The working procedure of human autopsy specimens

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In order to perform the Coordinated Research Program for the Reference Asian Man (phase 2): Ingestion and body content of trace elements of importance in Radiation Protection, study on elemental content in organs of normal Chinese has been worked by China Institute for Radiation Protection and Institute of Radiation Medicine – CAMS in recent two years.

1. Sampling and sample collection of human tissues

The principles of sampling and sample collection of human tissues are set up as follows:

(1) The persions? for analysis of elemental content or organs and tissues should be normal and health Chinese adult.

(2) For comparison the elements intake and tissues contents among populations from different food habit regions of China, the sampling regions for human tissues should correspond to the sampling regions for the first total diet study mentioned previously.

On the basis of above requests, an individual who had died suddenly and who had no apparent diseased condition at the time of death was considered to be a normal subject. Up to now, total seventy-two cases of adult victims of sudden death were examined postmortem within 24 hours after death from 1996 to 1998. These cases were taken from four geographical regions of China (Table 1). The Chinese tissues were taken at autopsy by coroner with special care to prevent contamination placed in individual polyethylene bags (with sign of serial number) and transported quickly to the laboratories where they were kept frozen (at -20°C) in the low temperature refrigerator until prepared for drying or ashing?. For large organs like liver, lungs, muscle samples of 50~100g were taken, for smaller organs like thyroid, the entire organ was taken. Then the coroner must fill in the record card in which including the items of name, sex, age, occupation, height, weight, nation, cause of death, date of death and autopsy. The record card should be identified by signature and date.

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2. The procedures of sample preparation

2.1 Bone

2.1.1 The quartz crucibles were soaked in a solution of 30% nitric acid for more than 24 hours, then rinsed with cleaning water and ion-free water. The crucibles were weighed respectively after drying in the air.

2.1.2 The bone (rib) samples were thawed, separated from extraneous muscles and cartilage with titanium knives and teasers, then rinsed with ion-free water and blotted with filter papers. After drying in the air (about 4 hours), the bone sample was placed in a crucible and weighed.

2.1.3 Put the quartz crucible (with bone sample) into a drying oven (in constant temperature) and drying for 24 hours at about 80°C.

2.1.4 Transferred the crucible (with cover) to a muffle furnace a more careful precaution against any dust from the inner walls of the furnace, increased temperate in step by step (50°C increment per 20 minutes) to avoid burning, up to the final temperature 550 °C for 12 hours or longer, then cut off the electricity supply.

2.1.5 About 12 hours later, the crucibles were put out from a muffle furnace, weighed, then the specific value of wet and ash weight could be calculated for each bone sample.

2.1.6 The bone ash, containing a small amount of grayish, is pulverized slowly in a clean agate mortar and pestle. This handling step is carried out in an ultra-clean working table or a sealed glove box, then weighed and made the ash sample into several aliquots to be needed for analysis.

2.1.7 The ash samples are placed in the cleaning polyethylene bags by heat sealing and each sample is serial numbered on the bag surface for identification. All ash samples are kept in sealed desiccators with a drying agent (high purity calcium-chloride).

2.1.8 All pre-cleaned PE bags were soaked in a solution of diluted nitric acid, rinsed with cleaning water and ion-free water, then drying in the air for reserve.

2.2 Soft tissue

2.2.1 The glass dishes were soaked in a solution of 30% nitric acid for more than 24 hours. Then rinsed with cleaning water and ion-free water. The dishes were weighed respectively after drying in the air (more than 24 hours).

2.2.2 The soft tissue samples were taken out from the low temperature refrigerator and thawed gradually in a refrigerator (in 4 °C). Then rinsed with cleaning water and ion-free water, separated from fat, connective tissues and blood vessels, and carrying out a segment about the size of 1 x 2 x 2cm with titanium knives and tweezers, blotting with filter papers.

After drying in the air (more than 6 hours), placed in a pre-cleaned glass dish and weighed. All operators must be worked by using power-free plastic gloves.

2.2.3 The following additional recommendations were made concerning the preparing of individual soft tissues.

Liver: Approximately 20~50 g should be taken from the right lobe, and any evidence of hepatitis or cirrhosis should be discarded.

Kidney: Approximately 20~50 g should be taken from right kidney in which including the three sections of cortex, medulla and pyramid.

Lung: Approximately 20~50 g should be taken from the lower half of the upper lobe (right) being careful to exclude any regions containing lymph nodes.

Muscle: Approximately 20~50 g should be taken from the psoas muscle (right side).

Thyroid: The whole right lobe should be taken from the two lobes and separated from the parathyroid and other extraneous tissues.

2.2.4 Put the glass dishes (with soft tissues) into a blast drying oven and drying for 48 hours at about 80°C to constant weight. Then weighted, and the specific value of wet and dry weight could be calculated for each soft tissue.

2.2.5 Be placed the dry-tissue sample into a cleaning PE bag. Then impacting with a iron mallet which is coated with polyethylene membrane and is pulverized slowly in a clean agate mortar and pestle. This handling step is carried out in an ultra-clean working table or a sealed glove box. Then weighed and made the dry-tissue sample into several aliquots to be needed for analysis.

2.2.6 The dry-tissue samples are placed in the cleaning PE bags by heat sealing and each sample is serial numbered on the bag surface for identification.

All dry-tissue samples are kept in a sealed desiccators with drying agent (high purity calcium chloride).

2.2.7 The fresh thyroid (one lobe) is weighed after drying in the air and irradiating by ultraviolet radiation for sterilizing. Then the fresh thyroid is heat sealed in a pre-cleaned PE-bag and doubled bagged by heat sealing the outer bag. Its still kept frozen (at -20°C) in the low temperature refrigerator for analyzing iodine by ENAA.

2.2.8 All of above handling steps must be prevented inadvertent contamination of samples and of microbial infection.

Table 1. The collection of human autopsy specimens in China

The regions of collection	Cases	Sex	Age (years)	The cause of death
South-A Jiansu Province (Shanghai)	7	Male	23~36 28.6 ± 4.2	Executed in accordance with the law
South-B Sichuang Province (Chengdu)	25	19(M), 6(F)	20~50 35.6 ± 10.5	Traumatic Shock *
North-B Shanxi Province (Taiyuan, Changzhi)	25	16(M), 9(F)	25~56 34.8 ± 7.1	Traumatic Shock *
North-A Hebei Province (Beijing, Tianjin)	15	9(M), 6(F)	15~59 33.9 ± 12.3	Traumatic Shock *
Total	72	51(M), 21(F)	15 ~59	Traumatic Shock *

* For the most part