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# INVESTIGATIONS ABOUT THE ACTION OF IONIZING RADIATION IN VITRO AND IN VIVO

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## ZUSAMMENFASSUNG

Die vorliegende Arbeit befaßt sich in einer Übersicht mit mehreren Studien aus dem Gebiet der natürlichen und künstlichen Radioaktivität und deren Wechselwirkung auf Zellen und auf den Menschen. Als natürlich ionisierende Strahlenquelle wird Radon-222 in seinen zellphysiologischen Effekten untersucht. Am Beispiel der künstlichen ionisierenden Strahlung durch den Fallout von Tschernobyl werden die drei Hauptkontaminationswege: externe Strahlenbelastung, Ingestion und Inhalation beschrieben, und in ihren Auswirkungen auf Mensch und Zellparameter erforscht.

## SUMMARY

The present review summarizes several studies, which deal with natural and artificial radioactivity and their action on cells and man. Radon-222 as natural ionizing radiation source will be investigated with respect to its cell physiological effects. Taking as example the artificial ionizing radiation due to the Chernobyl fallout, the three main contamination ways: external irradiation, ingestion and inhalation are described, and their action on man and cellular parameters is investigated.

## INTRODUCTION

Ionizing radiation affects living matter by damage induction ranging from reversible perturbation of cell physiological parameters to irreversible DNA mutation, which may ultimately lead to cancer development in man. The primary physico-chemical mechanism is based on the formation of radicals and other reactive intermediate species. While the indirect radiation effect implies damage to the target via water radiolysis, direct radical formation of the target molecules is caused by the direct interaction. Due to a limited repair capacity, the nucleic acids are the most sensitive targets.

Ionizing radiation can be classified into two sources:

1. Natural radiation as generated by Radon-222 in air and water and by other decay products of the uranium- and thorium-series in building material and natural soil. Cosmic rays contribute also to the natural radiation background.
2. Artificial ionizing radiation is caused mainly by medical diagnosis (e.g. thyroid analyis, x-rays,..) and radiotherapy, and by fallout products due to weapon tests or nuclear accidents, as in the case of Chernobyl.

Increased cancer risk can be caused by ionizing radiation in different ways: a) direct contamination (skin), b) external irradiation, c) ingestion and d) inhalation (lung).

## 1. The natural ionizing radiation source: Radon-222

Radon and its decay products (Rn-d) are presumed to be one of the strongest natural carcinogens in man's environment based on epidemiological evidence (F. Steinhäusler and E. Pohl, 1983), animal inhalation experiments (J. Chameaud and R. Perraud, 1980; F. Cross et al., 1980) and dosimetric calculations (W. Hofmann, 1984; W. Hofmann et al., 1990). Due to the lack of in vitro data about the carcinogenic potential of radon daughters, experiments have been carried out to investigate the physiologic and metabolic reaction of human lung cells, and of normal and neoplastic transformed lung tissue samples to different levels of exposure to radon and its decay products. The experimental methods used in this study are transmembrane resting potential (TMRP) measurements of the cell membrane by glass-microelectrodes and oxygen consumption measurements of tissue samples by polarographic Clark-style-electrodes. The glass microelectrodes, filled with KCl, with a tip diameter of < 0,5 µm and a resistance of more than 10 MOhm, close the circuit over preamplifier and amplifier with an Ag/AgCl-reference electrode (Licht and Jones 1985; Niemtzov 1985). The Rn-d atmosphere was generated by emanation of radon-222 from a radium-226 source in an incubator. Measurement of radon-222 and Rn-d was carried out by an ionizing chamber and a working- level-meter. While cell cultures were exposed for one week to an atmospheric radon concentration of 260 kBq/m$^3$ (high level exposure) and 37 kBq/m$^3$ (low level exposure), with a Rn-decay product concentration of 6 WL and 0,9 WL, resp. (RaA: 61 and 8,6 kBq/m$^3$, RaB: 25 and 3,6 kBq/m$^3$, RaC: 13 and 1,8 kBq/m$^3$), tissue samples were exposed for one day to the high level Rn-concentration. As a consequence of the high solubility of radon in aqueous solutions, about 17% of the atmospheric Rn-activity is retained in the medium at $37^{o}$ Celsius, causing an alpha particle exposure of the cells (W.A. Jennings and S.Russ, 1948). Saturation of Rn/ Rn-d in the medium was reached after several hours.

The analysis of the investigation of cellular parameters showed the following statistically significant (on the 5%-level, using the U-test) results: Decrease of the membrane potential during radon exposure; an increase of the oxygen consumption of lung carcinoma tissue after radon exposure and a decrease for normal tissue after the same treatment, compared to the corresponding unirradiated control tissues. All experiments may indicate extensive metabolic changes. Supplementary morphological investigations showed a deformation of the cell membrane and symptoms typical of heat treatment.

A depolarisation of the mean TMRP-values (4100 single measurements) during radon-222 and decay product exposure are shown in Figures 1 and 2 for high and low level exposure. After irradiation for 6 days, the TMRP increased again to values close to the control level, which occured in the case of low level exposure after reincubation in a radon-free atmosphere. This points to disturbances in the ratio of negatively and positively charged ions at the membrane due to radon exposure. A change from a log-normal frequency distribution of single TMRP measurements of untreated cells to a bimodal shape after radon treatment could be explained by a critical number of alpha hits, which affect only a fraction of the irradiated cells. Those cells receiving doses below the threshold, exhibit therefore values close to the control.

Figure 3 demonstrates the increase of the oxygen consumption due to radon exposure with 260 $kBq/m^3$ of lung tumor tissue as compared to the unirradiated control value. Four series with exposed normal and tumor lung tissue were carried out. Unirradiated control tissue was measured after the same storage time of 1 day as the treated one. Normal lung tissues reduced their oxygen consumption after radon treatment as compared to the control. This inverse effect may point to a reduction of mitochondria activity, while lung tumor tissue may exhibit stimulated cell energy metabolism (respiration) after radon exposure.

(For more detailed information see Reubel et al., 1987)

## 2. Artificial ionizing radiation caused by the Chernobyl accident

The reactor accident of Chernobyl increased significantly the radiation background due to artificial nuclides, especially in the region of Salzburg. Contamination of the inhabitants of the high fallout region occurred by external irradiation, inhalation and ingestion of radionuclides.

When the radioactive cloud of the reactor accident of Chernobyl reached Austria at the beginning of Mai 1986, a thunderstorm with rain washed out radionuclides in several regions. Short living nuclides as Te-132, 1-131 and 1-132 and long living nuclides as Cs-134 and Cs-137, Ru-103 and Ru-106 contaminated especially areas in Oberösterreich and Salzburg. This led to an increased external radiation burden for man and to a contamination of the food chain from soil via grass to animals. The latter caused an increased uptake of radionuclides by food into the body, leading to accumulation of mainly Cs-134 and Cs-137. Additionally, particles escaped from the reactor, which were composed of a variety of radionuclides. Called "Hot Particles" due to their high activities, they polluted the air, thereby increasing the probability of inhalation and cancer risk. Even in the region of Salzburg, some particles were found.

## 2.1 Artificial external radiation from contaminated soil and surfaces

The distribution and the environmental properties of the Chernobyl- fallout products during the primary phase of deposition was dependent on wind speed, amount of precipitation, topography, shielding structures and material surface characteristics.

Gamma dose rate measurements of several urban structures were carried out in the city of Salzburg as an example for high contamination during the two months after the initial deposition phase and during 6 - 12 months after the accident; included were houses, streets with different traffic frequency, pavements, parking lots, recreation areas and smaller urban structures, such as stone walls. Pre-Chernobyl-data of indoor/outdoor gamma dose rate measurements in the city of Salzburg were derived from a previous study on the natural radiation environment (Steinhäusler, 1982).

Fixed structures were measured by a gamma-dose-rate-meter and portable samples by gamma-spectrometry, using a Ge(Li)-detector (35% efficiency, resolution of 1,8 keV at the 1,33 MeV Co-60 peak).

The mean gamma dose rate of streets in the center of Salzburg with $5{,}7 \cdot (2{,}2)^{\pm 1}$ µR/h and of building material at the same sites with $7{,}6 \cdot (2{,}1)^{\pm 1}$ µR/h was the basis for further comparison with post-Chernobyl data. The contribution by cosmic rays to the external radiation values was 2 µR/h.

In the initial fallout deposition phase, total precipitation up to 40 mm, varying wind speed and shielding structures caused an inhomogeneous distribution of radionuclides, ranging from direct washout to dry fallout. The influence of varying micro-meteorological conditions was shown in the range of the mean gamma dose rate values on walls of houses in different regions in Salzburg one year later: $7{,}5 \cdot (1{,}6)^{\pm 1}$ µR/h up to $13{,}1 \cdot (0{,}8)^{\pm 1}$ µR/h. The extreme values differed statistically significant.

Washout on unshielded surfaces caused Cs-137-activities from 20 to 40 kBq/$m^2$. Dust and top soil values showed a range of Cs-137 values from 2,1 kBq/kg to 172 kBq/kg. Street dust could be higher still by a factor 10 to 50.

Within the following two months the gamma dose rate above soil decreased by a factor 5 due to radioactive decay of I-131 and above concrete by a factor 13 due to both, 1-131 decay and weathering effects (Figure 4). Precipitation in May und June 1986, cleaned urban surfaces from fractions of Cs-137 depending on the surface roughness. In areas with less precipitation the presence of small amounts of water caused the chemically reactive form of Cs-137 rather to bind to the material (Eggleton, 1987, Warming, 1982). The run-off by weathering was supported by washing and cleaning procedures and by mechanical removal of radionuclides, such as by traffic. This could be demonstrated by measurements of street profiles in the stable phase with a small remaining part of the Cs-137-component in the middle of streets and more than the double amount at the curb of the street as indicated by the relevant gamma dose rates of $5,7\cdot(1,1)^{\pm1}$ and $15,0\cdot(1,1)^{\pm1}$ µR/h, resp.

The fraction of Cs-137, removed by run-off from all urban structures during the first weeks after the accident, reached the sewage treatment plants via the drainage system, as indicated by increased Cs-137 values of the sewage sludge in the months from June to October.

Six months after deposition, those structures, which were cleaned from easily removable fractions, showed a chemically- bound radionuclide content of about 5% from the initial deposition. In contrast to this, unchanged or even increased fallout product levels could be detected in other areas. Snow and rain between November 1986 and June 1987 could not affect the surface-adherent fallout component as shown at selected control sites in the city. Different typical structures and types of material (grass, street, wall: 75 cm above ground, open terrace, wooden bridge, pavement) showed no significant temporal changes.

One year after initial deposition, the gamma dose rate of soil (20 µR/h) and of concrete (10 µR/h) at the control station remained elevated compared to pre-Chernobyl values by up to 500%. The remaining fallout products on different urban surfaces were investigated, categorized in vertical and horizontal structures.

Despite of having rough surfaces such as plaster or concrete, vertical walls showed low gamma dose rates ($9,6\cdot(1,3)^{\pm1}$ µR/h) similar to material with smooth surfaces ($8,8\cdot(1,3)^{\pm1}$ µR/h), but were still statistically significantly elevated by 50% over pre-Chernobyl values.

Amongst horizontal structures soil ($18,6\cdot(1,6)^{\pm1}$ µR/h), street curb, ($15,0\cdot(1,6)^{\pm1}$ µR/h) and wood ($14,9\cdot(1,4)^{\pm1}$ µR/h) showed the highest values because of an almost complete lack of run-off, i.e. the nuclides were integrated into the surface and could be found within the top layers.

The mean values of streets were about 80% of the values of soil, indicating a decontamination effect by traffic. Variation in the traffic frequency in different streets was reflected by a large factor of deviation.
The ratio of the mean gamma dose rate of "smooth" ($8{,}8 \cdot (1{,}3)^{\pm 1}$ µR/h) to "rough" ($11{,}1 \cdot (1{,}5)^{\pm 1}$ µR/h) material is about 0,8, since the surface of stone, concrete, and asbestos binds the radionuclides much stronger than glass, marble, plastic, and uncorroded metal. Weathering half-lifes of 350 d for concrete and of 250 d for soil indicate the strong binding of radionuclides to rough surfaces.

The mean values of the Cs-137 concentration of dirt- and dust-samples of main streets ($0{,}9 \cdot (2{,}4)^{\pm 1}$ kBq/kg), measured by gamma-spectrometry, were about 24% of the mean values of side streets, parking lots and garages ($3{,}8 \cdot (4{,}4)^{\pm 1}$ kBq/kg). The cesium fraction was more effectively removed (in addition to weathering effects) by street washing and increased traffic. The samples collected at streets curbs showed that these areas were partly unaffected by washing, weathering and traffic, with Cs-137-values of up to 46 kBq/kg.
Dirt samples of recreation areas show about 100% higher Cs-137 contents ($1{,}9 \cdot (3{,}8)^{\pm 1}$ kBq/kg) than main streets, mainly due to a mixture with soil and organic matter. Results of wipe tests indicated a wide range of possible values on balconies and window sills due to surface differences and cleaning procedures with surface activities ranging from 0,18 to 420 kBq/m$^2$

In summary, it can be said that in an urban environment radionuclides from fallout undergo the following processes: 1) removal (e.g. from the middle of main streets towards the curb; from smooth horizontal material towards the ground), 2) binding to microstructures (e.g. sheet metal, soil, wood, rough material), 3) redistribution in uncleaned parts of the urban environment, where run-off due to precipitation is prevented by water impervious structures.
The external radiation burden in Salzburg, without contribution from medical treatment, is still increased 2-3 times for the year 1991 due to the accident of Chernobyl.
(For more detailed information see Reubel, 1989)

## 2.2 Cesium-137 is enriched in human tissues due to ingestion.

The nuclear accident of Chernobyl in April 1986 increased not only the external radiation background, but contaminated the food chain for man and animals. Direct contamination affected both, the unshielded ground in gardens and fields by penetrating into earth a few centimeters and chemically binding to earth particles, as well as to plants including vegetables, which were cultivated at this time of spring. Contamination of plants, including grass, by the uptake of fallout products from the root system, supplied consumers with radionuclides via vegetables, fruit, meat and milk products. These fallout products were enriched within parts of the of the human body by different amounts and distributions (Dam et al., 1988). The aim of this study was to determine the distribution of the Cs-137 and its variation with time and age in six different human organs. Dose calculations for the different organs based on the UNSCEAR model are compared with values of the whole-body-measurements (UNSCEAR, 1982; Steinhäusler et al, 1988).

For this reason, tissue samples of fat, thyroid gland, lung, kidney, liver and muscle, originating from autopsy of male and female patients with ages ranging from 25 to 97 years, were taken. Tissues from 20 patients were received in the period from November 1986 to January 1987 and additional samples from 49 patients were analyzed in the time from June to August 1987. Alltogether 388 gamma-spectrometric measurements were carried out using a Ge(Li)-detector (see 2.1.).

First series (20 patients):

The geometric mean Cs-137-activities for the six selected organs are shown in Figure 5: fat has the lowest value with 19 Bq/kg, followed by kidney, lung and thyroid gland with 37 - 41 Bq/kg, liver with 47 Bq/kg and muscle with 7O Bq/kg. The histograms of all organs show log-normal distributions. All samples, either grouped according to age (<70 and >70 y), or according to sex, showed no statistically significant difference even with regard to the organ with largest relative difference.

Second series (49 patients):

The mean (geometric) Cs-137-activities for the selected organs of all patients are shown in Figure 5: fat has the lowest value (13 Bq/kg), followed by thyroid gland, kidney and lung (ranging from 39 - 44 Bq/kg), liver (57 Bq/kg) and muscle (76 Bq/kg). The histograms of all organs show also log-normal distributions. The extreme values of each organ could not be correlated with any kind of disease or cause of death. The high age of most of the patients made it unrealistic to search for a correlation of extreme values with a profession or occupation.
For the investigation of age- and sex-dependent tissue deposition of Cs-137, the organ groups were separated according to sex in the age categories < 50, 50 - 70 and > 70 years. The Cs-137 content for men and women could not be correlated to age.

When the mean organ values of men in each age group were compared with those of women in the corresponding age groups, a statistically significant difference resulted only for muscle in the age group 50 - 70 years (39 Bq/kg for women, 84 Bq/kg for men). The comparison of all mean values of all age groups - irrespective of sex - with one another showed only higher values for lung, liver and muscle for patients older than 70 years, which is based on high values for male and female lung and muscle and on an extremely high mean value for male liver.
The comparison between the summarized data of the first and the second series for each organ showed no statistically significantly differing values with the post-Chernobyl time period, with the exception of the thyroid gland. The rank of all organs is equal in both series, with the exception of a small difference for the thyroid gland.

Calculated whole body activities, using the organ mass fraction from ICRP 23 publications (1975), showed a typical log-normal distribution with a geometric mean value of $18 \cdot (2,1)^{\pm 1}$ Bq/kg. This distribution was compared with values derived from whole body measurements (geomentric mean: $40 \cdot (1,6)^{\pm 1}$ Bq/kg), taken during the same time period in the same region (Steinhäusler et al., 1988), with the result of a statistically significant difference. Assuming the dose-conversion factor from UNSCEAR (1982)
$P = 2,4 \times 10^{6}$ Gy/(Bq/kg),
the resulting mean dose per person, derived from the tissue samples is 0,04 mSv, in contrast to a dose of 0,10 mSv, obtained from multiplication of the values of the whole-body measurements with the above mentioned conversion factor.

**Following conclusions can be drawn from the results obtained:**

1. As expected from the literature, (Dam et al., 1988; Liden, 1964; Nay et al., 1964) muscle is the organ with highest values of Cs-137, which is taken up partly by the body instead of potassium, followed by the liver. The lower values of lung, kidney and thyroid gland are also in good agreement with the reported results. An exception is the low but measurable value of fatty tissue, which could not be detected by other groups (Dam et al., 1988).

2. While other investigators reported higher organ values for women than for men in most cases, our measurements resulted in statistically indifferent values for men and women with the exception of muscle: The Cs-137-activity of the female muscle is much lower in the age group of 50 -70 years than the male muscle (almost a factor 1:2), corresponding to the potassium uptake (Dam et al., 1988).

3. The statistically significant increase of the Cs-137-activity in lung, liver, muscle with the age of the patients, from 50 - 70 to 70 years - irrespective of sex - (second series), corresponds to results found before ( Legget, 1986; Liden, 1964) and is due to physiological changes.
4. Calculated whole body activities and resulting doses, using the data from measurements of organ samples, were compared with values gained from the whole body counter with the result of a significant difference by a factor 2,5. This discrepancy may be due to differences in individual living habits, food consumption, age, sex and health status.
5. Most of the results of Cs-137 measurements of human samples and individuals, which were published after the contamination with ,fallout products from the weapon tests about 1965, are gained from whole body measurements, excretion, blood, bone, bone marrow samples and deal with the metabolism of Cs-137. They are therefore not directly comparable with our results.
   (For more detailed information see Reubel et al., 1989)

### 2.3 Air pollultion with "Hot Beta Particles" could induce transformation of lung cells by inhalation.

Since it is known that "hot particles" with high specific activity have been dispersed into the atmosphere during the reactor accident at Chernobyl, it is of primary interest to assess the damage they may induce in lung tissue after inhalation. The particles found have diameters of a few to several hundred micrometers with a radioactive cover, consisting mainly of Ru-103 and Ru-106. For detailed description of other isotopes see the Studsvik report of Devell (1987). Depending on the particle size and activity and the number of particles in the air during a certain time interval, a finite probability per person exists for inhalation of such particles, which can induce cell death or neoplastic transformation (Hofmann et al., 1986).

In contrast to experimental results and models about the effect of hot alpha particles (Hofmann et al., 1988), only few theoretical calculations and experimental data about the effect of hot beta particles are available (Mayneord and Clarke, 1976; Burkart, 1986).

Using the model of Hofmann, it can be concluded that hot particles with extremely high activities induce mainly necrosis of the surrounding cell layer, while particles with lower surface activities are potentially more likely to induce cellular transformation. In order to confirm such theoretical calculations, human lung fibroblasts (WI 38) were exposed in a preliminary study to a Ru-106 hot particle with an initial surface activity of 2,4 kBq. The particle was either placed under a tissue culture plate with a defined amount of wells (50% passage of the beta particles), where the cells could be cultivated in different distances to the hot particle for growth investigation or under a petri-dish with a plastic bottom (75%

passage of the beta particles) for transmembrane resting potential (TMRP) measurements. Parameters of investigation were the vitality and growth rate of cells and any indication of transformation, measured by the TMRP (description see chapter 1.) and the cell proliferation activity. The latter can be determined by photometric methods: The light absorption of a monochromatic beam by stained cells in a monolayer in each single well of a tissue culture plate is proportional to the cell concentration. Transformation might be accompanied by an increase of the characteristic resting potential of a cell due to enhanced physiology, while cell death is indicated by TMRP decrease.

The results showed that cell death of WI38 cells could not be found as a consequence of the exposure to the Ru-106 hot particle.

While increased TMRP values, which might indicate cell transformation, were not observed, increased cell proliferation due to beta irradiation could not be excluded based on the preliminary photometric results: In 2 of 5 samples the cell density and concentration decreased significantly with the distance (1, 2 and 3 cm) from the particle (Figure 6).

(For more detailed information see Reubel and Muss, 1988).

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**Figure Captions:**

Figure 1: Depolarisation of the mean TMRP values of WI38 cells, exposed to an atmospheric radon-222 concentration of 260 kBq/m$^3$ The values are normalized to 100% control value.

Figure 2: Depolarisation of the mean TMRP values of WI38 cells, exposed to an atmospheric radon-222 concentration of 37 kBq/m$^3$ The values are normalized to 100% control value.

Figure 3: Oxygen consumption measurement of tumor lung tissue after exposure to an atmospheric radon-222 concentration of 260 kBq/m$^3$ C: control measurement.

Figure 4: Temporal changes of the gamma dose rate of soil and concrete in Salzburg city for the period of May 7, 1986 - May 25, 1987.

Figure 5: Geometric mean and standard deviation of the Cs-137 activities of human organ samples.

Figure 6: Measurement of the density of WI38 cells by light absorption, after exposure to a Ru-106 hot particle for 10 days, in different distances from the particle.

Figure 1: Depolarisation of the mean TMRP values of WI38 cells, exposed to an atmospheric radon-222 concentration of 260 kBq/m3. The values are normalized to 100% control value

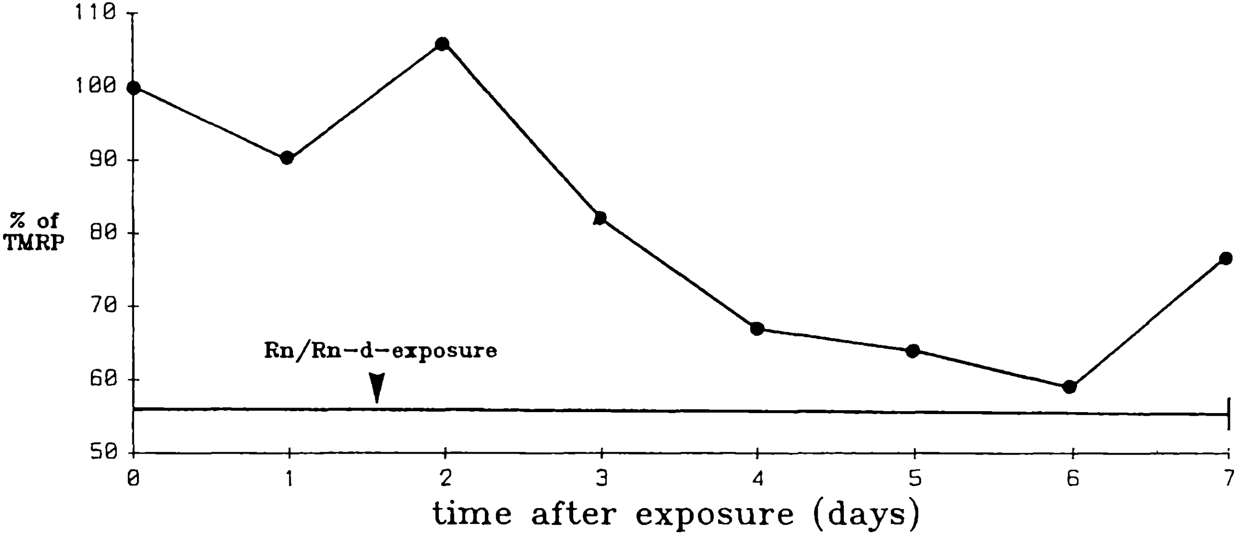


Figure 2: Depolarisation of the mean TMRP values of WI38 cells, exposed to an atmospheric radon−222 concentration of 37 kBq/m3. The values are normalized to 100% control value

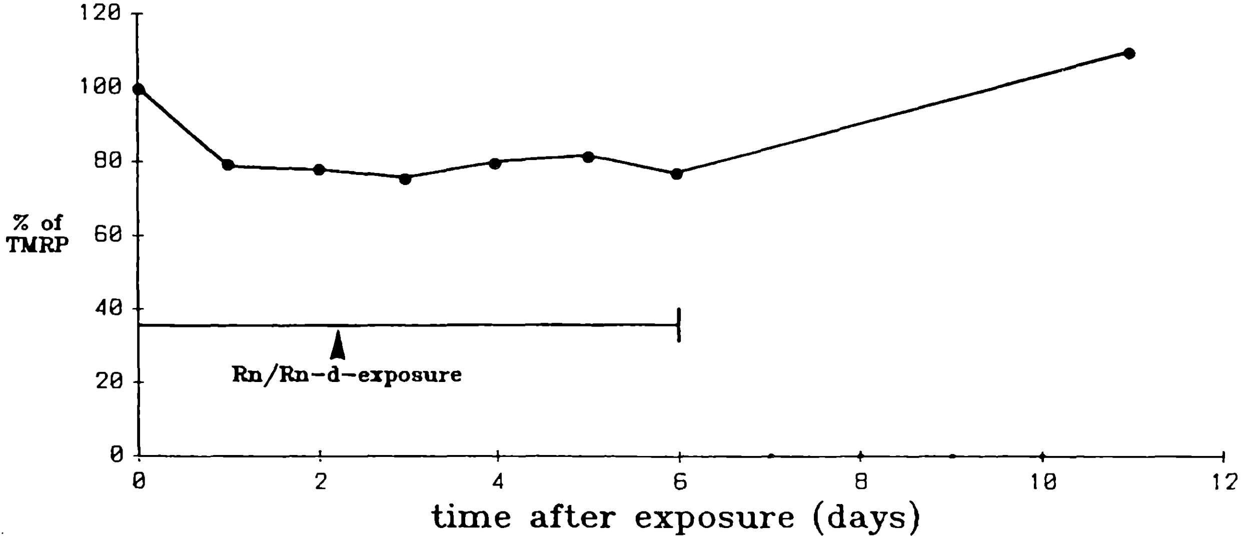


**Figure 3: Oxygen consumption measurement of tumor lung tissue after exposure to an atmospheric radon-222 concentration of 260 kBq/m3. C: control measurement.**

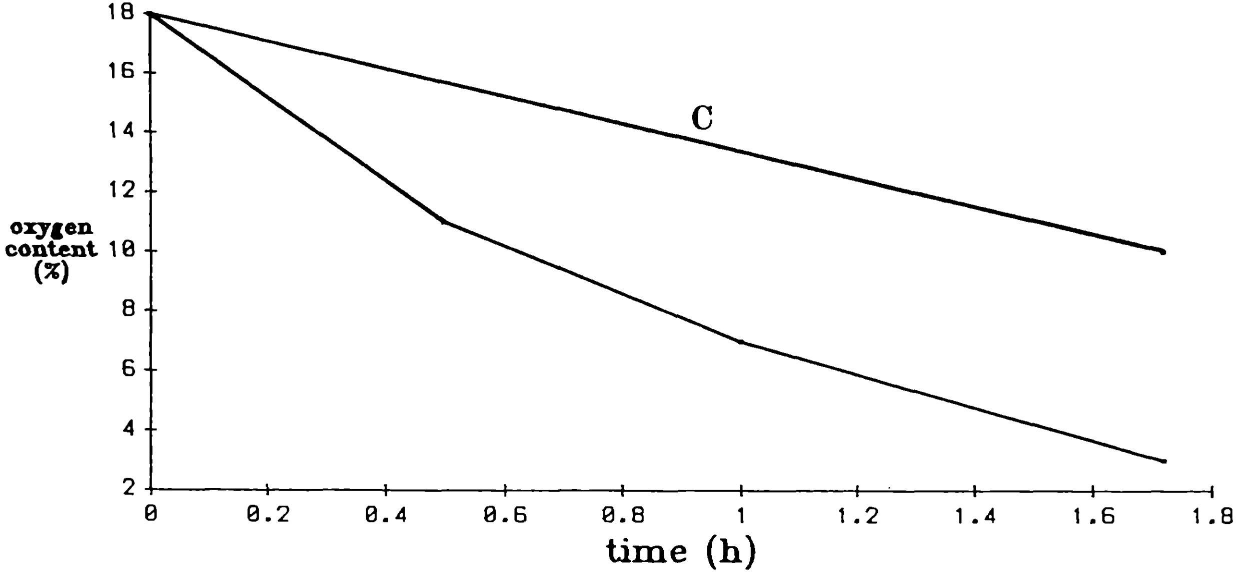


Figure 4: Temporal changes of the gamma dose rate of soil and concrete in Salzburg city for the period of May 7, 1986 - May 25, 1987

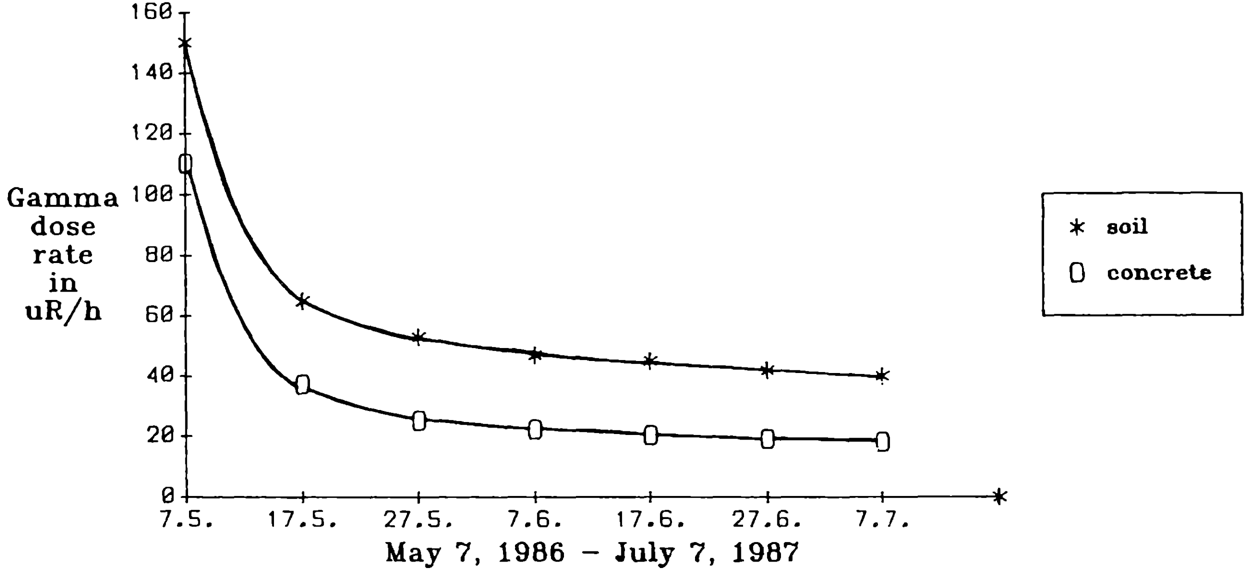


Figure 5: Geometric mean and standard deviation of the Cs−137 activities of human organ samples

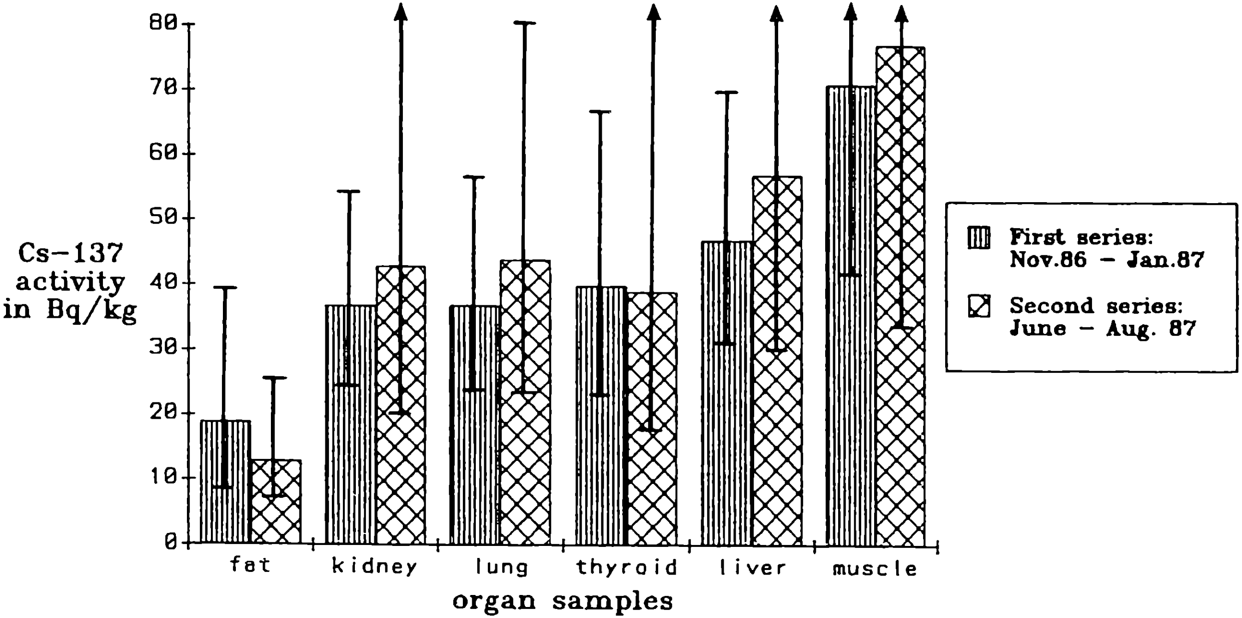
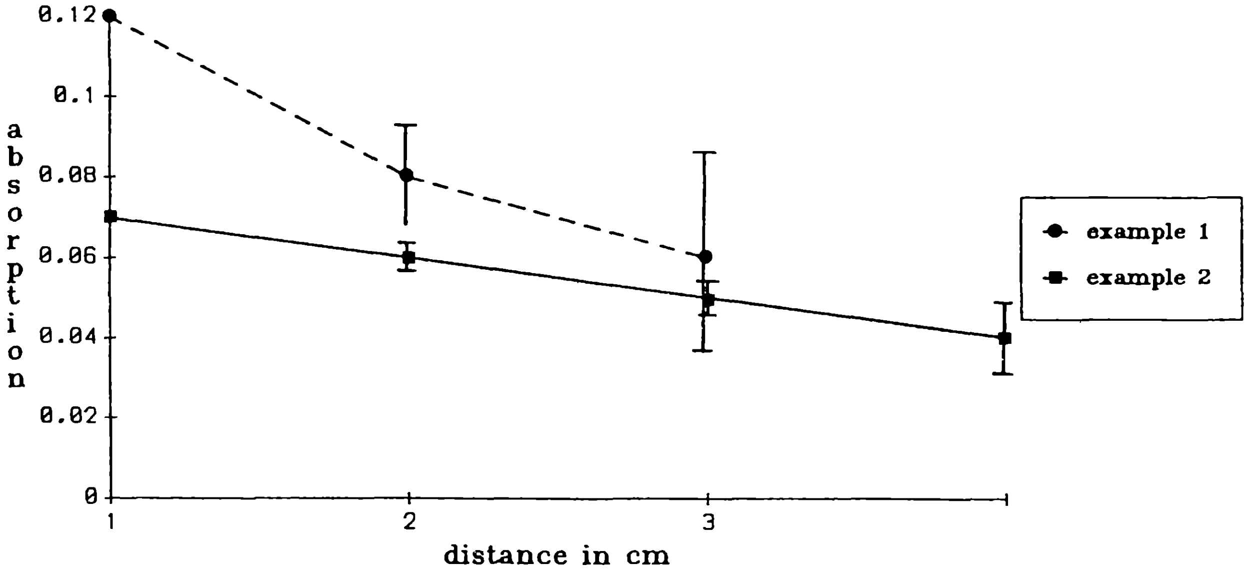


Figure 6: Measurement of the density of WI38 cells by light absorption, after exposure to a Ru-106 hot particle for 10 days, in different distances from the particle.



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