TRANSAT

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Report on acute and long-term toxicities, epi/genotoxic studies and transepithelial transfer of untritiated and tritiated particles on in vitro human models

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Summary

One of the objectives of the TRANSAT project WP3 was to improve the knowledge in the field of radiotoxicity of tritiated dusts that could be emitted during the dismantling of a nuclear power plant. Thus, the toxicity of tritiated cement and stainless steel particles was studied using in vitro methods applied to two human lung models: the BEAS-2B cell line and the 3D MucilAirTM model. Exposures of the BEAS-2B cell line to particles (2- and 24-hours) did not induce significant cytotoxicity. Nevertheless, DNA damage occurred upon exposure to stainless steel and cement particle whatever they were tritiated or not, as observed by alkaline comet assay. Tritiated particles induced cytostasis while the hydrogenated ones did not. Cement and stainless steel particles, whatever they were tritiated or not, induced a significant increase in chromosomal breakage as indicated by the increase in the centromere negative micronuclei formation, investigated by cytokinesis-block micronucleus assay. Particles were also assessed for their effects on cytotoxicity and its reversibility, epithelial integrity and metabolic activity using a 14-day kinetic mode. No effect of the particles on barrier integrity, metabolic activity or cell viability was noted following exposure to cement and stainless steel particles, whatever they were tritiated or not. Tritium transfer through the epithelium was observed, but no intracellular accumulation. Overall, these in vitro data indicate that tritiated or not stainless steel and cement particles were associated with moderate toxicity, in terms of cell death. However, these particles have a clastogenic potential to which tritium seems to contribute. These data may help in a better management of the hazard related to the inhalation of this type of particles.

Approval

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Abbreviations

BEGM	Bronchial Epithelial cell Growth Medium
BSA	bovine serum albumin
CBPI	cytokinesis-block proliferation index
HTO	Tritiated water
MMC	Mytomicin C
MN	Micronucleus
OBT	organically bound tritium
PFA	paraformaldehyde
ROS	reactive oxygen species
TEER	transepithelial electric resistance
SS316L	Stainless steel particle
WP	Work package

Summary

One of the objectives of the TRANSAT project WP3 was to improve the knowledge in the field of radiotoxicity of tritiated dusts that could be emitted during the dismantling of a nuclear power plant. Thus, the toxicity of tritiated cement and stainless steel particles was studied using *in vitro* methods applied to two human lung models: the BEAS-2B cell line and the 3D MucilAir™ model. Exposures of the BEAS-2B cell line to particles (2- and 24-hours) did not induce significant cytotoxicity. Nevertheless, DNA damage occurred upon exposure to stainless steel and cement particle whatever they were tritiated or not, as observed by alkaline comet assay.

Tritiated particles induced cytostasis while the hydrogenated ones did not. Cement and stainless steel particles, whatever they were tritiated or not, induced a significant increase in chromosomal breakage as indicated by the increase in the centromere negative micronuclei formation, investigated by cytokinesis-block micronucleus assay.

Particles were also assessed for their effects on cytotoxicity and its reversibility, epithelial integrity and metabolic activity using a 14-day kinetic mode. No effect of the particles on barrier integrity, metabolic activity or cell viability was noted following exposure to cement and stainless steel particles, whatever they were tritiated or not. Tritium transfer through the epithelium was observed, but no intracellular accumulation.

Overall, these *in vitro* data indicate that tritiated or not stainless steel and cement particles were associated with moderate toxicity, in terms of cell death. However, these particles have a clastogenic potential to which tritium seems to contribute. These data may help in a better management of the hazard related to the inhalation of this type of particles.

1 Introduction

Tritium is a radioactive isotope of hydrogen, with a physical half-life of 12.3 years. It is a beta emitter since its decay leads to the emission of an electron with a mean energy of 5.7 KeV. The main natural source of tritium results from the action of cosmic rays on the atmosphere, and the main anthropogenic source of tritium is associated with the nuclear industry[1]. Tritium is the main

radionuclide released by current nuclear power plants at an estimated level of 0.1 EBq per year [1]. Due to the growth of nuclear power generation worldwide and to the development of nuclear fusion technology, tritium releases into the environment are expected to increase. The beta particles emitted by the radioactive decay of tritium are not energetic enough to penetrate the outer layer of the human skin. Therefore, tritium poses a human health risk only after internal exposure through ingestion, inhalation and absorption through the skin. [1,2]. As an isotope of hydrogen, tritium forms HTO molecules that behave like water in the body. Once in the body, the biological half-life of tritium is estimated to be about 10 days, as HTO is incorporated into the body and eliminated relatively quickly, like H_2O [3]. A fraction of tritiated water may also be incorporated into organic molecules, forming organically bound tritium (OBT). OBT can remain in the body longer, and the biological half-life of a short-term component is about 40 days, and about a year for a long-term component [3]. Thus, the elimination of tritium could be slowed down, resulting in a higher absorbed radiation dose and more or less severe cell damage. Ingestion mostly contributes to exposure of general population while inhalation and absorption are the main routes of intake at the workplace.

During the decommissioning of nuclear facilities, operations are intended to remove or eliminate any tritiated material. These operations generate fine airborne dusts, namely aerosols, among them tritiated dust such as tritiated stainless steel and cement particles due to tritium interactions with building materials. Tritiated particles of micrometric and sub-micrometric size are respirable and deposit in the various compartments of respiratory tract. Workers may inhale particles during these operations and this may constitute a health risk for which there is currently a lack of information.

The health effects of aerosols being dependent on their physicochemical characteristics[4], it is therefore crucial to characterize this type of aerosol in order to determine the health risks in case of accidental or occupational human exposure. However, no data are available on this type of tritiated particles. In this context, one of the TRANSAT project goals was to improve the knowledge in the field of radiotoxicity, radiobiology, and dosimetry for tritiated dusts.

To gain insights on damage-inducing mechanisms related to inhalation of such particles, *in vitro* toxicological studies were accomplished using human lung models. Among the toxicological parameters of interest, genotoxicity is a key parameter because genetic damages play an essential role in the pathological consequences of ionizing radiation. [5]. Therefore, the genotoxicological impact of tritiated cement and stainless steel particles on lung cells has been extensively studied in the present work. The experimental strategy used and the results obtained are presented in this deliverable.

2 Experimental procedures

2.1 Tools and assays used for the characterization of the cyto-genotoxicity of particles on BEAS-2B cells

2.1.1 Cellular model: BEAS-2B

The BEAS-2B cell line is an immortalized but non-tumorigenic human cell line established from normal human bronchial epithelium obtained from a non-cancerous individual [6]. This cell line has been widely used for to determine various chemical, biological and particle agents with potential pulmonary toxicity [7–9].

Cells were cultured in culture flasks precoated with LHC basal medium supplemented with BSA (0.01 mg/mL), human fibronectin (0.01 mg/mL) and collagen (0.03 mg/mL). They were maintained in LHC-9 serum-free medium (Gibco) and BEGM™™ Medium (Lonza; Basel, Switzerland) in humidified atmosphere of 5% CO_2 at 37°C. Before confluence, passage was performed twice a week using trypsin (0.25%)-EDTA (2.6 mM).

2.1.2 Cells exposure to particles

2.1.2.1 Tritiated stainless steel particles

For logistical and safety reasons, it was not possible to collect particles from a dismantled nuclear power plant. Thus, stainless steel particles were produced at the laboratory, cutting 316L stainless steel pieces that representing decommissioning process within nuclear facility. Then the obtained aerosols were characterized. Because of a too low production rate of particles from cutting operations, a commercial powder (FF216030 Goodfellow Cambridge Limited, Huntingdon, England) with similar characteristics of home-made stainless steel particles was used. A tritium loading was performed on particles. Tritiation process consisted of two steps of reduction at 450°C under H_2 with high pressure for two hours, followed by the exposure of particles to tritium gas at 450°C during two hours. This method allowed to reach a specific radioactivity of 1MBq/mg. A degassing time was observed to eliminate the unbound tritium and improve the safety of tritiated sample. To assess the involvement of tritium in the various cyto-genotoxic endpoints following exposure to tritiated particles, all the experiments were also performed using particles loaded with hydrogen in the same conditions.

2.1.2.2 Tritiated cement particles

To mimic particles emitted during decommissioning, cement particles were produced at the laboratory, cutting a plate of hydrated Portland cement with a disk grinder. Particles were collected and characterized. Cement particles were exposed to tritium gas at room temperature to obtain a specific radioactivity of approximately 0.4 MBq/mg. To assess the involvement of tritium in the various cyto-genotoxic endpoints following exposure to tritiated particles, all the experiments were also performed using particles loaded with hydrogen in the same conditions.

2.1.2.3 Suspensions preparation and cell exposure

Particles suspensions were prepared extemporaneously. Stainless steel particles were first suspended in saline solution (NaCl 0.9%, $CaCl_2$ 1.25 mM and Hepes 10 mM) at the concentration 4 mg/mL. Cement particles were directly suspended in culture medium at the concentration 0.2 mg/mL. In both cases, dilutions were performed with culture medium in order to reach the chosen concentrations [0-200 µg/mL]. Cells were treated for 2 or 24 hours.

As a second control, cells were also exposed to tritiated water. The concentrations used were equal to the highest activity of the particles (8 kBq/mL for cell viability and comet assays performed with cement particles, 60 kBq/mL for the micronucleus assay performed with cement particles, and 100 kBq/mL for all the assays performed with SS316L particles).

2.1.3 Quantification of tritium released from tritiated particles

Tritiated stainless steel particles were suspended in cell culture medium (100 and 10 µg/mL) and incubated at 37°C with agitation (50 rpm). Two aliquots were collected at several time points over a 24-hour period. One aliquot was used to determine the total amount of tritium.This sample was mineralized by acid digestion using a mixture of HCl and HNO_3 (3 V / 1 V) and incubated for 24-hours at room temperature The second aliquot was centrifuged (10 min, 1635 g) to separate the soluble and particulate fractions and to determine the amount of tritium in supernatant (tritium released from particles).

In the case of cement suspensions, mineralization was performed by acid digestion using a mixture of HCl and HNO_3 (3 V / 1 V) and incubation for 48-hours at room temperature. the soluble fraction was obtained using Amicon® Ultra-0.5 10kDa centrifugal filter devices (Merck-Millipore) (centrifugation 14000g for 30 minutes).

Quantification was performed in the supernatant of stainless steel samples, the filtrate of cement samples as well as the mineralized samples of cement and stainless steel suspensions by liquid scintillation counting. Then, the following formula was applied: % tritium release= (amount of tritium in soluble fraction / amount of total tritium)*100

Experiment was performed in triplicates and results are expressed as Mean ± SD.

2.1.4 Confocal microscopy

Cells were seeded at 35 000 cells per well in 2-well chamber slides (Nunc™ Lab-Tek™ Chambered Coverglass™ System, Thermo Scientific). At 24-h post-seeding, a particle suspension (1 mL per well, final concentration 50 µg/mL) was added to each well. At the end of the 24-hour exposure, plasma membrane staining was performed by incubating the cells with fresh medium supplemented with wheat germ agglutinin-Alexa 488 at the concentration of 5 µg/ml. (Biotium, Fremont, USA) for 10 minutes at 37°C. Then, the wells were washed twice with PBS and fixed using 4 % (v/v) paraformaldehyde in PBS (Electron Microscopy Sciences, Hatfield, USA). After washing twice with PBS, cells were permeabilized with 0.05% Triton-X 100 solution. The cytoskeleton staining followed by nucleus staining were performed by incubating cells successively with phalloidin-tetramethylrhodamine B isothiocyanate (Sigma Aldrich Chimie Sarl; St. Quentin Fallavier, France) and DAPI. Finally, slides were mounted using ProLong® Gold antifade reagent (Fisher Scientific; Illkirch, France) and stored at 4 °C.

Slides were brought to room temperature prior to image collection on a Zeiss LSM 510 Meta confocal microscope (Carl Zeiss S.A.S; Marly Le Roi, France). Images were taken at 400× magnification and light reflection (543 nm laser) was used to visualize the particles. The images were processed using Image J software.

2.1.5 Cell viability assay: intracellular ATP quantification

The viability of cells was evaluated using CellTiter-Glo® Luminescence Cell Viability Assay according to manufacturer protocol (Promega; Charbonnières-les-Bains, France). This *in vitro* assay is based on an enzymatic reaction leading the conversion of luciferin, in the presence of ATP and Mg^{2+} into a luminescent compound, the oxyluciferin. The emitted luminescence was proportional to the amount of ATP, reflecting cells metabolic activity.

Twenty-four hours after seeding, cells were exposed to the particles for 2 or 24 hours. Luminescence signals were recorded using either a GloMax® Explorer Multimode Microplate Reader (Promega; Charbonnières-les-Bains, France) or a Spectra Max M5 Microplate Reader (Molecular Devices; San Jose, USA). For each experimental point, three independent assays were performed, each of them in triplicate (n = 9). The percentage of cellular viability was normalised to the unexposed control cells.

2.1.6 Genotoxicity studies

2.1.6.1 Comet assay

To detect the primary DNA damage induced by particles in BEAS-2B cells, the alkaline comet assay was performed as previously described[7]. BEAS-2B cells, seeded onto pre-coated 12-well plates (BD Falcon; Le Pont de Claix, France), were exposed to particles for 2 and 24 h.

Then, after washing, cells were trypsinized. The cell pellet was resuspended in low melting point agarose, and then spotted onto glass slides treated with successive coatings composed by 1.6% and 0.8% normal melting point agarose, respectively. Cells were then lysed, and the DNA denatured in a MilliQ water solution containing NaOH 300 mM and EDTA 1 mM. After electrophoresis (25 V and 300–315 mA), samples were neutralized and dehydrated. Air-dried slides were stained with propidium iodide (PI) before imaging and data acquisition. For negative control, cells were incubated only with culture medium, while slides treated with hydrogen peroxide (110 µM) for 5 minutes were used as positive controls. For each experimental condition, slides were prepared in duplicate. Samples were analysed using either a fluorescence Axio Imager A2 microscope (Carl Zeiss S.A.S; Marly Le Roi, France) or a BX60 microscope (Olympus; Rungis, France) at 400X magnification. Data processing was performed using the Komet 6.0 software (Andor Bioimaging, Nottingham, UK). Results were expressed as mean % tail DNA $\pm$ SEM.

2.1.6.2 Cytokinesis-Block Micronucleus assay

To identify chromosome breakage and chromosome loss following particles exposure, the Cytokinesis-Block MicroNucleus assay (CBMN) in combination with centromere labelling was performed as previously described [7] and in compliance with the OCDE487 guideline [10] .

Briefly, BEAS-2B cells were seeded onto a four-well chamber slide system (Lab-Tek™ II Nalgene Nunc International, Villebon sur Yvette, France) and treated with increasing concentrations of particles for 24 hours. Then, cells were washed and 3 µg/mL cytochalasin B (Sigma Aldrich Chimie Sarl; St. Quentin Fallavier, France) was added to the cultures to block cytokinesis. After 28 hours incubation, cells were fixed with 4% PFA. Mitomycin C (0.1 µg/mL) served as a positive control, whereas culture medium as the negative one. Upon permeabilization, the cytoskeleton was stained with Phalloidin-TRITC, while nuclei with DAPI. Centromere labelling was performed incubating successively cells with Crest serum and Alexa 488 anti-human antibody. Finally, slides were mounted using ProLong® Gold antifade reagent (Fisher Scientific; Illkirch, France).

CBMN was performed in duplicate, and slides were scored using either a fluorescence Axio Imager A2 microscope (Carl Zeiss S.A.S; Marly Le Roi, France) or a BX60 microscope (Olympus; Rungis, France) at 600x magnification. Micronuclei were only assessed in binucleated cells that had completed one nuclear division following exposure to the test compounds. For each experimental condition, the number of binucleated micronucleated cells was scored in 1000 binucleated cells.

To determine cytostasis, the Cytokinesis Block Proliferation Index (CBPI) was calculated by scoring mononucleated, binucleated, and multinucleated cells in the first 500 living cells analysed in each sample.

CBPI, which indicates the average number of cell divisions completed by the cells, was calculated as follows:

[(1 x number mononucleated) + (2 x number binucleated) + (3 x number multinucleated)]/(500 viable cells).

The percentage of cytostasis was calculated as recommended by OECD 487 test guideline:

$\{100 - 100 \times [(CBPI_{\text{exposed cells}}-1)/(CBPI_{\text{control cells}}-1)]\}$.

2.1.7 *Oxidative stress measurement*

Oxidative stress following exposure to the particles was evaluated using the GSH/GSSG-Glo Assay (Promega; Charbonnières-les-Bains, France) as previously described[7]. This assay allows the quantification of oxidized and reduced glutathione in cultured cells, through a luminescence-based system.

According to the manufacturer instructions, cells were cultured in a 96-well plate and then exposed for 30 min to the particles. After exposure, cells were lysed. Luciferin detection reagent was then added, and plates were further incubated for 15 min before luminescence was read at a GloMax® Explorer Multimode Microplate Reader (Promega; Charbonnières-les-Bains, France). Data were analyzed by subtracting the GSSG reaction signal from the total glutathione to obtain the value of reduced glutathione in the sample. Then, the GSH/GSSG ratio was calculated using this formula:

$(Luminescence_{\text{Total glutathione}} - Luminescence_{GSSG}) / [Luminescence_{GSSG} / 2]$

Data were expressed as % GSH/GSSG ratio (mean ± SD) related to the untreated cells.

2.2 Procedure used for the study of particle-induced toxicity in an in vitro 3D Human Airway Epithelia model

2.2.1 *Cellular model: MucilAir*

The MucilAir™ (Epithelix Sarl, Geneva, Switzerland) is an *in vitro* 3D model of Human Airway Epithelia. This model is characterized by a pseudostratified columnar epithelium presenting beating cilia and mucus production. These cells were isolated from the human nasal cavity of a pool of human

non-smokers donors without respiratory pathologies. Signed informed consent and ethical approval were obtained by the supplier.

The MucilAir™ model mimics the upper respiratory tract structure of the human lung, including basal, goblet and ciliated cells.

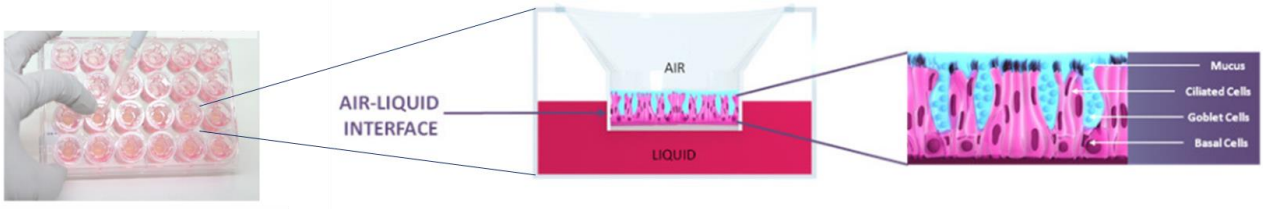


Figure 1 The three-dimensional lung model *in vitro,* MucilAir.

The lung model is composed of the three cell types: basal, ciliated and goblet cells, seeded on Transwell inserts (Picture from Epithelix).

Cells were supplied by the manufacturer ready to use in 24-well Transwell inserts of 0.4 µm pore size. Cells were cultivated with MucilAir™ serum-free culture medium (Epithelix Sarl; Geneva, Switzerland) and maintained at air-liquid interface configuration under standard conditions in a CO_2 incubator (37C and 5% CO_2) for up to three weeks (one week before the exposure and two weeks after exposure). The basolateral cell culture medium was changed twice a week, while the apical side was washed once a week with a sterile saline solution (NaCl 0.9%, $CaCl_2$ 1.25 mM and Hepes 10 mM). Renewing the basolateral media preserves the tissue homeostasis, and washing the apical compartment removes mucus and surface dead cells. Cellular morphology was assessed using optical microscopy twice a week.

2.2.2 Exposure protocol

One week after delivery, the MucilAir™ tissues were exposed to 50 µg/cm^2 (550 µg/mL) of particles for 24 hours. Particles stock solutions were diluted in saline solution and 30 µL of the suspension were applied on the apical compartment.

Basolateral medium was collected and replaced first at the end of the exposure period, then twice a week during 14 days. At the end of the treatment period, the apical side was washed and the mucus collected, then the procedure was repeated twice a week for two weeks after exposure.

Cells were also exposed to HTO at the concentrations of 165 and 550 kBq/mL (corresponding to the activities of tritiated cement and SS316L particles, respectively) as a control for studying tritium transfer through the epithelium. Trition-X 100 (1%) was used as a toxicity positive control and saline solution as a negative control. These controls were applied in the same manner (30 µL on the apical side).

2.2.3 Epithelial integrity (TEER Measurement)

In order to evaluate the integrity of MucilAir epithelium, transepithelial electric resistance (TEER) measurements were performed using the EVOM Epithelial Voltohmmeter (World Precision Instruments; Hertfordshire, United Kingdom). These measurements occurred after particles exposure and twice a week during 14 days.

Before measurement, 200 µL of saline solution were added onto the apical surface. Then, this volume was immediately removed in order to kept cells at the air-liquid interface. To calculate the TEER value of each insert, the mean resistance of a cell-free Transwell filter was subtracted from the obtained values.

2.2.4 Resazurin assay

To measure the cellular metabolism the resazurin assay was performed (Sigma-Aldrich; Saint-Quentin Fallavier, France). This test is based on the measurement of the fluorescent signal of resorufin produced by the reduction of resazurin by mitochondrial reductases.

The MucilAir— inserts were transferred in a new 24 wells plate containing 6 µM resazurin in saline solution. Resazurin solution (200 µL) was also applied on the apical surface and the plate was incubated for 1 h at 37 °C and 5% CO_2.. Then, 100 µL of the apical solution were distributed in a 96 wells plate for fluorescence measurement (excitation filter = 544 nm and emission filter = 590 nm). At the end of the experiment, the inserts were re-transferred in a new culture plate containing fresh MucilAir culture medium (700 µL per well). The remaining apical solution was removed without unsettling the epithelium and cells were put back into the incubator. The measurements were performed on four inserts per condition.

2.2.5 Evaluation of tritiated particle transfer

To estimate the cellular uptake of tritium and the transfer through the respiratory epithelium, the MucilAir tissues were exposed to 50 µg/cm^2 (550 µg/mL) of particles for 24 hours. After exposure, apical and basolateral media were collected and stored at -20°C. Cells were trypsinized and collected. Samples (apical and basolateral medium, cells) were mineralized and radioactivity was measured as described above.

3 Cyto-genotoxicity of tritiated stainless steel and cement particles in BEAS-2B cells

3.1 Behaviour of particles in cell culture medium

Tritiated SS316L particles are almost insoluble[11] in culture medium but 60% of tritium was released from the particles after 2 hours of incubation, probably as HTO.

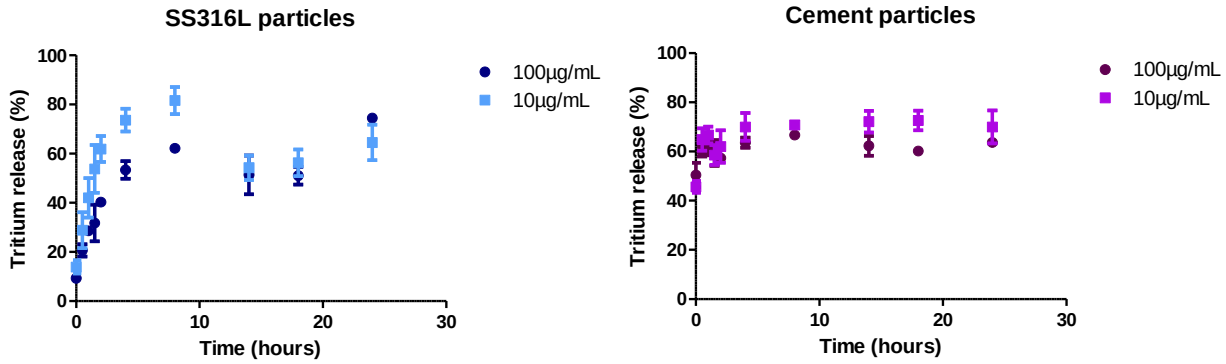


Figure 2 Quantification of tritium release from stainless steel and cement particles in cell culture medium

Tritiated particles were suspended in cell culture medium and incubated at 37°C under agitation. Samples were collected at several time points and then tritium amount was assessed in the supernatant and in the particles suspension using liquid scintillation counting.

In cell culture medium (37°C with agitation), cement particles are partially solubilized in culture medium and calcium oxide is released very quickly [11]. Cement particles also released a huge amount of tritium very quickly. Indeed, 70% of the tritium was released from the particles after 30 minutes of incubation. The remaining 30% of the activity remained associated with the particles until the end of the incubation period.

3.2 Observation of particles in cells

After exposure of cells to SS316L particles, some aggregates of different sizes were observed. The characterization of the particles suspension, conducted previously, showed that these particles formed aggregates when they were suspended in aqueous media [11]. Confocal microscopy pictures showed that the aggregates were often found in the vicinity of the cell nucleus, although internalization of particles by the cells were neither formally noted, nor excluded.

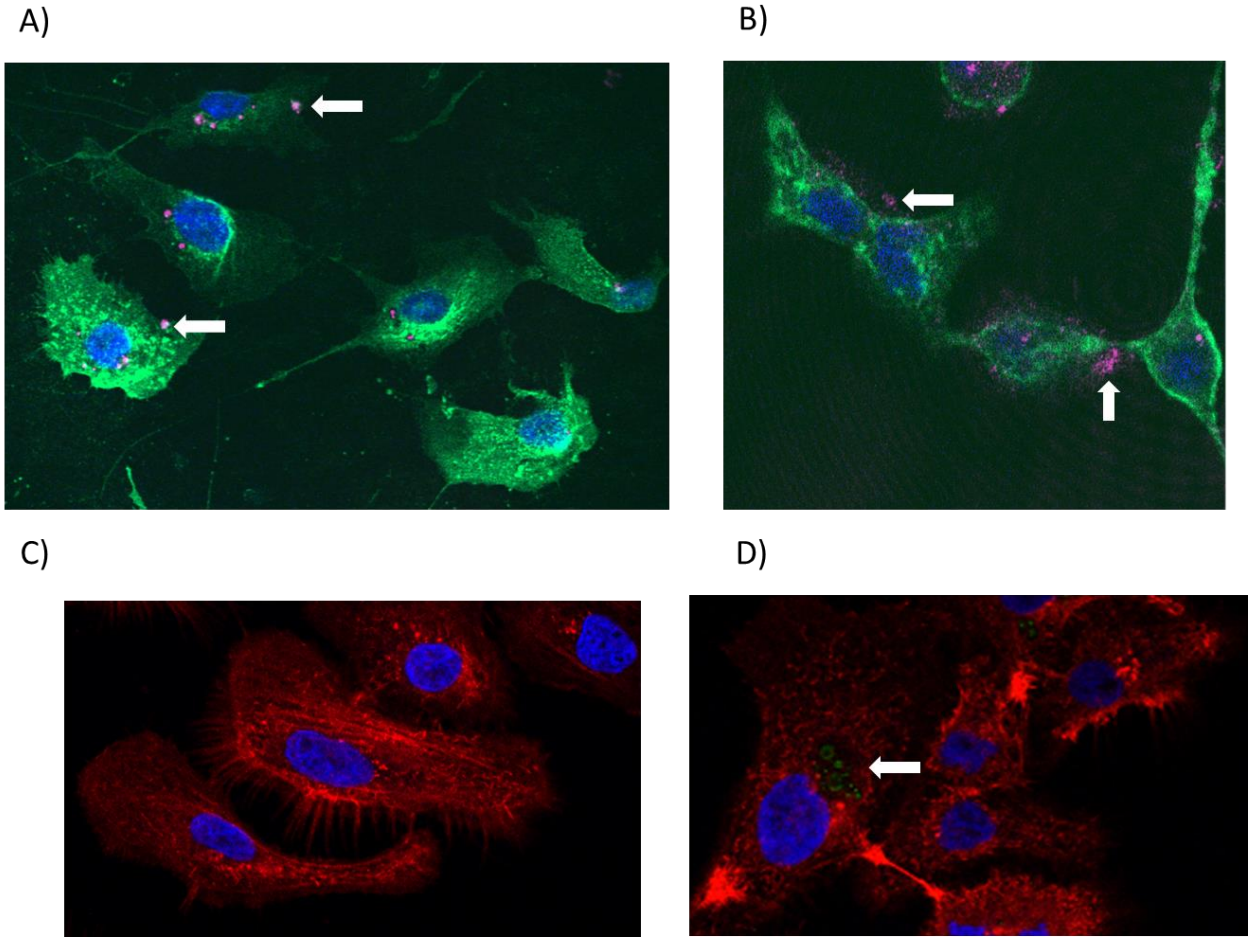


Figure 3 Confocal microscopy observation of BEAS-2B cells after exposure to hydrogenated particles.

A and B: After exposure of BEAS-2B cells to stainless steel (A) or cement (B) particles, staining of plasma membrane (green), nucleus (blue) and cytoskeleton (red) were performed. Images were acquired, using confocal microscopy. Particles, visualized by light reflection (pink), are indicated by white arrows.
C and D: Staining of nucleus (blue) and cytoskeleton (red) were performed on untreated cells and cells exposed to stainless steel particles. The SS316L particles (green and indicated by white arrow) seem to form depressions in the cells.

Changes in the cytoskeleton such as vesicle-like structures were also detected. These adaptations of the cell morphology were probably associated with the high density of the stainless steel particles. However, the possibility of cellular internalization of small aggregates of particles could not be excluded.

The cement particles behaved differently than the stainless steel ones. They were evenly distributed on the cell surface. The particles seemed to adhere to the plasma membrane. No morphological changes of cells were observed in presence of particles.

3.3 Cytotoxic effects of tritiated particles

Cytotoxicity, based on cellular ATP quantification, was investigated after exposure of BEAS-2B human lung cells to 0 to 200 µg/mL of hydrogenated and tritiated particles.

After 2-hours exposure to hydrogenated and tritiated SS316L particles, none of the tested conditions showed any cytotoxic effect (Figure 4A). Following 24-h exposure, no toxicity was noted, excepted for the highest tested concentration of hydrogenated SS316L particles (200 µg/mL) (Figure 4B).

Following exposure to hydrogenated and tritiated cement particles no cytotoxic effects were detected neither at 2-hours nor at 24-hours exposure duration (Figures 4C and 4D).

Figure 4E shows that the viability was unchanged when cells were exposed to at activities between 0 and 200 kBq/ mL *via* tritiated particles or tritiated water for 24 hours.

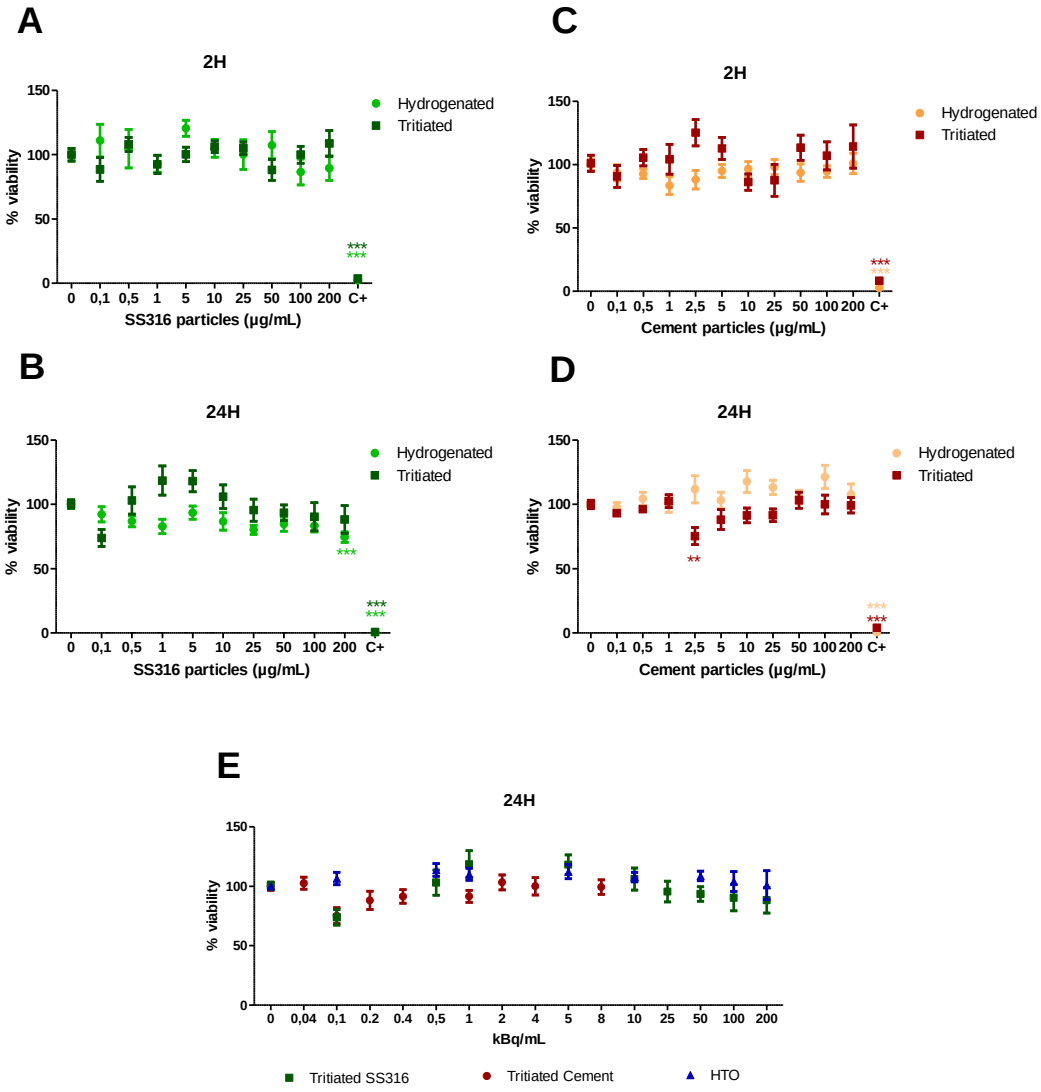


Figure 4 Cytotoxicity of hydrogenated and tritiated particles measured after 2 or 24-hours exposure in BEAS-2B cells.

As a positive control, cells were exposed to 9% Triton-X 100 diluted in culture medium. Data are presented as mean ± SEM of three independent experiments, each in triplicate. Statistical significance was evaluated by one way ANOVA followed by Dunnett's multiple comparisons test ** $p < 0.01$, *** $p < 0.001$.

These cytotoxicity data allowed us to select concentrations to be tested for genotoxicity experiments. We choose to eliminate the highest concentration of stainless steel particles even if the cytotoxicity induced did not exceed the threshold (55% ± 5%) recommended for these tests[10].

3.4 Genotoxic effects exerted by tritiated particles: DNA strand break

The alkaline version of the comet assay, measuring mainly DNA single strand breaks and alkaline labile sites, was used to assess particle-induced DNA damage in BEAS-2B cells. As shown in Figure 5, both types of particles induce DNA single-strand breaks compared to untreated cells.

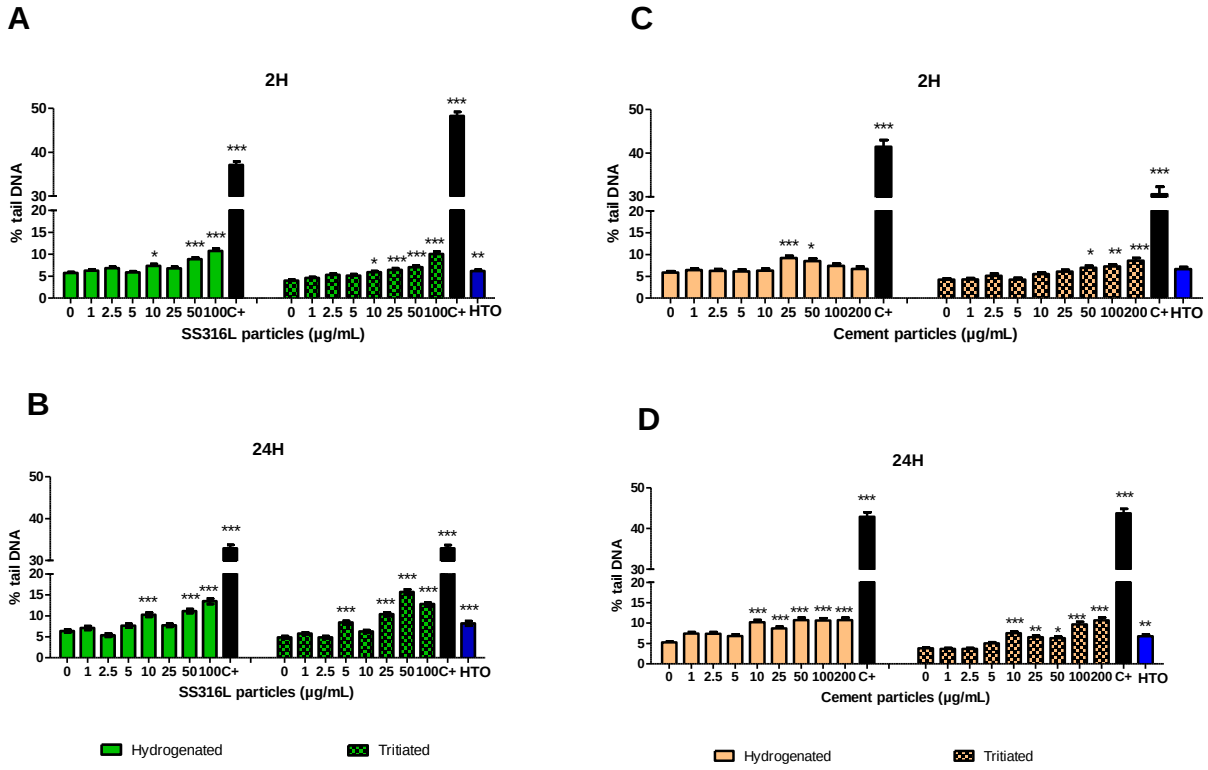


Figure 5 Evaluation of DNA damage induced by SS316L and cement particles, by alkaline comet assay.

Percentage of tail DNA was quantified after 2h and 24h of exposure to hydrogenated and tritiated SS316L or cement particles. As a positive control, cells were exposed to 110 µM hydrogen peroxide (black bars). Cells were also exposed to tritiated water with an activity corresponding to the activity of the highest tested concentration of particles (100 kBq/mL and 8 kBq/mL). Each bar represents the mean ± SEM of two to three independent experiments. Asterisks indicate statistically significant increase compared to untreated cells with p-value $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***).

Following 2-hours exposure, a statistical significantly increased amount of DNA damage was seen in cells exposed to hydrogenated SS316L at concentrations 10, 50 and 100 µg/mL and at concentrations ≥ 10 µg/mL with the tritiated ones (Figure 5A). The increases in primary DNA lesions induced by hydrogenated and tritiated stainless steel particles were similar following 24-hours exposure (Figure 5B).

Following 2-hours exposure, a statistical significantly increased amount of DNA damage was seen in cells exposed to hydrogenated cement particles at concentrations 25, 50 µg/mL and at concentrations ≥ 50 µg/mL with the tritiated ones (Figure 5C).

Following 24-hours exposure, cement particles induced a significant increase in DNA single strand breaks at concentrations ≥ 10 µg/mL. No difference in the induction of DNA damage was noted between hydrogenated and tritiated cement particles (Figure 5D).

Short and long exposure duration generated a similar amount of DNA breaks.

Tritium, in form of HTO, was able to induce an increase in DNA breaks in BEAS-2B cells, whatever the activity (100 kBq/mL and 8 kBq/mL). The increases in primary DNA lesions induced by hydrogenated particles were so marked that we cannot exclude nor concluded that additional DNA single strand breaks due to tritium would be evidenced in these experiments. Thus, the presence of

tritium could contribute or not to the DNA damage effect exerted by tritiated cement and SS316L particles.

3.5 Chromosomal damage exerted by tritiated particles: micronuclei formation

After 24-hours exposure of BEAS-2B cells to particles and controls (vehicle only and HTO), the cytokinesis-block proliferation index (CBPI) was determined (Figure 6). No difference was observed comparing the CBPI of hydrogenated particles at all tested concentrations to untreated cells indicating that stainless steel and cement particles were not cytostatic (Figures 6A and 6C). In contrast, tritiated particles impaired CBPI at the highest tested concentrations while HTO did not (Figures 6B and 6D).

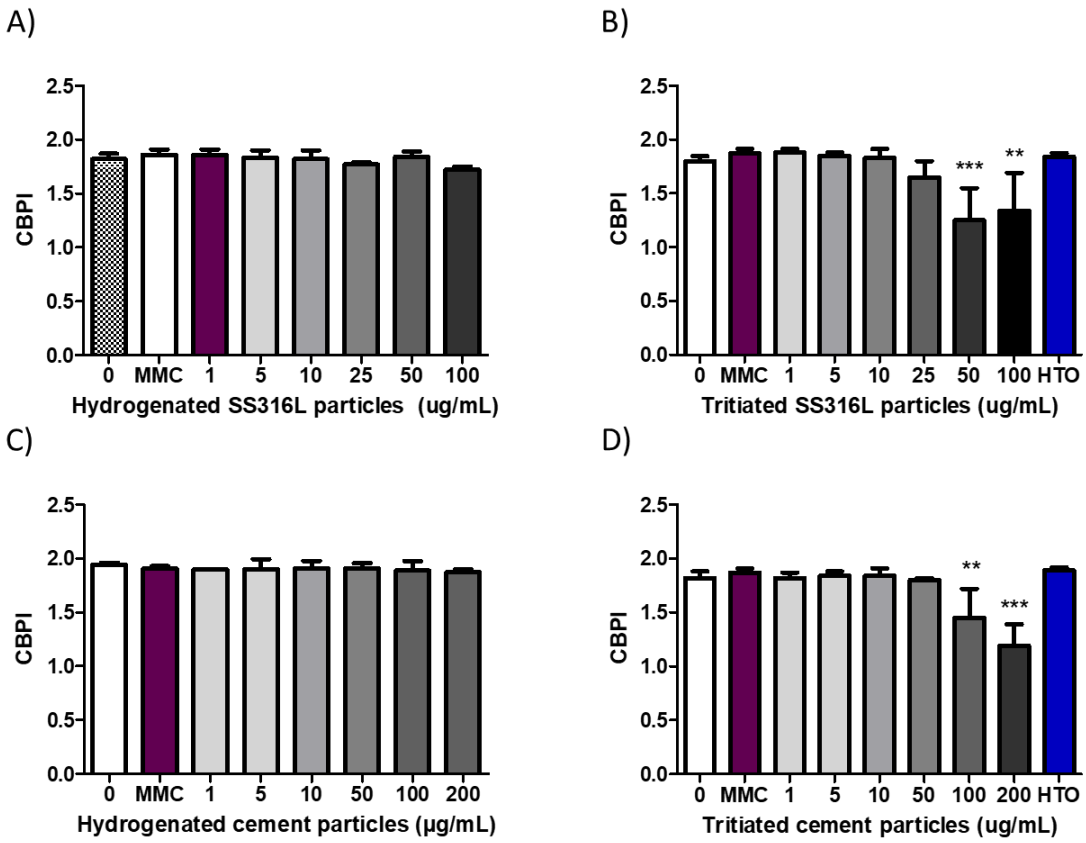


Figure 6 Cytokinesis Block Proliferation Index (CBPI) upon exposure to hydrogenated/tritiated SS316L (A and B) and cement (C and D) particles.

Cells were also exposed to tritiated water with an activity corresponding to the highest activity of particles (100 kBq/mL and 60 kBq/mL). Each bar represents the mean ± SD of two independent experiments. Asterisks indicate statistically significant difference compared to untreated cells evaluated by One way ANOVA, with p-value $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***).

Based on CBPI data, the effects of the exposure to particles on the cell proliferation rate were assessed by calculating the percentage of induced cytostasis (Table 1). Indeed, an inhibition of cell division could occur due to a toxic effect on various components necessary for cell division or as a result of the activation of cell cycle checkpoints following DNA or chromosome damage.

1)

SS316L	Hydrogenated		Tritiated	
	mean	sd	mean	sd
0	1.63	1.74	0.82	0.8
MMC	0.40	0.69	0.73	1.6
1	0.55	0.95	0.00	0.0
5	1.82	3.15	0.00	0.0
10	2.01	2.66	3.29	5.7
25	6.32	3.48	19.08	14.7
50	0.00	0.00	69.19	35.6
100	12.15	6.89	59.25	41.7
HTO			0.81	1.4

2)

Cement	Hydrogenated		Tritiated	
	mean	sd	mean	sd
0	0.5	0.8	1.8	2.7
MMC	3.8	1.5	1.7	2.8
1	2.9	0.3	2.2	3.9
5	6.3	6.4	0.6	1.1
10	4.7	4.7	2.1	3.7
50	3.7	3.2	3.8	3.4
100	6.5	7.1	43.4	34.0
200	7.3	3.3	75.3	25.6
HTO			0.0	0.0

Table 1 Cytostasis upon exposure of BEAS-2B cells to hydrogenated/tritiated SS316L (1) and cement (2) particles.

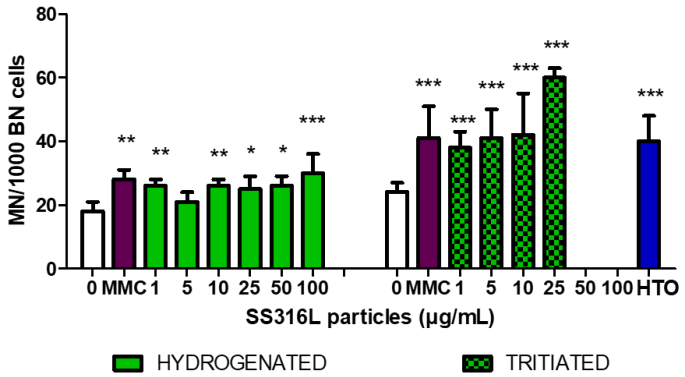
Each value represents the percentage of cytostasis expressed as mean ± SD of two independent experiments.

It is noteworthy that only tritiated stainless steel and cement particles induced a strong decrease in cytostasis at concentration above 50 and 100µg/mL, respectively. HTO, at activity corresponding to the highest tested concentrations of tritiated particles did not induce cytostasis.

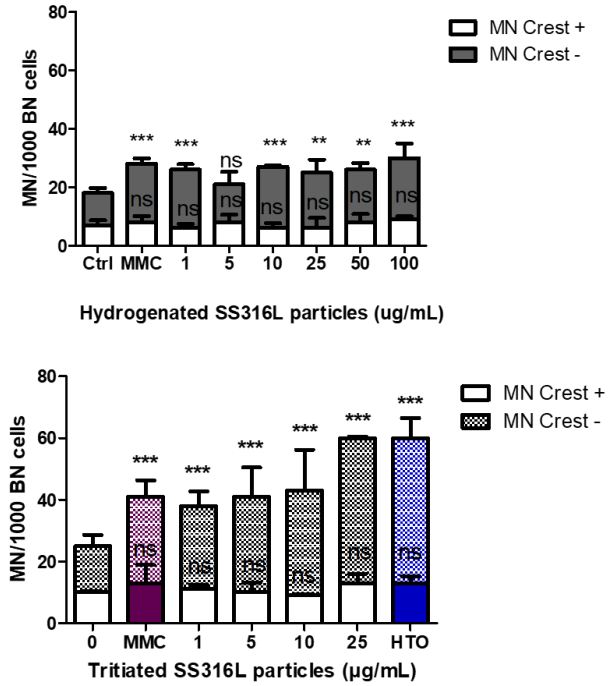
Using CBMN assay in combination with centromere labelling, particles-induced chromosomal damage were also investigated and among them, the discrimination between chromosome loss (leading to centromeric micronuclei; MN Crest +) and chromosome breakage (leading to acentromeric micronuclei; MN Crest -) was performed.

Mytomycin C (MMC) was used as a genotoxic positive control, causing a significant increase in the frequency of NMs compared to the control. As a clastogenic agent, MMC induces chromosomal breakage leading to the formation of acentromeric micronuclei (MN Crest -).

A)



B)



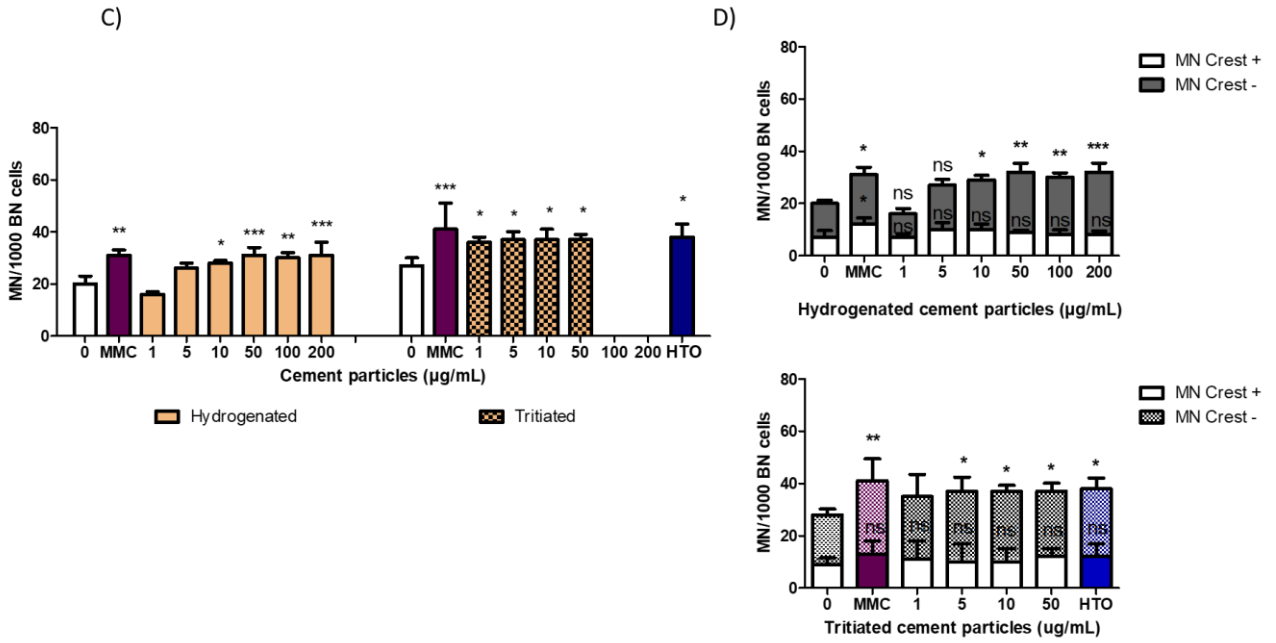


Figure 7 Micronuclei frequency in BEAS-2B cells exposed to SS316L and cement particles.

MMC (0.1 µg/mL) was used as clastogenic positive control. Cells were also exposed to HTO with an activity corresponding to the highest activity of particles (100 kBq/mL and 60 kBq/mL). Centromere labelling (Crest) was performed to discriminate between MN issued by a whole chromosome loss (MN Crest +) and MN resulting from chromosome breakage caused by DSB (MN Crest -). Each bar represents the mean ± SD of two independent experiments. Asterisks indicate statistically significant increase compared to untreated cells determined by Chi-square, with p-value $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***).

As shown in figure 7, a significant increase in MN frequency was detected, following exposure to hydrogenated and tritiated stainless steel and cement particles.

We can note that, following exposure to hydrogenated stainless steel particles, the increase in MN frequency was significant at 1 µg/mL and in the concentration range [10-100] µg/mL but no dose related effect was clearly evidenced. Following exposure to the tritiated stainless steel particles, a clear dose-dependent and strong increase ($p<0.001$) was evidenced in the concentration range [1-25] µg/mL (Figure 7A).

Following hydrogenated cement particles exposure, a significant and dose related increase in MN frequency was observed in the concentration range [10-200] µg/mL whereas tritiated cement particles induced a significant increase in MN frequency at lower concentrations, that is in the range [1-50] µg/mL.

Exposure of cells to HTO also resulted in a significant increase in MN frequency.

Cement particles as well as SS316L particles induced mainly MN Crest -, suggesting that such particles exerted genotoxic effects via the formation of double strand breaks (DSB) leading to chromosomal breakage. HTO display also clastogenic potential.

3.6 Oxidative stress

As oxidative stress is known to be one of the main pathways leading to DNA damage [12], the ability of particles to generate oxidative stress in cells was investigated. The ratio of reduced to oxidized glutathione was determined, following particles exposure. As seen in figure 8A, none of the tested conditions induced an oxidative stress able to disturb the GSH/GSSG ratio excepted of the positive control consisting in 20 µM menadione. Following exposure to cement particles (tritiated and hydrogenated), a moderate and non-significant increase in the GSH/GSSG ratio was observed.

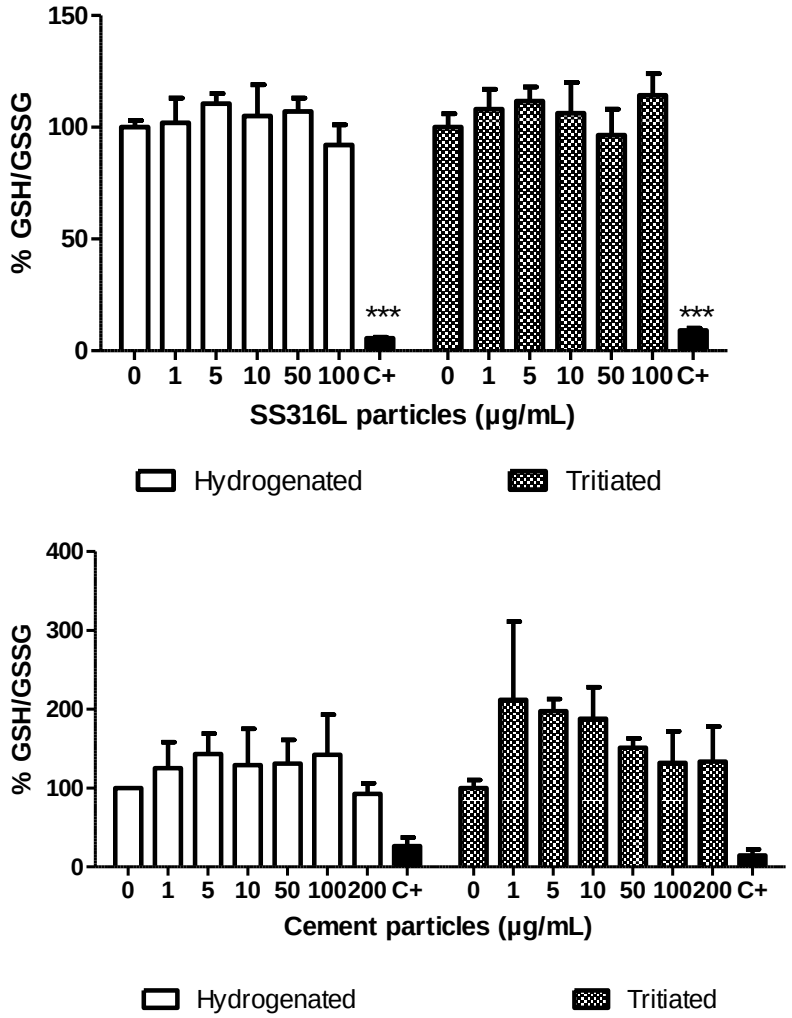


Figure 8 Oxidative stress evaluated by GSH/GSSG ratio in BEAS-2B cells, following SS316L and cement particles exposure.

Menadione (20µM) was used as positive control (C+). Data are presented as mean ± SEM of two independent experiments, each in duplicate. Statistical significance was evaluated by one way ANOVA followed by Dunnett's multiple comparisons test, *** $p < .001$.

4 Cytotoxicity of tritiated stainless steel and cement particles in an *in vitro* 3D Human Airway Epithelia model

A second lung cell model, the three-dimensional MucilAir model, was used to study and compare the toxicity of tritiated and non-tritiated particles and especially the transfer of tritium across the pulmonary epithelial barrier.

This model, which mimics the functioning of the human airway epithelium, has the potential to recapitulate toxicity responses through the respiratory function, when exposed to a variety of pathogens or chemicals [13]. We recently used such a model to assess the toxicity of tritiated tungsten particles [14] and to quantify cytotoxicity and tritium absorption after administration of HTO to the tissue for 24 h, at different activity levels (up to 33 kBq $\mu L^{-1} cm^{-2}$). A simple dosimetric model was developed to estimate the upper limits of the dose to cells, starting from the administered activity and considering water transport through the tissue [15] .

The MucilAir tissue model is characterized by morphology and functions comparable to those of a human epithelium. Obtained from human biopsies, MucilAir has a lifespan of one year, making it suitable for long-term *in vitro* studies. It also mimics accidental exposure to a hazard and allows

kinetics to be monitored for several weeks to determine short- and long-term toxic effects and their reversibility.

4.1 Epithelial Integrity and metabolic activity

TEER was measured upon exposure to particles and HTO at concentrations 165 and 550 kBq/mL (activity of tritiated cement and stainless steel respectively) at the end of exposure (day 1), then at day 4, 7, 10 and 14 post treatment.

As seen in Table 1, the positive control (1% Triton-X 100) exerted a severe and permanent reduction of TEER. At the other tested conditions, TEER was not decreased at the end of exposure and until day 14. A small and transient increase of TEER was detected for the lowest activity level of HTO, at day 7.

TEER	D1	D4	D7	D10	D14
C-	100 ± 30.1	100 ± 5.5	100 ± 3.1	100 ± 2.6	100 ± 6.2
C+	2.5 ± 0.4***	1.8 ± 0.3***	1.2 ± 0.3***	0.7 ± 0.3***	2 ± 0.3***
Hydrogenated cement	98.3 ± 16.1	103.4 ± 3.5	83 ± 2.8	82.4 ± 17.2	85 ± 21.5
Tritiated cement	83.9 ± 11.9	87.6 ± 21.9	114.2 ± 12.2	85.6 ± 5.1	118.5 ± 3.8
HTO 165kBq/ml	75.5 ± 19.5	121.2 ± 3.7	120.6 ± 4.2*	97.7 ± 7.5	103.1 ± 14
Hydrogenated SS316L	106.4 ± 6.3	78.6 ± 3.2	77.6 ± 20.7	102.8 ± 7.5	74.6 ± 17.3
Tritiated SS316L	82.3 ± 20.6	75.6 ± 32.4	80.5 ± 11.4	81.8 ± 16	97 ± 14.3
HTO 550kBq/ml	84.7 ± 42. 4	94.4 ± 44.1	104.4 ± 16.4	105.3 ± 2.2	91.2 ± 19.4

Table 2 Epithelial integrity of MucilAir following particles and HTO exposure, evaluated by TEER measurement.

The measurements were performed before (D0) and at the end of exposure (D1), and then at day 4, 7, 10 and 14 post exposure. The results are expressed as a percentage of TEER normalized to control conditions (C-) at the same time point. Triton-X 100 (1%), was used as a positive control (C+). Each value represents the mean ±SD of four individual inserts. Statistical significance was determined using one-way ANOVA followed by Dunnett's multiple comparisons test: $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***).

Cell viability was also evaluated, based on metabolic activity measurement. Viability was not decreased by the exposure to tritiated particles or HTO, neither at the end of the 24-h exposure, nor 7 or 14 days afterwards, while the positive control exerted a drastic decrease. Overall viability data confirms that culture conditions remained good over the entire investigated time frame.

RESAZURIN	D1	D7	D14
C-	100 ± 18.3	100 ± 5	100 ± 14.4
C+	3.6 ± 0.4***	0.4 ± 0.1***	0.1 ± 0.3***
Hydrogenated cement	92.4 ± 16.4	98.3 ± 4.7	82.4 ± 7.7*
Tritiated cement	161.7 ± 26.3*	92.7 ± 6.1	114.3 ± 2.1
HTO 165kBq/ml	128.2 ± 31.8	89.5 ± 5.6	117.2 ± 9.8*
Hydrogenated SS316L	83.2 ± 17.3	104.9 ± 4.2	119.3 ± 3.3*
Tritiated SS316L	153.9 ± 24.2	97.4 ± 10.2	122.5 ± 5.1**
HTO 550kBq/ml	147.1 ± 44.3	98.3 ± 4	120.3 ± 2.4*

Table 3 Metabolic activity of MucilAir following particles and HTO exposure, evaluated by resazurin assay.

Analyses were performed at the end of exposure (D1), 7 days (D7) and 14 days (D14) post exposure. Data are expressed as the percentage of cell viability (mean ± SD of four individual inserts) normalized to control conditions (C-) at the same time point. Triton-X 100 (1%), was used as a positive control (C+). Statistical significance was determined using one-way ANOVA followed by Dunnett's multiple comparisons test: $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***).

4.2 Transepithelial passage of tritium and cellular accumulation

To evaluate the absorption of tritium by the epithelial pulmonary MucilAir tissue, the extracellular and intracellular amount of tritium were quantified from day 1 to day 14 after exposure using liquid scintillation counting. The extracellular quantification was performed on mineralized apical and basolateral cell culture media.

% Tritium		D0	D1	D4	D7	D10	D14	D14 in cells
HTO 165kBq/ml	APICAL	<Q.L	3.56 ± 0.11	<Q.L	<Q.L	<Q.L	<Q.L	<Q.L
	BASOLATERAL	0.21 ± 0.03	71.73 ± 2.92	<Q.L	<Q.L	<Q.L	<Q.L	
Tritiated cement	APICAL	<Q.L	8.89 ± 1.19	<Q.L	<Q.L	<Q.L	<Q.L	0.73 ± 0.11
	BASOLATERAL	<Q.L	71.56 ± 3.68	<Q.L	<Q.L	<Q.L	<Q.L	
HTO 550kBq/ml	APICAL	<Q.L	3.88 ± 0.41	<Q.L	<Q.L	<Q.L	<Q.L	<Q.L
	BASOLATERAL	0.06 ± 0	66.57 ± 2.33	0.07 ± 0.01	<Q.L	<Q.L	<Q.L	
Tritiated SS316L	APICAL	<Q.L	53.63 ± 5.19	<Q.L	<Q.L	<Q.L	<Q.L	1.29 ± 0.37
	BASOLATERAL	<Q.L	25.06 ± 0.95	<Q.L	<Q.L	<Q.L	<Q.L	

Table 4 Transepithelial passage of tritium and cellular accumulation

Tritium content was measured in both apical and basal media of MucilAir, as well as in cells, using liquid scintillation counting after mineralization of the samples collected at the end of the 24-hours exposure (day 1), and at day 4, 7, 10 and 14 post exposure. The results are expressed as percentage of tritium in the exposure solution (mean±SD of four samples). <Q.L means that the obtained value was below the quantification limit.

At the end of exposure to tritiated cement and HTO, a low fraction of tritium (3% to 9% at day 1), remained in the apical media, while a high amount (67% to 72% at day 1) was able to translocate in the basolateral compartment. The behavior of SS316L particles was different; the majority of tritium amount (54%) was present in the apical media at day 1.

From day 1 to day 14 post exposure, the quantity of tritium still present in the apical and basolateral compartments severely and rapidly decreased up to less than 0.1% compared to day 1.

Finally, to quantify the intracellular level of tritium upon MucilAir exposure, tritium quantification was performed on mineralized cells at day 14. Tissue cells appear to retain a negligible amount of the total activity, as less than 0.05% of tritium was found in cells regardless of the time point. Also, no further release of such internalized activity appears to occur from cells to culture media.

5 Discussion/Conclusion

In order to assess the hazard of particles that could be emitted during the dismantling of a nuclear power plant, the toxicity of tritiated cement and stainless steel particles was investigated using *in vitro* methods. Hydrogenated particles were considered as the relevant controls to determine chemical stress to be compared to both chemical and radiative stress induced by tritiated particles.

During dismantling operations, the main route of exposure of workers to such particles is inhalation. Thus, we applied our *in vitro* methods on human lung cell models. The human non-cancerous lung cell line BEAS-2B and a 3D *in vitro* model of human respiratory epithelium MucilAir™ were chosen to study and compare the toxicity of hydrogenated and tritiated particles. These models are widely used to characterize the toxicity of different particles [16–20]. As a reference, BEAS-2B cells are among the most widely used immortalized cell lines for *in vitro* toxicology studies of agents for which the expected route of exposure is inhalation; this model that resembles airway basal epithelial cells, offers the advantage to be easy to handle, do not display inter-donor variability and have an extended life span.

The MucilAir™ model, was used to determine the toxicity and especially the transfer of tritium across the pulmonary epithelial barrier. The MucilAir™ tissue model allows kinetics to be monitored for several weeks to determine short- and long-term toxic effects and their reversibility.

Concerning stainless steel particle, a review on stainless steel toxicity concluded that steel is likely to exert very low toxicity to human [21]. For all the countless applications of stainless steel over many decades, harmful toxic effects have not been reported. In agreement with the literature [22,23], our results showed that an exposure of BEAS-2B cells to hydrogenated and tritiated stainless steel particles is associated to moderate to no cytotoxicity, assessed by the measurements of ATP content.

Among toxicological endpoints of interest, genotoxicity is a key parameter as the genetic damage plays a critical role in pathological consequences of ionizing irradiation [5]. The alkaline version of the comet assay, measuring mainly DNA single strand breaks and alkaline labile sites, was used to assess DNA damage after exposure of cells to hydrogenated and tritiated particles. An increase in DNA damage induced by hydrogenated and tritiated stainless steel particles was observed. Using the same technique, a similar genotoxic effect of stainless steel particles has been described in another lung cell model. Significantly higher amount of DNA damage was seen following exposure of A549 human lung cells to a concentration of 40 µg/cm^2 (80 µg/mL) SS316L particles for 4 hours compared to the controls [22].

Hydrogenated and tritiated stainless steel particles induced chromosomal damage. Indeed, a significant increase in the number of micronuclei in BEAS-2B cells exposed to hydrogenated and tritiated stainless steel particles was observed. The chromosome damage induction was dose related and highly significant with tritiated particles only. These observations suggest that a cumulative chemical and radiative stress could occur with tritiated particles. These micronuclei were predominantly negative to centromeric labeling, indicating that SS316L particles induced DNA double strand breaks (clastogenic potential).

Oxidative stress has been recognized as a key mechanism of particles-mediated toxicity including lipid peroxidation and DNA damage. Indeed, DNA damage formation associated with the generation of oxidative stress was suggested by the significant increase of sites of formamidopyrimidine DNA glycosylase detected in A549 cells following exposure to micrometric stainless steel particles [8]. Moreover, stainless steel particles are composed of metals such as Fe, which can generate reactive oxygen species by Fenton-type reactions.

The ability of particles to induce oxidative stress was thus assessed by quantifying cellular oxidized and reduced glutathione. Reduced glutathione (GSH), the most abundant low molecular weight thiol compound in cells, plays critical roles in protecting cells from oxidative damage and from the toxicity of electrophilic xenobiotic, and in maintaining redox homeostasis. Thus, an alteration in the balance between the reduced and oxidized forms of glutathione (GSH/GSSG) reveals oxidative stress at the cellular level.

Our results did not show any modification of the GSH/GSSG balance. As ROS were not assayed, we cannot exclude the possibility that ROS were synthesized and induced oxidative stress as well as oxidative damage without altering the GSH/GSSG balance. Nevertheless, the induced oxidative stress, if any, was not strong enough to modify the reduced /oxidized balance of the main thiol compound in the cells.

Concerning cement particles, it was demonstrated that respirable cement particles did not induce cytotoxicity as assessed by the lactate dehydrogenase (LDH) assay, in human primary epithelial cells from oropharyngeal mucosa exposed to concentrations up to 200 µg/cm^2 [24]. A non-cytotoxic effect of cement particles was also observed on the hamster ovary cell line CHO *in vitro* [25]. In agreement with these studies, no cytotoxicity, assessed by the measurements of ATP content, was observed after 2h and 24h of exposure of BEAS-2B cells to either hydrogenated and tritiated cement particle concentrations ranging from 0 to 200 µg/mL in our study.

Although no cytotoxic effect of cement particles were noted, DNA appears to be a privileged target of the toxicity exerted by cement particles. A study including cement warehouse workers reported

that continuous exposure to cement dust was associated with an increased frequency of nuclear aberrations, based on micronucleus cytome assay performed on exfoliated buccal cells [26]. An increase in micronuclei frequency was also found in lymphocytes collected from construction workers [27]. Recently, the ability of cement particles to induce micronucleus formation was investigated *in vitro*. A dose-dependent increase in micronuclei was observed in primary epithelial cells derived from the oropharyngeal mucosa following exposure to cement particles [28].

In our study, *in vitro* exposure of BEAS-2B cells to hydrogenated and tritiated cement particles also led to genotoxicity. A similar increase in the percentage of tail DNA (comet assay) was seen in cells exposed to hydrogenated and tritiated cement particles, compared to control. The effects of tritiated and hydrogenated particles being similar, the comet assay did not reveal, if any, additional DNA single strand breaks induced by the tritium bring and/or carried by tritiated particles (8kBq/mL at the highest tested concentration). However, the involvement of tritium in the induction of primary DNA lesions cannot be excluded as HTO also induced a significant increase in DNA damage as previously reported [29,30] .

Our results also showed that hydrogenated and tritiated cement particles induced chromosomal damage in cells as indicated by the significant increase in the micronuclei frequency, highlighted by the CBMN test. The presence of tritium seems to enhance the chromosome damaging effects of the particles because tritiated particles induced a significant increase in the frequency of micronuclei at lower concentrations than hydrogenated particles. These observations suggest that a cumulative chemical and radiative stress could occur with tritiated particles. These micronuclei were predominantly negative to centromeric labeling, suggesting that hydrogenated and tritiated cement particles induced double strand breaks (clastogenic potential), as well as HTO.

DNA and/or chromosome damage induced by particles are known to be frequently consecutive to oxidative stress. An exposure to cement particles was reported to be associated with oxidative stress in alveolar macrophages as indicated by the increase of intracellular ROS production and intracellular glutathione reduction following 12 hours of exposure to cement dust [31].

A slight increase of oxidative DNA damage (8-OHdG) was observed in fibroblasts exposed to soluble fraction of cement particles for 1 and 12 hours compared to control [32]. In our study, no significant increase in the GSH/GSSG ratio was noted. However, our results were associated with high variability that did not allow us to conclude on the ability of particles to induce the generation of oxidative stress.

Interestingly, a high level of ROS and an increase in the number of DNA double strand breaks were found in MCF-10 cells exposed to low doses of HTO [33]. Another study reported that DNA strand break induced by HTO in human umbilical vein endothelial cells, assessed by Comet assay and γ-H2AX immunostaining, were associated with modulations of DNA repair that could be regulated by the c-myc gene expression via miR34a [30]. One hypothesis is that tritium may enhance the genotoxic effects of tritiated particles by promoting the lifetime of the primary DNA lesions in cells via an inhibition of DNA repair systems.

Particles-induced cytotoxicity and its reversibility were assessed in MucilAir model using a 14-day kinetic mode. Our results showed that an exposure to high concentration (50 µg/cm^2) of tritiated stainless steel and cement particles did not result to any toxicity as indicated by TEER measurement and metabolic activity evaluation by resazurin assay. No long-term deleterious effects were observed as no difference were seen between TEER and metabolic activity measured before exposure and 14 days post-exposure. The activities associated with tritiated stainless steel and cement particles were respectively 16.5 kBq/well and 3.75 kBq/well. These results are in agreement with data obtained by Baiocco and colleagues showing a lack of toxicity following exposure of the Mucilair model to HTO at the activities up to 33 kBq/well [15].

The lack of toxicity could also be associated with the presence of a mucus layer on the surface of MucilAir, which reduced the interaction between the particles and cells, thus protecting them from severe damage.

The Mucilair model was also used to study the transfer of tritium across lung barrier. Following exposure to HTO and tritiated cement particles, the majority of the tritium was found in the basolateral

compartment. These data are in agreement with those from the study of tritium released into culture media, which show that about 60% is released rapidly from cement particles. On the contrary, in the case of exposure to tritiated stainless steel particles, the majority of tritium is present in the apical compartment probably attached to the particles. This behavior is different from that observed in the study of released tritium into culture media. Very low activities were measured in cells 14 days after the end of exposure, suggesting no cellular accumulation.

Overall, these results indicate that tritiated stainless steel and cement particles have little effect on cell viability. However, DNA appears to be a target of the adverse effects of these particles, cell exposure being associated with an increase in primary DNA damage as well as chromosomal breakage.

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