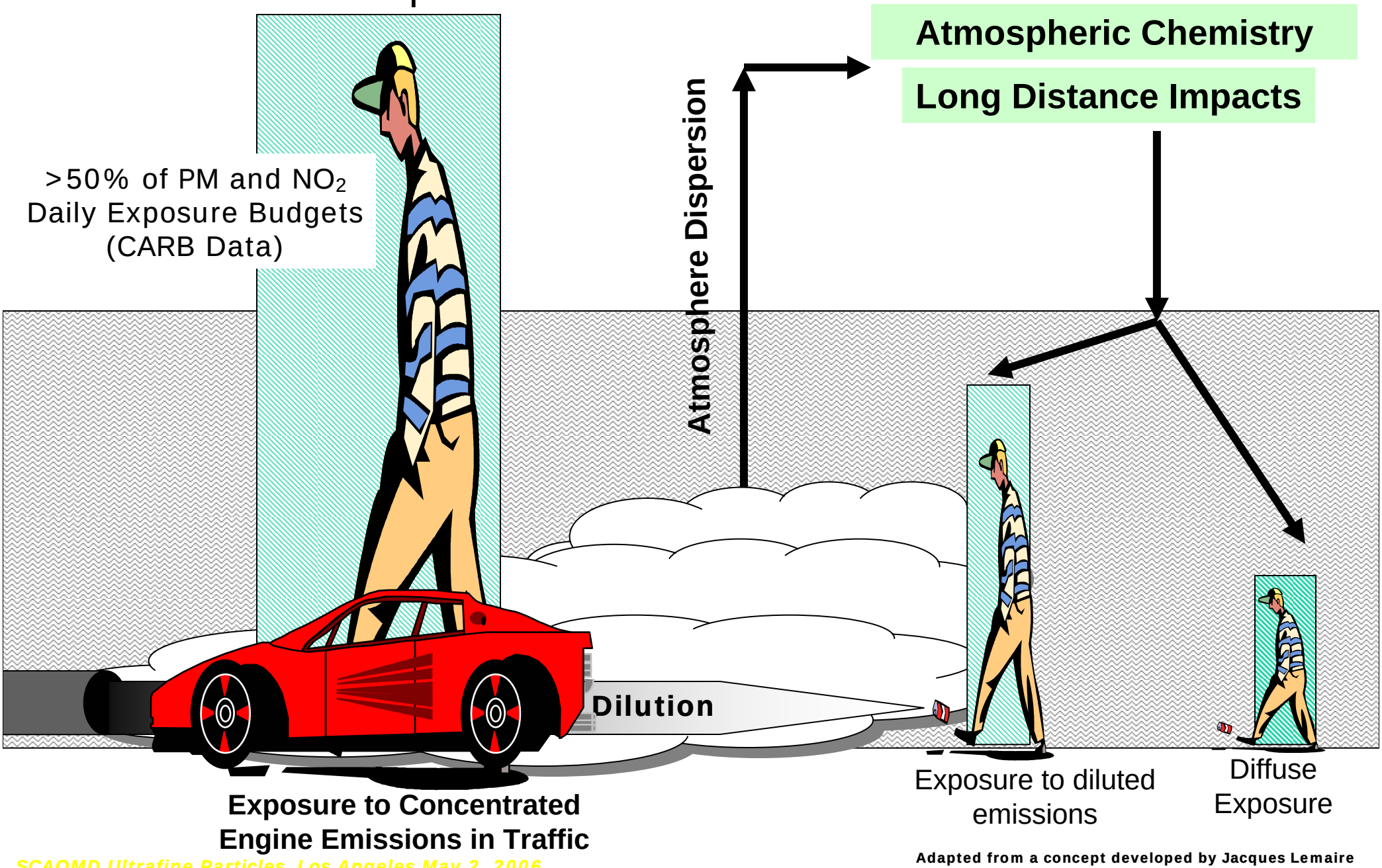
From Particles to NO_2 as main Diesel Emission Toxic Trigger : A link with After-Treatment Strategies

Jean-Paul MORIN
French National Institute for Health and Medical Research

Inserm
U644 ROUEN

Contact jean-paul.morin@univ-rouen.fr

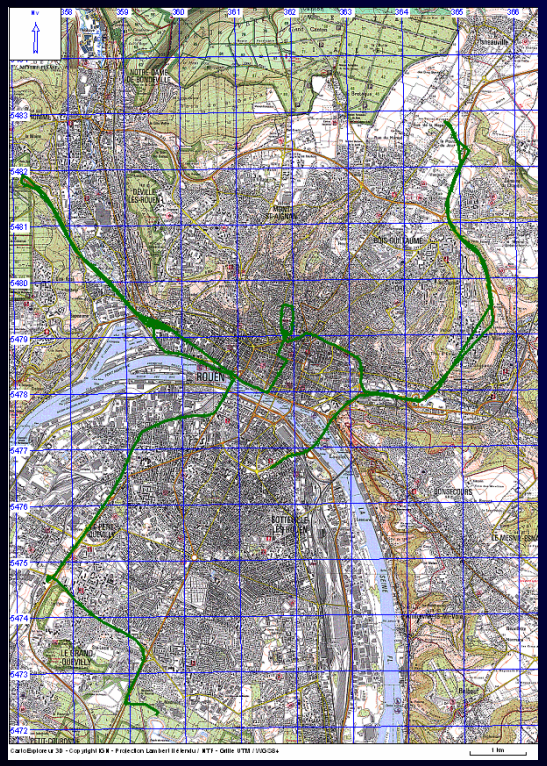
Exposure Levels to Traffic Emissions



Adapted from a concept developed by Jacques Lemaire

Inside car Ultrafine Particle Measurements P-Track (TSI)

GPS Track Recording
Localisation every 15 sec
Vehicle speed
Altitude



Software Carto Explorer Bayo

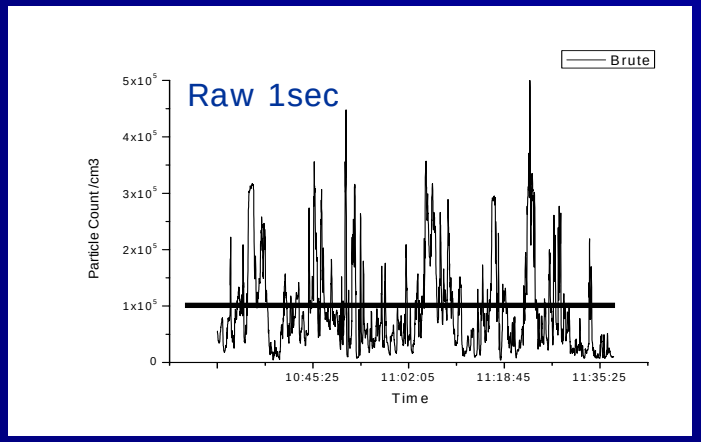
Urban Background
5.10^3 /cm3

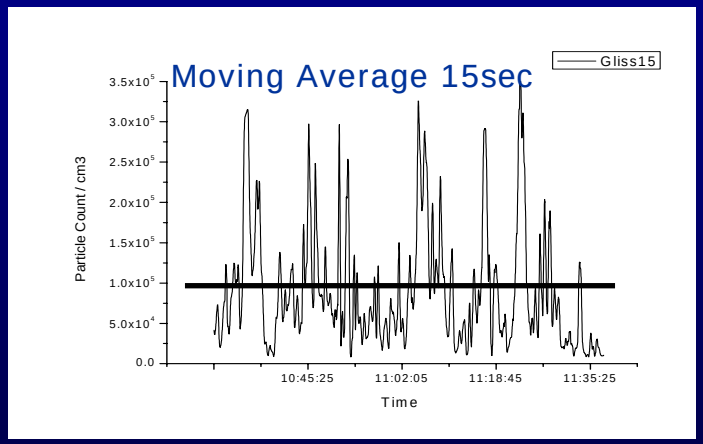
Raw value
Max 5.10^5 /cm3
Min 4.1 10^3 / cm3

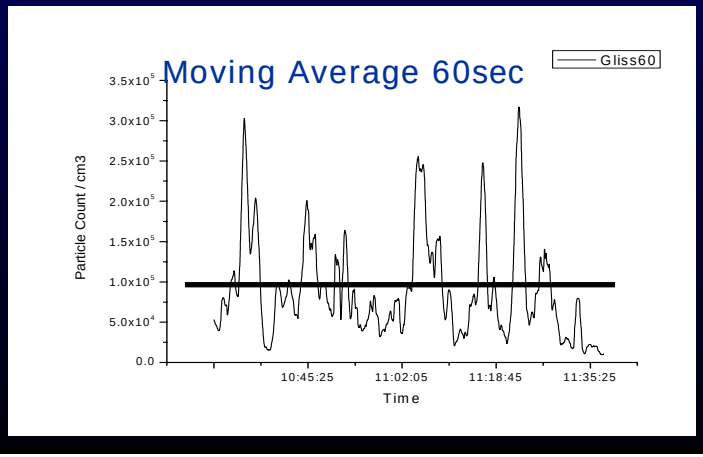
Moving Average 60sec
Max 3.2 10^5 /cm3
Min 9.5 1O^3 /cm3

Average over the period
9.10^4 /cm3

Rough mass Conversion
Based on Diesel PM
1O^3 /cm3 = 1 µg/m3







Statements :

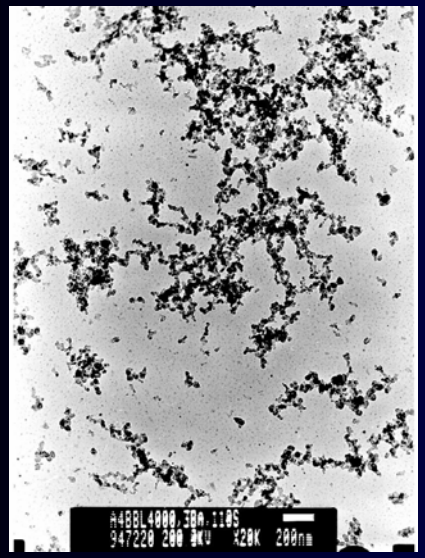
We do Inhale Complex Aerosols and Not Only Ultrafines
We do Inhale Freshly Emitted Tail Pipe Emissions

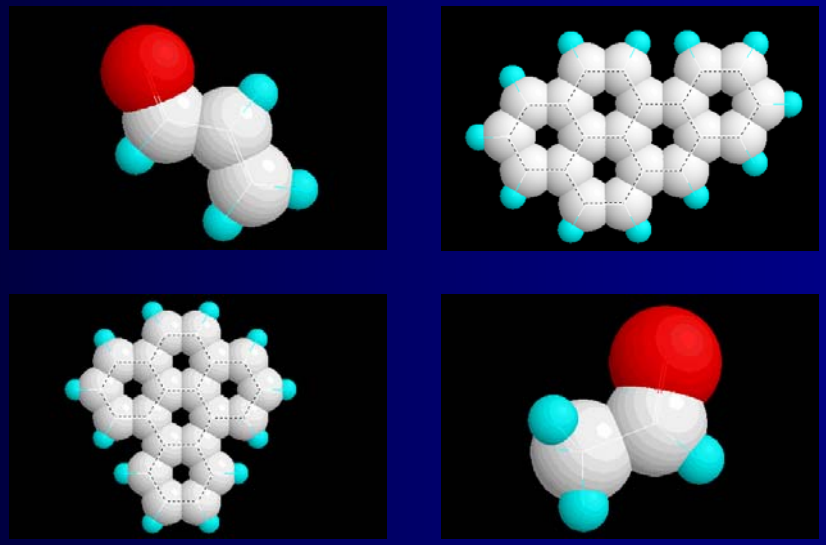
Objectives :

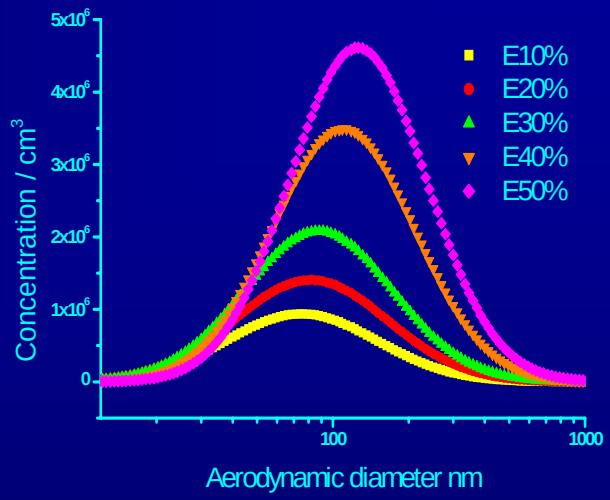
Development of pertinent toxicity models for screening the potential health benefits of new combustion emission depollution strategies using a wholistic approach of freshly generated engine emitted combustion aerosol impacts on cardiorespiratory endpoints

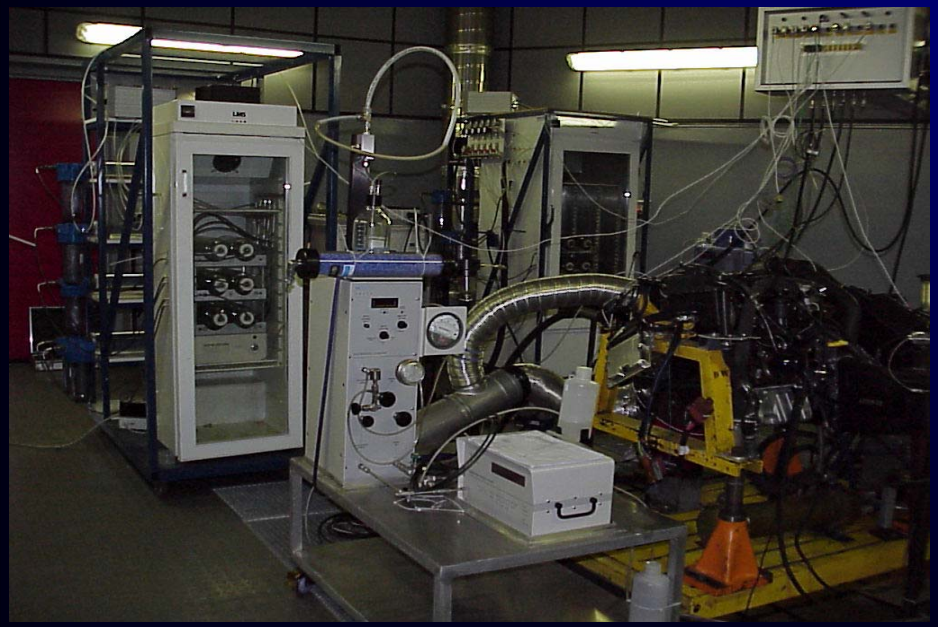
Contribution to the development of depollution and regulation strategies using a “health and safety” based rationale

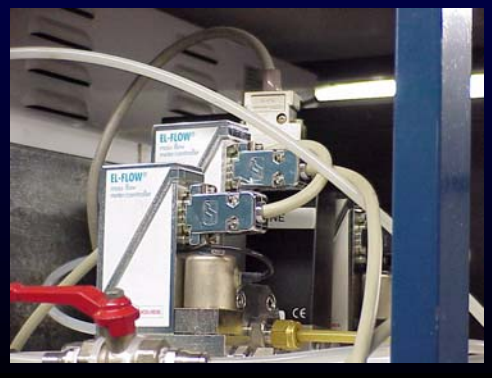
Impact of Complex Engine Emitted Aerosols on Lung Tissue in vitro

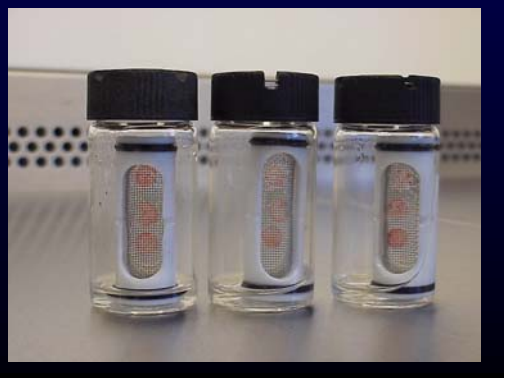












Lung Tissue Endpoints

-**Cell viability** :

Intracellular ATP content

- **Oxidative stress and Detoxication** :

Intracellular glutathione content (GSH)
Enzyme activity of SOD, Catalase, GPx, GST
8-hydroxy-2'-deoxyguanosin (histological staining)

- **Inflammatory response** :

TNFα (release in culture medium)
ICAM-1 (histological staining)

-**Apoptosis :**

-Nucleosome Assay
-TUNEL (histological staining)
-DNA Ladders

Modulation of Engine Emissions Quality

4 cylindrer Euro3 Diesel Engine

Fuel, Engine speed/load, DPF, « Catalytic Treatment »

Exhaust Characteristics (Gas Phase)

Post treat	NT		Oxycat +		Oxycat ++	
Sulphur	300ppm	0ppm	300ppm	0ppm	0ppm	0ppm
EGR	Yes	No	yes	yes	Yes	No
HC (ppm)	23	35	13	2	0	0
CO (ppm)	179	135	3	2	2	0
NOx (ppm)	448	780	422	468	465	755
NO_2 (ppm)	**23**	**45**	**106**	**191**	**230**	**440**
NO (ppm)	422	735	312	277	235	335
NO_2 / NOx	**0.06**	**0.0.6**	**0.25**	**0.45**	**0.5**	**0.6**

Oxidant Potential

Comparative Impacts on Lung Slices *In Vitro*

	Diesel Exhaust (Low NO_2/NO ratio)		Diesel Exhaust (High NO_2/NO ratio)		NO_2 Alone
	Total	Filtered	Total	Filtered	
ATP	=	=	--	-	-
GPx	+=	+=	+	+	+
GST	+	+	=	=	=
Catalase	+=	+=	++	+	++
GSH	--	-	---	---	--
SOD	+	+	-	-	-
TNFα	++	=	+ -©	+ -©	+ -©
Nucleosomes	++	=	=	=	=
TUNEL	+++	=	=	=	?
DNA Ladders	++	=	=	=	?
8-OxoG	+	+=	+++	+++	?

NO_2 Major Role ?

Gas Phase

Gas Phase

In vivo Inhalation Experimentations
Vigile unrestraint rats

Engine emissions 1:50 dilution
PM 500 µg/m3
NOx 8-10ppm

Exposure 3hours / Day
for 3 consecutive days

* Not treated emissions
* Oxycat DPF CRT type
* Fuel 50ppm Sulphur

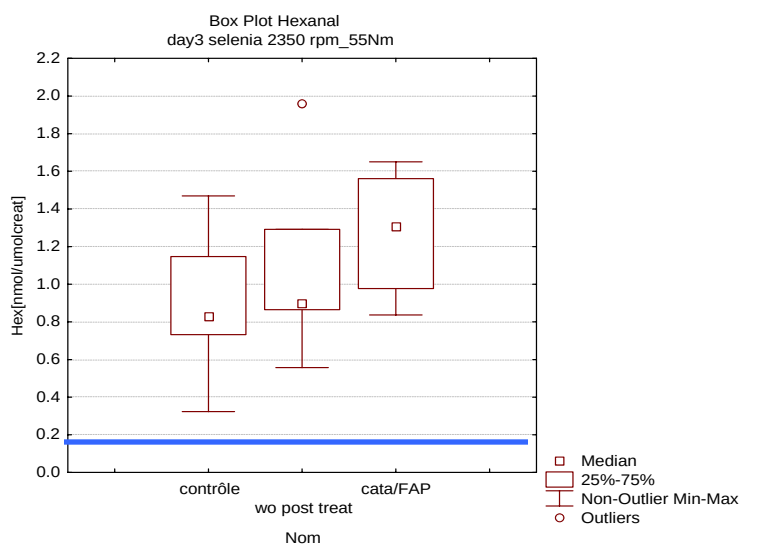
Evolution of Enzyme Activity in Organ Homogenates

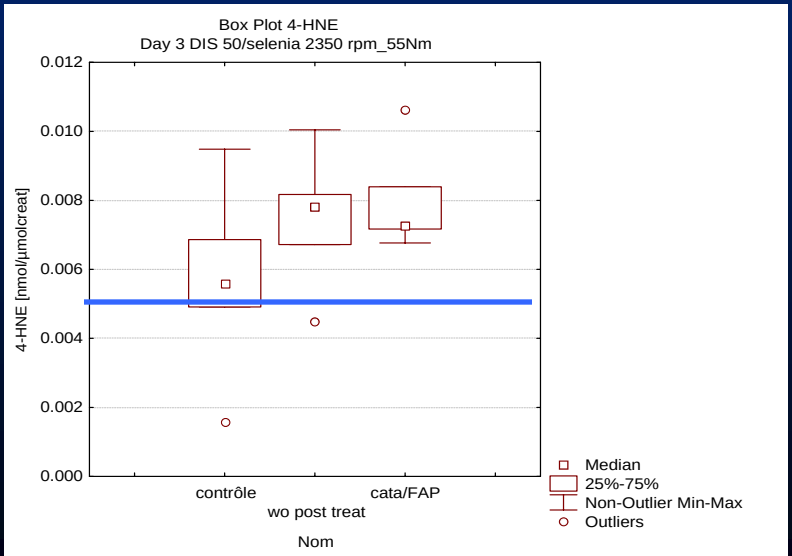
CAT	DIS50/10W40 2350/55	
	NT	OxyCat DPF
Lung	↘	↘
Heart	↗	↗(ns)
Liver	↗	↗↗
Kidney	↗	↗

GPX	DIS50/10W40 2350/55	
	NT	OxyCat DPF
Lung	=	=
Heart	↘	↘
Liver	↗	↗
Kidney	↗	=

SOD	DIS50/10W40 2350/55	
	NT	OxyCat DPF
Lung	=	↗
Heart	↘	↘
Liver	=	↘
Kidney	↗	↗↗

Urinary Excretion of Hexanal and 4-NHE



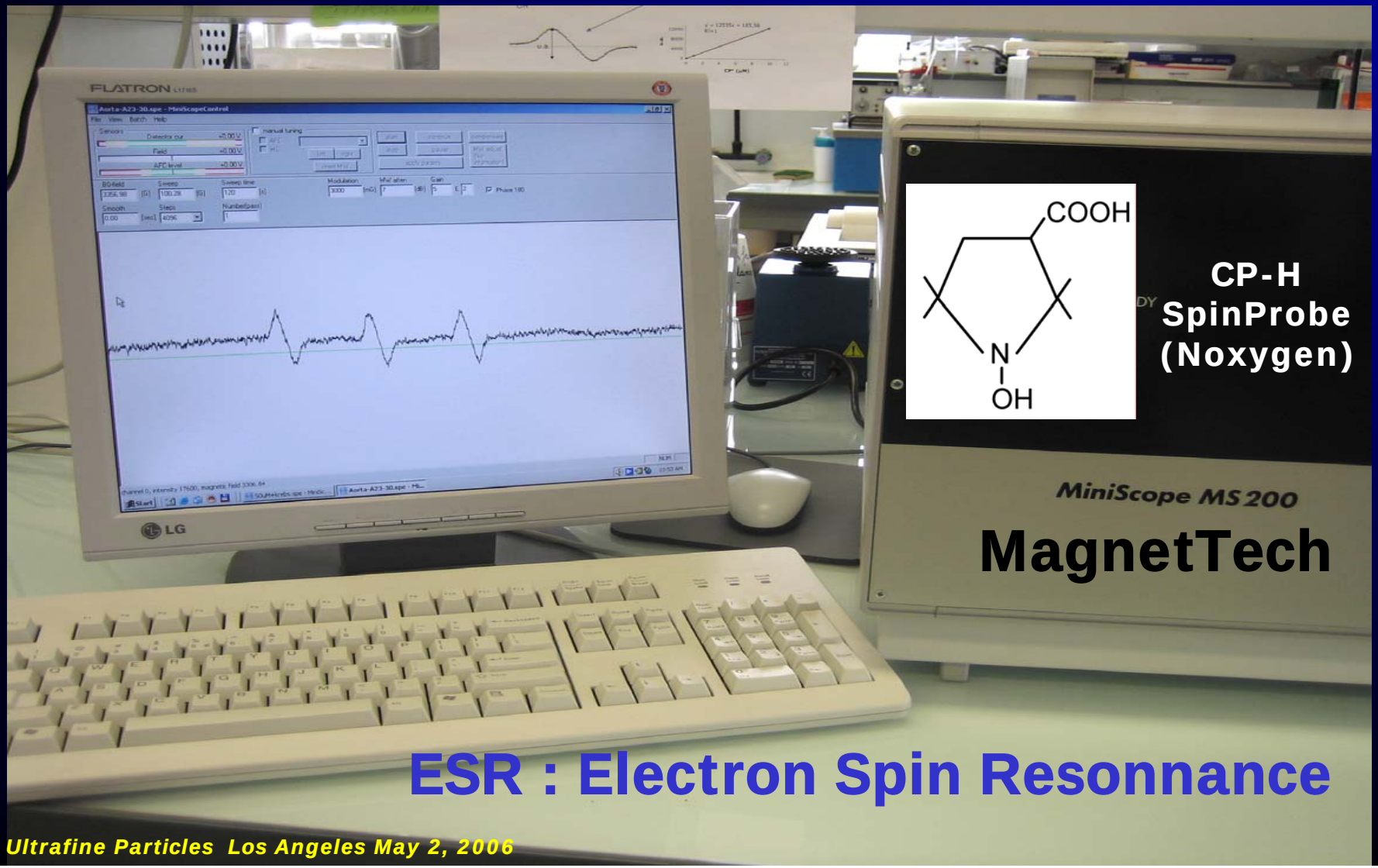


Conclusion

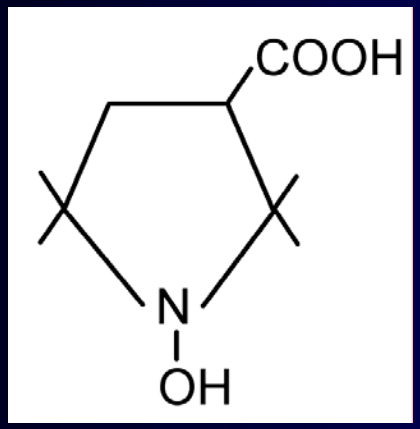
High NO_2/NOx Ratio is Associated
With Increased Oxidant Stress
both *in vitro* and *in vivo*

When Increasing NO_2/NOx Ratio
Emission Gaseous Phase Becomes
Predominant Toxic Trigger

Hypothesis of Reactive Oxygen Species (ROS) As candidates for triggering oxidant stress



ESR : Electron Spin Resonnance



CP-H Spin Probe from NOXYGEN

1-hydroxy-3-carboxy-2,2,5,5-tetramethylpyrrolidine
C9H17NO3 MW 223.7

Detection of OH and superoxide radicals, <u>peroxynitrite</u>

Oxidation of CP-H to CP*

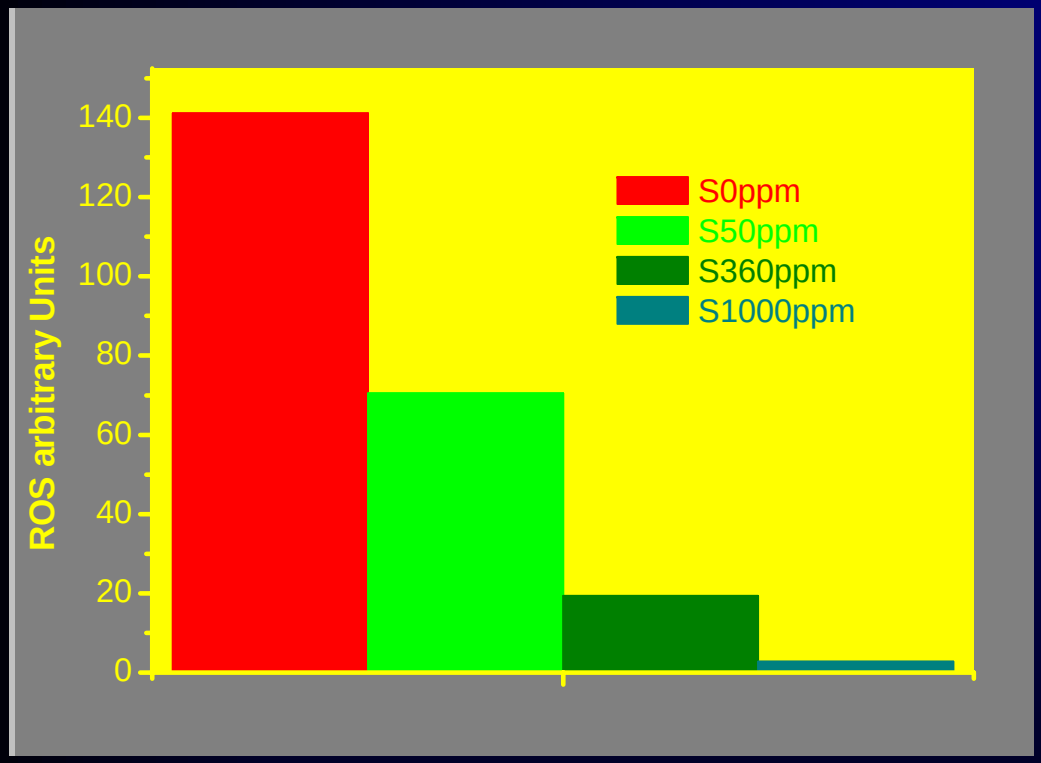
CP* Spin resistant to vitamin C and thiols reduction stable upon freezing

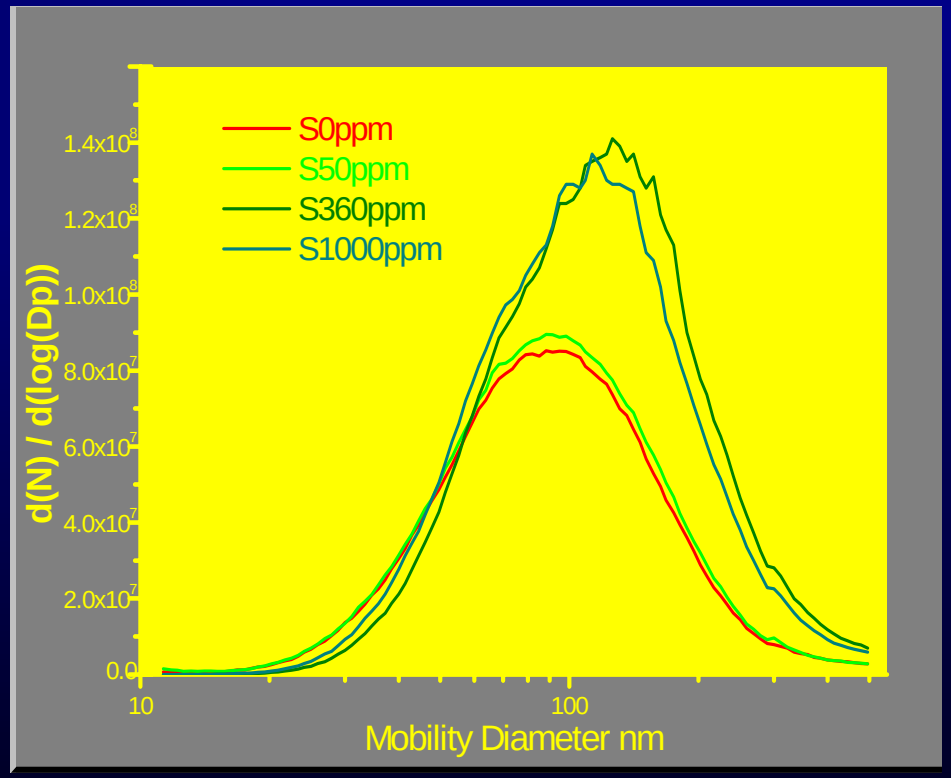
Study of ROS occurrence in Cell culture media exposed to diluted Diesel Engine emissions for 1 hour

Rotating continuous flow-through chambers
Vials with 1.5 ml of cell culture medium containing CPH
No biological samples in the vials
Medium frozen in liquid nitrogen and stored at –80°C untill ESR analysis

ROS trapping Followed by ESR Measurements
Impact of Sulfur in Fuel (Additivation of Dis0 fuel)

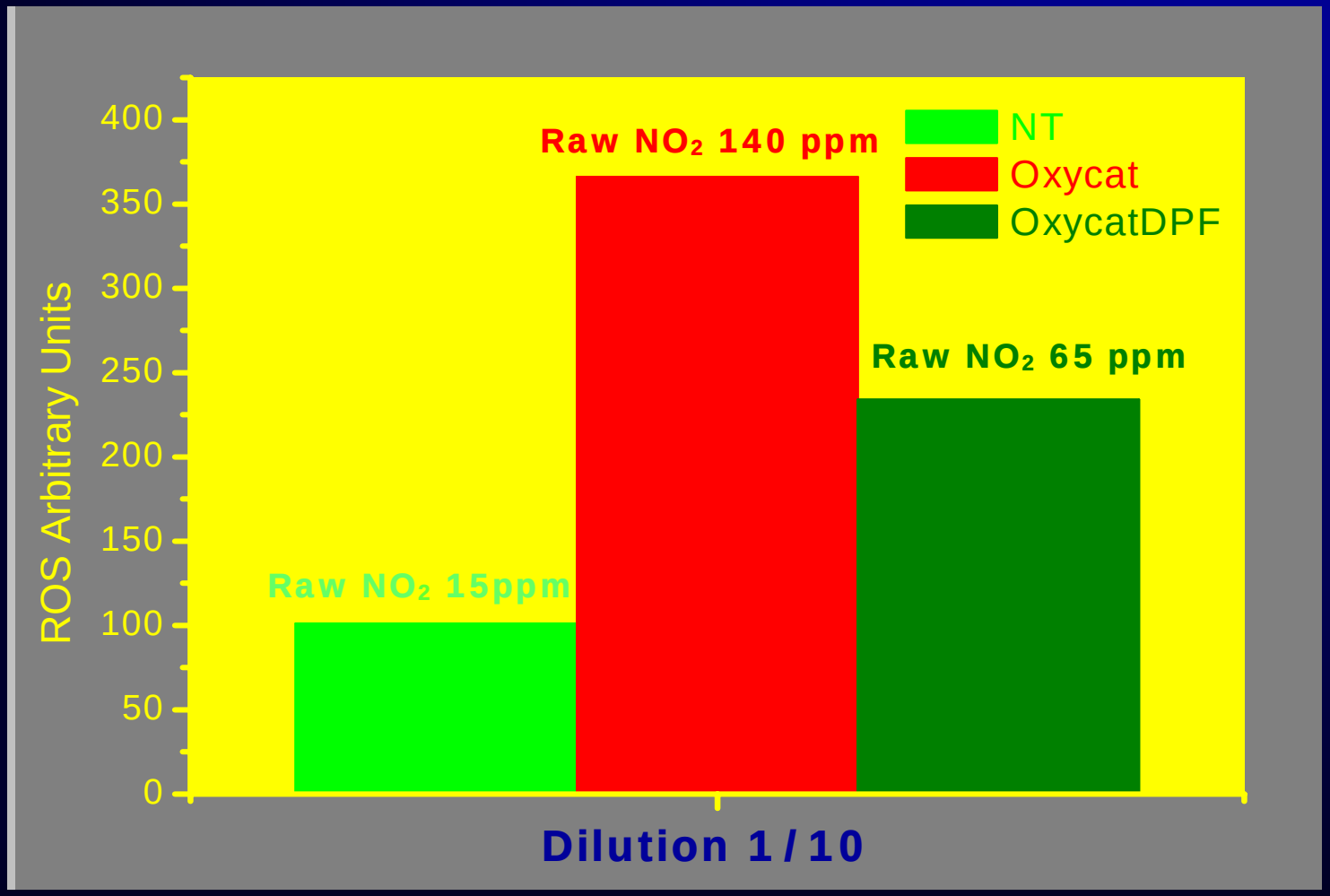
Sulfur Mix | Dibenzothiophene 38%
Benzothiophene 31%
Di-terbutyl-disulfide 31%



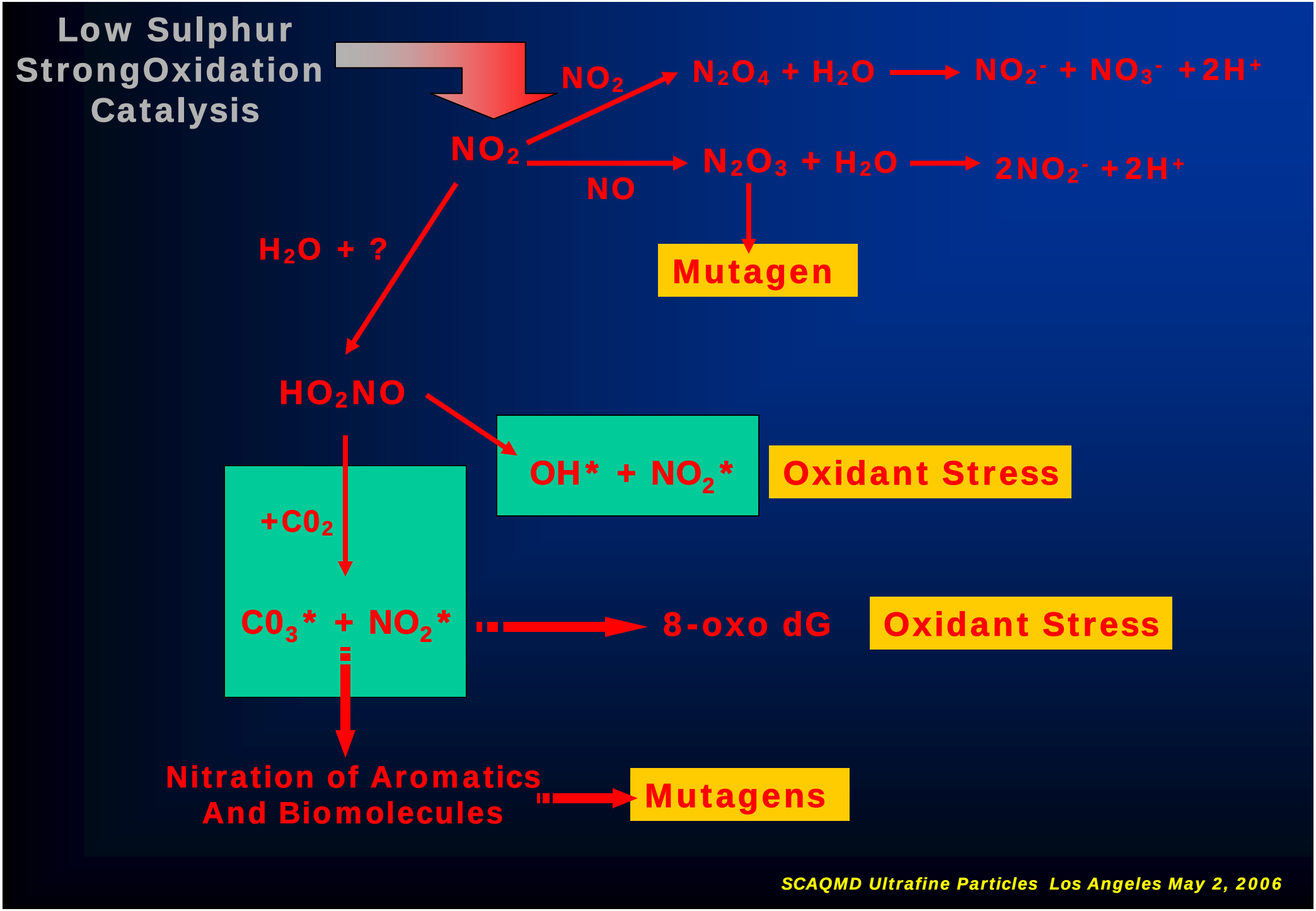


Monocylinder Diesel PowerEngine

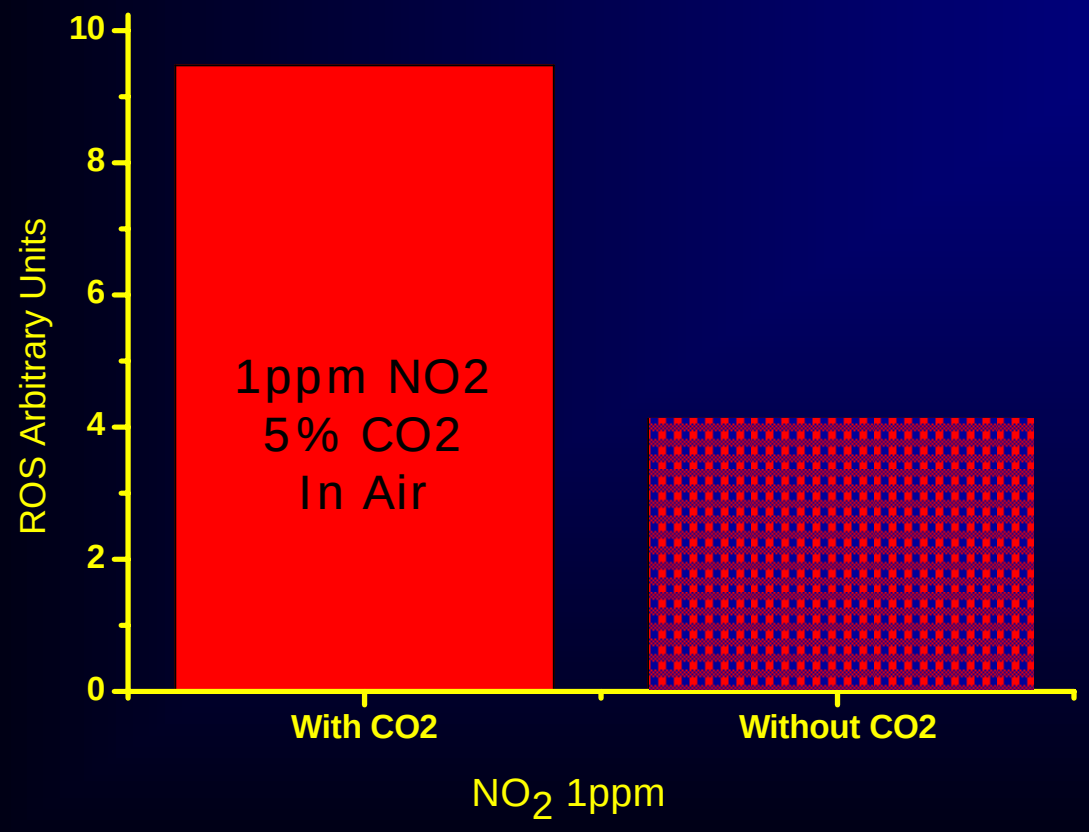
ROS trapping into liquids and ESR Measurements
Impact of After-Treatment (Dis0-LowSPash)



Euro 3 type 4 cylinder Engine


Low Sulphur
StrongOxidation
Catalysis
NO_2
$N_2O_4 + H_2O \longrightarrow NO_2^- + NO_3^- + 2H^+$
NO_2
$N_2O_3 + H_2O \longrightarrow 2NO_2^- + 2H^+$
NO
Mutagen
$H_2O + ?$
HO_2NO
$OH^* + NO_2^*$
Oxidant Stress
$+CO_2$
$CO_3^* + NO_2^*$
8-oxo dG
Oxidant Stress
Nitration of Aromatics
And Biomolecules
Mutagens

Can NO_2 Gas Produce ROS detected by CPH ?
Can CO_2 modulate NO_2 induced ROS Production?
Can NO_2 alone Account for ROS Oxycat Impact ?



NO_2 exerts a clear Oxidant Impact

CO_2 modulates NO_2 induced ROS Production

NO_2 might account for >50% of Oxycat induced ROS Occurence

Conclusion

ROS formation appears to be highly correlated to
NO_2 emission concentrations

Oxidant Damage appears to be highly correlated to
NO_2 emission concentrations

New Diesel Emission

PM Filtration on Sampling line <u>does not Modify</u> Toxic Profile
PM seem to account for « minor toxic impact » compared to gas phase

NO_2 : Marker of Diesel Emission Oxidant Potential ?
NO_2 : Main Toxic Trigger of New Technology Diesel Emissions ?

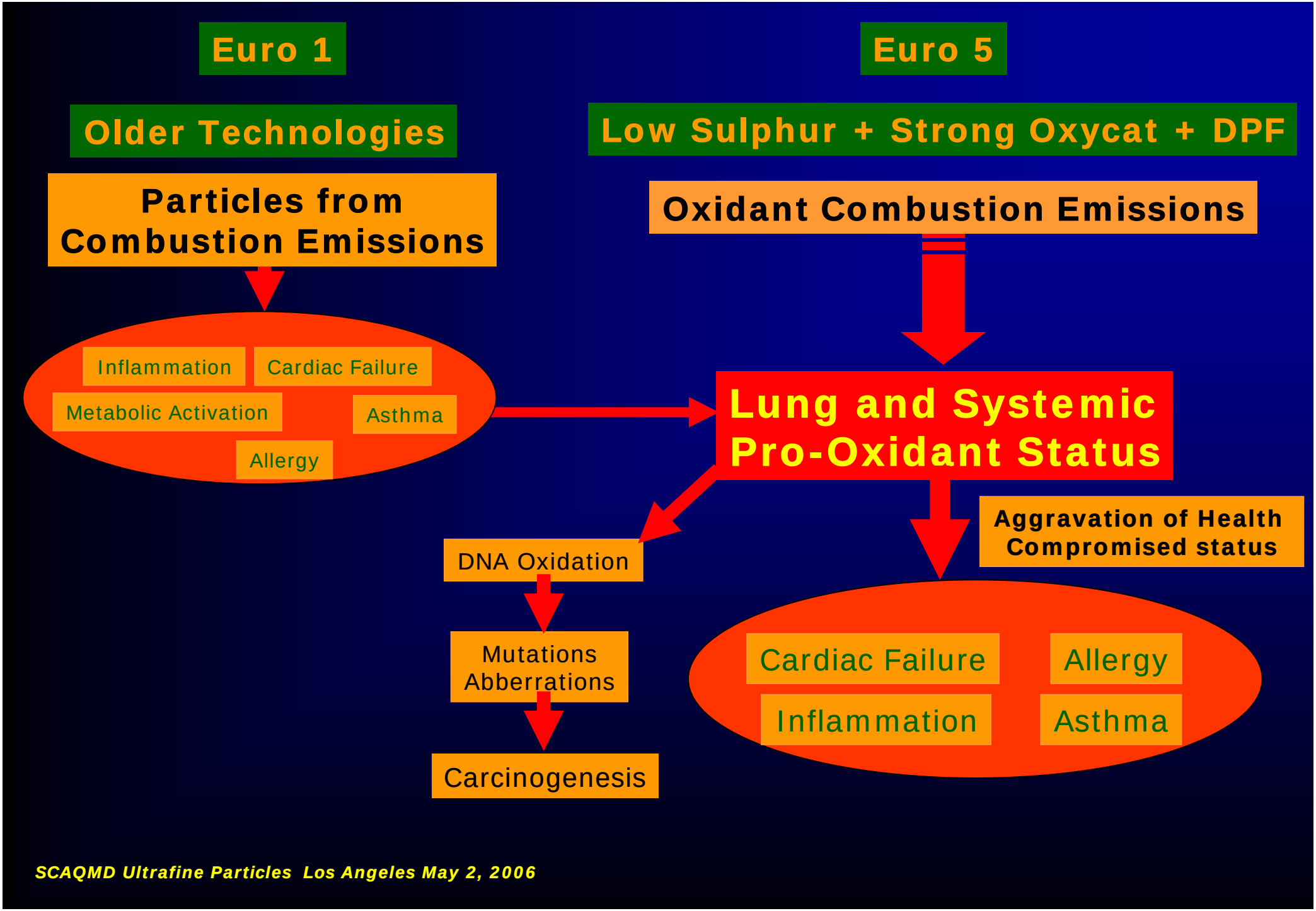
NO_2 increased emissions : a Multifactorial Process

Reducing fuel sulphur content
Using stronger oxidation catalysis
Engine Medium/Low Speeds/Loads

NO_2/Nox ratio
NO_2
ROS Transfer

	No After-T High Sulfur	Oxycat CO/HC Medium Sulfur		Enhanced Oxycat DPF Low Sulfur
Regulation	**Euro1**	**Euro3**		**Euro5**
NO_2/NOx	**<0.1**	**0.2**	**0.4**	**> 0.5**
NO_2	**<50ppm**			**Up to >500ppm**

Oxidant Potential And Toxic Injury


Euro 1
Euro 5
Older Technologies
Low Sulphur + Strong Oxycat + DPF
Particles from Combustion Emissions
Oxidant Combustion Emissions
Inflammation
Cardiac Failure
Metabolic Activation
Asthma
Allergy
Lung and Systemic Pro-Oxidant Status
Aggravation of Health Compromised status
DNA Oxidation
Mutations Abberrations
Carcinogenesis
Cardiac Failure
Allergy
Inflammation
Asthma

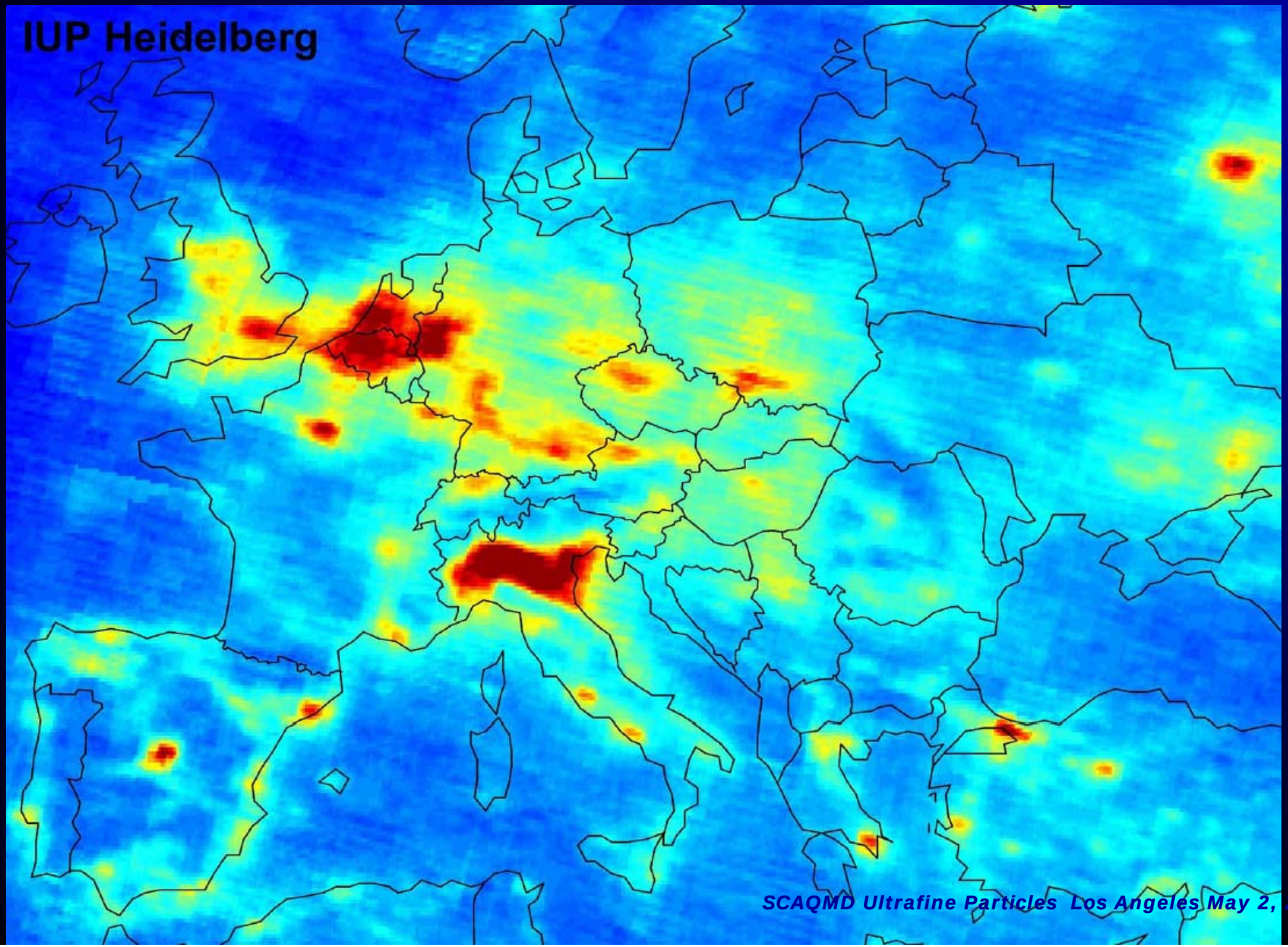
POLICY PROSPECTIVE

New Diesel Engine Emissions After-Treatment Comply
With PM and NOx Current Regulation and Trends

Increased NO_2 /NOx ratio and NO_2 in Emissions
Result from New Diesel Engine
after-Treatment Strategies (Oxidation Catalysis)
Which Trigger Health Impact Concerns
<u>not Addressed by NOx monitoring</u>

As for Air Quality Guidelines (WHO)
NO_2 Emissions Which become a Major Concern
should be monitored and included for Emission Regulation

NO2 over Europe Satellite View



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