# ETUDE DE LA REACTIVITE ET DU TRANSFERT DU TRIBUTYLETAIN ET DU MERCURE DANS LES ENVIRONNEMENTS AQUATIQUES

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# Problématiques scientifiques

## A PROBLEMATIQUES SCIENTIFIQUES

L'expansion et l'intensification des activités humaines sont à l'origine de l'accroissement de l'emission de polluants dans les milieux naturels. Leur dispersion peut s'effectuer dans l'atmosphère sous forme d'aérosols ou de gaz susceptibles de sédimenter ou de se déposer avec les précipitations; par infiltration à partir des lieux de stockage de déchets, d'épandage de fertilisants ou de pesticides en agriculture; par ruissellement ou par rejets directs dans les eaux de surface. Les hydrosystèmes continentaux, situés à la charnière entre les sources de contamination et les grands réservoirs de la ressource en eau (eaux souterraines et océans) sont donc particulièrement exposés à la pression anthropique et jouent en conséquence un rôle primordial dans l'amortissement et la dispersion des contaminants.

La contamination des milieux aquatiques par les éléments traces métalliques et leurs dérivés organométalliques est considérée depuis quelques décennies comme une des préoccupations premières en matière de protection de l'environnement. Leurs propriétés physicochimiques remarquables, sont à l'origine de nombreuses applications industrielles impliquant d'importants rejets directs ou indirects dans les écosystèmes ainsi qu'une modification significative du cycle biogéochimique de ces éléments. Parmi ces micropolluants majeurs, le tributylétain et le mercure ainsi que leurs dérivés (mono et dibutylétain, monométhylmercure et mercure élémentaire) sont devenus aujourd'hui des contaminants ubiquistes en raison de leur grande mobilité dans l'environnement. Ils présentent en outre la spécificité d'être particulièrement disponibles aux réactions chimiques et biologiques ayant lieu dans la biogéosphère. Ainsi les espèces introduites dans l'environnement ne conservent pas nécessairement leur forme initiale et sont sujettes à divers mécanismes naturels de transformation aboutissant à des produits parfois plus toxiques, à de très faibles concentrations et plus mobiles. Ils sont donc considérés, de part leur toxicité, leur mobilité et leur persistance, parmi les polluants métalliques les plus dangereux pour les systèmes aquatiques.

Dans ce contexte, des efforts particuliers, notamment dans le domaine analytique, doivent être entrepris afin de pouvoir estimer et quantifier l'impact environnemental de la contamination par le TBT et le mercure sur les écosystèmes aquatiques. Ainsi l'analyse consistant à déterminer la concentration totale d'un élément n'est plus suffisante pour comprendre quels sont les effets de ces métaux sur l'environnement. L'analyse de spéciation, c'est à dire la caractérisation des différentes formes chimiques d'un élément, permet de savoir quels dérivés de cet élément sont susceptibles d'engendrer l'impact le plus préjudiciable pour l'environnement et, par conséquent pour la santé. L'approche par spéciation est actuellement bien répandue et permet des progrès significatifs quant à la détermination des sources et des réservoirs environnementaux de la contamination. Néanmoins, si les résultats produits ont permis une meilleure évaluation du risque, la démarche reste essentiellement rétrospective et non préventive. Le développement d'outils analytiques et expérimentaux innovants est donc nécessaire afin de déterminer la spéciation biogéochimique, qui comprend en plus de la spéciation chimique,

l'étude de la réactivité et de la mobilité de chaque espèce chimique dans les écosystèmes naturels. Il s'agit donc d'anticiper le devenir de ces contaminants, d'appréhender le risque réel et de cibler les compartiments environnementaux particulièrement exposés.

Ainsi les mécanismes de transferts et de transformations dans les différents compartiments d'un écosystème aquatique déterminent l'impact écotoxicologique jouent donc un rôle important en terme de biodisponibilité et de toxicité des formes chimiques de ces éléments. Le cycle biogéochimique du mercure et des butylétains dépend des conditions physicochimiques du milieu, des formes chimiques sous lesquelles ils y parviennent et s'y trouvent, ainsi que de l'activité biologique. En effet, les cinétiques des réactions de transfert et de transformation dépendent non seulement des concentrations environnementales mais aussi de la disponibilité (ou labilité) des composés. La spéciation biogéochimique des différentes formes présentes dans l'environnement et leur réactivité sont donc indispensables afin d'appréhender correctement leur dynamique biogéochimique et d'évaluer le risque éco-toxicologique associé. De plus, l'amplitude des mécanismes biotiques et abiotiques dépend d'un ensemble de paramètres environnementaux difficilement reproductibles lors d'expériences en laboratoire d'où la nécessité d'expériences in situ afin de se rapprocher le plus possible des conditions naturelles. Le couplage de techniques analytiques de pointe pour les analyses de spéciation et les méthodes expérimentales avec une caractérisation des paramètres environnementaux, aussi bien physicochimiques que biologiques, est donc nécessaire afin de mieux caractériser ces processus.

Le présent travail s'articule autour des problématiques jumelles du TBT et du mercure dans les écosystèmes aquatiques. Les environnements d'eau douce ou marins se révèlent particulièrement exposés à la contamination par les butylétains et le mercure. Ces deux contaminants s'y trouvent présents à de très faibles concentrations, néanmoins suffisantes pour induire des implications écotoxicologiques graves. Ils sont ainsi sujets à divers mécanismes de transformations et de transferts, dépendant des conditions physicochimiques du milieu, des formes chimiques sous lesquelles ils y parviennent, ainsi que de l'activité biologique. Cette forte réactivité des butylétains et du mercure, en particulier vis-à-vis de la biota, conditionne leur dynamique biogéochimique et renforce leur qualification de polluant majeur. La première partie de ce travail porte sur l'étude mécanistique de la réactivité et du transfert du TBT et du mercure dans des écosystèmes aquatiques reconstitués. Ces expériences associent des techniques analytiques de spéciation innovantes et performantes, basées en partie sur la quantification par dilution isotopique, à des outils expérimentaux simulant les conditions environnementales. Les cinétiques de distribution (transformations et transferts) du TBT et du mercure ont ainsi été étudiées simultanément dans des microcosmes d'eau douce présentant une organisation simple et un contrôle satisfaisant des conditions. Dans une seconde étape, les principaux mécanismes de transformations et de transferts du TBT et du mercure ont été examinés dans des échantillons naturels. Les cinétiques simultanées de méthylation et déméthylation du mercure, ainsi que la production de formes volatiles ont été caractérisées au

moyen de traceurs isotopiques dans des sédiments estuariens. Enfin les processus de formation d'espèces volatiles des butylétains et leurs transferts aux différentes interfaces environnementales (sédiment-eau, eau-atmosphère) ont été déterminés *in situ*, en milieux estuarien et côtier. La représentativité de ces mécanismes au sein des cycles biogéochimiques a été évaluée et l'impact de ces voies de remobilisation des contaminants, via notamment leurs flux d'évasion à l'atmosphère, ont pu être estimés.

Ces couplages associant des techniques analytiques de pointe à des méthodes expérimentales permettant une bonne caractérisation des paramètres environnementaux, aussi bien physicochimiques que biologiques, permettent d'accéder à une compréhension accrue de la réactivité de ces contaminants et donc de proposer une estimation plus précise des risques environnementaux associés à la contamination des écosystèmes aquatiques par le TBT et la mercure.

Les travaux sur la réactivité du TBT et du mercure dans les écosystèmes d'eau douce reconstitués ont été réalisés dans le cadre d'une coopération entre le Laboratoire de Chimie Bio Inorganique et Environnement (CNRS, UPPA) et l'Institut National de l'Environnement Industriels et des Risques (INERIS), afin de développer des outils expérimentaux pratiques pour étudier l'impact des situations de contamination chronique par le TBT et le mercure dans les écosystèmes aquatiques. La caractérisation des formes volatiles des butylétains et l'étude de leur transfert aux interfaces s'inscrivent dans le projet BIOGEST (Biogas Transfer in Estuaries), dans le cadre du programme européen ELOISE (European Land Ocean Interaction Studies). Le projet BIOGEST, financé par la Communauté Européenne, a pour objectif l'étude des gaz d'origine biologique affectant le climat et la chimie atmosphérique, dans les eaux de surfaces des estuaires européens macrotidaux. Une approche transdisciplinaire a ainsi permis de déterminer et d'évaluer les flux atmosphériques de différents biogaz et leur impact dans les budgets globaux. L'étude simultanée des principaux processus biologiques responsables de leur production et distribution a également permis de développer des modèles prédictifs reliant les émissions de biogaz à des paramètres biogéochimiques tels que la charge en matière organique ou en nutriments. L'étude des cinétiques de méthylation et déméthylation du mercure dans des sédiments estuariens a été effectuée dans le cadre des programmes GIS-ECOBAG (Groupement d'intérêt Scientifique- Environnement ECOlogie et ECOnomie du Bassin Adour-Garonne) et PNEC (Programme National Environnements Côtiers), financés par le l'INSU, l'IFREMER, la DIREN aquitaine, l'Agence de l'eau Adour-Garonne et la région aquitaine. Le but de ces projets est d'aborder de manière transdisciplinaire (géochimie-chimie-microbiologie-biologie) le transfert des contaminants métalliques entre la colonne d'eau, les sédiments et les organismes vivants dans l'estuaire de l'Adour. Ces expérimentations ont fait l'objet d'une collaboration soutenue avec le Laboratoire d'Ecologie Moléculaire (UPPA), afin de caractériser les processus microbiens impliqués dans les biotransformations du mercure.

## A.1. Cycles biogéochimiques du tributylétain et du mercure dans les environnements aquatiques

### *A.1.1. Biogéochimie du Tributylétain*

Le tributylétain (TBT) a été très récemment recensé parmi la liste des substances dangereuses prioritaires figurant dans la Directive Cadre sur l'eau adoptée par le Parlement Européen et le Conseil de l'Europe en septembre 2000 et visant à l'établissement d'un cadre légal et méthodologique pour une hiérarchisation des substances polluantes pour les milieux aquatiques (eaux côtières et superficielles) (JOCE N° 2455/2001/CE). L'intérêt grandissant des instances européennes et internationales pour les composés organostanniques et leur impact environnemental représente un effort salutaire mais tardif quant à la reconnaissance de la dangerosité de ces dérivés organiques de l'étain et aux conséquences délétères induites par leur utilisation dans de nombreux secteurs industriels.

#### A.1.1.1. Sources anthropiques et dispersion dans l'environnement

L'étain est l'élément qui présente en effet le plus d'applications industrielles du fait des propriétés physicochimiques remarquables de ses dérivés organométalliques (Blunden & Chapman, 1986). Les organostanniques ont été depuis ces 40 dernières années et demeurent encore aujourd'hui abondamment utilisés comme stabilisateurs et catalyseurs pour la synthèse de nombreuses matières plastiques. Les dérivés trisubstitués, en tête desquels figurent le TBT et le triphénylétain (TPhT), ont également fait l'objet d'une utilisation intensive dans des domaines très variés en relation avec leurs propriétés biocides avérées (Hoch, 2001). Les utilisations biocides des triorganoétains représentent environ 20% de la consommation totale des organoétains (Rüdel, 2003) et la production mondiale annuelle de TBT a été estimée à 4000-5000 tonnes en 1989 (WHO IPCS, 1990). La principale utilisation du TBT reste néanmoins circonscrite aux peintures antisalissures pour bateaux et infrastructures portuaires immergées. Ce composé xénobiotique, uniquement introduit par voie anthropique dans les environnements aquatiques, se retrouve aujourd'hui distribué à l'échelle planétaire et s'affirme comme un polluant global persistant (Maguire, 1987 ; Fent & Hunn, 1995 ; Kannan et al., 1995 & 1997a ; Michel & Averty, 1999 ; Negria et al., 2004).

Les efforts de recherche et de compréhension du devenir de ce contaminant se sont pour l'essentiel concentrés sur les zones côtières et ont débouché dès le début des années 80 sur l'adoption d'une législation toujours plus restrictive quant à la commercialisation et l'utilisation des peintures antisalissures à base de TBT, à l'échelle européenne et internationale. Néanmoins, l'utilisation d'agents antisalissures organostanniques a eu et continue à avoir des effets étendus et parfois aigus sur l'environnement. L'ampleur géographique du problème du TBT ainsi que la disponibilité de ce composé vis-à-vis des réactions chimiques et biologiques naturelles ont été largement sous-estimées. Malheureusement, ces actions sont intervenues longtemps après que les

conséquences tragiques d'une utilisation continue du TBT aient été démontrées. Elles restent donc fondamentalement rétrospectives et ne constituent en rien des mesures préventives.

Si cette problématique associée au milieu marin semble avoir été bien entendue des législateurs, les hydrosystèmes continentaux sont très curieusement ignorés et les émissions de TBT et plus généralement d''organostanniques continueront, naturellement, puisqu'ils servent d'additifs dans de nombreux produits industriels, agricoles et domestiques. Les études menées en milieu dulcicole et dans les zones fortement anthropisées restent très parcellaires. Les données disponibles sur la production mondiale d'organostanniques et leurs utilisations montrent clairement que la majeure partie de ces composés est employée comme pesticides agricoles, agents de préservation et de protection du bois et entre dans la fabrication de nombreux produits manufacturés (verre, plastique, papier, cuir) (Blunden & chapman, 1986 ; Hoch, 2001 ; Maguire, 1991). Du fait de l'utilisation domestique de ces produits, les eaux usées et les boues d'épuration constituent donc des sources significatives d'organostanniques pour l'environnement et en conséquence, les écosystèmes aquatiques terrestres demeurent fortement exposés à leur contamination (Becker Van Slooten et al., 1994 ; Ebdon et al., 1998).

L'affinement des connaissances sur les modes de transfert et les interactions dans les milieux aquatiques, ainsi que la pleine appréhension de leur complexité et de leur nature indéterminée (Santillo et al., 1998), devraient nous aider à réduire, voire à prévenir, les risques futurs liés au TBT. La Figure 1 résume les principales voies d'entrée des organoétains dans les écosystèmes aquatiques ainsi que les différents mécanismes physiques (adsorption/désorption sur les particules en suspension, remise en suspension mécanique), chimiques (dégradation chimique et photochimique) et biologiques (biodégradation, bioaccumulation) régulant leur devenir.

### A.1.1.2. Distribution du tributylétain dans les environnements aquatiques

Un nombre considérable d'études portant sur la distribution du TBT dans les systèmes aquatiques sont disponibles dans la littérature, attestant ainsi du caractère ubiquiste de cette contamination. Les gammes de concentrations mesurées dans les principales matrices environnementales sont rassemblées dans la Figure 2. Cette distribution des concentrations illustre également les sources potentielles de TBT (sédiments) ainsi que les compartiments récepteurs et réservoirs de la contamination (sédiments et biota).

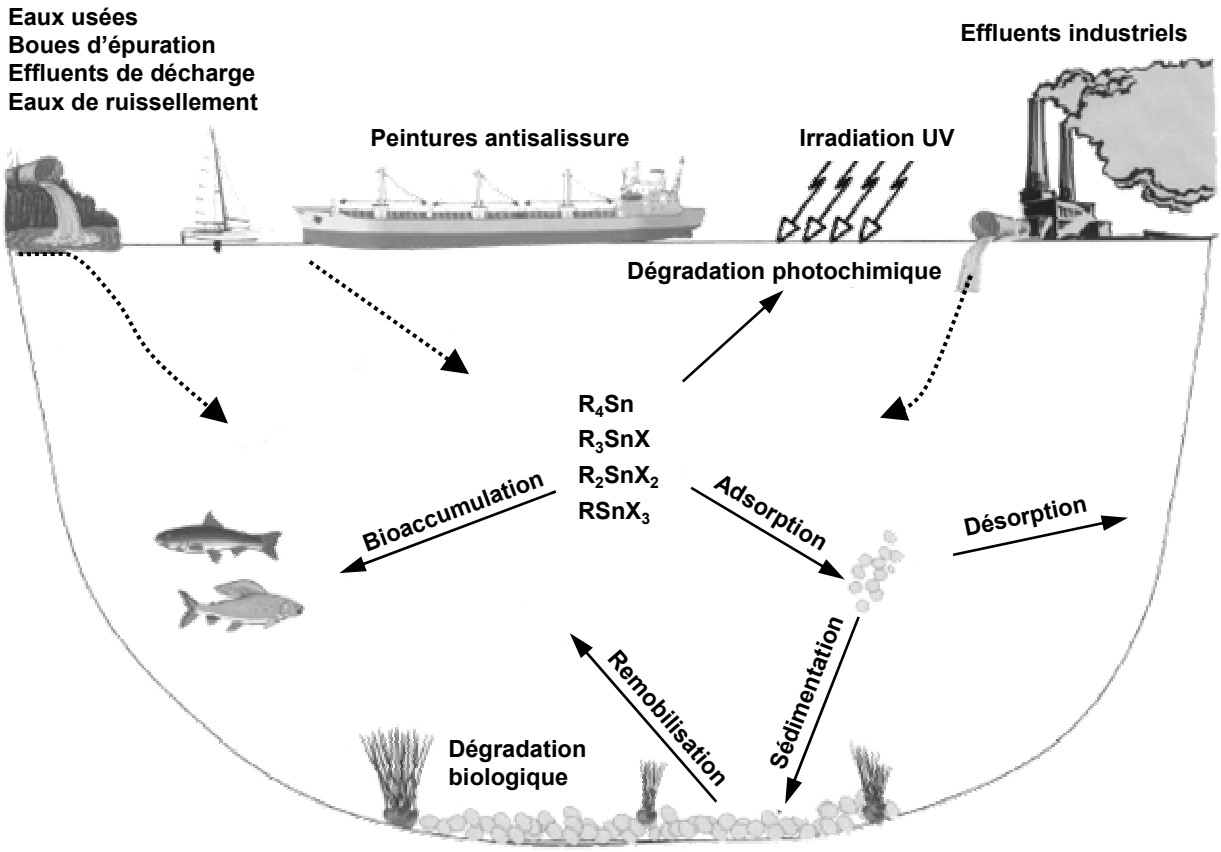


***Figure 1 –*** *Cycle biogéochimique des composés organostanniques en milieux aquatique*

*Eau*

Lorsqu'ils sont introduits dans l'eau, les principaux composés du TBT d'origine anthropique (i.e. chlorures, fluorures, oxydes et phosphates) forment des cations hydratés ($TBT^+$) pouvant se dissocier en hydroxo-complexes neutres (TBTOH) en fonction du pH (Fent, 1996 ; Hunziker et al., 2001). La forme cationique est stable pour des valeurs de pH inférieures à la constante d'acidité (p*K*a= 6,51) (Fent,1996) alors que la forme hydroxylée neutre prédomine pour des valeurs de pH supérieures au p*K*a. Outre le pH, les réactions de dissociations secondaires et la formation de complexes ou pairs d'ions sont influencées par la température de l'eau, la composition du milieu et sa force ionique. Dans l'eau de mer les hydroxydes, carbonates et chlorures de tributylétain sont les formes majoritairement rencontrées. Dans les eaux de lacs ou de rivières, les deux espèces $TBT^+$ et TBTOH sont susceptibles de former des liaisons fortes mais réversibles avec des ligands naturels présents dans la matière organique (groupements carboxylés, phénoxylés, thiols) ainsi qu'avec des constituants des membranes biologiques (Fent, 1996 ; Berg et al., 2001 ; Huzinker et al., 2001 ; Rüdel, 2003).

Le TBT est omniprésent dans les eaux de surface, quelque soit la zone géographique considérée. Les concentrations rapportées dans la littérature sont rarement supérieures au µg $l^{-1}$, mais dépassent fréquemment quelques dizaines de ng $l^{-1}$. Les valeurs observées dans les eaux fluviatiles et lacustres varient entre 1 et 50 ng $l^{-1}$. Dans les zones de plaisance ou proches de points de rejet (zones urbaines, agricoles, industrielles) les concentrations sont généralement comprises entre 100 et 500 ng $l^{-1}$ (Fent, 1996 ; Bancon, 2004 ; Hoch 2001).
La spéciation chimique du TBT dans l'eau apparaît de première importance car elle définit la biodisponibilité et donc la toxicité du TBT vis-à-vis des organismes aquatiques. Elle constitue un préalable indispensable à la compréhension de ses effets écotoxicologiques.

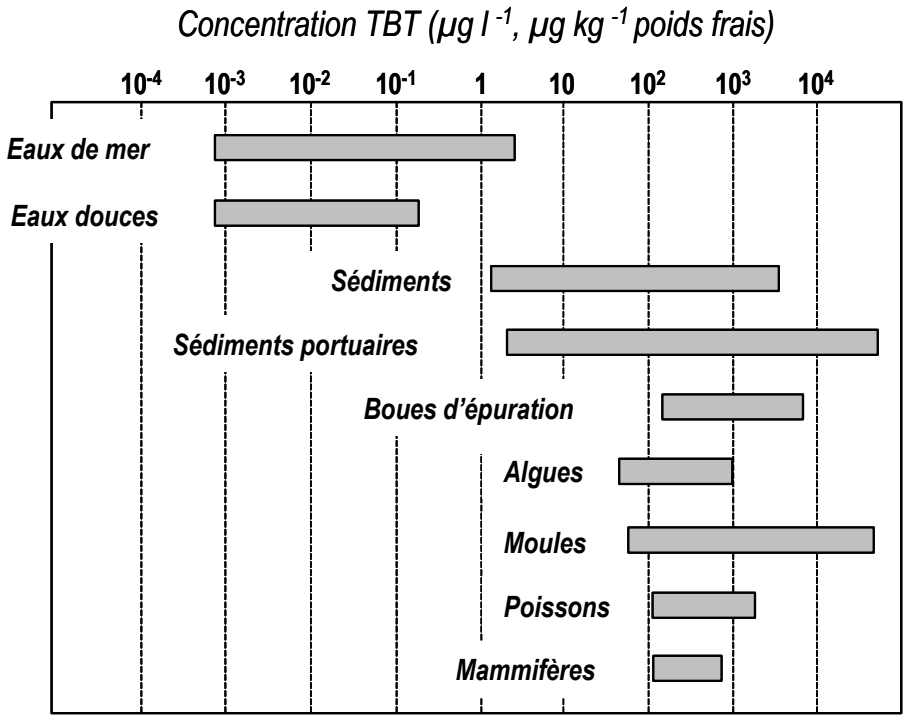


***Figure 2 –*** *Niveaux de concentration du TBT dans différentes matrices environnementales (d'après Hoch, 2001)*

*Sédiment et particules*

En raison de sa faible solubilité dans l'eau et de son caractère hydrophobe, il est généralement admis que le TBT présente une propension à s'adsorber sur les matières en suspension et le matériel sédimentaire. Néanmoins l'amplitude de cette partition dépend de nombreux facteurs environnementaux, tels que la salinité, la nature et la taille des particules ainsi que leur concentration, et la présence de matières organiques chélatantes à la fois particulaires et dissoutes (Unger et al., 1988 ; Randall & Weber ; 1986, Fent, 1996 ; Arnold et al., 1998). Dans la gamme de pH des eaux naturelles, le TBT forme également des complexes de surface stables avec les surfaces minérales du matériel sédimentaire (argiles, silice, hydroxydes d'aluminium et de fer) (Unger et al.,

1988 ; Weidenhaupt et al., 1997 ; Hoch & Schwesig, 2004). Bien que les mécanismes de sorption soient considérés comme le principal processus responsable de la diminution des teneurs en TBT dans l'eau, l'affinité du TBT pour la phase particulaire s'avère modérée en fonction de la nature des matières en suspension. Plusieurs auteurs concluent que le TBT, dans les eaux naturelles douces et marines, semble principalement présent dans la fraction dissoute avec environ 5% du TBT total associé aux particules (Maguire, 1996 ; Fent, 1996 ; Seligman et al., 1989). Néanmoins cette partition peut se déplacer de manière significative vers le solide dans certaines eaux superficielles continentales (lacs, estuaires, eaux usées) (Fent, 1996). Un autre aspect important de ces phénomènes de distribution du TBT est leur caractère réversible. Berg et al. (2001) ont montré que la sorption du TBT dans des sédiments lacustres est un processus rapide et réversible au cours duquel la matière organique particulaire joue un rôle pivot. C'est pourquoi l'adsorption du TBT sur les particules et les sédiments ne peut être considérée comme un piégeage absolu et toute resuspension de sédiments contaminés peut entraîner une augmentation significative des concentrations en TBT dans les eaux surjacentes ainsi qu'une mobilité et une distribution accrue du contaminant. Dans les sédiments, les teneurs en TBT sont généralement 100 à 1000 fois plus élevées que dans la colonne d'eau. Elles restent néanmoins très variables en fonction de l'activité maritime, des points de déversements localisés d'effluents industriels, agricoles ou domestiques. Elles sont également fortement influencées par les conditions d'échantillonnage (sédiment de surface, carottage) et la dynamique hydrologique. Pour les hydrosystèmes continentaux de surface, les concentrations varient en moyenne de 10 à 1000 µg $kg^{-1}$ (poids sec) (Fent, 1996 ; Hoch, 2001 ; Becker Van Slooten & Tarradellas, 1995). Les teneurs sont plus élevées dans les lacs et les zones portuaires avec des concentrations comprises entre plusieurs centaines de µg $kg^{-1}$ jusqu'à plusieurs mg $kg^{-1}$ (poids sec) pour les zones les plus contaminées (Maguire et al., 1986 ; Dowson et al., 1993a ; Landmeyer et al., 2004). A l'inverse les rivières présentent généralement des concentrations plus faibles, de l'ordre de plusieurs dizaines de µg $kg^{-1}$ (Bancon et al., 2004 ; Shebek et al., 1991 ; Dowson et al., 1992 ; Fent, 1996).

Les concentrations en TBT observées dans les sédiments rendent compte des apports passés et du caractère persistant de la contamination en TBT. Certaines études mettent également en évidence une légère diminution des teneurs dans les sédiments de surface, en réponse aux mesures de restriction ou d'interdiction promulguées par de nombreux états (Dowson et al., 1994 ; Evans, 1999 ; Champ, 2000). A l'inverse Becker Van Slooten et Tarradellas (1995) n'ont pas observé de diminution significative des niveaux de contamination dans les sédiments de surface de différents lacs suisses, quatre ans après l'interdiction des peintures antisalissures. Les systèmes aquatiques d'eaux douces restent, de manière globale, affectés par une contamination chronique et résiduelle des sédiments. Ce dernier compartiment représente le réservoir principal de TBT dans les environnements aquatiques et donc une source potentielle majeure de contamination pour la colonne d'eau et les organismes benthiques. Le problème actuel du TBT réside donc dans la contamination des sédiments et les

implications écotoxicologiques liées à sa remobilisation ou son transfert vers les autres compartiments environnementaux.

*Biota*

Les interactions du TBT avec les organismes aquatiques constituent, au même titre que les phénomènes d'adsorption sur les particules en suspension et le stockage dans les sédiments, un processus clé de son cycle biogéochimique dans les systèmes aquatiques continentaux et marins. Le TBT présente en effet une forte tendance à la bioconcentration dans la plupart les organismes aquatiques, à partir de l'eau et du sédiment, mais également à la bioamplification à travers le réseau trophique. Diverses études menées dans des écosystèmes limniques ont montrées l'omniprésence du TBT dans différents niveaux trophiques (algues, macrophytes, invertébrés benthiques et pélagiques, poissons omnivores ou fouisseurs, poissons carnivores et oiseaux) sans pour autant observer une bioamplification systématique au travers de la chaîne alimentaire (Fent & Hunn, 1995 ; Stäb et al., 1996). En milieu marin, la bioaccumulation ainsi que la biomagnification du TBT a été mise en évidence dans des organismes situés à des niveaux supérieurs de la chaîne, tels les thons, requins et cétacés (Iwata et al., 1995 ; Kannan et al., 1996 ; Kim et al., 1996a ; Tanabe et al., 1998). De manière générale, les mollusques bivalves filtreurs, de part leur régime alimentaire, leur sensibilité et leur faible potentiel métabolique pour les organostanniques, restent les espèces les plus exposées et sont très largement utilisés comme bioindicateurs de la contamination des écosystèmes aquatiques par le TBT (Fent ,1996 ; Hoch, 2001). Bien que la distribution du TBT dans les organismes aquatiques soit largement documentée pour un grand nombre de taxons et d'écosystèmes aquatiques, les mécanismes régulant la bioconcentration et la bioaccumulation sont quant à eux relativement peu compris. Ainsi, le caractère lipophile du TBT est généralement mis en relation avec sa capacité à être accumulé par la biota. Néanmoins, la partition de substances hydrophobes ionisables, comme le TBT, semble mal représentée par le simple partage octanol-eau. Huzinker et al. (2001) ont montré que le TBT a plus tendance à former des complexes avec certains ligands des membranes biologiques que de se partager entre phases lipidique et aqueuse. De plus, les mesures effectuées dans de nombreux organismes aquatiques d'eaux douces et marins montrent que les concentrations résiduelles dans les graisses sont beaucoup plus faibles que dans les autres tissus (Iwata et al., 1995, 1997 ; Kannan et al., 1996 & 1997b ; Stäb et al., 1996 ; Kim et al., 1996b ; Gurube et al., 1997 ; Tanabe et al., 1998). Le foie et les reins semblent être parmi les organes cibles de la contamination par le TBT, dans les organismes supérieurs (poissons, oiseaux et mammifères). Ces différents auteurs suggèrent que les liaisons TBT-protéines, dans le foie notamment, représentent le vecteur principal de la bioaccumulation.

De nombreux paramètres physicochimiques (concentration du contaminant, pH, force ionique, concentration et composition de la matière organique) et environnementaux/biologiques (groupe taxonomique, métabolisme, stade du développement, niveau trophique et régime alimentaire) influencent la biodisponibilité du TBT et

l'amplitude des processus d'accumulation (Tsuda et al., 1990 ; Fent & Looser, 1995 ; Looser et al., 1998, 2000). De plus, il convient de souligner l'intérêt fondamental de la spéciation chimique du contaminant pour accéder notamment à la compréhension des mécanismes de transferts à travers les membranes biologiques (Fent, 1996).

Enfin, les mécanismes de bioconcentration renforcent le caractère ubiquiste et persistant de la contamination des milieux aquatiques marins et continentaux par le TBT. Une fois fixé dans les organismes et subséquemment accumulé dans les réseaux trophiques, le TBT n'est alors plus disponible pour les processus de dégradation directe. Ceci est particulièrement vrai pour les mollusques bivalves, qui dominent écologiquement la plupart des habitats aquatiques et qui sont caractérisés par un fort potentiel d'accumulation (>5µg $g^{-1}$, Laughlin, 1996 ; Fent & Hunn, 1995) du fait de leurs mécanismes métaboliques et d'élimination limités. Ils représentent également une ressource alimentaire et économique avérée et constituent en outre une source d'exposition potentielle pour l'homme et un des substrats de la bioamplification à travers les réseaux trophiques aquatiques.

Ces processus d'accumulation sont donc particulièrement importants en terme d'évaluation des risques écotoxicologiques associés à ce contaminant. La distribution taxonomique de la contamination par le TBT des organismes aquatiques met également en évidence la contribution du matériel sédimentaire en tant que source et souligne encore une fois le risque potentiel lié au stockage du TBT dans ce compartiment.

### A.1.1.3. Devenir du tributylétain dans les environnements aquatiques

Le devenir du TBT et les implications écotoxicologiques liées à la présence de ce contaminant anthropique dans les milieux aquatiques sont directement fonction de sa persistance et donc de l'existence de mécanismes de dégradation, biotiques et abiotiques, pouvant se produire en milieu naturel. Le schéma principal de la dégradation du TBT, présenté dans la Figure 3, implique la rupture de liaisons carbone-étain, via des réactions de débutylation oxydatives successives. Les produits de cette dégradation sont les dérivés butylés di- et monosubstitués (DBT et MBT), jusqu'à l'obtention de l'étain inorganique (Blunden & Chapman, 1982).

Les principales voies de dégradation sont la photodécomposition, la dégradation biologique et la décomposition chimique. L'amplitude de ces processus dépend de nombreux paramètres environnementaux telles que la température, l'oxygénation, le degré d'ensoleillement, les teneurs en éléments minéraux, la présence de substances organiques biodégradables, la nature et la capacité d'adaptation des microorganismes (Maguire, 1996). De plus, les mécanismes de dégradation, assimilables à des réactions de désalkylation peuvent être concurrencés, en milieu naturel, par des réactions d'alkylation et notamment la méthylation biotique et abiotique (Weber, 1999) (Figure 3). Enfin, ces différents mécanismes, prenant place principalement dans les sédiments, modifient significativement la disponibilité et la mobilité des butylétains. Des études récentes ont ainsi démontré l'existence de flux diffusifs continus des dérivés organostanniques à l'interface sédiment-eau, en zone côtière

faiblement contaminée (Point et al., 2004 ; Amouroux et al., 2000). Ces résultats soulignent encore la dynamique, souvent sous-estimée, des butylétains en milieu aquatique. La Figure 4 rassemble les principales voies de transfert et de transformation illustrant la réactivité des composés organostanniques dans les écosystèmes aquatiques.

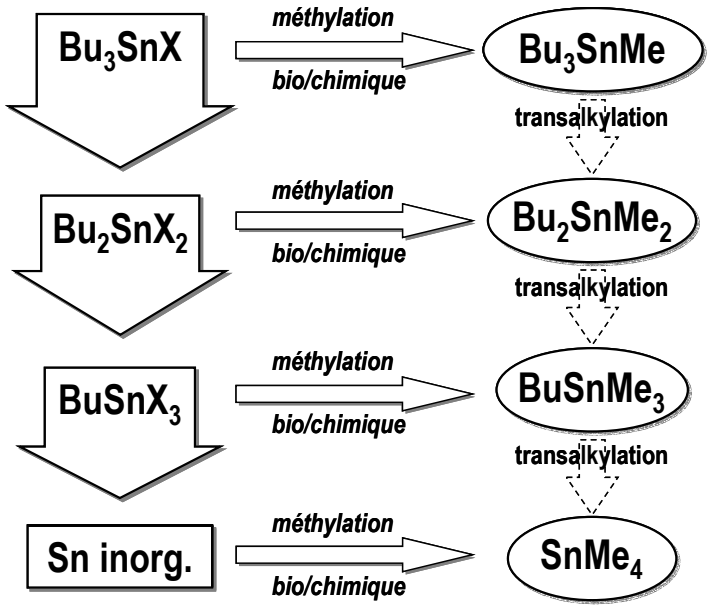


***Figure 3 –*** *Schéma de dégradation et de méthylation des butylétains*

*Persistance dans la colonne d'eau et mécanismes de dégradation*

Le TBT présente une persistance faible à modérée, dans la colonne d'eau des hydrosystèmes naturels. Les valeurs de temps de demi-vie ($t_{1/2}$), rassemblées dans le Tableau 1, varient de quelques jours à quelques semaines selon la nature du milieu aqueux et des conditions expérimentales et environnementales, comme précédemment citées. Le TBT étant chimiquement stable dans les eaux naturelles, les réactions d'hydrolyses restent négligeables. Quelque soit la nature des mécanismes de dégradation mis en jeux (biotique ou abiotique), la température et l'exposition aux radiations solaires apparaissent être des paramètres prépondérants. Néanmoins la photodégradation par la lumière du soleil ou les radiations ultraviolets (UV) demeure un processus lent caractérisé par des temps de demi-vie de quelques mois. (Maguire et al., 1983 ; Duhamel et al., 1987). Des expérimentations de photolyse du TBT sous irradiation UV artificielle (300-350 nm) ont montré une augmentation significative des vitesses de dégradation avec des temps de demi-vie de l'ordre de 1 à 18 jours. De plus, en présence d'acides fulviques (15 mg $l^{-1}$), ces valeurs sont réduites de moitié. (Maguire et al., 1983)

L'action photocatalytique du fer (III) sur la dégradation de TBT dans l'eau, sous irradiation UV (365 nm) ou sous irradiation solaire, s'avère également très efficace et abaisse les temps de demi-vie de l'ordre de quelques heures (Mailhot et al., 1999). Néanmoins, en conditions naturelles, ces mécanismes abiotiques de photodécomposition restent limités du fait de la gamme des longueurs d'onde du rayonnement solaire et de la faible pénétration des UV dans l'eau.

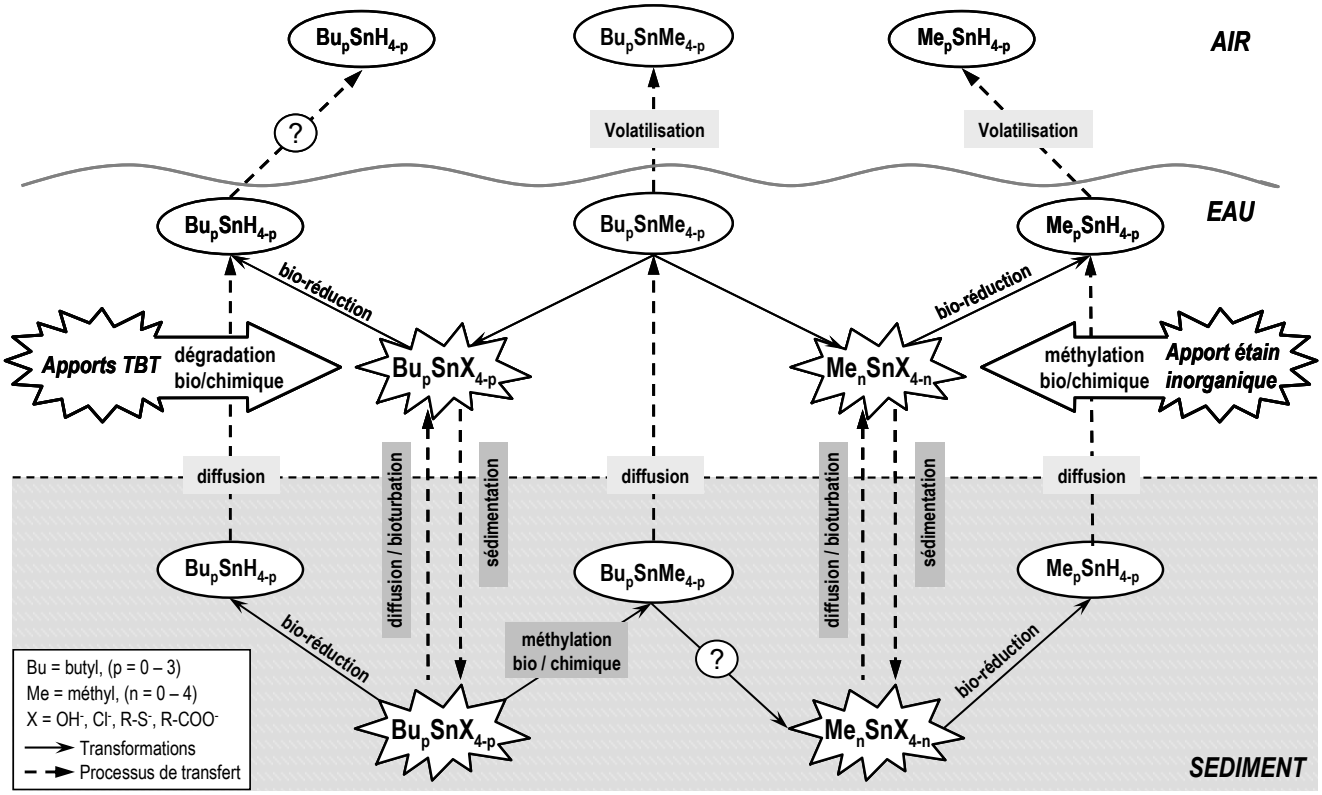


***Figure 4 –*** *Transferts et transformations des composés organostanniques en milieux aquatique*

De nombreux auteurs s'accordent sur le caractère prépondérant des mécanismes de dégradation du TBT par voie biologique (Maguire, 1987 ; Cooney, 1988 ; Seligman et al., 1988 ; Stewart & De Mora, 1990 ; Dowson et al., 1996). Ces processus de décomposition en milieu aquatique ont été notamment démontrés chez plusieurs espèces de microorganismes (algues et bactéries) (Maguire, 1984 ; St Louis et al., 1994 ; Gadd, 2000 ; Dubey & Roy, 2003). Certains auteurs indiquent que la stimulation de l'activité microbienne, en présence de lumière, induit/entraîne un accroissement de la dégradation du TBT dans la colonne d'eau, soulignant également le rôle des microorganismes photosynthétiques (Olson & Brinckman, 1986 ; Lee et al., 1989 ; Cooney & Wuertz, 1989 ; Huang et al., 1993).

***Tableau 1 –*** *Temps de demi-vie du TBT dans l'eau*

| Milieu | $[TBT]_0$ (µg Sn $l^{-1}$) | Conditions | Temps de demi-vie | Référence |
|---|---|---|---|---|
| eau douce | 1 (n)* | obscurité – 20°C | 20±5 semaines | Maguire & Tkacz 1985 |
| port Toronto, Canada | 1 (n) | lumière – KCN | > 11 mois | " |
| eau douce, marina, UK | 0,57(n) | 20°C – demi-jour | 6 jours | Thain *et al.* 1987 |
| eau de mer, UK | 0,8 (d)** | 5°C – " | 60 jours | " |
| eau estuarienne rivière Skidaway, USA | 0,5 – 1,6 (d) | obscurité – 28°C | 9-10 jours | Lee *et al.* 1987 |
| | " | " – 11°C | 11-13 jours | " |
| | " | lumière soleil – 28°C | 6 jours | " |
| | " | " – 11°C | 7-8 jours | " |
| | " | " – 19°C – 3 µg $l^{-1}$ chlorophylle | 9 jours | " |
| | " | " – 14°C – 12 µg $l^{-1}$ chlorophylle | 4 jours | " |
| | 1 (d) | " – 10 mg $l^{-1}$ $NaNO_3$ | 1-2 jours | Lee *et al.* 1989 |
| eau de mer baie de San Diego, USA | 2 (d) | obscurité – 16°C | 7 jours | Seligman *et al.* 1988 |
| | 0,23 – 2 (d) | lumière – " | 6 jours | " |
| | 0,23 (n) | " – " – formalin | 22 jours | " |
| | 2 (d) | " – " – " | 94 jours | " |
| | " | obscurité – " – " | 120 jours | " |
| eau de mer | 1 (d) | obscurité – 5°C | > 15 jours | Olson & Brinckman 1986 |
| baie de Chesapeake, USA | 0,3 – 1 (d) | lumière artificielle – 28°C | 6-7 jours | " |
| eau de mer | 0,62 (d) | mésocosme – 24°C | 3,5 jours | Hinga *et al.* 1987 |
| baie de Narrangansett, USA | 0,59 (d) | " – 22°C | 9 jours | Adelman *et al.* 1990 |

** échantillon naturel; ** échantillon dopé*

Lee et al. (1987) ont ainsi pu mettre en évidence l'absence de photodécomposition du TBT dans des eaux naturelles filtrées (0,22 µm) pendant une dizaine de jours. Mais une concentration élevée de phytoplankton (chlorophylle : 12 µg $l^{-1}$) ramène le temps demi-vie à 4 jours. L'ajout de nitrates, stimulant la croissance des algues et des bactéries (chlorophylle : 21 µg $l^{-1}$), réduit encore ce temps à 1-2 jours. (Lee et al., 1989). A l'inverse, l'inhibition de l'activité biologique, par ajout de formaline, entraîne une augmentation significative de la demi-vie du TBT de 6 à 94 jours (Seligman et al., 1988). De plus, les basses températures, en ralentissant la croissance et l'activité biologique des microorganismes, favorisent également la stabilité et la rémanence du TBT dans la colonne d'eau (Olson & brinckman,1986 ; Thain et al., 1987 ; Stewart & De Mora, 1990).

*Persistance dans les sédiments et mécanismes de dégradation*

La décomposition du TBT dans les sédiments d'eaux douces ou marins apparaît beaucoup plus lente que dans la colonne d'eau. Le Tableau 2 présente les résultats de plusieurs études cinétiques sur la dégradation du TBT dans des sédiments naturels. Les périodes de demi-vie obtenues à partir d'expériences d'incubation de sédiments en laboratoire sont de l'ordre de plusieurs mois. Elles peuvent être également déterminées *in situ* à partir de profils des concentrations dans des carottes sédimentaires. Les valeurs ainsi obtenues, de l'ordre de plusieurs années, soulignent le caractère persistant du TBT dans ce compartiment. Par ailleurs, la durée de vie des produits de dégradation du TBT (i.e. DBT et MBT) dans les sédiments, semble être du même ordre de grandeur que celle du composé parent (Dowson et al., 1993a, Sarradin et al., 1995). La variabilité des valeurs présentées dans le tableau 2 illustre la complexité des mécanismes mis en jeu et la diversité des facteurs environnementaux régissant le devenir du TBT dans les sédiments. La photolyse étant inexistante dans ce compartiment, il est généralement admis que la décomposition du TBT est régulée par des mécanismes de biodégradation impliquant des microorganismes (Maguire & Tkacz, 1985 ; Dowson et al., 1996 ; Dubey & Roy, 2003). Le caractère prépondérant de ces processus a été mis notamment en évidence par Maguire et Tkacz (1985), qui en bloquant l'activité biologique d'un sédiment par ajout de KCN, ont pu observer la stabilité des teneurs en TBT durant une période de 11 mois.

La biodisponibilité du contaminant, liée entre autre à la composition minéralogique du sédiment et à la teneur en matière organique, ainsi que la température, la biodiversité microbienne et l'oxygénation vont conditionner les mécanismes de décomposition (Fent, 1996 ; Dubey & Roy, 2003). Les conditions d'anoxie dans le sédiment semblent fortement limiter la biodégradation. Ainsi Dowson et al. (1993b) ont estimé que la demi-vie du TBT dans un sédiment anaérobie pouvait atteindre quelques dizaines d'années. Par ailleurs, si les mécanismes de dégradation du TBT dans le sédiment sont principalement d'origine biotique, il convient de souligner que la toxicité, induite par des teneurs élevés en TBT, est susceptible d'inhiber l'activité microbienne et donc de ralentir les processus de décomposition (Cooney & Wuertz, 1989 ; Avery et al., 1991 ; Gadd, 2000).

***Tableau 2 –*** *Temps de demi-vie du TBT dans les sédiments*

| Milieu | $[TBT]_0$ (µg Sn $kg^{-1}$) | Conditions | Temps de demi-vie | Référence |
|---|---|---|---|---|
| sédiment + eau douce<br>port Toronto, Canada | 1000 (n)* | obscurité – 20°C<br>" – KCN | 16±2 semaines<br>> 11 mois | Maguire & Tkacz 1985 |
| sédiment + eau de mer<br>baie de San Diego, USA | 171 (n) | obscurité – 15°C<br>aquarium ouvert | 23 semaines | Stang & Seligman 1986 |
| sédiment + eau douce, UK | 449 (n)<br>"<br>1290 (d)** | lumière/obscurité (12h/12h) – 14°C<br>aquarium ouvert<br>" | 51 semaines (surface)<br>$10^{aines}$ années (profondeur)<br>110 semaines (surface) | Dowson *et al.* 1993b<br>"<br>" |
| sédiment contaminé<br>ruisseau Red Bank, USA | 2368 – 14000 (n) | cinétique *in situ* | 25 – 50 semaines | Landmeyer *et al.* 2004 |
| sédiment eau douce<br>et estuarien, UK | 26 – 3927 (n) | carotte sédimentaire | 0,9 – 5,2 ans | Dowson *et al.* 1993a |
| sédiment portuaire<br>Boyardville, France | 19 – 195 (n) | " | 1,9 – 2,3 ans | Sarradin *et al.* 1995 |
| sédiment côtier<br>Baie de Chinhae, Corée | 1 – 69 (n) | " | 6,9 ans | Hwang *et al.* 1999 |
| sédiment portuaire<br>Nouvelle Zélande | 10 – 400 (n) | " | 1,3 – 4,4 ans | De Mora *et al.* 1995 |

** échantillon naturel; ** échantillon dopé*

La surestimation systématique des vitesses de dégradation dans les expérimentations en laboratoire s'explique par la difficulté à simuler les conditions naturelles, telles que l'apport continu de contaminant par sédimentation, les variations saisonnières de température, les conditions mixtes d'aérobiose et d'anaérobiose. La disparité des méthodes d'échantillonnage et des conditions expérimentales rendent également compte de la variabilité des cinétiques de dégradation rapportées dans la littérature.

Enfin, l'étude de mécanismes de décomposition abiotique dans le compartiment sédimentaire et leur importance relative par rapport aux processus microbiens reste très parcellaire et quelque peu contradictoire. Lors d'expériences en laboratoire sur un sédiment stérilisé riche en matière organique, Stang et al. (1992) ont ainsi mis en évidence une décomposition abiotique rapide de 85% du TBT ajouté au bout de 2 jours. A l'inverse, Landmeyer et al. (2004) n'observe pas de dégradation du TBT après autoclavage d'un sédiment de rivière. L'occurrence et l'importance de cette voie chimique de dégradation du TBT en milieu naturel demeure néanmoins incertaine et n'a pas été observé par ailleurs.

En conclusion, le TBT est peu persistant dans l'eau ($t_{1/2}$ de l'ordre du jour ou de la semaine) et sa décomposition y est assurée par voie principalement biologique en présence de lumière. Il ne s'agit pas d'une photolyse directe mais d'un accroissement de l'activité biologique sous l'influence de la lumière ou d'un processus photocatalytique. Dans les sédiments, la dégradation du TBT est lente ($t_{1/2}$ de quelques mois à quelques années) et essentiellement d'origine biotique. Le TBT y est plus stable et donc plus concentré que dans la colonne d'eau. Ce compartiment représente le réservoir privilégié du stockage du TBT dans les systèmes aquatiques continentaux et marins. La complexité et la variabilité des paramètres environnementaux régissant la biodisponibilité du TBT et donc sa stabilité font également de ce compartiment une source potentielle de contamination non maîtrisée.

*Biotransformation/Biométhylation*

Au même titre que la biodégradation, les biotransformations potentielles du TBT dans les environnements aquatiques, telles que la méthylation, jouent un rôle pivot dans son cycle biogéochimique en modifiant de manière significative à la fois sa biodisponibilité et sa mobilité. De même que pour le mercure, le sélénium ou l'arsenic, l'étain peut être méthylé dans l'environnement jusqu'à former des composés totalement substitués et donc potentiellement volatils (Thayer, 1995 ; Amouroux et al., 1998 ; Weber, 1999). En raison d'importantes limitations dans le domaine de l'échantillonnage et de l'analyse, l'existence formelle de ces espèces chimiques dans les environnements aquatiques, n'a été que très récemment rapportée dans la littérature (Weber, 1999, Amouroux et al., 2000). Les réactions de désalkylation du TBT peuvent donc être interférées par des mécanismes compétitifs de methylation d'origine essentiellement biologique, en milieu oxique ou anoxique (Weber, 1999 ; Ridley et al.,1977 ; Guard et al., 1981 ; Byrd & Andreae, 1982 ; Donard et al., 1987 ; Yozenawa

et al., 1994). De plus, dans les hydrosystèmes côtiers et continentaux, l'importance des gradients physicochimiques et du turnover biologique alliée à la contamination en TBT contribue à rendre ces processus de transformation significatifs quant au devenir de ce contaminant (Maguire & Tkacz, 1985 ; Yozenawa et al.,1994 ; Adelman et al., 1990).

Diverses études en laboratoire ont démontré que les bactéries sulfato-réductrices, présentes dans des sédiments anoxiques naturels, peuvent promouvoir activement, la méthylation de l'étain inorganique et organique (Yozenawa et al., 1994 ; Gilmour et al., 1985). La formation d'hydrures et de méthylhydrures d'étain ($Me_nSnH_{4-n}$ ; n=0-4) à partir d'étain inorganique a également pu être mise en évidence, en laboratoire, durant l'incubation de cultures de micro-organismes et d'algues, en conditions oxiques ou anoxiques (Donard et al., 1987 ; Jackson et al., 1982 ; Donard & Weber, 1988). Bien que les mécanismes de formation de ces espèces hydrurées ne soient pas totalement connus, ils sont fortement suspectés être d'origine biogénique et sont également susceptibles de se produire dans les environnements naturels (Jackson et al., 1982 ; Craig & Rapsomanikis, 1985). Par ailleurs, l'observation de formes mixtes méthylées de butylétains, telles que le méthyltributylétain ($MeSnBu_3$) et le diméthyldibutylétain ($Me_2SnBu_2$) dans des sédiments de surface contaminés a été rapportée par Maguire et al. (1984, 1986). Plus récemment, Amouroux et al. (2000) et Tessier et al. (2002) ont démontré la présence ubiquiste de ces dérivés méthylés du TBT ($Bu_nSnMe_{4-n}$ ; n = 1-3) dans des eaux et des sédiments côtiers et estuariens, à des niveaux de concentrations de l'ordre de la femtomole par litre. Des hydrures de butylétains ainsi que du stannane ($H_4Sn$) ont également été identifiés dans ces mêmes sédiments, mais seul le tétraméthylétain a pu être observé dans l'atmosphère surjacente.

Outre les mécanismes biologiques, Craig et Rapsomanikis (1985) suggèrent également que la méthylation abiotique peut avoir lieu dans les environnements naturels et expliquer la formation du tétraméthylétain. Ces mêmes auteurs montrent également que les dérivés trisubsitués sont plus facilement méthylés, que leurs homologues di- et monosubstitués. Des agents méthylants naturellement présents dans la colonne d'eau, comme le iodométhane ($CH_3I$), la méthylcobalamine ($CH_3CoB_{12}$) ou les substances humiques sont en effet capables de former, à partir des dérivés de l'étain, du mono-, di-, tri- ou tétraméthyle étain (Weber, 1999).

Ces différentes études démontrent l'origine principalement biogénique des dérivés méthylés de l'étain dans les systèmes aquatiques, ainsi que le rôle prépondérant du sédiment comme lieu privilégié de l'établissement de ces mécanismes de transformations. De plus, les processus naturels de méthylation affectent également les dérivés anthropiques de l'étain pour former des composés volatiles disponibles aux échanges entre les différents compartiments. Ils constituent, pour une fraction, un vecteur potentiel de la contamination des écosystèmes aquatiques par le TBT. Ces composés totalement substitués sont en effet, susceptibles d'être aisément redistribués dans la colonne d'eau, bioaccumulés par les organismes benthiques et pélagiques, voire de s'échanger avec l'atmosphère, via des phénomènes de bioturbation, de remise en suspension et de dégazage fonction du dynamisme hydrologique du milieu (turbulences, courants, houle).

### *A.1.2. Biogéochimie du Mercure*

Le mercure et ses composés, au même titre que le TBT, sont recensés par les instances européennes parmi les substances dangereuses prioritaires dans le domaine de l'eau (JOCE N° 2455/2001/CE). Les implications écotoxicologiques néfastes, dues à la présence de cet élément dans l'environnement, n'ont cessé de croître depuis plus d'un demi-siècle. (Craig, 1986 ; Wilken & Wallschläger, 1996 ; Kudo et al., 1998 ; Heaven et al., 2000). Le mercure est présent naturellement dans la biogéosphère sous formes d'espèces chimiques inorganiques ou organiques, à l'état dissous et particulaire et également en phase liquide et gazeuse. C'est le seul métal présent sous forme gazeuse dans l'atmosphère, pouvant ainsi circuler à l'échelle de la planète (Mason et al., 1994 ; Morel et al., 1998). Il est présent à l'état de traces voire d'ultra-traces dans l'environnement et peut être stocké dans les sols et les sédiments ou migrer vers les hydrosystèmes souterrains ou de surfaces. Sa dynamique dans l'environnement est également conditionnée par une propriété fondamentale de nature biologique : Le mercure et ses dérivés organiques en particulier sont hautement toxiques pour les organismes vivants et ils présentent une très forte capacité à la bioconcentration et à la bioamplification au sein des réseaux trophiques et particulièrement dans les écosystèmes aquatiques (Boudou & Ribeyre, 1997). Les quatre espèces chimiques principales sous lesquelles le mercure peut être présent naturellement dans l'environnement sont le mercure élémentaire Hg°, le mercure inorganique IHg, le monométhylmercure MMHg et le diméthylmercure DMHg (Stein et al., 1996). Il est important de noter que la dénomination ''mercure inorganique'' désigne un large spectre de composés, tels que des sels inorganiques, des chloro-, hydroxo- et thiocomplexes ainsi que des complexes fulviques et humiques (Stumm & Morgan, 1996 ; Meili, 1997). De plus, la spéciation chimique de cet élément dans les compartiments environnementaux est un paramètre indispensable pour accéder à la compréhension des mécanismes de transformations et de transferts auxquels il est soumis naturellement et pour ainsi prévenir des risques écotoxicologiques potentiellement associés (Clarkson, 1998 ; Boening, 2000).

#### A.1.2.1. Sources du mercure dans l'environnement

La dangerosité de ce métal vis-à-vis des écosystèmes aquatiques est étroitement liée au caractère dynamique marqué de son cycle biogéochimique. Tout d'abord, les voies d'entrée anthropiques mais également naturelles de ce contaminant sont multiples. Les grands réservoirs biogéochimiques (atmosphère, océans, sédiments et continents) sont tous plus ou moins impactés par la contamination au mercure et participent également activement à sa distribution à l'échelle globale. Le rôle central du compartiment atmosphérique et du mercure élémentaire comme vecteur de la contamination ont contribué notamment à une dispersion globale de ce contaminant dans des zones très éloignées des sources de pollution (Mierle, 1990 ; Lindqvist, 1991 ; Fitzgerald et al., 1998). La distribution du Hg dans l'environnement est complexe car cet élément, introduit par voie anthropique ou naturelle est continuellement recyclé dans la biogéosphère. Actuellement, les apports

anthropogéniques directs représentent seulement 20% des apports globaux à l'environnement. Les 80% restants, recouvrant essentiellement les émissions à l'atmosphère, illustrent l'accumulation et l'incorporation chronique du Hg anthropique dans le cycle naturel.

*Sources naturelles*

La principale entrée de mercure dans l'environnement a donc lieu sous forme de vapeur de mercure élémentaire et par transfert via l'atmosphère. Les émissions continentales naturelles, représentant environ 5 Mmol $an^{-1}$ (Lamborg et al., 2002), sont essentiellement liées aux phénomènes de dégazage de la croûte terrestres et à l'activité tectonique (éruptions volcaniques : 4,15 Mmol $an^{-1}$ ; sources géothermales : 0,3 Mmol $an^{-1}$) (Varekamp & Busek, 1986 ; Varekamp & Waibel, 1987). La seconde source naturelle de mercure à l'atmosphère est la production de Hg° dans les eaux de surface. L'émission de Hg° à partir des océans est actuellement estimée à 4 Mmol $an^{-1}$, valeur comparable à la volatilisation naturelle à partir des continents. Dans l'atmosphère, Hg° est soumis à des réactions d'oxydation lentes, avec des temps de demi-vie compris entre 0,3 et 2 ans (Fitzgerald et al., 1998 ; Schroeder & Munthe, 1998). Les dépôts atmosphériques de mercure inorganique ainsi formé constituent ainsi la source majeure de mercure pour les environnements aquatiques mais également terrestres (Bloom & Fitzgerald, 1988). Actuellement, les dépositions atmosphériques globales, sèches et humides, atteignent 11 Mmol $an^{-1}$ sur les continents et les océans 10 Mmol $an^{-1}$ dans les océans (Lamborg et al., 2002).

*Sources anthropiques*

Le flux anthropique global de mercure élémentaire vers l'atmosphère est estimé entre 9.5 et 13 Mmol $an^{-1}$ et contribue à 80% des apports anthropiques (Pacyna & Pacyna, 2002 ; Lamborg et al., 2002). Les rejets directs dans les sols par l'utilisation en agriculture de divers composés biocides et l'épandage de boues de stations d'épuration recouvrent environ 15% des apports anthropiques. Enfin les rejets industriels directs vers les eaux de surfaces continentales recouvrent les 5% restants (Stein et al., 1996). Les principales sources ponctuelles sont la combustion des énergies fossiles et principalement du charbon, l'incinération des déchets, l'industrie chimique (electrolyse chloro-alcaline) et la métallurgie non ferreuse (cuivre, nickel, plomb) et les productions d'acier et de ciment (Pirrone et al., 1996 ; Pacyna & Pacyna, 2002). De manière générale, les pays asiatiques (Chine, Inde, Corée) contribuent à environ 56% des émissions globales de Hg à l'atmosphère. L'Europe et le continent nord américain recouvrent moins de 25% de ces émissions (Pacyna & Pacyna, 2002). Par ailleurs, l'utilisation des composés organomécuriels, pour leurs propriétés biocides (insectides, fongicides), a été abandonnée dans les pays industrialisés.

Les émissions anthropiques ont fortement perturbées le cycle naturel du mercure, depuis l'avènement de l'ère industrielle. Actuellement, de par la forte dynamique biogéochimique de cet élément à l'échelle globale et malgré la réglementation de l'utilisation et des émissions directes de mercure, la principale source à l'environnement est la réémission de mercure anthropique déposé. Les émissions, dépôts, concentrations dans l'air et les eaux de surfaces sont en moyennes trois fois supérieurs à ceux de l'époque préindustrielle. Tous les compartiments environnementaux ont été contaminés par le mercure provenant des activités humaines (Mason et al., 1994).

### A.1.2.2. Devenir du mercure dans les environnements aquatiques

Le devenir du mercure dans les systèmes aquatiques est régi par les échanges entre les compartiments environnementaux et les transformations naturelles que peuvent subir les différentes formes chimiques mercurielles présentes dans le milieu. La Figure 5 illustre une vue générale de ces mécanismes en milieu aquatique. Les deux principales voies de transformation du mercure inorganique dans les environnements aquatiques sont les réactions de réduction et de méthylation. Ces processus s'avèrent primordiaux car ils modifient les propriétés physicochimiques du mercure et par conséquent sa dynamique environnementale et sa biodisponibilité. La réduction en mercure élémentaire entraîne ainsi une mobilité accrue du contaminant et son recyclage via l'atmosphère. La formation d'espèces alkylées, via la méthylation, accroît sa biodisponibilité et sa bioamplification dans les réseaux trophiques.

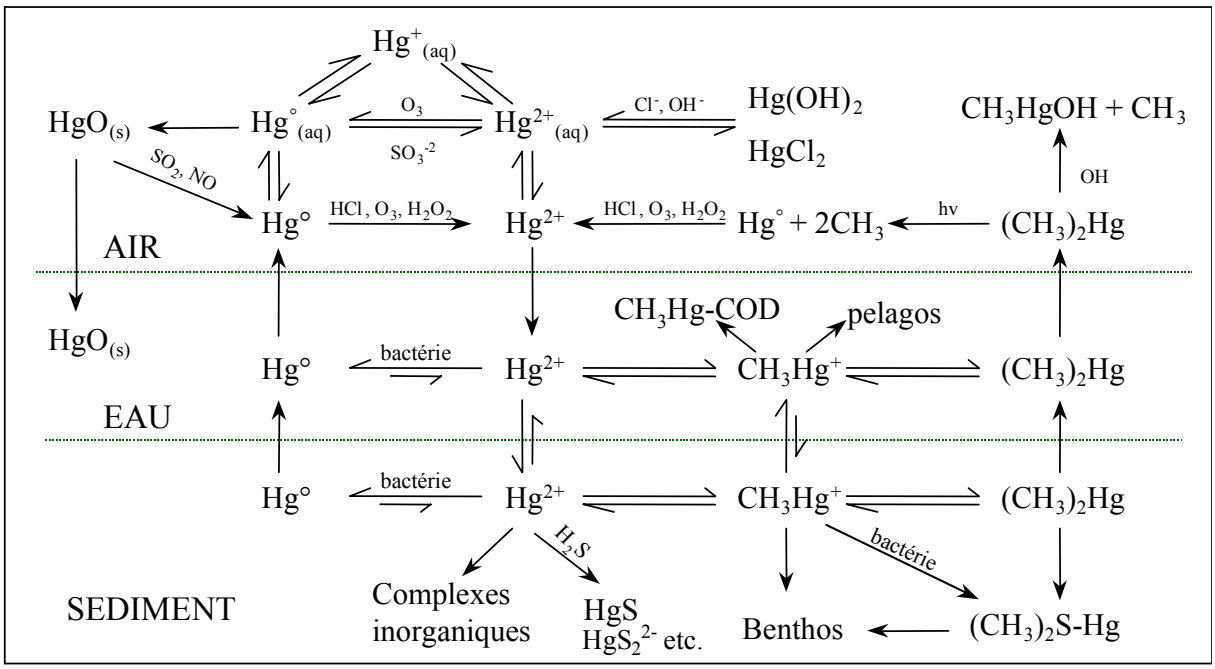


***Figure 5 -*** *Transferts et transformations des espèces chimiques du mercure en milieu aquatique (d'après Stein et al., 1996).*

Il est important de souligner que seule une fraction du mercure inorganique est disponible pour ces réactions de transformation. L'hypothèse du substrat, avancée par Mason et Fitzgerald (1990) et Fitzgerald et al. (1991), propose que seules les espèces labiles du mercure inorganique ou mercure réactif participent aux mécanismes de réduction et de méthylation en milieux aqueux. Les cinétiques et l'intensité de ces transformations devraient dépendre de la teneur en Hg réactif présent dans les eaux naturelles, plutôt que de la concentration totale en Hg dissous. Ce dernier point renforce encore l'intérêt indiscutable de la spéciation chimique du Hg dans les différentes matrices environnementales pour appréhender correctement son devenir et évaluer le risque écotoxicologique associé. Les concentrations ubiquitaires en Hg total (i.e. IHg + MMHg), et le pourcentage de MMHg rencontrées dans différents compartiments des systèmes aquatiques sont présentées dans la Figure 6.

*A.1.2.2.1. Réduction*

La réduction du mercure inorganique est considérée comme la source principale de mercure élémentaire dans les eaux marines (Mason & Fitzgerald, 1993) ou lacustres (Fitzgerald et al ., 1991 ; Amyot et al., 2000). Cette voie de transformation est d'importance car elle permet de réduire la quantité de mercure inorganique dans les environnements aquatiques et donc limiter le substrat pour la méthylation (Fitzgerald et al., 1991 ; Amyot et al., 2000). Les teneurs en mercure élémentaire dans les eaux résultent de la combinaison de processus photochimiques et biologiques.

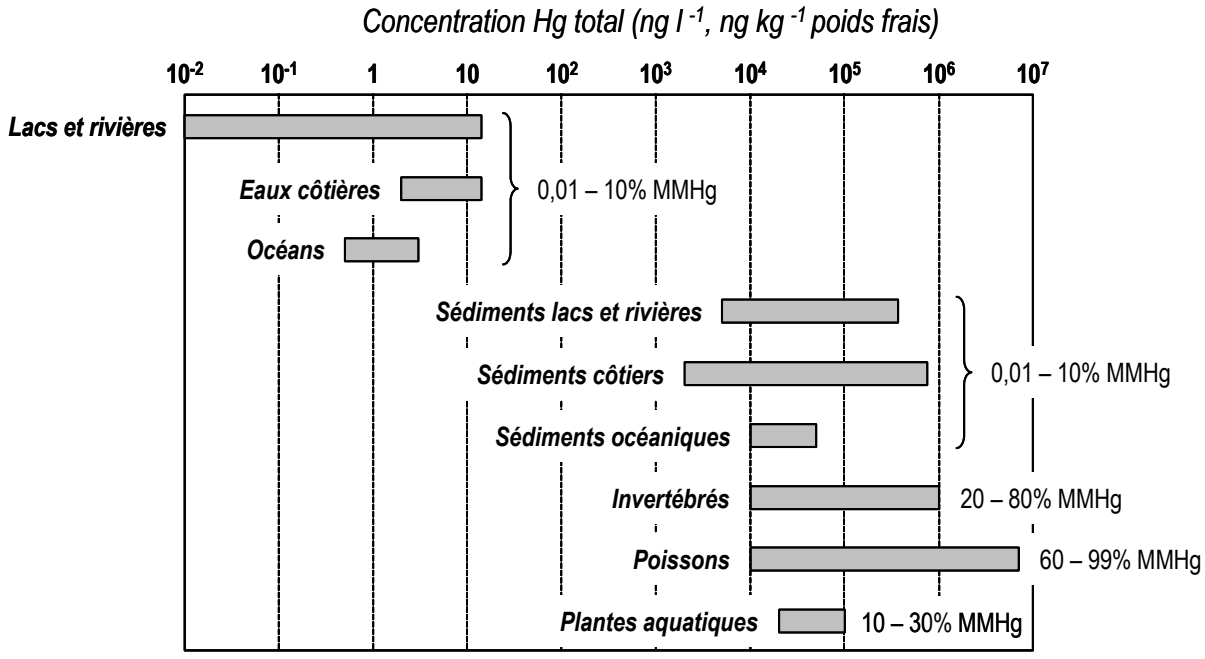


***Figure 6 -*** *Niveaux de concentrations en Hg total et % de MMHg en milieu aquatique (WHO IPCS, 1989 ; USEPA, 1997a ; INERIS, 2003 ; Slooff et al., 1995).*

*Réduction abiotique*

Dans les eaux de surface, la photoréduction du IHg en Hg° est induite par les radiations UV (Amyot et al., 1994 & 1997 ; Costa & Liss, 1999). Ce mécanisme est également stimulé par la réactivité photochimique de diverses composantes des eaux naturelles et de certaines formes complexées du mercure. De nombreuses études menées en laboratoire, à partir d'incubation d'échantillons naturels d'eaux douces ou marines, ont ainsi mis en évidence les mécanismes potentiels de réduction abiotique. Les acides humiques et fulviques sont capables de réduire IHg en Hg° (Allard & Arsenie, 1991 ; Matthiessen, 1996). La photoréduction de IHg, catalysée par le fer(III), a été démontrée par Zhang et Lindberg (2001). Les complexes organiques du mercure comportant des ligands photophores réactifs participent également à la réduction photochimique (Xiao et al., 1995 ; Weber, 1999 ; Nriagu, 1994). En conséquence, l'intensité lumineuse, la composition et la concentration de la matière organique dissoute (MOD), les teneurs en fer réactif ainsi que la disponibilité du mercure inorganique conditionnent la réduction photochimique (Zhang & Lindberg, 2001 ; Amyot et al., 2004). Cependant, si de nombreux mécanismes réactionnels ont pu être détaillés lors d'expérimentations en laboratoire, leur existence et leur contribution respective à la dynamique chimique du mercure dans les eaux naturelles demeurent mal estimées (Zhang & Lindgerg, 2001 ; O' Driscoll et al., 2004).

*Réduction biotique*

Les mécanismes d'origine biotique apparaissent néanmoins être les voies principales de la réduction du mercure inorganique dans les systèmes aquatiques marins et continentaux (Mason & Fitzgerald, 1993 ; Fitzgerald et al., 1991 ; Mason et al., 1993 ; Siciliano et al., 2002). Des concentrations élevées de mercure gazeux dissous (Hg°, DMHg) ont pu être observées dans des eaux profondes démontrant ainsi que les réactions photochimiques ne sont pas les seuls mécanismes impliqués dans la réduction du IHg (Mason et al., 1995a). Les bactéries hétérotrophes et le phytoplancton sont considérés comme les principaux acteurs de la réduction en milieu aquatique (Mason et al., 1995b ; Mason et al., 1998). Ces réactions sont essentiellement induites par les activités enzymatiques se produisant dans et à la surface des cellules des microorganismes (bactéries, algues) (Siciliano et al., 2002 ; Barkay et al., 2003 ; Poulain et al., 2004). Des expériences en laboratoire ou in situ, ont démontrées que certaines bactéries sont capables de réduire le mercure par une voix enzymatique codée génétiquement par le gène *mer A* (Barkay et al., 2003). Siciliano et al. (2002) ont également mis en évidence la complexité et l'interaction des processus enzymatiques microbiens de réduction et d'oxydation régulant les teneurs en Hg° dans des eaux douces de surface à l'échelle de la journée. Par ailleurs, Poulain et al. (2004) ont montré que des organismes phototrophes comme des algues sont capables de réduire le mercure et que ce processus est étroitement lié à l'activité photosynthétique. Dans les eaux de surface, la

lumière et les fortes températures favorisent généralement les émissions de Hg° en stimulant l'activité biologique des organismes participant à la réduction du IHg (Barkay et al., 2003).

La déméthylation du mercure par des processus biotiques et abiotiques peut également constituer une source de Hg° dans les eaux naturelles. Cette contribution semble néanmoins négligeable dans les eaux de surface (Mason et al., 1993 ; Mason et al., 1995a). Enfin la réoxydation du Hg° en IHg a souvent été ignorée, du fait de la faible réactivité supposée de Hg°. Des études récentes montrent que ce mécanisme induit par l'action de la MOD, des chlorures ou de l'activité enzymatique microbienne est à même de contrebalancer les réactions de réduction et d'avoir une influence significative sur le budget total du mercure dans les systèmes aquatiques (Siciliano et al., 2002 ; Lalonde et al., 2001).

*A.1.2.2.2. Méthylation*

La méthylation du mercure inorganique dans les milieux aquatiques s'effectue par le biais de processus naturels biotiques ou abiotiques. Les sédiments superficiels constituent de manière générale le site privilégié de la méthylation (Gilmour et al., 1998). Néanmoins ces processus peuvent également être observés, dans une moindre mesure, dans la colonne d'eau (Mason & Fitzgerald, 1990 ; Pongratz & heumann, 1998 ; Leermakers et al., 2001). Ces mécanismes de transformation aboutissent à la production d'espèces méthylées plus toxiques et biodisponibles (MMHg) mais également plus mobiles car volatiles (DMHg). Dans les eaux océaniques, les zones de déplétion en oxygène présentant une activité microbienne importante sont également le siège de la formation du DMHg (Mason & Sullivan, 1999 ; Cossa et al., 1994). De nombreuses études, menées en laboratoire à partir d'échantillons naturels, pu proposer divers schémas réactionnels potentiels, ainsi qu'une approche cinétique des mécanismes engagés. Néanmoins bon nombre d'entre eux demeurent incomplets et leur existence en conditions naturelles reste à confirmer. Enfin, ces processus doivent être considérés comme étant la résultante de mécanismes réversibles, simultanés et compétitifs de méthylation et de déméthylation. Le Tableau 3 rassemble quelques exemples de potentiels de méthylation et de déméthylation du mercure, calculés à partir d'incubations de sédiments dopés en IHg ou MMHg. Les proportions de MMHg par rapport aux teneurs en Hg total généralement observées dans diverses matrices environnementales sont présentées dans la Figure 6. Dans les écosystèmes aquatiques, MMHg représente ainsi de 0,01 à 10% du Hg total dans l'eau et les sédiments, de 10 à 30% dans les plantes, de 20 à 80% dans les invertébrés et de 60 à 99% dans les poissons et les prédateurs supérieurs (rapaces, mammifères).

***Tableau 3 –*** *Potentiels de méthylation et de déméthylation du mercure dans des sédiments*

| Site | Méthode | $[IHg]_0$ | $[MMHg]_0$ | Potentiel de méthylation | Potentiel de déméthylation | Référence |
|---|---|---|---|---|---|---|
| | | µg Hg $g^{-1}$ ou $ml^{-1}$ | µg Hg $g^{-1}$ ou $ml^{-1}$ | % $d^{-1}$ | % $d^{-1}$ | |
| Cheese quake, USA | slurry | 100 | 1 | 0,035 | 0,25 | Compeau & Bartha 1984 |
| Lac Ontario, Canada | slurry, $^{203}$IHg | 0,25 | | 0,001 - 0,22 | 0,02 - 0,27 | Ramlal *et al.* 1986 |
| Cadwell creek, USA | slurry | 0,25 | | 0,08 | | Gilmour & Capone 1987 |
| Pine Barrens, USA | slurry | 1 | 0,1 | 0,4 | 20 | Pak & Bartha 1998a |
| Rivière Minamata, Japon | aquarium | 1 | | 0,6 | | Ikingura & Akagi 1999 |
| Clear lake, USA | slurry | 125 | | 0,11 | | Macalady *et al.* 2000 |
| Lac Ontario, Canada | slurry | 3 | 0,3 | 1,6 | 0,5 | Hintelmann *et al.* 2000 |
| Estuaire de l'Adour, France | slurry, $^{199}$IHg, $^{201}$MMHg | 0,1 | 0,02 | 2,4 | 9 | Rodriguez *et al.* 2004 |
| Quabbin reservoir, USA | carotte | 0,5 | | 12 | | Gilmour *et al.* 1992 |
| Everglades, USA | carotte, $^{203}$IHg | 0,001 | | 0,12 | | Gilmour *et al.* 1998 |
| Skidaway river, USA | carotte | 1 | | 0,012 | | King *et al.* 2001 |
| Etang de Thau, France | carotte, $^{199}$IHg | 0,1 | | 0,75 - 0,90 | | Monperrus *et al.* 2003a |
| Long Island Sound, USA | carotte | | | 0,5 - 7 | | Hammerschmidt & Fitzgerald 2004 |

*Méthylation abiotique*

Plusieurs voies potentielles de méthylation du mercure par des processus physicochimiques ont été proposées (Weber, 1993). Ces mécanismes peuvent être observés dans l'eau et les sédiments. De nombreux agents méthylants, naturellement présents dans l'environnement sont susceptibles d'alkyler IHg par le transfert d'un carbanion méthyle ($CH_3^-$). La méthylcobalamine, les substances humiques, les halogénures de méthyle et certains composés métalliques méthylés peuvent participer à cette réaction. La méthylation abiotique par les substances humiques et fulviques a été démontrée par Weber (1993) en laboratoire. Néanmoins, en milieu naturel, la forte complexation de IHg avec les substances humiques prédomine sur la méthylation. Le iodométhane, synthétisé naturellement par certaines algues marines, est également susceptible de méthyler le mercure (Craig, 1982). Par ailleurs, des réactions de transméthylation entre IHg et des composés méthylés du plomb et de l'étain notamment, peuvent conduire à la formation de MMHg (Craig, 1986 ; Jewett et al., 1975 ; Rosenkranz et al., 1997). Cette voie de production semble notamment significative pour des sites présentant une pollution polymétallique conséquente (Ebinghaus et al., 1994). Néanmoins, ces agents méthylants organométalliques ne sont présents qu'à l'état d'ultra traces dans les systèmes non perturbés, limitant en conséquence l'importance des mécanismes de transméthylation.

En outre, la méthylation du mercure inorganique dans la colonne d'eau peut également être induite par voie photochimique. Cette réaction s'effectue sous l'action des radiations UV et par l'intermédiaire de produits de fin de métabolisme (acide acétique, méthanol, sulfure d'hydrogène, acides aminés) (Agaki et al., 1975 ; Gardfeldt et al., 2003).

Enfin les réactions abiotiques de formation de DMHg constituent également un processus important aboutissant à la production d'espèces volatiles susceptibles d'être dégazées. A pH supérieur à 7, la méthylation de IHg par la méthylcobalamine peut se poursuivre jusqu'à la formation de DMHg, bien que la seconde étape de méthylation soit très lente (Craig, 1986). Dans les environnements riches en sulfures, comme les sédiments, la diméthylation peut aussi résulter d'une réaction de dismutation du MMHg (Craig & Moreton 1984 ; Baldi et al., 1995). Le DMHg volatil ainsi formé peut être transféré à la colonne et potentiellement à l'atmosphère. Le sulfure mercurique (HgS) insoluble se dépose dans les sédiments et n'est alors plus disponible pour la méthylation. HgS constitue ainsi le puit principal de Hg dans les sédiments (Andersson et al., 1990).

*Méthylation biotique*

Dans les systèmes aquatiques non perturbés, la méthylation biotique est reconnue comme la principale voie de formation de MMHg (Berman & Bartha, 1986 ; Fitzgerald & Mason, 1997). Différents types d'organismes, tels que des bactéries (Jensen & Jernelov, 1969 ; Compeau & Bartha, 1985 ; Gilmour et al., 1992 ; Pak & Bartha, 1998a ; King et al., 2001), des macroalgues (Pongratz & Heumann, 1998) et des macrophytes (Mauro et al., 2002), ont été identifiés comme étant à l'origine de ces biotransformations. Les bactéries sulfato-réductrices

(BSR) sont reconnues comme étant les principaux médiateurs de la méthylation du mercure dans les sédiments anoxiques marins (King et al., 2000), estuariens (Compeau & Bartha, 1985) ou d'eau douce (Gilmour et al., 1992) ainsi que dans les eaux anoxiques lacustres (Matilainen, 1995). Diverses expériences d'inhibition spécifique de l'activité des BSR, par ajout de molybdate ($MoO_4^{2-}$), ont confirmé le rôle primordial de ces microorganismes pour la méthylation du Hg dans les sédiments (Compeau & Bartha, 1985 ; King et al., 1999). A l'inverse, la présence de sulfates stimule l'activité des BSR et accroît en conséquence la production de MMHg (Gilmour et al., 1992). Néanmoins, des teneurs en sulfures élevées, induites par la sulfato-réduction, sont susceptibles de limiter la disponibilité du Hg via des réactions de complexation et donc de diminuer significativement les taux de méthylation (Gilmour et al., 1998 ; Compeau & Bartha 1985).

Cependant, les métabolismes microbiens mis en œuvre lors de la méthylation du Hg demeurent peu connus. La biométhylation peut être enzymatique (Choi et al., 1994a&b ; King et al., 2000) ou non enzymatique (Matilainen, 1995). La première voie nécessite la participation active d'organismes métaboliseurs, alors que la seconde requiert uniquement la présence dans le milieu des produits de l'activité métabolique comme par exemple la production de méthylcobalamine (Wood et al., 1968). La méthylation enzymatique dépend de la physiologie des souches bactériennes dominantes ainsi que du métabolisme du carbone. King et al. (2000) ont ainsi montré que les bactéries utilisant l'acétate comme source de carbone semblent méthyler plus efficacement le Hg en raison de l'utilisation de l'enzyme méthyltransferase. Par ailleurs, dans des environnements dulcicoles, Pak et al. (1998b) ont pu observer l'établissement d'une synergie spécifique entre BSR et bactéries méthanogènes aboutissant à une méthylation significative du Hg.

Enfin Gilmour et Henry (1991) ont montré lors d'une étude comparative que les taux de méthylation du Hg associés à l'activité des BSR étaient plus faibles dans les sédiments d'eaux douces qu'en milieu marin. Dans les systèmes aquatiques naturels, l'amplitude des mécanismes de méthylation biotiques et abiotiques dépend d'un ensemble de paramètres physicochimiques et environnementaux difficilement reproductibles lors d'expérience en laboratoire, tels que le pH, les conditions redox, la biodiversité microbienne, les teneurs en sulfure et en matière organique et la présence d'agents méthylants biogéniques ou organométalliques. Bien que divers schémas réactionnels de méthylation abiotique aient été démontrés *in vitro* comme étant possibles et efficaces, la biomethylation dans les sédiments demeure la voie privilégiée de formation du MMHg dans les écosystèmes aquatiques naturels (Berman & Bartha, 1986).

*A.1.2.2.3. Déméthylation*

Simultanément à la méthylation, les processus de déméthylation du MMHg peuvent être de nature abiotique ou biotique et se dérouler dans l'eau et les sédiments. La déméthylation abiotique fait essentiellement intervenir la photodégradation du MMHg dans la colonne d'eau (Sellers et al., 1996). Dans les eaux de surface, ce mécanisme peut devenir significatif et représenter une voie de transfert non négligeable de Hg à l'atmosphère

en fonction des conditions saisonnières ou diurnes d'ensoleillement. (Gardfeldt et al., 2001). La dégradation du MMHg demeure néanmoins un processus principalement microbien s'effectuant soit par voie réductive, principalement en milieu aérobie (Omerland et al., 1991), soit par voie oxydative en conditions anoxiques (Marvin-Dipasquale et al., 2000). Diverses souches bactériennes, résistantes au Hg, ont ainsi développé des mécanismes enzymatiques de détoxification. La déméthylation réductive met en jeu l'enzyme organomercure lyase, codée par le gène *mer B*, capable de casser la liaison Hg-carbone pour former IHg qui est ensuite réduit en Hg° par l'enzyme mercure réductase, codée par le gène *mer A*. Les produits finaux de cette réaction sont le méthane et Hg° (Barkay et al., 1991). A l'inverse, la déméthylation oxydative génère du dioxyde de carbone et du IHg. (Oremland et al., 1991& 1995).

Ainsi la compréhension des processus de biotransformation du mercure s'affine continuellement, par la combinaison de la biologie moléculaire et de l'analyse de spéciation chimique. Des développements analytiques récents ont ainsi permis d'accéder à une meilleure connaissance des mécanismes antagonistes de méthylation et déméthylation. L'utilisation de traceurs radioactifs (Korthals & Windfrey, 1987) ou de traceurs isotopiques stables (Hintelmann et al., 2000) offre notamment la possibilité de distinguer et de caractériser leurs cinétiques respectives. De nombreuses études, basées sur des incubations d'échantillons naturels ont permis d'appréhender convenablement ces processus à partir de systèmes présentant des degrés d'organisation simples et contrôlables. La difficulté de ce challenge et l'erreur associée résident entre autre dans notre capacité à reproduire de manière correcte les conditions environnementales. L'étude mécanistique simultanée des processus de méthylation, de déméthylation et de volatilisation permet ainsi de déterminer la disponibilité du mercure vis-à-vis d'un écosystème donné et d'évaluer sa toxicité globale (Monperrus et al., 2004).

## A.2. Toxicité et réglementations

### *A.2.1. Toxicité du tributylétain et du mercure*

A.2.1.1. Cas du tributylétain et ses dérivés

Les dérivés organométalliques de l'étain et en particulier les formes trisubstituées utilisées comme biocides, présentent par définition une forte toxicité vis-à-vis des biocénoses terrestres et aquatiques. Si cette nocivité pour les organismes cibles (organismes salissants pour les carènes de bateaux, bactéries, champignons, etc) est intentionnelle, sa propension à avoir des incidences sur le milieu environnant a été largement sous-estimée. Les premières études sur les effets aigus et notamment la mortalité (Laughlin & Linden, 1987), n'avaient pas identifié les conséquences sublétales d'une exposition prolongée pour certains taxons. Le degré de toxicité des composés organostanniques dépend du nombre et de la nature du groupement alkyle lié à l'atome d'étain. Ainsi les composés trisubstitués induisent les effets les plus délétères (Blunden & Chapman, 1986). De plus, la toxicité vis-à-vis des différents groupes taxonomiques est fonction du nombre de carbone du groupement alkyle. Le Tableau 4 illustre la sensibilité spécifique de divers taxons pour les organostanniques trisubstitués. Pour cette famille de composés, le radical anionique associé n'a pas d'effet sur les propriétés toxiques à moins qu'il ne soit lui même biologiquement actif auquel cas la toxicité de la molécule est augmentée (Blunden & Chapman, 1986). Néanmoins la nature du radical anionique et donc la spéciation du contaminant vont conditionner sa disponibilité et sa capacité à traverser les membranes biologiques (Lascourrèges et al., 2000 ;Rüdel, 2003). Par ailleurs, les formes tetrasubstituées semblent présenter une toxicité retardée, du fait de leur conversion en composés trisubstitués, notamment dans le foie (Kimmel et al., 1977).

***Tableau 4 –*** *Susceptibilité spécifique aux dérivés organostanniques trisubstitués ($R_3SnX$) (d'après Blunden & Chapman, 1986)*

| R: Radical organique substitué | Espèces |
|---|---|
| $CH_3$ (méthyle) | Insectes |
| C2H5 (éthyle) | Mammifères |
| C3H7 (propyle) | Bactéries (Gram -) |
| C4H9 (butyle) | Bactéries (Gram +), poissons, mollusques, Algues |
| C6H5 (phényle) | Poissons, champignons, mollusques |
| Cyclo-C6H11 (hexyle) | Poissons, acariens |

Le TBT a été identifié comme le composé organostannique le plus toxique vis-à-vis des organismes aquatiques (Laughlin and Linden 1985; Laughlin et al., 1985). Le Tableau 5 rassemble les seuils de toxicité du TBT, c'est-à-dire les concentrations maximales acceptables dans l'eau pour assurer la reproduction ou la survie des espèces aquatiques les plus sensibles. Le TBT peut en effet induire à des niveaux d'exposition très faibles dans l'eau, de l'ordre de quelques ng $l^{-1}$, des effets chroniques de perturbation de croissance, de développement et de masculinisation chez certains mollusques (huîtres, gastéropodes) (Alzieu et al., 1986 ; Bryan et al., 1986 ; Ruiz et al., 1996 ; Alzieu, 1998). Les algues sont également sensibles au TBT, à des doses de quelques centaines de ng $l^{-1}$ (Blanck et al., 1984). Enfin chez les organismes supérieurs, comme les poissons, le TBT présente une toxicité aiguë à quelques mg $l^{-1}$ et des effets chroniques sur le système immunitaire à des concentrations proches du µg $l^{-1}$ (Hall & pinkey, 1985 ; Bushong et al., 1988).

***Tableau 5 –*** *Seuils de toxicité chronique du TBT et du Mercure pour différents organismes aquatiques (d'après INERIS, 2003 ; Alzieu, 2000 ; WHO IPCS, 1989)*

| Organismes | TBT | IHg | MMHg |
|---|---|---|---|
| Microorganismes | 1 ng $l^{-1}$ | 10aines µg $l^{-1}$ | < 100aines ng $l^{-1}$ |
| Plantes aquatiques | 100aines ng $l^{-1}$ | 1-100 µg $l^{-1}$ | 1 µg $l^{-1}$ |
| Mollusques | < 1 ng $l^{-1}$ | 1-100 µg $l^{-1}$ | 100aines ng $l^{-1}$ |
| Poissons | 1-100 µg $l^{-1}$ | 1 µg $l^{-1}$ | 100aines ng $l^{-1}$ |

La dangerosité du TBT vis-à-vis de la biota se traduit notamment par sa capacité à inhiber le transport transmembranaire d'éléments minéraux essentiels ($Ca^{2+}$, $Na^{+}$, $K^{+}$) (Selwyn, 1976) et à altérer les structures cellulaires des membranes (Gray et al., 1987). Mais l'effet nocif le plus souvent associé à ce contaminant demeure son rôle de perturbateur endocrinien ayant pour effet de bloquer certains systèmes enzymatiques, comme par exemple l'activité du cytochrome P-450 qui participe aux mécanismes de détoxication des substances xéniobiotiques ou exogènes chez de nombreux organismes vertébrés et invertébrés (Fish et al., 1976 ; Matthiessen & Gibbs, 1998). Le phénomène de surimposition de caractères sexuels mâles chez les femelles de nombreux gastéropdodes, connu sous le nom d'imposex, représente l'exemple le mieux documenté de perturbation endocrinienne induite par un composé xénobiotique (Bryan et al., 1986 ; Alzieu et al., 1998 ; Vos et al., 2000). L'inhibition du cytochrome P-450, par formation de complexes TBT-protéine, empêche la transformation de la testostérone en œstrogène et entraîne son accumulation chez la femelle. La masculinisation provoque une altération grave des fonctions reproductives allant jusqu'à la stérilité et pouvant également aboutir au déclin de populations spécifiques (Bryan et al., 1986).

Par ailleurs, il est important de souligner que les produits de dégradation du TBT, à savoir le DBT et MBT, sont susceptibles de présenter une toxicité plus élevée que le composé parent pour certaines espèces et vis-à-vis de systèmes enzymatiques spécifiques (Bouchard et al., 1999 ; Al-Ghais et al., 2000). Ainsi l'immunotoxicté du DBT apparaît particulièrement significative chez les poissons (O'Halloran et al., 1998). Enfin, les effets chroniques de l'exposition au TBT, sur la reproduction chez les mollusques n'ont pas de contrepartie chez les vertébrés aquatiques ou les mammifères terrestres. Le TBT et le DBT constituent néanmoins des agents immunitoxiques avérés pour les mammifères (Seinen et al., 1977). De même, pour ce groupe taxonomique, le TBT ainsi que les méthylétains semble également avoir des propriétés neurotoxiques attestées (Kobayashi et al., 1992).

Indépendamment des niveaux d'exposition, la susceptibilité des organismes aquatiques à la contamination ambiante par le TBT varie considérablement, en fonction de leurs caractéristiques génotypiques, métaboliques et ontogénétiques (USEPA, 2003). La toxicité aiguë et chronique de ce micropolluant a été attestée pour un grand nombre de taxons et d'écosystèmes aquatiques. Cependant, sa dimension chronique apparaît de la plus haut importance quant aux effets à long terme sur l'environnement, car induite à des concentrations de l'ordre de l'ultra-trace. De plus, le TBT introduit dans les écosystèmes aquatiques est sujet à un recyclage, via des mécanismes de dégradation ou de méthylation, pouvant être perçus comme des voies potentielles de détoxification du milieu. Néanmoins les dérivés issus de ces transformations revêtent également une toxicité significative pour le vivant, confirmant ainsi la nature ubiquiste et persistante de la contamination par le TBT et ses implications écotoxicologiques majeures pour les environnements aquatiques.

#### A.2.1.2. Cas du mercure et ses dérivés

La toxicité du mercure pour les organismes vivants en général et pour l'homme en particulier a été tragiquement illustrée par plusieurs épisodes d'empoisonnement à grande échelle de populations exposées au méthylmercure via l'alimentation (Japon 1950-1970, Irak 1971-1972) (Bakir et al., 1973 ; Tsubaki et al., 1977). La dynamique biogéochimique du mercure renforce également l'impact écotoxicologique néfaste de cet élément sur les écosystèmes naturels. Les principales voies d'exposition pour l'homme sont l'inhalation de mercure élémentaire et l'ingestion de nourriture contaminée par le méthylmercure et notamment les poissons. La bioamplification du méthylmercure et dans une moindre mesure du mercure inorganique au sein des réseaux trophiques aquatiques constitue le vecteur principal du risque d'intoxication jusqu'à l'homme (Figure 7). Le déversement d'une centaine de tonnes de mercure dans la baie de Minamata (Japon) a ainsi entraîné une pollution mercurielle générale et durable ce cet écosystème aquatique. L'ingestion de poissons hautement contaminés en MMHg par les populations locales a ainsi provoqué de nombreux décès et plusieurs milliers de cas d'empoisonnement entraînant des dommages physiologiques irréversibles pour les générations suivantes (USEPA, 1997b). Bien

que la contamination des organismes aquatiques et en particulier des poissons par le MMHg ne peut occasionner une toxicité létale pour l'homme que dans des cas extrêmes, le large spectre des pathologies humaines graves associées à une exposition au mercure sous forme inorganique ou organique illustrent la dangerosité de ce polluant (EPA, 1997b). L'exposition de l'homme au mercure peut entraîner des atteintes neurologiques, gastro-intestinales, rénales, dermatologiques, cardiovasculaire et immunitaires (Picot et al., 1998). L'organisation mondiale de la santé (WHO IPCS, 1990 & 1991) signale qu'à partir d'une concentration dans l'urine de 40 µg Hg $l^{-1}$, le risque de développer les symptômes précédemment cités devient important, alors que la valeur normale de référence a été estimé à 4µg Hg $l^{-1}$ (Tableau 6).

***Tableau 6 –*** *Valeurs de référence des concentrations totales en Hg dans différents tissus humains (WHO IPCS, 1990 &1991)*

| Milieux biologiques | Valeurs de référence |
|---|---|
| Sang | 5-10 µg Hg $l^{-1}$ |
| Urine | 4 µg Hg $l^{-1}$ |
| Cheveux | 1-2 mg µg Hg $kg^{-1}$ |
| Placenta | 10mg Hg $kg^{-1}$ |

La toxicité des espèces mercurielles est déterminée principalement par leur spéciation et leur stabilité chimique, leur propension à traverser les barrières biologiques et par le métabolisme des organismes récepteurs (équilibre absorption/excrétion). Ainsi les composés alkylés du mercure et principalement le MMHg sont extrêmement toxiques, lents à métaboliser et aisément bioaccumulables. Les seuils de toxicité de IHg et MMHg pour différents organismes aquatiques, présentés dans le Tableau 5, illustrent la dangerosité première du MMHg à des concentrations très faibles. Les concentrations létales du MMHg pour les organismes aquatiques sont généralement comprises entre 10 et 100 µg $l^{-1}$ (WHO IPCS, 1989).

Le MMHg, composé liposoluble, est stable dans la plupart des organismes et est capable de traverser facilement la barrière sang-système nerveux central (SNC), la barrière placentaire ainsi que les membranes cellulaires. Le temps de demi-vie du MMHg dans l'organisme est long et estimé à quelques 70 jours dans le sang contre 3 à 4 jours pour les formes inorganiques. Dans le SNC, il peut atteindre 270 jours ou plus (Picot et al., 1998). Ce caractère persistant et en conséquence toxique réside dans la très forte aptitude des composés mercuriels organiques et inorganiques pour former des complexes stables avec des ligands intracellulaires tels que les groupements thiol des protéines et des enzymes (Yoshino et al., 1966 ; Chang et al., 1972).

De la même manière, la compartimentation cellulaire du mercure chez les organismes inférieurs (bactéries, phytoplancton) est fonction de sa spéciation chimique et va conditionner son devenir dans les réseaux trophiques. Ces mécanismes semblent être à la base de sa bioamplification dans les systèmes trophiques aquatiques (Mason et al., 1996a&b ; Reinfelder & Fisher, 1991 ; Sunda 1998 ; Lawson & Mason, 1998). Alors que les concentrations en espèces mercurielles dans les eaux naturelles ne sont pas toxiques pour le phytoplancton, premier maillon de le chaîne alimentaire aquatique, le problème devient significatif pour les organismes supérieurs (Abreu et al., 2000 ; Hines et al., 2000). Le phytoplancton est ainsi capable de concentrer des métaux à partir de la phase dissoute (fraction ‹ 0,2 µm) jusqu'à plus de $10^5$ fois la concentration du milieu (Fisher & Reinfelder, 1995 ; Boudou & Ribeyre, 1997). Etant le premier maillon de la chaîne alimentaire, il sera en grande partie responsable de l'introduction du mercure dans les réseaux trophiques aquatiques (Figure 7).

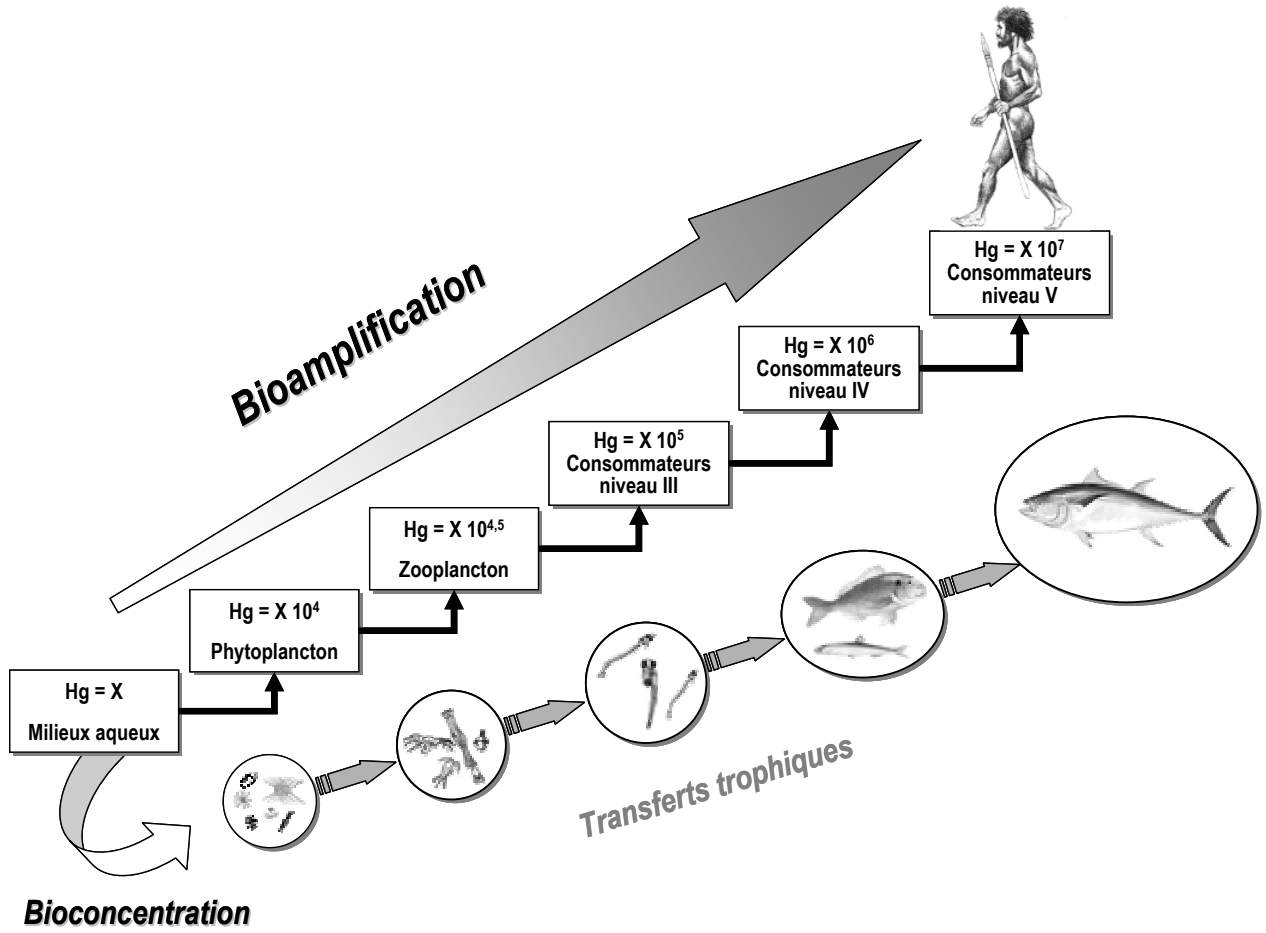


***Figure 7 –*** *Bioconcentration et bioamplification du mercure dans les réseaux trophiques aquatiques*

L'accumulation du mercure en solution par les organismes unicellulaires et le phytoplancton s'effectue tout d'abord par la diffusion passive des formes neutres et par le transport actif des formes ionisées à travers les menbranes cellulaires (Sunda, 1998 ; Miles et al., 2001 ; Anson moye et al., 2002). Dans une seconde étape, les espèces incorporées, vont interagir avec les structures cellulaires et se distribuer en fonction de leur spéciation chimique. Ainsi Mason et al. (1996a&b) ont mis en évidence chez la diatomée *Thalassiosira weisflogii*, que IHg semble se complexer majoritairement avec les groupements fonctionnels des membranes cytoplasmiques alors que MMHg demeure en solution dans le cytosol ou associé aux organites. Par ailleurs, Reinfelder et Fischer (1995) ont montré que les membranes phytoplanctoniques et leur matériel associé sont en grande partie excrétés par le zooplancton, tandis que la fraction cytoplasmique est majoritairement assimilée. Ainsi le MMHg sera majoritairement et efficacement transmis au prédateur.

Cette sélectivité du transfert trophique entre les espèces mercurielles, basée sur leur spéciation chimique et leur réactivité amplifie de manière dramatique la contamination des biocénoses aquatiques et le degré de toxicité induit pour les organismes prédateurs supérieurs. La toxicité spécifique du MMHg et les phénomènes de bioamplification soulignent encore une fois la nécessité de caractériser la spéciation chimique du contaminant dans le compartiment source et dans les entités biologiques. L'accès à ces informations permet la compréhension des mécanismes de transferts et transformation et autorise une estimation plus précise du risque. Il convient enfin de souligner que la plupart des études environnementales dédiées à la problématique mercure, notamment en milieux dulcicoles, sont axées sur des situations de contamination aiguë. De grandes incertitudes demeurent quant à la réactivité du mercure et ses incidences écotoxicologiques en relation avec la contamination chronique des écosystèmes aquatiques.

### *A.2.2. Réglementations dans les milieux aquatiques*

A.2.2.1. Réglementations relatives au tributylétain

Bien que l'utilisation des composés organostanniques et notamment du TBT comme agent antisalissure soit un phénomène assez récent, remontant à la fin des années soixante, les craintes sur l'impact écotoxicologique de ces substances ont rapidement abouti à l'adoption de diverses restrictions nationales et internationales. La dangerosité du TBT, vis-à-vis des écosystèmes aquatiques, a ainsi été reconnue dès le début des années quatre vingt en France (Alzieu et al., 1982). L'effet nocif le plus souvent associé à cette contamination, à savoir le développement de l'imposex chez les mollusques gastéropodes marins, représente un phénomène extrêmement sensible et spécifiquement chimique ayant nettement contribué à l'acceptation d'un lien direct de cause à effet et donc de la nécessité de contrôles. Le Tableau 7 retrace l'historique des signaux précurseurs et des actions engagées par diverses instances nationales et internationales pour prévenir des incidences écologiques de la contamination des écosystèmes aquatiques par le TBT.

***Tableau 7 –*** *Signes précoces de la contamination des écosystèmes aquatiques par le TBT et actions engagées*

| | |
|---|---|
| 1970-1971 | Accroissement rapide de l'emploi des peintures antisalissures à base de TBT sur les navires et premiers rapports faisant état d'imposex chez les escargots marins (Blaber, 1970 ; Smith, 1971). |
| 1976 - 1981 | L'échec répété de la fixation des larves provoque l'effondrement de l'ostréiculture dans le bassin d'Arcachon. |
| 1982 | La France introduit une législation interdisant l'emploi de peintures à base de TBT sur les petites embarcations |
| 1986 | Bryan et al. (1986) signalent de nombreux cas d'imposex chez les pourpres de la côte méridionale du Royaume-Uni et incriminent le TBT. |
| Mai 1987 | Le Royaume-Uni interdit la vente au détail de peinture au TBT pour les navires < 25 m et les cages de mariculture. |
| 1988-1989 | Restrictions introduites aux Etats-Unis, Canada, Australie et Nouvelle-Zélande. |
| 1991 | Interdiction harmonisée de la vente au détail de peinture au TBT à l'échelle de l'Union européenne. |
| 1994 | Premiers rapports établissant un lien entre l'imposex chez les bulots de la mer du Nord et le trafic maritime. |
| 1995 | La déclaration ministérielle de la quatrième conférence sur la mer du Nord (Esbjerg) comporte un engagement à œuvrer pour l'abandon progressif de la peinture au TBT à l'échelle mondiale, dans le cadre de l'Organisation Maritime Internationale (OMI). |
| 1997 | Approbation du principe de l'élimination planétaire des peintures contenant des organostanniques lors de la 40ème session du CPMM/OMI. |
| 1998 | L'OSPAR (convention pour la protection du milieu marin de l'Atlantique du Nord-Est) accorde la priorité aux organostanniques pour les actions visant à cesser tous les rejets. Fin de tous les rejets organostanniques dans le milieu marin en 2020, dans le cadre de la stratégie OSPAR pour les substances dangereuses. |
| Ocobre 2001 | Adoption par l'OMI de la Convention internationale sur le contrôle des systèmes antisalissures (Convention AFS) : Interdiction d'application de composés organostanniques sur tous les navires, à partir du 1er janvier 2003. Elimination des composés organostanniques de tous les navires, à partir du 1er janvier 2008 (à moins qu'un revêtement formant barrière empêche ces composés de s'échapper du système antisalissures non conforme sous-jacent). |
| Juillet 2002 | Directive 2002/62/CE de la Commission européenne portant 9ème adaptation au progrès technique de l'annexe I de la directive 76/769/CEE du Conseil concernant le rapprochement des dispositions législatives, réglementaires et administratives des États membres relatives à la limitation de la mise sur le marché et de l'emploi de certaines substances et préparations dangereuses (composés organostanniques). |
| Avril 2003 | Règlement n° 782/2003 du Parlement européen et du Conseil interdisant les composés organostanniques sur les navires à partir du 1er janvier 2003. |
| Septembre 2003 | France : Décret n° 2003-879 du 8 septembre 2003 relatif aux paraffines chlorées à chaîne courte et aux composés organostanniques et modifiant le décret n°92-1074 du 2 octobre 1992 relatif à la mise sur le marché, à l'utilisation et à l'élimination de certaines substances et préparations dangereuses. |

Les législations française et européenne ont récemment confirmé les efforts de réglementation adoptés par l'OMI en promulguant différents textes de loi visant à interdire la commercialisation et l'utilisation de peintures antisalissure à base de composés organostanniques. La directive européenne 2002/62/CE en interdit l'usage aux navires de toute longueur, aux appareillages de pisciculture et conchyliculture, ainsi qu'aux appareillages totalement ou partiellement immergés. Néanmoins il convient de souligner que ces mesures ne concernent pas les navires de guerre ou d'état. De plus la convention internationale AFS (Tableau 7) n'entrera en vigueur que 12 mois après que 25 États représentant 25 % du tonnage mondial de la marine marchande l'auront signée. Sept États étaient signataires au 31 janvier 2004 (près de 9 % du tonnage mondial).

Par ailleurs, il n'existe pas en France, à l'heure actuelle, de standard de qualité des eaux concernant ces substances. L'arrêté du 2 février 1998, relatif au prélèvement et à la consommation d'eau ainsi qu'aux émissions de toute nature des installations classées pour la protection de l'environnement soumise à autorisation, fixe pour l'oxyde de TBT (TBTO) une valeur limite de concentration dans les rejets (rejet en sortie d'atelier ou rejet final) de 0,05 mg $l^{-1}$ si le rejet dépasse 0,5 g $d^{-1}$. Cependant des études sont actuellement en cours pour établir des seuils de qualité des eaux superficielles pour le TBT (Babut et al., 2001). L'IFREMER propose ainsi un seuil sans effet inférieur à 1 ng $l^{-1}$ tous effets confondus (Alzieu et al., 1998). Le CEMAGREF s'oriente vers la valeur de 0,4 ng $l^{-1}$ (Tableau 8). Toutefois, les méthodes analytiques performantes susceptibles de répondre aux critères de qualité, imposés par le système d'évaluation de qualité des eaux superficielles (SEQ-Eau), existent mais demeurent difficiles à mettre en œuvre en routine. Certains seuils proposés restent donc invérifiables avec les méthodologies actuellement disponibles.

***Tableau 8 –*** *Définitions et valeurs provisoires des seuils de qualité de l'eau pour le TBT*

| Seuil | Définition | [TBT] (µg $l^{-1}$) |
|---|---|---|
| **1** | plus basse concentration chronique sans effet (NOEC) + facteur de sécurité de 10 ou plus basse valeur fiable aiguë (CE/LC50) + facteur de sécurité de 1000 | 0.0004 |
| **2** | plus basse concentration chronique sans effet (NOEC) sans facteur de sécurité ou plus basse valeur fiable aiguë (CE/LC50) + facteur de sécurité de 100 | 0.004 |
| **3** | plus basse valeur fiable aiguë (CE/LC50) sans facteur de sécurité | 0.05 |
| **4** | Moyenne géométrique des plus basses valeurs fiables aiguë (CE/LC50) pour trois niveaux trophiques (algues ou plantes, invertébrés et poissons) | 2.4 |

Enfin les mesures légales de restriction et d'interdiction dûment appliquées ainsi que l'établissement de seuils de qualité des eaux pourront représenter des progrès significatifs en terme de protection de l'environnement et de santé publique. Néanmoins l'impact écotoxicologique du TBT sur les écosystèmes aquatiques demeure entier du fait de son accumulation passée et de sa persistance dans les sédiments et les organismes. D'autre part, les autres sources d'utilisation des composés organostanniques, et du TBT en particulier, restent ignorées de ces réglementations bien qu'elles touchent en priorité les systèmes dulcicoles et que leurs incidences sur l'homme apparaissent encore plus directes.

### A.2.2.2. Réglementations relatives au mercure et ses dérivés

La toxicité élevée des différentes formes chimiques du Hg pour l'homme ainsi que ses multiples voies d'exposition ont amené les organismes nationaux et internationaux de santé publique à définir un cadre législatif contraignant visant à limiter son utilisation et ses rejets dans l'environnement. L'Annexe 1 rassemble, pour exemple, les nombreuses directives publiées à cet effet au niveau européen et concernant les eaux superficielles, l'atmosphère et les effluents et déchets toxiques. Les réglementations nationales et internationales convergent généralement vers des critères de qualité et des valeurs toxicologiques de références (VTR) similaires. Ainsi, La norme relative à la qualité des eaux destinées à la consommation humaines a été fixée à 1 µg Hg total $l^{-1}$ par la France en 1991 (Décret n° 91-257 07/04/91), harmonisée au niveau mondial par l'organisation mondiale de la santé (WHO ICPS, 1996) en 1996 et au niveau européen en 1998 (CE, 1998).

La dynamique biogéochimique du Hg dans les écosystèmes aquatiques et sa propension à la bioaccumulation au travers des réseaux trophiques ont également orienté les pouvoirs publics à réglementer les apports journaliers ou hebdomadaires en Hg (total ou méthylé) par l'alimentation et la consommation notamment de poisson. Les VTR, établissant la relation entre une dose externe d'exposition à différents composés mercuriels et la survenue d'un effet néfaste, définies par plusieurs organismes sont présentées dans le Tableau 9.

A partir de ces valeurs de référence, des propositions de concentrations limite chez le poisson destiné à la consommation humaine ont été établies par différents pays. Les teneurs ainsi estimées varient de 0,3 à 1,0 mg Hg total par kilogramme de chair humide. La directive européenne 93/351/CEE du 19 mai 1993 fixe la teneur maximale tolérable conditionnant la mise en marché des produits de la mer à 0,5 mg $kg^{-1}$ (p.h.: poids humide). La France a intégré cette directive à sa législation (JO du 21 juillet 1995) et a étendu ce critère aux coquillages, par arrêté du 21 mai 1999. Enfin il convient de souligner l'initiative éclairée du ministère de la Santé du Minnesota (Etats Unis) qui a proposé une valeur limite de 0,16 mg $kg^{-1}$ (p.h.) non plus exprimée en Hg total mais en Hg méthylé.

***Tableau 9 –*** *Valeurs toxicologiques de références pour différentes formes chimiques du Hg*

| Substance Chimique | Source | Voie d'exposition | Facteur d'incertitude | VTR | Année de révision |
|---|---|---|---|---|---|
| Chlorure mercurique | USEPA | orale | 1000 | RfD[a] = 3 $10^{-4}$ mg $kg^{-1}$ $j^{-1}$ | 1995 |
| | ATSDR | orale | 100 | MRL[b] = 7 $10^{-3}$ mg $kg^{-1}$ $j^{-1}$ (aiguë) | 2001 |
| | ATSDR | orale | 100 | MRL = 2 $10^{-3}$ mg $kg^{-1}$ $j^{-1}$ (sub chronique) | 2001 |
| Méthylmercure | USEPA | orale | 10 | RfD = $10^{-4}$ mg $kg^{-1}$ $j^{-1}$ | 2001 |
| | ATSDR | orale | 4,5 | MRL = 3 $10^{-4}$ mg $kg^{-1}$ $j^{-1}$ | 2001 |
| | WHO | orale | - | DHPT[c] = 1,6 $10^{-3}$ mg $kg^{-1}$ $j^{-1}$ | 2003 |
| Mercure total | WHO | orale | - | DJT[d] = 6 $10^{-4}$ mg $kg^{-1}$ $j^{-1}$ | 1996 |

[a]Reference Dose, [b]Minimun Risk Level, [c]Dose Hebdomadaire Provisoire Tolérable, [d] Dose Journalière Tolérable

De même, Euro Chlor (1999) et l'INERIS (2003) proposent des valeurs non officielles de concentrations sans effet prévisibles sur l'environnement (PNEC), obtenues par la méthode d'extrapolation statistique proposée par Aldenberg et Slob (1993) pour le compartiment aquatique et, pour les autres compartiments avec les facteurs d'incertitudes proposés dans le document guide publié par la Commission Européenne (TGD, 1997). Le Tableau 10 récapitule les valeurs PNEC établis par ces deux organismes. Ainsi, les efforts consentis pour intégrer le concept de spéciation chimique dans l'établissement de critères de qualités des milieux environnementaux, sont susceptibles d'aboutir à une évaluation plus précise des risques associés notamment à la contamination des écosystèmes aquatiques par le Hg.

***Tableau 10 –*** *Valeurs PNEC pour le mercure inorganique et organique*

| | INERIS | | Euro Chlor | |
|---|---|---|---|---|
| | Hg inorganique | Hg organique | Hg inorganique | Hg organique |
| PNEC eau (ng $l^{-1}$) | 240 | 10 | 470 | - |
| PNEC poissons (ng $g^{-1}$) | - | 25 | - | - |
| PNEC sédiments (µg $g^{-1}$) | 9,3 | 1,1 | 93 | - |
| PNEC sol (ng $g^{-1}$) | 27 | 23 | 300 | 23 |

## A.3. Méthodes analytiques sensibles pour l'étude de la réactivité du TBT et du Hg

L'étude de la contamination des environnements aquatiques par des éléments traces tels que l'étain et le mercure et de leur impact écotoxicologique ne peut être réalisée qu'au moyen de techniques analytiques fiables et performantes. L'analyse de spéciation, plutôt que la détermination de la teneur totale apparaît aujourd'hui comme un préalable indispensable pour une évaluation précise du comportement physicochimique, de la biodisponiblité, de la toxicité et donc du risque environnemental associé à la présence des contaminants métalliques dans les écosystèmes aquatiques. De plus, cette approche visant à déterminer la spécificité chimique d'éléments traces ou ultra traces dans l'environnement, au niveau moléculaire ou isotopique, repose sur l'établissement de protocoles rigoureux comprenant en amont le conditionnement du matériel (décontamination du matériel utilisé pour l'échantillonnage, le stockage et l'analyse et purification des réactifs d'analyse) et en aval la chaîne analytique proprement dite (préparation des échantillons, préconcentration, extraction, dérivatisation, séparation et quantification). Les différents réservoirs et sources des contaminants dans le milieu aquatique peuvent alors être identifiés et évalués convenablement. Dans une seconde étape, le développement d'outils analytiques toujours plus sensibles, permettant de travailler à des niveaux de concentrations réalistes et comparables aux teneurs ambiantes, ainsi que le développement d'outils expérimentaux, simulant les conditions du milieux, autorisent l'accès à une connaissance augmentée des processus clés de transfert et de transformation définissant la dynamique biogéochimique d'un contaminant.

### *A.3.1. Méthodes de couplage pour la spéciation et l'identification des métabolites de l'étain et du mercure à l'état de traces*

Les analyses de spéciation des composés organostanniques et mercuriels dans les matrices environnementales ont considérablement évoluées depuis les dernières décennies et ont permis d'abaisser les limites de détection en de ça du ppb (µg $l^{-1}$ ou ng $g^{-1}$) et pour les plus performantes du ppt (ng $l^{-1}$ ou pg $g^{-1}$). Ces techniques offrent ainsi la possibilité d'évaluer de manière juste et précise les problématiques de contamination des écosystèmes aquatiques naturels par le TBT et le Hg. Elles nécessitent tout d'abord une première étape délicate d'extraction et de préconcentration des analytes de la matrice environnementale tout en préservant leur structure chimique initiale. Elles font ensuite intervenir un couplage entre une technique de séparation et une détection spécifique de l'élément recherché. Le Tableau 11 rassemble quelques exemples de chaînes analytiques classiquement utilisées pour les analyses environnementales des butylétains et des différentes formes chimiques du Hg. Le couplage chromatographie en phase gazeuse (GC) et spectrométrie de masse couplée à un plasma induit (ICPMS) apparaît être la technique la plus performante bien que coûteuse.

***Tableau 11 –*** *Techniques analytiques pour la spéciation du mercure et des organoétains dans différentes matrices environnementales*

| Composés | Technique | Extraction | Dérivatisation | ADL ou MDL | Référence |
|---|---|---|---|---|---|
| | | **Tissus biologiques** | | | |
| IHg, MMHg | GC-MIP-AED | NaCl+HCl (agitation) | Grignard (BuMgCl) | 0,4 pg | Emteborg *et al.* 1994 |
| MMHg | GC-CV-AFS | KOH+méthanol (chauffage) | Ethylation ($NaBEt_4$) | 0,08 ng $g^{-1}$ | Liang *et al.* 1996 |
| IHg, MMHg | CT-GC-QFAAS | TMAH (micro-ondes) | ” | 3 ng $g^{-1}$ | Tseng *et al.*1998 |
| IHg, MMHg | GC-MIP-AED | ” | ” | 3 pg $g^{-1}$ | Gebersmann *et al.* 1997 |
| IHg, MMHg | ” | ” | ” | 0,1 ng $g^{-1}$ | Pereiro *et al.* 1998 |
| IHg, MMHg | GC-ICPMS | ” | ” | 0,15 pg | Wasik *et al.* 1998 |
| MBT, DBT, TBT, TPhT | GC-MIP-AED | ” | ” | 2-5 ng $g^{-1}$ | Schmitt *et al.* 1997 |
| Butyl-, phénylétains | GC-ICPMS | ” | ” | 0,05 pg | Rodriguez *et al.* 1999 |
| MMHg, TBT | ID-GC-ICPMS | ” | ” | 0,11 ng $g^{-1}$ Hg / 0,15 ng $g^{-1}$ Sn | Monperrus *et al.* 2003b |
| | | **Sédiments** | | | |
| IHg, MMHg | CT-GC-QFAAS | HCl 6N (micro-ondes) | Ethylation ($NaBEt_4$) | 0,5 ng $g^{-1}$ | Tseng *et al.* 1998 |
| IHg, MMHg | CT-GC-AFS | ” | ” | 0,2 ng $g^{-1}$ / 20 pg $g^{-1}$ | Stoichev *et al.* 2002 |
| MMT, MBT, DBT, TBT | CT-GC-AAS | Acide acétique (agitation) | ” | 1 ng $g^{-1}$ | Martin *et al.* 1994 |
| Méthyl-, butyl-, phényl-, octylétains | GC-FPD | Acide acétique (micro-ondes) | ” | 10 ng $g^{-1}$ | Lalère *et al.* 1995 |
| MBT, DBT, TBT | GC-AED | ” | ” | 2 ng $g^{-1}$ | Spuznar *et al.* 1996a |
| MMHg, IHg, MBT, DBT, TBT | GC-ICPMS | Acide acétique (Ultrasons) | Propylation ($NaBPr_4$) | 210 fg Hg / 52-170 fg Sn | De Smaele *et al.* 1998 |
| MBT, DBT, TBT | ID-GC-ICPMS | Acide acétique (micro-ondes) | Ethylation ($NaBEt_4$) | 0,09 ng $g^{-1}$ | Monperrus *et al.* 2003c |
| | | **Eaux** | | | |
| IHg, MMHg | PT-GC-MIP-AES | Purge | Ethylation ($NaBEt_4$) | 2 ng $l^{-1}$ / 0,6 ng $l^{-1}$ | Ceulemans & Adams 1996 |
| IHg, MMHg | CT-GC-ICPMS | ” | Hydruration ($NaBH_4$) | 0,1 ng $l^{-1}$ / 40 pg $l^{-1}$ | Tseng et *al.* 2000 |
| IHg, MMHg | CT-GC-AFS | ” | ” | 0,1 ng $l^{-1}$ / 10 pg $l^{-1}$ | Annexe 1 |
| MBT, DBT, TBT | GC-AED | Eau/Hexane | Propylation ($NaBPr_4$) | 7-12 ng $l^{-1}$ | Schubert *et al.* 2000 |
| Butyl-, phénylétains | GC-ICPMS | SPME | Ethylation ($NaBEt_4$) | 0,6 – 20 pg $l^{-1}$ | Aguerre *et al.* 2001 |
| MMHg, MBT, DBT, TBT | GC-ICPMS | ” | ” | 0,6 ng $l^{-1}$ Hg / 0,3-2 ng $l^{-1}$ Sn | Moens et *al.* 1997 |
| MMHg, IHg, MBT, DBT, TBT | GC-MIP-AES | ” | ” | 10 ng $l^{-1}$ Hg / 5 ng $l^{-1}$ BuSn | Carpinteiro et *al.* 2002 |
| MMHg, IHg, MBT, DBT, TBT | ID-GC-ICPMS | Eau/Isooctane | Propylation ($NaBPr_4$) | 10-60 pg $l^{-1}$ | Annexe 3 |
| Hg°, $Bu_nSnMe_{4-n}$ (n= 0-3) | CT-GC-ICPMS | Purge | - | 20 pg $l^{-1}$ Hg / 50 fg $l^{-1}$ Sn | Amouroux et *al.* 1998 |

Le couplage GC-ICPMS offre en effet une détection multiélémentaire et multiisotopique simultanée très sélective et sensible. La détection des espèces mercurielles par la spectrométrie de fluorescence atomique (AFS) représente également une méthode très largement éprouvée, alliant une détection spécifique très sensible à une robustesse avérée et des coûts abordables. Néanmoins, ce détecteur est sujet à certaines interférences spectrales pouvant limiter son application lors d'analyses de spéciation de certaines matrices environnementales (Annexe 2). Les différents couplages analytiques, dédiés à la spéciation de l'étain et du mercure, utilisés lors de ce travail, ont été développées en tout ou partie au sein du laboratoire (Pécheyran et al., 1998 ; Amouroux et al., 1998 ; Stoichev et al., 2002 ; Monperrus et al., 2003b&c; Annexe 2&3).

Les protocoles d'extraction des butylétains, et des composés mercuriels dans les échantillons solides (sédiments, particules et organismes vivants) et liquides sont résumés dans les Figures 8 et 9. Pour les analyses des formes non volatiles, ces méthodes mettent en œuvre la digestion sous champ micro ondes, pour extraire rapidement et efficacement les analytes de la matrice, suivie d'une étape de dérivatisation (hydruration ou éthylation) permettant de préconcentrer les analytes dans un petit volume de solvant et de réaliser leur séparation par chromatographie (Tseng et al., 1998 ; Szpunar et al., 1996b).

La spéciation des formes gazeuses dissoutes ($Bu_nSnMe_{4-n}$, n=0-3 ; Hg° ; DMHg) nécessite également une étape de prétraitement des échantillons. Il s'agit tout d'abord de purger les échantillons d'eau à l'aide d'un gaz inerte (Hélium), afin d'en extraire les formes volatiles dissoutes. Les analytes sont ensuite directement préconcentrés et piégés simultanément par cryogénie sur un support inerte (laine de verre silanisée) ou à température ambiante sur du sable enrobé d'or pour le Hg gazeux dissous spécifiquement. Les pièges cryogéniques et d'or sont enfin hermétiquement fermés et conservés à basse température (-196°C et +4°C, respectivement), jusqu'à l'analyse (Amouroux et al., 1998 ; Amouroux et al., 1999).

Les analyses de spéciation du mercure et des organoétains ont été réalisées au moyen de couplages associant la chromatographie en phase gazeuse, sur colonne remplie ou capillaire, à une détection spécifique par AFS ou ICPMS. Ces techniques permettent l'identification simultanée de plusieurs formes chimiques du mercure à savoir, le IHg et le MMHg ainsi que le Hg° et le DMHg et de l'étain telles que MBT, DBT, TBT (Figure 8 et 9).

Les limites de détection offertes par ces procédures analytiques sont présentées dans le Tableau 11 et les Annexes 2 et 3.

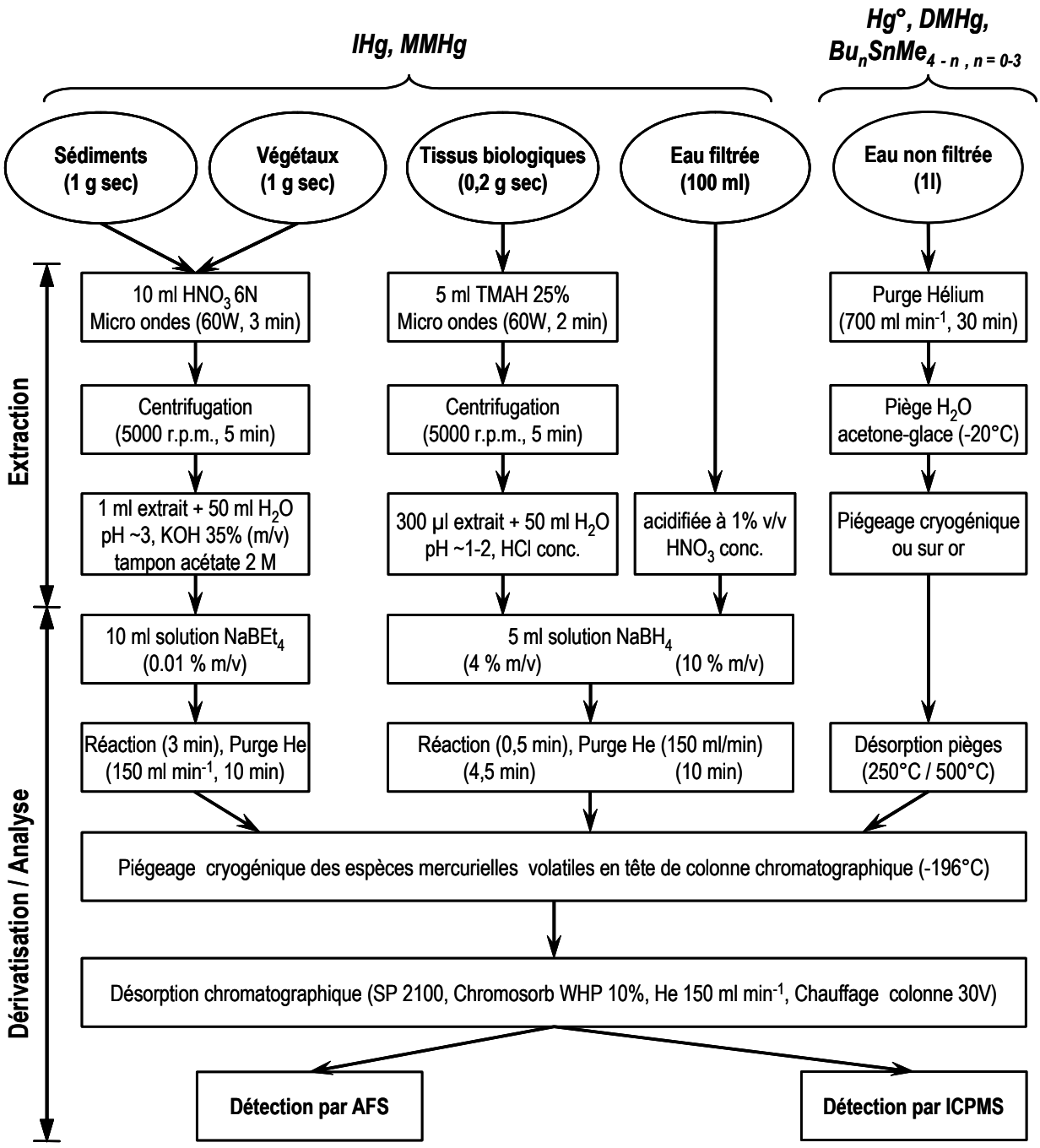


***Figure 8 -*** *Procédures analytiques de spéciation du mercure et de l'étain dans les matrices environnementales*

Par les couplages CT-GC-AFS et PT-CT-GC-ICPMS

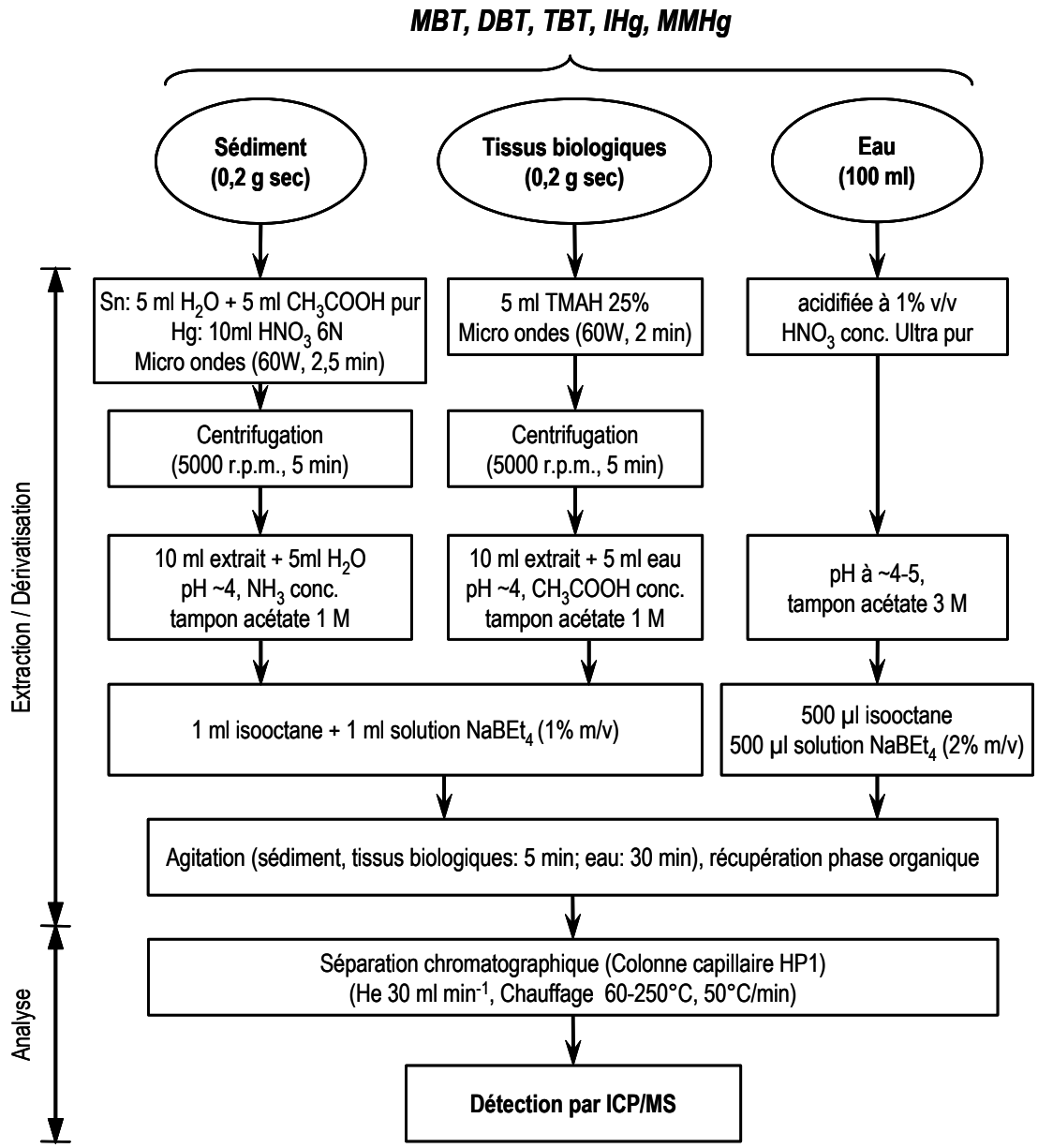


***Figure 9 -*** *Procédures analytiques de spéciation du mercure et de l'étain dans les matrices environnementales*

*Par le couplage GC-ICPMS*

Les différentes étapes d'extraction, de dérivatisation et de détection ont été rigoureusement contrôlées afin de s'affranchir de toute contamination de la chaîne analytique. La justesse et la précision de l'intégralité des protocoles de spéciation utilisés ont enfin été évaluées validées par des expériences de recouvrement

(Amouroux et al., 1998 ; Annexe 3) ou par l'analyse de matériaux de référence certifiés possédant des caractéristiques similaires aux échantillons (Annexe 2 ; Stoichev et al., 2002 ; Monperrus et al., 2003b&c).

Néanmoins la complexité de ces étapes de préparation demeure problématique et peut être source de nombreuses erreurs. L'analyse de spéciation des composés organométalliques dans les échantillons environnementaux représente en effet un réel défi analytique en raison, d'une part des très faibles concentrations ambiantes, et d'autre part de la complexité de matrices étudiées. Ainsi, cette analyse doit permettre la quantification précise de chaque espèce chimique sans contamination de l'échantillon ni dégradation ou transformation les entités organométalliques. Toutes les étapes des protocoles analytiques précédemment décrits représentent donc des phases particulièrement délicates. Les performances des couplages GC-ICPMS, offrant une analyse multiélémentaire et multiisotopique, ont ouverts la voie à des techniques innovantes, telles que l'analyse par dilution isotopique. Ces méthodes nouvelles de quantification par dilution isotopique, bien que limitées dans leurs applications par le faible nombre de standards isotopiquement enrichis disponibles commercialement, représentent une alternative efficace pour la validation et le contrôle des protocoles analytiques.

### *A.3.2. Méthodes de spéciation par dilution isotopique*

La dilution isotopique par spectrométrie de masse (IDMS) peut être appliquée aux analyses de spéciation dans le but de contrôler les pertes et les transformations des analytes pendant les différentes étapes du protocole analytique et donc de gagner en précision et en justesse sur la mesure. Les atouts de cette technique résident dans le fait qu'un recouvrement quantitatif n'est pas nécessaire à une bonne détermination de la concentration des analytes dans l'échantillon (Kingston et al. 1998, Ruiz et al. 2003) et que les réactions de dégradation ou de réarrangement (production d'artefact) des analytes sont facilement détectables (Hintelmann et al., 1997, Wilken & Falter 1998, Hammershmidt & Fitzgerald 2001, Rodriguez et al.2003).

Concernant l'étude de la spéciation du mercure et des butylétains dans l'environnement, l'utilisation des isotopes stables permet deux types de développement. Tout d'abord, une quantification plus précise des concentrations de chaque espèce, dans les différentes matrices environnementales, est rendue possible par un meilleur contrôle des protocoles analytiques. D'autre part les espèces enrichies isotopiquement offrent un potentiel important en tant que traceurs des processus environnementaux (transferts et transformations) de ces contaminants dans les écosystèmes.

A.3.2.1. Principe de la dilution isotopique

La dilution isotopique se base sur le dopage de l'échantillon par une quantité précise d'une forme isotopiquement marquée de l'analyte, de préférence avant tout traitement chimique. Cette molécule, utilisée comme traceur, est enrichie artificiellement avec un des isotopes de l'élément étudié de façon à obtenir une empreinte isotopique différente de l'espèce naturelle. L'analyse par spectrométrie de masse permet la détermination précise des abondances isotopiques du traceur, de l'espèce naturelle à doser et du rapport isotopique résultant pour l'échantillon dopé. La concentration des espèces peut être ainsi obtenue à partir de la mesure précise des abondances isotopiques de l'élément analysé dans l'ajout et dans l'échantillon, de la quantité de l'espèce isotopiquement marquée ajoutée à l'échantillon et de la modification du rapport isotopique. Les éléments étudiés doivent en outre posséder plus de un isotope stable : c'est le cas du mercure et de l'étain qui présentent respectivement 7 et 10 isotopes. La concentration de l'élément recherché peut alors être directement calculée à partir de la mesure des rapports isotopiques et de la quantité de traceur ajoutée, selon la formule présentée dans la Figure 10.

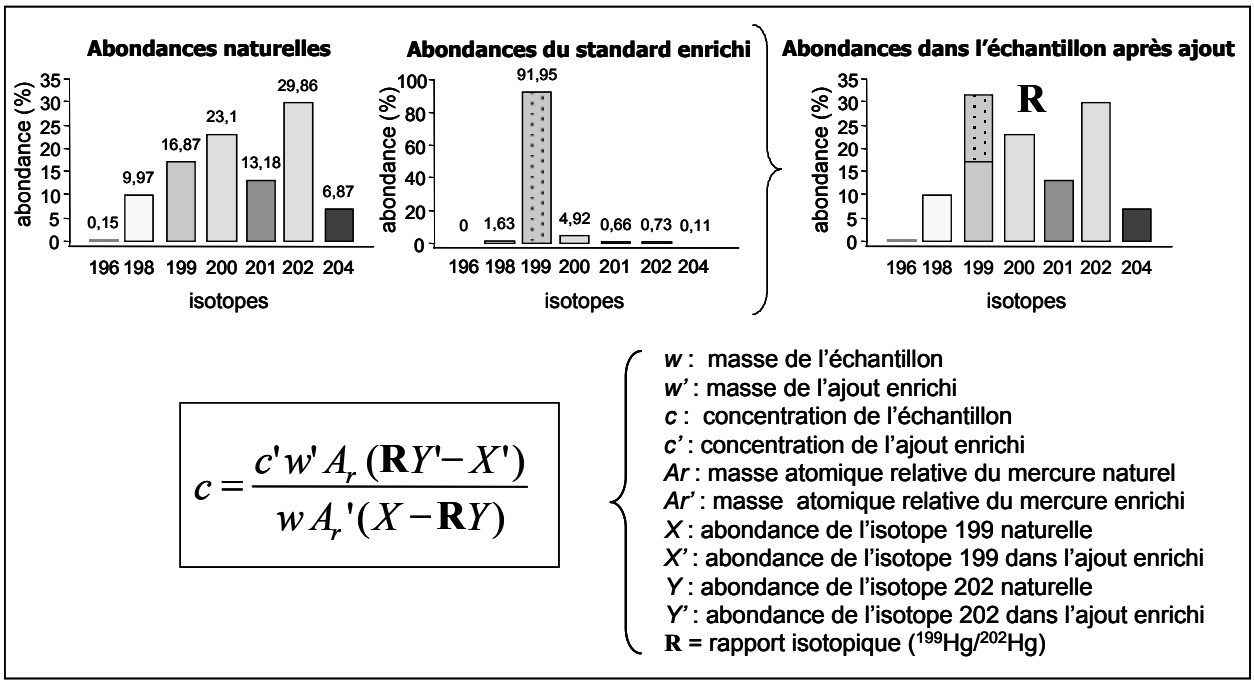


***Figure 10 –*** *Principe de la dilution isotopique - cas du mercure*

Le standard enrichi isotopiquement constitue de fait un étalon interne idéal. Les isotopes le caractérisant sont les mêmes que ceux de l'élément recherché et donc se comportent de manière identique dans la plupart des processus physicochimiques. Toute perte d'échantillon après marquage va affecter de la même façon les isotopes et donc ne va pas modifier le rapport isotopique. Le traitement chimique après marquage n'a plus

besoin d'être quantitatif. De même, les effets de matrice affectent en général de façon identique les isotopes d'un même élément et ne vont donc pas perturber le dosage par IDMS. L'analyse de spéciation par IDMS, en s'affranchissant des problèmes de pertes, de dilution, de recouvrements de dégradation et de transformation, inhérents aux protocoles de spéciation, permet d'augmenter de manière significative la précision et la justesse de la mesure.

Néanmoins, L'emploi de l'IDMS implique également qu'un équilibre complet s'établisse entre les espèces naturellement présentes dans l'échantillon et les espèces enrichies isotopiquement, afin que leur comportement soit identique au cours de la procédure analytique. Très peu d'applications de la dilution isotopique pour des analyses de spéciation ont été publiées (Hintelmann et al., 1995 ; Ruiz-Encinar et al., 2000 ; Hintelmann & Evans 1997 ; Demuth & Heuman, 2001 ; Christopher, 2001) mais la plupart d'entre elles préconisent que l'ajout doit être réalisé le plus tôt possible dans la chaîne analytique et qu'un temps d'équilibration suffisamment long soit respecté.

A.3.2.2. Isotopes stables : traceurs des processus environnementaux

Les traceurs isotopiques stables permettent de suivre et de caractériser les processus environnementaux dynamiques tels que les échanges et les transformations des composés du mercure et des butylétains dans les environnements aquatiques. En effet, afin de bien comprendre le cycle biogéochimique de ces polluants, il est indispensable de connaître les conditions environnementales nécessaires pour leur transformation ainsi que les taux et les cinétiques de ces transformations.

Les premières méthodes expérimentales utilisaient les espèces marquées radioactives mais du fait de leur faible activité, les concentrations a ajouter étaient souvent très grandes (Ramlal et al. 1986 ; Gilmour et al. 1998). Ces expériences ne reproduisaient pas réellement les processus naturels qui pouvaient être biaisés par une différence de partition ou de toxicité des espèces ajoutées par rapport aux espèces naturellement présentes dans l'environnement. De plus, la plupart des processus environnementaux sont des processus couplés et réversibles comme par exemple l'adsorption et la désorption, l'oxydation et la réduction, l'assimilation et la dépuration, la méthylation et la déméthylation. Afin de connaître la contribution de chacun des mécanismes sur le résultat net, il est nécessaire de pouvoir discriminer les deux processus antagonistes. En utilisant plusieurs espèces enrichies avec différents isotopes, il est alors possible de connaître le taux brut de chacun des processus. Enfin, la mise en œuvre de ces méthodes permet aussi de contrôler simultanément le devenir des espèces ambiantes qui sont naturellement présentes dans l'environnement.

*Principe et mise en oeuvre*

La transformation d'espèces marquées isotopiquement et ajoutées à l'échantillon est facilement détectable par la mesure des rapports isotopiques de chaque espèce chimique. L'utilisation de l'ICPMS, offrant une excellente précision sur ce type de mesure, permet de travailler à niveaux de concentration de traceur très faibles afin de ne pas perturber le système tout en détectant les transformations même minimes. La Figure 11 présente le principe de cette méthode appliquée à l'étude de la méthylation et de la déméthylation du Hg, telle que proposée par Hintelmann & Evans (1997). Les espèces marquées isotopiquement utilisées sont le $^{201}$MMHg pour suivre la déméthylation et le $^{199}$IHg pour suivre la méthylation. A la fin de la période d'incubation de l'échantillon dopé, des conversions d'espèces peuvent être observées modifiant les rapports isotopiques de chacune d'elles. Les équations sont données pour le calcul des concentrations des espèces formées. Les taux de méthylation et de déméthylation sont calculés directement en divisant la quantité d'espèce formée par la quantité d'espèce ajoutée.

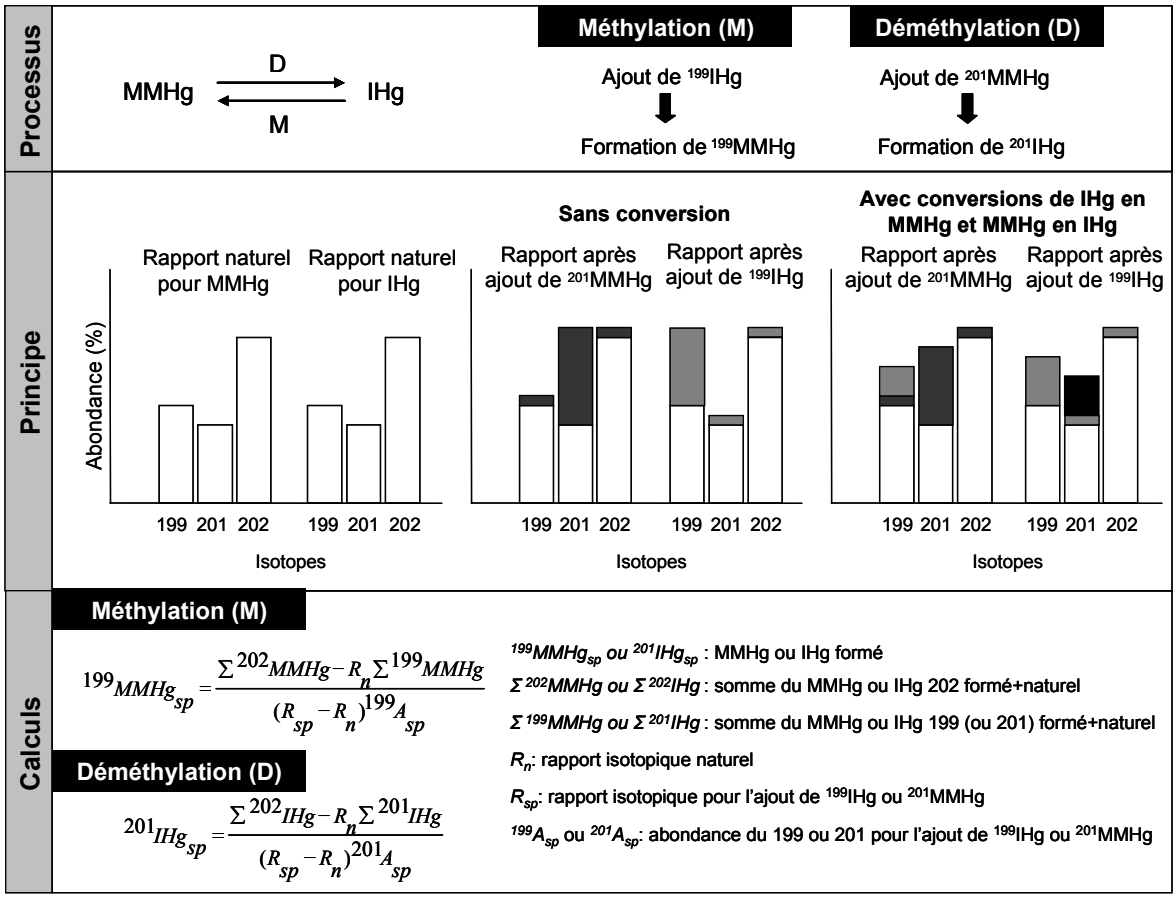


***Figure 11 -*** *Principe de l'utilisation des traceurs isotopiques stables pour l'étude de la méthylation/déméthylation du mercure*

Ces méthodes expérimentales utilisant les traceurs isotopiques peuvent être mises en œuvre pour les différentes techniques d'incubation en laboratoire (aquarium, mésocosmes,…) ou *in situ* (carottes de sédiment, enceintes…) et différents types d'échantillons (sédiments, eaux, biota).

### *A.3.3. Méthodes d'étude des processus de spéciation biogéochimique du tributylétain et du mercure en microcosmes*

*Etudes en microcosmes*

Si les sources et réservoirs des contaminants métalliques dans les écosystèmes aquatiques sont en grande partie connus, les cinétiques des processus élémentaires, à la fois physicochimiques et microbiologiques, concernant leur devenir ne sont que très peu caractérisées pour des milieux naturels représentatifs. L'étude mécanistique de la réactivité de contaminants métalliques tels que le TBT et le mercure dans ou à l'interface des différents compartiments environnementaux demeure un défi difficile à relever. En tant que réceptacles d'une grande partie des contaminants dans les environnements aquatiques, les sédiments et la colonne d'eau jouent en particulier un rôle primordial dans la dynamique des polluants métalliques. A partir d'un substrat plus ou moins hétérogène à la fois minéral et organique, les contaminants métalliques sont impliqués dans des processus réactionnels physicochimiques et microbiologiques multiphasiques. En conséquence, l'approche discrète d'un de ces processus (physicochimique ou bien microbiologique) ne suffit pas à comprendre les interactions voire les synergies du système dans son ensemble. De plus, une approche de ces processus à l'échelle d'un écosystème est irréalisable si l'on veut aborder certains mécanismes élémentaires. Une démarche intermédiaire consiste donc à étudier ces processus dans des réacteurs ou microcosmes permettant de contrôler un certains nombre de facteur forçant macroscopiques et d'effectuer des études cinétiques fines en vue de déterminer la réactivité du système et les échelles de temps caractéristiques des processus mis en jeu. Les expérimentations en milieu reconstitué permettent aussi d'éliminer les phénomènes physiques contrôlant de nombreux processus chimiques et biologiques tels que la diffusion moléculaire, les perturbations mécaniques (bioturbation, remise en suspension) et les hétérogénéités locales (inclusions oxiques ou anoxiques).

De plus, en raison d'importantes limitations dans le domaine de l'échantillonnage et de l'analyse, de nombreuses incertitudes subsistent quant à la caractérisation précise des processus de transformations de ces contaminants aux faibles niveaux de contamination chronique observés dans les environnements naturels. Les écosystèmes reconstitués ou microcosmes semblent constituer des outils expérimentaux efficaces et susceptibles de pouvoir pallier ces difficultés. Le Tableau 12 rassemble quelques exemples d'expérimentations en microcosmes sur le devenir du TBT et du mercure et simulant des conditions naturelles à différents degrés de complexité. La mise en œuvre conjointe de techniques analytiques très précises et sensibles, telles que les traceurs isotopiques, et d'études en systèmes contrôlés dûment paramétrés, semble être une voie privilégiée

pour déterminer la spéciation biogéochimique des micropolluants métalliques dans les écosystèmes aquatiques. De plus, la potentialité de ces outils en terme d'évaluation des risques écotoxicologiques semble également très prometteuse.

*Applications environnementales des traceurs isotopiques*

Actuellement, toutes les méthodes expérimentales utilisant les isotopes stables sont dédiées à l'étude des processus environnementaux des espèces du mercure. Quelques exemples d'applications sont présentés dans le Tableau 12. La plupart de ces investigations ont porté sur l'étude des processus de méthylation et déméthylation du mercure dans les sédiments au moyen d'incubations en laboratoire (suspension de sédiments, aquariums) ou *in situ* (carotte de sédiments).

***Tableau 12 -*** *Etudes sur les transformations et transferts du TBT et du mercure en microcosmes*

| Contaminant | Processus | Conditions | Références |
|---|---|---|---|
| TBT | dégradation | mésocosmes<br>eau, sédiments, benthos | Adelman *et al.* 1990 |
| TBT | dégradation<br>bioaccumulation | microcosmes<br>eau, sédiments, poissons | Dai *et al.* 1998 |
| IHg | méthylation<br>bioaccumulation | microcosmes<br>eau, sédiments, poissons | Ikingura & Akagi 1999 |
| $^{200}$IHg<br>$^{199}$MMHg | méthylation<br>déméthylation | microcosmes<br>eau, algues, périphyton | Mauro *et al. 2002* |
| $^{199}$IHg<br>$^{202}$MMHg | méthylation<br>déméthylation | suspension de sédiments<br>complexation nitrates, sulfures<br>acides fulviques | Hintelmann *et al.* 2000 |
| $^{200}$IHg | méthylation | carotte sédimentaire<br>partition eau interstitielle/phase solide | Hammerschmidt & Fitzgerald 2004 |
| $^{199}$IHg<br>$^{201}$MMHg | méthylation<br>déméthylation<br>volatilisation | suspension de sédiments<br>conditions oxiques/anoxiques<br>biotiques/abiotiques | Rodriguez *et al.* 2004 |

Tout d'abord, des incubations de suspensions de sédiments (ou « slurry ») ont été réalisées afin de déterminer les taux de méthylation et déméthylation dans des systèmes simples avec un contrôle précis des principaux

paramètres physicochimiques (température, oxygène, lumière...) et de l'activité biologique (Hintelmann et al. 2000, Rodriguez et al. 2004). De plus, cette approche expérimentale permet de caractériser les cinétiques de transformation, ainsi que l'influence de divers paramètres sur l'établissement et l'intensité de ces mécanismes.

La disponibilité du IHg pour la méthylation dans des suspensions de sédiments a ainsi été examinée par Hintelmann et al. (2000) en ajoutant du $^{199}$IHg complexé à différents ligands susceptibles d'être présents dans l'environnement tels que les nitrates, les acides fulviques et les sulfures. Les ligands organiques tels que les acides fulviques semblent limiter la disponibilité du IHg pour la méthylation alors que les sulfures semblent la stimuler. Par ailleurs, ils ont également réalisé une approche cinétique critique de l'utilisation des isotopes stables comme traceur, en comparant les taux de transformation spécifique des espèces marquées isotopiquement et de leurs analogues naturels. Ainsi, le traceur $^{199}$IHg semble être plus disponible pour la méthylation que le IHg ambiant alors que le $^{201}$MMHg semble quant à lui être dégradé de la même manière que le MMHg naturellement présent dans l'échantillon.

Le deuxième type d'applications environnementales est l'incubation de sédiment *in situ* (Hammerschmidt et Fitzgerald 2004). Pour cela le sédiment est échantillonné en carotte avec des tubes préalablement percés afin de pouvoir injecter les espèces marquées de mercure aux différentes profondeurs. Après une période d'incubation en conditions contrôlées (température, lumière), les potentiels de méthylation/déméthylation sont déterminés pour chaque tranche de sédiment. Le principal avantage de cette technique d'incubation, par rapport à la suspension de sédiment, est que les conditions d'incubation sont très proches des conditions environnementales car la structure du sédiment est préservée. Cette technique permet en outre d'étudier la répartition des processus en fonction de la profondeur. Ainsi, Hammerschmidt et Fitzgerald (2004) ont montré que la méthylation du mercure était maximale pour les sédiments de surface et variait en fonction des saisons.

D'autres applications des isotopes stables pour tracer les processus environnementaux ont été effectuées, à plus grande échelle, dans le cadre du projet METAALICUS (Mercury Experiment To Assess Atmospheric Loading in Canada and the United States) (Hintelmann et al. 2004, Amyot et al. 2004, Poulain et al. 2004). Le but de ce projet est de simuler un apport atmosphérique de mercure au dessus d'un écosystème forestier composé d'un lac, de sa zone humide et de son bassin versant et d'enregistrer l'effet de cette contamination artificielle au niveau de populations de poissons. Des ajouts distincts de $^{202}$IHg, $^{201}$IHg et $^{198}$IHg ont ainsi été réalisés dans les trois composantes de l'écosystème afin de tracer le cheminement du mercure vers le réservoir final. Le taux d'addition du mercure est de 25µg $m^{-2}$ $y^{-1}$ pour une surface totale de l'écosystème de 52 ha. Les premiers résultats portent sur la dynamique du mercure fraîchement déposé par aspergement sur la forêt boréale par rapport au mercure anciennement déposé dans l'écosystème (Hintelmann et al. 2004). Des expérimentations complémentaires, utilisant du $^{200}$IHg ont été réalisées à l'aide à des mésocosmes (enceintes enfermant le sédiment et la colonne d'eau) disposés dans un lac voisin du site expérimental afin d'étudier la formation et l'évasion d'espèces dissoutes gazeuses du mercure (Amyot et al. 2004, Poulain et al. 2004). Ce

projet unique illustre le grand potentiel des marqueurs isotopiques pour suivre le devenir d'un micropolluant à l'échelle d'un écosystème entier, en s'affranchissant du toute pollution significative de la zone expérimentale.

## A.4. Présentation du travail

Le travail présenté dans ce mémoire est centré sur l'étude de la réactivité et du transfert du TBT et du mercure dans les écosystèmes aquatiques. La distribution de ces micropolluants dans les écosystèmes naturels ainsi que leurs implications écotoxicologiques ont fait l'objet de nombreuses études depuis plusieurs décennies. Le TBT et le mercure sont considérés à juste titre comme des contaminants majeurs, persistants et ubiquistes. Néanmoins de nombreuses incertitudes demeurent quant à la caractérisation des mécanismes physicochimiques et biologiques régulant leur dynamique environnementale. Le développement récent d'outils analytiques toujours plus performants et sensibles, associés à des approches expérimentales adaptées et pluridisciplinaires, autorise une meilleure compréhension du cycle biogéochimique de ces éléments traces. Ces progrès nous permettent également d'appréhender ces problématiques dans le contexte difficile de situations de contamination chronique et ouvrent ainsi la voie à une meilleure évaluation des risques.

La première partie de ce mémoire présente un examen approfondi de la réactivité et de la dynamique biogéochimique du TBT dans des microcosmes d'eau douce ainsi que des études *in situ* axées sur la biométhylation et le transfert du TBT aux interfaces sédiment-eau et eau-atmosphère en milieux estuarien et côtier.

Tout d'abord la spéciation biogéochimique du TBT et de ses produits de dégradation a été réalisée dans les différents compartiments d'écosystèmes d'eau douce reconstitués présentant une organisation simple (eau, particules, sédiments, biota) et un contrôle satisfaisant des conditions expérimentales. Cette étude a été conduite au moyen de techniques analytiques justes et sensibles mettant en oeuvre des couplages GC-ICPMS ainsi que la quantification par dilution isotopique. Le devenir du TBT a été suivi en fonction du temps, permettant ainsi une approche cinétique des principaux mécanismes de transformation et de transfert des butylétains dans les microcosmes. Une attention particulière a été portée sur les processus de biodégradation et de bioaccumulation permettant de réaliser un bilan complet de la distribution du TBT et de ses métabolites dans ces écosystèmes modèles en vue de répondre aux problèmes d'impact et de risques toxicologiques dus à la pollution par ces composés organométalliques. Ces expérimentations sont présentées sous forme d'un article en anglais intitulé « (Tri)butyltin biotic degradation rates and pathways in different compartments of a freshwater model ecosystem », soumis à la revue Water Research.

Dans un second temps, des études réalisées en milieux estuarien et côtier centrées sur l'identification et la distribution des formes volatiles des organoétains dans les sédiments, la colonne d'eau et l'atmosphère sont présentées. Les voies potentielles de formation de ces espèces volatiles dissoutes, leurs transferts aux interfaces sédiment-eau et eau-atmosphère, ainsi que leur implication dans le cycle biogéochimique de l'étain en milieu aquatique sont discutés. Des modèles de transferts aux interfaces ont également été paramétrés afin

d'estimer l'amplitude de ces processus à plus grande échelle et de les comparer aux apports anthropiques direct d'étain à l'atmosphère. L'ensemble de ces résultats sont présentés sous forme de deux articles en anglais intitulés « Volatilization of organotin compounds from estuarine and coastal environments », publiés dans la revue Environmental Science and Technology et « Volatile organotin compounds (butylmethyltin) in three European estuaries (Gironde, Rhine, Scheldt) » publié dans la revue Biogeochemistry.

La deuxième partie de ce mémoire s'articule autour de la problématique du mercure dans les milieux aquatiques par le biais de deux études mécanistiques réalisées en laboratoire. La première approche se fait le pendant de la précédente étude menée en microcosmes sur le devenir du TBT. La réactivité du mercure et ses interactions avec les différentes composantes biogéochimiques constituant les écosystèmes modèles ont été examinées en détail. Les cinétiques de méthylation et de bioaccumulation, ainsi que la formation d'espèces gazeuses dissoutes du mercure ont ainsi été étudiés dans les différents compartiments des aquariums. La reproduction des principaux processus régissant la disponibilité du mercure vis-à-vis des réactions chimiques et biologiques observés dans les écosystèmes aquatiques naturels ont permis la validation des microcosmes en tant qu'outils potentiels pouvant aider à une meilleure évaluation des risques. Les résultats de cette étude sont exposés sous forme d'un article en anglais soumis à la revue Environmental Chemistry et intitulé « Mercury contamination pathways and bioaccumulation at various contamination levels in aquatic model ecosystems ».
Enfin, la seconde approche met en œuvre l'outil isotopique, utilisé en tant que traceur, afin de discriminer les mécanismes réactionnels couplés de la méthylation et de la déméthylation du mercure dans un sédiment estuarien. Cette démarche réductionniste, basée sur l'incubation d'une suspension de sédiment en laboratoire, permet d'appréhender plus simplement et en conditions contrôlées ces processus élémentaires gouvernant en partie la biodisponibilité et la mobilité du mercure dans les sédiments. La formation d'espèces volatiles du mercure a également pu être identifiée comme étant liée à la dégradation du méthylmercure. Ces expériences ont en outre démontré le potentiel important de l'utilisation d'espèces enrichies isotopiquement, en combinaison avec des techniques de mesure très sensibles, pour faciliter l'étude de la spéciation et de la réactivité du mercure. Ces travaux, ayant fait l'objet d'une publication récente dans la revue Marine Chemistry, sont présentés en anglais, au format d'édition.

Enfin un bilan de l'ensemble des résultats obtenus durant ce travail, présenté en conclusion, nous permet de faire une synthèse critique des approches analytiques et expérimentales nécessaires à une meilleure compréhension des situations de contamination chronique des écosystèmes aquatiques par le TBT et le mercure

En annexe, sont rapportés des informations complémentaires sur la réglementation du mercure et de ces composés au niveau européen ainsi que deux articles en anglais, portant sur le développement et l'optimisation de techniques analytiques pour la spéciation simulatnée des composés mercuriels et organostanniques dans les matrices environnementales. Enfin, l'étude du cycle biogéochimique du mercure en milieu tropical amazonien est présentée sous forme de deux articles en anglais.

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# CHAPITRE B

# Réactivité et transfert des organoétains dans les environnements aquatiques

# Chapitre B.1.

# Etude cinétique des mécanismes de dégradation du tributylétain dans des écosystèmes d'eau douce reconstitués

# Etude cinétique des mécanismes de dégradation du tributylétain dans des écosystèmes d'eau douce reconstitués

Le tributylétain (TBT) et les composés organostanniques, de manière générale, sont reconnus comme étant des micropolluants majeurs pour les environnements aquatiques du fait de leur haute toxicité vis-à-vis des organismes et de leur impact général sur les écosystèmes naturels. Les organostanniques, impliqués dans de nombreux procédés industriels ont ainsi été dispersés à très grande échelle dans l'environnement et représentent aujourd'hui des contaminants ubiquistes dans les milieux d'eaux douces et marins. Le devenir du TBT dans les écosystèmes aquatiques et les conséquences écotoxicologiques induites par sa présence sont directement dépendants de sa persistance dans le milieu ainsi que de la mise en place de mécanismes naturels de dégradation d'origine biotique et abiotique. Les processus de dégradation du TBT, dans la colonne d'eau et les sédiments, sont contrôlés par de nombreux paramètres clés tels que le pH, la température, l'irradiation solaire, les teneurs en matières organiques dissoutes, l'activité et la biodiversité microbienne. La biodégradation du TBT, initiée par les microorganismes (bactéries, algues), est considérée comme la voie préférentielle responsable de la diminution des teneurs en TBT dans la colonne d'eau et les sédiments. Néanmoins si les sources et les réservoirs du TBT dans les systèmes naturels sont bien discernés, les mécanismes élémentaires définissant sa réactivité vis-à-vis du milieu ainsi que leur importance relative demeurent empreints de nombreuses incertitudes et particulièrement en conditions naturelles de contamination chronique.

L'objectif de ce travail a été d'étudier en laboratoire, la persistance et la dynamique biogéochimique du TBT et de ses produits de dégradation entre les différents compartiments (air, eau, sédiments, plantes, gastéropodes) d'écosystèmes d'eau douce reconstitués. Cette approche expérimentale en milieu contrôlé, associée à des techniques analytiques de spéciation chimiques sensibles et précises, nous ont permis d'isoler et d'évaluer, sur une base cinétique, les mécanismes réactionnels régissant les transformations et transferts du TBT en milieu aquatique. Cette étude souligne le rôle primordial du compartiment sédimentaire en tant que réservoir de la contamination par le TBT mais également en tant que source potentielle vers la colonne d'eau. La représentativité environnementale des mécanismes identifiés confirme également l'utilité des microcosmes en tant qu'outil pour l'évaluation des risques écotoxicologiques liés aux situations de contamination chronique des écosystèmes aquatiques par le TBT.

# (Tri)Butyltin biotic degradation rates and pathways in different compartments of a freshwater model ecosystem

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**Abstract**

Experiments were conducted in controlled freshwater ecosystems (microcosms) to determine the persistence and biogeochemical dynamic of tributyltin (TBT) and its degradation products. TBT and its derivatives were monitored simultaneously for 23 days in sediment-water systems, with or without macroorganisms (macrophytes*: Elodea canadensis* and gastropods: *Lymnaea stagnalis*). Biphasic TBT removal from the water column was significantly enhanced by the presence of biota. The persistence of TBT in biota was assessed by a kinetic approach of the different bioaccumulation pathways and associated metabolisms adopted by the snails and the macrophytes in response to the TBT contamination. Furthermore, sediment acted for the final sink for butyltins in both types of microcosms, with more than 70% of TBT and its metabolites recovered in this compartment after two weeks of exposure. Degradation pathways in sediments of both biotic and abiotic microcosms appeared to represent a key process in TBT cycle and were characterized by half-lives in the range of one month. Specific transformation and transfer pathways of TBT as reactional mechanisms are discussed and modelled assessing in detail the role of each compartment with regards to the fate of TBT in the model aquatic ecosystems.

## 1- INTRODUCTION

Organotin compounds are known to be highly toxic micropollutants for aquatic ecosystems likely to seriously impair aquatic organisms (Bryan et al., 1986; Alzieu et al., 2000). They have been used in a variety of industrial processes for more than half a century and their subsequent discharge into the environment has became a topic of global concern. Organotins are ubiquitous contaminants widely spread in both marine and freshwater environments (Maguire, 1987; Fent & Hunn, 1995; Kannan et al., 1995; Michel & Averty, 1999; Negri et al., 2004). The fate of TBT in aquatic ecosystems and its ecotoxocological consequences are directly dependent on its persistence and thus on the occurrence of biotic and abiotic degradation mechanisms. TBT degradation involves the sequential removal of butyl groups from the tin atom, leading to the formation of di- (DBT) and monobutyltin (MBT). These desalkylation processes generally results in the reduction of toxicity (Blunden & Chapman, 1986; Cooney & Wuertz, 1989). It is worth mentioning that degradation products such as of DBT have been demonstrated to affect immuntoxicity in fishes (O'Halloran, 1998). Degradation can be achieved by either abiotic or biotic mechanisms. TBT degradation in sediments as well as in the water column is controlled by numerous key parameters, such as temperature, sunlight radiation, dissolved organic matter content and microorganism diversity and activity (Maguire, 1996). Chemical and UV degradation pathways are reported to be the most significant abiotic pathways in aquatic ecosystems (Maguire, 1996; Mailhot et al., 1999). Several inorganic and organic compounds naturally present in water or sediments, such as mineral and carboxylic acids or iron(III) act as photocatalysts and promote the abiotic degradation of TBT. Photodegradation alone generally is generally a slow process characterized by half-lives of several months (Maguire et al., 1983; Duhamel et al., 1987). On the contrary, several studies have reported that sunlight radiations enhance the degradation of TBT through the stimulation of biological activity (Olson & Brinckman, 1986; Lee et al., 1989; Cooney & Wuertz, 1989; Huang et al., 1993). In general, biodegradation mechanisms by microorganisms such bacteria and algae are assumed to be the preference process responsible for TBT breakdown in both water column and sediments (Maguire & Tkacz, 1985; Dowson et al., 1996; Dubey & Roy, 2003).

Further, desalkylation processes can also be offset to some extent by competing reaction pathways involving natural methylation (Byrd & Andreae, 1982; Guard, 1981; Weber, 1999). Methylation of butyltin derivatives is likely to occur in natural systems, leading to the formation of fully substituted and volatile compounds. Alkylation processes, generally assumed to be biologically mediated could thus represent a removal pathway for TBT in aquatic systems under both oxic and anoxic conditions (Guard et al., 1981; Ridley et al., 1977; Yozenawa et al., 1994; Gilmour et al., 1985; Donard et al., 1987).

An additional aspect controlling the fate and persistence of TBT in aquatic systems is directly linked to the partitioning processes between water, the sedimentary material and the biota via biotransformations. TBT half-

lives in water generally range from a few days to a few weeks whereas degradation rates are substantially lower in sediments with half-lives between several months to several years. Reported half-lives for TBT in environmental compartments are mainly related to laboratory experiments and are thus not comparable to real circumstances where rates of breakdown depends on numerous biogeochemical factors. Therefore, TBT persistence in natural sediments is often underestimated and half-lives of TBT, estimated from *in situ* measurements, can be stable for at least 2 decades in anoxic sediments (Dowson et al., 1993). The sediment appears therefore to act as a sink for TBT and represent a permanent threat for water contamination via desorption and remobilization processes or directly for benthic organisms via ingestion of particles. Furthermore, Amouroux et al. (1998 & 2000) and Tessier et al. (2002) recently reported on the formation of volatile methylated butyltin compounds in sediments and their resulting transfer to the water column of various estuarine and coastal environments. Point et al. (2004) have also evidenced the occurrence of continuous passive fluxes of butyltins at the sediment-water interface. These diffusion mechanisms are significant even in low impacted coastal environments and are directly related with the benthic biomass and activity. Biotransformation and transfer processes appear therefore to significantly modify the mobility and availability of TBT stored in sediments. Finally, these findings emphasize again the role of sediments as a secondary and significant source of TBT contamination for aquatic ecosystems.

Despite of the fact that both degradation and methylation mechanisms of TBT have been demonstrated to take place in natural aquatic systems, informations on mechanistic reactions involved are still limited. Estimations of the kinetics parameters forcing the transformations and the transfers of TBT are scarce and are often related to laboratory experiments focussing on single process. Simple laboratory experiments (e.g. batch adsorption or bioaccumulation studies) allow to isolate and assess individual fate processes but can also lead to their overestimation since they do not take into account other competing processes. On the other hand, field studies do not allow to integrate the whole complexity and heterogeneity of the system. Microcosms can thus be used as substitutes for field studies to better discriminate mechanistic reactions driving the biogeochemical dynamic of TBT in aquatic environments. They also allow to identify and validate the environmental significance of laboratory data derived from simpler systems (Pritchard et al., 1986).

The objective of this study was to determine the behavior of TBT and its degradation products under conditions representative of a temperate freshwater ecosystem. Indeed most studies conducted in mesocosms have mainly dealt with marine or coastal conditions. However, TBT has also been demonstrated to seriously affect fresh water biota. Therefore, this study was conducted under fresh water conditions to fully assess the reactivity and transfer or TBT between different compartments under low ionic strength conditions. TBT was added to the water column of freshwater microcosms in a single contamination pulse to allow a kinetically study of a transient toxicity regime from acute to chronic contamination. Microcosms were characterized by two different organization levels (sediment/water and sediment/water/plant/gastropod). A defined sampling strategy of all

compartment over time have allowed to closely assess the reactivity of TBT and its degradation products as well their kinetics of partitioning between the different compartments. The degradation kinetics of TBT and its metabolites in the different compartments of the aquaria and the derived reaction rates were determined over a 23 days incubation period. The bioaccumulation of TBT in both plants and snails was investigated as well as the metabolization processes leading to the production of DBT and MBT. Biotic degradation of TBT in sediments appears to be the major process affecting TBT behavior and fate in the microcosms on a mid-term basis. Finally, the environmental significance of the mechanisms observed are discussed to validate the use of the microcosms as practical tools to provide real insights for chronicle TBT contamination impact and ecotoxicological risk assessments.

## 2- MATERIALS AND METHODS

### 2-1- Microcosms experimental set up

The characteristics of the model ecosystems used for this study have been described in detail elsewhere (Tessier et al., 2004). Briefly five static microcosms were based on glass tanks (80×40×45 cm) and prepared in the following stages: (1) 25 kg dry weight of artificial sediment (sand 800-2500µm: 65%, Kaolin: 30%, cellulose: 4.75%, Tetramin: 0.15%, $CaCO_3$: 0.1%) were used for each experimental unit. (2) 110 litres of reconstituted fresh water (ISO 6341: $CaCl_2$, $2H_2O$ 294 mg $l^{-1}$, $MgSO_4$, $7H_2O$ 123.25 mg $l^{-1}$, $NaHCO_3$ 64.75 mg $l^{-1}$, KCl 5.75 mg $l^{-1}$ in deionised water) were gently added. Aeration was provided by airstones installed halfway in the water column to supply oxygen and support continuous mixing. (3) The experimental systems were then allowed to stabilize for one week to remove the excess of chloride and to permit settling of the suspended. The sediment obtained formed a uniform layer of ca. 6 cm depth on the bottom with an overlaying water column of 30 cm depth. (4) Biological organisms were introduced in three of the five microcosms (i.e. AQ1, AQ2 and AQ3) after oxygenation of the water column and the equilibration period. Fifty shoots of the macrophyte *Elodea canadensis* (i.e. 100g, fresh weight) were first planted when a minimum dissolved oxygen level of ca. 6 mg $l^{-1}$ was reached. Freshwater pulmonate snails (*Lymnaea stagnalis)* (350 g per aquarium) were introduced in the three biotic model ecosystems. All biological organisms originated from an aquaculture to avoid any growth of invasive and predator species. After one week, a biocenosis developed in the microcosms. The two remaining aquaria labelled AQ4 and AQ5 were received only sediments and water and were used as abiotic reference systems with regards to the presence of macroorganisms. All microcosms were kept in temperature-controlled room

(20°C) and exposed to a daily photoperiod of 16 hours during all the incubation experiments. Physico-chemical characteristics of the sediment and water are summarized in Table 1.

***Table 1 -*** *Microcosms composition and water quality parameters*

| **Microcosms composition** | **AQ1** | **AQ2** | **AQ3** | **AQ4** | **AQ5** |
|---|---|---|---|---|---|
| Water (110 l) | x | x | x | x | x |
| Sediment (25 kg dw) | x | x | x | x | x |
| Snails (350 g fw) | x | x | x | | |
| Waterweeds (100 g fw) | x | x | x | | |
| Spike concentration (nmol Sn $l^{-1}$) | 87.8 | 87.5 | control | 99.1 | 97.9 |
| **Water quality parameters** | | | | | |
| pH | 7.4 ± 0.3 | 7.3 ± 0.2 | 7.4 ± 0.2 | 7.5 ± 0.1 | 7.3 ± 0.2 |
| T (°C) | 18.8 ± 0.1 | 18.9 ± 0.1 | 18.9 ± 0.1 | 18.9 ± 0.1 | 18.7 ± 0.1 |
| $O_2$ (mg $l^{-1}$) | 4.2 ± 3.1 | 5.6 ± 2.8 | 2.8 ± 0.7 | 7.1 ± 0.6 | 7.6 ± 0.5 |
| $Cl^-$ (mg $l^{-1}$) | 125 ± 5 | 125 ± 5 | 120 ± 5 | 115 ± 5 | 120 ± 5 |
| $PO_4^{3-}$ (mg $l^{-1}$) | < 0.02 | < 0.02 | < 0.02 | < 0.02 | < 0.02 |
| $NO_3^-$ (mg $l^{-1}$) | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 |
| $NO_2^-$ (mg $l^{-1}$) | < 0.02 | < 0.02 | < 0.02 | < 0.02 | < 0.02 |
| $NH_4^+$ (mg $l^{-1}$) | 0.19 ± 0.06 | 0.12 ± 0.08 | < 0.05 | < 0.05 | < 0.05 |
| DOC (mg $l^{-1}$) | 27.0 ± 4.3 | 23.7 ± 6.5 | 42.0 ± 16.2 | 78.3 ± 30.4 | 109.5 ± 39.1 |
| POC (%) | 9.4 ± 3.1 | 14.9 ± 4.9 | 14.8 ± 5.0 | 1.5 ± 0.4 | 1.6 ± 0.6 |
| SPM (mg $l^{-1}$) | 11 ± 2 | 49 ± 25 | 24 ± 5 | 10 ± 2 | 18 ± 2 |

### 2-2- Microcosms contamination and sampling

Contamination of the different aquaria was achieved with one direct injection of TBT as TBT chloride to reach an initial toxic concentration of 10 µg Sn $l^{-1}$ in the water column of the four contaminated microcosms. This target concentration of TBT was selected to represent acute toxicity conditions of TBT to aquatic organisms and later chronic contamination levels of fresh water ecosystems. It represents a compromise to allow the analytical determination in all compartments. These initial conditions allowed to follow the different relationships between

the period of exposure, the potential losses of the contaminant by adsorption on the tank walls. It also permitted to obtain bioaccumulated concentrations and description of trophic transfers with regards to the remaining contamination levels. Finally, it demonstrated the differential cycling of TBT and its degradation products (DBT, MBT) in all compartments of the microcosms. The overall experimental setup consisted of two sets of biotic and abiotic systems prepared in duplicate and exposed to the same contamination and one TBT-free biotic control microcosm (AQ3) used as the reference. Contamination of the water column was achieved by the injection of a TBT chloride standard solution prepared in milli-Q water to give the initial concentrations of 10.42 (87.8), 10.39 (87.5), 11.76 (99.1) and 11.62 µg Sn $l^{-1}$ (97.9 nmol Sn $l^{-1}$) for AQ1, AQ2, AQ4 and AQ5, respectively.

The parameters for the water quality were monitored during both equilibration phase and incubation period in presence of the TBT. The water pH, the dissolved oxygen concentration and the temperature were measured weekly *in situ* using a multi probes recorder (WTW). The phosphate, chloride, nitrate, nitrite and ammonia concentrations were also monitored (Merck titration kits) weekly during the incubation experiments. The total and dissolved organic carbon contents (TOC and DOC) were measured by high temperature catalytic oxidation (Shimadzu TOC 5000) in bulk and filtered water (0.45 µm PTFE membrane) at the beginning of the experiments and after two weeks of incubation. The particulate organic carbon contents (POC) were obtained from the difference between the measured TOC and DOC values. The suspended particulate matter load (SPM) was obtained by filtering water samples on pre-weighted 0.45 µm PVDF membranes (47 mm d., Millipore, France). Filters were then dried at ambient temperature in a vertical laminar flow hood and accurately weighted.

Prior to the contamination of the aquaria, samples of water, sediment, snails and waterweeds were collected the day before the contamination to determine the background values of TBT and its derivatives (DBT, MBT) in each compartment. The different microcosms were then incubated in the presence of TBT during 23 days with a sampling frequency of 24 hours for the first three days of experiment. Later, the sample frequency was performed weekly. Once collected, the water samples were immediately filtered through pre-cleaned and pre-weighted 0.45-µm PVDF membranes. The dissolved fraction was then acidified to pH 4 with acetic acid (100%, extra pure, Merck, France) and stored in acid-cleaned Pyrex bottles at +4°C until analysis. Filters were conditionned in the same way than for the SPM measurement and stored at −20°C until analysis. Biological organisms were rinsed with milli-Q water (Millipore, France) after sampling and frozen, freeze-dried and stored at −20°C until analysis. Whole body tissues and shells were stored and analysed separately to discriminate between adsorption and absorption processes. Four sediment cores (ca. with a total of 50 g fresh weight) were manually collected using acid-cleaned polyethylene tubes at each sampling date. The cores were then mixed up in order to be more representative of the whole sediment compartment and finally conditioned and stored in the same way than that for biological samples.

Prior to analysis, the sediments were sieved at 2000 µm and 300 µm. Each fraction (i.e. clay particles: <300 µm, fine sand: 300 µm-2000 µm and coarse sand: >2000 µm) was analysed separately to obtain the

granulometric distribution of organotins in the sediment. During the experiment, an important biofilm was allowed to develop on the tank walls for both biotic and abiotic microcosms. This biofilm was sampled using glass slides and conditioned in a similar manner than for the biotissues. Additional sampling for the determination of volatile organotin species (i.e. tetramethyltin, mixed butylmethyltin derivatives and organotin hydrides) dissolved in water was also performed in all microcosms at the end of the incubation experiments. The purge and cryogenic trapping device developed for the extraction of volatile tin compounds from water samples is described in detail elsewhere (Amouroux et al., 1998). Briefly, 1 liter of water sample was continuously stripped for 30 min under Helium flow. The volatile species were subsequently cryofocused in a U-shaped glass trap filled with silanized glass wool (Supelco) and immersed in liquid nitrogen (−196°C).

### 2-3 Analytical procedures

The standard settings and methodologies used to achieve organotin compounds speciation in water, sediment and biological samples are described in details elsewhere (Monperrus et al., 2003a, 2004; Szpunar et al., 1996). All concentrations in the present paper are expressed with reference to the tin mass. The determination of TBT has been performed by species specific isotope dilution mass spectrometric analysis (SIDMS) using an inductively coupled plasma mass spectrometer as detector (Thermo Electron ICPMS, X7 series) after capillary gas chromatography (Thermo Electron CGC, Focus series). The isotope dilution is based on the addition of a precise amount of an isotopically labelled form of the analyte ([$^{117}$Sn]TBT) to the sample. The TBT concentration in the sample can be calculated from the observed isotope ratios when the natural and enriched isotope ratios, the mass of sample and the level of the spike are respectively known. The SIDMS-CGC-ICPMS approach offers an unmatched, accurate, selective and precise determination of TBT concentrations from ppt down to ppq levels routinely in environmental matrices. The MBT and DBT quantification was performed simultaneously by standard additions, using the same analytical chain.

An extraction-derivatization step was first required to obtain a complete alkylation of organotin compounds and to achieve speciation analysis by CGC-ICPMS. Briefly, for the filtered water, 100 ml of sample were accurately weighed in a flask and spiked with known amount of enriched [$^{117}$Sn]TBTCl solution (LGC Ltd, UK). The mixture was then buffered by addition of 5 ml of sodium acetate/acetic acid buffer (0.1 M). The pH was the adjusted to 5 using ammonium hydroxide solution (25% w/w). After addition of 0.5 ml of isooctane and 1 ml of sodium tetraehtylborate solution ($NaBEt_4$ : 0.5% w/w), the flask was immediately capped and vigorously hand shaken for 5 min. The organic phase was then transferred to an injection vial and stored at –20°C until measurement.

For the sediment, the particle, the biofilm and the snail samples, the organotin species were first extracted from the matrix by microwave digestion following the procedures optimized by Monperrus et al. (2003a&b) and

adapted from Szpunar et al. (1996) An aliquot of 0.25 g of dry sample or the entire filter was introduced in an extraction vessel and spiked with enriched [$^{117}$Sn]TBTCl. For the sediments, the particles, the shells and the biofilm extractions, 5 ml of acetic acid were added to the spiked samples. For the snail tissues, an alkaline extraction using tetramethylammonium hydroxide (25% w/w) instead of acetic acid was used. The mixture was then gently stirred, placed in the open microwave oven (Microdigest 301, Prolabo) and extracted for 2 min at 20% of irradiation power (40W). The extract was then centrifuged for 5 min at 2500 rpm and the supernatant was finally submitted to ethylation.

The organotins extraction from waterweed samples was achieved according to the procedure developed by Simon et al. (2002). An aliquot of 0.25 g of dry sample was first wetted with 2.5 ml of ethyl acetate and stirred for 1 h on elliptic table (400 rpm). The extraction was then completed by adding 6 ml of HCl solution (0.035 M in ethyl acetate) and stirring for 1 h on elliptic table (400 rpm).

The derivatization of solid sample extracts was then performed in a similar way than that used for the water samples. The aliquots of extracted solutions were buffered and the pH was set at 5. Two millitres of isooctane and 1 ml of 1 % (w/w) NaBEt4 solution were added and the mixture was manually shaken for 5 min.

The optimized operating conditions used for the GC separation and the ICPMS detection for the simultaneous speciation of organotin compounds has been described elsewhere (Monperrus et al., 2003, 2004). Parameters affecting the precision and accuracy of the isotopic ratio measurements such as detector dead time and mass bias were carefully evaluated. Extraction-derivatization procedures coupled to CGC-ICPMS analysis have been validated for organotins speciation in various environmental matrices as well as blank control of the whole analytical chain (Monperrus et al., 2003a&b, 2004). Detection limits (DL) were calculated as three times the standard deviation on 6 blanks measurements. DL of butyltin compounds in water were 39, 13, 14 pmol $l^{-1}$ for MBT, DBT, TBT, respectively.

For the sediment, the waterweed, the biofilm and the snail samples, the detection limits of butyltins were below 4.4, 2.9, 0.8 and 0.5 pmol $g^{-1}$ expressed in fresh weight respectively. For the particulate matter analyses, the DLs obtained were 0.2, 0.5 and 1.0 nmol $g^{-1}$ for MBT, DBT and TBT respectively.

Finally, the analytical set-up and methodology used for the speciation of the volatile organotin compounds are described in detail elsewhere (Amouroux et al., 1998). Briefly, the cryogenic traps used after stripping the volatile organotin compounds were desorbed at 250°C with Helium as carrier gas in a cryogenic gas chromatographic device directly connected to an ICP-MS. The detection limits offered by this method were lower than 0.4 fmol $l^{-1}$ for all the volatile organotin species identified.

The overall analytical reproducibility expressed as the relative standard deviation with regards to the different analyses made in replicate averaged 10% for the butyltin compounds identified in the various matrices investigated. Further, the biotic and abiotic microcosm experiments were conducted in duplicates and exhibited very reproducible results. The concentrations presented in this study are then expressed as mean values with

mean deviations for MBT, DBT and TBT of 18, 11 and 9% in water samples; 15, 10 and 8% in sediment samples; 11, 10 and 8% in waterweeds and 6, 5 and 5% in snails over the whole exposure experiments.

## 3- RESULTS & DISCUSSION

### 3-1- Evolution of water quality parameters

The mean values of the different physicochemical parameters monitored in water columns are presented in Table 1. The temperature and pH level monitored revealed only minor variations in all microcosms and over the whole exposure period. The average pH and water temperature for the five ecosystems along the incubation period were 7.4 ± 0.1, 18.8 ± 0.1°C, respectively. At the contrary, the dissolved oxygen concentrations displayed significant differences between the biotic and the abiotic systems with average values of 4.2 ± 0.4 and 7.3 ± 0.3 mg $l^{-1}$, respectively. The dissolved (DOC) and particulate organic carbon (POC) contents were measured in water after one day and two weeks of incubation. The DOC values remained stable in the biotic microcosms with average concentrations of 27 ± 4, 24 ± 8 and 42 ± 16 mg $l^{-1}$ in AQ1, AQ2 and AQ3, respectively. In the early stages of the sediment-water experiments (AQ4 and AQ5), the DOC concentrations were 2 to 3 times higher than in the biotic systems. Then, the DOC contents increased significantly reaching a mean concentration of 78 ± 30 and 109 ± 39 mg $l^{-1}$ in AQ4 and AQ5, respectively. The artificial sediment formed represented the main source of organic carbon to the water column. The POC values remained stable over the same exposure period and represented on average 13 ± 4% of the TOC in the biotic systems and 1.6 ± 04% in the abiotic ones. The model ecosystems finally exhibited higher DOC contents compared to typical freshwater environments and are representative of polluted or organic matter enriched areas.

The phosphate, nitrate and nitrite concentrations were always below 0.02, 0.05 and 0.02 mg $l^{-1}$, respectively. The chloride concentrations were quite high and ranged from 115 to 125 mg $l^{-1}$ in all aquaria. This excess resulted from the reconstituted fresh water media. The low recirculation of water induced by air bubbling did not remove efficiently the chloride levels via evasion. The ammonia concentrations in AQ1 and AQ2 displayed significant high levels. This has to be probably related to the decomposition of intoxicated snails.

The suspended particulate matter (SPM) concentrations were quite homogeneous during the incubation experiments except for AQ2 where the SPM load was 2 to 4 times higher than in AQ1 and AQ3 during the first week of exposure. The concentrations monitored averaged 11 ± 2, 49 ± 25, 10 ± 2 and 18 ± 2 mg $l^{-1}$ in AQ1, AQ2, AQ4 and AQ5 respectively. The overall SPM values remained nevertheless in the same order of magnitude and reflected the range of natural freshwater environments. The particles loads observed in the different microcosms mainly originated from resuspension of very fine clay particles (kaolinite) during the filling step and the introduction of biological organisms. These very fine clay particles were then not the subjected to

fast settling conditions. Also, the sediment sampling procedure caused little but repeated disturbances and remobilization of surface sediments resulting in a constant SPM load in the water column of the model ecosystems.

### 3-2- Overall organotin mass balance

The global butyltin distribution in each compartment of the microcosms is presented in Figure 1 for both biotic and abiotic experimental conditions. The contribution of adsorption on the tank walls could only be evidenced in the abiotic aquaria. In the biotic aquaria, this process cannot account for the loss of budget observed since OTs were preferentially maintained in solution of absorbed by the biotic compartment.

The mass balances obtained were calculated for the first day, and after the first and second week of exposure to illustrate the fate of butyltin compounds in the model ecosystems. For the abiotic microcosms, recoveries expressed as percentage of TOT amount determined for the whole system to that of spiked TBT reached on average 111%, 117% and 98% for the first day, the first week and the second week of experiment, respectively. Lower recoveries were obtained from the biotic systems. Only 69, 76 and 65% of the TBT introduced in the water column could be recovered in the different compartments after the first day and the first and second week of incubation. These values could either result from analytical underestimation with incomplete extraction efficiency and more specifically in the biotic aquaria. These incomplete recoveries could also be due other mechanisms contributing to the lower recoveries in the biotic aquaria. One of the processes to be mentioned is the potential volatilisation route via the formation of volatile $Me_4Sn$ or methylated butyltin species which could evade from the aquaria. Another route could also promote the complete degradation of TBT down to inorganic tin. The volatilization mechanisms should certainly be taken into account since in previous studies making reference to marine algae (*Enteromorpha sp.*) under both aerobic and anaerobic conditions and in the presence of inorganic tin resulted in significant loss of inorganic tin via volatilization as $Me_4Sn$ (Donard et al. 1987) or $SnH_4$ and associated methylated by products (Donard and Weber 1988). Despite of its occurrence, losses of volatile organotin compounds derived from the flux calculations did not appear to represent a significant transfer process in such closed and low disturbed model ecosystems. The volatile fraction only acted for 0.001% of the TOT budget. Nevertheless, the production of volatile methylated organotin compounds (i.e. $Me_4Sn$ and $Bu_3SnMe$) illustrated the complexity of the reconstituted ecosystems and evidenced the relevance of the studied microcosms with respect to the natural tin cycling.

The time course evolution of the TOT burdens in each compartment confirms that the sediments represented the main sink for butyltins in the abiotic microcosms and that particles played only a minor role in TOT distribution over time (Figure 1).

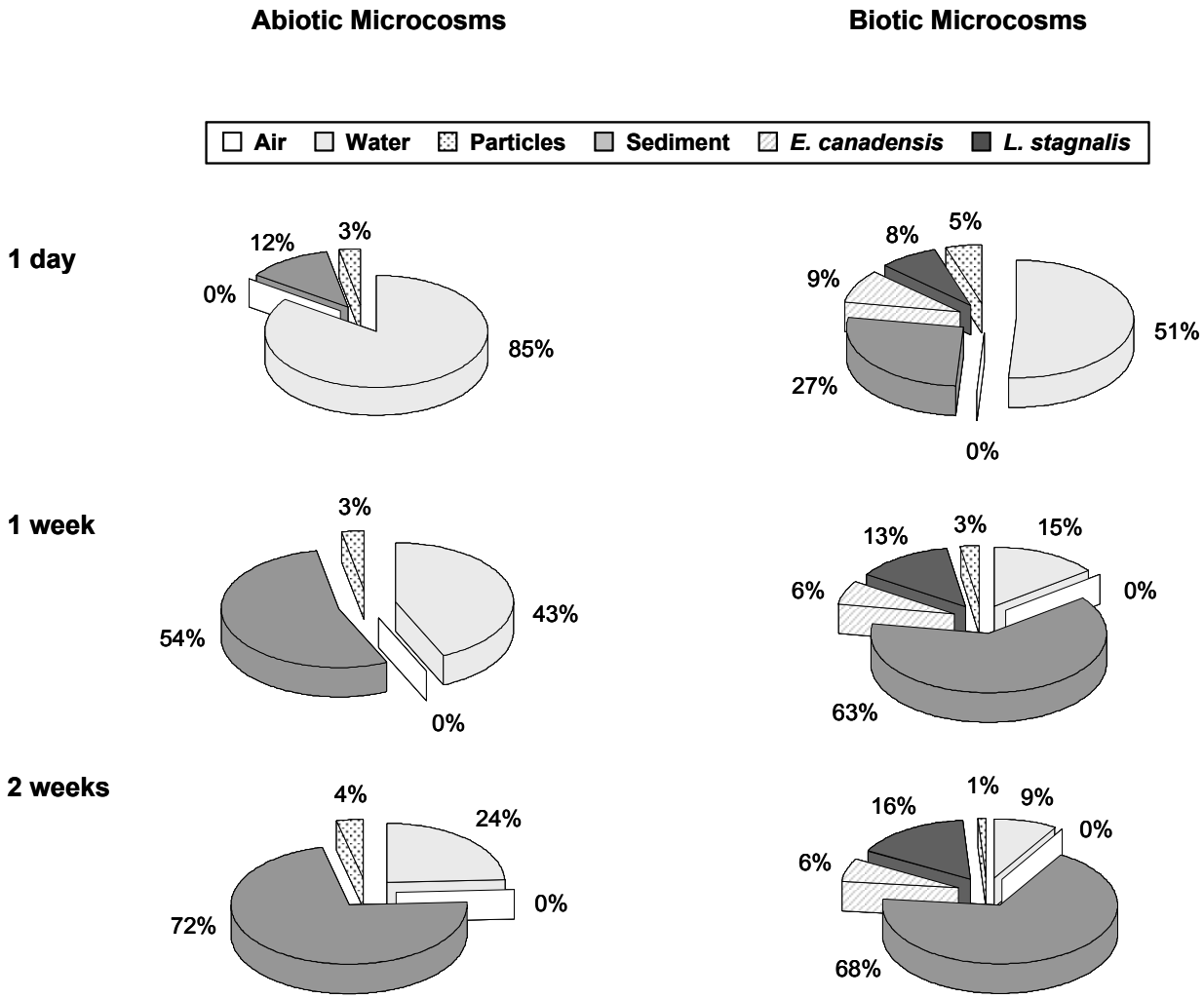


***Figure 5 -*** *Total butyltin burden distribution in the different compartments of the biotic and abiotic microcosms after 1 day, 1 week and 2 weeks of exposure*

As observed from the $K_d$ calculations, the TBT displayed a rather low affinity with particulate matter. For the whole contamination period, the TOT amounts determined on the particles represented on average 3.2 ± 0.4% of the total burden among which 82 ± 6% corresponded to TBT. After two weeks of exposure, 72% of the TOT burden was recovered in the sediment and 24% was still present in the water column. These results clearly demonstrate that the degradation of TBT via sequential debutylation is not the only route for butyltin removal in aquatic environments. The partitioning of the different butyltins in the different compartments of the biotic microcosms pointed out that biota represents an important sink for TBT in the first days of exposure. *L. stagnalis and E. canadensis* recovered respectively 8 and 9% of the total TOT budget after 24 hours of experiment. After, the proportion of TOT in the biota increased up to 16% after two weeks of exposure in *L. stagnalis*, while it decreased up to 6% in *E. canadensis*. The proportion of TBT metabolites as degradation products relatively to the TOT burden in the biota revealed also significant differences between snails and macrophytes. After two

weeks of exposure, the TBT metabolites represented 44% of the TOT burden measured in the snails, whereas in the macrophytes MBT and DBT accounted only for 27% of the TOT burden. These results illustrate the different bioaccumulation pathways and associated metabolisms adopted by the snails and the macrophytes in response to the TBT contamination and will be discussed later in the text in terms of preferential reactional pathways. Rapid biosorption on the macrophytes seems to be the first and privileged transfer process occurring in the biotic microcosms during the first hours of experiment. From the total biomass of macrophytes introduced in the biotic microcosms, a surface area of 7530 $cm^2$ can be derived by applying the mathematical relationship developed by Sher-kaul et al. (1995). Thus *E. canadensis* are likely to act as a significant interface for TBT transfer in the microcosms. This process appeared to be fast and reversible and corresponds to the fast removal rate of TBT from the water column as recorded during the first day of exposure. After 24 hours of exposure and until day 3rd, the TOT distribution in waterweeds contributed to the efficient removal of TBT reaching up to 35% of the maximum accumulated. For snail populations, accumulation of TBT was slower and reached a maximum only after three days of exposure. On the contrary to the results obtained with the waterweeds, the TBT elimination processes appeared to be less efficient despite of the fact that a significant amount of the contaminant was immediately uptaken by the biota compartment, the sediment remained the major and privileged sink for butyltins even in the biotic microcosms. Indeed, under these conditions the sediments trapped 27, 63% and 68% of the TOT burden after 24 hours, one week and two weeks of exposure respectively (Figure 1). Furthermore, TBT degradation pathways in sediments in both biotic and abiotic microcosms appeared to represent a key process in TBT cycle. The respective proportion of TBT derivatives (DBT, MBT) to the TOT load in sediment, must likely mainly originating from microbial degradation, continuously increased and reached on average 48 ± 2% after two weeks of exposure. These results emphasis the importance of TBT biotransformation processes in the reconstituted model ecosystems and the key role of sediments in the fate of contaminants. Specific bioaccumulation pathways as reactional pathways (concentration, degradation) will be discussed and modelled below assessing in detail the role of each compartment with regards to the fate of TBT in such systems.

### 3-3- Butyltins distribution in water

*Distribution in the dissolved phase*

The organotins background measured in the water column before the spike was found to be under the detection limits in the five microcosms for both dissolved and particulate phases. Furthermore, the water concentrations in the control microcosm (AQ3) remained negligible and below the detection limits during the whole incubation period. The time courses evolution of TBT, DBT, MBT and total organotin (i.e. TOT: sum of butyltin compounds)

concentrations in filtered water for biotic (AQ1 and AQ2) and abiotic (AQ4 and AQ5) experiments are described in Figure 2.

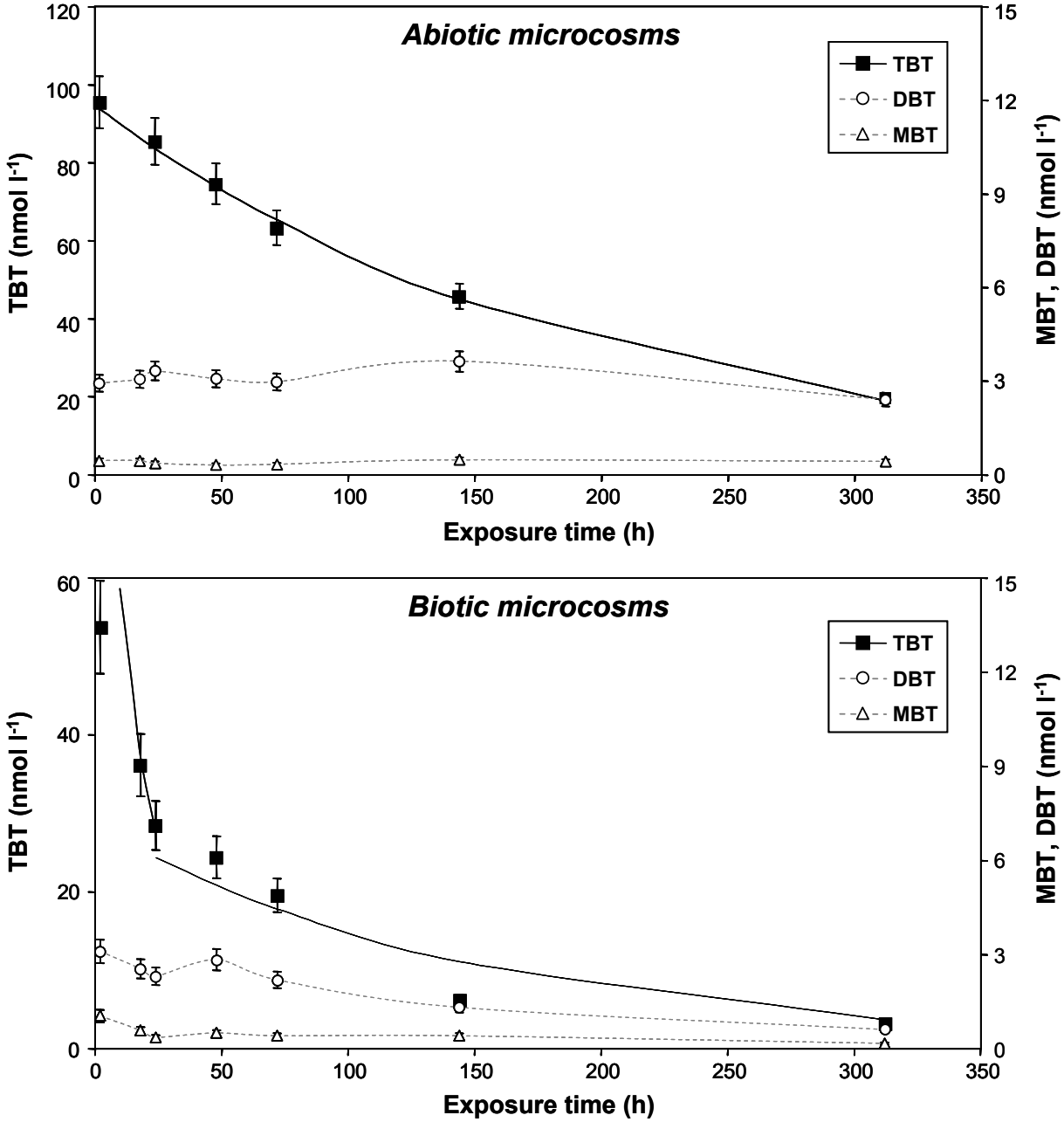


***Figure 2 –****Experimental and predicted (solid lines) distribution of butyltin concentrations in filtered waters of the abiotic and biotic microcosms. Errors bars represent the mean deviation obtained from duplicate microcosms*

Similar organotins concentration patterns were observed in both biotic and abiotic duplicate microcosms. The TBT concentrations in the filtered water samples of the AQ1 and AQ2 partitioned rapidly in the other compartments to reached a slow decrease averaging 28 nmol $l^{-1}$ (i.e. 32% of the initial spike) after the first day of exposure. Then, the rate of removal became slower. The TBT contents decreased to 6 nmol $l^{-1}$ after the first

week of incubation and stabilized around 3 nmol $l^{-1}$ after the second week (i.e. 7 and 4% of the initial spike, respectively). In the sediment-water microcosms, the TBT contents in the filtered water samples decreased much slower than in the biotic systems and the remaining concentrations were 86 nmol $l^{-1}$ after the first day of exposure and 56 nmol $l^{-1}$ after the first week (i.e. 57 and 87% of the initial contamination, respectively). Significant concentrations of TBT degradation products were observed after the first hours of exposure in all microcosms. The MBT concentrations in both biotic and abiotic microcosms remained constant and averaged 0.4 ± 0.1 nmol $l^{-1}$ during the whole period of incubation. The DBT concentrations in the abiotic systems averaged 3.1 ± 0.4 nmol $l^{-1}$ during the entire exposure period and 2.7 ± 0.3 nmol $l^{-1}$ in the biotic systems after the first 48 hours of incubation. The DBT concentrations then decreased to 0.6 nmol $l^{-1}$ after two weeks of incubation.

Small amounts of dissolved volatile organotin species were also measured during the whole incubation period in both biotic and abiotic systems. Organotin species such as tetramethyltin ($Me_4Sn$) and tributylmethyltin ($Bu_3SnMe$) were identified in unfiltered water samples. The $Me_4Sn$ concentrations averaged 0.52 ± 0.08 fmol $l^{-1}$ in all aquaria whereas the $Bu_3SnMe$ concentrations reached 1.4 ± 0.1 and 17.3 ± 1.9 fmol $l^{-1}$ in the biotic and abiotic microcosms respectively.

*Distribution in suspended particulate matter phase*

Neither TBT nor its degradation products (DBT and MBT) were detected in the particulate samples of the control microcosm during the exposure period. In aquaria AQ4 and AQ5, the TBT contents in the particles rapidly increased after two hours of exposure. After, the concentrations did not show any further significant variation and averaged 260 ± 64 nmol $g^{-1}$ and 176 ± 41 nmol $g^{-1}$, respectively. Similar patterns were observed for DBT but with concentrations 10 time lower reaching 29 ± 6 and 18 ± 4 nmol $g^{-1}$ in aquaria AQ4 and AQ5, respectively. Finally, the MBT concentrations were 20 times lower than for TBT and continuously increased from 6 to 23 nmol $g^{-1}$ in AQ4 and from 13 to 57 nmol $g^{-1}$ in AQ5.

The biotic microcosms revealed more variable behaviours. The overall TBT and DBT concentrations in AQ1 decreased from 179 to 50 nmol $g^{-1}$ and from 86 to 14 nmol $g^{-1}$ after two weeks of exposure, while the MBT concentrations first increased from 27 to 45 nmol $g^{-1}$ during the first three days of incubation and then decreased down to 17 nmol $g^{-1}$. On the contrary, the butyltin contents measured in AQ2 did not present any significant variations over the same period and averaged 65 ± 18, 8 ± 2 and 7 ± 1 nmol $g^{-1}$ for TBT, DBT and MBT, respectively. The significant differences in term of concentrations range observed in AQ1 and AQ4 when compared to AQ2 and AQ5 have to be related to the butyltin amounts available for partitioning in the dissolved phase as well as to a dilution effect due to the SPM load in each microcosm.

### 3-4- Butyltins distribution in sediments

After the to the grain size partitioning studies, 89% of the total organotins contents in the sediment (i.e. TOTs: sum of MBT, DBT, and TBT concentrations) were associated with the fine fraction of the sediment (<300 µm). For the silt and sandy fractions of the sediment (i.e. 300-2000 µm and >2000 µm) only 10% and 1% of the TOTs contents could be recovered. Therefore, most of the butyltins concentrations in sediments discussed below will make reference to the fine grain size fraction. The background values of butyltins measured in the sediments before the spike in all aquaria and throughout the incubation of the control microcosm reached on average 5.2 ± 1.8, 6.9 ± 1.6 and 8.4 ± 2.6 pmol $g^{-1}$ (fw) for MBT, DBT and TBT respectively. The average butyltin concentrations for both biotic and abiotic conditions and their distributions with regards to the time of exposure are displayed in Figure 3. The two sets of experimental conditions exhibited similar distribution patterns for the three organotin compounds. First, the TBT concentrations increased during the first week of exposure and reached a maximum of 239 pmol $g^{-1}$ and 405 pmol $g^{-1}$ in both biotic and abiotic microcosms. Then, between the first and the second week of incubation, the TBT contents decreased and reach a plateau value of 150 and 280 pmol $g^{-1}$ in biotic and abiotic systems. Simultaneously, the MBT and DBT concentrations continuously increased in all aquaria, with an apparent higher accumulation or production rate in the abiotic microcosms. After two weeks of exposure, the MBT and DBT contents appeared to reach equilibrium in the abiotic microcosms and both OTs species stabilized around 150 pmol $g^{-1}$. In contrast, the TBT degradation products concentrations in the biotic systems did not display any plateau trend and still increased after 23 days of exposure. The maximum concentrations observed were 67 ± 12 pmol $g^{-1}$ for MBT and 116 ± 17 pmol $g^{-1}$ for DBT.

### 3-5- Butyltins distribution in the biota

*Bioconcentration of TBT*

The butyltins concentrations and distribution over time in the waterweeds (*Elodea canadensis*) and the snails (*Lymnaea stagnalis*) are displayed in Figures 4 and 5. Prior to the contamination, the butyltins background in the snails and the waterweeds remained below the detection limits. No detectable level of butyltins in the biota could be recorded in the control microcosm during the exposure time.

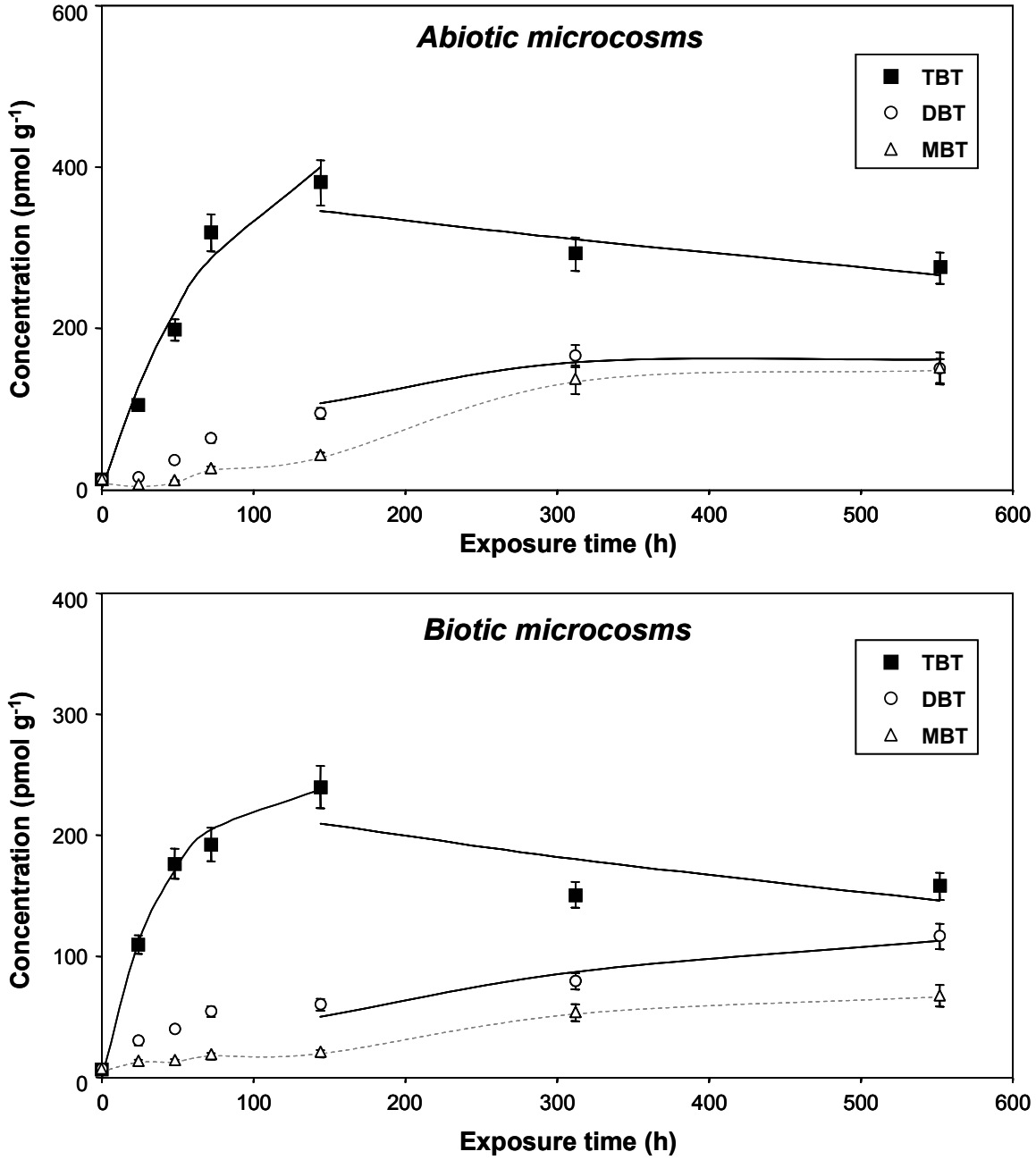


***Figure 3 –****Experimental and predicted (solid lines) distribution of butyltin concentrations in sediments of the abiotic and biotic microcosms. Errors bars represent the mean deviation obtained from duplicate microcosms*

Furthermore, the biotic microcosms realized in duplicate exhibited very similar time course evolutions for the butyltins concentrations for both the snails and the waterweeds.The TBT rapidly accumulated in the biota during the early stages of the exposure. A maximum in the TBT concentration was recorded after 24 h. in the waterweeds, 48 h. in the shell of the snail and after 72 h. in the snail tissues with average values of 4.8 ± 0.1, 1.9 ± 0.1 and 7.2 ± 0.5 nmol $g^{-1}$ expressed in fresh weight. After, the TBT decrease from the biotissues exhibited different patterns. The TBT concentrations in the *Elodea* diminished rapidly down to 3.1 nmol $g^{-1}$ from day 1 to day 3 and then continued with a slower removal rate. The final TBT concentrations reached 2.2 ± 0.2

nmol $g^{-1}$ after two weeks of exposure. The DBT contents in the waterweeds increased from non detectable level to a maximum of 1.0 ± 0.1 nmol $g^{-1}$ after 3 days of exposure and then decreased down to 0.5 ± 0.1 nmol $g^{-1}$ until the end of the incubation. The MBT concentrations on the other hand did not present significant variations during the exposure period and represented on average 10% of the TOT contents. The MBT concentrations stayed around 0.5 ± 0.1 nmol $g^{-1}$ during the first week of exposure and then decreased slightly down to 0.3 nmol $g^{-1}$.

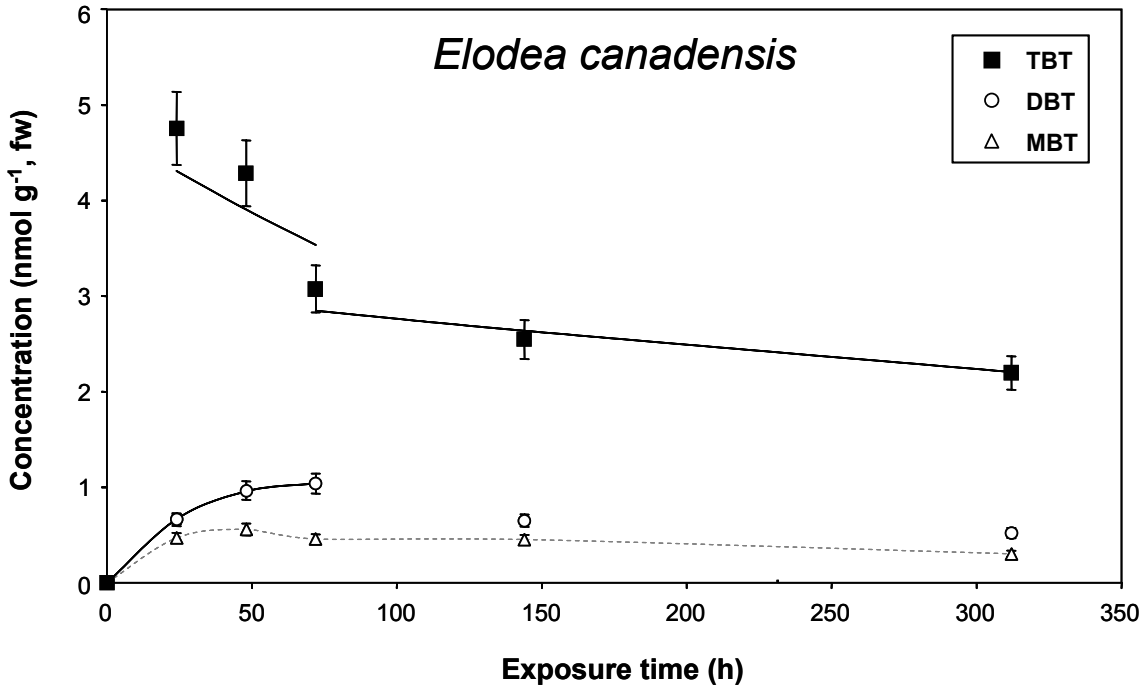


***Figure 4 –****Experimental and predicted (solid lines) distribution of butyltin concentrations in Elodea canadensis Errors bars represent the mean deviation obtained from duplicate microcosms*

The TBT distribution in the snail tissues and the shells was investigated separately to discriminate between sorption and bioconcentration processes. The butyltins fraction associated with the shells represented on average 20% of the TOT contents measured in the whole body. After two days of exposure, the TBT concentrations in the shells averaged around a plateau value of 1.6 ± 0.4 nmol $g^{-1}$ whereas the DBT and MBT levels increased continuously from non detectable levels to 0.73 ± 0.04 and 0.47 ± 0.03 nmol $g^{-1}$ respectively (data not shown). The proportion of TBT degradation products with regards to the TOT contents measured in the shells samples increased from 2 to 15% for MBT and from 10 to 23% for DBT during the entire exposure period. The TBT degradation or desorption mechanisms on the shell surface could account for the observed distribution

as well as adsorption of MBT and DBT from the water column. In the case of the snail tissues, the accumulation maximum was reached later than that obtained for the shells which confirms the differential pathways involved with TBT accumulation. TBT removal was then slower and concentrations slightly decreased from 7.2 to 5.3 nmol $g^{-1}$ whereas the TOT contents remained constant suggesting the occurrence of metabolisation mechanisms of TBT degrading it into DBT and MBT. The DBT concentrations in the tissues increased from non detectable levels to 2.1 ± 0.1 nmol $g^{-1}$ during the first week of exposure to stabilize around 2.4 ± 0.1 nmol $g^{-1}$ during the second week of experiment. During the same period, the MBT contents increased continuously up to 1.9 ± 0.1 nmol $g^{-1}$.

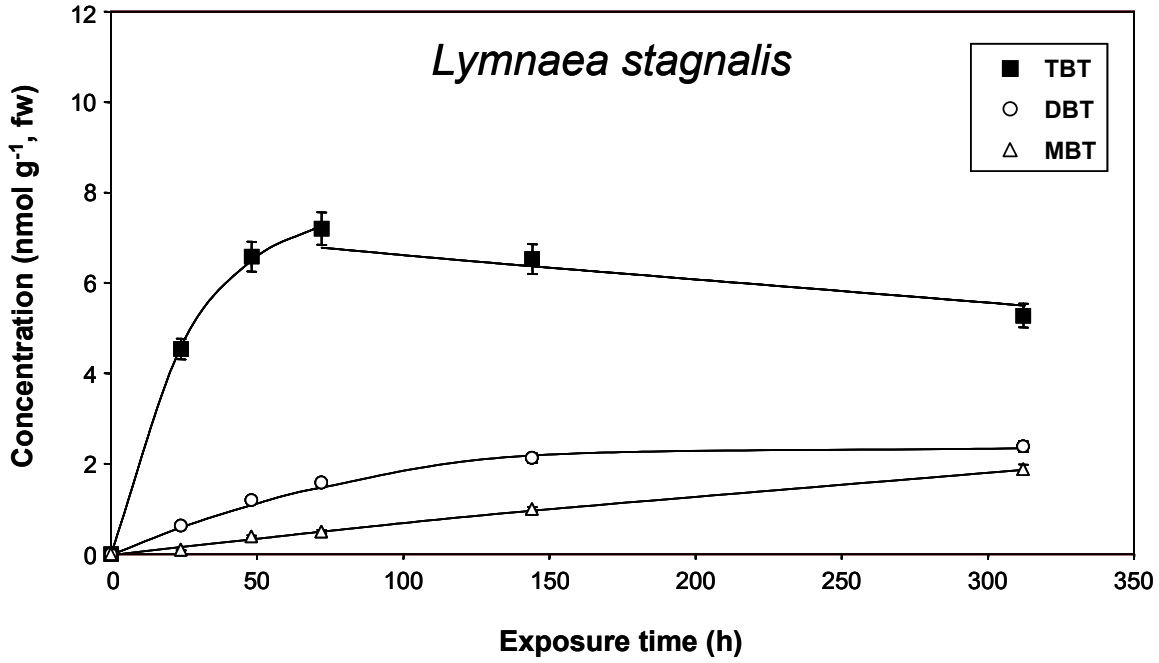


***Figure 5 –****Experimental and predicted (solid lines) distribution of butyltin concentrations in Lymnaea stagnalis Errors bars represent the mean deviation obtained from duplicate microcosms*

**3-6- Kinetic study: transformation and transfer mechanisms of butyltins**

The different rate constants of butyltin transfer and transformation pathways in water, sediments and biota of the studied microcosms are presented in Table 2.

*Degradation kinetics of TBT in filtered waters*

The TOT concentrations in the water column follow exactly the same time course evolution than for TBT in all microcosms. The TBT concentrations represented on average 90% and 95% of the TOT during the first week of exposure in both biotic and abiotic systems. During that time, the degradation products of TBT displayed constant concentrations over time. The respective proportions of MBT and DBT with regards to the TOT contents respectively increase up to 4% and 16% in the biotic microcosms and up to 2% and 11% in the abiotic ones. This result could be attributed to biodegradation by microorganisms which are assumed to play a major role in the breakdown of TBT in natural waters (Dowson et al., 1996; Cooney, 1988). This low degradation yield may be attributed to the high DOC contents, complexing the TBT present in solution or the fact that the biotic activity is reduced by the initial toxicity of the TBT injected in the aquaria (Waite et al., 1989; Looser et al., 1998; Rüdel 2003). Overall, TBT degradation in the water column did not appear to represent a significant mechanism under our experimental conditions. The changes in the TBT concentrations in the water appear to be mainly driven by the partition processes between the other compartments.

The observed breakdown of TBT concentrations in the water column over time can be described by using a first order exponential decay kinetic. The predicted distribution of TBT water concentrations in both types of microcosms are presented in Figure 2. In the abiotic systems, an average removal rate constant of 0.0052 ± 0.0006 $h^{-1}$ (half-life 5.6 days) was obtained for the entire exposure period. On the contrary, in the biotic microcosms, the TBT removal from the water was best described by using two successive first order exponential decay kinetics corresponding first to a fast removal of TBT during the first 24 hours and followed by a slower elimination rate starting from the first day after contamination up to the end of the exposure experiment. For the first kinetic, a rate constant of 0.048 ± 0.008 $h^{-1}$ (half-life, 14 hours) was obtained. The rate then slowed down to 0.0065 ± 0.0005 $h^{-1}$ (half-life, 4.4 days). The latter value is similar to the one obtained in the abiotic microcosms, suggesting the occurrence of similar removal processes delayed in time. The first kinetic rate can be related to rapid biosorption processes mainly through adsorption on the surface of the waterweed leaves. It we take into account the negligible biodegradation of TBT in water under our experimental conditions, the second mechanism appears to result mainly from TBT partitioning and adsorption in the sediments as showed in the abiotic microcosms.

The removal rate constants of TBT from the water recorded in the literature display a wide range of variations depending on the type of breakdown processes induced by our experimental conditions (Waite et al., 1989). Half-lives of TBT removal in natural waters, mainly via biodegradation pathways, ranged from several days to several weeks. (Maguire et al, 1985; Selingman et al., 1986; Waite et al., 1989; Ma et al., 2000). Adelman et al. (1990) reported a total removal rate of TBT in enclosed marine ecosystems to be of 0.008 $h^{-1}$ (half-life, 3.5 days) and obtained a scavenging rate of 0.002 $h^{-1}$ which is consistent with our transfer rates of TBT to the sediment calculated for both biotic and abiotic microcosms.

***Table 2 –*** *Transformation and transfer kinetics of butyltin in the studied microcosms*

| Compartment | Process | Rate constant | | Duration (h) |
|---|---|---|---|---|
| | | Abiotic microcosms | Biotic microcosms | |
| Water | TBT removal | | 0.048 ± 0.008 $h^{-1}$ | / 0–312 h |
| | | 0.0052 ± 0.0006 $h^{-1}$ | 0.0065 ± 0.0005 $h^{-1}$ | 0–24 h / 24–312 h |
| Sediment | TBT accumulation | 0.0061 ± 0.0005 $h^{-1}$ | 0.017 ± 0.008 $h^{-1}$ | 0-144 h |
| | TBT removal | 0.0008 ± 0.0001 $h^{-1}$ | 0.0009±0.0002 $h^{-1}$ | 144-312 h |
| | TBT degradation | 0.0027 ± 0.0009 $h^{-1}$ | 0.0018 ± 0.003 $h^{-1}$ | 144-312 h |
| *L. stagnalis* | TBT accumulation | - | 9.3 ± 2.3 ml $g^{-1}$ $h^{-1}$ | 0-72 h |
| | TBT depuration | - | 0.0009 ± 0.0001 $h^{-1}$ | 72-312 h |
| | TBT degradation | - | 0.0039 ± 0.0002 $h^{-1}$ | 72-312 h |
| | DBT accumulation | - | 14.3 ± 0.4 ml $g^{-1}$ $h^{-1}$ | 0–312 h |
| | MBT accumulation | - | 16.3 ± 1.1 ml $g^{-1}$ $h^{-1}$ | 0–312 h |
| *E. canadensis* | TBT accumulation | - | 6 $ml^{-1}$ $g^{-1}$ $h^{-1}$ | 0–24 h |
| | TBT removal | - | 0.0051 ± 0.0003 $h^{-1}$ | 24-72 h |
| | TBT metabolization | - | 0.0011 ± 0.0001 $h^{-1}$ | 72-312 h |
| | TBT degradation | - | 0.0082 ± 0.0005 $h^{-1}$ | 0-72 h |

*TBT partitioning between water and suspended matter*

When referring to the total TBT concentrations determined in bulk waters over the entire incubation time, 90% and 95% was present in the dissolved phase for the biotic and abiotic systems. This partition also agrees with the previous investigations reported on TBT partitioning in natural waters by Fent (1996) and Maguire (1996). TBT partitioning is strongly influenced by physicochemical properties of both dissolved and solid phases, among which parameters such as pH, particulate and dissolved organic matter contents as well as the mineralogical composition of the sorbent are of primary importance (Weidenhaupt et al., 1997; Arnold et al., 1998; Hoch et al., 2004). The partition coefficient (*K*d, l $kg^{-1}$) (i.e. the ratio between TBT concentrations in particles and water) gives an estimate of the relative affinity of TBT for the particulate phase. During the experimental period, the *K*d values for TBT ranged from $13\times10^3$ to $25\times10^3$ l $kg^{-1}$ in the biotic experiments and from $7\times10^3$ to $14\times10^3$ l $kg^{-1}$ in the abiotic ones. The published *K*d values for TBT on natural solid phases may vary by several orders of

magnitude from $10^2$ to $10^6$ l $kg^{-1}$ (Unger et al., 1988; Fent, 1996). The carbon contents in sediment and particulate material as well as the characteristics of the clay particles are likely to enhance the TBT sorption. Hoch et al. (2004) have demonstrated that a 50-fold increase of TBT adsorption could be evidenced when a total content of 5% organic matter was enriched on kaolinite particles. In our system, the level of DOC in the water column certainly binds the TBT or allows a partition of TBT in the dissolved organic carbon and prevent its sorption on the particles (Looser et al., 1998; Arnold et al., 1998) When complexed by organic matter in solution, TBT mostly maintained in the dissolved phases and also is prevented from degradation mechanisms. Adsorption equilibrium is reached within the first hours of contamination and no significant changes in butyltins distribution in particles are observed after.

*TBT partitioning between water and sediments*

After the first week of incubation, the TOT contents in the sediment remained constant under both biotic and abiotic experimental conditions and displayed a conservative behaviour at this time scale. The overall butyltin concentrations measured in the sediments were 2 times lower in the biotic microcosms than in the abiotic ones. This result was not unexpected due to the role of the biota in the uptake and sequestration of organotins both in the water column and in the sediments. Indeed, in the water column of the biotic microcosms, the remaining amount of TBT available for diffusion into the sediment was found to be 2 to 3 times lower than in the abiotic systems at the early stages of the exposures. Since no significant increase in the butyltin concentrations was recorded in the water columns after the first week of experiment, the TBT breakdown determined in the sediments must be driven by in situ biodegradation mechanisms. The net transfer of butyltin compounds from the sediment to the overlying water appears to be negligible. As previously mentioned for the suspended particles, hydrophobic partitioning mechanisms in the sediment should be the major sorption mechanism. However, on the contrary to what has been observed for the particles, the apparent distribution coefficients obtained were significantly lower and averaged 48 and 15 l kg-1 in the biotic and abiotic microcosms respectively. The partition coefficients obtained from these experiments are relatively low when compared to published values (Unger et al., 1988; Kram et al., 1989; Langston and Pope; 1995). This may be due to the incomplete partition equilibrium during the time of the experiment and simultaneous rapid degradation of TBT into DBT and MBT. Further, in our experimental closed systems, the high solid/solution ratios (about 250g l-1) could also explain the low Kd values obtained. Several authors have reported that TBT partitioning is drastically affected by the solid/solution ratio, resulting in decreasing Kd with increasing solid amounts (Unger et al., 1988; Langston and Pope, 1995; Harris et al., 1996). Hoch et al. (2004) studied the influence of the solid/solution ratio on TBT adsorption on Kaolinite at pH 6 and obtained similar Kd values ranging from 64 to 33 l kg-1 for high amounts of solids (20-100g l-1) due to both a dilution effect and a saturation of the sorbent. TBT partitioning between the different compartments of the biotic systems led to lower remaining water concentrations and thus

in higher Kd values, when compared with the abiotic systems. Finally the low turbulent conditions occurring in the microcosms regulating the water circulation and water-sediment interface perturbation due to the sampling, could also have contributed to limit exchanges between the water and sediment compartments, and to constrain them to simple passive diffusion processes leading to low distribution coefficients.

*Accumulation kinetics of TBT in sediments*

After the initial introduction of the contaminant as TBT, the OT rapidly partitioned in the sediments**.** This accumulation step was fitted by using a one compartment first-order kinetic model (Meador, 1997) with the assumption of a constant TBT concentration in the water during the first week of experiment. Despite of the fact that this requirement was not completely achieved in the experimental systems, the high remaining TBT concentrations in water did not appear to be a limiting factor for its accumulation in sediments in the early stages of the experiments. The geometrical mean of TBT levels in water during the first week was then retained for the calculations. During this period, the accumulation rate constants obtained in the abiotic microcosms averaged 0.0061 ± 0.0005 $h^{-1}$. This value is very similar to the rate constants previously obtained for the TBT removal from water. In the abiotic microcosms, the TBT distribution appears to be mainly driven by direct partition between water and the sediments.

In the biotic aquaria, the accumulation rate constant of TBT to the sediment were significantly higher and averaged 0.017 ± 0.008 $h^{-1}$. This significant difference could be attributed to additional contribution of the biota (E. Canadensis) which would actively absorb TBT from the water column and contribute to its transfer in the sediments. Reversible accumulation of metals by submerged macrophytes has indeed been previously reported by Everard and Denny (1985). These authors found that 90% of unbound lead taken up by *E. canadensis* in the first hour of exposure was first rapidly uptaken and returned to the water column within 14 days. However, further to their active uptake and transfer to sediment, the total amount of TBT accumulated by the waterweeds still remained low when compared to the quantities accumulated in sediment. The waterweeds appeared to induce a rapid passive transfer mechanism for TBT accumulation in the sediments with very low direct bioaccumulation by the plant themselves.

The maximum adsorption capacity of the sediment in the biotic aquaria was also probably reached faster than in the abiotic systems as evidenced by the lower TBT concentrations recorded in the filtered water samples. These biotic microcosms offered a more favourable media for the growth and diversification of micro-organisms in both water column and sediment. Under these experimental conditions, the biodegradation of TBT was certainly initiated earlier than in the abiotic systems. This will make the direct comparison of reaction kinetics between the 2 types of systems difficult since they are not fully synchronized on time.

*Degradation kinetics of TBT in sediments*

During the second week of exposure, the TBT concentrations were directly affected by degradation processes resulting in the simultaneous increase of its degradation metabolites DBT and MBT. Biologically induced mechanisms, involving micro-organisms can lead to successive debutylation reactions. These pathways have been demonstrated to be the most important route for TBT degradation in freshwater sediments (Dowson et al., 1996; Fent, 1996; Maguire, 1996). Under our experimental biotic and abiotic (with respect to macro-organisms) conditions, no major differences in TBT degradation and metabolites production could be evidenced when the concentration detected were normalized to the maximum amount of TBT accumulated in sediments. Indeed, after the first week of exposure, the average butyltin loads in the sediment compared to the TOT contents were 19%, 31% and 50% in the biotic microcosms and 24%, 27% and 49% in the abiotic ones, for MBT, DBT and TBT respectively. A simple first order exponential decay approach was then fitted to the data to describe the TBT losses in sediment when assuming a negligible resuspension and back transfer of TBT from sediment to water during the second week of incubation. The biodegradation rate constants obtained were 0.0008 ± 0.0001 $h^{-1}$ (Half-life, 38 days) and 0.0009±0.0002 $h^{-1}$ (half-life, 32 days) in the abiotic and biotic microcosms respectively. These values are consistent with the biodegradation rate constants found in organic-rich sediments from laboratory microcosm experiments (Landmeyers et al., 2004). However, the half-lives of TBT in the sediment, derived from the biodegradation rates, are much lower than other values reported for fresh and saline water systems. In natural environments, TBT is generally assumed to be much more persistent in sediment with half-lives ranging from a few months to several years (Maguire, 1985; Watanabe et al., 1985; Dowson et al., 1996). These rates determined in reconstituted media are likely to overestimate the biotransformation processes since microcosm experiments represent aerobic static conditions and fixed initial TBT concentrations. Further, real ecosystems represent a dynamic mixture of both aerobic and anaerobic microbial processes and potential changes in TBT concentrations from additional inputs since TBT has slowly banned for use as antifouling agent. Further, the constant high operating temperature (20°C) during the whole experiments could also account for the enhanced biodegradation processes.

During the second week of experiment, similar degradation rate constants were obtained in the all sets of aquaria suggesting that the degradation of TBT in the sediments involved the same types of mechanisms for both the biotic and abiotic conditions. These results would suggest that TBT removal in the sediment appears to be mainly driven by *in situ* biodegradation pathways. The presence of macrofauna did not led to any further enhanced degradation via the potential release of reactive organic molecules or with respect to the greater associated microbial diversity and activity.

The degradation rates of TBT through successive debutylations steps were modelled by fitting the TBT decrease in sediment with two first-order reactions in series. Degradation rate constants of 0.0027 ± 0.0009 $h^{-1}$ and 0.0018 ± 0.003 $h^{-1}$ were obtained describing the transformation of TBT into DBT in both abiotic and biotic

microcosms. However, we could not fit any model to follow the degradation rate between DBT to MBT. If we can assume that the DBT distribution in sediment could be well predicted by the model used, it results that whole TBT degradation processes are largely overestimated since they will not take into account the direct degradation reactions from TBT directly to MBT. This specific reactional pathway has been evidence with several forms of microbes (Barug, 1981). Further, this MBT can also be directly transformed into inorganic tin under these experimental conditions.

*Tin methylation and evasion fluxes of volatile organotin compounds*

Reactional pathways do not only occur between sediment and biota but will also include exchanges between sediment, water and air. The determination of volatile organotin species allowed us to estimate these evasion fluxes of tin from the microcosms. A simple diffusion model at the air-water interface was applied to our data (Vandal et al., 1991). Flux densities calculated for the total volatile organotin concentrations measured in water (i.e. TVT, sum of $Me_4Sn$ and $Bu_3SnMe$) were 0.07 pmol $m^{-2}$ $h^{-1}$ and 0.35 pmol $m^{-2}$ $h^{-1}$ in the biotic and abiotic microcosms, respectively. These results are comparable to those reported by Tessier et al. (2002) for low impacted estuarine systems. The $Me_4Sn$ detected in the aquaria is likely to originate from methylation of inorganic tin whereas the $Bu_3SnMe$ moieties are certainly produced through direct methylation of TBT (Maguire, 1984; Yozenawa, et al., 1994, Gilmour et al., 1985). Therefore, both degradation and biogenic methylaytion processes can be related to the formation of volatile and mobile tin compounds. Until the present sets of experiments they were mainly observed under reducing conditions in sediments (Amouroux et al., 2000; Yozenawa et al., 1994; Guard et al., 1981). Under our experimental conditions, simulating a non turbulent freshwater ecosystem, these conditions drastically limited the intensities of the volatilization processes. The determination of volatile tin compounds gives support the occurrence of active methylation mechanisms in the reconstituted freshwater ecosystems.

*Bioaccumulation of butyltins in Lymnaea stagnalis and Elodea canadensis*

Butyltin accumulation in biota was expressed as accumulation factors (AF) based on the following ratio: $[TBT]_{organism}/[TBT]_{water}$ (unit in ml water $g^{-1}$, fresh weight). This factor illustrates the potential of accumulation and affinity of an aquatic organism with regards to metals or molecules with respect to the equivalent quantity of water presenting the same quantity of entity considered (Baron et al., 1990). In our experiments, the accumulation capacities of TBT were found to be significantly higher for snails than for waterweeds. The AF values for MBT, DBT and TBT calculated after two weeks of exposure were 10711, 3874 and 1674 in *Lymnaea* and 1754, 851 and 696 in *Elodea* respectively. The important differences observed between the different compartments should be related to the specific and differential TBT metabolisms in the species studied. *Elodea* is a freshwater macrophyte, living completely submerged, adsorbing its mineral elements directly from the

aquatic medium through its wide leaf surfaces exchange capacities (Eugelink, 1998). Adsorption and absorption via the vegetation has been reported to represent an important removal process of TBT from water (Fent, 1996; Jensen et al., 2004). Further, if *Elodea* is likely to rapidly absorb TBT from the water via passive diffusion processes (Fent, 1996), it will also be likely to release some of the moieties absorbed in a significant proportion resulting in lower AF values. Similar results were also observed by Pflugmacher et al. (2000). They also reported on the efficiency of *E. canadensis* in accumulating TBT with significant impacts on its enzymatic system activity. It is well known that TBT exposure induces a significant inhibition of the cytochrome P-450 activity, a phase II detoxication enzyme system (glutathione S-transferase) which is in turn stimulated, resulting in enhanced metabolisation of TBT to DBT and MBT. The overall TBT removal over the whole exposure period averaged 54 ± 4% of the maximum TBT content accumulated in the waterweeds and only 27 ± 1% in the snails. The elimination of TBT from *Elodea* appears to follow a 2 steps kinetics corresponding first to a fast reversible biosorption/desorption process and second to a slower elimination of assimilated TBT through detoxification metabolisms. These results are consistent with previous studies reporting that *Elodea* not only absorbs heavy metals, but also releases them into the water column when they decay (Kähkönen and Manninen, 1998) and may even release them from living tissues (Everard and Denny, 1995).

Further to the absorption,desorption releases observed from the plant compartment, the presence of TBT drastically affected the snail population present in the aquaria. Indeed, the endocrine disrupting effect of TBT leading to the inhibition of detoxication enzymatic systems, such as the cytochrome P-450, have also been reported in various aquatic organisms such as fishes, bivalves and gastropods (Fish et al., 1976; Fent and Stegeman, 1993; Morcillo and Porte, 1997; Matthiessen and Gibbs, 1998). Freshwater gastropods are well known to be specifically and drastically impacted by organotins at low water concentrations. The RIVM (1989) reported a NOEC for *Lymnaea stagnalis* of 0.32 µg $l^{-1}$ for long-term tests and acute toxicity levels for adult snails are estimated to range from 30 to 400 µg $l^{-1}$ (WHO IPCS, 1990). Generally, the toxicity of TBT to freshwater snails depends on the species of snail considered, the age, the temperature, the pH, the exposure to water concentration and the length of time of exposure. The biodisponibility, bioavailability or uptake capabilities of the toxicant are also directly linked to its chemical speciation. TBT in the organisms can also be adsorbed via the suspended matter with is another routes of uptake since particles are ingested by the snail (Cardarelli and Evans, 1980). In the present study and our experimental conditions, the partitioning of TBT between the predominant compartments (i.e. water and sedimentary material) has led to induce high levels of exposure likely to induce acute toxicity conditions to the snail populations. Despite of the fact that we could describe a TBT metabolisation in snails, the full depuration processes were probably inhibited due to high remaining TBT concentrations in exposure water. This could in turn explain the higher accumulation factors found in *Lymnaea*.

*Uptake and elimination kinetics of TBT in Lymnaea stagnalis*

The uptake and elimination kinetics of TBT calculated in the snail population (*Lymnaea stagnalis*) do not allow to discriminate between 2 different types of models. Since degradation products of TBT such as DBT and MBT were very low in the tissues of the snail population studied in our experimental conditions, 2 types of approaches can be used. The first one will assume that the debutylation processes are minimal and negligible. The model will then assume only direct bioaccumulation-depuration processes. The second type of model will take into account the different metabolization moieties such as DBT and MBT, even if they are low and present the kinetics of slow metabolisation of TBT in this biotic compartment.

Bioaccumulation-depuration model: A first accumulation of TBT in snail tissues can be fitted to the results obtained during the first 72 hours of exposure using a simple first-order kinetic model (Fent & Looser, 1995). This model considers that the body burden of TBT in *L. stagnalis* behaves like a kinetically homogeneous unit. This approach enables the rate constants for uptake ($k_U$) to be calculated (**Table 2**). The bioconcentration curves can be plotted (Figure 5) to predict the TBT concentrations at different exposure times. The predicted TBT concentrations obtained are in good agreement with the experimental measurements ($R^2$= 0.999) indicating that the first-order kinetic model is fully applicable to the bioconcentration of dissolved TBT in *L. stagnalis*. The uptake rate constants for TBT accumulation in the snail tissues averaged 9.3 ± 2.3 ml $g^{-1}$ $h^{-1}$ from the duplicate biotic experiments. Despite of the fact that these accumulation kinetics are species dependent, the calculated $K_U$ values obtained are in the same order of magnitude of previous published values for other aquatic invertebrates (Fent and Looser, 1995; Meador, 1997). The breakdown products such as DBT and MBT were only present in low concentrations suggesting that the metabolization of TBT was slow under the experimental conditions. Therefore, the decay trend of the TBT contents in the tissues can also be described by first-order exponential kinetic and be attributed to a depuration process through direct TBT excretion. The resulting associated rate constants averaged 0.0009 ± 0.0001 $h^{-1}$ ($R^2$= 0.860) giving depuration half-lives ranging from 29 to 41 days and confirming the slow depuration of TBT in the snails.

Degradation model: The different modelling approach based on the fit of the time courses of DBT and MBT contents in the snail tissues after the first 72 hours of exposure could be fitted with two first-order elementary reactions in series. This modelling approach allows to integrate the fact that the evolution TBT burden on the organisms is related to the sequential debutylation process of TBT, possibly via detoxication metabolisms. Under our experimental conditions, only the first debutylation step of TBT into DBT could be fitted with the experimental data for DBT (Figure 5). The corresponding rate constant obtained was 0.0039 ± 0.0002 $h^{-1}$ ($R^2$= 0.955). This approach also leads to an overestimation of the TBT breakdown mechanisms but can be justified by the fact that steady state was still not reached. Since the TBT load was largely in excess in the water column compared to the accumulated snail body burden, it can be hypothesized that the snail compartment can accumulate TBT continuously faster than they can eliminate it.

The results obtained in our experimental conditions presented only low levels of DBT and MBT in the bodies of the *Lymnaea*. Further, the high toxic exposure concentration to which they were submitted as likely blocked the active detoxification metabolisms of the snails due to its acute toxicity. Since both DBT and MBT were also present in the water column via other degradation routes (from the sediments or via microbial degradation), the direct accumulation of the TBT derivatives such as DBT and MBT from the water column could account for the occurrence of these derivatives in snails. Therefore we believe that the first 1CFOK model based on the direct bioaccumulation-depuration allows the best description of the experimental data giving then passive accumulation rate constants of 16.3 ± 1.1 ml $g^{-1}$ $h^{-1}$ for MBT ($R^2$= 0.996) and 14.3 ± 0.4 ml $g^{-1}$ $h^{-1}$ for DBT ($R^2$= 0.999) (Figure 5). These findings support the hypothesis that TBT depuration in snails may only be a simple phase transfer process under our experimental conditions. The present calculated values are consistent with reported toxicokinetics parameters in various molluscs (Page et al., 1995; Gomez-Ariza et al., 1999) and illustrate the toxicity and persistence of TBT in benthic organisms.

*Uptake and elimination kinetics of TBT in Elodea canadensis*

The low resolution of the sampling and measurements of TBT contents in waterweeds during the early stages of exposure did not allow to fully describe the accumulation phase using the 1CFOK model. None the less, a $K_U$ value of 6 $ml^{-1}$ $g^{-1}$ $h^{-1}$ could be estimated after the first 24 hours of incubation. The fast TBT accumulation and subsequent breakdown in the *Elodea* compartment during the first 72 hours of exposure could be attributed to reversible adsorption/desorption mechanisms (Figure 4). The TBT removal trends in *Elodea* displays also a biphasic elimination process described previously following two first-order exponential decay kinetics. The two processes yield elimination rate constants of 0.0051 ± 0.0003 $h^{-1}$ and 0.0011 ± 0.0001 $h^{-1}$ corresponding to half-lives in this specific compartment of 6 and 26 days respectively. Then, the following slower decrease of TBT contents in the tissues could account for the sequestration of TBT within macromolecules and its result in the slow metabolization into DBT and MBT.

However, in this specific compartment, debutylation via metabolization could certainly be evidenced. Biological degradation of TBT by aquatic plant was first demonstrated by Pflugmacher et al. (2000). They investigated the effect of TBT on the enzymatic detoxification systems of *E. canadensis* with similar exposure concentration range. The results obtained demonstrated that the enzymatic systems were stimulated by the contamination and promoted the development of metabolization mechanisms leading to the formation of DBT and MBT after one week of experiment. In our case, the biphasic response observed for TBT removal from the *Elodea* compartment could illustrate the rapid depuration of the mobile TBT fraction adsorbed on the surface of the leaves. Compared to the data obtained on the snail populations, in the *Elodea* compartment, we are able to obtain significant DBT levels which allowed to use a first-order biotic degradation approach. The experimental data obtained on DBT and TBT in the *Elodea* tissues could indeed be fitted by two first-order reactions in series which resulted in a

TBT degradation rate constant of 0.0082 ± 0.0005 $h^{-1}$ translating into a half-live of 4 days ($R^2$= 0.999) (Figure 4). This result is totally consistent with the first TBT removal rate previously calculated and support the occurrence of an active debutylation metabolism of TBT into DBT during the acute toxicity regime (0-72h) by the plant compartments.

## 4- Conclusion

A kinetic study of the reactivity of TBT and its degradation products was achieved in reconstituted freshwater ecosystems, presenting a simple organization level: water-sediments-plants-gastropods. The single contamination pulse of the water column allowed to assess simultaneously the response of the different components of the microcosms to a transient toxicity regime from acute to chronic contamination. Although the kinetic parameters derived from such laboratory experiments are forced by the initial experimental conditions, this study confirms the relevance and usefulness of the controlled microcosms for studying TBT degradation and transfer pathways in target aquatic ecosystems when compared to previous model or field investigations. This study outlines the general scheme of TBT persistence and behaviour in aquatic ecosystems and pointed out the role of the sedimentary compartment as the major sink for TBT contamination as well as a secondary source of organotins for the water column, even in chronic contamination circumstances. TBT persistence in sediments as well as potential remobilization processes highlight the need for modifying the current regulation in order to integrate this compartment as a quality criteria for environmental risk assessment and monitoring.

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# Chapitre B.2.

# Processus de volatilisation des composés organostanniques dans des environnements estuariens et côtiers

# Processus de volatilisation des composés organostanniques dans des environnements estuariens et côtiers

Les composés organostanniques sont aujourd'hui considérés comme des polluants globaux, du fait de leur production et de leurs rejets dans l'environnement sans cesse augmentés depuis ces trente dernières années. L'utilisation des organostanniques trisubstitués et en particulier du tributylétain (TBT) comme agent biocide dans les peintures antisalissures des bateaux et autres infrastructures immergées a entraîné leur introduction directe dans les environnements aquatiques par lixiviation des surfaces traitées. Bien que des mécanismes naturels de dégradation du TBT aient été mis en évidence et que les concentrations dans la colonne d'eau aient sensiblement baissées en réponse à des mesures de réglementation, la contamination des écosystèmes aquatiques par le TBT demeurent l'un des plus importants problèmes écotoxicologiques de ces vingt dernières années. Comprendre le devenir de ces contaminants dans les milieux aquatiques est donc crucial, au regard de leur impact environnemental. Les composés organostanniques une fois introduits en milieu aquatique sont principalement associés aux sédiments, où ils peuvent subir des mécanismes de désalkylation par voie biologique ou chimique leur conférant ainsi une toxicité moindre vis-à-vis des organismes aquatiques. Néanmoins ces processus de dégradation peuvent être concurrencés par des mécanismes naturels d'alkylation et en particulier de méthylation, menant à la formation de composés totalement substitués, hydrophobes et volatils. Il apparaît donc critique d'évaluer ces mécanismes de transformation susceptibles de remobiliser le TBT stocké dans les sédiments, par la production d'espèces organostanniques plus mobiles et disponibles pour les transferts aux différentes interfaces environnementales (eau-sédiments, eau-air, eau-biota).

Le présent travail a ainsi pu mettre en évidence la présence ubiquiste de dérivés organostanniques volatils dans des environnements estuariens et côtiers. L'identification des différents composés volatils de l'étain dans la colonne d'eau et les sédiments a été réalisée au moyen de couplages analytiques extrêmement sensibles et sélectifs. Enfin des flux significatifs de transfert de ces espèces aux interfaces sédiment-eau et eau-air ont pu être calculés et extrapolés à l'échelle des écosystèmes étudiés afin d'évaluer l'importance de ces processus dans le cycle global du TBT en milieu aquatique.

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# Volatilization of Organotin Compounds from Estuarine and Coastal Environments

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The occurrence and speciation of volatile tin compounds (Sn) have been investigated in a contaminated area of the Arcachon Bay (SW France) and in the water column of the Scheldt (Belgium/Netherlands) and Gironde (SW France) estuaries. This paper describes the application of a multi-isotope analytical method, using gas chromatography and inductively coupled plasma-mass spectrometry. Analytes were collected by cryogenic trapping of the gaseous species. This trapping has allowed us to probe volatile tin compounds by detecting both $^{118}$Sn and $^{120}$Sn isotopes. Volatile organic tin compounds have been determined in both sediment and water. They could result from both natural methylation and hydridization processes of inorganic tin ($R_nR'_{4-n}Sn$; R = Me, R′ = H, $n = 0-4$) and from anthropogenic butyltin derivatives released from ship antifouling paintings which have accumulated in sediments ($R_nR'_{4-n}$ Sn; R = Bu, R′ = H or Me, $n = 0-3$). The most ubiquitous species were found to be the methylated forms of butyltin derivatives ($Bu_nSnMe_{4-n}$, $n = 0-3$). These results suggest that biological and/or chemical methylation mechanisms are likely to occur in sediments and to lead to remobilization of tin species into the water column and subsequently to the atmosphere. Finally, sediment−water and water−atmosphere fluxes have been calculated to assess the potential impact of these processes on the fate of organotin compounds in coastal environments.

## Introduction

The increasing production and release of organotin compounds to the environment over the last three decades has led to consider these species as "global pollutants" (*1*, *2*). The contamination generated by the direct introduction of biocides, mainly triorganotins, and more specifically of tributyltin (TBT) into the aquatic environment from the leaching of antifouling paints, is well established (*3*, *4*). Despite various degradation pathways and the evidence of decreasing concentrations in water column in some places around the world, TBT contamination is still considered to be one of the most important ecotoxicological problem of the last 2 decades (*5*−*7*).

Understanding the fate of tin compounds is of primary importance due to their impact on the environment (*8*−*14*). Organotin compounds are usually associated with the fine fraction (<63 $\mu$m) of the sediments (*1*, *15*). Transformation of organotin compounds can occur via different chemical and biological routes. The chemical fate of these compounds is known to be mainly driven by dealkylation processes in oxic or anoxic conditions (*7*, *9*, *16*). The dealkylation processes can be offset to some extent by competing reaction pathways involving natural methylation (*17*−*21*). Studies have indicated the occurrence of methyltin derivatives in the aquatic environments (*22*, *23*). Different natural methyl donor species, such as iodomethane, methyl-cobalamine or humic substances are indeed able to methylate tin compounds to yield mono-, di-, tri-, and tetramethyltin (*21*). In anoxic sediments, sulfate reducing bacteria can actively promote the methylation of inorganic or organic tin compounds (*16*, *24*). Such natural derivatization pathways may lead to the formation of fully substituted and volatile tin compounds in the aquatic environment (*18*, *23*, *25*). These results suggest that tin could be volatilized to the atmosphere from the marine environment (*19*). More recently Adelman et al. (*7*) came to the same conclusion after they studied the fate of TBT in laboratory experiments. They observed the degradation of radiolabeled TBT ($^{14}$C) during batch incubations in an enclosed marine ecosystem. At the end of the experiment, they were unable to recover all the $^{14}$C activity from the TBT introduced in the system. The authors related this loss in the radioactivity budget to the exchange of butyltin compounds at the air/water interface.

To date, there is no significant evidence of losses of organotin compounds to the atmosphere. Results reported for volatile tin compounds mainly suggest the occurrence of hydride forms of tin compounds. Tin hydride ($H_4Sn$) and methyltin hydrides ($Me_nSnH_{4-n}$, $n = 0-4$) have been detected in algae or microbial cultures (*21*, *22*, *26*). These hydride species were suspected to be formed biologically and are likely to occur naturally in the environment. Craig and Rapsomanikis (*20*) suggested that chemical methylation could also take place in the environment and explain the formation of tetramethyltin. The occurrence of methylated butyltin compounds, such as tributylmethyltin ($Bu_3SnMe$) and dibutyldimethyltin ($Bu_2SnMe_2$), in the surface of contaminated harbor sediments at concentrations below 50 ng/g (dry weight) have also been reported by Maguire et al. (*1*, *11*). Critical discussion has highlighted the care needed in the identification of such compounds when using atomic spectrometric detection methods, due to the lack of molecular information provided by such analytical techniques (*27*). $Bu_3SnMe$ was finally identified by GC/MS at concentrations up to 400 ng/g from incubated anoxic sediments spiked with TBT (*16*). Its formation was related to the specific activity of sulfate reducing bacteria.

While it is generally agreed that butyltin compounds inputs in coastal waters have been reduced, a major question remains, however, with regard to their accumulation and fate in sediments. It is now of major concern for risk assessment of butyltin contamination to know whether these compounds will be degraded or could be remobilized in the water column through natural diffusion, bioturbation processes, or by turbulent mixing due to the action of water.

The results presented in this paper report on the ubiquitous occurrence of volatile tin compounds in coastal areas. These results were obtained with highly sensitive and selective analytical methods. Several volatile tin species have been identified in all environments studied.

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## Materials and Methods

**Sampling Sites and Procedures.** Determination of the concentrations of total organotin compounds in the dissolved or suspended solid phase of the samples was not the objective of the present work.

**Sediment**. Sediments were collected at low tide or using a box corer in the main basin of the Arcachon harbor (France) and in the navigation channel of the Scheldt estuary (Belgium/Netherlands), respectively, where the organotin contamination from antifouling paints has been extensively reported (*12*−*15*). Sediments were sampled in the top 5 cm using a polyethylene cup and were immediately transferred to a gastight glass vial (100 mL) without any headspace. The vials were then stored in the dark at 4 °C and processed within 24 h. Sediment samples from the Arcachon harbor and the Scheldt estuary were from quasi anoxic conditions (black color). Sediment samples were further analyzed for butyltin compounds (*28*), total organic carbon and sulfur (LECO CS-125 analyzer), and water content.

**Water**. In the Arcachon harbor, water samples were directly collected into 1 L Pyrex bottles with gastight Teflon-lined caps and were processed like the sediments. Estuarine water samples were collected in two European estuaries selected for their different anthropogenic pressure during the EU BIOGEST (biogas transfer in estuaries) project cruises. Both the Scheldt and the Gironde estuaries are both macrotidal systems with long residence times for both water and suspended matter and very different anthropogenic contributions (*29*, *30*). The Scheldt estuary contains one of the largest harbors and industrial complexes in Europe and is bordered by a high population density (*29*). On the contrary, most of harbor and industrial activities in the Gironde estuary stopped more than 10 years ago, and the density of population along its banks is much lower (*30*). It is therefore considered to be one of the few large pristine estuarine area of Europe. In both estuaries, water samples were collected during four cruises on board the research vessels *Belgica* (SPPS/FN) and *Côte d'Aquitaine* (CNRS/INSU). These cruises took place in July 1996 and December 1996 for the Scheldt estuary and in November 1996 and June 1997 for the Gironde estuary. All samples were collected at 3 m depth, below the ship draught, using an acid-cleaned Teflon-coated Go-Flo sampler (GO, Miami, U.S.A.) or a custom 1 L ultra clean sampler (*4*). Both sampling devices gave the same results. Immediately after collection, the samples were transferred to a Teflon-lined capped 1 L Pyrex bottle for ship-board treatment within 30 min. Water samples were collected over a salinity gradient of 0−34 units (Practical Salinity Scale, International System of Units) during each cruise in both estuaries.

**Stripping Volatile Tin Compounds from Samples. Sediment**. Volatile tin compounds were extracted from the sediments collected using a purge and cryogenic trap setup. About 10 g of fresh sediment was transferred and weighed in a 50 mL purge glass vessel. A volume of 20 mL of milli-Q water prepurged with He (10 min at 300 mL/min) was added to the purging vessel. The suspension formed was continuously homogenized with a Teflon-coated magnetic stirrer. Gaseous species were stripped out the vessel and dried by passing them through a water trap at −20 °C (mixture of ice and acetone), finally cryofocused in a silanized glass trap (U-shaped, 6 mm i.d.) filled with acid-cleaned silanized glass wool (Supelco), and immersed in liquid nitrogen (−196 °C). After a 30-min purge, the cryotraps were closed with gastight Teflon caps and immediately transferred to a dry atmosphere cryogenic container (cooled with liquid nitrogen) for a maximum storage period of 1 week before analysis (*31*).

**Water**. Volatile tin compounds were extracted from water samples in the field using a purge and cryogenic trap setup described elsewhere (*31*). Within less than 30 min after sampling, the water sample was transferred directly from its 1 L Pyrex bottle to a 1.5 L purging vessel under He atmosphere. Water samples were continuously stripped for 1 h with a He flow rate of 700 mL/min to yield optimal extraction efficiency (*31*). The volatile compounds were then trapped and stored following the procedure described above for sediment.

**Chemicals and Standards.** All connections and tubing in contact with the samples were made of Teflon PTFE and PFA, respectively. All glass- and plasticware were previously cleaned with a biocide detergent and soaked with concentrated nitric acid (10%, Prolabo, France) for 3 days. Millipore milli-Q water was used for rinsing and preparing aqueous solutions. Reagents and standards were prepared with analytical grade chemicals, unless stated. Hexane (Fluka, 99.8%), methanol (Fluka, 99.8%), dichloromethane (Fluka, 99.8%), tropolone (Fluka, 99%), methylmagnesium chloride (Aldrich, 99%), sodium borohydride (Aldrich, 99%), and sodium sulfate (Prolabo, 99%) were used for the derivatization reactions and standard compound solution preparations. Tetramethyltin (Strem, 99%), tetraethyltin (Strem, 99%) and butyltin chloride species ($Bu_nSnCl_{4-n}$, $0 \leq n \leq 4$, Strem, 99%) were used as standard compounds for calibration and identification. Different volatile organotin compounds were synthesized for the identification of unknown species present in natural samples. Hydride forms of butyltin compounds were obtained from stock solutions of butyltin chloride compounds mixed with milli-Q water acidified to pH 2 with pure HCl (Prolabo) and 100 $\mu$L of 5% $NaBH_4$ (Merck) aqueous solution (*32*). Mixed methylated forms of butyltin compounds were obtained by dilution and extraction of the butyltin chloride stock solution by tropolone in hexane. The extract was dried over anhydrous $Na_2SO_4$ followed by classical Grignard derivatization reaction with MeMgCl (*33*). The volatile tin compounds, extracted into the organic phase, were dried with anhydrous $Na_2SO_4$ prior to injection into both analytical systems. Although recoveries of the volatile organotin species synthesis were less than 100%, these compounds were only extracted for qualitative identification in the natural samples.

**Determination of Volatile Tin Compounds.** The standard settings and methodologies used for both the ICP/MS and MIP/AED are discussed in details by Pécheyran et al. (*34*) and Szpunar et al. (*28*), respectively. Both techniques are independent of each other in both the chromatographic and detection aspects and are complementary for the identification of volatile tin compounds.

**Cryogenic Trapping−Gas Chromatography−Inductively Coupled Mass Spectrometry (CT-GC−ICP/MS)**. Most of the methodology is discussed in detail elsewhere (*31*, *34*). For most of the samples analyzed, direct injection was used for both the cryotraps and standards solutions into a cryotrapping−gas chromatography and ICP/MS (Elan 5000, Perkin-Elmer) system (*34*). This analytical procedure allows the samples to be trapped on inert materials, thus preventing chemical alteration. The high sensitivity and selectivity of the ICP/MS detector coupled with the cryofocusing technique permit operations which deliver detection limits lower than 50 femtogram for tin (as Sn).

**Capillary Gas Chromatography−Microwave Induced Plasma−Atomic Emission Detection (CGC-MIP/AED)**. To confirm and identify some of the unknown volatile compounds detected by CT-GC-ICP/MS, a second analytical technique was employed to take advantage of its high sensitivity of the overall system, the high separation efficiency provided by the capillary columns and the selectivity of the detector (CGC-MIP/AED, Hewlet-Packard Model 5921A) (*28*). With this approach, volatile species trapped in the field were also desorbed at 300 °C over 12 min under a helium flow rate at 10 mL/min. Volatile tin compounds were trapped in 10 mL Teflon-lined headspace vials filled with prepurged hexane.

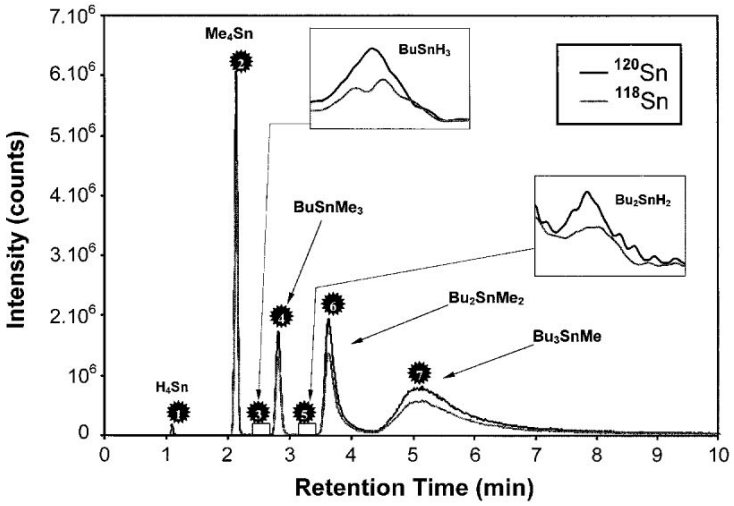


**FIGURE 1. Chromatogram of volatile tin compounds in a surface sediment sample from the harbor of Arcachon, obtained by purge, cryogenic trapping, gas chromatography, and ICP/MS detection (CT-GC-ICP/MS).**

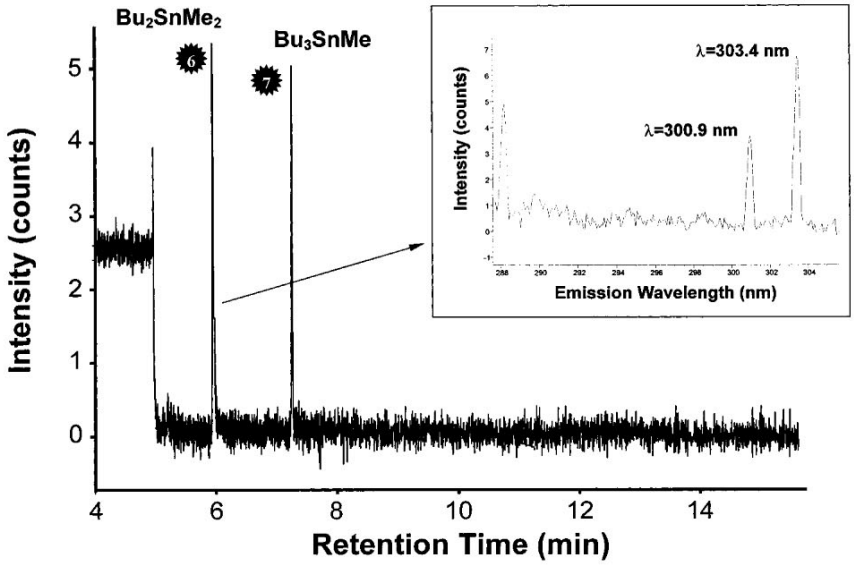


**FIGURE 2. Chromatogram of volatile tin compounds in a surface sediment sample from the harbor of Arcachon, obtained by cryogenic trapping followed by capillary gas chromatography and MIP/AES detection (CGC-MIP/AED).**

These solutions were then evaporated at room temperature under nitrogen flow (10 mL/min) to preconcentrate the less volatile species before their direct injection into the CGC using an automated sample injector (HP Model 7673A). This technique offers a detection limit below 1 pg as Sn.

## Results and Discussion

**Identification of Volatile Tin Compounds in Coastal Environments.** All analyses performed by CT-GC-ICP/MS on sediment and water samples from the Arcachon harbor and the Scheldt and Gironde estuaries presented similar chromatographic profiles. A typical CT-GC-ICP/MS chromatogram obtained for a contaminated sediment of Arcachon (silt) is presented in Figure 1 and is used as reference sample for the identification procedure applied in this work. On this chromatogram, seven peaks can be seen. The isotopic ratio $^{120}Sn/^{118}Sn$ (=1.35) for each peak was verified and confirmed the presence of tin-containing compounds (*31*). Peak identification for lower boiling point compounds was first based on a comparison of the retention times obtained with the CT-GC-ICP/MS system. Peaks corresponding to higher boiling points compounds underwent a complementary identification of retention time and detection using both CT-GC-ICP/MS and CGC-MIP/AED apparatus.

The peaks labeled from 1 to 5 (Figure 1) were accurately attributed to (1) stannane ($H_4Sn$), (2) tetramethyltin ($Me_4Sn$), (3) monobutyltin trihydride ($BuSnH_3$), (4) monobutyltrimethyltin ($BuSnMe_3$), and (5) dibutyltin dihydride ($Bu_2SnH_2$). Peaks 6 and 7 could not be identified by this approach, due to the large uncertainties of their retention time (RSD > 3%). The cryofocusing technique has low resolving separation capabilities for high boiling point compounds (*31*), and several organotin compounds could account for these peaks. Therefore, the same sediment sample was analyzed by CGC-MIP/AED; the chromatogram is shown in Figure 2. Only two peaks are present in both samples as a result of the poor recovery of light volatile species during the desorption step into the organic solvent. The spectral emission lines of these two peaks at 300.9 and 303.4 nm also confirmed the detection of tin-containing compounds. The precision of the retention

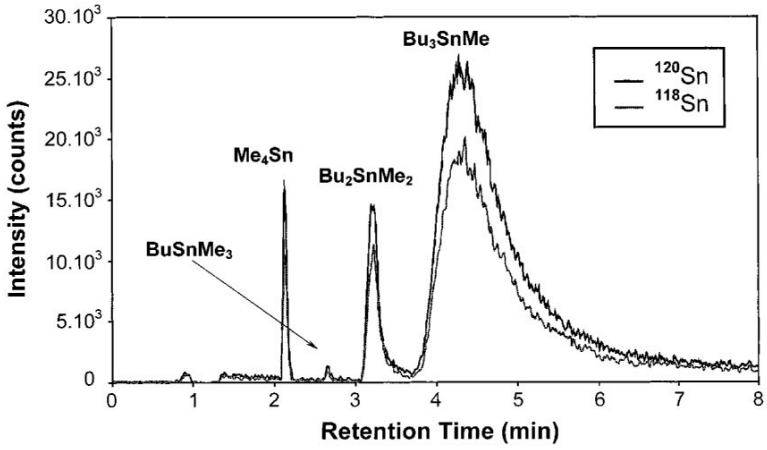


**FIGURE 3. Typical chromatogram of volatile organotin compounds in surface water samples from the Scheldt estuary and obtained by purge, cryogenic trapping, gas chromatography, and ICP/MS detection (CT-GC-ICP/MS). Elution conditions have been slightly changed by using faster heating programs than use for Figure 1 to improve the chromatographic elution.**

**TABLE 1: Organotin Compounds, Total Organic Carbon (TOC), Total Sulfur (TS), and Water Content in Different Sediments, Collected from Arcachon Harbor and Scheldt Estuary**

| | type | Sn inorg[a] (ng/g dw)[b] | MBT (ng/g dw) | DBT (ng/g dw) | TBT (ng/g dw) | ΣSn (ng/g dw) | TOC (%) | TS (%) | water (%) |
|---|---|---|---|---|---|---|---|---|---|
| Arcachon harbor (April 1997) | silt | 390 | 2460 | 530 | 600 | 3980 | 2.20 | 1.17 | 30 |
| Arcachon harbor (April 1997) | sand | 380 | 2720 | 2030 | 3340 | 8470 | 0.88 | 0.45 | 17 |
| Scheldt estuary (May 1998) | silt | 3 | 20 | 22 | 20 | 65 | 1.86 | 0.29 | 27 |
| Scheldt estuary (May 1998) | sand | 1 | 5 | 1 | 9 | 16 | 0.2 | 0.07 | 17 |

[a] Acid leachable inorganic tin. [b] Concentration in nanogram of tin per gram of sediment dry weight.

**TABLE 2: Volatile Tin Compounds Concentrations in Two Different Types of Surface Sediments in Arcachon Harbor and Scheldt Estuary**

| | | $H_4Sn$ | $Me_4Sn$ | $BuSnH_3$ | $BuSnMe_3$ | $Bu_2SnH_2$ | $Bu_2SnMe_2$ | $Bu_3SnMe$ | TVT[a] |
|---|---|---|---|---|---|---|---|---|---|
| Arcachon harbor silt | Sn (pg/g dw)[b] | 0.5 | 19.3 | n.d.[e] | 8.5 | n.d.[e] | 21.2 | 51.4 | 100.9 |
| | TVT (%)[c] | 0.5 | 19.1 | | 8.4 | | 21.0 | 50.9 | 100.0 |
| | MR (%)[d] | | 0.0049 | | 0.0003 | | 0.0040 | 0.0086 | 0.0025 |
| Arcachon harbor sand | Sn (pg/g dw) | n.d.[e] | 10.0 | 0.1 | 24.9 | 0.4 | 19.1 | 38.1 | 92.6 |
| | TVT (%) | | 10.8 | 0.1 | 26.9 | 0.4 | 20.6 | 41.1 | 100.0 |
| | MR (%) | | 0.0026 | | 0.0009 | | 0.0009 | 0.0011 | 0.0011 |
| Scheldt estuary silt | Sn (pg/g dw) | n.d.[e] | 0.3 | n.d.[e] | 0.4 | 0.1 | 2.7 | 18.8 | 22.4 |
| | TVT (%) | | 1.4 | | 1.8 | 0.5 | 12.0 | 84.3 | 100.0 |
| | MR (%) | | 0.0105 | | 0.0020 | | 0.0124 | 0.0938 | 0.0347 |
| Scheldt estuary sand | Sn (pg/g dw) | n.d.[e] | 0.5 | n.d.[e] | 0.02 | 0.1 | 0.6 | 13.6 | 14.9 |
| | TVT (%) | | 3.6 | | 0.1 | 0.8 | 3.9 | 91.5 | 100.0 |
| | MR (%) | | 0.0871 | | 0.0004 | | 0.0584 | 0.1601 | 0.1012 |

[a] Total volatile tin. [b] Concentration in picogram of tin per gram of sediment dry weight. [c] $[Bu_nSnR_{4-n}]100/[TVT]$, R = Me or H. [d] Methylation ratio: $[Bu_nSnMe_{4-n}]100/[Bu_nSnX_{4-n}]$, $n = 0-3$. [e] Not detected.

time allows much better possibilities for the identification of high molecular weight organotin compounds (boiling point > 200 °C). The retention times obtained for peaks 6 and 7 corresponded exactly to $Bu_2SnMe_2$ and $Bu_3SnMe$ species, respectively. $Bu_3SnH$ could coelute with $Bu_3SnMe$ in the samples investigated, but to date, except for extreme anoxic condition (*26*), methylation processes appear to override natural hydridization processes. The chromatographic peaks observed in sediments, corresponding to $Me_4Sn$, $BuSnMe_3$, $Bu_2SnMe_2$, and $Bu_3SnMe$, are present in most of the water samples collected. A typical sample chromatogram is shown in Figure 3.

**Distribution of Volatile Tin Compounds in Coastal Sediments and Waters. Sediment**. Total organotin concentrations and other characteristics have been determined in both Arcachon and Scheldt sediments as shown in Table 1. Arcachon sediments were found to be heavily contaminated with butyltin compounds when compared to Scheldt estuary samples. This difference can be partially explained not only by the enrichment of Arcachon samples in organic carbon and sulfur but also by the fact that Arcachon sediments were collected in the vicinity of a shipyard. The average values obtained for volatile tin compounds in the sediments are presented in Table 2. Most of the seven compounds used in this study were identified in the contaminated sediments investigated. Methylated forms are the major contributors to the total volatile tin (TVT) content. The hydride forms are found to account for only a small fraction of TVT (0.5−0.8%) and will not be discussed further. All the samples analyzed contained concentrations of TVT ranging from 15 to 100 pg/g (125−840 fmol/g) of dry sediment (Table 2). Concentrations in sediments represent therefore a very small proportion of the total organotin concentrations measured in the same samples and are highly variable, ranging between 0.001 and

**TABLE 3: Average Volatile Organotin Compound Concentrations in Surface Waters from the Arcachon Harbor and along the Scheldt and Gironde Estuaries[c]**

| | | *n* | $Me_4Sn$ (fmol/L)[a] | $BuSnMe_3$ (fmol/L) | $Bu_2SnMe_2$ (fmol/L) | $Bu_3SnMe$ (fmol/L) | TVT (fmol/L) |
|---|---|---|---|---|---|---|---|
| Arcachon harbor | (April 97) | 2 | 32.4 ± 3.2 | 11.0 ± 1.1 | 12.4 ± 2.5 | 63.1 ± 12.6 | 118.9 ± 19.4 |
| Scheldt estuary | (July 96) | 21 | 18.2 ± 10.2 | 1.6 ± 2.4 | 53.1 ± 87.1 | 470.0 ± 302.9 | 542.9 ± 402.6 |
| Scheldt estuary | (Dec 96) | 15 | 19.6 ± 3.2 | 3.4 ± 2.5 | 45.5 ± 38.8 | 526.2 ± 422.2 | 594.7 ± 479.5 |
| Gironde estuary | (Oct 96) | 17 | 0.5 ± 1.1 | 0.1 ± 0.1 | 0.6 ± 1.1 | 28.6 ± 15.8 | 29.8 ± 18.1 |
| Gironde estuary | (June 97) | 15 | n.d.[b] | n.d.[b] | n.d.[b] | 4.3 ± 5.6 | 4.3 ± 5.6 |

[a] Concentration in femtomole of tin per liter of water. [b] Not detected. [c] Mean ± SD; *n* is the number of samples.

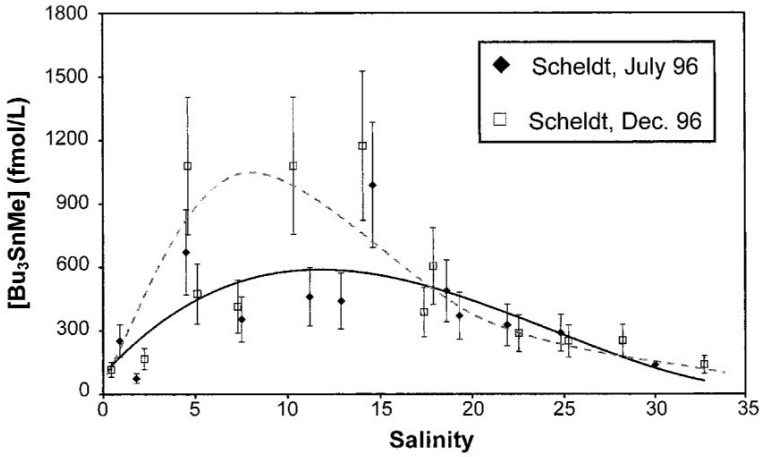


**FIGURE 4. Distribution of tributylmethyltin ($Bu_3MeSn$) in surface waters of the Scheldt estuary as a function of salinity in July and December 1996. Error bars represent a relative standard deviation of 25% obtained from duplicate analysis.**

0.003 to 0.03−0.1% dry weight in the Arcachon and Scheldt sediments, respectively (Table 2). The relative distribution of the volatile methylated tin species differs significantly between Arcachon and Scheldt samples. $Bu_3SnMe$ represents 41−51% of the TVT in Arcachon sediments and up to 84−92% in Scheldt sediments (Table 2).

**Water.** The average concentrations of the volatile tin species in surface waters obtained from different sampling campaigns are presented in Table 3. Concentrations recorded range from nondetect to 600 fmol/L for TVT. These concentrations are very low and could only be detected due to the sensitivity of the analytical method. However, they clearly demonstrate that these compounds are ubiquitous in all of the areas studied. Average TVT concentrations in Arcachon surface waters are higher than in the Gironde estuary with 120 and 4 to 30 fmol/L, respectively. They are however much lower than in the Scheldt estuary, which ranges from 540 to 600 fmol/L. No significant variations could be observed between the seasonal cruises on both estuaries. The fact that TVT concentrations in the Scheldt estuary are 50−100 times higher than in the Gironde estuary was expected. Both estuarine systems have a similar water residence times (a few weeks to some months) but present contrasting contamination patterns (*29*, *30*).

The ubiquitous and major species in all sample is $Bu_3SnMe$. It represents 50−100% of the TVT concentration in the samples. The distribution of $Bu_3SnMe$ for each cruise in both estuaries was plotted against salinity in Figures 4 and 5. Other volatile organotin compounds were present at much lower concentrations, and we could not find significant variability in either estuary. In both the Scheldt (Figure 4) and the Gironde (Figure 5) estuaries, the $Bu_3SnMe$ concentrations present a similar distribution and exhibit a maximum between salinity 5 and 15 at ca. 1200 and 40 fmol/L, respectively. This nonconservative distribution with maximum concentrations at intermediate salinity demonstrates that both estuaries are a continuous source of $Bu_3SnMe$ to the water column. Below salinity 5, $Bu_3SnMe$ concentrations decrease upstream but present significant concentrations in the river end-member of the Scheldt (100−250 fmol/L) and Gironde (10−20 fmol/L) estuaries. River inputs can thus partially contribute to the budget of volatile tin compounds in both estuaries. Above salinity 15, $Bu_3SnMe$ concentrations decrease seaward following a pseudolinear dilution curve showing a marine end-member concentration around 100 fmol/L and below the detection limit for the Scheldt and the Gironde estuaries, respectively. A fraction of the volatile tin compounds released in the estuary can thus be transported to coastal waters. A budget for volatile organotin compounds in the investigated estuaries will be discussed in detail elsewhere by Tessier et al. (manuscript in preparation).

**Formation of Volatile Organotin Compounds in Coastal Environments.** Tetramethyltin and three methylated and volatile forms of butyltin compounds in sediment and water are reported here for the first time. These findings demonstrate that natural chemical or biological methylation processes do not only involve inorganic species but also anthropogenic butyltin compounds. In the investigated sediments, the apparent methylation yield can be derived from the concentration ratio or "methylation ratio" (MR), between each methylated volatile tin compound and a potential precursor which has the same number of butyl groups ($Bu_nSn$, $n=0-3$). The MR presented in Table 2 suggest that the apparent methylation yield of tin compounds is generally higher in the Scheldt than in Arcachon sediments, with global values ranging from 0.001 to 0.003 and 0.035−0.100%, respectively. No significant trend was observed, however, between silt- and sand-type sediments in terms of volatile tin species distribution and for the MR. In each sediment, the MR for $Bu_3SnMe$ is also generally higher than for the other tin species. These results indicate that the methylation of tributyltin is more efficient or that $Bu_3SnMe$

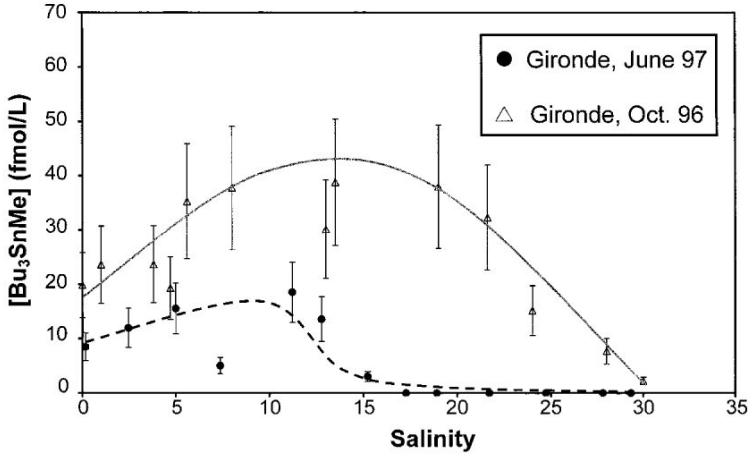


**FIGURE 5. Distribution of tributylmethyltin ($Bu_3MeSn$) in surface waters of the Gironde estuary as a function of salinity in October 1996 and June 1997. Error bars represent a relative standard deviation of 25% obtained from duplicate analysis.**

lifetime is longer than for other species for all type of sediments and contaminated sites. Moreover, previous investigations have demonstrated that trialkyltin species, such as trimethyltin, can be easily converted into tetramethyltin via chemical and biological pathways during incubations of natural sediments (*18*, *23*).

For water samples, the correlation coefficients were calculated between the concentrations of $Bu_3SnMe$ as a function of $Bu_2SnMe_2$ and $Me_4Sn$ during the four cruises performed on the Gironde and Scheldt estuaries. The two regression lines obtained with both $Bu_2SnMe_2$ and $Me_4Sn$ gave good correlation coefficients ($R^2 = 0.78$ and 0.72, respectively) and suggest that the occurrence of these compounds could be derived from the presence of $Bu_3SnMe$. The slopes of both regression curves indicate that $Bu_3SnMe$ concentrations are about 6 and 12 times higher than $Bu_2SnMe_2$ and $Me_4Sn$ concentrations, respectively. This ratio is in agreement with the relative distribution of total and volatile tin compounds observed in the sediments collected in the Scheldt (see Tables 1 and 2). The relative distribution is also similar between the Arcachon surface waters and total and volatile butyltin compounds observed in the collected sediments (see Tables 1 and 2). This result suggests that the various butyltin and inorganic tin species present in sediments follow identical methylation pathways in anoxic sediments and diffuse into the water column with similar transfer rates.

The degradation of butyltin compounds via sequential losses of butyl groups is therefore not the only route for butyltin removal in coastal sediments. Tributyltin and its degradation products can be involved in biological and/or chemical methylation pathways which lead to fully volatile substituted organic tin species. Yozenawa et al. (*16*) demonstrated that such mixed methylbutyltin species could be produced under sulfate-reducing activity during incubations with spiked sediments in the laboratory. However, it is not clear whether this process is due to intracellular mechanisms or to the reductive chemical conditions generated in the medium. The reaction pathway could follow either a reductive or an oxidative route for methyl group transfer from a carbanion or a carbocation donor, respectively (*21*). This result allows us to propose some hypotheses for the processes regulating the transformation and the transfer of tin in the coastal environment. It also demonstrates the overlap and interaction of natural and anthropogenic tin biogeochemical cycles in these environments.

Figure 6 presents a model of organotin cycling in coastal environments developed from this work. It demonstrates that volatile tin compounds occupy a central part of the chemical cycling of organotin compounds in estuarine and coastal environments. Butylmethyltin species result from both degradation of anthropogenic tributyltin biocide and natural methylation of tin species. Both processes contribute to the formation of volatile and mobile compounds. It is also reasonable that the formation of butylmethyltin species takes place when butyltin compounds disproportionate under reducing conditions in sediments. The relative distribution of the volatile organotin species in the different compartments also indicate that they can be transferred to the atmosphere under passive or turbulent diffusion processes.

**Transfer of Volatile Organotin Compounds to the Atmosphere.** A more detailed description of the saturation ratio and flux calculation method is found in the Supplementary Information. Prior to the determination of the transfer of volatile tin, the saturation ratio (SR) of these compounds must be determined by comparing their concentrations in sediment or water to water or atmosphere, respectively. At the sediment−water interface, the concentrations obtained by extracting the volatile compounds after stripping a sediment slurry may have led to an overestimate of the "real" porewater concentrations. This overestimation was assumed to be up to 10 times the "real" porewater concentration considering an identical dilution factor. For the water−atmosphere interface, the Henry's law constant for each volatile organotin compounds was required to calculate the SRs and was derived from literature data and molecular properties. If the SR is above 1, volatile compounds are then supersaturated in sediment or water and diffuse to the water or atmosphere, respectively. The different average SR, displayed in Table 4, demonstrate that volatile organotin compounds will systematically diffuse from surface sediment to the atmosphere.

**Sediment to Water Fluxes**. In a contaminated area such as the Arcachon harbor and the Scheldt estuary, total volatile tin compound concentrations in surface sediment per volume of pore water are from 80 to 30 000 times higher than in the overlying water. A simple modified diffusion model approach was used to evaluate fluxes of the compounds from surface sediments to the water column (*35*). For sediment to water exchanges, it was assumed that all volatile tin species were produced by chemical or biological mechanisms in bulk sediments and were passively released to pore waters. Volatile forms, extracted by stripping a sediment slurry, also strictly represent the water exchangeable fraction of the volatile tin compounds, which correspond to one to 10 times the "real" porewater concentrations. A large range of flux densities can

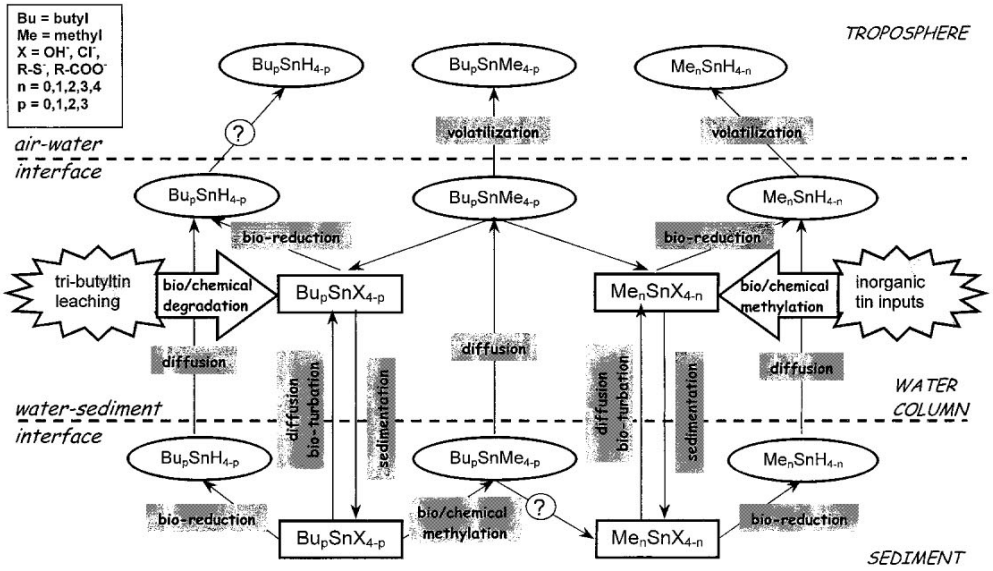


**FIGURE 6. Schematic diagram of organotin compounds chemical cycling in coastal and estuarine environments.**

**TABLE 4: Average Calculated Sediment to Water and Water to Sediment Saturation Ratios and Flux Densities of Volatile Organotin Compounds in the Coastal and Estuarine Environments Investigated[c]**

| | saturation ratio | | | | total volatile tin (TVT) flux density (nmol/m².yr) |
|---|---|---|---|---|---|
| | $Me_4Sn$ | $BuSnMe_3$ | $Bu_2SnMe_2$ | $Bu_3SnMe$ | |
| | | | Sediment to Water[a] | | |
| Arcachon harbor | 850−8520 | 2860−28590 | 3060−30600 | 1340−13360 | 50−470 |
| Scheldt estuary | 80−790 | 200−2010 | 90−880 | 100−1010 | 80−790 |
| | | | Water to Air[b] | | |
| Arcachon harbor | 22810 | 16080 | 9730 | 49520 | 90 |
| Scheldt estuary | 9190 | 3120 | 25660 | 262250 | 510 |
| Gironde estuary | 30 | 550 | 1180 | 13680 | 20 |

[a] SR sediment−water: $[Bu_nSnMe_{4-n}]_{pore\ water}/[Bu_nSnMe_{4-n}]_{water}$, $n = 0-3$, flux model adapted from ref *35*. [b] SR water−air: $[Bu_nSnMe_{4-n}]_{water} \times H/[Bu_nSnMe_{4-n}]_{air}$, $n = 0-3$, flux model adapted from ref *36*. [c] Methods and calculations are described in detail in the Supporting Information. The range obtained at the sediment−water interface corresponds to the potential overestimate in the porewater concentrations ("real") considering water exchangeable concentrations ("measured") to be 1−10 times higher.

be obtained but, by taking into account the hydrodynamic characteristic of the sites investigated, a better estimate can be determined. The Arcachon harbor is a tidal bay with low turbulent mixing, suggesting that mainly bioturbation will enhance sediment−water exchanges (*37*). On the other hand, the Scheldt estuary is a macrotidal channel with high turbulent mixing, where sediment resuspension and tidal current friction can strongly enhance the sediment−water exchanges (*35*). Flux calculations of the total volatile tin compounds at the sediment−water interfaces in the Arcachon harbor and Scheldt estuary are presented in Table 4. In Arcachon harbor, calculated flux densities range from 50 to 470 nmol/m².yr, considering low turbulent mixing. In the Scheldt estuary, flux densities obtained are higher and range from 80 to 790 nmol/m².yr, considering high turbulent mixing.

**Water to Air Fluxes.** During the two first cruises on the Gironde and the Scheldt, atmospheric samples were collected (*34*). Only $Me_4Sn$ was detected (detection limit ca. 20 fmol/m³) in a few samples, demonstrating that total volatile tin compounds were supersaturated in surface waters. To obtain accurate flux estimations, transfer velocities at the air−water interface were calculated using a model developed in an estuarine tidal environment (*36*). The turbulence at the interface is mainly driven by the wind speed in aquatic ecosystems, and it is difficult to predict average flux densities over a period of time within the range evaluated (*35*, *36*). However, in turbulent systems, due to high tidal current velocity, such as the Scheldt and the Gironde estuaries, wind speed has a less dominant influence and measured transfer velocities are generally high in any conditions (*38*). Atmospheric fluxes calculated for the different surface water samples collected in the Arcachon harbor and the Scheldt and Gironde estuaries are displayed in Table 4. The previous assumption led to TVT water to air fluxes at about 510 and 20 nmol/m².yr for the Scheldt and the Gironde estuaries, respectively, and 90 nmol/m².yr in the Arcachon harbor.

**Mass Balance of Volatile Organotin Compounds in the Water Column**. The TVT fluxes obtained in this work may allow the determination of the contribution of volatile organotin transfer to the total organotin budget in the respective environments (Arcachon, Scheldt). Such estimation remain very complex in the case of the Scheldt estuary and will be discussed elsewhere (Tessier et al., in preparation). For the Arcachon harbor, a large fraction of the TVT present in the sediment can reach the atmosphere. An average TVT flux of 10 g Sn/yr is emitted into the water column, from which about 50% enters the atmosphere. For a standing inventory of butyltin compounds in surface sediments, estimated to range from 1 to 10 kg as Sn (*13*−*15*), such a process would then require 100−1000 years to remove this toxic substances from the harbor sediments.

These results have further global implications for the fate of organotin compounds trapped in sediments. In turbulent

systems such as estuaries, diffusion processes of volatile organotin species to the water column and to the atmosphere may be a significant pathway to efficiently remove organotin compounds from coastal environments. Sediments have long been considered to be a sink for these highly toxic constituents. In the case of minimal external inputs resulting from increasingly efficient regulation, sediments should now be considered as a significant source of organotin compounds to overlying waters and a potential source to the atmosphere.

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## Supporting Information Available

Flux calculation methods for volatile organotin species at the water−air and sediment−water interface. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Supplementary Information for ES&T web site:**

**Flux calculation methods for volatile organotin species at the water-air and sediment-water interface**

*Saturation Ratio*

The saturation ratio (SR) estimation is necessary to validate the transfer of volatile species at natural interfaces. For the sediment to water exchange this ratio is expressed as:

$$SR = \frac{C_{pw}}{C_w}, \quad (1)$$

Where $C_{pw}$ is the pore water concentration. $C_{pw}$ was assumed to correspond to the water exchangeable fraction measured by stripping a slurry made of the sediment sample and milli-Q water. The concentration obtained represents the amount of this fraction reported to the volume of pore water in the collected sample. However, during the procedure the porewater is diluted about ten times which may lead to overestimate $C_{pw}$ by this factor to preserve a constant partitioning of the species between the solid and aqueous phase. In the last part of the manuscript, this potential overestimation was taken into account in the saturation ratio and flux results. $C_w$ is the measured water concentration, assuming an homogenous distribution in the water column, as it was observed in the studied environments. For the water to air exchange the saturation ratio (SR) is given by the following expression:

$$SR = \frac{C_w.H}{C_a}, \quad (2)$$

Where $C_w$ and $C_a$ are the concentrations measured in the water column and in overlying the air respectively. H represents the dimensionless Henry's law constant of the considered volatile compound. Only trace amounts of atmospheric $Me_4Sn$, near the detection limit (ca. 20 fmol/$m^3$), have been observed on the Gironde and the Scheldt

estuaries by Pécheyran *et al (1)*, and Tessier *et al* (manuscript in preparation). We therefore used this detection limit value as a reference for the atmospheric concentration of volatile tin compounds in our calculations. H values used in the paper were obtained from a quantitative structure-activity relationship (QSAR) between Henry's law constants available in the literature for tetraalkylated tin compounds *(2)* and molecular total surface area *(3)*. This simple method was found to provide a very good approximation of Henry's constant for volatile alkylated compounds *(4)*. Regarding to our results it is obvious that sediments and waters studied are supersaturated compared to the water column and the air above, respectively (SR>>1, see Table 4).

*Flux Calculation*

Flux calculations are based on the Fick's first law of diffusion with the assumption that the concentrations of the studied compounds in the sediment and the water column are in steady state compared to their transfer velocities at the interfaces.

*Sediment to water exchange*

To estimate the flux of volatile tin compounds, we used the Fick's first law modified for sediments by Li and Gregory *(5)* and Berner *(6)*. Middelburg *et al (7)* applied this method for gaseous exchange calculations in the central area of the Scheldt estuary, and provide us the parameters needed to implement this model. F, the flux density ($mol/m^2.yr$) is expressed as:

$$F = E.\phi.D_s.\left(\frac{\delta C}{\delta Z}\right)_{Z=0}, \qquad (3)$$

where E is a diffusion enhancement factor as explain below, $\phi$ is the porosity of the sediment which is about 0.6 for sand-type and 0.8 for silt-type sediments *(8)*; Ds is the

whole sediment diffusion coefficient ($m^2$/yr) and $(\delta C/\delta Z)_{Z=0}$ is the concentration gradient at zero depth. $D_s$ is calculated from the formula:

$$D_s = \frac{D_0}{1+n(1-\phi)}, \quad (4)$$

where $D_0$ is the free-solution diffusion coefficient ($m^2$/s), and n=3 *(9)*. $D_0$ was obtained from the Wilke & Chang equation *(10)* and was then corrected for the temperature and salinity accorded to Saltzman *et al (11)*. The concentration gradient at zero depth $(\delta C/\delta Z)_{Z=0}$ can be approximated by calculating $(C_{pw}-C_w)$/MLD, where MLD is the mixed layer depth in the surface sediment. A MLD value of 5 cm was estimated from Middelburg et al *(7)* data, which corresponds also to our total sampling depth. Despite tidal and seasonal variability of salinity and temperature within the water column, we took average values of 35.5 and 10 for the Arcachon, Scheldt-silt and Scheldt-sand samples, respectively, and average water temperature of 20 and 15°C for the Arcachon and Scheldt samples, respectively. The empirical diffusion enhancement factor (E) integrates the effects of mobile benthic fauna (bioturbation), the diffusion associated with methane and biogases ebullition and the physical disturbances by waves and tidal currents. Lower and upper values of E (ca. 4 and 28), validated by direct benthic flux chamber measurement *(7)*, were implemented to the model in order to describe low and high turbulent hydrological systems. Hartman & Hammond *(12)* also performed gas exchange rates experiments across the water-sediment interface in the San Francisco bay and found an enhancement factor ranging from 2 to 4, which can be considered as a lower limit for coastal environment (low turbulence).

*Water to air exchange*

F, the flux density (mol/$m^2$.yr) was obtained by applying the Fick's first law:

$$F = K\left(C_w - \frac{C_a}{H}\right), \quad (5)$$

where K is the transfer velocity (cm/h) at the air-water interface. Considering the low water solubility of the studied compounds we can assume that K is equal to $k_w$, the water gas transfer velocity *(13)*. We can then calculate the $k_w$ for the various volatile tin compounds with the model proposed by Clark *et al (14)*, developed for the estuarine system of the Hudson bay. The transfer velocity is given by the following expression:

$$k_w = \left(\frac{Sc}{600}\right)^{-\frac{1}{2}}.(2 + 0.24u^2), \quad (6)$$

where Sc is the solute Schmidt number, inversely proportional to the diffusion coefficient ($D_0$) and u is the wind speed (m/s) recorded at a height of 10 m. In order to simulate two different types of physical conditions at the water-air interface, we implemented the Clark's model with two wind speed values (ca. u=3 m/s and u=10 m/s ), which correspond to a smooth surface regime and rough surface regime, respectively *(13)*. This approach have allowed to estimate average transfer velocities for the various volatile tin compounds ranging between 2 and 13 cm/h for a low and high turbulence system, respectively. These results are in good agreement with the transfer velocities obtained for radon by Hartman & Hammond *(12)* in the San Francisco bay (ca. 4 cm/h), and those measured for $CO_2$ by Frankignoulle *et al (15)* in the Scheldt estuary (ca. 13.9 cm/h). In our both approaches of water-sediment and air-water exchanges, the lower value of our flux estimation seems then more appropriate to described a tidal bay such as in the Arcachon harbor. On the other hand, the upper flux estimation corresponds to a turbulent environment such as in the Scheldt estuary. These considerations are therefore used in the mass balance discussion section, in conclusion of the paper.

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# Chapitre B.3.

# Spéciation et distribution de composés organostanniques volatils (butylméthylétains) dans trois estuaires européens (Gironde, Rhine, Escaut)

# Spéciation et distribution de composés organostanniques volatils (butylméthylétains) dans trois estuaires européens (Gironde, Rhine, Escaut)

Les teneurs des composés organostanniques, et du tributylétain (TBT) en particulier, ont significativement baissé dans les environnements marins depuis la dernière décennie, en réponse à la réglementation de leur utilisation dans les peintures antisalissures, à la fin des années quatre vingt. Néanmoins leur accumulation passée dans les sédiments et leur réactivité au sein de ce compartiment représentent toujours une question préoccupante pour l'évaluation des risques actuels et futurs liés à la contamination des écosystèmes aquatiques par ces contaminants. Les processus de resuspension des sédiments, via des perturbations physiques naturelles ou anthropiques (bioturbation, hydrodynamisme, tempêtes, activités de dragage), sont susceptibles de provoquer la désorption directe des composés organostanniques et leur remobilisation vers la colonne d'eau.
Le devenir des composés organostanniques en milieu estuarien apparaît de ce point de vue particulièrement déterminant. Les estuaires représentent en effet des zones de transfert du matériel particulaire et dissous des continents vers les environnements marins. Ce sont, pour la plupart, des systèmes extrêmement dynamiques caractérisés par l'établissement de forts gradients physicochimiques. Ils présentent également une importante activité biologique et sont le théâtre d'un intense recyclage sédimentologique via des processus alternés de sédimentation et resuspension. Ils représentent ainsi un ''substrat'' très favorable pour la production et le transfert de composés organostanniques volatils.
Le présent travail se veut la continuité de l'étude précédemment développée dans le chapitre B.2. La production et la distribution des espèces volatiles des organoétains ainsi que leurs sources potentielles ont été examinées dans trois estuaires européens macrotidaux (la Gironde, le Rhin et l'Escaut) en relation avec leurs différents régimes hydrodynamiques, niveaux de contamination, et paramètres biogéochimiques. Les échanges à l'interface eau-atmosphère ont été quantifiés et intégrés à un simple modèle de mélange, afin d'estimer l'importance de ces processus par rapport aux flux de rivière et aux flux d'export aux eaux côtières adjacentes. La formation de composés volatils organostanniques en milieu estuarien apparaît principalement gouvernée par le temps de résidence de l'eau et des particules, le niveau de contamination des sédiments et l'activité microbiologique associée à l'établissement de zones d'anoxie dans le sédiment.

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# Volatile organotin compounds (butylmethyltin) in three European estuaries (Gironde, Rhine, Scheldt)

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**Key words:** air-water exchanges, European estuaries, organotin, volatile compound

**Abstract.** The occurrence of volatile organometallic species of tin was evidenced and investigated in three major European estuaries, such as the Gironde (F), the Rhine (NL) and the Scheldt (B/NL), along with the salinity gradient and for all seasons. The most ubiquitous species, observed in surface water, were found to be methylated forms of butyl-tin derivatives ($Bu_nSnMe_{4-n}$, $n$ = 0–3), with concentrations significantly higher in the Scheldt (ca. 75–2000 fmol $l^{-1}$) than in the Rhine (ca. 5–125 fmol $l^{-1}$) and the Gironde (ca. 5–90 fmol $l^{-1}$). Additionally, estuarine anoxic sediments were found to contain large amount of such volatile tin species.

The presence of volatile organic tin compounds is then supposed to result from natural methylation processes of both inorganic metal and anthropogenic derivatives accumulated in the sediments (i.e. tributyltin released from ship antifouling paintings and waste water discharges). These results suggest that microbial mediated and/or chemical methylation mechanisms are likely to produce volatile organotin species in anoxic estuarine environments. The production of volatile organotin species is also mainly dependent on the direct anthropogenic load of butyltin compounds within the estuary and on the residence time of such compounds in the system. Estuarine profiles along with the salinity gradient demonstrate that all investigated estuaries are continuous sources of volatile tin species, although these estuaries present different anthropogenic organotin discharges. In consequence, significant export of volatile tin species to the adjacent coastal waters were found. Finally, the evaluation of the seasonal fluxes of volatile tin species to the atmosphere establishes that volatilisation is a major sink for such compound in estuaries with long water residence time (i.e. Scheldt, Gironde).

## Introduction

The contamination of aquatic environments by organotin compounds (OTs) over the last three decades has led to consider these species as 'global pollutants' (Maguire et al. 1986; Chau et al. 1997). Triorganotin (TOTs)

compounds and more specifically tributyltin (TBT), widely used as biocides, are directly introduced into aquatic systems from the leaching of antifouling paints (Hugget et al. 1992; Quevauvillier & Donard 1996). Further, many other anthropogenic tin derivatives, extensively used in industrial processes, can significantly contribute to the contamination of natural waters via waste water and sewage sludge rejections (Carlier-Pinasseau, 1996).

Despite of the fact that the OTs concentrations in marine environments have decreased significantly during the last decade, as a result of their ban in the early nineties, their accumulation and fate in the sediments still remain a most critical issue for current and future risk assessment. Important sediment resuspension through large natural or anthropogenic physical perturbations (e.g. bioturbation, tide, storms, dredging activities) will directly results in desorption of the OTs and their remobilization to the overlaying water column (Berg et al. 2001). At present, we can consider that OTs are ubiquitous in all compartments of the environment and certainly originate from various and new sources especially in fresh water media (Donard et al. 2001).

As a result, the fate of OTs in estuarine systems is of major concern due to their impact on this specific environment (Donard & Weber 1985; Quevauvillier & Donard 1990). Estuaries are indeed important pathways for the transfer of dissolved and particulate material from the continent to the marine ecosystem. They are mostly highly dynamic systems characterised by the presence of strong physico-chemical gradients. They most often see high biological activity and are areas of strong sedimentological turn over via intense sedimentation and resuspension processes. In this highly variable environments, the transformation of OTs and production of volatile metabolites can occur via different chemical and biological mechanisms (Weber 1999). The chemical fate of these species is usually associated with that of the fine fraction ($<63$ $\mu$m) of the sediment (Maguire et al. 1986; Quevauvillier et al. 1990).

The strong physicochemical gradients and high biological turn over of these environments results in competition of natural dealkylation and methylation processes affecting the fate and behaviour of these species (Maguire & Tkacz 1985; Aldeman et al. 1990; Yozenawa et al. 1994). Laboratory studies have demonstrated the occurrence of methyltin derivatives in the aquatic environments and the potential biomethylation of both inorganic and organic tin by sulphate reducing bacteria in sediments (Gilmour et al. 1985; Yozenawa et al. 1994). Different natural methyl donor species, such as iodomethane, methyl-cobalamine or humic substances are also able to methylate tin compounds to yield to mono-, di-, tri- and tetramethyltin (Weber 1999). Such natural derivatization pathways could result in the formation of fully substituted and volatile tin compounds in the aquatic environment.

Tin hydride ($H_4Sn$) and methyltin hydrides ($Me_nSnH_{4-n}$; $n$ = 0–4) have been indeed detected in algae or microalgae cultures (Jackson et al. 1982; Donard & Weber 1988; Weber 1999). These hydride species were suspected to be formed biologically and are likely to occur naturally in the environment. Craig and Rapsomanikis (1985) suggested that chemical methylation could also take place in the environment and explain the formation of tetramethyltin. The occurrence of methylated butyltin compounds such as tributylmethyltin ($Bu_3SnMe$) and dibutyldimethyltin ($Bu_2SnMe_2$), in the surface of contaminated sediments have also been reported by Maguire et al. (1984, 1986). Recently, Amouroux et al. (2000) have demonstrated the ubiquitous occurrence of such mixed butylmethyltin compounds ($Bu_nSnMe_{4-n}$; $n$ = 1–3) in estuarine and coastal sediments, waters and atmosphere at femtomole per liter levels. These results indicate that natural alkylation processes can also affect anthropogenic tin derivatives and result in the formation of volatile tin compounds. These fully substituted species are then likely to see significant exchanges between the different environmental compartments such as sediment, water, air and in biota.

In this work, the occurrence of volatile tin compounds, their distribution and their potential sources were investigated in three macrotidal European estuaries. Concentrations profiles along with the salinity gradient were used to apply a simple mixing model to evaluate the biogeochemical fluxes of these volatile tin species in the investigated estuaries. Air-water exchange rates of the volatile tin species were also calculated in order to investigate the significance of their atmospheric fluxes versus their estuarine export to adjacent coastal waters.

## Materials and methods

### *Sampling sites and procedures*

Sediment, water and atmospheric samples were collected on board the research vessels *Belgica* (SPPS/FN), *Côte d'Aquitaine* (CNRS/INSU) and *Navicula* (NIOZ), during the EU BIOGEST project cruises on the Gironde (Oct. 1996, June and Sept. 1997, Feb. 1998), the Rhine (Oct. 1996, July and Nov. 1997, March 1998) and the Scheldt (July 1996, Dec. 1997, May and Oct. 1998) estuaries together with the hydrological parameters. These three estuaries are macrotidal systems with very different anthropogenic pressure (Wollast 1988; Kramer & Duinker 1988; IFREMER 1994). The Scheldt and the Rhine estuaries host some of the largest harbours and industrial complexes of Europe and are surrounded by a high population density (Wollast 1988; Kramer & Duinker 1988). On the contrary, most of shipping and industrial

activities taking place in the Gironde estuary have stopped more than 10 years ago. The density of population along its banks is also much lower. It is therefore considered to be one of the few large pristine estuarine area of Europe (IFREMER 1994). The hydrodynamic characteristics, biogeochemical properties and OT loads of the estuarine systems studied are displayed in Table 1.

*Sediments*

Sediments were collected using a box corer in the navigation channel of the Scheldt and the Gironde estuary. Sandy and muddy sediments were sampled in the top 5 cm using a polyethylene cup and were immediately transferred to a gas-tight glass vial (100 ml) without any headspace. The vials were then stored in dark refrigeration at 4 °C and processed on board within less than 2 hours. The water content were respectively about 30% and 15% for the muddy and sandy samples. A vertical fluid mud profile was also performed in the Maximum Turbidity Zone (MTZ) of the Gironde estuary in June 1997, using a specific sampler with a 20-cm vertical resolution. The detailed fluid mud sampling procedure are described elsewhere (Tseng et al. 2001).

*Water samples*

Surface water samples for each estuary were collected for every 2.5 increase of salinity unit with a total span ranging between 0 and 34 (Practical Salinity Scale, International System of Units). Surface waters were sampled at 3m depth with a PTFE coated Go-Flo non metallic sampler (General Oceanic, U.S.A.) to avoid ship and microlayer surface water contamination. After sampling, the collected water was immediately transferred through a silicone tubing into a gas tight PTFE lined 1 l Pyrex bottle until later ship-board treatment.

*Air samples*

Air sampling was performed all along the investigated estuaries during the first cruises, using a laboratory-made gas sampler developed by Pécheyran et al. (1998b). Air was pumped upwind from the ship for $^1/_2$ hour at c.a. 800 ml $min^{-1}$ from the top or the bow of the ship to avoid potential contamination from engine exhausts.

*Table 1.* Hydrodynamic characteristics and biogeochemical properties of the West European estuaries investigated

| Characteristics | | Gironde | Rhine | Scheldt |
|---|---|---|---|---|
| Location | | SW France (Gulf of Biscay) | SW The Netherlands (North sea) | SW The Netherlands/W Belgium (North sea) |
| Type | | Salt wedge to partially mixed | Modified by human impact | Partially to well mixed |
| Length (km) | | 70 | 30–40 | 160 |
| Surface ($km^2$) | | 600 | 100 | 300 |
| Residence time of water/particle | | 1–3 months/years | Days/days | 1–3 months/years |
| Mean Water temperature (°C)[a] | | 7–25 | 7–20 | 6–20 |
| Oxygen saturation range (%) | | 60–103 | 80–110 | 2–98 |
| DOC($\mu$M C) | | 159 (116–214)[b] | 194 (122–342) | 404 (92–857) |
| POC (%) | | 1.5 (0.7–7.5) | 4.3 (0.5–12.1) | 5.0 (1.3–12.3) |
| Mean seasonal | Winter | 1164 | 1068 | 96 |
| Discharge ($m^3/s$) | Spring | 506 | 1714 | 73 |
| | Summer | 498 | 2756 | 35 |
| | Fall | 288 | 1468 | 50 |
| Tidal range (m) | | 2–5 | 2–3 | 2–5 |
| SPM range ($mg\ l^{-1}$) | | 280 (14–1700) | 16 (2–30) | 50 (7–141) |
| TBT ($ng\ Sn\ g^{-1}$) | Sediment | – | 109–172[c] | 67–168[c] |
| TBT ($ng\ Sn\ l^{-1}$) | Water | nd–16[d] | 150[d] | 51 ± 48[e] |

[a]average value and level ranges of parameters listed according to the investigated cruises from 1996 to 1998.
[b]The average concentration shown by the mean value and concentration range listed in parenthesis.
[c]Qeuvauvllier et al. 1991; [d]Quevauvillier & Donard 1990; [e]Ritsema et al. 1998.
nd, not detected.

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**Extraction and cryogenic trapping of volatile tin compounds**

*Sediments*

Volatile tin compounds were extracted from the sediments and fluid mud collected using a purge and cryogenic trap set-up described elsewhere (Amouroux et al. 2000). About 10 g of fresh sediment was transferred in a 50 mL purge vessel made of glass with 20 ml of pre-purged milli-Q water (under He flow, 10 minutes at 300 ml $min^{-1}$). The suspension formed was continuously homogenized with a Teflon magnetic stirrer. The gaseous species were stripped out of the vessel and dried by carrying the gas stream through a moisture trap maintained at –20 °C (mixture of ice and acetone), finally cryofocused in a silanized U-shaped glass trap filled with acid-cleaned silanized glass wool (Supelco) and immersed in liquid nitrogen (–196 °C). Most of the volatile tin compounds extracted from the samples can therefore be concentrated and stabilised in the cryogenic trap. After 30 minutes of purge, the cryotraps were closed with gas tight Teflon caps and immediately transferred to a dry atmosphere cryogenic container (cooled with liquid nitrogen) for a maximum storage period of 1 week before analysis for quantitative recovery (Amouroux et al. 1998).

*Water samples*

The purge and cryogenic trapping device developed and optimized for the extraction of volatile metal compounds from water samples is described in detail elsewhere (Amouroux et al. 1998; Pécheyran et al. 1998a). Within less than 30 minutes after sampling, the water collected was directly transferred from its 1 l Pyrex bottle to a 1.5 l purging vessel under He atmosphere. The water samples were continuously stripped for 1 hour with an He flow rate at 700 ml $min^{-1}$. The volatile compounds were then cryocondensed and stored following the same procedure described above for the sediments.

*Air samples*

The aerosols and water vapour were removed from the air sample pumped using on-line 0.1 $\mu$m quartz filter (Millipore) and moisture trap (–20 °C), respectively. The volatile tin species are cryofocused on-line onto a silanised glass tube packed with silanized glass wool and maintained at –170 °C (Pécheyran et al. 1998b). The cryotraps were closed tightly with Teflon caps and stored in liquid $N_2$ until analysis as described above.

## Standards and reagents

All connections and tubing in contact with the samples were made of Teflon PTFE and PFA. All glass- and plastic-ware were previously cleaned with a biocide detergent and soaked with concentrated nitric acid (10%, PROLABO, France) for 3 days. Millipore milli-Q water was used for rinsing and preparing aqueous solutions. Reagents and standards were prepared with pure analytical grade chemicals, unless otherwise stated. Isooctane (FLUKA, 99.8%), methanol (FLUKA, 99.8%), acetic acid (Merck, 100%), sodium tetraethylborate ($NaBEt_4$, Aldrich, 99%), ammonium and acetate buffer solution (Merck) were used for the derivatization reactions and the preparation of the standard compound solutions. Pure tetramethyl-tin (STREM, 99%), tetraethyl-tin (STREM, 99%), tripropyl-tin chloride and butyl-tin chloride species ($Bu_nSnCl_{4-n}$, $0 \leq n \leq 4$, STREM, 99%) were used as standard compounds for calibration and identification.

## Determination of organotin compounds

The standard settings and methodologies used for both the GC-ICP/MS and CGC-ICP/MS are discussed in detail by Pécheyran et al. (1998b) and Rodriguez et al. (1999), respectively. These techniques present two different chromatographic resolutions with the same detection device. The approach was used to determine non volatile organotin compounds accumulated in sediments and fluid mud.

### *Cryogenic Trapping-Gas Chromatography-Inductively Coupled Mass Spectrometry (CT-GC-ICP/MS)*

Most of the analytical setup and methodology are discussed in detail elsewhere (Amouroux et al. 1998; Pécheyran et al. 1998b). For most of the samples analyzed, we used a direct injection of the cryotraps and standards solutions into the cryotrapping – gas chromatography and ICP/MS (Elan 5000/6000, Perkin-Elmer) system (Amouroux et al. 1998). The high sensitivity and selectivity of the ICP/MS detector coupled to the cryofocusing technique allows to yield method detection limits (DL) below 1 fmol $l^{-1}$ for tin in water samples. The identification of the volatile tin compounds observed in sediment, water and air samples is discussed in detail elsewhere by Amouroux et al. (2000) and Pécheyran et al. (1998b). Tetramethyltin ($Me_4Sn$), trimethylbutyltin ($BuSnMe_3$), dimethyldibutyltin ($Bu_2SnMe_2$) and methyltributyltin ($Bu_3SnMe$) were the major species identified. Hydride forms of butyltin compounds ($BuSnH_3$ and $Bu_2SnH_2$) were also observed by

Amouroux et al. (2000) in some of the samples. These species only account for a small fraction of the total volatile tin species and will not be further discussed in this paper. The purge and cryogenic trapping method, used for this work allowed to perform a physical trapping of all the gaseous tin species present in the sample. The volatile tin compounds trapped and identified can then be defined as the Total Volatile Tin fraction (TVT: total sum of tetramethyltin and methyl-butyltin compounds, $Bu_nSnMe_{4-n}$, $n = 0, \ldots, 3$)).

*Ethylation-Capillary Gas Chromatography- Inductively Coupled Mass Spectrometry (Et-CGC-ICP/MS)*

To evaluate the contamination level by total organotin compounds in the sediments of the investigated areas, a second analytical technique was used. The different steps of this analytical procedure are detailed elsewhere (Rodriguez et al. 1999). The analytes are first extracted from the natural matrix and then derivatized using a $NaBEt_4$ solution, prior to the separation by capillary gas chromatography, which allows a high separation efficiency. Finally, the detection by ICP/MS (HP 4500, Yokogawa) offers high sensitivity with a method detection limit below 1 ng $g^{-1}$ for tin in sediments.

## Results

*Distribution of volatile tin compounds in the different estuarine environments*

*Sediments*

Total organotin concentrations have been determined in both Scheldt and Gironde sediments. The Scheldt sediments were found to be more contaminated by butyltin compounds than in the Gironde with total butyltin concentrations (i.e. total sum of mono- di- and tributyltin concentrations) averaging 38 ng Sn $g^{-1}$ and 12 ng Sn $g^{-1}$ (dry weight), respectively. In a pristine area such as the Gironde estuary, the major organotin compound observed in the sediments was the monobutyltin (MBT, $8.0 \pm 0.4$ ng $g^{-1}$ dry weight) suggesting the efficient degradation of the TBT stored in these sediments, in dibutyltin (DBT) and then MBT. On the other hand, the distribution of the tin species, in the sediments of the Scheldt (Antwerpen Harbour) is characterised by significant amounts of TBT, DBT and MBT with values ranging around $20.2 \pm 0.3$, $21.7 \pm 1.5$, $19.7 \pm 1.5$ ng $g^{-1}$ dry weight respectively. The occurrence of butylated tin compounds mainly originating from ship antifouling paints and industrial effluents (De Mora & Phillips 1997; Bueno 1999) seems then directly related to the anthropogenic load of the investigated area. These values are in the same range than those reported by Ritsema et al. (1998) for the Scheldt estuary.

*Table 2.* Average volatile organotin compounds concentrations in estuarine sediments

| Estuary | *n* | $Me_4Sn$ | $BuSnMe_3$ | $Bu_2SnMe_2$ | $Bu_3SnMe$ |
|---|---|---|---|---|---|
| | | pg $g^{-1}$ dw | | | |
| *Gironde* | | | | | |
| muddy | 6 | n.d. | n.d. | n.d. | $0.005 \pm 0.002$ |
| *Scheldt* | | | | | |
| Sandy | 3 | 0.1–23.3 | 0.1–59.8 | 0.6–45.2 | 13.6–58.8 |
| muddy | 3 | 0.1–4.3 | 0.1–2.5 | 0.8–9.2 | 14.4–79.8 |

n.d. not detected.

The average concentrations obtained for volatile tin compounds in both sediments and fluid mud are presented in Table 2. The concentrations measured were also found to be higher in the Scheldt compared to the results of the Gironde estuary. For the volatile tin content, the methylated forms of butyltin compounds are the major contributors to the total fraction determined. TVT concentrations ranged from 15 to 188 pg $g^{-1}$ and from not detected to 8 fg $g^{-1}$ of dry weight in the Scheldt sediments and the Gironde fluid mud profile respectively. $Bu_3SnMe$ was only observed in the fluid mud profile, with higher values in the bottom of the sampling core. Volatile tin compounds account, nevertheless for only a very small proportion of the total butyltin concentrations recorded in the same samples (c.a. 0.2% and 0.00004% for the Scheldt and the Gironde, respectively).

*Water & air samples*

The average concentrations of the volatile tin species in estuarine surface waters and their seasonal variations are presented in Table 3. The average TVT concentrations are much higher in the Scheldt than in the Rhine and the Gironde and range respectively from 443 to 1120 fmol $l^{-1}$, 28 to 71 fmol $l^{-1}$ and 11 to 49 fmol $l^{-1}$. No significant seasonal trend was observed between the different cruises on the different estuaries. This distribution of TVT concentrations between the three estuaries was expected. Both the Scheldt and Rhine estuaries receive similar organotin contamination loads (Table 1) but the Rhine presents a very different hydrodynamic regime with high river discharge and hence short water residence time (few days). Therefore, the concentrations observed in the water of the Rhine were found to be in the same range than in the Gironde estuary, despite of the fact that this last hydrodynamic system is potentially less exposed to contamination sources. On the contrary, the hydrodynamic regime of the Scheldt and the Gironde estuaries

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*Table 3.* Average volatile tin compounds concentrations in water along European estuaries

| Estuary | *n* | $Me_4Sn$ fmol $l^{-1}$ | | Range | $BuSnMe_3$ fmol $l^{-1}$ | | Range | $Bu_2SnMe_2$ fmol $l^{-1}$ | | Range | $Bu_3SnMe$ fmol $l^{-1}$ | | Range | TVT fmol $l^{-1}$ | | Range |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Scheldt*** | | | | | | | | | | | | | | | | |
| Dec. 1996 | *13* | **20** | | 1–50 | **3** | | < DL[a]–6 | **32** | | 5–81 | **495** | | 117–1174 | **550** | | 131–1262 |
| May 1998 | *12* | **90** | | 19–140 | **7** | | 2–17 | **43** | | 2–28 | **843** | | 254–1983 | **983** | | 307–2214 |
| July 1996 | *14* | **19** | | 7–28 | **3** | | 1–5 | **16** | | 4–61 | **405** | | 75–989 | **443** | | 80–1074 |
| Oct. 1998 | *15* | **67** | | 1–145 | **12** | | 2–31 | **90** | | 19–141 | **951** | | 88–2162 | **1120** | | 190–2416 |
| ***Gironde*** | | | | | | | | | | | | | | | | |
| Feb. 1998 | *12* | **2** | | 2–4 | n.d. | | | n.d. | | | **36** | | 13–48 | **38** | | 2–76 |
| June 1997 | *7* | n.d. | | | n.d. | | | n.d. | | | **11** | | 3–19 | **11** | | 3–19 |
| Sept. 1997 | *11* | **3** | | 2–5 | **5** | | 2–7 | n.d. | | | **41** | | 10–87 | **49** | | 10–90 |
| Oct. 1996 | *17* | **1** | | < DL–4 | n.d. | | | **1** | | 1–2 | **26** | | 2–50 | **28** | | 3–53 |
| ***Rhine*** | | | | | | | | | | | | | | | | |
| Nov. 1997 | *15* | **3** | | 1–7 | **1** | | 1–2 | **2** | | < DL–5 | **38** | | 5–67 | **44** | | 5–65 |
| March 1998 | *13* | **5** | | 2–11 | **2** | | 1–4 | **3** | | 1–5 | **61** | | 22–125 | **71** | | 24–133 |
| Oct. 1996 | *9* | **1** | | 1–2 | **1** | | < DL–1 | **1** | | < DL–2 | **25** | | 8–45 | **28** | | 8–50 |

[a]DL: 0.4 fmol $l^{-1}$ (Amouroux et al. 1998); nd, not detected.

present similar characteristics with long water and particle residence times, which certainly favour the production of volatile tin species via methylation pathways. Nevertheless, due to the high industrialisation and urbanisation of the Scheldt watershed, TVT water concentrations were found more than ten times higher in the Scheldt estuary than in the Gironde. Moreover, these volatile tin species are ubiquitous in all areas studied, even in a pristine estuarine system such as the Gironde.

In the atmosphere above, volatile organotin compounds were determined for at least two season in each estuaries. Only $Me_4Sn$ was detectable in overlying atmosphere of the Scheldt, the Gironde and the Rhine estuaries with concentrations ranging between 1 pg $m^{-3}$ and 20 pg $m^{-3}$. This result demonstrates however that a net transfer of the volatile organotin species to the atmosphere can be observed, at least for the most volatile and probably most stable compounds. This is also a second confirmation of the occurrence of volatile organotin compounds in the natural atmosphere, after the observations made by Pécheyran et al (1998b) in the Gironde estuary.

*Distribution of volatile tin compounds in surface waters along the salinity gradient*

$Bu_3SnMe$ was found to be the major species for volatile tin in all water samples. This ubiquitous compound represents up to 85% of the TVT concentrations. The distribution of $Bu_3SnMe$ for both winter and spring cruises in the Scheldt and Rhine estuaries and winter and summer cruises in the Gironde estuary was plotted against salinity in Figures 1, 2 and 3. The $Bu_3SnMe$ distribution in all seasons displays the same pattern, for the Scheldt and Rhine estuaries, with concentration maxima between salinity 5 and 20 which then decrease at both end-members of the system (lowest and highest salinity). In the Gironde estuary, the $Bu_3SnMe$ concentrations also decrease seaward but without significant trend. Indeed, no significant concentration maxima could be observed in the upper and middle estuary.

These non-conservative distributions demonstrate that volatile organotin compounds are produced and released in the estuary within the intermediate salinity range at the estuarine maximum turbidity zone (MTZ). Below salinity 5, the $Bu_3SnMe$ concentrations decrease upstream but exhibit significant values in the river end-member of the Scheldt (100–300 fmol $l^{-1}$), the Rhine (5–25 fmol $l^{-1}$) and the Gironde (10–20 fmol $l^{-1}$) estuaries. For salinity levels higher than 20, the $Bu_3SnMe$ concentrations decrease seaward following traditional estuarine mixing processes. The average downstream concentrations of $Bu_3SnMe$ (marine end-member) are approximately 180 fmol $l^{-1}$, 10 fmol $l^{-1}$ for the Scheldt and the Rhine, respectively, and below the detection limit for the Gironde. The high SPM load measured in the Gironde and tidal

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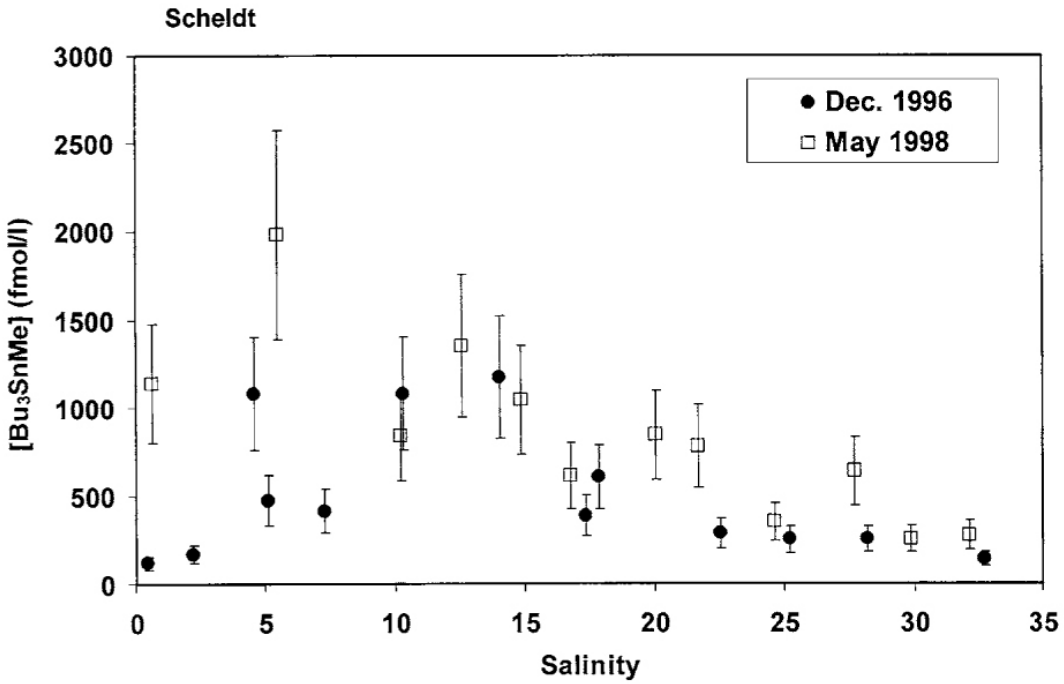


*Figure 1.* Distribution of tributylmethyltin ($Bu_3SnMe$) in surface waters of the Scheldt estuary as a function of salinity in December 1996 and May 1998. Error bars represent a relative standard deviation of 25% obtained from duplicate analysis.

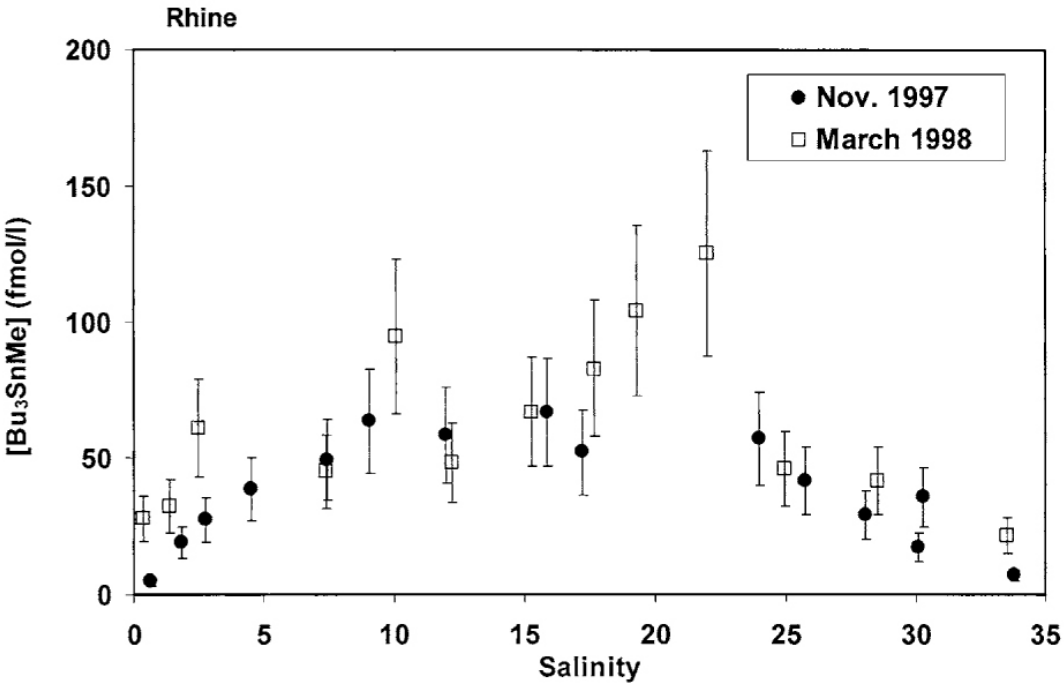


*Figure 2.* Distribution of tributylmethyltin ($Bu_3SnMe$) in surface waters of the Rhine estuary as a function of salinity in November 1997 and March 1998. Error bars represent a relative standard deviation of 25% obtained from duplicate analysis.

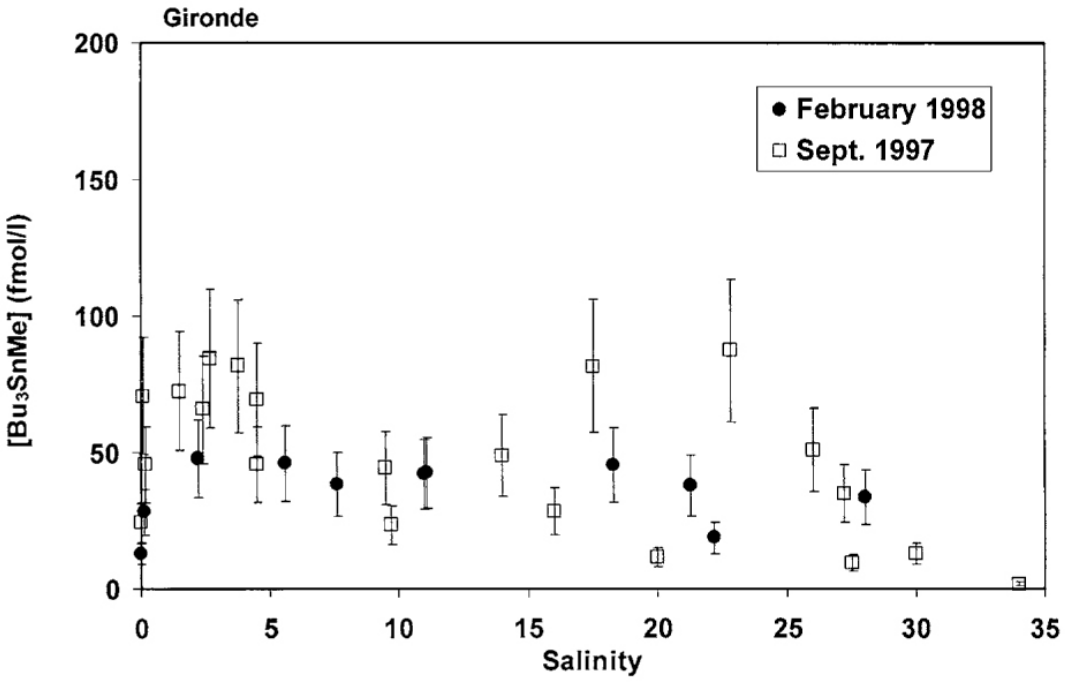


*Figure 3.* Distribution of tributylmethyltin ($Bu_3SnMe$) in surface waters of the Gironde estuary as a function of salinity in September 1997 and February 1998. Error bars represent a relative standard deviation of 25% obtained from duplicate analysis.

cycling could interfere in the $Bu_3SnMe$ distribution in the water column, by trapping and releasing successively volatile organotin compounds (Tseng et al. 2001). A fraction of the volatile tin compounds produced and released in the estuary can then be transported to coastal waters and also be transferred to the atmosphere.

## Discussion

*Sources and formation of volatile tin compounds in estuarine environments*

*Formation of volatile tin compounds in estuarine environments*

Tetramethyltin and three methylated and volatile forms of butyltin compounds were observed in both sediment and water column, with high concentrations in the underlying sediments. These results suggest that the volatile tin species can be produced in anoxic sediments from previously accumulated organotins and then passively diffuse to the water column (Amouroux et al. 2000). The presence of these species demonstrates that natural chemical or biological methylation processes do not only involve inorganic tin but also anthropogenic butyltin derivatives released in estuarine environments mainly by ship antifouling paintings and other urban and

industrial wastes (Carlier-Pinasseau 1996; Bueno 1999; Donard et al. 2001). Indeed, the methylation of trialkyltin species via chemical or biological pathways has been demonstrated during incubations of natural sediments (Guard et al. 1981; Craig et al. 1984). Yozenawa et al. (1994) also evidenced that such mixed methylbutyltin compounds could be produced under sulphate-reducing activity in incubated sediments. Therefore these mixed species could result from the degradation of anthropogenic tributyltin compounds via successive losses of butyl group in the sediment. Its degradation products can then be involved in biological and/or chemical methylation mechanisms (Amouroux et al. 2000).

The relative distribution of the volatile tin compounds observed in estuarine environments could then be derived from the presence of $Bu_3SnMe$. In the Rhine estuary, the extremely short residence time of the water and particles generates a conservative estuarine mixing of chemicals (i.e. dilution). Significant correlation concentrations were therefore found between the different volatile species (c.a. $Me_4Sn$ vs $Bu_3SnMe$: $R^2 = 0.62$; $BuSnMe_3$ vs $Bu_3SnMe$: $R^2 = 0.58$ and $Bu_2SnMe_2$ vs $Bu_3SnMe$: $R^2 = 0.54$). In more complex estuarine systems, with a long water and particles residence time such as the Scheldt and the Gironde, the same trend was observed, but was not statistically significant.

Significant correlation between the average $Bu_3SnMe$ concentrations and several biogeochemical parameters were also found and are presented in Table 1. The $Bu_3SnMe$ average concentrations were found to be anti correlated with the oxygen saturation ($R^2 = –0.76$) and correlated with particulate organic carbon ($R^2 = 0.53$), $CO_2$ partial pressure ($R^2 = 0.87$) and phaeopigments concentrations ($R^2 = 0.88$). These correlation suggest that the formation or the distribution of volatile tin compounds is potentially linked to the biogeochemistry of carbon in the estuary (e.g. respiration and/or degradation) and to the related microbial activities. Inputs of fresh organic carbon from the river and the watershed contribute to the heterotrophy of the estuarine system. Organic matter oxidation occurring in the MTZ leads then to reducing conditions in the surface sediments and in the water column which in turn enhance chemical and microbial mediated methylation mechanisms.

*Riverine inputs and production of volatile tin compounds*

In order to discuss the nature of the biogeochemical processes taking place in the the estuarine $Bu_3SnMe$ profiles, presented in Figures 1–3, a simple direct estuarine mixing model has been applied to the concentrations obtained on the three estuaries. This approach allows us to determine the apparent production and/or consumption of dissolved $Bu_3SnMe$ within the estuary. These calculations were performed assuming that the estuarine system is

*Table 4.* Seasonal river and estuarine $Bu_3SnMe$ fluxes

| Estuary | $F_{River}$ mmol $d^{-1}$ | $F_{Estuary}$ mmol $d^{-1}$ | $F_{Estuary} - F_{River}$ mmol $d^{-1}$ | $F_{Volatilisation}$ mmol $d^{-1}$ |
|---|---|---|---|---|
| *Scheldt* | | | | |
| Dec. 1996 | 0.4 | 5.0 | 4.6 | 143.2 |
| May 1998 | 6.5 | 10.9 | 4.4 | 240.3 |
| July 1996 | 0.6 | 2.8 | 2.2 | 170.6 |
| Oct. 1998 | 0.6 | 16.9 | 16.3 | 305.5 |
| *Gironde* | | | | |
| Feb. 1998 | 2.0 | 5.7 | 3.7 | 15.7 |
| Sept. 1997 | 1.9 | 7.1 | 5.2 | 29.0 |
| Oct. 1996 | 0.5 | 1.6 | 1.1 | 37.9 |
| *Rhine* | | | | |
| Nov. 1997 | 0.6 | 15.2 | 14.6 | 14.9 |
| March 1998 | 4.4 | 18.0 | 13.6 | 7.7 |
| Oct. 1996 | 1.5 | 10.5 | 9.0 | 2.2 |

in a steady state during the sampling period, and that lifetimes of dissolved gaseous tin compounds are in the order of their estuarine residence time. The shapes of estuarine profiles are then indicative of the behaviour of conservative mixing, removal or input and allow to provide estimates of internal production/consumption and export fluxes of dissolved constituents from the estuary (Boyle et al. 1974). In this part, volatilisation fluxes to the atmosphere are not considered and will be discussed in the following part.

In order to make these calculations, we have considered the fluvial flux into the estuary (river flux, $F_{river}$) which is given by $R \times C_o$ and where $R$ = river water discharge and $C_o$ = river end-member concentration. The flux expelled out of the estuary into the ocean (estuarine flux, $F_{estuary}$) is given by $R \times C_{*s}$, where $C_{*s}$ is the apparent river water concentration extrapolated from the $Bu_3SnMe$ concentration *vs.* salinity relationship at the seawater end-member. Under these considerations, the difference between river and estuarine flux would represent the net flux balance (removal or input) of $Bu_3SnMe$ in the estuary. The flux calculations from the mixing model are presented in Table 4.

The seasonal river fluxes obtained were found to be in the range of 1 mmol Sn $d^{-1}$, for the three estuaries investigated except in spring. During this period, the high river discharge generates elevated concentrations of

suspended particulate matter and could results in large riverine inputs of $Bu_3SnMe$ into the estuary as it can be observed (ca. 6.5 mmol Sn $d^{-1}$ and 4.4 mmol Sn $d^{-1}$ for the Scheldt and the Rhine, respectively). Therefore, the $Bu_3SnMe$ brought by the river inputs can not explain the differences in the water concentrations observed between the 3 estuaries (Table 3). Water column concentrations are indeed 10 times higher in the Scheldt estuary than in the Rhine and the Gironde estuaries despite of the fact that the fresh water fluxes are of the same magnitude.

The yields of seasonal estuarine fluxes were always found to be higher than river fluxes with similar values and are in the range of 1–20 mmol $d^{-1}$ for all estuaries and all seasons. These calculations suggest that estuarine systems are continuous sources of $Bu_3SnMe$ to the ocean, and besides that $Bu_3SnMe$ is also produced continuously within the estuary.

The water and particle residence times seem to influence significantly the concentration of volatile tin derivatives in each estuary. The water residence time varies from a few days for the Rhine estuary to several months to years for the Gironde and the Scheldt (Paucot & Wollast 1997; Irigoien & Castel 1997). For the three estuaries studied here, a decreasing trend of the overall average concentration of $Bu_3SnMe$ against increasing river discharge can be observed. The highest TVT concentrations were recorded in the estuary presenting the longest water residence time and with an important particulate material load. Factors such as industrial inputs, residence time and the total organotin concentration level in the sediment appear to simultaneously affect the TVT concentrations in these estuaries. These conditions certainly favour the formation (methylation) and transfer (diffusion) processes involved in the remobilization of volatile tin species in the water column. These reactions may take place on the bacteria associated on the particles of the estuarine system.

These suggestions are consistent with the overall distribution of $Bu_3SnMe$ concentrations against salinity and indicates that these compounds are formed within the MTZ in both the Scheldt and the Gironde estuaries. These estuarine systems display both a strong sedimentation rate and high potential remobilization of the particulate material (Figures 1 & 3) via various resuspension modes. The sediment-water exchanges affecting directly the concentration of volatile organotin compounds are certainly strongly enhanced by the resuspension of the surface sediments.

The three estuaries studied are indeed macrotidal systems with high turbulent mixing processes. The high tidal current friction on the surface sediments certainly contributes to the remobilization of the volatile tin species to the upper water column (Middelburg et al. 1995; Berg et al. 2001).

*Volatilisation fluxes of total volatile tin from European estuaries*

The method used for the saturation ratio and flux calculations is exposed in detail elsewhere (Amouroux et al. 2000).

The relative distribution of the volatile organotin species in the different compartments investigated indicate that they can be transferred to the atmosphere under passive or turbulent diffusion processes. Therefore, air and water measurements of volatile tin species allowed us to calculate fluxes of the TVT at the air-water interface. First, the saturation ratio (SR) estimation is necessary to validate the transfer of volatile species at natural interfaces. For the water to air exchange the saturation ratio (SR) is expressed as the water to air concentration ratio multiplied by the Henry's law constant of each volatile compounds. The Henry's law constants were derived from literature data and molecular properties (Amouroux et al. 2000). If the SR is above 1, volatile organotin compounds are supersaturated in water and are likely to diffuse to the atmosphere. Flux calculations are then based on the Fick's first law of diffusion with the assumption that the concentrations of the studied compounds in the water column are in steady state compared to their transfer velocities at the interface (Liss PS & Merlivat L, 1986). A model developed in an estuarine tidal environment was used in order to calculate accurately the transfer velocities (K) at the air-water interface. K is expressed as a function of the Schmidt number of the studied compounds and the wind speed (Clark et al. 1995). Sc was calculated and corrected for the temperature and salinity as explained by Amouroux et al. (2000). *In situ* wind speed values recorded during each cruise were then implemented to the Clark's model.

Seasonal fluxes of $Bu_3SnMe$ and average flux densities of $Bu_3SnMe$ are presented for each cruise in Table 4 and Table 5. The different volatile tin compounds were found largely supersaturated in all seasons for all estuaries. The flux density of $Bu_3SnMe$ follows the same overall distribution than the water concentrations and exhibits much higher values in the Scheldt estuary than the Rhine and the Gironde estuary. Estimated fluxes are then strongly dependent on the contamination levels of the estuaries and range from 4 to 416 nmol $m^{-2}$ $y^{-1}$ (as Sn). Annual water to atmosphere TVT flux is thus higher for the Scheldt (78 mol Sn $y^{-1}$) than for the Gironde (9 mol Sn $y^{-1}$) and the Rhine (3 mol Sn $y^{-1}$) estuary.

The seasonal $Bu_3SnMe$ volatilisation fluxes for the Rhine estuary are in the same range of order than the previous export flux estimates obtained from the mixing model. The production within the Rhine estuary seems then to be balanced between volatilisation processes and estuarine export to the coastal environment. On the contrary, for the Scheldt and Gironde estuary, the seasonal volatilisation fluxes are 20–80 and 4–30 times higher than the export of $Bu_3SnMe$ to the ocean. In such estuaries with long water residence time,

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*Table 5.* Average water to atmosphere saturation ratios and flux densities of volatile organotin compounds in European estuaries

| Estuary | Saturation ratio | | | | $Bu_3SnMe$ Flux Density |
|---|---|---|---|---|---|
| | $Me_4Sn$ | $BuSnMe_3$ | $Bu_2SnMe_2$ | $Bu_3SnMe$ | (nmol $m^{-2}$ $y^{-1}$) |
| *Scheldt* | | | | | |
| Dec. 1996 | 8260 | 2250 | 20460 | 232290 | 195 |
| May 1998 | 54430 | 8660 | 6850 | 586070 | 327 |
| July 1996 | 9930 | 4000 | 14850 | 310320 | 232 |
| Oct. 1998 | 38060 | 14350 | 91160 | 606530 | 416 |
| *Gironde* | | | | | |
| Feb. 1998 | 1070 | 4475 | 2980 | 17000 | 21 |
| June 1997 | | | | 9300 | 6 |
| Sept. 1997 | 2330 | 5730 | 2140 | 43880 | 16 |
| Oct. 1996 | 650 | 548 | 1180 | 18050 | 9 |
| *Rhine* | | | | | |
| Nov. 1997 | 1390 | 1020 | 1620 | 19183 | 28 |
| March 1998 | 2522 | 2290 | 2110 | 32473 | 14 |
| Oct. 1996 | 830 | 770 | 1100 | 16916 | 4 |

the evasion of $Bu_3SnMe$ to the atmosphere appears then to be the preferential elimination pathway of volatile tin species.

## Conclusion

Although their anthropogenic OT loads was significantly different, all investigated estuaries were found to be continuous sources of volatile organotin compounds to the water and atmosphere. The estuarine mixing model used did not allowed us to establish the effective production of such compounds in the estuaries and probably has led to underestimate the potential sources strengths. On the other hand, it seems that volatilisation pathways are major sink for volatile organotin compounds in estuaries, at least for those having long water residence time. In summary, production of volatile organotin compounds within the estuaries seems therefore mainly driven by the residence time of water and particles, the sediment contamination level and the *in situ* microbial activity related to the anoxic conditions occurring in the sediment. Further investigations are now necessary to describe the production sources and pathways of volatile organotin compounds in estuaries, but also their potential sinks within the system, especially their transfer to the biota.

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# CHAPITRE C

# Réactivité et transfert du mercure dans les environnements aquatiques

# Chapitre C.1.

# Etude des voies de contamination et de bioaccumulation du mercure dans des écosystèmes d'eau douce reconstitués

# Etude des voies de contamination et de bioaccumulation du mercure dans des écosystèmes d'eau douce reconstitués

Le monoéthylmercure (MMHg) est considéré comme un polluant hautement toxique pour l'ensemble des organismes vivants. Il peut être formé dans les écosystèmes aquatiques à partir d'espèces inorganiques du mercure (IHg), d'origine naturelle ou anthropique. Il est en outre sujet à une intense bioaccumulation au travers des réseaux trophiques, entraînant des incidences écotoxicologiques majeures au sein des milieux aquatiques continentaux et marins. En dépit de la dangerosité avérée de ce dérivé organique, les réglementations relatives à la qualité des ressources en eau sont essentiellement basées sur la teneur totale en mercure, ignorant la spéciation chimique de cet élément et sa réactivité potentielle conduisant à la formation du MMHg par des mécanismes naturels et dans des conditions de contamination ambiante chronique ou subchronique. La méthylation du mercure dans les eaux naturelles et les sédiments est principalement stimulée par l'activité microbiologique et notamment par les bactéries sulfatoréductrices. Divers schémas réactionnels de méthylation abiotiques, faisant intervenir des substances chimiques méthylantes d'origine biogénique ou minérale ont également été étudiés en laboratoire. A cela s'ajoutent les mécanismes de réduction du mercure, aboutissant à la formation d'espèces hydrophobes et volatiles telles le mercure élémentaire et le diméthylmercure. Le mercure présente donc une réactivité importante et un dynamisme biogéochimique intense entre les différents compartiments environnementaux. Néanmoins l'importance relative de ces processus et leur compétition en conditions naturelles ainsi que les réactions élémentaires impliquéés ne sont que peu maîtrisés.

L'objectif de ce travail a donc été de développer de manière combinée des outils expérimentaux et analytiques performants, pour tenter de déconvoluer les différents mécanismes chimiques et biologiques définissant la réactivité du mercure en milieu aquatique. Des écosystèmes modèles d'eau douce, réunissant quatre composantes (sédiment, eau, plante, gastéropode) ont ainsi été mis en œuvre et ont permis de réaliser, en conditions contrôlées, la spéciation biogéochimique du mercure à différents niveaux de contamination. Cette approche dynamique, en terme de contamination, nous a permis de discriminer et de caractériser les cinétiques chimiques impliquées dans la distribution et le devenir du mercure dans les microcosmes. Ces expériences soulignent en particulier l'importance des processus de transfert (bioaccumulation/dépuration, volatilisation) et de transformation (méthylation/déméthylation, réduction) du mercure entre les différents compartiments. L'observation de ces différents mécanismes confirme la pertinence et l'utilité de cette démarche réductionniste pour étudier les voies de contamination des écosystèmes aquatiques par le mercure.

# Mercury contamination pathways and bioaccumulation at various contamination levels in aquatic model ecosystems

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**Abstract**
Model aquatic ecosystems have been used to study the natural mechanisms involved in the distribution and transformation of inorganic mercury (IHg) in the different compartments and its interactions with the biota. The applicability of these models to provide real insights for pollution impact and ecotoxicological risk assessments has been demonstrated. Laboratory incubations in indoor freshwater microcosms, presenting a simple biological organization, were carried out at various spiked concentrations (3, 25 and 257 nmol $l^{-1}$ of IHg, as mercuric chloride) and from a single initial contamination of the water column. The different compartments of the model ecosystems (water, sediment, macrophytes *Elodea canadensis* and snails *Lymnaea stagnalis*) were investigated for mercury distribution and speciation during a two-month experimental period. The principal results obtained have evidenced different Hg biogeochemical pathways including biotic IHg methylation and reduction and transfer to the biota. A fast transfer of IHg from the water to the aquatic organisms and to the sediment was first observed. IHg methylation, clearly related to biogenic processes, was also demonstrated in all contaminated microcosms. Finally, gaseous mercury species were determined in the different microcosms and significant biological induced production of elemental Hg (Hg°) and dimethyl Hg (DMHg) was observed. This overall investigation, based on the time courses evolution of IHg and *in situ* produced monomethylmercury (MMHg) concentrations allows to determine uptake and elimination rate constants for IHg as well as the bioaccumulation kinetics of MMHg in macrophytes and snails.

## 1- INTRODUCTION

Monomethylmercury is known to be a very toxic pollutant, formed in the aquatic environments from anthropogenic inorganic Hg and likely to be bioaccumulated through the food web. However, Hg regulations in aquatic systems are mainly based on total Hg concentration, ignoring the speciation of the element and the related dangerousness of the derivatives likely to be produced under real environmental conditions. In order to accurately assess the risk associated with such pollutant, a simple ecotoxicological approach based on the determination of bioconcentration factors (BCF) is not pertinent enough. Toxicokinetic approaches based on batch experiments enable to isolate and measure individual fate processes under various constrained experimental conditions. However, they may also lead to biased estimates, since they focused on one pollutant associated with one single transfer process ignoring other interfering mechanisms likely to buffer or enhance it. Microcosms can thus be used as surrogates for field studies and to identify the environmental significance of laboratory data derived from simpler systems.[1] Furthermore, a large array of toxicity end points such as non observable effect concentrations (NOEC) and lethal concentrations (LC50) are available in the literature for Hg in aquatic species, but these parameters remain highly variable and are not relevant of real environmental conditions due to the various ecotoxicological methodologies used.[2] Moreover, Hg chemical forms present in hydrosystems have clearly distinct physicochemical properties and bioaccumulation capacities, also with considerable variations, according to the environmental factors and biological models studied.[3,4] A mechanistic understanding of the different processes regulating Hg cycle in aquatic environments represents thus a major issue to discriminate the various bioconcentration steps for Hg in aquatic food chains.

Model ecosystems appear thus to be a very attractive tool to better assess the fate and implications of mercury contamination in aquatic environments. The use of reconstituted ecosystems allows understanding in more simple way the natural mechanisms involved in the distribution and transformations of this contaminant in the different compartments and its interactions with the biota.[5,6] Models for metal transfer and transformation pathways can therefore be developed and further validated in real aquatic ecosystems.[7,8] These models can then be used as practical tools to provide real insights for pollution impact and ecotoxicological risk assessments.

In this study, an experimental approach based on the incubation of indoor microcosms was set up, to investigate Hg cycle in reconstructed fresh water ecosystems presenting different organisation level (sediment + water and sediment + water + biota). Experiments have been conducted at various contamination levels and from a single initial spike of inorganic Hg in the water column, ranging from 3.0 to 257.2 nmol $l^{-1}$. Hg species concentrations have been investigated in the different compartments of the model ecosystems, including sediment, water, overlaying air, pulmonate snails (*Lymnaea stagnalis*), and submerged macrophytes (*Elodea Canadensis*) during a two month incubation period. This dynamic approach, in term of contamination, allows then to investigate the

different Hg contamination pathways and to discriminate the chemical kinetics involved in Hg distribution and fate in the controlled model ecosystems. Specific rate constants of the main coupled mechanisms (methylation/demethylation, accumulation/elimination) were derived from this mechanistic approach. Finally, this article reports on the validation of the reconstituted microcosms for investigating Hg contamination pathways in freshwater ecosystems and the potential application for contaminant regulation. Microcosm incubation experiments highlighted Hg transfer and transformation between the different compartments and in particular Hg *in situ* methylation and bioaccumulation in waterweeds and snails.

## 2-MATERIALS AND METHODS

### 2-1 Microcosms experimental set up

Five rectangular glass aquaria (length 80cm, width 40cm, height 45 cm) were filled with 50 kg of artificial sediment (sand 800-2500µm: 65%, Kaolin: 30%, cellulose: 4.75%, Tetramin: 0.15%, $CaCO_3$: 0.1%) and 100 litres of reconstituted fresh water (ISO 6341: $CaCl_2\,2H_2O$ 294 mg $l^{-1}$, $MgSO_4\,7H_2O$ 123.25 mg $l^{-1}$, $NaHCO_3$ 64.75 mg $l^{-1}$, KCl 5.75 mg $l^{-1}$ in deionised water).[9] Sediment formed uniform layer of ca. 10 cm width on the bottom with a water column of 30cm depth. Physico-chemical characteristics of the water are summarized in Table 1. The water column was slightly aerated with bubblers and permanently homogenised using peristaltic pumps and silicon tubing (circulation flow rate of ca.100 ml $min^{-1}$). The systems were then allowed to stabilize for one week in order to remove chloride excess and suspended matter from the water column by bubbling and sedimentation, respectively. The aquaria were finally covered with a glass plate, in order to limit exchange with the atmosphere and to avoid potential contamination between the different systems. The microcosms were kept in temperature-controlled room (20°C) and exposed to a daily photoperiod of 16 hours during all the incubation experiments. Microcosms composition is indicated in Table 1. Biological organisms were introduced, in four of the five microcosms after the water column oxygenation and equilibration period (AQ1-4). The fifth one (AQ5) was incubated only with sediment and water and was defined as an abiotic system, with regard to macroorganisms. Fifty shoots of the macrophyte *Elodea canadensis*, collected in the Oise River (100km north of Paris, France), were planted in the aquaria when a minimum dissolved oxygen level of ca. 6 mg $l^{-1}$ was reached. A total of 230g of freshwater pulmonate snails (*Lymnaea stagnalis*, c.a. 700 individuals) also collected in the Oise River were introduced in each biotic model ecosystems. Over an acclimatisation period of one week, a biocenosis was allowed to develop in the microcosms.

***Table 1 -*** *Microcosms composition and water quality parameters*

| **Microcosms composition** | | **AQ1** | **AQ2** | **AQ3** | **AQ4** | **AQ5** |
|---|---|---|---|---|---|---|
| Water (100 l) | | x | x | x | x | x |
| Sediment (40 kg dw) | | x | x | x | x | x |
| Snails (230 g ww) | | x | x | x | x | |
| Waterweeds (500 g ww) | | x | x | x | x | |
| Spike concentration (nmol Hg $l^{-1}$) | | control | 3.0 | 25.4 | 257.2 | 255.7 |
| **Water quality parameters** | *n* | | | | | |
| pH | *35* | 7.8 ± 0.2 | 7.8 ± 0.2 | 7.8 ± 0.2 | 7.6 ± 0.2 | 7.9 ± 0.3 |
| T (°C) | *33* | 18.7 ± 0.1 | 18.7 ± 0.2 | 18.8 ± 0.1 | 18.9 ± 0.1 | 18.7 ± 0.2 |
| $O_2$ (mg $l^{-1}$) | *33* | 8.3 ± 0.7 | 8.4 ± 0.6 | 8.4 ± 0.6 | 7.8 ± 0.6 | 8.6 ± 0.6 |
| $Cl^-$ (mg $l^{-1}$) | | < 5 | < 5 | < 5 | < 5 | < 5 |
| $PO_4^{3-}$ (mg $l^{-1}$) | | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 |
| $NO_3^-$ (mg $l^{-1}$) | | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 |
| $NO_2^-$ (mg $l^{-1}$) | | < 0.07 | < 0.07 | < 0.07 | < 0.07 | < 0.07 |
| $NH_4^+$ (mg $l^{-1}$) | | < 0.6 | < 0.6 | < 0.6 | < 0.6 | < 0.6 |
| $Ca^{2+}$ + $Mg^{2+}$ (mg $l^{-1}$) | *4* | 57 ± 12 | 46 ± 10 | 50 ± 10 | 51 ± 10 | 37 ± 9 |

### 2-2 Microcosms contamination and sampling

Three different concentrations of inorganic mercury were allocated to the microcosms. The target concentrations were chosen in order to obtain realistic chronicle contamination levels similar to those observed in real freshwater environments. Contamination levels were also selected according to IHg toxicity, in order to preserve the exposed organisms. NOEC values reported in the literature were found to range from 5 to 500 nmol $l^{-1}$, for various aquatic organisms (algae, crustaceans, molluscs, fishes).[2] The experimental constraints, especially the relationships between exposure period, potential losses of contaminant by adsorption on the tank walls and evasion to the atmosphere as well as bioaccumulated concentrations led to remaining contamination levels much lower than the majority of experimental approaches set up using this metal. These experimental conditions allowed thus to reflect in a closer way the real Hg cycling in aquatic ecosystems. The setup consisted of one Hg-free biotic control microcosm labelled AQ1 and three unreplicated treatments with initial nominal IHg water concentrations of 3.0, 25.4 and 257.2 nmol Hg $l^{-1}$ for the three remaining biotic microcosms, AQ2, AQ3 and AQ4,

respectively. The abiotic system (AQ5), only composed of sediment and water, was treated with a nominal IHg concentration of 255.7 nmol Hg $l^{-1}$. IHg was applied as mercury chloride (Strem Chemical Inc) dissolved in milli-Q water. The contaminant solutions were added as a single pulse treatment in the water column. The water circulation was maintained only during the first hour after the contamination and was restarted before each sampling.

Water quality parameters were monitored during both preparatory phase of the experiment and incubation period. Water pH, dissolved oxygen and temperature were daily measured *in situ* using a multi probes recorder (WTW). Hardness, phosphate, chloride, nitrate, nitrite and ammonia, concentrations were monitored (Merck titration kits) daily during the stabilization period and weekly during the incubation experiments.

Samples of water, sediment, snails and waterweeds were collected initially: one day before the contamination and one day after the contamination. The microcosms were then incubated during a two months period, following a sampling frequency of 10 days. Bulk water samples were collected using acid-cleaned Pyrex bottles and were then acidified (1% Ultrapur $HNO_3$), prior to be stored at +4°C until analysis. One surface sediment core (ca. 3 cm) was manually collected using an acid-clean polyethylene tube at each sampling date. About 25 snails and 10 waterweed stems were collected in each microcosm and subsequently rinsed with milliQ water. All the solid samples were directly frozen, freeze-dried and stored at +4°C until analysis. Additional sampling for determining dissolved gaseous Hg species (i.e. Hg° and DMHg) was also carried out in all microcosms, at the end of the incubation experiments. The purge and cryogenic trapping device developed for the extraction of volatile Hg compounds from water samples is described in detail elsewhere.[10,11] Briefly, 1 liter of water sample was continuously stripped for 30 minutes under Helium flow. The volatile species were subsequently cryofocused in a U-shaped glass trap filled with silanized glass wool (Supelco) and immersed in liquid nitrogen (-196°C).

### 2-3 Analytical procedures

The analytical methods used to achieve Hg speciation in water, sediment and biological samples are described in details elsewhere.[12-14] Briefly, Hg speciation in water samples were carried out by direct derivatization with $NaBH_4$, coupled on-line with purge and cryogenic trapping gas chromatography and quartz furnace atomic fluorescence spectrometry detection (CT-GC-AFS). For the solid samples, Hg species were first extracted from the matrix under microwave field using nitric acid and TMAH for sediment and biological tissues digestion, respectively. The extracts were further analysed by CT-GC-AFS, with hydride generation and ethylation as derivatization procedures for biological and sediment samples, respectively. The validity of the analytical

procedures was checked during each series of dosages against certified reference materials (CRM) and spiked water samples. The extraction step and hydride generation method coupled to CT-GC-AFS was applied to a fish muscle CRM (DORM-1, NRCC) and an oyster tissue obtained from an intercomparison exercise (T-38). For sediment analyses, marine sediment CRM (IAEA 356) was employed to validate the extraction-ethylation-CT-GC-AFS procedure. IHg and MMHg values were consistently within the certified ranges for water,[13] sediment and biological matrices.[14] Detection limits of the method, defined as 3 times the standard deviation on blank analyses and expressed as pmol of Hg, were: 2.2 pmol $l^{-1}$ IHg, 0.5 pmol $l^{-1}$ MMHg for water samples; 2.0 pmol $g^{-1}$ IHg, 0.5 pmol $g^{-1}$ MMHg for sediments and 5.4 pmol $g^{-1}$ IHg, 0.4 pmol $g^{-1}$ MMHg for biological tissues (dry weight, dw).

Finally, the analytical set-up and methodology used for the speciation of the dissolved gaseous Hg species are described in detail elsewhere.[10] Briefly, cryogenic traps were desorbed at 250°C, under Helium carrier gas, into a cryogenic gas chromatographic device on-line connected to an inductively coupled plasma mass spectrometer (ICP-MS). Detection limits offered by this method were 100 fmol $l^{-1}$ and 1 fmol $l^{-1}$ for Hg° and DMHg, respectively.

## 3- RESULTS

### 3-1 Water quality parameters

Mean values of the different physicochemical parameters monitored in water columns are presented in Table 1. Water quality monitoring revealed only minor variations over the exposure period, with a good level of homogeneity over all microcosms and in the different layers of the water column. The average pH, water temperature and dissolved oxygen for the five ecosystems along the incubation period were 7.8 ± 0.1, 18.7 ± 0.1 °C and 8.3 ± 0.3 mg $l^{-1}$, respectively. Measured dissolved oxygen concentrations corresponded to average oxygen saturation of 90 ± 4% for the five microcosms. Observed pH conditions were in agreement with the recommended value for the reconstituted water based on ISO 6341 (i.e. pH 7.8 ± 0.2).[9] The different ancillary parameters measured in the water column didn't exhibit significant variations in concentrations over the whole incubation period. Measured phosphates, nitrates and chlorides contents lay in the lower concentration range commonly observed in lakes and rivers and were typical of low impacted freshwater environments (Table 1).[15,16] In addition, the water compartment of the studied laboratory microcosms was representative of weakly eutrophicated freshwater ecosystems characterized by a constant and equilibrated distribution of the nutrients.

### 3-2 Mercury species distribution in water

Hg background measured in the water column before the spike was found to be under the detection limits in the 5 microcosms for both IHg and MMHg (i.e. below 2.2 pmol $l^{-1}$ and 0.5 pmol $l^{-1}$, respectively). Furthermore, the control microcosm (AQ1) didn't exhibit significant increase of IHg content in water during the whole incubation period. Table 2 summarizes the IHg and MMHg concentrations measured in unfiltered water of the 5 microcosms during the two months exposure experiment.

***Table 2 -*** *Mercury species distribution in the water column of the studied model ecosystems*

| **Exposure time** | **AQ 1** | | **AQ 2** | | **AQ 3** | | **AQ 4** | | **AQ 5** | |
|---|---|---|---|---|---|---|---|---|---|---|
| (d) | IHg | MMHg | IHg | MMHg | IHg | MMHg | IHg | MMHg | IHg | MMHg |
| 0 | <dl* | <dl | <dl | <dl | <dl | <dl | <dl | <dl | <dl | <dl |
| 1 | <dl | <dl | 0.446 | <dl | 6.16 | <dl | 97.8 | <dl | 122 | <dl |
| 8 | <dl | <dl | 0.024 | 2.0 | 0.11 | 4.9 | 5.5 | <dl | 35.7 | <dl |
| 20 | 0.006 | <dl | 0.014 | <dl | 0.08 | 2.9 | 2.0 | <dl | 19.4 | <dl |
| 29 | 0.003 | <dl | 0.009 | <dl | 0.06 | 2.6 | 0.9 | 2.1 | 5.7 | <dl |
| 42 | <dl | <dl | <dl | <dl | 0.03 | 4.8 | 0.6 | 7.4 | 3.9 | <dl |
| 52 | <dl | <dl | 0.002 | <dl | 0.02 | 4.8 | 0.5 | 4.1 | 0.8 | 24 |
| | Hg° | DMHg | Hg° | DMHg | Hg° | DMHg | Hg° | DMHg | Hg° | DMHg |
| 52 | 0.6 | <dl | 0.5 | <dl | 1.9 | 0.04 | 12.1 | 0.13 | 5.9 | 0.04 |

* dl: detection limit (2.2, 0.5 and 0.001 pmol $l^{-1}$ for IHg, MMHg and DMHg)

Remaining IHg concentrations in water normalized with the initial contamination in the four spiked microcosms are displayed in Figure 1. Time courses evolutions of IHg contents in both biotic and abiotic systems demonstrate a fast removal of the contaminant from the water column during the first day of exposure. Afterwards, concentrations exhibit a slower decrease and tend to a final steady state value ranging from 0.1 to 0.3% of the initial spike. Irrespectively of the initial contamination, similar patterns of IHg decrease in water are observed in the 3 biotic microcosms (AQ2-4). After 8 days of exposure, less than 1% of the initial spike was

recovered in the water column of the biotic systems. At the opposite, in the abiotic microcosm (AQ5), IHg diminution was much slower, with 14% of the spike still remaining in the water column after one week of exposure (Table 2, Figure 1).

Significant MMHg concentrations were measured in biotic microcosms AQ3 and AQ4 after 8 and 29 days of exposure, respectively (Table 2). MMHg concentrations in water did not change significantly with incubation time and averaged 4.0 ± 1.2 (n=5) and 4.5 ± 2.7 pmol $l^{-1}$ (n=3) in AQ3 and AQ4, respectively. Significantly higher MMHg concentration reaching 23.5 pmol $l^{-1}$ was recorded in the abiotic microcosm (AQ5) after two months of experiment. Nevertheless, the analytical procedure did not allow a quantitative determination of MMHg in the presence of high IHg amount in the sample. Further analyses are thus required to really determine the occurrence of MMHg in water column of AQ4 and AQ5, during the first week of incubation.

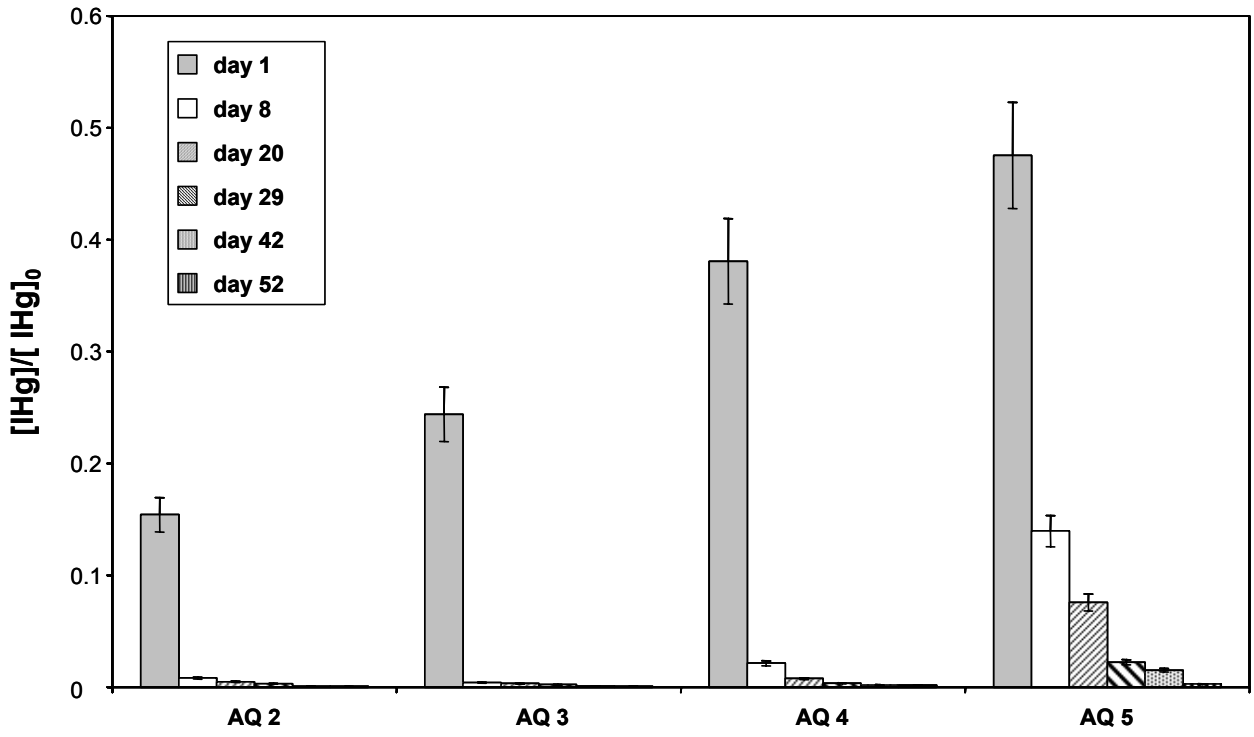


***Figure 1 -*** *Time course evolution of IHg concentrations normalized by initial spiked concentrations* in the water column of the microcosms

Significant amounts of dissolved gaseous mercury (DGM: Hg°+DMHg) were measured at the end of the incubation period, in both biotic and abiotic systems (Table 2). Concentrations ranged from 0.5 to 12.1 pmol $l^{-1}$ and from non detectable to 0.13 pmol $l^{-1}$ for Hg° and DMHg, respectively. The mean error, on duplicate samples, reached 8%, for both volatile compounds. Maximum DGM production was recorded in AQ4 and significant

amounts of Hg° and DMHg were also measured in the abiotic aquarium (AQ5) in the absence of macro-organisms.

### 3-3 Mercury species distribution in sediments

Before the initial spike, IHg background in the sediment averaged 30 ± 7 pmol $g^{-1}$ (dry wt.) in all aquaria and MMHg levels remained under the detection limit (i.e. 0.5 pmol $g^{-1}$). Increasing IHg concentrations, ranging from 60 to 102 pmol $g^{-1}$, were recorded in the sediment of the control microcosm over the whole incubation period (Table 3). After one month of incubation, IHg accumulation in sediment reached 200 pmol $g^{-1}$ in AQ2, 251 pmol $g^{-1}$ in AQ3, 984 pmol $g^{-1}$ in AQ4, and 1349 pmol $g^{-1}$ in AQ5 (Table 3). Accumulation in sediment was significantly higher in the microcosms with high mercury water contamination (256 nmol $l^{-1}$) and no significant difference was observed in the aquaria with lower water contaminations (3.0 and 25.4 nmol $l^{-1}$). IHg content in sediment of the abiotic microcosm (AQ5) tended rapidly to a maximum value at the end of the first week of exposure and then reached a plateau, suggesting that equilibrium was possibly attained within the sediments. In contrast, a tenfold lower accumulation was observed in the biotic system (AQ4) presenting the same initial contamination level after the first week of incubation.

***Table 3 -*** *Mercury species distribution in sediments of the studied model ecosystems concentrations expressed in pmol Hg $g^{-1}$, dw*

| Exposure time | AQ 1 | | AQ 2 | | AQ 3 | | AQ 4 | | AQ 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| (d) | IHg | MMHg | IHg | MMHg | IHg | MMHg | IHg | MMHg | IHg | MMHg |
| 0 | 27.9 | <dl* | 38.1 | <dl | 36.3 | <dl | 25.1 | <dl | 20.8 | <dl |
| 1 | 60.0 | <dl | 43.7 | <dl | 44.9 | <dl | 76.7 | <dl | 71.0 | <dl |
| 8 | 71.1 | <dl | 44.3 | <dl | 53.6 | <dl | 136 | <dl | 1164 | 8.4 |
| 29 | 102 | <dl | 199 | 4.0 | 251 | 8.4 | 984 | 32.1 | 1349 | 14.0 |

* dl: detection limit (2.0 and 0.5 pmol $g^{-1}$ for IHg and MMHg)

MMHg concentrations in sediment remained below the detection limit (< 0.5 pmol $g^{-1}$) during the first week of exposure for the biotic microcosms. In AQ5, significant MMHg concentration of 8.4 pmol $g^{-1}$ (dw) was measured after 8 days of experiment. Net methylmercury production in sediment was then observed in all spiked microcosms after one month of experiment and ranged from 4 to 32 pmol $g^{-1}$ (Table 3).

### 3-4 Mercury species distribution in biota

Hg species concentrations and distribution with exposure time in snails (*Lymnaea stagnalis*) and waterweeds (*Elodea canadensis*) are displayed in Table 4 and Figures 2 and 3. Before the microcosms contamination, IHg background in snails and waterweeds were in the range of 0.06 ± 0.03 (n=3) nmol $g^{-1}$ and 0.07 ± 0.03 (n=3) nmol $g^{-1}$, respectively. Detectable MMHg levels, averaging 0.017 ± 0.003 (n=3) nmol $g^{-1}$ were also measured in the plants of the biotic aquaria. Furthermore, after IHg spike biotic microcosms exhibited similar time courses evolution of IHg concentrations for both snails and plants (Figures 2 and 3). A maximum in IHg contents was recorded after the first week of exposure and then followed by a decrease of IHg concentrations in the organisms, from the first week to the termination of the experiment. The average maximum IHg concentrations in AQ2, AQ3 and AQ4 were 0.7, 3.5 and 25.3 nmol $g^{-1}$ (dw) for the snails and 1.1, 9.5 and 150.0 nmol $g^{-1}$ (dw) for the waterweeds, respectively.

***Table 4 –*** *Mercury species distribution in L. stagnalis and E. canadensis of the studied model ecosystems concentrations in nmol Hg $g^{-1}$, d.w.*

| **Exposure time** | **AQ 1** | | **AQ 2** | | **AQ 3** | | **AQ 4** | |
|---|---|---|---|---|---|---|---|---|
| (d) | IHg | MMHg | IHg | MMHg | IHg | MMHg | IHg | MMHg |
| *L. stagnalis* | | | | | | | | |
| 0 | 0.09 | <dl* | 0.04 | <dl | 0.07 | <dl | nd** | nd |
| 1 | 0.22 | 0.01 | 0.28 | <dl | 1.8 | 0.01 | 14.0 | <dl |
| 8 | 0.20 | <dl | 0.73 | <dl | 3.5 | <dl | 25.3 | <dl |
| 29 | 0.18 | <dl | nd | nd | 1.4 | 0.76 | 14.5 | 5.01 |
| 52 | 0.23 | <dl | 0.16 | 0.03 | 1.3 | 1.08 | 6.8 | 5.15 |
| *E. canadensis* | | | | | | | | |
| 0 | 0.11 | 0.02 | 0.05 | 0.02 | 0.06 | 0.01 | nd | nd |
| 1 | 0.14 | 0.01 | 1.52 | <dl | 9.8 | <dl | 12.7 | <dl |
| 8 | 0.22 | 0.03 | 1.11 | 0.04 | 9.5 | <dl | 150.0 | 0.32 |
| 29 | 0.18 | 0.01 | nd | nd | 2.4 | 0.24 | 53.1 | 0.78 |
| 52 | nd | nd | 0.42 | 0.04 | 2.9 | 0.22 | 22.4 | 0.88 |

* dl: detection limit (5.4 and 0.4 pmol $g^{-1}$ for IHg and MMHg), ** nd: not determined

Equilibrium seems to be reached in AQ3 after the first month, with an average plateau value of 1.4 nmol $g^{-1}$ and 2.6 nmol $g^{-1}$ for snails and waterweeds, respectively. Significant MMHg contents were also measured in the organisms from all exposed microcosms. Similar MMHg concentration patterns were observed in both snails and plants, with increasing MMHg production or accumulation from the first week to the termination of the experiment (Figures 2 and 3). MMHg concentrations in *Lymnaea* and *Elodea* were respectively 0.03 and 0.04 nmol $g^{-1}$ in AQ2, 1.08 and 0.22 nmol $g^{-1}$ in AQ3, and 5.15 and 0.88 nmol $g^{-1}$ in AQ4, after two months incubation period (Table 4).

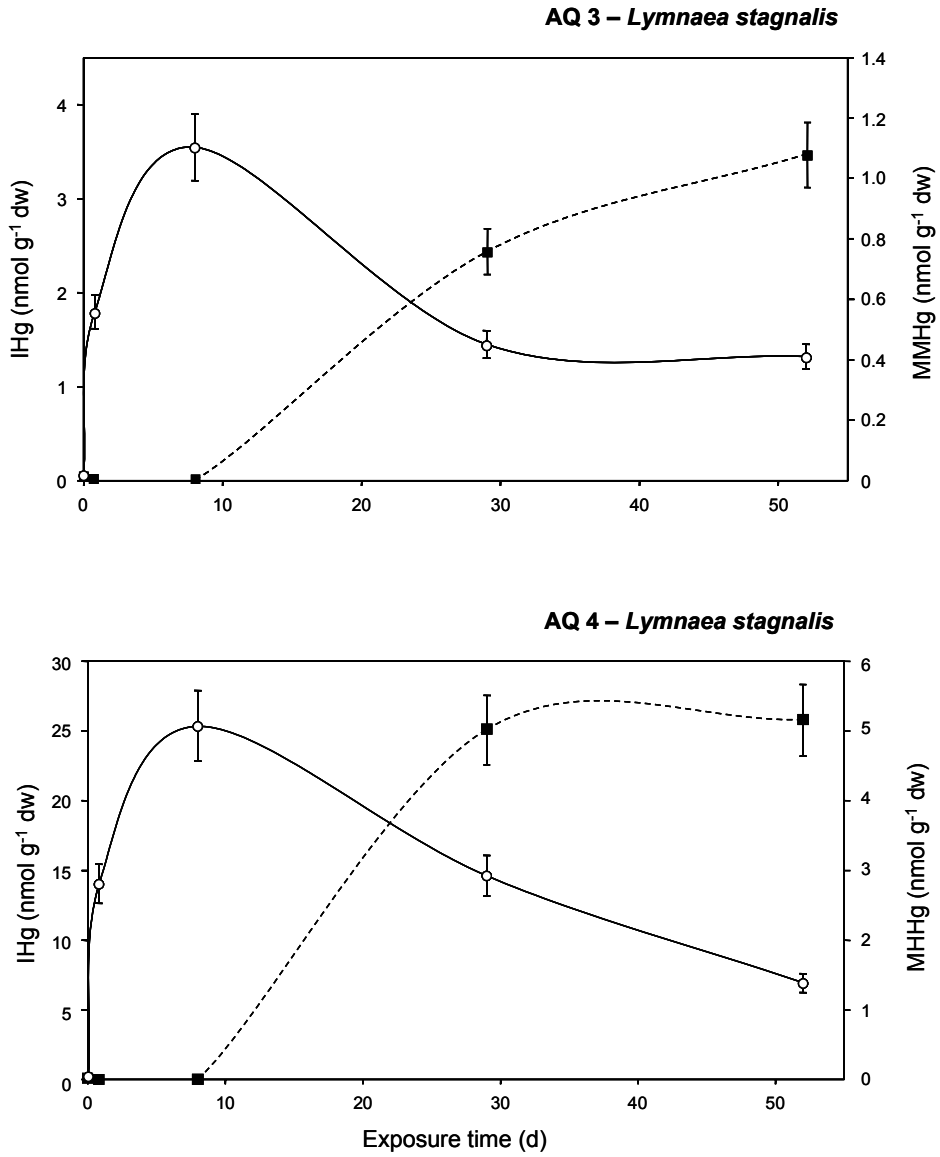


***Figure 2 -*** *Distribution of IHg (—○—) and MMHg (- -■- -·) concentrations in L. stagnalis of AQ3 and AQ4 Errors bars represent a relative standard deviation of 10% obtained from duplicate analyses*

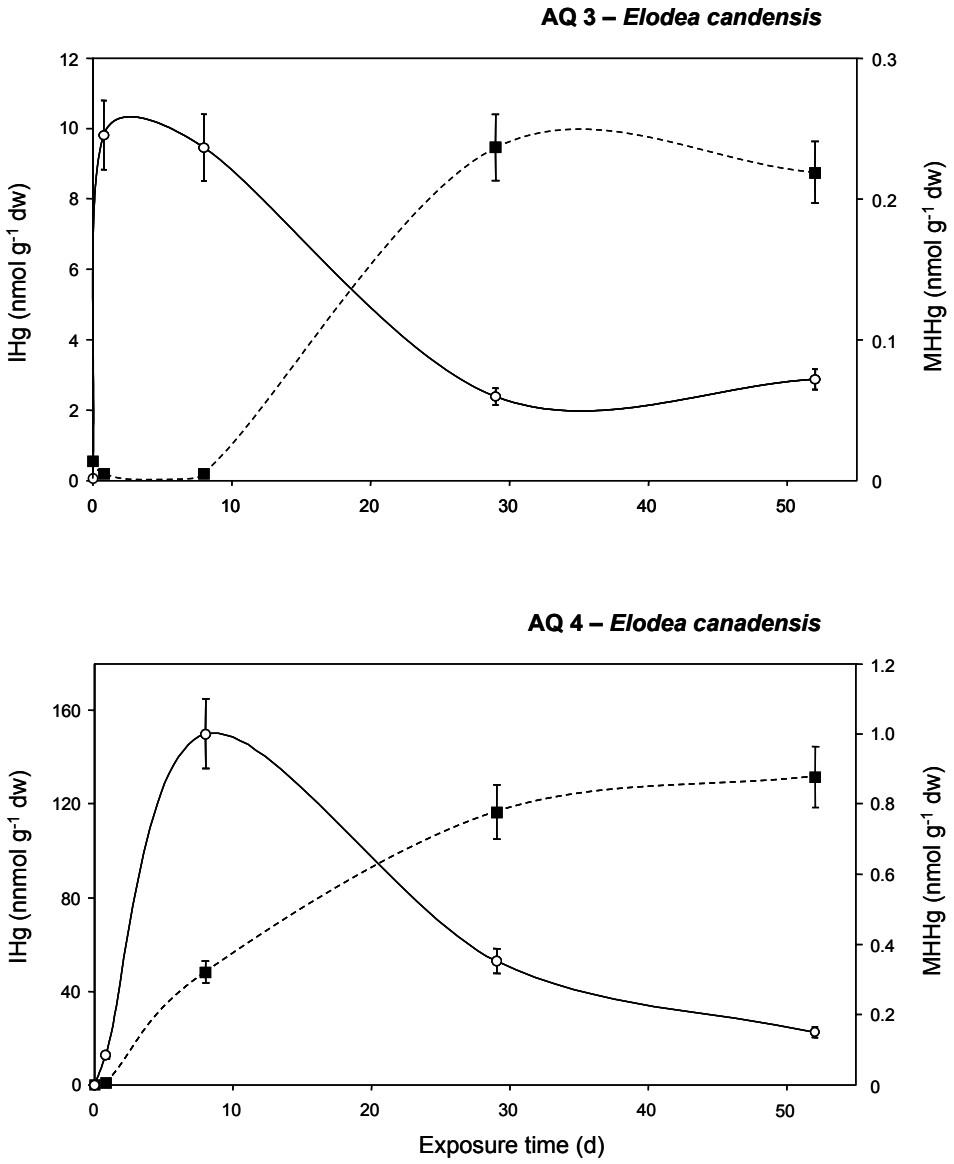


***Figure 3 -*** *Distribution of IHg (—○—) and MMHg (- -■- -·) concentrations in E.canadensis of AQ3 and AQ4. Errors bars represent a relative standard deviation of 10% obtained from duplicate analyses*

## 4- DISCUSSION

### 4-1 Overall mercury distribution

Mass balance calculations indicate that the total mercury budget was unfortunately not recovered in all microcosms. After the first day of exposure, only 50% of the spike was recovered in the contaminated systems, certainly due to significant adsorption processes on the tank walls or to reduction of IHg into Hg° leading to

potential losses by evasion. Nevertheless, since there was no turbulence of the water column during the experiment, losses by volatilization did not seem to be significant. After one month of incubation, AQ4 and AQ5 exhibit about 70% of recovery. Owing the poor resolution and representativeness of the sediment sampling (i.e. one single core at each sampling date), the Hg burden in this compartment appears then to be stained with important errors. However, the comparison of the Hg distribution in the different compartments of the studied ecosystems still remains correct since similar recoveries were obtained at the beginning of the exposure period. In addition, after one month of exposure, a change of one order of magnitude in the sediment concentrations did not modified drastically the Hg partitioning and the relative importance of the different compartments of the model ecosystems.

Figure 4 depicts the relative distribution of total mercury (i.e. HgT= IHg+MMHg) burdens in the different compartments of the microcosms AQ3 and AQ4. Mercury partitioning pointed out that biota represents the main sink for IHg within the first week of exposure. The contaminant is then partially removed from the biota or transformed *in vivo* and finally transferred to the sediment. After one week of exposure, HgT proportion taken up by the organisms to the total Hg budget reached 56% in AQ3 and 68% in AQ4, among which 43% and 60% were recovered in *E. canadensis*. Finally, after one month of experiment only 3.5% and 11.5% of the Hg burden were recovered in the biota of AQ3 and AQ4. Simultaneously to that, the HgT burdens in sediment increased with incubation time and represented up to 96% and 88% of the total Hg budget in AQ3 and AQ4 after one month of incubation. The same pattern, shifted in time with respect to the initial contamination pressure, was also observed in lowest contaminated microcosm (AQ2).

Nevertheless, significant accumulation and transformation processes of IHg were observed in both snails and waterweeds for the three exposed aquaria. MMHg proportion in *Elodea* after one month of exposure is 0.04, 0.19, 0.13% of the total Hg burden in AQ2, AQ3 and AQ4, respectively, and 0.03%, 0.47% and 0.64% in *Lymnaea*. Sediments were also identified as a significant methylation source, with MMHg content averaging 3.0 ± 0.6% of the Hg burden in the 3 biotic microcosms and 1.0% in the abiotic aquarium (AQ5), after one month of experiment. Finally, cumulated MMHg production measured after one month of incubation in all compartments reached 2.5, 4.3, 3.6 and 1.0% of Hg burden in AQ2, AQ3, AQ4 and AQ5, respectively.

Furthermore, the determination of volatile Hg species allowed us to estimate evasion fluxes of mercury from the microcosms. A simple diffusion model at the air-water interface was applied to our data[17] and led to flux densities of 6, 19, 121 and 59 pmol $m^{-2}$ $h^{-1}$ in AQ2, AQ3, AQ4 and AQ5, respectively. These results fell in the range generally observed for real freshwater environments such as lakes and rivers.[18-20] The high DGM concentrations recorded in AQ4 and AQ5 indicate that the experimental conditions (i.e. high IHg content and water temperature) are likely to balance the non-turbulent circulation regime of the water column, resulting in enhanced transfer rates. Overall, the volatilization mechanisms did not appear to be of primary importance in the overall Hg budget of the studied microcosms (Figure 4).

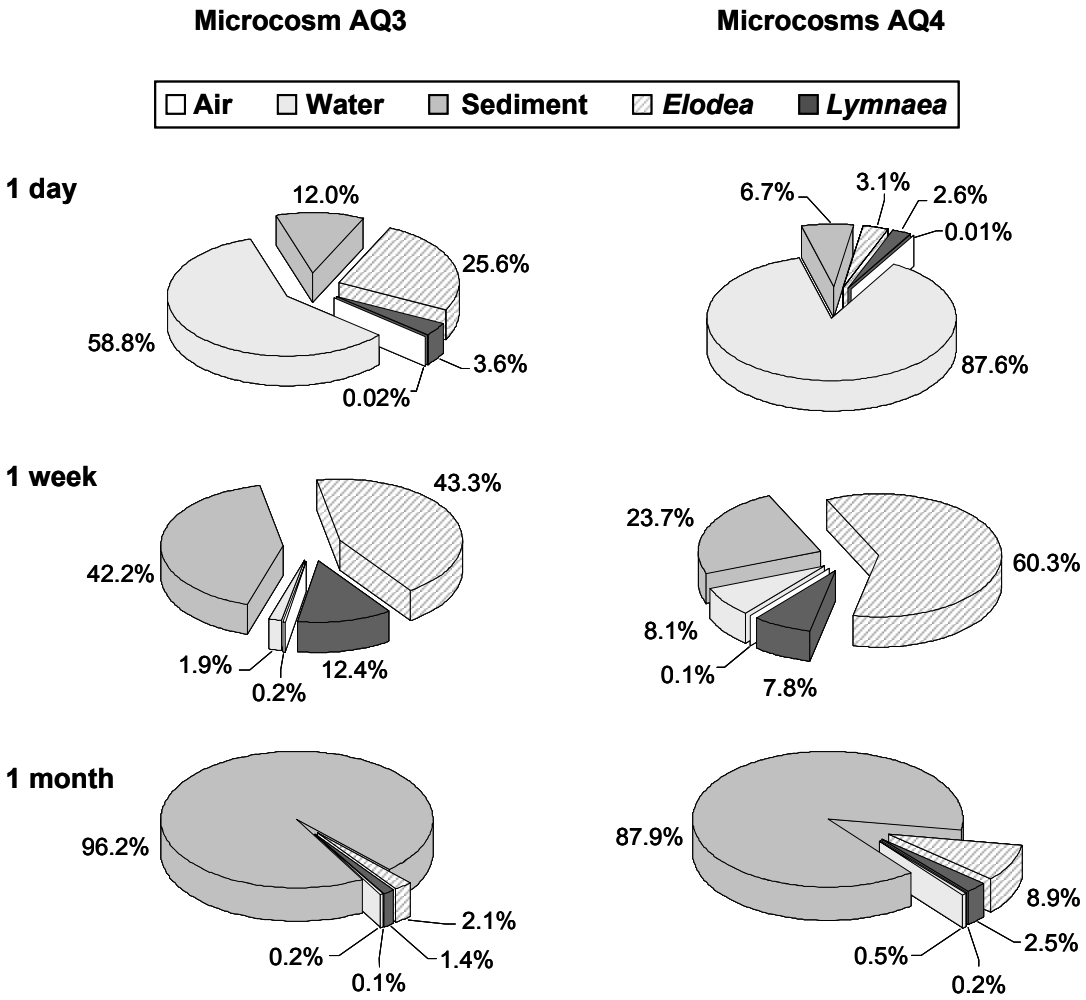


***Figure 4 -*** *Total Hg burden distribution in the different compartments of the microcosms AQ3 and AQ4 after 1 day, 1 week and 1 month of exposure*

Our experimental conditions, in simulating a non-turbulent freshwater ecosystem, probably constrained the volatilization processes to passive diffusion mechanisms. Mercury evasion fluxes at the air-water interface, integrated for the whole incubation period, only represented from 0.1 to 0.7% of the initial IHg spike in the exposed microcosms. Nevertheless, volatilization pathways still remain of importance since they are closely linked to the driving mechanisms in Hg cycle. DGM production is thought to be predominantly microbially mediated and linked to both methylation and demethylation mechanisms occurring at the water-sediment interface.[21,22] The occurrence of significant amounts of both Hg° and DMHg measured in the water column of the abiotic microcosm is also consistent with the assumption that DGM production in freshwater systems is

regulated by a combination of photochemical and biological processes.[20,23] Finally, the production of dissolved gaseous Hg species illustrated the complexity of the reconstituted ecosystems in term of Hg transformation and transfer and also evidenced the relevance of the studied microcosms with respect to the natural Hg cycling.

### 4-2 Inorganic mercury contamination pathways

The IHg fraction measured in the present work includes a pool of various inorganic and organic complexes. In the absence of sulphide, the speciation of inorganic mercury in freshwaters is usually dominated by uncharged chloride and hydroxide complexes.[15] However, organic complexes are readily formed through strong associations of Hg with humic matter and especially thiol groups (-RSH).[24,25] The MINEQL program (ERS, USA) was run in order to assess the theorical chemical speciation of Hg in the water column under our initial experimental conditions. According to water composition and quality parameters (Table 1), neutral Hg hydroxide ($Hg(OH)_2$) and Chloride complexes (HgClOH, $HgCl_2$) were found to be the predominant forms of the contaminant in the water column at the beginning of the experiments. Since no attempt was made to quantify the organic carbon contents in the water column of the microcosms, the contribution of Hg organic complexes was obviously underestimated.

*Inorganic mercury elimination from water*

Several biogeochemical mechanisms regulating the behaviour of the contaminant were evidenced in the studied microcosms. Despite of the lack of measurements during the early stages of the exposure experiments, IHg breakdown in water column of the biotic aquaria can be decomposed into two successive first-order decay kinetics Figure 5 compares the kinetics fitted by first-order exponential decay models for AQ4 and AQ5.

This simplified kinetic approach allowed to discriminate a biphasic removal process of IHg from the water column of the biotic microcosms (AQ2, AQ3 and AQ4). First, fast removal of IHg from the water column was observed within the first week of exposure, with a mean removal rate of ca. 0.025 ± 0.004 $h^{-1}$ and half-lives of IHg ranging from 23 to 33 hours. Thereafter, from the first week to the end of the first month of experiment, IHg release is characterized by a tenfold slower rate (i.e. 0.0023 ± 0.0007 $h^{-1}$). At the opposite in the abiotic aquarium (AQ5), it IHg could be preferentially binded to the particles and subsequently transferred to the sediment by settling. This transfer process can be described by a single first-order decay of IHg in water, with a removal rate of 0.0036 ± 0.0005 $h^{-1}$, similar to the second kinetics observed in the biotic microcosms.

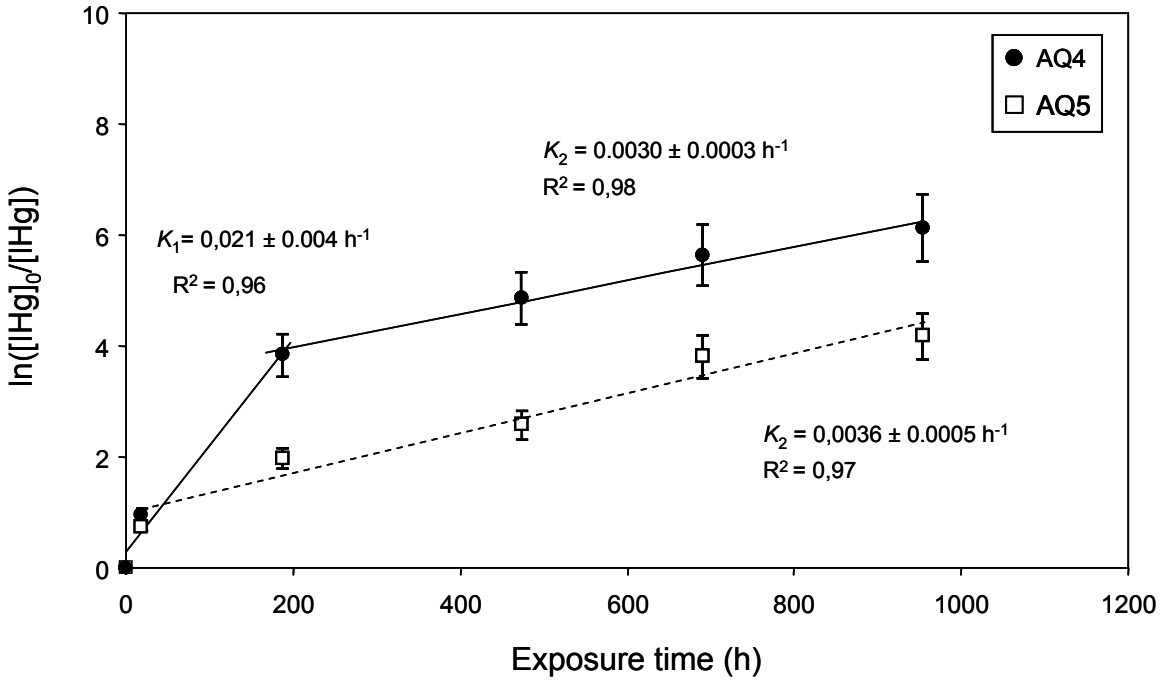


***Figure 5 -*** *Comparison of the removal kinetics of IHg from the water column in the biotic (AQ4) and abiotic (AQ5) microcosms (initial spiked concentrations $[IHg]_0$ at 257.2 and 255.7 nmol l$^{-1}$, respectively)*

*Inorganic mercury biosorption and bioaccumulation*

Rapid biosorption and bioaccumulation mechanisms could account for the observed non-linear kinetics of IHg decay from water column. IHg distribution in the biota always exhibit the same pattern characterized by rapid IHg uptake to a maximum level recorded after one week of exposure and thereafter followed by IHg elimination (Figures 2 and 3). Firstly, the contaminant was transferred from the water column to the biota. In a second time, biological regulation of IHg occurred and led to significant elimination of biogenic IHg and subsequent transfer to the particles and to the sediment. IHg accumulation in biota was expressed as an accumulation factor (AF), defined as the ratio between IHg concentrations in organisms and water. This factor, expressed in ml g$^{-1}$ fresh weight (fw), illustrates the affinity of an aquatic organism for a metal in terms of the equivalent quantity of water holding the same quantity of metal.[26] Accumulation capacities of IHg were found slightly higher for waterweeds than for snails. AF values calculated after the first week of exposure were 3084, 3258 and 460 for *Lymnaea* and 3823, 5423 and 1637 for *Elodea* in AQ2, AQ3 and AQ4, respectively. At the termination of the two months experiment AFs slightly increased and reached 6563, 5479 and 1342 for *Lymnaea* and 10560, 7184 and 2637 for *Elodea* in AQ2, AQ3 and AQ4, respectively. These results are consistent with previous study[27] showing high

bioaccumulation capacities of both IHg and MMHg by waterweeds (*Elodea densa*) and similar AF values for IHg (i.e. 5000 ml $g^{-1}$).

Furthermore, several authors[28-30] have demonstrated that rooted macrophytes are likely to take up heavy metals via their roots submerged in sediments but also readily absorb chemicals from the water column through their leaves and accumulate them to the cell walls and vacuoles. Accumulation rate constants in *Elodea* for the three biotic microcosms were then estimated from the initial data of the exposure experiments. IHg contents in the waterweeds were normalized by the IHg burden in water and plotted against incubation time. The slope finally represents the estimated accumulation rate constant expressed in $h^{-1}$. Thus *Elodea* efficiently accumulated IHg, with rate constants of 0.053 ± 0.005 $h^{-1}$, 0.025 ± 0.002 $h^{-1}$ and 0.046 ± 0.004 $h^{-1}$ for AQ2, AQ3 and AQ4, respectively. These values are in the same range of magnitude than the removal rate constants calculated for IHg in the water column (i.e. 0.025 ± 0.004 $h^{-1}$, on average), which in turn confirms the role of the waterweeds as the main sink for IHg over the first hours of exposure. Furthermore, similar accumulation rate constants of IHg in *Elodea* were found in the three biotic microcosms. This last point suggests that IHg transfer from the water column to the waterweeds is driven by the same mechanism independently of the initial contamination pressure.

For the snails, IHg accumulation rate constants calculated on the same basis were seven time lower and reached 0.0074 ± 0.0002 $h^{-1}$, 0.0034 ± 0.0002 $h^{-1}$ and 0.0058 ± 0.0004 $h^{-1}$ over the same exposure period in AQ2, AQ3 and AQ4, respectively. There was also a significant positive correlation between IHg concentrations (fw) in snails and waterweeds (average $R^2$= 0.95±0.02), over the three biotic microcosms and the whole incubation period. This suggests that IHg accumulation in *Lymnaea* may originate mainly from trophic transfer. Pieczynska[31] reported that *Lymnaea* not only feed on periphytic algae colonising *Elodea* surface but also significantly graze its leaves. Owing the high IHg concentrations recorded in *Elodea,* macrophytes and associated periphyton can represent a significant contamination source for *Lymnaea*. Thus bioaccumulation capacity can contribute to enhanced cumulative transfer of IHg into herbivorous snails predating on waterweeds.

Then, from the second week of exposure to the end of the experiment, significant amounts of IHg are removed from the biota or transformed within the organisms, suggesting the occurrence of reversible biosorption or elimination processes. For the three biotic microcosms, IHg removal averaged 76 ± 8% of the maximum IHg content accumulated in the waterweeds and 71 ± 8% in the snails. These results are consistent with previous studies reporting that *Elodea* not only absorbs heavy metals, but also releases them into the water column when they decay,[29] and may even release them from living tissues. For example, Everard and Denny[32] found 90% of unbound lead taken up by *E. canadensis* in the first hour of exposure and returned to the water column within 14 days. Non linear curve fitting of the data from day 8 to the termination of the experiment allowed to describe the IHg decrease in the organisms as a first-order exponential process. Elimination rate constants expressed in $h^{-1}$ were (9.9 ± 4.8)×$10^{-4}$ in AQ2, (9.1 ± 2.6)×$10^{-4}$ in AQ3, (8.5 ± 2.0)×$10^{-4}$ in AQ4 for *Lymnaea* and (11.3 ± 1.8)×$10^{-}$

$^{4}$ in AQ2, (12.8 ± 3.7)×10$^{-4}$ in AQ3, (12.6 ± 3.9)×10$^{-4}$ in AQ4 for *Elodea*. Half-lives of IHg in the organisms, calculated on the basis of regression analysis of IHg loss according to first-order kinetics, averaged 32 ± 2 days for the snails and 24 ± 2 days for the waterweeds in the three biotic microcosms. Despite the fact that similar rate constants were obtained for both *Lymnaea* and *Elodea*, mechanisms involved in the elimination of the contaminant in biota have to be different with respect to the physiology of the investigated species. IHg elimination in waterweeds may be due to reversible biosorption or physical diffusion mechanisms (e.g. osmosis) whereas depuration of IHg in snails may be related to excretion process but also to transformation of the contaminant through metabolisation mechanisms (e.g. methylation).

Nevertheless, owing the homogeneity of the calculated rate constants of accumulation and elimination for *Lymnaea* and *Elodea*, similar mechanisms were evidenced between the three biotic model ecosystems, irrespectively of the initial contamination pressure. This kinetic approach accounts for the development of reproducible environmental conditions controlling IHg distribution and speciation in the reconstituted ecosystems.

*Inorganic mercury adsorption in sediments*

During the first week of incubation, IHg concentration rapidly increased in the sediment of the abiotic microcosm (AQ5), simultaneously to the removal of the contaminant from the water column (Table 3). Afterwards and up to the first month of exposure, accumulation rate of IHg decreased sharply and an average plateau value at c.a. 1257 pmol g-1 in IHg concentrations seems to be reached over this period. At the opposite, IHg accumulation in the sediment of the biotic systems is shifted in time with respect to its removal in water, and became significant after one month of exposure. The relative affinity of IHg for water and sediment compartments can be described by the apparent partition coefficient ($K_p$) expressed as the ratio $[IHg]_{sediment} / [IHg]_{water}$ in l kg$^{-1}$. After one month of exposure, log $K_p$ values for IHg reached 4.3, 3.6, 3.0 and 2.4 in AQ2, AQ3, AQ4 and AQ5, respectively. For MMHg, log $K_p$ values averaged 4.0 ± 0.4 in the five microcosms. These values are in the same range of order but slightly lower for IHg, than average reported log $K_p$ of 5.0 ± 0.2 and 3.9 ± 0.1 for IHg and MMHg in freshwater ecosystems, respectively.[33,34] Since no discrimination between particulate and dissolved phases have been achieved for the water samples, the $K_p$ values reported here only give a rough indication of IHg partitioning. Furthermore, after one month of exposure, IHg partitioning between sediment and water in the biotic microcosms did not reach equilibrium due to the faster biosorption and bioaccumulation processes. Increasing IHg contents in sediment seems to be related to the removal of the contaminant from biota through excretion and elimination mechanisms. Owing the role of biota and particularly the waterweeds as the main sink for IHg in the water column, the transfer of the contaminant from the water compartment including living organisms to the sediment is strongly linked to biological turnover occurring in the model ecosystems.

### 4-3 Methymercury contamination pathways

*Inorganic mercury methylation in sediment and water*

Due to the high water temperature (c.a. 18°C) and the important biomass allowed to grow in the microcosms, relatively to the total volume of water, our experimental conditions favoured the development of anoxic sediment layer and were thus suitable for the occurrence of methylation mechanisms. The transformation of inorganic mercury to methylmercury in aquatic environment is known to be mediated by biological processes involving key methylators such as sulphate reducing bacteria (SRB).[35,36] Furthermore in aquatic systems, sediment and more precisely the oxic/anoxic interface represents the primary site of Hg methylation.[37-39] One of the mediators of this mechanism is the concentration of IHg substrate.

MMHg production was evidenced in the sediments of the five microcosms after one month of exposure (Table 3). Measured concentrations then corresponded to net methylation rates of 0.14, 0.29, 1.11 and 0.48 pmol $g^{-1}$ $d^{-1}$, for the two months exposure period in AQ2, AQ3, AQ4 and AQ5, respectively. MMHg production increased together with the contamination pressure in the biotic systems, whereas in the abiotic microcosm (AQ5) methylation potential was slightly lower than in AQ4 but occurred early. Nevertheless, microcosm AQ5 was not strictly abiotic with regards to micro-organisms and bacterial and algae biocenoses, mainly involved in Hg methylation, were allowed to develop in both sediment and water compartments as well as on the tank walls.

Despite the variability of MMHg concentrations recorded in the sediments, the percentages of MMHg to the total mercury (i.e. IHg+MMHg) were in the same range of order and reached 1.96, 3.24, 3.16 and 1.03%, in AQ2, AQ3, AQ4 and AQ5, respectively. These results are in agreement with previous studies, where a global relationship between IHg and MMHg concentrations in sediments from various aquatic environments has been established. It is usually admitted that about 1% of the total Hg is under the methylated form in natural unimpacted environments.[40]

Occurrence of MMHg was also evidenced in the water columns of both biotic and abiotic microcosms (Table 2). Although Hg methylation predominantly occurs in sediment, this mechanism has also been observed to a lesser extent in water by several authors.[21,36,41,42] Hence, biomethylation within the sediments and abiotic methylation in water column could both account for the observed levels. MMHg water concentrations measured in the biotic systems ranged from 0.5 to 7.4 pmol $l^{-1}$ and corresponded on average to 9% and 1% of the total water Hg content in AQ3 and AQ4, respectively. These results are in good agreement with typical values observed in natural freshwater systems, where MMHg levels generally range from 0.5 to 5.0 pmol $l^{-1}$ and represent from 1 to 10% of total Hg content.[43-46] Nevertheless, average MMHg water concentrations were similar in both AQ3 and AQ4, irrespectively of the contamination pressure, suggesting that equilibrium was possibly attained within the water columns. Remaining MMHg concentrations in water are thus the result of combined mechanisms such as

coupled methylation/demethylation reactions and bioaccumulation by snails and waterweeds. On the other hand, higher MMHg concentration was recorded in the water column of AQ5, in absence of snails and waterweeds (Table 2). Among the various factors influencing Hg methylation, the supply and availability of Hg are key parameters. Thus, higher IHg contents available for methylation were recorded in both sediment and water compartments of AQ5 with comparison to the biotic aquaria and could explain the increase in MMHg water concentration (Table 2 and 3). Under these experimental conditions (AQ5), microorganisms were allowed to grow up and contributed to enhanced MMHg production and then accumulation in the water column without any competitive bioaccumulation of organic mercury species by macroorganisms.

*Methylmercury bioaccumulation and trophic transfer*

Simultaneously to the removal of IHg from the biota, MMHg concentrations in both waterweeds and snails became significant after 1 month of experiment (Table 4). However MMHg burden accumulated in the biota at the end of the experiment exhibited pronounced differences between the two species, according to the biomagnification process of Hg through the food chain.[25,47,48] Thus, after two month of exposure MMHg represented from 17 to 45% of total Hg burden (i.e. IHg + MMHg) in *Lymnaea* and from 4 to 8% in *Elodea*, over the 3 biotic microcosms. Additionally, AF values for MMHg estimated at the end of the exposure period in the waterweeds were similar to those calculated for IHg and averaged 7070 ml $g^{-1}$, fw. Previous studies have demonstrated the higher affinity of rooted macrophytes for MMHg than for IHg.[27,28] In the present experiments, initial spiking conditions first favoured IHg uptake in macrophytes. Thereafter the occurrence of significant methylation mechanisms probably led to competitive accumulation processes in *Elodea* resulting in similar bioaccumulation capacities of IHg and MMHg. The proportion of MMHg in waterweeds at the end of the exposure experiment remained constant in the 3 biotic microcosms and represented on average 7 ± 3% of the IHg content. This last point suggests that IHg and MMHg distribution in *Elodea* have reached equilibrium after one month of exposure. Biotic and abiotic demethylation processes such as microbially mediated mechanisms in periphytic mats, photodemethylation in the upper layers of the water column and intracellular biotransformations could potentially reduce the net MMHg production and explain the observed plateau tendency for both IHg and MMHg distribution in waterweeds (Figure 3).

Moreover, rooted macrophytes have been identified as a relevant Hg methylation site in providing a suitable environment for dense periphyton growth including methylating microorganisms.[49] Thus *Elodea* potentially represented a significant MMHg source for *Lymnaea* mainly feeding on periphyton colonising macrophytes. Snails exhibited enhanced MMHg accumulation with AF values averaging 52000 ml $g^{-1}$ at the end of the 2 months exposure period. The MMHg burden measured in the water column can not only account for the observed contents in snails. MMHg was taken up by snails mainly through their diet. These results are

supported by a mean biomagnification factor (BMF= $[MMHg]_{Lymnaea} / [MMHg]_{Elodea}$ , fw) of 9 between snails and waterweeds, in AQ3 and AQ4. This value is consistent with reported BMFs for MMHg in typical freshwater ecosystems, where MMHg concentration will increase by a factor of 3 to 10 as it moves from one level to the next up the food chain.[50] Finally, experimental set up and sampling resolution did not allow to assess the Hg methylation and associated demethylation kinetics. Furthermore, investigation on Hg speciation and distribution in the reconstituted ecosystems still remains a challenge due to superimposition of accumulation and depuration mechanisms as well as biotransformation processes and trophic transfer.

**5- CONCLUSION**

Major Hg contamination pathways have been evidenced in the reconstructed freshwater ecosystems. IHg was rapidly removed from water through biosorption and uptake in the macrophyte dominant compartment. Biological turnover then governs IHg transformations and transfer and finally after two months of exposure partitioning to sediment appears to prevail. Associated kinetic parameters were quantified to assess impact and respective contribution of these mechanisms on IHg distribution within the microcosms. IHg was rapidly transferred from the water to the macrophytes, within the first day of exposure with average accumulation rate constant of 0.04 ± 0.01 $h^{-1}$ comparable to that calculated for the disappearance of the contaminant from the water column (i.e. 0.07 ± 0.02 $h^{-1}$). Bioaccumulation capacities of IHg in the grazing snails *L. stagnalis* were one order of magnitude lower than for the macrophytes *E. canadensis*, due to the indirect contamination via the trophic route ($K_{uptake}$= 0.006 ± 0.002 $h^{-1}$). After one month of exposure, significant elimination of the contaminant occurred in both living organisms according to first-order kinetics and resulting in IHg half-lives of 32 and 24 days in macrophytes and snails, respectively.

These experiments also revealed significant transformations of the contaminant in all compartments of the microcosms. MMHg production in the controlled ecosystems thus represents a key mechanism likely to remobilize the contaminant under a more toxic chemical form and then to lead to a significant risk of trophic transfer. The MMHg burdens accumulated in the organisms correspond to a much higher bioaccumulation capacity of this compound in *L. stagnalis* than in *E. canadensis* with AF at 52000 and 7000, respectively. According to the biomagnification processes of Hg through the food chain a BMF of 9 was found between snails and waterweeds.

Furthermore, the comparison of biotic and abiotic systems as well as the evidence of the gaseous Hg species production demonstrates the complexity of the geochemical and ecotoxicological mechanisms driving Hg bioavailability and transfer within aquatic systems. Finally, this overall investigation also confirms the relevance and usefulness of the controlled microcosms for studying Hg contamination pathways in target aquatic ecosystems when compared to previous model or field investigations. Nevertheless here lie the limitations of such experimental approach coupled to a mechanistic resolution. Despite the degree of reductionism in the model ecosystems with respect to natural environmental processes, it still remains difficult to clearly unravel overlaying biogeochemical mechanisms. Microcosms studies coupled to stable

enriched isotope tracers and simultaneous speciation analyses could offer a great potential for investigating dynamic environmental processes in order to provide real insights for pollution impact and ecotoxicological risk assessment.

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# Chapitre C.2.

# Caractérisation des mécanismes de méthylation/déméthylation et de volatilisation du mercure dans des suspensions de sédiments estuariens au moyen de traceurs isotopiques stables

# Caractérisation des mécanismes de méthylation/déméthylation et de volatilisation du mercure dans des suspensions de sédiments estuariens au moyen de traceurs isotopiques stables

Les processus de méthylation, de déméthylation et de volatilisation déterminent la disponibilité et la toxicité globale des polluants mercuriels dans les environnements aquatiques. Les processus aboutissant à la production de monométhylmercure (MMHg) dans les systèmes aquatiques naturels sont donc d'un intérêt fondamental et ont été très largement étudiés. Néanmoins des progrès demeurent encore à faire pour accéder à une compréhension améliorée de ces mécanismes. Bien que les microorganisms soient reconnus pour leur capacité à méthyler le mercure dans l'eau et les sédiments, la contribution des réactions de méthylation abiotique reste mal évaluée. De nombreuses études ont été dédiées a la caractérisation de ces processus de transformation, néanmoins la plupart des approches expérimentales ne permettent d'accéder qu'aux taux de méthylation nets, représentant la somme de différents mécanismes simultanés et compétitifs tels que la méthylation, la déméthylation, la réduction et la diméthylation. La compréhension du contrôle environnemental des niveaux de présence du MMHg dans les systèmes naturels nécessite donc de réaliser la spéciation du mercure dans les matrices étudiées et de discriminer et d'évaluer précisément les différentes cinétiques concurrentielles.

Le présent travail tente de répondre à cette demande par le développement de procédures expérimentales innovantes utilisant des traceurs isotopiques stables combinés à une détection sélective par spectrométrie de masse couplée à un plasma induit (ICPMS). L'ajout simultané de deux espèces chimiques du mercure marquées spécifiquement par un isotope ($^{199}$IHg et $^{201}$MMHg) à une suspension de sédiment estuarien et son incubation en conditions contrôlées nous ont ainsi permis de caractériser les cinétiques réactionnelles couplées de méthylation et de déméthylation en fonction de différents forçages environnementaux (conditions d'oxygénation du milieu, niveaux de contamination, inhibition de l'activité microbiologique). La formation d'espèces volatiles du mercure a également pu être identifiée comme étant principalement liée à la dégradation du MMHg. Ces expériences démontrent enfin le potentiel important des traceurs isotopiques en combinaison avec des techniques de mesure très sensibles afin d'offrir de nouvelles perspectives de recherche pour la compréhension du cycle biogéochimique du mercure dans les écosystèmes aquatiques.

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# Mercury methylation/demethylation and volatilization pathways in estuarine sediment slurries using species-specific enriched stable isotopes ☆

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**Abstract**

Species-specific enriched stable isotopes have been used to study mercury transformations (methylation, demethylation and volatilization) in estuarine sediments under different environmental conditions (both biotic and abiotic and oxic and anoxic). These experiments have demonstrated the potential for the isotopically enriched species in combination with highly sensitive measurement methods (ICP MS) to facilitate the study of mercury speciation and reactivity. Sediments (sterilized and nonsterilized) were spiked with both isotopically enriched inorganic ($^{199}$Hg) and monomethylmercury (MM$^{201}$Hg) at environmental levels to avoid perturbing the system and incubated under both aerobic and anaerobic conditions. The formation of MM$^{199}$Hg and the degradation of MM$^{201}$Hg were measured simultaneously during time series experiments by capillary gas chromatography-inductively coupled mass spectrometry. Specific methylation and demethylation rate constants ($K_m$ and $K_d$) were calculated. Results clearly showed that methylmercury levels in sediments are controlled by competing and simultaneous methylation and demethylation reactions. Operating conditions, likely to drastically modify the reactivity of the media, were of primary importance to assess the relative significance of each mechanism. In estuarine sediments, mercury methylation was enhanced under anaerobic nonsterile conditions, whereas other environmental conditions were either less favorable for monomethylmercury production or more effective for its degradation. The production of total gaseous mercury was found to be minimal, although it could be demonstrated that it was related to the fate and transformation of methylmercury.


*Keywords:* Mercury; Methylmercury; Estuarine sediments; Enriched stable isotopes; Methylation; Demethylation; Volatilization

## 1. Introduction

In estuarine sediments, processes of methylation, demethylation and volatilization determine the state and overall toxicity of mercury pollutants. Processes leading to monomethylmercury (MMHg) production in natural aquatic systems have been of tremendous

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interest; but improvement is still needed to understand the mechanisms involved. Although microorganisms are well recognized for their potential to methylate inorganic mercury in water and sediments, the contribution of abiotic mercury methylation is still unresolved.

Numerous studies have examined mercury methylation in aquatic ecosystems but most have addressed only the net rate of methylmercury production. The net amount of methylmercury produced in the aquatic environment, however, is dependent on simultaneous processes giving rise to different mercury species, such as elemental Hg ($Hg^0$), inorganic mercury (IHg), monomethylmercury (MMHg) and dimethylmercury (DMHg).

$$Hg^0 \Leftrightarrow Hg^{2+} \Leftrightarrow MMHg \Leftrightarrow DMHg \quad (1)$$

Studies aimed at understanding the environmental control of the concentration of methylmercury in natural environments require simultaneous measurements of both methylmercury and inorganic mercury as well as careful evaluation of the kinetics of demethylation and methylation.

Mercury methylation or methylmercury demethylation studies have mainly been carried out by means of radiochemical procedures (Korthals and Winfrey, 1987; Furutani and Rudd, 1980). Radioactive inorganic $^{203}Hg$ isotope is added to the sediment, and, after a pre-set anaerobic incubation time, methylmercury is extracted and the amount methylated of $^{203}Hg$ is quantified. This technique has several limitations. The major drawback lies in the elevated Hg levels required to observe detectable reaction yield. Indeed, due to the low specific activity of commercially available inorganic $^{203}Hg$ used in these studies, the tracer had to be added at concentrations at least 10 times higher than environmental Hg concentrations. This has an unknown effect on the microbial community and might favor the selection of mercury-tolerant strains of microorganisms. Because these experiments are based on the microbial response, the results observed are most of the times not comparable to those obtained with the initial bacterial distribution. Furthermore, with the $^{203}Hg$ approach, it is not possible to study demethylation and methylation reactions in the same sample. To overcome this limitation, labeled $^{14}CH_3Hg$ instead of $CH_3^{203}Hg$ was used to quantify the radioactive methane or carbon dioxide yield, which is known to be formed during demethylation of methylmercury (Ramlal et al., 1986). However, both methane and carbon dioxide can be incorporated into the microbial biomass, making the analytical protocol required extremely difficult and time consuming. Additional limitations of radiotracer techniques are associated with the necessary radioactive safety precautions and waste-handling procedures.

An improved procedure makes use of stable isotopes combined with inductively coupled plasma (ICP MS) detection. Indeed, to evaluate and follow methylation rates, this combination offers several potentials. First, the response is not calculated from the total metal concentration but is based on the changes in the isotope ratio of the metal of interest. Isotope ratio measurements are very precise even at very low concentration levels due to the sensitivity of the analytical technique used, thus allowing very low spike concentrations that should preserve the bacterial distribution in the samples. Second, in all these experiments, the fate of the tracer added but also the behavior of the naturally occurring metal already in the system can be monitored simply by following the different isotopes of that element. Third, demethylation rates can be measured together with methylation in the same subsample. To follow and unravel these coupled and often overlying processes, double-labeling tracers are necessary. Species-specific reactions are very sensitive, and the precision of speciated isotope dilution procedures can account for these difficult simultaneous mechanisms.

This approach has been recently used by Hintelmann et al. (2000), Lambertsson et al. (2001) and Mauro et al. (2002). Hintelmann et al. (2000) used GC–ICP MS and stable enriched isotope tracers for the simultaneous determination of methylation and demethylation rates of mercury species in anaerobic fresh water sediment slurries. The degree of artifactual formation of methylmercury during sample distillation and work-up was determined in separate experiments by adding $^{199}Hg(NO_3)_2$ to sediment samples, which were not incubated. Measured biotic methylmercury formation values were then corrected for the artifactual formation during sample work-up. Specific rate constants for the two processes were calculated and compared to the rate constants obtained by monitoring the changes in concentration

of the ambient methylmercury in the same sample. A major conclusion of this work was that added tracer $Hg^{2+}$ was better available for methylation reactions than ambient $Hg^{2+}$. On the other hand, the use of methylmercury isotopes seems valid to measure demethylation rates, as they did not observe differences in availability between added methylmercury and ambient methylmercury. Different Hg species were investigated with regards to their availability for methylation reactions. Compared to $Hg(NO_3)_2$, Hg-fulvate showed reduced availability and freshly precipitated HgS was hardly available.

This previous work demonstrates the potential of the use of enriched stable isotopes to simultaneously study methylation and demethylation reactions. However, the factors that influence the balance of the competing methylation and demethylation reactions that are responsible for the overall environmental methylmercury concentrations are yet insufficiently understood and little to no attempts have been made to determine the end products (Watras et al., 1995; Winfrey and Rudd, 1990; Park and Bartha, 1998).

Estuarine environments are of particular interest because they directly affect the speciation and partitioning of metals. In macrotidal estuaries, a series of oxic–anoxic oscillations following by alternate cycling of sedimentation and resuspension in these ecosystems directly affect the occurrence and speciation of mercury (Tseng et al., 2001). For these reasons, we conducted mercury methylation and demethylation experiments in an estuarine intertidal sediment, with the ultimate goal of correlating these activities with the prevailing environmental parameters. To link methylation and demethylation rates to other environmental conditions, like redox potential and microbiological activity, laboratory incubations of sediments spiked with enriched stable isotope tracers of methyl- and inorganic mercury have been carried out in the present study. An attempt has also been made to determine gaseous byproduct from the sediment slurries processes measuring mercury content released in the headspace during the experiments. This work is thus a first report on the use of species-specific enriched stable isotope tracers to investigate mercury reactivity in estuarine sediments. One of the main objectives was to validate the experimental setup to determine and model mercury biogeochemistry in estuarine and coastal environments.

## 2. Materials and methods

### 2.1. Instrumentation

A gas chromatograph (HP 6850) was equipped with a capillary column and coupled to an Agilent Model HP-7500 inductively coupled plasma mass spectrometer via a Silcosteel (Restek) transfer capillary. The instrumental configuration allows to work under mixed wet and dry plasma conditions. A detailed description has been previously published (Rodriguez Martin-Doimeadios et al., 2002a; Krupp et al., 2001).

Extraction of mercury species from solid samples was undertaken by a Microdigest A301 (2450 MHz, maximum power 200 W) microwave digester (Prolabo, Fontenay-sous-bois, France).

### 2.2. Reagents

Stock solutions of $Hg^{2+}$ (4.98 mmol $l^{-1}$) and MMHg (4.64 mmol $l^{-1}$) of natural isotopic composition were prepared by dissolving mercury(II) chloride (Strem Chemicals 99.9995% Hg) in 1% $HNO_3$ (Merck) and methylmercury chloride (Strem Chemicals) in methanol (Merck), respectively. Working standard solutions were prepared fresh daily by appropriate dilution of the stock standard solutions in 1% $HNO_3$ and stored in the fridge. Methylcobalamine (Sigma) used for synthesis was prepared by dissolution in an acetic acid-acetate buffer solution (0.1 M, pH 5). $^{201}HgO$ and $^{199}HgO$ were obtained from Oak Ridge National Laboratory (USA). Isotopically enriched mercury stock solutions were prepared by dissolving in 1% HCl (Merck).The sodium tetraethylborate (98%) was purchased from Strem Chemicals (Bisheheim, France).

All reagents were of analytical reagent grade. Ultrapure water was obtained from a Milli-Q system (Quantum EX, Millipore, USA).

### 2.3. Determination of mercury species in sediments and gold traps

Mercury species were determined in sediments after microwave-assisted extraction with 6 M $HNO_3$. The extracted species were derivatized by ethylation and then analyzed by CGC–ICP MS (Rodriguez

Martin-Doimeadios et al., 2003). Analytical setup and methodology used for the gold traps analysis are described in detail elsewhere (Amouroux et al., 1999). Briefly, gold-coated traps were desorbed at 500 °C, under pure He carrier gas, into a cryogenic gas chromatographic device on-line connected to an ICP MS.

Masses monitored were 199, 201 and 202. Reproducibility of $^{202}Hg/^{201}Hg$ isotope ratio measurements was 0.60% for MeEtHg and 0.69% for $Et_2Hg$. For $^{202}Hg/^{199}Hg$, reproducibilities were 0.43% and 0.46%, respectively. The detection limits for the CGC-ICPMS were estimated using three times the standard deviation of background noise next to the chromatographic peak, and were better than 0.129 fmol for $^{202}Hg$, 0.099 fmol for $^{201}Hg$ and 0.119 fmol for $^{199}Hg$.

### 2.4. Characterization and analysis of isotopically enriched spikes by reverse isotope dilution

At first, the actual abundances of the individual mercury isotopes in the initial inorganic mercury tracer solution were evaluated. As expected, significant enrichment in $^{201}Hg$ (found: $97.31 \pm 0.06\%$; supplier's value: 98.11%) and in $^{199}Hg$ (found: $91.08 \pm 0.05\%$; supplier's value: 91.95%) were found with respect to the natural isotopic distribution (13.18% and 16.87%, respectively) (IUPAC, 1998).

The enriched $MM^{201}Hg$ was synthesized by reaction with methylcobalamine. The optimization of the synthesis conditions is described elsewhere (Rodríguez Martin-Doimeadios et al., 2002b). The concentrations of the enriched $MM^{201}Hg$ and $^{199}Hg$ in the spike solutions were calculated using reverse isotope dilution analysis (this final solution was spiked with natural mercury standards of known concentration and purity). Two independent isotope dilution experiments were carried out. Each solution was injected four times. Average concentration of the $MM^{201}Hg$ spike solution turned out to be $4.61 \pm 0.17$ nmol $g^{-1}$ and enriched $^{199}Hg$ was $9.55 \pm 0.10$ nmol $g^{-1}$.

### 2.5. Collection and processing of sediment

Surface (0–5 cm) intertidal sediment was collected from the Adour River estuary (France) in October 2000. The location of the site was selected among other sites after an intense survey performed in the Adour estuary (Stoichev et al., 2004). This site was found to have one of the best potential to generate methylmercury presenting some of the highest MMHg concentrations in surface sediments together with high organic carbon content. The sample was hand collected with acid-cleaned polyethylene cup at low tide. It was immediately sieved through a clean Nylon sieve (2-mm) placed in precleaned polyethylene container and stored at 4 °C.

An aliquot of this sample was freeze-dried and then crushed in an acid-cleaned agate mortar. This part was used for the initial mercury species determination, organic carbon (Org C) and total sulfur ($S_T$) determinations. The concentrations of mercury species, organic C and total S, as well as the mercury species concentrations are summarized in Table 1.

### 2.6. Setup of methylation/demethylation experiment

A schematic diagram of the experimental design together with the sampling scheme is presented in Fig. 1. All materials used for the incubation experiments and sampling were thoroughly cleaned with concentrated nitric acid and subsequently sterilized before use. During the whole experiment, the biological and chemical integrity of the slurries was maintained using careful protocols. It is important to mention that the microbial diversity was monitored by molecular biology techniques (TRFLP method) for both nonsterile and sterile conditions (Duran et al., 2003).

Table 1
Characteristics of estuarine sediment and comparison of ambient mercury and monomethylmercury contents with added tracer contents

| Parameters | Values |
|---|---|
| Fine fraction (<50 μm) | 80% |
| Total sulfur content, $S_T$ (%dry weight) | 0.25 |
| Organic content, TOC (%dry weight) | 2.9 |
| Ambient total mercury | 1.19 nmol/g |
| Ambient methylmercury | 0.011 nmol/g |
| Increase in total mercury owing to the addition of $^{199}Hg^{2+}$ | 0.498 nmol/g |
| Increase in methylmercury owing to the addition of $MM^{201}Hg$ | 0.093 nmol/g |

TOC: total organic carbon.

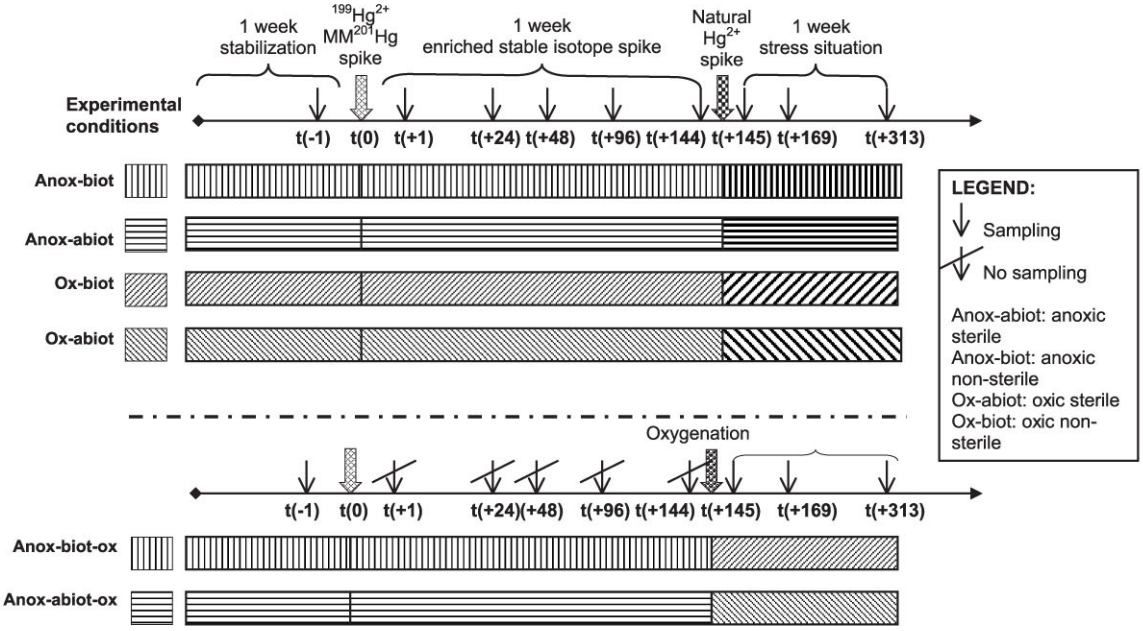


Fig. 1. Experimental design and sampling distribution along the time of study. Steps: stabilization; spike of enriched stable isotopes ($^{199}Hg^{2+}$ and $MM^{201}Hg$); incubation under different experimental conditions; high natural mercury spike and/or reoxygenation. *t* (+/− number): time in hours before (−) and after (+) the enriched species spike.

*2.6.1. Preconditioning*

For the methylation/demethylation study, a slurry was obtained with 500 g of the surface intertidal sediment that was mixed with filtered (<0.2 μm) estuarine water (salinity 5, Practical Salinity Scale) (UNESCO, 1985). The sediment slurry was divided in two batches under $N_2$ atmosphere. One batch was autoclaved at 121 °C for 20 min at 1 bar (abiotic). The other batch was left at its original state (biotic). The two batches were subdivided once more. Each slurry was poured in 1-l pyrex flask.

*2.6.2. Stabilization*

One flask was purged with $N_2$ to maintain anaerobic conditions, the other one with mercury-free laboratory air to keep the system aerobic. A duplicate for the anaerobic experiments was prepared. Sediment slurries were sealed gas-tight and were left for 1 week at 25 °C with continuous magnetic stirring for stabilization. After 1 week of stabilization, the bottles for anaerobic conditions were provided with a capped gold trap (referred as later Anox-abiot and Anox-biot experiments). The bottles for the aerobic experiment (Oxic-abiot and Oxic-biot experiments) were equipped with two consecutive gold traps. The first one was used to continuously trap the volatile species and the second one to avoid atmospheric mercury to enter the system.

*2.6.3. Enriched stable isotope incubation*

Mercury(II) chloride enriched in $^{199}Hg$ and monomethylmercury enriched in $^{201}Hg$ were added at a concentration at ca. 0.498 nmol of $^{199}Hg$ and 0.093 nmol of $MM^{201}Hg$ per gram of dry sediment, respectively. Incubations under aerobic and anaerobic conditions were performed at 25 °C in a thermostated incubator (model INFORS, Switzerland) in the dark with continuous elliptic agitation (160 rpm). As shown in Fig. 1, samples for analysis were taken after 1 week of stabilization period and before the spike ($t-1$), $t+1$ was 1 h after spike and the subsequent samples were taken each day after the spike for 1 week. Aliquots were kept in sealed polyethylene cryovials (Nalgene) and frozen at −196 °C until analysis. After each sediment sample collection, the gas phase headspace above the sediment was renewed by flushing it with the appropriate gas for 10 min (i.e., Hg free air and nitrogen for aerobic and anaerobic slurries, re-

spectively). This approach allowed all gaseous forms of mercury present in the headspace to be collected in the gold trap several times for 1 week. Gold trap from the aerobic and anaerobic systems were subsequently sealed with Teflon caps and kept in a mercury-free atmosphere before analysis.

*2.6.4. High natural mercury spike*

To study the response of the slurry system to a high contamination situation, each slurry was spiked with ca. 9.97 mmol of natural mercury per gram of dry sediment after the first week of incubation. Then, it was incubated under the same conditions (i.e., aerobic or anaerobic) for one additional week.

*2.6.5. Reoxygenation*

Two additional anaerobic experiments (biotic and abiotic) were simultaneously carried out (Anox-biot-ox and Anox-abiot-ox experiments). After the 2 weeks of experiments (i.e., 1 week stabilization and 1 week in presence of the spikes) and the collection of only two of the reference samples (i.e., $t-1$ and $t+144$ h), the system was oxygenated with mercury-free air. The aim of this experiment was to test the effect of reoxygenation of anaerobic sediments, similarly to naturally occurring redox oscillations observed in the environment.

*2.7. Data analysis*

*2.7.1. Calculation of methylmercury amount derived from enriched spikes*

To determine the amount of methylated and demethylated mercury, at least three isotopes of mercury must be monitored: one representing the newly produced methylmercury from the inorganic mercury tracer addition (isotope 199); another representing the demethylation of the monomethylmercury tracer addition (isotope 201); and the other demonstrating the changes in methylmercury concentrations derived from the mercury originally present in the sample (isotope 202). For each selected mercury isotope, the isotopic methylmercury concentration in the sediment sample was calculated separately by external calibration using the corresponding calibration curves.

The amount of methylated mercury deriving from the enriched isotope during the experiment can be calculated by using the next equation (Hintelmann et al., 1995):

$$^{1}\mathrm{MMHg_{sp}} = \frac{\sum^{2}\mathrm{MMHg} - \mathrm{R}_{n}\sum^{1}\mathrm{MMHg}}{R_{\mathrm{sp}} - R_{\mathrm{n}}} \qquad (2)$$

In this case, the superscript 1 is the isotope 199 and the superscript 2 is the isotope 202. The subscript $n$ refers to the natural methylmercury that was originally present in the sample before tracer addition, and the subscript "sp" is used to distinguish the newly produced methylmercury from the spike addition. $R_{\mathrm{n}}$ is the isotope ratio (corrected for mass bias) of the unspiked sediment sample and $R_{\mathrm{sp}}$ is calculated from the total mercury isotope ratio measurement in the tracer solution used for spiking.

$^{1}\mathrm{MMHg_{sp}}$ represents the total concentration of methylmercury produced in the sediment from the spike. $^{1}$MMHg represents the total concentration of the enriched isotope tracer coming from the sum of the newly produced and the originally present methylmercury. $^{2}$MMHg corresponds to the corrected concentration of natural monomethylmercury for produced MMHg from impurities in the tracer solution.

The amount of demethylated monomethylmercury from enriched spike is calculated in a similar way following rather the 201 isotope instead of 199. The demethylation is calculated by subtraction of the initial $MM^{201}Hg$ amount added to the final $MM^{201}Hg$ amount coming from the spike found.

*2.7.2. Calculation of methylation and demethylation rates*

The rate of demethylation of methylmercury in sediment can be expressed in pmol of $MM^{201}Hg$ demethylated per gram per hour (Ramlal et al., 1986). This in turn was used to calculate the specific rate of demethylation, i.e., the percentage of the added mercury demethylated per gram per hour.

Demethylation rate=(total pmol of $MM^{201}Hg$ demethylated)/(gram of dry sediment)*(hours incubated).

Specific demethylation rate = 100 *demethylation rate/(added pmol of $MM^{201}Hg$).

Specific rates of mercury methylation were calculated similarly to demethylation calculations.

Methylation/demethylation (M/D) ratios were calculated by dividing the methylation rate (pmol of produced $MM^{199}Hg$ per gram per hour) by the demethylation rate (pmol of lost $MM^{201}Hg$ per gram per hour). This ratio is a measure of the relative importance of methylating and demethylating activities and is useful in identifying potential conditions of accelerated methylmercury production (Ramlal et al., 1986).

*2.7.3. Calculation of specific methylation/demethylation rate constants*

A model based on first-order kinetics for both mercury methylation and methylmercury demethylation can be used to describe these transformation reactions. Considering bulk concentrations (aqueous and particulate phase) and negligible formation of gaseous mercury, net production of monomethylmercury can be written as follows:

$$\frac{d[MM^{199}Hg]}{dt} = K_m[^{199}Hg^{2+}] - K_d[MM^{199}Hg] \qquad (3)$$

where $MM^{199}Hg$ = concentration of $MM^{199}Hg$ newly generated from the $^{199}Hg^{2+}$ tracer in nmol $g^{-1}$; $K_m$ = specific methylation rate constant in $h^{-1}$; $[^{199}Hg^{2+}]$ = concentration of added $^{199}Hg^{2+}$ in nmol $g^{-1}$; $K_d$ = specific demethylation rate constant in $h^{-1}$; and $t$ = incubation time in h. Eq. (3), written here for the added inorganic tracer isotope, holds also for the added methylmercury ($MM^{201}Hg$) isotope and the ambient methylmercury isotopes (e.g., $MM^{202}Hg$).

The specific methylation and demethylation rate constants ($K_m$ and $K_d$) can be calculated using different approaches by simply taking into account the data obtained initially in the experiment or by using the whole data and a reversible reaction kinetic model. The first approach allows us to calculate the methylation constant for $^{199}Hg$ and the demethylation constant for $MM^{201}Hg$. By applying the kinetic model, it is then possible to obtain the methylation constant for $^{199}Hg$ and the demethylation constant for $MM^{199}Hg$ produced during the experiments. More information on calculations is included as *Supplementary electronic material*.

## 3. Results

The estuarine sediment characteristics of potential relevance to methylation and demethylation of mercury along with the initial inorganic and monomethylmercury contents are listed in Table 1. The experimental design for methylation and demethylation studies is displayed in Fig. 1. The first set of experiments was conducted to determine the effect of aerobic, anaerobic and biotic and abiotic conditions in the mercury transformation process referred as: Anox-biot (anaerobic nonsterile); Anox-abiot (anaerobic sterile); Ox-biot (aerobic nonsterile); Ox-abiot (aerobic sterile) experiments.

After the enriched stable isotopes spike, methylmercury and inorganic mercury concentrations were determined using the following isotopes (199, 201 and 202) separately (by external calibration using the corresponding calibration curves) in each sample collected at different times. Changes in the relative abundance of isotope 202 represents the evolution of natural mercury, that of the 201 isotope follows the evolution of spiked enriched monomethylmercury (demethylation) and the changes in the 199 isotope refers to the evolution of spiked enriched inorganic mercury (methylation).

The amount of methylated mercury deriving from the enriched 199 isotope during the experiments was calculated after the Eq. (2). The amount of demethylated mercury from the enriched $MM^{201}Hg$ spike was calculated with Eq. (2) modified to subtract the initial amount of $MM^{201}Hg$ added. In Fig. 2, the time-course evolution in the nmol $g^{-1}$ of methylmercury formed from $^{199}Hg$ and demethylated from $MM^{201}Hg$ for the different experimental conditions tested are plotted. In Fig. 3, a CGC–ICP MS chromatogram illustrates the occurrence of $MM^{199}Hg$ and $MM^{201}Hg$ and their evolution for the anaerobic nonsterile experiments. Both methylation and demethylation processes are evident.

The calculated percentage of methylated and demethylated mercury from the spikes at different selected times (1 and 24 h, and at the end of these experiments 1 week later) are displayed in Table 2. Table 3 summarizes the various model parameters calculated from time courses using different experimental conditions. In Table 4, the kinetic data obtained using different kinetic calculation approaches are shown.

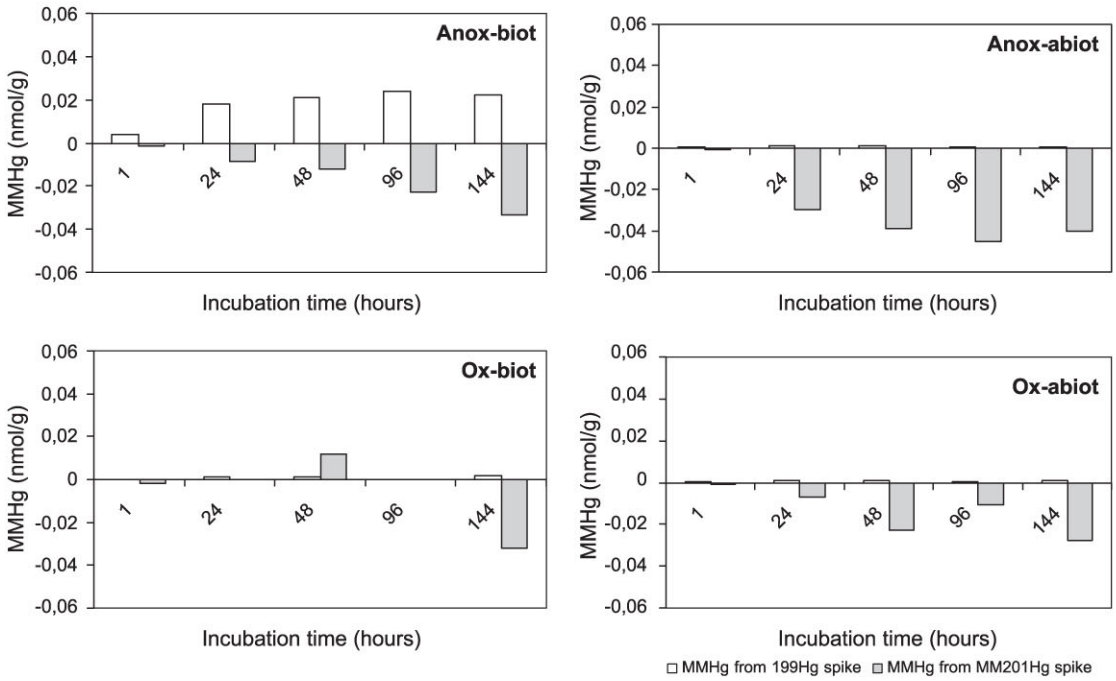


Fig. 2. Time course evolution in nmol $g^{-1}$ of monomethylmercury formed from $^{199}Hg^{2+}$ and demethylation from $MM^{201}Hg$ under different experimental conditions (Anox-biot: anoxic nonsterile; Anox-abiot: anoxic sterile; Ox-biot: Oxic nonsterile; Ox-abiot: oxic sterile).

### 3.1. Methylation

$^{199}Hg^{2+}$ methylation showed a strong dependence on redox conditions and biological activity. Of the different variables tested, the combination of anaerobic and biotic conditions was the most conducive to methylmercury production (Fig. 2). Only minor methylation was observed under the anaerobic sterile and aerobic sterile and nonsterile conditions. The highest methylation percentage (Table 2) corresponded to the anaerobic nonsterile conditions after 1 week of incubation (4.11%). The duplicate anaerobic experiments Anox-abiot-ox and Anox-biot-ox (Table 2) also confirmed that methylation occurs in the anaerobic nonsterile incubation conditions. In addition, among 18 bacterial strains isolated from the anaerobic nonsterile conditions, 6 of them were sulfate reducing bacteria belonging to the genera *Desulfovibrio*, *Desulfomicrobium* and *Desulfotomaculum*. Moreover, four isolates were sulfide and sulfur-oxidizers (anoxygenic phototrophs and lithotrophs) demonstrating the importance of anaerobic metabolisms linked to the sulfur cycle in the occurrence of significant methylation process in this microecosystems. The other eight isolates were strict fermentative bacteria (*Clostridium* sp.), or facultative anaerobes (R. Guyoneaud, personal communication). These results are in agreement with previous study (Compeau and Bartha, 1985), showing that sulfate-reducing bacteria appear to be the primary mercury methylators in anoxic sediments.

The time course studies for the anaerobic nonsterile (Anox-biot) incubation conditions (Fig. 2) clearly demonstrate that $MM^{199}Hg$ was rapidly formed from the $^{199}Hg^{2+}$ added during the first day of experiments. After, the production rate apparently decreased and the concentrations of methylmercury reached a steady state level after 1 week of incubation. For the anaerobic nonsterile conditions, the methylation rate constants observed were of the same order than that obtained using the kinetic model (Table 4). However, different values were obtained for the initial data approach and kinetic fitting for the other tested conditions. The $K_m$ and $K_d$ values are thus assigned to the calculation method used. The $K_m$ and $K_d$ parameters obtained from

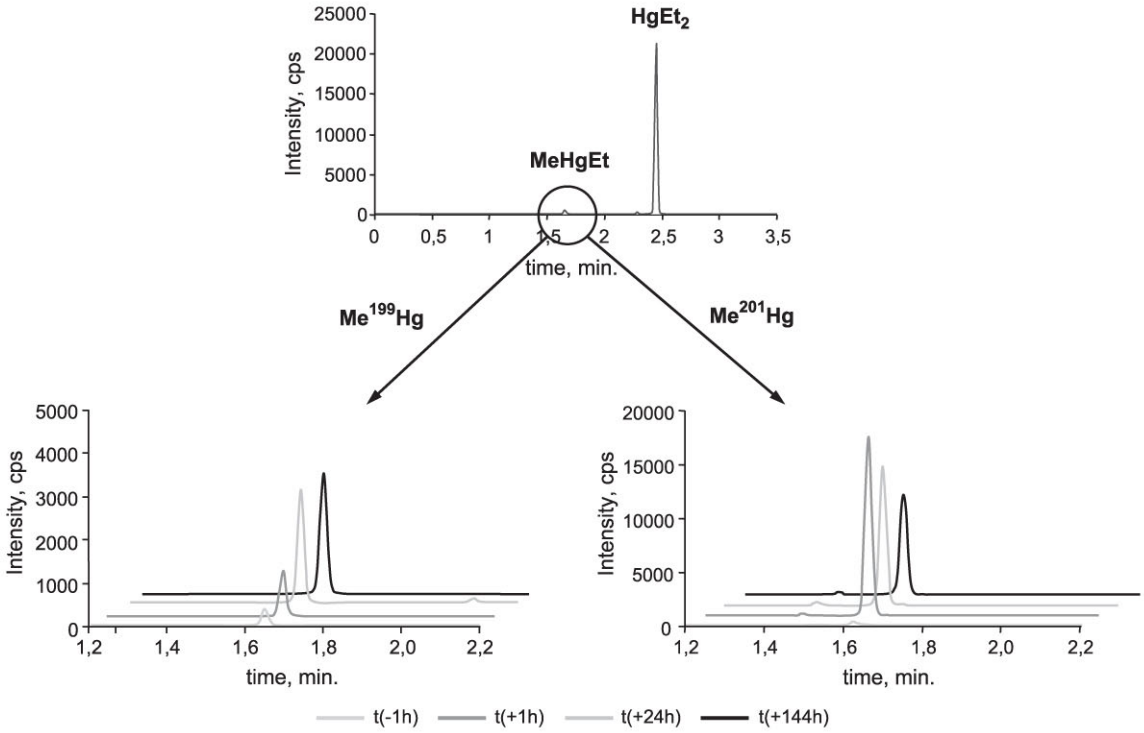


Fig. 3. Chromatograms obtained for the $MM^{199}Hg$ and $MM^{201}Hg$ evolution under anaerobic nonsterile incubation conditions. The chromatograms at different incubation times are shifted for clarity. $t(-1)$: 1 h before enriched species spike; $t$ (+1), (+24) and (+144): 1, 24 and 144 h, respectively, after the enriched species spike.

kinetic modeling of the $^{199}Hg$ data from the sterile anoxic, sterile oxic, and nonsterile oxic slurries are probably unreliable given the low $MM^{199}Hg$ concentration observed in the slurries.

Table 2
Total percentage of mercury methylated and demethylated from the spikes under the different experimental conditions (calculated by dividing the amount measure by the amount spiked × 100)

| | $^{199}Hg^{2+}$ methylated (%) | | | $MM^{201}Hg$ demethylated (%) | | |
|---|---|---|---|---|---|---|
| | 1 h | 24 h | 144 h | 1 h | 24 h | 144 h |
| Anox-biot | 0.74 | 3.34 | 4.11 | 1.42 | 9.10 | 35.11 |
| Anox-abiot | 0.09 | 0.178 | 0.123 | 0.86 | 32.45 | 43.50 |
| Ox-biot | n.d. | 0.196 | 0.259 | n.d. | 2.16 | 34.96 |
| Ox-abiot | 0.02 | 0.148 | 0.137 | 0.91 | 7.86 | 29.91 |
| | | 144 h | 313 h | | 144 h | 313 h |
| Anox-biot-ox | | 8.43 | 5.83 | | 77.63 | 103.3 |
| Anox-abiot-ox | | 0.012 | n.d. | | 32.12 | 19.28 |

n.d. not detected.

### 3.2. High inorganic mercury spike

After 1 week of incubation and when a steady state of methylmercury production and consumption had been reached, the slurries were spiked with high concentration of natural mercury (9.97 mmol of mercury per gram of sediment) and the incubation continued for another 1 week with new gold traps (Fig. 1). The samples were analyzed by using Eth-CT-GC-QAFS after microwave acid extraction (Tseng et al., 1997).

Firstly, the potential artifact MMHg formation due to the analytical procedure was checked. Abiotic methylation of inorganic mercury during the processing of sediment samples has been recently reported. The artifactual methylmercury formation results in significant systematic errors and it has been the subject of important controversy (Hintelmann et al., 1997; Quevauviller and Horvat, 1999; Falter, 1999; Rodríguez Martin-Doimeadios et al., 2003). Different analytical techniques were evaluated with respect to

Table 3
Comparison of specific mercury methylation and methylmercury demethylation rate constants calculated from tracer studies (calculated for $t=24$ h and $t=1$ week)

| Experimental conditions | Methylation rate (pmol $g^{-1}$ $h^{-1}$) | Demethylation rate (pmol $g^{-1}$ $h^{-1}$) | M/D | Specific methylation rate (pmol $g^{-1}$ $h^{-1}$)100/ (add Hg pmol $g^{-1}$) | Specific demethylation rate (pmol $g^{-1}$ $h^{-1}$)100/ (add MMHg pmol $g^{-1}$) |
|---|---|---|---|---|---|
| *1 day* | | | | | |
| Anox-biot | 0.816 | 0.360 | 2.268 | 0.1035 | 0.379 |
| Anox-abiot | 0.043 | 1.247 | 0.035 | 0.0056 | 1.339 |
| Ox-biot | 0.048 | 0.083 | 0.573 | 0.0062 | 0.090 |
| Ox-abiot | 0.036 | 0.302 | 0.119 | 0.0047 | 0.327 |
| *1 week* | | | | | |
| Anox-biot | 0.016 | 0.231 | 0.071 | 0.0212 | 0.244 |
| Anox-abiot | 0.005 | 0.281 | 0.018 | 0.0006 | 0.302 |
| Ox-biot | 0.010 | 0.221 | 0.047 | 0.0014 | 0.243 |
| Ox-abiot | 0.005 | 0.191 | 0.029 | 0.0007 | 0.208 |

this point and, in general, this process seems to be related with the amount of inorganic mercury available during the sample analysis (Hintelmann et al., 1997). In the present case, when high amount of natural inorganic mercury was added, an artifact due to the analytical procedure was detected. The concentration of MMHg (between 0.014 and 0.023 nmol $g^{-1}$) measured right after the addition of the $Hg^{2+}$ spike represents the accidental methylation during sample pretreatment. All measured concentrations from later time intervals (24 and 72 h) were corrected for this initial value. Results are shown in Table 5. The high inorganic mercury concentration added has no detectable inhibitory effect on the methylation process recorded. Under the anaerobic nonsterile conditions, a steady state of MMHg concentration was not attained until the termination of the experiments. The percentage of the total mercury converted to monomethylmercury declined with the highest inorganic mercury spiked. This phenomenon was noted also by other authors (Compeau and Bartha, 1984).

The experiments conducted with the low (0.498 nmol $g^{-1}$ of $^{199}Hg^{2+}$) and the high mercury contamination (9.97 mmol $g^{-1}$ of natural $Hg^{2+}$) lead to similar conclusions with respect to methylation process. Again, the methylation production was far more efficient under the anaerobic nonsterile conditions and, compared to the other conditions, it was again more significant for the aerobic nonsterile conditions.

For these particular set of experiments, the pore water of the slurry was extracted by centrifugation and filtration and was analyzed (Table 5). MMHg could only be found in the anaerobic nonsterile incubation conditions. The higher $Hg^{2+}$ values

Table 4
Comparison of specific mercury methylation and methylmercury demethylation rate constants ($K_m$ and $K_d$) calculated using different kinetic calculation approaches (rate constants in $h^{-1}$)

| Experimental conditions | Initial data approach | | | Reversible reaction model | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $K_m$ | $K_d$[a] | Half-life (hours) | $K_m$ | SD | $K_d$[b] | SD | $R^2$ | $MM^{199}Hg$ (pmol $g^{-1}$) |
| Anox-biot | $1.4\times10^{-3}$ | 0.0029 | 239 | $2.9\times10^{-3}$ | $9.6\times10^{-4}$ | 0.066 | 0.020 | 0.96 | 22.6 |
| Anox-abiot | $7.6\times10^{-5}$ | 0.0102 | 67.9 | $1.4\times10^{-3}$ | $9.2\times10^{-4}$ | 0.866 | 0.480 | 0.65 | 0.83 |
| Ox-biot | $8.2\times10^{-5}$ | 0.0007 | 990 | $1.3\times10^{-4}$ | $8.0\times10^{-5}$ | 0.052 | 0.025 | 0.94 | 1.34 |
| Ox-abiot | $6.3\times10^{-5}$ | 0.0072 | 96.3 | $2.6\times10^{-4}$ | $1.2\times10^{-4}$ | 0.182 | 0.080 | 0.98 | 0.74 |

[a] Rate constant calculate from changes in spiked $MM^{201}Hg$.
[b] Rate constant calculate from changes in the in situ generated $MM^{199}Hg$.

Table 5
Total amount of MMHg (pmol/g) (after artifact correction), total percentage of methylated mercury (amount measured divided by the amount spiked × 100) and pore water concentrations (nmol $l^{-1}$) after high natural mercury spike under the different experimental conditions

| Experimental conditions | MMHg (pmol $g^{-1}$) | | MMHg (%) | | Pore waters (nmol/L) | |
|---|---|---|---|---|---|---|
| | 24 h | 72 h | 24 h | 72 h | $Hg^{2+}$ | MMHg |
| Anox-biot | 50.0 | 152 | 0.35 | 1.08 | 0.223 | 0.044 |
| Anox-abiot | 6.04 | 6.96 | – | 0.06 | 1.147 | n.d. |
| Ox-biot | 12.0 | 19.0 | – | 0.19 | 0.208 | n.d. |
| Ox-abiot | 5.10 | 18.5 | 0.05 | – | 0.383 | n.d. |

obtained corresponded to the sterile (oxic and anoxic) experiments.

### 3.3. Demethylation

After 1 week of experiments, high demethylation percentages (between 30% and 43%) were obtained for all the conditions evaluated. The highest demethylation yield was obtained under the anaerobic sterile incubation conditions (Table 2).

Fig. 2 displays the time evolution for 1 week of experiments. The time course studies ensured that the specific rates of demethylation were linear during the anaerobic nonsterile incubation period and there was no evidence of changing demethylation kinetics over this period. This is in agreement with previous reported results, when rates were linear over a period of 24 h (Ramlal et al., 1986).

Results obtained also demonstrated very different kinetics for the nonsterile and sterile anaerobic incubations (Tables 2 and 3). In the sterile experimental conditions, the demethylation yield was very fast: after 24 h, 32.45% of spiked methylmercury had been demethylated, while in the nonsterile sediments only 9.10% had disappeared. For the aerobic experimental conditions, the trend obtained was much more erratic. Fastest processes were observed under the sterile conditions compared to the nonsterile incubations. After 1 week of experiments, values close to those obtained with the anaerobic conditions were obtained.

The measured methylmercury degradation rates for anaerobic–nonsterile incubations were similar to that found in earlier studies when expressed as a degradation yield. Under similar conditions, the yield of demethylated $MM^{201}Hg$ was about 9.1% while other studies found demethylation rates ranging from 5% to 0.3% per day per gram of sediment, despite much higher initial total spike concentration (Korthals and Winfrey, 1987; Ramlal et al., 1986). The specific demethylation rate found in our study (0.379% added MMHg $g^{-1}$ $h^{-1}$) is also similar to those reported in the literature (0.184–0.267% added MMHg $g^{-1}$ $h^{-1}$) for organic-rich sediments (Table 3) (Ramlal et al., 1986). However, Hintelmann et al. (2000) observed a decrease of almost 40% in methylmercury concentration over the first day in a similar experiment with isotopically enriched isotopes. The differences between this previous work and our experiments could be directly related with the different types of sediments studied (pristine soft water lakes vs. estuarine sediments), different organic carbon content [up to 49.9% in Hintelmann et al.'s (2000) study vs. 2.9% in the present work] and also with the spike levels (1.39–6.03 vs. 93 pmol $g^{-1}$ increase in monomethylmercury because of the addition of the spike).

For the demethylation kinetic calculations (Table 4), one order of magnitude lower values were obtained using the initial data approach. The same trend was observed for the different incubation conditions. Both oxic and anoxic sterile conditions were more effective in producing MMHg demethylation reactions.

### 3.4. Volatilization

The gaseous mercury detected in the gold traps can occur both as $Hg^0$ and DMHg, as any dimethylmercury formed in our closed test systems would be back converted to $Hg^0$ during this specific analytical procedure (see Materials and methods). We will then refer to as total gaseous mercury (TGM) later in the text. Results obtained for each series of experiments are given in Table 6. The TGM amounts ranged between 0.99 and 1.99 pmol of mercury after the first week of experiments (low isotopically enriched $^{199}Hg^{2+}$ and $MM^{201}Hg$ spike). TGM produced only represented a very small fraction of total mercury amount initially present in the matrix. Similar low values were obtained for all the experimental conditions tested. These results were also coherent with those reported by earlier studies (Steffan et al., 1998).

Table 6
Volatilization of mercury ($Hg^0$ and/or DMHg) from estuarine sediments spiked with $^{199}Hg^{2+}$ and $MM^{201}Hg$ and spiked with high amount of natural mercury (stress situation)

| Experimental conditions | Total amount (pmol) | Yield (%) |
|---|---|---|
| *After isotopically enriched spike* | | |
| Anox-biot | 1.15 | $3.3 \times 10^{-3}$ |
| Anox-abiot | 2.24 | $6.6 \times 10^{-3}$ |
| Ox-biot | 1.05 | $3.0 \times 10^{-3}$ |
| Ox-abiot | 1.15 | $3.4 \times 10^{-3}$ |
| Anox-biot-ox | 1.94 | $5.4 \times 10^{-3}$ |
| Anox-abiot-ox | 1.54 | $4.3 \times 10^{-3}$ |
| *After natural $Hg^{2+}$ spike* | | |
| Anox-biot | 8.47 | $4.3 \times 10^{-3}$ |
| Anox-abiot | 20.4 | $1.0 \times 10^{-2}$ |
| Ox-biot | 5.13 | $1.6 \times 10^{-3}$ |
| Ox-abiot | 3.14 | $2.6 \times 10^{-3}$ |
| *After reoxygenation* | | |
| Anox-biot-ox | 2.24 | – |
| Anox-abiot-ox | 1.89 | – |

After the second week of experiment (high inorganic Hg spike), enhanced TGM production was observed in all slurries, with amounts ranging from 3.14 to 20.4 pmol of mercury (Table 6). As previously reported by several authors (Xiao et al., 1994; Mason et al. 1995), this increase in TGM concentrations could be due to an artificial $Hg^0$ production, in relation with the addition of high Hg amount to the systems. Nevertheless, the volatilization rates calculated for the high $Hg^{2+}$ spike were found to be in the same range than those obtained for the low spike conditions. The highest values were observed in anaerobic and sterile conditions for both experiments (Table 6).

A triangular isotopic diagram, presented in Fig. 4, was built to trace the origin of TGM trapped on gold sand. Appropriate isotopic ratios (i.e., 202:199 vs. 202:201) of the three Hg sources (i.e., ambient Hg, $^{199}Hg^{2+}$ and $MM^{201}Hg$ spikes) were plotted. The diagram was then implemented with the measured isotopic ratios of the different TGM samples. The diagram clearly demonstrated the main contribution of both natural and $MM^{201}Hg$ spike to the produced TGM.

## 4. Discussion

### *4.1. Induced biotic methylation from spiked inorganic mercury*

When comparing the results from sterile and non-sterile incubation conditions (Table 2), it was clearly

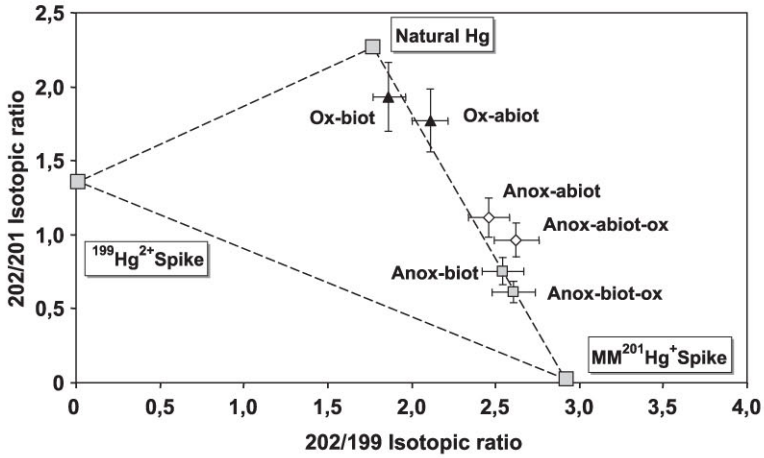


Fig. 4. Triangular isotopic diagram for total gaseous mercury. Appropriate isotope ratios (202:201 and 202:199) of the three mercury sources (natural mercury, $^{199}Hg^{2+}$ spike and $MM^{201}Hg$ spike). The measured isotope ratios for the different gold trap samples are represented. Error bars represent a relative standard deviation of 5% and 12% obtained from duplicate incubation for 202:199 and 202:201 ratios, respectively. (Experimental conditions: Anox-biot, anoxic nonsterile; Anox-abiot, anoxic sterile; Ox-biot, Oxic nonsterile; Ox-abiot, oxic sterile; Anox-abiot-ox: anoxic sterile reoxygenated; Anox-biot-ox: anoxic nonsterile reoxygenated).

found that methylation was significantly higher when samples were nonsterilized. These results confirm that biotic mechanisms are mainly responsible for methylation, and are in opposition to recently outlined potential for abiotic methylation of mercury (Weber, 1993).

In general, our results obtained for the methylation process in anaerobic and aerobic experiments were consistent with that of previous authors. Indeed, Fagerstrom and Jernelov (1972), working on fresh water sediment, and Olson and Cooper (1976), working on marine sediments of San Francisco Bay, found higher $Hg^{2+}$ methylation activity and higher persistence of MMHg under anaerobic conditions than under aerobic incubation conditions. Further, the results from Compeau and Bartha (1984) in estuarine sediments left no doubt that in estuarine sediments both the methylation of $Hg^{2+}$ and persistence of the MMHg found were favored under reducing ($-220$ mV) compared to oxidizing conditions ($+110$ mV).

The methylation kinetic rate constant values presented in Table 4 fit well with those previously reported by Hintelmann et al. (2000) with similar experiments also using enriched inorganic mercury spiked to lake sediments ($5.0\times10^{-4}$–$6.6\times10^{-4}h^{-1}$). These values were found for spiked mercury but they were much higher than those reported for endogenous Hg ($4.2\times10^{-5}h^{-1}$) (Hintelmann et al., 2000). This confirms that the methylation potential is related to free inorganic mercury spike, but not to the sediment equilibrated natural inorganic mercury. This comment implies that to generate or mimic the same speciation behavior as that of the naturally occurring $Hg^{2+}$, the added tracer obviously needs time to equilibrate with the sediment/pore water constituents and the spike should be added in a less available mercury specie.

### 4.2. Spiked and formed MMHg demethylation kinetic

The kinetic study could be helpful to elucidate the mechanisms associated with the results obtained. The first approach taking only into account the initial data allows calculating the demethylation rate constant of spiked $MM^{201}Hg$. On the other hand, the reversible reaction model allows calculating the demethylation rate constants of in situ formed $MM^{199}Hg$. The demethylation rate constants obtained by these different approaches, i.e., initial conditions and kinetic fitting, may correspond to the different pathways associated with different forms of MMHg occurring in the solution. There was a statistically significant difference for the $K_d$ values obtained with the initial data approach and the kinetic fittings model. This difference could indicate that they did not describe the same biochemical pathway (Table 4). Therefore, the $K_d$ obtained from $MM^{201}Hg$ signature would provide information on the degradation pathway from the spiked monomethylmercury as a labile form in the solution (aqueous form) while $K_d$ obtained with the $MM^{199}Hg$ would represent the degradation pathway of monomethylmercury formed into the cell (probably attached to the particle and other cellular constituents). In consequence, lower demethylation constant for spiked "extracellular MMHg" could be explained if demethylation is a microbially induced process under in situ conditions and requires MMHg uptake from the solution (spike), which would not be necessary for biologically formed "intracellular MMHg". Obviously, this explanation is valid only for the biotic anaerobic slurry experiments. For all the other tested conditions, demethylation rate constants for $MM^{199}Hg$ are probably overestimated using the kinetic model regression given the very low amounts of $MM^{199}Hg$ produced.

These results clearly demonstrate that kinetic experiments coupled to species-specific isotope enriched spikes is undoubtedly a critical method to discriminate chemical and biological pathways in sediments that must be further exploited.

### 4.3. Biotic and abiotic demethylation mechanisms

Results indicate that both biological and nonbiological mechanisms were responsible for MMHg demethylation in the sediment slurries. The nonbiological mechanisms were more conducive to the destruction of monomethylmercury.

Biological demethylation of mercury by microorganisms is already well known (Billen et al., 1974; Spangler et al., 1973). Oremland et al. (1991) investigated and identified the different bacterial populations involved in demethylation of monomethylmercury. On the basis of inhibition experiments, they concluded that in anoxic freshwater sediments, both methanogens and sulfidogens participated in methylmercury demethylation, but in estuarine and hypersa-

line sediments, only the contributions of sulfidogens were significant. Bacterial pathways include a two-step mechanism of demethylation followed by reduction to $Hg^0$ and an oxidative mechanism generating $Hg^{2+}$ as the end product (Robinson and Tuovinen, 1984; Oremland et al., 1991).

Abiological demethylation was previously considered to be unlikely because of the lack of activity in sterilized sample (Spangler et al., 1973; Korthals and Winfrey, 1987). However, a number of abiotic pathways in the natural environment may take place, such as the reaction with sulfide to form dimethylmercury (DMHg) and HgS (Deacon, 1978). The abiotic photochemical demethylation in surface waters has also been reported (Sellers et al., 1996).

Our results suggest that demethylation of MMHg is not exclusively a biological process and that potential abiotic processes for environmental demethylation of MMHg could be significant. The sterilization protocols are obviously changing the bulk chemistry of sediment material. The organic matter and other complexing agents can be destroyed or modified during autoclaving (121 °C). The reactivity of the medium against mercury may change by release of thiol groups, membrane polysaccharides, etc. Also, the efficiency of the sterilization should be checked. In these experiments, the analyses of bacteria communities show that the sterilization was effective only during the first 4–5 days of incubation (R. Guyoneaud, personal communication). After, specific spore-forming bacteria, which have not been destroyed by sterilization, grow again. Therefore, conclusions based on the robust sterilization approaches employed in this study are not definitive because of combined biological and chemical sediment modifications. Although further investigation is required to really constrain abiotic experimental conditions, methylation/demethylation kinetic results are directly related to the initial concentration trends that were thus under strict abiotic conditions.

### *4.4. Net methylation/demethylation process*

The specific rate measurements provide the means to determine whether certain environmental perturbations will tend to increase or decrease methylation or demethylation activity. Comparison of M/D ratios indicated the extent to which environmental disturbances may affect the net rate of mercury methylation and hence the methylmercury concentrations in aquatic environments. Under these conditions, the M/D ratio is a direct measure of the relative balance of methylating and demethylating activity.

The M/D ratios calculated for the different incubation conditions are presented in Table 3. After 24 h, the M/D ratio was >1 for the nonsterile anoxic conditions while the M/D ratio was <1 for all the conditions tested after one week of experiments. The nonsterile slurries conditions always produced the highest M/D ratios, indicating that these conditions had higher specific rates of net methylmercury production.

The general results from these experiments are that the conditions most conducive to net methylated species production in estuarine sediments were the anoxic zones under microbial mediating reducing conditions. General redox variations will therefore affect the mercury distribution and transfer with macrotidal estuaries (Tseng et al., 2001).

This effect was investigated in the following series of experiments. To integrate the importance of changing of redox conditions, the effect of a sudden change from anaerobic to aerobic conditions was investigated (Anox-abiot-ox and Anox-biot-ox experiments; Fig. 1). After reoxygenation, the demethylation yield was even more important under the biotic conditions than in the abiotic ones (Table 2). In the previously nonsterile anaerobic system, the demethylation process (both $MM^{199}Hg$ and $MM^{201}Hg$) overrode the methylation process. In the previously anaerobic sterile conditions, the demethylation rate had diminished and no methylation could be observed. This process might represent a natural environmental phenomenon such as resuspension of sediments during flood events or macrotidal cycles. This result implies that when reoxygenation occurs, the net methylmercury concentration will further diminish in the sediment system.

### *4.5. Formation of gaseous Hg from inorganic Hg and MMHg under oxic and anoxic conditions*

Volatilization of monomethylmercury under anaerobic conditions may be due to either disproportionation by $H_2S$ to volatile dimethylmercury or reduction to $Hg^0$ (Robinson and Tuovinen, 1984; Jeffries, 1982). In the presence of $H_2S$, $Hg^0$ would precipitate as HgS rather than volatilize (Compeau and Bartha, 1984).

Also, the yield of $Hg^{2+}$ via an oxidative demethylation pathway is also a possible end product. Therefore, recycling of the added $^{201}Hg$ (as $MM^{201}Hg$) tracer is very likely and the tracer does not necessarily leave the system, e.g., by evasion processes. Under oxic conditions, inorganic mercury reduction processes are also likely to take place through biotic (e.g., microbial reduction) (Gilmour and Henry, 1991; Tessier et al., 2003) or abiotic processes (e.g., photochemical reduction) (Amyot et al., 1997).

The major Hg sources involved in total gaseous mercury production were identified (Fig. 4). The TGM in all incubation media were found to be enriched with both $^{202}Hg$ and $^{201}Hg$ isotopes (natural Hg and $MM^{201}Hg$ signature, respectively).

The aerobic slurries displayed the lowest TGM values (Table 6), for which about 80% of the gaseous mercury originated from the ambient Hg and 20% from the $MM^{201}Hg$ spike (Fig. 4). However, biotic and abiotic aerobic systems did not exhibit significant difference in the TGM concentrations. Because no significant methylation was previously observed in aerobic incubations, volatile Hg must have originate mainly from the reduction of ambient $Hg^{2+}$ to Hg°, through both microbial and abiotic processes. Tseng et al. (2001) also provided evidence of higher $Hg^0$ concentrations in the oxic part of a fluid mud core in a macrotidal estuarine environment.

For the anaerobic experiments, the major contributor to TGM originated from the $MM^{201}Hg$ spike, with 60% and 70% of TGM amount enriched in $^{201}Hg$ for abiotic and biotic slurries, respectively. Therefore, the TGM yield could either result from biotic and abiotic demethylation or methylation of $MM^{201}Hg$ leading, respectively, to $^{201}Hg^0$ and $DM^{201}Hg$ production or from the direct reduction of natural $Hg^{2+}$ to $Hg^0$. Similar results have been observed in the fluid mud of an estuarine macrotidal environment. Tseng et al. (2001) have indeed reported the occurrence of DMHg in the dissolved oxygen depletion area of a fluid mud core. The production of DMHg from $MM^{201}Hg$ spike can then be identified as final product of the methylation pathway and may contribute also significantly to the production of TGM. Considering that demethylation and DMHg synthesis are thought to be predominantly microbially mediated (Robinson and Tuovinen, 1984; Jeffries, 1982), we can infer that TGM produced in the anaerobic nonsterile slurry is likely to have been originated from both $Hg^0$ produced during MMHg demethylation and DMHg produced during MMHg methylation. On the other hand, the TGM levels formed during anaerobic and abiotic incubations are more likely to be mainly in the $Hg^0$ form. This latter hypothesis is supported by the comparison of volatilization and demethylation rates. The highest volatilization rates were obtained under anaerobic sterile conditions, which, in turn, were found to be the most efficient to promote demethylation. Despite the fact that there are not enough data to establish a significant trend, the results confirm that the anaerobic ambient conditions favored both demethylation and volatilization reactions. Here again, the question about the impact of the sterilization process on the chemical properties of the incubation medium can be raised. The potential modification of organic matter and other complexing agents with regard to Hg complexation in the matrix could be hampered and therefore promote the availability of Hg to be reduced to $Hg^0$.

Despite the fact that the TGM amounts produced under the different incubation conditions were found to be significant, the volatilization mechanism did not appear to be of primary importance in the overall Hg budget, when compared with the competitive methylation/demethylation processes. Nevertheless, volatilization pathways are closely linked to these latter driving mechanisms in Hg cycle. Thus, further investigations on the gaseous Hg species, involving a speciation approach associated with enriched isotopic tracers in the liquid and gaseous phases, could offer a powerful tool to completely understand transfer and transformations of Hg between environmental compartments.

## 5. Conclusion

This work presents an original experimental method based on simultaneous speciation of mercury compounds by capillary GC-ICP MS and species-specific enriched stable isotopes. This experimental method has been for the first time applied to estuarine sediments, known as critical compartments for mercury biogeochemistry. The results demonstrate that kinetic investigations (rate constant, lifetime, steady state concentrations) and the analysis of the volatile fraction

can provide key information concerning environmental mercury transformations and mechanisms. As many key processes in mercury biogeochemical cycles are still unknown or poorly characterized, a large array of investigations can be now accomplished using such experiments to unravel mercury cycling in estuarine and marine environments.

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Supplementary electronic material

# "Mercury methylation/demethylation and volatilization pathways in estuarine sediment slurries using species specific enriched stable isotopes"

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## Kinetic calculations

A model based on first order kinetics for both mercury methylation and methylmercury demethylation can be used to describe these transformation reactions. Net production of monomethylmercury can be written as follows:

$$\frac{d\left[MM^{199}Hg^{+}\right]}{dt} = K_m\left[^{199}Hg^{2+}\right] - K_d\left[MM^{199}Hg^{+}\right] \qquad (1)$$

where $MM^{199}Hg^{+}$ = concentration of $MM^{199}Hg^{+}$ newly generated from the $^{199}Hg^{2+}$ tracer in nmol/g; $K_m$ = specific methylation rate constant in $hours^{-1}$; $[^{199}Hg^{2+}]$ = concentration of added $^{199}Hg^{2+}$ in nmol/g; $K_d$ = specific demethylation rate constant in $hours^{-1}$ and $t$ = incubation time in hours. Equation (1), written here for the added inorganic tracer isotope, holds also for the added methylmercury ($MM^{201}Hg$) isotope and the ambient methylmercury isotopes (e.g. $MM^{202}Hg$).

Using data obtained early in the experiments

From those data, $[MM^{199}Hg^{+}]$ is low enough so that the second term in Eq. (1) is much smaller than the first one. After integration, Eq. (1) reduces to:

$$K_m = \frac{\left[MM^{199}Hg^{+}\right]}{\left[^{199}Hg^{2+}\right]t} \qquad (2)$$

When $MM^{201}Hg$ is spiked to the sediment, the $^{201}Hg^{2+}$ resulting from the demethylation is virtually zero at the beginning of the experiment. Equation (1) reduces to:

$$\frac{d\left[MM^{201}Hg^{+}\right]}{dt} = -K_d\left[MM^{201}Hg^{+}\right] \qquad (3)$$

which is readily integrated to:

$$[MM^{201}Hg] = [MM^{201}Hg]_0 \, e^{-K_d t} \qquad (4)$$

where [MM$^{201}$Hg] is the initial concentration of MM$^{201}$Hg in the sediment. The specific demethylation rate constant $K_d$ is obtained by linear regression of $Ln[MM^{201}Hg]$ versus time ($t$).

Also the half-life time ($t_{1/2}$) of methylmercury in sediment can be calculated. The two constants are related by:

$$t_{1/2} = \frac{Ln(2)}{K_d} \qquad (5)$$

Reversible reaction model $\qquad {}^{199}Hg \underset{k_d}{\overset{k_m}{\rightleftharpoons}} MM^{199}Hg \qquad (6)$

The rates of change can be described by:

$$-\frac{d[^{199}Hg]}{dt} = \frac{d[MM^{199}Hg]}{dt} = K_m[^{199}Hg] - K_d[MM^{199}Hg] \qquad (7)$$

With the assumption that [MM$^{199}$Hg]$_0$=0 at the onset of the reaction, Eq. 7 can thus be integrated to obtain [MM$^{199}$Hg] as a function of time.

$$\frac{[MM^{199}Hg]}{[^{199}Hg]_0} = \frac{K_m}{K_m + K_d}(1 - e^{-(K_m + K_d)t}) \qquad (8)$$

[MM$^{199}$Hg]/[$^{199}$Hg]$_0$ ratios were then plotted versus incubation time (in hour) using Origin 6.1 software (OriginLab Corp., Northampton, USA). The Box Lucas 1 exponential fitting model was used to resolve equation (8) and to calculate the methylation ($K_m$) and demethylation ($K_d$) rate constants.

More precisely, the general expression of the equations that can be fitted with Box Lucas 1 is as follows:

$$y = a(1 - e^{bx}) \qquad (9)$$

with $a = K_m/(K_m + K_d)$ and $b = K_m + K_d$

thus $K_m$ ($= a \times b$) represents the tangent to the origin and $K_d = b - K_m$

The MM$^{199}$Hg equilibrium concentration can be calculated, by letting $t$ approach infinity:

$$[MM^{199}Hg]_{eq} = \frac{K_m[^{199}Hg]_0}{K_m + K_d} \qquad (10)$$

# Chapitre D

# Synthèse générale

# Synthèse générale

Les objectifs de ce travail de recherche étaient d'étudier la réactivité et le dynamisme biogéochimique du tributylétain et du mercure dans les écosystèmes aquatiques continentaux et marins. Les problématiques environnementales liées à la contamination des écosystèmes par ces micropolluants métalliques révèlent de nombreuses similitudes quant à leurs implications écotoxicologiques, justifiant cette approche bipolaire. Le tributylétain et le mercure présentent tout deux des caractéristiques de persistance et d'ubiquité dans l'environnement contribuant à les considérer à juste titre comme des substances dangereuses prioritaires. La toxicité du tributylétain et du mercure vis-à-vis des organismes biologiques est en effet effective à des niveaux de concentration extrêmement faibles, de l'ordre du ppt, faisant ainsi de leur surveillance et de l'évaluation des risques associés un défi difficile à relever. Leur devenir dans les environnements aquatiques sont également soumis à des cinétiques complexes de transformation et de transfert et leur disponibilité vis-à-vis des réactions élémentaires chimiques et biologiques demeurent peu connue ou bien mal évaluée en conditions naturelles. Enfin, si les réglementations actuelles, visant à réduire leur rejets anthropiques à l'environnement, on permis d'observer une diminution significative de leurs teneurs dans les eaux naturelles, les sédiments, la biota et l'atmosphère représentent les principaux réservoirs des apports accumulés par le passé, maintenant intégrés à la biogéosphère. Dans l'optique d'une démarche préventive quant à la contamination par le tributylétain et le mercure et à son transfert potentiel entre les différents compartiments environnementaux, il apparaît nécessaire d'orienter les efforts de recherche vers une compréhension accrue de leur cycle biogéochimique dans des situations de contamination chronique et subchronique. De plus il convient également de souligner que ces mêmes efforts de recherche et de surveillance sont principalement focalisés sur les environnements marins. Cette partition commence néanmoins à s'équilibrer, notamment dans le cas du mercure, du fait de son caractère atmophile et donc de sa mobilité plus grande dans l'environnement. Dans le cas du tributylétain, et plus généralement des organostanniques, les problématiques dulçaquicoles demeurent peu représentées, bien que de nombreuses sources anthropiques soient identifiées et contribuent à son introduction dans les hydrosystèmes continentaux. Le contexte législatif actuel ne réglemente également que très peu ces apports potentiels en milieux d'eau douce. Cette étude souligne enfin la nécessité d'appréhender et de caractériser, par le développement de techniques expérimentales et analytiques pluridisciplinaires, les processus réactionnels physicochimiques et microbiologiques multiphasiques régissant le devenir de ces contaminants en milieux aquatiques.

La première étape de ce travail a été de développer des techniques analytiques de spéciation performantes, justes et précises afin de déterminer les concentrations des composés mercuriels et organostanniques dans les

différentes matrices environnementales (air, eau, sédiments, tissus biologiques). Différents couplages analytiques associant la chromatographie en phase gazeuse et la détection par spectrométrie de masse couplée à un plasma inductif et de fluorescence atomique ont ainsi été optimisés afin de réaliser cet objectif. En particulier, la détermination des concentrations en mercure inorganique, monométhylmercure et tributylétain a été significativement améliorée par le développement et la mise en application de techniques innovantes de quantification par dilution isotopique. Cette méthodologie permet en outre de valider de chaque étape de la procédure analytique de spéciation et de s'affranchir des erreurs de quantification liées aux effets de matrices et aux éventuelles pertes ou réactions de conversion des analytes au cours du protocole d'extraction et d'analyse de l'échantillon. Enfin, les performances analytiques offertes par ces techniques, en terme de sensibilité, de précision et de justesse, répondent pleinement aux exigences formulées à savoir la quantification des composés mercuriels et organistanniques, dans les différents compartiments environnementaux, au niveau de la trace ou de l'ultra trace.

Pour réaliser l'étude de la réactivité du TBT et du Hg en milieu aquatique, différentes approches expérimentales simulant des écosystèmes naturels ont été mises en œuvre avec des degrés variables d'organisation et de contrôle des conditions expérimentales. Ce travail avait pour objectif de mieux caractériser les principaux mécanismes réactionnels affectant le devenir du TBT et du Hg, en milieu aquatique et de définir les compartiments cibles de la contamination. Tout d'abord, dans le cas du TBT, des écosystèmes d'eau douce présentant une organisation biologique simple (macrophytes + gastropodes) ont été mis en place au laboratoire. Ces aquariums, comportant un compartiment aqueux, sédimentaire et biologique, ont été incubés après contamination de la colonne d'eau par le TBT. Cette approche expérimentale en milieu contrôlé, associée à des techniques analytiques de spéciation chimique sensibles et précises, nous ont permis d'isoler et d'évaluer, sur une base cinétique, les mécanismes réactionnels régissant les transformations et transferts du TBT en milieu aquatique. L'intérêt majeur de cette approche réside dans l'observation simultanée des cinétiques de transformation et de transfert du contaminant dans et entre l'ensemble des compartiments constituant les microcosmes. Le dimensionnement cinétique des mécanismes réactionnels ainsi que leur importance relative, de même que la distribution du contaminant vers les compartiments cibles ont ainsi été réalisés. Cette étude souligne en particulier le rôle primordial du sédiment en tant que réservoir final pour le TBT et également en tant que site privilégié pour l'établissement des mécanismes de biodégradation. La bioaccumulation du TBT par *Elodea canadensis* et *Lymnaea stagnalis* ainsi que sa dégradation et dépuration *in vivo* ont également été mis en évidence et caractérisées par différents modèles cinétiques du premier ordre. Le compartiment biologique apparaît ainsi jouer un rôle significatif quant à la disparition du contaminant de la colonne d'eau, durant la phase initiale de l'incubation.

Bien que cette approche réductionniste, en microcosmes, ne puisse exactement simuler des conditions naturelles, elle permet de reproduire et de différencier les principaux mécanismes clés, via un modèle simplifié et contrôlé. La faible variabilité des concentrations en butylétains (erreur à la moyenne <15%) mesurées dans les duplicats d'aquariums et pour l'ensemble des matrices échantillonnées a également permis de valider les microcosmes comme étant des outils expérimentaux fiables et pertinents pour l'étude écotoxicologique des micropolluants métalliques en situation de contamination chronique.

Ces dispositifs expérimentaux peuvent être adaptés à de nombreux contaminants traces et offrent des perspectives prometteuses pour une meilleure évaluation des risques écotoxicologiques et la détermination de critères de qualité environnementale, intégrant la composante temporelle et la représentativité environnementale des mécanismes réactionnels décrits ainsi que l'importance relative des principaux compartiments du milieu aquatique où ils interviennent. Ils permettent en effet l'observation simultanée de la partition d'un contaminant entre les différentes composantes d'un écosystème ainsi qu'une paramétrisation précise des cinétiques mises en jeu. Ils semblent enfin pouvoir satisfaire à des tests de toxicité chronique, préalables et indispensables à l'introduction de toute substance chimique nouvelle dans l'environnement.

Le volet terrain de ces travaux est principalement orienté sur l'étude des mécanismes de transfert de composés organostanniques volatils aux interfaces naturelles : sédiment-eau et eau-air. Cette approche a pour but de compléter l'étude du cycle biogéochimique du TBT en milieu aquatique et de s'intéresser à l'établissement de mécanismes naturels de transformation susceptibles de modifier la mobilité du contaminant, représentant ainsi des voies potentielles de remobilisation et d'élimination du TBT. La première étape a été de développer des techniques d'échantillonnage ultra trace et de piègeage cryogénique des espèces volatiles. La mise en application d'un couplage GC-ICPMS a ensuite permis de réaliser la spéciation et l'identification des différents composés organostanniques volatils dans les sédiments et la colonne d'eau. Enfin, ces deux études dans des écosystèmes côtiers et estuariens ont permis de démontrer, pour la première fois, l'existence de ces formes méthylées des butylétains dans des milieux aquatiques naturels. Ces résultats mettent également en évidence l'établissement de mécanismes de transformation interférant le schéma classiquement admis de la dégradation séquentielle du tributylétain par des réactions de désalkylation. De plus, ces travaux ainsi que les approches en microcosmes mettent l'accent sur les transferts effectifs de ses composés volatils aux interfaces sédiment-eau et eau-air. La deuxième étape de ces travaux a été de caractériser les cinétiques de transfert par l'application et l'adaptation de différents modèles d'échange aux interfaces. Les grandeurs thermodynamiques associées à ces espèces mixtes butylées et méthylées de l'étain, telles que la constante de Henry et le nombre de Schmidt, ont été recalculées et corrigées en fonction des conditions environnementales (température, salinité). Les flux de volatilisation de ces espèces ont ainsi pu être quantifiés et extrapolées à l'ensemble des écosystèmes étudiés. Ces études montrent d'une part que les sédiments constituent le site principal de formation de ces espèces

volatiles mixtes, via la méthylation du TBT et de ses produits de dégradation (DBT et MBT) et d'autre part que l'intensité des processus de transfert est directement liée à la pression anthropique (niveau de contamination en TBT) et au régime hydrologique (courants, marée) du site étudié. Enfin, il convient à la lumière de ces résultats de réexaminer la réactivité et la mobilité des organostanniques en particulier dans les sédiments. Ce compartiment, très souvent considéré comme un site de séquestration de ces polluants à très long terme, représente une source potentielle de contamination pour la colonne d'eau et les biocénoses benthiques. L'intégration des sédiments dans les systèmes d'évaluation des critères de qualité environnementale apparaît ainsi très largement justifiée.

Le deuxième axe d'étude de ce mémoire a été focalisé sur la spéciation biogéochimique du mercure dans les environnements aquatiques. Des expérimentations en microcosmes, suivant le même schéma expérimental utilisé pour le TBT, ont été mises en place pour l'étude simultanée de la distribution et de la réactivité du Hg dans et entre les différentes composantes (sédiment, eau, plante, gastéropode) des aquariums. Cette approche en milieu simulé et contrôlé nous a permis de discriminer et de caractériser les cinétiques chimiques impliquées dans la distribution et le devenir du mercure après contamination de la colonne d'eau. Ces expériences soulignent en particulier l'importance des processus de transfert (bioaccumulation/dépuration, volatilisation) et de transformation (méthylation/déméthylation, réduction) du mercure entre les différents compartiments. En particulier, l'intensité des processus de méthylation mesurés en aquarium s'est avérée être très comparable à celle observée dans les environnements naturels. La production de MMHg représente le mécanisme clé régissant le cycle du Hg dans nos microcosmes ainsi que sa remobilisation sous une forme plus toxique et susceptible d'augmenter significativement le risque écotoxicologique via les processus bioconcentration et bioamplification. Enfin, l'observation et la caractérisation cinétique de ces différents mécanismes réactionnels confirment la pertinence et l'utilité de cette démarche réductionniste pour étudier les voies de contamination des écosystèmes aquatiques par le mercure.

L'étude de la réactivité du Hg a ensuite été poursuivie par des incubations de suspensions de sédiments estuariens, dans le but de caractériser finement les mécanismes couplés de méthylation et déméthylation du Hg au moins de traceurs isotopiques. La première étape de ce travail a été de valider cette technique expérimentale innovante combinée à une détection sélective par ICPMS. L'ajout simultané de deux espèces chimiques du mercure marquées spécifiquement par un isotope ($^{199}$IHg et $^{201}$MMHg) à une suspension de sédiment estuarien et son incubation en conditions contrôlées nous ont ainsi permis de travailler à des niveaux environnementaux de concentrations et de discriminer les cinétiques réactionnelles de méthylation et de déméthylation en fonction de différents forçages environnementaux (conditions d'oxygénation du milieu, niveaux de contamination, inhibition de l'activité microbiologique). Les méthodes d'incubation ont tout d'abord été évaluées en terme de sensibilité et de justesse pour tracer les processus environnementaux. Ce travail a permis la détermination des

constantes de vitesse de méthylation et déméthylation du Hg dans les sédiments, par l'application de modèles cinétiques du premier ordre. Les résultats ont en outre montrés que l'activité microbiologique dans le sédiment et les conditions d'anoxie étaient particulièrement propices à l'établissement de la méthylation du Hg. De plus, nos expérimentations ont également permis de mettre en évidence et de quantifier les processus de méthylation abiotique ainsi que l'existence, en toutes conditions, de mécanismes de déméthylation jusque là non observables par des méthodes classiques. Enfin, de manière générale, cette approche expérimentale permettant l'étude détaillée des cinétiques couplées de méthylation et de déméthylation dans des sédiments estuariens, au moyen de traceurs isotopiques stables, illustre les performances de ces techniques innovantes et particulièrement adaptées à la déconvolution des mécanismes réactionnels élémentaires et concurrentiels de transformation et de transfert du mercure dans les sédiments. Ces méthodes de marquage isotopique au niveau moléculaire couplées par exemple à des expériences de biochimie et d'écologie microbienne ouvrent la voie à des perspectives de recherches très prometteuses.

En conclusion, nous avons développé et validé, au cours de ces travaux, diverses approches expérimentales et analytiques afin de mieux caractériser la réactivité du TBT et du Hg dans les systèmes aquatiques. Cet objectif a été atteint par l'association de méthodes performantes de spéciation chimique et de détermination cinétique des mécanismes réactionnels. Les protocoles expérimentaux employés (microcosmes, traceurs isotopiques) ouvrent la voie à de nombreuses perspectives de recherche quant à la spéciation biogéochimique du mercure et de l'étain dans les écosystèmes naturels. Ces travaux préliminaires ont également contribués au développement d'outils susceptibles de répondre à une meilleure évaluation des risques écotoxicologiques et de satisfaire à la détermination précise de critères de qualité environnementale pour les écosystèmes aquatiques vis-à-vis de leur contamination chronique par ces deux micropolluants. Enfin divers projets de recherches, reprenant les bases de ces travaux et valorisant ces aquis, sont actuellement en cours au sein du laboratoire. Le projet ECODYN (Programme National ACI/Ecospère Continentale), vise à la mise en place d'un réacteur complexe simulant les oscillations oxiques-anoxiques dans les sédiments pour étudier la remobilisation et les transformations du Hg de traceurs isotopiques stables. L'impact des polluants métalliques sur les populations de civelles de l'estuaire de l'AdourLe GDR (UPPA-IFREMER). Dans le cadre du GDR UPPA-IFREMER, des expérimentations en aquariums vont être développées pour étudier l'impact des polluants métalliques sur les populations de civelles de l'estuaire de l'Adour. Enfin l'étude de la volatilisation des métaux et radionucléides dans les environnements côtiers ainsi que le rôle des métabolismes alguaires dans la production de ces composés seront approfondis au sein du programme pluridisciplinnaire ToxNuc-E (CEA-CNRS-INRA-INSERM).

# Chapitre E Annexes

# Chapitre E Annexe 1

# Législations et réglementations européennes

# Legislations et réglementations européennes

**74/63/CCE** Les limites sont fixées pour les teneurs en mercure dans les aliments des animaux.

**75/440/CEE** Valeur limite impérative 1 µg $l^{-1}$ de mercure dans les eaux superficielles destinées à la production d'eau alimentaire.

**76/160/CEE** Des vérifications périodiques des concentrations en mercure sont nécessaires dans les eaux de baignade.

**76/464/CEE** Le mercure est placé dans la liste des substances dangereuses pour le milieu aquatique.

**76/768/CEE** L'usage du mercure et de ses dérivés est interdit, à l'exception des composés phénylmercuriques et de l'éthylmercurithiosalicylate tolérés à faibles concentrations.

**78/319/CEE** Des règles sont préconisées concernant la production, la détention, le transport et l'élimination des déchets contenant de mercure.

**78/659/CEE** Le mercure est considéré comme essentiel pour la qualité des eaux douces aptes à la vie des poissons.

**79/117/CEE** L'usage des produits antiparasitaires mercuriels en l'agriculture est interdit.

**79/923/CEE** Les concentrations en mercure dans les eaux conchylicoles doivent assurer la bonne qualité des produits conchylicoles.

**80/068/CEE** Limitation de l'introduction dans les eaux souterraines du mercure et de ses dérivés.

**80/778/CEE** Valeur limite impérative 1 µg $L^{-1}$ de mercure dans les eaux, destinées à la consommation humaine.

**82/176/CEE** Les valeurs limites sont fixées pour les rejets en mercure (75 µg $L^{-1}$ en 1983 et 50 µg $L^{-1}$ après 01/07/1986) dans le milieu aquatique provenant de l'industrie chlore-alcaline.

**82/883/CEE** Surveillance nécessaire des teneurs en mercure dans l'eau, particules en suspension, sédiment et organismes vivants dans les milieux concernés de l'industrie de dioxyde de titane.

**84/156/CEE** Les rejets en mercure des secteurs autres que celui d'électrolyse chlore-alcaline sont considérés. Dans tous les cas la valeur limite en mercure (1 µg $L^{-1}$) est fixée pour les eaux de surface continentales.

**84/360/CEE** Un contrôle des émissions atmosphériques provenant des installations industrielles est imposé.

**84/631/CEE** Le transfert transfrontalier des déchets doit être contrôlé.

**Commission de Paris PARCOM** (05/06/1985) : Décision 85/1. Les valeurs limites sont fixées (100 µg $L^{-1}$ en 1986 et 50 µg $L^{-1}$ a partir de 01/07/1989) pour les rejets de mercure des secteurs autres que celui d'électrolyse chlore-alcaline. Les teneurs en mercure dans la chair des poissons ne doit pas excéder 0.8 mg $kg^{-1}$ (poids humide). La concentration de mercure dans les eaux des estuaires ne dois pas dépasser

0.5 µg $L^{-1}$. Des valeurs limites (poids sec) sont établies pour des niveaux de contamination des coquillages par le mercure: entre 0.6 et 1 mg $kg^{-1}$ les niveaux de contamination sont considérés comme moyens et au-delà de 1 mg $kg^{-1}$ ils sont importants.

**85/613/CEE** Directive concernant la prévention de la pollution marine d'origine tellurique telle qu'elle a été prévue par la Commission de Paris (PARCOM).

**86/278/CEE** Une valeur de mercure 16 mg $kg^{-1}$ (poids sec) est fixée pour les boues d'épuration lors de leurs usages en agriculture. La quantité de mercure pouvant être introduite dans un sol ne doit pas dépasser 0.1 kg $ha^{-1}an^{-1}$.

**89/869/CEE** Valeur limite de mercure (0.2 mg $m^{-3}$) est prévue pour les émissions atmosphériques en provenance d'incinération des déchets municipaux.

**89/677/CEE** Le mercure et ses dérivés sont interdits pour les usages suivants: des peintures antisalissures, de la préservation du bois, les traitements des textiles et eaux industrielles.

**Conférence de la Haye** (08/03/1990). Dès 1996 les émissions atmosphériques doivent être limitées à 2g de mercure par tonne de chlore produit dans les installations d'électrolyse chlore-alcaline. En 2010, les rejets en mercure provenant de telles installations seront interdits. La teneur en mercure dans les piles alcalines au manganèse doit être limitée à 0.025%. Les piles à usages spéciaux, pour lesquelles cette limite ne peut pas être atteinte ne doivent pas dépasser 2% de l'ensemble des piles. Des mesures doivent être prises pour remplacer le mercure des tubes fluorescents, des thermomètres, des plombages dentaires. Une réduction de l'ordre de 70% des apports riverains et atmosphériques du mercure à la mer du Nord doit être envisagée entre 1985 et 1995.

**91/118/CEE** Tout usage des composés organomercuriels est interdit pour le traitement des semences.

**91/157/CEE** Au 01 janvier 1993 est prévu l'interdiction de la mise sur le marché des piles alcalines au manganèse contenant plus de 0.025% en poids de mercure et plus de 0.05% dans le cas des piles destinées à un usage prolongé dans des conditions extrêmes. Il est recommandé de réduire l'élimination des piles avec les ordures ménagères.

**98/101/CEE** A partir du 01 janvier 2000 est interdite la mise sur le marché des piles contenant plus de 0.0005% en poids de mercure, les piles mises en circulation à partir du 18 septembre 1992 et contenant plus de 25 mg de mercure par élément et les piles alcalines au manganèse contenant plus de 0.025% en poids de mercure. Les piles de type bouton ne sont pas concernées par cette interdiction si elles contiennent moins de 2% en poids de mercure.

# Chapitre E Annexe 2

# Spéciation du mercure dans les matrices environnementales par chromatograpie en phase gazeuse couplée à un spectromètre de fluorescence atomique

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Short communication

# Improvement of analytical performances for mercury speciation by on-line derivatization, cryofocussing and atomic fluorescence spectrometry

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**Abstract**

A modified automated on-line hyphenated system for simultaneous inorganic ionic mercury ($Hg^{2+}$) and monomethylmercury ($MeHg^{+}$) analysis by hydride generation (HG) or ethylation (Eth), cryofocussing, gas chromatography (GC) separation and atomic fluorescence spectrometry (AFS) detection has been improved. Both derivatization methods are investigated with respect to the chromatographic and analytical performances. They can be both affected by interferences when the AFS detection system is used. Water vapor removal using a soda lime moisture trap improves significantly the chromatographic performances, the reproducibility and the detection limits for $Hg^{2+}$ and $MeHg^{+}$ analyzed with both methods. For ethylation (Eth) derivatization, a scattering interference generated from low-quality ethylation reagent has also been eliminated. For HG, improved detection limits are 0.13 ng $l^{-1}$ and 0.01 ng $l^{-1}$ for $Hg^{2+}$ and $MeHg^{+}$, respectively (0.1 l water sample), and reproducibility are 5% for $Hg^{2+}$ (20 ng $l^{-1}$) and $MeHg^{+}$ (5 ng $l^{-1}$). Improved detection limits for Eth are 0.22 ng $g^{-1}$ for $Hg^{2+}$ and 0.02 ng $g^{-1}$ for $MeHg^{+}$ (1 g dry sediment sample) and the reproducibility are 5–6% for $Hg^{2+}$ and $MeHg^{+}$ (1–2 ng $g^{-1}$).


*Keywords:* Mercury; Methylmercury; Cryofocussing hydride generation; Ethylation; Atomic fluorescence spectrometry

## 1. Introduction

The source of mercury (Hg) to the aquatic environment appears largely to be in the form of inorganic Hg compounds, either from direct atmospheric deposition or terrestrial runoff [1]. Once in the aquatic system, an interactive web of abiotic or biotic processes can convert inorganic ionic mercury ($Hg^{2+}$) to monomethylmercury ($MeHg^{+}$) [2]. The production of $MeHg^{+}$ is of particular importance, due to its extreme toxicity and biomagnification in the aquatic food chain [3]. Toxicological and biogeochemical studies require therefore speciation analysis of mercury species in various environmental matrices.

As a consequence, much effort has been made to develop analytical techniques for Hg speciation. Coupled techniques include separation by gas chromatography (GC; packed [4,5], capillary [6,7], or multicapillary [8–10]), or liquid chromatography [11] and detection by electron capture (ECD) [6], atomic absorption spectrometry (AAS) [4], atomic fluorescence spectrometry (AFS) [5,12,13], microwave induced plasma atomic emission spectrometry (MIPAES) [8,14] or inductively coupled plasma mass spectrometry (ICPMS) [7,9,10,15]. For sediment samples, questions have arisen about the artefact formation of $MeHg^{+}$ during sample preparation [16]. For water samples, the main problem is that concentration levels of the mercury species are low, especially for $MeHg^{+}$ [5,17].

Hyphenated techniques involving derivatization, cryogenic trapping (CT), gas chromatography and AAS

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detection has been found promising in order to achieve simultaneous determination of $Hg^{2+}$ and $MeHg^{+}$ at trace levels in different environmental matrices [4]. The hydride generation (HG) has been recommended for surface water samples while ethylation (Eth) is suitable for sediment extracts [18]. The AFS has been found to be one of the most sensitive detectors for mercury analysis in the environment providing as well low analytical costs [12]. While the AFS has never been used for the analysis of sediment extracts with cryofocussing Eth, a system HG–CT–GC–AFS has been described elsewhere [13]. However, clear separation between mercury species remains sometimes difficult to achieve in environmental matrices. An investigation of the HG conditions with factorial design has demonstrated that when more water enters the chromatographic column, the sensitivity of the system for the $Hg^{2+}$ is significantly decreased [19].

The objectives of this work are to evaluate HG–CT–GC–AFS and Eth–CT–GC–AFS for the simultaneous determination of $Hg^{2+}$ and $MeHg^{+}$ in environmental samples. We have investigated the potential analytical improvements provided by the addition of a moisture trap (i.e. Nafion drier, Soda lime) before the chromatographic column. Potential spectral interferences generated by both derivatization methods are also studied.

## 2. Experimental

### 2.1. Instrumentation

Mercury speciation analysis is performed using an automated on-line coupled derivatization (D), cryogenic trapping, gas chromatography and AFS detection (Merlin Millennium, PS Analytical, UK) set-up thoroughly controlled by a commercial software (Borwin, JMBS Developments, France) [5]. The general scheme of the system is presented in Fig. 1.

### 2.2. Reagents

Stock standard solutions ($1000\,\mu g\,ml^{-1}$) of $Hg^{2+}$ and $MeHg^{+}$ are prepared by dissolving mercury(II) chloride (Strem Chemicals 99.9995% Hg) in 1% $HNO_3$ (Merck) and methylmercury chloride (Strem Chemicals) in methanol (Merck), respectively. All stock solutions and standards are stored in a refrigerator and protected from light. Working standard solutions ($50\,ng\,ml^{-1}$) are prepared by appropriate dilution of the stock standard solutions with 1% $HNO_3$ and stored for maximum of 1 month in the refrigerator.

For HG, concentrated HCl (Baker) is used to keep the pH at about 1.5–2 during the hydride generation. A 4% $NaBH_4$ (Fluka) is freshly prepared and kept in an ice bath and protected from light. For Eth, Glacial acetic acid (Merck p.a.) and sodium acetate (Sigma) are used to prepare 2 M acetate buffer (HAc/Ac) solution in order to maintain the pH for the ethylation procedure at about 3.5. Solution (0.01%) of $NaBEt_4$ (98%, Strem Chemicals, France) is made daily under argon atmosphere.

All chemicals are of analytical reagent grade. Pure water ($18\,M\Omega\,cm^{-1}$) is obtained from a milli-Q system (Quantum EX, Millipore, USA).

### 2.3. Procedures

The conditions for the analytical procedure are adapted from Tseng et al. [4]. Both derivatization procedures coupled to atomic spectrometry have been validated for the speciation analysis in various environmental matrices [4,5]. Briefly, for HG, the pH of the sample is adjusted to 1.5 with HCl and final analysis is performed using HG–CT–GC–AFS system. For Eth, the pH is adjusted to about 3.3–3.6 with 2 M HAc/Ac buffer and samples are finally analyzed with ethylation as derivatization procedure.

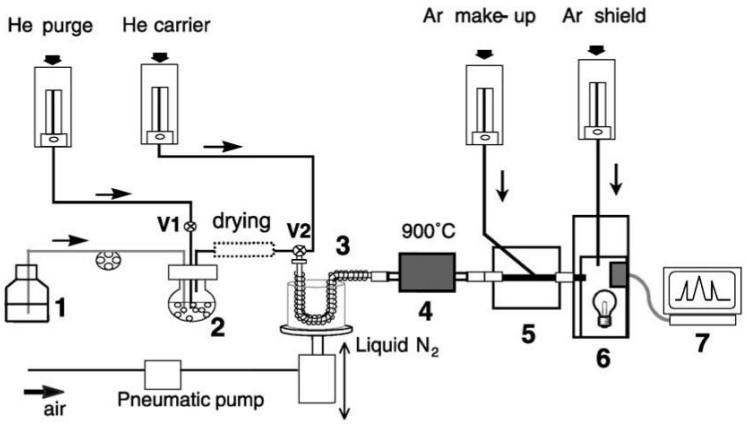


Fig. 1. Hg speciation in environmental matrices by D–CT–GC–AFS: (1) derivatizing solution; (2) reaction vessel; (3) chromatographic column; (4) quartz furnace; (5) PTFE interface; (6) atomic fluorescence detector; (7) computer (data acquisition); V1, V2, solenoid valves.

### 2.4. Moisture traps

A Nafion membrane drier [20] and a soda lime trap [21] have been found useful to improve the detection limit and the reproducibility for total mercury determination by cold vapor generation and atomic spectrometric detection. Similar dryers were connected between the derivatization reactor and the chromatographic column as shown in Fig. 1. The Nafion drier used (Perma Pure®) was described in details elsewhere [20]. The soda lime moisture trap is made of a pre-cleaned glass column packed with soda lime (>100 mesh, Aldrich) and connected between the reactor and the chromatographic column (Fig. 1). The trap is blanked every day prior its use, by heating at 100 °C for 30 min, under Hg free Argon flow (~1 l min$^{-1}$). The soda lime is replaced every 3 days, in order to avoid potential interferences due to its degradation.

## 3. Results and discussion

### 3.1. Improvements of the simultaneous determination of $Hg^{2+}$ and $MeHg^{+}$ by cryofocussing GC–AFS

#### 3.1.1. Influence of the moisture trap on the chromatographic separation

One of the disadvantages of the HG method is the generation of significant amount of water vapor during the derivatization reaction.

Firstly, Nafion drier was tested. The peak of $Hg^0$ became more symmetrical when the Nafion drier was used, but the signal for MeHgH was suppressed. The residence time in the drier was only about 0.9 s, but it was sufficient to decompose (or to adsorb) MeHgH. Similar decomposition of MeHgH in a Nafion drier has been reported in the literature [22]. In our conditions it was thus not possible to use it for mercury species determination with HG and this drier was not further used in our investigation.

Then, soda lime was used. In Fig. 2 presents typical chromatograms obtained with HG and Eth for standard mixtures of 1 ng $Hg^{2+}$ and 0.5 ng $MeHg^{+}$ in milli-Q water using the soda lime trap. The HG and Eth with soda lime trap allow simultaneous determination of $Hg^{2+}$ and $MeHg^{+}$. The influence of this moisture trap was thus investigated for both procedures. The peak width and asymmetry are compared in Table 1 for the systems without moisture trap and with soda lime trap. The peak width is taken at 50% of the maximal height. The peak asymmetry is calculated at 5% of the maximal height, according to the requirements of the European Pharmacopoeia (Waters Corporation, Milford, 2002).

*3.1.1.1. Hydride generation.* The peaks of $Hg^{2+}$ during HG for the system without moisture trap are usually much wider and less reproducible (Table 1). Their tailing is significantly higher (one-tailed *t*-test) at 99% confidence level compared to HG without moisture trap. This fact can lead to chromatographic resolution problems as $Hg^0$ peak appears first. On contrary, the asymmetry for $MeHg^{+}$ for the HG derivatization without moisture trap is significantly lower at 99% confidence level. Anyway, the utilization of soda lime moisture trap improves significantly the chromatographic performances of the analytical set-up for HG method.

*3.1.1.2. Ethylation.* As for the HG procedure, the influence of adding the soda lime trap on the peak width and asymmetry is studied also for Eth–CT–GC–AFS (Table 1). The elimination of the water vapor leads to more reproducible parameters, at least for $Hg^{2+}$. However, the chromatographic characteristics for both $Hg^{2+}$ and $MeHg^{+}$ are slightly worse in the system with soda lime trap. This is possibly due to higher dead volume produced by the moisture trap [23]. Consequently, the water does not seem to interfere with the chromatographic separation during the Eth procedure. Working with soda lime trap also requires frequent check of memory effects potentially generated by the condensation of high boiling points ethylated Hg derivatives. The peak of $Hg^0$ obtained with the Eth procedure (Fig. 2) originates from both ambient air contamination and from possible artefact formation from $Hg^{2+}$. This artefact depends on the pH and the quantity of added $NaBEt_4$ and

Table 1
Comparison of some chromatographic performances (peak asymmetry and width) of D–CT–GC–AFS system with and without soda lime trap

| Peak characteristics | With soda lime trap | | Without moisture trap | |
|---|---|---|---|---|
| | $Hg^{2+}$ | $MeHg^{+}$ | $Hg^{2+}$ | $MeHg^{+}$ |
| Asymmetry HG[a] | 2.68 ± 0.17 | 2.44 ± 0.18 | 3.73 ± 1.10 | 1.90 ± 0.39 |
| Width HG (min)[a] | 0.053 ± 0.004 | 0.056 ± 0.002 | 0.063 ± 0.027 | 0.048 ± 0.003 |
| Asymmetry Eth[b] | 2.41 ± 0.19 | 2.07 ± 0.22 | 2.05 ± 0.12 | 1.70 ± 0.18 |
| Width Eth (min)[b] | 0.070 ± 0.006 | 0.078 ± 0.005 | 0.059 ± 0.031 | 0.063 ± 0.004 |

The results are presented as mean value ± standard deviation.

[a] Chromatograms of standard mixtures ($Hg^{2+}$ and $MeHg^{+}$, 1 and 0.5 ng as Hg, respectively) are used for the calculation of the peak asymmetry and width for HG–CT–GC–AFS with soda lime trap ($n = 5$) and without moisture trap ($n = 10$). During the HG procedure the $Hg^{2+}$ is transformed into $Hg^0$ and $MeHg^{+}$ into MeHgH.

[b] Chromatograms of standard mixtures ($Hg^{2+}$ and $MeHg^{+}$, 2 and 0.5 ng as Hg, respectively) are used for the calculation of the peak asymmetry and width for Eth–CT–GC–AFS with soda lime trap ($n = 7$) and without moisture trap ($n = 5$). During the Eth procedure, the $Hg^{2+}$ is derivatized to $HgEt_2$ and $MeHg^{+}$ is derivatized to MeHgEt.

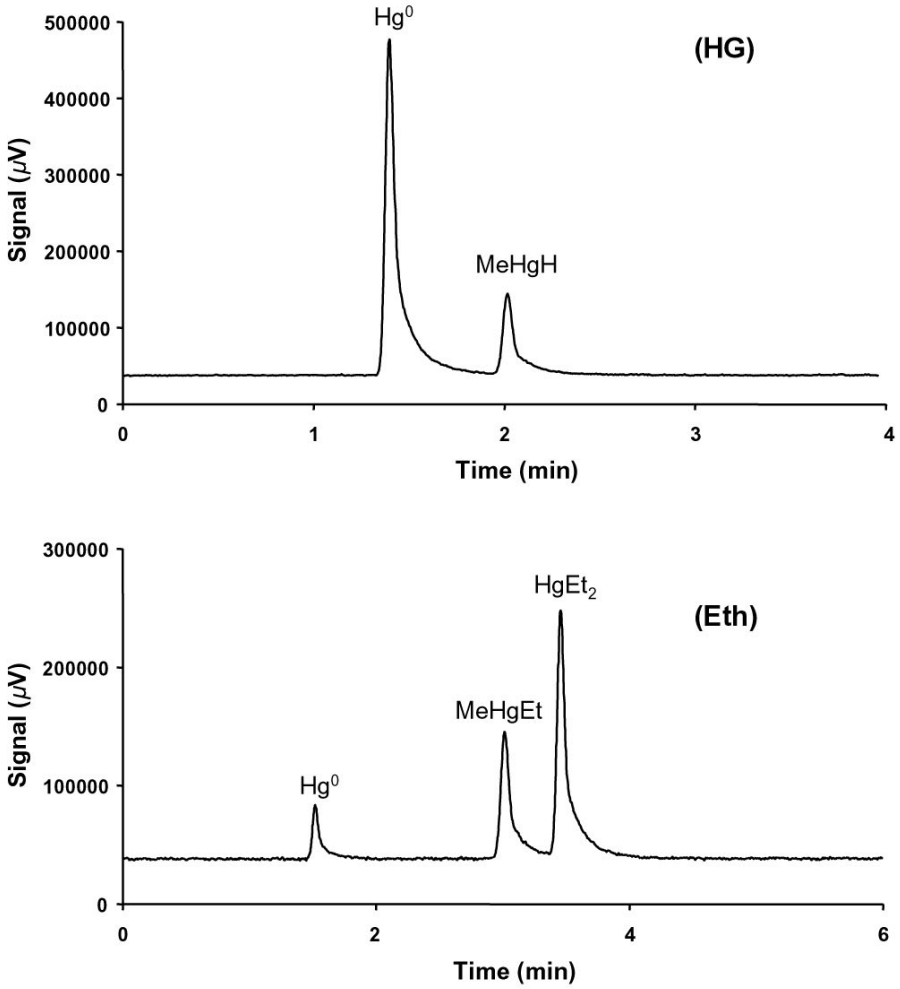


Fig. 2. Chromatograms obtained during the hydride generation (HG) and ethylation (Eth) procedures for a standard mixture of $Hg^{2+}$ and $MeHg^{+}$ (1 and 0.5 ng, respectively) in milli-Q water using soda lime trap.

at the selected conditions does not have influence on the determination [24].

*3.1.2. Removal of a scattering spectral interference with the ethylation procedure*

The critical condition during this procedure is the purity of the $NaBEt_4$, resulting in the appearance of a high peak at retention time 2.6 min, just before the MeHgEt peak. Thus, this peak would interfere with the determination of low concentration of $MeHg^{+}$ in sediments. It was found that this interference came from the utilization of certain lots of $NaBEt_4$. A purging of $NaBEt_4$ solution before use with argon (1 l $min^{-1}$ for 1 h), although useful to purify it from some mercury species [24], did not help to eliminate the observed new interference. It was found that the interfering peak was due to scattering resulting from the formation of fine particles after the quartz furnace (see Fig. 1 (4)). Thus further, when working with the ethylation procedure, a plug of sylanized glass wool was introduced into the interface to remove the particles from the gas stream.

*3.2. Improvements of the reproducibility and the detection limits by cryofocussing GC–AFS*

The analytical performances for Hg speciation are considered in relation to the influence of the soda lime trap for both HG and Eth procedures. The reproducibility is expressed as relative standard deviation (R.S.D. (%)). The absolute and method detection limits (ADL and MDL) are defined from three times the standard deviation from 10 working blank solutions. The results are presented in Table 2.

*3.2.1. Hydride generation*

For HG–CT–GC–AFS the reproducibility is calculated for solutions, containing 20 ng $l^{-1}$ $Hg^{2+}$ and 5 ng $l^{-1}$ $MeHg^{+}$. For the system without moisture trap it is about 12% for $Hg^{2+}$ and 8% for $MeHg^{+}$ ($n = 12$). It is improved when

Table 2
Analytical performances (detection limits and reproducibility) of D-CT-GC-AFS system with soda lime trap and without moisture trap

| | With soda lime trap | | Without moisture trap | |
|---|---|---|---|---|
| | $Hg^{2+}$ | $MeHg^{+}$ | $Hg^{2+}$ | $MeHg^{+}$ |
| Absolute detection limit HG (ng)[a] | 0.013 | 0.001 | 0.1 | 0.005 |
| Method detection limit HG ($ng\,l^{-1}$)[a] | 0.13 | 0.01 | 1.0 | 0.05 |
| Reproducibility HG (RSD, %)[b] | 5 | 5 | 12 | 8 |
| Absolute detection limit Eth (ng)[a] | 0.022 | 0.002 | 0.038 | 0.010 |
| Method detection limit Eth ($ng\,g^{-1}$)[a] | 0.22 | 0.02 | 0.38 | 0.10 |
| Reproducibility Eth (RSD, %)[c] | 5 | 6 | 6 | 6 |

[a] The detection limits (ADL and MDL) are defined from three times the standard deviation from 10 working blank solutions. In the case of Eth–CT–GC–AFS it is expressed for 1 g of sediment (dry weight).
[b] The reproducibility for HG–CT–GC–AFS is calculated for 20 $ng\,l^{-1}$ $Hg^{2+}$ and 5 $ng\,l^{-1}$ $MeHg^{+}$. For the system with soda lime trap 10 measurements were made, while for the system without moisture trap 12 measurements.
[c] The reproducibility for Eth–CT–GC–AFS with soda lime trap is calculated for 2 ng $Hg^{2+}$ and 1 ng $MeHg^{+}$ ($n = 11$). For Eth–CT–GC–AFS without moisture trap the reproducibility is given for 2 ng $Hg^{2+}$ and 2 ng $MeHg^{+}$ ($n = 10$).

the soda lime trap was added, especially for $Hg^{2+}$, reaching 5% for both mercury species ($n = 10$). The DL of the system without moisture trap are 1 $ng\,l^{-1}$ and 0.05 $ng\,l^{-1}$ for $Hg^{2+}$ and $MeHg^{+}$, respectively. With the soda lime, they are improved 10 times for $Hg^{2+}$ and five times for $MeHg^{+}$ (Table 2). This improvement, especially for $Hg^{2+}$, can be explained partly by better chromatographic performances. However, the absence of water entering the detection system resulted also in higher sensitivity of the AFS. It has already been demonstrated, that the water vapor is able to quench the fluorescence signal of elemental mercury [25].

### 3.2.2. Ethylation

The reproducibility for Eth–CT–GC–AFS (Table 2) is between 5 and 6% for both $Hg^{2+}$ and $MeHg^{+}$. There is no significant difference for the system with and without soda lime trap. The MDL of the system without moisture trap are 0.10 $ng\,g^{-1}$ for $MeHg^{+}$ and 0.38 $ng\,g^{-1}$ for $Hg^{2+}$. Additional improvement was noticed, however, for the system with soda lime trap, exhibiting MDL of 0.02 $ng\,g^{-1}$ for $MeHg^{+}$ and 0.22 $ng\,g^{-1}$ for $Hg^{2+}$ (Table 2). The lower DL are possibly due to the reduced water interference during the AFS detection because the chromatographic parameters were not improved by removing the water (see Table 1). The improvement in this case is not so drastic compared to cryofocussing HG, since the Eth procedure suffers to a lesser extend from water vapor interference [23].

### 3.2.3. Blanks

Irrespectively of the improvement of the DL found when the water interference was eliminated, the blank levels for both HG and Eth methods does not change with the addition of the soda lime trap. For HG, they are about 0.1 ng for $Hg^{2+}$ and 0.005 ng for $MeHg^{+}$. During Eth procedure, they are 0.06 ng and 0.01 ng for $Hg^{2+}$ and $MeHg^{+}$, respectively. The much higher blank levels for $Hg^{2+}$ compared to $MeHg^{+}$ explains the higher DL for $Hg^{2+}$ observed with both HG and Eth methods. In the case of Eth–CT–GC–AFS, this can only be due to $Hg^{2+}$ contamination of the reagents. For HG method, however, additional contamination source is found. Approximately 50% of the blank signal for $Hg^{2+}$ is due to $Hg^{0}$ in the headspace of the reaction flask coming from ambient air contamination. Working under ultra-clean conditions (e.g. clean room, laminar flow hood) could significantly reduce this latter contamination source and hence improve the analytical performances of the HG–CT–GC–AFS device.

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# Chapitre E Annexe 3

# Analyses simultanées des espèces du mercure et des butylétains par dilution isotopique dans des échantillons naturels d'eaux et de neiges

# Simultaneous speciation of mercury and butyltin compounds in natural waters and snow by Propylation and Species Specific Isotope Dilution Mass Spectrometry Analysis

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## ABSTRACT

A robust method for the simultaneous determination of mercury and butyltin compounds in aqueous samples has been developed. This method is capable of providing accurate results for analyte concentrations in the $pg.l^{-1}$ to $ng.l^{-1}$ range. The simultaneous determination of the mercury and tin compounds is proposed by species specific isotope dilution, derivatization and gas chromatography-ICPMS (GC-ICPMS). Derivatization by ethylation-propylation, reaction parameters such as pH and the chloride effect were carefully studied. Ethylation was found to be more sensitive to matrix effects, especially for mercury compounds. Propylation was thus the preferred derivatization method for the simultaneous determination of organo-mercury and –tin compounds in environmental samples. The analytical performance of this method provides a high level of accuracy and precision, with RSD values of 1 and 3% for analyte concentrations in the $pg.l^{-1}$ and $ng.l^{-1}$ range. Using cleaned protocols and SIDMS blank measurements, detection limits in the range of 10-60 $pg.l^{-1}$ were achieved, which are suitable for the determination of background levels of these contaminants in environmental samples. This has been demonstrated by the analysis of real snow and sea water samples using this method. This work illustrates the great advantage of species specific isotope dilution technique for the validation of speciation analytical method offering the possibility to overcome species transformations and non quantitative recoveries. Analysis time is saving by the simultaneous method allowing a single sample preparation and analysis.

## 1 INTRODUCTION

Butyltin and mercury compounds are the organometallic species most studied in the environment due to their high toxicity and their widespread distribution. They are found mainly in aquatic environments as a result of their anthropogenic uses, such as tin compounds in the shipping industry, and as a consequence of biotransformation, like methylation of inorganic mercury [1]. The important bioaccumulation ability of these compounds in the food chain is also of concern even for clean compartments of the biosphere. These contaminants can be transported via oceanic waters or atmospheric deposition and even at low concentration levels; these species can pose a real risk to marine organisms. High concentrations of butyltin compounds were observed in marine mammals and birds [2], even for non polluted ecosystems, methylmercury concentrations could be 1 million times the concentration found in the water [3]. The natural concentrations of these compounds in aquatic systems are typically in the $ng.l^{-1}$ range or less [2,3]. For these reasons, sensitive analytical methods are required to determine these low concentration levels.

Gas chromatography (GC) is a widely used separation technique for organo-mercury and –tin compounds [4,5]. GC hyphenation with an inductively coupled plasma mass spectrometer (ICPMS) is often used as a sensitive and multi-isotopic technique [5-7]. To date it is well established that ICPMS allows high sensitivity and multi-isotope capability for tin and mercury speciation analysis. In addition this technique allows the application of isotope dilution calibration [8,9]. For GC-ICPMS, the analyte compounds have to be transformed into volatile species prior to the analysis. Rapsomanikis et al. [10] have reviewed derivatization using $NaBEt_4$ showing its significant capabilities for the analysis of organo-mercury, -tin and –lead. Recently derivatization using $NaBPr_4$ has been reported [11-14] to exhibit promising results for organo-mercury and organo-tin species. De Smaele et al. [11] were the first to report the similar behaviour of $NaBPr_4$ than $NaBEt_4$ as derivatization reagent as well as the study of Schubert et al. [12] for the analysis of organo-tin and organo-lead. Demuth et al. [13] have demonstrated the advantages of $NaBPr_4$ for the determination of MMHg in aqueous samples avoiding MMHg artefactual transformations when using $NaBEt_4$.

Despite significant improvements in instrumentation, the quality of results is mainly associated with sample pre-treatment stages. There are traditional problems related to non-quantitative recoveries, and more recently, questions have arisen about the artefact formation and the transformations of species during sample preparation [13,15,16]. In the case of mercury in environmental samples, the detection of inorganic mercury and methylmercury needs to take into account the possible transformations by methylation, demethylation or reduction. For butyltin compounds, debutylation is possible. The use of isotope dilution techniques addresses these issues since quantitative recoveries are not necessary and rearrangement reactions can be easily

detected [13,15,16]. Several studies have shown the high potential of this technique for the speciation analysis of mercury [17-22], tin [21, 23-26], and chromium [27].

The objective of this work was to develop a robust method for the simultaneous detection of mercury and butyltin compounds in aqueous samples, and to provide accurate results even for very low concentration levels. Derivatization conditions were carefully studied for all the organo-metallic compounds taking into account environmental conditions (matrix interferences, concentration levels).

## 2 EXPERIMENTAL SECTION

### *2.1 Instrumentation*

A new interface developed in collaboration with Thermo Elemental has been used coupling a Thermo Electron GC (Focus) with a Thermo Electron ICP-MS (X7 series). The instrumental configuration permits a dual sample introduction that enables the system to operate under mixed wet and dry plasma conditions. The silcosteel capillary transfer line was inserted into a particular design torch injector allowing the introduction of the transfer line and the use of the impact ball nebulizer enabled continuous aspiration of standards (Tl + Sb) solution (10 μg/L). This configuration allowed optimization of the instrument performance and simultaneous measurement of $^{203}Tl/^{205}Tl$ and $^{121}Sb/^{123}Sb$ for mass bias correction during the chromatographic run. Operating conditions and instrumentation are listed in Table 1. An analytical balance Sartorius model BP211D with a precision of $10^{-5}$ g (Goettingen, Germany) was used for all the weighings.

1.1.1.1 2.2 Reagents

Ultrapure water was obtained from a Milli-Q system (Quantum EX, Millipore, USA). Analytical reagent grade isooctane, glacial acetic acid and sodium acetate were obtained from Sigma Aldrich (Belgium).

Buffer solution of pH 5 was prepared by dissolving sodium acetate in MQ water and adjusting to pH 5 with glacial acetic acid. Sodium tetraethylborate ($NaBEt_4$) (purity higher than 99%) and sodium tetrapropylborate ($NaBPr_4$) were purchased from GALAB (Germany). 0.5% (w/v) $NaBEt_4$ and $NaBPr_4$ solutions were prepared every 6 hours and kept in the dark.

### 2.3 Standards and samples

*Butytin standards* - Natural tributyltin chloride (TBTCl) and enriched [117Sn]TBTCl (90.5 μg $ml^{-1}$) were obtained from LGC Limited (United Kingdom). The purification and synthesis of these standards is described by Sutton et al. [28] A stock solution of 1000 mg (Sn) $l^{-1}$ of TBTCl was prepared by dissolving TBTCl in methanol and kept in

a fridge at dark. A 10 ng.ml$^{-1}$ as Sn dilution was prepared daily and used for the calibration. A dilution of the [117Sn]TBTCl spiking solution was prepared by weighing 0.1 g of a 90.5 mg g$^{-1}$ enriched solution and diluting into 10 g of water. Stock solutions of MBT and DBT (1000 mg (Sn) l$^{-1}$) of natural isotopic composition were prepared by dissolving $BuSnCl_3$ and $Bu_2SnCl_2$ (Sigma-Aldrich, Belgium) in methanol.

*Mercury standards* - Stock solutions of IHg and MMHg (1000 mg (Hg) l$^{-1}$) of natural isotopic composition were prepared by dissolving mercury (II) chloride (Strem Chemicals, USA) in 1% $HNO_3$ and methylmercury chloride (Strem Chemicals, USA) in methanol, respectively. Working standard solutions were prepared daily by appropriate dilution of the stock standard solutions in 1% $HNO_3$ and stored in the fridge until use.

| **GC parameters** | | | |
|---|---|---|---|
| Column | | MXT Silcosteel 30m id 0.53mm df 1µm | |
| Injection port | | Splitless | |
| Injection port temperature | | 250°C | |
| Injection volume | | 3 µl | |
| Carrier gas flow | | He 25 ml/min. | |
| Make up gas fbw | | Ar 300 ml/min. | |
| Oven program | | | |
| Initial temperature | | 60°C | |
| Initial time | | 0 min | |
| Ramp rate | | 60°C/min. | |
| Final temperature | | 250°C | |
| **Transfer line** | | | |
| Temperature | | 280°C | |
| Length | | 0.5 m | |
| Inner | | Silcosteel i.d. 0.28mm o.d., 0.53 mm | |
| Outer | | Silcosteel i.d. 1.0 mm. o.d. $^1/_{16}$" | |
| **ICP-MS parameters** | | | |
| Rf Power | | 1250 W | |
| Gas flow | Plasma | 15 L/min. | |
| | Auxiliary | 0.9 L/min. | |
| | Nebulizer | 0.6 L/min. | |
| Isotopes/dwell times | | Hg : 202. 201.199 | 30 ms |
| | | Sn : 117. 120 | 30 ms |
| | | Tl : 203. 205 | 5 ms |
| | | Sb : 121. 123 | 5 ms |

***Table 1 -*** Operating conditions for the GC/ICPMS coupling system.

Methylcobalamine (Sigma-Aldrich, Belgium) used for enriched monomethylmercury synthesis was prepared by dissolution in an acetic acid-acetate buffer solution (0.1 M, pH 5). $^{201}HgO$ was obtained from Oak Ridge National Laboratory (USA). The description of the methylmercury synthesis is described in a previous work [29]. $^{199}HgCl_2$ was prepared by dissolving $^{199}HgO$ (Oak Ridge National Laboratory, USA) in HCl.
The concentrations of the enriched TBT and MMHg solutions obtained were calculated by reverse isotope dilution mass spectrometry (RIDMS) at the same time as the sample spiking procedure. Three independent isotope dilution RIDMS analysis were carried out and each solution was injected five times.
WARNING : MMHg and TBT are highly toxic compounds and must be handled with appropriate personal protection.
Inorganic Antimony, tin and thallium were obtained from Spex Certiprep (USA). Inorganic antimony and thallium were used for the mass bias correction for tin and mercury, respectively. Inorganic tin was used for the detector dead time correction.

*Cleaning procedure* - Working under clean conditions is also an important consideration for the successful analysis at very low concentration levels. Special attention has been paid to the cleaning procedure of all vessels used for the sampling and the sample preparation. Briefly, all the containers were first washed using a detergent and rinse with MQ water. They were then cleaned in successive acid baths (nitric acid and hydrochloric acid). After rinsing with MQ water, they were dried under a laminar flow hood and stored in double sealed polyethylene bags until use. Blank determinations were also performed by SIDMS to check for any contamination during the sample preparation.

*Samples* - To date, no aqueous certified reference material exists for the speciation of mercury and tin compounds due to the instability of these compounds. To test the accuracy of the analytical procedure, the method was applied to synthetic and real aquatic samples (natural sea water and snow samples).
Synthetic sea water samples were obtained by dissolving NaCl in MQ water at 13 and 35 $g.l^{-1}$ concentration levels. Samples were then spiked with natural species (MMHg, IHg, MBT, DBT and TBT) and submitted to derivatization.

Fresh natural snow deposits samples were collected in the Pyrenees Mountain (South West, France) in February 2003 using cleaned HDPE bottles. Snow 1 was collected at an altitude of 788m and snow 2 at 800m. They were melted under a laminar flow hood, transferred to cleaned Teflon bottles, acidified with HCl 1% and stored at -20°C prior to the analysis.

Natural sea water samples were collected in the Thau Lagoon (Mediterranean Sea, France) in May 2003 using pre-cleaned Teflon bottles at two different locations. Water 1 was collected in the middle of the lagoon, water 2 near shellfish farming area. The samples was filtered through 0.45µm PVDF filter, acidified with HCl 1% and then stored at 4°C until measurements.

**2.4 Procedures**

In the proposed procedure, 100 ml of sample is accurately weighed in a flask and spiked with known amounts of the enriched [117Sn]TBTCl, [201Hg]MMHg and [199Hg]IHg solutions. The mixture is then directly submitted to derivatization. Each experiment is performed three times with three GC injections.

*Spiking procedure for isotope dilution* - Isotope dilution is based on the addition of a precise amount of an isotopically labeled form of the analyte to the sample. The concentration of the analyte in the sample can be calculated from the observed isotope ratios when the natural and enriched isotope ratios, the mass of sample and spike are respectively known. The spiking procedure is a critical stage that requires full equilibrium and the same behaviour for both the analyte and the analogue during the analytical procedure. For aqueous samples, equilibration step is less critical than for solid matrix for which the solubilization should be quantitative to be sure of the full equilibrium. To minimize errors on the isotope ratio in the final determination, the amount of enriched standard added to the sample is adjusted in order to obtain a spike to analyte isotopic ratio close to unity.

*Derivatization of the extract* - 5 ml of acetic acid / sodium acetate buffer (0.1M) was added to 100 ml of the water sample. The pH was adjusted at 5 using concentrated ammonium hydroxide. 0.2 ml of iso octane and 1 ml of 0.5% (w/v) sodium tetraethylborate (or sodium tetrapropylborate) were added, and the flask was immediately capped and vigorously hand shaken by hand for 5 min. The organic phase was then transferred to an injection vial and stored at – 18°C until measurement.

*SIDMS-GC-ICPMS analysis* - Optimized operating conditions used for the GC separation and the ICPMS detection for the simultaneous speciation of organomercury and organotin compounds has been described elsewhere [21]. Briefly, GC conditions were chosen in a way that the elution of the species are sufficiently away from the zone disturbed by the solvent elution (iso-octane). Peak shape was improved by heating the transfer line especially for the heavy species like TBT. The make up gas flow through the transfer line was optimized to obtain the best symmetric peak shape and the highest sensitivity. Parameters affecting the precision and accuracy of the isotopic ratio measurements such as detector dead time and mass bias were carefully evaluated. In previous works, mass bias for mercury has been corrected by using the $^{205}Tl/^{203}Tl$ isotope ratio [17]

and for tin by $^{123}Sb/^{121}Sb$ [26]. According to these methods, simultaneous isotope dilution analysis was performed under wet plasma conditions with a standard solution containing thallium and antimony (10 µg $l^{-1}$) introduced in the cyclonic chamber during all GC measurements. All GC/ICPMS parameters are presented in Table 1.

MMHg, IHg and TBT were analyzed by SIDMS whereas MBT and DBT were analyzed by external calibration. Each sample was injected three times and the chromatographic peak integration was performed using homemade software. Isotopes ratios were always measured as peak area ratios and corrected by mass bias factor.

*Blanks determination* - Blanks were also determined to check for any contamination which could be an important error source at low concentration levels. Each step of the procedure was carefully evaluated in terms of contamination. First, sample conditioning was tested by storing MQ water in the same condition as the real samples. For snow samples, blanks were carried through the storage and melting, and for water samples, blanks were carried through the storage and filtration. Steps of the entire sample preparation procedure were also checked carefully. Blanks were carried through the entire derivatization procedure with the analysis of samples. All these different blanks were quantified by species specific isotope dilution assuring accuracy and precision for these low values.

## 3 RESULTS AND DISCUSSION

### 3.1 Simultaneous determination of mercury and tin species in water samples

*Derivatization pH* - The derivatization of organometallic compounds with tetraalkylborates is known to be strongly pH dependent and needs to be optimized for each compound. Figure 1 shows the influence of the pH value on the relative derivatization efficiency of butyltin and mercury compounds by ethylation (1a) and propylation (1b). In the series of experiments, the pH was set between 3.8 to 5.2 for spiked solutions with 10 $ng.l^{-1}$ of each compound. For ethylation of mercury compounds, the intensity decreases slightly as the pH increases. For butyltin, the derivatization efficiency increases significantly as the pH increases. According to this pH optimization study, a pH value of 5 allows simultaneous derivatization of all investigated organotin and organomercury compounds with satisfactory derivatization yields. A similar series of experiments were carried out for derivatization using sodium tetrapropylborate and similar results were obtained. For this reason, derivatization for both ethylation and propylation were carried out under identical conditions.

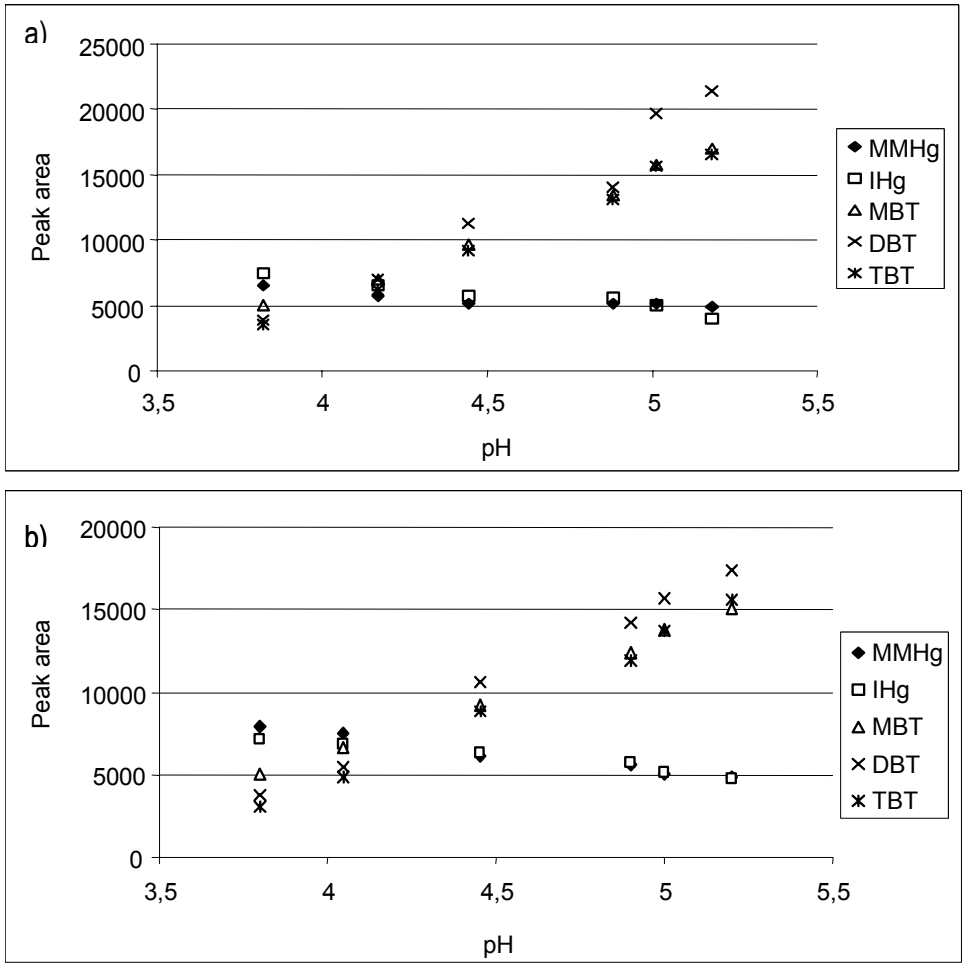


***Figure 1 -*** *Influence of pH on the peak area of organomercury and organotin compounds using $NaBEt_4$ (a) and $NaBPr_4$ (b) as derivatizating reagents.*

*Chloride effect* – De Diego et al. [30] mentioned that possible transformations of mercury species can be promoted by the presence of sodium chloride using ethylation. This attribution has been highlighted with the study of Demuth et al. [13] with the help of isotopically enriched species. Results have shown that methylmercury transformation into elemental mercury takes place exclusively with the presence of halide ions in the water even at low concentrations. Mercury and tin compounds responses were followed by varying the sodium chloride concentration. Table 2 gives recoveries for mercury and butyltin compounds in sodium chloride solutions (13 and 35 $g.l^{-1}$ of NaCl) containing 1 $ng.l^{-1}$ of each compound. Results are given for quantification both by external calibration, and specific isotope dilution, and show the strong dependence of the derivatization efficiency for mercury compounds on the presence of sodium chloride. Methylmercury is the most affected compound with only 30% recovery whereas inorganic mercury recoveries are greater than 80%. These results agree with the study of Carpinteiro et al. [31]. In this work, a calibration in sea water exhibits a slope 3 times lower than for the calibration in MQ water. De Diego et al. [30] attributed this chloride interference to the formation of highly stable chlorocomplexes of MMHg. A reduction of these complexes into elemental mercury has been demonstated by Demuth et al. [13] during ethylation in a salt water matrix.

| | NaCl 13 $g.l^{-1}$ | | | | NaCl 35 $g\ l^{-1}$ | | | |
|---|---|---|---|---|---|---|---|---|
| calibration | EC[1] | | SIDMS[2] | | EC | | SIDMS | |
| | Ethylation | Propylation | Ethylation | Propylation | Ethylation | Propylation | Ethylation | Propylation |
| MMHg | 30 | 96 | 101 | 100 | 30 | 94 | 101 | 100 |
| IHg | 83 | 98 | 100 | 99 | 81 | 95 | 100 | 99 |
| MBT | 101 | 102 | ND* | ND | 102 | 102 | ND | ND |
| DBT | 104 | 101 | ND | ND | 108 | 105 | ND | ND |
| TBT | 111 | 105 | 100 | 102 | 117 | 105 | 100 | 101 |

(All the RSD are below 5%), [1]External Calibration. [2]Species Specific isotope dilution, ND* : not determined

***Table 2 -*** *Chloride ions interferences on derivatization efficiency. Recoveries ± standard deviation (n=3).*

No interference by sodium chloride can be observed for butyltin compounds but on the contrary with mercury compounds, efficiency seems to be improved, especially for heavier species. TBT recoveries increase with the salinity and reach of 117% for a sodium chloride concentration of 35 $g.l^{-1}$. By adding sodium chloride, the solubility of butyltin compounds decrease. For pH 5, Inaba et al. [32] have determined solubility for TBTCl of 70 $mg.l^{-1}$ in distilled water and 2 $mg.l^{-1}$ in seawater. This allows a more efficient extraction of butyltin compounds in the organic phase for sodium chloride solutions.

Recovery values are also given for the analysis by specific isotope dilution for MMHg, IHg and TBT. The results show that isotope dilution allows correcting differences between derivatization efficiencies in MQ water and more complex matrix. Using species specific isotope dilution allows avoiding the calibration by standard addition which is also time consuming. Accuracy as well as the precision (RSD ranging from 0.9 to 2.1%) are simply and remarkably improved.

*Derivatization reagent*- An alternative for derivatization of such compounds in water containing salts has been proposed by several authors. Demuth et al. [13] have shown that propylation do not lead to artifact transformation of methylmercury in sea water. Schubert et al. [12] have compared derivatization using $NaBEt_4$ and $NaBPr_4$ for the analysis of organotin and organolead compounds and similar performances were observed. Lastly, propylation was applied by De Smaele et al. [11] for the simultaneous determination of organo-mercury, -lead and –tin compounds with equivalent efficiency than ethylation.
In this study, the identical series of experiments done by ethylation on salt effects has been repeated using propylation. Results are presented in Table 2. The same sensitivity for mercury compounds was achieved regardless of the sodium chloride concentration. As previously found by Demuth et al. [13], no methylmercury transformation takes place using sodium tetrapropylborate as derivatizating reagent. For butyl tin, the sensitivity is equivalent with those found by ethylation. This alternative for derivatization is thus preferential for the analysis of environmental samples which can exhibit high salinity and very low concentrations in the range of the $pg.l^{-1}$.

*Species specific isotope dilution analysis*- In addition to providing the highest level of accuracy and precidion, SIDMS also allows for the tracking and correction of possible species transformations during the analytical procedure. Figure 2 presents a chromatogram of a standard solution (2 $ng.l^{-1}$) spiked with enriched [117Sn]TBT, [201Hg]MMHg and [199Hg]IHg solutions, derivatizated by propylation. As reported by Demuth et al. [13], methylmercury can be reducted into Hg°. Alkyl addition or removal is also possible such as methylation of inorganic mercury and debutylation of butyltin compounds. Neither methylation of inorganic mercury, nor demethylation of methylmercury has been observed in standards and natural samples. Similarly, no debutylation of tributyltin has been detected. Species specific isotope dilution allows the correction of mercury species losses for matrix containing chloride ions.

**3.2 Performances of the analytical technique**

The performance of this method was investigated using spiked MQ water and synthetic sea water (35$g.l^{-1}$ NaCl) samples both for ethylation and propylation as derivatizating reactions. The LODs were determined by calculating 3 times the standard deviation for 5 blank solutions divided by the slope of the calibration curve (0.1-

5 ng.l$^{-1}$). Relative standard deviations were calculated from the standard deviation of 3 independent measurements of solutions containing 1 ng.l$^{-1}$ of each species. As Shown in Table 3, the characteristics for both derivatization methods in MQ water are comparable in terms of detection limits and precision. For sea water, propylation is the more sensitive derivatization technique, particularly for mercury species where detection limits were 3 times lower for MMHg. This is especially important for the analysis of natural samples from non polluted environments where MMHg concentration levels are not expected to exceed few tenth pg.l$^{-1}$. Analysis of real sea water samples will show that, in some cases, measurements of MMHg are feasible by propylation whereas no signal is detectable using ethylation.

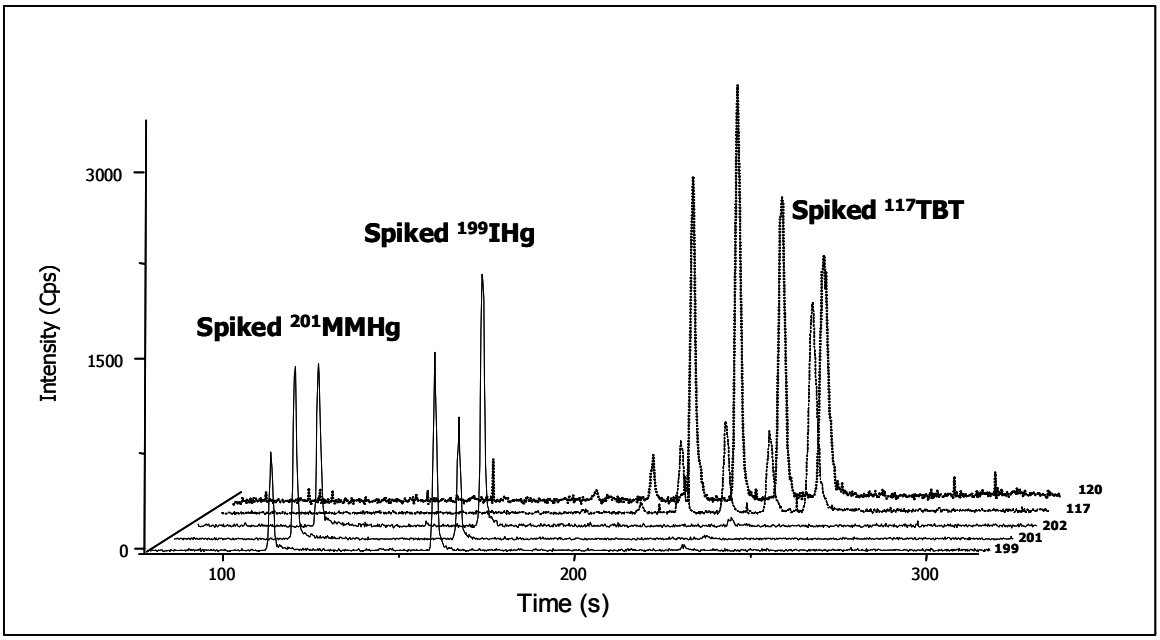


***Figure 2 -*** *Chromatogram obtained by GC-ICPMS for a standard solution containing mercury and butyltin compounds (2 ng.l$^{-1}$) spiked with enriched $^{201}$MMHg, $^{199}$IHg and $^{117}$TBT and derivatized with NaBPr$_4$.*

| **Ethylation** | | MMHg | IHg | MBT | DBT | TBT |
|---|---|---|---|---|---|---|
| MQ water | LOD* (ng l$^{-1}$) | 0.016 | 0.053 | 0.030 | 0.023 | 0.020 |
| | RSD%** | 1.6 | 1.9 | 1.8 | 4.2 | 1.2 |
| Sea water | LOD (ng l$^{-1}$) | 0.045 | 0.075 | 0.028 | 0.020 | 0.017 |
| | RSD% | 3.1 | 2.6 | 2.0 | 4.2 | 1.8 |
| **Propylation** | | MMHg | IHg | MBT | DBT | TBT |
| MQ water | LOD (ng l$^{-1}$) | 0.010 | 0.060 | 0.033 | 0.027 | 0.022 |
| | RSD% | 1.7 | 1.4 | 1.8 | 2.5 | 1.3 |
| Sea water | LOD (ng l$^{-1}$) | 0.012 | 0.063 | 0.032 | 0.024 | 0.019 |
| | RSD% | 1.1 | 2.3 | 1.6 | 2.1 | 1.8 |

*LOD=3*SD/calibration slope, **RSD% relative standard deviation (n=3. 1 ng l$^{-1}$)

***Table 3 -*** *Analytical performances for ethylation and propylation of spiked water (MQ water and sea water (NaCl 35g l$^{-1}$))*

### 3.3 Application to real aquatic samples

#### A- Snow samples

The described method was applied to the determination of mercury and tin compounds in natural environmental samples. Table 5 presents results for two snow samples collected in the Pyrenees (South West, France). Levels of MMHg, IHg and TBT in samples were obtained by species specific isotope dilution analysis. Values found by external calibration are also added for comparison. In addition, table 4 presents the assessment of derivatization by ethylation and propylation for mercury compounds and TBT.

*Blank controls* – As previously indicated, blank contamination could be the limiting factor for obtaining accurate results for low concentration levels. In the case of snow samples, blanks of the whole sample preparation (storage, melting, derivatization) were carefully investigated (see Table 4). For mercury compounds and TBT, quantification of blanks by SIDMS allows high reproducibility on blank values even for very low concentrations assuring very low detection limits (0.015, 0.108 and 0.138 ng $l^{-1}$ respectively). For MBT and DBT, blanks quantifications have shown an important and non-reproducible contamination certainly due to the storage in HDPE bottles. Results for MBT and DBT are not valid and are not presented here.

| Procedural blanks | | MMHg* | IHg* | MBT** | DBT** | TBT* |
|---|---|---|---|---|---|---|
| Snow sample (n=6) | Value (ng $l^{-1}$) | 0.028 | 0.774 | 0.250 | 1.45 | 1.02 |
| | SD (ng $l^{-1}$) | 0.005 | 0.036 | 0.046 | 0.47 | 0.29 |
| | LOD (ng $l^{-1}$) | 0.015 | 0.108 | 0.138 | 1.41 | 0.87 |
| Sea water (n=8) | Value (ng $l^{-1}$) | 0.056 | 0.775 | 0.218 | 0.22 | 0.19 |
| | SD (ng $l^{-1}$) | 0.007 | 0.042 | 0.014 | 0.03 | 0.04 |
| | LOD (ng $l^{-1}$) | 0.021 | 0.126 | 0.042 | 0.10 | 0.13 |

* determined by IDA, ** determined by external calibration

***Table 4 -*** *Blanks values of the entire sample preparation procedure for sea water and snow samples with associated standard deviations (SD) and detection limits (LOD).*

*Natural background concentrations* - The results by SIDMS for MMHg obtained by ethylation and propylation are in good agreement, with low relative standard deviations (2.6-6.3%) even at this low concentration levels. The results found by ethylation show that application of this derivatization method using an external calibration leads to inaccurate results (78 and 76% recoveries for snow 1 and 2 respectively). These results confirm that ethylation is more sensitive to such matrix effect than propylation, even when the matrix doesn't contain high concentrations of chloride ions. Further systematic investigations are necessary to identify other components that could promote transformation mechanisms such as humic acids or others halide ions.

| **MMHg** | | | | |
|---|---|---|---|---|
| Derivatization | Propylation | | Ethylation | |
| Calibration | EC[1] | SIDMS[2] | EC | SIDMS |
| Snow 1 | 0.416±0.021 *(5.0%)* | 0.386±0.018 *(2.6%)* | 0.305±0.027 *(8.8%)* | 0.390±0.016 *(4.1%)* |
| Snow 2 | 0.251±0.025 *(9.9%)* | 0.238±0.017 *(7.1%)* | 0.181±0.020 *(11.0%)* | 0.236±0.015 *(6.3%)* |

| **IHg** | | | | |
|---|---|---|---|---|
| Derivatization | Propylation | | Ethylation | |
| Calibration | EC | SIDMS | EC | SIDMS |
| Snow 1 | 0.446±0.025 *(5.6%)* | 0.423±0.014 *(3.3%)* | 0.482±0.053 *(10.9%)* | 0.412±0.014 *(3.3%)* |
| Snow 2 | 1.868±0.122 *(6.5%)* | 2.011±0.050 *(2.4%)* | 1.837±0.154 *(8.4%)* | 1.986±0.065 *(3.3%)* |

| **TBT** | | | | |
|---|---|---|---|---|
| Derivatization | Propylation | | Ethylation | |
| Calibration | EC | SIDMS | EC | SIDMS |
| Snow 1 | 0.400±0.023 *(5.7%)* | 0.399±0.005 *(1.2%)* | 0.412±0.019 *(4.6%)* | 0.409±0.006 *(1.5%)* |
| Snow 2 | 0.294±0.015 *(5.1%)* | 0.311±0.007 *(2.2%)* | 0.322±0.022 *(6.8%)* | 0.304±0.007 *(2.3%)* |

[1]External Calibration. [2]Species Specific isotope dilution

***Table 5- -****Determination of mercury species in snow samples using $NaBEt_4$ and $NaBPr_4$ as derivatization reagent. Mean values ($ng.l^{-1}$) ± standard deviation (n=3) and relative standard deviation.*

Results for IHg, values found by external calibration and SIDMS are in a good agreement both for ethylation and propylation. Precision on the results is improved with RSD ranging from 2.5 to 3.3% for SIDMS determination whereas with external calibration RSD were between 5.6 to 10.9%. As the same way, TBT concentrations found by ethylation and propylation well agree. Using SIDMS allow improving precision on the results with RSD ranging from 1.2 to 2.3% whereas by external calibration RSD are ranging from 4.6 to 6.8%.

**B- Sea water samples**

*Blank controls* – Blank contamination of the whole procedure for sea water analysis including filtration, storage and derivatization were carefully controlled (see Table 4). Coupling a meticulous cleaning procedure with blank quantification by SIDMS have led to very low and reproducible contamination permitting very low detection limits with 0.021, 0.126 and 0.042 ng $l^{-1}$ for MMHg, IHg and TBT, respectively.

*Natural background concentrations* - Table 6 presents concentrations of mercury and butyltin compounds found in the sea water samples both by ethylation and propylation. MMHg, IHg and TBT concentrations were determined by species specific isotope dilution, and MBT and DBT by external calibration.
In the case of mercury, some notable disparities can be observed between the two derivatization procedures. For IHg, low values were found by external calibration for the ethylation procedure with about 86% of recovery in comparison with SIDMS quantification. As mentioned previously, ethylation is more sensitive to matrix interferences but can be overcome by using isotope dilution. Propylation exhibits an excellent agreement between values found by external calibration and SIDMS but precision is greatly improved using SIDMS with RSD divided by a factor 2.
Using ethylation, methylmercury is below the detection limit whereas using propylation, it can be determined at very low concentration levels with a good precision. Figure 3 illustrates these results showing chromatograms obtained for the sea water sample (water 1) using ethylation and propylation. Ethylation suffers from matrix interferences for MMHg and no peak of MMHg is detected. Using propylation for the same sample, a significant peak of MMHg is detected allowing the quantification of MMHg in this sample. Regarding precision on the results, the use of propylation in connection with SIDMS quantification allow to drastically improve RSD with 24 and 32% for water 1 and 2 respectively instead of 62 and 76% found by external calibration.
Regarding results for butyltin compounds, values are in a good agreement between ethylation and propylation both by external calibration and SIDMS for TBT. Again, species specific isotope dilution allows a very good precision at very low concentrations with RSD ranging between 11 and 13% for TBT whereas using external

calibration RSD are ranging between 27 and 36%. For MBT and DBT, the use of SIDMS analysis should improve the poor precision found using external calibration with RSD ranging between 10 and 27%.

| **MMHg** | | | | |
|---|---|---|---|---|
| Derivatization | Propylation | | Ethylation | |
| Calibration | EC[1] | SIDMS[2] | EC | SIDMS |
| Water 1 | 0.053±0.033 *(62%)* | 0.042±0.010 *(24%)* | <LOD | <LOD |
| Water 2 | 0.033±0.025 *(76%)* | 0.037±0.012 *(32%)* | <LOD | <LOD |

| **IHg** | | | | |
|---|---|---|---|---|
| Derivatization | Propylation | | Ethylation | |
| Calibration | EC | SIDMS | EC | SIDMS |
| Water 1 | 2.14±0.15 *(7%)* | 2.31±0.06 *(2.6%)* | 1.89±0.22 *(11.6%)* | 2.19±0.11 *(5.0%)* |
| Water 2 | 1.09±0.10 *(9%)* | 1.11±0.05 *(4.5%)* | 0.88±0.11 *(12.5%)* | 1.03±0.07 *(6.8%)* |

| **TBT** | | | | |
|---|---|---|---|---|
| Derivatization | Propylation | | Ethylation | |
| Calibration | EC | SIDMS | EC | SIDMS |
| Water 1 | 0.45±0.12 *(27%)* | 0.47±0.05 *(11%)* | 0.46±0.15 *(33%)* | 0.46±0.06 *(13%)* |
| Water 2 | 0.25±0.09 *(36%)* | 0.27±0.03 *(11%)* | 0.23±0.08 *(35%)* | 0.25±0.03 *(12%)* |

| | **DBT** | | **MBT** | |
|---|---|---|---|---|
| Derivatization | Propylation | Ethylation | Propylation | Ethylation |
| Calibration | EC | EC | EC | EC |
| Water 1 | 1.42±0.36 *(25%)* | 1.57±0.33 *(21%)* | 0.92±0.18 *(19%)* | 1.19±0.25 *(21%)* |
| Water 2 | 1.12±0.25 *(22%)* | 1.09±0.30 *(27%)* | 0.72±0.09 *(12%)* | 0.69±0.07 *(10%)* |

[1]External Calibration. [2]Species Specific isotope dilution

**Table 6:** Determination of mercury species and butyltin compounds in sea water samples using $NaBEt_4$ and $NaBPr_4$ as derivatization reagent. Mean values ($ng.l^{-1}$) ± standard deviation (n=3)

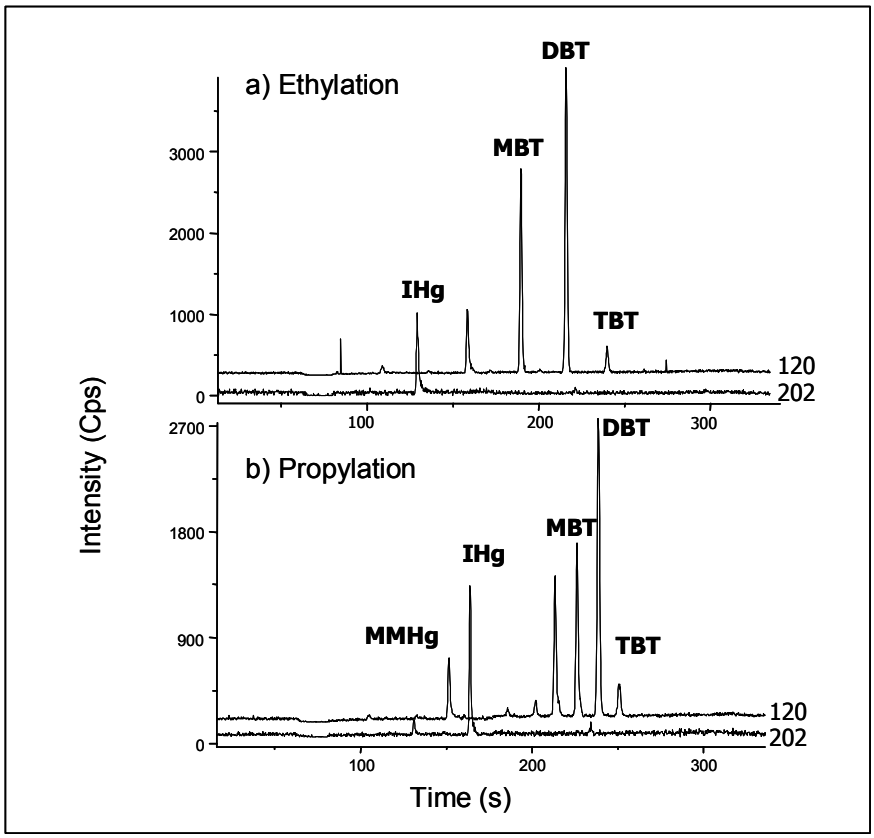


**Figure 3:** Chromatograms obtained by GC-ICPMS for a sea water sample using ethylation (a) and propylation (b) as derivatizating reagent.

## 4. CONCLUSIONS

An accurate and precise method for the simultaneous determination of mercury and tin species in aqueous samples by propylation CGC-ICPMS has been developed. For environmental samples, propylation is preferred over ethylation for derivatization since it is less affect by matrix effects. Precision values using SIDMS were significantly better than those obtained by external calibration, clearly demonstrating the superior capabilities of this approach for low level determinations. Taking into account that no certified reference material is available for the speciation of mercury and butyltin compounds in aqueous samples, SIDMS is considered as an absolute analytical method controlling species transformations and low recoveries. The proposed procedure could be used as a reference method in control laboratories.

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# Chapitre E Annexe 4

# Mercure élémentaire dans l'atmosphère d'un système forestier amazonien (Guyane française)

# Research Communications

## Elemental Mercury in the Atmosphere of a Tropical Amazonian Forest (French Guiana)

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Gaseous atmospheric mercury was investigated at two sites of a tropical Amazonian forest (French Guiana) in the Petit Inini River basin and the Petit Saut Lake in June, 1998. Gaseous atmospheric mercury was identified as elemental mercury ($Hg^0$). Diurnal variation of atmospheric $Hg^0$ in both studied aquatic environments were significantly correlated with air temperature and anticorrelated with relative humidity. Average $Hg^0$ concentrations were higher above the Petit Inini River (75 pmol $m^{-3}$/15.0 ng $m^{-3}$) than the Petit Saut Lake (14 pmol $m^{-3}$/2.8 ng $m^{-3}$). Background $Hg^0$ concentrations in the Petit Inini River basin were higher (27 pmol $m^{-3}$/5.4 ng $m^{-3}$) than those observed in remote environments. These data suggest that gold mining activity (i.e., Petit Inini River basin) may influence mercury mobilization in tropical forest ecosystems and that atmospheric transfer is a major pathway for mercury cycling in these environments.

### Introduction

Mercury (Hg) contamination in the Amazon ecosystem has been investigated since the early nineties (*1*, *2*). This sudden interest in Hg pollution and cycling follows the development of gold mining exploitation in the different Amazonian countries since the mid-seventies (*1*, *3*–*5*). The use of elemental mercury ($Hg^0$) to amalgamate and extract gold from river sediments or ore deposits by independent and mostly illegal gold miners ("garimpeiros") has led to the release of an important fraction of this pollutant into the environment (*6*, *7*). The low-cost procedures used by these small exploitations discharge between 1 and 2 kg of Hg per kg of extracted gold, either by volatilization resulting from the amalgam burning or by direct loss into the sediments or soils (*5*, *6*). Additionally, impact on human population has been observed in major gold exploitation areas of Brazil (*3*, *8*, *9*). High mercury concentrations were measured in urine or blood samples from gold miners and gold traders or fish-eating local populations, respectively (*3*, *8*). The fish-eating population are specially affected by Hg contamination because they ingest the harmful methylmercury (MeHg) which accumulates and reaches toxic levels. Malm et al. (*8*) found high concentrations of Hg (>50 $\mu g\ g^{-1}$, mostly as MeHg) in hair samples of populations living in the Tapajós River basin. In Amazonian river basins, where gold mining expands, carnivorous fishes exhibit high Hg concentrations (>0.5 $\mu g\ g^{-1}$), 10 times greater than noncarnivorous species (*6*). Bioaccumulation of MeHg within the aquatic food chain seems therefore to be the major pathway for Hg contamination.

A major issue for the assessment of Hg contamination from gold mining activities is to understand how Hg can be spread over a whole river basin and affect aquatic organisms. Although Hg can be transported by rivers over long distance, this metal is suspected to be principally emitted to the atmosphere as $Hg^0$ from gold extraction/purification processes in roasting devices (*5*, *6*). For Brazil, 77.9 t $yr^{-1}$ of Hg has been estimated to be released to the atmosphere from gold mining, representing 67.3% of that country's anthropogenic emissions (*7*). In the case of French Guiana, 3 t $yr^{-1}$ of Hg are believed to be emitted to the atmosphere as a result of gold mining activities (*10*). In the Amazonian atmosphere, gaseous Hg lifetime is expected to be shorter than in temperate environments (ca. 1 yr, *11*), because this rainforest ecosystem is emitting large amount of reactive gases and aerosols (*12*) which are able to enhance $Hg^0$ oxidation rate and therefore precipitation washout. Nevertheless, the scarce Hg speciation studies in the Amazonian environment do not permit understanding how Hg is transported, methylated, and finally transferred to aquatic organisms.

In this paper, we present the first gaseous Hg speciation measurements in the atmosphere and waters from a tropical Amazonian forest ecosystem. Diurnal variability and average site concentrations of gaseous Hg have been investigated in two remote areas in French Guiana suspected to be directly or indirectly affected by gold mining activities.

### Experimental Section

Air and water samples were collected at two distinct areas of the French Guiana, as shown in Figure 1, during a wet season (two weeks in June, 1998). Two sampling sites were studied in the Petit Inini river basin (200 km SW of Cayenne; latitude 3°45′ N/longitude 53°33′ W), which has been prospected for the last 100 years, but with a large development of illegal exploitations since the eighties. Air samples were collected at both sites: the Dorlin Camp Hill (DCH), in an open deforested area (altitude 250 m), and downhill from the Dorlin camp in the margin of the Petit Inini River (PIR) and beneath the forest canopy (altitude 130 m). The second sampling area is in the Petit Saut Lake (PSL; 50 km SW of Kourou; latitude 5°05′ N/longitude 53°03′ W), a large artificial lake covering a former tropical forest valley (310 $km^2$) that was formed 5 years ago due to the construction of a hydroelectric power plant dam. The lake is situated on the Sinnamary river basin which is also an area of past and present gold exploitations (upstream of the lake). Air samples were collected on a floating platform positioned in the central part of the lake (altitude 30 m), 20 km upstream of the dam of Petit Saut. It is important to mention that no rainfall occurred during the atmospheric sampling periods.

Sampling and storage equipment was made of Teflon, PVDF, PE, or PP, washed with detergent, soaked several days with 10% nitric acid, rinsed with milli-Q Millipore water and stored in double-zipped plastic bags. The sampling techniques and analytical methodologies used in this work are described below and are detailed elsewhere (*13*–*16*).

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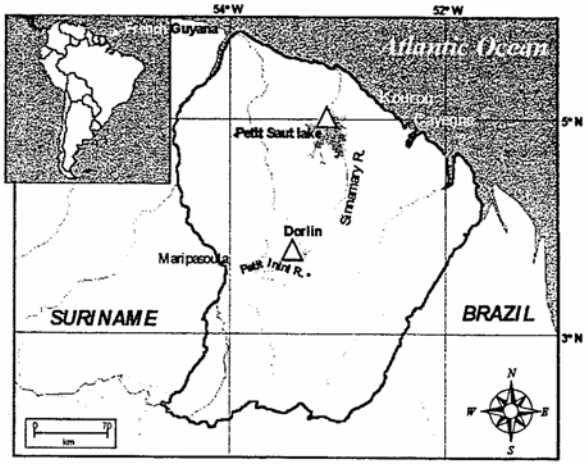


**FIGURE 1. Location of sampling sites in French Guiana (South America).**

Air samples were collected at 1.5 m height above the ground (soil or water). The air inlet was made of a filtration cartridge (Millipore) with a quartz microfiber filter (Whatmann) to separate aerosols from the air stream (pore size 0.1 $\mu$m). Air was pumped for 1 h with a vacuum membrane pump (KNF) through a Teflon tubing line (i.d. 1.6 mm; maximum length 5 m) to the gaseous Hg trapping device. Potential back diffusion contamination from the pump was avoided by placing an activated charcoal cartridge (Supelco) between the pump and the Hg trap. Air-pumping flow rate ranged from 0.5 to 1 L $min^{-1}$, which was continuously controlled during every 1 h sampling period by a calibrated gas flowmeter. Most of the samples were collected on single gold-coated quartz sand (Brooks) traps (borosilicate tube, o.d. 8 mm, length 120 mm). Dual sampling was also performed for the speciation study on both gold-coated traps and cryogenic traps. For cryogenic trapping, the air stream passed through a moisture trap, held at −20 °C, and subsequently through silanized glass wool (U-borosilicate tube, o.d. 8 mm, length 200 mm) maintained at −180 °C in a Dewar containing boiling liquid nitrogen. After collection, gold-coated and cryogenic traps were sealed with gas chromatographic end-caps (Alltech) and stored until analysis in a hermetically sealed plastic box filled with activated charcoal and in a transportable cryogenic container filled with liquid nitrogen (L'Air Liquide), respectively.

Water samples were collected and immediately transported in an opaque refrigerated box to the site where the equipment was installed. Analyses were always performed within less than 2 h of the sampling. Dissolved gaseous Hg was trapped by purging 1 L of surface water, with pure helium (L'Air Liquide) at 1 L $min^{-1}$ during 1 h (*16*). The gas stream was dried through a moisture trap, and gaseous Hg species were trapped either in a gold-coated or cryogenic trap in the same manner as for the air samples. Water samples were also acidified to 1% ultrapure nitric acid (Prochilab) for determination of total reducible Hg content, after direct hydride generation-cryofocusing gas chromatography and atomic absorption spectrometry (*17*).

Gold-coated and cryogenic traps were subsequently desorbed in the laboratory at 500 and 300 °C, respectively, under pure He carrier gas into a cryogenic gas chromatographic device connected to an ICP/MS (ELAN 6000, Perkin-Elmer-Sciex). The gaseous species are then cryofocused on the GC column to be subsequently separated and detected by choosing appropriate Hg stable isotopes (i.e., $^{200}$Hg and $^{202}$Hg) (*15*). Cryogenic traps were therefore used to investigate the speciation of gaseous Hg compounds in several atmospheric and aqueous samples (*15, 16*). Theoretically, a

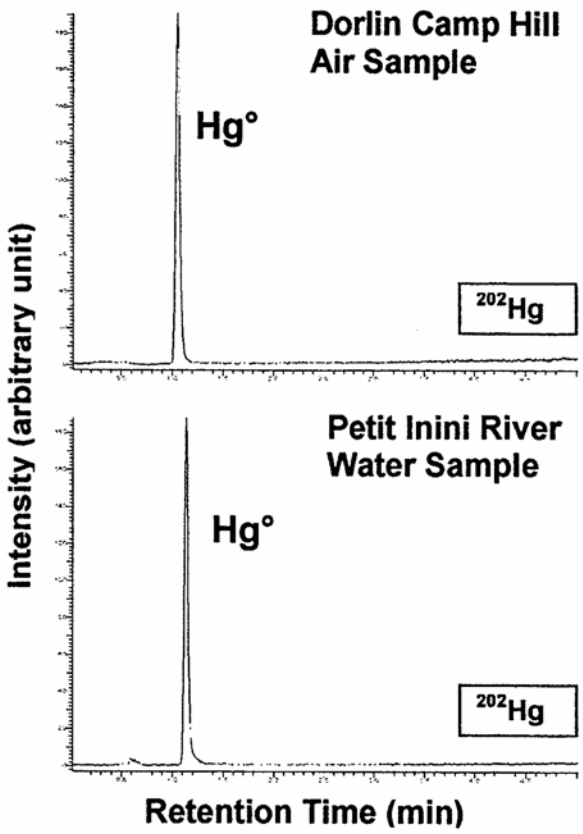


**FIGURE 2. Chromatograms of gaseous mercury speciation of air and water samples from Dorlin Camp Hill and Petit Inini River, respectively. Analyses were performed by cryofocusing gas chromatography, followed by inductively coupled plasma mass spectrometry detection *(15,16)*.**

number of organic and inorganic gaseous forms of mercury can be detected by this method, but only standards of $Hg^0$, dimethylmercury, and diethylmercury were tested. Nonetheless, the chromatograms in Figure 2 indicate that the unique form present in air and water samples is $Hg^0$. Quantitative data were based on gold-coated trapped samples.

For both trapping methods, several traps were used for blank field measurements following the same process as for sample traps. Average measured values of the blanks were subtracted from the sample measured values of the samples, and the standard deviation of the blank values allowed us to estimate the field detection limit for the method to be 150 fmol, which represents 3 pmol $m^{-3}$ (600 pg $m^{-3}$) in air samples and 150 pmol $m^{-3}$ (30 ng $m^{-3}$) in water samples.

Atmospheric relative humidity, air temperature, and direct UV−visible sunlight radiation were also measured at the air-sampling inlet using a data logging thermohygrometer (Hanna Instruments) and an UV−visible photocell (Applied Photophysics).

## Results and Discussion

**Speciation of Atmospheric Mercury.** Speciation analysis of volatile Hg compounds by cryotrapping air and purged water samples indicate that the only species identified is $Hg^0$ (Figure 2). The intercomparison between cryogenic trapping and gold amalgamation for atmospheric samples has been performed on four duplicates at the DCH site. Although cryogenic trapping quantification suffered from ice clogging due to high moisture content, the cryogenic trapping and gold amalgamation sampling methods show rather good

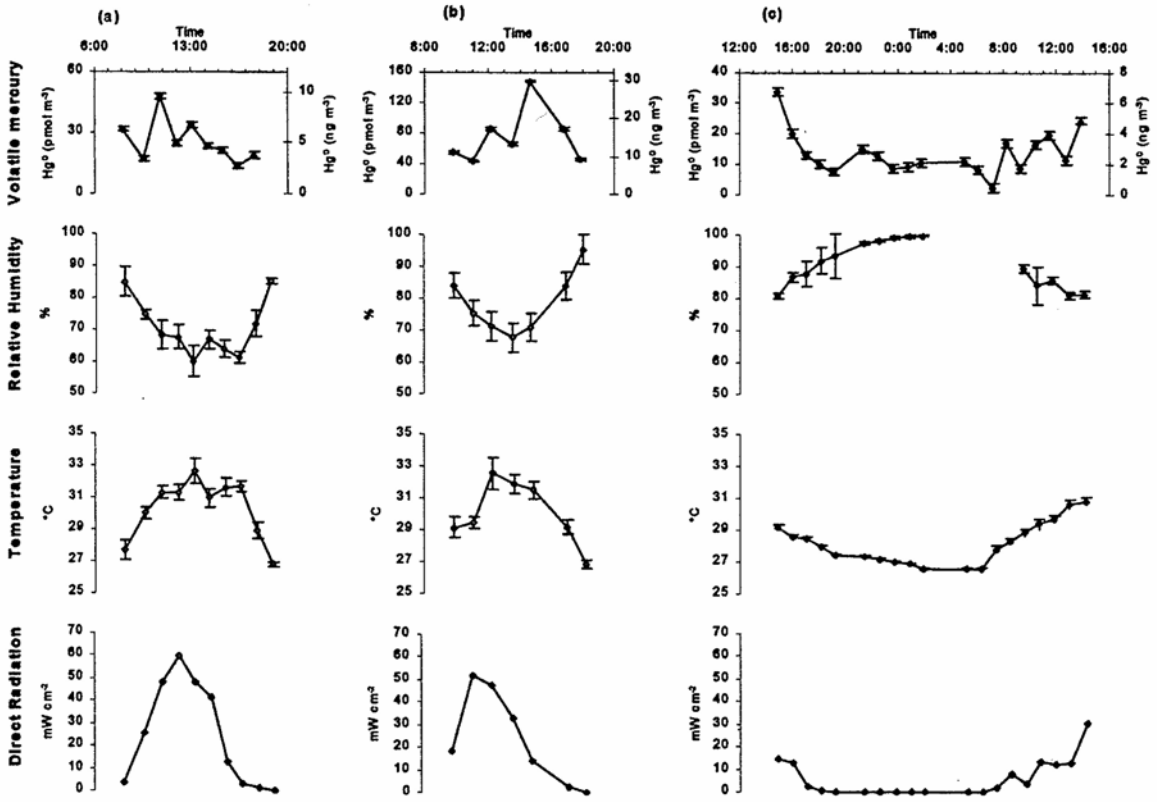


**FIGURE 3. Time series measurements of atmospheric elemental mercury concentrations at (a) Dorlin Camp Hill, (b) Petit Inini River, and (c) Petit Saut Lake, together with air temparature, relative humidity, and vertical sunlight UV–visible radiation. Time = UTC − 6 h.**

**TABLE 1. Linear Regression Equations for Elemental Mercury ($Hg^0$) vs Air Relative Humidity (RH) and Temperature (Tp) for the Various Atmospheric Sampling Sites in French Guiana (June, 1998)**

| regression | site | *n* | $r^2$ | slope | intercept |
|---|---|---|---|---|---|
| $Hg^0$ vs RH | DCH | 14 | 0.001 | 0.15 | 71.94 |
| | PIR | 7 | 0.210 | −0.62 | 87.64 |
| | PSL | 19 | 0.457 | −3.32 | 101.00 |
| $Hg^0$ vs Tp | DCH | 14 | 0.003 | 0.08 | 29.31 |
| | PIR | 7 | 0.299 | 0.15 | 27.62 |
| | PSL | 19 | 0.303 | 0.52 | 26.77 |

agreement with 26 ± 10 pmol $m^{-3}$ (5.0 ± 2.0 ng $m^{-3}$) and 29 ± 2 pmol $m^{-3}$ (5.8 ± 0.4 ng $m^{-3}$; $n = 4$), respectively. Higher average concentrations obtained with gold amalgamation probably illustrate the occurrence of inorganic or reactive gaseous Hg species which are suspected to be formed under such meteorological conditions (*18*). However, the difference between both methods was not statistically significant. We can therefore assume that gaseous Hg in the atmosphere was essentially in the form of $Hg^0$.

**Diurnal Variabilty of Atmospheric $Hg^0$.** Atmospheric concentrations of $Hg^0$ were investigated during a 2-day time period at DCH and PIR and one complete diurnal cycle at PSL. The results obtained are displayed on time series plots in Figure 3, along with atmospheric relative humidity (RH), air temperature (Tp), and direct UV–visible sunlight radiation (UVR). The variability of $Hg^0$ concentrations in all three sites seems to correlate with the meteorological parameters recorded, exhibiting a maximum concentration at noon (between 12:00 a.m. and 2:00 p.m.) and a minimum concentration in the evening and at night. Regressions between $Hg^0$ and RH, $Hg^0$ and Tp are shown in Table 1. No regression analysis was performed with UVR because they were not corresponding to total incident radiation and were recorded only every 15 min (each minute for RH and Tp). Good correlations were obtained with RH and Tp for both PIR and PSL sampling sites. On the other hand, no correlation was observed for both parameters at DCH site. These results indicate that atmospheric samples collected at DCH are more homogeneous and represent atmospheric $Hg^0$ concentrations of a well mixed atmosphere rather than mercury coming from soil evasion. The two sampling sites above aquatic environments have both wider ranges of RH and Tp throughout the day which lead to increase atmospheric $Hg^0$ variations. As surrounding environments such as surface waters and soils and vegetation are probably potential source of $Hg^0$ to the atmosphere, meteorological conditions are therefore playing a major role in the volatilization of $Hg^0$. Previous studies have demonstrated that temperature, sunlight, and air movement were significant parameters for surface exchanges of gaseous Hg with the atmosphere (*19–24*). Our results indicate that they apparently play a similar role in the tropical rainforest.

**Distribution and Potential Sources of Atmospheric $Hg^0$.** Table 2 shows the average concentrations of $Hg^0$ (± standard deviation) in the atmosphere at the three different sites investigated in French Guiana: Dorlin Camp Hill (DCH), Petit Inini River (PIR) and Petit Saut Lake (PSL). Additionally, $Hg^0$ concentrations measured in surface waters of the PIR and PSL are indicated.

PSL $Hg^0$ concentrations in the atmosphere are similar to values observed in most background sites at various latitudes (*25*). Average concentrations recorded at DCH and PIR are significantly higher than those obtained at PSL (Table 2). This indicates that $Hg^0$ measured in the DCH and PIR could originate partly from natural sources (*26*, *27*) but also from anthropogenic local sources (gold mining). DCH samples, collected in a higher and clearer point, represent the well-mixed layers over the rain forest canopy and could be considered as background concentration for the Petit Inini

**TABLE 2. Average Elemental Mercury Concentrations in the Atmosphere (Height: 1.5 m) and Surface Waters of French Guiana (June, 1998)**

| sample site | $Hg^0$-atmosphere pmol $m^{-3}$ (ng $m^{-3}$) | $Hg^0$-water[a] pmol $m^{-3}$ (ng $m^{-3}$) |
|---|---|---|
| Dorlin Camp Hill (DCH) 16-18/06/98 | 27 ± 8 (5.4 ± 1.6) ($n$ = 14) | n.s. |
| Petit Inini River (PIR) 17/06/98 | 75 ± 36 (15.0 ± 7.2) ($n$ = 7) | 2859 ± 90 (573.5 ± 18.1) ($n$ = 2) |
| Petit Saut Lake (PSL) 24-25/06/98 | 14 ± 7 (2.8 ± 1.4) ($n$ = 19) | 979 ± 50 (196.4 ± 10.0) ($n$ = 2) |
| background levels *(ref)* | 5–20 (1.0–4.0) (*22*) | 10–1000 (2.0–200.6) (*25*) |

[a] n.s.: no sample.

basin during the sampling period (June, 1998). On the other hand, higher concentrations at PIR could result from a sort of "greenhouse" effect retaining beneath the canopy the $Hg^0$ which is released by the river water or by the dense vegetation. The role of tropical vegetation in the volatilization of Hg is unknown and difficult to investigate. The atmosphere beneath the canopy is probably not well mixed with the overlaying troposphere, and the accumulation of $Hg^0$ from surface exchange processes may occur in this compartment (*26*).

$Hg^0$ concentrations in surface waters are about 3 times higher at PIR than at PSL (Table 2) and demonstrate that the Hg load at PIR is probably more important than at PSL. Natural biological and chemical pathways of $Hg^0$ production in surface waters (*28–30*) suggest that PSL site (sunlight, water temperature 31 °C) is more favorable for $Hg^0$ formation than PIR site (shaded, water temperature 25 °C). However, total reducible Hg concentrations (by sodium borohydride method, see ref *17*) were also found to be higher at PIR than at PSL, exhibiting 680 and 480 pmol $L^{-1}$ (136.4 and 96.3 ng $L^{-1}$), respectively. PIR seems therefore directly affected by gold exploitations that release their slurries upstream from the sampling point. In Brazilian gold mining areas, Roulet et al. (*31*) found that ferralitic soils enriched with Hg were a major source of Hg to the aquatic environment via erosion or resuspension processes. At both sites PSL and PIR, surface waters were found highly supersaturated with $Hg^0$ compared to the atmosphere with average saturation of 2937 and 1372%, respectively. Consequently, assuming an average water–air transfer coefficient for $Hg^0$ of 0.5 m $d^{-1}$ (typical for low turbulent surface water), Hg volatilization fluxes from both PSL and PIR surface waters are 473 and 1325 pmol $m^{-2}$ $d^{-1}$ (95.0 and 266 ng $m^{-2}$ $d^{-1}$), respectively. These fluxes are much higher than those measured in various lakes or marine environments (*25*) and indicate that aquatic environments, such as rivers, lakes, or flooded areas, in tropical rain forests are probably important sources for atmospheric Hg. Considering a simple mass balance model used by Mason et al. (*28*) for $Hg^0$ formation and volatilization in surface waters, and neglecting $Hg^0$ oxidation (*30*), we estimated that first-order Hg(II) reduction rate into $Hg^0$ would be approximately 20 and 40% $day^{-1}$ for PSL and PIR, respectively. The potential Hg(II) reduction capacity of these environments seems therefore extremely high and represents a major pathway for the dynamic recycling of Hg from gold mining areas, enhancing the atmospheric transfer in the Amazonian ecosystem.

The Hg sources responsible for the relatively high $Hg^0$ concentrations measured in Petit Inini River basin could be attributed to direct $Hg^0$ emission from amalgam combustion or mine tailing and indirect $Hg^0$ formation from reducible Hg released by atmospheric washout, soil weathering, or mining activity. Atmospheric Hg cycling in the tropical rain forest is probably more dynamic and critical than in previously investigated temperate environments and requires further investigations.

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# Chapitre E Annexe 5

# Mobilisation du mercure dans le sol d'un système forestier amazonien durant un évènement de pluie (Guyane française)

*J. Phys. IV France* **107** (2003)



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# Mercury mobilization in soil from a rainfall event in a Tropical forest (French Guyana)

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**Abstract.** Mercury (Hg) distribution was achieved in a pristine Amazonian forested area during a rainfall event along a basin thalweg. Hg concentrations were measured in both surface runoff and underground waters and soils. Total Hg concentrations in the particulate and dissolved phase were related to the main hydrological processes in order to trace the fate of mercury during the flood event. Rainfall waters were also collected to assess the amount of Hg from atmospheric deposition and foliage leaching. Total Hg concentrations were found relatively homogenous over the basin and were typical of remote area (9 ng $l^{-1}$). In addition, air samples collected at the same site, at the ground level and at different heights (1-40m), which allowed us to determine temporal and spatial variability of total gaseous atmospheric Hg. Finally, dynamic flux chambers experiments were carried out to directly determine the exchanges of gaseous Hg at the soil/atmosphere interface, in order to better understand the role of the atmospheric compartment within the forest ecosystem.

## 1. INTRODUCTION

Hg contamination in Amazonian ecosystems has been intensively investigated since the early nineties, due to the development of gold mining exploitation and deforestation activities[1,2]. Studying the mechanisms of Hg transport and exchange between environmental compartments is necessary for understanding its biogeochemical cycle in such tropical disturbed ecosystems. The use of elemental mercury (Hg°) to amalgamate and extract gold from river sediments or ore deposits has led to the release of an important fraction of this pollutant into the environment [3,4]. Nevertheless, Hg is also naturally present in Guyana soils, and under tropical climatic conditions can enhance drastically its remobilization from soils to the other environmental compartments. Hg can be spread over a whole watershed during intense rainfall events through atmospheric deposit, runoff and erosion processes. Furthermore, rainforest ecosystem can emit large amount of reactive gases and aerosols [5] which are able to enhance Hg oxidation and therefore precipitation washout. Evaporation mechanisms from soils can thus represent a significant source of Hg to the atmosphere.

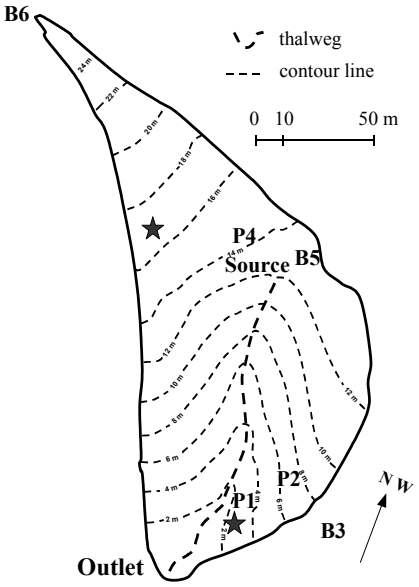
In this paper, we present Hg measurements in the different compartments of a forested watershed during a rainfall event. Mercury speciation and distribution have been investigated in rainfall, runoff waters and soils in order to assess on potential for Hg remobilization under the climate of tropical environments.

## 2. EXPERIMENTAL

### 2.1. Sample collection and handling

#### *2.1.1. Sampling site*

Experiments were carried out in an experimental scientific area (ECEREX) located in the north west of French Guyana (53.3°W, 5.2°N). The site has been set up in the seventies to study the impact of deforestation on hydrological and erosion processes and is composed of small hydrographic units of 2 ha and with average slope of 17%. Air, water and soil samples were collected in a pristine primary forest. The basin studied (Fig 1) is equipped with an overflow outlet in the downstream part, allowing one to record the surface waters flow discharge by way of a limnigraph. This watershed doesn't present a permanent water flow even during the rainfall season. Surface water flow appears in response to intense rainfall event when soils are wet and saturated with water. Two preferential water circulation paths have been previously evidence at the rainfall scale [6]. First a surface runoff that feeds the valley bottom of the watershed (thalweg), and second a subsurface flow following the basin slope and originating from a sheet of water formed at the basin summit.




| Samples | Total Hg ($ng.l^{-1}$) | Dissolved Hg ($ng.l^{-1}$) | Particulate Hg ($\mu g.g^{-1}$) |
|---|---|---|---|
| **Surface water** | | | |
| Source *(n=2)* | 42 *(40 – 43)* | 11 *(5 – 17)* | 0.7 *(0.2 – 1.2)* |
| Thalweg *(n=2)* | 653 *(471 – 835)* | 100 *(92 – 109)* | 12.6 *(10.6 - 14.6)* |
| Outlet *(n=4)* | 366 *(168 – 709)* | 67 *(33 – 137)* | 5.0 *(2.0 – 7.3)* |
| Non contaminated thalweg *(n=3)* | 13 | 5 *(3 – 7)* | 0.4 |
| **Subsurface waters** | | | |
| Pit 4 *(n=2)* | 23 *(16 – 29)* | - | - |
| Pit 2 *(n=2)* | 9 *(7 – 11)* | - | - |
| Pit 1 *(n=1)* | 6 | - | - |

**Figure 1**: Topography of the study basin and Hg concentrations in water samples. Location of the pit (P1, P2, P4), borehole (B6, B5, B3) and flux chamber stations (stars).

*2.1.2. Waters and soils sampling*

Rain, sub- and surface waters have been sampled during an intense rainfall event on the 29th of April 2001. Bulk rainwaters associated with foliage leaching were collected in four ultra clean collectors positioned from the top to the middle of the basin slope. Surface waters were sampled every 30 minutes at the watershed outlet, as well as in the thalweg at its source and at halfway. Three pedological pits were dug in order to collect subsurface waters above the thalweg source and in the downstream part of the basin (Fig 1). Immediately after sampling, all the rainfall and water samples were acidified (1% HCl) and stored in PTFE Teflon precleaned bottles at +4°C. Only surface waters were previously filtered at 0.45 µm before acidification and storage.

Several boreholes were performed at 3 m depth, between the top and the outlet of the watershed, in order to characterize the soils and the spatial distribution of total Hg.

*2.1.3. Atmospheric sampling*

Gaseous Hg measurements at the ground level and dynamic flux chambers (Polypropylene, ORNL type) experiments were carried out at 2 stations above the thalweg source and the basin outlet to directly determine the exchange between the soil and the atmosphere [7]. Furthermore, an

atmospheric profile of gaseous Hg (0-40m) was achieved in a clearing area located just above the studied basin. The sampling was conducted by using a tethered balloon to collect gaseous Hg on gold traps by pumping air at different heights.

### 2.2. Analytical procedure

Hg speciation and total Hg analyses were achieved using standard ultra traces techniques (CVAFS and GCAFS) in the dissolved and particulate phases and soils. Atmospheric gaseous Hg measurements were performed by a GC-ICPMS technique, after field trapping on gold coated sand [8].

## 3. RESULTS AND DISCUSSION

### 3.1. Atmospheric mercury inputs and evasion at the soil-air interface

At ground level and under the forest cover of the basin, gaseous Hg measurements exhibit concentrations ranging from 2 to 100 $ng.m^{-3}$. These values were found to be significantly higher than the atmospheric background previously observed above a lake, close to the study area [6]. Furthermore, the concentrations measured at the upstream station of the watershed, above the thalweg source, are relatively low and homogenous ($6 \pm 2$ $ng.m^{-3}$), whereas the downstream station exhibit higher and much heterogeneous concentrations ($41 \pm 30$ $ng.m^{-3}$). These experiments have been carried out under the same climatic conditions, during intense rainfall. Therefore this difference could be explained by the nature of the soils and the associated hydrological processes, such as hydromorphy and transfer to subsurface waters. Nevertheless it is critical to note that a local Hg contamination of the soil was evidenced at midslope, coming from the fall of a tree on a tensiometric station containing a Hg manometer.

An atmospheric profile was achieved in a clearing area just above the investigated watershed. Gaseous Hg concentrations measured at 40m height from the ground (overcanopy sampling), average 1.6 $ng.m^{-3}$ and correspond to the atmospheric background previously observed above the Petit Saut lake [6]. For lower relative altitudes, a significant decreasing concentration gradient was observed between 30 and 1m. Concentration maximum was measured at 30m and could be related to a gaseous

Hg source coming from the surrounding canopy summit. In order to assess the potential exchange between vegetation and atmosphere, additional sampling was achieved under the forest cover, nearby the glade. Concentrations obtained for relative altitudes of 10 and 1m, were very close to the ones measured in the clearing parcel. These results suggest that gaseous Hg exchange between the canopy and the free atmosphere are important, and certainly play a key role in the Hg biogeochemical cycle in tropical environments. In addition, dynamic flux chamber experiments were carried out under forest cover and for 2 distinct soils, simultaneously with the atmospheric sampling. Although these data have not been yet validated, they clearly demonstrate that the 2 sites are potential sources of gaseous Hg to the atmosphere. A first assessment of the volatilisation fluxes, measured using dynamic chambers, indicates that Hg evasion processes at the soil/atmosphere interface range between 10 to 1000 $ng.m^{-2}.h^{-1}$. These results also corroborate the atmospheric measurements with volatilisation fluxes significantly higher at the downstream station.

Total Hg concentrations in rainfall samples were found homogeneous over the whole watershed and are characteristic of remote environments with average value of 9 $ng.l^{-1}$. However, these concentrations remain significantly higher than the ones previously obtained in the clearing area of the Petit Saut lake (c.a. $5ng.l^{-1}$). This difference could be explained by the foliage leaching occurring during rainfall. Moreover, Hg speciation analyses performed on these samples exhibit significant methylmercury (MMHg) concentrations in the range of 0.2 $ng.l^{-1}$.

### 3.2. Mercury mobilisation in soils

Total Hg concentration maxima in soils were observed in the upstream part of the watershed (up to 500 $ng.g^{-1}$ in B6). In this area, soils present important amounts of clays, total iron (Fe) and carbon and are thus more likely to retain Hg. The lower total Hg values, ranging from 20 to 50 $ng.g^{-1}$, correspond to the downstream substrates (Boreholes 3 & 5). These soils, exposed to erosion and hydromorphic processes, exhibit low clay, Fe and carbon content, which induce a low retention of Hg and its potential transfer to the run off and underground waters.

### 3.3. Hg transfer at the interfaces during a flood event

Hg concentrations measured in the dissolved and particulate phases of surface and subsurface waters are summarized in Figure 1. Concentrations are very variable depending on the sampling point. At the

thalweg source they average 40 $ng.l^{-1}$, with 10 $ng.l^{-1}$ and 0.7 $\mu g.g^{-1}$ in the dissolved and particulate phase, respectively. At the downstream stations (mid-thalweg and outlet) total Hg concentrations exhibit much higher values ranging from 168 to 835 $ng.l^{-1}$. Important Hg amounts were also observed in both dissolved and particulate phases. These results confirm the accidental contamination of the soils at mid-thalweg, which generate high Hg concentrations in the runoff waters up to the watershed outlet. Additional sampling in an other basin during the same rainfall event has shown total Hg values in the range of 13 $ng.l^{-1}$, which indicates typical order of magnitude for a non contaminated site.

Water samples collected at the basin outlet show a decrease of total Hg concentrations from 709 $ng.l^{-1}$ at the beginning of the flood to 168 $ng.l^{-1}$ at the final fall of water discharge. The particulate total Hg contents follow the same pattern, whereas the dissolved total Hg concentrations were found quite homogeneous (33-59 $ng.l^{-1}$) during the flood and then increase during the fall, reaching up to 137 $ng.l^{-1}$. At the beginning of the flood event, erosive surface waters, enriched with particulate Hg, feed the basin outlet. A dilution process can then be observed initially at the flood peak and finally in the subsurface waters, resulting in higher dissolved Hg concentrations, which mainly contribute to the outlet flow.

In subsurface waters, total Hg concentrations are much lower than in the thalweg and range from 6 to 29 $ng.l^{-1}$. A significant decreasing gradient from up to downstream was observed. This diminution in Hg concentrations can be related to the decreasing Hg content in the soils intercepted by the water sheet. On the other hand, a concomitant decreasing gradient in dissolved Fe and Al concentrations was also observed. This last point certainly influence the mobility of Hg which can be easily associated to these elements in the mineral phase.

In conclusion, the whole results can be integrated in order to assess Hg inputs and evasion at the tropical watershed scale. The rainfall event, that induced the flood, has led to a total Hg deposit on the soil surface in the range of 10 mg. Volatilisation fluxes at the soil/atmosphere interface are related to the soil distribution. Preliminary estimations lead to emission fluxes ranging from 1 to 10 times the Hg deposit. Considering the flow discharge variations during the flood, Hg flux at the basin outlet was estimated to be around 10 times higher than the throughfall. Finally gaseous Hg concentrations and fluxes from the soil to the atmosphere clearly indicate that volatilisation processes play an important role in Hg cycling under tropical forest cover.

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# Chapitre E Annexe 6

# Curriculum Vitae

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## *FORMATION*

| | |
|---|---|
| 1999-2004 | Thèse de Chimie et Microbiologie de l'Eau, Université de Pau et des Pays de l'Adour, France |
| 1997-1998 | DEA de Chimie et Microbiologie de l'eau (Mention assez bien), Université de Pau et des Pays de l'Adour, France |
| 1996-1997 | Maîtrise de Sciences de l'Environnement (Mention assez bien), Université de Bordeaux I, France. |
| 1995-1996 | Licence de Chimie, Faculté des Sciences de Nantes, France. |
| 1992-1995 | DEUG A Physique-Chimie, Faculté des Sciences de Nantes, France. |
| 1990-1991 | Baccalauréat Série C (Mention bien), Lycée Pierre Mendès France, La Roche sur Yon, France. |

## *Thèse de Doctorat*

Titre: ***Etude de la réactivité et du transfert du tributylétain et du mercure dans les environnements aquatiques***
Directeur de thèse: O.F.X Donard
*Décembre 2004*

***RECHERCHE***

- **2000-2004** Travaux doctoraux

***Etude des biotransformations et mobilisation du mercure et du tributylétain dans les milieux aquatiques dynamiques.***
collaboration avec l'INERIS.

- Mise en œuvre et incubation de microcosmes (aquarium) contaminés pour l'étude du transfert et transformations du mercure et du tributylétain.
- Analyse de spéciation du mercure et de l'étain dans les microcosmes et modélisation.

***Etude du cycle biogéochimique du mercure en milieu tropical*** *(Programme CNRS Mercure en Guyane).*

- Campagnes de prélèvements et analyses de spéciation du mercure dans l'atmosphérique, les eaux de ruissellement et souterraines, les sols et les précipitations.
- Développement de chambre à flux dynamiques (type ONRL) pour l'étude du transfert du mercure à l'interface sol/atmosphère.
- Etude de la distribution et remobilisation du mercure au cours d'un événement pluvieux intense.

***Etude du cycle biogéochimique du mercure dans l'estuaire de l'Adour (PNEC 2000/2001 et Programme GISECOBAG).***

- Campagnes de prélèvements et analyses de spéciation du mercure dans les eaux, matières en suspension et les sédiments.
- Etude des mécanismes de méthylation et déméthylation du mercure dans des sédiments naturels incubés, par l'utilisation d'espèces mercurielles isotopiquement marquées.
- Développement de techniques analytiques d'extraction et d'analyse simultanées du mercure et des organostanniques.

***Etude des cycles biogéochimiques du mercure et de l'étain à l'interface sédiment/eau dans la lagune de Thau et impact sur la faune benthique (programme MICROBENT).***

- Campagne de prélèvements et utilisation de chambres benthiques.
- Analyse de spéciation des espèces volatiles et estimation de flux sédiment/eau.

***Etude de la volatilisation du mercure dans les lacs d'altitude pyrénéens (Programme PYROPOP).***

- campagne de prélèvements pour étudier le cycle diurne d'évasion du mercure.
- analyses de spéciation du mercure et estimation des flux eau/atmosphère.

- **1998-1999** Travaux de DEA, Service civil environnement au sein du LCABIE

***Détermination de formes volatiles de métal(loïdes), Hg, Sn, Pb, Se, en milieu estuarien côtier. Estimation de leur transfert à l'interface eau-atmospère.***

- Campagnes océanographiques de prélèvements d'air, d'eaux et de sédiments dans des estuaires européens macrotidaux (Programme européen BIOGEST).
- Traitement cryogénique des échantillons, analyses de spéciation et modélisation des flux d'évasion.
- Campagne de prélèvement d'eaux et de sédiments sur les estuaires de l'Adour et du Nervion et golfe de Gascogne (programme ECOMAN, Aquitaine-Euskadi).

***Etude du cycle biogéochimique du mercure en milieu tropical (Programme CNRS Mercure en Guyane).***

- Développement de techniques d'échantillonnage pour le piégeage du mercure gazeux dans l'eau et l'air.
- Campagnes de prélèvements d'air, d'eaux, de pluies et analyses de spéciation du mercure.

- **1997** Stage de maîtrise. LCABIE (3 mois)

***Détermination de formes volatiles de l'étain dans des sédiments portuaires contaminés.***

- Prélèvements terrain et piégeage cryogénique des analytes.
- Synthèse organique des analytes pour identification chromatographique.
- Analyse de spéciation et étude du transfert sédiment/eau.

***Techniques analytiques acquises***

- Extraction assistée sous champ micro-ondes des composés organomercuriels et organostanniques dans les sédiments, matières en suspension et tissus biologiques.
- Couplage chromatographie gazeuse – piégeage cryogénique - spectrométrie de masse quadripolaire couplée à un plasma inductif (CT-GC-ICP/MS) pour la spéciation multiélémentaire des espèces volatiles des métaux.
- Couplage dérivatisation - chromatographie gazeuse – piègeage cryogénique - spectrométrie de fluorescence atomique (HG/Eth-CT-GC-AFS).
- Couplage chromatographie gazeuse capillaire – ICP/MS.
- Analyse de spéciation par dilution isotopique

## ARTICLES PUBLIES OU ACCEPTES POUR PUBLICATION

**1.** *Sampling and probing volatile metal(loid) compounds in natural waters by in-situ purge and cryogenic trapping followed by gas chromatography and inductively coupled plasma mass spectrometry (P-CT-GC-ICP/MS)*.
D. Amouroux, **E. Tessier**, C. Pécheyran, O.F.X. Donard (1998).
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**2.** *Elemental mercury in the atmosphere of a tropical Amazonian Forest (French Guiana)*.
D. Amouroux, J.C. Wasserman, **E. Tessier**, O.F.X. Donard (1999).
**Environmental Science and Technology**, 33, 3044-3048.

**3.** *Volatilization of organotin compounds from coastal and estuarine environments.*
D. Amouroux, **E. Tessier**, O.F.X. Donard (2000).
**Environmental Science and Technology**, 34, 988-995.

**4.** *The role of the oceans in the global selenium cycle*.
D. Amouroux, P.S. Liss, **E. Tessier**, M. Hamren-Larsson, O.F.X. Donard (2001).
**Earth Planetary Sciences Letters**, 189, 277-283.

**5.** *Speciation of mercury in a fluid mud profile of a highly turbid macrotidal estuary (Gironde, France)*.
C.M. Tseng, D. Amouroux, G. Abril, **E. Tessier**, H. Etcheber, O.F.X. Donard (2001).
**Environmental Science and Technology**, 35, 2627-2633.

***6.*** *Cycling of volatile organotin compounds in three European estuaries (Gironde, Scheldt, Rhine).*
**E. Tessier**, D. Amouroux, O.F.X. Donard (2002).
**Biogeochemistry**, 59, 161-181.

***7.*** *Formation and volatilisation of alkyl-iodide and –selenide compounds in macrotidal estuaries.*
**E. Tessier**, D. Amouroux, E. Lemaire, H. Etcheber, O.F.X. Donard (2002)
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**8.** *Improvements of mercury species determination in environmental matrices involving on-line cryofocusing and atomic fluorescence spectrometry.*
T. Stoichev, R.C. Rodriguez Martin-Doimeadios, **E. Tessier**, D. Amouroux, O.F.X. Donard.
**Talanta** (sous presse).

**9.** *Mercury methylation/demethylation and volatilization pathways in estuarine sediment slurries using species specific enriched stable isotopes*.
R.C. Rodriguez Martin-Doimeadios, **E. Tessier**, D. Amouroux, R. Guyoneaud, R. Duran, P. Caumette, O.F.X. Donard.
**Marine Chemistry** (sous presse) **.**

## CHAPITRES PUBLIES DANS DES LIVRES

**1.** *Biogenic volatilization of trace elements from European estuaries.*
**E. Tessier**, D. Amouroux, O.F.X. Donard (2002).

In **Biogeochemistry of environentaly important trace elements**, ACS Symposium Series, Y. Cai Ed., American Chemical Society, 2002, pp. 151-165.

## PUBILCATIONS DANS DES ACTES DE CONGRES

**1.** *Atmospheric emission of gaseous biogenic selenium from coastal and marine environments.*
D. Amouroux, **E. Tessier**, P.S. Liss, M. Frankignoulle, O.F.X. Donard (1999)
**EOS Transactions**, American Geophysical Union (1999), 80, 2000 Ocean Sciences Meeting Supplement, p. OS139.

**2.** *European macrotidal estuaries as a source of atmospheric mercury.*
**E. Tessier**, D. Amouroux, C.M. Tseng, O.F.X. Donard (1999).
**EOS Transactions**, American Geophysical Union (1999), 80, 2000 Ocean Sciences Meeting Supplement, p. OS155.

**3.** *Interactions between selenium and sulfur biogeochemistry in the marine environment leading to bio-volatilization pathways.*
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**4.** *Investigation on mercury contamination pathways using model aquatic ecosystems.*
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**5.** *Volatilization of tin in the environment.*
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**6.** *Gaseous metal and metalloid species in the aquatic environment: biogeochemical cycles and pollution control.*
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**9.** *Mercury mobilization in soil, during a rainfall event, in a tropical forest area (French Guyana).*
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**12.** *Mercury species distribution in the water column of the Mediterranean Sea during summer 2003.*
**E. Tessier**, M. Monperrus, D. Amouroux, H. Pinaly, R. De Wit, A. Leynaert, O.F.X. Donard (2004).
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**1.** *European macrotidal estuaries as a source of atmospheric gaseous elemental mercury.*

D. Amouroux, **E. Tessier**, C.M. Tseng, M. Bueno, H. Pinaly, C. Pécheyran, M. Frankignoulle & O.F.X. Donard

En préparation pour **Atmospheric Environment**.

**2.** *Air-water exchanges of mercury along a river stream – hydroelectric reservoir transect in a tropical environment (French Guiana).*
**E. Tessier**, D. Amouroux, T. Stoichev, O.F.X. Donard.
En préparation pour **Atmospheric Environment**.

**3.** *Benthic fluxes of metals (Cu, Cd, Pb, Mn, U and IHg) and organometals (MMHg, DBT and TBT) in the eutrophicated Thau lagoon (Mediterranean coast, France) : role of biological activity.*
D. Point, M. Monperrus, **E. Tessier**, D. Amouroux, O.F.X. Donard, L. Chauvaud, G. Thouzeau, F. Jean, E. Amice, J. Grall, A. Leynaert and J. Clavier (2004).
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**4.** *Biogeochemistry of mercury at the sediment-water interface in the Thau lagoon. 2. Measurements of mercury methylation potential in sediment and water and fate of methylmercury.*
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6. *Mercury contamination pathways and bioaccumulation at various contamination levels in aquatic model ecosystems.*
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**1.** Détermination de formes volatiles de l'étain dans des sédiments portuaires contaminés.
**E. Tessier** (1997).
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**2.** Détermination de formes volatiles de métal(loïdes) (Hg, Pb, Se, Sn) en milieu estuarien côtier. Estimation de leur transfert à l'interface eau/atmosphère.
**E. Tessier** (1998).
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**3.** Cycle du mercure dans l'eau et l'air.
L. Charlet, D. Amouroux, **E. Tessier**, M. Bolte, T. Peretyazhko, M. Coquery, M.A. Mélières (2001).
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**4.** Apports atmosphériques de mercure et émissions par la canopée – Estimation de flux lors d'une averse au site ECEREX.
L. Charlet, **E. Tessier**, D. Amouroux, T. Stoichev, M. Grimaldi, C. Grimaldi, G. Dutin, O.F.X. Donard (2002).
**Programme Mercure en Guyane** – Rapport Final –Deuxième Partie, Programme Environnement, Vie et Société, ed. CNRS, pp. 16-25.

## CONFERENCES INVITEES

**1.** Formation and transfer of volatile metal and metalloid compounds in the environment.
D. Amouroux, C.M. Tseng, **E. Tessier**, O.F.X. Donard.
Bordeaux (France), 8th annual meeting of the Society of Environmental Toxicology and Chemistry – Europe April 1998.

**2.** Novel biogeochemical pathways for organotin compounds.
O.F.X. Donard, D. Amouroux, **E. Tessier** .
Odense (Danemark), 4th International Conference on environmental and biological aspect of main group organometals, Juin 1998.

**3.** La chimie bio-inorganique environnementale : un outil pour étudier le devenir des métaux et métalloïdes dans les milieux aquatiques.
D. Amouroux, G. Bareille, **E. Tessier**, C.M. Tseng, R. Rodriguez, T. Stoichev, D. Point, O.F.X. Donard.
Rennes (France), Congrès de la Société Française de Chimie SFC 2000, Septembre 2000.

**4.** Gaseous metal and metalloid species in the aquatic environment: biogeochemical cycles and pollution control.
D. Amouroux, **E. Tessier**.
Warsaw (Pologne), Chemistry Forum 2001 – 6th International Symposium, Mai 2001.

**5.** Improvements in sampling and sample preparation for speciation analysis to understand the environmental chemistry of trace metals.
D. Amouroux, R. Rodriguez, **E. Tessier**, M. Monperrus, T. Stoichev, S. Veschambre, D. Point, O. Zuloaga, J. Scancar, E. Krupp, C. Pécheyran, G. Bareille, O.F.X. Donard.
Portoroz (Slovénie), 4th Mediterranean Basin Conference on Analytical Chemistry, Septembre 2002.

**6.** Chemical versus biological experimental speciation approaches to investigate the biogeochemistry of metals and organometals in aquatic environments.
D. Amouroux, M. Monperrus, D. Point, **E. Tessier**, G. Bareille, O.F.X. Donard, L. Chauvaud, G. Thouzeau, J. Grall, F. Jean, A. Leynaert, J. Clavier, R. Guyoneaud, R. Duran, M. Goni, P. Caumette.
Almunecar (Espagne), 5th International Symposium on Speciation of Elements in Biological, Environmental and Toxicological Sciences, Septembre 2003.

## COMMUNICATIONS ORALES

**1.** Volatile organotin compounds in coastal waters related to methylation pathways in contaminated aquatic sediments.
D. Amouroux, **E. Tessier**, O.F.X. Donard.
Zurich (Suisse), Workshop on organotin compounds in the environment, Janvier 1998.

**2.** Mercury species distribution and cycling in three European estuaries (Gironde, Scheldt, Rhine).
D. Amouroux, C.M. Tseng, **E. Tessier**, M. Bueno, O.F.X. Donard.
Rio (Brésil), 5th International Conference on Mercury as a Global Pollutant, Mai 1999.

**3.** Fluxes of volatile selenium from ocean to atmosphere.
P.S. Liss, D. Amouroux, **E. Tessier**, M. Hamren-Larsson, O.F.X. Donard.
Bologna (Italie), 6th Scientific Conference of the International Global Atmospheric Chemistry Project, Septembre 1999.

**4.** Mercury emission to the atmosphere from European macrotidal estuaries.
D. Amouroux, **E. Tessier**, C.M. Tseng, O.F.X. Donard.
Noordwijkerhout (Pays-Bas), 3rd ELOISE Open Science Meeting, Decembre 1999.

**5.** Atmospheric emission of gaseous biogenic selenium from coastal and marine environments.
D. Amouroux, **E. Tessier**, P.S. Liss, M. Frankignoulle, O.F.X. Donard.
San Antonio (USA), 2000 Ocean Sciences Meeting AGU/ASLO, Janvier 2000.

**6.** Biogeochemistry of mercury in a macrotidal estuary : the Gironde, France.
D. Amouroux, C.M. Tseng, **E. Tessier**, O.F.X. Donard.
Biarritz (France), 7ème Colloque International d'Océanographie du Golfe de Gascogne, Avril 2000.

**7.** Biogenic volatile compounds emission of selected trace elements in three major European estuaries (Gironde, Rhine, Schledt).
**E. Tessier**, D. Amouroux, C.M. Tseng, O.F.X. Donard.
Visegrad (Hongrie), 4th Euroconference on Environmental Analytical Chemistry, Septembre 2000.

**8.** Interactions between selenium and sulfur biogeochemistry in the marine environment leading to bio-volatilization pathways.
D. Amouroux, **E. Tessier**, E. Krupp, P. Caumette, O.F.X. Donard.
San Diego (USA), 221st ACS National Meeting – Division of Environmental Chemistry, Avril 2001.

**9.** Investigation on mercury contamination pathways using model aquatic ecosystems.
**E. Tessier**, R.C. Rodriguez Martin-Doimeadios, D. Amouroux, A. Morin, E. Thybaud, E. Vindimian, O.F.X. Donard.
San Diego (USA), 221st ACS National Meeting – Division of Environmental Chemistry, Avril 2001.

**10.** Volatilization of tin in the environment.
O.F.X. Donard, **E. Tessier**, D. Amouroux.
San Diego (USA), 221st ACS National Meeting – Division of Environmental Chemistry, Avril 2001.

**11.** Mercury water-air exchanges along a stream – dam lake transect in a tropical forest area of French Guiana.
D. Amouroux, **E. Tessier**, T. Stoichev, O.F.X. Donard.
Minamata (Japon), 6th International Conference on Mercury as a Global Pollutant, Octobre 2001.

**12.** Investigation on mercury methylation and demethylation yield in an estuarine sediment using isotopically labelled inorganic and methyl mercury.
R.C. Rodriguez Martin-Doimeadios, **E. Tessier**, D. Amouroux, R. Guyoneaud, R. Duran, P. Caumette, O.F.X. Donard.
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**13.** Biogeochemistry of trace metals in the Adour estuary.
G. Bareille, D. Point, D. Amouroux, T. Stoichev, **E. Tessier**, O.F.X. Donard
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**14.** The use of enriched stable isotope to investigate biogeochemical pathways of metal species in estuarine environments.
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**17.** Mercury methylation rates in coastal sediments versus microbial diversity and specific activity.
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**18.** Transfer of metallic contaminants at the sediment-water interface in coastal environments: role of the biological and microbial activity and diversity.
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**19.** Monitoring bacterial communities adaptation to mercury contamination in estuarine sediments maintained in slurries.
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M. Monperrus, **E. Tessier**, D. Amouroux, O.F.X. Donard, L. Chauvaud, A. Leynaert, G. Thouzeau, F. Jean, J. Clavier.
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**25.** Sediments: sink or source for TBT ?.
C. Benoit, C. Caruesco, **E. Tessier**, D. Amouroux, S. Tellier, O.F.X. Donard.
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M. Monperrus, **E. Tessier**, D. Amouroux, A. Leynaert, R. De Wit, O.F.X. Donard.
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**27.** Mercury species benthic fluxes measurements in coastal environments as influenced by the biological activity.
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## COMMUNICATIONS PAR AFFICHE

**1.** Formation and transfer of volatile Hg, Se and Sn compounds in the marine coastal environment.
D. Amouroux, C.M. Tseng, **E. Tessier**, O.F.X. Donard.
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**3.** Determination of gaseous mercury and selenium species in the Amazonian forest : comparison of gold amalgamation and cryogenic trapping sampling techniques and detection by cryofocusing gas chromatography hyphenated to ICP/MS.
J.C. Wasserman, D. Amouroux, **E. Tessier**, O.F.X. Donard.
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**E. Tessier**, C.M. Tseng, D. Amouroux, O.F.X. Donard.
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**12.** Mercury pollution sources and interactions with microbial communities in estuarine sediments.
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**14.** Organometallic contaminants in benthic organisms of the Adour estuary and adjacent coastal areas.
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**16.** Adaptation of bacterial communities to mercury contamination in estuarine sediments maintained in slurries.
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**17.** Stratification of bacterial community assessed by T-RFLP in the water column of tidal estuaries (Adour, France).
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**23.** Trace metals (Cu, Cd, Zn, Pb, V, Mn, Co) and both Mercury and Tin species flux at the sediment water interface in a coastal environment as influenced by the biological activity: Bay of Brest (Northwestern, France).
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