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Réunions intégrées des groupes de correspondance de l'approche écosystémique sur la mise en œuvre de l'IMAP (CORMONs)

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 sédiments 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.

PNUE/PAM
Athènes, 2020

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- 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 de surveillance pour la préparation des échantillons et l'analyse des sédiments pour l'indicateur commun 17 de l'IMAP 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 échantillons de sédiments pour les métaux lourds, comprenant les six protocoles suivants : i) Protocole pour la digestion des sédiments à l'aide d'acide nitrique et d'acide fluorhydrique (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 en four graphite (GF-AAS) ; iv) Protocole pour l'analyse des métaux lourds par spectroscopie de masse à plasma à couplage inductif (ICP-MS) ; v) Protocole pour l'analyse du mercure total avec un analyseur de mercure solide ; vi) Protocole pour la normalisation des concentrations de métaux lourds à l'aide d'aluminium ; et Note technique pour l'analyse des échantillons de sédiments pour les contaminants organiques, comprenant les cinq protocoles suivants : i) Protocole pour l'analyse des pesticides organochlorés et des PCB dans les sédiments 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 sédiments par chromatographie en phase gazeuse – spectrométrie de masse (GC-MS) ; iii) Protocole pour l'analyse des HAP dans les sédiments par chromatographie en phase gazeuse – détecteur à ionisation de flamme (GC-FID) ; iv) Protocole pour l'analyse des HAP dans les sédiments par chromatographie en phase

gazeuse – spectrométrie de masse (GC-MS) ; v) Protocole pour la normalisation des concentrations de contaminants organiques par l'utilisation du carbone organique total (COT), 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 des sédiments pour l'indicateur commun 17 de l'IMAP, constituent une base solide pour une mise à jour régulière des pratiques de surveillance en vue de la réussite de l'IMAP.

Liste des abréviations/acronymes

CI	Indicateur commun
CU	Commission européenne
CdP	Conférence des parties
CORMON	Groupe de correspondance sur la surveillance
EcAp	Approche écosystémique
EEA	Agence environnementale européenne
EU	Union européenne
FAO	Organisation des Nations Unies pour l'alimentation et l'agriculture
HELCOM	Commission pour la protection du milieu marin dans la zone de la mer Baltique – Commission d'Helsinki
IAEA	Agence internationale de l'énergie atomique
IOC	Commission océanographique intergouvernementale
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 la qualité de la Méditerranée
OCDE	Organisation de coopération et de développement économiques
OSPAR	Convention pour la protection du milieu marin de l'Atlantique du nord-est
PAM	Plan d'action pour la Méditerranée
PdT	Programme de travail
PEBD	Polyéthylène basse densité
PNUE	Programme des Nations Unies pour l'Environnement
QA/QC	Assurance qualité / Contrôle qualité
QSR	Rapport sur la qualité
UE	Union Européenne
US EPA	Agence américaine de protection de l'environnement

Introduction

1. La détermination des concentrations de métaux lourds et de contaminants organiques ciblés dans différentes matrices marines est un élément clé de l'IMAP, car les résultats analytiques contribueront à l'évaluation de l'état environnemental de la masse d'eau considérée. Les sédiments sont l'une des matrices proposées pour l'analyse des métaux lourds et des contaminants organiques depuis la création du programme de surveillance PNUE/PAM – MED POL en 1981 (MED POL Phase II), car de nombreux métaux lourds et contaminants organiques persistants présents dans l'eau de mer ont tendance à devenir insolubles et à précipiter avec la fraction particulaire au fond de la mer. Par conséquent, puisque les sédiments sont le réservoir ultime de la plupart des métaux lourds et des contaminants organiques persistants qui sont introduits dans l'environnement marin, leur analyse fournira un aperçu clair de l'état de pollution de la masse d'eau examinée. De plus, dans les zones où les sédiments ne sont pas perturbés, le matériel sédimentaire déposé annuellement intègre la charge de pollution pendant cette période spécifique, et l'analyse des différentes couches sédimentaires fournit une tendance historique des processus de pollution dans la région.

2. Les contaminants peuvent pénétrer dans l'environnement marin à partir de sources terrestres et maritimes ainsi que par dépôt atmosphérique. Les sources terrestres affectent principalement les sédiments côtiers, où les concentrations les plus élevées de métaux et de contaminants organiques se trouvent généralement à proximité des « points chauds » de pollution (villes côtières et zones industrielles, embouchures de rivières drainant des bassins très peuplés et/ou industrialisés). Les sédiments offshore sont principalement influencés par les dépôts atmosphériques, qui jouent globalement un rôle très important, en particulier pour certains métaux (comme le Hg) et contaminants organiques (comme les HAP).

3. Parmi les sources de métaux lourds, on trouve à la fois des sources naturelles et des sources anthropiques. Il est donc important de pouvoir distinguer les enrichissements en métaux dus à des causes naturelles (telles que la minéralogie et la granulométrie des sédiments) et ceux qui sont dus à des activités humaines (urbaines, industrielles). À cette fin, la normalisation des données sur les métaux lourds est souvent utilisée, en vue de détecter l'empreinte humaine sur la distribution des métaux lourds dans les sédiments. En revanche, les sources de contaminants organiques persistants sont exclusivement anthropiques ; la charge totale des contaminants est donc d'origine anthropique.

4. Conformément aux exigences de l'IMAP (PNUE/PAM, 2019[1] ; PNUE/PAM, 2019a[2]), les contaminants devant obligatoirement être analysés dans les sédiments marins comprennent les métaux lourds [cadmium (Cd), plomb (Pb) et mercure total (THg)], les composés organochlorés (PCB, hexachlorobenzène, lindane et ΣDDT) et les hydrocarbures aromatiques polycycliques (16 composés HAP de référence de l'Agence des États-Unis pour la protection de l'environnement). De plus, d'autres paramètres doivent être analysés dans les sédiments, à savoir l'aluminium (Al), le carbone organique total (COT) et la taille des grains (< 2 mm et < 63 μm).

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 sédiments sont présentés dans l'annexe XII.

6. Les protocoles préparés dans le cadre des présentes lignes directrices de surveillance pour la préparation des échantillons et l'analyse des sédiments pour l'indicateur commun 17 de l'IMAP, présentés ci-dessous, décrivent des méthodologies appropriées pour l'analyse des sédiments marins aux fins de la détermination des métaux lourds et des contaminants organiques, l'objectif étant d'assurer la qualité des données. Ils 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.

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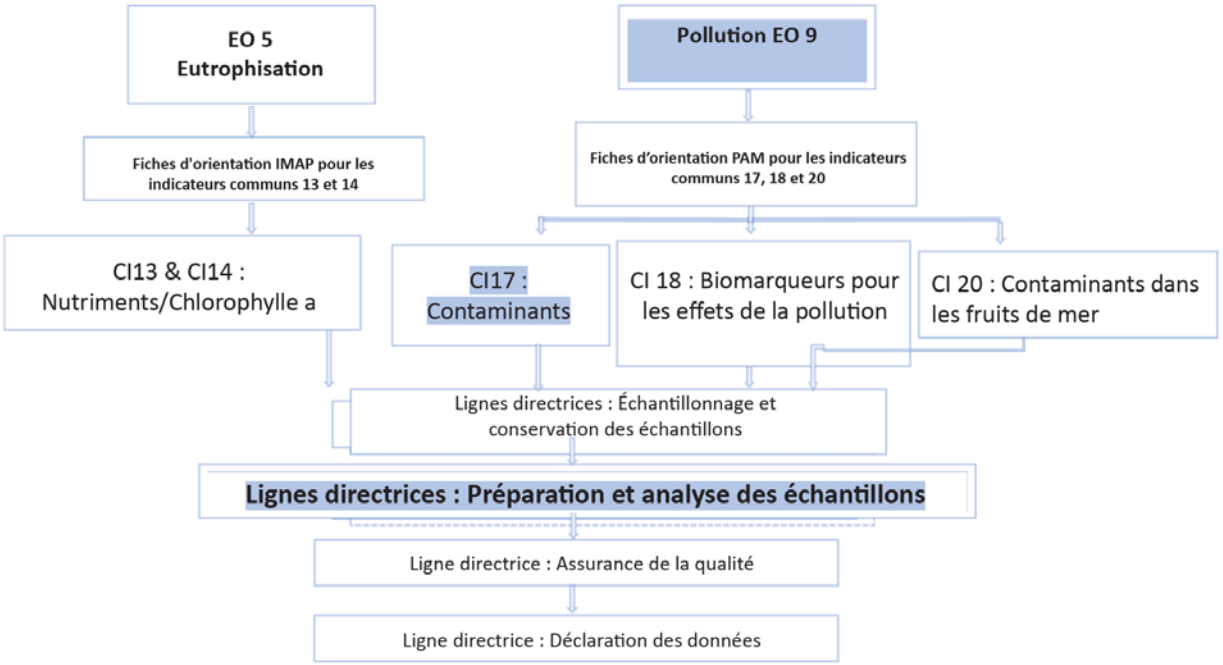
[1] UNEP/MAP (2019). UNEP/MED WG.467/5. IMAP Guidance Factsheets: Update for Common Indicators 13, 14, 17, 18, 20 and 21: New proposal for candidate indicators 26 and 27;
[2] UNEP (2019a). UNEP/MED WG.463/6. Monitoring Protocols for IMAP Common Indicators related to pollution;

7. Ces protocoles visent à rationaliser la préparation des échantillons de sédiments marins et l'analyse 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 et les différents programmes de surveillance nationaux. Ils fournissent des conseils, étape par étape, sur les méthodes à appliquer dans la zone méditerranéenne pour l'échantillonnage et la conservation des échantillons de sédiments.

8. 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. Ces protocoles 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 marins par décomposition thermique, amalgamation et spectrométrie d'absorption atomique (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 (2011), Méthode recommandée pour la détermination des hydrocarbures pétroliers dans les échantillons de sédiments (annexe IX). Ces méthodes ont été préparées dans le cadre du programme de surveillance MED POL. Les présentes lignes directrices sont également harmonisées avec les lignes directrices/protocoles similaires pour la préparation et l'analyse des échantillons de sédiments marins qui ont été élaborés par d'autres organisations régionales, telles qu'OSPAR (annexes II, V et X) et HELCOM (annexes VIII et XI). Par conséquent, toutes ces lignes directrices peuvent être appliquées dans le contexte de l'IMAP, ainsi que dans le cadre des travaux de l'Agence des États-Unis pour la protection de l'environnement (annexe III). Toutes ces lignes directrices étant adaptées au contexte de l'IMAP, elles pourraient permettre aux laboratoires méditerranéens compétents de l'IMAP de développer leurs propres méthodes d'échantillonnage et de traitement des échantillons. Les laboratoires des parties contractantes devraient adapter chaque étape des procédures et systématiquement les tester et les modifier pour en valider les résultats.

9. L'organigramme ci-dessous indique la catégorie des présentes lignes directrices de surveillance relatives à la préparation des échantillons et à l'analyse des sédiments pour l'indicateur commun 17 de l'IMAP au sein de la structure que forment toutes les directives de surveillance préparées pour les indicateurs communs 13, 14, 17, 18 et 20 de l'IMAP.



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

1 Note technique pour l'analyse des métaux lourds dans les sédiments

10. L'analyse des échantillons de sédiments marins pour la détermination des métaux lourds[3] comprend : i) la digestion des sédiments et ii) l'analyse de l'échantillon digéré pour les métaux lourds. Le Cd, le Pb et le THg sont les métaux qu'il est obligatoire de déterminer dans les échantillons de sédiments marins (PNUE/PAM, 2019). Toutefois, les Parties contractantes à la convention de Barcelone peuvent décider d'inclure dans leurs programmes de surveillance nationaux l'analyse de métaux lourds supplémentaires, en fonction de leurs priorités nationales.

11. Les laboratoires nationaux peuvent décider d'utiliser toute méthode analytique validée qu'ils jugent appropriée et qui répond à des critères de performance spécifiques (LOD, LOQ, précision, récupération et spécificité). Toutefois, afin d'aider les laboratoires d'analyse des Parties contractantes, les protocoles IMAP doivent servir de lignes directrices pour l'analyse des métaux lourds et des éléments traces dans les échantillons de sédiments marins. Les laboratoires analytiques 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.

12. 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

13. 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. 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.

14. La procédure d'étalonnage doit répondre à certains critères de base afin de fournir la meilleure estimation possible de la concentration réelle en élément de l'échantillon analysé (HELCOM, 2012[4]) :

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.

b) Éviter la contamination

15. 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 toute une nuit dans un récipient en plastique, dans une solution de savon (solution à 2 % dans l'eau du robinet) ;

[3] In the Guideline text the term "heavy metals" is used to designate both heavy metals and trace elements

[4] HELCOM (2012). Manual for marine monitoring in the COMBINE programme. Annex B-13 Appendix 3: Technical note on the determination of heavy metals and persistent organic compounds in marine sediment.

ii) rincer abondamment, d'abord à l'eau du robinet, puis à l'eau déionisée ultra-pure ;

iii) laisser les récipients reposer dans une solution concentrée de HNO_3 à 10 % (v/v), à température ambiante, pendant au moins 6 jours ;

iv) rincer abondamment à l'eau Milli-Q (au moins 4 fois) ;

v) laisser les récipients sécher sous une hotte à flux laminaire ;

vi) conserver les récipients dans des sacs plastiques en polyéthylène refermables par glissière pour éviter tout risque de contamination avant utilisation.

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

17. Dans le cadre de la présente note technique, les lignes directrices relatives à la préparation et à l'analyse des échantillons prévoit les protocoles IMAP suivants :

- Protocole pour la digestion des sédiments à l'aide d'acide nitrique et d'acide fluorhydrique (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 en four graphite (GF-AAS) ;
- Protocole pour l'analyse des métaux lourds par spectroscopie de masse à plasma à couplage inductif (ICP-MS) ;
- Protocole pour l'analyse du mercure total avec un analyseur de mercure solide ;
- Protocole pour la normalisation des concentrations de métaux lourds à l'aide d'aluminium.

18. 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 OSPAR (annexe II : Lignes directrices JAMP pour la surveillance des contaminants dans les sédiments ; annexe technique 6 : Détermination des métaux dans les sédiments – méthodes analytiques ; annexe V : Lignes directrices JAMP pour la surveillance des contaminants dans les sédiments ; annexe technique 5 : Normalisation des concentrations de contaminants dans les sédiments), et par l'Agence des États-Unis pour la protection de l'environnement (annexe III : Méthode 6020B de l'Agence des États-Unis pour la protection de l'environnement ; Méthode ICP-MS pour la détermination des éléments dans les échantillons d'eau et dans les extraits ou les produits digérés de déchets).

1.1 Protocole pour la digestion des sédiments à l'aide d'acide nitrique et d'acide fluorhydrique

19. Les échantillons de sédiments doivent être digérés (calcination humide) avant d'être analysés. La vitesse de digestion et l'efficacité de la décomposition 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.

20. L'IMAP exige la désintégration complète de la matière silicatée des sédiments à l'aide d'acide fluorhydrique (HF) pour permettre de mesurer la charge totale en métaux dans les sédiments, y compris en aluminium, nécessaire à des fins de normalisation. En outre, les matériaux de référence certifiés (MRC) des sédiments fournissent des valeurs certifiées pour les concentrations totales de métaux ; pour les utiliser aux fins du renforcement de l'assurance de la qualité des données, il convient donc de mesurer la teneur totale en métaux dans les échantillons de sédiments.

a) <u>Digestion acide par micro-ondes en systèmes fermés pour les métaux lourds, pour les analyses AAS, GFAAS et ICP-MS</u>

21. La digestion des sédiments peut être effectuée dans du téflon, ou dans des récipients de qualité équivalente, qui sont exempts de métaux et résistants aux acides forts, y compris le HF (Loring et Rantala, 1991[5]). Les échantillons de sédiments séchés (de 0,1 à 0,5 g) sont pesés dans le récipient à micro-ondes et placés dans une hotte laminaire compatible avec les fumées acides. Environ 5 ml d'acide nitrique de qualité analytique et 2 ml d'acide fluorhydrique de qualité analytique sont ajoutés, et il convient de laisser réagir chaque récipient pendant au moins 1 heure (ou plus si possible). Après la pré-digestion à température ambiante, 2 ml de peroxyde d'hydrogène (qualité analytique) sont ajoutés avec précaution, les récipients sont fermés et placés dans l'appareil à micro-ondes et les étapes de digestion sont effectuées, conformément à l'« Analyse des métaux traces dans les échantillons biologiques et sédimentaires : Livre de procédures de laboratoire » de l'AIEA (AIEA, 2012[6], annexe I). L'acide perchlorique et les matières organiques peuvent favoriser une réaction explosive ; cet acide doit donc être traité avec une grande prudence lorsqu'il est ajouté au sédiment. En outre, comme les récipients fermés retiennent le HF, de l'acide borique est ajouté après la digestion par le HF pour complexer le HF restant et rendre la solution résultante moins dangereuse, tout en empêchant la précipitation du fluorure d'aluminium. Après la digestion, les récipients sont retirés de l'appareil à micro-ondes et placés dans une hotte ventilée pour refroidir. Lorsque la pression est adéquate, les récipients sont ouverts et leur contenu est transféré dans des tubes gradués en polypropylène de 50 ml. Le réacteur en téflon doit être rincé 3 fois avec de l'eau Milli-Q. Il convient de laisser le contenu des tubes refroidir, puis de le diluer jusqu'à la marque (50 ml) avec de l'eau Milli-Q, avant de peser chaque tube et d'en noter le poids. Au moins un matériau de référence certifié doit être utilisé et préparé en double pour chaque lot de digestion. Ces digestions sont préparées de la même manière que les échantillons. Il convient d'utiliser un matériau de référence de plages de composition et de concentration similaires.

22. La digestion acide assistée par micro-ondes des sédiments est également proposée par OSPAR (2008[7]) (annexe 4.1.2), par HELCOM (2012) et par l'Agence des États-Unis pour la protection de l'environnement (1996[8], méthode 3052).

b) Digestion acide sur une plaque chauffante

23. 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 sans métal et résistant aux acides, permettant le traitement du HF et d'autres acides. Les échantillons de sédiments sont traités dans des cuves en téflon fermées avec de l'acide fluorhydrique (HF) en combinaison avec de l'eau régale afin de les décomposer. L'utilisation du HF est essentielle car c'est le seul acide qui dissout complètement les réseaux de silicates et libère tous les métaux.

24. L'annexe I contient une description détaillée de la procédure (AIEA, 2012). Un échantillon de sédiments secs (environ 0,2 g) est placé dans des récipients en téflon ; 1 ml d'eau régale (HNO_3 : HCl, 1:3 v/v) et 6 ml de HF concentré y sont ajoutés. Les récipients sont laissés à température ambiante pendant au moins 1 heure, puis ils sont fermés et placés sur un bloc d'aluminium sur une plaque chauffante à 120 °C pendant 2 h 30. Il convient ensuite de laisser les échantillons refroidir à température ambiante, avant d'ouvrir les tubes et d'ajouter de l'acide borique pour complexer le HF restant.

25. Outre la méthode de l'AIEA (annexe I), d'autres méthodes peuvent être utilisées pour la digestion des sédiments dans des systèmes ouverts, combinant le traitement HF avec l'acide perchlorique ou nitrique. Selon ces méthodes, le HF et l'acide perchlorique sont laissés au repos pendant une certaine période (entre 1 heure et une nuit) pour éviter les problèmes de réactions violentes qui peuvent être provoquées par la présence de matière organique dans le sédiment. Ensuite, le mélange acide perchlorique (ou acide nitrique)/HF est chauffé à 150 °C. Le HF est corrosif et

[5] Loring, DH and Rantala RTT (1991). Manual for the geochemical analyses of marine sediments and suspended particulate matter. Earth-Science Review, 32: 235:283. Elsevier Science Publishers B.V
[6] 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)
[7] OSPAR (2008). JAMP Guidelines for Monitoring Contaminants in Sediments. Technical Annex 6: Determination of metals in sediments – analytical methods
[8] US EPA (1996). Method 3052: Microwave assisted acid digestion of siliceous and organically based matrices.

toxique et il est nécessaire de l'éliminer ou de le rendre moins nocif pour les instruments de mesure. L'acide peut être éliminé par ébullition, ce qui nécessite des installations spécialisées pour extraire les fumées toxiques, ou neutralisé avec de l'acide borique (H_3BO_3) (OSPAR, 2008). Il convient de noter que la digestion dans les systèmes ouverts peut entraîner une perte de mercure (Delft et Vos, 1988[9]), et il importe d'éviter soigneusement les pertes de matière dues à de violentes réactions d'ébullition.

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

26. Dans la plupart des sédiments marins, Al, Cu, Cr, Fe, Ni, Zn et d'autres métaux peuvent être déterminés par spectroscopie d'absorption atomique à flamme, cette méthode présentant une sensibilité adéquate pour ces déterminations.

27. Dans la spectrométrie d'absorption atomique, la solution de l'échantillon est aspirée dans une flamme et atomisée. 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 cathodique creuse 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.

28. Les solutions étalons métalliques pour la courbe d'étalonnage sont préparées à partir de la solution étalon mère (1 000 ml/l, ou un étalon mère intermédiaire). Selon l'élément, il peut être nécessaire de préparer une solution étalon mère intermédiaire. Cette solution mère intermédiaire est préparée conformément aux instructions contenues dans la note technique, dans une matrice à 2 % de HNO_3. La courbe d'étalonnage est déterminée en fonction des concentrations attendues des échantillons, et la linéarité de la réponse AAS pour l'élément est prise en compte (courbe d'absorbance en fonction de la concentration donnée dans le livre des méthodes analytiques). En cas de probabilité d'ionisation ou d'interférences, il convient de choisir la bonne option selon le livre des méthodes analytiques, par exemple l'utilisation de la correction pour l'absorption non atomique au moyen d'un correcteur de fond de lampe au deutérium, l'utilisation d'une flamme oxydante air-acétylène, l'utilisation d'une flamme oxyde nitreux-acétylène, ou l'ajout d'un agent de libération ou d'un suppresseur d'ionisation.

29. Un protocole analytique détaillé pour l'analyse des métaux lourds dans les sédiments, préparé par l'AIEA (2012), est présenté à l'annexe I.

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

30. Dans les sédiments marins, Cd, Pb, Cu et d'autres métaux peuvent être identifiés par la spectroscopie d'absorption atomique en 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 sédiment, une fraction aliquote de la solution de l'échantillon (10-50 µl) est introduite dans un tube en graphite du 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 cathodique 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. Les réactifs utilisés comprennent l'argon, la solution étalon de l'élément visé (1 000 mg/l) et l'eau désionisée. Tous les réactifs doivent être de qualité analytique.

32. 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.

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[9] Delft W. van; Vos. G. (1988) Comparison of digestion procedures for the determination of mercury in soils by cold-vapor atomic absorption Spectrometry; Analytica Chimica Acta, 209, 147-156.

33. Le logiciel AAS donne généralement des programmes électrothermiques typiques pour chaque élément pour des échantillons de 10 µl dans du HNO_3 dilué (0,1 %) et des indications concernant les températures maximales de calcination 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 seront toujours pas satisfaisants (si, par exemple, la température maximale de calcination n'est pas suffisante pour éliminer le fond) ; la procédure devra alors être réalisée une nouvelle fois avec l'ajout d'un modificateur de matrice. Il est possible de devoir essayer différents modificateurs de matrice avant de trouver la meilleure solution.

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

35. La spectroscopie de masse à plasma à couplage inductif (ICP-MS) est actuellement l'un instrument 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 l'eau et les échantillons de sédiments digérés par l'acide.

36. Les limites de détection typiques pour la détermination des métaux traces par ICP-MS (en mg/kg dw), basées sur des tailles d'échantillon typiques (0,5-1 g), sont les suivantes (OSPAR, 2008) :

Al	Li	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
40	0.1	1	0.01	0.2	0.1	0.05	0.2	0.2	2

37. Le plasma à couplage inductif associé à un spectromètre de masse (ICP-MS) permet une analyse rapide d'une large gamme 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 doublement chargés 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. 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).

38. La méthode 6020B de l'Agence des États-Unis pour la protection de l'environnement (révision de 2014) décrit la détermination multi-élémentaire des métaux lourds par ICP-MS dans l'eau et les échantillons solides après digestion acide. Les espèces métalliques 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 6020B de l'Agence des États-Unis pour la protection de l'environnement est présentée à l'annexe III (US EPA, 2014[10]).

1.5 Protocole pour l'analyse du mercure total avec un analyseur de mercure solide

39. Le mercure total dans les sédiments peut être analysé par un analyseur de mercure solide, qui a une sensibilité adéquate pour cette détermination. L'échantillon est séché, puis décomposé

[10] USEPA (2014 revision) Method 6020B, ICP-MS. Environmental protection Agency, Washington, DC.

chimiquement sous oxygène dans le four de décomposition. Les produits de décomposition sont acheminés vers la section catalytique du four, où l'oxydation est achevée (les halogènes et les oxydes d'azote/soufre sont piégés). Le mercure présent dans les produits de décomposition restants est sélectivement piégé sur un amalgameur. Une fois le système rincé avec de l'oxygène, la vapeur de mercure est libérée par le chauffage rapide de l'amalgameur et transportée dans la cellule d'absorbance dans le trajet lumineux d'un spectrophotomètre d'absorption atomique à longueur d'onde unique. L'absorbance est mesurée à 253,7 nm en fonction de la quantité de mercure (ng). La plage de travail typique s'étend entre 0,1 et 500 ng. La vapeur de mercure est transportée dans des cellules d'absorption de longue (première) et de courte longueur. La même quantité de mercure est mesurée deux fois avec une sensibilité différente, ce qui donne une gamme dynamique qui s'étend sur quatre ordres de grandeur. La limite de détection typique est de 0,01 ng de mercure.

40. Les réactifs pour l'analyse comprennent l'eau ultra-pure (> 18 mΩ cm, par exemple MilliQ), l'acide nitrique à 65 % et la solution de $K_2Cr_2O_7$ de qualité analytique (10 % p/v).

41. Les étalons d'étalonnage doivent être préparés à partir de solutions mères étalons uniques ou d'étalons à éléments multiples par dilution de la solution mère à l'aide d'acide dilué, selon les besoins. Toutes les solutions étalons doivent être stockées dans des fioles jaugées en téflon, borosilicate ou quartz dans 0,5-1 % de HNO_3 et 0,1 % (v/v) de dichromate de potassium. Une autre courbe d'étalonnage peut être réalisée au moyen d'un matériau de référence solide certifié. Dans ce cas, un MRC approprié doit être pesé sur une coupelle d'échantillon tarée, l'instrument est réglé en fonction du type d'échantillon et l'absorbance est mesurée. La matrice du MRC doit être aussi semblable que possible à l'échantillon examiné. Cette procédure doit être répétée avec différents poids du MRC et/ou avec différents MRC, pour obtenir des résultats dans la plage de travail souhaitée. Ensuite, il convient de construire une courbe d'étalonnage en traçant l'absorbance en fonction des nanogrammes de mercure (en général, cette opération est effectuée automatiquement par le logiciel). Le type d'équation dépendra des niveaux, car la réponse n'est pas linéaire sur toute la plage de travail.

42. Un étalonnage quotidien doit être effectué chaque jour ouvrable avec un nombre minimum d'étalons pour garantir la validité de l'étalonnage primaire. Il est possible de réaliser cet étalonnage en utilisant soit un étalon liquide, soit un matériau de référence certifié (MRC) solide. L'étalonnage doit être effectué dans la gamme d'intérêt, avec au moins deux étalons (ou matrice MRC) et les résultats doivent être conformes aux critères d'acceptation. Les critères d'acceptation doivent être fixés à partir des données historiques, mais l'écart maximum ne doit pas dépasser 10 %.

Analyseur de mercure solide

43. Les paramètres analytiques de l'analyseur de mercure solide dépendent de la taille de l'échantillon et de la matrice, et sont spécifiques à l'instrument. Il est important de suivre les directives du fabricant de l'instrument. Il y a trois périodes à fixer : le séchage, la décomposition et l'attente. L'annexe IV contient une méthode détaillée décrivant le protocole pour la détermination du mercure total (inorganique et organique) dans les sédiments, préparée par l'AIEA (2012b[11]) (Méthode recommandée pour la détermination du mercure total dans les échantillons marins par décomposition thermique, amalgamation et spectrophotométrie d'absorption atomique). Avec cette méthode, le mercure total est déterminé sans aucun prétraitement chimique de l'échantillon, ce qui minimise les risques de contamination et/ou les erreurs supplémentaires dues à la manipulation de l'échantillon. La méthode est basée sur la méthode 7473 de l'Agence des États-Unis pour la protection de l'environnement (US EPA, 2007[12]).

1.6 Protocole pour la normalisation des concentrations de métaux lourds à l'aide d'aluminium

44. La normalisation est définie ici comme une procédure visant à ajuster les concentrations de métaux lourds en fonction de l'influence de la variabilité naturelle de la composition des sédiments, de

[11] IAEA (2012b). Recommended method on the determination of Total Mercury in marine samples by thermal decomposition, amalgamation and Atomic Absorption Spectrophotometry
[12] US EPA (2007). 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

la taille des grains et de la minéralogie. Dans les sédiments non pollués, les concentrations en métaux lourds augmentent généralement avec la diminution de la taille des grains du sédiment et, par conséquent, toute différence de concentration en métaux causée par des sources de pollution sera masquée par les différences de taille des grains. Une normalisation est donc appliquée pour différencier la variabilité naturelle et l'apport anthropique de contaminants.

45. En vertu d'une approche de normalisation, on considère que, dans les sédiments sableux, les concentrations de métaux lourds sont négligeables ; la concentration de métaux déterminée dans la fraction < 2 mm pourrait donc être normalisée ultérieurement pour un échantillon constitué à 100 % de la fraction < 63 µm. Toutefois, cette approche ne peut pas toujours compenser avec succès la variabilité des métaux, car les concentrations naturelles de métaux traces et leur variabilité dans les sédiments sont déterminées non seulement par la distribution granulométrique, mais aussi par la composition des minéraux et des composés secondaires.

46. Pour surmonter cet inconvénient, une approche de normalisation géochimique est souvent utilisée. Cette technique consiste à établir les relations mathématiques entre les concentrations de métaux et les concentrations d'un élément conservateur, qui représente une certaine fraction minérale du sédiment. Les éléments d'origine naturelle qui sont structurellement combinés à un ou plusieurs des principaux porteurs de métaux traces à grain fin sont considérés comme étant conservateurs et utilisés à des fins de normalisation. L'aluminium (Al) est l'élément le plus utilisé pour la normalisation, étant l'un des principaux constituants des aluminosilicates à grain fin auxquels sont associés la plupart des métaux traces. Toutefois, cette hypothèse n'est pas valable dans tous les cas, car certains composants des sédiments peuvent également servir d'hôtes à des contaminants qui ont une capacité de sorption encore plus élevée, mais ne contiennent ni silicium ni aluminium, comme les matières organiques, les oxydes de Fe/Mn ou les minéraux sulfurés (Kestern et Smedes, 2002[13]). De plus, lorsque les sédiments proviennent de l'érosion glaciaire de roches ignées, avec des quantités significatives d'aluminium présent dans les minéraux de feldspath contribuant à la fraction grossière, il est préférable d'utiliser le lithium comme élément conservateur pour la normalisation (Loring, 1991[14]).

47. La principale hypothèse pour l'application d'une normalisation géochimique à un élément conservateur est l'existence d'une relation linéaire entre le normalisateur et les autres métaux. Une telle relation indique que, dans les sédiments naturels d'une zone, la concentration du métal changera proportionnellement à la concentration du normalisateur. De plus, une relation linéaire doit exister entre la concentration du normalisateur et le pourcentage de la teneur en grains fins (limon et argile) des échantillons. Une telle relation permettrait d'utiliser les concentrations de normalisateur comme un indicateur de la variabilité granulométrique des sédiments, afin de distinguer l'enrichissement métallique lié à la pollution de l'enrichissement naturel causé par la variabilité de la taille des grains (Loring et Rantala, 1991).

Procédure de normalisation

48. Les concentrations de métaux lourds (Me) sont divisées par la concentration du normalisateur (ou cofacteur) dans chaque échantillon. Le rapport Me/Al dans les stations de référence représente la relation naturelle entre les deux métaux dans les sédiments de la zone, tandis que des rapports Me/Al plus élevés indiquent un enrichissement en métaux, qui ne peut pas être expliqué par la variabilité naturelle de la texture et doit être attribué aux apports anthropiques. Une approche plus détaillée permettra de calculer la droite de régression (et la pente) entre les concentrations de métal et de normalisateur (Al) dans les sédiments d'une zone. Pour veiller à ce que les changements de concentration du normalisateur reflètent les différences de teneur en matières fines, il est également nécessaire d'établir une régression statistiquement significative entre le normalisateur (Al) et la fraction fine des sédiments (c'est-à-dire argile < 2 µm, ou même limon + argile < 63 µm), afin de

[13] Kestern, M. and Smedes, F. (2002). Normalization procedures for sediment contaminants in spatial and temporal trend monitoring. J. Environ. Monit. 4, 109-115.
[14] Loring, DH (1991). Normalization of heavy metal data from estuarine and coastal sediments. ICES J. Mar. Sci. 48, 101-115

vérifier que le normalisateur est adapté pour être utilisé comme un indicateur du sédiment plus fin (Loring, 1991).

49. Kestern et Smedes (2002) proposent d'analyser également la fraction de sable (> 63 µm) afin de calculer les concentrations en aluminium (ou en lithium) dans les sédiments grossiers. Ils proposent un modèle présenté dans la figure 1. « C_X et N_X » représentent le cofacteur et les teneurs en contaminants éventuellement présents dans les matériaux grossiers(par exemple Al dans le feldspath) et peuvent être estimés à partir d'échantillons sans matière fine. La droite de régression entre le contaminant et le cofacteur aura ce point pour origine. Les régressions de séries de données co-génétiques, mais avec des niveaux de contamination différents auront ce point en commun, mais auront tendance à développer des pentes différentes à partir de ce « point décisif ». En principe, il suffit donc d'un seul échantillon supplémentaire pour estimer la pente d'un ensemble d'échantillons co-génétiques si ce point décisif est connu. La pente pour cet échantillon avec une teneur en contaminant C_S et une teneur en cofacteur N_S peut être exprimée comme suit :

$$P_L = \frac{dC}{dN} = \frac{C_S - C_X}{N_S - N_X}$$

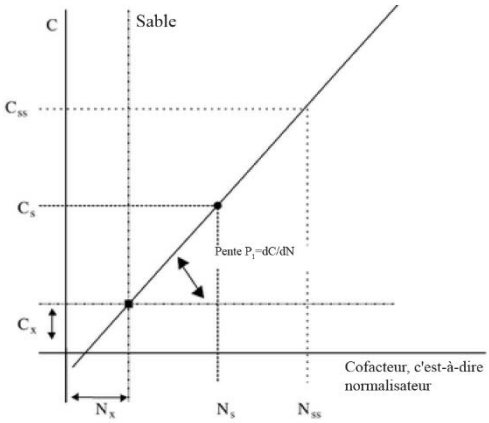

Figure 1. Teneur en contaminants C_S et teneur en cofacteurs N_S (Kestern and Smedes, 2002, in OSPAR, 2008b[15])

50. La pente de la régression représente la relation naturelle entre le métal et le cofacteur (normalisateur). « Les droites de régression tracées pour les échantillons de différentes zones peuvent ainsi être utilisées pour comparer leur degré de contamination. Plus le gradient est élevé, plus une zone est considérée comme étant contaminée. Les résidus positifs qui sont tracés au-dessus de cette droite indiquent que les concentrations sont plus élevées que ce qui serait prédit à partir de la relation contaminant/cofacteur, et peuvent représenter des échantillons de "points chauds". » (Kestern et Smedes, 2002, dans OSPAR, 2008b. Traduction libre.)

51. Kestern et Smedes (2002) soulignent également que « la précision du résultat dépend fortement de la variabilité naturelle (ou analytique) du N_X. Pour les échantillons à gros grains, un écart-type significatif du coefficient C_X et de la pente peut résulter de la propagation des erreurs de la variation analytique due aux faibles concentrations globales. Le coefficient C_X de la régression peut différer sensiblement d'un site à l'autre, en particulier lorsque l'on utilise des fractions granulométriques plus grossières. Dans certaines zones, la teneur en aluminium des fractions grossières est du même ordre que celle des fractions fines, et l'intersection N_X devient donc très élevée. Cela implique que le dénominateur est le résultat de la soustraction de deux nombres relativement importants, N_S et N_X. Par conséquent, en raison des incertitudes respectives qu'ils présentent, le résultat comporte une erreur extrême. » (OSPAR, 2008b. Traduction libre.) Dans le

[15] OSPAR (2008b). JAMP Guidelines for Monitoring Contaminants in Sediments. Technical Annex 5: Normalisation of contaminant concentrations in sediments.

cadre du programme de surveillance et d'évaluation intégrées MED POL, il a été décidé d'analyser la fraction < 2 mm du sédiment, il est donc possible que N_S et N_X soient relativement élevés.

52. Loring et Rantala (1992) présentent une approche similaire pour calculer la régression entre le métal et le normalisateur. En utilisant les données des stations non polluées (ou des stations de référence), on trace des diagrammes de dispersion des droites de régression entre les métaux et le normalisateur (Al ou Li) avec des bandes de confiance de 95 %. La droite de régression représente la variabilité naturelle des concentrations de métaux par rapport à la teneur en normalisateur (Al ou Li), et les stations situées dans les bandes de confiance peuvent être considérées comme non polluées. Au contraire, les stations situées au-dessus de la limite supérieure de la bande de confiance de 95 % peuvent être considérées comme polluées.

53. L'annexe technique 5 d'OSPAR, « Normalisation des concentrations de contaminants dans les sédiments » (OSPAR, 2008b) (annexe V), contient un examen détaillé des procédures de normalisation.

54. L'objectif de la normalisation est de réduire la variabilité entre les échantillons résultant des différences de propriétés des sédiments en vrac afin de tirer des conclusions sur le niveau de contamination par les métaux dans une zone spécifique, et/ou de comparer les niveaux de pollution entre différentes zones. Cependant, dans certaines zones, les corrélations entre les concentrations de contaminants et de cofacteurs peuvent être faibles, voire inexistantes. Par conséquent, il convient de recourir à la normalisation en tenant compte de ses limites et en ayant une bonne connaissance des caractéristiques des sédiments dans la zone étudiée.

2 Note technique pour l'analyse des contaminants organiques dans les sédiments marins

55. Les contaminants organiques qui doivent obligatoirement être analysés dans les sédiments 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 ΣDDT) et hydrocarbures aromatiques polycycliques (16 congénères individuels des HAP de l'Agence des États-Unis pour la protection de l'environnement – acénaphtène, acénaphtylène, anthracène, benz(a)anthracène, benzo(b)fluoranthène, benzo(k)fluoranthène, benzo(a)pyrène, benzo(ghi)pérylène, chrysène, dibenzo(a,h)anthracène, fluoranthène, indéno(1,2,3-cd)pyrène, naphtalène, phénanthrène, pyrène) (UNEP/MAP, 2019 ; PNUE/PAM, 2019a). Toutefois, les Parties peuvent décider d'inclure dans leurs programmes de surveillance nationaux l'analyse de composés organiques lourds supplémentaires, en fonction de leurs priorités nationales.

56. L'analyse des échantillons de sédiments 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.

57. Les laboratoires nationaux peuvent décider d'utiliser toute méthode analytique validée qu'ils jugent appropriée et qui répond à des critères de performance spécifiques (LOD, LOQ, précision, récupération et spécificité). Toutefois, afin d'aider les laboratoires d'analyse des Parties méditerranéennes, une liste de protocoles a été rédigée, devant servir de lignes directrices pour l'analyse des composés organiques dans les échantillons de sédiments marins. Les laboratoires analytiques 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.

58. Le laboratoire utilisé pour l'analyse des traces organiques doit être une installation dédiée, isolée des autres projets qui pourraient constituer des sources de contamination. Il doit être correctement élaboré avec des hottes et des tables munies de prises électriques qui ne présentent aucun danger en cas d'utilisation de solvants inflammables. Le laboratoire doit disposer d'extracteurs et d'évaporateurs rotatifs, ainsi que d'eau de refroidissement pour faire fonctionner les alambics. Dans les régions tropicales et dans les climats secs, un système de recirculation réfrigéré doit être utilisé pour réduire les températures aux niveaux requis et/ou pour conserver l'eau. L'acier inoxydable ou les carreaux de céramique constituent de bonnes surfaces non contaminantes. Si nécessaire, les tables

peuvent être recouverts 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. L'équipement de lutte contre l'incendie doit être installé dans des endroits évidents et le personnel de laboratoire doit être formé à son utilisation.

59. L'étalonnage des équipements pour l'analyse des contaminants organiques suit les mêmes procédures que pour l'analyse des métaux lourds (Note technique 2). 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 en ECD et leurs concentrations varient d'un lot à l'autre et selon le fournisseur. Les réactifs en poudre ou cristallins, tels que le sulfate de sodium anhydre (Na_2SO_4), l'hydroxyde de potassium (KOH) ou la laine de verre, doivent être extraits avec de l'hexane dans un appareil Soxhlet. Les adsorbants, tels que le gel de silice, l'alumine et le Florisil, doivent également être extraits par solvant. Toute la verrerie doit être vigoureusement frottée à l'aide de brosses dans de l'eau chaude et du détergent, puis rincée cinq fois à l'eau du robinet et deux fois à l'eau distillée. Ensuite, la verrerie doit être rincée à l'acétone ou au méthanol, puis à l'hexane ou à l'éther de pétrole, et cuite pendant la nuit dans un four à 300 °C. Lorsqu'elle n'est pas utilisée, toute la verrerie doit être stockée dans des armoires à l'abri de la poussière et fermée hermétiquement avec du papier en aluminium pré-nettoyé. Idéalement, la verrerie doit être nettoyée juste avant d'être utilisée. De plus amples informations sur les réactifs de nettoyage et la verrerie sont fournies dans le document PNUE/AIEA (2011[16]) (annexe VI).

60. Dans le cadre de la présente note technique, les lignes directrices fournissent les protocoles IMAP suivants pour l'analyse des composés organiques dans les échantillons de sédiments marins :

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

61. 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 dans le milieu marin. Méthodes de référence pour les études de pollution marine n° 71 ; annexe IX : Méthode recommandée pour la détermination des hydrocarbures pétroliers dans les échantillons de sédiments), par HELCOM (annexe VIII : Manuel pour le programme COMBINE de surveillance du milieu marin, annexe B-12, appendice 2. Note technique sur la détermination des biphényles chlorés et des pesticides organochlorés dans les sédiments marins ; annexe XI : Manuel pour le programme COMBINE de surveillance du milieu marin, annexe B-12, appendice 1. Note technique sur la détermination des hydrocarbures aromatiques polycycliques dans les sédiments) et par le CIEM/OSPAR (annexe VII : Directives JAMP pour la surveillance des contaminants dans les sédiments, annexe 2 : Analyse des PCB dans les sédiments ; annexe X : Lignes directrices JAMP pour la surveillance des contaminants dans les sédiments, annexe 3 : Détermination des HAP parents et alkylés dans les sédiments).

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

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

L'analyse des PCB et des pesticides organochlorés (POC) dans les échantillons de sédiments implique l'extraction de la matrice avec des solvants organiques, suivie d'un nettoyage et d'une séparation par chromatographie en phase gazeuse avec détection par capture d'électrons (GC-ECD) ou détection par spectrométrie de masse (GC-MS). Les échantillons peuvent être extraits à sec ou par voie humide. Les extraits sont ensuite concentrés dans un évaporateur rotatif à environ 15 ml, et nettoyés pour éliminer les lipides (lorsqu'ils sont présents en quantité importante) ainsi que le soufre élémentaire et les composés soufrés. Ces deux classes de composés peuvent interférer avec la séparation par chromatographie en phase gazeuse. Une étape de chromatographie d'adsorption (à l'aide de colonnes de Florisil) pourrait être utilisée pour éliminer les lipides interférents et pour fractionner l'extrait en classes de composés.

62. Pour réduire au minimum les erreurs systématiques dues à des conditions de chromatographie en phase gazeuse insuffisamment optimisées, à des pertes de déterminants (évaporation, rendement d'extraction insatisfaisant), et/ou à la contamination par la verrerie de laboratoire, les réactifs et l'environnement du laboratoire, il est essentiel que les sources d'erreurs systématiques soient identifiées et éliminées autant que possible (HELCOM, 2012b[17]). 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 en ECD ; 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.

63. 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 éluant ensemble 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 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, 2012b).

64. Pour l'analyse, les échantillons sont préparés pour l'extraction par solvant. Pour atteindre 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 des sédiments par Soxhlet à l'aide d'hexane et de dichlorométhane. Après les premiers traitements de nettoyage (élimination du soufre des extraits de sédiments), les extraits sont fractionnés par chromatographie sur colonne avec un détecteur à capture d'électrons (DCE). Il est recommandé, lors de l'utilisation de la GC-ECD (et, dans une certaine mesure, de la GC-MS), d'utiliser deux colonnes avec des phases stationnaires de polarité différente, car il peut y avoir une co-élution spécifique à la colonne des CB cibles avec d'autres CB ou composés organochlorés.

65. Un protocole pour la détermination des pesticides organochlorés et des polychlorobiphényles dans les échantillons de sédiments à l'aide de la CG-ECD, préparé par le PNUE/AIEA (2011), est

[17] HELCOM (2012b). Manual for marine monitoring in the COMBINE programme. Annex B-13 Technical note on the determination of heavy metals and persistent organic compounds in marine sediments. Appendix 2. Technical note on the determination of chlorinated biphenyls in marine sediment

présenté à l'annexe VI. Des protocoles analytiques similaires utilisant la GC-ECD sont également proposés par OSPAR (2008c[18]) (annexe VII) et par HELCOM (2012b) (annexe VIII).

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

66. L'analyse des PCB et des pesticides organochlorés (POC) dans les échantillons de sédiments implique une extraction de la matrice avec des solvants organiques polaires et non polaires, 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 atteindre 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 des sédiments par Soxhlet à l'aide d'hexane et de dichlorométhane. Après les premiers traitements de nettoyage (élimination du soufre des extraits de sédiments), les extraits sont fractionnés par chromatographie sur colonne.

67. L'analyse quantitative est réalisée en comparant le signal du détecteur produit par l'échantillon avec celui d'étalons définis, à l'aide d'un spectromètre de masse (MS). Souvent, en raison d'une séparation incomplète, plusieurs composés éluant ensemble 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 seront 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 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, 2012b).

68. Une méthode d'extraction, de concentration, de nettoyage et de fractionnement pour la détermination des pesticides organochlorés et des polychlorobiphényles dans les échantillons de sédiments 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. Tous les réactifs, y compris l'eau distillée, doivent être de qualité analytique. De plus, l'analyse des PCB et des pesticides organochlorés peut être effectuée par GC-ECD suivie d'une confirmation par GC-MS.

69. Des lignes directrices pour la détermination des pesticides organochlorés et des polychlorobiphényles dans les échantillons de sédiments à l'aide de la CG-MS sont proposées par OSPAR (2008c) (annexe VII) et par HELCOM (2012b) (annexe VIII).

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

70. 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.

[18] OSPAR (2008c) JAMP Guidelines for Monitoring Contaminants in Sediments. Technical Annex 2: technical annex on the analysis of PCBs in sediments.

71. Pour l'analyse des sédiments pour les HAP, les échantillons sont décongelés et préparés pour l'extraction par solvant. Pour atteindre une récupération satisfaisante des hydrocarbures pétroliers, les échantillons sont lyophilisés. Les sédiments sont ensuite extraits par Soxhlet à l'aide d'hexane et de dichlorométhane. Après les premiers traitements de nettoyage (élimination du soufre), les extraits sont fractionnés par chromatographie sur colonne avec de la silice et de l'alumine. La quantification est effectuée par GC-FID.

72. L'extraction est réalisée dans le Soxhlet avec 250 ml du mélange hexane/dichlorométhane (50:50) ; le cycle de siphon est d'environ 10 minutes pendant 8 heures. Lorsque l'extraction est terminée, l'extrait est évaporé à l'aide d'un évaporateur rotatif jusqu'à un volume d'environ 15 ml (la température du bain-marie ne dépasse pas 30 °C). L'extrait est séché avec du sulfate de sodium anhydre et transféré dans un tube gradué et concentré jusqu'à 4 à 5 ml au moyen d'un flux d'azote propre. Ensuite, un nettoyage est entrepris en vue d'éliminer les lipides, lorsqu'ils sont présents en quantité importante, ainsi que le soufre élémentaire et les composés soufrés. Ces deux classes de composés peuvent interférer avec la séparation par chromatographie en phase gazeuse. Le fractionnement des différents groupes de contaminants organochlorés peut être réalisé à l'aide d'une simple partition chromatographique sur colonne ou à l'aide de cartouches d'extraction en phase solide disponibles dans le commerce.

73. La quantification des HAP peut être 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 standard 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.

74. Une méthode détaillée pour la détermination par CG-FID des HAP dans les échantillons de sédiments, préparée par le PNUE/AIEA (2011b[19]), est présentée à l'annexe IX.

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

75. Les échantillons de sédiments sont décongelés et extraits par Soxhlet avec 250 ml du mélange hexane/dichlorométhane (50:50) ; le cycle du siphon est d'environ 10 minutes pendant 8 heures. Lorsque l'extraction est terminée, l'extrait est évaporé à l'aide d'un évaporateur rotatif jusqu'à un volume d'environ 15 ml (la température du bain-marie ne dépasse pas 30 °C). L'extrait est séché avec du sulfate de sodium anhydre et transféré dans un tube gradué et concentré jusqu'à 4 à 5 ml au moyen d'un flux d'azote propre. Ensuite, un nettoyage est entrepris en vue d'éliminer les lipides, lorsqu'ils sont présents en quantité importante, ainsi que le soufre élémentaire et les composés soufrés. Ces deux classes de composés peuvent interférer avec la séparation par chromatographie en phase gazeuse. Le fractionnement des différents groupes de contaminants organochlorés peut être réalisé à l'aide d'une simple partition chromatographique sur colonne ou à l'aide de cartouches d'extraction en phase solide disponibles dans le commerce. La quantification est effectuée par GC-MS.

76. Un protocole pour la détermination par GC-MS des HAP dans les échantillons de sédiments, préparé par le PNUE/AIEA (2011b), est présenté à l'annexe IX. Des protocoles analytiques détaillés

[19] UNEP/IAEA (2011b). Recommended method on the determination of petroleum hydrocarbons in sediment samples.

pour l'analyse des HAP par GC-MS sont également proposés par OSPAR (2008d[20]) (annexe X) et par HELCOM (2012c[21]) (annexe XI).

2.5 Protocole pour la normalisation des concentrations de contaminants organiques dans les sédiments par l'utilisation du carbone organique total (COT)

77. La normalisation est définie comme une procédure visant à ajuster les concentrations de contaminants en fonction de l'influence de la variabilité naturelle de la composition des sédiments, de la taille des grains, de la matière organique et de la minéralogie. La plupart des substances naturelles et anthropiques, les métaux et les contaminants organiques, présentent une affinité beaucoup plus élevée pour les particules fines que pour la fraction grossière. La taille des grains et la matière organique sont des facteurs importants qui contrôlent la répartition des composants naturels et anthropiques dans les sédiments. Par conséquent, la normalisation des données sur les contaminants pour les effets de la taille des grains ou du carbone organique est utilisée pour permettre des comparaisons significatives de la présence de substances dans des sédiments aux propriétés en vrac variables (OSPAR, 2008e[22]).

78. Dans le document d'orientation de la Commission européenne n° 25 sur la surveillance chimique des sédiments et des biotes en vertu de la directive-cadre sur l'eau (Commission européenne, 2010[23]), il est mentionné que les contaminants organiques dans les sédiments peuvent être normalisés à partir de la concentration de carbone organique total (COT), car les revêtements de matière organique de fines particules sont plus efficaces pour fixer les substances lipophiles telles que les composés chlorés et les HAP. Il est indiqué que les sédiments généralement plus grossiers (sable) sont des porteurs moins importants de substances lipophiles en raison de leur surface relative plus petite. Par conséquent, il est possible d'utiliser le rapport [concentration du composé organique]/[COT] en tant que valeur normalisée.

79. Dans de nombreux cas, la mobilité et la répartition des contaminants organiques dans l'environnement peuvent être prédites sur la base de leur répartition dans le carbone organique global dans les sédiments, qui peut être présenté à l'aide de différents normalisateurs (carbone organique total – COT ; carbone organique élémentaire – COÉ ; carbone organique particulaire – COP ; perte au feu – PF). Cependant, Kestern et Smedes (2002) soulignent que, puisque les contaminants organiques peuvent pénétrer dans l'environnement marin par différentes voies, « la question clé de la normalisation est donc la caractérisation correcte de la matière organique par le plus grand nombre de paramètres possible. Les types d'informations pouvant être obtenus par l'utilisation d'au moins quelques paramètres clés sont souvent complémentaires et extrêmement utiles, compte tenu de la complexité et de la diversité de la matière organique rencontrée dans le milieu sédimentaire. » (Traduction libre.) Ils notent également qu'« en raison de sa variabilité, la matière organique sera présente à la fois dans la fraction fine et dans la fraction grossière des sédiments. Contrairement à l'aluminium dans le cas des métaux, certaines matières organiques dans la fraction grossière peuvent contribuer à l'affinité pour les contaminants organiques en tant que cofacteur également, bien que ce cofacteur ait une importance environnementale limitée. » (Traduction libre.)

a) <u>Analyse du COT avec un analyseur de carbone</u>

80. Le carbone total (inorganique et organique) dans les sédiments peut être déterminé avec un analyseur de carbone. L'échantillon est injecté dans une chambre de réaction chauffée et garnie d'un catalyseur d'oxydation (Pt/Al_2O_3) ; l'eau est vaporisée et le carbone, tant organique qu'inorganique, est oxydé en CO_2, qui est mesuré au moyen d'un analyseur infrarouge (IR). Ensuite, le carbone inorganique est mesuré séparément, par l'acidification de l'échantillon de sédiments avec de l'acide HCl à un pH < 3, et tous les carbonates sont transformés en CO_2 mesuré dans l'analyseur IR. Le COT

[20] OSPAR (2008d). JAMP Guidelines for monitoring contaminants in sediments. Technical Annex 3: Determination of parent and alkylated PAHs in sediments
[21] HELCOM (2012c). Manual for marine monitoring in the COMBINE programme. Annex B-13, Appendix 1. Technical note on the determination of Polycyclic Aromatic Hydrocarbons (PAHs) in sediment
[22] OSPAR (2008e). JAMP Guidelines for Monitoring Contaminants in Sediments
[23] EC (2010). Guidance Document No: 25 Guidance on chemical monitoring of sediment and biota under the Water Framework Directive

peut être calculé par la différence entre le carbone total et le carbone inorganique. En variante, les carbonates inorganiques sont convertis en CO_2 avec un acide, qui est éliminé par purge avant l'injection de l'échantillon. L'échantillon restant ne contient que la fraction de carbone organique du carbone total, qui est mesurée dans l'analyseur IR.

b) Analyse du COT par oxydation humide

81. La technique d'oxydation humide est l'oxydation complète du carbone organique à l'aide de $K_2Cr_2O_7$ et de H_2SO_4 concentré et le titrage de l'excès de dichromate avec une solution de sulfate d'ammonium ferreux 0,5 N jusqu'au point d'équivalence (Schumacher, 2002[24]). Cette méthode est basée sur le protocole de Walkley et Black (1934[25]), tel que modifié et décrit par Nelson et Sommers (1996[26]).

82. Selon cette méthode, 0,5 g de sédiment séché est placé dans un erlenmeyer de 500 ml et 10 ml de solution de 1 N $K_2Cr_2O_7$ et 20 ml de H_2SO_4 concentré sont ajoutés et mélangés pendant 20 min. Le mélange est dilué à 200 ml de volume avec de l'eau distillée et 10 ml de H_3PO_4 à 85 %, 0,2 g de NaF et 15 gouttes d'indicateur diphénylamine. La solution est titrée en retour avec une solution ferreuse à 0,5 N.

[24] Schumacher, B.A (2002). Methods for the determination of Total Organic Carbon (TOC) in soils and sediments. EPA/600/R-02/069.
[25] Walkley, A. and Black, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Science, 37: 29-38
[26] Nelson, D.W and Sommers, L.E. (1996). Methods of Soil Analysis. Part 3. Chemical Methods. Soil Science Society of America Book Series no.5, pp. 961-1010.

Annex I

UNEP/MAP – IAEA (2012). Laboratory Procedure Book: Analysis of trace metals in biological and sediment samples (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

For further information on this method, please contact:

IAEA-Environment Laboratories
Marine Environmental Studies Laboratory
4a Quai Antoine 1er
MC-98000 Principality of Monaco

Tel. (377) 979 772 72; Fax. (377) 979 772 76
E-mail: NAEL-MESL.Contact-Point@iaea.org

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:

- HNO_3 (65%, Suprapur, Merck).
- HF (48%, analytical grade ISO, Merck).
- H_2O_2 (analytical grade), to be kept in the fridge after opening.
- Boric acid crystals, H_3BO_3 (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 HNO_3 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 H_2O_2
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:

- HNO_3 (65%, Suprapur, Merck).
- H_2O_2 (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 (HNO_3). Leave the samples at room temperature for at least 1 hour.
5. Add 2 ml of H_2O_2.
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:

- HNO_3 (65%, Suprapur, Merck).
- HF (48%, analytical grade ISO, Merck).
- HCl (30%, Suprapur, Merck).
- Boric acid crystals, H_3BO_3 (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 (HNO_3: 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:

- HNO_3 (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 (HNO_3). 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:

- HNO_3 (65%, analytical grade, low in mercury, Merck).
- Milli-Q deionised water (> 18MΩ cm, Millipore).
- 10% $K_2Cr_2O_7$ (w/v) solution (e.g. 10 g $K_2Cr_2O_7$ 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 V_2O_5 to each tube (including blanks).
6. Add 5 ml of concentrated Nitric acid (HNO_3). 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 $K_2Cr_2O_7$ 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 H_2O_2. 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% HNO_3 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%).

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

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 unbias 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 HNO_3 (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 aching 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 (Cstandard).

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 $$Cspike = \frac{Cstandard \times V2}{(V1+V2)}$$

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

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

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

Where:

C = Concentration of element in original sample (µg g^{-1} dry weight);

C_d = Concentration of element in sample solution (µg ml^{-1});

C_b = Mean concentration of element in reagent blanks (µg 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:

- $NH_4H_2PO_4$ (Suprapure, Merck) 25 g/l
- $Mg(NO_3)_2$ (Suprapure, Merck) 10 g/l

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

1000 µl $NH_4H_2PO_4$ solution

+ 50 µl $Mg(NO_3)_2$ solution

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

2) PALLADIUM NITRATE AND MAGNESIUM NITRATE

<u>SOLUTION (A):</u> $Pd(NO_3)$ (0.2%)

$Pd(NO_3)$ pure (1g)

- In a Teflon beaker, dissolve 1 g of $Pd(NO_3)$ 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 $Pd(NO_3)$ 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.

<u>SOLUTION (B)</u>: $Mg(NO_3)\ 6H_2O$ (1%)

$Mg(NO_3)\ 6H_2O$ Suprapure, Merck

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

<u>SOLUTION A+B:</u>

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

800 µl $Pd(NO_3)$ (0.2 %) + 200 µl $Mg(NO_3)\ 6H_2O$ (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:

- $NH_4H_2PO_4$ (Suprapure, Merck) 25 g/l
- $Mg(NO_3)_2$ (Suprapure, Merck) 10 g/l

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

In a plastic container make the folowing mixture every day:

2 ml $Pd(NO_3)$ + 1 ml $Mg(NO_3)$ $6H_2O$ + 400 µl $NH_4H_2PO_4$ + 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 hallow 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% HNO_3 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.

<u>Prepare a standard solution in 2% HNO_3 for optimization and sensitivity check. The concentration is given in the method book (Concentration for 0.2 abs).</u>

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

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

Where:

C = Concentration of element in original sample (µg g^{-1} dry weight);

C_d = Concentration of element in sample solution (µg ml^{-1});

C_b = Mean concentration of element in reagent blanks (µg 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 hallow 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:

- HNO_3 (65%, analytical grade, low in Hg, Merck).
- $K_2Cr_2O_7$ (analytical grade, low in Hg, Merck).
- $SnCl_2$ (analytical grade, Merck).
- HCl (30%, Suprapur, Merck)
- $HgCl_2$ (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:

<u>20% w/v $SnCl_2$ in 20 % v/v HCl (200 ml)</u>:

1. Weigh accurately 40 g of $SnCl_2$ into a clean glass beaker using a plastic spatula (beaker and spatula are used only for $SnCl_2$).
2. Add 40 ml of concentrated HCl directly to the $SnCl_2$ and transfer to a 200 ml volumetric flask. Mix and wait for complete dissolution of $SnCl_2$.

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.

$K_2Cr_2O_7$ 10% (w/v) in Milli-Q water:

1. Weigh 50 g of $K_2Cr_2O_7$ into a clean 500 ml glass volumetric flask.
2. Add about 250 ml of Milli-Q water and shake until $K_2Cr_2O_7$ 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 (+4º 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) $SnCl_2$ solution
   b) Milli-Q water
   c) Rinse solution (10% HNO_3)
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 $SnCl_2$ 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:

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

Where:

C = Concentration of total mercury in dry sample (µg g^{-1} dry);

Cd = Concentration of mercury in sample solution (µg ml^{-1});

Cb = Mean concentration of mercury in reagent blanks (µg 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:

- HNO_3 (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 HNO_3 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:

- HNO_3 (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 HNO_3 (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:

- HNO_3 (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 HNO_3 solution and heat at 60º 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:

- HNO_3 (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 HNO_3 solution.
4. Heat at 60ºC 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 HNO_3 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:

OSPAR (2008). JAMP Guidelines for Monitoring Contaminants in Sediments. Technical Annex 6: Determination of metals in sediments – analytical methods (4.1.2)

OSPAR COMMISSION

Protecting and conserving the North-East Atlantic and its resources

JAMP Guidelines for Monitoring Contaminants in Sediments

Technical Annex 6: Determination of metals in sediments – analytical methods

1. Introduction

This technical annex provides advice on the determination of metals in whole sediment and in sieved fractions. Determinations of trace metals can be achieved by acid digestion of the sediment followed by analysis of the digest solution by spectroscopic or spectrometric methods, or non-destructive techniques such as X-ray fluorescence analysis (XRF), instrumental neutron activation analysis (INAA) etc. The guidelines are intended to assist analytical chemists both in starting up metals analyses in sediments, and to those already performing such analyses. They do not provide full detail on specific laboratory procedures. Further guidance may be sought from specialised laboratories and publications (e.g. Loring and Rantala., 1991). Analyses should be carried out by experienced staff and the procedure validated.

Trace metals may occur in both fine and sand fractions of sediments. However, most natural and anthropogenic substances (metals and organic contaminants) show a much higher affinity to fine particulate matter than the coarse fraction. Iron and manganese oxy-hydroxide coatings, and constituents such as organic matter and clay minerals, contribute to the affinity for contaminants for this fine material.

Total methods, such as procedures involving total dissolution of sediment samples with hydrofluoric acid (HF) prior to analysis, or non-destructive methods without digestion such as neutron activation analysis (INAA) and X-ray fluorescence analysis, determine total trace metal contents in the whole sediment sample. In contrast, methods using a partial digestion with only strong acids, e.g. nitric acid or aqua regia, mainly measure trace metals in the fine fraction, and only extract small amounts of trace metals from the coarse fraction. For fine material, similar results have been obtained using both total and strong partial methods (Smedes et al, 2000; QUASH/QUASIMEME intercalibrations).

2. Sampling, pre-treatment and storage

Sampling sediments for metals analysis should preferably be done using cleaned plastic equipment, but this may not always be possible (e.g. at sea). Where metal sampling gear such as grabs must be used, care must be taken to avoid contamination of the sample, for instance by sub-sampling only sediment that has had no contact with the walls of the sampling device (maintain at least 1cm distance from sides).

Sediments can be stored in closed plastic or glass containers. Samples must be sieved at 2 mm as soon as possible after sampling to remove large debris as well as large detritus and benthic organisms. Otherwise during further sample handling like storage, freezing or ultrasonic treatment, biotic material will deteriorate and become part of the sediment sample. Samples may then be further wet sieved to a smaller size fraction. Further detail upon sieving procedures is available in the Technical Annex on Normalisation of Contaminant Concentrations in Sediments (OSPAR other agreement

number 1999-1). The sample can be stored at 4°C for several weeks and for extended periods when frozen at –20°C, although direct wet sieving is preferred. For prolonged storage freeze-drying of samples can be considered. In this case contamination and losses of contaminants during freeze-drying have to be checked. Air-drying is not appropriate due to high contamination risks. Besides, samples may be difficult to disaggregate and mineral structures may be affected.

Once sieved and dried, samples should be homogenised and ground to a fine powder in a non-contaminating mill (e.g. made of agate or silicon nitride), and stored in plastic or glass containers prior to analysis.

3. Blanks and contamination

Any contact between the samples and metals should be avoided. If metallic implements are required during sampling (e.g. grab jaws), they should be of stainless steel and contact between the sub-sample and metal should be minimised.

Plastic and glassware should be cleaned using a laboratory washing machine incorporating an acid wash, or by an equivalent cleaning procedure. Some plastic ware may not need to be cleaned before first use for metals work, but this feature must be thoroughly examined (e.g. using acid leaching tests) before proceeding with any real samples.

Blanks should be taken through the whole procedure. In practice, this will generally represent the time from acid addition to a sample container through to the final measurement. There should be at least one analytical blank in a batch of 10-20 samples, representing 5-10% of the sample number.

4. Digestion

Hydrofluoric acid digestion

HF digestions should be performed in polytetrafluorethylene (PTFE or PFA) vessels or equal quality, since the vessel must be metal-free and resist attack by the acid itself. Dried samples (normally 0,1-1g) should be accurately weighed into the vessel. Under fume extraction, the acid(s) are added. Some workers add HF first and leave the mixture to stand overnight, others add HF and nitric acid; others use a perchloric acid mixture etc. In general, the mixtures are left to stand for a period (1 hour – overnight) to avoid problems with violent reactions, which may be prompted by the presence of organic matter in the sediment. Note that perchloric acid and organic matter can promote an explosive reaction, so this acid must be treated with great caution if applied to sediments. Specially designed fume hoods should be used for HF and perchloric acid treatments.

HF is corrosive and toxic. It is therefore necessary to either remove the acid or render it less harmful to the measurement instruments. The acid may either be boiled off, which requires specialised facilities to extract the toxic fumes, or neutralised with boric acid (H_3BO_3), which is itself toxic.

Samples may be digested in a programmable heating block, with HF removal by evaporation. Alternatively, microwave digestions provide a rapid way to digest sediments. Some systems may allow the evaporation of HF, but in general microwaves use closed systems which allow pressure and temperature effects to rapidly dissolve the sediment. The most common methods use polytetrafluorethylene (PTFE or PFA) lined and sealed digestion vessels (Nakashima *et al*l 1988; Loring and Rantala, 1990). Since these closed systems retain the HF, boric acid is added after the HF digestion to complex remaining HF and make the resulting solution less hazardous, as well as preventing aluminium fluoride precipitation. The solution should be made up to volume with ultra pure water and left to stand for at least 24 hours prior to analysis to precipitate excess boric acid. Others use adjusted amounts of boric acid and head the digest to accelerate the process (Maham *et al* 1987). Typical methods are described, for example, in Cook *et al* (1997), Jones and Laslett (1994), Wu *et al* (1996).

If HF is to be removed by evaporation, care should be taken to ensure that mercury is not lost from sample solutions (Delft and Vos, 1988).
Strong acid digestion

Partial digestions follow broadly similar procedures to HF digestions, as above, for example using 10ml HNO3 and 10ml deionised water to 500mg sample. Microwave digestion is the preferred technique but alternative methods applying high pressure and temperature can be used. The method used needs to be checked. Adequate performance is achieved when digestion dissolves all the Al and Li from the clay fraction. It can easily be tested whether a method meets this requirement through parallel analyses of very fine grained samples by the partial method in use and a total method e.g. HF, INAA. If results for Al and Li do not differ significantly, the partial method used is sufficiently strong. It is important that the test samples do not contain any coarse material. To guarantee that the sample is sufficiently fine grained it is appropriate to sieve the sample over 20µm, or at least 63µm before analyses. When the partial method used results in lower contents than the total method, the conditions for the partial digestion such as time, temperature, acid concentration, etc., need to be adjusted. Usually boiling with aqua-regia is insufficient to a complete dissolution of Al. Historically, aqua regia has been used for strong acid digestions, but hydrochloric acid produces interferences for multi-element analysis by ICP so concentrated nitric acid alone may be used as a substitute (Christensen et al, 1982; Krumgalz and Fainshtein, 1989; Koopmann et al., 1991).

5. Analysis and detection

Analysis of metals in solution resulting from digestion may be performed by a variety of means, but usually involve spectrometric or spectroscopic detection. Flame or graphite furnace atomic absorption spectroscopy used to be the major method used for analysis of metals (Welz, 1985). Alternatively, non-destructive methods, i.e. XRF (e.g. Jenkins, 1999; Potts, 1992;, Williams, 1987; Bertin, 1984) and INAA (Alfassi 1990, 1994, 1998). De Soete *et al*. 1972; Kruger, 1971), which do not require a preceding digestion step, can be used. Recently, multi-element techniques like inductively coupled plasma attached to either an emission spectrometer (ICP-AES) or mass spectrometer (ICP-MS) now allow much more rapid analysis of a wide range of metals.

Interferences in analysis may arise through the presence of other components in the sample. Use of 3-point standard additions may highlight where these occur and can be used to correct for suppression or enhancement effects. Interferences occurring with multi-element analytical techniques can be complex and require skilled personnel to identify and minimise such effects (Cook *et al*, 1997).

Mercury can be detected by fluorescence spectrometry or cold vapour atomic absorption spectrometry, using tin (II) chloride in an acid solution to reduce Hg^{II} to the metallic Hg^{0} state. The gas is transported in a stream of argon to the detector. Alternative methods include pyrolysis combined with a gold trap and fluorescence detection. ICP-MS is also sufficiently sensitive to measure Hg directly in the digests

It should be ensured that the limits of detection of the analytical technique selected meets the requirements of the respective monitoring programme. Typical detection limits using different methods are given in Table 1.

Table 1: Typical limits of detection for the determination of trace metals with different techniques (in mg/kg d.w.) based on typical sample intakes (0,5 – 1 g)

	Al	Li	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
AAS / flame	5	0,2		0,5	5	2		5	5	10
AAS / graphite furnace, hydride technique, cold vapour	<1	<1-	0,2	0,02	<1	<1	0,05	<1	<1	-
ICP – AES with hydride generation	10	10	10 1	0,5	1	1	-	2	5	1
ICP – MS	40	0,1	1	0,01	0,2	0,1	0,05	0,2	0,2	2
X-ray fluorescence analysis (XRF)	1000	-	-	-	10	10	-	10	10	20
Neutron activation analysis (INAA)	-	-	0,3	1	0,8	-	0,1	-	-	2
Fluorescence spectrometry	-	-	-	-	-	-	0,1	-	-	-

6. Limits of detection

The limit of detection for each metal is normally determined by analysing a blank solution (containing acid to the dilution it is present in the sample) at least ten times. The limit of detection is calculated from 3 times the standard deviation of the blank taken through the whole procedure.

7. Calibration and standards

Calibrations are usually performed using multi-element stock solutions, using at least a 4-point calibration covering the range of concentrations expected. Multi-element solutions are commercially available, and may be used provided that they are of a similar matrix to the analyte. A crosscheck solution from a separate batch, or from a different supplier or an internal reference standard, should be used to check the calibration. Differences should not exceed 5%.

For non-destructive methods, appropriate certified reference sediments are required for calibration purposes.

8. Quality assurance

Every determinand should have its own Quality Control and Quality Assessment (QC – QA) scheme that includes regular blanks and calibration checks, the use of internal reference materials and certified reference materials and quality control charts. A system suitability check should be included in each batch to confirm that measuring instrument is operating correctly. In each batch of samples at least one standard addition (from the start of the digestion) should be included to demonstrate that matrix effects do not occur, and also a duplicate sample analysed in a different batch.

At least one laboratory reference material should be included in each batch of samples in order to check the long-term performance. A quality control Shewhart chart should be constructed for selected trace metals. If the warning limits are exceeded, the method should be checked for possible errors. When alarm limits are exceeded, the results should not be reported.

Certified reference materials (CRMs) for sediments are commercially available for both total methods and partial digestion methods. The data provided by such materials provide an independent check on the analytical performance.

Participation in an international proficiency-testing scheme e.g. QUASIMEME is highly recommended to improve comparability between laboratories. Relevant quality assurance data should be reported e.g. to ICES, together with concentration data.

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Annex III:

US EPA (2014 revision). Method 6020B. ICP-MS method for the determination of elements in water samples and in waste extracts or digests (4.1.3)

METHOD 6020B

INDUCTIVELY COUPLED PLASMA—MASS SPECTROMETRY

SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts who are formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required method use for the analysis of method-defined parameters, are intended to be guidance methods which contain general information on how to perform an analytical procedure or technique which a laboratory can use as a basic starting point for generating its own detailed standard operating procedure (SOP), either for its own general use or for a specific project application. The performance data included in this method are for guidance purposes only, and are not intended to be and must not be used as absolute quality control (QC) acceptance criteria for purposes of laboratory accreditation.

1.0 SCOPE AND APPLICATION

1.1 Inductively coupled plasma-mass spectrometry (ICP-MS) is applicable to the determination of sub-µg/L concentrations of a large number of elements in water samples and in waste extracts or digests (Refs. 1 and 2). When dissolved constituents are required, samples must be filtered and acid-preserved prior to analysis. No digestion is required prior to analysis for dissolved elements in water samples. Acid digestion prior to filtration and analysis is required for groundwater, aqueous samples, industrial wastes, soils, sludges, sediments, and other solid wastes for which total (acid-leachable) elements are required. The analyst should insure that a sample digestion method is chosen that is appropriate for each analyte and the intended use of the data. Refer to Chapter Three for the appropriate digestion procedures.

1.2 ICP-MS has been applied to the determination of over 60 elements in various matrices. Analytes for which the acceptability of Method 6020 has been demonstrated through multi-laboratory testing on solid and aqueous wastes are listed below.

Element	Symbol	CASRN[a]	Element	Symbol	CASRN[a]
Aluminum	Al	7429-90-5	Magnesium	Mg	7439-95-4
Antimony	Sb	7440-36-0	Manganese	Mn	7439-96-5
Arsenic	As	7440-38-2	Mercury	Hg	7439-97-6
Barium	Ba	7440-39-3	Nickel	Ni	7440-02-0
Beryllium	Be	7440-41-7	Potassium	K	7440-09-7

Element	Symbol	CASRN[a]	Element	Symbol	CASRN[a]
Cadmium	Cd	7440-43-9	Selenium	Se	7782-49-2
Calcium	Ca	7440-70-2	Silver	Ag	7440-22-4
Chromium	Cr	7440-47-3	Sodium	Na	7440-23-5
Cobalt	Co	7440-48-4	Thallium	Tl	7440-28-0
Copper	Cu	7440-50-8	Vanadium	V	7440-62-2
Iron	Fe	7439-89-6	Zinc	Zn	7440-66-6
Lead	Pb	7439-92-1			

[a]Chemical Abstract Service Registry Number

The performance acceptability of ICP-MS for the determination of the listed elements was based upon comparison of the multi-laboratory testing results with those obtained from either furnace atomic absorption spectrophotometry or inductively coupled plasma—optical emission spectrometry. It should be noted that one multi-laboratory study was conducted in 1988. As advances in ICP-MS instrumentation and software have been made since that time, other elements have been added through validation and with additional improvements in performance of the method. Performance, in general, presently exceeds the original multi-laboratory performance data for the listed elements (and others) that are provided in Sec. 13.0. Instrument detection limits (IDLs), lower limits of quantitation (LLOQs) and linear ranges will vary with the matrices, instrumentation, and operating conditions. In relatively simple matrices, IDLs will generally be < 0.1 µg/L. For less sensitive elements (e.g., Se and As) and desensitized major elements, IDLs may be ≥ 1.0 µg/L.

1.3 If Method 6020 is used to determine any analyte not listed in Sec. 1.2, it is the responsibility of the analyst to demonstrate the precision and bias of the method for the waste to be analyzed. The analyst must always monitor potential sources of interferences and take appropriate action to ensure data of known quality (see Sec. 9.0). Other elements and matrices may be analyzed by this method if performance is demonstrated for the analyte of interest, in the matrices of interest, at the concentration levels of interest in the same manner as the listed elements and matrices (see Sec. 9.0).

1.4 Use of this method should be restricted to spectroscopists who are knowledgeable in the recognition and correction of spectral, chemical, and physical interferences in ICP-MS analysis.

1.5 An appropriate internal standard is necessary for each analyte determined by ICP-MS. Recommended internal standards are ^{6}Li, ^{45}Sc, ^{89}Y, ^{103}Rh, ^{115}In, ^{159}Tb, ^{165}Ho, and ^{209}Bi. The lithium internal standard should have an enriched abundance of ^{6}Li, so that

interference from lithium native to the sample is minimized. Other elements may need to be used as internal standards when samples contain significant native amounts of the recommended internal standards as indicated by high bias of internal standard recoveries.

Note: Other potential causes of a high bias should also be considered before a final decision is made that the internal standard high bias is caused by an excessive concentration of the internal standard isotope in the sample.

1.6 Prior to employing this method, analysts are advised to consult the preparatory method for each type of procedure that may be employed in the overall analysis (e.g., Methods 3005, 3010, 3015, 3031, 3040, 3050, 3051, 3052, 7000, and 6800) for additional information on QC procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the manual and the information in Chapter Two for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies, and on the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly specified in a regulation, the use of SW-846 methods is *not* mandatory in response to Federal testing requirements. The information contained in this method is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives (DQOs) for the intended application.

1.7 This method is restricted to use by, or under supervision of, properly experienced and trained personnel. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 Prior to analysis, aqueous and solid samples are solubilized or digested using the appropriate sample preparation methods (see Chapter Three). When analyzing groundwater samples for dissolved constituents, acid digestion is not necessary, if the samples are filtered and acid-preserved prior to analysis (e.g., Methods 3005, 3010, 3015, 3031, 3050, 3051 and 3052). For oils, greases, or waxes, use the solvent dissolution procedure in method 3040 to prepare the samples.

2.2 This method describes multi-element determinations using ICP-MS in environmental samples. The method measures ions produced by a radio-frequency inductively coupled plasma. Analyte species in liquid are nebulized and the resulting aerosol is transported by argon gas into the plasma torch. The ions produced by high temperatures are entrained in the plasma gas and introduced, by means of an interface, into a mass spectrometer. The ions produced in the plasma are sorted according to their mass-to-charge (*m/z*) ratios and quantified with a channel electron multiplier. Interferences must be assessed and valid corrections applied or the data flagged to indicate problems. Interference correction must include compensation for background ions contributed by the plasma gas, reagents, and constituents of the sample matrix.

3.0 DEFINITIONS

Refer to Chapter One, Chapter Three, and the manufacturer's instructions for definitions that may be relevant to this procedure.

4.0 INTERFERENCES

4.1 Isobaric elemental interferences in ICP-MS are caused by isotopes of different elements forming atomic ions with the same nominal *m/z* ratio. A data system must be used to correct for these interferences. This involves determining the signal for another isotope of the interfering element and subtracting the appropriate signal from the analyte isotope signal.

4.2 Isobaric molecular and doubly charged ion interferences in ICP-MS are caused by ions consisting of more than one atom or charge, respectively. Most isobaric interferences that could affect ICP-MS determinations have been identified in the literature (Refs. 3 and 4). Examples include $^{75}ArCl^{+}$ ion on the ^{75}As signal and MoO^{+} ions on the cadmium isotopes. While the approach used to correct for molecular isobaric interferences is demonstrated below using the natural isotope abundances from the literature (Ref. 5), the most precise coefficients for an instrument can be determined from the ratio of the net isotope signals *observed* for a standard solution of the interfering element at a concentration which produces sufficient interference at the isotopes of interest that a reliable measurement can be made. Because the ^{35}Cl natural abundance of 75.77% is 3.13 times the ^{37}Cl abundance of 24.23%, the chloride correction for arsenic can be calculated (approximately) as follows (where the $^{38}Ar^{37}Cl^{+}$ contribution at *m/z* 75 is a negligible 0.06% of the $^{40}Ar^{35}Cl^{+}$ signal):

Corrected arsenic signal (using the abundances of natural isotopes for coefficient approximations) =

(*m/z* 75 signal) - (3.13) [(*m/z* 77 signal) - (0.87) (*m/z* 82 signal)]

where, the final term adjusts for any selenium contribution at 77 *m/z*,

<u>NOTE</u>: Arsenic values can be biased high by this type of equation when the net signal at *m/z* 82 is caused by ions other than $^{82}Se^{+}$, (e.g., $^{81}BrH^{+}$ from bromine wastes [Ref. 6]).

<u>NOTE</u>: The coefficients should be verified experimentally using the procedures or coefficients provided by the instrument manufacturer.

Similarly,

Corrected cadmium signal (using the abundances of natural isotopes for coefficient approximations) =

(*m/z* 114 signal) - (0.027)(*m/z* 118 signal) - (1.63)(*m/z* 108 signal)

where, the last 2 terms adjust for any $^{114}Sn^{+}$ or $^{114}MoO^{+}$ contributions at *m/z* 114.

NOTE: Cadmium values will be biased low by this type of equation when $^{92}ZrO^{+}$ ions contribute at *m/z* 108, but use of *m/z* 111 for Cd is even subject to direct ($^{94}ZrOH^{+}$) and indirect ($^{90}ZrO^{+}$) additive interferences when Zr is present.

NOTE: With respect to the arsenic equation above, the coefficients could be improved. For example, the coefficient to modify "3.13" (in the equation above) for a particular instrument can be determined from the observed ratio of the m/z 75 to the m/z 77 net isotope signals for a solution of hydrochloric acid. The concentration of HCl used should provide enough signal at the measured isotopes to ensure that a reliable measurement can be made, while not exceeding the linear range of the detector.

The accuracy of these types of equations is based upon the constancy of the *observed* isotopic ratios for the interfering species. Corrections that presume a constant fraction of a molecular ion relative to the "parent" ion have not been found (Ref. 7) to be reliable, e.g., oxide levels can vary with operating conditions. If a correction for an oxide ion is based upon the ratio of parent-to-oxide ion intensities, the correction must be adjusted for the degree of oxide formation by the use of an appropriate oxide internal standard previously demonstrated to form a similar level of oxide as the interferent. For example, this type of correction has been reported (Ref. 7) for oxide-ion corrections using ThO^{+}/Th^{+} for the determination of rare earth elements. The use of aerosol desolvation and/or mixed gas plasmas have been shown to greatly reduce molecular interferences (Ref. 8). These techniques can be used, provided that IDL, bias, and precision specifications for analysis of the samples can be met.

4.3 As technology continues to develop, modifications to existing ICP-MS instrumentation can reduce or completely remove common interferences thus eliminating the need for reliance on correction equations. Instruments must be able to demonstrate successful freedom from interferences. Examples of such modifications are discussed in more detail below:

4.3.1 Recent ICP-MS instruments may include collision or reaction cells for removal of molecular isobaric interferences. This type of interference removal is effective, and highly recommended for complex and/or varying matrices. The systems work either by collision of molecular species with an inert gas (usually helium) or by reaction of molecular species or the target analyte with reactive gases (e.g., ammonia or methane). Manufacturer recommendations should be followed for the configuration of the collision/reaction cell. This technique may eliminate the need for most correction equations, but freedom from interference still needs to be demonstrated using the spectral interference check (SIC) solutions described in sections 7.23 and 9.9.

4.3.2 High resolution ICP-MS instruments are available based on several mass analyzer designs with much higher mass resolution within the mass range of traditional ICP-MS instruments. These mass analyzers are not based on quadrupole mass analyzers and have orders of magnitude resolution above quadrupoles, which helps reduce or eliminate interference from polyatomic ions with the same nominal mass. These mass analyzers reduce or eliminate the need for most correction equations, but the instrument needs to be operated at sufficient resolution to remove the expected

interference. For example, resolving ^{52}Cr from $^{40}Ar^{12}C$ requires a resolution of around 4000, while resolving ^{75}As from $^{40}Ar^{35}Cl$ requires a resolution of around 8000. Freedom from interferences needs to be demonstrated for the particular higher resolution mass analyzers ICP-MS.

4.4 Additionally, solid-phase chelation may be used to eliminate isobaric interferences from both element and molecular sources. An on-line method has been demonstrated for environmental waters such as sea water, drinking water and acid decomposed samples. Acid decomposed samples refer to samples decomposed by methods similar to methods 3052, 3051, 3050 or 3015. Samples with% levels of iron and aluminum should be avoided. The method also provides a method for preconcentration to enhance detection limits simultaneously with elimination of isobaric interferences. The method relies on chelating resins such as imminodiacetate or other appropriate resins and selectively concentrates the elements of interest while eliminating interfering elements from the sample matrix. By eliminating the elements that are direct isobaric interferences or those that form isobaric interfering molecular masses, the mass region is simplified and these interferences cannot occur. The method has been proven effective for the certification of reference materials and validated using reference materials (Refs. 13-15). The method has the potential to be used on-line or off-line as an effective sample preparation method specifically designed to address interference problems.

4.5 Since commercial quadrupole ICP-MS instruments nominally provide unit resolution at 10% of the peak height, very high ion currents at adjacent masses can also contribute to ion signals at the mass of interest. Although this type of interference is uncommon, it is not easily corrected, and samples exhibiting a significant problem of this type could need resolution improvement, matrix separation, or analysis using another verified and documented isotope, or otherwise the use of another method.

4.6 Physical interferences are associated with the sample nebulization and transport processes as well as with ion-transmission efficiencies. Nebulization and transport processes can be affected if a matrix component causes a change in surface tension or viscosity. Changes in matrix composition can cause significant signal suppression or enhancement (Ref. 9). Dissolved solids can deposit on the nebulizer tip of a pneumatic nebulizer and on the interface skimmers (reducing the orifice size and the instrument performance). Dissolved solid levels below 0.2% (2,000 mg/L) have been currently recommended (Ref. 10) to minimize solid deposition, although currently-available ICP-MS systems may be able to tolerate much higher levels. An internal standard can be used to correct for physical interferences, if it is carefully matched to the analyte so that the two elements are similarly affected by matrix changes (Ref. 11). When intolerable physical interferences are present in a sample, a significant suppression of the internal standard signals (to less than 30% of the signals in the calibrations standard) will be observed. Dilution of the sample five-fold (i.e., dilute one part sample with four parts diluent [1:5 = 1+4]) will usually eliminate the problem.

4.7 Memory interferences or carry-over can occur when there are large concentration differences between samples or standards which are analyzed sequentially. Sample deposition on the sampler and skimmer cones, spray chamber design, and the type of nebulizer affect the extent of the memory interferences which are observed. The rinse period between samples must be long enough to eliminate significant memory interference.

4.8 Reagents and sample processing hardware may yield artifacts and/or interferences to sample analysis. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents may be necessary. Refer to each method to be used for specific guidance on QC procedures.

5.0 SAFETY

5.1 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

5.2 Concentrated nitric and hydrochloric acids are moderately toxic and extremely irritating to skin and mucus membranes. Use these reagents in a hood and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield for eye protection when working with these reagents.

5.3 **Hydrofluoric acid is a very toxic acid and penetrates the skin and tissues deeply if not treated immediately.** Injury occurs in two stages: firstly, by hydration that induces tissue necrosis; and secondly, by penetration of fluoride ions deep into the tissue and thereby reacting with calcium. Boric acid and/or other complexing reagents and appropriate treatment agents should be administered immediately.

WARNING: Consult appropriate safety literature for determining the proper protective eyewear, clothing and gloves to use when handling hydrofluoric acid. **Always have appropriate treatment materials readily available prior to working with this acid.** See Method 3052 for additional recommendations for handling hydrofluoric acid from a safety and an instrument standpoint.

5.4 Many metal salts, are extremely toxic if inhaled or swallowed.

WARNING: Exercise extreme care to ensure that samples and standards are handled safely and properly and that all exhaust gases are properly vented. Wash hands thoroughly after handling.

6.0 EQUIPMENT AND SUPPLIES

6.1 Inductively coupled plasma-mass spectrometer:

6.1.1 The system must be capable of providing resolution, better than or equal to 1.0 u (unified atomic mass unit) at 10% peak height. The system must have a mass range from at least 6 to 240 u and a data system that allows corrections for isobaric interferences and the application of the internal standard technique. Use of a mass-flow controller for the nebulizer argon and a peristaltic pump for the sample solution are recommended.

6.1.2 Argon gas, high-purity grade (99.99%).

6.2 Volumetric flasks of suitable material composition, precision and accuracy

6.3 Volumetric pipets of suitable material composition, precision and accuracy

This section does not list all common laboratory ware (e.g., beakers) that might be used.

7.0 REAGENTS AND STANDARDS

7.1 Reagent-grade, and whenever necessary, ultra-high purity-grade chemicals, must be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 Reagent water - Reagent water must be interference free. All references to water in this method refer to reagent water unless otherwise specified.

7.3 Ultra high-purity or equivalent acids must be used in the preparation of standards and for sample processing. Redistilled acids are recommended because of the high sensitivity of ICP-MS. Nitric acid at less than 2% (v/v) is necessary for ICP-MS to minimize damage to the interface and to minimize isobaric molecular-ion interferences with the analytes. Many more molecular-ion interferences are observed when hydrochloric and sulfuric acids are used (Refs. 3 and 4). The use of 1% (v/v) HCl is necessary for the stability of antimony and silver concentrations in the range of 50 - 500 µg/L. For concentrations greater than 500 µg/L silver, additional HCl will be needed. As a consequence, the accuracy of analytes that need significant chloride molecular-ion corrections (e.g., As and V) will degrade.

7.3.1 Nitric acid (concentrated), HNO_3

7.3.2 Nitric acid (50% [v/v]), HNO_3 - Prepare by adding 500 mL concentrated HNO_3 to 400 mL water and diluting to 1 L.

7.3.3 Nitric acid (1% [v/v]), HNO_3 - Prepare by adding 10 mL concentrated HNO_3 to 400 mL water and diluting to 1 L.

7.3.4 Hydrochloric acid (concentrated), HCl

7.3.5 Hydrochloric acid (37%), HCl - Prepare by adding 370 mL concentrated HCl to 400 mL water and diluting to 1L.

7.3.6 Hydrofluoric acid (concentrated), HF

7.3.7 Phosphoric acid (concentrated), H_3PO_4

7.3.8 Phosphoric acid (85% [v/v]), H_3PO_4 - Prepare by adding 850 mL concentrated H_3PO_4 to 100 mL water and diluting to 1 L.

7.3.9 Sulfuric acid (concentrated), H_2SO_4

7.3.10 Sulfuric acid (96% [v/v]) H_2SO_4, - Prepare by adding 40 mL water to a 2 L glass beaker. While gently stirring, carefully add 960 mL concentrated H_2SO_4 to the beaker. Mix until combined. Allow to cool. Carefully, quantitatively transfer solution to a 1-L volumetric flask. Bring to volume with additional water if necessary. Mix thoroughly through inversion to combine.

WARNING: Considerable heat is generated upon combining sulfuric acid and water. The use of appropriate personal protection (e.g. proper gloves, safety glasses and protective clothing) is necessary to avoid personal injury such as thermal burns or acid burns due to solution splatter. Also, always add acid to water (rather than water to acid) to reduce splatter.

7.3.11 Citric acid, $HO_2CCH_2C(OH)(CO_2H)CH_2CO_2H$

7.4 Bismuth(III) oxide, Bi_2O_3

7.5 Holmium(III) carbonate pentahydrate, $Ho_2(CO_3)_3 \cdot 5H_2O$

7.6 Indium (powder), In

7.7 Lithium[^{6}Li] carbonate (95 atom % ^{6}Li), $^6Li_2CO_3$

7.8 Ammonium hexachlororhodate(III), $(NH_4)_3RhCl_6$

7.9 Scandium(III) oxide, Sc_2O_3

7.10 Terbium(III) carbonate pentahydrate, $Tb_2(CO_3)_3 \cdot 5H_2O$

7.11 Yttrium(III) carbonate, $Y_2(CO_3)_3 \cdot 3H_2O$

7.12 Ammonium hexafluorotitanate(IV), $(NH_4)_2TiF_6$

7.13 Ammonium molybdate(VI) $(NH_4)_2MoO_4$

7.14 Aluminum(III) nitrate nonahydrate, $Al(NO_3)_3 \cdot 9H_2O$

7.15 Calcium carbonate, $CaCO_3$

7.16 Iron powder, Fe

7.17 Magnesium oxide, MgO

7.18 Sodium carbonate, Na_2CO_3

7.19 Potassium carbonate, K_2CO_3

7.20 Standard stock solutions - Purchase standard stock solutions from an appropriate commercial source. Otherwise, prepare them manually in the laboratory using only ultra, high-purity grade chemicals or metals (≥ 99.99% purity). See Method 6010 for instructions on preparing standard solutions from solids. Replace stock standards when succeeding dilutions for the preparation of calibration standards cannot be verified.

7.20.1 Bismuth internal standard stock solution (100 µg/mL Bi) - Dissolve 0.1115 g Bi_2O_3 in a minimum amount of dilute HNO_3. Add 10 mL concentrated HNO_3 and dilute to 1 L with reagent water.

7.20.2 Holmium internal standard stock solution (100 µg/mL Ho) - Dissolve 0.1757 g $Ho_2(CO_3)_3 \cdot 5H_2O$ in 10 mL reagent water and 10 mL concentrated HNO_3. After dissolution is complete, warm the solution to degas. Add 10 mL concentrated HNO_3 and dilute to 1 L with reagent water.

7.20.3 Indium internal standard stock solution (100 µg/mL In) - Dissolve 0.1000 g indium in 10 mL concentrated HNO_3. Dilute to 1 L with reagent water.

7.20.4 Lithium internal standard stock solution (100 µg/mL ^6Li) - Dissolve 0.6312 g $^6Li_2CO_3$ (95% atomic abundance) in 10 mL of reagent water and 10 mL concentrated HNO_3. After dissolution is complete, warm the solution to degas. Add 10 mL concentrated HNO_3 and dilute to 1 L with reagent water.

7.20.5 Rhodium internal standard stock solution (100 µg/mL Rh) - Dissolve 0.3593 g $(NH_4)_3RhCl_6$ in 10 mL reagent water. Add 100 mL concentrated HCl and dilute to 1 L with reagent water.

7.20.6 Scandium internal standard stock solution (100 µg/mL Sc) - Dissolve 0.15343 g Sc_2O_3 in 10 mL 50% hot HNO_3. Add 5 mL concentrated HNO_3 and dilute to 1 L with reagent water.

7.20.7 Terbium internal standard stock solution (100 µg/mL Tb) - Dissolve 0.1828 g $Tb_2(CO_3)_3 \cdot 5H_2O$ in 10 mL 50% HNO_3. After dissolution is complete, warm the solution to degas. Add 5 mL concentrated HNO_3 and dilute to 1 L with reagent water.

7.20.8 Yttrium internal standard stock solution (100 µg/mL Y) - Dissolve 0.2316 g $Y_2(CO_3)_3 \cdot 3H_2O$ in 10 mL 50% HNO_3. Add 5 mL concentrated HNO_3 and dilute to 1 L with reagent water.

7.20.9 Titanium interference stock solution (100 µg/mL Ti) - Dissolve 0.4133 g $(NH_4)_2TiF_6$ in reagent water. Add 2 drops concentrated HF and dilute to 1 L with reagent water.

7.20.10 Molybdenum interference stock solution (100 µg/mL Mo) - Dissolve 0.2043 g $(NH_4)_2MoO_4$ in reagent water. Dilute to 1 L with reagent water.

7.20.11 Gold preservative stock solution for mercury (100 µg/mL Au) - Purchase as a commercially prepared, high-purity solution of $AuCl_3$ in dilute HCl matrix.

7.21 Mixed-calibration standard solutions - Prepare by diluting stock standard solutions to levels in the linear range for the instrument, using the same combination and concentrations of acids used in the preparation of the sample digestates (approximately 1% HNO_3). The calibration standard solutions must contain a suitable concentration of an appropriate internal standard for each analyte. Internal standards may be added on-line at the time of analysis using a second channel of the peristaltic pump and an appropriate mixing manifold. Generally, an internal standard should be no more than 50 u removed from the analyte. Recommended internal standards include ^{6}Li, ^{45}Sc, ^{89}Y, ^{103}Rh, ^{115}In, ^{159}Tb, ^{169}Ho, and ^{209}Bi. Prior to preparing the mixed standards, each stock standard solution must be analyzed separately to determine possible spectral interferences or the presence of impurities.

NOTE: Care should be taken when preparing the calibration standards to ensure that the elements are compatible and stable when mixed together. Standards which interfere with another analyte, or which are contaminated with another analyte, may not be included in the same calibration standard as that analyte.

Transfer the mixed-standard solutions to an appropriate container for storage. Freshly mixed standards must be prepared as needed with the realization that concentrations can change upon aging. Calibration standards must be initially verified using a QC standard (see Sec. 7.24).

7.22 Blanks - Three types of blanks are necessary for analysis: (1) the calibration blank, which is used in establishing the calibration curve; (2) the method blank, which is used to monitor for possible contamination resulting from the sample preparation procedure; and (3) the rinse blank, which is used to flush the system between all samples and standards.

7.22.1 Calibration blank - Prepare by acidifying reagent water using the same combination and concentrations of acids used in the preparation of the matrix-matched calibration standards (Sec. 7.21) along with the selected concentrations of internal standards, such that there is an appropriate internal standard element for each of the target analytes. The use of HCl for antimony and silver is discussed in Sec. 7.3. The calibration blank will also be used for all initial calibration blank (ICB) and continuing calibration blank (CCB) determinations.

7.22.2 Method blank — Prepare by a processing either a volume of reagent water equal to that used for actual aqueous samples, or, otherwise, a clean, empty container, equivalent to that used for actual solid samples through all of the preparatory and instrument determination steps used for making ICP-MS determinations in samples. These steps may include, but are not limited to, pre-filtering, digestion, dilution, filtering, and analysis (refer to Sec. 9.5).

7.22.3 Rinse blank - Prepare as a 1 - 2% HNO_3 solution. Prepare a sufficient quantity such that it may be used to flush the system in between standards and samples. If mercury is to be analyzed, the rinse blank should also contain 2 µg/mL $AuCl_3$.

7.23 Spectral interference check (SIC) solutions - Prepare so as to contain known concentrations of interfering elements that will demonstrate the appropriate magnitude of interferences and provide an adequate test of any corrections. Chloride in the SIC solution provides a means to evaluate software corrections for chloride-related interferences such as $^{35}Cl^{16}O^{+}$ on $^{51}V^{+}$ and $^{40}Ar^{35}Cl^{+}$ on $^{75}As^{+}$. Iron is used to demonstrate adequate resolution of the spectrometer for the determination of manganese. Molybdenum serves to indicate oxide effects on cadmium isotopes. The other components are present to evaluate the ability of the measurement system to correct for various molecular-ion isobaric interferences. The SIC is used to verify that the interference levels are corrected by the data system within appropriate QC limits.

NOTE: The final SIC solution concentrations in Table 1 are intended to evaluate corrections for known interferences on only the analytes identified in Sec. 1.0. If the test method is to be used to determine other element(s), it is the responsibility of the analyst to modify the SIC solution accordingly, or prepare an alternative SIC solution, so as to allow adequate verification of interference corrections on the additional element(s) (see Sec. 9.9).

7.23.1 Mixed stock SIC solutions - Prepare the SIC stock solutions using only ultra-pure reagents. They can be obtained commercially or prepared using the following procedures:

7.23.1.1 Mixed SIC stock solution I - Prepare by adding 13.903 g $Al(NO_3)_3 \cdot 9H_2O$, 2.498 g $CaCO_3$ (previously dried at 180 EC for 1 hr), 1.000 g Fe, 1.658 g MgO, 2.305 g Na_2CO_3 and 1.767 g K_2CO_3 to 25 mL of reagent water. Slowly add 40 mL of (50%) HNO_3. After dissolution is complete, warm the solution to degas. Cool and dilute to 1 L with reagent water.

7.23.1.2 Mixed SIC stock solution II - Prepare by slowly adding 7.444 g 85% H_3PO_4, 6.373 g 96% H_2SO_4, 40.024 g 37% HCl, and 10.664 g citric acid ($C_6O_7H_8$) to 100 mL of reagent water. Dilute to 1 L with reagent water.

7.23.2 Mixed working SIC solution - Prepare by combining 10.0 mL of SIC stock solution I, 2.0 mL each of 100-µg/mL titanium stock solution and 100-µg/mL molybdenum stock solution, and 5.0 mL of SIC stock solution II. Dilute to 100 mL with reagent water. Prepare fresh weekly.

7.24 Initial calibration verification (ICV) standard - Prepare by combining compatible metals from standard stock solution sources that differ from those used for the preparation of the calibration standards. The ICV should be prepared so as to contain metal concentrations that are near, but not equal to, the midpoint concentration level of the calibration curve.

7.25 Continuing calibration verification (CCV) standard - Prepare using the same acid matrix and stock standards employed when preparing the calibration standards. The CCV should be prepared so as to contain metal concentrations equal or nearly equivalent to the midpoint concentration of the calibration curve.

7.26 Mass spectrometer tuning solution - Prepare so as to contain elements that represent all of the mass regions of interest (i.e., 10 µg/L Li, Co, In, and Tl) in order to verify that

the resolution and mass calibration of the instrument are within the designated specifications (see Sec. 10.1).

7.27 If the determination of one or more metals using a non-aqueous solvent is required, then all standards and quality control samples must be prepared on a weight/weight basis in the non-aqueous solvent since the density of non-aqueous solvents is not uniform. Standards and quality control materials containing organometallic materials that are soluble in non-aqueous solvents are available from a variety of vendors.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Sample collection, preservation and storage requirements may vary by EPA program and may be specified in a regulation or project planning document that requires compliance monitoring for a given contaminant. Where such requirements are specified in the regulation, follow those requirements. In the absence of specific regulatory requirements, use the following information as guidance in determining the sample collection, preservation and storage requirements.

See Chapter Three, Inorganic Analytes, for sample collection and preservation instructions.

9.0 QUALITY CONTROL

9.1 Refer to Chapter One for guidance on quality assurance (QA) and QC protocols. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over those criteria given in Chapter One. Any effort involving the collection of analytical data should include development of a structured and systematic planning document, such as a Quality Assurance Project Plan (QAPP) or a Sampling and Analysis Plan (SAP), which translates project objectives and specifications into directions for those that will implement the project and assess the results. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated. All data sheets and QC data should be maintained for reference or inspection.

9.2 Refer to Methods 3005, 3010, 3015, 3031, 3040, 3050, 3051, 3052, 7000, and 6800 for QC procedures to ensure the proper operation of the various sample preparation techniques. Any more specific QC procedures provided in this method will supersede those noted in Methods 3005, 3010, 3015, 3031, 3040, 3050, 3051, 3052, 7000, and 6800.

9.3 Instrument Detection Limits

Instrument detection limits (IDLs) are useful means to evaluate the instrument noise level and response changes over time for each analyte from a series of reagent blank analyses to obtain a calculated concentration. They are not to be confused with the lower limit of quantitation, nor should they be used in establishing this limit. It may be helpful to compare the calculated IDLs to the established lower limit of quantitation, however, it should be understood that the lower limit of quantitation needs to be verified according to the guidance in Sec. 9.8. IDLs in µg/L can be estimated as the mean of the blank result plus three times the standard

deviation of 10 replicate analyses of the reagent blank solution. (Use zero for the mean if the mean is negative). Each measurement should be performed as though it were a separate analytical sample (i.e., each measurement must be followed by a rinse and/or any other procedure normally performed between the analysis of separate samples). IDLs should be determined at least once using new equipment, after major instrument maintenance such as changing the detector, and/or at a frequency designated by the project. An instrument log book should be kept with the dates and information pertaining to each IDL performed.

9.4 Initial demonstration of proficiency

Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination by generating data of acceptable precision and bias for target analytes in a clean matrix. If an autosampler is used to perform sample dilutions, before using the autosampler to dilute samples, the laboratory should satisfy itself that those dilutions are of equivalent or better accuracy than is achieved by an experienced analyst performing manual dilutions. It is recommended that the laboratory should repeat the demonstration of proficiency whenever new staff members are trained or significant changes in instrumentation are made.

9.5 Initially, before processing any samples, the analyst should demonstrate that all parts of the equipment that come into direct contact with the sample and reagents are interference-free. This is accomplished through the analysis of a method blank. As a continuing check, each time samples are digested and analyzed, and when there is a change in reagents, a method blank should be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination. If an interference is observed that would prevent the determination of the target analyte, determine the source and eliminate it, if possible, before processing the samples. The method blank should be carried through all stages of sample preparation and instrument determination procedures. When new reagents or chemicals are received, the laboratory should monitor the preparation and/or analysis blanks associated with samples for any signs of contamination. It is not necessary to test every new batch of reagents or chemicals prior to sample preparation if the source shows no prior problems. However, if reagents are changed during a preparation batch, separate blanks need to be prepared for each set of reagents.

9.6 Linear range

The linear range establishes the highest concentration that may be reported without diluting the sample. Following calibration, the laboratory may choose to analyze a standard at a higher concentration than the high standard in the calibration. The standard must recover within 10% of the true value, and if successful, establishes the linear range. The linear range standards must be analyzed in the same instrument run as the calibration they are associated with (i.e., on a daily basis) but may be analyzed anywhere within that run. If a linear range standard is not analyzed for any specific element, the highest standard in the calibration becomes the linear range.

9.7 Sample QC for preparation and analysis

The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, bias, and sensitivity). At a minimum, this should include the

analysis of QC samples including a method blank, a matrix spike (MS), a laboratory control sample (LCS), and a duplicate sample in each analytical batch. Any method blanks, LCS, MS samples, and duplicate samples should be subjected to the same preparatory and instrument determination procedures as those used on actual samples (see Sec. 11.0).

9.7.1 For each batch of samples analyzed, at least one method blank must be carried throughout the entire sample preparation and instrument determination process, as described in Chapter One. The importance of the method blank is to aid in identifying when and/or if sample contamination is occurring. The method blank is considered to be acceptable if it does not contain the target analytes at concentration levels that exceed the acceptance limits defined in Chapter One or in the project-specific DQOs. The laboratory should not subtract the results of the method blank from those of any associated samples. Such "blank subtraction" is not reliable because it is based on a single method blank value rather than a statistically determined blank concentration.

Blanks are generally considered to be acceptable if target analyte concentrations are less than ½ the LLOQ or are less than project-specific requirements. Blanks may contain analyte concentrations greater than acceptance limits if the associated samples in the batch are unaffected (i.e. targets are not present in samples or sample concentrations **are ≥10X** the blank). Other criteria may be used depending on the needs of the project.

If the method blank fails to meet the necessary acceptance criteria, it should be re-analyzed once. If still unacceptable, then all samples associated with the method blank must be re-prepared and re-analyzed, along with all other appropriate analysis batch QC samples. If the method blank results do not meet the acceptance criteria and reanalysis is not practical, then the laboratory should report the sample results along with the method blank results, and provide a discussion of the potential impact of the contamination on the sample results. However, if an analyte of interest is found in a sample in the batch near its concentration confirmed in the blank, the presence and/or concentration of that analyte should be considered suspect and may require qualification. Refer to Chapter One for additional guidance regarding the proper protocol when analyzing method blanks.

9.7.2 Documenting the effect of the matrix should include the analysis of at least one MS and one duplicate unspiked sample or one matrix spike/matrix spike duplicate (MS/MSD) pair for each batch of samples processed, at a minimum frequency of one per every 20 samples, as described in Chapter One. An MS/MSD pair is used to document the bias and precision of a method in a given sample matrix. The decision on whether to prepare and analyze duplicate samples or an MS/MSD pair must be based on knowledge of the samples in the analysis batch. If samples are expected to contain target analytes above the LLOQ, laboratories may choose to use an MS and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes above the LLOQ, the laboratories should use an MS/MSD pair.

MS/MSD samples should be spiked with each target element at the project-specific action levels, or, when lacking project-specific action levels, between the low- and mid-level standards, as appropriate. Acceptance criteria should be set at laboratory-derived limits, developed through the use of historical analyses, for each matrix type being analyzed. However, historically derived acceptance limits must not exceed ± 25%

recovery of the target element spike values for bias, and ≤ 20 relative percent difference (RPD) for precision. In the absence of historical data, MS/MSD acceptance limits should be set at ± 25% recovery and ≤ 20 RPD. Refer to Sec. 4.0 of Chapter One for further guidance. If the bias and precision indicators in an analytical batch fail to meet the acceptance criteria, then the interference test discussed in Sec. 9.10 should be performed. Refer to the definitions of bias and precision, in Chapter One, for the proper data reduction protocols.

NOTE: If the background sample concentration is very low or non-detect, a spike of greater than 5 times the background concentration is still acceptable. To assess data precision with duplicate analyses, it is preferable to use a high concentration field sample to prepare unspiked laboratory duplicates for metals analyses.

Calculate the RPD between duplicate or MS determinations as follows:

$$RPD = \frac{|D_1 - D_2|}{\left(\frac{|D_1 + D_2|}{2}\right)} \times 100$$

where:

RPD = relative percent difference
D_1 = MS or first sample analysis value
D_2 = MSD or duplicate sample analysis value

9.7.3 At least one LCS should be prepared and analyzed with each batch of analytical samples processed, at a minimum frequency of one LCS per every 20 samples, as described in Chapter One. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS should be spiked at the same levels and using the same spiking materials as the corresponding MS/MSD (see above Sec. 9.7.2). When the results of the MS analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can acceptably perform the analysis in a clean matrix.

LCS acceptance criteria should be set at laboratory-derived limits, developed through the use of historical analyses. However, historically derived acceptance limits must not exceed ± 20% of the target element spike values. In the absence of historical data, LCS acceptance limits should be set at ± 20%. If the result of an LCS does not meet the established acceptance criteria, it should be re-analyzed once. If still unacceptable, then all samples associated with the LCS must be re-prepared and re-analyzed, along with all other appropriate analysis batch QC samples.

9.7.4 Reference materials containing known amounts of target elements are recommended when an appropriately similar medium of interest are available as one type of QC after appropriate sample preparation. The reference material may be used as the LCS. For soil reference materials, the manufacturers' established acceptance criterion should be used. For solid reference materials, ± 20% (see Sec. 9.7.3) recovery of the reported manufacturers' target element values may not be achievable. Refer to Chapters One and Three for additional information.

9.8 Lower Limit of Quantitation (LLOQ) check standard

9.8.1 The laboratory should establish the LLOQ as the lowest point of quantitation which, in most cases, is the lowest concentration in the calibration curve. The LLOQ is initially verified by the analysis of at least 7 replicate samples, spiked at the LLOQ and processed through all preparation and analysis steps of the method. The mean recovery and relative standard deviation of these samples provide an initial statement of precision and accuracy at the LLOQ. In most cases the mean recovery should be +/- 35% of the true value and RSD should be ≤ 20%. In-house limits may be calculated when sufficient data points exist. Monitoring recovery of LLOQ over time is useful for assessing precision and bias. Refer to a scientifically valid and published method such as Chapter 9 of Quality Assurance of Chemical Measurements (Taylor 1987) or the Report of the Federal Advisory Committee on Detection and Quantitation Approaches and Uses in Clean Water Act Programs (http://water.epa.gov/scitech/methods/cwa/det/index.cfm) for calculating precision and bias for LLOQ.

9.8.2 Ongoing LLOQ verification, at a minimum, is on a quarterly basis to validate quantitation capability at low analyte concentration levels. This verification may be accomplished either with clean control material (e.g., reagent water, method blanks, Ottawa sand, diatomaceous earth, etc.) or a representative sample matrix (free of target compounds). Optimally, the LLOQ should be less than the desired regulatory action levels based on the stated project-specific requirements.

9.9 Verify the magnitude of elemental and molecular-ion isobaric interferences and the adequacy of any corrections at the beginning of an analytical run or once every 12 hours of continuing sample analysis, whichever is more frequent. Do this by analyzing the SIC solution. Results for the unspiked elements in the SIC solution should be less than 2 times the LLOQ. Note that it may not be possible to obtain SIC spiking solutions that are completely free of the unspiked elements. If the presence and concentration of an unspiked element can be confirmed via vendor documentation and/or determination of multiple isotopes of the element in the correct ratios, the concentration actually present may be subtracted from the determined value prior to comparing to the LLOQ limits. Refer to Sec. 4.0 for a discussion on interferences and potential solutions to those interferences if additional guidance is needed.

9.10 The intensities of each internal standard must be monitored for every analysis to ensure that it does not decrease below 30%, with respect to its intensity during the initial calibration. If this occurs, a significant matrix effect must be suspected. Under these conditions, the IDL has degraded, and therefore the correction capability of the internal-standardization technique must then be questioned. If this happens, perform the following procedure:

9.10.1 Make sure the instrument has not drifted by observing the internal standard intensities in the nearest clean matrix, i.e., the calibration blank. If the low internal standard intensities are also observed in the nearby calibration blank, terminate the analysis, correct the problem, recalibrate the instrument, verify the new calibration, and reanalyze the affected samples.

9.10.2 If drift has not been demonstrated to occur as outlined in Sec. 9.10.1, matrix effects need to be removed by diluting the affected sample. Dilute the sample five-

fold (1:5), taking into consideration the need to add the appropriate amounts of internal standards, and reanalyze. If the first dilution does not eliminate the problem, repeat the dilution procedure in an iterative fashion, using ever-increasing dilutions, until the internal-standard intensities exceed the 30% acceptance limit. Correct the reported results using the appropriate dilution factors.

9.11 To obtain analyte data of known quality, it is necessary to measure more than the analytes of interest in order to apply corrections or to determine whether interference corrections are necessary. For example, tungsten oxide molecular-ion species can be very difficult to distinguish from mercury isotopes. If the concentrations of interference sources (such as C, Cl, Mo, Zr, W) are such that, at the correction factor, the analyte is less than the LLOQ and the concentration of interferents are insignificant, then the data may go uncorrected.

NOTE: Monitoring the interference sources does not inevitably necessitate monitoring of the interferant itself, but that a molecular species may be monitored to indicate the presence of the interferent.

When correction equations are used, all QC criteria must also be met. Extensive QC for interference corrections is needed at all times. The monitored masses must include those elements whose hydrogen, oxygen, hydroxyl, chlorine, nitrogen, carbon and sulfur molecular ions could impact the analytes of interest. Unsuspected interferences may be detected by adding pure major matrix components to a sample to observe any impact on the analyte signals. When an interference source is present, the sample elements impacted must be flagged to indicate (a) the percentage interference correction applied to the data; or (b) an uncorrected interference, by virtue of the elemental equation used for quantitation. The isotope proportions for an element or molecular-ion cluster provide information useful for QA.

NOTE: Only isobaric elemental, molecular, and doubly charged interference corrections, which employ the observed isotopic-response ratios or parent-to-oxide ratios (provided an oxide internal standard is used as described in Sec. 4.2) for each instrument system, are acceptable corrections for use in this method.

9.12 It is recommended that the laboratory adopt additional QA practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze reference materials and participate in relevant performance evaluation (PE) studies.

9.13 If less than acceptable bias and precision data are generated for the matrix spike(s), the additional QC protocols in Sections 9.13.1 and/or 9.13.2 should be performed prior to reporting concentration data for the elements in this method. At a minimum these tests should be performed with each batch of samples prepared/analyzed with corresponding unacceptable data quality results. If matrix interference effects are confirmed, then an alternative test method should be considered or the current test method modified, so that the analysis is not affected by the same interference. The use of a standard-addition analysis procedure may also be used to compensate for this effect (refer to Method 7000).

9.13.1 Dilution test

If the analyte concentration is within the linear range of the instrument and sufficiently high (minimally, a factor of 25 times greater than the LLOQ), an analysis of a 1:5 dilution should agree to within ± 20% of the original determination. If not, then a chemical or physical interference effect must be suspected. The matrix spike is often a good choice of sample for the dilution test, since reasonable concentrations of most analytes are present. Elements that fail the dilution test are reported as estimated values.

9.13.2 Post-digestion MS

If a high concentration sample is not available for performing the dilution test, then a post-digestion MS should be performed. The test only needs to be performed for the specific elements that failed original matrix spike limits, and only if the spike concentration added was greater than the concentration determined in the unspiked sample. Following preparation, which may include, but is not limited to, pre-filtration, digestion, dilution and filtration, an aliquot, or dilution thereof, should be obtained from the final aqueous, unspiked-analytical sample, and spiked with a known quantity of target elements. The spike addition should be based on the indigenous concentration of each element of interest in the sample. The recovery of the post-digestion MS should fall within a ± 25 % acceptance range, relative to the known true value, or otherwise within the laboratory-derived acceptance limits. If the post-digestion MS recovery fails to meet the acceptance criteria, the sample results must be reported as estimated values.

9.14 Ultra-trace analysis necessitates the use of clean chemistry practices. Several suggestions for the reduction of contaminants in the analytical blank are provided in Chapter Three, Inorganic Analytes.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Conduct mass calibration and resolution verification checks in the mass regions of interest using the mass spectrometer tuning solution (Sec. 7.26). The mass calibration and resolution verification acceptance criteria must be met prior to the analysis of samples. If the mass calibration differs by more than 0.1 u from the true value, then the mass calibration must be adjusted to the correct value. The resolution must also be verified to be less than 0.9 u full width at 10% peak height.

10.2 At a minimum, the elements required for the project plus any required for interference correction must be calibrated. Recommended isotopes for the analytes in Sec. 1.2 are provided in Table 2. Flush the system in between each standard and sample using the rinse blank (Sec. 7.22.3). The rinse time needs to be sufficient to ensure that analytes present in the linear range are effectively cleaned out prior to analysis of the subsequent sample. Use the average of at least three readings (of a single injection) for both calibration standard and sample analyses.

10.3 Calibration standards should be prepared on an as-needed basis unless stability warrants preparing fresh daily, (or each time a batch of samples is analyzed). If the ICV standard is prepared daily and the results of the ICV analyses meet the acceptance criteria,

then the calibration standards do not need to be prepared daily and may be prepared and stored for as long as the calibration standard viability can be verified through the use of the ICV. If the ICV fails to meet the acceptance criteria, trouble shoot the situation, and then prepare a new set of calibration standards if needed and recalibrate the instrument

10.4 A calibration curve must be analyzed daily. The instrument may be calibrated using a single point standard and a calibration blank (ICB) or a multipoint calibration curve. If a multipoint curve is used a minimum of three standards are required and the correlation coefficient (r) should be $\geq$ 0.995 or the coefficient of determination (r^2) should be $\geq$ 0.990. Relative Standard Error may be used as an alternative to r or r^2, and should be $\leq$ 20%. If a multipoint calibration is used the low standard must be at or below the LLOQ.

NOTE: Inversely weighted linear regressions or other methods may be used in order to minimize curve fitting errors at the low end of the calibration curve.

10.5 After the initial calibration is completed it is verified using several checks.

10.5.1 Initial Calibration Verification (ICV) - The ICV is a standard prepared from a different source than the initial calibration standards. It is analyzed at approximately the mid-level of the calibration and serves as a check that the initial calibration standards are at the correct concentrations. The acceptance range is 90-110% of the true value.

10.5.2 Low-level readback or verification - For a multi-point calibration, the low level standard should quantitate to within 80-120% of the true value. For a single point calibration, a standard from the same source as the calibration standard and at or below the LLOQ is analyzed and should recover within 80-120% of the true value.

10.5.3 Mid-level readback or verification - For a multi-point calibration, the mid-level standard should quantitate to within 90-110% of the true value. For a single point calibration, a standard from the same source as the calibration standard and at the mid-point of the linear range is analyzed and should recover within 90-110% of the true value.

10.5.4 Initial Calibration blank (ICB) - If a multi-level calibration is used, an ICB is analyzed immediately after the calibration (or after the ICV) and must not contain target analytes above half the LLOQ. If a single point calibration is used, the calibration is forced through the ICB, but a second ICB is analyzed as a check and must not contain target analytes above half the LLOQ. If the ICB consistently has target analyte concentrations greater than half the LLOQ, the LLOQ should be re-evaluated.

NOTE: After cleaning the sampler and skimmer cones, improved performance in calibration stability has been observed by method users if the instrument is exposed to the SIC solution. Improved performance has also been observed if the instrument is allowed to rinse for 5 - 10 minutes before starting the calibration process.

10.5.5 Verify the ongoing validity of the calibration curve after every 10 samples, and at the end of each analysis batch run, through the analysis of a CCV standard (Sec. 7.25) and a CCB (Sec. 7.22.1). For the curve to be considered valid the analysis result of the CCV standard must be within ± 10% of its true value and the CCB must not contain target analytes above the LLOQ. If the calibration cannot be verified, sample analysis

must be discontinued, the cause of the problem determined and the instrument recalibrated. All samples following the last acceptable CCV standard must be reanalyzed Flow-injection systems may be used as long as they can meet the performance criteria of the method.

11.0 PROCEDURE

11.1 Preliminary treatment of most samples is necessary because of the complexity and variability of sample matrices. Groundwater samples which have been pre-filtered and acidified will not need acid digestion. Samples which are not digested must either use an internal standard or be matrix-matched with the standards (i.e., acid concentrations should match). Solubilization and digestion procedures are presented in Chapter Three, Inorganic Analytes.

NOTE: If mercury is to be analyzed, the digestion procedure must use mixed nitric and hydrochloric acids through all steps of the digestion. Mercury will be lost if the sample is digested when hydrochloric acid is not present. If it has not already been added to the sample as a preservative, Au should be added to give a final concentration of 2 mg/L (use 2.0 mL of gold preservative stock (Sec. 7.20.11) per 100 mL of sample) to preserve the mercury and to prevent it from plating out in the sample introduction system.

11.2 Initiate an appropriate operating configuration of the instrument computer according to the instrument manufacturer's instructions.

11.3 Set up the instrument with the proper operating parameters according to the instrument manufacturer's instructions.

11.4 Operating conditions

Tune the instrument by following the instructions provided by the instrument manufacturer. Allow at least 30 minutes for the instrument to equilibrate before analyzing samples.

NOTE: The instrument should have features that protect it from high ion currents. If not, precautions must be taken to protect the detector. A channel electron multiplier or active film multiplier will suffer from fatigue after being exposed to high ion currents. This fatigue can last from several seconds to hours depending on the extent of exposure. During this time period, response factors are constantly changing, which invalidates the calibration curve, causes instability, and invalidates sample analyses.

11.5 Calibrate the instrument following the procedure outlined in Sec. 10.0.

11.6 Flush the system with the rinse blank solution (Sec. 7.22.3) until the signal levels return to the data quality objectives or method LLOQs (usually about 30 seconds) before the analysis of each sample. Nebulize each sample until a steady-state signal is achieved (usually about 30 seconds) prior to collecting data.

11.7 Dilute and reanalyze samples that exceed the linear range for an analyte (or species needed for a correction) or measure an alternate, but less-abundant, isotope. The

linearity at the alternate mass must be confirmed by appropriate calibration (see Sec. 10.4). Alternatively apply solid-phase chelation chromatography to eliminate the matrix as described in Sec. 4.3.

11.8 Determination of percent dry weight

When sample results are to be calculated on a dry-weight basis, a separate portion of sample for this determination should be weighed out at the same time as the portion used for analytical determination.

CAUTION: The drying oven should be contained in a hood or vented. Significant laboratory contamination may result from a heavily contaminated hazardous waste sample.

11.8.1 Immediately after weighing the sample aliquot to be digested, weigh an additional 5- to 10-g aliquot of the sample to the nearest 0.01g into a tared crucible. Dry this aliquot overnight at 105 EC. Allow the sample to cool in a desiccator before weighing.

11.8.2 Calculate the % dry weight as follows:

$$\%\,\text{dry weight} = \frac{\text{g of dry sample}}{\text{g of sample}} \times 100$$

This oven-dried aliquot is not used for the extraction and should be appropriately disposed of once the dry weight is determined.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 If dilutions were performed, apply the appropriate corrections to the sample values.

12.2 If appropriate, or required by the project or regulation for data reporting, calculate results for solids on a dry-weight basis as follows:

$$Concentration_{DW} = \frac{C \times V}{W \times S}$$

where:

$Concentration_{DW}$ = Concentration on a dry weight basis (mg/kg)
C = Digest concentration (mg/L)
V = Final volume after sample preparation (L)
W =Wet sample mass (kg)
S = % Solids/100 = % dry weight/100

Calculations must include appropriate interference corrections (see Sec. 4.2 for examples), internal-standard normalization, and the summation of signals at 206, 207, and 208 *m/z* for lead (to compensate for any differences in the abundances of these isotopes between samples and standards).

13.0 METHOD PERFORMANCE

Performance data and related information are provided in SW-846 methods only as examples and guidance. The data do not represent required performance criteria for users of the methods. Instead, performance criteria should be developed on a project-specific basis, and the laboratory should establish in-house QC performance criteria for the application of this method. These performance data are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.

Table 3 summarizes the method performance data for aqueous and sea water samples with interfering elements removed and samples preconcentrated prior to analysis. Table 4 summarizes the performance data for a simulated drinking water standard. These data are provided for guidance purposes only.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operations. 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*, a free publication available from the American Chemical Society (ACS), Committee on Chemical Safety,
http://portal.acs.org/portal/fileFetch/C/WPCP_012290/pdf/WPCP_012290.pdf.

15.0 WASTE MANAGEMENT

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 ACS publication listed in Sec. 14.2.

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17.0 TABLES, DIAGRAMS, FLOW CHARTS, AND VALIDATION DATA

The pages to follow contain the tables, and figures referenced by this method.

TABLE 1

RECOMMENDED SPECTRAL INTERFERENCE CHECK (SIC) SOLUTION COMPONENTS AND CONCENTRATIONS

Solution Component	SIC Concentration (mg/L)
Al	100.0
Ca	300.0
Fe	250.0
Mg	100.0
Na	250.0
P	100.0
K	100.0
S	100.0
C	200.0
Cl	2000.0
Mo	2.0
Ti	2.0

TABLE 2

RECOMMENDED ELEMENTAL ISOTOPES FOR SELECTED ELEMENTS

Element of Interest	Mass of Isotope
Aluminum	**27**
Antimony	121, **123**
Arsenic	**75**
Barium	138, 137, 136, **135**, 134
Beryllium	**9**
Bismuth (IS)	209
Cadmium	**114**, 112, **111**, 110, 113, 116, 106
Calcium (I)	42, 43, **44**, 46, 48
Chlorine (I)	35, 37, (77, 82)[a]
Chromium	**52**, **53**, **50**, 54
Cobalt	**59**
Copper	**63**, **65**
Holmium (IS)	165
Indium (IS)	**115**, 113
Iron (I)	**56**, **54**, **57**, 58
Lanthanum (I)	139
Lead	**208**, **207**, **206**, 204
Lithium (IS)	6[b], 7
Magnesium (I)	24, **25**, **26**
Manganese	**55**
Mercury	202, **200**, 199, 201
Molybdenum (I)	98, 95, 96, 92, **97**, 94, (108)[a]
Nickel	58, **60**, 62, **61**, 64
Potassium (I)	**39**
Rhodium (IS)	103
Scandium (IS)	45
Selenium	80, **78**, **82**, **76**, **77**, 74
Silver	**107**, **109**
Sodium (I)	**23**
Terbium (IS)	159
Thallium	**205**, 203
Vanadium	**51**, **50**
Tin (I)	120, **118**
Yttrium (IS)	89
Zinc	64, **66**, **68**, **67**, 70

NOTE: Method 6020 is recommended for only those analytes listed in Sec.1.2. Other elements are included in this table because they are potential interferents (labeled I) in the determination of recommended analytes, or because they are commonly used internal standards (labeled IS). Isotopes are listed in descending order of natural abundance. The most generally useful isotopes are underlined and in boldface, although certain matrices may necessitate the use of alternative isotopes.

[a] These masses are also useful for interference correction (Sec. 4.2).

[b] Internal standard must be enriched in the ^{6}Li isotope. This minimizes interference from indigenous lithium.

TABLE 3

METHOD PERFORMANCE DATA FOR AQUEOUS AND SEA WATER SAMPLES [a]
WITH INTERFERING ELEMENTS REMOVED AND SAMPLES PRECONCENTRATED PRIOR TO ANALYSIS

ELEMENT	ISOTOPE	CONCENTRATION (ng/mL) [b]		
		9.0 mL	27.0 mL	CERTIFIED
Manganese	55	1.8±0.05	1.9±0.2	1.99±0.15
Nickel	58	0.32±0.018	0.32±0.04	0.30±0.04
Cobalt	59	0.033±0.002	0.028±0.003	0.025±0.006
Copper	63	0.68±0.03	0.63±0.03	0.68±0.04
Zinc	64	1.6±0.05	1.8±0.15	1.97±0.12
Copper	65	0.67±0.03	0.6±0.05	0.68±0.04
Zinc	66	1.6±0.06	1.8±0.2	1.97±0.12
Cadmium	112	0.020±0.0015	0.019±0.0018	0.019±0.004
Cadmium	114	0.020±0.0009	0.019±0.002	0.019±0.004
Lead	206	0.013±0.0009	0.019±0.0011	0.019±0.006
Lead	207	0.014±0.0005	0.019±0.004	0.019±0.006
Lead	208	0.014±0.0006	0.019±0.002	0.019±0.006

NOTE: Data obtained from Ref. 12.

[a] The dilution of the sea-water during the adjustment of pH produced 10 mL samples containing 9 mL of sea-water and 30 mL samples containing 27 mL of sea-water. Samples containing 9.0 mL of CASS-2, n=5; samples containing 27.0 mL of CASS-2, n=3.

[b] 95% confidence limits

TABLE 4

ANALYSIS OF NIST SRM 1643b - TRACE METALS IN WATER [a]

ELEMENT	ISOTOPE	CONCENTRATION (ng/mL) [b]	
		DETERMINED	CERTFIED
Manganese	55	30±1.3	28±2
Nickel	58	50±2	49±3
Cobalt	59	27±1.3	26±1
Nickel	60	51±2	49±3
Copper	63	23±1.0	21.9±0.4
Zinc	64	67±1.4	66±2
Copper	65	22±0.9	21.9±0.4
Zinc	66	67±1.8	66±2
Cadmium	111	20±0.5	20±1
Cadmium	112	19.9±0.3	20±1
Cadmium	114	19.8±0.4	20±1
Lead	206	23±0.5	23.7±0.7
Lead	207	23.9±0.4	23.7±0.7
Lead	208	24.2±0.4	23.7±0.7

NOTE: Data obtained from Ref. 12.

[a] 5.0 mL samples, n=5

[b] 95% confidence limits

TABLE 5

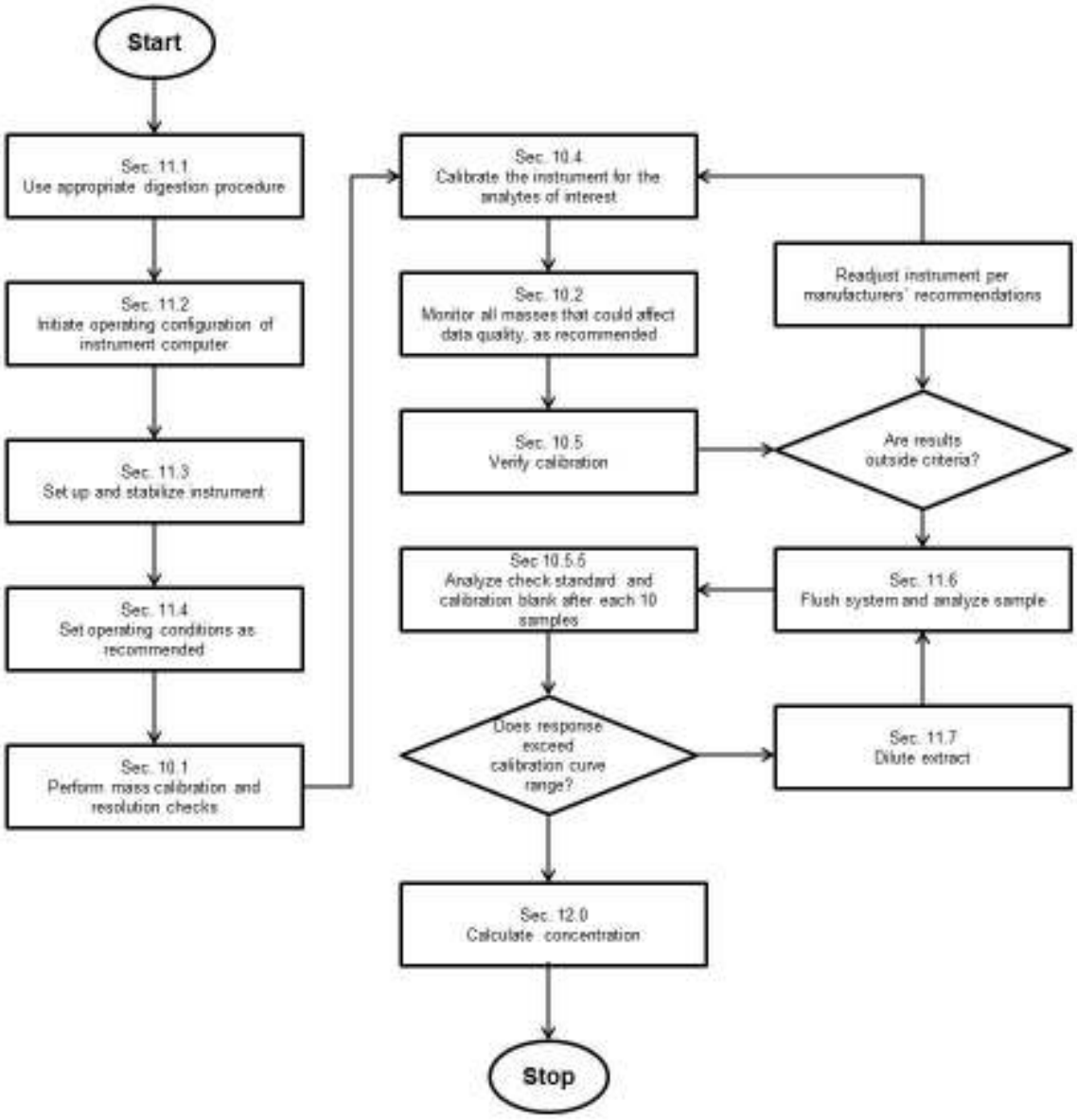
COMPARISON OF TOTAL MERCURY RESULTS IN HEAVILY CONTAMINATED SOILS

Soil Sample	Mercury in µg/g	
	ICP-MS	CVAA
1	27.8	29.2
2	442	376
3	64.7	58.2
4	339	589
5	281	454
6	23.8	21.4
7	217	183
8	157	129
9	1670	1360
10	73.5	64.8
11	2090	1830
12	96.4	85.8
13	1080	1190
14	294	258
15	3300	2850
16	301	281
17	2130	2020
18	247	226
19	2630	2080

NOTE: Data obtained from Ref. 16.

METHOD 6020A

INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY



Appendix A

Summary of Revisions to Method 6020 (From Revision 1, February 2007):

1. Improved overall method formatting for consistency with new SW-846 methods style guidance.
2. Section 1.2 – Changed “Inductively coupled plasma—atomic emission spectrometry” to “Inductively coupled plasma—optical emission spectrometry”.
3. Section 1.6 - inserted references to additional 3000 series preparatory methods to ICP analysis. Also added method 6800 to sections 1.6 and 9.2 as a preparatory method.
4. Inserted additional safety guidance regarding the use of HF.
5. Inserted new section (7.27) regarding analysis of non-aqueous solvents.
6. Reformatted certain paragraphs with the heading "NOTE" or "WARNING" to better denote the importance of the recommendations provided therein.
7. Extensively reformatted “REAGENTS AND STANDARDS” section and to meet current SW-846 method guidelines.
8. Significantly updated and expanded “QUALITY CONTROL” section for better adherence to current SW-846 method guidelines and for improved alignment with current universal practices for published analytical methods.
9. Inserted new sections (Sections 7.23 and 9.9) to describe the preparation and use of the spectral interference check (SIC) solution; also added instructions to match the matrix of this solution to that of the calibration standards.
10. Renamed "QC standard" as "ICV standard" in Sec. 7.24.
11. Added new Sec. 7.25 describing the preparation of a "CCV" standard, consistent with the equivalent section in 6010.
12. Replaced the term “unity” with “uniform” in Section 7.27.
13. Removed all references to method 7000 except for guidance regarding the method of standard addition.
14. The term “accuracy” was replaced by “bias” where appropriate.
15. In Section 9.4, the requirement to repeat the demonstration of proficiency for new staff and instrumentation changes was changed to a recommendation.
16. Section 9.7.2 – Added a note regarding MS/MSD spike concentrations and unspiked laboratory duplicates.
17. The section regarding analysis of reference materials (Sec. 9.7.4) was revised for clarity and the term “Standard Reference Material” was replaced with “reference material” throughout the method.
18. Inserted new section (Sec. 9.8) describing the preparation and use of an LLOQ standard. This section includes two new references for guidance on assessing precision and bias.
19. The section describing matrix interference check samples (Sec. 9.13) has been revised for clarity. The post-digestion MS is only recommended if a high concentration sample is not available for performing the dilution test.
20. Substituted certain terms with new terms (i.e. “must” in place of “shall”) to conform with the Performance-based Methods Approach goal of flexibility.
21. Removed reference to “linear dynamic range” as noted by the Inorganic Methods Work Group. Section 9.6 regarding the linear range was added.
22. Mid-level read back or verification standard added to Section 10.5.3.
23. Moved the sentence “If the ICB consistently has target analyte concentrations greater than half the LLOQ, the LLOQ should be re-evaluated.” From Section 10.5.5 to Section 10.5.4.
24. Added 95 as mass of isotope for molybdenum.

25. Tables 3 and 4 from 6020A presenting example precision and accuracy data for aqueous and solid matrices were removed.
26. Language was updated in Section 9.7.1 regarding method blanks.

Annex IV:

IAEA (2012). Recommended method on the determination of Total Mercury in marine samples by Thermal Decomposition Amalgamation and Atomic Absorption Spectrophotometry (4.1.4)

RECOMMENDED METHOD ON THE DETERMINATION OF TOTAL MERCURY IN MARINE SAMPLES BY THERMAL DECOMPOSITION AMALGAMATION AND ATOMIC ABSORPTION SPECTROPHOTOMETRY

Marine Environmental Studies Laboratory in co-operation with MED POL

December 2012

For further information on this method, please contact:

IAEA Environment Laboratories, Monaco
Marine Environmental Studies Laboratory
4a Quai Antoine 1er, MC 98000
Principality of Monaco

RECOMMENDED METHOD ON THE DETERMINATION OF TOTAL MERCURY IN MARINE SAMPLES BY THERMAL DECOMPOSITION AMALGAMATION AND ATOMIC ABSORPTION SPECTROPHOTOMETRY

TABLE OF CONTENT

1. SCOPE 1
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3. SAMPLE PRE-TREATMENT 1
4. REAGENTS 2
5. MATERIAL 2
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7. PROCEDURE 4
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9. CALCULATION OF RESULTS 7
10. EXPRESSION OF RESULTS 8
11. REFERENCES 8

<u>NOTE:</u> This recommended method is not intended to be an analytical training manual. Therefore, this method is written with the assumption that it will be performed by formally trained analytical chemist.

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 shall not be used as absolute QC acceptance criteria.

1. SCOPE

The recommended method hereinafter describes the protocol for the determination of total mercury (inorganic and organic) in sediment and biological material.

By using this method, the total mercury in solid samples can be determined without sample chemical pre-treatment.

The recommended protocol is mainly based on the EPA 7473 method; users are encouraged to consult this document (EPA, 2007).

2. PRINCIPLE

The sample is dried and then chemically decomposed under oxygen in the decomposition furnace. The decomposition products are carried out to the catalytic section of the furnace, where oxidation is completed (halogens and nitrogen/sulfur oxides are trapped). The mercury present in the remaining decomposition products is selectively trapped on an amalgamator. After flushing the system with oxygen, the mercury vapour is released by rapid heating of the amalgamator, and carried through the absorbance cell in the light path of a single wavelength atomic absorption spectrophotometer. The absorbance is measured at 253.7 nm as a function of mercury quantity (ng).

The typical working range is 0.1–500 ng. The mercury vapour is carried through a long (first) and a short path length absorbance cell. The same quantity of mercury is measured twice with different sensitivity resulting in a dynamic range that spans four orders of magnitude.

The typical detection limit is 0.01 ng of mercury.

3. SAMPLE PRE-TREATMENT

The sediment samples are prepared following the recommendations of UNEP (2005);

The marine organisms are prepared following the recommendations of UNEP (1984, 1994).

4. REAGENTS

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

4.1. ULTRAPUR WATER (type MilliQ)

4.2. NITRIC ACID 65%

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

Weight 25 g of $K_2Cr_2O_7$ in 250 ml glass bottle, fill it up to 250 ml with water, and shake until total dissolution of solids. 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.

4.4. COMMERCIAL STANDARD SOLUTION 1000 µg ml^{-1} MERCURY

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.

5. MATERIAL

5.1. SOLID MERCURY ANALYZER

Optionally equipped with an auto-sampler.

5.2. ANALYTICAL BALANCE

With a 0.001 g precision at least.

5.3. VOLUMETRIC CONTAINERS

Preferably in Teflon or glass.

5.4. PIPETTES

Some microliter pipettes sized 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.

5.5. METAL SPATULA (inox).

5.6. SAMPLE BOAT

Metal or metal alloy. Before measurement, sample boats are cleaned by heating over a flame until constant “red” to remove mercury.

5.7. OXYGEN

It should be of high purity and free of mercury. If there is a possible mercury contamination from oxygen, install a gold mesh filter between the cylinder and the instrument to prevent any mercury from entering the instrument.

6. CALIBRATION

6.1. PRIMARY CALIBRATION. This is the calibration of the instrument working range. This calibration is performed initially (usually done by the manufacturer and stored in the instrument), and/or when any significant instrumental parameters are changed (i.e. after maintenance).

6.2. PREPARE STANDARD SOLUTIONS of appropriate concentration by dilution of a commercial standard (see 4.4). It is recommended to prepare standard solution in Teflon or glass container, in 1 or 0.5% HNO_3 (see 4.2) and 0.1% (v/v) potassium dichromate (see 4.3). Fresh mercury standard should be prepared daily. Prepare a zero calibration solution using the same quantity of acid and potassium dichromate.

6.3. START THE INSTRUMENT according to the manufacturer recommendations.

6.4. CLEAN THE SYSTEM. Inject 100 µl of water and start the measurement with the recommended parameters (see 7.1). Repeat the cleaning until the absorbance is below 0.001ABS.

6.5. SET THE INSTRUMENT PARAMETERS (see 7.1) for selected volume (usually 100 µl) and inject the zero calibration, at least three measurements should be done. The zero solution serves to correct the amount of mercury in water and reagent used for preparing the calibration curve, hence the important of keeping the injected volume equal at all points of the calibration curve. If the amount of mercury in the zero calibration is high (i.e. more than 0.01 ng), it is recommended to check for contamination sources and to prepare new standard solution with clean acid.

6.6. STANDARDS ARE MEASURED from the lowest to the highest at least twice. The maximum relative standard deviation between readings should be 3% (except for zero calibration); if higher it is recommended to carry out more measurements.

6.7. EXAMPLE OF AMOUNTS used for recalibration (primary):

<u>First Range:</u>

Standard (ng ml^{-1})	1	3	10	30	100	300
Volume injected (µl)	100	100	100	100	100	100
Quantity of Hg (ng)	0.1	0.3	1	3	10	30

Second Range:

Standard (µg ml^{-1})	1	2	3	4	5	6
Volume injected (µl)	100	100	100	100	100	100
Quantity of Hg (ng)	100	200	300	400	500	600

Note: The calibration of the second range might induce problems for subsequent analysis, due to the relatively high quantity of mercury introduced (especially with memory effect). It should be performed only if there is a probability of using it (i.e. measuring samples with high mercury level > 1µg g^{-1}). After the reading of the last calibration point, clean the system (see 6.4).

6.8. ALTERNATIVE CALIBRATION CURVE can be performed using a solid certified reference material. In this case, weigh accurately a CRM onto a tare sample boat, set up the instrument according to the sample type (see 7.1) and measure the absorbance. The matrix of the CRM should be as similar as possible to the sample of interest. Repeat this procedure with different weights of the CRM and/or with different CRM, to get results in the desired working range.

6.9. CONSTRUCT A CALIBRATION CURVE by plotting the absorbance against Nano grams of mercury (this could be done automatically by the software). The type of equation will depend on the levels, as the response is not linear over the entire working range.

6.10. DAILY CALIBRATION: calibration performed every day with a minimum number of standards to ensure that the primary calibration is valid. It can be performed by using either liquid standard (see 6.2) or solid certified reference material (CRM) see 6.8. It should be performed in the range of interest, with at least two standards (or matrix CRM) and the results should agree within the acceptance criteria. The acceptance criteria should be set through the use of historical data, but the maximum deviation should not exceed 10%.

7. PROCEDURE

7.1. GENERAL ANALYTICAL PARAMETERS

The analytical parameters will depend on the sample size and matrix, and are instrument specific. It is important to follow the guidelines from the instrument manufacturer. There are three time to set: drying, decomposition and waiting.

Some typical recommended conditions below:

Drying time:

Sample type	Dry (s)	Comments
Liquid	0.7 x injected Volume (µl)	
Dry inorganic	10	
Organic liquid	50–300	To be optimized[1]
Dry organic (i.e. fat)	50–200	To be optimized[1]
Wet (i.e. fresh)	0.7 x weight x % moisture	Example: 100 mg with 45% moisture 0.7 x 100 x 0.45= 31.5s (35)

[1] In the case of organic, there is a risk of explosion especially with organic liquid; to optimize set the instrument at: 300s dry/ 150s decomposition/ 45s wait, do the measurement and check for possible small explosion, note the time of the phenomenon and add to the drying time 10s more.

Decomposition time:

Sample type	Decomposition (s)	Comments
Liquid	150–400	To be optimized[1]
Solid inorganic	120 + 0.4 x sample (mg)	To be optimized[1]
Solid organic	120	

[1] Set the instrument to XX (see above) dry/ 400s decomposition/ 45s wait, run a sample and observe the results. Decrease the decomposition time by 30s and repeat measurement. Continue until you observe a significant decrease, note that time and add to the decomposition time 30s more.

Waiting time:

It is recommended to use 40–45s, except for long decomposition time (over 200s) when it is beneficial to add 10s of waiting for every 100s of decomposition.

Note: These indications above are recommended by ALTECH (AMA 254).

7.2. ANALYSIS OF A SOLID SAMPLE

Weight a sample accurately onto a tare boat, insert the boat into the instrument, set the appropriate parameters (see 7.1) and start the measurements. The results can be records on absorbance, quantity or concentration depending on the instrument software. See 9: Calculation of results.

7.3. ANALYSIS OF BLANK FOR SOLID MEASUREMENT

Analyse an empty sample boat using the same instrument settings than for the sample.

7.4. ANALYSIS OF A LIQUID SAMPLE

Dose a known volume of the sample onto a sample boat, set the appropriate parameters (see 7.1) and start the measurements. The results can be records on absorbance, quantity or concentration depending on the instrument software. See the calculation section (see 9).

7.5. ANALYSIS OF BLANK FOR LIQUID

Repeat 7.4 with the same volume of blank solution (solution that contain the same reagent and chemical than the sample).

8. QUALITY CONTROL

8.1. For every day of analysis, the CALIBRATION SHOULD BE VALIDATED by doing a daily calibration (see 6.10) before starting the measurements. The results of the daily calibration should be recorded for quality control purposes.

8.2. CERTIFIED REFERENCE MATERIAL

At least one certified reference material of a representative matrix should be measured with each batch of the sample, the calculated results should fall in the value of the certificate and within the coverage uncertainty (Linsinger, 2010), to show evidence of unbiased results. The results for the CRM should be recorded for quality control purpose and plotted in a control chart (UNEP/IOC/IAEA 1994).

8.3. A DUPLICATE OR TRIPLICATE SAMPLE should be processed on a routine basis.

A duplicate sample should be processed with each analytical batch or for every 10 samples.

8.4. A SPIKED SAMPLE should also be included, whenever a new sample matrix is being analysed, especially if no certified reference material is available for that matrix. Measure a spiked sample by adding a known volume of standard solution (prepared as in paragraph 6.2) to the sample in the boat. Keep the spike volume small enough not to overspill. The recovery of spike calculated with the equation 2 should be 85–115% (this limits should be reset after collection of historical data). If the test fails, it is recommended to check the calibration (see 6.10) and/or to revise the instrument parameters (see 7.1).

$$\text{Spike (ng)} = \text{Concentration of standard (ng/ml)} \times \text{Volume of spike (ml)} \qquad \text{Equation 1}$$

$$\text{Recovery (\%)} = \frac{\text{Spiked sample (ng)} - \text{Unspiked sample (ng)}}{\text{Spike (ng)}} \times 100 \qquad \text{Equation 2}$$

To be valid the quantity of Spike (equation 1) should be in the range of 50–150% the quantity of unspiked sample.

9. CALCULATION OF RESULTS

9.1. SOLID SAMPLE RESULTS are calculated using equation 3

$$w(Hg) = \frac{(\rho 1 - \rho 0)}{\mathrm{m}} \times R \qquad \text{Equation 3}$$

Where:

w(Hg) is the mass fraction of element m in the sample, expressed in mg kg^{-1};

ρ1 is the quantity of mercury, expressed in ng as measured in the sample;

ρ0 is the quantity of mercury expressed in ng as measured in the blank (see 7.3);

R is the recovery calculated using the CRM (see 8.2) or spike (see 8.4);

m is the amount of sample in mg.

Note: ρ1 and ρ0 are calculated using calibration curve equation (usually done by software).

9.2. LIQUID SAMPLE RESULTS are calculated using equation 4

$$w(Hg) = \frac{\frac{(\rho 1 - \rho 0)}{Vi} \times V}{\mathrm{m}} \times f \times R \qquad \text{Equation 4}$$

Where:

w(Hg) is the mass fraction of mercury in the sample, expressed in mg kg^{-1};

ρ1 is the quantity of mercury, expressed in ng as measured in the sample solution;

ρ0 is the quantity of mercury expressed in ng as measured in the blank solution (see 7.4);

R is the recovery calculated using the CRM (see 8.2) or spike (see 8.4);

Vi is the injected volume (should be the same in sample and blank solution) in ml;

m is the amount of sample in mg;

V is the volume of solution in ml;

f is the dilution factor.

Note: ρ1 and ρ0 are calculated using calibration curve equation (usually done by software).

10. EXPRESSION OF RESULTS

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

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

Example: w(Hg) = 0.512 ± 0.065 mg kg^{-1}.

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Annex V:

OSPAR (2008). JAMP Guidelines for Monitoring Contaminants in Sediments. Technical Annex 5: Normalisation of contaminant concentrations in sediments (4.1.5)

OSPAR COMMISSION

Protecting and conserving the North-East Atlantic and its resources

JAMP Guidelines for Monitoring Contaminants in Sediments

Technical Annex 5: Normalisation of contaminant concentrations in sediments

1. Introduction

As contaminant concentrations may vary due to differences in bulk sediment composition, e.g. differences in particle size distribution, organic matter content, results from comparisons of observed data to assessment criteria or trend assessments may be obscured. In order to reduce variances of contaminant concentrations due to differences in bulk sediment composition and to increase the power of monitoring programmes to address the objectives of the JAMP, procedures for normalisation of the concentrations of contaminants in sediment have been developed and used in OSPAR assessments of monitoring data.

2. Purposes

This annex provides guidance on the application of methods to normalise contaminant concentrations in sediments. Normalisation is defined here as a procedure to adjust contaminant concentrations for the influence of the natural variability in sediment composition, grain size, organic matter and mineralogy. Most natural and anthropogenic substances, metals and organic contaminants, show a much higher affinity to fine particulate matter compared to the coarse fraction. Constituents such as organic matter and clay minerals contribute to the affinity to contaminants in this fine material.

Fine material, both inorganic and organic, and associated contaminants are preferentially deposited in areas of low hydrodynamic energy, while in areas of higher energy, fine particulate matter is mixed with coarser sediment particles which generally have smaller binding capacity for contaminants. This dilution effect will cause lower and variable contaminant concentrations in the resulting sediment. Obviously, grain size and organic matter are important factors controlling the distribution of natural and anthropogenic components in sediments. It is, therefore, essential to normalise for the effects of grain size or organic carbon in order to provide a basis for reliable assessments of temporal trends and for meaningful comparisons of the occurrence of substances in sediments of variable bulk properties with background (assessment) criteria and environmental assessment criteria derived for a defined sediment composition.

In sediment of varying bulk properties, contaminant concentrations will be closely related to the distribution of fine grained material, and any effects of other sources of contaminants, for example anthropogenic sources, will be at least partly obscured by grain size differences. Also in temporal trend monitoring, differences in sediment bulk properties can obscure trends, but if samples have a considerable and constant percentage of fine material, the influence of grain size distribution is of minor importance and may probably be neglected.

3. Normalisation procedures

Two different approaches to correct for variable sediment compositions are widely used:

a. Isolation of the fine fraction by sieving, e.g. <20 µm, <63 µm, can be regarded as means to reduce the differences in sediment granulometric compositions and is applicable to both metals and organic contaminants (e.g. Ackermann *et al*. 1983; Klamer *et al*. 1990; QUASH, 2000). Consequently the coarse particles, which usually do not bind anthropogenic contaminants and dilute their concentrations, are removed from the sample. Then, contaminant concentrations measured in these fine fractions can be directly compared. Subsequently, the differences in sediment composition due to geochemical nature remaining after sieving can be further corrected for by the use of co-factors. Thus, sieving is a powerful first step in normalisation;

b. Normalisation can be performed by relating the contaminant concentration with components of the sediment that represents its affinity for contaminants, i.e. binding capacity. Normalisation of contaminant concentrations can be performed by linear regression against cofactors (Cato, 1977; Smedes, 1997; Smedes *et al.,* 1997). Another procedure takes into account that the coarse sediment fraction contains natural metal concentrations in the crystal structure before the normalisation is performed (see section 4). Combinations of co-factors, possibly identified from multiple regression analysis, can be used.

4. Normalisation using co-factors

a. The binding capacity of the sediments can be related to the content of fines, primary factor, in the sediments. Normalisation can be achieved by calculating the concentration of a contaminant with respect to a specific **grain-size fraction** such as <2 µm, i.e. the clay factor, <20 µm or <63 µm;

b. As the content of fines is represented by the contents of major elements of the clay fraction such as **aluminium** (Windom *et al*. 1989) or an appropriate trace element enriched in that fraction such as **lithium** (Loring 1991), these can also be used as co-factors, secondary factors. Both, aluminium and lithium behave conservatively, as they are not significantly affected by, for instance, the early diagenetic processes and strong redox effects frequently observed in sediments. Problems may occur when the sediment is derived from glacial erosion of igneous rocks, with significant amounts of aluminium present in feldspar minerals contributing to the coarse fraction. In such cases, lithium may be the preferable co-factor (Loring 1991);

c. Organic matter, usually represented by organic carbon, is the most common co-factor for organic contaminants due to their strong affinity to this sediment component. In some environments, trace metal concentrations can also be normalised using organic carbon content especially in surface sediments (Cato 1977). However, due to the non-conservative nature of organic matter, its suitability as co-factor has to be checked prior to an assessment.

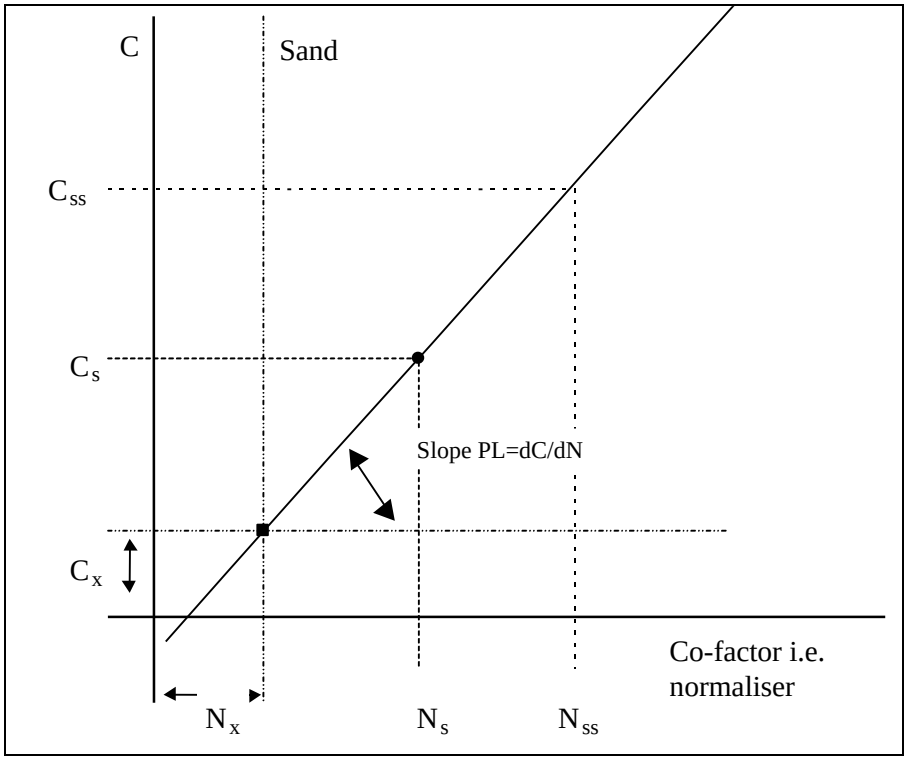

Figure 1: Relationship between the contaminant C and the cofactor N (from Smedes, 1997).

5. Theory

The general model for normalisation taking into account the possible presence of contaminants and cofactors in the coarse material is given in figure 1 (Cato, 1977; Smedes *et al*.1997; Kersten and Smedes, 2002). C_x and N_x represent the contaminant and the co-factor contents, respectively, in pure sand. These "intercepts" can be estimated from samples without fines and organic material. The line of regression between the contaminant and co-factor will originate from that point. That means that regression lines of sample sets with a different pollution level and consequently different slopes will have this point in common (i.e. pivot point) (OSPAR 2008). When this pivot point is known, only one sample is required to estimate the slope. This allows determination of the contaminant content for any agreed (preselected) co-factor content (N_{ss}) by interpolation or extrapolation. The slope (PL) for a sample with a contaminant content C_s and a cofactor content of N_s can be expressed as follows:

$$PL = \frac{dC}{dN} = \frac{C_s - C_x}{N_s - N_x} \qquad (1)$$

The extrapolation to an agreed co-factor content, N_{ss}, follows the same slope:

$$PL = \frac{dC}{dN} = \frac{C_s - C_x}{N_s - N_x} = \frac{C_{ss} - C_x}{N_{ss} - N_x} \qquad (2)$$

Rewriting gives the contaminant content, C_{ss}, that is normalised to N_{ss}:

$$C_{ss} = (C_s - C_x)\frac{N_{ss} - N_x}{N_s - N_x} + C_x \qquad (3)$$

Results of different samples normalised to the agreed N_{ss} can be compared directly.

Normalisation by this model can be applied with different cofactors. Here primary and secondary cofactors can be distinguished. A primary cofactor like the clay fraction or organic carbon is not

present in the coarse fraction and consequently has no intercept (N_x=0). Al and Li are present in the coarse fraction and therefore are considered to be secondary cofactors. Provided N_x and C_x are known, the model allows recalculation of total samples to a co-factor content usually found in sieved fractions, either <20 or <63µm. However such an extrapolation for a coarse grained sample will be associated with a large error due to the uncertainty of the intercepts and the analysed parameters. For a more fine grained sample, or a sieved fraction, the uncertainty of the normalised result is much lower than for normalisation of a coarse grained sample to the agreed cofactor content and will result in a more accurate result. The model presented also applies to the normalisation of organic contaminants using organic carbon but in that case the intercepts N_x and C_x will not differ significantly from zero.

Principally, the result allows comparison of data of total and sieved samples, irrespective of the sieving diameter but the error has to be taken into account. Through propagation of errors the standard error of the result can be calculated from the analytical variation and the natural variation of the intercept N_x (Smedes *et al*., 2005). Results can therefore always be reported with a standard deviation.

6. Considerations on co-factors

The **clay mineral content** is the most important cofactor for trace metals. In the model above the N_x will be zero for clay and only the intercept due to the content of the trace metal in the coarse fraction (C_x) has to be taken into account. However, current intercomparison exercises do not include this parameter. Presently other parameters such as aluminium or lithium are used to represent the clay content.

The **aluminium** content in the sandy fraction may vary from area to area. For some areas aluminium contents in the sandy fractions are found at the same level as found in the fines (Loring, 1991) and therefore the intercept N_x becomes very high. In equation (3) this implies that the denominator is the result of subtracting two large numbers, that is the normaliser content in the sample (N_s) and the normaliser content in only sand (N_x). Consequently, due to their individual uncertainties, the result has an extremely high error. Obviously, normalisation with low intercepts is more accurate. Much lower intercepts are found if partial digestion methods are used that digest the clay minerals, but not the coarse minerals. Using partial digestion, the spatial variability of the results of aluminium analyses in the sandy fraction has been found to be much smaller than with total methods. Although normalising concentrations of contaminants in fine grained material will always give more accurate results, an error calculation will identify whether using coarse samples (and total methods, e.g. HF, X-ray fluorescence, litium tetraborate fusion) allows the requirements of the programme to be met.

For most areas the **lithium** content in the sandy fraction is much lower than in the fine fraction. In addition, results from partial digestion and total methods do not differ significantly. There is only little spatial variability of the lithium content in the sandy fraction. Generally, compared to aluminium, more accurate normalised data can be expected using lithium.

As for clay, no intercept (N_x) applies for organic matter, which is usually represented by **organic carbon**. Organic matter also occurs in the coarse fraction but is even then a cofactor that contributes to the affinity for contaminants, whereas the aluminium in the coarse fraction does not. Furthermore, organic matter in a sample is not always well defined as it can be composed of material with different properties. The most variable properties will be found in the organic matter present in the coarse fraction and not associated with the fines. In **fine sediments** or in the sieved fine fractions the majority of the organic matter is associated with the mineral particles and it is assumed to be of more constant composition than in the total sample. In addition, the nature of the organic matter may show spatial and temporal variations. For samples with low organic carbon content close to the detection limit, normalisation using this cofactor suffers from a large relative error. This results from the detection

limit and the insufficient homogeneity that cannot be improved due to the limited intake mass for analysis.

For further interpretation of data the **proportion of fines** determined by sieving can be useful. Provided, there are no significant amounts of organic matter in coarse fractions, the proportion can be used as a co-factor, particularly for organic contaminants. The error in the determination of fines has to be taken into account and will be relatively high for coarse samples.

7. Considerations on contaminants

Almost all trace metals, except mercury and in general also cadmium, are present in the coarse mineral matrix of samples. The metal concentrations show a spatial variability depending on the origin of the sandy material. In sandy sediments, partial digestion techniques result in lower values than are obtained from total digestion techniques. This implies that partial digestion results in lower intercepts (pivot point is closer to the zero). However, the partial digestion must be strong enough so the clay will be totally digested (as is the case with HF digestion techniques), and the measured aluminium content remains representative for the clay. It was demonstrated that analyses of fine material gave similar results for several trace elements using both total and strong partial methods (Smedes *et al*. 2000; Kersten and Smedes, 2002, cf. Technical Annex 6)

For organic contaminants the situation is more complex. In general, correlations of organic contaminants with organic carbon have no significant intercept, i.e. the contaminants are primarily associated with organic matter. Thus, for sediment samples that contain low concentrations of organic carbon (e.g. very sandy sediments), concentrations of these contaminants can be below or very close to the analytical detection limit. Application of the normalisation procedure using organic carbon to such samples is inappropriate, since it will greatly magnify the analytical error. The influence of these errors can be minimised by analysing muddy sediments, or by analysing a fine fraction sieved from the bulk sandy sediment.

In some cases, PAHs in sediment are found associated with materials such as soot or ash. Concentrations of PAHs can be quite high in these materials, and this can result in high concentrations of PAHs in grain size fractions where soot, ash, etc. are concentrated. These materials generally are present in small quantities, and the PAHs associated with them have little biological activity, and therefore are of limited environmental significance. Although the available data are not comprehensive, existing information indicates that PAH concentrations in sieved fine fractions (e.g. <63 um) are not significantly affected by the presence of small amounts of soot, ash etc.

8. Isolation of fine fractions for analyses

The Sample preparation

Samples should be sieved at 2 mm as soon as possible after sampling to remove large detritus and benthic organisms. Otherwise during further sample handling like storage, freezing or ultrasonic treatment, biotic material will deteriorate and become part of the sediment sample. Until the final sieving procedure that isolates the fines, the sample can be stored at 4°C for about a week and up to 3 months when frozen at –20°C, although direct wet sieving is preferred. For prolonged storage freeze-drying of samples can be considered. In this case contamination and losses of contaminants during freeze-drying have to be checked. Air-drying is not appropriate due to high contamination risks and checks are needed. Besides, samples may be difficult to be disaggregated and mineral structures may be affected.

Requirements for Sieving

A wet sieving procedure is required to isolate the fine-grained fractions, <63 µm or <20 µm. Wet sieving re-suspends fine particles that would otherwise remain attached to coarser particles in the sample. Sediments should be agitated during sieving to prevent to disaggregate agglomerates of fines and to prevent clogging of the mesh. Freeze-dried samples need to be re-suspended using ultrasonic treatment. Seawater, preferably from the sampling site, should be used for sieving as it reduces the risk of physico-chemical changes in the sample i.e. losses through leaching or contamination. Furthermore seawater assists the settling of fine particles after the sieving. If water from the sampling site is not available, then seawater of an unpolluted site, diluted with deionised water to the required salinity, can be used. The amount of water used for sieving should be kept to a minimum and be reused for sieving subsequent batches.

To minimise or prevent contamination it is recommended to use large sample amounts of sediment for sieving. No significant contaminant losses or contamination was detected when at least 25 g of fine fraction is isolated.

Methodology

Both automated and manual methods are available for sieving. A video presentation of these methods can be provided by the QUASH Project (QUASH 1999).

- The automatic sieving method pumps seawater over a sieve that is clamped on a vibrating table (Klamer *et al*. 1990). The water passing the sieve is led to a flow-through centrifuge that retains the sieved particles and the effluent of the centrifuge is returned to the sieve by a peristaltic pump. Large sample amounts, up to 500 g, can be handled easily.
- The second method is a manual system sieving small portions 20-60 g using an 8-cm sieve in a glass beaker placed in an ultrasonic bath (Ackermann *et al*. 1983). Particles are isolated from the water passing the sieve by batch wise centrifugation. The water can be reused for a subsequent batch of sediment. In case of sandy samples, when large amounts of sediments have to be sieved, removal of the coarse material by a pre-sieving over e.g. 200-µm mesh can facilitate the sieving process.

Isolated fine fractions have to be homogenised thoroughly, preferably by a ball mill, as centrifugation produces inhomogeneous samples due to differences in settling speed of different grain-size fractions.

9. Limitations of normalisation

The purpose of normalisation is to reduce the variability between samples arising from differences in bulk sediment properties. However, it has been observed in some areas that the correlations between contaminant and cofactor concentrations may be weak or even absent. This may happen, e.g., if the cofactor used is inappropriate for the contaminant of concern, the degree of contamination is very variable with time or space, or there is significant additional variance arising from the measurements of the concentration of the chosen cofactor.

Contracting Parties may specify additional cofactors other than Al, Li or TOC to be used for the normalisation of concentrations of particular contaminants in their monitoring data. The effectiveness of the normalisation should be accessed through the effect of application of normalisation on the residual variance about time series, as described above. When making proposals, it will be necessary for Contracting Parties to ensure that pivot values and Background Concentrations expressed in relation to the same cofactors are also available.

Current procedures applied in OSPAR trend assessments include the application of smoothers or, for short time series, linear regressions. Normalisation using cofactors should be applied if this results in

a reduction of the residual variance around the fitted smoother or regression in time series, but should not be applied if the residual variance is not reduced. In case the residual variance can be reduced for time series, normalisation should also be applied to check whether observed concentrations of contaminants are at or close to Background (Assessment) Concentrations and whether they comply with the Environmental Assessment Criteria.

Furthermore, as the composition of sand-sized material may differ significantly between different parts of the Convention area, **pivot values** (cf. Section 5) can vary too. In addition, they can vary with the analytical method, i.e. with partial or total digestion for metals analysis. The use of inappropriate pivot values could have significant impact on the calculated normalised concentrations (cf. Section 5), particularly for sediment samples containing relatively small proportions of fine grained material. Therefore, Contracting Parties may propose derived pivot values appropriate to particular parts of the Convention area to OSPAR/ICES for review. Such regionalised pivot values should be applicable over large parts of the Convention area, for example across entire (sub-)Regions

The current **Background (Assessment) Concentrations** may be inappropriate for application throughout the Convention area, as they were derived from a data set that emphasises the northern part of the Convention area. In addition, the Background Concentrations are currently expressed as normalised values (to 5% aluminium for metals and 2.5% TOC), and these "reference" values for the cofactors may not be appropriate for all areas. The use of inappropriate values for Background Concentrations could result in misleading assessments, e.g., as to whether concentrations in sediment are at or close to background. Therefore, Contracting Parties may propose derived Background Concentrations and associated cofactor values that they consider to be appropriate to particular parts of the Convention area. OSPAR/ICES will review if the combinations of Background Concentrations and associated cofactor values are consistent with the way in which pivot values to be used in the assessment of the field data are expressed, to allow the construction of straight lines joining pivot values and Background Concentrations. Such regionalised Background Concentrations should be applicable over large parts of the Convention area, for example across entire (sub-)Regions

10. Recommendations

1. For monitoring, it would be ideal to analyse samples with equal composition. This could be confirmed by determination of co-factors Al, Li, TOC and parameters of the grain size distribution (e.g. clay content, proportion <20µm, proportion <63µm). However, this situation will seldom occur.

2. New temporal trend programs should be carried out by the analysis of fine sediments or a fine-grained fraction, isolated by sieving. Existing temporal trend programs could be continued using existing procedures, provided that assessment of the data indicates that the statistical power of the programs is adequate for the overall objectives.

3. Contaminant concentrations in whole sediments can be subjected to normalisation using co-factors for organic matter, clay minerals etc., by taking into account the presence of both co-factors and target contaminants in the mineral structure of the sand fraction of the sediment. Taking into account these non-zero intercepts of regressions of contaminant concentrations with co-factors, normalisation to preselected co-factor content will reduce the variance arising from different grain sizes. Normalised values for sandy sediments will have greater uncertainties than for muddy sediments. The propagated error of the variables used for normalisation may be unacceptably high for sandy sediments, if both contaminant and co-factor concentrations are low, particularly when approaching detection limits. In that case, in order to reduce the overall uncertainty, alternative procedures, such as sieving, need to be used to minimise the impact of this error structure.

4. The natural variance of sample composition will be smaller in the fraction <20 µm than in the fraction <63 µm. Therefore, for trace metals, the fraction <20 µm should be preferred over the fraction <63 µm. However, separation of the fraction <20 µm can be considerably more laborious than the separation of the fraction <63 µm and might be an obstacle to its wide application. For this practical reason, the fraction <63 µm is an acceptable compromise for monitoring programmes. For organic contaminants, the fraction < 63 µm should be used for analyses, as it may be difficult to incorporate the organic matter with the highest binding capacity for organic contaminants in the fine grained fraction < 20 µm completely. Thus, variances due to separating the fine fraction can be reduced.

5. There will still be some residual variance arising from differences in the composition (mineralogy and organic carbon content) of the sediments. Therefore, the preferred approach is analyses of contaminants in fine sediments or in the fraction <63 µm, followed by normalisation of analytical results using cofactors (see section 3). Current scientific knowledge indicates that this procedure minimises the variances arising from differences in grain size, mineralogy and organic matter content. Application of this two-tiered approach to fractions < 20 µm gives results that can be directly compared to results found by normalisation of concentrations measured in fractions < 63 µm.

6. In order to clarify aspects of data interpretation, analytical data for field samples should be accompanied by information on limits of detection and long term precision. In order to contribute to environmental assessment, data for field samples should include the grain size distribution, as a minimum the proportion of the analysed fraction in the original whole sediment. Aluminium (Al) and total organic (TOC) concentrations should be reported for use as potential cofactors. If possible, the determination of Li as an additional potential cofactor is recommended.

7. In order to take into consideration potential regional differences in sediment composition in monitoring contaminants in sediments and its assessment, cofactors others than those mentioned in section 3 may be used. Furthermore, regionalised pivot points for calculating normalised contaminant concentrations as well as regionalised Background (Assessment) Concentrations may be derived for different regions.

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12. Appendix: Testing normalisation methods

As normalisation should correct for sediment composition, a criterion for an adequate normaliser is that after normalisation of equally polluted sediment samples with different grain size distributions, the results should not differ significantly. However, sample sets to test normalisation approaches for this criterion are scarce. An alternative approach is to take one sample and to produce subsamples with varying grain size distributions (Smedes 1997, Smedes *et al*. 1997, Smedes *et al*. 2000). Both the fine and coarse subsamples are analysed for contaminants and potential normalisers. In this way a higher variability for the normaliser concentrations, i.e. a worst case than ever will occur in nature, can be obtained which provides a sensitive test for the usefulness of potential normalisers.

Normalisation is intended to correct for sediment composition for sediments that are equally polluted. Here equally polluted means that the sediments are in equilibrium with the same water. Normalised results should not significantly differ for sediment samples with different grain size distributions
To test which parameters, i.e. co-factors, are suitable for a certain area, a set of equally polluted samples should be collected. In practice this is often problematic as often pollution is not homogeneous in the area and/or the range in grain-size that can be collected is too limited to properly demonstrate relations between co-factors and contaminants.

However, this can also be addressed through an active approach which is applicable to all areas, excepting areas where sediment is dominated by only sand or gravel. Smedes *et al*. 1997 used pairs of sieved and un-sieved samples to test co-factors. In the EU QUASH project, survey and intercalibration samples were actively separated in different grain-size fractions (Smedes *et al*., 2000). To adopt this approach, the following procedure is suggested. A large sediment sample, 3 litre or more, containing sand as well as fine material, is taken. This sample is transferred to a glass bottle and liquefied using local water and then shaken, tumbled or mixed for at least one month. The sediment is then separated into subsamples with different grain-size compositions by sieving and decantation. A range of fractions can be separated, for example <20µm, <63µm, >63µm etc and, of course, also the un-fractionated sediment is part of the set.

A decantation procedure will give another type of sample. Suspend the sediment in the local water used for shaking and wait a short time to allow the coarse material to settle. Then quickly pour off

part of the upper water into a second container. Let the particles settle and pour the water back, with the residual coarse material, and repeat until sufficient fine material has been separated for analysis. It is also possible to sieve only at 20µm and mix the <20µm and the >20µm in different proportions creating a series of equidistant compositions.

It is suggested that the approach described above also includes the estimation of pivot values. Therefore a portion of coarse sediment, i.e. >63µm, is treated with ultrasonic so fine material attached to the coarse sediment is released. This fine material is washed out and if sufficient can be analysed also. The coarse sample is added to the sample set.

Results from such an exercise are given below. Here 10 kg sediment from 6 different positions was equilibrated by tumbling for 3 months in excess water. In this research project, organotin compounds were added to investigate their distribution over grain-size fractions (Smedes and Nummerdor, 2003[1]). In Figure 1–4, the relations of co-factors and some metals are given for several stations. The extreme differences in composition caused by the separation process allow demonstration of the relations over large concentration ranges. Also, some rather extreme samples (like very coarse floating material that was sometimes present in low quantities, typically 0.1–0.5%) were isolated when present. This material had a very high organic carbon content, very low mineral cofactors and a higher OC/N ratio than the rest of the fractions. In Figure 4, this sample is the outlier (open symbols) in the relations. Basically these fractions have no meaning as they are of very low abundance but they give some indication of whether target elements or compounds show a preference for organic carbon, although it should be considered it is not the typical OC as is normally found from humic and fulvic residues.

Values close to the origin also allow derivation of pivot values, although this was not the focus of the QUASH project. Figure 5 shows cofactor and zinc data for all stations in the fraction >63µm; only a few of these were ultrasonically treated. For the Dutch coastal area, the pivot value for Zn can be estimated at about 14–15 mg/kg and for Al and Li at around 4 g/kg and 4 mg/kg respectively.

Application of this process will provide robust information and allow optimization of normalisation for a certain location and will show what bias or variability may be expected from the use of non-regionalised values for pivot points in the procedure for normalisation.

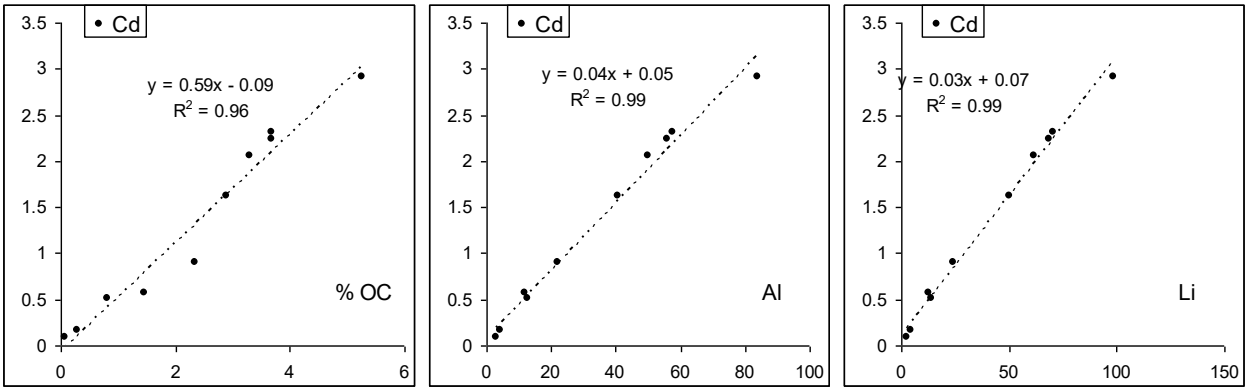

Figure 1 Cd and cofactors in Rotterdam harbour area.

[1] Smedes, F., and Nummerdor, G.A.,. 2003. Grain-size correction for the contents of butyl compounds in sediment. RIKZ\2003.035. ISBN 03693477x, National Institute of Coastal and Marine Management (Rijksinstituut voor Kust en Zee), RIKZ, PO Box 20907, 2500 EX, The Hague, The Netherlands.

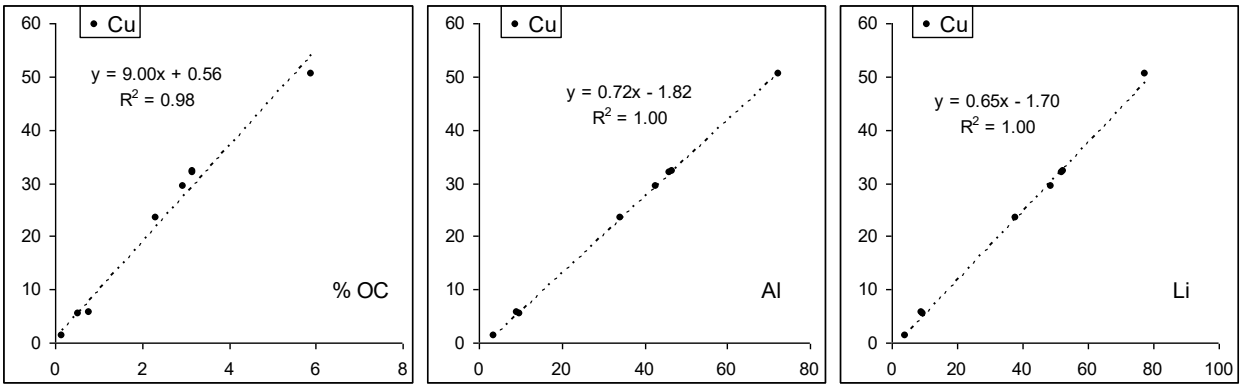

Figure 2. Cu and cofactors in Nieuwe Waterweg towards Sea.

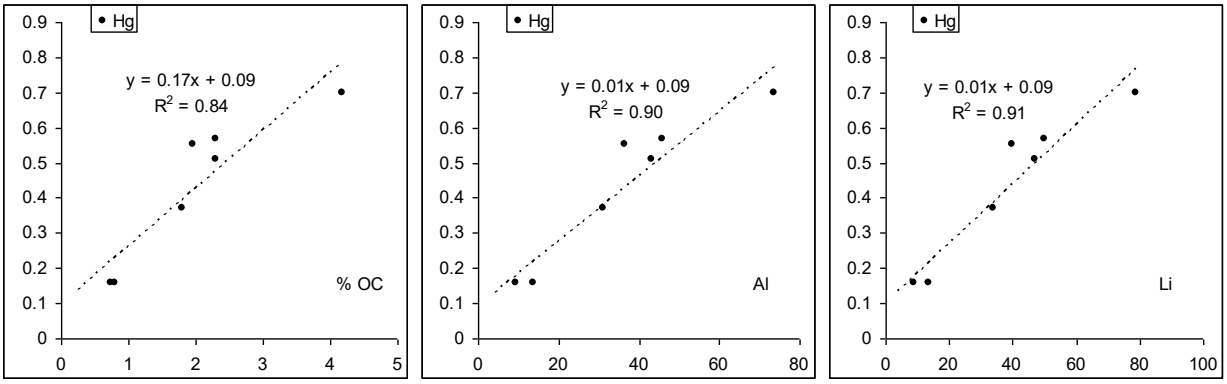

Figure 3. Hg and cofactors in sludge dump site at Sea.

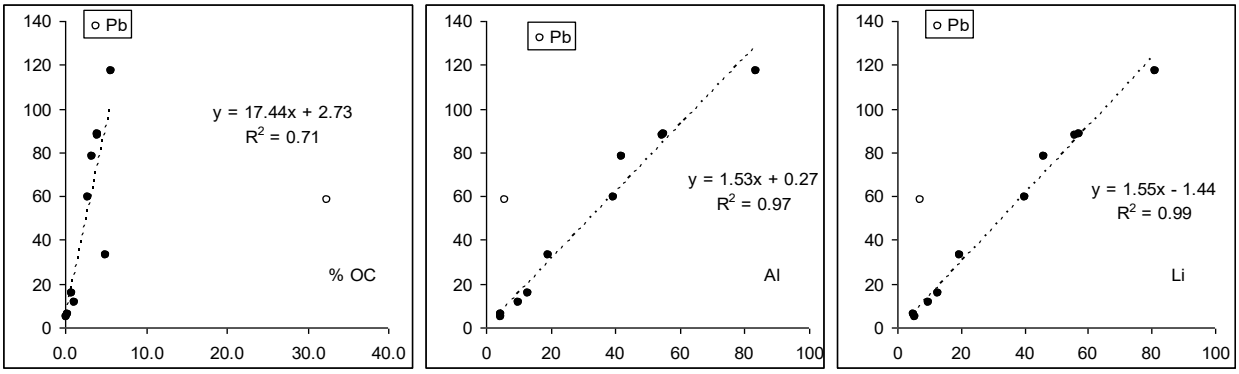

Figure 4. Pb and cofactors in front of IJmuiden Harbor. Note that one outlier is omitted. This is from coarse floating material with high OC content and representing less than 0.2% of the sample weight.

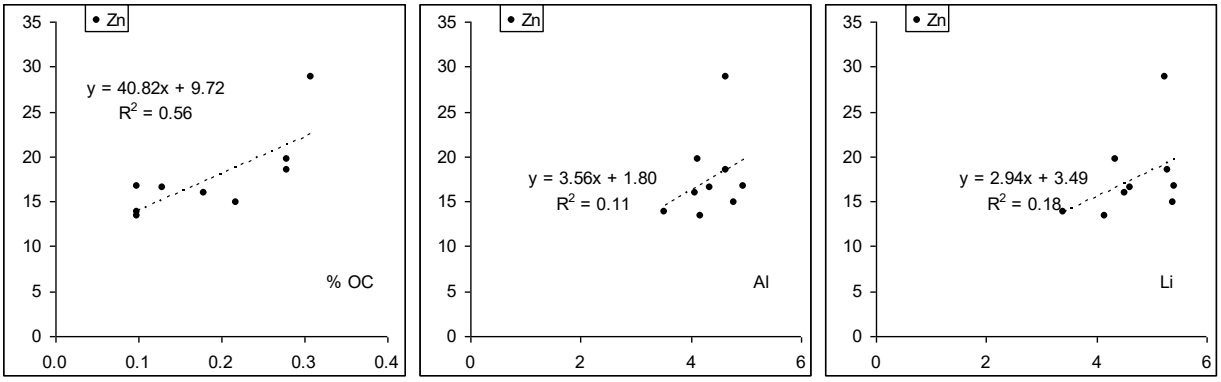

Figure 5. Zn and cofactors in only the >63 samples.

Annex VI:

UNEP/IAEA (2011). Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment. Reference Methods for Marine Pollution Studies No 71 (4.2.1)

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 1er
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 $KMnO_4$) or equivalent quality, demonstrated to be free from interfering substances.
- Detergent.
- Potassium dichromate.
- HCl. 32%.
- Concentrated H_2SO_4 (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 Id_4.
- n-C_{14} d_{30}, n-C_{19} d_{40}, n-C_{32} d_{66}.
- Naphthalene d_8.
- 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 mg/100 ml ⇒ 0.52 mg/ml ⇒ 520 µg/ml ⇒ 520 ng/µl

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 (Na_2SO_4)*, 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.

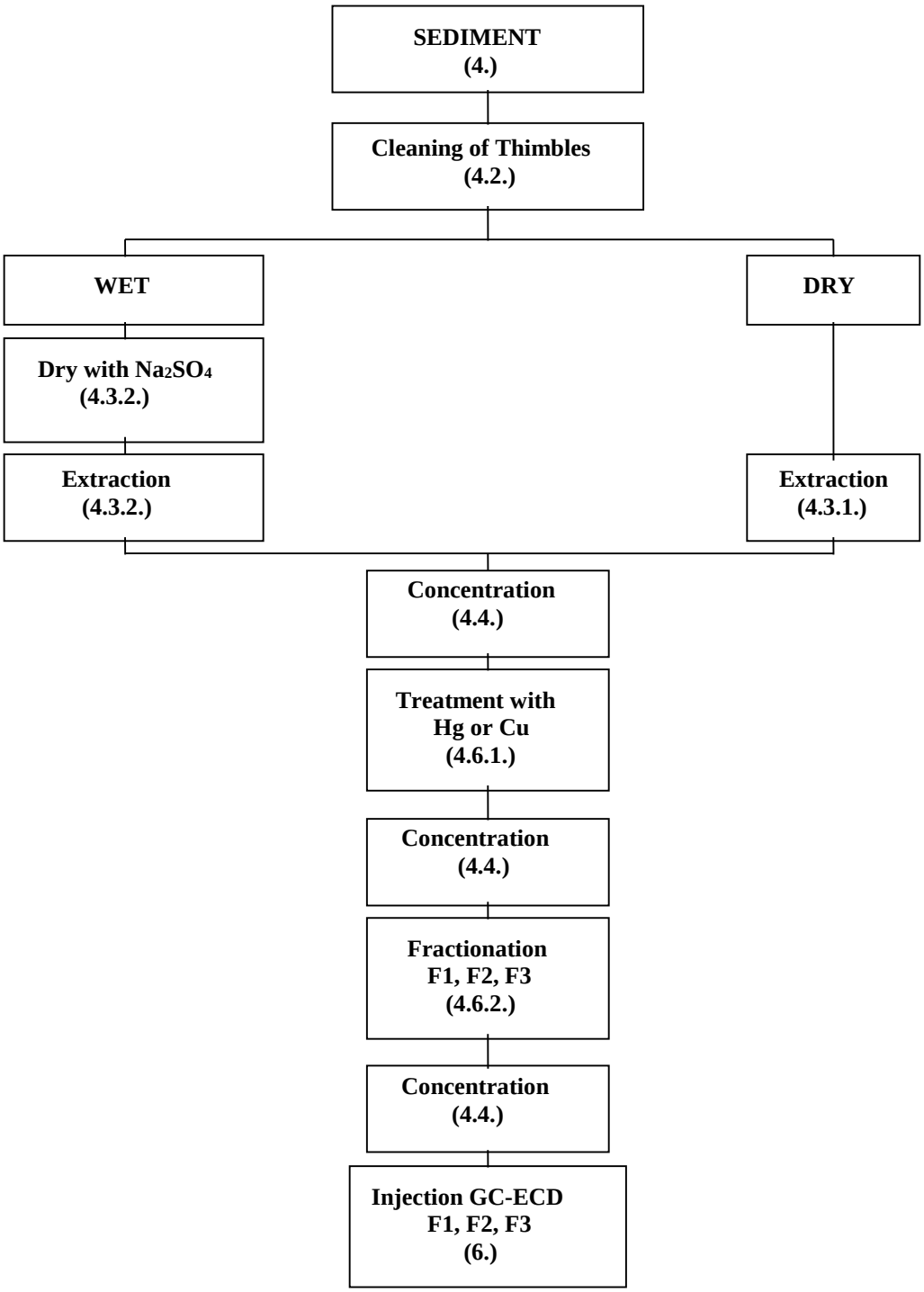


Diagram of the extraction procedure for sediment samples.

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 Id_4 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.

$$\% \text{ Sample weight} = \frac{\text{Sample dry weight}}{\text{Sample wet weight}} \text{ X } 100$$

$$= \frac{0.4979}{1.8555} \text{ X } 100 = 26.8 \%$$

Calculate the percent moisture.

Water content = wet weight - dry weight
= 1.855 g - 0.4979 g = 1.3576 g

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

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

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:

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

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:

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

With 4.443 g of sample extracted:
80.5 mg/ 4.443 g = 18.1 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

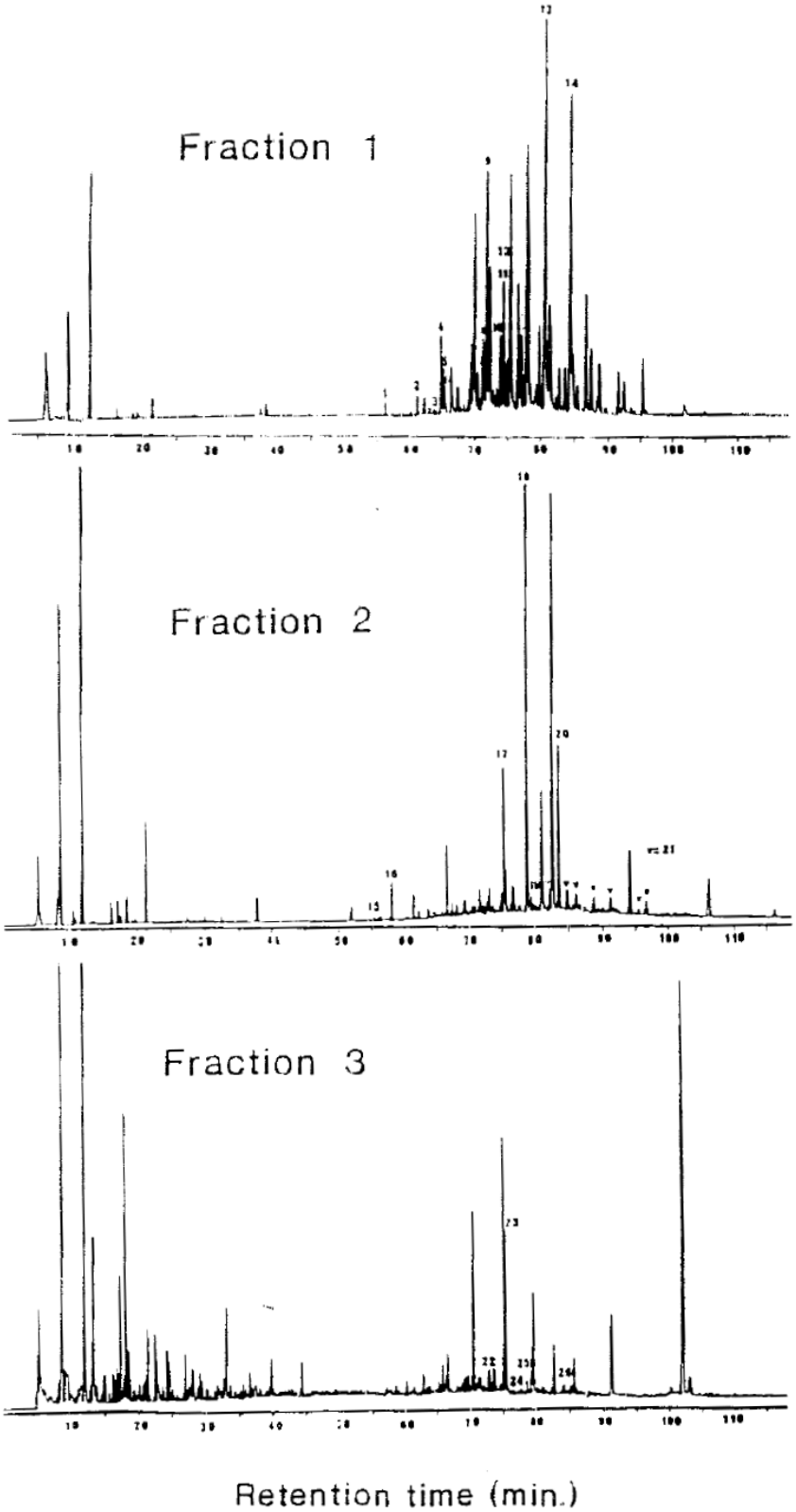
A Florisil column is used for this fractionation, which is prepared in the following way. The Florisil should be pre-extracted in the Soxhlet apparatus to remove any contaminants, using methanol or dichloromethane for 8 hours, followed by hexane for another 8 hours. It is then dried in an oven. Activation 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.



Peak Number	Compounds
1	H.C.B
2	Internal Standard 2, 4, 5 trichlorobiphenyl (IUPAC No : 29)
3	Heptachlor
4	2,2',5,5' tetrachlorobiphenyl (IUPAC No : 52)
5	2,2',4,5' tetrachlorobiphenyl (IUPAC No : 49)
6	Aldrin
7	DDMU
8	op DDE
9	2,2',4,5,5'pentachlorobiphenyl (IUPAC No : 101)
10	2,2',3',4,5 pentachlorobiphenyl (IUPAC No : 97)
11	pp' DDE
12	2,2',3,3',6,6' hexachlorobiphenyl (IUPAC No : 136)
13	2,2',4,4',5,5' hexachlorobiphenyl (IUPAC No : 153)
14	2,2',3,4,4',5' hexachlorobiphenyl (IUPAC No : 138)
15	Alpha-HCH
16	Lindane
17	op DDD
18	pp' DDD
19	op DDT
20	pp' DDT
21	Toxaphene
22	Alpha-endosulfan
23	Dieldrin
24	Endrin
25	Beta-endosulfan
26	Endosulfan sulfate

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.

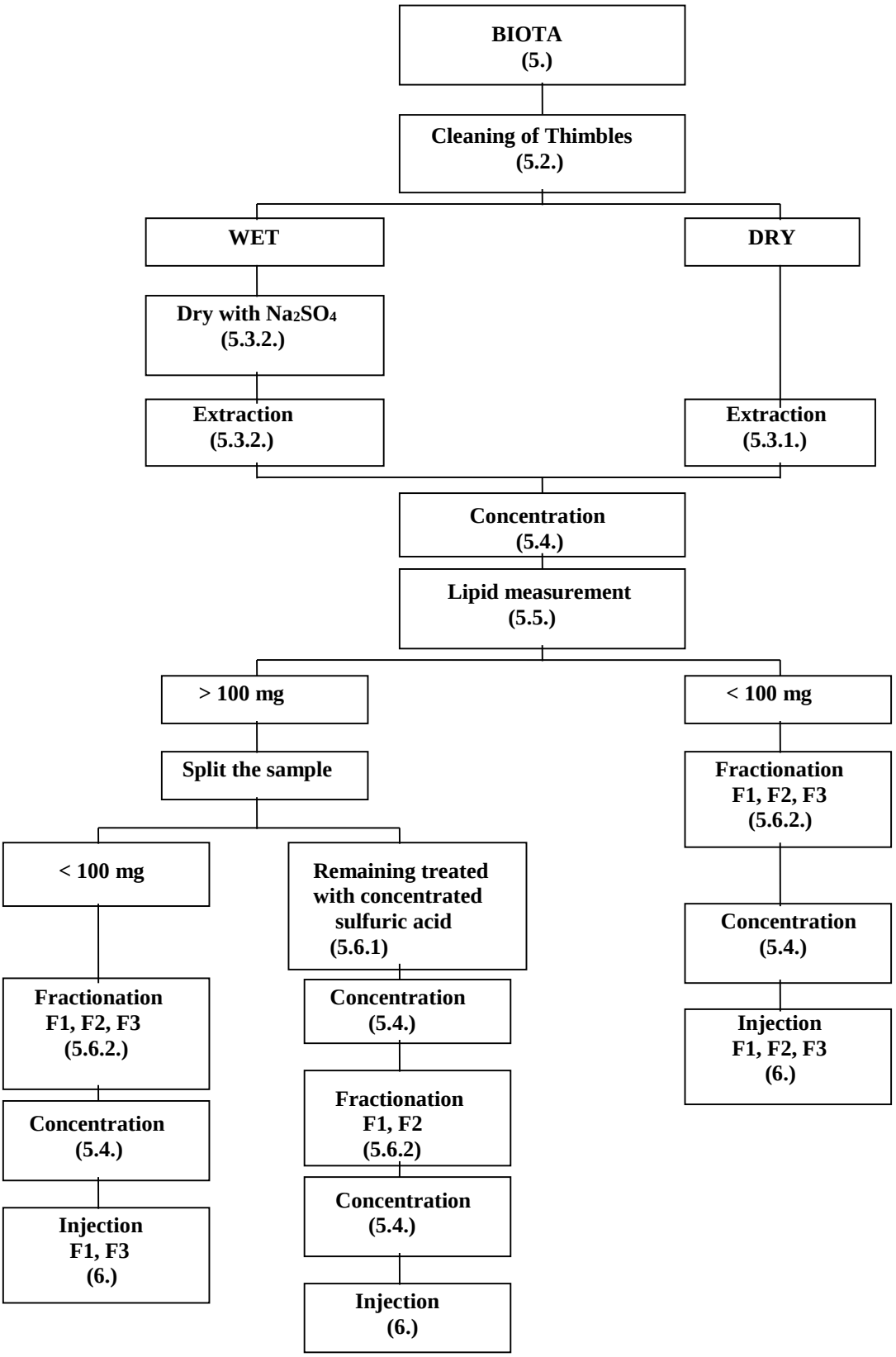


Figure 3: Diagram of the extraction procedure for biota samples.

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 Na_2SO_4 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 H_2. 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:

- H_2 or He carrier gas at inlet pressure of 0.5 to 1 Kg/cm^2 to achieve a flow rate of 1 to 2 ml/min.

- Make-up gas N_2 or Ar/CH_4 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 H_2 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:

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

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

$$\mathrm{HEPT} = \frac{\mathrm{L}}{\mathrm{N}}$$

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, Phenoclors) 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
Operator    : jpv
Acquired    : 12 Jul 95   8:02 am using AcqMethod OC
Instrument  :   5989B
Sample Name: standard ar1254
Misc Info   :
Vial Number: 1
```

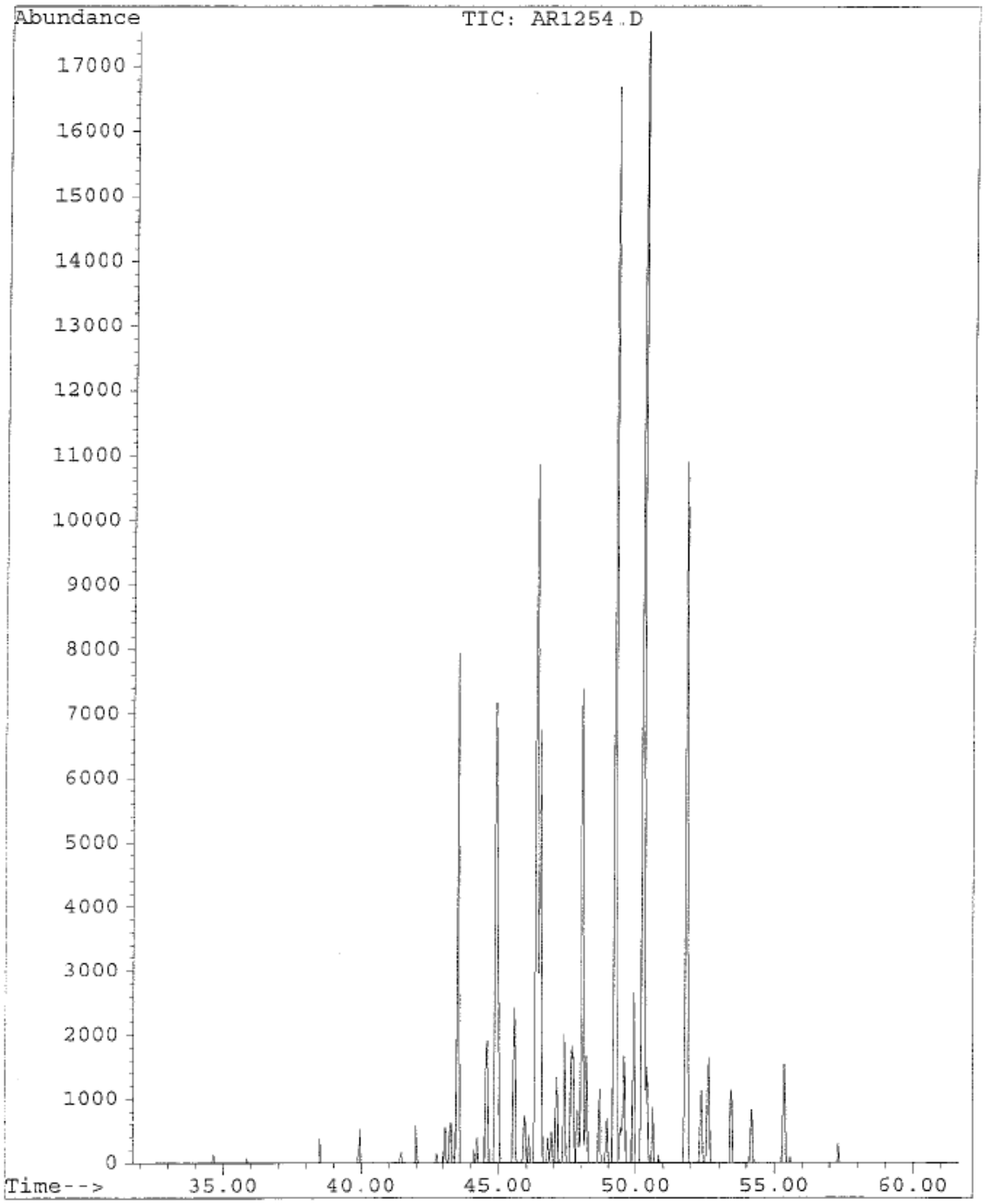


Figure 4: TIC of Aroclor 1254

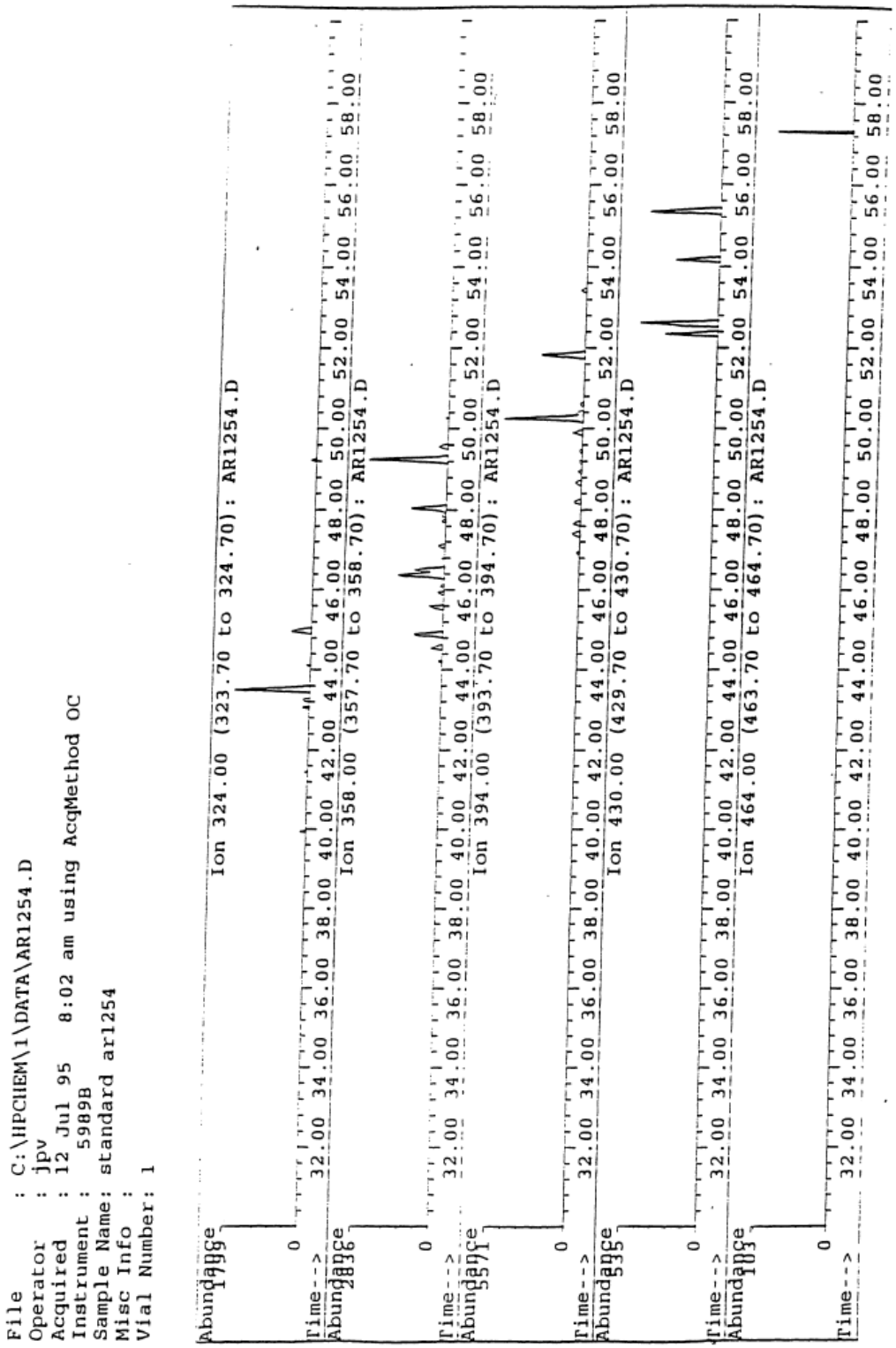


Figure 5: RIC of Aroclor 1254 main compounds

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

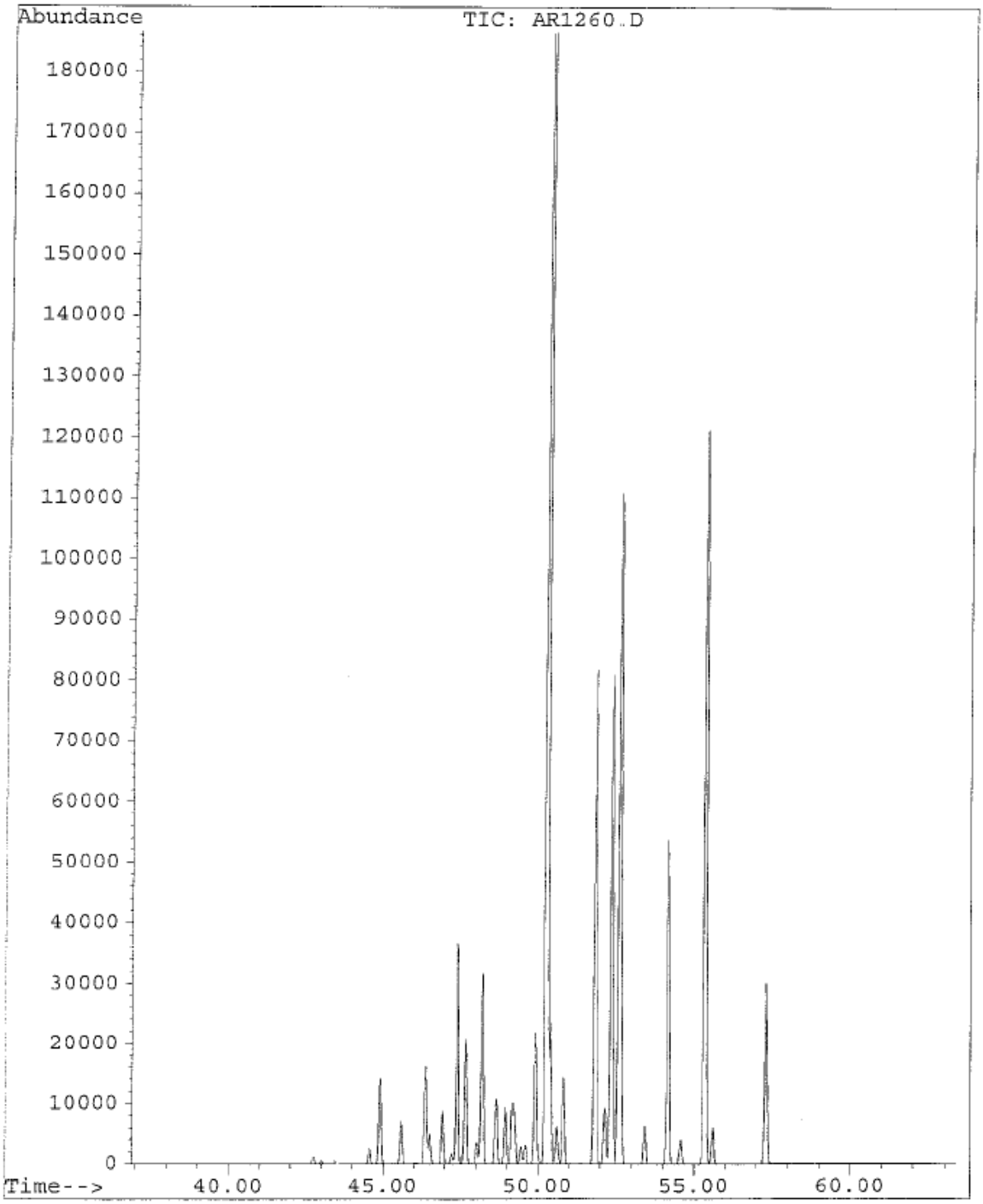


Figure 6: TIC of Aroclor 1260

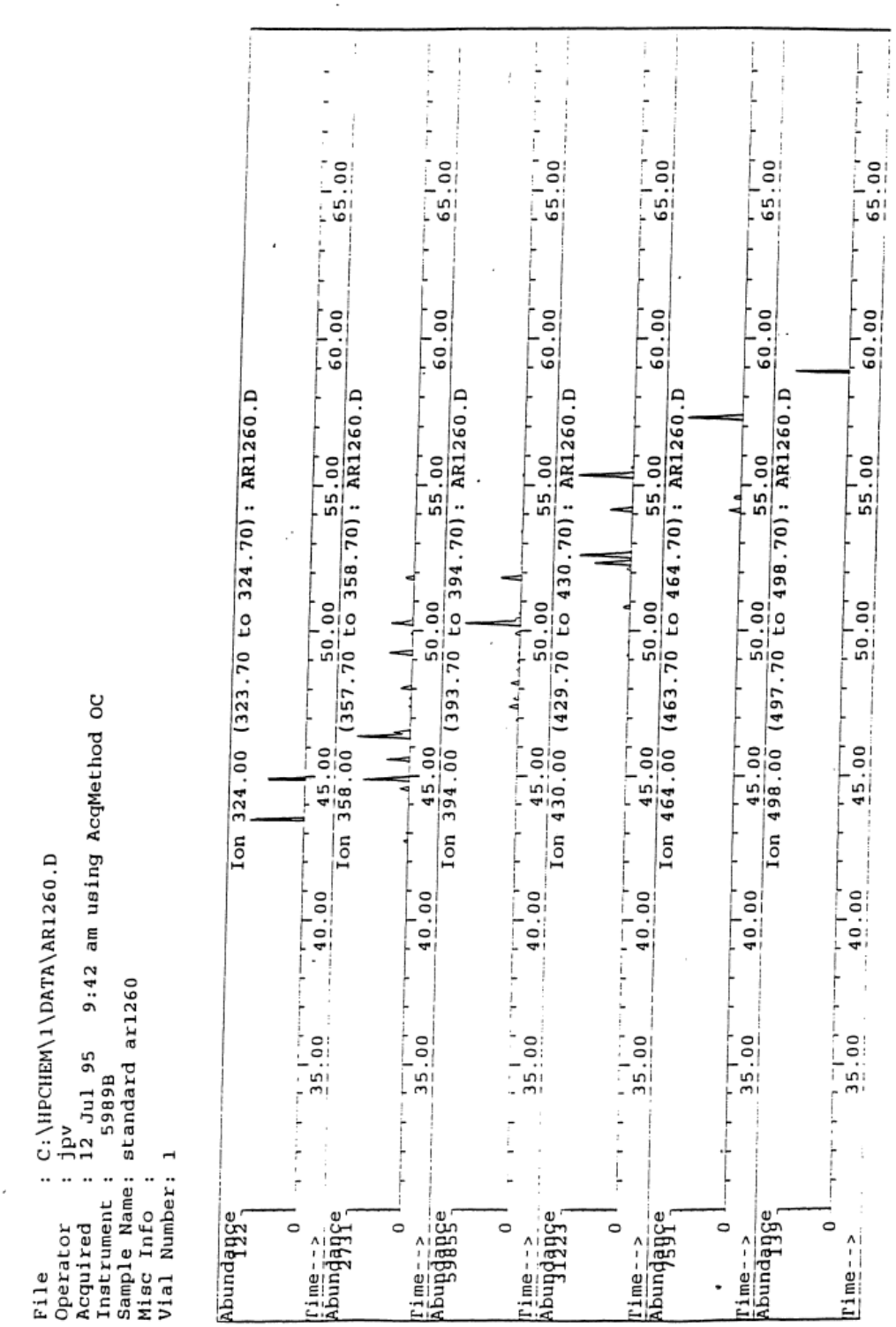


Figure 7: RIC of Aroclor 1260 main compounds

7.2. Example of a selected ion monitoring programme useful for quantitative analysis of chlorinated compounds.

Compounds	Fraction Nº 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-C_{14} d_{30}, n-C_{19} d_{40}, n-C_{32} d_{66}, 2) polycyclic aromatic hydrocarbons: Naphthalene d_8,

Hexamethylbenzene, Cadalene (deuterated PAHs are also useful), 3) organochlorine compounds: PCB congeners 29, 30, 121 or 198, ε HCH and Endosulfan Id_4, 4) sterols: 5 α (H)-androstan-3β-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. N_2O and CO_2) 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 CO_2 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 CO_2. 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.

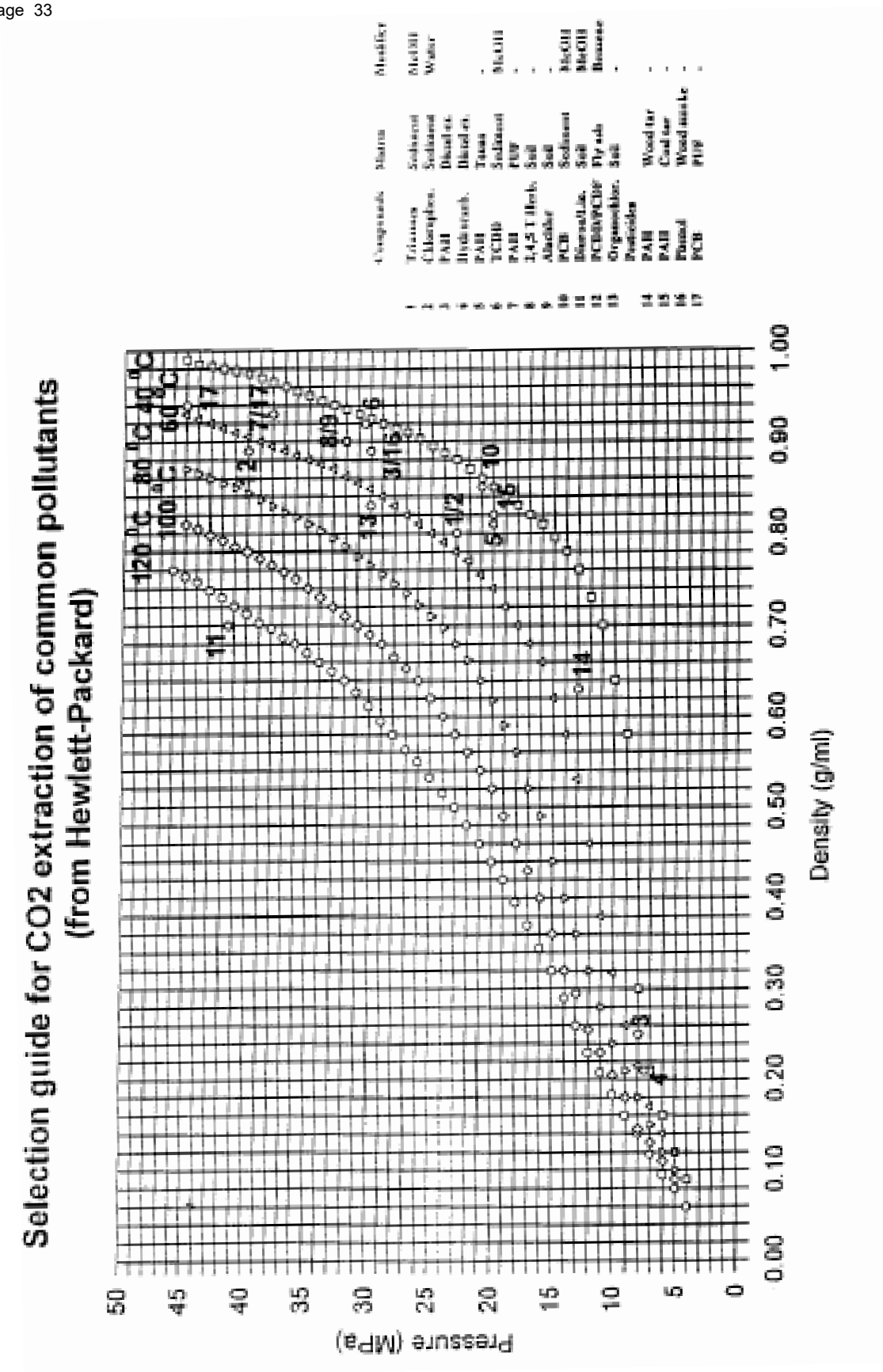


Figure 8: Guide for CO_2 extractions

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, see10.1.) 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 carrousel 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 carrousel 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

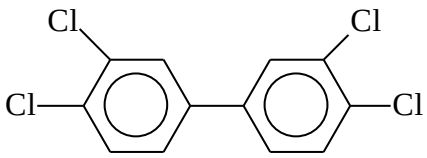
The residence time of total DDT in the environment is relatively short (t1/2 = 3-5 years), so, at least 75-80 % of the current total DDT should be in the form of DDE or DDD if it was introduced into the environment before the 1975 ban. Values of Henry's law constant indicate that these compounds can 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.

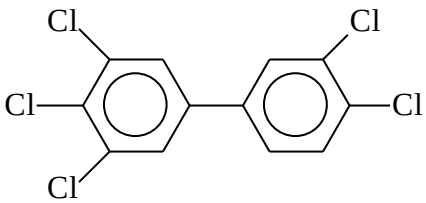
10.2. PCBs congeners

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.

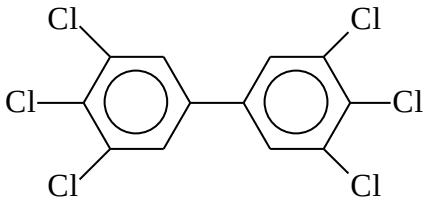
Recently, attention has been paid to congeners having 2 para-chlorines and at least 1 meta-chlorine. These congeners are called "coplanar" PCBs. Among the 209 congeners, 20 members attain coplanarity due to non-ortho chlorine substitution in the biphenyl ring. Three of these show the same range of toxicity as the 2,3,7,8 tetrachlorodibenzo-p-dioxin and the 2,3,7,8 tetrachlorodibenzofuran, these are the IUPAC Nº: 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).



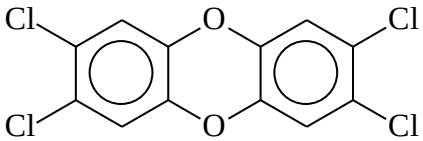
3,3',4,4' tetrachlorobiphenyl
IUPAC Nº: 77



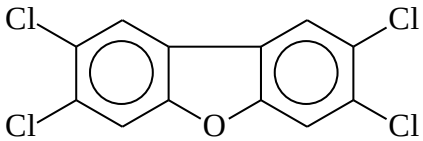
3,3',4,4',5 pentachlorobiphenyl
IUPAC Nº: 126



3,3',4,4',5,5' hexachlorobiphenyl
IUPAC Nº: 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 Nº	Clophen A50	Aroclor 1254	PCB Nº	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 Nº 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 Nº29, ...pg PCB Nº198, ...pg ε HCH and ….. pg Endosulfan Id_4 were added as internal standard.

The .....ml extract was reduced by rotary evaporator to approximately .....ml.

This was treated with sodium sulfate to dry the extract. Then treated with mercury to remove sulphur. This was further reduced to .....ml for lipid determinations. Corrected dry wt. : .....g.

Lipid determinations:

.....ml total extract;

10 µl 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 Nº29 : .....ng recovered in F1 : .....% Recovery.

PCB Nº198 : .....ng recovered in F1 : .....% Recovery.

ε HCH : .....ng recovered in F2 : .....% Recovery.

Endosulfan Id_4: .....ng recovered in F3 : .....% Recovery.

Attach tabulation of individual compounds quantified in sample.

Sample worksheet for analysis of chlorinated compounds in marine sediments.

12. REFERENCES

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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/µl) 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/µl of PCB No 29

Stock Solution of Endosulfan I d4:

1 ml from the original vial (250ng/µl) 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/µl of Endosulfan I d_4

Working solution of internal standards:

0.5 ml from the stock solution of PCB No 29 (2.5 ng/µl) should be transferred into a 50 ml volumetric flask, then, 0.5 ml from the stock solution of Endosulfan I d4 (2.5 ng/µl) should be transferred into the volumetric flask, then 1 ml from the original vial (1ng/µl) of ε HCH should be transferred into that volumetric flask, then 0.5 ml from the concentrated solution (2ng/µl) of PCB No 198, and the volume adjusted to 50 ml with hexane. This working solution contains:

25 pg/µl of PCB No 29
20 pg/µl of PCB No 198
20 pg/µl of ε HCH
25 pg/µl 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, Diedrin 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

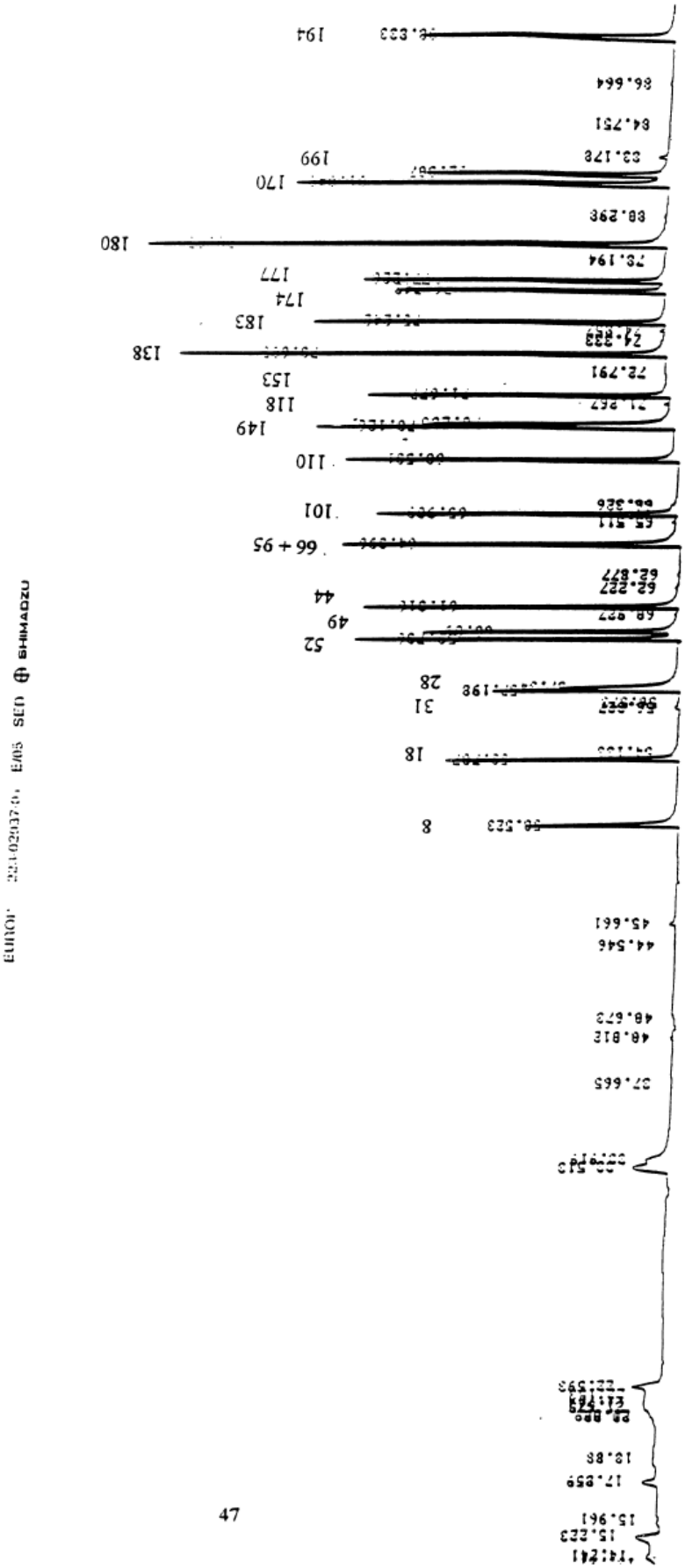
Preparation of the PCB congeners solution

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 Nº:	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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Annex VII:

OSPAR (2008). JAMP Guidelines for Monitoring Contaminants in Sediments. Technical Annex 2: technical annex on the analysis of PCBs in sediments related to Protocol (4.2.2)

OSPAR COMMISSION

Protecting and conserving the North-East Atlantic and its resources

JAMP Guidelines for Monitoring Contaminants in Sediments

Technical Annex 2: technical annex on the analysis of PCBs in sediments

Determination of chlorobiphenyls in sediments - analytical method

1. Introduction

This annex provides advice on (chlorinated biphenyl) CB analysis for all sediment fractions and suspended particulate matter (*e.g.* < 2mm fraction and < 20µm fraction). The guideline is an update of the earlier version (Smedes and de Boer, 1994 and 1997) taking into account evolutions in the field of analytical chemistry and also covering the determination of planar CBs. Basically, these consist of mono-*ortho* (CB105, CB114, CB118, CB123, CB156, CB157, CB167 and CB189) and non-*ortho* substituted CBs (CB81, CB77, CB126 and CB169). When reviewing the literature, it should be noted that planar, coplanar and dioxin-like CBs / PCBs are all equivalent terms. OSPAR SIME has advised that monitoring for planar CBs in sediments should only take place when the concentrations of marker (non-planar) CBs are e.g. 100 times higher than the Background Assessment Concentrations for those compounds.

The analysis of CBs in sediments generally involves extraction with organic solvents, clean-up (removal of sulphur and column fractionation), and gas chromatographic separation with electron-capture or mass spectrometric detection. All stages of the procedure are susceptible to insufficient recovery of analytes and/or contamination. Quality control procedures are recommended in order to check the method's performance. These guidelines are intended to encourage and assist analytical chemists to reconsider their methods and to improve their procedures and/or the associated quality control measures where necessary. Due to the low concentrations of, particularly, non-*ortho* substituted CBs in sediments compared to those of other CBs, their determination requires an additional separation and concentration step. Therefore, in the relevant sections a distinction will be made between the non-*ortho* substituted CBs and the others.

These guidelines can also be used for several other groups of organochlorine compounds, *e.g.* DDTs and their metabolites, chlorobenzenes and hexachlorocyclohexanes. Recoveries in the clean-up procedures must be checked carefully. In particular, treatment with H_2SO_4 results in a loss of some compounds (*e.g.* dieldrin and endosulfanes (de Boer and Wells, 1996). Also, the clean-up procedure with silver ions can result in low recoveries for some pesticides (*e.g.* hexachlorocyclohexanes).

These guidelines are not intended as a complete laboratory manual. If necessary, guidance should be sought from highly specialised research laboratories. Whichever analytical procedure is adopted, each laboratory must demonstrate the validity of each step in the procedure. In addition, the use of a second (and different) method, carried out concurrently with the routine procedure is recommended for validation. Analyses must be carried out by experienced staff.

2. Sampling and storage

Plastic materials (except polyethylene or polytetrafluorethene) must not be used for sampling due to the possible adsorption of contaminants onto the container material. Samples should be stored in solvent washed aluminium cans or glass jars. Aluminium cans are preferred, as glass jars are more susceptible to breakage. Samples should be transported in closed containers; a temperature of 25°C should not be exceeded. If samples are not analysed within 48 h after sampling, they must be stored in the short term at 4°C. Storage over several months or longer should be limited to those samples which have been frozen (< -20°C) and dried samples.

3. Precautionary measures

Solvents, chemicals and adsorption materials must be free of CBs 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 determining the presence of CBs and other interfering compounds by GC analysis. If necessary, the solvents can be purified by re-distillation but this practice is not favoured by most analytical laboratories as they generally opt to buy high quality solvents directly. Chemicals and adsorption materials should be purified by extraction and/or heating. 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. Alternatively, glass thimbles with a G1 glass filter at the bottom can be used. Generally, paper filters should be avoided and substituted by appropriate glass filters. As all super cleaned materials are prone to contamination (*e.g.* by the adsorption of CBs and other compounds from laboratory air), materials ready for use should be held in sealed containers and should not be stored for long periods. All containers, tools, 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. In addition, all glassware should preferably be covered with aluminium foil and stored in cupboards to keep out any dust. Old and scratched glassware is more likely to cause blank problems because of the larger surface and therefore greater chance of adsorption. Furthermore, scratched glassware can be more difficult to clean. All glassware should be stored in clean cupboards, ensuring dust cannot enter (QUASIMEME, 2007)

4. Pre-treatment

Before taking a subsample for analysis, the samples should be sufficiently homogenised.

CBs can be extracted from wet or dried samples, although storage, homogenisation and extraction are much easier when the samples are dry. Drying the samples, however, may alter the concentrations *e.g.* by the loss of compounds through evaporation or by contamination (Smedes and de Boer, 1994 and 1997). Losses and contamination must be accounted for.

Chemical drying can be performed by grinding with Na_2SO_4 or $MgSO_4$ until the sample reaches a free-flowing consistency. It is essential that there are at least several hours between grinding and extraction to allow for complete dehydration of the sample; any residual water will decrease the extraction efficiency.

Freeze-drying is becoming a more popular technique, although its application should be carefully considered. Possible losses or contamination must be checked. Losses through evaporation are diminished by keeping the temperature in the evaporation chamber below 0°C. Contamination during

freeze-drying is reduced by putting a lid, with a hole of about 3 mm in diameter, on the sample container.

5. Extraction

The target compounds must be extracted from the sediment with an organic solvent prior to analysis. Extraction methods do not differ for planar CBs but, because of the low concentrations, a substantially larger sample intake has to be considered. Generally, at least a 100 g sample of freeze-dried sediment is required.

5.1 Wet sediments

Wet sediments are extracted in a step-wise procedure by mixing them with organic solvents. Extraction is enhanced by shaking, Ultra Turrax mixing, ball mill tumbler or ultrasonic treatment. Water miscible solvents are used (especially in the first step) such as methanol, acetone, acetonitrile, *etc.* The extraction efficiency of the first step is low as there will be a considerable amount of water in the liquid phase at that stage. The extraction is continued with a mixture of polar and apolar solvents (*e.g.* acetone/hexane or methanol/dichloromethane). For adequate extraction of target compounds, wet sediments must be extracted with organic solvents at least three times. The contact time with the solvent should be sufficient to complete the desorption of the CBs from the sediment.

When using a Soxhlet, extraction of wet sediments should be done in two steps. A polar solvent, such as acetone, is first used to extract the water from the sediment and then the flask is replaced and the extraction continued with a polar/apolar mixture such as acetone/hexane.

In both cases water must be added to the combined extracts and the CBs must be extracted to an apolar solvent such as hexane.

5.2 Dry sediments

For dried sediments, Soxhlet extraction is the most frequently used technique. A mixture of a polar and an apolar solvent (*e.g.* acetone/hexane) is recommended for efficient extraction; a good choice is 25% acetone in hexane. A greater proportion of polar solvent increases the extraction efficiency, but the polar solvent must be removed prior to gas chromatographic analysis. Extraction can be carried out with a normal Soxhlet or a hot Soxhlet apparatus. A sufficient number of extraction cycles must be performed (ca. 8 h for the hot Soxhlet and ca. 12 to 24 h for normal Soxhlet extraction). The extraction efficiency must be checked for different types of sediments by a second extraction step. These extracts should be analysed separately.

Although the use of binary non-polar/polar solvent mixtures and Soxhlet is still the benchmark for CB extraction, there have been numerous attempts to find alternative procedures, which are less time-consuming, use less solvent and/or enable miniaturisation. Amongst these novel approaches are pressurized liquid extraction (PLE) and related subcritical water extraction (SWE), microwave-assisted extraction (MAE), matrix solid-phase dispersion (MSPD), ultrasound extraction (US) and supercritical fluid extraction (SFE).

From among the techniques mentioned, PLE or Accelerated Solvent Extraction (ASE) has – so far – been most successful. Soxhlet methods are easily translated into PLE as the same solvent compositions can be used. The method further allows interesting modifications that include in-cell clean-up of samples by adding fat retainers, such as florisil or alumina, to the cell, and the use of a small carbon column in the extraction cell, which selectively adsorbs dioxin-like compounds (subsequently isolated by back-flushing with toluene) (Sporring *et al*., 2003). PLE and MAE have the shared advantage over SFE that they are matrix-independent, which facilitates method development and changing-over from

the classical Soxhlet extraction. Recent years have also seen an increased use of ultrasound-based techniques for analytes isolation from solid samples. With most applications, extraction efficiency is fully satisfactory, and sonication time often is 30 min or less (Roose and Brinkman, 2005).

All the methods described above are in principle suitable for extracting CBs from sediments. However, Soxhlet extraction is still the reference for alternative approaches.

6. Clean-up

6.1 Removal of sulphur and sulphur-containing compounds

An aqueous saturated Na_2SO_3 solution is added to a hexane extract. In order to allow the transfer of the HSO_3^- ions to the organic phase, tetrabutylammonium salts (TBA) and *iso*-propanol are then added to the mixture. Water is subsequently added to remove the *iso*-propanol. The aqueous phase must then be quantitatively extracted with hexane (Jensen *et al*., 1977). If the extraction was performed by a polar solvent miscible with water, then a Na_2SO_3 solution can be added directly after extraction. If the extraction mixture also contains an apolar solvent, then depending on the ratio of the solvents, the addition of TBA and *iso*-propanol may or may not be necessary. Any excess Na_2SO_3 and reaction products can be removed by the addition of water and thus partitioning between apolar solvent and water.

Japenga *et al*. (1987) developed a column method for the removal of sulphur and sulphur-containing compounds. The column material is made by mixing an aqueous solution of Na_2SO_3 with Al_2O_3. Some NaOH is also added to improve the reaction with sulphur. Subsequently the material is dried under nitrogen until a level of deactivation equivalent to 10 % water is reached. Storage must be under nitrogen because sulphite in this form may easily be oxidised to sulphate. Eluting the extract (hexane) through a column filled with this material results in removal of the sulphur in combination with further clean-up of the sediment extract. The sulphur removal properties are somewhat difficult to control.

Mercury, activated copper powder, wire or gauze (Smedes and de Boer, 1994 and 1997; Wade and Cantillo, 1996) remove the sulphur directly from an organic solvent. Although mercury is appropriate for removing sulphur, it should be avoided for environmental reasons. Copper can be applied during or after Soxhlet extraction. Ultrasonic treatment might improve the removal of sulphur. If sulphur appears to be present in the final extract the amount of copper or mercury used was insufficient and the clean-up procedure must be repeated.

Silver ions strongly bind sulphur and sulphur compounds. Loaded onto silica, $AgNO_3$ is a very efficient sulphur removing agent. It can be prepared by mixing dissolved $AgNO_3$ with silica and subsequently drying under nitrogen. Compounds containing aromatic rings are strongly retained, but for CBs this retention is reduced, probably due to shielding of the rings by the chlorine atoms. Retained compounds can easily be eluted by using cyclohexene, or another solvent with double bonds, as a modifier (Eganhouse, 1986; Japenga *et al*., 1987).

Elemental sulphur is strongly retained on a polystyrene-divinylbenzene copolymer column as generally applied for gel permeation chromatography (GPC). In addition, GPC combines sulphur removal with a clean-up stage.

All these methods have advantages and disadvantages. For different samples, the use of multiple methods may sometimes prove necessary. Several of the methods leave some aromatic sulphur compounds in the extract. These compounds elute from the GC column at similar retention times to some of the lower-chlorinated CBs. The major part of these compounds can be removed by eluting an apolar extract over a column with silica loaded with concentrated H_2SO_4. Other interfering compounds (*e.g.* phthalates and fatty acid esters) are also removed by using this procedure.

6.2 Further clean-up

The extraction procedures above will result in the co-extraction of many compounds other than CBs. The extract may be coloured due to pigments extracted from sediment, and may also contain sulphur and sulphur-containing compounds, oil, PAHs and many other natural and anthropogenic compounds which will need to be removed from the extract. Different clean-up 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. Most CBs are stable under acid conditions; therefore treatment with sulphuric acid or acid impregnated silica columns may be used in the clean-up.

The most commonly used clean-up methods involve the use of alumina or silica adsorption chromatography, but gel permeation chromatography (GPC) is also employed.

As CBs are apolar, clean-up using normal-phase chromatography is the most appropriate technique for the separation from other compounds. Using an apolar solvent (*e.g.* hexane or *iso*-octane) as an eluent, CBs normally elute very rapidly. All polar solvents used in the extraction or sulphur removal step should be removed before further clean-up. The last concentration step is usually performed by evaporation with a gentle stream of nitrogen. Evaporation to dryness should always be avoided.

Deactivated Al_2O_3 (with 5 - 10% water) is often used as a primary clean-up method. Provided that sulphur has been removed beforehand, Al_2O_3 clean-up sometimes yields a sufficiently clean extract for a GC-ECD analysis of the sample to be performed. Al_2O_3 removes lipid compounds from the extracts (although samples with a very high lipid content and low CB concentrations may require additional clean-up).

Deactivated silica (with 1 - 5% water) does not retain CBs (including planar CBs) and only slightly retains polycyclic aromatic hydrocarbons (PAHs) when eluted with hexane or *iso*-octane. When organochlorine pesticides are also to be determined in the same extract, deactivation of the silica with a few percent of water is essential.

For high activity silica (overnight at 180°C) the retention of CBs is negligible, while PAHs are more strongly retained. The CBs and a few other organochlorine compounds are eluted with apolar solvents. More polar solvents (*e.g.* hexane/acetone) should be avoided as some interfering organochlorine pesticides would be eluted.

For the separation of CBs from lipids or oil components, reversed-phase HPLC can be used. In reversed-phase chromatography CBs elute during a solvent gradient of 80 to 90% methanol, together with numerous other compounds of similar polarity. Most of the above mentioned extraction methods and clean-up procedures yield an extract containing an apolar solvent. These cannot be injected directly for reversed-phase chromatography, and so compounds must be transferred between solvents several times *e.g.* before injection and after elution. When using polar solvents for extraction (*e.g.* for wet sediments) reversed-phase columns could be used directly for clean-up. When eluting an acetonitrile extract from a C_{18} solid phase extraction (SPE) column with acetonitrile, high molecular hydrocarbons are strongly retained while CBs elute in the first few column volumes.

The above mentioned normal-phase chromatographic procedures on silica and Al_2O_3 can be transferred to HPLC having the advantages of higher resolution and better reproducibility.

When using GPC the elution of CBs should be carefully checked. When applying GPC, two serial columns are often used for improved lipid separation. Solvent mixtures such as dichloromethane/hexane or cyclohexane/ethyl acetate can be used as eluents for GPC. However, a second clean-up step is often required to separate the CBs from other organohalogenated compounds.

One advantage of using PLE extraction is that it is possible to combine the clean up with the extraction, especially when mass spectrometry will be used as the detection method. If Soxhlet extraction is used for biota, then there is a much greater quantity of residual lipid to be removed than in the case of PLE with fat retainers. An additional clean-up stage may therefore be necessary. Methods have been developed for online clean-up and fractionation of dioxins, furans and CBs with PLE for food, feed and environmental samples (Sporring *et al*., 2003). The first method utilises a fat retainer for the on-line clean-up of fat. Silica impregnated with sulphuric acid, alumina and Florisil have all been used as fat retainers. A non-polar extraction solvent such as hexane should be used if fat retainers are used during PLE.

Non-*ortho* CBs require a more specialised clean-up, similar to that which is generally associated with the analysis of dioxins and furans. Although initial clean-up may very well proceed along the lines described above, the larger sample intake results in even larger amounts of co-extractives and care has to be taken that the capacity of the adsorption columns is not exceeded and/or that sulphur is adequately removed. Often, more rigorous procedures are applied to remove the excess material by e.g. shaking the sample with concentrated sulphuric acid. A more efficient and safer alternative is to elute the sample over a silica column impregnated with sulphuric acid (40 % w/w).

Non-*ortho* CBs are nearly always separated from the other CBs using advanced separation techniques. A very efficient method is to inject the extracts (after concentrating them) into a HPLC system coupled to PYE (2-(1-pyrenyl) ethyldimethylsilylated silica) column. Column dimensions are typically 4.6 x 150 mm column, but combinations of several columns in-line are sometimes used. PYE columns not only allow the separation of *ortho,* mono-*ortho* and non-*ortho* CBs on the basis of structural polarity from each other but also from dibenzo-*p*-dioxins and dibenzofurans. The eluting solvent is an apolar solvent such as *iso*-hexane. When coupled to a fraction collector, the use of a HPLC system allows the automatic clean-up of a considerable number of samples. Alternatively, HPLC systems equipped with porous graphite carbon. Column sizes are in the order of 50 x 4.7 mm and care has to be taken that the column is not overloaded. Similarly to PYE columns, they will separate non-*ortho* CBs from the others and from dioxins and furans. Fully automated systems, such as Powerprep, that combine several steps are routinely used (Focant and De Pauw, 2002).

7. Pre-concentration

Evaporation of solvents with a rotary-film evaporator was up until recent the common method. However, evaporation of solvents using this technique should be performed at low temperature (water bath temperature of ≤ 30°C) and under controlled pressure conditions, in order to prevent losses of the more volatile CBs. To reduce the sample to the final volume, solvents can be removed by blowing-down with gently streaming nitrogen. Only nitrogen of a controlled high quality should be used. As a solvent for the final solution to be injected into the GC, *iso*-octane is recommended.

Turbovap sample concentrators can also be used to reduce solvent volume. This is a rapid technique, but needs to be carefully optimised and monitored to prevent both losses (both of volatiles and solvent aerosols) and cross-contamination. The use of rotary-film evaporators is more time consuming but more controllable. Here also, evaporation to dryness should be avoided at all costs. Syncore parallel evaporators (Buchi, Switzerland) can be used with careful optimisation of the evaporation parameters. 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. Again water-bath temperatures should be minimised to prevent losses. When reducing the sample to the required final volume, solvents can be

removed by a stream of clean nitrogen gas. Suitable solvents for injection into the gas chromatograph (GC) include hexane, heptane, toluene and *iso*-octane.

7.1 Calibration and preparation of calibrant solutions

Internal standards (recovery and quantification standards) should be added in a fixed volume or weight to all standards and samples. The ideal internal standard is a CB which is not found in the samples. All CBs with a 2,4,6-substitution (e.g. CB115, CB155, CB198) are, in principle, suitable. Alternatively, 1,2,3,4-tetrachloronaphthalene or homologues of dichloroalkylbenzylether can be used. For GC analysis with mass selective detection (GC-MS), ^{13}C labelled CBs should be used for each degree of chlorination. This especially critical for the non-*ortho* CBs. If possible, the labelled calibrant solutions should correspond to the unlabelled determinants. For the non-*ortho* CBs a labelled standard is available for each congener and use of all of them is recommended. When preparing a calibration solution for a new determinant for the first time, two independent stock solutions of different concentrations should always be prepared simultaneously to allow cross checking. A new calibration solution should also be cross-checked to the old standard solution. Crystalline CBs of known purity should always be used for preparing calibration solutions. If the quality of the standard materials is not guaranteed (*e.g.* as in the case for a Certified Reference Material) by the producer or supplier, it should be checked by GC preferably with mass spectrometric detection. Solid standards should be weighed to a precision of 10^{-5} grams. In recent years, a lot of certified commercial custom made standards have become available and laboratories have been switching to these. Calibration solutions should preferably be stored in ampoules in a cool and dark place. When stored in containers the weight loss during storage should be recorded.

8. Instrumental determination

8.1 Injection techniques

The two modes commonly used are splitless and on-column injection. In split injection, strong discrimination effects may occur. The liner should possess sufficient capacity with respect to the injected volume after evaporation, but should not be oversized to avoid poor transfer to the column and losses by adsorption. Liners with light packing of (silylated) glass wool may improve the performance for CBs, but may degrade some organochlorine compounds like DDT, which are often included in national monitoring programmes.

Recently, other techniques such as temperature-programmed or pressure-programmed injection have become more prominent. They offer additional advantages such as an increased injection volume without the negative effects previously associated with that, but should be thoroughly optimised before use. Increasing the injection volume will allow either or both the elimination of an extra evaporation step and lowering the detection limits.

8.2 Carrier gas

Hydrogen is the preferred carrier gas and is indispensable for columns with very small inner diameters. Helium is also acceptable and the standard carrier for GC-MS.

8.3 Columns

Only capillary columns should be used. The following parameters are recommended:

Minimum Length	50 m (for microcolumns of internal diameter <0.1 mm, shorter columns can be suitable).
Maximum internal diameter	0.25 mm. Note that for diameters <0.15 mm the elevated pressure of the carrier gas needs special instrumental equipment as most of the instruments are limited to 400 kPa.
Film thickness	0.2-0.4 µm.

Columns which do not fulfil these requirements generally do not offer sufficient resolution to separate CB28, CB105 and CB156 from closely eluting CBs. A wide range of stationary phases can be used for CB separation. The chemical composition is different for many producers and depends on the maximum temperature at which the column can be operated. Further advice may be found in the producer's catalogues, where compositions, applications and tables to compare products from different manufacturers are included.

In recent years, new chromatographic phases have become available that result in an improved separation of critical CB pairs. A good example is the HT-8 phase (1,7-dicarba-closo-dodecarborane phenylmethyl siloxane) (Larsen *et al*., 1995) that shows a remarkable selectivity for CBs. This column is currently recommended for CB analysis. Examples of the retention times for various CBs are given in Table 1.

8.4 Detection

The electron capture detector (ECD) is still frequently used for CB analysis. Injection of chlorinated solvents or oxygen-containing solvents should therefore be avoided due to the generation of large interfering signals. When using mass selective detectors (MSD) negative chemical ionisation mode (NCI) is extremely sensitive for pentachlorinated to decachlorinated CBs and is approximately ten fold better than ECD. However, MS systems have improved considerable allowing analysis by Electron impact ionisation (EI), whereas before, electron-capture negative ion chemical ionisation (ECNICI) was often necessary in order to detect the low concentrations of, in particular the planar CBs. Suggested target and qualifier ions for *ortho* CBs (including non-*ortho* CBs) are shown in Table 1 and in Table 2 for non-*ortho* CBs.

Table 1 Example of retention times for selected CB congeners using a 50 m HT8 column (0.25 mm i.d. and 0.25 µm film), along with possible target and qualifier ions. Temperature programme: 80°C, hold for 1 minute, ramp 20°C/minute, to 170 °C, hold 7.5 minutes, ramp 3 °C/minute to 300 °C, hold for 10 minutes.

CB congener	MW	RT	Target Ion	Qualifier Ion	Number of chlorines
^{13}C-CB28	**270**	28.371	268	270	3
CB31	**258**	28.071	256	258	3
CB28	**258**	28.388	256	258	3
^{13}C-CB52	**304**	30.317	304	302	4
CB52	**292**	30.336	292	290	4
CB49	**292**	30.698	292	290	4
CB44	**292**	32.024	292	290	4
CB74	**292**	34.881	292	290	4
CB70	**292**	35.199	292	290	4
^{13}C-CB101	**340**	36.612	338	340	5
CB101	**326**	36.630	326	328	5
CB99	**326**	37.062	326	328	5
CB97	**326**	38.267	326	328	5
CB110	**326**	39.277	326	328	5
CB123*	**326**	41.2	326	328	5
CB118*	**326**	41.563	326	328	5
CB105*	**326**	43.443	326	328	5
CB114*	**326**	42.2	326	328	5
^{13}C-CB153	**374**	42.567	372	374	6
CB149	**362**	40.328	360	362	6
CB153	**362**	42.584	360	362	6
CB132	**362**	42.236	360	362	6
CB137	**362**	43.744	360	362	6
13C-CB138	**374**	44.437	372	374	6
CB138	**362**	44.487	360	362	6
CB158	**362**	44.663	360	362	6
CB128	**362**	46.307	360	362	6
^{13}C-CB156	**374**	48.406	372	374	6
CB156*	**362**	48.366	360	362	6
CB167*	**362**	46.4**	360	362	6
CB157*	**362**	48.698	360	362	6
^{13}C-CB180	**408**	48.829	406	408	7
CB187	**396**	44.787	394	396	7
CB183	**396**	45.264	394	396	7
CB180	**396**	48.846	394	396	7
CB170	**396**	50.684	394	396	7
^{13}C-CB189	**406**	53.182	406	408	7
CB189*	**396**	53.196	394	396	7
^{13}C - CB194	**442**	57.504	442	440	8
CB198	**430**	50.347	430	428	8
CB194	**430**	57.514	430	428	8

***mono-ortho CBs, ** to be checked**

Next to conventional GC-MS, the use of ion-trap with its tandem MS^2 option - i.e., yielding improved selectivity - is receiving increased attention. The use of GC-ITMS provides a less expensive alternative to high-resolution mass spectrometry (HRMS), which is commonly used to determine PCDD/F and, as such, also ideally suited for all CB groups (Eppe *et al*., 2004).

Table 2 Possible target and qualifier ions for non-*ortho* CBs, including labelled internal standards

CB	Target ion (*m/z)*	Qualifier (*m/z*)	Qualifier (*m/z*)	Qualifier (*m/z*)
^{13}CB81	304	302	NA	NA
CB81	292	290	220	222
^{13}CB77	304	302	NA	NA
CB77	292	290	220	222
^{13}CB126	338	340	NA	NA
CB126	326	328	254	256
^{13}CB169	372	374	NA	NA
CB169	360	362	218	220

8.5 Separation, identification and quantification

When using GC-ECD and to a certain extent GC-MS, two columns with stationary phases of different polarity should be used, as column-specific coelution of the target CBs with other CBs or organochlorine compounds occurs. The temperature programme must be optimised for each column to achieve sufficient separation of the CB congeners to be determined. An isothermal period in the programme around 200-220°C of approximately 30 minutes is recommended. Care should be taken that CBs of interest do not co-elute with other CB congeners (for example CB28 and CB31). When using GC-ECD, compounds are identified by their retention time in relation to the standard solutions under the same conditions. Therefore GC conditions should be constant. Shifts in retention times should be checked for different areas of the chromatogram by identifying characteristic, unmistakable peaks (*e.g.* originating from the internal standard or higher concentrated CBs such as CB153 and CB138. When using a GC-MS system, the molecular mass or characteristic mass fragments or the ratio of two ion masses can be used to confirm the identity of separated CBs. Since calibration curves of most CBs normally non-linear when using a GC-ECD, but should be linear for GC-MS, a multilevel calibration of at least five concentrations is recommended. The calibration curve must be controlled and the best fit must be applied for the relevant concentration range. Otherwise, one should strive to work in the linear range of the detector must. Analysis of the calibration solutions should be carried out in a mode encompassing the concentrations of the sample solutions (or alternatively by injecting matrix-containing sample solutions and matrix-free standard solutions distributed regularly over the series). When the chromatogram is processed with the help of automated integrators the baseline is not always set unambiguously and always needs to be inspected visually. When using GC-ECD, peak height is preferable to peak area for quantification purposes. From the two columns of different polarity the more reliable result should be reported.

Recent years have witnessed the emergence of so-called comprehensive two-dimensional gas chromatography (GC x GC) – a technique that can be used to considerably improve analyte/matrix as well as analyte/analyte separation. Briefly, a non-polar x (semi-)polar column combination is used, with a conventional 25-30 m long first-dimension, and a short, 0.5-1 m long, second-dimension column. The columns are connected via an interface called a modulator. The latter device serves to trap, and focus, each subsequent small effluent fraction from the first-dimension column and, then, to launch it into the second column. The main advantages of the comprehensive approach are that

the entire sample (and not one or a few heart-cuts, as in conventional multidimensional GC (Dallüge *et al*. 2003) is subjected to a completely different separation, that the two-dimensional separation does not take any more time than the first-dimension run, and that the re-focusing in the modulator helps to increase analyte detectability. The most interesting additional benefit for CBs is, that structurally related as CB congeners show up as so-called ordered structures in the two-dimensional GC x GC plane. The very rapid second-dimension separation requires the use of detectors with sufficiently high data acquisition rates. Initially, only flame ionisation detectors could meet this requirement. However, today there is also a micro-ECD on the market that is widely used for GC x GC-µECD of halogenated compound classes. Even more importantly, analyte identification can be performed by using a time-of-flight mass spectrometer [Dallüge *et al*., 2002] or - with a modest loss of performance, but at a much lower price - one of the very recently introduced rapid-scanning quadrupole mass spectrometers [Korytar *et al*., 2005; Adachour *et al*., 2005). So far, the use of GC x GC has been limited to qualitative applications and still seems inappropriate for routine quantification of analytes.

9. Quality assurance

Planners of monitoring programmes must decide on the accuracy, precision, repeatability, and limits of detection and determination which they consider acceptable. The limit of determination should depend on the purpose of the investigation. A limit of at least 0.1 ng/g (dry weight, fraction < 2mm) should be reached, but detection limits of 0.01 ng/g are achievable nowadays. The method for calculating the limit of determination should reflect QUASIMEME advice (Topping *et al*., 1992). The limit of determination that can be achieved depends on the blank, on the sample matrix, on concentrations of interfering compounds and on the mass of sediment taken for analysis. References of relevance to QA procedures include HELCOM, 1988; QUASIMEME 1992; Wells *et al*., 1992; Oehlenschläger, 1994; Smedes *et al.*, 1994 and ICES, 1996.

9.1 System performance

The performance of the GC system should be monitored by regularly checking the resolution of two closely eluting CBs. A decrease in resolution points to deteriorating GC conditions. The signal-to-noise ratio yields information on the condition of the detector. A dirty ECD detector or MS ion 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.

9.2 Recovery

The recovery should be checked and reported. One method is to add an internal (recovery) standard to each sample immediately before extraction and a second (quantification) standard immediately prior to injection. If smaller losses occur in extraction or clean-up or solutions are concentrated by uncontrolled evaporation of solvents (*e.g.* because vials are not perfectly capped) losses can be compensated for by normalisation. If major losses are recognised and the reasons are unknown, the results should not be reported, as recoveries are likely to be irreproducible. A control for the recovery standard is recommended by adding the calibration solution to a real sample. Recoveries should be between 70 and 120%, if not, samples should be repeated.

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Annex VIII:

HELCOM (2012). Manual for marine monitoring in the COMBINE programme. Annex B-13, Appendix 2. Technical note on the determination of chlorinated biphenyls in marine sediment (4.2.3)

Guidelines on the determination of chlorinated hydrocarbons in sediment

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1. Introduction

These guidelines are based on the review from Smedes and de Boer (1994, 1998) and Eljarrat and Barceló (2009).

The analysis of chlorinated hydrocarbons in sediments generally involves extraction with organic solvents, clean-up, removal of sulphur, column fractionation and gas chromatographic separation, mostly with electron capture or mass-spectrometric detection.

All steps of the procedure are susceptible to insufficient recovery and contamination. Quality control measures are recommended in order to regularly monitor the performance of the method. These guidelines are intended to encourage and assist analytical chemists to critically review their methods and to improve their procedures and quality assurance measures, if necessary.

These guidelines can be applied for the determination of several types of chlorinated hydrocarbons, e.g., chlorinated biphenyls (CB), chlorobenzenes, DDT and its metabolites and hexachlorocyclohexanes. It should be noted that these guidelines do not cover the determination of non-*ortho* substituted CB. Due to the low concentrations of non-*ortho* CB in sediments comparing to those of other CB, their determination requires an additional separation and concentration step similar to the analysis of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F).

These guidelines are not intended as complete laboratory manual. If necessary, guidance should be sought from specialized laboratories. Laboratories should demonstrate validity of each methodological step. Moreover, use of an alternative method, carried out concurrently to the routine procedure, is recommended for validation.

Contracting parties should follow the HELCOM monitoring guideline but minor deviations from this are acceptable if the method achieves comparable results. Validation of the adopted method needs to be performed on the relevant matrix and concentration range e.g. by taking part in intercomparison studies or proficiency testing schemes.

2. Sampling and storage

The major criterion for successful sediment sampling is to ensure undisturbed sample stratification. (For further details about sampling, see Annex B-13, Appendix 3 “Technical note on the determination of heavy metals in marine sediments” of the COMBINE manual.)

Plastic materials (except polytetra-fluorethene, PTFE) should not be used for sampling and storage due to the risk of adsorption of target compounds onto the container material. Samples should be transported in closed containers and preferentially at temperatures below 10 °C. The samples should be stored at 4 °C as soon as possible, but at least if they have not been analysed within 48 hours after collection (short-term storage). For long-term storage over several months the samples should be frozen below -20 °C or dried (Law and de Boer, 1995). When drying, avoid methods with substantial risk of losing volatile substances (see Chapter 4: Pretreatment).

3. Blanks and contamination

Basically, care should be taken to avoid contaminations during all steps of the analytical chain, including sampling, extraction and clean-up.

In order to reduce blank and sample contaminations to a minimum it is strongly recommended to pretreat all used glassware, solvents, chemicals, adsorption materials, etc., as follows:

- Glassware should be thoroughly washed with detergents and can be furthered cleaned, other than calibrated instruments, by heating at temperatures > 250 °C. The glassware should be rinsed with an organic solvent prior to use.

- All solvents should be analyzed for impurities by concentrating to 10 % of the regular final volume. This concentrate is then analysed similarly to a sample by HPLC or GC. The solvent blank should not contain target analytes or other interfering compounds in higher concentrations than specified by the laboratory.

- All chemicals and adsorption materials should be analyzed for impurities and purified (e.g., by heating or extraction), if necessary. Soxhlet thimbles should be pre-extracted. Glass fiber thimbles are preferred over paper thimbles. Alternatively, full glass Soxhlet thimbles, with a G1 glass filter at the bottom, can be used.

Storage of these supercleaned materials for a longer period is not recommended, as laboratory air might contain target compounds which can adsorb onto these materials. Therefore, contaminated blank samples might occur despite precautionary measures due to contamination from the air. Volatile compounds are usually the most common contaminants in blanksamples (Gremm and Frimmel, 1990). Therefore, if possible, critical steps should be done in a clean bench.

4. Pretreatment

The samples should be thoroughly homogenized before subsampling for analysis. The amount of samples usually depends on the expected concentrations. For the marine environment, the amount of sample should be equal to an amount representing 50–100 mg of organic carbon.

Chlorinated hydrocarbons can be extracted from wet or dried samples. However, storage, homogenization and extraction are easier to handle with dried samples.

Drying the samples at ambient or elevated temperatures as well as freeze-drying may alter the concentrations, e.g., by contamination or loss of compounds through evaporation (Law *et al.,* 1994). Therefore, potential losses and contaminations should be analyzed in advance, e.g. by exposing 1–2 g ClS-bonded silica to the drying conditions and subsequent extraction and analysis (clean-up can be omitted) (Smedes and de Boer, 1998). For evaluation of potential losses, analytes identical or similar to chlorinated hydrocarbons need to be added to the material. However, bear in mind that added analytes can behave differently from analytes that have interacted longer with the matrix material and therefore may be sorbed more strongly. To avoid contamination during freeze-drying, placing a lid with a hole of about 3 mm in diameter on the sample container is suggested.

Chemical drying of samples can be performed by grinding with Na_2SO_4, or $MgSO_4$ until the sample reaches a sandy consistency. It is essential that several hours elapse between grinding and extraction to allow for complete dehydration of the sample. Residual water will decrease extraction efficiency.

5. Extraction and clean-up

The target compounds must be extracted from the sediment with an organic solvent prior to further analysis.

Other extraction and clean-up methods than those described below may be used, provided that the methods have been tested and found equivalent to established methods regarding e.g. recovery.

5.1. Extraction of wet sediments

Wet sediments are extracted by mixing with organic solvents. Extraction is enhanced by shaking, ultra-turrax mixing, ball mill tumbler or ultrasonic treatment. Water-miscible solvents such as methanol, acetone, and acetonitrile, are used, in particular in the first step. The extraction efficiency of the first step is

low as there will be a considerable amount of water in the liquid phase. Extraction will be continued with a mixture of polar and apolar solvents such as acetone/ hexane or methanol/ dichloromethane. It has to be kept in mind that hexane and dichloromethane is a lot more toxic than similar solvents such as pentane, heptane, cyclohexane, isohexane. For complete extraction at least three subsequent extractions are required and a contact time of up to 24 hours with the solvent should be sufficient to complete the desorption of the chlorinated hydrocarbons from the sediment.

Soxhlet extraction or extraction by pressurized liquid extraction such as ASE 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. In a second step the collecting flask is replaced and the extraction will be continued using a less polar solvent or solvent mixture such as acetone/hexane or toluene. Thereafter, the extracts will be combined.

To separate the water and keep the chlorinated hydrocarbons in a solvent that is compatible with the continued analysis different methods can be used. For example, water will be added to the combined extracts and the chlorinated hydrocarbon compounds will be extracted to a non-polar solvent. Another possibility is to add Na_2SO_4 to bind water.

Extraction should be conducted with a sufficient number of extraction cycles. Extraction efficiency should be analyzed for different types of sediments through a second extraction step. The extracts will be analysed separately and compared. A recovery of more than 90 % during the first extraction step is considered adequate.

5.2. Extraction of dry sediments

For dried sediments pressurized liquid extraction (e.g. ASE) is frequently applied to extract chlorinated hydrocarbons. The use of a mixture of a polar and a non-polar solvent, e.g., 25 % (v/v) acetone/hexane is recommended for sufficient extraction efficiency. A higher content of polar solvent increases extraction efficiency, but it has to be removed prior to gas chromatographic analysis.

Alternatively to ASE, extraction can be conducted with a regular Soxhlet, a hot Soxhlet with at least 50 to 60 extraction cycles (approximately 8 hours for the hot Soxhlet) or by microwave extraction. Supercritical fluid extractions have also been demonstrated, but have not found wide application due to low reproducibility compared to the other technique (Law et al, 2011).

Extraction should be conducted with a sufficient number of extraction cycles. Extraction efficiency should be analyzed for different types of sediments through a second extraction step. The extracts will be analysed separately and compared. A recovery of more than 90 % during the first extraction step is considered adequate.

Prior to any concentration steps, a keeper (high-boiling solvent, e.g. a high-boiling alkane or toluene) should be added. Make sure that the keeper does not interfere with the analytes of interest in the instrumental analysis.

5.3. Removal of sulphur and sulphur-containing compounds

The crude extracts usually require clean-up to remove co-extracted compounds (Wise *et al.,* 1995). Due to chlorophyll-like compounds extracted from the sediment, the raw extract is usually colored and also contains sulphur and sulphur-containing compounds, oil, PAH compounds and other natural and anthropogenic compounds. Selection of the appropriate clean-up method depends on the subsequent instrumental method to be used for analysis. Copper powder, wires, or gauze are the most common ways to remove the sulphur directly from an organic solvent. Copper can be applied during or after sediment extraction. Ultrasonic treatment might improve the removal of sulphur. If sulphur appears to be present in the final extract, the amount of copper used was insufficient and the clean-up procedure must be repeated.

Be aware that a prolonged contact between the sample and the copper may degrade some chlorinated pesticides.

Another possibility to remove sulphur is to add an aqueous saturated Na_2SO_3 solution to a hexane extract. In order to allow transfer of the HSO_3-ions to the organic phase, tetrabutylammonium (TBA) salts and isopropanol are added to the mixture. Subsequently, water is added to remove the isopropanol. The aqueous phase is then quantitatively extracted with hexane (Jensen *et al.,* 1977). If the extraction is performed with a polar solvent which is miscible with water, the Na_2SO_3 solution can be added directly after the extraction. If the extraction mixture also contains a non-polar solvent, then, depending on the ratio of the solvents, the addition of TBA and isopropanol may not be necessary. Any excess $Na_2SO_3$and reaction products can be removed by the addition of water and partitioning between the non-polar solvent and water.

Japenga *et al.* (1987) developed a column method for the removal of sulphur and sulphur-containing compounds. The column material is made by mixing an aqueous solution of Na_2SO_3 with Al_2O_3. Some NaOH is also added to improve the reaction with sulphur. Subsequently, the material is dried under nitrogen until a level of deactivation equivalent to 10 % water is reached. Storage must be under nitrogen because sulphite in this form may easily be oxidized to sulphate. Eluting the extract (hexane) through a column filled with this material results in removal of the sulphur in combination with further clean-up of the sediment extract.

Silver ions strongly bind sulphur and sulphur compounds. Loaded on silica, $AgNO_3$ is a very efficient sulphur-removing agent. It can be prepared by mixing dissolved $AgNO_3$ with silica and subsequently drying under nitrogen. Compounds containing aromatic rings are strongly retained, but for chlorinated hydrocarbons retention is reduced, probably due to shielding of the rings by the chlorine atoms. Retained compounds can easily be eluted by using cyclohexene, or another solvent with double bonds, as a modifier.

Elemental sulphur is strongly retained on a polystyrene divinylbenzene copolymer column as generally applied for gel permeation chromatography (GPC). In addition, this method combines the removal of sulphur with a clean-up.

Sometimes the use of multiple methods may be necessary for different samples. Several methods leave aromatic sulphur compounds in the extract which will elute from the GC column at the same retention time as the lower chlorinated biphenyls. The major part of these compounds can be removed by eluting a non-polar extract over a column containing silica loaded with concentrated sulphuric acid.

The recovery during clean-up should be analyzed carefully. In particular, treatment with H_2SO_4 results in loss of, e.g., dieldrin and endosulfanes. Also, the clean-up procedure with silver ions or copper can result in low recoveries for certain pesticides.

5.4. Further clean-up

Clean-up using normal phase chromatography is the most appropriate technique for the separation of the chlorinated hydrocarbons from other compounds. Using non-polar solvent, e.g., hexane or *iso*-octane, chlorinated hydrocarbons usually elute very rapidly.

All polar solvents used during extraction or sulphur-removal should be removed before further clean-up. The last concentration step is usually performed by evaporation with a gentle stream of nitrogen. Evaporation to dryness should always be avoided.

Deactivated Al_2O_3 (5–10 % water) is often used as the primary clean-up step through which usually a sufficiently clean extract for a gas chromatography- electron capture detector (GC-ECD) analysis of the sample is achieved, given that sulphur has been removed.

Deactivated SiO_2 (1–5 % water) does not retain chlorinated hydrocarbons (including planar CB) and only slightly retains polycyclic hydrocarbons when eluted with hexane or *iso*-octane.

For high activity silica (overnight at 180 °C), the retention of chlorinated hydrocarbons is negligible while PAH compounds are more strongly retained. The chlorinated hydrocarbons are eluted with non-polar solvents. Upon using more polar solvents (e.g., hexane/acetone) some interfering organochlorine pesticides might become eluted**.**

When GPC is used for removing sulphur (see 5.3 REMOVAL OF SULPHUR AND SULPHUR-CONTAINING COMPOUNDS) the removal of high molecular weight material can also be incorporated into the procedure. GPC does not separate chlorinated hydrocarbons from other compounds in the same molecular range (such as organochlorine pesticides), so additional clean-up is usually required.

For the separation of chlorinated hydrocarbons from lipids or oil components reverse-phase high-performance liquid chromatography (RP-HPLC) can be used. Due to the use of aqueous solvents in RP- HPLC solvents need to be changed from polar to non-polar and *vice versa*. Another option is the use of strong acid (e.g. H_2SO_4) to degrade the lipids; however, it may also degrade some pesticides.

6. Gas chromatography

In particular, for the large number of CB congeners (a total of 209) high-resolution capillary gas chromatography (GC) is the method of choices. However, the analysis of CBs in sediments should focus on the determination of selected individual congeners as it is currently impossible to separate all CBs in technical mixtures and from other ECD-detectable compounds. For example, the seven common indicator-PCBs should be analysed. If there is a desire to separate and analyse all congeners, it is recommended to use multidimensional gas chromatography (MGC) that makes use of two successive columns of different selectivity or polarity. However, the optimization is difficult (co-elution of some PCBs) and it is not routinely applied at commercial laboratories. Alternatively two ECD detectors and two parallel columns with different selectivity or polarity can be used, reducing the detection limit by a factor of 2 but improving the selectivity of co-elution PCBs by choosing the column with least overlap for suspected co-elutions.

Another option is to use GC-MS instrumentation for more selective determination.

For all GC methods, parameters have to be optimized.

6.1 Column dimensions

Column dimensions for the determination of chlorinated hydrocarbons are:

- length: minimum 50 m, and
- inner diameter (i. d.): maximum 0.25 mm.
- film thickness: 0.2 µm to 0.4 µm

Greater resolution can be obtained by reducing the inner diameter to 0.20 mm or less. Below a diameter of 0.15 mm the carrier gas pressure rises to values above 500 kPa, which are often not compatible with regular GC instruments. Also, the risk of leakage increases.

6.2 Stationary phases

A wide range of stationary phases can be used for the separation of chlorinated hydrocarbons (e.g., 94 % dimethyl-, 5 % phenyl-, 1 % vinyl polysiloxane, or 7 % phenyl-, 7 % cyanopropyl-, 86 % methyl-siloxane).

The use of more polar phases is sometimes limited as their maximum temperatures are not as high as for non-polar, chemically bonded phases. Stationary phases that separate chlorinated hydrocarbons on the

basis of molecular size, such as the liquid crystal phase, should not be used for monitoring purposes since they do not provide sufficient reproducibility.

6.3 Carrier gas

Preferentially, hydrogen should be used as the GC carrier gas. When using columns with very small inner diameters, the use of hydrogen is essential. The linear gas velocity should be optimized.

Appropriate settings for 0.25 mm i.d. columns range from 20–40 cm s^{-1} and for 0.15 mm i.d. columns from 30–50 cm s^{-1}.

6.4 Injection techniques

The two systems commonly used are splitless and on-column injection. Split injection should not be used due to strong discrimination effects. Other techniques such as temperature-programmed or pressure-programmed injection may have additional advantages, but should be thoroughly optimized before use.

The volume of the liner should be large enough to contain the gas volume of the evaporated injected solvent. When the liner is too small, memory effects may occur due to contamination of the gas tubing attached to the injector. Very large liner volumes can cause a poor transfer of early eluting components, so that peaks due to those analytes will be reduced or even disappear. In addition, the use of a light packing of (silylated) glass wool in the liner improves the response and reproducibility of the injection, but some organochlorine pesticides such as DDT may be degraded when this technique is applied.

An auto-sampler should be used.

6.5 Temperature programming

The temperature programme must be optimized for sufficient separation of the chlorinated hydrocarbons. A separation time of 60 to 120 minutes can be neccessary. In addition to a reproducible temperature programme, a fixed equilibration time is important for a correct analysis and constant retention times.

For further details and recommendations see Smedes and de Boer (1998).

6.6 Detection

The use of a mass spectrometer (MS) or tandem mass spectrometer (MS/MS) is highly recommended. MS gives the possibility to use ^{13}C labelled internal standards. Different ionization methods have been reported: Electron impact ionization (EI), Negative chemical ionization (NCI) or electron capture negative ionization (ECNI). Another used detector for the analysis of chlorinated hydrocarbons is the electron capture detector (ECD), but injection of chlorinated or oxygen-containing solvents should be avoided. NCI and ECNI is extremely sensitive for penta- to decachlorinated CBs (approximately ten-fold better than ECD), but can be less sensitive for less chlorinated PCBs (Law et al, 2011).

6.7 Identification

Usually, the compounds in the sample are identified based on their retention times as compared to those of the standard compounds analyzed under the same conditions. Moreover, upon using GC-MS compound characteristic mass fragments serve as additional identifiers.

7. Quantification

Automatically processed chromatograms should be reviewed if, e.g., the baseline is set correctly.

For calibration purposes a multilevel calibration with at least five concentration levels is recommended. The calibration curve should be linear and cover the working range. Obtained calibrations should be regularly validated in terms of precision and accuracy.

Prior to running a series of samples and standards, the GC or HPLC systems should be equilibrated by injecting at least one sample extract. In addition, standards used for multilevel calibrations should be

regularly distributed over the sample series so that matrix-and non-matrix-containing injections alternate. A sample series should include:

- a procedural blank;
- a laboratory reference material;
- at least five standards;
- one standard sample treated similarly to the samples for determination of the recovery.

The limit of quantification usually depends on the purpose of the investigation. The limit of quantification that can be achieved depends on the blank sample, the sample matrix, concentrations of interfering compounds, and the amount of sample. However, a limit of quantification of 0.1 ng g^{-1} (dry weight, fraction < 2 mm) or better should be attained for single compound analysis. The method for calculating the limit of determination should follow the advice in Part B-4.2.3 (COMBINE manual).

8. Quality Assurance

A number of measures should be taken to ensure sufficient quality of the analysis. Six main areas can be identified:

1. extraction efficiency and clean-up;
2. calibrant and calibration;
3. system performance;
4. long-term stability;
5. internal standards; and
6. frequent participation in interlaboratory proficiency testing schemes (e.g. QUASIMEME two times a year, www.quasimeme.org)

8.1. Extraction efficiency and clean-up

Extraction efficiency and clean-up can be controlled by analysing reference materials (Annex B-7). To determine the recovery rates of the clean-up and concentration steps, it is recommended to pass a standard solution (see 8.5. INTERNAL STANDARDS) through the entire procedure. The addition of corresponding internal standards to the samples is preferred.

If major losses have occurred, the results should not be reported.

8.2 Calibrant and calibration

Basically, calibration solutions should be stored in ampoules at a cool, dark place. Weight loss during storage should be recorded for all standards.

Calibration solutions from certified crystalline compounds should be used. However, the laboratory should have the appropriate equipment and expertise to handle these hazardous crystalline substances. Alternatively, certified compound solutions can be used. Preparation of two independent stock solutions allows cross-checks of the standard solutions if necessary.

8.3 System performance

The performance of the GC system can be monitored through regularly analyzing the resolution of two closely eluting compounds. A decrease in resolution indicates deteriorating GC conditions.

The signal-to-noise ratio of a low concentrated standard can give information on the condition of the detector. For example, a dirty MS-source can be recognized by the presence of a higher background signal, together with a reduced signal-to-noise ratio if not used in the SIM mode.

8.4 Long-term stability

One laboratory reference sample should be included in each series of samples. A quality control chart should be recorded for selected chlorinated hydrocarbons. If the warning limits are exceeded, the method should be checked for possible errors. When alarm limits are exceeded, the results obtained should not be reported.

A certified reference material should be analysed at least twice a year, and each time the procedure is changed.

8.5 Internal standards

Internal standards should be added to all standards and samples either in a fixed volume or by weight and should not interfere with the target analytes.

If possible, it is preferable to have internal standards corresponding as much as possible to each analyte, e.g. using isotopically labeled compounds combined with mass spectrometry as detection technique (e.g. pp-DDT-D8, isotopically labelled CBd).

After clean-up and before GC analysis, an additional internal standard can be added to evaluate the recovery of the internal standards added before clean-up.

8.6 Interlaboratory proficiency testing schemes

Each laboratory analysing sediments should participate in interlaboratory studies on the determination of chlorinated hydrocarbons in sediments on a regular basis (e.g. QUASIMEME offers the possibility to take part twice a year, www.quasimeme.org).

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Annex IX:

UNEP/IAEA (2011). Recommended method on the determination of petroleum hydrocarbons in sediment samples (4.2.4)

RECOMMENDED METHOD ON THE DETERMINATION OF PETROLEUM HYDROCARBONS IN SEDIMENT SAMPLES

NAEL/Marine Environmental Studies Laboratory in co-operation with MED POL

November 2011

For further information on this method, please contact:

IAEA-Environment Laboratories
Marine Environmental Studies Laboratory
4a Quai Antoine 1er
MC-98000 Principality of Monaco

Tel. (377) 979 772 72; Fax. (377) 979 772 76
E-mail: NAEL-MESL.Contact-Point@iaea.org

RECOMMENDED METHOD ON THE DETERMINATION OF PETROLEUM HYDROCARBONS IN SEDIMENT SAMPLES

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<u>NOTE:</u> 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.

1. SAMPLING

Detailed guidelines for collecting the sediment samples are available in UNEP(DEC)/MEDWG.282/Inf.5/Rev.1

2. GENERAL DISCUSSION

Following collection of sediment 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. Sediments are then Soxhlet extracted using hexane and dichloromethane. Following initial clean-up treatments (removal of sulfur), extracts are fractionated using column chromatography with silica and alumina. Quantification is done by GC-FID and GC-MS.

3. APPARATUS

- 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, 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.
- 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.
- Solid Phase Extraction glass columns: Upti-Clean SPE Glass Columns Si/CN-S (1g/0.5g)/6ml-PTFE Frits
- 12 or 24 ports Glass Vacuum manifold for simultaneous use of multiple SPE columns. Manifold must be fitted with flow control valves to allow a fine adjustment of flow through the SPE column.

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 H_2SO_4 (d 20°C: 1.84 g/ml).
- Potassium dichromate.
- HCl, 32 % (Merck).
- Hexane," distilled in glass" quality.

- Dichloromethane, “distilled in glass” quality.
- Methanol, “distilled in glass” quality.
- Acetone, “distilled in glass” quality.
- Anhydrous sodium sulfate.
- Carborundum.
- Copper powder (Merck, <63µm, 99 % purity).
- Glass wool
- 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-C_{14} d30, n-C_{19} d40, n-C_{32} d66.
- Hexamethylbenzene, Cadalene: 1,6-dimethyl-4-(1-methylethyl)naphthalene, Naphthalene-d8.
- 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 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.

4.2.2. Cleaning of adsorbents

Silica gel and Alumina are treated chemically. Reagents are 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 in a rotary evaporator at a low speed, until the sorbent starts falling down as fine particles. Reagents are then dried in a drying oven at 120 °C for 4 hours. Silica and alumina are activated at 200 °C for 4 hours. Sorbents are allowed to cool in the oven. As active sorbents attract water and contaminants from the atmosphere, controlled deactivation is carried out by adding water to the fully active sorbent (5 % by weight).

The deactivation procedure is carried out by adding the water to the sorbent, and mixing by gentle shaking for a few minutes. The equilibration takes one day.

4.2.3. 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.

5. PROCEDURE

5.1. EXTRACTION OF FREEZE-DRIED SAMPLES

10 to 20 grams of freeze-dried sediment sample, ground and sieved at 250 µm, are extracted in a Soxhlet extractor with a mixture of hexane and dichloromethane (50:50). Internal standards are added to the sample for recovery: 50 µl of a mixture containing: 30ng/ µl of n-C_{14} d30, 32.444 ng/µl of n-C_{19} d40, 40 ng/µl of n-C_{32} d66 for the first fraction and 30 ng/µl of Hexamethylbenzene and 32.688 ng/µl of Cadalene and 33.3588 ng/µl of Naphthalene-d8 for the second fraction.

Extraction is realized in the Soxhlet with 250 ml of the mixture hexane/dichloromethane (50:50), the siphon cycle is about 10 minutes during 8 hours.

5.1.1. 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.

<u>Alternative method</u>:

Using microwave oven: 10-15 g of sediment is placed in a glass tube with 40 ml of mixture hexane/methylene chloride (50:50), 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 30 min. (a

combined extraction could be performed for both OC and PH if proper internal standards are added before extraction starts).

5.2. CONCENTRATION OF THE EXTRACT

When the extraction is completed, the extract is evaporated with a rotary evaporator to a volume of about 15 ml (the temperature of the water bath does not exceed 30 °C).

The extract is dried with anhydrous sodium sulfate and transferred in a graduated tube and concentrated down to 4 to 5 ml using a flow of clean nitrogen.

5.3. EXTRACTABLE ORGANIC MATTER (EOM)

The EOM is determined in the following manner. On the weighing pan of an electro-balance, a known volume of the sediment extract is evaporated (up to 100 µl) and the residue is weighted 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:

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

5.4. CLEAN-UP PROCEDURE AND FRACTIONATION

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

5.4.1. Sulfur and sulfur compounds removal

<u>Preparation of Copper:</u>

Transfer about 20 g 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, does it 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 sulfur compounds in the sample will be detected by the tarnishing of the copper powder.

5.4.2. Fractionation

The clean-up and separation are achieved by a simple column chromatographic partition as follows:

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 60 °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.

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.

<u>Separation of compounds:</u>

The sample (maximum 100 mg lipids for sediment) 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 unsatured and aromatic hydrocarbons.

5.4.3. Fractionation: Alternative Method using SPE columns

An alternative method using commercially available Solid Phase Extraction cartridges have been implemented.

This method requires Solid Phase Extraction glass columns (Upti-Clean SPE Glass Columns Si/CN-S 1g/0.5g/6ml-PTFE Frits) and a 12 or 24 ports Glass Vacuum manifold for simultaneous separation on multiple SPE columns. Manifold must be fitted with flow control valves to allow a fine adjustment of flow through the SPE column and disposable Teflon liners.

Once placed onto the manifold, SPE columns are rinsed with 10 ml of n-Hexane.

The sample is applied on top of the Solid Phase Extraction columns. The first fraction containing aliphatics compounds is obtained by eluting with 4 ml of n-Hexane. The second fraction containing PAHs is obtained by eluting 5 ml of a n-Hexane: Dichloromethane (1:1) solution.

6. GAS CHROMATOGRAPHY CONDITIONS

6.1. QUANTIFICATION OF PETROLEUM HYDROCARBONS

Gas Chromatograph:	FISONS GC 8000
Detector:	FID
Injection Technique:	On column
Injector temperature:	30 °C
Injection Volume:	1 µl
Carrier gas:	Helium
Flow rate:	1.9 ml/min.
Column used:	
Type of column:	Capillary
Length:	30 m
Diameter:	0.32 mm
Phase:	HP-5MS
Film thickness:	0.25 µm
Temperature program:	
Initial temperature:	60 °C
Rate:	3.5 °C/min.
Final temperature:	300 °C
Isothermal:	22 min.
Detector temperature:	310 °C
Air flow:	320 ml/min.
Hydrogen flow:	27 ml/min.

6.2. QUANTIFICATION OF PAHs

GC/MS:	Agilent MSD5975
Detector:	MS - SIM
Injection Technique:	Splitless
Injector temperature:	270 °C
Injection Volume:	1 µl
Splitter closing time:	1 min.
Carrier gas:	Helium
Flow rate:	1.6 ml/min.

Column used:	
Type of column:	Capillary
Length:	30 m
Diameter:	0.25 mm
Phase:	DB-XLBMS
Film thickness:	0.25 µm
Temperature program:	
Initial temperature:	60 °C
Isothermal:	1 min.
First rate:	10 °C/min.
To:	100 °C
Second rate:	3.5 °C/min.
To:	290 °C
Isothermal:	10 min.
Interface temperature:	290 °C
Source temperature:	230 °C

6.3. TARGET AND CONFIRMATION IONS 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		
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
d_{10} - diphenyl	164	162	32
Acenaphthylene	152	151	20
Acenaphthene	154	153	86

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	98
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
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	21
Perylene	252	253	22
Benzo[b or k]fluoranthene	252	253	23
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.

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:

$$Amount = \frac{\text{Peak Area}}{\text{Response Factor}}$$

The method based on the external calibration doesn't take into account any variance in gas chromatograph performance.

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:

$$RRF(X) = \frac{\text{Area (X)}}{\text{Area (IS)}} \text{ x } \frac{\text{Qty (IS)}}{\text{Qty (X)}}$$

Where:

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.

$$\%RSD = \frac{\text{Standard deviation of the RRFs}}{\text{Average of the RFs}} \text{ x } 100$$

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.

$$Qty\,(\text{X}) = \text{Qty (IS) x } \frac{\text{Area (X)}}{\text{Area (IS)}} \text{ x } \frac{1}{\text{mRR}F(X)}$$

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 of course. Recoveries below 70% are to be considered unacceptable. Recoveries in excess of 100 % may indicate the presence of interference.

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. REFERENCES

Review Meeting of MED POL – Phase III Monitoring Activities. Palermo (Sicily), Italy 12-15 December, 2005. UNEP(DEC)/MEDWG.282/Inf.5/Rev.1. Methods for Sediments Sampling and Analysis.

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Annex X

OSPAR (2008). JAMP Guidelines for monitoring contaminants in sediments. Technical Annex 3: Determination of parent and alkylated PAHs in sediments (4.2.5)

OSPAR COMMISSION

Protecting and conserving the North-East Atlantic and its resources

JAMP Guidelines for Monitoring Contaminants in Sediments

Technical Annex 3: 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.

2.2 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-*d*]thiophene	234
C_1-Naphthalenes	142	C_1-benzonaphthothiophenes	248
C_2-Naphthalenes	156	C_2-Fluoranthenes/Pyrenes	230
C_3-Naphthalenes	170	Benz[*a*]anthracene	228
C_4-Naphthalenes	184	Chrysene	228
Acenaphthylene	152	2,3-Benzanthracene	228
Acenaphthene	154	C_1- Benz[*a*]anthracene/ Chrysene	242
Biphenyl	154	C_2- Benz[*a*]anthracene/ Chrysene	256
Fluorene	166	C_3- Benz[*a*]anthracene/ Chrysene	270
C_1-Fluorenes	180	Benzo[*a*]fluoranthene	252
C_2-Fluorenes	*194*	Benzo[*b*]fluoranthene	252
C_3-Fluorenes	*208*	Benzo[*j*]fluoranthene	252
Dibenzothiophene	184	Benzo[*k*]fluoranthene	252
C_1-Dibenzothiophenes	198	Benzo[*e*]pyrene	252
C_2-Dibenzothiophenes	212	Benzo[*a*]pyrene	252
C_3-Dibenzothiophenes	226	Perylene	252
Phenanthrene	178	Indeno[1,2,3-*cd*]pyrene	276
Anthracene	178	Benzo[*ghi*]perylene	276
C_1-Phenanthrenes/Anthracenes	192	Dibenz[*a,h*]anthracene	278
C_2-Phenanthrenes/Anthracenes	206	Benzo[*k*]fluoranthene	252
C_3-Phenanthrenes/Anthracenes	220	Cyclopenta[*cd*]pyrene	226
Fluoranthene	202	Naphtho[2,1-*a*]pyrene	302
Pyrene	202	Dibenz[*a,e*]pyrene	302
C_1-Fluoranthenes/Pyrenes	216	Dibenz[*a,i*]pyrene	302
Benzo[*b*]naphtho[2,1-*d*]thiophene	234	Dibenz[*a,l*]pyrene	302
Benzo[*b*]naphtho[1,2-*d*]thiophene	234	Dibenz[*a,h*]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 quadropole 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.

7.2 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.

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 µg 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.

[1] More info on https://srmors.nist.gov/view_detail.cfm?srm=1941B

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.

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Annex XI

HELCOM (2012). Manual for marine monitoring in the COMBINE programme. Annex B-13, Appendix 1. Technical note on the determination of Polycyclic Aromatic Hydrocarbons (PAHs) in sediment (4.2.6)

Guidelines for the determination of polycyclic aromatic hydrocarbons (PAH) in sediment

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1. Introduction

This Technical note provides advice on the analysis of polycyclic aromatic hydrocarbons (PAH) in total marine sediments, sieved fractions, and suspended particulate matter. The analysis of PAH compounds in sediments basically includes extraction with organic solvents, clean-up, and separation through high performance liquid chromatography (HPLC) with ultraviolet (UV) or fluorescence detection or gas chromatographic separation (GC) with flame ionization (FID) or mass spectrometric (MS) detection (Kassim & Barcelo, 2009, 1989; Wise *et al.,* 1995).

All steps of the procedure are susceptible to insufficient recovery and contamination. Quality control measures are recommended in order to regularly monitor the performance of the method. These guidelines are intended to encourage and assist analytical chemists to critically review their methods and to improve their procedures and quality assurance measures, if necessary.

These guidelines are not intended as complete laboratory manual. If necessary, guidance should be sought from specialized laboratories. Laboratories should demonstrate validity of each methodological step.

Moreover, use of an alternative method, carried out concurrently to the routine procedure, is recommended for validation.

Contracting parties should follow the HELCOM monitoring guideline but minor deviations from this are acceptable if the method achieves comparable results. Validation of the adopted method needs to be performed on the relevant matrix and concentration range e.g. by taking part in intercomparison studies or proficiency testing schemes.

2. Sampling and storage

The major criterion for successful sediment sampling is to ensure undisturbed sample stratification. (For further details about sampling, see Annex B-13, Appendix 3 “Technical note on the determination of heavy metals in marine sediments” of the HELCOM COMBINE manual.)

Plastic materials should not be used for sampling and storage due to the risk of adsorption of PAH compounds onto the container material. Samples should be transported in closed containers and preferentially at temperatures below 10 °C. The samples should be stored at 4 °C as soon as possible, but at least if they have not been analysed within 48 hours after collection (short-term storage). For long-term storage over several months the samples should be frozen below -20 °C or dried (Law and de Boer, 1995). When drying, avoid methods with substantial risk of losing volatile substances (see Chapter 4: Pretreatment).

PAH compounds are sensitive to photo-degradation and, thus, exposure to direct sunlight or other light sources should be avoided during storage as well as during all steps of sample preparation (Law and Biscaya, 1994). The use of amber glassware is strongly recommended.

3. Blanks and contamination

Basically, care should be taken to avoid contaminations during all steps of the analytical chain, including sampling, extraction and clean-up.

In order to reduce blank and sample contaminations to a minimum it is strongly recommended to pretreat all used glassware, solvents, chemicals, adsorption materials, etc., as follows:

- Glassware should be thoroughly washed with detergents and can be furthered cleaned, other than calibrated instruments, by heating at temperatures > 250 °C. The glassware should be rinsed with an organic solvent prior to use.
- All solvents should be analyzed for impurities by concentrating to 10 % of the regular final volume. This concentrate is then analysed similarly to a sample by HPLC or GC. The solvent blank should not contain target analytes or other interfering compounds in higher concentrations than specified by the laboratory.
- All chemicals and adsorption materials should be analyzed for impurities and purified (e.g., by heating or extraction), if necessary. Soxhlet thimbles should be pre-extracted. Glass fiber thimbles are preferred over paper thimbles. Alternatively, full glass Soxhlet thimbles, with a G1 glass filter at the bottom, can be used.

Storage of these supercleaned materials for a longer period is not recommended, as laboratory air might contain PAH compounds which can adsorb onto these materials. Therefore, contaminated blank samples might occur despite precautionary measures due to contamination from the air. Volatile compounds, in particular naphthalene and phenanthrene, are usually the most common contaminants in blank samples (Gremm and Frimmel, 1990). Therefore, if possible, critical steps should be done in a clean bench.

4. Pretreatment

The samples should be thoroughly homogenized before subsampling for analysis. The amount of samples usually depends on the expected concentrations. For the marine environment, the amount of sample should be equal to an amount representing 50–100 mg of organic carbon.

PAHs can be extracted from wet or dried samples. However, storage, homogenization and extraction are easier to handle with dried samples.

Drying the samples at ambient or elevated temperatures as well as freeze-drying may alter the concentrations, e.g., by contamination or loss of compounds through evaporation (Law *et al.,* 1994). Therefore, potential losses and contaminations should be analyzed in advance, e.g. by exposing 1–2 g CIS-bonded silica to the drying conditions and subsequent extraction and analysis (clean-up can be omitted) (Smedes and de Boer, 1998). For evaluation of potential losses, analytes identical or similar to PAHs need to be added to the material. However, bear in mind that added analytes can behave differently from analytes that have interacted longer with the matrix material and therefore may be sorbed more strongly. To avoid contamination during freeze-drying, placing a lid with a hole of about 3 mm in diameter on the sample container is suggested.

Chemical drying of samples can be performed by grinding with Na_2SO_4, or $MgSO_4$ until the sample reaches a sandy consistency. It is essential that several hours elapse between grinding and extraction to allow for complete dehydration of the sample. Residual water will decrease extraction efficiency.

5. 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). The most photo-sensitive PAH is benzo[*a*]pyrene, followed by anthracene. Photo-degradation occurs more rapidly in the absence of a sample matrix. Therefore, the PAH standard solution should be regularly analyzed for their PAH content.

Other extraction and clean-up methods than those described below may be used, provided that the methods have been tested and found equivalent to established methods regarding e.g. recovery. For naphthalene, which can easily be lost in several steps during sample preparation, headspace or purge and trap analysis might provide a suitable alternative to extraction methods.

5.1 Extraction of wet sediments

A commonly used and very efficient method for PAH extraction from sediments is alkaline saponification. This method requires only a short extraction time (approximately 1.5 hrs under the reflux) and it also eliminates organic sulphur and other interfering compounds such as lipids. The resulting extract is easy to clean up.

Wet sediments could also 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. In a second step a less polar solvent / solvent mixture such as acetone/hexane should be used. It has to be kept in mind that hexane is a lot more toxic than similar solvents such as pentane, heptane, cyclohexane, isohexane. For sufficient extraction at least three subsequent extractions are needed. The contact time with the solvent should be long enough to allow complete desorption of the PAH compounds from the sediment pores. The contact time might be up to 24 hours which basically depends on the type of sediment.

The required contact time of the sediment with the solvent can be reduced by using microwave extraction, supercritical fluid extraction, Soxhlet extraction or pressured liquid extraction (e.g. ASE). Soxhlet or ASE 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. In a second step the collecting flask is replaced and the extraction will be continued using a less polar solvent or solvent mixture such as acetone/hexane or toluene. Thereafter, the extracts will be combined.

To separate the water and keep the PAHs in a solvent that is compatible with the continued analysis different methods can be used. For example, water will be added to the combined extracts and the PAH compounds will be extracted to a non-polar solvent. Another possibility is to add Na_2SO_4 to bind water.

Extraction should be conducted with a sufficient number of extraction cycles. Extraction efficiency should be analyzed for different types of sediments through a second extraction step. The extracts will be analysed separately and compared. A recovery of more than 90 % during the first extraction step is considered adequate.

5.2 Extraction of dry sediments

The methods described above can also be used for dried sediments. However, pressurized liquid extraction (PLE) is the most frequently applied technique to extract PAH compounds from dried sediments and it is recommended over mixing methods, in particular for dry samples.

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. Although toluene is not favored due to its high boiling point, it should be chosen when sediment samples could contain soot particles. For routine marine samples, the use of a mixture of a polar and a non-polar solvent such as acetone/hexane (1/3, v/v) is recommended.

Extraction should be conducted with a sufficient number of extraction cycles. Extraction efficiency should be analyzed for different types of sediments through a second extraction step. The extracts will be analysed separately and compared. A recovery of more than 90 % during the first extraction step is considered adequate.

5.3 Clean-up

The crude extracts usually require clean-up to remove co-extracted compounds (Wise *et al.,* 1995). Due to chlorophyll-like compounds extracted from the sediment, the raw extract is usually colored and also contains sulphur and sulphur-containing compounds, oil and 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 polar solvents used during extraction should be removed. The recommended acetone/hexane mixture will result in hexane after evaporation due to the formation of an azeotrope if hexane is abundant. Evaporation can be done using either a Kuderna-Danish,a rotary evaporator or other evaporation system (e.g. parallel evaporation). In particular, upon using rotary or parallel evaporation, ambient or mild vacuum conditions and a water bath temperature of not more than 30 °C should be applied and care should be taken to stop evaporation at a sample volume of about 2 ml or by using automatic systems. For further volume reduction a gentle stream of nitrogen can be applied. The extract should never be evaporated to dryness.

To remove polar interferences from the extract the following chromatographic procedures can be used:

- desactivated aluminium oxide (10 % water), eluted with hexane – in particular upon using HPLC-Fluorescence for subsequent analysis
- silica or modified silica columns, e.g., aminopropylsilane or cyanopropyl phase eluted with toluene or a semipolar solvent mixture such as hexane/acetone (95/5, v/v) or hexane/dichloromethane (98/2, v/v)

- Gel permeation chromatography (LC-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 organic solvent extraction. Ultrasonic treatment might improve the removal of sulphur. Alternative methods to the use of copper were reported by (Smedes and de Boer, 1998).

Analysis by GC-FID or HPLC-UV requires a more elaborate clean-up. Aliphatic hydrocarbons originating from mineral oil interfere with the flame ionization detection. They can be removed from the extract by fractionation over columns filled with activated aluminium oxide or silica as described above. However, a first fraction is eluted with only hexane and then rejected. The PAHs elute in the second fraction with a more polar solvent, e.g., diethylether or acetone/hexane. When applying fractionation, the elution pattern has to be checked frequently and in the presence of sample matrix, as this can partially deactivate the clean-up column resulting in earlier elution of the PAH compounds than in the standard solution.

Alkylated PAHs are difficult to remove from extracts by column clean-up. When excessive amounts of these compounds are present, they may interfere with HPLC analysis and such samples should be better analysed by GC-MS. An alternative could be preparative HPLC fractionation using a normal phase silica, cyanopropyl or aminopropyl column.

After clean-up, the eluate or fractions must be concentrated, to e.g. 1 ml. Any concentration method should be conducted carefully as described above as high volatility of the PAH compounds may result in losses during evaporation. HPLC and GC require different solvents for injection of the extract. With the methods suggested, obtained extracts are usually in non-polar solvents. However, for HPLC analysis even small amounts of non-polar solvents may result in a shift of retention time and broadening of the peaks (Reupert and Brausen, 1994). Acetonitrile should be used preferentially as the PAH exhibit higher stability in acetonitrile as compared to e.g., methanol. Hexane can be removed by the addition of 5 ml acetonitrile for each ml of extract and subsequent evaporation to 1–2 ml. Azeotropic evaporation leaves only acetonitrile. During solvent exchange, evaporation to dryness should be avoided.

Azeotropic exchange can also be applied the other way around. In that case, 5 ml hexane must be added for each ml of acetonitrile.

For GC methods, *iso*-octane or toluene are suitable solvents for injection and can be added as keeper before evaporation to the required volume.

6. Chromatographic determination

The separation of PAHs should be optimized for at least the compounds listed in Annex B-13 (Appendix 1, Table 1) (Keith and Telliard, 1979). Separation should not only be optimized for a standard solution but also for a sample, as samples often contain several non-target PAHs that should be separated from the target compounds, if possible. In addition, sample extracts can be affected by remaining matrix effects, despite clean-up.

Basically and in particular for the parent PAH both HPLC-Fluorescence and GC-MS analyses are considered to be equally valid methods. However, with respect to the alkylated PAH species satisfactory analysis is often not obtained using HPLC. This is particularly relevant as alkylated PAH compounds are of increasing interest. Therefore, use of GC-MS analysis is recommended.

Table 1 Compounds of interest for environmental monitoring for which the guideline applies.

Compound	MW	Compound	MW

Naphtalene	128	Fluoranthene	202
C1-Naphtalenes	142	Pyrene	202
C2-Napthalenes	156	Benzo[a]anthracene	228
C3-Napthalenes	170	Chrysene	228
Acenaphthylene	152	Benzo[a]pyrene	252
Acenaphthene	154	Perylene	252
Fluorene	166	Indeno[1,2,3-cd]pyrene	276
Phenanthrene	178	Benzo[k]fluoranthene	252
Anthracene	178	Benzo[ghi]perylene	276
C1-Phenanthrene/Anthracene	196	Dibenzo[ah]anthracene	278
C2-Phenanthrene/Anthracene	206		
C3-Phenanthrene/Anthracene	220		

6.1 High performance liquid chromatography

For HPLC analysis of PAH, a binary gradient is necessary to achieve proper compound separation.

Using HPLC and measuring concentrations with the peak height, a 50 % valley should be considered as adequate separation.

Solvents should be degassed through an online degassing system in order to allow proper operation of the high pressure pump. Sample injection should be carried out with an autosampler. In addition, a thermostated column compartment (10–30 °C) should be used as retention time and resolution can be affected by varying the temperature.

6.1.1 Columns

The column specifications are:

- stationary phases: e.g., octadecylsilane (RP-18), or special PAH column material;
- length: 15–25 cm;
- inner diameter: 4.6 mm or less;
- particle size: 5 µm or less.

If the dimensions of the detector cell and the tubings are appropriate, columns with diameters smaller than 4.6 mm can be chosen in order to reduce the flow of the eluent and, thus, to save solvent. In this case the amount of sample injected should also be reduced to e.g. 25–50 µl for a 4.6 mm column or 10 to 20 µl for a 3 mm column.

6.1.2 Gradient Elution

For elution, e.g. methanol/water or acetonitrile/water can be applied. The use of acetonitrile allows higher flow rates, with the disadvantage of having higher health risks than methanol.

A typical elution gradient with a flow rate of 1–1.5 ml min-1 for a 4.6 mm column is:

- start at initially 50 % methanol/water or acetonitrile/water

- run to 100 % methanol or acetonitrile in 40 minutes
- remain for 20 minutes
- back to the initial conditions for about 5 minutes
- equilibrium time of about 5 to10 minutes (3–5 times the dead volume) prior to the next injection,

100 % methanol or acetonitrile may not be sufficient to elute all non-target compounds from the column. In this respect, a further elution step using acetone/methanol (1/1) or acetonitrile/acetone (1/1) can be applied. A ternary gradient is then necessary.

In order to obtain reproducible retention times, the equilibrium time after each run should be constant. Therefore, automatic injection is strongly recommended.

6.1.3 Detection

For the detection of PAHs, the more sensitive and selective fluorescence detector is preferred over a UV detector. The excitation and emission wavelengths should be programmable to allow the detection of PAHs at their optimum wavelength (Reupert and Brausen, 1994; ISO, 1995).

However, when PAHs elute close to each other, wavelength switching cannot be carried out between these peaks and a wavelength pair appropriate for the respective compounds has to be chosen. The use of two detectors in series, or running the analysis twice with different wavelength programmes, can minimize the need for such compromises.

As the fluorescence signals of some PAHs can decrease by up to a factor of ten in the presence of oxygen, the eluents must be degassed thoroughly. Therefore, instruments with online degassing systems are strongly recommended. In addition, PTFE tubings should not be used as this material is permeable to oxygen and allows oxygen to enter the system again. The use of stainless steel or PEEK (polyetheretherketone) tubing is recommended.

Acenaphthylene is not detectable with fluorescence and, therefore, a UV or diode-array detector should be used for detection.

Another possible detection technique is mass spectrometry, where isotopically labeled compounds are used as internal standards.

6.1.4 Identification

The individual PAHs are identified by comparing the retention time of the substance in a sample with that of the respective compound in a standard solution analyzed under the same conditions. It is recommended to confirm the results by using other suitable wavelength for UV-absorption or excitation and emission wavelengths for fluorescence detection.For HPLC analysis, reproducibility of retention times should be within ±1 minute.

6.2 Gas chromatography

6.2.1 Columns

Column dimensions for the determination of PAHs should be the following:

- length: minimum 25 m;
- inner diameter (i. d.): maximum 0.25 mm;
- film thickness: between 0.2 µm and 0.4 µm;
- stationary phases: A wide range of non-polar or slightly polar stationary phases can be used for the separation of PAHs, e.g., a 5 % phenyl-substituted methyl polysiloxane phase.

Better resolution can be obtained by increasing the length and reducing the inner diameter to 0.20 mm or less. However, below a diameter of 0.15 mm, the carrier gas pressure rises to values above 500 kPa, which are often not compatible with regular GC equipment. Also, the risk of leakages increases.

6.2.2 Carrier gas

Preferentially, helium should be used as carrier gas for GC-MS. Upon using columns with very small inner diameters, the use of hydrogen is essential.

The linear gas velocity should be optimized. Appropriate settings for 0.25 mm i.d. columns range from 20 to 40 cm s^{-1} and for 0.15 mm i.d. columns from 30 to 50 cm s^{-1}.

6.2.3 Injection techniques

Sample injection should be carried out with an autosampler. The two systems commonly used are splitless and on-column injection. Other techniques such as temperature-programmed or pressure-programmed injection may have additional advantages, but should be thoroughly optimized before use. Due to their high boiling points, on-column injection is recommended for separation of the PAH compounds.

6.2.4 Temperature programming

The temperature program must be optimized for sufficient separation of the PAH compounds. For GC-MS analysis peak areas are generally used and a 10 % valley would represent a good separation. Less resolved peaks may also be quantified - e.g. by dropping perpendiculars to the baseline-, but increasing errors may result.

In addition to a reproducible temperature program, a fixed equilibration time is important for a correct analysis and constant retention times.

6.2.5 Detection

The most frequently used detector for GC analysis of the PAH compounds is a mass spectrometric detector operating in the Selected Ion Monitoring (SIM) mode and with electron impact ionization (EI) as the ionization method. The selectivity of a mass spectrometric detector is excellent and the chromatographic noise of a standard is similar to that of a sample. However, major drawbacks are the matrix-dependent response and the convex calibration curves that both often occur and make quantification difficult which, however, can be overcame with tandem mass spectrometry (GC-MS/MS).

Another technique for PAH identification is the full-scan MS using an ion trap operating with the same sensitivity as SIM, but in full scan to give the best detection limits and compound identification for methylated PAHs, compared to quadrupole MS with multiple ion monitoring mode (Law et al, 2011). The use of a flame ionization detector (FID) is also possible, but since the selectivity of the FID is low, it is not recommended. In addition, isotopically labeled internal standards (see 8.5) cannot be used in combination with a FID.

6.2.6 Identification

For GC-MS analysis the presence of the characteristic mass fragments or mass transitions (GC-MS/MS) prove the presence of the particular PAH compound. Retention times should be reproducible within ±0.05 minutes.

7. Quantification

Automatically processed chromatograms should be reviewed if, e.g., the baseline is set correctly. Because the separation of the peaks is often incomplete in HPLC analysis, the use of peak heights is recommended for quantification. In case of GC techniques, the use of peak areas is recommended.

For calibration purposes a multilevel calibration with at least five concentration levels is recommended. The calibration curve should be linear and cover the working range. Usually, the response of FID, UV and fluorescence detectors exhibit linearity over a large range.

Since mass spectrometric detectors often lack sufficient linear response, the use of stable isotopes is a prerequisite. Furthermore, the response of PAHs in standard solutions is often much lower than in sample extracts. A combination of different methods, e.g., use of internal standards and standard addition, might give quantitative results.

Obtained calibrations should be regularly validated in terms of precision and accuracy.

Prior to running a series of samples and standards, the GC or HPLC systems should be equilibrated by injecting at least one sample extract. In addition, standards used for multilevel calibrations should be regularly distributed over the sample series so that matrix-and non-matrix-containing injections alternate. A sample series should include:

- a procedural blank;
- a laboratory reference material;
- at least five standards;
- one standard sample treated similarly to the samples for determination of the recovery.

The method for calculating the limit of determination should reflect the advice in Part B-4.2.3 (COMBINE manual).

The limit of quantification usually depends on the purpose of the investigation. The limit of quantification that can be achieved depends on the blank sample, the sample matrix, concentrations of interfering compounds, and the amount of sample. However, a limit of quantification of 2 ng g^{-1} (dry weight) or better should be attained for single compound analysis.

8. Quality Assurance

A number of measures should be taken to ensure sufficient quality of the analysis. Six main areas can be identified:

1. extraction efficiency and clean-up;
2. calibrant and calibration;
3. system performance;
4. long-term stability;
5. internal standards; and
6. Frequent participation in interlaboratory proficiency testing schemes (e.g. QUASIMEME two times a year, www.quasimeme.org).

8.1 Extraction efficiency and clean-up

Extraction efficiency and clean-up can be controlled by analysing reference materials (Annex B-7). To determine the recovery rates of the clean-up and concentration steps, it is recommended to pass a standard solution (see 8.5. INTERNAL STANDARDS) through the entire procedure. The addition of corresponding internal standards to the samples is preferred.

If major losses have occurred, the results should not be reported.

8.2 Calibrant and calibration

Basically, calibration solutions should be stored in ampoules at a cool, dark place. Weight loss during storage should be recorded for all standards.

For PAH determination preferentially calibration solutions from certified crystalline PAHs should be used. However, the laboratory should have the appropriate equipment and expertise to handle these hazardous crystalline substances. Alternatively, certified PAH solutions can be used. Preparation of two independent stock solutions allows cross-checks of the standard solutions if necessary.

8.3 System performance

The performance of the HPLC or GC system can be monitored through regularly analyzing the resolution of two closely eluting PAHs or chlorinated biphenyl compounds. A decrease in resolution indicates deteriorating HPLC or GC conditions.

The signal-to-noise ratio of a low concentrated standard can give information on the condition of the detector. For example, a dirty MS-source can be recognized by the presence of a higher background signal, together with a reduced signal-to-noise ratio if not used in the SIM mode.

8.4 Long-term stability

One laboratory reference sample should be included in each series of samples. A quality control chart should be recorded for selected PAH compounds, e.g., fluoranthene (stable results), pyrene (sensitive to quenching), benzo[a]pyrene (sensitive to light). If warning limits are exceeded, the method should be checked for possible errors and the obtained sample results should not be reported.

If available, a certified reference material (CRM) should be analysed regularly and in particular, if the procedure was changed.

8.5 Internal standards

Internal standards should be added to all standards and samples either in a fixed volume or by weight and should not interfere with the target analytes.

A number of deuterated PAH compounds were proven to be suitable for GC-MS as well as for HPLC analysis. For GC-MS analysis it is recommended to have internal standards corresponding to each analyte, e.g. by using isotopically labeled compounds. Otherwise, at least four internal standards representing the different ring-sizes of the PAH compounds should be added.

The following compounds can be used (Wise et al., 1995):

- for HPLC analysis: phenanthrene-d10, fluoranthene-d10, perylene-d12, 6-methyl-chrysene;
- for GC-MS analysis: naphthalene-d8, phenanthrene-d10, chrysene-d12, perylene-d12;
- for GC-FID analysis: 1-butylpropylene, m-tetraphenyl

After clean-up and before GC analysis, an additional internal standard can be added to evaluate the recovery of the internal standards added before clean-up.

8.6 Interlaboratory proficiency testing schemes

Each laboratory analysing sediments should participate in interlaboratory studies on the determination of PAH in sediments on a regular basis (e.g. QUASIMEME offers the possibility to take part twice a year, www.quasimeme.org).

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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)

Table of the proposed assessment criteria for trace metals (TMs)

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^{-1} d.w.			Fish (MB) µg kg^{-1} f.w.			Sediment µg kg^{-1} 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

[a]Cd value is below the detection limit (<BDL) and Pb presents a majority of non-detected values in monitoring datasets.
[b]estimated 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 of the proposed assessment criteria for polycyclic aromatic hydrocarbons (PAHs)

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^{-1} d.w.			Sediment µg kg^{-1} 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, Acenaphtylene, 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.
[a]OSPAR 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;
[c]ERL: Effect Range Low

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	Musselμg kg^{-1} d.w.			Fish μg kg^{-1} w.w.			[d]Sediment μg kg^{-1} 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*

[1]OSPAR Commission, 2013.
[a]OSPAR Commission, CEMP: 2008/2009 Assessment of trends and concentrations of selected hazardous substances in sediments and biota, Monitoring and Assessment Series
[b]OSPAR Commission, Background document on CEMP assessment criteria for the QSR 2010, Monitoring and Assessment Series
[c]LC: Low concentrations calculated from QUASIMEME; However, BC values should be considered as zero for OCs
[d]Total 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)

Annex XIII

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