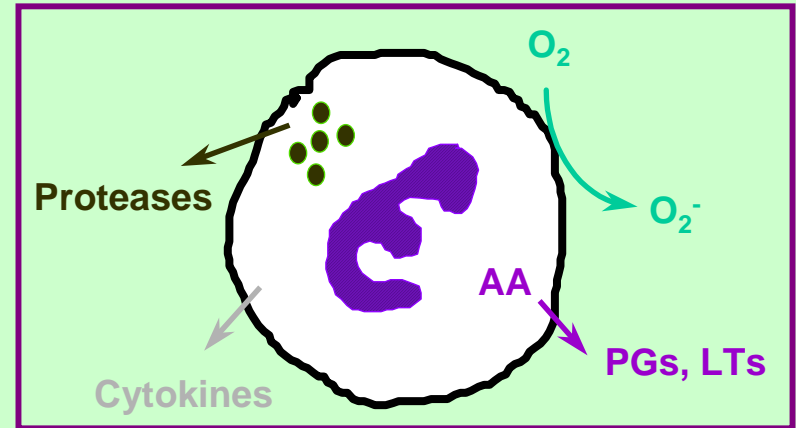


Upregulation of cyclooxygenase-2 in neutrophils by polychlorinated biphenyls

Patricia Ganey, Ph.D.
Michigan State University

A little about neutrophils

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



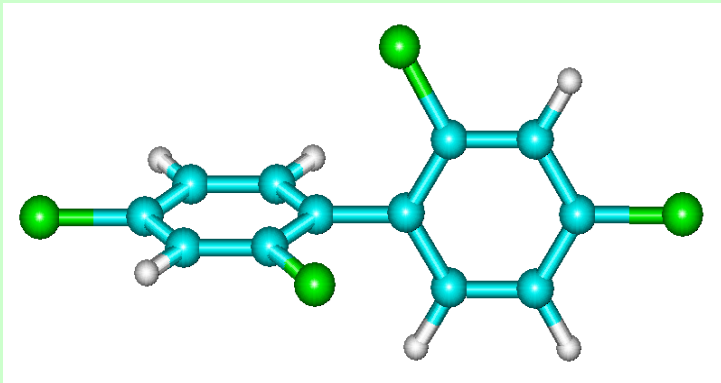
✧ Activation is a requirement for effective pathogen destruction.

✧ Inhibition of neutrophil activation may impair host defense.

✧ Inappropriate activation of neutrophils may contribute to tissue injury.

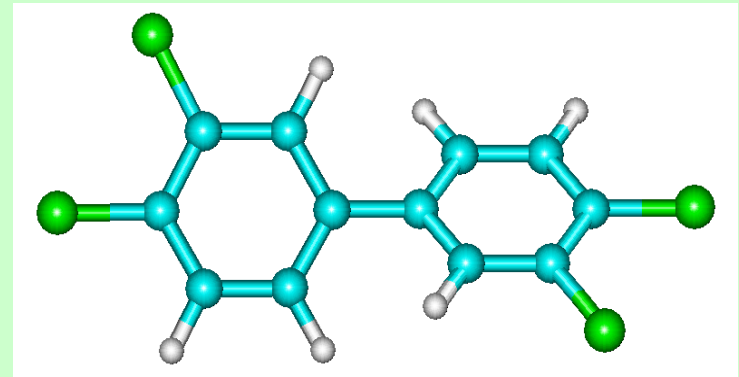
✧ Neutrophil-dependent damage in models of inflammation-mediated injury.

Polychlorinated Biphenyls (PCBs)



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

Activates neutrophils



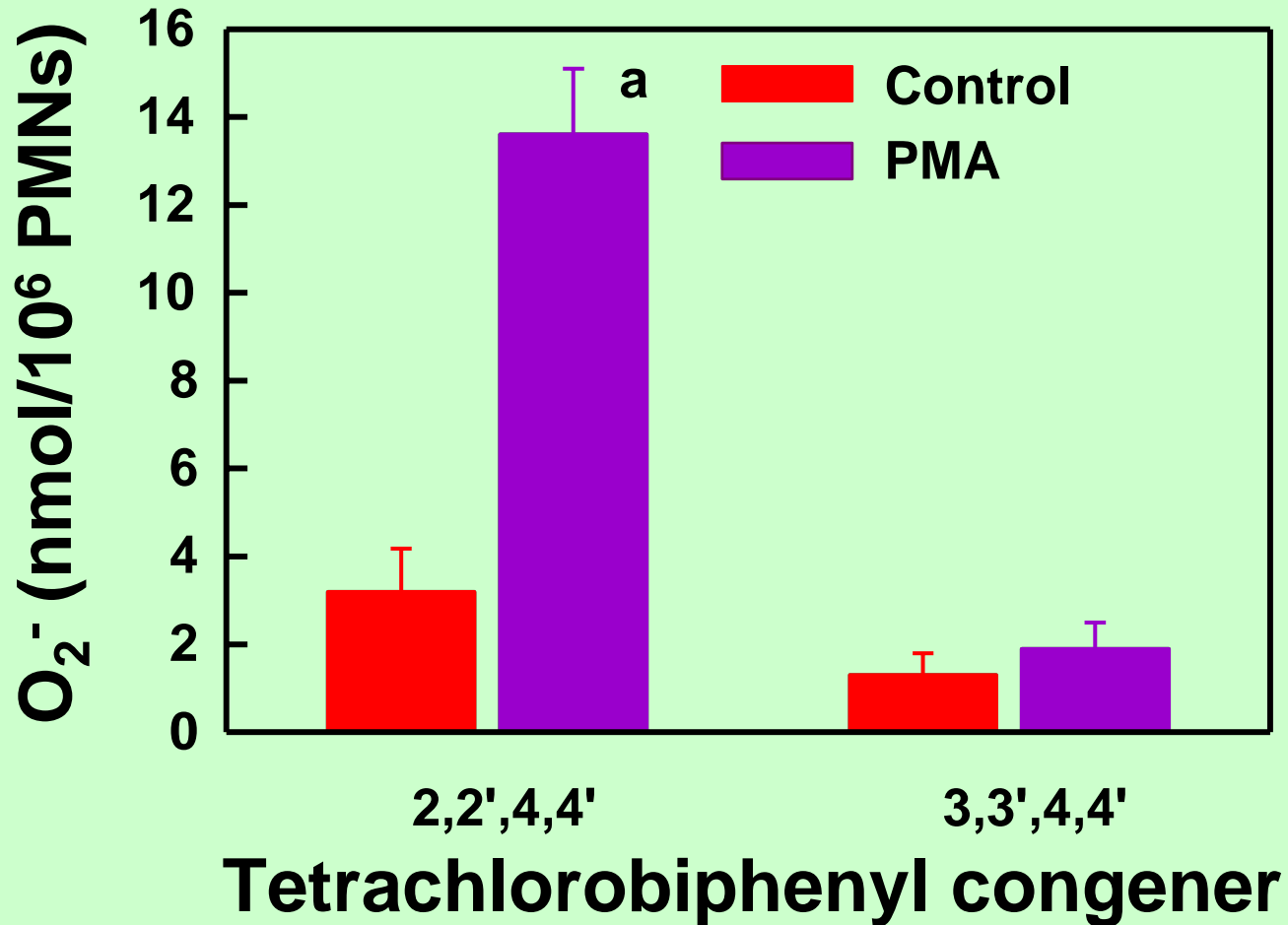
3,3',4,4'-Tetrachlorobiphenyl
Coplanar
Not ortho-substituted
High affinity for the AhR

Does not activate neutrophils

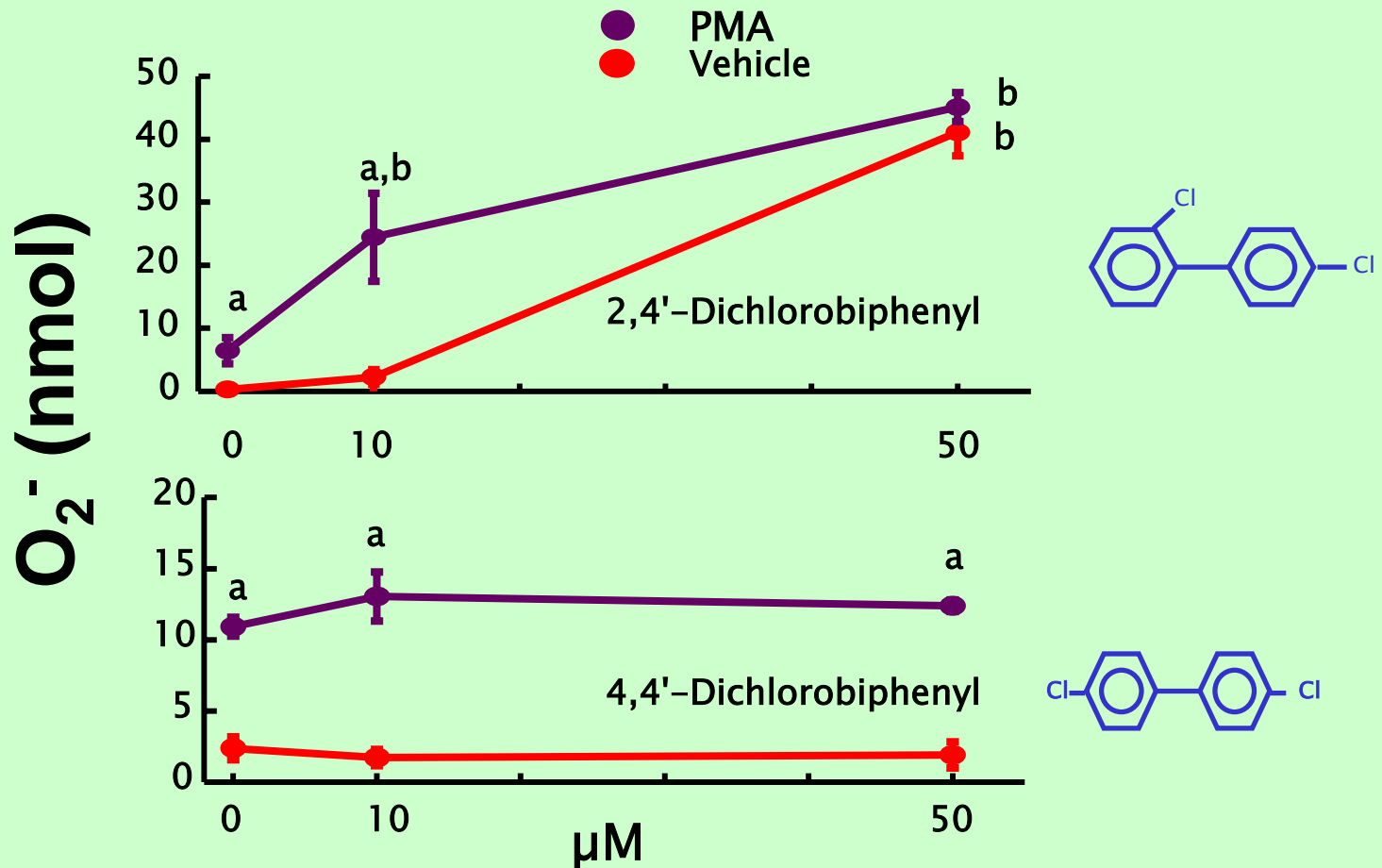
Early Observations

- * 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

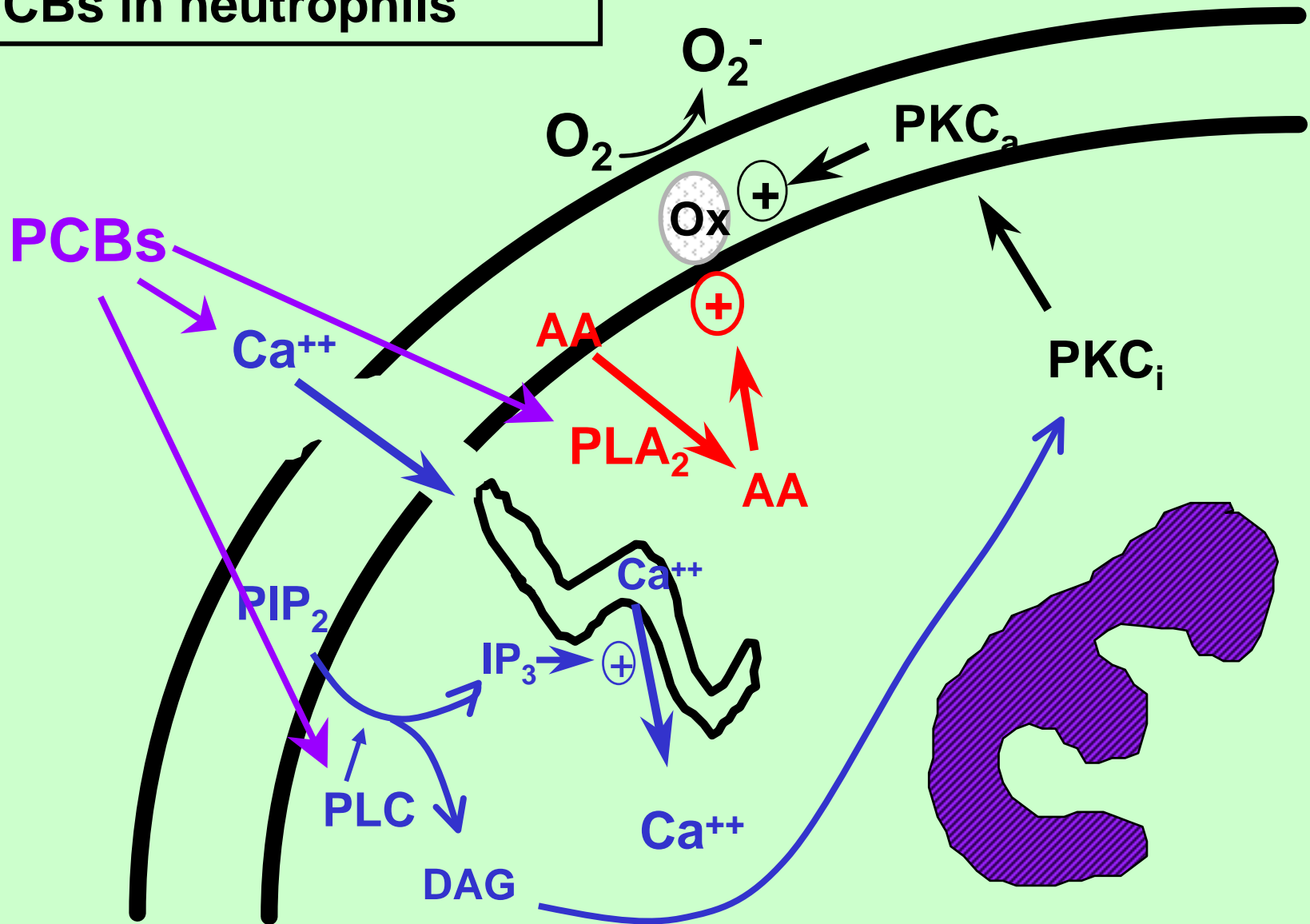


Ortho-substituted PCBs activate neutrophils

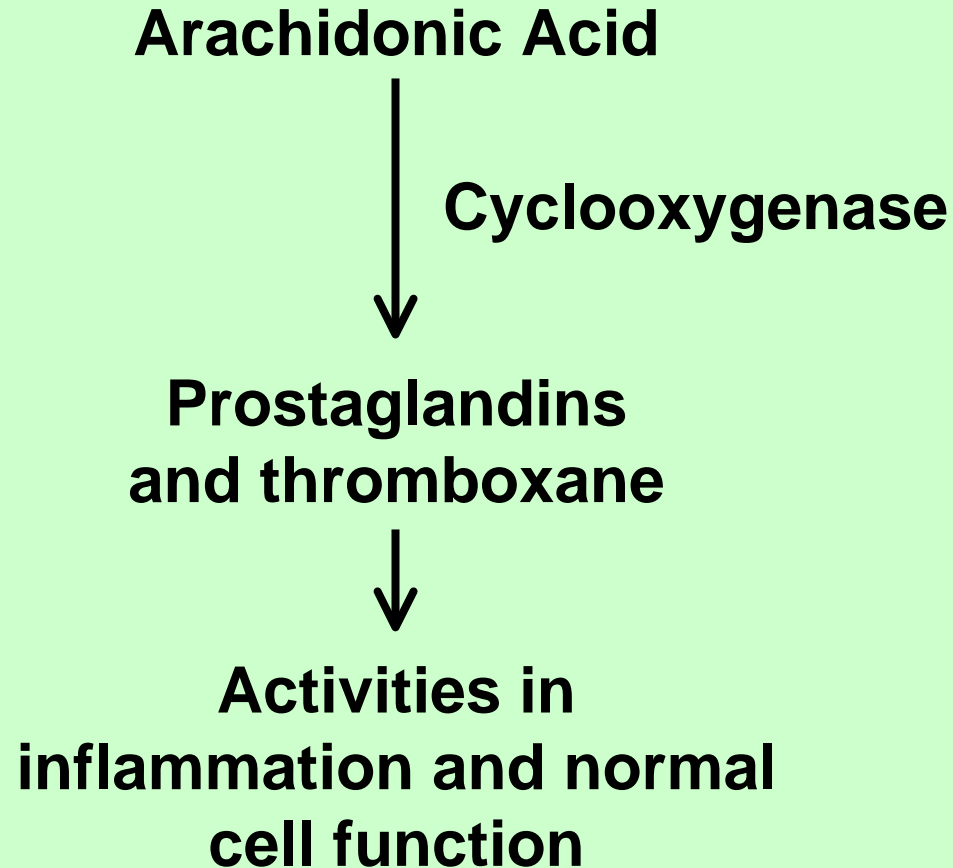


What mechanisms are involved in activation of neutrophils by ortho-substituted PCBs?

Some pathways activated by PCBs in neutrophils



Arachidonic acid metabolism, simplified

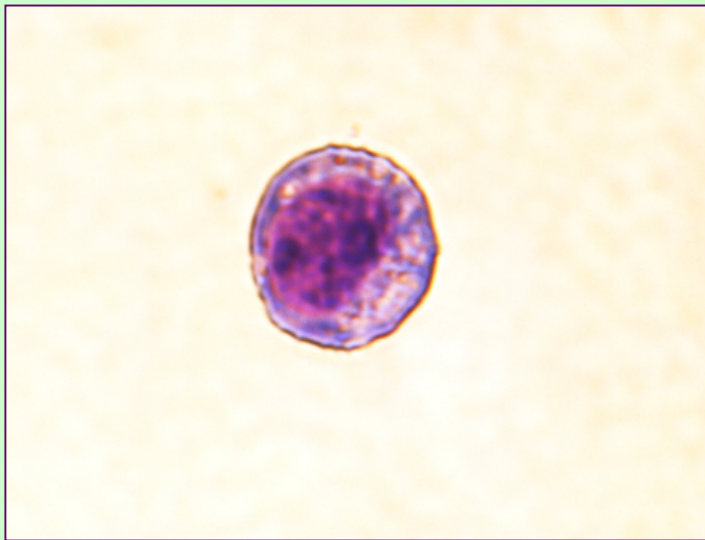


Cyclooxygenase-2 (COX-2)

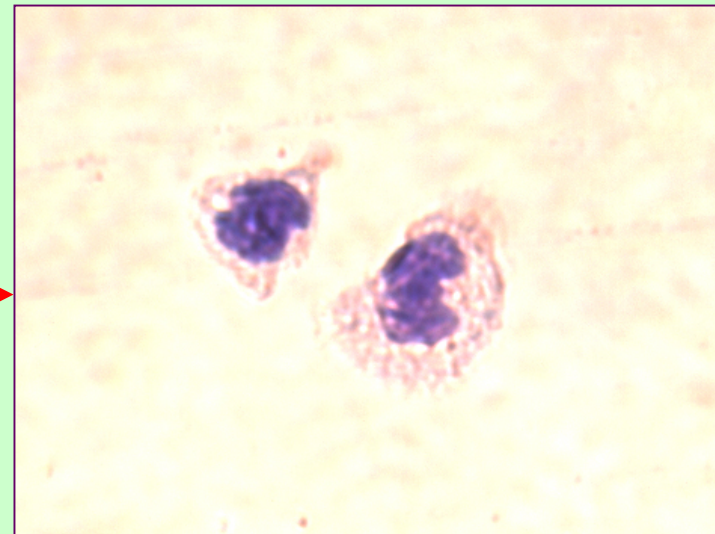
- * Enzyme produced by gene of the same name
- * Immediate early gene
- * Upregulated in neutrophils during inflammation
- * **Hypothesis: COX-2 is upregulated in neutrophils by ortho-substituted PCBs.**

HL-60 cells

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

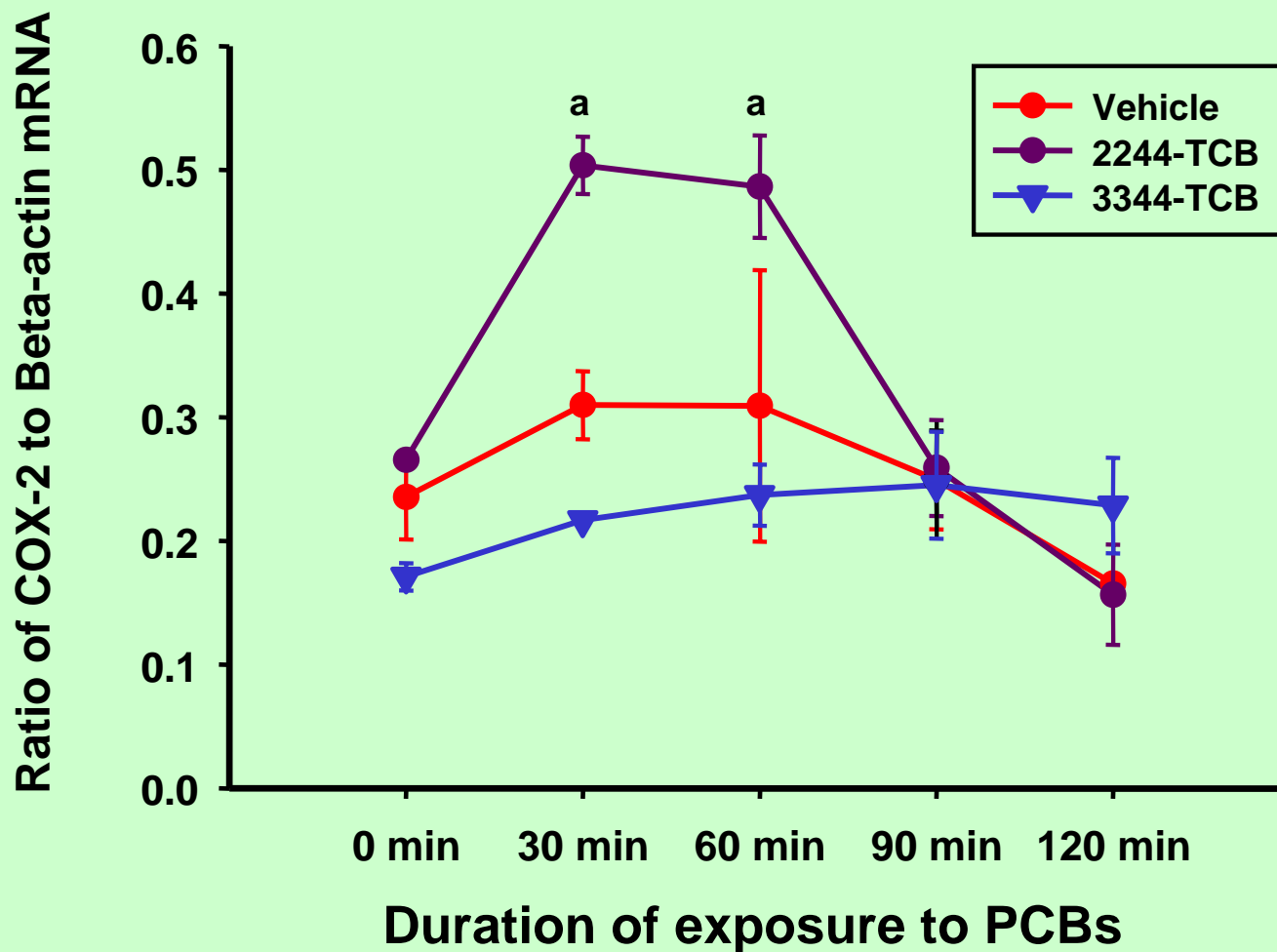


Undifferentiated

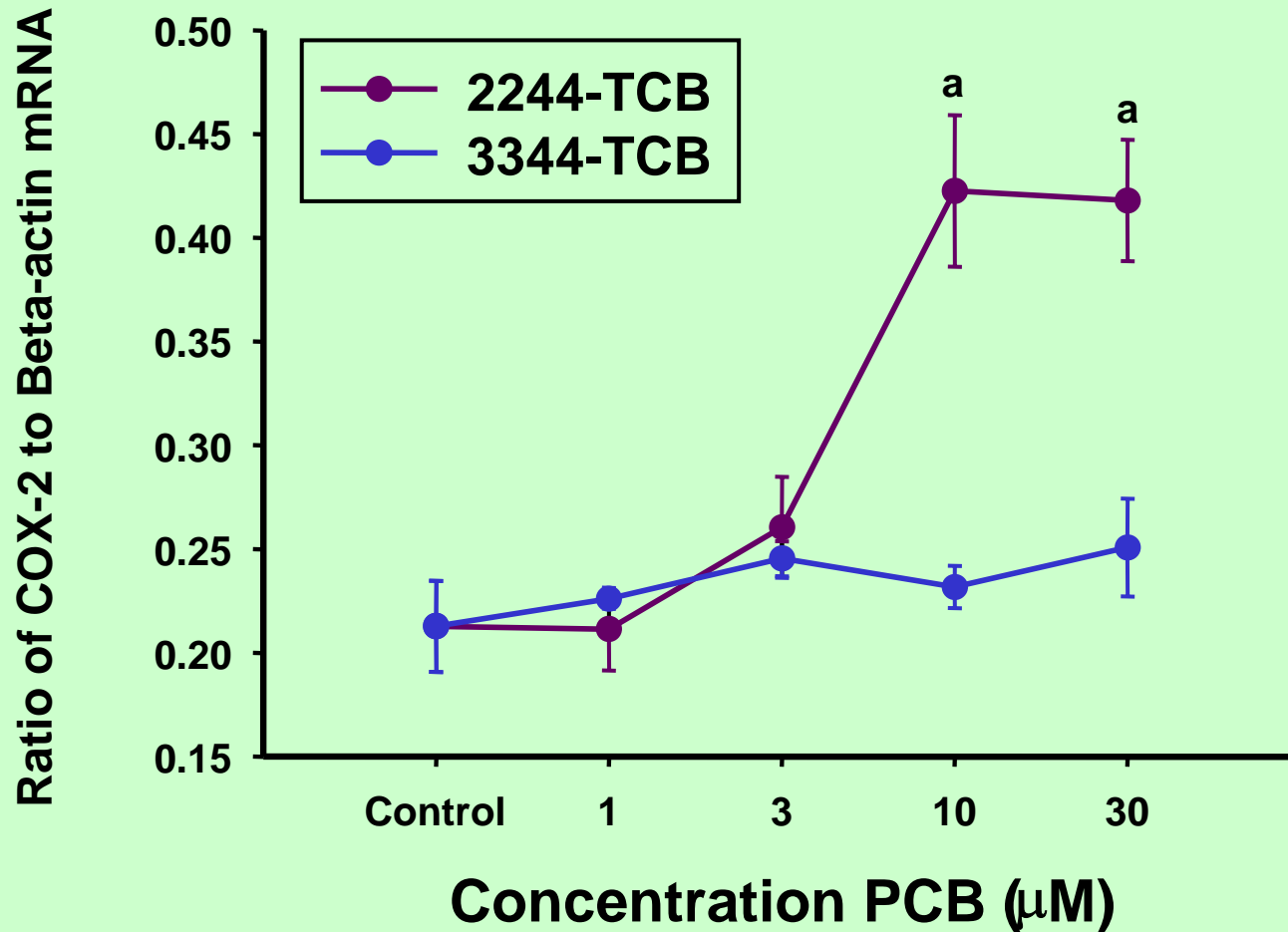


Differentiated

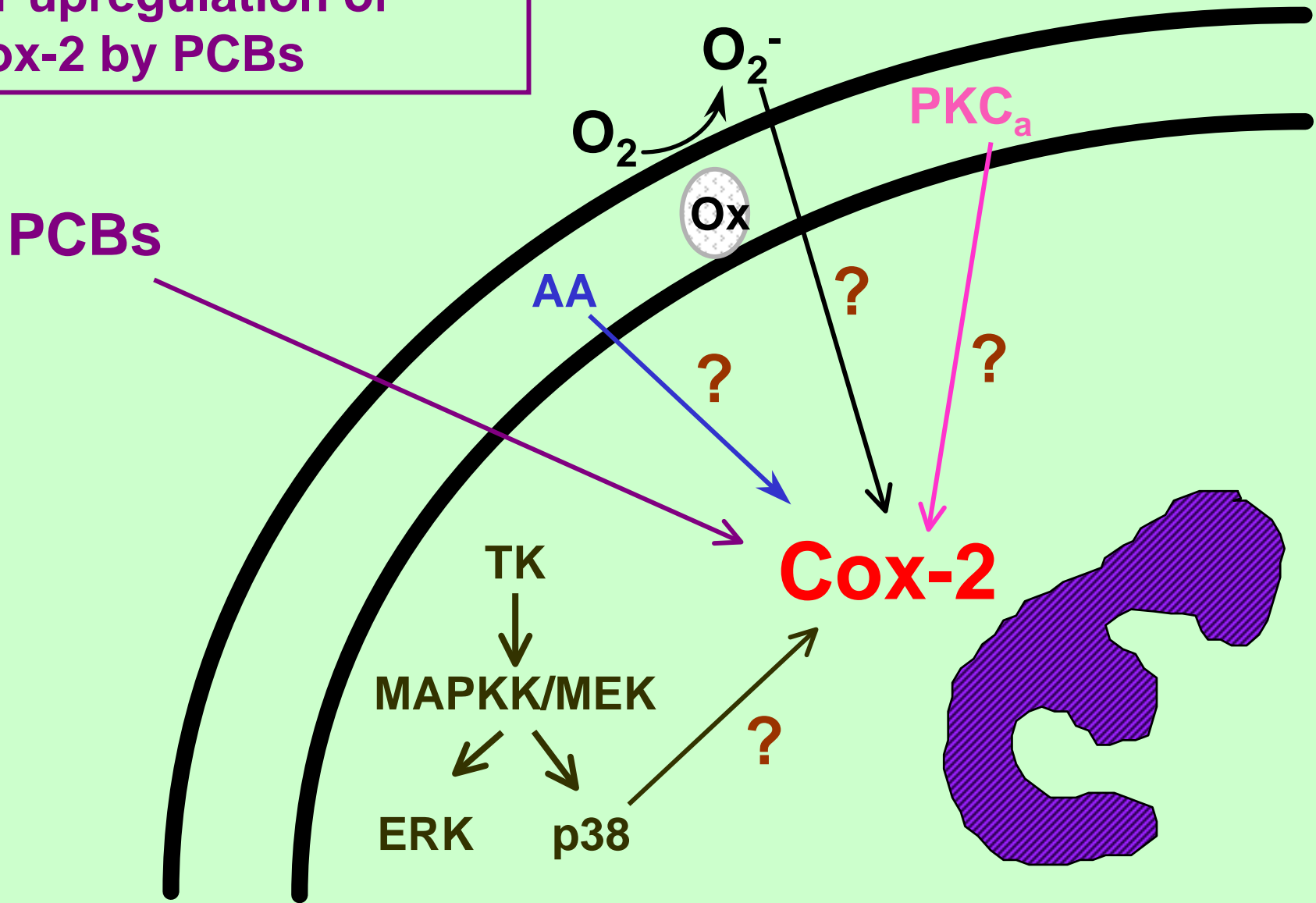
An ortho-substituted PCB congener increases COX-2 mRNA in HL60 cells



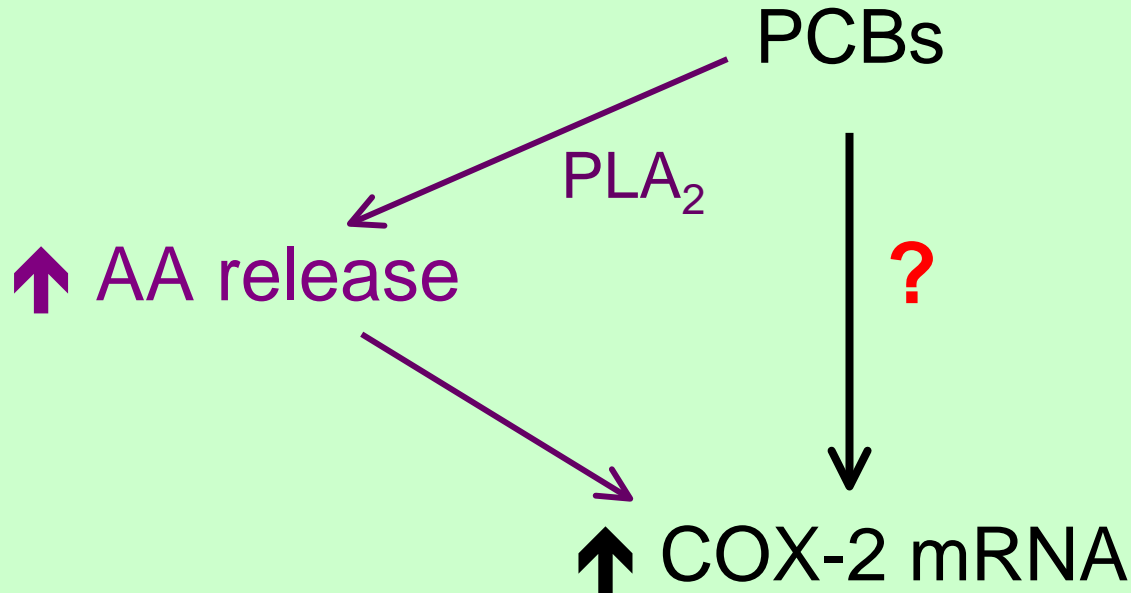
PCB-induced upregulation of COX-2 is concentration-dependent



Potential mechanisms for upregulation of Cox-2 by PCBs



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



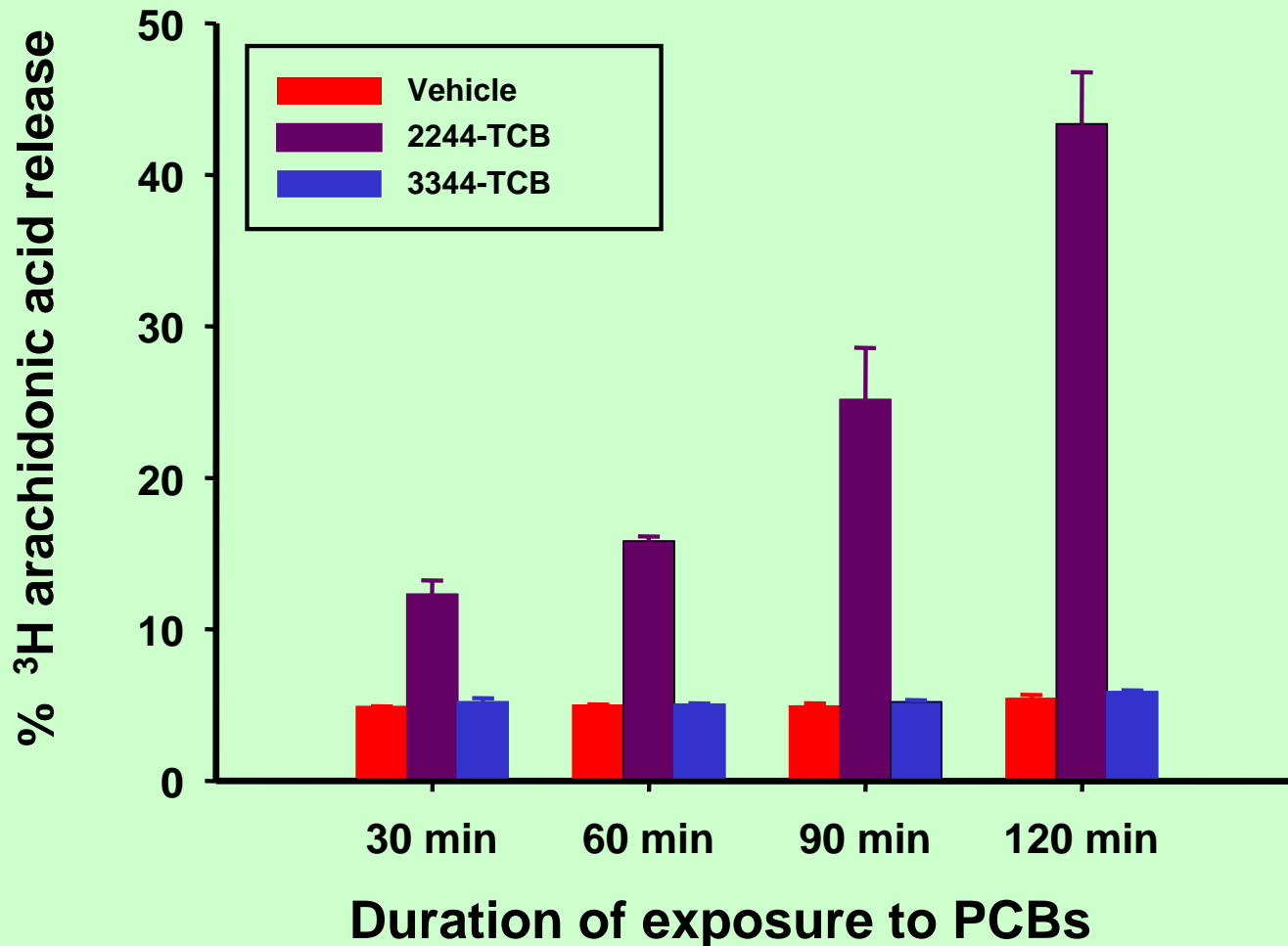
* PLA₂ contributes to upregulation of COX-2 mRNA in macrophages

* *Balsinde et al., 1999*

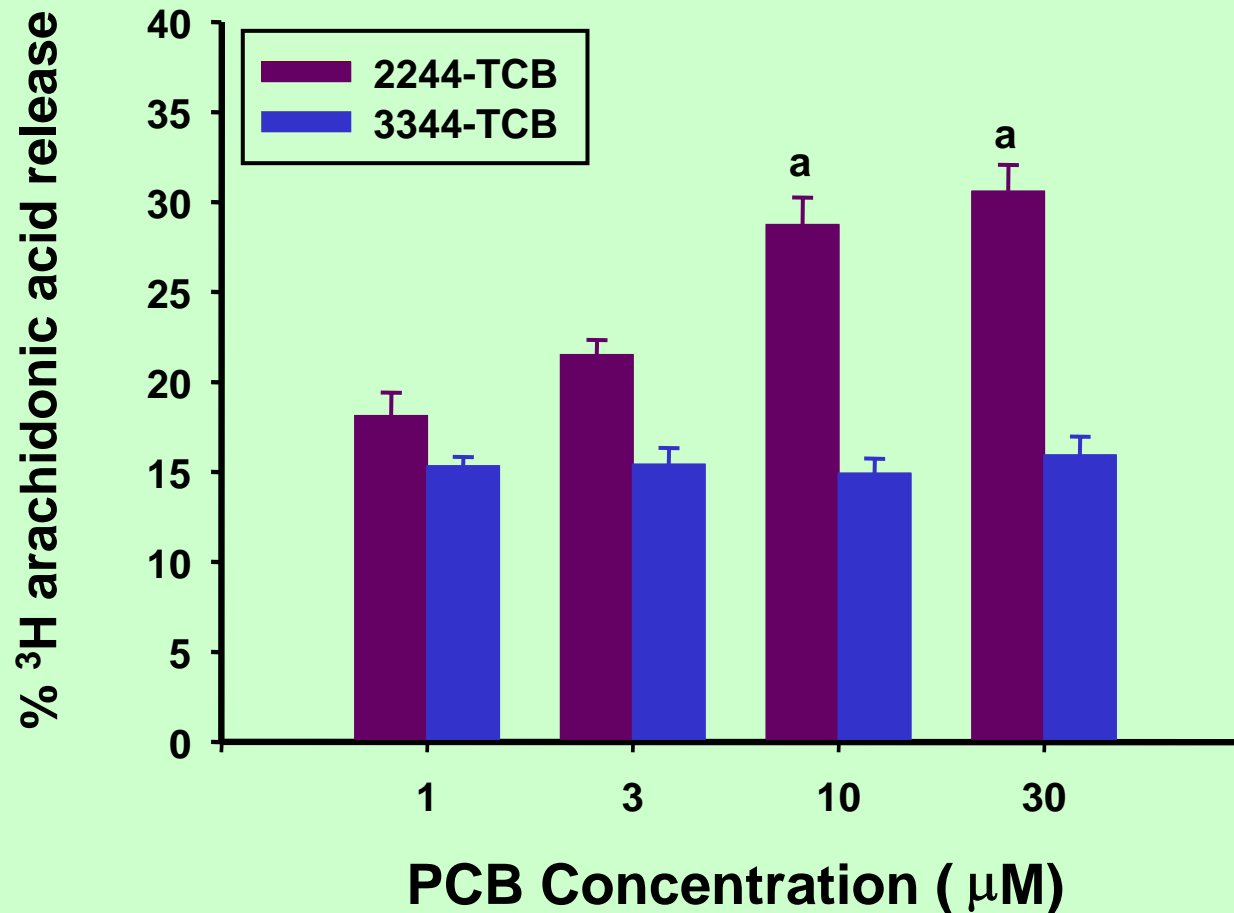
* Expression of COX-2 in brains of PLA₂-deficient mice was decreased relative to wild-type mice

* *Bosetti and Weerasinghe, 2003*

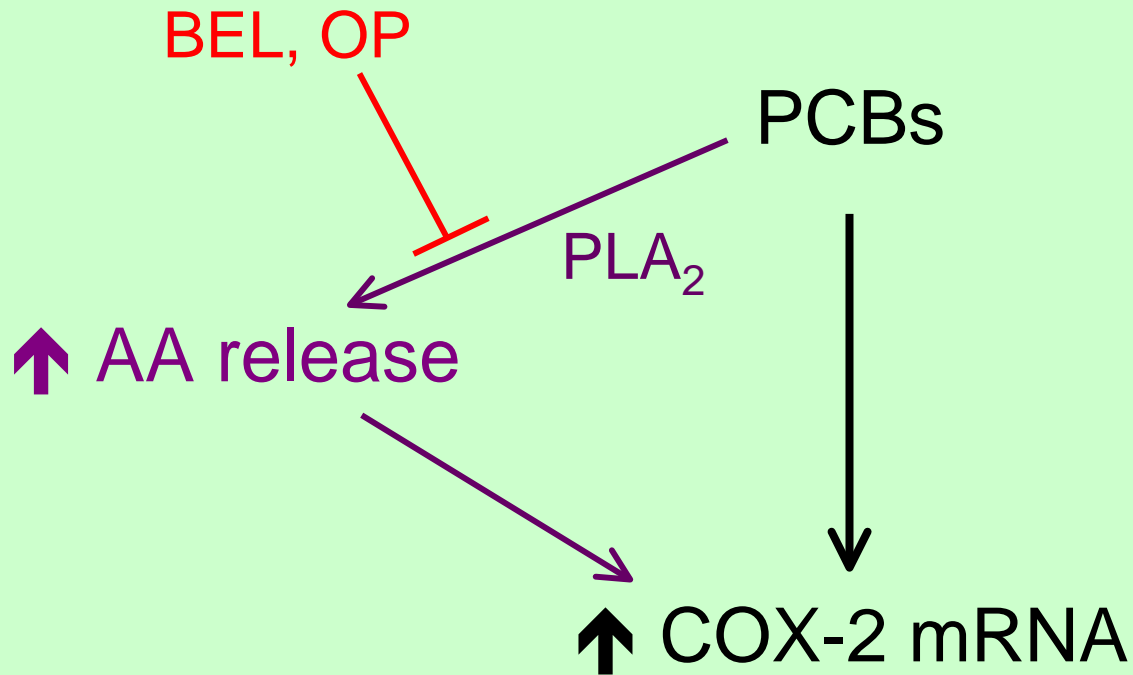
The time-course of 2,2',4,4'-TCB-stimulated release of arachidonic acid is similar to that for the upregulation of COX-2



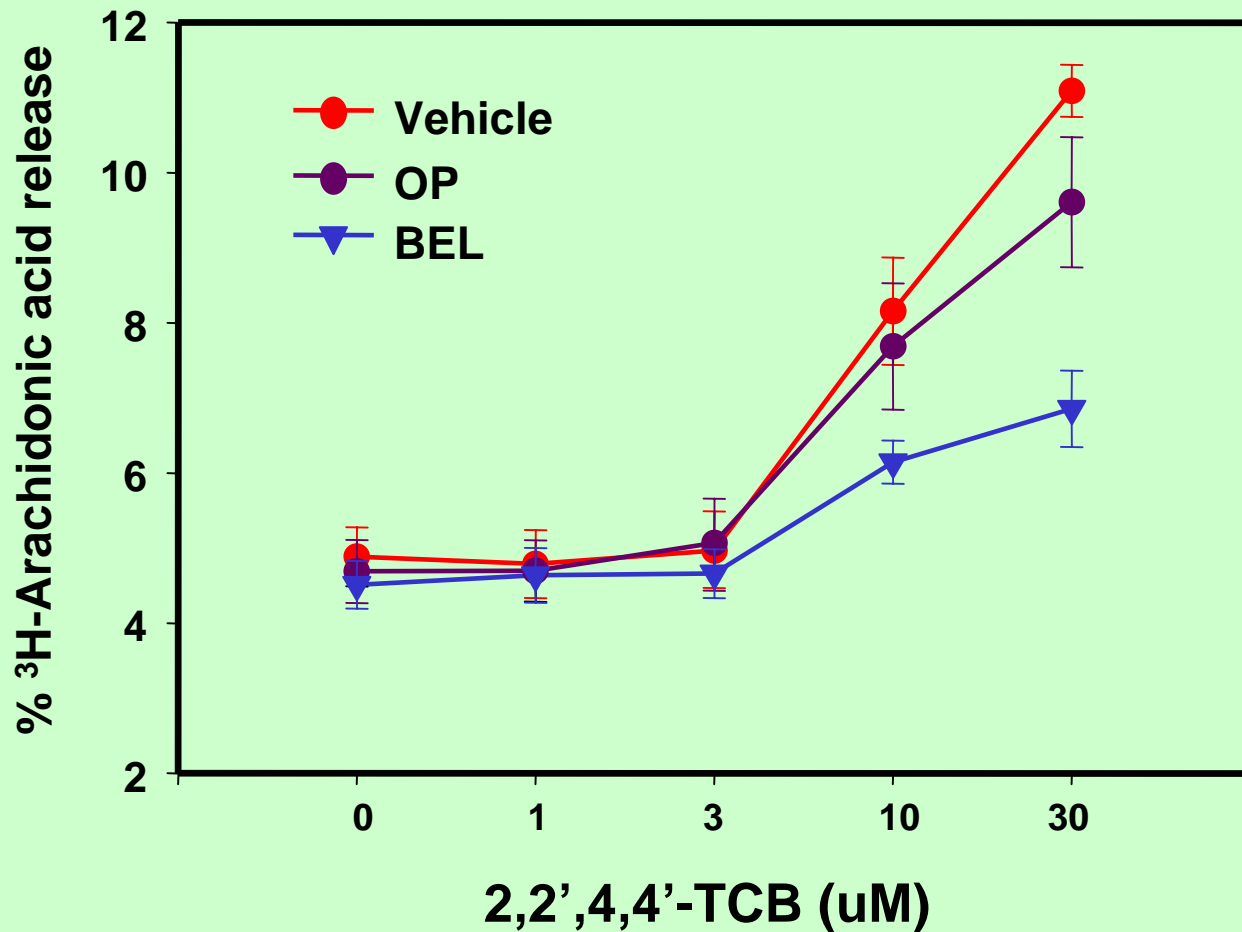
The concentration-response relationship for 2,2',4,4'-TCB-stimulated release of arachidonic acid is similar to that for upregulation of COX-2



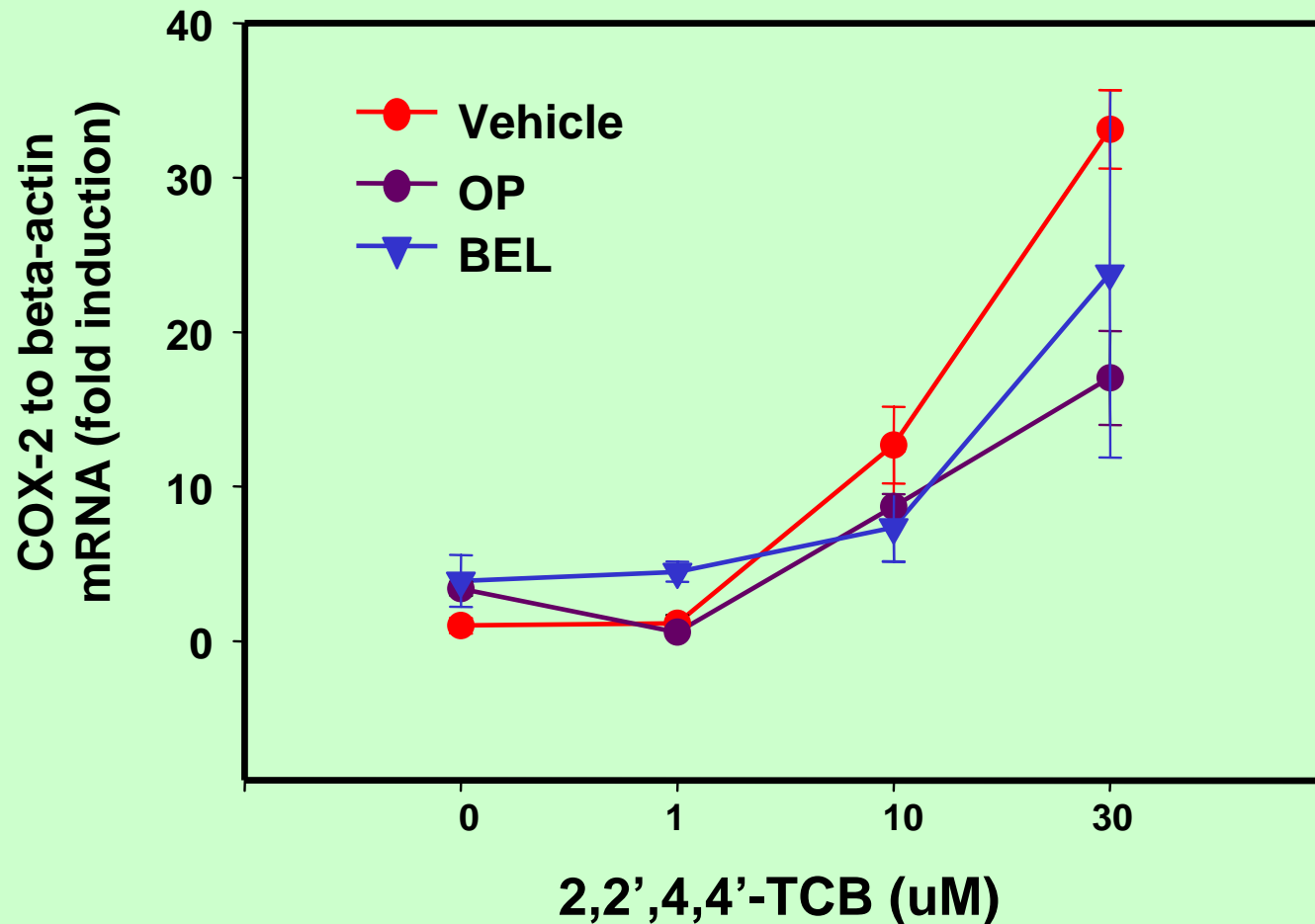
What signaling mechanisms are involved in PCB-mediated increased expression of COX-2 mRNA?



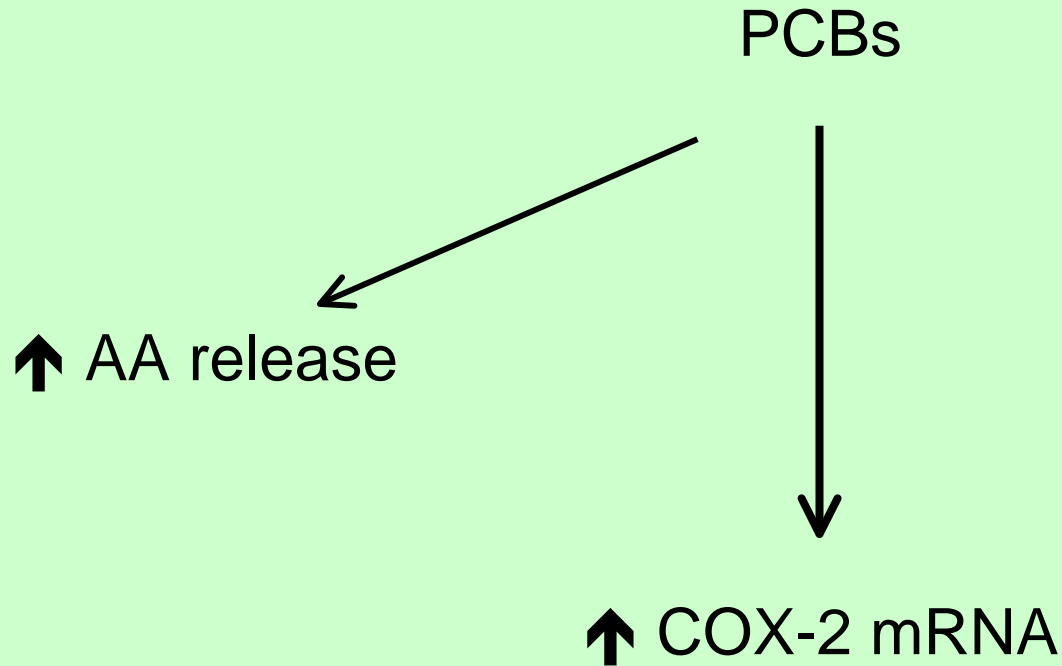
BEL inhibits PCB-mediated release of arachidonic acid



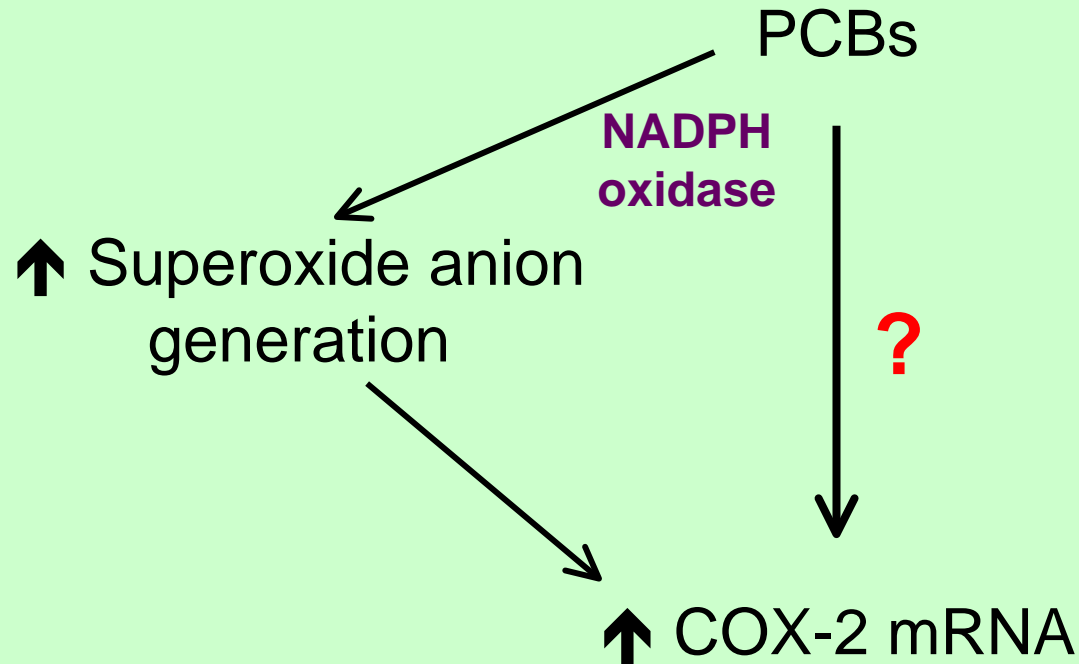
Inhibition of arachidonic acid release does not abolish PCB-induced upregulation of COX-2



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

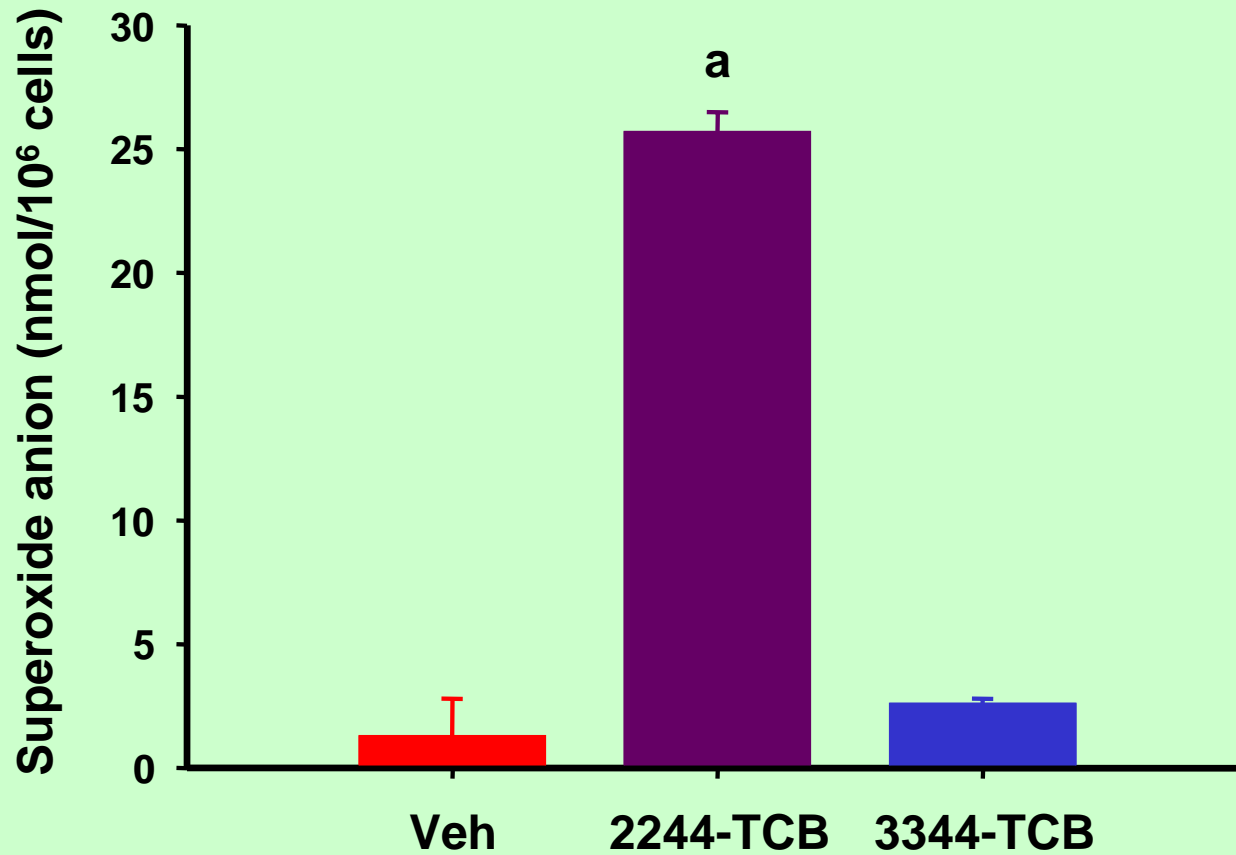


What signaling mechanisms are involved in PCB-mediated increased expression of COX-2 mRNA?

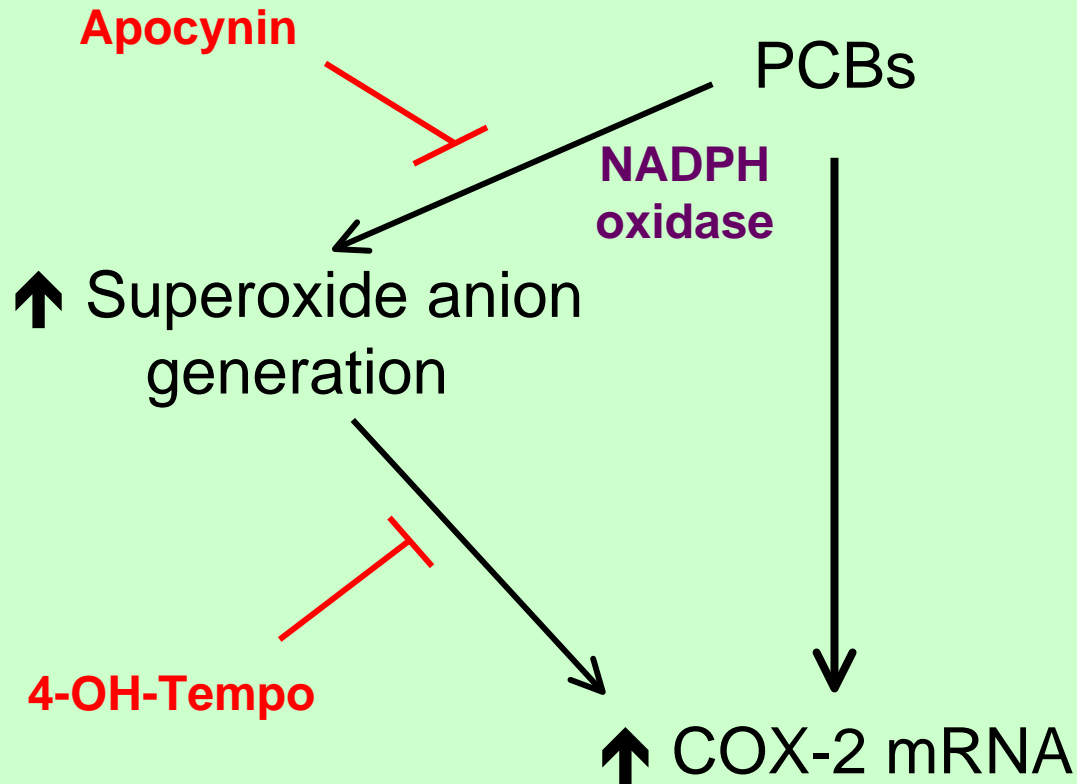


- * Increased expression of COX-2 was dependent on generation of ROS in
 - * human mesangial cells (high glucose) *Kiritoshi et al, 2003*
 - * human monocytes (differentiation) *Barbieri et al, 2003*
 - * human keratinocyte cell line (AAPH) *Cui et al, 2004*

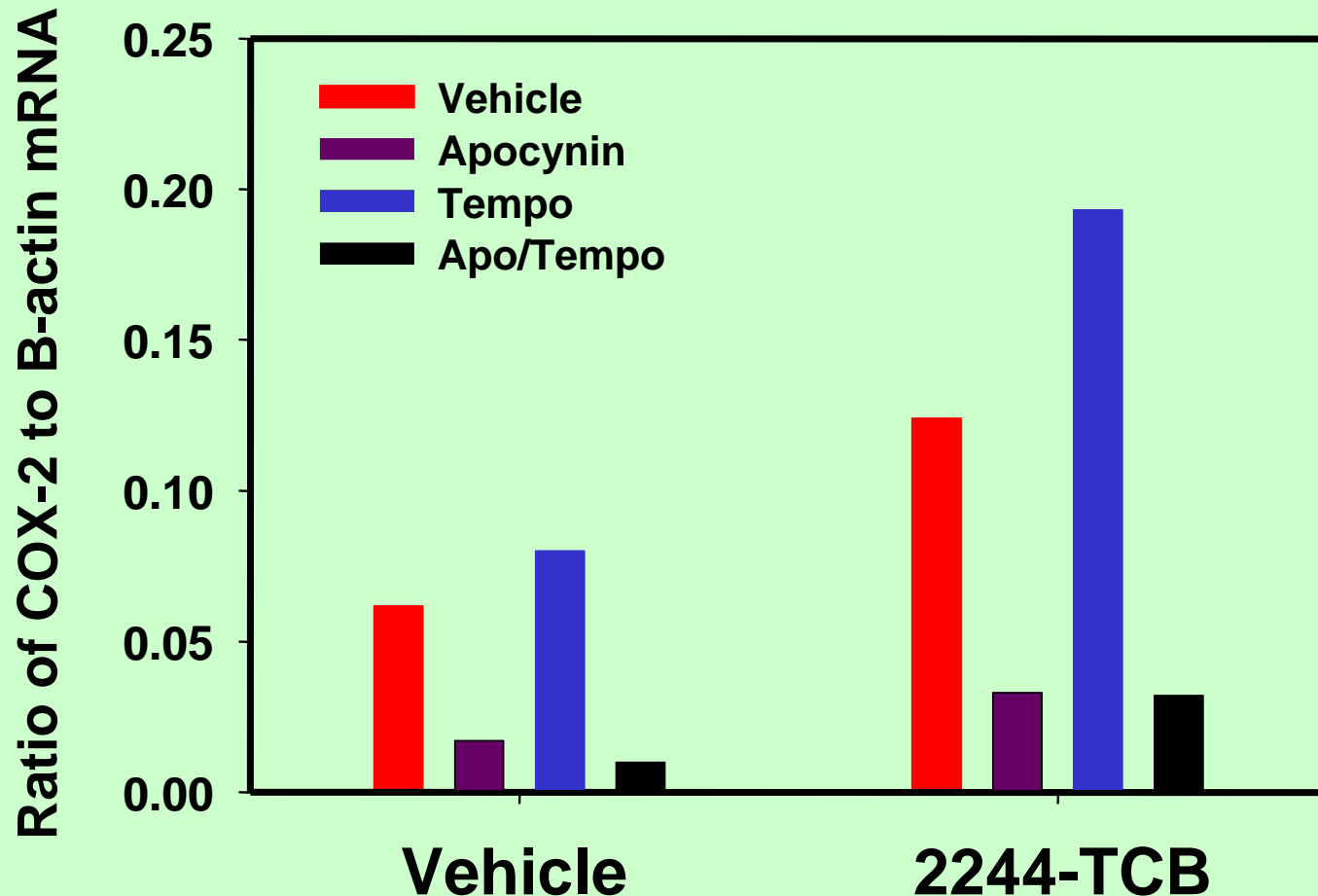
PCBs that increase expression of COX-2 mRNA also increase production of superoxide anion



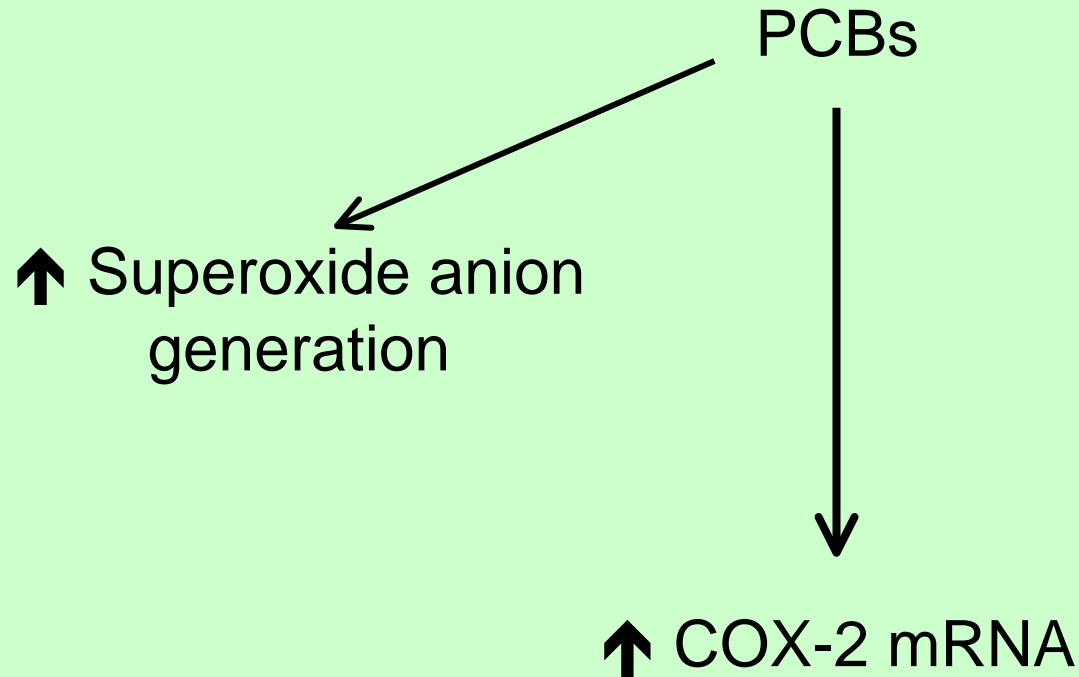
What signaling mechanisms are involved in PCB-mediated increased expression of COX-2 mRNA?



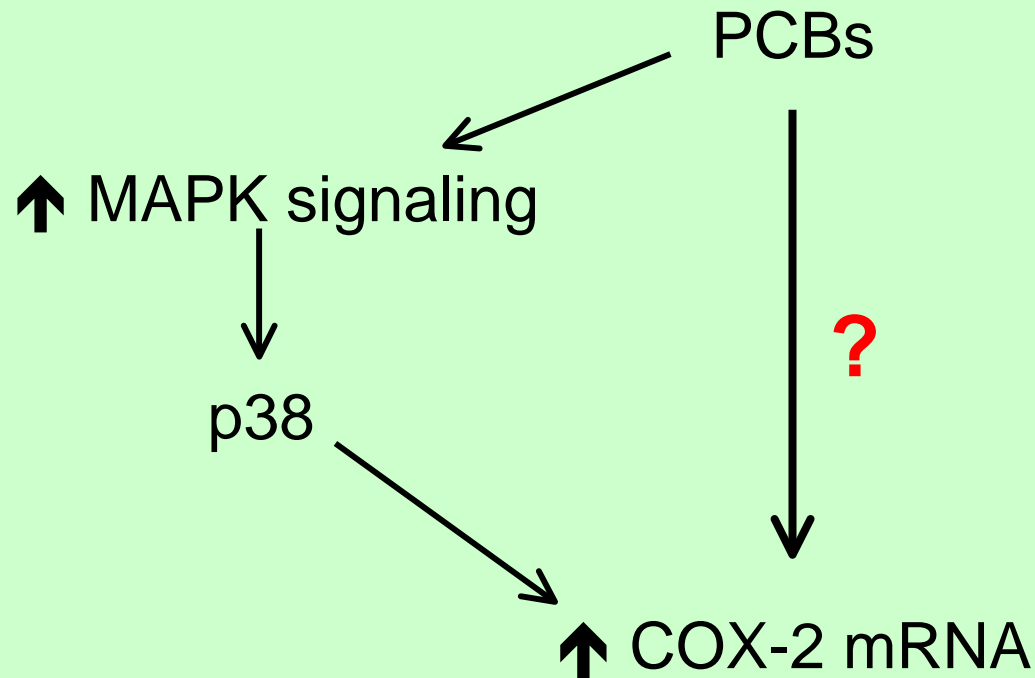
Apocynin, but not Tempo, decreases the upregulation of COX-2 mRNA



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?



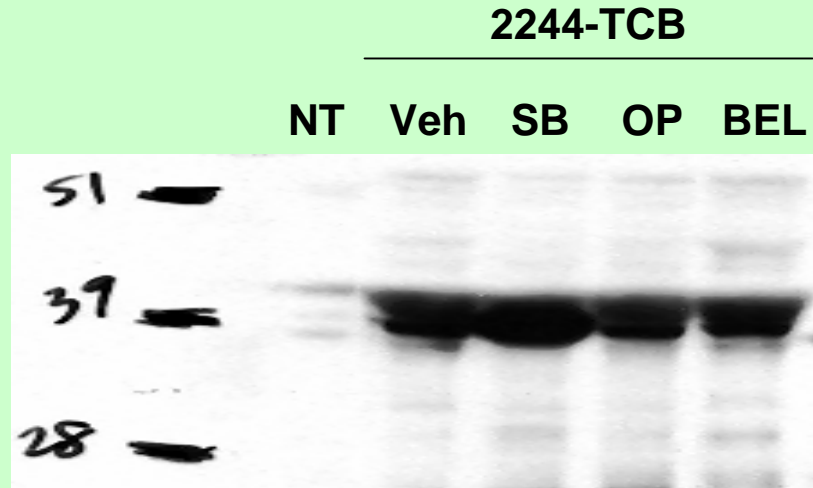
* **p38 MAPK contributes to increased expression of COX-2**

* *Souza et al., 2004; Westra et al., 2004; Grishin et al., 2004, Cui et al., 2004*

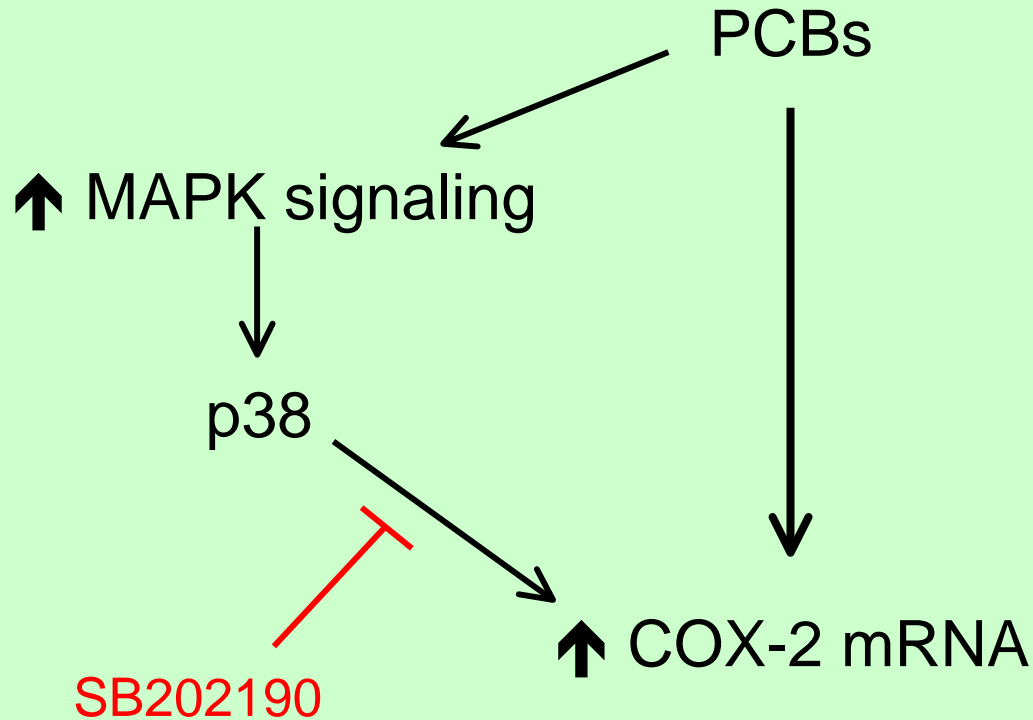
* **p38 MAPK stabilizes COX-2 mRNA**

* *Mifflin et al., 2004; Hendrickx et al., 2003*

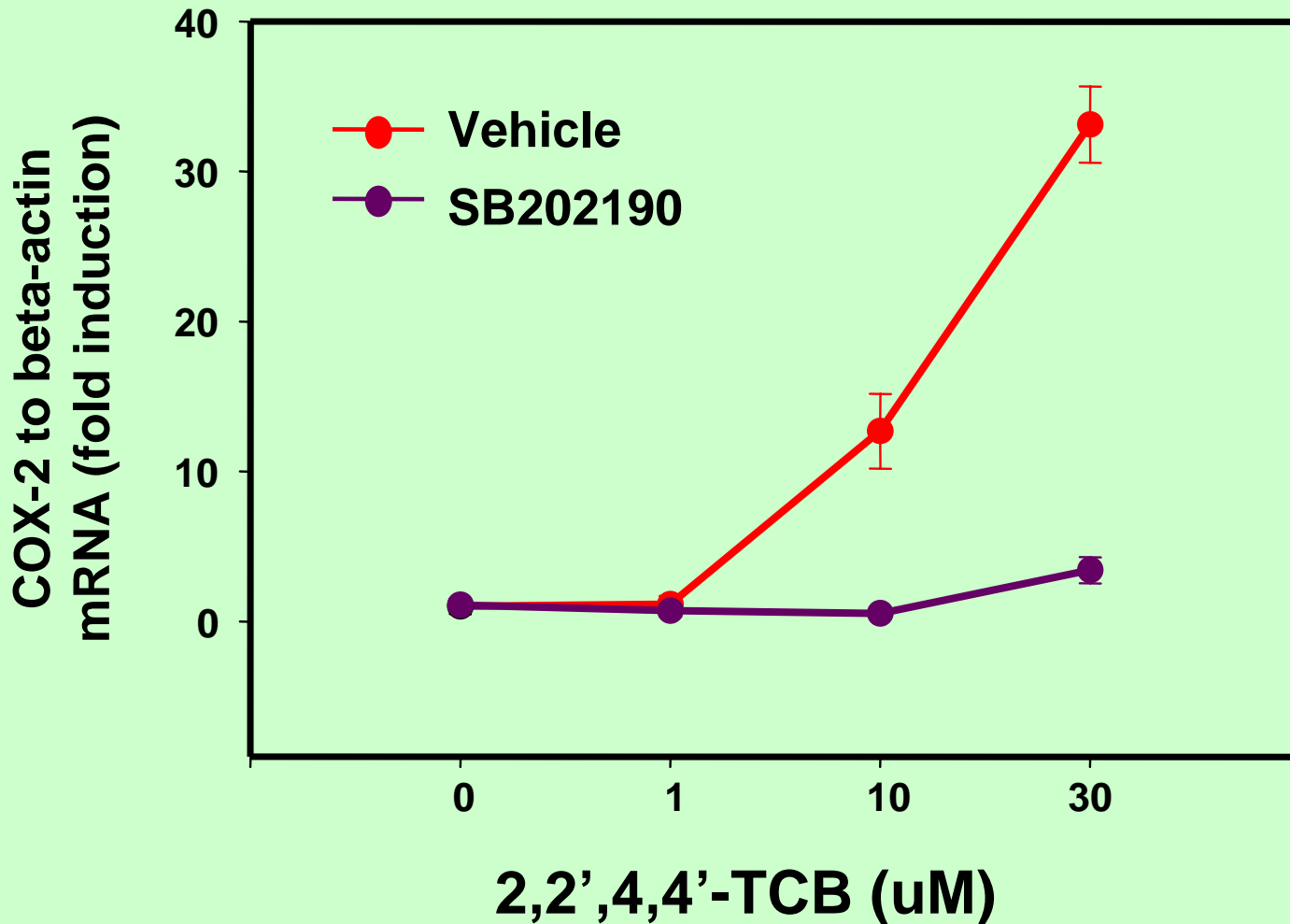
2,2',4,4'-TCB increases p38 in HL-60 cells



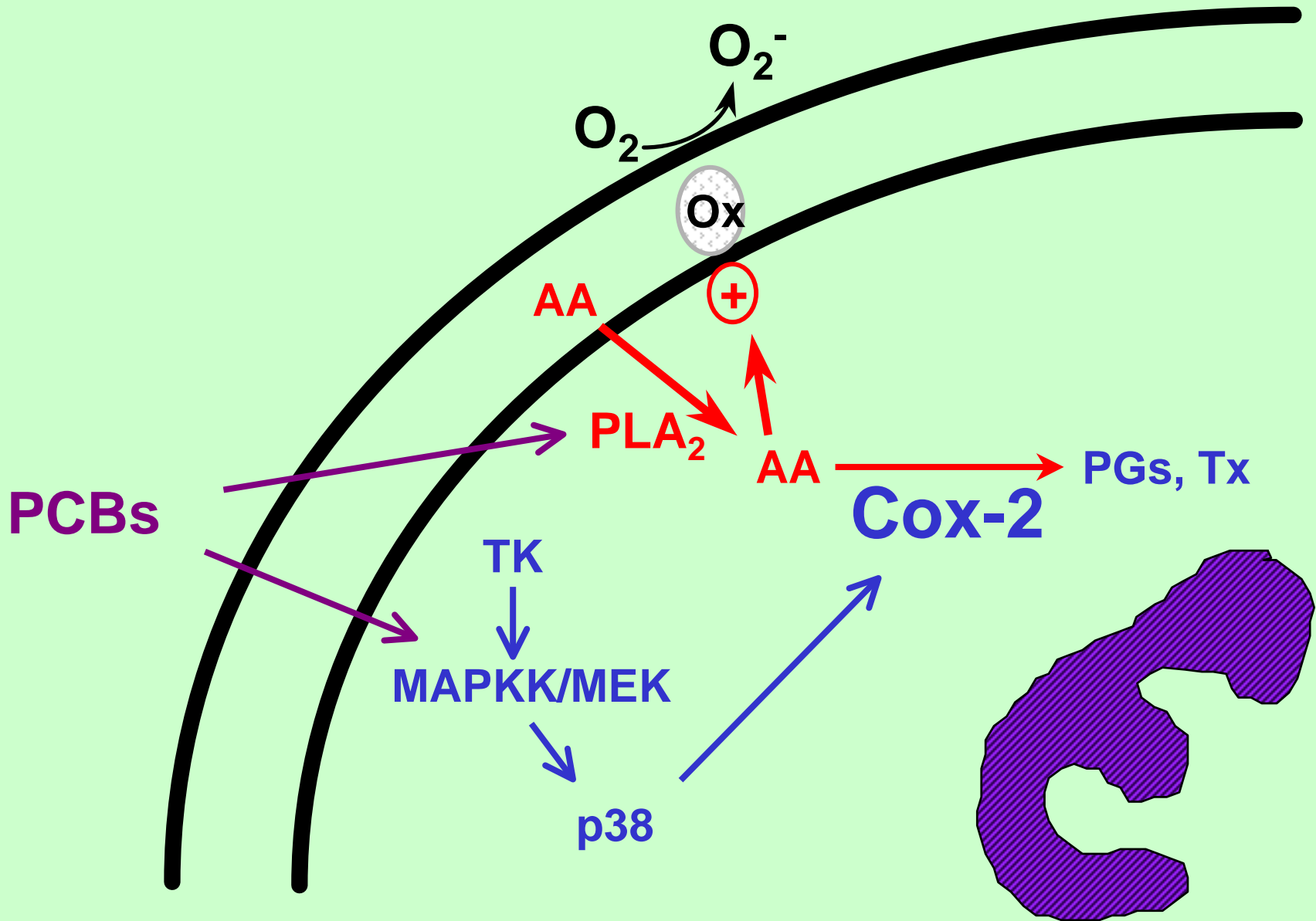
What signaling mechanisms are involved in PCB-mediated increased expression of COX-2?



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



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



Acknowledgments

✧ **Steve Bezdecny**

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