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Bioassay of ^{210}Po in human urine and internal contamination of man

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The deliberate poisoning of A. Litvinenko in London in late 2006 with ^{210}Po, attracted attention to the difficulties in identifying internal contamination with alpha emitting radionuclides and to the limited knowledge available on the cycling of many naturally occurring radioisotopes in the body and their baseline concentration values in humans. To cope with the emergency caused by the spread of high ^{210}Po activity, which contaminated several people and places in London, we were called upon to analyze urine samples in potentially contaminated people. A reference group of adult humans was also selected for determination of baseline ^{210}Po values to be used for comparative purposes. Concentrations of ^{210}Po in urine samples from three Portuguese citizens that have been at contaminated places, in London, ranged from 2.3 to 4.1 mBq·L^{-1} while in the reference group ^{210}Po concentrations ranged from 0.5 to 4.8 mBq·L^{-1}. Analytical quality of results was ensured through participation in an international inter laboratory comparison exercise on ^{210}Po determination in aqueous samples. Results indicated that people potentially exposed to ^{210}Po in London were not internally contaminated with the radionuclide used as a poisoning agent, and the levels of this radionuclide measured in the urine were similar to the naturally occurring levels in the reference group. Polonium levels in urine and in man are discussed in the light of ^{210}Po levels in the human diet.

Introduction

Following the poisoning of A. LITVINENKO with polonium (^{210}Po), in London, presumably occurred on the 1st November 2006, there has been confirmation that several public places, including hotels, restaurants, airplanes and other vehicles, were contaminated with the same radioactive isotope.[1] British radiation protection authorities reported on the radioactive contamination and informed health and radiation protection authorities of several countries when citizens from there were identified to have been at contaminated places. Following this information there was the need to proceed immediately with radioactivity analyses in order to check whether people, Portuguese citizens in our case, had been contaminated.

Polonium-210, the radioactive substance identified as the poisoning agent present in the body of LITVINENKO and in contaminated public places, is a naturally-occurring radiosotope ($T_{1/2}$ = 138.4 d), of the uranium radioactive decay series. Therefore, ^{210}Po is a common and widespread radionuclide in our environment and, generally, it is present in air, water, food and even in the human body.[2] On a mass basis ^{210}Po is a very rare element on Earth but it has a very high specific activity, $1.7 \cdot 10^{14}$ Bq·g^{-1}. When ^{210}Po atoms decay, alpha particle radiation ($E\alpha = 5.305$ MeV) is emitted. As to the activity of 1 Bq of ^{210}Po corresponds $5.9 \cdot 10^{-15}$ g, even a small amount of polonium on mass basis emits a high radiation dose and it is, therefore, very radiotoxic to human beings.

Faced with the request to assess internal contamination of people that had been in London at the time of the poisoning events, we had to proceed rapidly in order to come out with results useful to support medical judgment and radiation protection measures. However, assessing internal contamination of people or reassuring them though the bioassay of ^{210}Po faced one major difficulty. Indeed, although ^{210}Po can easily be quantified through the measurement of alpha radiation emission, there is a lack of reference data on ^{210}Po in human tissues and in body fluids that could be used as a baseline to assess the eventual contamination of persons. Therefore, a reference group was selected for use in a comparative procedure with the exposed group.

Material and methods

Sampling

Several Portuguese citizens were identified as having been at the contaminated places in London, as tourists and as workers, during the period of the radioactive contamination. Post-factum they were individually contacted by the Portuguese Health Authorities in order to be informed about the potential contamination risk and to organize a bioassay of ^{210}Po in urine. Three out of those citizens, residents in the Lisbon area, volunteered to be analyzed for ^{210}Po and the others declined the analysis.

A reference group of citizens that had not been in London during the period or after the poisoning event, and residents in the Lisbon area as well, were invited to collaborate as volunteers to the ^{210}Po bioassay in the organism.

A 24 h urine sample was collected by each individual in a plastic can. Urine was immediately acidified in the can to pH<2 by addition of 2 mL concentrated HNO_3 and brought to the laboratory for analysis.

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Analysis

In the laboratory the pH of urine sample was checked, adjusted as needed to pH<2, and 80±1 mBq of ^{209}Po in 1M HNO_3 solution was added to the urine sample. Po-209 ($T_{1/2}$ = 103 y) is an artificial isotope of polonium, not existing in the environment, and as other polonium isotopes, it is radioactive and decays with the emission of alpha particle radiation with the energy Eα = 4.882 MeV. A known amount of ^{209}Po is added to the sample for use as an internal isotopic tracer allowing computing the radiochemical yield of the analysis. After careful mixing of the isotopic tracer with the sample, $MnCl_2$ was added along with potassium permanganate ($KMnO_4$) and the sample stirred with continuous nitrogen bubbling for 4–5 hours. After addition of ammonia (NH_4) to raise pH to 8.5–9.0, the MnO_2 precipitate (brown flock) was allowed to settle overnight. The next morning the overlying liquid was discarded and the precipitate collected by centrifugation. The MnO_2 precipitate was dissolved in a small volume of HCl and H_2O_2 and pH adjusted to 2. After addition of 200 mg of ascorbic acid, polonium isotopes were plated onto the surface of a silver disc during three hours with continuous magnetic stirring. Polonium in hydrochloric solution plates spontaneously and selectively onto the Ag metal disc, allowing the preparation of a radioactive source of virtually no thickness and, thus, suitable for high resolution alpha-spectrometry.[3]

Ag discs with polonium isotopes were measured with 450 mm^2 active surface ion implanted silicium detectors, connected to an OCTETEPlus, ORTEC EG&G multichannel pulse analyzer. The energies of polonium isotopes are well resolved and allow for straightforward quantitative determination of ^{210}Po in the sample (Fig. 1).

Analytical quality assurance

Quantitative determination of ^{210}Po in urine samples in order to assess the internal contamination of human beings was not at the time a routine analysis in many countries. Even the analytical techniques for polonium, despite their relative simplicity, were not of current use in many laboratories. Therefore, there was a need to intercompare results and ensure the accuracy of results. The organization of an intercomparison exercise on the analysis of ^{210}Po in urine samples was suggested to the International Atomic Energy Agency (IAEA). This exercise was implemented in April 2007, using HCl acidified water samples as a surrogate for human urine. For testing the ability to analyze ^{210}Po at different concentration levels, the IAEA prepared a sample material by ^{210}Po addition to acidified water samples in amounts not disclosed to the participating laboratories. Each participating laboratory received 5 Nalgene bottles, numbered from 1 to 5, each one containing about 50–60 g of liquid sample. Laboratories were requested to determine ^{210}Po concentration in the content of each bottle. A reporting deadline was established, and the data reported by the laboratories was processed by the IAEA.[4]

Results and discussion

Urine samples

Results of ^{210}Po analyses in urine samples, including those from the Exposed group and those from the Reference group are shown in Table 1. Concentrations in the Reference group ranged from 0.5 to 4.8 mBq·L^{-1}, averaging 2.0±1.4 mBq·L^{-1}. Concentrations of ^{210}Po measured in Exposed group, averaging 3.4±0.8 mBq·L^{-1}, all fell in that reference range. Applying a t-test to compare the mean values and associated standard errors of the two groups it was concluded that they were not significantly different ($p<0.05$).

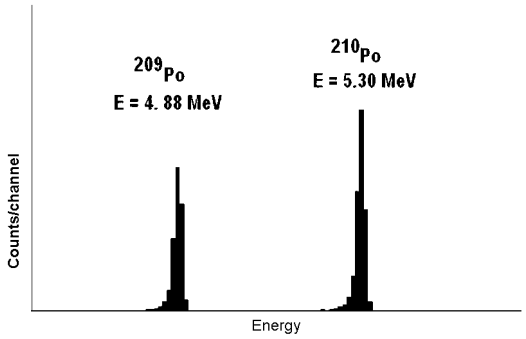

Fig. 1. Alpha-spectrogram of polonium in a urine sample

Table 1. Polonium-210 measured in urine samples

	Concentration	Daily excretion rate	
	mBq·L^{-1} ± 1SD	Volume (L)	mBq·d^{-1} ± 1 SD
Exposed group			
1	4.1 ± 0.3	2.38	9.76 ± 0.71
2	3.8 ± 0.6	1.28	4.86 ± 0.77
3	2.3 ± 0.4	2.63	6.05 ± 1.05
Average ± 1 SD	3.4 ± 0.8		6.89 ± 2.08
Min–Max	2.3 – 4.1		6.05 – 9.76
Reference group			
1	0.5 ± 0.1	2.10	1.09 ± 0.21
2	0.9 ± 0.2	1.98	1.70 ± 0.40
3	3.5 ± 0.3	1.77	6.20 ± 0.53
4	4.8 ± 0.6	1.05	5.06 ± 0.63
5	1.3 ± 0.3	0.88	1.14 ± 0.26
6	1.6 ± 0.2	1.72	2.75 ± 0.34
7	1.6 ± 0.6	2.15	3.44 ± 1.29
Average ± 1 SD	2.0 ± 1.4		3.06 ± 1.83
Min–Max	0.5 – 4.8		1.09 – 6.20

Comparison of ^{210}Po daily excretion rates between the two groups of individuals is heavily dependent upon the amount of urine collected during the 24 hours period. There are differences between groups in the 24 hours urine volumes offered to the laboratory for analysis, suggesting that some urine samples in the Reference group could correspond to a collection time shorter than 24 hours. In this case, comparison between groups based on the ^{210}Po concentration in urine is more reliable than a comparison based on 24 hours total ^{210}Po activity excreted. Still, the comparison of the average ^{210}Po daily excretion rates of both groups with associated standard errors did show that the two groups are not significantly different ($p<0.05$).

Observation of individual ^{210}Po concentration values in each group shows that an important variability do exist amongst adults. It is known that ^{210}Po is absorbed by man from inhaled air and from the food and water ingested. In the Portuguese population the average daily intake of ^{210}Po and ^{210}Pb from the diet was measured as 1.3 and 0.59 Bq·d^{-1}, respectively.[3] Most of the ^{210}Po intake takes place with the ingestion of food and the inhalation of air and cigarette smoke give smaller contributions.[3]

Using the ^{210}Po average daily intake in the Portuguese population, 1.3 Bq·d^{-1}, and assuming an average ^{210}Po gut absorption efficiency of 0.30, the daily absorption rate of ^{210}Po into the systemic circulation is 0.39 Bq·d^{-1}. The contribution from air inhalation to ^{210}Po in the blood stream is $1.2\cdot10^{-4}$ Bq·d^{-1} and tobacco smoke gives an additional contribution of about $6\cdot10^{-3}$ Bq·d^{-1} to the total ^{210}Po absorbed in the blood stream.[5] In the Portuguese population with about 1/3 smokers and different diets, some based on sea food and others based on agriculture products, the intake of ^{210}Po certainly varies and, thus, the ^{210}Po amount daily excreted in urine may vary from one person to the next.

Most of the ^{210}Po in the systemic circulation has a biological half-life of 50 d and it is mainly excreted with the feces. About one third is excreted with the urine at a rate of about 1.5% per day,[6] which accounts for the excretion of about 6 mBq·d^{-1} in this case. This estimated ^{210}Po urinary excretion rate can be compared to the daily excretion rates measured in both groups (Table 1).

Unfortunately, no studies were conducted to assess ^{210}Po in human fluids and in human tissues in the Portuguese population that could provide a more robust estimate of baseline levels. Data from other countries is scarce also and most of it were obtained with analytical techniques and nuclear instruments considered obsolete today.[7] Nevertheless, data compiled from the literature suggests that ^{210}Po daily excretion rate in the urine varies from about 5 to 15 mBq·d^{-1} although these rates are not tied to dietary habits and regions.[8,9] Assuming this range of values as an acceptable estimate of ^{210}Po urinary excretion by man at baseline level, than all values determined by us in Portuguese citizens are at the baseline (Table 1). Therefore, no additional intake of ^{210}Po from the poisoning act in London had occurred with the Exposed group of people.

In assessing contamination of members of the public in London areas in relationship with the ^{210}Po contamination, the UK Health Protection Agency considered the following screening levels. In people with ^{210}Po daily excretion rate determined at or below 30 mBq·d^{-1}, the computed committed effective dose is below 1 mSv·y^{-1}, which is the maximum allowed dose increment to members of the public[10] and this is considered as of no concern from the radiation protection viewpoint. People with bioassay results indicating ^{210}Po daily excretion rates above 30 mBq·d^{-1} were assigned for a more detailed radiation dose assessment that would lead in several cases to doses above 6 mSv·y^{-1}.[9]

The Portuguese citizens in the Exposed group were all in the category under 30 mBq·d^{-1} and were of no concern regarding internal radioactive contamination and absorbed radiation dose.

Intercomparison exercise

Aqueous samples received from the IAEA were shaken to ensure homogeneity, and the amount of liquid determined by weighing. From each bottle, three aliquots of 5 to 10 g each, determined by weighting, were used in replicate analysis. Each was spiked with 0.080±0.001 Bq of ^{209}Po from a HNO_3 solution, and thoroughly mixed. Samples were processed as described above and the results averaged and reported to the IAEA before the deadline. Table 2 summarizes the IAEA values, disclosed after the closure of the exercise, and our values as reported to the IAEA. They match the IAEA values and are accepted by statistical tests at the most demanding level, i.e., within 95% uncertainty level, and demonstrated the accuracy of our routine analytical procedure for ^{210}Po analysis in aqueous samples.[10]

Table 2. Results of an international intercomparison exercise for determination of ^{210}Po in aqueous media, organized in April 2007 (IAEA, 2008)

Sample	IAEA nominal value (spike), Bq·kg^{-1} ± 1 SD	Our determination, Bq·kg^{-1} ± 1 SD
No. 1	52.8 ± 1.4	47.9 ± 1.7
No. 2	101.6 ± 2.8	93.6 ± 3.2
No. 3	52.8 ±1.4	48.4 ± 1.6
No. 4	101.6 ± 2.8	90.6 ± 3.0
No. 5	Blank (<0.1)	0.0092 ± 0.0030

This type of exercise allowed the laboratories to check their own ability to measure ^{210}Po at different activity levels and at blank (but not zero) level. The range of concentrations tested encompassed the concentrations found in human urine samples.

Conclusions

The group of Portuguese citizens potentially exposed to ^{210}Po contamination during their stay in London in November-December 2006, was not internally contaminated with ^{210}Po above normal (baseline) levels. The values of ^{210}Po in their urine samples were comparable with the values measured in the Reference group.

The international intercomparison exercise organized by the IAEA for ^{210}Po analysis in aqueous samples, as a surrogate for urine, allowed checking that the accuracy and precision of our analytical procedure in the analysis of ^{210}Po was highly satisfactory and leading to reliable results even at low levels of radioactivity.

The poisoning act in London using ^{210}Po as a poisoning agent is the type of malevolent and criminal use of radioisotopes, fortunately rare. Besides the targeted victim of poisoning, many other people and several public places were contaminated with the radioactive substance Amongst the consequences of this event, the need to screen members of the public for internal contamination by ^{210}Po posed a challenge to radioanalytical laboratories. Indeed, there was a need for a rapid response in assessing contamination of people, through using tested radioanalytical techniques able to providing accurate results. The events have shown that it is not always granted that the laboratories can respond timely and accurately, especially if there has been no early warning and preparation time. Furthermore, as radioactive substances such as ^{210}Po naturally occur in our planet, they are present in the human body and it is a needed to know better the baseline levels in the population in order to decide what contamination is and what is not.

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