BABEŞ-BOLYAI UNIVERSITY

FACULTY of ENVIRONMENTAL SCIENCES

PhD THESIS
(Summary)

MODELS FOR THE ASSESSMENT OF LUNG CANCER RISK

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CONTENTS

Key words: Transformation Frequency-Tissue Response model (TF-TR), radon, lung cancer, risk, exposure, dose-response relationship, hot spots, direct and non-targeted effects, radon-smoking synergism.

INTRODUCTION

The main radioactive element contaminating the human environment is radon (^{222}Rn). Radon is a radioactive gas, naturally occurring from radium decay, in the uranium series, which is ubiquitous in soils and rocks, in variable amounts (BEIR VI, 1999; **Truţă-Popa**, Cosma, 2009). Radon seeps out easily from the soil into outdoor air and also into the air in homes, decaying into its short -lived products called radon daughters or progeny (Alpen, 1998; BEIR, 1999). The International Commission on Radiological Protection (ICRP, 1981) estimated that almost half of the total effective dose received by the people of the USA, from all sources of ionizing radiation is attributed to radon ^{222}Rn and its short-lived decaying products (^{218}Po, ^{214}Pb, ^{214}Bi and ^{214}Po). This proportion is variable, and in Romania it counts for about 57% (Iacob and Botezatu, 2000). Radon and its short-lived progeny are inhaled and, while radon itself is generally exhaled immediately after inhalation, its short-lived progeny- which are solid elements deposit on the surfaces of the bronchial epithelium, thus exposing sensitive cells to alpha radiation (BEIR VI). Indeed, the significant dose contribution to the lung is not from radon, but from its short-lived progeny (Alpen, 1998). The alpha particle hits damages cell's DNA that can result in mutations in genes that control certain behaviors, such as growing of the cancer cell and its uncontrolled division. thus leading to cancer. Radon is the second cause of lung cancer, after smoking. Lung cancer is the world's most common cancer, and the second cause of death, after coronary heart disease (Ferlay et al, 2007). This facts, in addition to the numerous epidemiologic indoor and underground miner studies on radon exposure, prompted the International Agency for Research on Cancer in 1988 to classify radon as a human carcinogen. In 1986, the USA Environmental Protection Agency (EPA, 1992) established an "action level" for indoor radon exposures of 4 pCi/ Litre of air (160 Bq/m^3).

Radon concentration measurements would not have a significant outcome for the general population if they weren't processed, analysed and interpreted through some radiation carcinogenesis models, so that the health risk induced by a certain exposure be assessed as rigurous as possible. The correct quantification of this risk is of vital importance, both for occupational and residential exposures, for radioprotection purposes and the consequences on human health. The biological effects caused by exposure to ionizing radiation, particularly radon, are the result of a complex series of physical, biological and physiological interactions (Kotecki, 1998). These interaction may depend upon factors such as the type of radiation, the lduration and intensity of exposure and the nature of the biological system implied (Mebust et al., 2002). One of the fundamental tools of radiation biology is a formalism describing these interactions as dose-response and time-dose relationships (Fleishman, 2004). During the last decade, the

carcinogenesis theory was marked by the report of an interactive relationship betweeen experimental observations and modeling efforts (Fleishman, 2004).

The effect of single and multiple alpha particle intersections of bronchial cells during a given exposure period, on radon-induced lung cancer risk, was simulated by a Transformation Frequency – Tissue Response model (TF-TR) based on experimentally observed cellular transformation and survival functions that represents a simplified version of the SVM of radiation carcinogenesis.

The TF-TR model is biologically motivated and successfully fitted sets of representative data. In the initiation sub-process, it represents an important step in the study of neoplastic transformations, *in vitro,* and cancer.

CHAPTER 1.

INTERACTION OF IONIZING RADIATION WITH LIVING MATTER

This chapter was structured in two subchapters, i.e.: ***Fundamental concepts in radiation physics*** and ***Principles of general carcinogenesis***. First of these subchapters contains information regarding: ***ionizing radiation***, ***radon source*** and ***mitigation***, a concise description of the way alpha particles emitted particularly by radon short-lived progeny deposited on the bronchial epithelium can cause cancer. Introduction of ***dosimetric concepts***, ***quantities and units*** was also necessary to be presented in this subchapter, along with: ***units specific to radon dosimetry***, information on ***radionuclide distribution***, ***homogeneity of the composition*** and ***the value of absorbed dose***, analysis of ***lung cancer risk extrapolation from miners to general population***, ***equivalent exposure units for miner and residential studies***, and also ***comparative dosimetry for homes and mines***, ***estimation of F factor.***

The second subchapter contains, as could be deduced from the title, principles of general carcinogenesis, including concepts that are absolutely necessary in approaching the lung cancer risk, such as: indicators used in cancer epidemiology (incidence, mortality, surviving ratio), epidemiology-in general and analitic epidemiology, particularly, fenotipic characteristics of tumor cells, the stages of tumor development (tumoral proliferation, local invasion and angiogenesis), about malign and bening tumors and conversion from benign adenoms to neoplasms in metastasis. This subchapter treats one of the most important aspect of the radon-induced lung cancer risk approach, i.e. the fundamental stages of radiation carcinogenesis: initiation, promotion and progression (with latency), but also reviews concepts like cellular proliferation, immune response, induction of apoptosis and radiotherapy.

CHAPTER 2.

MODELS AVAILABLE IN LITERATURE FOR THE ASSESSMENT OF RADON-INDUCED LUNG CANCER RISK

Chapter 2 reviews ***Models available in literature for the assessment of radon-induced lung cancer risk*** and presents a history of radiation carcinogenesis model, along with concept that are fundamental when studying the issue of the thesis, i.e.: ***risc measurement units*** (Relative Risk, Absolute Risk and Excess Relative Risk), ***approaches of risk estimation*** (empiric, dosimetric and mechanistic approach) and the original, novel element: the ***Transformation Frequency-Tissue Response (TF-TR)*** model we proposed, ***for the assessment of lung canecr risk induced by radon***. Taking into consideration the great volume of laborious calculations assumed by modeling, we facilitated an ***automatic processing of data***, for the possible situations, with Fortran programms we developed, and the details of this process are described in more detail in the last subchapter of chapter 2.

THE TRANSFORMATION-FREQUENCY TISSUE RESPONSE (TF-TR) MODEL PROPOSED FOR THE ASSESSMENT OF LUNG CANCER RISK INDUCED BY RADON

Transformation Frequency-Tissue Response (TF-TR) model, formely termed Initiation-Promotion (IP) is a mechanistic, biology-based model (Hofmann, **Truta-Popa**, Balásházy I, Fakir H, Crawford-Brown DJ, 2004; Hofmann, **Truţă-Popa**, Fakir H, 2006; **Truţă-Popa** et al., 2003 a, b; 2005; 2007; 2008; 2009 a, b), derived from the State Vector Model (Crawford-Brown and Hofmann, 1990; 1993). In this model, lung cancer risk R(D) is expressed as the product of the transformation frequency (TF) and tissue response (TR) functions, while dose is expressed in terms of single (n=1) and multiple (n >1) alpha particles (Hofmann, **Truţă-Popa**, Fakir H, 2006; **Truţă-Popa** et al., 2008, a). While oncogenic transformation is assumed to be the primary initiation step, stimulated mitosis by killing adjacent cells is interpreted as the necessary radiological promotion event (Hofmann, **Truţă-Popa**, Balásházy I, Fakir H, Crawford-Brown DJ, 2004; Hofmann, **Truţă-Popa**, Fakir H, 2006; **Truţă-Popa** et al., 2003 a, b; 2005; 2007; 2008, a; 2009, a, b).

Transformation Frequency (TF)

TF(D) and TR(D) functions were derived from experimentally observed *in vitro* oncogenic transformation and survival data for C3H 10T1/2 mouse cells exposed to charged particles of varying LET (Bettega et al., 1992; Miller et al., 1995) and rat tracheal epithelial (RTE) cells irradiated with ^{241}Am alpha particles (Kugel et al., 2002; Poncy et al., 2002). The LETs of the charged particles used in both experimental studies correspond to the LET spectrum

of radon progeny alpha particles in bronchial epithelium. The transformation data, i.e. the transformation frequencies per surviving cell (TFS), were expressed as a linear function of dose, αD, and the corresponding survival data were fitted by an exponential function, exp(-γD), where the coefficient α was obtained by fitting the above experimental transformation and survival data and γ represents the cell killing probability. In the present study the value of 1.298 reported by Kugel (2002) was assumed for the cell killing probability (γ) because it corresponds to epithelial lung cells which are the target cells for cancer induction. While *in vitro* transformation results are expressed as transformation frequencies per surviving cell (TFS), *in vivo* exposures per exposed cell (TFE(D)) are required for human lung cancer predictions (Hofmann, **Truţă-Popa**, Fakir H, 2006; **Truţă-Popa** et al., 2008, a). The relationship between these two transformation frequencies is given by:

$$TFE(D) = TFS(D) \cdot \exp(-\gamma \cdot D) = (\alpha \cdot D) \cdot \exp(-\gamma \cdot D)$$

Tissue Response (TR)

This step relates in-vitro transformation in single cells to in-vivo transformation in tissue, i.e. in a stem cell system. First, the transition from in-vitro mitotic rates λ_2 (1 day^{-1}) to in-vivo mitotic rates λ_1 (1/30 day^{-1}) has to be considered (**Truţă-Popa** şi colab., 2009, b).. Second, the transition from single cells in a Petri dish to a stem cell system which is characterized by the replacement of radiation-induced cell killing of neighboring cells by stimulated division of stem cells, which increases λ_1 (30 days cycle time) to λ_2 (one day cycle time),

$$TR(D) = \{\lambda_1 + p \cdot \lambda_2 \cdot [1 - \exp(-\gamma \cdot D)]\} / \lambda_1,$$

where

- λ_1 is the normal mitotic rate of lung cells, equivalent to a cycle time of 30 days (BEIR VI, 1999) ($\lambda_1 = \frac{1}{\tau}$, where τ is the cell cycle duration, $\lambda_1 = \frac{1}{30} = 0.033/day$), which may increase to λ_2, the rate of division under conditions of extensive tissue damage and cellular replacement, corresponding to a cycle time of approximately one day (λ_2=1/day)
- p denotes the probability that a progenitor cell will divide as a direct result of the inactivation of an adjacent epithelial cell (**Truta-Popa** et al. 2008, a). It was assumed here that p = 1, i.e. each dead epithelial cell would force a stem cell to divide (Hofmann, **Truţă-Popa**, Fakir H, 2006; **Truţă-Popa** et al., 2008, a; 2009, b).

In other words, the TR function represents promotion, expressed as the possibility that a cell undergoes fixation of lesions from the initiation phase during division, *in vivo*, due to stimulated mitosis.

Single and multiple cellular traversals

Epidemiological studies on lung cancer risk suggest that in cases of protracted exposures the crucial quantity for cellular radiation effects is the dose per cell cycle (Chadwick et al., 2003). Model predictions in these thesis were performed for a cell cycle time considered of 30 days (BEIR VI, 1999). While the number of cells hit increases with the average tissue or organ dose, the average dose received by the traversed cells remains constant until multiple alpha particle hits start playing a greater role (**Truţă-Popa** et al., 2008, a). This observation questions the applicability of average doses for alpha radiations at the cellular level, where radiobiological effects originate, at sufficiently low doses and thus average dose *(D)* was replaced by the frequency of single and multiple alpha particle hits. For a given average number of cellular hits ($\overline{H}$) per cell cycle time during the total exposure period *(T)*, equivalent to the average dose (*D)*, the actual number of single and multiple hits (P_n), delivering an average dose (D_n) to a traversed nucleus of a basal or secretory cell during the lifetime of these cells, were selected from a Poisson distribution:

$$P_n = \frac{\overline{H}^n \cdot e^{-\overline{H}}}{n!}$$

$$\overline{H} = \frac{N_h}{N_0}$$

where: $N_h = \frac{D}{\overline{D_c}}$ is the number of hits, N_0 is the number of cell cycles in the exposure period considered (T), $N_0 = \frac{T}{\tau}$ and $\overline{D_c}$ is the mean cellular dose per single hit (Hofmann, **Truţă-Popa** and Fakir, 2006; **Truţă-Popa** et al., 2008, a, b; 2009, b). Formulated in terms of cellular hits, the TF-TR model thus simulates the random alpha particle intersections of bronchial cells during a given exposure period (Hofmann, **Truţă-Popa**, Fakir H, 2006; **Truţă-Popa** et al., 2008, a).

Radiation carcinogenesis model (TF-TR)

Considering all aspects discussed above, lung cancer risk simulated by the TF-TR model is given by:

$$R(D) = C \cdot \sum_{i=1}^{n} TFS(D_n) \cdot \exp(-\gamma \cdot D_n) \cdot \left[\{\lambda_1 + \lambda_2 \cdot p \cdot [1 - \exp(-\gamma \cdot D_n)]\} / \lambda_1\right] \cdot P_n$$

where

- C is a constant scaling factor relating the number of transformed cells at dose *D* to the occurrence of an observable bronchial tumor, derived from epidemiological studies.
- $D_n = n \cdot \overline{D_c}$. Am considerat α=1, γ=1.298 Gy^{-1} (Kugel şi colab, 2002) şi $\overline{D_c}$ =0.33 for cells with a nuclear diameter of 9 μm and an LET of 130 keV μm^{-1} (Hofmann, **Truţă-Popa**, Fakir H, 2006; **Truţă-Popa** et al., 2008, a; 2009, b).

The application of this equation to lung cancer risk is based on the assumption that there are no significant differences in the shapes of the transformation and cell killing functions between in-vitro and in-vivo irradiation conditions, although their relative values may differ significantly.

CHAPTER 3.

EFECTS OCCURED AS A RESULT OF THE INTERACTION OF ALPHA RADIATION WITH LUNGS

3.1. DIRECT CELLULAR EFFECTS

The objectives of the ”Direct cellular effects” chapter were three-folded: 1*) to explore the role of single and multiple alpha particle hits in bronchial epithelial cells exposed to alpha particles emitted from the short-lived radon progeny ^{218}Po and ^{214}Po,* 2) *to relate the frequency of alpha particle hits to the resulting lung cancer incidence in the framework of a transformation-frequency-tissue response (TF-TR) model based on experimental cellular radiobiological effects* and 3*) to investigate the non-uniform distribution of alpha particle deposition in carenal ridges,* by integrating the hot spots effects in the model TF-TR .

3.1.1. Simulation of cellular alpha particle hits in lung cells and tissue volumes

At the tissue level, low doses of alpha particles are characterized by a relatively small number of cells traversed by alpha particles, although each nucleus of an intersected cell receives a rather high amount of energy (Hofmann, **Truţă-Popa**, Fakir H, 2006, **Truţă-Popa** et al., 2008, a). In contrast to low linear energy transfer (LET) radiations, where many, or even all cells receive a relatively low cellular energy, low dose effects of high LET radiations, such as alpha particles, are related to a small number of cells affected, while most cells are not hit at all (Hofmann et al., 2004).. While the number of cells hit increases with the average tissue or organ dose, the average dose received by the traversed cells remains constant until multiple alpha particle hits start playing a greater role (**Truţă-Popa** et al., 2008). Thus, the average dose *(D)* was replaced by the frequency of single and multiple alpha particle hits (see Chapter 2, details of TR-TR model). For a given average number of cellular hits per cell cycle time during the total exposure period, equivalent to the average dose (*D),* the actual number of single and multiple

hits delivering an average dose to a traversed nucleus of a basal or secretory cell during the lifetime of these cells, were selected from a Poisson distribution (Hofmann, **Truţă-Popa** and Fakir, 2006). Cellular alpha particle hits were assumed to be uniformly distributed throughout the whole exposure period reported in the epidemiological data used in this work (Hornung and Meinhardt, 1987; Darby et al., 2006; Monchaux, 2004; Tomasek et al., 2008).

A fundamental role in the assessment of lung cancer induced by low radon exposures is the biological effect of a single alpha particle, that is why we used the Poisson distribution in the formulation of the model, applied to the selected dose-response experimental data. This method allows the assessment of lung cancer risk (proportional to the oncogenic transformation frequency) as a function of average number of cellular alpha particle hits.

The contribution of single and multiple cellular hits to carcinogenic risk is illustrated in Figure 1 for transformation frequencies, representing the initiation step.

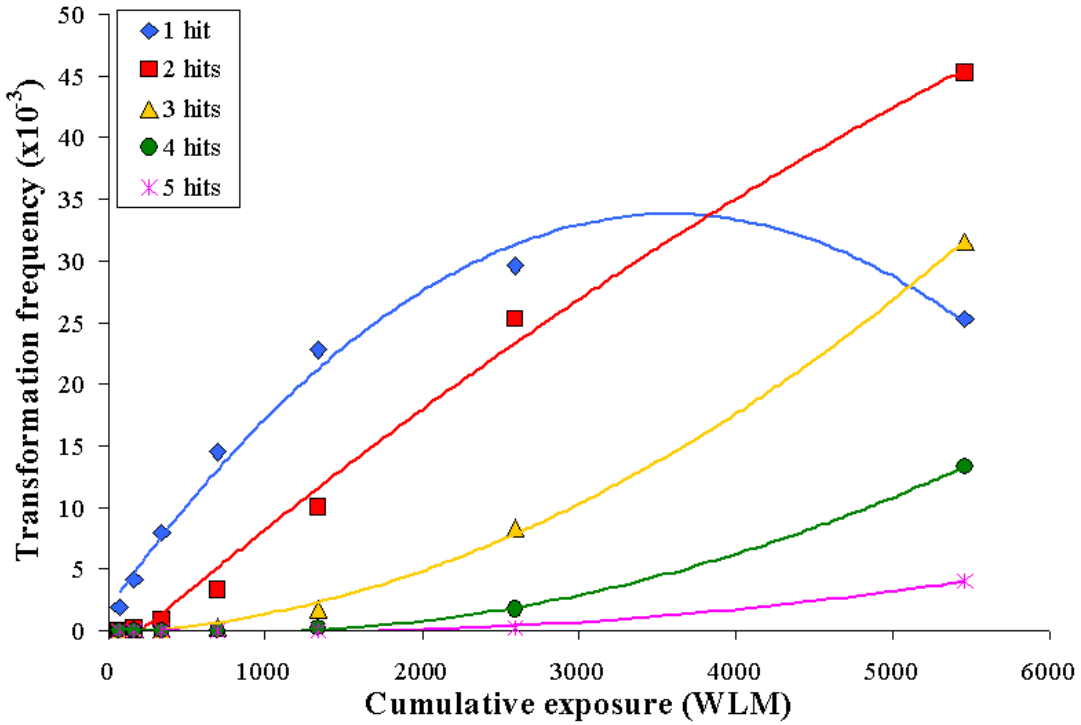

Figure 1. *Transformation frequencies (TF), arising from single and multiple cellular hits, plotted as functions of the cumulative exposure*

While single hits produce a linear dose-response relationship at low radon exposure levels (see Figure 1), multiple hits may play an increasing role at higher cumulative exposures, eventually leading to a nearly linear dose-response relationship over the whole exposure range (Hofmann, **Truţă-Popa**, Fakir H, 2006). If, however, multiple hits are required for cancer initiation (Brenner, 1992), then a sublinear dose-effect curve may be expected in the low exposure range.

Predicted transformation frequencies due to multiple hits rise in a sublinear fashion with increasing number of hits, consistent with experimental data from micro-beam experiments (Miller et al., 1999).

To consider potential bystander effects, which have been observed in cellular *in vitro* studies (Sawant et al., 2001, a) alpha particle interactions were also simulated for larger sensitive target volumes in bronchial epithelium, consisting of a collection of cells, thereby increasing significantly the number of multiple hits.

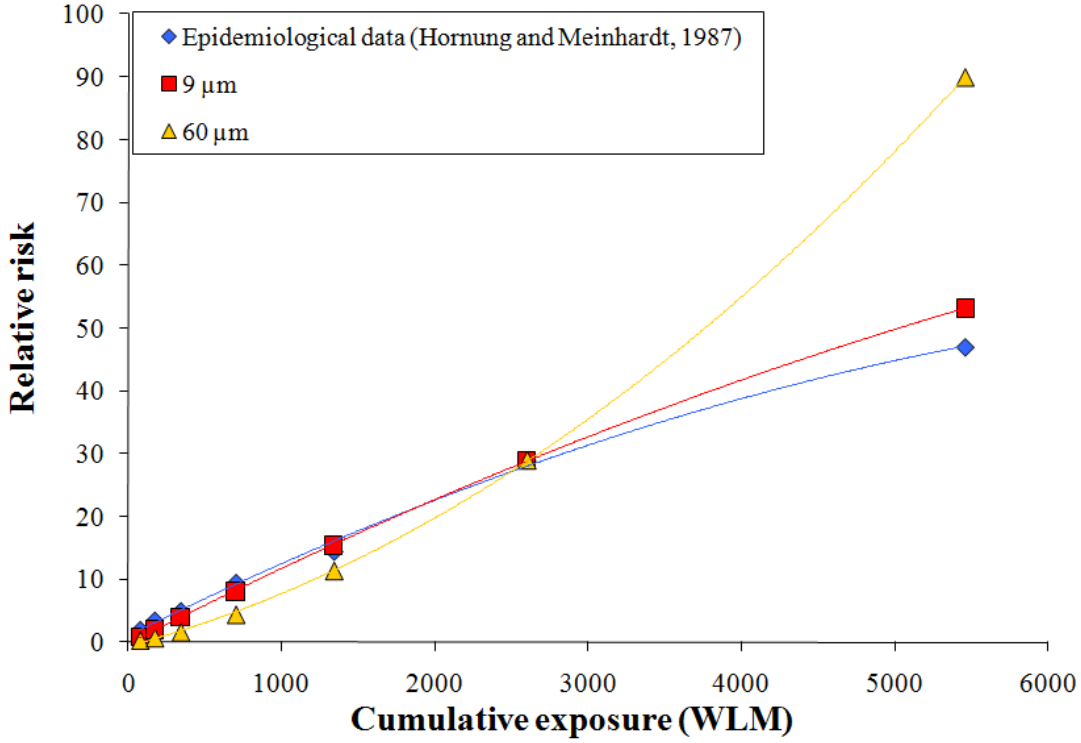

***Figure 2**. Comparison of predicted lung cancer risk between single cells (9 µm diameter) and a collection of these cells in a spherical tissue volume of 60 µm diameter.*

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Lung cancer risk predictions for a collection of cells within a spherical tissue volume with 60 µm diameter are compared in Figure 2 with the predictions for single cells (Hofmann, **Truţă-Popa** and Fakir, 2006). As illustrated in Figure 1, a higher number of multiple hits in this larger tissue volume produce a sublinear response, eventually leading to a linear-quadratic response over the whole exposure range. However, the excellent agreement of the predictions for single cells with the epidemiological data (Figure 2), suggests that bystander effects, if operating at all under *in vivo* conditions, may be restricted to a small number of adjacent cells (Little şi Wakeford, 2002).

3.1.2. The role of the multiplicity of cellular hits by radon progeny alpha particles for lung cancer incidence

To explore the role of the multiplicity of cellular hits for lung cancer incidence, the number of single and multiple alpha particle hits were computed for basal and secretory cells in

the bronchial epithelium of human airway bifurcations employing Monte Carlo methods (Crawford-Brown and Hofmann, 1990). In the present study, two characteristic exposure conditions were investigated: (i) a lifetime residential indoor exposure of 20 Working Level Months (WLM) (BRER, 1991), and (ii) a cumulative exposure of 578.6 WLM over a 4 year working period, representing the average exposure measured in Colorado mines (BEIR VI, 1999). The surface source densities of alpha particle emissions were: 3.3 x 10^4 (^{218}Po) and 3.9 x 10^4 (^{214}Po) alpha particles per cm^2 for residential chronic exposure, and 1.5 x 10^4 (^{218}Po) and 2.2 x 10^4 (^{214}Po) alpha particles per cm^2 for occupational uranium miner exposures, normalized to a cumulative exposure of 1 WLM (Hofmann et al., 2000). Microdosimetry calculations, i.e. number of hits and cellular doses, were performed for spherical cell nuclei of 9 μm diameter, located at 20 μm (secretory cells) and 40 μm (basal cells) depth in bronchial epithelium of the airway generations 3-4 bifurcation (Mercer et al., 1991), which is representative of the bronchial region where most bronchial carcinomas have been observed (Hofmann, **Truţă-Popa** and Fakir, 2006).

TAB. 1. Number of nuclear hits in target secretory and basal cells in bronchial epithelium at locations T, R_1 and R_2 of a symmetric bifurcation model, for both indoor and miner exposures (Hofmann, **Truţă-Popa** and Fakir, 2006).

Location	Depth	Number of hits			
		20 WLM, 70 yrs		578.6 WLM, 4 yrs	
(μm)		Uniform	Non-uniform	Uniform	Non-uniform
T	20	1.5×10^{-3}	1.9×10^{-2}	4.2×10^{-1}	5.0
	40	6.0×10^{-4}	7.6×10^{-3}	1.7×10^{-1}	2.1
R_1	20	8.5×10^{-4}	7.2×10^{-5}	2.3×10^{-1}	2.0×10^{-2}
	40	3.1×10^{-4}	2.6×10^{-5}	8.5×10^{-2}	7.6×10^{-3}
R_2	20	9.6×10^{-4}	6.5×10^{-4}	2.6×10^{-1}	1.8×10^{-1}
	40	3.5×10^{-4}	1.5×10^{-4}	9.7×10^{-2}	4.2×10^{-2}

Hit numbers refer to individual cells with a cycle time of 30 days irradiated over a given cumulative exposure period (Hofmann, **Truţă-Popa** and Fakir, 2006). The total activity of alpha-emitting radon progeny within the bifurcation is the same for both uniform and non-uniform distributions (Hofmann, **Truţă-Popa** and Fakir, 2006). Even in the case of a uniform activity distribution within the bifurcation, hit numbers differ among the three selected positions due to the varying contributions from the opposite wall surfaces (Hofmann, **Truţă-Popa** and Fakir, 2006).

In case of non-uniform distributions, it is the distance of the target cells from the activity accumulations at the carinal ridge which causes the predicted differences in hit numbers at the three sites (Hofmann, **Truţă-Popa** and Fakir, 2006).

3.1.3. Epidemiological data used and the results predicted by TF-TR model, based on direct alpha particle hits

The epidemiological miner data selected for this study were:

1) the ones reported by Tomasek and colleagues (2008) for the investigation of cellular effects occuring at low radon exposure,

2) the Colorado Plateau uranium miner data raported by Hornung and Meinhardt (1987) for the analysis of hot spots effect occuring at high radon exposures. These epidemiological data were compared to theoretical predictions of the model for the validation of this model, and the results were in excellent agreement (Figure 3 and 4).

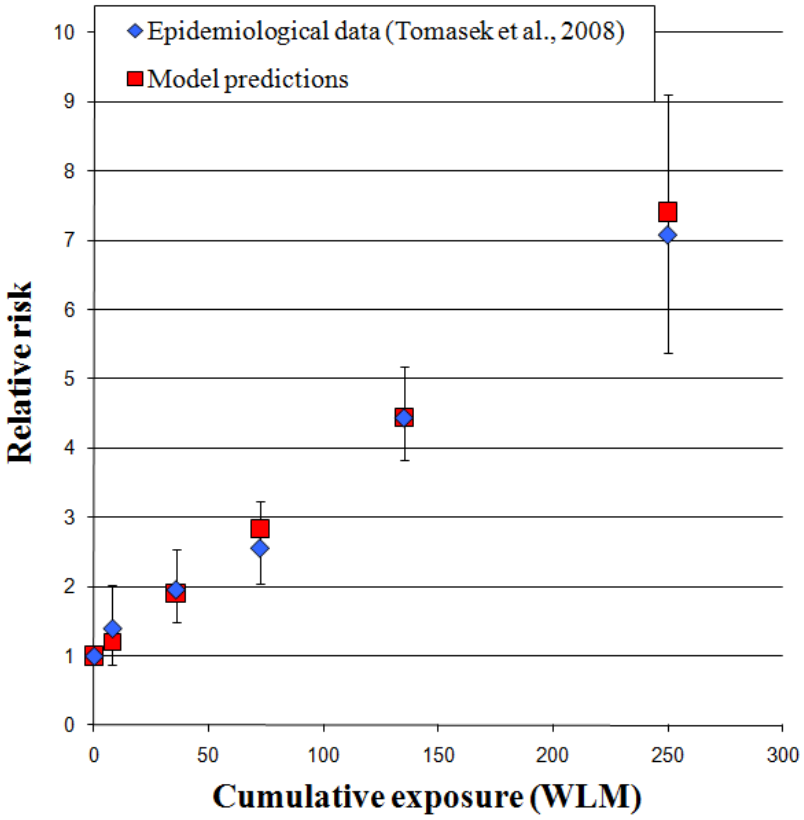


Figure 3. *TF-TR model predictions compared to the epidemiological data reported byTomasek et al. (2008) for the Czeck miners*

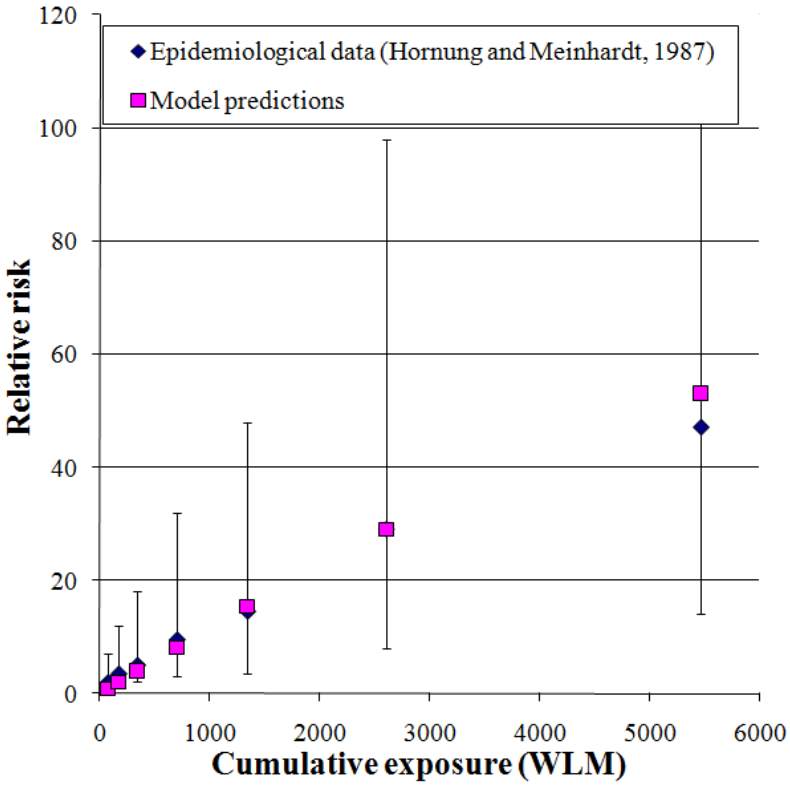


Figure 4. *TF-TR model predictions compared to the epidemiological data reported by Hornung and Meinhardt (1987) for the Colorado Plateau uranium miners*

Vertical bars represent 95% confidence limits (Hornung and Meinhardt, 1987).

To facilitate the comparison between the values predicted with the TF-TR model and epidemiological data, we adopted the following methods:

1) for converting the epidemiological data expressed as functions of dose, in WLM, a dose-exposure conversion factor (CF) of 5 mGy/WLM was applied (Hofmann, **Truţă-Popa** and Fakir, 2006), and the convertion from WLM in Bq/m^3 was based on the relationship 1WLM=230 Bq/m^3 (ICRP 65, 1994);
2) to facilitate the comparison between theoretical predictions and observations on Colorado Plateau miners all risk functions were normalized to a relative risk of 29, for cumulative exposure of 2600 WLM (13 Gy), and for the Czech miners, all risk functions were normalized to a relative risk of 4.44, at cumulative exposure of 135 WLM (0.675 Gy).

This normalization determines the scaling factor C from the model equation The normalization was introdued because the primary goal of this study was to examine the shape of dose-effect curve, at different exposure conditions, and not the absolute number of lung cancer cases.

3.1.4. Effect of dose versus dose -rate

Simulations of lung cancer risk indicated that cancer induction for continuous exposures is related to the cell cycle time of the irradiated cell, thus showing a distinct dose-rate effect. (Hofmann, **Truţă-Popa** and Fakir, 2006). For the comparison of the present lung cancer predictions with available epidemiological information, the data on relative lung cancer risk in US uranium miners reported by Hornung and Meinhardt (1987) were chosen (Figure 2). In order to facilitate the comparison of the theoretical predictions with these epidemiological findings, all risk functions are normalized to a relative risk of 29 at 2600 WLM.

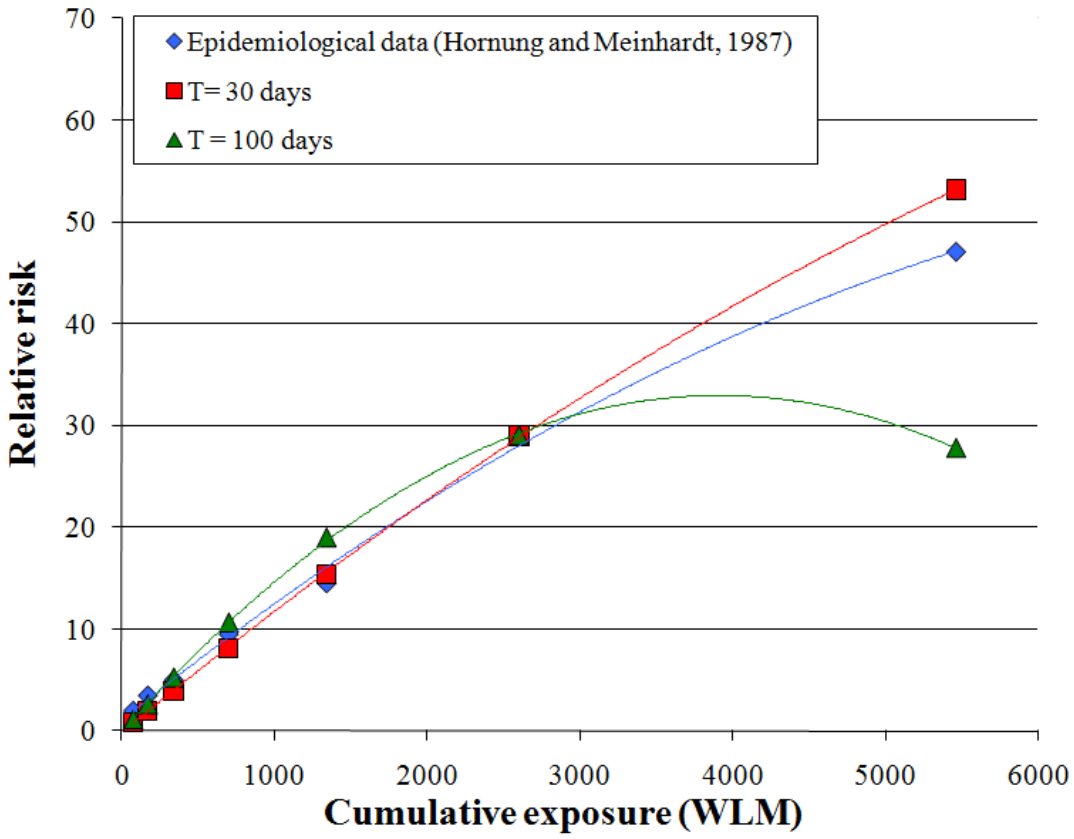
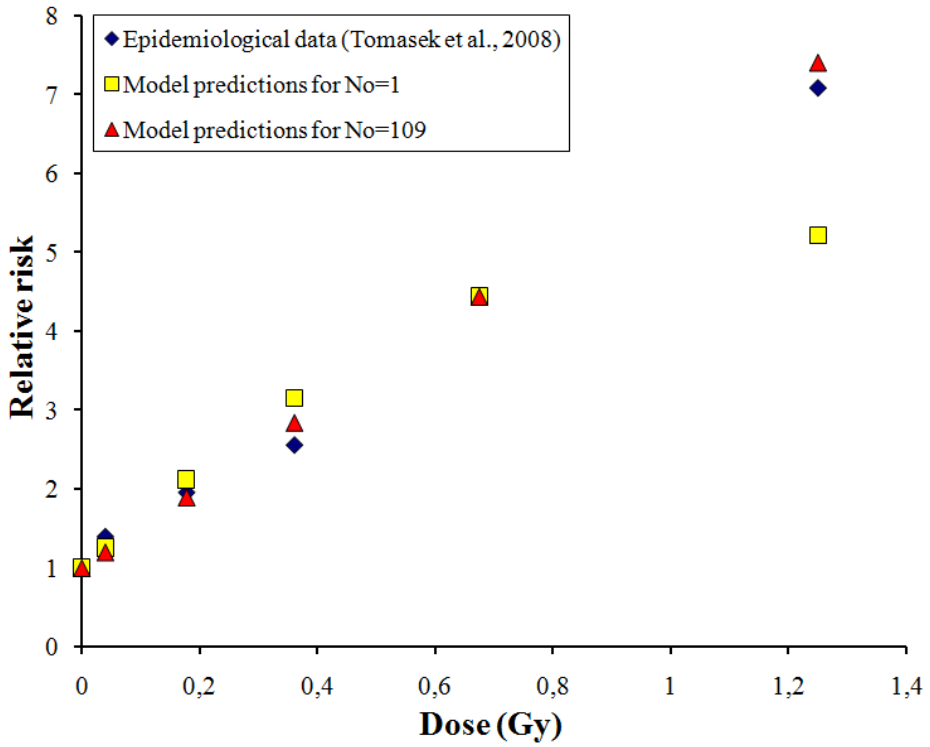


Figure 5. *Comparison of theoretical TF-TR model predictions with the epidemiological data of Hornung and Meinhardt (1987) on US uranium miners for target cell cycle times of 30 and 100 days, respectively.*

This comparison indicates that the dose per cycle time of bronchial epithelial cells is the appropriate dosimetric quantity for protracted exposures, while the total dose, equivalent to an acute exposure, would greatly reduce lung cancer risk at higher exposures (as shown by the 100 days cycle time), phenomenon called the inverse dose-rate effect (Hofmann, **Truţă-Popa** and Fakir, 2006). Indeed, the assumed cycle time of bronchial target cells of 30 days provides an excellent fit to the epidemiological data for the Coloradon Plateau uranium miners.

For lower occupational exposures, such as the ones for the Czeck miners, the same inverse dose-rate effect was observed: for cumulative exposures higher than 135 WLM (D=0.675), the

predicted risc with TF-TR model for 109 cell cycles (each cell cycle of 30 days) are very similar to the epidemiological data reported by Tomasek et al. (2008) but decreases for a longer cell cycle (assumed to cover all the 9 years duration of exposure) (Figure 6).



Figuea 6*. Epidemiological RR in Tomasek data (2008) compared to the RR predicted with the TF-TR model for No=1 and No=109, inverse dose-rate effect*

This comparison indicates that the dose per cycle time of bronchial epithelial cells is the appropriate dosimetric quantity for protracted exposures, while the total dose, equivalent to an acute exposure, would greatly reduce lung cancer risk at higher exposures (as shown by the one, longer cell cycle).

The phenomenon of risk decreasing with increasing dose rate for higher cumulative exposures –as indicated by the results above- is commonly called the inverse dose-rate effect or protraction enhancement effect, and it was reported both in epidemiologic and animal data for these exposure conditions (Lubin et al., 1995, a; Monchaux et al., 1994; 2002; 2004).

Relative risk values predicted by TF-TR model for cumulative exposures to radon and different dose-rates for rats are in good agreement with the experimental ones (Monchaux, 2004), showing the same invese dose-rate effect, just that the cumulative exposure value corresponding to the maximum lung cancer risk is different (1.16 at 200 WLM predicted, compared to 1.20 at 3000 WLM, experimentally observed), but the shape of the dose-response relationship is quite similar (Figure 7). Anyway, risk predictions for the inverse dose-rate effect are in agreement also with the results reported by Lubin et al. (1995, a), indicating the fact that this effect occurs only at cumulative exposures higher than 50 WLM.

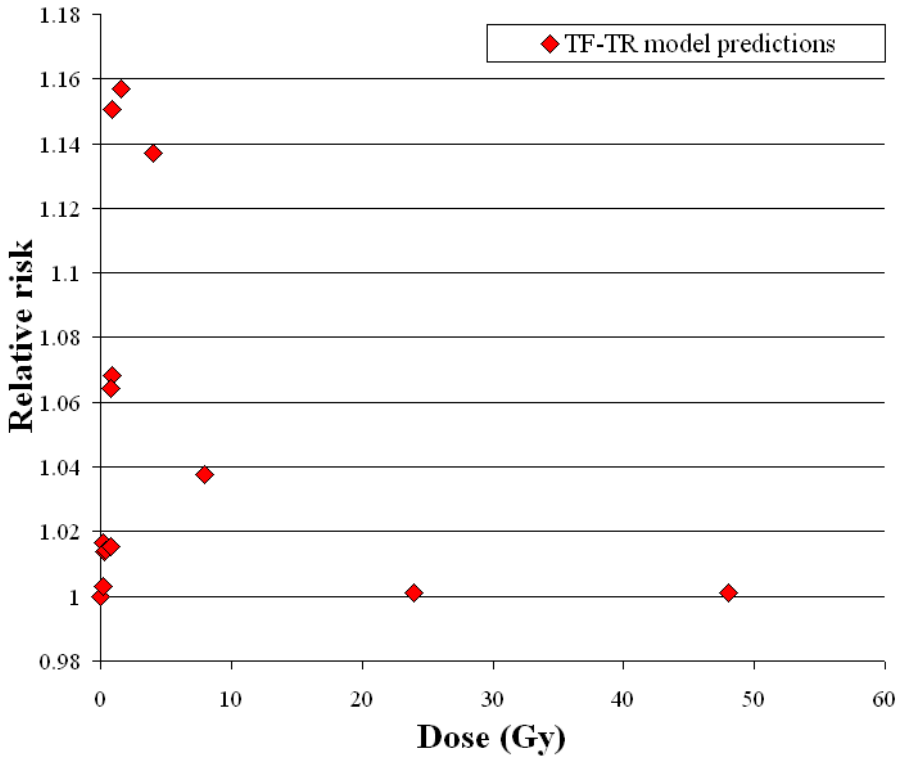


Figure 7*. The inverse dose-rate effect predicted by the TF-TR model for ratin vivo data, reported by Monchaux (2004)*

For low cumulative exposures (lower than 50 WLM) characteristic of residential exposures, the results obtained showed no inverse dose-rate effect since at very low doses the probability that more than one alpha particle would traverse a cell is very small and there would be no possibility for interactions from multiple hits (Lubin et al., 1995, a). This supports the hypothesis that at low doses, lung cancer risk is governed by the rate at which dose is delivered, and not by total cumulative dose alone. However, the possibility that radiation risks at low doses and dose rates are not predicted realistically by the current no-threshold theory should not be neglected, as observed in an experiment where the lung cancer incidence was slightly lower in rats exposed at low exposure rate (25 WLM) than in the control group (Morlier et al., 1994; Monchaux and Morlier, 2002).

Extrapolation of experimental in vitro cellular data to in vitro exposure conditions, extrapolation from high to low doses, corelated versus uncorelated oncogenic transformation probabilities, as well as ***exposure-dosimetry parameters*** were also studied in chapter 3 of the thesis.

Extrapolation from high to lower radon exposures is also influenced by **the inverse dose-rate effect** occuring at cumulative exposures higher than 100 WLM that correspond to multiple alpha particle traversals and is responsible for the increase of oncogenic transformation probability *in vitro* or carcinogenesis *in vivo* when a given total dose is given for a protracted

period of time (BEIR VI, 1999). For low, residential exposures, the inverse dose-rate effect does not occur because, in these exposure range the single alpha particle tyraversals dominate (Hofmann, **Truţă-Popa** and Fakir, 2006), fact that is illustrated in the following graph:

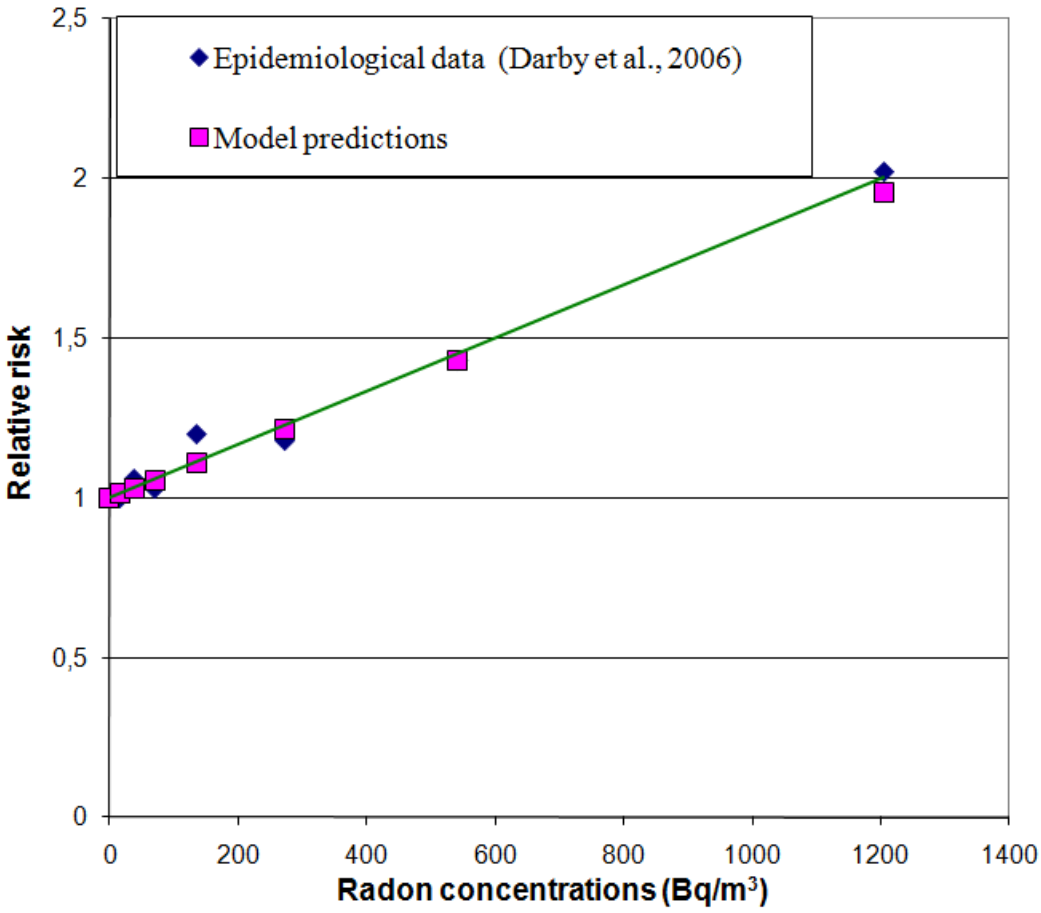


Figure 8. *Model predictions compared to the European residential data and the liniar non-threshold hypothesis*

European residential data (Darby et al., 2006), TF-TR model predictions and the linear no-threshold hypothesis (LNT) plotted in Figure 8 indicate a linear relationship between the lung cancer relative risk and radon exposure (concentration). To facilitate the comparison between TF-TR model predictions with these epidemiological data, all risk functions were normalized to a relative rosk of de 1.43 at 542 Bq/m^3. The excellent agreement between the risk observed and the one predicted with TR-TR model illustrates the dominat role of single alpha particle hits in case of residential exposures.

Impact of non-unifor distribution of dose and the hot spots effect

The difficulties in determining or quantifying the effects of internal sources also include the problem of non-uniform distribution of dose in organs and tissues (WHO, 2001). The deposition of the inhaled radon progeny within the airways exhibits a very inhomogeneous pattern. It was observed that the activity accumulations at the branching points of bronchial

airway bifurcations produce hot spots of alpha particle hits (Hofmann, **Truţă-Popa** and Fakir, 2006). Thus, a small volume of basal and secretory cells in this region may receive substantially higher doses (as high as several orders of magnitude) than cells located at other sites of the bronchial airways, suggesting that multiple alpha particle hits may occur primarily at carinal ridges, especially in the case of occupational exposures of uranium miners (Hofmann, **Truţă-Popa** and Fakir, 2006; Szoke et al., 2007).

The present research analyses, among other aspect, the hot spots effect on the radon-induced transformation frequencies in the assessment of lung cancer risk. Thus, it was assumed that the average dose D received by cells located at other sites of the straight (tubular) and Y-shaped bronchial airways, is 10 times greater in hot spots (D_{hs}), inducing higher transformation frequencies in cells in the hot spots. The combined effect of these doses on TF (TF_{comb}) may be the most appropriate value to be used in calculating the lung cancer risk. Two approaches were studied, in this respect: (1) D_{hs} (the dose in the hot spots) is not considered in the dose-WLM relationship ($D = CF \cdot WLM$) so TF_{comb} will depend on the epidemiological doses (D_{epid}); (2) D_{hs} is already considered in the dose-WLM relationship, so TF_{comb} will depend on the tubular doses (D_{tub}). Thus, the combined effect of hot spots on TF induced by exposure to epidemiological doses (TF_{comb}) will be:

$$\text{for a) } TF_{comb} = TF(D_{epid}) \cdot (1 - f) + TF(D_{hs}) \cdot f$$

$$\text{for b) } TF_{comb} = TF(D_{tub}) \cdot (1 - f) + TF(D_{hs}) \cdot f$$

where f represents the fraction of hot spot effects. Assuming approach (1), and comparing f=0.1 and 0.2, it was noticed that TF_{comb} increased (at doses below 13 Gy) and subsequently decreased less steeply (at higher doses than 13 Gy) for f=0.1 than for f=0.2 relative to the TF_{epid}. Changing the value of p will not make a difference in the shape of the final relative risk (Hofmann, **Truţă-Popa** and Fakir, 2006). .

For the comparison of epidemiological data with TF-TR model predictions including the hot spots effect, the Colorado Plateau uranium miner data (Hotnung and Meinhardt, 1987) were selected because the hot spots effect on TF is better discernible over this large range of doses (from low to very high).

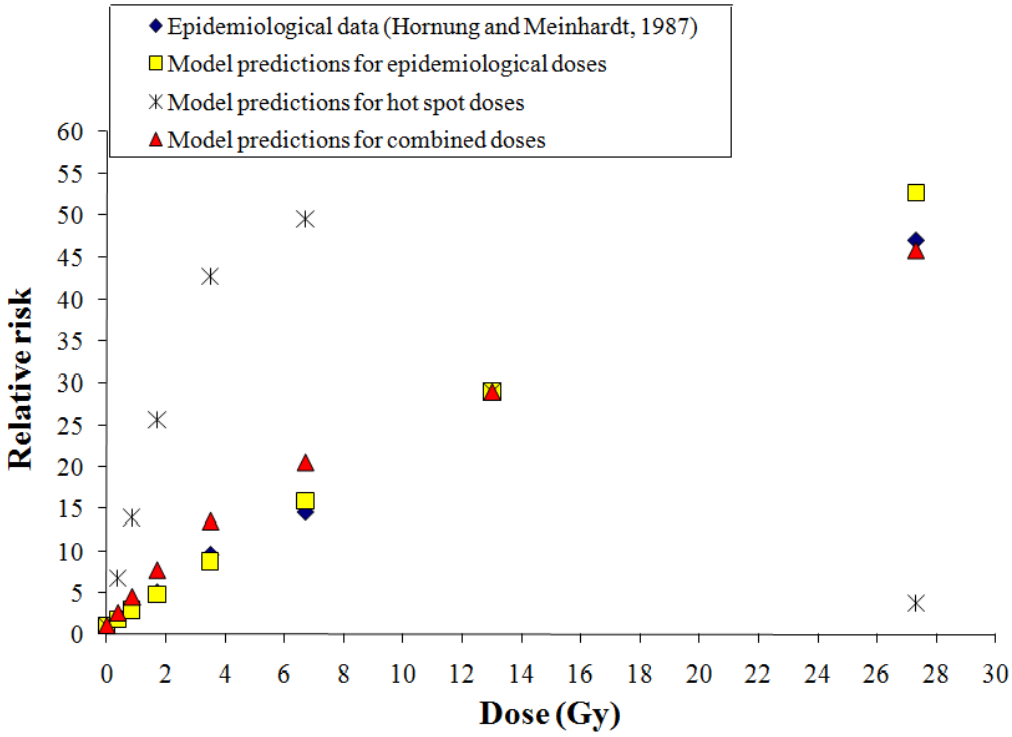


***Figure 9**. Epidemiological RR (Hornung and Meinhardt, 1987) compared to model predictions for epidemiological, hot spots and combined doses, with approach (1), for f=0.1*

When D_{hs} is already considered in the D-WLM relationship (approach (2)), and compare the f=0.1 and 0.2 values, it was noticed that for doses below 13 Gy, the TF_{comb} had the same values, and for doses higher than 13 Gy the TF_{comb} is higher for f=0.1 than for f=0.2 relative to the $TF_{epid.}$.

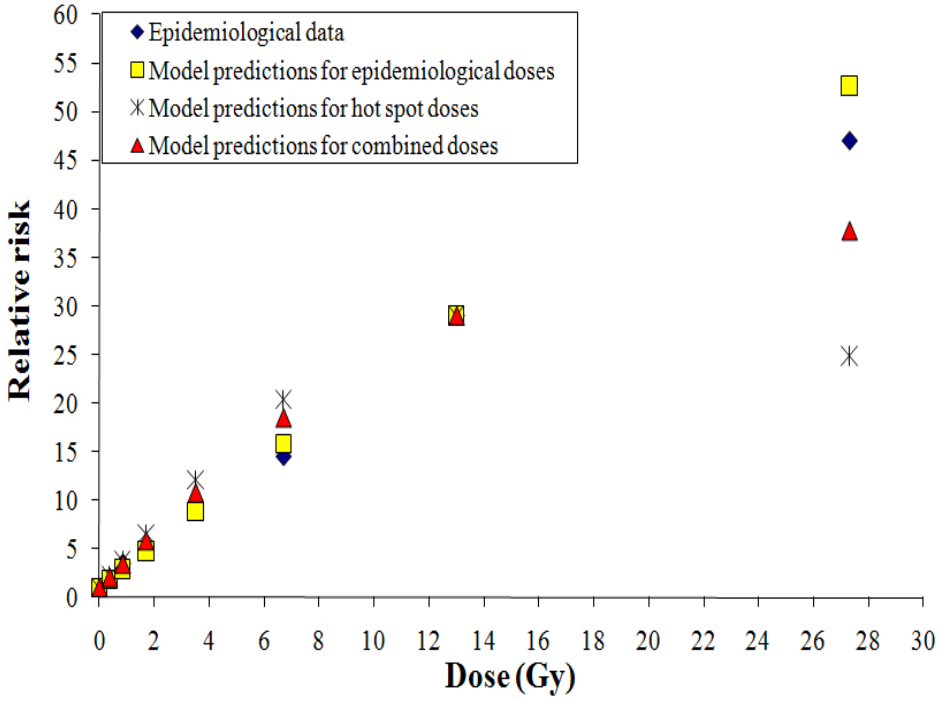


***Figure 10**. Epidemiological RR (Hornung and Meinhardt, 1987) compared to model predictions for epidemiological, hot spot and combined doses, with approach (2), for f=0.2*

Thus using either approach, for a hot spot dose contribution of 10% of the total dose, would give much closer values to the epidemiological results than for a 20% contribution. The anatomical regions of hot spots from the carenal ridge sites - where the exposure, and corresponding given dose to the epithelial cells is much higher - were clinically observed as preferred locations for the occurrence of bronchial carcinomas (Martonen and Hofmann, 1991).

3.2. IMPACT OF NON-TARGETED EFFECTS OCCURRING AT LOW DOSES, ON LUNG CANCER RISC PREDICTIONS WITH TF-TR MODEL

For many decades, high LET radiation effects observed at the organ level were assumed to be related to radiobiological effects in cells or cell nuclei directly hit by alpha particles. In recent years, however, radiobiological effects were also observed in cells not traversed by an alpha particle, termed non-targeted effects. The objective of this subchapter was to explore the role of non-targeted cellular effects (bystander mechamisms, adaptative response, genomic instability and induced apoptosis) on the shape of the dose-effect curve in the low dose region, i.e. to investigate whether these mechanisms will increase or decrease lung cancer risk at radon exposure levels characteristic of indoor exposures, based on available information and the TF-TR model.

3. 2.1. Bystander mechanisms

A general definition for bystander effects refers to damage that occurs in cells that were not traversed by radiation, but were in the neighborhood of an irradiated cell, i.e. they were bystanders at the time of irradiation. A "detrimental" bystander effect amplifies the biological effectiveness of a given radiation dose by effectively increasing the number of cells that experience adverse effects over that directly exposed to the radiation (Brenner et al., 2001). For alpha particles, Sawant et al. [35)] reported that the resulting transformation frequency when 10% of the C3H 10T1/2 cells were exposed to alpha particles was not less than in the case when all the cells on a dish were exposed to the same number of alpha particles. These data for transformation frequencies in mouse fibroblast cells were analyzed in terms of a microdosimetric bystander model (Fakir et al., 2009). At low dose exposures, where the average number of hits is less than 1, the bystander induced foci exceed the direct contribution by almost an order of magnitude, while for higher doses, when the average number of hits is greater than 2, the bystander effect is less efficient. The ratios of the bystander-initiated transformations to the direct effect for a given average number of cellular hits were implemented into the TF-TR model. (**Truţă-Popa** et al., 2008, a, c).

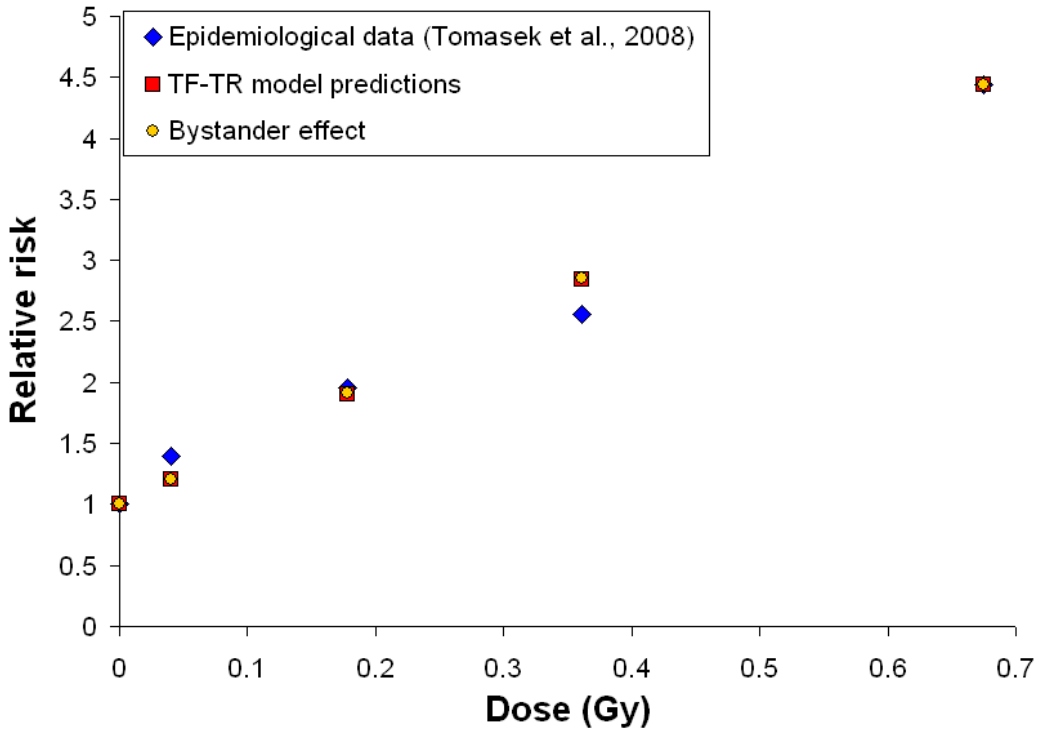


Figure 11. *Detrimental bystander impact on dose-effect relationship*

Since the average number of hits calculated for the Czech miner data is less than 1, the bystander effect significantly increases the risk values. However, the average number of hits is so small that the shape of the dose-effect curve is hardly affected (**Truţă-Popa** şi colab., 2008, b, c) (Figure 11). In contrast to the above discussed detrimental bystander effects, "protective" bystander responses have also been reported (Belyakov şi colab., 2006). Potentially damaged or sensitive cells may be removed by either apoptosis or by premature differentiation. Belyakov and colleagues (2006) observed that even if only one single region of the multicellular tissue section of the epithelial layer was hit by alpha particles, thousands of additional cells were were found to undergo differentiation in the explant outgrowth (and thus do not contribute to cancer formation). However, these experimental evidence (Belyakov et al., 2006) does not provide any information on a potential dose dependency and thus will not be modeled here.

3. 2.2. Adaptative response

Adaptive response is another natural defense mechanism of the organism against oncogenesis (**Truţă-Popa** şi colab., 2008, c). It refers to the cells first exposed to low levels of DNA stress, e.g. by low LET radiation (priming or conditioning dose), rendering cells resistant to a subsequent exposure low doses of radiation (challenging dose), thus reducing the effect of low doses of radiation (Sawant et al., 2001, b). In the study of Iyer and Lehnert (2002), non-irradiated human fibroblast cells (HFL-1) were able to adapt if grown in a medium with supernatants

transferred from HFL-1 cells, irradiated with 1 cGy of α-particles. The adaptation was manifested through increased clonogenic survival after subsequent exposure to 10 and 19 cGy of α (Iyer and Lehnert, 2002). Since this study is currently the only example in the available literature on adaptive response involving alpha particles as priming and challenging dose, these results were used to assess their impact on lung cancer risk at low, chronic radon exposures.

Since adaptive response affects cell killing [36], the impact of this mechanism on lung cancer risk computed with the TF-TR model was considered by replacing the term $\exp(-\gamma \cdot D)$ with $\exp(-k \cdot \gamma \cdot D)$, where k is a coefficient representing the ratio between directly irradiated and treated cells at the same dose D:

$$k = \frac{\gamma'}{\gamma},\ \gamma' = -\frac{\ln SF'}{D}, \gamma = -\frac{\ln SF}{D},$$

where γ' and γ are the cell killing probabilities and SF' and SF are the surviving fractions reported in the study of Iyer and Lehnert (2002) for treated and not treated cells, respectively. Based on the experimental data, an average value of k = 0.28 was obtained.

To incorporate the adaptive response mechanism into the analysis of the epidemiological data of Tomasek et al. (2008), two scenarios were considered i) only doses smaller than 0.2 Gy (the highest dose value used in the Iyer and Lehnert study, and, ii) all doses smaller than the normalizing dose of 0.675 Gy are affected by the adaptive response.

In the first case, shown in Figure 12, adaptive response significantly reduces lung cancer risk at low radon levels, exhibiting a sublinear response, while the risk above 0.2 Gy remains unaffected. In the second case, also shown in Figure 12, lung cancer risk is reduced for all doses below the normalizing dose. Since the same factor applies to all doses, normalization by a constant factor does not affect the linear dose-effect relationship predicted by the model for direct radiation effects.

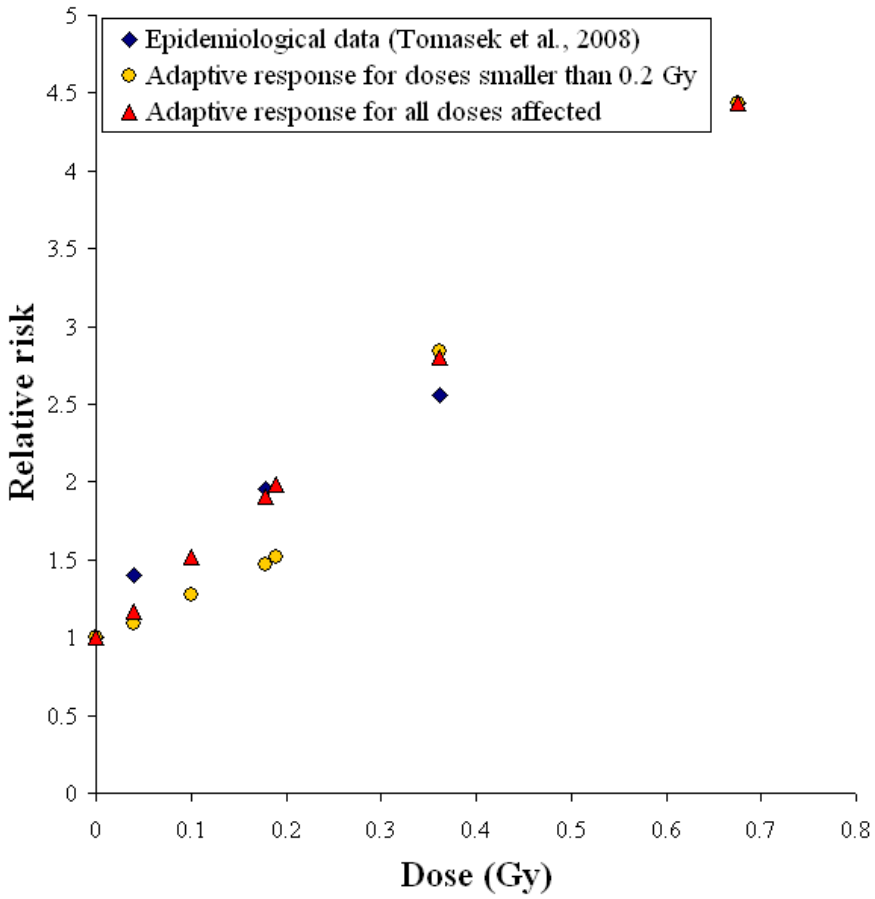


Figure 12. *The impact of adaptive response on dose-effect relationship*

3. 2.3. Instabilitatea genomică

Genomic instabilioty is transmitted to descendent cells, inducing lesions in the progeny of the irradiated cells, even after many generations (Hall and Hei, 2003). Thus, for protracted exposure, each generation is affectet both by direct exposure to ionizing radiation and also by the genetic radiation-induced modifications of the previous generations. Due to the rather qualitative nature of the available experimental data regarding the duration of this effect and its dependence on dose, two different scenarios were assumed:

(a) the effect of genomic instability decreases in an exponential fashion for a specified number of cellular generations independent of cellular dose, and

(b) the number of cellular generations affected by genomic instability depends linearly on dose.

If genomic instability is incorporated into the TF-TR model, predicted risks for both scenarios are higher than estimated for direct radiation-induced damage at all exposure levels. However, if normalized to a RR of 4.4 at 135 WLM (0.675 Gy) (**Truţă-Popa** et al., 2008, a, b), then the two scenarios produce different results.

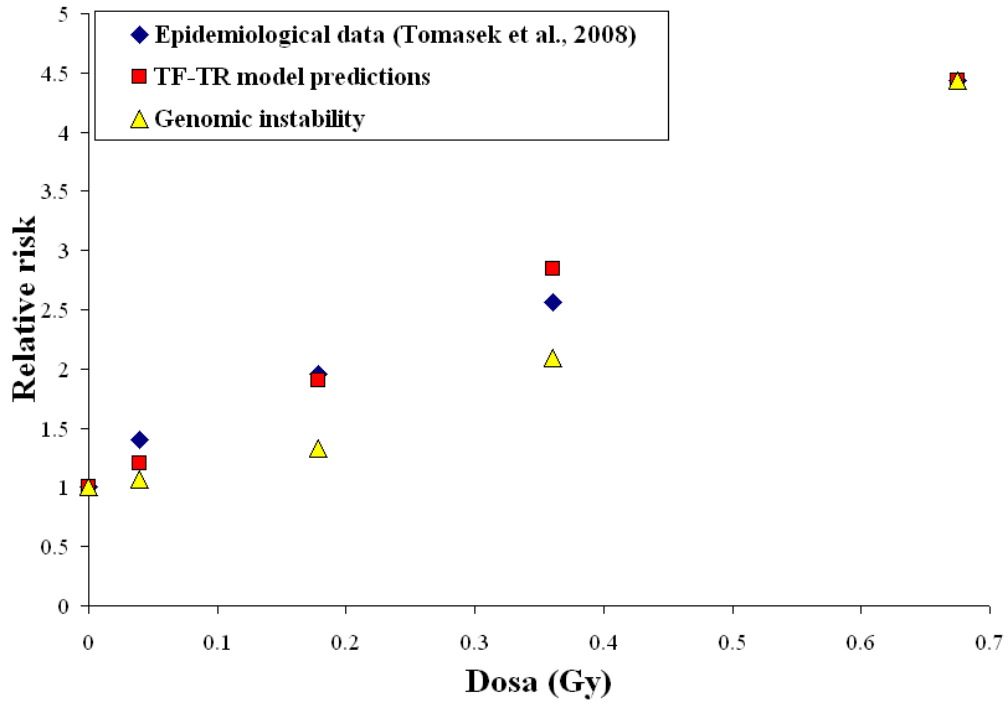


Figure 13. *The impact of genomic instability on dose-effect relationship*

In case of independence of dose (a), the shape of the dose-effect curve is not affected at all, while the dose dependency (b) reduces the risk in the low dose region relative to the higher doses (Figure 13) (**Truţă-Popa** et al., 2008, a, b). Hence both cases may be regarded as the upper and lower bounds of the effect of genomic instability on lung cancer risk.

3. 2.4. Induction of apoptosis by surrounding cells

Transformed cells produce superoxide anions in the surrounding microenvironment that participate in intercellular signaling. These signals can be eliminated by their non-transformed neighbors through intercellular induction of apoptosis (Bauer, 2000). Cell deletion by apoptosis in renewing tissues is considered a protective mechanism for the organism in removing mutated cells from renewing tissues in which tumours could arise (Hendry, 1999).

The experimental results reported by Portess et al. (2007) were chosen to explore the effect of induced apoptosis on oncogenic transformation. In this study, transformed cells were forced into apoptosis by surrounding non-transformed cells, stimulated by exposures to low doses of ionizing radiation, notably alpha particles (Portess et al., 2007). The number of apoptotic cells in non-irradiated transformed cells increased with dose in the low dose range, approaching a plateau value of about 20% at around 100 mGy (Portess şi colab., 2007). While this effect decreases the carcinogenic risk at doses above 100 mGy by 20%, this also implies that induced apoptosis is less effective at lower doses.

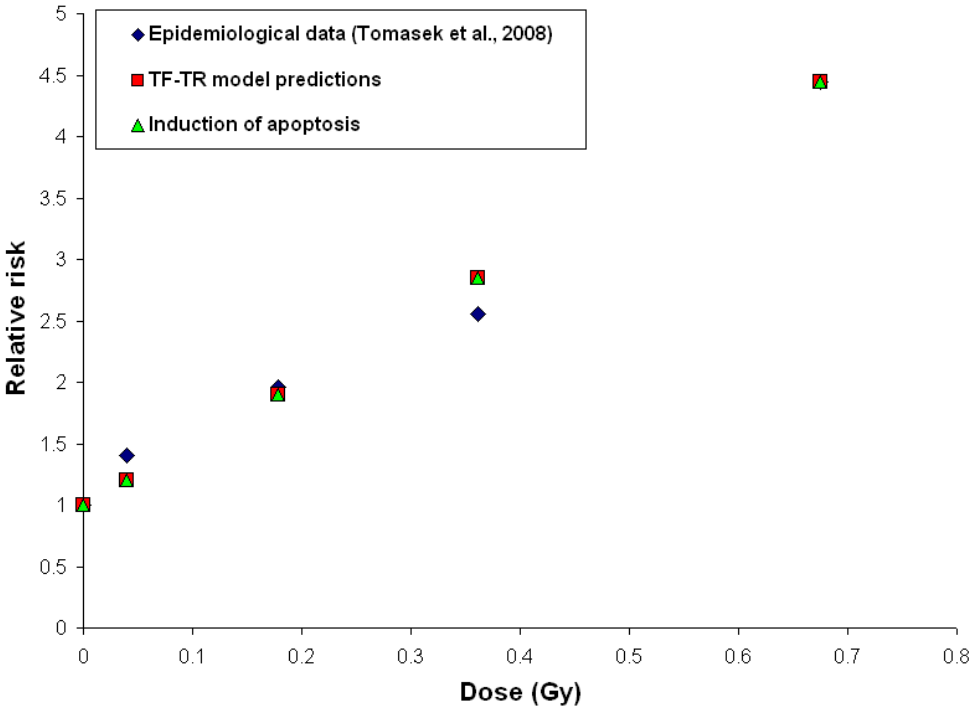


Figure 14. *The impact of induction of apoptosis on dose-effect relationship*

Hence, if normalized to the risk at 135 WLM = 0.675 Gy, induced apoptosis slightly increases the risk at the lowest doses relative to higher doses (Figure 14) (**Truţă-Popa** et al., 2008, b).

CHAPTER 4.

IMPACT OF SMOKING AND THE ŞI ASSESSMENT OF THE NUMBER OF LUNG CANCERS ATTRIBUTED TO RADON RADONULUI

4.1. EXPLORING THE POTENTIAL SYNERGISTIC EFECT OF SMOKING ON RADON-INDUCED LUNG CANCER RISK

The interaction between radon progeny and smoking has been intensively studied, but the mechanisms by which these two carcinogenic elements from the human environment interact are still largely unknown. The most common relationship describing this interaction, which is also assumed in this approach, is that their joint effect is synergistic and multiplicative. Smoking, the first cause, and radon, the second recognized cause of lung cancer, were analyzed in this subchapter, trying to find an explanation for their synergistic effect in lung cancer induction, and the optimum versions of some models based on the specific biological effects of these two carcinogens. While approaching the combination of models proposed, models such as the ones adopted by the National Academy of Science on this issue along with other models anaysing the hot spots effect were taken into consideration. The results predicted with these models proposed are in good aggreement with the epidemiological or experimental evidence.

In subchapter 4.1 I described different types of lung cancer and the frequency of their occurrence, the history of the research in modeling the radon-smoking synergism (Crawford-Brown, 1992 and BEIR IV, 1988 approaches), sources of errors in the studies assessing the lung cancer risk induced by radon and smoking, radioactive components of tobacco and cigarette smoke, information on passive smoking (ETS) in the assessment of lung cancer risk induced by radon and smoking, the comparison bewteen BEIR IV and BEIR VI model for the assessment of radon-smoking synergistic effect and I proposed the following models for the assessment of this effect:

4.1.1 TF-TR model combined with BEIR IV model for the estimation of the synergistic effect of radon and smoking

The model to predict lung cancer risk induced by the synergistic action of radon and smoking was derived from the TF-TR (former Initiation-Promotion) model (Hofmann, **Truţă-Popa** and Fakir, 2006; **Truţă-Popa** et al., 2008, 2009) and the BEIR IV model (1988)

$$RR(D,CS) = RR(D) \cdot RR(CS) = RR(D) \cdot (1 + b \cdot CS)$$

where the first term RR(D) was calculated with the TF-TR model and the second term, RR(CS) was calculated according to the BEIR IV model, *CS* represents the number of cigarettes assumed to be smoked per day, and *b* is a constant equal to 0.3/cigarette/day (value adopted by the BEIR IV Committee, 1988) (**Truţă-Popa** et al., 2010).

The combination of the TF and BEIR IV models suggests the possibility of interpreting cigarette smoke as promoter of the cells initiated by radon (Hofmann et al., 1993). The reason for this choice is based on the fact that the high dose of alpha radiation at bifurcations from ^{210}Po in ^{210}Pb-enriched smoke particles is supplemented by the radon and thoron progeny ^{218}Po, ^{214}Po, ^{212}Po, and ^{212}Bi, inhaled in the normal breathing process between smoking cigarettes (Martell, 1983), (all radioactive particled being deposited mostly in branching sites of the airways) (**Truţă-Popa** et al., 2010). The aim of this study is to investigate the dependence of lung cancer risk induced by radon and smoking on the exposure duration and intensity.

Epidemiological data used and the results predicted with the TF-TR model, combined with the BEIR IV model

In order to validate the combined model (TF-TR with BEIR IV) we compared the risk predictions with epidemiological data reported for uranium miners, both for high (Hornung and Meinhardt, 1987) and low cumulative exposures (Tomasek et al., 2008). We compared the the lung cancer risk of low, protracted exposures to these two carcinogens with shorter exposures and with radon exposures only (**Truţă-Popa** et al., 2010). First, we considered the high exposure

data of Colorado Plateau uranium miners (Hornung and Meinhardt, 1987) comparing different values for CS, starting with the value adopted by Crawford-Browwn (1982) for non-smokers (0.008 cig./day). The results are illustrated below (**Truţă-Popa** et al., 2010):

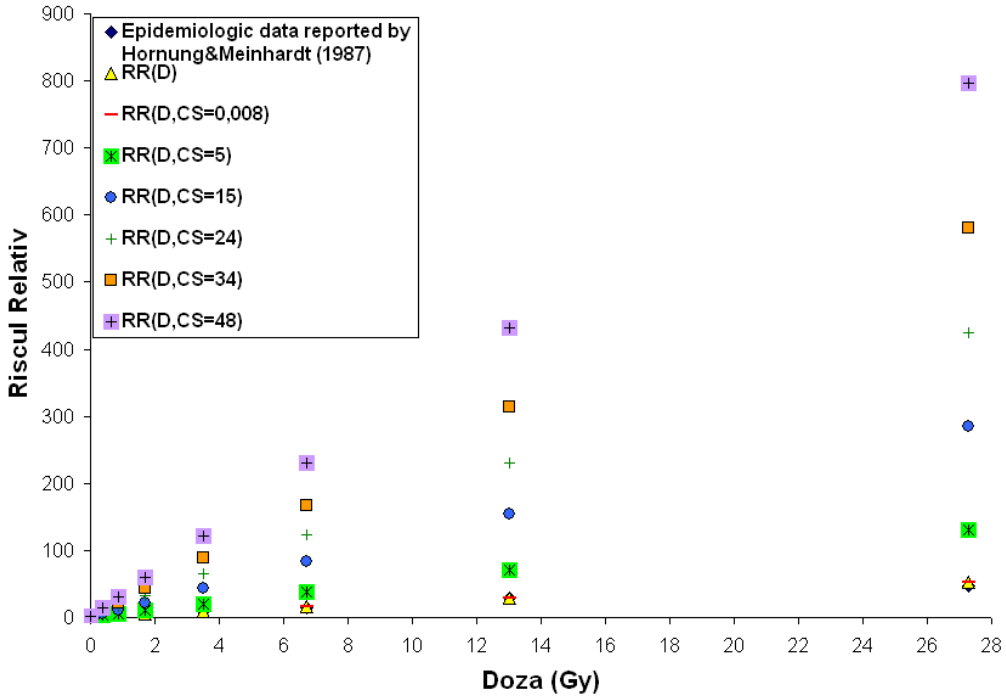


Figure 15. *Lung cancer risk induced by radon and smoking for different CS values, compared to epidemiological data for high cumulative radon exposures (Hornung and Meinhardt, 1987)*

Lung cancer risk values induced by exposure to both carcinogens increased with increasing CS values (number of cigarettes smoked/day), which is in excellent agreement with observations of other studies (Hofmann et al., 1993; Lee et al., 1999; BEIR IV, 1988). Without contribution from smoking, lung cancer risk is much smaller.

If we consider the same CS values for low cumulative radon exposures (Tomasek et al., 2008), the same lung cancer risk increase with increasing number of cigarettes smoked/day could be observed (see Figure 16):

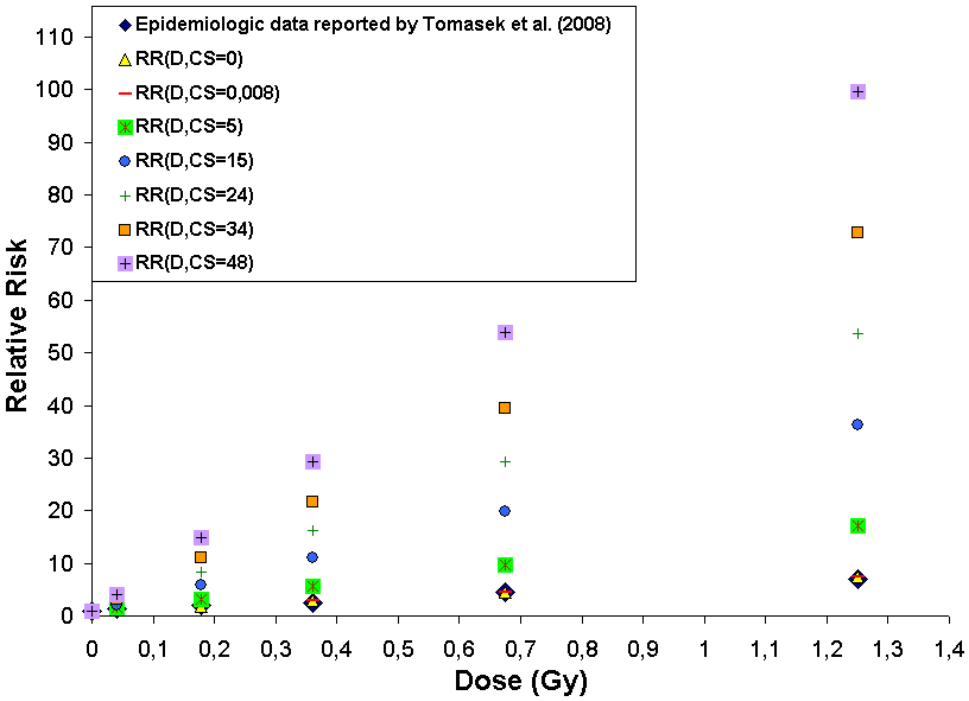


Figure 16. *Lung cancer risk induced by radon and smoking for different CS values, compared to epidemiological data for low cumulative radon exposures (Tomasek et al., 2008)*

By comparing the lung cancer relative risk (RR) predictions of the combined model (1) for similar doses (D) reported in both epidemiological data sets (Hornung and Meinhardt, 1987; Tomasek et al., 2008), the inverse dose-rate effect can be clearly observed (**Truţă-Popa** et al., 2010):

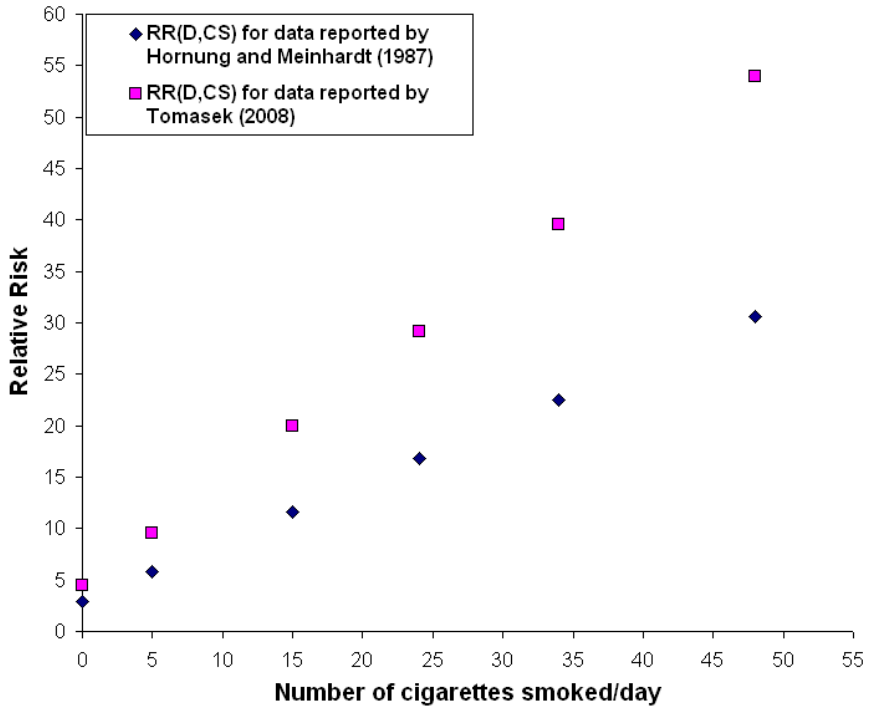


Figure 17. *Lung cancer risk induced by radon and smoking for similar doses of the two epidemiological data sets (Hornung and Meinhardt, 1987; Tomasek et al., 2008), exhibiting an inverse dose-rate effect*

In the case we considered, the reference dose was 0.85 Gy for the epidemiological data of Hornung and Meinhardt (1987), which is close to the dose of 0.675 Gy reported by Tomasek et al. (2008). For the first data set, the average exposure duration was 4 years, and cumulative exposures were high (up to 5460 WLM, equivalent to 27.3 Gy assuming a conversion factor CF=0.005Gy/WLM) **Truţă-Popa** et al., 2008, a). For the second epidemiological data set (Tomasek et al., 2008), the average exposure duration was longer, about 9 years, and cumulative exposures were much smaller than the ones for Colorado Plateau uranium miners, smaller than 250 WLM (equivalent to 1.25 Gy based on the same conversion factor, CF=0.005Gy/WLM). As illustrated in Figure 17, even if the dose-rate is smaller for the Czech uranium miners (Tomasek et al., 2008), the lung cancer relative risk values increase with decreasing dose-rate. (**Truţă-Popa** et al., 2010). This fact confirms the hypothesis that protracted exposures at lower doses are more dangerous for health than acute exposures at high doses, thus suggesting that residential radon exposures of general population should be seriously considered because this could imply significant health risks.

4.1.2 Association between the combined exposure to radon and smoking, and the hots spots

The mechanism through which smoking interacts synergistically with radon could be explained by the increase of submicronic particle concentration of radon progeny attached to high dimension particles in the main cigarette smoke. They are selectively deposited in the so-called ”hot spots” localised at the carinal bridges of the bronchial airway bifurcations (Hofmann et al., 1993, b; Hofmann, **Truţă-Popa** and Fakir, 2006; **Truţă-Popa** et al., 2009, a). The smoke tars are hardly disolved by the lung fluids and they fix radon-progeny particularly in the bifurcation sites. In this conditions, a substantial decay will take place before radon-progeny are cleaned by the specific lung clearance processes (Martell, 1983; Baias et al., 2009).

Radon progeny inhaled during normal breathing between cigarettes make an even larger contribution to the alpha-radiation dose at bifurcations that, together with the one due to cigarette smoke, causes progresive lesions to the sites where the smoke particles enriched by insoluble ^{210}Pb are easily trapped (Martell, 1983).

From the considerations listed above, the risk assessment of lung cancer induced by radon and smoking could be performed with the Tf-TR model adopted for hot spots (described in Chapter 3), model that is based on the excessive alpha particle contribution to dose, the new element being only the cause of this hot spots (due to these two carcinogens, not only from radon). The influence of hot spots on the total risk is the same, the dose-effect relationship will not change.

4.2. APPLICATION OF RADON CARCINOGENESIS MODELS

4.2.1. Estimation of lung cancer cases induced by radon for the population of some areas of Transylvania, based on Darby's model

The objective of the present subchapter was to assess the lung cancer risk induced by exposures to radon progeny of people living in some areas of Transylvania. Romania. Indoor radon concentrations were measured in 667 dwellings of Stei area, Cluj, Bistrita-Nasaud, Sibiu and Alba counties. Measurements were performed using CR-39 track detectors, exposed for minimum 3 months. Average annual radon concentrations were 232, 114, 71, 62 and 161 Bq m^{-3} for Stei area, Cluj, Bistrita-Nasaud, Sibiu and Alba, respectively (**Truţă-Popa** et al., 2009, b; c).

The liniar model of Darby was used to simulate the dose-effect relationship and the relative lung cancer risk at low doses of alpha particles, specific indoors. The relative risks predicted for the measured exposure levels, together with information on the total number of lung cancer deaths reported and the number of inhabitants of these areas allowed us to estimate the fraction of lung cancers attributed to radon, for each area. These fractions are: 16.67% for Stei area, 9.09% for Cluj , 5.66% for Bistriţa-Năsăud, 4.76% for Sibiu and 12.28% for Alba county, for never-smokers (**Truţă-Popa** et al., 2009, c).

By comparing the percentage of lung cancers attributed to radon based on Darby's model (16.7%) (**Truţă-Popa** et al., 2009, c) with the one predicted with TF-TR for Ştei area (15.4%) (**Truţă-Popa** et al., 2009, b), it could be noticed that TF-TR model predictions are smaller than with Darby's model, but are still very close. Assuming that smoking rates are similar for the investigated areas (10.72% smoking man and 5.95% smoking women) (Sainz-Fernandez et al., 2009), about 64 to 69% of the total annual number of lung cancer deaths, stratified for gender, would be attributed to radon and occurr among smoking mail population and about 35 to 44% would be attributed to radon among smoking popularion (**Truţă-Popa** et al., 2009, c). For residential radon exposures the dose-effect relationship is linear, in agreement with many other epidemiological studies (eg. Darby et al., 2006).

The number of total lung cancer deaths due to indoor exposure to ^{222}Rn ($N_{Rn,a}$) was estimated with the following relation, derived from the other available studies (Catelinois et al. 2006; Pirard et al. 2007):

$$N_{Rn,a} = \frac{(RR-1)}{RR} \cdot N_{T,a}$$

where $\frac{(RR-1)}{RR}$ is the fraction of risk attributed to radon (FRA), *a* is the area where population is exposed, *RR* is the relative risk, predicted by the model, and $N_{T,a}$ is the total annual number of lung cancer deaths in the area *a*, where radon concentrations were measured

(see Table 1) (**Truţă-Popa** şi colab., 2009, c; d). The demographic data were provided by The National Institute of Statistics, Romania (INSSE, 2009), for each county. For these calculations, it was assumed that the populations of the different regions as well as the measured sites were homogeneously distributed among these regions (**Truţă-Popa** şi colab., 2009, c).

Based on the radon concentration measurements in Transylvania, lung cancer risk predictions using Darby's model are summarized in Table 2 for the different exposure categories in the selected regions.

TAB. 2. Radon concentrations measured in the investigated areas, annual average radon concentrations and RR risk predictions with Darby's model (**Truţă-Popa** et al., 2009, c; Cosma et al., 2009)

Area/ County	Intervals of measured values	Average Measured Rn concentrations [Bq m^{-3}]	Frequency [%]	Annual Rn concentrations [Bq m^{-3}]	RR predictions, with Darby's model
Stei area	0-99	57	36	**232**	1.05
	100-199	139	34		1.12
	200-399	268	17		1.23
	400-599	471	4		1.40
	600-799	694	3		1.59
	800-1000	857	2		1.72
	>1000	1550	4		2.31
Cluj	<25	20	10	**114**	1.02
	25-49	38	23		1.03
	50-99	69	32		1.06
	100-199	140	20		1.12
	200-399	266	11		1.22
	400-799	592	4		1.50
Bistrita-N	<25	16	15	**71**	1.01
	25-49	38	33		1.03
	50-99	69	30		1.06
	100-199	128	16		1.11
	200-399	250	6		1.21
Sibiu	<25	21	18	**62**	1.02
	25-49	40	36		1.03
	50-99	68	31		1.06
	100-199	144	16		1.12
Alba	<50	38	11	**161**	1.03
	50-99	65	11		1.05
	100-199	127	44		1.11
	200-399	281	33		1.24

The average annual radon concentrations for the investigated areas/counties were: 232, 114, 71, 62 and 161 Bq m^{-3} for Stei area, Cluj, Bistrita-Nasaud, Sibiu, and Alba county, respectively (**Truţă-Popa** et al., 2009, c).

Assuming that all the regions where radon concentration was measured have similar smoking history, and that the RR for smokers is about 25 times greater than that for lifetime non-smokers (Darby et al. 2006), the predicted RR and number of lung cancers for lifetime non-smokers, males and females smokers, estimated according to $N_{Rn,a}$ equation are listed in Table 3:

TAB. 3 . Relative risk and the number of lung cancers attributed to radon, for ever smiokers, current mail and female smokers (**Truţă-Popa** et al., 2009, c)

Area/ County	RR_{Rn} for never-smokers	RR for smo-kers	Frecq. smok. in male. pop. [%]	Frecqs mok. in female. pop. [%]	$RR_{Rn,sm}$ for man	$RR_{Rn,sm}$ for women	N_T aver., annual	N_{Rn} among never-smok..	N_{Rn} among male-smokers	N_{Rn} printre female-smokers
Stei	1.20	30.0	10.72	5.95	3.22	1.79	5	0.83	3.45	2.20
Cluj	1.10	27.5	10.72	5.95	2.95	1.64	303	27.55	200.22	117.82
Bistrita Nasaud	1.06	26.5	10.72	5.95	2.84	1.58	92	5.21	59.61	33.65
Sibiu	1.05	26.25	10.72	5.95	2.81	1.56	173	8.24	111.52	62.24
Alba	1.14	28.50	10.72	5.95	3.06	1.70	144	17.68	96.87	59.08

The percentages of lung cancer deaths attributed to radon exposure were derived from the total, average annual number of lung cancer deaths for the areas where Rn-222 concentrations were measured (NT) and the number of lung cancers deaths attributed to radon, both for lifetime nonsmokers and smokers. The estimated 64-69% percentage of lung cancers attributed to radon among smokers was based on the fraction of smokers among the investigated population (10.72%) (Sainz et al., 2009). The SSRR99- 1999 study reported than in 1999, in Romania, 39% of the women were smokers and 72% of the men were smokers (Eurespir, 2009). For the male population where about 72% are smokers, the percentage of lung cancers due to exposure to radon among smokers would then be about 95-96%, and for the female population where about 39% are smokers, the percentage of lung cancers due to exposure to radon among smokers would then be about 90- 91%, values that are in excellent agreement with the BEIR VI estimations (BEIR VI, 1999). The average annual lung cancer mortality rates per 100,000 inhabitants provided by the National Institute of Statistics for the investigated counties since 2006 until 2008 were: 44, 43, 29, 41 and 38 for Cluj, Bihor, Bistrita-Nasaud, Sibiu and Alba counties, respectively. (**Truţă-Popa** et al., 2009, c). If we consider the Cluj county, which has 689523 inhabitants (INSSE) and an average annual mortality rate of 44 (per 100,000 inhabitants), the annual average number of lung cancer deaths is 303. (**Truţă-Popa** et al., 2009, c). The RR estimated with Darby's model, for never smokers in Cluj county, based on the

measured radon concentrations and frequency of measurements in defined exposure intervals, was 1.10. (**Truţă-Popa** et al., 2009, c). Thus, the number of lung cancer deaths attributed to radon, computed with N_{Rn} equation for lifetime nonsmokers (NRn) will be 27.55, which represents 9.09% of the total, annual number of lung cancers deaths for Cluj county (**Truţă-Popa** et al., 2009, c). A simpler method for estimating the percentage of radon-induced lung cancers (for which we used the notation %LC), could be derived from the equation for N_{Rn}, and we formulated it as:

$$\%LC_{Rn} = (FRA) \times 100,$$

The formula proposed in this study for assessing the percentage of lung cancers attributed to radon has the advantage that it requires only the RR value, but this must be very precisely estimated. This method is particularly relevant in cases where the demographic data (regarding the number of total lung cancer cases or deaths, the number of inhabitants) are not available, are difficult to get from medical records or National Institutes of Statistics, or where not all persons who die of lung cancer are registered accordingly, especially in the rural sites. Hence, the quantitative estimates of the lung cancer risk caused by radon will no longer be affected by the uncertainties from the demographic and lung cancer data, a fact that is really important in using the risk projections as a basis for making risk-management decisions (BEIR VI,1999).

For smokers, the RR values were reported to be about 25 times higher than for non-smokers (Darby et al., 2006). This would mean that smokers exposed at the same concentrations measured in Cluj county would have a RR of 27.5, which, multiplied by the frequency of male smokers (10.72%) would give $RR_{Rn,sm}$ of 2.95 (**Truţă-Popa** et al.., 2009, c). By replacing this value in N_{Rn} equation, the total, annual number of lung cancer deaths attributed to radon exposure among male smokers would be 200.22 (**Truţă-Popa** et al., 2009, c). Thus, the percentage of lung cancer deaths caused by simultaneous exposure to radon concentrations measured in Cluj county and smoking represents 66.08% of the annual lung cancer deaths (**Truţă-Popa** et al., 2009, c), the rest being attributed to other causes than smoking and radon exposure, such as: passive smoking, occupational exposure to certain chemicals and ionizing radiation, diet, and family history of cancer (Neuberger and Gesell, 2002). The estimated percentages of lung cancer deaths attributed to radon in each investigated area, calculated in the manner described above, were represented in Figure18.

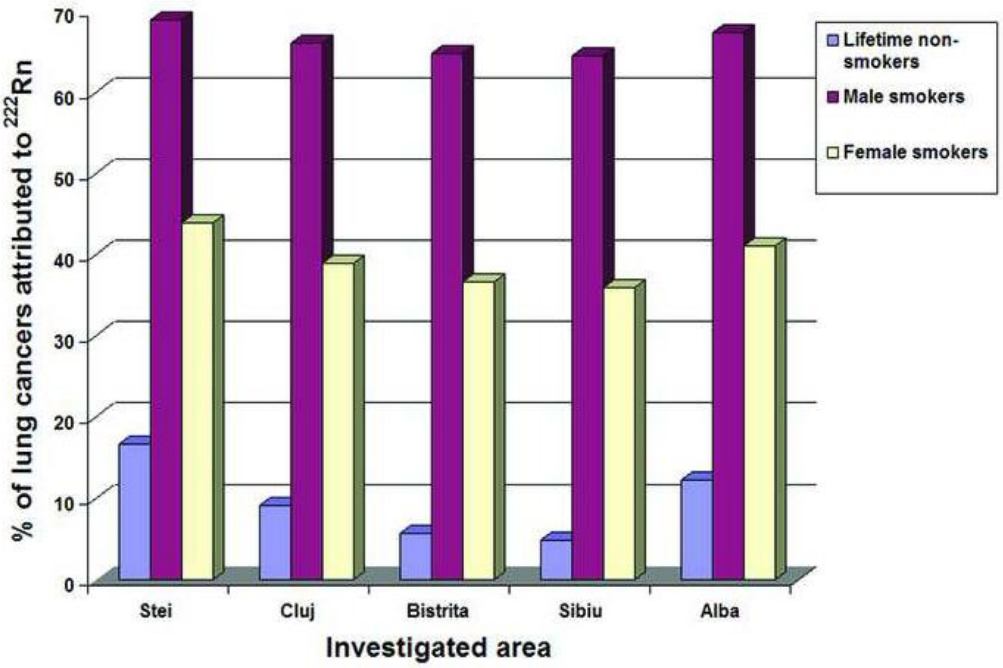


***Figure 18**. Percentage of lung cancer deaths due to exposure to radon for the investigated areas, for lifetime non-smokers, male and female smokers. (Truţă-Popa şi colab., 2009)*

The proposed method for the assessment of the percentage of lung cancers attributed to radon exposure was validated by comparison with the epidemiological data reported by Darby et al. (2006). In her study, RR values, frequency and radon concentrations, as well as the number and percentages of lung cancers for each concentration interval were presented. Based on this information and equation for N_{Rn}, the absolute number of lung cancer cases corresponding to measured radon concentration and frequency of measurements was computed and then plotted in Figure 19:

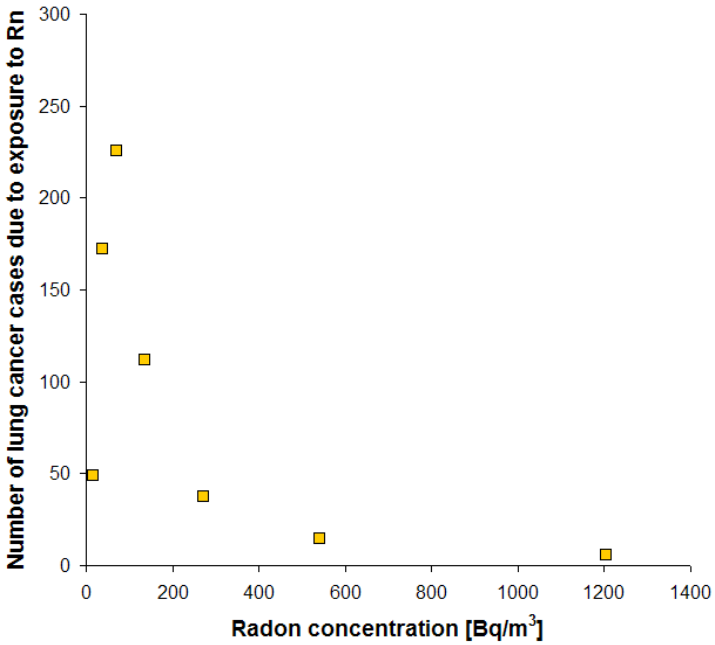


***Figure 19** Numărul de cazuri de cancer pulmonar induse de expunerea la radon pentru studiul lui Darby şi colab., (2006).*

The highest number of lung cancer cases due to radon exposure occurred below 100 Bq/m^3, because 72.5% of the total number of lung cancers also occurred in this range. This coincides with the highest frequency of measurements in radon concentration intervals below 100 Bq/m^3 (Figure 19). Based on the computed weighted average RR of 1.094, for the entire population, and applying the original formulation of Catelinois et al. (2006), the number of lung cancers attributed to radon is 616, out of 21,356 people, for the whole exposure time considered. This corresponds to a percentage of lung cancers due to exposure to radon (%LC_{Rn}) of 8.6%. The same number was also predicted by using the proposed simplified formulation (for %LC_{Rn}), which if rounded, is exactly the 9% of lung cancers attributable to indoor radon exposure reported by Darby et al. (2006), thus exhibiting excellent agreement with this but also with other epidemiological studies, too (Catelinois et al., 2006; Lubin and Steindorf, 1995, b).

4.2.2. Estimation of radon-induced lung cancer incidence for rats, based on TF-TR model

To predict the number of radon-attributed lung cancers for the rat experimental data of Monchaux (2004) with the TF-TR model, the dose-exposure conversion factor 1WLM=0.008Gy was used (Hofmann et al., 1993, b) and the time of exposure T (days) was derived from the experimental data (Monchaux, 2004). The following table summarizes the TF-TR model predictions of the lung cancer relative risk (RR) for the experimental doses (D):

TAB 4. Radon-induced lung cancer relative risk predicted by TF-TR model for experimental doses reported by Monchaux (2004)

WLM	Gy	RR(D)
0.25	0.002	1.00002
25	0.2	1.00292
25	0.2	1.01662
42	0.336	1.01396
50	0.4	1.01424
105	0.84	1.15047
107	0.856	1.06843
100	0.8	1.06432
100	0.8	1.01534
200	1.6	1.15713
500	4	1.13697
1000	8	1.03785
3000	24	1.00114
6000	48	1.00114

Monchaux (2004) reports the total number of rats in each xposure cathegory and the corresponding number of lung cancers. From these data, we estimated the lung cancer

frequency for each cathegory. By multiplying the lung cancer RR predicted by TF-TR model with the lung cancer frequency, a lung cancer RR of 1.7247 for the whole rat population under study was obtained. With this information and the N_{Rn} formula we assessed the number of lung cancers attributed to radon, for each rat group, based on TF-TR model predictions.(**Truţă-Popa** et al., 2009, d). By comparing these theoretical predictions with experimental data (Figure 20), it could be observed that they are in great agreement, the shape of dose-effect relationship is the same, first increasing to a peak value, and then decreasing, probably due to the cell killing occuring at such high doses.

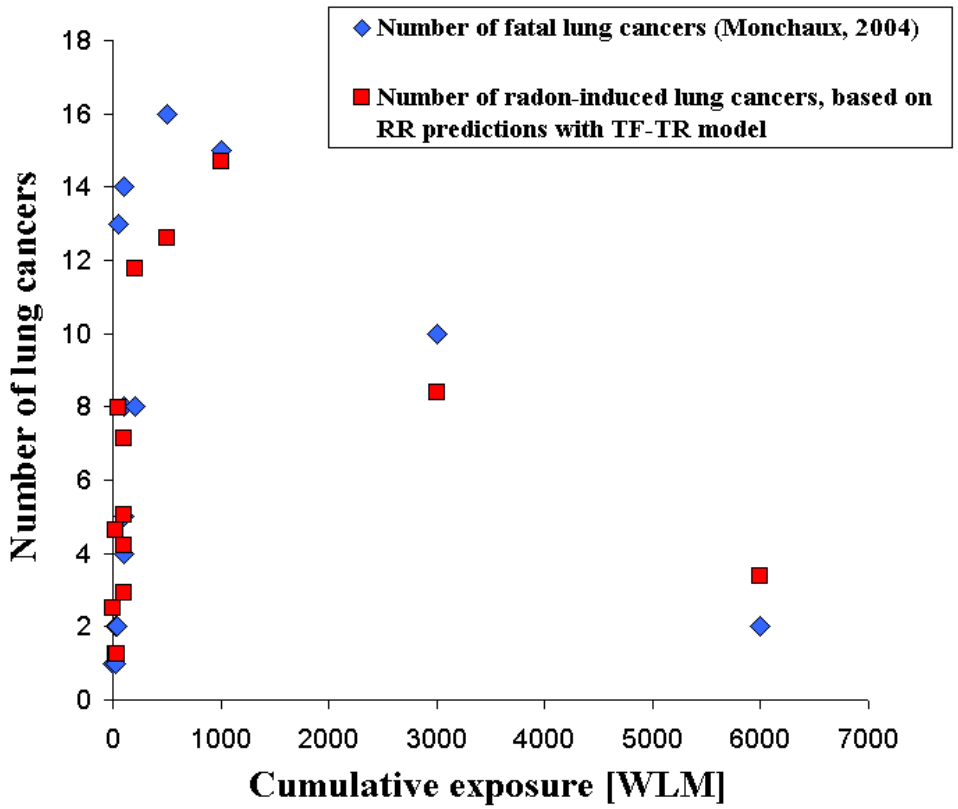


Figure 20 *Comparison between the number of lung cancers predicted by dintre estimările numărului de cancere pulmonare prezise cu modelul TF-TR şi numărul de cancere pulmonare fatale atribuite radonului, raportat de Monchaux (2004)*

The differences may be included in the confidence limits. The fact that the number of lung cancers predicted by TF-TR model is so simmilar to the number of fatal cancers reported experimentally could be explained by the fact that the rats under study exposed only at one carcinogenic agents, i.e. radon, and not others, that could also cause lung cancer (for example cigarette smoke). The great aggreement between the TF-TR model predictions and the experimental observations without normalization to be needed (as in the epidemiological cases) represents a cery important stage of this study, validating the model proposed.

CHAPTER 5.

CONCLUSIONS

- While single hits produce a linear dose-response relationship at low radon exposure levels, multiple hits may play an increasing role at higher cumulative exposures, eventually leading to a nearly linear dose-response relationship over the whole exposure range. Predicted transformation frequencies due to multiple hits increase in a sublinear fashion with increasing multiplicity of hits, consistent with experimental *in vitro* data from micro-beam experiments. Thus, if multiple hits are required for cancer initiation, then a sublinear dose-effect curve may be expected in the low exposure range.
- Lung cancer risk simulations based on experimentally obtained transformation data indicate that cancer induction for continuous exposures is related to the cycle time of an irradiated cell, thus exhibiting a distinct dose-rate effect. This finding is consistent with rat inhalation experiments where an inverse dose rate effect has been observed at intermediate exposure levels (Cross, 1988). While the dominant role of single hits leads to a linear dose-response relationship at low radon exposure levels, predicted lung cancer risk for a collection of interacting cells exhibits a linear-quadratic response.
- Integrating the hot spots effect in the initial TF-TR model, for a hot spot dose contribution of 10% of the total dose, would give much closer values to the epidemiological results than for a 20% contribution. Thus, assuming that about 10% of the bronchial cells located at the airways' bifurcations receive a 10 times greater dose, the lung cancer relative risk for the same average dose is only a little higher at low exposures, thus suggesting that this hypothesis would better illustrate the real conditions.
- For low doses of ionizing radiation, for example radon, the lung cancer risk predictions of TF-TR model also take into consideration non-targeted effects such as bystander mechanisms, genomic instability, induction of apoptosis and adaptive response,occurring particularly at low level exposures. In general, non-targeted effects like genomic instability and bystander effects amplify the biological effectiveness of a given radiation dose by effectively increasing the number of cells that experience effects over those directly exposed to the radiation. On the other hand, induction of apoptosis and adaptive response will decrease the risk values and thus can be considered as defense mechanisms against oncogenesis. While these observations are related to the absolute number of lung cancer cases, the results of the present calculations suggest that their effect on the shape of the dose-response relationship may be different, due to normalization process. Indeed, genomic instability and adaptive response cause a substantial reduction of the risk at low doses, while induction of apoptosis and detrimental bystander effects slightly increase

the risk. The non-targeted effects analysed in this work suggest that the relevant target for the detrimental radiation effects described in this study may be much greater than single cells, i.e. a collection of cells or small tissue volumes (Hofmann, Truţă-Popa, and Fakir, 2006).

- The lung cancer risk predictions of the TF-TR combined with BEIR IV model as well as the TF-TR model integrating the hots spots effect are in great agreement to the available epidemiological data, the interaction between radon progeny and smoking having a synergistic, multiplicative effect. The increase of lung cancer risk values with the number of cigarettes smoked/day (CS), for the case of simultaneous exposure to radon and smoking, is obvious and in excellent aggreement to the observations of other studies (Hofmann et al., 1993; Lee and Lichtenstein, 1999; BEIR IV, 1988). For the same number of cigarettes smoked/day (CS), although dose rate is smaller in the case of Czeck miners (compared to the Colorado Plateau), the risk values are higher, for the same dose (**Truţă-Popa** et al., 2010), effect interpreted as inverse dose-rate effect or protraction enhancement effect, reported in other studies, also. This fact confirms the hypothesis that protracted exposures at lower doses are more dangerous for health than acute exposures at high doses, thus suggesting that residential radon exposures of general population should be seriously considered because this could imply significant health risks.
- RR predicted by TF-TR was successfully used in the assessment of fatal lung cancer incidence as a result of radon exposures for rats (Monchaux, 2004). The theoretical and experimental results were in excellent agreement, even after normalization, fact that represents a very important stage of this work, validating the model proposed. By comparing these theoretical predictions with experimental data, it could be observed that the shape of dose-effect relationship is the same, first increasing to a peak value, and then decreasing, probably due to the cell killing occuring at such high doses. The fact that the number of lung cancers predicted by TF-TR model is so simmilar to the number of fatal cancers reported experimentally could be explained by the fact that the rats under study exposed only at one carcinogenic agents, i.e. radon, and not others, that could also cause lung cancer (for example cigarette smoke).
- The linear risk model of Darby was used to simulate the dose-effect relationship and relative lung cancer risk at low doses of alpha particles specific to residential radon exposures. Predicted relative risks at the measured exposure levels, together with information on the total number of reported lung cancer deaths and the number of people living in these regions enabled us to estimate the fraction of lung cancer cases in each area attributable to radon. These percentages are 16.67% for Stei area, 9.09% for Cluj, 5.66% for Bistrita-Nasaud, 4.76% for Sibiu, and 12.28% for Alba county among lifetime non-smokers. Assuming that the smoking rates are similar for the investigated regions (10.72% smokers among man and 5.95% among women), around 64 to 69% of the total number of annual lung cancer deaths, stratified by sex,

would be attributed to radon and occur among smoking male population, and around 35 to 44% would be attributed to radon and occur among smoking female population (Truţă-Popa et al., 2009).

- The method we proposed for the computation of percentage of radon-induced lung cancers was validated by comparison with the epidemiological data of Darby and colleagues (2006). The predicted percentage of lung cancers due to exposure to radon ($\%LC_{Rn}$) of 8.6% is exactly the same as the epidemiologically reported one (Darby et al., 2006) of 9%, if we round it, and is in good agreement to other studies (Catelinois, 2006; Lubin and Steindorf 1995, b). This means that the method we proposed is good.

In conclusion, the study represents an important step towards a comprehensive model that integrates essential biological mechanisms influencing the dose-response relationship at low, but also at high doses of alpha particles. The models proposed in this study offer, among others, the posssibility of lung cancer risk estimation in any exposure conditions to radon (and radon-cigarette smoke), or facilitate the estimation of the number of lung cancers (cases or deaths) attributed to radon exposure. Model predictions could thus represent a base for elaborating rules and regulations for human safety, outlined in radio-protection guides.

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