



MOLECULAR AND CELLULAR
TOXICOLOGY AND NEUROPATHOLOGY CORE LAB

PM_{2.5}-Induced Imbalance of Intracellular Reactive Oxygen Species Cause Vascular Dysfunction and Developmental Disorder

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<http://ctlab501.com>

2018.11@SOT-AACT



FB Page



Chao Lab

Air quality in Taiwan

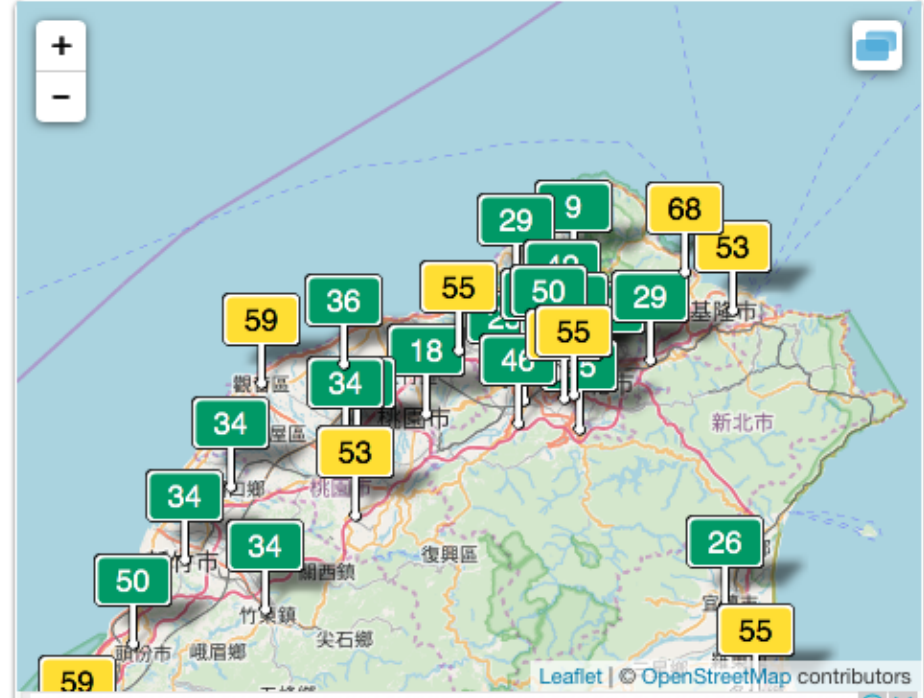


Taoyuan AQI: Taoyuan Real-time Air Quality Index (AQI).

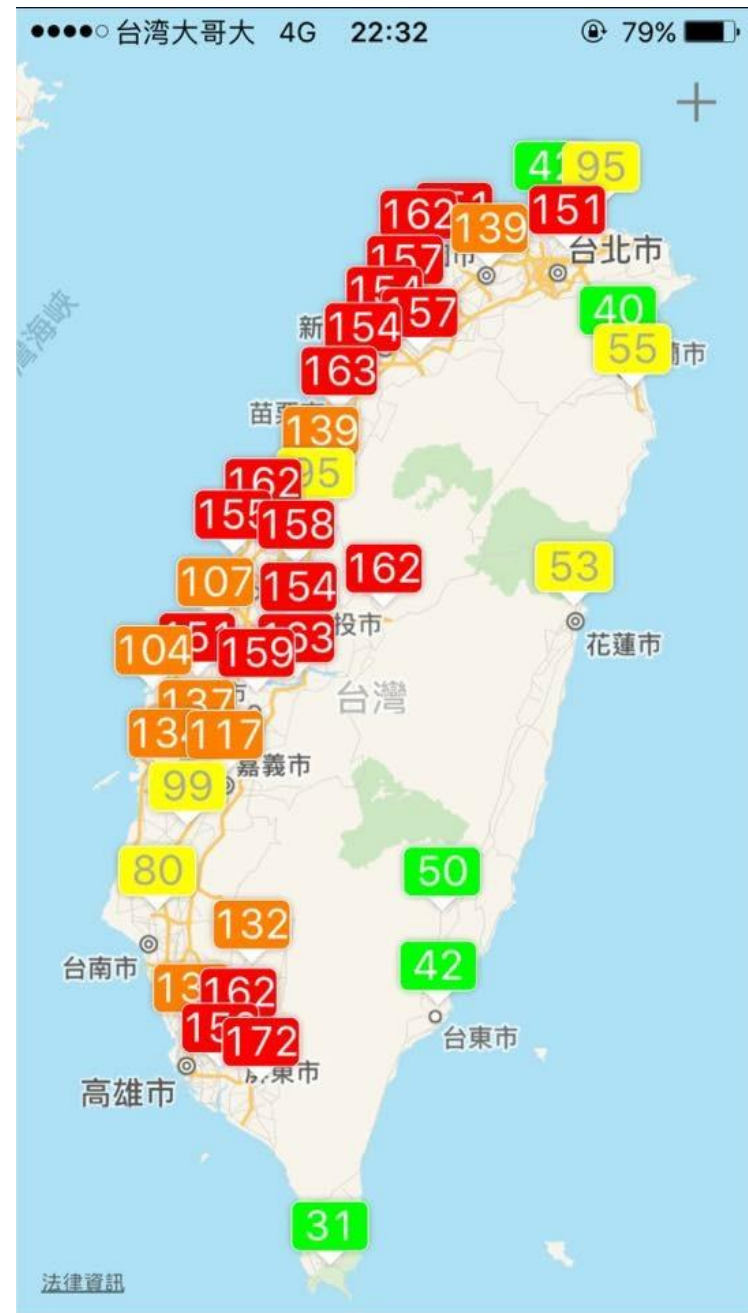
18 **Good**
 Updated on Thursday 21:00
 Primary pollutant: o3

Current Past 48 hours data

	Wednesday 6	12	18	Thursday 6	12	18	Min	Max
PM2.5 AQI	13	13	13	13	13	13	9	59
PM10 AQI	16	16	16	16	16	16	5	35
O3 AQI	18	18	18	18	18	18	11	33
NO2 AQI	15	15	15	15	15	15	4	23
SO2 AQI	6	6	6	6	6	6	3	6
CO AQI	4	4	4	4	4	4	2	6
UVI	1	1	1	1	1	1	1	3
Temp.	24	24	24	24	24	24	24	26

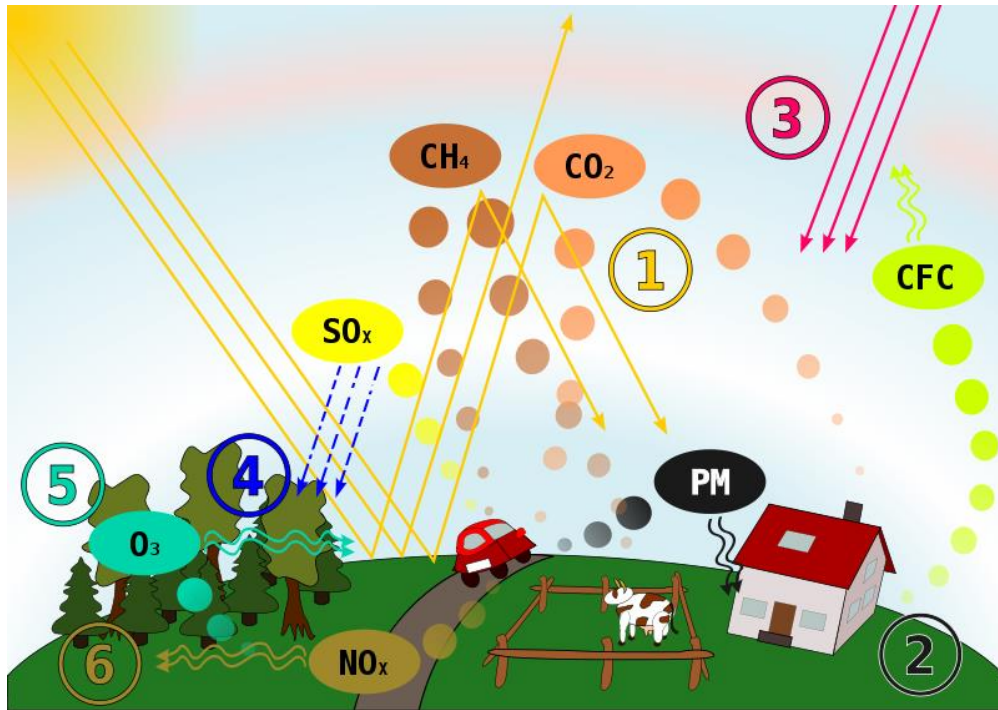


PM @ 2018/11/14

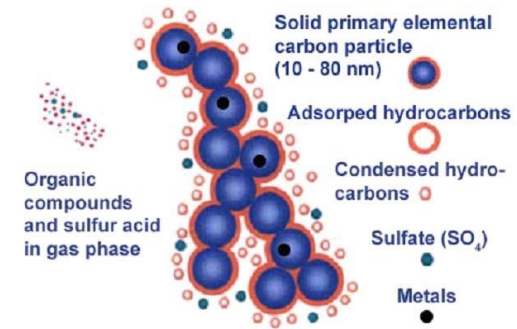


<http://aqicn.org/map/>

Air pollutants contain Particulate Matter



Agglomerated diesel particle

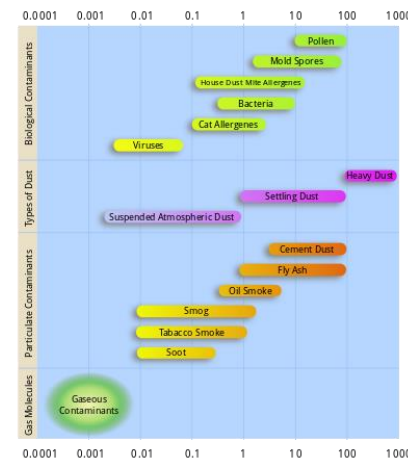
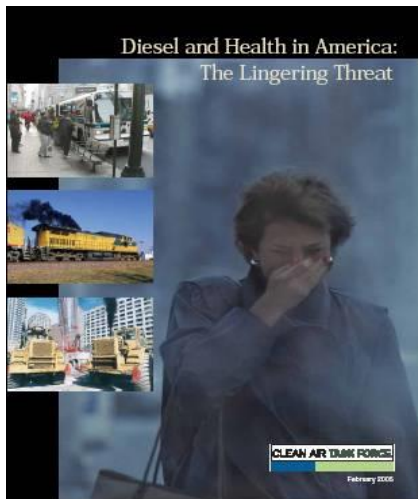


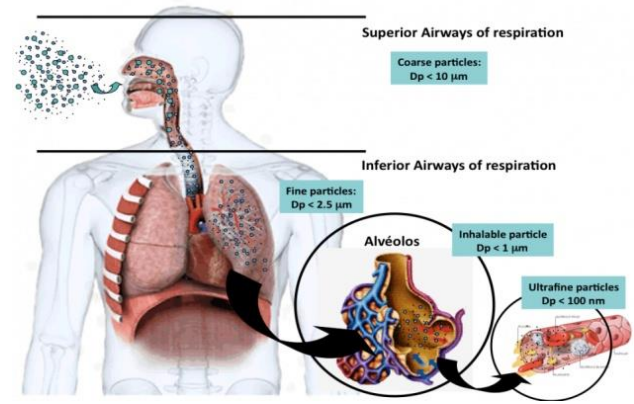
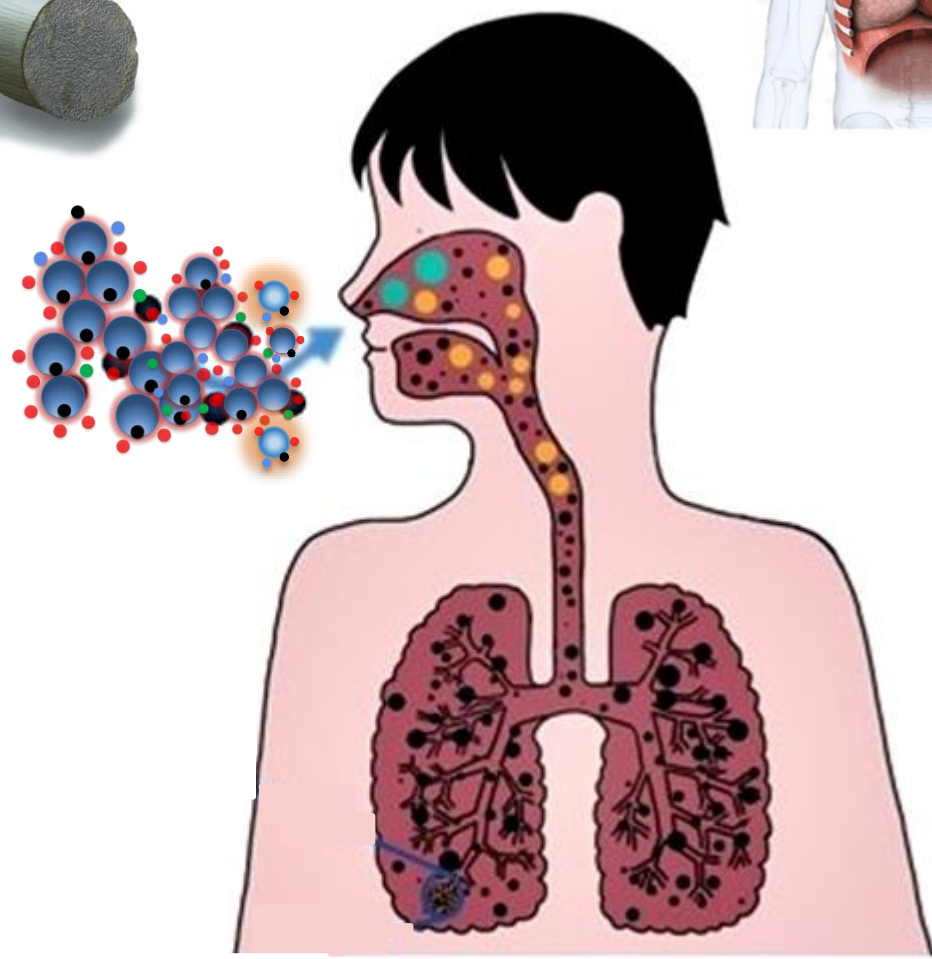
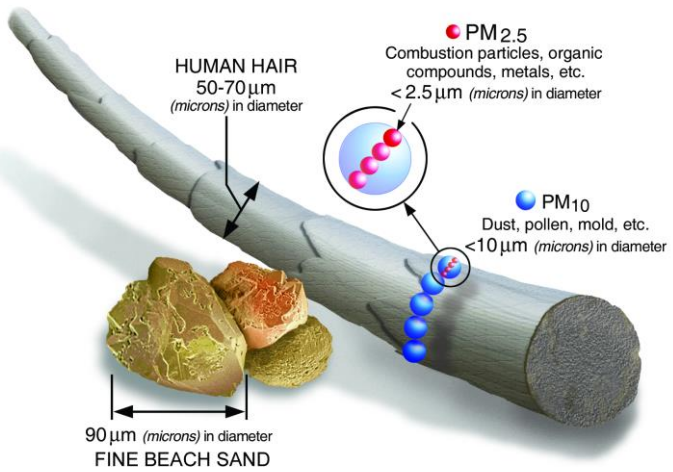
Typical chemical composition of fine particulate matter in the united states

Diesel PM2.5

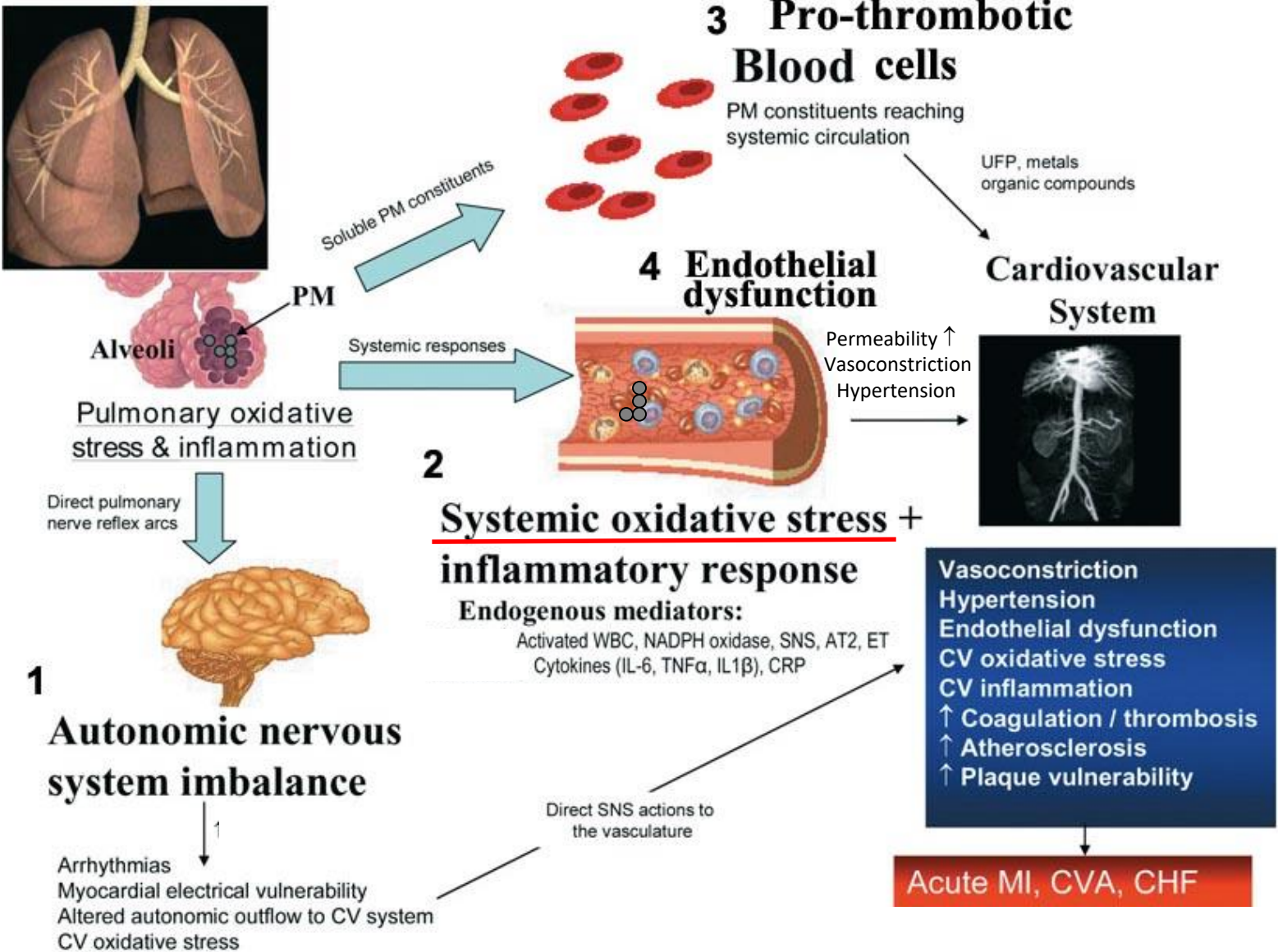
Elemental Carbon	Organic Carbon	Sulfate, Nitrate, Ammonium	Minerals	Unknown
75%	19%	1%	2%	3%

Reproduced from U.S. EPA (2002) Health effects assessment document for diesel engine exhaust. EPA/600/8-90/0-57F, May 2002



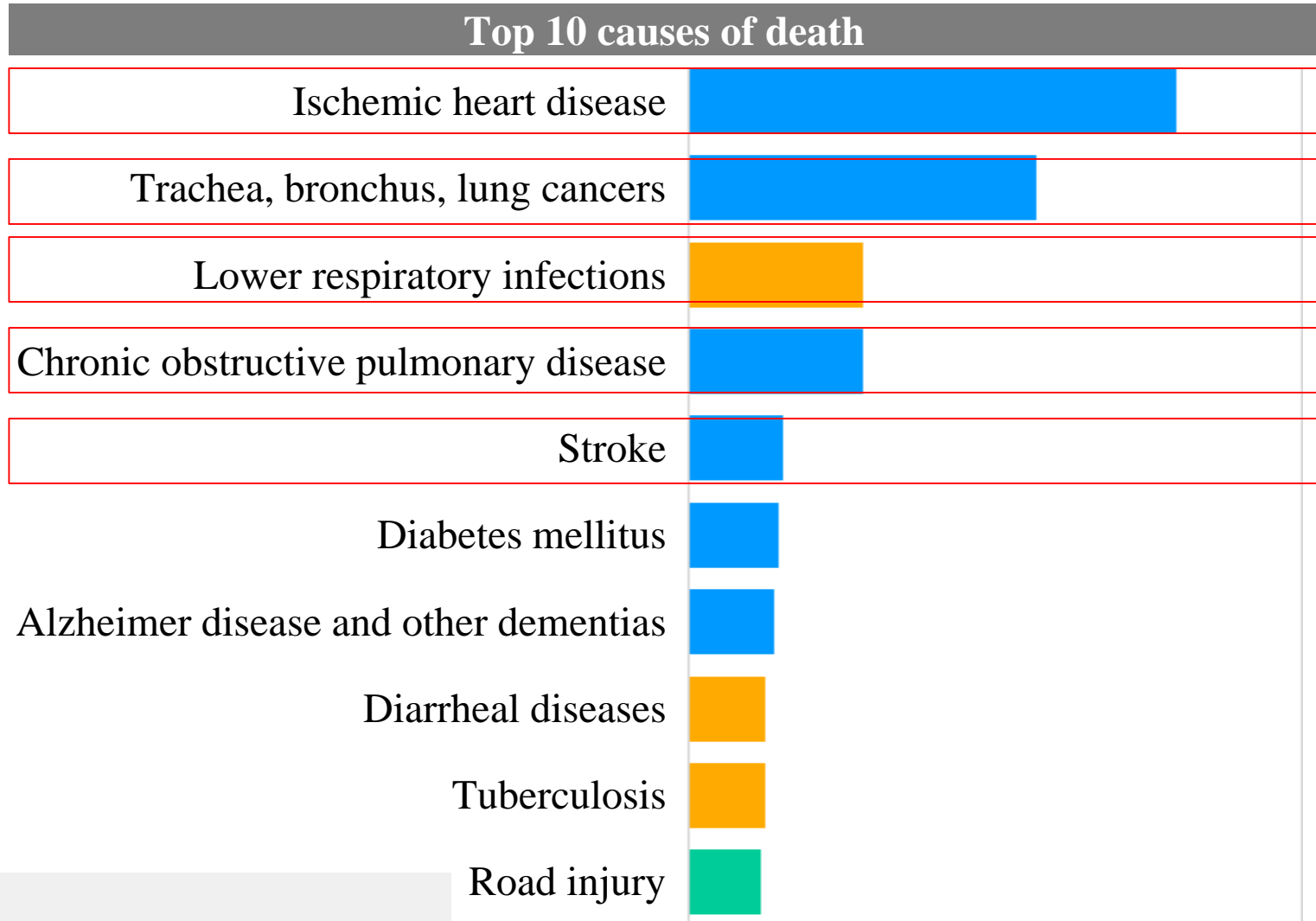


PM_{2.5} cause cardiovascular and systemic effects



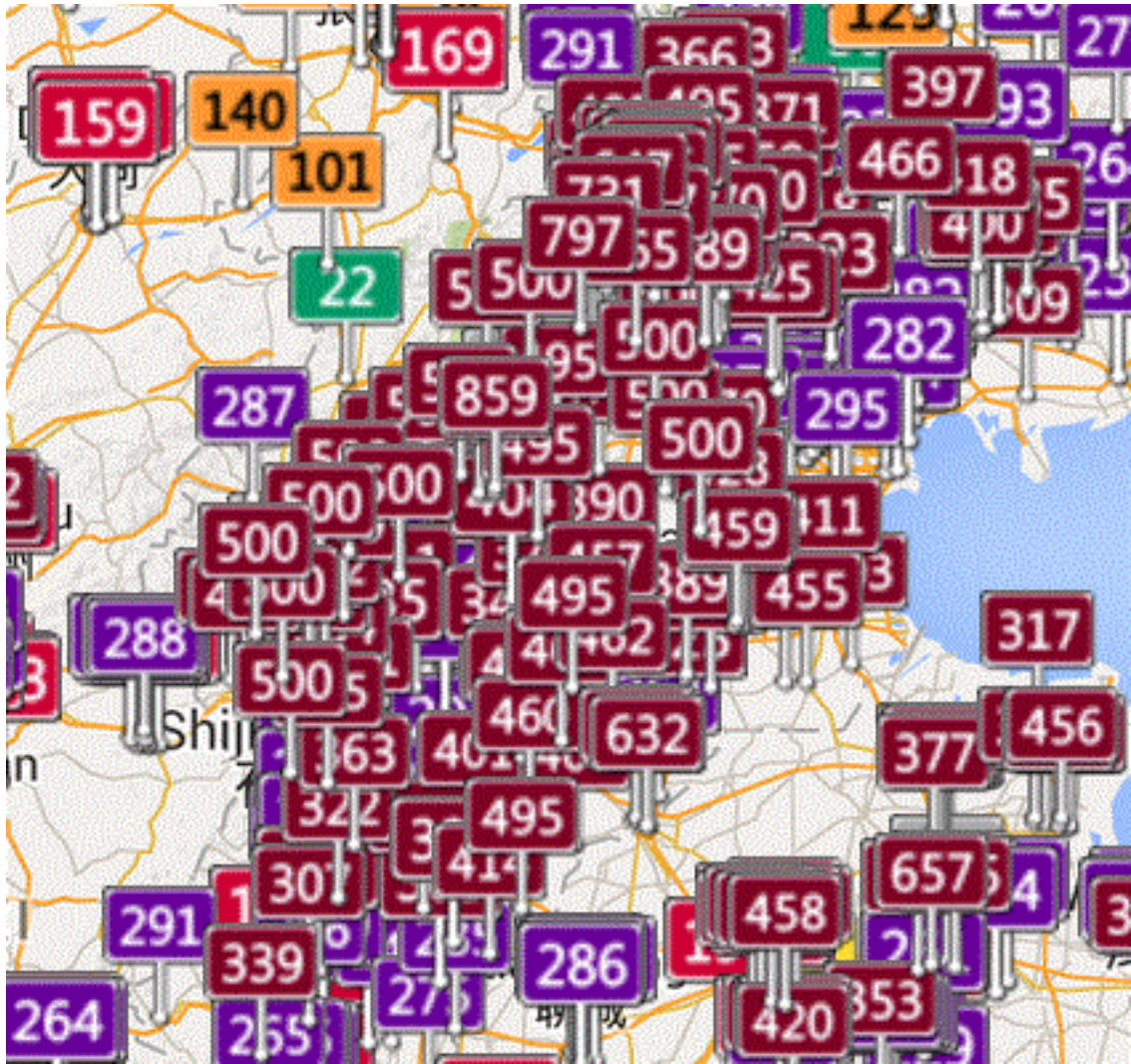
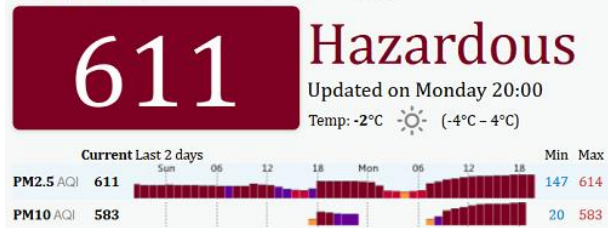
Modified from Brook, *Clinical Sci*, 115, 175-187, 2008

Top 10 cause of death relation to PM_{2.5}



Beijing PM_{2.5}

Beijing AQI: Beijing Real-time Air Quality Index (AQI).



PM in Beijing @ 2018/11/14



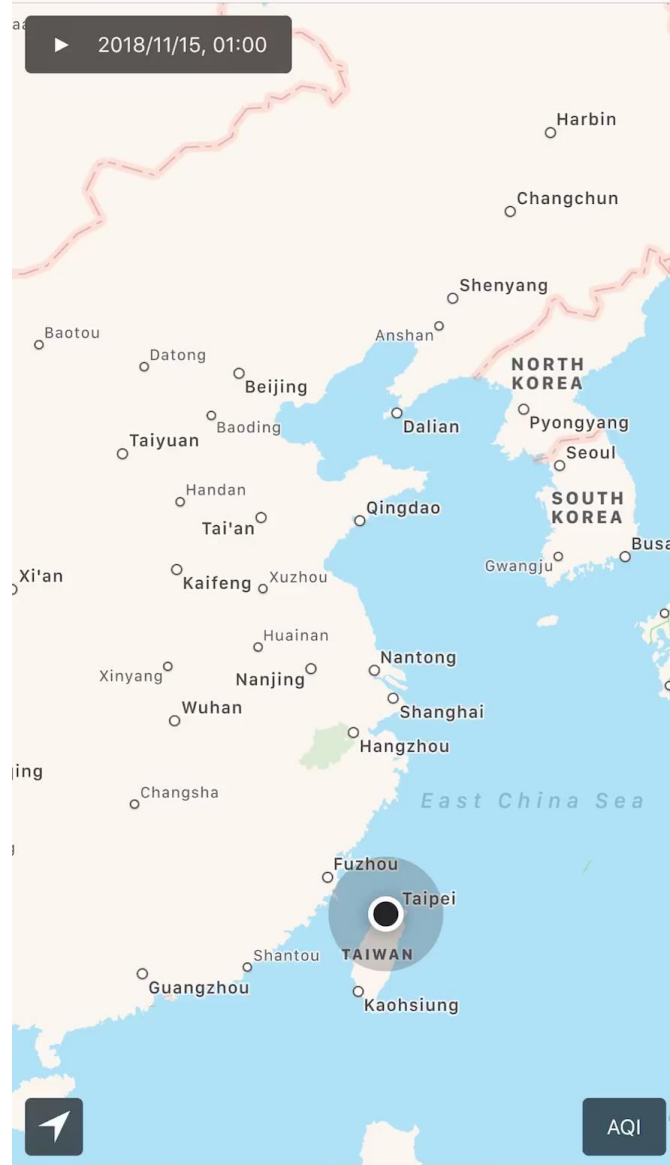
01:27



Air Quality Map



▶ 2018/11/15, 01:00



AQI



Dashboard



Map



Ranking

Daily deaths in the 1952 London smog

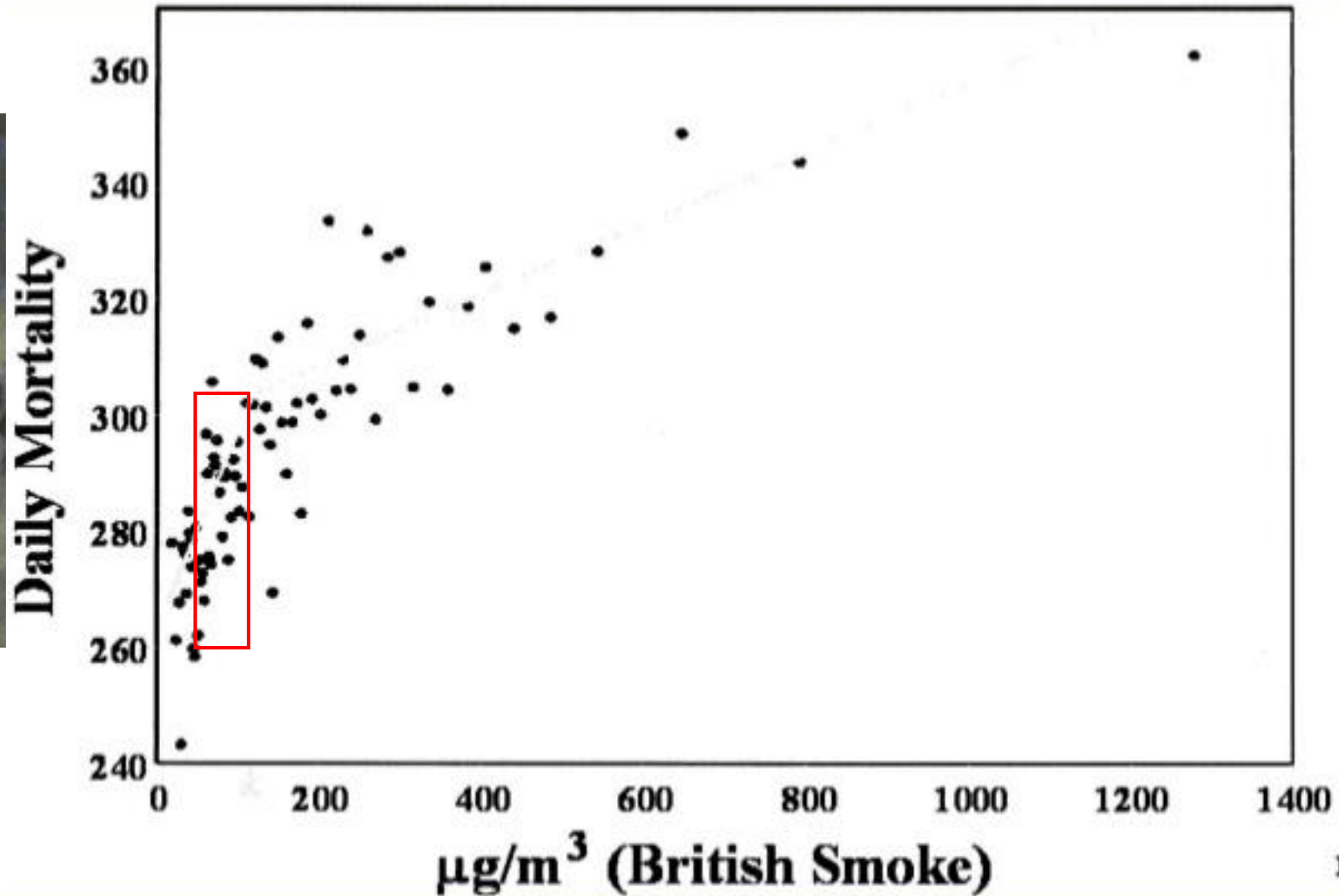
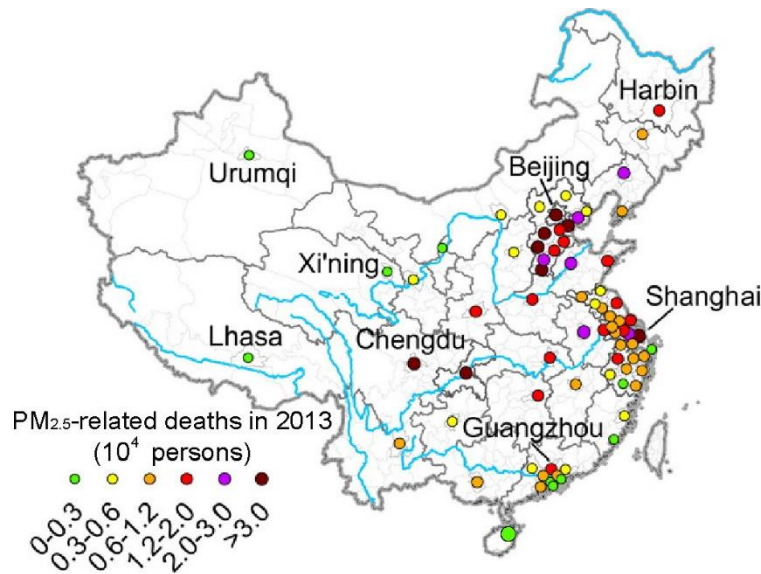
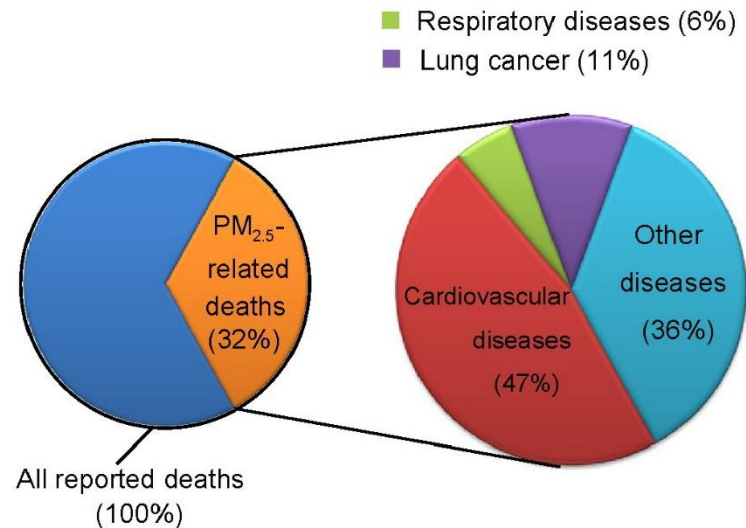


Figure 1

The smog caused people dead in China (2013)



PM_{2.5}-related deaths in the 74 leading cities of China (2013)

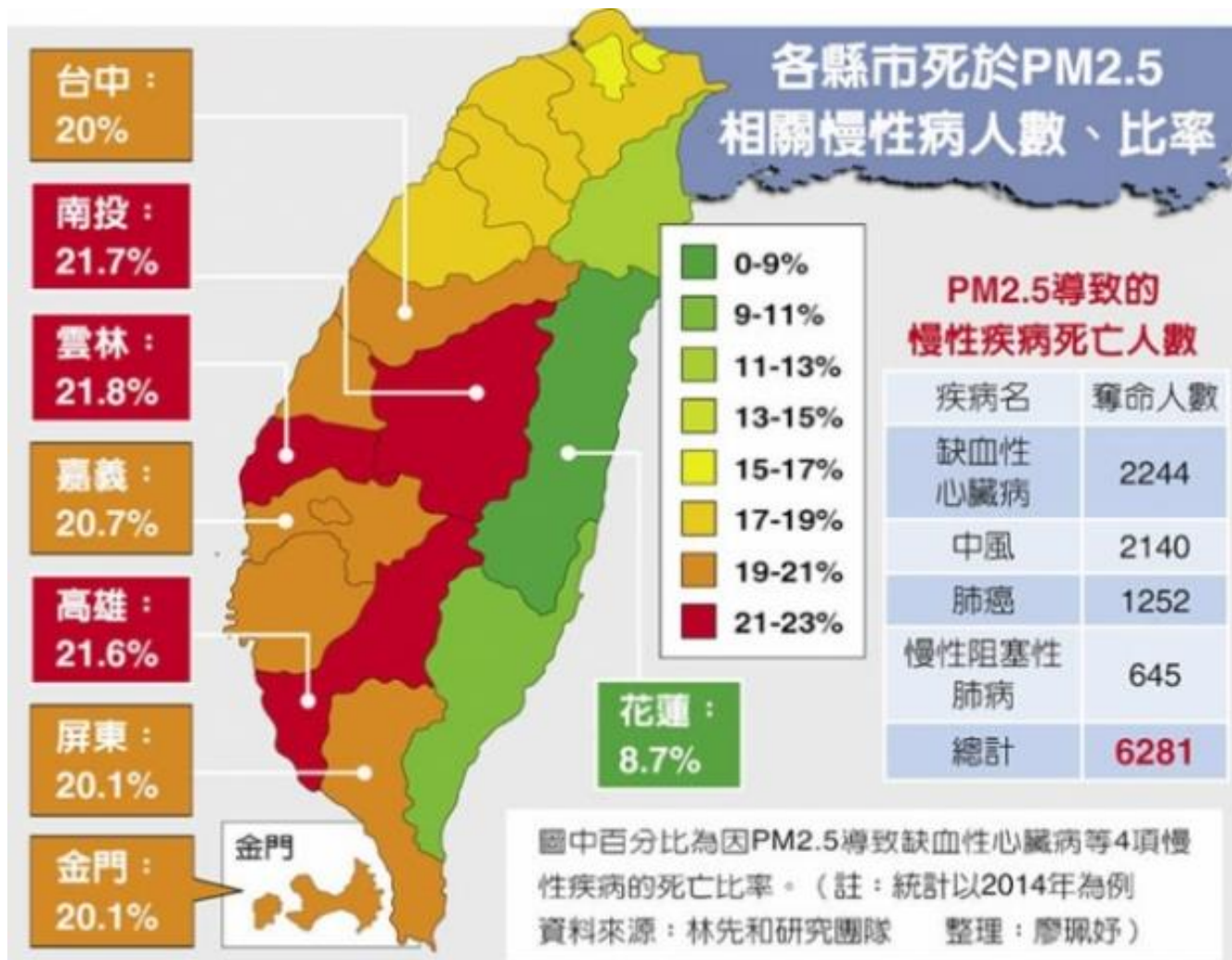


Relative contribution of PM_{2.5}-related deaths in the 74 leading cities of China (2013)

Exposure-response coefficients (ERCs) for the long-term mortality effects of PM_{2.5}.

Health outcomes	β (%) ^a
All-cause mortality	5.37 (3.48,7.27)
Cardiovascular mortality	5.91 (3.63,8.18)
Respiratory mortality	2.54 (0.95,4.12)
Lung-cancer mortality	8.93 (4.32,13.55)

^a Defined as the percent change of mortality per 10 $\mu\text{g}/\text{m}^3$ increase of PM_{2.5} concentration with 95% confidence intervals.



- Cardiovascular issue is the main disorder that PM_{2.5} might cause

10 years Air quality change in Taiwan

測項	PM ₁₀ ($\mu\text{g}/\text{m}^3$)	SO ₂ (ppb)	NO ₂ (ppb)	CO (ppm)	O _{3,avg} (ppb)	O _{3,8hr} (ppb)	PM _{2.5} ($\mu\text{g}/\text{m}^3$)		
							自動	手動	
							69~76 站次	30~31 站次	
2008	97 年	58.9	4.55	17.49	0.50	29.24	46.51	33.7	—
2009	98 年	59.7	4.23	16.76	0.48	30.74	47.89	33.3	—
2010	99 年	57.2	4.32	17.53	0.50	27.94	45.26	31.2	—
2011	100 年	54.9	3.98	16.65	0.48	29.18	45.53	32.3	—
2012	101 年	51.2	3.44	15.41	0.47	29.42	45.41	28.4	—
2013	102 年	53.9	3.59	15.18	0.46	29.96	46.42	30.3	24.0
2014	103 年	52.9	3.54	15.24	0.45	30.17	46.86	25.1	23.6
2015	104 年	47.7	3.18	14.21	0.44	29.48	45.25	21.8	22.0
2016	105 年	43.5	3.03	14.11	0.43	27.92	43.03	20.9	20.0
2017	106 年	44.7	2.95	13.48	0.39	30.49	45.80	20.7	18.3

備註 1: 細懸浮微粒手動監測濃度，於 101 年 11 月 29 日起開始執行採樣，總測站數為 30 站次，104 年增加至 31 站次。

備註 2: 歷年自動監測站濃度總計測站數，97~99 年為 69 站次；100 年為 70 站次；101~103 年為 74 站次；104~106 年為 76 站次。

備註 3: 103~106 年細懸浮微粒自動數據係經過手動監測站迴歸式校正數值。

備註 4: 本表統計資料未扣除受境外污染傳輸及特殊天氣型態影響之數據。









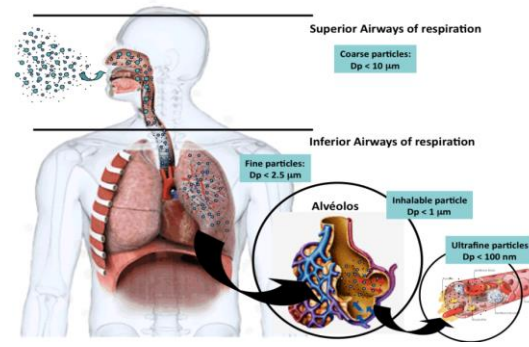
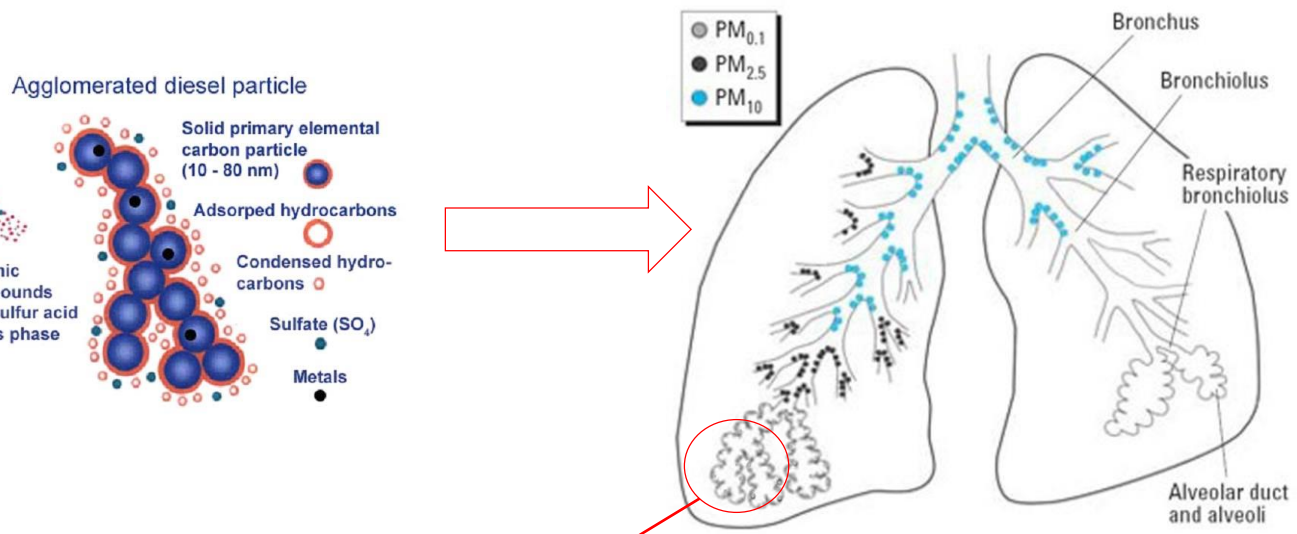






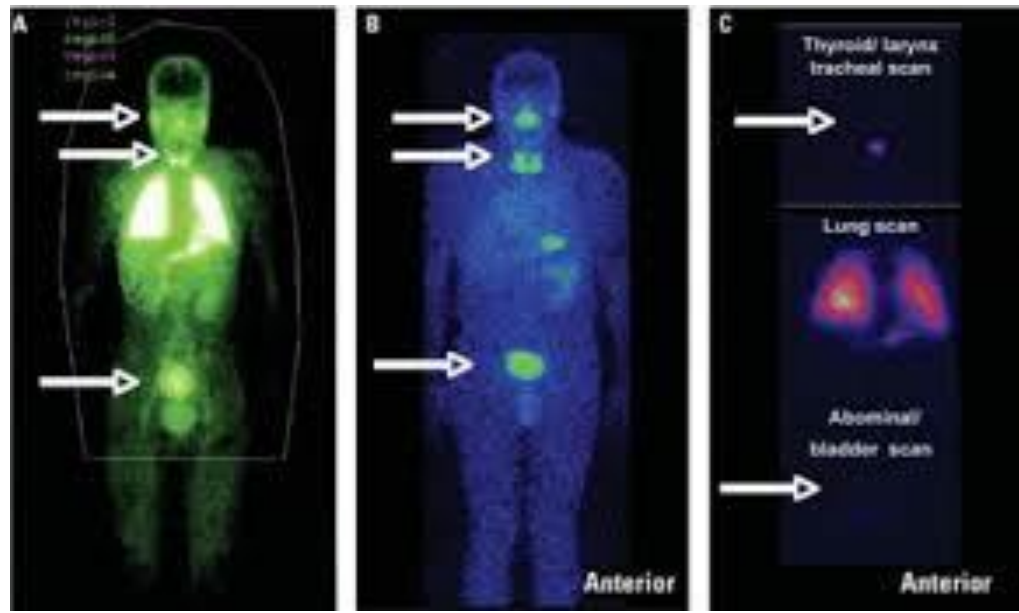
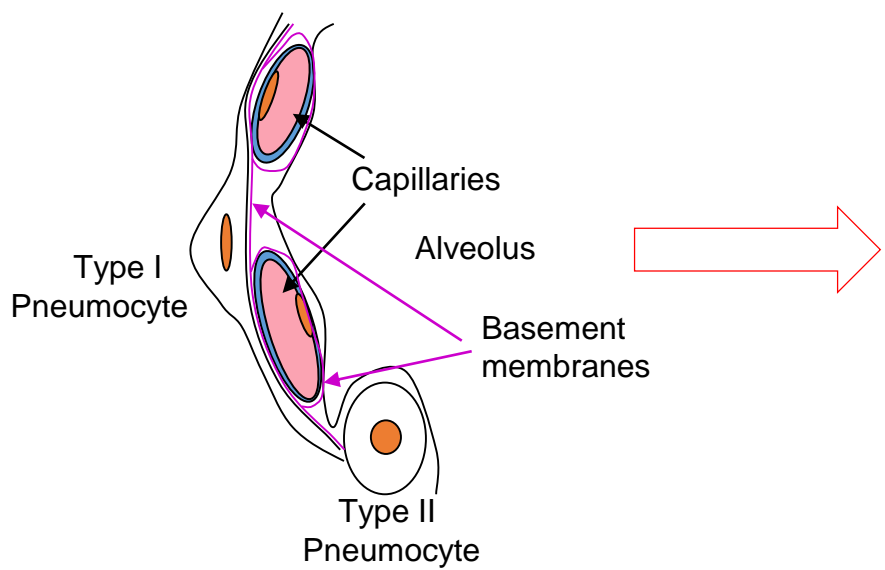


PM_{2.5} translocate into the circulation and cause damages



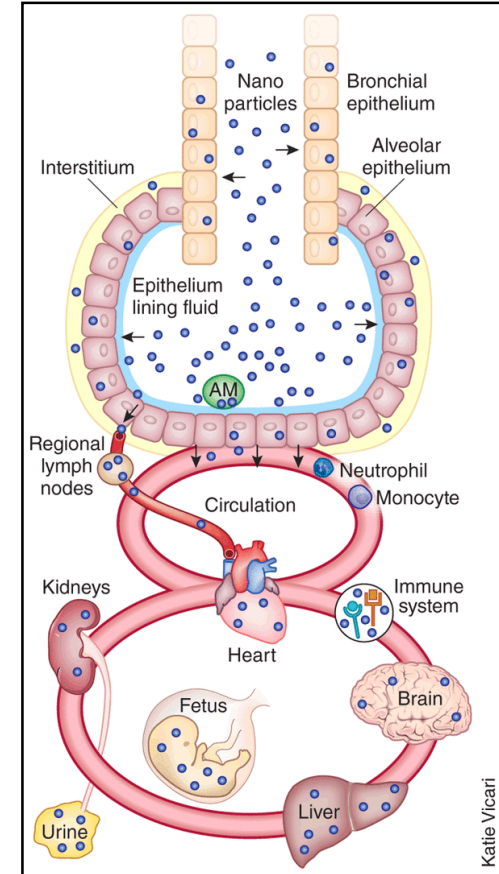
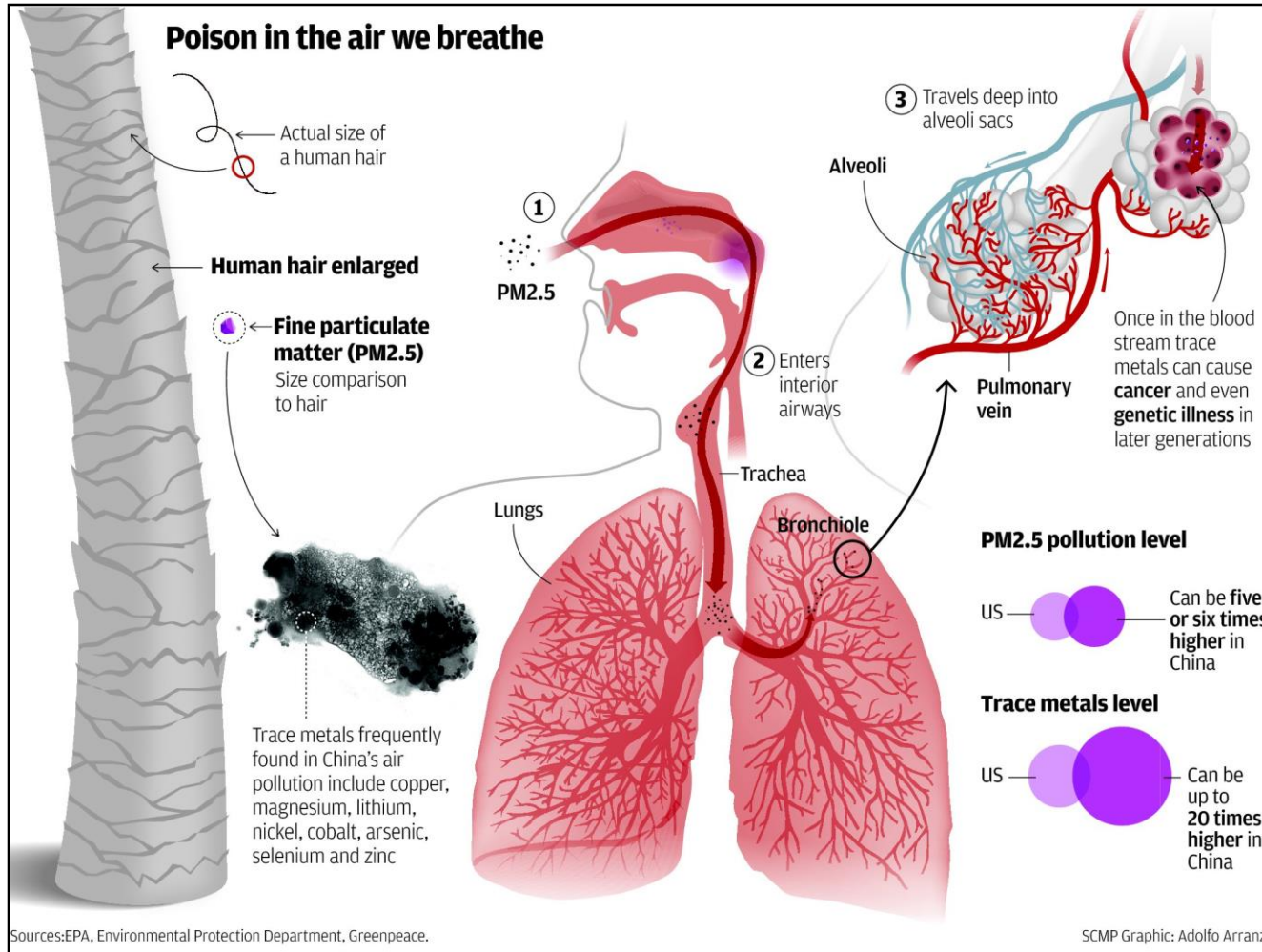
Adapted from *Environ Health Perspect*, 2006,114:6,810–817

Alveolar Wall: Blood-air barrier



Nemmar et al, *Brief Rapid Comm*, 2002

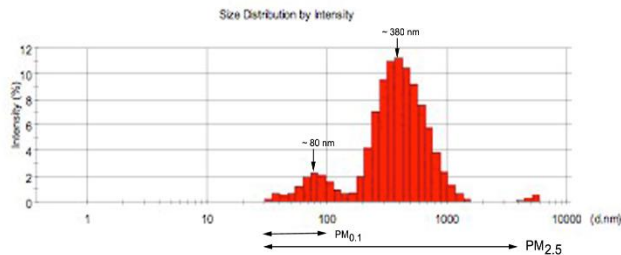
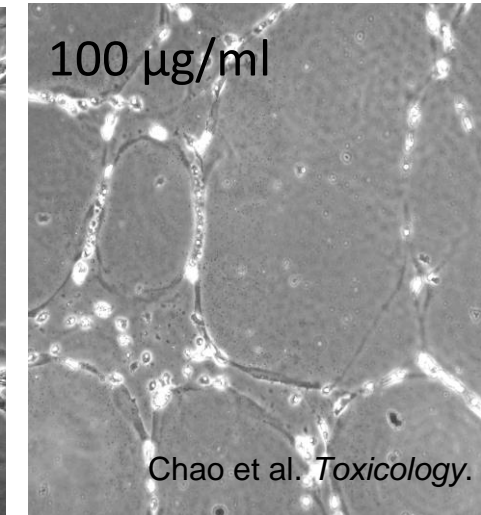
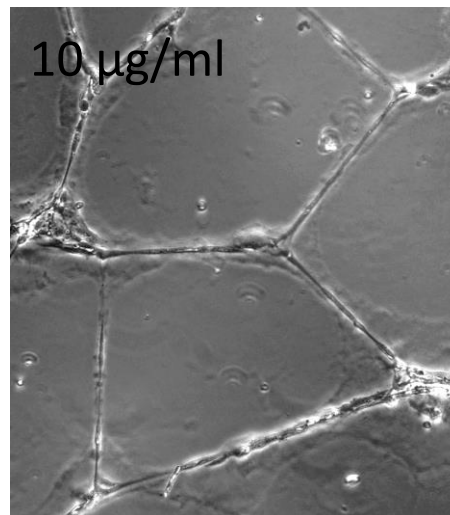
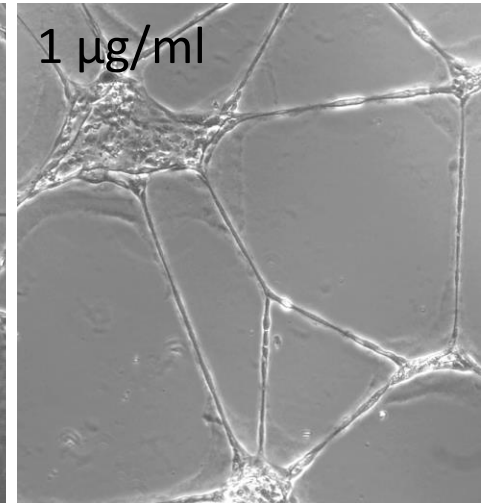
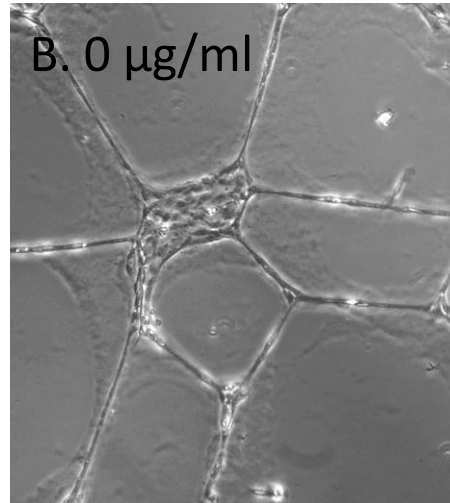
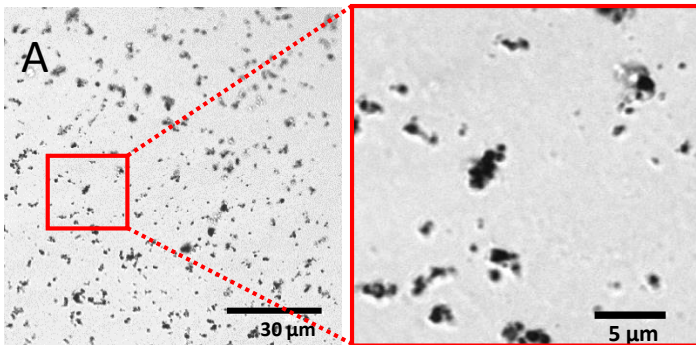
By what mechanism that $PM_{2.5}$ translocate into the circulation?



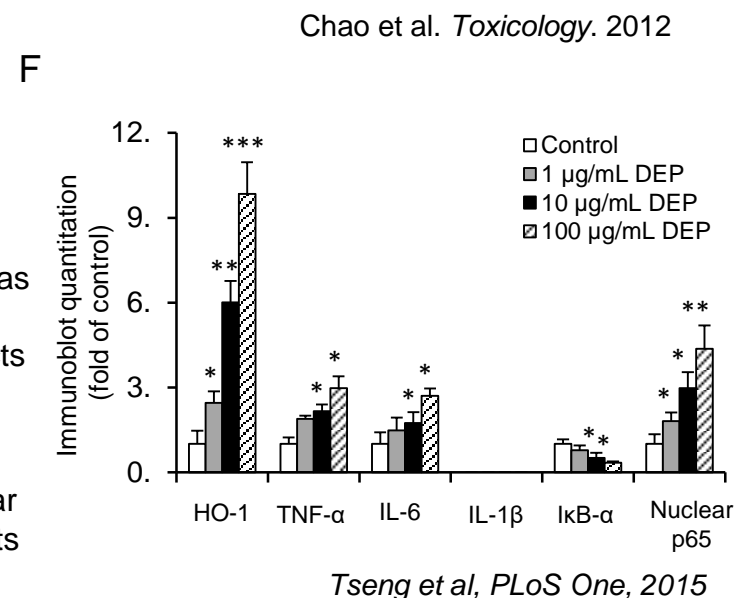
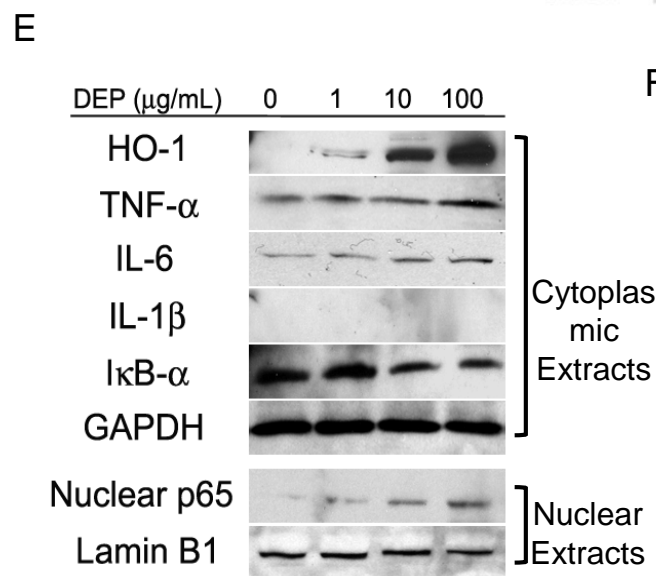
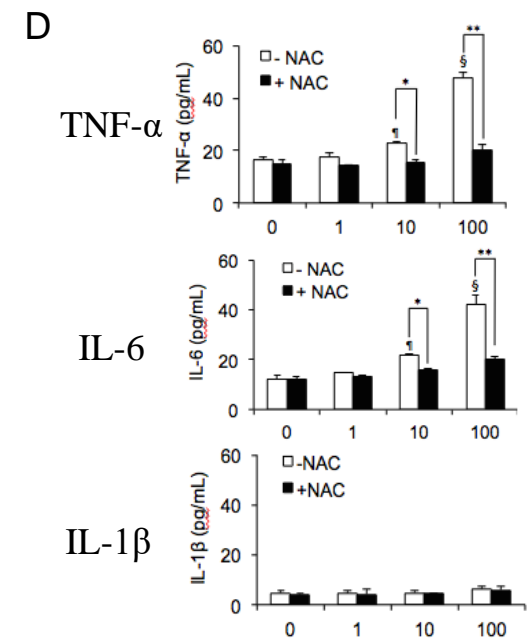
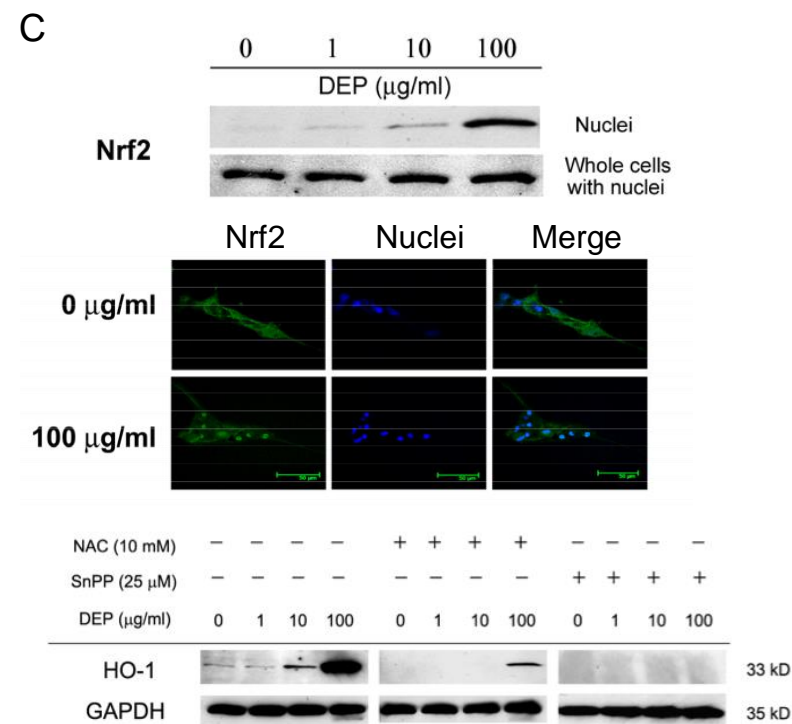
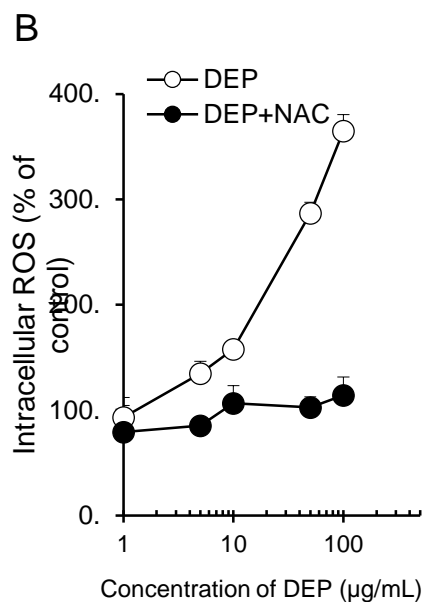
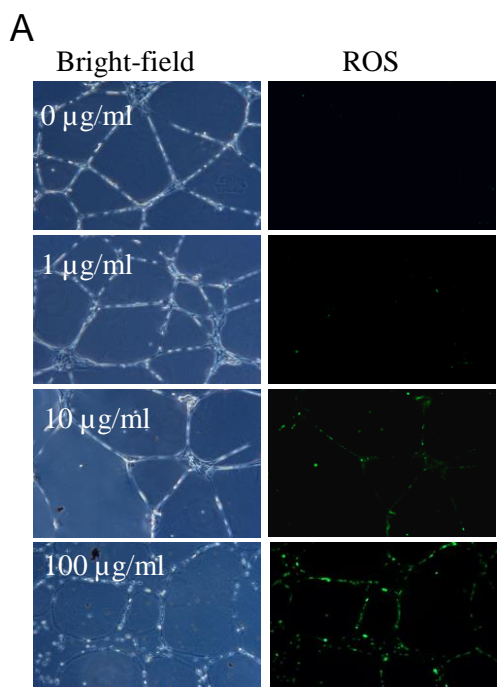
The endothelial in vitro model experiment:

A. The PM_{2.5} particle

B. The model treated with PM_{2.5}

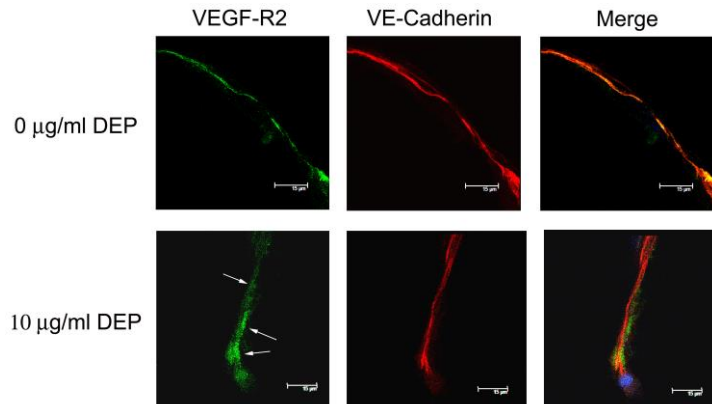
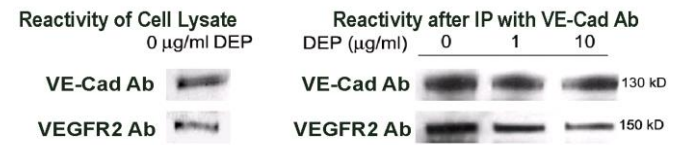
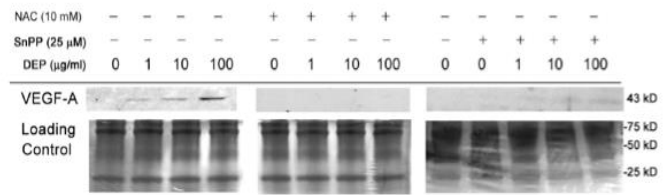


Antioxidative mechanism: Nrf-2 and HO-1 activation in PM_{2.5} treated capillary-tube cells



PM_{2.5} disrupt our vascular junction with going paracellular route

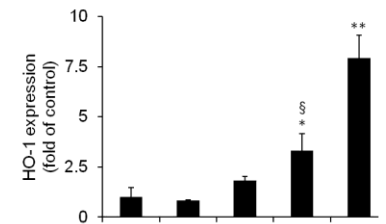
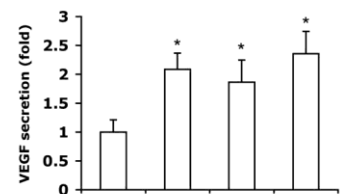
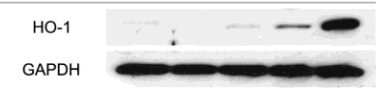
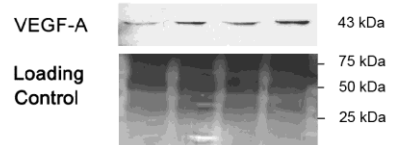
A.



Chao et al, Toxicology, 2012

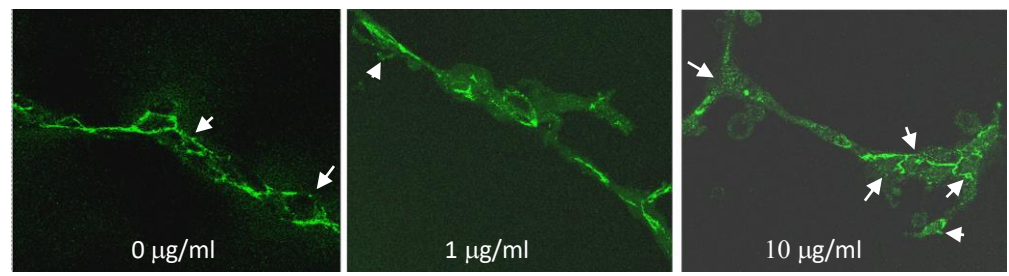
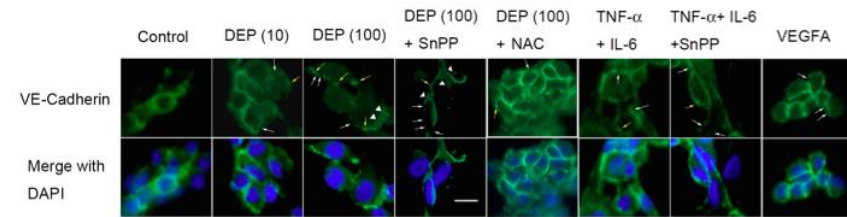
DEP (100 μg/mL)	-	-	-	+
TNF-α (47.5 pg/mL)	-	+	+	-
IL-6 (42.4 pg/mL)	-	+	+	-
SnPP (25 μM)	-	-	+	-

DEP (100 μg/mL)	-	-	-	+	+
TNF-α (47.5 pg/mL)	-	+	+	-	-
IL-6 (42.4 pg/mL)	-	+	+	-	-
SnPP (25 μM)	-	+	-	-	-
NAC (10 mM)	-	-	-	+	-

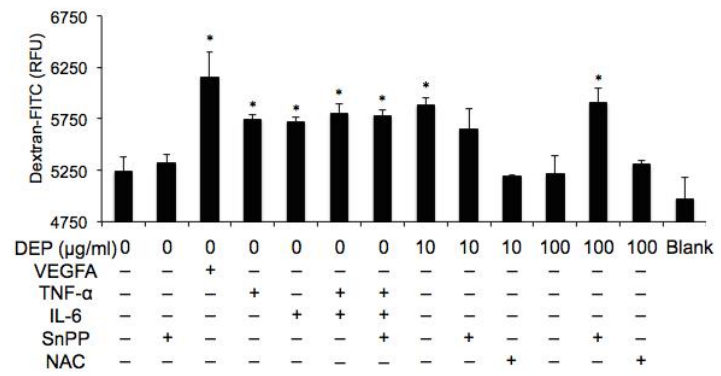


DEP (100 μg/mL)	-	-	-	+	+
TNF-α (47.5 pg/mL)	-	+	+	-	-
IL-6 (42.4 pg/mL)	-	+	+	-	-
SnPP (25 μM)	-	+	-	-	-
NAC (10 mM)	-	-	-	+	-

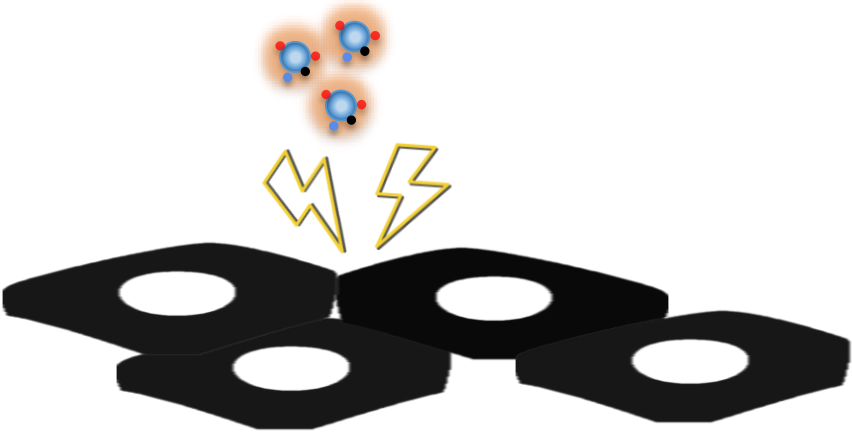
DEP (100 μg/mL)	-	-	-	+
TNF-α (47.5 pg/mL)	-	+	+	-
IL-6 (42.4 pg/mL)	-	+	+	-
SnPP (25 μM)	-	-	+	-

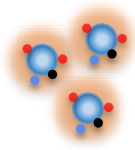


Chao et al, Toxicology, 2011



Tseng et al, PLoS One, 2015

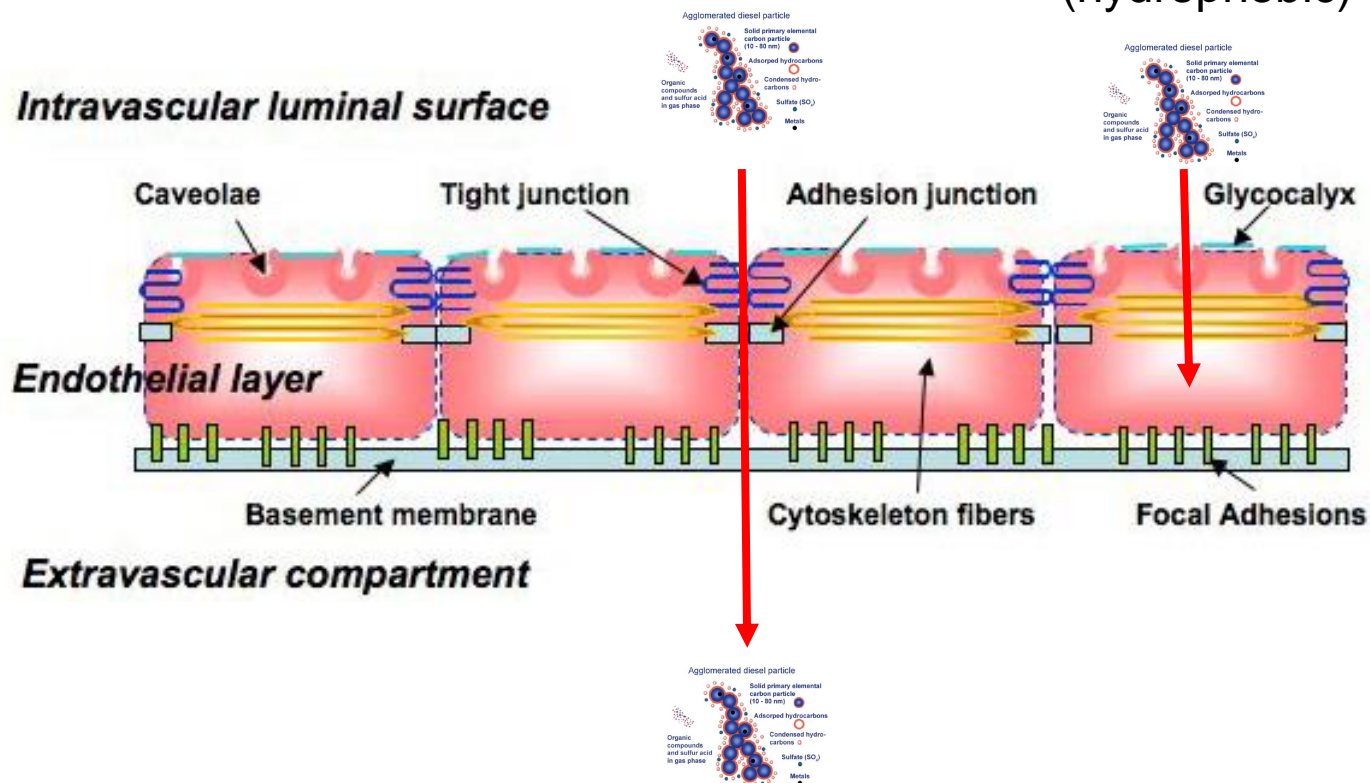




The mechanism that $PM_{2.5}$ translocate into the circulation and cause damages

1. Para-cellular (hydrophilic)

2. Trans-cellular (hydrophobic)



Transcellular mechanism - Vesiculo-vacuolar organelle (VVO)

J Histochem Cytochem. 2001 Apr;49(4):419-32.

The vesiculo-vacuolar organelle (VVO). A new endothelial cell permeability organelle.

Dvorak AM¹, Feng D.

⊕ Author information

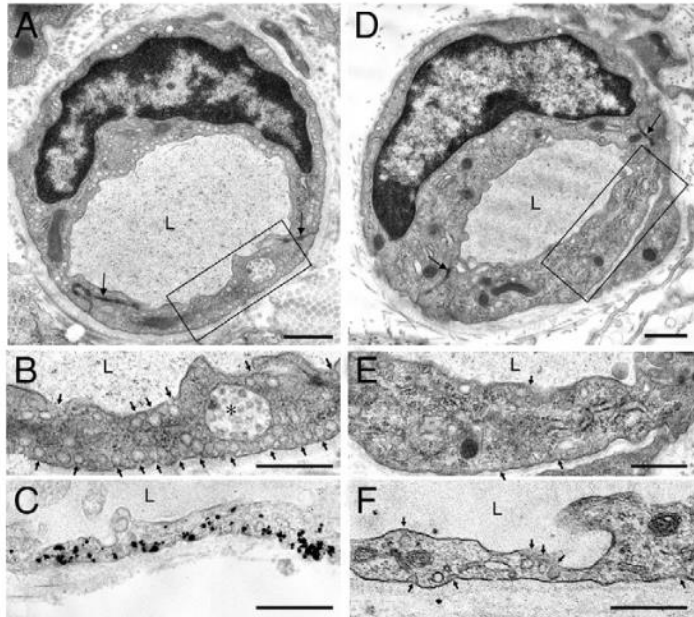
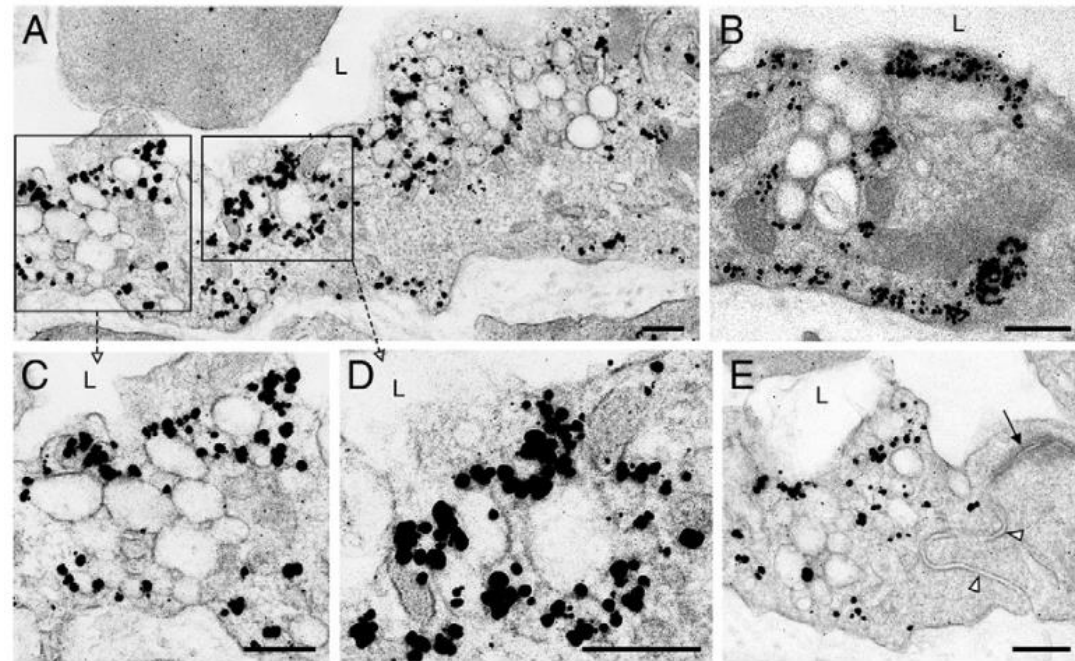
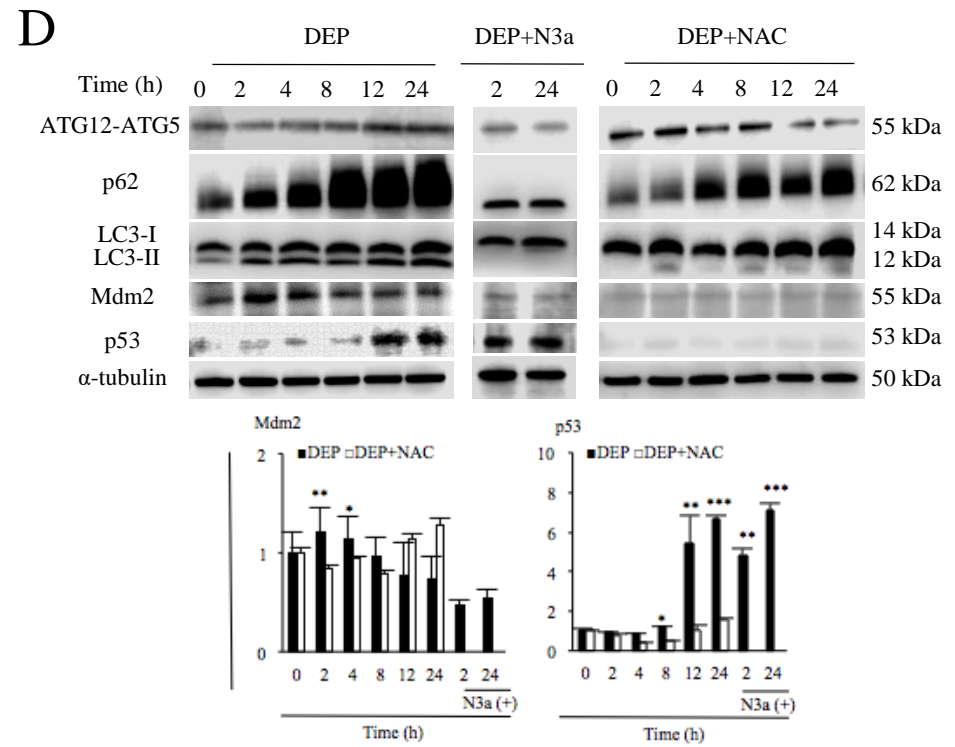
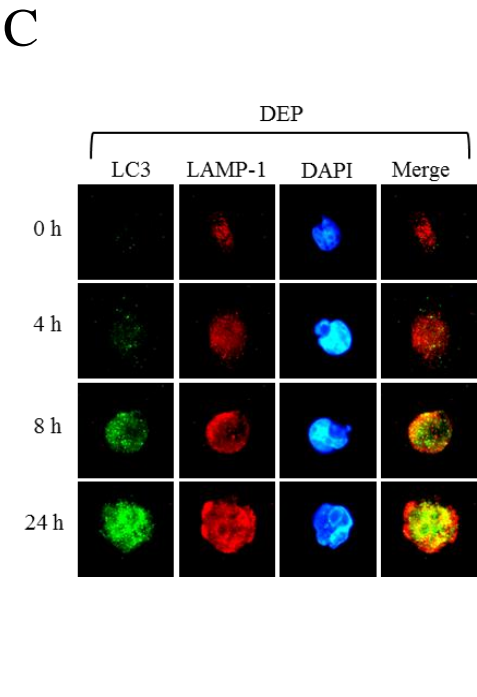
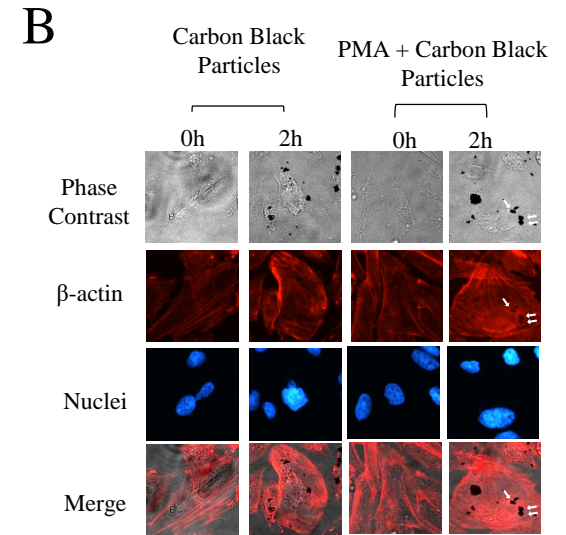
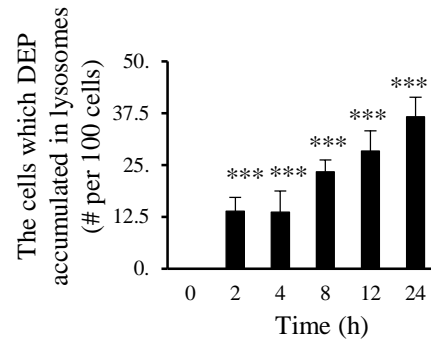
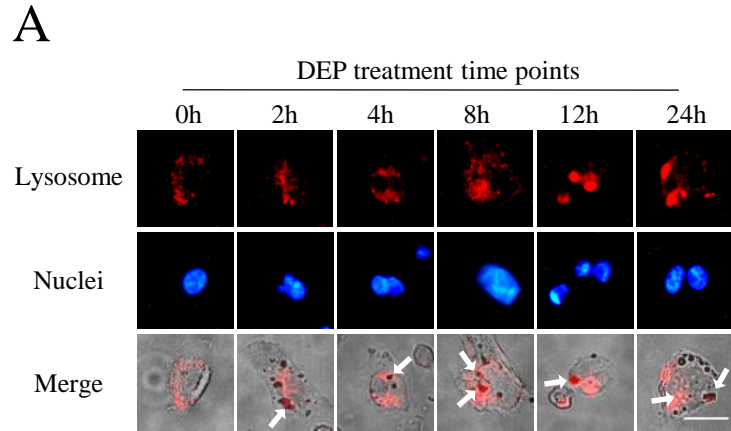


Figure 2. Electron micrographs of flank skin capillaries of wild-type (A–C) and *cav-1*^{-/-} mice (D–F). **Boxes** in (A) and (D) indicate portions enlarged in (B) and (E), respectively. Numerous 50- to 100-nm vesicles (caveolae), some indicated by **arrows**, are present in the cytoplasm and attached to both luminal and abluminal plasma membranes of wild-type capillary endothelial cells (A and B), whereas caveolae are largely, although not entirely, absent from capillaries of *cav-1*^{-/-} mice (D and E). Immuno-nanogold staining for caveolin-1 demonstrates strong labeling of caveolae in wild-type capillaries (C) and absence of labeling in their *cav-1*^{-/-} counterparts (F). L, lumen; **asterisk**, multivesicular body. Magnification bars = 1 μm .

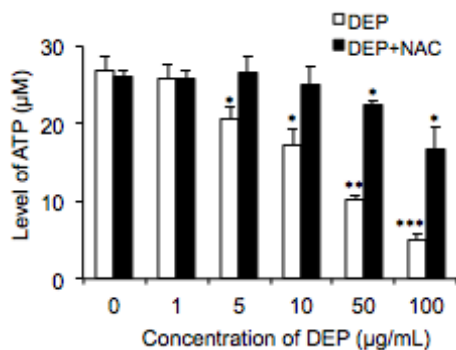


PM_{2.5} cause autophagy in endothelial capillary

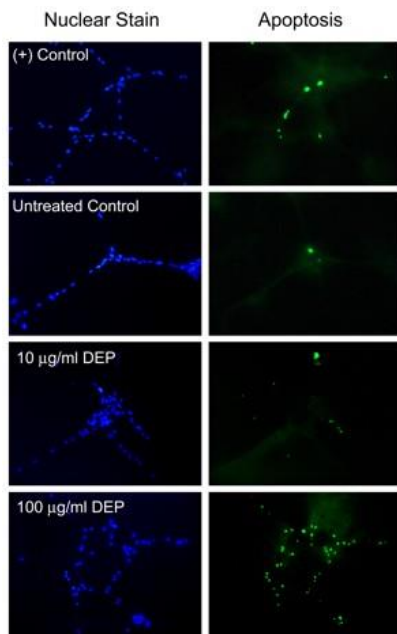


PM_{2.5}-caused endothelial apoptosis might lead to atherosclerosis

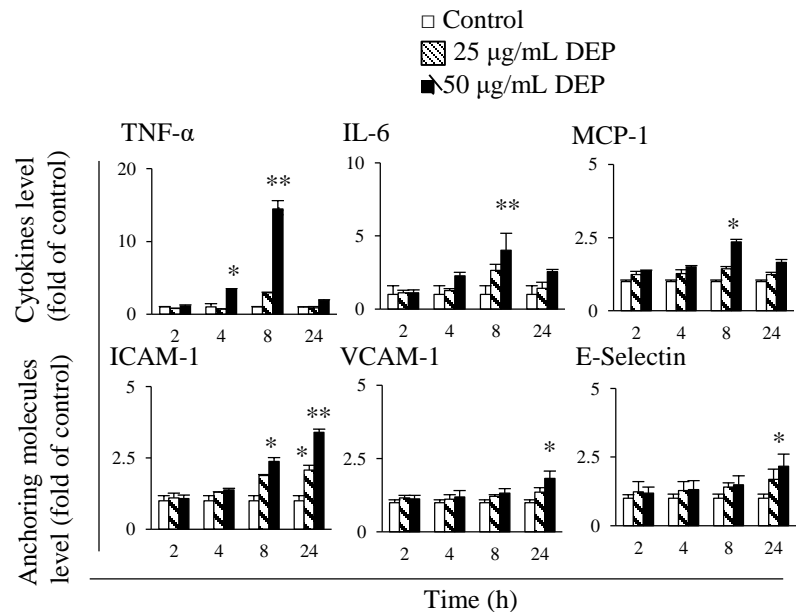
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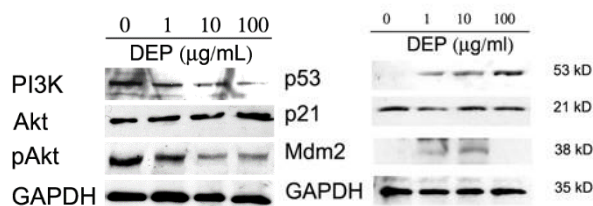
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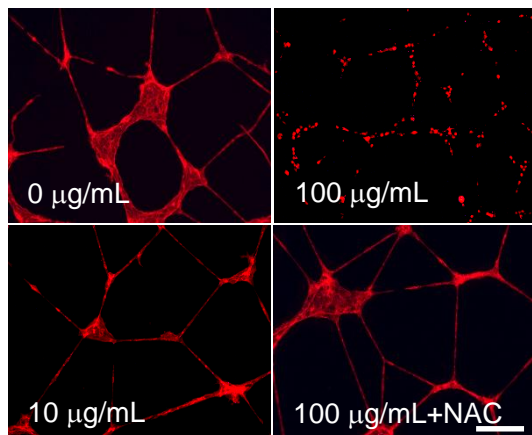
E



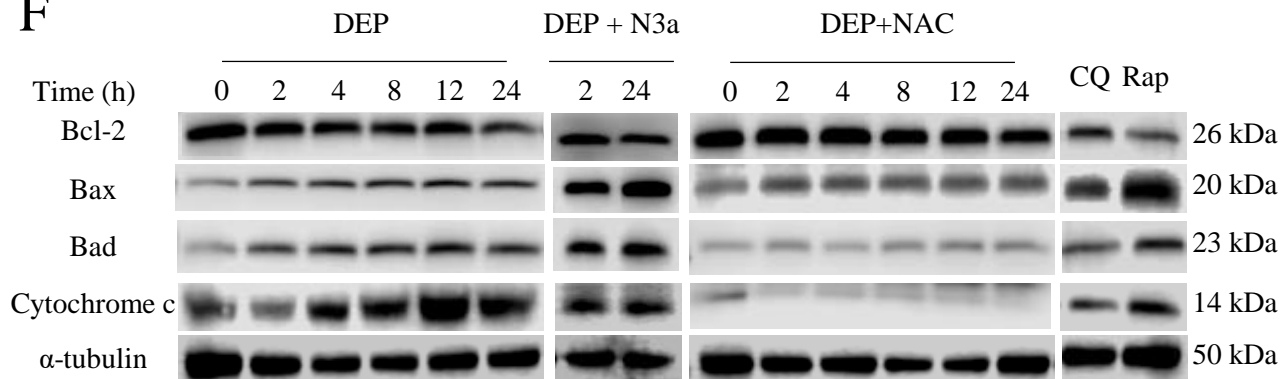
B



C

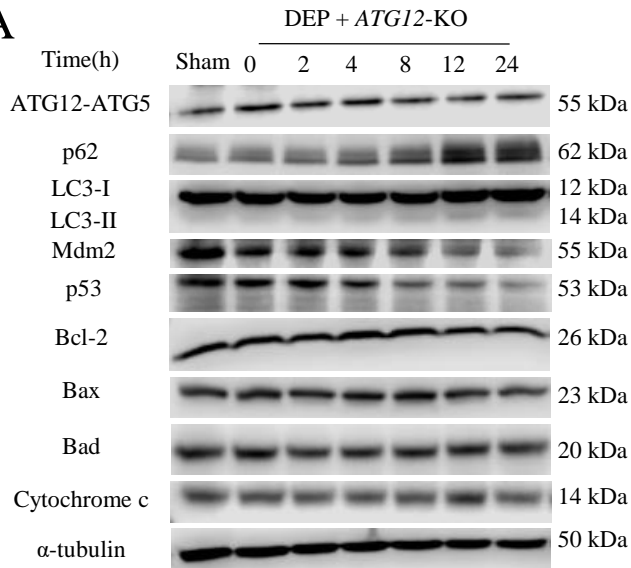


F

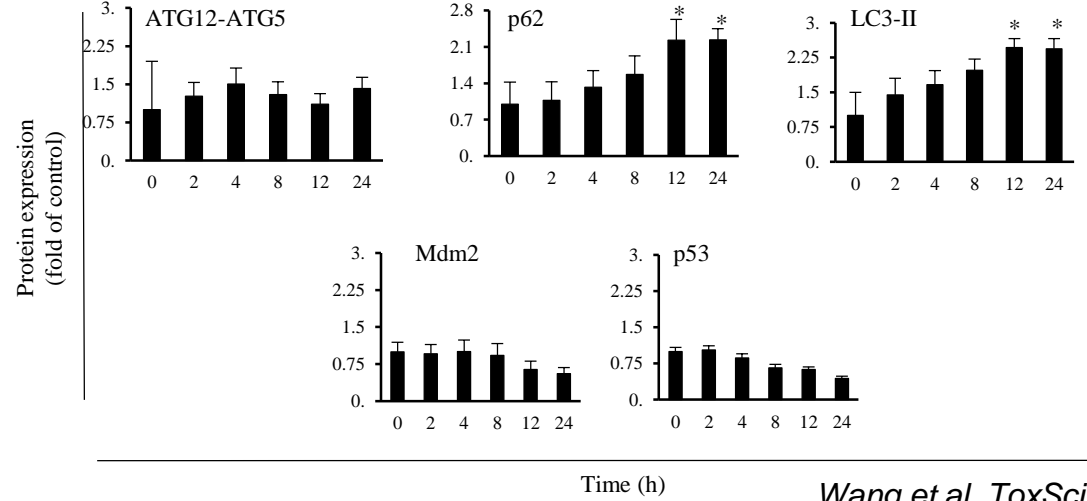


Blockage of autophagy might stop PM_{2.5}-induced endothelial apoptosis

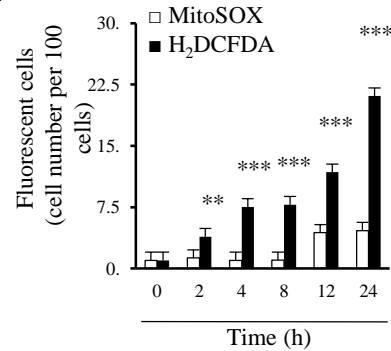
A



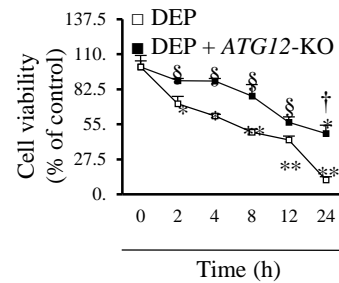
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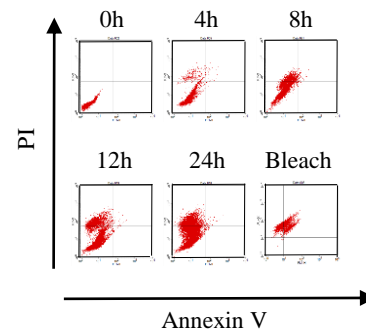
C



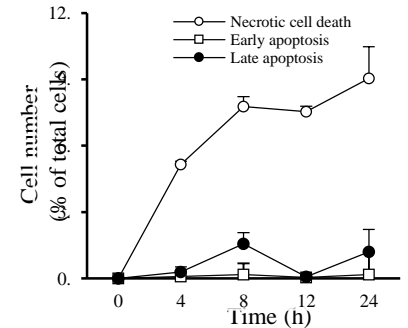
D



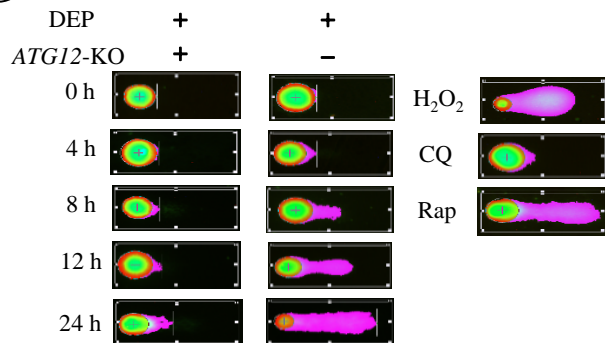
E



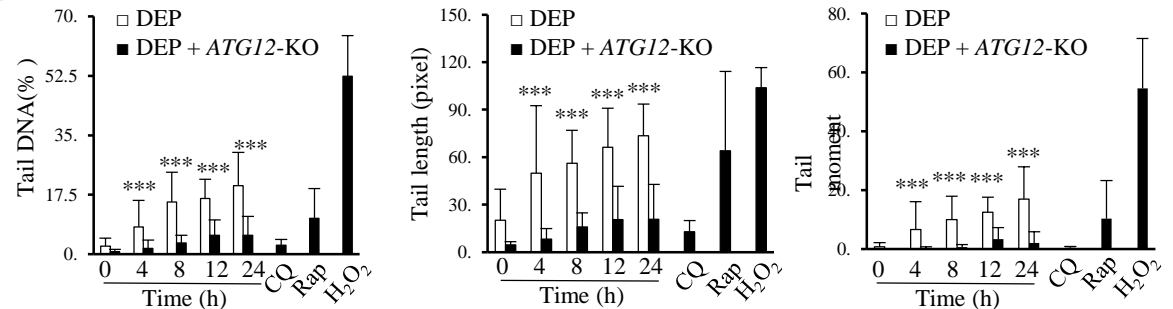
F



G

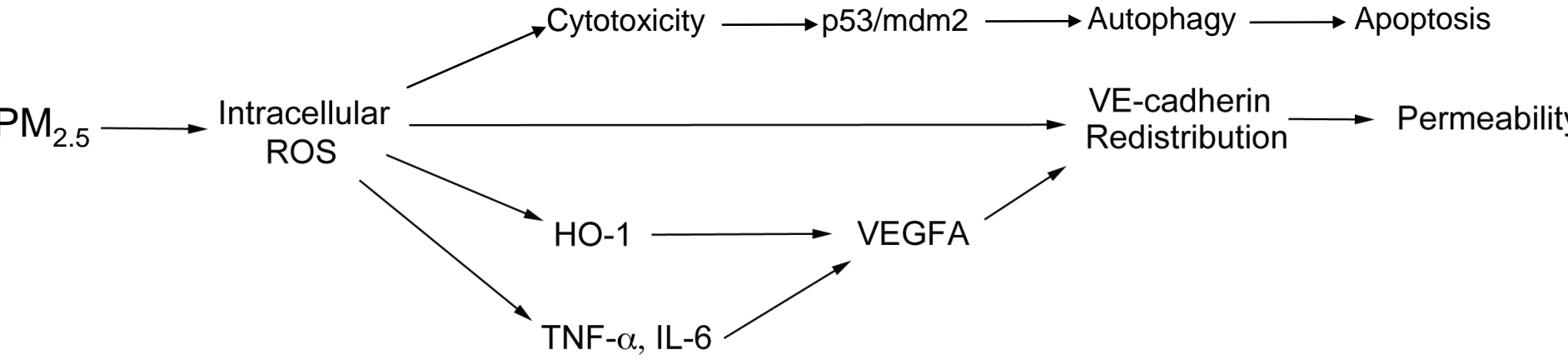


H



Wang et al, ToxSci, 2017

Summary




Tseng et al, Cardiovascular Toxicology, 2017







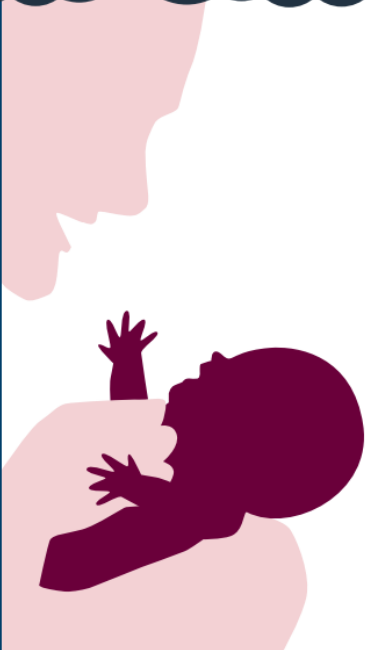
In the womb **Baby**

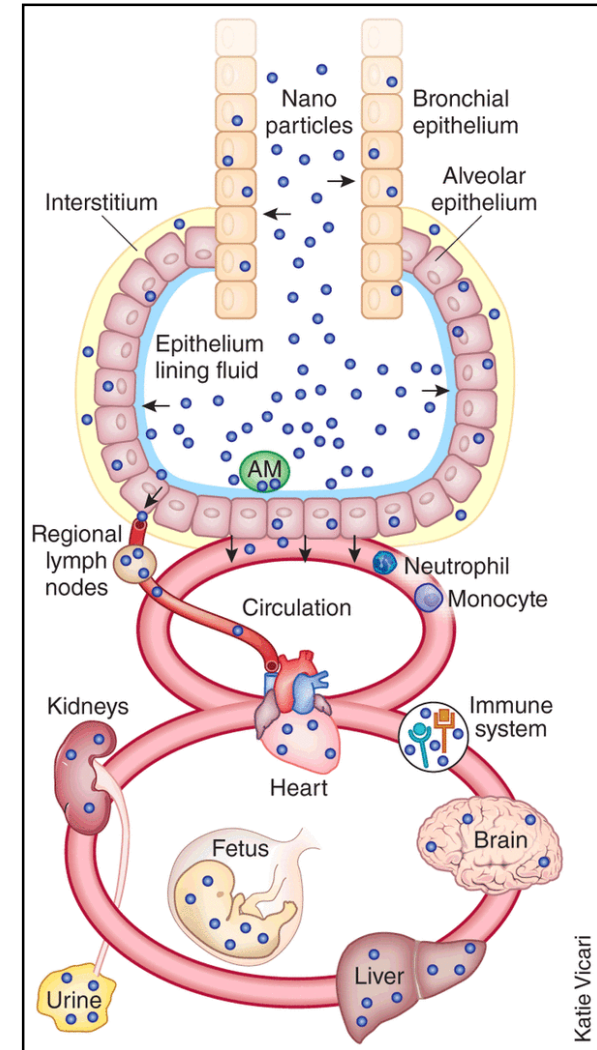
Outdoor pollution: vehicle exhaust, industrial emissions



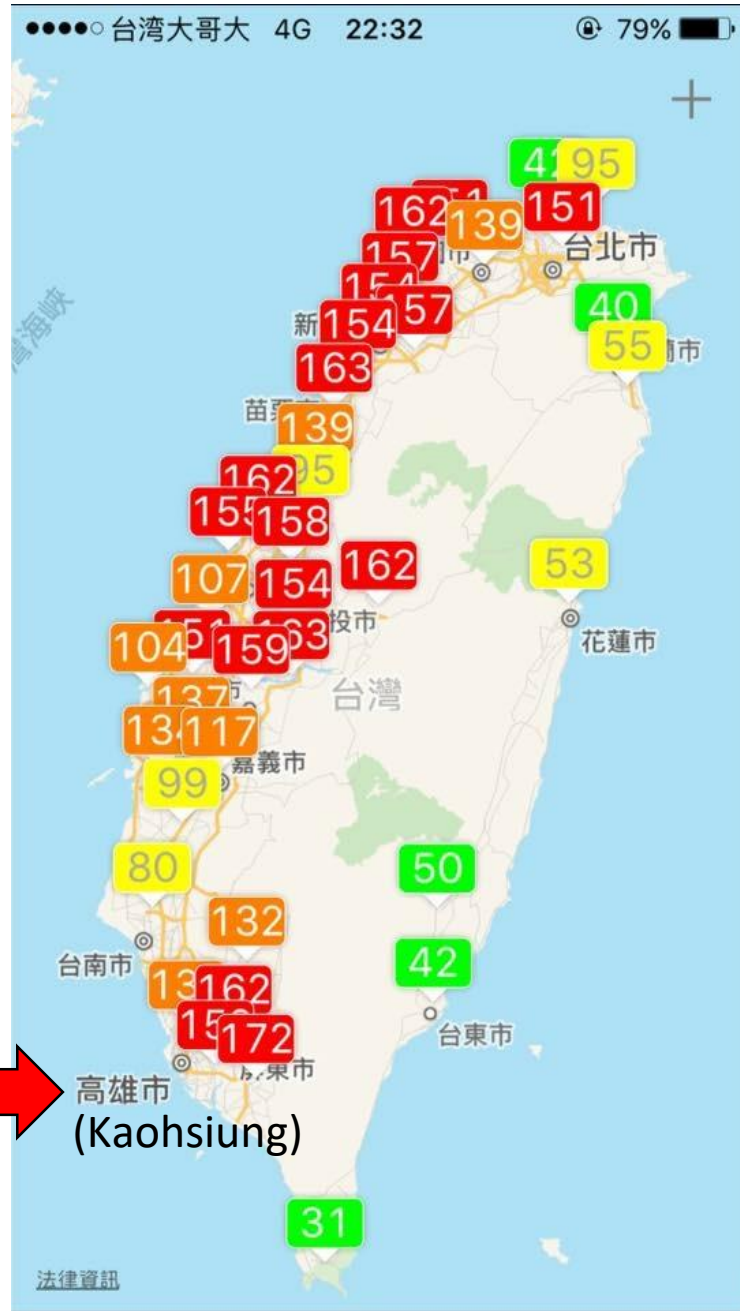
! Harms from high pollution

-  Low birth weight
-  Premature birth
-  Stillbirth
-  Organ damage



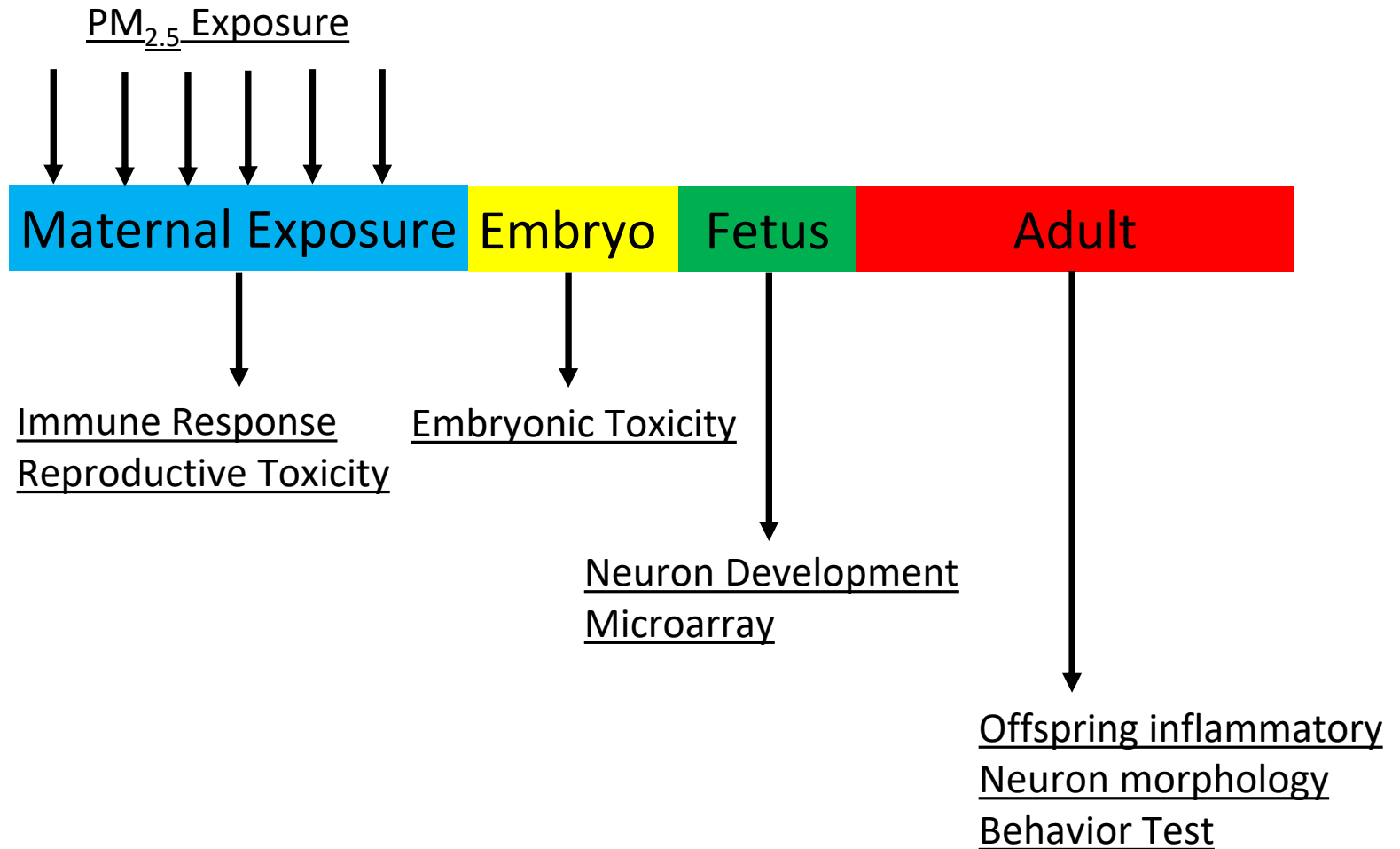


*Includes exhaust gases from cooking, heating and burning solid fuels, use of household cleaners and other chemicals, VOCs, etc

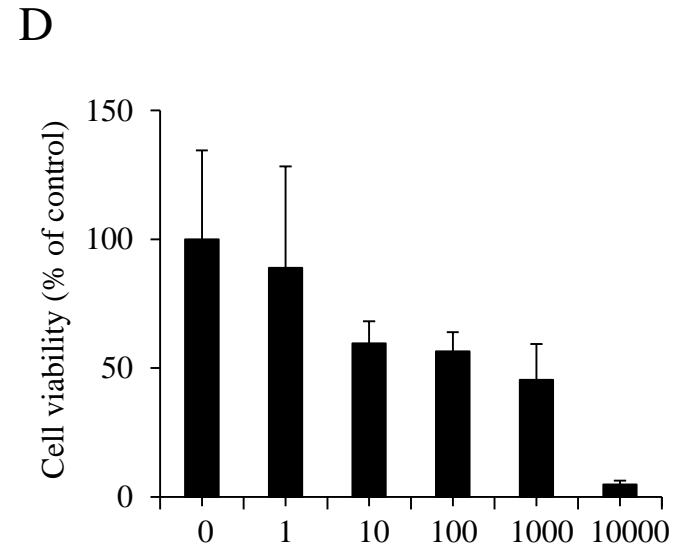
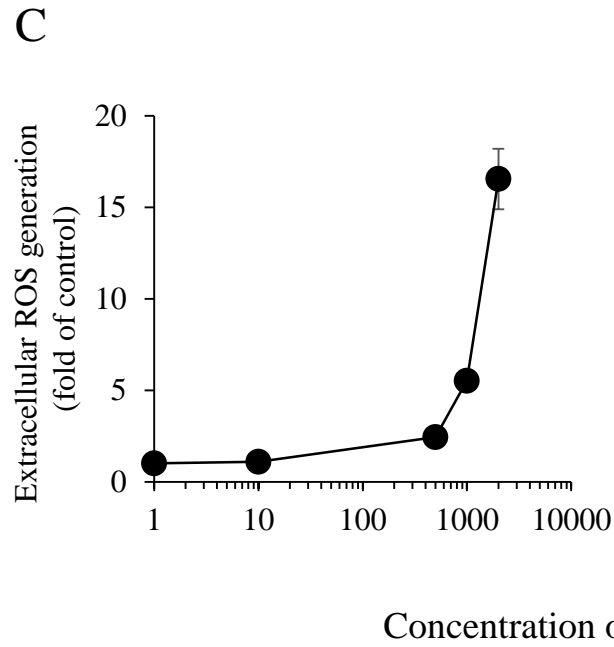
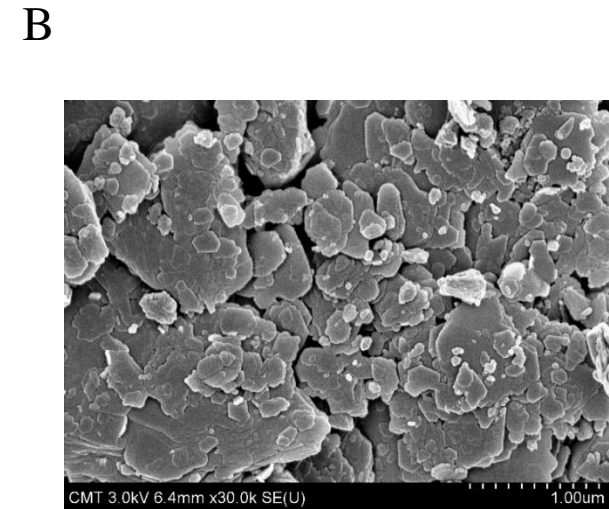
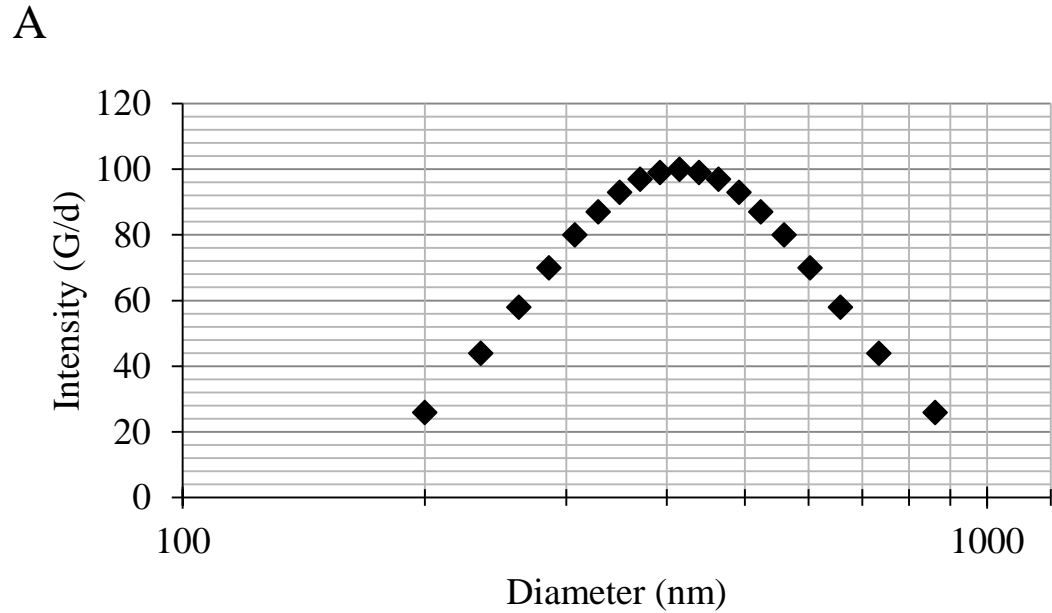



 高雄市
 (Kaohsiung)

In vivo model studies



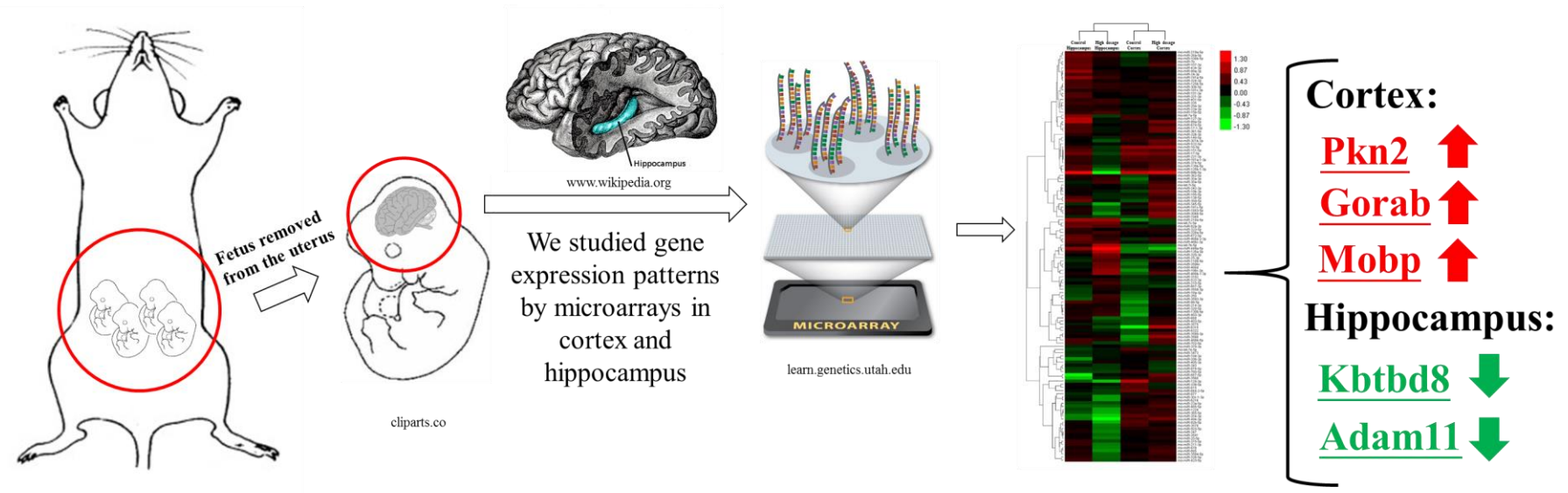
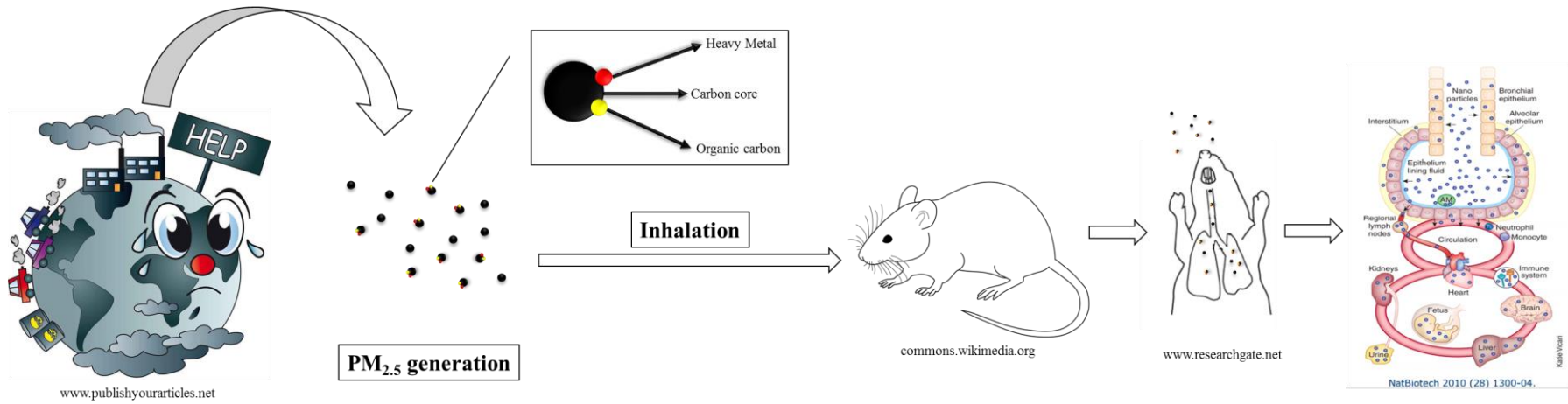
The PM_{2.5} size is about 400 nm



Concentration of PM_{2.5} (μg/mL)

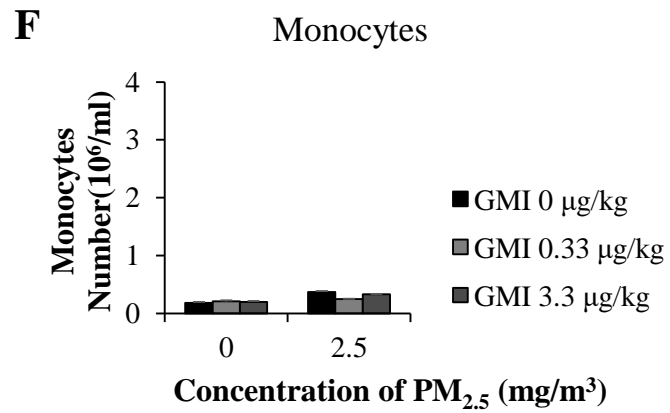
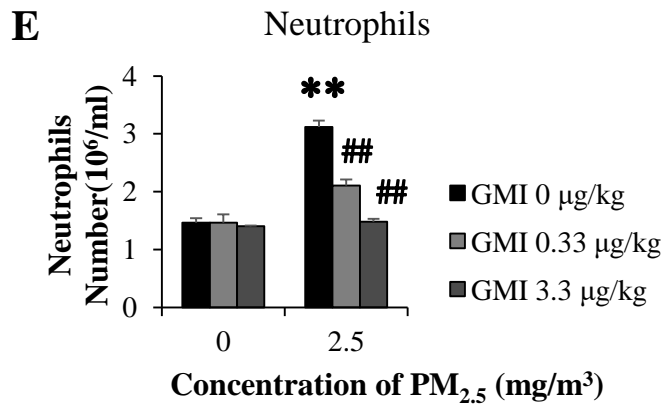
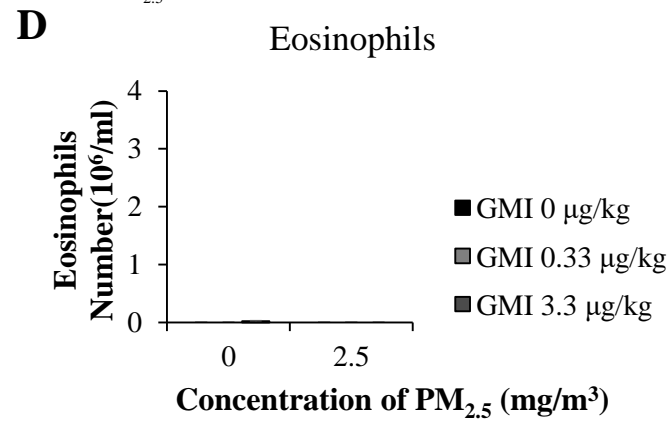
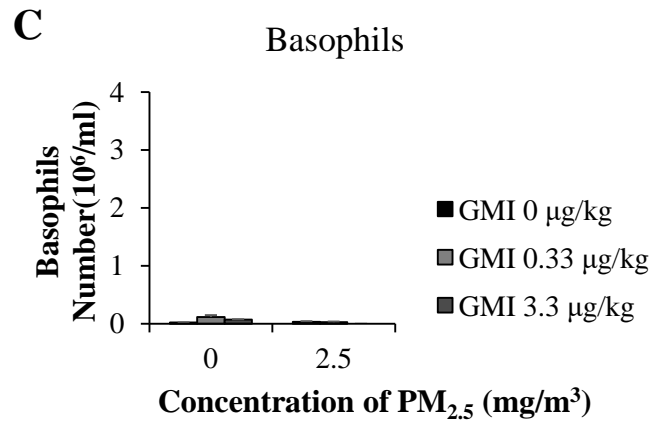
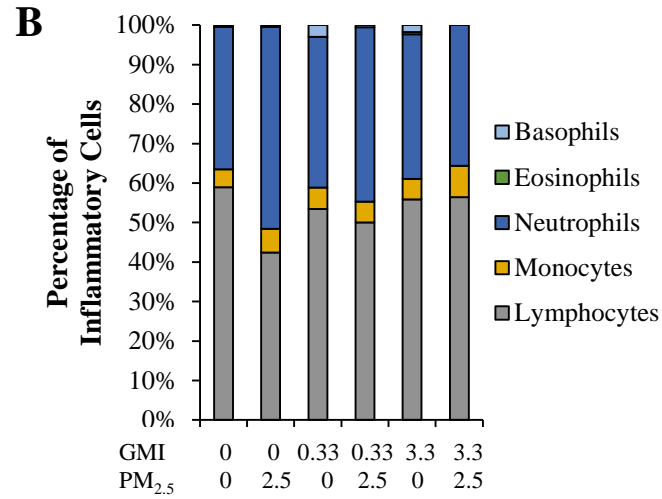
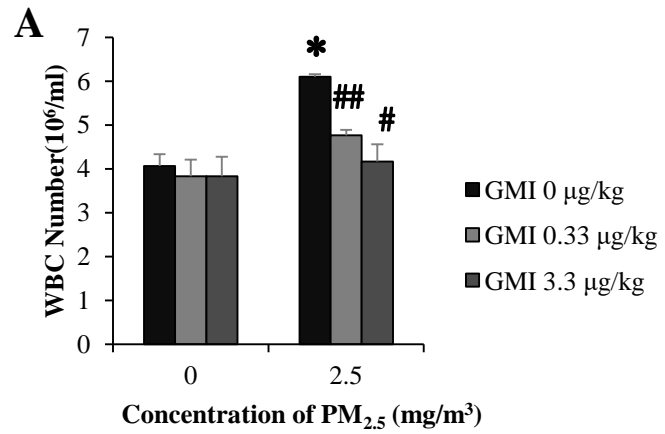
Tseng et al, Am J Chin Med, 2016

PM_{2.5} cause Fetal Brain Malformation



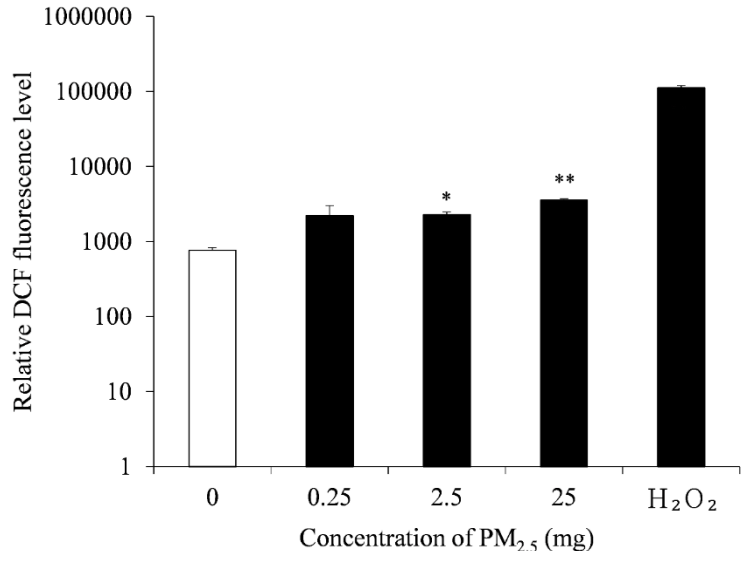
Global gene expression changes in fetal brain induced by PM_{2.5}.

PM_{2.5} induce maternal inflammation responses

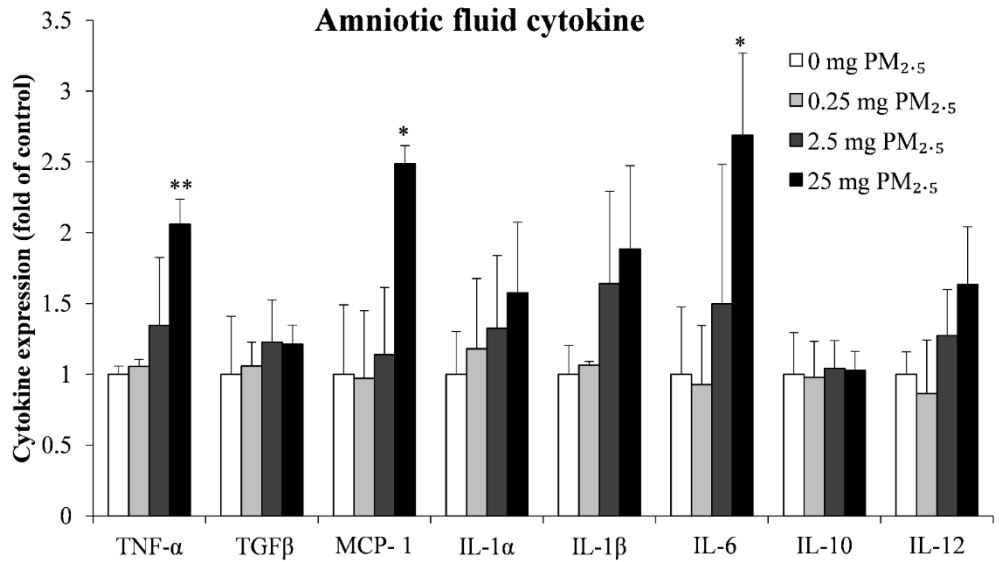


PM_{2.5} induce maternal inflammation responses

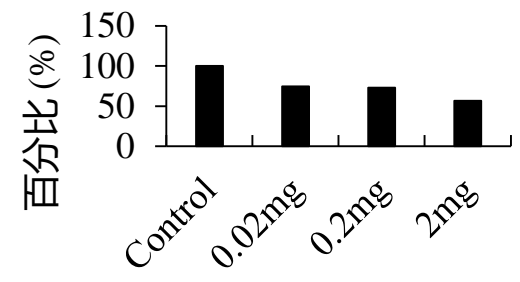
(a)



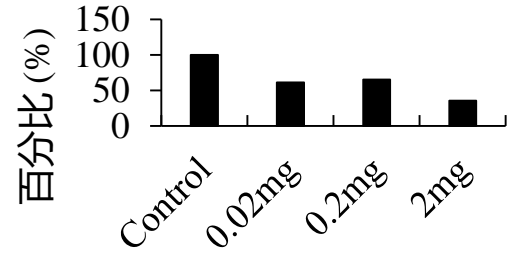
(b)



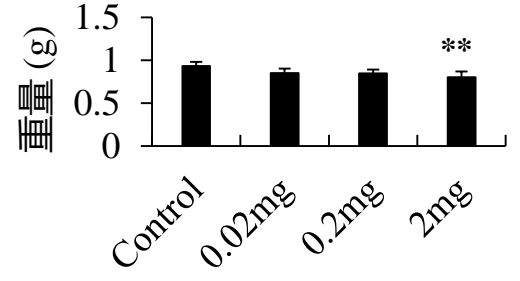
懷孕率



出生率

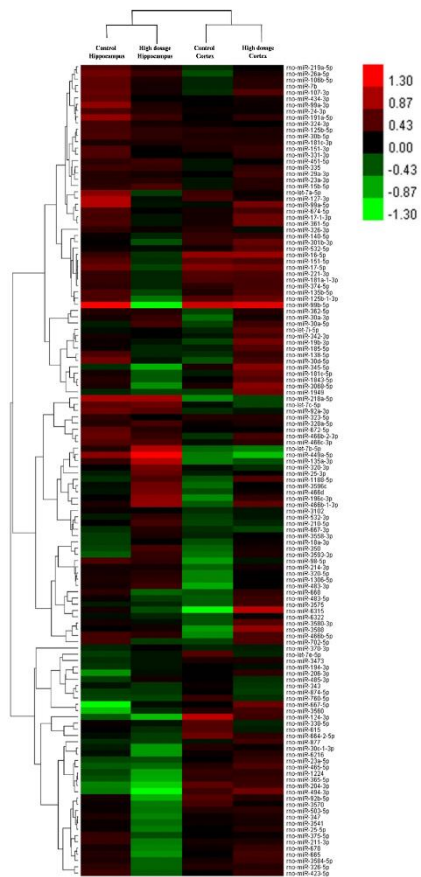


胎鼠體重



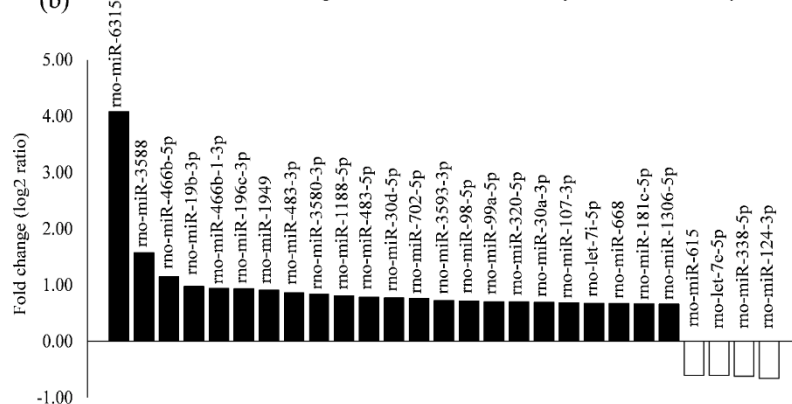
PM_{2.5} affect miRNAs in fetal brain

(a)



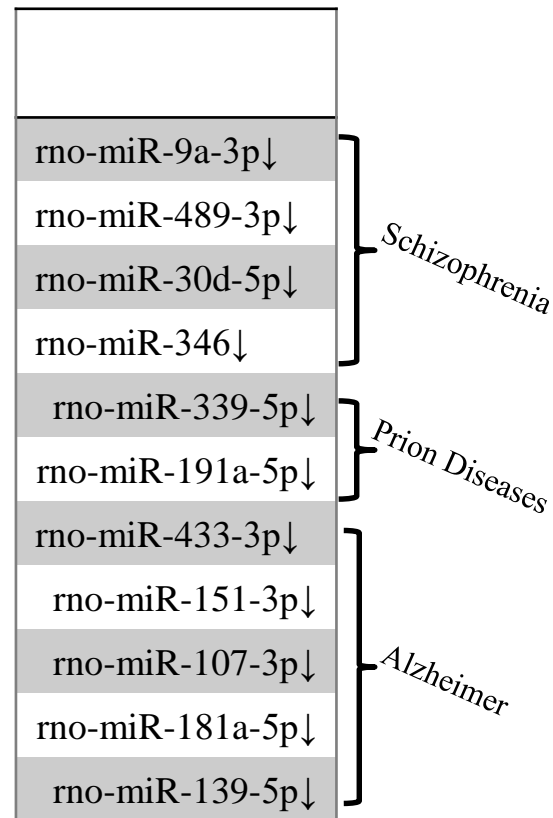
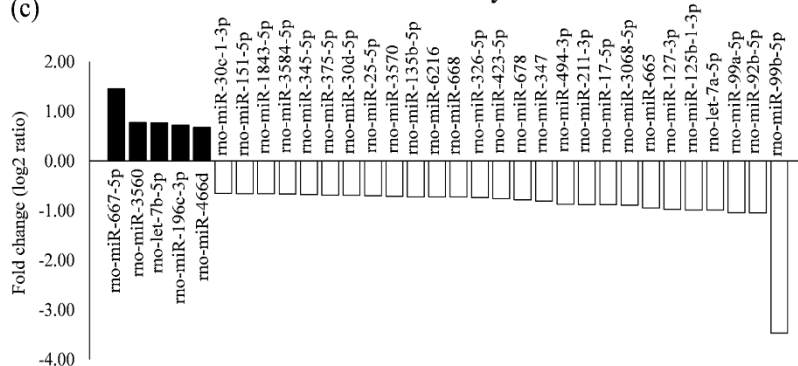
(b)

miRNA fold changes in fetus cortex detected by miRNA microarray

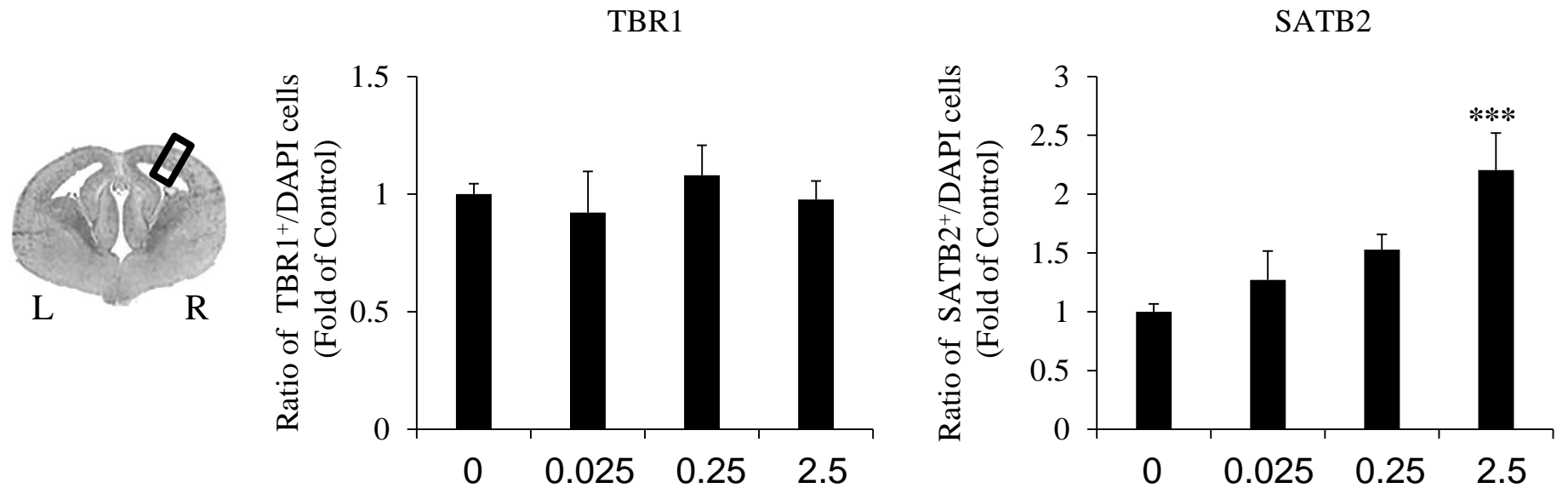
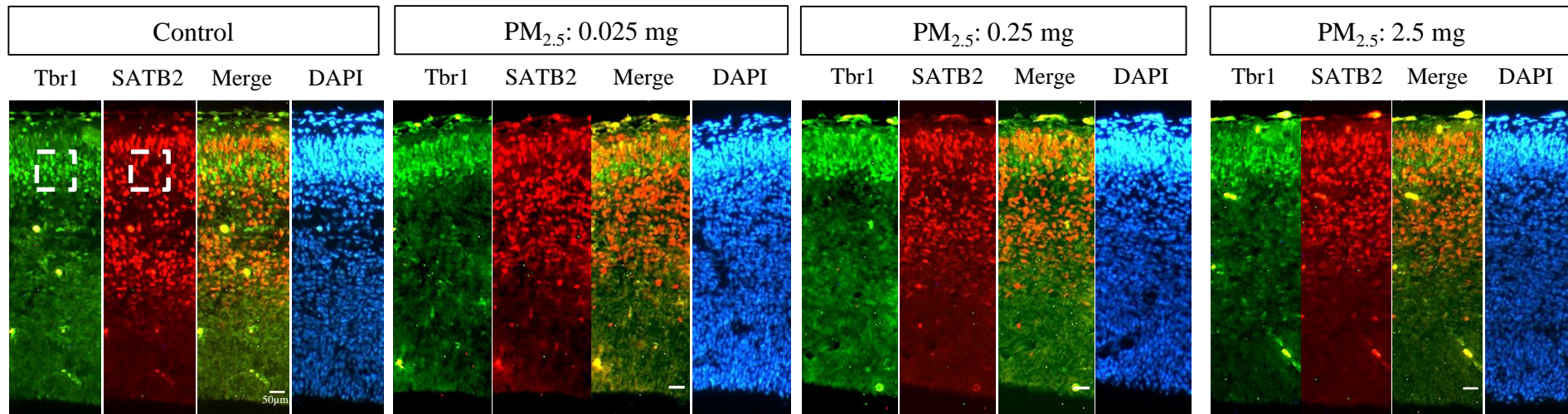


(c)

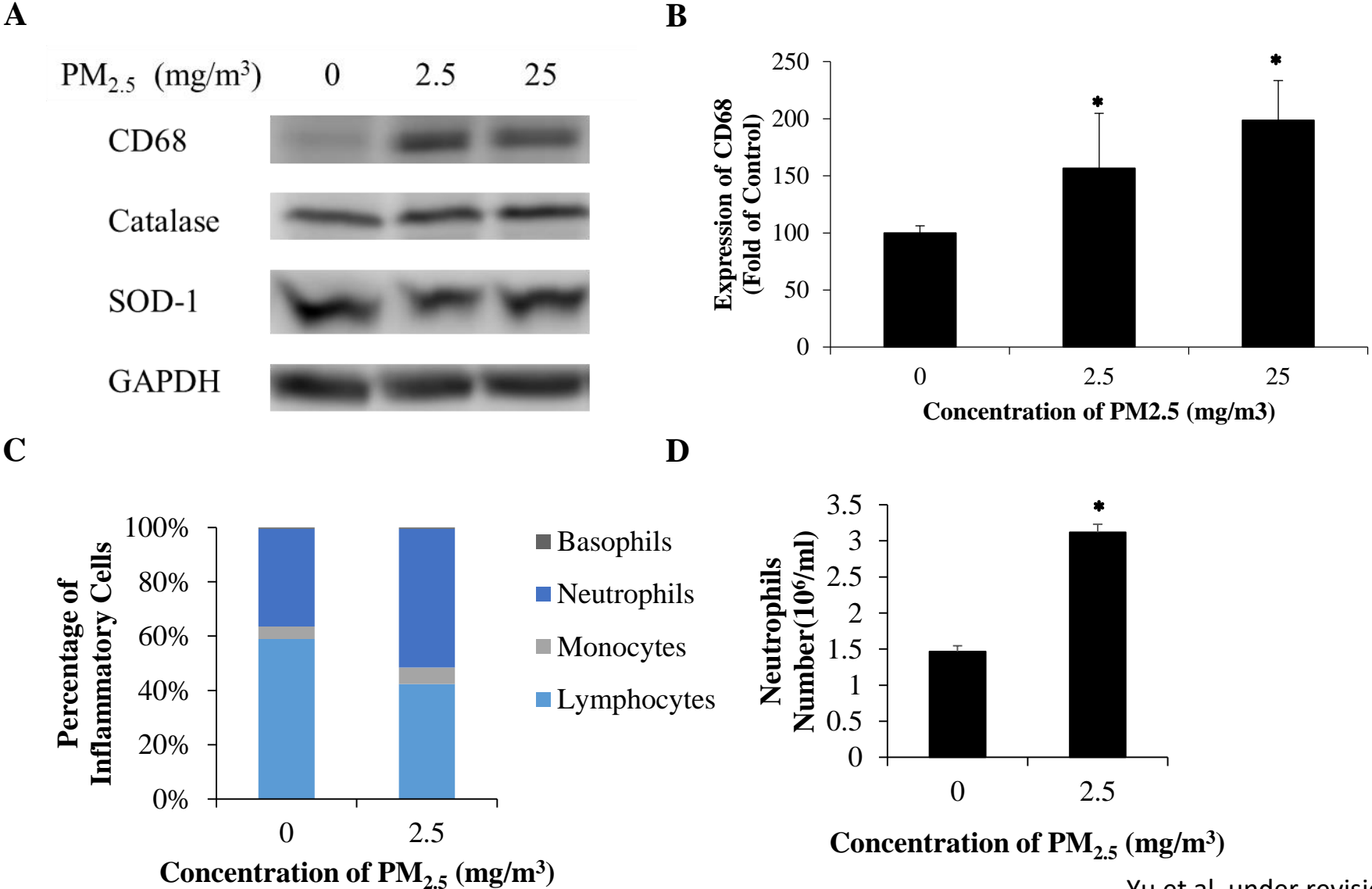
miRNA fold changes in fetus hippocampus detected by miRNA microarray



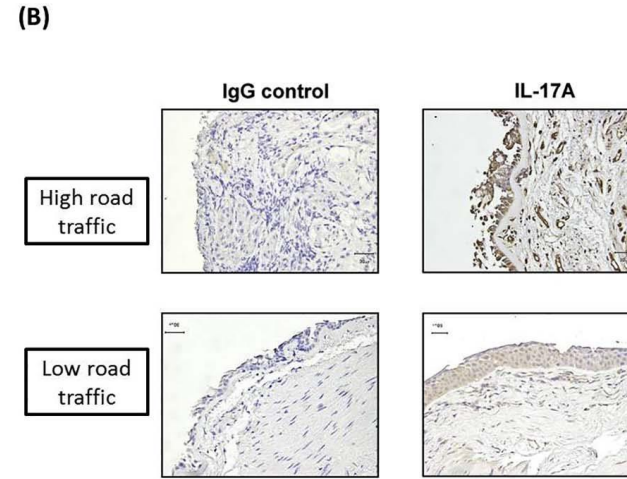
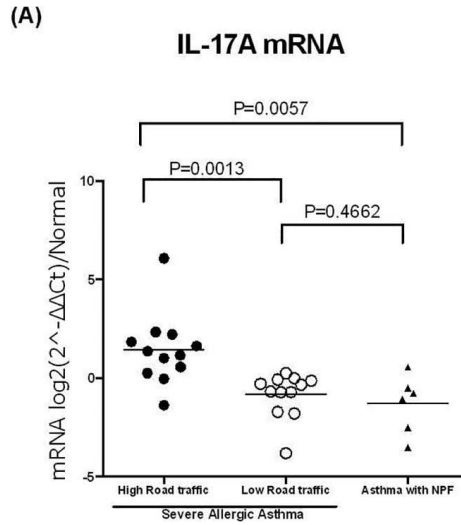
PM_{2.5} cased fetal brain cortex development delay



PM_{2.5} increase brain inflammation in the offspring

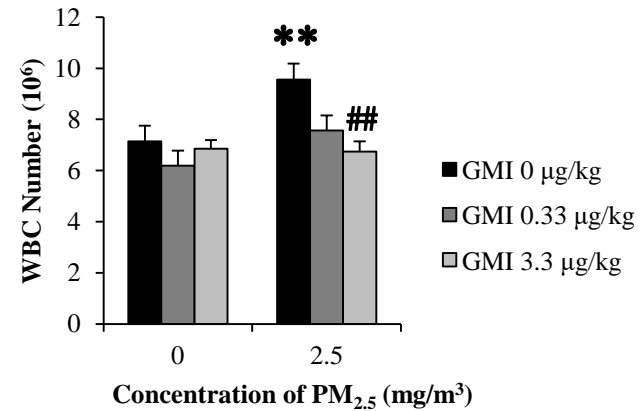


PM_{2.5} increase inflammatory & asthma ratio in offspring

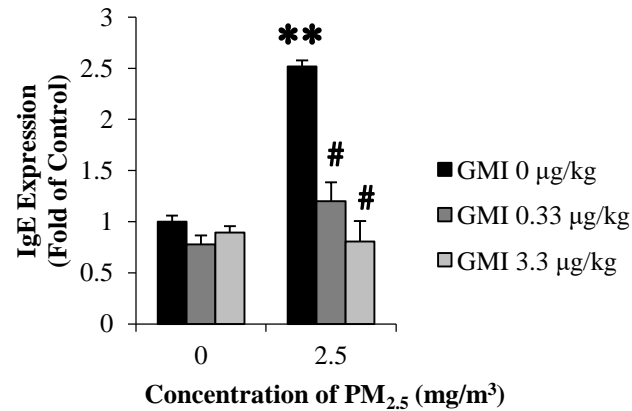


Weng et al, *Biochemical Pharmacology*, 2018

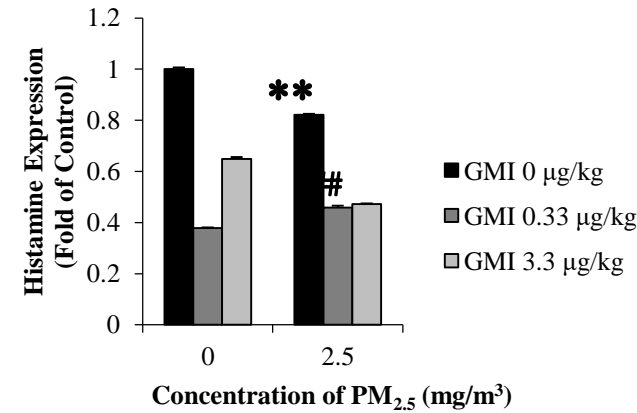
A



B

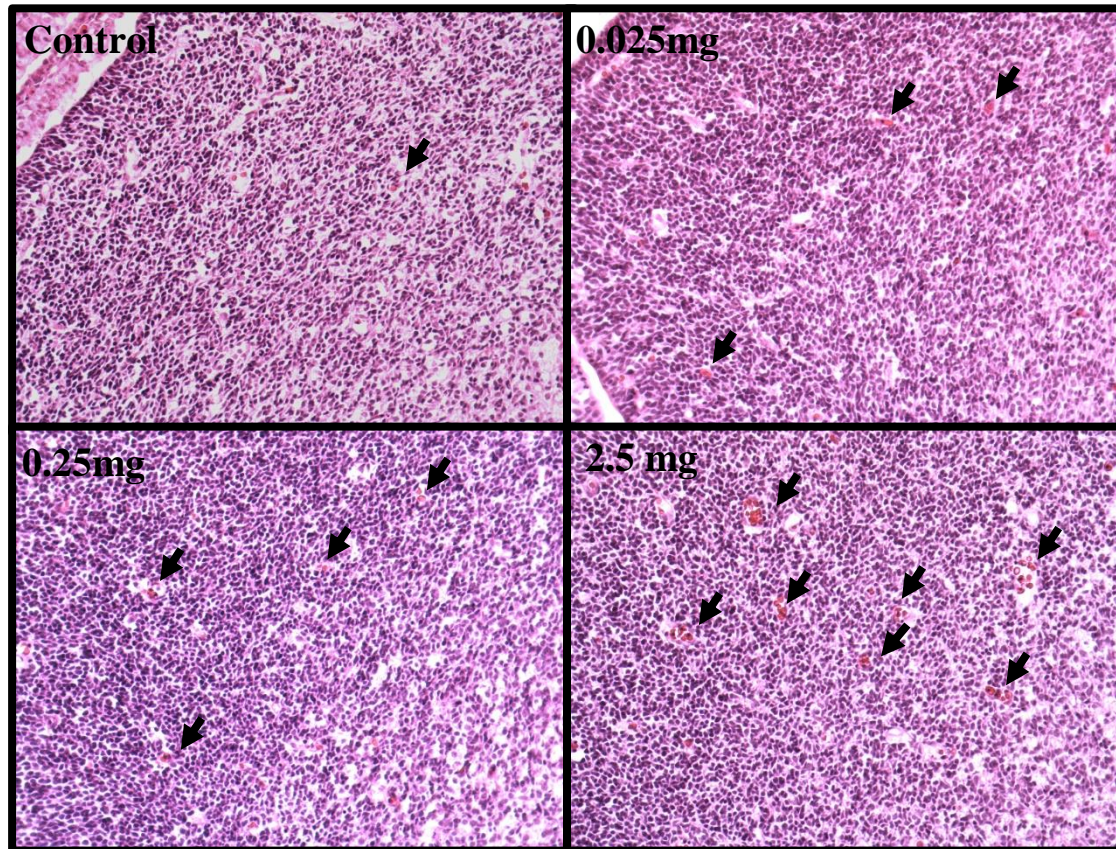
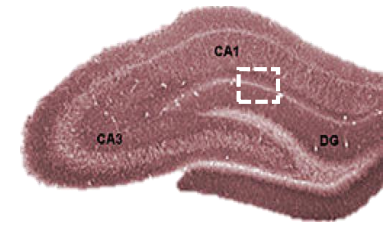


C

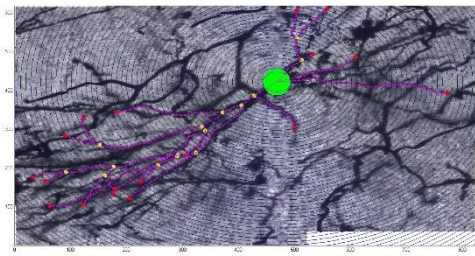


Yu et al, under revision

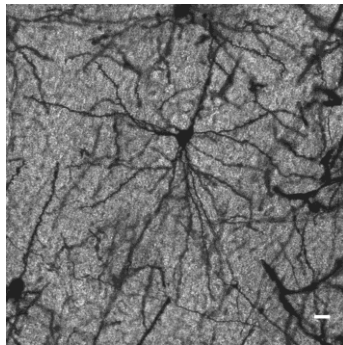
PM increases the amounts of blood vessels in the offspring dentate gyrus



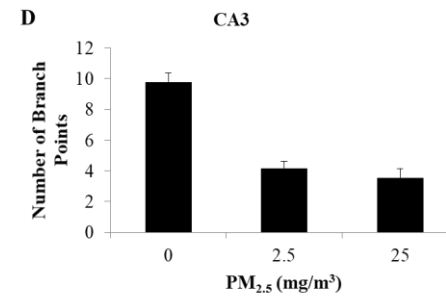
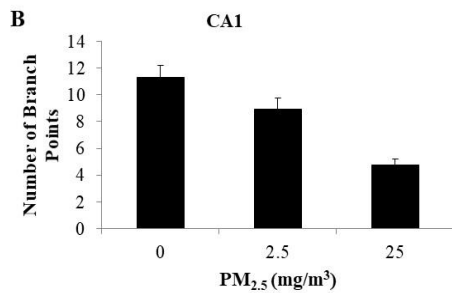
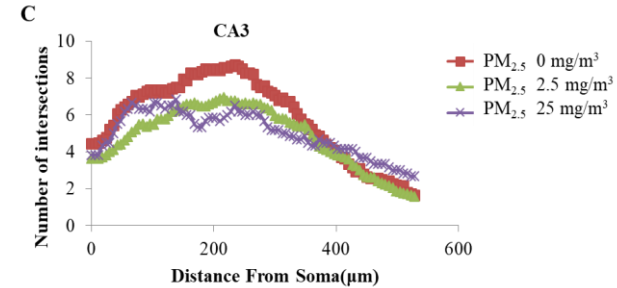
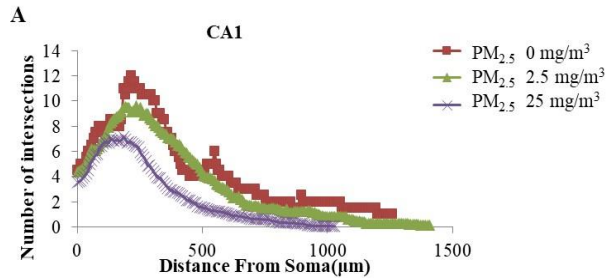
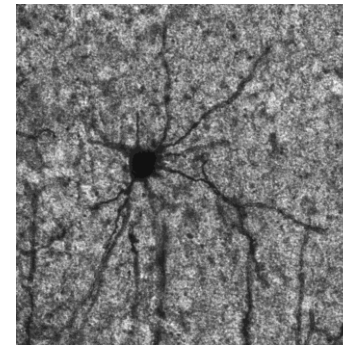
Neural cell morphology: spine spots decrease (MATLab Bonfire software analysis)



Control

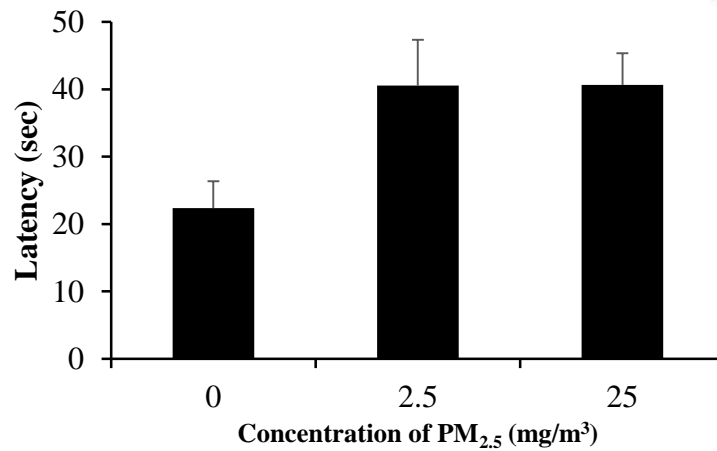
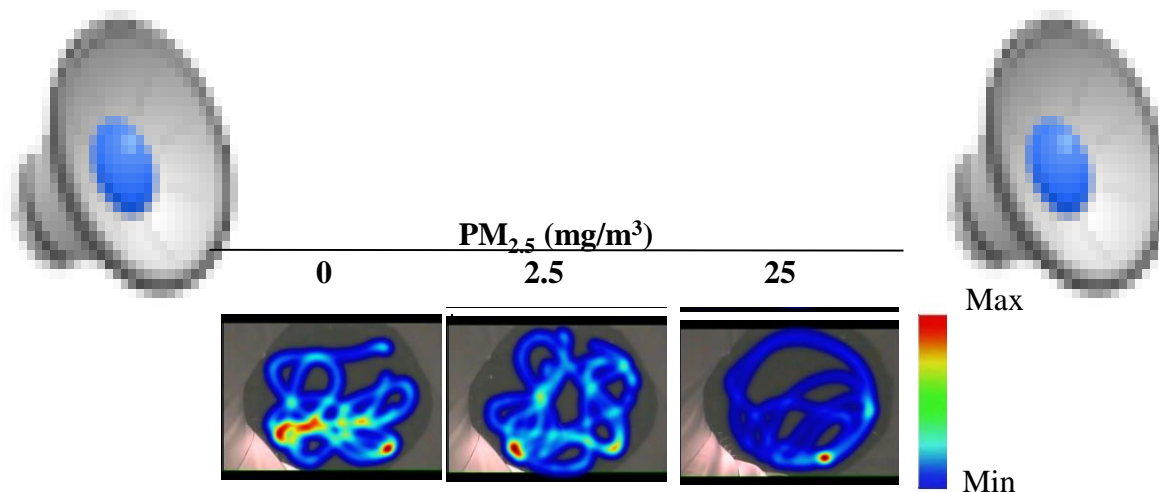


PM_{2.5}

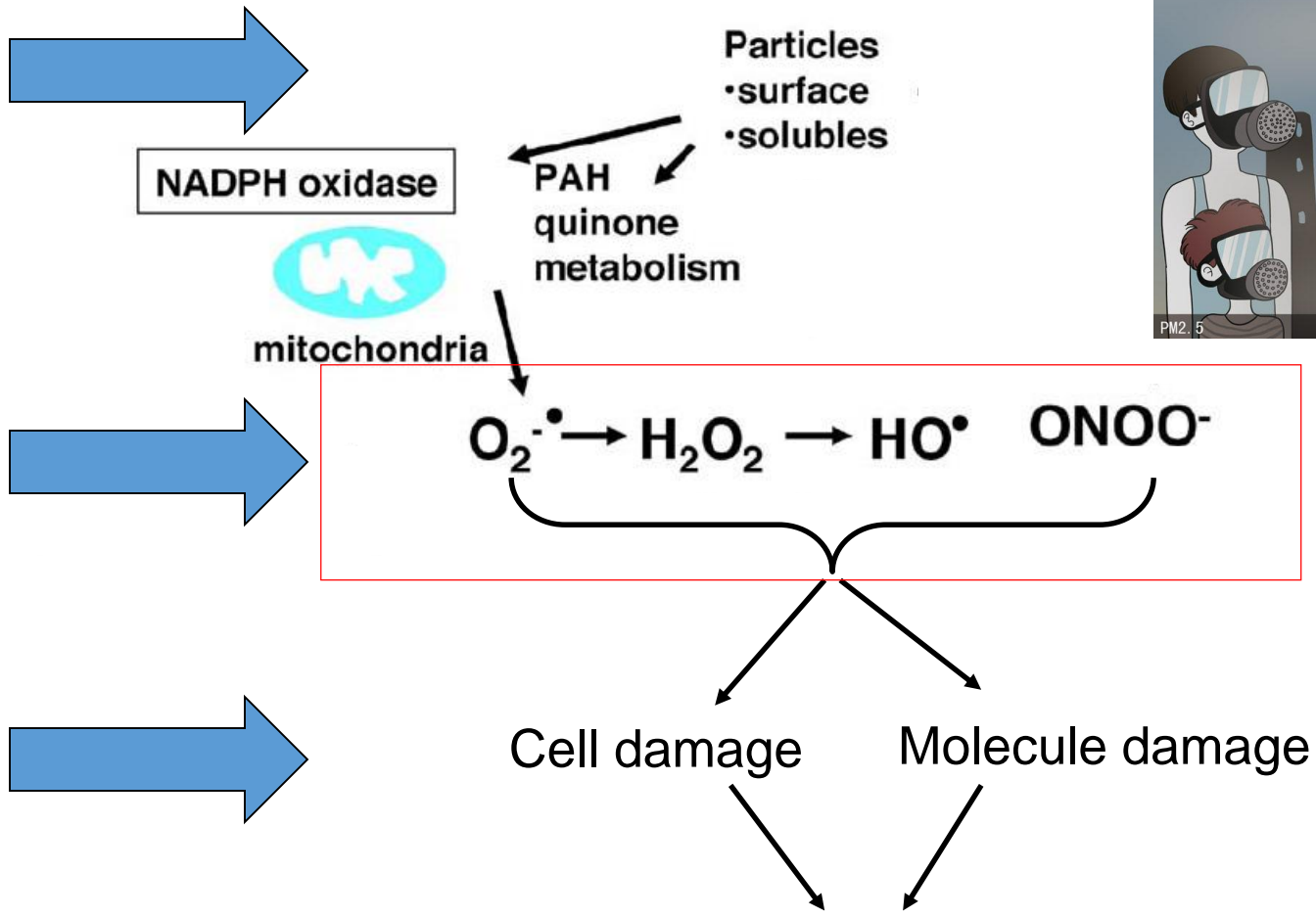


Behavior Test: 對照組

Behavior Test: PM_{2.5}組



What is the prevention against PM_{2.5}??

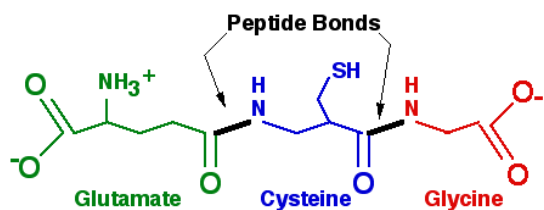
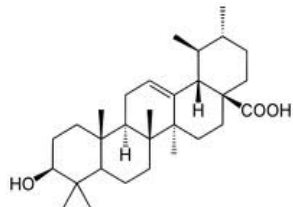


How to protect ?

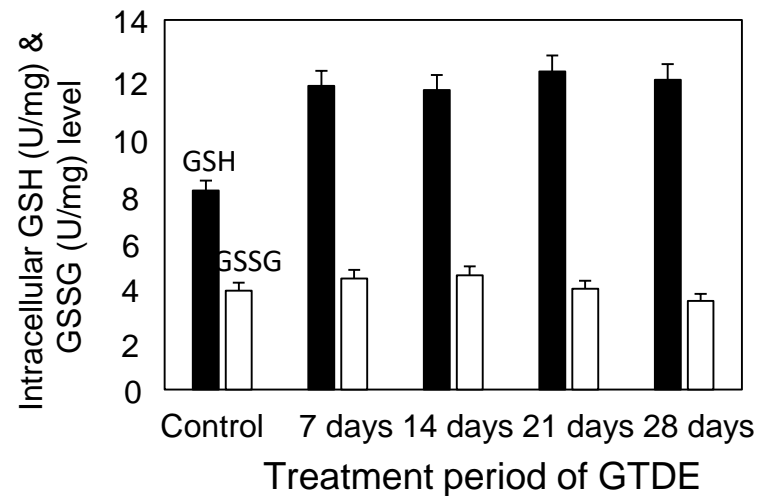
Is that going through by anti-oxidative stress?

Upregulation of endogenous GSH arises protection against ROS

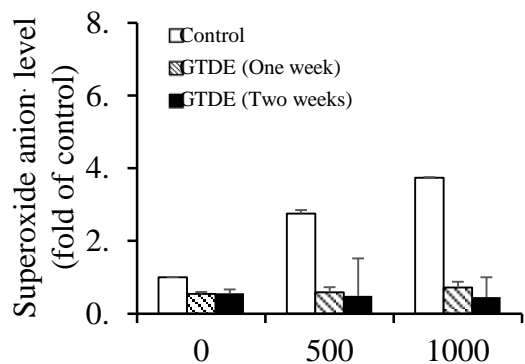
A



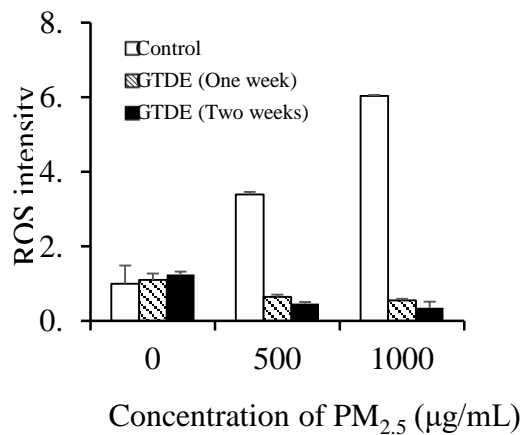
B



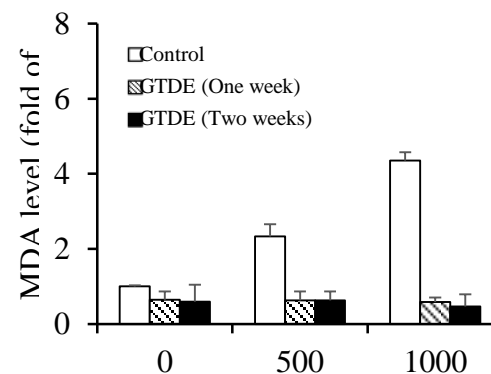
C



D

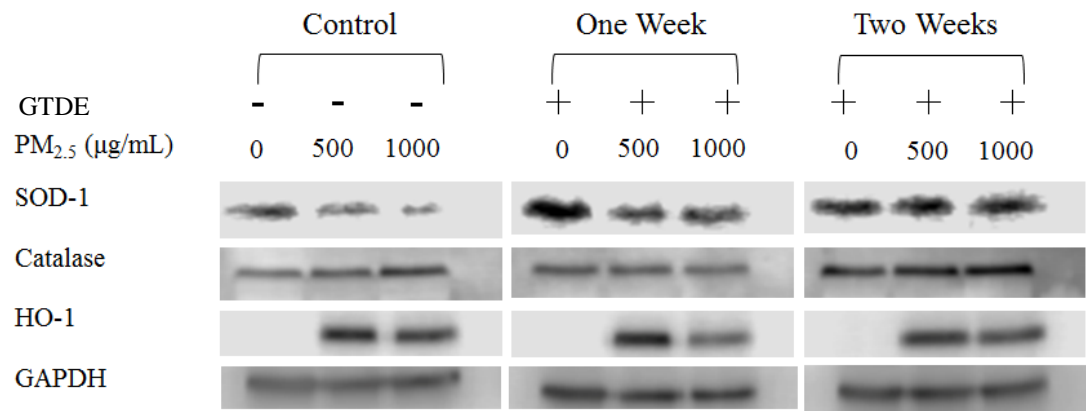


E

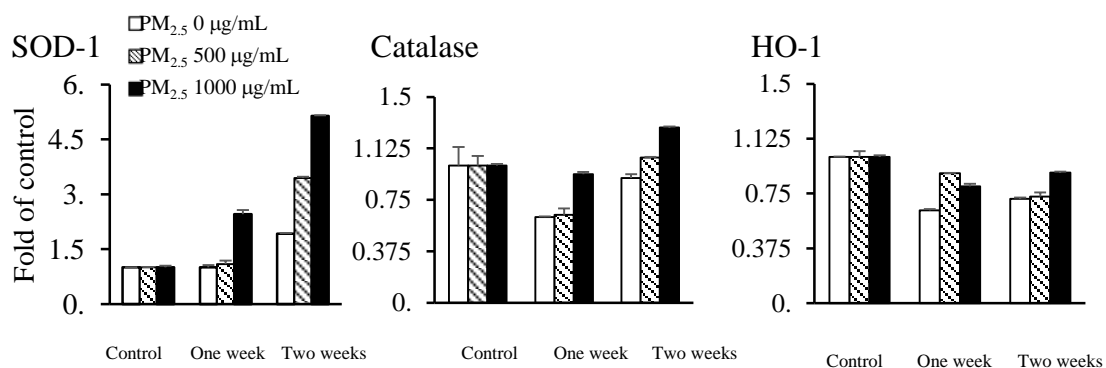


G. tsugae blocks ROS generation and its sequential DNA damages

A

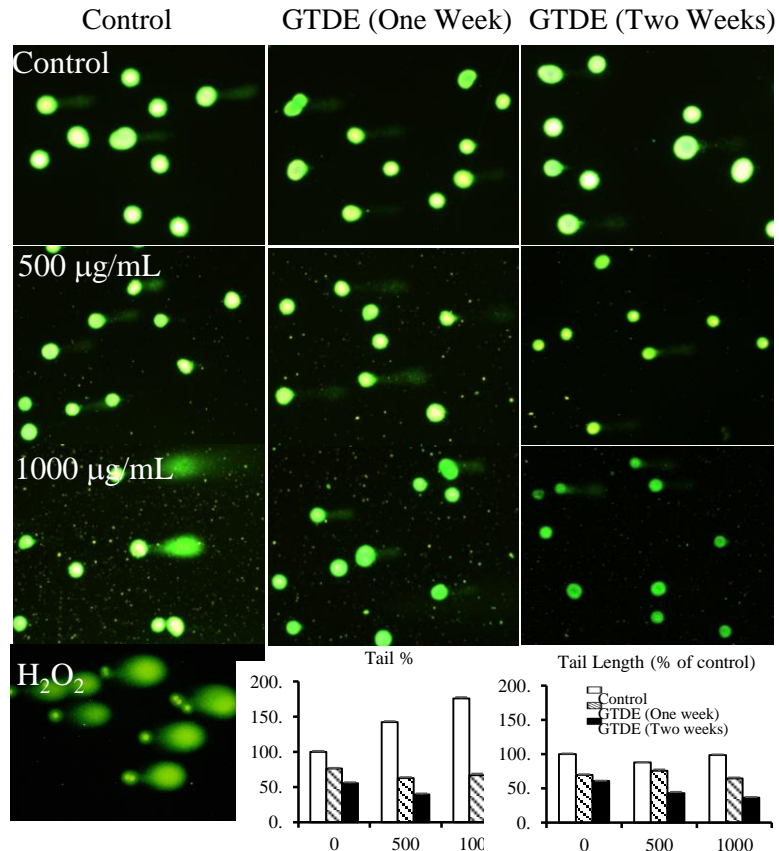


B

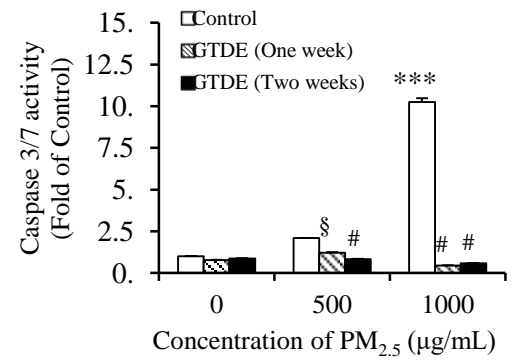


Tseng et al, Am J Chin Med, 2016

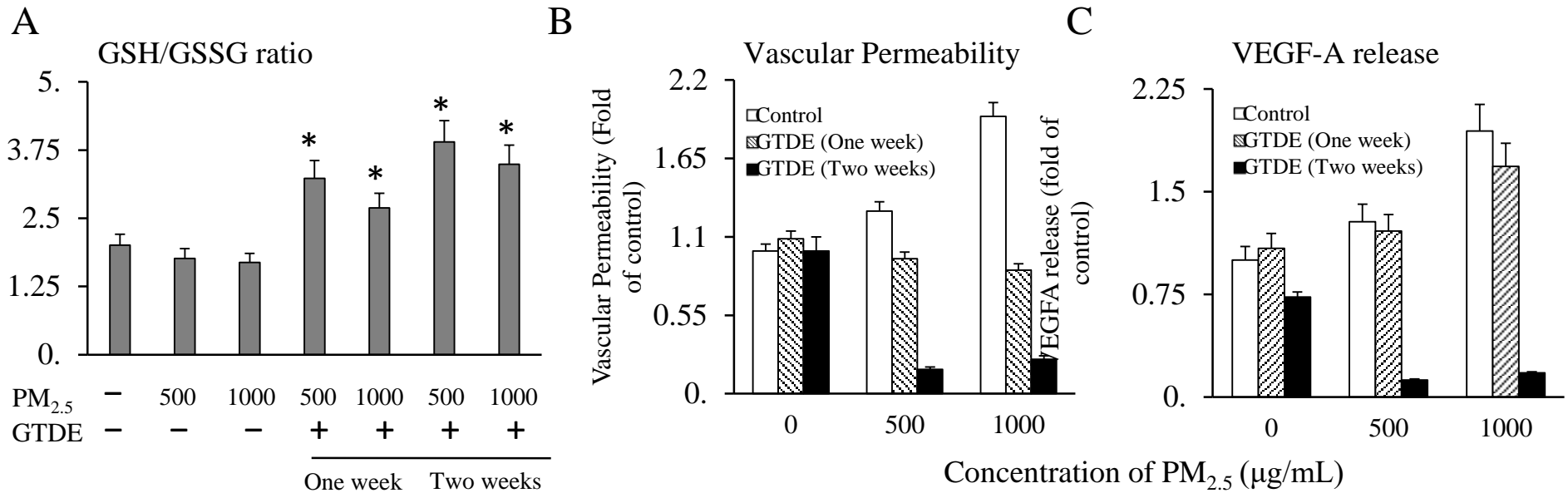
C



D



GTDE restores PM_{2.5}-induced vascular permeability

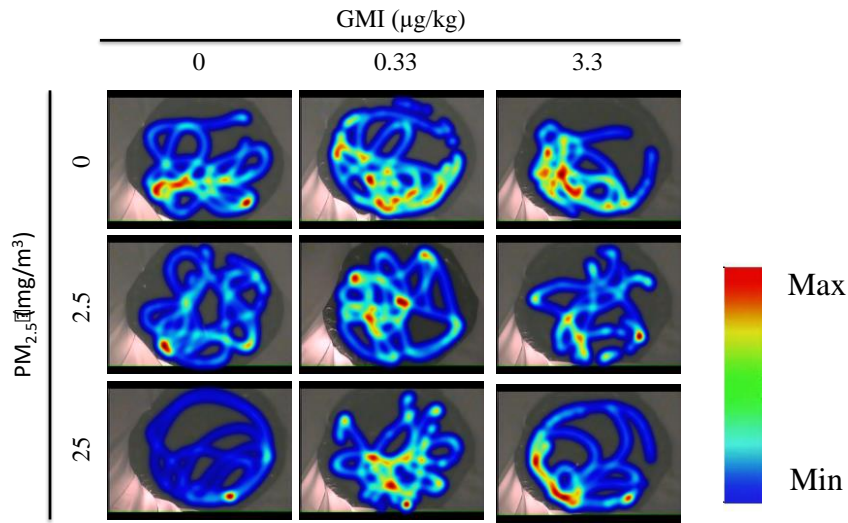
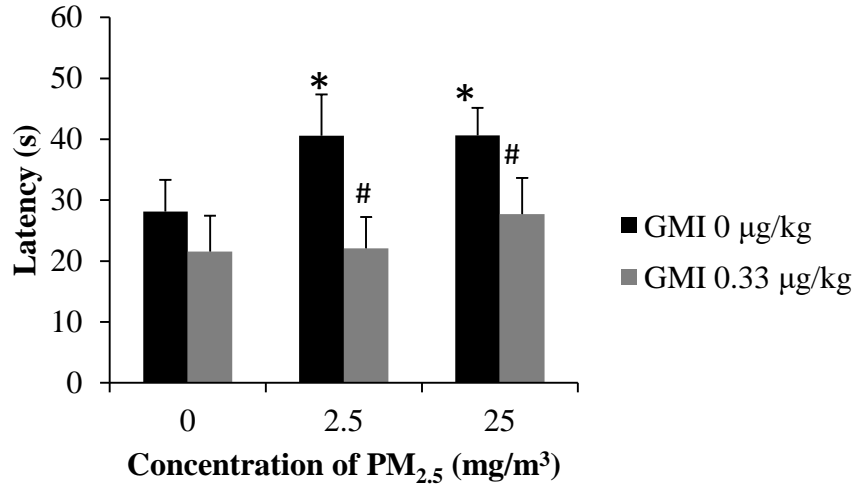


Tseng et al, Am J Chin Med, 2016

Table. In vivo physiological conditions in samples treated 2 weeks GTDE and PM_{2.5}

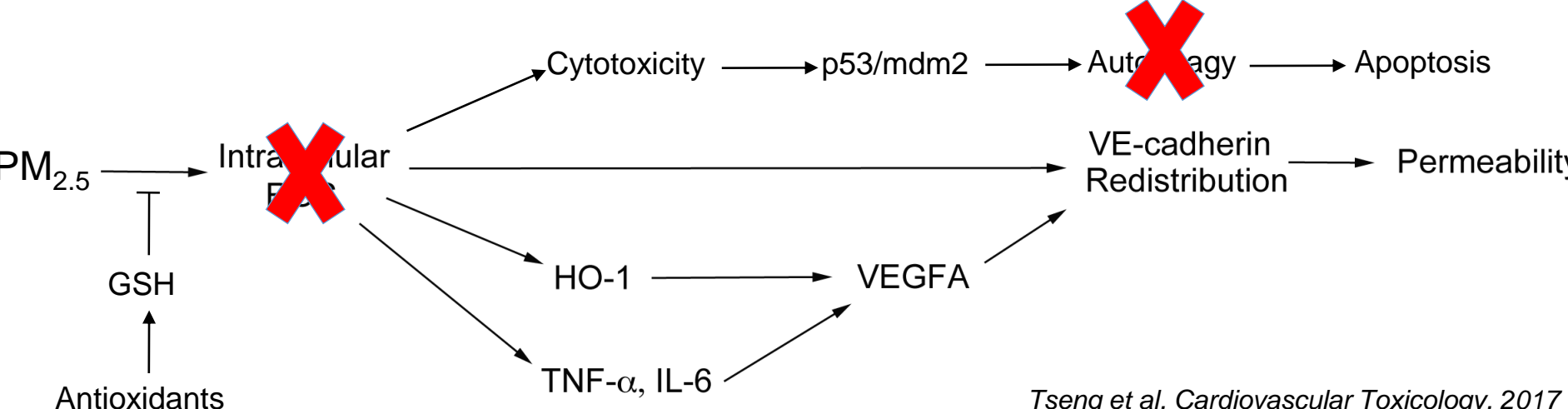
PM _{2.5} (mg/m ³)	Body Weight (g)	Hemoglobin (g/dL)	White Blood Cell (cells/mL)	Red Blood Cell (cells/mL)
0	272.2	17.83	1.17E+07	7.58E+09
0.2	294.8	18.67	1.20E+07	8.62E+09
2	305.5	16.67	2.32E+07	5.53E+09
20	268.8	19.67	3.01E+07 [#]	1.83E+09 [#]
0+GTDE	268.1	19.69	1.06E+07	6.54E+09
0.25+GTDE	259.4	22.67	8.13E+06	5.23E+09
2.5+GTDE	274.2	20.33	7.13E+06	7.20E+09
25+GTDE	292.4	19.5	9.85E+06 ^{**}	5.80E+09 [*]

記憶測試：靈芝增加子代老鼠的長期記憶能力

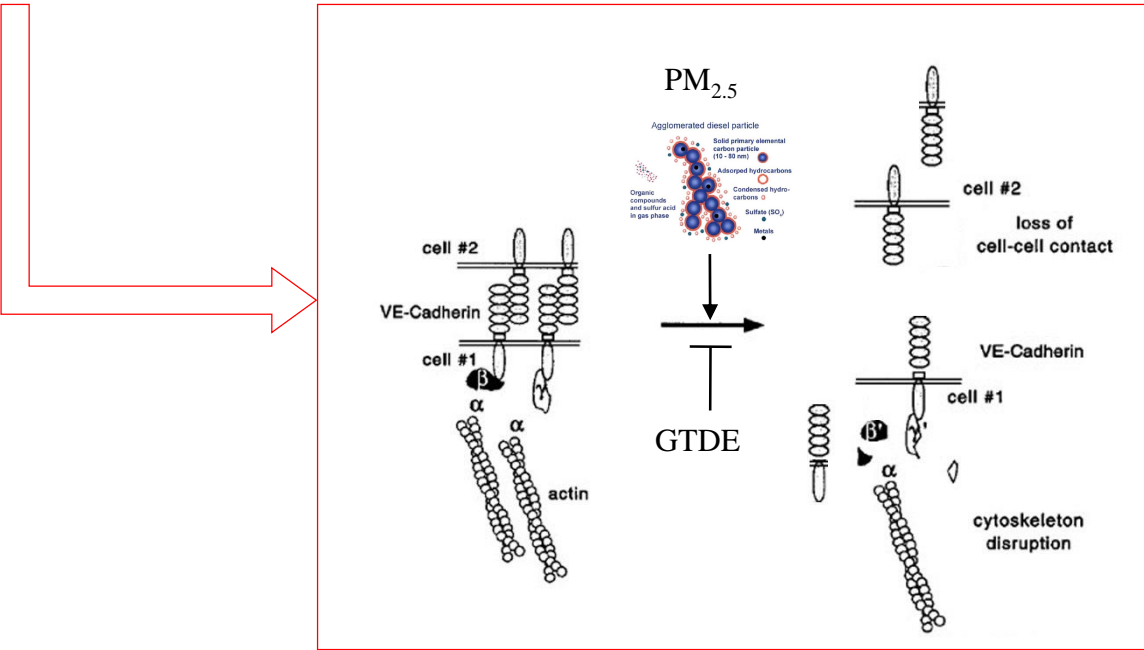


rno-miR-9a-3p↓	rno-miR-9a-3p↓	Schizophrenia
rno-miR-489-3p↓		
rno-miR-30d-5p↓		
rno-miR-346↓		
rno-miR-339-5p↓		Prion Diseases
rno-miR-191a-5p↓		
rno-miR-433-3p↓		Alzheimer
rno-miR-151-3p↓		
rno-miR-107-3p↓		
rno-miR-181a-5p↓		
rno-miR-139-5p↓		

Summary



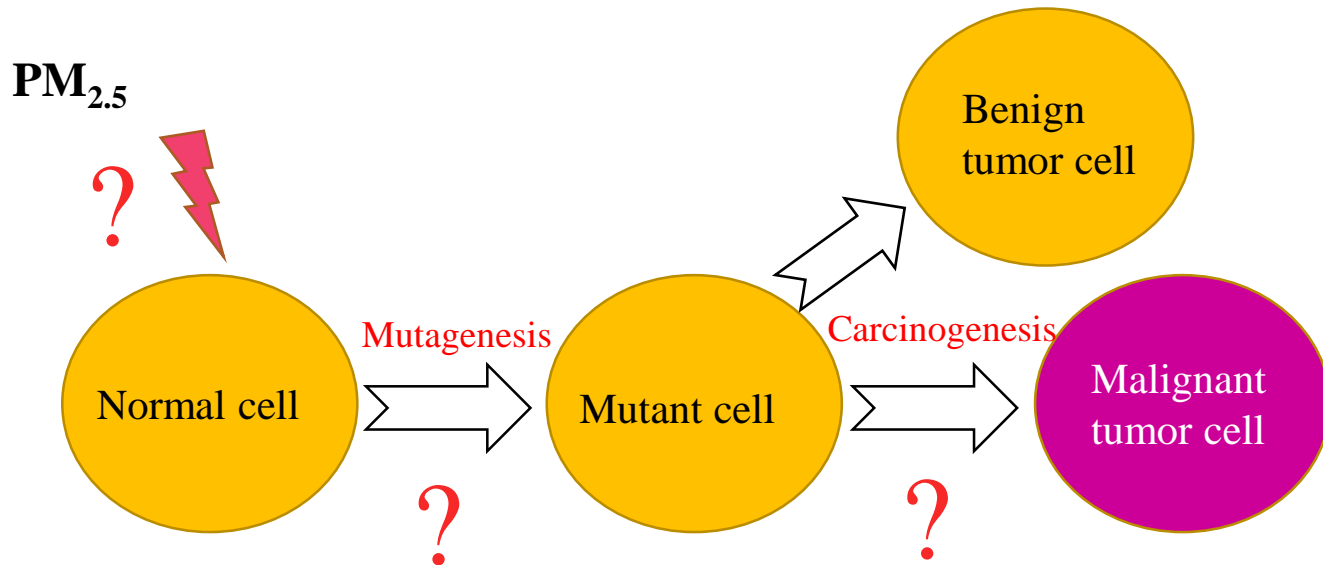
Tseng et al, Cardiovascular Toxicology, 2017



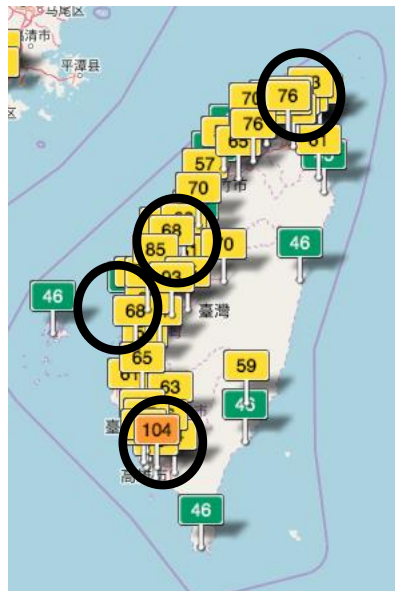
Tseng et al, Am J Chin Med, 2016

Aim:

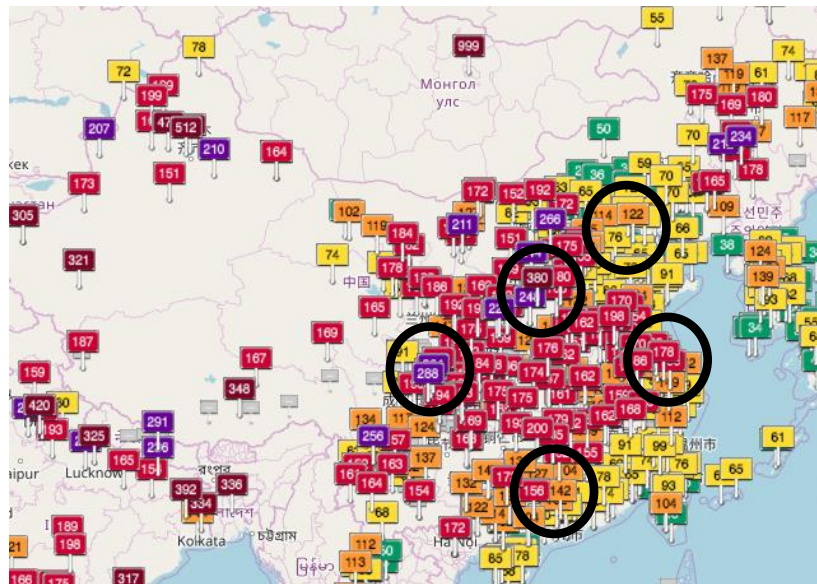
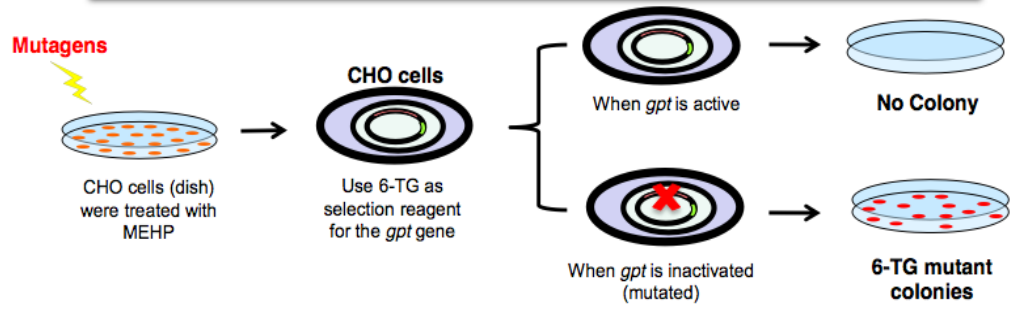
Chronic Exposure to PM_{2.5} or Its Major Component Cause Genotoxicity, Mutagenesis and Carcinogenicity in Mammalian Cells



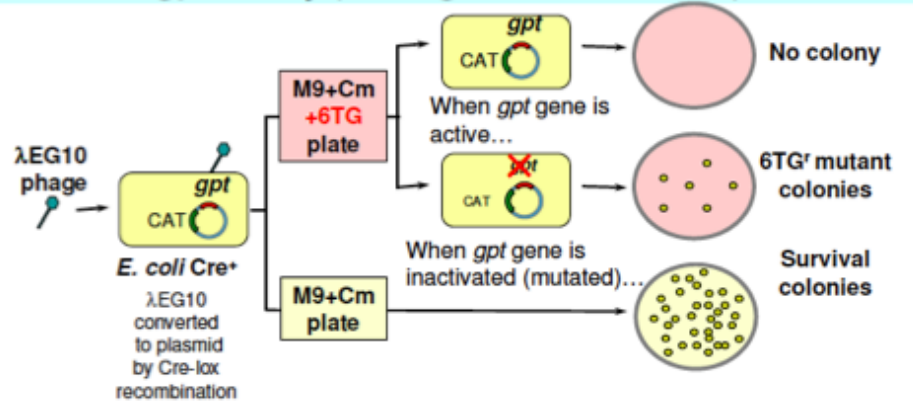
The impact of Chinese PM_{2.5} in mutagenesis and carcinogenesis



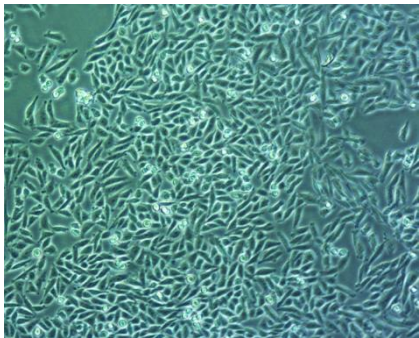
gpt Assay (6-Thioguanine selection) for mutagenicity



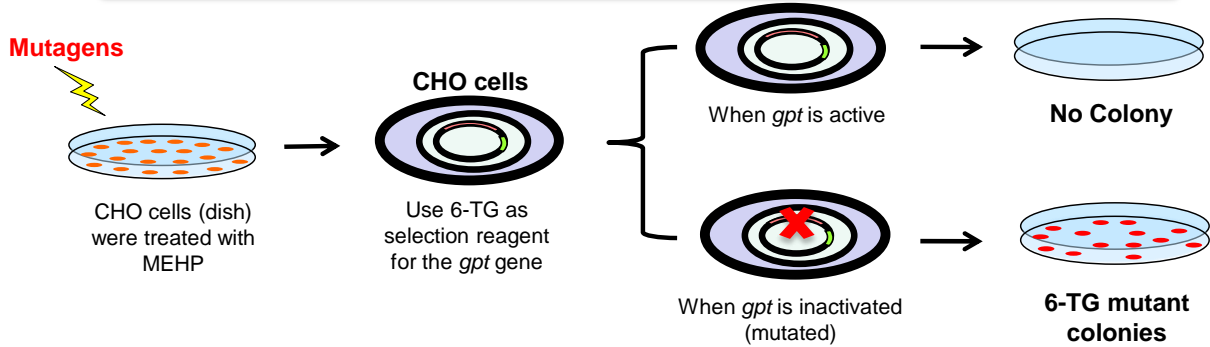
gpt Assay (6-thioguanine Selection)



In Vitro model: *gpt*-transgenic CHO AS52 cell line with 6-TG selection



gpt Assay (6-Thioguanine selection) for mutagenicity



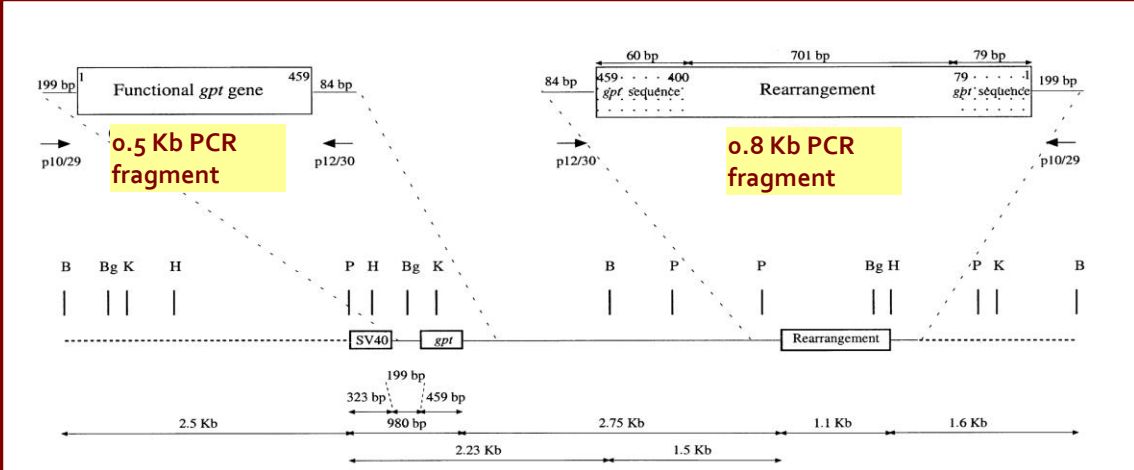
Mutagens: AFB1, TCDD, PAH, BPA, PM_{2.5}, Aniline, Aminophenol, DEHP...

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Food and Chemical Toxicology
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Cytoplasmic and nuclear toxicity of 3,5-dimethylaminophenol and potential protection by selenocompounds
 Pinar Erkekoglu^{a,b,*}, Ming-Wei Chao^{a,c,1}, Wenjie Ye^a, Jing Ge^a, Laura J. Trudel^a, Paul L. Skipper^a, Belma Kocer-Gumuslu^b, Bevin P. Engelward^a, Gerald N. Wogan^a, Steven R. Tannenbaum^a

Research Article
 Received 12 May 2014, Revised 6 June 2014, Accepted 11 June 2014, Published online 19 July 2014
 www.elsevier.com/locate/jfct
 DOI 10.1002/jfct.3046
Protective effects of ascorbic acid against the genetic and epigenetic alterations induced by 3,5-dimethylaminophenol in AA8 cells
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Intracellular Generation of ROS by 3,5-Dimethylaminophenol: Persistence, Cellular Response, and Impact of Molecular Toxicity
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The *gpt* gene and linked *gpt* rearrangement in AS52 cells
 Kelecsenyi, Z. et al. Mutagenesis (2000) 15:25-31

Chao et al., *Tox Sci*, 2012

Genotoxicity of 2,6- and 3,5-Dimethylaniline in Cultured Mammalian Cells: The Role of Reactive Oxygen Species
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PM_{2.5} contains Dimethylaniline

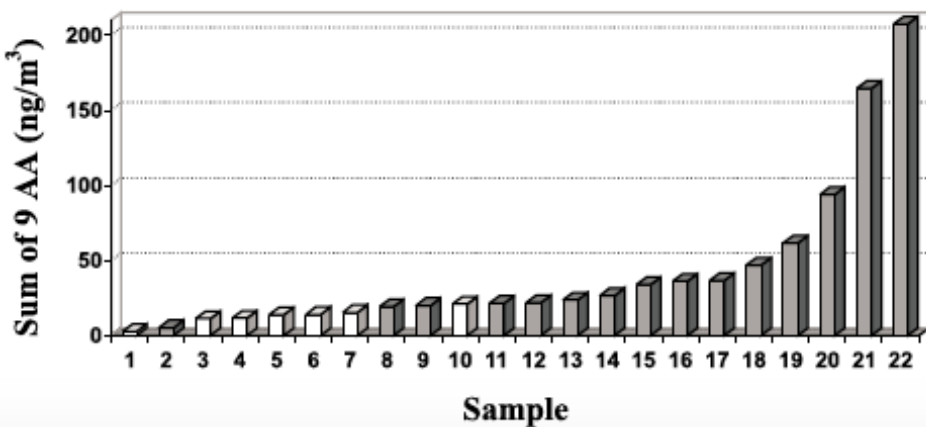


Fig. 2. Levels of 9 AA (excluding aniline) in the air of public buildings: 1: hospital ward I, 2: bank, 3: train compartment (no smoking), 4: hospital ward II, 5: public library, 6: newspaper office I, 7: office of non-smokers, 8: office with one smoker, 9: hospital waiting room I, 10: dentist office, 11: private club I, 12: newspaper office II, 13: school, 14: private club II, 15: hospital waiting room II, 16: office with two smokers, 17: bar, 18: police office, 19: hairdresser lounge, 20: computer centre, 21: private club III, 22: discotheque.

Palmiotta et al, Chemosphere, 2001

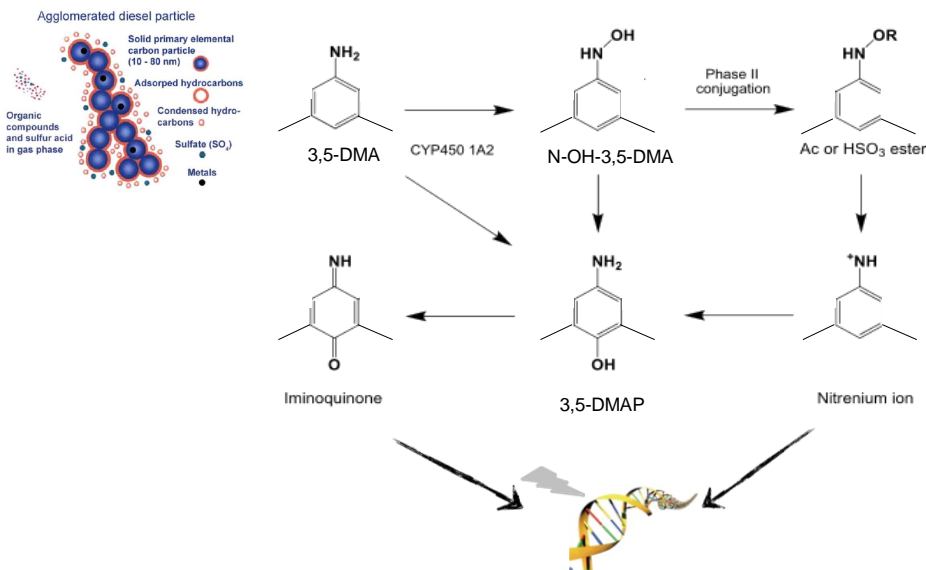


Table I. Formation of DNA adducts by three [¹⁴C]alkylanilines^a

		3-EA	3,5-DMA	2,6-DMA
Human	Control	15 ± 6	36 ± 11	13 ± 1
	Experiment	15 ± 3	1392 ± 74	258 ± 187
Rat	Control	39 ± 12	5 ± 1	11 ± 1
	Experiment	30 ± 5	1101 ± 204	50 ± 39
Dog	Control	15 ± 8	21 ± 14	35 ± 21
	Experiment	22 ± 1	727 ± 37	180 ± 53
Mouse	Control	68 ± 16	77 ± 23	33 ± 2
	Experiment	162 ± 9	139 ± 35	146 ± 22

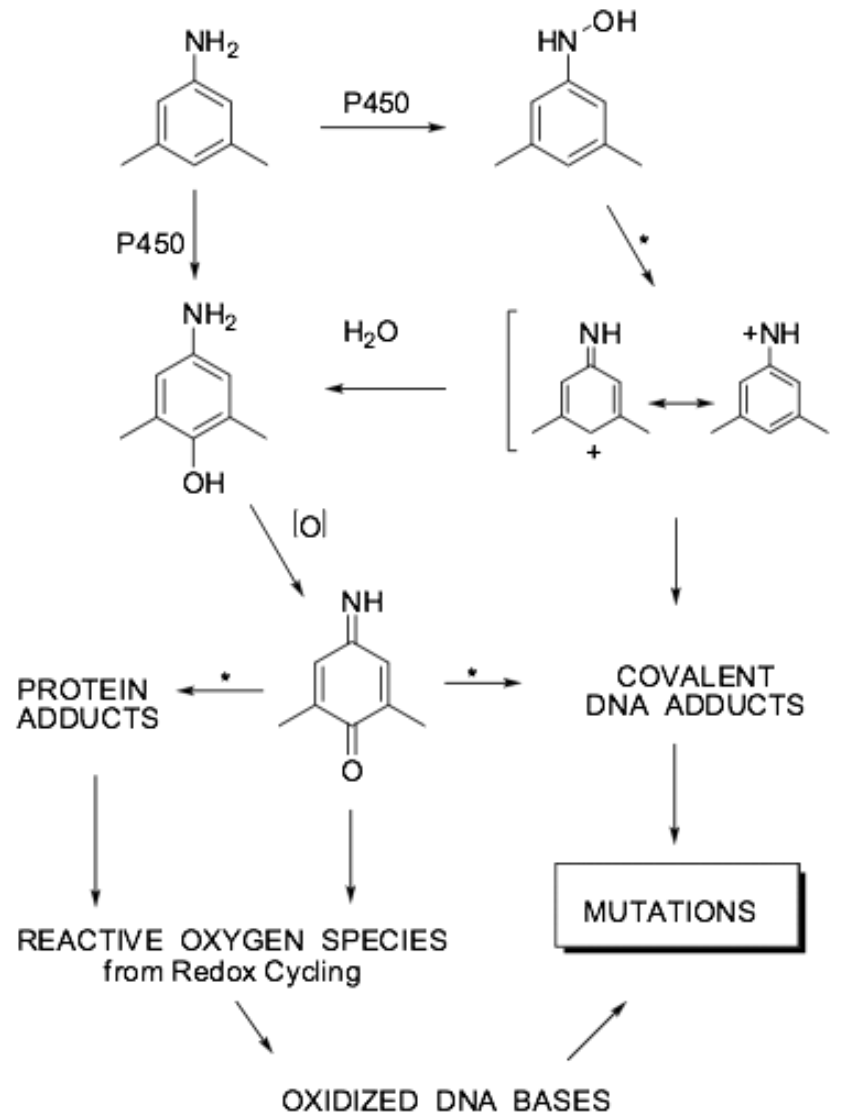
Skipper et al, *Chem Res Toxicol*. 2006

Cui et al, *Chem Res Toxicol*. 2007

Mutation type	Number of mutations (% of total)			
	Control	3,5-DMA+S9	N-OH-3,5-DMA	3,5-DMAP
		- NAC	- NAC	- NAC
Single base pair substitution	12 (52)	19 (54)	21 (40)	25 (54)
Transversions	G:C to T:A	6 (26)	4 (11)	8 (15)
	G:C to C:G	3 (13)	1 (3)	0 (0)
	A:T to T:A	2 (9)	2 (6)	0 (0)
Transitions	A:T to C:G	0 (0)	1 (3)	0 (0)
	G:C to A:T	1 (4)	4 (11)	7 (13)
	A:T to G:C	0 (0)	7 (20)	6 (11)
Insertion	4 (17.5)	6 (17)	5 (10)	4 (8)
Deletion	1 bp	4 (17.5)	4 (11)	10 (19)
	2 bp	0 (0)	5 (15)	0 (0)
	3 bp	2 (9)	0 (0)	10 (19)
Multiple sequence change	1 (4)	1 (3)	6 (11)	1 (2)
Total	23 (100)	35 (100)	52 (100)	46 (100)

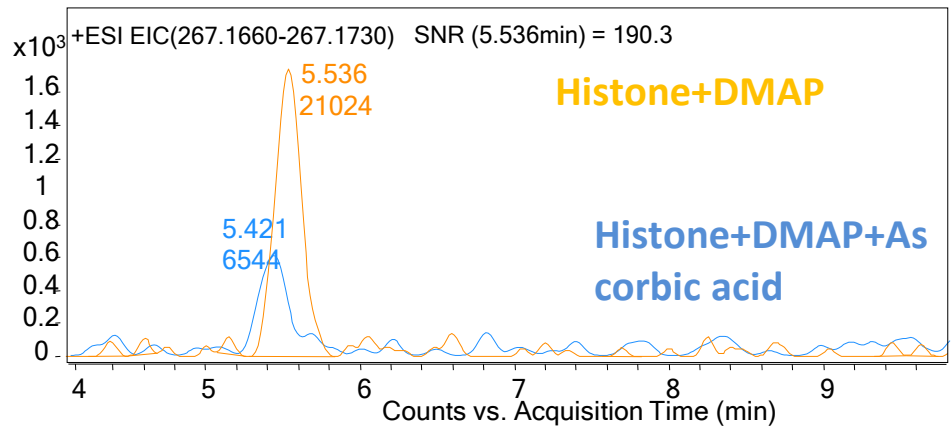
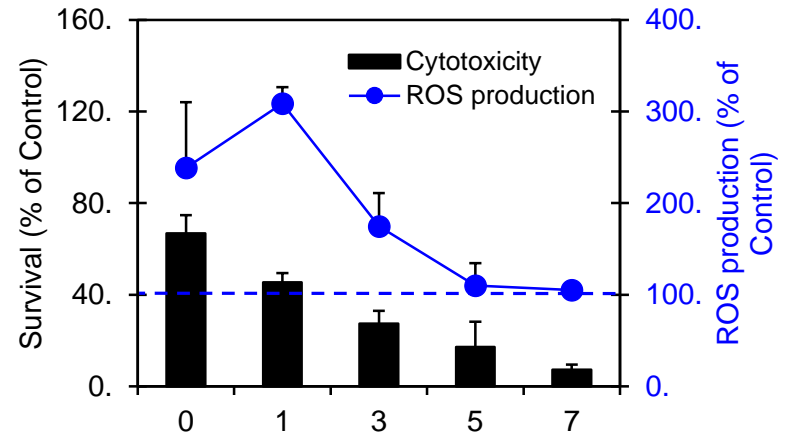
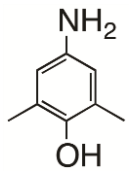
Chao et al, ToxSci, 2012

The idea for Long-term ROS generation in the cells

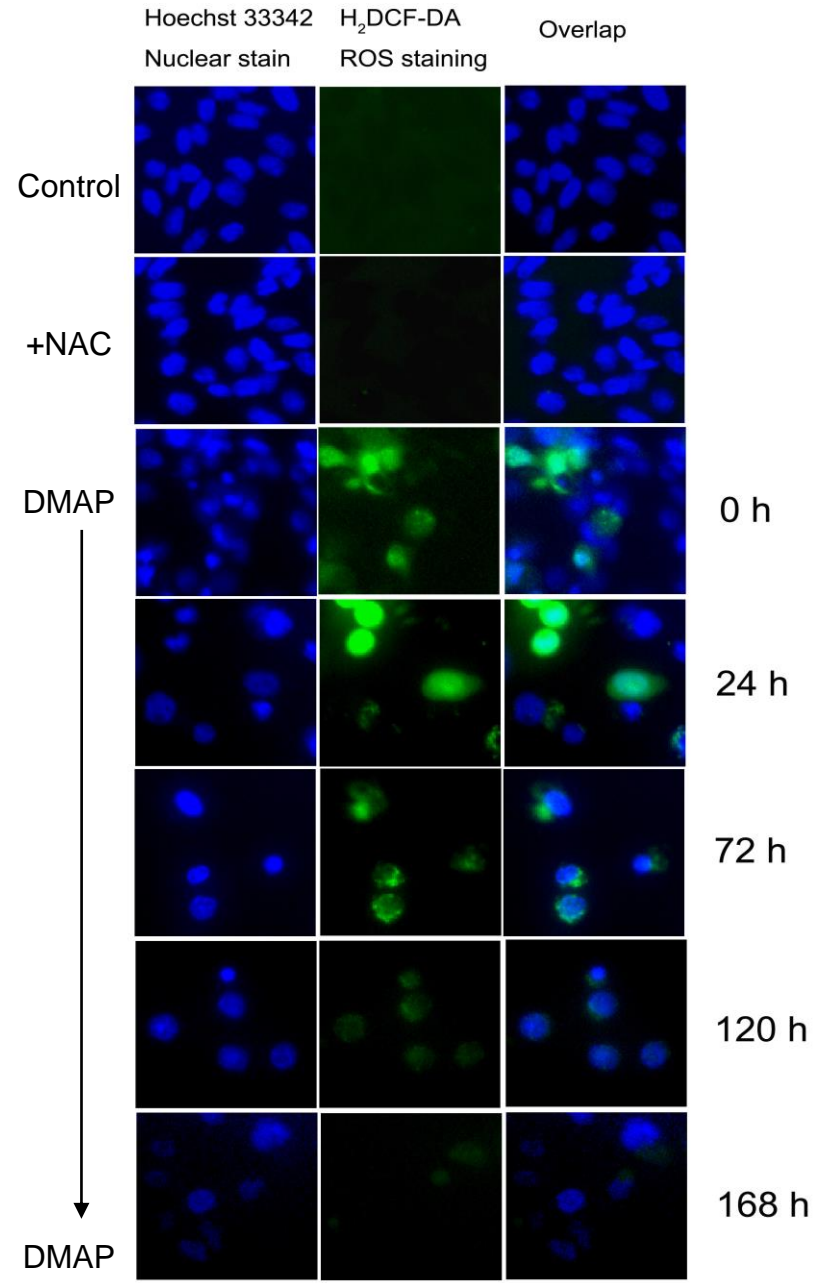


Monocyclic aromatic amine-induced ROS can be detected within 5 days

3,5-DMAP



Ye et al, Chemical Research Toxicology, 2012



Chao et al, ToxSci, 2014

Acknowledgements



Chia-Yi Tseng



王志軒



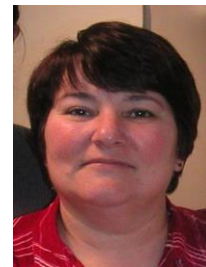
廖家巍



林佩穎



童思瑜



Marion Gordon



Gerald Wogan



Wen-Hsung Chan



莊妤甄



余佳語



林欽鴻



吳均豪



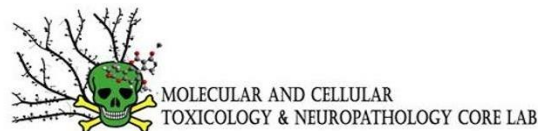
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