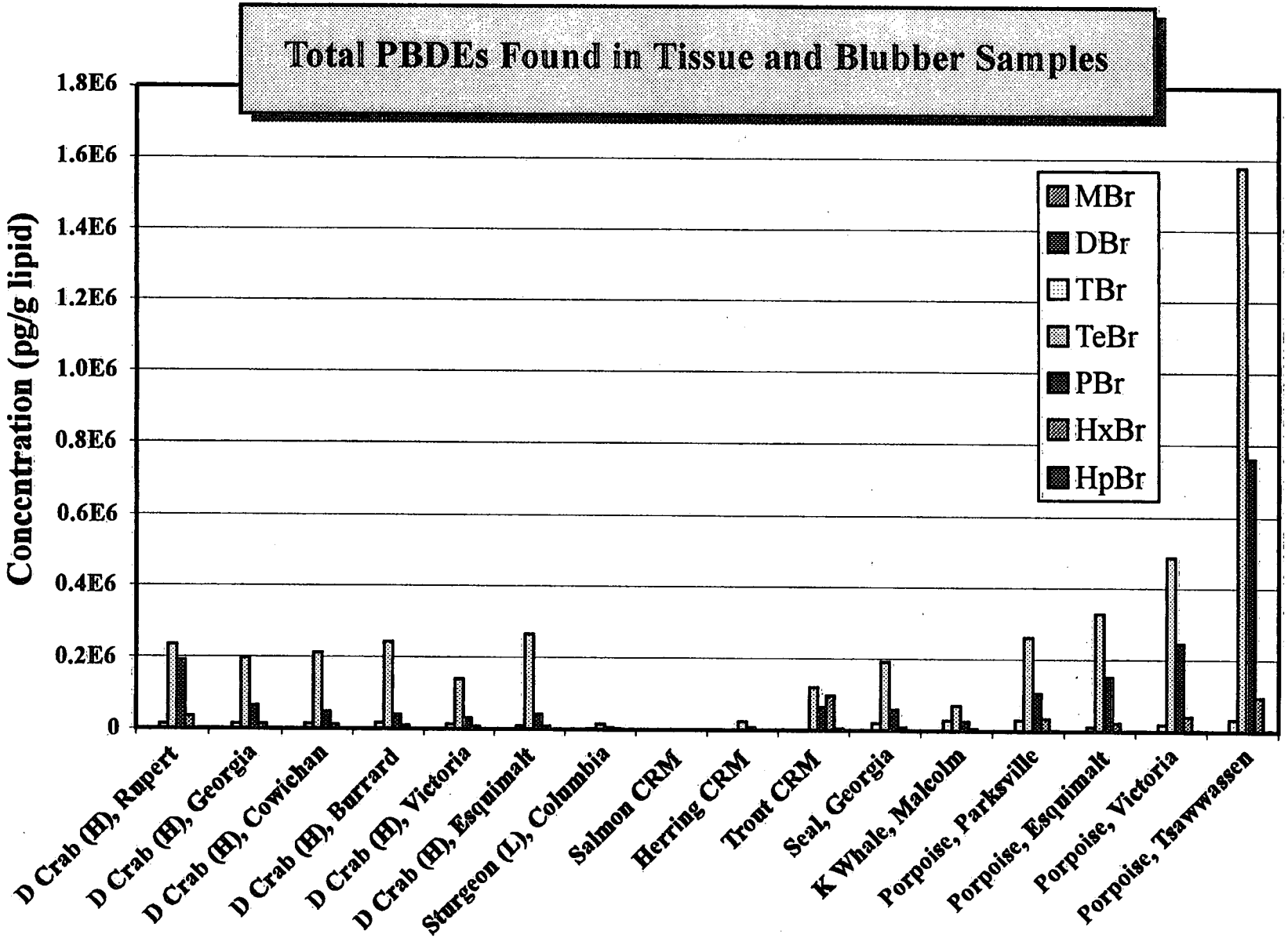
Elution Order of PBDEs, Experimental vs Predicted from 1/2 rrts of PCDEs

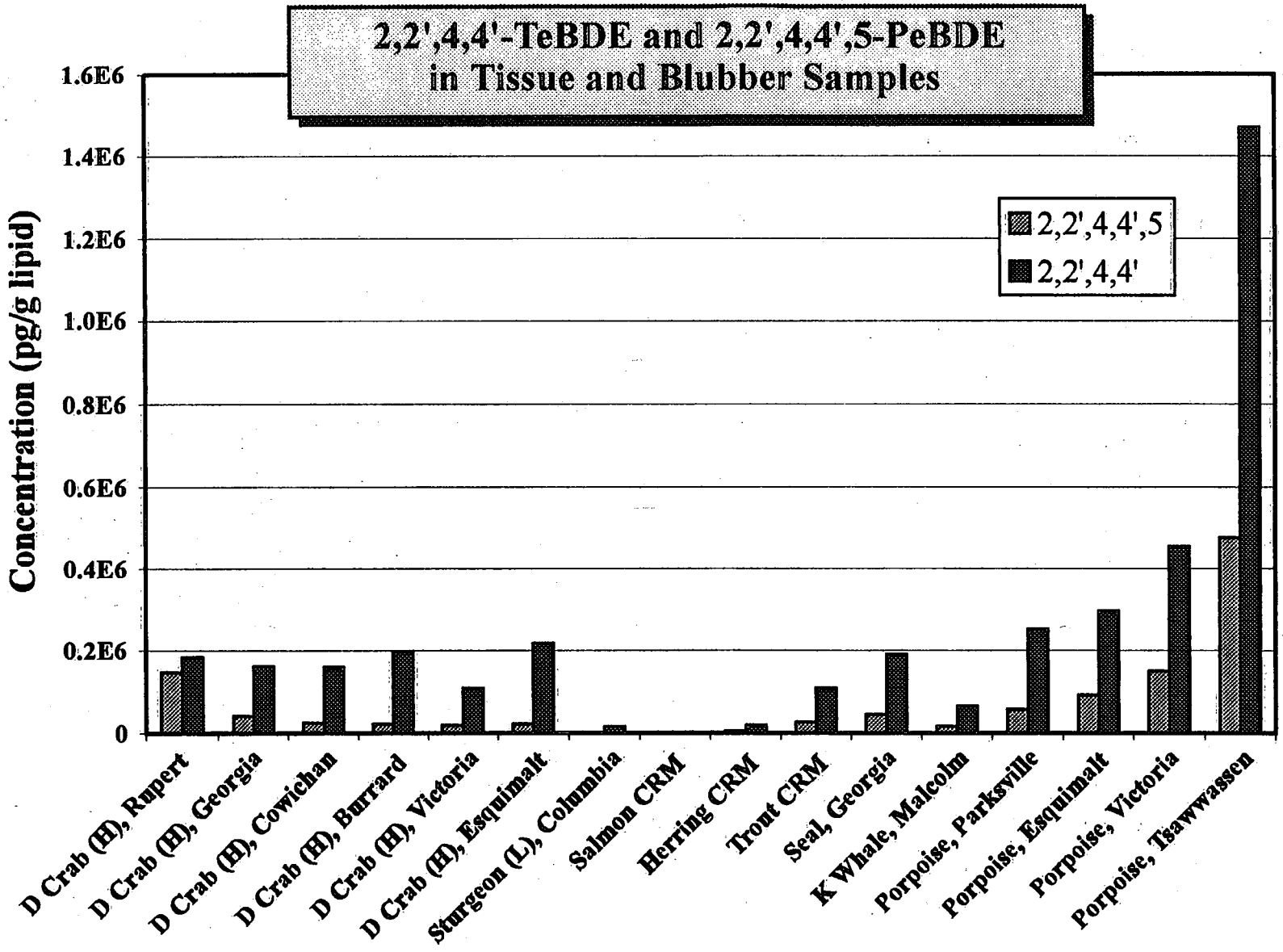
PBDE Congener	Expt. rrt			Based on 1/2 rrts		Based on IOS Experimental rrts	
	1/2 rrt*	IOS**	exptl rts	pred rts	pred-explt (s)	pred rts	pred-explt (s)
1	0.223	0.496	17:07	17:56	49	17:09	2
2	0.229		17:30	18:06	36		
10	0.374		22:43	22:17	-26		
7	0.417	0.644	24:12	23:31	-41	23:41	-31
8	0.512	0.672	24:55	26:15	80	24:55	0
12	0.453		25:17	24:33	-44		
13	0.518	0.676	25:21	26:26	65	25:05	-16
15	0.542	0.694	25:53	27:07	74	25:53	0
30	0.507		28:38	26:07	-151		
32	0.663	0.790	30:18	30:37	19	30:07	-11
33	0.712	0.827	31:37	32:01	24	31:45	8
35	0.718	0.875	32:07	32:12	5	33:53	106
37	0.742	0.856	32:43	32:53	10	33:02	19
75	0.796	0.892	35:45	34:27	-78	34:38	-67
71	0.863	0.933	36:24	36:23	-1	36:26	2
47	0.870	0.940	37:04	36:35	-29	36:45	-19
66	0.906	0.961	37:47	37:37	-10	37:40	-7
77	0.942	0.001	38:56	38:56	0	38:56	0
119	0.996		41:21	38:56	-145		
99	1.033	1.054	42:05	41:17	-48	41:47	-18
85	1.152	1.112	44:02	44:42	40	44:21	19
153	1.196	1.157	46:35	45:59	-36	46:20	-15
189	1.539		53:24	55:52	148		

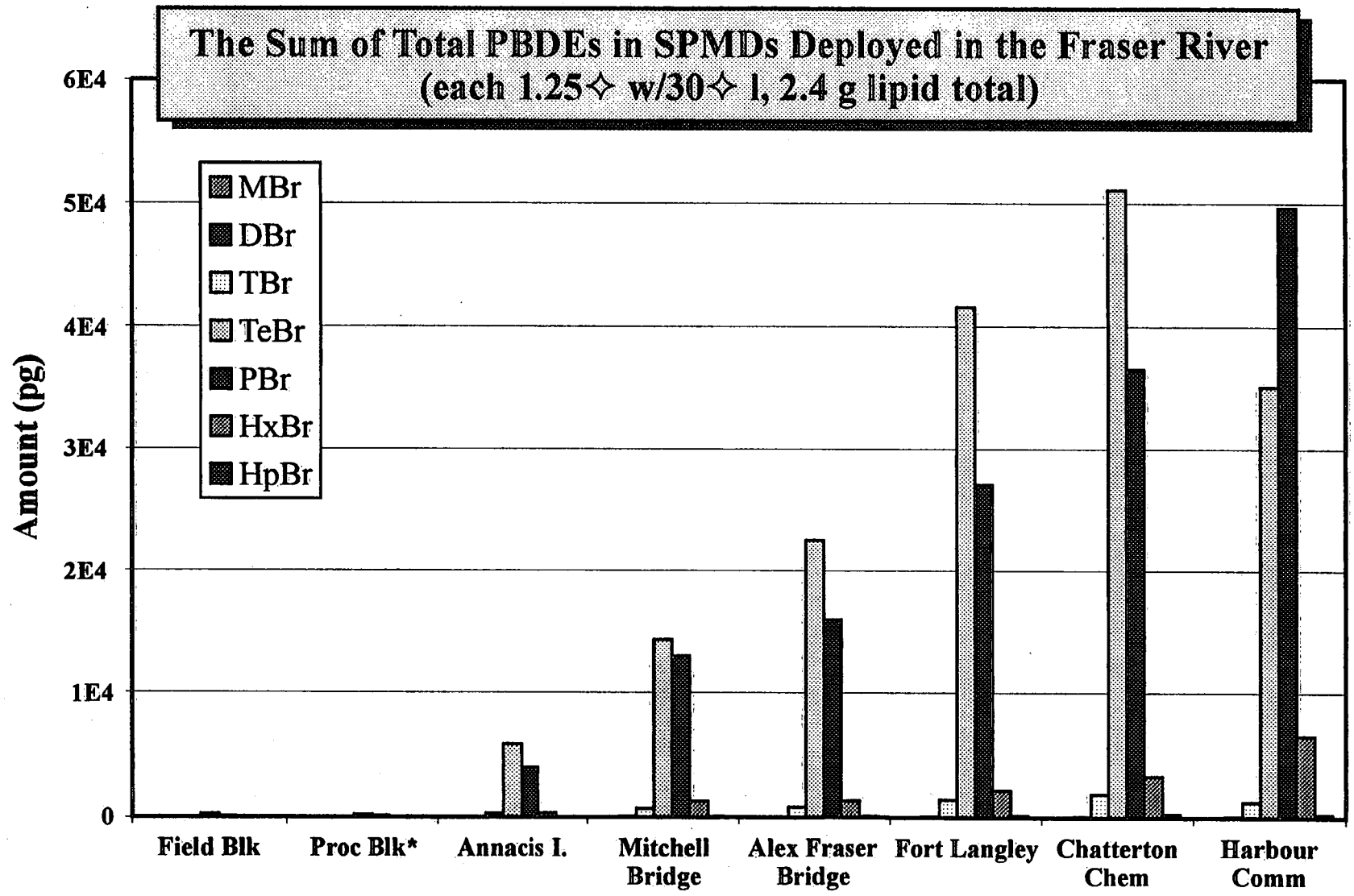
**from Nevalainen et al. Environ. Sci. Technol. 1994, 28, 1341-1347 **from IOS standards RRT vs PCDE 77*

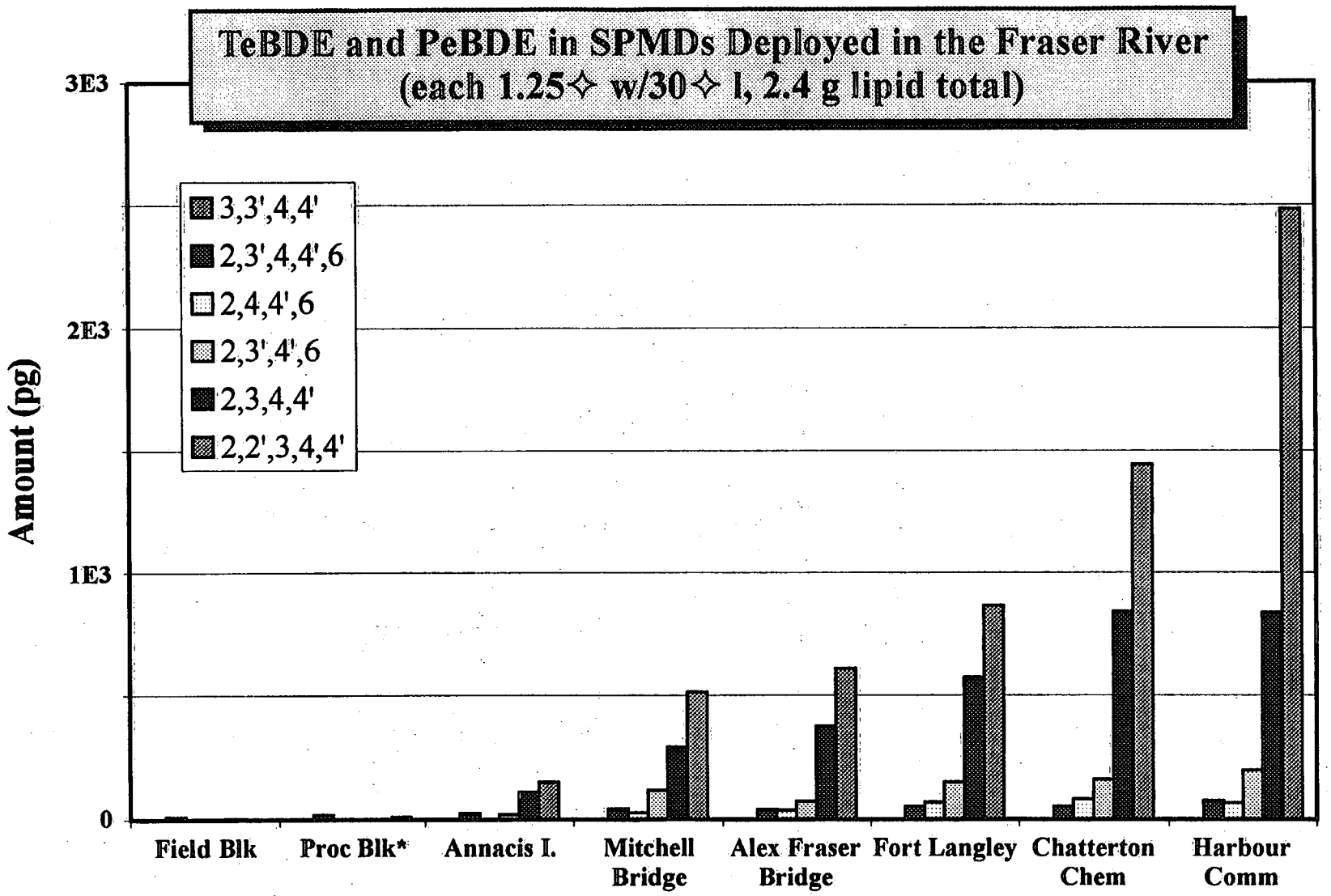
Multiresidue Analysis



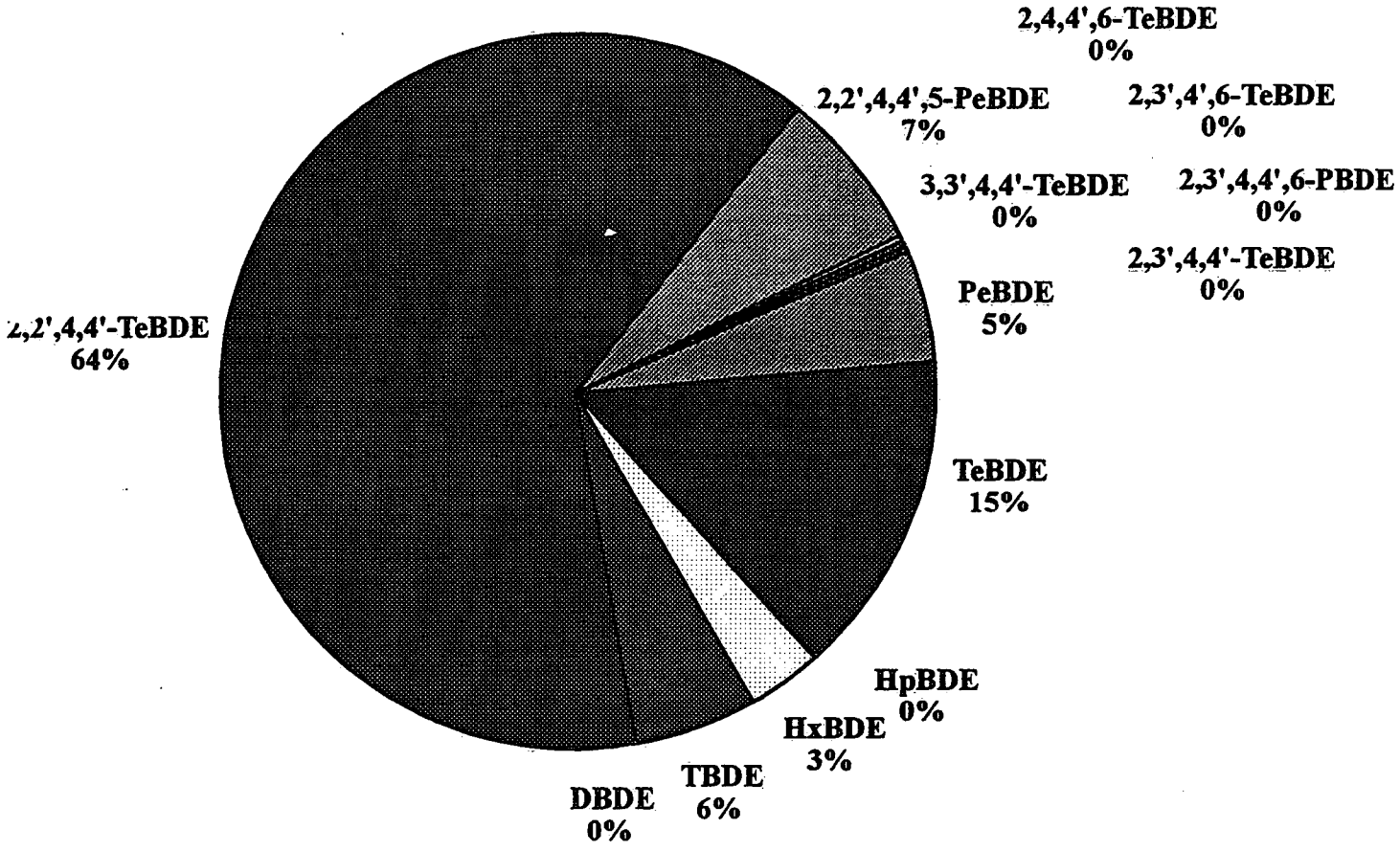

Total PBDEs Found in Tissue and Blubber Samples
Concentration (pg/g lipid)
1.8E6
1.6E6
1.4E6
1.2E6
1.0E6
0.8E6
0.6E6
0.4E6
0.2E6
0
MBr
DBr
TBr
TeBr
PBr
HxBr
HpBr
D Crab (H), Rupert
D Crab (H), Georgia
D Crab (H), Cowichan
D Crab (H), Burrard
D Crab (H), Victoria
D Crab (H), Esquimalt
Sturgeon (L), Columbia
Salmon CRM
Herring CRM
Trout CRM
Seal, Georgia
K Whale, Malcolm
Porpoise, Parksville
Porpoise, Esquimalt
Porpoise, Victoria
Porpoise, Tsawwassen


2,2',4,4'-TeBDE and 2,2',4,4',5-PeBDE
in Tissue and Blubber Samples
Concentration (pg/g lipid)
1.6E6
1.4E6
1.2E6
1.0E6
0.8E6
0.6E6
0.4E6
0.2E6
0
2,2',4,4',5
2,2',4,4'
D Crab (H), Rupert
D Crab (H), Georgia
D Crab (H), Cowichan
D Crab (H), Burrard
D Crab (H), Victoria
D Crab (H), Esquimalt
Sturgeon (L), Columbia
Salmon CRM
Herring CRM
Trout CRM
Seal, Georgia
K Whale, Malcolm
Porpoise, Parksville
Porpoise, Esquimalt
Porpoise, Victoria
Porpoise, Tsawwassen

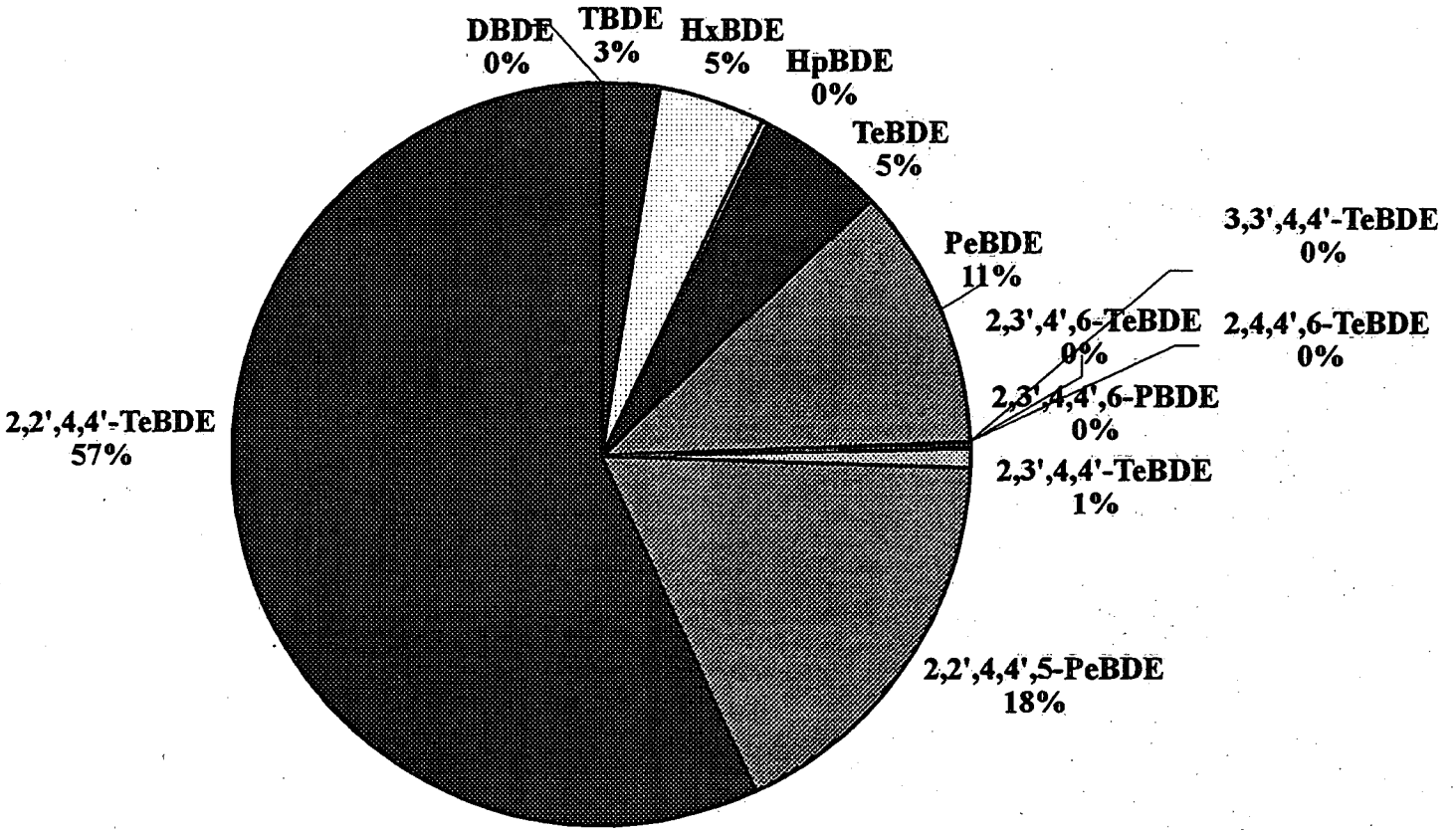

The Sum of Total PBDEs in SPMDs Deployed in the Fraser River
(each 1.25✧ w/30✧ l, 2.4 g lipid total)
Amount (pg)
6E4
5E4
4E4
3E4
2E4
1E4
0
MBr
DBr
TBr
TeBr
PBr
HxBr
HpBr
Field Blk
Proc Blk*
Annacis I.
Mitchell Bridge
Alex Fraser Bridge
Fort Langley
Chatterton Chem
Harbour Comm


TeBDE and PeBDE in SPMDs Deployed in the Fraser River
(each 1.25✧ w/30✧ l, 2.4 g lipid total)
3E3
2E3
1E3
0
Amount (pg)
3,3',4,4'
2,3',4,4',6
2,4,4',6
2,3',4',6
2,3,4,4'
2,2',3,4,4'
Field Blk
Proc Blk*
Annacis I.
Mitchell Bridge
Alex Fraser Bridge
Fort Langley
Chatterton Chem
Harbour Comm

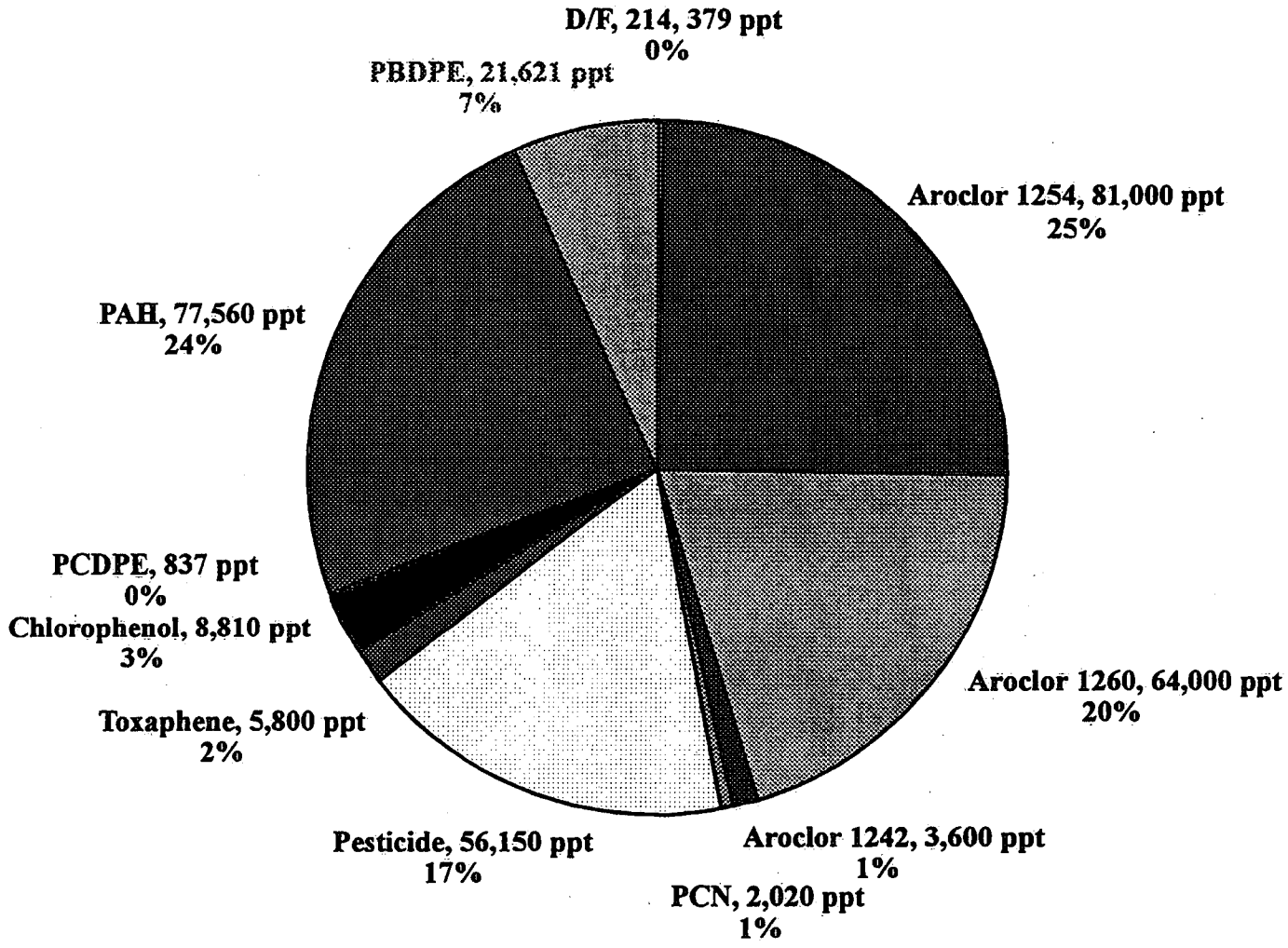
PBDEs in Dungeness Crab Hepatopancreas (Vancouver Harbour, Burrard)



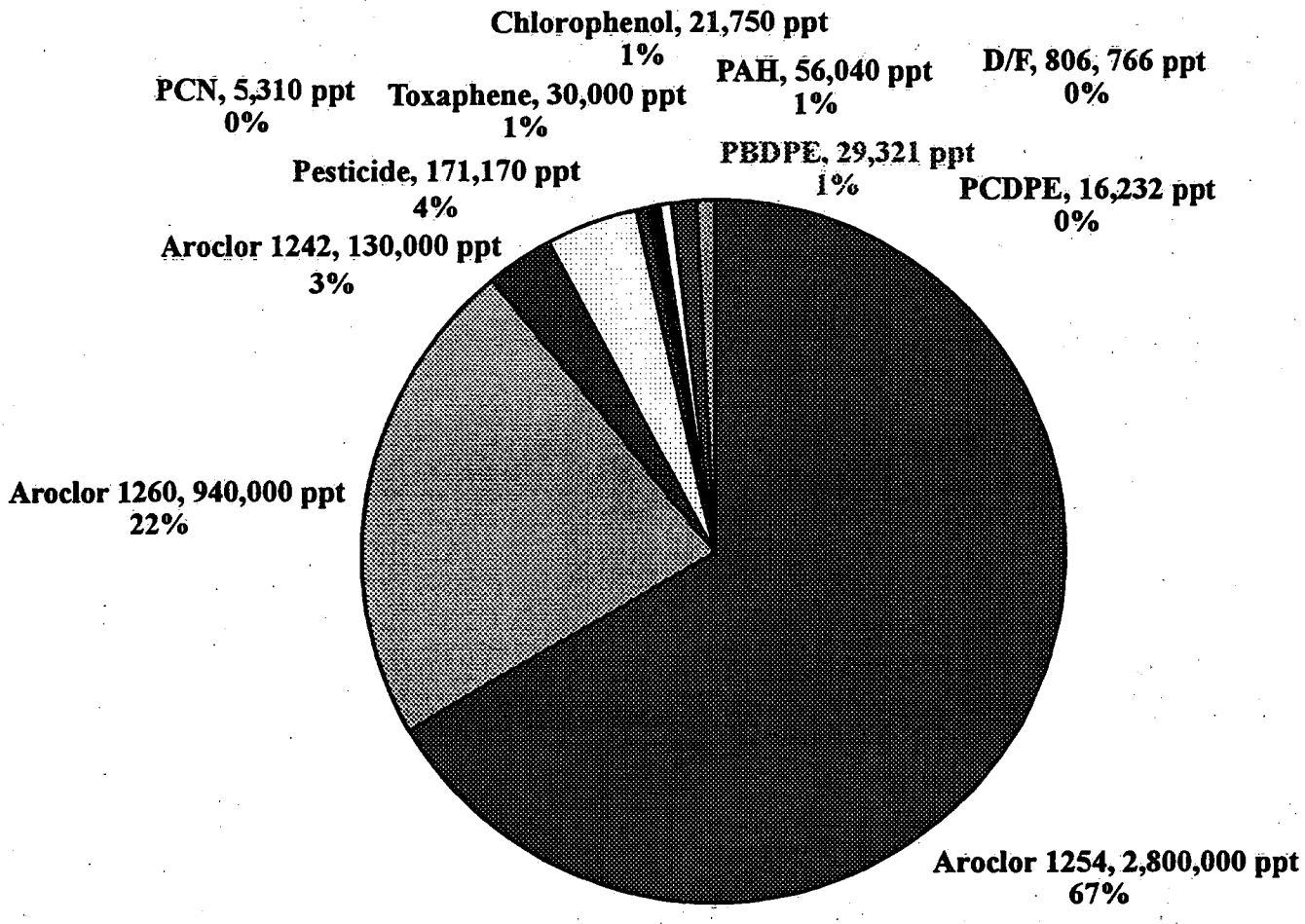
PBDEs in a Porpoise Blubber Sample (Esquimalt Harbour)



Contaminant Totals in Dungeness Crab from Cowichan Bay (pg/g wet)



Contaminant Totals in Dungeness Crab from Victoria Harbour (pg/g wet)



Improvements needed in PBDEs Analysis

- Unequivocal assignments of congeners in calibration solutions
- Consensus about the congener concentrations in calibration solutions, especially when there are variations in instrumental analysis techniques among the laboratories.
- Undefined Linearity and Dynamic range.
- Identification of major congeners not present in the calibration solutions. Are homologue totals important?
- Lacking SRMs and CRMs. IOS uses a spiked CRM (lake trout) as an in-house CRM.
- How accurate are the calibration standards?
- Identify potential interferences that can not be resolved at 10,000 RP. We can not always monitor molecular ions and characteristic fragments due to HRMS voltage scan limitations.

DISTRIBUTION OF PBDES IN BIOTA AND SEDIMENTS FROM THE ESTUARY AND GULF OF ST. LAWRENCE (PROPOSED STUDY)

Michel Lebeuf

The Estuary and Gulf of St. Lawrence are key habitats for marine biota in Canada as well as supporting several commercial fisheries. The Estuary-Gulf of St. Lawrence system is the principle outlet of the Great Lakes and receives the run-off of the St. Lawrence basin. As such, the Estuary-Gulf of St. Lawrence system is the recipient of considerable pollution from the Great Lakes-St. Lawrence basin, one of the most heavily urbanised and industrialised drainage basins in North America. The Lower St. Lawrence Estuary is a major zone downstream of Lake Ontario for sedimentation of suspended particles and associated contaminants. Significant quantities of both old and new organic contaminants have been detected in various compartments of this system.

The presentation will begin with an overview of our previous work on persistent organic pollutants (POPs) in the Estuary-Gulf of St. Lawrence, summarising the present and, where possible, the historic levels of POPs in this system. Based on this experience and knowledge, the proposed study of the distribution of PBDEs is designed with two areas of research - one focusing on the biota and the other on sediments.

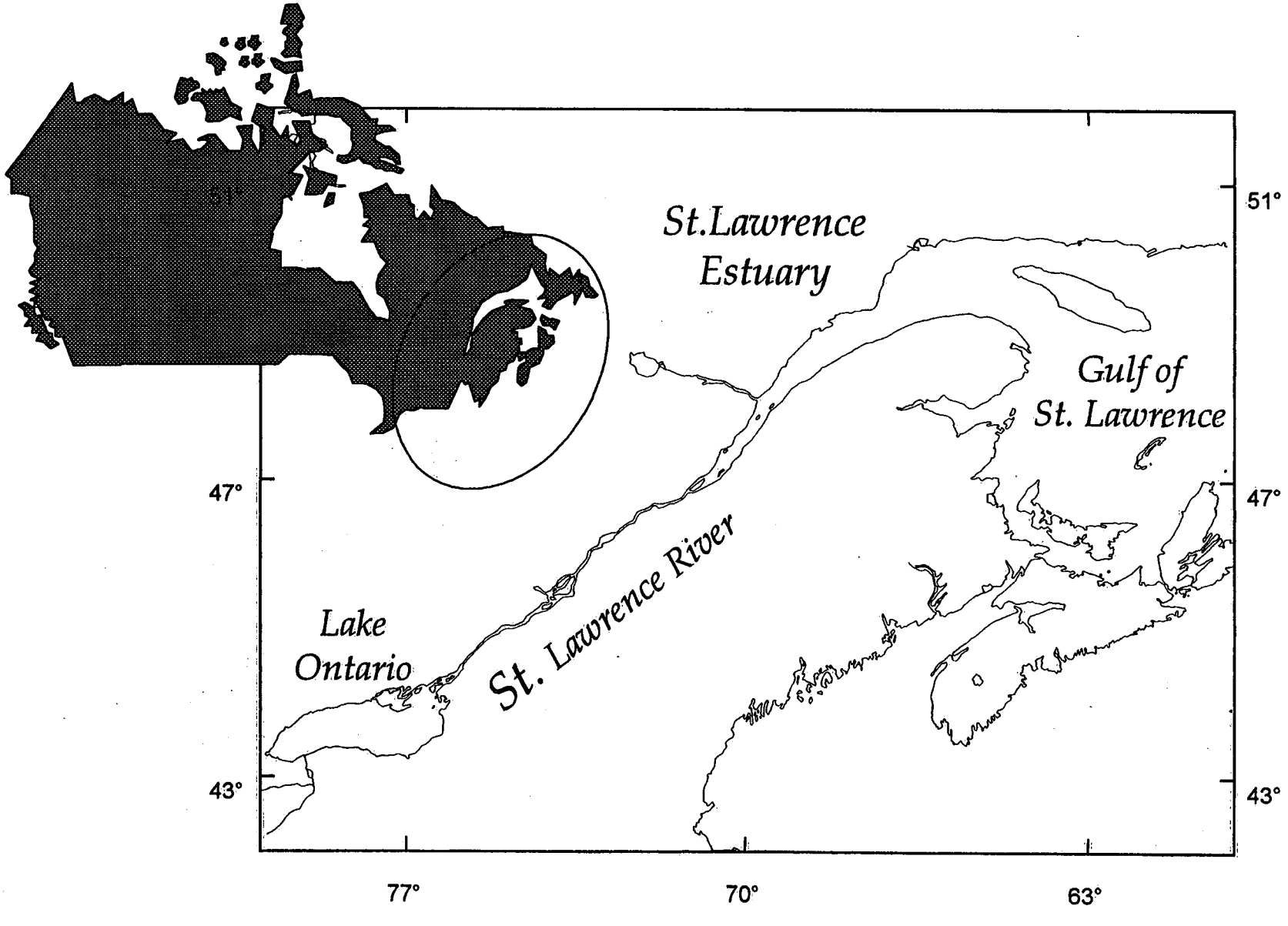
There have been previous studies reporting high levels of POPs in beluga whales, the largest marine mammal in the Estuary-Gulf of St. Lawrence. Thus it is particularly important to investigate the bioaccumulation of PBDEs in this system. The first aspect of this proposed study will focus on the bioaccumulation of PBDEs through a marine food web, which will include plankton, benthic invertebrates, pelagic and benthic fishes, and marine mammals. The second aspect of the research will examine the accumulation of PBDEs in marine sediments. We have successfully used sedimentary records of contaminants to establish the historic inputs of other POPs within the Lower St. Lawrence Estuary. The same approach will be applied to determine both the magnitude and the trend of PBDE inputs to the marine portion of the St. Lawrence system.

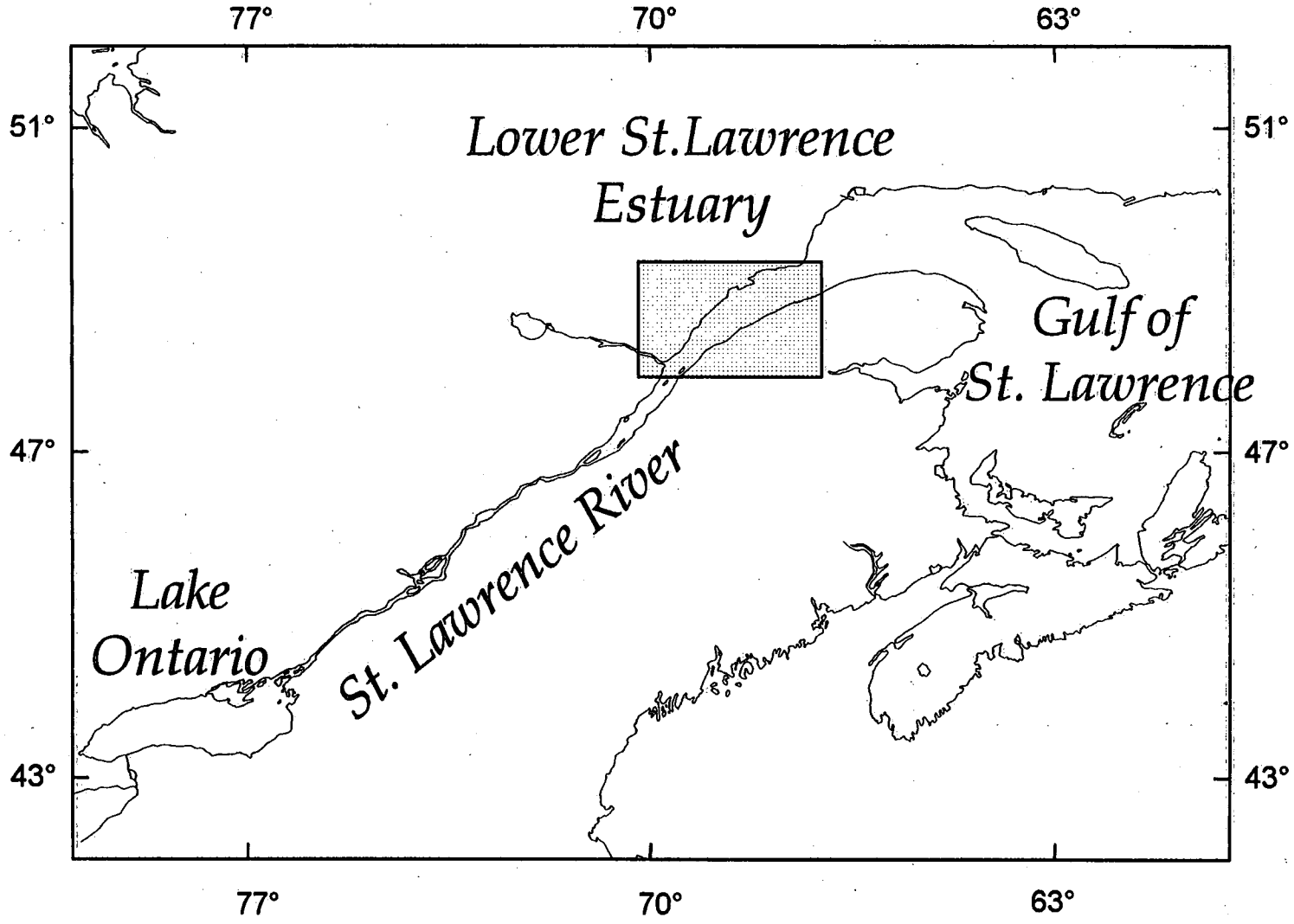
Distribution of PBDEs in biota and sediments from the Estuary and Gulf of St. Lawrence (proposed study)

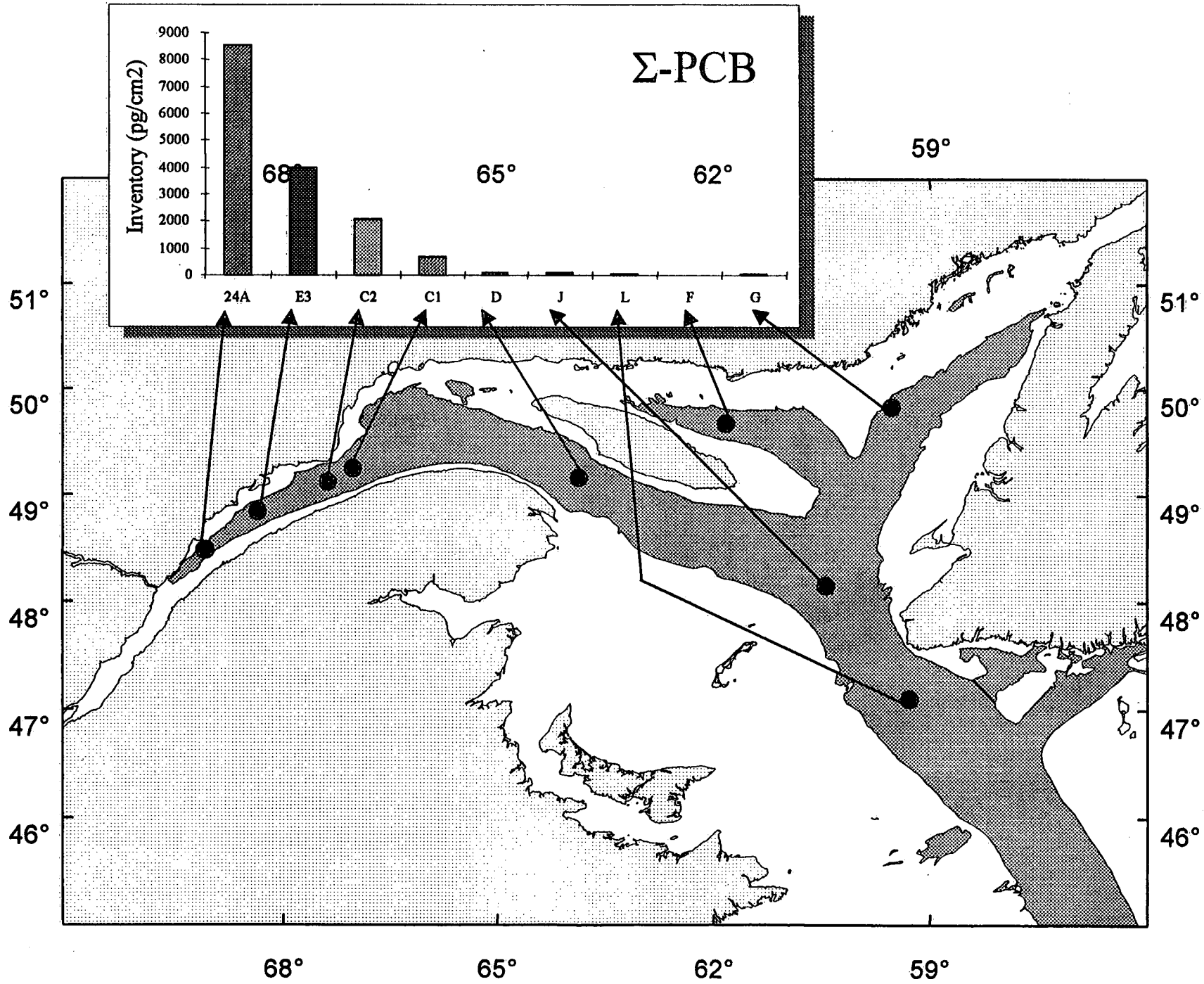
Michel Lebeuf
Maurice Lamontagne Institute
Fisheries and Oceans, Canada

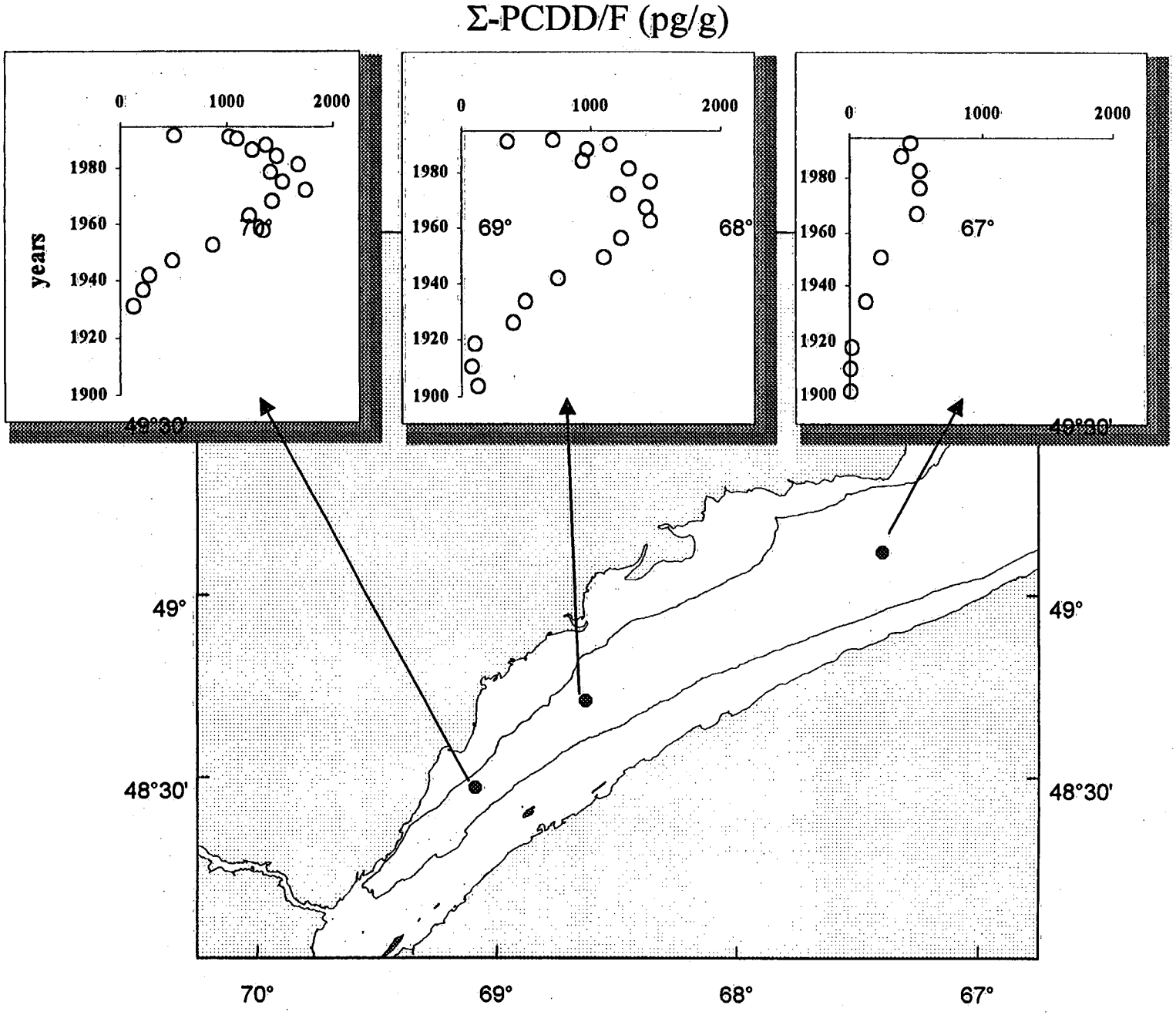
Plan

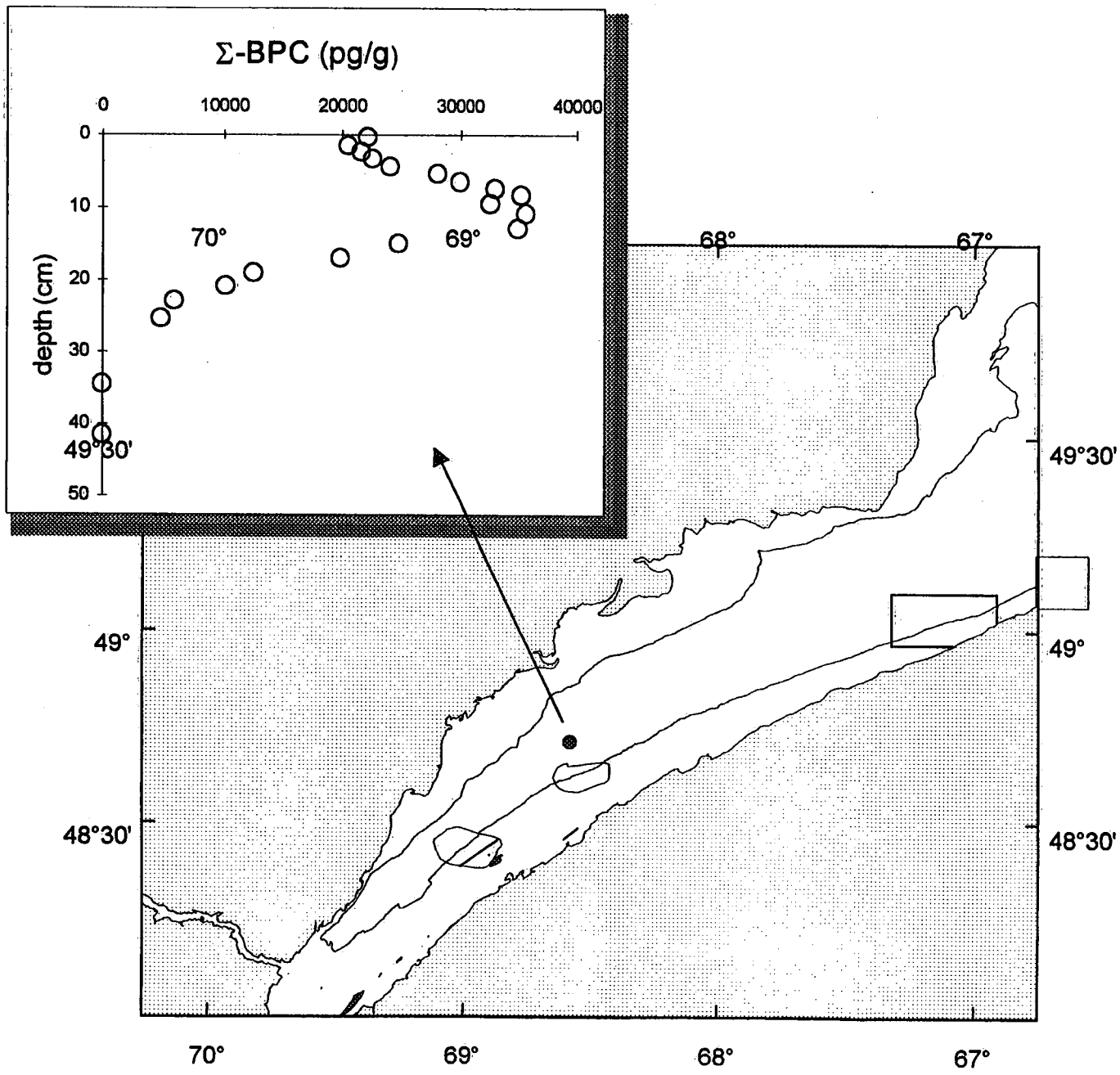
- Site: Estuary and Gulf of St. Lawrence
- POPs in sediments (proposed study)
- POPs in biota (proposed study)
- Analytical method for PBDEs


St.Lawrence
Estuary
Gulf of
St. Lawrence
St. Lawrence River
Lake
Ontario
51°
47°
43°
77°
70°
63°


77°
70°
63°
51°
47°
43°
Lower St.Lawrence
Estuary
Gulf of
St. Lawrence
St. Lawrence River
Lake
Ontario


Σ-PCB
Inventory (pg/cm2)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
24A
E3
C2
C1
D
J
L
F
G
68°
65°
62°
59°
51°
50°
49°
48°
47°
46°

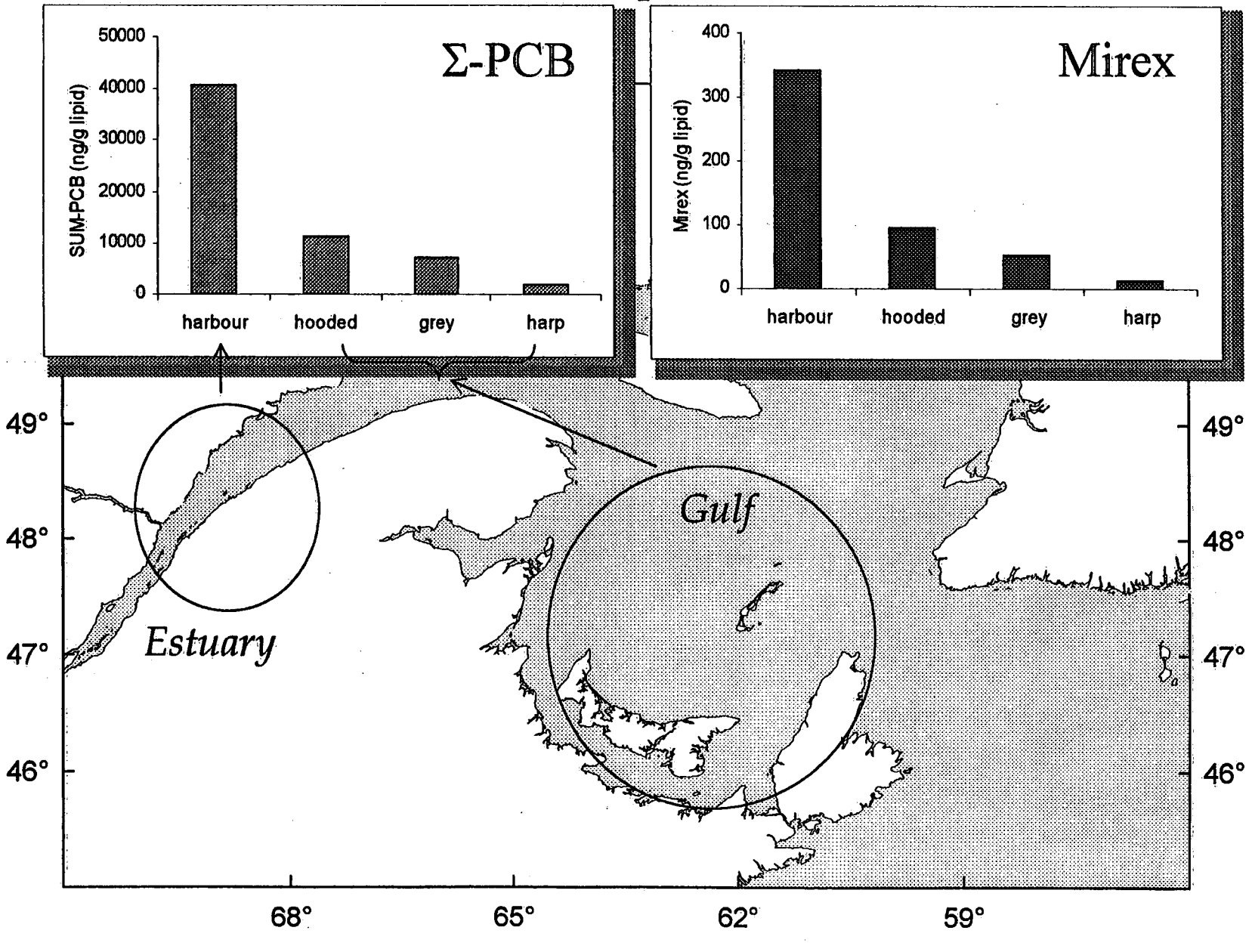

Σ-PCDD/F (pg/g)
0
1000
2000
1980
1960
1940
1920
1900
years
70°
69°
68°
67°
49°30'
49°
48°30'
70°
69°
68°
67°

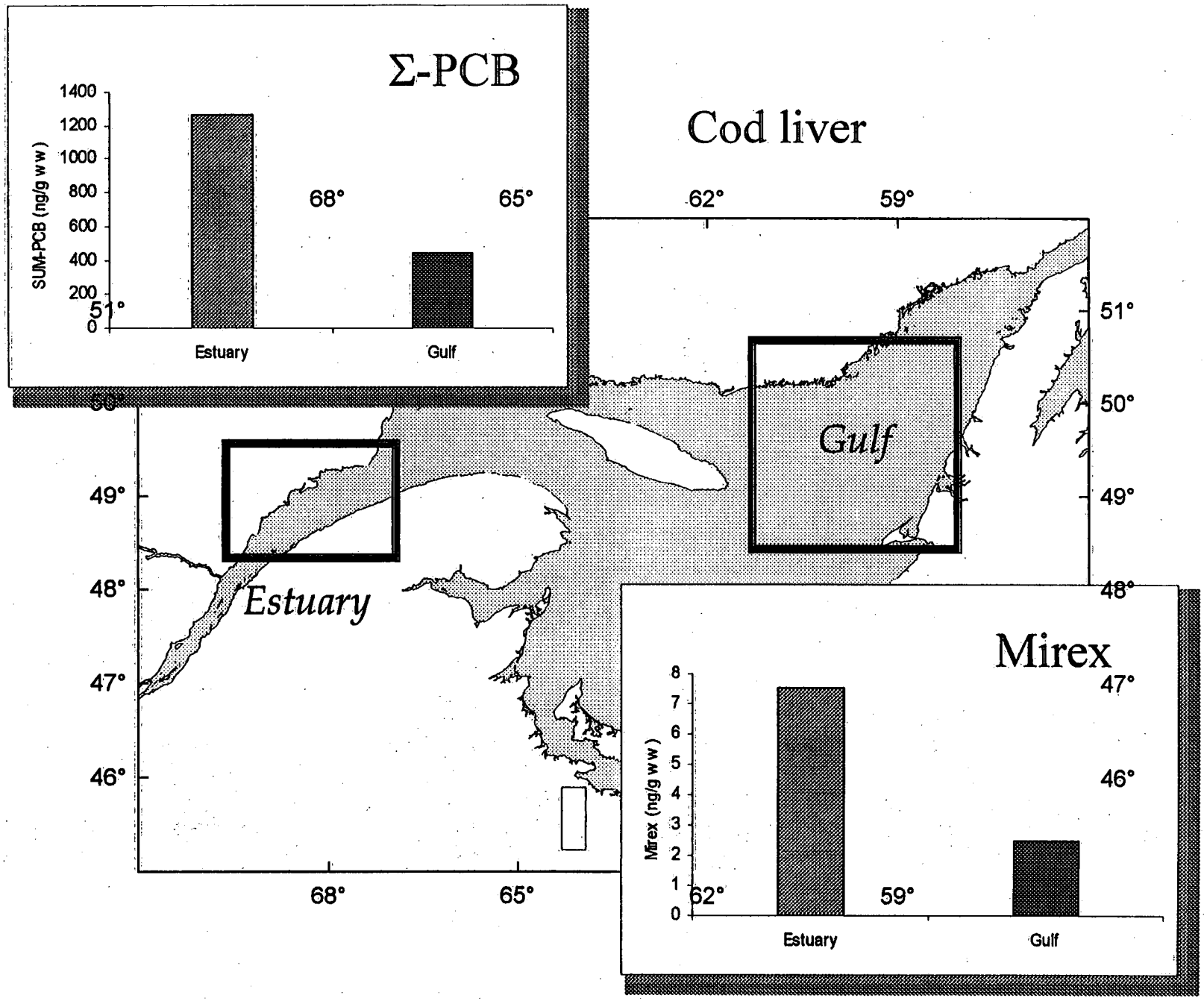

Σ-BPC (pg/g)
0
10000
20000
30000
40000
depth (cm)
0
10
20
30
40
50
70°
69°
68°
67°
49°30'
49°
48°30'

Sediments (Proposed study)

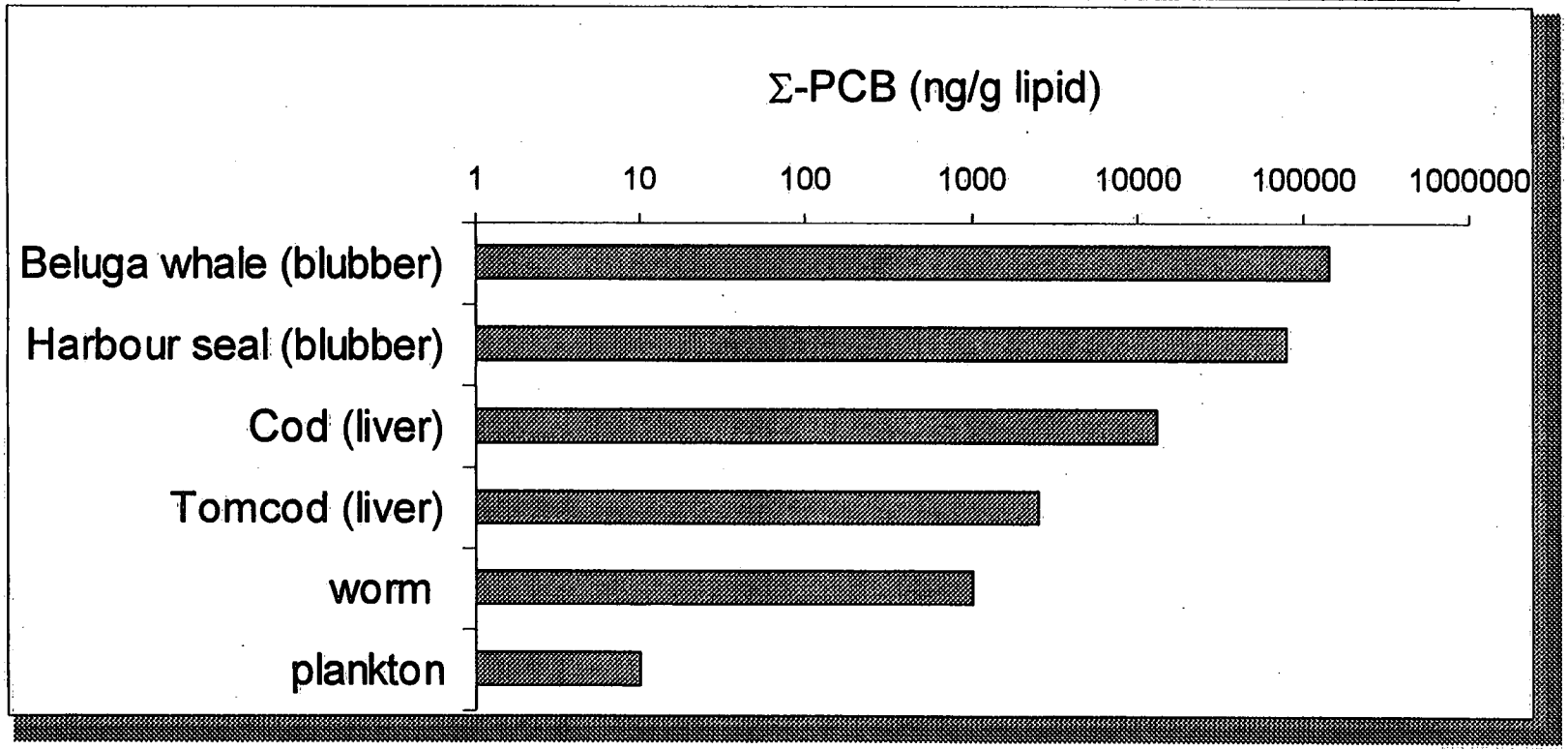
- Measure PBDE levels in about 30 sediment samples; one (or two) sediment core from the Lower St. Lawrence Estuary
- Date sediment layers using ^{210}Pb method
- Report levels, the history of sedimentary accumulation and flux of PBDEs

Seal species




Σ-PCB
SUM-PCB (ng/g w w)
1400
1200
1000
800
600
400
200
0
Estuary
Gulf
Cod liver
62°
59°
51°
50°
49°
48°
47°
46°
68°
65°
Gulf
Estuary
Mirex
Mirex (ng/g w w)
8
7
6
5
4
3
2
1
0
Estuary
Gulf

Bioaccumulation of PCBs by organisms from the St. Lawrence Estuary



Biota (Proposed study)

- Measure PBDE levels in about 60 samples of biota collected in the St. Lawrence Estuary
- Evaluate the trophic level of the organisms using $\delta^{15}N$ method
- Report levels, patterns of congeners and BAF

Analytical method

extraction

↓

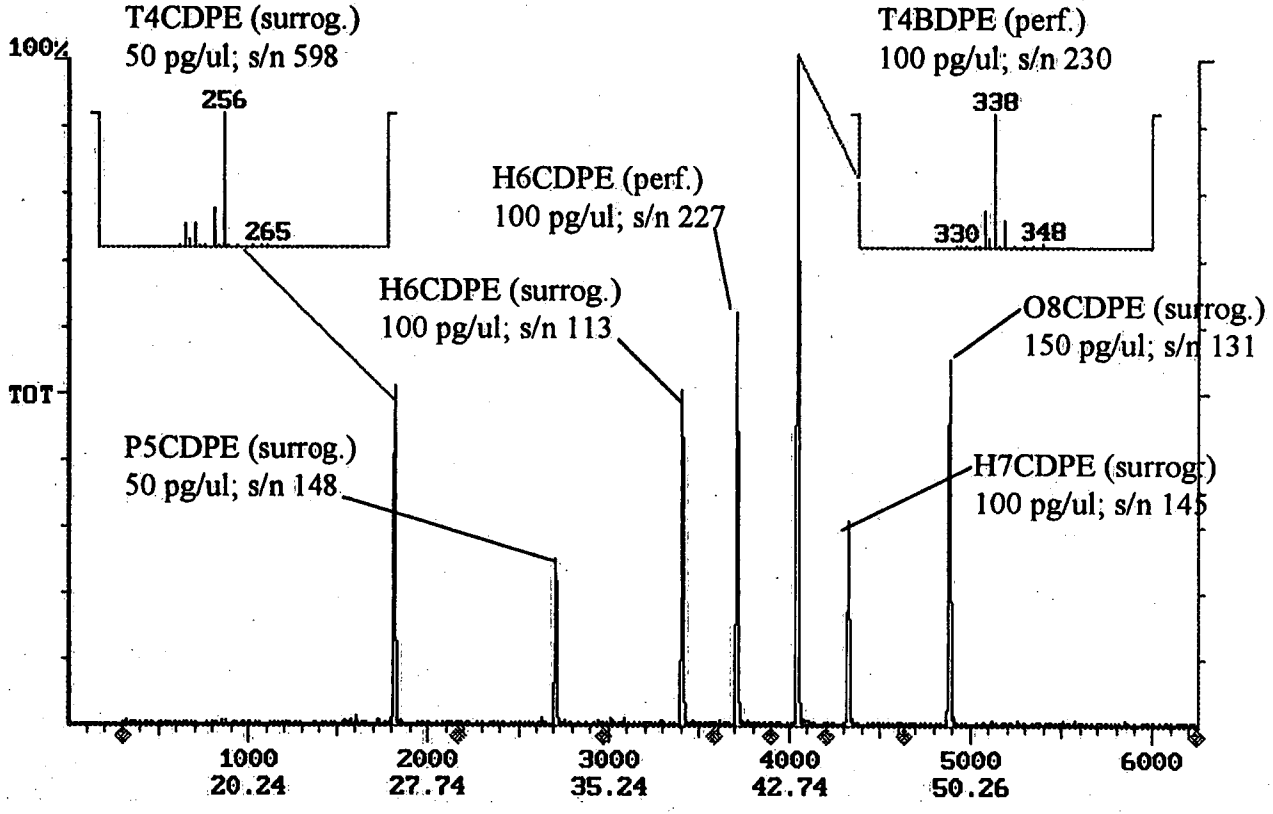
clean up

- (QA/QC)
- (preliminary quantification) by **GC-MS/MS**

↓

Confirmation by **GC-HRMS**

Surrogate spiking solution and performance standard mix (compounds at 50-150 pg/ul)



What is next ...

- Method development and validation
- Sampling
- PBDEs analysis
- Complementary analysis (^{210}Pb, $\delta\ ^{15}N$)

POLYBROMINATED DIPHENYL ETHERS (PBDES) AND METHOXY POLYBROMINATED DIPHENYL ETHERS (ME-O-PBDES) IN SELECTED WILDLIFE TISSUES

Mary Simon and Bryan Wakeford

A variety of species (Herring Gull, Osprey, Bald Eagle, Peregrine Falcon, Snapping Turtle, Dungeness Crab, Mudpuppy, Polar Bear, Harbour Seal, Pilot Whale, Fish, Mink and River Otter), and tissues (such as eggs, egg yolk sacs, liver, blubber, oil, hepatopancreas, blood plasma) were analyzed for Polybrominated Diphenyl Ethers (PBDEs) and Methoxy-Polybrominated Diphenyl Ethers (MeO-PBDEs). The samples were selected on the basis that they had relatively elevated levels of PCDDs/PCDFs and/or PCBs/OCs when previously analyzed at NWRC.

Identification of the compounds were produced by comparison of full scan (m/z 50 to m/z 900) mass spectral patterns with the characteristic mass fragmentation patterns and the GC retention times of the available PBDE standards. The mass spectrometer scan mass resolution was set at 1000. Quantitation of the PBDEs were done at 10000 resolution by using Selected Ion Monitoring Chromatography (SIM) analysis. The method for determining semi-quantitative levels for MeO-TBDPEs was devised using SIM chromatograms for tetra MeO-BDEs and comparing the integrated areas with those of the 2,2',4,4'-BDE standard. The identity of the Me-O-DPE was confirmed by using accurate mass determinations at 10000 resolution. The analytical standards available at NWRC were supplied through courtesy of Dr. Ake Bergman (University of Stockholm): 2,2',4,4'-TetraBDE; 2,2',4,4',5-PentaBDE ; 2,2',3,4,4'-PentaBDE ; 2,2',4,4',5,5'-HexaBDE and 2,2',3,4,4',5-HexaBDE.

The major Tetra BDE congener was 2,2',4,4'-BDE and it was detected in all samples analyzed in the range from < 1 ppb to >3,000 ppb), MeO-Br4-DEs (2 congeners) were detected in three samples. Up to three Penta Bromo Diphenyl Ether congeners were detected in 14 of the 18 specimens in ranges from 1 to >700 ng/g. Up to five Hexa BDE congeners were detected in 14 of the specimens in range up to 200 ng/g. Six of the specimens showed detectable amounts of up to three Hepta BDE congeners.

Polybrominated Diphenyl ethers (PBDEs) and Methoxy Polybrominated Diphenyl ethers (Me-O-PBDEs) in Selected wildlife Tissues

Mary Simon and Bryan Wakeford
National Wildlife Research Centre
Canadian Wildlife Service

Introduction

- First detection at NWRC -1996
  - New Brunswick tree swallows contaminated marsh
  - whole body homogenate - elevated PCDDs
  - full MS scan on extract
    - brominated Diphenyl ethers
    - brominated Methoxy Diphenyl ethers
    - chlorinated Diphenyl ethers

1998 Work - Brominated DPEs

- Selection of Specimens and tissues
- Identification of compounds
- Method of Quantitation of compounds
- Summary of results

BrDPEs - Specimen Selection

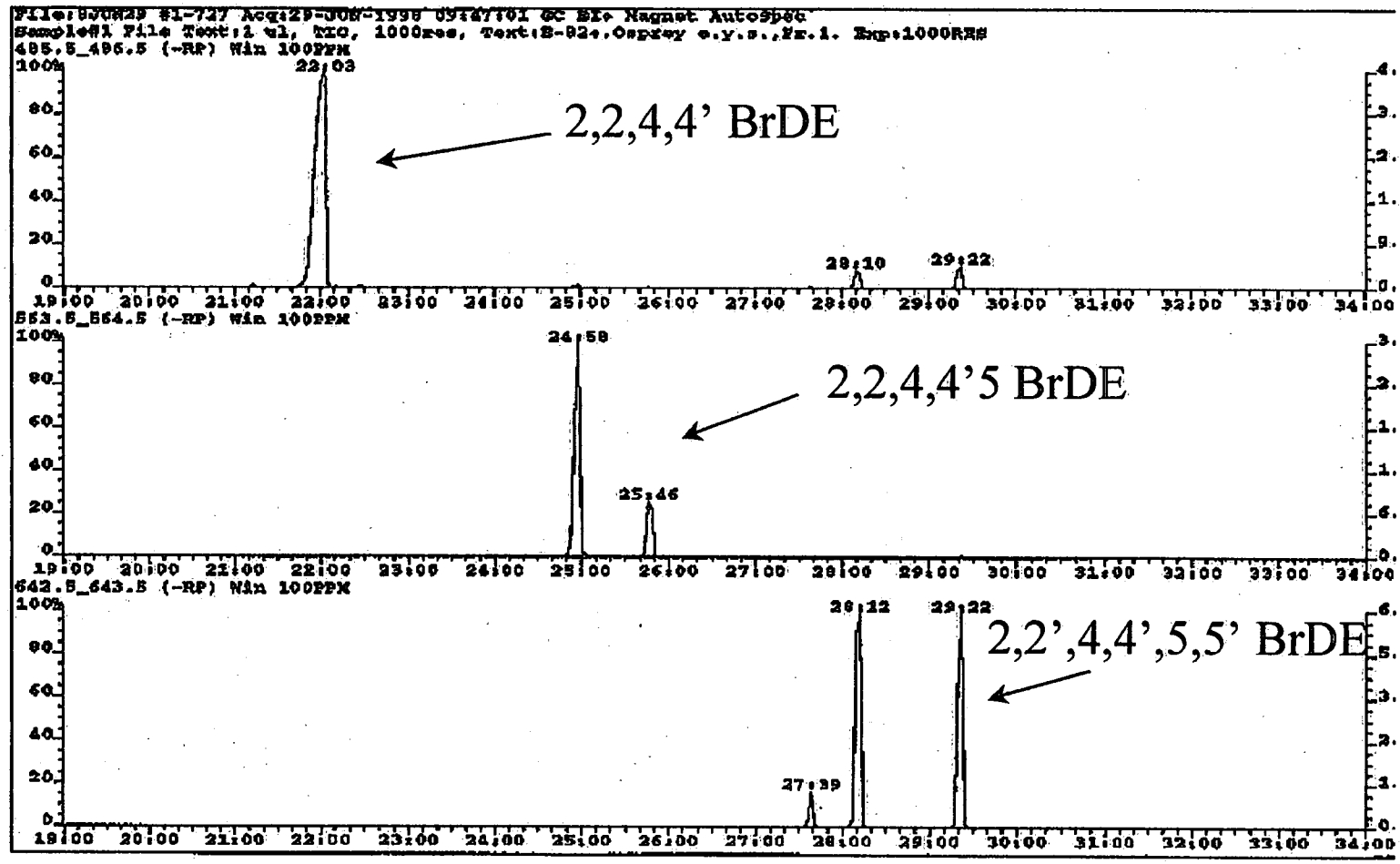
- Survey done on side - minimized work
- GC/MS ready extracts for PCDD/Fs - stored in dark cupboards up to several years
- Criteria
  - had relatively elevated PCDD
  - various species - avian, mammal, fish amphibian
  - various tissues - eggs, liver, blubber, blood plasma

Specimens analyzed

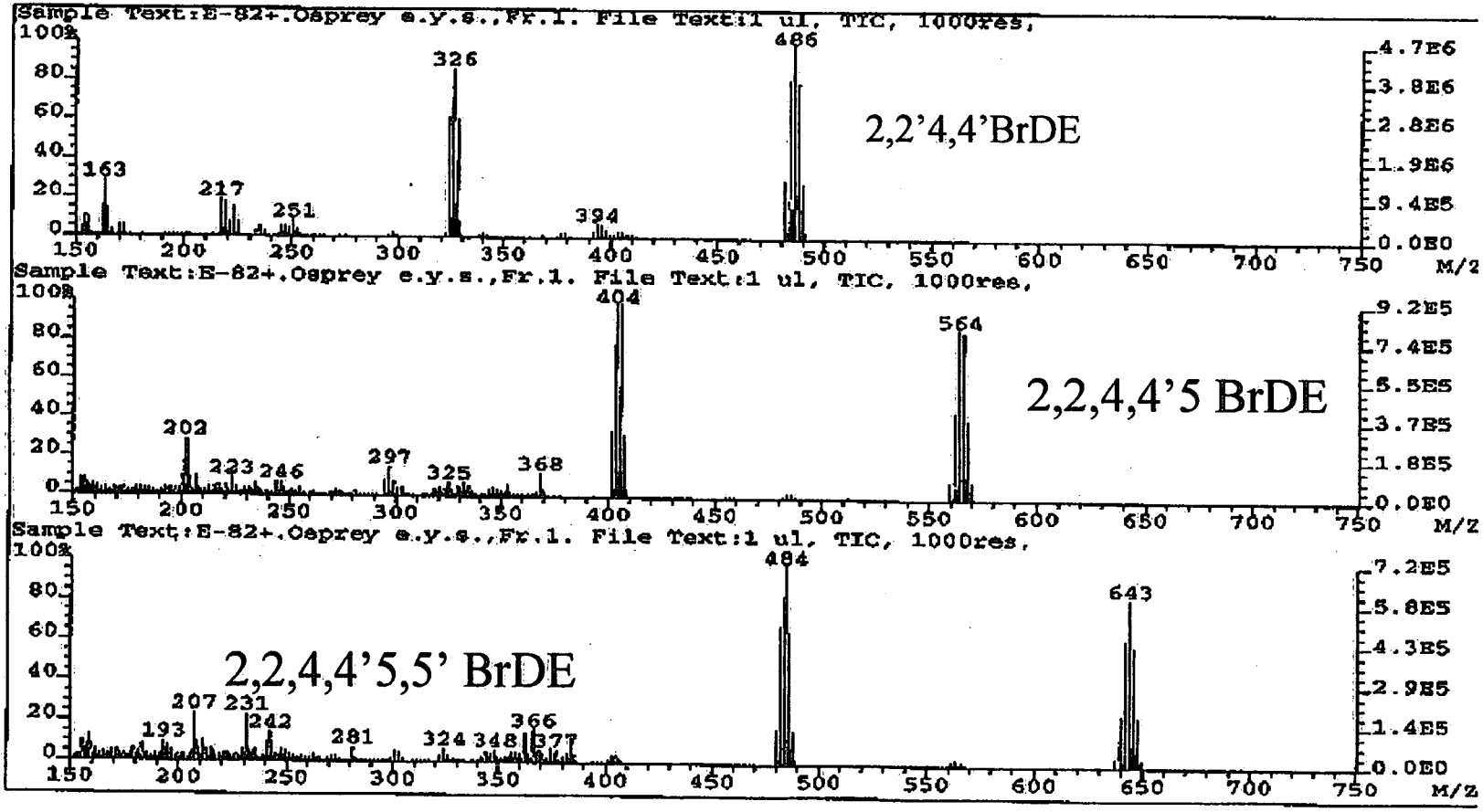
■ Herring Gull egg	Lake Ontario - 1989, 95, 97
■ Bald Eagle egg yolk sac	B. C. - 1992
■ Osprey egg	B.C. - 1995, 96, 97
■ Snapping Turtle egg	Ontario - 1991
■ Mudpuppy egg	Ontario - 1993
■ Dungenese Crab h. pancreas	B.C. - 1997
■ Peregrine Falcon egg	Labrador - 1997
■ Elephant Seal blubber	USA - 1994
■ Polar Bear plasma	Manitoba - 1996
■ Mink liver	Maritimes - 1996
■ Pilot Whale blubber	Mass USA - 1990
■ Fish oil	USA - 1997

Identification BrDEs - Osprey egg yolk sac

-extracted ion chromatograms from full scans (1000 res..)



Identification of Br_x DEs 1000 Res. Osprey egg yolk sac

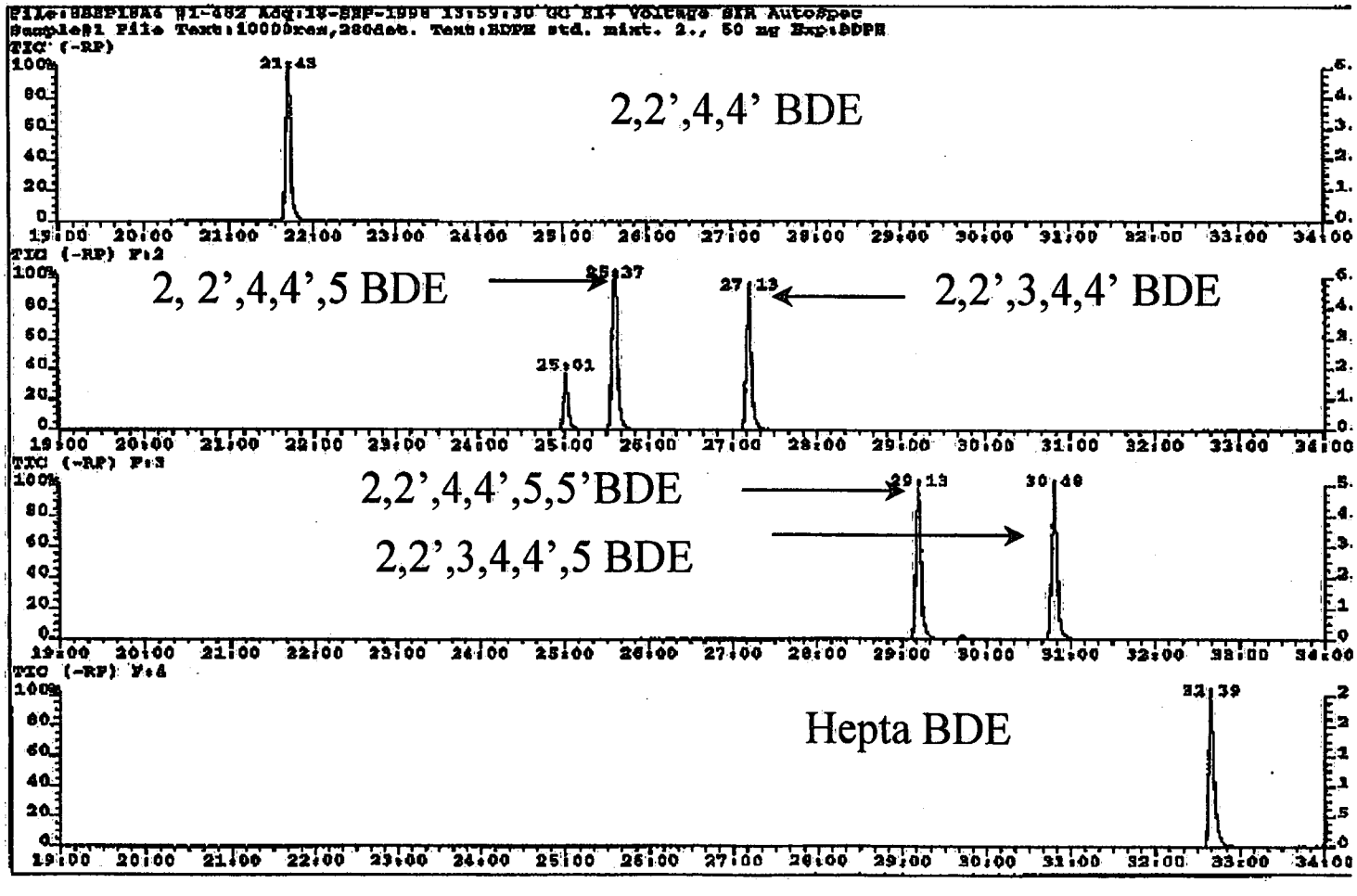


Quantitation of PBDEs

- High resolution Mass Spectrometer at 10,000 - selected ion monitoring
- Selected ions for each group
  - TBDEs m/z 483.713, 485.711, 325.877
  - PBDEs m/z 563.622, 565.620, 403.787
  - HxBDEs m/z 641.532, 643.530, 483.696
  - HpBDEs m/z 721.441, 723.439, 561.606

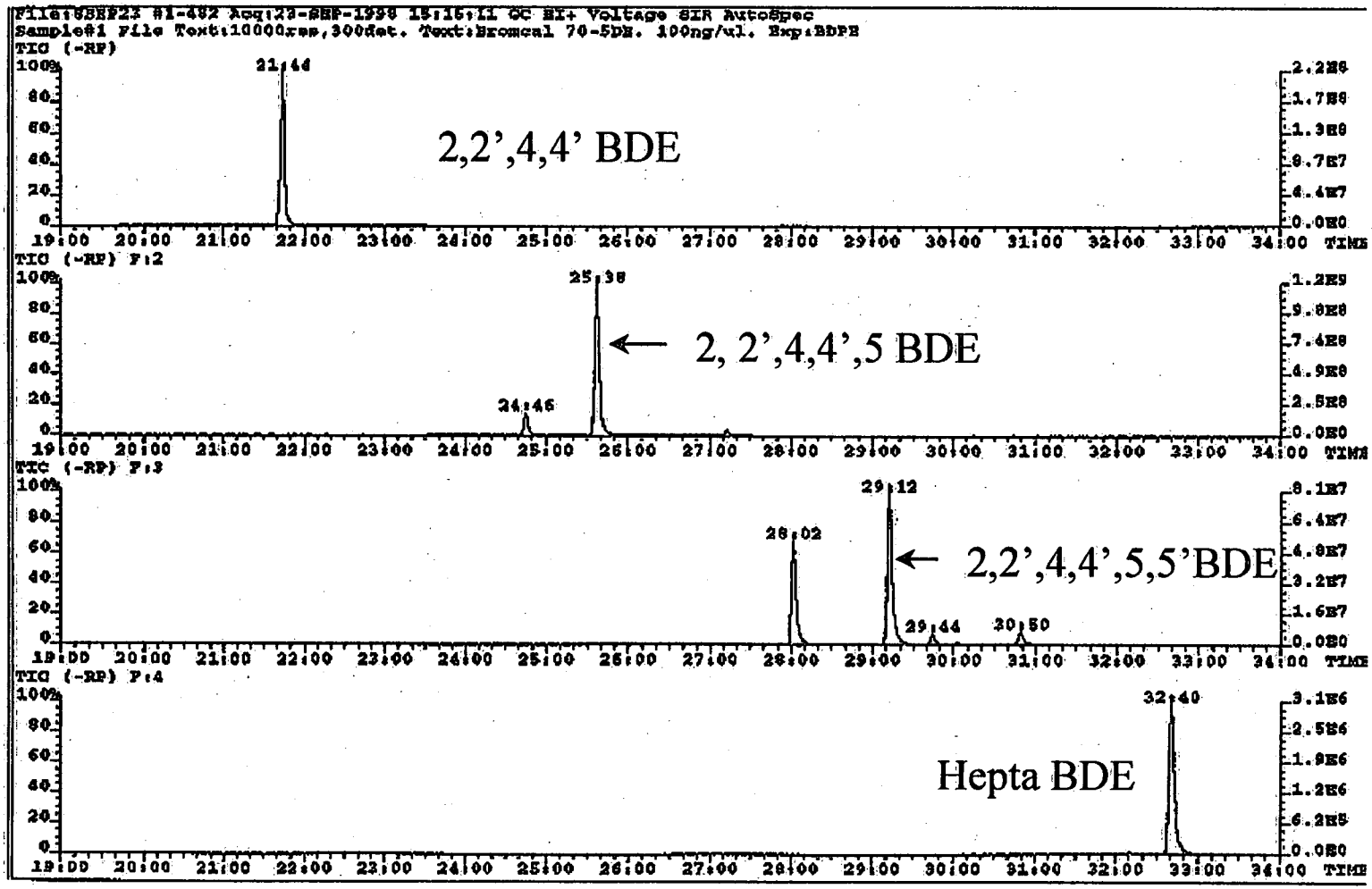
Quantitation by SIM (10000 res.)
BDPE Std. mix from Ake Bergman

Bromkal compounds in purple colour

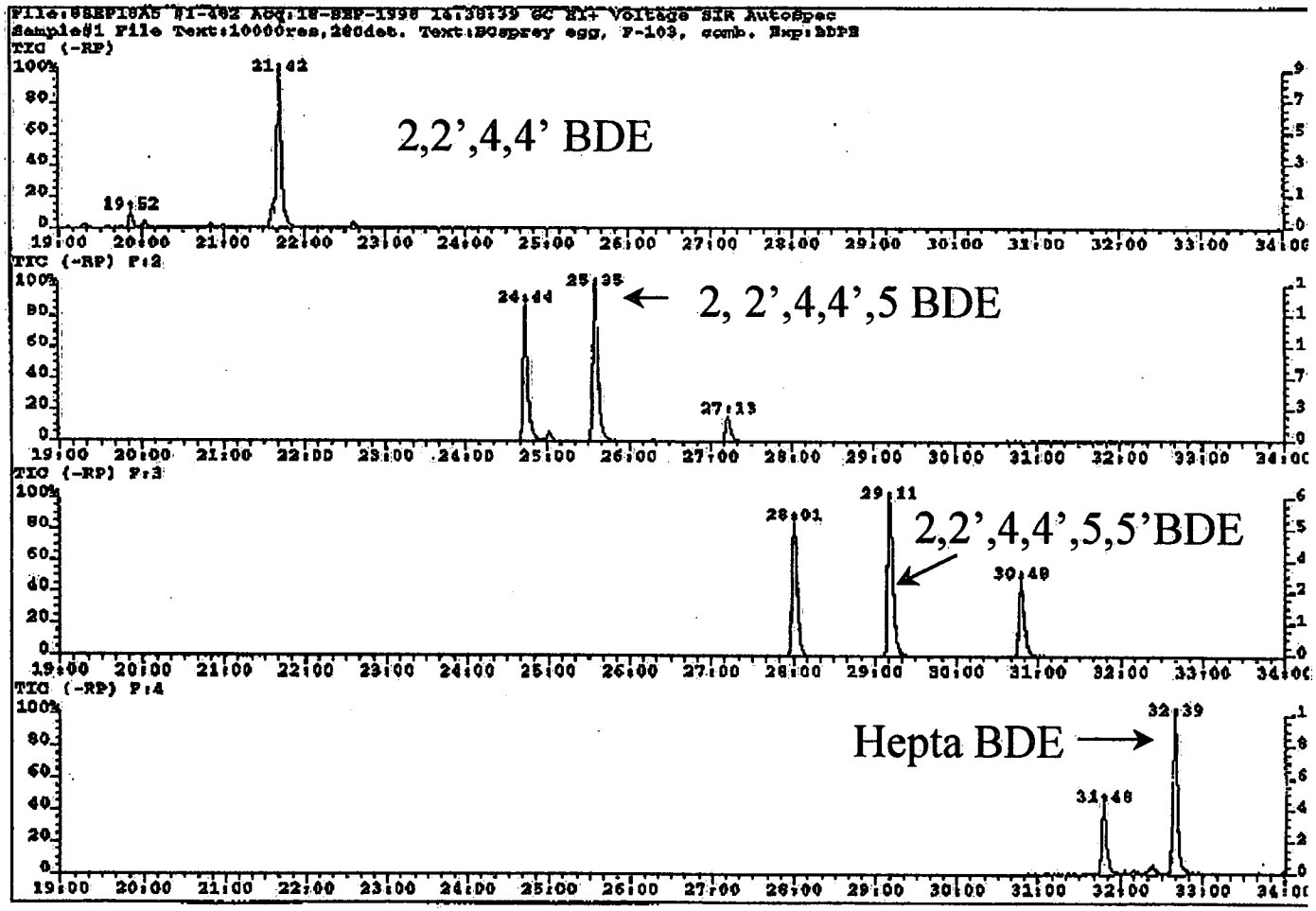


Quantitation by SIM (10000 res.)

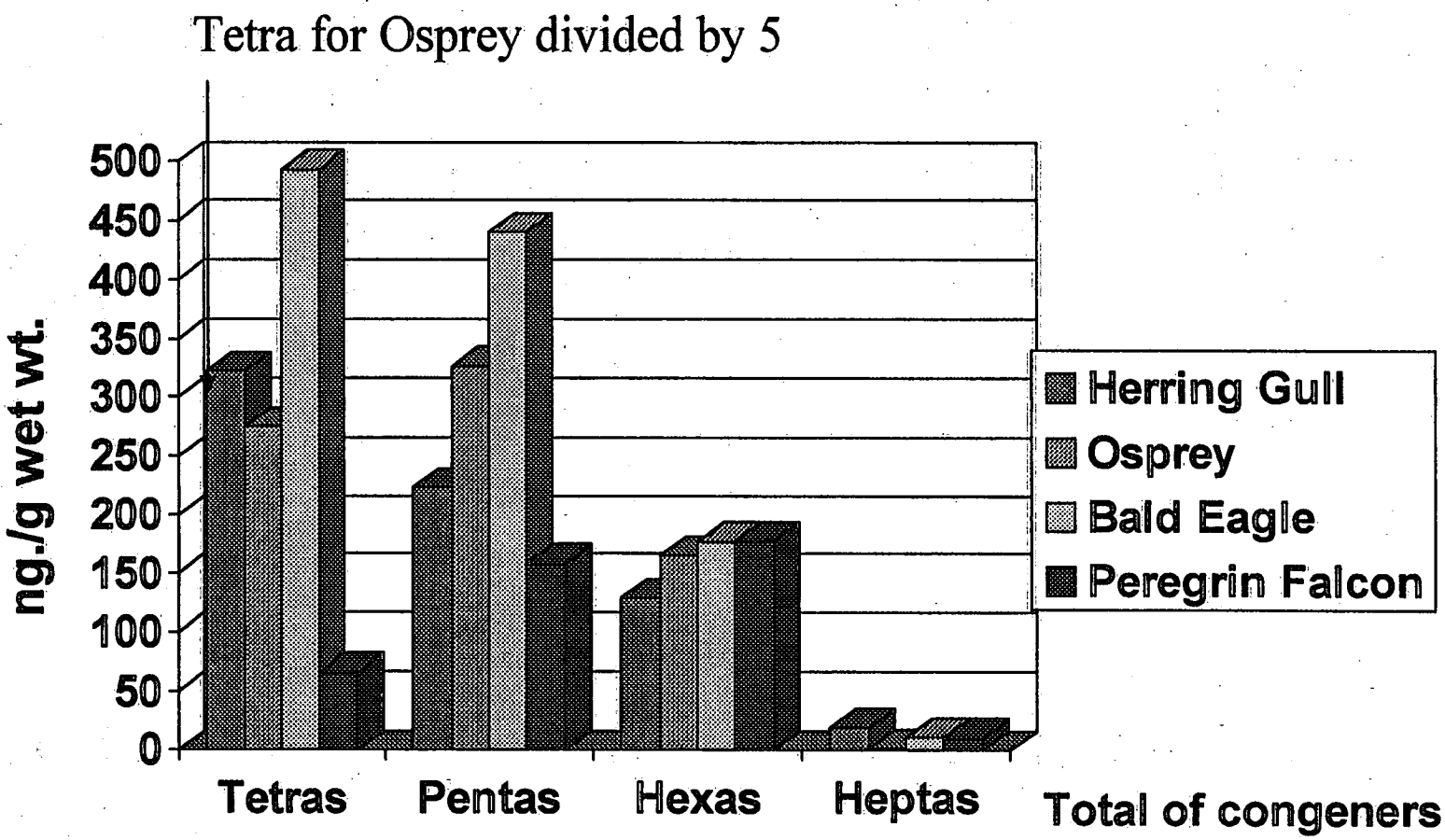
Bromkal 70-5DE



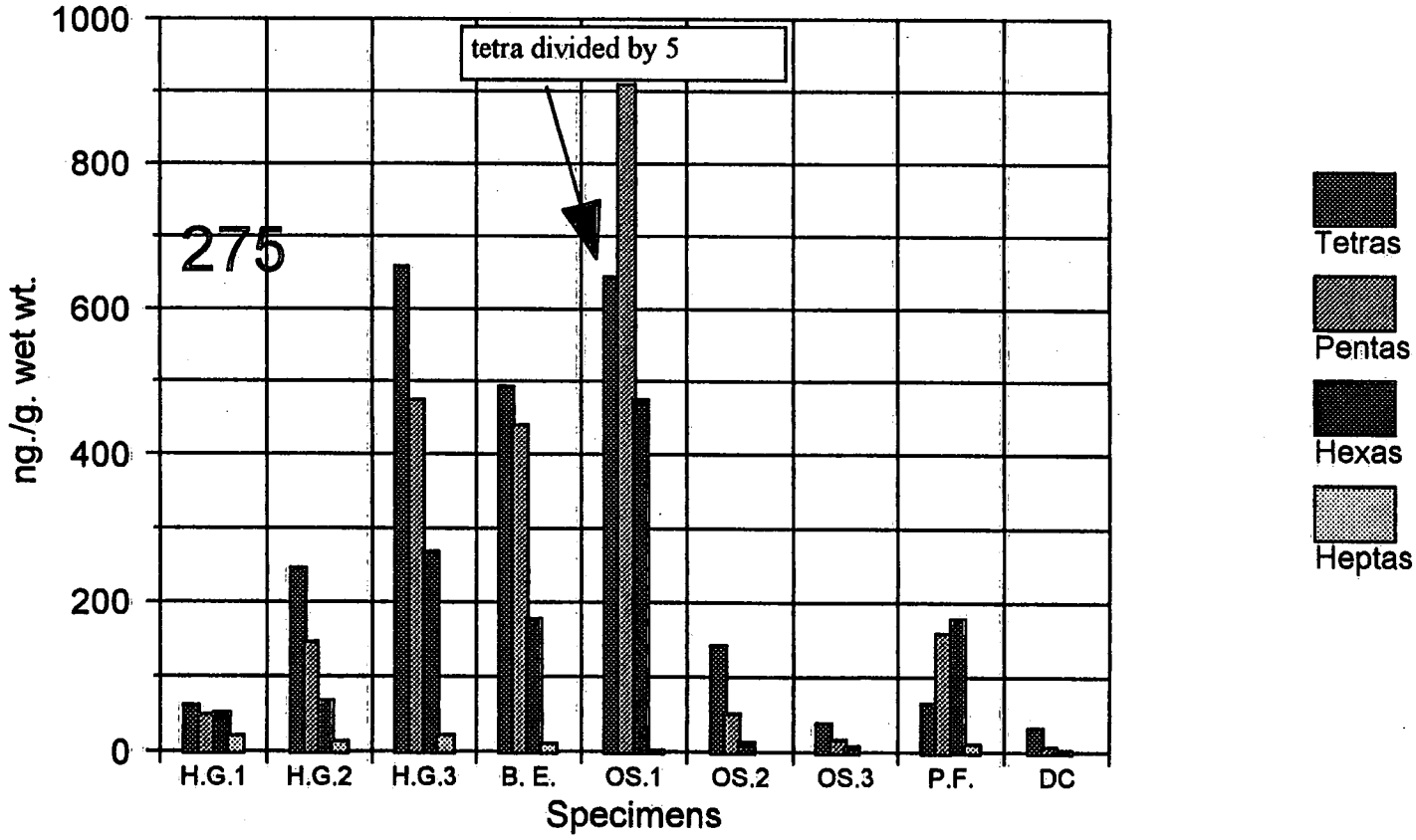
Quantitation by SIM (10000 res.) in Osprey egg



Br_xDEs in avian egg specimens



BrDEs results in individual specimens



Number of Brominated Diphenyl Ether congeners detected in various wildlife tissues

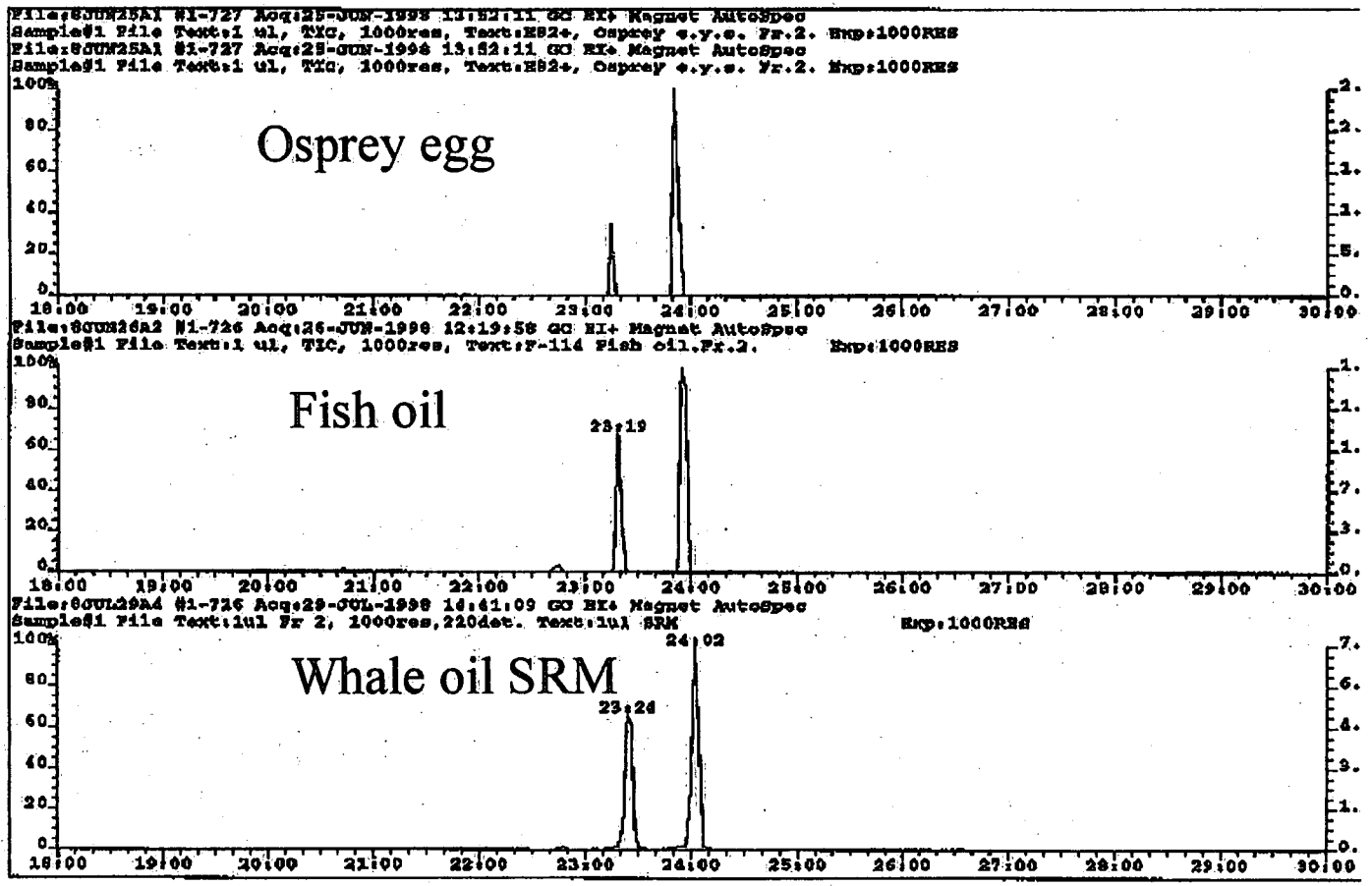
	Tetras	Pentas	Hexas	Heptas
Herring Gull egg	2	3	4	2
Osprey egg	3	3	4	1
Bald Eagle egg	2	2	2	3
Snapping turtle egg	1	2	2	1
Mudpuppy egg	1	2	2	0
Dungenese Crab pancreas	2	2	2	0
Peregrin falcon	1	2	2	2
Seal blubber	1	2	1	0
Whale Blubber SRM	2	2	2	1
Fish oil - food supplement	2	2	2	0

Methoxy Tetra Bromo Diphenyl Ether - semi quantitation

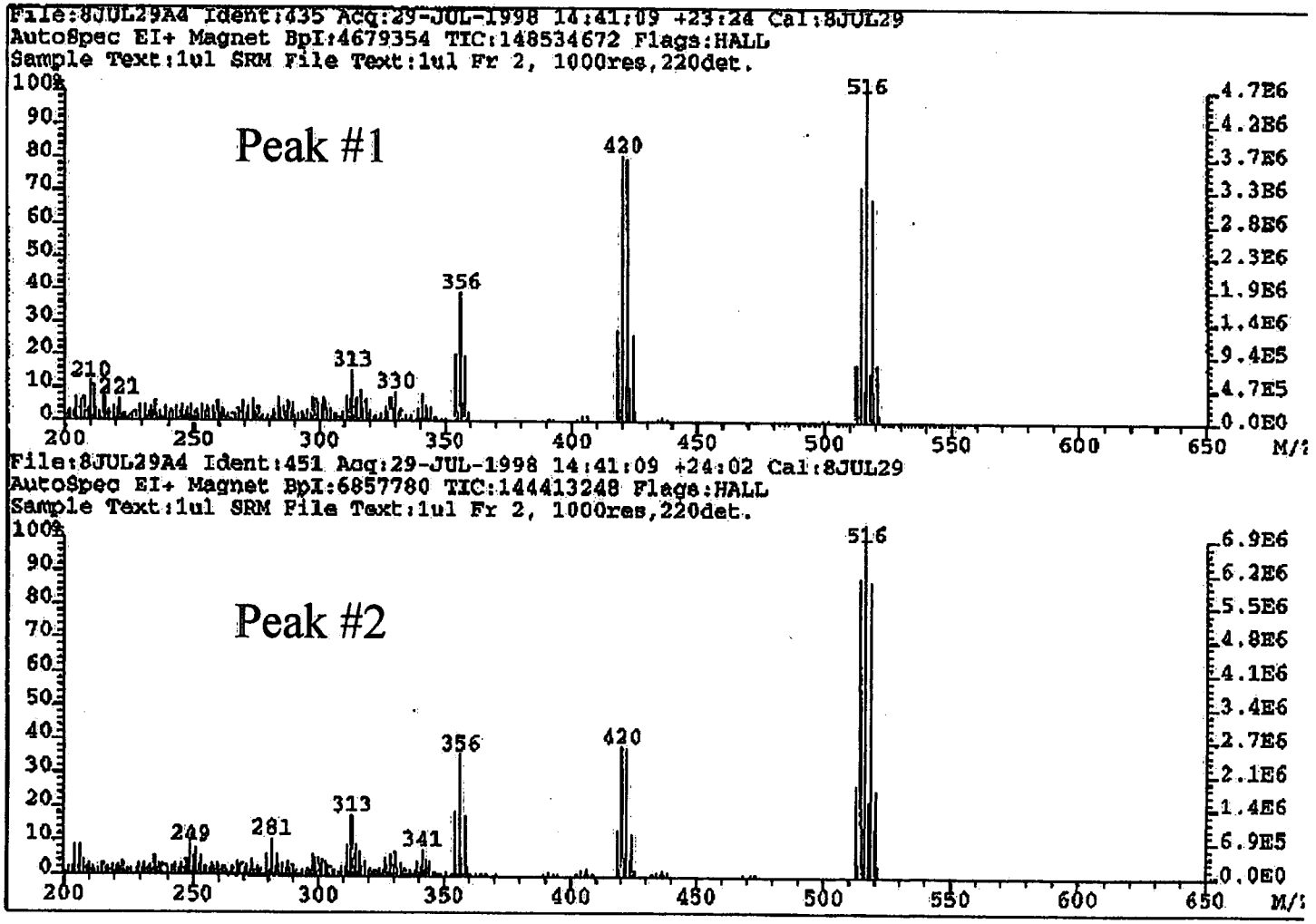
- Full mass spectral scan for determining strongest ion in molecular cluster
- Integrated area of selected ion compared the response with integrated area of the major ion from 2, 2', 4, 4'- BDE in same GC injection

MeO-Br_4DEs in three tissues
Osprey Egg, Fish oil and Whale blubber SRM

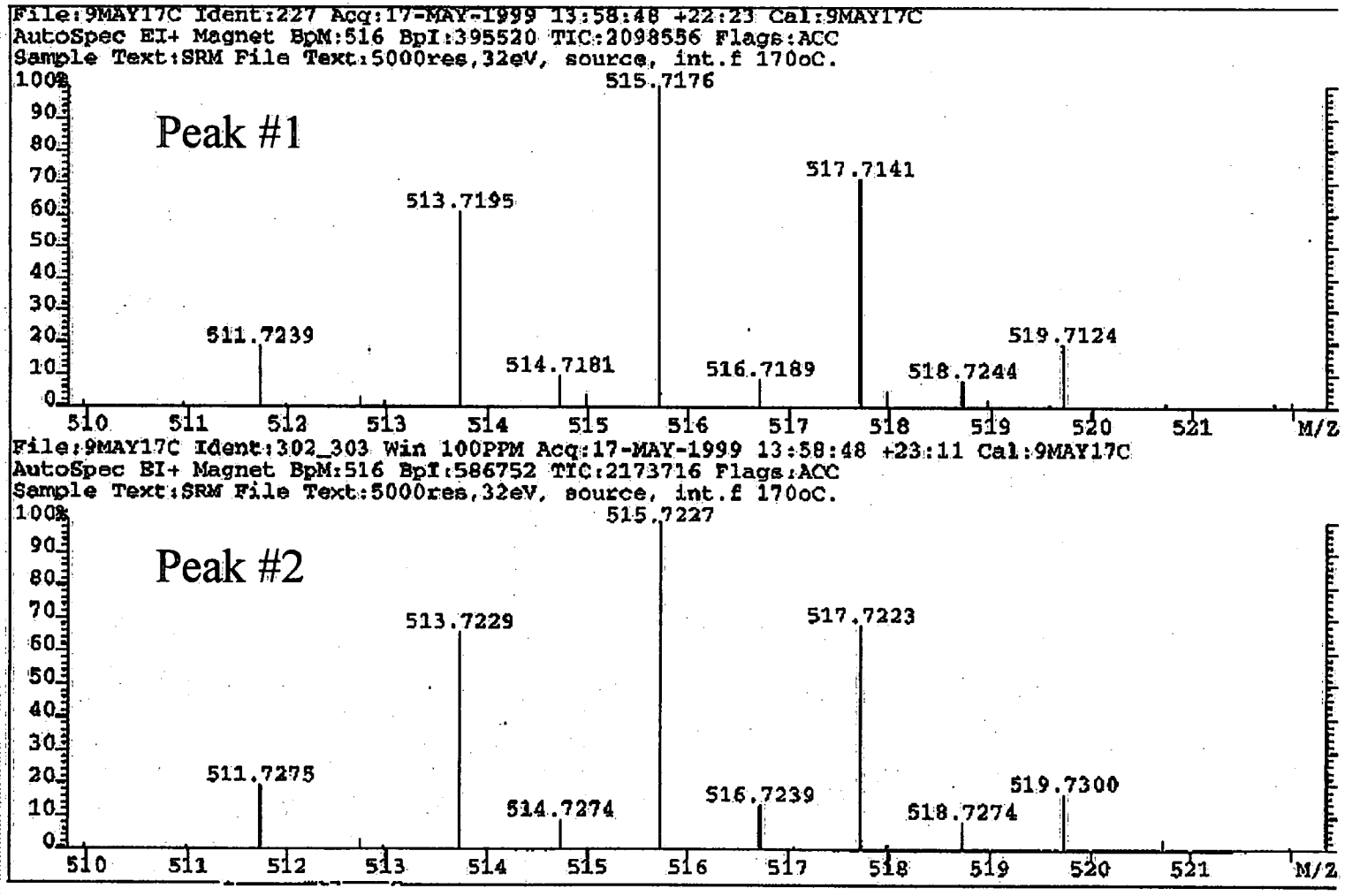
-extracted ion chromatograms from full scans (1000 res.)



Identification of MeO- Br_4 DE - full scan in whale blubber SRM - 1000 resolution



High resolution confirmation of MeO-Br_4DEs at 10,000 resolution



Semi- Quantitative results for Methoxy Br_4 DEs

	Peak A	Peak B
Whale blubber	212.5	260.1
Fish oil	1.04	2.27
Osprey egg	.09	.37

ng./ g. wet weight

Summary of results

- BDEs detected
  - in several species
  - in different tissues
  - from several locations in Canada and the US

TSRI contribution

- Year one - focus on Herring gull egg in Great Lakes
  - Levels of three specimens in Lake Ontario are detectable
  - Large Specimen Bank collection - Temporal trends can be assessed
  - Comparison with other contaminant data and potentially support through NWRC Herring Gull model

TSRI Contribution - cont.

- Year 2 and 3 - current plan is to focus on various species - mainly avian
- Make use of specimen bank and in-house expertise on selection of tissues and interpretation of results.

Acknowledgments

- Technical staff - NWRC
  - Abde Idrissi
  - Masresha Asrat
  - Karen Timm
  - Guy Savard
- Prof Ake Bergman - standards
- Ross Norstrom - advice and support

HALOGENATED DIMETHYL BIPYRROLES - IDENTIFICATION, DISTRIBUTION, AND TOXICOLOGICAL ACTIVITY

Sheryl Tittlemier

Halogenated dimethyl bipyrroles (HDBPs) are a series of brominated chlorinated nitrogen-containing heterocyclic compounds. They were first observed in 1988 in a Leach's storm-petrel egg sample from BC. HDBPs observed in wildlife have an N,N'-dimethyl-2,2'-bipyrrole skeletal structure. They are hexahalogenated, containing varying amounts of bromine and chlorine atoms. Physical properties such as water solubility, vapour pressure, and octanol/water partition coefficient are similar to other highly halogenated organic compounds such as the octa- to decachlorinated PCBs.

HDBPs have been found in northern elephant seal, pilot and minke whale, bald eagle, and various seabird samples from the Atlantic and Pacific coasts of North America. HDBPs were not detected in eggs from freshwater bird species that were sampled from the Great Lakes. Concentrations of the major HDBP congener, $C_{10}H_6N_2Br_4Cl_2$, ranged from 0.61 ppb in herring gull eggs from Newfoundland to 5 ppm in bald eagle liver from BC.

In preliminary toxicity tests, one of the minor HDBP congeners, $C_{10}H_6N_2Br_6$, was found to have CYP1A inducing capabilities in chicken embryo hepatocytes. The source of HDBPs is not yet known, although evidence suggests that they may be naturally-produced by marine organisms. Their strict marine occurrence and structural similarity to a known marine bacterium product support the natural production hypothesis.

Halogenated Dimethyl Bipyrroles - Identification, Distribution, and Toxicological Activity

Sheryl Tittlemier, Ross Norstrom

Carleton University, Ottawa, Ontario

Canadian Wildlife Service, Hull, Quebec

Overview

- introduction
- identification of HDBPs
- physical properties
- distribution and levels in wildlife
- toxicology
- future work

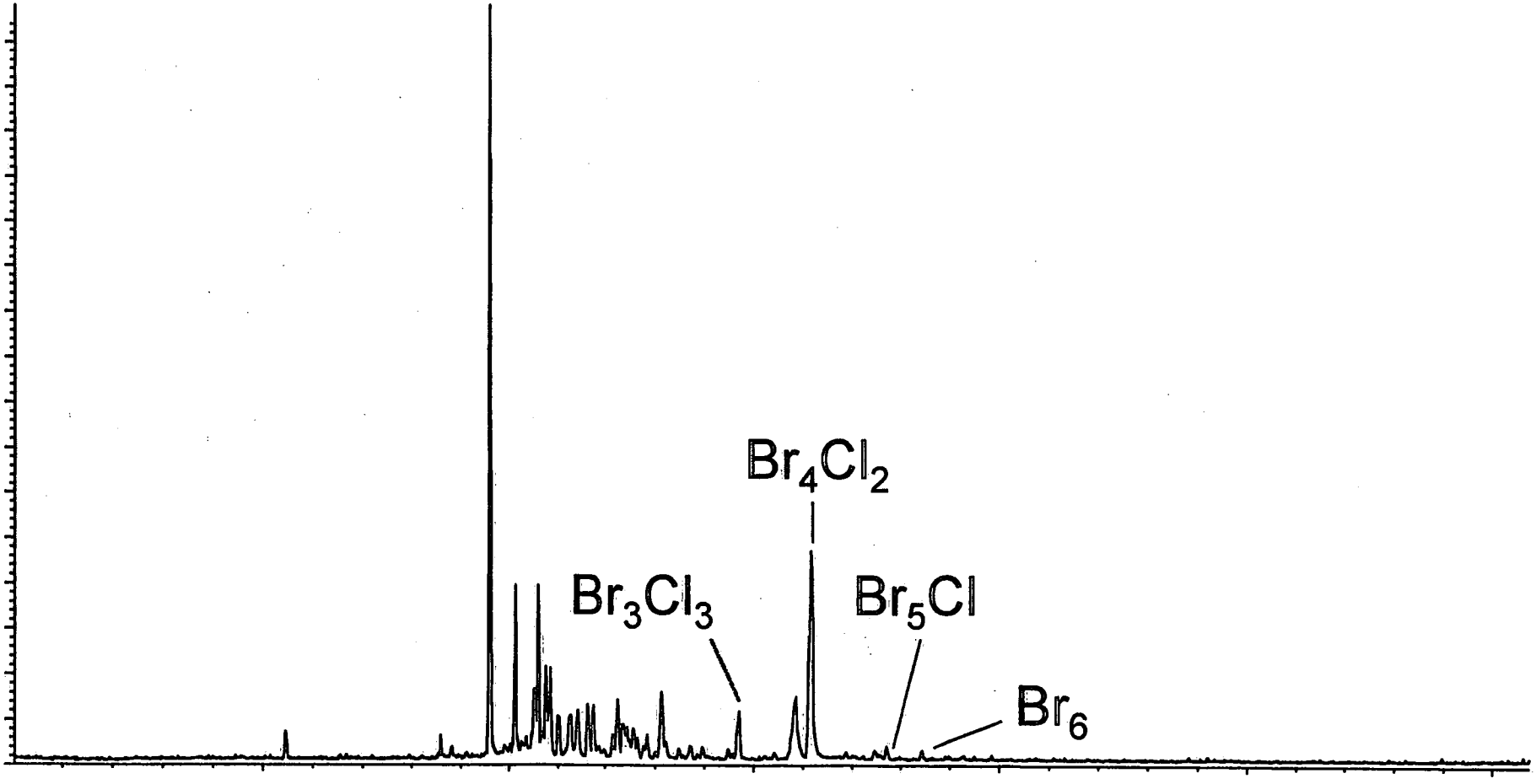
Introduction

- first observed in 1988, in Leach's storm-petrel egg sample from British Columbia
- could not be identified by MS
- compound was halogenated

Introduction

- series of brominated and chlorinated compounds
  - major compound: Br_4Cl_2
  - minor compounds: Br_3Cl_3, Br_5Cl, Br_6

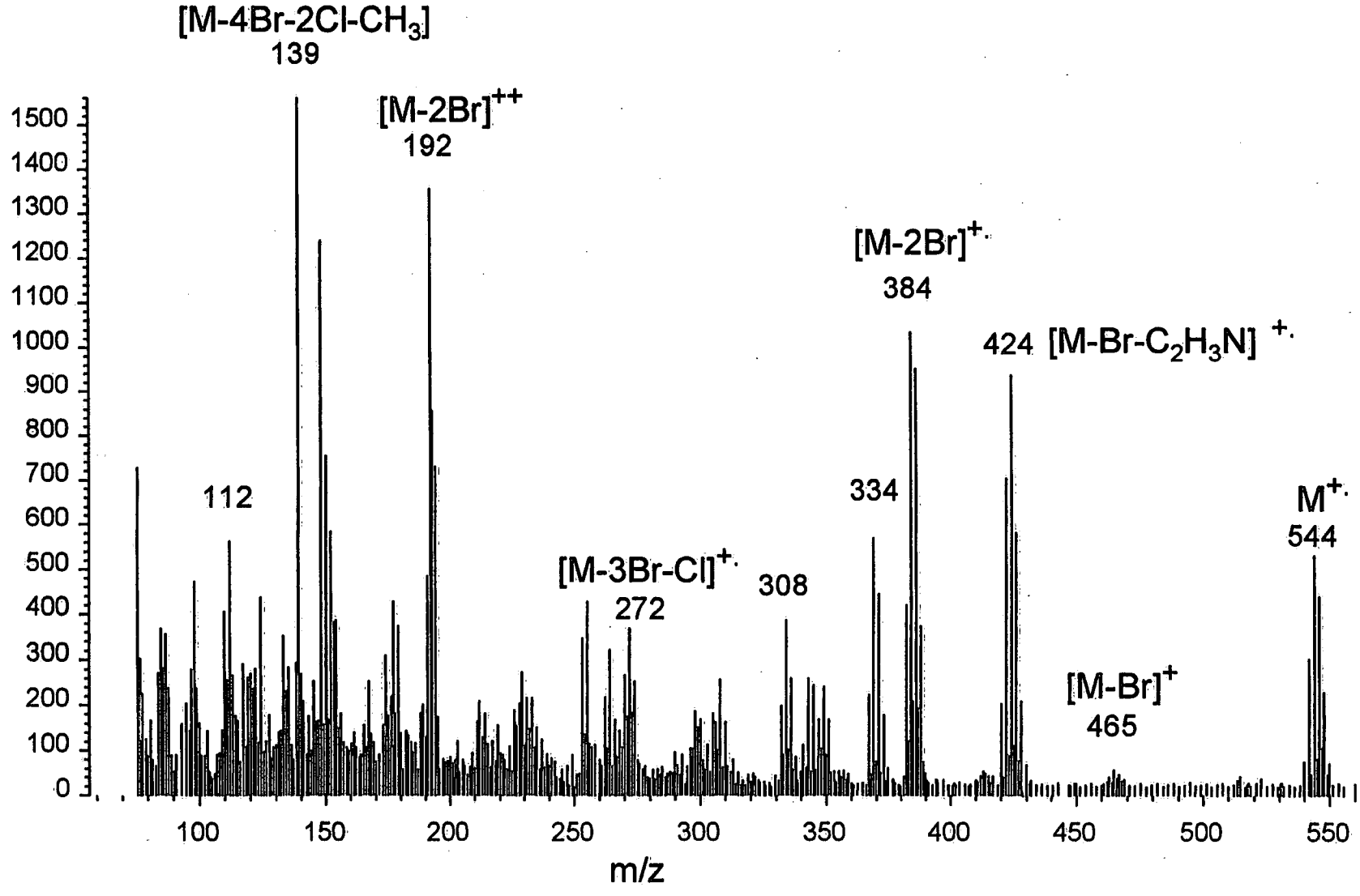
GC-ECNI-MS TIC



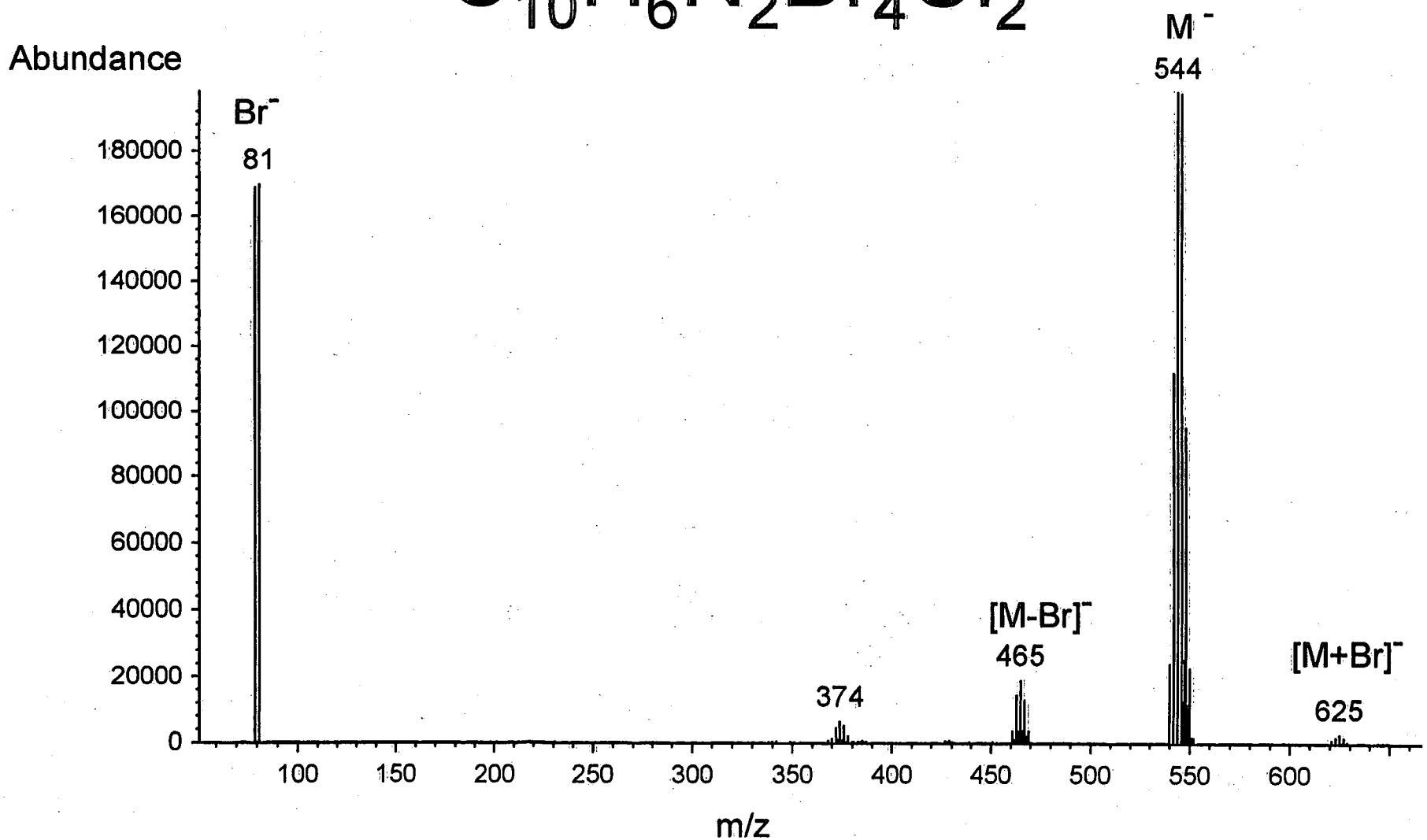
Identification

- EI, ECNI, PCI-MS
  - halogen content, molecular ion
- high resolution MS
  - molecular formula: $C_{10}H_6N_2Br_4Cl_2$
- NH_3/ND_3 isotope exchange PCI-MS
  - two 3° nitrogen atoms

EI spectrum - $C_{10}H_6N_2Br_4Cl_2$

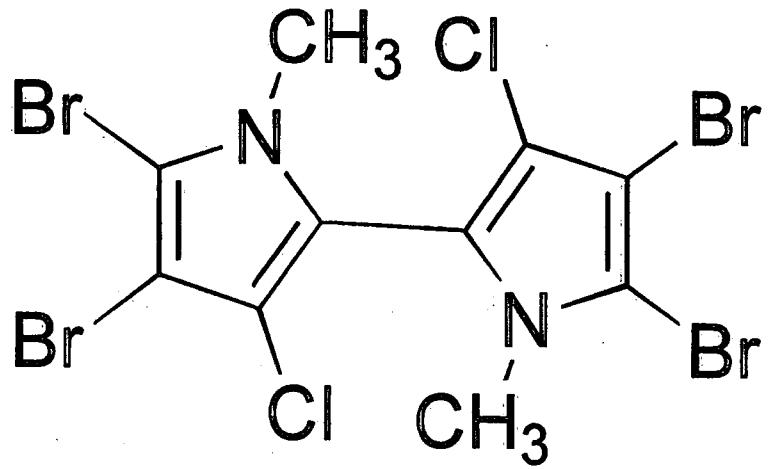


ECNI spectrum - $C_{10}H_6N_2Br_4Cl_2$



Identification

- structure
  - comparison to synthesized products



Physical Properties

- vapour pressure (GC-RT)
  - 1.4×10^{-4} to 2.65×10^{-5} Pa
- water solubility (generator column)
  - 8.2×10^{-8} g/L for $C_{10}H_6N_2Br_6$
- log Kow (FCM estimated)
  - 7.3 for $C_{10}H_6N_2Br_6$
- similar to higher chlorinated PCBs

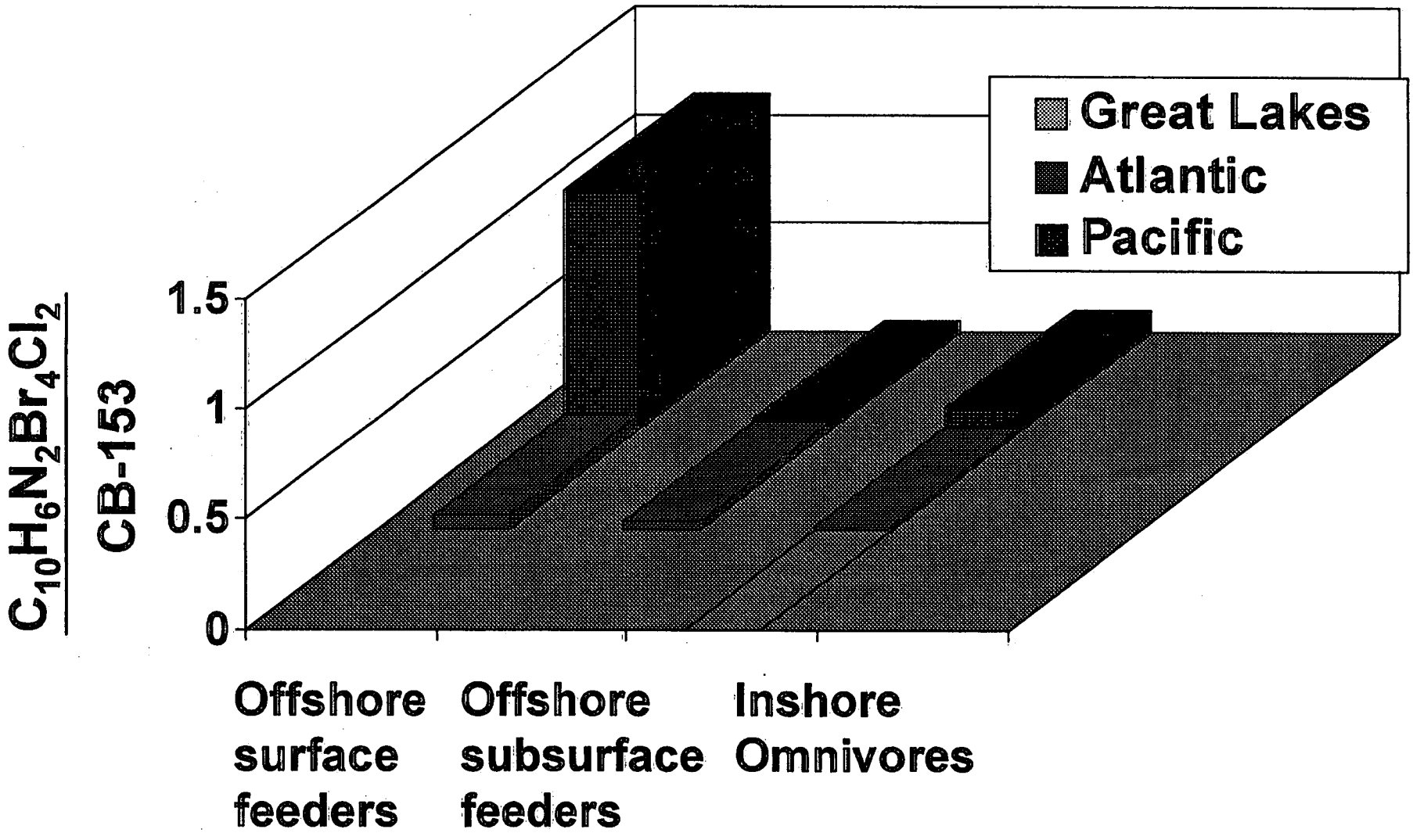
Distribution of $C_{10}H_6N_2Br_4Cl_2$

- found in
  - elephant seal (150 ppb)
  - gray whale
  - minke whale
  - killer whale
  - sperm whale (~ 2 ppb)
  - pilot whale (~ 100 ppb)
  - bald eagle liver (5000-5 ppb)
  - great blue heron eggs (~ 0.4 ppb)
  - double-crested cormorant eggs (~0.4 ppb)

Distribution of $C_{10}H_6N_2Br_4Cl_2$

- measured $C_{10}H_6N_2Br_4Cl_2$ and CB-153 in eggs from Great Lakes, Pacific, and Atlantic regions of Canada (ES&T, 1999, *33*, 26-33)
- took ratio of $C_{10}H_6N_2Br_4Cl_2$ to CB-153 to normalize to levels of other halogenated compounds

Mean relative concentrations



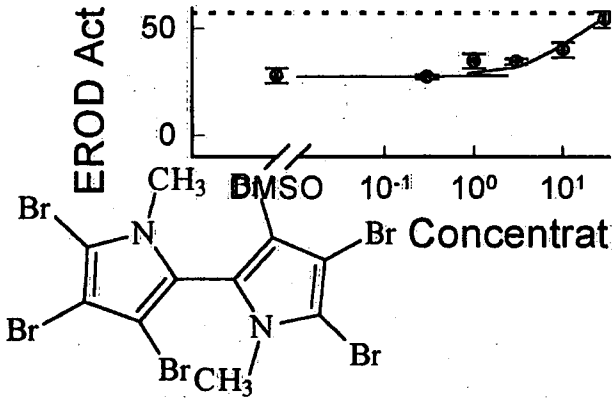
Results

- $C_{10}H_6N_2Br_4Cl_2$ not detected in any Great Lakes samples
- offshore surface feeders had higher concentrations than other two groups in Pacific and Atlantic samples
- concentrations were higher in offshore surface feeder samples from Pacific than Atlantic

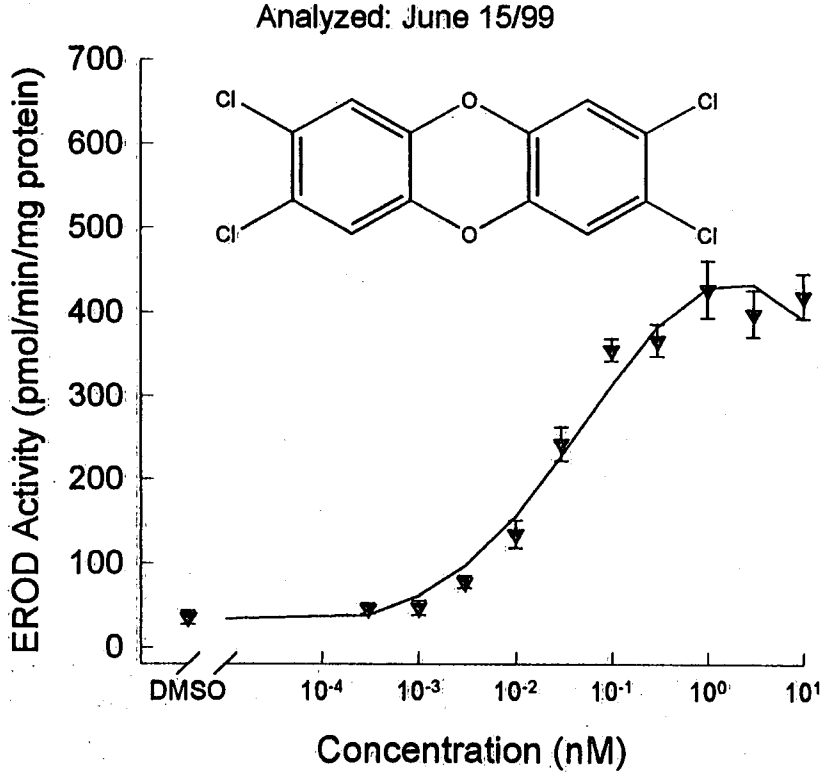
Toxicology

- tested CYP1A inducing capabilities of $C_{10}H_6N_2Br_6$
- CYP1A activity estimated by measuring EROD activity
- bioassay uses chicken embryo hepatocyte (CEH) cultures

CEH EROD assay



EC_{50} = 8.2



EC_{50} = 0.0303

CEH EROD assay results

	TCDD EQ
TCDD	1.0
CB-105	0.005
$C_{10}H_6N_2Br_6$	0.004
CB-118	0.0008

Future work

- worldwide distribution of HDBPs in marine mammals
- synthesis and purification of HDBP congeners
- toxicity testing on purified HDBP congeners
  - aquatic, mammalian

Acknowledgements

- lab work and analyses performed at NWRC
- water solubility measurements performed at Carleton University
- Mary Simon, CWS
- David Blank, Gordon Gribble, Dartmouth College
- John Elliot, Neil Burgess, Chip Weseloh, CWS
- Wally Jarman, U of Utah
- John Giesy, Michigan State University
- Richard Jeffries, Sean Kennedy, NWRC

ESTIMATION OF HUMAN EXPOSURE IN CANADA TO POLYBROMINATED DIPHENYL ETHERS (PBDPES)

John Jake Ryan, W. Harvey Newsome, and Benoit Pâtry

PBDPEs have recently been shown to occur in both environmental and biotic media in significant amounts particularly in Europe. Current data indicates that concentrations of these persistent organic pollutants (POPs) are increasing whereas those of most of the other POPs appear to be decreasing. In North America, some data exists on wildlife but no information is available on levels in consumable foods (human intake) or human body burdens (uptake). Both of these parameters are essential in order to define health effects and to judge the possible risk. It is proposed to study the exposure question in Canada by the analysis of human milk and fatty animal foods.

This issue will be addressed firstly by developing and modifying existing trace organic analytical methodology presently in use at Health Canada for the specific and reliable determination of the PBDPE congeners in biotic samples. These methods involve sample extraction, fat removal with either strong acid or gel permeation, followed by further purification with silica and magnesium silicate (Florisil) adsorbants. Determination is by gas chromatography with either mass spectrometry or electron capture detection. Other techniques already researched by Fisheries and Oceans Canada will be adapted to the in-house Health Canada methods along with the identical carbon-12 primary and carbon-13 surrogate standards commonly employed by all laboratories in the TSRI project. Validity of analytical results will be assessed by comparison of analytical data from common samples analysed by all participating laboratories i.e. an interlaboratory check sample program.

The established analytical methodology will then be applied to two types of biotic samples. PBDPEs will be measured in human milk obtained from across Canada in the 1990's. These samples have been collected from all provinces with a view of obtaining an overview of the general population exposure and body burdens. Lipid adjusted levels of persistent organochlorines in humans give reliable measurements of body burdens. From previous work with other organochlorine compounds, it is to be expected that levels in this tissue type will be relatively elevated and not too difficult to measure. In fact, an attempt will be made to carry out some of the determinations with gas chromatographic separation and electron capture detection. Further biotic sample examination will be carried out on fatty food composites (mostly dairy and meat products) taken from the Health Canada total diet program. This program, as applied to exposure from POPs, targets fatty animal foods of high consumption as purchased commercially across Canada and prepared in normal kitchen practise. A combination of level in food and its consumption amount gives an estimate of the daily intake. In the total diet samples, measurement will be made by necessity with gas chromatography-mass spectrometry.

Completion of this three part approach, methodology development, analysis of human milk and common foods should give human intake and exposure data. This information will allow regulators the tools to make informed decisions about the occurrence and human health risks of these brominated compounds. Future work will focus on the exposure of selected populations, the analysis of specialized foods, and the application of the methodology to the analysis of human blood.

Estimation of human exposure in Canada to polybrominated diphenyl ethers (PBDPEs)

Proposed study

John Jake Ryan, W. Harvey Newsome, and Benoit Patry,

Bureau of Chemical Safety, Food Directorate, Health Protection Branch,
Health Canada, Ottawa, Ontario.

Introduction:

- Recent studies show significant amounts of PBDE in environment and biotics in Europe.
- Concentrations appear to be increasing contrary to most other POPs (Persistent Organic Pollutants)
- Little or no information in North America for amount in foods (intake) and human body burdens (uptake).
- This information is essential to define health effects and possible risk.

Definition of POPs

- A- Bioaccumulate - solubility low in water and high in lipid.
- B- Travel Long Distances - semi-volatile.
- C- Persist - resistant to physical, chemical and biological agents.
- D- Adverse effects - affects organisms in a dose related manner.

- Ex: Chlordane, DDT, Dioxins/Furans, PCBs, HCB, etc…

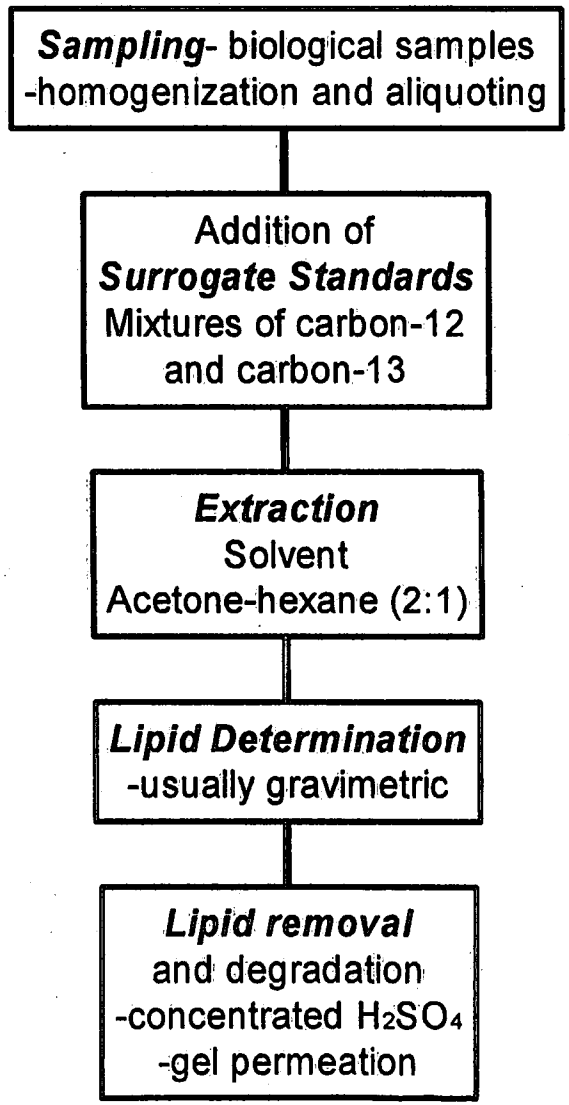
- Other Possible POPs: Hexachlorocyclohexanes (HCH), Polyaromatic hydrocarbons (PAHs), PBDE etc...

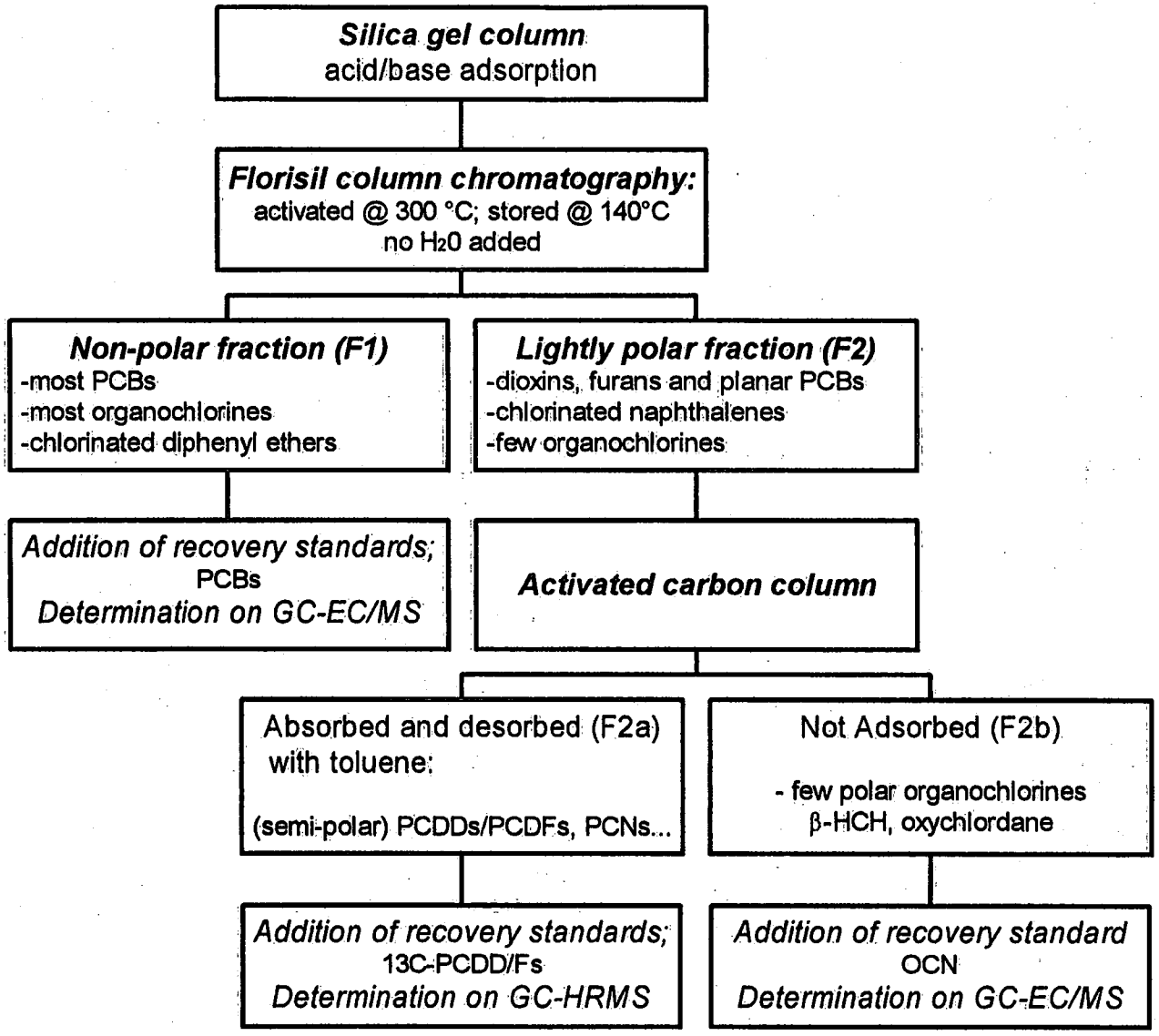
To study the exposure in Canada, we propose to analyze Canadian human milk and fatty animal foods;

- By developing and modifying our analytical methodology for trace POPs, to perform specific and reliable determination of PBDPE in biotic samples.

- Apply validated method to determine levels in the Canadian milk and foods samples.

Health Protection Branch method for Persistent Organic Pollutants




Silica gel column
acid/base adsorption
Florisil column chromatography:
activated @ 300 °C; stored @ 140°C
no H20 added
Non-polar fraction (F1)
-most PCBs
-most organochlorines
-chlorinated diphenyl ethers
Lightly polar fraction (F2)
-dioxins, furans and planar PCBs
-chlorinated naphthalenes
-few organochlorines
Addition of recovery standards;
PCBs
Determination on GC-EC/MS
Activated carbon column
Absorbed and desorbed (F2a)
with toluene:
(semi-polar) PCDDs/PCDFs, PCNs...
Not Adsorbed (F2b)
- few polar organochlorines
β-HCH, oxychlordane
Addition of recovery standards;
13C-PCDD/Fs
Determination on GC-HRMS
Addition of recovery standard
OCN
Determination on GC-EC/MS

Method Development/Validation

- Common ^{12}C primary and ^{13}C surrogate standards solution for all participating laboratories.

- Results validated by comparing data from check samples analyzed by all participating laboratories.

Human Exposure

A- Human Milk

- 60 human milk samples collected in 1992 from all provinces to evaluate exposure and body burdens.

- Levels expected to be easy to detect due to relatively high fat content in milk and lipophilic properties of PBDE.

- Lipid adjusted levels (fat based concentrations) for organohalogenated compounds give reliable measurements of human body burdens.

Human Intake

B- Foods

- 60 Fatty foods samples (mostly dairy and meats) taken from the Health Canada Total Diet Program, collected in 1996 across Canada.

- This program applied to exposure from POPs in fatty animal foods, collected across Canada, prepared in normal kitchen practice.

- Food levels X consumption amount = daily intake

Conclusion

- After the method is developed, human milk and food samples analyzed, PBDPE human intake and exposure data will be available for regulatory purposes.

- Informed decisions about occurrence and human health risks of PBDPEs.

Future Work

- Exposure of selected populations.
- Analysis of specialized foods.
- Application of the methodology to human blood.

TOXICOLOGICAL ASSAYS FOR POLYBROMINATED DIPHENYL ETHERS

Nigel J. Bunce

As halogenated aromatic compounds, PBDEs suggest the possibility of Ah receptor-mediated toxicity, as occurs for chlorinated aromatic compounds such as PCBs, PCDDs, and chlorinated diphenyl ethers. We shall carry out a suite of *in vitro* bioassays on individual PBDEs, commercial PBDE mixtures, and environmental samples. One focus will be the steps leading to the induction of monooxygenase enzymes (cytochrome P-450 1A1). Although not in itself a toxic response, this acts as a well-established marker of exposure of wild life to aromatic xenobiotic compounds. The assays to be used are all running in our laboratory; however, we hope to be able to bring improved analytical methodology to them as part of this research program.

AhR Binding Assay: "Dioxin-like" activity is a multi-step response that is initiated by binding the toxicant to the Ah receptor. Not all ligands actually cause toxicity; some are antagonistic and others simply unproductive. The Ah receptor binding activity monitors all these possibilities. Currently our laboratory uses the traditional method of competition between the ligand(s) and radiolabelled reference ligand for a fixed aliquot of Ah receptor preparation; the experiment consists of measuring the *decrease* in radioactivity that is bound to the receptor as the concentration of the unlabelled competitor increases. In our experiments we shall use 1 nM radiolabelled 2,3,7,8-TCDD as the reference radioligand; PDBEs will be used as unlabelled competitors. We are exploring the possibility of replacing the use of radiolabelled TCDD with a fluorescently labelled TCDD analog, in which case, the assay could be carried out more simply by means of analysis of samples using capillary electrophoresis.

Post AhR binding Assays: The gel shift assay monitors the association of ligand-bound Ah receptor to specific recognition sites (dioxin response enhancers, or DREs) on DNA) to determine the effectiveness of PBDEs in activating the Ah receptor to the DNA-binding state. The assay employs a synthetic 32-mer double-stranded oligonucleotide that contains the consensus DRE recognition sequence and that is terminally bonded to ^{32}P-phosphate. We presently carry out this assay by slab gel electrophoresis and autoradiography. This method is time consuming and difficult to quantitate. We are currently attempting to develop a capillary electrophoretic method to carry out this assay faster and with greater reproducibility through the use of a fluorescently labelled oligonucleotide. We are also developing a kinase assay to monitor the effectiveness of Ah receptor ligands with respect to endpoints that do not involve activation of the DRE-binding transcription factor. Kinase activation appears to be a major second pathway to account for non-P-450 toxic endpoints upon exposure to halogenated aromatic compounds. This assay is not yet in place, but will be used as and when it is available.

EROD Assay: EROD (ethoxyresorufin-O-deethylase) activity is an enzyme assay that monitors the production of cytochrome P450 1A1. This Ah receptor-mediated response is an endpoint of the DRE transcriptional activation pathway that is often taken as a proxy for toxicity. Primary rat hepatocytes are incubated with the PBDE congener; the activity of the cells in causing the deethylation of the substrate ethoxyresorufin to the fluorescent product resorufin is measured 24 h later.

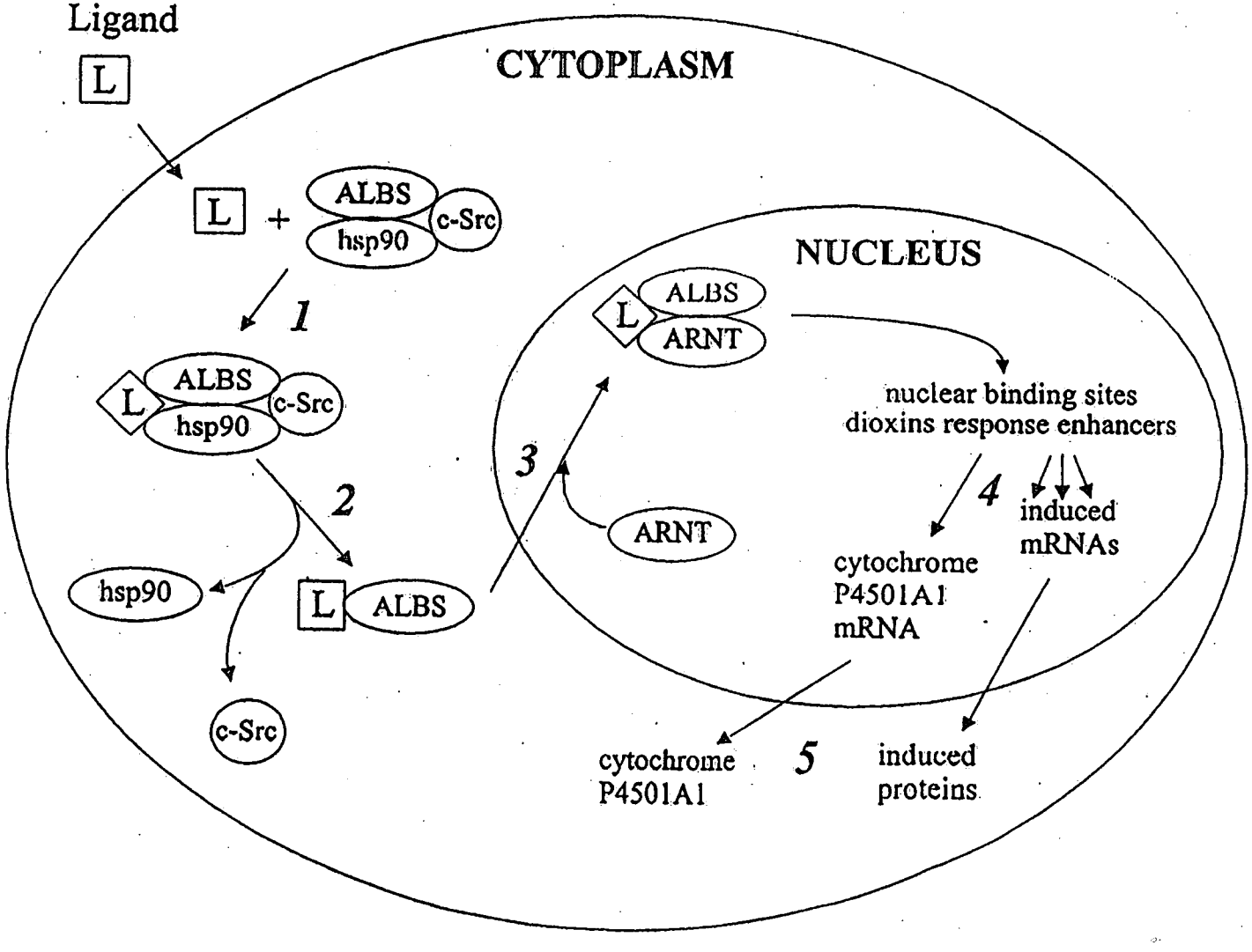
In vitro toxicological assays for PBDPEs

Nigel J. Bunce
Department of Chemistry and Biochemistry
University of Guelph

Overall goals

- Suite of in vitro bioassays:
  - Ah receptor binding
  - post AhR binding assays
  - EROD induction
- Used with:
  - individual PBDPE congeners
  - synthetic and commercial mixtures
  - environmental samples

Ah receptor-mediated mechanism of action of HAHs



PBDPEs as halogenated aromatic compounds

- Do PBDPEs have dioxin-like or PCB-like properties:
  - Ah receptor binding?
  - P-450 induction?
- Polychlorinated diphenyl ethers do show AhR binding

Ah receptor binding

- Varying concentrations of target congener(s) compete with fixed concentration of radiolabelled TCDD and fixed aliquot of AhR
- Obtain relative binding affinities by probit method
- No binding = no AhR mediated toxicity

AhR binding of PCDPEs

Relative binding affinity: 2,3,7,8-TCDD = 1

3,3',4,4'-tetrachloro	0.010
3,3'4,4'5-pentachloro	0.014
2,3',4,4',5-pentachloro	0.0077
2,3'4',6-tetrachloro-	0.00012

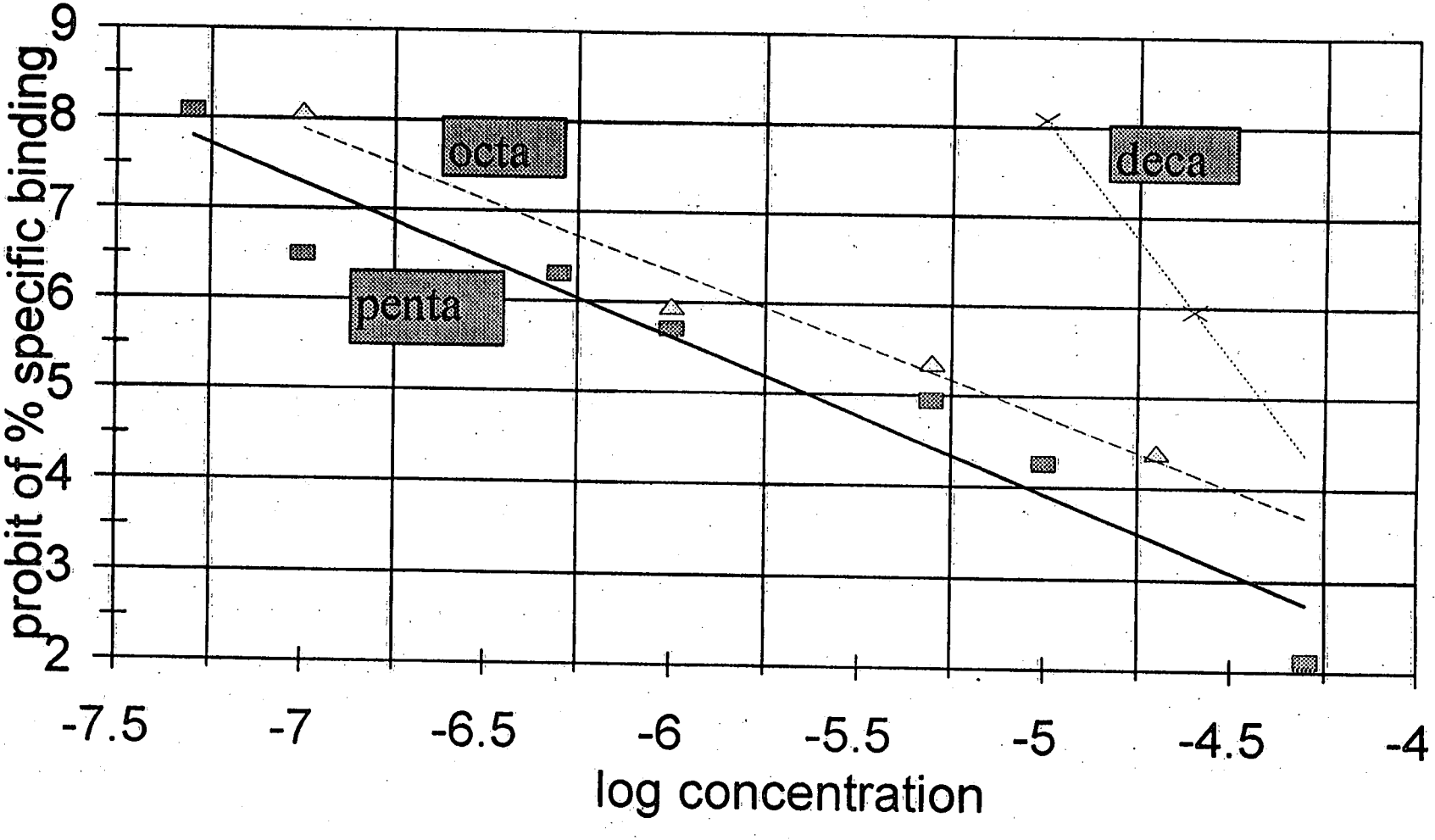
(unpublished observations)

Preliminary experiments with commercial PBDPEs

- Ah receptor binding observed
- RBAs are in the same range as PCDPEs
  - penta 4.3×10^{-4}
  - octa 1.4×10^{-4}
  - deca 2.6×10^{-5}

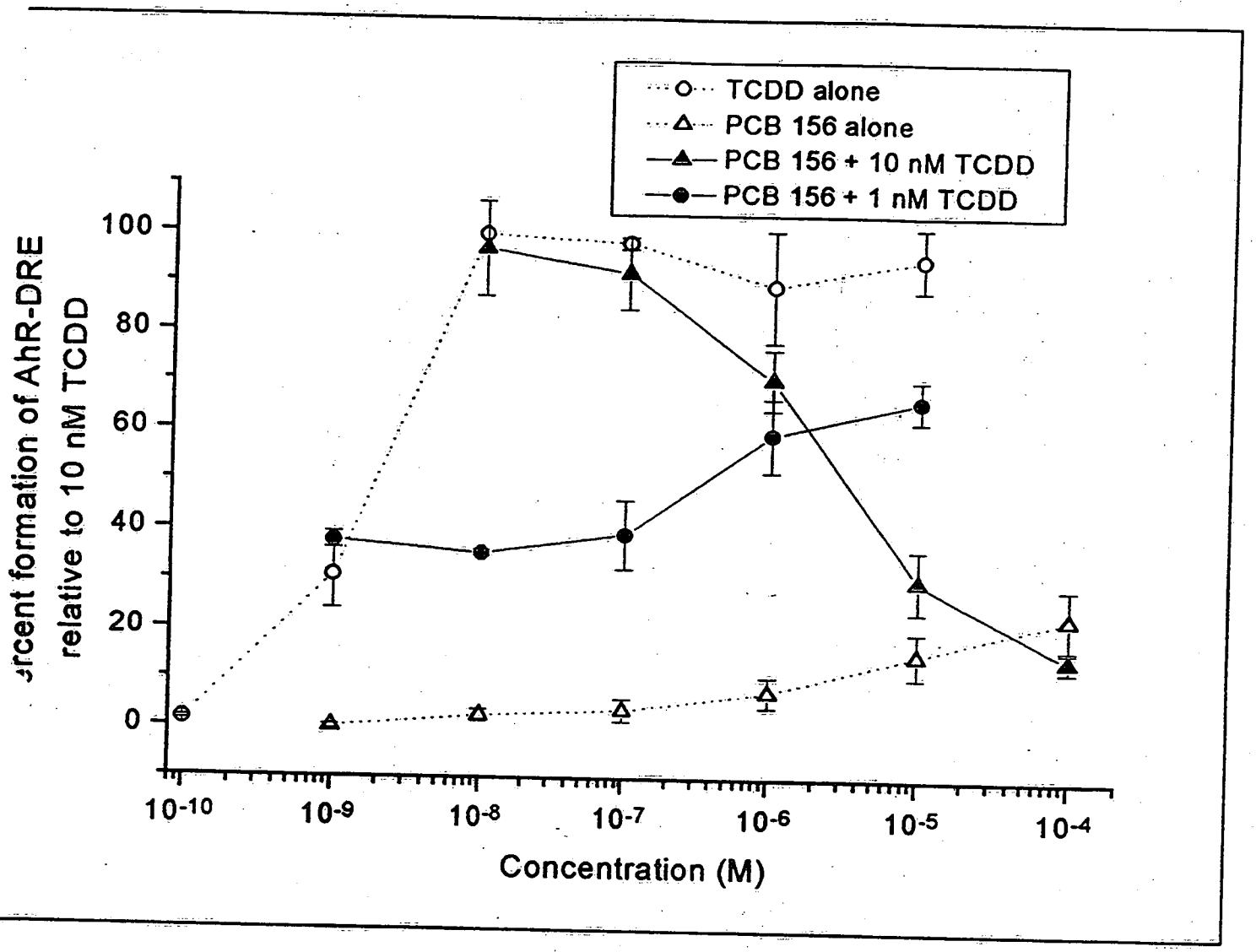
- G. Chen, unpublished

Commercial PBDPEs: AhR Binding



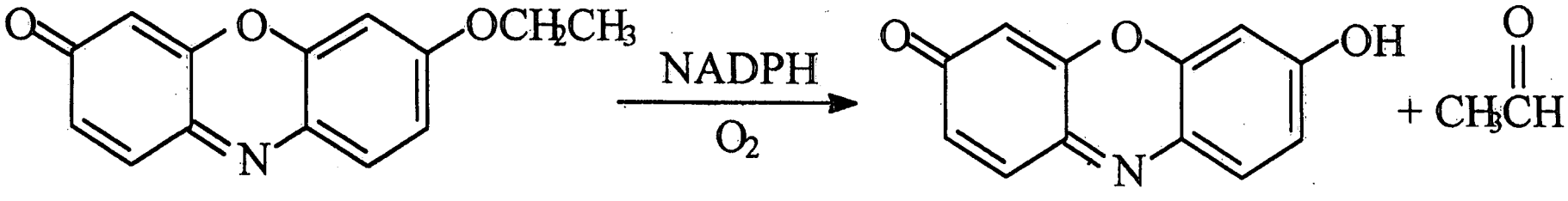
Post-binding Assay I: Gel shift

- Objective: does bound AhR-PBDPE complex transform to DRE binding form?
- Incubate with ^{32}P-labelled 32-mer oligonucleotide; look for the presence of a shifted band; measure intensity (densitometry)
- Single PBDPE congener ± TCDD; commercial PBDPE ± TCDD
- Plans: modify to use fluorescent label and CE analysis


TCDD alone
PCB 156 alone
PCB 156 + 10 nM TCDD
PCB 156 + 1 nM TCDD
rcent formation of AhR-DRE
relative to 10 nM TCDD
100
80
60
40
20
0
10-10
10-9
10-8
10-7
10-6
10-5
10-4
Concentration (M)

Post-binding Assay II: EROD Assay

- CYP 1A1-catalyzed conversion of ethoxyresorufin to fluorescent resorufin
- CYP 1A1 activity α I_F

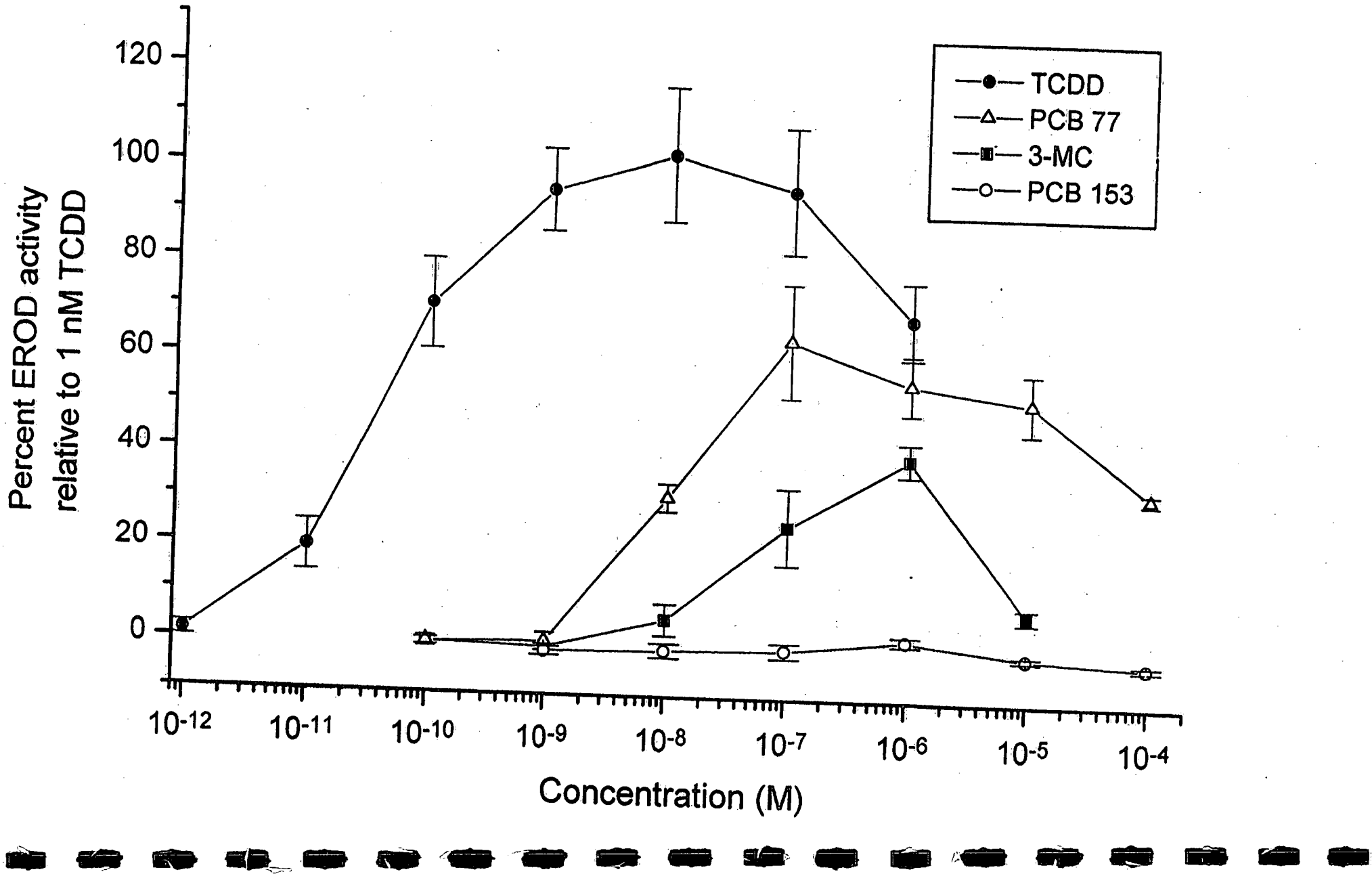


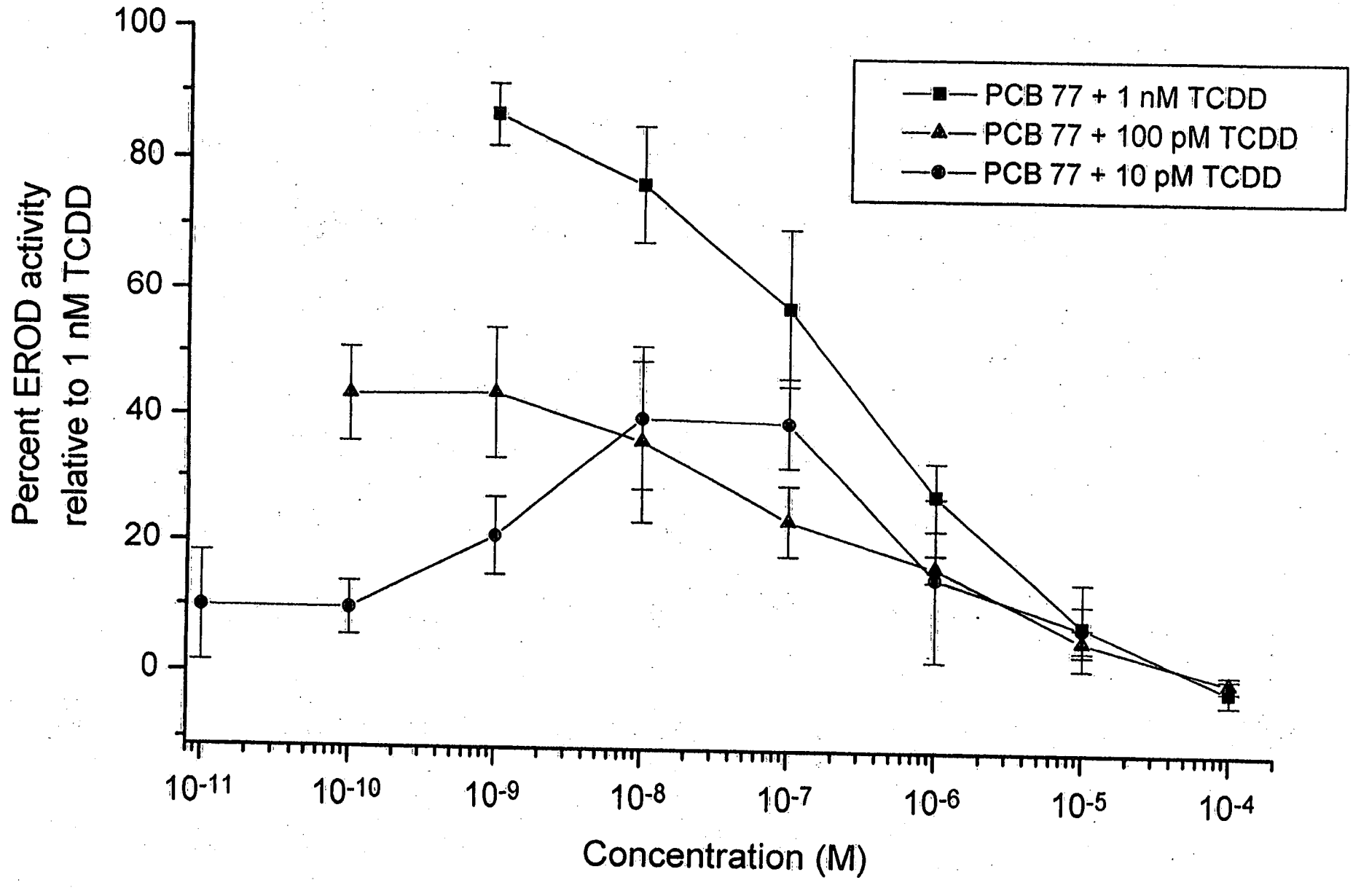
7-ethoxyresorufin

resorufin

EROD Assay

- Incubate primary rat hepatocytes with PBDPE congener or mixture; after 24 h, add ethoxyresorufin and measure fluorescence intensity after brief incubation
- Must check for inhibition of EROD reaction
- Plan to examine the production of P-450 enzyme


Percent EROD activity
relative to 1 nM TCDD
120
100
80
60
40
20
0
TCDD
PCB 77
3-MC
PCB 153
10-12
10-11
10-10
10-9
10-8
10-7
10-6
10-5
10-4
Concentration (M)


PCB 77 + 1 nM TCDD
PCB 77 + 100 pM TCDD
PCB 77 + 10 pM TCDD
Percent EROD activity
relative to 1 nM TCDD
100
80
60
40
20
0
10-11
10-10
10-9
10-8
10-7
10-6
10-5
10-4
Concentration (M)

Post-binding Assay III: c-src Kinase

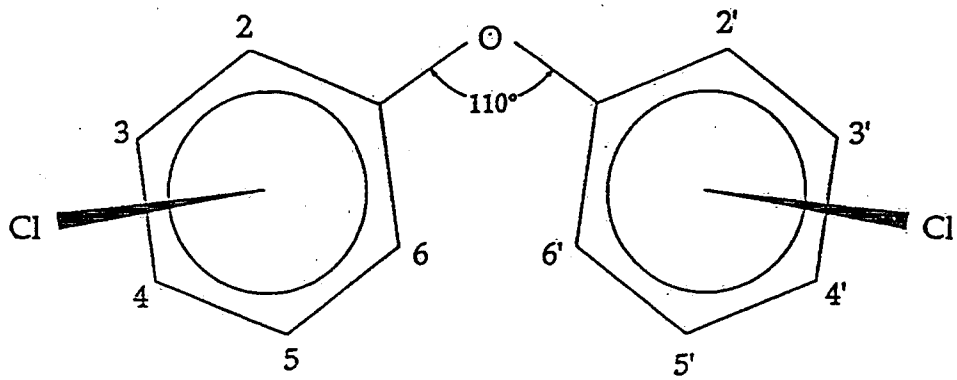
- Probes the non-P-450 mechanisms
- Measure kinase activity following incubation of cytosol with HAC, using ^{32}P-ATP and synthetic peptide that mimics c-src binding site
- Single PBDPE congener ± TCDD; commercial PBDPE ± TCDD
- Plans: modify to use fluorescently labelled synthetic peptide and CE analysis

TOXICITY OF PBDEs TO AQUATIC ORGANISMS: LESSONS LEARNED FROM PCDEs.

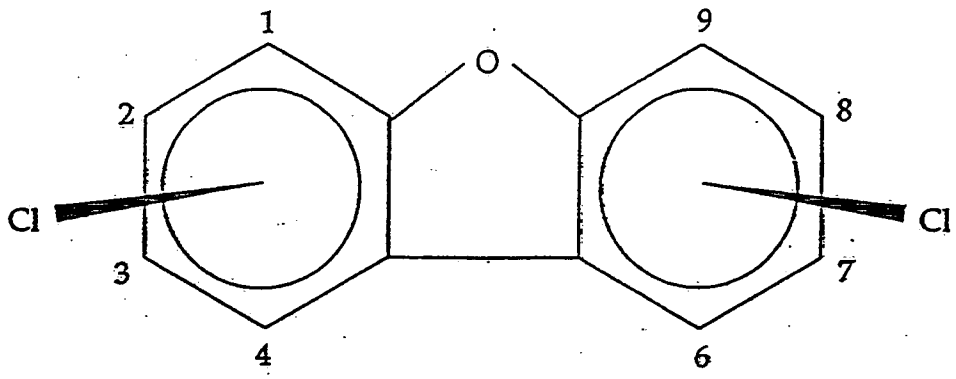
Chris Metcalfe

In previous research conducted in my laboratory, several polychlorinated diphenyl ether (PCDEs) congeners were synthesized and tested for embryotoxic potency in an in vivo assay with early life stages of the aquarium fish, the Japanese medaka, and several of these congeners induced mortalities with moderate potency. However, these compounds did not induce lesions in medaka indicative of toxicity through Ah-receptor mediated mechanims, such as edematous lesions. These results with PCDEs may indicate that PBDEs do not induce biological responses in fish through Ah-receptor mediated mechanisms, although this hypothesis requires testing. Because of the close structural similarity between halogenated diphenyl ethers and thyroxine (T3), this class of contaminants may act as thyroid agonists. Few assay systems are available to screen for thyroid hormone disruptors in aquatic species, but I propose to expose fish (i.e. medaka, trout) to commercial PBDE mixtures and single congeners and screen for induction of hypo- or hyperthydroidism by histological evaluation of the thyroid gland. I also propose screening these compounds for thyroid disruption by determining whether they accelerate or inhibit regression of the tails of Xenopus tadpoles; a process that is under the control of thyroid hormones in amphibians.

Chlorinated Diphenyl Ethers



Chlorinated Dibenzofurans



SOURCES OF CHLORINATED DIPHENYL ETHERS (CDPEs)

a) Impurities in chlorophenol products (100-1000 ppm).
b) Impurities in phenoxy herbicides.
c) Incinerator fly ash.
d) Used PCB transformer fluids.
e) Heat transfer fluids (Dowtherm).

CHLORINATED DIPHENYL ETHERS IN GREAT LAKES FISH

Location	Species	CDPE Conc. (ppb)
L. Superior	Lake trout	3.8 (1.7-6.9)[a]
L. Huron	Lake trout	16.0 (6.1-29.3)[a]
L. Erie	Walleye	56.6 (34.9-105.6)[a]
L. Ontario	Lake trout	126.5 (54.2-303.4)[a]
Whitby Hbr, Ontario	Pike	2445 (1053-4959)[b]

a) Niimi, Metcalfe and Huestis, 1994.

b) Huestis and Sergeant, 1992.

Relative Concentrations of PCDEs and PCDFs in Fish

Samples	Concentration (ng/g wet weight)
L. Ontario Lake Trout:	
PCDEs	126.5
PCDFs	1.2
Whitby Hbr. Pike:	
PCDEs	14,000
PCDFs	11.5

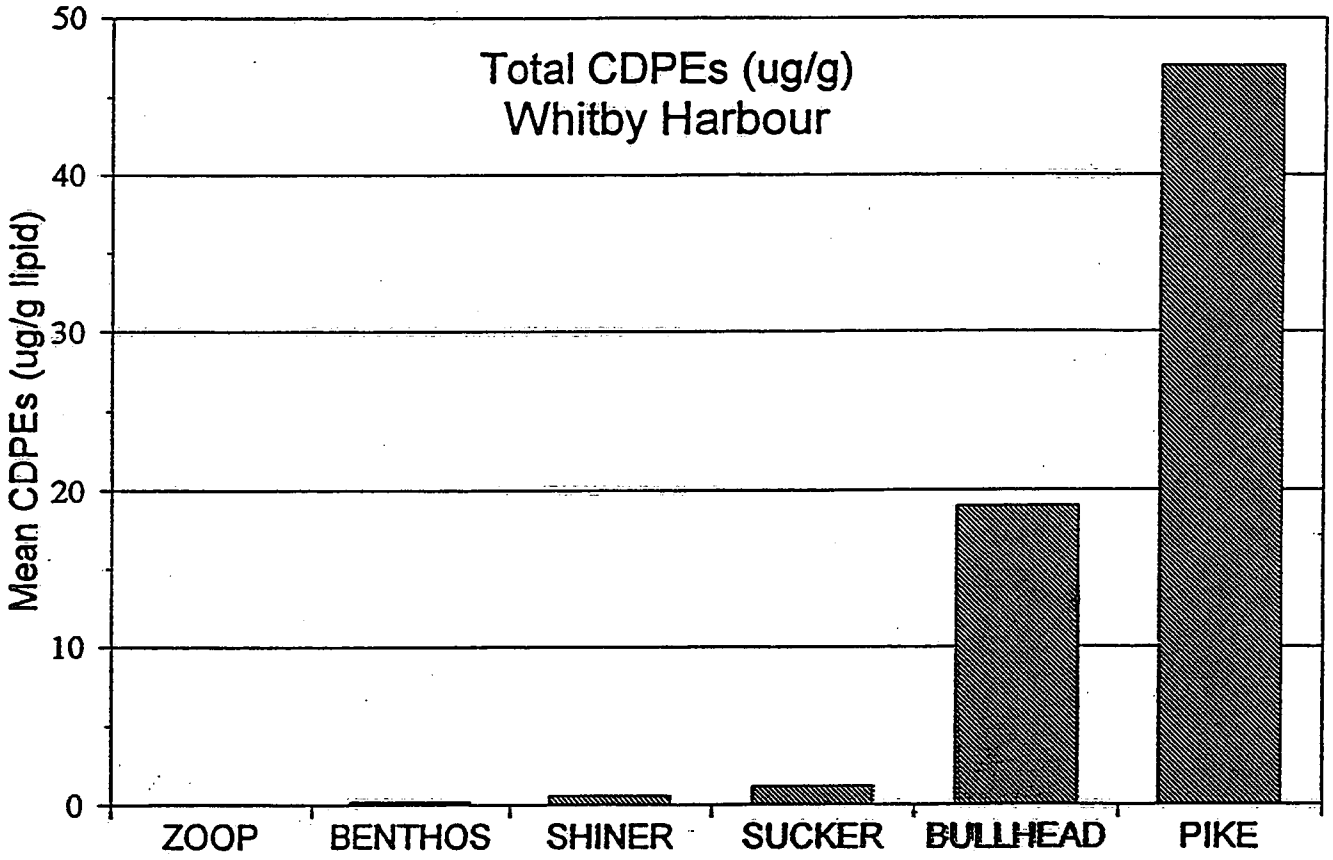


Figure 2: Mean concentrations (µg/g lipid) of total CDPEs in biota from the food-web in Whitby Harbour, Lake Ontario.

JAPANESE MEDAKA
Embryotoxic End-Points

1) Cumulative mortality (Day 17)
2) Hatching success
3) Swim-bladder inflation
4) Developmental abnormalities
5) Toxicopathic lesions

EMBRYOTOXICITY TO MEDAKA

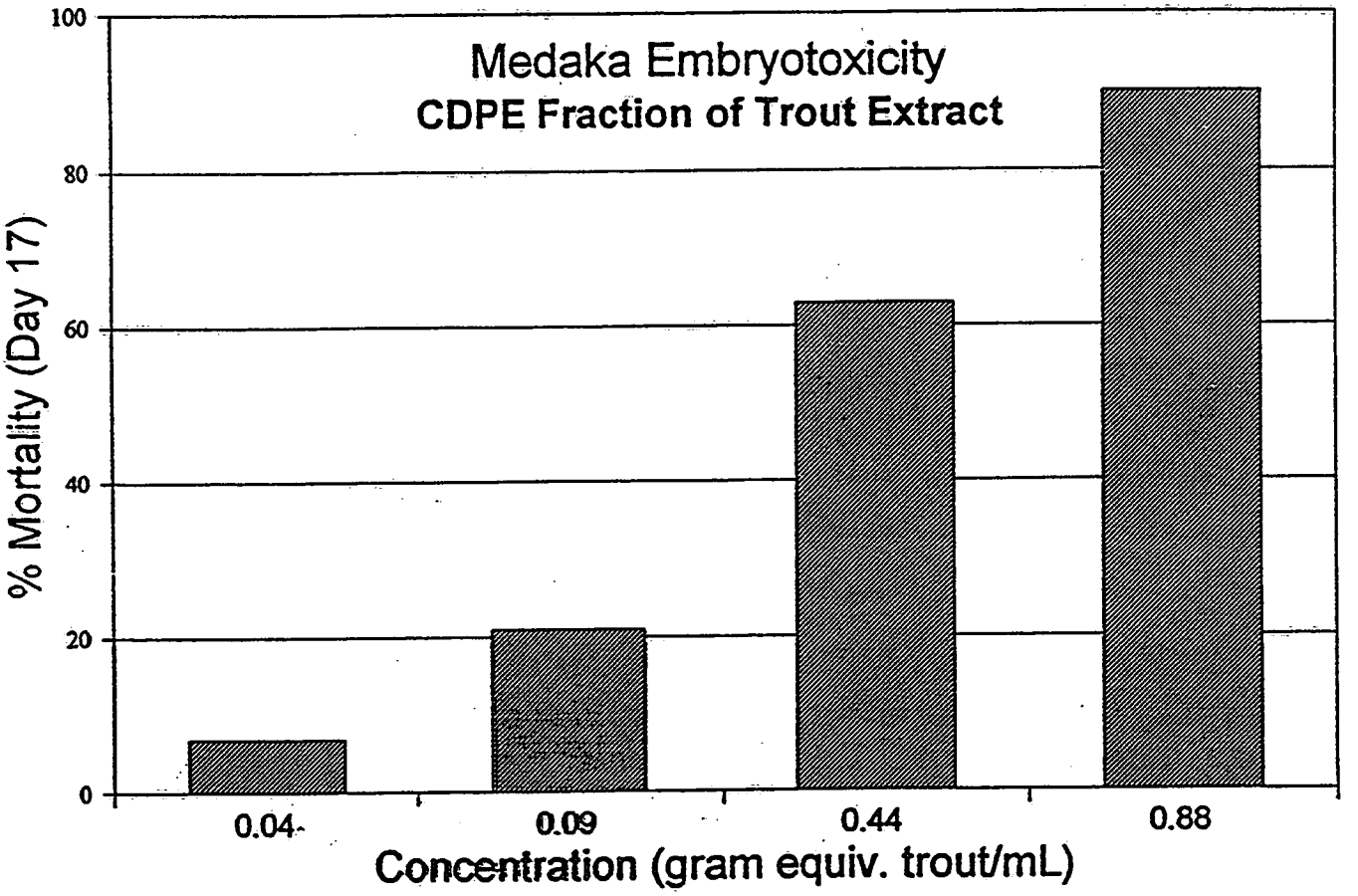
Compound	LC50 (ng/mL)	TEF
2,3,7,8-TCDD	0.0057	1
PCDEs:		
105 (2,3,3',4,4'-Penta)	10.8	0.00056
77 (3,3',4,4'-Tetra)	170.6	0.00003
118 (2,3',4,4',5-Penta)	605.2	0.00001
71 (2,3',4',6-Tetra)	>2,500	-

TIE APPROACH

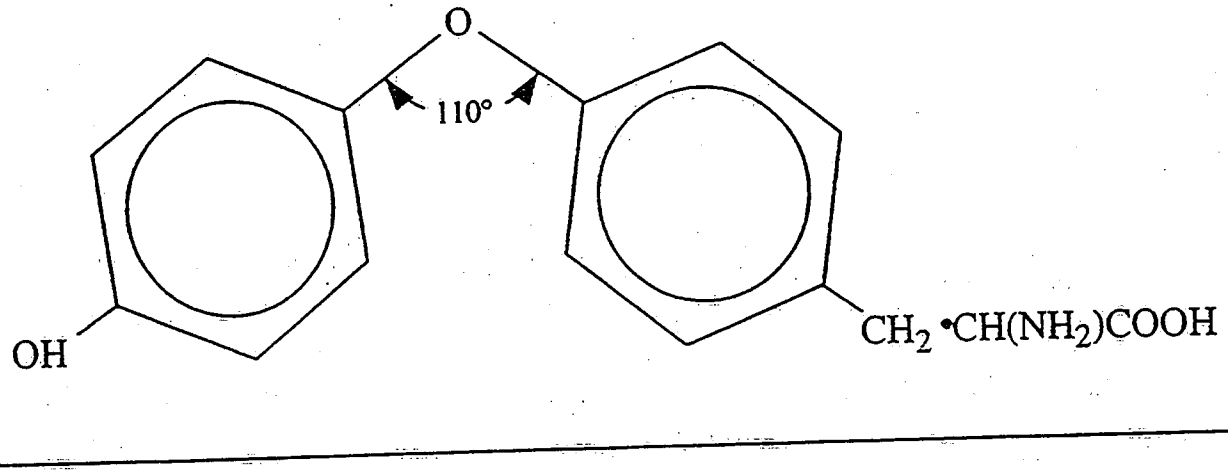
Subfractionation of Extract from Lake Ontario Lake Trout

↕

Medaka Embryoxicity


Medaka Embryotoxicity
CDPE Fraction of Trout Extract
% Mortality (Day 17)
100
80
60
40
20
0
0.04
0.09
0.44
0.88
Concentration (gram equiv. trout/mL)

Thyroxine



THYROID HORMONES
(Thyroxine)

Affects more physiological and developmental pathways than any other hormone:

- Metamorphosis of amphibians & some fish

- Enhances:
  - Growth rate
  - Bone & tooth growth
  - Fin ray development in fish
  - Moulting
  - Limb regeneration
  - Reproductive development
  - CNS development

- Controls:
  - Nervous system function
  - Uptake of glucose, Vitamin A
  - Nitrogen metabolism

ENDOCRINE DISRUPTORS OF THYROID FUNCTION

1. Thyroxine agonists (e.g. PCDDs)

2. Binding to carrier proteins (e.g. PCBs)

Hypothalamus

↕

TRH

↕

Pituitary (Hypophesis)

↕

TSH

↕

Thyroid

↕

T3 (& T4)

↕

Binding Protein

Polybrominated Diphenyl Ethers

In vivo Test Protocols

Medaka embryotoxicity - TEFs

Thyroid histology

- ☛ Medaka (aqueous exposure)
- ☛ Trout (i.p. exposure)

Amphibian metamorphosis

- ☛ Xenopus (aqueous exposure)

THYROID HISTOLOGY
(Medaka and Trout)

Endpoints:

Hypothyroidism

Hyperthyroidism (Goiter)

AMPHIBIAN METAMORPHOSIS
(Xenopus laevis)

Endpoints:

Tail resorption

Hind-limb formation

CHEMICAL DISRUPTION AND MEASUREMENT OF THYROIDAL STATUS IN FISH

Scott Brown

The most commonly used indices of fish thyroidal status are based on thyroxine (T4) secretion by thyroid tissue under control of the central brain-pituitary-thyroid axis. However, much of the control of the fish thyroid system also occurs in peripheral tissues, such as liver, by regulating T4 prohormone conversion to biologically active 3,5,3'-triiodothyronine (T3) or to biologically inactive 3,3',5'-triiodothyronine and by regulating T3 conversion to inactive 3,3'-diiodothyronine. Some basic differences between fish and mammalian thyroid systems are described. The importance of peripheral indices of fish thyroid status are illustrated by examining the effects of co-planar PCB and Al exposure in trout. It is recommended that peripheral indices (deiodination and possibly excretion pathways) be included in a minimum suite of assays to detect xenobiotic effects on the fish thyroid system.

Chemical Disruption and Measurement of Thyroid Status in Fish

Scott Brown

National Water Research Institute

Multiplicity of Interactions between Polyhalogenated Aromatic Hydrocarbons (PHAH) and the Mammalian Thyroid System

Effects on:

- **Thyroid Gland**
- **Plasma Hormone Levels and Transport**
- **Thyroid Hormone Metabolism**
  - **deiodination**
  - **conjugation**
- **Receptor Binding**

Recent Review:

Brouwer et al. 1998 Toxicol. & Industrial Health 14

PHAHs and Thyroid Status in Fish

Author	Journal	Species	Parameter	Chemical
Alkindi et al.	J. Fish Biol. 1996	flounder	T4, T3	PAH
Besselink et al.	Environ. Toxicol. Chem. 1997	flounder	T4, FT4, T3	TCDD
Besselink et al.	Environ. Pollution 1996	flounder	T4, FT4, T3	Clophen A50
Brown et al.	Environ. Toxicol. Chem. 1998	rainbow trout	T4, T3, histo	PCDF
Folmar et al.	Aquat. Toxicol. 1982	coho salmon	T4, T3	Aroclor 1254
Leatherland & Sonstegard	J. Fish. Res. Board Can. 1978	coho salmon	T4, T3, histo	Aroclors 1242 & 1254
Leatherland & Sonstegard	J. Fish Diseases 1979	rainbow trout	T4, T3, histo	Aroclor 1254& Mirex
Singh	Mar. Environ. Res. 1989	*Monopterus*	T4, T3, TPO, histo	3-methylcholanthrene
Stephens et al.	J. Fish Biol.	Turbot	WB T4, T3	PAH

1. Briefly Review Teleost Thyroid System

2. Describe Some Fundamental Differences between Teleosts and Higher Vertebrates

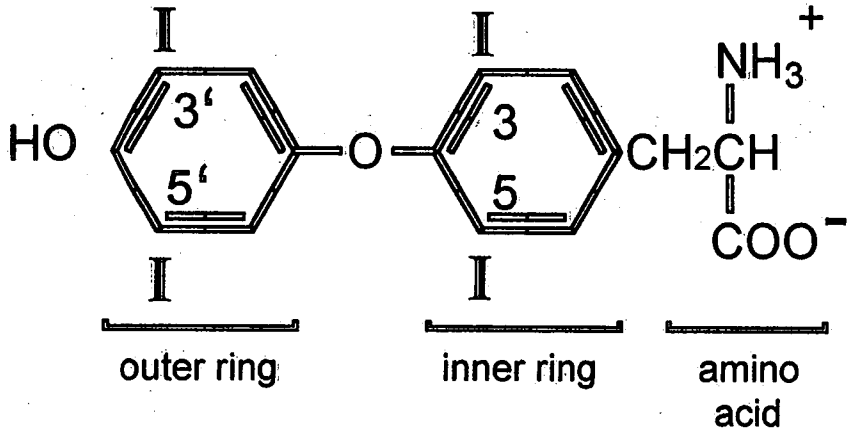
3. Review Effects of Coplanar PCB on Teleost Thyroid Status

4. Discuss Requirements to Assess Thyroidal Status in Teleosts

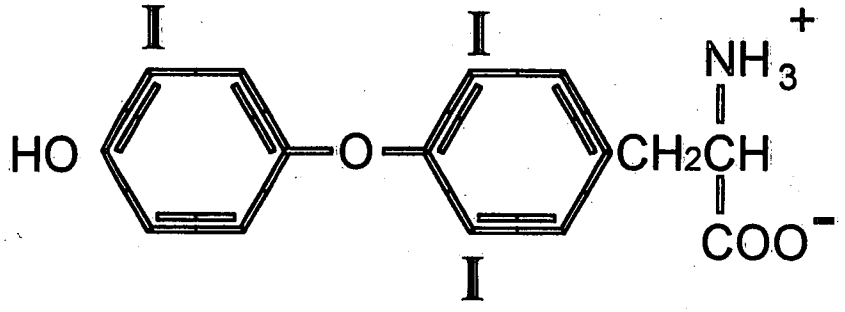
Eales, J.G., and S.B. Brown 1993
Measurement and regulation of thyroid status in teleost fish.
Rev. Fish Biol. 3: 299-347.

Eales, J.G., Brown, S.B., Cyr, D.G., Adams, B.A. and Finnson, K.R. 1999
Deiodination as an Index of Chemical Disruption of Thyroid Hormone Homeostasis and Thyroidal Status in Fish.
Environmental Toxicology and Risk Assessment: Standardization of Biomarkers for Endocrine Disruption and Environmental Assessment: Eighth Volume, ASTM STP 1364,
D.S. Henshel, M.C. Black, and M.C. Harrass, Eds., American Society for Testing and Materials, West Conshohocken, PA.

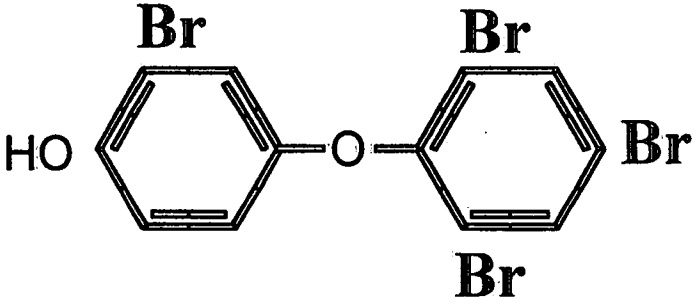
THYROXINE = T4



3,5,3'-TRIIODOTHYRONNE = T3

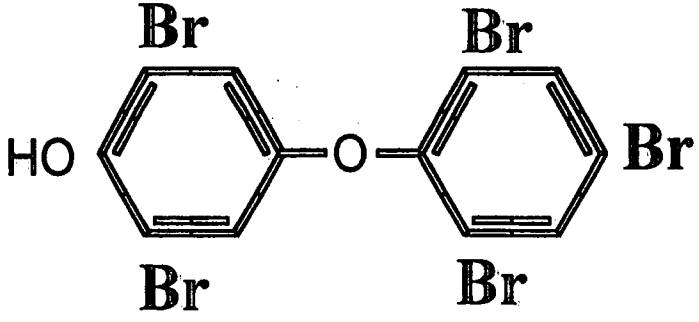


4'-hydroxy-1,3,3',5-tetrabromodiphenyl ether

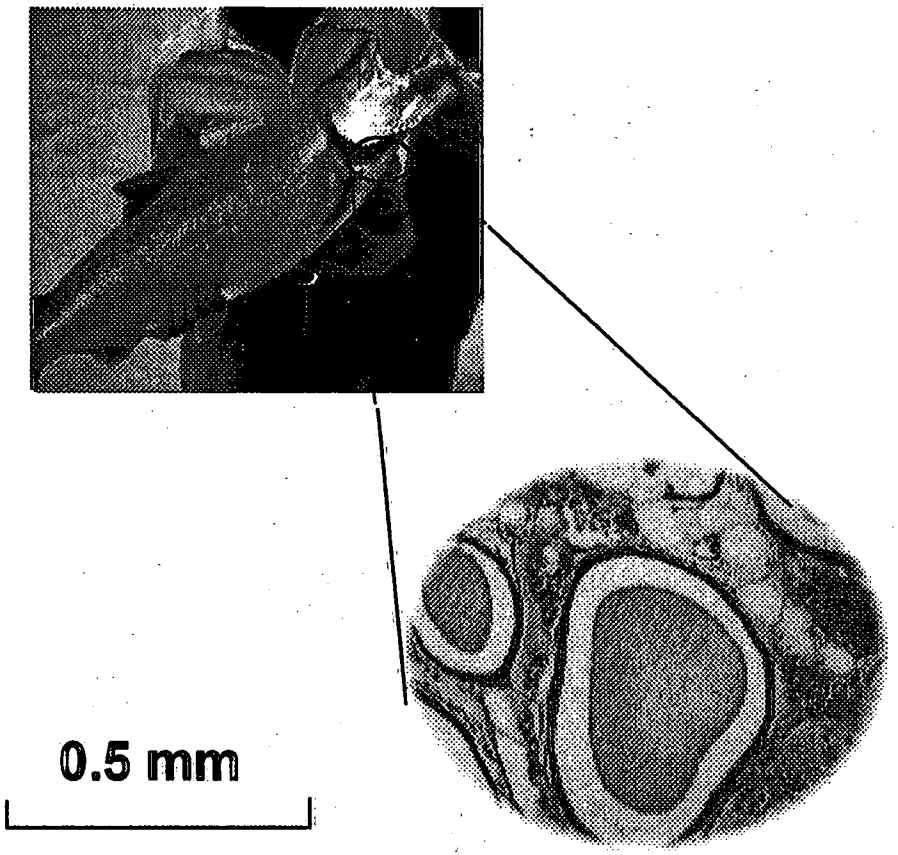


T3 analogue

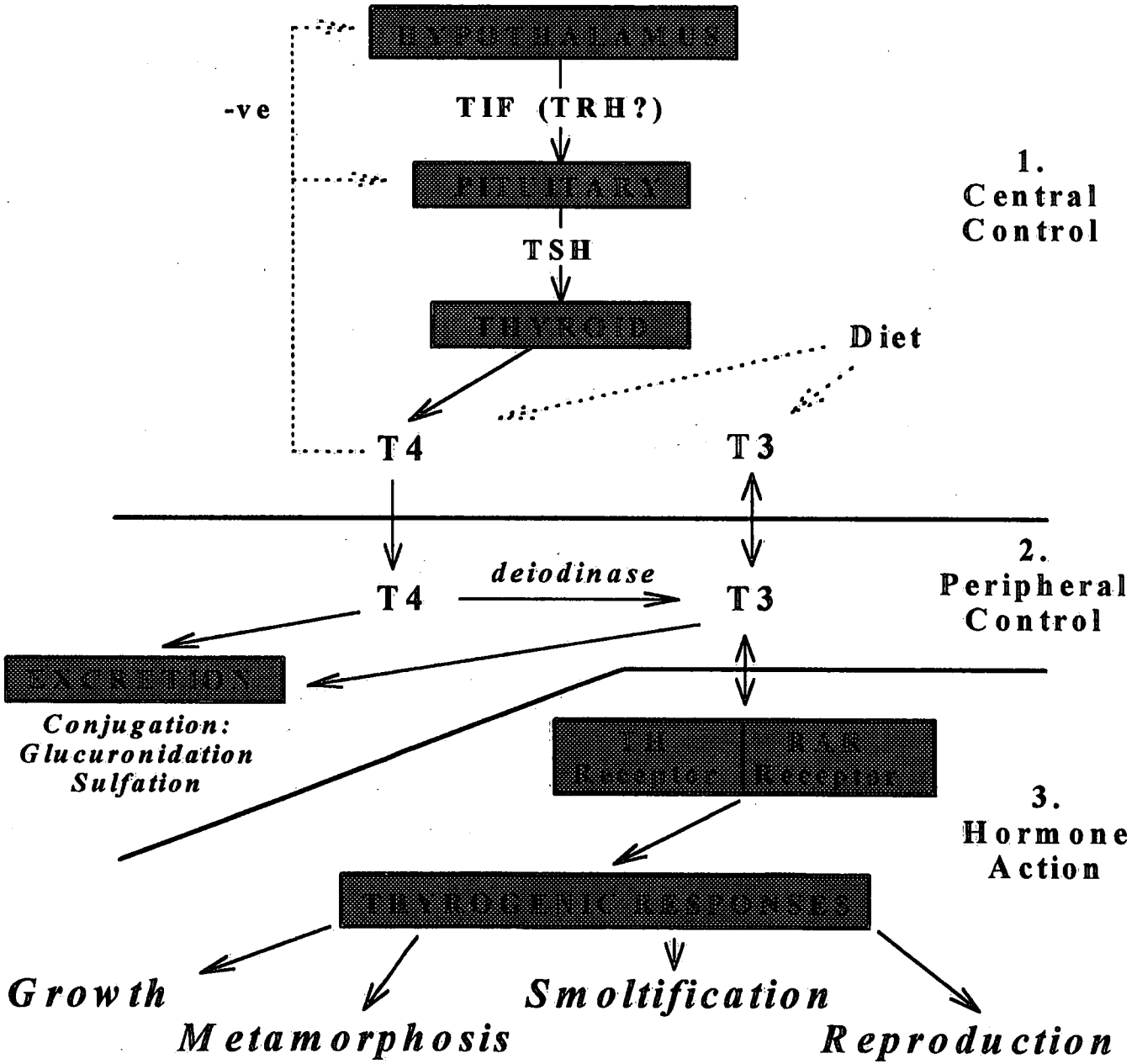
4'-hydroxy-1,3,3',5,5'-pentabromodiphenyl ether



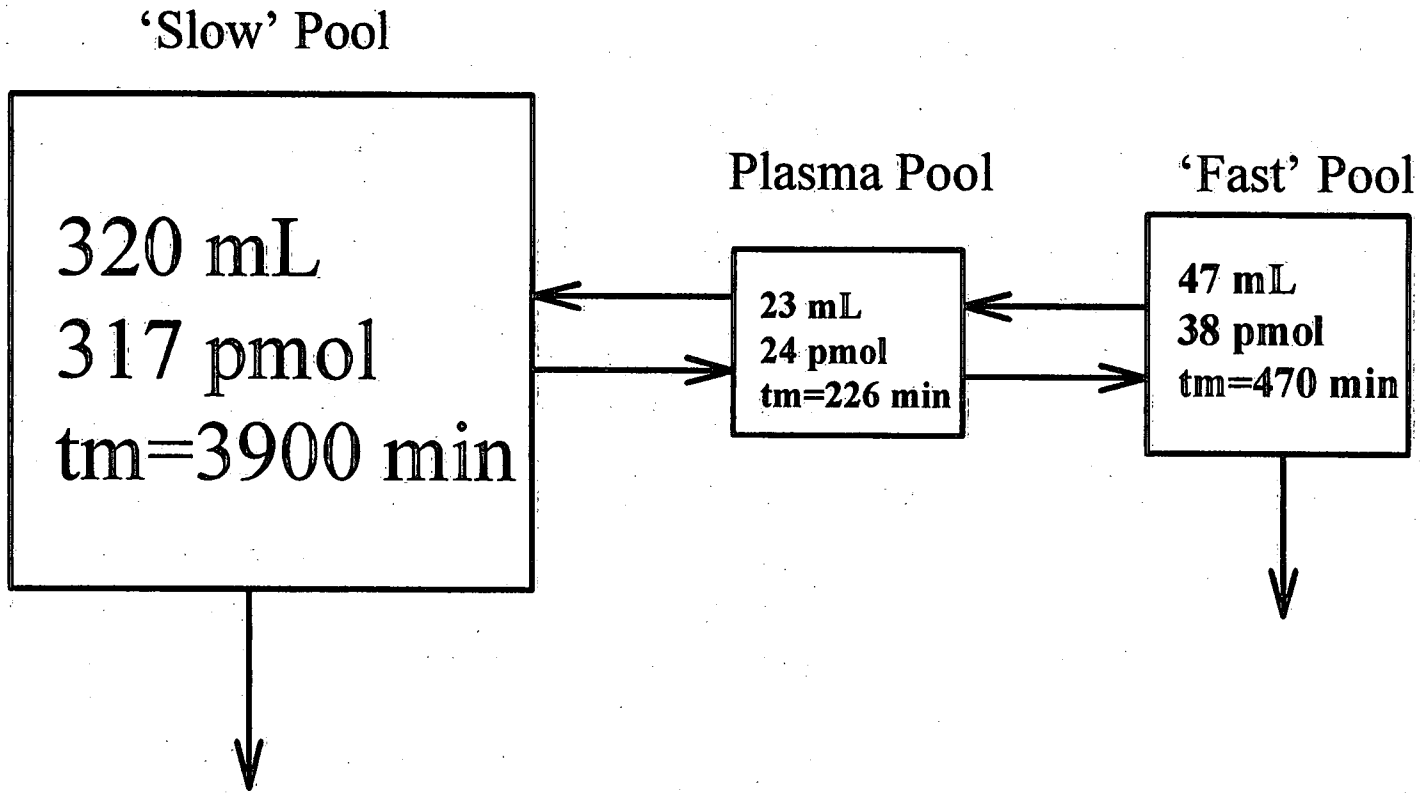
T4 analogue

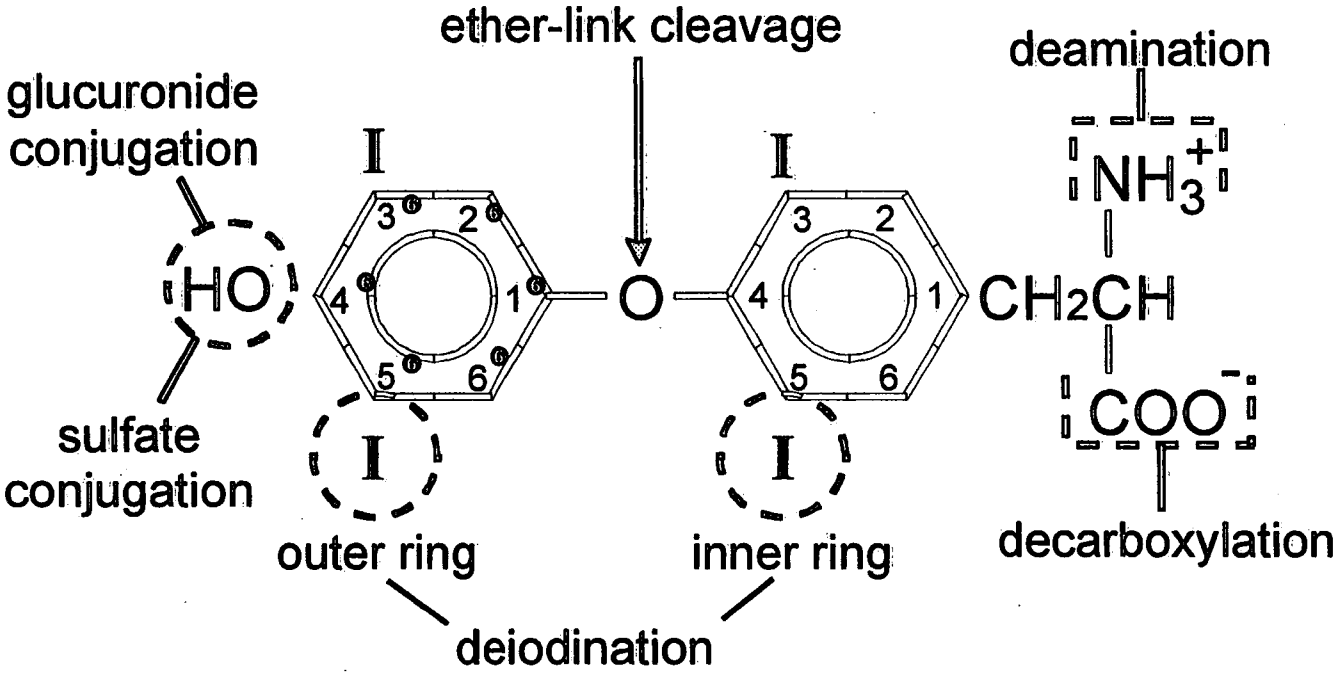


Location of Thyroid Tissue in Trout

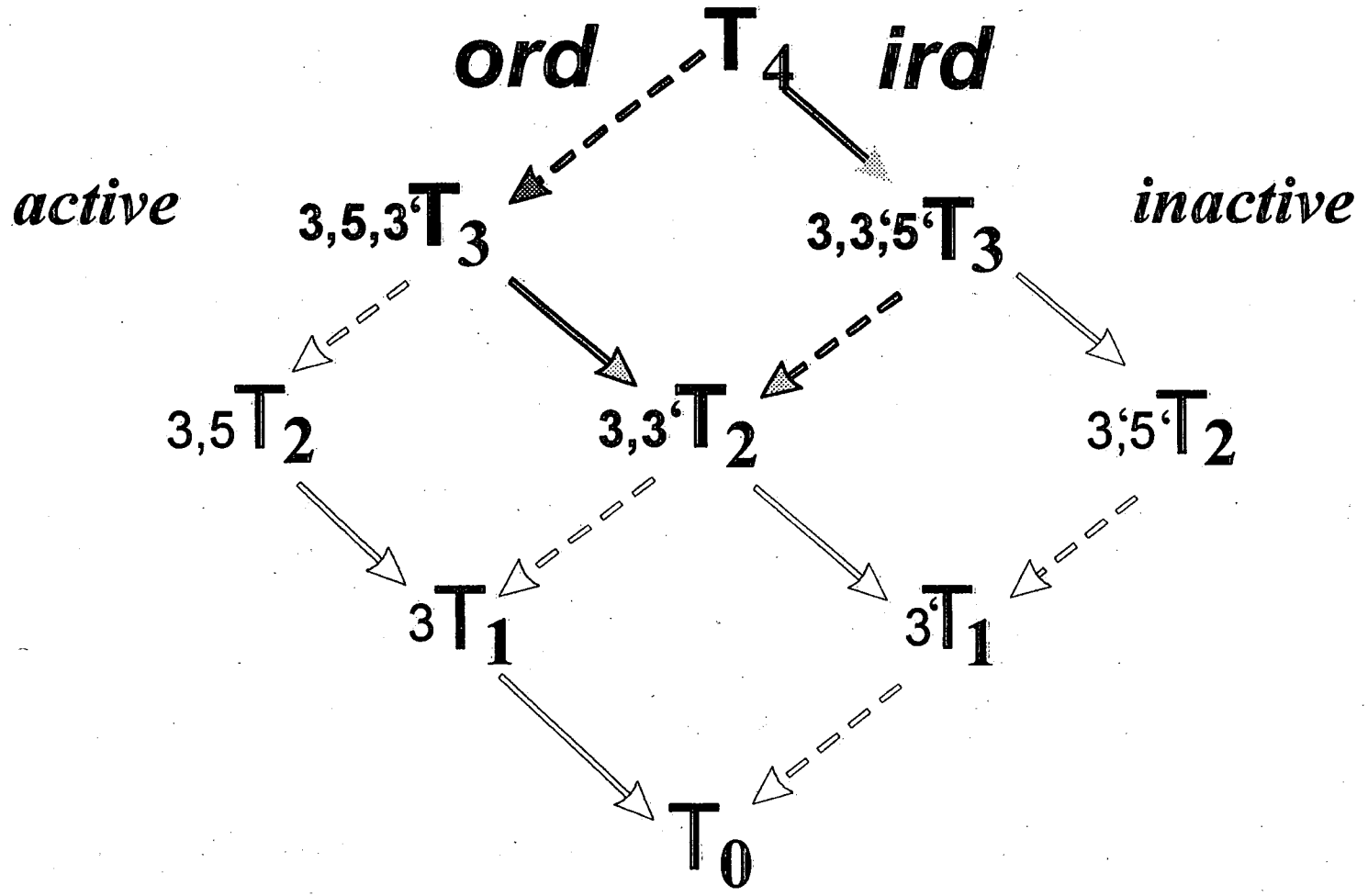

HYPOTHALAMUS
-ve
TIF (TRH?)
PITUITARY
TSH
THYROID
Diet
1.
Central
Control
T4
T3
T4
deiodinase
T3
2.
Peripheral
Control
EXCRETION
Conjugation:
Glucuronidation
Sulfation
TH
Receptor
RAR
Receptor
3.
Hormone
Action
THYROGENIC RESPONSES
Growth
Metamorphosis
Smoltification
Reproduction

T3 Distribution In Rainbow Trout



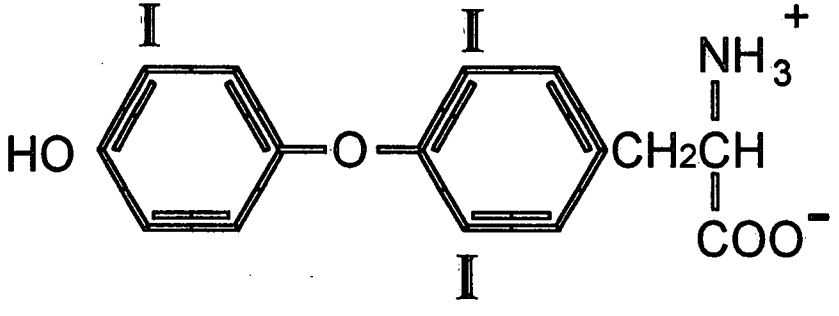


Possible Chemical Conversions of T4

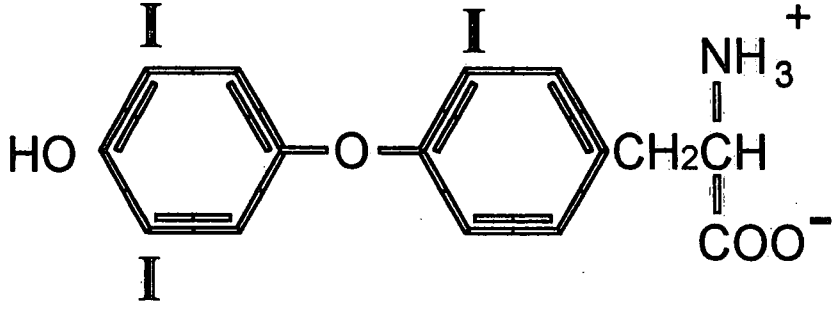


Potential Stepwise Monodeiodination Pathways for Thyroid Hormones

3,5,3'-TRIIODOTHYRONNE = T3



3,5',3'-TRIIODOTHYRONNE = Reverse T3



Type I Deiodinase:

Non-autoregulated ORD with fairly wide tissue distribution (liver, lung, kidney, brain) in mammals. Catalyzes the ORD of T4, rT3 and the sulfated metabolites of T4 and T3. Not well documented in fish.

Type II Deiodinase:

Autoregulated ORD that produces T3. In mammals found in brain, placenta and brown adipose tissue. Also important in the fetus. Widespread in fish tissues but particularly liver where it provides the bulk of circulating T3. In fish T4ORD responsive to physiologic state.

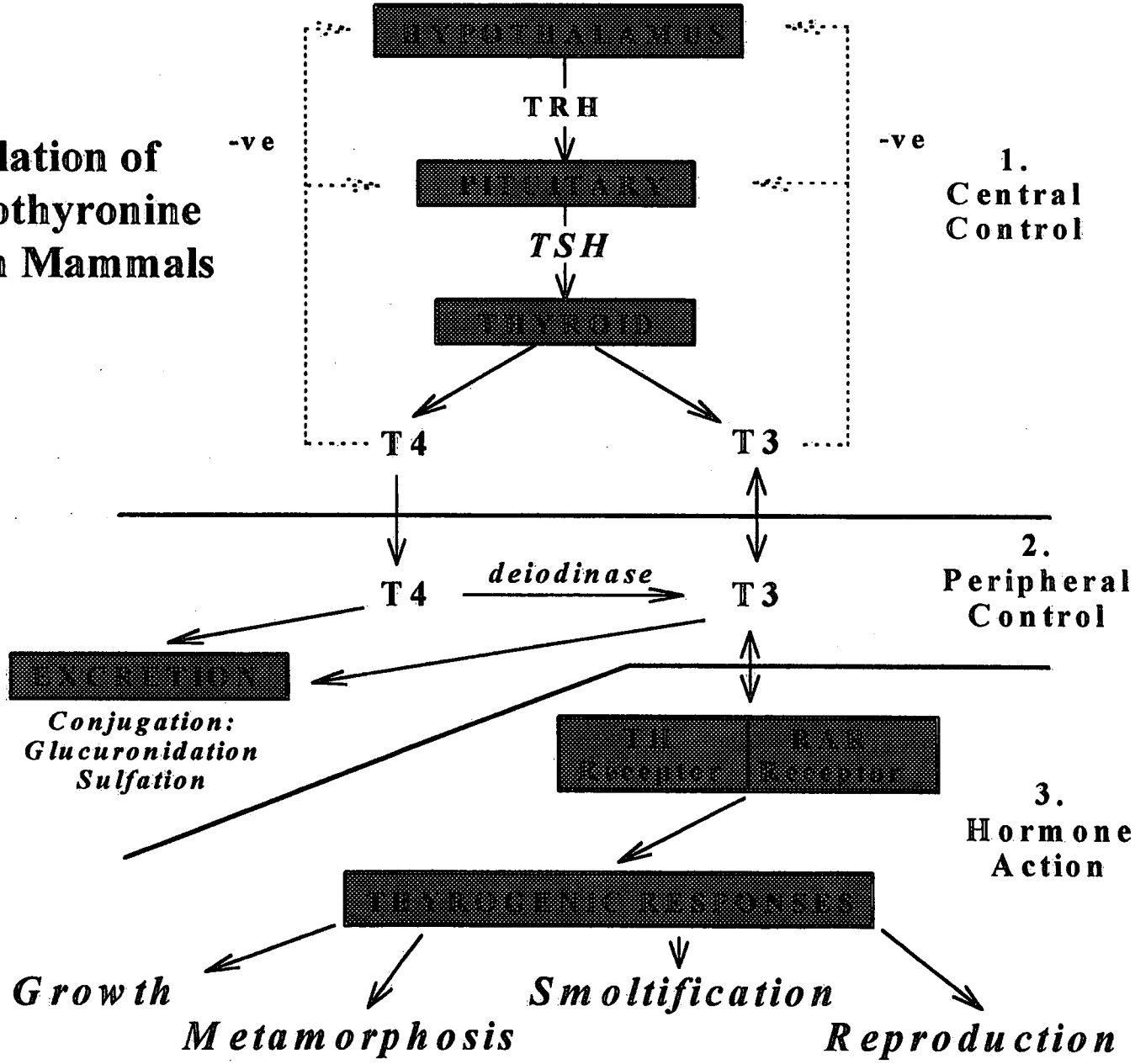
- **Activated by nutritional state - particularly protein increases Vmax**
- **Increased by growth hormone and testosterone treatment**
- **Decreased by stress, estradiol, Pb, Al, Cd.**
- **Affected by temperature, season and salinity**

Type III Deiodinase:

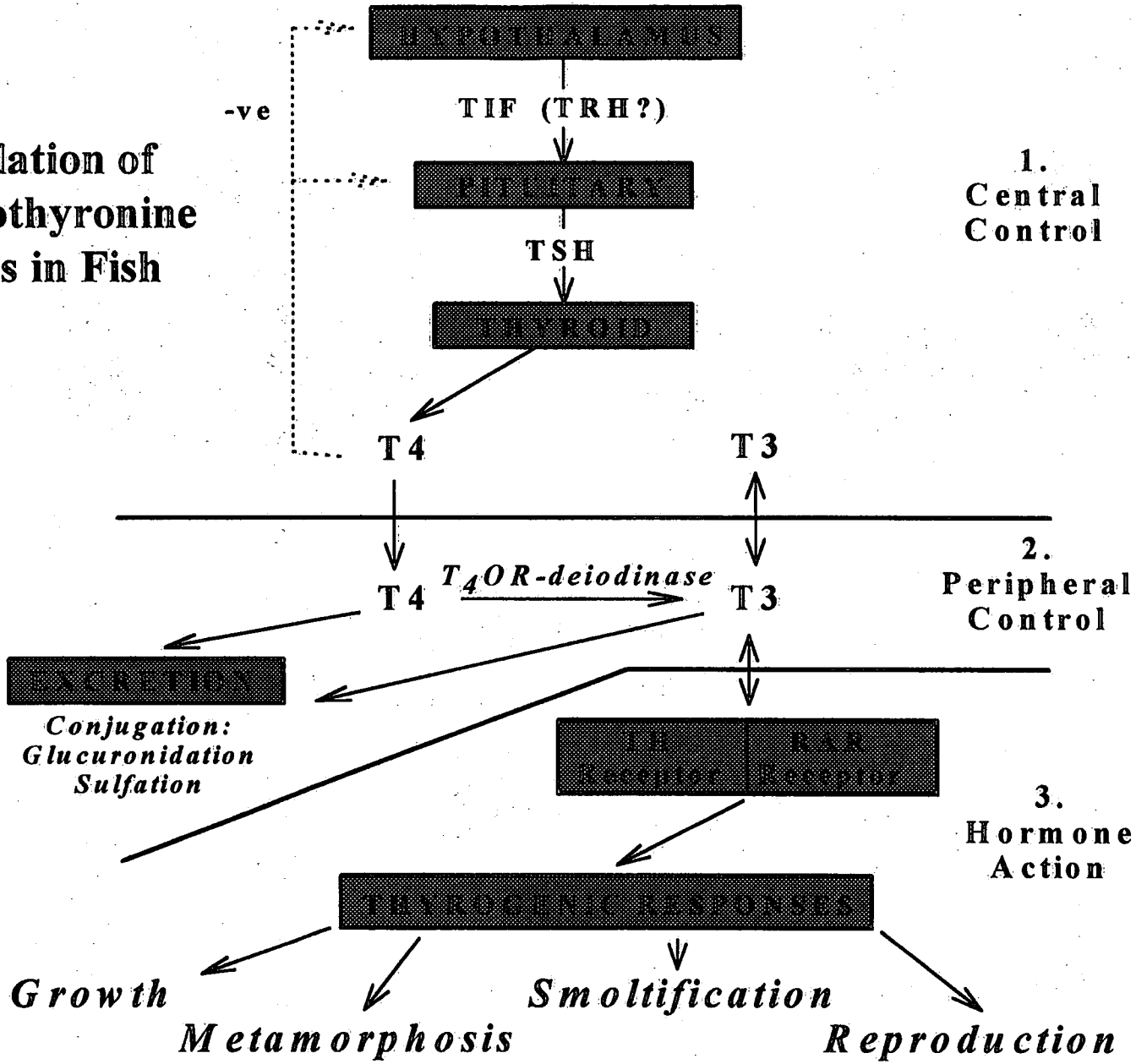
IRD that catalyzes the conversion of T3 and T4 to 33'T2 and rT3 respectively. Inactivating pathway.

- **Induced by T3 challenge, physical stress and PCB treatment in fish**

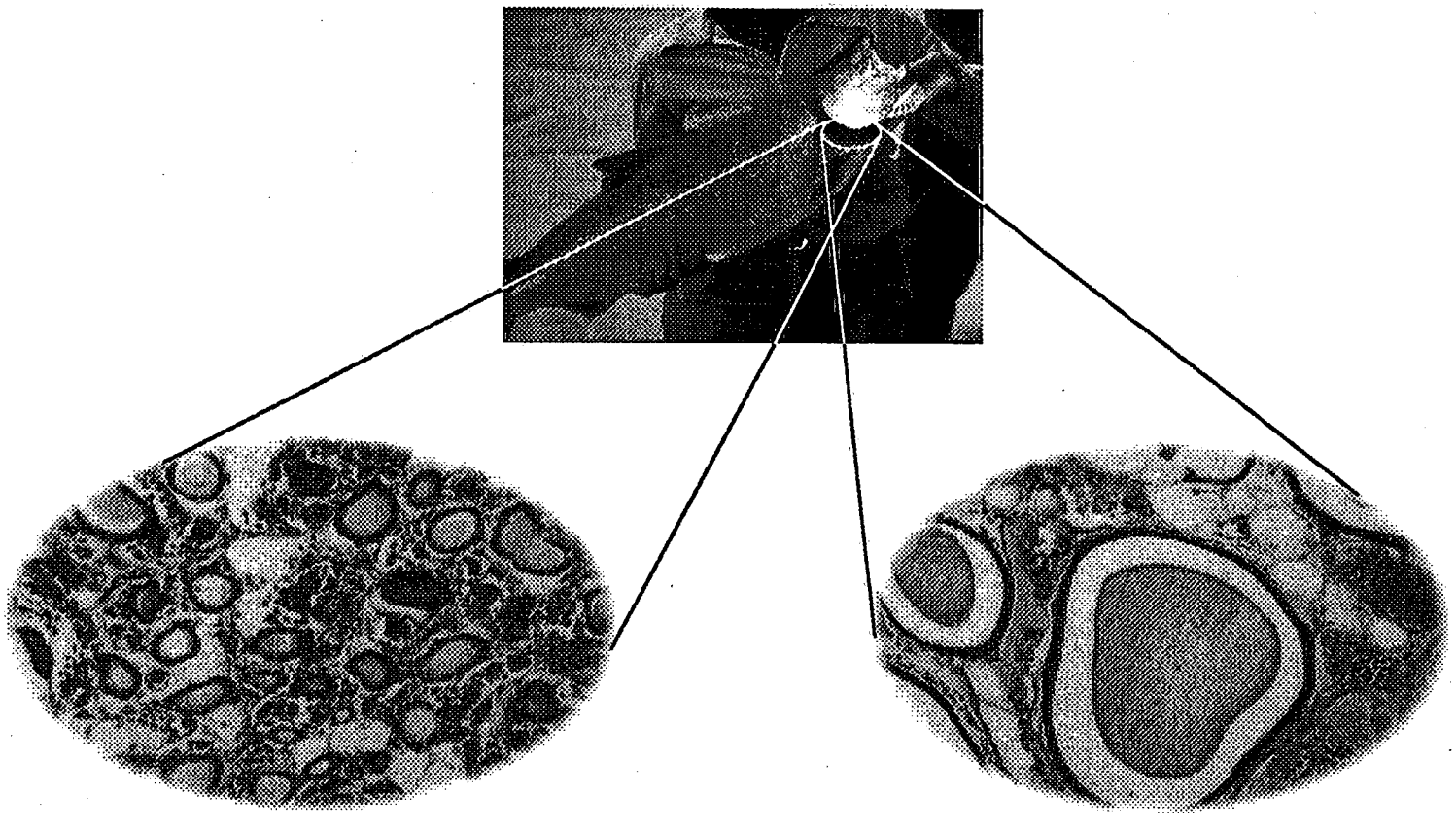
Regulation of Triiodothyronine Status in Mammals



Regulation of Triiodothyronine Status in Fish



Lake Trout Thyroid Gland



Microfollicular Hyperplasia in Trout from Lake Ontario

Normal Thyroid Follicules in Trout from Inland Lake

In vivo Exposure to Co-Planar PCB

• Transient effect thyroid histology • Transient effect plasma T4?	Central Measures
• Increased T4 PCR • Increased T4 Glucuronidation • No effect plasma T3 • No effect T3 PAR • No effect hepatic T4ORD • No effect T3 Glucuronidation	Peripheral Measures
• No effects on growth	Tertiary Response

In vivo Exposure to Aluminum

• No effect thyroid histology • No effect plasma T4	Central Measures
• No effect T4 PCR • +/- effect plasma T3 • T3 PAR decreased by 40% • Hepatic T4ORD inhibited (both Km and Vmax altered) • Muscle T3 lowered to 14% of control	Peripheral Measures
• Impaired growth	Tertiary Response

Conclusion:

No single parameter is capable of representing thyroid status in fish

Suite of measures required that covers:

1. Measures of Centrally Controlled Secretion/Biosynthesis:

- thyroid gland histology
- plasma T4

2. Measures of Peripherally Controlled T3 Production:

- deiodination - both ORD and IRD
- plasma/Tissue T3

- conjugation/excretion

3. Receptor and Post-Receptor Events:

- binding and reporter gene assays
- TH mediated responses
  - metamorphosis
- TH mediated neurological effects?
  - orientation
  - social hierarchy

PROBABILISTIC RISK ASSESSMENT OF POLYBROMINATED DIPHENYL ETHERS

Keith Solomon and Paul K. Sibley

Probabilistic risk assessment (PRA) has received increasing attention in recent years as a method for determining the risk of contaminants in aquatic environments. PRA is based on comparisons of distributions of exposure data (generally environmental concentrations measured in the water) and effect data (toxicity responses which are typically generated in laboratory studies). The degree of overlap of the two distributions is used as a measure of the probable risk; distributions exhibiting greater overlap indicate higher risk associated with the compound in question. Quantitatively, this risk is expressed as the probability of exceeding an assessment criterion (referred to as centiles) which is determined from the toxicity distribution.

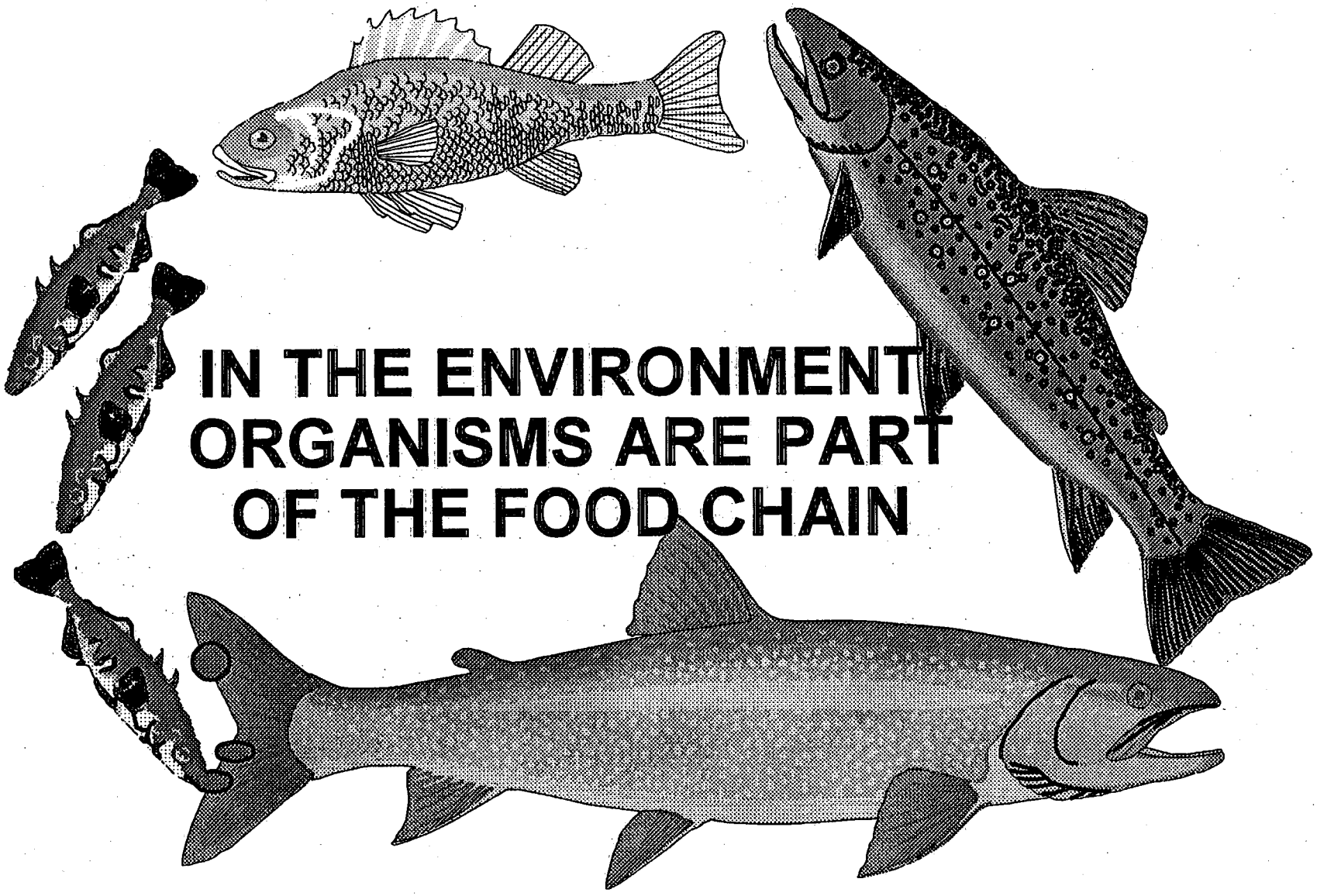
In practice, PRA is applicable in situations for which exposure and effect(s) for a given compound can be directly related. This typically includes compounds which do not bioaccumulate and which have a definitive mode of toxic action. Application of PRA to bioaccumulative substances has proven difficult because of uncertainty in matching exposure and toxicity. Specifically, exposure to bioaccumulative substances may be indirect, via the food chain, rather than direct. One potential approach to circumvent this difficulty is to use tissue residue measurements (as opposed to environmental measurements) as a basis for constructing the exposure distribution in PRA. This approach could be applied to bioaccumulative compounds, such as polybrominated diphenyl ethers (PBDE's), for which there appears to be a specific mode of toxic action (e.g., binding at the Ah receptor).

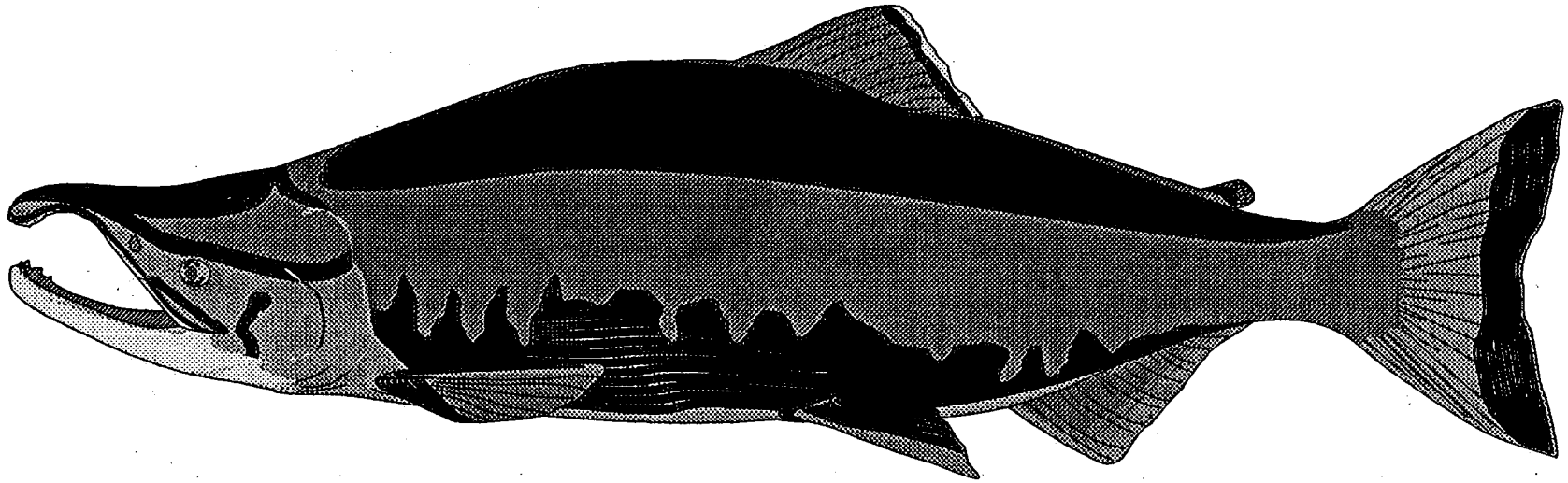
In this presentation, we illustrate and discuss the application PRA to bioaccumulative substances using PBDE's as an example. Where PBDE's are concerned, a critical shortcoming is the lack of toxicity information with which to compare exposure (tissue residue) information. Thus, an important aspect of future research, from a PRA perspective, will be the generation of an adequate toxicity database for these compounds.

PROBABILISTIC RISK ASSESSMENT OF AGROCHEMICALS

Keith R. Solomon & Paul K. Sibley,,

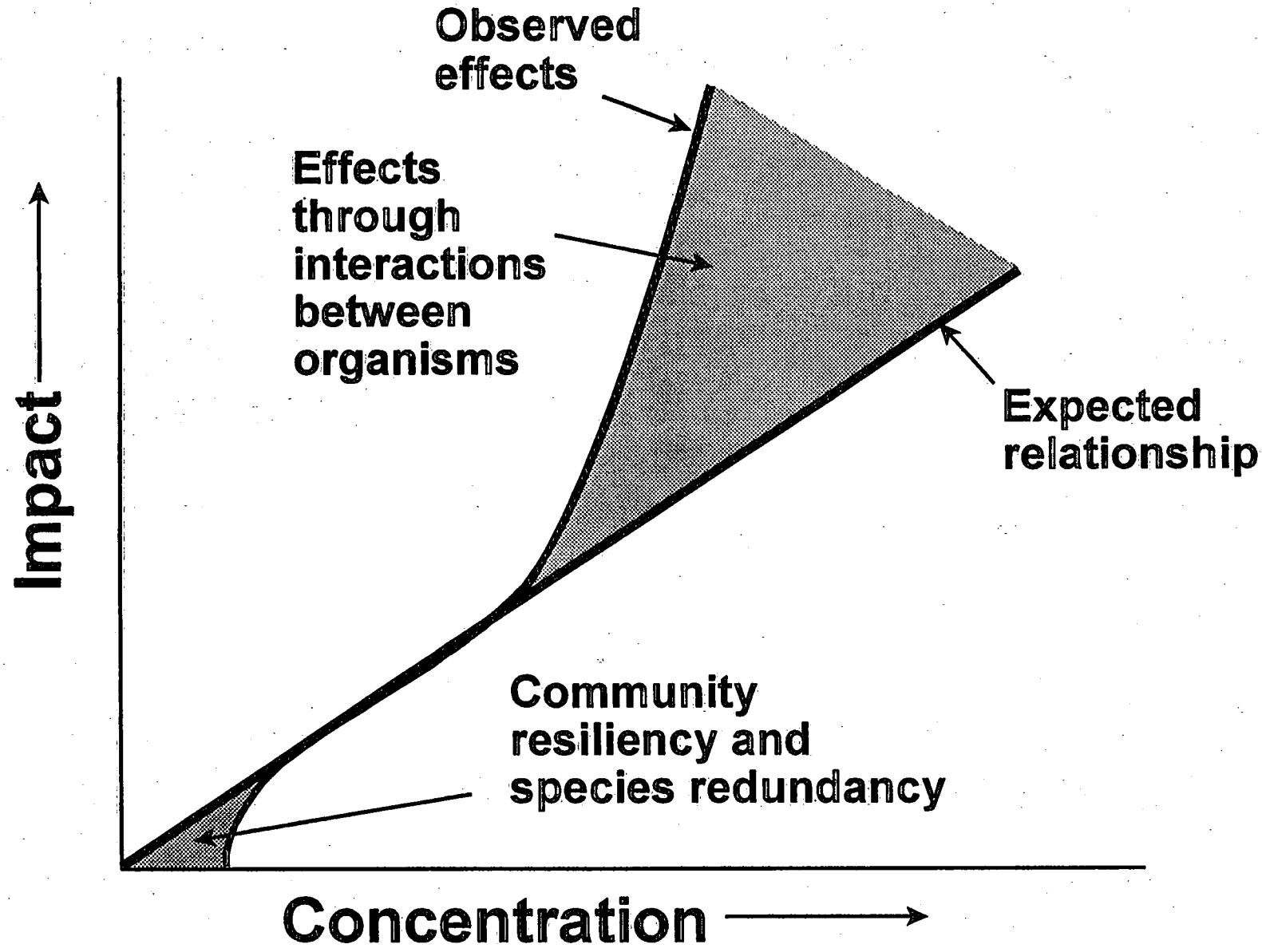
Centre for Toxicology and Department of Environmental Biology,, University of Guelph,, Guelph, ON, N1G 2W1, Canada
ksolomon@tox.uoguelph.ca


IN THE ENVIRONMENT
ORGANISMS ARE PART
OF THE FOOD CHAIN



AND SOME, AS INDIVIDUALS, ARE THEREFORE EXPENDABLE

ECOLOGICAL CONCEPT



From David Allen, Personal communication

EXPERIENCE WITH PEST MANAGEMENT

Studies on pests have shown that organisms can tolerate high mortality without suffering population-level effects

- *Acyrthosiphon pisum* response to imidacloprid,
- Populations exposed to the 72-h LC60 were able to maintain rates of population increase similar to untreated controls
- Compensatory mechanisms where the unaffected individuals are able to maintain heightened rates of reproduction due to decreased competition for limiting resources

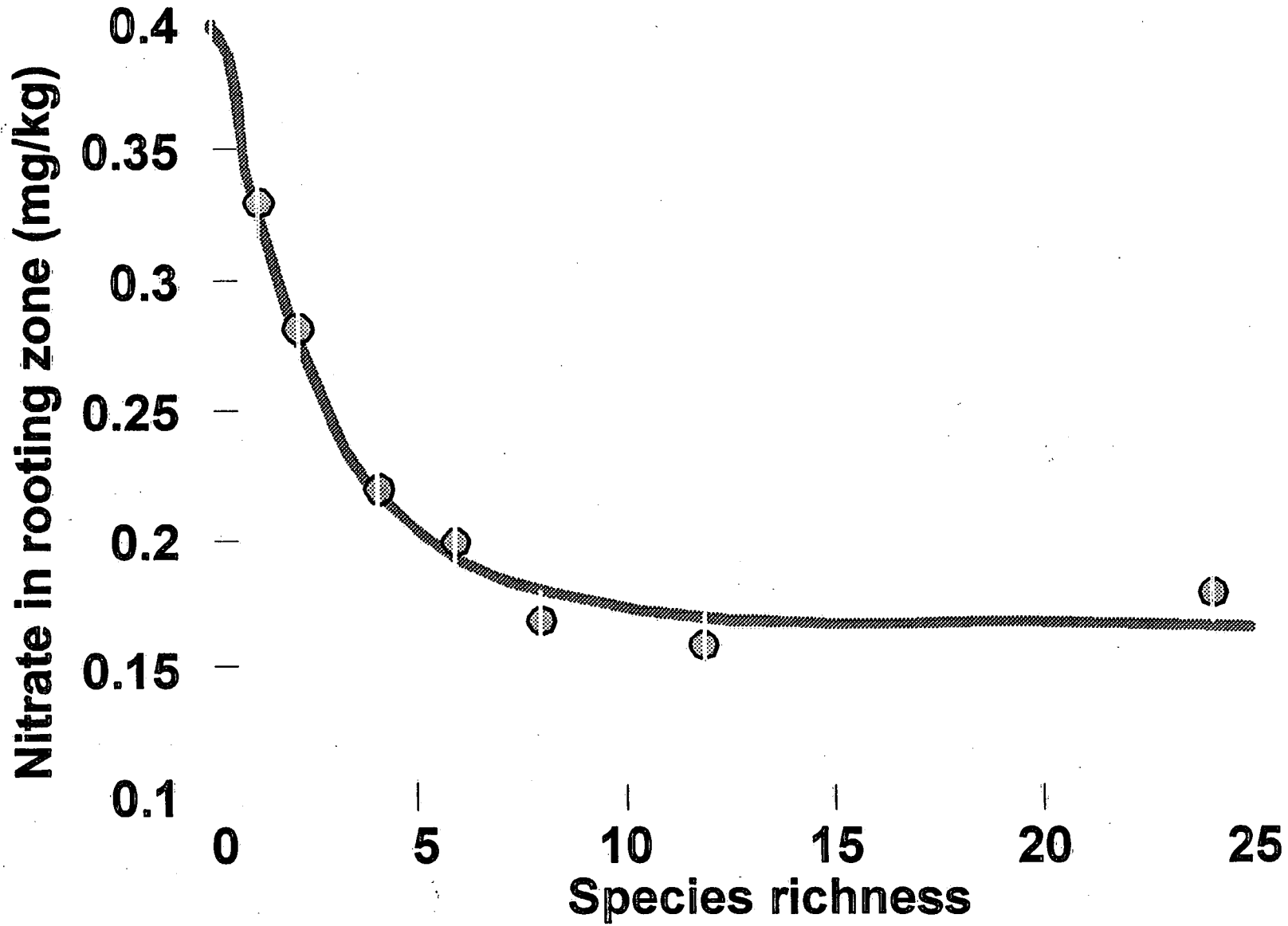
(Walthall and Stark, 1997).

STRUCTURE AND FUNCTION

Studies in North American Prairie ecosystems

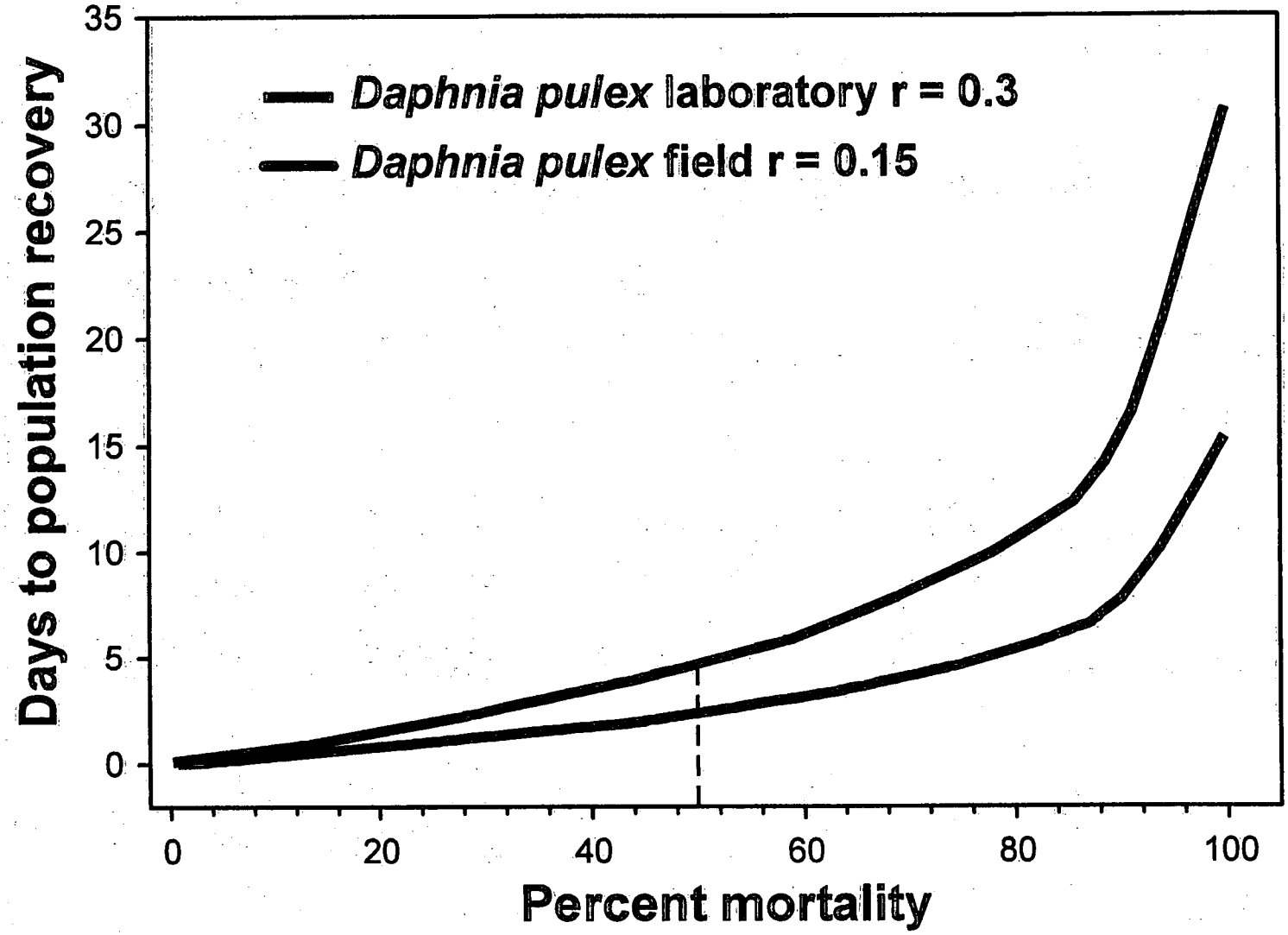
- Community function, measured by nutrient cycling, less easily disrupted when diversity high.
- Community function showed little change when diversity ranged from 10 to the maximum of 24 species and only decreased when the number of species was less than ten.
- With drought as environmental stressor sustainability of production was maintained at expense of diversity.
- In terms of community productivity, diversity acted as a buffer.

Tillman 1996



Tillman et al 1996

Time for Population Recovery



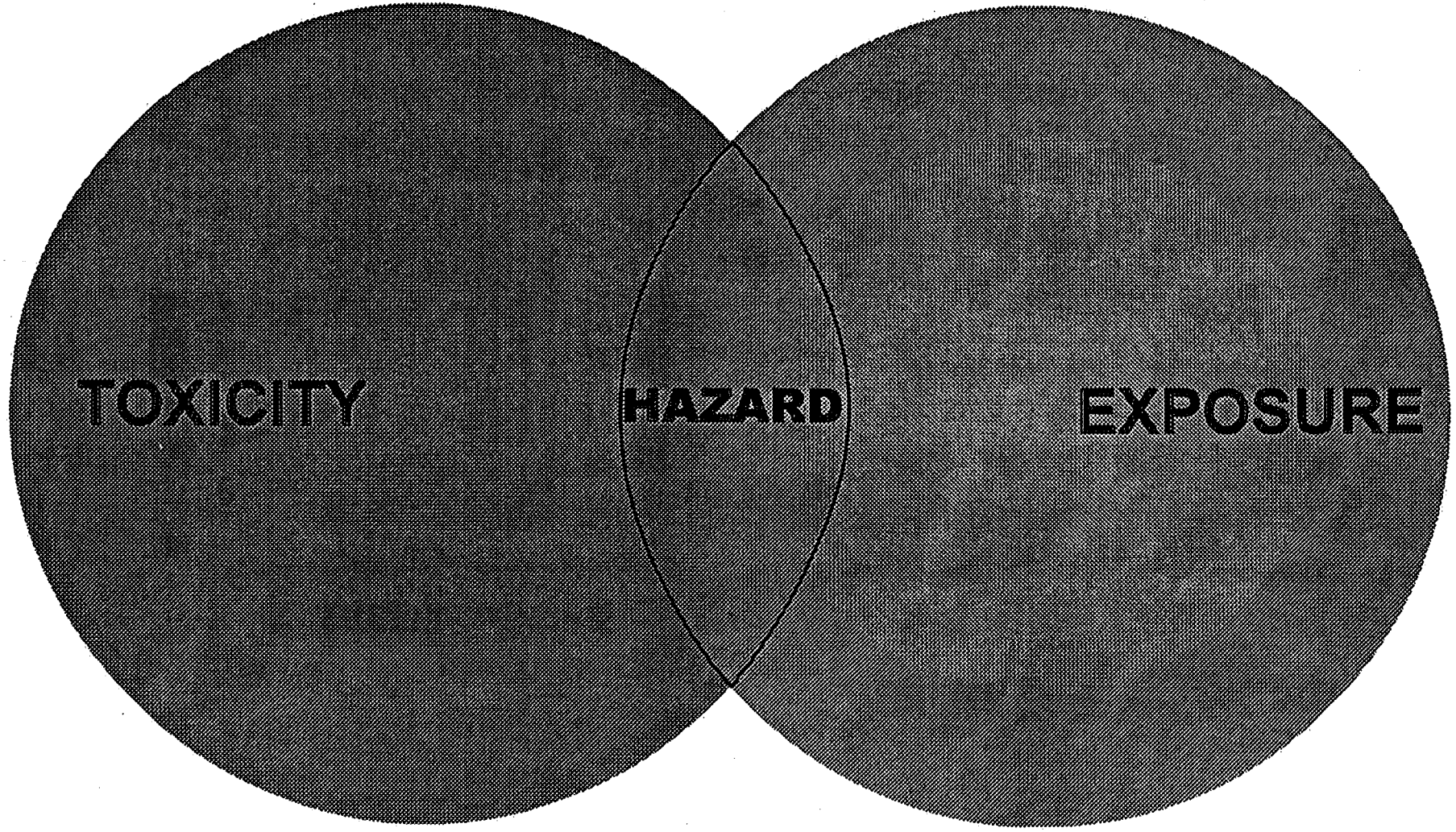
From Larry Barnthouse

Frequency of Effects

- In assessing ecotoxicological risks, the return frequency protected against should be consistent with the resiliency of vulnerable populations.
- Low return frequencies, for example, once in two or three years for fish.
- Higher return frequencies, days or weeks, for zooplankton.

Ecotoxicological Risk Assessment

- Some effects at the organism- and population-level can be tolerated
- Provided that
  - effects are restricted on the spatial and temporal scale
  - keystone organisms are not adversely affected
- Local extinction (pseudo extinction) may be tolerated
  - provided that functions of these organisms can be taken over by other organisms, or
  - repopulation can occur from nearby unaffected populations or from (propagules)


TOXICITY
HAZARD
EXPOSURE

QUOTIENTS

$$\text{HAZARD} \approx \frac{\text{EXPOSURE CONCENTRATION}}{\text{EFFECT CONCENTRATION}}$$

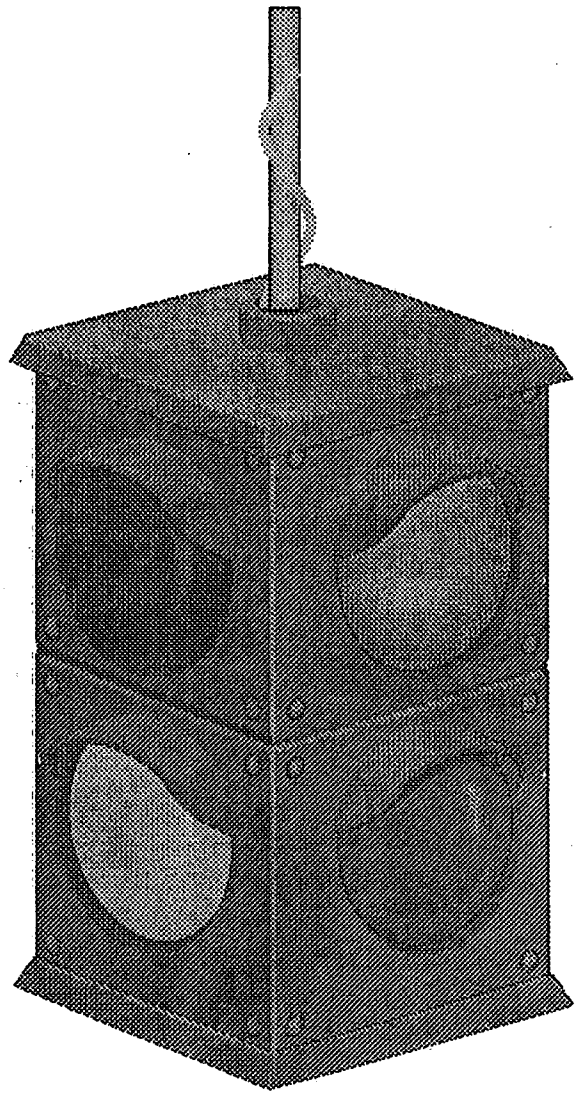
(LOC)

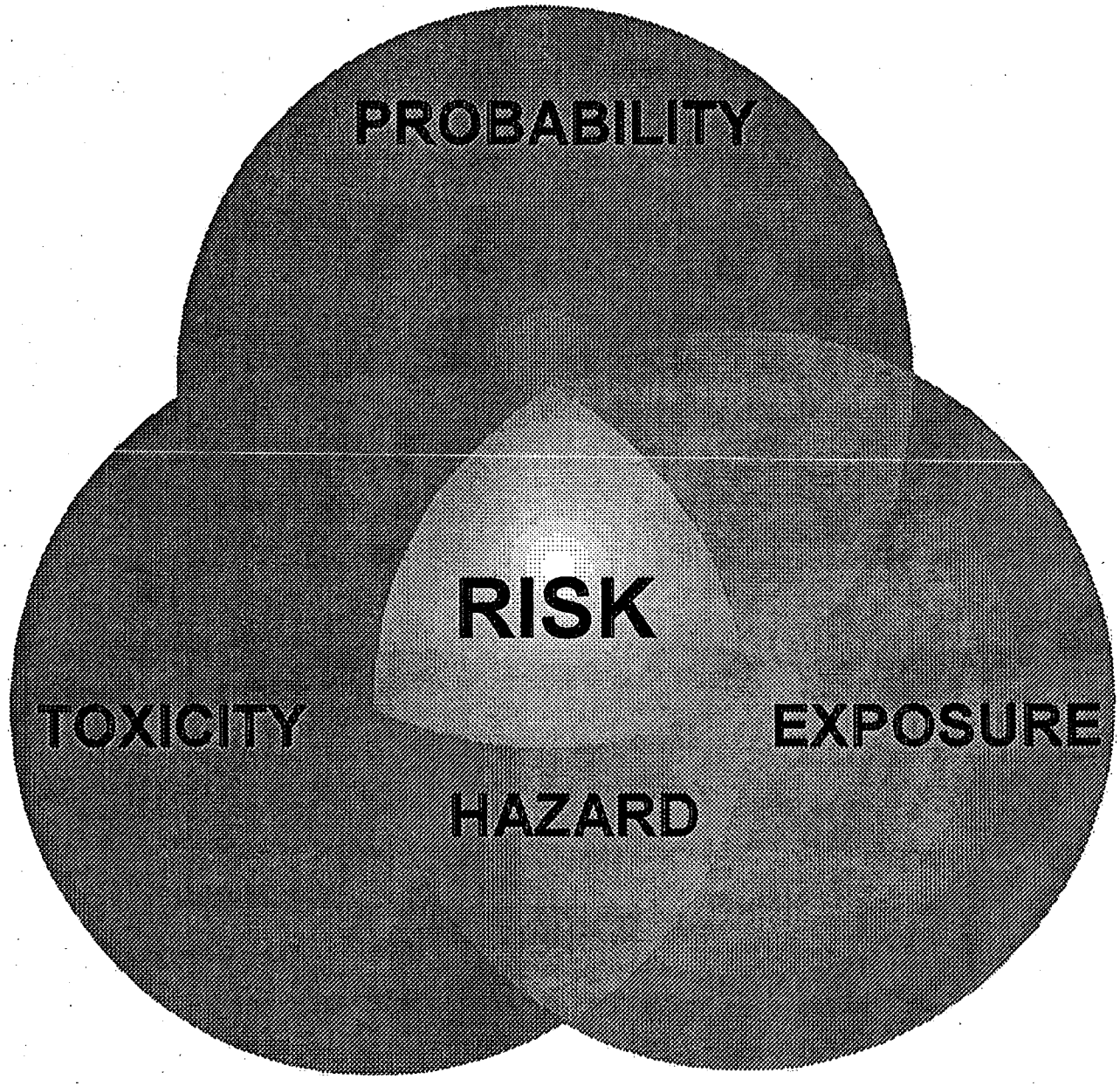
$$\text{MARGIN OF SAFETY} \approx \frac{\text{EFFECT CONCENTRATION}}{\text{EXPOSURE CONCENTRATION}}$$

(TER)

Quotient Method

- The quotient approach is designed to be protective, not predictive.

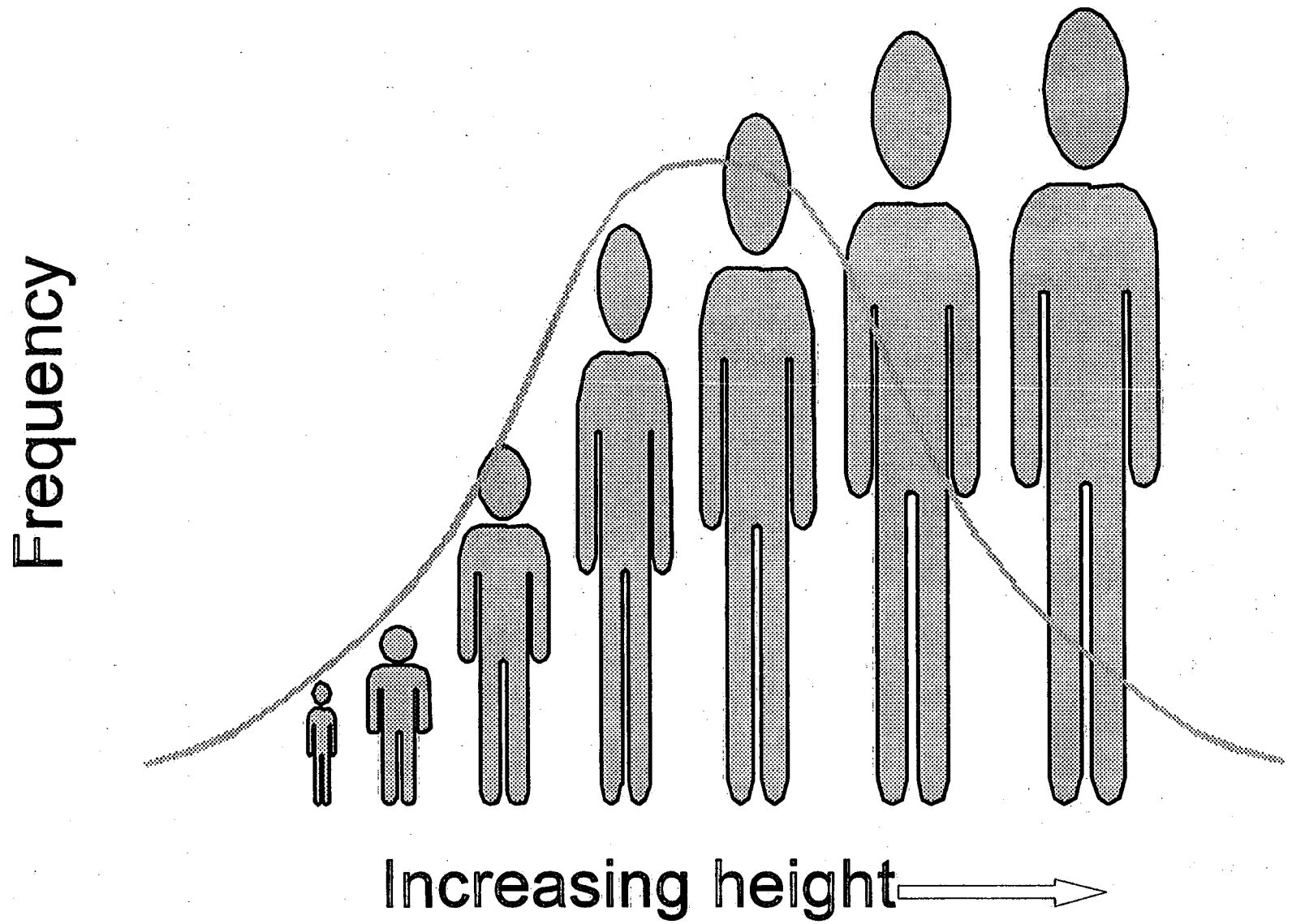


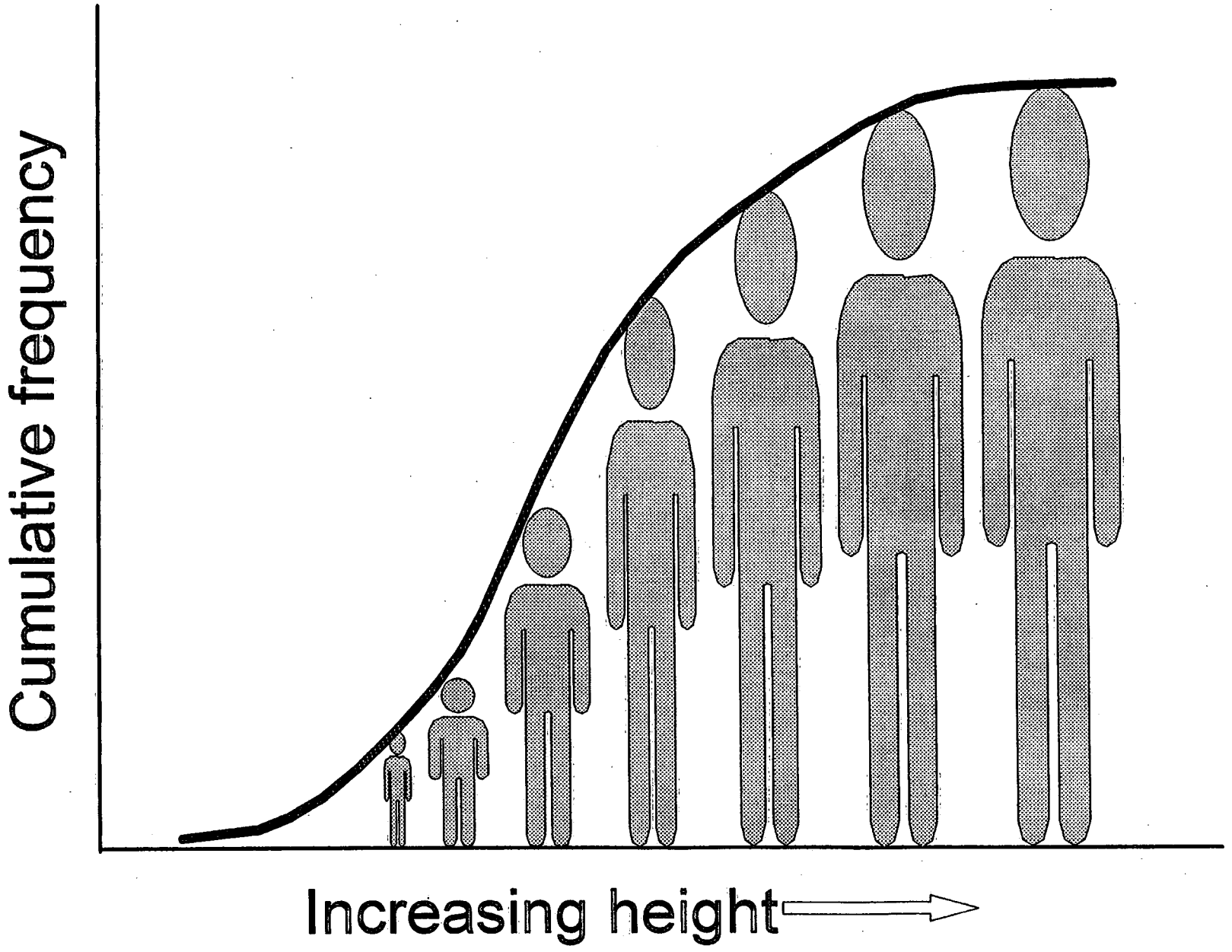

PROBABILITY
RISK
TOXICITY
EXPOSURE
HAZARD

Probabilistic Approaches

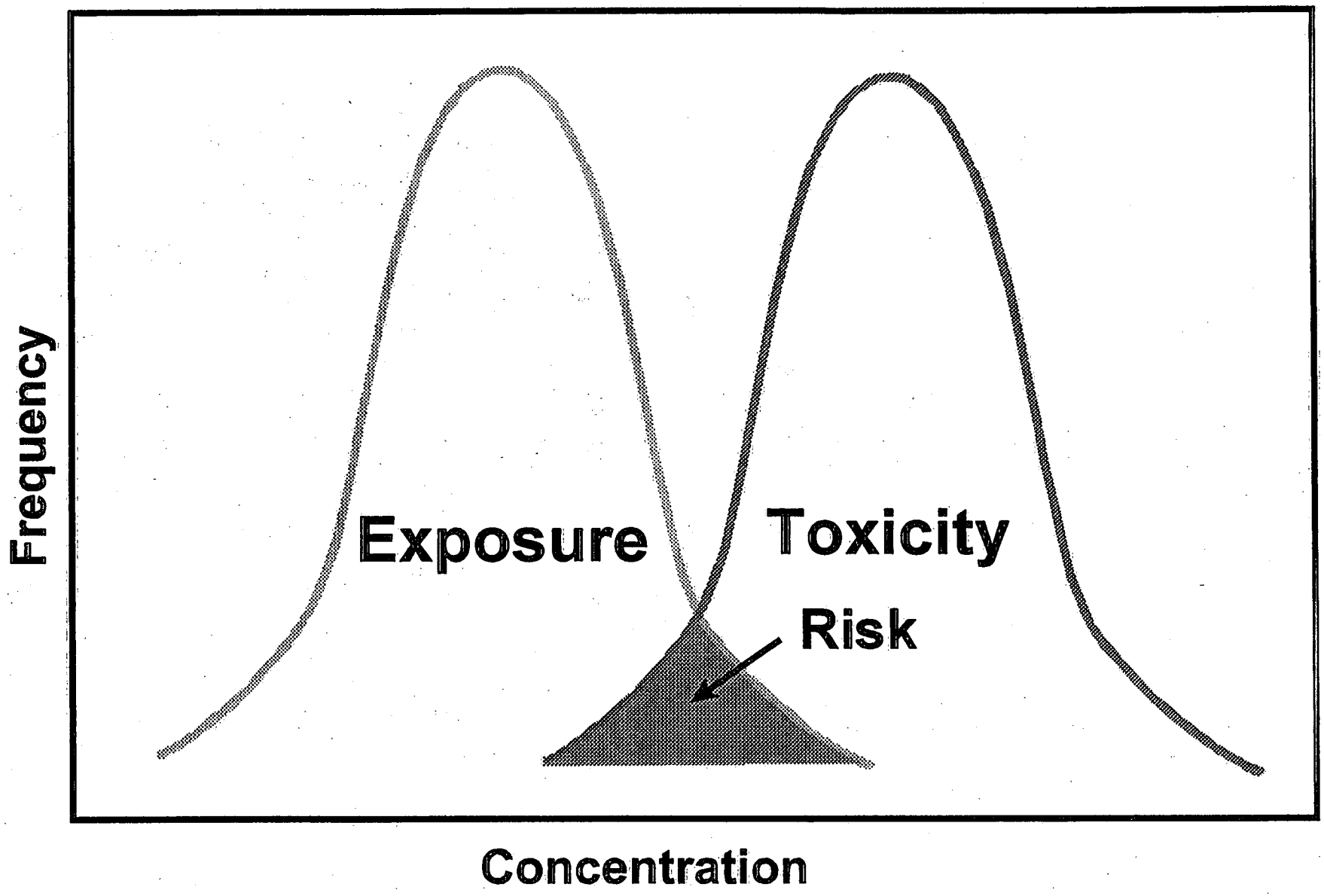
- The probability of occurrence is widely used in the characterization of risk from events in human society
  - insurance industry
  - protection against failure in mechanical and civil engineering projects
- Probabilistic approaches have also been suggested for establishing thresholds of concern for human health risk assessment[1].

[1] I.C. Munro R.A. Ford E. Kennepohl and J.G. Sprenger, *Food Chem. Toxicol.*, 1996, **34**, 829

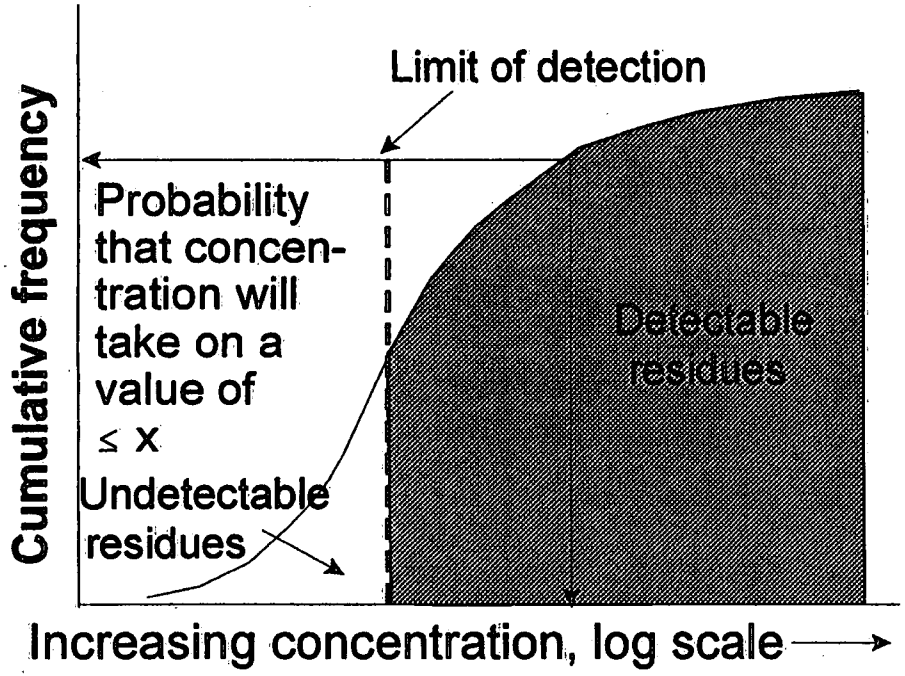

Frequency
Increasing height


Cumulative frequency
Increasing height

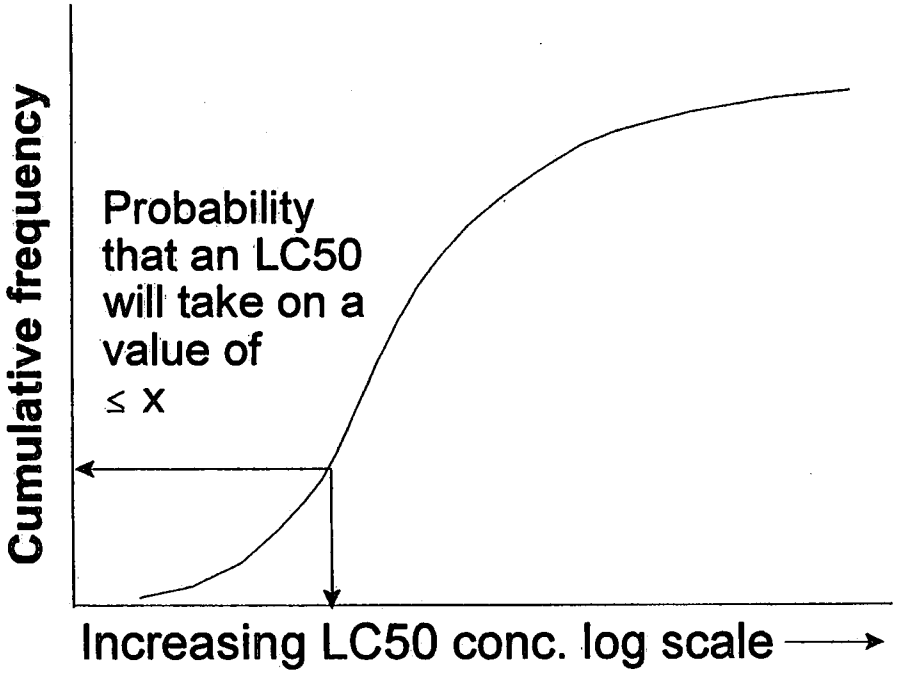
CHARACTERIZING RISK



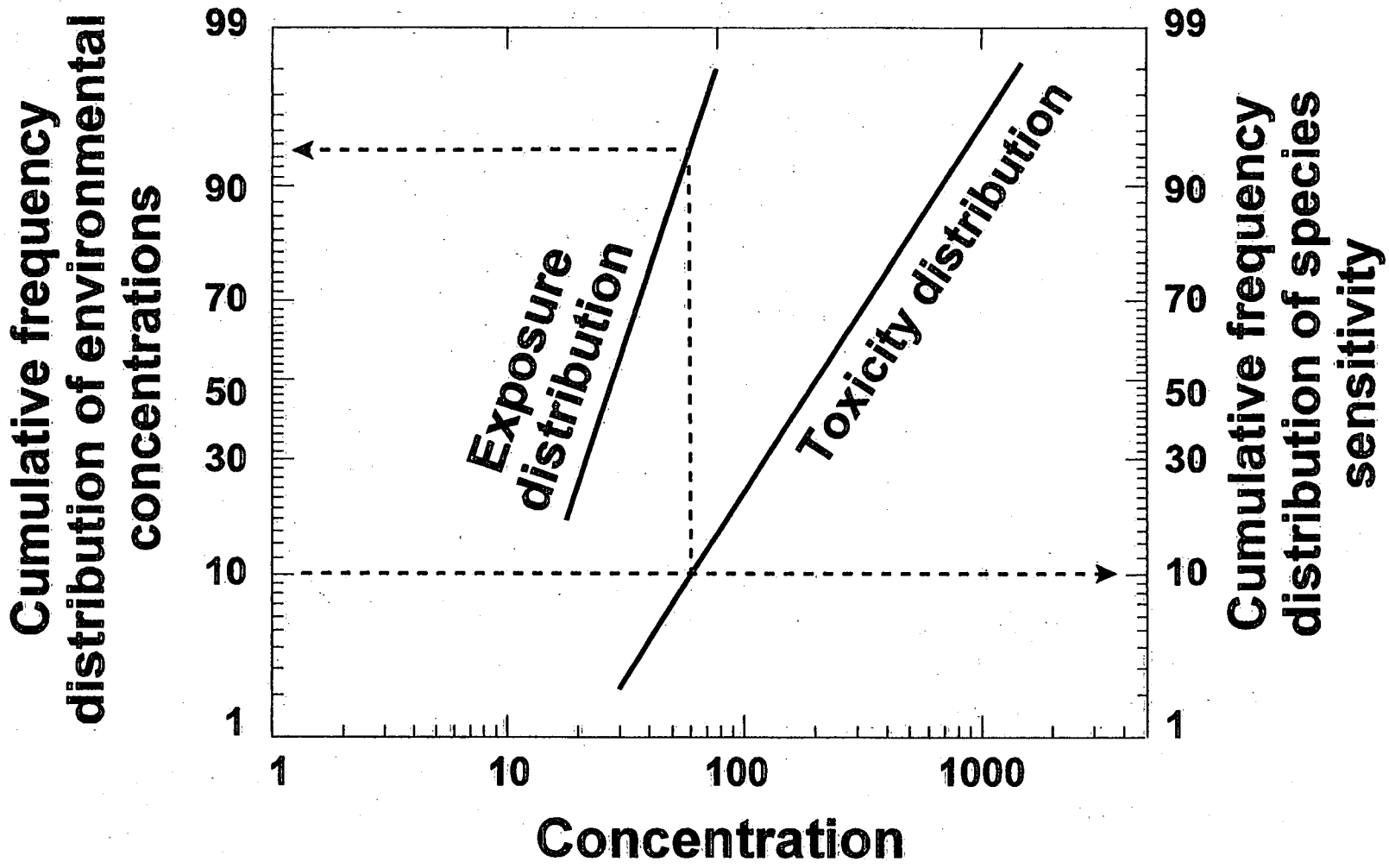
Exposure

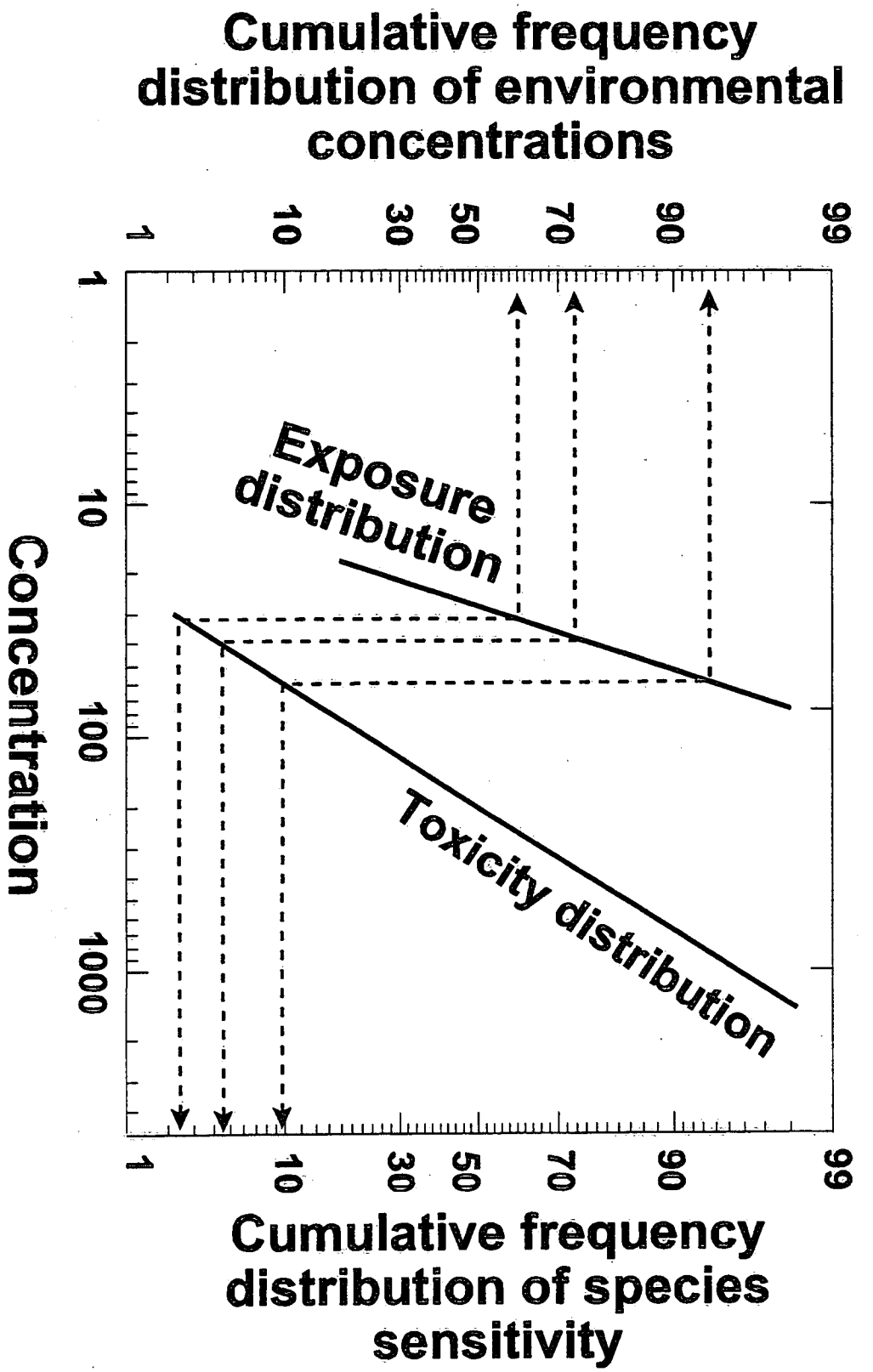


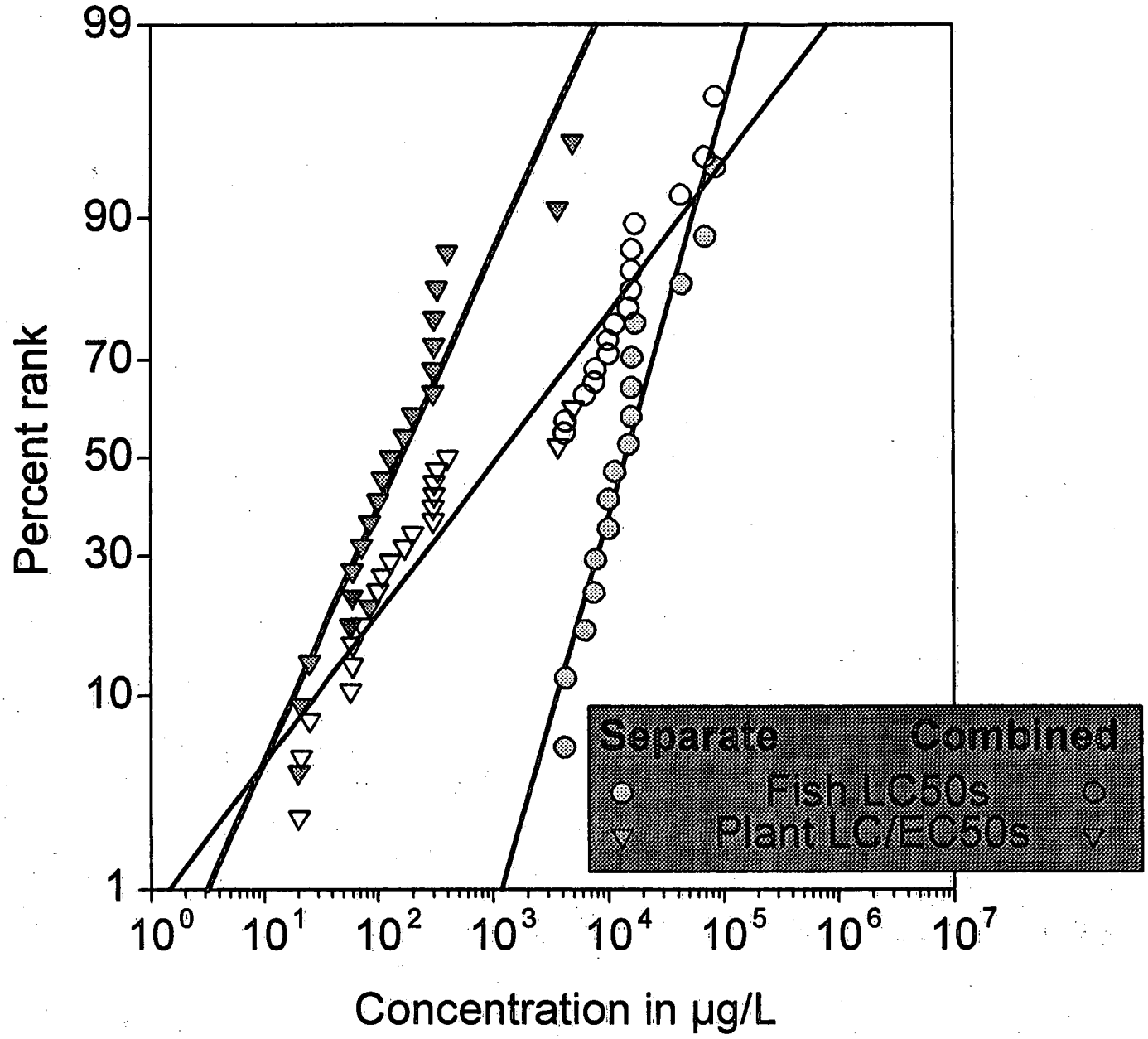
Toxicity



PROBABILISITC APPROACH




Cumulative frequency distribution of environmental concentrations
1
10
30
50
70
90
99
Exposure distribution
Toxicity distribution
Concentration
1
10
100
1000
Cumulative frequency distribution of species sensitivity


99
90
70
50
30
10
1
Percent rank
10^0 10^1 10^2 10^3 10^4 10^5 10^6 10^7
Concentration in µg/L
Separate
Combined
Fish LC50s
Plant LC/EC50s

PRESENTATION OF RISK INFORMATION

Focus on communicating the risk to the risk managers.

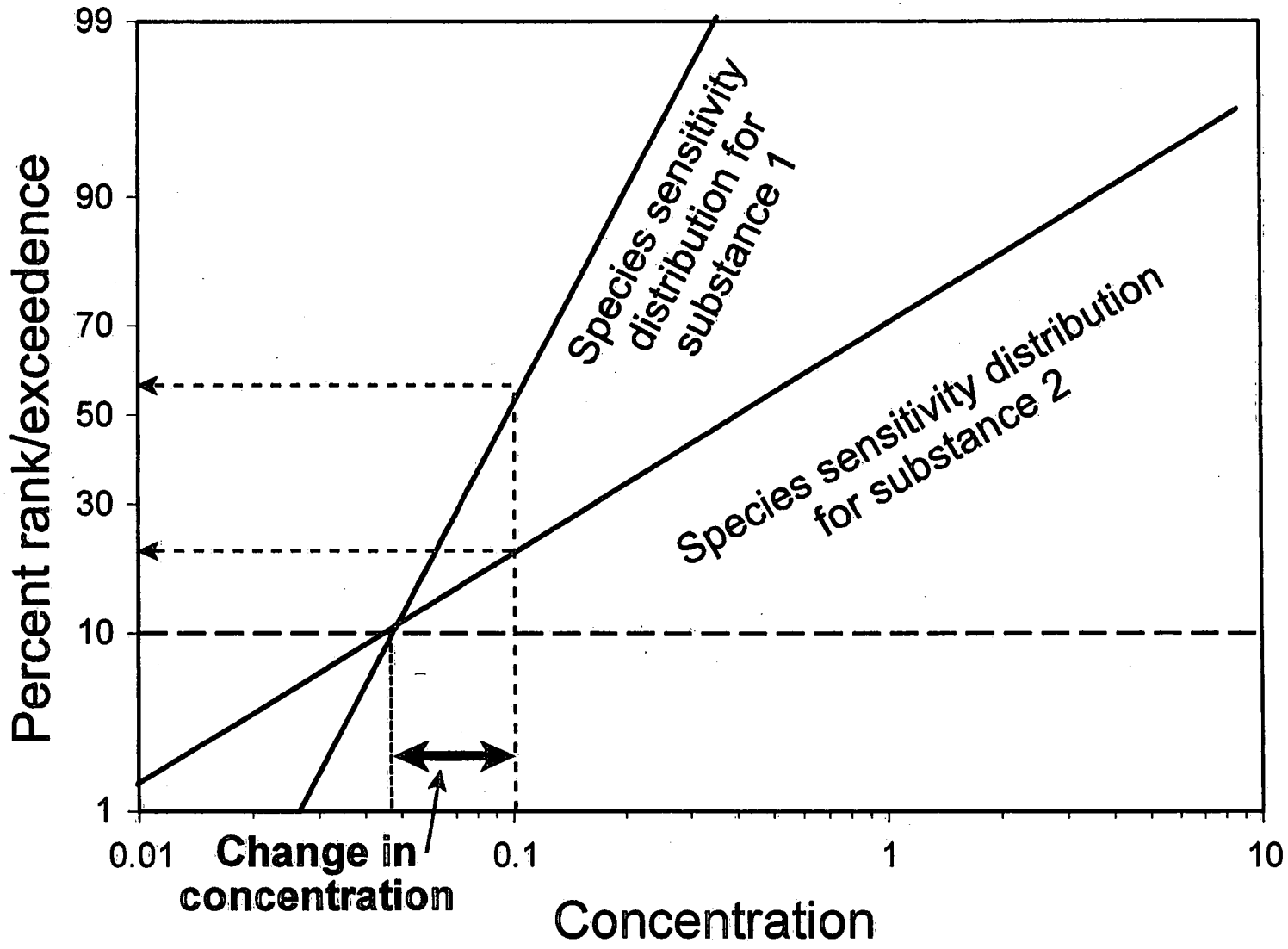
COMMUNICATION OF RISK

It is important to carry information up to the decision making level so that ecological relevance and recovery potential can be judged.

Graphical displays = rates of change, what if?

Tabular displays = remember who the critters are

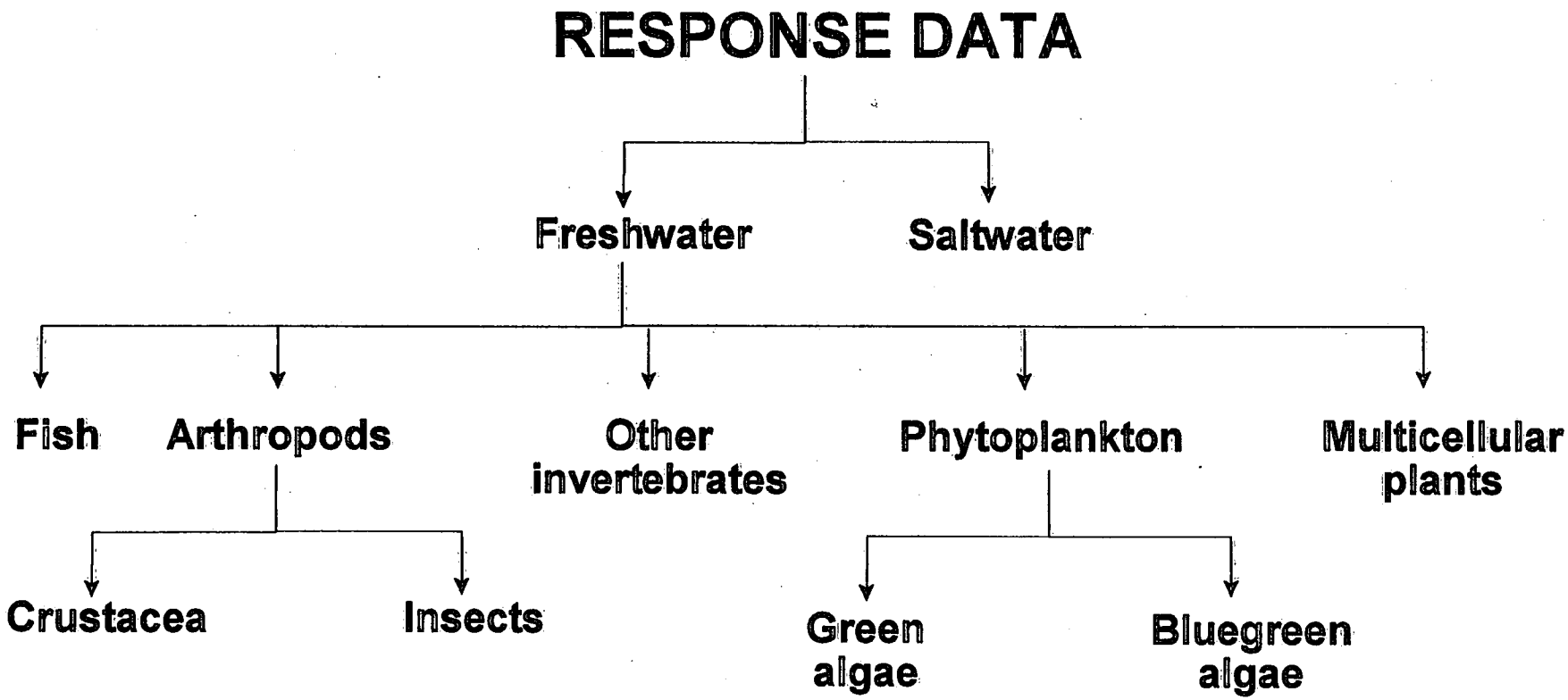
GRAPHICAL DISPLAY



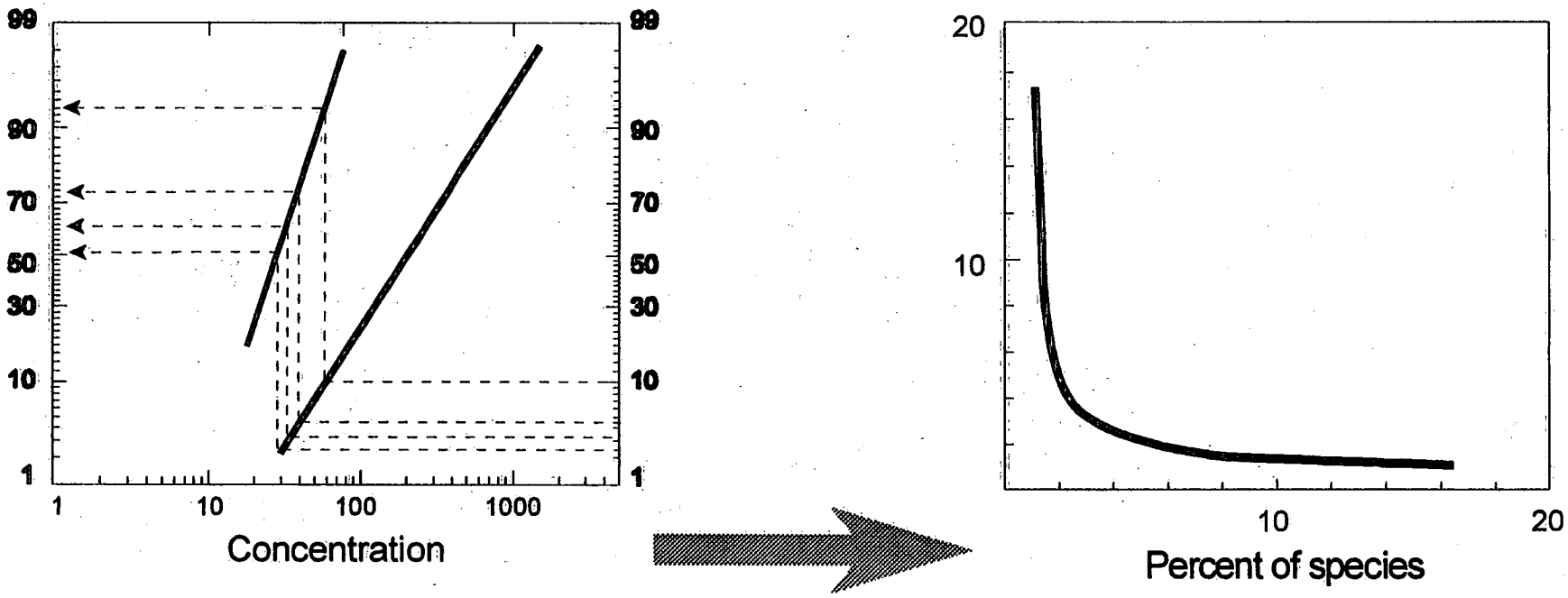
TABULAR DISPLAY

Group	Species	48 h LC50
Mosquito	various species	0.5
Mosquito	*Aedes aegypti*	10
Mosquito	*Culex pipiens*	22
Dragonfly	*Pseudagrim spp.*	50
Cladoceran	*Ceriodaphnia sp*	110
Amphipod	*Gammarus pulex*	140
Mosquito	*Culex quinquefaciatus*	150
Cladoceran	*Ceriodaphnia du*	160
Cladoceran	*Daphnia sp.*	180
Cladoceran	*Daphnia pulex*	210
Midge	(Various species)	210
Mosquito	*Culicoides pipiens*	250

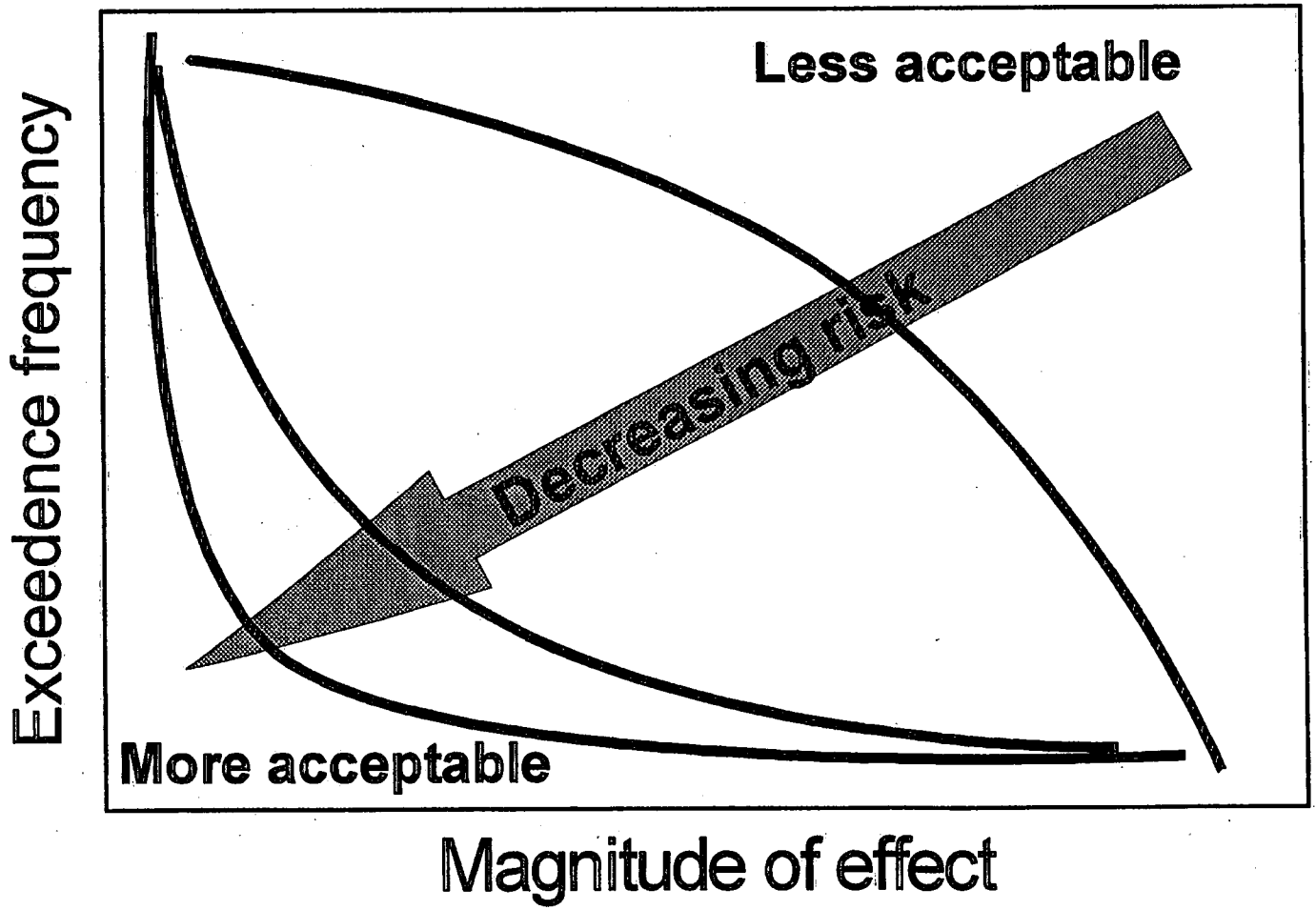
SEGREGATION OF DATA



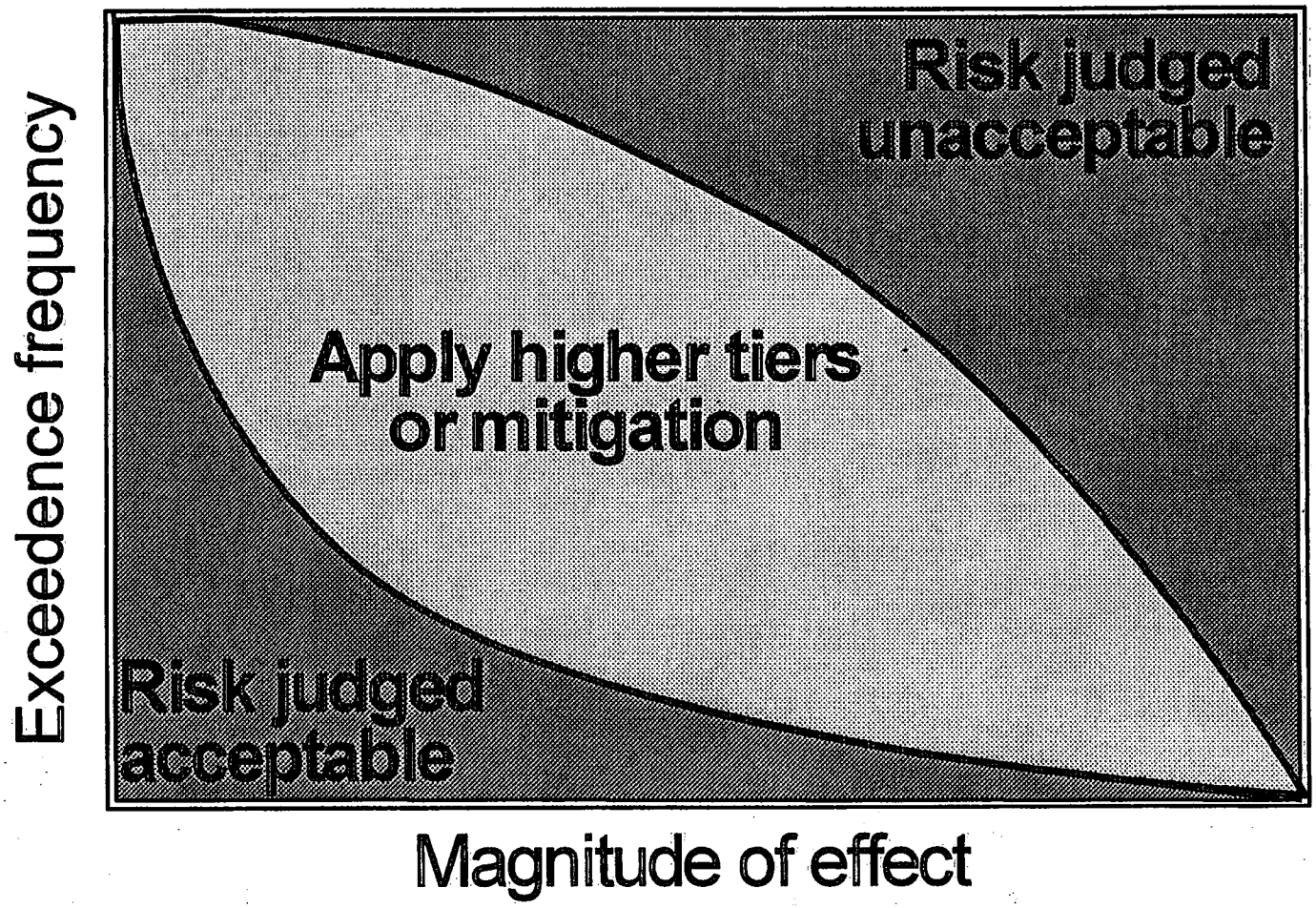
DERIVING THE EXCEEDENCE PROFILE



USING JOINT PROBABILITY CURVES TO EVALUATE RISK



USING JOINT PROBABILITY CURVES TO MAKE RISK MANAGEMENT DECISIONS



Ecological Committee On FIFRA Risk Assessment Methods

- USEPA-Industry-academia
  - Development of new methods
  - Distributional approaches to toxicity and exposure
  - Old and new pesticides
- Reviewed in June 1999
- Will be implemented in the next few years

Appendix 1

AGENDA

August 19, 1999

8:00 Registration (North Seminar Room)

8:50 Opening Remarks by *John Lawrence*, Director, AECB and *Vic Cairns*, Manager, GLLFAS

9:10 Overview of TSRI, *David Kane*, TSRI Secretariat, Health Canada

9:30 Key Note Lecture, Brominated Flame Retardants- An Environmental Issue of Particular Concern? *Ake Bergman*, Stockholm University

10:30 **Coffee Break**

10:40 PBDE Standards, *Terry Grim*, Cambridge Isotope Laboratory

11:00 Distribution of PBDEs and PCDEs in fish CRMs, *Dave Sergeant*, Great Lakes Laboratory for Fisheries and Aquatic Sciences, Department of Fisheries and Oceans

11:20 Distribution of PBDEs in the Canadian Environment, *Jennifer Luross*, University of Guelph

11:40 PBDEs in biota samples from Coastal British Columbia, *Michael Ikonomou*, Institute for Ocean Sciences, Department of Fisheries and Oceans

12:00 **Lunch Break**

1:30 Distribution of PBDEs in biota and sediments from the Estuary and Gulf of St. Lawrence (proposed study), *Michel Lebeuf*, Maurice Lamontagne Institute, Department of Fisheries and Oceans

1:50 PBDEs and Methoxy-PBDEs in selected wildlife tissues, *Mary Simon and Bryan Wakeford*, Canadian Wildlife Services, Environment Canada

2:10 Halogenated Dimethyl Bipyrroles- Identification, Distribution, and Toxicological Activity, *Sheryl Tittlemier*, Canadian Wildlife Services, Environment Canada

2:30 Estimation of Human Exposure in Canada to PBDEs, *Jake Ryan*, Health Protection Branch, Health Canada

2:50 **Coffee Break**

3:10 Toxicological Assays for PBDEs, *Nigel J. Bunce*, University of Guelph

3:30 Toxicity of PBDEs to aquatic organisms: Lessons learned from PCDEs, *Chris Metcalfe*, Trent University

3:50 Chemical Disruption and Measurement of Thyroidal Status in Fish, *Scott Brown*, National Water Research Institute, Environment Canada

4:10 Probabilistic Risk Assessment of PBDEs, *Keith Solomon and Paul Sibley*, University of Guelph

5:30 **Reception, Library Guest Lounge**

AGENDA

August 20, 1999

9:00 Workshop A: Determination, Distribution, and Fate

Workshop B: Toxicology, Risk Assessment

12:00 **Lunch Break**

1:00 Workshop Reports

1:30 Open Discussion

2:30 Closing Remarks

Appendix 2

Participants List
PBDE Workshop, 1999

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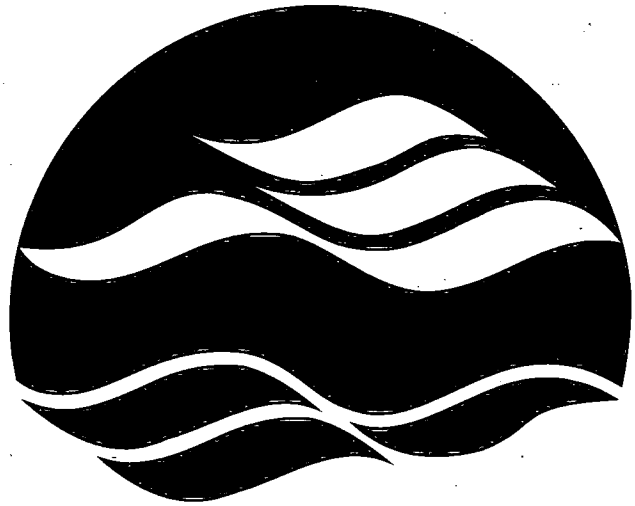

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