Nutritional Modulation of Pro-Inflammatory Responses Induced by Co-Planar PCBs

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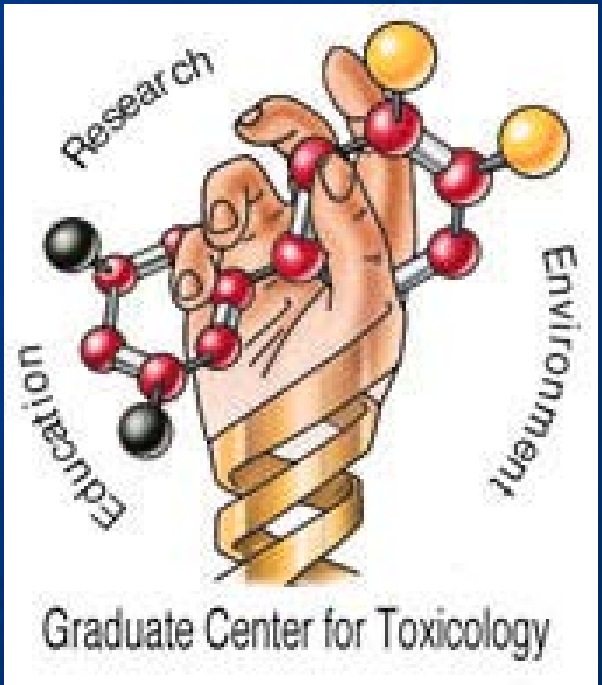
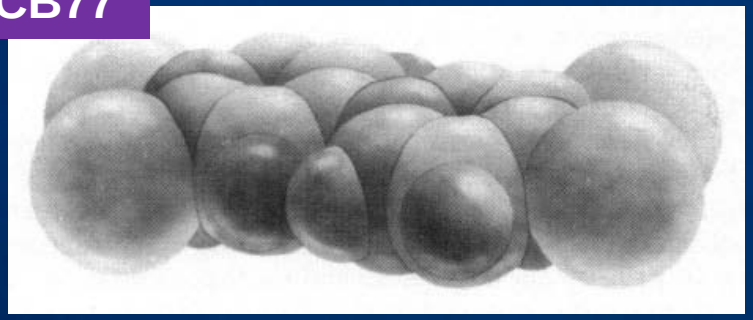


PRO-INFLAMMATORY PROPERTIES OF POLYCHLORINATED BIPHENYLS (PCBS)

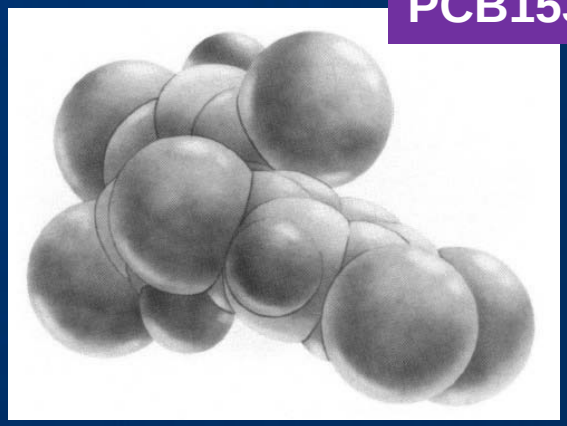
Polychlorinated biphenyls (PCBs)

- Man-made persistent organic environmental pollutants (in the US manufactured 1929 until 1979)
- Sources: dielectric fluids in transformers and capacitors, caulking materials and paints
- Complex mixtures of 209 congeners, biological activity depends on the pattern of chlorine substitution

PCB77



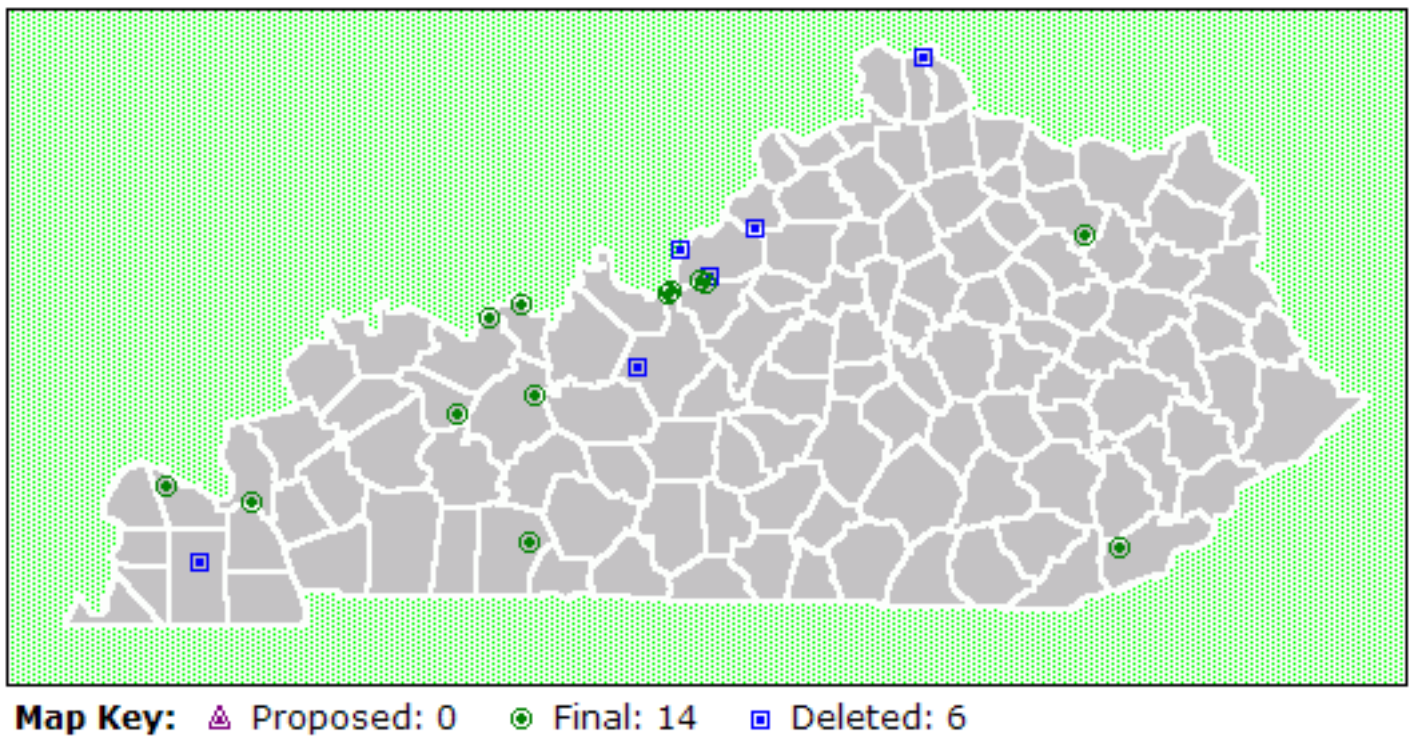
PCB153



(McFarland and J U Clarke. 1989. *Environ Health Perspect*)

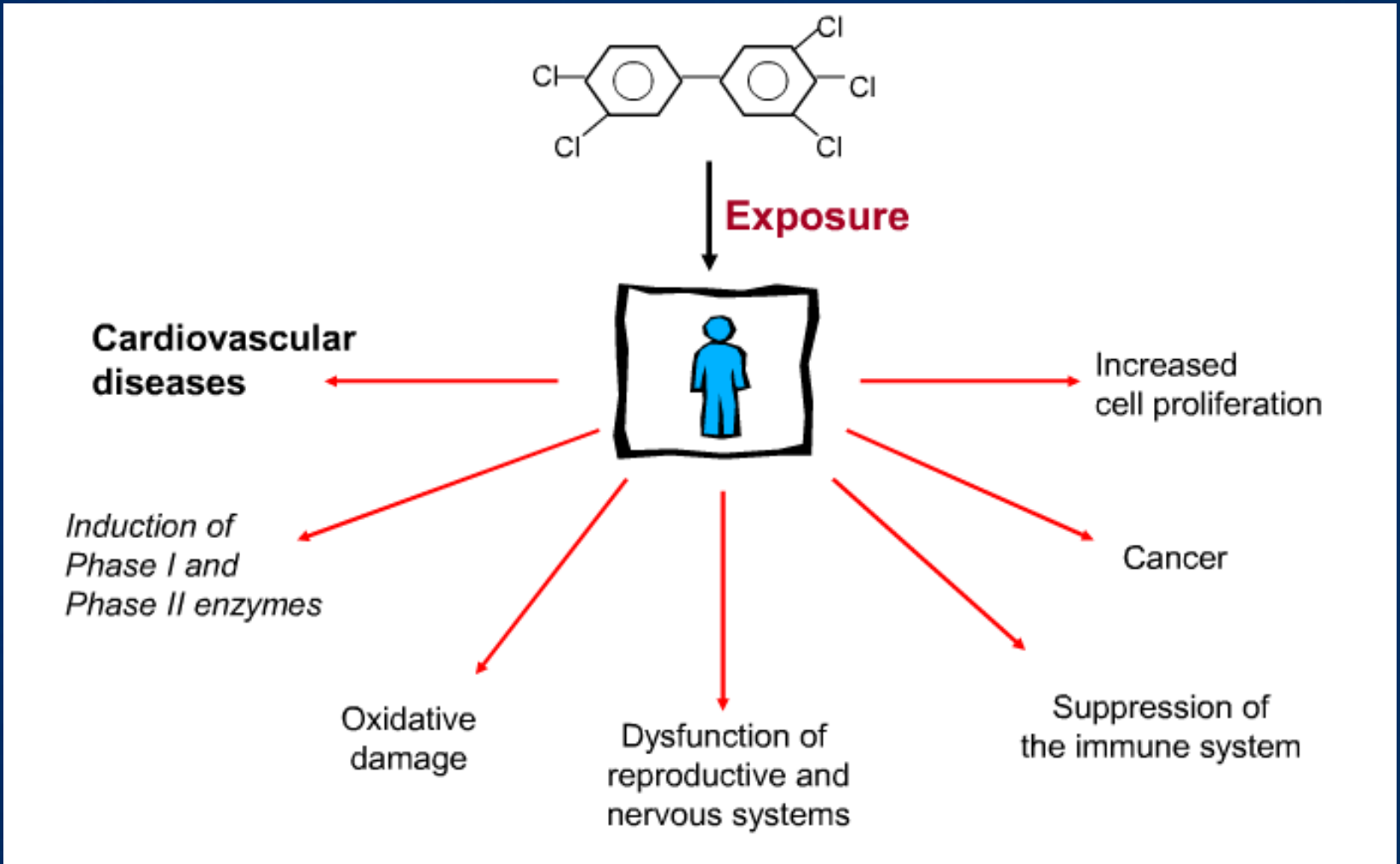
Superfund sites in Kentucky (KY)

- Out of 14 active Superfund sites in KY, 4 are contaminated by PCBs



(http://www.epa.gov/region4/waste/npl/index.htm#KY)

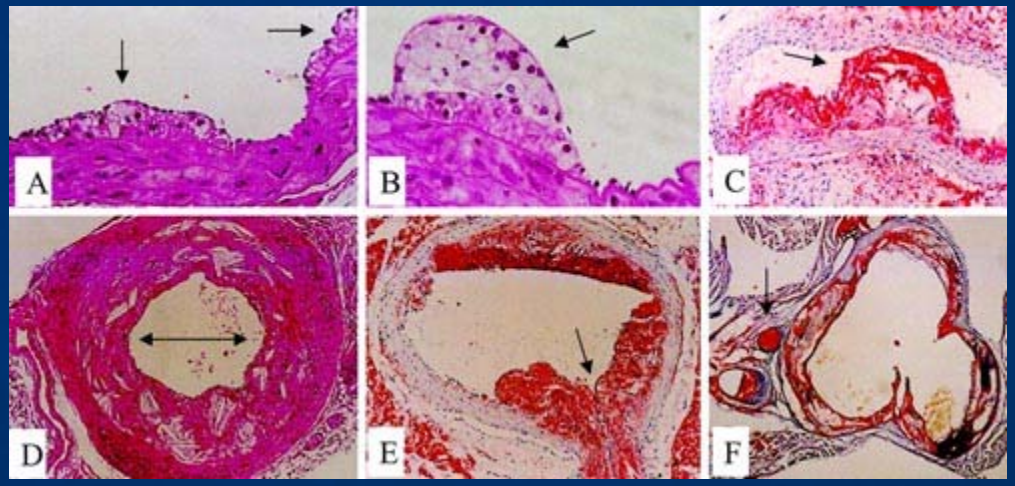
Toxic effects of PCBs



(Adopted from http://www.epa.gov by Dr. Xabier Arzuaga)

Atherosclerosis

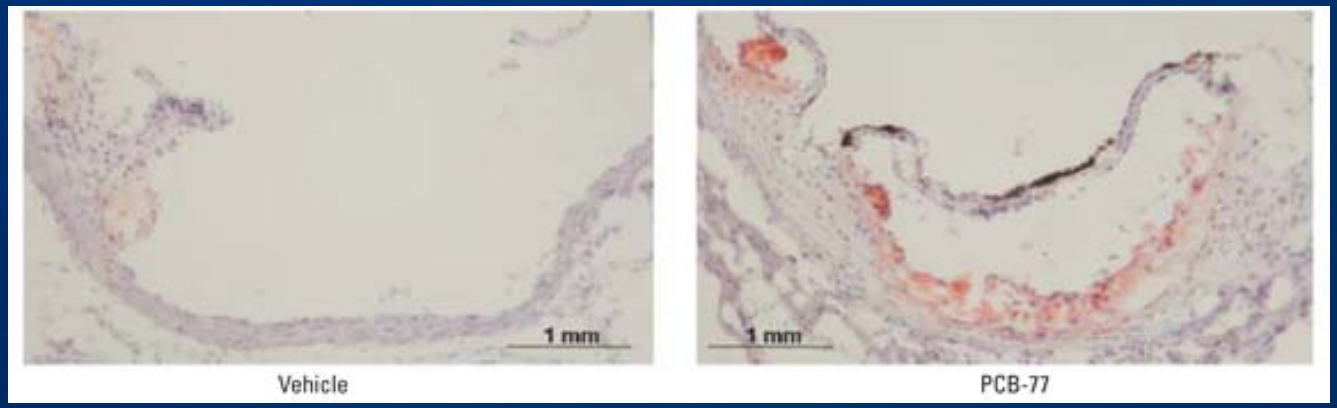
- Cardiovascular diseases are a leading cause of death in the US (Lloyd-Jones et al, 2008. *Circulation*)
- Atherosclerosis is an inflammatory disease (Libby et al, 2002. *Circulation*)



(Moghadasian et al, 2001. *Lab Invest*)

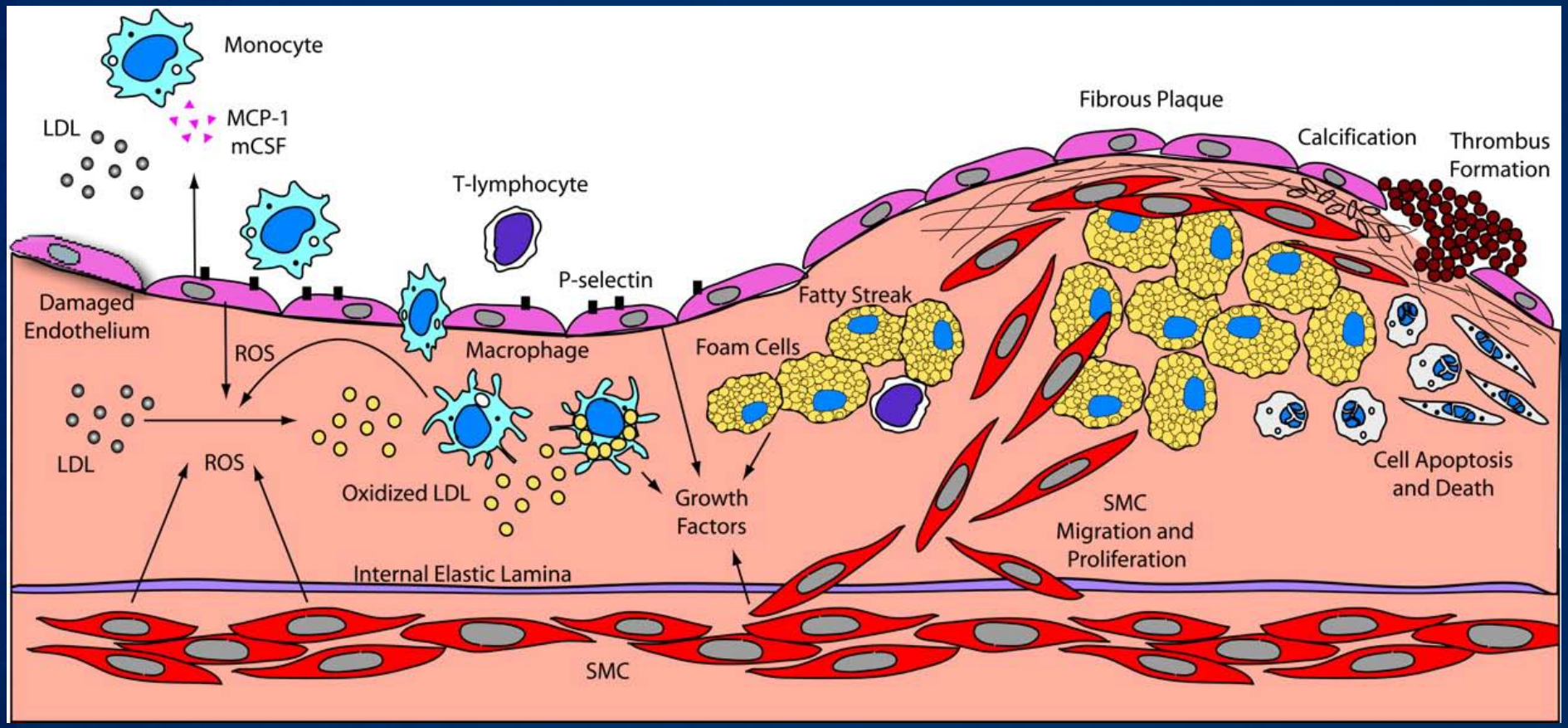
Cardiovascular toxicity of PCBs

- Increased mortality from cardiovascular diseases was observed among Swedish capacitor workers (Gustavsson et al., 1997. *Am J Ind Med*)
- Coplanar PCBs cause endothelial cell dysfunction (Toborek et al., 1995. *J Biochem Toxicol*)
- PCB77 increased atherosclerosis in apolipoprotein E-deficient (ApoE$^{-/-}$) mice



(Arsenescu et al., 2008. *Environ Health Perspect*)

Development of atherosclerosis

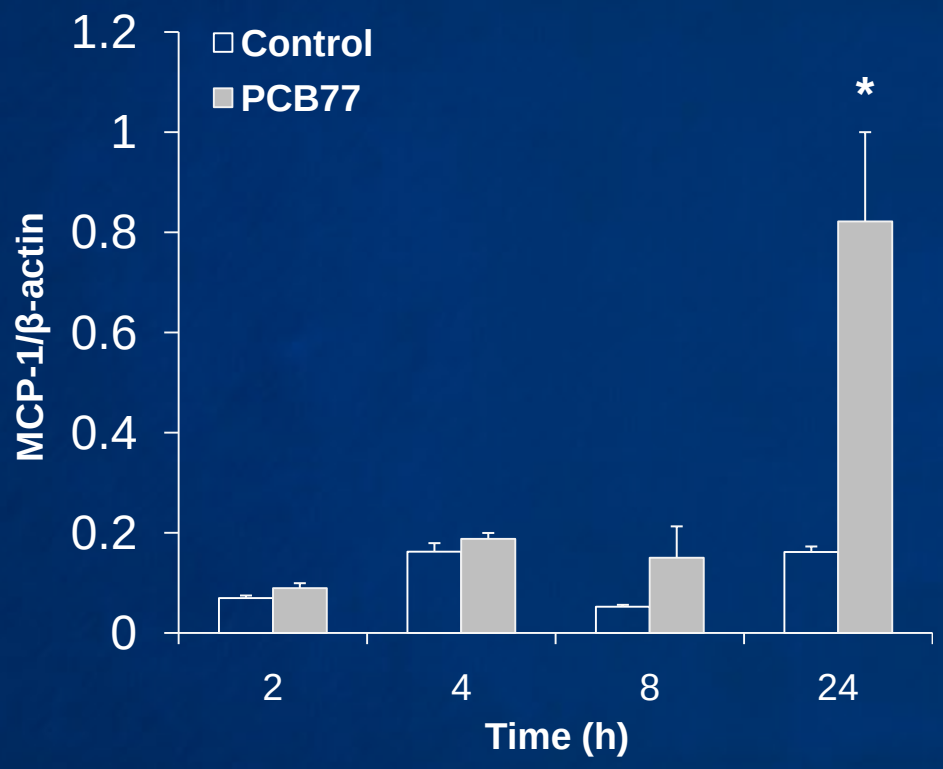


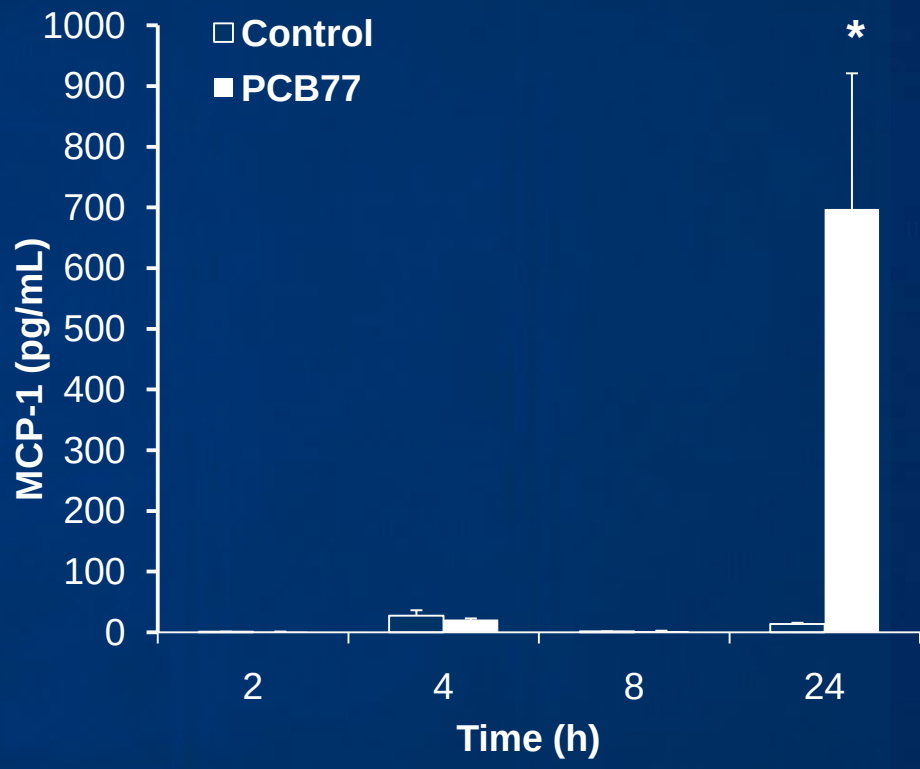
(Madamanchi et al., 2005. *Arterioscler Thromb Vasc Biol*)

Monocyte chemoattractant protein-1 (MCP-1) /CCL2

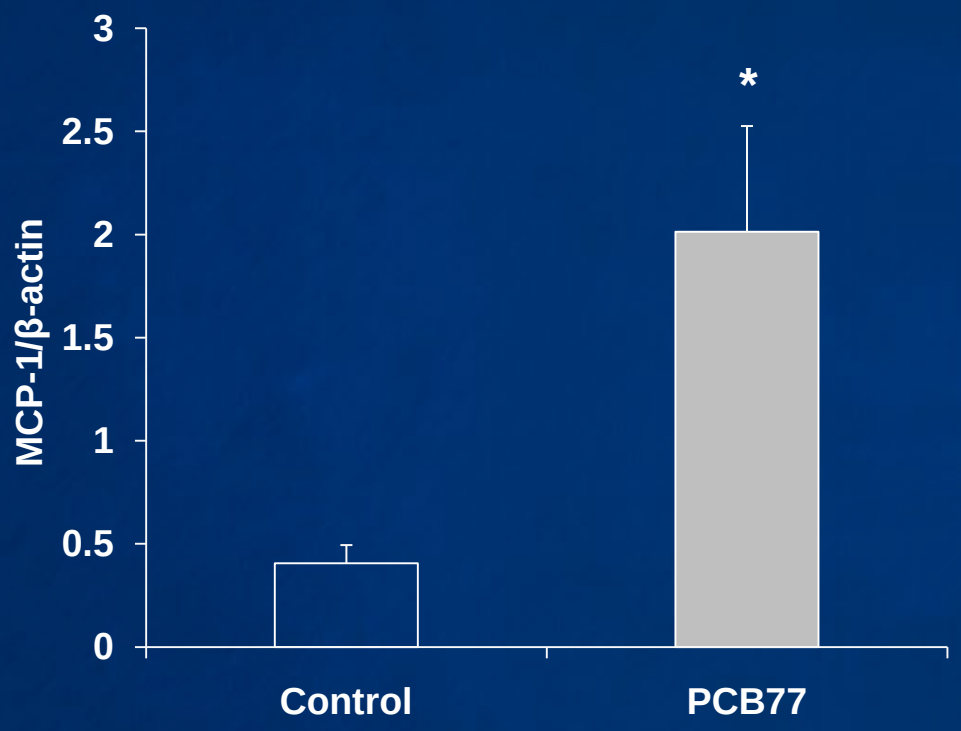
- Mononuclear cell attractant
- High MCP-1 levels present in atherosclerotic plaques in humans (Nelken et al., 1991. *J Clin Invest*)
- Plasma MCP-1 levels correlate with a risk of coronary heart disease (Hoogeveen et al., 2005. *Atherosclerosis*)
- MCP-1 deletion attenuates atherosclerosis formation (Gu et al., 1998, *Mol Cell*)
- MCP-1 over-expression accelerate lesion formation (Aiello et al., 1999. *Arterioscler Thromb Vasc Biol*)

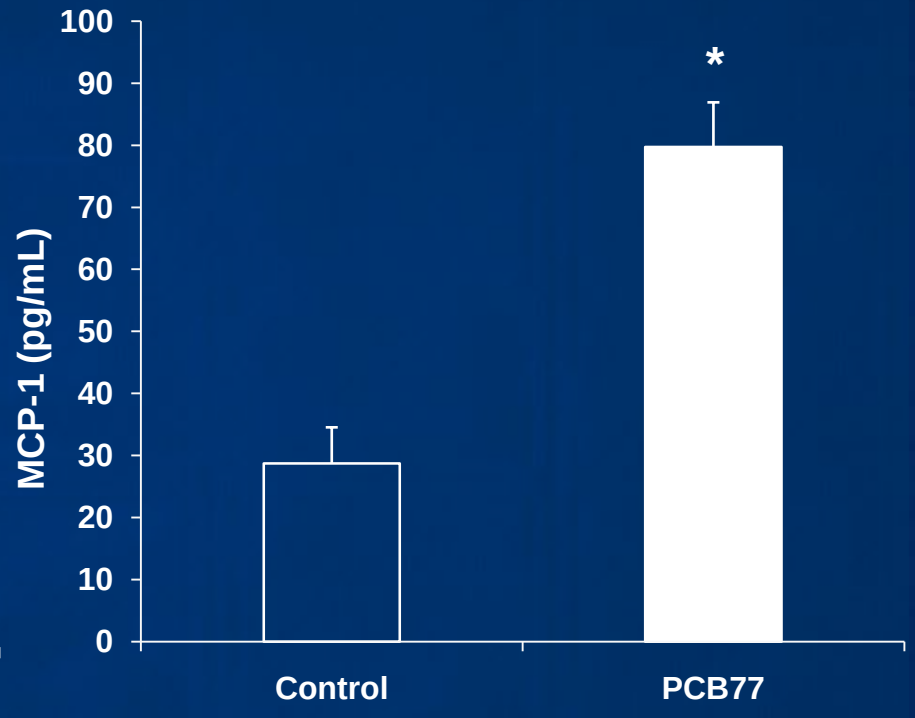
MCP-1 up-regulation by PCB77 in primary endothelial cells

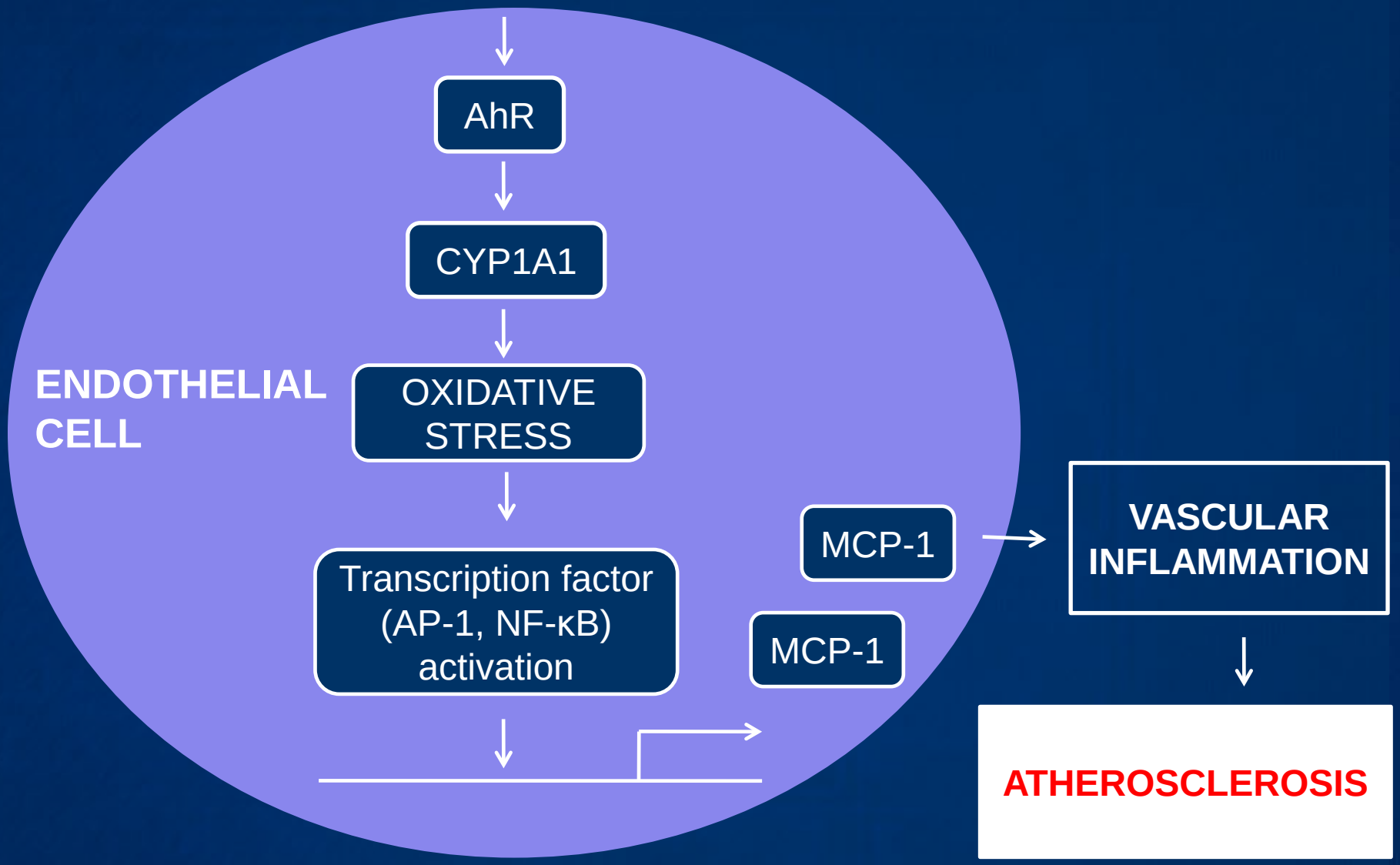




PCB77 induces MCP-1 mRNA and protein expression *in vivo*







AhR = aryl hydrocarbon receptor, AP-1 = activator protein-1, CYP1A1 = cytochrome P450 1A1, NF-κB = nuclear factor-κB

PREVENTION OF PCB TOXICITY BY METABOLITES OF DOCOSAHEXAENOIC ACID

Nutritional modulation of PCB toxicity

PCBs

Omega-6 polyunsaturated fatty acids (linoleic acid)

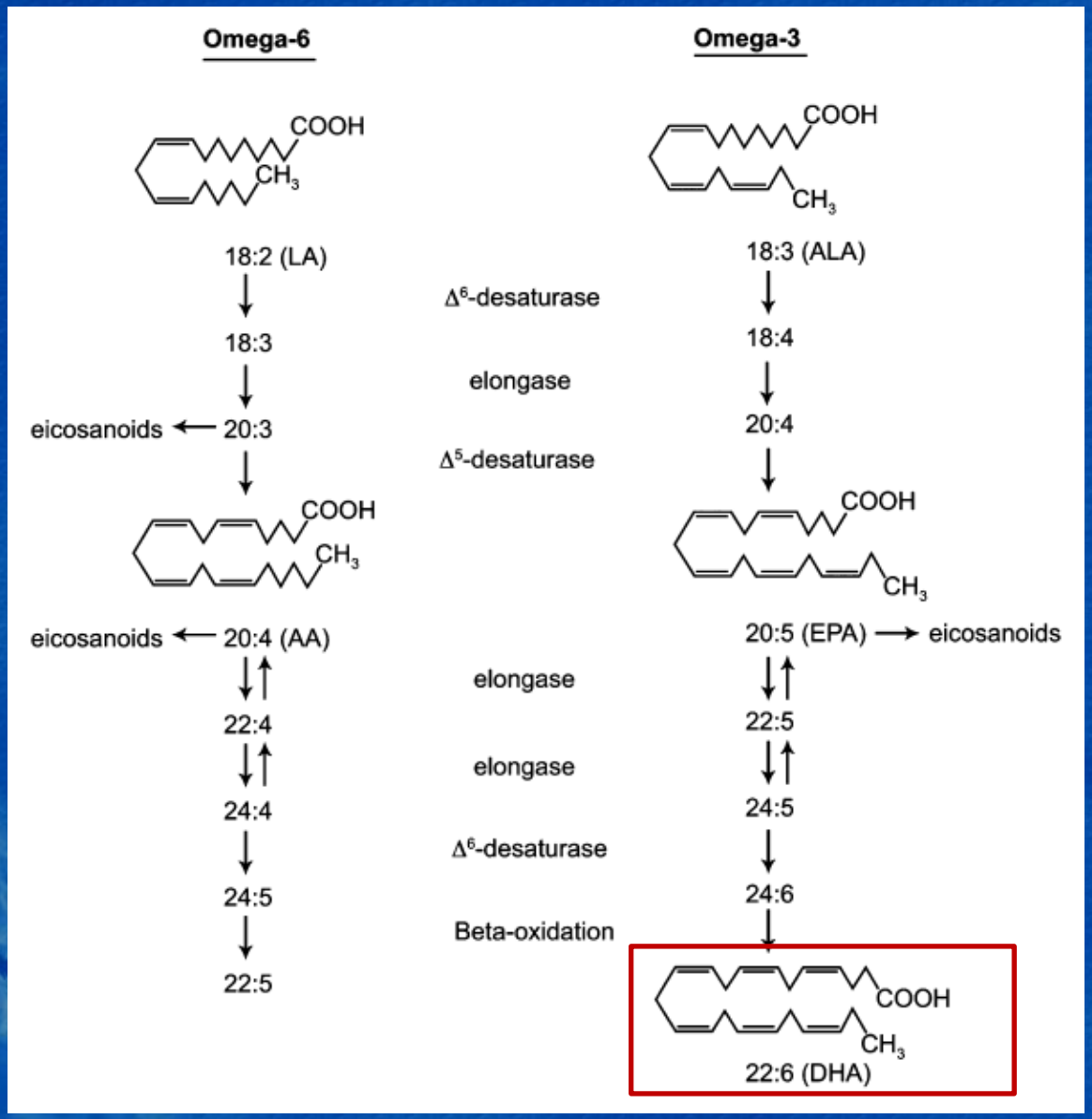
Antioxidant vitamins (C and E)
Phenolic compounds (quercetin, EGCG, resveratrol)
Essential minerals (Zn)
Omega-3 polyunsaturated fatty acids (α-linolenic acid)

ENDOTHELIAL INFLAMMATORY RESPONSE

Dietary antioxidants

DIRECT ANTIOXIDANTS	ANTIOXIDANT RESPONSE INDUCERS
•Vitamins C and E •Dietary phenolics (quercetin, EGCG, resveratrol)	•Stimulate nuclear factor-erythroid 2-related factor 2 (Nrf2) binding to antioxidant response element (ARE) •Sulforaphane, cinnamaldeyde

Polyunsaturated fatty acids (PUFAs)

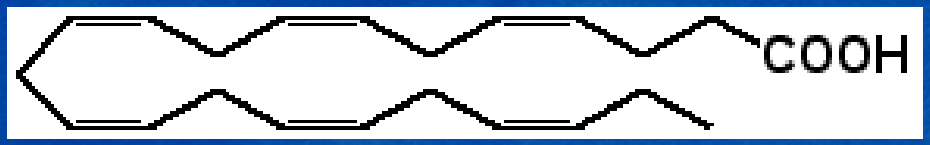


(Harris et al, 2008, *Atherosclerosis*)

Omega-3 PUFAs in prevention of cardiovascular diseases

- Positively affect the risk of coronary heart disease (Lee et al., 2008. *Mayo Clin Proc*)
- Fish-derived docosahexaenoic acid (DHA, 22:6ω-3) and eicosapentaenoic acid (EPA, 20:5ω-3)
- Incorporate into atherosclerotic plaque and increase plaque stability, improve arrhythmia, prevent platelet aggregation, decrease blood pressure and plasma triglycerides…

DHA metabolism



Enzymatic

Acylated
cyclooxygenase-2
Lipoxygenases
Cytochromes P450

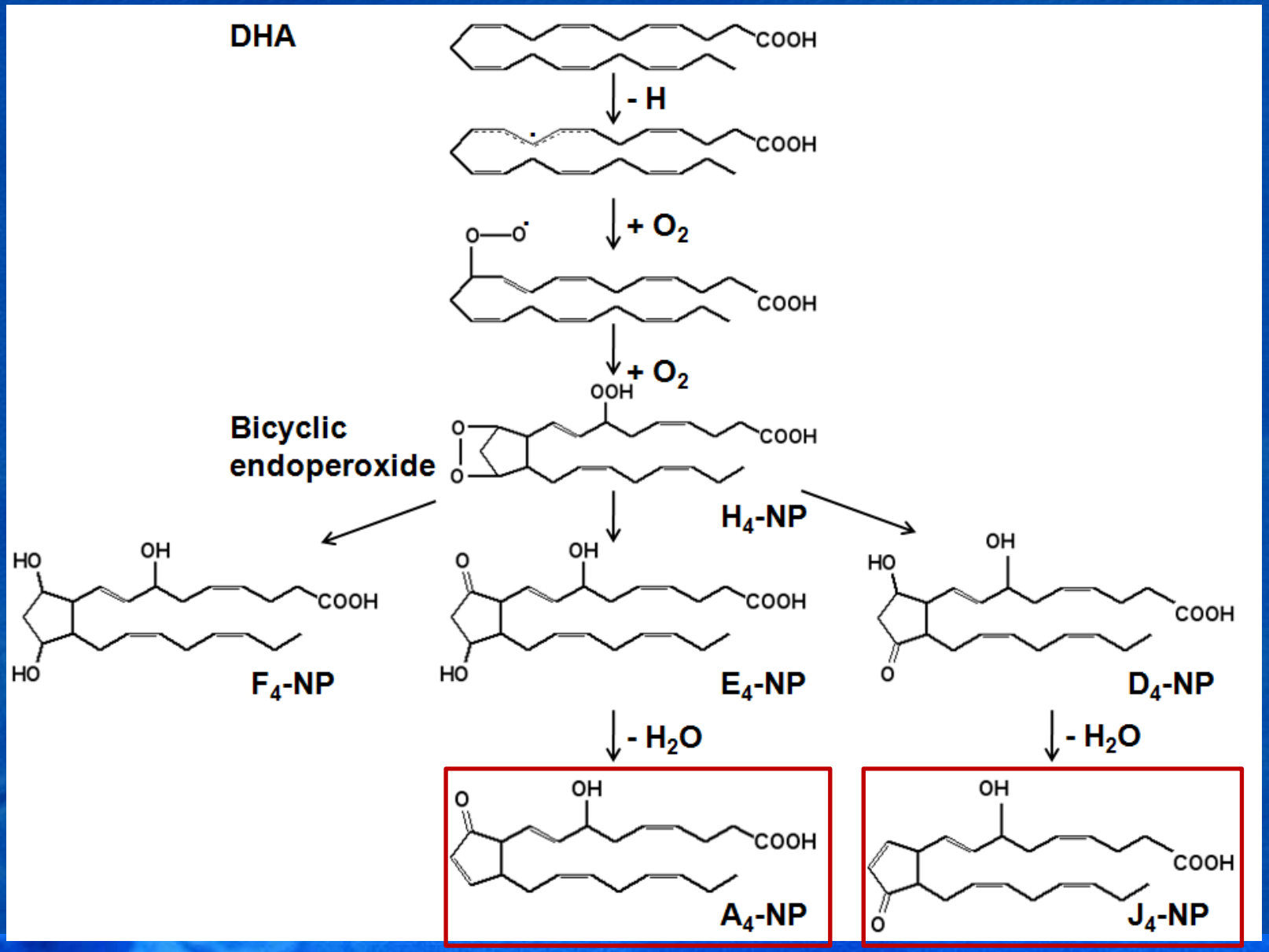
17-hydroxy-DHA
Protectins
Resolvins

Non-enzymatic

Free radical-initiated

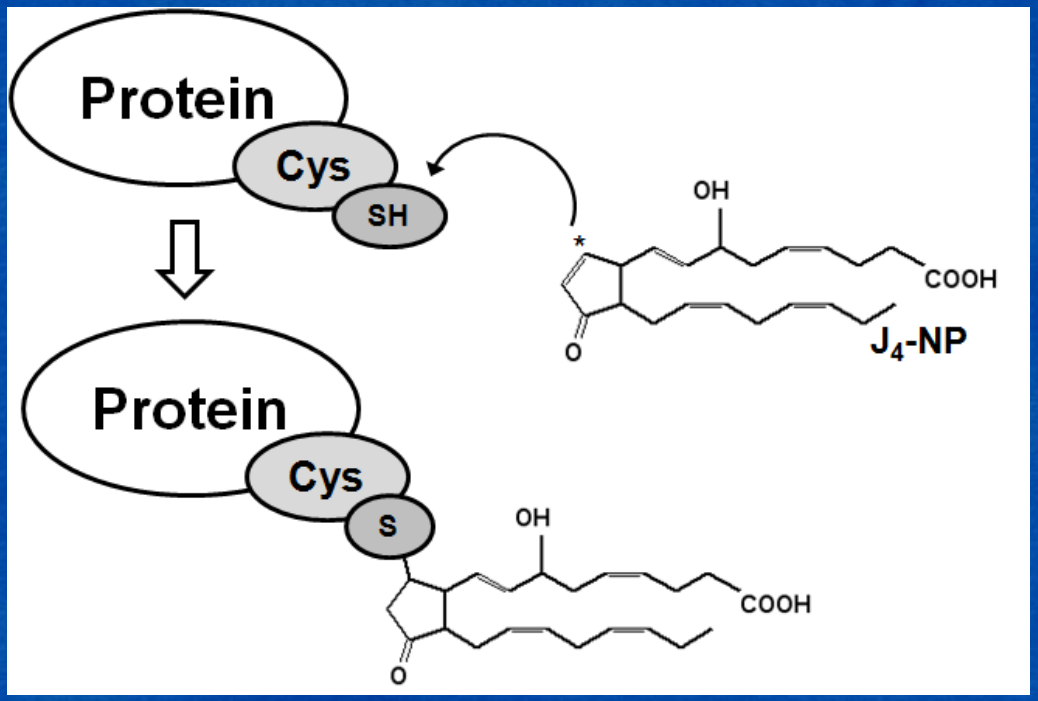
Neuroprostanes

Non-enzymatic oxidation of DHA – neuroprostane (NP) formation

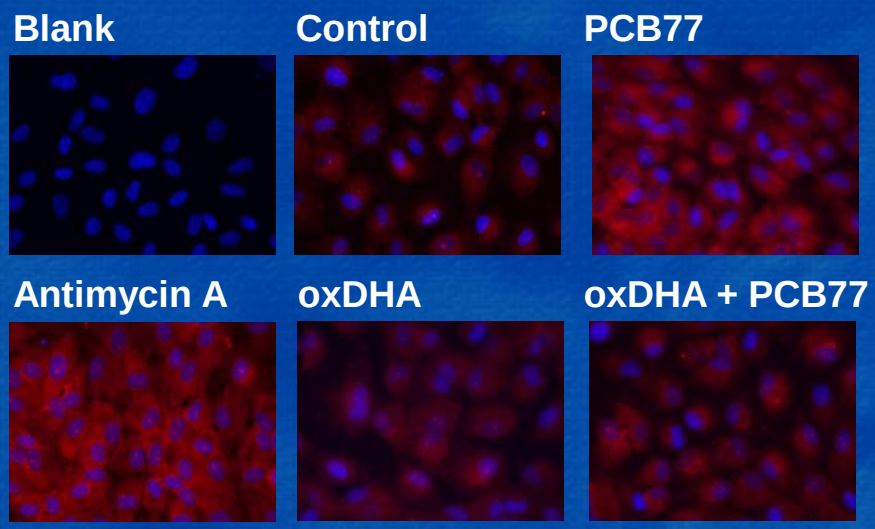


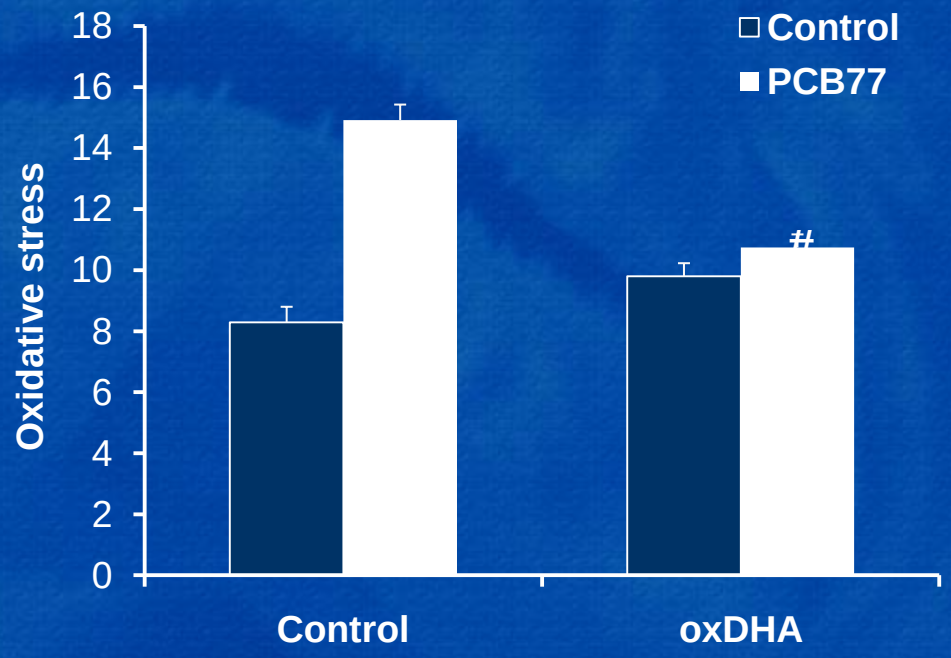
Anti-inflammatory properties of cyclopentenone-containing lipid metabolites

- Cyclopentenone prostaglandins directly inhibit I-κB kinase (Rossi et al., Nature. 2000)
- A_4/J_4 neuroprostanes prevent NF-κB activation (Musiek et al., J Biol Chem. 2008)

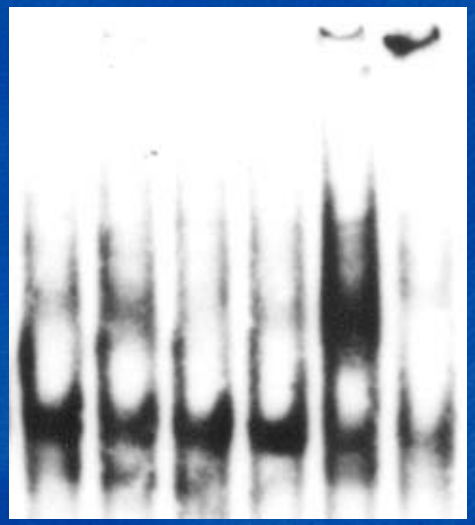


Oxidized DHA (oxDHA) prevents PCB77-induced superoxide production



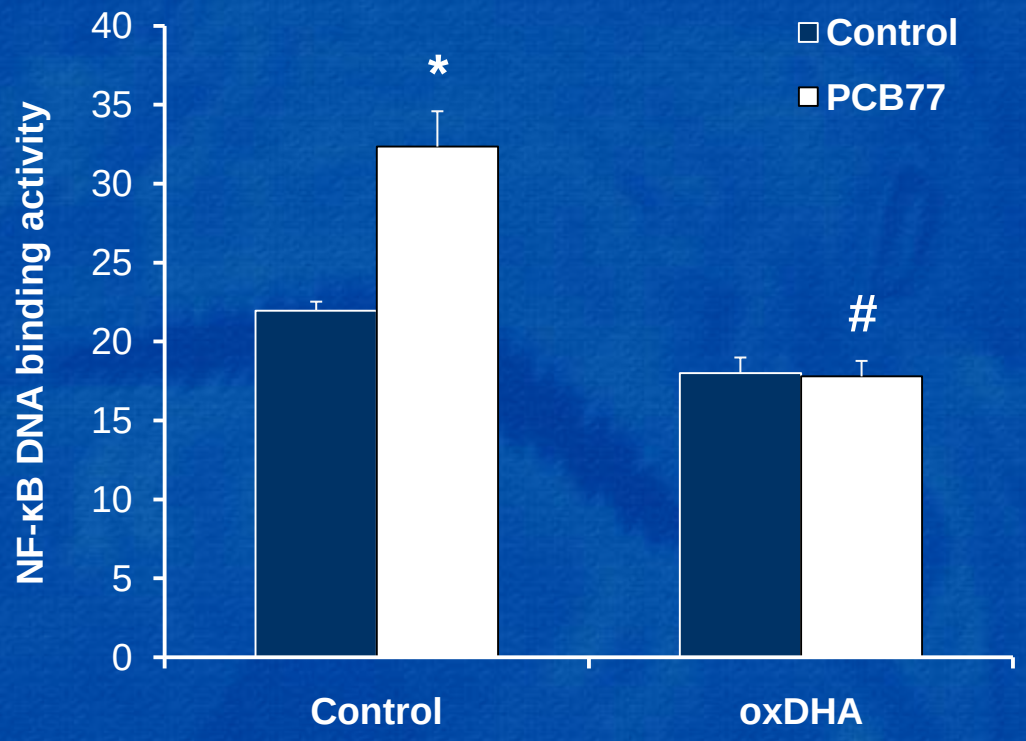


Oxidized DHA prevents an increase in DNA-binding activity of nuclear factor-κB (NF-κB) induced by PCB77



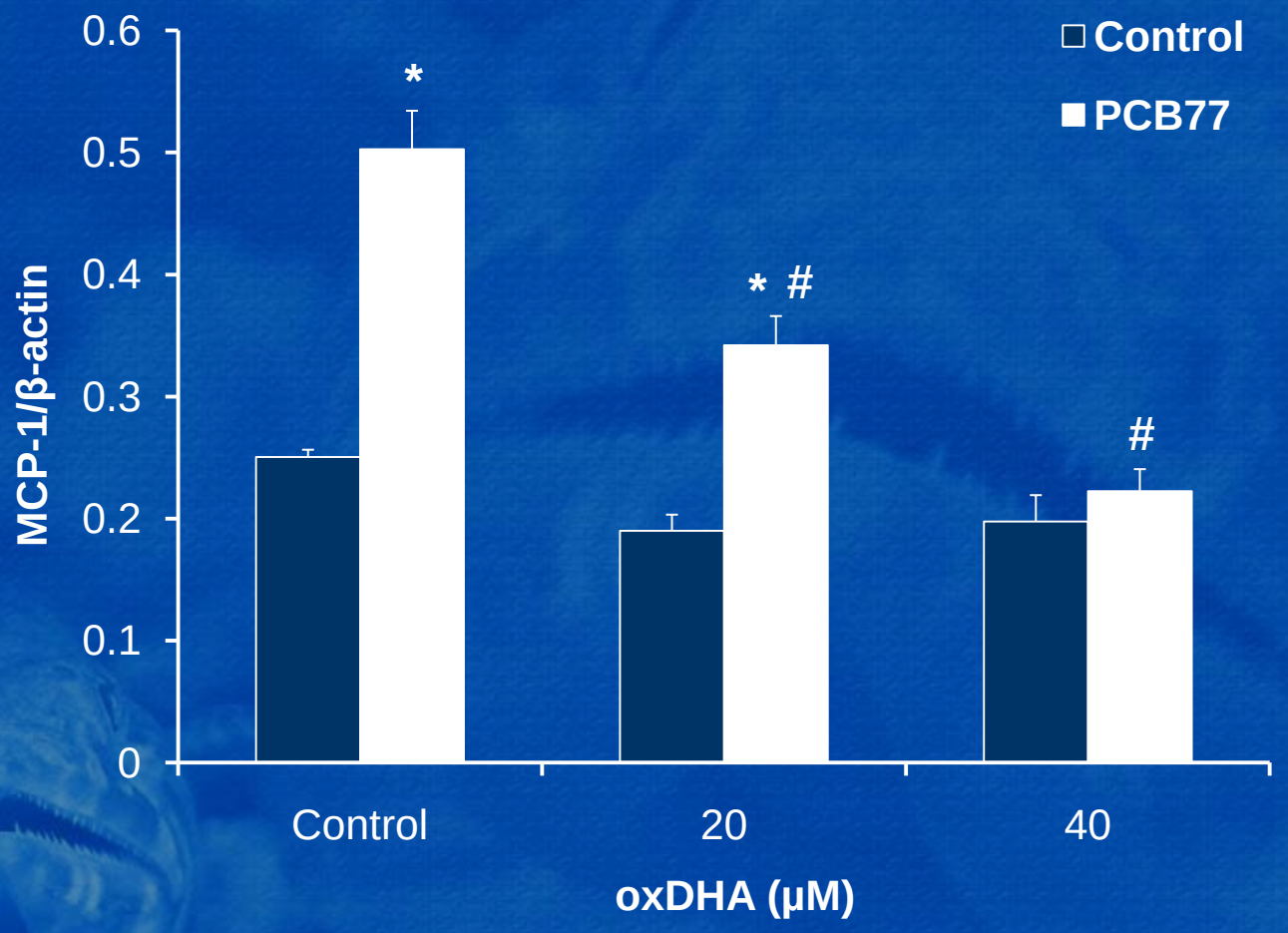
1 2 3 4 5 6

1 - control
2 - PCB77
3 – oxDHA
4 - oxDHA + PCB77
5 - TNF-α
6 - p65 supershift

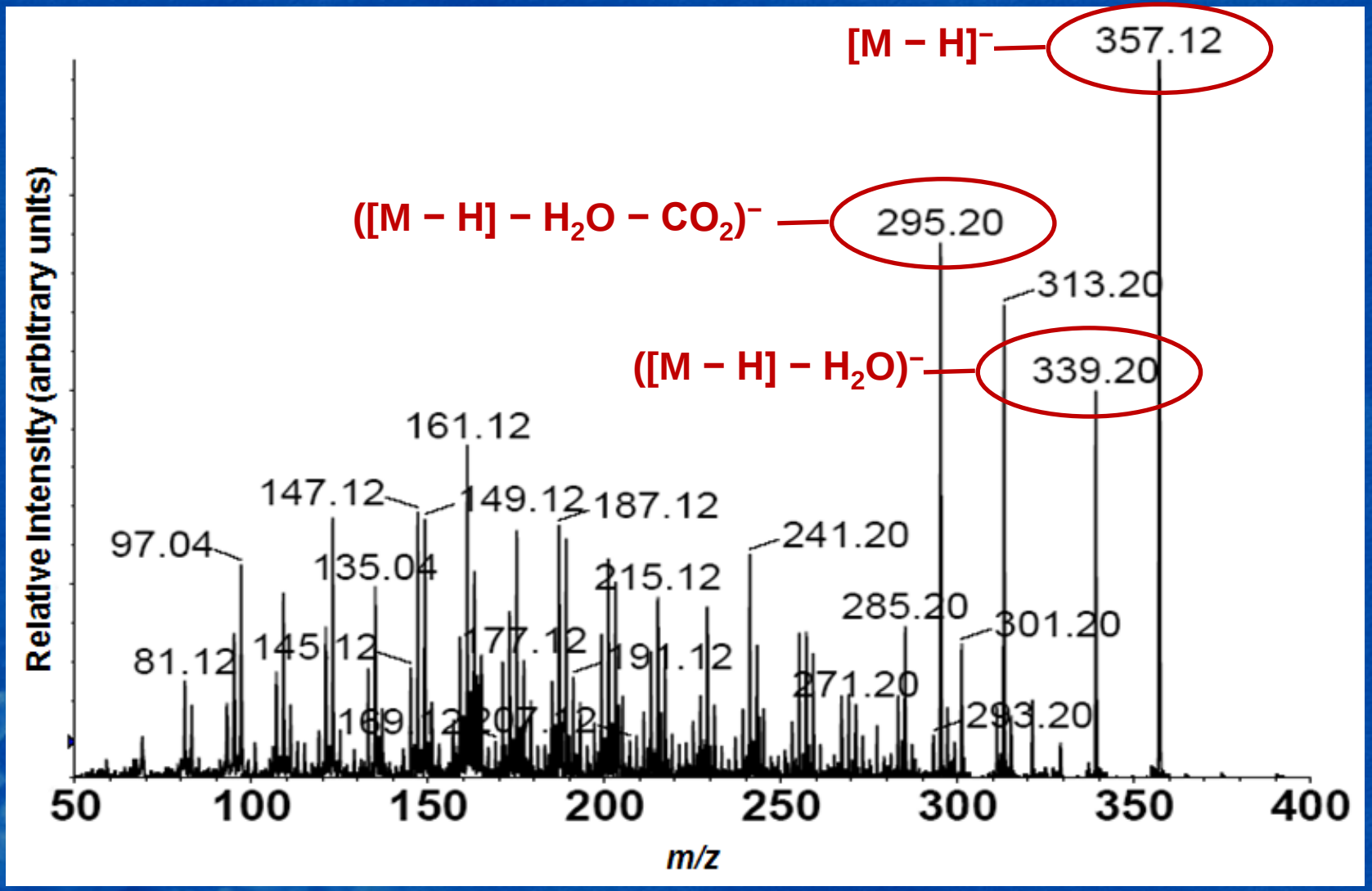


TNF-α = tumor necrosis factor-α (NF-κB inducer)

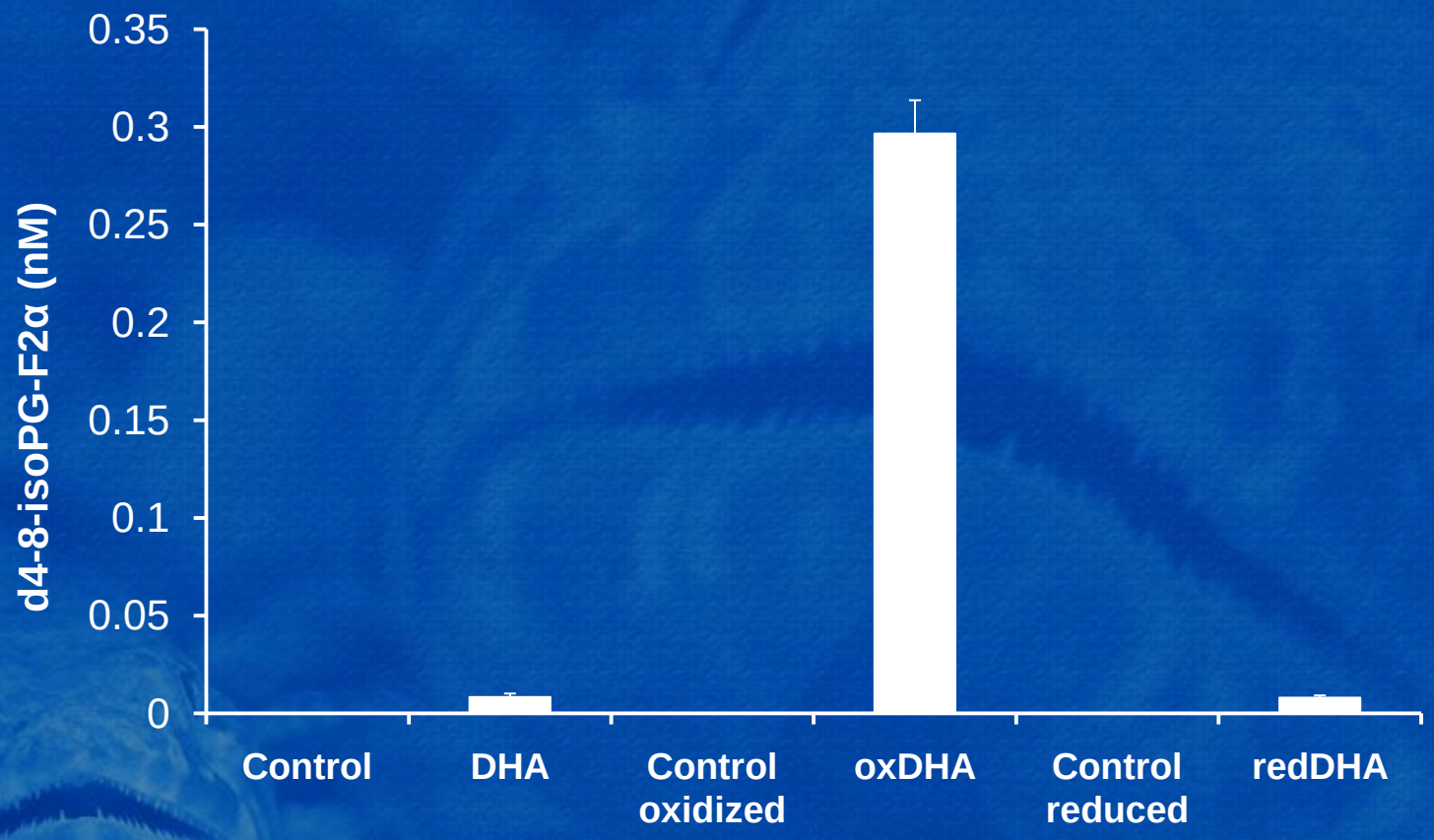
Oxidized DHA prevents MCP-1 mRNA up-regulation by PCB77



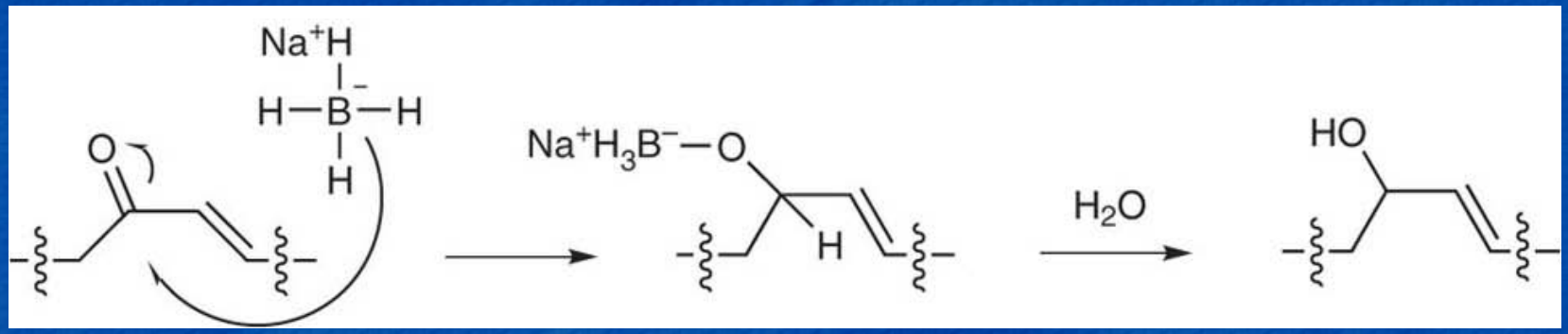
Tandem mass spectrometry analysis of A_4/J_4-NPs (*m/z* 357)



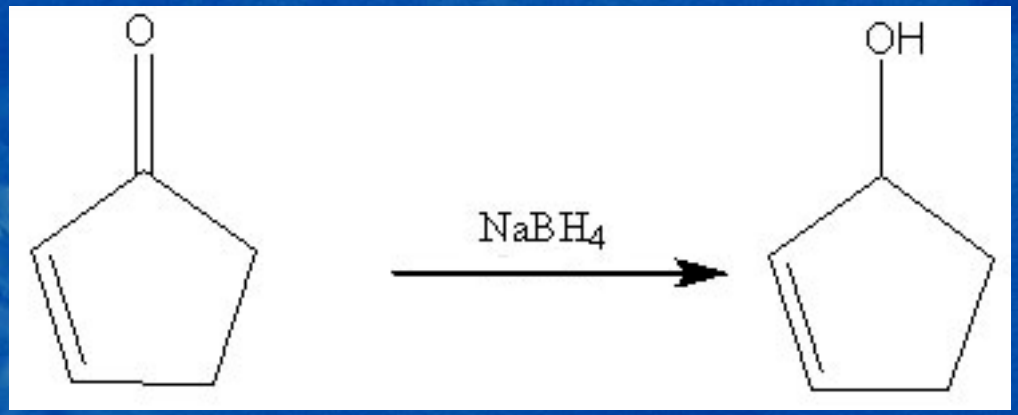
A_4/J_4-NP levels in the treatment samples



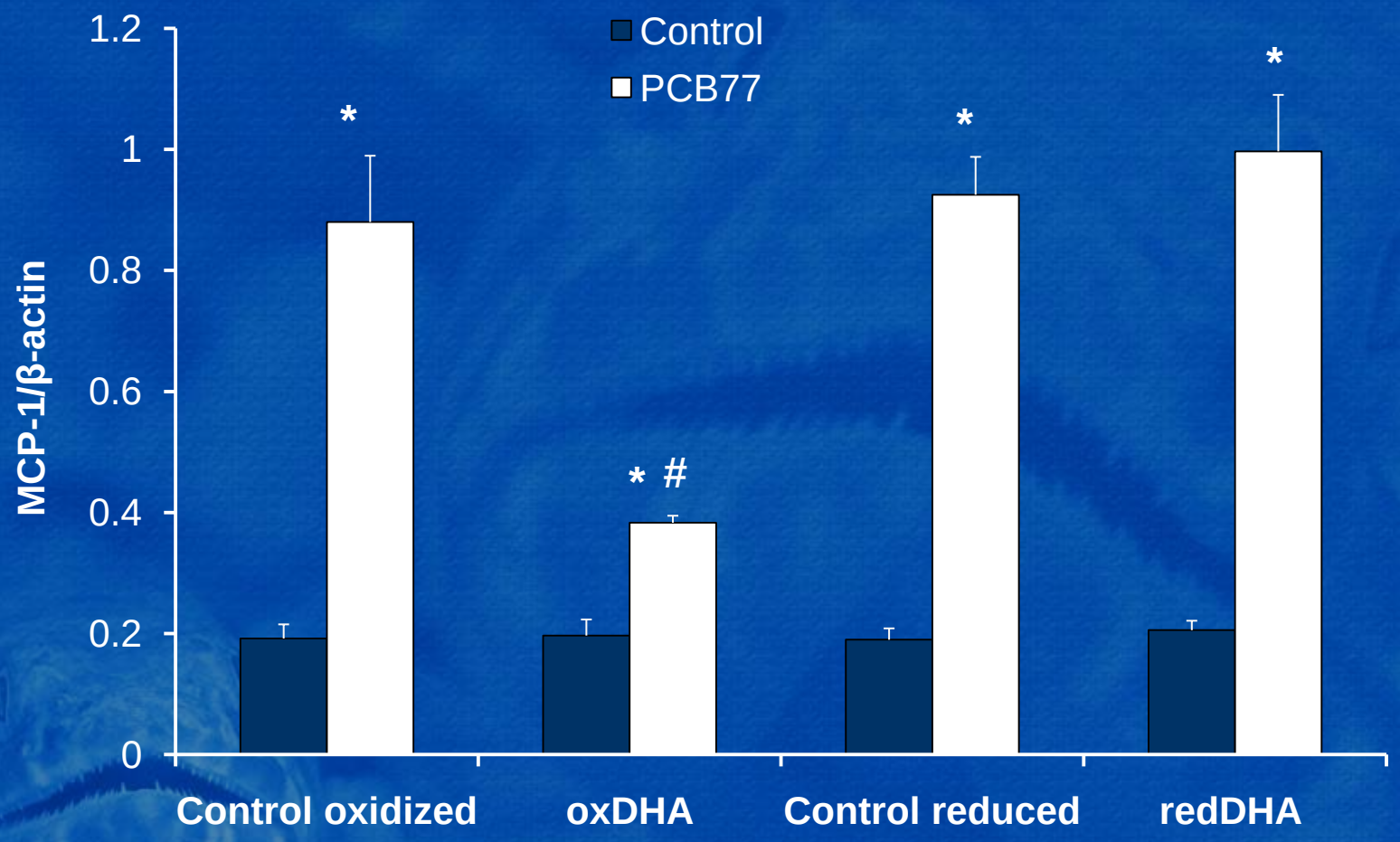
Sodium borohydride ($NaBH_4$)-mediated reduction of A_4/J_4-NPs



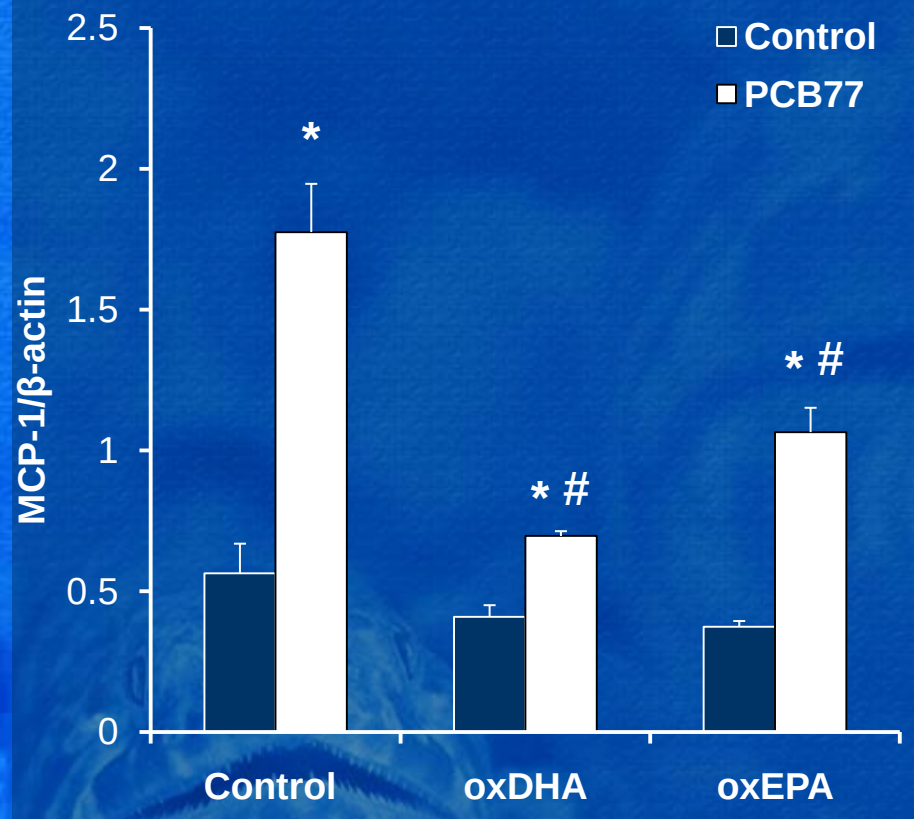
(Groeger et al, Nat Chem Biol. 2010)

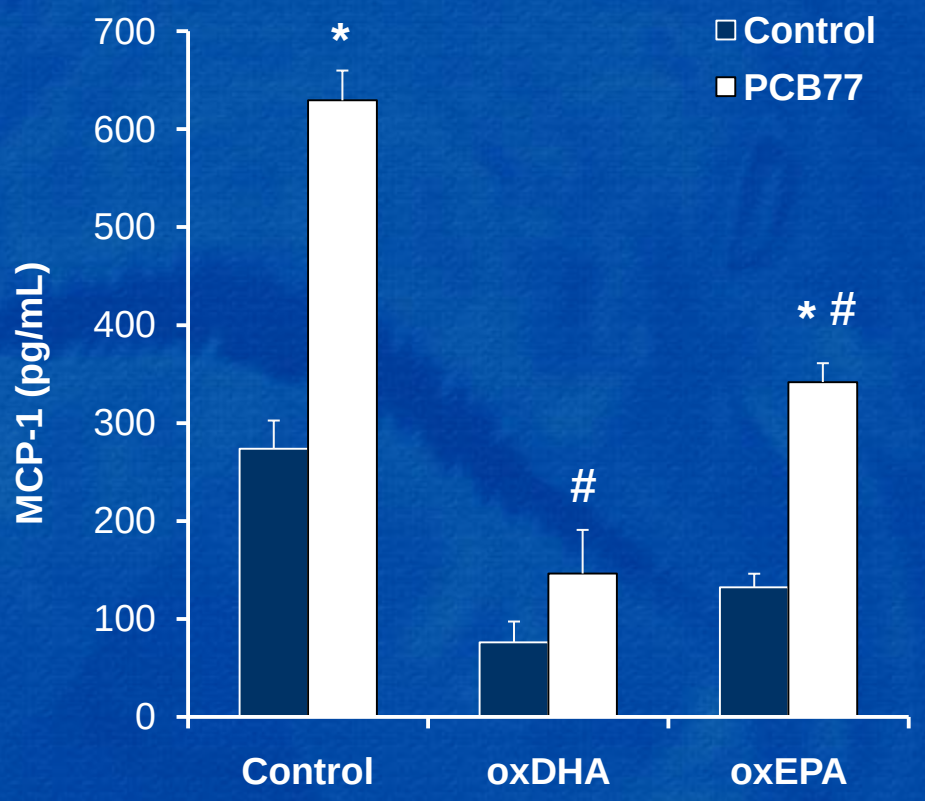


Reduced DHA is ineffective in preventing MCP-1 mRNA up-regulation by PCB77



Inhibition of PCB77-induced MCP-1 mRNA up-regulation by oxDHA and oxEPA

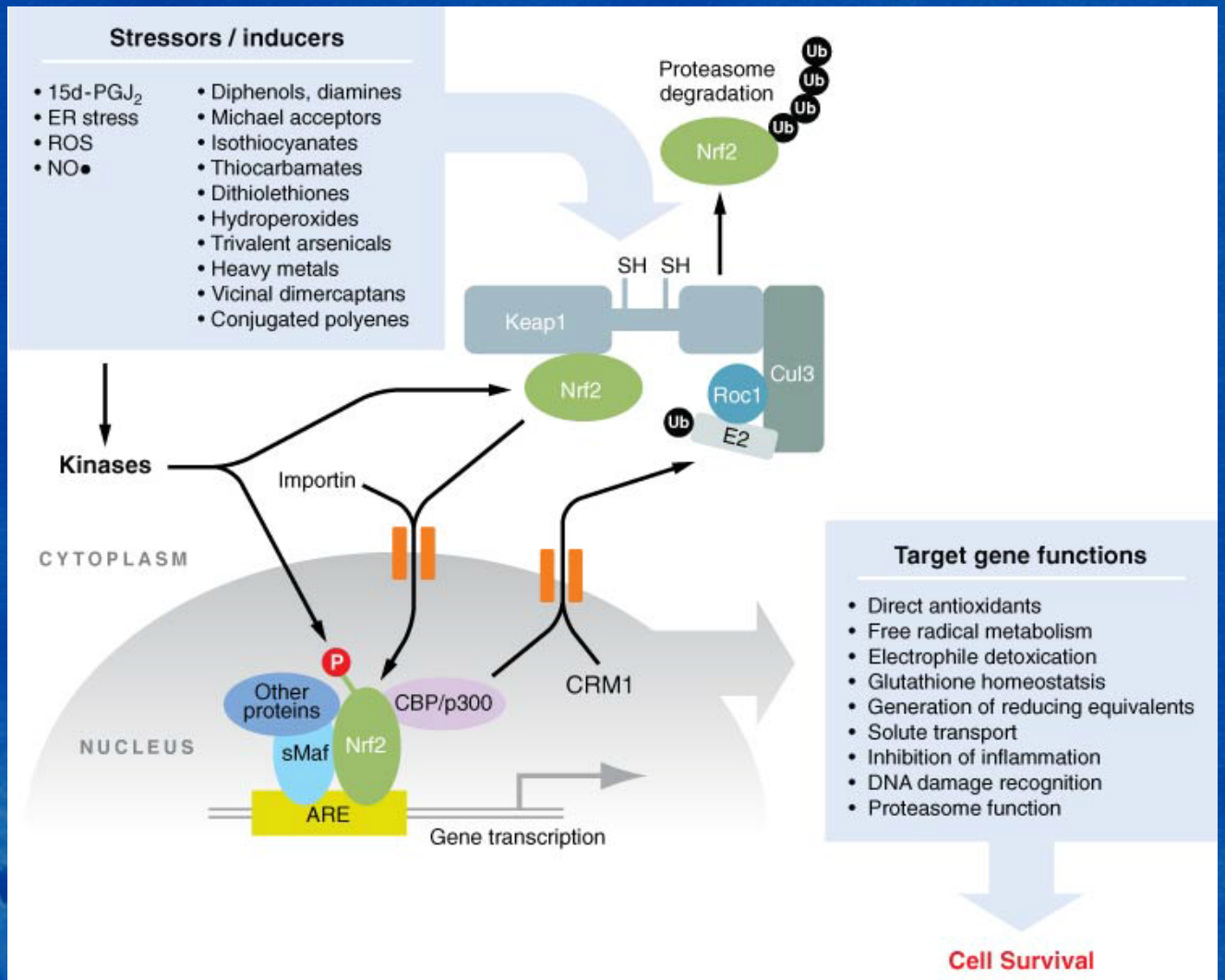




Nuclear factor-erythroid 2-related factor 2 (Nrf2)

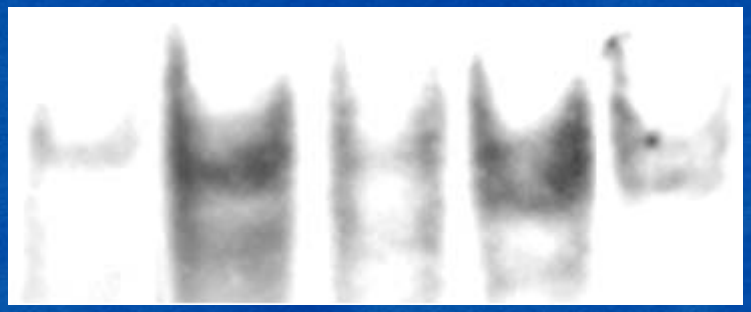
- Targets antioxidant response element (glutahione synthesis and metabolism, heme oxygenases, NAD(P)H:quinone oxidoreductase)
- Activation associated with reduced inflammation in aortic endothelial cells (Zakkar et al., 2009. *Arterioscler Thromb Vasc Biol*)
- Cyclopentenone metabolites of DHA and EPA activate Nrf-2 by Keap-1 binding (Gao et al., 2007. *J Biol Chem*)

Nrf2 signaling pathway



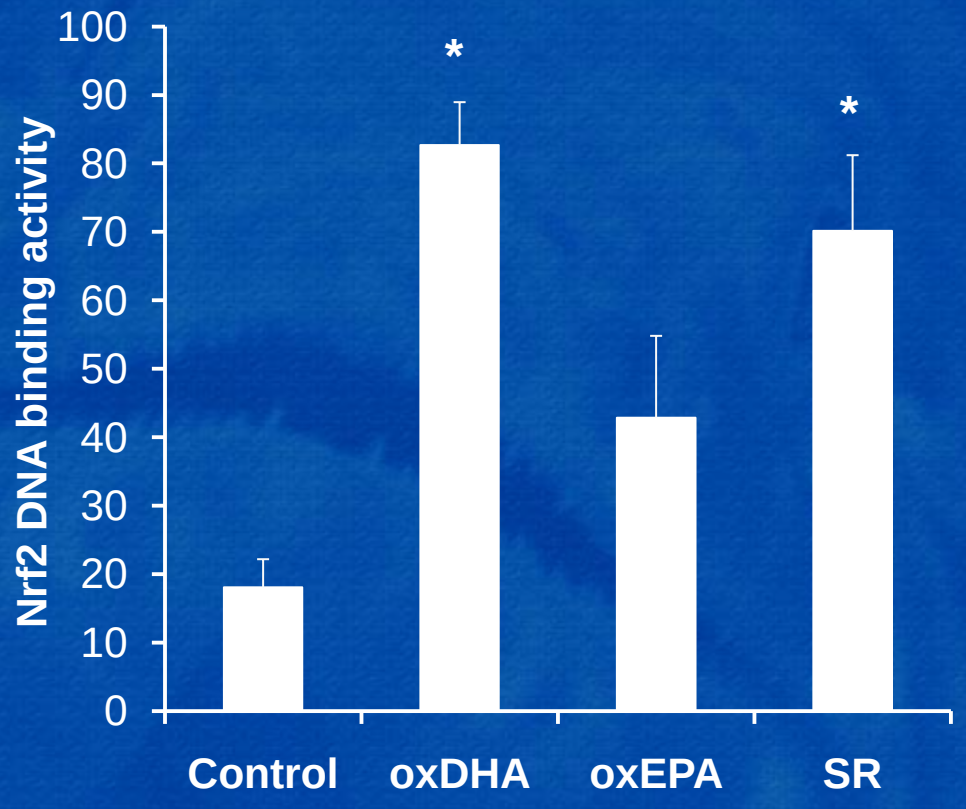
Kensler TW, et al. 2007.
Annu. Rev. Pharmacol. Toxicol. 47:89–116

Oxidized omega-3 fatty acids up-regulate Nrf2 DNA-binding activity



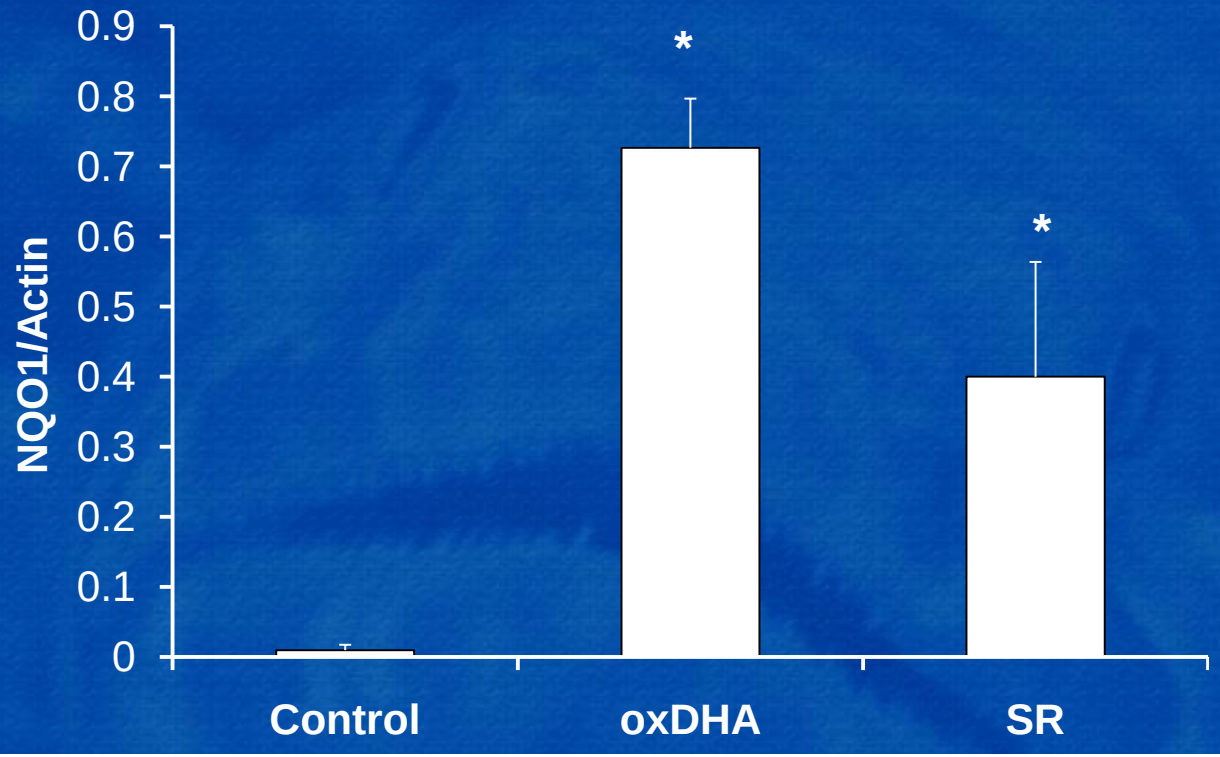
1 2 3 4 5

1 - control
2 - oxDHA
3 – oxEPA
4 - SR
5 - Nrf2 supershift



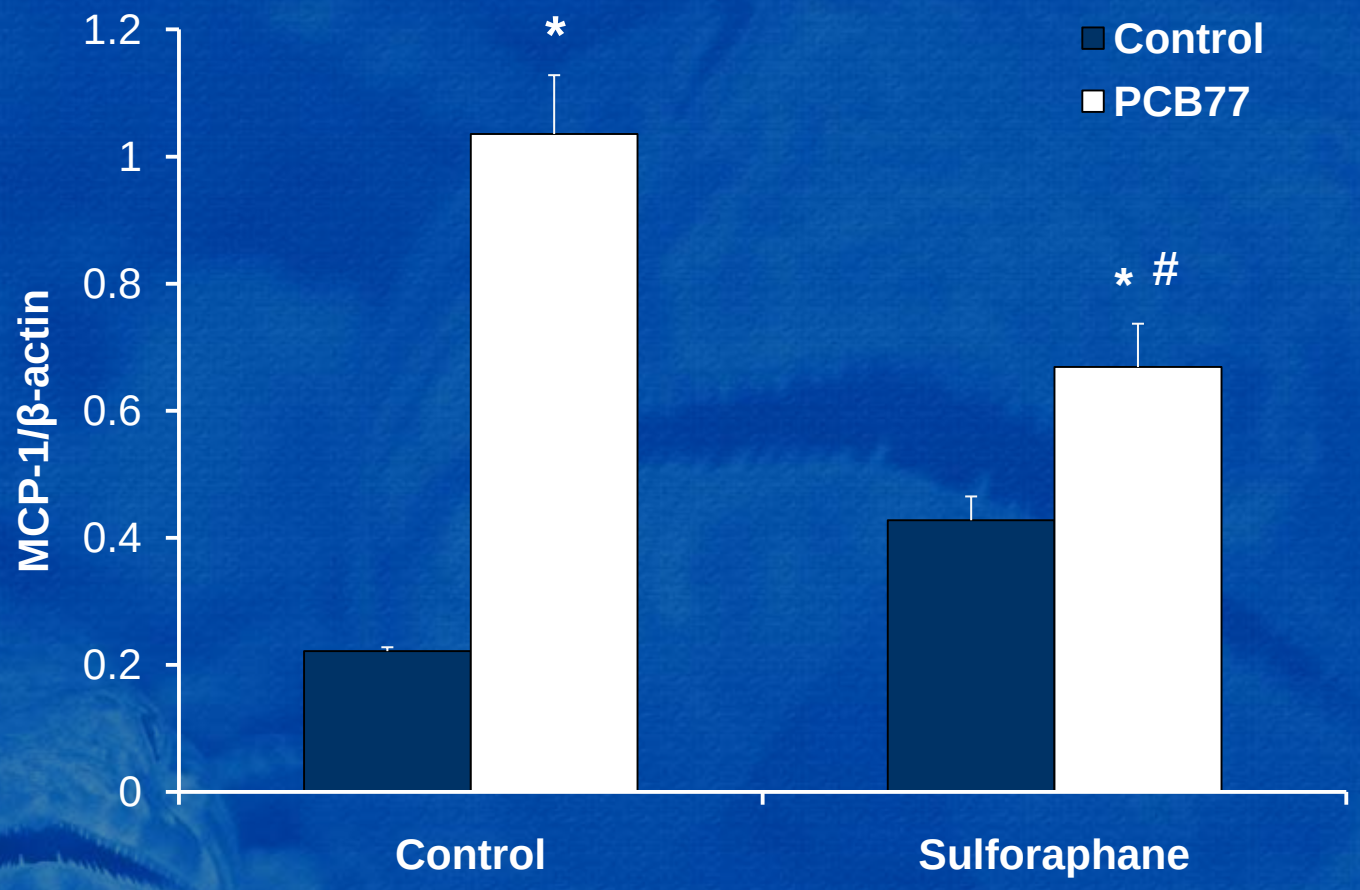
SR = sulforaphane (Nrf2 inducer)

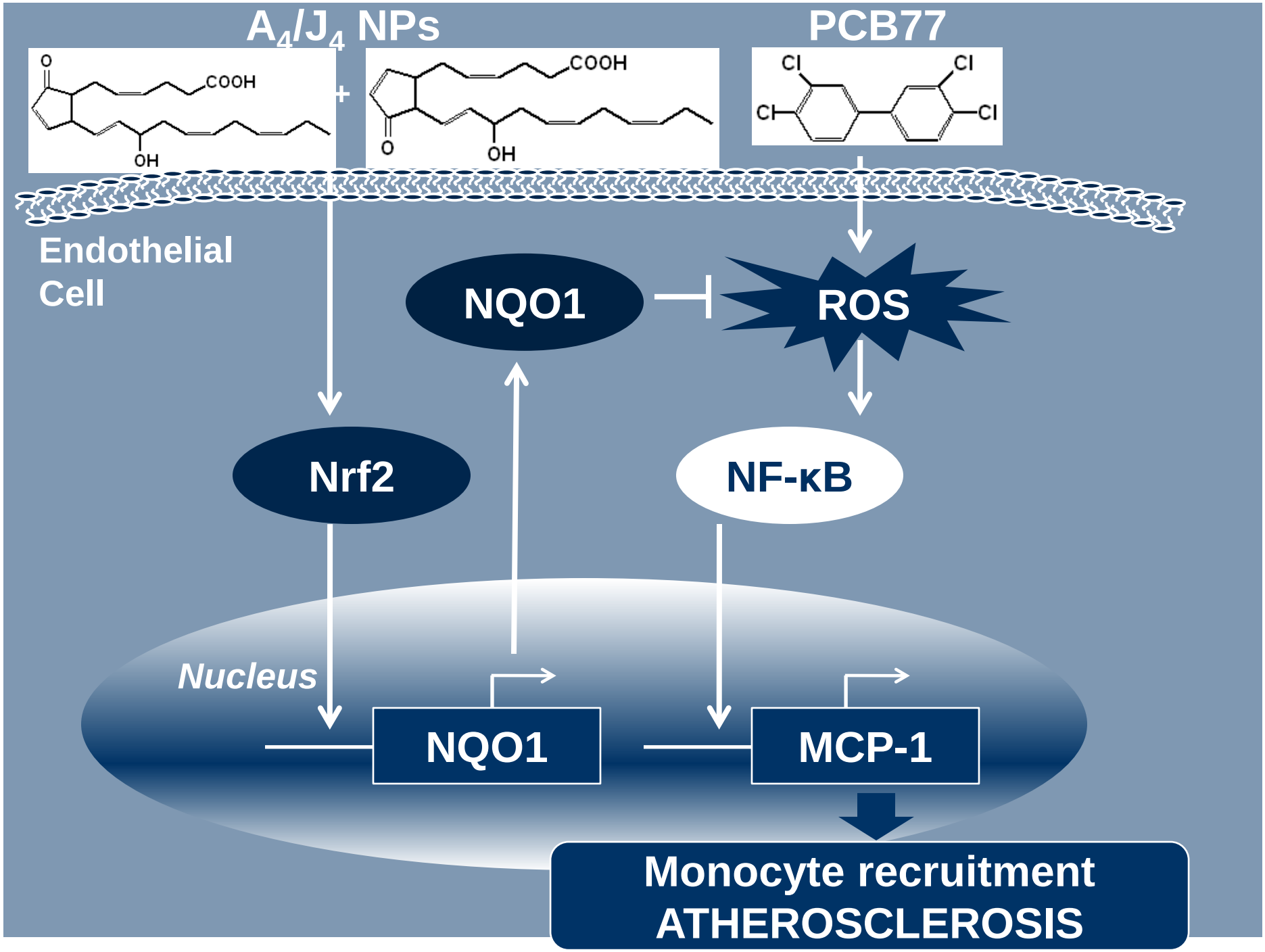
Oxidized DHA up-regulates NAD(P)H:quinone oxidoreductase (NQO1) protein expression



SR = sulforaphane (Nrf2 inducer)

Sulforaphane prevents PCB77-induced MCP-1 mRNA expression




A4/J4 NPs
PCB77
COOH
OH
O
+
Cl
Endothelial Cell
NQO1
ROS
Nrf2
NF-κB
Nucleus
NQO1
MCP-1
Monocyte recruitment
ATHEROSCLEROSIS

Conclusions

- Coplanar PCB77 up-regulates MCP-1 expression in endothelial cells and in mouse vasculature
- Metabolites of DHA have capacity to activate Nrf2 pathway and antioxidant responses and thus prevent endothelial toxicity of coplanar PCB77

Future studies

- *In vivo* demonstration of alleviated PCB toxicity after dietary enrichment with fish oil

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