NANOČÁSTICE – JAK IDEÁLNĚ MONITOROVAT EFEKT OXIDAČNÍHO STRESU U PROFESIONÁLNÍ EXPOZICE?

NANOPARTICLES – HOW TO BEST MONITOR OXIDATIVE STRESS EFFECT IN OCCUPATIONAL EXPOSURE?

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Toxikologické informační středisko
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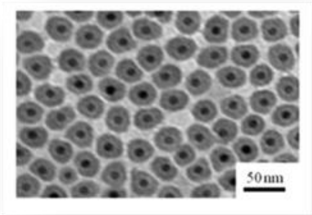
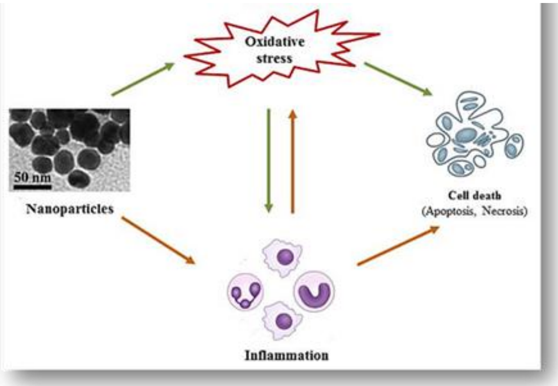
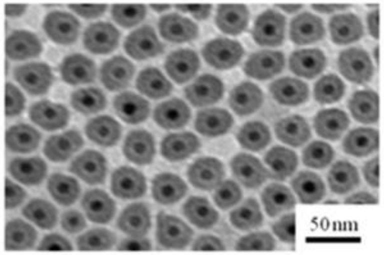


Nanoparticles

- The number of applications of nanomaterials increases enormously
- **Workers and researchers** are engaged in the development, and production of nano-enabled composites
- **Limited data** available on exposures and health effects
- **Experimental studies** – oxidative stress, inflammation, lung fibrosis, cardiovascular disorders, cancer *(Huang 2017, Runa 2017)*
- Unique physical and chemical properties
- Higher reactivity and cytotoxicity
- What markers could be used in workers?





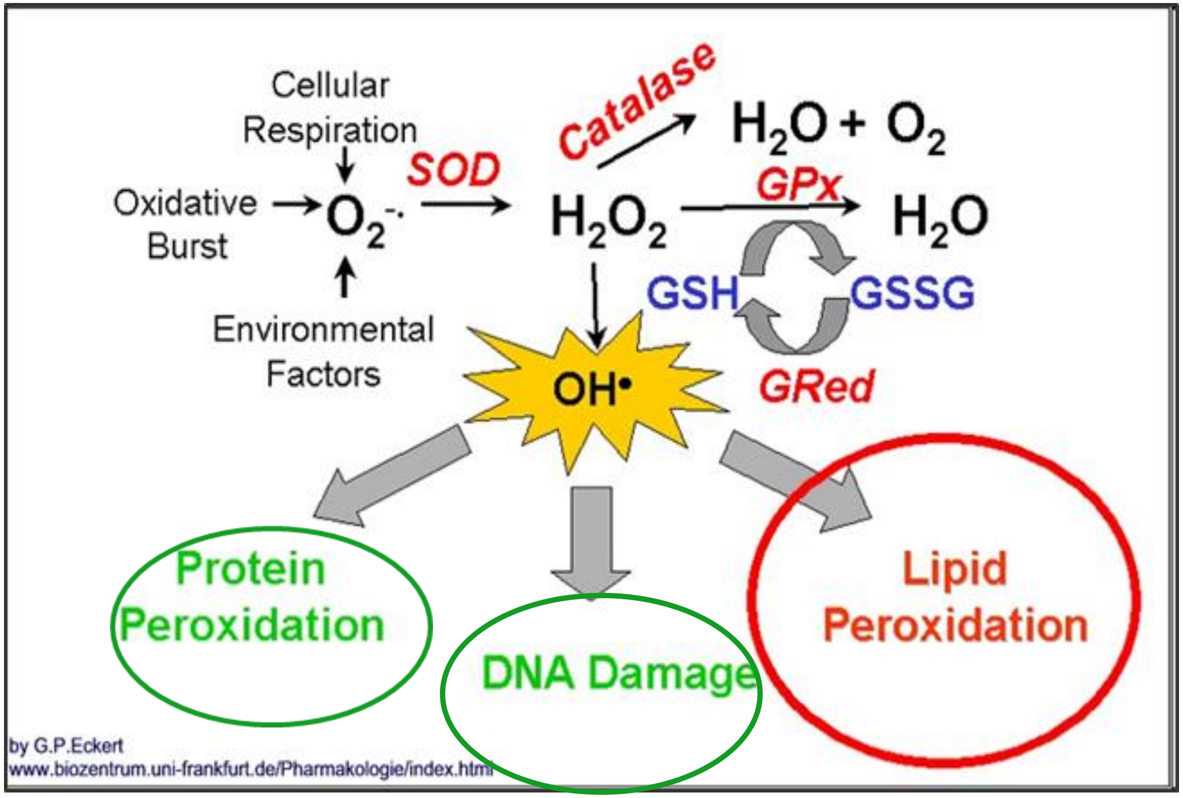
NANOPARTICLES

- 1 - Workers exposed to nanoTiO_2 - 2012, 2013
- 2 - Office employees from nanoTiO_2 plant - 2013
- 3 - Workers exposed to nano Fe-oxides - 2013

- 4 – Researchers handling nanocomposites - 2015-2020

NANOPARTICLES

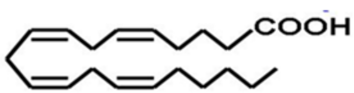
- **Cause oxidative stress, inflammation and cell death**
- **Proteins, nucleic acids and lipids damage**



Lipid peroxidation – direct (by ROS)

polyunsaturated acids of the cellular membranes

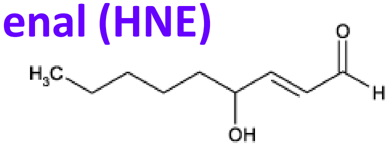
Arachidonic acid



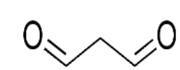
n-hexanal (C_6), n-heptanal (C_7), C_8,..- C_{12}

hydroxy-*trans*-2-hexenal (HHE)

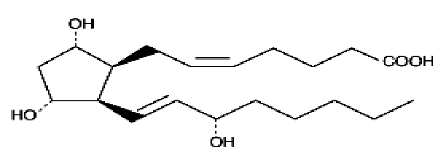
hydroxy-*trans*-2-nonenal (HNE)



malondialdehyde (MDA)

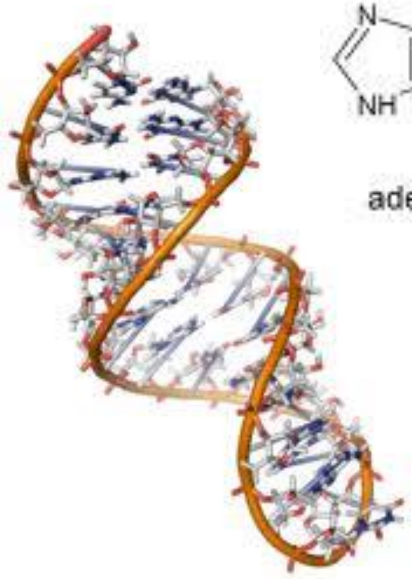


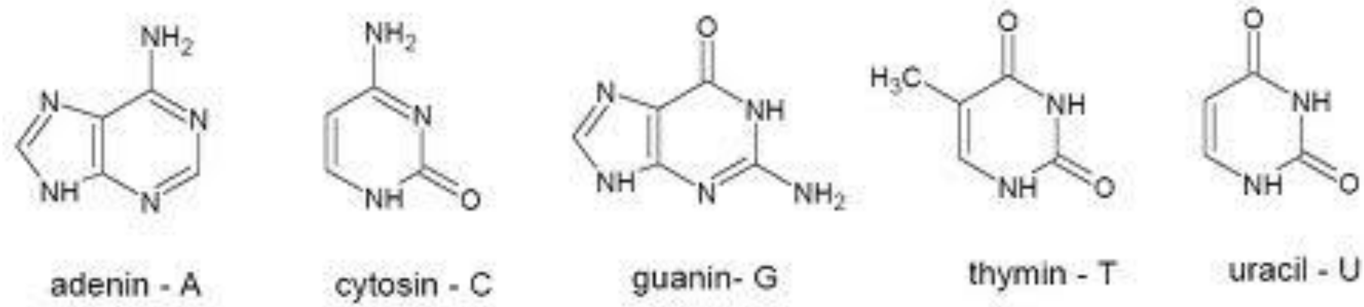
isoprostanes (8-isoprostane)



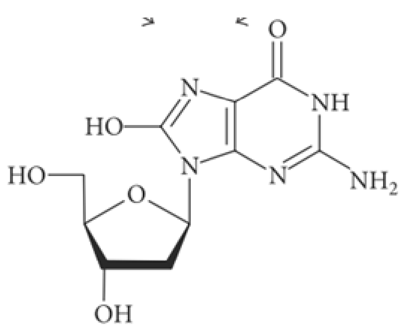
biologically active aldehydes (C_6-C_{12}..., HHE, HNE, MDA)

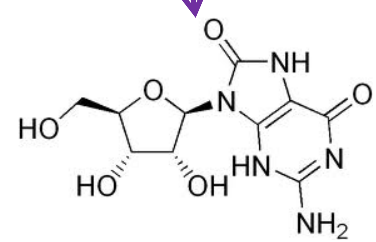
Markers of oxidation of NUCLEIC ACIDS

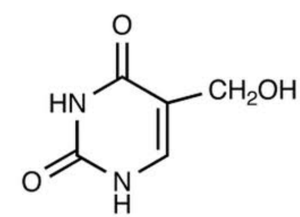




Oxidation products





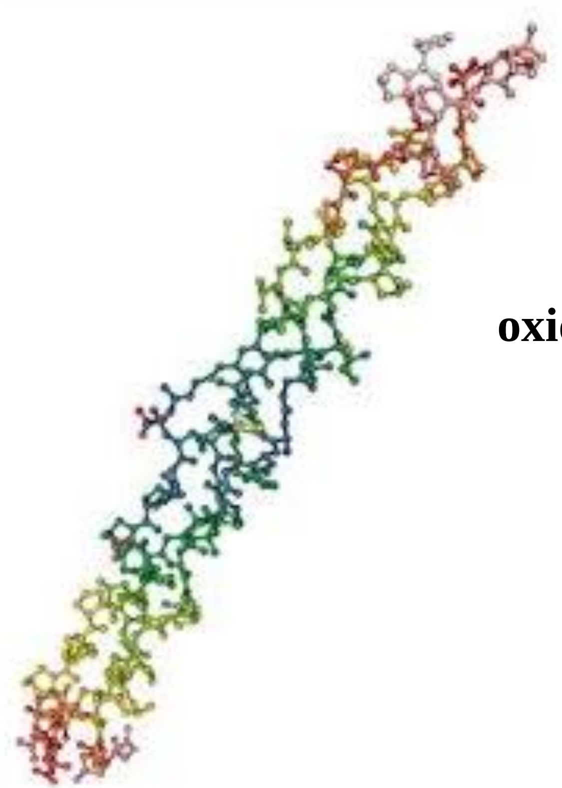


8-hydroxy-2'-deoxyguanosine
8-OHdG (DNA)

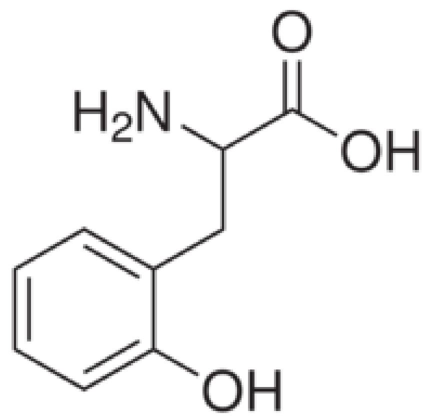
8-hydroxyguanosine
8-OHG (DNA)

5-hydroxymethyluracile
5-OHMeU (RNA)

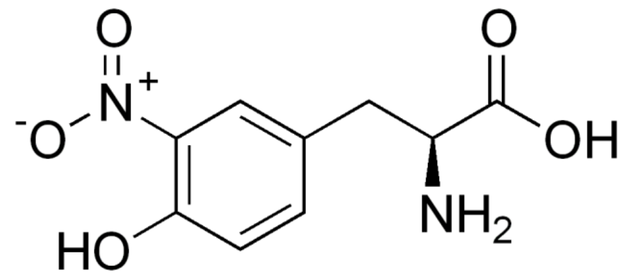
Oxidation of PROTEINS



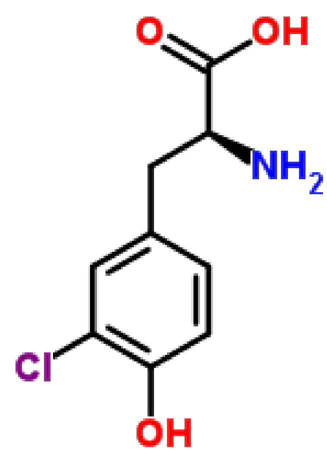
Biotransformation products



***o*-Tyrosine –**
oxidation of phenyalanine

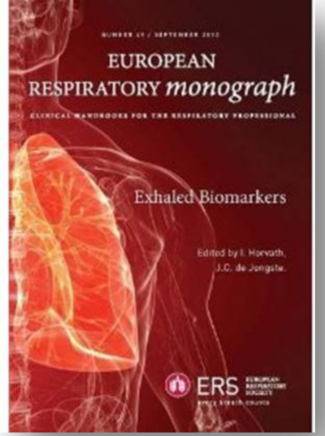


Nitrotyrosine –nitration of tyrosine

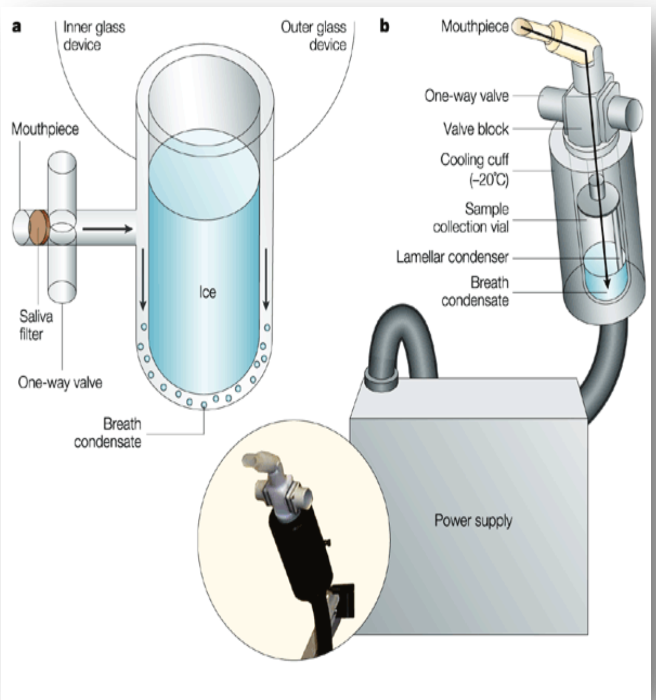


3-Chlorotyrosine –oxidized by HOCl formed by myeloperoxidase from leukocytes

Exhaled breath condensate (EBC) collection *Horváth et al. 2017*



EcoScreen (Jaeger)



Research method -
non-invasive collection (15 min) of substances from the respiratory system (120 L air) after cooling to -10°C.

EBC liquid contains:

1) condensed H_2O - 99%
2) water soluble particles
3) non-soluble particles from the droplets released from the bronchoalveolar lining fluid during expiration and contraction of respiratory bronchioles

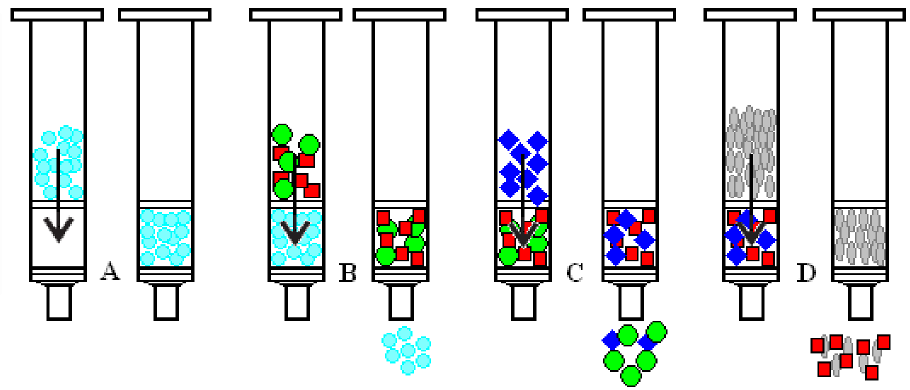
ANALYSIS OF THE SAMPLES
liquid chromatography- electrospray ionization - tandem mass spectrometry (LC/ESI/MS-MS)

Samples - SPE

LC/ESI/MS-MS ANALYSIS

QUANTITATIVE AND QUALITATIVE DATA EVALUATION

Desalting and increasing concentration of the samples
Solid phase extraction (SPE)



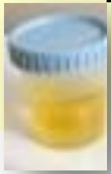
Analysis using LC/ESI/MS-MS
TSQ Vantage, LTQ Orbitrab

Examination – workers + controls

- Questionnaire
- Occupational history – years of exposure, daily exposure, PPE, latency since last shift,
- Personal history - diseases, medication, smoking, alcohol intake, regular physical activity,
- Diet, last meal, last smoking.
- Family history
- Physical examination, temperature,
- Blood pressure,
- Body mass index
- Spirometry
- Monitoring local data of environmental pollution (SO_2, CO, NOx, $PM_{2.5}$, PM_{10})

Markers measured in EBC, urine, plasma (2016-2020)

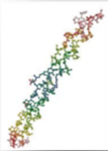
LC-ESI-MS/MS analysis



- **aldehydes C_6-C_{12}**
- **malondialdehyde (MDA)**
- **4-hydroxy-trans-hexenal (HHE)** **oxidation of lipids**
- **4-hydroxy-trans-nonenal (HNE)**
- **8-isoProstaglandin $F_{2\alpha}$ (8-isoprostane)**

- **8-hydroxy-2-deoxyguanosine (8-OHdG)**
- **8-hydroxyguanosine (8-OHG)** **oxidation of nucleic acids**
- **5-hydroxymethyl uracil (5-OHMeU)**

- **o-tyrosine (o-Tyr)**
- **3-chloro-tyrosine (3-Cl-Tyr)** **oxidation of proteins**
- **nitrotyrosine (NO-Tyr)**



- *leukotrienes LTB_4, LTC_4, LTD_4, LTE_4* *markers of inflammation*
- *interleukins - IL 4, 5, 9, 10, 13, lipoxins (LXA, LXB),*
- *tumor necrosis factor (TNF)*
- *FeNO – fractional exhaled nitric oxide - inflammation*

Aerosol Measurement at the workplace

- 8-h shifts measurements, background measurements
- **Berner Low-Pressure Cascade Impactor** (BLPI) - separation into the size fractions and chemical analysis,
- Scanning Mobility **Particle Sizer** (SMPS),
- Aerodynamic **Particle Sizer** (APS),
- Condensation **Particle Counter** (CPC)
- Optical Particle Sizer (OPS).
- Personal nanoparticle samplers (Pluto Technology Taiwan) 2019, 2020

Exposure and Groups of Workers

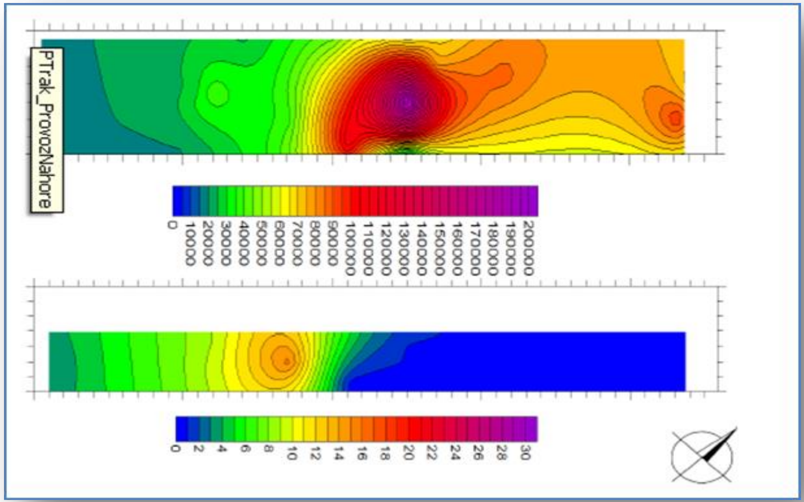
* France (ANSES) recommends occupation exposure limit (8h OEL) for nanoTiO_2 of 0.80 µg/m^3 and for 15 min 4 µg/m^3 i. e. these limits are exceeded 3 – 6 x

	N	Age	Proportion of nano particles	Exposure time/day	Median Mass Concentration mg/m^3	Median Particles number /cm^3
TiO_2 2012* 	20	34±8	80%	7.5 h	0.65	19 800
TiO_2 2013* 	14	34±5	80%	7.5 h	0.40	23 200
TiO_2 2013 office 	22	44±4	80%	15 min	0.40	23 200
Fe-oxides 2013 	14	43±8	80%	7.5 h	0.083	66 800
Nanocomposites 2016-2018 	61	40±12	40-95%	3.0 h	0.12-1.84	48 000-540 000
Controls 2012-2018	Comparable number, age and gender			No nano- exposure		

Group 1 WORKERS IN PRODUCTION of TiO_2 pigments 2012 pre-shift, post-shift and 2013 post-shift

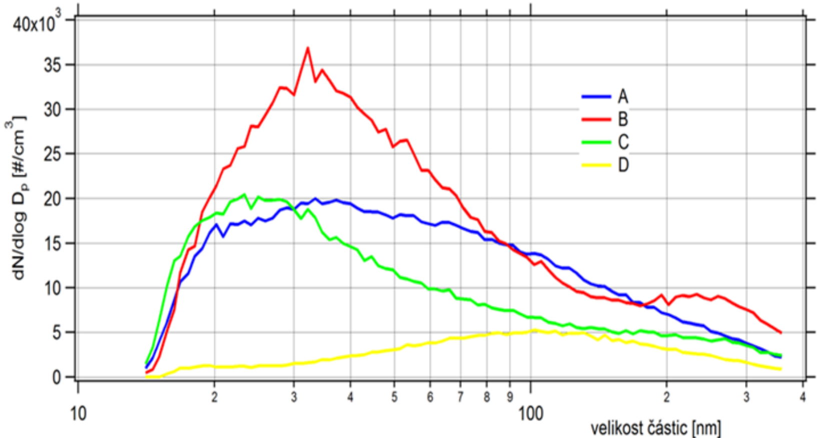
80 % nano size

Ilmenite - iron titanium oxide, $FeTiO_3$

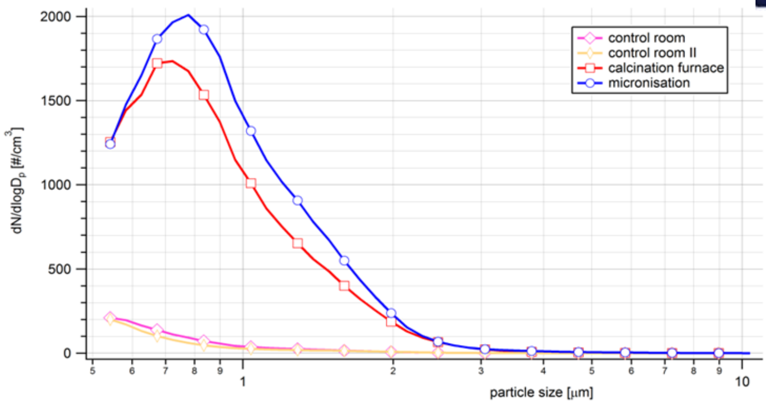


Titania – TiO_2

SMPS - scanning mobility particle sizer - particles from 15 to 350 nm



APS - aerodynamic particle sizer - particles from 500 nm to 10 µm

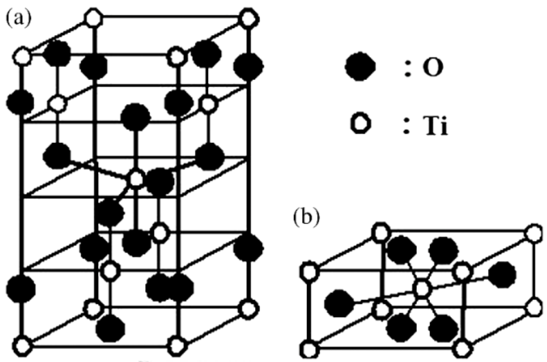


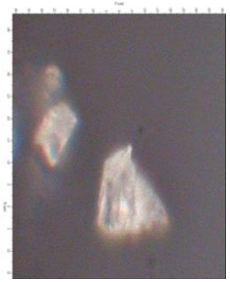
Raman microspectroscopy found TiO_2 in EBC (2012)

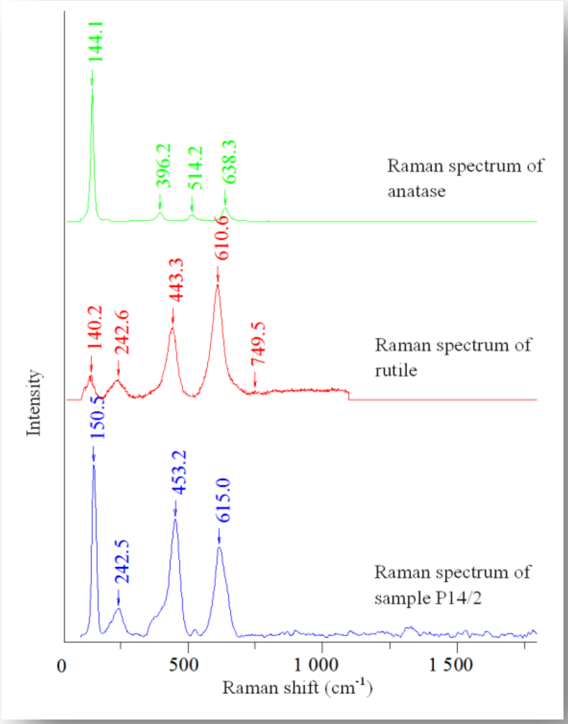
pre-shift in 40 % workers

post-shift in 70 % workers

a) anatas b) rutile

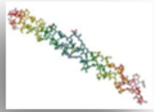


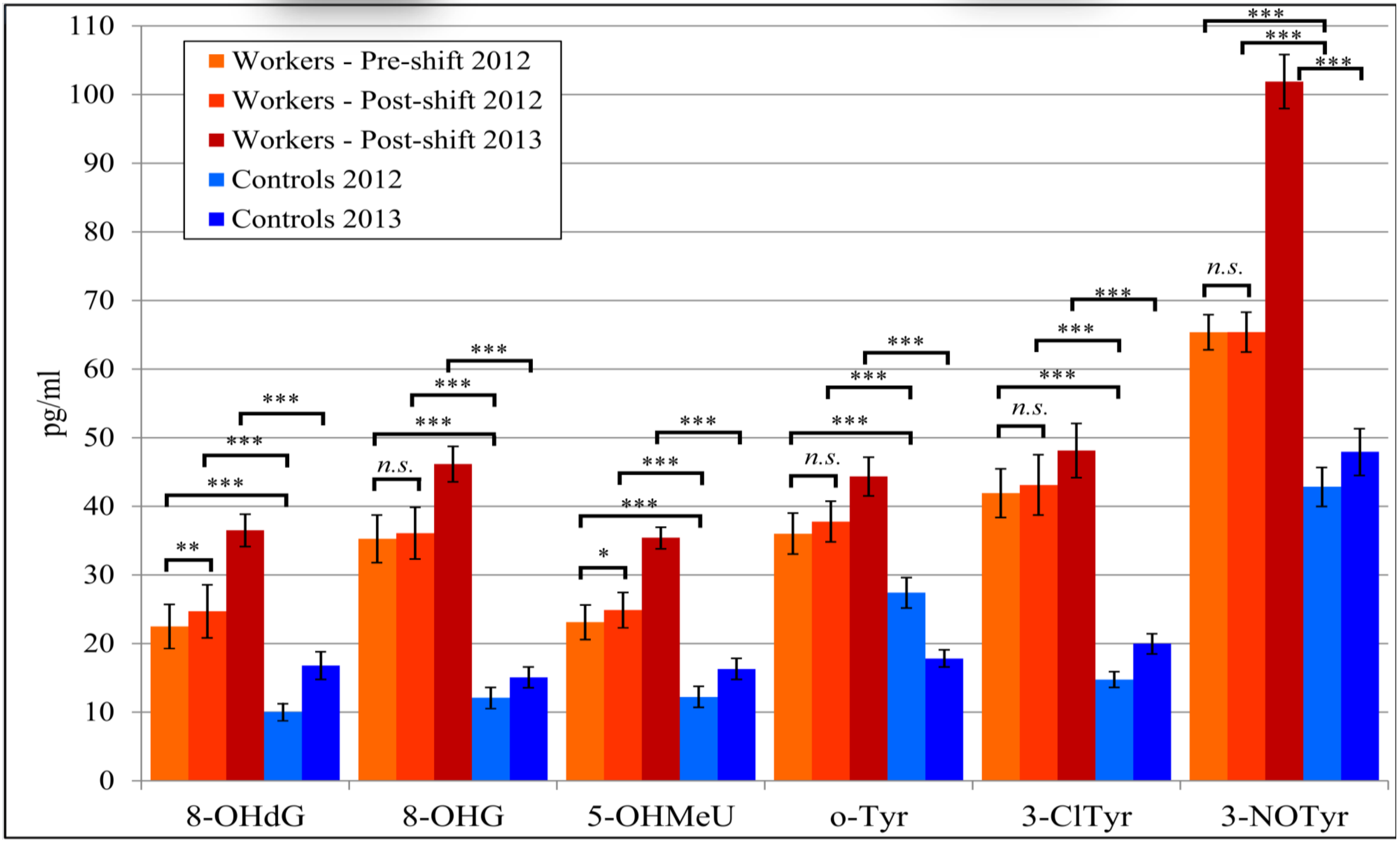




Pelclova D, Barosova H, Kukutschova J, Zdimal V, Navratil T, Fenclova Z, Vlckova S, Schwarz J, Zikova N, Kacer P, Komarc M, Belacek J, Zakharov S.: Raman microspectroscopy of exhaled breath condensate and urine in workers exposed to fine and nano TiO_2 particles: a cross-sectional study. ***J Breath Research 2015***

TiO_2 Production Workers and Controls





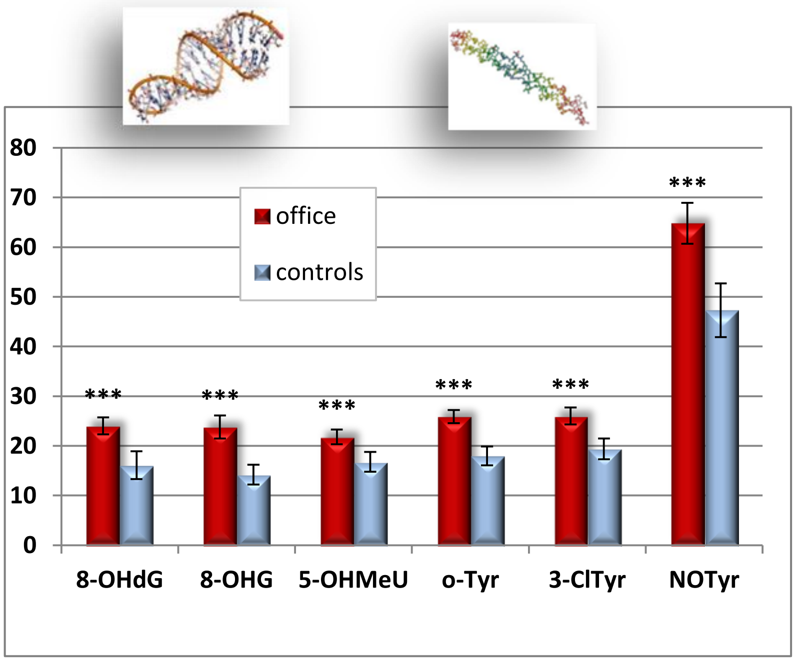
Pelclova D, Zdimal V, Fenclova Z, Vlckova S, Turci F, Corazzari I, Kacer P, Schwarz J, Zikova N, Makes O, Syslova K, Komarc M, Belacek J, Navratil T, Machajova M, Zakharov S. Markers of oxidative damage of nucleic acids and proteins among workers exposed to TiO2(nano) particles. ***Occup Environ Medicine 2016***

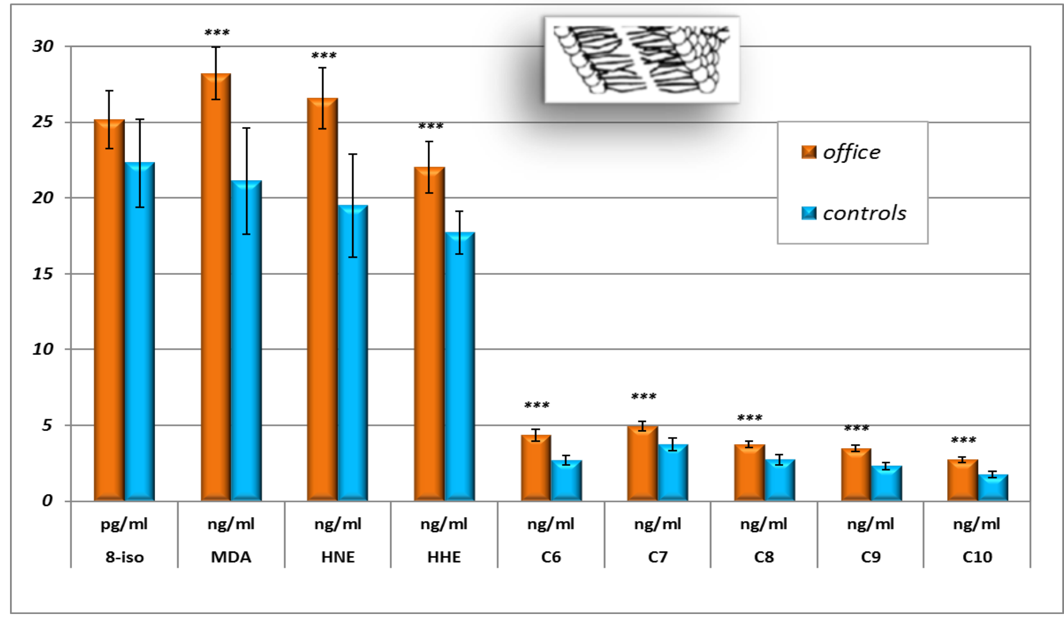
Multiple regression analysis – the job is the key (TiO_2)

	8-OHdG (pg/ml)	8-OHG (pg/ml)	5-OHMeU (pg/ml)	o-Tyr (pg/ml)	3-ClTyr (pg/ml)	3-NOTyr (pg/ml)
TiO_2 Production exposure (Yes/No)	**19.20***** **(14.75, 23.66)**	**30.37***** **(26.75, 34.00)**	**19.35***** **(16.30, 22.40)**	**28.95***** **(25.51, 32.38)**	**28.43***** **(23.71, 33.14)**	**51.68***** **(44.31, 59.04)**
Age (years)	0.02 (-0.17, 0.21)	-0.02 (-0.17, 0.13)	0.06 (-0.06, 0.19)	-0.02 (-0.16, 0.13)	0.13 (-0.07, 0.32)	-0.06 (-0.36, 0.25)
Smoking (Yes/No)	-0.29 (-3.65, 3.07)	0.70 (-2.04, 3.43)	0.38 (-1.92, 2.69)	-0.46 (-3.05, 2.14)	-1.41 (-4.97, 2.14)	1.62 (-3.94, 7.17)
SO_2 (μg/m^3) (CO, NO_x) environmental	0.02 (-0.13, 0.17)	-0.02 (-0.14, 0.10)	-0.04 (-0.15, 0.06)	-0.13* (-0.24, -0.01)	-0.06 (-0.22, 0.10)	0.16 (-0.09, 0.41)

Pelclova D, Zdimal V, Fenclova Z, Vlckova S, Turci F, Corazzari I, Kacer P, Schwarz J, Zikova N, Makes O, Syslova K, Komarc M, Belacek J, Navratil T, Machajova M, Zakharov S. Markers of oxidative damage of nucleic acids and proteins among workers exposed to TiO2(nano) particles. ***Occup Environ Medicine 2016***

Group 2 Office employees TiO_2 and Controls (2013)



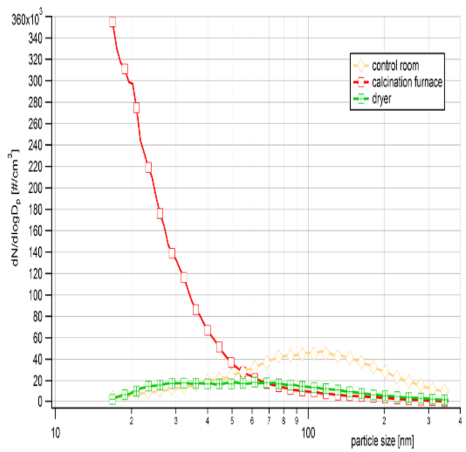


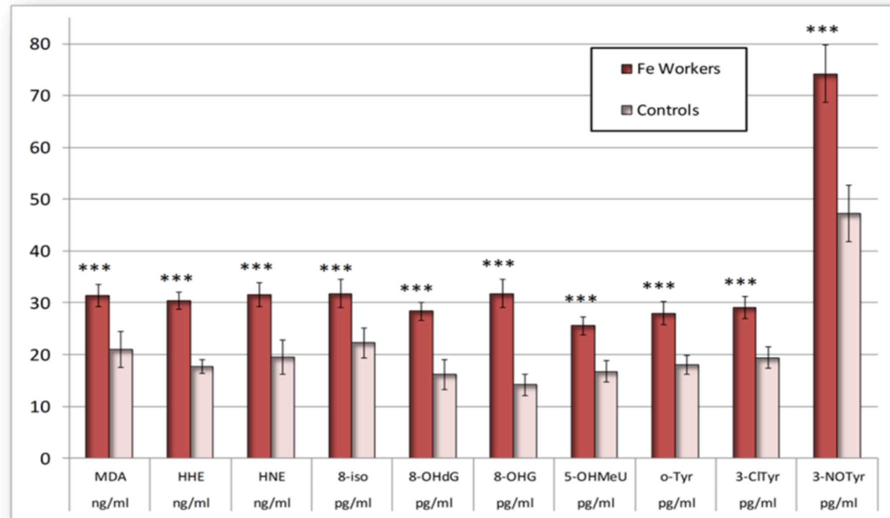
Pelclova D, Zdimal V, Kacer P, Vlckova S, Fenclova Z, Navratil T, Komarc M, Schwarz J, Zikova N, Makes O, Zakharov S. Markers of nucleic acids and proteins oxidation among office workers exposed to air pollutants including (nano)TiO2 particles. ***Neuro Endocrinol Lett. 2016***

Pelclova D, Zdimal V, Kacer P, Komarc M, Fenclova Z, Vlckova S, Zikova N, Schwarz J, Makes O, Navratil T, Zakharov S, Bello D. Markers of lipid oxidative damage among office workers exposed intermittently to air pollutants including nanoTiO2 particles. ***Rev Environ Health 2017***

GROUP 3 Fe oxides (+nano) pigments production 2013

80% particles in nano size





J Breath Res. 2016 Feb 1;10(1):016004. doi: 10.1088/1752-7155/10/1/016004.

Oxidative stress markers are elevated in exhaled breath condensate of workers exposed to nanoparticles during iron oxide pigment production.

Pelclova D[1], Zdimal V, Kacer P, Fenclova Z, Vlckova S, Syslova K, Navratil T, Schwarz J, Zikova N, Barosova H, Turci F, Komarc M, Pelcl T, Belacek J, Kukutschova J, Zakharov S.

Group 4 - NANOCOMPOSITES PRODUCING RESEARCH WORKERS IN 2016, 2017, 2018 (2019, 2020)

Examination of 61 workers pre-shift and post-shift

+ 62 controls

Research plant for **new resistant nanocomposites**

- metals and geopolymers (nano SiO_2 filler)

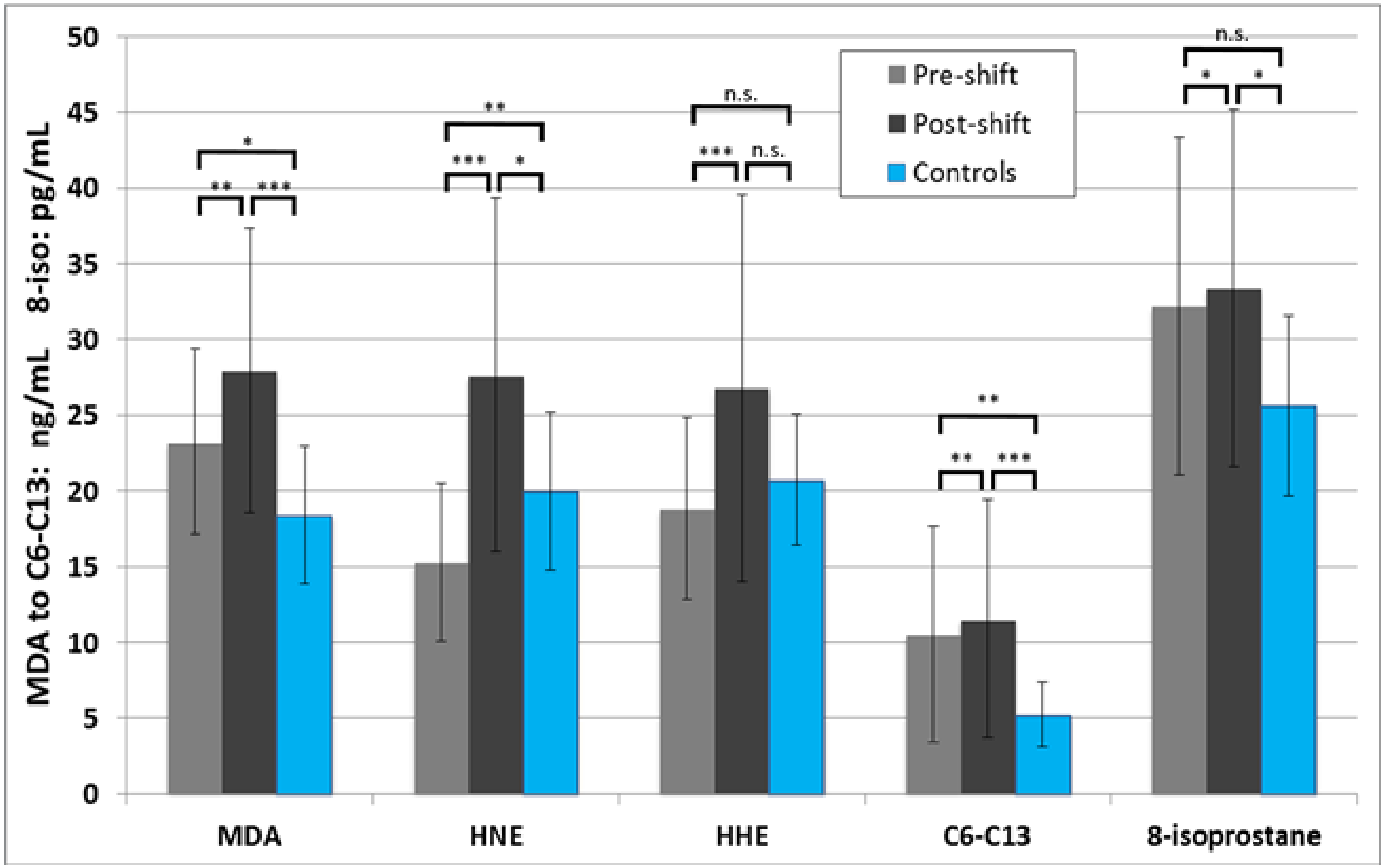
by welding and machining (grinding) technology.

Mass concentrations
- higher at WELDING

Proportion (%) of nanoparticles
– higher at MACHINING

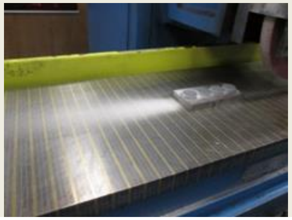
Markers of oxidation of lipids - nanocomposites 2016

Workers Pre-shift and Post-shift vs. Controls



*** (p<0.001) **(p<0.01) *(p<0.05)

Lung functions– post-shift decrease p<0.05



Chronic bronchitis more frequent:

4 (20%) nanocomposites, 0 % controls p=0.033*

Duration of exposure (but not age) correlated negatively with FEV1/FVC ($p<0.05$)

LUNG FUNCTIONS	%FVC	%VCIN	%FEV1	FEV1/FVC	%PEF
Pre-shift	94.7±13.3	92.2±13.0	102.2±13.5	0.89±0.06	110.2±14.3
Post-shift	95.0±11.6	93.1±11.0	↓99.0±12.0*	↓0.86±0.06*	106.8±15.2
Controls	100.8±13.6	98.7±13.0	106.1±14.0	0.89±0.06	111.8±20.2

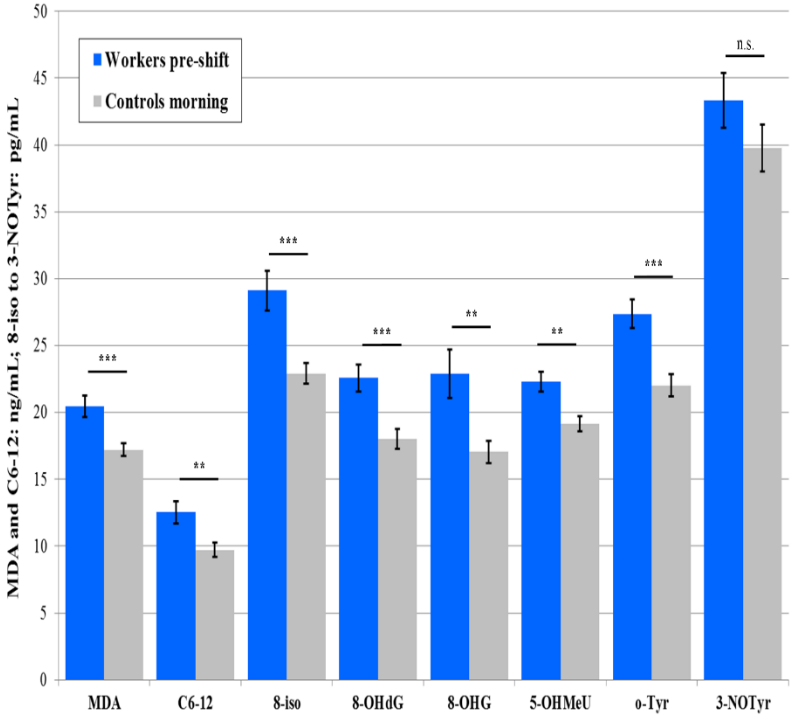
nanomaterials 2020

MDPI

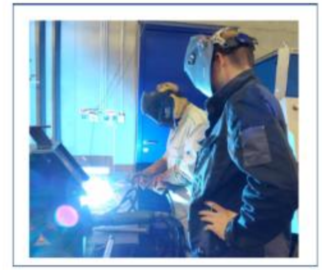
Article

Three-Year Study of Markers of Oxidative Stress in Exhaled Breath Condensate in Workers Producing Nanocomposites, Extended by Plasma and Urine Analysis in Last Two Years

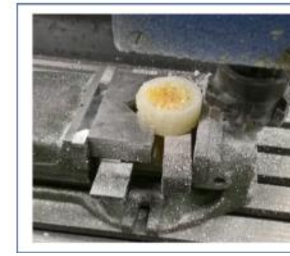
Daniela Pelclova [1,*], Vladimir Zdimal [2], Martin Komarc [3,4], Jaroslav Schwarz [2], Jakub Ondracek [2], Lucie Ondrackova [2], Martin Kostejn [2], Stepanka Vlckova [1], Zdenka Fenclova [1], Stepanka Dvorackova [5], Lucie Lischkova [1], Pavlina Klusackova [1], Viktoriia Kolesnikova [1], Andrea Rossnerova [6] and Tomas Navratil [7]

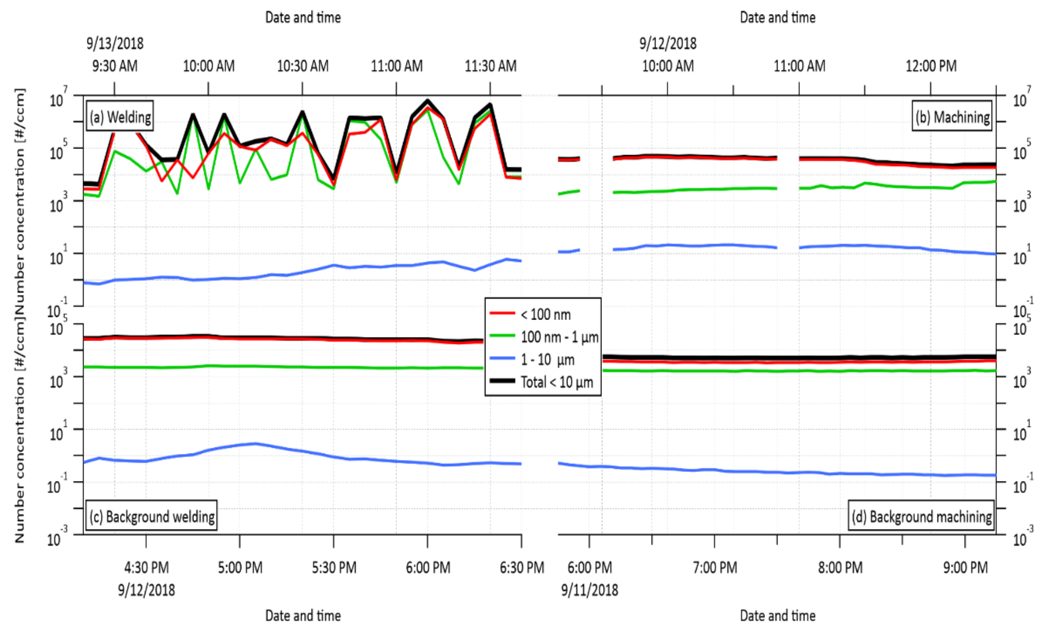


WELDING



MACHINING





Mean±SD 2016, 2017, 2018

all workers **Pre-shift** and all controls morning

EBC markers –

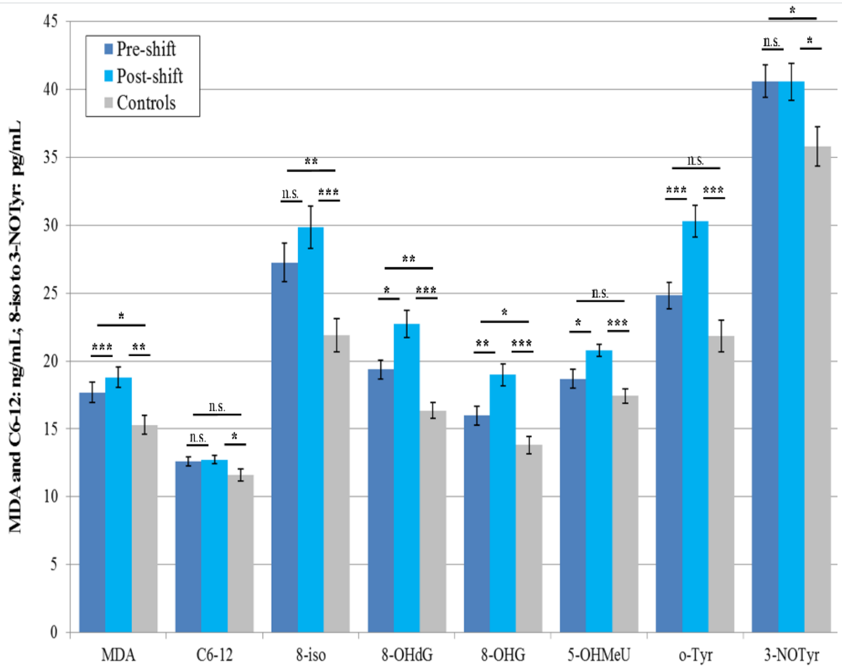
highest significance

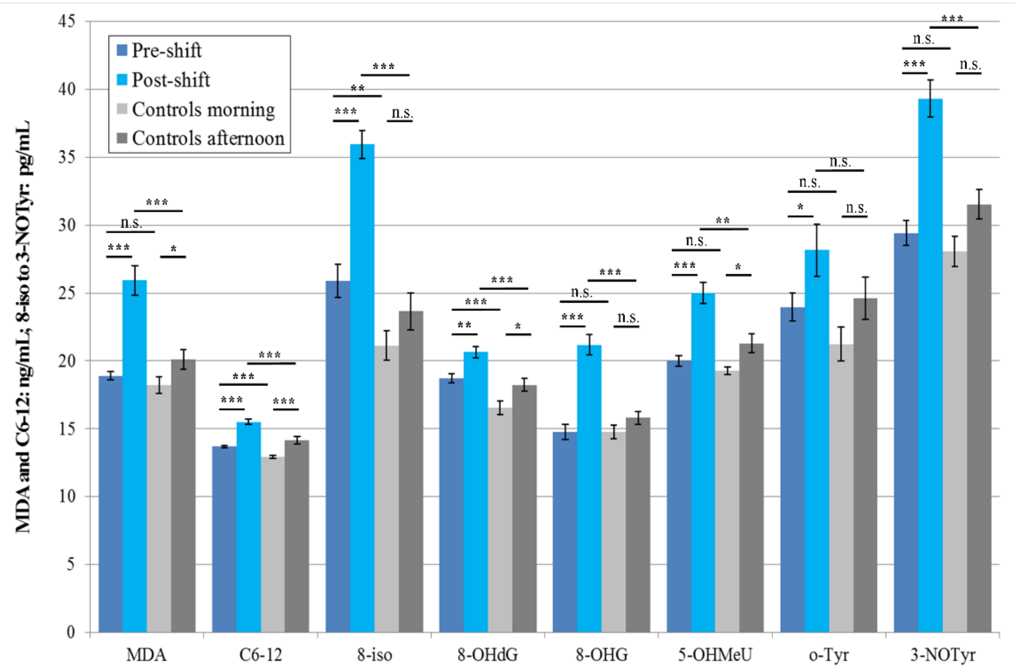
2017

5 pre-shift ↑ 5 shift effect ↑
8 postshift ↑ vs. morning controls

2018

3 pre-shift ↑ 8 shift effect ↑
7 postshift ↑ vs afternoon controls

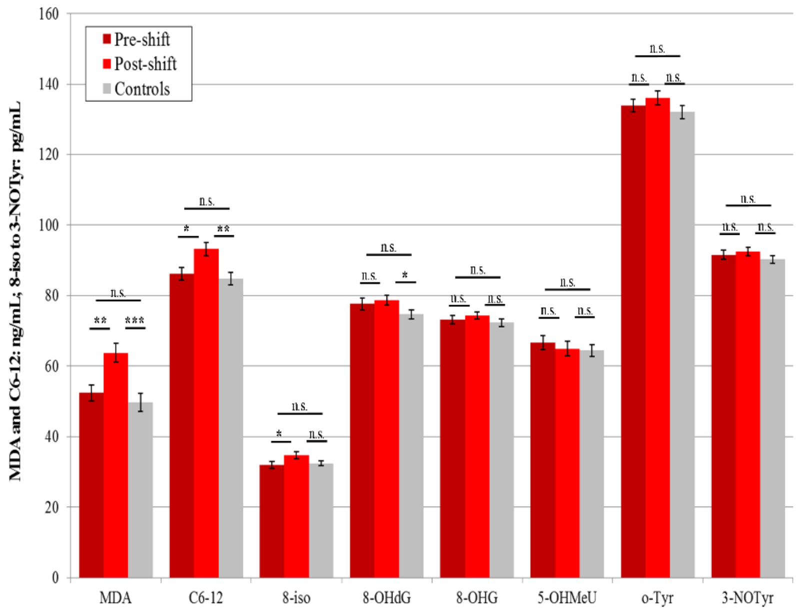


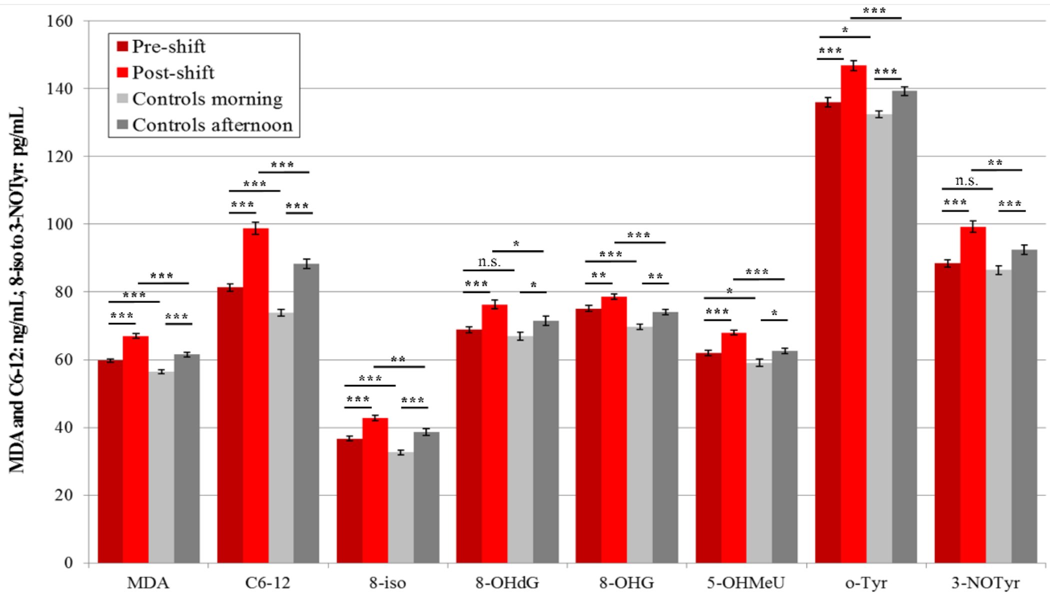


Plasma samples

2017
0 pre-shift ↑ 3 shift effect ↑
3 postshift ↑ vs morning controls

2018
6 pre-shift ↑ 8 shift effect ↑
8 postshift ↑ vs afternoon controls





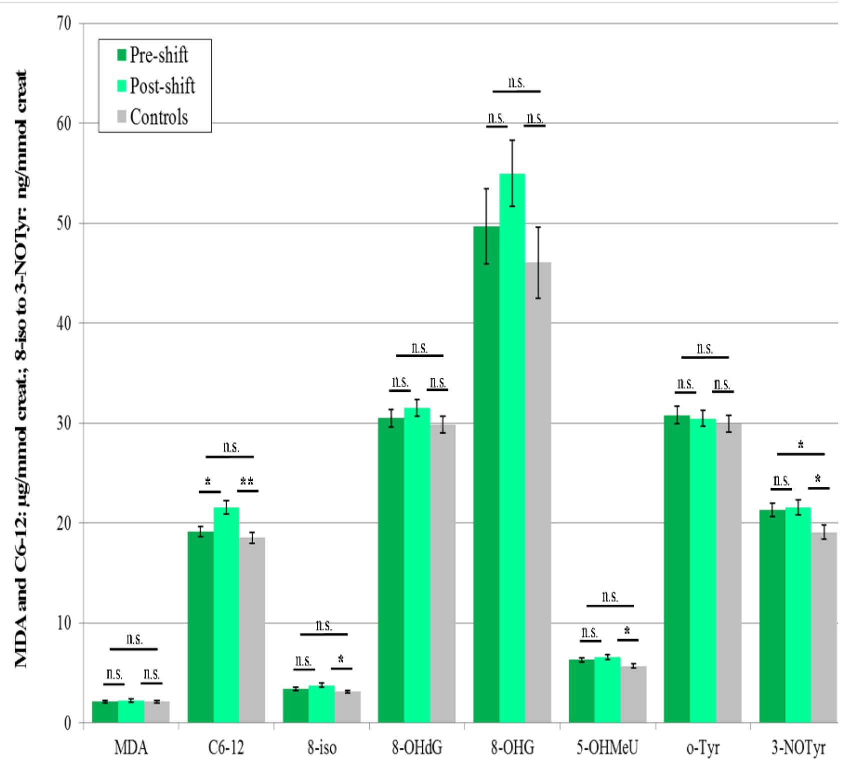
Urine samples –

same trends, but more diversity

2017

1 pre-shift ↑ 1 shift effect ↑

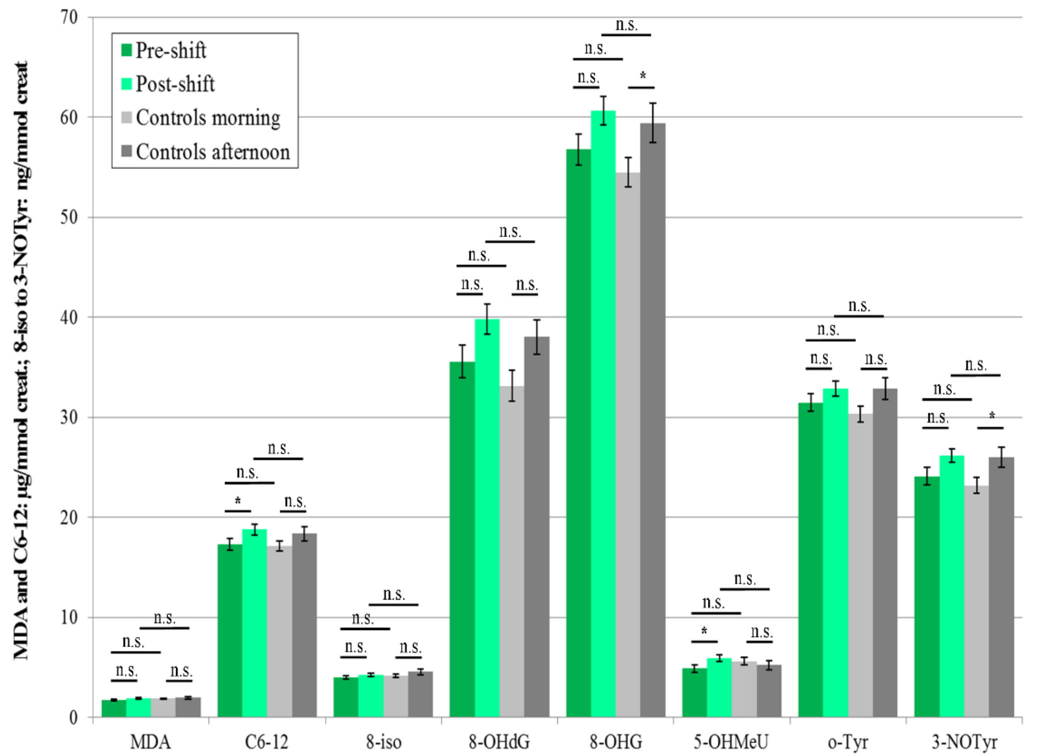
4 postshift ↑ vs morning controls



2018

0 pre-shift ↑ 0 shift effect ↑

2 postshift ↑ vs afternoon controls



Li 2013

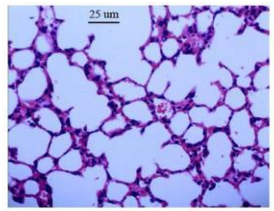
Molecular Mechanisms of Nanosized Titanium Dioxide-Induced Pulmonary Injury in Mice

Bing Li[1¶], Yuguan Ze[1¶], Qingqing Sun[1¶], Ting Zhang[2,3¶], Xuezi Sang[1], Yaling Cui[1], Xiaochun Wang[1], Suxin Gui[1], Danlin Tan[1], Min Zhu[1], Xiaoyang Zhao[1], Lei Sheng[1], Ling Wang[1], Fashui Hong[1*], Meng Tang[2,3*]

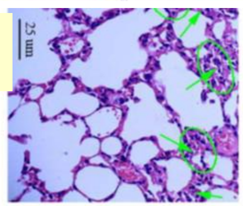
Nasal application of nano-TiO_2 suspension (6 nm) daily for 90 days

2.5-10 mg/kg b.w.

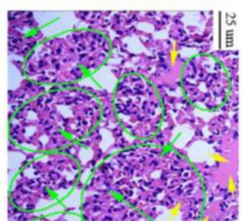
controls



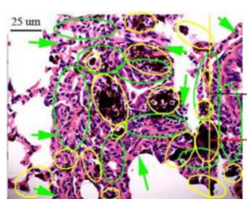
2.5 mg/kg



5 mg/kg



10 mg/kg



- *interstitium thickening*
- *inflammatory infiltration*
- *apoptosis*
- *oedema*
- *deposits of agglomerated TiO2*

Table 3. Oxidative stress in the mouse lung after nasal administration with nano-TiO_2 for 90 consecutive days.

Oxidative stress	TiO_2 NPs (mg/kg BW)			
	0	2.5	5	10
$O_2^{\cdot-}$ (nmol/mg prot. min)	23±1.15a	30.27±1.51b	39.18±1.96c	50±2.50d
H_2O_2 (nmol/mg prot. min)	43±2.15a	61.22±3.06b	78.96±3.95c	110±5.50d
MDA (μmol/ mg prot)	1.08±0.05a	1.59±0.08b	2.89±0.15c	5.15±0.26d
Carbonyl (μmol/mg prot)	0.54±0.03a	0.98±0.05b	1.85±0.09c	3.04±0.15d
8-OhdG (mg/g tissue)	0.42±0.02a	2.26±0.11b	4.25±0.21c	7.12±0.36d

Letters indicate significant differences between groups ($p<0.05$). Values represent means ± SE (N = 5).

MDA -malondialdehyde, **PC-** protein carbonyl, **8-OHdG** 8-hydroxy-2-deoxyguanosine

Toxicol. Res.
Vol. 28, No. 4, pp. 217-224 (2012)
Open Access http://dx.doi.org/10.5487/TR.2012.28.4.217
pISSN: 1976-8257 eISSN: 2234-2753

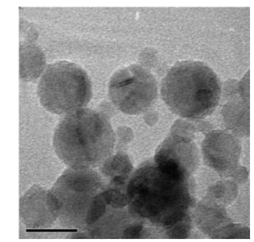
Toxicological Research
Official Journal of
Korean Society of Toxicology
Available online at http://www.toxmut.or.kr

Kwon 2012

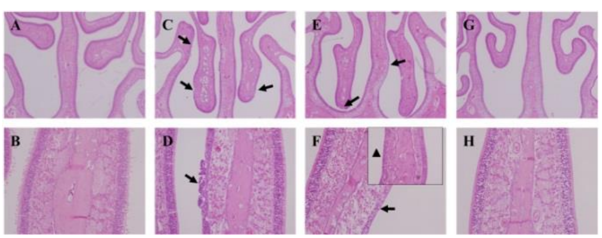
Nasal and Pulmonary Toxicity of Titanium Dioxide Nanoparticles in Rats

Soonjin Kwon[1,2], Young-Su Yang[1], Hyo-Seon Yang[1], Jinsoo Lee[1,2], Min-Sung Kang[1],
Byoung-seok Lee[3], Kyuhong Lee[1] and Chang-Woo Song[1]

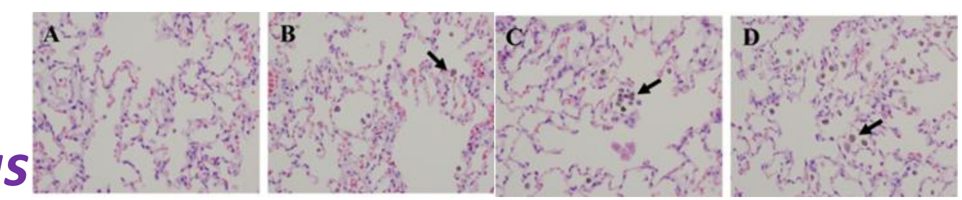
- TiO_2 nanoparticles **inhaled** (nose) for **2 weeks**
- 6 h/day, 5 days /week
- concentration 10 mg/m^3
- histopathological changes in nasal mucosa
- nanoparticles in the lung tissue
- toxic effects in the tissues - REVERSIBILITY



nose



lungs



control **day 1** **day 7** **day 15**

control **day 1** **day 7** **day 15**

J Nanopart Res. 2015 Oct;17:413. Epub 2015 Oct 19.

Assessing the first wave of epidemiological studies of nanomaterial workers.

Liou SH[1], Tsai CS[2], Pelclova D[3], Schubauer-Berigan MK[4], Schulte PA[4].

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[3]Department of Occupational Medicine, First Faculty of Medicine, Charles University in Prague, Prague, Czech Republic.
[4]National Institute for Occupational Safety and Health, Cincinnati, OH, USA.

- **1 682 commercial products with nanoparticles**
- Price 10^{12} (trillions) USD
- Only 6 published Occupational studies until 2015, + conference abstracts
- MATERIALS: Nanomaterials (Taiwan), TiO_2 (Czech Rep, China), carbon nanotubes (Russia, South Korea, Netherlands, Japan), Fe oxides(Czech Rep), $CaCO_3$ (China), nanoAg (Korea)

- **International Agency for Research on Cancer (IARC) classified TiO_2 as „possibly carcinogenic to humans“ (group 2B)**

BIOMARKERS, 2016
http://dx.doi.org/10.3109/1354750X.2016.1160432

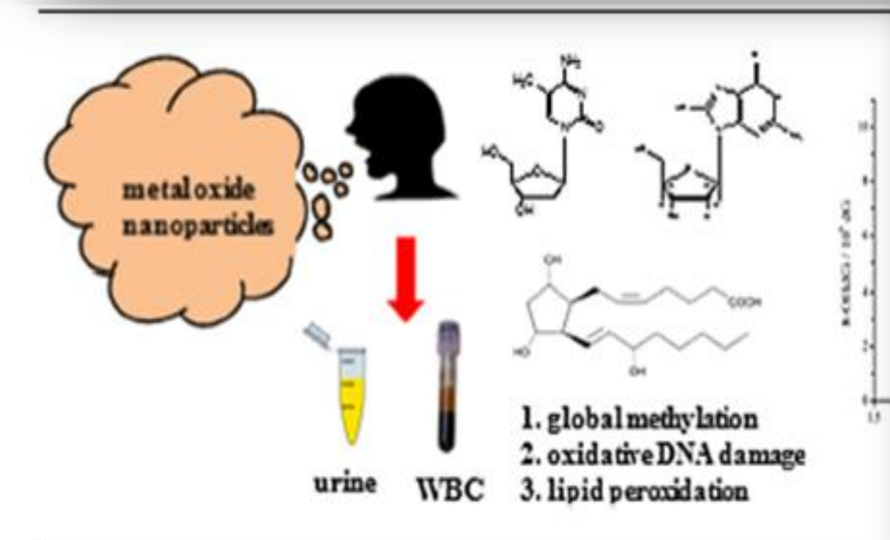
RESEARCH ARTICLE

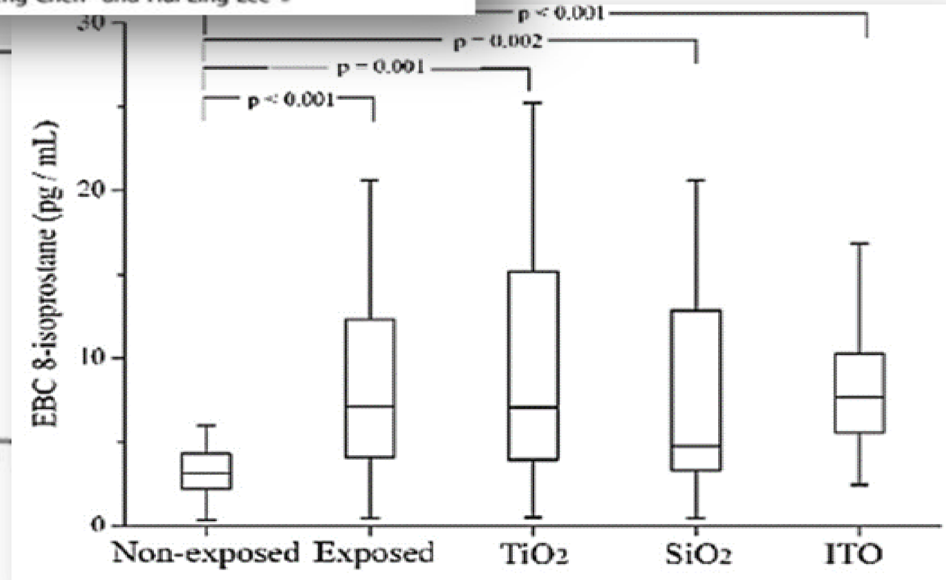
Increased levels of oxidative stress biomarkers in metal oxides nanomaterial-handling workers

Saou-Hsing Liou[a]#, Yu-Cheng Chen[b], Hui-Yi Liao[a]#, Chien-Jen Wang[a], Jhih-Sheng Chen[b] and Hui-Ling Lee[b]#

Taylor & Francis

Liou 2016





↑ 8-OHdG in urine ↑8-isoprostane in EBC

130 workers 26xTiO_2, 31xSiO_2, 30xIndium Tin Oxide (*ITO* of display technologies, electroluminescent, and electrochromatic displays, touch screen technologies).

DNA damage, lipids peroxidation, DNA hypomethylation, and genomic instability – oncogenesis,….

NANOTOXICOLOGY, 2018
https://doi.org/10.1080/17435390.2018.1425502

Taylor & Francis
Taylor & Francis Group

Check for updates

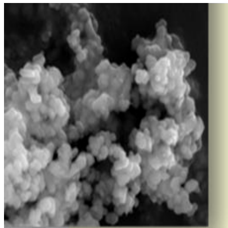
ORIGINAL ARTICLE

Cardiopulmonary effects induced by occupational exposure to titanium dioxide nanoparticles

Zhao 2018

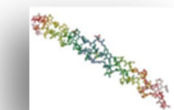
Lin Zhao[a], Yifang Zhu[b], Zhangjian Chen[a], Huadong Xu[a], Jingwen Zhou[c], Shichuan Tang[d], Zhizhen Xu[d], Fanling Kong[e], Xinwei Li[c], Yifei Zhang[f], Xianzuo Li[f], Ji Zhang[c] and Guang Jia[a]

- TiO_2 production plant in China
- 85 TiO_2 packers 3.17 mg/m^3, 39% nano TiO_2
- Cardiovascular disease markers (cell adhesion molecules VCAM, ICAM)
- Blood malondialdehyde (MDA), TNF, IL-10, ….
- **Lung functions** impaired ($p < 0.05$)
- **X-ray** – 43% increased interstitial pattern in workers
- ***All markers associated with exposure to*** $\boldsymbol{TiO_2}$



Conclusions

- Results consistent with oxidative stress hypothesis
- Suggest a lung injury at the molecular level
- Collective test – minimum 20 exposed workers
- Two or more body fluids – EBC, plasma, urine
- Several biomarkers – all groups



- Timing – post-shift vs. controls same time may reflect both chronic and acute effect and prevents diurnal variations
- Control group of the same size from the same location
- Post-shift spirometry may reveal impairments after higher exposure
- *X-ray after long-term intense exposure*
- Similarity of findings in EBC in silicosis and asbestos-exposed patients

Pelclová D et al. 8-isoprostane and leukotrienes in EBC in Czech subjects with silicosis. Ind Health. 2007

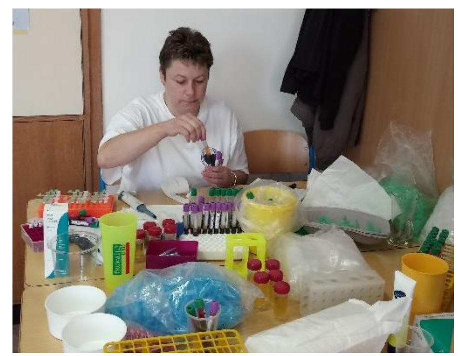
Pelclová D et al. Increased 8-isoprostane, marker of oxidative stress in EBC in asbestos exposure. Ind Health. 2008

Further plans

- Follow-up was organized in Sept 2019, and Sept 2020
- Personal samplers also used – PENs, individual data available
- Frozen EBC, plasma, urine samples (-80 °C) – *stored also from previous years*
- Nanoparticles in the samples 2016-2020 to be detected (Nizamov 2017) and correlated with the markers
- Antioxidant capacity to be measured, GSH, ferric reducing antioxidant power (FRAP)
- Further analyses possible – co-operation with D. Bello (2021) from UMassachusetts in Lowell (fractalkine, ILs.)
- We are open for further collaboration

Děkuji kolegům a děkuji za pozornost

Charles University in Prague, Department of Occupational Medicine,
and General University Hospital Prague, Czech Republic,
Technical University in Liberec, Faculty of Mechanical Engineering, Department of Machining and Assembly, Department of Material Science
Institute of Chemical Process Fundamentals of the CAS, v.v.i., Prague
Heyrovsky Institute of Physical Chemistry of the CAS, v.v.i., Prague



Project of the Charles University in Prague PROGRES Q25/LF1, Q29/LF1

daniela@pelclova.cz