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Report on predicted effectiveness of tritiated products

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Summary

Aim of the WP3 of the TRANSAT project is to study the impact of tritiated products on environment and human health. In this framework, in order to investigate the possible biological damage induced by radiation emitted by tritiated particles at the cellular level (Task 4), we created a software replica of the setup used for in--vitro experiments, with the BEAS--2B cell line being exposed to tritiated steel particles. The activity in Task 4 that is described in this Deliverable has been focused on the modeling of the radiation field due to the particle presence in proximity of cells, to estimate the dosimetric quantities of interest, namely the radiation dose absorbed by cells and, more specifically, by cell nuclei, the main critical target for radiation action. The dosimetric analysis also sets the basis to make predictions on tritium--induced DNA damage, as an indicator of the biological effectiveness of these peculiar tritiated products, to be further correlated with biological data.

Approval

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TRANSAT
TRANSversal Actions for Tritium

Report on predicted effectiveness of tritiated products

TRANSAT DELIVERABLE D3.9

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Table of contents

Index of Tables

Table of figures

Abbreviations

WP	Work package
AMU	Aix Marseille University
IAEA	International Atomic Energy Agency
CEA	Commisariat à l'Énergie Atomique et aux Éenergies Alternatives
IRSN	Institut de Radioprotection et de Sûreté Nucleáré
LET	Linear Energy Transfer
RBE	Relative Biological Effectiveness
HTO	tritiated water
OBT	Organically Bound Tritium

Summary

Aim of the WP3 of the TRANSAT project is to study the impact of tritiated products on environment and human health. In this framework, in order to investigate the possible biological damage induced by radiation emitted by tritiated particles at the cellular level (Task 4), we created a software replica of the setup used for *in-vitro* experiments, with the BEAS-2B cell line being exposed to tritiated steel particles. The activity in Task 4 that is described in this Deliverable has been focused on the modeling of the radiation field due to the particle presence in proximity of cells, to estimate the dosimetric quantities of interest, namely the radiation dose absorbed by cells and, more specifically, by cell nuclei, the main critical target for radiation action. The dosimetric analysis also sets the basis to make predictions on tritium-induced DNA damage, as an indicator of the biological effectiveness of these peculiar tritiated products, to be further correlated with biological data.

1 Introduction

Tritium (^{3}H) is a beta emitter and a radioactive isotope of hydrogen with a physical half-life of 12.3 years. There exist two main sources of tritium: the natural source is the action of cosmic rays on nitrogen, oxygen and argon in the atmosphere (stable inventory of around 3.5 kg on Earth and a natural production of nearly 200 g/year), while non-natural sources are related to industrial activities in the nuclear power field, as later discussed.
Tritium is very mobile and released mainly as tritiated water in the environment, directly from any of the above-mentioned sources, or from tritiated waste storage or treatments. This has led to environmental and health impact issues. At present, due to Deuterium-Tritium fusion reactors development studies as well as to the decommissioning of old nuclear power facilities the tritium release in the environment is expected to increase. These additional releases combined with authority and public acceptance pressure lead to the need for new tritium release impact mitigation strategies to be developed, as well as calling for a better understanding of tritium impacts on health and the environment.
In this context, one of the TRANSAT project goals is to improve the knowledge in the field of radiotoxicity, radiobiology, dosimetry for specific tritiated products. In the nuclear field, tritium can be released into the atmosphere as tritium gas or tritiated water. Some of the tritium release can be then transformed in organically bound tritium (OBT). The radiotoxicological consequences of a contamination by tritiated water or organically bound tritium in animals or cells have been identified during experiments mainly at high tritium concentrations [1]. Moreover, epidemiological studies conducted on workers, who may have been exposed to tritium, have included doses due to tritium exposure, but tritium-specific doses have not been the subject of a separate assessment [2]. As such, at present these studies provide only limited indications of the risks to health posed by tritium exposure, besides being also limited to the more common tritiated products only.
During the decommissioning of nuclear facilities, operations are intended to remove or eliminate any tritiated material. These operations generate fine airborne dusts, namely aerosols, with the possibility of having tritiated products with peculiar chemical speciation due to tritium interactions with building materials [1]. The same holds for tritium interactions with elements of new fusion reactors. It is therefore proposed here to study the consequence of the release of specific tritiated particles in terms of radiotoxicology and ecotoxicology. The cross-cutting materials identified in the TRANSAT project as interesting in both the above-mentioned scenarios (hence for fission and fusion activities) are stainless steel and cement particles. The outcomes foreseen in this project will help radiation protection authorities, IAEA and other nuclear safety advisory organizations to assess more precisely the radiobiology, dosimetry, genotoxicology and ecotoxicology of such specific tritiated particles of micrometric and sub-micrometric size. Accordingly, new safety rules and radiation protection approaches should emerge for safe handling of tritium, especially during dismantling activities.

In the framework of the TRANSAT project relevant particles, such as stainless steel and cement ones, have been produced in sufficient quantities, well characterized and then tritiated. After these steps, studies on radiobiology, radiotoxicology and ecotoxicology have been undertaken. The biological effectiveness of tritiated particles in inducing damage at the cell level have been investigated with in vitro measurements using a non-tumorigenic lung epithelial cell line, to gain insights on damage-inducing mechanisms related to inhalation of such particles. As a general strategy, non-tritiated (hydrogenated) particles have been used as a control condition, in order to assess the entity of damage (if any) related to the contamination of cells with such particles only. On top of that, tritiated particles might lead to radiation-induced damage. In order to establish to what extent radiation plays a role in the possible biological damage induced by such particles, it becomes essential to provide an estimate of the radiation dose (energy deposited by radiation per target mass) absorbed by cells, and more specifically, cell nuclei containing the genomic material as the main critical target for radiation action. Within this context, the purpose of Task 3.4 of the TRANSAT project was to perform such dosimetric estimate reproducing the experimental setup used for these *in-vitro* studies, as well as to provide indications on the expected biological effects induced by radiation. This document is presenting the strategy that was developed to achieve such objectives and the results achieved in this Task.

2 Modelling cell cultures and exposure conditions

This work concerns the modelling of tritium concentration/dose deposition at the cellular level in the experimental setup used, an *in-vitro* human bronchial epithelial cell line (BEAS-2B) model (cultured in adherent conditions). Modelling allows to evaluate the dosimetric quantities and make considerations about the tritium biological effectiveness and the associated radiaton-induced damage to the cell and, in particular, the cell nucleus.
The simulations have been performed using the Monte Carlo radiation transport code PHITS (version 3.22) for the construction of the cell model, dosimetric assessment and characterization of the radiation field in cell nuclei, and using a database of results obtained with the biophysical code PARTRAC to estimate biological effectiveness based on DNA damage induction.

2.1 Tritiated steel particle source

In order to develop a model able to reproduce the experimental conditions, we need to study the cell morphology. Confocal microscopy images of bronchial epithelial cells (BEAS-2B) treated with steel particles have been acquired and provided by the AMU partner of the project and successively analysed with the software ImageJ, to extract the cell component sizes. Figure 1 shows an example of a cell analysed and reconstructed in 3D, whose dimensions are reported in Table 1 together with the steel particle radius R, taken as an average value from particle characterization conducted by the IRSN and CEA partner [3]. r_n is the radius of the cell nucleus, l_1 and l_2 refer to the cytoplasm, whose shape is irregular and can vary a lot from cell to cell, and h is the cell thickness. Another important information that has to be extracted from microscopy images is the position of steel particles relative to cells. In particular, the image analysis has led to the conclusion that steel particles are not internalized in cells (as it might be expected because of their size), but rather deposited on the cell surface, at different possible distances from cell nuclei. In some cases, some sort of "depression" in the cell membrane could be observed, due to the weight of the particle, which is possibly bringing the particle, though not internalized, closer to the nucleus. Also, from the confocal images it was not possible to estimate the thickness of the cytoplasm layer above the nucleus. For these reasons a very thin cytoplasm layer (0.1 µm) was assumed and included in the cell model above the nucleus, as later detailed.

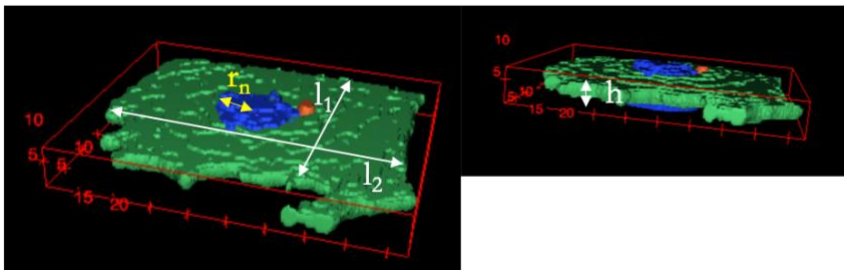

Figure 1: 3D reconstruction from confocal microscopy images. A bronchial epithelial (BEAS-2B) cell treated with steel particles.

Table 1: Typical dimensions of a cell and a steel particle. rn, l_1, l_2 and h refer to Figure 1 and are measured from confocal microscopy images.

cell	[µm]
r_n	8
l_1	47
l_2	49
h	16
steel part.	**[µm]**
R	2.35

Starting from the image analysis and taking into account the culture experimental conditions (see later Table 4), we built a representative software replica of a cell model, as shown in Figure 2. The

cell, whose cytoplasm (in green) is shaped as a rhomboid prism and the nucleus (in blue) as a cylinder, are modelled as water equivalent. The dimensions of each component are reported in Table 2. The water volume V_u, containing exactly a single cell and a tritiated steel particle deposited on the upper surface of the cell, represents our reference volume unit. Considering this configuration, one steel particle per V_u, we are assuming in our volume a steel particle concentration of $1.33 \cdot 10^4$ µg/ml. It is worth noting that such concentration is much higher than the experimental one, which is in the range 1-100 µg/ml, and a rescaling of results to concentrations used for the experiments will be needed, but we will address this issue later in the text.
To estimate the dose to the cell nucleus, we simulated the tritium decay within the model. Tritium decays into ^{3}He by β-decay as:

$$^3_1H \rightarrow ^3_2He + e^- + \bar{\nu_e}. \qquad (i)$$

The full energy distribution of emitted electrons has been considered in the simulation. Electrons have an average energy of 5.7 keV, corresponding to a range in water/tissue of 0.5 µm, which is much smaller than the nuclear diameter. As it will be later discussed, this makes the positioning of the particle relative to the nucleus a key information to perform a dosimetric assessment.

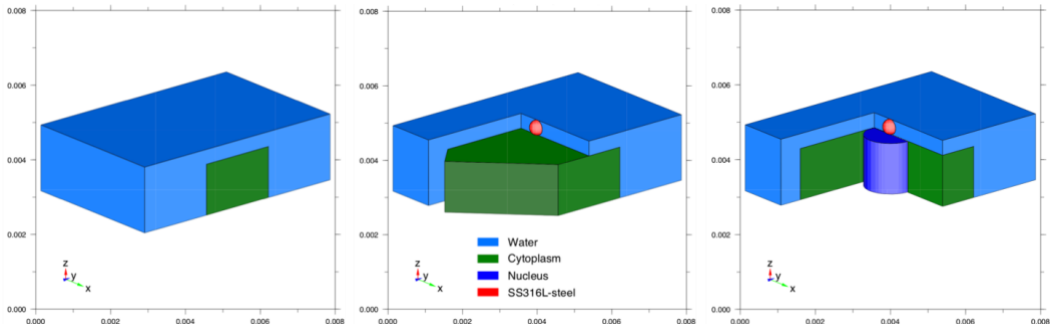

Figure 2: The cell model used for simulations (Monte Carlo transport code PHITS v.3.22). Left panel: whole water volume containing a single cell. Central and right panels: two inner views highlighting the cell (central) and the cell nucleus (right).

Table 2: Cell and steel particle dimensions and density used for the model. Cell: r_n and h_n are the nuclear radius and height, <l> and h_{cyto} are the cytoplasm mean-dimension and ρ_{cell} is the water-like density of the cell. Steel particle: R is the steel particle radius and ρ_{steel} is the mean SS316-steel density. Water volume is indicated with V_u.

cell	
r_n	8.00 µm
<l>	60.00 µm
h_n	16.00 µm
h_{cyto}	16.20 µm
ρ_{cell}	1.06 g/cm^3
steel part.	
R	2.35 µm
ρ_{steel}	7.75 g/cm^3
water volume	
V_u	$7.22 \cdot 10^4$ µm^3

In the present case, the tritium source is the tritiated steel particle. In the process of particle tritiation, tritium can be deposited only on the particle surface or permeate beneath the surface, possibly being distributed in the whole particle volume. This information is not easy to access experimentally, and, due to the limited range of tritium decay electrons, has an important impact on the chance that electrons reach the cell nucleus. We have therefore considered the following two different kinds of tritiated particle sources: a surface one, where tritium is only on the particle surface, and a volumetric one, with tritium in the whole particle volume. We have made dose calculations for both these two source types, since they represent the two extreme cases for a tritiated particle.

Figure 3 shows a visual representation (in color scale for particle flux, for illustrative purposes) of electron tracks emitted from the particle for the surface (left panel) and volumetric (right panel) sources.

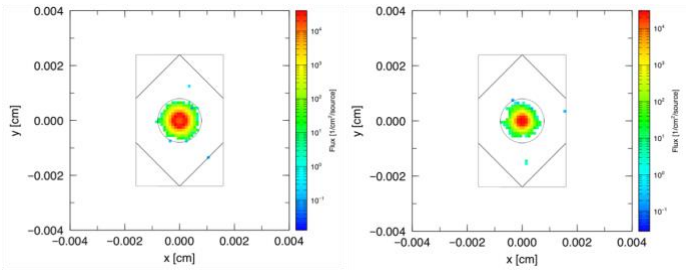

Figure 3: Upper view of the cell with, in colour, tracks of the electrons emitted during tritium decay considering a surface source (left panel) or a volumetric source (right panel).

In order to fully characterize the dose absorption by the cell nucleus, we performed different simulations varying the particle-nucleus distance, i.e. moving the steel particle on the upper surface of the cell, from the nucleus centre along a radial axis with coordinate r (see Figure 4).

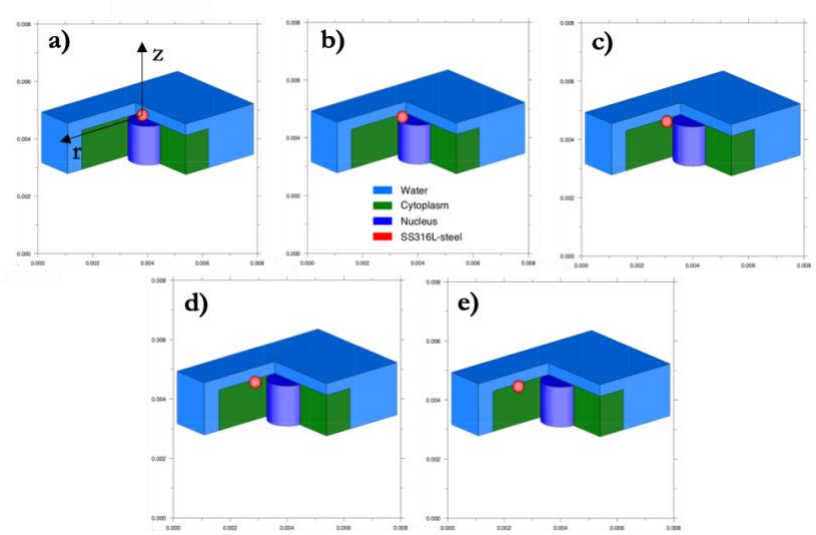

Figure 4: Different simulated configurations for the dose evaluation: the steel particle position changes along the r-direction.

The S-value, i.e. the absorbed dose per decay, for the nucleus for each configuration (distance) has been reported in the graph of Figure 5, for both the surface (blue curve) and volumetric (red curve) sources. The dose per decay to the nucleus is maximum when the particle lies over the nucleus, and it rapidly decreases when it is moved away. In particular, for both the two source kinds, the S-value goes approximately to zero when the particle-source distance is higher than 11 μm. This means that only one nucleus at the time can be reached from electrons emitted by one tritiated particle. The dose per decay to the nucleus is about one order of magnitude lower for the case of volumetric source, since electrons emitted from the inner volume of the particle lose part of their energy or are stopped already inside the particle itself.
It should be noted that the dose is not homogeneously distributed inside the nucleus, since the range of electrons is much shorter than the nuclear dimensions and the electrons are thus stopped in the upper part of the nucleus. “Local-doses” to cell nuclei regions in closer proximity to tritiated particles are expected to be higher and this could have consequences on the spatial distributions of DNA damage, as later discussed.

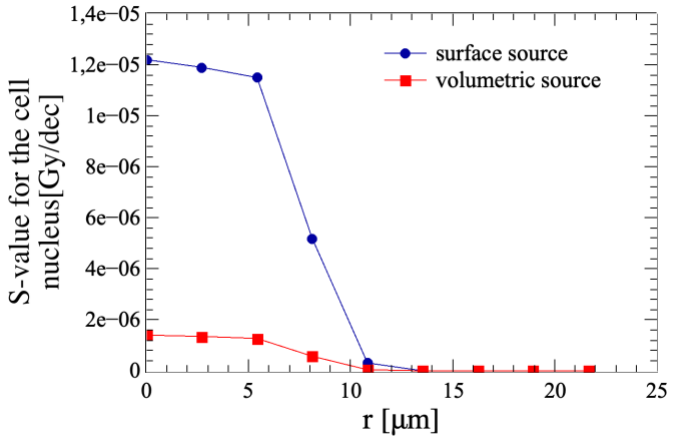

Figure 5: Simulated S-value for the cell nucleus [Gy/dec] as a function of the steel particle - cell nucleus distance (r[µm]) for the case of a surface source (blue line) and of a volumetric source (red line).

2.2 Tritiated water (HTO)

It is interesting to compare the cellular dosimetry for a tritiated particle source to the tritiated water (HTO) scenario. The simulation has been therefore repeated considering the whole volume V_u as source for tritium decays (hence the volume filled with tritiated water). The underlying assumption is that, due to water exchange between the cell and the environment, the whole cell volume is also available for the transport and decay of HTO molecules. The S-value for the nucleus in this case (HTO) is reported in Table 3, together with the two averages of the dose per decay for surface and volumetric tritiated particle sources. Such average values have been calculated using S-values of Figure 5 in the range $0 < r < 11$ µm.

Table 3: Mean dose to the cell nucleus for the three different kinds of sources.

source	S-value (nucleus) [Gy/dec]
HTO	$1.16 \cdot 10^{-5}$
Surface	$8.20 \cdot 10^{-6}$
Volumetric	$9.27 \cdot 10^{-7}$

One can notice that the S-value for HTO is 1.4 times higher than for the surface source. At a first glance this seems to be a low value, since in the HTO scenario the whole volume and thus the whole nucleus itself, which is our target, is a tritium source. However, we are looking at the dose per decay (not at the total dose) to the nucleus, which, for HTO, is dependent on the chosen unitary volume V_u: the bigger the volume surrounding the nucleus, the lower the S-value.

3 From the model to the experimental setup

As mentioned in Section 2.1, in our software model we are considering the presence of a steel particle on the cell surface in the volume unit, which means a steel-particle concentration $C_{mod} = 1.33 \cdot 10^4$ µg/ml, much higher than the experimental one, $1 \leqq C_{exp} \leqq 100$ µg/ml. This means that not all cells will come in contact with a steel particle in real experimental conditions, as later discussed.
A method to go from our software model to the experimental conditions, specified in Table 4, is therefore needed. In particular, as a representative case, we present calculations for the experimental conditions that have been adopted to perform the micronuclei assay. This assay was conducted on cells exposed to tritiated particles for 24 hours. What we want to calculate is therefore the total cumulative dose to the cell population in this time interval.

Table 4: Experimental conditions as provided by the AMU partner for the micronuclei assay on BEAS-2B cells.

cells per well	40000
well surface	1,8 cm^2
volume of medium in a well	800 µl
confluency	60-70%
steel particle concentration range	1-100 µg/ml
specific activity	1 kBq/µg
length of exposure	24 hours

It has also to be considered that, in real experimental conditions, it is possible that part of the tritium carried by steel particles is released to the cell culture medium. Due to the medium composition, it can be assumed that released tritium is then distributed in the whole volume of medium in form of HTO. The amount of tritium release will necessarily affect the dosimetry. It is also expected that such release process has a specific kinetics, meaning that the release might be continuously happening during the 24h exposure period. Also, the particle deposition on the cell surface will presumably be time-dependent, starting at the beginning of the exposure.
For the sake of simplicity, we will make the following assumptions, later discussed in light of experimental data: i) steel particles immediately deposit on the cell surface at the beginning of the exposure; ii) tritium release in form of HTO is immediate and saturates at a given fraction of the total activity of tritiated particles.
At a first stage, the dose calculations in the experimental conditions have been performed assuming two extreme cases: no tritium release from the particles (Section 3.1) and complete tritium release (HTO, Section 3.2). The real conditions, i.e. a combination of these two scenarios, have been then considered (Section 3.3).
We report here the dose evaluation for three different C_{exp} values: 1, 50 and 100 µg/ml, thus including the lowest and highest limits and an intermediate value.

3.1 Dose evaluation in case of no tritium release from the particle

To estimate the dose absorbed by the cell nuclei population in 24 hours, starting from our cell model, we need to determine the dose absorbed by a single nucleus and the fraction of nuclei within the well that are actually irradiated by the tritiated steel particles.
To calculate this latter quantity, first of all we should translate the three concentrations of steel particles into particle numbers per well. Multiplying C_{exp}[µg/ml] by the volume of medium in the well [µl], we obtain the total steel mass per well. To obtain the particle number (n_{part}), reported in Table 5 for each C_{exp}, the total steel mass per well is divided by the one-steel-particle mass.

Table 5: Steel particles per well corresponding to three different experimental steel particle concentrations.

C_{exp} [µg/ml]	n_{part} [steel part. per well]
1	1900
50	95000
100	190000

Now, it is useful to make some considerations on our geometry. Since steel particles deposit only on the cell upper surface, we can refer to a two-dimensional geometry and consider only the upper view of the well. Assuming the steel particles and the cells evenly distributed within the well, the well surface fraction, $A_{\%}$, which receives dose from the n_{part} is given by:

$$A_{\%} = \frac{A_{eff}}{A_{well}} \qquad (ii)$$

where $A_{well}$is the well surface and A_{eff} is an “effective area”. Such area corresponds to the total area within which the nuclei can receive dose from particles, i.e.:

$$A_{eff} = \pi r^2 \cdot n_{part} \quad (iii)$$

We know from Figure 5 that a nucleus receives a significant dose if r, the nucleus-particle distance (centre to centre), is less than 11 µm. Thus, in this case we have assumed r = 11 µm to calculate the effective area. It follows that only M of the total N_{nucl} nuclei in the well receive dose, where:

$$M = \frac{A_{eff}}{A_{well}} \cdot N_{nucl} \quad (iv)$$

Table 6 shows the percentages of nuclei irradiated $M/N_{nucl}[\%]$ for the three steel particle concentrations considered.

Table 6: Percentages of nuclei receiving dose from tritiated steel particles, M/N_{nucl}, for the three concentration values C_{exp}.

C_{exp} [µg/ml]	M/N_{nucl} [%]
1	0.4
50	20.1
100	40.1

The second important quantity we need to estimate is the dose absorbed by a single cell nucleus in a certain time range due to a single steel tritiated particle, d. It can be calculated as:

$$d[Gy] = S - value \cdot A_{source} \cdot m_{steel} \cdot t_{exp}\ , \quad (v)$$

where A_{source} [dec/(s·g)] is the steel particle specific activity, m_{steel} [g] is the mass of a steel particle and t_{exp} [s] is the exposure length (24 hours in this case). Table 7 shows the lower and the upper limits of d, d_{low} and d_{up}, together with its average value $< d >$, for surface and volumetric sources. They have been calculated using the S-value for r = 11 µm, r = 0 µm and the average S-value over the range 0 <r< 11 µm (see Table 3) respectively. On average, if a particle lies at less than 11 µm from its centre, a nucleus absorbs a dose of 0.3 Gy or 3 cGy considering a surface or a volumetric source respectively.

Table 7: Dose to a single cell nucleus due to a tritiated steel particle (eq. 4): d_{low} and d_{up} are the dose lower and upper limits respectively and <d> is the dose average value.

	d_{low} [Gy]	<d> [Gy]	d_{up} [Gy]
surface	0.011	0.30	0.44
volumetric	0.002	0.03	0.05

At this point, the dose to the cell nuclei population D, i.e. the dose absorbed by the whole nuclei population within the well in 24 hours, is obtained by combining the two information just calculated:

$$D\ [Gy] = d\ \cdot M/N_{nucl}. \quad (vi)$$

The average dose to the cell nuclei population, $< D >$ and the lower and upper limits, D_{low} and D_{up}, have been obtained by using in eq. *(v)* $< d >$, d_{low} and d_{up} respectively. $< D >$, D_{low} and D_{up} values for the three different C_{exp} and for the two kinds of particle sources are reported in Table 8, Table 9 and Table 10. As one can see, the average dose in case of surface particle source goes from some mGy up to hundreds of mGy for the concentration range considered, and in case of volumetric particle source it is one order of magnitude lower in the same range.

Table 8: Average dose to the cell nuclei population (eq. (v)) for different steel particle concentrations calculated assuming no tritium release.

	surface source	volumetric source
C_{exp} [µg/ml]	<D> [Gy]	
1	$1.20 \cdot 10^{-3}$	$1.35 \cdot 10^{-4}$
50	$5.99 \cdot 10^{-2}$	$6.77 \cdot 10^{-3}$
100	$1.20 \cdot 10^{-1}$	$1.35 \cdot 10^{-2}$

Table 9: Lower limit of dose to the cell nuclei population (eq. (v)) for different steel particle concentrations calculated assuming no tritium release.

	surface source	volumetric source
C_{exp} [µg/ml]	D_{low} [Gy]	
1	$4.55 \cdot 10^{-5}$	$6.38 \cdot 10^{-6}$
50	$2.27 \cdot 10^{-3}$	$3.19 \cdot 10^{-4}$
100	$4.55 \cdot 10^{-3}$	$6.38 \cdot 10^{-4}$

Table 10: Upper limit of dose to the cell nuclei population (eq. (v)) for different steel particle concentrations calculated assuming no tritium release.

	surface source	volumetric source
C_{exp} [µg/ml]	D_{up} [Gy]	
1	$1.78 \cdot 10^{-3}$	$2.04 \cdot 10^{-4}$
50	$8.88 \cdot 10^{-2}$	$1.02 \cdot 10^{-2}$
100	$1.78 \cdot 10^{-1}$	$2.04 \cdot 10^{-2}$

3.2 Dose evaluation in case of complete tritium release (HTO)

An analogous approach has been adopted to calculate the dose absorbed by the cell nuclei population in 24 hours assuming complete and immediate tritium release by the steel particles or, in other words, an HTO scenario.
In this case, the percentage $M/N_{nucl}[\%]$ of nuclei receiving dose is 100%, since tritium becomes present in the whole well medium, including cell volumes. We need therefore to calculate only the dose to a single cell nucleus d, which, for HTO conditions, can be defined as the dose absorbed by a single nucleus in a certain time range for a given amount of tritium released by the tritiated particles in the volume V_u. It is reasonable to assume that such amount depends on the steel particle concentration, and, more specifically, is proportional to the number of particles that could be found in the volume V_u, n_{Vu}, if particles were distributed homogeneously in the whole medium instead of depositing at the bottom of the well. These values are reported in Table 11 for the three concentrations considered.

Table 11: Steel particle numbers n_{Vu} in the chosen unitary volume V_u for three different steel particle concentrations.

C_{exp} [µg/ml]	n_{Vu}
1	$1.71 \cdot 10^{-4}$
50	$8.57 \cdot 10^{-3}$
100	$1.71 \cdot 10^{-2}$

The dose to a single cell nucleus has then been calculated using eq. *(v)*, assuming that the activity of the tritium released by the n_{Vu} particles is the same as the activity of n_{Vu}tritiated steel particles. Since M/N_{nucl}= 100%, the dose to the cell nuclei population coincides with the dose to a single cell nucleus D. Average dose values for the three C_{exp} are listed in Table 12.

Table 12: Dose to the cell nuclei populations for different steel particle concentrations calculated assuming complete tritium release (HTO).

C_{exp} [µg/ml]	D [Gy]
1	$7.23 \cdot 10^{-5}$
50	$3.61 \cdot 10^{-3}$
100	$7.23 \cdot 10^{-3}$

3.3 Real experimental conditions

We know from experimental tests conducted by the AMU partner of the project (see Figure 6) that, in our condition, the tritiated steel particles release in ~2 hours a percentage of the initial activity that tends later on to stabilize at a ~60% value. Such "saturation" value seems not to vary a lot with particle concentration. Given that ~2 hours is a short time interval with respect to the duration of the exposure, and that the available data do not allow to assess a specific dependence of the amount of tritium release on particle concentration, we suppose that an average percentage of 60% of the total activity is immediately released in the medium in form of HTO, as above introduced.

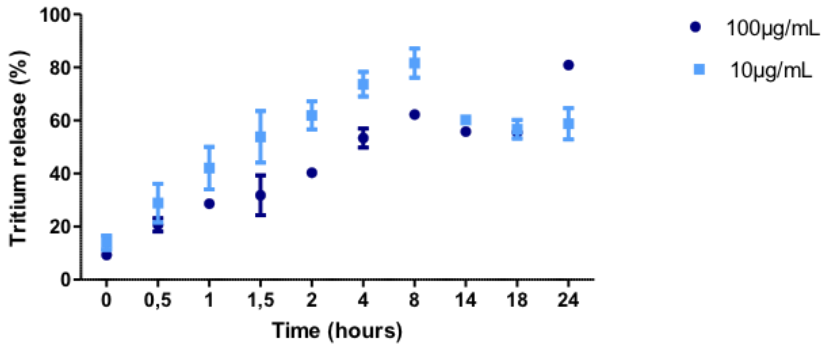

Figure 6: Data from AMU - release of tritium activity in cell culture medium as a function of time (percentage of total initial activity) for two tested concentrations.

For this reason, the dose to the nuclei population in real experimental conditions can be estimated combining 40% of dose for the "no tritium release" scenario and 60% of dose for the full HTO scenario. The average dose values for the real scenario, i.e. values obtained considering $< D >$ values for "no tritium release", are reported in Table 13. The upper and lower dose limits for the real scenario, calculated using D_{low} and D_{up} for "no tritium release", are listed in Table 14 and Table 15 respectively.

Table 13: Average dose to the cell nuclei population in "real conditions": 40% of "no-tritium release" and 60% of HTO.

	surface source	volumetric source
C_{exp} [µg/ml]	<D> [Gy]	
1	$5.23 \cdot 10^{-4}$	$9.75 \cdot 10^{-5}$
50	$2.61 \cdot 10^{-2}$	$4.88 \cdot 10^{-3}$
100	$5.23 \cdot 10^{-2}$	$9.75 \cdot 10^{-3}$

Table 14: Lower limit of dose to the cell nuclei population in "real conditions": 40% of "no-tritium release" and 60% of HTO.

	surface source	volumetric source
C_{exp} [µg/ml]	D_{low} [Gy]	
1	$6.15 \cdot 10^{-5}$	$4.59 \cdot 10^{-5}$
50	$3.08 \cdot 10^{-3}$	$2.30 \cdot 10^{-3}$
100	$6.15 \cdot 10^{-3}$	$4.59 \cdot 10^{-3}$

Table 15: Upper limit of dose to the cell nuclei population in "real conditions": 40% of "no-tritium release" and 60% of HTO.

	surface source	volumetric source
C_{exp} [µg/ml]	D_{up} [Gy]	
1	$7.54 \cdot 10^{-4}$	$1.25 \cdot 10^{-5}$
50	$3.77 \cdot 10^{-2}$	$6.24 \cdot 10^{-3}$
100	$7.54 \cdot 10^{-2}$	$1.25 \cdot 10^{-2}$

3.4 Biological effectiveness

Some considerations can now be made about the tritium-induced DNA damage, as an indicator of the tritium biological effectiveness. Following the approach proposed in [2,3], initial DNA damage yield can be quantified using analytical functions that reproduce damage as a function of linear energy transfer (LET) in cell nuclei, this latter being estimated in its restricted form, conceptually equivalent to the dose mean lineal energy $\bar{y}_D$ (basically, a dose-average of the microdosimetric equivalent for LET). Such functions have been studied to reproduce full results of Monte Carlo calculations with the code PARTRAC, a biophysical code that simulates DNA damage induction starting from initial interactions of particles in a water-equivalent cell model including a software replica of its genomic content as a target. DNA damage can be induced both directly (direct interactions of particles with the DNA structure) or in an indirect way (energy deposition to water molecules surrounding the DNA, with formation and diffusion of radicals, that in turn can interact with DNA and damage it). Different kinds of DNA damage results from radiation action and can be predicted with the code.

In particular, the $\bar{y}_D$-dependent yield of double-strand breaks (DSBs), clusters of DSB (DSB clusters, defined as two or more DSBs within a genomic distance of less than 25 bp) and sites of DSB (DSB sites, a more general definition including isolated DSBs and DSB clusters) is reproduced by:

$$Yield\ per\ cell\ [Gy^{-1}] = 6.6 \cdot (p1 + (p2\ \bar{y}_D)^{p3})/(1\ +\ (p4\ \bar{y}_D)^{p5})\ , \qquad (vii)$$

where $p1$-$p5$ are radiation-dependent parameters.
To apply this formula to predict DNA damage induction in our case, we need to have a characterization of the $\bar{y}_D$ quantity in cell nuclei. This is also possible with the code PHITS, that has a dedicated tally function to calculate the distribution of microdosimetric quantities. In particular, we simulated with PHITS the dose distribution of the lineal energy $d(y)$ in cell nuclei and obtained its first moment $\bar{y}_D$ in different exposure conditions. Simulation results indicate that $\bar{y}_D$is mostly independent on particle position with respect to the cell nucleus, and also on the kind of source considered: as far as the cell nucleus is reached by tritium decay electrons, a constant $\bar{y}_D$~ 10 keV/µm in the cell nucleus is obtained for any of the considered scenarios.
Considering such low $\bar{y}_D$ value, the yield for associated DNA damages have then been calculated using $\bar{y}_D$ and the $p1$-$p5$ parameters for protons in [4] that are assumed to reproduce data for electrons and photons at the low-LET limits.
Under the assumption of the linearity of the DNA damages considered with dose, we can then obtain an estimate of tritium-RBE, radiation biological effectiveness, for each damage, as the ratio of the damage yield following tritium irradiation to the damage yield following photon irradiation at the same dose. The yields and the RBE values associated with DSB, DSB clusters and DSB sites obtained with such methodology are reported in Table 16.

Table 16: Damage yields per cell per unit dose and RBE for double-strand breaks (DSB), their cluster (DSB clusters) and DSB sites.

	Yield per cell [Gy^{-1}]	RBE
DSB	57.75	1.3
DSB cluster	0.55	1.2
DSB sites	57.11	1.3

It is then interesting to consider how these theoretical predictions actually translate into an empirical observation of DNA damage. Considering a single cell, the damage yield to the nucleus can be obtained by multiplying the yield of Table 16 by the dose to its nucleus, as previously estimated.
When a cell is hit by a tritiated particle in experimental conditions, we also have to remember that 40% of the particle activity is still effective in inducing damage directly to the cell, while 60% of tritium was immediately release within the medium. The yield associated with the dose from the tritiated particle itself is therefore:

$$Yield\ per\ cell =\ 40\%\ \cdot d[Gy] \cdot Yield[Gy^{-1}Gbp^{-1}]. \qquad (viii)$$

Table 17 shows the average values of the yield for DSBs, DSB clusters and DSB sites and their lower and upper limits, obtained using the doses of Table 7 for the surface particle source.

Table 17: Yield per cell (average value and limits) of three DSB damages for a single cell nucleus hit by a steel particle.

	$Yield_{low}$ per cell	<Yield per cell>	$Yield_{up}$ per cell
DSB cluster	0.003	0.066	0.098
DSB sites	0.259	6.821	10.113
DSB	0.262	6.897	10.226

If a cell is not in direct contact with a particle, it can only receive dose from HTO, due to the tritium release from particles within the whole medium. The damage yield for a non-hit cell can be calculated

using the 60% of the dose of Table 12. The values for DSBs, DSB clusters and DSB sites associated with the highest particle concentration, C_{exp} = 100 µg/ml, are reported in Table 18.

Table 18: Yield per cell of three DSB damages for a single cell nucleus not in contact with a steel particle (only HTO).

	<Yield>
DSB cluster	0.002
DSB sites	0.248
DSB	0.251

These latter are therefore the damage yields that all the cell nuclei within the well experience for a steel particle concentration of 100 µg/ml. Moreover, at this concentration, ~40% of the nuclei in the well are hit by a steel particle (see Table 6) and thus they undergo additional damage yield, described by *eq. (viii).*

4 Conclusions

In this work, a software model reproducing the experimental setup used for *in-vitro* study, a human lung cell line (BEAS-2B) exposed to tritiated steel particles, has been developed. The tritium concentration/deposition has been modelled to characterize the radiation field produced by the tritium decay, to perform a dosimetry analysis and eventually to provide indications on the expected biological effects induced by radiation. Reference experimental conditions used for the analysis are those used to perform the micronuclei assay on BEAS-2B cells exposed at different particle concentration for 24 hours, as informed by the AMU partner of the project. For the sake of simplicity, we have assumed that particles immediately deposit at the bottom of the well used for cell-culturing, that they are homogeneously distributed within the well surface and that they immediately release part of the activity to the medium (in form of HTO) as indicated by dedicated measurements. The dose to the cell nuclei population was found to be generally low for the tritium concentration analyzed but, more precisely, two dose ranges should be considered. The first one interests cells not directly in contact with a tritiated particle, receiving dose only from tritium released to the medium, and it is not significant as well as the damage yield associated. The second dose range interests cells directly in contact with a tritiated particle and it start to be significant. The tritium concentration plays here an important role, since it determines the percentage of cells in contact with a particle. Such percentage is only 0.4 for the lowest concentration considered (1 µg/ml) but it rises to 40 for 100 µg/ml.

The new modeling strategy presented in this work is meant to be general and it can be used for a broad range of cases. Possible refinements, also depending on the availability of experimental data for benchmark, are possible. As an example, for this work we have simulated all the steel particles with the same diameter, corresponding to the average value of the experimental distribution (see [3]), but the distribution itself could be employed. Moreover, it would be possible to investigate the impact on the dosimetry related to the presence of particle aggregates, which has also been observed experimentally. Finally, other kinds of particles could be considered, including cement particles used in the project, hence with different elemental composition, dimensions and interactions with the biological medium in terms of tritium release or particle solubility.
Results presented in this document also suggest to include in future radiobiological measurements DNA damage markers that can be better related to the initial damage, as well as to its spatial distribution, as for example DNA repair foci, also considering shorter exposures (maybe at a higher particle concentration or activity to compensate for the lower cumulative dose). This would allow to make a better bridge between radiobiological data and initial DNA damage predictions.

5 Bibliography

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