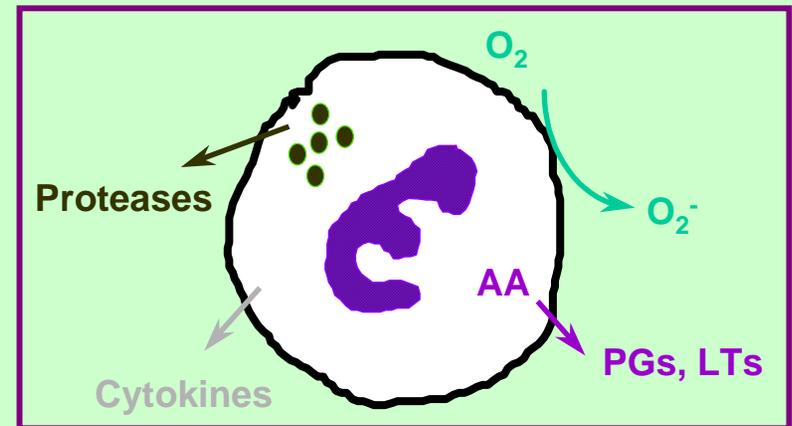


Upregulation of cyclooxygenase-2 in neutrophils by polychlorinated biphenyls

Patricia Ganey, Ph.D.
Michigan State University

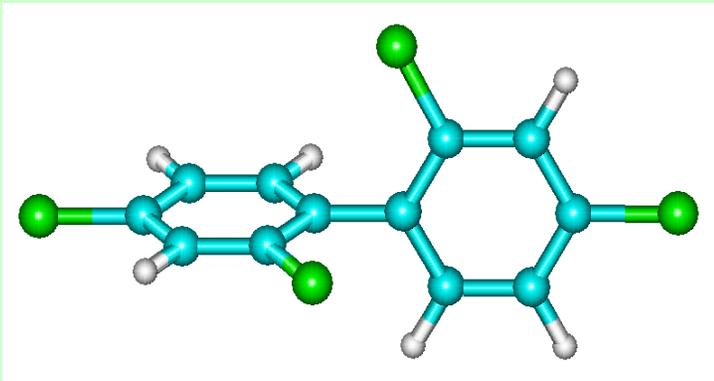
A little about neutrophils

- Neutrophils are the first line of defense against invading pathogens.



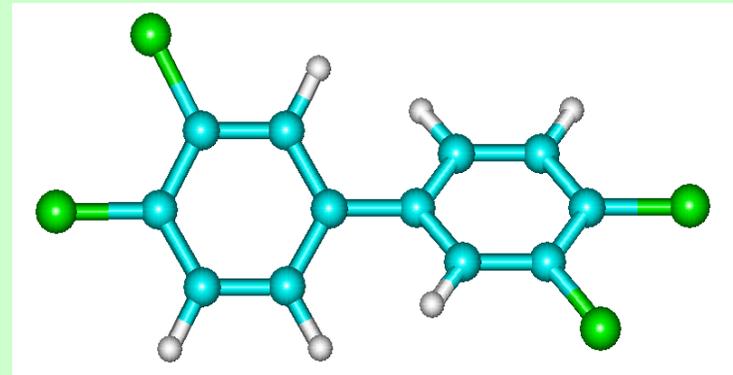
- Activation is a requirement for effective pathogen destruction.
 - Inhibition of neutrophil activation may impair host defense.
- Inappropriate activation of neutrophils may contribute to tissue injury.
 - Neutrophil-dependent damage in models of inflammation-mediated injury.

Polychlorinated Biphenyls (PCBs)



2,2',4,4'-Tetrachlorobiphenyl
Non-coplanar
Ortho-substituted
Poor ligand for the AhR

Activates neutrophils



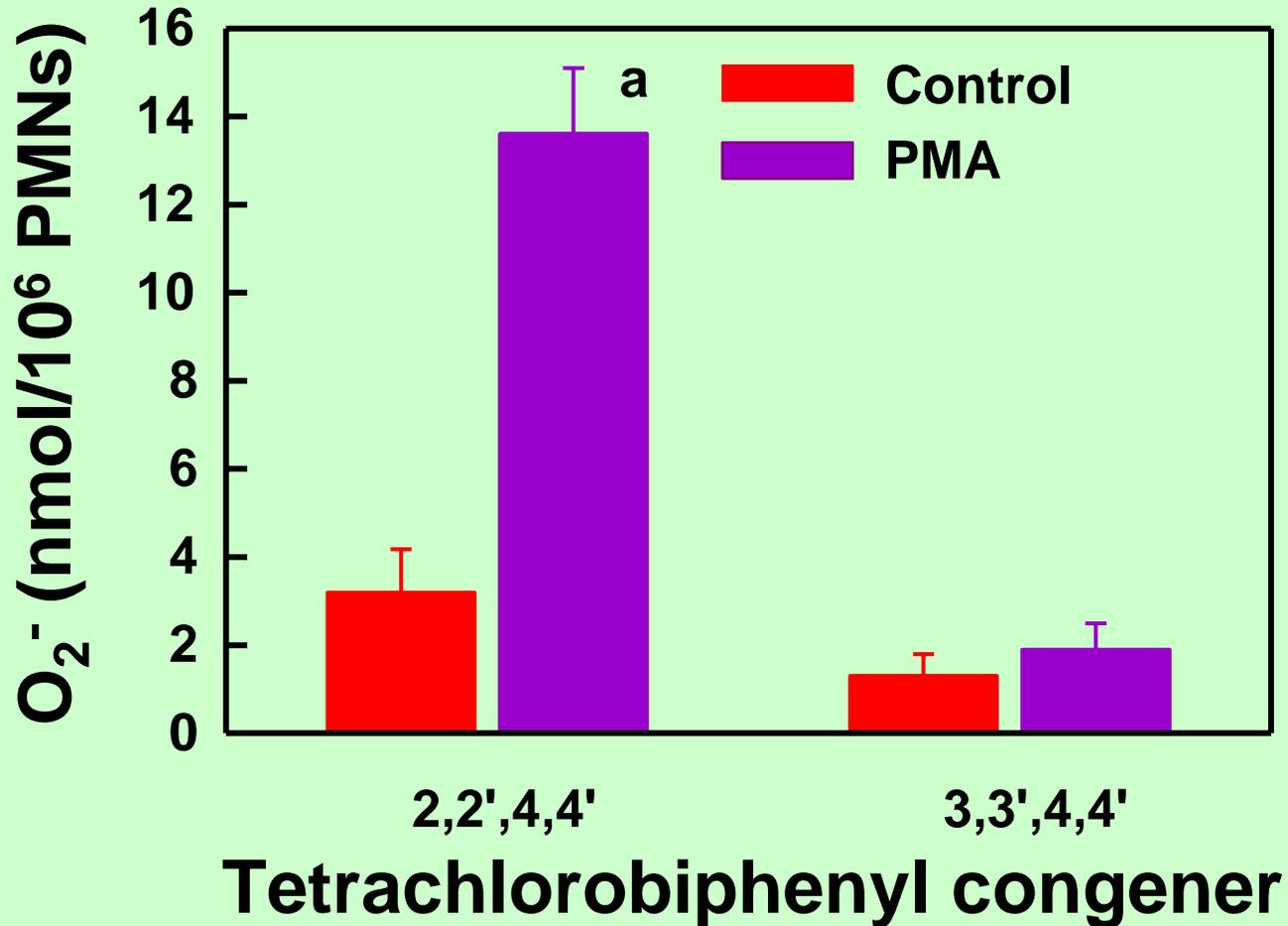
3,3',4,4'-Tetrachlorobiphenyl
Coplanar
Not ortho-substituted
High affinity for the AhR

Does not activate neutrophils

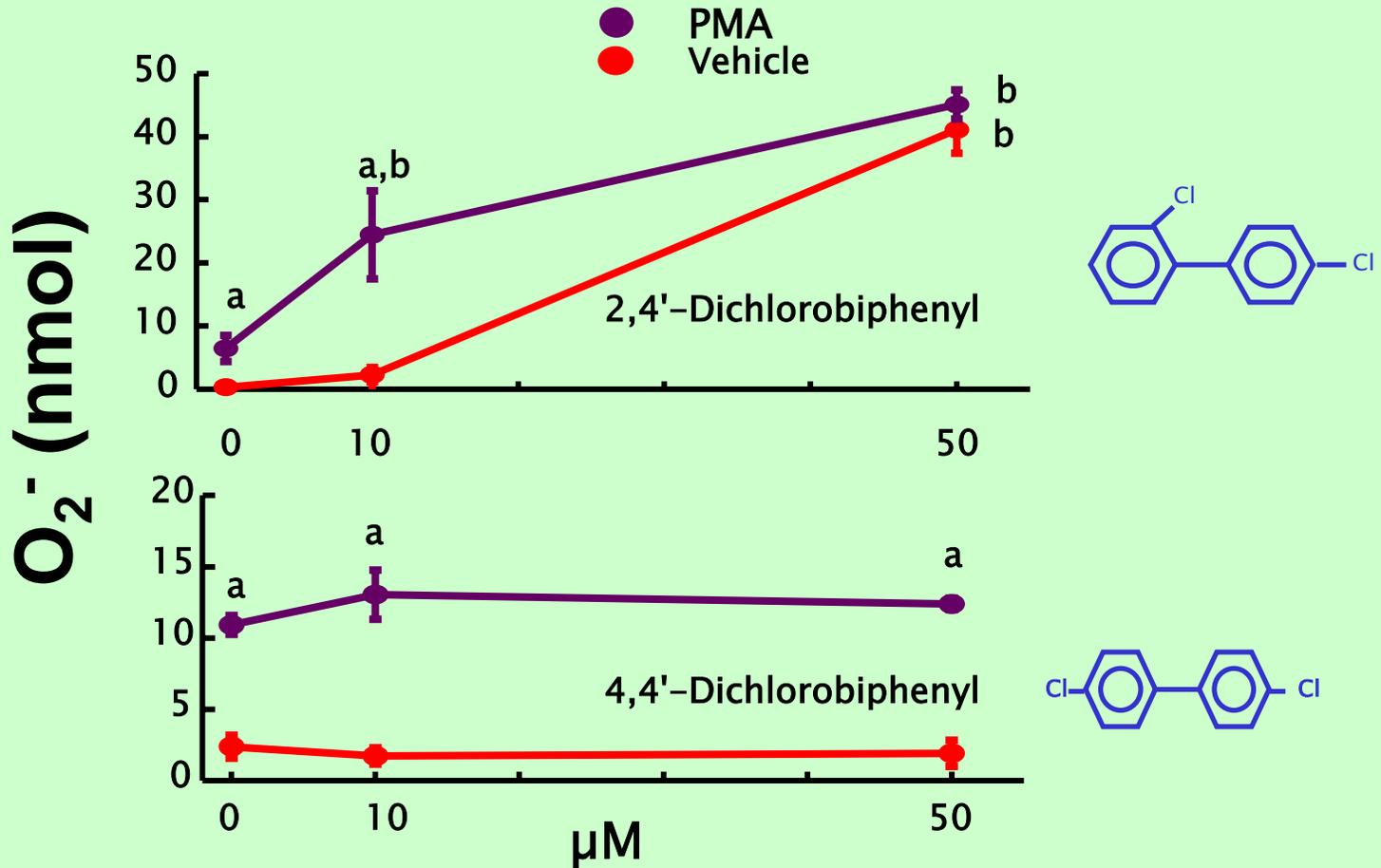
Early Observations

- **PCBs activate neutrophils**
- **PCBs alter activation in response to other stimuli**
- **Effects in neutrophils are structure-dependent**
 - **Appear to be Ah receptor-independent**

Activation of neutrophils by PCBs is structure-dependent

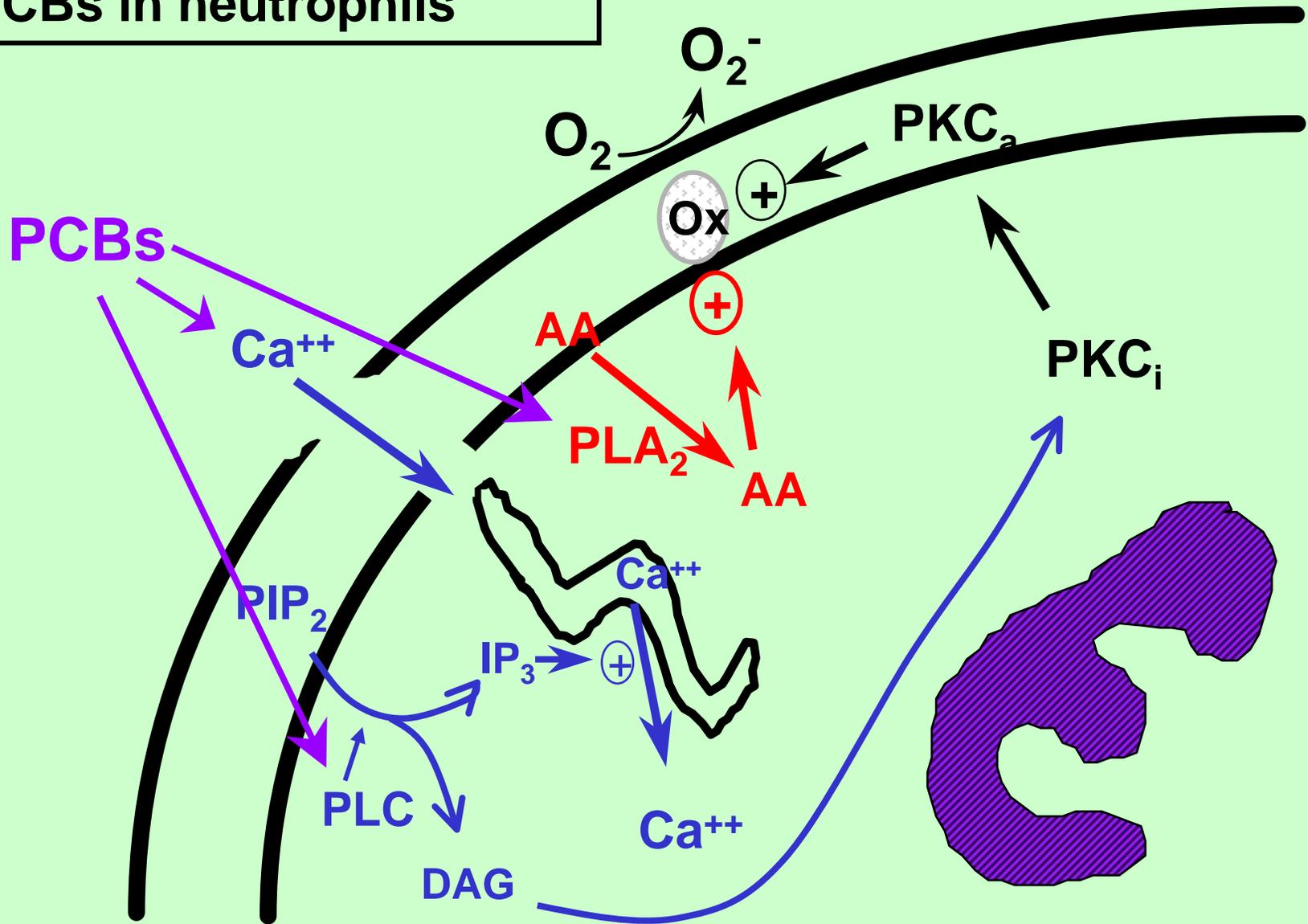


Ortho-substituted PCBs activate neutrophils

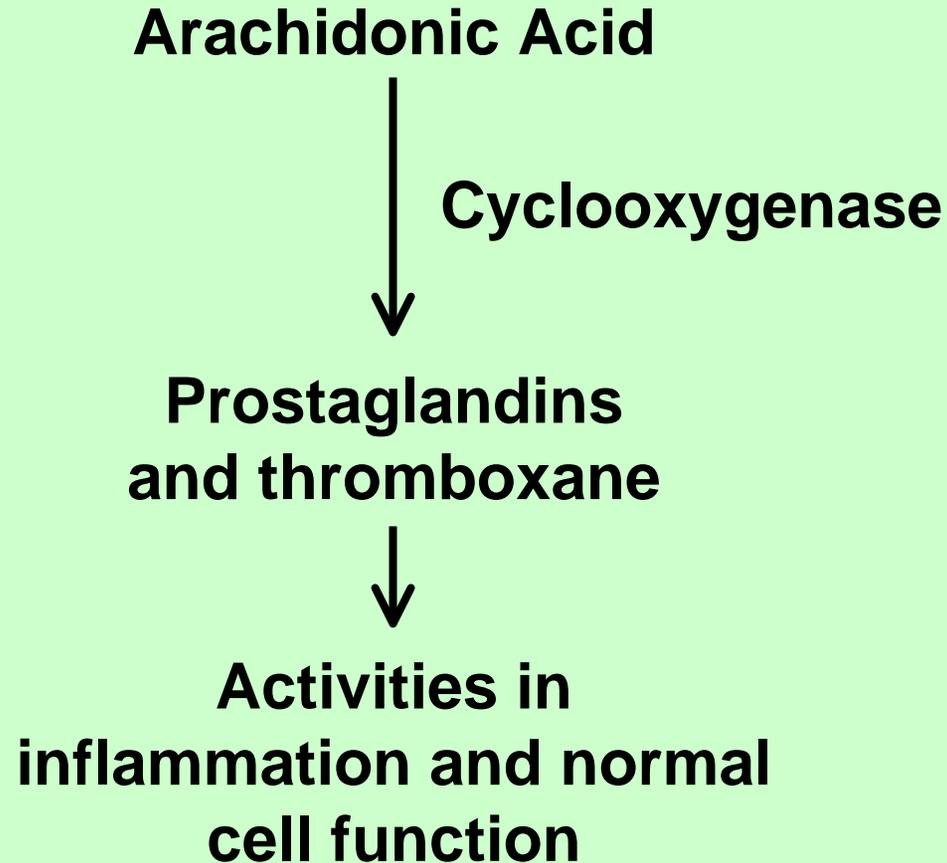


What mechanisms are involved in activation of neutrophils by ortho-substituted PCBs?

Some pathways activated by PCBs in neutrophils



Arachidonic acid metabolism, simplified

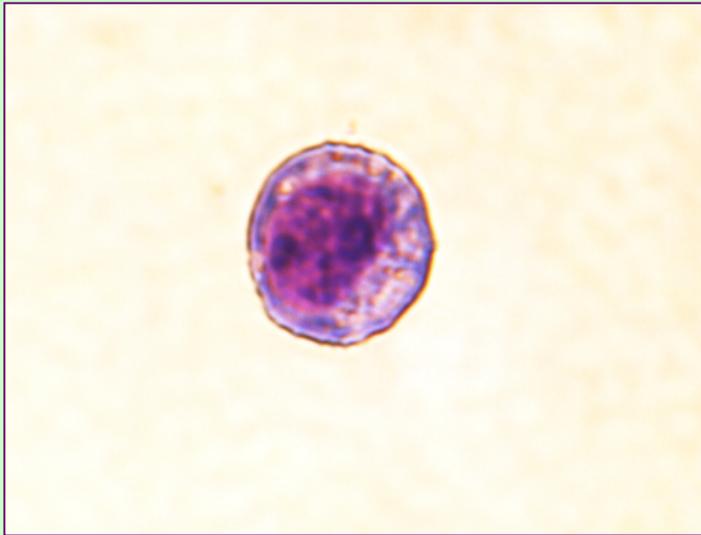


Cyclooxygenase-2 (COX-2)

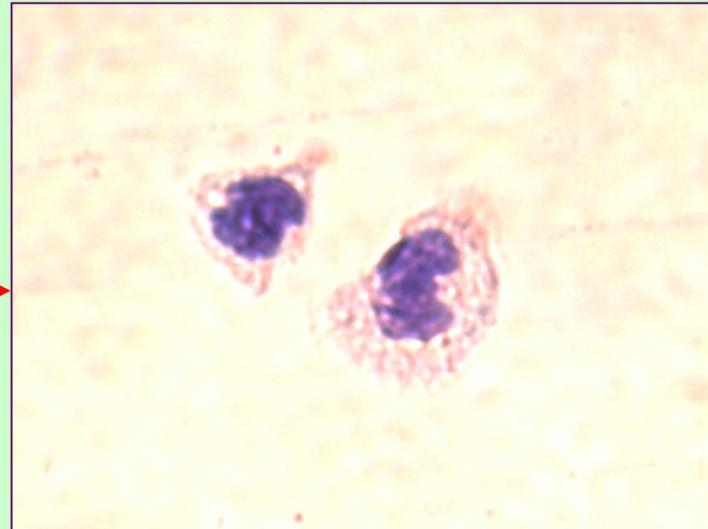
- Enzyme produced by gene of the same name
- Immediate early gene
- Upregulated in neutrophils during inflammation
- **Hypothesis: COX-2 is upregulated in neutrophils by ortho-substituted PCBs.**

HL-60 cells

- A human, promyelocytic cell line
- On treatment with dimethylsulfoxide, differentiate to a neutrophil-like phenotype

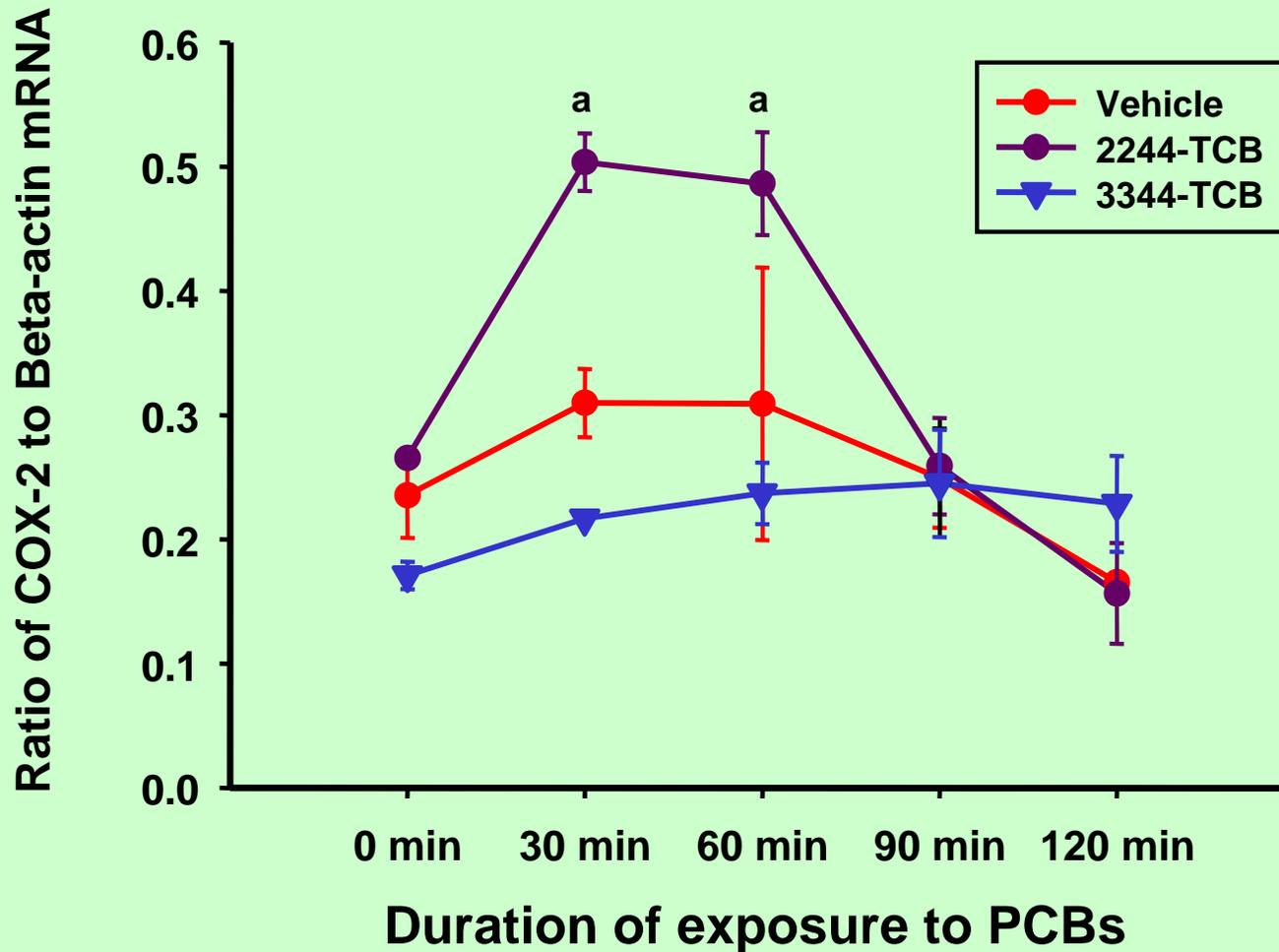


Undifferentiated

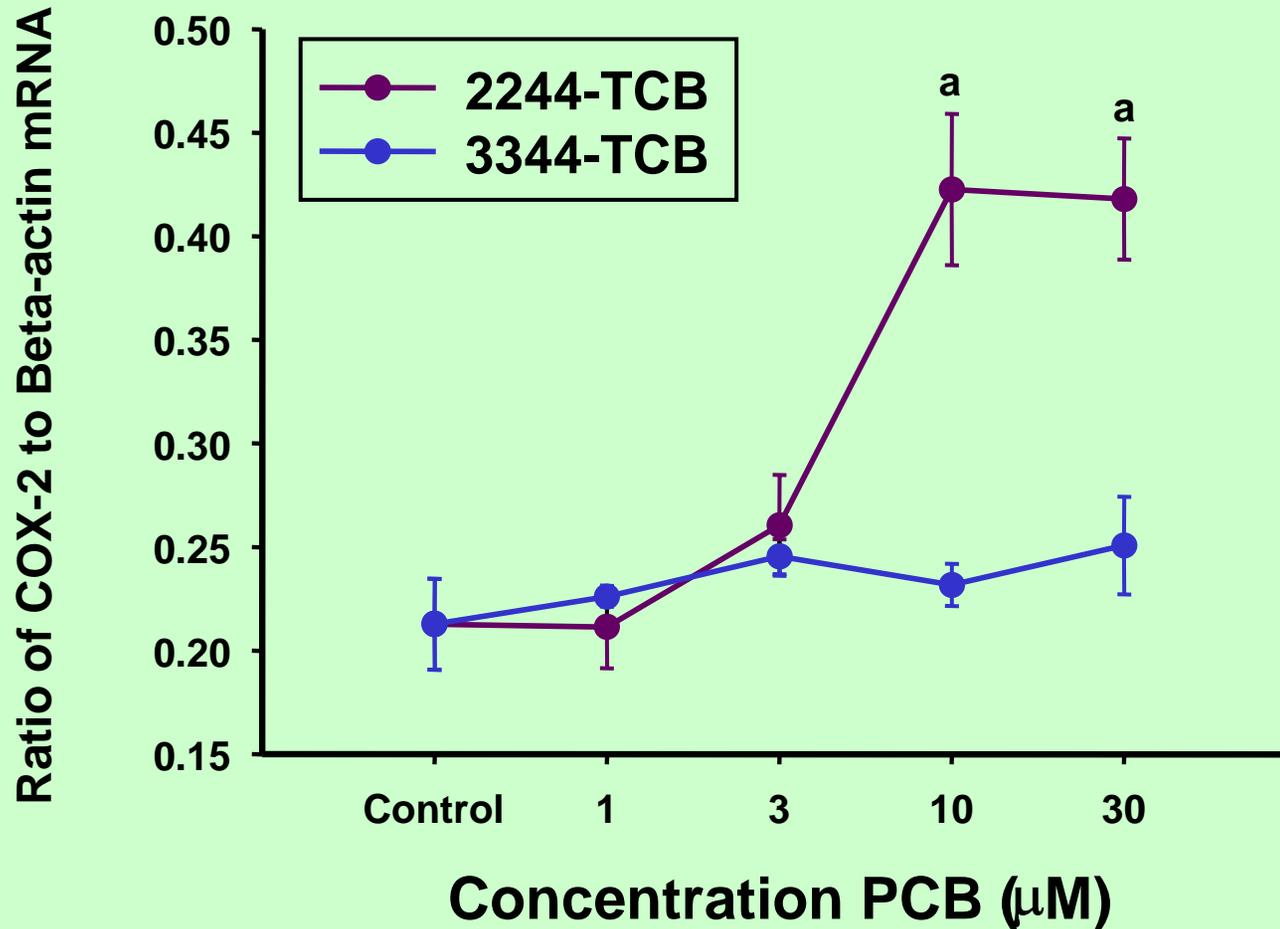


Differentiated

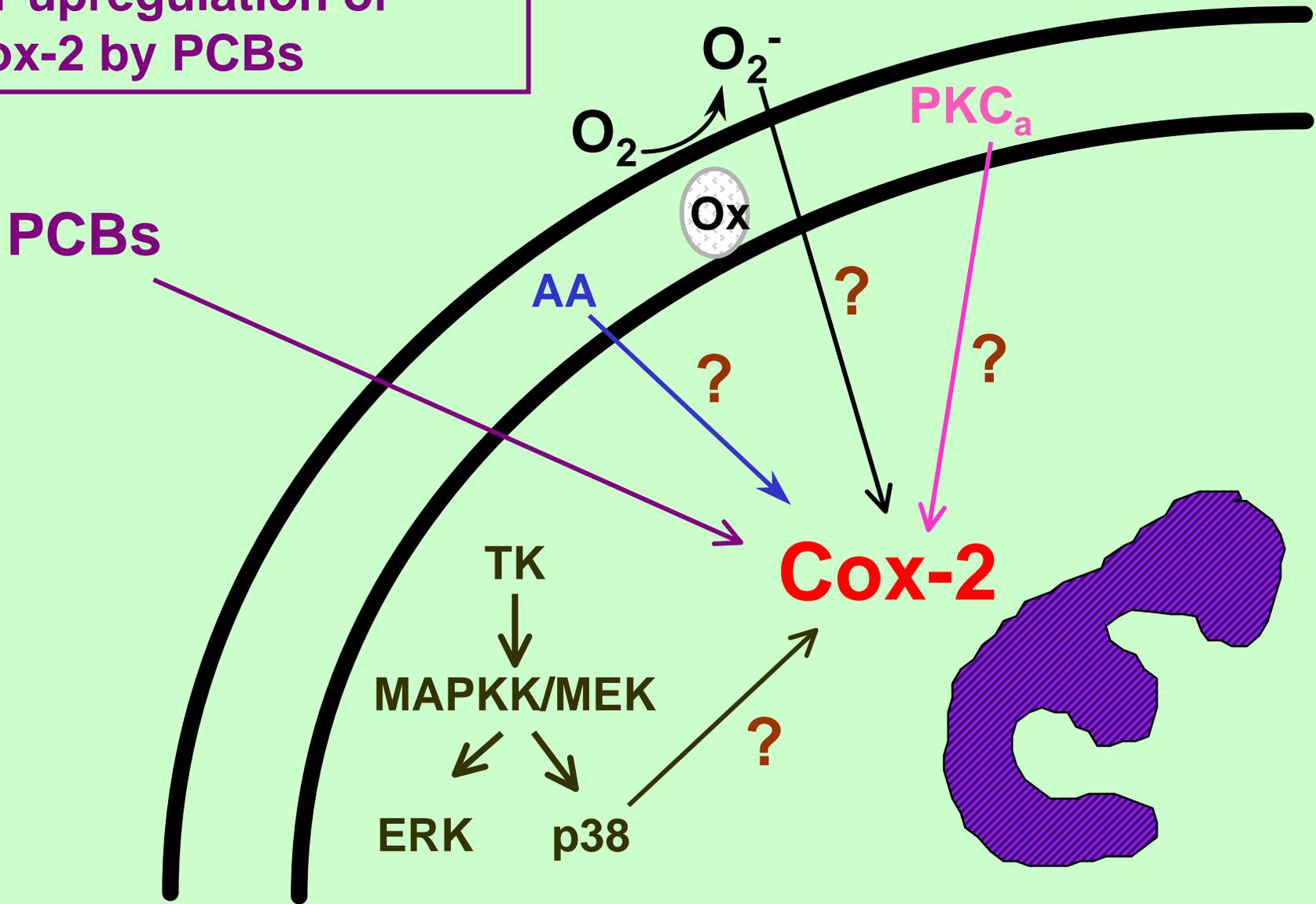
An ortho-substituted PCB congener increases COX-2 mRNA in HL60 cells



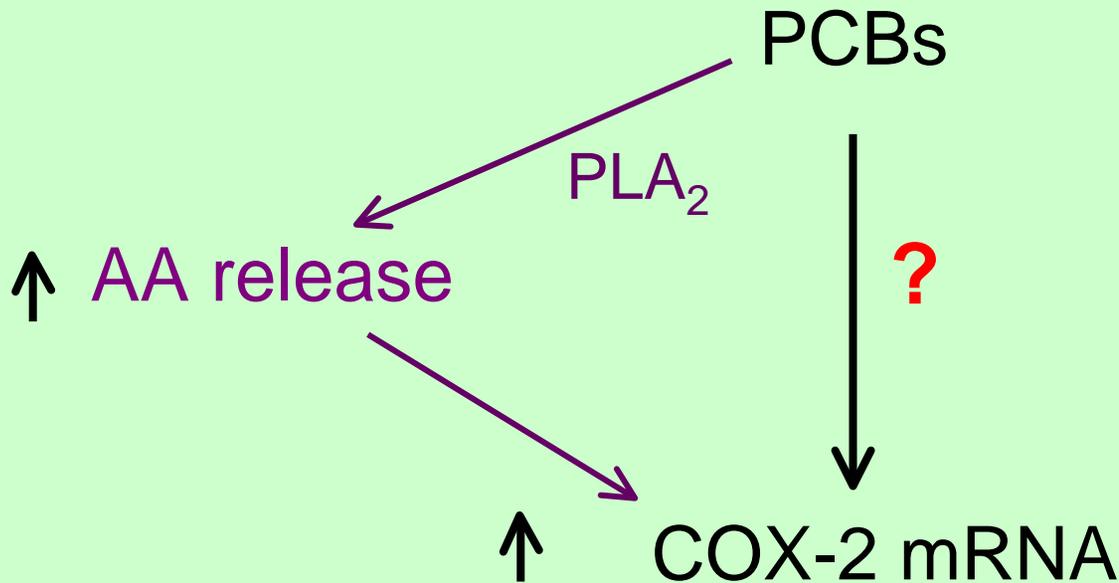
PCB-induced upregulation of COX-2 is concentration-dependent



Potential mechanisms for upregulation of Cox-2 by PCBs

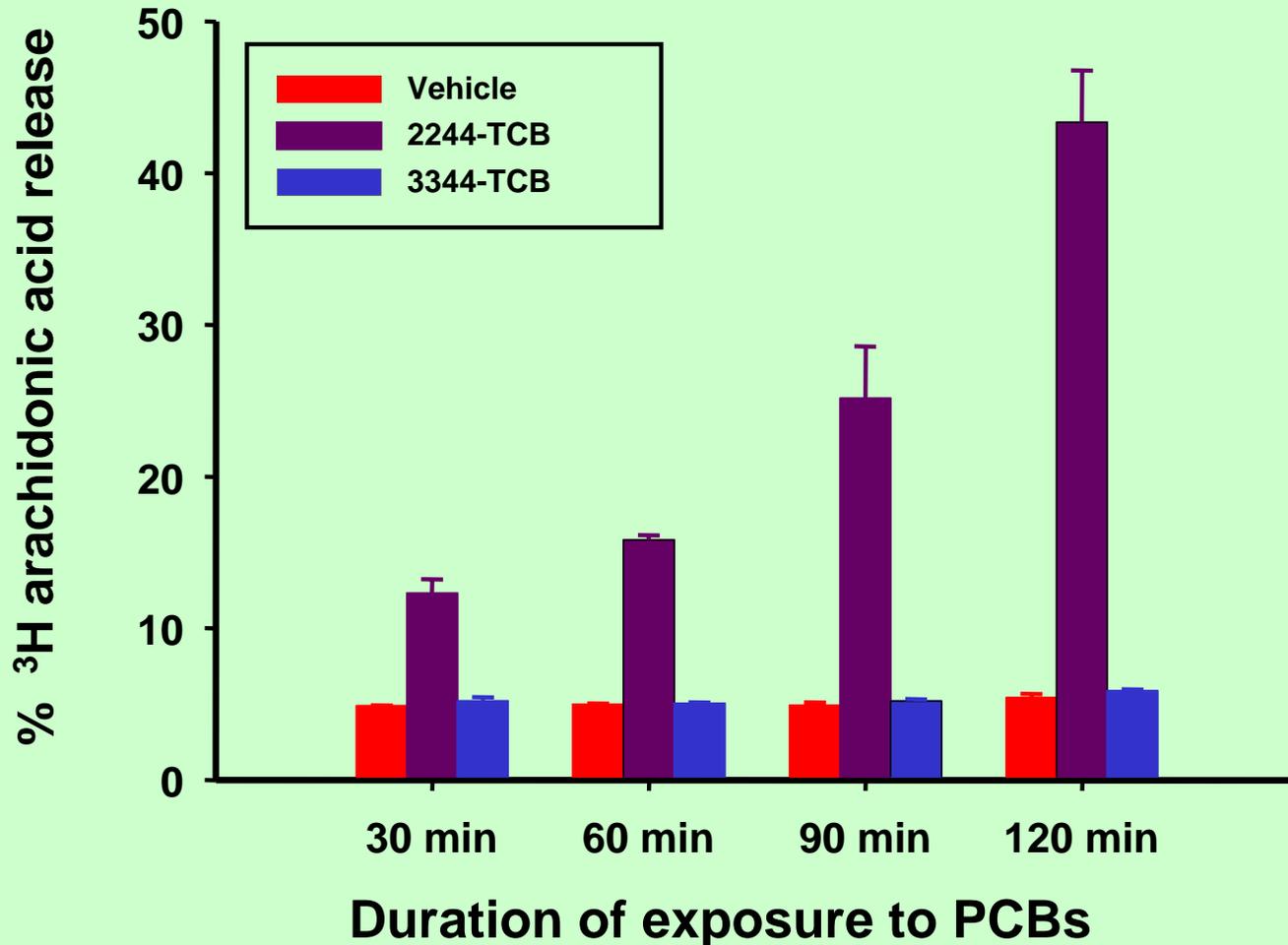


What signaling mechanisms are involved in PCB-mediated increased expression of COX-2 mRNA?

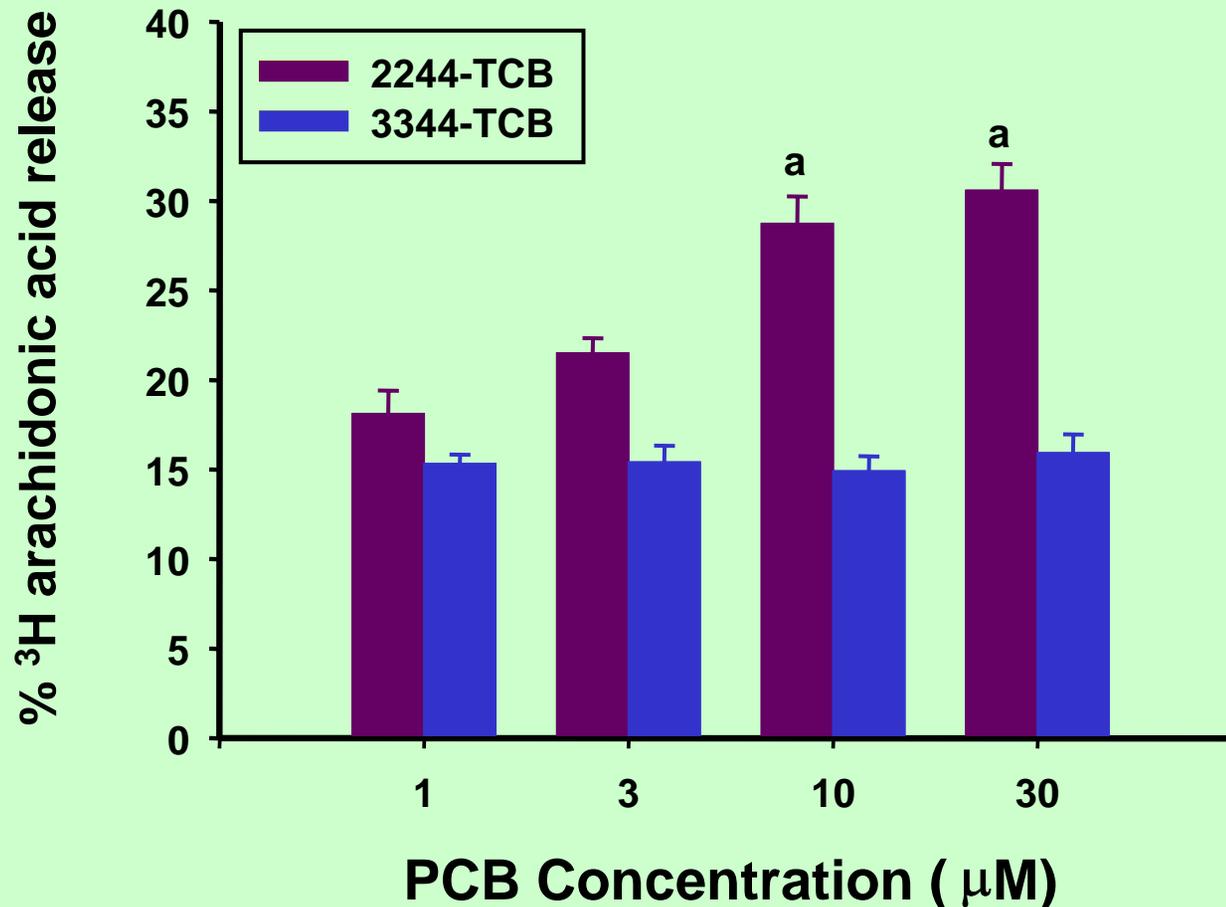


- PLA₂ contributes to upregulation of COX-2 mRNA in macrophages
 - *Balsinde et al., 1999*
- Expression of COX-2 in brains of PLA₂-deficient mice was decreased relative to wild-type mice
 - *Bosetti and Weerasinghe, 2003*

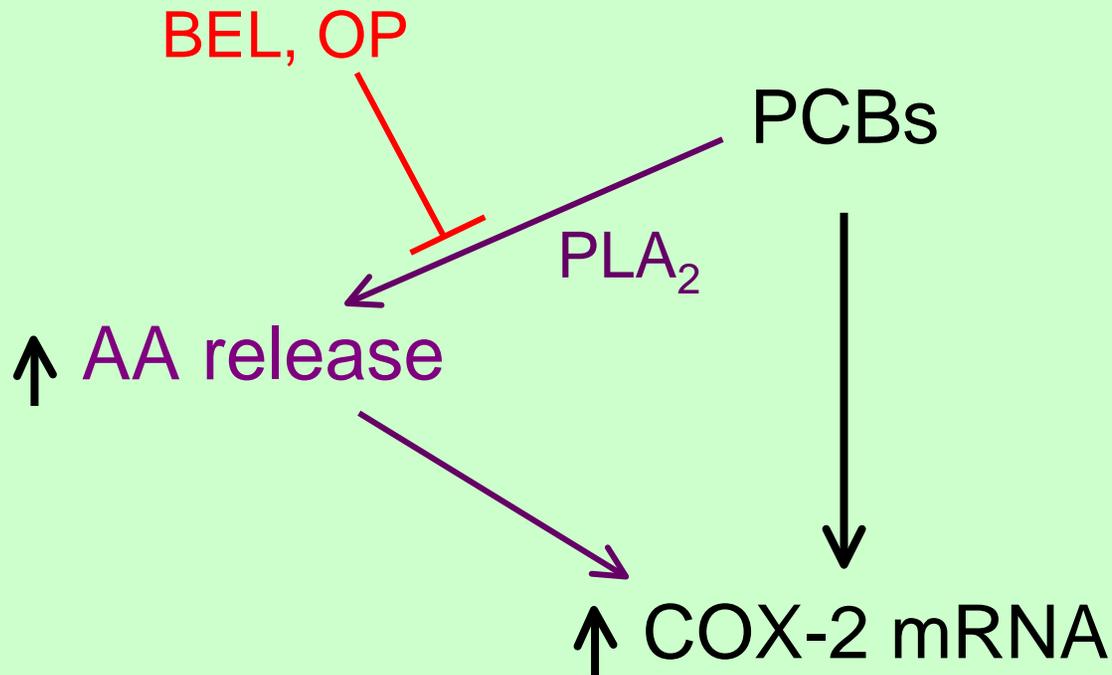
The time-course of 2,2',4,4'-TCB-stimulated release of arachidonic acid is similar to that for the upregulation of COX-2



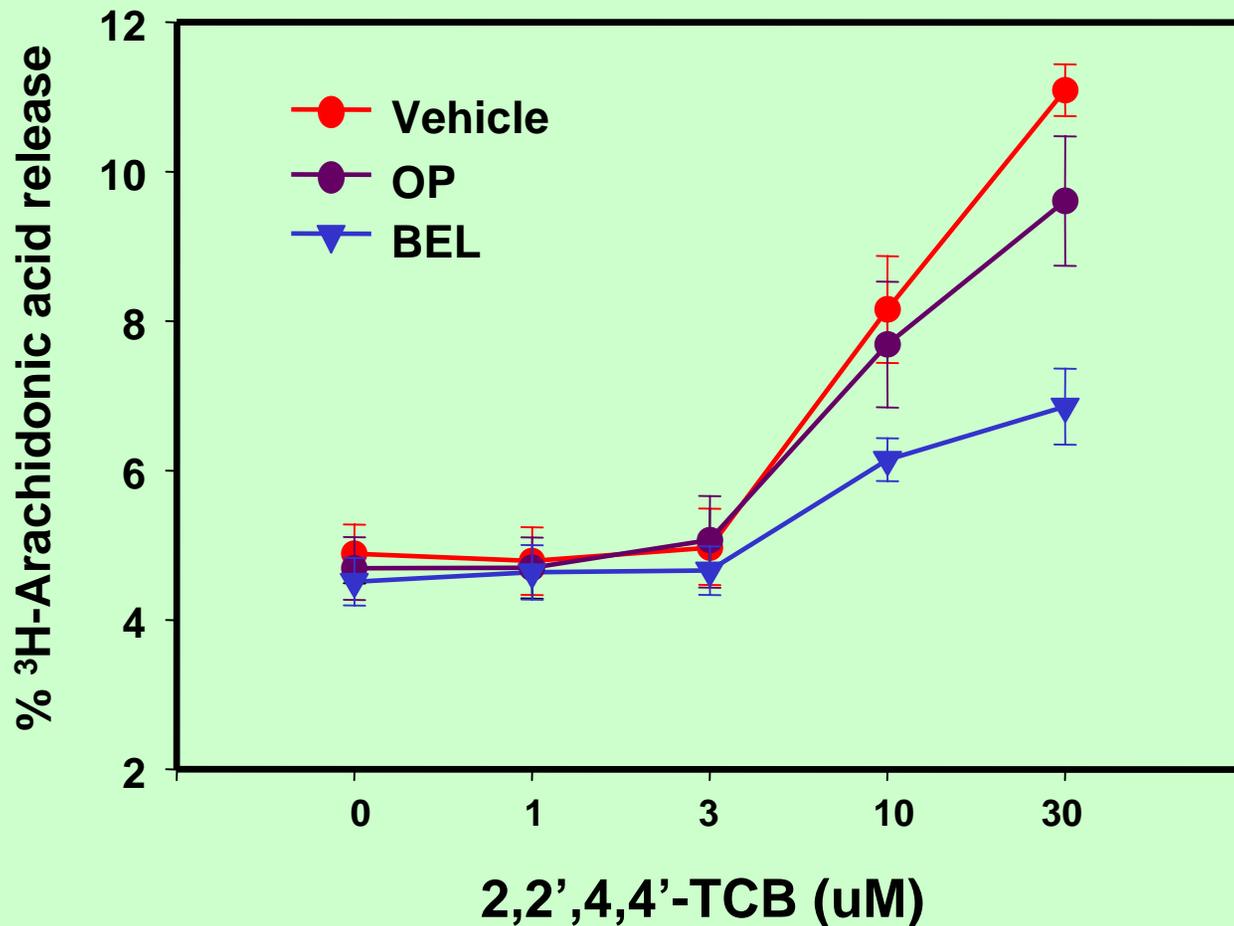
The concentration-response relationship for 2,2',4,4'-TCB-stimulated release of arachidonic acid is similar to that for upregulation of COX-2



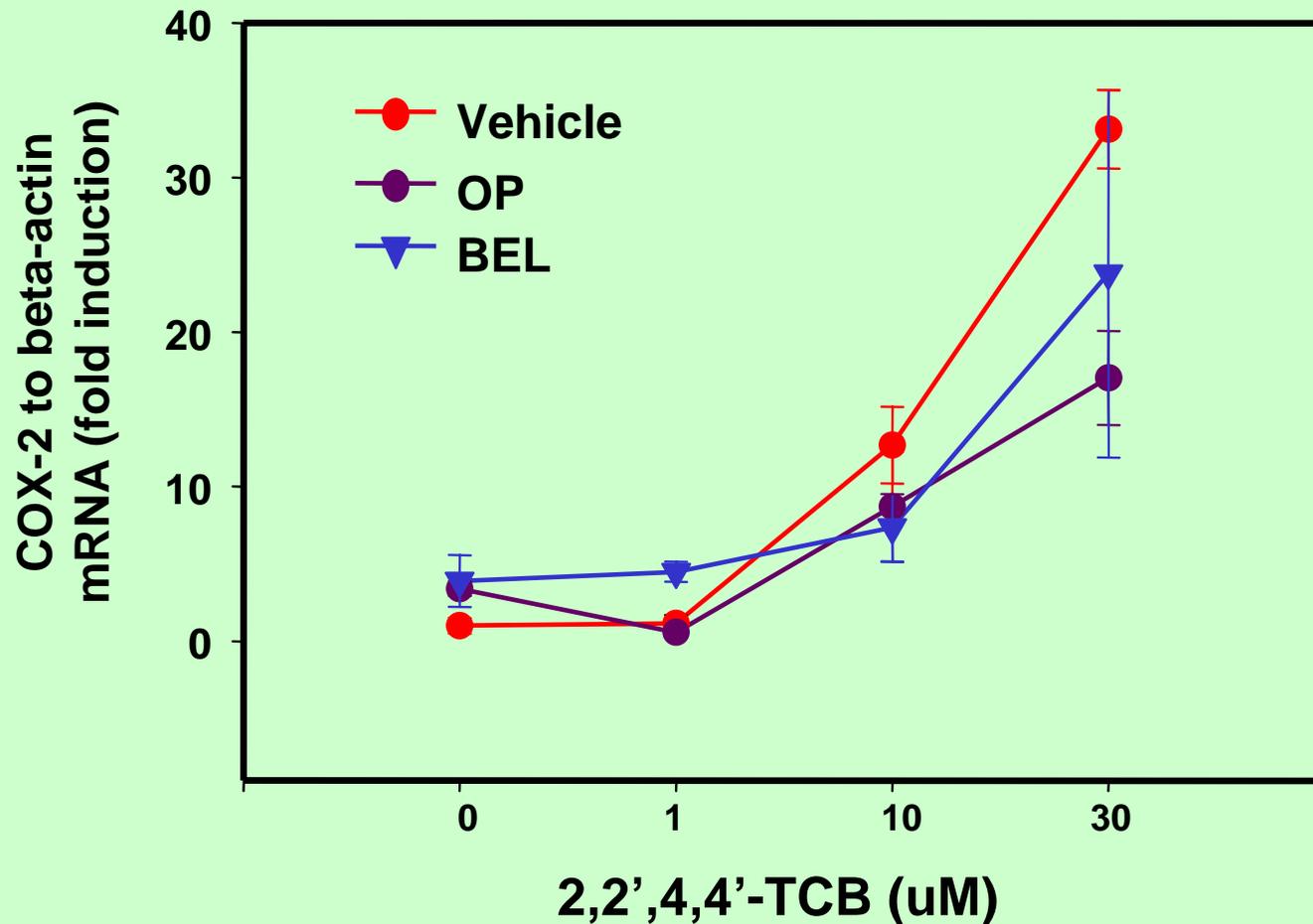
What signaling mechanisms are involved in PCB-mediated increased expression of COX-2 mRNA?



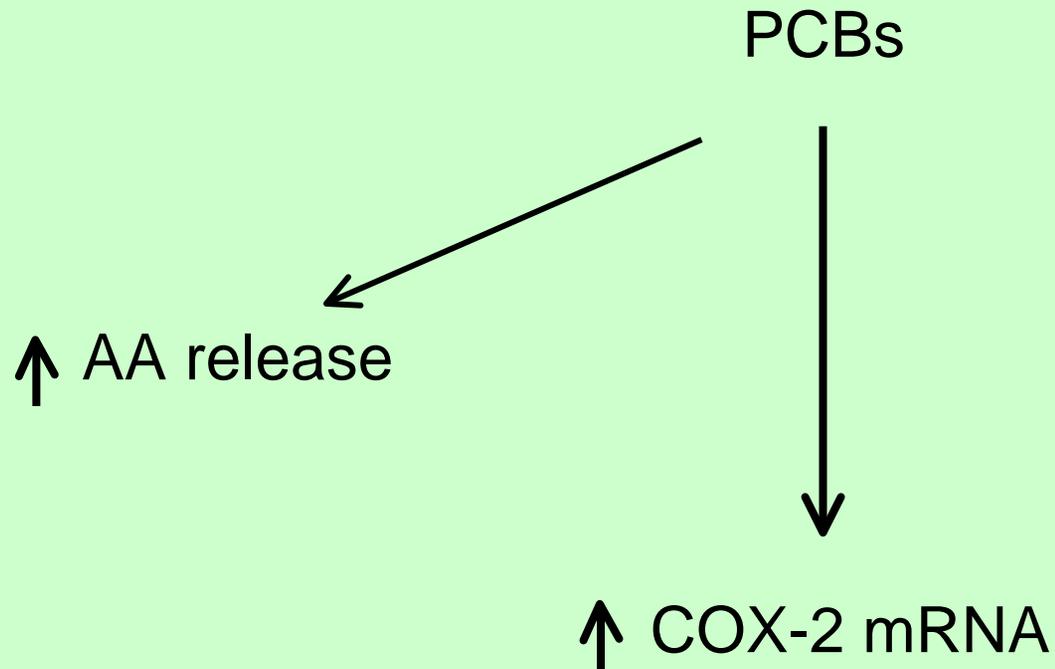
BEL inhibits PCB-mediated release of arachidonic acid



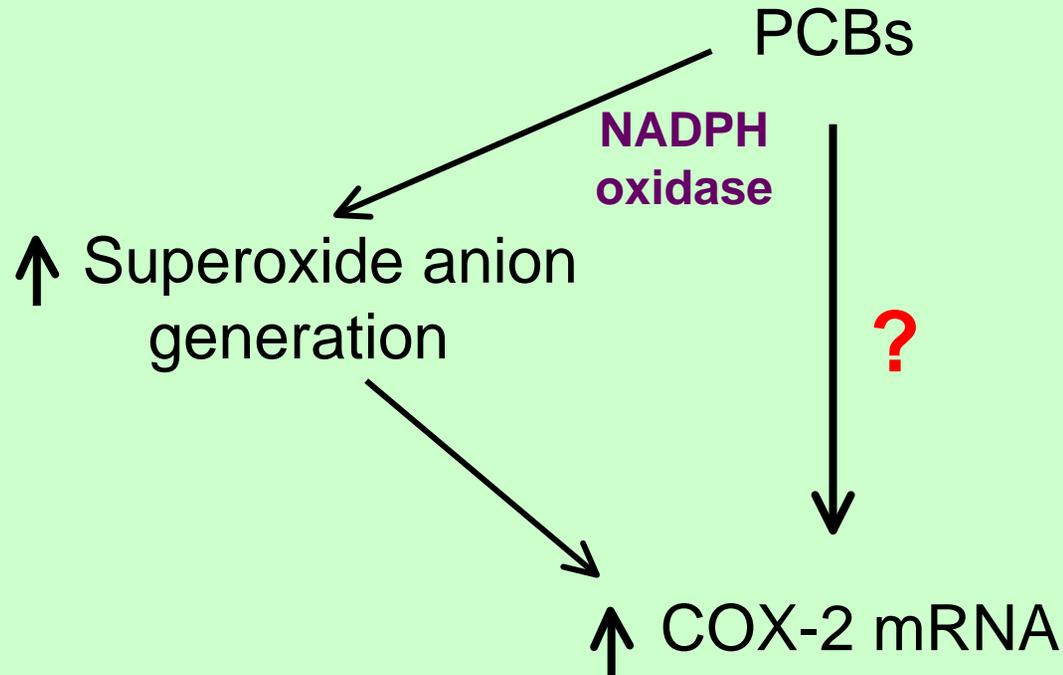
Inhibition of arachidonic acid release does not abolish PCB-induced upregulation of COX-2



What signaling mechanisms are involved in PCB-mediated increased expression of COX-2 mRNA?

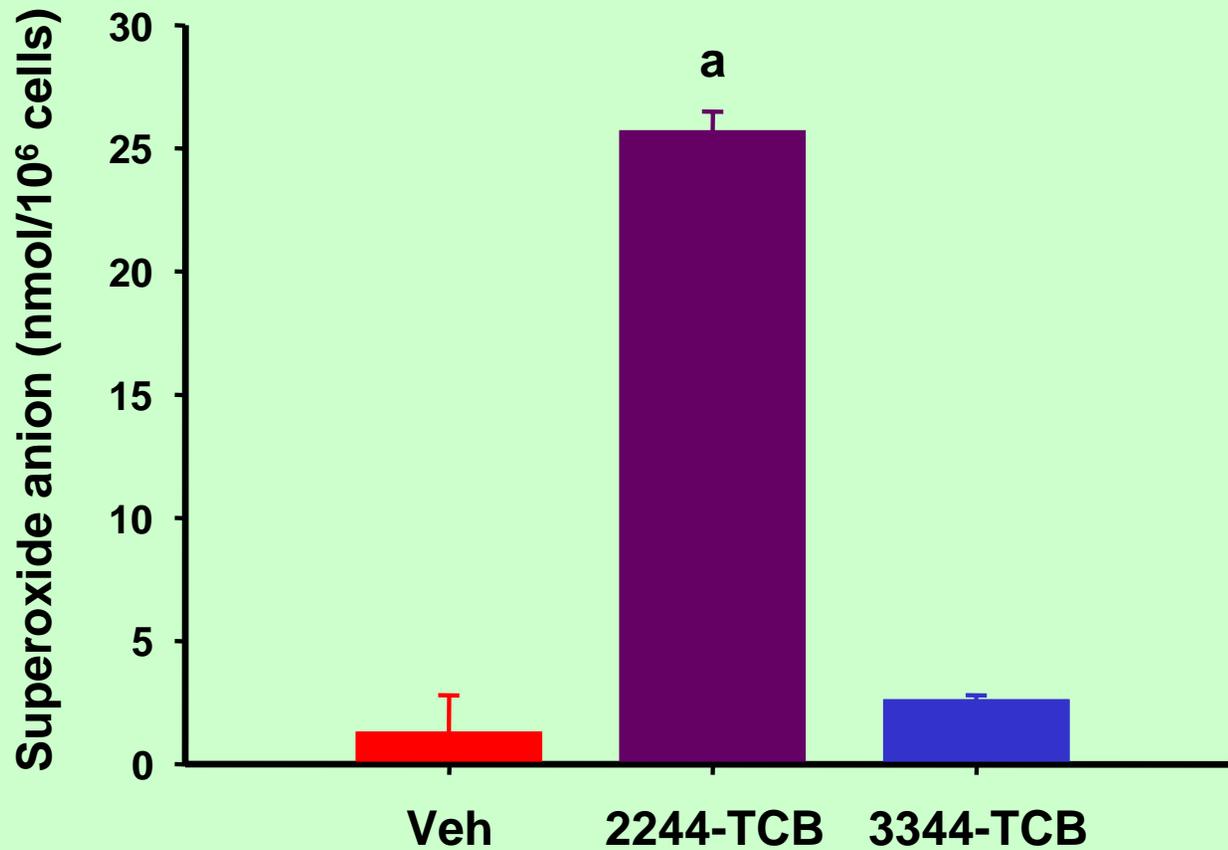


What signaling mechanisms are involved in PCB-mediated increased expression of COX-2 mRNA?

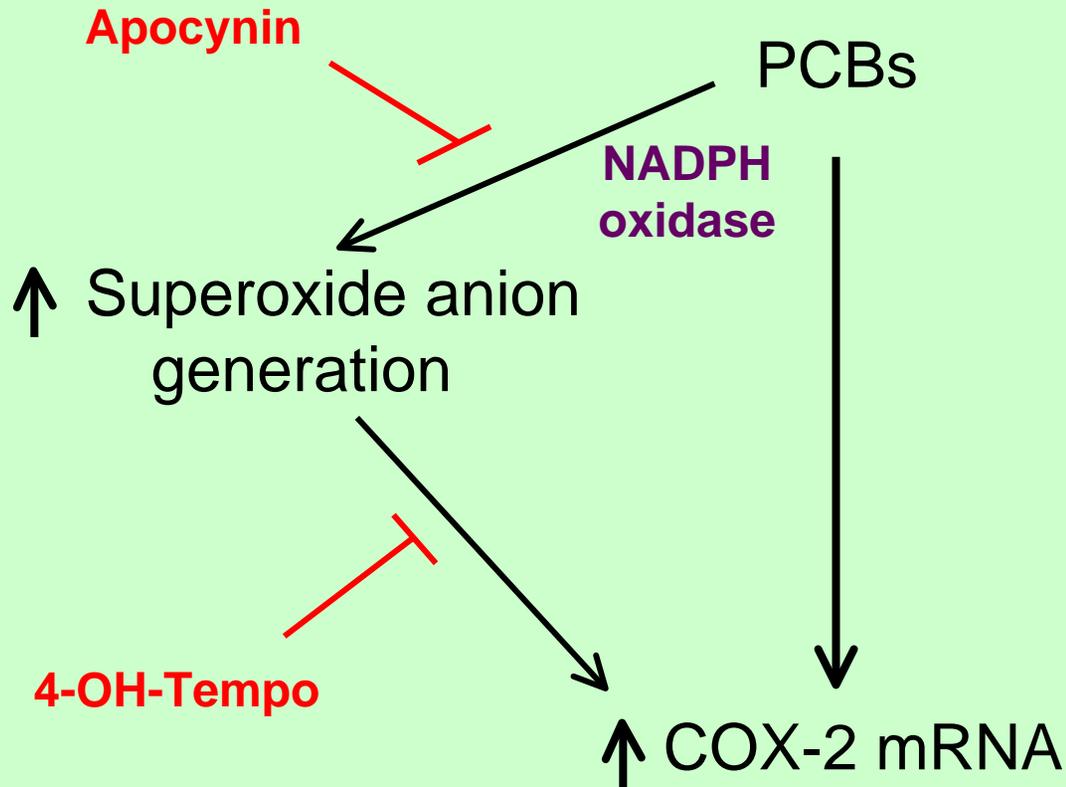


- Increased expression of COX-2 was dependent on generation of ROS in
 - human mesangial cells (high glucose) *Kiritoshi et al, 2003*
 - human monocytes (differentiation) *Barbieri et al, 2003*
 - human keratinocyte cell line (AAPH) *Cui et al, 2004*

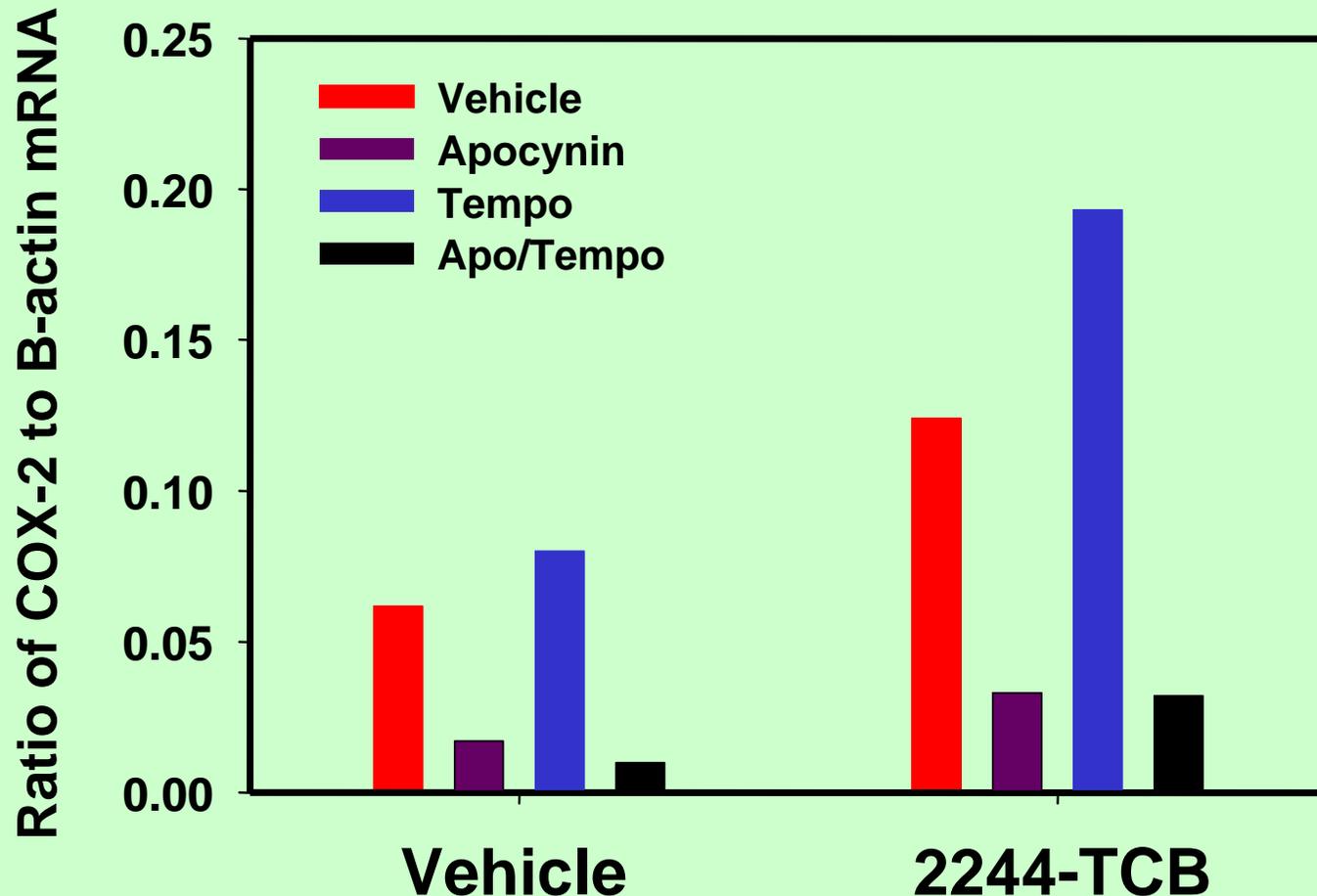
PCBs that increase expression of COX-2 mRNA also increase production of superoxide anion



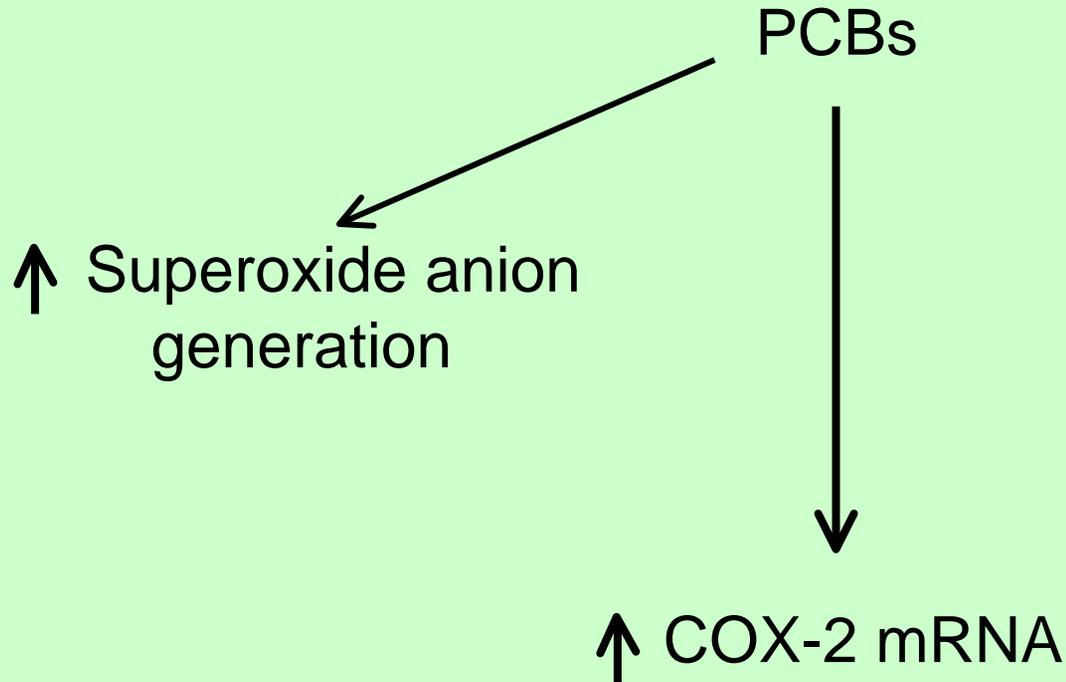
What signaling mechanisms are involved in PCB-mediated increased expression of COX-2 mRNA?



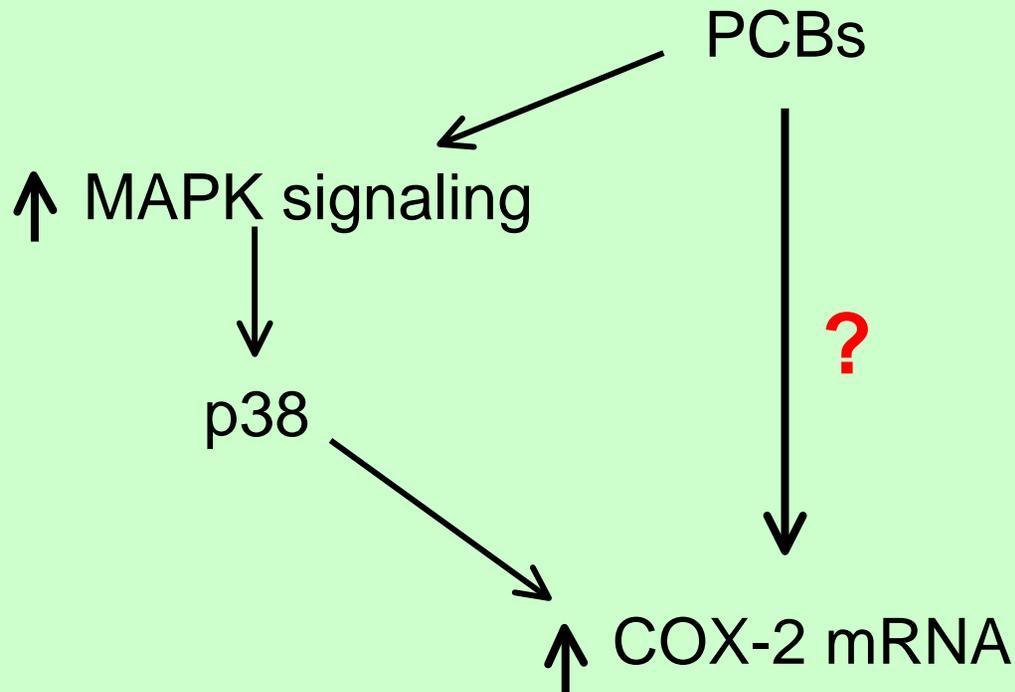
Apocynin, but not Tempo, decreases the upregulation of COX-2 mRNA



What signaling mechanisms are involved in PCB-mediated increased expression of COX-2?

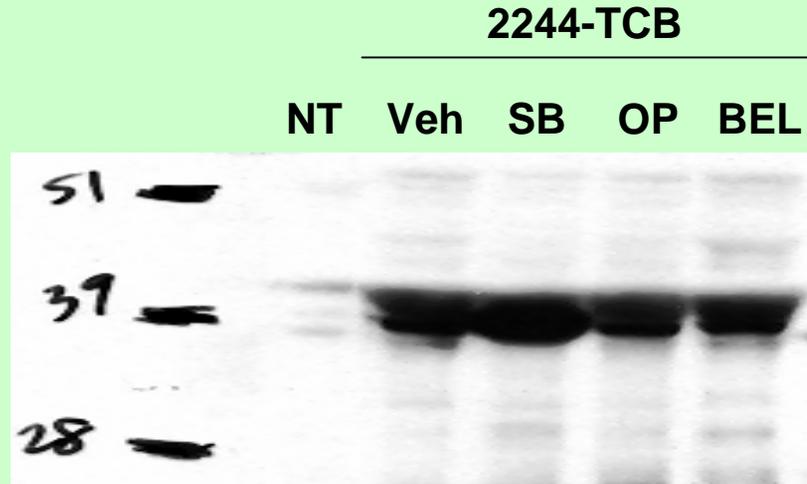


What signaling mechanisms are involved in PCB-mediated increased expression of COX-2?

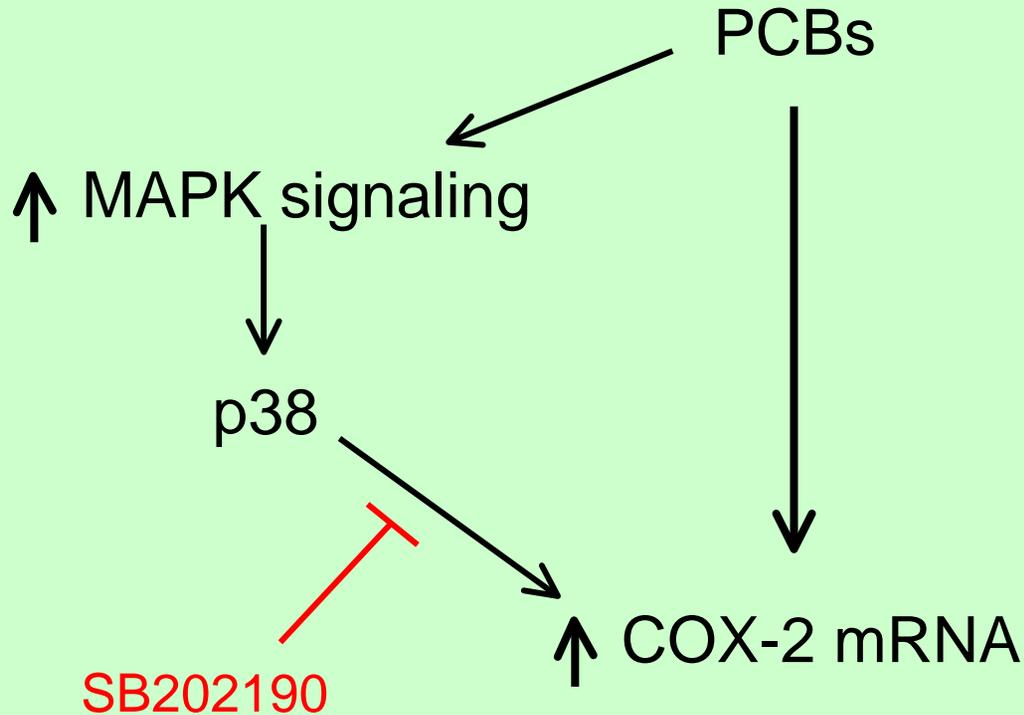


- **p38 MAPK contributes to increased expression of COX-2**
 - *Souza et al., 2004; Westra et al., 2004; Grishin et al., 2004, Cui et al., 2004*
- **p38 MAPK stabilizes COX-2 mRNA**
 - *Mifflin et al., 2004; Hendrickx et al., 2003*

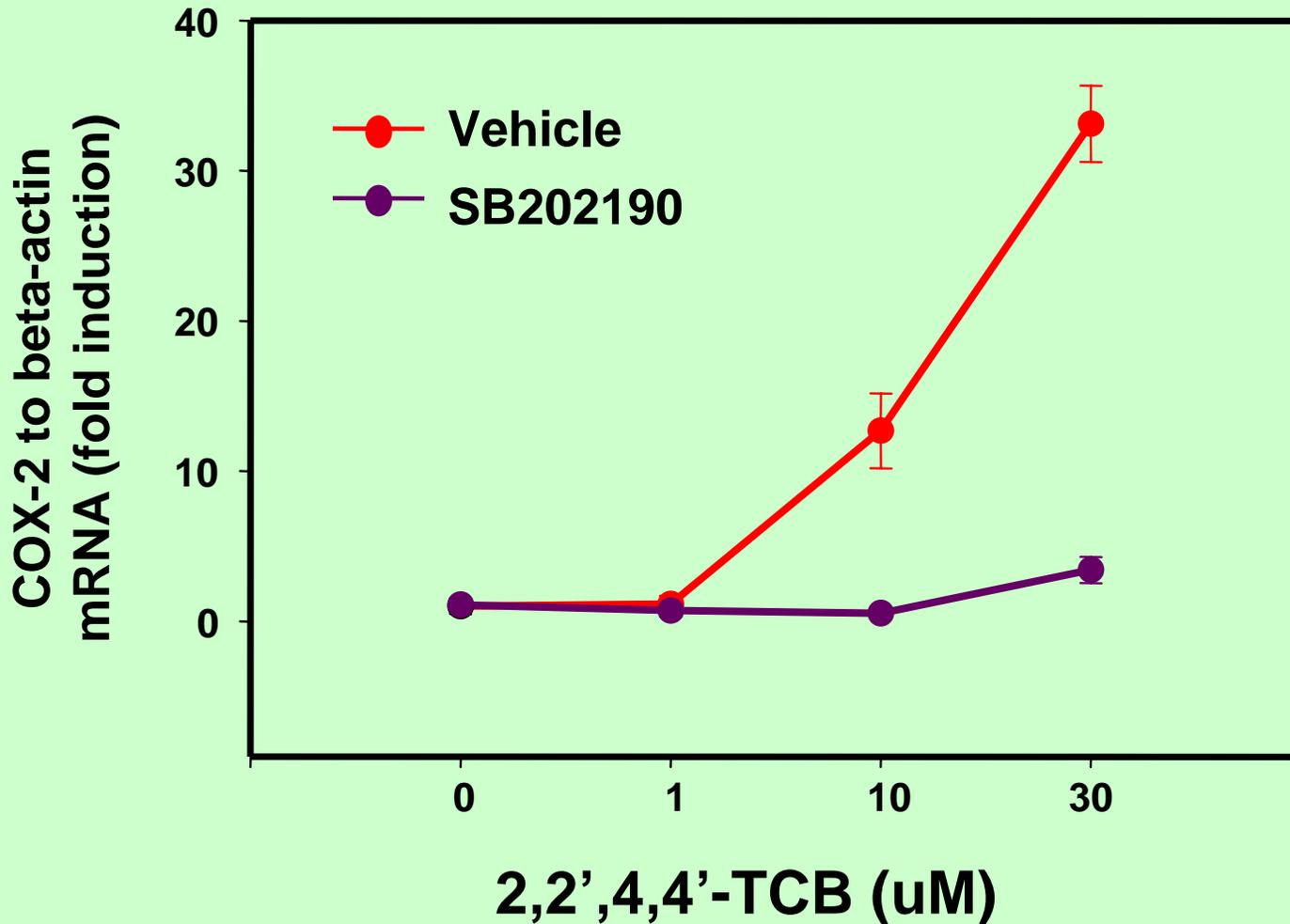
2,2',4,4'-TCB increases p38 in HL-60 cells



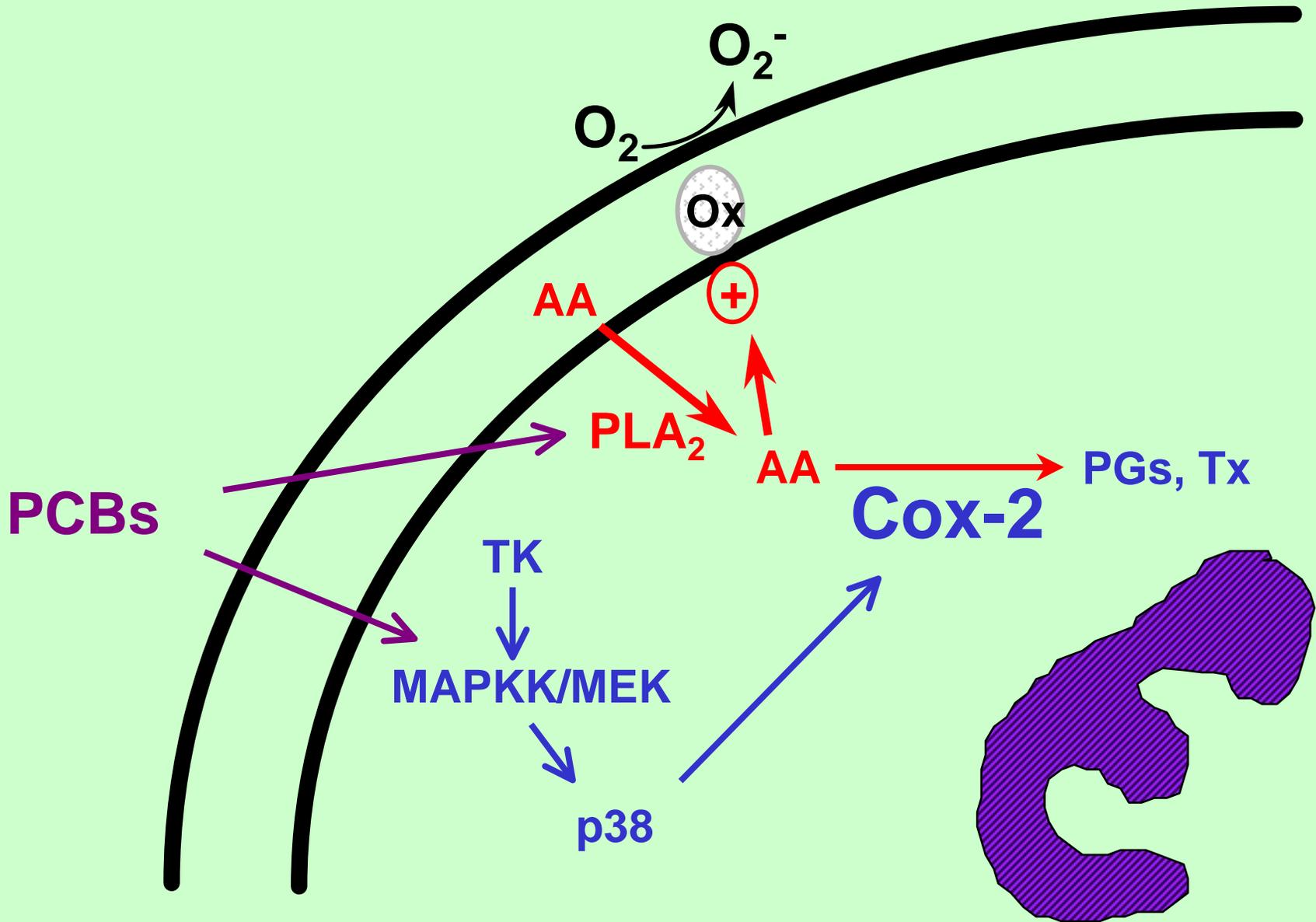
What signaling mechanisms are involved in PCB-mediated increased expression of COX-2?



An inhibitor of p38 reduces PCB-mediated upregulation of COX-2



Upregulation of COX-2 by PCBs in HL-60 cells



Acknowledgments

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