

## TESTING OF INDIVIDUAL SENSITIVITY TO RADON AND THORON EXPOSURE BY *IN VITRO* IRRADIATION OF LYMPHOCYTES CULTURE

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**ABSTRACT.** Identify the factors that affect individual sensitivity is a very important step in cancer risk assessment at low doses or low doses rates, according with study of dose-response relationship. DNA damage response processes are likely to play an important role in radiation by cancer risk association. This paper tries to quantify the individual sensitivity to alpha particles exposure using an in vitro exposure model. Quantify of DNA lesions by comet assay, in lymphocytes cultures exposed to the same doses of radiation was made. The irradiation was performed using the pitchblende ore during a 72 hours period, intermittent exposure with dose rates of 89.15 kBq/m<sup>3</sup>. The lowest and highest Radon Thoron concentrations computed in this study were 89.15 kBq/m<sup>3</sup> and 212.9 kBq/m<sup>3</sup>, respectively.

The study was done for healthy donors, comparatively with chronic obstructive pulmonary diseases donors. Ageing in vitro of lymphocytes cultures exposed to low doses of ionizing radiation is the biological model used, which are presented here. Based on these results, a standard test should be develop and applied for study of sensitivities in average population groups.

**Key words:** *individual sensitivity; low doses exposure model*

### INTRODUCTION

Radon is a ubiquitous gas and an indoor air pollutant in homes (Darby et al., 2005; PHE, 2014). Based on the evidences from human and animal exposures, the International Agency for Research in Cancer (IARC) has classified the radon as a group 1 carcinogen (IARC 2001). When is inhaled, radon 222 can have a carcinogenic effect on lung tissue, because of the emission of alpha particles upon decay (Hofmann et al., 1986; Fabrikant, 1990).

Our disease donors were patients with chronic obstructive pulmonary diseases (COPD). Individuals with respiratory diseases like COPD are especially sensitive to air pollution, and should be a vulnerable group to radon exposure, when the concentrations of the alpha particles from air are increased. When lung cancer cases were compared with controls, history of any previous lung disease was associated with a significant increased risk of lung cancer. Several lung diseases, including asthma, chronic bronchitis, pneumonia, and tuberculosis, were reported more often by lung cancer cases than by controls, and the difference was statistically significant for asthma, and chronic bronchitis (Wu et al., 1995; Turner et al., 2007).

Usually, the individual sensitivities were treated in relation with genetic factors, age, sex, nutrition, disease, and drug. As could be observed, the epidemiology studies, means the most directly way to estimate human risks to Radon, and often, is masked by confounding factors such as age, smoking, life style (Muirhead, 2008; Greenland, 2001; Stram and Kopecky, 2003) etc. Testing of individual sensitivities to Radon exposure could be a good point in environmental Radon studies, because the Radon toxicity is a certainty (Au et al., 1995; ATSDR, 2010). While the main low-dose risk is currently assessed to be from cancer induction and, to a lesser extent hereditary effects, some non-cancer effects may also be of concern even at low doses (NAS, 1999).

Although high radon concentrations are associated to increased risk of lung cancer by both experimental studies and investigations of underground miners, epidemiologic studies of residential radon exposure display inconsistencies. In many cases, such extrapolations may either over or underestimate the risk (Barros-Dios et al., 2002; Sa`inz et al., 2008).

In order to have a real risk of environmental assessment to low doses and low dose rates of radon exposure, it is very important to know the individual susceptibility (HLE, 2008). In the low-dose region it is easily to assume that each increment of dose produce a directly proportionate increment in biological and/or health effect. Knowing of the dose – response relationship is necessary for testing of individual sensitivity to ionizing radiation. An in vivo exposure test couldn't be available direct, through human model exposure, so, in vitro methods, must be more efficiently for risk estimation in radiation protection, and need arose to conduct experiments using in vitro irradiation methods.

The cells human radiosensitivity was observed not only for a series of genetic disorders (Friedberg, 1978), but also for a wide range of cancers as a consequence of exposure to different genotoxic agents from environment (Ron, 1998; Scott et al., 1999; Leong et al., 2000).

In the present rapid and reliable tests are used for prediction of normal tissue responses to radiotherapy (Burnet et al., 1994; Barbera et al., 2000). The comet assay is a simple technique for quantification of low levels of DNA damage in individual cells, very sensitive, using the fluorescence microscopic method (Fairbairn et al., 1995; Singh et al., 1988; Pereira et al., 1988; Tice, et al., 2000; Møller et al., 2000; Brock and Tucker, 2000; Rosslar et al., 2006).

The present study tries to classify the individual sensitivity, by in vitro exposure method of the lymphocytes cultures, according with individual susceptibility to radiation. The exposure marker will be DNA damage, quantified by comet head and comet

tail. The irradiation was performed using the pitchblende ore during a 72 hours period, intermittent exposure with dose rates of  $89.15 \text{ kBq/m}^3$ . The lowest and highest activities computed in this study were  $89.15 \text{ kBq/m}^3$  and  $212.9 \text{ kBq/m}^3$ , respectively.

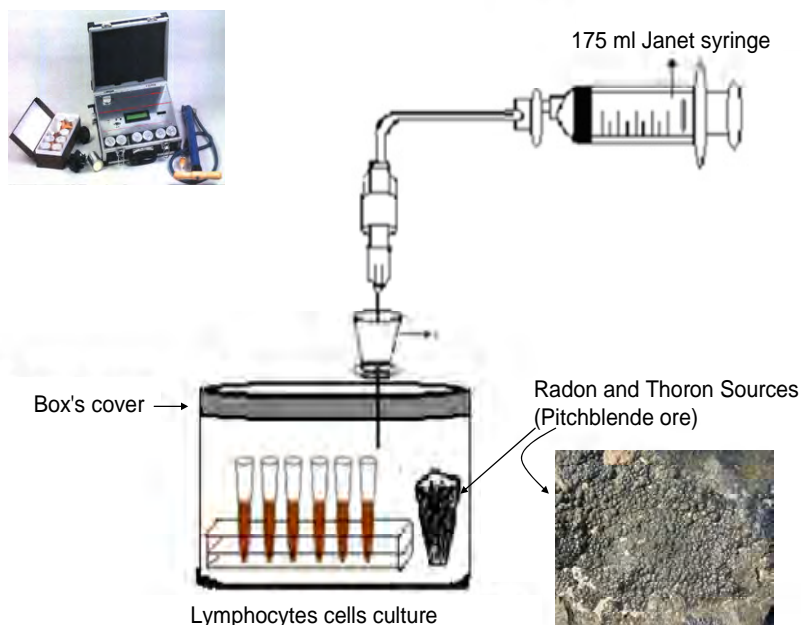
## MATERIAL AND METHOD

The study took place during May-July 2008.

### In vitro Irradiation System using Pitchblende Ore

Pitchblende ore ( $\text{UO}_3$ ,  $\text{U}_2\text{O}_5$ ,  $\text{U}_3\text{O}_8$ ), is a uranium ore. Is a radioactive ore, which contain also oxides of lead, thorium, and rare earth elements. The name came from pitch, because of its black color, and blende, a term used by German miners to denote minerals whose density suggested metal content, but whose exploitation was, at the time they were named, either impossible or not economically feasible. The natural airborne radioactivity results by disintegration of "parent-progeny", so-called "radon-thoron" (RnTn). These radioactive isotopes are the main contributors to the dose from natural radiation sources.

Using the Hamza' example (Hamza et al., 2008), we have done our particularly exposure system, figure 1.



**Fig. 1.** Assembly for in vitro irradiation, using pitchblende ore

## Estimation of radon activity

The Radon-Thoron gas radioactivity inside the experiment box, having 1000 ml capacity, figure1, was measured with Lucas cell scintillation, according with the recommended methodology (Plch, 1997; Eappen et al., 1980). The structure of this instrument is based on the Lucas cell principle, and can be used for Radon and Thoron content determinations in soil, water and for medium or high indoor RnTn concentrations (more than 200 Bq/m<sup>3</sup>).

A quantity of gas from the chamber (50 ml) was extracted using a Janet syringe and then this air was introduced in the Lucas cell of the LUK 3A, figure 1, device previously emptied and calibrated (Cosma et al., 2008).

The extracting operation was possible due a special valve in the upper part of the radon exposure boxes. The radon activity estimation was very well done, because in 2007, this method was included an intercompared experiment between laboratories, Chiba (Japan), when was apply for two different concentrations (Janik et al., 2009).

The airborne radioactivity was monitored after each day of exposure, during 72 h, when lymphocytes cells culture was kept inside of the experimental box, at 22°C. After 24 hours an exact volume of radon gas was counted immediately as well as after 3 h, being the time when radon progeny build-up is roughly completed and a state of transient equilibrium reached. The alpha particle counted was converted in Bequerel/m<sup>3</sup>, by using the equation 1:

$$C_{Rn} = N/V_s \cdot k, \text{ (eq. 1)}$$

where:  $C_{Rn}$  – radon concentration for indoor experimental box's air

$N$  – counted number of scintillation / second

$V_s$  – Janet syringe volume (175 ml capacity)

$k$  – means a constants, according with the used apparatus ( 1.38)

Radon, Thoron and their progeny, comprise a complex radiation source emitting alpha, beta and gamma particles, but when compared to the alpha dose, the others are negligible (Jostes, 1996). So, we assuming that the majority of exposure is due to alpha particle emitted to radon rather than the rest of progeny. The measured activity of pitchblende ore are presented in the next table 1 (figure 2).

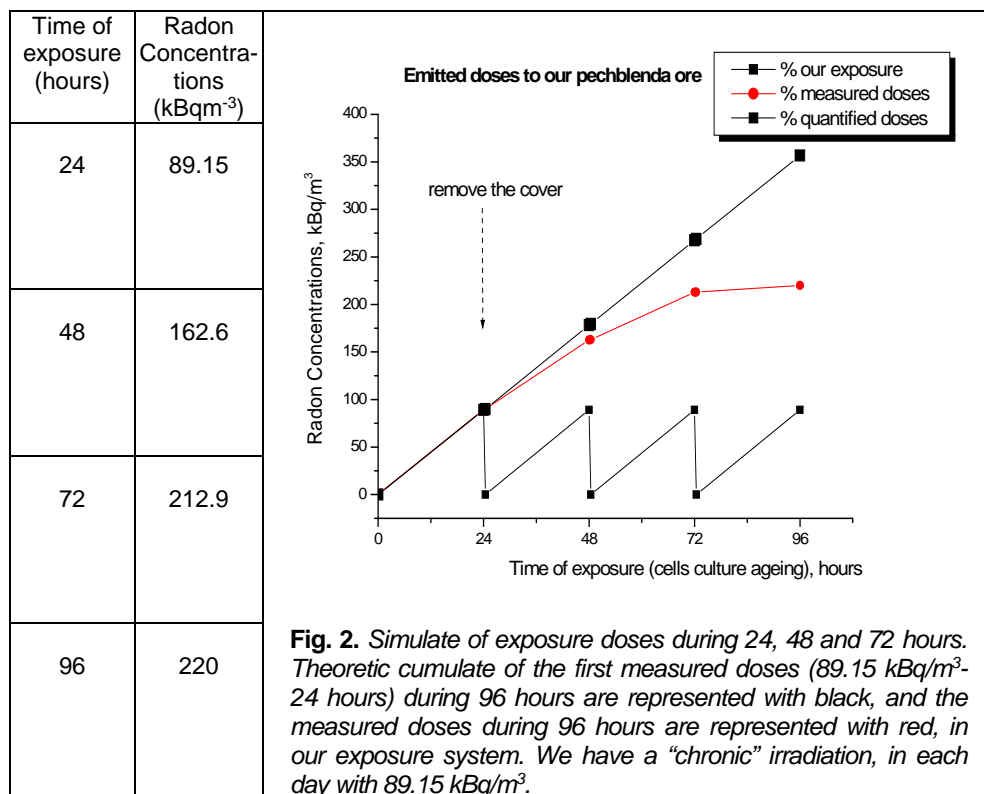
## Irradiation sample

10 ml of blood was taken from 3 healthy donors and 3 diseases donors by venipuncture, and were collected in heparin's vacuette tubes (Greiner Labortechnik, Austria). Blood donations come from volunteer donors and a safety protocol has been respected, according with Medical College Recommendations.

Lymphocytes are separated using the density gradient protocol, on Histopaque-1077. Briefly, blood is diluted 1:1 with PBS and layered over 600  $\mu$ l Histopaque and centrifuged at 800 X g for 20 minutes. The 'buffy' coat, an opalescent layer containing

mononuclear cells is aspirated into 3-5 ml of PBS and centrifuged at 250 X g for 10 minutes to pellet the lymphocytes and counted over a haemocytometer and viability tested. The pellet is resuspended in 1 ml of (RPMI + 15% fetal serum, + 10% glutamine + antibiotics) in test tubes. Aliquots of  $2 \times 10^6$  cells per 100  $\mu\text{L}$  of medium are taken for each dose of the test material.

**Table 1.** The irradiation doses during *in vitro* exposure,  $t = 22^\circ\text{C}$ , constant



Aliquots containing lymphocytes were diluted with PBS 1:10 and tubes are inverted to mix the cells and test material in order to obtain a homogenous suspension.

The cells cultures suspension was distributed equally, in three test tubes, for each sample donor (healthy respectively diseases). Than, were closed to irradiation in our experimental box, at room temperature ( $22^\circ\text{C}$ ). In each day, one test tube from each sample was analyzed.

### Study of genotoxicity using (SCGE, “comet assay”)

We performed the single cell gel electrophoresis after each day of irradiation, 24, 48, 72 hours respectively. The comet assay is a relatively novel technique based on electrophoresis migration of damaged DNA. With this assay, effects such as DNA single-strand breaks, incomplete excision repair sites and alkali-labile sites can be easily analyzed. The alkaline comet assay was performed according to Tice’s protocol, 2000; Collins, 2004 and modified after Brie et al., 2004.

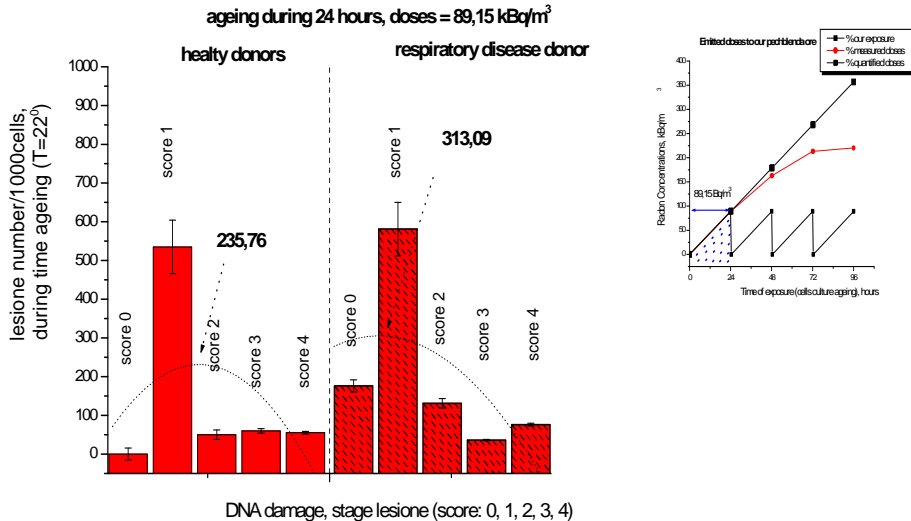
Inducted DNA damage quantification: Cells were scored with a Zeiss Axiolmager M1 Microscope using an image program acquisition with automated integration time adjustment, automated thresholding of cells ('head') and Comet tails, and measurement of cell features.

### RESULTS

DNA damage response processes are likely to play an important role in radiation-associated cancer risk. Also, a variety of less well understood epigenetic factors and non-targeted effects may also be involved.

#### After 24 hours of irradiation

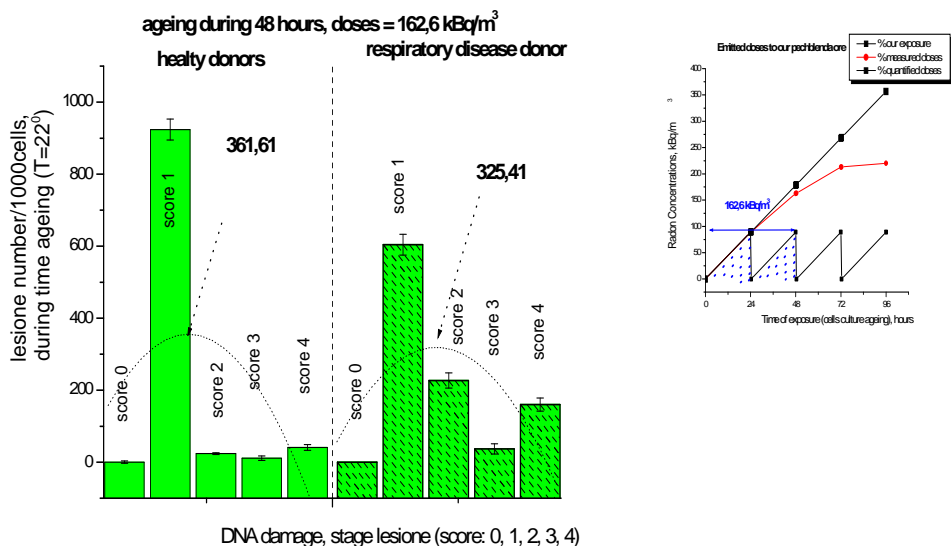
Could be observed the significantly increased number of Score1 lesions, both for healthy and disease donor with exposure, but not an important difference between, figure 3.



**Fig. 3.** Representation of cells number according with stage lesions (Score 0, 1, 2, 3, 4) after 24 hour of irradiation and fit polynomial analysis of the data points represented.

### After 48 hours of irradiation

Could be observed the significantly increased of lesions number with Score 1, both for healthy and disease donor with exposure, figure 4. Also, are presented the increase of the lesions with Score 2 and 4 for disease donor.

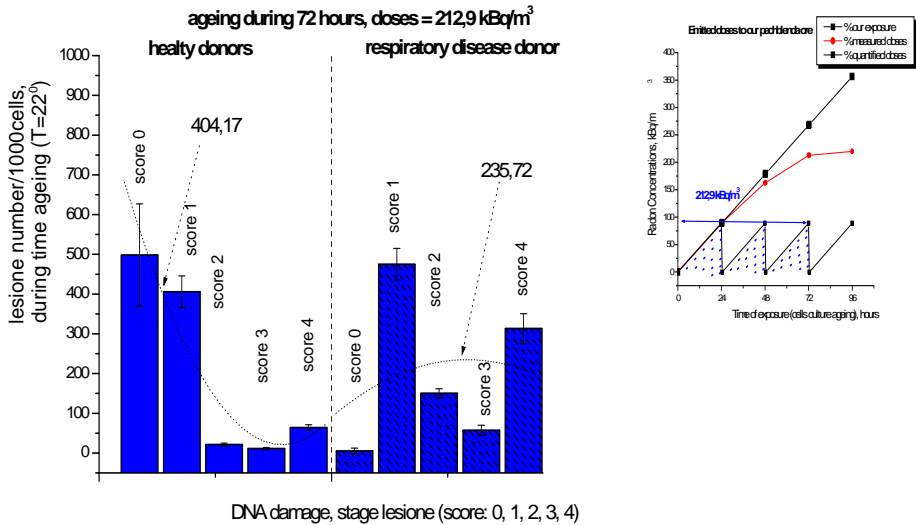


**Fig. 4.** Representation of cells number according with stage lesions (Score 0, 1, 2, 3, 4) after 48 hour of irradiation and fit polynomial analysis of the data points represented.

### After 72 hours of irradiation

We observed the significantly increased of lesions number with Score 1, both for healthy and disease donor with exposure, figure 5. Also, are presented the increase of the lesions with Score 2 and much more Score 4 for disease donor. The increased score 0, for healthy donor could be an artifact and must be verified again.

**The overall evaluation of DNA damage** was made through two parameters: lesions score (SL), figure 7, 8, 9 table 2, 3 and tail factor (TF), figure 10, 11, 12 and table 4, 5. The results are presented below.



**Fig. 5.** Representation of cells number according with stage lesions (Score 0, 1, 2, 3, 4) after 72 hour of irradiation and fit polynomial analysis of the data points represented.

### A) Score Lesions (SL) and Individual Sensitivity

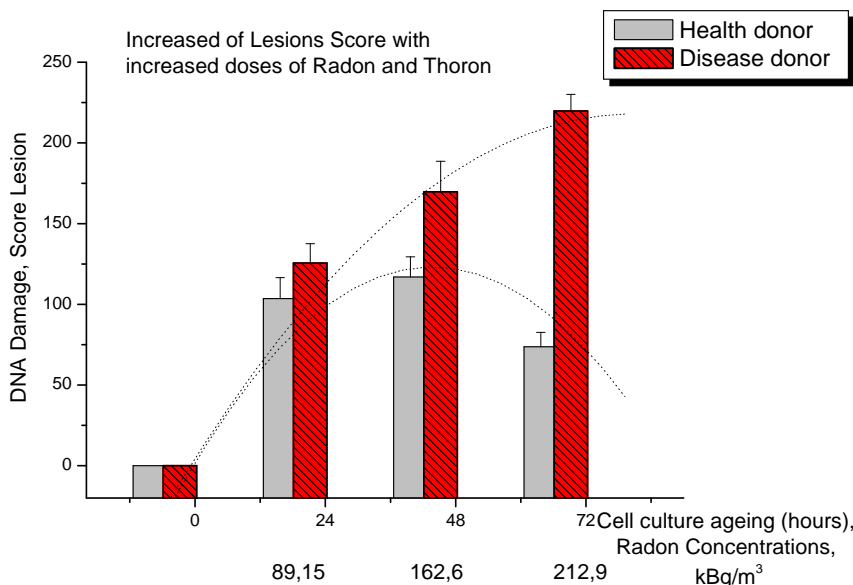
Have been registered the significantly increase of Score Lesions of DNA for the donors with chronic obstructive pulmonary diseases, figure 6. The individual sensitivity was estimated by induced DNA damage to lymphocytes cells, which has quantified according with the percent of induced effect, table 2.

**Table 2.** Induced effects in time, according with individual sensitivity, using as exposure marker the Score Lesion, SL

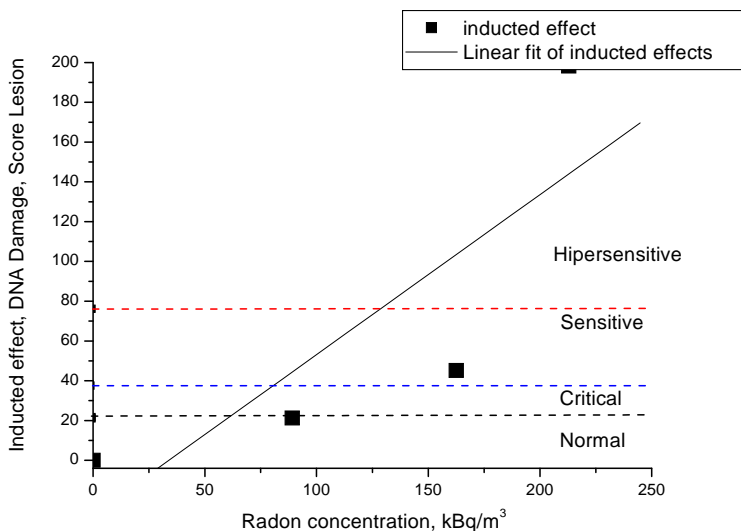
Score Lesions Healthy Donor (H)	Score Lesions Disease Donor (D)	Time of exposure	Radon Concentrations kBq/m <sup>3</sup>	Effect (%) (D-H)/H x 100
103.5	125.5	24 hour	89.15	21.25
116.9	169.7	48 hour	162.6	45.16
73.7	219.8	72 hour	212.9	198.23

Representing the best linear fit of induced effects, figure 7 and overlapping the graphic representation of score lesions, figure 8, was possibly to classify the individual sensitivity.

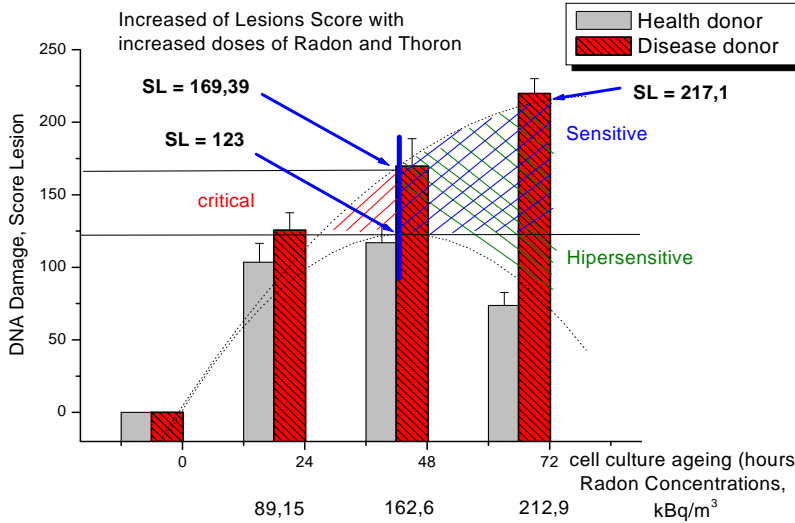




**Fig. 6.** The overall dependence of the Score Lesions with *in vitro* exposure doses of Radon and Thoron, and the fit polynomial analysis of data points represented.



**Fig. 7.** Dependence of Individual Sensitivity according with exposure doses for *in vitro* cells culture, by quantify of Score Lesions. (Data points are the mean for two independent experiments; best linear fit is drawn. \* Statistically significant difference from control at  $p = 0.05$ .)



**Fig. 8.** Overlapping the linear fit of induced effect with overall representation of score lesions

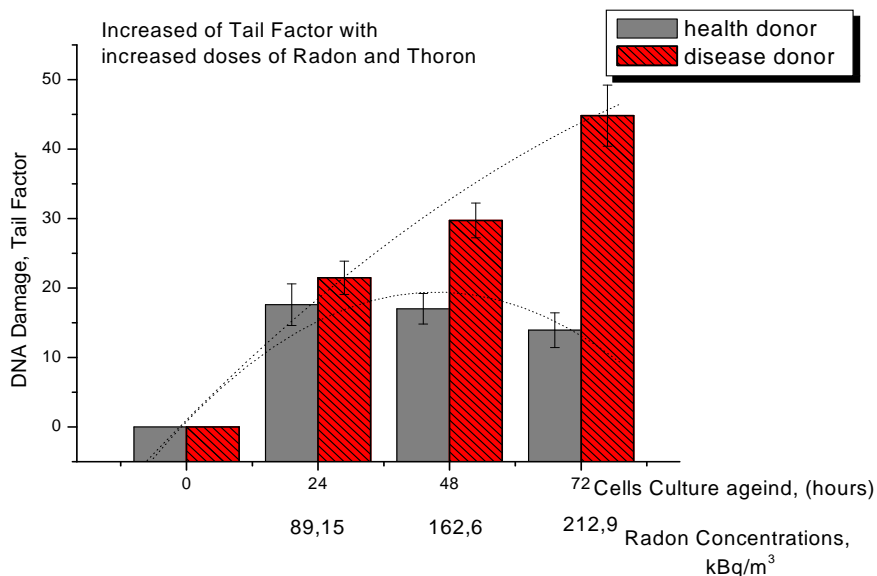
From figures 6, 7, 8 and table 2 have been possible to quantify the individual sensitivity, according with Score Lesions, which are represented in the below table 3.

**Table 3.** Inducted effect in time, according with individual sensitivity, using as exposure marker the Score Lesion, SL

Time of exposure	Inducted Effect, %	Individual Sensitivity Characterization according with Score Lesions
24 h	21.25	Normal
	> 21.25	Critic
48h	21.25 – 37.71	Critic
	> 37.71	Sensitive
	>76.50	Hipersensitive

**B) Tail factor (TF) and Individual Sensitivity**

In a similar way, we quantified the individual sensitivity using as exposure marker the Tail factor. In figure 10, have been represented the overall dependence of Tail Factor with *in vitro* exposure doses of Radon and Thoron. Have been registered the significantly increase of Tail Factor to donors with chronic obstructive pulmonary diseases, figure 9.



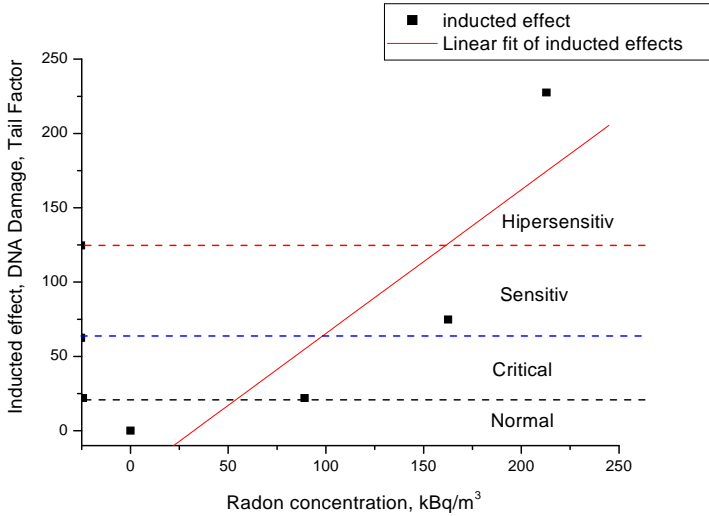
**Fig. 9.** The overall dependence of Tail Factor with *in vitro*, exposure doses of Radon and Thoron and the fit polynomial analysis of data points represented.

The individual sensitivity was estimated by induced damage effects to DNA, for lymphocytes cells irradiated, quantification being in accord with the percent of induced effects, table 4.

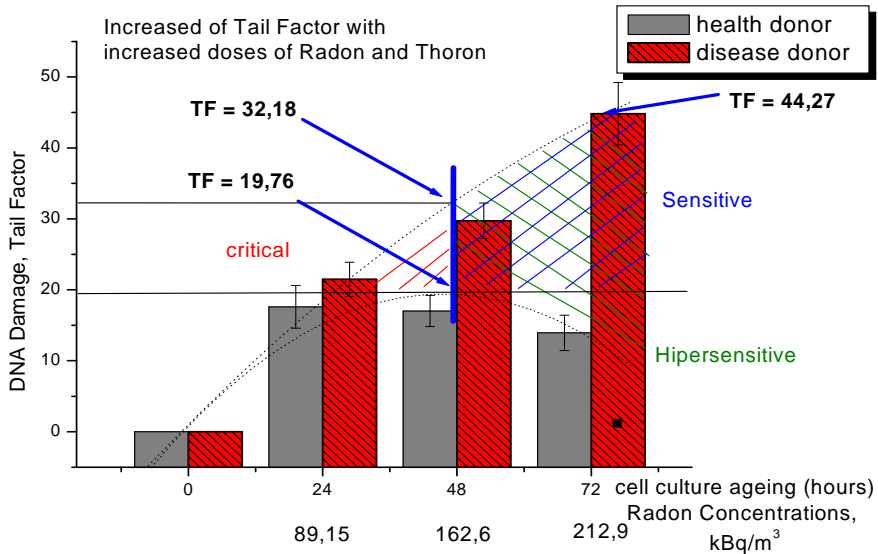
**Table 4.** Induced effects in time, according with individual sensitivity, using Tail Factor (TF) as exposure marker

Tail Factor Healthy Donor (H)	Tail Factor Disease Donor (D)	Time of exposure	Radon Concentrations kBq/m <sup>3</sup>	Effect (%) (D-H)/H x 100
17.6	21.47	24 hour	89.15	21.98
17.01	29.73	48 hour	162.6	74.77
13.93	44.82	72 hour	212.9	227.516

Representing the best linear fit of induced effects (TF), figure 10 and overlapping the graphic representation of tail factor, figure 11, was possibly to classify the individual sensitivity, table 5.



**Fig. 10.** Dependence of Individual Sensitivity according with exposure doses of *in vitro* cells culture, by quantify of Tail Factor. (Data points are the mean for two independent experiments; best linear fit is drawn. \*Statistically significant difference from control at  $p = 0.05$ .)



**Fig. 11.** Cell sensitivity representation according with Tail Factor. Overlapping the linear fit of inducted effect with overall representation of Tail Factor

From figures 10, 11, 12 and table 4 have been possible to quantify the individual sensitivity, according with Tail Factor which are represented in the below table 5.

**Table 5.** *Inducted effect in time, according with individual sensitivity, when the exposure marker is the Tail Factor*

Time of exposure	Inducted Effect (%)	Individual Sensitivity Characterization according with Tail Factor
24 hour	21.98	Normal
	> 21.98	Critic
48 hour	21.98 – 62.85	Critic
	62.85 – 124.03	Sensitive
	> 124.03	Hipersensitive

**The individual sensitivity**, using in vitro exposure to alpha particle emitted from Radon and Thoron, should be appreciated, already after 48 hour, from A) and B), when have been registered, the significantly effects.

## DISCUSSION

Studies in vitro, about Radon toxicity, were often signaled (Purrot et al., 1980; Edwards et al., 1980; Pohl-Ruling and Pohl, 1990; Jostes et al., 1991; Jostes 1996; Petitot et al., 1997; Pohl-Ruling et al., 2000; Hamza et Mohankumar, 2009). Hamza Z and her collaborators in 2009, using a similar in vitro model has observed the dicentric aberrations in lymphocytes, healthy donors, from the irradiated blood with Radon, for the next doses, table 6:

**Table 6.** *Our results (irradiation with alpha particle emitted to pitchblende ore) comparative with Hamza results (irradiation with alpha particle from a Radium sources, with an 98.6 kBq activity)*

Our Conc. of alpha particle, kBq/m <sup>3</sup>	Hamza's Conc. of radon, kBq/m <sup>3</sup>	Radon Dose Estimated (Hamza) (mGy)	DC/cells yield dicentrics Hamza's Experiment	Our Inducted effect (%), DNA Damage, SL Score Lesions	Our Inducted effect (%), DNA Damage, TF Tail Factor
89.15	89.3	7.14	0.0052	21.25	21.98
	112.34	8.98	0.0057		
162.6				45.16	74.77
212.9	216.10	17.288	0.0073	198.23	227.516
	424. 132	33.93	0.01020		

Correlate, our DNA damage could be associated with induction of chromosome aberrations, in irradiated sample. DNA damage after the irradiation must be more prominent because the cells ageing, was amplified at 22°C, a temperature not properly, for cells divisions process, even the cells was kept in a culture medium. According with our experiment, the dose–response should be different between donors, due to interindividual variations. Also, a significantly difference was observed between healthy and diseases donors, for low irradiation doses of Radon, by association, between 7.14 mGy -17.28 mGy respectively. We consider that donors with respiratory diseases, are especially sensitive to radon exposure, and represent a vulnerable group, with an increased lung cancer risk. The increased of lymphocytes lesions number reflected between Score Lesions (45.16 %, after 48 hours) and Tail Factor (74.77%, after 48 hours) represent a really evidence of their vulnerability.

Personal exposure to Radon varies, depending on the concentrations present in homes or in the occupational environment. Bauchinger et al., in 1994 has done a biodosimetric study about environmental exposure. According with their research, the domestic exposure to radon, in some houses was possible to induce an increased number of chromosomes aberrations at very low doses and dose-rates (Bauchinger et al., 1994). The similarly studies was reported to Zhou et al., 2001, and Mihalache et al., 2007.

Although high radon concentrations are associated to increased risk of lung cancer by both experimental studies and investigations of underground miners, epidemiologic studies of residential radon exposure display inconsistencies. In many cases, such extrapolations may be either over or underestimate the risk (Stram and Kopecky, 2003; Muirhead, 2008).

Comparing our in vitro irradiation Radon concentrations, with those from database, Darby and his col., 2005, has estimate the lung risk cancer, for lower concentrations, ~104 Bq/m<sup>3</sup>. Radon concentrations around 60 and 330 Bq/m<sup>3</sup>, have been reported to Friedmann et al.,1996, for Austrian people exposure, also, 280 Bq/m<sup>3</sup>, Cosma and col., 2007 in Stei region (Bihor county) Romania, in association with risk lung cancer incidence, or 4.6 kBq/m<sup>3</sup>, Lubin et al., in 1997, like an association with lung cancer mortality residential radon exposure.

Lettner and colab. in 1996, have been reported similarly Radon concentrations, up to 100 kBq/m<sup>3</sup>, in a thermal gallery.

Results obtained from ecologic studies have the inherent problem that conclusions are subject to the ecologic fallacy. The validity of individual risk estimates based on group data is not known and cannot be reliably determined from an ecologic study design. The ecologic study is primarily designed for generating hypotheses. Testing the hypothesis, assessing the validity of the association, and obtaining reliable estimates of the exposure-response relationships require independent testing by individual-level study designs having personal exposure measures, as well as individual health data.

The lowest radiation dose associated with statistically significant increased risk remains controversial. Current understanding and quantification of risk at low doses is limited by the uncertainties of the available scientific methods and by a lack of understanding of the basic biological mechanisms. Epidemiological studies

are not powerful enough to detect risks at doses approximating 1 cGy in the general population, because the necessary large populations are not available. The published data that have been used to estimate low-dose risks are often equivocal. In evaluating risks at small doses, all published studies need to be considered unless there are scientifically defensible reasons for exclusion. Although unequivocal evidence of risk is unavailable at very low doses, this does not mean that increased risks do or do not exist. However, if there is a risk below 1 cGy, it is very small for any given individual – the controversial issue being the risk to a large population potentially exposed to these small risks (Brenner and Mossman, 2005).

Studies based on individual sensitivities in estimates of exposures should generally be more informative if the aim is to quantify a dose-response relationship, in radiation protection dosimetry. According to Mezei and Kavet (2002) risk estimates are applicable only to subjects or groups of subjects with various characteristics who are at risk of developing cancer rather than to cases that already developed cancer (Mezei and Kavet 2002).

Four basic model options on low dose response tend to be considered following exposure of the whole body or of individual tissues: linear-no-threshold, linear but with a zero-effect interval below a given threshold dose, supralinear (hypersensitivity), or more complex bi-modal relationships (including beneficial health effects or hormesis at low doses) (UNSCEAR, 2000; CERRIE, 2004; NRC, 2006; French Academy, 2005; ICRP, 2007). According with our results, studies about Low-Dose Risk Extrapolation should be associated with individual sensitivities, so: normal – hormesis; critic – threshold; Sensitive – LNT and hypersensitive – Hypersensitive.

The setting of dose constraints can take account of individual variability in radiation response. So, understanding the cellular mechanisms of carcinogenesis is increasingly important to assess the biologic risks.

The next appeared problem will be to correlate the observed sensitivities between the individuals, or groups. Only knowing the spread of sensitivities in average population groups, could have realistic estimates of radiation risk.

## CONCLUSION

The result of cell's irradiation combined with ageing process is an increase of DNA damage. For estimation of induced DNA lesions, the comet assay is a very properly and sensitive test.

A great difference in response to irradiation has been observed between healthy and chronic obstructive pulmonary diseases donor, using comet head and comet tail as exposure markers. The quantifications of DNA damage on the lymphocytes cultures expose in vitro, to different concentrations of Radon and Thoron, intermittent exposure with dose rates of 89.15 kBq/m<sup>3</sup> revealed a significantly increasing of induced lesions, for disease donors, which has been classified as hypersensitive. Also, this donors represent a vulnerable group with an increased risk in lung cancer apparition, according with our results, the aspects of dose–response relationship being a really evidence.

In conclusion, the individual sensitivity could be assessed after 48 hours, using our in vitro model. The sensitivity was proportional estimated with individual susceptibility to radiation, by induced effects to DNA level. This sensitivity has been classified as: normal, critic, sensitive and hypersensitive individuals.

This model must be combined with analysis of environmental Radon concentrations (exposure assessment) and epidemiologic studies, epidemiological cohorts of populations (uranium miners, nuclear workers, medically exposed groups, residential radon exposures, etc.) potentially informative for low dose risk research. At present there is insufficient information to establish how large these various differences in sensitivity may be between individuals or between groups of individuals both in the sizes of the variations and also in the proportions of the population that are affected with consequent on risk estimates at low dose.

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