Riunione PRIN 29.06.21

Unità di Ancona: UNIVPM

Silvia Di Valerio, S. Vaiasicca, D. Ramini, S. Fantone, G. Tossetta, D. Marzioni, A. D. Procopio, A.Pugnaloni

UNIMORE
UNIVERSITÀ DEGLI STUDI DI MODENA E REGGIO EMILIA

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Fibre Asbestiformi:
Caratterizzazione e Analisi della vitalità cellulare

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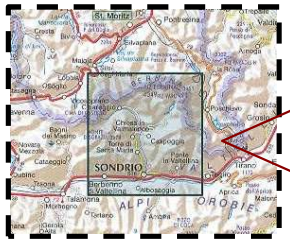
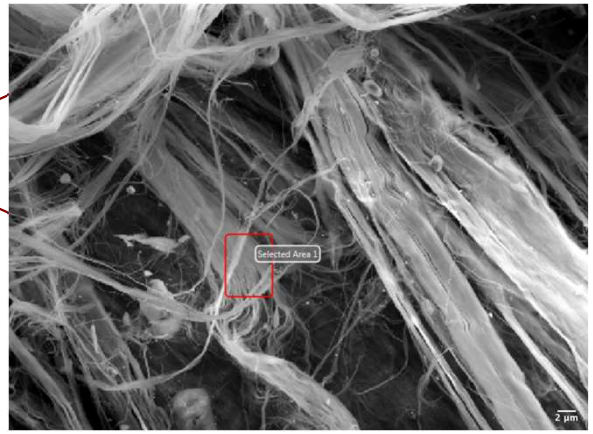
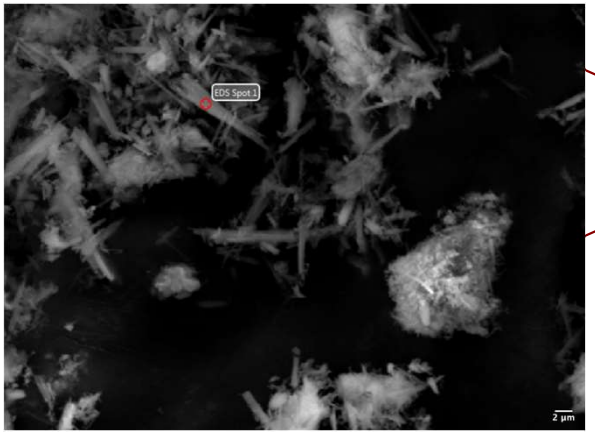


Valmalenco (Central Alps, Italy)



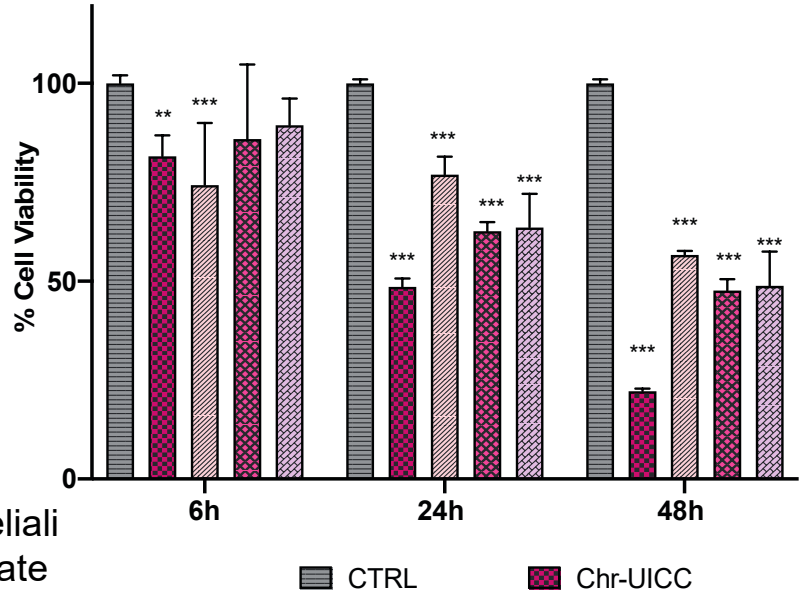
SEM microscopy



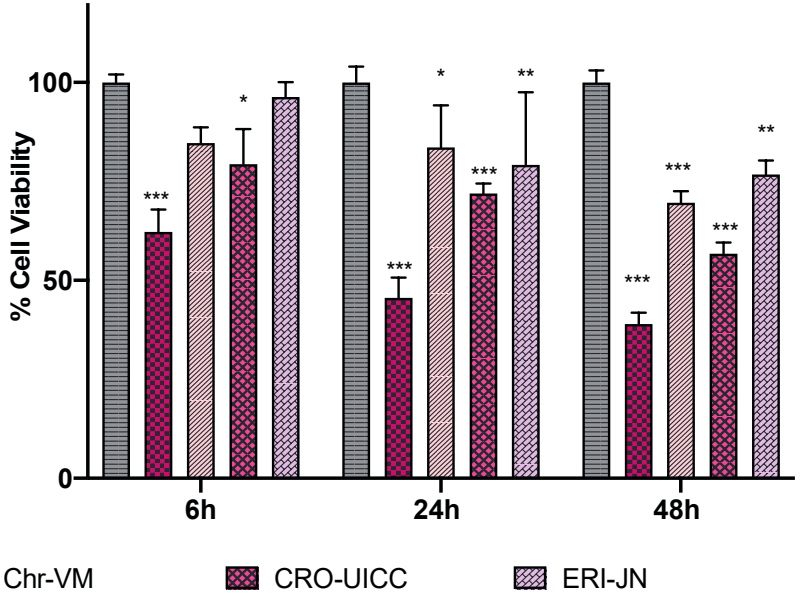
Jersey Nevada (USA)

MTT

MeT5A



A549



Cellule mesoteliali virus trasformate

Cellule di adenocarcinoma polmonare

Fibre Asbestiformi:
DCF(Diclorofluoresceina) Radicali liberi

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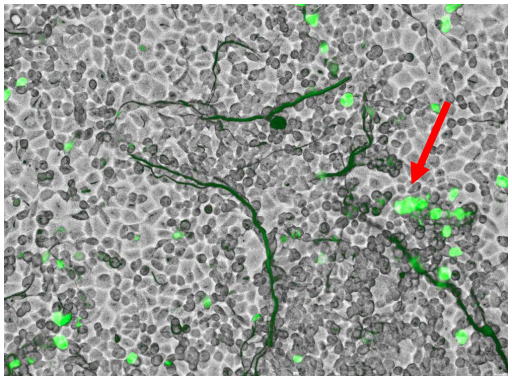
Chr-UICC



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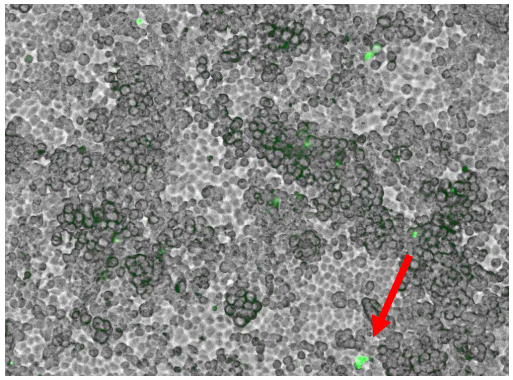
CTRL



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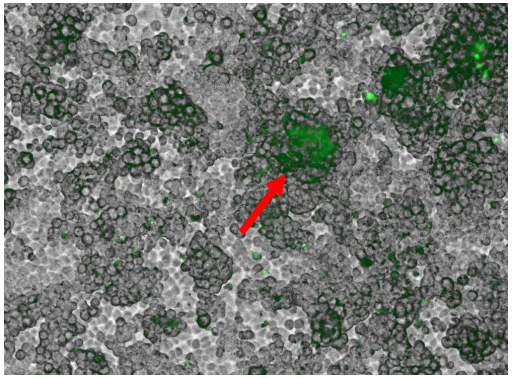
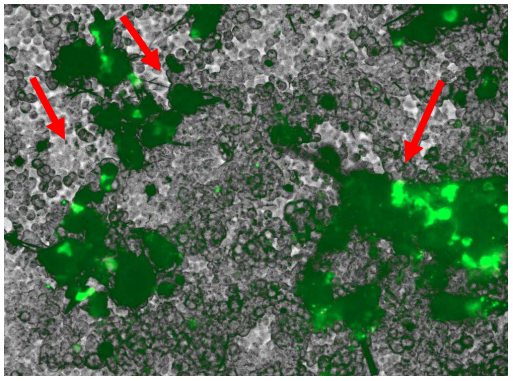
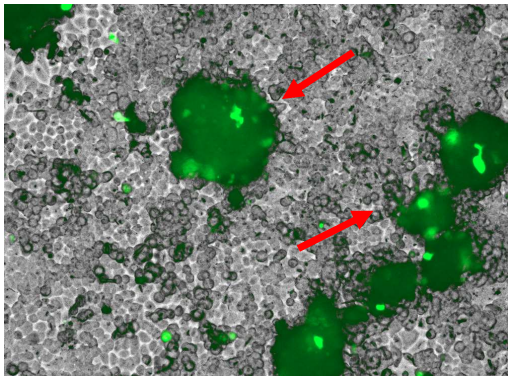




CRO-UICC



ERI-JN

Analisi del danno al DNA:
Comet assay

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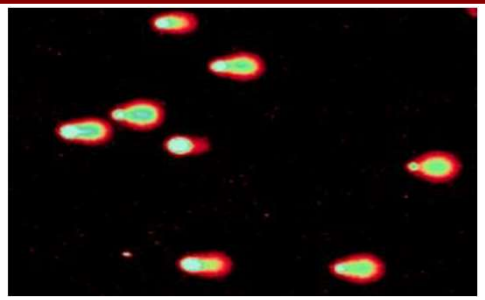

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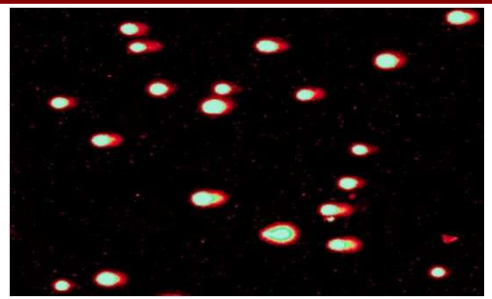

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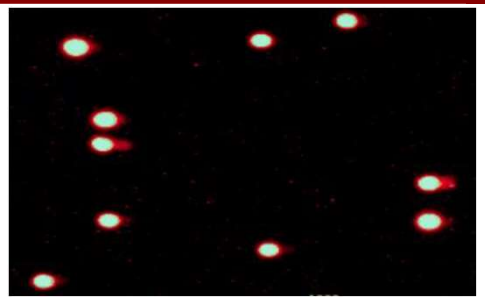
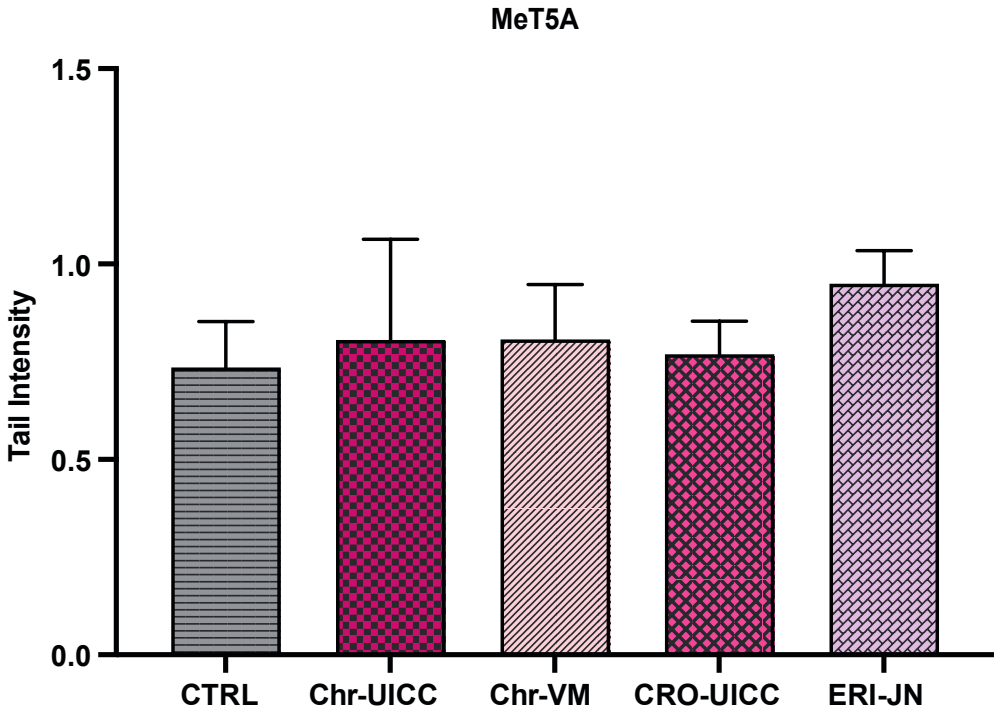
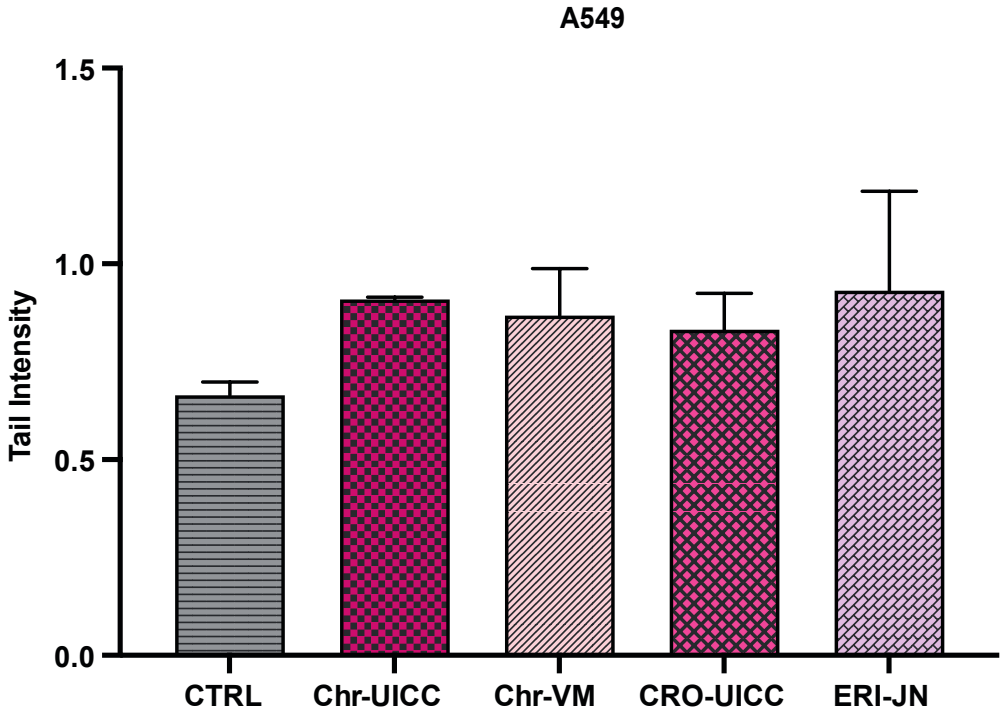


CHR-VM

ERI-JN





Analisi del danno al DNA:
Immunofluorescenza foci γ–H2AX(Ser139)

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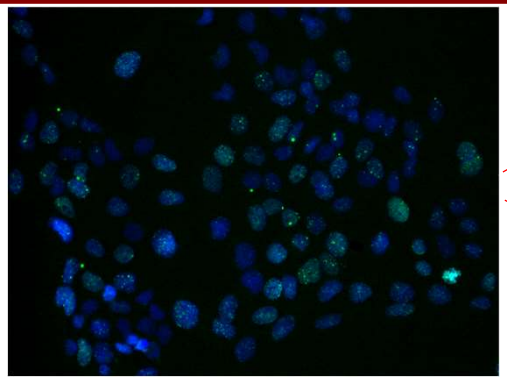
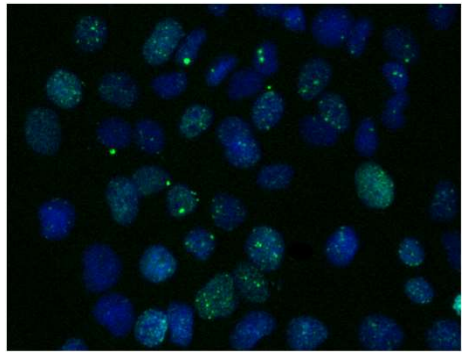
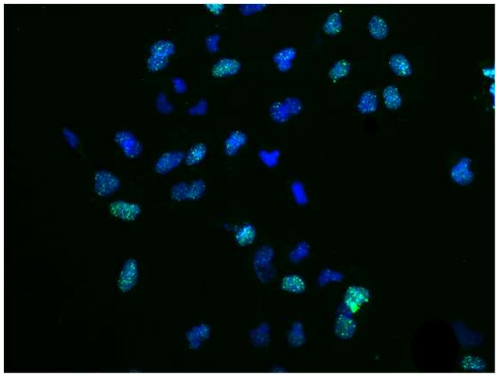


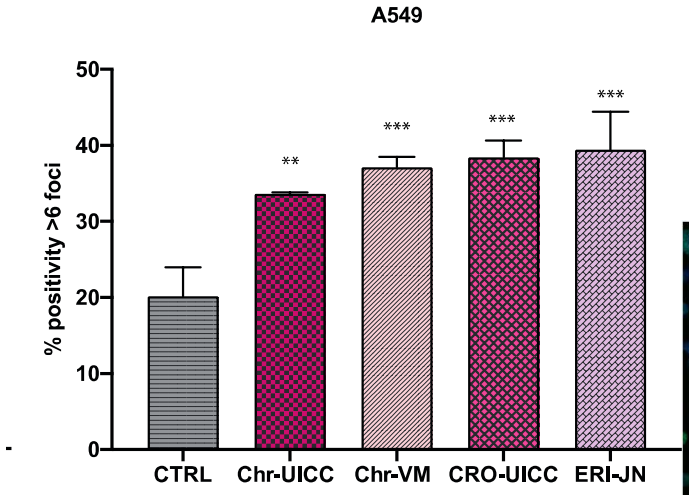
CHR-VM

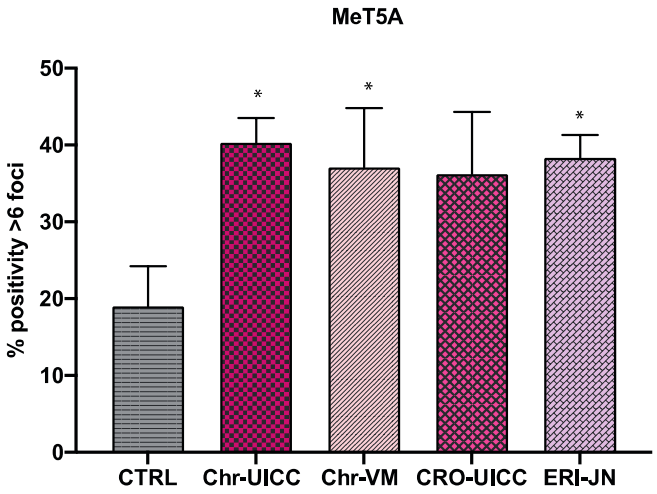




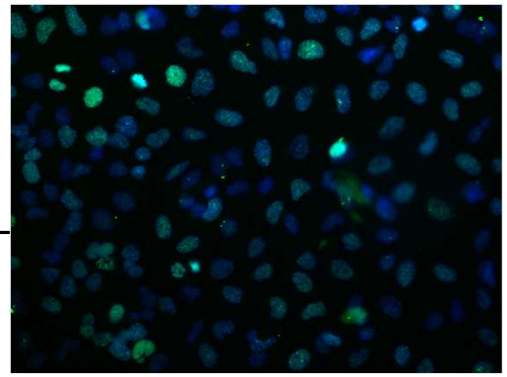
ERI-JN

MeT5A





A549



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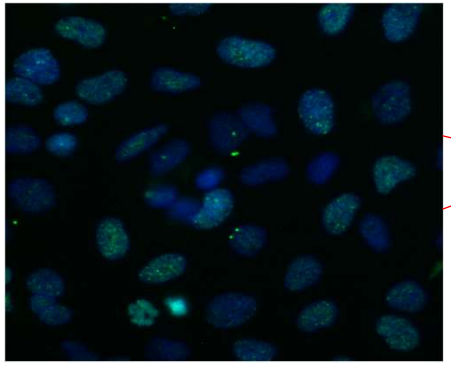

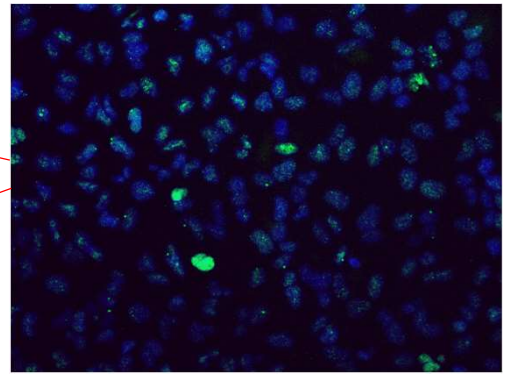

ERI-JN

Cellule positive >6 foci

Meccanismi di riparo:
Livelli di glutatione e RAD 51 WB

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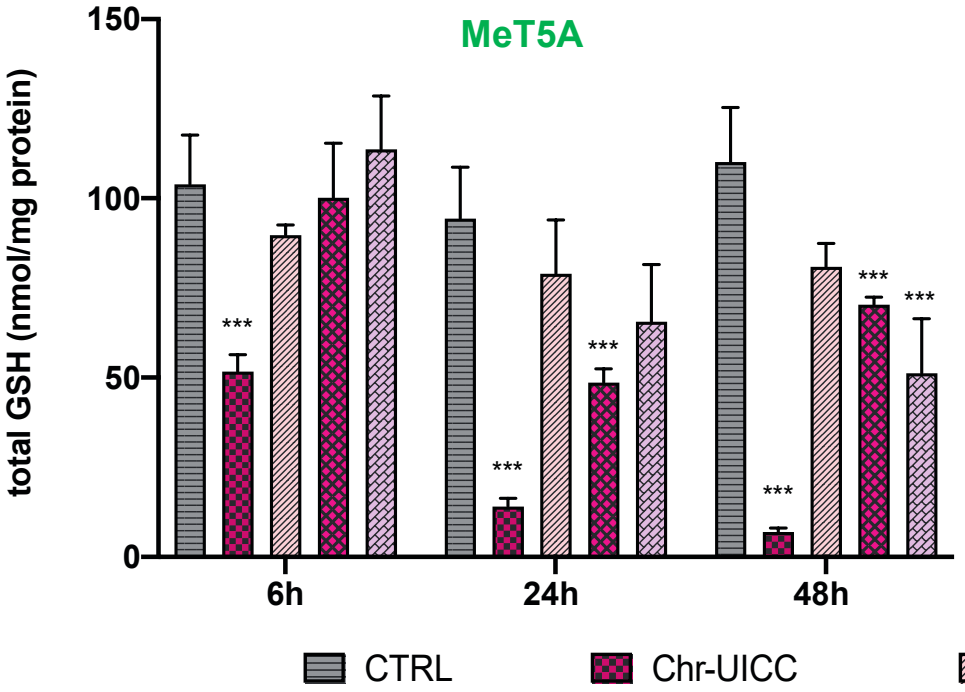
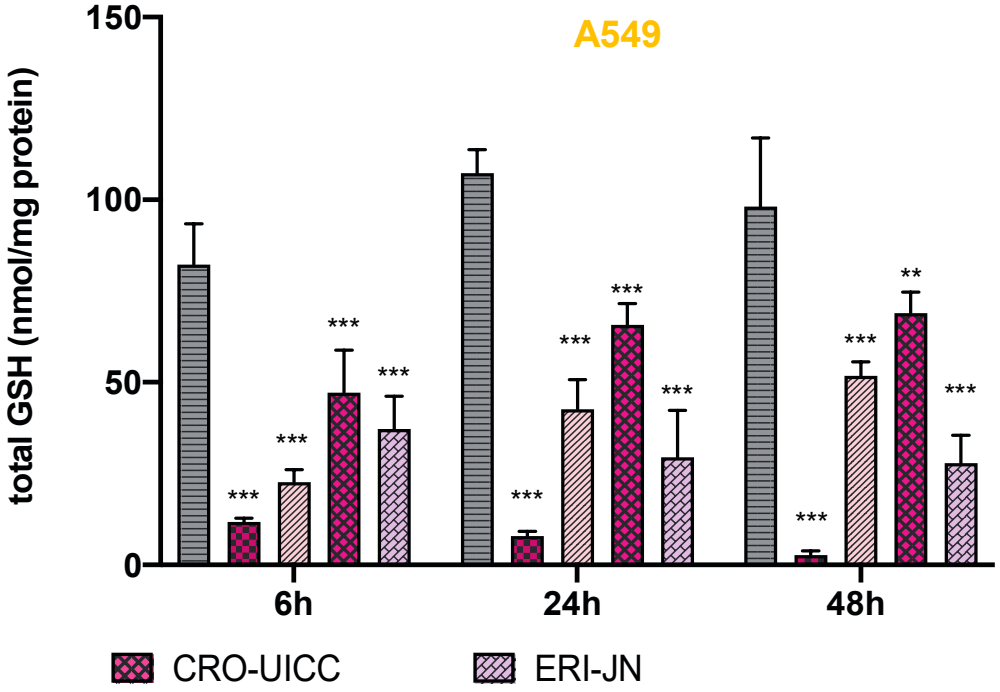
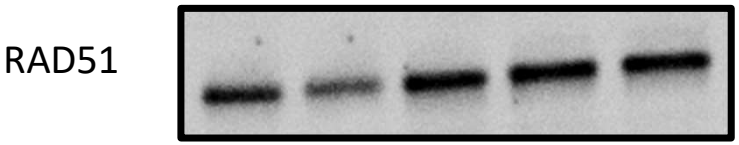
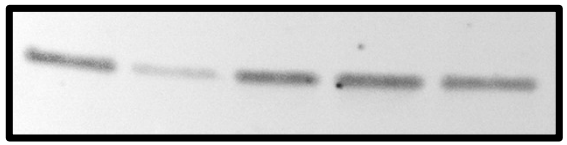






37 KDa



Infiammazione/Metilazione: Real-Time PCR

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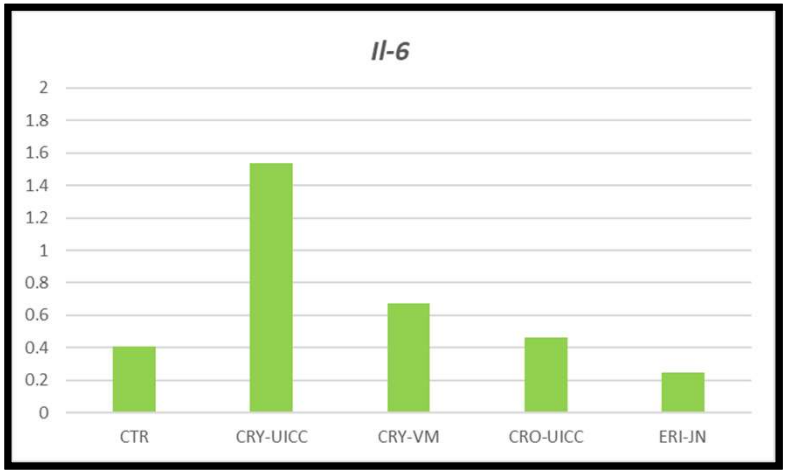
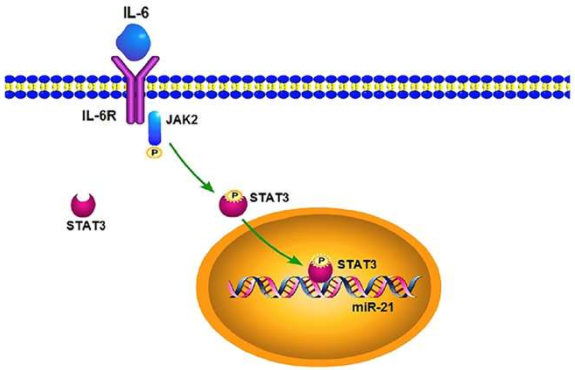
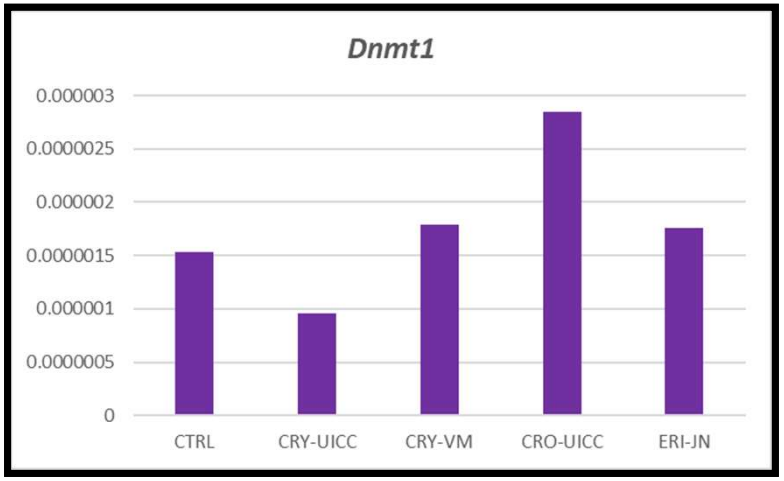
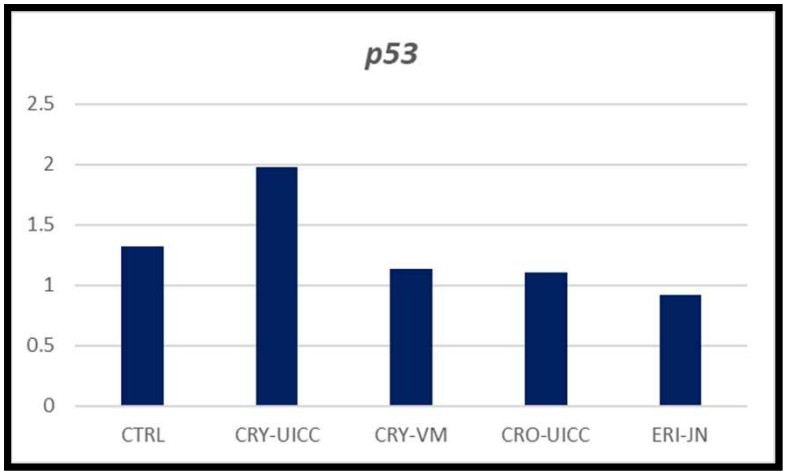
MeT5A 24h









Conclusioni

- **VM CHRY** e **JN ERI** esercitano rispettivamente un effetto citotossico attraverso una riduzione di vitalità e genotossico attraverso induzione di danno al DNA *in vitro*;
- I meccanismi di riparo risultano compromessi;
- E’ necessario prestare la giusta sorveglianza su questo tipo di particolati presenti in natura

Fibre Asbestiformi/Microscopio ottico MeT5A
Crisotilo russo (Chr-Ru), Wollastonite;

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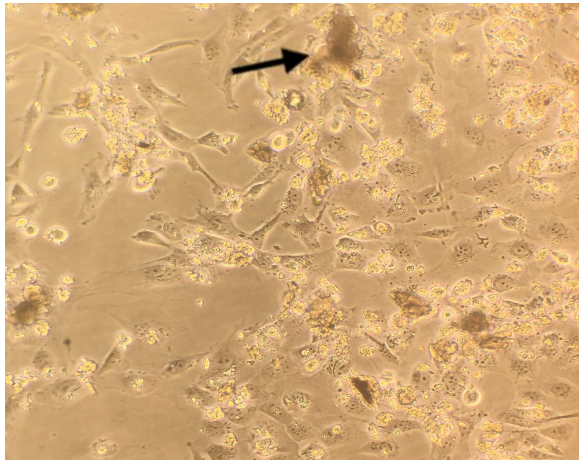
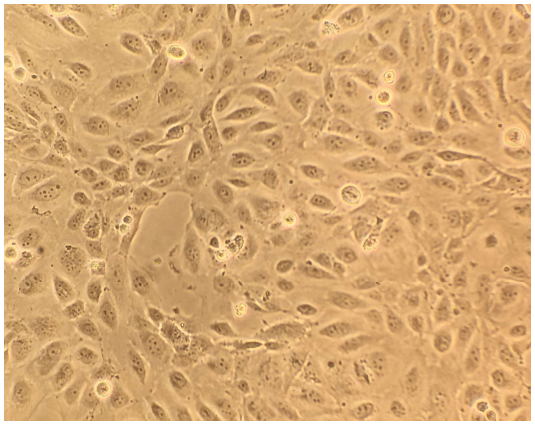
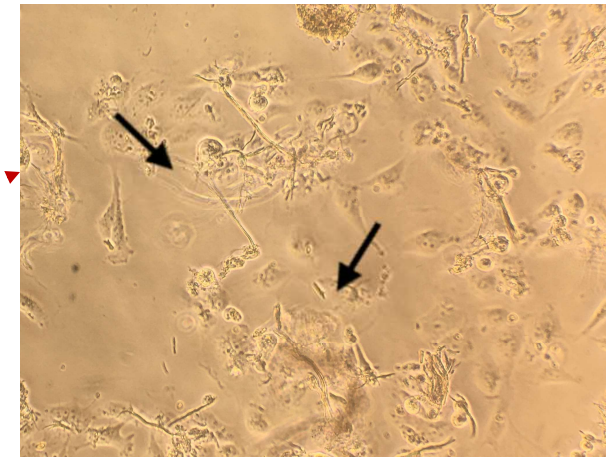


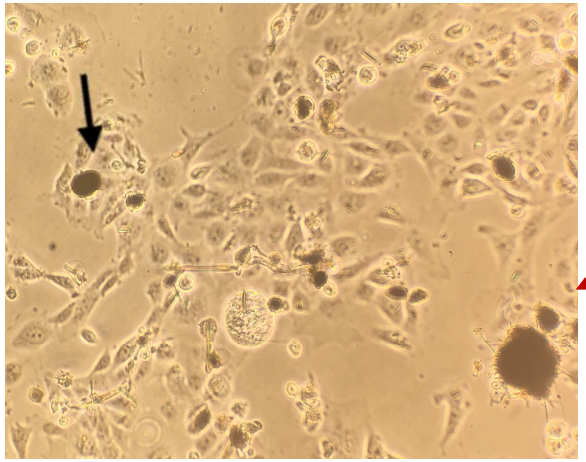
R1

20X
CTR

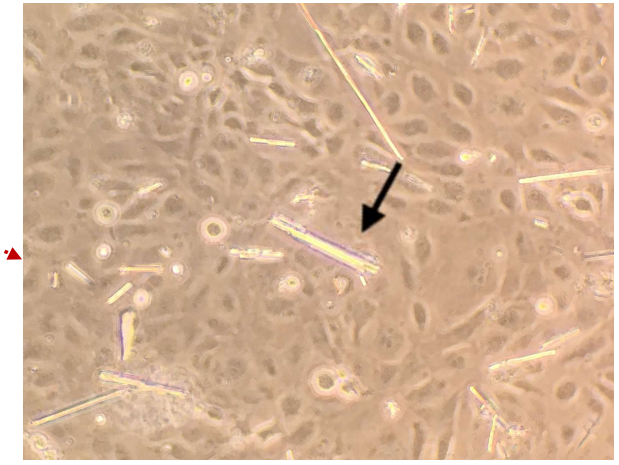




R2



CRO



W

Fibre Asbestiformi/Microscopio ottico A549
Crisotilo russo(Chr-Ru), Wollastonite;

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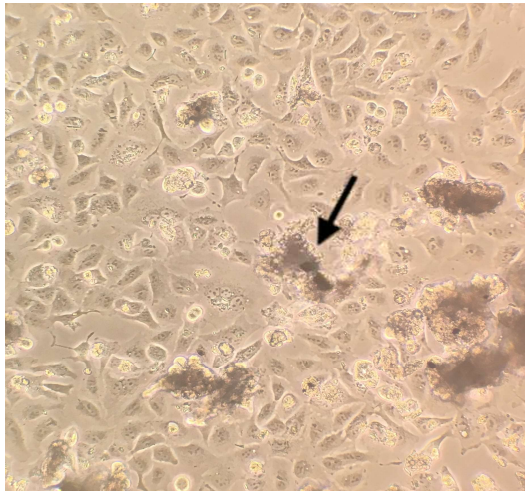
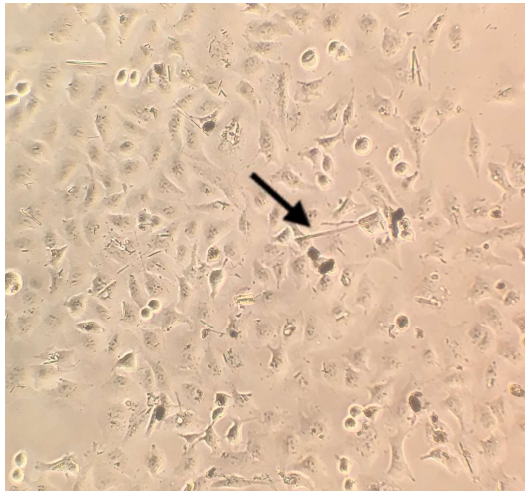


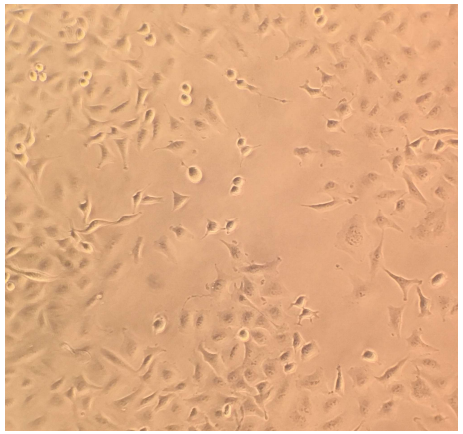
R1

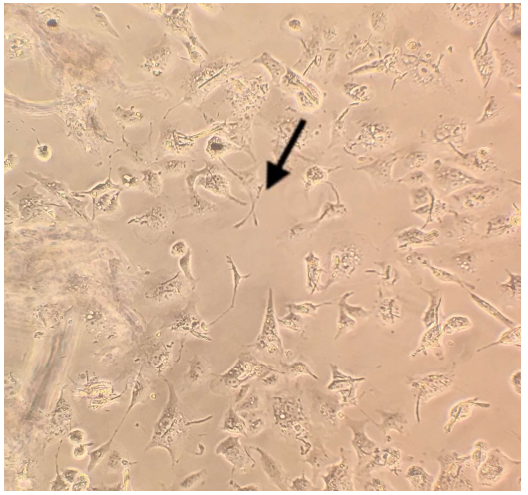


CRO

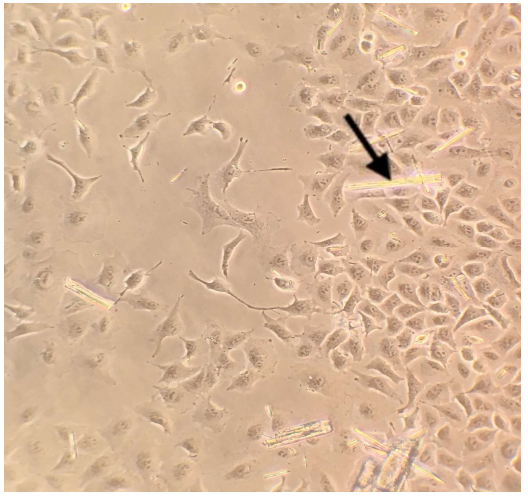
20X

CTR





R2



W

Fibre Asbestiformi/Microscopio ottico:
Crisotilo russo(Chr-Ru), Wollastonite; A549

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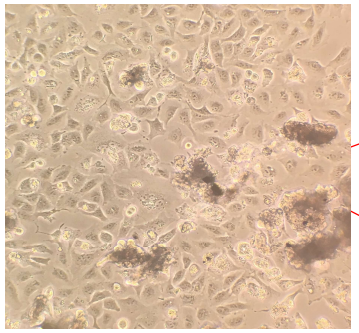
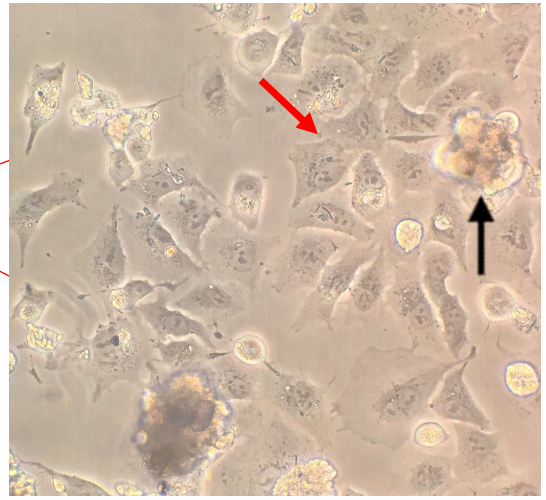
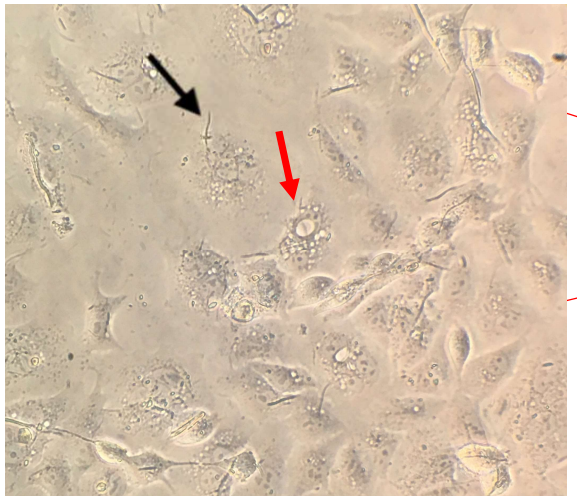


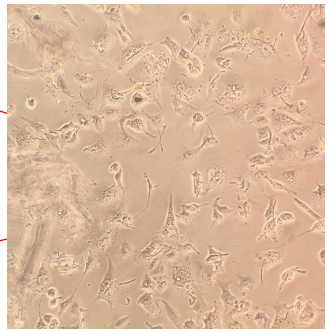
R1 20X



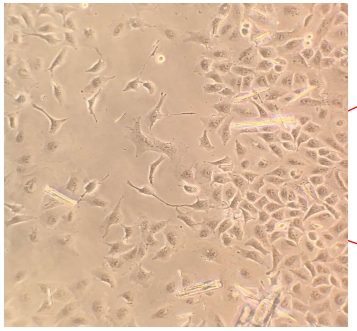
R1 40X



R2 40X



R2 20X



W 20X

W 40X

Crisotilo Russo(Chr-Ru):
Caratterizzazione e Analisi della vitalità cellulare

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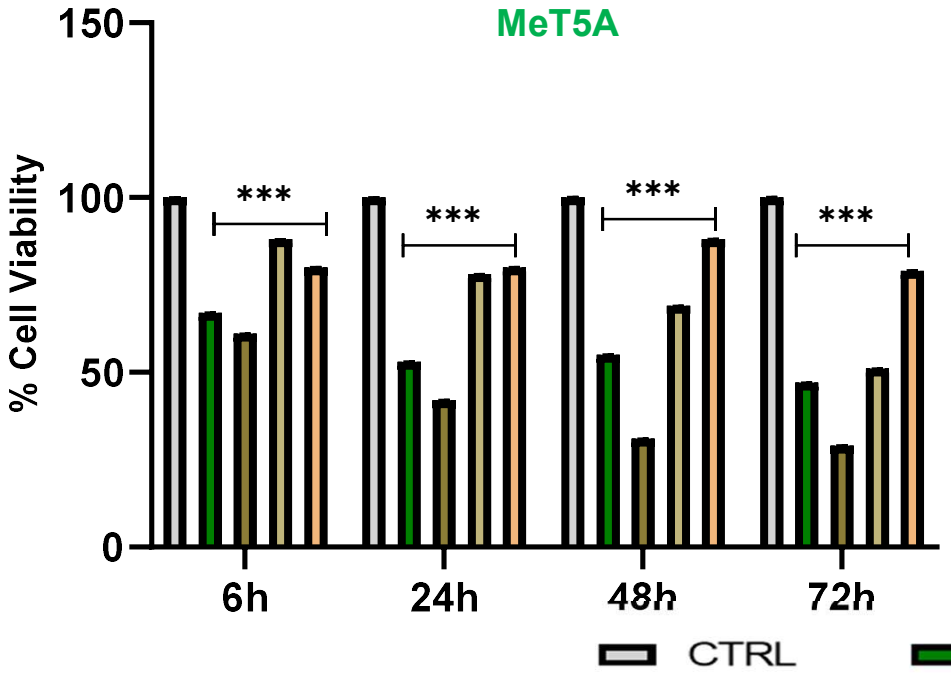

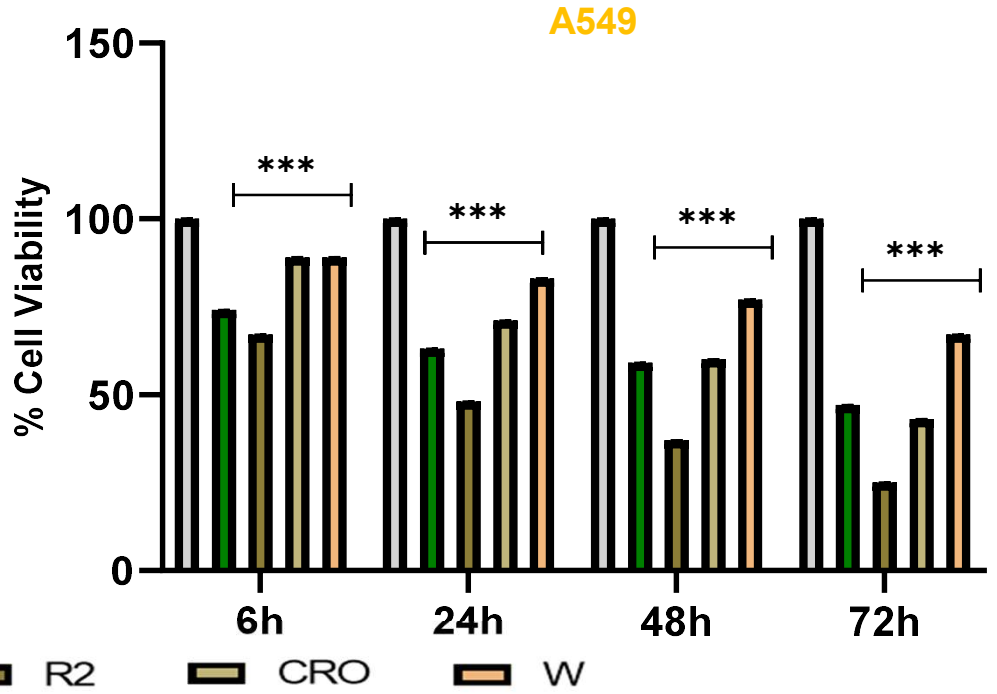

Met5a	Media 6h	Media 24h	Media 48h	Media 72h
1.CTRL	100±0.034	100±0.031	100±0.061	100±0.067
2.R1	74±0.017	63±0.008	59±0.012	47±0.067
3.R2	67±0.014	48±0.011	37±0.023	25±0.031
4.CRO	89±0.024	71±0.019	60±0.039	43±0.045
5.W	89±0.036	83±0.038	77±0.048	67±0.071

ds: 1-2; 1-3; 1-4; 1-5; 2-3; 2-4; 2-5; 3-4; 3-5; 4-5

A549	Media6h	Media 24h	Media 48h	Media 72h
1.CTRL	100±0.053	100±0.034	100±0.14	100±0.14
2.R1	67±0.022	53±0.029	55±0.041	47±0.036
3.R2	61±0.019	42±0.028	31±0.027	29±0.027
4.CRO	88±0.029	78±0.026	69±0.054	51±0.055
5.W	80±0.015	80±0.054	88±0.15	79±0.16

ds: 1-2; 1-3; 1-4; 1-5; 2-3; 2-4; 2-5; 3-4; 3-5; 4-5

CTRL=controllo; R1= Chr-Ru <5µm; R2= Chr-Ru > 5µm; CRO=crocidolite; W=wollastonite

Conclusioni

- **CHR-RU** esercita un effetto citotossico tempo dipendente *in vitro*; a partire dalle 6h sino alle 72h(tramite saggio MTT)
- Osservazione di una maggiore citotossicità di **R2 (Chr-Ru >5µm)** rispetto **R1 (Chr-Ru <5µm)**
- Il metasilicato di calcio: **WOLLASTONITE (W)** non risulta avere un effetto citotossico rilevante *in vitro*
- Risulta importante continuare ad osservare gli effetti ed i meccanismi che questa fibra attua nei modelli *in vitro*

Prospettive future

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- Studio dei meccanismi molecolari ed epigenetici coinvolti dopo l'esposizione a fibre minerali asbestiformi (mRNA, miRNA tramite qPCR e WB)

- Investigare il tipo di risposta scatenata dal Sistema immunitario in seguito all'esposizione a tali sostanze (studio citochine tramite Bioplex)

- Analisi dei segnali paracrini rilasciati dale cellule (vescicole extracellulari)

Acknowledgments

UNIVERSITÀ POLITECNICA DELLE MARCHE

Prof. Antonio D. Procopio

Prof. Armanda Pugnaloni

Prof. Daniela Marzioni

Prof. Francesca Fazioli

Dr. Salvatore Vaiasicca

Dr. Deborah Ramini

Dr. Sonia Fantone

Dr. Giovanni Tossetta

UNIMORE
UNIVERSITÀ DEGLI STUDI DI MODENA E REGGIO EMILIA

Prof. Alessandro F. Gualtieri

PRIN 2017

UNIVERSITÀ POLITECNICA DELLE MARCHE

I dati esposti saranno presentati dal Dr. Salvatore Vaiasicca quale invited speacker al Goldschmidt 2021 Conference, Lyon (France) and online 4 July 2021 - 9 July 2021

GOLDSCHMIDT VIRTUAL· 2021 4–9 JULY

geochemical society

Cytotoxic and Genotoxic potential of asbestos fibers from environmental outcrops

Salvatore Vaiasicca, S.Di Valerio, L.Cianfruglia, D.Ramini, T.Armeni, F.Fazioli, A.D.Procopio, A.Pugnaloni.

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UNIVERSITÀ DEGLI STUDI DI MODENA E REGGIO EMILIA

UNIVERSITÀ POLITECNICA DELLE MARCHE

FIBRES: a multidisciplinary mineralogical, crystal-chemical and biological project to amend the paradigm of toxicity and cancerogenicity of mineral fibres.

Role of mineral fibers on the materno-fetal placental barrier

Prof. Daniela Marzioni
Dott.ssa Sonia Fantone
Dott. Giovanni Tossetta

Published: 10 May 1974

Placental transfer of asbestos

H. M. CUNNINGHAM & R. D. PONTEFRACT

Nature **249**, 177–178 (1974) | Cite this article

Abstract

ASBESTOS fibres have been reported to penetrate the walls of the stomach and intestine of animals[1,2]. Earlier work at this institution showed that asbestos fibres can pass through the walls of the digestive tract and travel throughout the body[3,4]. Additional work with neutron-activated asbestos also indicated that asbestos could cross the placenta but definite confirmation of this was not obtained[4]. Here we show, by electron microscopy, that asbestos fibres can cross the placenta but that the extent to which this occurs is highly variable.

Published: 01 October 1998

Assessment of Asbestos Burden in the Placenta and Tissue Digests of Stillborn Infants in South Texas

A. K. Haque, D. M. Vrazel & T. Uchida

Archives of Environmental Contamination and Toxicology **35**, 532–538 (1998) | Cite this article

Abstract.

The primary aim of this prospective study was to examine the tissues and placentas of autopsied stillborn infants for presence of asbestos fibers. Asbestos burden of lung, liver, skeletal muscle, and placenta digests of 82 stillborn infants was determined using standard bleach digestion technique. The digests were examined by electron microscopy, and the types of fibers determined using energy dispersive x-ray analysis and selected area diffraction analysis. Digests of 45 placentas collected from deliveries of liveborn healthy infants were processed and examined similarly as controls. Asbestos fibers were detected in 50% of the fetal digests and 23% of the placental digests of stillborn infants. Of the fibers present, 88% were chrysotile, 10% were tremolite, and 2% were actinolite and anthophyllite. Fibers measured

› Pediatr Pathol Lab Med. Nov-Dec 1996;16(6):877-92. doi: 10.1080/15513819609168711.

Is there transplacental transfer of asbestos? A study of 40 stillborn infants

A K Haque [1], D M Vrazel, K D Burau, S P Cooper, T Downs

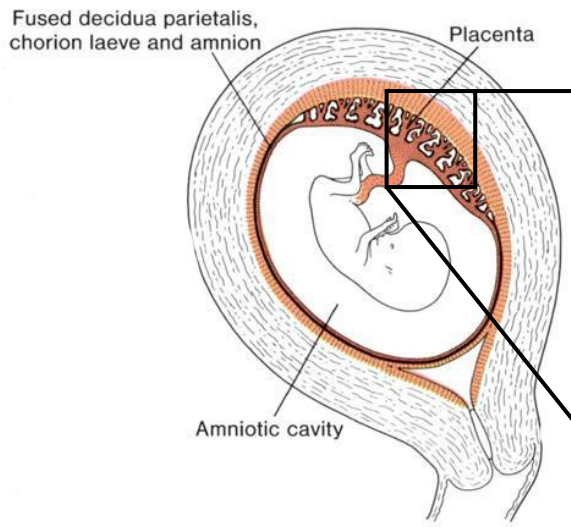
Affiliations + expand

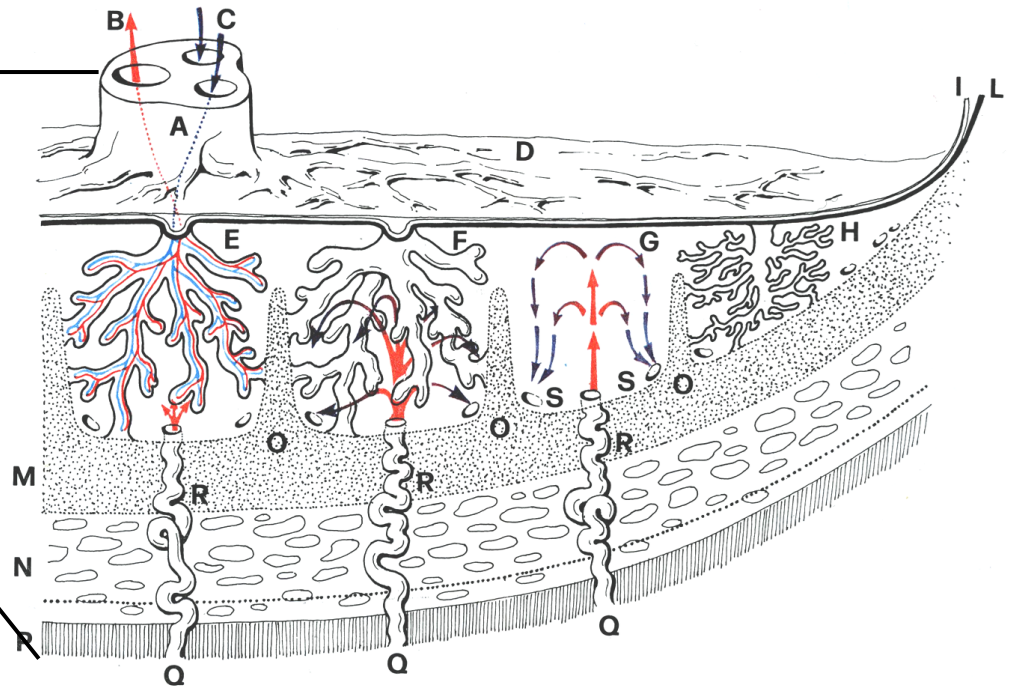
PMID: 9025886 DOI: 10.1080/15513819609168711

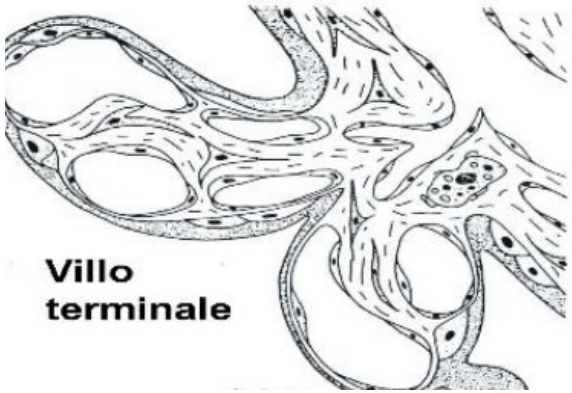
Abstract

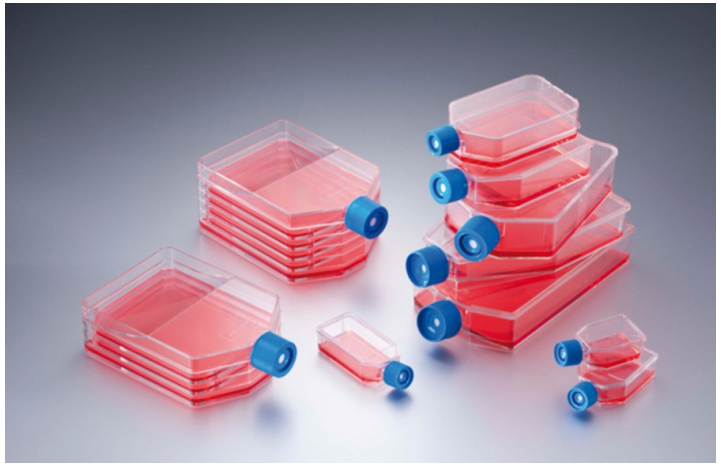
An autopsy study was conducted to investigate whether there is transplacental transfer of asbestos in humans. The asbestos burden of lung, liver, skeletal muscle, and placenta digests of 40 stillborn infants was determined using a bleach digestion method. The fibers detected in the tissue digests were characterized as to the type of asbestos, using electron microscopy, energy-dispersive x-ray analysis, and selected-area diffraction analysis. Placental digests of 45 full-term, liveborn infants were similarly processed as controls. Low levels of small, thin, uncoated asbestos fibers were detected in the placentas and organs of 37.5% of the stillborn infants (15 of 40). The fiber sizes ranged from 0.05 to 5.0 microns in length and 0.03 to 0.3 micron in width, with a mean length of 1.15 microns and a mean width of 0.069 micron. Maximum numbers of fibers were found in the lungs (mean 235,400 fibers/g; n = 10), followed by liver (mean 212,833 fibers/g; n = 6), placenta (mean 164,500 fibers/g; n = 4), and skeletal muscle (80,000 fibers/g; n = 1). The fibers were detected at all stages of gestation and showed no association with gestational age. A significant association was found between fiber presence and working mothers, and positive but nonsignificant associations were found with maternal history of drug abuse, previous abortions, and fetal maceration. No association was found between

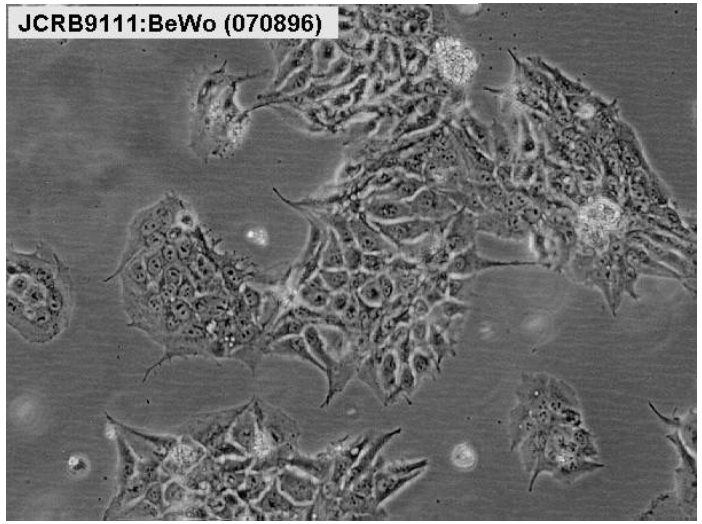
The Materno-Fetal Barrier



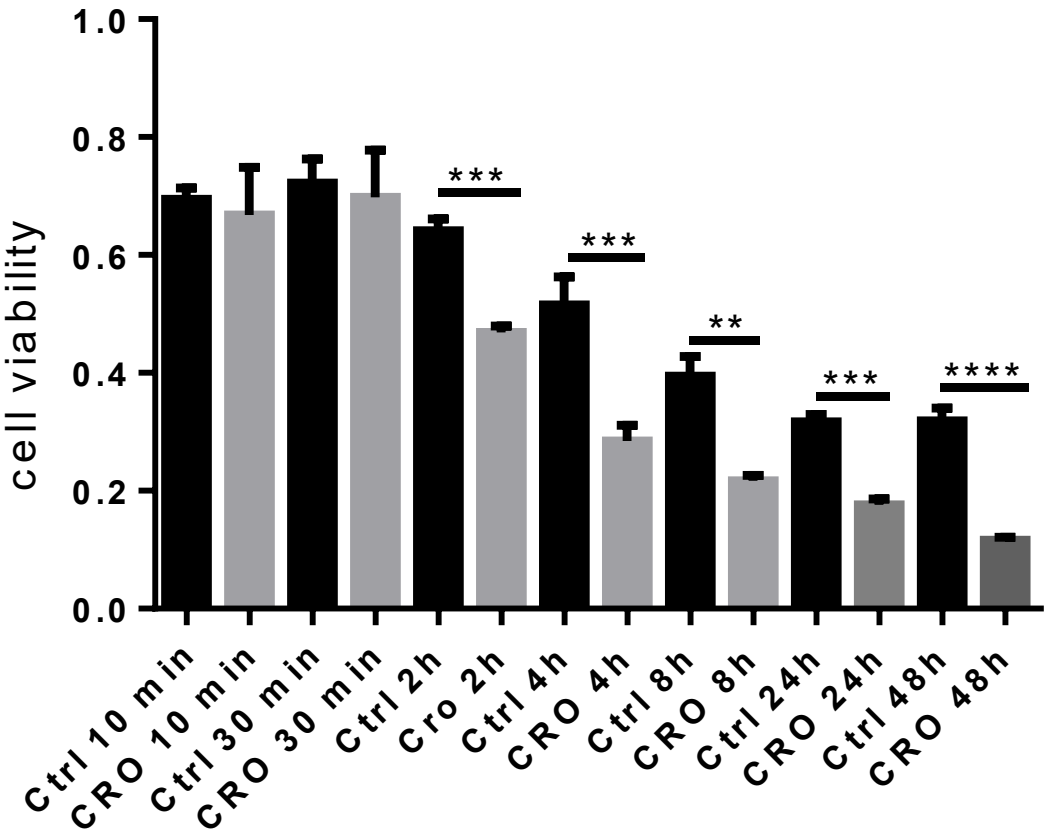


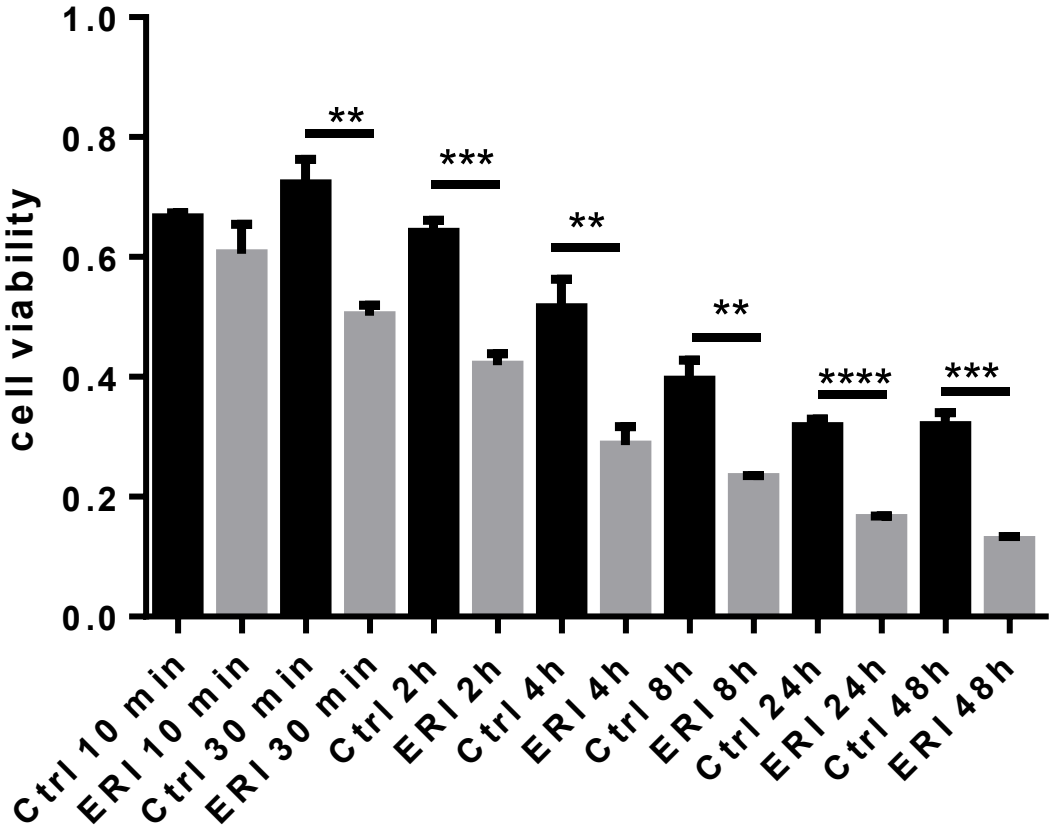




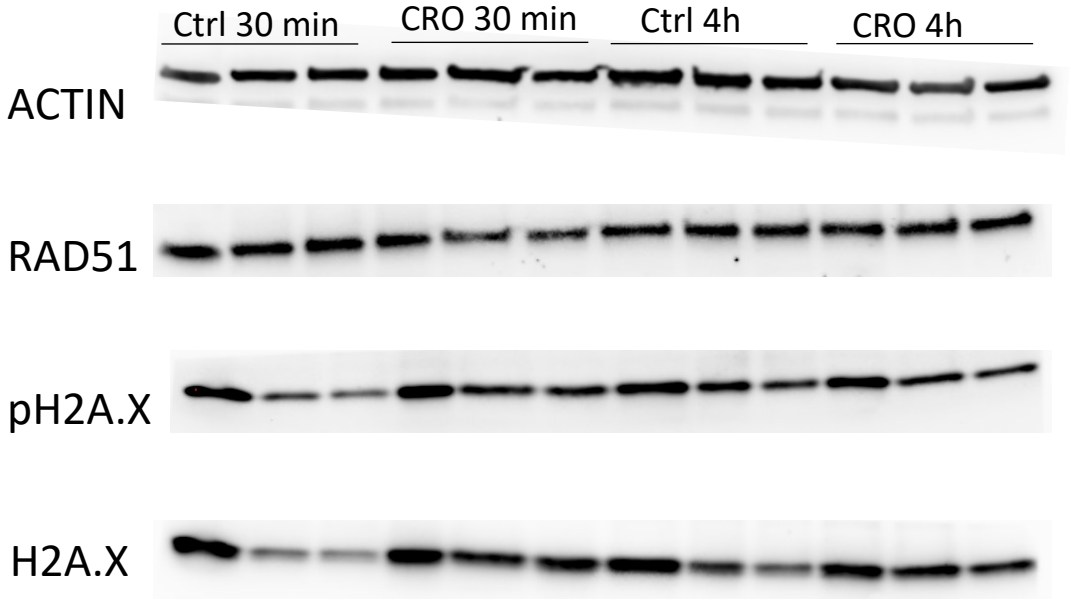


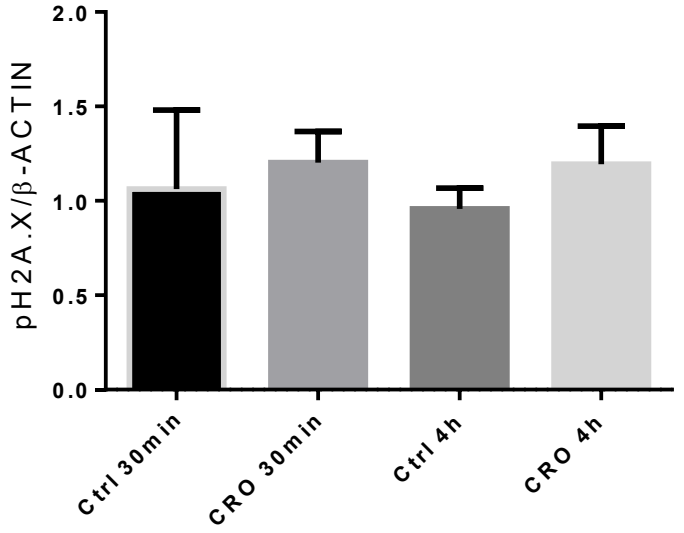
MTT ASSAY in BeWo cell line treated with Amphibole Asbestos: Crocidolite and Erionite

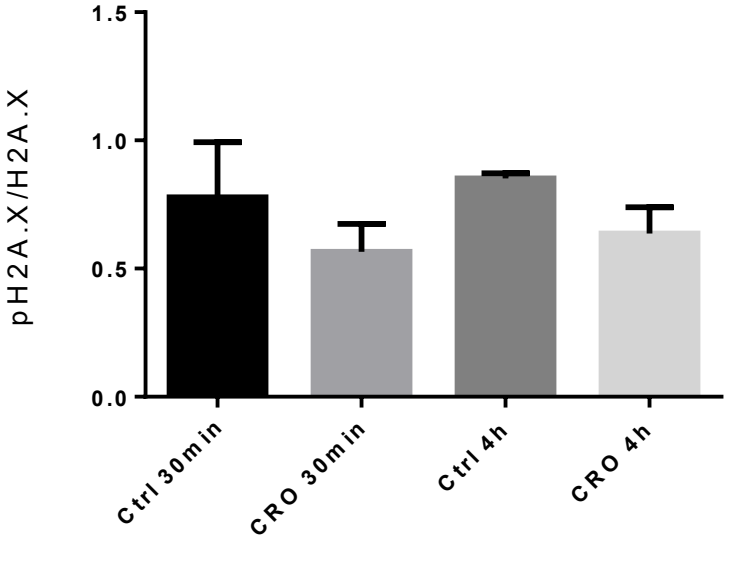


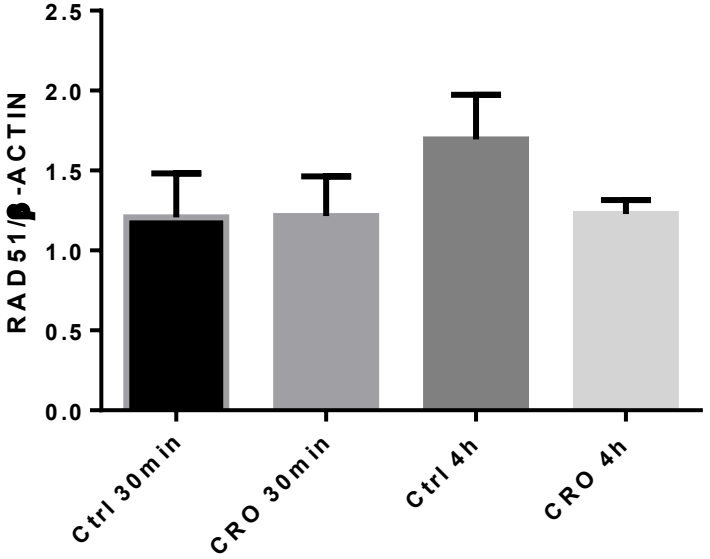


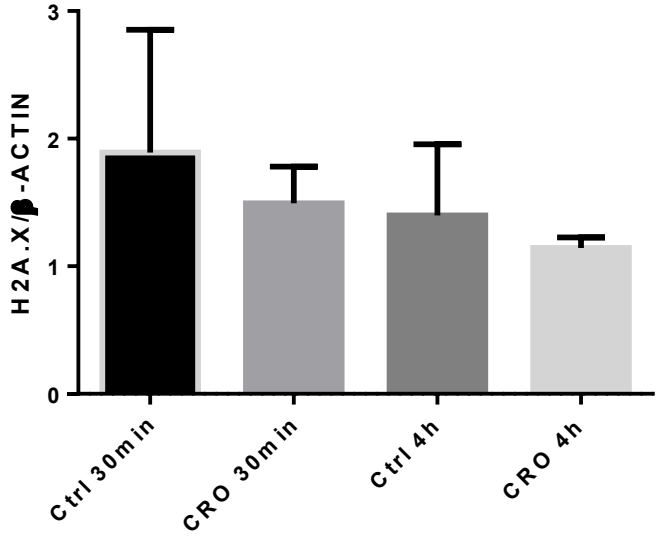
BeWo cell line treated with Crocidolite



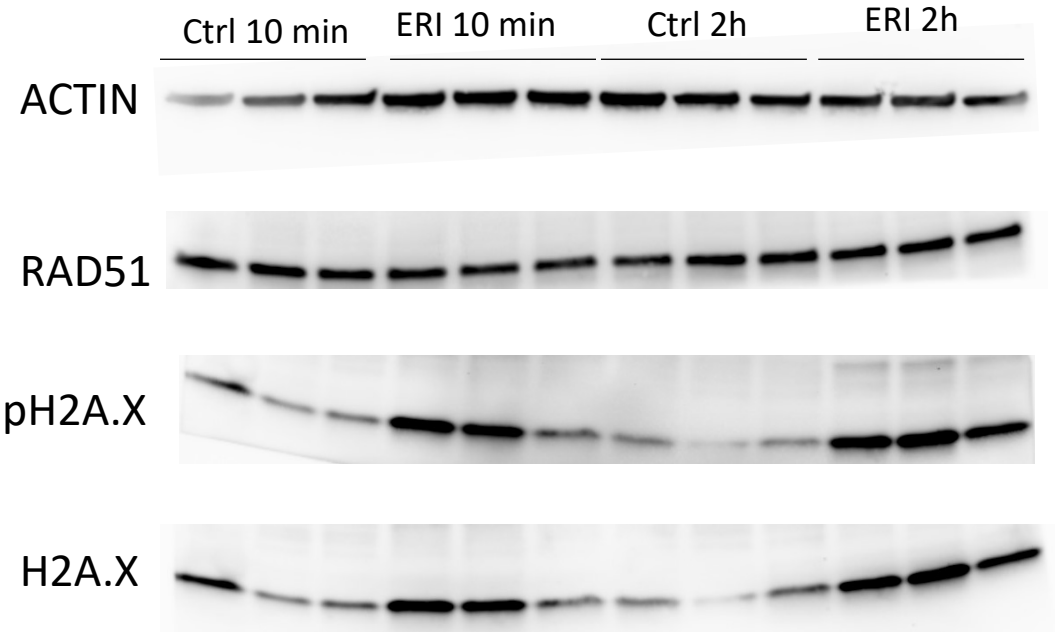


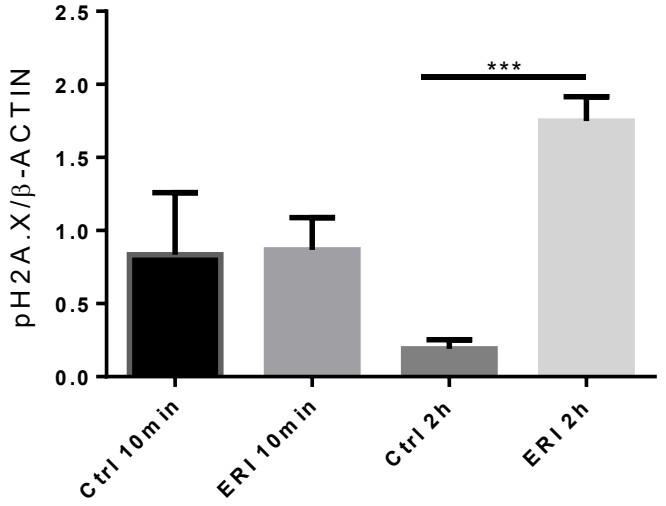


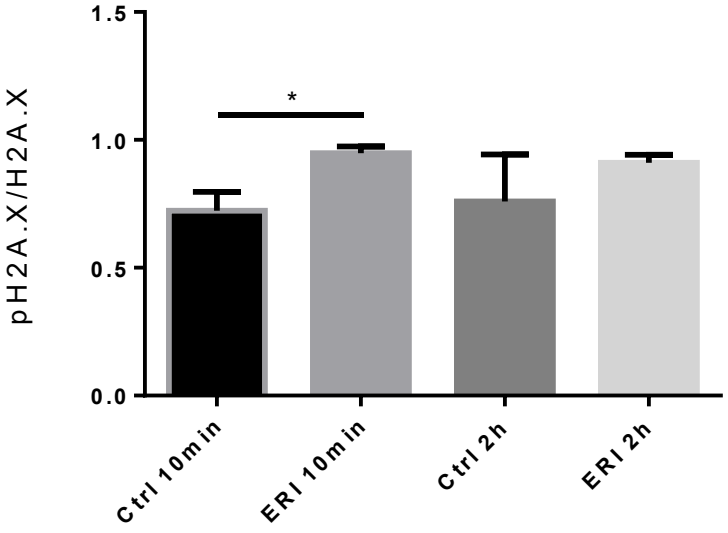


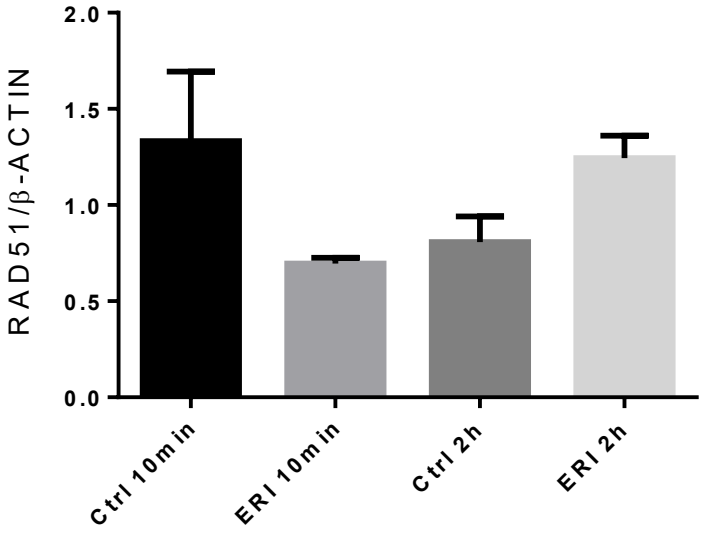


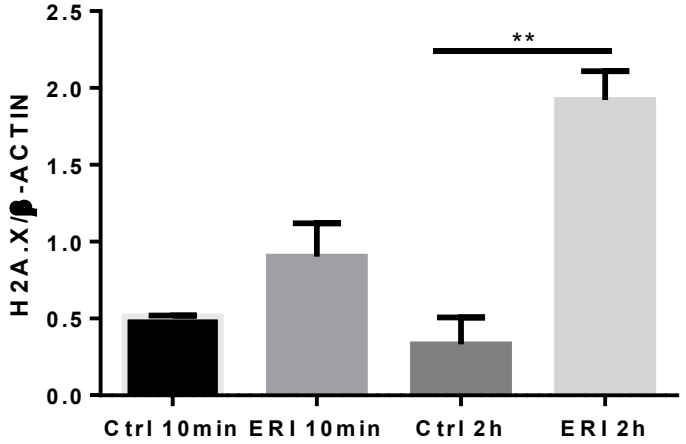
BeWo cell line treated with Erionite



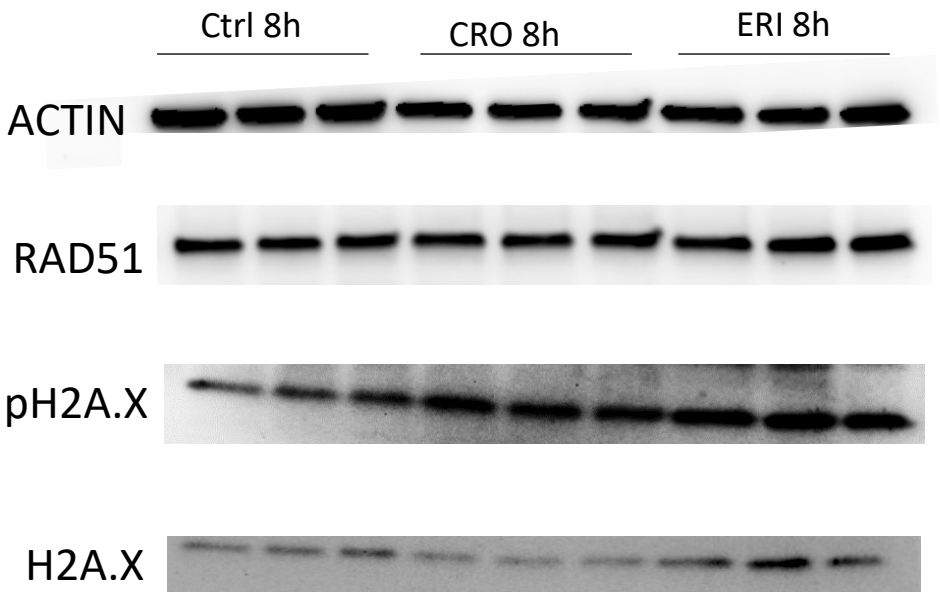


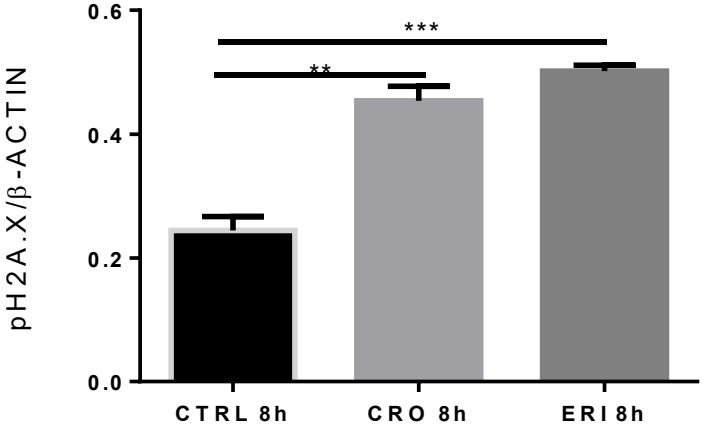


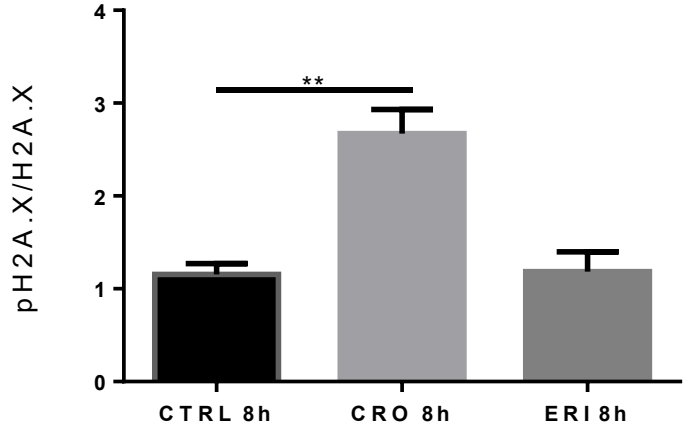


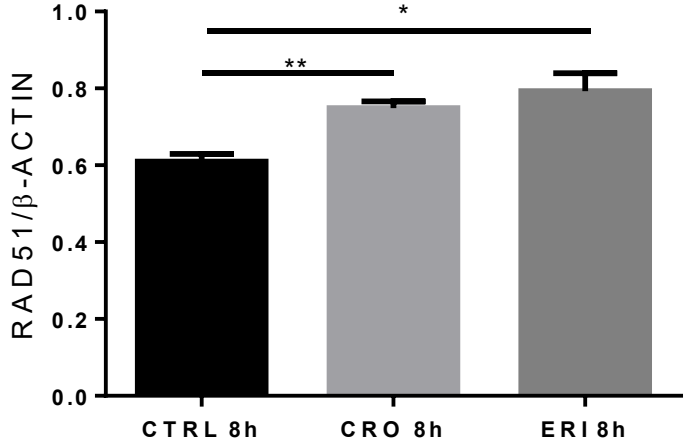


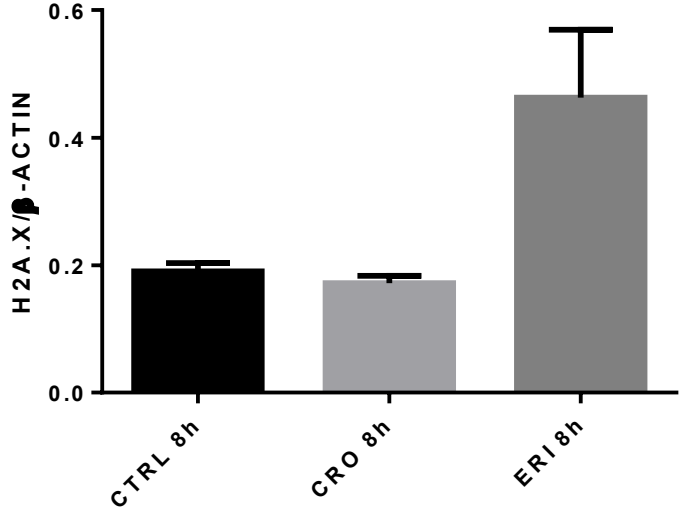
BeWo cell line treated with Crocidolite and Erionite







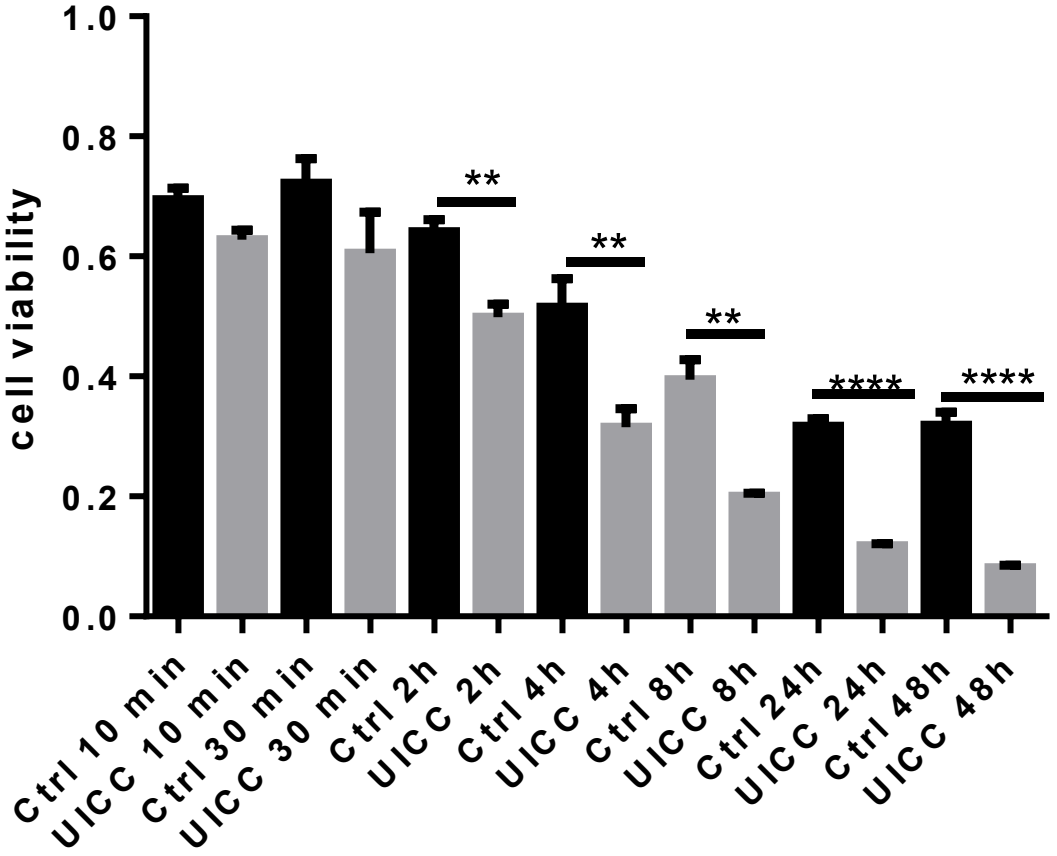


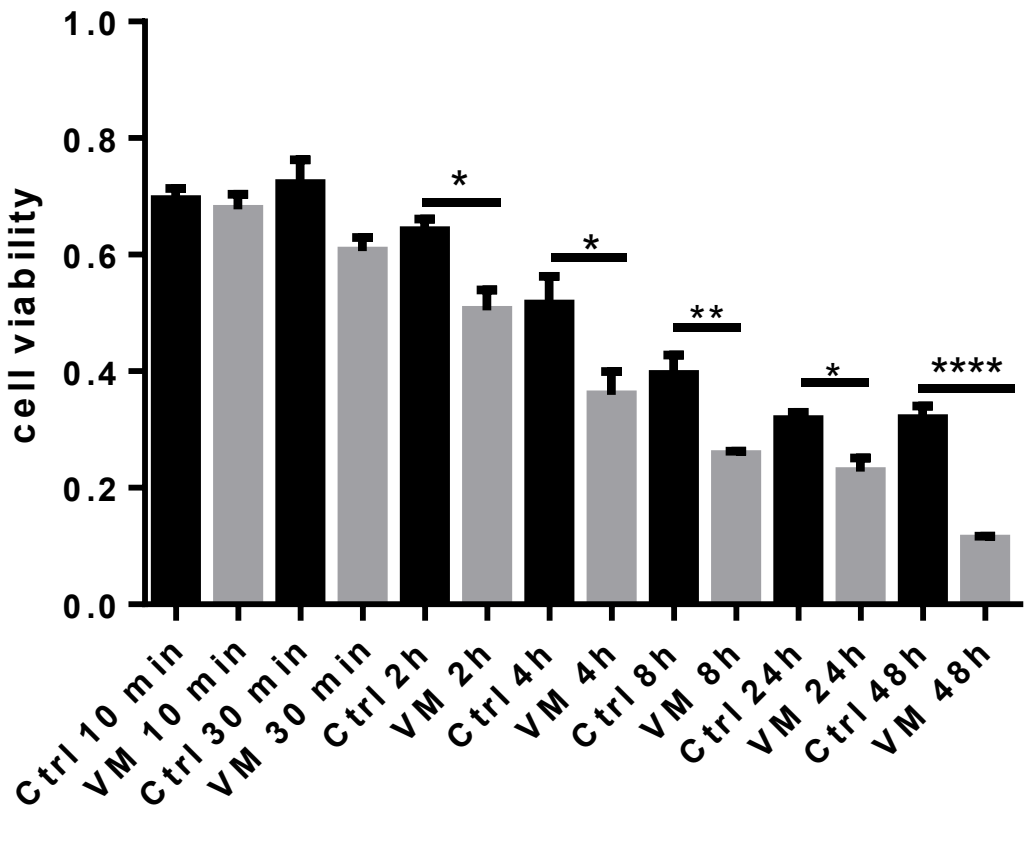


Results

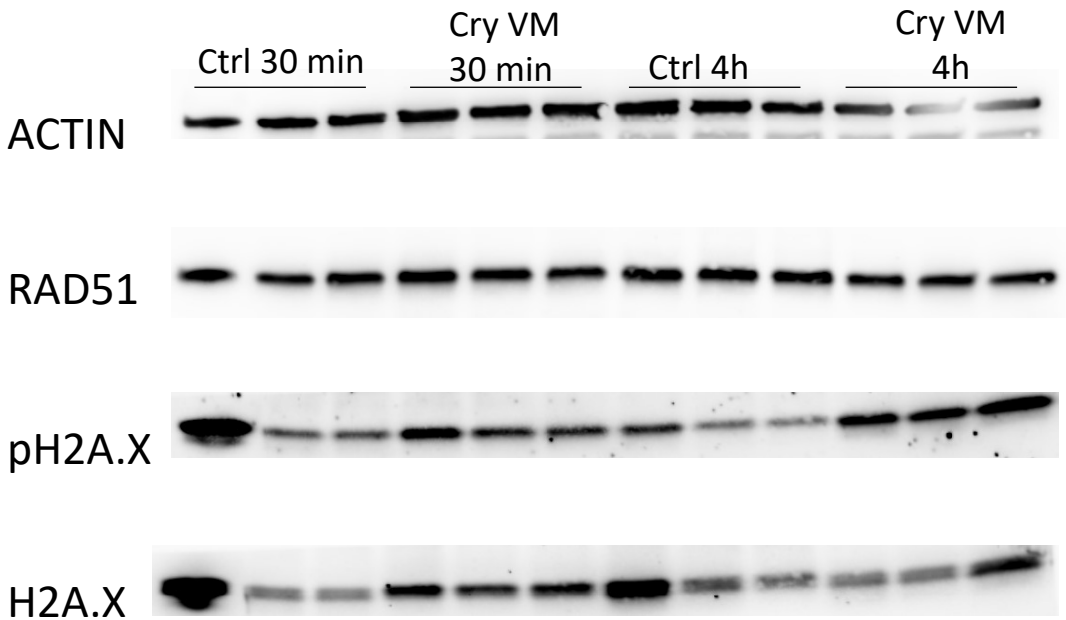
- Erionite is more cytotoxic than Crocidolite (MTT assay)
- At 30 min and 4 h, cells treated with Crocidolite did not show any damage
- At 10 min, cells treated with Erionite did not show any damage
- At 2 h, cells treated with Erionite showed DNA damage without repair
- At 8 h, both fibers showed DNA damage with consequent repair

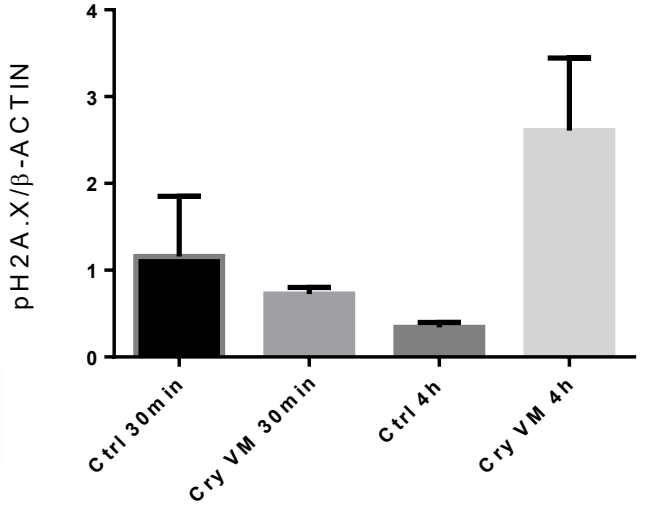
MTT ASSAY BeWo cell line treated with Serpentine Asbestos: Chrysotile VM and Chrysotile UICC

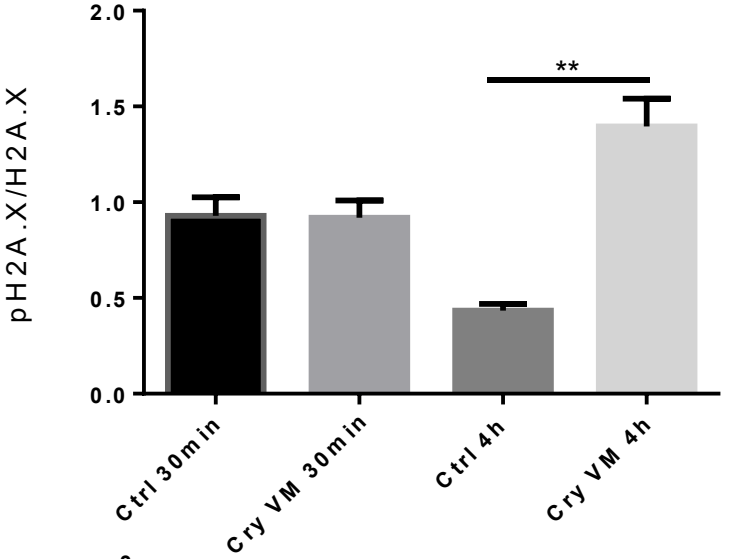


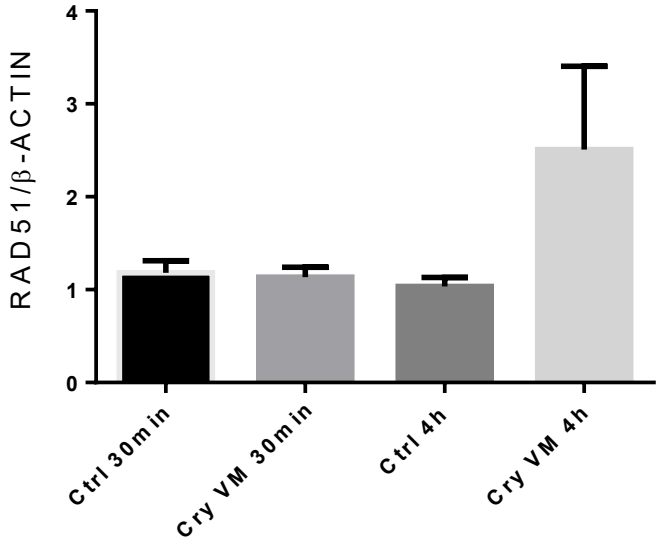


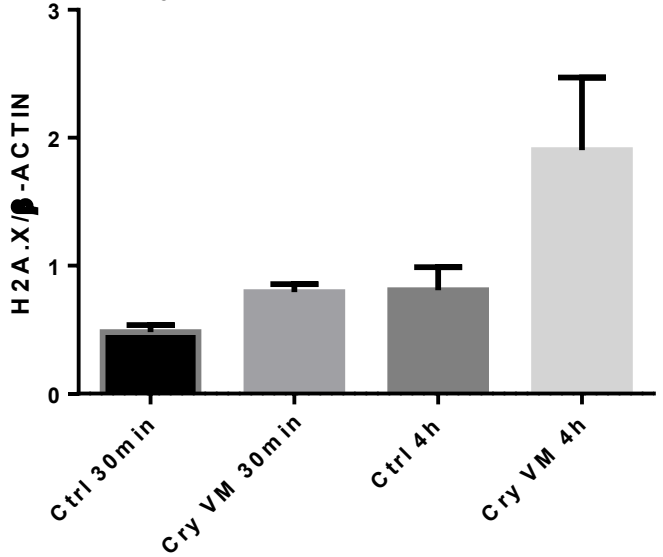
BeWo cell line treated with Chrysotile VM



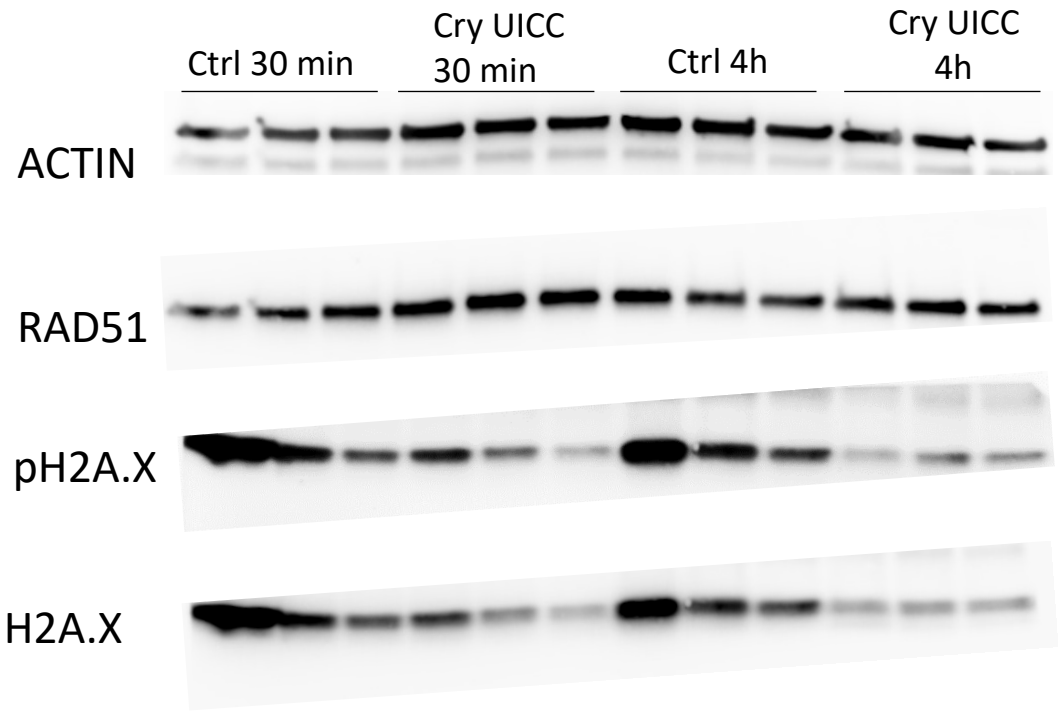


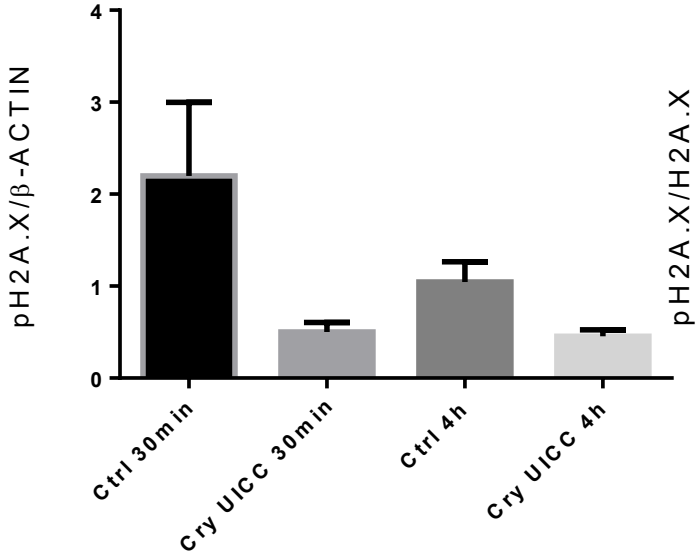


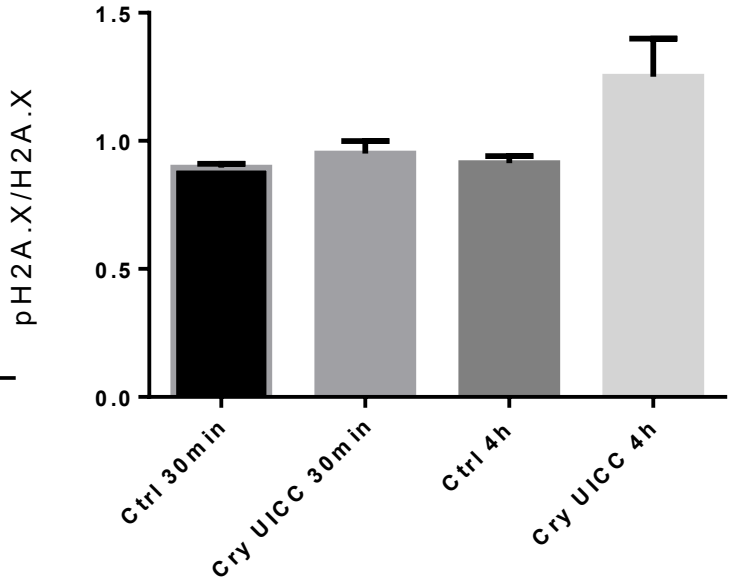


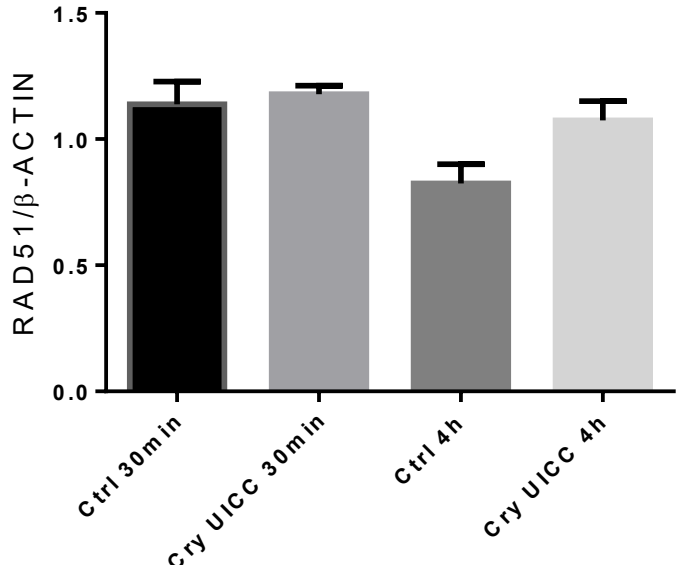


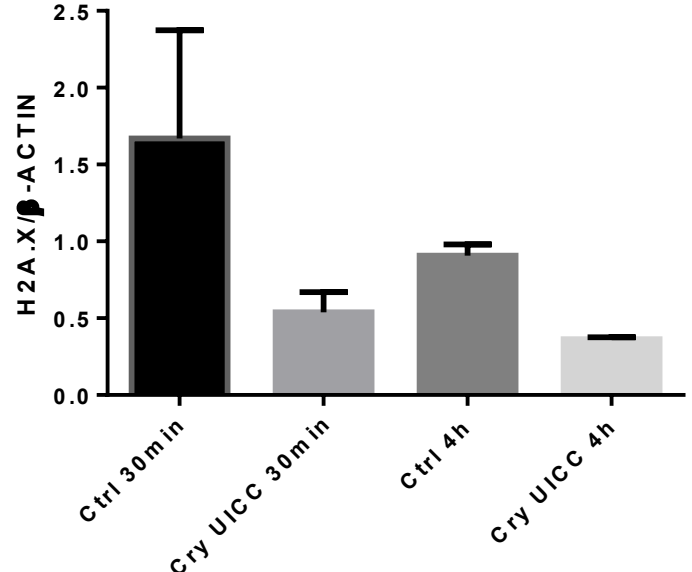
BeWo cell line treated with Chrysotile UICC



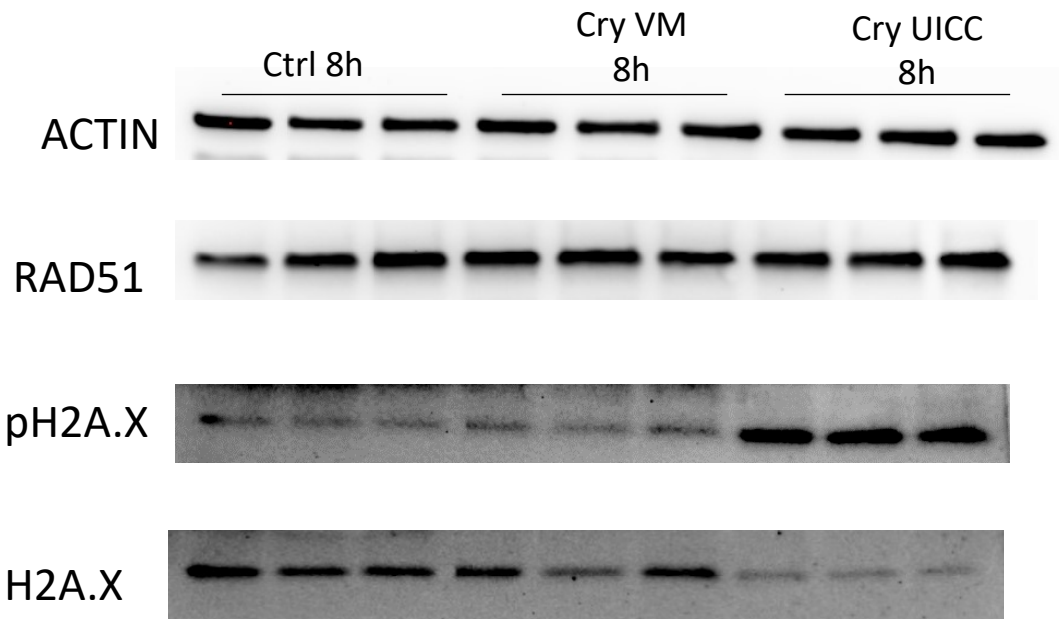


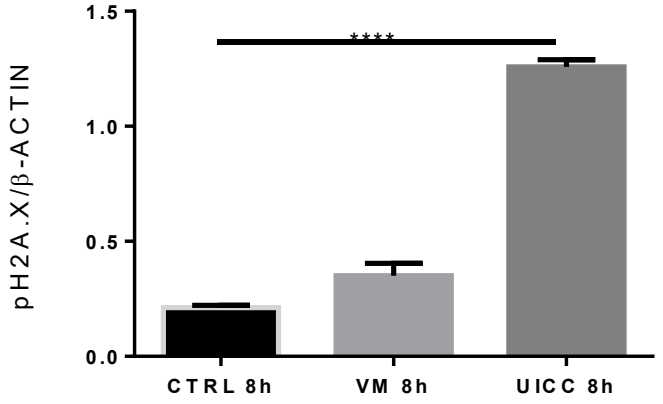


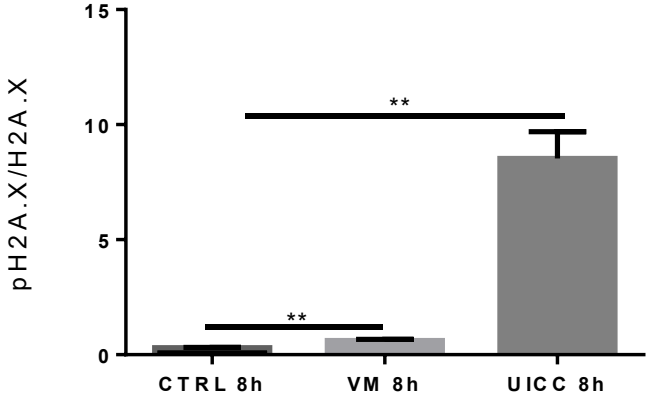


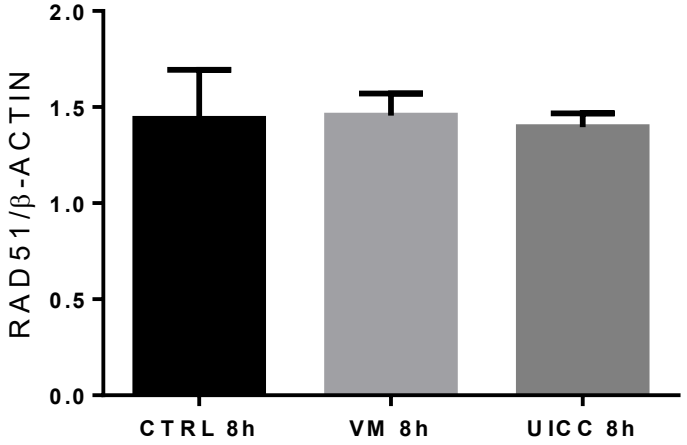


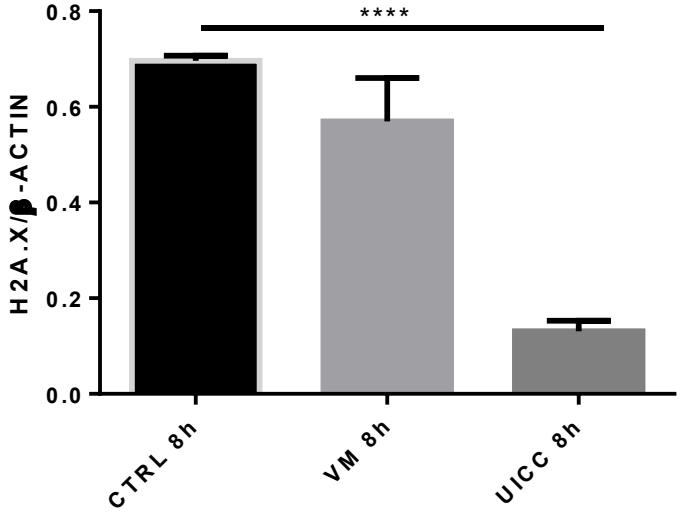
BeWo cell line treated with Chrysotile VM and Chrysotile UICC







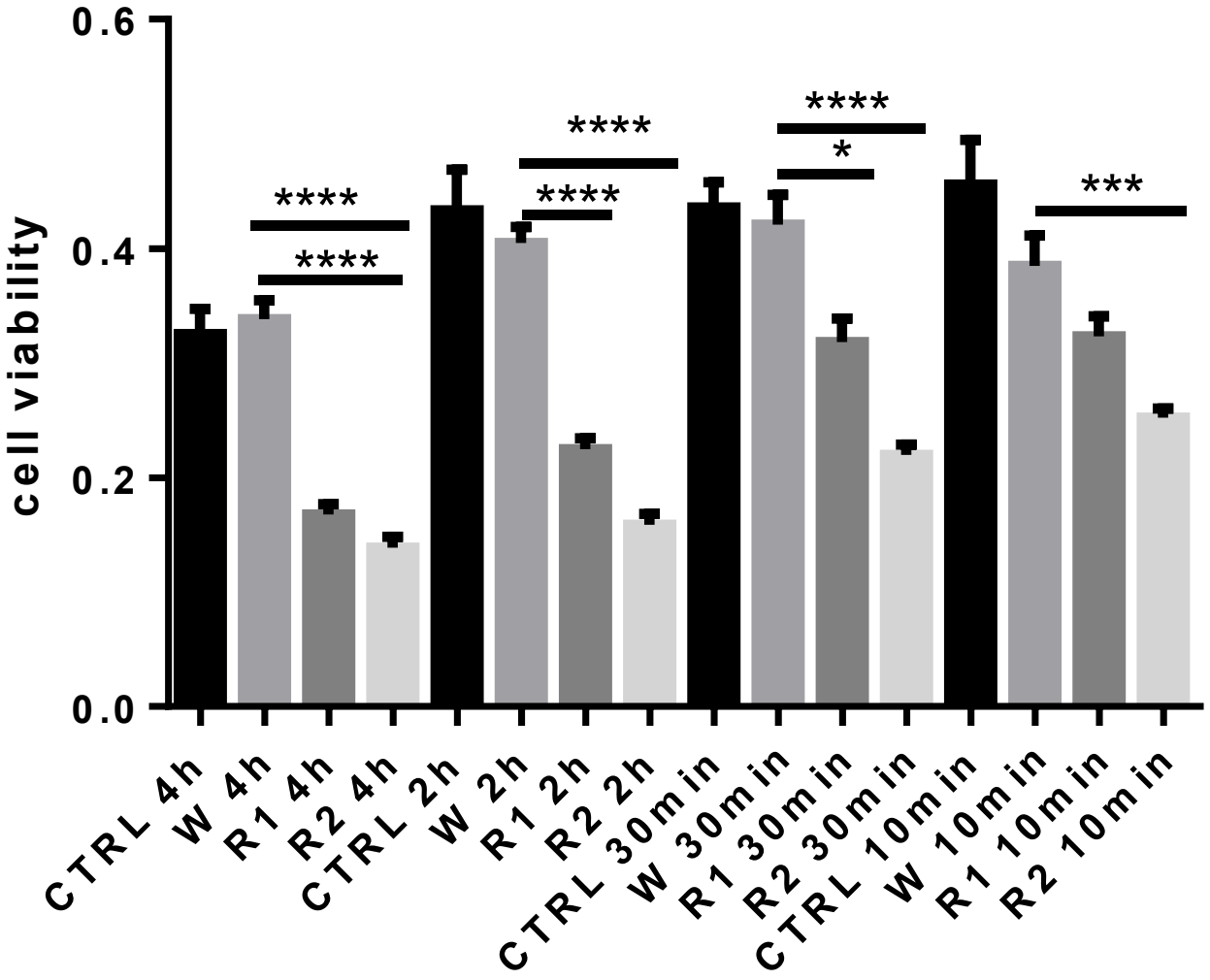




Results

- Both Chrisotyle VM and Chrisotyle UICC have been shown cytotoxic at 2 h (MTT assay)
- At 30 min and 4 h, cells treated with Chrysotile UICC did not show DNA damage
- At 30 min and 4 h, cells treated with Chrysotile VM showed DNA damage without repair
- At 8 h, both fibers showed DNA damage without repair

MTT ASSAY BeWo cell line treated with Serpentine Asbestos: Chrysotile R1 and Chrysotile R2



Future experiments

- Cell treatments with W, R1,R2 to evaluate pH2A.X, H2A.X and RAD51 expression
- Evaluation of cell death (Caspase3, Bax and Bcl2)
- Comet assay
- Micronuclei assay