

## HEAVY METAL CONTAMINATION (MERCURY AND LEAD) IN AYURVEDA PREPARATIONS. INTRODUCTORY REMARKS.

---

**AUTHORS:** DR. CERIANI D. \* - DR. CERNUSCHI M. \* - DR. GRAMMINGER K. \*\*  
DR. LORINI G. \*\* - P.Ch. TAGLIABUE A. \*

**KEYWORDS:** Ayurveda, Quality Assurance (HACCP), Atomic Absorption (Heavy Metals)

**SUMMARY:** Over a period of three years (1994-1997) the authors have examined 150 Ayurveda products marketed in Italy. A total of 222 atomic absorption spectrophotometric (AAS) analyses were completed for the detection of any Hg and Pb contamination. This report illustrates the analysis techniques, the contamination results (43% of all analyzed products), as well as causes and possible remedies.

### INTRODUCTION

Over the past few years, Ayurveda, an ancient Indian science, has been enjoying an increasing success in Western countries, although it has been introduced only relatively recently (10-15 years ago). The Ayurveda practice [1] was started some 5000 years ago and consists in a systematic and scientific set of recommendations concerning each person's life, with the aim of prolonging it in perfect health. In this respect, a key role is played by suggestions on food and daily life habits, connected with the different psycho-physic constitutions (PRAKRITI). In order for the constitution [2] (which is always the same from birth to death) to remain healthy and balanced, different types of food programs are also suggested, based on fruit, roots, leaves, plant flowers, either in their natural form (traditional Ayurveda) or in the form of extract (incorrectly called modern Ayurveda and more suitably defined as Indian Phytotherapy). Another group of preparations is based on spices (e.g. ginger, cloves, black pepper, long pepper, tumeric, etc.), either in their natural form or as extracts.

The third and last group of preparations [3] is more strictly medicinal (and are recognized as medicines in

India) and includes modern or traditional herbal compositions with the addition of Heavy Metals, detoxified (i.e. made non toxic for the human body according to the Ayurveda tradition) Minerals (in oxide form).

The latter products are not dealt with in our research as they are strictly medicinal and as such their Import to Italy is banned if they do not comply with the law provisions on drugs.

According to the Ayurveda rules, the preparations falling within the first two groups (the subject of our research) are specially indicated for people in good health as they are aimed at maintaining such healthy Status and this differentiates them from medicinal preparations that are indicated only for people affected by some pathology. For this reason the first two groups of Ayurveda preparations fall in - and are tolerated -the general category of FOOD PREPARATIONS or NATURAL FOOD under the Italian law provisions (still showing a poor phytopharmacological identification of Asiatic plants that are unknown and thus not included in the western pharmacopoeia). As such, they are therefore subject to food regulations. [4]

### SURVEY

The research was carried out from June 1994 to June 1997 on a sample of 150 Ayurveda preparations marketed in Italy as tablets, liquids and jams, under the definition of "food preparations" or "herbal preparations" of different Indian manufacturers of Ayurveda products.

The analyses performed at the CHEMICAL LAB of U.S.S.L.\* (Local Health Care Unit) No. 1 in Varese were focused on establishing any HEAVY METAL - namely MERCURY and LEAD - contamination and, on a small control sample, on detecting the presence (in products available in the form of liquids or capsules) of preservatives such as Sorbic Acid, Benzoic Acid and

Parabens that are commonly used in India for the above-mentioned preparations, which however shall be dealt with in a future publication. **Analysis methods for mercury and lead**

#### **MERCURY**

Analysis technique: ATOMIC ABSORPTION SPECTROPHOTOMETRY WITH DEVELOPMENT OF COED VAPORS - MHS

Equipment and machines: AA Spectrophotometer "5100 PERKIN-ELMER"; microwave mineralizer "MILESTONE mis 1200"; Mercury hollow-cathode Eamp PERK.IN EEMER - Class A calibrated pipettes and matrasses; Hydride development System MHS-10 PERKIN EEMER.

Glass treatment: The glass devices used must be treated with acid washing with a 0.1N nitric acid solution prior to their use.

Diluting agent: 18 MOhm (milli-O) de-ionized water

Nitric acid 65%: Extra-pure - (Baker 9598-00) - with a low Mercury percentage (max 0.5 ppb)

Hydrochloric acid 35%: Extra-pure - (Baker 9530-51) - with a low Mercury percentage (max 0.5 ppb)

Sodium-Boron Hydride: (Baker 9161) FIA degree

Sodium Hydrate: (RPE Carlo Erba) Mercury content max 0.1 ppm. Sampling System: manual

Reference Standard: **1g/L** Standard mercury solution (MERCK art. 19795)

Calibrating Standard: 10 and 20 u.g/E Standard solutions obtained through cascading dilutions from the reference Standard; taking 2 ml and thinning to 100 ml a 20 mg/E solution is obtained. By diluting 5 ml to 100 a 1 mg/E solution is obtained; finally, 100 and 200 uL respectively are sampled and added to 10 ml of system White - Eine gas: Nitrogen (for transfer to the Hg vapor cell)

Sample White: HNO<sub>3</sub>:H<sub>2</sub>O<sub>2</sub>:H<sub>2</sub>O=40:2:58 solution simulating the sample's preparation.

System White: 1.5% HNO<sub>3</sub> + 1.5% HCl solution

Reducing solution: 3% NaBH<sub>4</sub> + 1% NaOH solution (manual 20 sec \* 0.2 ml/sec approximately)

Sample's volume: 10ml

#### **Preparation of the sample:**

A percentage of the sample (0.2-0.5 grams) is precisely measured to the fourth decimal (**weight**). 4 ml of extra-pure HNO<sub>3</sub> and 0.2 ml of 30% H<sub>2</sub>O<sub>2</sub> are added and then inserted in the microwave mineralizer. The mineralization process is to be undertaken with a suitable programmed formula depending on the

sample's characteristics (for instance, for pieces of desiccated plants the following is used: 5 min \* 300w, 30 sec \* 600w, 4 min \* 240w). 10 uL of 5% KMnO<sub>4</sub> (stabilizing agent) are added to the mineralized material, which is then transferred to a 10, 20 or 25 ml matrass (**volume**) and taken to the desired volume with distilled water. For highly contaminated samples further dilutions are performed. (Note: persistence of the coloring due to permanganate indicates a good mineralization).

#### **Procedure:**

Open the Nitrogen valve and check for the correct dripping of the reducing solution (approximately 0.2 ml/sec). Open the working file XAEIMHG.GEE. Then proceed in sequence with the preliminary auto-zero function with the "system white" solution (checking for reproducibility at least two more times) and then proceed with the Reslope Operation with a 10 ug/E Standard. If reslope points are obtained (at least two) matching the previous calibration (maximum tolerance = 10%), then you can proceed with the analysis without having to perform the entire calibration again. Then, before reading the samples, the reset Operation must be performed with "sample white".

#### **Method sensitivity - measurability limit**

As far as a generic solution to be analyzed is concerned, the measurability limit equals 0.05 u.g/L. The term "solution to be analyzed" refers to the mineralized sample. Now, if we were to consider, for instance, that 0.5 g of the sample were mineralized and brought to 20 ml, we would immediately notice that the measurability limit that could be related to the sample equals 2 ppb (0.002 ppm) and the higher one equals 0.8 ppm. Any sample showing correct concentrations > 0.8 ppm are diluted.

#### **Calculations and expression of the results**

The instrument's **reading** obtained through interpolation gives the result referred to the analyzed solution expressed in u.g/L. In order to obtain the numeric result referred to the sample (in mg/kg) (correct concentration), the following expression must be applied, where f is the dilution factor (if any):

$$Hg(sample) = \frac{reading * volume * f}{weight * 1000}$$

## LEAD

Analysis technique: ATOMIC ABSORPTION SPECTROPHOTOMETRY WITH GRAPHITE FURNACE - ZEEMAN EFFECT - ADDITION METHOD.

Equipment and machines: AA Spectrophotometer "5100 PERKIN-ELMER" with automatic sampler; microwave mineralizer "MILESTONE mis 1200";

Lead hollow-cathode lamp PERKIN ELMER.

Class A calibrated pipettes and matrasses, 1.5 ml disposable beakers for the automatic sampler.

Glass treatment: The glass devices used must be treated with acid washing with a 0.1/v nitric acid solution prior to their use.

Diluting agent: 18 MOhm (milli-Q) de-ionized water

Nitric acid 65%: Extra-pure - (Baker 9598-00) - with a low Eead percentage (max 0.5 ppb)

Reference Standard: 1g/L Standard lead solution (MERCK art. 19776).

Calibrating Standard: 80 ug/E Standard solution obtained through cascading dilutions from the reference Standard, taking 2 ml and thinning to 100 ml a 20 mg/L solution is obtained. By diluting 20 ml to 100 a 4 mg/E solution is obtained, which is finally diluted to 100 thus allowing to obtain the Standard desired.

Eine gas: Argon

White: HNO<sub>3</sub>/H<sub>2</sub>O=2/3, which simulates the sample's preparation.

Matrix modifier: 1% NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> + 0.05% Mg(NO<sub>3</sub>)<sub>2</sub> solution

### Preparation of the sample

A percentage of the sample (0.2-0.5 g) is weighted exactly (**weight**) to the fourth decimal, 4 ml of extra-pure HNO<sub>3</sub> and 0.2 ml of 30% H<sub>2</sub>O<sub>2</sub> are to be added and then insert the whole in the microwave mineralizer. The mineralization process is to be undertaken with a suitable programmed formula depending on the sample's characteristics (for instance, for pieces of desiccated plants the following is used: 5 min \* 300w, 30 sec \* 600w, 4 min \* 240w). The mineralized material is then transferred to a 10, 20 or 25 ml matrass (**volume**) and taken to the desired volume with distilled water. For highly contaminated samples further dilutions are performed.

### Procedure:

Open the Argon valve and the one supplying cooling water. Open working file AGGPBDIE.GEE; place the

80 ng/E Standard solution in position 36 of the self-sampler; place the matrix modifier in position 39, the diluent (distilled water) in position 0 and the white in position 40. Then, the samples to be analyzed are generally loaded in positions 1,2,...,#,...N. Type "RUN SAMPEE" to proceed. First, the instrument automatically reads the white as AUTOZERO and then reads the sample. Each sample is supposed to undergo three readings ("as it is", "first 50 ppb addition", "second 80 ppb addition"). As you can notice in the table below, 28 uE are always taken from the furnace, of which 12 uL are for the modifier, 8 uE for the sample and the remaining 8 uE for the additions.

position	39	0	36	#	40
white	12	8	0	0	8
as it is	12	8	0	8	0
50 ppb addition	12	3	5	8	0
80 ppb addition	12	0	8	8	0

### Method sensitivity - measurability limit

As far as a generic solution to be analyzed is concerned, the measurability limit equals 1 ug/E. Moreover, since the addition method is used, it is best to work with solutions to be analyzed having a lead content not exceeding 100 ug/E so as to be able to work in a range of instrumental linearity. The term "solution to be analyzed" refers to the mineralized sample. Now, if we were to consider, for instance, that 0.5 g of the sample were mineralized and brought to 10 ml, we would immediately notice that the measurability limit that could be related to the sample equals 20 ppb (0.020 ppm) and the higher one equals 2 ppm. Any sample showing correct concentrations > 2 ppm are diluted.

### Calculations and expression of the results

The instrument's **reading** obtained through linear regression (minimum squares) gives the result referred to the analyzed solution expressed in ug/E. In order to obtain the numeric result referred to the sample (in mg/kg) (correct concentration), the following expression must be applied, where f is the dilution factor (if any):

$$Pb(sample) = \frac{reading * volume * f}{weight * 1000}$$

The ZEEMAN effect, as is widely known [5], is extremely useful for correcting background absorptions; at the same time, the addition method becomes essential in reducing the matrix effect. These two techniques matched together allow to obtain optimum results also on complex matrixes such as products based on herb, dried extracts, etc. In fact, regression lines with correlation coefficients usually > 0.99 are obtained

### Results of research on mercury

105 mercury survey analyses were carried out on 150 preparations analyzed.

As at present no specific law provisions exist for these matrixes, we referred to the existing commonly accepted literature:

1) FAO/WHO guideline.

The PTW! (PROVISIONAL TOLERABLE WEEKLY INTAKE) value [6] established by the JECFA (JOINT FAO/WHO EXPERT COMMITTEE on FOOD ADDITIVES) committee for MERCURY is 0.005 mg/kg of body weight.

2) Guideline established by the Italian law (with small changes within the EU) on food: the maximum tolerance level referred to shark species is 0.7 mg/kg of fish. [7,8,9]

A comparison of the two sets of regulations allows us to establish that they are the same for an adult having a body weight of approximately 60 Kg consuming approximately 420 g of fish food in a week. This weekly fish intake value can be compared to an average consumption of three fish plates a week. It is obvious that the average consumption of Ayurveda food preparations is remarkably lower than this quantity. Thus, the reference of 0.7 mg/kg of mercury used by us to assess the preparations' eligibility falls well within the safety limits.

In our 105 mercury survey analyses we found as many as 42 values higher than 0.7 mg/kg (ranging from a minimum value of 0.8 mg/kg to a maximum value of 200 mg/kg) and 63 values lower than 0.7 mg/kg.

**TABLE 1 - MERCURY SURVEY**

Hg	1994	1995	1996	1997	TOT.
< 0.7 mg/kg	20	24	8	11	63
> 0.7 mg/kg	37	5	0	0	42
total analyses	57	29	8	11	105

% eligible	35	83	100	100	60
% non eligible	65	17	0	0	40

The table shows that in the years following 1994 -when our research was started - the percentage of mercury-contaminated products decreased. The research and thus the possibility to eliminate the possible causes of mercury contamination of products were made possible thanks to the active cooperation of an Indian sample factory that, upon our Suggestion, accepted to adopt a few changes in the production methods, enforcing strict quality controls as well as pre-selecting the raw material through chemical analyses based on international Standards carried out at the Calcutta university. It immediately emerged that the critical point where the highest mercury contamination occurred was the production and packaging department. We then inferred that mercury contamination of Ayurveda products coming from India is mainly connected with an incorrect packaging procedure, as the raw material is of good quality.

### Results of research on lead

117 lead survey analyses were performed on 150 products analyzed. The law provisions for food [10,11,12] generally indicated a tolerance limit of 1 mg of lead for 1 kg of preparation.

In our 117 lead survey analyses we found as many as 54 values higher than 1 mg/kg (ranging from a minimum value of 1.03 mg/kg to a maximum value of 56.9 mg/kg) and 63 values lower than 1 mg/kg.

**TABLE 2 -LEAD SURVEY**

Pb	1994	1995	1996	1997	TOT.
< 1 mg/kg	12	24	14	13	63
> 1 mg/kg	18	16	13	7	54
total analyses	30	40	27	20	117
% eligible	40	60	52	65	54
% non eligible	60	40	48	35	46

Differently from the mercury analyses, the first lead analyses (1994) were limited because interest (and problems) were focused on mercury research. Obviously, due to economic reasons, once mercury contamination was established, no research on lead was carried out.

Later on, starting in 1995, we focused more on lead and cadmium contamination because the percentage of mercury-contaminated samples was decreasing due to the strict quality controls enforced in the Indian pilot factory. Incidentally, Cd has always turned out to be lower than 0.1 mg/kg and thus the control over this heavy metal was discontinued almost immediately. Despite the efforts made by the pilot factory for improving production and packaging at a local level, lead contamination remained high (35%) and thus it can be correlated to a raw-material contamination added to possible subsequent contamination during production, treatment and packaging, especially in the case of tablets.

### CONCLUSIONS

Besides the evaluation of validity and usefulness of Ayurveda, confirmed by over 4000 years of history, the authors stress the quality and control method for Ayurveda products.

Of the 222 analyses performed (both on mercury and on lead) on Ayurveda (non medicinal) products marketed in Italy, 43% of the products turned out to be non eligible, which is a high percentage, especially considering that, thanks to the cooperation of the Indian pilot factory starting from 1995 (otherwise the percentage would have been even higher), a drop in the contamination percentages was recorded in the global count. It is desirable that importers pay more attention to analytical preliminary controls on Heavy Metals (especially mercury and lead) to be carried out in Indian University and/or Public Labs based on International Standard techniques (as in our research) to be followed by a further verification at European official chemical labs before import. The authors also suggest a further official control at the Italian customs before entry (at airport, sea and ground facilities) not just for products directly coming from India, but also for those indirectly coming from other EU countries such as Great Britain and Holland, so as to perform a further selection of such products in order to safeguard health and at the same time to avoid rejection and exclusion from the market for these preparations that for thousands of years have been considered as valuable in maintainin" and

### REFERENCES

- [1] "Selected medical plants of India". Chemexcil, Bombay, India [2] Svoboda: "Prakriti, la costituzione individuale" (Prakriti, the individual constitution), edizioni Mediterranee [3] Svoboda: "La medicina ayurvedica" (Ayurveda medicine), Armenia editore [4] Rizzati: "Tutela igienica sanitaria degli alimenti e bevande e dei consumatori" (Hygiene and health protection of food, beverages and consumers) 22nd edition - Pirola editore [5] Minoia. Caroli: "Applicazioni dell'ETA-AAS Zeeman nel laboratorio chimico e tossicologico" (Applications of ETA-AAS Zeeman in the chemical and toxicological lab), volumes I and II. edizioni Cortina [6] FAO [7] Circular letter of the Health Ministry No. 25 of 24th March 1975(1)
- [8] Ministerial Decree of 14th December 1971 (1) [9] Ministerial Decree of 29th March 1974 (1) [10] Decree of the President of the Italian Republic 180/88(1)
- [11] Law decree 64/93 (1) [12] Decree of the Health Ministry 106/97 (1)

(1) = Italian laws