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Vidéoconférence, 1-3 décembre 2020

Point 5 de l'ordre du jour : Sessions CORMON parallèles (Pollution et déchets marins, et Biodiversité et pêche)

Directives/Protocoles de contrôle concernant la préparation et l'analyse des échantillons de biote marin pour l'indicateur commun 17 de l'IMAP métaux lourds, éléments traces et polluants organiques

Pour des raisons environnementales et économiques, le tirage du présent document a été restreint. Les participants sont priés d'apporter leur copie à la réunion et de ne pas demander de copies supplémentaires.

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- Annex I: IAEA (2012). Analysis of trace metals in biological and sediment samples: Laboratory procedure book (IAEA/Marine Environmental Studies Laboratory in co-operation with UNEP/MAP MED POL) (4.1.1.);
- Annex II: HELCOM (2012). Manual for marine monitoring in the COMBINE programme. Annex B-12, Appendix 4: Technical note on the determination of trace metallic elements in biota (5.1.1);
- Annex III: US EPA (1994) US-EPA Method 200.8: Determination of trace elements in waters and wastes by inductively coupled plasma-mass spectrometry (5.1.2.);
- Annex IV: IAEA (2012). Recommended method on the determination of Total Hg in samples of marine origin by Cold Vapour Atomic Absorption Spectrometry (5.1.3);
- Annex V: HELCOM (2012). COMBINE Annex B-12, Appendix 4, Attachment 1. Technical note on the determination of Total Mercury in marine biota by Cold Vapour Atomic Absorption Spectroscopy (5.1.4);
- Annex VI: UNEP/IAEA (2011). Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment. Recommended Methods for Marine Pollution Studies No 71. (4.2.1);
- Annex VII: HELCOM (2012). Manual for marine monitoring in the COMBINE programme. Annex B-12, Appendix 3. Technical note on the determination of chlorinated biphenyls and organochlorine pesticides in biota (5.2.1);
- Annex VIII: IAEA (2013). Recommended method for the determination of petroleum hydrocarbons in biological samples (5.2.2);

- Annex IX: HELCOM (2012b). Manual for marine monitoring in the COMBINE programme. Annex B-12, Appendix 2. Technical Note on the determination of Polycyclic Aromatic Hydrocarbons in Biota (5.2.3);
- Annex X: ICES/OSPAR (2008). JAMP Guidelines for monitoring contaminants in biota and sediments. Annex 1: Polyaromatic hydrocarbons in biota - Determination of parent and alkylated PAHs in biological materials (5.2.4);
- Annex XI: European Commission (2014). Common implementation strategy for the Water Framework Directive (2000/60/EC). Guidance Document No. 32 on Biota Monitoring (the Implementation of EQSbiota) under the Water Framework Directive. Technical Report - 2014 – 083. (5.2.5);
- Annex XII: Background Assessment Criteria recommended to be used to assess concentrations in Mediterranean sediments, mussel (*Mytilus galloprovincialis*) and fish (*Mullus barbatus*) (UNEP(DEPI)/MED WG.444/12, 6th Meeting of the Ecosystem Approach Coordination Group 2017);
- Annex XIII: References.

Note du Secrétariat

Conformément au programme de travail 2020-2021 adopté par la COP21, le programme MED POL a préparé les lignes directrices de surveillance relatives aux indicateurs communs 13, 14, 17 et 20 de l'IMAP en vue de leur examen lors de la réunion intégrée des groupes de correspondance sur la surveillance de l'approche écosystémique (CORMON) (décembre 2020), tandis que les lignes directrices de surveillance pour l'indicateur commun 18 ainsi que les lignes directrices de surveillance relatives à l'assurance qualité et à la communication des données sont en cours de finalisation en vue de leur examen lors de la réunion du CORMON sur la surveillance de la pollution prévue en avril 2021.

Ces lignes directrices de surveillance contiennent des manuels cohérents destinés à guider le personnel technique des laboratoires compétents IMAP des Parties contractantes pour la mise en œuvre des pratiques de surveillance normalisées et harmonisées liées à un indicateur commun IMAP spécifique (c'est-à-dire l'échantillonnage, la conservation et le transport des échantillons, la préparation et l'analyse des échantillons, ainsi que l'assurance qualité et la communication des données de surveillance). Pour la première fois, ces lignes directrices présentent un résumé des meilleures pratiques connues disponibles et utilisées dans la surveillance du milieu marin, en exposant des pratiques analytiques globales intégrées qui pourront être appliquées afin de garantir la représentativité et l'exactitude des résultats analytiques nécessaires à la production de données de surveillance de qualité assurée.

Les lignes directrices/protocoles de surveillance s'appuient sur les connaissances et les pratiques acquises au cours des 40 années de mise en œuvre de la surveillance du MED POL et sur des publications récentes, mettant en évidence les pratiques actuelles des laboratoires maritimes des Parties contractantes ainsi que d'autres pratiques issues des conventions sur les mers régionales et de l'Union européenne. Une analyse approfondie des pratiques actuellement disponibles du PNUE/PAM, du PNUE et de l'AIEA ainsi que d'HELCOM, d'OSPAR et du Centre commun de recherche de la Commission européenne a été entreprise afin de contribuer à une approche novatrice pour la préparation des lignes directrices/protocoles de surveillance de l'IMAP.

Les lignes directrices/protocoles de surveillance abordent également les problèmes identifiés lors de la réalisation des épreuves de compétence organisées par l'UNEP/MAP-MEDPOL et l'AIEA depuis deux décennies maintenant, les nombreux résultats insatisfaisants dans le cadre des tests inter-laboratoires pouvant être liés à des pratiques inadéquates au sein des laboratoires compétents de l'IMAP/MEDPOL.

En vue de soutenir les efforts nationaux, les présentes lignes directrices pour la surveillance fournissent deux notes techniques pour la préparation des échantillons et l'analyse du biote marin pour l'indicateur commun 17 de l'IMAP : a) Note technique pour l'analyse des métaux lourds dans les échantillons de biote, comprenant les protocoles suivants : i) Protocole pour la digestion des tissus du biote à l'aide d'acide nitrique (digestion assistée par micro-ondes en système fermé et digestion sur plaque chauffante) ; ii) Protocole pour l'analyse des métaux lourds par spectroscopie d'absorption atomique à flamme (F-AAS) ; iii) Protocole pour l'analyse des métaux lourds par spectroscopie d'absorption atomique à four graphite (GF-AAS) ; iv) Protocole pour l'analyse des métaux lourds par spectroscopie de masse à plasma inductif (ICP-MS) ; v) Protocole pour l'analyse du mercure total par spectrométrie d'absorption atomique à vapeur froide (CV-AAS) ; b) Note technique pour l'analyse des contaminants organiques dans les échantillons de biote, comprenant les protocoles suivants : i) Protocole pour l'analyse des pesticides organochlorés et des PCB dans les biotes par chromatographie en phase gazeuse – détecteur à capture d'électrons (GC-ECD) ; ii) Protocole pour l'analyse des pesticides organochlorés et des PCB dans les biotes par chromatographie en phase gazeuse – spectrométrie de masse (GC-MS) ; iii) Protocole pour l'analyse des HAP dans les biotes par chromatographie en phase gazeuse – détecteur à ionisation de flamme (GC-FID) ; iv) Protocole pour l'analyse des HAP dans les biotes par chromatographie en phase gazeuse – spectrométrie de masse (GC-MS) ; v) Protocole pour la normalisation des concentrations de contaminants organiques par

l'utilisation de la teneur en lipides, en vue de la réunion intégrée des groupes de correspondance sur la surveillance de l'approche écosystémique (CORMON) Biodiversité et pêche, Pollution et déchets marins, et Côte et hydrographie.

Les lignes directrices/protocoles de surveillance, y compris les présentes lignes directrices relatives à la préparation des échantillons et à l'analyse du biote marin pour l'indicateur commun 17 de l'IMAP, établissent une base solide pour une mise à jour régulière des pratiques de surveillance en vue d'une mise en œuvre réussie de l'IMAP.

Liste des abréviations/Acronymes

CdP	Conférence des parties
CE	Commission Européenne
CI	Indicateur commun
COI	Commission océanographique internationale
CORMON	Groupe de correspondance sur la surveillance
EcAp	Approche écosystémique
EEA	l'Agence européenne pour l'environnement
EFSA	Autorité européenne de sécurité des aliments
FAO	L'Organisation pour l'alimentation et l'agriculture des Nations Unies
HELCOM	Convention sur la protection de l'environnement marin de la zone de la mer Baltique (convention d'Helsinki)
IAEA	Agence Internationale de l'Energie Atomique
IMAP	Programme de surveillance et d'évaluation intégrées de la mer et des côtes méditerranéennes et les critères d'évaluation connexes
MED POL	Programme coordonné de surveillance continue et de recherche en matière de pollution dans la Méditerranée
MED QSR	Rapport sur l'état de la qualité de la Méditerranée
OECD	L'Organisation de coopération et de développement économiques (OCDE)
OSPAR	Convention pour la protection du milieu marin de l'Atlantique du Nord-Est ("Convention OSPAR")
PAM	Plan d'action pour la Méditerranée
PdT	Programme de Travail
QA/QC	Assurance Qualité / Contrôle Qualité
QSR	Le rapport sur l'état de la Mer
UE	Union Européenne
US EPA	Agence de protection de l'environnement des États-Unis

1. Introduction

1. Le biote marin absorbe les contaminants du milieu marin par l'intermédiaire de la nourriture et du milieu aquatique (respiration, échange cutané). En fonction de leurs propriétés physico-chimiques et du métabolisme de l'organisme, les contaminants peuvent être bioaccumulés dans le corps de l'organisme et, dans certains cas (comme pour le mercure et les polluants organiques persistants), ils peuvent être bioamplifiés dans les niveaux supérieurs de la chaîne alimentaire marine. Depuis la création du programme de surveillance PNUE/PAM - MED POL en 1981 (MED POL Phase II), le poisson benthique *Mullus barbatus* et le bivalve *Mytilus galloprovincialis* sont utilisés en tant qu'espèces sentinelles pour évaluer l'accumulation de contaminants dans les organismes marins de la mer Méditerranée. Dans le cadre de l'indicateur commun 17 du Programme intégré de surveillance et d'évaluation (IMAP), il est recommandé d'analyser les mêmes organismes, à savoir le poisson benthique *Mullus barbatus* (tissu musculaire) et les bivalves *Mytilus galloprovincialis* ou *Donnax trunculus* (corps entier). Les Parties peuvent décider d'inclure dans leurs programmes de surveillance nationaux la collecte et l'analyse d'autres espèces d'intérêt national. Dans tous les cas, il convient de communiquer les données sur les contaminants ainsi que les métadonnées pertinentes au Secrétariat du PNUE/PAM en utilisant le format approprié.

2. Les deux organismes susmentionnés sont présents dans l'environnement marin côtier et absents des zones marines offshore. Ils ne peuvent donc pas servir d'organismes sentinelles pour évaluer l'impact des contaminants dans l'environnement marin offshore. Pour ces zones offshore, les espèces sentinelles appropriées à utiliser pour l'évaluation de la pollution seront désignées par les Parties à un stade ultérieur.

3. Conformément aux exigences de l'IMAP^{1,2}, les contaminants devant obligatoirement être analysés dans le muscle des poissons et dans l'ensemble du corps des bivalves sont les suivants : métaux lourds [cadmium (Cd), plomb (Pb) et mercure (Hg)], composés organochlorés (PCB, hexachlorobenzène, lindane et ΣDDTs) et hydrocarbures aromatiques polycycliques (16 composés HAP de référence de l'Agence des États-Unis pour la protection de l'environnement). D'autres paramètres doivent également être mesurés, tels que la longueur, le sexe et le poids humide total de l'organisme, ainsi que la teneur en lipides du tissu à analyser.

4. Les métaux lourds et les contaminants organiques sont présents dans le biote marin à l'état de traces (ng/kg – mg/kg) ; il est donc primordial d'éviter toute contamination croisée provenant de l'environnement du laboratoire (particules de poussière et contamination par l'analyste), des récipients ou des matériaux d'emballage des échantillons, des instruments utilisés lors du prétraitement et de la préparation des échantillons, et des réactifs chimiques utilisés pour l'analyse. Dès lors, la manipulation et l'analyse des échantillons doivent être effectuées dans un laboratoire propre, afin d'éliminer la contamination croisée et de contrôler le blanc analytique total. À cette fin, si le laboratoire n'est pas spécifiquement conçu comme un « laboratoire propre » (classe 100 de la norme fédérale 209 des États-Unis), il doit être équipé de salles à flux laminaire appropriées, de tables propres et de hottes spécialement conçus pour l'analyse des métaux à l'état de traces.

5. Les critères d'évaluation proposés par le PNUE/PAM (critères d'évaluation de base – BAC, et critères d'évaluation environnementale – EAC) pour les métaux lourds et les contaminants organiques ciblés dans les poissons et les bivalves sont présentés dans l'annexe XII .

6. Les protocoles associés aux présentes lignes directrices, tels qu'ils sont présentés ci-dessous, visent à rationaliser la préparation des échantillons du biote marin et l'analyse des métaux lourds et des contaminants organiques, y compris en présentant des orientations, étape par étape, sur les méthodes à appliquer dans la zone méditerranéenne pour la préparation des échantillons et l'analyse

¹ (UNEP 2019). UNEP/MED WG.467/5. IMAP Guidance Factsheets: Update for Common Indicators 13, 14, 17, 18, 20 and 21: New proposal for candidate indicators 26 and 27;

² UNEP (2019a). UNEP/MED WG.463/6. Monitoring Protocols for IMAP Common Indicators related to pollution; UNEP/MAP (2019)

des tissus du biote marin en vue de la détermination des métaux lourds et des contaminants organiques, afin de garantir la comparabilité de l'assurance qualité des données ainsi que la comparabilité entre les zones d'échantillonnage des différents programmes de surveillance nationaux. Ces protocoles sont conçus pour être non pas des manuels de formation analytique, mais des lignes directrices pour les laboratoires méditerranéens, qu'il convient de tester et de modifier afin d'en valider les résultats finaux.

7. En vue d'éviter les répétitions inutiles, il est également fait référence aux protocoles déjà publiés et accessibles au public, qui peuvent également être utilisés par les laboratoires compétents des Parties contractantes participant à la mise en œuvre de l'IMAP. Les protocoles IMAP exposés ci-après s'appuient sur les méthodes recommandées par le PNUE/PAM et l'AIEA pour l'analyse des métaux lourds et des contaminants organiques, comme les suivantes : AIEA (2012), Analyse des métaux traces dans les échantillons biologiques et sédimentaires : Livre de procédures de laboratoire, Méthodes de référence (annexe I) ; AIEA (2012), Méthode recommandée pour la détermination du Hg total dans les échantillons d'origine marine par spectrométrie d'absorption atomique à vapeur froide (annexe IV) ; PNUE/AIEA (2011), Préparation d'échantillons pour l'analyse de certains hydrocarbures chlorés dans le milieu marin : Méthodes de référence pour les études de pollution marine n° 71 (annexe VI) ; AIEA (2013), Méthode recommandée pour la détermination des hydrocarbures pétroliers dans les échantillons biologiques (annexe VIII). Ces méthodes ont été préparées dans le cadre du programme de surveillance MED POL. Les présents protocoles sont également harmonisés avec des lignes directrices/protocoles similaires pour la préparation et l'analyse des échantillons du biote marin qui ont été élaborés par d'autres organisations des mers régionales, telles qu'OSPAR (annexe X) et HECLOM (annexes II, V, VII, IX). La méthode d'analyse développée par l'Agence des États-Unis pour la protection de l'environnement est également prise en compte (annexe III). Toutes ces lignes directrices étant adaptées au contexte de l'IMAP, elles peuvent permettre aux laboratoires méditerranéens compétents de développer leurs propres méthodes d'échantillonnage et de traitement des échantillons. Les laboratoires des Parties devraient adapter chaque étape des procédures et systématiquement les tester et les modifier pour en valider les résultats.

8. Le diagramme ci-dessous indique la catégorie des présentes lignes directrices de surveillance relatives à la préparation et à l'analyse des échantillons du biote marin pour l'indicateur commun 17 de l'IMAP au sein de la structure que forment toutes les lignes directrices de surveillance préparées pour les indicateurs communs 13, 14, 17, 18 et 20 de l'IMAP.

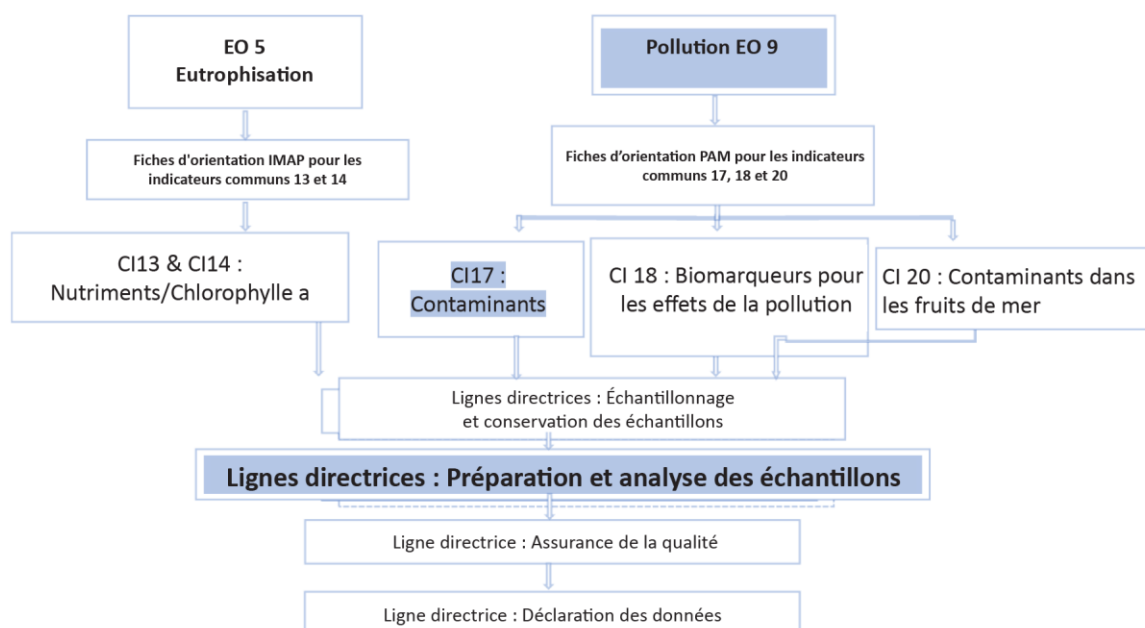


Diagramme de flux : lignes directrices pour la surveillance des objectifs écologiques 5 et 9 de l'IMAP

2. Note technique pour l'analyse des métaux lourds dans les échantillons de biote

9. L'analyse des échantillons de biote marin pour la détermination des métaux lourds comprend :
i) la digestion des tissus du biote et ii) l'analyse de l'échantillon digéré pour les métaux lourds.

10. Les laboratoires nationaux peuvent décider d'utiliser toute méthode d'analyse validée qu'ils jugent appropriée et qui répond à des critères de performance spécifiques (LD, LOQ, précision, récupération et spécificité). Toutefois, afin d'aider les laboratoires d'analyse des Parties méditerranéennes, une liste non-exclusive de protocoles a été rédigée pour servir de lignes directrices pour l'analyse des métaux lourds dans les échantillons du biote marin. Les laboratoires d'analyse devraient adapter, tester et modifier chaque étape des procédures présentées dans les protocoles afin de valider leurs résultats finaux. La liste des méthodes et du matériel d'analyse n'est pas exhaustive, et les laboratoires sont encouragés à utiliser leurs propres matériels/méthodes qu'ils jugent adéquats pour les analyses requises.

11. Quelle que soit la méthode d'analyse utilisée, l'analyse des métaux lourds suit certaines procédures communes à toutes les méthodes d'analyse, telles que l'étalonnage des équipements d'analyse ainsi que les procédures de nettoyage et de manipulation visant à éviter la contamination des échantillons par l'environnement du laboratoire et par les outils et récipients utilisés pour l'analyse.

a) Étalonnage

12. Étalons préparés à partir de solutions mères standard uniques ou d'étalons multi-éléments, par dilution de la solution mère à l'aide d'acide dilué, selon les besoins. Toutes les solutions standard doivent être stockées dans des flacons volumétriques en polyéthylène, en borosilicate ou en quartz, selon la meilleure adéquation aux analytes respectifs. Les solutions étalons à faible concentration, si elles sont préparées correctement et contrôlées dans le cadre d'un système d'assurance qualité (vérification des anciens étalons par rapport aux nouveaux étalons, et comparaison avec des étalons provenant d'une autre source), peuvent être conservées pendant une période maximale d'un mois.

13. La procédure d'étalonnage doit répondre à certains critères de base afin de donner la meilleure estimation possible de la concentration réelle en éléments de l'échantillon analysé (HELCOM, 2012³) :

- i) les concentrations des étalons pour la préparation de la courbe d'étalonnage doivent couvrir la gamme des concentrations prévues ;
- ii) la précision analytique requise doit être connue et réalisable dans toute la gamme des concentrations ;
- iii) la valeur mesurée à l'extrémité inférieure de la gamme doit être significativement différente du blanc analytique de procédure ;
- iv) les propriétés chimiques et physiques des étalons doivent ressembler étroitement à celles de l'échantillon étudié ;
- v) les instruments d'analyse doivent être réétalonnés régulièrement (tous les 10 à 20 échantillons) pour permettre de corriger la dérive instrumentale et l'efficacité analytique.

b) Éviter la contamination

³ HELCOM (2012). Manual for marine monitoring in the COMBINE programme. Annex B-12, Appendix 4: Technical note on the determination of trace metallic elements in biota.

14. Pour éviter la contamination par les métaux dans le laboratoire, tous les récipients en verre et en plastique utilisés doivent être soigneusement nettoyés. Les directives générales pour le nettoyage comprennent les orientations suivantes :

- i) laisser les récipients tremper pendant la nuit dans un contenant en plastique dans une solution alcaline de tensioactifs (par exemple une micro-solution à 2 % dans de l'eau du robinet ou, mieux encore, dans de l'eau distillée) ;
- ii) rincer minutieusement les récipients, d'abord à l'eau du robinet ou, mieux encore, à l'eau distillée, puis à l'eau déionisée ultra-pure (18 M Ω cm) ;
- iii) laisser les récipients au repos dans une solution de HNO₃ concentrée à 10 % (en volume) (qualité analytique) à température ambiante pendant au moins 6 jours ;
- iv) rincer soigneusement les récipients à l'eau déionisée ultra-pure (au moins 4 fois) ;
- v) laisser sécher les récipients sous une hotte à flux laminaire ;
- vi) conserver les récipients dans des sacs en polyéthylène fermés (par exemple avec fermeture à glissière) pour éviter tout risque de contamination avant utilisation.

15. Cette procédure doit être utilisée pour tous les articles en plastique utilisés dans les laboratoires, comme les embouts, les gobelets pour échantillonneur automatique et les récipients en plastique.

16. Dans le cadre de la présente note technique, les lignes directrices pour la préparation des échantillons et l'analyse du biote marin pour l'indicateur commun 17 de l'IMAP fournissent les cinq protocoles suivants de l'IMAP pour l'analyse des métaux lourds dans les échantillons du biote marin :

- Protocole pour la digestion des tissus du biote à l'aide d'acide nitrique (digestion assistée par micro-ondes en système fermé et digestion sur plaque chauffante) ;
- Protocole pour l'analyse des métaux lourds par spectroscopie d'absorption atomique à flamme (F-AAS) ;
- Protocole pour l'analyse des métaux lourds par spectroscopie d'absorption atomique à four graphite (GF-AAS) ;
- Protocole pour l'analyse des métaux lourds par spectroscopie de masse à plasma inductif (ICP-MS) ;
- Protocole pour l'analyse du mercure total par spectrométrie d'absorption atomique à vapeur froide (CV-AAS).

17. Ces protocoles sont basés sur les méthodes analytiques développées par l'AIEA (annexe I : Analyse des métaux traces dans les échantillons biologiques et sédimentaires ; annexe IV : Méthode recommandée pour la détermination du mercure total dans les échantillons d'origine marine par spectrométrie d'absorption atomique à vapeur froide), par HELCOM (annexe II : Manuel pour le programme COMBINE de surveillance du milieu marin : Note technique sur la détermination des éléments métalliques à l'état de traces dans les biotes ; annexe V : Programme COMBINE : Note technique sur la détermination du mercure total dans le biote marin par spectroscopie d'absorption atomique à vapeur froide) et par l'Agence des États-Unis pour la protection de l'environnement (annexe III : Méthode 200.8 de l'Agence des États-Unis pour la protection de l'environnement : Détermination des éléments traces dans les eaux et les déchets par spectrométrie de masse à plasma inductif).

2.1. Protocole pour la digestion des tissus du biote à l'aide d'acide nitrique

18. Les échantillons de tissus du biote doivent être digérés (incinération humide) avant l'analyse. La vitesse de digestion et l'efficacité de la décomposition de l'acide augmentent considérablement avec des températures et une pression élevées ; c'est pourquoi la digestion par micro-ondes dans des récipients fermés est la méthode privilégiée. Toutefois, si un tel équipement n'est pas disponible, la digestion des échantillons dans des récipients ouverts sur une plaque chauffante est une méthode alternative. Les échantillons de biote peuvent être digérés à l'état humide ou sec, mais, quelle que soit

la méthode appliquée, il est extrêmement important de garantir la destruction complète de toute la matière organique de l'échantillon, ainsi que d'éviter les pertes de métaux et la contamination de l'échantillon (HELCOM, 2012).

19. L'existence de composés de carbone organique dissous résiduels dans l'échantillon digéré modifierait la viscosité de la solution et pourrait donc fausser les résultats lorsque l'étalonnage de l'instrument AAS est effectué à l'aide de solutions étalons aquatiques. De plus, dans le spectromètre GF-AAS, le carbone organique résiduel peut subir des réactions secondaires avec l'analyte avant ou pendant le processus d'atomisation, provoquant des interférences avec la matrice (Harms, 1985⁴).

a) Digestion acide par micro-ondes en systèmes fermés (pour l'analyse des métaux lourds avec analyse AAS, GFAAS et ICP-MS)

20. La digestion des tissus du biote peut être effectuée dans du téflon, ou dans des récipients de qualité équivalente en matériau pur, qui sont exempts de métal et résistants aux acides forts, ce qui réduit considérablement les pertes d'éléments par volatilisation et la contamination par désorption des impuretés de la surface du récipient. De plus, comme seules de petites quantités d'acide nitrique de grande pureté sont utilisées, il est possible d'obtenir des blancs d'analyse extrêmement faibles. Les systèmes à micro-ondes permettent un transfert d'énergie très rapide vers l'échantillon et une montée en température et en pression interne très rapide de la cuve, ce qui a pour avantage une réduction énorme du temps de digestion (HELCOM, 2012).

Réactifs de digestion pour l'analyse du cadmium, du plomb et d'autres métaux lourds

21. Les réactifs suivants sont nécessaires :

- i) HNO₃ (65 %, par exemple Suprapur) ;
- ii) H₂O₂ (qualité analytique) à conserver au réfrigérateur après ouverture ;
- iii) eau désionisée ultra-pure (> 18 MΩ cm, par exemple Millipore).

22. Peser les échantillons de tissus de biote séchés (environ 0,2 g) dans le récipient à micro-ondes et les placer dans une hotte laminaire compatible avec les fumées acides. Ajouter environ 5 ml d'acide nitrique (HNO₃) et laisser chaque récipient réagir pendant au moins une heure (ou plus si possible). Après la pré-digestion à température ambiante, ajouter avec précaution 2 ml de peroxyde d'hydrogène (H₂O₂), fermer les récipients et les placer dans l'appareil à micro-ondes, puis effectuer les étapes de digestion, conformément à l'« Analyse des métaux traces dans les échantillons biologiques et sédimentaires : Livre de procédures de laboratoire » de l'AIEA (AIEA, 2012⁵, annexe I).

23. Veiller à ce que tous les produits chimiques utilisés dans l'analyse restent extrêmement propres une fois leurs contenants ouverts. Les placer dans un double sachet et ne les ouvrir que sur une table ou dans une salle propre. Il est également fortement conseillé de ne pas utiliser de pipettes ou d'autres dispositifs pour prélever des produits chimiques dans le récipient principal, mais d'en transférer des échantillons dans des récipients pré-nettoyés pour un usage quotidien. Cela est primordial pour éviter la contamination des produits chimiques ultra-propres très coûteux nécessaires à cette analyse.

Réactifs de digestion pour l'analyse du mercure

24. Pour l'analyse du mercure, les réactifs suivants sont nécessaires :

- i) HNO₃ (65 %, qualité analytique, certifié faible en mercure) ;
- ii) eau désionisée ultra-pure (> 18 MΩ cm) ;

⁴ Harms, U. 1985. Possibilities of improving the determination of extremely low lead concentrations in marine fish by graphite furnace atomic absorption spectrometry. *Fresenius Journal of Analytical Chemistry*, 322: 53-56.

⁵ IAEA (2012). Analysis of trace metals in biological and sediment samples: Laboratory procedure book (IAEA/Marine Environmental Studies Laboratory in co-operation with UNEP/MAP MED POL)

- iii) solution de $K_2Cr_2O_7$ à 10 % (en poids) (par exemple 10 g de $K_2Cr_2O_7$ de qualité analytique dilués dans 100 ml avec de l'eau déionisée ultra-pure) ;
- iv) V_2O_5 de qualité analytique.

25. Peser les échantillons de tissus de biote séchés (environ 0,2 à 1,5 g selon la concentration prévue) dans le récipient à micro-ondes et les placer dans une hotte laminaire compatible avec les fumées acides. En cas de traitement de bivalves de poids élevé (> 1 g), ajouter 40 mg de V_2O_5 à chaque tube (y compris les blancs). Ajouter 5 ml d'acide nitrique concentré (HNO_3) et laisser réagir pendant au moins une heure. En cas d'utilisation d'une grande quantité d'échantillon, ajouter plus d'acide jusqu'à ce que le mélange devienne liquide. Pour contrôler le rendement de la procédure de digestion, au moins 2 blancs doivent être préparés de la même manière que les échantillons pour chaque lot d'analyse. De plus, au moins un matériau de référence certifié doit être utilisé et préparé en double pour chaque lot de digestion. Préparer ces digestions de la même manière que les échantillons. Il convient d'utiliser un matériau de référence de composition et de concentration similaires. Après la digestion, retirer les récipients de l'appareil à micro-ondes et les placer dans une hotte ventilée pour qu'ils refroidissent. Lorsque la pression est suffisante, ouvrir les récipients, ajouter 1 ml de solution de $K_2Cr_2O_7$ (la concentration finale doit être de 2 % en volume), transférer leur contenu dans un flacon jaugé, de préférence en téflon, mais qui peut également être en verre, et le porter à un volume connu.

b) Digestion acide dans des systèmes ouverts

26. Si aucun système de digestion par micro-ondes n'est disponible, il est possible d'effectuer une digestion sur une plaque chauffante programmable placée à l'intérieur d'une hotte spécialement conçue à cet effet, permettant le traitement des acides. Cependant, pour la destruction complète de la matière organique, il faut généralement de grandes quantités de réactifs et des appareils volumineux avec de grandes surfaces, et la méthode est sujette à des problèmes de contamination (valeurs à blanc trop élevées) en cas d'utilisation d'acides insuffisamment purifiés. De plus, la vitesse de réaction et l'efficacité de la décomposition de l'acide dans des récipients ouverts sont plus faibles que dans des récipients fermés sous pression.

27. Peser les échantillons de tissus de biote séchés (environ 0,2 g) dans le récipient à micro-ondes et les placer dans une hotte à flux laminaire compatible avec les fumées acides. Ajouter environ 5 ml d'acide nitrique concentré (HNO_3) dans chaque récipient et laisser réagir à température ambiante pendant au moins 1 heure. Fermer les tubes et les placer dans un bloc d'aluminium sur une plaque chauffante à 90 °C pendant 3 heures. Laisser les échantillons refroidir à température ambiante, ouvrir les tubes avec précaution et transférer les échantillons dans les tubes gradués ou les flacons jaugés de 50 ml en polypropylène étiquetés.

28. Une description détaillée de la procédure est présentée dans le document de l'AIEA intitulé « Analyse des métaux traces dans les échantillons biologiques et sédimentaires : Livre de procédures de laboratoire » (AIEA, 2012, annexe I).

29. En plus de la méthode de l'AIEA, d'autres méthodes ont été élaborées pour la digestion des tissus du biote dans des systèmes ouverts, au moyen d'aqua regia ou de $HNO_3/HClO_4$ (Black et al., 2013⁶).

2.2. Protocole pour l'analyse des métaux lourds par spectroscopie d'absorption atomique à flamme (F-AAS)

30. La spectroscopie d'absorption atomique à flamme (F-AAS) a une sensibilité adéquate pour la détermination d'un large éventail de métaux dans les tissus du biote marin. La solution de l'échantillon

⁶ Black, K., Kalantzi, I., Karakassis, I., Papageorgiou, N., Pergantis, S., Shimmield, T. (2013). Heavy metals, trace elements and sediment geochemistry at four mediterranean fish farms, Science of the Total Environment. Elsevier, 444, 128–137.

est aspirée dans une flamme et atomisée. Dans le cas de l'AAS à flamme, un faisceau lumineux est dirigé à travers la flamme, dans un monochromateur, et sur un détecteur qui mesure la quantité de lumière absorbée par l'élément dans la flamme. Chaque métal ayant sa propre longueur d'onde caractéristique, on utilise une lampe à cathode creuse source composée de cet élément. La quantité d'énergie absorbée à la longueur d'onde caractéristique est proportionnelle à la concentration de l'élément en question dans l'échantillon.

31. Un protocole analytique détaillé pour l'analyse des métaux lourds dans les échantillons de tissus du biote, préparé par l'AIEA (2012), est présenté à l'annexe I (Livre de procédures de laboratoire – Analyse des métaux traces dans les échantillons biologiques et sédimentaires, chapitre III-3 : Détermination des métaux traces dans les sédiments et les matériaux biologiques par F-AAS).

2.3. Protocole pour l'analyse des métaux lourds par spectroscopie d'absorption atomique en four graphite (GF-AAS)

32. Dans les tissus du biote marin, le cadmium, le plomb ainsi que d'autres métaux lourds peuvent être déterminés par spectroscopie d'absorption atomique à four graphite (GF-AAS), cette méthode présentant une sensibilité adéquate pour ces déterminations. Dans le cadre de l'analyse par GF-AAS, après la digestion de l'échantillon de biote, une aliquote de la solution d'échantillon (10-50 µl) est introduite dans un tube en graphite du spectromètre GF-AAS et atomisée par chauffage rapide à haute température. Un faisceau lumineux est dirigé à travers le tube en graphite, dans un monochromateur, et sur un détecteur qui mesure la quantité de lumière absorbée par l'élément atomisé dans le tube. Chaque métal ayant sa propre longueur d'onde caractéristique, on utilise une lampe à cathode creuse source composée de cet élément. La quantité d'énergie absorbée à la longueur d'onde caractéristique est proportionnelle à la concentration de l'élément en question dans l'échantillon.

33. Le logiciel AAS donne généralement des programmes électrothermiques typiques pour chaque élément pour 10 µl d'échantillon dans du HNO₃ dilué (0,1 %) et des indications concernant les températures maximales d'incinération et d'atomisation. Des informations plus spécifiques peuvent également être trouvées dans la littérature, telles que des recommandations concernant les modificateurs de matrice et l'utilisation de tubes de séparation ou de tubes avec plateforme. Lorsqu'un programme est optimisé pour la détermination d'un élément dans une matrice spécifique, toutes les informations doivent être consignées dans le journal des méthodes du laboratoire.

34. Pour certains éléments et certaines matrices, les résultats obtenus ne sont toujours pas satisfaisants (par exemple, la température maximale d'incinération n'est pas suffisante pour éliminer le fond) ; il convient alors d'effectuer à nouveau cette procédure en ajoutant un modificateur de matrice. Il est possible de devoir essayer différents modificateurs de matrice avant de trouver la meilleure solution.

35. Un protocole analytique détaillé pour l'analyse des métaux lourds dans les sédiments par GF-AAS, préparé par l'AIEA (2012), est présenté à l'annexe I (Livre de procédures de laboratoire – Analyse des métaux traces dans les échantillons biologiques et sédimentaires, chapitre III-2 : Détermination des métaux traces dans les sédiments et les matériaux biologiques par GF-AAS).

2.4. Protocole pour l'analyse des métaux lourds par spectroscopie de masse à plasma à couplage inductif (ICP-MS)

36. La spectroscopie de masse à plasma à couplage inductif (ICP-MS) est actuellement l'un des instruments les plus avancés pour l'analyse des métaux ; elle permet de déterminer des concentrations inférieures au µg/l d'un grand nombre d'éléments dans les échantillons de tissus du biote digérés par l'acide. L'ICP-MS permet une analyse rapide d'un large éventail de métaux lourds. La plupart des instruments de routine utilisent un spectromètre de masse quadripolaire, de sorte que la résolution de

masse n'est pas assez élevée pour éviter le chevauchement des éléments à double charge ou des ions multi-éléments (principalement des hydrures, des oxydes et des hydroxydes) formés dans le plasma. La principale préoccupation concerne les interférences avec l'argon, car le plasma est généralement un plasma d'argon, qui chevauche l'arsenic. Certains éléments sont sujets à des effets de mémoire (en particulier le mercure) et nécessitent des précautions supplémentaires pour éviter les effets de report. Le logiciel des instruments modernes de l'ICP-MS comprend toutes les formules de réglage et de correction nécessaires, décrites ci-dessus, pour effectuer l'analyse (HELCOM, 2012).

37. La méthode 200.8 de l'Agence des États-Unis pour la protection de l'environnement (1994⁷) décrit la détermination multi-élémentaire des métaux lourds par ICP-MS dans l'eau et les échantillons solides après digestion acide. Cette méthode est initialement destinée aux échantillons solides inorganiques (sols et sédiments), mais elle peut également être appliquée directement aux échantillons organiques. Selon Enamorado-Baez et al. (2015⁸), pour les tissus du biote, il serait possible de réaliser l'étape de digestion en utilisant uniquement de l'acide nitrique (similaire à la méthode 3051 de l'Agence des États-Unis pour la protection de l'environnement établie pour les sédiments, les boues, les sols et les huiles), mais en augmentant le rapport masse d'échantillon/volume d'acide.

38. Les espèces de métaux provenant d'un liquide sont nébulisées et l'aérosol qui en résulte est transporté par le gaz argon dans la torche à plasma. Les ions produits par les hautes températures sont entraînés dans le gaz du plasma et introduits, par le biais d'une interface, dans un spectromètre de masse. Les ions produits dans le plasma sont triés en fonction de leur rapport masse/charge et quantifiés à l'aide d'un multiplicateur d'électrons à canaux. Les interférences doivent être évaluées et des corrections valables doivent être appliquées. La correction des interférences doit inclure la compensation des ions de fond apportés par le gaz du plasma, les réactifs et les constituants de la matrice de l'échantillon. La méthode 200.8 de l'Agence des États-Unis pour la protection de l'environnement est présentée à l'annexe III.

2.5. Protocole pour l'analyse du mercure total dans les échantillons d'origine marine par spectrométrie d'absorption atomique à vapeur froide (CV-AAS)

39. Cette méthode, largement utilisée pour la détermination du mercure total dans les tissus biologiques, est simple, rapide et applicable à un grand nombre d'échantillons environnementaux. La gamme de travail typique est de 0,25-100 ng mL⁻¹ pour l'injection directe de vapeur froide, avec utilisation du système « batch » (AIEA, 2012b⁹). L'analyse CV-AAS peut être effectuée manuellement à l'aide du spectromètre CV-AAS par lots ou automatiquement à l'aide de techniques d'injection de flux (FIAS). La technique FIAS est une approche très efficace pour introduire et traiter des échantillons liquides en spectrométrie d'absorption atomique ; elle réduit la consommation d'échantillons et de réactifs et présente une plus grande tolérance aux interférences, des limites de détermination plus basses et une meilleure précision par rapport aux techniques classiques utilisant la vapeur froide (HELCOM, 2012b¹⁰).

40. Les échantillons de tissus du biote sont digérés avec des acides forts et le mercure inorganique est réduit à sa forme élémentaire avec du chlorure stanneux. La vapeur de mercure froide passe ensuite dans la cellule d'absorption à quartz d'un spectromètre d'absorption atomique (SAA), où sa concentration est mesurée.

⁷ US EPA (1994) US-EPA Method 200.8: Determination of trace elements in waters and wastes by inductively coupled plasma-mass spectrometry.

⁸ Enamorado-Báez, S.M., Abril, JM and Gómez-Guzmán, JM (2013) Determination of 25 Trace Element Concentrations in Biological Reference Materials by ICP-MS following Different Microwave-Assisted Acid Digestion Methods Based on Scaling Masses of Digested Samples. Hindawi Publishing Corporation, ISRN Analytical Chemistry, Volume 2013, Article ID 851713, 14 pages. <http://dx.doi.org/10.1155/2013/851713>

⁹ IAEA (2012b). Recommended method on the determination of Total Hg in samples of marine origin by Cold Vapour Atomic Absorption Spectrometry.

¹⁰ HELCOM (2012b). COMBINE Annex B-12, Appendix 4, Attachment 1. Technical note on the determination of Total Mercury in marine biota by Cold Vapour Atomic Absorption Spectroscopy.

41. Dans le cadre de la méthode CV-AAS, le mercure inorganique est réduit à sa forme élémentaire avec du chlorure stanneux. La vapeur de mercure froide passe ensuite dans la cellule d'absorption à quartz d'un instrument AAS, où sa concentration est mesurée. Le faisceau lumineux de la lampe à cathode creuse au mercure est dirigé à travers la cellule à quartz, dans un monochromateur et sur un détecteur qui mesure la quantité de lumière absorbée par la vapeur atomisée dans la cellule. La quantité d'énergie absorbée à la longueur d'onde caractéristique est proportionnelle à la concentration de l'élément en question dans l'échantillon.

42. Une méthode recommandée décrivant le protocole pour la détermination du mercure total dans les biotes, préparée par l'AIEA (2012b), est présentée à l'annexe IV (Méthode recommandée pour la détermination du mercure total dans les échantillons d'origine marine par spectrométrie d'absorption atomique à vapeur froide). HELCOM (2012b) propose également une méthode pour la détermination du mercure total dans le biote marin à l'aide de la CV-AAS (annexe IV), de même que l'Agence des États-Unis pour la protection de l'environnement (2007b¹¹).

3. Note technique pour l'analyse des contaminants organiques dans le biote marin

43. Conformément aux exigences de l'IMAP (PNUE/PAM, 2019 ; PNUE/PAM, 2019a), les contaminants organiques dont la présence dans le biote marin doit obligatoirement être surveillée dans le cadre de l'IMAP sont les suivants : composés organochlorés (PCB [28, 31, 52, 101, 105, 118, 138, 153, 156, 180], hexachlorobenzène, lindane et Σ DDT) et hydrocarbures aromatiques polycycliques (16 congénères individuels des HAP de l'Agence des États-Unis pour la protection de l'environnement – acénaphthène, acénaphthylène, anthracène, benz(a)anthracène, benzo(b)fluoranthène,

44. benzo(k)fluoranthène, benzo(a)pyrène, benzo(ghi)pérylène, chrysène, dibenzo(a,h)anthracène, fluoranthène, indéno(1,2,3-cd)pyrène, naphthalène, phénanthrène, pyrène). Toutefois, les Parties contractantes à la convention de Barcelone peuvent décider d'inclure dans leurs programmes de surveillance nationaux l'analyse de composés organiques lourds supplémentaires, en fonction de leurs priorités nationales.

45. L'analyse des échantillons de biote marin pour la détermination des contaminants organiques comprend : i) l'extraction ; ii) la concentration ; iii) le nettoyage ; iv) le fractionnement ; et v) la quantification des contaminants.

46. Les laboratoires nationaux peuvent décider d'utiliser toute méthode d'analyse validée qu'ils jugent appropriée et qui répond à des critères de performance spécifiques (LD, LOQ, précision, récupération et spécificité). Toutefois, afin d'aider les laboratoires d'analyse des Parties contractantes, les protocoles IMAP ont été rédigés pour servir de lignes directrices pour l'analyse des composés organiques dans les échantillons de biotes marins. Les laboratoires d'analyse devraient adapter, tester et modifier chaque étape des procédures présentées dans les protocoles IMAP afin de valider leurs résultats finaux. La liste des méthodes et du matériel d'analyse n'est pas exhaustive, et les laboratoires sont encouragés à utiliser leurs propres matériels/méthodes qu'ils jugent adéquats pour les analyses requises.

47. Dans le cadre de la présente note technique, les lignes directrices pour la préparation d'échantillons et l'analyse du biote marin pour l'indicateur commun 17 de l'IMAP fournissent les cinq protocoles suivants de l'IMAP :

¹¹ US EPA (2007b). U.S. Environmental Protection Agency, EPA method 7473, Mercury in solids and solutions by thermal decomposition, amalgamation and atomic absorption spectrophotometry Rev 0.
<http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/7473.pdf>

- Protocole pour l'analyse des pesticides organochlorés et des PCB dans les biotes par chromatographie en phase gazeuse – détecteur à capture d'électrons (GC-ECD) ;
- Protocole pour l'analyse des pesticides organochlorés et des PCB dans les biotes par chromatographie en phase gazeuse – spectrométrie de masse (GC-MS) ;
- Protocole pour l'analyse des HAP dans les biotes par chromatographie en phase gazeuse – détecteur à ionisation de flamme (GC-FID) ;
- Protocole pour l'analyse des HAP dans les biotes par chromatographie en phase gazeuse – spectrométrie de masse (GC-MS) ;
- Protocole pour la normalisation des concentrations de contaminants organiques par l'utilisation de la teneur en lipides.

48. Ces protocoles sont basés sur les méthodes analytiques développées par le PNUE/AIEA (annexe VI : Préparation d'échantillons pour l'analyse de certains hydrocarbures chlorés sélectionnés dans le milieu marin. Méthodes de référence pour les études de pollution marine n° 71 ; annexe VIII : Méthode recommandée pour la détermination des hydrocarbures pétroliers dans des échantillons biologiques), par HELCOM (annexe VII : Manuel pour le programme COMBINE de surveillance du milieu marin, annexe B-12, appendice 3 : Note technique sur la détermination des biphényles chlorés et des pesticides organochlorés dans les biotes ; annexe IX : Manuel pour le programme COMBINE de surveillance du milieu marin : annexe B-12, appendice 2 : Note technique sur la détermination des hydrocarbures aromatiques polycycliques dans les biotes) et par le CIEM/OSPAR (annexe X : Lignes directrices JAMP pour la surveillance des contaminants dans le biote et les sédiments, annexe 1 : Hydrocarbures polyaromatiques dans le biote – Détermination des HAP parents et alkylés dans les matériels biologiques).

3.1. Protocole pour l'analyse des pesticides organochlorés et des PCB dans les biotes par chromatographie en phase gazeuse – détecteur à capture d'électrons (GC-ECD)

49. L'analyse des PCB et des pesticides organochlorés (PCO) dans les échantillons de biotes marins (muscles de poissons et corps entier de bivalves) implique une extraction de la matrice avec des solvants organiques, suivie d'un nettoyage et d'une séparation par chromatographie en phase gazeuse avec capture d'électrons (GC-ECD) ou détection par spectrométrie de masse (GC-MS). Pour réduire au minimum les erreurs systématiques dues à des conditions de chromatographie en phase gazeuse insuffisamment optimisées, à des pertes déterminantes (évaporation, rendement d'extraction insatisfaisant) et/ou à la contamination par des articles de laboratoire, des réactifs et l'environnement du laboratoire, il est essentiel que les sources d'erreurs systématiques soient identifiées et éliminées dans la mesure du possible (HELCOM, 2012c¹²).

50. Pour l'analyse, les échantillons sont préparés pour l'extraction par solvant. Pour obtenir une récupération satisfaisante des hydrocarbures chlorés, les échantillons sont séchés soit par dessiccation avec du sulfate de sodium anhydre, soit par lyophilisation. Les lipides sont ensuite extraits du biote par Soxhlet à l'aide d'hexane ou d'éther de pétrole. Après les premiers traitements de nettoyage (traitement des extraits de biote avec de l'acide sulfurique concentré pour détruire certains lipides interférents), les extraits sont fractionnés par chromatographie sur colonne.

51. Tous les réactifs, y compris l'eau distillée, doivent être de qualité analytique. Les solvants disponibles dans le commerce comme l'acétone, l'acétonitrile, le dichlorométhane, l'hexane et le pentane sont invariablement contaminés par des substances actives par DCE ; leurs concentrations varient d'un lot à l'autre et selon le fournisseur. La qualité des réactifs doit être vérifiée par injection de 2 µl d'un lot de 100 ml de solvant, après concentration à 50 µl dans un évaporateur rotatif. Aucun pic dans le chromatogramme GC-ECD (90-250 °C) ne devrait être supérieur à celui correspondant à 1 pg de lindane. Sinon, le solvant doit être distillé.

¹² HELCOM (2012c). Manual for marine monitoring in the COMBINE programme. Annex B-12, Appendix 3. Technical note on the determination of chlorinated biphenyls and organochlorine pesticides in biota.

52. Le laboratoire utilisé pour l'analyse des traces organiques doit être une installation spécialisée, isolée des autres projets qui pourraient constituer des sources de contamination. Il doit être correctement construit avec des hottes et des tables munies de prises électriques qui ne présentent aucun danger pour l'utilisation de solvants inflammables. Le laboratoire doit disposer d'extracteurs et d'évaporateurs rotatifs refroidissant l'eau pour faire fonctionner les alambics. Dans les régions tropicales et dans les climats secs, un système de recirculation réfrigéré doit être utilisé pour abaisser les températures aux niveaux requis et/ou pour conserver l'eau. L'acier inoxydable et les carreaux de céramique constituent de bonnes surfaces non contaminantes. Si nécessaire, les tables peuvent être recouvertes d'une résine époxy dure et les murs peuvent être peints avec de la peinture époxy. Une feuille d'aluminium sur la table de travail offre une surface nettoyable avec un solvant. Il est essentiel de disposer d'une installation de stockage ventilée pour les solvants. Les tables doivent être équipées de supports pour la fixation des alambics, des extracteurs, etc. L'interrupteur d'urgence doit être accessible tant depuis l'intérieur que depuis l'extérieur du laboratoire. Le matériel de lutte contre l'incendie doit être installé dans des endroits évidents et le personnel de laboratoire doit être formé à son utilisation.

53. L'analyse quantitative avec le détecteur à capture d'électrons (ECD) est effectuée par une comparaison du signal du détecteur produit par l'échantillon avec celui produit par des étalons définis. En raison d'une séparation incomplète, plusieurs composés de co-élution peuvent être présents sous un seul signal de détecteur ; c'est pourquoi la forme et la taille du signal doivent être examinées de manière critique. Le temps de rétention relatif et la taille du signal doivent être confirmés sur des colonnes dont les phases stationnaires sont de polarité différente, ou par l'utilisation de techniques de GC multidimensionnelles. La GC doit être étalonnée avant chaque lot de mesures. Comme l'ECD a une courbe de réponse non linéaire, un étalonnage à plusieurs niveaux est fortement conseillé. Pour déterminer les taux de récupération, il convient d'ajouter un étalon interne approprié à chaque échantillon au début de la procédure d'analyse. L'étalon interne idéal est un PCB qui n'est pas présent dans l'échantillon et qui n'interfère pas avec d'autres PCB. Tous les congénères de PCB substitués en 2,4,6 sont, en principe, appropriés. Il est également possible d'utiliser le 1,2,3,4-tétrachloronaphtalène ou les homologues du dichloroalkylbenzyléther (HELCOM, 2012c).

54. Une méthode étape par étape pour la détermination des pesticides organochlorés et des polychlorobiphényles dans les échantillons biologiques a été préparée par le PNUE/AIEA (2011¹³) (annexe VI), comprenant la liste des réactifs, des solvants, des étalons et des exemples pour la préparation des solutions de base, intermédiaires et de travail. Une méthode pour l'analyse des pesticides organochlorés et des PCB dans les tissus des biotes est également proposée par HELCOM (2012c) (annexe VII).

3.2. Protocole pour l'analyse des pesticides organochlorés et des PCB dans les biotes par chromatographie en phase gazeuse – spectrométrie de masse (GC-MS)

55. L'analyse des PCB et des pesticides organochlorés (PCO) dans les échantillons de biotes marins (muscles de poissons et corps entier de bivalves) implique une extraction de la matrice avec des solvants organiques, suivie d'un nettoyage et d'une séparation par chromatographie en phase gazeuse avec détection par spectrométrie de masse (GC-MS). Pour l'analyse, les échantillons sont préparés pour l'extraction par solvant. Pour obtenir une récupération satisfaisante des hydrocarbures chlorés, les échantillons sont séchés soit par dessiccation avec du sulfate de sodium anhydre, soit par lyophilisation. Les lipides sont ensuite extraits du biote par Soxhlet à l'aide d'hexane ou d'éther de pétrole. Après les premiers traitements de nettoyage (traitement des extraits de biote avec de l'acide sulfurique concentré pour détruire certains lipides interférents), les extraits sont fractionnés par chromatographie sur colonne (PNUE/AIEA, 2011).

¹³ IAEA (2013). Recommended method for the determination of petroleum hydrocarbons in biological samples

56. L'analyse quantitative est réalisée par une comparaison du signal du détecteur produit par l'échantillon avec celui produit par des étalons définis, à l'aide d'un spectromètre de masse (MS). Souvent, en raison d'une séparation incomplète, plusieurs composés de co-élution peuvent être présents sous un seul signal de détecteur. Par conséquent, la forme et la taille du signal doivent être examinées de manière critique. Avec un détecteur MS, la masse moléculaire ou les fragments de masse caractéristiques devraient être enregistrés à cette fin. La GC doit être étalonnée avant chaque lot de mesures. Comme le MS a une courbe de réponse non linéaire, un étalonnage à plusieurs niveaux est conseillé. Pour déterminer les taux de récupération, il convient d'ajouter un étalon interne approprié à chaque échantillon au début de la procédure d'analyse. L'étalon interne idéal est un PCB qui n'est pas présent dans l'échantillon et qui n'interfère pas avec d'autres PCB. Tous les congénères de PCB substitués en 2,4,6 sont, en principe, appropriés. Il est également possible d'utiliser le 1,2,3,4-tétrachloronaphtalène ou les homologues du dichloroalkylbenzyléther. Pour la GC-MS, il convient d'utiliser pour étalons internes, de préférence, des PCB marqués ¹³C (HELCOM, 2012c).

57. Une méthode d'extraction, de concentration, de nettoyage et de fractionnement pour la détermination des pesticides organochlorés et des polychlorobiphényles dans les échantillons biologiques a été préparée par le PNUE/AIEA (2011) (annexe VI), comprenant la liste des réactifs, des solvants, des étalons et des exemples pour la préparation des solutions de base, intermédiaires et de travail. L'analyse des PCB et des pesticides organochlorés peut être effectuée par GC-ECD suivie d'une confirmation par GC-MS. Une méthode d'analyse des pesticides organochlorés et des PCB dans les tissus des biotes par GC-MS est également proposée par HELCOM (2012c) (annexe VII).

3.3. Protocole pour l'analyse des HAP dans les biotes par chromatographie en phase gazeuse – détecteur à ionisation de flamme (GC-FID)

58. Les HAP sont lipophiles et se concentrent donc dans les lipides d'un organisme. Ils doivent dès lors être extraits par extraction Soxhlet, ou digestion alcaline suivie d'une extraction liquide-liquide avec un solvant organique. Les échantillons congelés sont décongelés et extraits par Soxhlet à l'aide de méthanol. Des extraits de tissus contiendront toujours de nombreux composés autres que les HAP, et un nettoyage est nécessaire pour éliminer les composés qui pourraient interférer avec l'analyse ultérieure. Pour réduire le volume de l'échantillon à 2 cm³, les solvants sont évaporés à l'aide d'un évaporateur à film rotatif à basse température (température du bain-marie de 30 °C ou moins) et dans des conditions de pression contrôlées afin d'éviter les pertes des HAP les plus volatils, tels que les naphthalènes. Il convient d'éviter l'évaporation jusqu'à la sécheresse. Lors de la réduction de l'échantillon au volume final, les solvants peuvent être éliminés par un flux d'azote gazeux propre. Les solvants et les matériaux adsorbants doivent tous être contrôlés pour détecter la présence de HAP et d'autres composés interférents. Si l'on trouve de tels composés, les solvants, les réactifs et les matériaux adsorbants doivent être purifiés ou nettoyés à l'aide de méthodes appropriées (HELCOM, 2012d¹⁴).

59. Après nettoyage et concentration, les extraits sont fractionnés par chromatographie sur colonne avec de la silice et de l'alumine. Le nettoyage et la séparation sont réalisés par une simple partition chromatographique sur colonne, comme suit : Une colonne de chromatographie est préparée à l'aide d'une burette de 50 ml, dans laquelle un morceau de laine de verre est ajouté près du robinet d'arrêt pour maintenir le matériau de garnissage. Ensuite, 5 g de silice sont transférés dans la colonne, puis 10 g d'alumine et, enfin, 1 g de sulfate de sodium sont ajoutés afin d'éviter de perturber la première couche lorsque les solvants sont versés dans la colonne. Pour la séparation des composés, l'échantillon (maximum 300 mg de lipides non saponifiés) est appliqué sur le dessus de la colonne. Une première fraction est obtenue par élution de l'échantillon avec 20 ml d'hexane (F1) ; cette fraction contiendra les aliphatiques saturés. La deuxième fraction (F2) est obtenue par élution avec 30 ml d'un mélange d'hexane et de dichlorométhane (90:10) ; cette fraction contiendra les hydrocarbures insaturés et aromatiques (AIEA, 2013¹⁵).

¹⁴ HELCOM (2012d). Manual for marine monitoring in the COMBINE programme. Annxe B-12, Appendix 2. Technical Note on the determination of Polycyclic Aromatic Hydrocarbons in Biota.

¹⁵ IAEA (2013). Recommended method for the determination of petroleum hydrocarbons in biological samples.

60. La quantification est effectuée par GC-FID. L'information la plus utilisée pour identifier un pic est son temps de rétention, ou son temps de rétention relatif (c'est-à-dire le temps de rétention ajusté par rapport à celui d'un composé de référence sélectionné). Le comportement de rétention dépend de la température, et la comparaison des temps de rétention obtenus à deux températures ou plus peut aider à déterminer l'identité d'un pic. Cependant, les temps de rétention ne sont pas spécifiques et, malgré la haute résolution offerte par les colonnes capillaires, deux composés intéressants dans le même échantillon peuvent avoir des temps de rétention identiques. En chromatographie en phase gazeuse, les résultats sont généralement quantifiés par un étalonnage externe ou un étalonnage interne. Un étalonnage externe est effectué par l'injection d'échantillons étalons contenant des concentrations variables du composé à analyser et par la création d'une courbe d'étalonnage (surface en fonction de la concentration). Un étalonnage interne est basé sur l'utilisation d'un étalon interne, qui est défini comme un composé non interférent ajouté à un échantillon en concentration connue afin d'éliminer la nécessité de mesurer la taille de l'échantillon dans l'analyse quantitative et de corriger la variation instrumentale. Dans cette méthode, l'étalon interne est ajouté à chaque échantillon et à chaque solution étalon.

61. Une méthode étape par étape pour la détermination des hydrocarbures de pétrole dans les échantillons biologiques, préparée par l'AIEA (2013) (annexe VIII), comprend une liste de réactifs et de matériel de laboratoire ainsi que des procédures de nettoyage pour la verrerie, les réactifs, les adsorbants et les cartouches d'extraction. Des lignes directrices pour la préparation d'échantillons de biote pour l'analyse des HAP ont également été préparées par HELCOM (2012d) (annexe IX).

3.4. Protocole pour l'analyse des HAP dans les biotes par chromatographie en phase gazeuse – spectrométrie de masse (GC-MS)

62. Les HAP présents dans le milieu marin peuvent provenir de processus de combustion et de rejets de pétrole et de produits pétroliers. Les HAP de combustion sont principalement des composés parents (non substitués), alors que le pétrole et ses produits contiennent une gamme beaucoup plus large de composés alkylés en plus des HAP parents. Cela a des implications pour la détermination analytique, car les techniques basées sur la CLHP et la GC sont toutes deux adéquates pour la détermination d'une gamme limitée de HAP parents dans des échantillons influencés par les processus de combustion, alors que dans les zones de contamination importante par les hydrocarbures et à la suite de déversements d'hydrocarbures, seule la GC-MS a une sélectivité suffisante pour déterminer toute la gamme des HAP présents (CIEM/OSPAR, 200816).

63. Les HAP sont lipophiles et se concentrent donc dans les lipides d'un organisme. Ils doivent dès lors être extraits par extraction Soxhlet, ou digestion alcaline suivie d'une extraction liquide-liquide avec un solvant organique. Les échantillons sont extraits par Soxhlet à l'aide de méthanol. Des extraits de tissus contiendront toujours de nombreux composés autres que les HAP, et un nettoyage est nécessaire pour éliminer les composés qui pourraient interférer avec l'analyse ultérieure. Pour réduire le volume de l'échantillon à 2 ml, les solvants sont évaporés à l'aide d'un évaporateur à film rotatif à basse température (température du bain-marie de 30 °C ou moins) et dans des conditions de pression contrôlées afin d'éviter les pertes des HAP les plus volatils, tels que les naphthalènes. Il convient d'éviter l'évaporation jusqu'à la sécheresse. Lors de la réduction de l'échantillon au volume final, les solvants peuvent être éliminés par un flux d'azote gazeux propre. Les solvants et les matériaux adsorbants doivent tous être contrôlés pour détecter la présence de HAP et d'autres composés interférents. Si un tel composé est trouvé, alors les solvants, les réactifs et les matériaux adsorbants doivent être purifiés ou nettoyés à l'aide de méthodes appropriées.

64. Après nettoyage et concentration, les extraits sont fractionnés par chromatographie sur colonne avec de la silice et de l'alumine. Le nettoyage et la séparation sont réalisés par une simple partition chromatographique sur colonne, comme suit : Une colonne de chromatographie est préparée

¹⁶ ICES/OSPAR (2008). JAMP Guidelines for monitoring contaminants in biota and sediments. ANNEX 1: Polyaromatic hydrocarbons in biota - Determination of parent and alkylated PAHs in biological materials.

à l'aide d'une burette de 50 ml, dans laquelle un morceau de laine de verre est ajouté près du robinet d'arrêt pour maintenir le matériau de garnissage. Ensuite, 5 g de silice sont transférés dans la colonne, puis 10 g d'alumine et, enfin, 1 g de sulfate de sodium sont ajoutés afin d'éviter de perturber la première couche lorsque les solvants sont versés dans la colonne. Pour la séparation des composés, l'échantillon (maximum 300 mg de lipides non saponifiés) est appliqué sur le dessus de la colonne. Une première fraction est obtenue par élution de l'échantillon avec 20 ml d'hexane (F1) ; cette fraction contiendra les aliphatiques saturés. La deuxième fraction (F2) est obtenue par élution avec 30 ml d'un mélange d'hexane et de dichlorométhane (90:10) ; cette fraction contiendra les hydrocarbures non saturés et aromatiques (AIEA, 2013).

65. La quantification est effectuée par GC-MS. Les deux modes d'injection couramment utilisés sont l'injection sans fractionnement et l'injection sur colonne. L'injection automatique des échantillons doit être utilisée dans la mesure du possible pour améliorer la reproductibilité de l'injection et la précision de la méthode dans son ensemble. En cas d'utilisation de l'injection fractionnée, le revêtement doit avoir une capacité suffisante pour contenir le volume de solvant injecté après évaporation. Pour l'analyse des HAP, la propreté du revêtement est également très importante si l'on veut éviter les effets d'adsorption et de discrimination, et la colonne d'analyse ne doit pas contenir de sites actifs sur lesquels les HAP peuvent être adsorbés. L'hélium est le gaz vecteur privilégié, et seules des colonnes capillaires doivent être utilisées (HELCOM, 2012d).

66. Une méthode étape par étape pour la détermination des hydrocarbures de pétrole dans les échantillons biologiques, préparée par l'AIEA (2013) (annexe VIII), comprend une liste de réactifs et de matériel de laboratoire ainsi que des procédures de nettoyage pour la verrerie, les réactifs, les adsorbants et les cartouches d'extraction. Des méthodes d'analyse par GC-MS des HAP dans les tissus des biotes sont également proposées par HELCOM (2012d) (annexe IX) et par le CIEM/OSPAR (2008) (annexe X).

3.5. Protocole pour la normalisation des concentrations de contaminants organiques par l'utilisation de la teneur en lipides

67. La normalisation de la teneur totale en lipides du biote marin est un moyen de réduire la variabilité du niveau de pollution. Pour les contaminants organiques qui s'accumulent dans les lipides des organismes par le biais de la séparation hydrophobe, les concentrations mesurées de contaminants dans les biotes peuvent être normalisées pour les poissons dont la teneur en lipides est de 5 % (Commission européenne, 2014¹⁷). Cette teneur en lipides par défaut de 5 % a été incorporée dans la ligne directrice 305 de l'OCDE (1996¹⁸) sur la bioconcentration afin d'assurer la comparabilité des résultats des essais de bioconcentration. Le raisonnement qui sous-tend cette normalisation lipidique est que la concentration dans le biote du corps entier est en corrélation linéaire avec la teneur en lipides de l'espèce (Commission européenne, 2014).

68. D'autres groupes taxonomiques, tels que les bivalves, ont des teneurs en lipides différentes de celles des poissons. Pour les bivalves marins, une teneur en lipides d'environ 1 % est proposée (Autorité européenne de sécurité des aliments, 2009¹⁹).

69. La normalisation des données mesurées en fonction de la teneur en lipides est décrite à l'annexe A.7 du document d'orientation n° 32 de la Commission européenne (Commission européenne, 2014) (annexe XI). La Commission européenne recommande que les concentrations de contaminants soient normalisées à des teneurs en lipides de 5 % dans les poissons et de 1 % dans les bivalves, ou à des teneurs en poids sec de 26 % dans les poissons et de 8,3 % dans les bivalves, sur la base de la teneur en lipides mesurée ou du poids sec, ou sur la base de valeurs génériques pour la

¹⁷ European Commission (2014). Common implementation strategy for the Water Framework Directive (2000/60/EC). Guidance Document No. 32 on Biota Monitoring (the Implementation of EQSbiota) under the Water Framework Directive. Technical Report - 2014 – 083.

¹⁸ OECD (1996). OECD Guidelines for Testing Chemicals: Proposal for Updating Guideline 305. Bioconcentration: Flow-Through Fish Test Paris 1996.

¹⁹ EFSA. 2009 Guidance Document on Risk Assessment for Birds and Mammals. Parma, Italy: European Food Safety Authority. Authority EFS.358 pp.

teneur en lipides ou le poids sec pour les espèces concernées, obtenues à partir du système mondial d'information sur les poissons FishBase²⁰.

70. Pour calculer les concentrations normalisées *concnorm, lipides* ou *concnorm, poids sec* à partir des concentrations mesurées *concmeas* pour une espèce de poisson x, les équations suivantes peuvent être utilisées (teneur en lipides et teneur en poids sec exprimées en fractions massiques) :

$$\text{concnorm, lipides} = \text{concmeas} - 0,05/\text{teneur en lipides}_x$$

ou

$$\text{concnorm, poids sec} = \text{concmeas} - 0,26/\text{poids sec}_x$$

71. De même, pour calculer les concentrations normalisées *concnorm, lipides* ou *concnorm, poids sec* à partir des concentrations mesurées *concmeas* pour une espèce de bivalve x, les équations suivantes peuvent être utilisées (teneur en lipide et teneur en poids sec exprimées en fractions massiques) :

$$\text{concnorm, lipides} = \text{concmeas} - 0,01/\text{teneur en lipides}_x$$

ou

$$\text{concnorm, poids sec} = \text{concmeas} - 0,083/\text{poids sec}_x$$

72. Il convient également de noter que l'utilisation de la teneur exacte en lipides ou du poids sec des échantillons de biote est toujours préférable à l'utilisation de valeurs génériques pour l'espèce (telles que celles disponibles sur FishBase).

73. La teneur totale en lipides des poissons ou des bivalves peut être déterminée par la méthode de Bligh et Dyer (1959²¹), utilisant des techniques d'extraction au chloroforme/méthanol (OCDE, 1996), ou par la méthode proposée par Smedes (1999²²), qui a une efficacité d'extraction comparable et une grande précision, mais qui utilise des solvants organiques moins toxiques [mélange propan-2-ol-cyclohexane-eau (8 + 10 + 11 en volume) pour éviter l'utilisation de chloroforme].

²⁰ FishBase: A global information system on fishes (www.fishbase.in)

²¹ Bligh EG, Dyer WJ: A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959, 37:911-917.

²² Smedes F (1999). Determination of total lipid using non-chlorinated solvents. Analyst, 124:1711-1718.

Annex I

IAEA (2012). Analysis of trace metals in biological and sediment samples: Laboratory procedure book (IAEA/Marine Environmental Studies Laboratory in co-operation with UNEP/ MAP MED POL) (4.1.1)



REPORT

Laboratory Procedure Book ANALYSIS OF TRACE METALS IN BIOLOGICAL AND SEDIMENT SAMPLES

**IAEA/NAEL Marine Environmental Studies Laboratory in co-operation with
UNEP/MAP MED POL**

November 2012

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Laboratory Procedure Book
ANALYSIS OF TRACE METALS IN BIOLOGICAL AND SEDIMENT SAMPLES

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I. MICROWAVE-OVEN DIGESTION PROCEDURES

I-1. DIGESTION OF SEDIMENT FOR THE DETERMINATION OF TRACE METAL

Principle:

The sediment samples are treated in closed Teflon vessels with hydrofluoric acid (HF) in combination with nitric acid, in order to decompose the samples. The use of HF is essential as it is the only acid that completely dissolves the silicate lattices and releases all the metals.

Reagents:

- HNO₃ (65%, Suprapur, Merck).
- HF (48%, analytical grade ISO, Merck).
- H₂O₂ (analytical grade), to be kept in the fridge after opening.
- Boric acid crystals, H₃BO₃ (analytical grade ISO, Merck).
- Milli-Q deionised water (> 18MΩ cm, Millipore).

Procedure:

1. Shake the sample bottles for about 2 min. for homogenization.
2. Wait a few minutes before opening the bottles.
3. Weigh accurately about 0.2 g of dry sample in labeled Teflon reactor (CEM)
4. Slowly add 5 ml of HNO₃ and 2 ml of concentrated hydrofluoric acid (HF). If the samples are strongly reactive, leave them at room temperature for at least 1 hour.
5. After room temperature digestion add 2 ml of H₂O₂
6. Close the reactor and put them in a microwave oven.
7. Set up the correct program:

Step	Power (W)	% Power	Ramp time (min sec)	PSI	°C	Hold time (min sec)
1	1200	100	10.00	600	190	12.00

8. Allow for the samples to cool to room temperature, release pressure carefully by opening the valve, and then open the reactor.
9. Weigh 0.8 g of boric acid into a polyethylene weighing boat, transfer it to the reactor, then add about 15 ml of Milli-Q water

10. Close the reactor and put them in a microwave oven.

11. Set up the correct program:

Step	Power (W)	% Power	Ramp time (min sec)	PSI	°C	Hold time (min sec)
1	1200	100	10.00	600	170	12.00

12. Allow for the samples to cool to room temperature, release pressure carefully by opening the valve, and then open the reactor.

13. Label some polyethylene 50 ml tubes and record the weight of the empty tubes.

14. Transfer the samples into 50 ml polypropylene graduated tubes. Rinse the Teflon reactor with Milli-Q water 3 times.

15. Shake the tubes.

16. Allow them to cool and then dilute to the mark (50 ml) with Milli-Q water, weigh each tube and record the weight.

Reagent blanks:

At least 2 blanks should be prepared for each batch of analysis. They are prepared in a similar manner as the samples, except that no sample is added to the digestion vessels.

Reference materials:

At least one certified reference material should be used and prepared in duplicate for each digestion batch. These digestions are prepared in a similar manner as the samples. A reference material of similar composition and concentration range should be used.

I-2. DIGESTION OF BIOTA FOR THE DETERMINATION OF TRACE METAL

Principle:

The biological samples are treated with concentrated nitric acid in order to decompose the samples and solubilize all metals.

Reagents:

- HNO₃ (65%, Suprapur, Merck).
- H₂O₂ (analytical grade) to be kept in the fridge after opening.
- Milli-Q deionised water (> 18MΩ cm, Millipore).

Procedure:

1. Shake the samples bottles for about 2 min. for homogenization.
2. Wait a few minutes before opening the bottles.
3. Weigh accurately about 0.2 g of dry sample in labeled Teflon reactor (CEM)
4. Add 5 ml of concentrated Nitric acid (HNO₃). Leave the samples at room temperature for at least 1 hour.
5. Add 2 ml of H₂O₂.
6. Close the reactor and place them in a microwave oven.
7. Run the appropriate program:

Step	Power (W)	% Power	Ramp time (min sec)	PSI	°C	Hold time (min sec)
1	1200	100	5 00	600	50	5 00
2	1200	100	5 00	600	100	5 00
3	1200	100	10 00	600	200	8 00

8. Allow for the samples to cool to room temperature, release pressure carefully by opening the valve, and then open the reactor.
9. Label some polyethylene 50 ml tubes and record the weight of the empty tubes.
10. Transfer samples into the labeled 50 ml polypropylene graduated tubes. Rinse the Teflon tubes with Milli-Q water 3 times.
11. Dilute to the mark (50 ml) with Milli-Q water and shake.

12. Allow them to cool and then dilute to the mark (50 ml) with Milli-Q water, weigh each tube and record the weight.

Reagent blanks:

At least 2 blanks should be prepared for each batch of analysis. They are prepared in a similar manner as the samples, except that no sample is added to the digestion vessels.

Reference materials:

At least one certified reference material should be used and prepared in duplicate for each digestion batch. These digestions are prepared in a similar manner as the samples. A reference material of similar composition and concentration range should be used.

II. HOT PLATE DIGESTION PROCEDURES

II-1. DIGESTION OF SEDIMENT FOR THE DETERMINATION OF TRACE METAL

Principle:

The sediment samples are treated in closed Teflon vessels with hydrofluoric acid (HF) in combination with aqua regia, in order to decompose the samples. The use of HF is essential as it is the only acid that completely dissolves the silicate lattices and releases all the metals.

Reagents:

- HNO₃ (65%, Suprapur, Merck).
- HF (48%, analytical grade ISO, Merck).
- HCl (30%, Suprapur, Merck).
- Boric acid crystals, H₃BO₃ (analytical grade ISO, Merck).
- Milli-Q deionised water (> 18MΩ cm, Millipore).

Procedure:

1. Shake the sample bottles for about 2 min. for homogenization.
2. Wait a few minutes before opening the bottles.
3. Weigh accurately about 0.2 g of dry sample in labeled Teflon tubes (FEP, 50 ml, Nalgene)
4. Slowly add 1 ml of aqua regia (HNO₃: HCl, 1:3 v/v) and 6 ml of concentrated hydrofluoric acid (HF). Leave the samples at room temperature for at least 1 hour.
5. Close the tubes and place them in an aluminum block on a hot plate at 120°C for 2hrs 30min.
6. Weigh 2.70 g of boric acid into the labeled 50 ml polypropylene graduated tubes or volumetric flask, then add about 20 ml of Milli-Q water and shake.
7. Allow samples to cool to room temperature then open the tubes.
8. Transfer the samples into the 50 ml polypropylene graduated tubes (containing the boric acid). Rinse the Teflon tubes with Milli-Q water 3 times.
9. Put in ultrasonic bath (at 60°C) for at least 30 minutes, until all the boric acid is dissolved.

10. Allow them to cool to room temperature and then dilute to the mark (50 ml) with Milli-Q water. If using glass, transfer the solution in plastic container. Allow particles to settle before analysis.

Reagent blanks:

At least 2 blanks should be prepared for each batch of analysis. They are prepared in a similar manner as the samples, except that no sample is added to the digestion vials.

Reference materials:

At least one certified reference material should be used and prepared in duplicate for each digestion batch. These digestions are prepared in a similar manner as the samples. A reference material of similar composition and concentration range should be used.

II-2. DIGESTION OF BIOTA FOR THE DETERMINATION OF TRACE METAL

Principle:

The biological samples are treated with concentrated nitric acid, in order to decompose the samples and solubilize all metals.

Reagents:

- HNO₃ (65%, Suprapur, Merck).
- Milli-Q deionised water (> 18MΩ cm, Millipore).

Procedure:

1. Shake the samples bottles for about 2 min. for homogenization.
2. Wait a few minutes before opening the bottles.
3. Weigh accurately about 0.2 g of dry sample in labeled Teflon tubes (FEP, 50 ml, Nalgene)
4. Add 5 ml of concentrated Nitric acid (HNO₃). Leave samples at room temperature for at least 1 hour.
5. Close the tubes and place them in an aluminum block on a hot plate at 90°C for 3hrs.
6. Allow the samples to cool to room temperature then open the tubes carefully.
7. Transfer the samples in the labeled 50 ml polypropylene graduated tubes or volumetric flask. Rinse the Teflon tubes with Milli-Q water 3 times.
8. Dilute to the mark (50 ml) with Milli-Q water and shake. If using glass transfer the solution in plastic container.

Reagent blanks:

At least 2 blanks should be prepared for each batch of analysis. They are prepared in a similar manner as samples, except that no sample is added to the digestion vials.

Reference materials:

At least one certified reference material should be used and prepared in duplicate for each digestion batch. These digestions are prepared in a similar manner as the samples. A reference material of similar composition and concentration range should be used.

II-3. DIGESTION OF BIOTA OR SEDIMENT FOR THE DETERMINATION OF TOTAL MERCURY BY Cold vapour-AAS

Principle:

The biological or sediment samples are treated with concentrated nitric acid, in order to decompose the samples and solubilize all metals.

Reagents:

- HNO₃ (65%, analytical grade, low in mercury, Merck).
- Milli-Q deionised water (> 18MΩ cm, Millipore).
- 10% K₂Cr₂O₇ (w/v) solution (e.g. 10 g K₂Cr₂O₇ diluted into 100 ml with Milli-Q water).

Procedure:

1. Shake the samples bottles for about 2 min. for homogenization.
2. Wait a few minutes before opening the bottles.
3. Number the Teflon tubes.
4. Weigh accurately about 0.2 g to 1.5 g of dry sample in Teflon tubes (FEP, 50 ml, Nalgene) depending of the expected concentration.
5. If processing plants or high weight of bivalve (> 1g), add 40 mg of V₂O₅ to each tube (including blanks).
6. Add 5 ml of concentrated Nitric acid (HNO₃). If large amount of sample is used add more acid until the mixture becomes liquid.
7. Leave the samples at room temperature for at least 1 hour.
8. Close the tubes and place them in an aluminum block on a hot plate at 90°C for 3hrs.
9. Allow for the samples to cool to room temperature then open the tubes carefully.
10. Add about 20 ml of Milli-Q water
11. Add 1 ml of K₂Cr₂O₇ solution (*NOTE*: final concentration should be 2% v/v).
12. Dilute to 50 ml preferably in Teflon, but glass is also good.

Reagent blanks:

At least 2 blanks should be prepared for each batch of analysis. They are prepared in a similar manner as the samples, except that no sample is added to the digestion vials.

Reference materials:

At least one certified reference material should be used and prepared in duplicate for each digestion batch. These digestions are prepared in a similar manner as the samples. A reference material of similar composition and concentration range should be used.

III. INSTRUMENTAL TECHNIQUES

III.1. PREPARATION OF METAL STANDARD SOLUTIONS FOR THE CALIBRATION CURVE

Principle:

The calibration curve must be made by at least 3 points (standard solutions of different concentration) plus a zero calibration. The concentration of the standard solutions must be calculated so that they bracket the concentrations of the samples and the Reference Materials.

If the concentration of the samples is unknown, the calibration curve will be centered on the Reference Materials. If the concentration of the samples exceeds the limit of the calibration curve, either the samples must be diluted to the appropriate concentration, or the calibration curve must be extended with a higher concentration standard. If, on the contrary, the concentration of the samples is lower than the lowest calibration curve's point, a new calibration curve must be prepared.

Reagent:

- Milli-Q deionised water (> 18MΩ cm, Millipore).
- Commercial standard solution 1000 µg ml⁻¹: Use a certified reference material solution; this solution should be accompanied by a certificate stipulating at the minimum the traceability of the certified concentration as well as the expiration date. The density of the solution or the certified content in mg kg⁻¹ should also be defined.
- Acid solutions used for sample preparation.

Standards preparation:

1. Put approximately 10 ml of Milli-Q water into clean polypropylene tubes (50 ml)
2. MATRIX MATCH the standards: add reagents in order to obtain a similar matrix as in the samples. Ex: for BIOTA: 5 ml of concentrated nitric acid and 2 ml of H₂O₂. For SEDIMENTS (hot plate digestion): 2.7 g Boric acid, 1 ml of aqua regia, 6 ml of HF.
3. Add the appropriate quantity of standard solution with a micropipette.
4. Dilute to the mark (50 ml) with Milli-Q water.
5. Shake well.

External Calibration Verification (ECV):

In order to check the accuracy of the prepared curve an independent standard is prepared. The concentration of this ECV should be in the calibration curve. This solution is prepared as describe above but using a second source of stock standard solution.

NOTE:

Some standard producers are selling specific multi-element solution for ECV purpose.

III-2. DETERMINATION OF TRACE METALS IN SEDIMENT AND BIOLOGICAL MATERIALS BY GF-AAS

Principle:

The samples are digested with strong acids (see Digestion Procedures).

For graphite furnace (GF) AAS, an aliquot of sample solution (10-50 µl) is introduced into a graphite tube of the GF-AAS and atomized by rapid heating at high temperature. A light beam is directed through the graphite tube, into a monochromator, and onto a detector that measures the amount of light absorbed by the atomized element in the tube. Each metal has its own characteristic wavelength, so a source hollow cathode lamp composed of that element is used. The amount of energy absorbed at the characteristic wavelength is proportional to the concentration of the element in the sample.

Reagents:

- Argon.
- Standard solution of the element of interest 1000 mg l⁻¹ (Merck).
- Milli-Q deionized water (>18 MΩ cm, Millipore).

Materials:

- Volumetric material, polypropylene tubes with caps (50 ml) cleaned according to Cleaning Procedures or glass volumetric flask and plastic container (for transferring).
- Atomic Absorption Spectrometer.
- Micropipettes.
- Polypropylene cups for automatic sampler.

Reagent solutions:

Metal standard solutions for the calibration curve: (See procedure III.1)

1. Put approximately 10 ml of Milli-Q water into clean polypropylene tubes (50 ml) or in volumetric flasks.
2. Add reagents in order to obtain a similar matrix as the sample (e.g. if sample is in 10% nitric acid add 5 ml of nitric acid).

3. Add the appropriate quantity of stock standard solution (1000 mg l⁻¹ or an intermediate stock standard) with a micropipette.
4. Dilute to the mark (50 ml) with Milli-Q water.
5. Shake well.
6. If glass is used then transfer the solution into a polypropylene container.

These solutions can be kept for a few days if stored in the refrigerator (+4°C).

Depending on the element, it may be necessary to make an intermediate stock standard solution. This intermediate stock is prepared as described above in a 2% HNO₃ matrix.

Before analysis:

Analytical conditions may change for each element, so it is necessary to first carefully read the relevant manufacturer manual before starting. Nowadays instrument software have integrated cookbook and already develop program to be used as starting point. Example of working conditions is given in table 1.

Determine the calibration curve according to the expected concentrations of the samples and the linearity of the AAS response for the element considered, software will usually provide recommended working range.

ANALYSIS BY GF-AAS

General operation:

1. Switch on the instrument (make sure the lamp of interest is on).
2. Make sure the rinsing the bottle is filled with fresh Milli-Q water (as this bottle is under argon pressure it should be disconnected before opening the gas).
3. Switch on argon and cooling system.
4. Open the furnace and take out the graphite tube.
5. Clean the inside, outside and quartz window with alcohol.
6. Install an appropriate graphite tube and close the furnace.
7. Optimize the lamp position and record the gain in the instrument logbook.
8. Install the auto sampler.
9. Make sure there is no air inside the syringe system.
10. Set up the capillary position (including length).
11. Run a “tube clean” cycle.

Operation when using a develop program:

Calibration curve:

The automatic sampler can make the calibration curve by mixing an appropriate volume of standard and zero calibration solutions, so only one standard solution needs to be prepared. It can be the highest standard solution of the calibration curve, or a solution more concentrated in case of standard additions. The solution must be chosen so that the volumes pipetted by the automatic sampler to make the standards are not lower than 2 µl. The calibration curve can also be prepared manually.

Sequence:

At least one blank, one reference material and one check standard (ECV, See procedure III.1) are measured before the samples, so it is possible to check that the system is under control before allowing the instrument to work automatically.

A reslope should be measured every 5 to 10 samples, as well as either a reference material or the ECV or a sample. This allows checking the accuracy of the reslope and the precision of the instrument over the run.

The instrument is recalibrated regularly (every 10-20 samples) to correct for instrumental drift and graphite tube efficiency.

Running a sequence:

1. Fill the carousel with samples, standard, zero calibration and matrix modifier if needed.
2. Select the program needed and carefully check all parameters (type of measurements, matrix modifier, the temperature program, reslope standard and rate, type of calibration, etc....)
3. Check that the number of fires from the graphite tube in use is low enough to allow for the full sequence to be run.
4. Program the auto sampler and the sequence.
5. Make an instrument zero.
6. Measure the zero calibration as a sample and record the absorbance in the logbook. It should be low or comparable with previous data.
7. Inject a known volume one standard solution, calculate the M_0 (quantity in pg to get a signal of 0.0044ABS) and record it in the logbook. Compare with previous records. Check the peak shape and the RSD of the reading (should be <5%).

$$M_{0(\text{pg})} = \frac{C_{\text{standard}} (\text{ng ml}^{-1}) \times Q_{\text{ut standard injected}} (\mu\text{l})}{\text{ABS standard}} \times 0.0044$$

8. Inject a reference material solution and check if the concentration is correct. Check the peak shape and the RSD of the reading (should be <5%).
9. Run the sequence.
10. Even if the instrument is all automatic, stay around to check the beginning of the sequence (calibration curve, procedure blank, reference material and check std ECV), and ideally return regularly to check the reslope, so that the sequence can be stopped if needed.

Minimum quality control checks

The ECV should be within 10% of the true value, in case of failure any results obtained after the last acceptable ECV should be rejected. The samples can be measured again after the ECV is under acceptable limit again (i.e. changing graphite tube, verifying calibration curve...)

The Zero calibration blanks measured during the run stay under acceptable limit (to be defined during the method validation), in case of failure the calibration should be redone and all results obtained after last acceptable blank should be re-measured.

The sample blanks measured during the run stay under acceptable limit (to be defined during the method validation), in case of failure all samples prepared along the failing blanks should be redone (prepared again).

The Certified Reference Material: At least one certified reference material of a representative matrix will be prepared with each batch of sample; the calculated result should fall in the value of the certificate and within the coverage uncertainty, to show evidence of unbiased result. The results for the CRM should be recorded for quality control purpose and plotted on a control chart

Verify the RSD of reading (<5%).

Check that all samples were within the concentration limits of the calibration curve. If not, take the appropriate action (dilution or new calibration curve) and restart the sequence.

Developing a program:

The AAS software generally gives typical electrothermal programs for each element for 10 µl of sample in diluted HNO₃ (0.1%) and indications concerning maximum ashing and atomization temperatures. More specific information may also be found in the literature, such as recommendations regarding matrix modifiers and the use of partition tubes or tubes with platform. Some examples of working conditions are listed in table 1.

When a program is optimized for the determination of an element in a specific matrix, all information should be reported in the logbook of methods of the laboratory, with all needed information such as:

- Matrix
- Type of tube
- Volume of injection
- Type of calibration (direct or standard addition)
- Matrix modifier used and quantity
- Examples of a typical sample and standard peak
- Maximum number of fires

A program is ideally optimized when:

The sensitivity is correct (comparable to the one in the literature)

The background is minimal

The peak shape is correct and comparable in the standard and the sample

It is possible to have a reference material of the same matrix and the same concentration as the sample, and the concentration found in the reference material is acceptable.

NOTE:

The optimization is done first on the sample solution (reference material can be a good one to start with).

Some software has the option of automatic program optimization where ashing and atomization temperature can be varied automatically, it is highly recommended to use those options with each new matrix or new element.

Optimization of drying stage:

The drop of sample should be dry before beginning the ashing stage to avoid boiling, which would spread the sample through the entire graphite tube.

A typical drying stage would bring the solution close to 100°C slowly, and then just above 100°C.

The drying is correct when no noise can be heard when ashing stage starts.

The signal can be measured from the beginning of the temperature program; if the drying stage is correctly set, no perturbations should be seen before ashing stage.

Optimization of ashing stage:

The ashing temperature should be set so that no element is lost.

This stage is separated into three steps: ramping (time to optimize), staying (time to optimize) and staying without gas (generally 2 seconds).

To find this optimal temperature, fix the atomization T° at the recommended T° and increase ashing T° by increments of 50°C until the absorbance decreases.

When the optimum T° is found, the time can be optimized the same way: increase the ashing time (ramp and stay) until the ratio between Abs and Background is maximum.

Matrix modifier:

For some elements and some matrices, the results obtained are still not satisfactory (e.g. maximum ashing T° is not sufficient to eliminate the background), this procedure should be redone with the addition of a matrix modifier. Different matrix modifiers could be tried before finding the best solution.

Often the matrix modifier solution is added to the injection (e.g. 2 or 4 µl for 20 µl injection). If the total volume of injection changes, it is necessary to check that the drying stage is still correct.

The absence of analyte of interest in the matrix modifier should be checked.

The main matrix modifiers are listed in section III.3.

The ashing temperature optimization protocol will be repeated with the addition of matrix modifier, to define the optimum temperature using a specific matrix modifier.

Optimization of atomization stage:

Before the atomization stage, the argon must be stopped. There are two steps in the atomization stage: ramping and staying. The read command should be on during these two-steps.

WARNING: if the Zeeman correction is on, the reading time cannot exceed 4s.

The T° of ashing is fixed at the T° found in the optimization procedure, and the atomization T° is increased. The best T° is the lowest one that gives the best signal.

The ramping should also be optimized.

Cleaning stage:

Add a cleaning stage after the atomization, by increasing the T° to 100-200°C and opening the argon. To increase the lifetime of the graphite tube, it is recommended to do this gradually, in two steps. First open the argon at 0.5 ml/min, and second open argon at maximum gas flow (3 ml/min).

Cooling stage:

It is highly recommended to impose the cooling stage to increase the lifetime of the graphite furnace. It can also be helpful to add a last step at injection T° for 2 or 3 second to stabilize the T° before the next injection.

Check for matrix effect:

When developing a program for a new matrix it is necessary to evaluate the accuracy of the method.

Each unknown type of samples should be spike to check for potential matrix effect.

This spike is considered as a single point standard addition, and should be performed with a minimum dilution factor. The recovery for spike calculated using equation 2 should be 85-115%. If this test fails, it is recommended to run analyses with standard addition method.

Spike solution: mix a fixed volume (V1) of the sample solution with a known volume (V2) of a standard solution of a known concentration (C_{standard}).

Unspike solution: mix the same fixed volume (V1) of the sample solution with the same volume (V2) of reagent water.

Measure concentration C (mg l⁻¹) in both solutions on the calibration curve, and calculate recovery as:

Equation 1
$$C_{spike} = \frac{C_{standard} \times V_2}{(V_1 + V_2)}$$

Equation 2
$$R = \frac{C_{Spike\ Solution} - C_{Unspike\ solution}}{C_{spike}} \times 100$$

To be valid, the concentrations of spike and unspike solutions should be in the linearity range of the calibration curve and Spike concentration (equation 1) should be in the range of 50-150% of the concentration of unspike solution.

When the program is ready, save all information and run it as described in the previous section.

Standard addition:

Main points to check before standard addition run:

Determine the linearity of the instrument.

Make sure the last point of the curve is in the linearity range (quantity of analyte in sample + quantity of analyte in last addition).

The zero addition should be above the DL. Generally the quantity of sample injected is smaller to permit the addition.

The curve should contain at least 3 points plus zero addition, adequately chosen. Best results will be obtained using additions representing 50, 100, 150 and 200% of the expected concentration of sample.

The standard addition curve should be done for each matrix; a fish should not be quantified on a mussel calibration curve!

Switching off the instrument

Print and save the results.

Verify that all needed information is recorded in the logbook.

Switch off the gas, cooling system and instrument.

Empty the carousel and the waste bottle.

Calculation:

The software can calculate the final concentrations. Alternatively, it can be done by hand using the following formula. If the same volume is always injected

$$C(\mu\text{g} / \text{g}) = \frac{(C_d - C_b) \times V \times F}{W}$$

Where:

C = Concentration of element in original sample ($\mu\text{g g}^{-1}$ dry weight);

C_d = Concentration of element in sample solution ($\mu\text{g ml}^{-1}$);

C_b = Mean concentration of element in reagent blanks ($\mu\text{g ml}^{-1}$);

V = Volume of dilution of digested solution (ml);

W = Dry weight of sample;

F = Dilution factor, if needed (=1 in case of no additional dilution other than that resulting from digestion procedure).

III-3. MATRIX MODIFIERS

1) AMMONIUM PHOSPHATE AND MAGNESIUM NITRATE

Make the following 2 solutions in ultra pure deionized water:

- $\text{NH}_4\text{H}_2\text{PO}_4$ (Suprapure, Merck) 25 g/l
- $\text{Mg}(\text{NO}_3)_2$ (Suprapure, Merck) 10 g/l

In a polyethylene cup (for AAS autosampler) make a solution with:

1000 μl $\text{NH}_4\text{H}_2\text{PO}_4$ solution

+ 50 μl $\text{Mg}(\text{NO}_3)_2$ solution

Add about 4 μl of modifier solution for 20 μl of sample.

2) PALLADIUM NITRATE AND MAGNESIUM NITRATE

SOLUTION (A): $\text{Pd}(\text{NO}_3)$ (0.2%)

$\text{Pd}(\text{NO}_3)$ pure (1g)

- In a Teflon beaker, dissolve 1 g of $\text{Pd}(\text{NO}_3)$ in aqua regia on a hot plate using a minimum amount of acid.
- Transfer into a 100 ml volumetric flask and complete to 100 ml with ultrapure deionized water. Keep this solution (1%) in the refrigerator (+4 °C).
- Dilute the $\text{Pd}(\text{NO}_3)$ solution (1%) with ultrapure deionized water to make a 0.2% solution:

Add 20 ml of solution in a 100 ml volumetric flask and complete to the volume.

- This 0.2% solution can be kept in the refrigerator (+4°C) for 6 months.

SOLUTION (B): $\text{Mg}(\text{NO}_3) 6\text{H}_2\text{O}$ (1%)

$\text{Mg}(\text{NO}_3) 6\text{H}_2\text{O}$ Suprapure, Merck

Make a 10 g/l solution in ultra pure deionized water.

SOLUTION A+B:

In a polyethylene cup (for AAS autosampler) make the following mixture every day of analysis:

800 μl $\text{Pd}(\text{NO}_3)$ (0.2 %) + 200 μl $\text{Mg}(\text{NO}_3) 6\text{H}_2\text{O}$ (1%)

Use about 4 µl of this solution for 20 µl sample.

3) PALLADIUM NITRATE, MAGNESIUM NITRATE AND AMMONIUM PHOSPHATE:

Make the following 2 solutions in ultrapure deionized water:

- $\text{NH}_4\text{H}_2\text{PO}_4$ (Suprapure, Merck) 25 g/l
- $\text{Mg}(\text{NO}_3)_2$ (Suprapure, Merck) 10 g/l

And a palladium nitrate solution (1%) as described in 2)

In a plastic container make the following mixture every day:

2 ml $\text{Pd}(\text{NO}_3)_2$ + 1 ml $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ + 400 µl $\text{NH}_4\text{H}_2\text{PO}_4$ + 6.6 ml of Milli-Q water.

Use about 4 µl of this solution for 20 µl sample.

4) Permanent modification with Iridium:

Use commercial solution of iridium 1000 µg ml⁻¹

- Inject 50 µl of the solution and run the temperature program below
- Repeat this 3 times
- The coating is stable for about 200 injections and can be repeated

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)
1	100	5	30
2	1200	20	5
3	100	5	2
4	2500	2	10

TABLE 1. EXAMPLES OF GRAPHITE FURNACE CONDITIONS

Element	Cu	Cu	Cd	Cd	Pb	Pb	As	As	Cr	Cr
Sample type	Sediment	Biota	Sediment	Biota	Sediment	Biota	Sediment	Biota	Sediment	Biota
Wavelength (nm)	327.4	327.4	228.8	228.8	283.3	283.3	193.7	193.7	357.9	357.9
Lamp current (mA)	4	4	4	4	5	5	10	10	7	7
Slit	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5R	0.5R
Graphite tube	Partition Tube	Partition Tube	platform	platform	platform	platform	platform	platform	Partition Tube	Partition Tube
Matrix Modifier	none	none	none	Pd, Mg, Amonium Phosphate	none	Pd, Mg, Amonium Phosphate	Pd, Mg	Pd, Mg	none	none
Peak Measurement	area	area	area	area	area	area	area	area	area	area
M ₀ (pg/0.0044 UA) on standard	13	13	1	1	16	16	15	15	2.5	2.5
Ashing T° (C°)	700	700	300	700	400	925	1400	1400	1100	1100
Atomisation T° (C°)	2300	2300	1800	1900	2100	2200	2600	2600	2600	2600
Remark							Data for Ultra Lamp only!! Number of Fire is critical	Data for Ultra Lamp only!! Standard Addition often required. Number of fire is critical	Use peak Height for lower concentration (peak shape)	Standard Addition often required. Use peak Height for lower concentration (peak shape)

III-4. DETERMINATION OF TRACE METALS IN SEDIMENT AND BIOLOGICAL MATERIALS BY FLAME-AAS

Principle:

The samples are digested with strong acids (see procedure). Atomic absorption spectrometry resembles emission flame photometry in the fact that the sample solution is aspirated into a flame and atomized. In case of flame-AAS, a light beam is directed through the flame, into a monochromator, and onto a detector that measures the amount of light absorbed by the element in the flame. Each metal has its own characteristic wavelength so a source hollow cathode lamp composed of that element is used. The amount of energy absorbed at the characteristic wavelength is proportional to the concentration of the element in the sample.

In case of flame emission, the amount of light emitted at the characteristic wavelength for the element analyzed is measured.

Reagents:

- Acetylene (pure quality).
- Air (pure quality).
- Standard solution of the element of interest 1000 mg l⁻¹ (Merck).
- Milli-Q deionised water (>18 MΩ cm, Millipore).

Material:

- Volumetric material, polypropylene tubes with caps (50 ml, Sarstedt), cleaned according to Cleaning Procedures or glass volumetric flask and plastic container (for transferring).
- AAS Varian Spectra-AA10.
- Micropipettes (Finnpipette).
- 1 polyethylene bottle (500 ml) for Milli-Q water.

Reagent solutions:

Metal standard solutions for the calibration curve (See procedure III-1):

1. Put about 10 ml of Milli-Q water into clean polypropylene tubes (50 ml) or in volumetric flask.

2. Add reagents in order to obtain a similar matrix as in the sample (e.g. if sample is in 10% nitric acid add 5 ml of nitric acid).
3. Add the appropriate quantity of stock standard solution (1000 mg l⁻¹ or an intermediate stock standard) with a micropipette.
4. Dilute to the mark (50 ml) with Milli-Q water.
5. Shake well.
6. If glass is used then transfer the solution into a polypropylene container.

These solutions can be kept for a few days if stored in the refrigerator (+4°C).

Depending on the element, it may be necessary to make an intermediate stock standard solution. This intermediate stock is prepared as described above in a 2% HNO₃ matrix.

Before analysis:

Analytical conditions may change for each element, so it is necessary to first carefully read the analytical methods book of the AAS before starting an analysis.

Determine the calibration curve according to the expected concentrations of the samples, and the linearity of the AAS response for the element considered (absorbance versus concentration curve given in the analytical methods book).

If ionization or interferences are likely, choose the right option according to the analytical method book, e.g. use of correction for non atomic absorption by using deuterium lamp background corrector, use of oxidizing air-acetylene flame; use of nitrous oxide-acetylene flame; addition of a releasing agent or ionization suppressant.

Prepare a standard solution in 2% HNO₃ for optimization and sensitivity check. The concentration is given in the method book (Concentration for 0.2 abs).

ANALYSIS BY FLAME-AAS:

Calibration curve:

Prepare standards with at least three concentrations plus zero. The zero calibration solution is prepared as other standard solutions without adding analyte.

If the samples are not within the calibration range, dilute them in the same matrix, or prepare a new calibration curve.

General operation:

1. Switch on the instrument and the gas.
2. Make sure the rinsing bottle is filled with fresh water.
3. Make sure the lamp of interest is on.
4. Before beginning optimization, wait approximately 15 minutes so that the lamp is stable.
5. Optimize the lamp position in order to get maximum energy. Record the gain in the logbook.
6. Use a card to optimize the burner position.
7. Switch the flame on.
8. Make instrument zero with **no solution**.
9. Aspirate the sensitivity standard solution.
10. Adjust the burner position slightly in order to get the maximum signal.

WARNING: make sure that the burner is not in the light !! The signal should be zero when no solution is aspirated.

11. Adjust flame composition in order to get the maximum signal.
12. Put the capillary back in the rinsing solution.

Running a sequence:

1. Make an instrument zero while aspirating **NO SOLUTION**.
2. **MEASURE THE ZERO CALIBRATION AS A SAMPLE** and record the absorbance in the logbook. It should be low or comparable with previous data. This should be done before calibration, because while the zero calibration is set up, the instrument automatically subtracts

it from all measurements! If the absorbance of the zero solution is high, it is necessary to check for the source of contamination before beginning an analysis.

3. Run a calibration curve.
4. At least one blank, one reference material and one check standard (ECV See procedure III-1) are measured before any samples, so that it is possible to verify that the system is under control before running the samples.
5. Run the samples, a zero calibration and reslope should be measured every 5 to 10 samples, as well as either a reference material or the ECV or a sample. This allows to check the accuracy of the reslope and the precision of the instrument over the run, as well as to see if the instrument is still under control.
6. During the run verify that the RSD between reading (abs) is below 5%, if it increases the nebulizer should be checked.

Switching off:

1. Save and print out results.
2. Rinse the flame with at least 500 ml of Milli-Q water (by aspirating)
3. Switch off the flame, the instrument and the computer
4. Empty the waste bottle
5. Switch off the gas

Calculation:

The software can calculate the final concentration. Alternatively, it can be done by hand using the following formula. If the same volume is always injected

$$C(\mu\text{g} / \text{g}) = \frac{(C_d - C_b) \times V \times F}{W}$$

Where:

C = Concentration of element in original sample ($\mu\text{g g}^{-1}$ dry weight);

C_d = Concentration of element in sample solution ($\mu\text{g ml}^{-1}$);

C_b = Mean concentration of element in reagent blanks ($\mu\text{g ml}^{-1}$);

V = Volume of dilution of digested solution (ml);

W = Dry weight of sample;

F = Dilution factor, if needed (=1 in case of no additional dilution other than that resulting from digestion procedure).

III-5. DETERMINATION OF TOTAL MERCURY IN SEDIMENT AND BIOLOGICAL SAMPLES BY VGA-AAS

Principle and application:

The sediment or biological samples are mineralized with strong acids. The inorganic mercury is reduced to its elemental form with stannous chloride. The cold mercury vapor is then passed through the quartz absorption cell of an AAS where its concentration is measured. The light beam of Hg hollow cathode lamp is directed through the quartz cell, into a monochromator and onto a detector that measures the amount of light absorbed by the atomized vapor in the cell. The amount of energy absorbed at the characteristic wavelength is proportional to the concentration of the element in the sample.

Reagents:

- HNO₃ (65%, analytical grade, low in Hg, Merck).
- K₂Cr₂O₇ (analytical grade, low in Hg, Merck).
- SnCl₂ (analytical grade, Merck).
- HCl (30%, Suprapur, Merck)
- HgCl₂ (salt, Merck) or standard Hg solution (1000 mg l⁻¹, Merck).
- Milli-Q deionised water (>18 MΩ cm, Millipore).
- Argon (pure quality).

Material:

- AAS Varian-Spectra AA-10 and VGA-76.
- Glass volumetric flasks from 50 to 1000 ml (Class A),
- Micropipettes (Finnpipette).

Reagent solutions:

20% w/v SnCl₂ in 20 % v/v HCl (200 ml):

1. Weigh accurately 40 g of SnCl₂ into a clean glass beaker using a plastic spatula (beaker and spatula are used only for SnCl₂).
2. Add 40 ml of concentrated HCl directly to the SnCl₂ and transfer to a 200 ml volumetric flask. Mix and wait for complete dissolution of SnCl₂.

3. Add Milli-Q water to the mark (200 ml).
4. With older stock of SnCl_2 it may be necessary to warm up the solution on a hot plate to obtain complete dissolution of SnCl_2 (do not allow to boil).
5. If SnCl_2 is found to be contaminated, it should be purged with nitrogen for 30 minutes before use.

This solution should be made fresh for each day of analysis.

NOTE:

All glassware used for preparation of SnCl_2 solution should be kept separately from remaining laboratory ware in order to avoid cross contamination of ware for trace element determination.

Nitric acid 10% v/v (500 ml):

1. Put about 400 ml of Milli-Q water into a 500 ml volumetric flask.
2. Add carefully 50 ml of concentrated nitric acid.
3. Make up to the mark with Milli-Q water.
4. Shake well.
5. This solution can be stored if kept in a tightly closed flask.

$\text{K}_2\text{Cr}_2\text{O}_7$ 10% (w/v) in Milli-Q water:

1. Weigh 50 g of $\text{K}_2\text{Cr}_2\text{O}_7$ into a clean 500 ml glass volumetric flask.
2. Add about 250 ml of Milli-Q water and shake until $\text{K}_2\text{Cr}_2\text{O}_7$ is dissolved.
3. Make up to the mark with Milli-Q water.

Mercury standards

Preferably use a commercial stock of Hg

Solution stock 1: 1 mg ml⁻¹ Hg in 10% nitric acid

1. Weigh exactly 1.354 g of HgCl_2 into a 1 liter glass volumetric flask.
2. Add about 500 ml of Milli-Q water.
3. Add 10 ml of concentrated nitric acid (low in Hg).

4. Complete to the mark with Milli-Q water
5. Shake well until complete dissolution is achieved.
6. Transfer into a 1 liter Teflon bottle.

Closed tightly with a torque wrench and keep in the refrigerator (+4° C).

Calibration curve (at least 3 standards and zero calibration) (See procedure III-1):

1. Put about 10 ml of Milli-Q water into a clean 50 ml glass volumetric (or plastic tube).
2. Add reagents as in the digested samples.
3. Add the appropriate quantity of stock standard solution (stock 1 or stock 2 depending on the samples concentrations) with a micropipette.
4. 1 ml of $K_2Cr_2O_7$ solution.
5. Dilute to the mark (50 ml) with Milli-Q water.
6. Shake well.

These solutions should be done fresh every day of analysis.

Sample digestion procedure:

It is strongly recommended to use the digestion procedure for Hg.

In case you use the digestion prepared by microwave oven for trace metal determination, it is strongly recommended that an aliquot of the solution be treated with 2% v/v $K_2Cr_2O_7$ solution as a preservative. Or that Hg is measured in the day following the digestion.

For sediment, the blank as to be checked as generally boric acid is not clean enough! It might be better to use Suprapur boric acid if mercury has to be measured in the sediment digestion solution.

ANALYSIS BY CV-AAS:

Calibration curve:

Prepare standard solutions with at least three standard concentrations plus one zero. The zero calibration is prepared as standard solutions without adding the mercury standard.

If the samples are not within the calibration curve, dilute them in the same matrix, or prepare a new calibration curve.

General operation:

1. Switch on the instrument.
2. Make sure the mercury lamp is on.
3. Before beginning optimization, wait approximately 15 minutes so that the lamp is stable.
4. Optimize the lamp position **without the cell** in order to get maximum energy. Record the gain in the logbook.
5. Optimize the burner position with the cell, the maximum energy should be read.
6. Make instrument zero.

Operation of the VGA:

1. Switch on the argon.
2. Put each of the 3 Teflon capillary tubes into the appropriate solutions:
 - a) SnCl₂ solution
 - b) Milli-Q water
 - c) Rinse solution (10% HNO₃)
3. Switch on the VGA and slowly tighten the pressure adjusting screw on the peristaltic pump until the liquids are pumped (do not over tighten as this will shorten the life of the pump tubes).
4. Check that there are no leaks.
5. Let the system running for about 10 min. in order to clean the system. Disconnect the black tube from the quartz absorption cell if the system has not been running for a while (to prevent contamination of the cell).

Running a sequence:

1. Make an instrument zero without connecting the VGA to the cell.
2. Connect the VGA to the cell.
3. Set up the delay time (about 50s for VGA Varian), this can be optimized under the optimized signal, aspirate a standard solution and measure the time needed to reach the maximum (stable) signal.

NOTE: this is for online determination system.

4. Measure **AS SAMPLE** the signal, obtained when only SnCl₂ and Milli-Q water are aspirating. It should be zero.
5. Measure **AS SAMPLE** the signal, obtained when all three solution are measured, it should be zero, so the next instrument zero can be done on that.
6. **MEASURE THE ZERO CALIBRATION AS A SAMPLE** and record the absorbance in the logbook. It should be low or comparable with previous data. This should be done before calibration, because while the zero calibration is set up, the instrument automatically subtracts it from all measurements! If the absorbance of the zero solution is high, it is necessary to check for the source of contamination before beginning an analysis.
7. Run a calibration curve.
8. At least one blank, one reference material and one check standard (ECV See procedure III-1) are measured before any samples, so that it is possible to verify that the system is under control before running the samples.
9. Run the samples, a zero calibration and reslope should be measured every 5 to 10 samples, as well as either a reference material or the ECV or a sample. This allows to check the accuracy of the reslope and the precision of the instrument over the run, as well as to see if the instrument is still under control.

Shutdown procedure:

1. Rinse all tubing with Milli-Q water for about 20 min. (make sure to keep separate the tube for the SnCl₂ solution from the other tubes).
2. Turn off the VGA system.
3. Release the tension from the tubing.
4. Turn off the gas and instrument.
5. Empty the waste bottle.

Calculation:

$$C(\mu\text{g} / \text{g}) = \frac{(C_d - C_b) \times V \times F}{W}$$

Where:

C = Concentration of total mercury in dry sample ($\mu\text{g g}^{-1}$ dry);

C_d = Concentration of mercury in sample solution ($\mu\text{g ml}^{-1}$);

C_b = Mean concentration of mercury in reagent blanks ($\mu\text{g ml}^{-1}$);

V = Volume of dilution of digested samples (ml)=57.5 ml;

W = Dry weight of sample (g);

F = Dilution factor, if needed (=1 in case of no additional dilution other than that resulting from digestion procedure).

IV. CLEANING PROCEDURES

IV-1. CLEANING GENERAL LABWARE FOR THE DETERMINATION OF TRACE ELEMENTS

Reagents:

- HNO₃ (65% analytical grade, ISO, Merck).
- Special laboratory detergent (Micro).
- Milli-Q deionised water (>18 MΩ cm, Millipore).

Procedure:

1. Allow the vessels to soak overnight in a plastic container in a soap solution (Micro solution 2% in tap water).
2. Rinse thoroughly first with tap water then with Milli-Q water.
3. Leave the vessels to stand in 10% (v/v) concentrated HNO₃ solution at room temperature for at least 6 days.
4. Rinse thoroughly with Milli-Q water (at least 4 times).
5. Allow the vessels to dry under a laminar flow hood.
6. Store the vessels in closed plastic polyethylene bags to prevent the risk of contamination prior to use.

This procedure should be used for all plastic ware use in the laboratory as tips, cup for autosampler, plastic container.....

IV-2. CLEANING OF DIGESTION TEFLON VESSELS FOR THE DETERMINATION OF TRACE ELEMENTS

Reagents:

- HNO₃ (65% analytical grade, ISO, Merck).
- HCl (25% analytical grade, Merck).
- Special laboratory detergent (Micro).
- Milli-Q deionised water (>18 MΩ cm, Millipore).

Procedure:

1. Soak the vessels (Teflon reactors, CEM) and their caps overnight in a detergent solution (Micro solution 2% in tap water) in a plastic container.
2. Rinse thoroughly first with tap water then with Milli-Q water.
3. Fill the Teflon reactor with 5 ml of HNO₃ (conc), close the reactor and put them in the microwave oven.
4. Set up the correct program:

Step	Power (W)	% Power	Ramp time (min sec)	PSI	°C	Hold time (min sec)
2	1200	100	10.00	600	100	5 00
3	1200	100	10 00	600	200	10.00

5. Allow the samples to cool to room temperature, release pressure carefully by opening the valve, and then open the reactor.
6. Empty the reactor (acid can be kept for some run of cleaning) and rinse them carefully with Milli-Q water.
7. Put them to dry under a laminar flow hood.
8. Once dry, the vessels should be closed and put into polyethylene bags to prevent the risk of contamination prior to use.

IV-3. CLEANING TEFLON LABWARE FOR THE DETERMINATION OF MERCURY AND METHYL MERCURY

Reagents:

- HNO₃ (65% analytical grade, ISO, Merck).
- HCl (25% analytical grade, Merck).
- HCl (30%, Suprapur, Merck).
- Special laboratory detergent (Micro).
- Milli-Q deionised water (>18 MΩ cm, Millipore).

Procedure:

1. Allow the vessels to soak overnight in a plastic container in a soap solution (Micro solution 2% in tap water).
2. Rinse thoroughly first with tap water then with Milli-Q water.
3. Put the vessels in 50% (v/v) concentrated HNO₃ solution and heat at 60° C for 2 days.
4. Rinse thoroughly with Milli-Q water (at least 4 times).
5. Transfer the vessels into 10% (v/v) concentrated HCl solution for a further 3 days (at least) at room temperature.
6. Rinse thoroughly with Milli-Q water (at least 4 times).
7. Allow the vessels to dry in a laminar flow hood.
8. All vessels are stored in polyethylene plastic bags. When possible (especially for Teflon bottles), the vessels are filled with 1% HCl (Suprapur, Merck) heated on a hot plate for one night and hermetically closed with a torque wrench.

IV-4. CLEANING LABWARE FOR THE DETERMINATION OF MERCURY BY VGA-CV-AAS; SIMPLIFIED PROCEDURE FOR TEFLON AND GLASSWARE

Reagents:

- HNO₃ (65% analytical grade, ISO, Merck).
- Special laboratory detergent (Micro).
- Milli-Q deionised water (>18 MΩ cm, Millipore).

Procedure:

1. Allow the vessels to soak overnight in a plastic container in a soap solution (Micro solution 2% in tap water).
2. Rinse thoroughly first with tap water then with Milli-Q water.
3. Fill the glass or Teflon vessels with 10% (v/v) concentrated HNO₃ solution.
4. Heat at 60°C for 2 days. In case of volumetric flasks, let stand for 6 days at room temperature.
5. Rinse thoroughly with Milli-Q water (at least 4 times).
6. Allow the vessels to dry in a laminar flow hood.
7. All vessels are stored in polyethylene plastic bags. Clean volumetric flasks are filled with Milli-Q water.

NOTE:

For contaminated labware, a precleaning step with 50% (v/v) concentrated HNO₃ solution should be used. In this case, steps 3) to 5) should be done twice: first with 50% acid solution, then with 10% acid solution.

Annex II.

HELCOM (2012). Manual for marine monitoring in the COMBINE programme. Annex B-12, Appendix 4: Technical note on the determination of trace metallic elements in biota (5.1.1)

HELCOM Manual for marine monitoring in the COMBINE programme

ANNEX B-12, APPENDIX 4: TECHNICAL NOTE ON THE DETERMINATION OF TRACE METALLIC ELEMENTS IN BIOTA

1. INTRODUCTION

Metallic elements appear in different marine biological matrices in trace concentrations, ranging from the mg/kg through the $\mu\text{g}/\text{kg}$ to the ng/kg level. Stoeppler (1991) provided a comprehensive review of the most frequently used techniques for quantitative analysis of metallic trace elements, such as optical atomic absorption, fluorescence or emission spectrometry, anodic, cathodic or adsorptive stripping voltammetry, isotope dilution mass spectrometry and total reflection X-ray fluorescence, respectively. In spite of the powerful instrumental techniques presently in use, various analytical error sources have to be taken into consideration that may significantly influence the accuracy of the analytical data.

2. WORKING CONDITIONS

For each step of the analytical procedure, contamination of the sample may occur from the environment (laboratory air dust particles and the analyst), from sample containers or packing materials, from instruments used during sample pre-treatment and sample preparation, and from the chemical reagents used for analysis. The predominant purpose of the analytical clean laboratory is to eliminate contamination, which may be airborne or laboratory-induced, as far as possible and to control the total analytical blank.

Contamination by particles from the laboratory air may be controlled by a high-efficiency particulate filter. (A clean room is designed to maintain air with 100 particles per ft^3 or 3.6.103 per m^3 of 0.5 μm particles (class 100 of U.S. Federal Standards 209), or better, preferably with a minimum of activity in the room.) U.S. Federal Standards 209 describes designs for complete laminar flow rooms, clean benches, and fume hoods, and contains information on design, testing, and maintenance of clean rooms, and should be considered an essential reference for those interested in a clean laboratory.

To control the analytical blank for analysis of metallic trace elements, one must not only maintain good laboratory air quality, but also select the appropriate composition and type of construction materials used to build the laboratory. Principally, contaminants must be effectively removed at the source to minimize their uncontrolled distribution in the analytical clean laboratory. Accordingly, the laboratory's walls should be cleaned easily and therefore painted with special metal-free wipe-resistant paints. Surfaces of working areas should be protected with, for example, disposable plastic (polyethylene, PTFE) foils. The floors should, for example, be covered with adhesive plastic mats. Details of the design that are essential for obtaining a working laboratory with low trace element blanks are described by Moody (1982), Mitchell (1982a), Boutron (1990), and Schmidt and Gerwinski (1994).

3. PRETREATMENT OF LABORATORY WARE AND REAGENTS, CONTAMINATION CONTROL

Chemically resistant materials, used in the production of high-quality laboratory ware appropriate for metallic trace element analysis, include low- and high-density polyethylene (LDPE and HDPE), polypropylene (PP), polytetrafluorethylene (PTFE), perfluoralkoxy (PFA), ethylenetetrafluorethylene (ETFE), tetrafluorethylenepoly- fluorpropylene (FEP), borosilicate and quartz glass, respectively. With appropriate pretreatment and handling, these materials meet the requirements of purity necessary for the required analytical investigations. Cleaning procedures for plastic and glass laboratory ware were comprehensively dealt with by Moody and Lindstrom (1977), Tschopel et al. (1980), Kosta (1982) and Boutron (1990). Generally, immersion in diluted (10-25 % v/v) high-purity nitric acid at room temperature for a period of one to three days, followed by repeated rinsing with high-purity water, is recommended. Steaming in high-purity acids (predominantly nitric acid) is also very effective to remove impurities from container surfaces and condition them for subsequent analysis.

The materials mentioned above for the production of laboratory ware exhibit some adsorptive or exchange properties. Boundary-surface interactions can be important, particularly when very dilute analytical solutions are being handled, since uncontrollable losses through sorption of element ions can occur (Tschopel et al., 1980; Harms, 1985). Based on this information, it is imperative that volumetric flasks, reagent vessels, pipette tips, etc., for handling samples, sample solutions and low-level reference or analyte solutions must never be used for transferring or processing stock calibration solutions, analytes solutions or concentrated reagents. Considerable quantities of analytes may be adsorbed from such solutions by the respective container surfaces, residuals of which may be leached later when dilute sample or analyte solutions are handled.

The availability of high-purity reagents is a key condition for reliable investigations of metallic trace element concentrations. For many analytical problems, the level of a specific contaminant can adequately be controlled only by applying specific purification methods. The first order of priority in regard to high-purity reagents is a sufficient supply of high-purity water. Ion-exchange units are universally accepted as an effective means of removing dissolved ionic species from water. Since high-purity water is frequently used in metallic trace element analysis, equipment for sustainable production of high-purity water by high-purity mixed-bed ion exchange resins should be available. The next most important group of reagents are mineral acids. Contamination of the sample by residual concentrations of metallic trace elements in the acids used for dissolution or decomposition represents a major problem. Purification of the acids is essential to ensure acceptable blanks. Isothermal (isopiestic) distillation can produce volatile acids (and ammonia) of medium concentration in high-purity form. For example, pure hydrochloric acid (and ammonia) can be generated by placing an open container of concentrated reagent-grade acid adjacent to a

container of high-purity water, within a closed system (such as a desiccator) at room temperature. Acid vapours are continuously transferred into the water until equilibrium is obtained. Purification by sub-boiling distillation is based on motionless evaporation of the liquid by infrared heating at the surface to prevent violent boiling. Different purification systems are described in detail by Matthinson (1972), Kuehner et al. (1972), Dabeka et al. (1976), Tschopel et al. (1980), Mitchell (1982b), Moody and Beary (1982), Moody et al. (1989), and Paulsen et al. (1989). Acids of extremely high purity are produced by multiple batchwise distillation of reagent-grade acids in a silica apparatus, which is placed in a laminar-flow hood.

4. SAMPLE PRETREATMENT

If the determinands are heterogeneously distributed in the sample material, it may be preferable to homogenize prior to taking subsamples for analysis. However, this procedural step is problematic, since uncontrollable contamination through the homogenizing tool may occur. Cryogenic homogenization at liquid nitrogen temperature and application of high-purity material such as quartz, PTFE, titanium or stainless steel for the construction of homogenizing devices may help to minimize contamination (Iyengar, 1976; Iyengar and Kasperek, 1977; Klusmann et al., 1985).

5. SAMPLE DECOMPOSITION

For accurate direct measurements of metallic trace element contents in biological matrices, appropriate calibration (reference) standards are lacking in most instances. Therefore, multi-stage, easy to calibrate methods are still necessary, which include decomposition procedures and transformation of biological material into solution.

As a general rule wet sample is to be subject to decomposition procedures to avoid contamination or loss of determinands. A general sample decomposition procedure cannot be recommended due to the diverse composition of materials to be analysed, as well as to the different elements to be determined, and also because of the variety of possible analytical methods applied. However, the following minimum requirements should be met:

- complete destruction of all organic material of the sample,
- avoidance of determinand losses,
- avoidance of contamination.

Complete decomposition of the organic matrix is a prerequisite for a variety of the subsequently used instrumental determination techniques. Residual dissolved organic carbon from biological materials incompletely disintegrated after decomposition with nitric acid causes problems particularly in voltammetric and polarographic determinations. Both are sensitive to interference from chelating and electroactive organic components coexisting in incompletely decomposed samples during analysis (Pratt et al., 1988; Wurfels

et al., 1987, 1989). Residual dissolved organic carbon compounds even of low molecular weight can change the equilibria in the spray chambers for sample introduction in atomic emission spectrometry (AES), optical emission spectrometry (OES), and atomic absorption spectrophotometry (AAS) by changing the viscosity of the sample solution. In such cases, comparison with pure aquatic calibration standard solutions can lead to erroneous results. In graphite furnace atomic absorption spectrophotometry (GFAAS), residual organic carbon may undergo complicated secondary reactions with the analyte prior to or during the atomization process. Such 'matrix interferences' alter the rate at which atoms enter the optical path relative to that obtained for an undisturbed element standard (Harms, 1985; and other references cited there).

The comparatively simple dry ashing method using a muffle furnace is problematic, since both uncontrollable losses of the determinands and contamination through contact with the furnace material may occur.

Both, application of a carefully developed and controlled temperature programme and modifying the matrix prior to the ashing procedure (addition of ashing aids agents) may be suitable to prevent losses of volatile elements (special analytical problems concerning mercury determination are described in Attachment 1). The use of special materials (quartz, titanium, stainless steel) for the construction of sample containers may be helpful to minimise contamination.

In the widely applied wet ashing procedure in open systems, the sample is treated with acids, mainly nitric, sulphuric and perchloric acids, in different ratios and under different conditions. Usually large quantities of reagents and voluminous apparatus with large surfaces are needed for complete destruction of the organic material. Serious contamination problems (too high blank values) may arise, if insufficiently purified acids are used.

The rate of reaction and efficiency of acid decomposition increase substantially with elevated temperatures. Accordingly, closed-vessel techniques, using conventional heating or microwave energy, have an advantage over open systems. As a result of the closed systems with vessels manufactured of dense and very pure material (PTFE, PFA, quartz), loss of elements through volatilisation and contamination by desorption of impurities from the vessel surface are significantly reduced. In addition, since only small quantities of high-purity acid (usually nitric acid) need to be used, extremely low analytical blanks can be obtained. Kingston and Jassie (1986, 1988) comprehensively considered the fundamental parameters governing closed vessel acid decomposition at elevated temperatures using a microwave radiation field. Microwave systems enable a very fast energy transfer to the sample and a very rapid build up of high internal vessel temperature and pressure, with the advantage of an enormous reduction in digestion time occurs. Furthermore, a reduction of acid volume

(McCarthy and Ellis, 1991) and contamination reduction during the decomposition process were found (Dunemann, 1994; Sheppard et al., 1994).

The application of microwave energy must be carefully controlled to avoid explosions; a pressure-relief system is recommended for safe operation (Gilman and Grooms, 1988). At this stage of development, it can be concluded that advances in pressure and temperature feedback control features have contributed to the acceptance of microwave sample decomposition in analytical chemistry.

6. CALIBRATION

For calibration purposes, single element standard stock solutions at a concentration of 1000 mg/l, purchased from a qualified manufacturer, should be available. The actual concentration of the named element should be stated on the label together with the date of the preparation of the standard solution.

Fresh stock standard solutions should be compared with the old standard solutions. Traceability can be ensured by the use of CRM(s) or participation in intercomparison exercises (EURACHEM, 2003).

Single or mixed element working standard solutions for calibration purposes are prepared by dilution of the standard stock solutions using dilute acid, as required.

Both stock standard and working standard solutions are stored in polyethylene, borosilicate or quartz volumetric flasks. Working standard solutions at concentrations less than 100 µg/l should be freshly prepared for every batch of samples and kept no longer than two weeks. The calibration procedure must meet some basic criteria in order to give the best estimate of the true (but unknown) element concentration of the sample analysed. These criteria are as follows:

- The amounts or concentrations of standards for the establishment of the calibration function must cover the range as related to practical conditions. The mean of the range should be roughly equal to the expected analyte concentration in the sample.
- The required analytical precision must be achievable and known throughout the entire range.
- The measured value (response) at the lower end of the range must be significantly different from the procedural analytical blank.
- The chemical and physical properties of the calibration standards must closely resemble those of the sample under investigation.
- The calibration standards must be processed through the entire analytical procedure in the same manner as the sample.

- The standard addition technique should be used only under very special circumstances (Cardone, 1986a, 1986b).

7. DETERMINATION

In an analytical series, especially with the number of samples >10, the control of calibration settings should be carried out with 2-3 calibration solution between environmental 10 samples. The analytical series should contain also a control sample of LRM or CRM.

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Annex III.

US EPA (1994) US-EPA Method 200.8: Determination of trace elements in waters and wastes by inductively coupled plasma-mass spectrometry (5.1.2)

METHOD 200.8

**DETERMINATION OF TRACE ELEMENTS IN WATERS AND WASTES
BY INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY**

**Revision 5.4
EMMC Version**

S.E. Long (Technology Applications Inc.), T.D. Martin, and E.R. Martin - Method 200.8,
Revisions 4.2 and 4.3 (1990)

S.E. Long (Technology Applications Inc.) and T.D. Martin - Method 200.8, Revision 4.4 (1991)

J.T. Creed, C.A. Brockhoff, and T.D. Martin - Method 200.8, Revision 5.4 (1994)

**ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268
METHOD 200.8**

200.8-1

DETERMINATION OF TRACE ELEMENTS IN WATERS AND WASTES BY INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY

1.0 SCOPE AND APPLICATION

- 1.1 This method provides procedures for determination of dissolved elements in ground waters, surface waters and drinking water. It may also be used for determination of total recoverable element concentrations in these waters as well as wastewaters, sludges and soils samples. This method is applicable to the following elements:

Analyte		Chemical Abstract Services Registry Number (CASRN)
Aluminum	(Al)	7429-90-5
Antimony	(Sb)	7440-36-0
Arsenic	(As)	7440-38-2
Barium	(Ba)	7440-39-3
Beryllium	(Be)	7440-41-7
Cadmium	(Cd)	7440-43-9
Chromium	(Cr)	7440-47-3
Cobalt	(Co)	7440-48-4
Copper	(Cu)	7440-50-8
Lead	(Pb)	7439-92-1
Manganese	(Mn)	7439-96-5
Mercury	(Hg)	7439-97-6
Molybdenum	(Mo)	7439-98-7
Nickel	(Ni)	7440-02-0
Selenium	(Se)	7782-49-2
Silver	(Ag)	7440-22-4
Thallium	(Tl)	7440-28-0
Thorium	(Th)	7440-29-1
Uranium	(U)	7440-61-1
Vanadium	(V)	7440-62-2
Zinc	(Zn)	7440-66-6

Estimated instrument detection limits (IDLs) for these elements are listed in Table 1. These are intended as a guide to instrumental limits typical of a system optimized for multielement determinations and employing commercial instrumentation and pneumatic nebulization sample introduction. However, actual method detection limits (MDLs) and linear working ranges will be dependent on the sample matrix, instrumentation and selected operating conditions. Given in Table 7 are typical MDLs for both total recoverable determinations by "direct analysis" and where sample digestion is employed.

- 1.2 For reference where this method is approved for use in compliance monitoring programs [e.g., Clean Water Act (NPDES) or Safe Drinking Water Act (SDWA)] consult both the appropriate sections of the Code of Federal Regulation (40 CFR Part 136 Table 1B for NPDES, and Part 141 § 141.23 for drinking water), and the latest Federal Register announcements.
- 1.3 Dissolved elements are determined after suitable filtration and acid preservation. In order to reduce potential interferences, dissolved solids should not exceed 0.2% (w/v) (Section 4.1.4).
- 1.4 With the exception of silver, where this method is approved for the determination of certain metal and metalloid contaminants in drinking water, samples may be analyzed directly by pneumatic nebulization without acid digestion if the samples have been properly preserved with acid and have turbidity of <1 NTU at the time of analysis. This total recoverable determination procedure is referred to as "direct analysis".
- 1.5 For the determination of total recoverable analytes in aqueous and solid samples a digestion/extraction is required prior to analysis when the elements are not in solution (e.g., soils, sludges, sediments and aqueous samples that may contain particulate and suspended solids). Aqueous samples containing suspended or particulate material $\geq 1\%$ (w/v) should be extracted as a solid type sample (Section 11.2.2).
- 1.6 The total recoverable sample digestion procedure given in this method is not suitable for the determination of volatile organo-mercury compounds. However, for "direct analysis" of drinking water (turbidity <1 NTU), the combined concentrations of inorganic and organo-mercury in solution can be determined by "direct analysis" pneumatic nebulization provided gold is added to both samples and standards alike to eliminate memory interference effects.
- 1.7 Silver is only slightly soluble in the presence of chloride unless there is a sufficient chloride concentration to form the soluble chloride complex. Therefore, low recoveries of silver may occur in samples, fortified sample matrices and even fortified blanks if determined as a dissolved analyte or by "direct analysis" where the sample has not been processed using the total recoverable mixed acid digestion. For this reason it is recommended that samples be digested prior to the determination of silver. The total recoverable sample digestion procedure given in this method is suitable for the determination of silver in aqueous samples containing concentrations up to 0.1 mg/L. For the analysis of wastewater samples containing higher concentrations of silver, succeeding smaller volume, well mixed sample aliquots must be prepared until the analysis solution contains <0.1 mg/L silver. The extraction of solid samples containing concentrations of silver >50 mg/kg should be treated in a similar manner.
- 1.8 The total recoverable sample digestion procedure given in this method will solubilize and hold in solution only minimal concentrations of barium in the presence of free sulfate. For the analysis of barium in samples having varying

and unknown concentrations of sulfate, analysis should be completed as soon as possible after sample preparation.

- 1.9 This method should be used by analysts experienced in the use of inductively coupled plasma mass spectrometry (ICP-MS), the interpretation of spectral and matrix interferences and procedures for their correction. A minimum of six months experience with commercial instrumentation is recommended.
- 1.10 Users of the method data should state the data-quality objectives prior to analysis. Users of the method must document and have on file the required initial demonstration performance data described in Section 9.2 prior to using the method for analysis.

2.0 SUMMARY OF METHOD

- 2.1 An aliquot of a well mixed, homogeneous aqueous or solid sample is accurately weighed or measured for sample processing. For total recoverable analysis of a solid or an aqueous sample containing undissolved material, analytes are first solubilized by gentle refluxing with nitric and hydrochloric acids. After cooling, the sample is made up to volume, is mixed and centrifuged or allowed to settle overnight prior to analysis. For the determination of dissolved analytes in a filtered aqueous sample aliquot, or for the "direct analysis" total recoverable determination of analytes in drinking water where sample turbidity is <1 NTU, the sample is made ready for analysis by the appropriate addition of nitric acid, and then diluted to a predetermined volume and mixed before analysis.
- 2.2 The method describes the multi-element determination of trace elements by ICP-MS.¹⁻³ Sample material in solution is introduced by pneumatic nebulization into a radiofrequency plasma where energy transfer processes cause desolvation, atomization and ionization. The ions are extracted from the plasma through a differentially pumped vacuum interface and separated on the basis of their mass-to-charge ratio by a quadrupole mass spectrometer having a minimum resolution capability of 1 amu peak width at 5% peak height. The ions transmitted through the quadrupole are detected by an electron multiplier or Faraday detector and the ion information processed by a data handling system. Interferences relating to the technique (Section 4.0) must be recognized and corrected for. Such corrections must include compensation for isobaric elemental interferences and interferences from polyatomic ions derived from the plasma gas, reagents or sample matrix. Instrumental drift as well as suppressions or enhancements of instrument response caused by the sample matrix must be corrected for by the use of internal standards.

3.0 DEFINITIONS

- 3.1 **Calibration Blank** - A volume of reagent water acidified with the same acid matrix as in the calibration standards. The calibration blank is a zero standard and is used to calibrate the ICP instrument (Section 7.6.1).

- 3.2 **Calibration Standard (CAL)** - A solution prepared from the dilution of stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration (Section 7.4).
- 3.3 **Dissolved Analyte** - The concentration of analyte in an aqueous sample that will pass through a 0.45 µm membrane filter assembly prior to sample acidification (Section 11.1).
- 3.4 **Field Reagent Blank (FRB)** - An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to the sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment (Section 8.5).
- 3.5 **Instrument Detection Limit (IDL)** - The concentration equivalent to the analyte signal which is equal to three times the standard deviation of a series of 10 replicate measurements of the calibration blank signal at the selected analytical mass(es). (Table 1).
- 3.6 **Internal Standard** - Pure analyte(s) added to a sample, extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes that are components of the same sample or solution. The internal standard must be an analyte that is not a sample component (Sections 7.5 and 9.4.5).
- 3.7 **Laboratory Duplicates (LD1 and LD2)** - Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicates precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.
- 3.8 **Laboratory Fortified Blank (LFB)** - An aliquot of LRB to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements (Sections 7.9 and 9.3.2).
- 3.9 **Laboratory Fortified Sample Matrix (LFM)** - An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations (Section 9.4).
- 3.10 **Laboratory Reagent Blank (LRB)** - An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, and internal standards that are used with other samples. The LRB is used to determine if method analytes or other interferences

are present in the laboratory environment, reagents, or apparatus (Sections 7.6.2 and 9.3.1).

- 3.11 **Linear Dynamic Range (LDR)** - The concentration range over which the instrument response to an analyte is linear (Section 9.2.2).
- 3.12 **Method Detection Limit (MDL)** - The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero (Section 9.2.4 and Table 7).
- 3.13 **Quality Control Sample (QCS)** - A solution of method analytes of known concentrations which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check either laboratory or instrument performance (Sections 7.8 and 9.2.3).
- 3.14 **Solid Sample** - For the purpose of this method, a sample taken from material classified as either soil, sediment or sludge.
- 3.15 **Stock Standard Solution** - A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source (Section 7.3).
- 3.16 **Total Recoverable Analyte** - The concentration of analyte determined either by "direct analysis" of an unfiltered acid preserved drinking water sample with turbidity of <1 NTU (Section 11.2.1), or by analysis of the solution extract of a solid sample or an unfiltered aqueous sample following digestion by refluxing with hot dilute mineral acid(s) as specified in the method (Sections 11.2 and 11.3).
- 3.17 **Tuning Solution** - A solution which is used to determine acceptable instrument performance prior to calibration and sample analyses (Section 7.7).
- 3.18 **Water Sample** - For the purpose of this method, a sample taken from one of the following sources: drinking, surface, ground, storm runoff, industrial or domestic wastewater.

4.0 INTERFERENCES

- 4.1 Several interference sources may cause inaccuracies in the determination of trace elements by ICP-MS. These are:
 - 4.1.1 **Isobaric elemental interferences** - Are caused by isotopes of different elements which form singly or doubly charged ions of the same nominal mass-to-charge ratio and which cannot be resolved by the mass spectrometer in use. All elements determined by this method have, at a minimum, one isotope free of isobaric elemental interference. Of the analytical isotopes recommended for use with this method (Table 4), only molybdenum-98 (ruthenium) and selenium-82 (krypton) have isobaric elemental interferences. If alternative analytical isotopes having higher

natural abundance are selected in order to achieve greater sensitivity, an isobaric interference may occur. All data obtained under such conditions must be corrected by measuring the signal from another isotope of the interfering element and subtracting the appropriate signal ratio from the isotope of interest. A record of this correction process should be included with the report of the data. It should be noted that such corrections will only be as accurate as the accuracy of the isotope ratio used in the elemental equation for data calculations. Relevant isotope ratios should be established prior to the application of any corrections.

- 4.1.2 Abundance sensitivity - Is a property defining the degree to which the wings of a mass peak contribute to adjacent masses. The abundance sensitivity is affected by ion energy and quadrupole operating pressure. Wing overlap interferences may result when a small ion peak is being measured adjacent to a large one. The potential for these interferences should be recognized and the spectrometer resolution adjusted to minimize them.
- 4.1.3 Isobaric polyatomic ion interferences - Are caused by ions consisting of more than one atom which have the same nominal mass-to-charge ratio as the isotope of interest, and which cannot be resolved by the mass spectrometer in use. These ions are commonly formed in the plasma or interface system from support gases or sample components. Most of the common interferences have been identified³, and these are listed in Table 2 together with the method elements affected. Such interferences must be recognized, and when they cannot be avoided by the selection of alternative analytical isotopes, appropriate corrections must be made to the data. Equations for the correction of data should be established at the time of the analytical run sequence as the polyatomic ion interferences will be highly dependent on the sample matrix and chosen instrument conditions. In particular, the common ⁸²Kr interference that affects the determination of both arsenic and selenium, can be greatly reduced with the use of high purity krypton free argon.
- 4.1.4 Physical interferences - Are associated with the physical processes which govern the transport of sample into the plasma, sample conversion processes in the plasma, and the transmission of ions through the plasma-mass spectrometer interface. These interferences may result in differences between instrument responses for the sample and the calibration standards. Physical interferences may occur in the transfer of solution to the nebulizer (e.g., viscosity effects), at the point of aerosol formation and transport to the plasma (e.g., surface tension), or during excitation and ionization processes within the plasma itself. High levels of dissolved solids in the sample may contribute deposits of material on the extraction and/or skimmer cones reducing the effective diameter of the orifices and therefore ion transmission. Dissolved solids levels not exceeding 0.2% (w/v) have been recommended³ to reduce such effects. Internal standardization may be effectively used to compensate for many physical interference effects.⁴ Internal standards ideally should have similar

analytical behavior to the elements being determined.

- 4.1.5 Memory interferences - Result when isotopes of elements in a previous sample contribute to the signals measured in a new sample. Memory effects can result from sample deposition on the sampler and skimmer cones, and from the buildup of sample material in the plasma torch and spray chamber. The site where these effects occur is dependent on the element and can be minimized by flushing the system with a rinse blank between samples (Section 7.6.3). The possibility of memory interferences should be recognized within an analytical run and suitable rinse times should be used to reduce them. The rinse times necessary for a particular element should be estimated prior to analysis. This may be achieved by aspirating a standard containing elements corresponding to 10 times the upper end of the linear range for a normal sample analysis period, followed by analysis of the rinse blank at designated intervals. The length of time required to reduce analyte signals to within a factor of 10 of the method detection limit, should be noted. Memory interferences may also be assessed within an analytical run by using a minimum of three replicate integrations for data acquisition. If the integrated signal values drop consecutively, the analyst should be alerted to the possibility of a memory effect, and should examine the analyte concentration in the previous sample to identify if this was high. If a memory interference is suspected, the sample should be reanalyzed after a long rinse period. In the determination of mercury, which suffers from severe memory effects, the addition of 100 µg/L gold will effectively rinse 5 µg/L mercury in approximately two minutes. Higher concentrations will require a longer rinse time.

5.0 SAFETY

- 5.1 The toxicity or carcinogenicity of reagents used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method.^{5,8} A reference file of material data handling sheets should also be available to all personnel involved in the chemical analysis. Specifically, concentrated nitric and hydrochloric acids present various hazards and are moderately toxic and extremely irritating to skin and mucus membranes. Use these reagents in a fume hood whenever possible and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield for eye protection, protective clothing and observe proper mixing when working with these reagents.
- 5.2 The acidification of samples containing reactive materials may result in the release of toxic gases, such as cyanides or sulfides. Acidification of samples should be done in a fume hood.
- 5.3 All personnel handling environmental samples known to contain or to have been

in contact with human waste should be immunized against known disease causative agents.

- 5.4 Analytical plasma sources emit radiofrequency radiation in addition to intense UV radiation. Suitable precautions should be taken to protect personnel from such hazards. The inductively coupled plasma should only be viewed with proper eye protection from UV emissions.
- 5.5 It is the responsibility of the user of this method to comply with relevant disposal and waste regulations. For guidance see Sections 14.0 and 15.0.

6.0 EQUIPMENT AND SUPPLIES

6.1 Inductively coupled plasma mass spectrometer:

- 6.1.1 Instrument capable of scanning the mass range 5-250 amu with a minimum resolution capability of 1 amu peak width at 5% peak height. Instrument may be fitted with a conventional or extended dynamic range detection system.

Note: If an electron multiplier detector is being used, precautions should be taken, where necessary, to prevent exposure to high ion flux. Otherwise changes in instrument response or damage to the multiplier may result.

- 6.1.2 Radio-frequency generator compliant with FCC regulations.
 - 6.1.3 Argon gas supply - High purity grade (99.99%). When analyses are conducted frequently, liquid argon is more economical and requires less frequent replacement of tanks than compressed argon in conventional cylinders (Section 4.1.3).
 - 6.1.4 A variable-speed peristaltic pump is required for solution delivery to the nebulizer.
 - 6.1.5 A mass-flow controller on the nebulizer gas supply is required. A water-cooled spray chamber may be of benefit in reducing some types of interferences (e.g., from polyatomic oxide species).
 - 6.1.6 If an electron multiplier detector is being used, precautions should be taken, where necessary, to prevent exposure to high ion flux. Otherwise changes in instrument response or damage to the multiplier may result. Samples having high concentrations of elements beyond the linear range of the instrument and with isotopes falling within scanning windows should be diluted prior to analysis.
- 6.2 Analytical balance, with capability to measure to 0.1 mg, for use in weighing solids, for preparing standards, and for determining dissolved solids in digests or extracts.

- 6.3 A temperature adjustable hot plate capable of maintaining a temperature of 95°C.
- 6.4 (Optional) A temperature adjustable block digester capable of maintaining a temperature of 95°C and equipped with 250 mL constricted digestion tubes.
- 6.5 (Optional) A steel cabinet centrifuge with guard bowl, electric timer and brake.
- 6.6 A gravity convection drying oven with thermostatic control capable of maintaining 105°C ± 5°C.
- 6.7 (Optional) An air displacement pipetter capable of delivering volumes ranging from 0.1-2500 µL with an assortment of high quality disposable pipet tips.
- 6.8 Mortar and pestle, ceramic or nonmetallic material.
- 6.9 Polypropylene sieve, 5-mesh (4 mm opening).
- 6.10 Labware - For determination of trace levels of elements, contamination and loss are of prime consideration. Potential contamination sources include improperly cleaned laboratory apparatus and general contamination within the laboratory environment from dust, etc. A clean laboratory work area designated for trace element sample handling must be used. Sample containers can introduce positive and negative errors in the determination of trace elements by (1) contributing contaminants through surface desorption or leaching, (2) depleting element concentrations through adsorption processes. All reusable labware (glass, quartz, polyethylene, PTFE, FEP, etc.) should be sufficiently clean for the task objectives. Several procedures found to provide clean labware include soaking overnight and thoroughly washing with laboratory-grade detergent and water, rinsing with tap water, and soaking for four hours or more in 20% (V/V) nitric acid or a mixture of dilute nitric and hydrochloric acid (1+2+9), followed by rinsing with reagent grade water and storing clean.

Note: Chromic acid must not be used for cleaning glassware.

- 6.10.1 Glassware - Volumetric flasks, graduated cylinders, funnels and centrifuge tubes (glass and/or metal free plastic).
- 6.10.2 Assorted calibrated pipettes.
- 6.10.3 Conical Phillips beakers (Corning 1080-250 or equivalent), 250 mL with 50 mm watch glasses.
- 6.10.4 Griffin beakers, 250 mL with 75 mm watch glasses and (optional) 75 mm ribbed watch glasses.
- 6.10.5 (Optional) PTFE and/or quartz beakers, 250 mL with PTFE covers.
- 6.10.6 Evaporating dishes or high-form crucibles, porcelain, 100 mL capacity.

6.10.7 Narrow-mouth storage bottles, FEP (fluorinated ethylene propylene) with ETFE (ethylene tetrafluorethylene) screw closure, 125-250 mL capacities.

6.10.8 One-piece stem FEP wash bottle with screw closure, 125 mL capacity.

7.0 **REAGENTS AND STANDARDS**

7.1 Reagents may contain elemental impurities that might affect the integrity of analytical data. Owing to the high sensitivity of ICP-MS, high-purity reagents should be used whenever possible. All acids used for this method must be of ultra high-purity grade. Suitable acids are available from a number of manufacturers or may be prepared by sub-boiling distillation. Nitric acid is preferred for ICP-MS in order to minimize polyatomic ion interferences. Several polyatomic ion interferences result when hydrochloric acid is used (Table 2), however, it should be noted that hydrochloric acid is required to maintain stability in solutions containing antimony and silver. When hydrochloric acid is used, corrections for the chloride polyatomic ion interferences must be applied to all data.

7.1.1 Nitric acid, concentrated (sp.gr. 1.41).

7.1.2 Nitric acid (1+1) - Add 500 mL conc. nitric acid to 400 mL of reagent grade water and dilute to 1 L.

7.1.3 Nitric acid (1+9) - Add 100 mL conc. nitric acid to 400 mL of reagent grade water and dilute to 1 L.

7.1.4 Hydrochloric acid, concentrated (sp.gr. 1.19).

7.1.5 Hydrochloric acid (1+1) - Add 500 mL conc. hydrochloric acid to 400 mL of reagent grade water and dilute to 1 L.

7.1.6 Hydrochloric acid (1+4) - Add 200 mL conc. hydrochloric acid to 400 mL of reagent grade water and dilute to 1 L.

7.1.7 Ammonium hydroxide, concentrated (sp.gr. 0.902).

7.1.8 Tartaric acid (CASRN 87-69-4).

7.2 Reagent water - All references to reagent grade water in this method refer to ASTM Type I water (ASTM D1193).⁹ Suitable water may be prepared by passing distilled water through a mixed bed of anion and cation exchange resins.

7.3 Standard Stock Solutions - Stock standards may be purchased from a reputable commercial source or prepared from ultra high-purity grade chemicals or metals (99.99-99.999% pure). All salts should be dried for one hour at 105°C, unless otherwise specified. Stock solutions should be stored in FEP bottles. Replace stock standards when succeeding dilutions for preparation of the multielement stock standards can not be verified.

CAUTION: Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.

The following procedures may be used for preparing standard stock solutions:

Note: Some metals, particularly those which form surface oxides require cleaning prior to being weighed. This may be achieved by pickling the surface of the metal in acid. An amount in excess of the desired weight should be pickled repeatedly, rinsed with water, dried and weighed until the desired weight is achieved.

- 7.3.1 Aluminum solution, stock 1 mL = 1000 µg Al: Pickle aluminum metal in warm (1+1) HCl to an exact weight of 0.100 g. Dissolve in 10 mL conc. HCl and 2 mL conc. nitric acid, heating to effect solution. Continue heating until volume is reduced to 4 mL. Cool and add 4 mL reagent grade water. Heat until the volume is reduced to 2 mL. Cool and dilute to 100 mL with reagent grade water.
- 7.3.2 Antimony solution, stock 1 mL = 1000 µg Sb: Dissolve 0.100 g antimony powder in 2 mL (1+1) nitric acid and 0.5 mL conc. hydrochloric acid, heating to effect solution. Cool, add 20 mL reagent grade water and 0.15 g tartaric acid. Warm the solution to dissolve the white precipitate. Cool and dilute to 100 mL with reagent grade water.
- 7.3.3 Arsenic solution, stock 1 mL = 1000 µg As: Dissolve 0.1320 g As₂O₃ in a mixture of 50 mL reagent grade water and 1 mL conc. ammonium hydroxide. Heat gently to dissolve. Cool and acidify the solution with 2 mL conc. nitric acid. Dilute to 100 mL with reagent grade water.
- 7.3.4 Barium solution, stock 1 mL = 1000 µg Ba: Dissolve 0.1437 g BaCO₃ in a solution mixture of 10 mL reagent grade water and 2 mL conc. nitric acid. Heat and stir to effect solution and degassing. Dilute to 100 mL with reagent grade water.
- 7.3.5 Beryllium solution, stock 1 mL = 1000 µg Be: Dissolve 1.965 g BeSO₄•4H₂O (DO NOT DRY) in 50 mL reagent grade water. Add 1 mL conc. nitric acid. Dilute to 100 mL with reagent grade water.
- 7.3.6 Bismuth solution, stock 1 mL = 1000 µg Bi: Dissolve 0.1115 g Bi₂O₃ in 5 mL conc. nitric acid. Heat to effect solution. Cool and dilute to 100 mL with reagent grade water.
- 7.3.7 Cadmium solution, stock 1 mL = 1000 µg Cd: Pickle cadmium metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.
- 7.3.8 Chromium solution, stock 1 mL = 1000 µg Cr: Dissolve 0.1923 g CrO₃ in a solution mixture of 10 mL reagent grade water and 1 mL conc. nitric

acid. Dilute to 100 mL with reagent grade water.

- 7.3.9 Cobalt solution, stock 1 mL = 1000 µg Co: Pickle cobalt metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.
- 7.3.10 Copper solution, stock 1 mL = 1000 µg Cu: Pickle copper metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.
- 7.3.11 Gold solution, stock 1 mL = 1000 µg Au: Dissolve 0.100 g high purity (99.9999%) Au shot in 10 mL of hot conc. nitric acid by dropwise addition of 5 mL conc. HCl and then reflux to expel oxides of nitrogen and chlorine. Cool and dilute to 100 mL with reagent grade water.
- 7.3.12 Indium solution, stock 1 mL = 1000 µg In: Pickle indium metal in (1+1) nitric acid to an exact weight of 0.100 g. Dissolve in 10 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.
- 7.3.13 Lead solution, stock 1 mL = 1000 µg Pb: Dissolve 0.1599 g PbNO₃ in 5 mL (1+1) nitric acid. Dilute to 100 mL with reagent grade water.
- 7.3.14 Magnesium solution, stock 1 mL = 1000 µg Mg: Dissolve 0.1658 g MgO in 10 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.
- 7.3.15 Manganese solution, stock 1 mL = 1000 µg Mn: Pickle manganese flake in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.
- 7.3.16 Mercury solution, stock, 1 mL = 1000 µg Hg: DO NOT DRY. **CAUTION:** highly toxic element. Dissolve 0.1354 g HgCl₂ in reagent water. Add 5.0 mL concentrated HNO₃ and dilute to 100 mL with reagent water.
- 7.3.17 Molybdenum solution, stock 1 mL = 1000 µg Mo: Dissolve 0.1500 g MoO₃ in a solution mixture of 10 mL reagent grade water and 1 mL conc. ammonium hydroxide., heating to effect solution. Cool and dilute to 100 mL with reagent grade water.
- 7.3.18 Nickel solution, stock 1 mL = 1000 µg Ni: Dissolve 0.100 g nickel powder in 5 mL conc. nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.
- 7.3.19 Scandium solution, stock 1 mL = 1000 µg Sc: Dissolve 0.1534 g Sc₂O₃ in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to

100 mL with reagent grade water.

- 7.3.20 Selenium solution, stock 1 mL = 1000 µg Se: Dissolve 0.1405 g SeO_2 in 20 mL ASTM Type I water. Dilute to 100 mL with reagent grade water.
- 7.3.21 Silver solution, stock 1 mL = 1000 µg Ag: Dissolve 0.100 g silver metal in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water. Store in dark container.
- 7.3.22 Terbium solution, stock 1 mL = 1000 µg Tb: Dissolve 0.1176 g Tb_4O_7 in 5 mL conc. nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.
- 7.3.23 Thallium solution, stock 1 mL = 1000 µg Tl: Dissolve 0.1303 g TlNO_3 in a solution mixture of 10 mL reagent grade water and 1 mL conc. nitric acid. Dilute to 100 mL with reagent grade water.
- 7.3.24 Thorium solution, stock 1 mL = 1000 µg Th: Dissolve 0.2380 g $\text{Th}(\text{NO}_3)_4 \cdot 4\text{H}_2\text{O}$ (DO NOT DRY) in 20 mL reagent grade water. Dilute to 100 mL with reagent grade water.
- 7.3.25 Uranium solution, stock 1 mL = 1000 µg U: Dissolve 0.2110 g $\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (DO NOT DRY) in 20 mL reagent grade water and dilute to 100 mL with reagent grade water.
- 7.3.26 Vanadium solution, stock 1 mL = 1000 µg V: Pickle vanadium metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.
- 7.3.27 Yttrium solution, stock 1 mL = 1000 µg Y: Dissolve 0.1270 g Y_2O_3 in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.
- 7.3.28 Zinc solution, stock 1 mL = 1000 µg Zn: Pickle zinc metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.
- 7.4 Multielement Stock Standard Solutions - Care must be taken in the preparation of multielement stock standards that the elements are compatible and stable. Originating element stocks should be checked for the presence of impurities which might influence the accuracy of the standard. Freshly prepared standards should be transferred to acid cleaned, not previously used FEP fluorocarbon bottles for storage and monitored periodically for stability. The following combinations of elements are suggested:

Standard Solution A		Standard Solution B
Aluminum	Mercury	Barium
Antimony	Molybdenum	Silver
Arsenic	Nickel	
Beryllium	Selenium	
Cadmium	Thallium	
Chromium	Thorium	
Cobalt	Uranium	
Copper	Vanadium	
Lead	Zinc	
Manganese		

Except for selenium and mercury, multielement stock standard solutions A and B (1 mL = 10 µg) may be prepared by diluting 1.0 mL of each single element stock standard in the combination list to 100 mL with reagent water containing 1% (v/v) nitric acid. For mercury and selenium in solution A, aliquots of 0.05 mL and 5.0 mL of the respective stock standards should be diluted to the specified 100 mL (1 mL = 0.5 µg Hg and 50 µg Se). Replace the multielement stock standards when succeeding dilutions for preparation of the calibration standards cannot be verified with the quality control sample.

7.4.1 Preparation of calibration standards - fresh multielement calibration standards should be prepared every two weeks or as needed. Dilute each of the stock multielement standard solutions A and B to levels appropriate to the operating range of the instrument using reagent water containing 1% (v/v) nitric acid. The element concentrations in the standards should be sufficiently high to produce good measurement precision and to accurately define the slope of the response curve. Depending on the sensitivity of the instrument, concentrations ranging from 10-200 µg/L are suggested, except mercury, which should be limited to ≤5 µg/L. It should be noted the selenium concentration is always a factor of 5 greater than the other analytes. If the direct addition procedure is being used (Method A, Section 10.3), add internal standards (Section 7.5) to the calibration standards and store in FEP bottles. Calibration standards should be verified initially using a quality control sample (Section 7.8).

7.5 Internal Standards Stock Solution - 1 mL = 100 µg. Dilute 10 mL of scandium, yttrium, indium, terbium and bismuth stock standards (Section 7.3) to 100 mL with reagent water, and store in a FEP bottle. Use this solution concentrate for addition to blanks, calibration standards and samples, or dilute by an appropriate amount using 1% (v/v) nitric acid, if the internal standards are being added by peristaltic pump (Method B, Section 10.3).

Note: If mercury is to be determined by the "direct analysis" procedure, add an aliquot of the gold stock standard (Section 7.3.11) to the internal standard solution sufficient to provide a concentration of 100 µg/L in final the dilution of all blanks, calibration standards, and samples.

- 7.6 Blanks - Three types of blanks are required for this method. A calibration blank is used to establish the analytical calibration curve, the laboratory reagent blank is used to assess possible contamination from the sample preparation procedure and to assess spectral background and the rinse blank is used to flush the instrument between samples in order to reduce memory interferences.
- 7.6.1 Calibration blank - Consists of 1% (v/v) nitric acid in reagent grade water. If the direct addition procedure (Method A, Section 10.3) is being used, add internal standards.
- 7.6.2 Laboratory reagent blank (LRB) - Must contain all the reagents in the same volumes as used in processing the samples. The LRB must be carried through the same entire preparation scheme as the samples including digestion, when applicable. If the direct addition procedure (Method A, Section 10.3) is being used, add internal standards to the solution after preparation is complete.
- 7.6.3 Rinse blank - Consists of 2% (v/v) nitric acid in reagent grade water.
- Note:** If mercury is to be determined by the "direct analysis" procedure, add gold (Section 7.3.11) to the rinse blank to a concentration of 100 µg/L.
- 7.7 Tuning Solution - This solution is used for instrument tuning and mass calibration prior to analysis. The solution is prepared by mixing beryllium, magnesium, cobalt, indium and lead stock solutions (Section 7.3) in 1% (v/v) nitric acid to produce a concentration of 100 µg/L of each element. Internal standards are not added to this solution. (Depending on the sensitivity of the instrument, this solution may need to be diluted 10-fold.)
- 7.8 Quality Control Sample (QCS) - The QCS should be obtained from a source outside the laboratory. The concentration of the QCS solution analyzed will depend on the sensitivity of the instrument. To prepare the QCS dilute an appropriate aliquot of analytes to a concentration ≤100 µg/L in 1% (v/v) nitric acid. Because of lower sensitivity, selenium may be diluted to a concentration of <500 µg/L, however, in all cases, mercury should be limited to a concentration of ≤5 µg/L. If the direct addition procedure (Method A, Section 10.3) is being used, add internal standards after dilution, mix and store in a FEP bottle. The QCS should be analyzed as needed to meet data-quality needs and a fresh solution should be prepared quarterly or more frequently as needed.
- 7.9 Laboratory Fortified Blank (LFB) - To an aliquot of LRB, add aliquots from multielement stock standards A and B (Section 7.4) to prepared the LFB. Depending on the sensitivity of the instrument, the fortified concentration used should range from 40-100 µg/L for each analyte, except selenium and mercury. For selenium the concentration should range from 200-500 µg/L, while the concentration range mercury should be limited to 2-5 µg/L. The LFB must be carried through the same entire preparation scheme as the samples including sample digestion, when applicable. If the direct addition procedure (Method A, Section 10.3) is being used, add internal standards to this solution after

preparation has been completed.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

- 8.1 Prior to the collection of an aqueous sample, consideration should be given to the type of data required, (i.e., dissolved or total recoverable), so that appropriate preservation and pretreatment steps can be taken. The pH of all aqueous samples **must** be tested immediately prior to aliquoting for processing or "direct analysis" to ensure the sample has been properly preserved. If properly acid preserved, the sample can be held up to 6 months before analysis.
- 8.2 For the determination of dissolved elements, the sample must be filtered through a 0.45 µm pore diameter membrane filter at the time of collection or as soon thereafter as practically possible. Use a portion of the sample to rinse the filter flask, discard this portion and collect the required volume of filtrate. Acidify the filtrate with (1+1) nitric acid immediately following filtration to pH <2.
- 8.3 For the determination of total recoverable elements in aqueous samples, samples are **not** filtered, but acidified with (1+1) nitric acid to pH <2 (normally, 3 mL of (1+1) acid per liter of sample is sufficient for most ambient and drinking water samples). Preservation may be done at the time of collection, however, to avoid the hazards of strong acids in the field, transport restrictions, and possible contamination it is recommended that the samples be returned to the laboratory within two weeks of collection and acid preserved upon receipt in the laboratory. Following acidification, the sample should be mixed, held for 16 hours, and then verified to be pH <2 just prior withdrawing an aliquot for processing or "direct analysis". If for some reason such as high alkalinity the sample pH is verified to be >2, more acid must be added and the sample held for 16 hours until verified to be pH <2. See Section 8.1.
- Note:** When the nature of the sample is either unknown or known to be hazardous, acidification should be done in a fume hood. See Section 5.2.
- 8.4 Solid samples require no preservation prior to analysis other than storage at 4°C. There is no established holding time limitation for solid samples.
- 8.5 For aqueous samples, a field blank should be prepared and analyzed as required by the data user. Use the same container and acid as used in sample collection.

9.0 QUALITY CONTROL

- 9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and calibration solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data thus generated.
- 9.2 Initial Demonstration of Performance (mandatory)

- 9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of linear calibration ranges and analysis of quality control samples) and laboratory performance (determination of method detection limits) prior to analyses conducted by this method.
- 9.2.2 Linear calibration ranges - Linear calibration ranges are primarily detector limited. The upper limit of the linear calibration range should be established for each analyte by determining the signal responses from a minimum of three different concentration standards, one of which is close to the upper limit of the linear range. Care should be taken to avoid potential damage to the detector during this process. The linear calibration range which may be used for the analysis of samples should be judged by the analyst from the resulting data. The upper LDR limit should be an observed signal no more than 10% below the level extrapolated from lower standards. Determined sample analyte concentrations that are greater than 90% of the determined upper LDR limit must be diluted and reanalyzed. The LDRs should be verified whenever, in the judgement of the analyst, a change in analytical performance caused by either a change in instrument hardware or operating conditions would dictate they be redetermined.
- 9.2.3 Quality control sample (QCS) - When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS (Section 7.8). To verify the calibration standards the determined mean concentration from three analyses of the QCS must be within $\pm 10\%$ of the stated QCS value. If the QCS is used for determining acceptable on-going instrument performance, analysis of the QCS prepared to a concentration of 100 $\mu\text{g/L}$ must be within $\pm 10\%$ of the stated value or within the acceptance limits listed in Table 8, whichever is the greater. (If the QCS is not within the required limits, an immediate second analysis of the QCS is recommended to confirm unacceptable performance.) If the calibration standards and/or acceptable instrument performance cannot be verified, the source of the problem must be identified and corrected before either proceeding on with the initial determination of method detection limits or continuing with on-going analyses.
- 9.2.4 Method detection limits (MDL) should be established for all analytes, using reagent water (blank) fortified at a concentration of two to five times the estimated detection limit.⁷ To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$\text{MDL} = (t) \times (S)$$

where:

- t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates]
- S = standard deviation of the replicate analyses

Note: If additional confirmation is desired, reanalyze the seven replicate aliquots on two more nonconsecutive days and again calculate the MDL values for each day. An average of the three MDL values for each analyte may provide for a more appropriate MDL estimate. If the relative standard deviation (RSD) from the analyses of the seven aliquots is <10%, the concentration used to determine the analyte MDL may have been inappropriately high for the determination. If so, this could result in the calculation of an unrealistically low MDL. Concurrently, determination of MDL in reagent water represents a best case situation and does not reflect possible matrix effects of real world samples. However, successful analyses of LFMs (Section 9.4) can give confidence to the MDL value determined in reagent water. Typical single laboratory MDL values using this method are given in Table 7.

The MDLs must be sufficient to detect analytes at the required levels according to compliance monitoring regulation (Section 1.2). MDLs should be determined annually, when a new operator begins work or whenever, in the judgement of the analyst, a change in analytical performance caused by either a change in instrument hardware or operating conditions would dictate they be redetermined.

9.3 Assessing Laboratory Performance (mandatory)

- 9.3.1 Laboratory reagent blank (LRB) - The laboratory must analyze at least one LRB (Section 7.6.2) with each batch of 20 or fewer of samples of the same matrix. LRB data are used to assess contamination from the laboratory environment and to characterize spectral background from the reagents used in sample processing. LRB values that exceed the MDL indicate laboratory or reagent contamination should be suspected. When LRB values constitute 10% or more of the analyte level determined for a sample or is 2.2 times the analyte MDL whichever is greater, fresh aliquots of the samples must be prepared and analyzed again for the affected analytes after the source of contamination has been corrected and acceptable LRB values have been obtained.
- 9.3.2 Laboratory fortified blank (LFB) - The laboratory must analyze at least one LFB (Section 7.9) with each batch of samples. Calculate accuracy as percent recovery using the following equation:

$$R = \frac{LFB - LRB}{s} \times 100$$

where:

- R = percent recovery
- LFB = laboratory fortified blank
- LRB = laboratory reagent blank
- s = concentration equivalent of analyte added to fortify the LBR solution

If the recovery of any analyte falls outside the required control limits of 85-115%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

- 9.3.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 85-115% (Section 9.3.2). When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the mean percent recovery (\bar{x}) and the standard deviation (S) of the mean percent recovery. These data can be used to establish the upper and lower control limits as follows:

$$\begin{aligned}\text{UPPER CONTROL LIMIT} &= \bar{x} + 3S \\ \text{LOWER CONTROL LIMIT} &= \bar{x} - 3S\end{aligned}$$

The optional control limits must be equal to or better than the required control limits of 85-115%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to establish an on-going precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

- 9.3.4 Instrument performance - For all determinations the laboratory must check instrument performance and verify that the instrument is properly calibrated on a continuing basis. To verify calibration run the calibration blank and calibration standards as surrogate samples immediately following each calibration routine, after every ten analyses and at the end of the sample run. The results of the analyses of the standards will indicate whether the calibration remains valid. The analysis of all analytes within the standard solutions must be within $\pm 10\%$ of calibration. If the calibration cannot be verified within the specified limits, the instrument must be recalibrated. (The instrument responses from the calibration check may be used for recalibration purposes, however, it must be verified before continuing sample analysis.) If the continuing calibration check is not confirmed within $\pm 15\%$, the previous 10 samples must be reanalyzed after recalibration. If the sample matrix is responsible for the calibration drift, it is recommended that the previous 10 samples are reanalyzed in groups of five between calibration checks to prevent a similar drift situation from occurring.

9.4 Assessing Analyte Recovery and Data Quality

- 9.4.1 Sample homogeneity and the chemical nature of the sample matrix can affect analyte recovery and the quality of the data. Taking separate aliquots from the sample for replicate and fortified analyses can in some cases assess the effect. Unless otherwise specified by the data user, laboratory or program, the following laboratory fortified matrix (LFM) procedure (Section 9.4.2) is required.
- 9.4.2 The laboratory must add a known amount of analyte to a minimum of 10% of the routine samples. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis and for total recoverable determinations added prior to sample preparation. For water samples, the added analyte concentration must be the same as that used in the laboratory fortified blank (Section 7.9). For solid samples, the concentration added should be 100 mg/kg equivalent (200 µg/L in the analysis solution) except silver which should be limited to 50 mg/kg (Section 1.8). Over time, samples from all routine sample sources should be fortified.
- 9.4.3 Calculate the percent recovery for each analyte, corrected for background concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range of 70-130%. Recovery calculations are not required if the concentration of the analyte added is less than 30% of the sample background concentration. Percent recovery may be calculated in units appropriate to the matrix, using the following equation:

$$R = \frac{C_s - C}{s} \times 100$$

where:

R = percent recovery

C_s = fortified sample concentration

C = sample background concentration

s = concentration equivalent of analyte added to fortify the sample

- 9.4.4 If recovery of any analyte falls outside the designated range and laboratory performance for that analyte is shown to be in control (Section 9.3), the recovery problem encountered with the fortified sample is judged to be matrix related, not system related. The data user should be informed that the result for that analyte in the unfortified sample is suspect due to either the heterogeneous nature of the sample or an uncorrected matrix effect.
- 9.4.5 Internal standards responses - The analyst is expected to monitor the responses from the internal standards throughout the sample set being

analyzed. Ratios of the internal standards responses against each other should also be monitored routinely. This information may be used to detect potential problems caused by mass dependent drift, errors incurred in adding the internal standards or increases in the concentrations of individual internal standards caused by background contributions from the sample. The absolute response of any one internal standard must not deviate more than 60-125% of the original response in the calibration blank. If deviations greater than these are observed, flush the instrument with the rinse blank and monitor the responses in the calibration blank. If the responses of the internal standards are now within the limit, take a fresh aliquot of the sample, dilute by a further factor of two, add the internal standards and reanalyze. If after flushing the response of the internal standards in the calibration blank are out of limits, terminate the analysis and determine the cause of the drift. Possible causes of drift may be a partially blocked sampling cone or a change in the tuning condition of the instrument.

10.0 CALIBRATION AND STANDARDIZATION

- 10.1 Operating conditions - Because of the diversity of instrument hardware, no detailed instrument operating conditions are provided. The analyst is advised to follow the recommended operating conditions provided by the manufacturer. It is the responsibility of the analyst to verify that the instrument configuration and operating conditions satisfy the analytical requirements and to maintain quality control data verifying instrument performance and analytical results. Instrument operating conditions which were used to generate precision and recovery data for this method (Section 13.0) are included in Table 6.
- 10.2 Precalibration routine - The following precalibration routine must be completed prior to calibrating the instrument until such time it can be documented with periodic performance data that the instrument meets the criteria listed below without daily tuning.
 - 10.2.1 Initiate proper operating configuration of instrument and data system. Allow a period of not less than 30 minutes for the instrument to warm up. During this process conduct mass calibration and resolution checks using the tuning solution. Resolution at low mass is indicated by magnesium isotopes 24, 25, and 26. Resolution at high mass is indicated by lead isotopes 206, 207, and 208. For good performance adjust spectrometer resolution to produce a peak width of approximately 0.75 amu at 5% peak height. Adjust mass calibration if it has shifted by more than 0.1 amu from unit mass.
 - 10.2.2 Instrument stability must be demonstrated by running the tuning solution (Section 7.7) a minimum of five times with resulting relative standard deviations of absolute signals for all analytes of less than 5%.
- 10.3 Internal Standardization - Internal standardization must be used in all analyses to correct for instrument drift and physical interferences. A list of acceptable

internal standards is provided in Table 3. For full mass range scans, a minimum of three internal standards must be used. Procedures described in this method for general application, detail the use of five internal standards; scandium, yttrium, indium, terbium and bismuth. These were used to generate the precision and recovery data attached to this method. Internal standards must be present in all samples, standards and blanks at identical levels. This may be achieved by directly adding an aliquot of the internal standards to the CAL standard, blank or sample solution (Method A, Section 10.3), or alternatively by mixing with the solution prior to nebulization using a second channel of the peristaltic pump and a mixing coil (Method B, Section 10.3). The concentration of the internal standard should be sufficiently high that good precision is obtained in the measurement of the isotope used for data correction and to minimize the possibility of correction errors if the internal standard is naturally present in the sample. Depending on the sensitivity of the instrument, a concentration range of 20-200 µg/L of each internal standard is recommended. Internal standards should be added to blanks, samples and standards in a like manner, so that dilution effects resulting from the addition may be disregarded.

- 10.4 Calibration - Prior to initial calibration, set up proper instrument software routines for quantitative analysis. The instrument must be calibrated using one of the internal standard routines (Method A or B) described in Section 10.3. The instrument must be calibrated for the analytes to be determined using the calibration blank (Section 7.6.1) and calibration standards A and B (Section 7.4.1) prepared at one or more concentration levels. A minimum of three replicate integrations are required for data acquisition. Use the average of the integrations for instrument calibration and data reporting.
- 10.5 The rinse blank should be used to flush the system between solution changes for blanks, standards and samples. Allow sufficient rinse time to remove traces of the previous sample (Section 4.1.5). Solutions should be aspirated for 30 seconds prior to the acquisition of data to allow equilibrium to be established.

11.0 PROCEDURE

11.1 Aqueous Sample Preparation - Dissolved Analytes

11.1.1 For the determination of dissolved analytes in ground and surface waters, pipet an aliquot (≥ 20 mL) of the filtered, acid preserved sample into a 50 mL polypropylene centrifuge tube. Add an appropriate volume of (1+1) nitric acid to adjust the acid concentration of the aliquot to approximate a 1% (v/v) nitric acid solution (e.g., add 0.4 mL (1+1) HNO₃ to a 20 mL aliquot of sample). If the direct addition procedure (Method A, Section 10.3) is being used, add internal standards, cap the tube and mix. The sample is now ready for analysis (Section 1.2). Allowance for sample dilution should be made in the calculations.

Note: If a precipitate is formed during acidification, transport, or storage, the sample aliquot must be treated using the procedure in Section 11.2 prior to analysis.

11.2 Aqueous Sample Preparation - Total Recoverable Analytes

11.2.1 For the "direct analysis" of total recoverable analytes in drinking water samples containing turbidity <1 NTU, treat an unfiltered acid preserved sample aliquot using the sample preparation procedure described in Section 11.1.1 while making allowance for sample dilution in the data calculation. For the determination of total recoverable analytes in all other aqueous samples or for preconcentrating drinking water samples prior to analysis follow the procedure given in Sections 11.2.2 through 11.2.8.

11.2.2 For the determination of total recoverable analytes in aqueous samples (other than drinking water with <1 NTU turbidity), transfer a 100 mL (± 1 mL) aliquot from a well mixed, acid preserved sample to a 250 mL Griffin beaker (Sections 1.2, 1.3, 1.7, and 1.8). (When necessary, smaller sample aliquot volumes may be used.)

Note: If the sample contains undissolved solids >1%, a well mixed, acid preserved aliquot containing no more than 1 g particulate material should be cautiously evaporated to near 10 mL and extracted using the acid-mixture procedure described in Sections 11.3.3 through 11.3.7.

11.2.3 Add 2 mL (1+1) nitric acid and 1.0 mL of (1+1) hydrochloric acid to the beaker containing the measured volume of sample. Place the beaker on the hot plate for solution evaporation. The hot plate should be located in a fume hood and previously adjusted to provide evaporation at a temperature of approximately but no higher than 85°C. (See the following note.) The beaker should be covered with an elevated watch glass or other necessary steps should be taken to prevent sample contamination from the fume hood environment.

Note: For proper heating adjust the temperature control of the hot plate such that an uncovered Griffin beaker containing 50 mL of water placed in the center of the hot plate can be maintained at a temperature approximately but no higher than 85°C. (Once the beaker is covered with a watch glass the temperature of the water will rise to approximately 95°C.)

11.2.4 Reduce the volume of the sample aliquot to about 20 mL by gentle heating at 85°C. DO NOT BOIL. This step takes about two hours for a 100 mL aliquot with the rate of evaporation rapidly increasing as the sample volume approaches 20 mL. (A spare beaker containing 20 mL of water can be used as a gauge.)

11.2.5 Cover the lip of the beaker with a watch glass to reduce additional evaporation and gently reflux the sample for 30 minutes. (Slight boiling may occur, but vigorous boiling must be avoided to prevent loss of the HCl-H₂O azeotrope.)

11.2.6 Allow the beaker to cool. Quantitatively transfer the sample solution to

a 50 mL volumetric flask or 50 mL class A stoppered graduated cylinder, make to volume with reagent water, stopper and mix.

- 11.2.7 Allow any undissolved material to settle overnight, or centrifuge a portion of the prepared sample until clear. (If after centrifuging or standing overnight the sample contains suspended solids that would clog the nebulizer, a portion of the sample may be filtered for their removal prior to analysis. However, care should be exercised to avoid potential contamination from filtration.)
- 11.2.8 Prior to analysis, adjust the chloride concentration by pipetting 20 mL of the prepared solution into a 50 mL volumetric flask, dilute to volume with reagent water and mix. (If the dissolved solids in this solution are >0.2%, additional dilution may be required to prevent clogging of the extraction and/or skimmer cones. If the direct addition procedure (Method A, Section 10.3) is being used, add internal standards and mix. The sample is now ready for analysis. Because the effects of various matrices on the stability of diluted samples cannot be characterized, all analyses should be performed as soon as possible after the completed preparation.

11.3 Solid Sample Preparation - Total Recoverable Analytes

- 11.3.1 For the determination of total recoverable analytes in solid samples, mix the sample thoroughly and transfer a portion (>20 g) to tared weighing dish, weigh the sample and record the wet weight (WW). (For samples with <35% moisture a 20 g portion is sufficient. For samples with moisture >35% a larger aliquot 50-100 g is required.) Dry the sample to a constant weight at 60°C and record the dry weight (DW) for calculation of percent solids (Section 12.6). (The sample is dried at 60°C to prevent the loss of mercury and other possible volatile metallic compounds, to facilitate sieving, and to ready the sample for grinding.)
- 11.3.2 To achieve homogeneity, sieve the dried sample using a 5-mesh polypropylene sieve and grind in a mortar and pestle. (The sieve, mortar and pestle should be cleaned between samples.) From the dried, ground material weigh accurately a representative 1.0 ± 0.01 g aliquot (W) of the sample and transfer to a 250 mL Phillips beaker for acid extraction.
- 11.3.3 To the beaker add 4 mL of (1+1) HNO₃ and 10 mL of (1+4) HCl. Cover the lip of the beaker with a watch glass. Place the beaker on a hot plate for reflux extraction of the analytes. The hot plate should be located in a fume hood and previously adjusted to provide a reflux temperature of approximately 95°C. (See the following note.)

Note: For proper heating adjust the temperature control of the hot plate such that an uncovered Griffin beaker containing 50 mL of water placed in the center of the hot plate can be maintained at a temperature approximately but no higher than 85°C. (Once the beaker is covered with a watch glass the temperature of the water will rise to approximately

95°C.) Also, a block digester capable of maintaining a temperature of 95°C and equipped with 250 mL constricted volumetric digestion tubes may be substituted for the hot plate and conical beakers in the extraction step.

- 11.3.4 Heat the sample and gently reflux for 30 minutes. Very slight boiling may occur, however vigorous boiling must be avoided to prevent loss of the HCl-H₂O azeotrope. Some solution evaporation will occur (3-4 mL).
- 11.3.5 Allow the sample to cool and quantitatively transfer the extract to a 100 mL volumetric flask. Dilute to volume with reagent water, stopper and mix.
- 11.3.6 Allow the sample extract solution to stand overnight to separate insoluble material or centrifuge a portion of the sample solution until clear. (If after centrifuging or standing overnight the extract solution contains suspended solids that would clog the nebulizer, a portion of the extract solution may be filtered for their removal prior to analysis. However, care should be exercised to avoid potential contamination from filtration.)
- 11.3.7 Prior to analysis, adjust the chloride concentration by pipetting 20 mL of the prepared solution into a 100 mL volumetric flask, dilute to volume with reagent water and mix. (If the dissolved solids in this solution are >0.2%, additional dilution may be required to prevent clogging of the extraction and/or skimmer cones. If the direct addition procedure (Method A, Section 10.3) is being used, add internal standards and mix. The sample extract is now ready for analysis. Because the effects of various matrices on the stability of diluted samples cannot be characterized, all analyses should be performed as soon as possible after the completed preparation.

Note: Determine the percent solids in the sample for use in calculations and for reporting data on a dry weight basis.

11.4 Sample Analysis

- 11.4.1 For every new or unusual matrix, it is highly recommended that a semi-quantitative analysis be carried out to screen the sample for elements at high concentration. Information gained from this may be used to prevent potential damage to the detector during sample analysis and to identify elements which may be higher than the linear range. Matrix screening may be carried out by using intelligent software, if available, or by diluting the sample by a factor of 500 and analyzing in a semi-quantitative mode. The sample should also be screened for background levels of all elements chosen for use as internal standards in order to prevent bias in the calculation of the analytical data.
- 11.4.2 Initiate instrument operating configuration. Tune and calibrate the instrument for the analytes of interest (Section 10.0).

- 11.4.3 Establish instrument software run procedures for quantitative analysis. For all sample analyses, a minimum of three replicate integrations are required for data acquisition. Use the average of the integrations for data reporting.
- 11.4.4 All masses which might affect data quality must be monitored during the analytical run. As a minimum, those masses prescribed in Table 4 must be monitored in the same scan as is used for the collection of the data. This information should be used to correct the data for identified interferences.
- 11.4.5 During the analysis of samples, the laboratory must comply with the required quality control described in Sections 9.3 and 9.4. Only for the determination of dissolved analytes or the "direct analysis" of drinking water with turbidity of <1 NTU is the sample digestion step of the LRB, LFB, and LFM not required.
- 11.4.6 The rinse blank should be used to flush the system between samples. Allow sufficient time to remove traces of the previous sample or a minimum of one minute (Section 4.1.5). Samples should be aspirated for 30 seconds prior to the collection of data.
- 11.4.7 Samples having concentrations higher than the established linear dynamic range should be diluted into range and reanalyzed. The sample should first be analyzed for the trace elements in the sample, protecting the detector from the high concentration elements, if necessary, by the selection of appropriate scanning windows. The sample should then be diluted for the determination of the remaining elements. Alternatively, the dynamic range may be adjusted by selecting an alternative isotope of lower natural abundance, provided quality control data for that isotope have been established. The dynamic range must not be adjusted by altering instrument conditions to an uncharacterized state.

12.0 DATA ANALYSIS AND CALCULATIONS

- 12.1 Elemental equations recommended for sample data calculations are listed in Table 5. Sample data should be reported in units of $\mu\text{g}/\text{L}$ for aqueous samples or mg/kg dry weight for solid samples. Do not report element concentrations below the determined MDL.
- 12.2 For data values less than 10, two significant figures should be used for reporting element concentrations. For data values greater than or equal to 10, three significant figures should be used.
- 12.3 For aqueous samples prepared by total recoverable procedure (Section 11.2), multiply solution concentrations by the dilution factor 1.25. If additional dilutions were made to any samples or an aqueous sample was prepared using the acid-mixture procedure described in Section 11.3, the appropriate factor should be applied to the calculated sample concentrations.

- 12.4 For total recoverable analytes in solid samples (Section 11.3), round the solution analyte concentrations ($\mu\text{g/L}$ in the analysis solution) as instructed in Section 12.2. Multiply the $\mu\text{g/L}$ concentrations in the analysis solution by the factor 0.005 to calculate the mg/L analyte concentration in the 100 mL extract solution. (If additional dilutions were made to any samples, the appropriate factor should be applied to calculate analyte concentrations in the extract solution.) Report the data up to three significant figures as mg/kg dry-weight basis unless specified otherwise by the program or data user. Calculate the concentration using the equation below:

$$\text{Sample Conc. (mg/kg)} = \frac{C \times V}{W}$$

dry-weight basis

where:

- C = Concentration in the extract (mg/L)
V = Volume of extract (L, 100 mL = 0.1L)
W = Weight of sample aliquot extracted ($\text{g} \times 0.001 = \text{kg}$)

Do not report analyte data below the estimated solids MDL or an adjusted MDL because of additional dilutions required to complete the analysis.

- 12.5 To report percent solids in solid samples (Sect. 11.3) calculate as follows:

$$\% \text{ solids (S)} = \frac{DW}{WW} \times 100$$

where:

- DW = Sample weight (g) dried at 60°C
WW = Sample weight (g) before drying

Note: If the data user, program or laboratory requires that the reported percent solids be determined by drying at 105°C , repeat the procedure given in Section 11.3 using a separate portion ($>20 \text{ g}$) of the sample and dry to constant weight at $103\text{-}105^\circ\text{C}$.

- 12.6 Data values should be corrected for instrument drift or sample matrix induced interferences by the application of internal standardization. Corrections for characterized spectral interferences should be applied to the data. Chloride interference corrections should be made on all samples, regardless of the addition of hydrochloric acid, as the chloride ion is a common constituent of environmental samples.
- 12.7 If an element has more than one monitored isotope, examination of the concentration calculated for each isotope, or the isotope ratios, will provide useful information for the analyst in detecting a possible spectral interference.

Consideration should therefore be given to both primary and secondary isotopes in the evaluation of the element concentration. In some cases, secondary isotopes may be less sensitive or more prone to interferences than the primary recommended isotopes, therefore differences between the results do not necessarily indicate a problem with data calculated for the primary isotopes.

- 12.8 The QC data obtained during the analyses provide an indication of the quality of the sample data and should be provided with the sample results.

13.0 METHOD PERFORMANCE

- 13.1 Instrument operating conditions used for single laboratory testing of the method are summarized in Table 6. Total recoverable digestion and "direct analysis" MDLs determined using the procedure described in Section 9.2.4, are listed in Table 7.
- 13.2 Data obtained from single laboratory testing of the method are summarized in Table 9 for five water samples representing drinking water, surface water, ground water and waste effluent. Samples were prepared using the procedure described in Section 11.2. For each matrix, five replicates were analyzed and the average of the replicates used for determining the sample background concentration for each element. Two further pairs of duplicates were fortified at different concentration levels. For each method element, the sample background concentration, mean percent recovery, the standard deviation of the percent recovery and the relative percent difference between the duplicate fortified samples are listed in Table 8.
- 13.3 Data obtained from single laboratory testing of the method are summarized in Table 10 for three solid samples consisting of SRM 1645 River Sediment, EPA Hazardous Soil and EPA Electroplating Sludge. Samples were prepared using the procedure described in Section 11.3. For each method element, the sample background concentration, mean percent recovery, the standard deviation of the percent recovery and the relative percent difference between the duplicate fortified samples were determined as for Section 13.2.
- 13.4 Data obtained from single laboratory testing of the method for drinking water analysis using the "direct analysis" procedure (Section 11.2.1) are given in Table 11. Three drinking water samples of varying hardness collected from Regions 4, 6, and 10 were fortified to contain 1 µg/L of all metal primary contaminants, except selenium, which was added to a concentration of 20 µg/L. For each matrix, four replicate aliquots were analyzed to determine the sample background concentration of each analyte and four fortified aliquots were analyzed to determine mean percent recovery in each matrix. Listed in the Table 11 are the average mean percent recovery of each analyte in the three matrices and the standard deviation of the mean percent recoveries.
- 13.5 Listed in Table 12 are the regression equations for precision and bias developed from the joint USEPA/Association of Official Analytical Chemists (AOAC) multilaboratory validation study conducted on this method. These equations

were developed from data received from 13 laboratories on reagent water, drinking water and ground water. Listed in Tables 13 and 14, respectively, are the precision and recovery data from a wastewater digestate supplied to all laboratories and from a wastewater of the participant's choice. For a complete review of the study see Reference 11, Section 16.0 of this method.

14.0 POLLUTION PREVENTION

- 14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.
- 14.2 For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Management for Waste Reduction", available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington D.C. 20036, (202)872-4477.

15.0 WASTE MANAGEMENT

- 15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult "The Waste Management Manual for Laboratory Personnel", available from the American Chemical Society at the address listed in the Section 14.2.

16.0 REFERENCES

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17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1: ESTIMATED INSTRUMENT DETECTION LIMITS

Element	Recommended Analytical Mass	Scanning Mode ¹	Selection Ion Monitoring Mode ^{2,3}
Aluminum	27	0.05	0.02
Antimony	123	0.08	0.008
Arsenic ⁽³⁾	75	0.9	0.02
Barium	137	0.5	0.03
Beryllium	9	0.1	0.02
Cadmium	111	0.1	0.02
Chromium	52	0.07	0.04
Cobalt	59	0.03	0.002
Copper	63	0.03	0.004
Lead	206, 207, 208	0.08	0.015
Manganese	55	0.1	0.007
Mercury	202	n.a	0.2
Molybdenum	98	0.1	0.005
Nickel	60	0.2	0.07
Selenium ⁽³⁾	82	5	1.3
Silver	107	0.05	0.004
Thallium	205	0.09	0.014
Thorium	232	0.03	0.005
Uranium	238	0.02	0.005
Vanadium	51	0.02	0.006
Zinc	66	0.2	0.07

Instrument detection limits (3σ) estimated from seven replicate integrations of the blank (1% v/v nitric acid) following calibration of the instrument with three replicate integrations of a multi-element standard.

¹Instrument operating conditions and data acquisition mode are given in Table 6.

²IDLs determined using state-of-the-art instrumentation (1994). Data for ⁷⁵As, ⁷⁷Se, and ⁸²Se were acquired using a dwell time of 4.096 seconds with 1500 area count per sec ⁸³Kr present in argon supply. All other data were acquired using a dwell time of 1.024 seconds per AMU monitored.

TABLE 2: COMMON MOLECULAR ION INTERFERENCES IN ICP-MS

BACKGROUND MOLECULAR IONS

Molecular Ion	Mass	Element Interference^a
NH ⁺	15	
OH ⁺	17	
OH ₂ ⁺	18	
C ₂ ⁺	24	
CN ⁺	26	
CO ⁺	28	
N ₂ ⁺	28	
N ₂ H ⁺	29	
NO ⁺	30	
NOH ⁺	31	
O ₂ ⁺	32	
O ₂ H ⁺	33	
³⁶ ArH ⁺	37	
³⁸ ArH ⁺	39	
⁴⁰ ArH ⁺	41	
CO ₂ ⁺	44	
CO ₂ H ⁺	45	Sc
ArC ⁺ , ArO ⁺	52	Cr
ArN ⁺	54	Cr
ArNH ⁺	55	Mn
ArO ⁺	56	
ArOH ⁺	57	
⁴⁰ Ar ³⁶ Ar ⁺	76	Se
⁴⁰ Ar ³⁸ Ar ⁺	78	Se
⁴⁰ Ar ⁺	80	Se

^amethod elements or internal standards affected by the molecular ions.

TABLE 2: COMMON MOLECULAR ION INTERFERENCES IN ICP-MS (Cont'd)

MATRIX MOLECULAR IONS		
Molecular Ion	Mass	Element Interference^a
Bromide¹²		
$^{81}\text{BrH}^+$	82	Se
$^{79}\text{BrO}^+$	95	Mo
$^{81}\text{BrO}^+$	97	Mo
$^{81}\text{BrOH}^+$	98	Mo
$\text{Ar}^{81}\text{Br}^+$	121	Sb
Chloride		
$^{35}\text{ClO}^+$	51	V
$^{35}\text{ClOH}^+$	52	Cr
$^{37}\text{ClO}^+$	53	Cr
$^{37}\text{ClOH}^+$	54	Cr
$\text{Ar}^{35}\text{Cl}^+$	75	As
$\text{Ar}^{37}\text{Cl}^+$	77	Se
Sulphate		
$^{32}\text{SO}^+$	48	
$^{32}\text{SOH}^+$	49	
$^{34}\text{SO}^+$	50	V, Cr
$^{34}\text{SOH}^+$	51	V
$\text{SO}_2^+, \text{S}_2^+$	64	Zn
Ar^{32}S^+	72	
Ar^{34}S^+	74	
Phosphate		
PO^+	47	
POH^+	48	
PO_2^+	63	Cu
ArP^+	71	
Group I, II Metals		
ArNa^+	63	Cu
ArK^+	79	
ArCa^+	80	

TABLE 2: COMMON MOLECULAR ION INTERFERENCES IN ICP-MS (Cont'd)

MATRIX MOLECULAR IONS		
Molecular Ion	Mass	Element Interference^a
Matrix Oxides [*]		
TiO	62-66	Ni, Cu, Zn
ZrO	106-112	Ag, Cd
MoO	108-116	Cd

^{*}Oxide interferences will normally be very small and will only impact the method elements when present at relatively high concentrations. Some examples of matrix oxides are listed of which the analyst should be aware. It is recommended that Ti and Zr isotopes are monitored in solid waste samples, which are likely to contain high levels of these elements. Mo is monitored as a method analyte.

TABLE 3: INTERNAL STANDARDS AND LIMITATIONS OF USE

Internal Standard	Mass	Possible Limitation
⁶ Lithium	6	a
Scandium	45	polyatomic ion interference
Yttrium	89	a,b
Rhodium	103	
Indium	115	isobaric interference by Sn
Terbium	159	
Holmium	165	
Lutetium	175	
Bismuth	209	a

a May be present in environmental samples.

b In some instruments Yttrium may form measurable amounts of YO⁺ (105 amu) and YOH⁺ (106 amu). If this is the case, care should be taken in the use of the cadmium elemental correction equation.

Internal standards recommended for use with this method are shown in bold face. Preparation procedures for these are included in Section 7.3.

TABLE 4: RECOMMENDED ANALYTICAL ISOTOPES AND ADDITIONAL MASSES WHICH MUST BE MONITORED

Isotope	Element of Interest
<u>27</u>	Aluminum
121, <u>123</u>	Antimony
<u>75</u>	Arsenic
135, <u>137</u>	Barium
<u>9</u>	Beryllium
106, 108, <u>111</u> , 114	Cadmium
<u>52</u> , 53	Chromium
<u>59</u>	Cobalt
<u>63</u> , 65	Copper
<u>206</u> , <u>207</u> , <u>208</u>	Lead
<u>55</u>	Manganese
95, 97, <u>98</u>	Molybdenum
<u>60</u> , 62	Nickel
77, <u>82</u>	Selenium
<u>107</u> , 109	Silver
<u>203</u> , <u>205</u>	Thallium
<u>232</u>	Thorium
<u>238</u>	Uranium
<u>51</u>	Vanadium
<u>66</u> , 67, 68	Zinc
83	Krypton
99	Ruthenium
105	Palladium
118	Tin

NOTE: Isotopes recommended for analytical determination are underlined.

TABLE 5: RECOMMENDED ELEMENTAL EQUATIONS FOR DATA CALCULATIONS

Element	Elemental Equation	Note
Al	(1.000) (²⁷ C)	
Sb	(1.000) (¹²³ C)	
As	(1.000) (⁷⁵ C)-(3.127) [(⁷⁷ C)-(0.815) (⁸² C)]	(1)
Ba	(1.000) (¹³⁷ C)	
Be	(1.000) (⁹ C)	
Cd	(1.000) (¹¹¹ C)-(1.073) [(¹⁰⁸ C)-(0.712) (¹⁰⁶ C)]	(2)
Cr	(1.000) (⁵² C)	(3)
Co	(1.000) (⁵⁹ C)	
Cu	(1.000) (⁶³ C)	
Pb	(1.000) (²⁰⁶ C)+(1.000) [(²⁰⁷ C)+(1.000) (²⁰⁸ C)]	(4)
Mn	(1.000) (⁵⁵ C)	
Mo	(1.000) (⁹⁸ C)-(0.146) (⁹⁹ C)	(5)
Ni	(1.000) (⁶⁰ C)	
Se	(1.000) (⁸² C)	(6)
Ag	(1.000) (¹⁰⁷ C)	
Tl	(1.000) (²⁰⁵ C)	
Th	(1.000) (²³² C)	
U	(1.000) (²³⁸ C)	
V	(1.000) (⁵¹ C)-(3.127) [(⁵³ C)-(0.113) (⁶² C)]	(7)
Zn	(1.000) (⁶⁶ C)	

TABLE 5: RECOMMENDED ELEMENTAL EQUATIONS FOR DATA CALCULATIONS

Element	Elemental Equation	Note
Bi	(1.000) (²⁰⁹ C)	
In	(1.000) (²⁰⁹ C)-(0.016) (¹¹⁸ C)	(8)
Sc	(1.000) (⁴⁵ C)	
Tb	(1.000) (¹⁵⁹ C)	
Y	(1.000) (⁸⁹ C)	

C - Calibration blank subtracted counts at specified mass.

(1) - Correction for chloride interference with adjustment for ⁷⁷Se. ArCl 75/77 ratio may be determined from the reagent blank. Isobaric mass 82 must be from Se only and not BrH⁺.

(2) - Correction for MoO interference. Isobaric mass 106 must be from Cd only not ZrO⁺. An additional isobaric elemental correction should be made if palladium is present.

(3) - In 0.4% v/v HCl, the background from ClOH will normally be small. However the contribution may be estimated from the reagent blank. Isobaric mass must be from Cr only not ArC⁺.

(4) - Allowance for isotopic variability of lead isotopes.

(5) - Isobaric elemental correction for ruthenium.

(6) - Some argon supplies contain krypton as an impurity. Selenium is corrected for ⁸²Kr by background subtraction.

(7) - Correction for chloride interference with adjustment for ⁵³Cr. ClO 51/53 ratio may be determined from the reagent blank. Isobaric mass 52 must be from Cr only not ArC⁺.

(8) - Isobaric elemental correction for tin.

TABLE 6: INSTRUMENT OPERATING CONDITIONS FOR PRECISION AND RECOVERY DATA¹

Instrument	VG PlasmaQuad Type I
Plasma forward power	1.35 kW
Coolant flow rate	13.5 L/min.
Auxillary flow rate	0.6 L/min.
Nebulizer flow rate	0.78 L/min.
Solution uptake rate	0.6 mL/min.
Spray chamber temperature	15°C
Data Acquisition	
Detector mode	Pulse counting
Replicate integrations	3
Mass range	8-240 amu
Dwell time	320 µs
Number of MCA channels	2048
Number of scan sweeps	85
Total acquisition time	3 minutes per sample

¹The described instrument and operating conditions were used to determine the scanning mode MDL data listed in Table 7 and the precision and recovery data given in Tables 9 and 10.

TABLE 7: METHOD DETECTION LIMITS

AMU	Element	Scanning Mode ¹		Selection Ion Monitoring Mode ²	
		Total Recoverable		Total Recoverable	Direct Analysis ³
		Aqueous µg/L	Solids mg/kg	Aqueous µg/L	Aqueous µg/L
27	Al	1.0	0.4	1.7	0.04
123	Sb	0.4	0.2	0.04	0.02
75	As	1.4	0.6	0.4	0.1
137	Ba	0.8	0.4	0.04	0.04
9	Be	0.3	0.1	0.02	0.03
111	Cd	0.5	0.2	0.03	0.03
52	Cr	0.9	0.4	0.08	0.08
59	Co	0.09	0.04	0.004	0.003
63	Cu	0.5	0.2	0.02	0.01
206,207,208	Pb	0.6	0.3	0.05	0.02
55	Mn	0.1	0.05	0.02	0.04
202	Hg	n.a.	n.a.	n.a	0.2
98	Mo	0.3	0.1	0.01	0.01
60	Ni	0.5	0.2	0.06	0.03
82	Se	7.9	3.2	2.1	0.5
107	Ag	0.1	0.05	0.005	0.005
205	Tl	0.3	0.1	0.02	0.01
232	Th	0.1	0.05	0.02	0.01
238	U	0.1	0.05	0.01	0.01
51	V	2.5	1.0	0.9	0.05
66	Zn	1.8	0.7	0.1	0.2

¹Data acquisition mode given in Table 6. Total recoverable MDL concentrations are computed for original matrix with allowance for sample dilution during preparation. Listed MDLs for solids calculated from determined aqueous MDLs.

²MDLs determined using state-of-the-art instrumentation (1994). Data for ⁷⁵As, ⁷⁷Se, and ⁸²Se were acquired using a dwell time of 4.096 seconds with 1500 area count per seconds ⁸³Kr present in argon supply. All other data were acquired using a dwell time of 1.024 seconds per AMU monitored.

³MDLs were determined from analysis of seven undigested aqueous sample aliquots.

n.a. - Not applicable. Total recoverable digestion not suitable for organo-mercury compounds.

TABLE 8: ACCEPTANCE LIMITS FOR QC CHECK SAMPLE
METHOD PERFORMANCE ($\mu\text{g/L}$)¹

Element	QC Check Sample Conc.	Average Recovery	Standard Deviation² (S_r)	Acceptance Limits³ $\mu\text{g/L}$
Aluminum	100	100.4	5.49	84-117
Antimony	100	99.9	2.40	93-107
Arsenic	100	101.6	3.66	91-113
Barium	100	99.7	2.64	92-108
Beryllium	100	105.9	4.13	88-112 ⁴
Cadmium	100	100.8	2.32	94-108
Chromium	100	102.3	3.91	91-114
Cobalt	100	97.7	2.66	90-106
Copper	100	100.3	2.11	94-107
Lead	100	104.0	3.42	94-114
Manganese	100	98.3	2.71	90-106
Molybdenum	100	101.0	2.21	94-108
Nickel	100	100.1	2.10	94-106
Selenium	100	103.5	5.67	86-121
Silver	100	101.1	3.29	91-111 ⁵
Thallium	100	98.5	2.79	90-107
Thorium	100	101.4	2.60	94-109
Uranium	100	102.6	2.82	94-111
Vanadium	100	100.3	3.26	90-110
Zinc	100	105.1	4.57	91-119

¹Method performance characteristics calculated using regression equations from collaborative study, Reference 11.

²Single-analyst standard deviation, S_r.

³Acceptance limits calculated as average recovery \pm three standard deviations.

⁴Acceptance limits centered at 100% recovery.

⁵Statistics estimated from summary statistics at 48 and 64 $\mu\text{g/L}$.

TABLE 9: PRECISION AND RECOVERY DATA IN AQUEOUS MATRICES

DRINKING WATER

Element	Sample	Low	Average			High	Average		
	Conc. µg/L	Spike µg/L	Recovery R (%)	S (R)	RPD	Spike µg/L	Recovery R (%)	S (R)	RPD
Al	175	50	115.8	5.9	0.4	200	102.7	1.6	1.1
Sb	<0.4	10	99.1	0.7	2.0	100	100.8	0.7	2.0
As	<1.4	50	99.7	0.8	2.2	200	102.5	1.1	2.9
Ba	43.8	50	94.8	3.9	5.8	200	95.6	0.8	1.7
Be	<0.3	10	113.5	0.4	0.9	100	111.0	0.7	1.8
Cd	<0.5	10	97.0	2.8	8.3	100	101.5	0.4	1.0
Cr	<0.9	10	111.0	3.5	9.0	100	99.5	0.1	0.2
Co	0.11	10	94.4	0.4	1.1	100	93.6	0.5	1.4
Cu	3.6	10	101.8	8.8	17.4	100	91.6	0.3	0.3
Pb	0.87	10	97.8	2.0	2.8	100	99.0	0.8	2.2
Mn	0.96	10	96.9	1.8	4.7	100	95.8	0.6	1.8
Mo	1.9	10	99.4	1.6	3.4	100	98.6	0.4	1.0
Ni	1.9	10	100.2	5.7	13.5	100	95.2	0.5	1.3
Se	<7.9	50	99.0	1.8	5.3	200	93.5	3.5	10.7
Ag	<0.1	50	100.7	1.5	4.2	200	99.0	0.4	1.0
Tl	<0.3	10	97.5	0.4	1.0	100	98.5	1.7	4.9
Th	<0.1	10	109.0	0.7	1.8	100	106.0	1.4	3.8
U	0.23	10	110.7	1.4	3.5	100	107.8	0.7	1.9
V	<2.5	50	101.4	0.1	0.4	200	97.5	0.7	2.1
Zn	5.2	50	103.4	3.3	7.7	200	96.4	0.5	1.0

S (R) Standard deviation of percent recovery.

RPD Relative percent difference between duplicate spike determinations.

< Sample concentration below established method detection limit.

TABLE 9: PRECISION AND RECOVERY DATA IN AQUEOUS MATRICES (Cont'd)

<u>WELL WATER</u>									
Element	Sample	Low	Average			High	Average		
	Conc. µg/L	Spike µg/L	Recovery R (%)	S (R)	RPD	Spike µg/L	Recovery R (%)	S (R)	RPD
Al	34.3	50	100.1	3.9	0.8	200	102.6	1.1	1.3
Sb	0.46	10	98.4	0.9	1.9	100	102.5	0.7	1.9
As	<1.4	50	110.0	6.4	16.4	200	101.3	0.2	0.5
Ba	106	50	95.4	3.9	3.3	200	104.9	1.0	1.6
Be	<0.3	10	104.5	0.4	1.0	100	101.4	1.2	3.3
Cd	106	10	88.6	1.7	3.8	100	98.6	0.6	1.6
Cr	<0.9	10	111.0	0.0	0.0	100	103.5	0.4	1.0
Co	2.4	10	100.6	1.0	1.6	100	104.1	0.4	0.9
Cu	37.4	10	104.3	5.1	1.5	100	100.6	0.8	1.5
Pb	3.5	10	95.2	2.5	1.5	100	99.5	1.4	3.9
Mn	2770	10	*	*	1.8	100	*	*	0.7
Mo	2.1	10	103.8	1.1	1.6	100	102.9	0.7	1.9
Ni	11.4	10	116.5	6.3	6.5	100	99.6	0.3	0.0
Se	<7.9	50	127.3	8.4	18.7	200	101.3	0.2	0.5
Ag	<0.1	50	99.2	0.4	1.0	200	101.5	1.4	3.9
Tl	<0.3	10	93.9	0.1	0.0	100	100.4	1.8	5.0
Th	<0.1	10	103.0	0.7	1.9	100	104.5	1.8	4.8
U	1.8	10	106.0	1.1	1.6	100	109.7	2.5	6.3
V	<2.5	50	105.3	0.8	2.1	200	105.8	0.2	0.5
Zn	554	50	*	*	1.2	200	102.1	5.5	3.2

S (R) Standard deviation of percent recovery.

RPD Relative percent difference between duplicate spike determinations.

< Sample concentration below established method detection limit.

* Spike concentration <10% of sample background concentration.

TABLE 9: PRECISION AND RECOVERY DATA IN AQUEOUS MATRICES (Cont'd)

POND WATER									
Element	Sample Conc.	Low Spike	Average Recovery			High Spike	Average Recovery		
	µg/L	µg/L	R (%)	S (R)	RPD	µg/L	R (%)	S (R)	RPD
Al	610	50	*	*	1.7	200	78.2	9.2	5.5
Sb	<0.4	10	101.1	1.1	2.9	100	101.5	3.0	8.4
As	<1.4	50	100.8	2.0	5.6	200	96.8	0.9	2.6
Ba	28.7	50	102.1	1.8	2.4	200	102.9	3.7	9.0
Be	<0.3	10	109.1	0.4	0.9	100	114.4	3.9	9.6
Cd	<0.5	10	106.6	3.2	8.3	100	105.8	2.8	7.6
Cr	2.0	10	107.0	1.0	1.6	100	100.0	1.4	3.9
Co	0.79	10	101.6	1.1	2.7	100	101.7	1.8	4.9
Cu	5.4	10	107.5	1.4	1.9	100	98.1	2.5	6.8
Pb	1.9	10	108.4	1.5	3.2	100	106.1	0.0	0.0
Mn	617	10	*	*	1.1	100	139.0	11.1	4.0
Mo	0.98	10	104.2	1.4	3.5	100	104.0	2.1	5.7
Ni	2.5	10	102.0	2.3	4.7	100	102.5	2.1	5.7
Se	<7.9	50	102.7	5.6	15.4	200	105.5	1.4	3.8
Ag	0.12	50	102.5	0.8	2.1	200	105.2	2.7	7.1
Tl	<0.3	10	108.5	3.2	8.3	100	105.0	2.8	7.6
Th	0.19	10	93.1	3.5	10.5	100	93.9	1.6	4.8
U	0.30	10	107.0	2.8	7.3	100	107.2	1.8	4.7
V	3.5	50	96.1	5.2	14.2	200	101.5	0.2	0.5
Zn	6.8	50	99.8	1.7	3.7	200	100.1	2.8	7.7

S (R) Standard deviation of percent recovery.

RPD Relative percent difference between duplicate spike determinations.

< Sample concentration below established method detection limit.

* Spike concentration <10% of sample background concentration.

TABLE 9: PRECISION AND RECOVERY DATA IN AQUEOUS MATRICES (Cont'd)

SEWAGE TREATMENT PRIMARY EFFLUENT

Element	Sample	Low	Average			High	Average		
	Conc. µg/L	Spike µg/L	Recovery R (%)	S (R)	RPD	Spike µg/L	Recovery R (%)	S (R)	RPD
Al	1150	50	*	*	3.5	200	100.0	13.8	1.5
Sb	1.5	10	95.7	0.4	0.9	100	104.5	0.7	1.9
As	<1.4	50	104.2	4.5	12.3	200	101.5	0.7	2.0
Ba	202	50	79.2	9.9	2.5	200	108.6	4.6	5.5
Be	<0.3	10	110.5	1.8	4.5	100	106.4	0.4	0.9
Cd	9.2	10	101.2	1.3	0.0	100	102.3	0.4	0.9
Cr	128	10	*	*	1.5	100	102.1	1.7	0.4
Co	13.4	10	95.1	2.7	2.2	100	99.1	1.1	2.7
Cu	171	10	*	*	2.4	100	105.2	7.1	0.7
Pb	17.8	10	95.7	3.8	1.1	100	102.7	1.1	2.5
Mn	199	10	*	*	1.5	100	103.4	2.1	0.7
Mo	136	10	*	*	1.4	100	105.7	2.4	2.1
Ni	84.0	10	88.4	16.3	4.1	100	98.0	0.9	0.0
Se	<7.9	50	112.0	10.9	27.5	200	108.8	3.0	7.8
Ag	10.9	50	97.1	0.7	1.5	200	102.6	1.4	3.7
Tl	<0.3	10	97.5	0.4	1.0	100	102.0	0.0	0.0
Th	0.11	10	15.4	1.8	30.3	100	29.3	0.8	8.2
U	0.71	10	109.4	1.8	4.3	100	109.3	0.7	1.8
V	<2.5	50	90.9	0.9	0.6	200	99.4	2.1	6.0
Zn	163	50	85.8	3.3	0.5	200	102.0	1.5	1.9

S (R) Standard deviation of percent recovery.

RPD Relative percent difference between duplicate spike determinations.

< Sample concentration below established method detection limit.

* Spike concentration <10% of sample background concentration.

TABLE 9: PRECISION AND RECOVERY DATA IN AQUEOUS MATRICES (Cont'd)

INDUSTRIAL EFFLUENT									
Element	Sample Conc.	Low Spike	Average Recovery			High Spike	Average Recovery		
	µg/L	µg/L	R (%)	S (R)	RPD	µg/L	R (%)	S (R)	RPD
Al	44.7	50	98.8	8.7	5.7	200	90.4	2.1	2.2
Sb	2990	10	*	*	0.3	100	*	*	0.0
As	<1.4	50	75.1	1.8	6.7	200	75.0	0.0	0.0
Ba	100	50	96.7	5.5	3.4	200	102.9	1.1	0.7
Be	<0.3	10	103.5	1.8	4.8	100	100.0	0.0	0.0
Cd	10.1	10	106.5	4.4	2.4	100	97.4	1.1	2.8
Cr	171	10	*	*	0.0	100	127.7	2.4	1.7
Co	1.3	10	90.5	3.2	8.7	100	90.5	0.4	1.3
Cu	101	10	*	*	0.9	100	92.5	2.0	1.6
Pb	294	10	*	*	2.6	100	108.4	2.1	0.0
Mn	154	10	*	*	2.8	100	103.6	3.7	1.6
Mo	1370	10	*	*	1.4	100	*	*	0.7
Ni	17.3	10	107.4	7.4	5.0	100	88.2	0.7	1.0
Se	15.0	50	129.5	9.3	15.1	200	118.3	1.9	3.6
Ag	<0.1	50	91.8	0.6	1.7	200	87.0	4.9	16.1
Tl	<0.3	10	90.5	1.8	5.5	100	98.3	1.0	2.8
Th	0.29	10	109.6	1.2	2.7	100	108.7	0.0	0.0
U	0.17	10	104.8	2.5	6.6	100	109.3	0.4	0.9
V	<2.5	50	74.9	0.1	0.3	200	72.0	0.0	0.0
Zn	43.4	50	85.0	4.0	0.6	200	97.6	1.0	0.4

S (R) Standard deviation of percent recovery.

RPD Relative percent difference between duplicate spike determinations.

< Sample concentration below established method detection limit.

* Spike concentration <10% of sample background concentration.

TABLE 10: PRECISION AND RECOVERY DATA IN SOLID MATRICES

EPA HAZARDOUS SOIL #884

Element	Sample Conc. (mg/kg)	Low ⁺ Spike (mg/kg)	Average Recovery			High ⁺ Spike (mg/kg)	Average Recovery		
			R (%)	S (R)	RPD		R (%)	S (R)	RPD
Al	5170	20	*	*	-	100	*	*	-
Sb	5.4	20	69.8	2.5	4.7	100	70.4	1.8	6.5
As	8.8	20	104.7	5.4	9.1	100	102.2	2.2	5.4
Ba	113	20	54.9	63.6	18.6	100	91.0	9.8	0.5
Be	0.6	20	100.1	0.6	1.5	100	102.9	0.4	1.0
Cd	1.8	20	97.3	1.0	1.4	100	101.7	0.4	1.0
Cr	83.5	20	86.7	16.1	8.3	100	105.5	1.3	0.0
Co	7.1	20	98.8	1.2	1.9	100	102.9	0.7	1.8
Cu	115	20	86.3	13.8	3.4	100	151.7	4.2	4.6
Pb	152	20	85.0	45.0	13.9	100	85.2	25.7	23.7
Mn	370	20	*	*	12.7	100	95.2	10.4	2.2
Mo	4.8	20	95.4	1.5	2.9	100	102.3	0.7	2.0
Ni	19.2	20	101.7	3.8	1.0	100	100.7	0.8	0.8
Se	<3.2	20	79.5	7.4	26.4	100	94.8	9.4	26.5
Ag	1.1	20	96.1	0.6	0.5	100	97.9	0.8	2.3
Tl	0.24	20	94.3	1.1	3.1	100	76.0	1.0	2.9
Th	1.0	20	69.8	0.6	1.3	100	102.9	2.2	7.9
U	1.1	20	100.1	0.2	0.0	100	106.7	0.0	0.0
V	17.8	20	109.2	4.2	2.3	100	113.4	1.3	2.4
Zn	128	20	87.0	27.7	5.5	100		12.9	14.1

S (R) Standard deviation of percent recovery.

RPD Relative percent difference between duplicate spike determinations.

< Sample concentration below established method detection limit.

* Spike concentration <10% of sample background concentration.

- Not determined.

+ Equivalent.

TABLE 10: PRECISION AND RECOVERY DATA IN SOLID MATRICES

NBS 1645 RIVER SEDIMENT

Element	Sample	Low ⁺	Average			High ⁺	Average		
	Conc. (mg/kg)	Spike (mg/kg)	Recovery R (%)	S (R)	RPD	Spike (mg/kg)	Recovery R (%)	S (R)	RPD
Al	5060	20	*	*	-	100	*	*	-
Sb	21.8	20	73.9	6.5	9.3	100	81.2	1.5	3.9
As	67.2	20	104.3	13.0	7.6	100	107.3	2.1	2.9
Ba	54.4	20	105.6	4.9	2.8	100	98.6	2.2	3.9
Be	0.59	20	88.8	0.2	0.5	100	87.9	0.1	0.2
Cd	8.3	20	92.9	0.4	0.0	100	95.7	1.4	3.9
Cr	29100	20	*	*	-	100	*	*	-
Co	7.9	20	97.6	1.3	2.6	100	103.1	0.0	0.0
Cu	112	20	121.0	9.1	1.5	100	105.2	2.2	1.8
Pb	742	20	*	*	-	100	-	-	-
Mn	717	20	*	*	-	100	-	-	-
Mo	17.1	20	89.8	8.1	12.0	100	98.4	0.7	0.9
Ni	41.8	20	103.7	6.5	4.8	100	102.2	0.8	0.0
Se	<3.2	20	108.3	14.3	37.4	100	93.9	5.0	15.1
Ag	1.8	20	94.8	1.6	4.3	100	96.2	0.7	1.9
Tl	1.2	20	91.2	1.3	3.6	100	94.4	0.4	1.3
Th	0.90	20	91.3	0.9	2.6	100	92.3	0.9	2.8
U	0.79	20	95.6	1.8	5.0	100	98.5	1.2	3.5
V	21.8	20	91.8	4.6	5.7	100	100.7	0.6	0.8
Zn	1780	20	*	*	-	100	*	*	-

S (R) Standard deviation of percent recovery.

RPD Relative percent difference between duplicate spike determinations.

< Sample concentration below established method detection limit.

* Spike concentration <10% of sample background concentration.

- Not determined.

⁺ Equivalent.

TABLE 10: PRECISION AND RECOVERY DATA IN SOLID MATRICES

EPA ELECTROPLATING SLUDGE #286

Element	Sample Conc. (mg/kg)	Low ⁺ Spike (mg/kg)	Average Recovery			High ⁺ Spike (mg/kg)	Average Recovery		
			R (%)	S (R)	RPD		R (%)	S (R)	RPD
Al	5110	20	*	*	-	100	*	*	-
Sb	8.4	20	55.4	1.5	4.1	100	61.0	0.2	0.9
As	41.8	20	91.0	2.3	1.7	100	94.2	0.8	1.5
Ba	27.3	20	1.8	7.1	8.3	100	0	1.5	10.0
Be	0.25	20	92.0	0.9	2.7	100	93.4	0.3	0.9
Cd	112	20	85.0	5.2	1.6	100	88.5	0.8	0.5
Cr	7980	20	*	*	-	100	*	*	-
Co	4.1	20	89.2	1.8	4.6	100	88.7	1.5	4.6
Cu	740	20	*	*	6.0	100	61.7	20.4	5.4
Pb	1480	20	*	*	-	100	*	*	-
Mn	295	20	*	*	-	100	-	-	-
Mo	13.3	20	82.9	1.2	1.3	100	89.2	0.4	1.0
Ni	450	20	*	*	6.8	100	83.0	10.0	4.5
Se	3.5	20	89.7	3.7	4.2	100	91.0	6.0	18.0
Ag	5.9	20	89.8	2.1	4.6	100	85.1	0.4	1.1
Tl	1.9	20	96.9	0.9	2.4	100	98.9	0.9	2.4
Th	3.6	20	91.5	1.3	3.2	100	97.4	0.7	2.0
U	2.4	20	107.7	2.0	4.6	100	109.6	0.7	1.8
V	21.1	20	105.6	1.8	2.1	100	97.4	1.1	2.5
Zn	13300	20	*	*	-	100	*	*	-

S (R) Standard deviation of percent recovery.

RPD Relative percent difference between duplicate spike determinations.

< Sample concentration below established method detection limit.

* Spike concentration <10% of sample background concentration.

- Not determined.

+ Equivalent.

**TABLE 11: PRIMARY DRINKING WATER CONTAMINANTS
PRECISION AND RECOVERY DATA**

Analyte	Regional Sample Background Concentration, µg/L			Average Mean ¹ % Recovery	S (R)
	(IV)	(VI)	(X)		
Antimony	0.16	0.07	0.03	114%	1.9
Arsenic	< MDL	2.4	1.0	93	8.5
Barium	4.6	280	14.3	(*)	–
Beryllium	< MDL	< MDL	< MDL	100%	8.2
Cadmium	0.05	0.05	0.03	81	4.0
Chromium	0.71	5.1	0.10	94	2.5
Copper	208	130	14.3	(*)	–
Lead	1.2	1.2	2.5	91	2.6
Mercury	< MDL	0.23	< MDL	86	11.4
Nickel	1.7	3.6	0.52	101%	11.5
Selenium	< MDL	4.3	< MDL	98	8.4
Thallium	< MDL	0.01	< MDL	100	1.4

¹The three regional waters were fortified with 1.0 µg/L of all analytes listed, except selenium, which was fortified to 20 µg/L.

(*) Recovery of barium and copper was not calculated because the analyte addition was <20% the sample background concentration in all waters. (Recovery calculations are not required if the concentration of the analyte added is less than 30% of the sample background concentration. Section 9.4.3).

S (R) Standard deviation of the mean percent recoveries.

TABLE 12: SUMMARY STATISTICS AND DESCRIPTIVE EQUATIONS FOR THE 20 ANALYTES TESTED IN THE COLLABORATIVE STUDY

Analyte	Reagent Water					Finished Drinking Water					Ground Water				
	C ^a	\bar{X}^b	S _R	S _T	Regr. Equations	\bar{X}	S _R	S _T	Regr. Equations	\bar{X}	S _R	S _T	Regr. Equations		
Aluminum	8.00	10.01	2.33	1.74	$\bar{X} = 0.992C + 1.19$	11.18	9.02	6.34	$\bar{X} = 0.954C + 2.38$	9.86	7.10	2.70	$\bar{X} = 0.946C + 2.20$		
	12.00	10.98	5.16		$S_R = 0.056\bar{X} + 2.59^c$	11.02	3.02		$S_R = 7.70^d$	13.40	10.27		$S_R = 0.169\bar{X} + 6.22^e$		
	56.00	59.13	5.55	4.19	$S_T = 0.042X + 1.27$	56.97	7.14	6.18	$S_T = 0.013X + 6.17$	51.75	10.78	16.92	$S_T = 0.172X + 0.75^f$		
	80.00	82.59	4.92			82.73	8.01			82.83	33.37				
	160.00	158.95	11.82	8.90		159.89	11.94	10.59		155.40	15.39	19.27			
	200.00	200.89	8.61			189.98	12.97			189.64	31.46				
Antimony	2.80	2.75	0.27	0.27	$\bar{X} = 0.999C + 0.04$	2.73	0.29	0.17	$\bar{X} = 0.983C + 0.03$	2.82	0.19	0.22	$\bar{X} = 1.003C + 0.01$		
	4.00	4.22	0.46		$S_R = 0.013\bar{X} + 0.61^a$	4.10	0.47		$S_R = 0.049\bar{X} + 0.19$	4.02	0.35		$S_R = 0.059\bar{X} + 0.04$		
	20.00	19.76	1.09	0.85	$S_T = 0.022X + 0.20$	19.17	1.37	0.66	$S_T = 0.026X + 0.08$	20.12	0.82	0.97	$S_T = 0.058X + 0.02$		
	28.00	27.48	1.38			26.48	1.72			27.77	1.38				
	80.00	82.52	2.24	1.76		83.43	2.05	2.46		80.34	9.14	6.80			
	100.00	98.06	1.34			97.19	5.31			101.09	2.89				
Arsenic	8.00	8.64	3.01	3.02	$\bar{X} = 1.013C + 0.50$	9.00	3.13	1.96	$\bar{X} = 0.993C + 0.57$	10.40	5.17	4.90	$\bar{X} = 0.949C + 0.91$		
	12.00	12.58	3.18		$S_R = 0.031\bar{X} + 2.74$	11.37	1.77		$S_R = 0.018\bar{X} + 2.55$	7.85	4.62		$S_R = 0.048\bar{X} + 4.52$		
	56.00	55.44	4.64	3.51	$S_T = 0.007X + 2.95$	53.77	4.12	4.07	$S_T = 0.031X + 1.65$	53.25	3.49	7.88	$S_T = 0.059X + 4.29$		
	80.00	85.15	2.54			87.72	4.14			83.60	12.46				
	160.00	161.80	11.15	3.96		157.56	4.83	6.30		159.86	11.67	14.94			
	200.00	201.52	10.81			197.99	10.66			194.41	18.24				
Barium	8.01	7.58	0.50	0.48	$\bar{X} = 1.001C - 0.36$	8.21	1.21	1.11	$\bar{X} = 0.995C + 0.37$	8.04	2.60	2.24	$\bar{X} = 1.055C - 0.21$		
	12.00	11.81	1.05		$S_R = 0.039\bar{X} + 0.31$	12.56	1.79		$S_R = 0.045\bar{X} + 0.97^c$	12.85	1.45		$S_R = 0.020\bar{X} + 2.05$		
	48.00	47.32	1.60	1.82	$S_T = 0.024X + 0.25$	49.13	3.72	3.77	$S_T = 0.040X + 0.72^c$	50.12	2.98	2.19	$S_T = 0.014X + 2.08$		
	64.00	65.52	2.90			65.30	4.16			69.53	2.66				
	160.00	157.09	6.53	4.07		155.25	7.82	5.67		164.44	8.81	6.61			
	200.00	198.53	8.28			196.52	5.70			208.32	9.22				
Beryllium	2.80	3.31	0.81	0.26	$\bar{X} = 1.056C + 0.32$	3.15	0.47	0.31	$\bar{X} = 1.055C + 0.20$	3.02	0.46	0.22	$\bar{X} = 1.049C + 0.08$		
	4.00	4.45	0.73		$S_R = 0.067\bar{X} + 0.55$	4.45	0.51		$S_R = 0.057\bar{X} + 0.28$	4.27	0.44		$S_R = 0.084\bar{X} + 0.16$		
	20.00	22.38	2.76	1.00	$S_T = 0.038X + 0.11$	21.27	1.23	0.63	$S_T = 0.016X + 0.25$	21.55	1.72	1.10	$S_T = 0.043X + 0.06$		
	28.00	30.02	2.86			29.57	1.67			29.24	2.09				
	80.00	84.18	4.79	4.02		87.59	6.89	1.88		84.23	9.05	4.32			
	100.00	102.88	5.90			102.64	6.27			103.39	10.17				
Cadmium	4.00	4.01	0.34	0.20	$\bar{X} = 1.007C + 0.07$	4.11	0.88	0.71	$\bar{X} = 0.985C + 0.10$	3.98	0.48	0.14	$\bar{X} = 0.944C + 0.11$		
	6.00	6.32	0.49		$S_R = 0.041\bar{X} + 0.19$	5.87	0.58		$S_R = 0.031\bar{X} + 0.65$	5.62	0.73		$S_R = 0.017\bar{X} + 1.09^a$		
	20.00	19.81	1.12	0.86	$S_T = 0.022X + 0.10^c$	19.57	1.45	1.26	$S_T = 0.021X + 0.61$	18.15	1.73	0.88	$S_T = 0.029X + 0.01$		
	28.00	28.33	0.94			27.68	1.27			26.86	2.59				
	80.00	81.28	4.91	1.33		80.62	4.45	2.02		77.83	3.05	1.88			
	100.00	100.11	3.24			98.15	3.60			95.31	2.04				

TABLE 12: SUMMARY STATISTICS AND DESCRIPTIVE EQUATIONS FOR THE 20 ANALYTES TESTED IN THE COLLABORATIVE STUDY

Analyte	Reagent Water					Finished Drinking Water				Ground Water			
	C ^a	\bar{X}^b	S _R	S _T	Regr. Equations	\bar{X}	S _R	S _T	Regr. Equations	\bar{X}	S _R	S _T	Regr. Equations
Chromium	8.00	8.27	0.32	1.54	$\bar{X} = 1.017C + 0.62$	9.46	2.34	2.08	$\bar{X} = 0.990C + 1.45$	8.98	1.47	0.37	$\bar{X} = 1.026C + 0.89$
	12.00	13.88	3.10		$S_R = 0.066\bar{X} + 0.48$	13.10	2.39		$S_R = 0.015\bar{X} + 2.19$	13.42	1.13		$S_R = 0.067\bar{X} + 0.68$
	56.00	57.86	4.03	2.68	$S_T = 0.026\bar{X} + 1.25$	56.04	2.24	1.29	$S_T = 2.18^d$	59.35	5.99	5.42	$S_T = 0.068\bar{X} - 0.37$
	80.00	84.73	2.65			84.38	3.18			83.90	5.70		
	160.00	157.66	13.62	6.97		158.24	5.12	3.16		164.58	14.11	9.80	
	200.00	197.43	9.47			196.72	7.47			199.88	11.19		
Cobalt	0.80	0.88	0.10	0.05	$\bar{X} = 0.977C + 0.01$	0.92	0.45	0.31	$\bar{X} = 0.964C + 0.06$	0.85	0.13	0.09	$\bar{X} = 0.989C - 0.01$
	1.21	0.98	0.04		$S_R = 0.028\bar{X} + 0.06$	1.02	0.10		$S_R = 0.019\bar{X} + 0.32$	1.04	0.18		$S_R = 0.057\bar{X} + 0.09$
	20.10	20.77	0.74	0.67	$S_T = 0.027\bar{X} + 0.02$	20.45	0.91	0.53	$S_T = 0.014\bar{X} + 0.30$	20.81	1.11	1.12	$S_T = 0.012\bar{X} + 0.40^e$
	28.20	27.75	0.96			27.29	1.22			28.07	2.16		
	80.50	78.59	2.29	2.31		78.04	3.72	1.84		79.26	4.66	1.34	
	101.00	98.79	2.94			97.62	4.62			99.41	4.22		
Copper	4.00	3.88	0.73	0.59	$\bar{X} = 1.003C - 0.05$	3.33	0.85	0.99	$\bar{X} = 0.976C - 0.38$	3.86	1.40	0.71	$\bar{X} = 0.977C - 0.01$
	6.00	6.14	1.00		$S_R = 0.037\bar{X} + 0.64$	5.95	1.78		$S_R = 0.063\bar{X} + 0.86$	5.96	0.95		$S_R = 0.073\bar{X} + 0.92$
	20.00	20.07	1.08	0.92	$S_T = 0.016\bar{X} + 0.51$	18.90	1.64	1.51	$S_T = 0.029\bar{X} + 0.86$	18.97	1.68	2.32	$S_T = 0.077\bar{X} + 0.35$
	28.00	27.97	1.94			27.21	2.76			27.44	2.58		
	80.00	79.80	3.22	1.91		76.64	5.30	3.42		79.30	9.05	6.54	
	100.00	99.57	4.42			96.17	5.64			97.54	11.16		
Lead	4.00	4.00	1.57	1.62	$\bar{X} = 1.043C - 0.31$	3.44	1.15	1.18	$\bar{X} = 1.032C - 0.30$	4.20	1.13	1.76	$\bar{X} = 1.012C + 0.15$
	6.00	5.56	2.00		$S_R = 0.064\bar{X} + 1.43^c$	6.84	1.10		$S_R = 0.015\bar{X} + 1.06$	6.27	2.38		$S_R = 0.048\bar{X} + 1.27$
	20.00	20.54	2.91	4.36	$S_T = 3.42^d$	20.18	1.20	1.44	$S_T = 0.011\bar{X} + 1.13$	19.57	2.72	0.88	$S_T = 1.78^d$
	28.00	30.90	4.58			28.08	1.57			28.55	1.73		
	80.00	80.57	3.13	4.29		80.92	2.30	2.07		82.47	4.38	2.69	
	100.00	102.93	6.62			101.60	3.23			102.47	3.58		
Manganese	0.80	0.86	0.15	0.09	$\bar{X} = 0.983C + 0.02$	0.96	0.32	0.42	$\bar{X} = 0.989C + 0.10$	0.64	0.22	0.17	$\bar{X} = 0.954C - 0.16$
	1.20	1.09	0.12		$S_R = 0.026\bar{X} + 0.11$	1.13	0.38		$S_R = 0.047\bar{X} + 0.29$	0.90	0.21		$S_R = 0.103\bar{X} + 0.14$
	20.00	20.43	0.89	0.72	$S_T = 0.027\bar{X} + 0.06$	21.06	1.32	0.96	$S_T = 0.021\bar{X} + 0.40$	19.61	2.60	2.62	$S_T = 0.025\bar{X} + 0.09^e$
	28.00	27.53	0.41			27.60	1.47			25.65	4.10		
	80.00	79.00	3.16	2.38		79.57	4.18	2.01		77.38	6.13	2.90	
	100.00	97.60	2.51			97.97	4.10			95.86	6.74		

TABLE 12: SUMMARY STATISTICS AND DESCRIPTIVE EQUATIONS FOR THE 20 ANALYTES TESTED IN THE COLLABORATIVE STUDY

Analyte	Reagent Water				Finished Drinking Water				Ground Water				
	C ^a	\bar{X}^b	S _R	S _T	Regr. Equations	\bar{X}	S _R	S _T	Regr. Equations	\bar{X}	S _R	S _T	Regr. Equations
Molybdenum	2.80	2.63	0.32	0.16	$\bar{X} = 1.012C - 0.20$	2.80	0.20	0.32	$\bar{X} = 1.013C - 0.07$	3.00	0.47	0.42	$\bar{X} = 1.032C - 0.09$
	4.00	3.85	0.31		$S_R = 0.032X + 0.22$	3.95	0.47		$S_R = 0.037X + 0.17$	3.60	0.90		$S_R = 0.55X + 0.43$
	20.00	19.75	0.64	0.64	$S_T = 0.021X + 0.09$	19.78	0.60	1.16	$S_T = 0.035X + 0.20$	20.69	1.37	1.11	$S_T = 0.042X + 0.27$
	28.00	27.87	1.07			27.87	1.51			28.80	2.01		
	80.00	83.07	3.07	1.78		85.65	3.50	3.07		84.26	4.13	4.81	
100.00	100.08	4.32			99.06	2.89			103.57	6.10			
Nickel	4.00	4.02	0.41	0.50	$\bar{X} = 1.000C + 0.12$	3.66	0.53	1.03	$\bar{X} = 0.953C - 0.19$	4.81	2.06	2.82	$\bar{X} = 1.022C + 0.66$
	6.00	6.36	0.91		$S_R = 0.051X + 0.31$	5.44	1.32		$S_R = 0.046X + 0.56$	6.67	3.66		$S_R = 0.091X + 2.03$
	20.00	19.93	1.30	0.63	$S_T = 0.017X + 0.40$	18.42	0.87	1.11	$S_T = 0.023X + 0.91$	20.58	3.71	2.37	$S_T = 0.008X + 2.75^c$
	28.00	28.02	1.25			27.09	1.68			30.73	3.75		
	80.00	79.29	2.95	2.55		75.84	4.40	3.94		82.71	9.49	5.42	
100.00	100.87	7.20			95.83	4.41			101.00	9.89			
Selenium	32.00	33.54	4.63	1.57	$\bar{X} = 1.036C - 0.06$	32.57	4.37	3.65	$\bar{X} = 1.022C + 0.14$	32.46	4.95	3.24	$\bar{X} = 1.045C - 0.83$
	40.00	41.03	6.04		$S_R = 0.051X + 3.24$	42.18	3.71		$S_R = 0.056X + 2.10$	41.46	3.30		$S_R = 0.037X + 2.97$
	80.00	81.40	5.86	5.44	$S_T = 0.061X - 0.64$	79.97	6.66	5.28	$S_T = 0.040X + 2.15$	81.63	6.94	5.65	$S_T = 0.058X + 1.02$
	96.10	98.34	8.57			94.94	7.90			98.92	4.39		
	160.00	163.58	15.69	9.86		163.48	9.17	10.06		167.54	8.69	12.98	
200.00	214.30	10.57			212.19	16.49			209.21	14.65			
Silver	0.80	0.93	0.09	0.14	$\bar{X} = 0.917C + 0.26$	0.70	0.34	0.34	$\bar{X} = 0.888C + 0.09$	0.70	0.26	0.10	$\bar{X} = 0.858C - 0.00$
	1.20	1.51	0.23		$S_R = 0.196X - 0.09$	1.37	0.33		$S_R = 0.186X + 0.17$	0.98	0.28		$S_R = 0.169X + 0.14$
	48.00	49.39	3.25	1.81	$S_T = 0.053X + 0.08$	45.43	6.78	5.15	$S_T = 0.164X + 0.18$	45.59	4.27	2.70	$S_T = 0.120X - 0.01$
	64.00	63.54	2.75			60.35	2.22			59.71	6.58		
	160.00	136.42	48.31	12.19		119.06	55.28	36.34		121.43	42.55	28.19	
200.00	153.74	57.34			172.15	31.92			160.69	27.15			
Thallium	2.80	2.89	0.23	0.22	$\bar{X} = 0.984X + 0.08$	2.88	0.40	0.16	$\bar{X} = 1.010C + 0.01$	2.88	0.14	0.12	$\bar{X} = 1.023C - 0.06$
	4.00	3.92	0.15		$S_R = 0.035X + 0.09$	3.96	0.21		$S_R = 0.040X + 0.21$	3.88	0.37		$S_R = 0.056X + 0.04$
	20.00	19.27	0.99	0.67	$S_T = 0.027X + 0.13$	19.77	1.13	0.83	$S_T = 0.039X + 0.02$	20.22	1.05	0.65	$S_T = 0.049X - 0.06$
	28.00	28.08	0.83			27.61	1.24			28.65	1.50		
	80.00	81.29	3.65	2.86		85.32	4.08	4.05		83.97	6.10	6.05	
100.00	96.69	2.86			100.07	4.33			101.09	4.15			
Thorium	0.80	0.93	0.16	0.09	$\bar{X} = 1.013C + 0.08$	0.78	0.13	0.07	$\bar{X} = 1.019C - 0.06$	0.87	0.17	0.07	$\bar{X} = 1.069C - 0.03$
	1.20	1.22	0.19		$S_R = 0.036X + 0.13$	1.09	0.19		$S_R = 0.035X + 0.12$	1.15	0.17		$S_R = 0.041X + 0.13$
	20.00	20.88	0.90	0.71	$S_T = 0.025X + 0.07$	21.66	0.94	0.54	$S_T = 0.024X + 0.05$	21.78	0.90	0.94	$S_T = 0.027X + 0.04$
	28.00	27.97	1.11			28.09	0.83			29.86	1.65		
	80.10	81.14	2.99	2.14		79.99	2.03	2.60		86.00	3.43	1.95	
100.00	102.64	3.39			100.50	4.56			107.35	4.72			

TABLE 12: SUMMARY STATISTICS AND DESCRIPTIVE EQUATIONS FOR THE 20 ANALYTES TESTED IN THE COLLABORATIVE STUDY

Analyte	Reagent Water				Finished Drinking Water				Ground Water				
	C ^a	\bar{X}^b	S _R	S _r	Regr. Equations	\bar{X}	S _R	S _r	Regr. Equations	\bar{X}	S _R	S _r	Regr. Equations
Uranium	0.80	0.86	0.05	0.08	$\bar{X} = 1.026C - 0.02$	0.85	0.15	0.09	$\bar{X} = 1.026C - 0.04$	0.84	0.23	0.19	$\bar{X} = 1.058C - 0.06$
	1.20	1.10	0.11		$S_R = 0.048\bar{X} + 0.02$	1.05	0.13		$S_R = 0.044\bar{X} + 0.11$	1.10	0.14		$S_R = 0.039\bar{X} + 0.17$
	20.10	21.38	0.99	0.82	$S_r = 0.027\bar{X} + 0.05$	22.30	1.40	0.46	$S_r = 0.022\bar{X} + 0.07$	21.56	1.11	1.08	$S_r = 0.028\bar{X} + 0.16$
	28.10	28.36	1.10			28.89	1.47			29.86	1.83		
	80.30	82.47	4.03	2.16		80.31	2.00	2.71		85.01	3.76	2.00	
	100.00	103.49	5.24			100.70	5.30			106.47	3.74		
Vanadium	32.00	31.02	2.68	2.19	$\bar{X} = 1.025C - 2.21$	33.15	2.51	2.28	$\bar{X} = 1.022C - 0.30$	33.25	3.83	1.87	$\bar{X} = 1.076C - 1.87$
	40.00	38.54	2.94		$S_R = 3.79^d$	40.20	1.88		$S_R = 0.023\bar{X} + 1.45$	40.34	3.08		$S_R = 0.033\bar{X} + 2.25$
	80.00	79.14	4.94	4.29	$S_r = 3.26^d$	77.83	4.18	2.75	$S_r = 0.023\bar{X} + 1.38$	84.42	3.97	2.93	$S_r = 0.049\bar{X} - 0.09$
	96.00	93.47	3.85			96.32	1.34			98.70	5.03		
	160.00	162.43	5.67	3.30		161.89	7.63	6.56		170.94	9.09	11.55	
	200.00	208.20	2.65			214.91	5.89			217.90	11.36		
Zinc	8.00	8.33	2.56	1.78	$\bar{X} = 1.042C + 0.87$	11.60	6.18	5.72	$\bar{X} = 0.943C + 2.54$	7.29	1.12	2.20	$\bar{X} = 0.962C + 0.07$
	12.00	15.49	4.18		$S_R = 0.041\bar{X} + 2.60$	10.21	4.96		$S_R = 0.048\bar{X} + 5.27$	12.66	3.24		$S_R = 0.093\bar{X} + 0.92$
	56.00	56.07	2.91	2.47	$S_r = 0.030\bar{X} + 1.42$	56.83	7.66	4.56	$S_r = 0.004\bar{X} + 5.66^e$	54.86	5.12	7.24	$S_r = 0.069\bar{X} + 1.55$
	80.00	85.53	5.81			82.88	8.34			78.62	8.56		
	160.00	165.17	7.78	9.87		156.69	17.01	9.48		150.12	12.52	10.84	
	200.00	207.27	14.61			191.59	17.21			184.37	16.59		

^a True Value for the concentration added (µg/L)

^b Mean Recovery (µg/L)

^c COD_r < 0.5 - Use of regression equation outside study concentration range not recommended.

^d COD_r < 0 - Mean precision is reported.

^e COD_r < 0 - Unweighted linear regression equation presented.

TABLE 13: BACKGROUND AND SPIKE MEASUREMENTS IN WASTEWATER DIGESTATE^a

	Background		Concentrate 1					Concentrate 2					
	Conc. µg/L	Std Dev µg/L	Spike µg/L	Found µg/L	Std Dev µg/L	% Rec %	RSD %	Spike µg/L	Found µg/L	Std Dev µg/L	% Rec %	RSD %	RSD_r %
Be	0.0	0.0	100	94.5	11.8	94.5	12.5	125	118.1	14.7	94.5	12.4	3.5
Al	78.2	12.4	200	260.9	41.2	91.4	15.8	250	309.1	48.5	92.4	15.7	2.7
Cr	19.5	8.1	200	222.2	23.3	101.4	10.5	250	274.3	26.6	101.9	9.7	2.0
V	1.9	2.8	250	271.8	36.5	108.0	13.4	200	219.3	30.1	108.7	13.7	2.6
Mn	296.6	24.7	125	419.0	35.7	97.9	8.5	100	397.4	34.8	100.8	8.8	1.0
Co	2.5	0.4	125	124.7	12.3	97.8	9.9	101	100.7	9.4	97.2	9.3	2.8
Ni	47.3	5.0	125	161.7	4.9	91.5	3.0	100	142.7	5.6	95.4	3.9	2.1
cu	77.4	13.2	125	194.5	29.5	93.7	15.2	100	172.3	26.6	94.9	15.4	2.2
Zn	77.4	4.9	200	257.4	16.3	90.0	6.3	250	302.5	21.1	90.0	7.0	1.8
As	0.8	1.1	200	194.9	8.0	97.1	4.1	250	244.7	12.8	97.6	5.2	3.4
Se	4.5	6.2	250	236.8	14.2	92.9	6.0	200	194.3	9.3	94.9	4.8	3.8
Mo	166.1	9.4	100	269.8	19.0	103.7	7.0	125	302.0	18.0	108.7	6.0	1.5
Ag	0.6	0.7	200	176.0	14.6	87.7	8.3	250	214.6	17.8	85.6	8.3	2.3
Cd	2.7	1.1	125	117.0	4.8	91.4	4.1	100	96.6	3.2	93.9	3.3	2.9
Sb	3.3	0.2	100	100.2	4.8	96.9	4.8	125	125.9	4.3	98.1	3.4	1.8
Ba	68.6	3.3	250	321.0	19.4	101.0	6.0	200	279.3	17.2	105.4	6.2	2.5
Tl	0.1	0.1	100	103.3	8.0	103.2	7.7	125	129.2	8.9	103.3	6.9	2.1
Pb	6.9	0.5	125	135.1	7.8	102.6	5.8	100	110.3	6.3	103.4	5.7	1.8
Th	0.1	0.1	125	140.2	19.5	112.1	13.9	100	113.3	15.4	113.2	13.6	2.7
U	0.4	0.2	125	141.2	19.3	112.6	13.7	100	113.6	16.0	113.2	14.1	2.5

^aResults from 10 participating laboratories. Wastewater digestate supplied with the study materials. Mean background concentrations determined by the participants.

TABLE 14: SPIKE MEASUREMENTS IN PARTICIPANTS WASTEWATER^a

	Concentrate 1					Concentrate 2					
	Spike µg/L	Found µg/L	Std Dev µg/L	% Rec %	RSD %	Spike µg/L	Found µg/L	Std Dev µg/L	% Rec %	RSD %	RSD _r %
Be	101	103.4	12.0	103.4	11.6	125	128.2	13.6	102.6	10.6	2.4
Al	200	198.7	23.9	99.4	12.0	250	252.4	15.5	101.0	6.1	2.9
Cr	200	205.4	12.3	102.7	6.0	250	253.4	15.4	101.4	6.1	1.1
V	250	246.5	4.4	98.6	1.8	200	196.8	2.8	98.4	1.4	2.0
Mn	125	119.0	5.4	95.2	4.5	100	95.5	4.3	95.5	4.5	0.8
Co	125	125.8	7.0	100.6	5.6	101	99.5	5.3	98.5	5.3	1.8
Ni	125	127.4	9.7	101.9	7.6	100	101.0	7.5	101.0	7.4	1.7
cu	125	126.8	5.3	101.4	4.2	100	105.3	3.6	105.3	3.4	2.8
Zn	200	201.4	36.7	100.7	18.2	250	246.4	29.7	98.6	12.1	2.6
As	200	207.3	11.9	103.7	5.7	250	263.0	2.6	105.2	1.0	3.2
Se	250	256.8	26.4	102.7	10.3	200	214.0	18.7	107.3	8.7	3.6
Mo	100	98.6	4.6	98.6	4.7	125	123.2	6.7	98.6	5.4	2.2
Ag	200	200.7	48.9	100.4	24.4	250	231.2	63.5	92.5	27.5	8.2
Cd	125	123.2	11.5	98.6	9.3	100	95.8	2.9	95.8	3.0	5.8
Sb	100	92.2	4.4	92.2	4.8	125	119.0	1.0	95.2	0.8	2.8
Ba	250	245.2	12.8	98.1	5.2	200	204.7	12.1	102.4	5.9	2.1
Tl	100	100.0	0.9	100.0	0.9	125	128.0	6.0	102.4	4.7	3.5
Pb	125	125.8	5.1	100.6	4.1	100	100.8	2.7	100.8	2.7	2.2
Th	125	124.2	7.6	99.4	6.1	100	99.8	5.7	99.8	5.7	3.2
U	125	130.4	10.3	104.3	7.9	100	106.4	6.8	106.4	6.4	2.3

^aResults from five participating laboratories. Mean concentrations before spiking are not listed because they varied considerably among the different wastewaters.

Annex IV.

IAEA (2012). Recommended method on the determination of Total Hg in samples of marine origin by Cold Vapour Atomic Absorption Spectrometry (IAEA/Marine Environmental Studies Laboratory in co-operation with UNEP/MAP MED POL) (5.1.3)



REPORT

RECOMMENDED METHOD FOR THE DETERMINATION OF TOTAL MERCURY IN SAMPLES OF MARINE ORIGIN BY COLD VAPOUR ATOMIC ABSORPTION SPECTROMETRY

**IAEA/NAEL Marine Environmental Studies Laboratory in co-operation with
UNEP/MAP MED POL**

December 2012

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RECOMMENDED METHOD ON THE DETERMINATION OF TOTAL MERCURY
IN SAMPLES OF MARINE ORIGIN BY COLD VAPOUR ATOMIC ABSORPTION
SPECTROMETRY

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NOTE: This method is not intended to be an analytical training manual. Therefore, the method is written with the assumption that it will be used by formally trained analytical chemists.

In addition, the IAEA recommended methods are intended to be guidance methods that can be used by laboratories as a starting point for generating their own standard operating procedure. If performance data are included in the method they must not be used as absolute QC acceptance criteria.

1. SCOPE

This method describes a protocol for measurement of total mercury by cold vapour atomic absorption spectrometry (CV-AAS). The method is simple, rapid and applicable to a large number of environmental samples. This method is applicable when the element content in the digested solution is above the method limit (~ 0.15 ng ml⁻¹ depending on instrument). The typical working range is 0.25–100 ng ml⁻¹ for direct injection of cold vapour, using “batch system”; FIAS or amalgamation accessory will give better sensitivity.

2. PRINCIPLE

The sediment or biological samples are mineralized with strong acids. The inorganic mercury is reduced to its elemental form with stannous chloride. The cold mercury vapour is then passed through the quartz absorption cell of an atomic absorption spectrometer (AAS), where its concentration is measured. The light beam of Hg hollow cathode lamp is directed through the quartz cell, into a monochromator and onto a detector that measures the amount of light absorbed by the atomized vapour in the cell. The amount of energy absorbed at the characteristic wavelength is proportional to the concentration of the element in the sample.

3. REAGENT

The reagents used shall meet the purity requirement of the subsequent analysis:

3.1. WATER

Reagent water (referenced also as water in the text) should be free of contamination.

3.2. NITRIC ACID 65%

3.3. HYDROCHLORIC ACID (37%)

3.4. HYDROGEN PEROXIDE

3.5. VANADIUM PENTOXIDE (V_2O_5)

3.6. SILICON ANTI-FOAMING

3.7. HYDROXYLAMINE HYDROCHLORIDE ($NH_2OH.HCl$)

Dissolve 12.0 g of $NH_2OH.HCl$ in 100 ml reagent water. This solution may be purified by the addition of 0.1 ml of $SnCl_2$ solution and purging 1 hour with Hg-free argon.

3.8. POTASSIUM DICHROMATE OXIDIZING SOLUTION (10% w/v)

Weight 25 g of $K_2Cr_2O_7$ in a 250 ml glass bottle, fill it up to 250 ml with water, and shake until total dissolution of the solid. Keep the bottle tightly closed in a double plastic bag, and in an Hg free environment (i.e. laminar flow hood). This solution is stable indefinitely and rarely becomes contaminated.

3.9. $BrCl$ OXIDIZING SOLUTION

Weigh accurately 11 g of $KBrO_3$ and 15 g of KBr into a clean 1 liter glass bottle. Add 200 ml of Milli-Q water; add carefully 800 ml of concentrated HCl . The dilution has to be carried out in a well-ventilated fume hood to prevent exposure to toxic fumes released during dissolution of $KBrO_3$. Keep the bottle wrapped in aluminium foil, tightly closed in a double plastic bag, and in an Hg free environment (i.e. laminar flow hood). This solution is stable indefinitely but can become contaminated.

3.10. STANNOUS CHLORINE SOLUTION 20% (w/v) in 20% (w/v) HCl

Weigh 20 g of $SnCl_2$ in a 100 ml volumetric flask; add 20 ml of concentrated HCl ; dissolve the $SnCl_2$ (if needed heat at $60^\circ C$ for a few minutes on a hot plate); complete to 100 ml with water. This solution might be purified by bubbling with Hg-free argon for 15 minutes. The obtained solution should be clear and transparent, cloudy or yellow solution indicates a bad quality $SnCl_2$. This solution should be prepared fresh every day preferably, if not it should be kept in the fridge.

Note: The concentration of this solution is dependent on the type of accessory use for vapour generation, and can vary between 5 and 30%, the recommendation of the manufacturer

should be followed (i.e. the solution above is recommended for a VGA-70 from Varian). The validity of the solution (i.e. shelf-life) should be defined during method validation.

3.11. COMMERCIAL STANDARD SOLUTION 1000 $\mu\text{g ml}^{-1}$

Use a certified reference material solution; this solution should be accompanied by a certificate stipulating at minimum the traceability of the certified concentration, as well as the expiry date. The density of the solution, or the certified content in mg kg^{-1} should also be defined, to allow for the preparation of the calibration solution by weighing. Stock solutions should be kept at 5°C.

3.12. ARGON

Use of a gas purifier cartridge for removing mercury, oxygen and organic compounds is recommended.

4. MATERIAL

This section does not list the common laboratory glassware.

4.1. ATOMIC ABSORPTION SPECTROPHOTOMETER

Instrument equipped with an appropriate cold vapour generation system and a quartz or glass tube atomizer. Use a hollow cathode lamp or, preferably, an electrodeless discharge lamp (which gives a greater and more stable light intensity), operated at a current recommended for the lamp and by the instrument manufacturer. An AAS system with background correction device is recommended.

4.2. GLASSWARE

All the glassware, polypropylene, or fluorocarbon (PFA or TFM) containers, including the sample bottles, flasks and pipettes tips, should be washed in the following sequence:

- 24 hrs soaking in a laboratory soap (or 10% alcohol);
- followed by 24 hrs soaking in 10% nitric acid;
- followed by 10% soaking in water;
- final rinse in water; and
- drying under a laminar flow hood.

The cleaned items should be kept in a double sealed plastic bag. It is better to avoid storage of low level ($< 5 \text{ ng ml}^{-1}$) solution in plastic, and for this purpose glass or Teflon is recommended.

If it can be documented, through an active analytical quality control program, using spiked samples and method blanks, then certain steps in the cleaning procedure would not be needed for routine samples, those steps may be eliminated from the procedure (i.e. for the levels

measured by flame AAS, some sterile plastic containers are sufficiently free of contamination for certain analytes).

4.3. PIPETTES

Some microliter pipettes size ranging from 50 to 10000 µl are needed. The accuracy and precision of the pipettes used should be checked as a routine every 6 months, and the results obtained should be compared with the individual certificates.

4.4. VOLUMETRIC CONTAINERS of suitable precision and accuracy.

5. INTERFERENCES

- 5.1. IODIDE, GOLD AND SILVER are known interferences for mercury determination by cold vapour. In samples from marine origin (biota or sediment), the levels of those elements are low, and consequently, do not interfere in the measurement process.
- 5.2. WATER VAPOUR (moisture) should be avoided in the measurement cell, always follow the manufacturer's protocol (e.g. use of membrane drying tube, correct position of gas separator...) and check for absence of moisture in the measurement cell.
- 5.3. When using GOLD AMALGAMATION, and with certain batch systems, the excess of oxidant can cause interference or damage the gold amalgamator, it is then recommended to pre-reduce the samples with hydroxylamine ammonium (see 3.7). This is important when using large amount of digested solution in "batch system".
- 5.4. Some samples (i.e. plants or large amount of mussels) might produce FOAM during the reduction reaction. If the amount of foam is important, it can interfere with gas liquid separation, and/or leak in the measurement cell, this phenomenon can be overcome by using silicon anti-foaming inside the gas liquid separator and/or in the "batch" system. Another option is to use vanadium pentoxide during digestion (see 6.4).
- 5.5. REDUCTION of inorganic mercury will induce loss, so it is important to stabilise all the solutions by using a strong oxidant as dichromate or BrCl (see 3.8 or 3.9).

6. SAMPLE PREPARATION

- 6.1. The sample should be prepared according to the recommended method for digestion of marine samples for the determination of trace metal (IAEA, 2011), but before diluting to the final volume (or weight) with water, add an adequate volume of potassium dichromate or BrCl to get the final concentration of 2% or 1% respectively. All the samples from marine origin (sediment or biota) can be prepared using the acid mixture recommended for fish, as Hg is not attached to silicates. For microwave digestion of sample size above 0.8 g, it is strongly recommended to do cold digestion for at least 5 hours and to use a long ramping time (i.e. 25 minutes) to avoid strong reactions in the microwave vessels.
- 6.2. If other trace elements have to be determined in the digested solution prepared according to the recommended method for digestion of marine samples for the determination of trace metal (IAEA, 2011), following the dilution to the final volume or weight, transfer a quantitatively sufficient amount of digested solution (i.e. at least 10 ml) into a separate container (preferably glass or Teflon), and add an oxidising solution 1% (v/v) of BrCl (see 3.9) or 2% (v/v) of potassium dichromate (see 3.8). Record the amount of oxidising solution added in order to calculate the dilution factor (i.e. dilution factor = 1.01 for 0.1 ml of dichromate in 10 ml).
- 6.3. Alternatively, the samples can be digested using a mixture of 5 ml of HNO₃ and 2 ml of H₂O₂ at 90°C for 4 hours on a hot plate. It is recommended to leave the samples in acid at room temperature, for at least 1 hour before heating. The digestion can be performed either in a Teflon or glass closed containers. After cooling, add an adequate volume of potassium dichromate or BrCl to get the final concentration of 2% or 1% respectively, and dilute to the final volume with water (i.e. for 50 ml final volume, add 1 ml of potassium dichromate or 0.5 ml of BrCl solution). This procedure can be used with bigger sample size if needed (i.e. 2 g); in this case, the volume of nitric acid should be increased to obtain a liquid mixture.
- 6.4. In the case that the digested solution produces foam during the reduction process (see 5.4), 45 mg of vanadium pentoxide should be added in the digestion vessels before addition of the acid mixture, then follow either paragraph 6.1 or 6.3.

7. PROCEDURE

7.1. SAMPLE SOLUTION

Use the sample prepared with one option as described in section 6.

7.2. BLANK SOLUTION

Prepare at least two blank solutions with each batch of sample, using the same procedure than for the samples.

7.3. PREPARATION OF CALIBRATION SOLUTIONS

7.3.1. Before each batch of determination, prepare by the appropriate dilution of 1000 $\mu\text{g ml}^{-1}$ stock standard solution (see 3.11), at least 4 standard solutions and one calibration blank solution, covering the appropriate range of the linear part of the curve. The calibration standards and calibration blank should be prepared using the same type of acid and oxidising solution than in the test portion (the final concentration should be similar).

7.3.2. Calibration solutions should be prepared fresh each day.

7.3.3. If the necessary intermediate stock standard solutions can be prepared in 5% nitric acid and 1% BrCl or 2% $\text{K}_2\text{Cr}_2\text{O}_7$, these solutions should be prepared monthly.

7.3.4. All volumetric material (pipettes and containers) should be of appropriate precision and accuracy, if not available standard solution can be prepared by weighing.

7.4. INSTALLATION OF VAPOUR GENERATOR ACCESSORY

7.4.1. Install the accessory according to the manufacturer's instructions. Certain systems (i.e. VGA from Varian) are designed to be used for hydride generation as well, and require in the instructions to aspirate an extra HCl solution, in the case of stannous chlorine reduction this solution is to be replaced by water. It is recommended to separate the systems used for hydride and for SnCl_2 (i.e. use a spare gas liquid separator and Teflon tubing).

7.4.2. Switch on the argon. For on-line system: start the pump, check the aspiration, and verify the gas liquid separator. If needed replace the pump tubing, clean the gas liquid separator by sonication in diluted detergent.

7.4.3. Clean the system by aspirating reagent and 10% nitric acid as a sample for about 10 minutes. For batch system, perform two cycles with 10% nitric acid.

7.4.4. Set up the atomic absorption spectrometer according to the manufacturer's instructions, at the appropriate wavelength, using the appropriate conditions, and with the suitable background correction system in operation.

7.4.5. Optimise the position of the measurement cell to get the maximum signal.

7.4.6. Connect the vapour generation system to the measurement cell.

7.5. CALIBRATION

7.5.1. Adjust the response of the instrument to zero absorbance whilst aspirating water.

NOTE: if the instrument zero reading is more than 0.002 ABS, the system should be clean again and reagent should be checked.

7.5.2. Aspirate the set of calibration solutions in ascending order, and as a zero member, the blank calibration solution. After the last standard, aspirate 10% nitric acid for 1 minute to rinse the system.

NOTE: The calibration curve is automatically plotted by the instrument software. The obtained curve should be linear with $r > 0.995$.

To correct for the instrumental drift, the calibration should be performed every 20 samples or if the calibration verification has failed (see 7.8.1).

7.6. ASPIRATE SAMPLE BLANK (see 7.2) AND SAMPLE SOLUTIONS (see 7.1)

Record their concentrations as calculated by the software using the calibration curve. Rinse the system by aspirating 10% nitric acid for at least 30 s between samples.

7.7. IF THE CONCENTRATION OF THE TEST PORTION EXCEEDS THE CALIBRATION RANGE, dilute the test portion with the blank solution accordingly.

NOTE: After the measurement of high level (or over calibration) sample, measure a sample blank or water to check the absence of memory effect. If necessary, clean the system for 1 minute with 10% nitric acid.

7.8. QUALITY CONTROL SOLUTIONS

The quality control solutions as described below should be measured during the run.

7.8.1. Calibration Verification CV

After the initial calibration, the calibration curve must be verified by the use of initial calibration verification (CV) standard.

The CV standard is a standard solution made from an independent (second source) material, at/or near midrange. This solution as a calibration standard should be prepared using the same type of acid and oxidising solution than in the test portion (the final concentration should be similar).

The acceptance criteria for the CV standard must be $\pm 10\%$ of its true value.

If the calibration curve cannot be verified within the specified limits, the causes must be determined and the instrument recalibrated before the samples are analysed. The analysis data for the CV must be kept on file with the sample analysis.

The calibration curve must also be verified at the end of each analysis batch and/or after every 10 samples. If the calibration cannot be verified within the specified limits, the sample analysis must be discontinued, the causes must be determined and the instrument recalibrated. All samples following the last acceptable test must be reanalysed.

7.8.2. Blank solution (see 7.2)

The maximum allowed blank concentration should be well documented, and if the blank solution exceeds this value all samples prepared along the contaminated blank should be prepared again and reanalysed.

7.8.3. Post digestion spike

Each unknown type of sample should be spiked to check for potential matrix effect.

This spike is considered as a single point standard addition, and should be performed with a minimum dilution factor. The recovery of spike calculated with equation 1 should be 85-115%. If this test fails, it is recommended to run analysis with standard addition method.

Spike solution: mix a fix volume (V1) of the sample solution, and a known volume (V2) of a standard solution with known concentrations (Cstandard).

Unspike solution: mix the same fix volume (V1) of sample solution, and the same volume (V2) of reagent water.

Measure the concentration C (mg l⁻¹) in both solutions on the calibration curve (see 7.6), and calculate recovery as:

$$C_{spike} = \frac{C_{standard} \times V_2}{(V_1 + V_2)} \quad \text{Equation 1}$$

$$R = \frac{C_{Spike\ Solution} - C_{Unspike\ solution}}{C_{spike}} \times 100 \quad \text{Equation 2}$$

To be valid, the concentrations of spiked and unspiked solutions should be in the linearity range of the calibration curve, and the spiked concentration (equation 1) should be in the range of 50-150% of the concentration of the unspiked solution.

7.8.4. Dilution test

If the analyte concentration is sufficiently high (minimally, a factor of 10 above the lower limit of the quantitation following dilution), an analysis of a 1:5 dilution should agree within

±10% of the original determination. If not, then a chemical or physical interference effect should be suspected, and method of standard addition is recommended.

7.8.5. Certified Reference Material

At least one certified reference material of a representative matrix should be prepared with each batch of sample, the calculated result should fall in the value of the certificate and within the coverage uncertainty (Linsinger, 2010), to show evidence of an unbiased result.

The results for the CRM should be recorded for quality control purpose and plotted on a control chart (UNEP/IOC/IAEA 1994).

An example of sequence order with recommended criteria and actions is given in table 1.

TABLE 1. EXAMPLE OF AN ANALYTICAL SEQUENCE:

Solutions Description	Performance	Action
Calibration blank	< maximum allowed calibration blank value	Stop until resolve
Standard solution 1–4	$r > 0.995$	recalibrate in the linearity range
CV	±10% of the true value	Stop until resolve
Sample blank	< maximum allowed blank value	
CRM	Fall in the certificate value within coverage uncertainty, or fall within acceptable criteria of the QC chart	Stop until resolve, check Matrix spike and run again with standard addition method if necessary
Matrix Spike	recovery $100\% \pm 15\%$	switch to standard addition, keep records for future analysis of the same matrix
Dilution Test	sample 1 = 5x sample 1 diluted 5x within 10%	switch to standard addition, keep records for future analysis of the same matrix
Unknown Sample 1–10	should \geq standard 1 and \leq standard 4	report as <minimum quantification limit or dilute
CV	±10% of the true value	Stop until resolve
Unknown Sample 11–20	should \geq standard 1 and \leq standard 4	report as <minimum quantification limit or dilute
Calibration blank	< maximum allowed calibration blank value	Stop until resolve
Standard solution 1–4	$r > 0.995$	recalibrate in the linearity range
CV	±10% of the true value	Stop until resolve
Etc....		

8. CALCULATION OF RESULTS

Results are calculated using equation 3

$$w(m) = \frac{(\rho_1 - \rho_0)}{m} \times f \times V \times R \quad \text{Equation 3}$$

Where:

w(m) is the mass fraction of element m in the sample, expressed in mg kg⁻¹;

ρ1 is the concentration of element m, expressed in mg/l as measured in the sample solution;

ρ0 is the concentration of element m expressed in mg/l as measured in the blank solution;

F is the dilution factor calculated as follow:

$$f = \frac{\text{final volume}}{\text{initial volume}}$$

or equal to 1 if ρ1 is determined in undiluted solution;

R is the recovery calculated using the CRM (see 7.8.5) or the post digestion spike.

m is the mass of sample in g

V is the volume of solution in ml

9. EXPRESSION OF RESULTS

The rounding of values will depend on the uncertainty reported with the result; in general for this method two or three significant figures should be reported.

Uncertainty component should be reported with all results. (ISO 2005, Nordtest 2004)

Example: w(Hg) = 0.512 ± 0.065 mg kg⁻¹.

10. REFERENCES

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Annex V.

HELCOM (2012). COMBINE Annex B-12, Appendix 4, Attachment 1. Technical note on the determination of Total Mercury in marine biota by Cold Vapour Atomic Absorption Spectroscopy (5.1.4)

HELCOM Manual for marine monitoring in the COMBINE programme

ATTACHMENT 1. TECHNICAL NOTE ON THE DETERMINATION OF TOTAL MERCURY IN MARINE BIOTA BY COLD VAPOUR ATOMIC ABSORPTION SPECTROSCOPY

1. POSSIBILITIES OF USING COLD VAPOUR ATOMIC ABSORPTION SPECTROMETRY IN TOTAL MERCURY ANALYSIS

The most widely used method for the determination of total mercury in biological tissues is cold vapour atomic absorption spectrometry (CV-AAS), based on a technique elaborated in detail by Hatch and Ott (1968). In this method, (divalent) ionic mercury is reduced to its metallic form (Hg⁰) in acidic solution using a powerful reducing agent. Subsequently, the elemental mercury is volatilized (purged) by a carrier gas and transported into an absorption cell, where the 253.65 nm wavelength absorbance of mercury atoms is measured.

CV-AAS analysis can be performed manually using batch CV-AAS or automatically using flow injection (FI) techniques. FI is a very efficient approach for introducing and processing liquid samples in atomic absorption spectrometry. The FI technique, combined with a built-in atomic absorption spectrometer optimised for mercury determination, reduces sample and reagent consumption, has a higher tolerance of interferences, lower determination limits and improved precision compared with conventional cold vapour techniques.

The efficiency of various flow injection mercury systems has been reported by several groups (Tsalev *et al.*, 1992a, 1992b; Welz *et al.*, 1992; Guo and Baasner, 1993; Hanna and McIntosh, 1995; Kingston and McIntosh, 1995; Lippo *et al.*, 1997). Better sensitivities of both conventional CV-AAS and FI-CV-AAS can be obtained by collecting mercury vapour released from the sample solution on a gold adsorber (Welz and Melcher, 1984). This so-called amalgamation technique eliminates kinetic interferences due to a different vaporization rate or a different distribution function of the elemental mercury between the liquid and the gaseous phases. The amalgamation ability of the gold adsorber must be carefully and regularly checked. Volatile compounds (in particular sulfur-containing compounds) evaporating together with the elemental mercury from the sample solution may deactivate the adsorber surface. This means an increased risk of underestimation, as unknown quantities of mercury are not collected by the adsorber.

2. SAMPLE PRETREATMENT

It is generally agreed that oxidative conversion of all forms of mercury in the sample to ionic Hg(II) is necessary prior to reduction to elemental Hg and its subsequent measurement by CV-AAS. Therefore, the initial procedural step in mercury analysis is a sample pretreatment, which is aimed at liberating the analyte element from its chemical bonding to the organic matrix and thus transforming all of the analyte species into a well-defined oxidation state. For this purpose, a wide variety of combinations of strong acids (HCl, H₂SO₄, HNO₃) and oxidants (H₂O₂, KMnO₄, K₂Cr₂O₇, K₂S₂O₈) have been tested and recommended (Kaiser *et al.*, 1978; Harms, 1988; Vermeiret *et al.*, 1989; Ping and Dasgupta, 1989; Baxter and Frech, 1990; Landi *et al.*, 1990; Navarro *et al.*, 1992; Lippo *et al.*, 1997).

A suitable sample pretreatment, which implies the complete transformation of all organomercury species into inorganic mercury ions, requires the following:

- oxidation mixtures with a high oxidation potential;
- rapid oxidation (usually promoted by high reaction temperatures), preferably in closed systems;

- compatibility with CV-AAS techniques;
- stability of sample solutions during storage (at least short term);
- no formation of solid reaction products.

On-line sample pretreatment is of particular interest in total mercury determinations because it allows reduction of the well-known problems associated with the inherent risk of contamination, and volatilization and adsorption losses. At present, suitable procedures for on-line pretreatment of solid biological samples are lacking. However, several authors (Tsalev *et al.*, 1992a 1992b; Welz *et al.*, 1992; Guo and Baasner, 1993) have demonstrated that microwave digestion coupled with FI-CV-AAS can successfully be applied to the analysis of liquid samples.

3. CONTROL OF CONTAMINATION AND ANALYTE LOSSES

Major difficulties arise due to the mobility and reactivity of mercury and its compounds, respectively, during sample preparation, sample pretreatment, and analysis. Therefore, the stability of samples and standard solutions is of prime importance, and it is advisable to test the stability of typical standard and sample solutions under typical laboratory conditions.

Mercury can disappear from solution due to several mechanisms, including volatilization of mercury compounds, reduction of such compounds followed by volatilization of elemental (metallic) mercury, adsorption on container walls, adsorption onto colloids or particles, incorporation into stable chemical complexes, or incorporation, upon reduction, into stable amalgams.

Thermodynamic considerations of Toribara *et al.* (1970) showed that loss of mercury from a solution containing the element in the monovalent form may occur readily through disproportion and subsequent loss of metallic mercury. Because of the high oxidation potential of the mercury(II)-mercury(I) system, almost any reducing substance could convert some divalent mercury ions into monovalent mercury ions, which then spontaneously disproportionate into mercury(II) and mercury(0). The latter escape as metallic vapour from the solution into the gas phase. Because of the almost impossibility of preventing the introduction of small amounts of reducing substances by reagents or solvents, the more dilute mercury(II) solutions would be less stable and lose mercury more readily. The only practical method for stabilizing such solutions is to add a small excess of an oxidising substance (such as permanganate), which has a higher oxidation potential than the mercury(II)-mercury(I) system.

Similarly, Feldman (1974) concluded from his experiments that solutions with 0.1 µg divalent Hg dm⁻³ in distilled water could be stored in glass vials for as long as five months without deteriorating if the solutions contained 5 % (v/v) HNO₃ and 0.01 % Cr₂O₇²⁻. Storage of such solutions was safe in polyethylene vials for at least 10 days if the solutions contained 5 % (v/v) HNO₃ and 0.05 % Cr₂O₇²⁻. The efficiency of this mixture was probably due to its ability to prevent the hydrolysis of dissolved mercury and prevent its reduction to valencies lower than +2.

4. REDUCING REAGENTS

Tin(II) chloride and sodium tetrahydroborate are predominantly used as reducing reagents for the determination of total mercury by CV-AAS. Sodium tetrahydroborate has been found advantageous for several applications owing to its higher reducing power and faster reaction (Toffaletti and Savory, 1975). In addition, this reductant has been successfully used even in the presence of interfering agents such as iodide and selenium (Kaiser *et al.*, 1978). However, potential interferences can occur from metal ions (e.g., Ag(I), Cu(II), Ni(II)), which are themselves reduced to the metallic state and so may occlude mercury through amalgamation.

Welz and Melcher (1984) showed that sodium tetrahydroborate could more readily attack those organic mercury compounds which were not reduced to metallic mercury by tin(II) chloride.

However, they stated that sodium tetrahydroborate could not be recommended as the reducing reagent for the amalgamation technique. They found that, due to the rather violent reaction with sodium tetrahydroborate, fine droplets of the sample solution were carried by the gas stream and contaminated or deactivated the adsorber surface. Further, they considered even more important the fact that not only mercury but all gaseous hydride-forming elements (e.g., arsenic, antimony, selenium) were volatilized when sodium tetrahydroborate was used as reductant. These hydrides reacted with the adsorber material and deactivated its surface, thus no longer permitting a sensitive and reproducible determination of mercury.

5. INTERFERENCES

Interferences by volatile nitrogen oxides in the determination of mercury by FI-CV-AAS were studied by Rokkjaer et al. (1993). The main symptom of the interference effects was a suppression, broadening or even splitting of the mercury signal. The authors postulated that volatile nitrogen oxides formed as reaction products of nitric acid during sample decomposition scavenged the reducing agent and concomitantly inhibited the reduction of mercury(II). The rate of the reaction of nitrogen oxides with the reducing agent was considered to be so fast that it was consumed before the reduction of mercury was complete. Rokkjaer et al. (1993) demonstrated that the interference could easily be remedied by purging the sample solution with an inert gas prior to the introduction of the reducing agent. Lippo et al. (1997) concluded from their experiments that nitrogen mono- and dioxide, having molecular absorption bands at 253.63 nm and 253.85 nm, respectively, might cause unspecific absorption at the specific mercury wavelength of 253.65 nm, leading to enhanced and broadened mercury signals if not properly compensated for by adequate instrumental background correction.

6. INTERNAL (ROUTINE) QUALITY CONTROL

In order to demonstrate that the analytical method applied is fit for the purpose of the investigations to be carried out, control materials should be regularly analysed alongside the test materials (cf. Chapter B.5 of the Manual).

The control materials - preferably certified reference materials (CRM) - should be typical of the test materials under investigation in terms of chemical composition, physical properties and analyte concentration. Fitness for purpose is achieved if the results obtained from the analysis of the control materials are within the defined limits of permissible tolerances in analytical error (see Chapters B.3.5, B.4.2.5 and B.4.2.5.2b of the Manual).

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Annex VI.

UNEP/IAEA (2011). Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment. Recommended Methods for Marine Pollution Studies No 71



UNITED NATIONS ENVIRONMENT PROGRAMME

November 2011

Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment

Recommended Methods For Marine Pollution Studies 71

Prepared in co-operation with



NOTE: This recommended method is not intended to be an analytical training manual. Therefore, the method is written with the assumption that it will be used by formally trained analytical chemists. Several stages of this procedure are potentially hazardous; users should be familiar with the necessary safety precautions.

For bibliographic purposes this document may be cited as:

UNEP/IAEA: Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment. Reference Methods for Marine Pollution Studies No 71, UNEP, 2011.

PREFACE

The Regional Seas Programme was initiated by UNEP in 1974. Since then, the Governing Council of UNEP has repeatedly endorsed a regional approach to the control of marine pollution and the management of marine and coastal resources and has requested the development of regional action plans. The Regional Seas Programme at present includes thirteen regions and has over 140 coastal States participating in it (1).

One of the basic components of the action plans sponsored by UNEP in the framework of the Regional Seas Programme is the assessment of the state of the marine environment, its resources and the sources and trends of the pollution and its impact on human health, marine ecosystems and amenities. In order to assist those participating in this activity and to ensure that the data obtained through this assessment can be compared on a world-wide basis and thus contribute to the Global Environment Monitoring System (GEMS) of UNEP, a set of Reference Methods and Guidelines for marine pollution studies are being developed as part of a programme of comprehensive technical support which includes the provision of expert advice, reference methods and materials, training and data quality assurance (2). The Methods recommended for adoption by Governments participating in the Regional Seas Programme.

The methods and guidelines are prepared in co-operation with the relevant specialised bodies of the United Nations system as well as other organisations and are tested by a number of experts competent in the field relevant to the methods described.

In the description of the methods and guidelines, the style used by the International Organisation for Standardisation (ISO) has been followed as closely as possible.

The methods and guidelines published in UNEP's series of Reference Methods for Marine Pollution Studies are not considered as definitive. They are planned to be periodically revised taking into account the new developments in analytical instrumentation, our understanding of the problems and the actual need of the users. In order to facilitate these revisions, the users are invited to convey their comments and suggestions to:

Marine Environmental Studies Laboratory
IAEA Environment Laboratories
4, Quai Antoine 1^{er}
MC 98000 MONACO

which is responsible for the technical co-ordination of the development, testing and inter-calibration of Reference Methods.

References:

(1) www.unep.org/regionalseas (2011)

(2) UNEP/IAEA/IOC: Reference Methods and Materials: A Programme of comprehensive support for regional and global marine pollution assessment. UNEP, 1990.

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1. SCOPE AND FIELD OF APPLICATION

This reference method is intended for use in monitoring programmes and pilot research studies. The document describes procedures for the isolation of purified fractions amenable for the determination of DDTs and PCBs in marine sediments and marine organisms by capillary GC/ECD. It is assumed that most of the participants in the UNEP Regional Seas Programmes are equipped with advanced high resolution capillary gas chromatographs and will be able to implement most, if not all, of the procedures described in Reference Method No 40, "Determination of DDTs and PCBs by capillary gas chromatography and electron capture detection" (UNEP 1988). Assuming consistent results are routinely being obtained with these methods by the analytical laboratory, the determination of specific compounds (as opposed to generic mixture of PCBs) opens up the possibility not only of identifying environmental "hot spots", but also for characterising sources, elucidating transport pathways and developing data of greater toxicological relevance. The organisation and content of this document, however, deserves further comment. Under the sections devoted to SEDIMENTS and ORGANISMS, subsections are provided relating to procedures for: 1) Sampling, 2) Extraction and 3) Clean-up and fractionation. In each subsection, several alternative procedures are described. These various procedures have been previously tested and are provided to accommodate the range of capabilities in participating laboratories. For example, laboratories which have access to an HPLC may consider the benefits of using HPLC fractionation procedures in lieu of more conventional low pressure column chromatographic method. Participants are generally encouraged to implement the most effective procedures within the constraints of their individual laboratories.

Several other halogenated pesticides and other electron capturing organic compounds may be present in environmental samples and many of these compounds could also be isolated by the methods described here. However, not all residues will be stable to the clean-up procedures applied for the determination of PCBs and DDTs. Consequently, every analyst must test for analyte recovery and analytical reproducibility prior to applying these methods for other analytes on a routine basis. Primary emphasis should be placed on obtaining the cleanest possible purified fraction for capillary GC/ECD analysis so that interferences and misidentification are minimised, if not eliminated.

2. PRINCIPLES

Following collection of sediment or biota samples using appropriate techniques, samples are stored in trace organic free vessels at -20°C until analysis. For analysis, the samples are prepared for solvent extraction. To achieve a satisfactory recovery of the chlorinated hydrocarbons, samples are dried by either desiccation with anhydrous sodium sulphate or by freeze-drying. Lipids are then Soxhlet extracted from sediments using hexane and dichloromethane, and from biota using hexane or petroleum ether. Following initial clean-up treatments (removal of sulphur from sediment extracts and treatment of biota extracts with concentrated sulphuric acid to destroy some interfering lipids), extracts are fractionated using column chromatography. Detailed protocols for absorption chromatographic fractionation are described for both low and high pressure systems, using Florisil and silica gel respectively. (Additional information concerning alternative techniques including gel permeation chromatography is provided).

3. REAGENTS, SOLVENTS, STANDARDS

3.1. Reagents

3.1.1. List of reagents

- Demineralized distilled water produced by distillation over potassium permanganate (0.1 g/l KMnO₄) or equivalent quality, demonstrated to be free from interfering substances.
- Detergent.
- Potassium dichromate.
- HCl. 32%.
- Concentrated H₂SO₄ (d 20°C: 1.84 g/ml).
- Sulfochromic cleaning solution made from concentrated sulphuric acid and potassium

- dichromate.
- KOH.
- Anhydrous sodium sulphate.
- Copper fine powder (particle size 63µm).
- Carborundum boiling chips.
- Hg.
- Glass wool
- Alumina (200-240).
- Silica gel (60-100).
- Florisil PR (60-100).
- Bio-Beads SX-3 (200-400).
- Sephadex LX-20.

Solvents:

- Hexane, Dichloromethane, Methanol, Pentane, Cyclohexane, Toluene and Ethyl Acetate, all “distilled in glass” quality.

Standards:

- PCB congeners: 29, 30, 121, 198.
- ε HCH.
- Endosulfan Id₄.
- n-C₁₄ d₃₀, n-C₁₉ d₄₀, n-C₃₂ d₆₆.
- Naphthalene d₈.
- Hexamethylbenzene.
- Cadalene: 1, 6-dimethyl-4-(1-methylethyl)naphthalene.
- DDT reference solutions - Prepare a stock solution of the DDT series (pp' DDT, op DDT, pp' DDD, op DDD, pp' DDE, op DDE) by dissolving 50 mg of each compound in 100 ml of hexane. Store stock solution in sealed glass ampoules.
- Other reference solutions - should be prepared if other residues are to be quantified in these procedures.

NOTES:

Working solutions obtained from the stock reference solutions should be prepared on a regular basis depending on their use and stored in clean glass volumetric flasks tightly capped with non-contaminating materials such as Teflon or glass. Extreme care must be taken to ensure that the concentrations of the standards have not altered due to solvent evaporation.

In order to achieve acceptable accuracy for the standard solutions, at least 50 mg of pure individual compound should be weighed and dissolved into 100 ml of hexane. This will give stock solutions of 500ng/µl.

Example of preparation of stock solutions:

Preparation of a stock solution of pp' DDE at approximately 500ng/µl:

The pp' DDE stock solution is prepared by dissolving approximately (but weighed accurately) 50 mg of pp' DDE in hexane in a 100 ml volumetric flask and bringing the volume to exactly 100 ml with hexane. If the actual weight of pp' DDE is 52 mg, then

$$\frac{52 \text{ mg DDE}}{100 \text{ ml solvent}} \times \frac{1000 \mu\text{g}}{\text{mg}} \times \frac{\text{ml}}{1000 \mu\text{l}} = \frac{52 \text{ mg DDE}}{100 \text{ ml of solution}}$$

$$52 \text{ mg}/100 \text{ ml} \Rightarrow 0.52 \text{ mg/ml} \Rightarrow 520 \mu\text{g/ml} \Rightarrow 520 \text{ ng}/\mu\text{l}$$

The concentration of the stock solution will be: 520ng/µl

Preparation of an intermediate solution:

Use the stock solution to prepare the intermediate solution. The concentration of pp' DDE intermediate solution should be approximately 5ng/μl. To prepare the 5ng/μl intermediate solution, transfer 1 ml of the pp' DDE stock solution into a 100 ml volumetric flask and dilute with hexane to 100 ml.

$$\frac{1 \text{ ml DDE stock solution}}{100 \text{ ml final volume}} \times \frac{520 \text{ ng DDE}}{\mu\text{l}} = \frac{5.2 \text{ ng}}{\mu\text{l intermediate solution}}$$

The concentration of the intermediate solution will be: 5.2 ng/μl

Preparation of the working solution:

Use the intermediate solution to prepare the working solution. The concentration of pp' DDE in the working solution could be approximately 50pg/μl.

To prepare the 50 pg/μl working solution, transfer 1 ml of the pp' DDE intermediate solution into a 100 ml volumetric flask and dilute with hexane to 100 ml.

$$\frac{1 \text{ ml DDE intermediate solution}}{100 \text{ ml final volume}} \times \frac{5.2 \text{ ng}}{\mu\text{l}} \times \frac{1000 \text{ pg}}{\text{ng}} = \frac{52 \text{ pg}}{\mu\text{l working solution}}$$

The concentration of the working solution will be: 52 pg/μl

3.1.2. Cleaning of solvents

All reagents, including the distilled water should be of analytical quality. Commercially available solvents like acetone, acetonitrile, dichloromethane, hexane and pentane are invariably contaminated with ECD-active substances; their concentrations vary from batch to batch and with supplier. Reagent quality should be checked by injection of 2 μl of a 100 ml batch of solvent, after concentration to 50 μl in a rotary evaporator. No peak in the GC-ECD chromatogram (90 - 250 °C) should be larger than that for 1pg of lindane. Otherwise, the solvent must be distilled. The following procedure has been found to be both efficient and cost effective, as it allows the use of technical grade solvents as the basic material (reducing the cost by one order of magnitude). 130 - 150 cm height columns are required; the packing material must be glass (to allow subsequent cleaning with an oxidising acid). The entire equipment is cleaned prior to use by 2 consecutive distillation procedures with 500 ml water in each case. It is essential that a current of nitrogen gas (15 ml/min) flows from the distillation flask during distillation of the organic solvents: the condenser serves as exhaust. Ambient air is not in contact with the solvent in this way. Problems are associated with other methods of excluding room air (e.g., active carbon or molecular sieves), the most important one being discontinuity. The condensate is distilled into a 1 litre flask at a 1:20 ratio. This large volume allows for direct transfer into the appropriate solvent containers which should be made of glass and of a sufficient size to provide solvent for not more than 6 analyses. A bottle with sufficient solvent for 10 - 15 analysis has to be opened and closed many times and even when kept closed, when not in use, contamination from the surrounding atmosphere takes place. For more detailed information, consult the Reference Method No 65: UNEP/IOC/IAEA: Reagent and laboratory ware clean-up procedures for low-level contaminant monitoring.

3.1.3. Cleaning of reagents and adsorbents

3.1.3.1. Cleaning of reagents

Powdered or crystalline reagents, such as anhydrous sodium sulfate (Na₂SO₄)*, potassium hydroxide (KOH), glass wool * and carbon or carborundum boiling chips *, must be thoroughly cleaned before use. They should be extracted with hexane in a Soxhlet apparatus for 8 hours and then with methanol or dichloromethane for another 8 hours. For those items indicated by an *, this will require pre-combustion in a muffle furnace at approximately 400°C.

3.1.3.2. Cleaning of adsorbents

Silica gel, alumina and Florisil have to be solvent extracted. Each reagent is first refluxed with methanol or dichloromethane in a Soxhlet apparatus for 8 hours, then with n-hexane for the same period. The solvent is removed by a rotary evaporator operating at low speed, until the sorbent starts falling down as fine particles. Reagents are dried in a drying oven at 0.01 mbar. If this is not available, they are dried in a normal oven at 120°C for 4 hours. This serves to activate silica and alumina. Florisil has to be activated at 130°C for 12 hours. The sorbent is allowed to cool in the oven (if possible under vacuum to avoid uptake of contaminants from the atmosphere) or alternatively, in a dessicator. As active sorbents attract water and contaminants from the atmosphere, controlled deactivation should be carried out by adding water to the fully active sorbent (5% by weight to silica, 2% by weight to alumina, and 0.5% by weight to Florisil). The deactivation procedure should be carried out by adding the water to the sorbent and mixing by gentle shaking for a few minutes. The equilibration takes one day. The activity can be maintained for longer periods of time by sealing the required amount of sorbent in glass ampoules. Otherwise, the activation/deactivation has to be done the day before use.

3.2. Apparatus and equipment

The laboratory used for organic trace analysis must be a dedicated facility, isolated from other projects that could be sources of contamination. It must be properly constructed with fume hoods and benches with electric sockets that are safe for use with flammable solvents. The laboratory must have extractors and rotary evaporators cooling water to run the stills. In tropical regions and in dry climates, a refrigerated re-circulating system should be used to reduce temperatures to the required levels and/or to conserve water. Stainless steel or ceramic tiles make good non-contaminating surfaces. If necessary, benches can be coated with a hard epoxy resin and walls can be painted with epoxy paint. A sheet of aluminium foil on the workbench provides a surface which can be cleaned with solvent. A vented storage facility for solvents is essential. Benches must be fitted with frames to hold stills, extractors, etc. The emergency cut-off switch should be accessible from both inside and outside the laboratory. Fire fighting equipment should be mounted in obvious places and laboratory personnel trained in their use.

3.2.1. List of materials

- A coring device with liners and plunger or a grab sampler (thoroughly cleaned with detergents and solvents before use).
- Glass jars and aluminium foil, stainless steel knives, scoops, forceps, labels, marking pens, logbook.
- Insulated plastic boxes for transporting samples. Ice or dry ice.
- Deep freezer (-18 to -20°C) for sample preservation (frost free type freezers heat to above zero during frost removal cycles and they cannot be used for long term storage).
- Rotary evaporator.
- Kuderna-Danish (or similar) concentrator and heater.
- Soxhlet extraction apparatus and heaters.
- Glassware including boiling flasks, ground glass stoppers, beakers, Erlenmeyer flasks, separatory funnels, centrifuge tubes, weighing bottles, pipettes, tissue grinders.
- Drying oven (temperature range up to at least 300°C) for determining sample dry weights, baking of contaminant residues from glassware and reagents.

Note: A muffle furnace is better for baking materials at greater than 300°C, if required.

- Centrifuge and tubes.
- Freeze-dryer and porcelain pestle and mortar.
- Analytical balance with an accuracy of 0.1 mg and an electro-balance with an accuracy of at least 1 µg.
- Stainless steel tweezers and spatulas.

- Dessicator - completely free of organic contamination and with no grease applied to sealing edges.
- Supply of clean, dry nitrogen.
- Columns for silica gel, alumina and Florisil chromatography.
- Mechanical blender (food mixer).
- Vacuum pump (water-jet air pump).

3.2.2. Cleaning of glassware

Scrub all glassware vigorously with brushes in hot water and detergent. Rinse five times with tap water and twice with distilled water. Rinse with acetone or methanol followed by hexane or petroleum ether. Bake overnight in an oven at 300 °C. All glassware should be stored in dust free cabinets and tightly sealed with pre-cleaned aluminium foil when not in use. Ideally glassware should be cleaned just before use.

For more detailed information, consult Reference Method No 65: UNEP/IOC/IAEA: Reagent and laboratory ware clean-up procedures for low level contaminant monitoring.

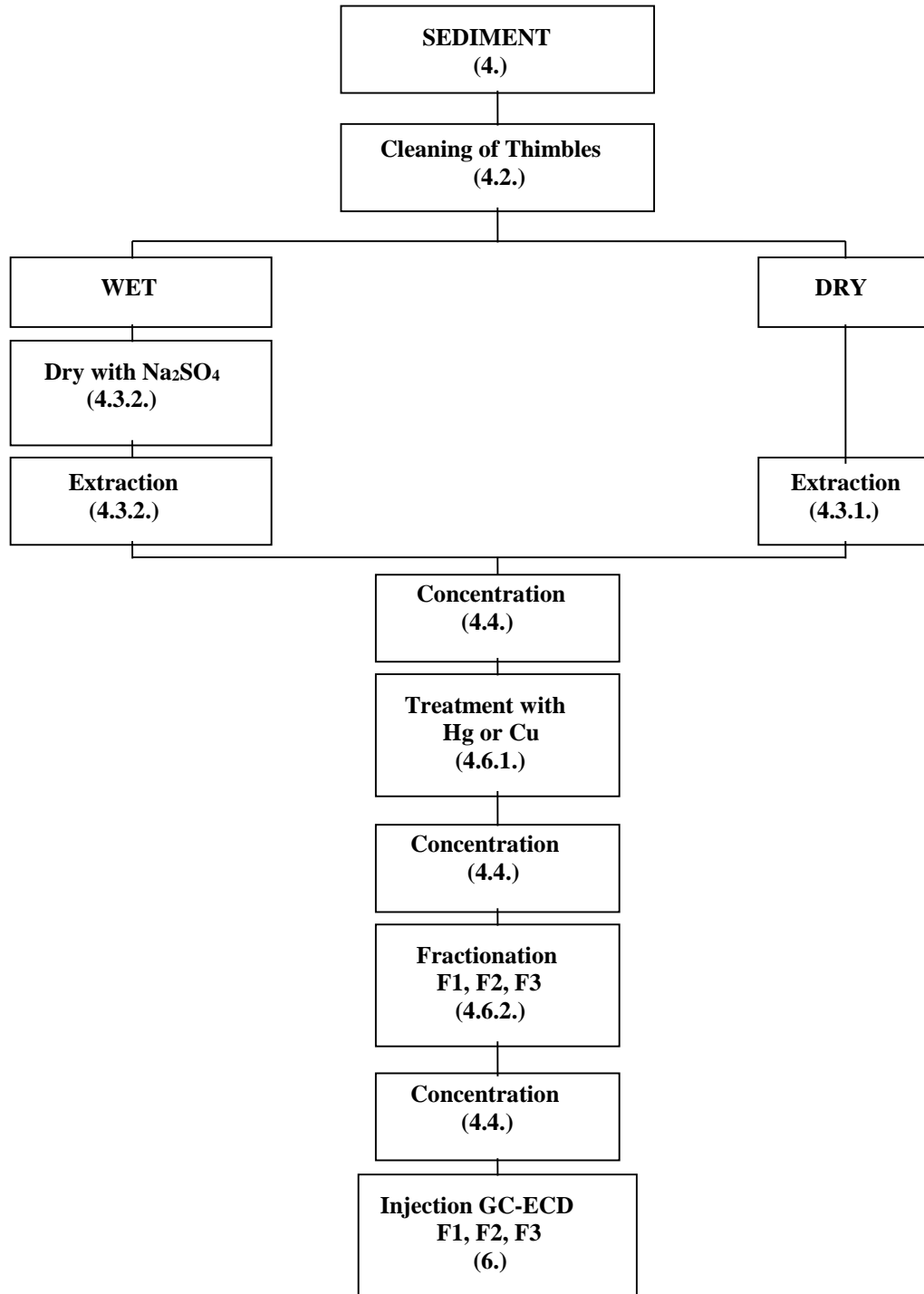


Diagram of the extraction procedure for sediment samples.

4. SEDIMENTS

4.1. Sampling

For the preparation of the samples (including selection of sites, collection of samples and storage) the reader should refer to the Reference Method N° 58: Guidelines for the use of sediments for the marine pollution monitoring programmes, to the Reference Method N° 20: UNEP/IOC/IAEA: Monitoring of petroleum hydrocarbons in sediments and to UNEP(DEC)/MEDW.C282/Inf.5/Rev1: Methods for sediment sampling and analysis (2006).

4.2. Cleaning of extraction thimbles

Paper extraction thimbles should be cleaned prior to sample extraction. For use in the extraction of sediment samples, the extraction can be performed in the Soxhlet apparatus with 250 ml of a mixture hexane / dichloromethane (50:50) for 8 hours cycling the solvent through at a rate of 4 to 5 cycles per hour. Add into the solvent a few carborundum boiling chips to get a regular ebullition.

The use of disposable paper thimbles for the extraction procedure rather than re-usable glass fibre thimbles is recommended due to the difficulties encountered in cleaning the latter.

4.3. Extraction of sediments

4.3.1. Extraction of freeze-dried samples

Select a 50-100 g sub-sample of the sediment, weigh this sub-sample and freeze-dry it. When dried, re-weigh it and calculate the dry to wet ratio. Then pulverise the sample using a pestle and mortar and sieve it using a 250 µm stainless steel sieve. Accurately weigh about 20 g of ground sample and place it in the pre-cleaned extraction thimble. Add 1 ml of a solution of 25 pg/µl of 2,4,5 trichlorobiphenyl (PCB N° 29), 20.9 pg/µl of 2,2',3,3',4,5,5',6 octachlorobiphenyl (PCB N° 198), 20 pg/µl of ε HCH and 21 pg/µl of Endosulfan Id₄ as internal standards and extract for 8 hours in a Soxhlet apparatus with 250 ml of a mixture hexane / dichloromethane (50:50), cycling the solvent through at a rate of 4 to 5 cycles per hour, add into the solvent a few carborundum boiling chips to get a regular ebullition. Alternatively (or in addition), PCB congeners No 30, 121, or octachloronaphthalene and PCB congeners can be used as internal standards. Prepare a procedural blank by extracting an empty thimble using the same procedure as for the samples.

4.3.2. Extraction of wet samples

The sediment is thawed, sieved at 250 µm and homogenised manually with a stainless steel spatula or clean glass rod. A sub-sample of 1-2 g is weighed into a flask and placed in a drying oven at 105 °C for 24 hours, then allowed to cool to room temperature and re-weighed. Calculate the dry to wet ratio and discard the dry sediment (unless it is being used for other analysis e.g. TOC, total organic carbon).

Place a 30-40 g sub-sample of thawed, homogenised sediment into a blender. Slowly, add 100g of anhydrous sodium sulphate (desiccant) and blend the mixture at high speed for 10 minutes. Transfer the dried sample quantitatively to the pre-cleaned extraction thimble in the Soxhlet apparatus, add the internal standard solution (see above) and apply the same extraction procedure as above. Extract the same amount of sodium sulphate as a procedural blank, making sure to add an appropriate amount of internal standard solution.

4.3.3. Example of determination of percent moisture

Many environmental measurements require the results to be reported on a dry weight basis. The percent moisture or water content in the sample is determined by weighing an aliquot, not used for analysis, of the sample before and after drying. The drying can be done by heating a few grams (1-2 g) of the sample in an oven to constant weight.

Weigh an empty glass beaker that will be used to hold the sample while it is dried.
Empty beaker weight = 10.4417 g

Add the wet sample to the beaker and reweigh. Calculate the wet weight of the sample.
Empty beaker weight + wet sample = 12.2972 g
Wet sample weight = 12.2972 g - 10.4417 g = 1.8555 g

Dry the sample to constant weight: dry the sample for 24 hours, weigh it, dry again for 12 hours, re-weigh it, when the difference in weight is less than 5%, it means that the sample is dried.

Empty beaker weight + dry sample weight = 10.9396 g
Dry sample weight = 10.9396 g - Empty beaker weight
Dry sample weight = 10.9396 g - 10.4417 g = 0.4979 g

Calculate the percent dry sample weight.

$$\begin{aligned}\% \text{ Sample weight} &= \frac{\text{Sample dry weight}}{\text{Sample wet weight}} \times 100 \\ &= \frac{0.4979}{1.8555} \times 100 = 26.8 \%\end{aligned}$$

Calculate the percent moisture.

$$\begin{aligned}\text{Water content} &= \text{wet weight} - \text{dry weight} \\ &= 1.855 \text{ g} - 0.4979 \text{ g} = 1.3576 \text{ g}\end{aligned}$$

$$\% \text{ Moisture} = \frac{\text{Sample water weight}}{\text{Sample wet weight}} \times 100$$

$$\% \text{ moisture} = \frac{1.3576}{1.8555} \times 100 = 73.2 \%$$

4.4. Concentration of the extract

For both extraction procedures, the extracts are concentrated in a rotary evaporator to about 15 ml. Under good vacuum conditions the temperature of the water bath must not exceed 30 °C. Dry the extract with anhydrous sodium sulphate (when the sodium sulphate moves freely in the flask it means that the extract is dried). Collect the dried extract in the graduated tube of a Kuderna-Danish concentrator. Concentrate the extract to approximately 5 ml with the Kuderna-Danish concentrator and adjust the volume to exactly 1 ml by evaporating excess solvent under a gentle stream of clean dry nitrogen. The sample extract will be analysed gravimetrically for extractable organic matter (EOM) content at the 1 ml volume as a starting point. If measurements of the EOM are outside the calibration range of the balance, the total volume of the extract is adjusted accordingly using either dilution with hexane or evaporating under a stream of nitrogen gas.

4.5. Extractable organic matter

Before carrying out the clean-up procedure, it is advisable to determine the extractable organic matter.

The EOM is determined in the following manner. On the weighing pan of an electro-balance, evaporate a known volume of the sediment or biota extract (up to 100 µl) and weigh the residue with a precision of about ± 1 µg. If the residue is less than 2 µg, pre-concentration of the original extract is required. The quantity of EOM is:

$$\text{EOM } (\mu\text{g/g}) = \frac{\text{Weight of residue } (\mu\text{g}) \times \text{volume of the extract (ml)} \times 1000}{\text{Volume evaporated } (\mu\text{l}) \times \text{quantity of sample extracted (g)}}$$

Note that extreme care must be taken to ensure balance and pans are clean, dry and stable to obtain accurate readings at the ± 1 µg level. A small hot plate is used to warm pans and forceps and thus keep these instruments dry after solvent cleaning. If no electro-balance is available, a known volume of the extract can be transferred into a clean pre-weighed beaker. The solvent is evaporated with dry and clean nitrogen until a constant weight of about 1 mg is reached. Calculate the amount of “lipids” in the sample taking into account the volume of the lipid extract which was dried.

Example of calculation of E.O.M.

The extractable organic matter content of a sample is operationally defined as the weight of material extracted with the solvent employed (H.E.O.M. in case hexane is used as solvent). An aliquot of the sample extract is taken (few µl), the solvent is evaporated and the residue is weighed to determine the quantity of lipids extracted in the aliquot and from it to the total sample. The results are normally reported in mg lipids per gram dry weight extracted.

A 1 µl aliquot is removed from a 2.5 ml sample extract for determination of E.O.M. The 1 µl aliquot is evaporated on the pan of an electro-balance and the residue is weighed. Three determinations are made and the average taken.

Measurements:

Sample dry weight extracted: 4.443 g

Total volume of the extract: 2.5 ml

Sample aliquot removed: 1 µl

(1) Weight of a 1 µl aliquot after solvent evaporation: 32.2 µg

(2) Weight of a 1 µl aliquot after solvent evaporation: 32.1 µg

(3) Weight of a 1 µl aliquot after solvent evaporation: 32.3 µg

Average weight of a 1 µl aliquot : 32.2 µg

Total volume of the extract: 2.5 ml

Total quantity of lipids in the sample:

$$32.2 \mu\text{g}/\mu\text{l} \times 2.5 \text{ ml} \times \frac{1000 \mu\text{l}}{\text{ml}} = 80500 \mu\text{g} \text{ or: } 80.5 \text{ mg}$$

With 4.443 g of sample extracted:

$$80.5 \text{ mg} / 4.443 \text{ g} = 18.1 \text{ mg lipids/g}$$

4.6. Clean-up procedure and fractionation

Purposes of the clean-up: removal of lipids, whenever present at a significant amount; removal of elementary sulphur and sulphur compounds. Both these compound classes can interfere with the gas-chromatographic separation.

4.6.1. Sulphur and sulphur compounds removal

Elementary sulphur and sulphur compounds such as mercaptans should be removed from the extract. This could be done by using either mercury or activated copper.

a) Mercury method.

Add one drop (a few ml) of mercury to the sediment extract and shake vigorously for one minute. Centrifuge and carefully recover and transfer the extract in another tube with a Pasteur pipette. If the mercury is still tarnished, repeat the treatment with another drop of mercury, shake, transfer the hexane into another tube. Repeat this treatment until the mercury stays brilliant in the extract. Rinse the mercury with 5 ml of hexane and combine the extracts. Then, concentrate the resulting solution to ca. 1 ml with a gentle stream of nitrogen.

Cleaning of mercury:

Caution: When removing mercury from the sample, always use a plastic tray to keep the glassware in and work under a fume hood.

Fit a folded filter paper in a 10 cm diameter conical glass funnel and fix the funnel over a 250 ml glass beaker. Using a needle, make a small hole in the bottom of the filter paper. Carefully put the mercury onto the funnel. The mercury flows through the small hole in the filter paper leaving the solid impurities on its surface. The mercury collected is washed three times by shaking it carefully with dichloromethane and by removing dichloromethane layer with the help of a clean glass syringe. Allow the rest of dichloromethane evaporate and store the clean mercury in a thick walled glass bottle with a ground glass stopper. In order to avoid escape of mercury vapour, store the mercury under methanol.

Another way of cleaning the mercury involves sucking the dirty mercury through a capillary tube, such as a Pasteur pipette, connected to a guard-flask and then to a vacuum pump. The mercury will pass through the Pasteur pipette and will be collected and cleaned in the guard-flask. Then it should be transferred into a thick wall glass bottle with a ground glass stopper. The mercury is covered with a layer of methanol to protect it from oxidation.

b) Activated copper method.

Transfer about 20 grams of the copper powder in an Erlenmeyer. Add enough concentrated HCl to cover the copper powder, agitate. Sonicate for 10 min., agitate, put again in ultrasonic bath and sonicate for 10 min. Throw the used HCl, add some fresh HCl, transfer in ultrasonic bath and sonicate for 20 min. repeat that procedure four times in total. Wash with distilled water, agitate, discard, add water again, transfer in ultrasonic bath and sonicate for 15 min., discard the used water, repeat that procedure again, up to pH neutral. Wash with acetone, agitate, transfer in ultrasonic bath and sonicate for 15 min. repeat that procedure four times in total. Then use the same procedure with hexane as a solvent.

Keep in hexane (use it immediately, avoids Cu to be in contact with air).

Transfer 3 to 4 Pasteur pipettes per sample in the flasks containing the hexane extracts. Let the copper react all night. The presence of sulphur compounds in the sample will be detected by the tarnishing of the copper powder. Then, concentrate the resulting solution to ca. 1 ml with a gentle stream of pure nitrogen.

4.6.2. Fractionation

An adsorption chromatography step is used to remove interfering lipids and to fractionate the extract into classes of compounds. Many variations of adsorption chromatography clean-up procedures have been published to date. Four procedures are reported here in order of increasing complexity.

Preparation of the columns: Glass burettes (1 cm diameter) with Teflon stopcocks make convenient adsorption columns. The column is plugged with pre-cleaned cotton or glass wool. Prepare separate columns for each sample and blank determination. The column is partially filled with hexane. The appropriate amount of sorbent is mixed with hexane in a small beaker to form a slurry. A glass funnel and a glass rod are used to pour the adsorbent into the column. Several rinses with hexane are necessary to fill the column to the desired height. Tap with a pencil or a hard silicone tube against the column in order to settle the adsorbent into an even bed. Flush the material adhering to the wall of the column down to the bed with solvent. Prepare each column freshly immediately before use. Never let the column get dry.

4.6.2.1. Florisil

A Florisil column is used for this fractionation, which is prepared in the following way. The Florisil should be pre-extracted in the Soxhlet apparatus to remove any contaminants, using methanol or dichloromethane for 8 hours, followed by hexane for another 8 hours. It is then dried in an oven. Activation is achieved by heating the dried Florisil at 130°C for 12 hours. It is then partially deactivated with 0.5% water by weight and stored in a tightly sealed glass jar with ground glass stopper. The water should be well mixed into the Florisil and the mixture should be allowed to equilibrate for one day before use. The activation/deactivation procedure should be carried out one day before use. A 1 cm burette with Teflon stopcock is plugged with pre-cleaned glass wool. A column with a sintered glass disk could also be used. 17 grams of Florisil are weighed out in a beaker and covered with hexane. A slurry is made by agitation and poured into the glass column. The Florisil is allowed to settle into an even bed and any Florisil adhering to the column is rinsed down with hexane. The solvent is drained to just above the Florisil bed. It should be rinsed with a further 5 ml of hexane; one gram of anhydrous sodium sulphate is added to the top of the column in order to protect the surface of the Florisil from any disturbance. The column should never run dry. Individual columns should be prepared immediately before use and a new column of Florisil used for each sample.

The extract, reduced to 1 ml, is put onto the Florisil column. It is carefully eluted with 65 ml of hexane and the first fraction collected. Then the column is eluted with 45 ml of a mixture containing 70 % of hexane and 30 % of dichloromethane and the second fraction collected. The third fraction will be eluted with 60 ml of pure dichloromethane.

Fraction one will contain the PCBs, pp' and op DDE and some other pesticides such as HCB, aldrin, heptachlor, DDMU.

Fraction two will contain the DDTs, DDDs, most of the toxaphene, and some pesticides such as the HCH isomers and chlordane components.

Fraction three will contain mainly dieldrin, endrin, heptachlor epoxide and endosulfan components. Typical chromatograms obtained are shown below.

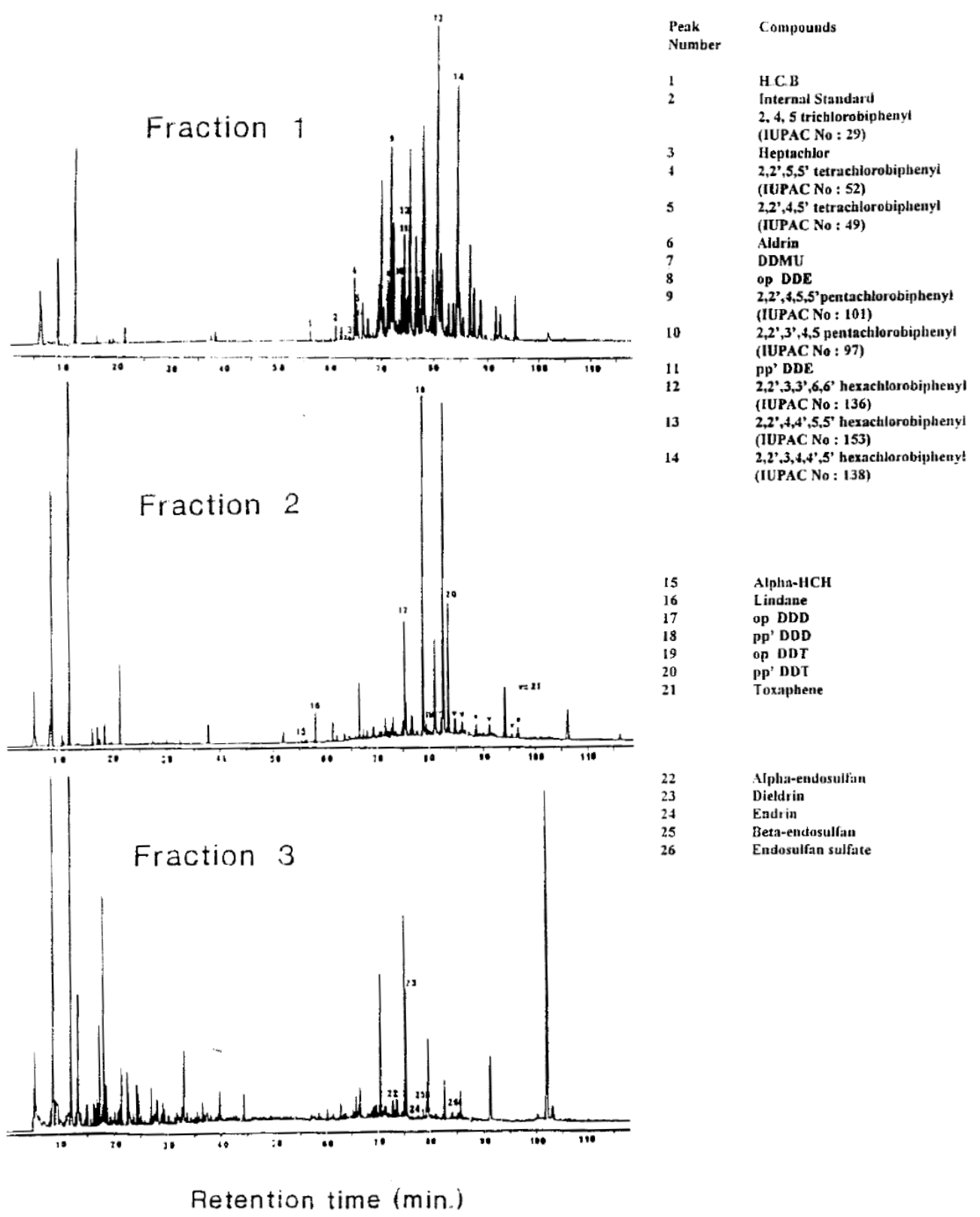


Figure 2: GC-ECD organohalogen analyses

4.6.2.2. Gel permeation chromatography

Low pressure GPC can be used as an alternative clean-up technique to remove high molecular weight co-extractable lipidic material from polycyclic aromatic compounds and halogenated aromatics. Concurrently, elemental sulphur could be also removed from the whole organic extract.

The main feature of the semi-preparative-GPC as a clean-up technique relies on the compatibility of this analytical procedure with labile components of the extract (i.e. DDTs, chlorinated cyclohexadiene derivatives), which are not stable in other types of extract clean-up procedures. Further, GPC as a clean-up technique has already been automated, enabling a high sample throughput, taking into account the short analysis time involved.

The GPC retention mechanism may involve adsorption, partition and size exclusion mechanisms. The predominance of one mechanism over the others is largely determined by the choice of the mobile phase and the pore size of the packing. In the case of GPC packings with large pore size (1000-2000 daltons) size exclusion and adsorption mechanisms prevail (Bio-Beads SX-3 using cyclohexane, dichloromethane-hexane, dichloromethane-cyclohexane, toluene-ethylacetate and ethylacetate-cyclohexane) (Ericksson *et al.*, 1986). On the other hand, when smaller pore sizes (400 daltons) are used in combination with highly polar solvents, (THF, DMF) size exclusion predominates (Lee *et al.*, 1981). While using the first approach, a chemical class fractionation could be obtained, however, if smaller pore sizes are used it should be combined with another fractionation technique (i.e. adsorption chromatography) to achieve this selectivity. It has yet to be demonstrated that using GPC as a single clean-up step produces a completely clean extract for GC-ECD determination. Nevertheless, taking into account the increasing availability of high-resolution low molecular weight exclusion packings, they could definitively integrate fractionation and clean-up in a single step.

Low resolution packing (Sephadex LH and Bio-Beads SX, 200-400 mesh size) are the most widely used because they are inexpensive and afford relatively high sample loading (500 mg in 10 mm i.d. columns). The implementation of low resolution GPC requires a solvent delivery system and a UV detector and may be useful. For method development, it is advisable to inject a broad range of standard compounds covering the whole range of molecular weights of the analytes to be determined in order to determine the cut-off points to fractionate real samples. Reported recoveries of PCBs and PAHs range from 60 to 80 % for the concentration level (ng) injected. (Fernandez and Bayona, 1992).

4.6.2.3. Alumina and HPLC (silica column)

The first step in this clean-up procedure is an adsorption step using an alumina column to remove most of the lipid material. Prepare an alumina column (4 x 0.5 cm i.d., made from a Pasteur pipette). Apply the concentrated extract to the top of the column and elute with 10 ml hexane. Concentrate the eluate to about 200 µl. It is followed by a second step to more completely remove interfering compounds and at the same time to separate the compounds of interest into different fractions, containing aliphatics, PCBs, PAHs, pesticides and toxaphene. Between 20 and 200 µl of the extract (after alumina clean-up) are eluted on a stainless steel column (200 x 4 mm i.d.), packed with Nucleosil 100-5 with n-pentane, 20 % dichloromethane in n-pentane and finally dichloromethane. The eluate is collected in fractions containing 1) n-hydrocarbons, 2) PCBs, 3) PAHs and toxaphene, 4) pesticides and toxaphene and 5) acids, etc. (polar compounds). The size of the fractions has to be determined with standard solutions containing the compounds of interest, collecting the eluate in 0.5 ml fractions. Each fraction is then analysed by GC-ECD. Full details have been given in the literature (Petrick *et al.*, 1988 and IOC, 1993).

4.6.2.4. High pressure chromatography

High pressure liquid chromatography (HPLC) columns packed with microparticles are available and have the advantages of high reproducibility, low consumption of solvents, high efficiency and high sample loading capacity.

This method can be used to separate fractions containing aliphatic hydrocarbons, PCBs and aromatic hydrocarbons from interfering compounds. These fractions can then be analysed separately for their constituents by GC-FID and/or GC-ECD.

HPLC methods have been developed using synthetic solutions of n-alkanes, PAHs, pesticides, PCBs and toxaphene and have been applied to samples in which interfering substances were present in such high concentrations as to render the analysis of HC and PCBs extremely difficult without this clean-up procedure (e.g. sediments and biological tissues with OCs in the ng/g range). The samples are eluted with n-hexane, subjected to clean-up over alumina, concentrated down to 20-200 µl and treated by HPLC. With the use of n-hexane, n-pentane and 10 %, 20 % and 50 % dichloromethane in n-hexane, respectively, the following five fractions are obtained : 1) n-hydrocarbons and alkenes, 2) PCBs and alkylbenzenes, 3) PAHs and toxaphene, 4) pesticides, 5) acids, etc.(polar compounds). (Petrick *et al.* 1988).

5. BIOTA

5.1. Sampling

Organisms accumulate many contaminants from their environment (i.e., from sea water, suspended particulate matter, sediment and food). Field and laboratory studies have shown that contaminant concentrations in some marine plants and animals reflect concentrations in their environment. Scientists use this process (termed bio-accumulation) to assess marine contamination resulting from human activity (e.g., pipeline discharges, dumping from ships).

There are problems with using biota as bio-accumulators (bio-indicators). For example, tissues from individuals of a species exposed to the same contaminant concentration may contain different levels of contamination after the same exposure time. These deviations reflect individual differences in factors such as age, sex, size, and physiological and nutritional states. Also, various species show different contaminant concentrations following identical exposure; differences in elimination rates may partially account for this. These factors must be considered when planning a monitoring programme in order to control their effects on the precision of the analysis (by reducing the variances). Variance reduction is necessary in order to detect smaller differences in mean contaminant concentrations observed in monitoring programmes.

For proper sampling and sample preparation, refer to Reference Method No 6 “Guidelines for monitoring chemical contaminants in the sea using marine organisms” and Reference Method No 12 Rev.2 “Sampling of selected marine organisms and sample preparation for the analysis of chlorinated hydrocarbons”.

5.2. Cleaning of extraction thimbles

As for extraction of sediment samples, thimbles should be extracted first with the same solvent used for the extraction of the sample. As the extraction of biota sample is achieved with hexane, a pre-extraction of these thimbles is made with 250 ml of hexane for 8 hours in the Soxhlet apparatus, cycling the solvent through at a rate of 4 to 5 cycles per hour. Add into the solvent a few carborundum boiling chips to get a regular ebullition.

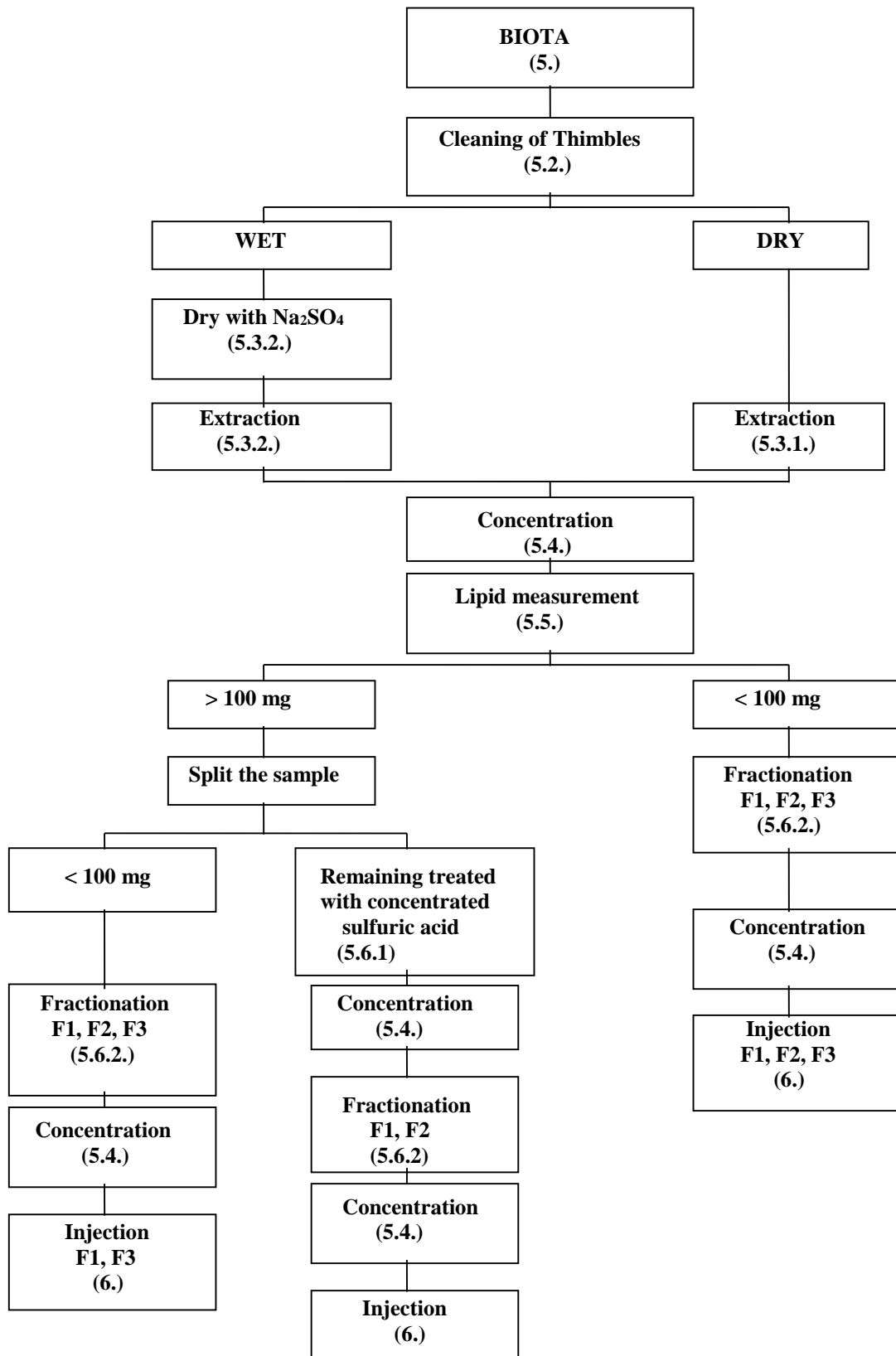


Figure 3: Diagram of the extraction procedure for biota samples.

5.3. Extraction of tissues

5.3.1. Extraction procedure for freeze-dried samples.

Take a 50 to 100 g fresh weight sub-sample from the sample. Weigh this sub-sample and freeze-dry it. When the sub-sample appears to be dry, re-weigh it and freeze-dry it for a further 24 hours and then re-weigh it. If the difference between the two dry weights is greater than 5%, continue the freeze-drying process. Special care must be taken to ensure that the freeze-drier is clean and does not contaminate the samples. The freeze drying procedure should be tested by drying 100 g Na₂SO₄ as a blank and extracting this as a sample. Pulverise the freeze-dried sub-sample carefully using a cleaned pestle and mortar. Accurately weigh about 5 to 10 g of this pulverised material, note the exact weight to be extracted, and place it into a pre-cleaned extraction thimble in a Soxhlet apparatus. The size of the sub-sample should be adjusted so that about 100 mg of extractable organic matter ("lipid") will be obtained. Smaller sub-samples should be used if residue concentrations are expected to be high. Add a known amount of internal standard to the sub-sample in the thimble before Soxhlet extraction. It is important to spike the sample at levels that are near to that of the analyte concentrations in the samples. If, in the end, the analyte and the internal standard concentrations do not fall within the established calibration range of the GC-ECD, the analysis must be repeated. Consequently, it may be advisable to perform range-finding analysis for samples of unknown character beforehand. Candidate internal standards are the same as for sediment samples (see 5.3.). Add about 200 ml of hexane or petroleum ether to the extraction flask with a few carborundum boiling chips, and extract the sample for 8 hours cycling the solvent through at a rate of 4 to 5 cycles per hour. Extract an empty thimble as a procedural blank, making sure to spike it with internal standards in the same fashion as the sample. If unacceptable procedural blanks are found, the source of contamination must be identified and eliminated rather than subtracting high blank values from the analytical results.

5.3.2. Extraction procedure without freeze-drying

Select a 25 to 100 g fresh weight sub-sample and place in a blender. Add anhydrous sodium sulphate to the sample, manually homogenise and determine whether the sample is adequately dried. If not, more sodium sulphate should be added until a dry mixture is obtained. Normally, 3 times by the sample weight used should be enough. Once this has been achieved, blend the mixture at high speed for 1 or 2 minutes until the mixture is well homogenised and the sample appears to be dry. Transfer the mixture to a pre-cleaned extraction thimble, add internal standards as described above and extract the dehydrated tissue with about 200 ml hexane or petroleum ether for 8 hours in a Soxhlet apparatus, cycling 4 to 5 times per hour. Extract the same amount of sodium sulphate as the procedural blank, making sure to add internal standards in the same fashion as the sample.

5.4. Concentration of the extract

Refer to section (4.4.)

5.5. Extractable Organic Matter (EOM)

Refer to section (4.5.)

5.6. Clean-up procedure and fractionation

5.6.1. Removal of lipids by concentrated sulphuric acid

If the lipid content of the extracts is higher than 100-150 mg, a preliminary step for the removal of the lipids is necessary before further sample purification. This can be carried out by using concentrated sulphuric acid. Treatment with sulphuric acid is used when chlorinated hydrocarbons are to be determined. However, sulphuric acid will destroy dieldrin and endrin so that an aliquot of the untreated extract must be set aside for the determination of these compounds.

CAUTION: During all this procedure it is very important to wear safety glasses.

Take an aliquot of the concentrated extract, containing about 200 mg of “lipids”, transfer into a separatory funnel and add to this extract enough hexane in order to dilute the sample (40 to 50 ml should be enough), this will allow recovery of the hexane after acid treatment, because if the sample is too concentrated, the destroyed “lipids” will become almost solid and it will be difficult then to recover the hexane from this solid mass. Add 5 ml concentrated sulphuric acid to the extract and tightly fit the glass stopper and shake vigorously. Invert the funnel and carefully vent the vapours out through the stopcock. Repeat this procedure for several minutes. Place the separatory funnel in a rack and allow the phases to separate. Four or five samples and a spiked blank are convenient to process at one time. The extract should be colourless. Recover the hexane phase into a glass beaker. Dry with sodium sulphate and transfer the hexane into a Kuderna-Danish concentrator. Reduce the volume of the extract by evaporating the solvent with a gentle stream of pure nitrogen to about 1 ml.

5.6.2. Fractionation

Refer to section (4.6.2.)

6. CAPILLARY GAS CHROMATOGRAPHIC DETERMINATIONS

6.1. Gas chromatographic conditions

- Gas chromatograph with a split/splitless injection system, separate regulation system for inlet and column pressures and temperatures; multi-ramp temperature programming facilities (preferably microprocessor controlled), electron capture detector interfaced with the column with electronic control unit and pulsed mode facilities. An integrator with a short response time (0.25 s) is essential.
- Narrow-bore (0.22 mm internal diameter), 25 m long, fused silica open tubular column, coated with SE-54 (0.17 µm film thickness, preferably chemically bonded) with sufficient resolution to separate the relevant peaks in the standards provided for PCB analysis.
- Carrier gas should be high purity H₂. If this is not available or if the GC is not equipped with a special security system for hydrogen leak, He may be used. Gas purification traps should be used with molecular sieves to remove oxygen, moisture and other interfering substances.
- High purity nitrogen gas (99.995 %) as ECD make-up gas can be used (Argon/methane high purity gas is another option).

Conditions:

- H₂ or He carrier gas at inlet pressure of 0.5 to 1 Kg/cm² to achieve a flow rate of 1 to 2 ml/min.
- Make-up gas N₂ or Ar/CH₄ at the flow rate recommended by the manufacturer (between 30 and 60 ml/min.).
- ECD temperature: 300°C

6.2. Column preparation

Fused silica columns are the columns of choice for their inertness and durability (they are extremely flexible). They are made of material that is stable up to 360 °C. The 5 % phenyl methyl silicone gum (SE-54) liquid phase, is present as a thin, (0.17 µm), uniform film which can tolerate temperatures up to 300 °C. SE-54 is relatively resistant to the detrimental effects of solvents, oxygen and water, at least at low temperatures. These columns are even more resistant and durable if the liquid phase is chemically bonded to the support by the manufacturer.

For GC/MS work, it is advised to restrict the film thickness to 0.17 µm because with thicker films some of the phase could be released, resulting in an increase of the noise signal in the GC/MS.

The flexible fused silica columns can be conveniently connected directly to the inlet and outlet systems without the transfer lines used in conventional glass capillary chromatography which often lead to increased dead volume. Low bleed graphite or vespel ferrules provide a good seal.

The presence of extraneous peaks and elevated baseline drift will result in poor detector performance. This can be caused by components which elute from the column, such as residual solvents and low molecular weight liquid phase fractions on new columns and build-up of later eluting compounds on old columns. Conditioning is a necessary step to remove these contaminants. New columns are connected to the inlet (while left unconnected to the detector). Columns are flushed with carrier gas at low temperature for 15 min. to remove the oxygen, then heated at 70-100 °C for 30 min. and finally at 170 °C overnight. The column can be then connected to the detector. Old columns can be heated directly to elevated temperatures overnight. The final temperature is selected as a compromise between time required to develop a stable baseline and expected column life. Thus, it may be necessary for older columns to be heated to the maximum temperature of the liquid phase resulting in shorter column life. The temperature of the ECD, when connected to the column, should always be at least 50 °C higher than the column, in order to avoid condensation of the material onto the detector foil. It is essential that carrier gas flows through the column at all times when at elevated temperatures. Even short exposure of the column to higher temperature without sufficient flow will ruin the column.

CAUTION: if H₂ is used as a carrier gas, position the column end outside of the oven to avoid explosion risk.

6.3. Column test

When the column has been connected to the detector, the carrier gas flow is set to 30 ml per minute for a column with 4 mm internal diameter. The column performance is then measured according to the criteria of the “number of theoretical plates” for a specific compound and can be achieved according to the following procedure.

- Set injector and detector temperatures at 200 and 300°C respectively and the column oven temperature at 180 °C.
- Inject pp’ DDT standard and measure the retention time (Tr). Adjust the column temperature to get a pp’ DDT retention time relative to Aldrin of 3.03.
- Measure the width of the pp’ DDT peak at its half height ($b_{1/2}$), in minutes and the retention time (Tr) also in minutes.
- Calculate the number of theoretical plates using the formula:

$$N = 5.54 \left(\frac{Tr}{b_{1/2}} \right)^2$$

- A parameter which is independent of the column length is the height equivalent to a theoretical plate (HETP):

$$HETP = \frac{L}{N}$$

Where L is the column length. Adjust the flow rate of the carrier gas to obtain optimum performance. The HETP should be as low as possible (i.e. the number of theoretical plates should be as great as possible).

The column remains in optimum condition as long as the liquid phase exists as a thin, uniform film. The quality of the film at the inlet side may be degraded as a result of repeated splitless

injections. Decreased column quality may be remedied by the removal of the end of the column (10 to 20 cm) at the inlet side. Chemically bonded liquid phases require less maintenance.

6.4. Electron capture detector

High-energy electrons, emitted by a radioactive source within the detector (e.g. a ^{63}Ni foil), are subject to repeated collisions with carrier gas molecules, producing secondary electrons. These electrons, upon returning to their normal state, can be captured by sample molecules, eluting from a GC column. The resulting reduction in cell current is the operating principle of an electron capture detector. The detector current produced is actually a non-linear function of the concentration of electron-capturing material. However, the useful linear range of an ECD may be greatly improved if the instrument is operated at a constant current, but in a pulsed mode, i.e. with short voltage pulses being applied to the cell electrodes. The current in the cell is kept constant by varying the frequency of the pulses.

Contamination of the detector (and thus lower sensitivity) may result from high-boiling organic compounds eluting from the column. Periodic heating to 350°C may overcome this problem. The ^{63}Ni ECD can be used at 320°C under normal operational conditions, in order to limit such contamination.

The optimum flow for an ECD (30 to 60 ml/min.) is much higher than carrier gas flow through the column of one or two ml/min. Thus an additional detector purge flow is necessary (N_2 or Ar/CH_4). Once leaving the outlet of the column, the compounds have to be taken up into an increased gas flow in order to avoid extra-volume band broadening within the detector. Thus, the detector purge flow also serves as the sweep gas.

6.5. Quantification

The most widely used information for identification of a peak is its retention time, or its relative retention time (i.e., the adjusted retention time relative to that of a selected reference compound). Retention behaviour is temperature dependent and comparison of retention times obtained at two or more temperatures may aid in determining a peak's identity. However, retention times are not specific and despite the high resolution offered by capillary columns, two compounds of interest in the same sample may have identical retention times.

One way of using retention indexes could be to inject di-n-alkyl-phthalates such as a mixture containing di-n-methyl-phthalate, di-n-ethyl-phthalate, di-n-propyl-phthalate, di-n-butyl-phthalate, di-n-hexyl-phthalate and di-n-heptyl-phthalate, which will cover the elution range from 70°C to 260°C . An arbitrary index of 100 is given to the di-n-methyl phthalate, 200 to the di-n-ethyl phthalate, and so on up to 700 to the di-n-heptyl phthalate; it is possible to identify all chlorinated pesticides by a proper retention index. This will be used also for unknown compounds which can be found easily on the GC/MS using the same index and so, identified. (Villeneuve J.P. 1986).

PCBs represent a complex mixture of compounds that cannot all be resolved on a packed column. Also there is no simple standard available for their quantification. Each peak in a sample chromatogram might correspond to a mixture of more than one individual compound. These difficulties have led to the recommendation of various quantification procedures. The usual method to quantify PCBs is to compare packed-column chromatograms of commercially available industrial formulations (Aroclors, Clophens, Phenoclor) with the sample chromatogram. Most commonly, it is possible to match one single formulation, such as Aroclor 1254 or Aroclor 1260 with the sample chromatogram. An industrial formulation (or mixture of formulations) should be chosen to be as close a match as possible and in the case of sample extracts from sediment or organisms, Aroclor 1254 and Aroclor 1260 are most frequently chosen.

For the second fraction obtained on Florisil separation, it is possible to quantify DDTs after comparison with the retention times of peaks in the sample chromatogram to those in the corresponding standard, the peak heights (or peak areas) are measured and related to the peak height (or peak area) in the standard according to the formula:

$$[\text{Concentration}] = \frac{h \times C \times V \times 1000}{h' \times V(\text{inj}) \times M \times R} \text{ ng/g (or pg/g)}$$

Where:

- V = total extract volume (ml)
- M = weight of sample extracted (g)
- H = peak height of the compound in the sample
- h' = peak height of the compound in the standard
- C = quantity of standard injected (ng or pg)
- V (inj) = volume of sample injected (µl)
- R = Recovery of the sample

7. COMPUTERIZED GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS)

7.1. Operating conditions

The chemical ionisation source of a mass spectrometer can be used to produce negative ions by electron capture reactions (CI-NI-MS) using a non-reactive enhancement gas such as methane or argon. CI-NI has the advantage of being highly selective, permitting the detection of specific compounds in complex matrices. Under CI-NI conditions, methane (99.99 %) is used as the reagent gas. Samples are introduced through a SE-54, 30 m x 0.25 mm i.d., fused silica column. The film thickness used is 0.17 µm in order to minimise the bleeding of the phase into the system. Helium is used as carrier gas with an inlet pressure of 13 psi, which gives a carrier flow of 1.5 ml/min. or a gas velocity of 44 cm/sec.

The temperature of the injection port is held at 250°C.

The temperature of the source is set at 240°C, the quadrupole at 100°C and the interface at 285°C.

Injections of 1-3 µl are made in the splitless mode.

The temperature programme of the oven starts at 70°C, for 2 minutes, then it is increased at 3°C/min. to 260°C and kept under isothermal conditions for 40 minutes.

File : C:\HPCHEM\1\DATA\AR1254.D
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Acquired : 12 Jul 95 8:02 am using AcqMethod OC
Instrument : 5989B
Sample Name: standard ar1254
Misc Info :
Vial Number: 1

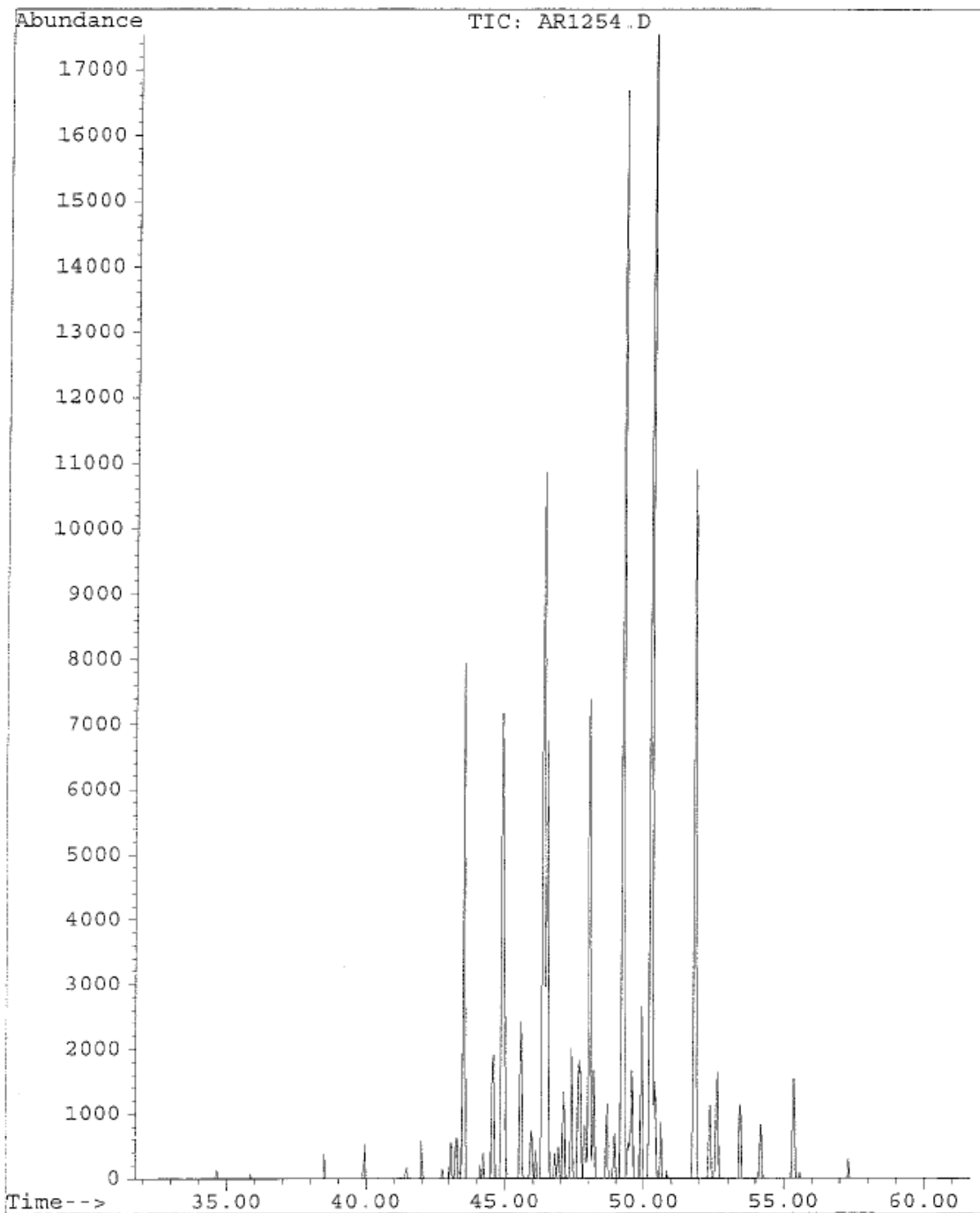


Figure 4: TIC of Aroclor 1254

File : C:\HPCHEM\1\DATA\AR1254.D
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Acquired : 12 Jul 95 8:02 am using AcqMethod OC
Instrument : 5989B
Sample Name: standard ar1254
Misc Info :
Vial Number: 1

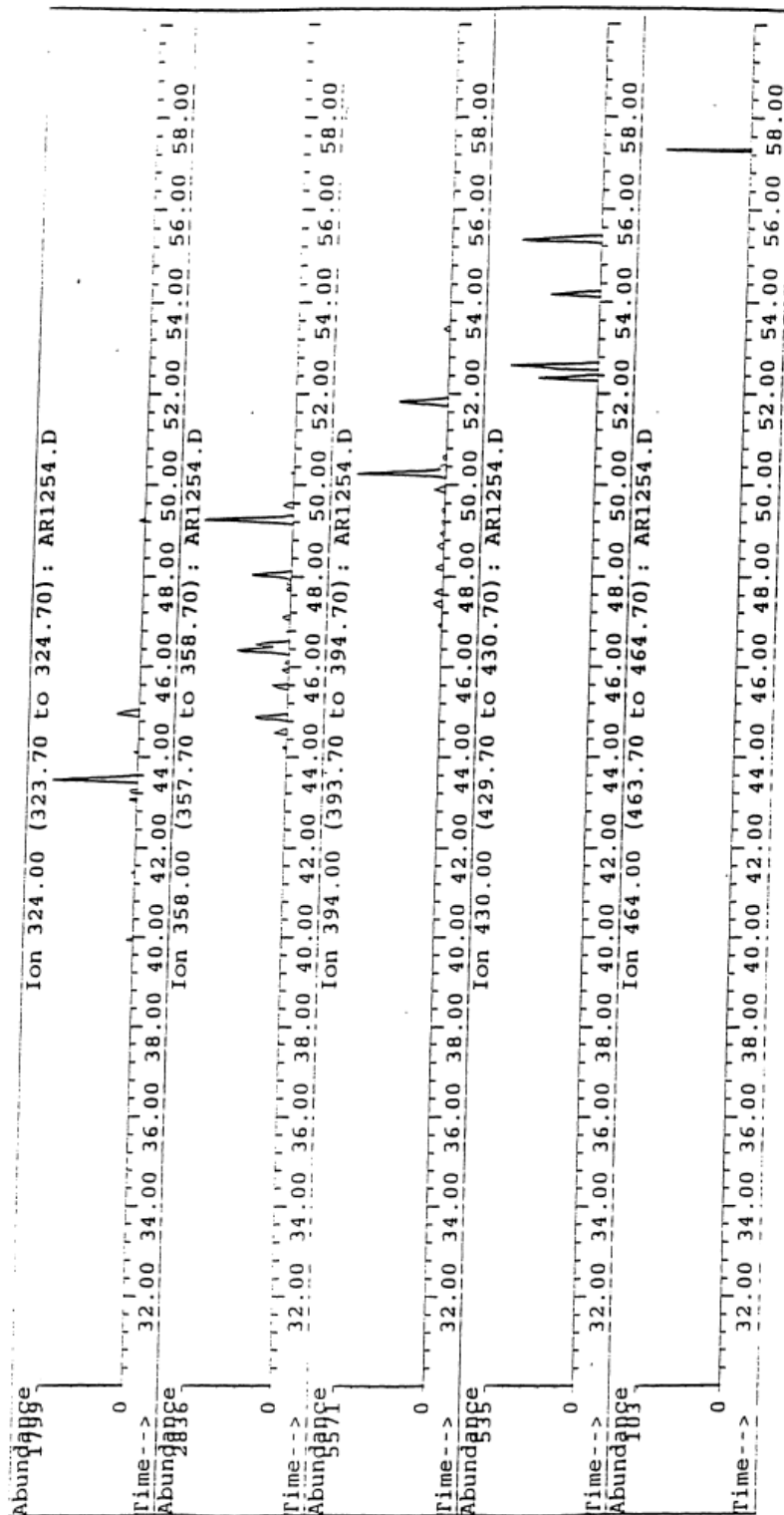


Figure 5: RIC of Aroclor 1254 main compounds

File : C:\HPCHEM\1\DATA\AR1260.D
Operator : jpv
Acquired : 12 Jul 95 9:42 am using AcqMethod OC
Instrument : 5989B
Sample Name: standard ar1260
Misc Info :
Vial Number: 1

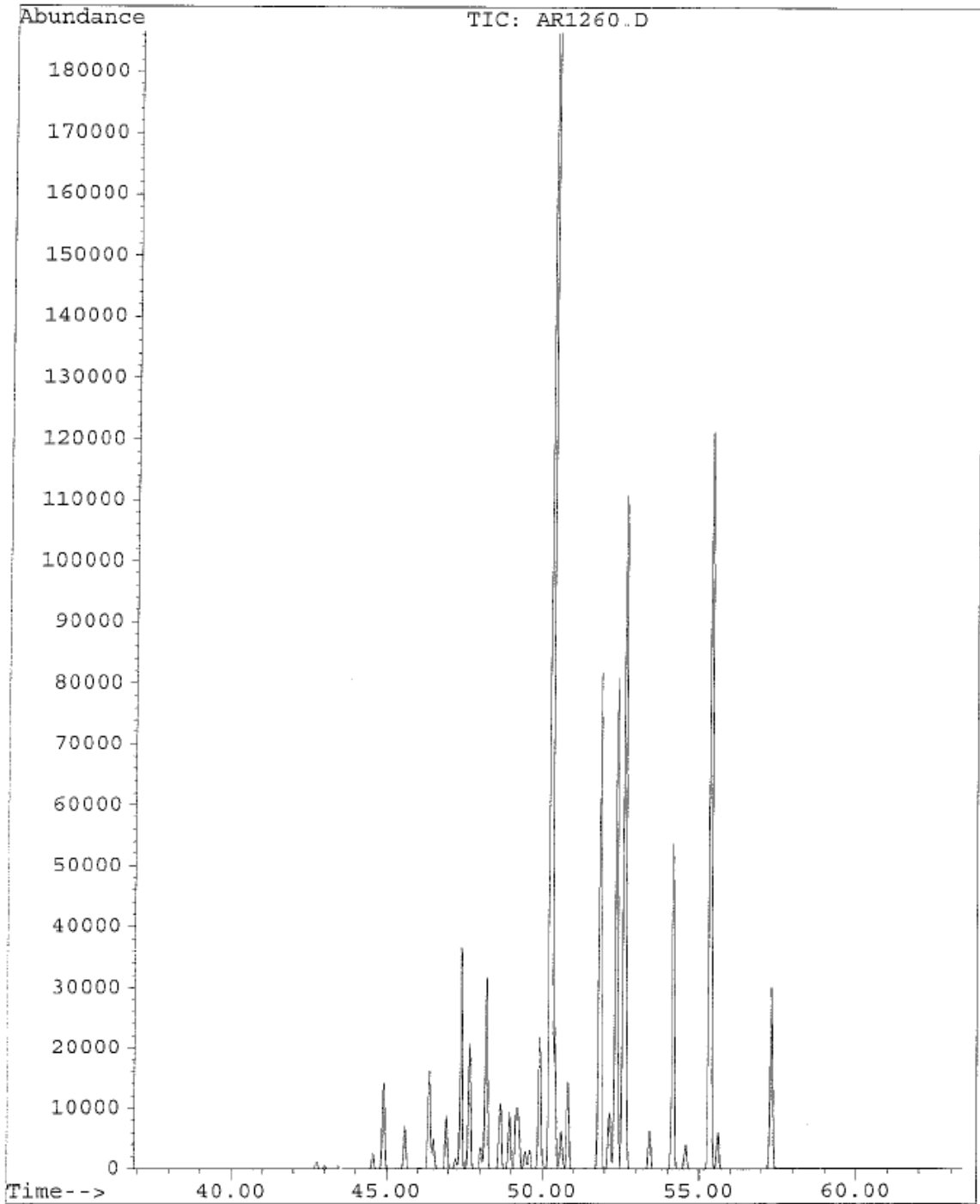


Figure 6: TIC of Aroclor 1260

File : C:\HPCHEM\1\DATA\AR1260.D
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Acquired : 12 Jul 95 9:42 am using AcqMethod OC
Instrument : 5989B
Sample Name: standard ar1260
Misc Info :
Vial Number: 1

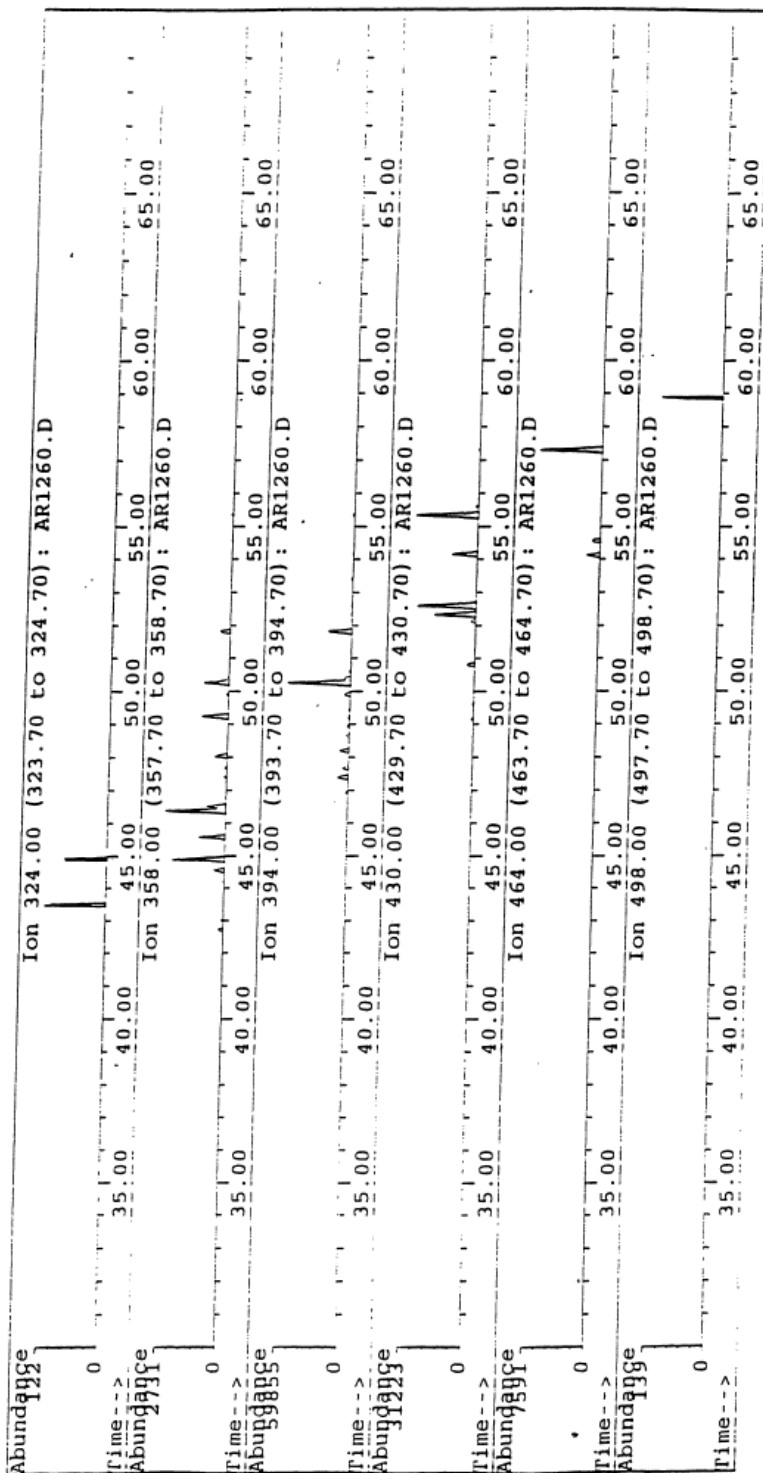


Figure 7: RIC of Aroclor 1260 main compounds

7.2. Example of a selected ion monitoring programme useful for quantitative analysis of chlorinated compounds.

Compounds	Fraction N° on Florisil	Retention Time (min.)	Target Ion (daltons)
HCB	1	37-38	284
Heptachlor	1	44-45	266
Aldrin	1	46-48	237
op DDE	1	51-53	246
Transnonachlor	1	52-54	444
pp' DDE	1	53-55	281
PCBs			
3 Cl	1		258
4 Cl	1		292
5 Cl	1	40-55	324
6 Cl	1	40-55	358
7 Cl	1	45-55	394
8 Cl	1	45-60	430
9 Cl	1	50-60	464
10 Cl	1	58-60	498
α HCH	2	37-39	255
β HCH	2	39-41	255
γ HCH (Lindane)	2	39-41	255
δ HCH	2	41-43	255
γ Chlordane	2	51-53	410
α Chlordane	2	52-54	266
op DDD	2	54-56	248
pp' DDD	2	56-58	248
op DDT	2	56-58	246
pp' DDT	2	58-60	283
Heptachlor epoxide	3	49-51	318
α Endosulfan	3	52-54	406
Dieldrin	3	53-55	346
Endrin	3	55-57	346
β Endosulfan	3	55-57	406
Endosulfan sulfate	3	58-60	386

8. NOTES ON WATER ANALYSIS

The levels of lipophilic compounds in tissues of aquatic organisms and organic fractions of sediments are determined to a large extent by the levels of these compounds in the surrounding water (marine mammals are an obvious exception). Data for CBs and hydrocarbons in sea water is therefore extremely useful for an understanding of the levels in organisms. However, the levels in sea water are extremely low and consequently, their determination needs considerable experience. Large volumes of water are required and extreme care has to be taken in order to avoid contamination during sampling, extraction and clean-up of the samples. Details are described in Manual and guide No 27 of IOC, 1993 and Villeneuve J.P. (1986).

9. ALTERNATIVE PROCEDURES

9.1. Combining sample preparation and extraction for chlorinated and petroleum hydrocarbons in sediment samples.

In the event that analyses for petroleum hydrocarbons and chlorinated compounds (and/or sterols) are of interest, the following extraction procedure can be used. To the freeze-dried sample introduce internal standards for each compound class. The following are suggested: 1) aliphatic hydrocarbons: - n-C₁₄ d₃₀, n-C₁₉ d₄₀, n-C₃₂ d₆₆, 2) polycyclic aromatic hydrocarbons: Naphthalene d₈,

Hexamethylbenzene, Cadalene (deuterated PAHs are also useful), 3) organochlorine compounds: PCB congeners 29, 30, 121 or 198, ϵ HCH and Endosulfan Id₄, 4) sterols: 5 α (H)-androstan-3 β -ol. These standards are used for quantifying the recovery of the total procedure. Samples are Soxhlet extracted for 8 hours with 250 ml of a mixture hexane / dichloromethane (50:50), cycling the solvent through at a rate of 4 to 5 cycles per hour. The solvent extract is concentrated by rotary evaporation down to 15 ml and transferred to a Kuderna-Danish tube. It is then further concentrated down to 5-6 ml under nitrogen gas. Following removal of sulphur and water, the extract is separated into aliquots: 1/3 for petroleum hydrocarbons and sterols and 2/3 for chlorinated hydrocarbons.

Note: Mercury method should be used only if chlorinated pesticides and PCBs are analysed. If the combined method is used for petroleum and chlorinated hydrocarbons, then the copper method should be used instead of mercury that will destroy some of the PAHs.

9.2. Supercritical fluid extraction (SFE) of marine samples

Sample preparation is probably the most time-consuming and labor-intensive analytical task performed in a laboratory. Studies shows that 60 % of the overall sample analysis time is spent in sample preparation which is the main source of error and of contamination. In addition, the amount of hazardous chemicals used for sample preparation is a continuous source of concern. Due to safe handling and disposal requirements, the reduction of their use is a priority for laboratories worldwide.

Supercritical fluids are gases (i.e. N₂O and CO₂) at room temperature and pressures above the critical point. The SFE technique allows an efficient extraction of a variety of contaminants with considerable reduction in the analysis cost, sample amount and allows the extraction of the thermal sensitive substances, reducing the amount of environmentally hazardous solvents.

A small change in the pressure of a supercritical fluid results in a big change in its density and the solvent strength of the fluid changes with changing density. As a result, one supercritical fluid easily performs the work of many solvents. If this is not enough, it is possible to add a modifier, such as methanol (a few per cent) to increase the solvating range of the fluid. Therefore, SFE should speed up the sample preparation process, minimising the wastes associated with the analysis.

Until now, the main fields of analytical applications of SFE are related to environmental studies and to the food-processing industry (Hawthorne, 1990, Bayona, 1993). A method using carbon dioxide (80°C-340 atm) for the extraction of total petroleum hydrocarbons has been approved as an EPA standard method. The extraction efficiency of modified CO₂ for the recovery of 41 organochlorine and 47 organophosphorus pesticides spiked on sand at different pressures and temperatures were higher than 80%. Furthermore, by increasing the extraction temperature up to 200°C, PCBs and PAHs can be extracted from naturally occurring samples with neat CO₂. Nam *et al.* (1991), have developed a method for rapid determination of polychlorinated organics in complex matrices. The method is based on direct coupling of supercritical fluid extraction with tandem supercritical fluid chromatography and gas chromatography. The on-line system permits simultaneous extraction and analysis with high reproducibility and accuracy.

Selection guide for CO₂ extraction of common pollutants (from Hewlett-Packard)

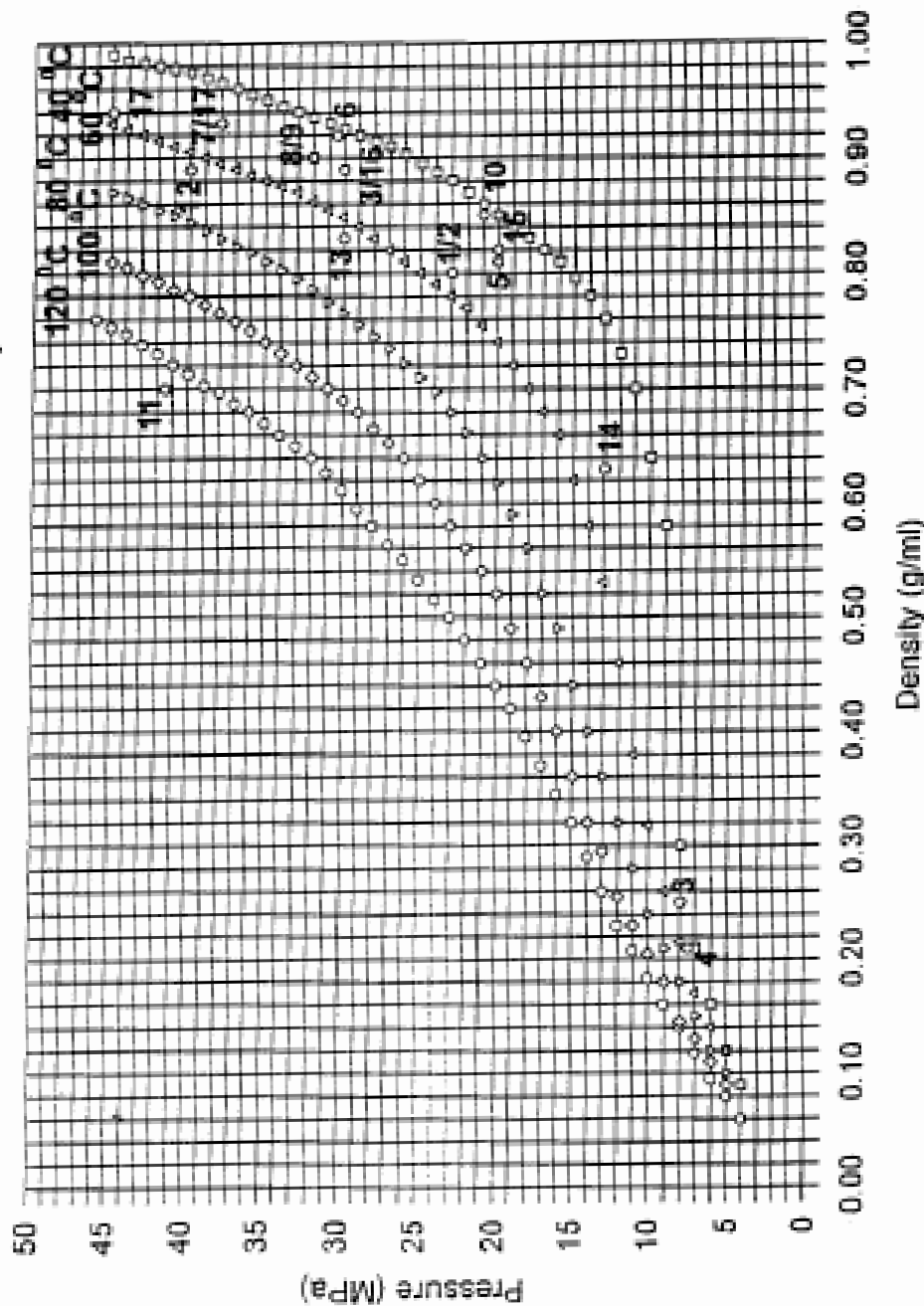


Figure 8: Guide for CO₂ extractions

9.3. Microwave assisted extraction for marine samples

9.3.1 Sediment

Another alternative method for the extraction of chlorinated hydrocarbons in sediment samples (or combined extraction for chlorinated hydrocarbons and petroleum hydrocarbons) is the use of the Microwave oven instead of the Soxhlet extractor. The main advantage of the microwave oven is the fact that, for one sample, only 40 ml of solvent mixture are used instead of 250 ml for clean-up of extraction thimbles and 250 ml for the extraction itself.

10 to 15 grams of freeze-dried sediment sample, ground and sieved at 250 µm, are put in the glass tube of the reactor. Appropriate internal standards (for OCs and/or PHs, see 10.1.) are added to the sample for recovery and samples are extracted with 40 ml of a mixture of hexane / dichloromethane (50:50).

Extraction is realised within the following cycle:

- Power of the microwaves: 1200 watts
- Temperature increase to 115 °C in 10 minutes.
- Extraction maintained at 115 °C for 30 minutes
- Cooling to ambient temperature within one hour.

The carousel containing 14 reactors, 12 samples could be extracted together with one blank and one Reference Material within 1 and half hour and with 10 times less solvent mixture than the standard Soxhlet extraction.

After cooling down to room temperature the solvent mixture is recovered in a 100 ml glass flask. The sediment is poured in a glass funnel containing a plug made of glass wool. The extracted sediment is washed with 10 - 20 ml of hexane. The extract follows then the procedure of clean-up and fractionation.

9.3.2 Biota

3 to 8 grams of freeze-dried biota sample is accurately weighted, the weight to be extracted is noted, and it is placed into the pre-cleaned glass tube of the reactor. A known amount of internal standard is added to the sub-sample in the tube before extraction. Candidate internal standards are the same than for sediment samples refers to section (5.3.1.)

Extraction is realized with 30 ml of a mixture hexane / acetone (90:10) within the following cycle:

- Power of the microwaves: 1200 watts
- Temperature increase to 115 °C in 10 minutes.
- Extraction maintained at 115 °C for 20 minutes
- Cooling to ambient temperature within one hour.

The carousel containing 14 reactors, 12 samples could be extracted with one blank and one Reference Material within 1 and half hour and with 10 times less solvent mixture than the standard Soxhlet extraction.

After cooling down to room temperature the solvent mixture is recovered in a 100 ml glass flask. The powder of biota is poured in a glass funnel containing a plug made of glass wool. The extracted biota is washed with 10 - 20 ml of hexane. The extract is then concentrated with rotary evaporator and ready for E.O.M, clean-up and fractionation procedure.

10. DATA INTERPRETATION

10.1. DDT

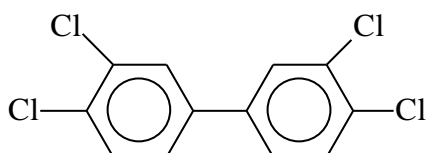
The residence time of total DDT in the environment is relatively short ($t_{1/2} = 3-5$ years), so, at least 75-80 % of the current total DDT should be in the form of DDE or DDD if it was introduced into the environment before the 1975 ban. Values of Henry's law constant indicate that these compounds can reach the troposphere as vapour. These vapours are little adsorbed by airborne particulate matter and represent the major component in atmospheric chlorinated hydrocarbon levels. Vapour movements of these pollutants suggest that restrictions and regulations operating in the more technically advanced countries could only be partially effective on a worldwide basis.

The presence of the *op* DDT together with anomalous *pp'* DDT values in environmental samples indicates a recent treatment with this insecticide.

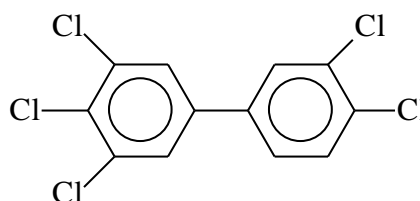
10.2. PCBs congeners

Among the 209 possible PCB congeners, seven of them: 28, 52, 101, 118, 138, 153 and 180, were selected as the most relevant because of their distribution in the chromatogram and in the chlorination range.

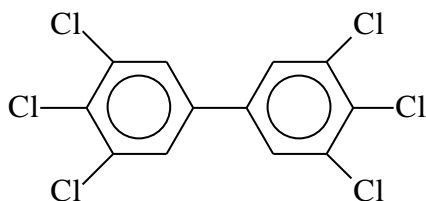
Recently, attention has been paid to congeners having 2 *para*-chlorines and at least 1 *meta*-chlorine. These congeners are called "coplanar" PCBs. Among the 209 congeners, 20 members attain coplanarity due to non-ortho chlorine substitution in the biphenyl ring. Three of these show the same range of toxicity as the 2,3,7,8 tetrachlorodibenzo-*p*-dioxin and the 2,3,7,8 tetrachlorodibenzofuran, these are the IUPAC N^o: 77, 126 and 169. These compounds should be identified and quantified in the environmental samples with high priority. They can be separated using fractionation with carbon chromatography (Tanabe *et al.*, 1986).



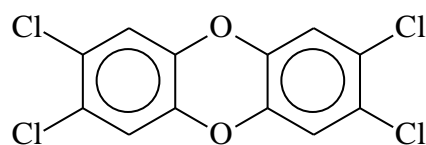
3,3',4,4' tetrachlorobiphenyl
IUPAC N^o: 77



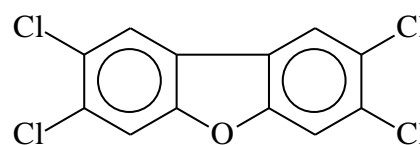
3,3',4,4',5 pentachlorobiphenyl
IUPAC N^o: 126



3,3',4,4',5,5' hexachlorobiphenyl
IUPAC N^o: 169



2,3,7,8 tetrachlorodibenzo-*p*-dioxin



2,3,7,8 tetrachlorodibenzofuran

10.3. Typical profiles of commercial mixtures

Formulations available in different countries are slightly different in their composition (Aroclor in USA, Kanechlor in Japan, Clophen in Germany, Phenoclor in France, Fenclor in Italy or Sovol in Russia). For the same global composition, such as Aroclor 1254, KC-500 or Phenoclor DP-5, the composition of individual congeners differs by 5-10 %. If a sample is collected on the French coast (therefore, contaminated with DP-5), and is quantified with DP-5 and Aroclor 1254, the difference observed in concentration could be in the order of 5-10 %. This shows the importance of choosing one common standard for the quantification of global industrial formulations or the importance of quantifying with individual congeners.

Percent contribution of individual chlorobiphenyls to Clophen A 50 and Aroclor 1254.

PCB N°	Clophen A50	Aroclor 1254	PCB N°	Clophen A50	Aroclor 1254
17	0	0.19	115	0.28	0.3
18	0	0.41	118	10.9	6.39
28	0.05	0.25	119	0.19	0.14
31	0.05	0.22	122	0.19	0.5
33	0.11	0.14	123	0.85	0.81
40	0.28	0.2	126	0.08	0
41	0.83	0.64	128	3.04	2.07
42	0.13	0.23	129	0.83	0.23
44	2.46	2.03	130	0.83	0.63
47	0.18	0.11	131	0.06	0.16
48	0.17	0.14	132	2.57	1.98
49	1.96	1.64	134	0.52	0.49
52	5.53	5.18	135	1.61	1.62
53	0.06	0.09	136	0.91	1.12
56	0.44	0.58	137	0.25	0.25
60	0.34	0.54	138	3.61	3.2
63	0.15	0.05	141	0.98	1.04
64	0.71	0.45	146	0.8	0.83
66	0.5	0.59	149	4.5	2.21
67	0.13	0.09	151	1.22	1.17
70	3.85	3.21	153	4.17	4.26
74	1.35	0.78	156	1.43	1.62
82	1.05	0.95	157	0.31	0
83	0.53	0.45	158	0.98	0.77
84	2.08	1.95	167	0.35	0.21
85	1.85	1.66	170	0.65	0.31
87	4.22	3.78	171	0.5	0.5
90	0.85	0.93	172	0.09	0.05
91	0.92	0.83	173	0.09	0.09
92	1.53	1.58	174	0.37	0.34
95	6	6.02	175	0.11	0.05
96	0.05	0.08	176	0.43	0.32
97	2.8	2.55	177	0.21	0.21
99	4.06	3.6	178	0.19	1.35
100	0.15	0.1	179	0.2	0.21
101	7.72	7.94	180	0.53	0.38
105	1.9	3.83	183	0.21	0.17
107	0.94	0.72	187	0.3	0.32
110	6.27	5.85	190	0.05	0.08
			201	0.6	0.68

11. QUALITY ASSURANCE / QUALITY CONTROL

Guidelines on the QA/QC requirements for analysis of sediments and marine organisms are detailed in Reference Method No 57, "Contaminant monitoring programs using marine organisms: Quality assurance and good laboratory practice". Brief descriptions of issues that must be addressed in the course of understanding the procedures described here are given below.

11.1. Precision

The precision of the method should be established by replicate analysis of samples of the appropriate matrix. Estimate the precision of the entire analytical procedure by extracting five sub-samples from the same sample after homogenisation. Alternatively, perform replicate analysis of an appropriate certified reference material (RM; see below) containing the analytes of interest. The principal advantage of using a RM is that the material permits the simultaneous evaluation of accuracy while offering a well homogenised sample. Precision should be evaluated as a matter of course during the initial implementation procedure just before initiation of sample analysis.

11.2. Accuracy

The accuracy of the methods described here must be confirmed by analysis of a suitable RM (i.e. appropriate matrix, analytes) prior to initiation of sample analysis. Agreement between measured and certified concentrations for any individual analyte should be within 35 % and on average within 25%. It is advisable to introduce RMs on a regular basis (e.g. every 10-20 samples) as a method of checking the procedure. Further description of the preparation of control charts and criteria for data acceptance are discussed in Reference Method N° 57.

11.3. Blanks

Blanks represent an opportunity to evaluate and monitor the potential introduction of contaminants into samples during processing. Contributions to the analyte signal can arise from contaminants in the reagents, those arising from passive contact between the sample and the environment (e.g. the atmosphere) and those introduced during sample handling by hands, implements or glassware. It is essential to establish a consistently low (i.e. with respect to analytes) blank prior to initiating analysis or even the determination of the method detection limit. In addition, it is necessary to perform blank determinations on a regular basis (e.g. every batch of samples).

11.4. Recovery

Recovery reflects the ability of the analyst to fully recover surrogate compounds introduced to the sample matrix or blank at the beginning of the procedure. The primary criteria for selection of compounds to be used for testing recovery are that they: 1) have physical (i.e. chromatographic/partitioning) properties similar to and if necessary spanning those of the analytes of interest, 2) do not suffer from interferences during gas chromatographic analysis, 3) are baseline resolved from the analytes of interest.

Recovery should be tested on all samples and blanks as a routine matter of course. Recoveries below 70% are to be considered unacceptable. Recoveries in excess of 100 % may indicate the presence of interference.

11.5. Archiving and reporting of results

Every sample should have an associated worksheet which follows the samples and the extracts through the various stages of the procedure and upon which the analyst notes all relevant details. An example of such a worksheet is given below. Each laboratory should construct and complete such a worksheet. Relevant chromatograms should be attached to the worksheet. Analyses should be grouped

and composite or summary analysis sheets archived with each group. Final disposal of the data will depend on the reasons for which it was collected but should follow the overall plan model.

All processed samples should be archived at all steps of the procedure:

- deep frozen (in the deep-freezer as it was received).
- freeze-dried (in sealed glass container kept in a dark place).
- extracted (after injection on the GC, sample extracts should be concentrated down to 1 ml and transferred into sealed glass vials, a Pasteur pipette sealed with a butane burner is adequate and cheap).

Sample: IAEA-357 : Marine Sediment

wet wt.

----- =, % water in freeze dried sample determined by drying at 105°C :
dry wt.

.....g freeze-dried wt. extracted with hexane in Soxhlet extractor for 8 hours.

.....pg PCB N°29,pg PCB N°198,pg ϵ HCH and pg Endosulfan Id₄ were added as internal standard.

Theml extract was reduced by rotary evaporator to approximatelyml.

This was treated with sodium sulfate to dry the extract. Then treated with mercury to remove sulphur. This was further reduced toml for lipid determinations. Corrected dry wt. :g.

Lipid determinations:

.....ml total extract;

10 μ l aliquots weighed on micro-balance:mg;mg;mg.

HEOM =mg/g dry weight.

.....mg lipid subjected to column chromatography fractionation on Florisil.

F1:ml hexane

F2:ml hexane/dichloromethane (70:30)

F3:ml dichloromethane

GC determinations:

PCB N°29 :ng recovered in F1 :% Recovery.

PCB N°198 :ng recovered in F1 :% Recovery.

ϵ HCH :ng recovered in F2 :% Recovery.

Endosulfan Id₄:ng recovered in F3 :% Recovery.

Attach tabulation of individual compounds quantified in sample.

Sample worksheet for analysis of chlorinated compounds in marine sediments.

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ANNEX

**PREPARATION OF THE SOLUTION OF INTERNAL STANDARDS:
PCB No 29, PCB No 198, ϵ HCH and Endosulfan I d4**

Stock Solution of PCB No 29:

1 ml from the original vial (250ng/ μ l) should be transferred into a 100 ml volumetric flask, and then the volume is adjusted to 100 ml with hexane. This stock solution contains:

2.5 ng/ μ l of PCB No 29

Stock Solution of Endosulfan I d4:

1 ml from the original vial (250ng/ μ l) should be transferred into a 100 ml volumetric flask, and then the volume is adjusted to 100 ml with hexane. This stock solution contains:

2.5 ng/ μ l of Endosulfan I d4

Working solution of internal standards:

0.5 ml from the stock solution of PCB No 29 (2.5 ng/ μ l) should be transferred into a 50 ml volumetric flask, then, 0.5 ml from the stock solution of Endosulfan I d4 (2.5 ng/ μ l) should be transferred into the volumetric flask, then 1 ml from the original vial (1ng/ μ l) of ϵ HCH should be transferred into that volumetric flask, then 0.5 ml from the concentrated solution (2ng/ μ l) of PCB No 198, and the volume adjusted to 50 ml with hexane. This working solution contains:

25 pg/ μ l of PCB No 29

20 pg/ μ l of PCB No 198

20 pg/ μ l of ϵ HCH

25 pg/ μ l of Endosulfan I d4

CAUTION: VIALS SHOULD BE COOLED AT 20°C PRIOR TO OPENING

Preparation of the Aroclor 1254 solution

Preparation of the stock solution:

1 ml from the original vial should be transferred into a 100 ml volumetric flask, then, the volume is adjusted to 100 ml with hexane. This stock solution contains:

6.5 ng/μl of Aroclor 1254

Preparation of the working solution:

1 ml from this stock solution should be transferred into a 50 ml volumetric flask and the volume adjusted to 50 ml with hexane. This working solution contains :

0.13 ng/μl of Aroclor 1254

CAUTION : VIAL SHOULD BE COOLED TO 20 °C PRIOR TO OPENING

Preparation of the Aroclor 1260 solution

Preparation of the stock solution:

1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains:

5.44 ng/μl of Aroclor 1260

Preparation of the working solution:

1 ml from the stock solution should be transferred into a 50 ml volumetric flask, then the volume is adjusted to 50 ml with hexane. This working solution contains

0.1088 ng/μl of Aroclor 1260

CAUTION: VIAL SHOULD BE COOLED TO 20 °C PRIOR TO OPENING

Preparation of the pp' DDE, pp' DDD and pp' DDT solution

pp' DDE:

Stock solution: 1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This stock solution contains:

5 ng/μl of pp' DDE

pp' DDD:

Stock solution: 1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains:

5 ng/μl of pp' DDD

pp' DDT:

Stock solution: 1 ml of the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This stock solution contains:

5 ng/μl of pp' DDT

Working solution: pp' DDE, pp' DDD and pp' DDT together.

1 ml from the stock solution of pp' DDE, 2 ml of the stock solution of pp' DDD and 3 ml of the stock solution of pp' DDT should be transferred into a 100 ml volumetric flask and the volume adjusted to 100 ml with hexane. This solution contains

- pp' DDE : 50 pg/μl
- pp' DDD : 100 pg/μl
- pp' DDT : 150 pg/μl

NOTE: Further dilution may be necessary depending on the sensitivity of the EC Detector.

CAUTION: VIAL SHOULD BE COOLED TO 20 °C PRIOR TO OPENING

Preparation of Aldrin, Dieldrin and Endrin standard solutions:

Aldrin:

Stock solution: 1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains:

5 ng/μl of Aldrin

Dieldrin:

Stock solution: 1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains:

5 ng/μl of Dieldrin

Endrin:

Stock solution: 1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains:

5 ng/μl of Endrin

Working solution: Aldrin, Dieldrin and Endrin together.

1 ml from the stock solution of Aldrin, 1 ml from the stock solution of Dieldrin and 1 ml from the stock solution of Endrin are transferred into a 100 ml volumetric flask and the volume is adjusted to 100 ml with hexane. This working solution contains:

Aldrin : 50 pg/μl
Dieldrin : 50 pg/μl
Endrin : 50 pg/μl

NOTE: Further dilution may be necessary depending on the sensitivity of the detector.

CAUTION: VIALS SHOULD BE COOLED TO 20 °C PRIOR TO OPENING

Preparation of the HCB and Lindane standard solutions:

HCB:

Stock solution: 1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains:

5 ng/μl of HCB

Lindane:

Stock solution: 1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains:

5 ng/μl of lindane

Working solution:

1 ml from the stock solution of HCB and 1 ml from the stock solution of Lindane are transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains:

HCB : 50 pg/μl

Lindane : 50 pg/μl

NOTE: further dilution may be necessary depending on the sensitivity of the EC Detector.

CAUTION: VIALS SHOULD BE COOLED TO 20 °C PRIOR TO OPENING

Preparation of the PCB congeners solution

In a 100 ml volumetric flask, transfer 1 ml from the original vial. Adjust to 100 ml with hexane in order to obtain the working solution with the following concentrations:

CB N°:	Compounds:	Concentrations (pg/μl)
8	2,4'	17.50
18	2,2',5	12
31	2,4',5	10.6
28	2,4,4'	4.6
52	2,2',5,5'	8.6
49	2,2',4,5'	12.1
44	2,2',3,5'	10.7
66	2,3',4,4'	5.5
95	2,2',3,5',6	5.7
101	2,2',4,5,5'	9.3
110	2,3,3',4',6	11.1
149	2,2',3,4',5',6	12.1
118	2,3',4,4',5	8.5
153	2,2',4,4',5,5'	8.4
138	2,2',3,4,4',5'	13.8
183	2,2',3,4,4',5',6	10.3
174	2,2',3,3',4',5,6'	9.4
177	2,2',3,3',4',5,6	9.5
180	2,2',3,4,4',5,5'	16.3
170	2,2',3,3',4,4',5	13.4
199	2,2',3,3',4,5,5',6'	9.3
194	2,2',3,3',4,4',5,5'	12.6

Separate into 10 volumetric flasks of 10 ml, seal with Teflon tape and keep in refrigerated place in order not to evaporate them.

CAUTION: VIAL SHOULD BE COOLED TO 20 °C PRIOR TO OPENING

EUNOCI 221-02937-0, E05 SED @ BHIMADZU

14:241	15,223	
15,961		
17,859		
19,88		
21,880		
21,728		
22,598		
27,665		
48,812		
48,673		
44,546		
45,661		
50,523	8	
54,133	18	
56,927	31	
58,927	28	
59,591	52	
68,927	49	
69,811	101	
69,927	66 + 95	
69,927	44	
69,927	49	
69,927	52	
69,927	49	
71,267	118	
71,267	153	
72,791	138	
74,828	183	
75,747	174	
76,194	177	
76,194	180	
88,298	170	
88,298	170	
89,178	199	
84,751		
86,664		
86,664	194	

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Annex VII.

HELCOM (2012). Manual for marine monitoring in the COMBINE programme. Annex B-12, Appendix 3. Technical note on the determination of chlorinated biphenyls and organochlorine pesticides in biota (5.2.1)

HELCOM Manual for marine monitoring in the COMBINE programme

ANNEX B-12, APPENDIX 3. TECHNICAL NOTE ON THE DETERMINATION OF CHLORINATED BIPHENYLS AND ORGANOCHLORINE PESTICIDES IN BIOTA

1. INTRODUCTION

The analysis of chlorinated biphenyls (CBs) and organochlorine pesticides (OCPs) in fish samples generally involves extraction from the respective matrix with organic solvents, followed by clean-up and gas chromatographic separation with electron capture (GC-ECD) or mass spectrometric (GC-MS) detection.

The analytical procedure is liable to systematic errors due to insufficiently optimized gas chromatographic conditions, determinant losses (evaporation, unsatisfactory extraction yield), and/or contamination from laboratory ware, reagents and the laboratory environment. It is therefore essential that the sources of systematic errors are identified and eliminated as far as possible.

In the following paragraphs, the guidelines drafted by the OSPAR Ad Hoc Working Group on Monitoring (OSPAR, 1996) have been taken into consideration.

2. PRE-TREATMENT OF LABORATORY WARE AND REAGENTS; CONTAMINATION CONTROL

Glassware, reagents, solvents, column adsorption materials and other laboratory equipment that come into contact with the sample material to be analysed should be free of impurities that interfere with the quantitative determination of CBs and OCPs.

For cleaning purposes, the following procedures should be followed:

1. Glassware should be thoroughly washed with detergents, dried with acetone and rinsed with a non-polar solvent such as *n*-pentane, and heated to > 100 °C prior to use.
2. Glass fibre Soxhlet thimbles should be pre-extracted with an organic solvent. The use of paper Soxhlet thimbles should be avoided. Alternatively, glass fibre thimbles or full glass Soxhlet thimbles, with a G1 glass filter at the bottom, are recommended.
3. Solvents should be checked for impurities using GC after concentrating the volume normally used in the procedure to 10 % of the final volume. If necessary, solvents can be purified by controlled re-distillation and rectification over KOH in an all-glass distillation column.
4. Reagents and column adsorption materials should be checked for contamination before use by extraction with an organic solvent (e.g., *n*-pentane) and analysis by GC, using the detector which will also be used for the final determination (ECD or MS).
5. Laboratory air can also be contaminated with CBs, OCPs or compounds interfering with the CB/OCP analysis. A good estimation of the contamination of the air can be found by placing a petri dish with 2 grams of C18-bonded silica for two weeks in the laboratory. After this period, the material is transferred to a glass column and eluted with 10 ml of 10% diethylether in hexane. After concentrating the eluate, the CB concentrations can be measured. Absolute amounts of <1 ng show that the contamination of the air is at an acceptably low level in that laboratory (Smedes and de Boer, 1994).

3. SAMPLE PRETREATMENT

To ensure complete extraction of the lipophilic CBs and OCPs from biological sample matrices, it is essential to dry the material and disrupt the cell walls of the biological matrix to be analysed. This can be achieved by Ultra Turrax mixing or grinding of the sample with a dehydrating reagent, such as Na₂SO₄, followed by multiple solid/liquid extraction with a mixture of polar and non-polar solvents (e.g., acetone/hexane or methanol/dichloromethane). It is essential to allow complete binding of the water present in the sample with the dehydrating reagent (this requires at least several hours) prior to starting the extraction step. The extraction efficiency must be checked for different types and amounts of biological matrices to be investigated (see 'recovery section').

4. CLEAN-UP

The crude extract obtained from sample pretreatment requires a clean-up in order to remove co-extracted lipophilic compounds that interfere with the gas chromatographic determination of CBs and OCPs. Normal-phase solid/liquid chromatography, using deactivated Al₂O₃ or deactivated silica as adsorbents and hexane or iso-octane as solvents, is an appropriate technique for the separation of the determinands from lipids or other interfering compounds. Effective removal of high molecular weight compounds can be achieved by gel permeation chromatography (GPC). However, GPC does not separate CBs from other compounds in the same molecular range, such as organochlorine pesticides (OCPs). Therefore, additional clean-up may be required. Treatment of the OCP fraction with concentrated H₂SO₄ can improve the quality of the subsequent gas chromatogram. However, this treatment is not recommended if determinands of the dieldrin type or heptachloroepoxides, which are easily broken down by H₂SO₄, are to be determined.

5. DETERMINATION BY GAS CHROMATOGRAPHY

Because of the large number of organochlorine compounds to be determined, high resolution gas chromatography (GC) using, preferably narrow bore, fused silica wall-coated open-tubular (capillary) columns is necessary.

Carrier gas

Hydrogen is the preferred carrier gas and is indispensable for columns with very small inner diameters. For safety reasons, hydrogen should not be used without a safety module which is able to check for small hydrogen concentrations inside the GC oven coming from possible leakages. As a compromise to safety aspects, helium is also acceptable.

Columns

In order to achieve sufficient separation, capillary columns should have a length of >60 m, an internal diameter of < 0.25 mm (for diameters below 0.18 mm the elevated pressure of the carrier gas needs special instrumentation) and a film thickness of the stationary phase of < 0.25 µm. For routine work, the SE 54 (Ultra 2, DB 5, RTx 5, CP-Sil 8) phase (94 % dimethyl-, 5 % phenyl-, 1 % vinyl-polysiloxane) or medium polar columns (CP-Sil 19, OV-17, OV 1701, DB 17) have been shown to give satisfactory chromatograms. A second column with a stationary phase different, from that used in the first column, may be used for confirmation of the peak identification.

Injection

Splitless and on-column injection techniques may both be used. Split injection is not recommended because strong discrimination effects may occur. Other techniques such as temperature-programmed or pressure-programmed injection may have additional advantages, but should be thoroughly optimized before use.

In splitless injection, the volume of the liner should be large enough to contain the gas volume of the evaporated injected solvent. If the liner is too small, memory effects can occur due to contamination of the gas tubing attached to the injector. Very large liner volumes, in contrast, can cause a poor transfer of early eluting components.

A 1 µl injection normally requires a ca. 1 ml liner. The occurrence of memory effects should be tested by injection of iso-octane after analysis of a CB or OCP standard. The use of a light packing of silylated glass wool in the liner improves the response and reproducibility of the injection. However, some organochlorine pesticides such as DDT may disintegrate when this technique is used. In splitless injection, discrimination effects can occur.

The splitless injection time should therefore be optimized to avoid discrimination. This can be done by injecting a solution containing an early-eluting and a late-eluting CB, e.g., CB28 and CB180. Starting with a splitless injection time of 0.5 minutes, the peak height of the late-eluting compound will presumably increase relative to that of the first compound. The optimum is found at the time when the increase does not continue any further. The split ratio is normally set at 1:25 and is not really critical. The septum purge, normally approximately 2 ml min⁻¹, should be stopped during injection. This option is not standard in all GCs.

Due to the variety of on-column injectors, a detailed optimization procedure cannot be given. More information on the optimization of on-column parameters may be obtained from Snell *et al.* (1987).

The reproducibility of injection is controlled by the use of an internal standard not present in the sample.

Detector

Quantitative analysis is performed by comparing the detector signal produced by the sample with that of defined standards. The use of an electron capture detector (ECD) sensitive to chlorinated compounds or - more generally applicable - a mass selective detector (MSD) or (even) a mass spectrometer (MS) is essential.

Due to incomplete separation, several co-eluting compounds can be present under a single detector signal. Therefore, the shape and size of the signal have to be critically examined. With a MSD or MS used as detector, either the molecular mass or characteristic mass fragments should be recorded for that purpose. If only an ECD is available, the relative retention time and the signal size should be confirmed on columns with different polarity of their stationary phases, or by the use of multi-dimensional GC techniques (de Boer *et al.*, 1995; de Geus *et al.*, 1996).

Calibration

Stock solutions of individual organohalogen compounds should be prepared using iso-octane as the solvent and weighed solid individual standard compounds of high purity (> 99 %). Stock solutions can be stored in measuring flasks in a refrigerator or in a dessicator with a saturated atmosphere of iso-octane, but losses can easily occur, particularly when storing in refrigerators (Law and de Boer, 1995). Loss of solvents in stock solutions can be controlled by recording the weight and filling up the missing amount before a new aliquot is taken. However, aliquots stored in sealed glass ampoules are much more appropriate and can normally be stored for several years. Fresh stock standard solutions should be prepared in duplicate and compared with the old standard solutions. Working standards should be prepared gravimetrically from stock solutions for each sample series. All manipulations with solvents, including pipetting, diluting and concentrating, should preferably be checked by weighing. Due to day-to-day and season-to-season temperature differences in laboratories and due to the heating of glassware after cleaning, considerable errors can be made when using volumetric glassware as a basis for all calculations.

The GC should be calibrated before each batch of measurements. Since the ECD has a non-linear response curve, a multilevel calibration is strongly advised. Megginson *et al.* (1994) recommend a set of six standard solutions for CB determination or five standard solutions for OCP determination. Standards used for multilevel calibration should be regularly distributed over the sample series, so that matrix and non-matrix containing injections alternate. When concentrations of compounds in the sample fall outside either side of the calibration curve, a new dilution or concentrate should be made and the measurement repeated. Considerable errors can be made when measuring concentrations which fall outside the calibration curve.

For MS detection, a multi-level calibration is also recommended.

Recovery

For the purpose of determining recovery rates, an appropriate internal standard should be added to each sample at the beginning of the analytical procedure. The ideal internal standard is a CB which is not present in the sample and which does not interfere with other CBs. All 2,4,6-substituted CB congeners are, in principle, suitable. Alternatively, 1,2,3,4-tetrachloronaphthalene or the homologues of dichloroalkylbenzylether can be used. For GC with mass selective detection (GC-MSD), ¹³C-labelled CBs must be used as internal standards. With GC/MS, ¹³C-labelled CBs should preferably be used as internal standards.

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Annex VIII.

IAEA (2013). Recommended method for the determination of petroleum hydrocarbons in biological samples (IAEA/Marine Environmental Studies Laboratory in co-operation with UNEP/MAP MED POL) (5.2.2)



REPORT

**RECOMMENDED METHOD FOR THE
DETERMINATION OF
PETROLEUM HYDROCARBONS IN BIOLOGICAL
SAMPLES**

**IAEA/NAEL Marine Environmental Studies Laboratory in co-operation with
UNEP/MAP MED POL**

November 2013

For further information on this method, please contact:

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RECOMMENDED METHOD FOR THE DETERMINATION OF PETROLEUM
HYDROCARBONS IN BIOLOGICAL SAMPLES

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NOTE: This method is not intended to be an analytical training manual. Therefore the method is written with the assumption that it will be used by formally trained analytical chemists. Several stages of this procedure are potentially hazardous; user should be familiar with the necessary safety precautions.

In addition, the IAEA recommended methods are intended to be guidance methods that can be used by laboratories as a starting point for generating their own standard operating procedure. If performance data are included in the method they must not be used as absolute QC acceptance criteria.

1. Sampling

Detailed guidelines for collecting biological samples are available in Reference Method No 12 Rev.2, UNEP/FAO/IAEA/IOC.

2. General discussion

Following collection of biological samples using appropriate techniques, samples are stored in non-contaminating jars at -20 °C until analysis. For analysis, the samples are defrosted and prepared for solvent extraction. To achieve a satisfactory recovery of the petroleum hydrocarbons, samples are freeze-dried. Samples are then Soxhlet extracted using methanol. Following initial clean-up treatments (partial removal of lipids by saponification), extracts are fractionated using column chromatography with silica and alumina. Quantification is done by GC-FID and GC-MS. Complementary guidelines for the analytical procedures are available in the Reference Method No 20.

3. Apparatus

- Glass jars and aluminium foil, stainless steel knives, scoops, forceps, labels, marking pens, log book.
- Insulated plastic boxes for transporting samples. Ice or dry ice.

- Deep freezer (-18 to -20 °C) for sample preservation (frost free type freezers heat to above zero during frost removal cycles and they cannot be used for long term storage).
- Rotary evaporator.
- Soxhlet extraction apparatus and heaters or Microwave oven
- Glassware including boiling flasks, ground glass stoppers, beakers, Erlenmeyer flasks, separatory funnels, centrifuge tubes, weighing bottles, pipettes, tissue grinders.
- Drying oven (temperature range up to at least 300 °C) for determining sample dry weights, baking of contaminant residues from glassware and reagents.
- Centrifuge and tubes.
- Freeze-dryer and porcelain mortar and pestle.
- Analytical balance with a precision of 0.1 mg and an electro-balance with a precision of at least 1 µg.
- Stainless steel tweezers and spatulas.
- Dessicator - completely cleaned and with no grease applied to sealing edges.
- Supply of clean dry nitrogen.
- Columns for the silica/alumina chromatography.
- Mechanical blender (food mixer).
- Vacuum pump (water-jet air pump).
- Ultrasonic bath.

4. Reagents

4.1. List of reagents

- Demineralized distilled water produced by distillation over potassium permanganate (0.1 g KMnO_4 per liter) or equivalent quality, demonstrated free from interfering substances.
- Detergent.
- Sulfochromic cleaning solution made from concentrated sulfuric acid and potassium dichromate.

- Concentrated H₂SO₄ (d 20°C: 1.84 g/ml).
- H₂SO₄ 1M
- KOH
- Potassium dichromate.
- Hexane, "distilled in glass" quality.
- Dichloromethane, "distilled in glass" quality.
- Methanol, "distilled in glass" quality.
- Acetone, "distilled in glass" quality.
- Anhydrous sodium sulfate.
- Carborundum.
- Glass wool.
- pH Paper.
- Silica gel Merck Kieselgel 60 (0.04-0.063 mm, 230-400 mesh).
- Aluminium oxide neutral Merck 90 Active (0.063-0.200 mm, 70-230 mesh).
- *n*-C₂₄-d₅₀, Friedeline, Hexamethylbenzene , Naphthalene-d₈, Acenaphtene-d₁₀, Phenanthrene-d₁₀, Chrysene-d₁₀, Perylene-d₁₂, Fluorene-d₁₀, Benzo(a)pyrene-d₁₂.
- Standard solutions of aliphatic and aromatic hydrocarbons.

Working solutions from the stock reference solutions are prepared on a regular basis and stored in clean glass volumetric flasks tightly capped with non-contaminating materials such as Teflon or glass. Extreme care must be taken to ensure that standards have not changed their concentrations through solvent evaporation.

4.2. Cleaning of reagents and adsorbents

4.2.1. Cleaning of glassware

Scrub all glassware vigorously with brushes in hot water and detergent. Rinse with tap water and with distilled water. Rinse with acetone followed by hexane or alternatively bake overnight in an oven at 450 °C. All glassware should be stored in dust free cabinets and tightly sealed with pre-cleaned aluminum foil when not in use. Ideally glassware should be rinsed with the same solvent just before use.

For more detailed information, consult Reference Method No 65: UNEP/IOC/IAEA: Reagent and laboratory ware clean-up procedures for low level contaminant monitoring.

4.2.2. Cleaning of reagents

Powdered or crystalline reagents, such as anhydrous sodium sulfate (Na_2SO_4)*, glass wool* and carborundum boiling chips*, are thoroughly cleaned before use. They are extracted with hexane in a Soxhlet apparatus for 8 hours and then with methanol or dichloromethane for another 8 hours. For those indicated by an *, this will require pre-combustion in a muffle furnace at approximately 400 °C.

4.2.3. Cleaning of adsorbents

Preparation of silica and alumina: silica gel and alumina are pre-cleaned by Soxhlet extraction, first for 8 hours with methanol and then for 8 hours with hexane. They are dried at 50 °C to remove the solvent, then at 200 °C for 8 hours and then stored in amber bottle.

Before use, they are activated at 200 °C for 4 hours and partially deactivated with 5 % water.

The deactivation procedure is carried out by adding the water to the sorbent, and mixing by gentle shaking for a few minutes. The equilibration is reached overnight.

4.2.4. Cleaning of extraction thimbles

Paper extraction thimbles should be cleaned prior to sample extraction. For use in the extraction of biological samples, the extraction can be performed in the Soxhlet apparatus with 250 ml of a mixture methanol / dichloromethane (50:50) for 8 hours cycling the solvent through at a rate of 4 to 5 cycles per hour. Add into the solvent a few carborundum boiling chips to get a regular ebullition.

The use of disposable paper thimbles for the extraction procedure rather than reusable glass fiber thimbles is recommended due to the difficulties encountered in cleaning the latter.

5. Procedure

5.1. Extraction of freeze-dried samples

A 50 to 100 g fresh weight sub-sample is selected from the sample. This sub-sample is weighed and freeze-dried. When the sub-sample appears to be dry, it is weighed again and the dry to wet ratio is calculated.

Just before starting analysis, as results have to be reported on a dry weight basis, the percent moisture or water content in a sample can be determined by weighing an aliquot, not used for analysis, of the sample before and after drying. The drying is done by heating a few grams (1-2 g) of the sample in an oven at 105°C for 24 hours to a constant weight.

The freeze-dried sub-sample is carefully pulverized in a cleaned pestle and mortar.

5 to 10 grams of freeze-dried sample are extracted with a Soxhlet extractor with 200 ml of methanol. Internal standards are added to the sample in the extraction thimble before extraction starts:

- *n*-C₂₄-d₅₀ for the aliphatic hydrocarbon fraction (Friedeline as GC internal standard, spiked right before GC injection)
- Hexamethylbenzene for the unresolved compounds from the aromatic hydrocarbon fraction (Friedeline as GC internal standard, spiked right before GC injection)
- Naphthalene-d₈, Acenaphtene-d₁₀, Phenanthrene-d₁₀, Chrysene-d₁₂, Perylene-d₁₂ for the aromatic hydrocarbon fraction (Fluorene-d₁₀, Benzo(a)pyrene-d₁₂ as GC internal standard spiked right before GC injection)

After the extraction is completed, 20 ml of 2 M KOH are added to the flask and the extraction is continued for 2 more hours in order to saponify the lipids.

The content of the extraction flask is then transferred into a separatory funnel with 30 ml of water (distilled and extracted with hexane) and extracted with 90 ml of

hexane and re-extracted again twice with 50 ml of hexane. Then all hexane extracts are combined, filtered through glass wool and dried with anhydrous sodium sulfate.

The aqueous phase is not discarded as it is used for total lipid weight determination, it's acidified with 1 M sulfuric acid and extracted 3 times in a separatory funnel with 30 ml of hexane. Then all hexane extracts are combined, filtered through glass wool and dried with anhydrous sodium sulfate.

Alternative method:

Using the microwave oven: 3-5 g of biota is placed in a glass tube with 30 ml of methanol, the oven is set at 1200 Watts, the temperature is programmed to reach 115°C in 10 min. and then isothermal at 115°C for 20 min. (internal standards are added before extraction starts).

After cooling 5 ml of 2 M KOH are added to the glass tube and the oven is set at 1200 Watts, the temperature is programmed to reach 90°C in 5 min. and then isothermal at 90°C for 10 min. in order to saponify lipids.

The content of the glass tube is filtered through glass wool and transferred into a separatory funnel with 5 ml of water (distilled and extracted with hexane) and extracted with 20 ml of hexane and re-extracted again twice with 15 ml of hexane. Then all hexane extracts are combined, filtered through glass wool and dried with anhydrous sodium sulfate.

The aqueous phase is not discarded as it is used for total lipid weight determination, it's acidified with 2 ml of 1 M sulfuric acid and extracted 3 times in a separatory funnel with 10 ml of hexane. Then all hexane extracts are combined, filtered through glass wool and dried with anhydrous sodium sulfate.

5.2. Concentration of the extract

The hexane fraction (50 ml), containing the non-saponifiable lipids and consequently the petroleum hydrocarbons is concentrated with a rotary evaporator down to about 15 ml (maximum temperature: 30°C). Then transferred in a graduated tube and concentrated with nitrogen down to a volume corresponding to 1 ml/ gram of freeze-dried sample extracted (this will avoid the precipitation of the lipids in the tube).

The hexane fraction (30 ml) containing the saponifiable lipids is concentrated with a rotary evaporator down to about 15 ml and then transferred in a graduated tube and concentrated with nitrogen.

The lipids are weighed with the electro-balance. The total lipid content is the sum of the lipid found in the first hexane fraction and this one. Then this fraction is discarded.

5.3. Extractable organic matter (EOM)

Solvent extractable organic matter (E.O.M.) is determined in the following manner. On the weighing pan of an electrobalance, a known volume of the extract (up to 100 µl) is evaporated and the residue weighed to about ± 1 µg. If the residue is less than 2 µg, pre-concentration of the original extract is required.

The quantity of E.O.M. is

$$\text{E.O.M. } (\mu\text{g/g}) = \frac{\text{Weight of residue } (\mu\text{g}) \times \text{Volume of extract (ml)} \times 1000}{\text{Volume evaporated } (\mu\text{l}) \times \text{Quantity of sample extracted (g)}}$$

The total E.O.M. is the sum of both non-saponified and saponified lipids.

5.4. Clean-up procedure and fractionation

Especially in the case of biota samples, it is necessary to clean-up the extract before proceeding with the analysis. The clean-up should remove non-petroleum hydrocarbons material that fluoresces under certain conditions. Furthermore, materials that may cause quenching will be removed simultaneously.

5.4.1. Fractionation

The clean-up and separation are achieved by a simple column chromatographic partition as follows:

A chromatography column is prepared using 50 ml burette in which a piece of glass wool is added near the stopcock to maintain the packing material. Then, 5 g of silica are transferred into the column, then 10 g of alumina and on top 1 g of sodium sulfate is added in order to avoid the disturbance of the first layer when solvents are poured into the column.

Separation of compounds:

The sample (maximum 300 mg of non-saponified lipids) is applied on top of the column. A first fraction is obtained by eluting the sample with 20 ml of hexane (F1), this fraction will contain the saturated aliphatics. The second fraction (F2) is obtained by eluting with 30 ml of a mixture of hexane and dichloromethane (90:10), this fraction will contain the unsaturated and aromatic hydrocarbons.

6. Gas Chromatography Conditions

6.1. Quantification of petroleum hydrocarbons

Gas Chromatograph	Agilent 7890
Detector	Flame Ionization Detector (FID)
Injection mode	Splitless
Carrier gas	Helium 1.2 ml min ⁻¹
Column	HP-5 (crosslinked 5% Ph Me Silicone) 30 m x 0.25 mm i.d. x 0.25 µm film thickness
Injector temperature	270°C
Detector temperature	300°C
Oven temperature program	60°C initial for 1 min., 60°C to 290°C at 4°C min ⁻¹ , 290°C for 40 min.

6.2. Quantification of PAHs

Gas Chromatograph	Agilent 6890 N
Detector	MSD 5975
Injection mode	Splitless
Carrier gas	Helium 1.5 ml min ⁻¹
Column	DB-XLBMSD 30 m x 0.25 mm i.d. x 0.25 µm film thickness
Injection specifications	inj. press.: 13 psi, Constant flow on 13 psi at 60°C, Temp. injector 270°C
Transfer line	280°C
Ion source	240°C
Analyzer	100°C
Oven temperature program	60°C initial, 60°C to 100°C at 10°C min ⁻¹ , 100°C to 285°C at 4°C min ⁻¹ , 285°C for 20 min.

6.3. Target ions to use for quantification and confirmation ions and their relative abundance for GC/MS analyses of PAHs

Compound	Target	Confirming	% Abundance
Benzene	78		
C ₁ - benzene	92		
C ₂ - benzene	106		
C ₃ - benzene	120		
C ₄ - benzene	134		
d ₈ - Naphthalene	136	134	8
Naphthalene	128	127	10
C ₁ - naphthalene	142	141	80
C ₂ - naphthalene	156	141	47 - 95
C ₃ - naphthalene	170	155	61 - 300
C ₄ - naphthalene	184	169	189
Acenaphthylene	152	151	20
d ₁₀ - Acenaphthene	164	162	97
Acenaphthene	154	153	86
d ₁₀ - Fluorene	176	174	93
Fluorene	166	165	80
C ₁ - fluorene	180	165	95 - 144
C ₂ - fluorene	194	179	25
C ₃ - fluorene	208	193	
d ₁₀ - phenanthrene	188	187	22
Phenanthrene	178	179	16
Anthracene	178	176	20
C ₁ - phenanthrene/anthracene	192	191	39 - 66
C ₂ - phenanthrene/anthracene	206	191	16 - 150
C ₃ - phenanthrene/anthracene	220	205	
C ₄ - phenanthrene/anthracene	234	219, 191	73 - 297
Dibenzothiophene	184	185	14
C ₁ - dibenzothiophene	198	197	53
C ₂ - dibenzothiophene	212	211	

C ₃ - dibenzothiophene	226	211	
C ₄ - dibenzothiophene	240	211	
Fluoranthene	202	200	17
Pyrene	202	200	21
C ₁ - fluoranthene/pyrene	216	215	36 - 64
Benz[a]anthracene	228	226	19
d ₁₂ - Chrysene	240	236	26
Chrysene	228	226	21
C ₁ - benzanthracene/chrysene	242	243	20
C ₂ - benzanthracene/chrysene	256	241	75 - 131
C ₃ - benzanthracene/chrysene	270	255	
C ₄ - benzanthracene/chrysene	284	269, 241	
d ₁₂ - perylene	264	260	24
Perylene	252	253	22
Benzo[b or k]fluoranthene	252	253	23
d ₁₂ - Benzo[a]pyrene	264	260	20
Benzo[a or e]pyrene	252	253	22
Indeno[1,2,3-c,d]pyrene	276	138	50
Dibenz[a,h]anthracene	278	279	24
Benzo[g,h,i]perylene	276	138	37

7. Quantification

The most widely used information for identification of a peak is its retention time, or its relative retention time (i.e., the adjusted retention time relative to that of a selected reference compound). Retention behavior is temperature dependent and comparison of retention times obtained at two or more temperatures may aid in determining a peak's identity. However, retention times are not specific and despite the high resolution offered by capillary columns, two compounds of interest in the same sample may have identical retention times.

In gas chromatography, results are usually quantified by either external calibration or internal calibration. Compounds identification is confirmed by GC-MS.

7.1. External Calibration

An external calibration is performed by injecting standard samples containing varying concentrations of the compound to be analyzed and creating a calibration curve (area vs. concentration). A response factor (RF) is calculated, for each target compounds, using the following equation:

$$RF = \frac{\text{Peak Area}}{\text{Sample Amount}}$$

The unknown samples are injected and the amounts of target compounds are then calculated with the following equation:

$$\text{Amount} = \frac{\text{Peak Area}}{\text{Response Factor}}$$

The method based on the external calibration doesn't take into account any variance in gas chromatograph performance and it requires the final volume of sample injected and the final volume of the extract.

7.2. Internal Calibration

This method is based on the use of an *internal standard* which is defined as a non-interfering compound added to a sample in known concentration in order to eliminate the need to measure the sample size in quantitative analysis and for correction of instrumental variation.

In this method, the internal standard is added to each sample and standard solution.

In a multiple point internal calibration each analyses contains the internal standard whose total amount is kept constant and the analyte of interest whose amount covers the range of concentrations expected. The ratio of the areas of the internal standard and analyte are then used to construct the calibration curve.

A multiple points relative response factor (RRF) calibration curve is established for analytes of interest for each working batch. A RRF is determined, for each analyte, for each calibration level using the following equation:

Where:

$$RRF(X) = \frac{\text{Area}(X)}{\text{Area}(IS)} \times \frac{\text{Qty}(IS)}{\text{Qty}(X)}$$

Area (X) = the area of the analyte to be measured (target compound)

Area (IS) = the area of the specific internal standard

Qty (X) = the known quantity of the analyte in the calibration solution

Qty (IS) = the known quantity of the internal standard in the calibration solution

The relative response factors determined for each calibration level are averaged to produce a mean relative response factor (mRRF) for each analyte. The percent relative standard deviation (%RSD) for the 3 response factor must be less than or equal to 15%, for each analyte.

$$\% RSD = \frac{\text{Standard deviation of the RRFs}}{\text{Average of the RFs}} \times 100$$

Sample analyte concentrations are calculated based on the quantity and response of the internal standard.

The following equation gives the amount of analyte in the solution analysed.

$$Qty(X) = Qty(IS) \times \frac{\text{Area}(X)}{\text{Area}(IS)} \times \frac{1}{mRRF(X)}$$

Where:

Qty (X) = the unknown quantity of the analyte in the sample

Qty (IS) = the known quantity of the internal standard added to the sample

Area (X) = the area of the analyte

Area (IS) = the area of the internal standard

mRRF (X) = the average response factor of the analyte

Sample analyte concentrations are then calculated by dividing the amount found (Qty) by the grams of samples extracted.

8. Quality assurance/quality control

Guidelines on the QA/QC requirements for analysis of sediments and marine organisms are detailed in Reference Method No 57, "Contaminant monitoring programs using marine organisms: Quality assurance and good laboratory practice".

The precision of the method is established by the replicate analysis of samples of the appropriate matrix. The precision of the entire analytical procedure is estimated by extracting five sub-samples from the same sample after homogenization. Precision is evaluated as a matter of course during the initial implementation procedure just before initiation of sample analysis.

8.1. Accuracy

The accuracy of the methods is confirmed by analysis of a suitable RM (i.e. appropriate matrix, analytes) prior to initiation of sample analysis. Agreement between measured and certified concentrations for any individual analyte should be within 35 % and on average within 25 %. Reference Materials are introduced on a regular basis (e.g. every 10-15 samples) as a method of checking the procedure. Further description of the preparation of control charts and criteria for data acceptance is elaborated in Reference Method No 57.

8.2. Blanks

Blanks represent an opportunity to evaluate and monitor the potential introduction of contaminants into samples during processing. Contributions to the analyte signal can arise from contaminants in the reagents, those arising from passive contact between the sample and the environment (e.g. the atmosphere) and those introduced during sample handling by hands, implements or glassware. It is essential to establish a consistently low (i.e. with respect to analytes) blank prior to initiating analysis or even the

determination of the method detection limit. In addition, it is necessary to perform blank determinations on a regular basis (e.g. every batch of samples).

8.3. Recovery

Recovery reflects the ability of the analyst to fully recover surrogate compounds introduced to the sample matrix or blank at the beginning of the procedure. The primary criteria for selection of compounds to be used for testing recovery are that they: 1) have physical (i.e. chromatographic/partitioning) properties similar to and if necessary spanning those of the analytes of interest, 2) do not suffer from interferences during gas chromatographic analysis, 3) are baseline resolved from the analytes of interest.

Recovery should be tested on all samples and blanks as a routine matter. Recoveries should be within 60% - 125%. However lower recoveries might be expected for low molecular weight PAHs (d₈-Naphthalene for example) due to their higher volatility. Recoveries higher than 100 % may indicate the presence of interferences.

8.4. Archiving and reporting of results

Every sample should have an associated worksheet which follows the samples and the extracts through the various stages of the procedure and upon which the analyst notes all relevant details. An example of such a worksheet is given below. Each laboratory should construct and complete such a worksheet. Relevant chromatograms should be attached to the worksheet. Analyses should be grouped and composite or summary analysis sheets archived with each group. Final disposal of the data will depend on the reasons for which it was collected but should follow the overall plan model.

All processed samples should be archived at all steps of the procedure:

- deep frozen (in the deep-freezer as it was received).
- freeze-dried (in sealed glass container kept in a dark place).

- extracted (after injection on the GC, sample extracts should be concentrated down to 1 ml and transferred into sealed glass vials, a Pasteur pipette sealed with a butane burner is adequate and cheap).

9. Bibliography

- Reference Method No 20, UNEP/IOC/IAEA: Determination of petroleum hydrocarbons in sediments. UNEP, 1992.
- Reference Method No 65, UNEP/IOC/IAEA: Reagent and laboratory ware clean-up procedures for low-level contaminants monitoring. UNEP, 1995.
- Reference Method No 57, UNEP/IOC/IAEA/FAO: Contaminant monitoring programs using marine organisms: Quality assurance and good laboratory practice. UNEP, 1990.
- Reference Method No 6, UNEP/FAO/IOC/IAEA: Guidelines for monitoring chemical contaminants in the sea using marine organisms. UNEP, 1993.
- Reference Method No 12 Rev.2, UNEP/FAO/IAEA/IOC: Sampling of selected marine organisms and sample preparation for the analysis of chlorinated hydrocarbons. UNEP, 1991.

Annex IX.

HELCOM (2012b). Manual for marine monitoring in the COMBINE programme. Annex B-12, Appendix 2. Technical Note on the determination of Polycyclic Aromatic Hydrocarbons in Biota (5.2.3)

HELCOM Manual for marine monitoring in the COMBINE programme

ANNEX B-12, APPENDIX 2. TECHNICAL NOTE ON THE DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN BIOTA

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) consist of a variable number of fused aromatic rings. By definition, PAHs contain at least three fused rings, although in practice related compounds with two fused rings (such as naphthalene and its alkylated derivatives) are often determined and will be considered in these guidelines. PAHs arise from incomplete combustion processes and from both natural and anthropogenic sources, although the latter generally predominate. PAHs are also found in oil and oil products, and these include a wide range of alkylated PAHs formed as a result of diagenetic processes, whereas PAHs from combustion sources comprise mainly parent (non-alkylated) PAHs. PAHs are of concern in the marine environment for two main reasons: firstly, low-molecular weight (MW) PAHs can be directly toxic to marine animals; secondly, metabolites of some of the high-MW PAHs are potent animal and human carcinogens, benzo[a]pyrene is the prime example. Carcinogenic activity is closely related to structure, however, and benzo[e]pyrene and four benzofluoranthene isomers (all six compounds have a molecular weight of 252 Da) are much less potent. Some compounds (e.g., heterocyclic compounds containing sulphur, such as benzothiophenes and dibenzo-thiophenes) may also cause taint in commercially exploited fish and shellfish and render them unfit for sale. PAHs are readily taken up by marine animals both across gill surfaces and from their diet, and may bioaccumulate, particularly in shellfish. Filter-feeding organisms such as bivalve molluscs can accumulate high concentrations of PAHs, both from chronic discharges to the sea (e.g., of sewage) and following oil spills. Fish are exposed to PAHs both via uptake across gill surfaces and from their diet, but do not generally accumulate high concentrations of PAHs as they possess an effective mixed-function oxygenase (MFO) system which allows them to metabolize PAHs and to excrete them in bile. An assessment of the exposure of fish to PAHs therefore requires also the determination of PAH-metabolite concentrations in bile samples, as turnover times can be extremely rapid. Thus, the analysis of PAHs in fish muscle tissue should normally only be undertaken for food quality assurance purposes (Law and Biscaya, 1994).

There are marked differences in the behaviour of PAHs in the aquatic environment between the low-MW compounds (such as naphthalene; 128 Da) and the high-MW compounds (such as benzo[ghi]perylene; 276 Da) as a consequence of their differing physico-chemical properties. The low-MW compounds are appreciably water soluble and can be bioaccumulated from the "dissolved" phase by transfer across gill surfaces, whereas the high-MW compounds are relatively insoluble and hydrophobic, and can attach to both organic and inorganic particulates within the water column. PAHs derived from combustion sources may actually be deposited to the sea already adsorbed to atmospheric particulates, such as soot particles. The majority of PAHs in the water column will eventually be either taken up by biota or transported to the sediments, and deep-water depositional areas may generally be regarded as sinks for PAHs, particularly when they are anoxic.

2. APPROPRIATE SPECIES FOR ANALYSIS OF PAHS

2.1 Benthic fish and shellfish

All teleost fish have the capacity for rapid metabolism of PAHs, thereby limiting their usefulness for monitoring temporal or spatial trends of PAHs. Shellfish (particularly molluscs) generally

have a lesser metabolic capacity towards PAHs, and so they are preferred because PAH concentrations are generally higher in their tissues.

For the purposes of temporal trend monitoring, it is essential that long time series with either a single species or a limited number of species are obtained. Care should be taken that the sample is representative of the population and that sampling can be repeated annually. There are advantages in the use of molluscs for this purpose as they are sessile, and so reflect the degree of contamination in the local area to a greater degree than fish which are mobile. The analysis of fish tissues is often undertaken in conjunction with biomarker and disease studies, and associations have been shown between the incidence of some diseases (e.g., liver neoplasia) in flatfish and the concentrations of PAHs in the sediments over which they live and feed (Malins et al., 1988; Vethaak and Rheinallt, 1992). The exposure of fish to PAHs can be assessed by the analysis of PAH-metabolites in bile, and by measuring the induction of mixed-function oxygenase enzymes which affect the formation of these metabolites. At offshore locations, the collection of appropriate shellfish samples may be problematic if populations are absent, sparse or scattered, and the collection of fish samples may be simpler. Generally, the analysis of PAHs in fish muscle tissue should only be considered for the purposes of food quality assurance.

Recent monitoring studies have indicated a seasonal cycle in PAH concentrations (particularly for combustion-derived PAHs) in mussels, with maximum concentrations in the winter prior to spawning and minimum concentrations in the summer. It is particularly important, therefore, that samples selected for trend monitoring and spatial comparisons are collected at the same time of year, and preferably in the first months of the year before spawning.

2.2 Fish

Fish are not recommended for spatial or temporal trend monitoring of PAHs, but can be useful as part of biological effects studies or for food quality assurance purposes. The sampling strategy for biological effects monitoring is described in the OSPAR Joint Assessment and Monitoring Programme (JAMP).

3. TRANSPORTATION

Live mussels should be transported to the laboratory for sample preparation. They should be transported in closed containers at temperatures between 5 °C and 15 °C, preferably below 10 °C. For live animals it is important that the transport time is short and controlled (e.g., maximum of 24 hours).

Fish samples should be kept cool or frozen (at a temperature of -20 °C or lower) as soon as possible after collection. Frozen fish samples should be transported in closed containers at temperatures below -20 °C. If biomarker determinations are to be made, then it will be necessary to store tissue samples at lower temperatures, for example, in liquid nitrogen at -196 °C.

4. PRETREATMENT AND STORAGE

4.1 Contamination

Sample contamination may occur during sampling, sample handling, pretreatment and analysis, due to the environment, the containers or packing materials used, the instruments used during sample preparation, and from the solvents and reagents used during the analytical procedures. Controlled conditions are therefore required for all procedures, including the dissection of fish organs on-board a ship (see ANNEX B-13, Appendix 1). In the case of PAHs, particular care must be taken to avoid contamination at sea. On ships there are multiple sources of PAHs, such as the

oils used for fuel and lubrication, and the exhaust from the ship's engines. It is important that the likely sources of contamination are identified and steps taken to preclude sample handling in areas where contamination can occur. A ship is a working vessel and there can always be procedures occurring as a result of the day-to-day operations (deck cleaning, automatic overboard bilge discharges, etc.) which could affect the sampling process. One way of minimizing the risk is to conduct dissection in a clean area, such as within a laminar-flow hood away from the deck areas of the vessel. It is also advisable to collect samples of the ship's fuel, bilge water, and oils and greases used on winches, etc., which can be used as fingerprinting samples at a later date, if there are suspicions of contamination in particular instances.

4.2 Shellfish

4.2.1 Depuration

Depending upon the situation, it may be desirable to depurate shellfish so as to void the gut contents and any associated contaminants before freezing or sample preparation. This is usually applied close to point sources, where the gut contents may contain significant quantities of PAHs associated with food and sediment particles which are not truly assimilated into the tissues of the mussels. Depuration should be undertaken under controlled conditions and in filtered sea water; depuration over a period of 24 hours is usually sufficient. The aquarium should be aerated and the temperature and salinity of the water should be similar to that from which the animals were removed.

4.2.2 Dissection and storage

When samples are processed, both at sea and onshore, the dissection must be undertaken by trained personnel on a clean bench wearing clean gloves and using clean stainless steel knives and scalpels. Stainless steel tweezers are recommended for holding tissues during dissection. After each sample has been prepared, all tools and equipment (such as homogenizers) should be cleaned.

4.3 Fish

4.3.1 Dissection and storage

The dissection of fish muscle and internal organs should be carried as soon as possible after collection. The details of fish muscle and liver dissection are given in ANNEX B-13, Appendix 1. If possible, the entire right side dorsal lateral fillet should be homogenized and sub samples taken for replicate PAH determinations. If, however, the amount of material to be homogenized would be too large, a specific portion of the dorsal musculature should be chosen. It is recommended that the portion of the muscle lying directly under the first dorsal fin is used in this case.

When dissecting the liver, care should be taken to avoid contamination from the other organs. If bile samples are to be taken for PAH-metabolite determinations, then they should be collected first. If the whole liver is not to be homogenized, a specific portion should be chosen in order to ensure comparability. Freeze-drying of tissue samples cannot be recommended for PAH determination, due to the contamination which may result from back-streaming of oil from the rotary pumps used to generate the vacuum.

If plastic bags or boxes are used, then they should be used as outer containers only, and should not come into contact with tissues. Organ samples (e.g., livers) should be stored in pre-cleaned containers made of glass, stainless steel or aluminium, or should be wrapped in pre-cleaned aluminium foil and shock-frozen quickly in liquid nitrogen or in a blast freezer. In the latter case, care should be taken that the capacity of the freezer is not exceeded (Law and de Boer,

1995). Cold air should be able to circulate between the samples in order that the minimum freezing time can be attained (maximum 12 hours). The individual samples should be clearly and indelibly labelled and stored together in a suitable container at a temperature of -20 °C until analysis. If the samples are to be transported during this period (e.g., from the ship to the laboratory), then arrangements must be made which ensure that the samples do not thaw out during transport. Sub samples for biomarker determinations should be collected immediately after death in order to minimize post-mortem changes in enzymatic and somatic activities, and stored in suitable vials in liquid nitrogen until analysis.

When samples are processed, both at sea and onshore, the dissection must be undertaken by trained personnel on a clean bench wearing clean gloves and using clean stainless steel knives and scalpels. Stainless steel tweezers are recommended for holding tissues during dissection. After each sample has been prepared, all tools and equipment (such as homogenizers) should be cleaned.

When pooling of tissues is necessary, an equivalent quantity of tissue should be taken from each fish, e.g., 10 % from each whole fillet.

5. ANALYSIS

5.1 Preparation of materials

Solvents, reagents, and adsorptive materials must be free of PAHs and other interfering compounds. If not, then they must be purified using appropriate methods. Reagents and absorptive materials should be purified by solvent extraction and/or by heating in a muffle oven, as appropriate. Glass fibre materials (e.g., Soxhlet thimbles) are preferred over filter papers and should be cleaned by solvent extraction. It should be borne in mind that clean materials can be re-contaminated by exposure to laboratory air, particularly in urban locations, and so storage after cleaning is of critical importance. Ideally, materials should be prepared immediately before use, but if they are to be stored, then the conditions should be considered critically. All containers which come into contact with the sample should be made of glass, and should be pre-cleaned before use. Appropriate cleaning methods would include washing with detergents, rinsing with water, and finally solvent-rinsing immediately before use. Heating of glassware in an oven (e.g., at 400°C for 24 hours) can also be useful in removing PAH contamination.

5.2 Lipid determination

Although PAH data are not usually expressed on a lipid basis, the determination of the lipid content of tissues can be of use in characterizing the samples. The lipid content should be determined on a separate subsample of the tissue homogenate, as some of the extraction techniques used routinely for PAH determination (e.g., alkaline saponification) destroy lipid materials. The total fat weight should be determined using the method of Smedes (1999) or an equivalent method.

5.3 Dry weight determination

Generally PAH data are expressed on a wet weight basis, but sometimes it can be desirable to consider them on a dry weight basis. Again, the dry weight determination should be conducted on a separate sub sample of the tissue homogenate, which should be air-dried to constant weight at 105 °C.

5.4 Extraction and clean-up

PAHs are lipophilic and so are concentrated in the lipids of an organism, and a number of methods have been described for PAH extraction (see, e.g., Ehrhardt *et al.*, 1991). The preferred methods generally utilize either Soxhlet extraction, or alkaline digestion followed by liquid-liquid extraction with an organic solvent. Microwave-assisted solvent extraction can be mentioned as one of the modern techniques being applied to PAH analysis (Budzinski *et al.*, 2000; During and Gaath, 2000; Vázquez Blanco *et al.*, 2000; Ramil Criado *et al.*, 2002). In the case of Soxhlet extraction, the wet tissue must be dried by mixing with a chemical agent (e.g., anhydrous sodium sulphate), in which case a time period of several hours is required between mixing and extraction in order to allow complete binding of the water in the sample. Alkaline digestion is conducted on wet tissue samples, so this procedure is unnecessary. In neither case can the freeze-drying of the tissue prior to extraction be recommended, owing to the danger of contamination from oil back-streaming from the rotary pump (which provides the vacuum) into the sample. Non-polar solvents alone will not effectively extract all the PAHs from tissues when using Soxhlet extraction, and mixtures such as hexane/dichloromethane may be effective in place of solvents such as benzene and toluene, used historically for this purpose. Alkaline digestion has been extensively used in the determination of PAHs and hydrocarbons and is well documented. It is usually conducted in alcohol (methanol or ethanol), which should contain at least 10 % water, and combines disruption of the cellular matrix, lipid extraction and saponification within a single procedure, thereby reducing sample handling and treatment. For these reasons, it should be the method of choice. Solvents used for liquid-liquid extraction of the homogenate are usually non-polar, such as pentane or hexane, and they will effectively extract all PAHs.

Tissue extracts will always contain many compounds other than PAHs, and a suitable clean-up is necessary to remove those compounds which may interfere with the subsequent analysis. Different techniques may be used, both singly or in combination, and the choice will be influenced by the selectivity and sensitivity of the final measurement technique and also by the extraction method employed. If Soxhlet extraction was used, then there is a much greater quantity of residual lipid to be removed before the analytical determination can be made than in the case of alkaline digestion. An additional clean-up stage may therefore be necessary. The most commonly used clean-up methods involve the use of alumina or silica adsorption chromatography, but gel permeation chromatography and similar high performance liquid chromatography (HPLC) based methods are also employed (Nondek *et al.*, 1993; Nyman *et al.*, 1993; Perfetti *et al.*, 1992). The major advantages of using HPLC-based clean-up methods are their ease of automation and reproducibility.

5.5 Pre-concentration

The sample volume should be 2 cm³ or greater to avoid errors when transferring solvents during the clean-up stages. Evaporation of solvents using a rotary-film evaporator should be performed at low temperature (water bath temperature of 30 °C or lower) and under controlled pressure conditions, in order to prevent losses of the more volatile PAHs such as naphthalenes. For the same reasons, evaporation to dryness should be avoided. When reducing the sample to final volume, solvents can be removed by a stream of clean nitrogen gas. Suitable solvents for injection into the gas chromatograph (GC) or GC-MS include pentane, hexane, heptane and *iso*-octane, whereas for HPLC analyses acetonitrile and methanol are commonly used.

5.6 Selection of PAHs to be determined

The choice of PAHs to be analysed is not straightforward, both because of differences in the range of PAH compounds resulting from combustion processes and from oil and oil products, and also because the aims of specific monitoring programmes can require the analysis of different representative groups of compounds. PAHs arising from combustion processes are predominantly parent (unsubstituted) compounds, whereas oil and its products contain a much

wider range of alkylated compounds in addition to the parent PAHs. This has implications for the analytical determination, as both HPLC-based and GC-based techniques are adequate for the determination of a limited range of parent PAHs in samples influenced by combustion processes, whereas in areas of significant oil contamination and following oil spills only GC-MS has sufficient selectivity to determine the full range of PAHs present. The availability of pure individual PAHs for the preparation of standards is problematic and limits both the choice of determinands and, to some degree, the quantification procedures which can be used. The availability of reference materials certified for PAHs is also rather limited. A list of target parent and alkylated PAHs suitable for environmental monitoring is given in Table 1. In both cases, the list was concentrated on a subset of parent (predominantly combustion-derived) PAHs due to analytical limitations. This approach completely neglects the determination of alkylated PAHs, which allows the interpretation of PAH accumulation from multiple sources including those due to oil inputs. It will not be necessary for all of these PAH compounds and groups to be analysed in all cases, but an appropriate selection can be made from this list depending on the specific aims of the monitoring programme to be undertaken.

Table 1: Compounds of interest for environmental monitoring for which the guidelines apply			
Compound	MW	Compound	MW
Naphthalene	128	C2-Phenanthrenes/Anthracenes	206
C1-Naphthalenes	142	C3-Phenanthrenes/Anthracenes	220
C2-Naphthalenes	156	Fluoranthene	202
C3-Naphthalenes	170	Pyrene	202
C4-Naphthalenes	184	C1-Fluoranthenes/Pyrenes	216
Acenaphthylene	152	C2-Fluoranthenes/Pyrenes	230
Acenaphthene	154	Benz[a]anthracene	228
Biphenyl	154	Chrysene	228
Fluorene	166	2,3-Benzanthracene	228
C1-Fluorenes	180	Benzo[a]fluoranthene	252
C2-Fluorenes	194	Benzo[b]fluoranthene	252
C3-Fluorenes	208	Benzo[j]fluoranthene	252
Dibenzothiophene	184	Benzo[k]fluoranthene	252
C1-Dibenzothiophenes	198	Benzo[e]pyrene	252
C2-Dibenzothiophenes	212	Benzo[a]pyrene	252
C3-Dibenzothiophenes	226	Perylene	252
Phenanthrene	178	Indeno[1,2,3-cd]pyrene	276
Anthracene	178	Benzo[ghi]perylene	276
C1-Phenanthrenes/ Anthracenes	192	Dibenz[ah]anthracene	278

5.7 Instrumental determination of PAHs

Unlike the situation for chlorobiphenyls (CBs), where GC techniques (particularly GC-ECD) are used exclusively, two major approaches based on GC and HPLC are followed to an equal extent in the analysis of PAHs. The greatest sensitivity and selectivity in routine analyses are achieved by combining HPLC with fluorescence detection (HPLC-UVF) and capillary gas chromatography with mass spectrometry (GC-MS). In terms of flexibility, GC-MS is the most capable technique, as in principle it does not limit the selection of determinands in any way, while HPLC is suited only to the analysis of parent PAHs. In the past, analyses have also been conducted using HPLC with UV-absorption detection and GC with flame-ionization detection, but neither can be recommended because of their relatively poor selectivity.

Intercomparison exercises have demonstrated a serious lack of comparability between specific hydrocarbon concentrations measured in different laboratories and using both analytical approaches described above (Farrington *et al.*, 1986). An interlaboratory performance study has been carried out within the QUASIMEME laboratory testing scheme in order to assess the level of comparability among laboratories conducting PAH analyses and to identify improvements in methodology (Law and Klungsøyr, 1996; Law *et al.*, 1998, QUASIMEME). Limits of determination within the range of 0.2 to 10 µg kg⁻¹ wet weight for individual PAH compounds should be achievable by both GC-MS and HPLC-UVF techniques.

5.8 HPLC

Reversed-phase columns (e.g., octadecylsilane (RP-18)) 15–30 cm in length are used almost exclusively, in conjunction with gradient elution using mixtures of acetonitrile/water or methanol/water. A typical gradient may start as a 50 % mixture, changing to 100 % acetonitrile or methanol in 40 minutes. This flow is maintained for 20 minutes, followed by a return to the original conditions in 5 minutes and 5–10 minutes' equilibration before the next injection. The use of an automatic injector is strongly recommended. Also, the column should be maintained in a column oven heated to 10–30°C. The systems yielding the best sensitivity and selectivity utilize fluorescence detection. As different PAH compounds yield their maximum fluorescence at different wavelengths, for optimum detection of PAHs the wavelengths of the detector should be programmed so that the excitation/emission wavelengths detected are changed at pre-set times during the analytical determination. For closely eluting peaks, it may be necessary to use two detectors in series utilizing different wavelength pairs, or to affect a compromise in the selected wavelengths if a single detector is used. As the fluorescence signals of some PAHs (e.g., pyrene) are quenched by oxygen, the eluents must be degassed thoroughly. This is usually achieved by continuously bubbling a gentle stream of helium through the eluent reservoirs, but a vacuum degasser can also be used. Polytetrafluorethylene (PTFE) tubing must not then be used downstream of the reservoirs as this material is permeable to oxygen; stainless steel or polyether-etherketone (PEEK) tubing is preferred.

5.9 GC-MS

The two injection modes commonly used are splitless and on-column injection. Automatic sample injection should be used wherever possible to improve the reproducibility of injection and the precision of the overall method. If splitless injection is used, the liner should be of sufficient capacity to contain the injected solvent volume after evaporation. For PAH analysis, the cleanliness of the liner is also very important if adsorption effects and discrimination are to be avoided, and the analytical column should not contain active sites to which PAHs can be adsorbed. Helium is the preferred carrier gas, and only capillary columns should be used. Because of the wide boiling range of the PAHs to be determined and the surface-active properties of the higher PAHs, the preferred column length is 25–30 m, with an internal diameter of 0.15 mm to 0.3 mm. Film thicknesses of 0.3 µm to 1 µm are generally used; this choice has little impact on critical resolution, but thicker films are often used when one-ring aromatic compounds are to be determined alongside PAHs, or where a high sample loading is

needed. No stationary phase has been found on which all PAH isomers can be resolved; the most commonly used stationary phase for PAH analysis is 5 % phenyl methylsilicone (DB-5 or equivalent). This will not, however, resolve critical isomers such as benzo[*b*], [*j*] and [*k*]fluoranthenes, or chrysene from triphenylene. These separations can be made on other columns, if necessary. For PAHs there is no sensitivity gain from the use of chemical ionization (either positive or negative ion), so analyses are usually conducted in electron-impact mode at 70 eV. The choice of full-scan or multiple-ion detection is usually made in terms of sensitivity. Some instruments such as ion-trap mass spectrometers exhibit the same sensitivity in both modes, so full-scan spectra are collected, whereas for quadrupole instruments greater sensitivity is obtained if the number of ions scanned is limited. In that case, the masses to be detected are programmed to change during the analysis as different PAHs elute from the capillary column.

6. CALIBRATION AND QUANTIFICATION

6.1 Standards

A range of fully deuterated parent PAHs is available for use as standards in PAH analysis. The availability of pure PAH compounds is limited (Annex B-7). Although most of the parent compounds can be purchased as pure compounds, the range of possible alkyl-substituted PAHs is vast and only a limited selection of them can be obtained. In HPLC, where the resolving power of the columns is limited and the selectivity less than that which can be obtained using MS detection, only a single internal standard is normally used (e.g., phenanthrene-d10), although fluoranthene-d10 and 6-methyl chrysene, among others, have also been used. If GC-MS is used, then a wider range of deuterated PAHs can be utilized, both because of the wide boiling range of PAHs present and because that allows the use of both recovery and quantification standards. Suitable standards could range from naphthalene-d8 to perylene-d10. It is always recommended to use at least two and preferably three internal standards of hydrocarbons of small, medium, and high molecular weight (e.g., naphthalene-d8, phenanthrene-d10, perylene-d12). Crystalline PAHs of known purity should be used for the preparation of calibration standards. If the quality of the standard materials is not guaranteed by the producer or supplier (as for certified reference materials), then it should be checked by GC-MS analysis. Solid standards should be weighed to a precision of 10⁻⁵ grams. Calibration standards should be stored in the dark because some PAHs are photosensitive, and ideally solutions to be stored should be sealed in amber glass ampoules. Otherwise, they can be stored in a refrigerator in stoppered measuring cylinders or flasks that are gas tight to avoid evaporation of the solvent during storage.

6.2 Calibration

Multilevel calibration with at least five calibration levels is preferred to adequately define the calibration curve. In general, GC-MS calibration is linear over a considerable concentration range but exhibits non-linear behaviour when the mass of a compound injected is low due to adsorption. Quantification should be conducted in the linear region of the calibration curve, or the non-linear region must be well characterized during the calibration procedure. For HPLC-UVF, the linear range of the detection system should be large, and quantification should be made within the linear range. External standardization is often used with HPLC due to the relatively limited resolution obtainable with this technique as generally employed.

6.3 Recovery

The recovery of analytes should be checked and reported. Given the wide boiling range of the PAHs to be determined, the recovery may vary with compound group, from the volatile PAHs of low molecular weight to the larger compounds. For GC-MS analysis, deuterated standards can

be added in two groups: those to be used for quantification are added at the start of the analytical procedure, whilst those from which the absolute recovery will be assessed are added prior to GC-MS injection. This ensures that the calculated PAH concentrations are corrected for the recovery obtained in each case. In the case of HPLC, where only a single deuterated PAH standard is used, it is more common to assess recovery periodically by carrying a standard solution through the whole analytical procedure, then assessing recovery by reference to an external standard. This technique does not, however, correct for matrix effects, and so may be used in conjunction with the spiking of real samples.

7. ANALYTICAL QUALITY CONTROL

Planners of monitoring programmes must decide on the accuracy, precision, repeatability, and limits of detection and determination which they consider acceptable. Achievable limits of determination for each individual component are as follows:

- for GC-MS measurements: 0.2 µg kg⁻¹ ww;
- for HPLC measurements: 0.5–10 µg kg⁻¹ ww.

Further information on analytical quality control procedures for PAHs can be found elsewhere (Law and de Boer, 1995). A procedural blank should be measured with each sample batch, and should be prepared simultaneously using the same chemical reagents and solvents as for the samples. Its purpose is to indicate sample contamination by interfering compounds, which will result in errors in quantification. The procedural blank is also very important in the calculation of limits of detection and limits of quantification for the analytical method. In addition, a laboratory reference material (LRM) should be analysed within each sample batch. Test materials from the former runs of QUASIMEME Laboratory Proficiency Testing can be used as Laboratory Reference Material. The LRM must be homogeneous and well characterized for the determinands of interest within the analytical laboratory. Ideally, stability tests should have been undertaken to show that the LRM yields consistent results over time. The LRM should be of the same matrix type (e.g., liver, muscle, mussel tissue) as the samples, and the determinand concentrations should be in the same range as those in the samples. Realistically, and given the wide range of PAH concentrations encountered, particularly in oil spill investigations, this is bound to involve some compromise. The data produced for the LRM in successive sample batches should be used to prepare control charts. It is also useful to analyse the LRM in duplicate from time to time to check within-batch analytical variability. The analysis of an LRM is primarily intended as a check that the analytical method is under control and yields acceptable precision, but a certified reference material (CRM) of a similar matrix should be analysed periodically in order to check the method bias. The availability of biota CRMs certified for PAHs is very limited (Annex B-7; QUASIMEME), and in all cases the number of PAHs for which certified values are provided is small. At regular intervals, the laboratory should participate in an intercomparison or proficiency exercise in order to provide an independent check on the performance.

8. DATA REPORTING

The calculation of results and the reporting of data can represent major sources of error, as has been shown in intercomparison studies for PAHs. Control procedures should be established in order to ensure that data are correct and to obviate transcription errors. Data stored in databases should be checked and validated, and checks are also necessary when data are transferred between databases. Data should be reported in accordance with the latest ICES reporting formats.

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Annex X.

**ICES/OSPAR (2008). JAMP Guidelines for monitoring contaminants in biota and sediments.
Annex 1: Polycyclic aromatic hydrocarbons in biota - Determination of parent and alkylated PAHs
in biological materials (5.2.4)**

1.5.5.16 JAMP Guidelines for monitoring contaminants in biota and sediments

Request

ICES has received the following request from OSPAR:

Development of JAMP monitoring guidelines (OSPAR no. 2-2007)

To carry out the following development work with regard to the JAMP Guidelines for monitoring Contaminants in Sediments (OSPAR agreement 2002-16) and JAMP Guidelines for monitoring Contaminants in Biota (OSPAR agreement 1999-2) to ensure that monitoring guidance is in place to support a revised Co-ordinated Environmental Monitoring Programme.

- a. develop draft technical annexes on monitoring of polybrominated diphenyl ethers and hexabromocycladodecane in sediments and biota following the structure of the existing technical annexes. SIME 2007 will be invited to clarify the congeners and compartments that are relevant for the development of monitoring guidance for brominated flame retardants.*
- b. review the existing technical annexes on PAHs to see whether they are adequate for monitoring of the alkylated PAHs and, as appropriate, prepare advice on any revisions that are necessary.*
- c. to develop a draft technical annex on monitoring of TBT and its breakdown products in biota”*

Advice on point (a) has been provided previously.

Summary

Alkyl PAH in sediment and biota

The current OSPAR technical annex for PAH analyses required updating as clear guidance was required to ensure quantification of alkyl homologues of PAHs and alkyl substituted sulphur-heterocyclic PAHs in biota and sediment. The proposed updated technical guidelines are presented in annexes 1 and 2 and are recommended to OSPAR for adoption as part of their JAMP guidelines for monitoring contaminants.

The technical annex for the analyses of parent and alkylated PAHs in biota contains information for the selection of species, sampling techniques, sample transport, conservation, and sample treatment (including extractions, clean-up, and pre-concentration).

The analytical protocol follows the same technical principles as for the analysis of unsubstituted, parent PAHs. However, HPLC with fluorescence detection (HPLC-UVF) cannot be used for the detailed analysis of individual alkylated PAHs. Gas chromatography with mass spectrometry (GC-MS) is presently the preferred analytical technique for the analysis of both parent and alkylated PAHs.

Organotins in biota

The technical annex for organotins in biota, as appended at annex 3 is proposed for adoption by OSPAR as part of the Joint Monitoring and Assessment Programme (JAMP) Guidelines for Monitoring Contaminants in Biota.

Explanation

The full text of the response is found in the attached technical annexes.

Sources of information

ICES. 2008a. Report of the Working Group on Marine Sediments (WGMS 2008). ICES CM 2008/MHC:03.
ICES. 2008b. Report of the Marine Chemistry Working Group (MCWG 2008).

ANNEX 1: Polyaromatic hydrocarbons in biota

Determination of parent and alkylated PAHs in biological materials

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) consist of a variable number of fused benzene rings. By definition, PAHs contain at least two fused rings. PAHs arise from incomplete combustion processes and from both natural and anthropogenic sources, although the latter generally predominate. PAHs are also found in oil and oil products, and these include a wide range of alkylated PAHs formed as a result of diagenetic processes, whereas PAHs from combustion sources comprise mainly parent (non-alkylated) compounds. PAHs are of concern in the marine environment for two main reasons: firstly, low molecular weight (MW) PAHs can cause tainting of fish and shellfish and render them unfit for sale; secondly, metabolites of some of the high MW PAHs are potent animal and human carcinogens — benzo[*a*]pyrene is the prime example. Carcinogenic activity is closely related to structure. Benzo[*e*]pyrene and the four benzofluoranthene isomers all have a molecular weight of 252 Da; however, they are much less potent than benzo[*a*]pyrene. Less is known about toxicity of alkylated PAHs. However, one study has demonstrated that alkylated PAHs may have increased toxicity compared to the parent compound (Marvanova *et al.*, 2008).

PAHs are readily taken up by marine animals both across gill surfaces (lower MW PAHs) and from their diet. They may bioaccumulate, particularly in shellfish. Filter-feeding organisms such as bivalve molluscs can accumulate high concentrations of PAHs, both from chronic discharges to the sea (e.g., of sewage) and following oil spills. Fish are exposed to PAHs both via uptake across gill surfaces and from their diet, but do not generally accumulate high concentrations of PAHs as they possess an effective mixed-function oxygenase (MFO) system which allows them to metabolise PAHs and to excrete them in bile. Other marine vertebrate and marine mammals also metabolise PAHs efficiently. An assessment of the exposure of fish to PAHs therefore requires the determination of PAH metabolite concentrations in bile, as turnover times can be extremely rapid.

There are marked differences in the behaviour of PAHs in the aquatic environment between the low MW compounds (such as naphthalene; 128 Da) and the high MW compounds (such as benzo[*ghi*]perylene; 276 Da) as a consequence of their differing physico-chemical properties. The low MW compounds are appreciably water soluble (e.g. naphthalene) and can be bioaccumulated from the dissolved phase by transfer across gill surfaces, whereas the high MW compounds are relatively insoluble and hydrophobic, and can attach to both organic and inorganic particulates within the water column. PAHs derived from combustion sources may actually be deposited to the sea already adsorbed to atmospheric particulates, such as soot particles. The sediment will act as a sink for PAHs in the marine environment.

2. Appropriate species for analysis of parent and alkylated PAHs

2.1 Benthic fish and shellfish

Guidance on the selection of appropriate species for contaminant monitoring is given in the OSPAR Joint Assessment and Monitoring Programme guidelines. All teleost fish have the capacity for rapid metabolism of PAHs, thereby limiting their usefulness for monitoring temporal or spatial trends of PAHs. Shellfish (particularly molluscs) generally have a lesser metabolic capacity towards PAHs, and so they are preferred because PAH concentrations are generally higher in their tissues. The blue mussel (*Mytilus edulis*) occurs in shallow waters along almost all coasts of the Northeast Atlantic. It is therefore suitable for monitoring in near shore waters. No distinction is made between *M. edulis* and *M. galloprovincialis* because the latter species, which may occur along Spanish and Portuguese coasts, fills a similar ecological niche. A sampling size range of 30–70 mm shell length is specified to ensure availability throughout the whole maritime area. In some areas (e.g., the Barents Sea), other species may be considered. Recent monitoring studies have indicated a seasonal cycle in PAH concentrations (particularly for combustion-derived PAHs) in mussels, with maximum concentrations in the winter prior to spawning and minimum concentrations in the summer. It is particularly important, therefore, that samples selected for trend monitoring and spatial comparisons are collected at the same time of year, and preferably in the first months of the year prior to spawning.

For the purposes of temporal trend monitoring, it is essential that long time-series with either a single species or a limited number of species be obtained. Care should be taken that the sample is representative of the population and that it can be sampled annually. There are advantages in the use of molluscs for this purpose as they are sessile, and so reflect the degree of contamination in the local area to a greater degree than fish which are mobile and metabolise PAHs relatively efficiently. The analysis of fish tissues is often undertaken in conjunction with biomarker and disease studies, and associations have been shown between the incidence of some diseases (e.g., liver neoplasia) in flatfish and the concentrations of PAHs in the sediments over which they live and feed (Malins *et al.*, 1988; Vethaak and Rheinallt, 1992). The exposure of fish to PAHs can be assessed by the analysis of PAH metabolites in bile, and by measuring the induction of mixed-function oxygenase enzymes which catalyse the formation of these metabolites.

3. Transportation

Live biota should be transported in closed containers at temperatures between 5°C and 10°C. For live animals it is important that the transport time is short and controlled (e.g., maximum of 24 hours). If biomarker determinations are to be made, then it will be necessary to store tissue samples at lower temperatures, for example, in liquid nitrogen at -196°C.

4. Pre-treatment and storage

4.1 Contamination

Sample contamination may occur during sampling, sample handling, pre-treatment, and analysis, due to the environment, the containers or packing materials used, the instruments used during sample preparation, and from the solvents and reagents used during the analytical procedures. Controlled conditions are therefore required for all procedures. In the case of PAHs, particular care must be taken to avoid contamination at sea. On ships there are multiple sources of PAHs, such as the oils used for fuel and lubrication, and the exhaust from the ship's engines. It is important that the likely sources of contamination are identified and steps taken to preclude sample handling in areas where contamination can occur. A ship is a working vessel and there can always be procedures occurring as a result of the day-to-day operations (deck cleaning, automatic overboard bilge discharges, etc.) which could affect the sampling process. One way of minimizing the risk is to conduct dissection in a clean area, such as within a laminar-flow hood away from the deck areas of the vessel. It is also advisable to collect samples of the ship's fuel, bilge water, and oils and greases used on winches, etc., which can be used as fingerprinting samples at a later date, if there are suspicions of contamination in particular instances.

Freeze-drying of tissue samples may be a source of contamination due to the back-streaming of oil vapours from the rotary vacuum pumps. Furthermore, drying the samples may result in losses of the lower molecular weight and more volatile PAHs through evaporation (Law and Biscaya, 1994).

4.2 Shellfish

4.2.1 Depuration

Depending upon the situation, it may be desirable to depurate shellfish so as to void the gut contents and any associated contaminants before freezing or sample preparation. This is usually applied close to point sources, where the gut contents may contain significant quantities of PAHs associated with food and sediment particles which are not truly assimilated into the tissues of the mussels. Depuration should be undertaken in controlled conditions and in clean seawater; depuration over a period of 24 hours is usually sufficient. The aquarium should be aerated and the temperature and salinity of the water should be similar to that from which the animals were removed.

4.2.2 Dissection and storage

Mussels should be shucked live and opened with minimal tissue damage by detaching the adductor muscles from the interior of at least one valve. The soft tissues should be removed and homogenised as soon as possible, and frozen in glass jars or aluminium cans at -20°C until analysis. Plastic materials must not be used for sampling and storage owing to possible adsorption of the PAHs onto the container material. As PAHs are sensitive to photo-degradation, exposure to direct sunlight or other strong light must be avoided during storage of the samples as well as during all steps of sample preparation, including extraction and storage of the extracts (Law and Biscaya, 1994). The use of amber glassware is strongly recommended.

When samples are processed, both at sea and onshore, the dissection must be undertaken by trained personnel on a clean bench wearing clean gloves and using PAH-free stainless steel knives and scalpels. Stainless steel tweezers are recommended for holding tissues during dissection. After each sample has been prepared, all tools and equipment (such as homogenisers) should be cleaned by wiping with tissue and rinsing with solvent.

5. Analysis

5.1 Preparation of materials

Solvents and adsorptive materials must all be checked for the presence of PAHs and other interfering compounds. If found then the solvents, reagents, and adsorptive materials must be purified or cleaned using appropriate methods. Adsorptive materials should be cleaned by solvent extraction and/or by heating in a muffle oven as appropriate. Glass fibre materials (e.g. Soxhlet thimbles and filter papers used in pressurised liquid extraction (PLE)) should be cleaned by solvent extraction or pre-baked at 450°C overnight. It should be borne in mind that clean materials can be re-

contaminated by exposure to laboratory air, particularly in urban locations, and so the method of storage after cleaning is of critical importance. Ideally, materials should be prepared immediately before use, but if they are to be stored, then the conditions should be considered critically. All containers which come into contact with the sample should be made of glass or aluminium, and should be pre-cleaned before use. Appropriate cleaning methods would include washing with detergents, rinsing with water of known quality, and finally solvent rinsing immediately before use.

5.2 Lipid determination

Although PAH data are not usually expressed on a lipid basis, the determination of the lipid content of tissues can be of use in characterising the samples. This will enable reporting concentrations on a wet weight or lipid weight basis. The lipid content should be determined on a separate subsample of the tissue homogenate, as some of the extraction techniques used routinely for PAHs determination (e.g., PLE with fat retainers, alkaline saponification) destroy or remove lipid materials. The total lipid content of fish or shellfish should be determined using the method of Bligh and Dyer (1959) as modified by Hanson and Olley (1963) or an equivalent method such as Smedes (1999). Extractable lipid may be used, particularly if the sample size is small and lipid content is high. It has been shown that if the lipid content is high (>5%) then extractable lipid will be comparable to the total lipid.

5.3 Extraction

PAHs are lipophilic and so are concentrated in the lipids of an organism, and a number of methods have been described for PAH extraction (Ehrhardt *et al.*, 1991). These methods generally utilise either Soxhlet extraction, or alkaline digestion followed by liquid-liquid extraction with an organic solvent. In the case of Soxhlet extraction, the wet tissue must be dried by mixing with a chemical drying agent (e.g., anhydrous sodium sulphate), in which case a time period of several hours is required between mixing and extraction in order to allow complete binding of the water in the sample. Samples are spiked with recovery standard and should be left overnight to equilibrate. Alkaline digestion is conducted on wet tissue samples, so this procedure is unnecessary.

Apolar solvents alone will not effectively extract all the PAHs from tissues when using Soxhlet extraction, and mixtures such as hexane/dichloromethane may be effective in place of solvents such as benzene and toluene, used historically for this purpose. Alkaline digestion has been extensively used in the determination of PAHs and hydrocarbons and is well documented. It is usually conducted in alcohol (methanol or ethanol), which should contain at least 10% water, and combines disruption of the cellular matrix, lipid extraction and saponification within a single procedure, thereby reducing sample handling and treatment. Solvents used for liquid-liquid extraction of the homogenate are usually apolar, such as pentane or hexane, and they will effectively extract all PAHs.

Alternatively extraction of wet or dry samples of biota may be carried out by pressurised liquid extraction (PLE). This is a more recent method, requiring less solvent and time for the extraction process. The wet biota sample is dried by mixing with sufficient anhydrous sodium sulphate to form a free flowing mixture and is packed into stainless steel extraction cells containing a glass fibre filter and sodium sulphate or glass powder to fill the cell. To ensure a better recovery samples may be extracted twice and extractions are performed at elevated temperatures and pressure.

5.4 Clean-up

Tissue extracts will always contain many compounds other than PAHs, and a suitable clean up is necessary to remove those compounds which may interfere with the subsequent analysis. Different techniques may be used, either singly or in combination, and the choice will be influenced by the selectivity and sensitivity of the final measurement technique and also by the extraction method employed. If Soxhlet extraction was used, then there is a much greater quantity of residual lipid to be removed before the analytical determination can be made than in the case of alkaline digestion. An additional clean-up stage may therefore be necessary. The most commonly used clean-up methods involve the use of deactivated alumina or silica adsorption chromatography. When applying fractionation, the elution pattern has to be checked frequently. This should be carried out in the presence of sample matrix, as that can partially deactivate the clean-up column, resulting in earlier elution of the PAHs than in a standard solution.

Gel permeation chromatography (GPC) and high performance liquid chromatography (HPLC) based methods are also employed (Nondek *et al.*, 1993; Nyman *et al.*, 1993; Perfetti *et al.*, 1992). The major advantages of using HPLC-based clean-up methods are their ease of automation and reproducibility.

Isocratic HPLC fractionation of the extract can be used to give separate aliphatic and aromatic fractions (Webster *et al.*, 2002). A metal-free silica column is used for the clean-up/fractionation as dibenzothiophene (DBT) can be retained on ordinary silica columns. The split time is determined by injection of a solution containing representative aliphatic and PAH standards. The silica column is regenerated by a cleaning cycle after a set number of samples. If PAHs are to be analysed by HPLC and there are significant amounts of alkylated PAHs present then the removal of the alkylated PAHs may be difficult.

5.5 Pre-concentration

In the methods suggested above, all result in an extract in which non-polar solvents are dominant. The sample volume should be 2 ml or greater to avoid errors when transferring solvents during the clean-up stages. Syncore parallel evaporators can be used with careful optimisation of the evaporation parameters. Evaporation of solvents using a rotary-film evaporator should be performed at low temperature (water bath temperature of 30°C or lower) and under controlled pressure conditions, in order to prevent losses of the more volatile PAHs such as naphthalenes. For the same reasons, evaporation to dryness must be avoided. When reducing the sample to final volume, solvents can be removed by a stream of clean nitrogen gas. Suitable solvents for injection into the GC-MS include pentane, hexane, heptane, *iso*-hexane and *iso*-octane.

5.6 Selection of PAHs to be determined

The choice of PAHs to be analysed is not straightforward, both because of differences in the range of PAH compounds resulting from combustion processes and from oil and oil products, and also because the aims of specific monitoring programmes can require the analysis of different representative groups of compounds. PAHs arising from combustion processes are predominantly parent (unsubstituted) compounds, whereas oil and its products contain a much wider range of alkylated compounds in addition to the parent PAHs. This has implications for the analytical determination, as both HPLC-based and GC-based techniques are adequate for the determination of a limited range of parent PAHs in samples influenced by combustion processes, whereas in areas of significant oil contamination and following oil spills only GC-MS has sufficient selectivity to determine the full range of PAHs present. The availability of pure individual PAHs for the preparation of standards is problematic and limits both the choice of determinands and, to some degree, the quantification procedures which can be used. The availability of reference materials certified for PAHs is also rather limited. A list of target parent and alkylated PAHs suitable for environmental monitoring is given in Table A1.1. This differs both from the list previously developed within ICES specifically for intercomparison purposes, and the historic list of Borneff. In both cases, the lists were concentrated on a subset of parent (predominantly combustion-derived) PAHs due to analytical limitations. This approach completely neglects the determination of alkylated PAHs, which allows the interpretation of PAH accumulation from multiple sources including those due to oil inputs. It will not be necessary for all of these PAH compounds and groups to be analysed in all cases, but an appropriate selection can be made from this list depending on the specific aims of the monitoring programme to be undertaken.

Table A1.1 Compounds of interest for environmental monitoring for which the guidelines apply. For compounds in italics standards are not available for any isomers in this group.

Compound	MW	Compound	MW
Naphthalene	128	2, 3d-benzonaphthothiophene	234
C ₁ -Naphthalenes	142	C ₁ -234	248
C ₂ -Naphthalenes	156	C ₂ -Fluoranthenes/Pyrenes	230
C ₃ -Naphthalenes	170	Benz[<i>a</i>]anthracene	228
<i>C₄-Naphthalenes</i>	184	Chrysene	228
Acenaphthylene	152	2,3-Benzanthracene	228
Acenaphthene	154	C ₁ - Benz[<i>a</i>]anthracene/ Chrysene	242
Biphenyl	154	C ₂ - Benz[<i>a</i>]anthracene/ Chrysene	256
Fluorene	166	C ₃ - Benz[<i>a</i>]anthracene/ Chrysene	270
C ₁ -Fluorenes	180	Benzo[<i>a</i>]fluoranthene	252
<i>C₂-Fluorenes</i>	194	Benzo[<i>b</i>]fluoranthene	252
<i>C₃-Fluorenes</i>	208	Benzo[<i>j</i>]fluoranthene	252
Dibenzothiophene	184	Benzo[<i>k</i>]fluoranthene	252
C ₁ -Dibenzothiophenes	198	Benzo[<i>e</i>]pyrene	252
<i>C₂-Dibenzothiophenes</i>	212	Benzo[<i>a</i>]pyrene	252
<i>C₃-Dibenzothiophenes</i>	226	Perylene	252
Phenanthrene	178	Indeno[1,2,3- <i>cd</i>]pyrene	276
Anthracene	178	Benzo[<i>ghi</i>]perylene	276
C ₁ -Phenanthrenes/Anthracenes	192	Dibenz[<i>a,h</i>]anthracene	278
C ₂ -Phenanthrenes/Anthracenes	206	Benzo[<i>k</i>]fluoranthene	252
C ₃ -Phenanthrenes/Anthracenes	220	Cyclopenta[<i>cd</i>]pyrene	226
Fluoranthene	202	Naphtho[2,1- <i>a</i>]pyrene	302
Pyrene	202	Dibenz[<i>a,e</i>]pyrene	302
C ₁ -Fluoranthenes/Pyrenes	216	Dibenz[<i>a,i</i>]pyrene	302
2, 1d-benzonaphthothiophene	234	Dibenz[<i>a,l</i>]pyrene	302
1,2d-benzonaphthothiophene	234	Dibenz[<i>a,h</i>]pyrene	302

5.7 Instrumental determination of PAHs

The greatest sensitivity and selectivity in routine analysis for parent PAH is achieved by combining HPLC with fluorescence detection (HPLC-UVF) or capillary gas chromatography with mass spectrometry (GC-MS). However, for the analysis of parent and alkylated PAHs GC-MS is the method of choice. In terms of flexibility, GC-MS is the most capable technique, as in principle it does not limit the selection of determinands in any way, while HPLC is suited only to the analysis of parent PAHs. In the past, analyses have also been conducted using HPLC with UV-absorption detection and GC with flame-ionisation detection, but neither can be recommended for alkylated PAHs because of their relatively poor selectivity. Both in terms of the initial capital cost of the instrumentation, and the cost per sample analysed, HPLC-UVF is cheaper than GC-MS. With the advent of high-sensitivity benchtop GC-MS systems, however, this cost advantage is now not as marked as in the past, and the additional information regarding sources available makes GC-MS the method of choice.

Limits of determination within the range of 0.05 to 0.5 $\mu\text{g kg}^{-1}$ wet weight for individual PAH compounds should be achievable by GC-MS. However this limit can be lowered in routine analysis.

5.7.1 GC-MS

The three injection modes commonly used are splitless, on-column and PTV (programmed temperature vaporiser). Automatic sample injection should be used wherever possible to improve the reproducibility of injection and the precision of the overall method. If splitless injection is used, the liner should be of sufficient capacity to contain the injected solvent volume after evaporation. For PAH analysis, the cleanliness of the liner is also very important if adsorption effects and discrimination are to be avoided, and the analytical column should not contain active sites to which PAHs can be adsorbed. Helium is the preferred carrier gas, and only capillary columns should be used. Because of the wide boiling range of the PAHs to be determined and the surface-active properties of the higher PAHs, the preferred column length is 25–50 m, with an internal diameter of 0.15 mm to 0.3 mm. Film thicknesses of 0.2 μm to 1 μm are generally used; this choice has little impact on critical resolution, but thicker films are often used when one-ring aromatic compounds are to be determined alongside PAHs, or where a high sample loading is needed. No stationary phase has been found on which all PAH isomers can be resolved; the most commonly used stationary phase for PAH analysis is 5% phenyl methylsilicone (DB-5 or equivalent). This will not, however, resolve critical isomers such as benzo[*b*], [*j*] and [*k*]fluoranthenes, or chrysene from triphenylene. Chrysene and triphenylene can be separated on other columns, if necessary such as a 60 m non polar column such as DB5MS. For PAHs there is no sensitivity gain from the use of chemical ionisation (either positive or negative ion), so analyses are usually conducted in electron-impact mode at 70eV. Quadrupole instruments are used in single ion monitoring to achieve greater sensitivity. The masses to be detected are programmed to change during the analysis as different PAHs elute from the capillary column. In SIM the molecular ion is used for quantification. Qualifier ions can be used to confirm identification but they are limited for PAHs. Triple quadrupole mass spectrometry can also be used and will give greater sensitivity. Some instruments such as ion-trap and time of flight mass spectrometers exhibit the same sensitivity in both modes, so full scan spectra can be used for quantification.

An example of mass spectrometer operating conditions in SIM mode is given in Table A1.2. The ions are grouped and screened within GC time windows of the compounds. In general the number of ions should not be greater than 20. The dwell time is important parameter and should be close for each ion. For GC capillary column analysis a dwell time should not be shorter than 20 ms, while a sum of a dwell in each retention time windows should not be greater than 500 ms. An example of conditions that can be used along with dwell times are shown in Table A1.2.

Table A.1.2 Example of operational conditions for the GC-MS analysis of parent and alkylated PAHs.

Group N°	Retention time (min)	Dwell time (ms)	Ions in group (AMU)					
			128	136	142			
1	8.00	100	128	136	142			
2	21.00	100	152	156	160			
3	23.70	100	154	164	168	170		
4	26.80	80	166	176	180	182	184	
5	31.60	80	178	184	188	194	196	198
6	35.30	100	192	198				
7	36.60	100	206	212				
8	39.40	80	202	206	212	216	220	226
9	44.65	100	216	220				
10	45.30	100	226	228	230	234	240	
11	48.58	90	242	248				
12	52.00	100	252	256	264	266		
13	59.00	100	266	276	278	288		

Alkylated homologues of PAHs (C1–C4), mainly associated with petrogenic sources, contain a number of different isomers that can give very complex but distinct distribution profiles when analysed by GC-MS. Integration of each isomer separately is difficult for most alkylated PAHs. 1- and 2-Methyl naphthalene give well resolved peaks that can be quantified separately. C1-Phenanthrene/anthracene gives five distinct peaks corresponding to 3-methyl phenanthrene, 2-methyl phenanthrene, 2-methyl anthracene, 4- and 9-methyl phenanthrene and 1-methyl phenanthrene. These may be integrated as a group or as separate isomers. For all other alkylated PAHs the area for all isomers may be summed and quantified against a single representative isomer. This method will lead, however, to an overestimation of the concentration as may include non alkylated PAHs. Examples of integrations of both parent and alkylated PAHs are shown in Appendix 1.

6. Calibration and quantification

6.1 Standards

The availability of pure PAH compounds are limited. Although most of the parent compounds can be purchased as pure compounds, the range of possible alkyl-substituted PAHs is vast and only a limited selection of them can be obtained. PAH standards are available for at least one isomer of most alkyl group listed in Table A1.1. A range of deuterated PAHs (normally 5 to 7) should be used as internal standards to cover the range of PAHs being analysed in samples. A range of fully-deuterated parent PAHs is available for use as standards in PAH analysis. Suitable standards could range from d₈-naphthalene to d₁₄-dibenz[*a,h*]anthracene. Crystalline PAHs of known purity should be used for the preparation of calibration standards. If the quality of the standard materials is not guaranteed by the producer or supplier (as for certified reference materials), then it should be checked by GC-MS analysis. Solid standards should be weighed to a precision of 10⁻⁵ grams. Calibration standards should be stored in the dark because some PAHs are photosensitive, and ideally solutions to be stored should be sealed in amber glass ampoules or sealed GC vials. Otherwise, they can be stored in a refrigerator in stoppered measuring cylinders or flasks that are gas tight to avoid evaporation of the solvent during storage.

6.2 Calibration

Multilevel calibration with at least five calibration levels is preferred to adequately define the calibration curve. In general, GC-MS calibration is linear over a considerable concentration range but may exhibit a change of slope at very low concentrations. Quantification should be conducted in the linear region of the calibration curve. A separate calibration curve may be used where sample concentrations are very low. An internal standard method should be employed, using a range of deuterated PAHs as internal standards.

6.3 Recovery

The recovery of analytes should be checked and reported. Given the wide boiling range of the PAHs to be determined, the recovery may vary with compound group, from the volatile PAHs of low molecular weight to the larger compounds. Deuterated standards can be added in two groups: those to be used for quantification are added at the start of the analytical procedure, whilst those from which the absolute recovery will be assessed are added prior to GC-MS injection. This allows the recovery to be calculated.

7. Analytical Quality Control

Planners of monitoring programmes must decide on the accuracy, precision, repeatability, and limits of detection and determination which they consider acceptable. Achievable limits of determination for each individual component are as follows:

- for GC-MS measurements: 0.05 µg kg⁻¹ ww;
- Further information on analytical quality control procedures for PAHs can be found elsewhere (Law and de Boer, 1995). A procedural blank should be measured with each sample batch, and should be prepared simultaneously using the same chemical reagents and solvents as for the samples. Its purpose is to indicate sample contamination by interfering compounds, which will result in errors in quantification. The procedural blank is also very important in the calculation of limits of detection and limits of quantification for the analytical method. In addition, a laboratory reference material (LRM) should be analysed within each sample batch. The LRM must be homogeneous and well-characterised for the determinands of interest within the analytical laboratory. Ideally, stability tests should have been undertaken to show that the LRM yields consistent results over time. The LRM should be of the same matrix type (e.g. mussels) as the samples, and the determinand concentrations should be in the same range as those in the samples. Realistically, and given the wide range of PAH concentrations encountered, particularly in oil spill investigations, this is bound to involve some compromise. The data produced for the LRM in successive sample batches should be used to prepare control charts. It is also useful to analyse the LRM in duplicate from time to time to check within-batch analytical variability. The analysis of an LRM is primarily intended as a check that the analytical method is under control and yields acceptable precision, but a certified reference material (CRM) of a similar matrix should be analysed periodically in order to check the method bias. The availability of biota CRMs certified for PAHs is very limited, and in all cases the number of PAHs for which certified values are provided is small. At present, only NIST 1974a (a frozen wet mussel tissue) and NIST 2974 (a freeze-dried mussel tissue) are available. At regular intervals, the laboratory should participate in an intercomparison or proficiency exercise in which samples are circulated without knowledge of the determinand concentrations, in order to provide an independent check on performance.

8. Data reporting

The calculation of results and the reporting of data can represent major sources of error, as has been shown in intercomparison studies for PAHs. Control procedures should be established in order to ensure that data are correct and to obviate transcription errors. Data stored on databases should be checked and validated, and checks are also necessary when data are transferred between databases. Data should be reported in accordance with the latest ICES reporting formats.

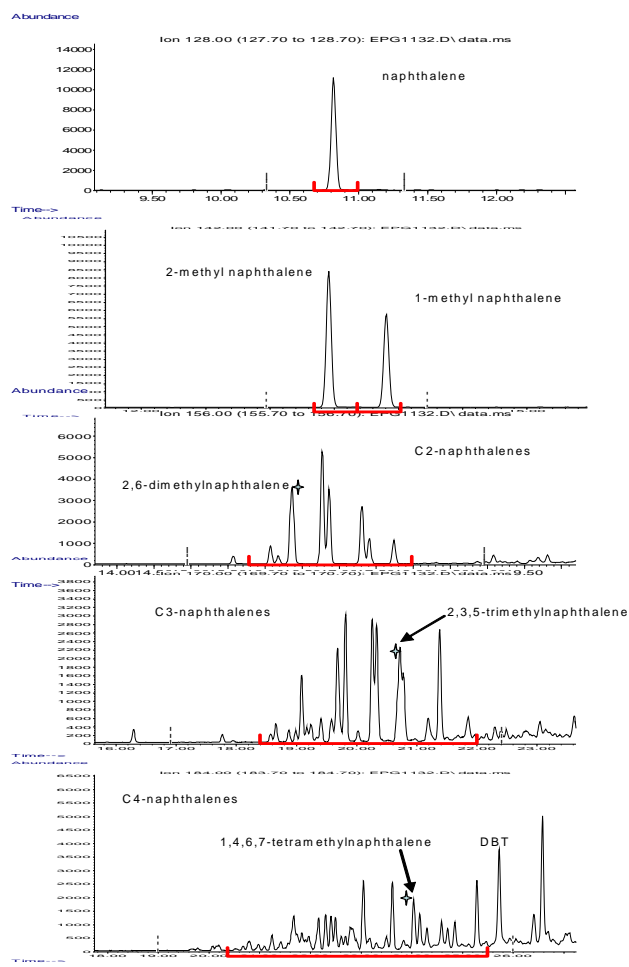
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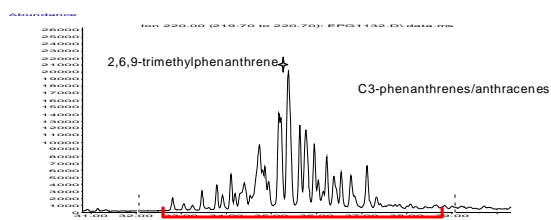
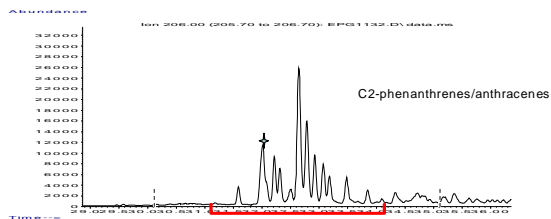
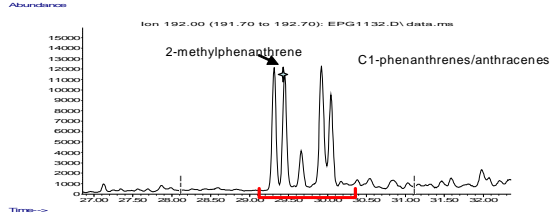
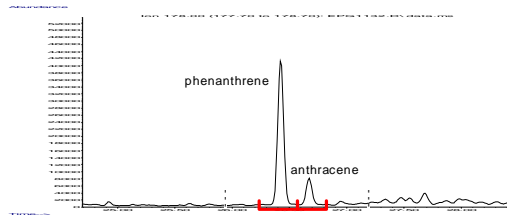
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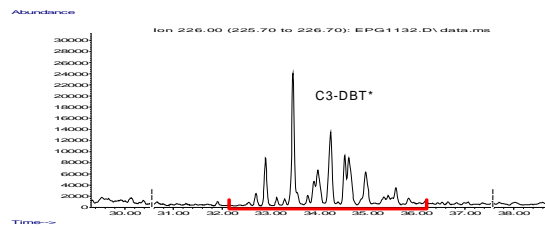
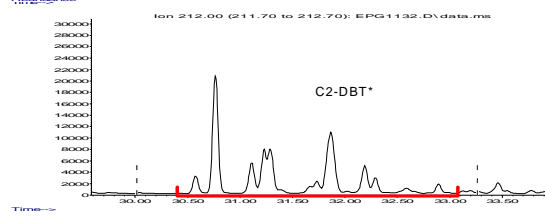
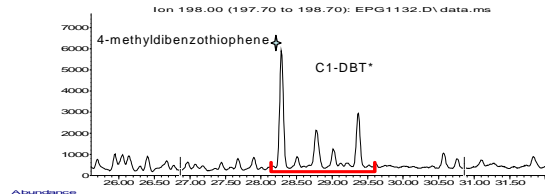
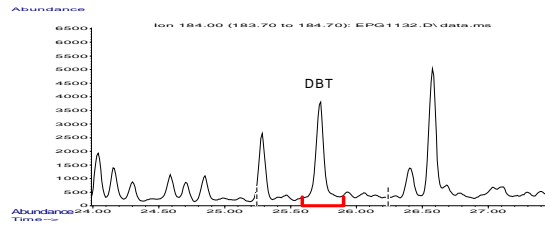
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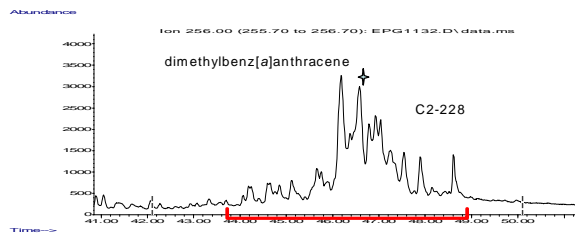
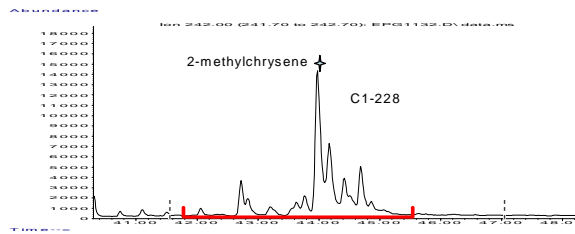
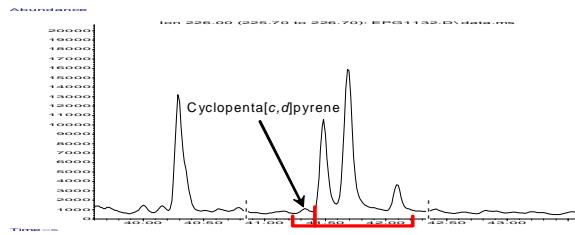
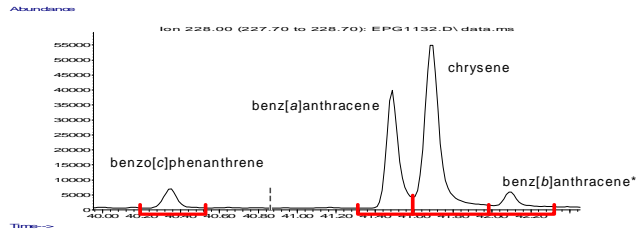
ANNEX 1 – APPENDIX 1

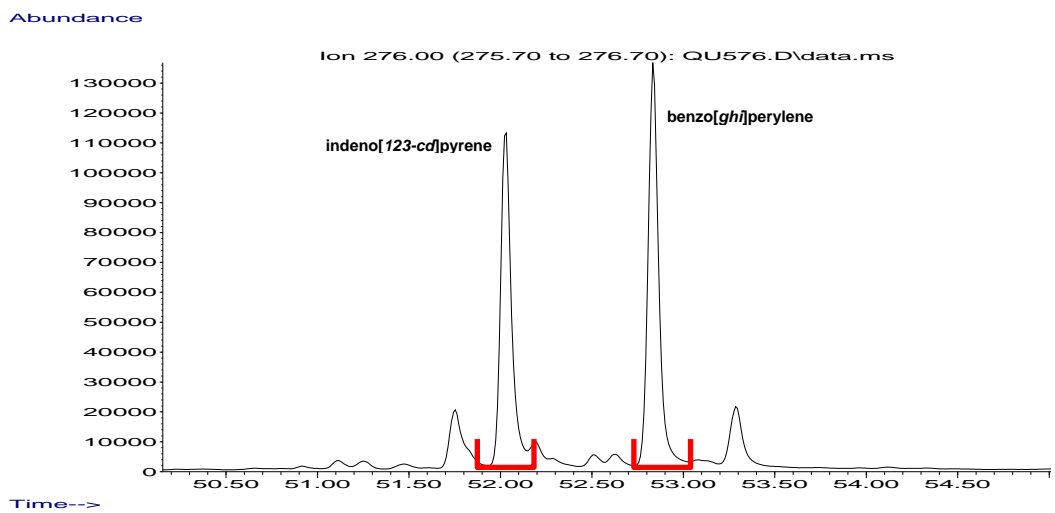
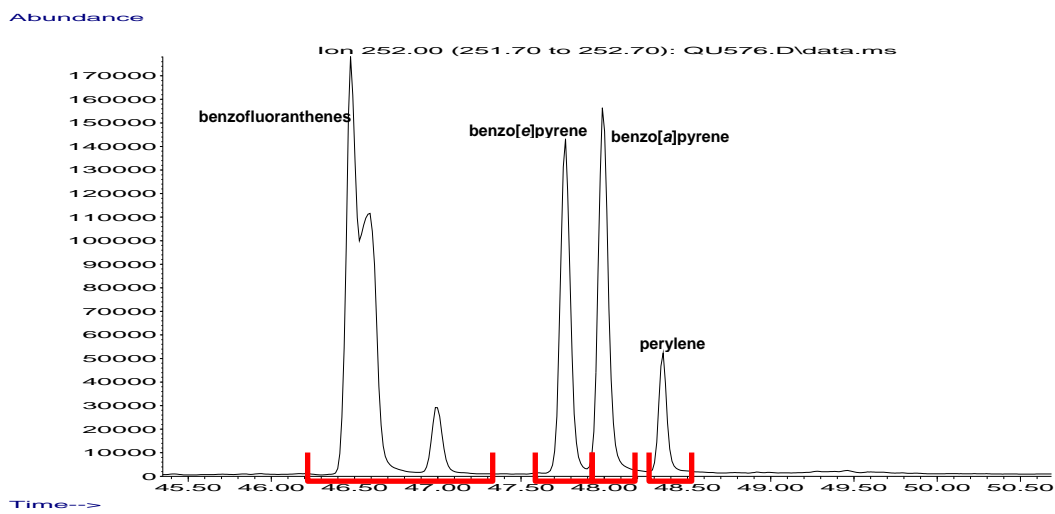
Examples of integration of parent and alkylated PAHs analysed by GC-MS. The standards used for the calibration of the alkylated PAHs are asterixed.











ANNEX 2

Technical annex: Polyaromatic hydrocarbons in sediments

Determination of parent and alkylated PAHs in sediments

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) consist of a variable number of fused aromatic rings. By definition, PAHs contain at least two fused rings. PAHs arise from incomplete combustion processes and from both natural and anthropogenic sources, although the latter generally predominate. PAHs are also found in oil and oil products, and these include a wide range of alkylated PAHs formed as a result of diagenetic processes, whereas PAHs from combustion sources comprise mainly parent (non-alkylated) PAHs. Metabolites of some of the high MW PAHs are potent animal and human carcinogens – benzo[*a*]pyrene is the prime example. Carcinogenic activity is closely related to structure. Benzo[*e*]pyrene and the four benzofluoranthene isomers all have a molecular weight of 252 Da, however they are much less potent than benzo[*a*]pyrene. Less is known about toxicity of alkylated PAHs. However, one study has demonstrated that alkylated PAHs may have increased toxicity compared to the parent compound (Marvanova *et al.*, 2008).

This Technical Annex provides advice on the analysis of parent and alkylated polycyclic aromatic hydrocarbons (PAH) in total sediment, sieved fractions, and suspended particulate matter. The analysis of in sediments generally includes extraction with organic solvents, clean-up, high performance liquid chromatography (HPLC) with ultraviolet or fluorescence detection or gas chromatographic (GC) separation with flame ionisation (FID) or mass spectrometric (MS) detection (e.g., Fetzer and Vo-Dinh, 1989; Wise *et al.*, 1995). All steps in the procedure are susceptible to insufficient recovery and/or contamination. Quality control procedures are recommended in order to check the performance of the method. These guidelines are intended to encourage and assist analytical chemists to critically reconsider their methods and to improve their procedures and/or the associated quality control measures, where necessary.

These guidelines are not intended as a complete laboratory manual. If necessary, guidance should be sought from highly specialised research laboratories. Whichever procedure is adopted, each laboratory must demonstrate the validity of each step of its procedure. In addition, the use of a second (and different method), carried out concurrently to the routine procedure, is recommended for validation. The analyses must be carried out by experienced staff.

2. Pre-treatment and Storage

2.1 Contamination

Sample contamination may occur during sampling, sample handling, pre-treatment and analysis, due to the environment, the containers or packing materials used, the instruments used during sample preparation, and from the solvents and reagents used during the analytical procedures. Controlled conditions are therefore required for all procedures. In the case of PAHs, particular care must be taken to avoid contamination at sea. On ships there are multiple sources of PAHs, such as the oils used for fuel and lubrication, and the exhaust from the ship's engines. It is important that the likely sources of contamination are identified and steps taken to preclude sample handling in areas where contamination can occur. A ship is a working vessel and there can always be procedures occurring as a result of the day-to-day operations (deck cleaning, automatic overboard bilge discharges, etc.) that could affect the sampling process. It is advisable to collect samples of the ship's fuel, bilge water, and oils and greases used on winches, etc., which can be used as fingerprinting samples at a later date, if there are suspicions of contamination in particular instances.

Freeze-drying of sediment samples may be a source of contamination due to the back-streaming of oil vapours from the rotary vacuum pumps. Furthermore drying the samples may result in losses of the lower molecular weight, more volatile PAHs through evaporation (Law *et al.*, 1994).

Plastic materials must not be used for sampling and storage owing to possible adsorption of the PAHs onto the container material. Samples should be transported in closed containers; a temperature of 25°C should not be exceeded. If the samples are not analysed within 48 hours after sampling, they must be stored at 4°C (short-term storage). Storage over several months is only possible for frozen, (i.e., below –20°C) and/or dried samples (Law and de Boer, 1995).

As PAHs are sensitive to photo-degradation, exposure to direct sunlight or other strong light must be avoided during storage of the samples as well as during all steps of sample preparation, including extraction and storage of the extracts (Law and Biscaya, 1994). The use of amber glassware is strongly recommended.

2.3 Blanks

The procedural detection limit is determined by the blank value. In order to keep the blank value as low as possible, PAHs or other interfering compounds should be removed from all glassware, solvents, chemicals, adsorption materials, etc., that are used in the analysis. The following procedures should be used:

- glassware should be thoroughly washed with detergents and rinsed with an organic solvent prior to use. Further cleaning of the glassware, other than calibrated instruments, can be carried out by heating at temperatures >250°C;
- all solvents should be checked for impurities by concentrating the amount normally used to 10% of the normal end volume. This concentrate can then be analysed by GC and should not contain significant amounts of PAHs or other interfering compounds;
- all chemicals and adsorption materials should be checked for impurities and purified (e.g., by heating or extraction), if necessary. Soxhlet thimbles should be pre-extracted. Glassfibre thimbles are preferred over paper thimbles. Alternatively, full glass Soxhlet thimbles, with a G1 glass filter at the bottom, can be used. The storage of these supercleaned materials for a long period is not recommended, as laboratory air can contain PAHs that will be absorbed by these materials. Blank values occurring despite all the above-mentioned precautions may be due to contamination from the air. The most volatile compounds will usually show the highest blanks (Gremm and Frimmel, 1990).
- Glassfibre filters used for the PLE (pressurised liquid extraction) method should be heated at 450°C overnight.

3. Pre-treatment

Before taking a subsample for analysis, the samples should be sufficiently homogenised. The intake mass is dependent on the expected concentrations. For the marine environment, as a rule of thumb, the mass of sample taken for analysis can be equal to an amount representing 50–100 mg organic carbon. PAHs can be extracted from wet or dried samples. However, storage, homogenisation and extraction are much easier when the samples are dry. Care must be taken if freeze-drying samples for the reasons described in 2.1. Possible losses and contamination have to be checked. Contamination can be checked by exposing 1–2 g C18-bonded silica to drying conditions and analysing it as a sample (clean-up can be omitted) (Smedes and de Boer, 1997). Contamination during freeze-drying can be reduced by placing a lid, with a hole about 3 mm in diameter, on the sample container, while evaporation of the water is not hindered.

4. Extraction and clean-up

Exposure to light must be kept to a minimum during extraction and further handling of the extracts (Law and Biscaya, 1994). Since photo-degradation occurs more rapidly in the absence of a sample matrix, first of all the standard solution used for checking the recovery of the procedure will be affected, allowing a proper detection of the influence of light. The most photo-sensitive PAH is benzo[*a*]pyrene, followed by anthracene.

4.1 Wet sediments

Wet sediments should be extracted using a stepwise procedure by mixing with organic solvents. Extraction is enhanced by shaking, Ultra Turrax mixing, ball mill tumbling or ultrasonic treatment. Water-miscible solvents, such as acetone, methanol, or acetonitrile, are used in the first step. The extraction efficiency of the first step will be low as there is a considerable amount of water in the liquid phase. For sufficient extraction, at least three subsequent extractions are needed. The contact time with the solvent should be sufficient to complete the desorption of the PAHs out of the sediment pores. Heating by microwave or refluxing will accelerate this process.

When utilising a Soxhlet, the extraction of wet sediments should be conducted in two steps. First, a polar solvent, such as acetone, is used to extract the water from the sediment, then the flask is replaced and the extraction continued with a less polar solvent or solvent mixture (e.g., acetone/hexane). Thereafter, the extracts must be combined. For both batch and Soxhlet extraction, water must be added to the combined extracts and the PAHs must be extracted to a non-polar solvent.

Extraction of wet sediments by pressurised liquid extraction (PLE) is a more recent method, requiring less solvent and time for the extraction process. Wet sediment is dried by mixing with sufficient anhydrous sodium sulphate to form a free flowing mixture and is packed into stainless steel tubes for extraction. Extractions are performed at elevated temperatures and pressures. Various extracting solvents (DCM, acetone, methanol, acetonitrile, hexane, DCM: acetone [1:1], hexane:acetone [1:1]) were investigated by Saim *et al.* (1998) and as long as the solvent polarity was >1.89 (*i.e.* all

except hexane) no significant differences were noted. Extraction temperatures can be manipulated to suit the analytical requirements.

4.2 Dry sediments

Although all the methods mentioned above can also be used for dried sediments, Soxhlet extraction is the most frequently applied technique to extract PAHs from dried sediments. Medium-polar solvents such as dichloromethane or toluene, or mixtures of polar and non-polar solvents can be used. When using dichloromethane, losses of PAHs have occasionally been observed (Baker, 1993). Although toluene is not favoured because of its high boiling point, it should be chosen as solvent when it is expected that sediment samples contain soot particles. For routine marine samples, the use of a mixture of a polar and a non-polar solvent (e.g., acetone/hexane (1/3, v/v)) is recommended.

The extraction can be carried out with a regular or a hot Soxhlet (Smedes and de Boer, 1997). A sufficient number of extraction cycles must be performed (approximately 8 hours for the hot Soxhlet and 12 to 24 hours for normal Soxhlet). The extraction efficiency has to be checked for different types of sediments by a second extraction step. These extracts should be analysed separately.

PLE can also be used for the extraction of freeze-dried sediments. Instead of anhydrous sodium sulphate to dry the sediment the sample is mixed with a clean sand or diatomaceous earth to increase the surface area of the sediment. The same solvent mixtures detailed above for wet sediment extraction can be used for the dry sediments. Supercritical fluid extraction (SFE) has also been used for the extraction of organic compounds. The optimum conditions may vary for specific sediments (e.g., Dean *et al.*, 1995; Reimer and Suarez, 1995).

4.3 Clean-up

The crude extract requires a clean-up to remove the many other compounds which are co-extracted (e.g., Wise *et al.*, 1995). Due to chlorophyll-like compounds extracted from the sediment, the raw extract will be coloured and also contain sulphur and sulphur-containing compounds, oil, and many other natural and anthropogenic compounds. Selection of the appropriate clean-up method depends on the subsequent instrumental method to be used for analysis. Prior to the clean-up, the sample must be concentrated and any polar solvents used in the extraction step should be removed. The recommended acetone/hexane mixture will end in hexane when evaporated because of the formation of an azeotrope. Evaporation can be done either using a rotary evaporator or parallel evaporating systems such as Syncore. Especially for the rotary evaporator, care should be taken to stop the evaporation in time at about 5 ml. For further reducing the volume, a gentle stream of nitrogen should be applied. The extract should never be evaporated to dryness. The drawback of the rotary evaporator is that more volatile components may be lost during the nitrogen drying stage whilst the heavier components stick to the glassware. The Buchi Syncore Analyst also uses glass tubes but the system is sealed, avoiding contamination from the lab air during evaporation. It does not use a nitrogen stream, thus reducing the loss of volatiles and if the flushback module is fitted the sides of the tubes are rinsed automatically thus reducing the loss of the heavier components.

For removing more polar interferences from the extract, deactivated aluminium oxide (10 % water), eluted with hexane, as well as silica or modified silica columns, e.g., aminopropylsilane, eluted with toluene or a semipolar solvent mixture such as hexane/acetone (95/5, v/v) or hexane/dichloromethane (98/2, v/v), can be used. Gel permeation chromatography (GPC) can be used to remove high molecular weight material and sulphur from the extracts.

For GC-MS analysis, sulphur should be removed from the extracts, in order to protect the detector. This can be achieved by the addition of copper powder, wire or gauze during or after Soxhlet extraction. Copper can also be added to the PLE cell, however, this is not always sufficient and further treatment with copper may be required following extraction. Ultrasonic treatment might improve the removal of sulphur. As an alternative to copper, other methods can be used (Smedes and de Boer, 1997).

Aliphatic hydrocarbons originating from mineral oil interfere with the flame ionisation detection. They can be removed from the extract by fractionation over columns filled with activated aluminium oxide or silica. The first fraction eluting with hexane is rejected. The PAHs elute in a second fraction with a more polar solvent, e.g., diethylether or acetone/hexane. When applying fractionation, the elution pattern has to be checked frequently. This should be carried out in the presence of sample matrix, as that can partially deactivate the clean-up column, resulting in earlier elution of the PAHs than in a standard solution.

Gel permeation chromatography (GPC) and high performance liquid chromatography (HPLC) based methods are also employed (Nondek *et al.*, 1993; Nyman *et al.*, 1993; Perfetti *et al.*, 1992). The major advantages of using HPLC-based clean-up methods are their ease of automation and reproducibility.

Isocratic HPLC fractionation of the extract can be used to give separate aliphatic and aromatic fractions (Webster *et al.*, 2002). A metal free silica column is used for the clean up/fractionation as dibenzothiophene (DBT) can be retained on ordinary silica columns. The split time is determined by injection of a solution containing representative aliphatic and PAH standards. The silica column is regenerated by a cleaning cycle after a set number of samples. If PAHs are to be analysed by HPLC and there are significant amounts of alkylated PAHs present then the removal of the alkylated PAHs may be difficult.

4.4 Pre-concentration

In the methods suggested above, all result in an extract in which non-polar solvents are dominant. The sample volume should be 2 ml or greater to avoid errors when transferring solvents during the clean-up stages. Syncore parallel evaporators can be used with careful optimisation of the evaporation parameters. Evaporation of solvents using a rotary-film evaporator should be performed at low temperature (water bath temperature of 30°C or lower) and under controlled pressure conditions, in order to prevent losses of the more volatile PAHs such as naphthalenes. For the same reasons, evaporation to dryness must be avoided. When reducing the sample to final volume, solvents can be removed by a stream of clean nitrogen gas. Suitable solvents for injection into the GC-MS include pentane, hexane, heptane, *iso*-hexane, and *iso*-octane.

5. Selection of PAHs to be determined

The choice of PAHs to be analysed is not straightforward, both because of differences in the range of PAH compounds resulting from combustion processes and from oil and oil products, and also because the aims of specific monitoring programmes can require the analysis of different representative groups of compounds. PAHs arising from combustion processes are predominantly parent (unsubstituted) compounds, whereas oil and its products contain a much wider range of alkylated compounds in addition to the parent PAHs. This has implications for the analytical determination, as both HPLC-based and GC-based techniques are adequate for the determination of a limited range of parent PAHs in samples influenced by combustion processes, whereas in areas of significant oil contamination and following oil spills only GC-MS has sufficient selectivity to determine the full range of PAHs present. The availability of pure individual PAHs for the preparation of standards is problematic and limits both the choice of determinands and, to some degree, the quantification procedures that can be used. The availability of reference materials certified for PAHs is also rather limited. A list of target parent and alkylated PAHs suitable for environmental monitoring is given in Table A2.1, and this differs both from the list previously developed within ICES specifically for intercomparison purposes, and the historic list of Borneff. In both cases, the lists were concentrated on a subset of parent (predominantly combustion-derived) PAHs due to analytical limitations. This approach completely neglects the determination of alkylated PAHs, which allows the interpretation of PAH accumulation from multiple sources including those due to oil inputs. It will not be necessary for all of these PAH compounds and groups to be analysed in all cases, but an appropriate selection can be made from this list depending on the specific aims of the monitoring programme to be undertaken.

Table A2.1 Compounds of interest for environmental monitoring for which the guidelines apply. For compounds in italics standards are not available for any isomers in this group.

Compound	MW	Compound	MW
Naphthalene	128	Benzo[b]naphtho[2,3- <i>d</i>]thiophene	234
<i>C</i> ₁ -Naphthalenes	142	<i>C</i> ₁ -benzonaphthothiophenes	248
<i>C</i> ₂ -Naphthalenes	156	<i>C</i> ₂ -Fluoranthenes/Pyrenes	230
<i>C</i> ₃ -Naphthalenes	170	Benz[<i>a</i>]anthracene	228
<i>C</i> ₄ -Naphthalenes	184	Chrysene	228
Acenaphthylene	152	2,3-Benzanthracene	228
Acenaphthene	154	<i>C</i> ₁ - Benz[<i>a</i>]anthracene/ Chrysene	242
Biphenyl	154	<i>C</i> ₂ - Benz[<i>a</i>]anthracene/ Chrysene	256
Fluorene	166	<i>C</i> ₃ - Benz[<i>a</i>]anthracene/ Chrysene	270
<i>C</i> ₁ -Fluorenes	180	Benzo[<i>a</i>]fluoranthene	252
<i>C</i> ₂ -Fluorenes	194	Benzo[<i>b</i>]fluoranthene	252
<i>C</i> ₃ -Fluorenes	208	Benzo[<i>j</i>]fluoranthene	252
Dibenzothiophene	184	Benzo[<i>k</i>]fluoranthene	252
<i>C</i> ₁ -Dibenzothiophenes	198	Benzo[<i>e</i>]pyrene	252
<i>C</i> ₂ -Dibenzothiophenes	212	Benzo[<i>a</i>]pyrene	252
<i>C</i> ₃ -Dibenzothiophenes	226	Perylene	252
Phenanthrene	178	Indeno[1,2,3- <i>cd</i>]pyrene	276
Anthracene	178	Benzo[<i>ghi</i>]perylene	276
<i>C</i> ₁ -Phenanthrenes/Anthracenes	192	Dibenz[<i>a,h</i>]anthracene	278
<i>C</i> ₂ -Phenanthrenes/Anthracenes	206	Benzo[<i>k</i>]fluoranthene	252
<i>C</i> ₃ -Phenanthrenes/Anthracenes	220	Cyclopenta[<i>cd</i>]pyrene	226
Fluoranthene	202	Naphtho[2,1- <i>a</i>]pyrene	302
Pyrene	202	Dibenz[<i>a,e</i>]pyrene	302
<i>C</i> ₁ -Fluoranthenes/Pyrenes	216	Dibenz[<i>a,i</i>]pyrene	302
Benzo[<i>b</i>]naphtho[2,1- <i>d</i>]thiophene	234	Dibenz[<i>a,l</i>]pyrene	302
Benzo[<i>b</i>]naphtho[1,2- <i>d</i>]thiophene	234	Dibenz[<i>a,h</i>]pyrene	302

6. Instrumental determination of PAHs

The greatest sensitivity and selectivity in routine analysis for parent PAH is achieved by combining HPLC with fluorescence detection (HPLC-UVF) or capillary gas chromatography with mass spectrometry (GC-MS). However, for the analysis of parent and alkylated PAHs GC-MS is the method of choice. In terms of flexibility, GC-MS is the most capable technique, as in principle it does not limit the selection of determinands in any way, while HPLC is suited only to the analysis of parent PAHs. In the past, analyses have also been conducted using HPLC with UV-absorption detection and GC with flame-ionisation detection, but neither can be recommended for alkylated PAHs because of their relatively poor selectivity. Both in terms of the initial capital cost of the instrumentation, and the cost per sample analysed, HPLC-UVF is cheaper than GC-MS. With the advent of high-sensitivity benchtop GC-MS systems, however, this cost advantage is now not as marked as in the past, and the additional information regarding sources available makes GC-MS the method of choice.

Limits of determination within the range of 0.05 µg kg⁻¹ dry weight for individual PAH compounds should be achievable by GC-MS.

6.1 GC-MS

The three injection modes commonly used are splitless, on-column and PTV (programmed temperature vaporiser). Automatic sample injection should be used wherever possible to improve the reproducibility of injection and the precision of the overall method. If splitless injection is used, the liner should be of sufficient capacity to contain the injected solvent volume after evaporation. For PAH analysis, the cleanliness of the liner is also very important if adsorption effects and discrimination are to be avoided, and the analytical column should not contain active sites to which PAHs can be adsorbed. Helium is the preferred carrier gas, and only capillary columns should be used. Because of the wide boiling range of the PAHs to be determined and the surface-active properties of the higher PAHs, the preferred column length is 25–50 m, with an internal diameter of 0.15 mm to 0.3 mm. Film thicknesses of 0.2 µm to 1 µm are generally used; this choice has little impact on critical resolution, but thicker films are often used when one-ring aromatic compounds are to be determined alongside PAHs, or where a high sample loading is needed. No stationary phase has been found on which all PAH isomers can be resolved; the most commonly used stationary phase for PAH analysis is 5% phenyl methylsilicone (DB-5 or equivalent). This will not, however, resolve critical isomers such as

benzo[*b*], [*j*] and [*k*]fluoranthenes, or chrysene from triphenylene. Chrysene and triphenylene can be separated on other columns, if necessary such as a 60 m non-polar column such a DB5MS. For PAHs there is no sensitivity gain from the use of chemical ionisation (either positive or negative ion), so analyses are usually conducted in electron-impact mode at 70eV. Quadrupole instruments are used in single ion monitoring to achieve greater sensitivity. The masses to be detected are programmed to change during the analysis as different PAHs elute from the capillary column. In SIM the molecular ion is used for quantification. Qualifier ions can be used to confirm identification but they are limited for PAHs. Triple quadrupole mass spectrometry can also be used and will give greater sensitivity. Some instruments such as ion-trap and time of flight mass spectrometers exhibit the same sensitivity in both modes, so full scan spectra can be used for quantification.

An example of mass spectrometer operating conditions in SIM mode is given in Table A2.2. The ions are grouped and screened within GC time windows of the compounds. In general the number of ions should not be greater than 20. The dwell time is an important parameter and should be close for each ion. For GC capillary column analysis a dwell time should not be shorter than 20 ms, while a sum of a dwell in each retention time windows should not be greater than 500 ms. An example of conditions that can be used along with dwell times are shown in Table A2.2.

Table A.2.2 Example of operational conditions for the GC-MS analysis of parent and alkylated PAHs.

Group N°	Retention time (min)	Dwell time (ms)	Ions in group (AMU)					
1	8.00	100	128	136	142			
2	21.00	100	152	156	160			
3	23.70	100	154	164	168	170		
4	26.80	80	166	176	180	182	184	
5	31.60	80	178	184	188	194	196	198
6	35.30	100	192	198				
7	36.60	100	206	212				
8	39.40	80	202	206	212	216	220	226
9	44.65	100	216	220				
10	45.30	100	226	228	230	234	240	
11	48.58	90	242	248				
12	52.00	100	252	256	264	266		
13	59.00	100	266	276	278	288		

Alkylated homologues of PAHs (C1–C4), mainly associated with petrogenic sources, contain a number of different isomers that can give very complex but distinct distribution profiles when analysed by GC-MS. Integration of each isomer separately is difficult for most alkylated PAHs. 1- and 2-Methyl naphthalene give well resolved peaks that can be quantified separately. C1-Phenanthrene/anthracene gives five distinct peaks corresponding to 3-methyl phenanthrene, 2-methyl phenanthrene, 2-methyl anthracene, 4- and 9-methyl phenanthrene and 1-methyl phenanthrene. These may be integrated as a group or as separate isomers. For all other alkylated PAHs the area for all isomers may be summed and quantified against a single representative isomer. This method will, however, lead to an overestimation of the concentration as may include non alkylated PAHs. Examples of integrations of both parent and alkylated PAHs are shown in Appendix 1.

7. Calibration and quantification

7.1 Standards

The availability of pure PAH compounds are limited. Although most of the parent compounds can be purchased as pure compounds, the range of possible alkyl-substituted PAHs is vast and only a limited selection of them can be obtained. PAH standards are available for at least one isomer of most alkyl group listed in Table A2.1. A range of deuterated PAHs (normally 5 to 7) should be used as internal standards to cover the range of PAHs being analysed in samples. A range of fully-deuterated parent PAHs is available for use as standards in PAH analysis. Suitable standards could range from d_8 -naphthalene to d_{14} -dibenz[*a,h*]anthracene. Crystalline PAHs of known purity should be used for the preparation of calibration standards. If the quality of the standard materials is not guaranteed by the producer or supplier (as for certified reference materials), then it should be checked by GC-MS analysis. Solid standards should be weighed to a precision of 10^{-5} grams. Calibration standards should be stored in the dark because some PAHs are photosensitive, and ideally solutions to be stored should be sealed in amber glass ampoules or sealed GC vials. Otherwise, they can be stored in a refrigerator in stoppered measuring cylinders or flasks that are gas tight to avoid evaporation of the solvent during storage.

Calibration

Multilevel calibration with at least five calibration levels is preferred to adequately define the calibration curve. In general, GC-MS calibration is linear over a considerable concentration range but may exhibit a change of slope at very low concentrations. Quantification should be conducted in the linear region of the calibration curve. A separate calibration curve may be used where sample concentrations are very low. An internal standard method should be employed, using a range of deuterated PAHs as internal standards.

7.3 Recovery

The recovery of analytes should be checked and reported. Given the wide boiling range of the PAHs to be determined, the recovery may vary with compound group, from the volatile PAHs of low molecular weight to the larger compounds. Deuterated standards can be added in two groups: those to be used for quantification are added at the start of the analytical procedure, whilst those from which the absolute recovery will be assessed are added prior to GC-MS injection. This allows the recovery to be calculated.

8. Analytical quality control

Planners of monitoring programmes must decide on the accuracy, precision, repeatability, and limits of detection and determination, which they consider acceptable. Achievable limits of determination for each individual component using GC-MS are $0.05 \mu\text{g kg}^{-1}$ dry weight.

Further information on analytical quality control procedures for PAHs can be found elsewhere (Law and de Boer, 1995). A procedural blank should be measured with each sample batch, and should be prepared simultaneously using the same chemical reagents and solvents as for the samples. Its purpose is to indicate sample contamination by interfering compounds, which will result in errors in quantification. The procedural blank is also very important in the calculation of limits of detection and limits of quantification for the analytical method. In addition, a laboratory reference material (LRM) should be analysed within each sample batch. The LRM must be homogeneous and well characterised for the determinands of interest within the analytical laboratory. Ideally, stability tests should have been undertaken to show that the LRM yields consistent results over time. The LRM should be of the same matrix type (e.g., liver, muscle, mussel tissue) as the samples, and the determinand concentrations should be in the same range as those in the samples. Realistically, and given the wide range of PAH concentrations encountered, particularly in oil spill investigations, this is bound to involve some compromise. The data produced for the LRM in successive sample batches should be used to prepare control charts. It is also useful to analyse the LRM in duplicate from time to time to check within-batch analytical variability. The analysis of an LRM is primarily intended as a check that the analytical method is under control and yields acceptable precision, but a certified reference material (CRM) of a similar matrix should be analysed periodically in order to check the method bias. A marine sediment (NIST SRM 1941b)¹ is available, with certified values for 24 PAHs and a further 44 as reference (non-certified) values. At regular intervals, the laboratory should participate in an intercomparison or proficiency exercise in which samples are circulated without knowledge of the determinand concentrations, in order to provide an independent check on performance.

9. Data reporting

The calculation of results and the reporting of data can represent major sources of error, as has been shown in intercomparison studies for PAHs. Control procedures should be established in order to ensure that data are correct and to obviate transcription errors. Data stored on databases should be checked and validated, and checks are also necessary when data are transferred between databases. Data should be reported in accordance with the latest ICES reporting formats.

10. References

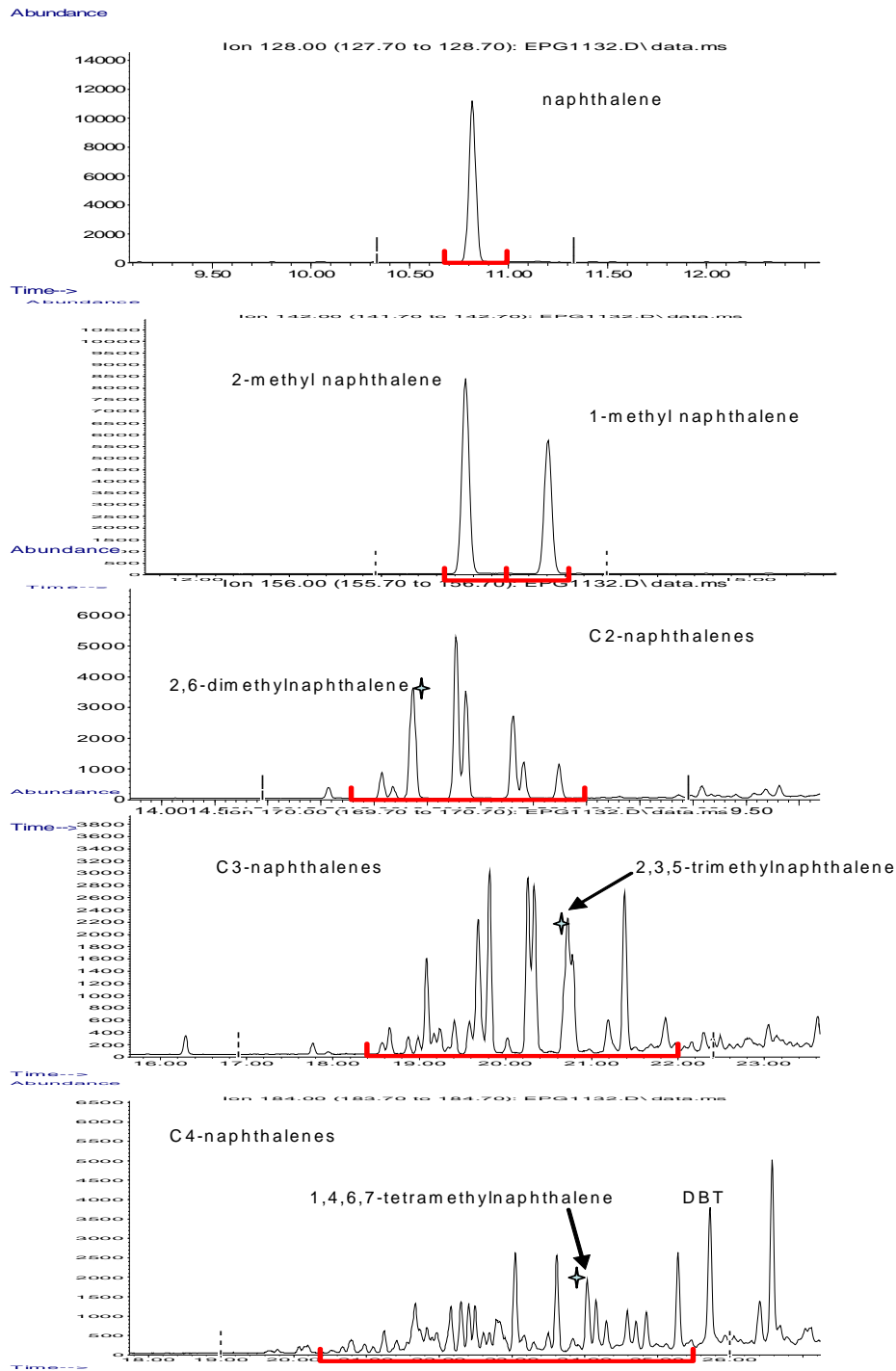
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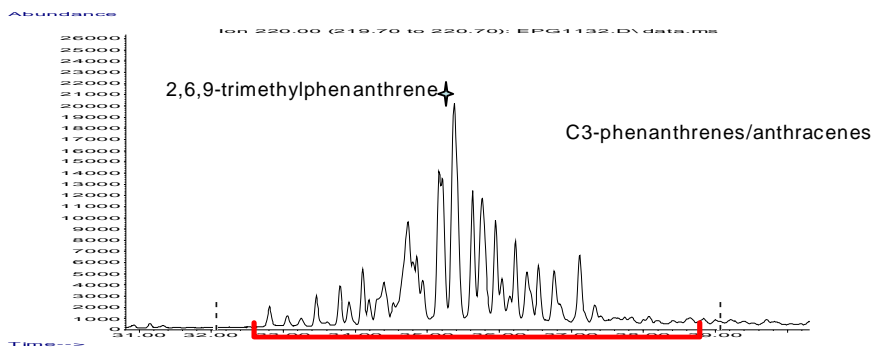
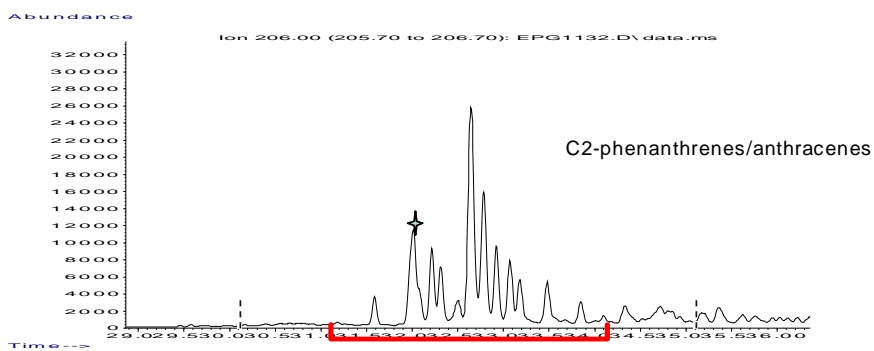
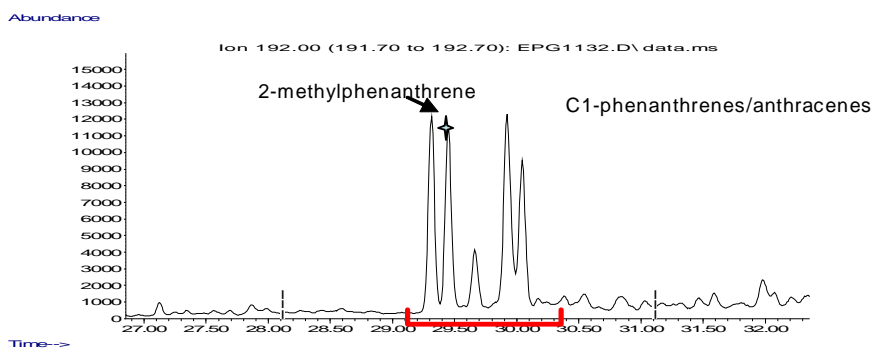
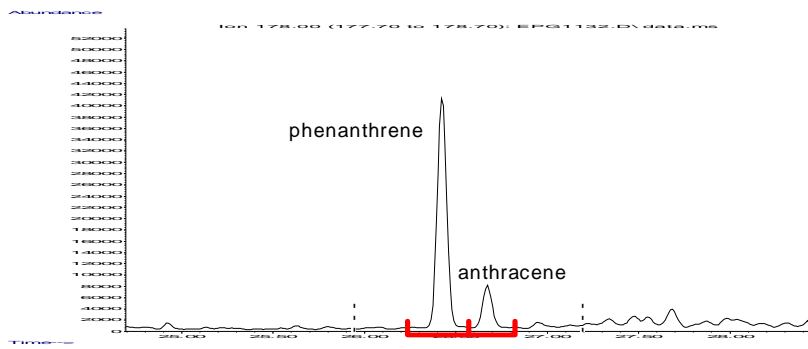
¹ More info on https://srmors.nist.gov/view_detail.cfm?srm=1941B

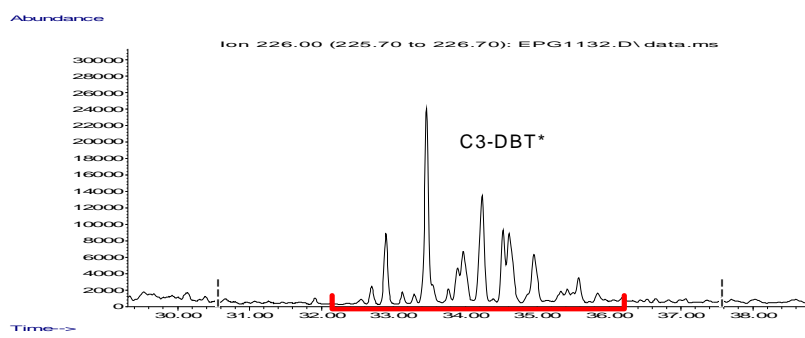
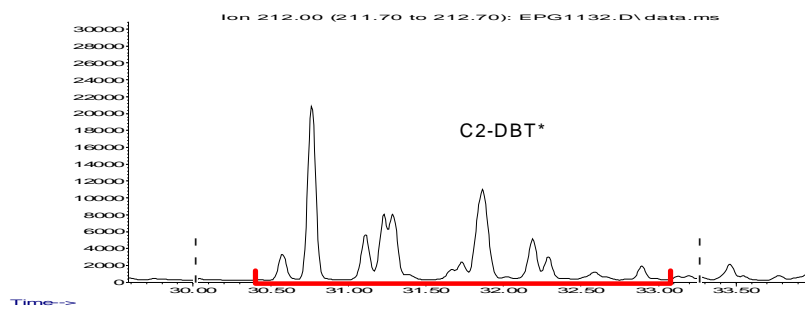
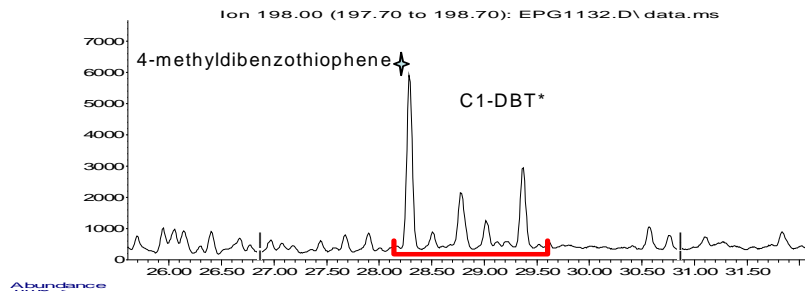
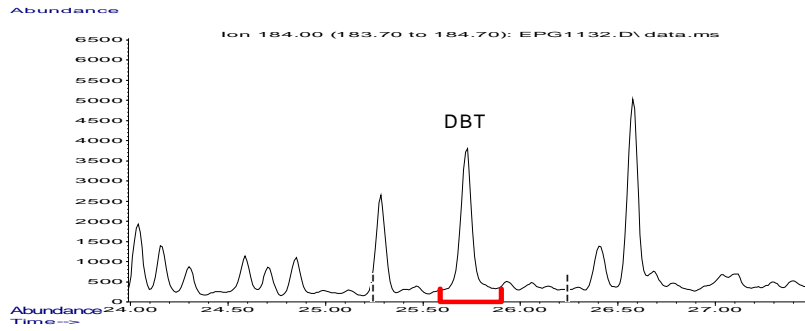
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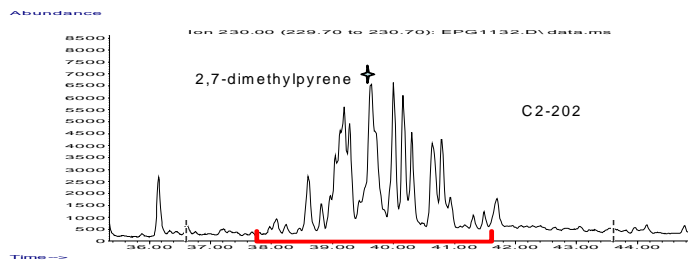
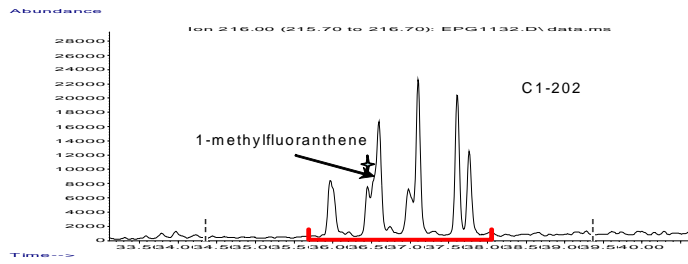
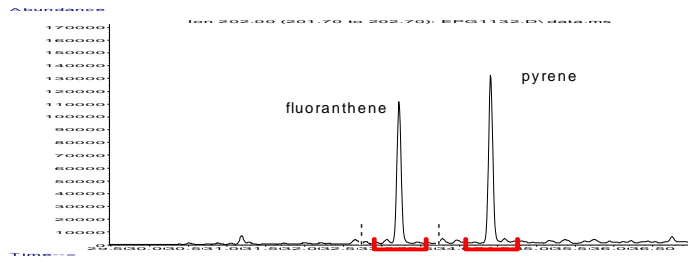
ANNEX 2 – APPENDIX 1

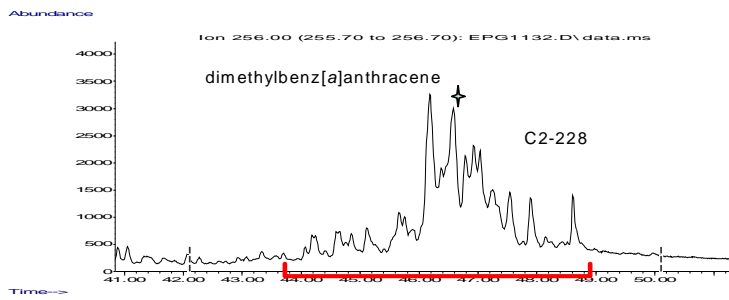
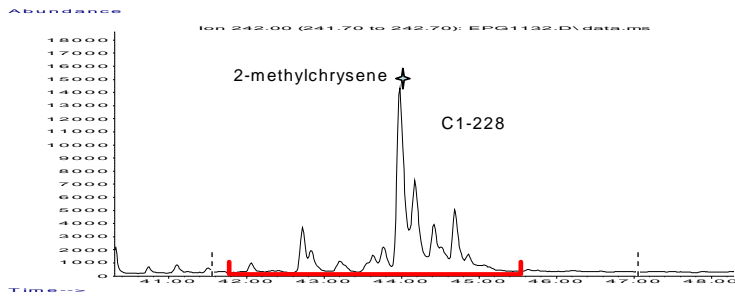
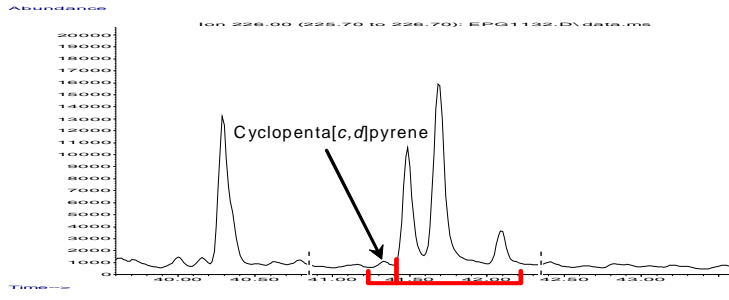
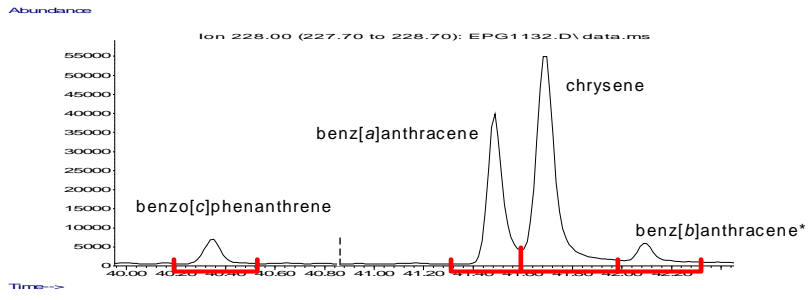
Example integrations of parent and alkylated PAHs analysed by GC-MS. The standards used for the calibration of the alkylated PAHs are asterixed.



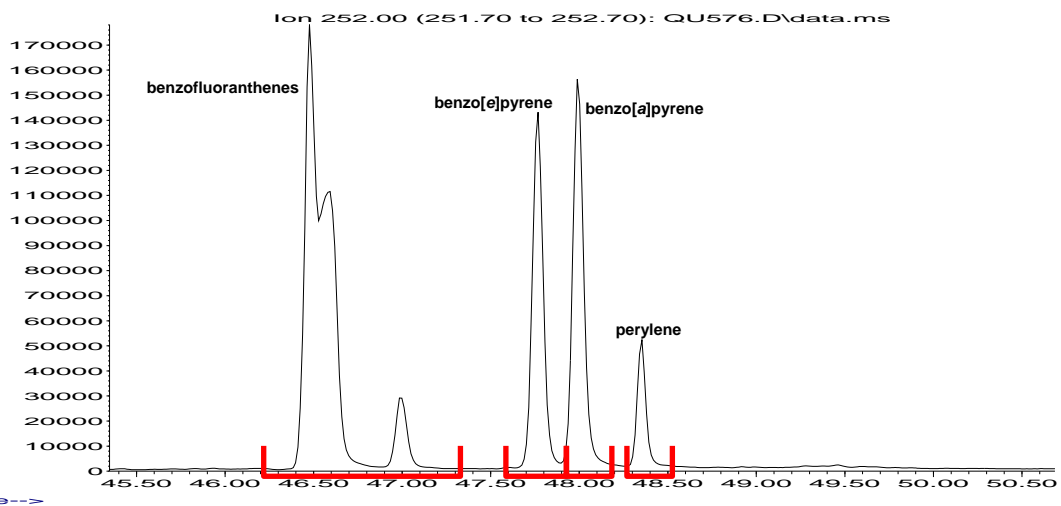




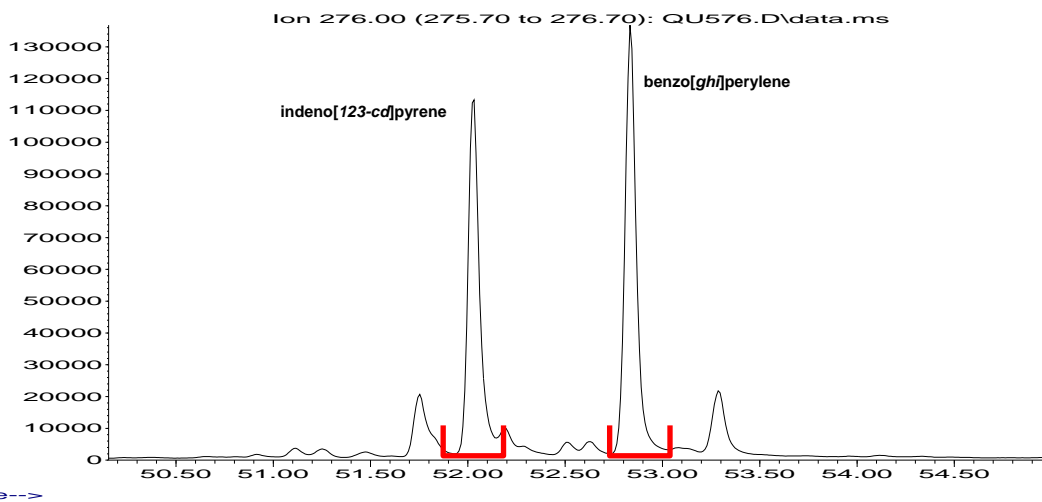




Abundance



Abundance



ANNEX 3

Technical Annex: Organotin compounds in biota

This annex is intended as a supplement to the general guidelines. It is not a complete description or a substitute for detailed analytical instructions. The annex provides guidelines for the measurement of organotins, in marine biota in monitoring programmes. Target compounds include tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT) and also triphenyltin (TPhT), diphenyltin (DPhT), and monophenyltin (MPhT).

1. Species

Target species for the monitoring of organotin compounds are shellfish, in particular bivalves like *Mytilus edulis* or *Mytilus galloprovincialis*. *Mytilus edulis* occurs in shallow waters along almost all coasts of the Contracting Parties. It is therefore suitable for monitoring in nearshore waters. No distinction is made between *M. edulis* and *M. galloprovincialis* because the latter, which may occur along the coast from Spain and Portugal to the southern coasts of UK, cannot easily be discerned from *M. edulis*. A sampling size range of 3-6 cm is specified to ensure availability throughout the whole maritime area. The Pacific oyster (*Crassostrea gigas*) should be sampled in areas where *Mytilus sp.* is not available. The sampling size should be within the length range 9-14 cm to ensure individuals of the 2 year age class.

Gastropods can also be used for TBT indicators, for instance in relation to biological effect monitoring. However, gastropods do not feed as continuously as bivalves and have a higher capacity of TBT metabolism, possibly resulting in a higher variability of TBT body burdens in gastropods compared with bivalves. In addition, correlation between imposex and TBT body burdens in the environment can be low, because of a time-lag between current TBT levels and imposex induced irreversibly in the early life stages and also because of non-continuous feeding strategies. In some sensitive gastropod species, imposex can also be induced by TBT at lower levels than analytical detection limits generally achieved.

2. Sampling

Two alternative sampling strategies are described: sampling to minimise natural variability and length-stratified sampling. References of relevance to sampling and statistics include Gilbert (1987); Bignert *et al.* (1993 and 1994); Nicholson and Fryer (1996); and Nicholson *et al.* (1997). Advice on sampling strategies for temporal trend and spatial monitoring in shellfish are provided in OSPAR's general JAMP Guidelines for Monitoring Contaminants in Biota and in Technical Annex 1: Organic Contaminants.

3. Transportation

Samples should be kept cool and frozen at $<-20^{\circ}\text{C}$ as soon as possible after collection. Length and weight should be determined before freezing. Live mussels should be transported in closed containers at temperatures between $5-15^{\circ}\text{C}$, preferably $<10^{\circ}\text{C}$. Frozen samples should be transported in closed containers at temperatures $<-20^{\circ}\text{C}$. More rigorous conditions will be necessary for samples for biological effects monitoring, *e.g.* storage in liquid nitrogen.

4. Pre-treatment and storage

4.1 Contamination

Sample contamination may occur during sampling, sample handling, pre-treatment, and analysis (Oehlenschläger, 1994), due to the environment, the containers or packing material used, the instruments used during sample preparation or from the chemical reagents used during the analytical procedures. Controlled conditions are therefore required for all procedures, including the dissection of organisms on board ship.

4.2 Depuration

Mussels should be placed on a polyethylene tray elevated above the bottom of a glass aquarium. The aquarium should be filled with sub-surface sea water collected from the same site as the samples and which has not been subject to contamination from point sources if possible. The aquarium should be aerated and the mussels left for 20-24 hours at water temperatures and salinity close to those from which the samples were removed.

4.3 Opening of the shells

Mussels should be shucked live and opened with minimum tissue damage by detaching the adductor muscles from the interior of one valve. The mussels should be inverted and allowed to drain on a clean towel or funnel for at least 5 minutes in order to minimise influence on dry weight determinations.

4.4 Dissection and storage

The soft tissues should be removed and deep frozen (-20°C) as soon as possible in containers appropriate to the intended analysis. TBT is stable in cockles and oysters stored at -20°C in the dark over a 7 month period. Longer storage can cause significant loss of TBT due to degradation (Gomez-Ariza *et al.*, 1999). The dissection of the soft tissue must be done under clean conditions on a clean bench by scientific personnel, wearing clean gloves and using clean stainless steel knives. After each sample has been prepared, the tools should be cleaned regularly. Washing in acetone or alcohol and high purity water is recommended. When the analysis is eventually undertaken, all fluids that may initially separate on thawing should be included with the materials homogenised. Homogenisation should be performed immediately prior to any sub-dividing of the sample.

5. Analysis

5.1 Preparation of materials

Solvents, chemicals and adsorption materials must be free of organotin compounds or other interfering compounds. If not they should be purified using appropriate methods. Solvents should be checked by concentrating the volume normally used in the procedure to 10% of the final volume and then analysing for the presence of organotin compounds and other interfering compounds using a GC. If necessary, the solvents can be purified by redistillation. Chemicals and adsorption materials should be purified by extraction and/or heating. Glass fibre materials (*e.g.* thimbles for Soxhlet extraction) should be pre-extracted. Alternatively, full glass thimbles with a G1 glass filter at the bottom can be used. Generally, paper filters should be avoided in filtration and substituted for by appropriate glass filters. As all super cleaned materials are prone to contamination (*e.g.* by the adsorption of organotin compounds and other compounds from laboratory air), materials ready for use should not be stored for long periods. All containers, skills, glassware *etc.* which come into contact with the sample must be made of appropriate material and must have been thoroughly pre-cleaned. Glassware should be extensively washed with detergents, heated at >250°C and rinsed immediately before use with organic solvents or mixtures such as hexane/acetone. Alternatively, all glassware can be washed in 10% HCl (or even in concentrated HCl) and then rinsed with distilled water.

5.2 Lipid determination

Organotin data are not usually expressed on a lipid basis. Lipid content is not a good normaliser because of poor correlations to organotin content. However, the determination of the lipid content of tissues can be of use in characterising the samples. If required, the lipid content should be determined on a separate subsample of the tissue homogenate, as some of the extraction techniques used routinely for organotin determination may destroy lipid materials. The total fat weight should be determined using the method of Bligh and Dyer (1959) or Smedes (1999).

5.3 Dry weight determination

Dry weight determinations should be carried out by air-drying homogenised sub-samples of the material to be analysed to constant weight at 105°C.

5.4 Determination of organotins by gas chromatography

5.4.1 Calibration and preparation of calibrant solutions

5.4.1.1 External calibration

When using an external calibration, multilevel calibration with at least five calibration points is preferred to adequately define the calibration curve. Standards preparation can be done in two ways depending on the methods of extraction/derivatisation used:

- i) by using alkyltins salts then proceed to the derivatisation step as for samples (for hydridisation or ethylation followed by purge-and-trap analysis, there is no other appropriate way than using alkyltin salts);
- ii) by using commercially readily available derivatised standards (*e.g.* Quasimeme <http://www.quasimeme.org/>).

Standard solutions can be prepared in (m)ethanol or another solvent depending on the instrumental method used. Addition of an internal standard (tripropyltin chloride TPrTCl or ¹³C labelled or deuterated TBT if using GC analysis with mass selective detection) to all standard and samples solutions is recommended. When using tripropyltin chloride, which is an underderivatised standard, the recovery efficiency of the whole procedure can be determined.

A new calibration solution should always be cross-checked to the old standard solution.

Calibrant solutions should be stored in a refrigerator in gas tight containers to avoid evaporation of solvent during storage. It is important to determine the expiry date of standard dilutions in order to avoid a concentration shift due to deterioration of analytes or evaporation of solvents.

5.4.1.2 Isotope Dilution-Mass Spectrometry

When using Isotope Dilution-Mass Spectrometry technique (IDMS), external calibration is not required.

5.4.2 Homogenisation and drying

Homogenisation should be carried out on fresh tissue. Care should be taken that the sample integrity is maintained during the actual homogenisation and during drying. When the analysis is undertaken, all fluids that may initially separate on thawing should be included with the materials homogenised. Homogenisation should be performed immediately prior to succeeding procedures. When grinding samples after drying, classical techniques using a ball mill can be used. Cryogenic homogenisation of dried or fresh materials at liquid nitrogen temperatures using a PTFE device (*cf.* Iyengar and Kasperek, 1977) or similar techniques can be applied (*cf.* Iyengar, 1976; Klussmann *et al.*, 1985).

5.4.3 Extraction

Release of organotin compounds from the biological matrix is a critical step, due to the strong matrix binding of the analytes and possible species degradation. Recovery standards should be added prior to extraction, however correction procedures should be used with care as equilibration between the spiked and the target compounds is not always guaranteed. Different extraction techniques are commonly used, such as microwave assisted extraction, mechanical shaking and digestion. Microwave assisted extraction (MAE) as well as mechanical shaking provide quantitative recoveries with negligible degradation of the TBT compounds (Centineo *et al.*, 2004). However, it must be taken into account that considerable loss of DBT, due to degradation was reported for MAE. Digestion techniques can be used to extract butyltins, though species degradation is not always under control using this technique. Mechanical shaking seems to be a suitable technique. Alternatively, pressurised liquid extraction (accelerated solvent extraction) can be used to extract organotin compounds. Extraction usually takes place in an aqueous methanolic acidic environment, with subsequent extraction to an organic phase, such as pentane or hexane. Acidic conditions enhance the extraction efficiency, acetic acid is usually preferred to other acids to ensure stability of butyltins compounds. Complexing agents such as tropolone are often employed. Extraction can be performed on wet as well as on freeze-dried samples. Wet tissue must be dried by mixing with anhydrous sodium sulphate or other anhydrous materials.

5.4.4 Derivatisation

5.4.4.1 Alkylation

Grignard reagent: A variety of Grignard reagents is used for alkylation reactions in derivatisation. The smaller the alkylation group, the more volatile the products of derivatisation, and the greater the losses during the transfer and work up. This method is time-consuming and requires very dry conditions and non-protic solvents. The use of Grignard reagents is hazardous as they react violently with protic solvents such as water, acid, alcohol, ketones and appropriate safety precautions must be taken. A liquid-liquid extraction step is necessary to isolate the derivatised organotins. However, unlike hydride derivatives of butyltins which may degrade in hours or days, the tetraalkyl derivatives formed with Grignard reagents are very stable (Morabito *et al.*, 2000). Derivatisation with Grignard reagents include extra steps in the analytical procedure as clean up of excess Grignard reagent with acid is required.

Sodium Tetraethylborate (NaBEt₄): Derivatisation with this complexing agent has been developed to minimise the analysis time. The NaBEt₄ procedure allows a simultaneous extraction-derivatisation in a buffered medium (optimum pH 4-5). NaBEt₄ derivatisation produces more thermally stable derivatives. However, NaBEt₄ is extremely air sensitive, since it is considered as pyrophoric, care must be taken to keep its chemical integrity. Although solutions in water have been shown to be stable for about 1 month at 4 °C, it is recommended to prepare them freshly for use. Solutions of the reagent in an organic solvent (e.g. tetrahydrofuran, methanol or ethanol) seem to be more stable (Smedes *et al.*, 2000). The determination of organotin compound in complex matrices, such as biological matrices with high lipid content, has led to several problems, including low recovery and low derivatisation efficiency. A clean-up step might be subsequently required.

Sodium Diethyldithiocarbamate (NaDDTC): NaDDTC is preferable to Grignard reagents as it does not require anhydrous conditions but it does not simultaneously derivatise and extract like NaBEt₄. Yet this step can be combined with Grignard reagent to provide better derivatisation for a wider spectrum of organotins.

5.4.4.2 Hydride generation

The butyltin species are converted to an hydride form by sodium tetrahydroborate (NaBH₄). Hydride generation produces a large volume of hydrogen as a by-product, which facilitates the purging of butyltin hydrides from a large volume of sample.

5.4.5 Clean-up

The most commonly used clean-up methods involve the use of alumina or silica adsorption chromatography. For the latter, phenyltin compounds like triphenyltin may not co-elute with butyltins. Gel permeation chromatography and similar high performance liquid chromatography (HPLC) based methods are also employed. The major advantages of using HPLC-based clean-up methods are their ease of automation and reproducibility.

5.4.6 Pre-concentration

Evaporation of solvents using a rotary evaporator should be performed under controlled temperature and pressure conditions, and the sample volume should be kept above 2 ml. Evaporation to total dryness should be avoided. To reduce the sample volume even more, e.g. to a final volume of 100 µl, solvents like pentane or hexane can be removed by concentration with a gentle stream of nitrogen. Only nitrogen of a controlled high quality should be used. Iso-octane is recommended as a keeper for the final solution to be injected into the GC.

5.5 Instrumental determination

Most of the analytical techniques developed for the speciation of organotin compounds are based on GC. GC remains the preferred separation technique owing to its high resolution and the availability of sensitive detectors such as (pulsed) flame photometry ((P)FPD), mass spectrometry (MS) or inductively coupled plasma- mass spectrometry (ICP-MS)

As an alternative approach, high performance liquid chromatography has become a popular technique. It mainly uses fluorescence, ultraviolet, and more recently inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), and mass spectrometry detectors such as atmospheric pressure chemical ionisation mass spectrometry (APCI-MS-MS) and electrospray ionisation mass spectrometry (ESI-MS). ICP-MS and (P)FPD detectors have been applied widely because of their inherent selectivity and sensitivity. (P)FPD has been shown to have greater selectivity and lower detection limits (by a factor of 25 to 50 times) for organotin compounds than those obtained with conventional FPD (Bravo *et al.*, 2004).

5.5.1 Gas chromatography

The two injection modes commonly used are splitless and on-column injection. Automatic sample injection should be used wherever possible to improve the reproducibility of injection and the precision of the overall method. If splitless injection is used, the liner should be of sufficient capacity to contain the injected solvent volume after evaporation. Helium must be used for GC-MS, GC-FPD and GC-ICP-MS. The preferred column length is 25–30 m, with an internal diameter of 0.15 mm to 0.3 mm. Film thicknesses of 0.3 µm to 1 µm are generally used. The most commonly used stationary phase for organotin analysis is 5% phenyl methyl siloxane. Mass spectrometric analyses are usually conducted in electron-impact mode at 70eV.

5.5.2 High Performance Liquid Chromatography

All stainless steel parts of the HPLC system that come into contact with the sample should be replaced by polyether ketone (PEEK) components. Reverse phase columns (e.g. octadecylsilane C18) are commonly used (Wahlen and Catterick, 2003) and the mobile phase can consist, for example, of a mixture of acetonitrile, water and acetic acid with 0.05% triethylamine, pH 3.1–3.4 (65:25:10 variable depending on columns used).

5.5.3 Detection

Flame photometry (FPD), equipped with a 610 nm band-pass filter, selective for tin compounds), mass spectrometry (MS) or inductively coupled plasma-mass spectrometry (ICP-MS) are mainly used as detectors for gas chromatography and high performance liquid chromatography.

6. Quality assurance

References of relevance to QA procedures include HELCOM (1988); HELCOM COMBINE manual, QUASIMEME (1992); Oehlenschläger (1994); ICES (1996); and Morabito *et al.* (1999).

6.1 System performance

The performance of the instrumentation should be monitored by regularly checking the resolution of two closely eluting organotin compounds. A decrease in resolution points to deteriorating instrumental conditions. A dirty MS-source can

be recognised by the presence of an elevated background signal together with a reduced signal-to-noise ratio. Chromatograms should be inspected visually by a trained operator.

6.2 Recovery

The recovery should be checked and reported. One method is to add an internal (recovery) standard to each sample immediately before extraction (e.g. tripropyltin) and a second (quantification) standard immediately prior to injection (e.g. tetrapropyltin). The recovery of MBT may be lower than for other organotin compounds, probably because of a lower derivatisation efficiency.

When using Isotope Dilution-Mass Spectrometry technique, the loss of target analytes is compensated. However, the recovery should still be calculated and should be between 50% and 150%.

6.3 Calibrant solutions and calibration

See Section 5.4.1.

6.4 Blanks

A procedural blank should be measured for each sample series and should be prepared simultaneously using the same chemicals and solvents as for the samples. Its purpose is to indicate sample contamination by interfering compounds, which will lead to errors in quantification. Even if an internal standard has been added to the blank at the beginning of the procedure, a quantification of peaks in the blank and subtraction from the values obtained for the determinands must not be performed, as the added internal standard cannot be adsorbed by a matrix.

6.5 Accuracy and precision

A Laboratory Reference Material (LRM) should be included, at least one sample for each series of identically prepared samples. The LRM must be homogeneous, well characterised for the determinands in question and stability tests must have shown that it produces consistent results over time. The LRM should be of the same type of matrix (e.g. liver, muscle tissue, fat or lean fish) as the samples, and the determinand concentrations should occur in a comparable range to those of the samples. If the range of determinand concentrations in the samples is large (> factor of 5) two reference materials should be included in each batch of analyses to cover the lower and upper concentrations. The data produced for the LRM in successive sample batches should be used to prepare control charts. It is also useful to analyse the LRM in duplicate from time to time to check within-batch analytical variability. The analysis of an LRM is primarily intended as a check that the analytical method is under control and yields acceptable precision, but a certified reference material (CRM such as ERM-CE 477 (mussel, certified for TBT, DBT, MBT) or NIES No. 11 (fish tissue certified for TBT and non certified reference value for TPhT)) of a similar matrix should be analysed periodically in order to check the method bias. Additionally a duplicate of at least one sample should be run with every batch of samples. Each laboratory should participate in interlaboratory comparison studies and proficiency testing schemes on a regular basis, preferably at an international level.

6.6 Data collection and transfer

Data collection, handling and transfer must take place using quality controlled procedures.

7. Data recording and reporting parameters

The calculation of results and the reporting of data can represent major sources of error, as has been shown in intercomparison studies for organotin compounds. Control procedures should be established in order to ensure that data are correct and to avoid transcription errors. Data stored in databases should be checked and validated, and checks are also necessary when data are transferred between databases.

Data reporting should be in accordance with the requirements of the monitoring programme and with the latest ICES reporting formats. Results should be reported according to the precision required for the programme. In practice, the number of significant figures is defined by the performance of the procedure.

The following parameters should be recorded:

7.1 Sampling and biological parameters

Shellfish

- location of sampling site (name, latitude, and longitude);
- date and time of sampling (GMT);
- sampling depth with respect to low tide (for sub-tidal sites only);
- irregularities and unusual conditions;
- name and institution of sampling personnel;
- number of pooled samples;
- number of individuals in pool;
- mean, minimum and maximum length and standard deviation;
- mean dry shell weight;
- mean soft tissue weight (wet weight);
- condition index.

7.2 Analytical and quality assurance parameters

- LRM and CRM results for a set of organotin compounds, reported on a wet weight basis;
- descriptions of the extraction, cleaning and instrumental determination methods;
- mean tissue lipid weight and method of extraction;
- the mean soft dry weight and method of determining water content if this differs from air drying to constant weight at 105°C (if sufficient material is available);
- the detection limit for each organotin compound. Specific performance criteria, including detection limits and precision, are usually set by the programme. A typical detection limit for single contaminants is 1 µg/kg wet weight, although this might be difficult to achieve for phenyltins compounds.
- QA information according to the requirements specified in the programme.

7.3 Lipids

- total lipids (*e.g.* Bligh and Dyer, 1959; or Smedes, 1999) (expressed as % or g/kg wet weight).

7.4 Parameters

- organic contaminants of interest to monitoring programmes for which these guidelines apply: organotin compounds suite required for analysis
- Butyltin compounds: Tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT)
- Phenyltin compounds: Triphenyltin (TPhT), diphenyltin (DPhT) and monophenyltin (MPhT)

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Annex XI.

European Commission (2014). Common implementation strategy for the Water Framework Directive (2000/60/EC). Guidance Document No. 32 on Biota Monitoring (the Implementation of EQSbiota) under the Water Framework Directive. Technical Report - 2014 – 083. (5.2.5)

European Commission Guidance Document No. 32. On Biota Monitoring

Technical Report - 2014 - 083

(The implementation of EQSBIOTA) under the Water Framework Directive

ANNEX A.7 Normalisation of measured data with respect to lipid and dry weight content

The appropriate metric to use for normalisation of contaminant concentrations in biota will usually follow from the normalisation used in the bioaccumulation studies used to derive the biota EQS.

For substances that accumulate through hydrophobic partitioning into the lipids of organisms, measured concentrations in fish should be normalised to fish with a lipid content of 5% (EC 2011). The energy content for mussels of 19.3 kJ/g dw (Smit 2005; EFSA 2009) corresponds to a lipid content of approximately 1% for freshwater and marine bivalves (Bruner et al. 1994; Lazzara et al. 2012; Pleissner et al. 2012), and measured concentrations in bivalves should therefore be normalised to bivalves with a lipid content of 1%. The rationale behind this lipid normalisation is that the whole body biota concentration is linearly correlated with the lipid content of the species for those substances.

For a substance that does not accumulate by hydrophobic partitioning into lipids, but via another mechanism of accumulation, normalisation against another parameter, such as dry weight (e.g. for mercury), may be appropriate. The default dry weight content for fish is approximately 26% (Smit 2005; EFSA 2009). For mussels, EFSA has suggested a default dry weight content of 8.3% (Smit 2005; EFSA 2009).

Based on the above, contaminant concentrations should be normalised to lipid contents of 5% in fish and 1% in bivalves, or to dry weight contents of 26% in fish and 8.3% in bivalves, on the basis of the measured lipid content or dry weight, or on the basis of generic values for lipid content or dry weight for the relevant species obtained from FishBase, for example.

To calculate the normalised concentrations $\text{conc}_{\text{norm, lipid}}$ or $\text{conc}_{\text{norm, dry weight}}$ from measured concentrations $\text{conc}_{\text{meas}}$ for a fish species x, the following equations can be used (lipid content and dry weight content expressed as mass fractions):

$$\text{conc}_{\text{norm, lipid}} = \text{conc}_{\text{meas}} \cdot 0.05/\text{lipid content}_x$$

or

$$\text{conc}_{\text{norm, dry weight}} = \text{conc}_{\text{meas}} \cdot 0.26/\text{dry weight}_x$$

Similarly, to calculate the normalised concentrations $\text{conc}_{\text{norm, lipid}}$ or $\text{conc}_{\text{norm, dry weight}}$ from measured concentrations $\text{conc}_{\text{meas}}$ for a bivalve species x, the following equations can be used (lipid content and dry weight content expressed as mass fractions):

$$\text{conc}_{\text{norm, lipid}} = \text{conc}_{\text{meas}} \cdot 0.01/\text{lipid content}_x$$

or

$$\text{conc}_{\text{norm, dry weight}} = \text{conc}_{\text{meas}} \cdot 0.083/\text{dry weight}_x$$

Using the exact lipid or dry weight content of the biota samples is always preferred over generic values for the species (such as those available from FishBase).

It is acknowledged that for the organic priority substances, e.g. dioxins, covered by both the WFD and food legislation, lipid normalisation may result in different conclusions under the MSFD for descriptors 8 and 9, even when human health is the protection goal in both cases. The discrepancy will depend upon whether the actual lipid content is greater or less than the 5% benchmark. The results should therefore be interpreted with appropriate qualification.

Annex XII.

Background Assessment Criteria recommended to be used to assess concentrations in Mediterranean sediments, mussel (*Mytilus galloprovincialis*) and fish (*Mullus barbatus*) (UNEP(DEPI)/MED WG.444/12, 6th Meeting of the Ecosystem Approach Coordination Group 2017)



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**UNITED NATIONS
ENVIRONMENT PROGRAMME
MEDITERRANEAN ACTION PLAN**

11 July 2017
Original: English

6th Meeting of the Ecosystem Approach Coordination Group

Athens, Greece, 11 September 2017

Agenda item 4: Review of Quality Status Report (QSR)

Pollution Assessment Criteria and Thresholds

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**UNITED NATIONS
ENVIRONMENT PROGRAMME
MEDITERRANEAN ACTION PLAN**

28 April 2017
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Meeting of the MED POL Focal Points

Rome, Italy, 29 – 31 May 2017

Agenda item 8: Implementation of Decision IG 22/7 on IMAP and Articles 7 and 8 of the LBS Protocol

Pollution Assessment Criteria and Thresholds

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List of Acronyms and key definitions

ADR	Adriatic Sea eco-region
AEL	Aegean and Levantine Seas
BAC	Background Assessment Criteria
BC	Background Concentration
CEN	Central Mediterranean Sea
EAC	Environmental Assessment Criteria
GES	Good Environmental Status
IQR	Interquartile range as a measure of the data dispersion (non-parametric distributions)
Mean	Arithmetic mean as central tendency estimator
Med BAC	Mediterranean BAC (MEDPOL datasets)
Median	Midpoint value (50th percentile) of the datasets
Med BC	Mediterranean BC (MEDPOL datasets)
N	Number of individual data
Percentile(s)	Value(s) below a given % of data of the ordered datasets can be found
WMS	Western Mediterranean Sea eco-region
λ (%)	% of data of the aggregated datasets in the primary normal component
Cd	Total cadmium
Hg/HgT	Total mercury
Pb	Total lead
PAHs	Polycyclic Aromatic Hydrocarbons (group of petroleum hydrocarbons compounds)
OCs	Organochlorinated Compounds (group of compounds including PCBs and Pesticides)
AChE	Acetylcholinesterase biomarker
MT	Metallothioneins biomarker
MN	Micronuclei Frequency
LMS	Lysosomal Membrane Stability
SoS	Stress on Stress

Explanatory Note by the Secretariat

1. The first estimates of Mediterranean background concentrations (BCs) and both background and environmental assessment criteria (BACs/EACs) were made for trace metals in sediments and biota and PAHs in sediments in 2011, following the OSPAR (Oslo Paris Convention) methodology approach (UNEP(DEPI)/MED WG.365/Inf.8). Later in 2014, an informal online expert group on contaminants was established and delivered its first report on assessment criteria in March 2015 and it was discussed at the MEDPOL Focal Points Meeting in June 2015 (UNEP(DEPI)/MED WG.417/Inf.15).

2. The online group made a preliminary proposal regarding the Mediterranean BACs for major chemical pollutants (in sediment and biota) and biomarkers and recommended as a first step the use of a number of BAC and EAC values both adopted by OSPAR and developed by scientific studies in the Mediterranean Sea. The group pointed out the need to undertake a data analysis of additional data sets from Reference Stations in order to adjust (or to develop) as appropriate threshold assessment criteria for the Mediterranean Sea region.

3. In February 2016, Decision IG. 22/7 at the 19th Meeting of Contracting Parties (COP19) agreed on the Integrated Monitoring and Assessment Programme (IMAP) of the Mediterranean Sea and Coast and Related Assessment Criteria. The main outputs during the initial phase of IMAP will include further update of GES definitions, refinement of assessment criteria and development of national level integrated monitoring and assessment programmes.

4. Therefore, this document with refined assessment criteria will contribute as an instrument to both assess and monitor the achievement of GES (2017-2021). This assessment criteria includes Background Concentrations (BCs), Background Assessment Criteria (BACs) and Environmental Assessment Criteria (EACs), for hazardous chemical substances and biomarkers for the Mediterranean Sea as a whole. For the first time, background concentrations (BCs) have been also calculated at a regional and sub-regional scales.

5. The methodology and results contained in this document are fully elaborated in the information document (UNEP(DEPI)/MED WG.427/Inf.3), which contains all the detailed data/metadata information, datasets characteristics, statistical results and scientific rationale of the performed analysis and assessment.

6. This proposal was presented at the Meeting of the Ecosystem Approach Correspondence Group on Pollution Monitoring held in Marseille, France, 19-21 October 2016. During the meeting it was noted that true natural background concentrations were difficult to assess and even reference stations might be contaminated to a certain extent. Also it was recommended to follow closely the recent and evolving work within the MSFD so as to be aligned if appropriate. Other suggestions included the need for more data on biomarkers, to consider how baselines may vary significantly even within one sub-region, and how to attain the most accurate baseline value for sediments. Among the revisions include the addition of AChE activity (nmol/min mg protein in gills) for France as well as Spain.

7. In addition and for future elaboration of assessment criteria the general proposed changes and considerations include:

- a) Nine new values for Cd, Hg and Pb in mussel, fish and sediment are proposed as new Mediterranean Background Assessment Criteria (Med BACs), as well as the adjustment of the fish EACs for trace metals. Further, the Annex to Decision 22/7 (IMAP) should be considered for EACs already imported from EU Directives (EU/1881/2006 and EU/629/2008) and OSPAR, despite taking into account the refinements proposed in this document (see Annex I). It is worth to mention, that for Cd and Pb in fish fillet tissue, datasets exhibits analytical issues, such as they are up to 100% below the detection limits (<BDLs). Contracting Parties

may consider, whether to continue reporting on these metals in fish flesh tissue, or instead report on these metals in liver tissue.

- b) The Med BACs for organic compounds have been proposed solely for PAHs in biota, thus no more datasets were available. Twelve Med BACs plus three Med BAC estimations are proposed as a new assessment criteria (see Annex I). For PAHs in sediment and organochlorinated compounds (OCs) no data was available (either sufficiently available or quality assured) to perform data analysis to derive Med BACs. Nevertheless, a revision has been provided for OCs (see Annex I). It is suggested that the Annex to Decision 22/7 (IMAP) for EACs (imported from adopted OSPAR values) should be considered for reference, as revised and reported in the present document (see Annex I). Further, it is recommended that Contracting Parties consider regular sediment sampling and the determination of organic contaminants, as limited studies are available for the Mediterranean Sea basin and sub-regions to establish proper assessment criteria.
- c) New four Med BACs are proposed for four biomarkers (see Annex I). Nevertheless, the assessment criteria (BACs and EACs) for biomarkers are based on limited geographical data (i.e. mainly from Croatia, Spain and Italy) and multiple methodologies and reference values have been used (in particular for Lysosomal Membrane Stability, LMS). It is therefore suggested to ensure more comparable and precise results and standard methodologies to be adopted for all Mediterranean laboratories. Further, the Annex to Decision 22/7 (IMAP) for biomarkers assessment criteria in the Mediterranean should consider the observations mentioned above and the proposed amendments in this document.

8. The present document was reviewed by the Meeting of the MED POL Focal Points, held in Rome, Italy, 29-31 May 2017.

1. Database Sources

1. This document uses the datasets for the MEDPOL Reference Stations updated until 2012, as well as the datasets submitted by Contracting Parties through the informal online group on contaminants during the period 2014-2015. The later, constitutes a selection of reference stations undertaken by national experts that has made available more recent data (including 2014) from national monitoring networks. This data was provided in the majority of cases for trace metals, petroleum hydrocarbons and biological markers (in the MEDPOL database format) for different biota species (fish and bivalves) and marine sediments all over the Mediterranean coastal environments. However, the online group data sets were not representative of Reference Stations/Areas for all the countries datasets submitted, and historical datasets, as well as from coastal and polluted areas were also submitted which would influence the results if computed straight. Therefore, a data selection process was found to be mandatory. Both databases needed a selection, aggregation and quality assurance processes (based on model-based clustering, see Section 3.6 in UNEP(DEPI)/MED WG.427/Inf.3) in order to select the reference stations before the statistical data analysis and assessments could be performed.

2. We have undertaken a synoptic approach to develop the calculations of the Mediterranean BCs (Med BCs) and BACs (MedBACs) at different spatial scales with the available merged datasets from both sources. Therefore, we assigned and grouped the selected stations by eco-regions as shown in the Figure 2.1 and further detailed in Table 2.1. It has been recognised that differences between regions and sub-regions within the Mediterranean Sea basins are likely to occur and should be taken into account for the environmental and pollution assessments as will be shown later in this document. The calculation of the Med BACs have been performed for the Mediterranean Sea as a whole with the calculated Med BCs (either corresponding to the median (50th percentile) for hazardous chemical substances or the 10th or 90th percentile for selected biomarkers), including BCs developed both for Mediterranean eco-regions and sub-regional seas. For, EACs, those adopted within OSPAR and the EU Directives (EU/1881/2006 and EU/629/2008) are further suggested.

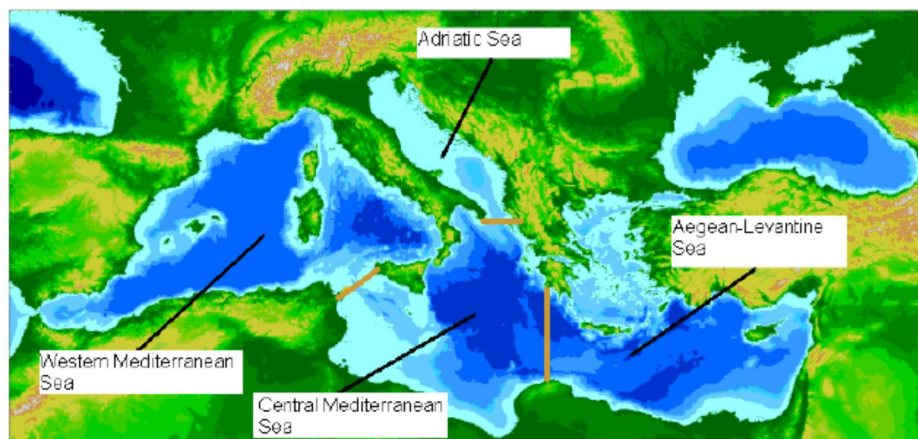


Figure 2.1. The four Mediterranean MEDPOL eco-regions (WMS, Western Mediterranean Sea; ADR, Adriatic Sea; CEN, Central Mediterranean and AEL, Aegean and Levantine Seas)

Table 2.1. The Mediterranean eco-regions and sub-regions aggregation according the database sources and availability within this document.

Eco-regions	Sub-regional seas/basins*
Western Mediterranean Sea (WMS)	Alboran Sea (ALBS) North Western Mediterranean Sea (NWMS) Tyrrhenian Sea (TYRS) Western Mediterranean Islands and Archipelago (WMIA)
Adriatic Sea (ADR)	North Adriatic (NADR) Middle Adriatic (MADR) South Adriatic (SADR)
Central Mediterranean (CEN)	Central Mediterranean(CEN) Ionian Sea (IONS)
Aegean and Levantine Seas (AEL)	Aegean Sea (AEGS) Levantine (LEVS)

*all available MED POL data was used (seeUNEP(DEPI)/MED WG.427/Inf.3)

2. Scientific assessment of Background Concentrations (BCs) and Background Assessment Criteria (BACs)

2.1. Assessment criteria for trace metals (Cd, Hg, Pb) in mussels

3. For *Mytilusgalloprovincialis* species (MG), the tables below (Table 3.1 and Table 3.2) show the Med BCs and Med BACs calculated for the Mediterranean Sea basin, and the BCs for each eco-region except for the Central Mediterranean basin (no data available). The table also compares the determined Med BACs with the median value (50% of the data) of the MEDPOL Database for each eco-region earlier assessed (UNEP(DEPI)/MED WG.365/Inf.4), which included coastal and hotspot stations.

Table 3.1. Mediterranean BCs and BACs (Med BACs) for *Mytilusgalloprovincialis* ($\mu\text{g}/\text{kg}$ d.w.)

Trace metal	Mediterranean Sea Basin		Western Mediterranean (WMS)		Adriatic Sea (ADR)		Central Mediterranean (CEN)		Aegean-Levantine Seas (AEL)	
	MedB Cs	Med BACs	WMS BCs	*50% MEDPOL Database	ADR BCs	*50% MEDPOL Database	CEN BCs	*50% MEDPOL Database	AEL BCs	*50% MEDPOL Database
Cd	730.0	1095.0	660.5	660 <MedBAC	782.0	800 <MedBAC	-	430 <MedBAC	942.0	750 <MedBAC
HgT	115.5	173.2	109.4	130 MedBAC	126.0	140 <MedBAC	-	160 <MedBAC	110.0	80 <MedBAC
Pb	1542	2313	1585	2000 <MedBAC	1381	1530 <MedBAC	-	810 <MedBAC	2300	2280 <MedBAC

*median value of the MEDPOL Database from UNEP(DEPI)/MED WG.365/Inf.4 Report (2011); BAC=BCx1.5

4. It should be noticed that some calculated BCs for eco-regions (with solely reference stations datasets) are above the Med BCs, although below Med BACs. For example, cadmium and lead in the AEL eco-region (Aegean-Levantine Sea) in the Eastern Mediterranean Sea. Here, the explanation is that the major contributor to this eco-region (Turkey) presents quite higher background values for MG than the average for the reference stations in the Mediterranean Sea (see Information Document).

Table 3.2. Summary of statistical results of the Background Concentrations for trace metals (TM) in mussel by eco-regions in the Mediterranean Sea ($\mu\text{g}/\text{kg dw}$)

Trace metal	Eco-region	N ¹	Mean ²	10 ^{th 5}	25 ^{th 5}	Median ³ (BCs)	IQR ⁴	75 ^{th 5}	90 ^{th 5}
	ADR	151	1565.7	725.0	952.1	1381.0	1037.0	1989.1	2688.1
Pb	AEL	27	2174.8	944.4	1430.0	2300.0	1500.0	2930.0	3730.0
	WMS	157	1541.0	500.0	1075.4	1585.2	824.6	1900.0	2374.0
	ADR	154	196.2	85.6	104.6	126.0	71.4	176.0	409.0
HgT	AEL	31	123.4	74.2	80.0	110.0	79.0	159.0	177.8
	WMS	174	117.3	70.0	90.8	109.4	43.3	134.0	170.0
	ADR	151	753.8	413.0	510.5	782.0	470.5	981.0	1099.9
Cd	AEL	32	1269.1	297.1	737.7	942.0	1199.0	1936.7	3132.8
	WMS	174	731.5	364.0	520.0	660.5	370.0	890.0	1218.1

¹ N=number of individual data, ² Mean=arithmetic mean as central tendency estimator ³Median is the midpoint value (50th percentile) of the datasets, ⁴IQR= interquartile range as a measure of the data dispersion (non-parametric distributions), ⁵Percentile(s) = indicative value below a given % of the ordered datasets can be found.

2.2. Assessment criteria for trace metals (Cd, Hg, Pb) in sediment

5. The tables below (Table 3.3 and 3.4) shows the calculated Med BCs and BACs for the Mediterranean Sea and the BCs for each eco-region, except for the Central Mediterranean basin due to the shorten datasets in this eco-region. The calculated background concentrations (BCs) for each eco-region (aggregated datasets) exhibit values under the calculated Med BACs. The aggregated datasets considered solely the size fractions from <63 μm to 2mm (see UNEP(DEPI)/MED WG.427/Inf.3).

Table 3.3. Mediterranean BCs and BACs (Med BCs and BACs) in surface sediments ($\mu\text{g}/\text{Kg d.w.}$)

Trace metal	<i>Mediterranean Sea Basin</i>		Western Mediterranean (WMS)		Adriatic Sea (ADR)		Central Mediterranean (CEN)		Aegean-Levantine Seas (AEL)	
	<i>MedB Cs</i>	<i>Med BACs</i>	<i>WMS BCs</i>	*50% MEDPOL Database	<i>ADR BCs</i>	*50% MEDPOL Database	<i>CEN BCs</i>	*50% MEDPOL Database	<i>AEL BCs</i>	*50% MEDPOL Database
Cd	85.0	127.5	91.2	1600 >MedBAC	92.3	210 >MedBAC	-	90 <MedBAC	56.0	100 <MedBAC
HgT	53.0	79.5	60.0	160 >MedBAC	106.8 >MedBAC	100 >MedBAC	-	50 <MedBAC	31.2	150 >MedBAC
Pb	16950	25425	20465	19400 <MedBAC	13932	9830 <MedBAC	-	4390 <MedBAC	4920	16890 <MedBAC >AELBAC**

*median value of the MEDPOL Database from UNEP(DEPI)/MED WG.365/inf.4 Report (2011); BAC=BCx1.5; **see text

6. Despite unbalanced datasets for each eco-region in the MEDPOL Database, this should be interpreted as preliminary information for the surface sediments contamination originated mostly from highly impacted coastal sites and known hotspots, particularly, in the Western Mediterranean Sea. Similarly as for mussels, it should be noticed that some calculated BCs for eco-regions with reference stations datasets are above the calculated Med BCs, although below Med BACs (except for HgT). Further, to correctly assess Pb in sediments in the AEL eco-region an AEL BAC should be considered.

Table 3.4. Summary of statistics for TM BCs in the Mediterranean eco-regions ($\mu\text{g}/\text{kg}$ dw sediment).

Trace metal	Eco-region	N ¹	Mean ²	10 th 5	25 th 5	Median ³ (BCs)	IQR ⁴	75 th 5	90 th 5
	ADR	37	16543	3429	7513	13932	19711	27223	32098
Pb	AEL	85	13897	2123	3727	4920	13223	16950	41200
	CEN	2	2761	-	-	2761	-	-	-
	WMS	132	18792	6020	15935	20465	9135	25070	28845
	ADR	27	119.6	28.9	37.8	106.8	149.9	187.7	240.8
HgT	AEL	84	43.4	1.8	5.4	31.2	59.6	65.0	118.5
	CEN	1	58.0	-	-	58.0	-	-	-
	WMS	122	70.3	24.8	40.0	60.0	40.0	80.0	138.4
	ADR	32	125.8	60.0	70.6	92.3	75.4	146.1	268.1
Cd	AEL	20	114.5	35.4	45.7	56.0	42.8	88.5	387.1
	CEN	2	25.5	-	-	25.5	-	-	-
	WMS	85	94.4	50.0	71.5	91.2	32.5	104.0	128.4

¹ N=number of individual data, ² Mean= arithmetic mean as central tendency estimator ³ Median is the midpoint value (50th percentile) of the datasets, ⁴ IQR= interquartile range as a measure of the data dispersion (non-parametric distributions), ⁵ Percentile(s) = indicative value below a given % of the ordered datasets can be found.

2.3. Assessment criteria for trace metals (Cd, Hg, Pb) in fish

7. Trace metals in fish are determined in several species in the Mediterranean Sea, such as *Mullus barbatus* (MB), *Boopsboops* (BB), *Mullussurmuletus* (MS) and *Upneusmollucensis* (UM). These species have been selected in the framework of MEDPOL according their geographical distribution within the national monitoring programs, with some countries monitoring more than one species. The majority of monitoring datasets are available for *Mullus barbatus* (MB), and therefore, this species has been chosen as the reference species to calculate the Mediterranean BCs and BACs for trace metals, although the statistical analysis has been undertaken for all (see UNEP(DEPI)/MED WG.427/Inf.3). The countries with available datasets for reference stations for MB were Cyprus, Greece, Italy, Spain, Turkey and Israel.

8. In terms of quality assurance, the datasets for Cd and Pb in fillet tissue of MB presents some analytical issues which impede to determine consistent Med BCs and BACs. Particularly, for Cd, the majority of the countries datasets reported over a 90% of the data as BDLs (below detections limit), if not a 100%. Similarly, for Pb, a majority of datasets are reported as either as BDLs or with large values pointing to sample contamination or reporting issues. This is valid for reference stations, but also for coastal and hotspot stations within the MEDPOL Database, and therefore, it should be concluded that the MB species is not a good proxy for the evaluation of Cd and Pb in Mediterranean fish (fillet tissue). More, the organic contaminant determinations in MB are almost all 100% reported as BDLs (eg. OCs). Obviously, this impedes to correlate any biological effects with the concentrations of hazardous chemical contaminants in MB sampled from the environment. The table below (Table 3.5) presents the calculated Med BCs and BACs for fish (fresh weight), despite the considerations explained above need to be observed.

Table 3.5. Mediterranean BCs and BACs (Med BACs) in fish ($\mu\text{g}/\text{kg}$ f.w.)

Trace metal	<i>Mediterranean Sea Basin</i>		Western Mediterranean (WMS)	Adriatic Sea (ADR)	Aegean-Levantine Seas (AEL)
	<i>MedBCs</i>	<i>Med BACs</i>	<i>WMS BCs</i>	<i>ADR BCs</i>	<i>AEL BCs</i>
Cd	(3.7) ^a	(16.0) ^b	-	-	-
Hg	50.6	101.2	68.0	150.5 >MedBAC	44.6
Pb	(31) ^a	(40) ^b	38	-	20

^aCd value is below the detection limit (<BDL) and Pb presents a majority of non-detected values in monitoring datasets.

^bestimated BACs from reliable limits of detection (BAC=1.5 x LOD) using both analytical data and certified reference material information (DORM-2). However, liver tissue matrix should be recommended for Cd and Pb as within OSPAR Convention.

;BAC=BCx2.0

2.4. Assessment criteria for polycyclic aromatic hydrocarbons (PAHs) in mussels

9. In order to develop Mediterranean BC and BACs for PAHs in mussels a limited number of datasets from MEDPOL countries for reference stations were available (Table 3.6). The contributions were from France, Greece, Italy, Spain and Turkey. The polycyclic aromatic hydrocarbons were determined in mussel samples of similar length; despite each country followed different strategies to pool the samples (see UNEP(DEPI)/MED WG.427/Inf.3).

Table 3.6. Mediterranean BCs and BACs (Med BACs) in mussel samples ($\mu\text{g}/\text{kg}$ d.w.)

PAH*	<i>Mediterranean Sea Basin</i>		Western Mediterranean (WMS)	Adriatic Sea (ADR)	Aegean-Levantine Seas (AEL)
	<i>MedBCs</i>	<i>Med BACs</i>	<i>WMS BCs</i>	<i>ADR BCs</i>	<i>AEL BCs</i>
F	1.0	2.5	0.96	1.07	0.60
P	7.1	17.8	4.93	9.04	7.55
A	0.5	1.2	0.52	0.38	0.30
FL	3.0	7.4	3.38	2.03	6.60
PY	2.0	5.0	3.02	0.85	5.90 >MedBAC
BaA	0.8	1.9	1.20	0.53	1.60
C	1.0	2.4	1.24	0.27	5.20 >MedBAC
BkF	0.6	1.4	1.27	0.29	1.50 >MedBAC

BaP	0.5	1.2	0.60	0.32	0.70
GHI	0.9	2.3	0.90	-	1.20
DA	0.5	1.3	0.53	-	-
ID	1.2	2.9	1.23	-	0.90

*Not included: Naphthalene, Acenaphthylene, Acenaphthene are below detection limits (BDLs) or had a limited monitoring datasets, and therefore BACs are preliminary estimations. Benz(e)pyrene and Benzo(b)fluoranthene had not enough datasets.

10. In terms of quality assurance checks, the primary normal component (λ) for PAHs ranged between values from 58% to 96% for the aggregated reference stations datasets indicating from sufficient to excellent aggregated datasets. In the Table 3.7 below, the medians (BCs) for individual PAHs are shown for each Mediterranean eco-region. It should be noticed, that some eco-region medians (BCs) are above the calculated Med BACs. In this case, as mentioned before, this responds to the effect of grouped data by geographical areas with a scarce number of high data in reference stations. Further, when the medians (BCs) and confidence intervals are above the Med BACs for a sub-regional sea, the number and magnitude of the data should be further examined in detail and related to low λ values (see UNEP(DEPI)/MED WG.427/Inf.3).

Table 3.7. Summary of statistics for PAHs BCs in the Mediterranean eco-regions ($\mu\text{g}/\text{kg}$ dw mussel).

PAHs	Eco-region	N ¹	Mean ²	10 ^{th 5}	25 ^{th 5}	Median ³ (BCs)	IQR ⁴	75 ^{th 5}	90 ^{th 5}
F	ADR	60	1.13	0.66	0.77	1.07	0.59	1.36	1.73
	AEL	3	0.93	0.50	0.50	0.60	-	-	-
	WMS	76	1.50	0.49	0.60	0.96	1.19	1.78	3.23
P	ADR	60	9.25	5.94	7.87	9.04	2.73	10.60	13.92
	AEL	2	7.55	5.60	5.60	7.55	-	-	-
	WMS	90	7.17	2.35	3.70	4.93	3.76	7.46	11.59
A	ADR	55	0.77	0.21	0.25	0.38	0.33	0.58	2.09
	AEL	3	0.43	0.30	0.30	0.30	-	-	-
	WMS	53	0.88	0.29	0.38	0.52	0.33	0.71	1.36
FL	ADR	60	2.66	1.23	1.41	2.03	2.32	3.73	4.65
	AEL	3	5.50	1.90	1.90	6.60	-	-	-
	WMS	90	5.42	1.71	2.03	3.38	3.91	5.94	12.51
PY	ADR	60	2.82	0.38	0.51	0.85	1.22	1.73	4.43
	AEL	3	6.53	3.40	3.40	5.90	-	-	-
	WMS	90	5.17	0.97	1.77	3.02	4.75	6.52	13.20
BaA	ADR	60	1.02	0.19	0.28	0.53	0.94	1.22	3.17
	AEL	3	1.70	1.40	1.40	1.60	-	-	-
	WMS	40	2.82	0.29	0.57	1.20	3.70	4.27	7.34
C	ADR	50	0.74	0.12	0.19	0.27	0.93	1.11	2.27
	AEL	3	4.63	2.70	2.70	5.20	-	-	-
	WMS	68	4.20	0.55	0.77	1.24	4.12	4.90	12.83
BeP	ADR	-	-	-	-	-	-	-	-
	AEL	3	2.63	1.90	1.90	2.80	-	-	-
	WMS	42	1.12	0.36	0.51	0.79	0.79	1.30	2.41
BbF	ADR	30	0.59	0.13	0.35	0.43	0.30	0.65	1.46
	AEL	3	3.93	1.20	1.20	5.30	-	-	-
	WMS	59	2.43	0.18	0.26	0.49	3.44	3.70	7.40
BkF	ADR	24	0.28	0.09	0.14	0.29	0.20	0.33	0.53
	AEL	3	1.10	0.30	0.30	1.50	-	-	-
	WMS	46	2.07	0.32	0.50	1.27	2.23	2.73	5.26
BaP	ADR	27	0.38	0.10	0.11	0.32	0.32	0.43	1.09
	AEL	3	0.60	0.30	0.30	0.70	-	-	-
	WMS	45	1.17	0.21	0.38	0.60	0.72	1.10	3.00
GHI	AEL	3	1.13	0.70	0.70	1.20	-	-	-
	WMS	50	1.37	0.31	0.40	0.90	1.23	1.63	2.73
DA	WMS	24	0.76	0.32	0.38	0.53	0.21	0.60	0.88
ID	AEL	3	0.80	0.40	0.40	0.90	-	-	-
	WMS	25	1.74	0.50	0.61	1.23	1.59	2.20	4.47

¹ N=number of individual data, ² Mean= arithmetic mean as central tendency estimator ³ Median is the midpoint value (50th percentile) of the datasets, ⁴ IQR= interquartile range as a measure of the data dispersion (non-parametric distributions), ⁵Percentile(s) = indicative value below a given % of the ordered datasets can be found.

2.5. Assessment criteria for biomarkers (AChE, MT, MN, LMS and SOS) in mussels

11. The developments of the assessment criteria using the information from the MEDPOL pilot biomonitoring programmes were too limited with datasets from Croatia, Italy and Spain. Some datasets from Greece were also available from 2005. Therefore, the Western Mediterranean Sea and the Adriatic Sea eco-regions were evaluated for the majority of biomarkers, whilst the Aegean-Levantine Sea eco-region was only evaluated for one biomarker (LMS-LP) but not finally used. The following table (Table 3.8) shows the calculated Mediterranean BCs and BACs for selected biomarkers (see UNEP(DEPI)/MED WG.427/Inf.3 for full details and plots) using the WMS region data, thus data from the Adriatic Sea should be further investigated and could be of use solely at local level. Therefore, it should be noted that the proposed assessment criteria (see Annex I in this document) is applicable to a sub-regional scale according the countries datasets origin (see further details in UNEP(DEPI)/MED WG.427/Inf.3) and previous information.

Table 3.8. Mediterranean BCs and BACs calculated for biomarkers in mussel samples.

Biomarker	<i>Mediterranean Sea Basin</i>		Western Mediterranean (WMS)	Adriatic Sea (ADR)
	<i>MedBCs (median)</i>	<i>^aMed BACs</i>	<i>WMS BCs</i>	<i>ADR BCs</i>
AChE activity (nmol/min mg protein in gills) ^b	21	15	20.86	12.20 <MedBAC
Metallothioneins (µg/g digestive gland (DG))	192	247	191.3	200.5
Lysosomal membrane stability (LMS-Neutral red retention (NRR), minutes)	(45)	120*	45.0 <Standard	47.4 <Standard
Lysosomal membrane stability (LMS-Liabilisation period (LP), minutes)	(13)	20*	-	16.8 <Standard
Micronuclei frequency (per 1000 in haemocytes)	0.0	1.0	0.0	0.5
Stress on stress (days)	7	11	-	-

^aeither the 10th percentile or the 90th percentile are considered for biomarkers to establish the Background Assessment Criteria (BACs), see UNEP(DEPI)/MED WG.427/Inf.3;^bfrom available datasets from Spain; *adopted ICES/OSPAR standard

Annex I
Tables of the proposed assessment criteria

A. Table of the proposed assessment criteria for trace metals (TMs)

The tables below (Tables A.1.1 and A.1.2) compare the new proposed/revised BCs, BACs and EACs in this document (using Reference Stations datasets) with the earlier proposed threshold values in the Mediterranean Sea. Further details can be found in the information document (UNEP(DEPI)/MED WG.427/Inf.3).

Table A.1.1. Mediterranean Sea: Background Concentrations (Med BCs), Med BACs and EACs; Calculation =>BC = 50th (median); BAC=1.5 x BC (mussel, sediment); BAC=2.0 x BC (fish)

Trace metal	Mussel (MG) µg/kg d.w.			Fish (MB) µg/kg f.w.			Sediment µg/kg d.w.		
	BC	Med BAC	EC*	BC	Med BAC	EC*	BC	Med BAC	ERL**
Cd	730.0	1095.0	5000	(3.7) ^a	(16.0) ^b	50	85.0	127.5	1200
Hg	115.5	173.2	2500	50.6	101.2	1000	53.0	79.5	150
Pb	1542	2313	7500	(31) ^a	(40) ^b	300	16950	25425	46700

^aCd value is below the detection limit (<BDL) and Pb presents a majority of non-detected values in monitoring datasets.

^bestimated BACs from reliable limits of detection (BAC=1.5 x LOD) using analytical data and certified reference material information (DORM-2) (see also text). However, liver tissue matrix should be recommended in fish for Cd and Pb as within OSPAR Convention.

*EC/EU 1881/2006 and 629/2008 Directives for maximum levels for certain contaminants in foodstuffs

** Long et al. 1995 (idem OSPAR adopted values)

Table A.1.2. Earlier data (2011-2015) from UNEP(DEPI)/MED WG.365/Inf.8, UNEP(DEPI)/MED WG.417/inf.15 Part3 and Annex to UNEP(DEPI)/MED IG.22/7Decision.

Trace metal	^a Mussel (MG) µg/kg d.w.			^b Mussel µg/kg d.w.	^c Fish (MB) µg/kg d.w. ^f			Sediment µg/kg d.w.		
	BC	Med BAC	EC	BAC	BC	Med BAC	(EC)	BC	^e Med BAC	ERL
Cd	725	1088	5000	1000	4	8/16 ^d	207	-	150	1200
Hg	125	188	2500	170	296	600	4150	-	45	150
Pb	2500	3800	7500	1000	279	558	1245	-	30000	46700

^a preliminary data for the NW Mediterranean (Spain);

^b additional BAC data provided by Lebanon for *Brachidontesvariabilis* species;

^c preliminary data for the NW Mediterranean (Spain);

^d earlier estimation wet weight;

^e estimated from sediment cores (UNEP(DEPI)/MED WG.365/Inf.8, 2011);

^f a dry/wet ratio of 20 should be used to convert units for MG (f.w. units = d.w. units / 5)

B. Table of the proposed assessment criteria for polycyclic aromatic hydrocarbons (PAHs)

The tables below (Tables A.2.1 and A.2.2) compare the new proposed/revised BCs, BACs and EACs with the earlier proposed threshold values in the Mediterranean Sea. Further details can be found in the information document (UNEP(DEPI)/MED WG.427/Inf.3).

Table A.2.1. Mediterranean Sea Background Concentrations (BCs), Med BACs and EACs; Calculation =>BC = 50th (median); BAC=2.5 x BC (mussel); no data for sediment available

PAH compound	Mussel (MG) µg/kg d.w.			Sediment µg/kg d.w.		
	Med BC	Med BAC	^a OSPAR EAC	^a OSPAR BC	^a OSPAR BAC	^c ERL
F	1.0	2.5	-	-	-	-
P	7.1	17.8	1700	4.0	7.3	240
A	0.5	1.2	290	1.0	1.8	85
FL	3.0	7.4	110	7.5	14.4	600
PY	2.0	5.0	100	6.0	11.3	665
BaA	0.8	1.9	80	3.5	7.1	261
C	1.0	2.4	-	4.0	8.0	384
BkF	0.6	1.4	260	-	-	-
BaP	0.5	1.2	600	4.0	8.2	430
GHI	0.9	2.3	110	3.5	6.9	85
DA	0.5	1.3	-	-	-	-
ID	1.2	2.9	-	4.0	8.3	240

^{*}Naphthalene, Acenaphthylene, Acenaphthene, Benz(e)pyrene and Benzo(b)fluoranthene are below detection limits (BDLs) or have limited monitoring datasets, and therefore their BACs are preliminary estimations.

^aOSPAR Commission, CEMP: 2008/2009 Assessment of trends and concentrations of selected hazardous substances in sediments and biota (OSPAR PAHs sediment datasets from Spain, not TOC corrected; ^cERL: Effect Range Low

Table A.2.2. Earlier data (2011-2015) from UNEP(DEPI)/MED WG.365/Inf.8, UNEP(DEPI)/MED WG.417/inf.15 Part3 and Annex to UNEP(DEPI)/MED IG.22/7Decision.

PAH compound	Mussel (MG) µg/kg d.w.			Sediment µg/kg d.w.		
	Med BC	Med BAC	^a OSPAR EAC	^a OSPAR BC	^a OSPAR BAC	^a ERL
P		24.3	1700		7.3	240
A		4.1	290		1.8	85
FL		6.8	110		14.4	600
PY		6.1	100		11.3	665
BaA		1.3	80		7.1	261
C		2.4	-		8.0	384
BkF		1.8	260		-	-
BaP		1.3	600		8.2	430
GHI		1.3	110		6.9	85
ID		0.8	-		8.3	240

C. Table of the proposed assessment criteria for organochlorinated compounds (OCs)

(Summary of OSPAR values to be used in the Mediterranean Sea)

Table A.3.1. OSPAR Region (Background Concentrations (BCs), BACs and EACs)¹

OCs compound	Mussel µg/kg d.w.			Fish µg/kg w.w.			^d Sediment µg/kg d.w.		
	BC/LC ^c	BAC	EAC	BC/LC ^c	BAC	EAC (lipid w.)	BC/LC ^c	BAC	EAC/ERL
CB28 ^a	0.25	0.75	3.2	0.05	0.10	64	0.05	0.22	1.7
CB52 ^a	0.25	0.75	5.4	0.05	0.08	108	0.05	0.12	2.7
CB101 ^a	0.25	0.70	6.0	0.05	0.08	120	0.05	0.14	3.0
CB105 ^a	0.25	0.75	-	0.05	0.08	-	0.05	-	-
CB118 ^a	0.25	0.60	1.2	0.05	0.10	24	0.05	0.17	0.6
CB138 ^a	0.25	0.60	15.8	0.05	0.09	316	0.05	0.15	7.9
CB153 ^a	0.25	0.60	80	0.05	0.10	1600	0.05	0.19	40
CB156 ^a	0.25	0.60	-	0.05	0.08	-	0.05	-	-
CB180 ^a	0.25	0.60	24	0.05	0.11	480	0.05	0.10	12
Σ7CBs ICES ^b	-	-	-	-	-	-	0.20	0.46	11.5*
Lindane ^a	0.25	0.97	1.45	-	-	11**	0.05	0.13 ⁺	3.0*
α-HCH ^a	0.25	0.64	-	-	-	-	-	-	-
pp'DDE ^a	0.25	0.63	5-50***	0.05	0.10	-	0.05	0.09 ⁺	2.2*
HCB ^a	0.25	0.63	-	0.05	0.09	-	0.05	0.16 ⁺	20.0*
Dieldrin ^a	-	-	5-50***	-	-	-	0.05	0.19 ⁺	2.0*

¹OSPAR Commission, 2013.

^aOSPAR Commission, CEMP: 2008/2009 Assessment of trends and concentrations of selected hazardous substances in sediments and biota, Monitoring and Assessment Series

^bOSPAR Commission, Background document on CEMP assessment criteria for the QSR 2010, Monitoring and Assessment Series

^cLC: Low concentrations calculated from QUASIMEME; However, BC values should be considered as zero for OCs

^dTotal organic carbon (TOC) corrected values; ⁺LC from Spain (OSPAR, 2013)

*ERLs values instead EACs: Effect Range Low (Long et al. 1995); ERL for ICES Σ7CB is total CB concentration/2

**EAC for fish liver derived by applying a conversion factor of 10 on EAC for whole fish (CEMP 2008/2009)

***Ecotoxicological assessment criteria (earlier data from the QSR2000 Report-Chapter 4)

It should be noted that at present, no quality assured or sufficient datasets exist in the MEDPOL Database to calculate the threshold values for the Mediterranean Sea.

D. Table of the proposed assessment criteria for biological markers in mussels

The tables below (Tables A.4.1 and A.4.2) compare the new proposed/revised BCs, BACs and EACs with the earlier proposed threshold values in the Mediterranean Sea. Further details can be found in the information document (UNEP(DEPI)/MED WG.427/Inf.3).

Table A.4.1. Mediterranean Sea and standard reference values; Calculation => BAC = 10th or 90th percentile depending on the parameter.

Biomarkers	Mussel (<i>Mytilusgalloprovincialis</i>)	
	Med BAC	EAC
Stress on Stress (SOS, days)	11	5 ^a
Metallothioneins (µg/g digestive gland)	247	-
Lysosomal membrane stability (LMS-NNR, neutral red retention method, minutes)	120 ^{a*}	50 ^{a*}
Lysosomal membrane stability (LMS-LP, Cytochemical method, labilisation period minutes)	20 ^{a*}	10 ^{a*}
AChE activity (nmol/min mg protein in gills) ^b -France	29	20
AChE activity (nmol/min mg protein in gills) ^b -Spain	15	10 ^a
Micronuclei frequency (per 1000 in haemocytes)	1.0	-

^aTechnical annex: assessment criteria for biological effects measurements. Integrated monitoring of chemicals and their effects. ICES Cooperative Research Report No. 315. Davies, I.M. and Vethaak, A.D.Eds.

^bsubregional differences between assessment criteria are observed by countries

*Moore et al., 2006 (Standard values adopted by ICES)

Table A.4.2. Earlier data (2015) from UNEP(DEPI)/MED WG.417/inf.15 Part3 and Annex to UNEP(DEPI)/MED IG.22/7Decision.

Biomarkers	Mussel (<i>Mytilusgalloprovincialis</i>)	
	Med BAC	EAC ^a
Stress on Stress (SoS, days)	10	5
Lysosomal membrane stability (LMS-NNR, neutral red retention method, minutes)	120	50
Lysosomal membrane stability (LMS-LP, Cytochemical method, labilisation period minutes)	20	10
AChE activity (nmol/min mg protein in gills) - France	29	20
AChE activity (nmol/min mg protein in gills) - Spain	15	10
Micronuclei frequency (per 1000 in haemocytes)	3.9	-

Annex II
References

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Annex XIII.

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