DETERMINATION OF TOTAL MERCURY AND METHYLMERCURY IN HUMAN HEAD HAIR BY RADIOCHEMICAL METHODS OF ANALYSIS

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ABSTRACT

In continuation of the studies previously started in the framework of the CRP on Assessment of Environmental Exposure to Mercury in Selected Human Populations as Studied by Nuclear and Other Techniques, four additional groups of Indians living in the Xingu Park, in the Amazonic region of Brazil, were studied for their content of mercury in hair.

Part of the samples were analyzed for total mercury by instrumental neutron activation analysis at IPEN-CNEN/SP (São Paulo, Brazil) and part were analyzed for total mercury and methylmercury by cold vapour atomic absorption spectroscopy at the Jozef Stefan Institute, Ljubljana, Slovenia.

The trend observed was similar to that found for the six other Indian tribes studied up to now and presented in previous RCMs, i.e., the concentrations of total mercury found were several orders of magnitude higher than the average concentration found for the control population. Also the data obtained for methylmercury show that most part of the element is present in the hair of the Indians in the organic form.

To assess the contamination by mercury of these populations, hair was chosen as the biological monitor.

Human hair samples have received much attention lately for studying mercury and other trace elements in vivo. They can be used for monitoring environmental exposure to pollutants as well as for evaluating poisoning by heavy metals.

Hair samples have the advantage of being easier to collect and prepare than other biological materials, like blood, urine, tissues, organs and others.

Elements such as mercury are absorbed by hair and retained over a period of time in which they remain in the head, while blood and urine levels indicate transient concentrations[3].

In the first part of the present work, results have been presented of the analysis of total mercury in head hair of the control group, the group of the Billings Dam and one Indian tribe living in the Xingu Park[4]. The method employed for analysis of mercury was instrumental neutron activation analysis, measuring the activity of the radioisotope ^{197}Hg ($t_{1/2}$ = 64.1h).

It was verified that the concentrations of mercury in the hair of the Indians were several orders of magnitude higher than the concentrations found for the control group. On the contrary, no such trend was found in the hair of the individuals living near the Billings Dam, in São Paulo.

The collection of hair samples was then extended to nine other tribes living inside the Xingu Park. For this collection it was very important the collaboration of physicians from the School of Medicine of São Paulo, who have been for many years responsible for the health care of these Indians. In most of these tribes, total mercury in hair was determined by instrumental neutron activation analysis.

Besides determining total mercury in this kind of study, it is also very important to analyze the particularly toxic methylmercury in hair and in other human tissues. Inorganic mercury can be methylated in the environment, specially by some organisms like fish and the resulting methylmercury is taken up by some organisms more readily than inorganic mercury.

Prenatal life is specially sensitive to the toxic effects of methylmercury. Incorporation of methylmercury by the foetus via placenta can affect normal neural development, leading to unbalanced brain architecture and decreased brain size[5].

In the present work, total mercury and methylmercury have been determined in the hair of some of the Indian tribes of the Xingu Park by cold vapour atomic absorption spectroscopy (CVAAS), with the collaboration of

the Department of Environmental Sciences of the Jozef Stefan Institute (Ljubljana, Slovenia), which is considered as the reference laboratory for the IAEA Programme, due to its large experience and high quality results on analysis of mercury and methylmercury in environmental samples.

2. EXPERIMENTAL

2.1. Collection and Washing of Hair Samples

The hair samples are collected and washed according to the protocol recommended by the IAEA[6].

The samples are cut using stainless steel scissors, from the occipital area of the head, and as close as possible to the scalp, in an amount corresponding to about 2 grams.

The hair is then cut with the scissors into segments as short as possible and transferred to a glass vial, for washing with acetone. The samples are covered completely with the solvent and stirred at frequent intervals, for 10 minutes and the solvent carefully decanted. After drying of the solvent at room temperature, the hair is homogenized and washed three times with distilled water. A final washing step with acetone is then carried out and the samples are left to dry in the open, being at this point ready for analysis.

2.2. Determination of total mercury in hair by instrumental neutron activation analysis

About 100 to 200 mg of the prepared hair samples and of the reference material Chinese Human Hair, GBW 09101 (SHINR-HH), are weighed in polyethylene envelopes previously washed with diluted nitric acid and desionized water. For each set of five samples, one reference material is analysed.

Irradiations are carried out for a period of one hour, in a pneumatic station, under a thermal neutron flux of about 10^{12} $n.cm^{-2}.s^{-1}$.

The standards are prepared by pippeting about 1μg of mercury, in the nitrate form, onto sheets of Whatman No. 40 filter paper, previously

impregnated with a solution of thioacetamide, to prevent mercury losses by volatilization before and during irradiation, as recommended by Noguchi et al[7].

After a decay period of about 70 hours, samples, reference materials and mercury standards are measured in a GMX 20195 ORTEC Ge detector, with a resolution of 1.9 keV in the 1332 keV peak of ^{60}Co. The detector is coupled to an ADCAM 918A Multichannel Buffer and associated electronics.

Spectrum analysis is performed by means of the VISPECT2 software, developed by D. Piccot, from Saclay,France[8].

For calculation of mercury concentrations, the 77 keV peak of ^{197}Hg ($t_{1/2}$ = 64.1h) is used.

2.3. Determination of total mercury and methylmercury in hair by cold vapour atomic absorption spectroscopy

A part of the hair samples collected from the Indians of the Xingu Park was sent to the Department of Environmental Sciences of the Jozef Stefan Institute (Ljubljana, Slovenia), for analysis of total mercury and methylmercury. This laboratory is considered as a reference laboratory for the IAEA Programme, due to its large experience and high quality in the analysis of mercury in biological and environmental samples.

The method used for hair analysis is basically the technique described by May et al.[9], which uses an anion exchange separation of extracted inorganic from organic mercury species, followed by destruction of organic species by UV irradiation, with the usual CV-AAS finish.

About 100 mg of hair is shaken with 10 ml 6 M HCl for 24 h in the dark and centrifuged. Protected from the light, the sediment is washed twice, recentrifuged and the washings combined with the centrifugate, which is then passed down a Cl^- form Dowex-1 anion exchange column to absorb inorganic Hg^{++}. The presence of Hg^{++} in the eluate is tested for by reduction with $SnCl_2$ and CV-AAS. The eluate is then subjected to 24 h irradiation from a UV lamp to decompose MeHg to Hg(II), and Hg(II) determined by CV-AAS.

Total mercury in hair is determined by destruction of up to 100 mg of hair with 2 ml conc. HNO_3 in a sealed tube by heating in a block for

several hours (or preferably overnight) at 90°C, followed by CV-AAS determination.

2.4. Determination of methylmercury in hair by neutron activation analysis

Experiments were carried out aiming the determination of methylmercury by neutron activation analysis, after separation by the method of volatilization of methylmercury cianide, as described by Zelenko and Kosta[10], using Conway cells for the procedure.

2.4.1. Separation of methylmercury

Whatman nº 40 or 41 filter paper is cut in rings of 2.0 cm of internal diameter and 3.0 cm of external diameter. Each ring is placed in the outer part of a Conway cell.

A volume of 150 μL of L-cystein hydrochloride or thioacetamide is pipetted onto the filter paper ring, which is allowed to dry at room temperature for 20 to 30 minutes.

About 70 mg of the hair sample is placed in the inner part of the cell followed by 250 μL of a solution of potassium hexacyanoferrate.

After homogeneization, 500 μL of a solution of 50% H_2SO_4 is added to the hair sample.

Each Conway cell is covered, after moistening of the cover with H_2SO_4 and put in an oven at 75°C, for a period of 14 to 16 hours.

The cell is then allowed to cool to room temperature and the filter paper ring is taken out.

2.4.2. Irradiation

The filter papers are sealed in double polyethylene envelopes and irradiated, together with mercury standards, for a period of 45 to 50 min, under a thermal neutron flux of 2×10^{12} n.cm^{-2}. s^{-1}.

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A volume of 150 μL of L-cystein hydrochloride or thioacetamide is pipetted onto the filter paper ring, which is allowed to dry at room temperature for 20 to 30 minutes.

About 70 mg of the hair sample is placed in the inner part of the cell followed by 250 μL of a solution of potassium hexacyanoferrate.

After homogeneization, 500 μL of a solution of 50% H_2SO_4 is added to the hair sample.

Each Conway cell is covered, after moistening of the cover with H_2SO_4 and put in an oven at 75°C, for a period of 14 to 16 hours.

The cell is then allowed to cool to room temperature and the filter paper ring is taken out.

2.4.2. Irradiation

The filter papers are sealed in double polyethylene envelopes and irradiated, together with mercury standards, for a period of 45 to 50 min, under a thermal neutron flux of 2×10^{12} $n.cm^{-2}.s^{-1}$.

The standards utilized are 50 μL of a solution of mercury nitrate, pipetted onto filterpaper, corresponding to a mass of 1 μg of mercury.

2.4.3. Measurements

After a decay time of about 48 hours, samples and standards are measured, in the same way as described in item 2.2.

3. RESULTS AND DISCUSSION

3.1. Analysis of reference materials

Besides analyzing the reference materials Fish Flesh Homogenate (IAEA), MA-A-2/TM (Hg = 0.47 ± 0.02 ppm) and Chinese Human Hair, GBW 09101, SHINR-HH (Hg = 2.16 ± 0.21 ppm), also the candidate IAEA reference materials IAEA-085 and IAEA-086 were analyzed, after the 3rd RCM meeting in Monaco.

For Fish Flesh, the results obtained were: 5.7% relative error and 9.4% relative standard deviation and for Chinese Human Hair: 1.8% relative error and 6.9% relative standard deviation.

Tables I and II show the results obtained for IAEA085 and IAEA086. The relative standard deviations obtained were, respectively of 8.8 and 13.6%.

3.2. Analysis of hair samples from the Indians of the Xingu Park

At the 3rd RCM Meeting in Monaco, results were presented for the analysis of mercury in hair of the Indians living in the Xingu Park up to the 6th group.

In the present paper, results are presented for the groups from 7 to 10. The analysis of the 11th group is still proceeding.

Tables III and IV present the results of the analysis of total mercury in the 7th and 8th group of Indians, obtained by instrumental neutron activation analysis.

In Table V, the results are shown of the analysis of total mercury and methylmercury in hair of the 9th group of Indians by cold vapour atomic absorption spectroscopy.

Table VI presents the results obtained for the 10th group of Indians, for total mercury and methyl-mercury, by cold vapour atomic absorption spectroscopy.

In Table VII, are the results for the determination of total mercury in the 10th group of Indians, by instrumental neutron activation analysis.

In Table VIII, the results of the experiments of determination of methylmercury by neutron activation analysis, after separation by volatilization of methylmercury cyanide, are presented. The hair samples analyzed belong to the second group of Indians, whose hair samples have been already analyzed for total mercury and methylmercury in Ljubljana.

In Table IX, a summary is presented of the results obtained for the whole duration of the Research Contract, for total mercury and methylmercury in hair of the Indians living in the Xingu Park.

It can be observed that for the ten groups of Indians studied, all of them presented concentrations of total mercury several orders of magnitude higher than the control population.

The arithmetic means, geometric means and medians of the ten groups varied respectively from: 4.7 to 20.6 ppm, 3.2 to 19.0 ppm and 2.4 to 18.8 ppm. The corresponding values for the control group were: 1.06, 0.93 and 0.96.

In the groups where the methylmercury concentration was also determined, it can be observed that most part of the mercury is present in the organic form, with concentrations varying from about 70 to 100%.

Given such very high total mercury and methylmercury concentrations in the hair of the Indians, it can be concluded that these populations could be at risk as regards contamination by mercury.

The most probable source of this contamination is fish, since they are consumed very frequently by the Indians and since it is well known that fish and fish products are the dominant sources of methylmercury in the diet[12].

Lacerda and Pfeiffer[2] have verified that the mercury concentrations in Amazonian fish are, in various sites, nearly five times the maximum permissible levels for human consumption. On the other hand, the region where the Xingu Park is situated is far from the sites of gold exploration activity and is still suposedly free from contamination.

It would be necessary to analyze fish, water, sediments and other materials from the region of the Park, but this was not done up to now, due to the remoteness of the region.

According to the physicians of the São Paulo School of Medicine, who are responsible for the health care of the Indians, they have not up to now shown any symptoms of contamination by mercury.

As to the experiments of determination of methylmercury by neutron activation analysis, after separation as methyl-mercury cyanide in the Conway cells, the results were not reproducible, as can be seen in Table VIII. A few of them yielded results close to the ones obtained in Slovenia, but most of them were lower, due probably to losses of MeHg during irradiation.

4. CONCLUSIONS

With relation to the work developed in our Research Contract in the last year of the CRP, the following main conclusions can be drawn:

1. The results obtained for total mercury in the hair of the Indians belonging to the last four tribes analyzed were several orders of magnitude higher than the ones obtained for the control population.

2. Mercury is present in the hair of the Indians mainly as methylmercury, with concentrations from about 70 to 100%.

3. The main source of contamination is probably fish, due to the fact that the Indian populations living in the Xingu Park consume fish very frequently.

4. It would be necessary in any case to analyze samples of water, fish and aerosols from the Xingu Park, to investigate further the sources of contamination.

5. As to the analysis of methylmercury by neutron activation, the results were not reproducible, as can be seen in Table VIII. A few of them yielded results close to the ones obtained in Slovenia, but most of them were lower, due probably to losses of MeHg during irradiation.

5. PLANS FOR FUTURE WORK

The work on analysis of mercury and methylmercury in hair will proceed in the Radiochemistry Division of IPEN-CNEN/SP, even after the end of the CRP of the IAEA on assessment of environmental exposure to mercury.

There are several groups in Brazil working in this field and it is felt that due to the extension and seriousness of the problem, all possible contributions are welcome.

Of the 17 tribes living in the Xingu Park, hair samples have been collected from 11 of them. Ten of them have already been analyzed and the last one is in the process of analysis by INAA. The experiments on determination of methylmercury by NAA will also proceed.

The Brazilian Government is financing a Project that aims studying the cicle of mercury in the ecosystem of the Amazonic forest. It is a multi-disciplinary project, with the collaboration of researchers from several institutions. Mercury will be analyzed in samples of rocks, soils, water, aerossols, sediments, plants, human hair and other biological samples. Our Institute will be responsible for the analysis of hair samples. The project will be centered in the region of the Cupixi River (State of Amapá, Northern region of Brazil), where there are several sites of gold extraction using mercury for amalgamation. In this region it is also possible to find still zones of forest that are preserved from anthropogenic contribution and could serve as a background for comparison purposes.

The collaboration with the Jozef Stefan Institute is considered as very important for the continuation of the Project. A fellowship is being asked for one of the researchers of the Radiochemistry Division, to work with Dr. A.R. Byrne on analysis of total mercury and methylmercury.

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TABLE I

RESULTS OF THE ANALYSIS OF TOTAL MERCURY IN THE REFERENCE MATERIAL IAEA-085 BY INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS.

ALIQUOT	TOTAL Hg (ppm)
1	29.8
2	29.4
3	25.1
4	29.5
5	30.1
6	28.8
7	26.5
8	26.3
9	24.4
10	24.7
11	26.7
12	25.5
13	21.9
14	27.1
15	26.7
16	28.2
17	26.2
18	30.6
19	25.4
20	23.7

$x = 26.83$

$s = 2.36$

$s_{rel} = 8.8\%$

TABLE II

RESULTS OF THE ANALYSIS OF TOTAL MERCURY IN THE REFERENCE MATERIAL IAEA-086 BY INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS

ALIQUOT	TOTAL Hg (ppm)
1	0.55
2	0.62
3	0.77
4	0.64
5	0.74

$x = 0.664$

$s = 0.090$

$s_{rel} = 13.6\%$

TABLE III

RESULTS OF THE ANALYSIS OF TOTAL MERCURY IN HAIR OF THE INDIANS FROM THE JURUNA TRIBE (7TH GROUP) FROM THE XINGU PARK, BY INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS.

SAMPLE CODE NUMBER	TOTAL MERCURY (ppm)
1154	23.5
1155	12.6
1156	28.2
1159	25.0
1160	28.4
1161	14.1
1163	16.2
1167	18.4
1167-2	21.8
1169	14.9
1170	19.1
1172	19.0
1173	11.7
1175	12.6
1176	19.4
1181	12.7
1182	17.0
1183	18.4
1184	16.2
1186	15.8
1189	30.2
1190	19.9
1201	9.9
1207	14.9
1222	14.0
1223	18.0
1224	13.2
1334	13.8
1365	11.1
1494	15.4
1645	21.8
1754	2.48
1996	13.0
2280	16.0
2335	20.6
2336	9.1
2337	12.0
2369	13.3
6056	12.6

$n = 39$ range = 2.48 - 30.2

$x = 16.5$

$s = 5.5$

median = 15.8

$x_G = 15.5$

TABLE IV

RESULTS OF THE ANALYSIS OF TOTAL MERCURY IN HAIR OF THE INDIANS FROM THE XINGU PARK (8TH GROUP), BY INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS.

SAMPLE CODE NUMBER	TOTAL MERCURY (ppm)
1	13.9
2	16.0
3	12.5
4	27.6
5	24.5
6	17.9
7	31.7
8	30.2
9	29.1
10	48.6
11	19.6
12	18.4
13	18.7
14	12.5
15	13.1
16	13.7
17	19.3
18	13.9
19	11.8
20	14.5
21	18.2
22	6.7
23	17.6
24	11.7
25	14.1
26	18.8
27	16.6
28	15.9
29	-
30	14.3
31	19.1
32	28.9
33	14.0
34	13.7
35	11.5
36	16.2
37	14.1
38	21.3
39	2.10
40	17.3
41	20.4
42	14.7

$n = 41$; $x = 17.20$; $s = 6.05$; $x_G = 16.3$; median = 16.24
range = 2.10 - 31.7

TABLE V

RESULTS OF THE ANALYSIS OF TOTAL MERCURY AND METHYLMERCURY IN HAIR OF THE INDIANS FROM THE XINGU PARK (9TH GROUP) BY COLD VAPOUR ATOMIC ABSORPTION SPECTROSCOPY.

SAMPLE CODE NUMBER	TOTAL Hg (ppm)	MeHg (ppm)	% MeHg
2	13.2	12.0	91
6	25.0	22.8	91
7	23.7	23.7	100
8	10.9	10.5	96
9	21.5	20.8	97
10	16.0	14.9	93
11	15.7	14.4	92
12	16.3	13.4	82
17	21.8	16.9	78
18	16.3	14.7	90
21	21.8	18.0	83
22	15.4	14.5	94
23	17.2	17.2	100
25	19.9	15.8	79
26	12.1	10.0	83
28	17.0	15.3	90

$n = 16$	$n = 16$
$x = 17.7$	$x = 15.9$
$s = 4.1$	$s = 3.9$
$x_G = 17.3$	$x_G = 15.5$
Median = 16.6	Median = 15.1
range = 10.9 - 25.0	range = 10.0 - 22.8

TABLE VI

RESULTS OF THE ANALYSIS OF TOTAL MERCURY AND METHYLMERCURY IN HAIR OF THE INDIANS FROM THE XINGU PARK (10TH GROUP) BY COLD VAPOUR ATOMIC ABSORPTION SPECTROSCOPY

SAMPLE: CODE NUMBER	TOTAL Hg (ppm)	MeHg (as Hg) ppm
267	2.32	-
1375	3.41	-
1377	2.82	-
1381	2.33	-
1382	1.62	-
1387	1.98	-
1407	1.69	-
1509	1.48	-
1531	9.19	7.92
1555	4.24	-
1558	5.99	5.46
1577	1.49	-
1859	26.1	24.2
1904	4.05	-
2031	4.12	-
2189	2.41	-
2225	1.50	-
2243	2.19	-
2258	2.27	-
3309	12.6	12.0

Quality control analyses:

IAEA Hair 085	25.1 ± 1.21 (5)	23.9 ± 1.13 (4)
	Hg total	MeHg
	$x = 4.69$	$x = 12.40$
	$s = 5.78$	$s = 8.32$
	$x_G = 3.22$	$x_G = 10.58$
	$s_G = 2.17$	$s_G = 1.89$
	Median = 2.37	Median = 9.96
	Range = 1.48 - 26.1	Range = 5.46 - 24.2

TABLE VII

RESULTS OF THE ANALYSIS OF TOTAL MERCURY IN HAIR OF THE INDIANS FROM THE XINGU PARK (10TH GROUP) BY INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS.

SAMPLE: CODE NUMBER	TOTAL Hg (ppm)
72	26.03
273	16.70
290	21.04
409	18.55
410	33.14
780	1.48
924	17.00
1383	1.52
1405	1.94
1414	4.50
1428	1.68
1429	1.78
1447	7.14
1540	2.05
1541	2.11
1542	2.68
1574	2.75
1588	2.27
1605	2.18
1606	9.29
1883	2.72
1978	2.84
2006	4.59
2254	3.28
2257	4.45
5269	21.72
7510	2.36

$x = 8.07$ $x_G = 4.72$

$s = 9.04$ $s_G = 2.76$

$s_{rel} = 112.03\%$ Range = 1.48 - 33.14

Median = 2.84

TABLE VIII

RESULTS OF THE EXPERIMENTS OF ANALYSIS OF MeHg BY THE VOLATILIZATION METHOD FOLLOWED BY NAA

SAMPLE CODE NUMBER	MeHg (ppm) SLOVENIA	MeHg (ppm) IPEN	DIFFERENCE
1225 - Gl	16.3	7.02 ± 1.62	- 56.9 %
1225 - Gl	16.3	2.92 ± 0.66	- 82.1 %
1225 - Gl	16.3	14.34 ± 1.64	- 11.5 %
1228 - Gl	16.7	15.02 ± 1.79	- 10.0 %
1230 - Gl	12.4	6.66 ± 0.20	- 46.3 %
1234 - Gl	18.4	4.32 ± 1.17	- 76.5 %
1241 - Gl	14.9	15.30 ± 2.04	+ 2.6 %
1242 - Gl	13.7	0.675 ± 0.007	- 95.0 %
1245 - Gl	14.4	7.82 ± 1.44	- 45.7 %
1251 - Gl	9.47	5.57 ± 0.00	- 58.8 %
1269 - Gl	10.1	1.58 ± 0.20	- 84.4 %
1277 - Gl	4.79	3.61 ± 0.29	- 24.6 %
1281 - Gl	15.4	12.81 ± 2.00	- 16.8 %
1285 - Gl	18.4	0.60 ± 0.06	- 96.7 %
1293 - Gl	10.2	5.50 ± 0.92	- 46.0 %
1293 - Gl	10.2	6.77 ± 0.37	- 33.6 %
1324 - Gl	25.7	18.79 ± 2.48	- 26.9 %
1341 - Gl	15.4	7.09 ± 0.45	- 54.0 %
1341 - Gl	15.4	2.90 ± 0.52	- 81.2 %
1652 - Gl	22.8	19.84 ± 2.56	- 0.87 %

TABLE IX

SUMMARY OF THE RESULTS OBTAINED FOR MERCURY CONTENTS IN THE HAIR OF THE BRAZILIAN POPULATIONAL GROUPS STUDIED.

POPULATIONAL GROUP	x	s	MEDIAN	x_G	RANGE
CONTROLS	1.06	0.55	0.96	0.93	0.20-2.5
INDIAN GROUP 1	18.50	5.9	18.0	17.1	6.87-34.3
INDIAN GROUP 2	12.0	4.0	10.7	11.4	6.54-21.6
INDIAN GROUP 3	8.7	3.0	8.2	8.2	4.5-18.5
INDIAN GROUP 4	13.2	3.8	13.0	12.7	4.8-25.3
INDIAN GROUP 5	10.6	3.9	11.5	9.4	1.7-15.1
INDIAN GROUP 6	20.6	10.0	18.8	19.0	8.1-57.3
INDIAN GROUP 7	16.5	5.5	15.8	15.5	2.5-30.2
INDIAN GROUP 8	17.2	6.0	16.2	16.3	2.10-31.7
INDIAN GROUP 9	17.7	4.1	16.6	17.3	10.9-25.0
INDIAN GROUP 10*	4.7	5.8	2.4	3.2	1.5-26.1
INDIAN GROUP10**	8.1	9.0	2.8	4.7	1.5-33.1

* Results obtained by CVAAS

** Results obtained by INAA