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INTERCALIBRATION OF ANALYTICAL METHODS ON MARINE ENVIRONMENTAL SAMPLES

Results of MEDPOL-II Exercise for the Intercalibration of Chlorinated Hydrocarbon Measurements on Mussel Homogenate (MA-M-2/OC)

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1. Introduction

It is well known today that molluscs are able to concentrate micropollutants (e.g. chlorinated hydrocarbons) in their tissues from the surrounding sea-water. When animals of the same species are living in the same conditions, the average concentration of a contaminant determined in their tissues after a sufficiently long exposure time should reflect the mean concentration of this substance in their environment. On the other hand, chlorinated hydrocarbons are normally present in sea water at very low concentration levels and it is much easier and less expensive to determine them in biological tissues than directly in sea water. Mussels, in particular, have been considered as good indicators of chlorinated hydrocarbon pollution of the marine environment and this led to the development of mussel watch programmes in many countries in the late seventies (1).

The present intercomparison had a double aim: first, it was intended to give to laboratories dealing with chlorinated hydrocarbon analyses of mussel tissues an opportunity for checking their analytical performance. Then, it was judged highly suitable for these laboratories to have at their disposal a reference material made of mussel tissue with robust estimations of the "true values" with respect to several chlorinated hydrocarbons. Such a material would allow chemists to check the validity of new analytical procedures.

2. Scope of the Intercomparison

Each participating laboratory received a sample accompanied by an information sheet and a report form. Participants were requested to determine as many as they could from among the following 9 components: Aroclor 1254, Aroclor 1260, Lindane, Hexachlorobenzene (HCB), Aldrin, Dieldrin, pp'DDT, pp'DDD and pp'DDE. The International Laboratory of Marine Radioactivity, however, expressed its interest in receiving results of individual isomers of PCBs instead of results in terms of industrial mixtures.

In total, 42 laboratories from 17 countries expressed their willingness to participate either in the Worldwide or in the Mediterranean Intercalibration exercises.

At the end of this exercise 27 laboratories from both exercises had submitted their results, the list of these laboratories is set out by country in the end of this report.

The analytical methods used by the different laboratories are listed in Table 4 with the lab code numbers.

In order to have more confidence in the concentrations in this sample, we report here the results obtained by participants in the two different exercises.

3. Description of the Material

About 600kg of Mediterranean mussels (Mytilus galloprovincialis) were collected from a local supplier. Soft tissues were separated from the shells which were discarded. After freezing, soft tissues were lyophilized under a vacuum of 10^{-1} Torr.

Lyophilized tissues were ground in a mixer made of stainless steel and glass only. The fraction of the material passing through a 150 micron sieve was collected while the residue was again ground in a porcelain ball mill. After grinding, the resulting powder was then sieved and the fraction passing through a 150 micron sieve was added to the first portion of powder.

Homogeneization was carried out by mixing the entire quantity of powder having a particle size of less than 150 microns in a stainless steel rotating drum for 100 hours. Then, aliquots of 35g were packed into glass bottles equipped with aluminum screw caps (labelled MA-M-2/OC). Teflon tape was then wound around the cap to minimize the contact between the sample and the outside atmosphere.

The homogeneity of the material for chlorinated hydrocarbon content was checked by determining the concentration of Aroclor 1254, HCB, Lindane, pp'DDE, pp'DDD, pp'DDT and Toxaphene in ten subsamples taken randomly from the bulk of the powder before packing. Determinations were performed by gas chromatography using both "packed" and "capillary" column techniques. The variance between samples could be explained by the analytical variance for each element determined. This material was, therefore, considered as homogeneous for the purpose of the intercalibration.

The water content of the lyophilized material as determined by drying to a constant weight at 85°C was found to be about 6.5%. Since, however, the moisture content can vary with changes in ambient humidity and temperature, it was recommended that the water content of this material always be determined in a separate subsample (not that taken for the analysis) by drying for 48 hours at 85°C.

The participants were requested to make at least one, and if possible multiple determinations of each chlorinated hydrocarbon and to complete the corresponding report form attached to the information sheet.

The concentrations reported by participating laboratories are calculated on a dry-weight basis. Participants were requested to give their limit of detection rather than the statement "not detected" when this situation arose.

4. Evaluation of Results

The data provided by laboratories participating in this intercomparison exercise are listed in Tables 1 and 2. The treatment of the raw data involves the application of Chauvenet's and Dixon's test for the identification and rejection of outlying values.

The data provided by participating laboratories were processed by a special statistical computer program which has been used already in previous intercomparisons organized by the IAEA. This programme uses non-parametric techniques and hence does not assume a particular form for the data distribution. Outlying results were eliminated by a distribution-free procedure (2,3). The medians and their confidence intervals were accepted as the most robust estimations of the true values. The confidence intervals of the medians were estimated from a Table given by Remington and Schork (4). The evaluation procedure which was used is described in Appendix 1. The results are listed in Table 3.

5. Discussion

The range of concentrations of chlorinated compounds in this particular sample of mussel tissue is between the concentration ranges observed for Copepod (MA-A-1/OC) and Fish (MA-A-2/OC). The relative uncertainties obtained by participants, expressed as the ratios between the % standard deviations and the means for different compounds, are also between those obtained for the two previous samples. It is also apparent that the lower the concentrations are, the higher are the discrepancies between results reported by the laboratories.

Intercalibration exercises arranged in order to increase the quality of analytical capabilities of environmental laboratories demand samples with organochlorine concentrations of the same order or higher than those found in sample MA-M-2/OC, if any further comparisons on the techniques be made possible.

Note

The IAEA will appreciate all remarks and comments from analysts using the mussel homogenate MA-M-2/OC on the basis of this intercomparison. If a sufficient number of new results are received in the future, the data base will be revised and a new report will be issued.

References

(1) GOLDBERG, E.D. et.al., The Mussel Watch. Environmental Conservation, Vol. 5, No. 2 (1978)

(2) VEGLIA, A: A nonparametric statistical method for the determination of a confidence interval for the mean of a set of results obtained in a laboratory intercomparison. Report IAEA/RL/84 (August 1981)

(3) PSZONICKI, L., HANNA, A.N., SUSCHNY, O.: Report on Intercomparison V-9 of the Determination of Trace Elements in Cotton Cellulose. Report IAEA/RL/97 (March 1983)

(4) REMINGTON, R.D., SCHORK, M.A.: Statistics with Application to the Biological and Health Sciences. Prentice Hall, Inc., Engelwood, Cliffs, N.J. (1970).

APPENDIX 1

Data handling and statistical evaluation

The participants were requested to make at least one, preferably more separate determinations of each compound and to report the results of all determinations as net values, i.e., after correcting for the blanks.

The main stages of the general statistical procedure used for evaluation of data were as follows:

1. Laboratory means were calculated on the basis of the reported individual determinations.
2. All laboratory means for one compound were treated as a set of data points and arranged by their ascending values.
3. The set of data was tested for outlying results and the outliers were rejected, using the following procedure:

   - the data points most distant from the mean of the set were tested sequentially one after the other:
   - for every tested point an h-value was calculated:

$$h = (x_j - \bar{x}_{n-1})\ (S_{n-1})^{-1} \left(\frac{n}{n-1}\right)^{-1/2}$$

   where: x_j - value of the point to be tested
   $\bar{x}_{n-1}$ - arithmetic mean of the set without x_j
   S_{n-1} - standard deviation of the set without x_j
   n - total number of the data points in the set

   - if the h-value was found to be larger than 3.162, then x_j was rejected as an outlier at the significance level of 0.05, and the testing procedure was continued for the next most distant point;
   - if h was smaller than 3.162, then the point was provisionally excluded from the set of data and the next point was tested. If for this next point the h-value was also smaller than 3.162, both points were accepted, and the procedure was finished, however, if for the second points h was larger than 3.162, both points were rejected as outliers and the testing was continued for the next data points of the set.
4. The overall median was calculated in the usual way and its confidence limits were found in the table given by Remington and Schork (4).

Table 1.: Organochlorine compound concentrations reported by participating laboratories for MA-M-2/OC (ng/g dry weight)

Lab. Code No.	Lindane	HCB	pp'DDE	pp'DDD	pp'DDT	Aroclor1254	Alpha-HCH	Aroclor1260	Dieldrin
1	2.1	0.55	42.3	41.5	32	490	1.7	n.r.	n.r.
2	n.r.	n.r.	19.3	15.3	15.3	205	2	140	1.3
3	3.4	n.r	16.7	48.7	147.5	1418	0.17	n.r.	n.r.
9	2.0	n.r	n.r	49.7	57.5	368	1.3	n.r.	n.r.
11	3.5	1	50	43	31	604	2.9	n.r.	n.r.
12	2.78	n.r.	22.25	36.4	26.3	235	1.38	164	0.57
14	0.52	n.r.	9.39	7.63	3.44	342.4	n.r	n.r	n.r
15	6.94	n.r.	n.r.	25.2	51.7	516.3	n.r.	n.r.	n.r.
16	n.r.	n.r.	80.1	n.r.	14.3	901	n.r.	n.r.	n.r.
17	32.36	184.24	202.04	344.88	41.89	575.69	n.r.	n.r.	n.r.
19	0.93	0.67	n.r.	n.r.	n.r.	135	n.r.	n.r.	n.r.
20	2.3	2	43.3	n.r.	35.5	465	n.r.	n.r.	4.5
21	n.r.	n.r.	34.03	46.476	67.823	n.r.	n.r.	242.202	n.r.
25	1.7	0.2	55	52	45	270	n.r.	n.r.	n.r.
31	220	27.5	53	n.r.	75	600	n.r.	n.r.	n.r.
32	7	1.5	35	n.r.	41	997	n.r.	n.r.	n.r.
34	1.07	0.5	32.8	32.9	30.8	270	n.r.	n.r.	n.r.
36	134	160	1680	n.r.	290	n.r.	n.r.	n.r.	850
37	16	n.r.	12.9	35	1	3620	n.r.	1560	16.8
39	3.1	n.r.	50.1	44.5	n.r.	n.r.	n.r.	n.r.	2.6
40	1.05	0.5	12.5	n.r.	16.4	780	n.r.	n.r.	n.r.
42	1	1	56	n.r.	16	1138	n.r.	n.r.	n.r.
43	4	2	52	43	29	915	n.r.	n.r.	8.5
46	3.5	0.5	11	3	47	200	8.5	n.r.	2.5
51	2.4	1.4	62	n.r.	34.7	1255	n.r.	n.r.	n.r.
52	n.r.	n.r.	50.7	48.3	27.9	n.r.	n.r.	n.r.	n.r.
62	0.7	n.r.	17.5	n.r.	17.5	189.35	n.r.	n.r.	n.r.

n.r.: No results reported.

Table 2: Organochlorine compound concentrations in Mussel MA-M-2/OC
(reported values in ng/g dry weight)

	Lindane	HCB	pp'DDE	pp'DDD	pp'DDT	Aroclor1254	Aroclor1260	Alpha-HCH	Dieldrin
No of results	23	15	24	17	25	23	4	7	8
Max. Value	220	184.24	1680	344.88	290	3620	1560	8.5	850
Min. Value	0.52	0.2	9.39	3	< 1	135	140	0.17	0.57
Average	20	26	110	54	48	720	530	2.56	111
Stand. Dev.	±52	±60	±340	±76	±58	±730	±690	±2.74	±300
(%)	(260%)	(230%)	(310%)	(140%)	(120%)	(100%)	(130%)	(107%)	(270%)
No of results after Chauvenet's test	17	12	22	16	23	22	4	6	5
Chauvenet's range	0.52--4	0.2--2	9.39--80.1	3--52	1--75	135--1418	140--1560	0.17--2.9	0.57--4.5
Average	2.1	1	37	36	33	590	530	1.6	2.3
Stand. Dev.	+1.1	+0.6	+20	+15	+19	+380	+690	+0.9	+1.5
(%)	(52%)	(60%)	(54%)	(42%)	(58%)	(64%)	(130%)	(56%)	(66%)
No of results after Dixon's test	17	12	22	16	23	22	3	6	7
Dixon's range	0.52--4	0.2--2	9.39--80.1	3--52	1--75	135--1418	140--242.202	0.17--2.9	0.57--16.8
Average	2.1	1	37	36	33	590	180	1.6	5.3
Stand. Dev.	±1.1	±0.6	±20	±15	±19	±380	±53	±0.9	±5.7
(%)	(52%)	(60%)	54%)	(42%)	(58%)	(64%)	(29%)	(56%)	(110%)

Table 3: Most robust estimations of the "true values" of organochlorine residues in the mussel sample MA-M-2/OC.

Compounds	Number of results accepted	Median	Confidence interval (α =0.05)
Lindane	16	2.3	1.0 - 3.4
H.C.B.	7	0.67	0.2 - 1.5
pp'DDE	22	42	19 - 52
pp'DDD	16	42	33 - 46
pp'DDT	22	32	26.3 - 45
Alpha-HCH	4	1.5	- (1)
Aroclor 1254	22	490	270 - 900
Aroclor 1260	4	200	- (1)
Dieldrin	5	2.5	0.6 - 4.5

(1): Impossible to apply the test (less than 5 accepted values)

Table 4. Methods of analysis reported by the participating laboratories

Lab Code Number	Extraction	Confirmation	Work-up Procedures	GC Conditions
1	Hexane in Soxhlet	MeOH, KOH and GC with 2 columns	Separation PCBs and DDTs Silica Gel Column Clean-up: Elution with hexane on microscale Florisil column	Det: 230°C Inj: 230°C 5% DC 200 on Gas Chrom Q at 200°C 5% QF-1 on Chromosorb W at 180°C
2	Grinder w/ Na_2SO_4 Petroleum ether	MeOH, KOH	Clean-up: Alumina Separation: Silica gel elution w/ Pentane and benzene	Det: 300°C Inj: 240°C Col: 203°C 10% QF1+5% SF96 on Chromosorb WHP
3	Hexane in Soxhlet	MeOH, KOH	PCBs and DDTs Silica Gel Clean-up: Sulphuric Acid 15% Ether/Hexane on Florisil column	Det: 240°C Inj: 220°C Col: 180°C 5% QF-1 on Chromosorb W
9	Hexane $MeCl_2$ (1:1) in Soxhlet	MeOH, KOH	Clean-up: Alumina Separation: Silica gel elution w/Pentane	Det: 230 Ing: 240°C Col: 203°C 10% QF-1+5% SF96 on Chromosorb WHP
11	Hexane in Soxhlet	Silica Gel F1: Hexane F2: Hexane/Ethyl Ether (75:25)	Clean-up: Sulphuric Acid	Det: 350°C Inj: 250°C OV 17/QF-1 (1.5%, 1.95%) on Supelcoport at 220°C

12	Ultra sonic Methylene Chloride		Clean-up: Florisil F1: Hexane F2: Hexane: Methylene Chloride (1:1)	Fused Silica SE 54, 25m T Prog. 175°C to 280°C at 4°C/min Det: 300°C Inj: 275°C
14	Soxhlet Hexane	MeOH, KOH	Clean-up: Sulphuric Acid	Det: 300°C Inj: 250°C OV 17, QF-1 (1.5%, 1.95%) on Chromosorb W
15	Soxhlet Hexane	MeOH, KOH	Clean-up: Sulphuric Acid	Det: 300°C Inj: 250°C OV 17, QF-1 (1.5%, 1.95%) on Chromosorb W at 210°C
16	Soxhlet Hexane	MeOH, KOH	Clean-up: Sulphuric Acid	Det: 235°C Inj: 235°C OV 17, QF-1 (1.5%, 1.95%) on Chromosorb W at 200°C
17	Petroleum Ether: Acetone (1:1) Ultrasonic	MeOH, KOH	Clean-up: Sulphuric Acid	Det: 320°C 10% DC 200 on Chromosorb at 210°C
19	Ultrasonic Methylene Chloride: Methanol (2:1)		Silica-Alumina (1:1)	OV 17, QF-1 (1.5%,1.95%) on Supelcoport at 200°C

21	Soxhlet Hexane	MeOH, KOH	Silica Gel Chroma- tography F1: Hexane F2: 25% Diethyl Ether in Hexane	Det: 260°C Inj: 210°C QF-1, DC 200 (2.5%, 2.5%) at 190°C
25	Soxhlet Hexane and Methylene Chloride	Sulfuric Acid MeOH, KOH	Florisil Chroma- tography F1: Hexane *F2: Hexane/Methylene F3: Methylene Chloride F4: Diethyl Ether *chloride (70:30)	Det: 300°C Inj: 210°C Fused Silica SE 54, 25m T Prog. 70°C for 2 min, then up to 260°C at 3°C/min.
31	Steam distil- lation solvant extraction iso- octane/Toluene (1:1)	Standard addition	15g dried sample in 2 l water pH 9 (KOH) for 7 hrs. (no clean-up)	Single ion monitoring in negative ion CH4-CI mode SE-52 (25m x 0.32mm i.d.) 60°C to 140°C at 20°C/min 140°C to 250°C at 6°C/min On-column injection
32	Extraction Ethyl acetate		Clean-up and separation of PCBs and DDE from other O.C. pesticides on Florisil	Detector: 300°C Injector: 220-250°C 2% OV1 + 4% OV 210 on Chromosorb W 2% OV 17 + 2.6% OV 210 on Chromosorb W
34	Ultrasonic acetone		Clean-up: H2S04	Detector: 300°C Injector: 250°C Oven: 160°C (2min) then up to 220°C at 4°C/min Fused silica OV 1 (25m)

36	Soxhlet Methanol/Chloroform (1.1)		Florisil, 6%, 15% and 50% Ether/hexane	Fused silica SE-52 (25m 0.2mm i.d.) 100°C up to 260°C at 4°C/min.
37	Solvant equilibration (hexane) for 6 days at room temperature	pp'DDE: UV Photo- lysis	Chromatography column with Na2S04 and silica gel	Detector: 300°C 1.5% OV 17 + 1.95% OV 210 on Chromosorb W at 190°C
39	Soxhlet Pentane 6 hrs.		Clean-up: Al203	25m CpSil 8 CB column 60°C (2 min) up to 180°C at 20°C/min (6 min) then up to 220°C at 4°C/min then up to 240°C at 4° C/min (5 min) then up to 270°C at 4°C /min.
40	Hexane/acetone (2:1) in glass column, 1 cm i.d.		Clean-up: Al203	120°C (36 min) then up to 150°C at 1°C/min (20 min) then up to 240°C at 2°C/min.
42	Blender 60 sec hexane/acetone (1:1)	Chemical derivation and 2 GC columns	Separation Florisil F1: Hexane F2: Hexane/ACN/MeCl2 (50:35:15) Clean-up: Florisil	10m x 0.53mm i.d. column 50% phenylmethyl silicone 160°C (1 min) up to 190°C at 25°C/min (5 min) up to 230°C at 25°C/min.

43	Blender acetone/ petroleum ether	2 G.C. Columns (packed) and capillary	Florisil: DCM/Petroleum ether (20:80) Clean-up and separation on Florisil with hexane	Detector: 300°C 5% QF 1 at 175°C 5% Dow 11 at 130°C
46	Soxhlet hexane		Clean-up: Sulphuric Acid	Fused silica 12.5m OV 1 120°C up to 275°C at 16°C/min.
51	Soxhlet hexane	capillary column SE 54 (50m)	Florisil Clean-up: Sulphuric on Florisil Acid	Detector: 280°C Injector: 220°C 200°C (10 min) up to 240°C at 1°C/min
52	Ultrasonic with hexane	a) H2S04 b) H2S04, saponified c) H2S04, saponified oxidation		2.8% QF 1 + 0.6% SF96 on Chromosorbin Detector: 300°C Injector: 300°C 180°C to 210°C at 1°C/min
62	hexane/acetone (1:3)	Alcoholic KOH	Clean-up: Sulphuric Acid hexane/diethyl ether (9:1)	Detector: 300°C Injector: 250°C 2.8% QF 1 + 0.6% SF 96 on Chromosorb AW DMCS 165°C (8 min) up to 190°C at 2°C/min

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