Hazard Evaluation of Air Pollution by Using the Key Characteristics Approach

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Introduction

The WHO-IARC monograph was adopted the Key Characteristics (KCs) method to evaluate carcinogens at 2018, which is the new approach to considering mechanistic evidence. Because air pollution contains a variety of carcinogens, different carcinogens are likely to exhibit different spectra of these important properties. Air pollution is one of the most important concerns for human health for people living in Asia. In this study, we investigated the KC of air pollution mixtures to evaluate human carcinogens with scientific accuracy.

Cancers Contributed to Extrinsic factors
By Wu et al., Nature 2016.

	Intrinsic MS	Extrinsic MS - Known	Extrinsic MS - Unknown	Extrinsic MS - Total
ALL	65.8	34.2	0	34.2
Lung Adenocarcinoma	9.1	73.8	17.1	90.9
Lung - Small Cell	0	92.8	7.2	100
Lung-Squamous	0	47	53	100
Colorectal	17.1	66	16.9	82.9
Stomach	22.3	6.1	71.6	77.7
Pancreatic	49.9	50.1	0	50.1
Liver	10.9	21.3	67.8	89.1
Breast	35.5	60.1	4.4	64.5

2014 Cancer Registries in Japan

	Mortality / Incidence Rate		
	Total	Male	Female
Lung	1 / 3	1 / 2	2 / 4
Colorectal	2 / 1	3 / 3	1 / 2
Stomach	3 / 2	2 / 1	4 / 3
Pancreatic	4 / 7	5 / 7	3 / 6
Liver	5 / 6	4 / 5	8 / 7
Breast			5 / 1
Prostate		7 / 4	

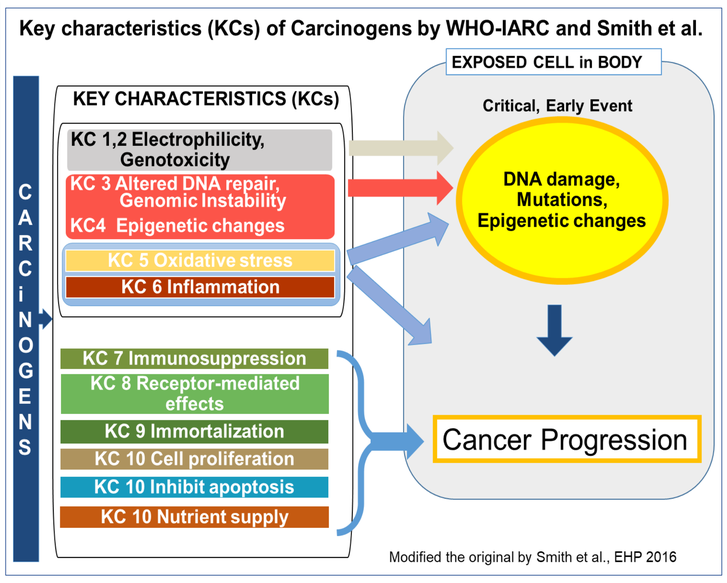
https://ganjoho.jp/reg_stat/statistics/stat/summary.html

Cross-validation between Target Pathways in Hallmarks of Cancer by Hanahan & Weinberg (Cell, 2000 and 2011) and KCs

Priority targets	KC 10 Deregulated metabolism	KC 8, 10 Evasion of anti-growth signaling	KC 5, 6 Angio-genesis	KC 3 Genetic instability	KC 10 Resistance to cell death	KC 9 Replicative immortality	KC 10 Tissue invasion and metastasis	KC 6 Tumor promoting inflammation	KC 10 Tumor microenvironment
H-Ras	±	+	+	+	±	–	+	+	+
Jun/Fos/AP 1	±	–	0	+	±	±	+	+	+
Cyclin D, IL8, CXCL	±	0	+	+	0	±	+	+	0
PPAR	+	+	–	0	–	+	+	+	0
AhR	+	+	+	+	±	+	±	+	+
EGF receptor	+	0	+	+	±	+	+	+	0
Steroid hormone receptors	+	0	0	+	0	±	+	+	+

Wilhelm Engström et al. Carcinogenesis 2015;36:S38-S60

Key characteristics (KCs) of Carcinogens by WHO-IARC and Smith et al.



Modified the original by Smith et al., EHP 2016

International Comparison of Particulate Matter Environmental Standards
Cited from IEEI. 0r.jp, 2013

	International Guidelines	Annnual Average μg/m^3	Daily Average μg/m^3
PM_{10}	Japan	-	100
	U.S.	-	150
	China	70	150
	WHO	20	50
$PM_{2.5}$	Japan	15	35
	U.S.	12	35
	China	35	75
	WHO	10	25

Realistic Exposures to Diesel and Gasoline Engine Exhausts
2007 technology engines (mg/bhp-h)

Metals

Antimony compounds	< 0.001
Arsenic	< 0.0002
Beryllium compounds	< 0.0003
Cadmium	< 0.00003
Chromium compounds	0.0007 ± 0.0003
Cobalt compounds	< 0.0001
Inorganic lead	< 0.0001
Manganese	< 0.00022
Mercury	< 0.00016
Nickel	0.0002 ± 0.0001
Selenium	< 0.0001

Organic compounds

Acetaldehyde	0.61 ± 0.27
Acrolein	< 0.01
Aniline	0.000150 ± 0.000075
Benzene	< 0.01
Biphenyl	0.013780 ± 0.001716
Bis(ethylhexyl)phthalate	NR
1,3-Butadiene	< 0.01
Chlorine(chloride)	< 0.007
Chlobenzene and derivatives	NR
Cresol isomers	0.02727 ± 0.01233
Cyanide compounds	< 0.05
dl-*n*-Butylphthalate	NR
Dioxins and dibenzofurans	0.00000066 ± 0.000000055
Ethyl benzene	0.05 ± 0.04
Formaldehyde	1.90 ± 1.01
Hexane	< 0.01
Methanol	0.07 ± 0.13
Methyl ethyl ketone	< 0.01
Naphthalene	0.0982 ± 0.0423
4-Nitrobiphenyl	< 0.00000001
Phenol	0.00905 ± 0.00414
Phosphorus	0.0130 ± 0.0064
Propionaldehyde	0.01
Styrene	< 0.01
Toluene	0.26 ± 0.28
Xylene isomers and mixtures	0.35 ± 0.10
ortho-Xylene	0.13 ± 0.07
meta-and *para*-Xylenes	0.20 ± 0.08

41 organic compounds Meet the TOX21 database

Result and Discussion

We first conducted a KC analysis of 40 organic compounds of diesel exhaust in IARC monograph 105 using data of biological test results from the PubChem database. As a result, it was found that some PAH is highly responsive among those compounds. Similarly, hazardous chemicals in Air quality data provided by the Japan Atmospheric Environmental Regional Observation System and the Kanagawa Prefectural Environmental Science Research Center was analyzed by the KCs approach. The result showed that the contribution of genotoxic substances was large.

The completed 31 Assay battery: Multiple Key Characteristics for Carcinogens and non-carcinogens in Diesel and Gasoline Engine Exhausts

33 Organic compounds — 31 Assays — Scoring : negative 1, inconclusive 2, positive 3

Chemical name	AHR – aryl hydrocarbon receptor (human)	ATAD5 – ATPase family AAA domain containing 5 (human)	CASP7 – caspase 7 (human)	CYP19A1 – cytochrome P450 family 19 subfamily A member 1 (human)	GLI3 – GLI family zinc finger 3 (human)	HDAC9 – histone deacetylase 9 (human)	HIF1A – hypoxia inducible factor 1 subunit alpha (human)	HSPB1 – heat shock protein family B (small) member 1 (human)	JUN – Jun proto-oncogene, AP-1 transcription factor subunit (human)	LOC100 757539 – histone H2AX (Chinese hamster)	NFE2L2 – nuclear factor, erythroid 2 like 2 (human)	NFKB1 – nuclear factor kappa B subunit 1 (human)	NR1H4 – nuclear receptor subfamily 1 group H member 4 (human)	NR1I2 – nuclear receptor subfamily 1 group I member 2 (human)	NR1I3 – nuclear receptor subfamily 1 group I member 3 (human)	NR3C1 – nuclear receptor subfamily 3 group C member 1 (human)	PPARA – peroxisome proliferator activated receptor alpha (human)	PPARD – peroxisome proliferator activated receptor delta (human)	PPARG – peroxisome proliferator activated receptor gamma (human)	RARA – retinoic acid receptor alpha (human)	Rorc – RAR-related orphan receptor gamma (mouse)	RXRA – retinoid X receptor alpha (human)	SMAD2 – SMAD family member 2 (human)	SMAD3 – SMAD family member 3 (human)	THRB – thyroid hormone receptor beta (human)	Thrb – thyroid hormone receptor beta (Mouse, rat)	TP53 – tumor protein p53 (human)	THRA – thyroid hormone receptor alpha (human)	THRA – thyroid hormone receptor alpha (human)	VDR – vitamin D receptor (human)	sum
1-Nitropyrene	3	1	1	1	1	1	1	1	1	1	3	1	1	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	50
2-Nitroanthracene	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	2	1	1	1	1	1	1	1	1	1	1	1	39
9-Nitroanthracene	2	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1	2	1	1	2	1	1	2	2	1	1	1	1	1	1	1	39
Acenaphthene	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	25
Acenaphthylene	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	2	1	1	1	1	1	1	1	1	1	1	1	25
Acetaldehyde	1	1	1	2	2	1	1	1	1	1	1	1	1	1	2	2	1	1	1	1	1	1	3	2	1	1	1	3	1	2	1	39
Acrolein	1	1	1	1	1	2	1	1	1	1	1	1	1	1	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	36
Aniline	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	26
anthracene	1	1	1	1	1	2	1	1	1	1	1	1	1	1	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	39
Benz(a)anthracene	3	2	1	1	1	2	2	1	1	1	1	1	1	1	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	41
Benzene	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	25
Benzo(a)pyrene	3	2	1	1	1	2	2	1	1	1	3	1	1	2	2	2	2	1	1	2	1	1	2	2	1	1	1	2	1	1	1	47
Benzo(b)fluoranthene	3	2	1	1	1	2	2	1	1	1	2	1	1	2	2	2	2	1	1	2	1	1	2	2	1	1	1	2	1	1	1	50
Benzo(ghi)perylene	1	1	1	1	1	2	1	1	1	1	2	1	1	2	2	2	2	1	1	2	1	1	2	2	1	1	1	2	1	1	1	49
Benzo(k)fluoranthene	3	2	1	1	1	2	2	1	1	1	2	1	1	2	2	2	2	1	1	2	1	1	2	2	1	1	1	2	1	1	1	49
Biphenyl	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	25
Chrysene	3	2	1	1	1	3	1	1	1	1	3	1	1	1	1	1	2	1	1	1	1	1	2	1	1	1	1	2	1	1	1	34
Chrysene	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	25
Dibenz(a,h)anthracene	3	2	1	1	1	2	2	1	1	1	2	1	1	2	2	2	2	1	1	2	1	1	2	2	1	1	1	2	1	1	1	49
Fluoranthene	1	1	1	1	1	2	1	1	1	1	2	1	1	2	2	2	2	1	1	2	1	1	2	2	1	1	1	2	1	1	1	48
Fluorene	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	25
Formaldehyde	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	24
Hexane	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	24
Methanol	1	1	1	1	1	2	1	1	1	1	2	1	1	2	2	2	2	1	1	2	1	1	2	2	1	1	1	2	1	1	1	48
Methyl ethyl ketone	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	25
Naphthalene	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	25
ortho-Xylene (O-Xylene)	3	2	1	1	1	2	2	1	1	1	2	1	1	2	2	2	2	1	1	2	1	1	2	2	1	1	1	2	1	1	1	49
Phenanthrene	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	25
Phenol	1	1	1	1	1	2	1	1	1	1	2	1	1	2	2	2	2	1	1	2	1	1	2	2	1	1	1	2	1	1	1	48
Propionaldehyde	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	25
Pyrene	1	1	1	1	1	2	1	1	1	1	2	1	1	2	2	2	2	1	1	2	1	1	2	2	1	1	1	2	1	1	1	48
Styrene	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	25
Toluene	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	25
	50	39	37	35	34	38	37	38	35	32	49	40	38	48	46	42	41	30	36	38	39	32	39	33	30	33	36	41	35	35	34	

Multiple Key Characteristics

10 Carcinogens of the IARC Group 1 in Diesel and Gasoline Engine

#	Names	KC1 Is Electrophilic: Electrophilic structure	KC1 Is Electrophilic: Protein adducts	KC1 Is Electrophilic: DNA adducts	KC2 Is Genotoxic: Bacterial mutation (Ames)	KC2 Is Genotoxic: Nonmamalian chromosomal abberation	KC2 Is Genotoxic: Mammalian mutation assay (rodents, human)	KC3 Alters DNA Repair or Causes Genomic Instability	KC4 Induces Epigenetic Alterations	KC5 Induces Oxidative Stress	KC6 Induces Chronic Inflammation (modificate inflammation)	KC7 Is Immuno-suppressive	KC8 Modulates Receptor - mediated Effects	KC9 Causes Immortalization	KC10 Alters Cell Proliferation, Cell Death or Nutrient Supply
1	Arsenic and inorganic arsenic	-	Yes	no report	no report	positive (compounds)	no report	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	Beryllium and beryllium	-	no report	no report	no report	positive (compounds)	no report	Yes	no report	Yes	Yes	Yes	no report	Yes	Yes
3	Cadmium and cadmium	-	no report	interact with DNA (ADP)	no report	positive (compounds)	positive	Yes	Yes	Yes	inconclusive	Yes	Yes	Yes	Yes
4	Chromium VI	-	no report	interact with DNA	inconclusive	inconclusive	inconclusive	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	Nickel compounds (Nickel chloride)	-	no report	interact with DNA	negative	positive	positive	Yes	inconclusive	Yes	Yes	Yes	no report	Yes	Yes
6	1,3-butadiene	Yes	Yes (metabolites)	metabolites or GSH-metabolites conjugated	positive (metabolic)	positive	no report	inconclusive	inconclusive	inconclusive	Yes	inconclusive	Yes	Yes	Yes
7	Benzene	Yes	Yes	metabolites conjugated	no report	positive	positive	Yes	Yes	Yes	inconclusive	Yes	Yes	Yes	Yes
8	Benzo[a]pyrene	Yes	Yes	metabolites conjugated	positive (metabolic)	positive (metabolic)	positive (metabolic)	inconclusive	inconclusive	Yes	inconclusive	Yes	Yes	Yes	Yes
9	Formaldehyde	Yes	Yes	Yes	positive	positive	positive	inconclusive	inconclusive	Yes	inconclusive	Yes	Yes	Yes	Yes
10	Dioxin/dibenzofurans	Yes	no report	no report	inconclusive	inconclusive	inconclusive	inconclusive	inconclusive	Yes	inconclusive	Yes	Yes	Yes	Yes

5 Metals and its compounds shown in brown.
5 others are organic compounds including VOCs, PAH and dioxin.

Multiple Key Characteristics of Chemicals with High Score

Benz(a)anthracene
Benzo(a)pyrene
Benzo(b)fluoranthene
Benzo(k)fluoranthene

Target	#	KCs
TP53 - tumor protein p53	2	Is Genotoxic
HDAC9 - histone deacetylase 9	4	Induces Epigenetic Alterations
HSPB1 - heat shock protein family B (small) member 1	5	Induces Oxidative Stress
NFE2L2 - nuclear factor, erythroid 2 like 2		
SMAD2 - SMAD family member 2		
NFKB1 - nuclear factor kappa B subunit 1	6	Induces chronic inflammation
AHR - aryl hydrocarbon receptor	8	Modulates receptor-mediated effects
HIF1A - hypoxia inducible factor 1 subunit alpha		
JUN - Jun proto-oncogene, AP-1 transcription factor subunit		
NR1H4 - nuclear receptor subfamily 1 group H member 4 (FXR)		
NR1I2 - nuclear receptor subfamily 1 group I member 2 (PXR_VDR)		
NR1I3 - nuclear receptor subfamily 1 group I member 3 (CAR)		
NR3C1 - nuclear receptor subfamily 3 group C member 1 (GR)		
PGR - progesterone receptor (PR)		
RXRA - retinoid X receptor alpha		
ATAD5 - ATPase family AAA domain containing 5	10	Alters cell proliferation, cell death or nutrient supply
CASP7 - caspase 7		

Biossays with High Score (show in red)

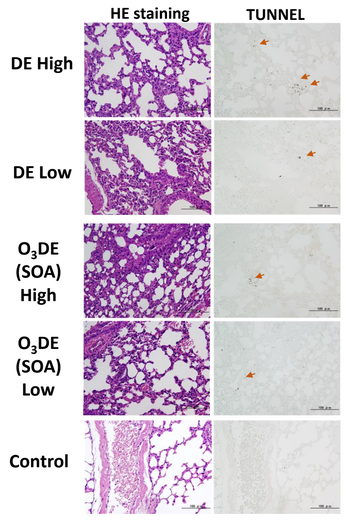
	AHR – aryl hydrocarbon receptor (human)	ATAD5 – ATPase family AAA domain containing 5 (human)	CASP7 – caspase 7 (human)	CYP19A1 – cytochrome P450 family 19 subfamily A member 1 (human)	GLI3 – GLI family zinc finger 3 (human)	HDAC9 – histone deacetylase 9 (human)	HIF1A – hypoxia inducible factor 1 subunit alpha (human)	HSPB1 – heat shock protein family B (small) member 1 (human)	JUN – Jun proto-oncogene, AP-1 transcription factor subunit (human)	LOC100 757539 – histone H2AX (Chinese hamster)	NFE2L2 – nuclear factor, erythroid 2 like 2 (human)	NFKB1 – nuclear factor kappa B subunit 1 (human)	NR1H4 – nuclear receptor subfamily 1 group H member 4 (human)	NR1I2 – nuclear receptor subfamily 1 group I member 2 (human)	NR1I3 – nuclear receptor subfamily 1 group I member 3 (human)
Score numbers out of 99	50	39	37	35	34	38	37	38	35	32	49	40	38	48	46

AHR - aryl hydrocarbon receptor (human)
NFE2L2 - nuclear factor, erythroid 2 like 2 (human)
NR1I2 - nuclear receptor subfamily 1 group I member 2 (human) PXR
NR1I3 - nuclear receptor subfamily 1 group I member 3 (human) CAR

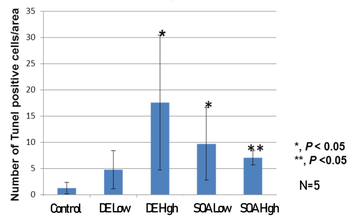
Positive result chemicals

AHR	NFE2L2	NR1I2	NR1I3
2-Nitrofluorene	1-Nitropyrene	9-Nitroanthracene	Benzo(a)pyrene
Benz(a)anthracene	Benz(a)anthracene	Anthracene	
Benzo(a)pyrene	Benzo(b)fluoranthene	Benzo(a)pyrene	
Benzo(k)fluoranthene	Benzo(k)fluoranthene	Benzo(b)fluoranthene	
Chrysene			
ortho-Xylene (O-Xylene)			

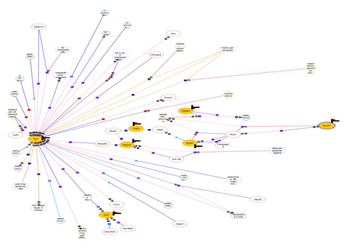
Effects of Exposures to Diesel Exhaust (DE) and Secondary Organic Aerosol (SOA) in Lungs of the 4-weeks exposed Mouse

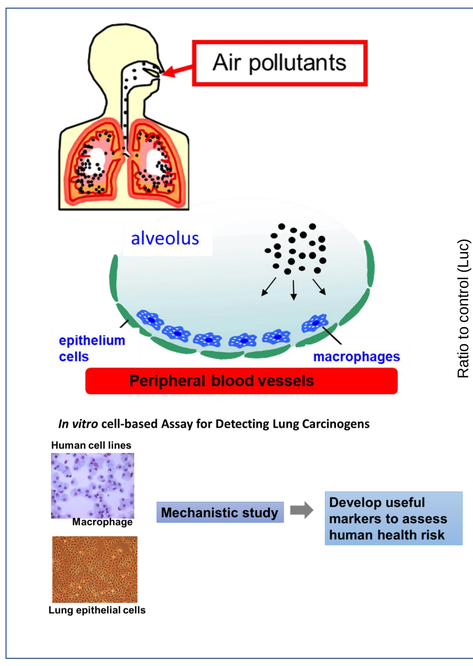


Increase of apoptosis in TUNNEL assay

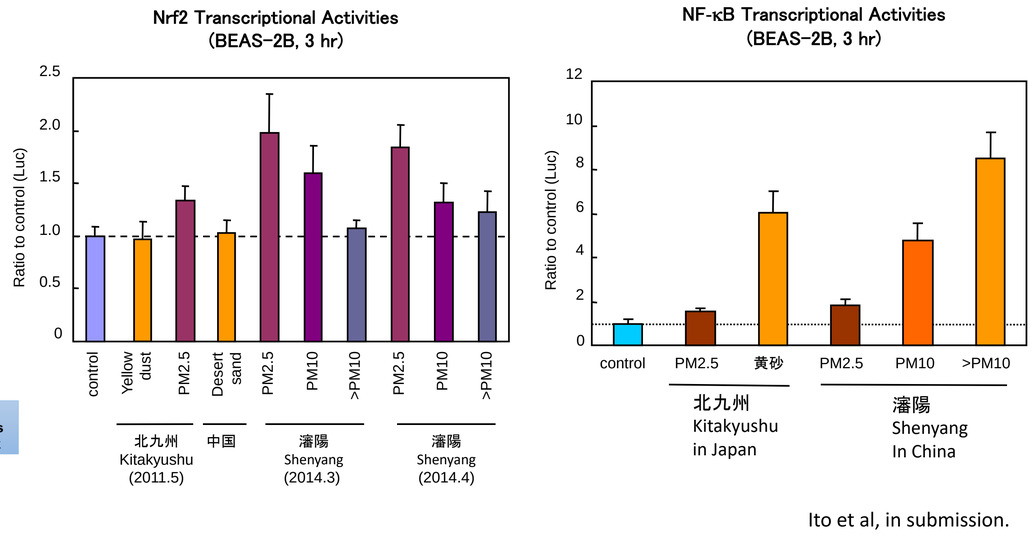


Effects on TNFα-BCl2 pathway with Gene Expression Profiling





Nrf2 and NF-kB Activation by Chinese Airborne Particles in Human Epithelial Cells

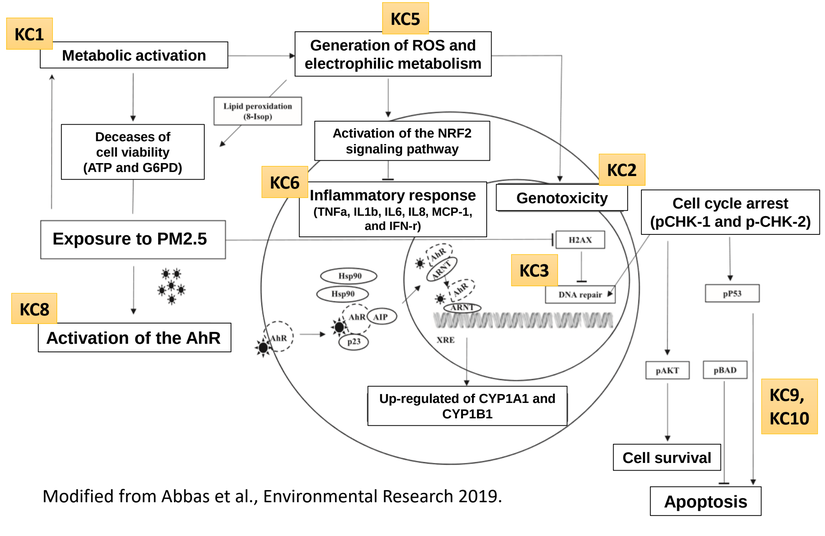


Ito et al, in submission.

Conclusion

> Diesel and gasoline engine exhausts exists carcinogens with multiple KCs.
> KC5 oxidative stress and KC8 nuclear receptors were detected as more important characters from diesel and gasoline engine exhausts. PAHs were detected as more important chemicals
> KC5 oxidative stress and KC6 chronic inflammation are critical role in carcinogenesis of DE in cells.
> We should pay attention on mechanistic-related biomarkers of KCs to evaluate air pollution.

Organic Extracts from Air pollution PM2.5 exhibits Multiple Key Characteristics



Modified from Abbas et al., Environmental Research 2019.