DIVISION DES RESSOURCES EN EAU

PROGRAMME DES NATIONS UNIES POUR L'ENVIRONNEMENT

PRESENTATION DE LA CONTRIBUTION DE LA TUNISIE ET DES MODES D'ÉCHANTILLONNAGE ET DE TRAITEMENT SUGGERES PAR L'U.N.E.S.C.O.

FEVRIER 1977.

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REPUBLIQUE TUNISIENNE
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Service Hydrologique

PROGRAMME DES NATIONS UNIES POUR

L'ENVIRONNEMENT

POLLUTION D'ORIGINE TELLURIQUE TRANSPORTÉE EN MÉDITERRANEE

PRESENTATION DE LA CONTRIBUTION DE LA TUNISIE ET DES MODES D'ÉCHANTILLONNAGE ET DE TRAITEMENT SUGGERES PAR L'U.N.E.S.C.O.

FEVRIER 1977.-

SLAH BOUZAIANE
Ingénieur Principal
Responsable du Secteur Centre

PROGRAMME DES NATIONS UNIES POUR

L'ENVIRONNEMENT

Pollution d'origine Tellurique transportée
en Méditerranée

- Resume des décision prises par un groupe d'expert des pays méditérranées réunis sous l'égide de L'UNESCO

  correspondance UNESCO page 1 à 7

- Présentation générale de la contribution de la Tunisie au projet d'évaluation de la pollution transportées par les rivière à la Méditerranée.

  Document DRE - page 8 à 10

- Suggestions pour l'échantillonnage et le traitement avant analyse chimique des matières en suspension.

  Document UNEP30 - page 11 à 19

- Analyse chimique. Document UNESCO - page 19 à 23

  - Spectrophotométrie d'absorption atomique * page 24 à 41
  - chromatographie en phase gazeuses * page 41 à 51

* tiré du "FAO MANUAL of Méthods in Aquatic Environment Research part 3 sampling and analysis of biological Matérial."

Resumé des décisions prises par un groupe d'experts
des pays méditerranéens réunis sous l'Egide de
l'UNESCO en Mai 76 à Paris[1] et en Décembre 76 à Rome[2]

(1) voir rapport de Mission
ZEBIDI BIB. EG 368

(2) voir rapport de Mission
BOUZAÏANE BIB EG 373

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15 février 1977

Cher Monsieur,

Comme vous le savez sans doute l'Unesco est l'une des Agences travaillant sur le Plan d'Action pour la Méditerranée coordonné par l'UNEP. L'action de l'Unesco s'effectue au travers de deux projets, le premier sur les polluants transportés par les sédiments des rivières (Projet MED-IX), le second sur les polluants d'origine terrestre (Projet MED X) dans lequel l'Unesco est responsable du problème des apports de polluants en solution par les rivières.

Deux réunions d'un groupe d'experts des Pays Méditerranéens, tenues en mai 1976 à Paris et en décembre 1976 à Rome, ont développé une méthodologie commune afin d'harmoniser leur approche du problème et ont préparé des recommandations aux Gouvernements en ce qui concerne la sélection des rivières à surveiller et des polluants à analyser. Veuillez trouver ci-joint les rapports de ces deux sessions.

En ce qui concerne les polluants en solution (Projet MED X), le groupe d'experts a recommandé une liste de polluants à analyser (Appendice I). Dans votre pays les rivières suivantes ont été proposées pour cet inventaire :

Med jordan

En premier lieu il a été recommandé de compiler les données antérieures, c'est-à-dire d'avant 1976, en utilisant un formulaire standard (Appendice II) page 5 et 6

En second lieu, le groupe d'experts a proposé que le programme de surveillance, recommandé à sa première réunion et qui avait déjà débuté dans certains pays, soit poursuivi ou commencé en 1977 jusqu'à ce qu'une année hydrologique complète soit étudiée. Cependant, vu les délais imposés par l'UNEP, les premiers résultats de ce programme sont déjà requis pour juin 1977.

En ce qui concerne les polluants particulaires (Projet MED IX), le groupe d'experts a remarqué qu'il y avait un manque de données général pour le Bassin Méditerranéen, sauf sur quelques rares rivières, en dépit de l'importance primordiale de ces éléments dans l'estimation de la charge totale en polluants. Il a été recommandé qu'une campagne de surveillance préliminaire des polluants particulaires figurant à l'Appendice III page 7 soit

effectuée sur certaines rivières sélectionnées. Dans votre pays les rivières proposées sont :

Medjerdah

Pour unifier autant que possible les méthodes utilisées, une description des procédures simplifiées d'échantillonnage et de prétraitement de la matière en suspension a été préparée par le Secrétariat avec l'aide et les conseils des spécialistes du Laboratoire de Radioactivité Marine de l'IAEA à Monaco (Appendice IV) Voir page 11 à 23

Au cas où certains pays auraient des difficultés analytiques le Laboratoire de l'IAEA à Monaco est disposé à fournir une assistance sous forme d'analyses sur un nombre de rivières restreint. Si cette aide est requise les contacts doivent être pris directement avec le Docteur Fukai dans ce Laboratoire. Les spécialistes de l'IAEA proposent d'effectuer les analyses en mai 1977 et renverront ensuite les résultats aux pays concernés.

Au cas où des pays le demanderaient, l'Unesco leur fournira une aide financière modeste pour l'échantillonnage et l'analyse de la matière en suspension et/où une aide technique par des experts.

En raison des délais très stricts imposés par l'UNEP, une première estimation des apports polluants à la Méditerranée doit être entreprise avant juillet 1977. Je vous serais reconnaissant si vous pouviez suivre le calendrier suivant :

1 - Compilation des données antérieures (avant 1976) sur les polluants en solution : veuillez remplir le formulaire standard (Appendice II) et le renvoyer à la Division des Sciences de l'Eau de l'Unesco avant le 30 avril 1977.

2 - Surveillance actuelle des polluants en solution (1976/1977): veuillez utiliser le même formulaire (Appendice II) et le renvoyer rempli à l'Unesco avant le 31 mai 1977.

3 - Surveillance des polluants dans la matière en suspension : les résultats des analyses des polluants figurant à l'Appendice III devront être reportés sur le formulaire standard (Appendice V) et envoyés à l'Unesco avant le 15 juin 1977. Au cas où une aide analytique de la part du Laboratoire de l'IAEA à Monaco serait nécessaire, les sédiments en suspension, préparés suivant les procédures recommandées à l'Appendice IV, devront être reçus par le Docteur Fukai (Laboratoire International de Radioactivité Marine, IAEA, Musée Océanographique, Monaco-Ville, Principauté de Monaco) avant le 30 avril 1977.

Je vous prie d'agréer, Cher Monsieur, l'expression de mes sentiments distingués.

pour J.A. Da Costa
pour S. Dumitrescu,
Directeur,
Division des Sciences de l'Eau.

Appendice I

UNEP PROJET MED X

LISTE DES POLLUANTS DE L'INVENTAIRE DES POLLUANTS EN SOLUTION

1. Polluants extrêmement dangereux (liste noire)
   - Arsenic
   - Mercure
   - Plomb
   - Cadmium
   - PCB (chlorodiphényles)
   - Autres composés organo-chlorés

2. Autres polluants significatifs (liste grise)

Matière organique :	DBO
	DCO, Org. total
Substances organiques :	Phénols
	détergents (MBAS)
	huiles minérales (extrait à l'hexane)
	composés organophosphorés
Métaux lourds :	cuivre
	chrome
	nickel
	zinc
Eléments nutritifs :	phosphore total
	azote total (Kjeldahl)
	nitrates
	ammoniaque
Température :	
Polluants microbiens :	bactéries coliformes
Solides en suspension :	solides en suspensions
	solides volatiles

PROJET SUR LES POLLUANTS D'ORIGINE TERRESTRE ABOUTISSANT A LA MEDITERRANEE

INVENTAIRE DES RIVIERES PRINCIPALES

Pays :
Rivière :

INFORMATIONS GENERALES

A. INFORMATION GEOGRAPHIQUE

Superficie totale du bassin : ..... km^2

Nom et emplacement de la station : .....

Distance de l'embouchure : ..... km

Superficie du bassin à la station : ..... km^2

Barrage en amont : oui / non

Barrage en aval : oui / non

Autres informations : .....

B. INFORMATION HYDROLOGIQUE

Débit moyen annuel (longue période): ..... m^3/sec

Période d'observation correspondante: ..... année

Débits moyens mensuels typiques :

J	F	M	A	M	J	J	A	S	O	N	D

Autres informations sur le régime : .....

.....

.....

C. INFORMATION SUR LA QUALITE DE L'EAU

1. Echantillonage

L'échantillonage est-il intégré dans l'espace :

- horizontalement : oui / non
- verticalement : oui / non

L'échantillonage est-il intégré dans le temps ? oui / non

Si oui sur quelle période ? .....

Les échantillons sont-il filtrés ? oui / non

Si oui indiquez le type et le diamètre des pores du filtre utilisé :

.... micron

2. Résultats analytiques

Les valeurs moyennes sont rapportées dans la page suivante, indiquez ici la méthode de calcul utilisée :

- moyenne arithmétique : oui / non
- moyenne pondérée : oui / non

Si oui sur quelle base : .....

NOM, TELEPHONE ET ADRESSE DE L'INSTITUTION : .....

.....

PERSONNE AYANT REMPLI LE QUESTIONNAIRE (téléphone) .....

.....

RESULTATS ANALYTIQUES

(Veuillez remplir une feuille différente pour chaque année hydrologique)

PAYS : .....
RIVIERE : .....
STATION : .....

Année d'observation : .....

Débit moyen correspondant : .....

Débits moyens mensuels :

J	F	M	A	M	J	J	A	S	O	N	D

Paramètre	Concentration moyenne	Variation		Nombre d'échantillons	Mois prélevés
		min.	max.		
Liste noire :					
Arsenic	µg/l				
Mercure	µg/l				
Plomb	µg/l				
Cadmium	µg/l				
PCB	µg/l				
Autres comp. org.chlorés	µg/l				
Liste grise :					
P total	mgP/l				
N total kjeldahl	mgN/l				
N-NO_3	mgN/l				
N-NH_4	mgN/l				
Cuivre	µg/l				
Chrome	µg/l				
Nickel	µg/l				
Zinc	µg/l				
Comp. org. phosph.	µg/l				
Phénols	µg/l				
DBO_5	mg/l				
DCO	mg/l				
Corg. total	mg/l				
Détergents (MBAS)	mg/l				
Huiles minérales (solubles dans l'hexane)	mg/l				
Bactéries coliformes	col/100ml				

Paramètres généraux : (information générale ne concernant pas l'inventaire des polluants)

Température :
Matière en suspension :
Oxygène dissous :
pH :
Conductivité µS (microohms/cm) à °C

Remarques sur l'année étudiée :

PROJET UNEP MED X

Liste des Polluants de l'inventaire des polluants particulaires

1. Polluants extrèmement dangereux (liste noire)

Arsenic
Mercure
Plomb
Cadmium
Chlorodiphenyles (PCB)
Autres composes organo-chlorés

2. Autres polluants significatif (liste grise)

Cuivre
Chrome
Nickel
Zinc

Présentation générale de la contribution de la Tunisie au projet d'évaluation de la pollution transportée par les rivières à la méditérranée

PROGRAMME DES NATIONS UNIS POUR L'ENVIRONNEMENT (P.N.U.E)

Contribution de la Tunisie
Procedure d'évaluation de la pollution transportée par
la Medjerdah à la Méditerranée

-/-

La Tunisie participe par le canal de la D.R.E.S (D.R.E) au groupe de travail constitué par l'UNESCO, en vue de l'étude de la pollution d'origine Tellurique transporté par les rivières à la méditerranée.

Conformement aux termes de reference établis par le groupe de travail pour le choix des rivières (voir note DRE - E.C 368) seule la Medjerdah a été retenue pour ce travail avec comme station de reference, Djedeïdah.

CARACTERISTIQUES DU BASSIN VERSANT LIMITE A CETTE STATION :

La station de Djedeïdah est située à 50 km de la mer et à 300 km de la station la plus en amont du tronçon Tunisien du cours d'eau.

Le Bassin limité est de 22100 km^2 soit 96% de la totalité du Bassin de la Medjerdah, évalué à 23000 km^2.

LES APPORTS D'EAU :

L'apport moyen annuel d'eau du Bassin à la mer est de 990 M de m^3, le débit moyen interannuel est de 31 m^3/s.

LES APPORTS DE SEL EN SOLUTION :

Quant aux apports de sel ils s'élèvent à 1,16 M. t/an soit une salinité moyenne de 1,17 g/l.

La Medjerdah fournirait alors 0,042 % des apports totaux de sel à la mer et 0,003 % des apports liquides : ceux ci étant évalué respectivement à $2,735 \times 10^9$ tonnes/an (E.A.R.V.E.I - 1945) et $3,75 \times 10^{13}$ m^3/an.

LES APPORTS EN SUSPENSION :

Les apports de sédiments en suspension de la Medjerdah à la station de Medjez-El-Bab (BV : 21200 km^2, située à 66 km en amont de Djedeïdah sont évalués à 21,5 M.t pour un apport d'eau de 715 M. m^3 soit une turbidité moyenne de 30/ g/l.

Il convient de remarquer que ces valeurs sont surement sous estimées, étant donné que le mode de prélèvement ponctuel dans le temps et l'espace n'integre pas les transports dans toute la section de mesure.

Quant à la nature des sédiments transportés, les analyses granulométriques n'ont été faites que pour les sédiments transportés lors de la crue de Mars-Avril 1973. Il en ressort le tableau suivant.

POURCENTAGE DES DIFFERENTS MATERIAUX EN SUSPENSION

DATE	ARGILE 2	LIMON 2-20	SABLE TRES FIN 20-50	SABLE FIN 50-200	MATIERE ORGANIQUE
30/3/73	44,1	40,1	13,1	1	1,3
31/3/73	37,2	31,1	25,1	5,2	1,3
1/4/73	16,0	29,8	31,9	21,0	1,3

Malgré le caractére très localisé de ce résultat il convient de remarquer la prépondérance des éléments fin dans les matériaux transportés.

PROCEDURE ARRETE POUR L'ESTIMATION DES POLLUANTS TRANSPORTES PAR LA RIVIERE MEDJERDAH :

1°/ Fréquence des prélèvements

Elle sera de 4 fois par an correspondant à deux prélèvements en crue et deux prélèvements en étiage, l'échantillon prélevé en crue sera la composition de trois échantillons prelevés en montée de crue, au maximum et en decrue.

2°/ Mode de prélèvement

Les prélèvements se feront au milieu de la section de la rivière et à 20 cm de la surface de l'eau à l'aide de preleveur automatique type OTT les bouteilles de prélèvement sont en verre et de capacité 2 l.

- Suggestions pour l'échantillonnage et le pretraitement avant analyse chimique des matières en suspension dans l'eau des rivières.

- ANALYSE chimique

Documents UNESCO.

- Spectrométrie d'absorption atomique *

- Chromatographie en phase gazeuse *

* tiré du "FAO Manual of méthods in a quatic environnement Reseserch part 3 Snapling and analysis of biological matérial".

PROJET UNESCO/UNEP

"Rôle de la sédimentation dans la pollution de la mer Méditerranée"

APPENDICE IV

Suggestions pour l'échantillonnage et le prétraitement

avant analyse chimique des matières en suspension dans l'eau des rivières

I. INTRODUCTION

Les méthodes d'échantillonnage et de prétraitement avant analyse chimique des matières en suspension dans les rivières sont suggérées ici à la requête finale de la réunion d'experts des pays méditerranéens sur les polluants d'origine terrestre en Méditerranée, tenue à Rome en décembre 1976. Ces notes ont été rédigées après consultation de membres du Laboratoire International de Radioactivité Marine de Monaco (AIEA) et avec leurs conseils.

Il est clair que les méthodes présentées ici peuvent ne pas être les meilleures d'un point de vue scientifique ; toutefois elles ont été choisies pour faciliter et simplifier l'obtention et le traitement des échantillons. Cependant, comme il est urgent d'acquérir sur la quantité de polluants transportés par les suspensions autant de données que possible avant juillet 1977 avec une erreur raisonnable, il semble nécessaire d'unifier les méthodes d'échantillonnage et de prétraitement qui seront utilisées par les laboratoires concernés. Dans ce contexte, il est donc recommandé que les méthodes décrites ici soient utilisées à titre expérimental.

On sait par exemple, que la détermination de la quantité de métaux introduits dans la Méditerranée par les diverses activités humaines nécessite la connaissance des niveaux naturels de ces éléments dans les sédiments en suspension. Nous proposons pour cela d'effectuer une attaque ménagée libérant les éléments provenant de la pollution, mais cette procédure est actuellement délicate à mettre en oeuvre. L'utilisation, recommandée ici, d'HCl dilué pour effectuer cette attaque ménagée est probablement la méthode la plus simple, mais ceci aura également pour effet d'attaquer partiellement les minéraux eux-mêmes en plus des oligo-éléments métalliques qui y sont associés. On aura ainsi une limite supérieure des apports d'origine humaine. Les considérations pratiques ne permettent cependant pas, pour l'instant, d'employer des procédures plus élaborées.

Dans l'hypothèse d'un projet à long terme sur le même sujet - après 1977 - les détails techniques des méthodes à utiliser pour un tel projet devraient être revus une nouvelle fois pour établir une méthodologie plus satisfaisante. Si d'autres méthodes sont déjà

.../...

utilisées par certains laboratoires, il serait nécessaire qu'elles soient comparées avec celles décrites ici par des inter-comparaisons qui seraient d'une grande valeur pour établir les méthodes à utiliser ultérieurement pour un programme à long terme.

Les descriptions détaillées des méthodes analytiques ne sont pas données ici puisqu'il existe déjà de nombreuses méthodes valables qu'on peut trouver dans la littérature publiée. Cependant, on doit insister sur le fait que toute méthode prétendant à une certaine précision devra être utilisée avec un contrôle analytique approprié, sans lequel il y a toujours la possibilité d'une erreur pouvant même dépasser l'ordre de grandeur. Il est donc très important, dans l'éventualité d'un projet à long terme, que des moyens de contrôle analytique appropriés et facilem accessibles soient fournis aux laboratoires, soit par la mise sur pied d'exercices d'intercalibration des méthodes analytiques ou par la distribution de standards de référence. Malheureusement, il n'est pas possible maintenant de réaliser ce type de programme qui requiert beaucoup de temps.

Bien que les résultats analytiques puissent être exprimés par rapport au volume d'eau filtré, g/l, mg/l, etc ... il est aussi nécessaire de connaître la contamination de la matière particulaire elle-même - g/g, mg/g - c'est pourquoi la marche à suivre pour la mesure de la charge en suspension est aussi présentée ici avec les procédés d'échantillonnage et d'analyse des métaux traces et des hydrocarbures chlorés.

II. MESURE DE LA CHARGE EN SUSPENSION

Cette mesure peut être réalisée de façon simple en pesant le filtr avant et après la filtration ; dans chaque cas, le filtre doit être séch au dessicateur dans des conditions identiques. Bien que relativement imprécise, cette méthode est suffisante pour le problème considéré. La quantité d'eau à filtrer pour cette mesure dépend de la turbidité de l'eau : pour des eaux claires, 10 litres sont nécessaires, mais, dans le cas d'eaux particulièrement turbides, 1 litre peut être suffisant La teneur en suspension des rivières de la Méditerranée est généralement comprise entre 10 et 1.000 mg/l). Cette mesure doit être effectuée à la

.../...

même place et au même endroit que l'échantillonnage pour l'analyse des polluants en suspension, mais ne requiert pas d'échantillonneur spécial (on peut donc utiliser l'eau prélevée pour l'analyse).

III. PRISE DE L'ECHANTILLON ET PRETRAITEMENT

1. Eléments traces métalliques

a) Echantillonnage

La principale précaution est d'éviter toute contamination par l'élément à analyser du dispositif d'échantillonnage et du récipient contenant l'eau. Tout dispositif d'échantillonnage non-métallique peut convenir à condition qu'il ait été auparavant soigneusement nettoyé à l'acide chlorhydrique (ou HNO_3) dilué et rincé plusieurs fois à l'eau bidistillée. Pour éviter la contamination par les films superficiels, il est recommandé de prélever sous la surface de l'eau à 20 cm par exemple. Si le dispositif d'échantillonnage contient des parties en caoutchouc, ce qui n'est pas recommandé, il faut éviter tout contact de l'eau avec elles, et une corde en nylon sera utilisée de préférence pour descendre le dispositif dans l'eau.

Dans le cas où un dispositif spécial d'échantillonnage en rivière n'est pas disponible, un seau en plastique (nettoyé à l'acide) peut être utilisé. L'échantillonnage peut aussi être réalisé directement à la main avec une bouteille en plastique (nettoyée à l'acide).

Le volume d'eau à prendre dépend des conditions hydrologiques. La turbidité variant dans les fleuves méditerranéens entre 10 et 1.000 mg/l, une quantité de 10 litres semble suffisante. Bien sûr, pour des rivières peu chargées, des quantités plus grandes devront être prises puisqu'en général on prend 500 mg de matière en suspension sèche pour l'analyse, 100 mg étant le minimum acceptable.

b) Stockage

L'eau brute recueillie ne devrait pas être longtemps stockée dans des récipients avant la filtration. De toutes façons, les récipients devront être exempts de toute contamination. Pour la mesure des métaux en trace, des bouteilles en polyéthylène lavées à l'acide nitrique

.../...

ou chlorhydrique sont recommandées. Le polyéthylène dur (linéaire) est préférable au polyéthylène souple (molécules croisées). Les bouteilles en verre sont, en général, moins conseillées en raison de la force ionique peu élevée des eaux douces.

c) Filtration

L'eau recueillie devra être filtrée aussitôt que possible. Si la filtration ne peut être réalisée dans les vingt quatre heures, il est nécessaire de noter l'intervalle de temps écoulé et de le reporter avec les résultats analytiques. Bien que la taille standard de l'échantillon ait été fixée arbitrairement à 10 litres, il est conseillé de choisir le volume à filtrer de façon à obtenir de l'ordre de 500 mg de suspensions (100 mg minimum).

On utilise, en général, un système de filtration sous vide en verre. Le contact entre l'eau et les parties en caoutchouc doit être évité à tout prix. Afin d'homogénéiser l'échantillon, il est nécessaire d'agiter vigoureusement le récipient d'eau brute avant la filtration, en particulier si une partie seulement de l'eau prélevée est filtrée.

Les filtres utilisés ont un diamètre de pore proche de 0,5 micron et doivent être décontaminés, si nécessaire, par immersion dans une solution diluée d'acide pour analyse et rincés avec de l'eau bidistillée. Le diamètre des filtres du commerce est variable mais, si l'on considère les turbidités rencontrées, il est conseillé de prendre des filtres de diamètre égal ou supérieur à 4,5 cm dans le but d'éviter l'emploi d'un trop grand nombre de filtres.

Les filtres disponibles dans le commerce sont très variés (millipore, nucléopore, membrane). N'importe lequel d'entre eux ayant le diamètre de pores requis convient à condition que son type soit rapporté dans les feuilles de résultats. On choisira de préférence des filtres dont la teneur en métaux-trace est minimum.

Il faut noter que si la turbidité de la rivière échantillonnée est forte la filtration prendra beaucoup de temps et le filtre devra être changé plusieurs fois avec une pince en plastique. On peut alors opérer sur plusieurs installations de filtration en même temps.

.../...

Quand la filtration est terminée, le filtre est soigneusement séché en maintenant le vide quelques instants, puis il est retiré et séché à l'air ou dans un dessicateur (ne pas sécher au four) en prenant soin d'éviter les contaminations.

Si les analyses chimiques sont effectuées par un autre laboratoire que celui qui a filtré, les filtres doivent aussitôt être mis dans des petites boîtes en plastique (lavées) et expédiés, ainsi que cinq filtres vierges qui serviront pour la détermination du "blanc" par le laboratoire d'analyses.

2. Chlorodiphényles (PCB) et autres hydrocarbures chlorés

a) Recommandation générale

Afin d'éviter toute contamination des échantillons pendant l'échantillonnage, le prétraitement et l'analyse, tous les appareils d'échantillonnage, verrerie, installation de filtration, etc ... devront être prénettoyés à l'hexane ou par un autre solvant organique ne contenant pas d'hydrocarbures halogénés, puis chauffés à 300° C pendant quatre heures pour détruire la matière organique. Les solvants sans PCB ni pesticides sont disponibles dans le commerce (par exemple auprès de Solvent Documentation Synthesis) mais l'eau décontaminée doit être préparée par l'analyste lui-même.

Il est également essentiel de travailler dans un laboratoire décontaminé, sans fibre de verre ni particules de peinture, et qui n'a pas subi de pulvérisation d'aérosols, tous ces produits contenant des hydrocarbures halogénés. Même l'air conditionné peut causer une contamination. C'est pourquoi le niveau de contamination du laboratoire par les hydrocarbures halogénés doit être vérifié périodiquement.

b) Echantillonnage

L'appareil utilisé doit être en métal ou en verre (le plastique et le caoutchouc sont à proscrire totalement pendant tout le processus d'analyse des hydrocarbures chlorés) préalablement nettoyés (voir plus haut). A défaut d'appareil ad hoc l'échantillonnage peut être effectué avec un seau en métal prénettoyé. On peut aussi procéder manuellement

.../...

avec une bouteille métallique ou en verre prénettoyée. Afin d'éviter une contamination par la pellicule superficielle, un échantillonnage à 20 cm de la furface de l'eau est recommandé.

D'une façon générale, l'analyse requiert environ 200 mg, cette quantité dépendant du niveau de contamination de la matière en suspension. C'est pourquoi il est recommandé de prélever entre 1 litre pour les eaux très turbides (plus de 200 mg/l), ou très polluées, et 10 litres pour les eaux moins turbides ou moins contaminées.

c) Stockage

L'eau brute est gardée dans des récipients prénettoyés en verre ou en métal et transportée dès que possible au laboratoire pour filtration. Pour éviter tout contact avec des matériaux en plastique ou en caoutchouc, il est recommandé que les récipients soient scellés avec des couvercles en feuille d'aluminium prénettoyée.

d) Filtration

Comme pour les métaux, la filtration doit être réalisée dans les vingt quatre heures si possible, sinon veuillez noter l'intervalle de temps entre l'échantillonnage et la filtration. L'installation de filtration, le porte filtre et le récipient des eaux filtrées doivent être en verre ou en métal (prénettoyé). Il est recommandé de filtrer sous vide.

Le diamètre du filtre peut varier de 2,5 cm à 10 cm, mais, dans tous les cas, le filtre doit être en fibre de verre avec une taille de pores de l'ordre de 0,3 micron. Ces filtres sont facilement disponibles (par exemple, auprès des compagnies Whatman, type fibre de verre GF/C, ou Gelman, type fibre de verre 0,3 μm) et doivent être préalablement nettoyés au solvant et chauffés à 300° C.

Après filtration, si les filtres doivent être stockés avant analyse, ils devront être conservés dans des capsules prénettoyées en verre ou en métal, scellées par une feuille d'aluminium ou par un bouchon à vis.

.../...

On doit noter que les filtres en fibre de verre peuvent adsorber des hydrocarbures chlorés dissous. C'est pourquoi, dans les cas de faibles turbidités, on doit déterminer ainsi la quantité de polluant adsorbé sur le filtre :

- après la première filtration, déterminer la teneur en solution dans l'eau filtrée
- refiltrer cette eau à travers un nouveau filtre en fibre de verre (prénettoyé) et déterminer la quantité d'hydrocarbures chlorés retenue sur ce second filtre puis la soustraire de la quantité trouvée auparavant.

IV. ANALYSE

1. Oligoéléments métalliques

a) Attaque ménagée

Il existe plusieurs types d'attaques ménagées pour lessiver les éléments métalliques liés aux particules en suspension : chacune a ses avantages et ses inconvénients.

C'est une méthode simple, l'attaque à l'acide chlorhydrique 1M, qui a été choisie ici.

On doit garder à l'esprit que la quantité de métaux traces mis en solution ainsi ne provient pas entièrement des activités humaines, mais inclut aussi des apports naturels provenant de l'altération, de l'érosion etc ... Une partie des métaux contenus dans les minéraux eux-mêmes peut également être libérée ainsi. Aussi les données obtenues par cette méthode seront-elles une limite supérieure des métaux traces provenant des activités humaines.

L'acide chlorhydrique utilisé doit être pur et peut être trouvé dans le commerce. L'acide dilué 1M sera préparé au laboratoire avec de l'eau bidistillée.

La matière en suspension obtenue après filtration est placée avec le filtre dans un bécher dans lequel on verse lentement 50 ml d'acide dilué. Suivant la teneur en carbonate et la quantité de matière en suspension, jusqu'à 5 ml d'acide peuvent être nécessaires pour dissoudre les carbonates, aussi procède-t-on lentement. Après l'addition d'acide

.../...

le bécher est couvert avec une capsule de verre et chauffé pendant une à deux heures sur une plaque chauffante.

Dans le cas d'une mesure de mercure, la température de la solution d'attaque ne doit pas dépasser 60° C. Après refroidissement la solution est filtrée au travers d'un filtre nettoyé, de préférence en fibre de verre. Pour l'analyse de mercure, il est recommandé d'effectuer une analyse directe par spectrométrie d'absorption atomique sans flamme (ou par activation neutronique). Si cet élément n'est pas recherché, le filtrat est évaporé presque complètement puis le résidu est repris par 20 ml d'HCl 0,1M. Cette dernière solution est normalement utilisée pour l'analyse de Cu, Zn, Cd, Pb, etc ... par spectrométrie d'absorption atomique avec flamme, bien qu'une petite partie du Pb puisse être perdue pendant l'évaporation. Dans tous les cas, il est nécessaire d'effectuer des blancs sur toute la procédure suivie.

b) Commentaire sur les méthodes analytiques

Dans la mesure du possible, il est recommandé d'utiliser la spectrométrie d'absorption atomique avec flamme pour sa simplicité. Par contre, pour l'arsenic son emploi est contestable en raison de sa sensibilité, beaucoup plus faible pour cet élément que pour les autres. Pour le mercure, la méthode sans flamme doit être utilisée.

Les procédures analytiques détaillées pour la spectrométrie d'absorption atomique peuvent être trouvées dans le FAO Manual of Methods in Aquatic Environmental Research Part 3: Sampling and Analysis of Biological Materials (p. 69/86) (FAO Fisheries Technical Paper 158, 1976, 124 p)*. Comme il a déjà été dit, il est très important de tester la méthode employée par des contrôles analytiques sans lesquels la valeur scientifique des résultats obtenus serait douteuse.

Dans le tableau suivant sont donnés des exemples de sensibilité de la méthode avec flamme (concentration qui correspond à 1 % d'absorption lue au spectrophotomètre). Pour effectuer des mesures avec une bonne marge de sécurité, les concentrations mesurées devraient être au moins dix fois supérieures aux sensibilités données.

.../...

* Voir page

Sensibilités de la spectrométrie d'absorption atomique avec flamme pour divers éléments traces (IAEA, Laboratoire de Monaco).

Elément	Sensibilité (ng/ml)
Cr	100
Ni	150
Cu	90
Zn	20
As	800
Cd	40
Pb	500

2. Chlorodiphényles et autres hydrocarbures chlorés

a) Prétraitement

Les filtres sont séchés à la température ambiante, la chaleur pouvant faire évaporer les hydrocarbures chlorés, puis sont écrasés avec un mortier - prénettoyé - ou broyés dans un mélangeur prénettoyé. Dans le cas du broyage l'extraction par solvant - l'acétonitrile est recommandé - peut être réalisée par l'ajout direct du solvant dans le broyeur. Si les filtres sont broyés au mortier l'extraction doit être faite ensuite par un "extracteur à solvant".

L'extrait est ensuite dilué à l'eau décontaminée, dans un rapport de 1 à 5 volumes. On effectue ensuite une extraction à l'hexane pur (sans hydrocarbures chlorés) sur la solution eau-acétonitrile. L'extrait à l'hexane est ensuite purifié par chromatographie sur microcolonne de florisil.

L'extrait purifié est concentré par évaporation jusqu'à un volume adéquat pour l'analyse, par exemple par un évaporateur rotatif - prénettoyé - suivi d'un concentrateur du type Kuderna-Danish.

b) Analyse

L'extrait final est analysé par chromatographie en phase gazeuse. La procédure analytique détaillée est décrite également dans le FAO Manual of Methods in Aquatic Environment Research Part 3: Sampling and Analysis of Biological Material pp. 86/95 (FAO Fisheries Technical Paper 158, 1976, 124 p).

On n'oubliera pas que des blancs devront être effectués pendant tout le prétraitement afin de dépister toute contamination.

DIAGRAMME DE L'ANALYSE DES POLLUANTS DANS LA MATIERE EN SUSPENSION

	Eléments traces	Hydrocarbures chlorés	Matière en suspension (turbidité)
pré-nettoyage (dispositif d'échantillonnage et de filtration, récipients)	lavage à HNO_3 dil conc.	lavage aux solvants purs (hexane) et chauffage à 300°C	lavage eau pure
pré-pesée			pré-pesée des filtres
échantillonnage - dispositif :	échantillonneur en plastique ou seau en plastique ou bouteille en plastique	échantillonneur ou seau ou bouteille en métal ou en verre	indifférent
- volume :	1 - 10 l	1 - 10 l	1 - 5 l
- situation :	20cm sous la surface	20cm sous la surface	20cm sous la surface
filtration	filtre en cellulose nettoyé à l'acide dilué ≃ 0.5 µ	filtre en fibre de verre nettoyé au solvant et chauffé à 300°C. ≃ 0.3 µ	filtre en cellulose prépesé
prétraitement	Hg | Autres éléments attaque ménagée à H Cl + plaque chauffante <60°C | >60°C filtration | (fibre de verre) ↓ | ↓ Abs.Atom. sans flamme | évaporation ↓ HCl ↓ Abs.Atom.	broyage du filtre ↓ extraction au solvant ↓ purification de l'extrait ↓ évaporation ↓	dessication du filtre
analyse		chromatographie en phase gazeuse	pesée

Référence pour la technique analytique :

FAO Manual of methods in aquatic environment research. Part. 3 - Sampling and Analysis of Biological Material. P. 69-95 (FAO Fisheries Technical Paper, 158)

APPENDICE V

FORMULAIRE D'ECHANTILLONNAGE ET D'ANALYSE DES POLLUANTS PARTICULAIRES

PAYS : .....
RIVIERE : .....
STATION : ..... distance de l'embouchure .....km
VILLE OU INDUSTRIE EN AVAL DE LA STATION : oui / non
PERSONNE QUI A REMPLI LE FORMULAIRE :
Nom .....
Adresse et Téléphone .....

	Eléments traces	Hydrocarbures chlorés	Turbidité
Echantillonnage . date . dispositif utilisé . volume prélevé . situation (rive gauche, droite, milieu) . profondeur			
Filtration . date . type d'installation . type de filtre . volume			
Prétraitement et analyses			

Veuillez indiquer sur une feuille additionnelle le diagramme général de la procédure suivie.

Résultats			
	As µg/g Cd Cu Hg Pb Zn	Chlorodiphenyles (PCB) µg/g Autres hydrocarbures chlorés µg/g	Turbidité mg/l

Remarque - Les résultats peuvent également être exprimés en µg par litre d'eau brute, dans ce cas la turbidité est absolument nécessaire.

Commentaire - Veuillez reporter sur une feuille additionnelle toutes les remarques se rapportant aux conditions hydrologiques du prélèvement, sur la nature des matières en suspension, sur les rejets éventuels de polluants particulaires en amont etc...

6.4 Atomic Absorption Spectrophotometry

The application of atomic absorption spectrophotometry (AAS) for the determination of elements in biological materials consists in introducing, usually after wet combustion, a digest of the material through a burner of an atomic absorption spectrophotometer where the sample is atomised by a flame (Flame AAS). A light beam emitted by a hollow cathode lamp with a cathode made from the element to be analysed is passed through the flame. When a sample is evaporised in the flame, its atoms absorb the lines emitted by the cathode and thus reduces the signal of the spectral line projected with the aid of a monochromator into a phototube. The reduction of the signal is proportional to the amount of the element in the sample. However, non-atomic absorption of the line emitted by the hollow cathode lamp can also occur, especially when samples containing high concentrations of other substances are burned in a low temperature flame. This non-atomic absorption is caused by two processes: molecular absorption and light scattering.

Molecular absorption occurs when the flame temperature is not high enough to transform all molecules into atoms. Higher flame temperature causes more molecules to disintegrate but they also produce a higher number of ions thus reducing the atomic absorption and hence the sensitivity.

Light scattering is caused by particles present in the sample solution. These particles are produced in the flame when the organic material is not completely destroyed in the previous combustion or by the high solid content in the aspirated sample. They reduce the signal which arrives at the photomultiplier. Both types of non-atomic absorption occur over a wide range of wavelength and are greatly increased at shorter wavelength.

Three methods may be employed to reduce or compensate for the non-atomic absorption. One is a chemical separation before introducing the sample solution into the flame. Another method consists in applying a correction (background correction) to the atomic absorption measurement and the third requires that the standards are prepared in solutions which approximate the matrix concentrations. The absorption measurement of the line source (e.g. the hollow cathode lamp) is the sum of the atomic absorption and the non-atomic absorption. The background correction of the absorption due to light scattering and molecular absorption can be achieved by determining the absorption at the selected wavelength of a continuous spectrum emitting light source (hydrogen filled hollow cathode lamp or deuterium arc lamp). In practice this means that both (i) the absorption of the selected line emitted from the cathode lamp and (ii) the absorption of the continuous spectrum is determined.

In modern double beam instruments these two measurements are carried out simultaneously so that in very short succession (20 to 150 times per second) either light of the cathode lamp or the continuous source pass through the flame. The data processing unit which receives the output from the photomultiplier is synchronised with the modulated light sources so that the signal from the cathode lamp can be distinguished from the signal of the continuous source making an automatic correction for non-atomic absorption possible (automatic background correction).

The third methodology used in correcting for non-atomic absorption consists in preparing the standards in solutions which closely resemble the chemical composition of the sample under investigation or using the standard addition method for calibration. This requires that the concentration of potential interfering elements is known (e.g. Windom, 1972), or that known amounts of the element to be analysed are (after pretreatment) added to subsamples of the original sample. From the data obtained, a calibration curve is constructed which, when extrapolated to zero absorption, allows the estimation of the concentration of the element in the sample.

Preparing a solution which simulates the often complicated matrix of environmental samples is not easy, since viscosity, surface tension and complex chemical composition can often not be matched accurately enough. It also requires additional chemical analyses, expecially of alkali and alkaline earth metals which are present in high concentrations in marine samples. Also the chemicals used to simulate the matrix constitute contamination hasards. An indication of the approximate concentrations of Na, K, Ca, Mg, Cl, S, P and Fe in marine organisms can be obtained from Table III.

Besides the normal flame AAS two other AAS techniques are of interest. Elements and compounds which are volatile at room temperature such as metallic Hg, and the hydrides of As, Se, Pb, Sb, etc. can be introduced, after the necessary transformation to volatile state, into the light beam. The absorption of metallic Hg is then determined without a flame while the hydrides are atomized by a flame. This chemical transformation, which eliminates many matrix interferences, is used mainly for the determination of Hg, As and Se.

Another flameless AAS technique relies on the volatilization of elements at high temperature. This is achieved by heating a small sample (5-100 µl) in a graphite furnace in the absorption light pass. Comparing the sensitivities of the different methods show that the determination with the flameless AAS have lower detection limits (DL) except when matrix interferences in the graphite furnace occur. Another advantage of the graphite furnace is the small sample size. In Table IV an example is given of the DLs of the different AAS methods together with the concentration range which are typical for marine organisms. In many cases the sensitivity of the flame AAS should be sufficient for the determinations.

In conclusion it can be said that despite the fact that many authors have without apparent difficulties analysed many different elements with flameless and flame AAS, often a wide spread of data is observed for the same elements in intercalibration exercises (see e.g. ICES, 1974). The determination of trace elements is not necessarily an easy undertaking and many factors can influence the quality of the results. A good reproducibility or precision of the results obtained in one laboratory is an indication that the many factors which may influence the outcome of the determination are under control, but still systematic errors may be a problem. These systematic errors can only be determined by intercalibration, reference to a certified standard, or analysis with different independent methodologies.

Table III

Major constituents (Na, K, Ca, Mg, Cl, S, P) in some marine organisms in µg element/g FW

	Na	K	Ca	Mg	Cl	S	P	Ref.
sea water 36‰ S	10600	380	400	1300	18000	800	10^{-3}	(6)
Ulva lactuca*	3100–5600	4100–4700	3500–17200	4100–4500	200	800–34300	–	(1)
Calanus finmarchicus	5400–6500	2900	360– 400	240– 300	10500–11800	1400– 1500	1300–1500	(2)
Plankton, 76 µ-net*	11500	7900	1200	1300	–	–	–	(8)
Plankton, 360 µ-net*	8000	1000	2000	900	–	–		(8)
Euphausids *	5600	1800	900	800	–	–		(8)
Copepods *	7400	1200	800	850	–	–	–	(8)
Radiolarians *	10300	1100	850	1100	–	–	–	(8)
Fucus sp. soft pt.	1000–1100	3800–4100	500–1400	400– 500	–	–	150	(3)
Mytilus edulis, muscle	1400	9000	130	700	1100	100	3600	(4)
Cardium edulis, muscle	5100	3050	410	930	–	–	–	(4)
Patella vulgaris, podium	4200	2400	1000	760	–	–	–	(4)
Sepia officinalis, muscle	600	5260	60	310	550	50	2190	(4)
Ostrea edulis	6500	2580	1850	410	–	–	–	(4)
Engraulis encrasicolus	1300	2700	3000	540	2100	2600	2200	(5)
Sardina pilchardus	930	5000	2300	460	1700	2700	2200	(5)
Merluccius vulgaris	750	5000	750	4500	2200	2100	1000	(5)
Mugil cephalus	1000	3200	300	300	–	–	1700	(5)
Scomber scombrus	1000	4200	200	400	–	–	2700	(5)
Pandalus borealis *	1160–2100	3320–3930	11900–25000	530–800	–	–	–	(7)

* estimates from IN or AN

Ref: Vinogradov (1953) (1) page 65, (2) p. 387, (3) p. 383, (4) p. 291–292, (5) p. 506–509, (6) Sverdrup et al. (1942) (7) Fisler and La Roche (1972), (8) Martin and Knauer (1973)

Table IV

Typical concentration (ng/g FW) in marine organisms and detection limits (DL) in flame and flameless AAS (ng/ml)

Element	conc. in organisms	flame relative	graphite furnace relative	graphite furnace absolute	aerat.-reduction relative
Hg	100– 3000				0.05–0.1
Cd	10– 1500	2	0.003–0.02	0.0003–0.0005	
Zn	3000–100000	1 – 3	0.001–0.02	0.0001–0.00008	
Pb	50– 5000	10 – 30	0.05 –1	0.005	
Cu	200–50000	1 – 8	0.05 –1	0.005 –0.007	
Cr	200–10000	3 – 8	0.1 –1	0.01 –0.005	
Mn	100–20000	2 – 8	0.01–0.1	0.001 –0.0005	
As	200–10000				0.15–0.2
Se	300–2000				0.15–0.3
Na		< 0.2–1			
K		< 2 – 4			
Ca		< 2 – 2			
Mg		< 2 – 4			

6.4.1 Determination of Hg

Mercury occurs in the environment both in inorganic and organic forms. Since the organic forms of mercury are more toxic and in many organisms 60 to 95% of the mercury is organically bound (Knauer and Martin, 1972, Cumont et al., 1972) information on both the inorganic and organic mercury concentrations are needed. The determination of the organic mercurials can be carried out with gas chromatography or with AAS. Using AAS the organic mercury concentrations are obtained from the difference between the total and the inorganic mercury. Inorganic mercury is released in the presence of organic mercurials by addition of tin(II) chloride alone (Magos, 1971). On the other hand if the tin chloride is added together with a cadmium or copper salt, inorganic plus methyl-Hg and other organomercurials are released. One has, therefore, different possibilities to determine inorganic and organic Hg separately: Either by determining inorganic mercury and subtracting this value from a total Hg determination after wet digestion or from the differential release of first inorganic and then organic Hg.

6.4.1.1 Determination of total Hg

Due to the recent development of the flameless AAS methods, especially the reduction-aeration techniques, auxilary equipment kits are now commercially available (Stux and Rothery, 1971; Klemm and Fetter, 1972; Wolber and Doshart, 1972; Kahn, 1971; Anon., 1972; etc.). These kits have greatly facilitated the Hg analysis, but, although the reduction-aeration technique is in principle simple and sensitive, in practice an accurate determination of Hg in natural samples in difficult (Ure, 1975). This is partly due to volatility of the organic mercury compounds, to contamination of the samples from air, storage and sampling equipment and to several parameters which influence the actual analytic methods.

The determination of the total mercury (Hg_t) in biological samples with AAS requires:

(1) Transformation of all organic mercurials into inorganic Hg, either by wet combustion or through the addition of Cd or Cu salt during the reduction process with $SnCl_2$.

(2) The reduction of the mercuric ion to metallic Hg with an excess of $SnCl_2$ or $SnSO_4$

(3) The volatilisation of metallic Hg at room temperature by aeration and its measurement by flameless AAS.

Step (2) and (3) describe the so-called reduction-aeration technique.

Several publications deal with the various versions of this method.

(a) Aeration with recirculation. Here the air used for the volatilisation is passed several times through the reaction vessel containing the sample in a closed system. This system consists of the reaction vessel, the AAS absorption cell, a pump and often a drier to avoid condensation of water in absorption cell (e.g. Uthe et al., 1970). This configuration has the advantages that Hg can be liberated from large volume samples, and that the

recorded absorbance will reach a plateau after some time, but often memory effects are caused by the many components of the system.

(b) Aeration without recycling. (Armstrong and Uthe, 1973, Inkander et al., 1972). In this configuration the air-Hg mixture, after passing through the AAS absorption cell, is discharged from the system. It has the advantage of a simpler system with less components in contact with air-Hg mixture, but the partition between Hg in solution and in air is not optimal. However, the memory effect is neglectable and the system can easily be rendered automatic (Armstrong and Uthe, 1973).

(c) With and without air drying. In order to avoid water condensation in the AAS absorption tube the air-Hg mixture is often dried with magnesium perchlorate, anhydrous calcium sulphate or silica gel (Hatch and Ott, 1968, Ure and Shand, 1974). The disadvantages of the driers are memory effect, loss of Hg and contamination hazards due to the chemicals used for drying. Gilbert and Hume (1973) have used heated absorption cells to prevent water condensation. Stux and Rothery (1971) let 15 to 20% of the air used for aeration bypass the reaction vessel and go directly to the absorption vessel. In this way the dry air will prevent the condensation of the absorption cell.

(d) Agitation and stirring the sample before aeration or instead of aeration. Ure and Shand (1974) agitated the sample with a fixed volume of air instead of aeration with bubbles and then passed the air-Hg mixture into the measuring cell. Under this condition no driers are needed. Stux and Rothery (1971) combined stirring with bubbling. After addition of the $SnCl_2$ or $SnSO_4$ the sample solution was stirred for 90 seconds before bubbling. The peak height becomes approximately five times greater than when the bubbling is started immediately after the addition of the Sn-salts.

Armstrong and Uthe (1973) proposed a semi-automatical Hg-determination in which the samples are first digested manually with a mixture of nitric and sulphuric acid, oxidised with $KMnO_4$ and cleared with H_2O_2. A series of treated samples are then automatically analysed with the aid of Technicon Auto-Analyser Equipment which carries out the reduction of Hg with hydroxylamine and $SnSO_4$ and equilibrate the Hg vaper with air before passing it through AAS. Also Bailey and Lo (1971), Lindstedt and Skare (1971) and others have published automated Hg analyses. The digestion is, however, always carried out manually. Automated analyses should be preferred to manual ones since they increase reproducibility. Armstrong and Uthe (1973) report a relative standard deviation of 3-8% in a concentration range of 0.100-5.000 ppm = 100-9000 ng/kg FW.

Several publications deal with possible interferences (see Ure, 1975 for a review). During the digestion of tissues from marine organisms bromides and iodines can be formed which produce interferences in the flameless Hg determination (Omang, 1973). The interference of the anions of the commonly used acids in the digestion process is neglectable, if the digested solutions are diluted. Chloride causes depression of the signal when above 4 N. In most digested samples the interferences are not serious provided that the standard solutions are made up in similar matrix. The concentration procedures for Hg from solutions reviewed by Ure (1975) may be used to collect the products of a preliminary reduction-aeration, if doubts about matrix interferences may arise. Recent reviews of Koch and Manning (1973), Westöö (1974) and Ure (1975) can be consulted for additional information.

In the following the procedures of two versions of the reduction-aeration method are described in detail. An open system using the commercial kit supplied by Varian (Stux and Rothery, 1971) following a digestion in a closed Teflon crucible or plastic bottle as described under 6.3 and a closed system following the same digestion method as published by Manns

and Holland (1971) and recommended by the 'FAO/WHO expert consultation to identify the food contaminants to be monitored and to recommend sampling plans and methodology' (FAO/WHO, 1973). The closed system version has also been adopted as official first action by the American Association of Official Analytical Chemists for the determination of Hg_T in sea food (Krantz and Holck, 1974).

Before describing the Hg methods in detail a few general precautions and suggestions are to be mentioned. The air used in the aeration-reduction method to transport the volatile Hg into the absorption cell must be cleaned by passing it through two washing bottles filled with an acidic potassium permanganate solution prepared by mixing equal volumes of a 2% K-permanganate solution and 50% (v/v) H_2SO_4 (Topping and Pirie, 1972).

When K-permanganate has not been used in the digestion the digested sample can be reduced directly with $SnSO_4$ to elementary Hg. Unstoppered bottles containing acidic K- permanganate solution used for preparing digestion mixtures collect Hg from the contaminated ambient air (Ure, 1975).

6.4.1.1.1 Flameless Hg-determination in a closed system (Munns and Holland, 1971)

Apparatus:

a) AA-spectrophotometer with Hg lamp and gas flow-through cell complete with quartz windows, continuous spectrum lamp and pen recorder.

b) Diaphragma pump internally coated with acrylic-type plastic spray, or similar.

c) 16 gage Teflon tubing for connections.

d) Gas inlet adapter $\overline{\$}$ 24/40 (Kontes Glass Co. K-18100 or similar).

e) Boiling flasks 250 ml flat bottom boiling flasks with $\overline{\$}$ 24/40 joint, or similar

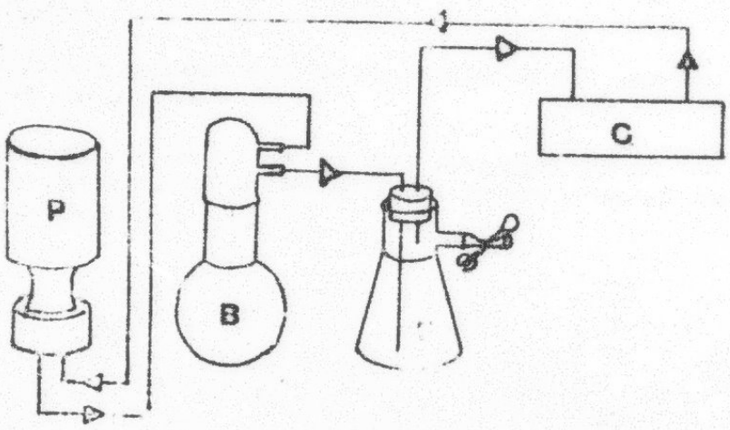

Fig. 28 Apparatus for the flameless determination of mercury
P: pump; B: boiling flask; F: filtering flask; C: absorption cell
(after Munns and Holland, 1971)

Reagents

a) Reducing solution: mix 50 ml H_2SO_4 with about 300 ml H_2O. After letting cool to room temperature dissolve 15 g NaCl, 15 g hydroxylamine sulfate, and 25 g $SnCl_2$ in the diluted H_2SO_4 and bring the volume to 500 ml with H_2O.

b) Diluting solution: add 58 ml HNO_3 and 67 ml H_2SO_4 to a volumetric flask (1 liter) containing about 500 ml H_2O and bring up to volume with H_2O.

c) Drying agent to be placed into filtering flasks (see Fig. 28): $Mg(ClO_4)_2$

Caution (Mg-perclorate may explode, if it comes into contact with organic substances)

d) Hg-stock solution for preparing standards: dissolve 0.1354 g $HgCl_2$ in 100 ml volumetric flask and bring up to volume with H_2O.

Hg-standards

Prepare internal standards by spiking the subsamples taken from a digested sample of the same matrix or prepare a model matrix taking into consideration the chemical composition of marine organisms (see Tab. III) and the digestion procedure used. Spike the standards with aliquotes from the Hg stock solution.

Procedure:

1) Transfer cold digested sample into a volumetric flask of appropriate size (e.g. 50 or 100 ml) rinse the digestion vessel with small amounts of distilled water, add to the volumetric flask and bring with H_2O to volume.

2) Transfer an aliquote (e.g. 20 ml) into the reaction vessel.

3) Adjust the output of the pump to circa 2 litres/minute.

4) Connect aeration-reduction apparatus as shown in Fig. 28 except for the gas inlet adapter.

5) Zero the AA spectrophotometer,

6) Add 20 ml of reducing solution and immediately connect the gas inlet adapter. Aerate for about three minutes and record the signal. Adjust the aeration time to obtain an optimal signal.

7) Disconnect pressure hose on the outlet of the pump and open vent on the filtering flask to flush the system.

The system is now ready for the next determination or for the calibration with standards. From the calibration curve and the dilution of the original sample calculate the Hg concentration in the sample.

6.4.1.1.2 Flameless Hg-determination in an open system (Parker, 1972)

The apparatus for determining mercury by flameless atomic absorption (Fig. 29) consists of an absorption tube (Vycor with Vitreosil end windows), tube holder, six reaction vessels, stand and stopper. Incorporated within the stopper is the air by-pass system which permits approximately 20% of the carrier gas to by-pass the reaction vessel. The balancing of the air flows between by-pass and reaction vessel is achieved through two orifices, one of which is the tip of the bubbler. Note that changing the size of this orifice may result in reduced sensitivity or ineffectiveness of the by-pass.

The by-pass is included to ensure that air entering the absorption tube is not saturated with water vapor. This prevents condensation on the end windows of the tube, which would result in a prolonged absorption signal with increased non-atomic component. A drying tube is not required when this system is used.

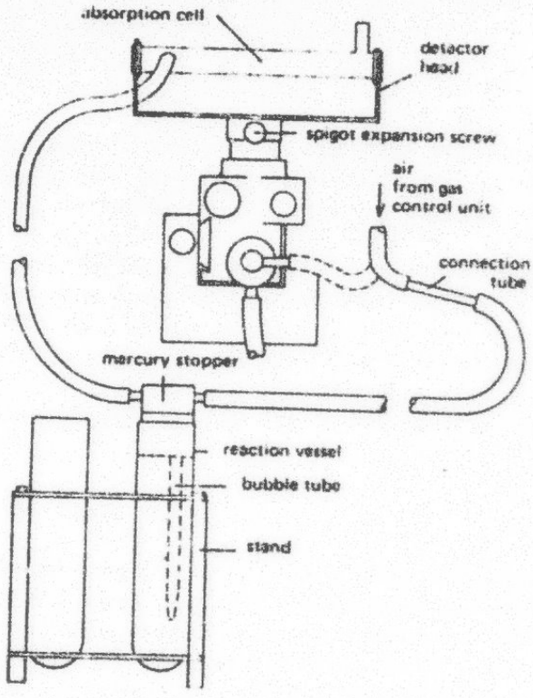

Fig. 29 General arrangement of Hg kit (Parker, 1972)
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Parameters Affecting the Signal

Stirring

The effectiveness of the stirring operation is the most important factor in obtaining good reproducibility and sensitivity.

As the solution is stirred, mercury diffuses from the liquid into the air space in the reaction vessel. The importance of proper stirring lies in the fact that the height of the recorded peak is entirely dependent on the concentration of mercury in the air phase. Consequently, it is important that the mercury content of the air should be consistently representative of the mercury content of the sample. This can only be achieved by ensuring that the mercury distribution between the air and liquid phases is in equilibrium. If the stirring is not sufficiently vigorous, or the time allowed is too short, the required equilibrium will not be achieved and reproducibility and sensitivity will suffer accordingly.

It is recommended that stirring be sufficiently vigorous to produce a vortex which descends to the bottom of the reaction vessel. Under these conditions the distribution is near equilibrium after 60 seconds, the concentration of mercury in the gas phase changes only slowly, and acceptable results are obtained.

Gas Flow Rate

Under normal circumstances, large changes in flow rate result in very small changes in peak height. This is because the peak height depends on the concentration of mercury in the air, and this is not greatly affected by the rate of purging through the cells.

A flow rate of 4 litres/minute will result in a fast, sharp peak, and is recommended for normal use. If the response time of the recorder or electronics system is greater than approximately one second for full scale deflection, curved calibration and some reduction in accuracy at higher absorbances will result. These problems can be overcome without compromise by reducing the air flow rate - for example use 1.5 - 2 litres/minute. The residence time of the mercury-laden air in the light path is increased and the peak is recorded more slowly.

Bubbling Through or Over the Solution

Provided that the solution is thoroughly stirred, and equilibrium distribution of mercury between the phases has been achieved, the purge gas may be passed over or through the solution. The same result is obtained by each method. Advantages of not passing the purge gas through the solution are that very little water vapor is carried to the absorption tube and foaming of samples is prevented. It is possible to pass the purge gas over the surface of even large samples by continuing the stirring while purging.

Apparatus

a) AA spectrophotometer with Hg lamp, continuous spectrum lamp and pen recorder.

b) Varian Hg determination kit or similar (Fig. 29).

c) Two washing bottles to eliminate Hg from the air (see above).

Reagents

Reducing solution: Dissolve 10 g hydroxylamine sulphate and 15 g $SnCl_2 \cdot H_2O$ in 40 ml 1:6 H_2SO_4 and bring to 100 ml with H_2O.

Hg Standard

Prepare Hg standards as described in the previous method.

Procedure

1) After assembling the kit following the instruction supplied by the manufacturer, set the air flow to the desired value consulting the remarks made about the gas flow above. Turn off at the 'Z' valve.

2) Transfer the cold digested sample into a volumetric flask of convenient size (50, 100 ml) with distilled water and bring to volume.

3) Add 5 ml of diluted digestion solution and 5 ml of distilled water to a reaction vessel.

4) Add 1 ml of reducing solution and insert immediately the stopper.

5) Stir for 90 seconds and measure the peak height after turning the air on by means of the 'Z' valve.

6) Remove reaction vessel after turning off the air.

The apparatus is now ready for a new determination; for the calibration curve and the dilution of the original sample calculate the Hg content.

6.4.1.2 Determination on inorganic and total Hg without wet combustion

The following methods have not found wide application and, therefore, difficulties may arise. Since, however, these methods will allow the determination of organic mercurials with AAS, the detailed procedure is given below. Yield determinations are to be carried out before using them for routine analysis.

The methods are based on the observation that inorganic Hg can be determined in the presence of organic Hg after complexing the inorganic Hg with cysteine in an acid medium from which the inorganic Hg complexed with cysteine can be released after addition of $SnCl_2$ and NaOH to make the solution alkaline (Magos, 1971). Total Hg can be determined by adding either Cd or Cu salts during the reduction step which will cause the release of Hg from both inorganic and organic compounds.

Baltisberger and Knudson (1974) have utilised the part of the method involving the selective liberation of inorganic Hg by tin (II) sulfate in sulfuric acid medium in the presence of organic Hg compounds to determine Hg in fresh water. The Hg_T was determined after treatment with H_2O_2.

Methodology according to Magos (1971)

Apparatus

AA spectrometer complete with kit for reduction-aeration method.

Reagents

a) Cysteine hydrochloride, 1% (w/v) high purity

b) NaCl 1% high purity

c) H_2SO_4 16 N high purity

d) $SnCl_2$, for each determination 100 mg are needed

e) $SnCl_2$ - $CdCl_2$ reagent: Add 25 g of $SnCl_2$ and 5 g of $CdCl_2$ to about 40 ml of distilled water, heat until boiling. Bring to 50 ml volume in a volumetric flask after cooling.

f) NaOH 45% high purity

g) Silicone MS antifoam: Occasionally this material had to be employed to avoid foaming of the material in the reaction vessel.

Hg standards

a) Inorganic standard: Dissolve 0.6767 g $HgCl_2$ in 1% H_2SO_4 and bring it with the H_2SO_4 to 1000 ml in a volumetric flask. Add 1 ml of this solution, 9 g NaCl, 0.7545 Na-EDTA and 0.063 g L-cysteine hydrochloride to a 1000 ml volumetric flask, dissolve and bring up to volume (In the refrigerator this solution remains unchanged for at least six months).

b) Methylmercury standard: Dissolve 60.08 mg methylmercury chloride (e.g. AB Casco, Stockholm, Sweden) in a 100 ml volumetric flask in acetone and bring up to volume. Make from this solution a 1 : 1000 dilution in a volumetric flask with distilled water.

In an alternative way the standards can be prepared from 36.96 mg of methylmercury dicyandiamide (e.g. AB Casco, Stockholm, Sweden) with distilled water; bring it first to 500 ml in a volumetric flask and then dilute 1 : 100. These solutions are not very stable and the Hg content must be checked, since losses occur due to volatilization and precipitation.

Sample preparation

Magos (1971) proposed a sample preparation which is a similar procedure to the one suggested by Skare (1972). Skare found that fish meat could be easily homogenized, if it were dissolved in excess alkaline with occasional shaking and standing overnight at room temperature. This homogenate was stable for a long time, if stored in a refrigerator.

The samples may be homogenized in a 1% NaCl solution with a stainless steel, glass or Teflon homogenizer. In many cases the following procedure may be used which is simpler, but it presents contamination hazards. The relative high concentration of NaOH will dissolve the glass surface in contact with the NaOH and the impurities contained in the glass will be released (Adams, 1972).

Fishmeal: 0.5 g are mixed with 1 ml of the 1% cysteine solution, 1 ml of 20% NaCl solution and 1 ml of the 45% NaOH solution. Heat to boiling point and transfer the solution with distilled water into the reaction vessel of the Hg apparatus.

Whole specimen: Bring a 40% NaOH solution of twice the weight in g of the specimen in ml to boil. Drop the specimen into the NaOH and let it dissolve (20 minutes). After boiling dilute with distilled water so that the final NaOH concentration will be 20% w/v based on the weight of the specimen.

a) Determination of inorganic Hg

Procedure :

1) Prepare instrument according to the method chosen.

2) Transfer with a pipette an aliquot of the sample (1 - 20 ml) or of the standard into the reaction vessel.

3) Add 1 ml of the cysteine solution and make up to 21 - 23 ml with the 1% NaCl solution.

4) Add 10 ml of 15 N H_2SO_4

5) Add 100 mg of $SnCl_2$ to the reaction vessel and 20 ml of the 45% NaOH quickly and close the reaction vessel immediately.

6) Record the peak height according to the reduction-aeration method used.

b) Determination of total Hg

Procedure:

Proceed as above except for step 5 as follows:

5) Add 1 ml of the $SnCl_2$ - $CdCl_2$ reagent (Reagent 'c')

c) Determination of first inorganic and then organic Hg in the same reaction vessel

Follow procedure (b) until step 6. One to three minutes after the addition of the NaOH add 10 ml of 15 N H_2SO_4 and 1 ml of the $SnCl_2$ - $CdCl_2$ reagent and 20 ml of 45% NaOH and read the peak height.

6.4.2 Determination of cadmium

Since the lower typical concentration of Cd in marine organisms is near the DL (Tab. IV) flame techniques, as recommended by the manufacturer of the instrument, should only be employed for the higher concentrations.

Should lower concentrations be encountered, the recommendations of the Analytical Methods Committee (1975) of the Chemical Society could be employed. This included an extraction with a liquid ion-exchanger (Amberlite LA-2) in 4-methylpentan-2-one after wet digestion and transformation of the Cd into iodocadmate. Julshamn and Braekkan (1975) and Childs and Gaffke (1974) concentrated the trace metals Cd, Fe, Cu, Mn, Zn and Pb after complexing with Na-diethyl-dithiocarbamate and extraction with methyl-isobutyl after wet digestion. In this way they could determine approximately 0.05 µg Cd/g FW.

If a graphite furnace is available its lower DL (Tab. IV) will allow analyses without any additional concentration processes. Details on the use of the graphite furnace can be obtained consulting the manufacturers instructions and the papers of Sperling (1975) and Slavin et al. (1975).

6.4.3 Determination of total arsenic

Arsenic is one of the 'difficult' elements to determine with AAS because its absorption lines are both below 200 nm and direct aspiration of arsenic containing solutions into the flame suffer from high background absorbance and noise level resulting in a low signal to noise ratio. Several remedies have been proposed such as the use of different flame combustion mixtures and burner constructions (Kirkbright et al., 1969, Kahn and Schallis, 1968), but many of the problems can be eliminated when gaseous arsine is generated from the sample and analysed with an argon-hydrogen flame.

Outline of the determination of As by arsine generation:

After wet digestion of the organic substance with nitric acid or nitric acid mixture the solution is purged of the interfering nitrogen oxides with CO_2 or by boiling. Then the sample is diluted with a mixture of HCl and H_2SO_4. The arsenic present is reduced to As^{+3} with KI and $SnCl_2$. Finally the arsine gas is produced by the addition of zinc powder, and the arsine gas transported with argon into the flame, where the arsine is atomised. Several different arsine generation systems have been proposed and later improved, ranging from collecting As in a liquid nitrogen cold trap to collecting the arsine in a rubber balloon before releasing the accumulated gasses into the AAS flame. (Holak, 1969, Manning, 1971, Dalton and Malmoski, 1971, Thompson and Thomerson, 1974, Freeman and Uthe, 1974). Only a few As determinations employing AAS have been carried out on marine organisms (e.g. Windom, 1972).

An investigation on the efficiency of different reductants showed that the strongest absorption signal was obtained when KI, $SnCl_2$ and zinc powder was used (Maruta and Sudoh, 1975). Within a certain range the amount of the KI and $SnCl_2$ added is not critical (Maruta and Sudoh, 1975). Vijan and Wood (1974) have published an automated method which works without a flame and sweeps the generated hydrides of As, Sb, Bi, Se, Te and Ge into a heated, windowless quartz cell, using a proportional pump. Maruta and Sudoh (1975) studied several factors which may interfer with the determination. They conclude that serious interferences arise only from nitric acid, lead, chromium and selenium, while solutions of arsenic containing 0.05 - 1 M sulfonic, perchloric or phosphoric acid in the presence of 1 N HCl did not interfer. If HNO_3 was used for digestion of biological samples all interfering oxides of nitrogen can be eliminated by boiling the samples prior to the arsine generation. Less than 5% change of the signal was observed after addition of 80 µg of Na, K, Mg, Ca, Mn, Co, Ni, Cu, Al, Fe, and V to a sample solution containing 0.8 µg of As. Under these conditions Cr, Pb and Se reduced the signal by 30%, 13% and 10% respectively. Cr and Pb interfered in the generation of arsine, but not in the atomination process, while Se reduced the signal after it was introduced into the flame following formation of H_2Se. Adding 8 µg of Cr, Pb or Se did not interfer.

Stux and Parker (1972) observed that the zinc powder used may contain high As impurities resulting in high blank values. It is, therefore, necessary to test several batches of zinc from different suppliers in order to select a zinc powder with a low As concentration.

All of the above mentioned methods with the exception of Maruta and Sudoh's accumulate the arsine generated over a certain time period and transfers after the generation, the arsine in a very short time interval into the flame. Stux and Parker (1972) have studied the parameters involved in the arsine generation in order to eliminate these holding devices. Nearly all firms which manufacture AAS instruments offer kits for the determination of As and Se. Their instructions should be followed when applying their kits. For an illustration, the methodology of Stux and Parker (1972) are given here.

Apparatus

Varian Techtron atomic absorption spectrophotometer with hollow cathode lamp for As and hydrogen continuum lamp or similar apparatus.

Burner for nitrogen-hydrogen entrained air flame.

As and Se kit (Fig. 30).

Reagents

a) hydrochloric acid, high purity

b) sulphuric acid, high purity

c) 15% KI solution, high purity

d) 20% $SnCl_2$ in concentrated HCl, high purity

e) As - free zinc powder

Procedure

If nitric acid has been used in the wet digestion, it must be removed either by boiling under a fume hood or by purging the sample with CO_2.

Using a nitrogen-hydrogen-entrained air flame with a nitrogen flow of 7.5 units (approximately 11 litres/minute), optimize the burner position and flame while aspirating an aqueous arsinic solution. Note that the optimum conditions obtained are also suitable for selenium determinations. Remove the nebulizer and nebulizer bung from the spray chamber and fit a plain inlet bung equipped with a barb type hose connector which will accept ¼ inch laboratory tubing. Connect the special stopper as shown in Figure 32 and set the control valve at "BY-PASS".

Adjust the support pressure to approximately 15 psi to give a nitrogen flow of 5.5 units (8 litres/minute). Now use the auxiliary support control to give a nitrogen flow of 7.5 units (11 litres/minute). Ensure that the hydrogen flow remains at the optimum rate established when originally setting the flame.

For the analysis, 20 ml of sample solution, which should be 20% in hydrochloric acid and 5% in sulphuric acid and contain up to 250 ng arsenic is placed in the reaction vessel. Add 1.0 ml of 15% potassium iodide solution, and 1.0 ml of 20% stannous chloride in concentrated hydrochloric acid. Insert the special stopper in the reaction vessel, switch the valve to SAMPLE and record the air peak. When the pen has returned to zero, rapidly inject 2.0 ml of a zinc slurry (1 g/ml) through the septum. A vigorous reaction will now occur and the peak will appear within 1-2 seconds. When the recorder pen has returned almost to the baseline, switch the valve back to "BY-PASS" and immediately remove the stopper from the reaction vessel. The system is now ready for the next sample.

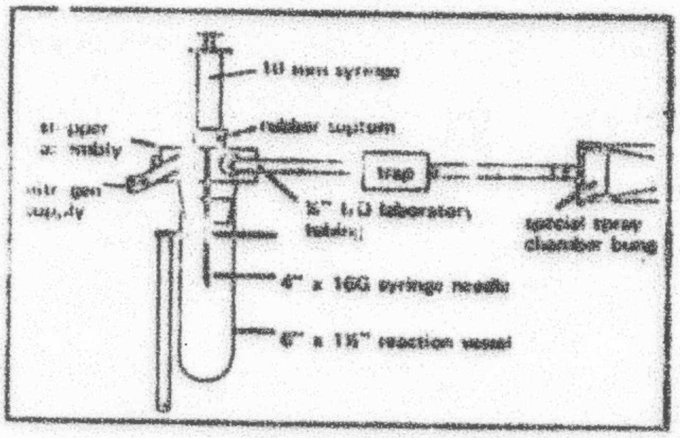

Fig. 30 Apparatus for the As and Se determination
(Stux and Parker, 1972)
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6.4.4 Determination of total selenium

Selenium can be determined with the same kit which is used for the As determinations (see section 6.4.3) following with a slight modification the same procedure. However, it has been observed that when reducing Se (II), (IV) or (VI) with KI and $SnCl_2$, once the element was formed, it rapidly changed into a more stable form which was not readily available for further reduction (Stux and Parker, 1972). Stux and Parker overcame this difficulty by increasing the acid strength to 40% HCl and 10% sulfuric acid. Thompson and Thomerson (1974) observed that solutions of Se (VI) gave negligible response in comparison with equivalent amounts of Se (IV). Treating the sample solutions with 'aqua regia' did improve the Se (VI) detection but the efficiency was still less than 50%. It seems, therefore, that if the valence state of Se (VI) occurs analytical problems may be encountered.

6.4.5 Determination of total copper

Concentration of copper in marine organisms range between 200 and 50000 µg Cu/kg FW (Tab. III). Since the DLs for Cu with flame AAS are about 0.01 µg Cu/ml for the most sensitive line 324.7 nm, diluting or selecting a less sensitive line, e.g. 222.6 or 244.2 may bring the concentration into the optimal range (Tab. IV). Topping (1973), Windom et al. (1973) and Segar et al. (1971) among others have used AAS for the determination of Cu in marine organisms without reporting difficulties. In the ICES intercalibration exercise the results of the Cu-determination were in very good agreement indicating that no difficulties occurred. If concentrations are to be determined which are smaller than the DLs, Cu may be extracted with ammonium pyrrolidine-dithiocarbamate (Analytical Methods Committee, 1971) or with sodium diethyl dithiocarbamate (Julshamn and Braekkan, 1975). If a graphite furnace is available its lower DLs will make extraction unnecessary. Indications on the operating conditions can be found in the operation instructions and by Slavin et al. (1975).

6.4.6 Determination of total zinc

Due to the high zinc concentrations in marine organisms and the low DLs of the flame AAS no difficulties are anticipated when the digested samples are analysed under the standard conditions suggested by the manufacturer of the AAS instrument (Windom et al., 1973; Topping, 1973; Cutshall and Holton, 1972; ICES, 1974). Dilutions may be necessary in order to bring the high concentrations into the optimal range (Tab. IV), or about 1000-times less sensitive 307.6 line may be used avoiding zinc contamination with the diluting solution.

If on the other hand, very small zinc concentrations have to be determined, extraction of zinc with ammonium pyrrolidine-dithiocarbate may be employed (Analytical Methods Committee, 1973), or, if available, a graphite furnace can be used (Clark et al., 1973).

6.4.7 Determination of total manganese

DLs are low enough to allow direct determination with flame AAS. Bradfield (1974) studied the possible interferences in the determination of plant materials. He observed interference of Ca and Mg on the absorbance of Mn in the presence of sulfate. No interferences were observed from Fe, K and Cl. The addition of Lanthanium chloride or NaCl (1 mg/ml) reduced the interference practically to zero. Other remedies are: measure the absorbance higher in the flame, decrease the droplet size and hence the size of solid particles in the flame with the addition on an alcohol. Bradfield suggests, therefore, to avoid wet combustion with H_2SO_4. At any rate these interferences can be controlled by using the standard addition method in subsamples of the original sample in order to match the matrix adequately. Bradfield's remedies may be used if the sensitivity is insufficient for the Mn determination.

Shigematsu et al. (1975) studied optimal conditions of temperature, different gases, etc. for the Mn determination with a graphite atomizer. No decrease of absorbance was observed from HCl, HNO_3, $HClO_4$ and H_2SO_4 at low concentrations (0.05 M) on 25 µg Mn/l. Only H_3PO_4 reduced the signal by 15%. The effect of salt at 40 times the Mn concentration was negligible except for Na_2SiO_3. At 400 and 4000 times, however, the signal was significantly reduced by $CaCl_2$ (48 and 77% respectively) showing the same interference already mentioned above. Reduction of the Mn absorbance is to be expected, since marine organisms contain relatively large amounts of Ca and Mg and also of SO_4 in relation to trace elements (Tab. III).

6.4.8 Determination of total chromium

The determination of chromium (e.g. Slavin et al., 1975) should not present problems with respect to DLs, since the typical concentration of Cr in marine organisms is about 25 times higher (Tab. III). However, matrix effects are to be anticipated and, this has therefore to be taken into consideration, during the preparation of standards. Yanagisawa et al. (1970) report small interferences of Cu, Fe, Na, K, Zn and others in concentrations 5 times that of Cr (250 µg/ml). The temperature of the flame contributes considerably to the magnitude of the effects observed. Green (1975) demonstrated the importance of the valence state of Cr in perchloric acid solutions. He suggests that all Cr be converted into Cr (III) by a hydrogen peroxide treatment. Furthermore, an addition of NH_4Cl can increase the response by up to 30%. If standard determination with the air acetylene flames are not sensitive enough, extraction with ammonium pyrrolidine dithiocarbamate into methyl isobutyl ketone may be employed (Gilbert and Clay, 1973).

6.4.9 Determination of total lead

From a strictly analytical point of view, Pb determinations should not present special problems. The DLs are low enough to allow a direct analysis with flame AAS. However, severe contamination problems are to be anticipated, since Pb is ubiquitously present in air, water, on glass surfaces, etc. In fact, Patterson (1974), a specialist on Pb determination, maintained only recently that most Pb determinations are not valid because of Pb contamination. It is obvious, therefore, that much effort during the sample treatment and analyses has to be made to prevent contamination of Pb. Reagent blanks must be carefully checked and the air used in the AAS should be passed through washing bottles, especially if the laboratory is located in areas with intensive car traffic.

Interference of nitric acid, sulfuric acid and tin in the digestion of samples has been reported (Roschnik, 1973) thus, nitric acid digestions should be avoided or after digestion the nitric acid must be eliminated by boiling.

If the standard flame technique is not sufficiently sensitive, a preconcentration with ammonium pyrrolidine dithiocarbamate (Analytical Methods Committee, 1975a) or with diethylammonium diethyldithiocarbamate (Roschnik, 1973) may be applied. If a graphite furnace is available, its higher sensitivity may be of advantage (see Table III). Some indications on the programming of the furnace can be obtained by consulting the manufacturer's manual and the paper by Slavin et al., 1975.

6.5 Gas Chromatographic Analysis of Chlorinated Hydrocarbons

Gas-liquid chromatography (GLC) uses the differences in partition coefficients of various components of a mixture between a liquid stationary phase and a moving gas phase for separating the different components. The liquid phase is adsorbed on inert material. The inert material impregnated with the liquid phase is then placed in a glass, metal or plastic tube (chromatographic column) through which the "carrier gas" is passed. For analysis a sample containing analyte(s) is volatilized at an elevated temperature in the injection leak located at one end of the chromatographic column which in maintained at a given temperature in an oven (Isothermic GLC). The carrier gas (e.g. nitrogen) passing transports the sample through the column, causing a continuous dissolution of the analytes in the liquid phase along the tube and their continuous eluation from the liquid phase. Analytes which are less soluble in the liquid phase will be eluated before those which are more soluble, allowing a chromatographic separation of the different components of a sample. When the analytes leave the column they enter a suitable detector - in the Chl-HC analyses usually an electron capture detector (ECD) - which records eluation curves on a recorder (chromatogram). The amount of analyte is quantified from the peak area or, under certain conditions, from the peak height. Although GLC is a very sensitive technique, both identification and quantification raise several problems (Jensen et al., 1973; Chau and Sampson, 1975). Known mixtures of analytes can, in most cases, be securely identified and quantified by a comparison with standards and by their retention times relative to a reference substance (e.g. HCB, aldrine, etc.), but, in environmental samples, the nature of the substances cannot always be predicted. Even when predictions can be made, different substances may possess the same retention time. For instance, the peaks of p,p'-DDE and p,p'-DDD coincide with the PCB peaks number 4 and 9 respectively, while p,p-HCH lies very near to the PCB peak number 10 (Figs. 31a and 31b).

As a remedy, the different analytes can be separated by solvent partition, column chromatography, thin-layer chromatography, selective destruction during pre-treatment, etc. Repetition of the gas chromatographic analysis after chemical transformation can also be used for identification.

In recent years, a characterization by mass spectrometry has been increasingly employed to supply additional information on the analytes examined. The mass spectrometric analysis consists in bombarding the analyte with an electron beam which causes electron loss and

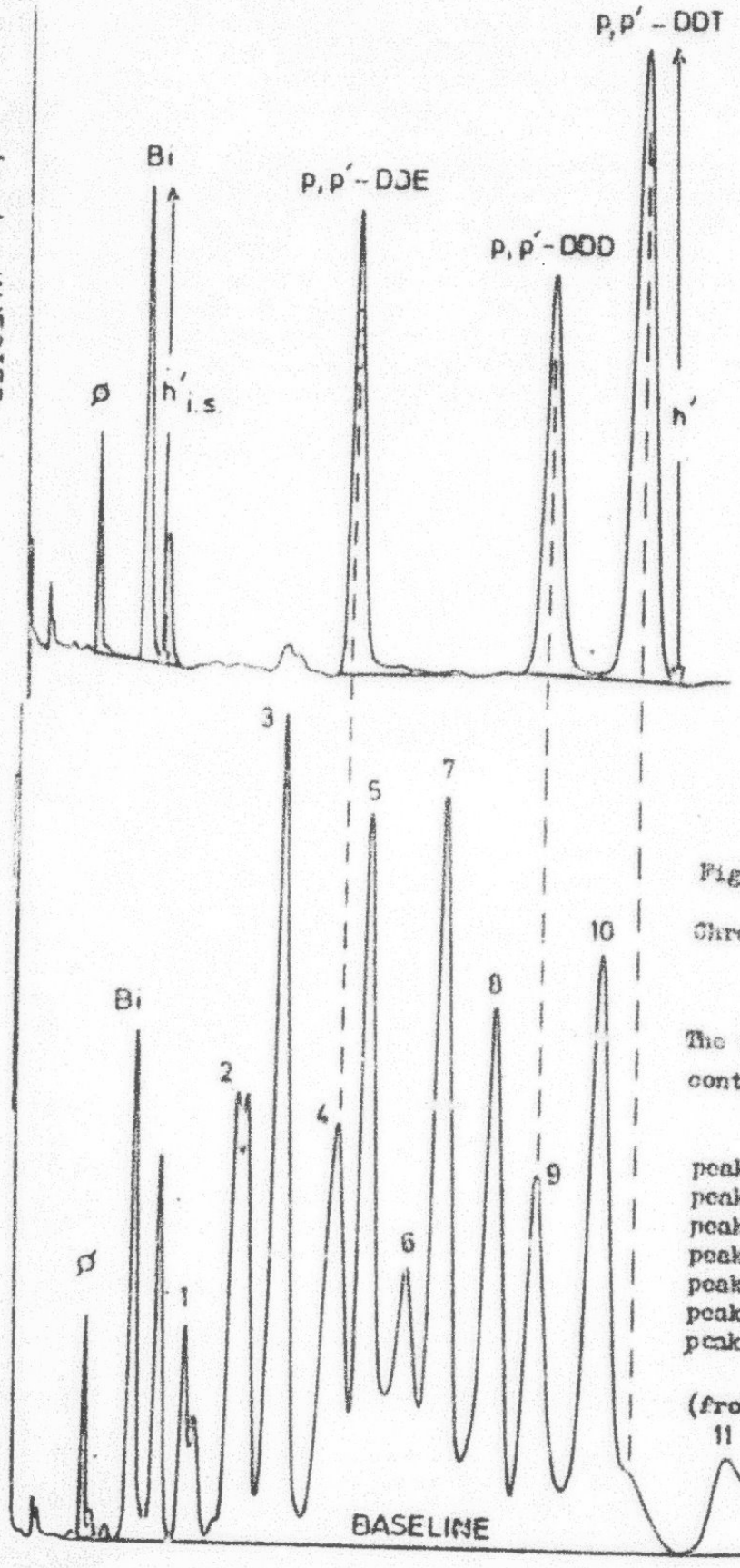

Fig. 31a

Chromatogram of DDT standard

The Standard contains:

p,p'- DDE	0.1 ng/µl
p,p'- DDD	0.2 ng/µl
p,p'- DDT	0.3 ng/µl

(from Jensen et al., 1975a)

Fig. 31b

Chromatogram of PCB standard Chlophen A50

The standard with a concentration of 1 ng/µl contains:

peak 1	0.074 ng/µl	peak 8	0.127 ng/µl
peak 2	0.110 ng/µl	peak 9	0.036 ng/µl
peak 3	0.169 ng/µl	peak 10	0.030 ng/µl
peak 4	0.058 ng/µl	peak 11	0.022 ng/µl
peak 5	0.115 ng/µl	peak 12	0.025 ng/µl
peak 6	0.053 ng/µl	peak 13	0.020 ng/µl
peak 7	0.100 ng/µl	peak 14	0.010 ng/µl

(from Jensen et al., 1975a)

fragmentation of the molecule. The fragmentation and the pattern of the spectra of the different fragments resulting from bond fission and rearrangement of atoms are highly characteristic of the original molecular structure and can, therefore, be used for an identification of the unknown analyte. This can be done either simultaneously with the gas chromatographic analysis or in a batch treatment, i.e. after collection of the fractions eluated from the gas chromatograph.

In combined gas chromatography/mass spectrometry, the gas chromatograph is coupled directly with a mass spectrograph, allowing the continuous introduction of the eluated compounds into the mass spectrograph which, during the registration of the eluation curve, scans the mass spectrum of the fragments repeatedly. The mass spectrum obtained is analysed by comparing it with the spectra of known substances either manually or with the aid of a computer (Stenhagen et al., 1973). More details may be obtained from reviews by Biros (1971) and Fenselau (1974). The major drawback of Chl-HC identification with a mass spectrograph is its relative high instrument cost as compared with that of a gas chromatograph and the need for additional specialized personnel for its operation. This will make it mandatory for most participating centres to rely on chemical separation and transformation for analyte identification.

For the purpose of the pilot projects, the screening will be limited to certain persistent pesticides which will allow the use of much simpler procedures, especially during the pre-treatment of the samples.

In a recent review on the aspects of organic marine pollution, Duursma and Marchand (1974) point out that non-persistent pesticides are very unlikely to contaminate significantly the marine environment since they will be degraded before reaching it. In fact, only the PCBs and pesticides such as dieldrin, endrine, the DDTs, heptachlor, aldrin, lindane, chlordane, toxaphene, hexachlorocyclohexane, endosulfan, methoxychlor and wastes from the PVC production (aliphatic Chl-HCs) have been detected in marine organisms (National Academy of Sciences, 1973).

Before Chl-HCs can be analysed in a gas chromatograph, interfering organic substances, specially lipids, must be eliminated in a pretreatment. For a complete analysis, rather complicated pretreatments are necessary. Several multi-residue procedures have been developed which are described in detail in publications such as the Pesticide Analytical Manual (U.S. Department of Health and Education, 1975), the EPA Pesticide Manual (Thompson, 1974), and the Official Methods of the AOAC (Horwitz, 1975). The Pesticide Analytical Manual will be supplied to all participants carrying out Chl-HC analyses thanks to the courtesy of the U.S. Food and Drug Administration.

Somers (1974) reviewed recently pesticide analyses of various foods, including fish, for the FAO/WHO Expert Consultation to Identify the Food Contaminants to be Monitored and to Recommend Sampling Plans and Methodology (Rome, 7-11 October 1974). This review may be consulted for a comparison of the different methodologies used in pesticide analyses in different methodologies used in pesticide analyses in different countries.

It should also be mentioned that the extraction of large environmental samples is expensive, not only in work time, but also because pesticide grade solvents are costly. Whenever possible, small sub-samples should be studied and miniature equipment used, such as micro-blenders and micro-soxhlets (from Fisher Scientific or Karl Kolb Scientific Technical Supply, Buchschlag-Frankfurt, Federal Republic of Germany).

The above-mentioned manual considers that a screening for all possible pesticides, and hence their methodology, can often be simplified. For example, if the monitoring is limited to the PCBs, DDTs and lindane (γ BHC), a simple clean up after lipid extraction with concentrated H_2SO_4 is sufficient. If, however, dieldrin (a first priority Chl-HC) is also to be monitored, a more complicated pre-treatment is necessary. Since during complicated sample preparation procedures loss and contamination occur, sample preparation should be kept to a

minimum and yield determinations are essential. The above-mentioned simple acid clean-up may also be used to determine separately the PCBs, DDTs and lindane, thus allowing a check on complicated pre-treatment.

6.5.1 Cleaning of glassware, instruments and reagents

Detailed instructions on how to carry out the cleaning and avoid contamination of various origins are given in the Pesticide Analytical Manual, and should be followed scrupulously. Giam and Wong (1972) investigated, especially, the problems of background contamination in the analysis of open ocean biota. They recommended to heat all materials which can withstand a high temperature (glassware, glasswool, aluminium foils, Florisil, sodium sulfate and sodium chloride, etc.) in an oven at 300-350°C overnight. All other materials, including blenders, which are not heat resistent can be cleaned by solvent extration, first with acetone and then with petroleum ether or hexane. Distilled water should be distilled twice in the presence of 0.1-0.2 g of potassium permanganate for every 3 litres in a special distillation apparatus set aside for this purpose only.

6.5.2 Pre-treatment

Three steps can be identified in the pre-treatment:

(a) Extraction of the lipids (fat) plus Chl-HCs;

(b) determination of the amount of lipid (fat content)

(c) clean-up and separation of interfering substances.

Principle of the lipid extraction procedure

The tissue is first homogenized in a high speed blender in the presence of anhydrous Na_2SO_4. Then the homogenate is extracted in the same blender (cold extraction) or a Soxhlet extractor (warm extraction) several times with the solvent selected (e.g. petroleum ether hexane); the various extracts are combined, dried with anhydrous Na_2SO_4 and finally concentrated e.g. in a Kuderna-Danish concentrator.

Detailed procedures are given in the Pesticide Analytical Manual (section 211ff, especially 211, 13f), in Official Methods of AOAC (Horwitz, 1975) for Fish under procedure 29.012(e), in the EPA Pesticide Manual (Thompson, 1974) under section 5. A(1), and in Farrington et al. (1972).

Lipids have been extracted from fish and other aquatic organisms with hexane (e.g. Harvey et al., 1974; Addison et al., 1972; Murphy, 1972), with hexane/acetone (Bourne and Bogan, 1972), with petroleum ether (Nimmo et al., 1971, Pesticide Analytical Manual), with acetonitrile (Smith and Cole, 1970), with hot perchloric-acetic acid mixture (Stanley and Le Favoure, 1965) and with other solvents. The hot perchloric-acetic acid mixture used by Stanley and Le Favoure may be unsuitable because it destroys several "pesticides", for instance dieldrin, malathion, parathion, and reduce the recovery of aldrin, heptachlor, epoxide and endrin.

Extraction mixtures containing dehydrating solvents such as acetone, isopropanol, etc., make the cell membranes more permeable for lipids and thus increase the amount of fats extracted (Jensen et al., 1973).

According to the EPA Pesticide Manual, an extraction with petroleum ether should be preferred to other solvents because, so far, no collaborative study has been carried out to show that different extractions are really equivalent. In fact, recently, Hattula (1974) compared different cold column extractions and different hot Soxhlet extractions of Na_2SO_4 analysing dried fish homogenate of 3 fish species containing different fat contents. Four different solvents were employed: diethylether, diethylether and n-pentane (1:1), 25%

n-hexane in acetone and 10% diethylether in petroleum ether (1:1) and chloroform and methanol (1:1). Hattula could show that cold extractions yield, in general, lower (50-90%) amounts of extractable lipids than the hot Soxhlet extraction, but not in all cases. The most erratic results were obtained with "classical" chloroform/methanol extraction which extracted also proteinaceous material. Recalculating Hattula's data, it was found that the different methods will not even extract similar lipid fractions from fish of different fat content nor extract with the different solvents similar fractions of Chl-HCs from the three fish species studied.

Similar large differences in the analytical results were observed by Duursma (1976) when comparing 6 different methodologies for the determination of "pesticides" in aquatic organism and sediment samples.

Although it seems reasonable to expect that different solvent mixtures and methodologies would extract different lipid fractions, there are several publications which show that different methodologies yield similar results (e.g. Pettinati and Swift, 1975). However, these results were obtained by comparing homogeneous materials such as fish meal of similar fat content or different forms of meat. If, on the other hand, meat, vegetables and various composite diets with or without acid hydrolysis were used to compare different extraction methodologies, considerable differences in lipid yields are observed (Conway and Adams, 1975).

Recommended reference extraction methodology

In the light of these results, it would be advisable to use in the pilot studies only one type of solvent, i.e. petroleum ether (30-60°C), employing the blender method as described in the Pesticide Analytical Manual, section 211.13f. In very hot climates, however, hexane, which has a high boiling point, may be better. If other methodologies are being used or are favoured, it is highly recommended to compare them on each species with the petroleum-ether blender method in order to allow a comparison of the results from different laboratories.

Clean-up and separation of interfering substances

Multi-residual analyses: Clean-up and serations for multi-residual analyses are complicated. They rely on partitions between hexane and acetonitrile. Further purification and separation are achieved with chromatographic columns, such as Florisil, silica gel, alumina, magnesia, celite, etc., and combinations of these absorbents. Detailed procedures are given in section 211ff of the Pesticide Analytical Manual, the EPA Pesticide Manual, section 5.A, and in the Official Methods AOAC, under procedure 29.014-7.

Simplified pre-treatment for the analysis of DDTs, PCBs and lindane

Very simple clean-up and separation procedures can be employed if the analyses are restricted to the DDTs, PCBs and lindane. Murphy (1972) has shown that a simple treatment with concentrated H_2SO_4 after the extraction of the lipids is sufficient for the gas chromatographic analysis of these substances. Duursma (1976 and personal communication) suggests, after petroleum or hexane blender extraction (see Pesticide Analytical Manual, section 211.13f), three simple clean-ups for (A) acid stable Chl-HCs (PCBs, DDT + metabolites), BHC and 2,4 D-esters without and with pre-concentration, (B) for the acid-stable Chl-HCs plus aldrin, dieldrin and endrin, and (C) weak and strong alkaline saponification which will transform certain Chl-HCs by dehydrochlorination and thus may serve as a clean-up and as an identification at the same time (see below).

A. Clean-up for acid resistant Chl-HCs without extract pre-concentration

Procedure:

1. Add 8 drops of concentrated H_2SO_4 to 2 ml subsample of the extract contained in a small centrifuge tube.

2. Shake vigorously for 10 minutes on a vibrator and centrifuge.

3. Inject 10 µl from the surface layer into the GLC.

A'. Clean-up for acid-resistant Chl-HCs with extract pre-concentration (to be employed if the chromatographic response after the previous clean-up is too small).

Procedure:

1. Evaporate 10 ml of the original extract in a graduated centrifuge tube by placing the tube into a water bath of ± 40°C and blow clean air or N_2 through a pipette onto the surface of the extract until the volume is reduced to exactly 1 ml.

2. Add 4 drops of concentrated H_2SO_4, shake vigorously for 10 minutes on a vibrator and centrifuge.

3. Inject 10 µl of the supernatant hexane into the GLC.

B. Clean-up for acid stable Chl-HCs plus aldrin, dieldrin and endrin (Florisil chromatography)

Florisil eluation can be carried out with a hexane ethyl-ether mixture or a petroleum ethyl-ether mixture. The analyst should be aware that quantitative Florisil eluations are not easy to achieve and require considerable experience. Therefore, before treating unreplaceable actual samples, trial runs with yield determination using spare samples spiked with relevant Chl-HCs should be carried out.

6% ethyl-ether in petroleum ether will eluate:

Aldrin	Heptachlor epoxide	BHC
Lindane	DDD	PCBs
Heptachlor	DDE	Disyston
Methoxychlor	DDT	

15% ethyl-ether in petroleum ether:

Endrin	Parathion
Dieldrin	Methyl parathion
Thiodan I	Malathion (trace)

and 50% ethyl-ether in petroleum ether:

Thiodan II	Malathion

For the detailed procedure see the Pesticide Analytical Manual section 211.14d. For a hexane ethyl-ether eluation follow the following instructions (Duursma, personal communication):

Procedure:

1. Prepare a Florisil chromatographic column in a 12 cm long 6 mm diameter glass tube closed at one end with a sintered glass disc or glasswool cleaned by high temperature heating by adding pre-conditioned Florisil (see Pesticide

Analytical Manual section 121.3 and 211.14d) so that a 2 cm long column results; place a small centrifuge tube under the column.

2. Pour 2 ml of the extract through the column and collect in the centrifuge tube.

3. Add subsequently 8 ml of a hexane/diethyl-ether mixture (9/1; v/v) and collect always in the same column. Evaporate; eluate to 2 ml exactly as described under A1.

4. Inject 10 µl into GLC. Chromatogram will include dieldrin, aldrin and endrin, if present.

5. Follow procedure A1-A3 and the H_2SO_4 will destroy all Chl-HCs except the acid-resistant ones. Note that aldrin will not be completely destroyed.

6. Inject 10 µl into GLC for acid-resistant Chl-HCs and compare the two chromatograms obtained.

C. Weak and strong alkaline saponification

Often peaks of different Chl-HCs, especially the ones of PCBs, overlap with those of the DDTs (see Figure 31). A saponification with alcoholic KOH can serve as a clean-up since the fats will be hydrolysed and, at the same time, dechlorinates certain DDTs, for instance pp'DDT is transformed into pp'DDE and pp'TDE into pp'MDE, etc., while the stable PCBs will resist this treatment unchanged. This allows the employment of alkaline saponification, both as a confirmation test for the PCBs and an elimination of interference in the quantitative determination of the PCBs. The Pesticide Analytical Manual procedure is described under section 211.15d and also mentioned under 251.16(1).

Duursma (personal communication) suggests the following procedure:

Weak saponification:

1. To 2 ml hexane extract in a small centrifuge tube add 1 pellet of KOH dissolved in 0.2 ml of distilled water.

2. Shake slightly, centrifuge and inject 10 µl of the hexane supernatant into the GLC. The chromatogram represents a weak saponification.

3. Wait 6-24 hours, and inject again 10 µl into the GLC. Compare the peaks of the two chromatograms obtained.

Strong saponification:

1. To 2 ml of extract in a small centrifuge tube add 1 pellet of KOH dissolved in 1 ml of ethyl-alcohol (ca. 2% alcoholic KOH).

2. Shake for 10 minutes and inject the hexane supernatant.

3. Compare chromatograms obtained with those obtained through the other procedures.

The effect of alcoholic saponification is illustrated in Figure 32. A precise clean-up for different matrixes is difficult to prescribe and therefore the above procedures are to be modified according to the analyst's experience. Spiking sub-samples with the various Chl-HCs expected and running them through the whole procedure is recommended to increase accuracy in the interpretation of the chromatograms.

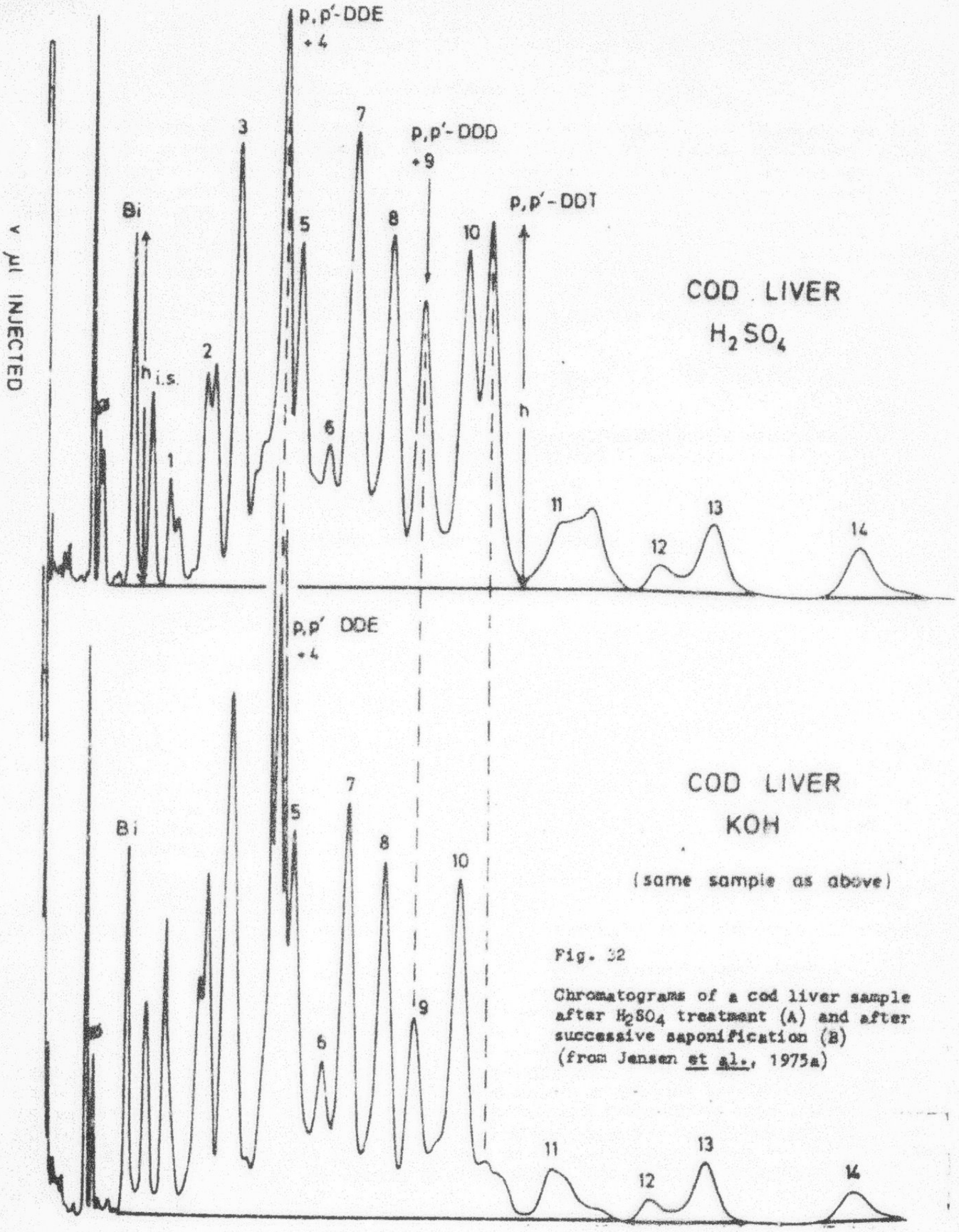

Fig. 32

Chromatograms of a cod liver sample after H_2SO_4 treatment (A) and after successive saponification (B) (from Jensen et al., 1975a)

Quantification of chromatograms

The different methods and various aspects of quantification of the chromatograms are discussed in the Pesticide Analytical Manual, section 302.4ff. Special difficulties arise in the PCB analysis (Jensen et al., 1973). Commercially available PCBs are mixtures of PCBs of various degrees of chlorination and the code implies only the average number of Cl-atoms. In Figure 31, 14 different PCBs can be identified in a Chlophen A 50 sample, but many more peaks are obtained if highly sophisticated gas-liquid chromatography is employed. One of the most common ways to quantify chromatograms of PCBs consists in matchin the peaks with peaks of known PCB standards. However, this procedure is not very successful since degradation and selective uptake of the PCBs by environmental samples has considerably changed the relative distribution of the PCB peaks so that the environmental sample do not resemble the available standards.

Chau and Sampson (1975) have conducted a comparison of 7 of the most widely used methods in PCB analysis:

1. Peak by peak quantification based on the isomeric distribution and weight-percent of PCB represented in each peak (Webb/McCall method).

2. Mean of all peaks as Aroclor 1254.

3. Mean of all peaks eluated after p,p'-DDE as Aroclor 1254.

4. Total peak height of all peaks as Aroclor 1254.

5. Mean of all peaks as Aroclor 1260.

6. Mean of all peaks eluated after p,p'-DDE as Aroclor 1260.

7. Total peak height of all peaks as Aroclor 1260.

They found that the precision using a certain method was usually sufficient but that the accuracy in measuring the PCB content of environmental samples appeared to be virtually unknown. PCB analyses of materials which had undergone little change from the time of PCB treatment to analysis, could easily be matched with commercial standards, but not environmental samples which have undergone photo-alterations or discrimination of the various PCBs during accumulation.

The Webb/McCall method proved to be the best method, i.e. the one with a minimum spread of values. Reasonable results were also obtained with methods 2 and 5. As expected, no single peak or group of peaks could be selected as representative of the total PCB content.

In addition to the seven methods mentioned above, the quantification based on a single peak after total chlorination of the PCBs present in the sample is worth mentioning. In this methodology all PCB components are perchlorinated to decachloro-biphenyl (DCB) with antimony pentachloride (Armour, 1973). Although this method will not allow the identification of the single components of the PCB, it has the advantage of supplying a single peak, thus increasing the sensitivity and facilitating quantification of the total PCB content. It might further be used to check the total PCB concentration obtained from the other quantification techniques discussed above. Recently, Trotter and Young (1975) have observed that different batches of $SbCl_5$ are contaminated to various degrees with DCB and also bromononachlor-biphenyl, which requires careful checking of the contaminated level of the $SbCl_5$ used. If nonchlorinated biphenyls are present in the sample, they will, of course, also be perchlorinated by this treatment.

Identification by chemical transformation

Since several substances can have the same retention time, a confirmation of the identity is necessary. Rerunning the sample with different GLC columns (ICES, 1974) and comparing retention times before and after a chemical transformation allows the identification of certain substances on the chromatogram. For example, a treatment with an alcoholic NaOH or KOH solution will dehydrochlorinate DDT and DDD and transform them into DDE and DDMU respectively. At the same time, the lipids are hydrolysed without changing the PCBs, so the method can also be used as a clean-up for a PCB analysis.

Chau and Lanouette (1972) proposed a solid matrix for the alkaline transformation confirmation of DDT, DDD and several other pesticides. Miles (1972) reports on an easy dehydrochlorination with 1.5 diazobicyclo (5.4 D) undec-5-ene (DBU) and a successive oxidation by chromic acid to dichlorobenzophenones as a means for both confirmation of the DDTs and their separation from the PCBs. Treatment with K-dichromate in sulphuric acid is reported to remove DDE without altering the PCBs (Westöö and Norén, 1970). DDE can also be oxidized with chromic trioxide in acetic acid to dichloro-benzo-phenone (Mulhern et al., 1971). The previously mentioned conversion of all PCBs by total chlorination to DCB can also be used as a confirmation test (Armour, 1973).

6.6 Gas Chromatographic Analysis of Methyl Mercury

Gas chromatography is usually employed to determine methyl mercury (Hg_M). An alternative method, not yet sufficiently tested utilizing AAS is described under section 6.4.1.

The procedure of GLC analysis takes the following steps:

1. Homogenize the sample (0.5-5.0 FW).
2. Liberate the Hg_M from its protein bond with a strong acid (2.3N, HCl, HBr, alkali bromide salt in H_2SO_4, NaBr in HCl).
3. Extract the Hg_M with an organic solvent (benzene or toluene).
4. Separate Hg_M from interfering impurities by extraction with an aqueous or ethanolic solution containing a thiol compound (cysteine, glutathione, thiosulphate).
5. Liberate Hg_M from the thiol compound with an acid as in step 2.
6. Extract again with an organic solvent.
7. Analyse in gas-liquid chromatograph.

Detection limit is 1 µg/kg FW with a standard deviation of 2% at concentrations above 50 µg Hg_M/kg FW.

Recovery is about 95% of Hg.

In a recent review prepared as a working paper for the FAO/WHO Expert Consultation to Identify the Food Conaminants to be Monitored and to Recommend Sampling Plans and Methodology, Westöö (1974) recommended the following Hg_M method as reference method (Fig. 33). Jensen et al. (1975) describe a similar procedure.

The following description of the analytical procedure proposed by Westöö (1974) has been reproduced by kind permission of the author:

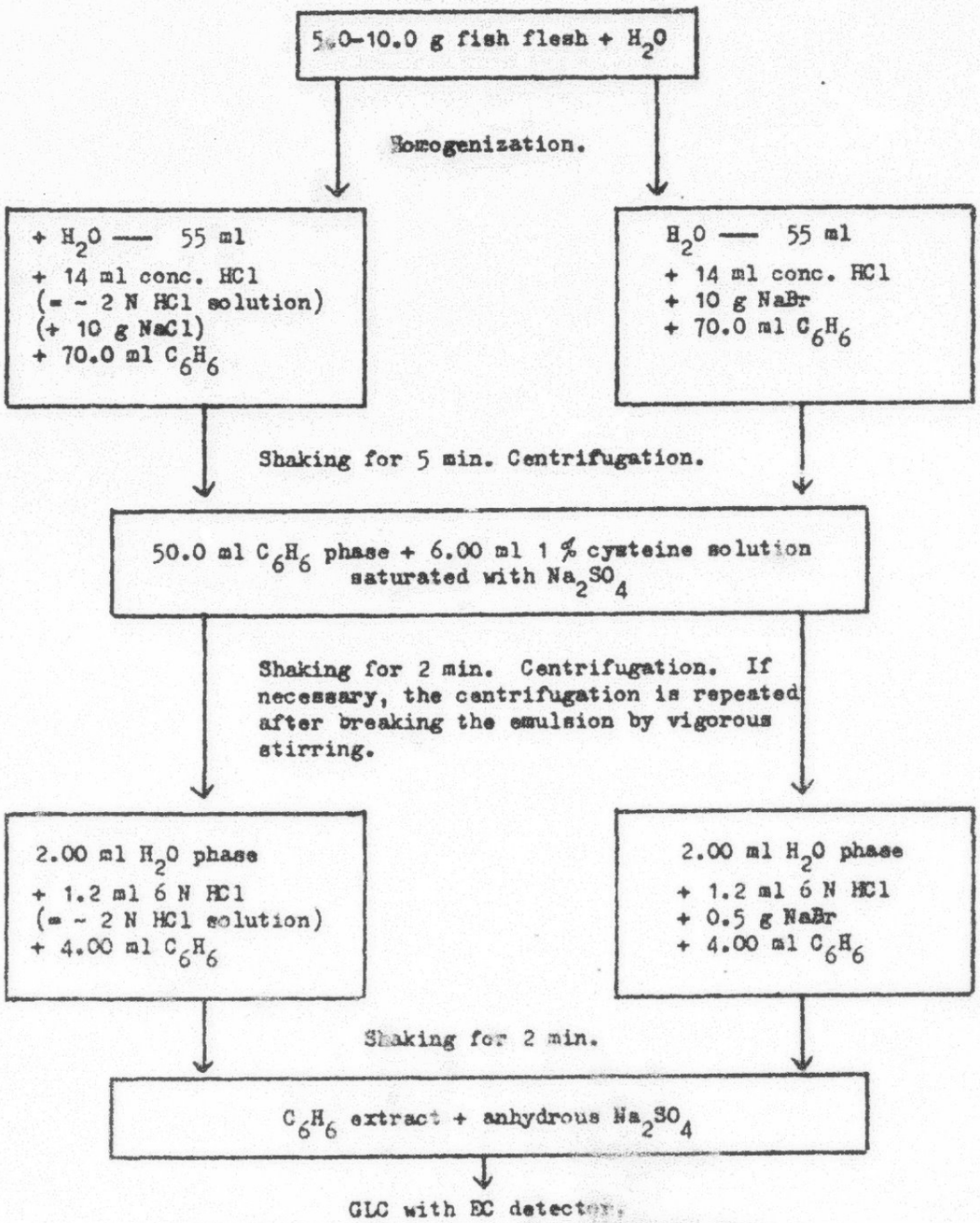

Fig. 33 Analysis of methylmercury in fish flesh (Westöö, 1974)

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