

The health impact of Diesel engine emissions is most frequently associated with the sole impact of particulate matter (PM) which have been classified as air toxic. In this respect, a vast majority of Diesel emission health impact studies do not consider the gaseous phase of the aerosol as a potential toxicity trigger.

The aim of the present study was to assess the respective lung toxicity contributions of PM phase and gaseous phase of Diesel engine emissions. This study was conducted in vitro on rat lung tissue in organotypic cultures exposed in a bi-phasic system to continuously sampled and diluted Diesel engine emissions with highly preserved physicochemical properties as described by Morin et al. (1999) LePrieur et al. (2000) and Bion et al. (2002).

Methods :

The engine used for these experiments is a 2l common rail direct injection turbocharged intercooled Diesel engine which is operated at various speeds, and loads, using after treatment devices and different fuels (sulphur contents) in order to modulate emission physicochemical compositions as assessed by regulated emission measurements. Soot content and size distribution were measured using an AVL415 reflectometer and SMPS respectively. CO, CO₂, NO, NO₂, HC and O₂ were measured using a Horiba Mexa 7000 analyser.

As previously described, rat lung slices organotypic cultures maintained in flow through rotating chambers were exposed in parallel to continuous flows of either clean air (control) or diluted emissions (10 to 30%).

To evaluate the respective effects of gases and PM on rat lung slices, more than 99.99% of DEPs are removed from the test atmosphere thanks to filter placed on the sampling line downstream the sampling line. This filtration which allowed to remove soot from the emissions did not modify the gaseous fraction characteristics.

Typical exposure duration to diluted emissions was 3 hours as in previously published studies. Three main biological pathways and DNA alterations were assessed in this study.

ATP and total glutathione intracellular levels were assayed from lung slice perchloric extracts using ATP Bioluminescence HSII kit (Roche Diagnostics) and according to Mistry et al. 1991 respectively.

Enzyme activity assessment : Slices were washed twice in ice cold Tris/NaCl buffer and homogenized in 0.5ml of buffer using a glas/glas Kontess tissue grinder. Superoxide dismutase (SOD EC-1.15.1.1), glutathione peroxidase (GPX EC-1.11.1.9), catalase (CAT EC-1.11.1.6) and glutathione-S-transferase (GST EC-2.5.1.18) activities were assayed according to established methods (Crapo (1978), Wendel (1981), Aebi et al. (1974) and Habig et al. (1974) respectively).

Tumor necrosis factor alpha (TNF α) concentrations were assayed in culture medium using the rat TNF α ELISA kit (Endogen)

Tissue nucleosome content were assayed in lung slices using the Rat Nucleosome ELISA kit (Amersham).

Results :

While total NO_x did not differ to a large extent according to the experimental condition, the most striking feature was the wide range of NO₂/NO ratio variations from 0.06 up to 1.16. Although NO₂ may act per se, we have considered the NO₂/NO ratio as a marker of the emission oxidizing potential and have classified experimental situation as exhibiting low, moderate and high oxidizing potentials, highest oxidizing potential being evidenced downstream highest Oxicat potency.

Characterization of ROS occurrence during contact of liquid medium containing CPH (spinprobe) with continuous flow of diluted emissions confirmed this emission oxidizing potential which we found to be highly correlated with NO₂ concentrations in the emissions.

We analysed the NO₂ potency to mimick diluted emission impact on ROS occurrence and showed that NO₂ diluted in air could promote the occurrence of ROS in a similar way, which could be modulated by CO₂, thus pointing according to the chemistry of NO₂ to the occurrence of peroxynitrite degradation with OH^{*}, NO₂^{*}, and CO₃^{*} formation which are highly oxidizing ROS that could play a very active role in observed oxidant injury.

Lung slices

Lung slices experiments showed toxicity pattern variation according to emission oxidant potential. The highest NO₂ and NO₂/NO_x ratio being more toxic leading to high oxidant stress and lung tissue viability loss. With high NO₂ and NO₂/NO_x, sample filtration did not change the tox profile, meaning that gas phase components and not PM was then triggering toxicity independent of PM content.

Experiments performed with NO₂ in air showed that NO₂ could mimick high oxidant emission toxicity pattern, with however only a partial intensity compared to Diluted Diesel emissions.

In vivo Oxidant stress

We compared the impacts of NO₂, non catalyzed emissions doped with NO₂ and catalysed emission impacts on systemic inflammation (TNF α), systemic oxidant stress (liver, lung and heart antioxidant defenses) and electrocardiogram. Two animal models were used : Healthy rats (Sham) and rats undergoing chronic heart failure due to myocardial infarction (MI).

Impacts were clearly more severe in MI rats compared with sham rats for inflammation, Concerning oxidant stress, impacts on Superoxide dismutase were higher downstream oxicat for sham rats where NO₂ alone or doped emissions appear to be less active than oxicat emissions. For MI rats, NO₂ doped emissions and oxicat emissions were more efficient than NO₂ alone. This points that NO₂ is probably not the only trigger in these situation.

As far as Glutathione peroxidase is concerned, NO₂ alone, NO₂ doped emissions and oxycat emissions had roughly similar impacts taking into account that basal levels in unexposed rats were higher in MI rats than in sham rats. NO₂ could be assigned for most of the impact on GPX.

It is of interest to notice that oxidant injury is not restricted to respiratory system, but also tackles other organs, primarily heart and to a lesser extent liver.

In vivo Cardiac impacts in chronic heart failure :

A clear impact of NO₂ and engine emissions can be observed on heart rate variability parameters. Reduced RMSSD is observed with untreated emissions while increased RMSSD could be evidenced downstream oxicat and NO₂ doped emissions.

Oxycat emissions were inducing bradycardia while this was not observed in other experimental situation. Air containing NO₂ was producing an intermediary but not statistically significant impact on Heart rate and RMSSD.

Concerning arrhythmia induction, a clear impact on premature ventricular beats was observed downstream doped emissions and oxicat emissions which was barely sen with NO₂. A major impact of Oxycat was seen on bigeminy induction, tachycardia and bradycardia events, which were not observed with NO₂ in air and NO₂ doped emissions.

Interstingly, these responses were of high amplitude at day one of exposure and “adaptative process is progressively taking place at day 2 and 3, appearing to be able to take over the injury. This observation suggests the occurrence of some adaptation which could result from the antioxidant defense tuning which is described in both lung and heart tissue.

Discussion

Oxidation catalysis appears to induce detrimental cardiorespiratory health impacts that can to some extent be mimicked by NO₂ as far as inflammation, and systemic oxidant stress are considered. As far as electrocardiography is concerned in myocardial infarcted rats, the impact of oxycat treated emissions appear to be far more detrimental, these injuries could not be directly reproduced by NO₂ to a significant extent. The ECG impacts are similar to those we could induce by either mitochondrial blockers or strong oxidants like ozone and suggest that strong oxidant acting molecules might be generated upon exposure to Oxycat treated emissions which might have been probed by ESR experiments.

It appears clearly that due to technical requirements for oxidation catalysis for the attainment of emission regulatory levels from Diesel engines, a cleracut impact on emission oxidant potential shows up which leads to unexpected and not anticipated primary NO₂/NO_x shares. Numerous reports are pointing to increased NO₂ atmospheric concentrations close to the traffic which in the view of our results may bring new health related issues for the population living close to high traffic density areas. We are also pointing to population exposure when being inserted in the traffic either when driving cars or being bus passengers. Preliminary experiments from our group measuring air quality in car cabins showed NO₂ concentrations/minute entering cars up to 1500 µg/m³, mean hour NO₂ concentrations for mixed traffic typology routes including urban, periurban, highway and rural contributions of 250-300 µg/m³ which are above thresholds of air quality guidelines.

Taking into account the carpark high penetration with light duty Diesel cars and vans, the prospective evolution of oxidation catalysis trends towards higher efficiency for DPF handling and the absence of mature technologies for NO_x nor NO₂ reduction at least for light duty engines, we are warning to new prospective health impacts of Diesel emissions in close correlation with the technical choices rendered necessary for the attainments of successive regulated pollutant emission levels reductions from Euro1 to Euro4 and prospectively Euro 5. This should be taken into account by regulatory authorities in order to take measures to not only control NO_x, but control NO₂ emissions or NO₂/NO_x shares from Diesel engine emissions. For both light duty and heavy duty vehicles.

**From Particulates to NO₂ as health concern triggers
from Diesel engine emissions.
A link with emission after-treatment strategies.**

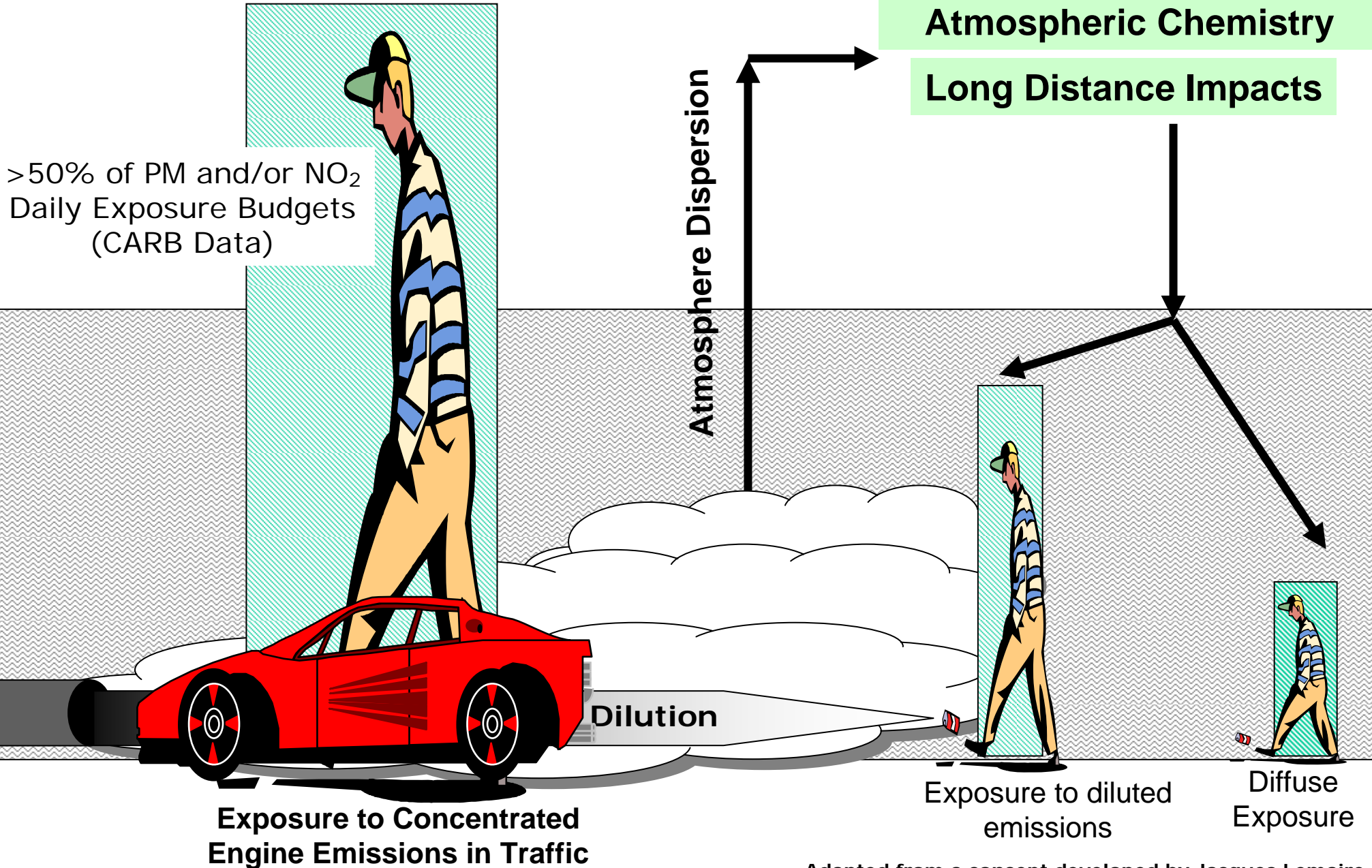
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Xth ETH Zurich August 2006

Exposure Levels to Traffic Emissions



Statements :

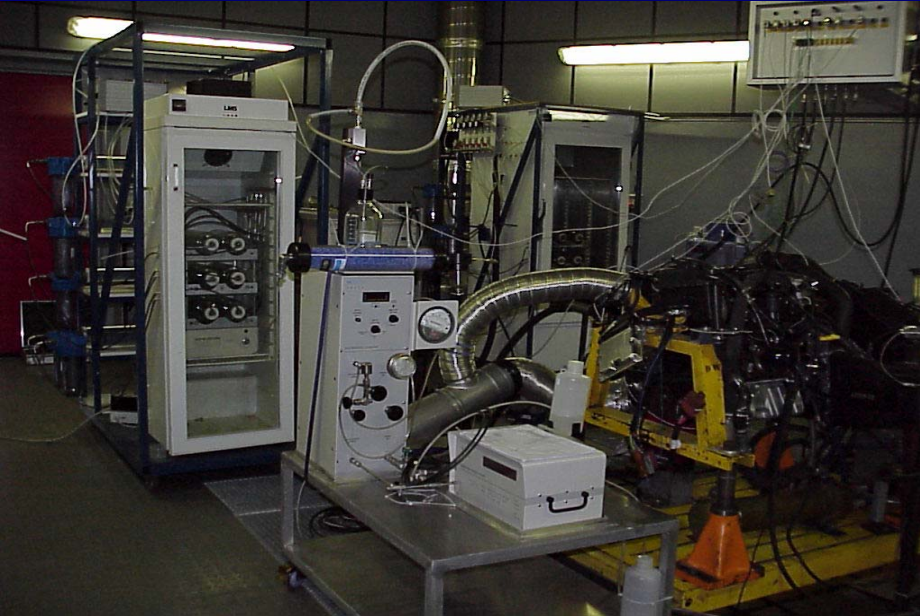
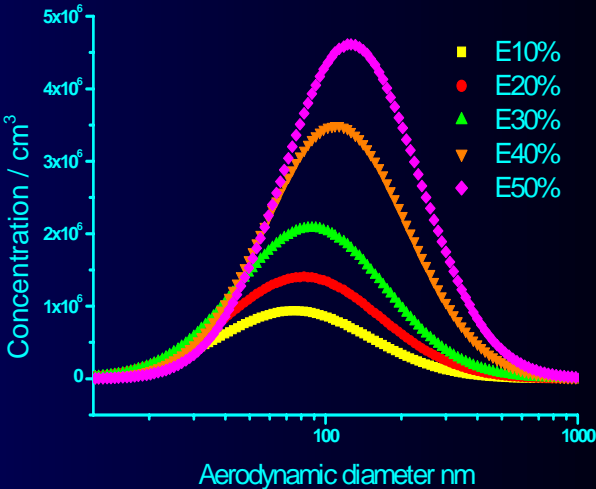
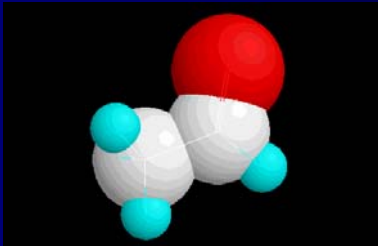
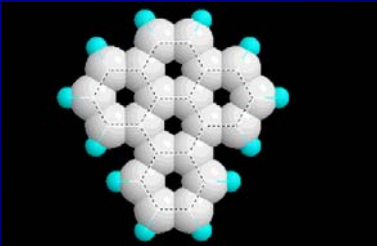
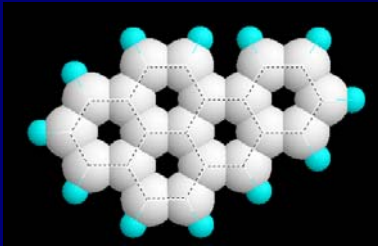
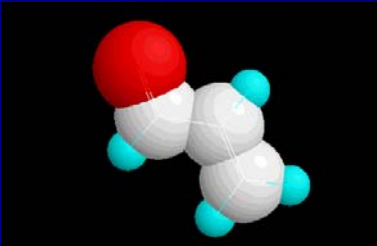
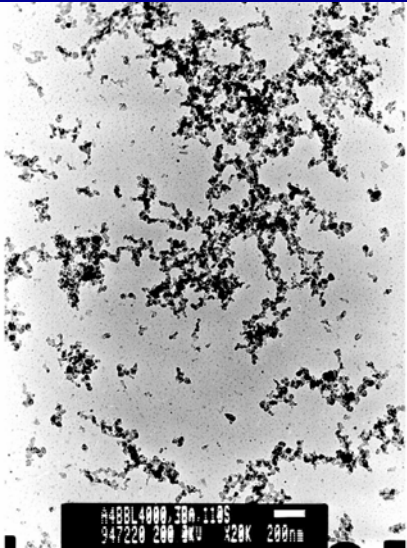
We do Inhale Complex Aerosols and Not Only Ultrafines
We do Inhale Freshly Emitted Tail Pipe Emissions
accounting for significant daily exposure contribution

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



2 | Euro3 Common Rail DI steady state operation 2250 rpm Half load Exhaust Characteristics (Gas Phase)

Post treat	NT		Oxycat +		Oxycat ++ (CRT)	
	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	668	465	755
NO ₂ (ppm)	23	45	106	291	230	440
NO (ppm)	422	735	312	377	235	335

NO₂/NOx

0.06

0.0.6

0.25

0.45

0.5

0.6

Oxidant
Potential



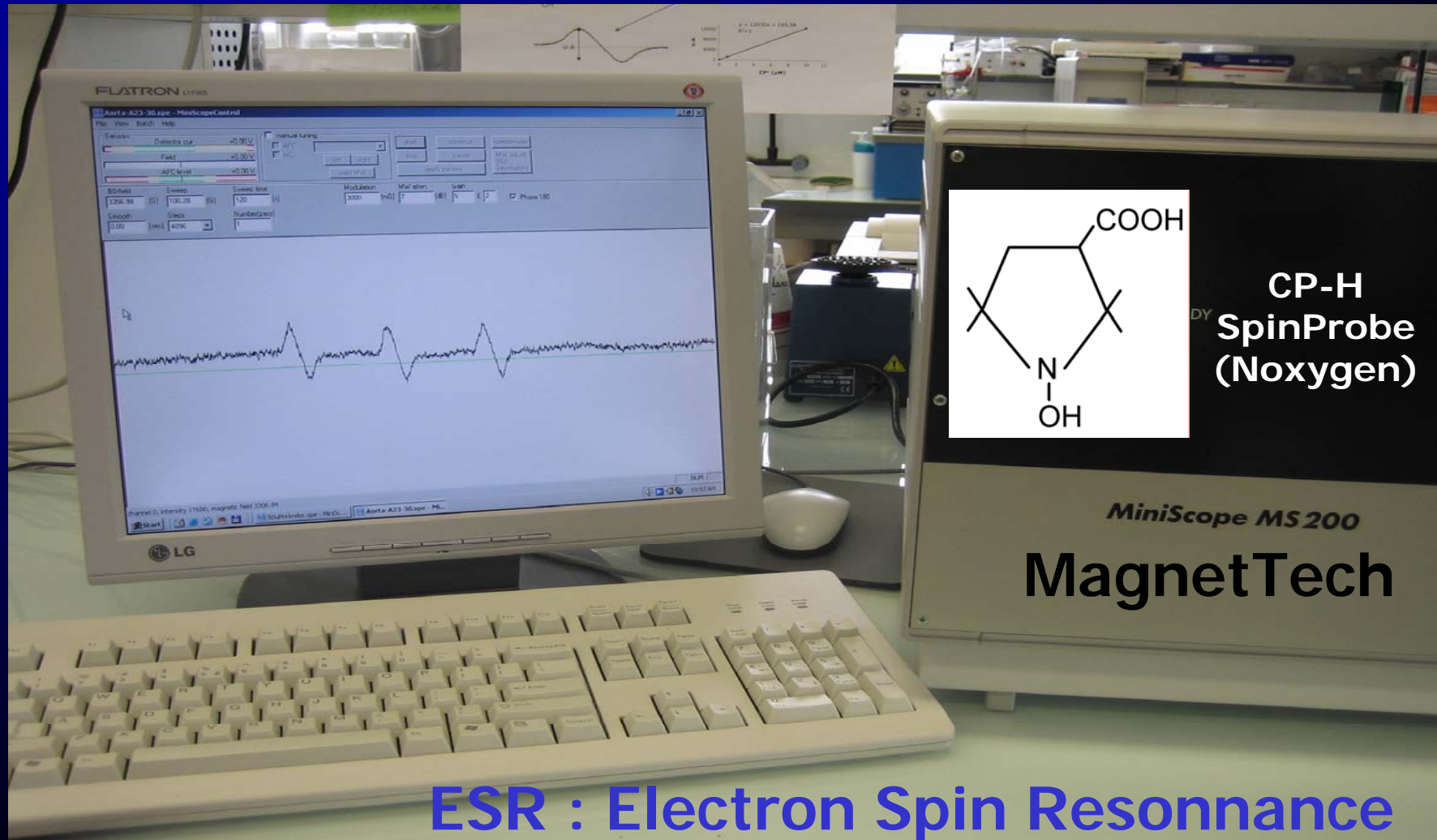
Comparative Impacts on Lung Slices *In Vitro*

	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	+	+	-	-	-
TNF _α	++	=	+ -Ⓢ	+ -Ⓢ	+ -Ⓢ
Nucleosomes	++	=	=	=	=
TUNEL	+++	=	=	=	=
DNA Ladders	++	=	=	=	=
8-OxoG	+	+=	+++	+++	++

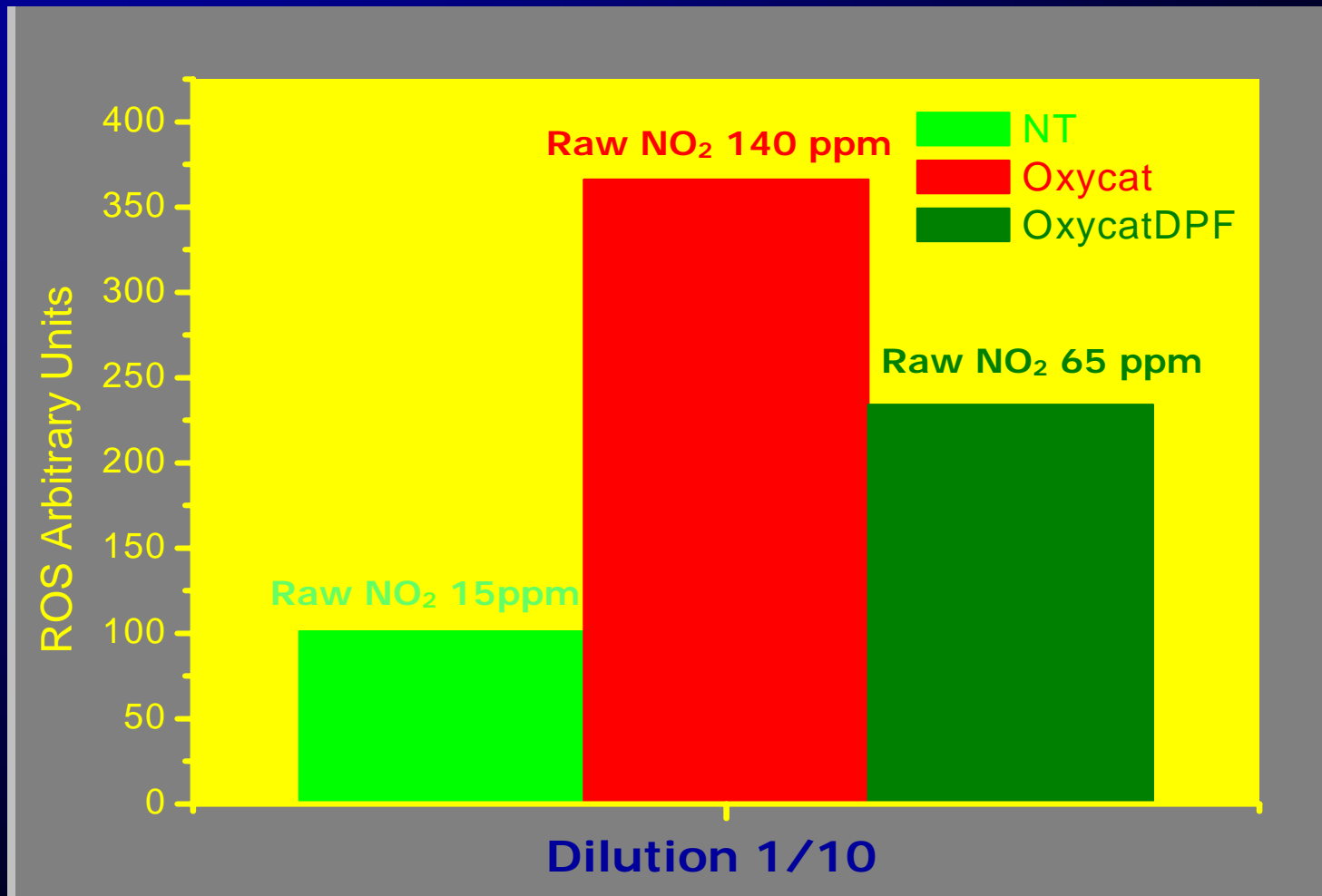
	Total	Filtered	Role
			NO ₂
			Major
			Role
			PM ?

	Gas Phase	Gas Phase
	↑	↑

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

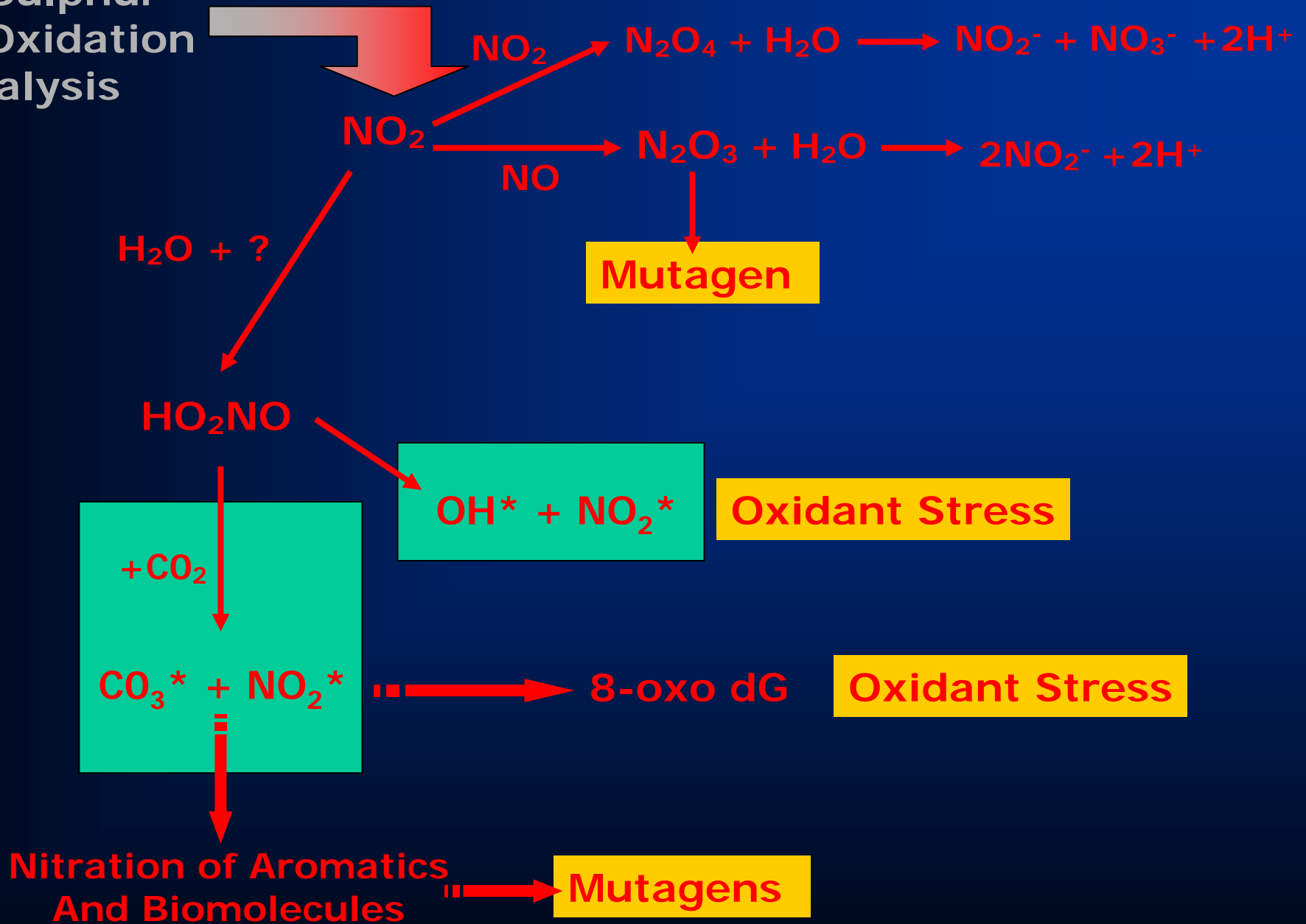


ROS trapping into liquids and ESR Measurements Impact of After-Treatment (Dis0-LowSPash)

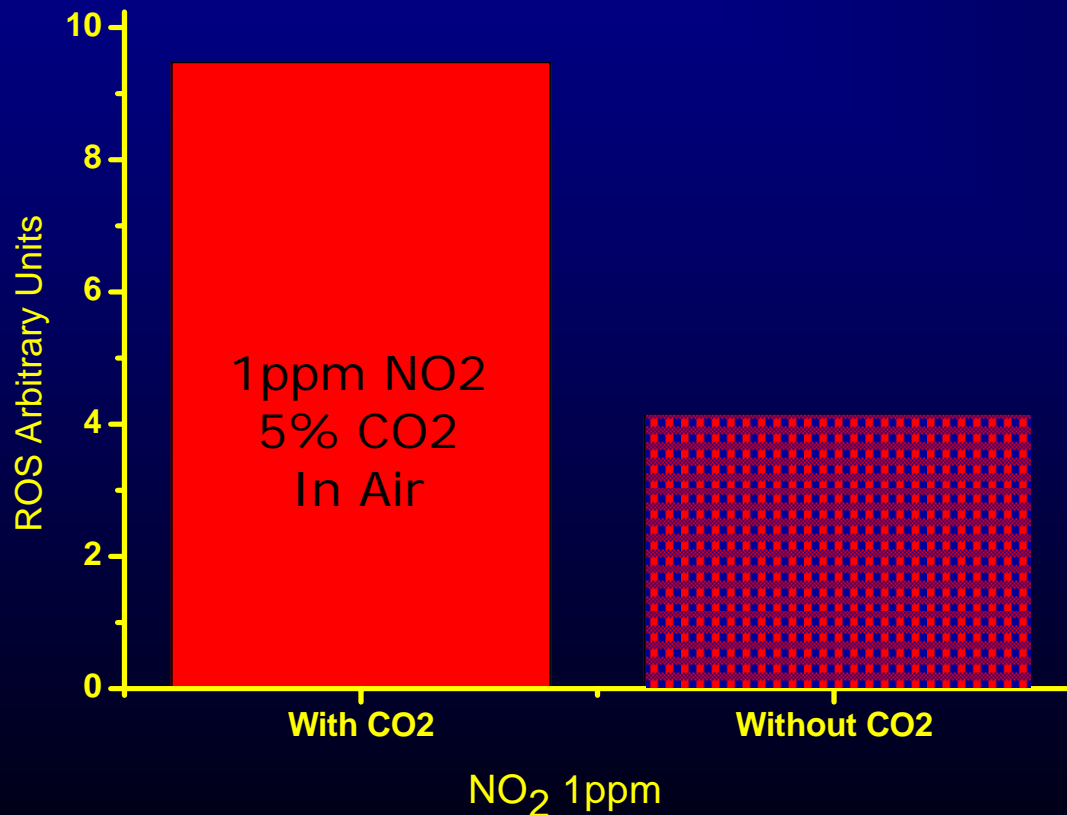


Euro 3 type 4 cylinder Engine

Low Sulphur Strong Oxidation Catalysis



Can NO₂ Gas Produce ROS detected by CPH ?
Can CO₂ modulate NO₂ induced ROS Production?
Can NO₂ alone Account for ROS Oxycat Impact ?

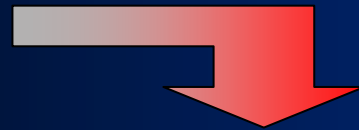


NO₂ exerts a clear Oxidant Impact

CO₂ modulates NO₂ induced ROS Production

NO₂ might account for >50% of Oxycat induced ROS Occurrence

Low Sulphur
Strong Oxidation
Catalysis



NO_2

Hypothesis for NO_2
Interaction with
Biological Fluids

H_2O

Yes

HO_2NO

$\text{OH}^* + \text{NO}_2^*$

Oxidant Stress

$+\text{CO}_2$

$\text{CO}_3^* + \text{NO}_2^*$

8-oxo dG

Oxidant Stress

Nitration of Aromatics
And Biomolecules

Mutagens

Conclusion

ROS formation appears to be highly correlated to
NO₂ emission concentrations

Oxidant Damage appears to be highly correlated to
NO₂ emission concentrations

New Diesel Emission With DOC

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

NO₂ : Marker of Diesel Emission Oxidant Potential ?

NO₂ : Main Toxic Trigger of New Technology Diesel Emissions ?

In vivo Inhalation Experimentations

Vigile unrestraint rats

- * 2 liters Euro3 Common rail DI engine
- * Steady state operation
- * Oxycat CRT type no DPF
- * Fuel 50ppm Sulphur

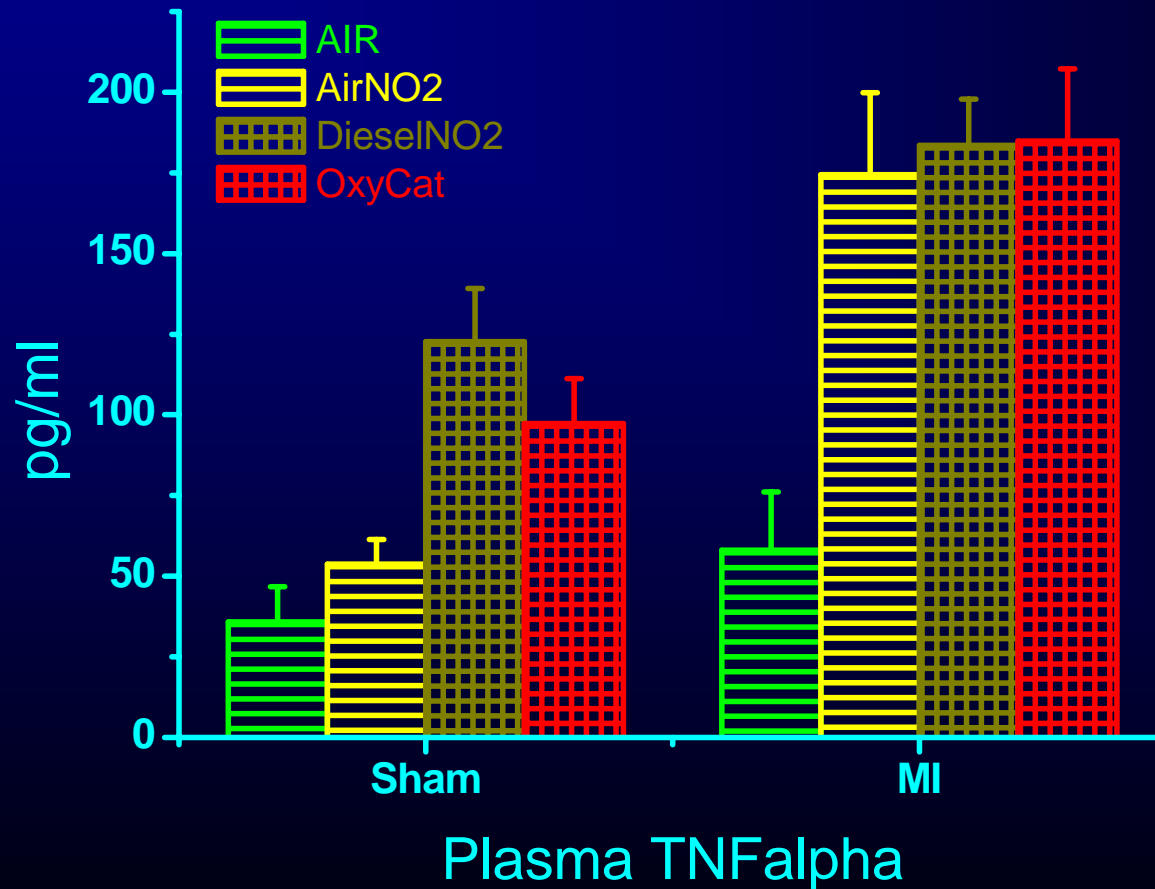
Engine emissions 1:50 dilution
PM 500 $\mu\text{g}/\text{m}^3$

Sham and CHF rats
Inhalation Exposure 3hours / Day
for 3 consecutive days

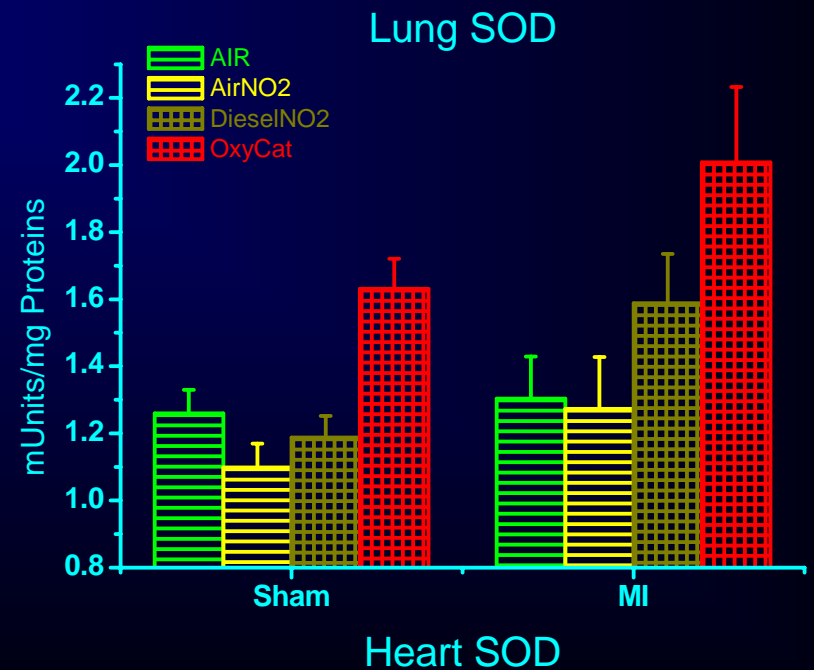
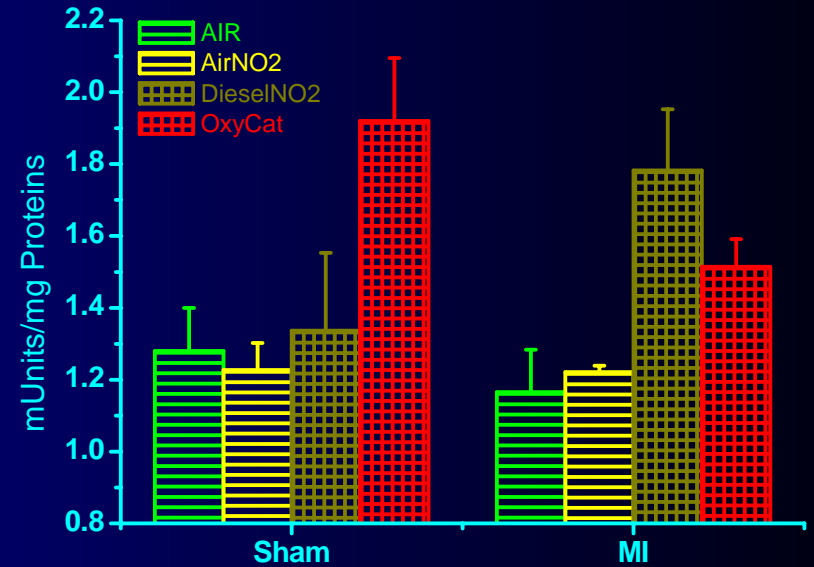
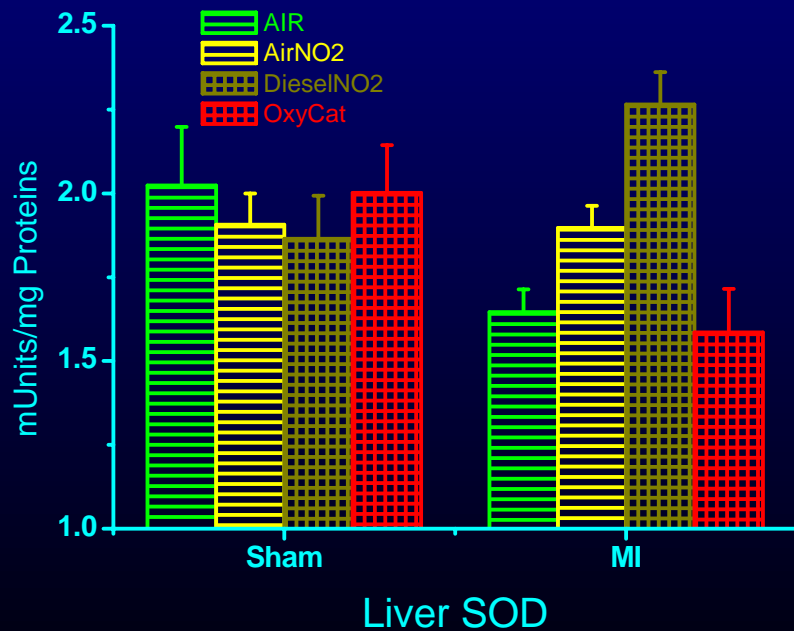
- Not treated emissions
- Oxycat
- Not treated emissions + NO₂
- Air + NO₂



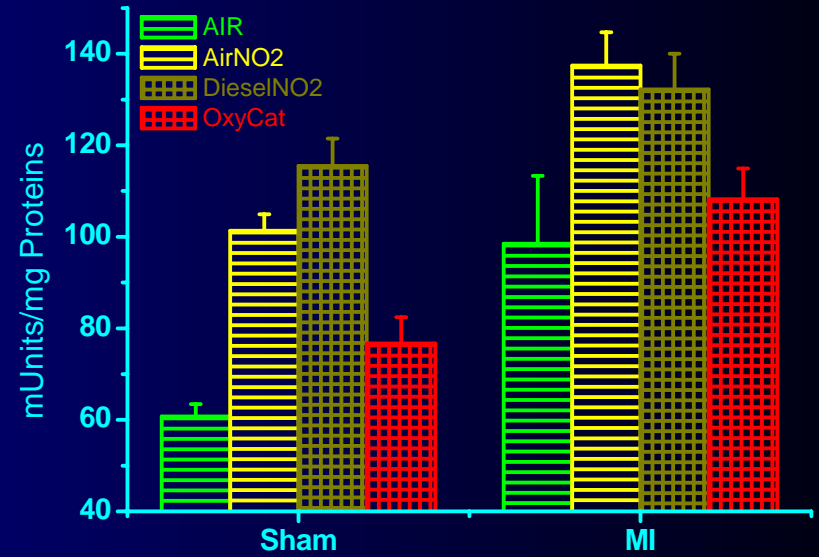
Impacts of NO₂, and Oxycat On Diesel Emission Inhalation Induced Plasma TNFalpha levels



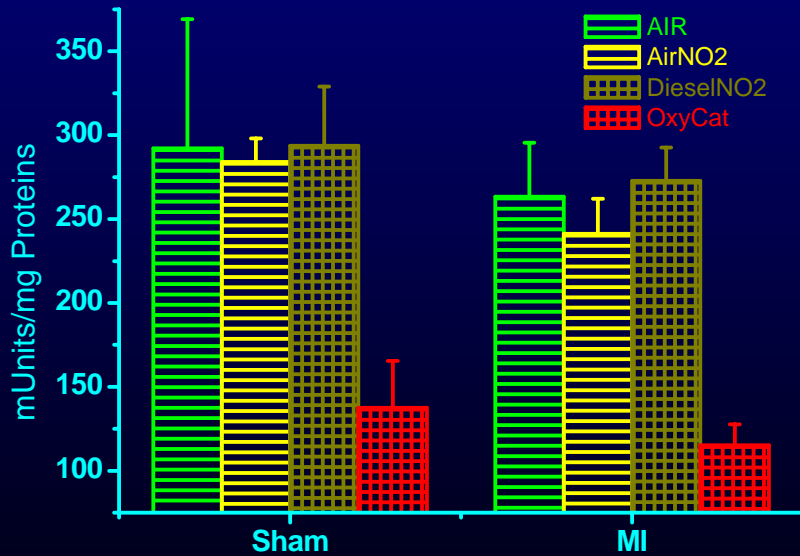
Impacts of NO₂, and Oxycat On Diesel Emission Inhalation Induced Systemic Oxydant Stress day3



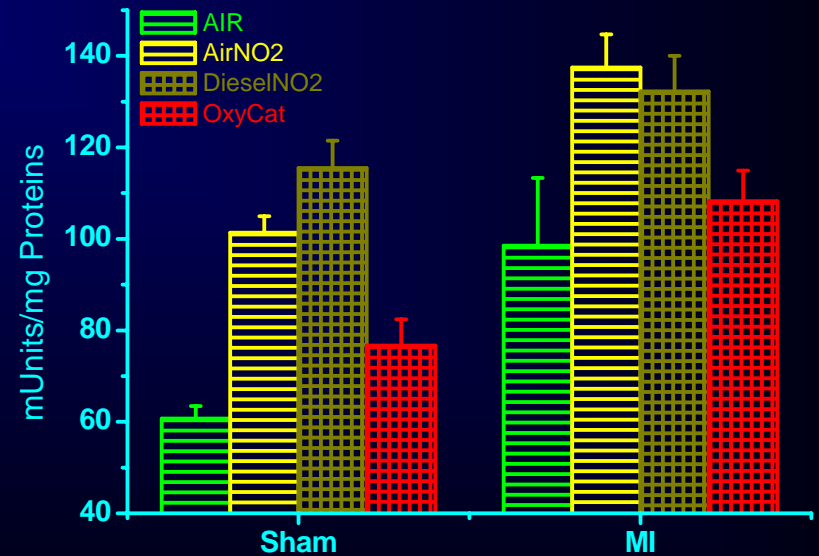
Impacts of NO₂, and Oxycat On Diesel Emission Inhalation Induced Systemic Oxydant Stress Day 3



Lung GPX



Liver GPX



Heart GPX

Impact on Cardiac Endpoints :

Chronic Heart Failure (MI >2months)

Continuous Electrocardiography Recordings by Telemetry

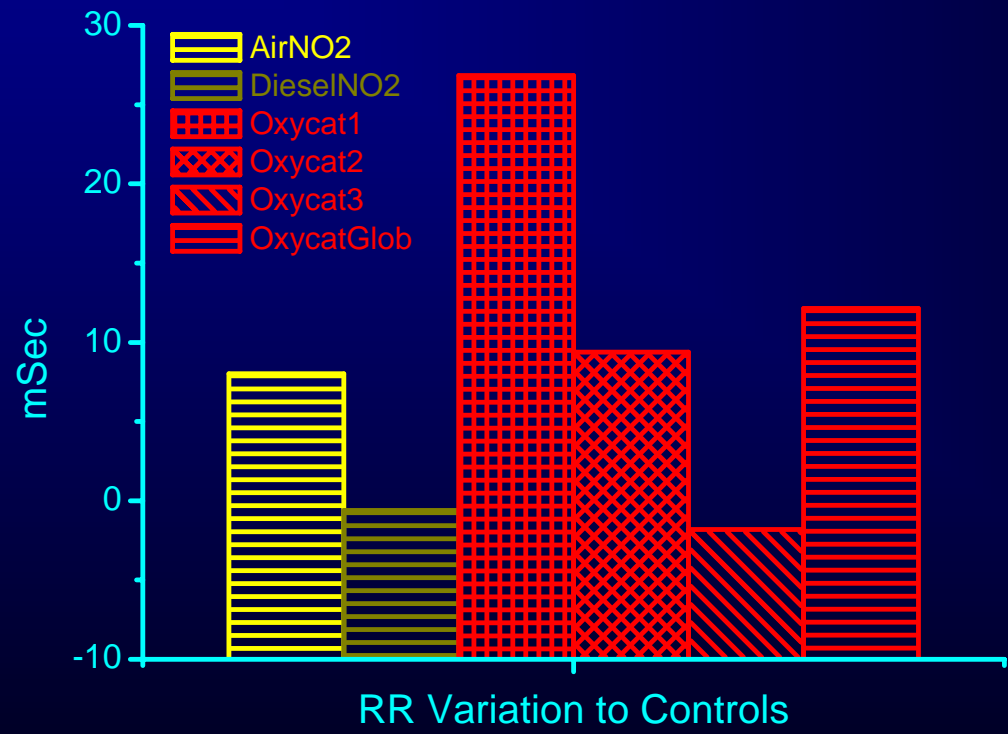
Heart rate by RR intervals

Heart rate variability assessment on sinus beats

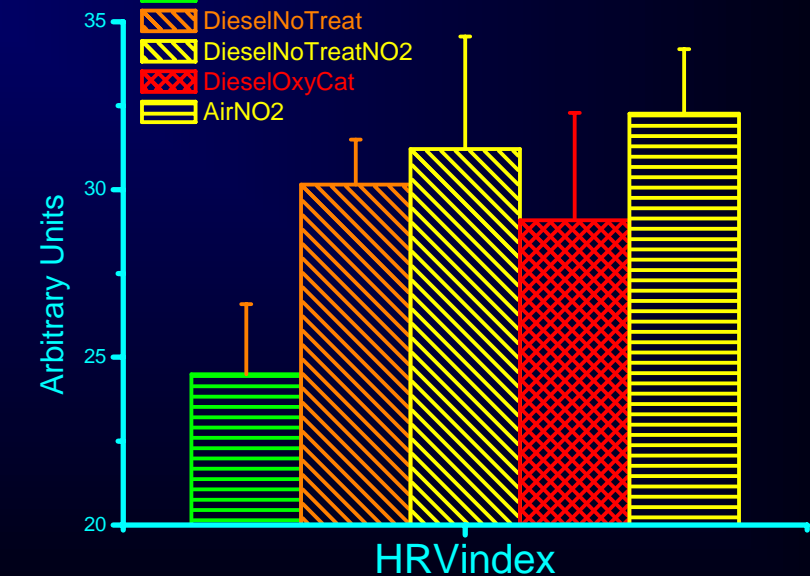
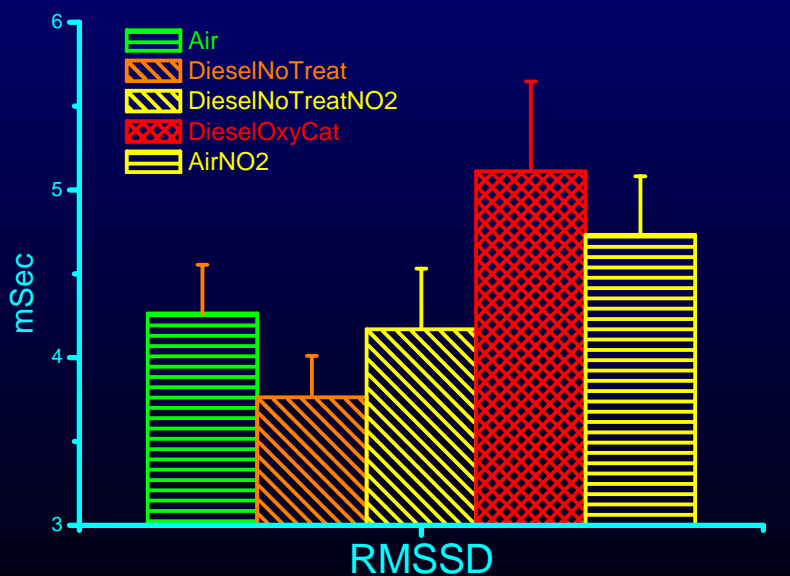
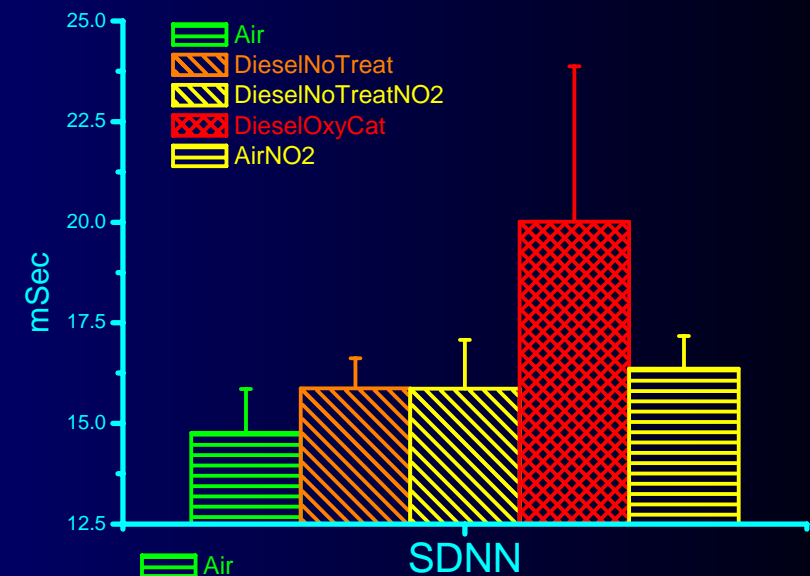
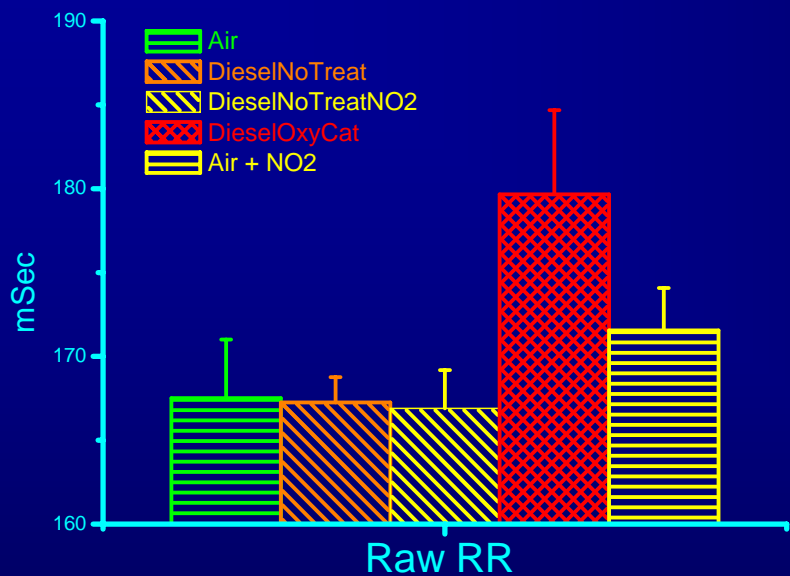
Search and quantification of arrhythmia events

(symbol dynamic coding approach in house software)

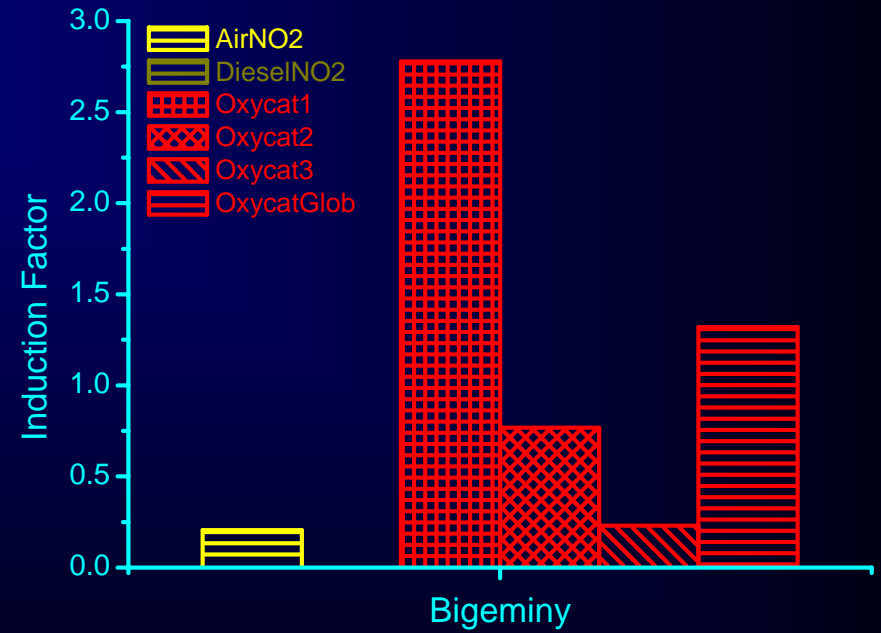
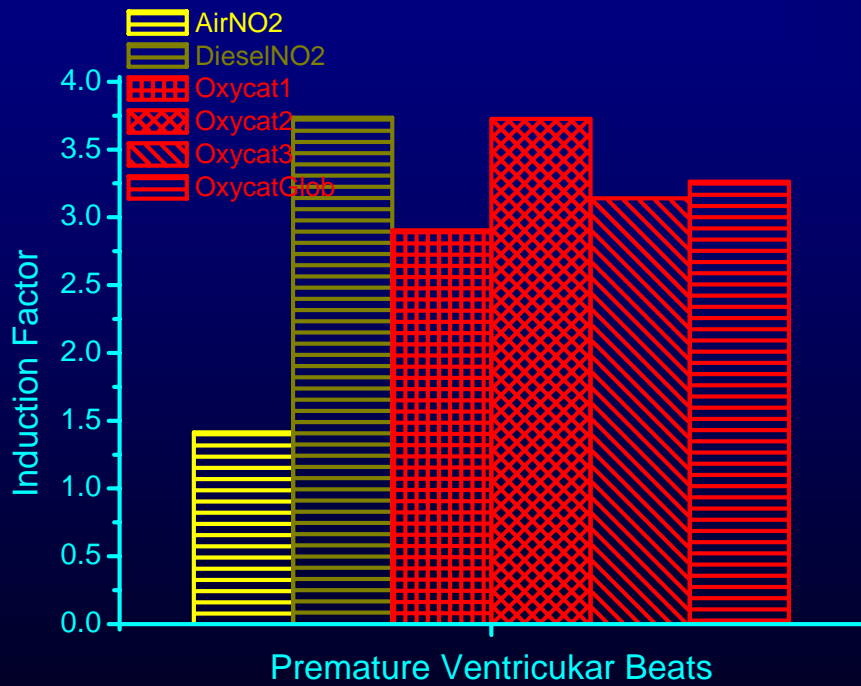
Impacts of NO2, and Oxycat on Diesel Emission Inhalation Induced Heart Rate Variations



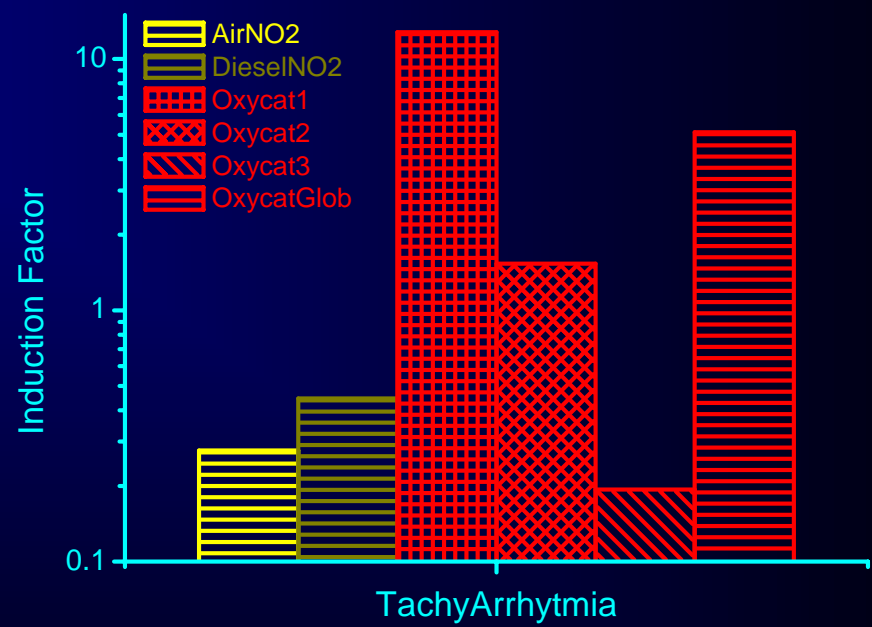
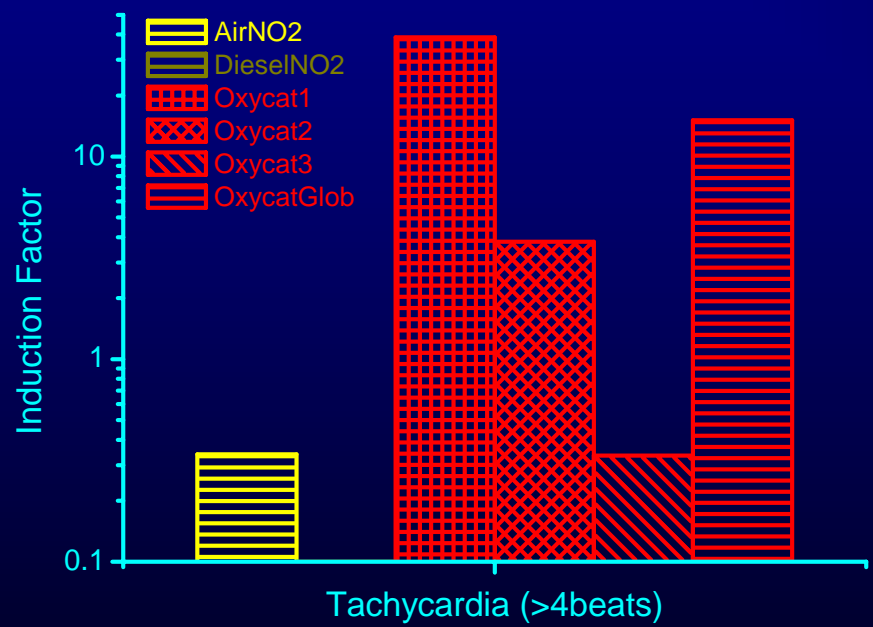
Impacts of NO2, and Oxycat on Diesel Emission Inhalation Induced Heart Rate Variability



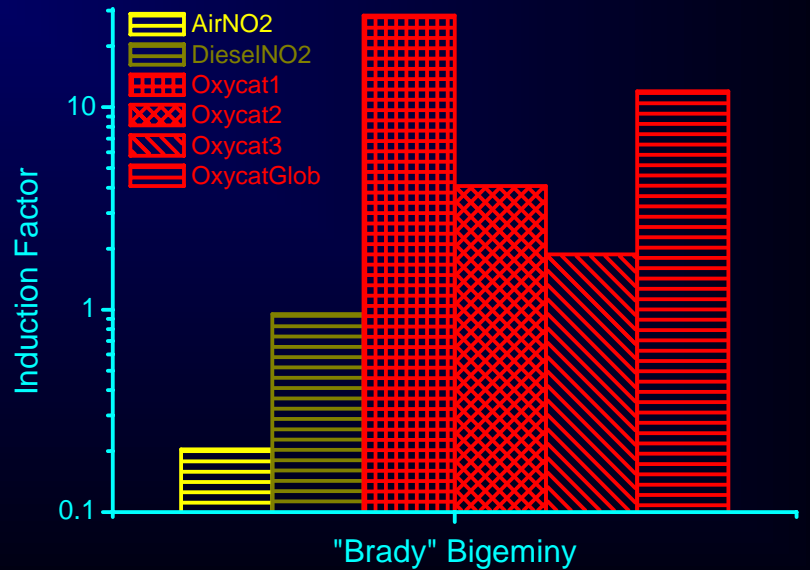
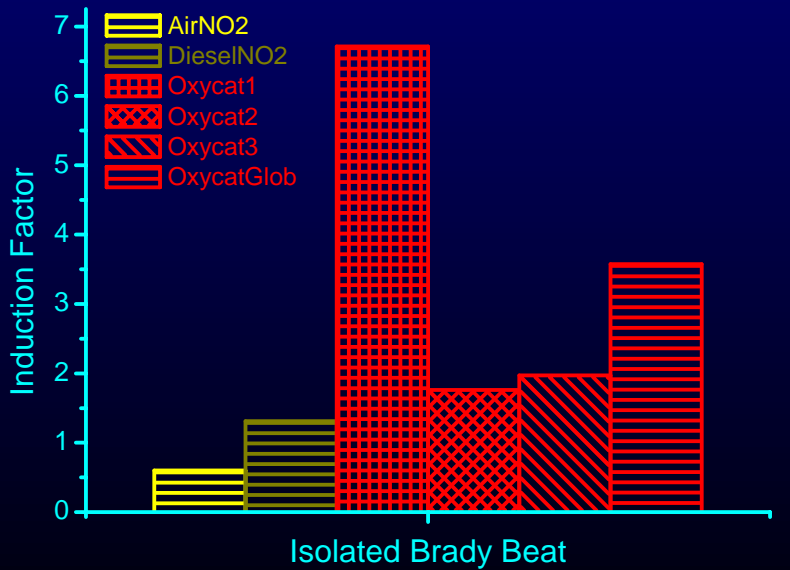
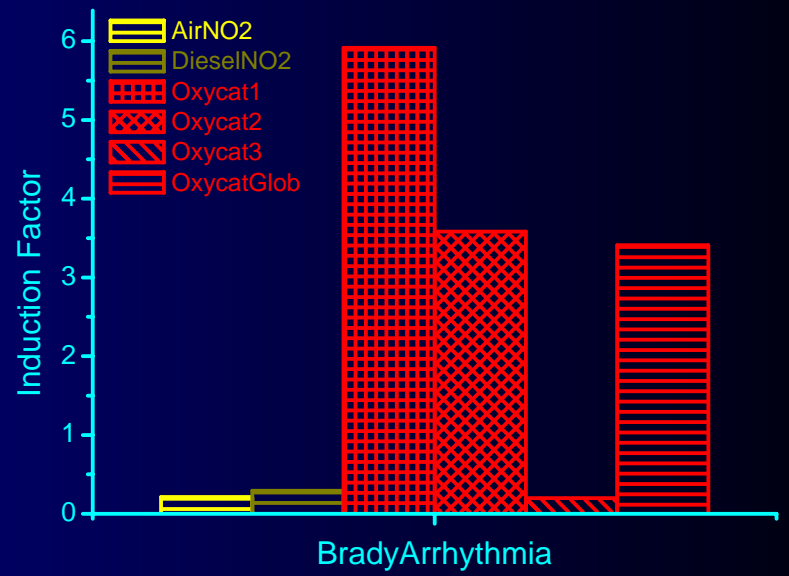
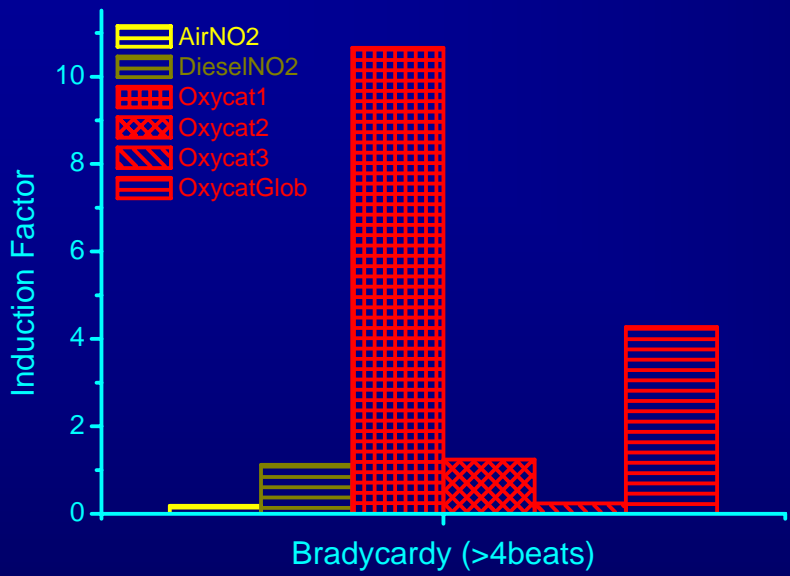
Impacts of NO₂, and Oxycat on Diesel Emission Inhalation Induced Heart Premature beats



Impacts of NO2, and Oxycat on Diesel Emission Inhalation Induced Heart Tachycardia Events



Impacts of NO2, and Oxycat on Diesel Emission Inhalation Induced Heart Bradycardia Events



Conclusion

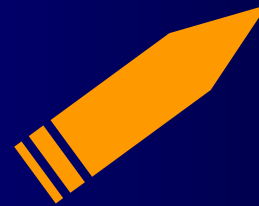
Diesel + Oxycat appears to induce most deleterious impact on electrocardiograms of exposed Chronic heart failure rats

While with untreated emissions, NO₂ alone, NO₂ doped emissions no impact of repeated exposure were observed, with Oxycat first exposure was more deleterious than the following ones suggesting adaptative process

Similar cardiac observations were made with other oxidant gases like ozone suggesting by analogy a very high oxidant potential in Oxycat treated Diesel emissions

NO₂ / Oxidant increased emissions : a Multifactorial Process

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



NO₂/Nox ratio
NO₂
ROS Transfer

Regulation	No After-T High Sulfur Euro1	Oxycat CO/HC Medium Sulfur Euro3	Enhanced Oxycat DPF Low Sulfur 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

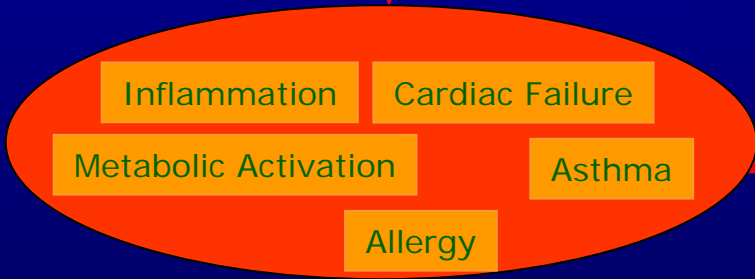
**Older Technologies
No after-Treatment**

**Particles from
Combustion Emissions**

Euro 5

Low Sulphur + Strong Oxycat + DPF

Oxidant Emissions



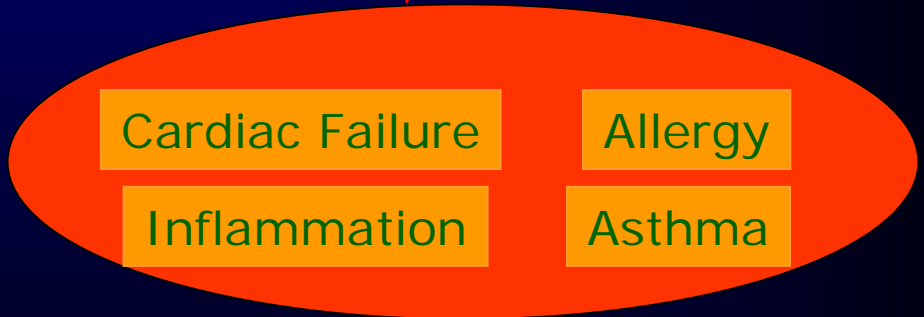
**Lung and Systemic
Pro-Oxidant Status**

DNA Oxidation

Mutations
Aberrations

Carcinogenesis

**Aggravation of Health
Compromised status**



Acknowledgements to Coworkers

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