

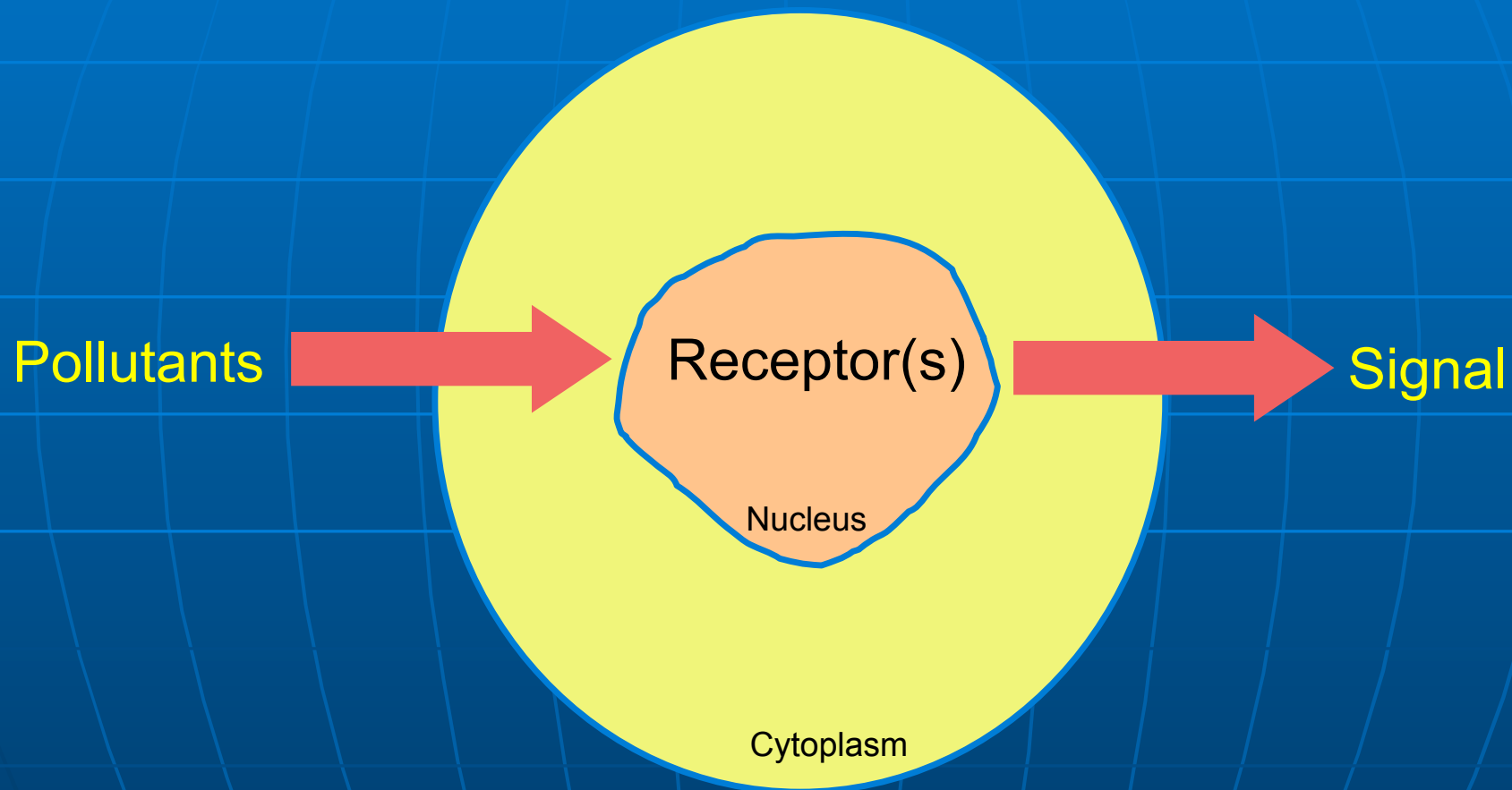
# Cell-based assays

Application to the detection of diesel  
exhaust pollutants

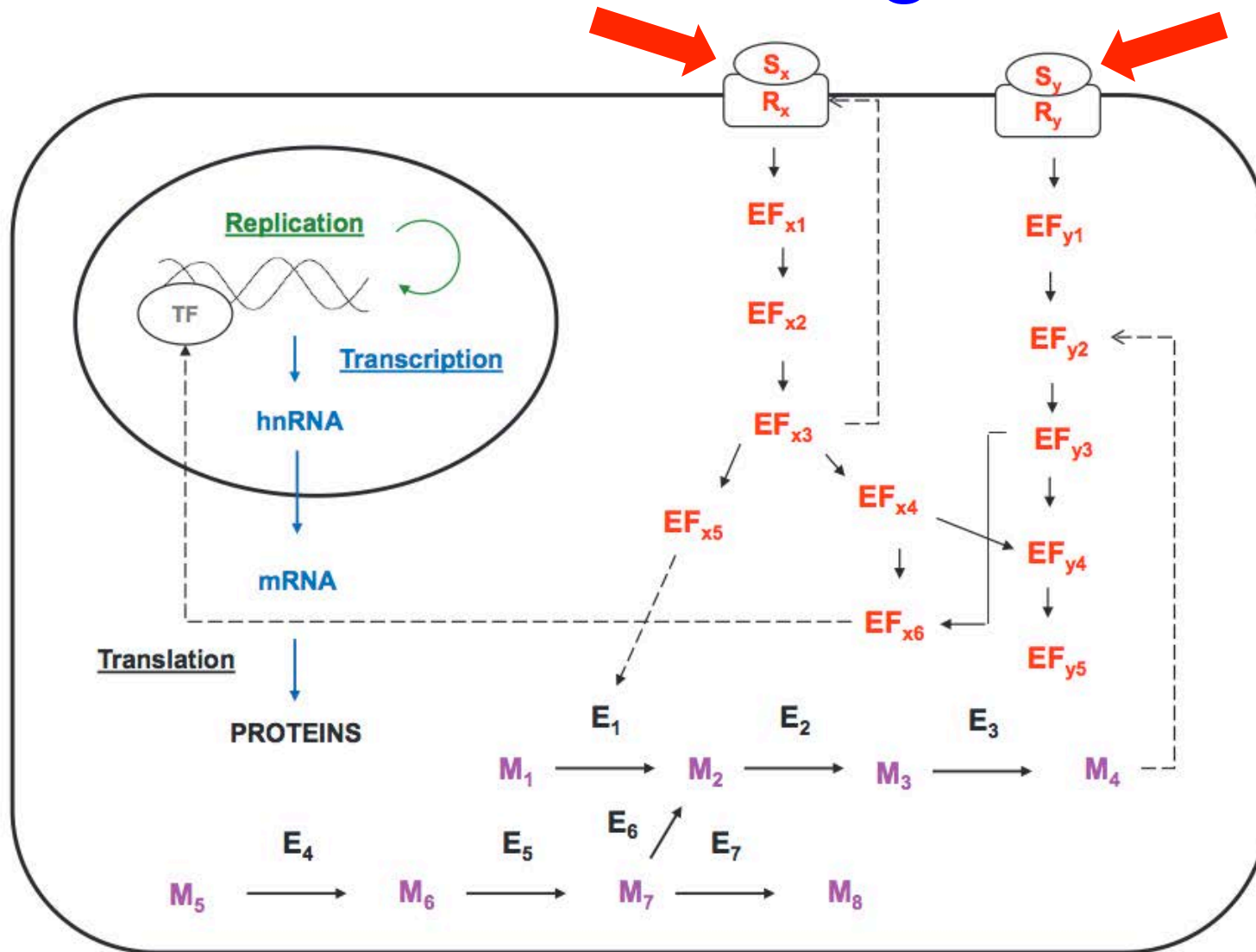
*Hanspeter Naegeli*

University of Zurich Switzerland

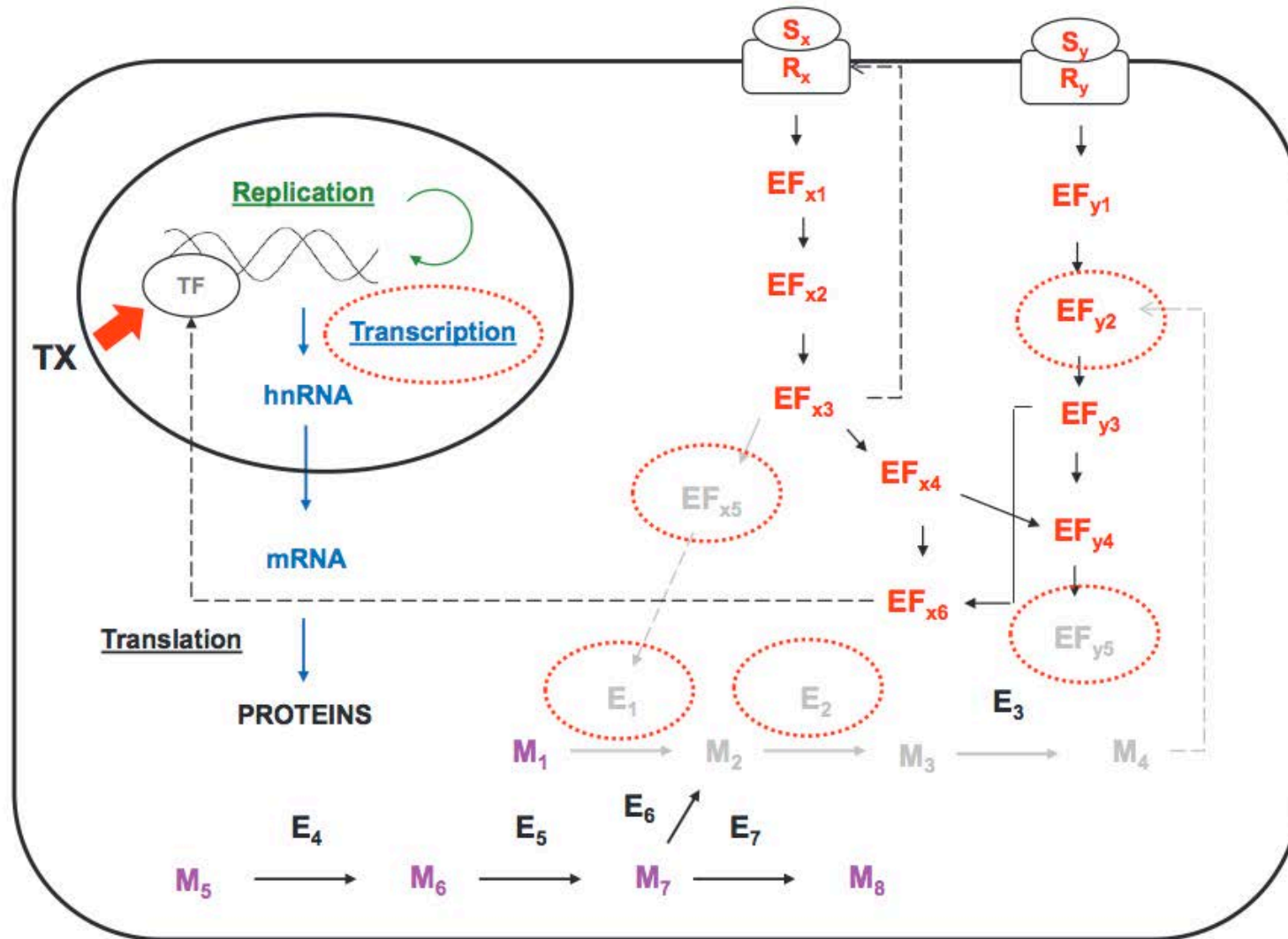
# Principle of cell-based assays



# “Fast” cellular signals



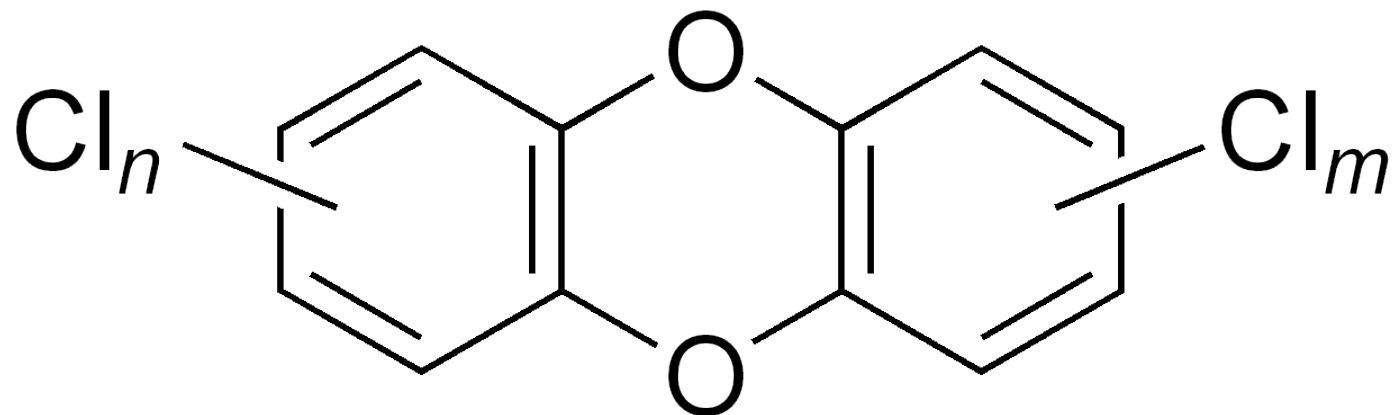
# “Slow” cellular signals



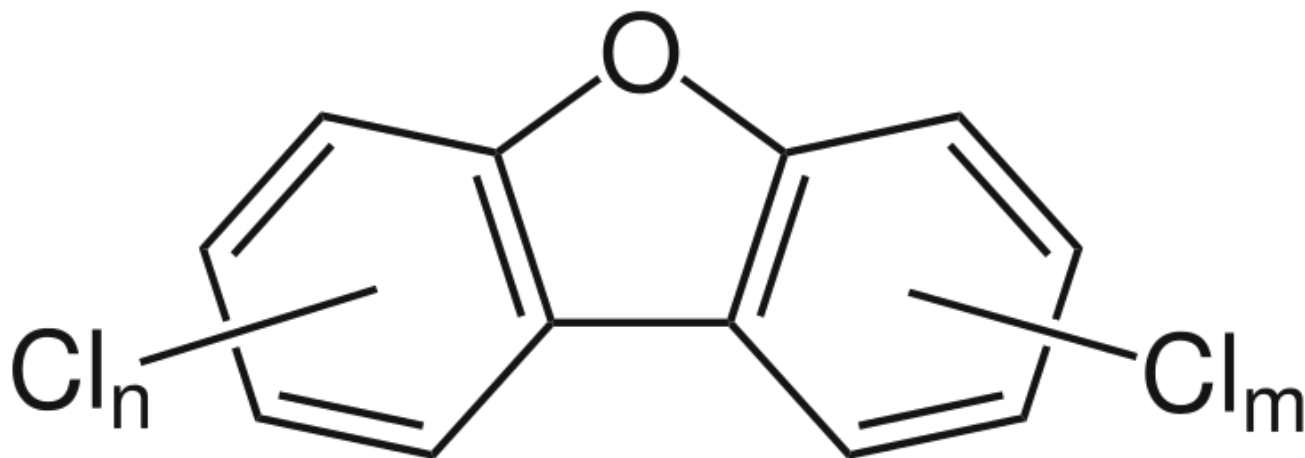
# Response to diesel exhaust pollutants



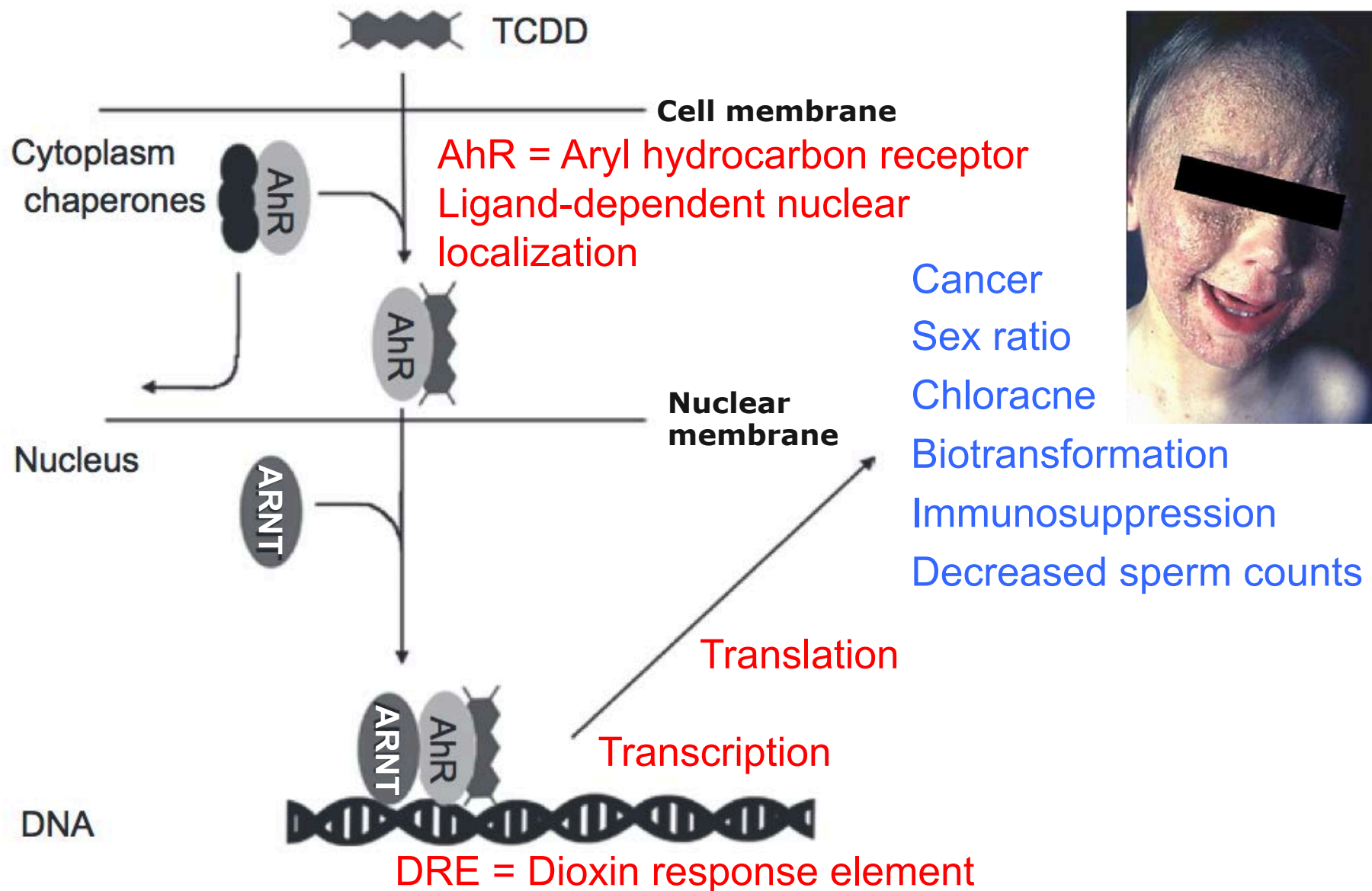
## Polychlorinated dibenzodioxins



## Polychlorinated dibenzofurans



# Biological activity of “dioxins”

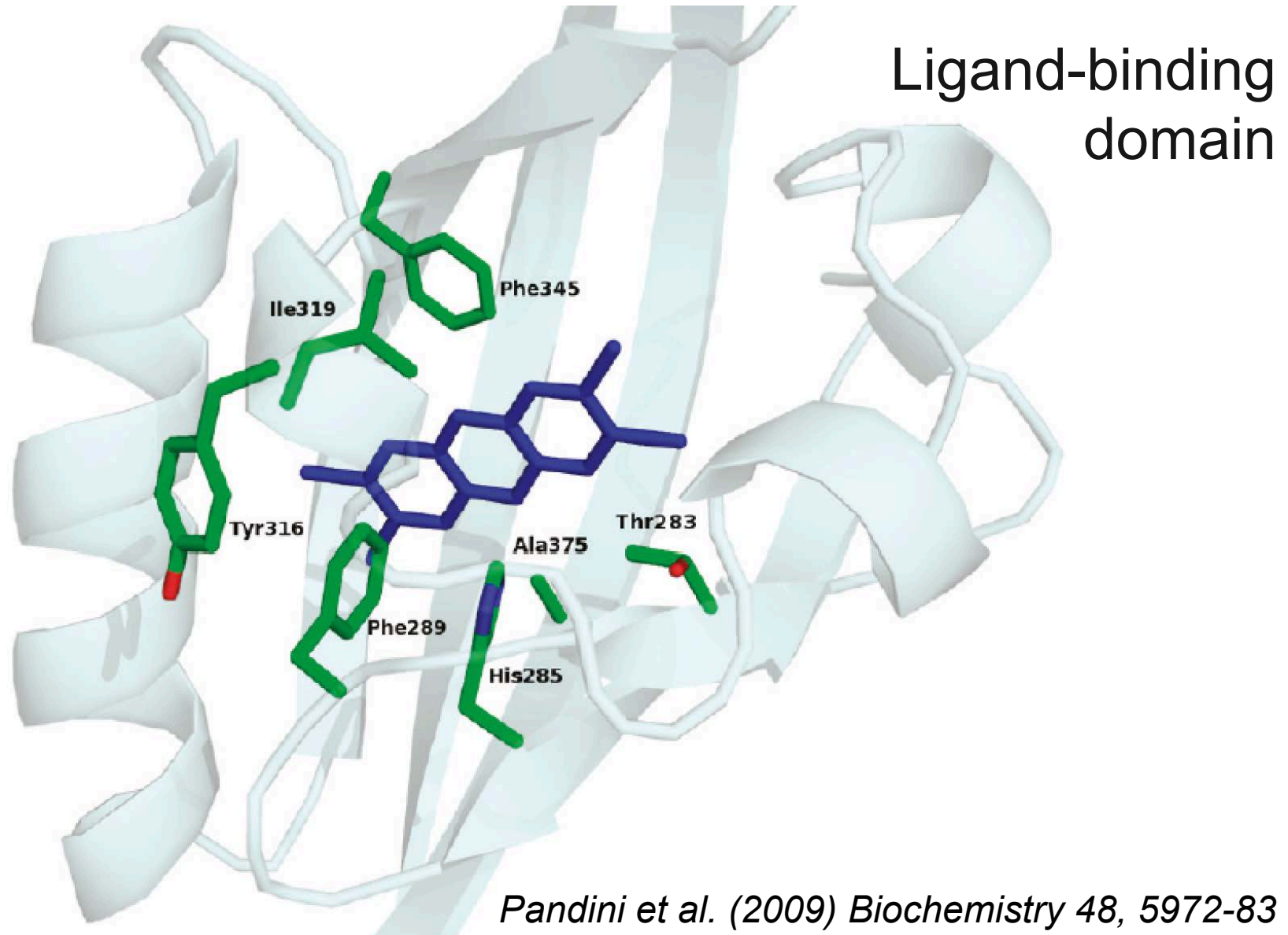


# Low-dose adverse effects in offspring

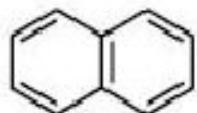
Effect in offspring	Maternal body burden ( <b>ng/kg</b> body weight)	Equivalent estimated human daily intake ( <b>pg/kg</b> )
Long Evans rats: accelerated eye opening and decreased sperm count in male offspring	LOAEL: 80	40
Holzman rats: decreased sperm count in male offspring	LOAEL: 100	50
Wistar rats: decreased sperm count and altered sexual behaviour in male offspring	LOAEL: 40	20
Holzman rats: decreased anogenital distance in male offspring	NOAEL: 20 LOAEL: 80	10 40



# Aryl hydrocarbon (Ah) receptor



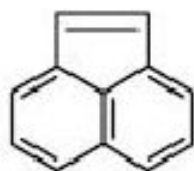
# Polycyclic aromatic hydrocarbons



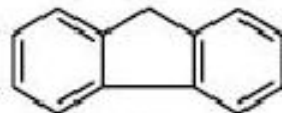
Naphthalene



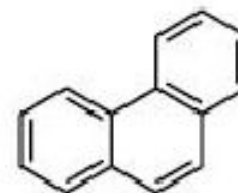
Acenaphthene



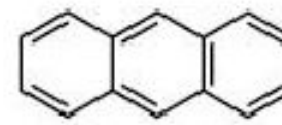
Acenaphthylene



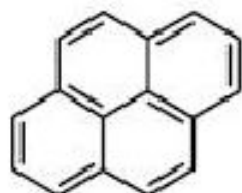
Fluorene



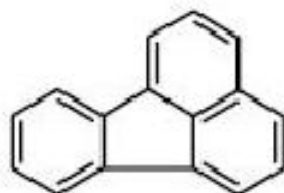
Phenanthrene



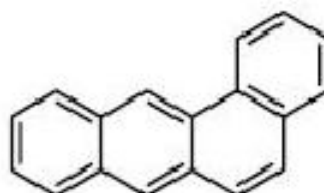
Anthracene



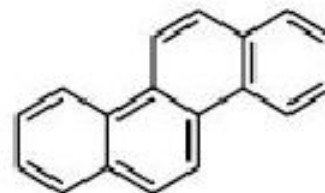
Pyrene



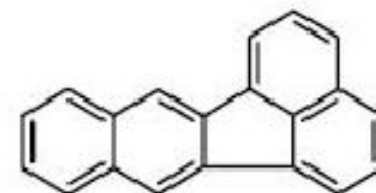
Fluoranthene



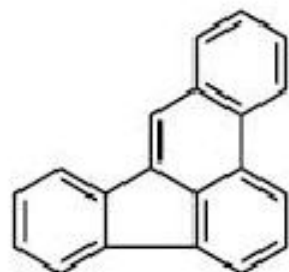
Benzo[a]anthracene



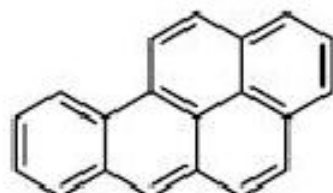
Chrysene



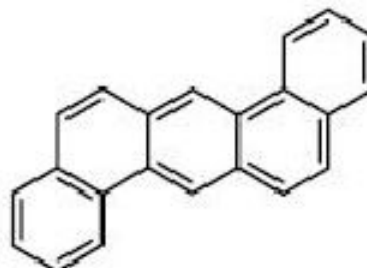
Benzo[k]fluoranthene



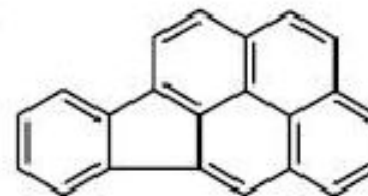
Benzo[b]fluoranthene



Benzo[a]pyrene



Dibenz[a,h]anthracene

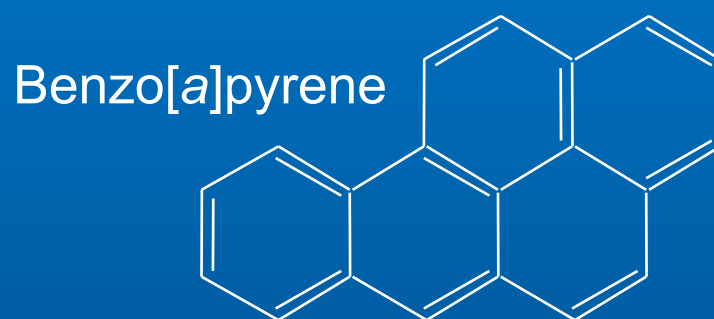


Indeno[1,2,3-cd]pyrene

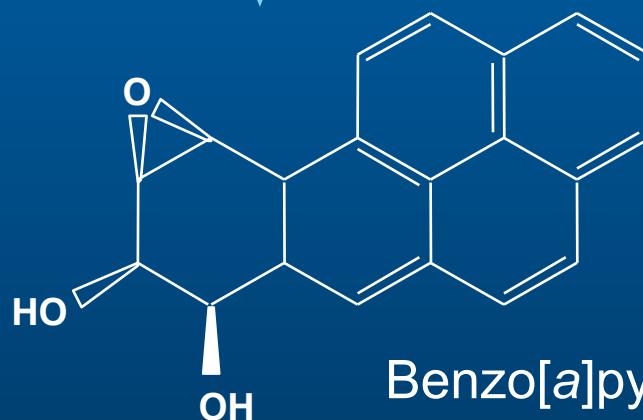


Benzo[ghi]perylene

# Polycyclic aromatic hydrocarbons

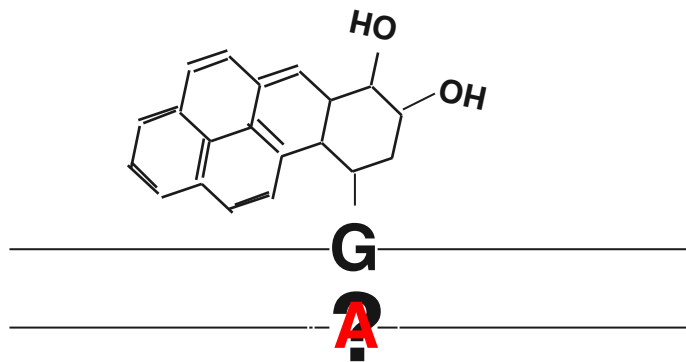
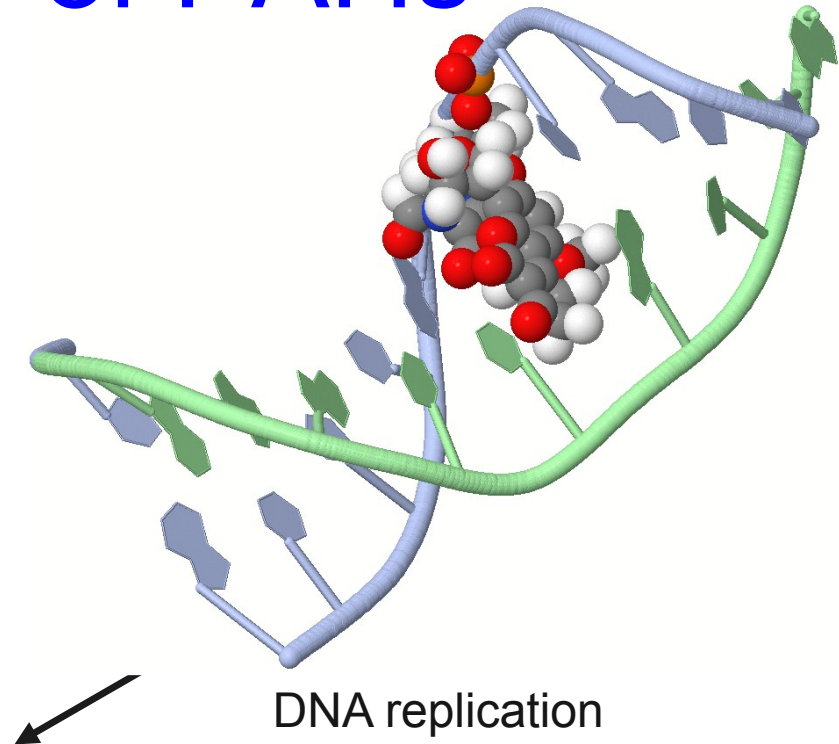
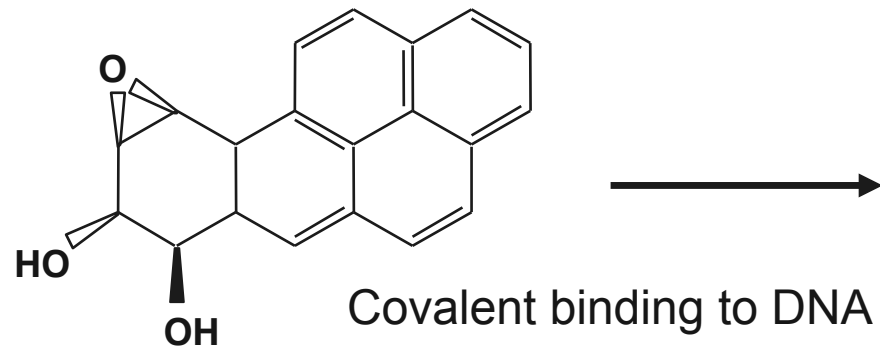


Biotransformation by cytochrome P450  
monooxygenases (CYP1A1, CYP1B1)



Benzo[a]pyrene diol epoxide

# Genotoxicity of PAHs

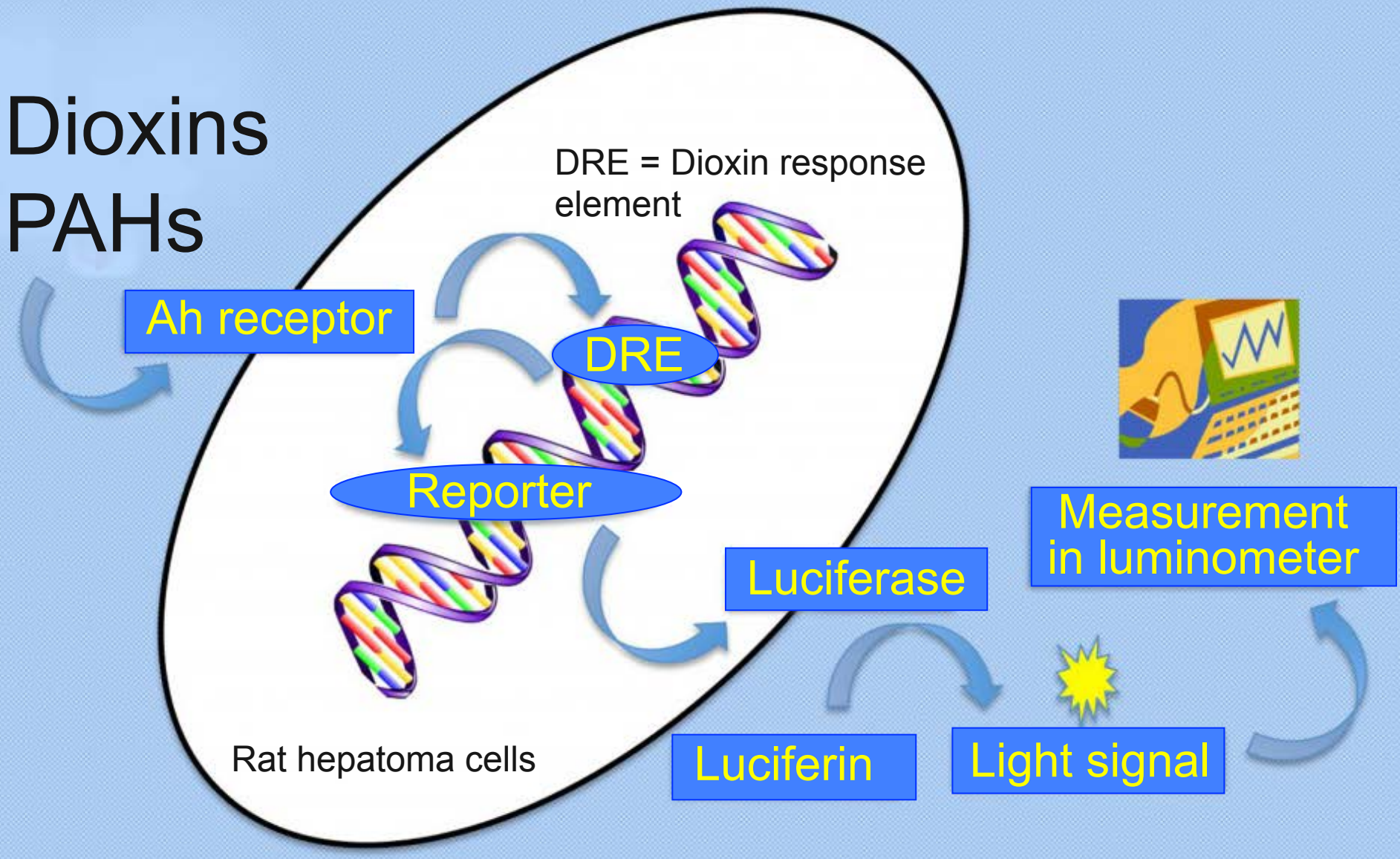


Mutations → → → → → Cancer

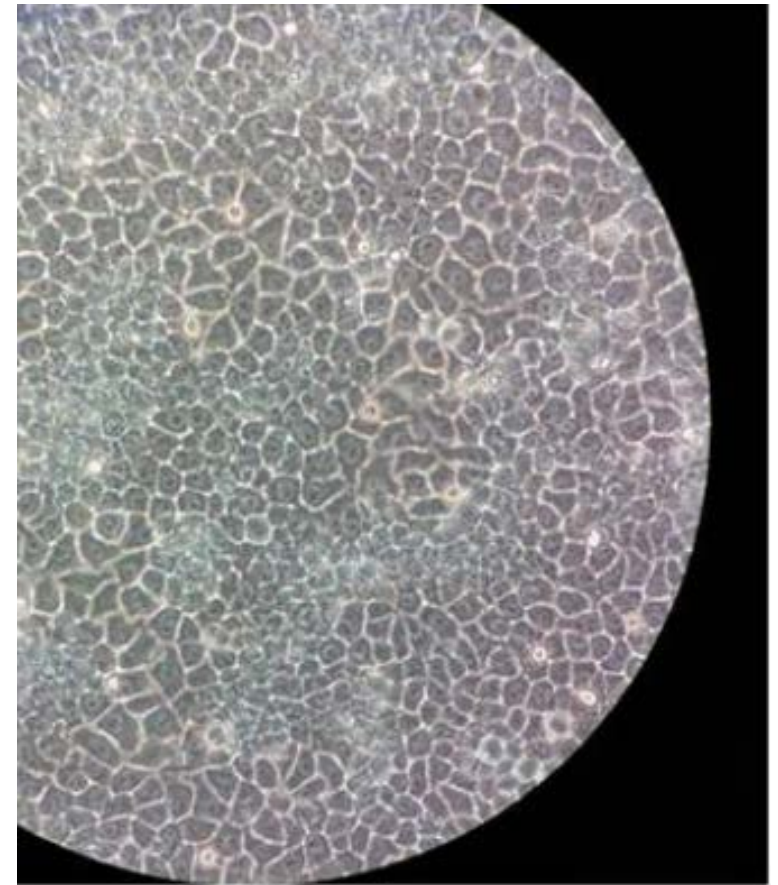
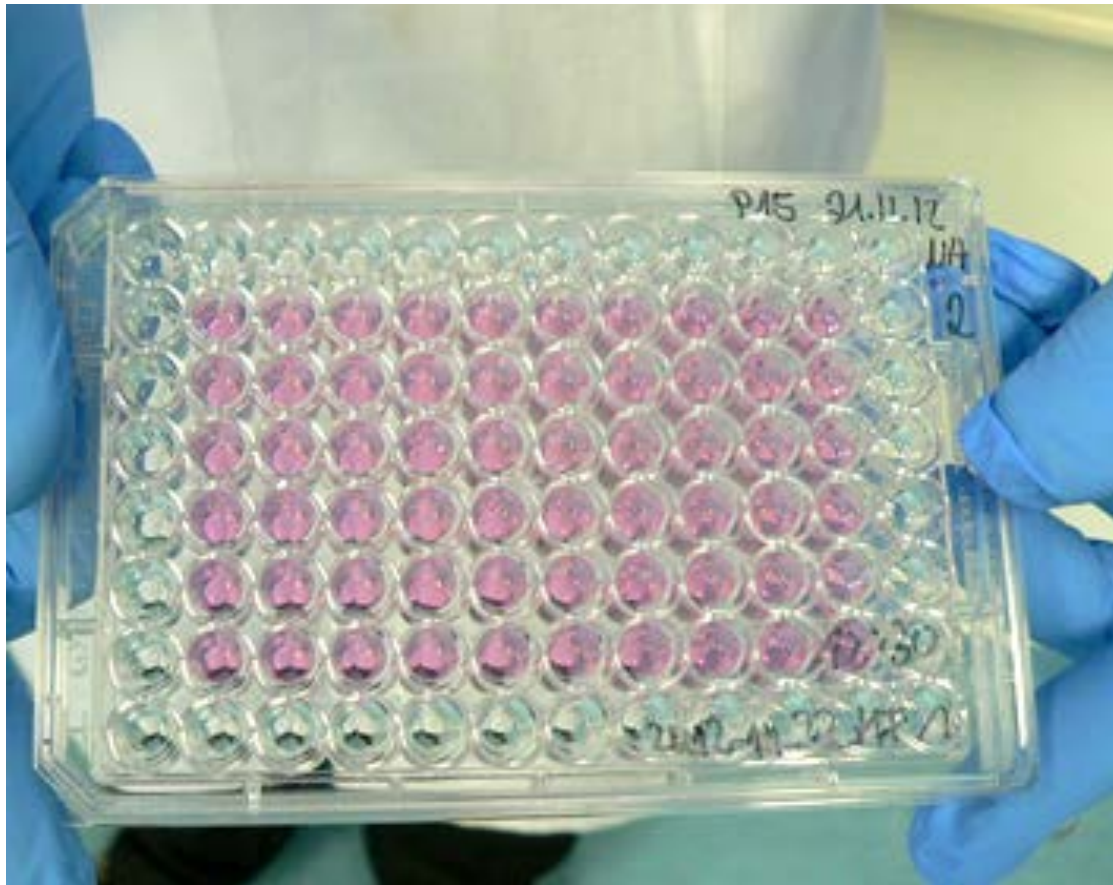


# Dioxin Responsive-Chemically Activated Luciferase eXpression (DR-CALUX) assay

Dioxins  
PAHs



# Rat hepatoma cells in culture



# Application: monitoring of pollutants

Sampling of airborne PM1 particles ( $< 1 \mu\text{m}$ ) on quartz fiber filters



Extraction with dichloromethane



Reconstitution in dimethyl sulfoxide

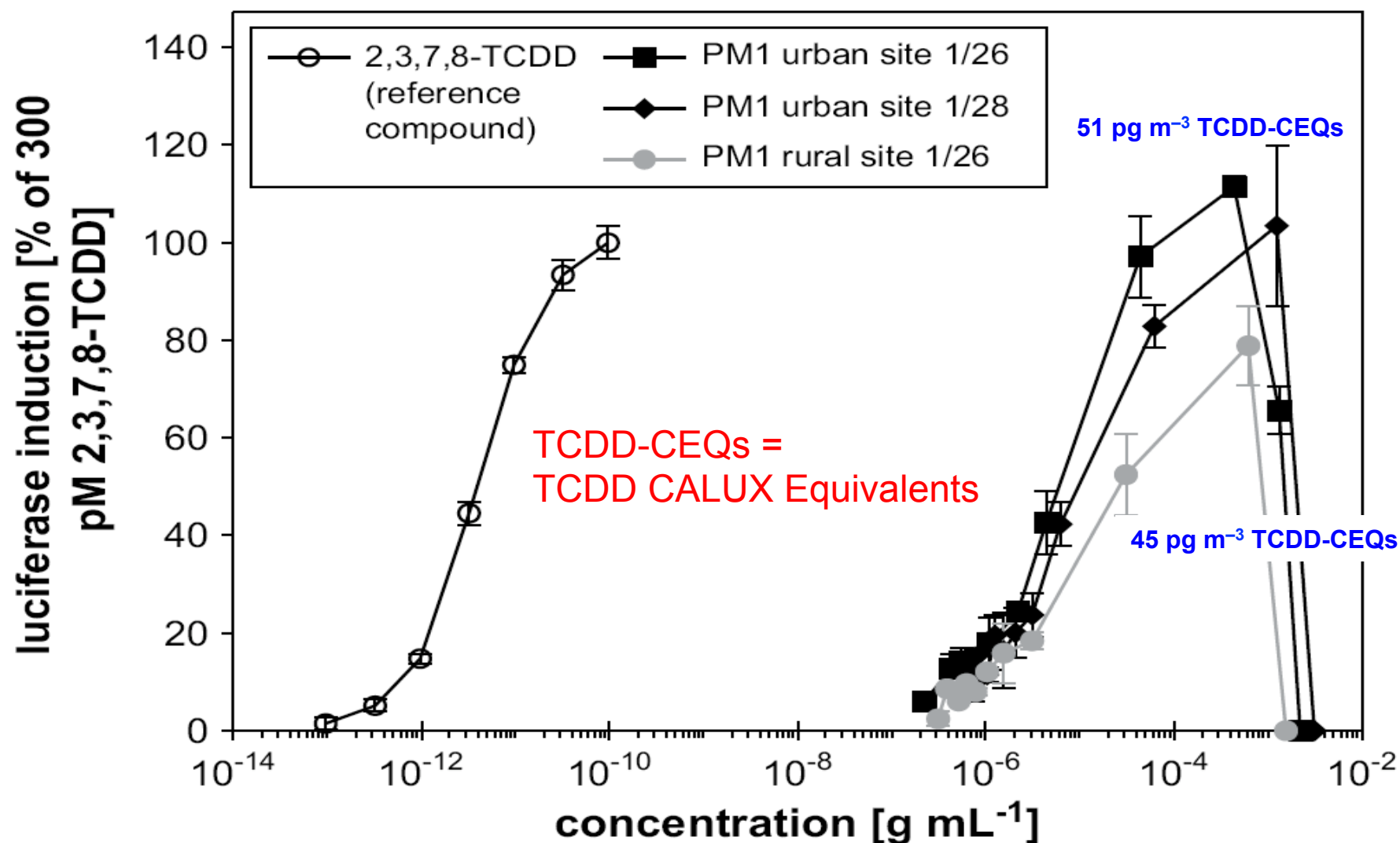


**DR-CALUX assay (24 h)**

*Wenger et al. (2009) Atmos. Environ. 43, 3556-62*



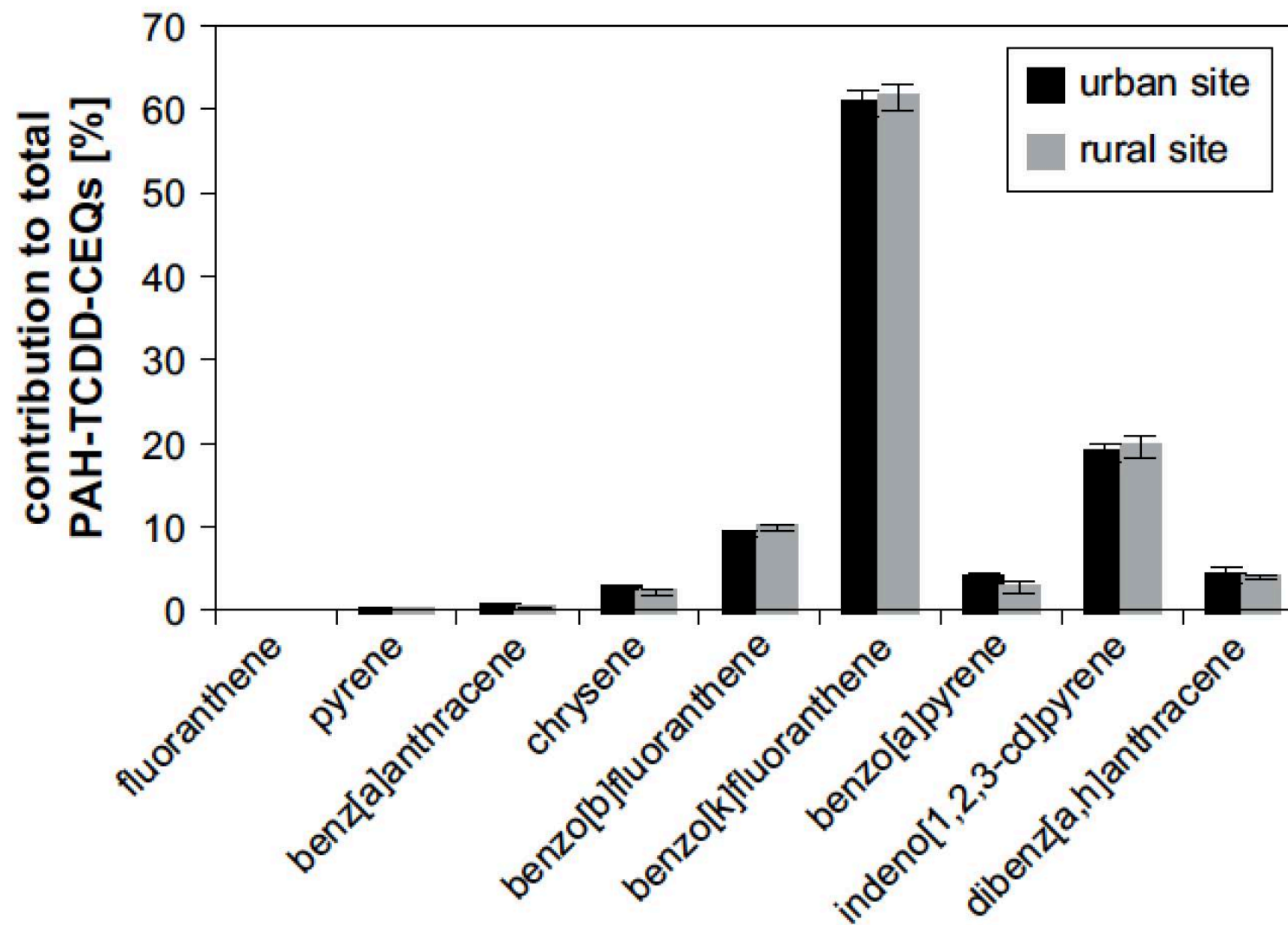
# Monitoring of atmospheric air



Wenger et al. (2009) *Atmos. Environ.* 43, 3556-62

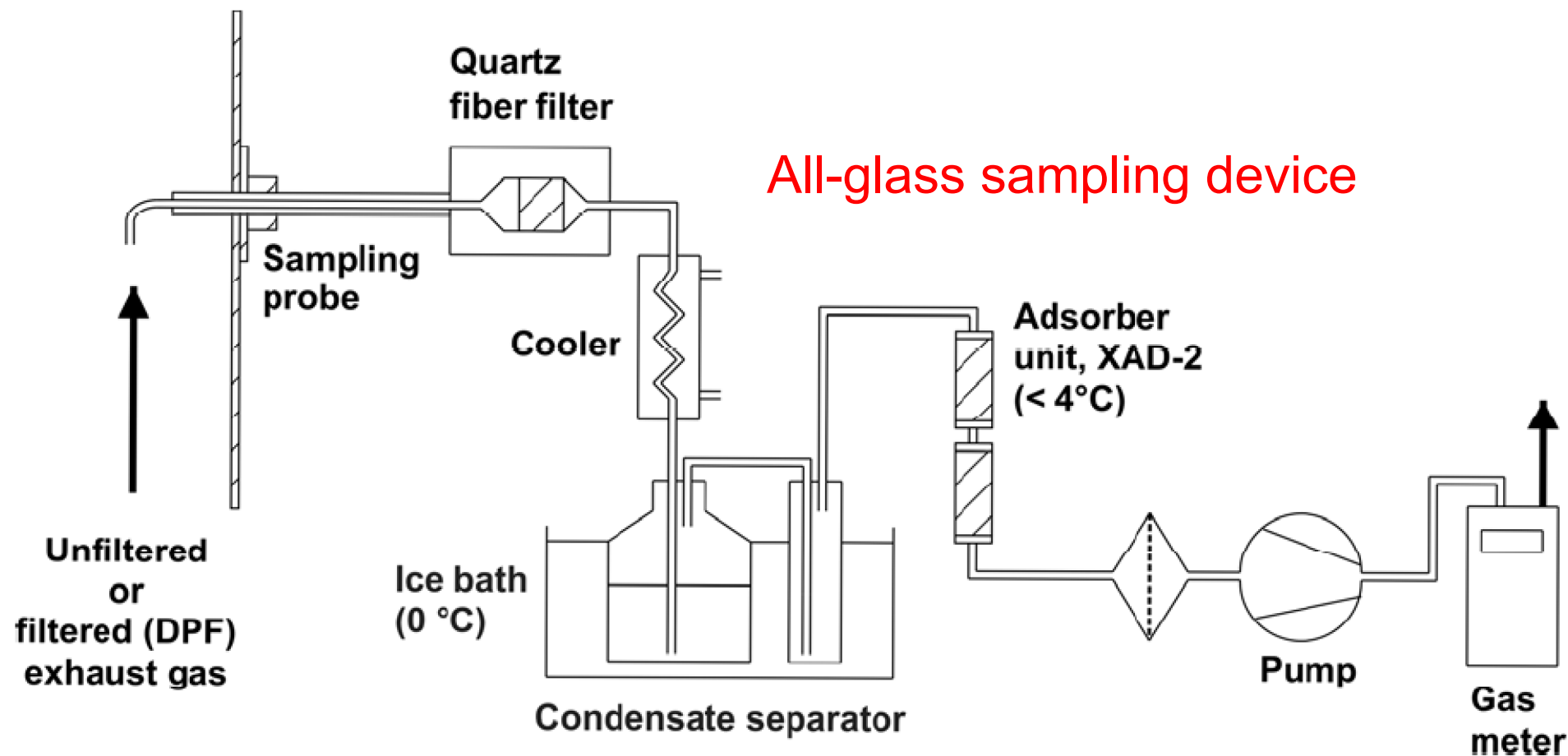


# Monitoring of atmospheric air



Wenger et al. (2009) Atmos. Environ. 43, 3556-62

# Application: Efficiency of diesel particulate filters (DPF)



# Diesel exhaust samples

sample code	chlorine ( $\mu\text{g g}^{-1}$ ) <sup>a</sup>	fuel additive ( $\mu\text{g g}^{-1}$ ) <sup>b</sup>	DPF <sup>c</sup>
ref <sup>d</sup>	none (<2)	none (<0.1/<0.1)	none
Fe	none (<2)	Fe (4.5)	none
FeF	none (<2)	Fe (4.5)	F
Cu	none (<2)	Cu (9)/Fe (7.5)	none
CuF	none (<2)	Cu (9)/Fe (7.5)	F
Cl	Cl (14)	none (<0.1/<0.1)	none
ClFe	Cl (14)	Fe (4.5)	none
ClFeF	Cl (14)	Fe (4.5)	F
ClCu	nc	nc	nc
ClCuF	Cl (14)	Cu (9)/Fe (7.5)	F
xCl	nc	nc	nc
xClFe	nc	nc	nc
xClFeF	nc	nc	nc
xClCu	nc	nc	nc
xClCuF	xCl (110)	Cu (9)/Fe (7.5)	F

nc, not collected

Wenger et al. (2008) *Environ. Sci. Technol.* 42, 2992-8

# Application: Efficiency of Diesel particulate filters (DPF)

Particle-bound and semivolatile compounds



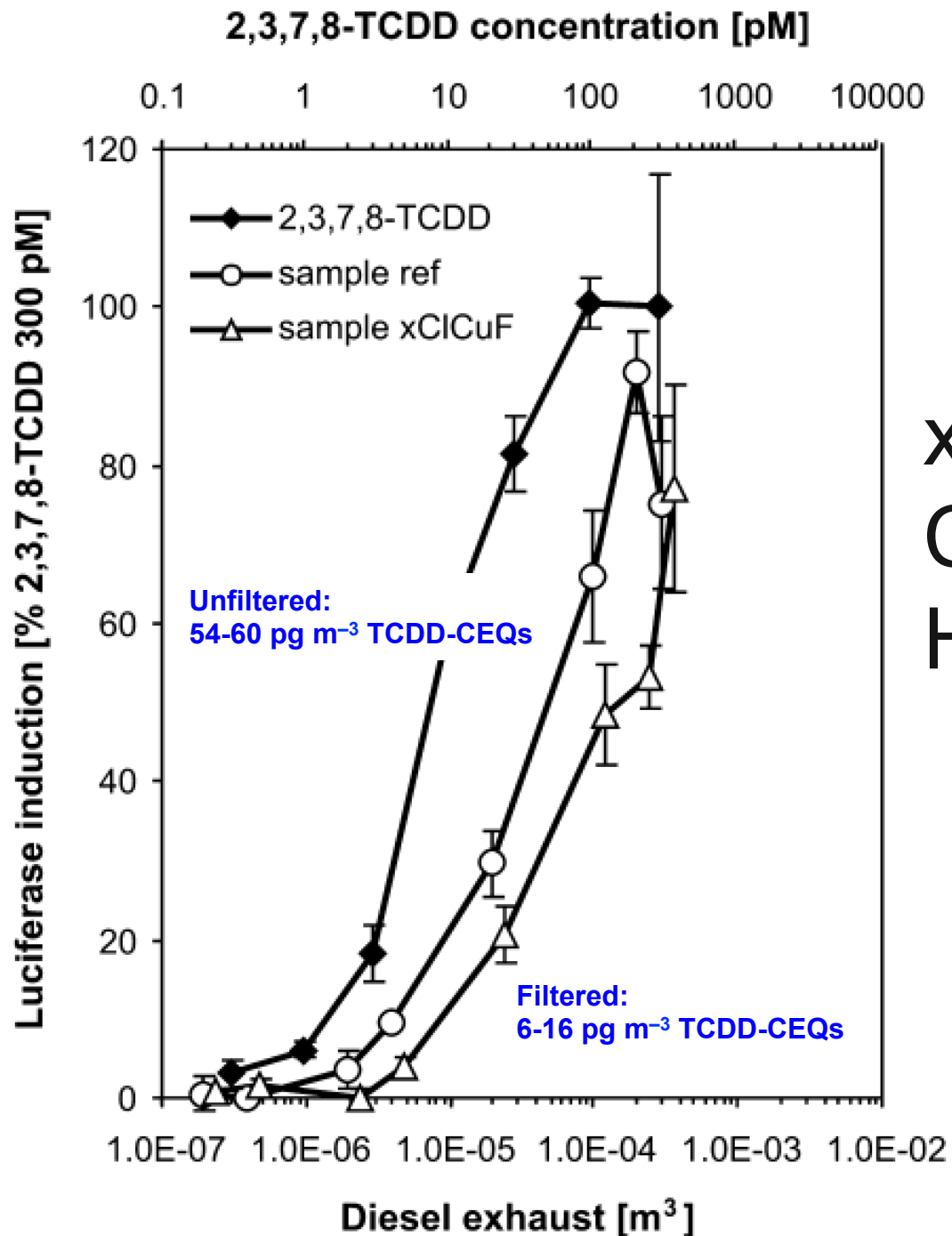
Extraction with dichloromethane/hexane/toluene



Reconstitution in dimethyl sulfoxide



**DR-CALUX assay (24 h)**



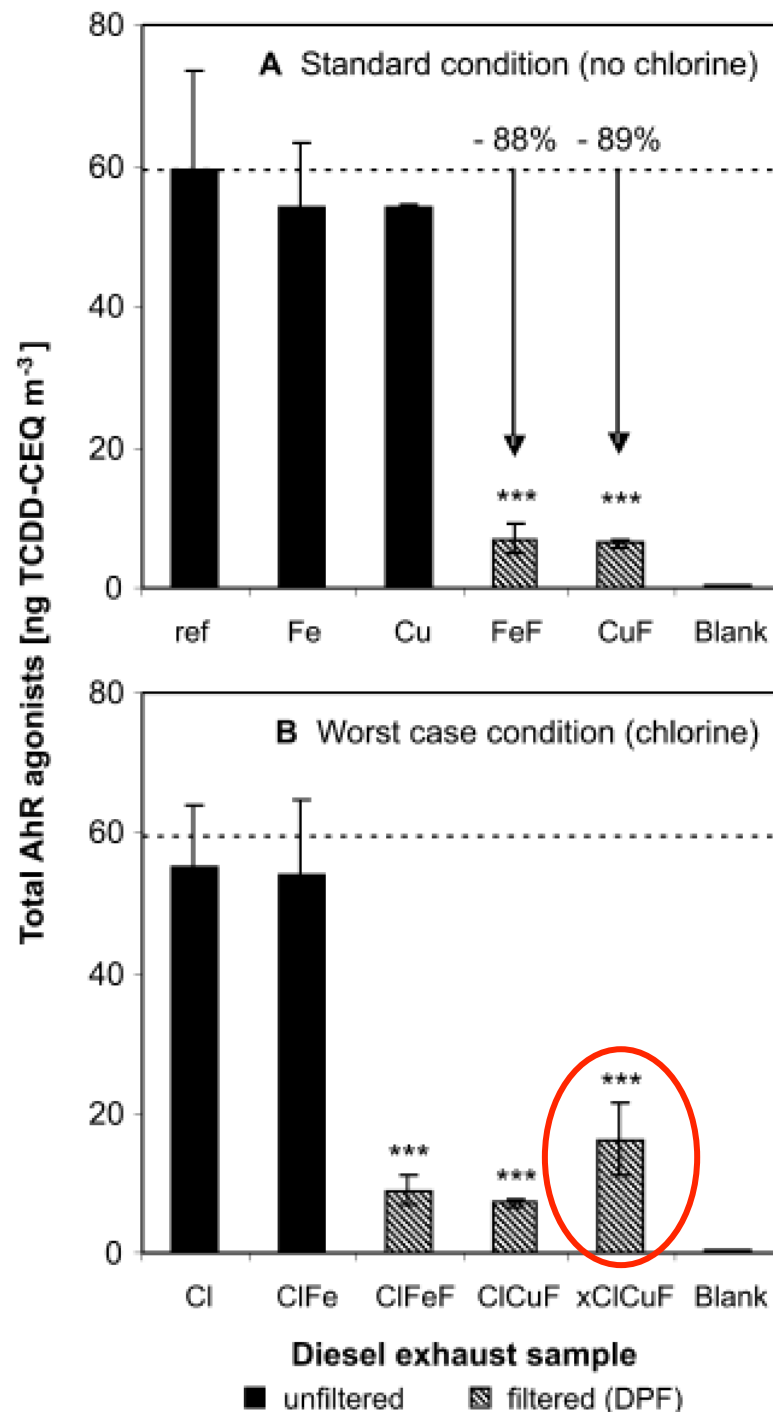
## Filter effect

xClCuF:  
Cu/Fe fuel additive  
High chlorine

Wenger et al. (2008) *Environ.  
Sci. Technol.* 42, 2992-8

# Filter effect

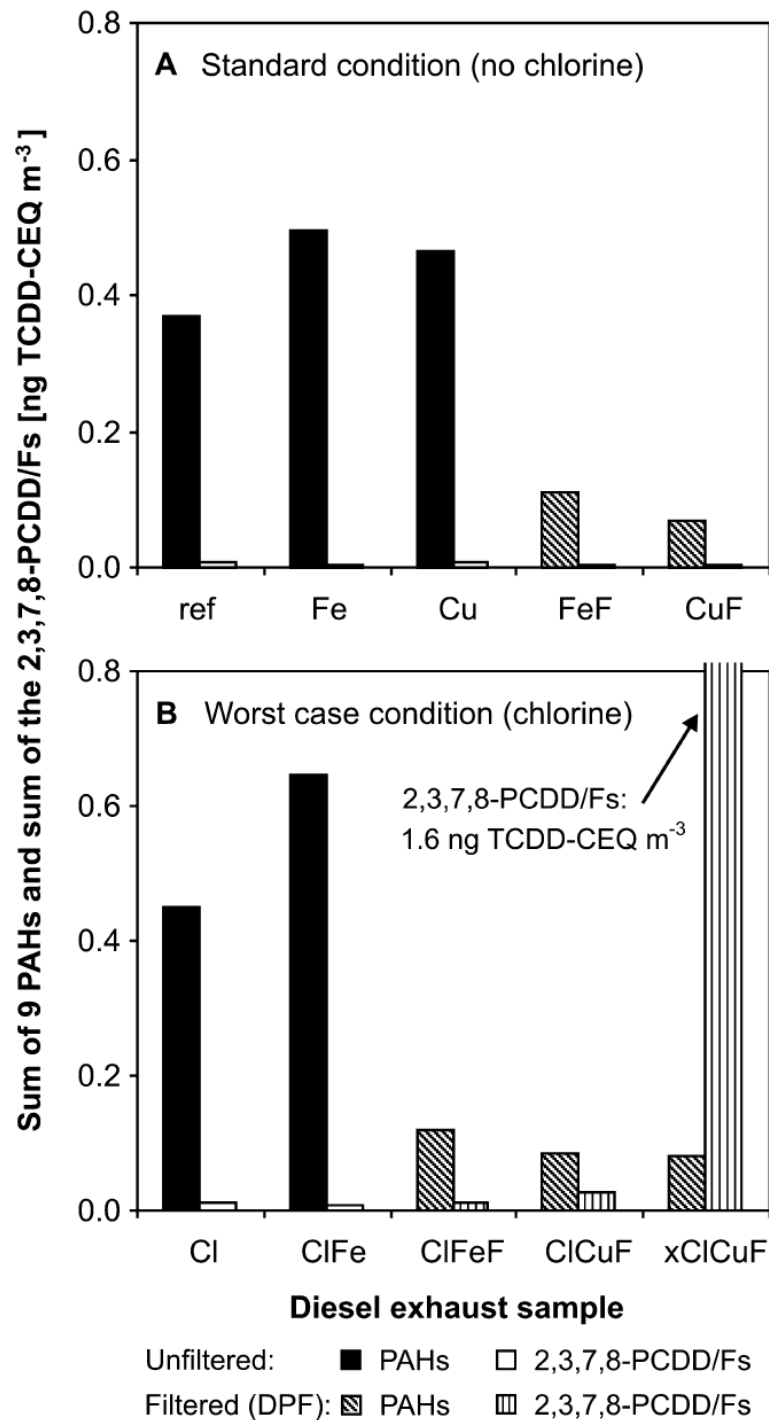
Fe: Fe fuel additive  
Cu: Cu fuel additive



Wenger et al. (2008) *Environ. Sci. Technol.* 42, 2992-8

# Filter effect

Fe: Fe fuel additive  
Cu: Cu fuel additive



Wenger et al. (2008) *Environ. Sci. Technol.* 42, 2992-8

# Conclusions

- Principle of cell-based assays: easy-to-grow cells provide sensors of hazardous compounds
- Screening assay; Output: activity equivalents like TCDD equivalents
- Increasing repertoire of commercially available reporter assays
- Automatic and fast detection of luminescent or fluorescent reporter products
- Application: Screening assays and functional evaluation of complex mixtures



# ACKNOWLEDGEMENTS

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- **Renato Zenobi**  
ETH Zurich, Department of Chemistry & Applied Biosciences