

# HOW DO ANTIOXIDANTS WORK IN PLASTICS AND PEOPLE?

Garry R. Buettner  
&  
Freya Q. Schafer

## I. Introduction:

### A. What is a Free Radical?

"It is an atom or group of atoms possessing one or more unpaired electrons.". The word "free" in front of "radical" is considered unnecessary.

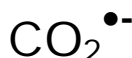
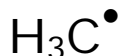
## II. Notation

### A. Superscript dot to the right, usually

### B. Examples (Note: dot, then charge)



$\text{O}_2^{\bullet\bullet}$  or  $\text{O}_2^{2\bullet}$  dioxygen,  
the  $\text{O}_2$  you are breathing now.



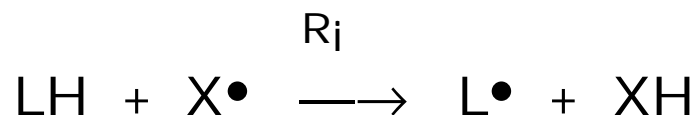
### III. Types of radicals we have:

1. Sigma,  $\sigma$
2. pi - delocalized,  $\pi$
3. Mixture of sigma and pi
4. carbon-centered  $\text{H}_3\text{C}\bullet$
5.  $\text{O}_2$  - centered  $\text{H}_3\text{COO}\bullet$
6. Sulfur - centered  $\text{GS}\bullet$
7. Nitrogen - centered  $\text{R}_2\bullet\text{NO}$
8. Metals:  $\text{Cu}^{2+}$ ,  $\text{Fe}^{2+,3+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Mo}$ ,  $\text{Co}^{2+}$
9. Reducing radicals,  $\text{CO}_2^{\bullet-}$ ,  $\text{PQ}^{\bullet+}$
10. Oxidizing radicals,  $\text{HO}\bullet$ ,  $\text{LOO}\bullet$
11. ...

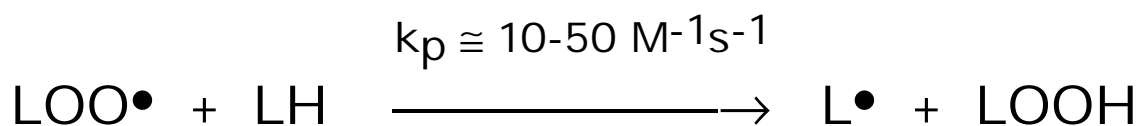
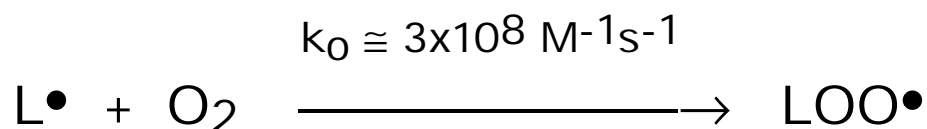
## IV. Classic Lipid Peroxidation

### A. The Basics (one-electron Processes)

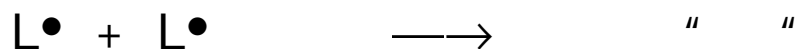
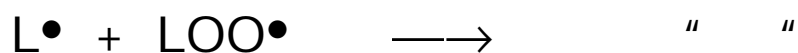
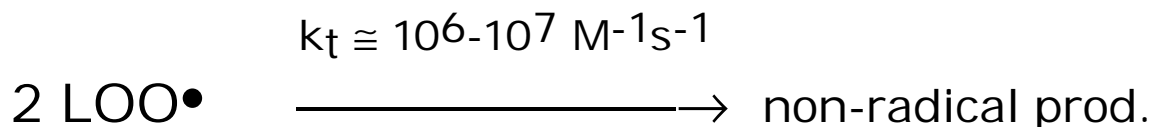
*Initiation:*



*Propagation* (a cycle of destruction):



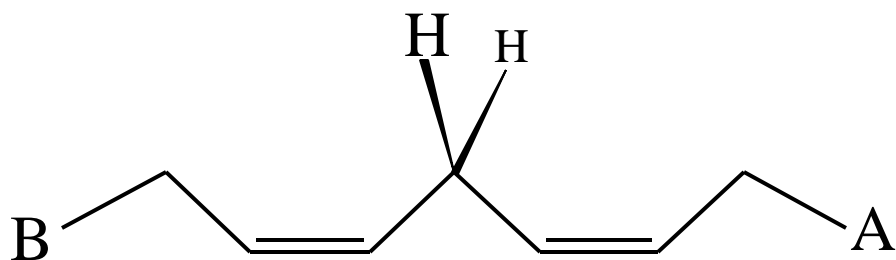
*Termination:*



## B. Oxidizability

of PUFA is dependent on the number

of *bis*-allylic methylene positions

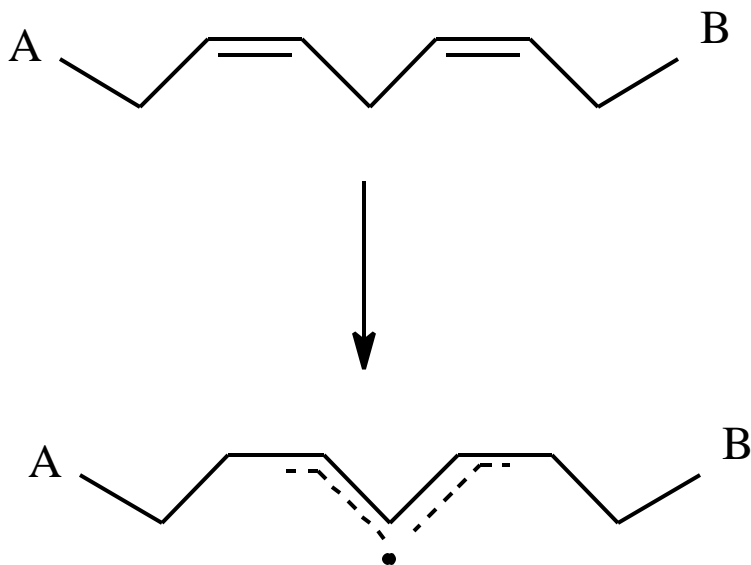


*bis*-allylic  
*bis* = double

allyl  $\longrightarrow$  C-C=C

Bond Dissociation Energy for an alkyl C-H  $\approx$  104 kcal/mol;  
for a *bis*-allylic C-H 75 – 80 kcal/mol

## C. The Lipid Radical, a pentadienyl-like radical



## V. ANTIOXIDANTS

### A. Definition

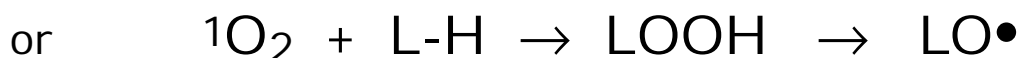
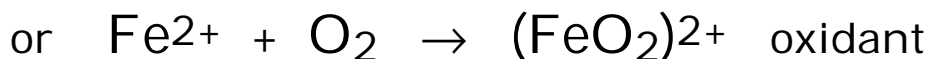
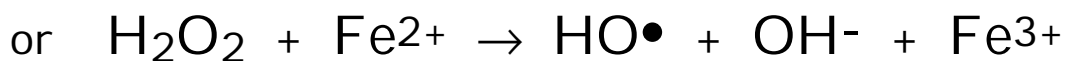
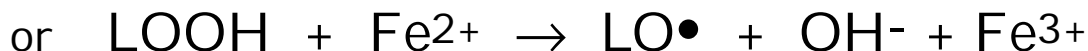
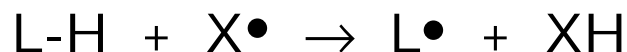
**A substance when present in trace (small) amounts inhibits oxidation of the bulk.**

There are two broad classes:

1. *Preventative*
2. *Chain-breaking*

### B. Preventative Antioxidants reduce the rate of chain initiation

*Initiation:*



# 1. Targets for preventative antioxidants

## a. Metals - Fe, Cu

### *i.* Chelates - EDTA

DETAPAC

Desferal® ≡ deferrioximine

Phytate ?

### *ii.* Proteins & metals

Transferrin Fe

Ferritin Fe

Hemes

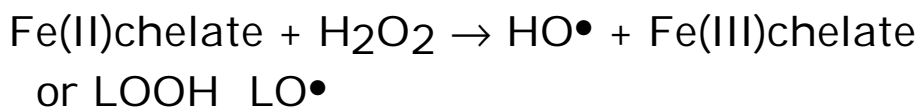
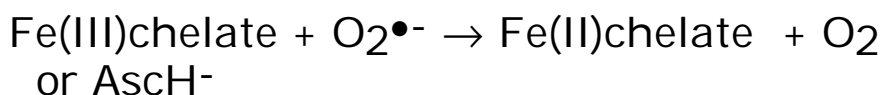
Hemoglobin

Myoglobin

:

Caeruloplasmin Cu

### *iii.* Key aspect is:

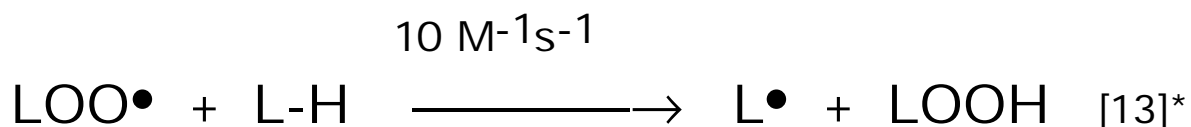
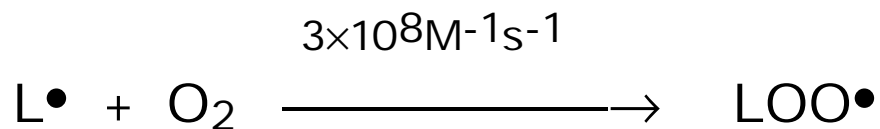


### *iv.* Peroxides: $\text{H}_2\text{O}_2$ , $\text{LOOH}$

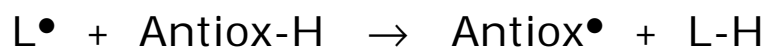
CAT, GPx, PhGPx, SOD

## C. Chain-breaking Antioxidants

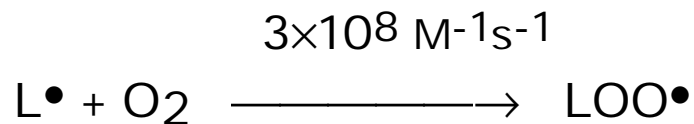
*Propagation:*



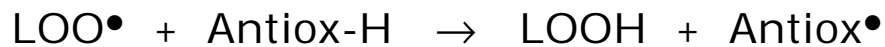
*i.* Intercept  $\text{L}\bullet$  ?



Not likely! Why = k's



*ii.* Repair LOO•



$$k = 10^4 \rightarrow 10^8 \text{ M}^{-1}\text{s}^{-1}$$

It can be successful.

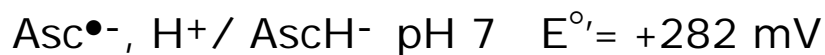
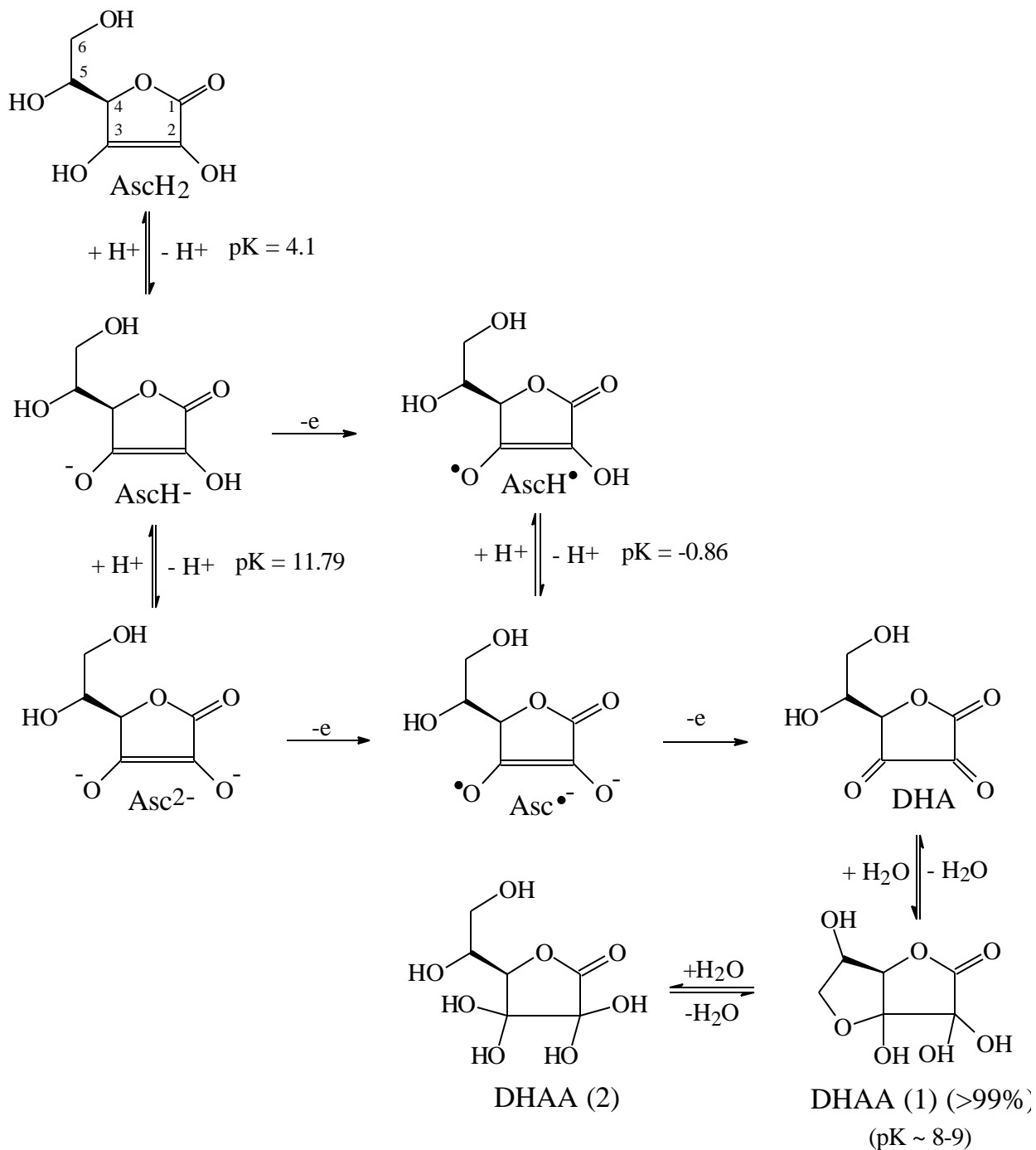
*iii.* Good antioxidant (chain-breaking)

- a. relatively UNreactive  
Antioxidant & Antiox•
- b. Antiox• - decays to harmless products
- c. Does not add O<sub>2</sub>.
- d. Recycled - somehow.



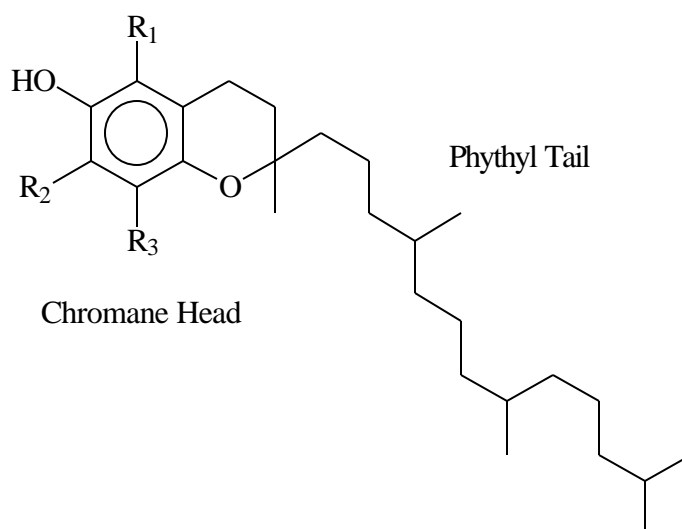
## VI. Vitamins C & E. (Donor Antioxidants)

### A. Vitamin C (ascorbate)



## B. Vitamin E

### $\alpha$ , $\beta$ , $\gamma$ , $\delta$ - Tocopherols



**Table 1** Forms of Tocopherol

	$R_1$	$R_2$	$R_3$
$\alpha$	$\text{CH}_3$	$\text{CH}_3$	$\text{CH}_3$
$\beta$	$\text{CH}_3$	H	$\text{CH}_3$
$\gamma$	H	$\text{CH}_3$	$\text{CH}_3$
$\delta$	H	H	$\text{CH}_3$

## C. The Big Picture: (Buettner, GR. *Arch. Biochem. Biophys.* 300:535-543,1993)

**Figure:** Membrane lipid peroxidation. Only one leaflet of the bilayer is represented.

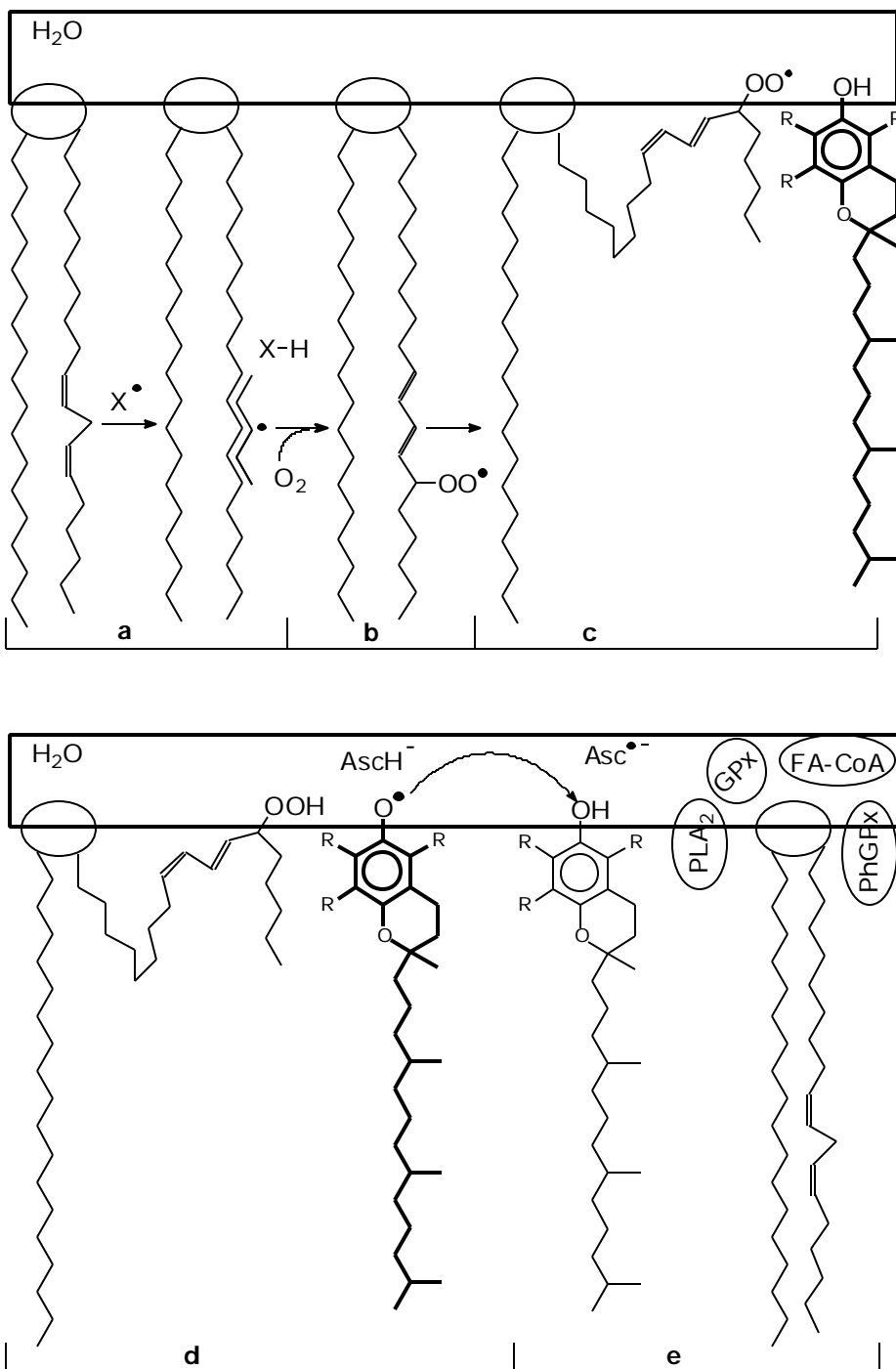
a) Initiation of the peroxidation process by an oxidizing radical,  $X^\bullet$ , by abstraction of a *bis*-allylic hydrogen, thereby forming a pentadienyl radical.

b) Oxygenation to form a peroxy radical and a conjugated diene.

c) The peroxy radical moiety partitions to the water-membrane interface where it is poised for repair by tocopherol.

d) The tocopheroxyl radical can be repaired by ascorbate.

e) Tocopherol has been recycled by ascorbate; the resulting ascorbate radical can be recycled by enzyme systems. The enzymes phospholipase  $A_2$  (PLA<sub>2</sub>), phospholipid hydroperoxide glutathione peroxidase (PhGPx), glutathione peroxidase (GPx), and fatty acyl-coenzyme A (FA-CoA), cooperate to detoxify and repair the oxidized fatty acid chain of the phospholipid. This cartoon cannot show the dynamic aspects of this process. TOH in the membrane will undoubtedly be bobbing "up and down" so that the position of the "OH" is variable. In addition, TOH and TO<sup>•</sup> may have somewhat differing positions at the interface. The chemical aspects of this process will undoubtedly also describe the free radical oxidation of LDL.



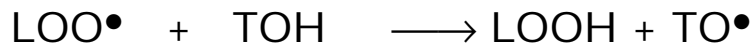
## D. Energetics

### Table 1

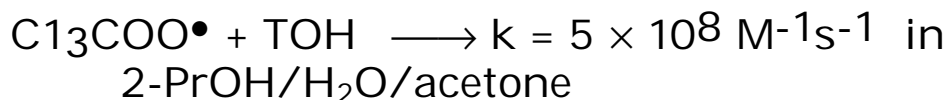
Redox Couple	$E^{\circ'}/\text{mV}$
$\text{HO}\bullet, \text{H}^+/\text{H}_2\text{O}$	+ 2310
$\text{RO}\bullet, \text{H}^+/\text{ROH}$ (aliphatic alkoxy radical)	+ 1600
$\text{ROO}\bullet, \text{H}^+/\text{ROOH}$ (alkyl peroxy radical)	+ 1000
$\text{GS}\bullet/\text{GS}^-$ (glutathione)	+ 920
$\text{PUFA}\bullet, \text{H}^+/\text{PUFA-H}$ (bis-allylic-H)	+ 600
$\text{TO}\bullet, \text{H}^+/\text{TOH}$	+ 480
$\text{H}_2\text{O}_2, \text{H}^+/\text{H}_2\text{O}, \text{HO}\bullet$	+ 320
Ascorbate $\bullet^-$ , $\text{H}^+/\text{Ascorbate monoanion}$	+ 282
Fe(III) EDTA/ Fe(II) EDTA	+ 120
$\text{O}_2/\text{O}_2\bullet^-$	- 330
Paraquat/ Paraquat $\bullet^-$	- 448
Fe(III) DFO/ Fe(II) DFO	- 450
$\text{RSSR}/\text{RSSR}\bullet^-$ (GSH)	- 1500
$\text{H}_2\text{O}/\text{e}^-_{\text{aq}}$	- 2870

---

## E. Kinetics

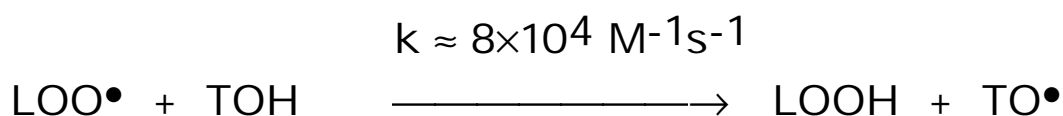
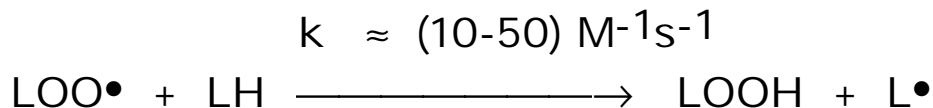


$$k = 2.5 \times 10^6 \text{ M}^{-1}\text{s}^{-1} \quad \text{oleic acid solution}$$



But Larry Patterson Rad. Lab Notre Dame  
 Linoleate micelles  $k = 8 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$

Thus, how does vitamin E protect? Kinetics



Thus,

$$\text{Rate (LOO}\bullet + \text{TOH)} = 8 \times 10^4 \text{ M}^{-1}\text{s}^{-1} [\text{TOH}] [\text{LOO}\bullet]$$

---


$$\text{Rate (LOO}\bullet + \text{LH)} = 10-50 \text{ M}^{-1}\text{s}^{-1} [\text{LH}] [\text{LOO}\bullet]$$

$$\frac{R_{inh}}{R_{prop}} = \frac{8 \times 10^3 [TOH]}{[LH]}$$

$$1:1 \quad \Rightarrow \quad [TOH]:[LH] = 1:8000$$

But

$$9:1 \quad \Rightarrow \quad [TOH]:[LH] = 1:900$$

$$99:1 \quad \Rightarrow \quad [TOH]:[LH] = 1:90$$

How much vit E is there in membranes?

Ratio  $\approx$  1:1000

**F. Gey KF.** (1998) Vitamins E plus C and interacting conutrients required for optimal health. A critical and constructive review of epidemiology and supplementation data regarding cardiovascular disease and cancer. *Biofactors*. **7(1-2):**113-74, 1998.

**Abstract:** Antioxidants are crucial components of fruit/vegetable rich diets preventing cardiovascular disease (CVD) and cancer: plasma vitamins C, E, carotenoids from diet correlate prevalence of CVD and cancer inversely, low levels predict an increased risk of individuals which is potentiated by combined inadequacy (e.g., vitamins C + E, C + carotene, A + carotene); self-prescribed rectification of vitamins C and E at adequacy of other micronutrients reduce forthcoming CVD, of vitamins A, C, E, carotene and conutrients also cancer; randomized exclusive supplementation of beta-carotene +/- vitamin A or E lack benefits except prostate cancer reduction by vitamin E, and overall cancer reduction by selenium; randomized intervention with synchronous rectification of vitamins A + C + E + B + minerals reduces CVD and counteracts precancerous lesions; high vitamin E supplements reveal potentials in secondary CVD prevention.

*Plasma values desirable for primary prevention:*

≥ 30 μmol/L lipid-standardized vitamin E, *i.e.*,  
α-tocopherol/cholesterol ≥ 5.0 μmol/mmol;

≥ 50 μmol/L vitamin C aiming at **vitamin C/vitamin E**  
**> 1.3-1.5;**

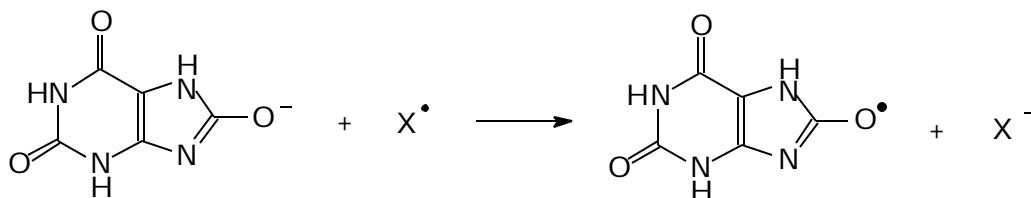
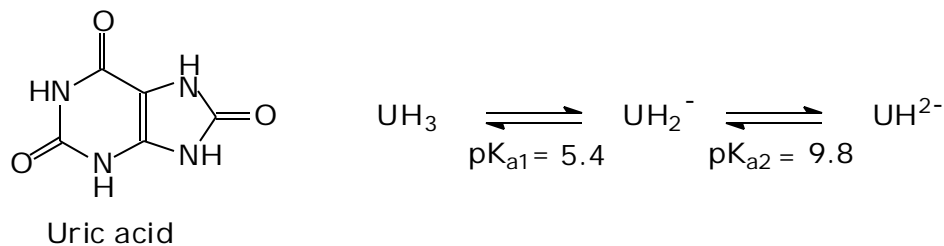
≥ 0.4 μmol/L beta- (≥ 0.5 μmol/l alpha+ beta-) carotene.

**CONCLUSIONS:** In CVD vitamin E acts as first risk discriminator, vitamin C as second one; optimal health requires synchronously optimized vitamins C + E, A, carotenoids and vegetable conutrients.





## VII. Uric Acid; an antioxidant?



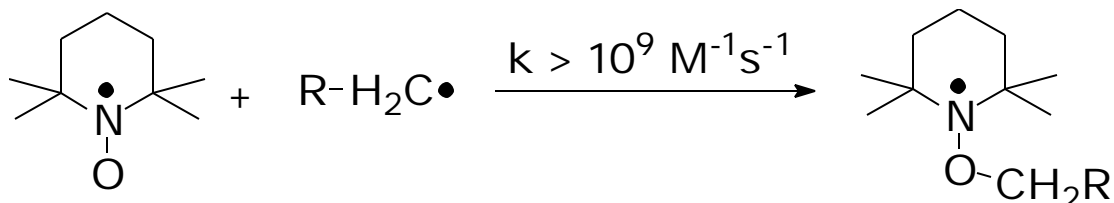
$E^{\circ} = +590 \text{ mV}$  for  $\text{UH}^{\bullet}, \text{H}^+ / \text{UH}_2^-$ ; because the reduction potential of the urate radical (+590 mV) is above that of  $\text{Asc}^{\bullet-}$  (+282 mV) at neutral pH, ascorbate would be expected to repair the urate radical<sup>1</sup>.

<b>Reaction</b>	<b>rate constant/<math>\text{M}^{-1}\text{s}^{-1}</math></b>
$\text{UH}^{\bullet} + \text{O}_2$	$< 10^{-2}$
$\text{UH}^{\bullet} + \text{AscH}^- \rightarrow \text{UH}_2^- + \text{Asc}^{\bullet-}$	$1 \times 10^6$
$\text{ROO}^{\bullet} + \text{UH}_2^- \rightarrow \text{ROOH} + \text{UH}^{\bullet-}$	$3 \times 10^6$
$\text{Cl}_3\text{COO}^{\bullet} + \text{UH}_2^- \rightarrow \text{Cl}_3\text{COOH} + \text{UH}^{\bullet-}$	$3.2 \times 10^8$
$\cdot\text{NO}_2 + \text{UH}_2^- \rightarrow \text{NO}_2^- + \text{UH}^{\bullet-} + \text{H}^+$	$1.8 \times 10^7$
$\text{R} - \text{G}^{\bullet} (-\text{H}) + \text{UH}_2^- \rightarrow \text{R} - \text{G} + \text{UH}^{\bullet-}$ (G = guanosine)	$1.2 \times 10^9$

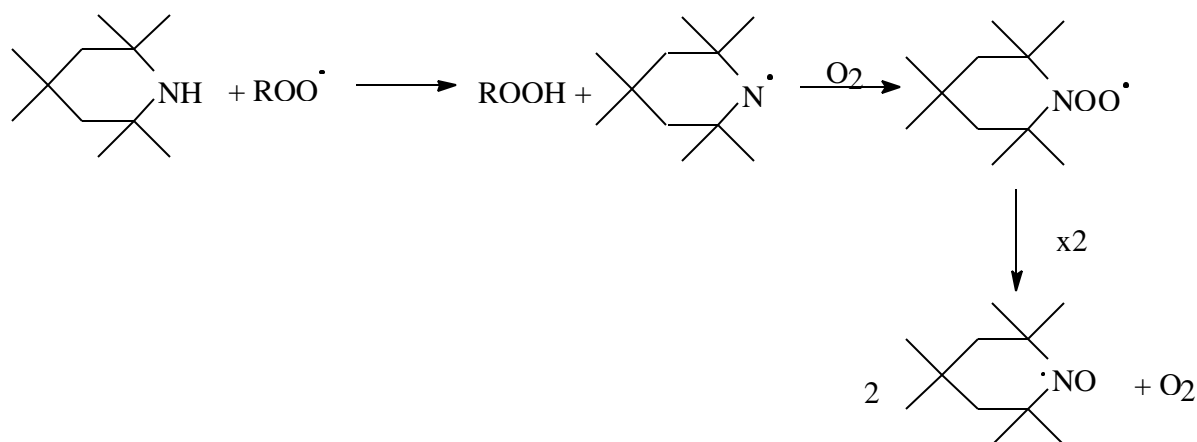
<sup>1</sup> Simic MG, Jovanovic SV (1989) Antioxidation mechanisms of uric acid. *J Am Chem Soc* **111**: 5778-5782.



## Nitroxide reactions with Carbon-centered radicals<sup>5</sup>



### C. HALS Hindered Amine Light Stabilizers Syringe nitroxide<sup>6 7</sup> and other plasticware<sup>8</sup>



### D. Auto paint, UV protection and radiation protection

#### Tempo, Ford Motor Co. & Topcoat enamels<sup>9</sup> Protection of skin in radiation therapy<sup>10 11 12</sup>

<sup>5</sup> Moad G, Solomon DH (1995) The Chemistry of Free Radical Polymerization. Ed.1 Pergamon press

<sup>6</sup> Buettner GR, Scott BD, Kerber RE, Mügge A. (1991) Free radicals from plastic syringes. *Free Rad Biol Med* **11**: 69-70.

<sup>7</sup> Buettner GR, Sharma MK. (1993) The syringe nitroxide free radical - Part II. *Free Rad Res Comm* **19**: S227-S230.

<sup>8</sup> Miller CW, Chen GM, Janzen EG. (1999) Detection of free radicals in reperfused dog skin flaps using electron paramagnetic resonance spectroscopy: A pilot study. *Microsurgery*. **19(4)**:171-175.

<sup>9</sup> Gerlock JL, Bauer DR, Mielewski DF. (1990) Using nitroxide decay to study the photooxidation kinetics of automotive topcoat enamels. *Free Radical Res Comms*. **10**:123-33.

<sup>10</sup> Goffman T, Cuscata D, Glass J, Hahn S, Krishna CM, Lupton G, Mitchell JB. (1992) Topical application of nitroxide protects radiation-induced alopecia in guinea pigs. *Int J Radiation Oncology Biol Phys* **22**: 803-806.

## IX. Nitric Oxide:

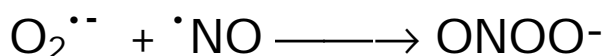
### Pro-oxidant

1. Rxn with superoxide

$t_{1/2} (\cdot\text{NO}) \approx \text{seconds in biological setting}$  i.e.  $\approx < 1 \text{ s}$

Rxn with  $\text{O}_2^{\cdot-}$

$$k = 6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$$



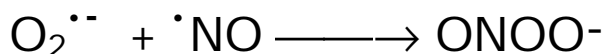
peroxynitrite

2. Peroxynitrite (Oxoperoxonitrate (1-))



$$\epsilon_{240} (\text{ONOOH}) = 770 \pm 50 \text{ M}^{-1} \text{ cm}^{-1}$$

$\text{ONOOH} \rightarrow \text{HNO}_3$  proceeds through a first order isomerization.

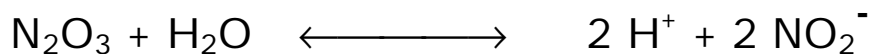
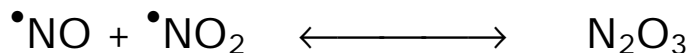
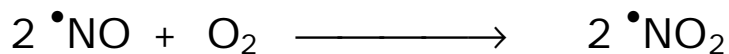


$\text{ONOO}^-$  - powerful one- and two-electron oxidant!

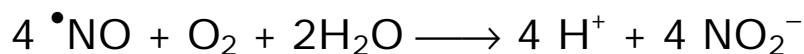
<sup>11</sup> Hahn SM, Tochner Z, Krishna CM, Glass J, Wilson L, Samuni A, Sprague M, Venzon D, Glatstein E, Mitchell JB, Russo A. (1992) Tempol, a stable free radical, is a novel murine radiation protector. *Cancer Research* **52**: 1750-1753.

<sup>12</sup> DeGraff WG, Krishna MC, Kaufman D, Mitchell JB. (1992) Nitroxide-mediated protection against x-ray- and neocarzinostatin-induced DNA damage. *Free Rad Biol Med* **13**: 479-487.  
Oxygen Society Annual Meeting, New Orleans, LA Nov 19-22, 1999

### 3. Reactions of $\bullet\text{NO}$ with $\text{O}_2$



The net reaction is:

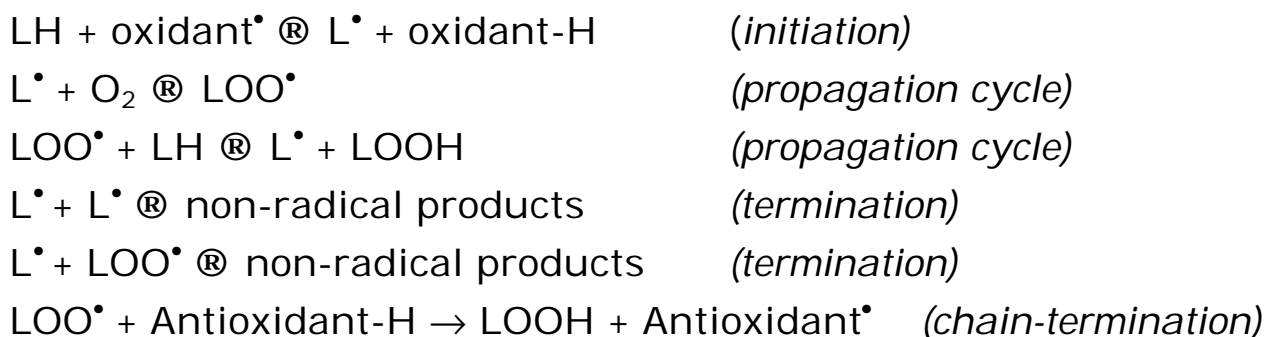


The rate of this process is governed by a rare, third-order rate constant where  $k = 2.4 \times 10^6 \text{ M}^{-2} \text{ s}^{-1}$  at  $37^\circ\text{C}$ <sup>13</sup>. This  $k$  is the same at pH 4.9 and 7.4. Nitrate is not formed. The activation energy is 2 kcal/mol.<sup>14</sup>

$$-d[\bullet\text{NO}]/dt = +d[\text{NO}_2^-]/dt = 4k[\bullet\text{NO}]^2[\text{O}_2]$$

$$t_{1/2}(\bullet\text{NO}) \approx \text{seconds, but in a biological setting} \approx < 1 \text{ s}$$

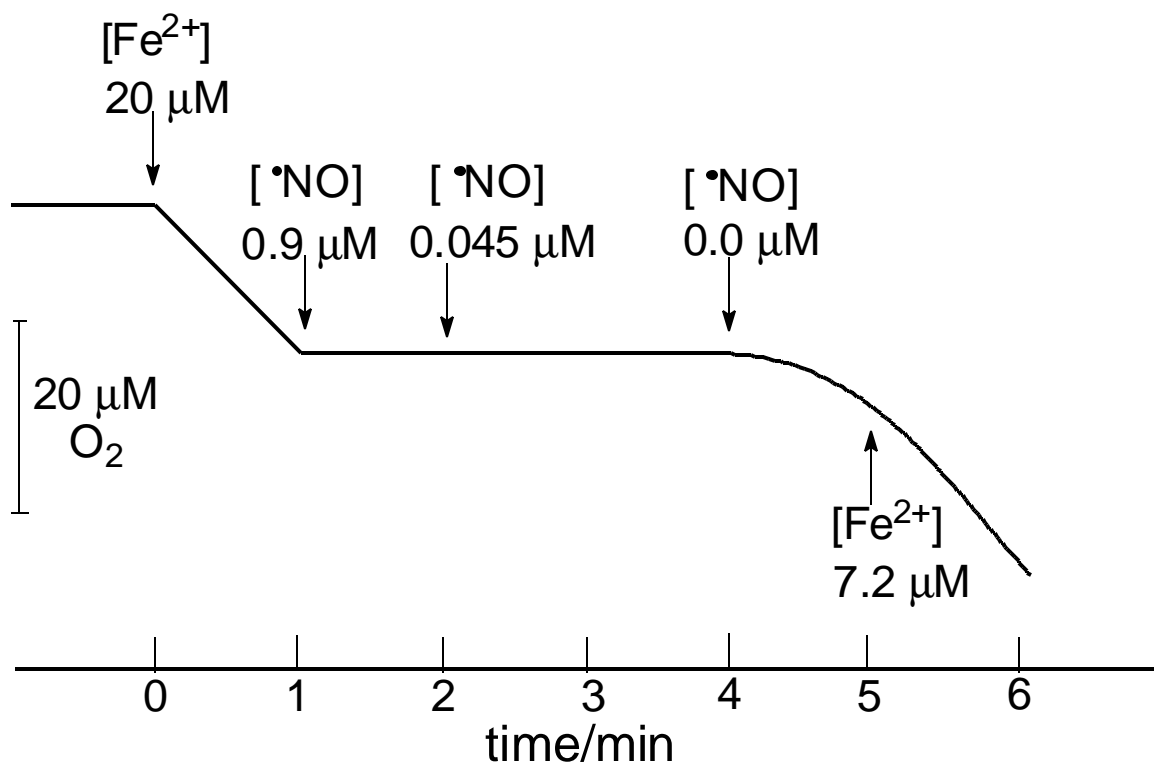
Nitric oxide can serve as a chain-terminating antioxidant during lipid peroxidation. Lipid peroxidation occurs in a three-step process: initiation, propagation and termination.



<sup>13</sup> Ford *et al.* *FEBS Lett* **326**:1-3, 199

<sup>14</sup> Lewis & Deen, *Chem. Res. Toxicol.* **7**:568-574, 1994.

It is in the termination process that  $\cdot\text{NO}$  may play a crucial role;  $\cdot\text{NO}$  could serve as an antioxidant *via* the following chain-termination reactions:



**Figure: Changes in concentration of  $\cdot\text{NO}$  and  $\text{Fe}^{2+}$  during cellular lipid peroxidation and its inhibition by  $\cdot\text{NO}$ .** Shown are the concentrations of  $\cdot\text{NO}$  and  $\text{Fe}^{2+}$  at key time points. Cellular (HL-60) lipid peroxidation was initiated with 20  $\mu\text{M}$   $\text{Fe}^{2+}$ . At 1 min after the addition of  $\text{Fe}^{2+}$ , 0.9  $\mu\text{M}$   $\cdot\text{NO}$  was introduced.  $\cdot\text{NO}$  was rapidly depleted and is below the limit of detection at about 4 min. At the time of  $\cdot\text{NO}$  depletion, rapid  $\text{O}_2$  uptake resumes. This reinitiation of  $\text{O}_2$  consumption is due to  $\text{Fe}^{2+}$  that is still present at 7.2  $\mu\text{M}$  or about 36% of its original value.<sup>15</sup>

<sup>15</sup> Kelley EE, Wagner BA, Buettner GR, Burns CP. (1999) Nitric oxide inhibits iron-induced lipid peroxidation in HL-60 cells. *Arch Biochem Biophys* **370**: 97-104.  
Oxygen Society Annual Meeting, New Orleans, LA Nov 19-22, 1999

Traditionally vitamin E (TOH) has been thought to be the principal chain-breaking antioxidant in blood and in lipid structures of cells.<sup>16</sup> However, the experiments done that lead to this conclusion were done in the absence of  $\bullet\text{NO}$ .

From the data presented above in conjunction with kinetic information from the literature, it is possible to make a kinetic estimate of the importance of TOH vs  $\bullet\text{NO}$  as a chain-breaking antioxidant in our experiments with cells. A typical TOH level in cells is 1000-2000 PUFA/1 TOH.<sup>17</sup> If PUFA constitutes about 22% of the lipids in a cell membrane,<sup>18, 19</sup> then there are about 5,000-10,000 total fatty acid chains/1 TOH. As a first approximation, the mole fraction of TOH in lipid regions of membranes will be

$$\begin{aligned} \text{Mole fraction (TOH)} & \gg 1/(5,000 \text{ to } 10,000) \\ & \gg 1/7,500 \\ & \gg 1.3 \times 10^{-4}. \end{aligned}$$

Because TOH and the various fatty acids have similar properties, we assume, for simplicity, that they have the same partial molar volume in the lipid regions of cell membranes. Using a density of  $\approx 0.9$  g/mL and an average molecular weight of 300 g/mole for the fatty acyl chains of the lipids in a cell membrane, then the effective molarity of the fatty acyl chains in the lipid regions of membranes will be  $\approx 3$  M. The molarity of TOH will then be about

$$\text{M(TOH) will be } 3 \text{ M} \times 1.3 \times 10^{-4} \gg 400 \text{ nM.}$$

<sup>16</sup> Burton GW, Joyce A, Ingold KU. (1983) Is vitamin E the only lipid soluble, chain breaking antioxidant in human blood plasma and erythrocyte membranes? *Arch Biochem Biophys* **221**: 281-290.

<sup>17</sup> Kelley EE, Buettner GR, Burns CP. (1995) Relative  $\alpha$ -tocopherol deficiency in cultured tumor cells: Free radical-mediated lipid peroxidation, lipid oxidizability, and cellular polyunsaturated fatty acid content. *Arch Biochem Biophys* **319**: 102-109.

<sup>18</sup> Wagner BA, Buettner G R, Oberley LW, Burns CP. (1998) Sensitivity of K562 and HL-60 cells to edelfosin, an ether lipid drug, correlates with production of active oxygen species. *Cancer Res* **58**: 2809-2816.

<sup>19</sup> Kelley EE, Buettner GR, Burns CP. (1995) Relative  $\alpha$ -tocopherol deficiency in cultured tumor cells: Free radical-mediated lipid peroxidation, lipid oxidizability, and cellular polyunsaturated fatty acid content. *Arch Biochem Biophys* **319**: 102-109.

The rate at which  $\cdot\text{NO}$  would terminate the chain propagation reactions in lipid peroxidation by reacting with the chain-carrying peroxy radical,  $\text{LOO}\cdot$ , will be:

$$\text{rate inhibition } (\cdot\text{NO}) = k_{\text{NO}} [\cdot\text{NO}] [\text{LOO}\cdot]$$

where  $k_{\text{NO}} = 2 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ <sup>20</sup> and  $[\cdot\text{NO}] = 8 \times 45 \text{ nM} = 360 \text{ nM}$ . Here 8 is the estimated membrane/water partition coefficient for  $\cdot\text{NO}$ ;<sup>21</sup> 45 nM is the measured  $\cdot\text{NO}$  concentration in the aqueous phase of our cell suspension during the inhibition phase of lipid peroxidation. Thus

$$\text{rate inhibition } (\cdot\text{NO}) = 2 \times 10^9 \text{ M}^{-1}\text{s}^{-1} \cdot 360 \cdot 10^{-9} \text{ M} [\text{LOO}\cdot]$$

The rate at which TOH would terminate these reactions would be:

$$\text{rate inhibition } (\text{TOH}) = k_{\text{TOH}} [\text{TOH}] [\text{LOO}\cdot]$$

where  $k_{\text{TOH}} = 8 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$ <sup>23</sup> and  $[\text{TOH}] = 400 \text{ }\mu\text{M}$ . Thus, rate inhibition (TOH) =  $8 \times 10^4 \text{ M}^{-1}\text{s}^{-1} \times 400 \times 10^{-6} \text{ M} [\text{LOO}\cdot]$  The ratio of these rates is

$$\text{rate } (\cdot\text{NO})/\text{rate } (\text{TOH}) = 20/1$$

in these experiments.

<sup>20</sup> Padmaja S, Huie RE. (1993) The reaction of nitric oxide with organic peroxy radicals. *Biochem Biophys Res Commun* **195**: 539-544.

<sup>21</sup> Rubbo H, Radi R, Trujillo M, Telleri R, Kalyanaraman B, Barnes S, Kirk M, Freeman BA. (1994) Nitric oxide regulation of superoxide and peroxynitrite-dependent lipid peroxidation. Formation of novel nitrogen-containing oxidized lipid derivatives. *J Biol Chem* **269**: 26066-26075.

<sup>22</sup> Liu X, Miller MJS, Joshi M S, Thomas DD and Lancaster JR Jr. (1998) Accelerated reaction of nitric oxide with O<sub>2</sub> within the hydrophobic interior of biological membranes. *Proc Natl Acad Sci USA* **95**: 2175-2179.

<sup>23</sup> Buettner GR. (1993) The pecking order of free radicals and antioxidants: Lipid peroxidation,  $\alpha$ -tocopherol, and ascorbate. *Arch Biochem Biophys* **300**: 535-543.



Comparing rate constants for the reaction of  $\cdot\text{NO}$  and TOH with lipid peroxy radicals we have:

In fact, if the concentration and kinetic parameters used to make this estimate were to accurately represent these processes in cells, then an aqueous concentration of  $\cdot\text{NO}$  of only 2 nM would provide antioxidant protection for membrane lipids equal to that of vitamin E. This is a remarkably low concentration of  $\cdot\text{NO}$  and is easily achievable in many cells and tissues.

When comparing estimated rate constants

$$k_{\text{NO}} = 2 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{\text{TOH}} = 8 \cdot 10^4 \text{ M}^{-1}\text{s}^{-1}$$

$$k_{\text{NO}} / k_{\text{TOH}} = 2.5 \cdot 10^4$$

No matter how you slice it,  $\cdot\text{NO}$  appears to be a great antioxidant.