

# Metabolism of Trichloroethylene & Covalent Binding of Reaction Products

**F. P. Guengerich**

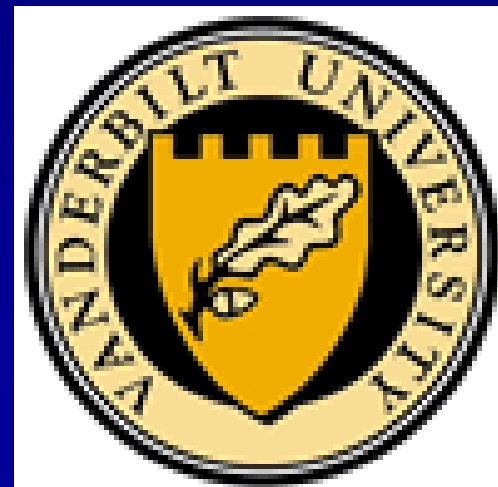
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<http://www.toxicology.mc.vanderbilt.edu/CenterInvest/guengerich>

<http://isihighlycited.com/author.cgi?&link1=Browse&link2=Results&id>

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# Trichloroethylene in Dickson (TN), 2003

<http://www.toxicology.mc.vanderbilt.edu/TCE>

## Recent Articles on TCE in Dickson

[Chemicals, illnesses hard to link, federal agency says](#)

[Family blames health woes on Dickson's landfill](#)

[Water assurances were lies, commissioners told](#)

[Dickson landfill area will be warned](#)

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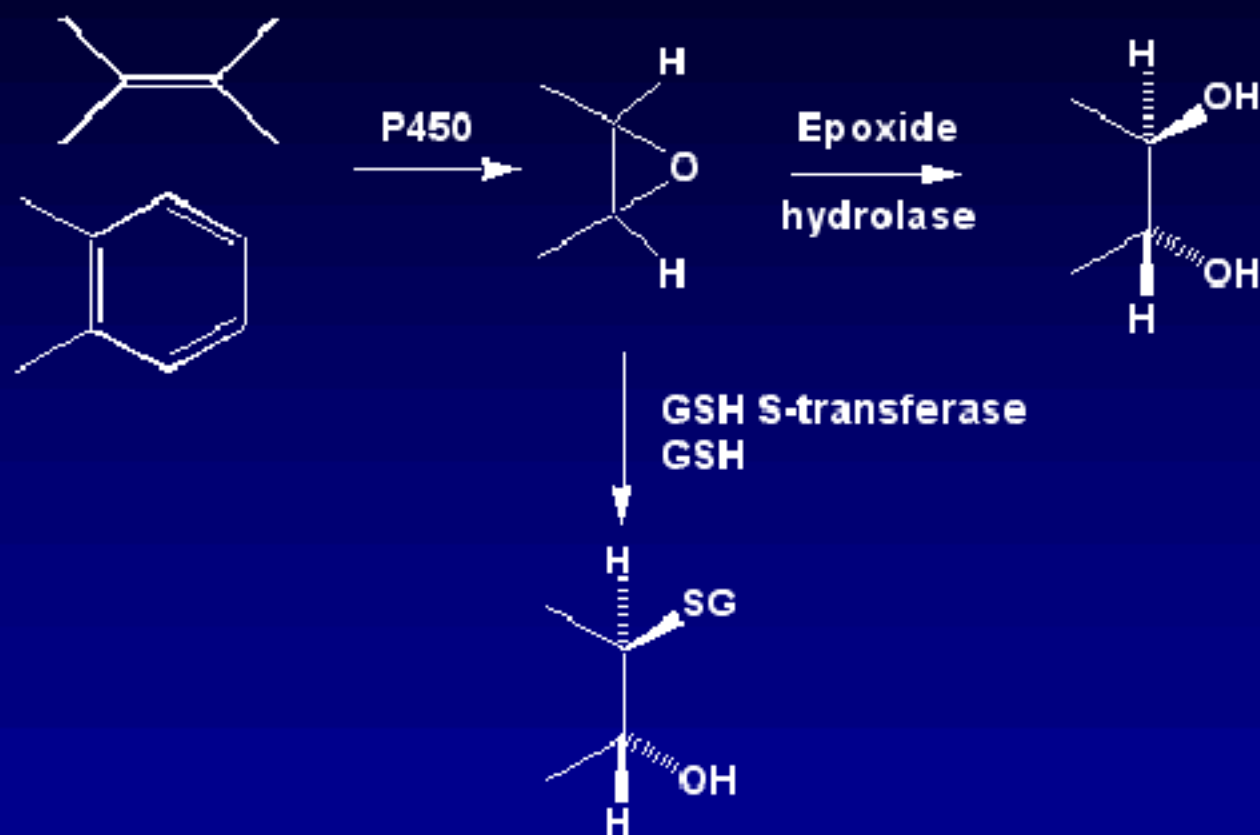
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# Enzymatic formation & reactions of epoxides









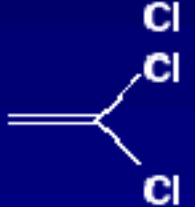

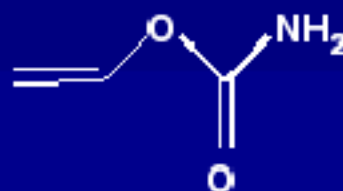
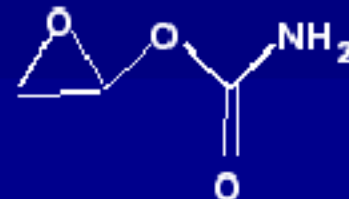


# Epoxide stability: relation to biological activity

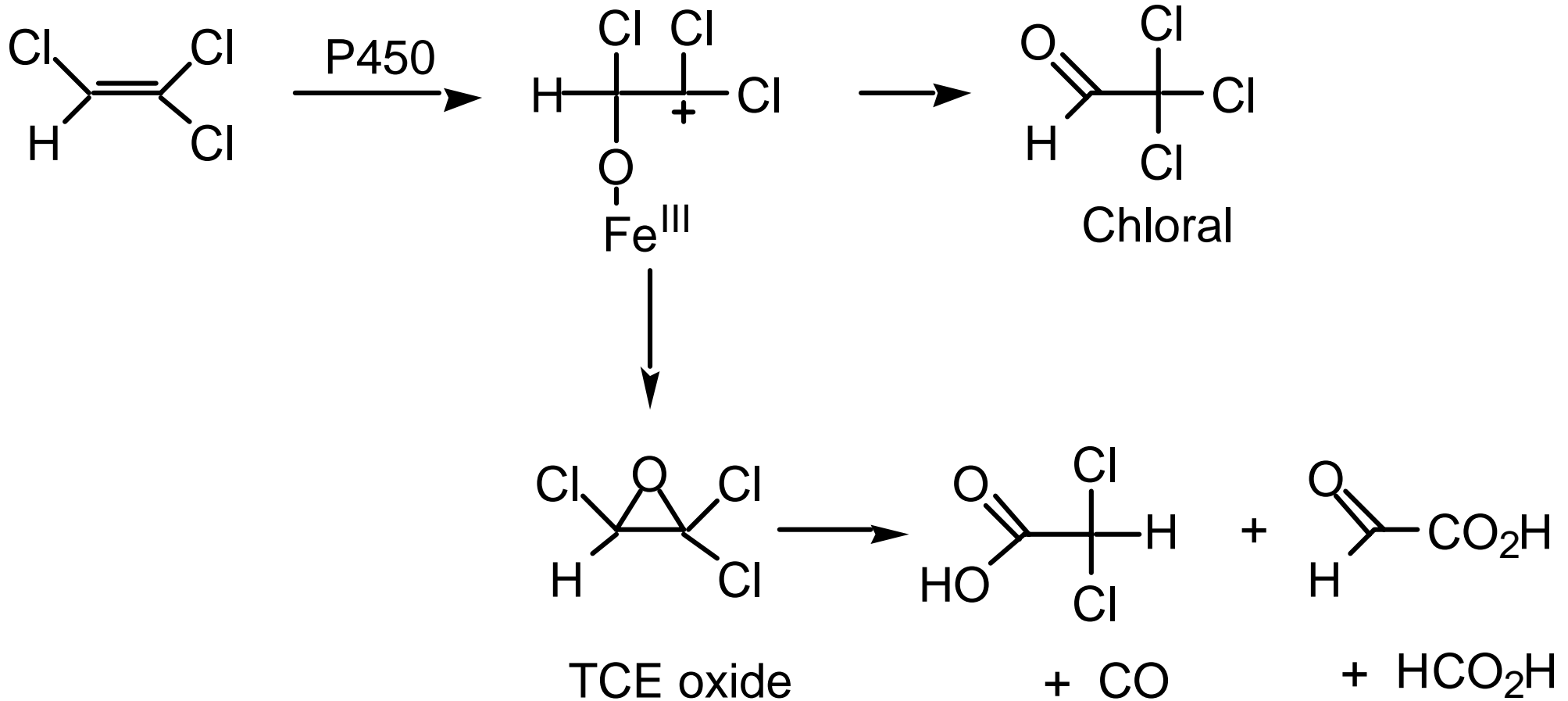
Drinkwater *et al.* (1978) *Cancer Res.* 38, 3247-3255

	$t_{1/2}$ , min	Conc. needed to unwind superhelical DNA, mM	<i>S. typh.</i> TA98 revertants/ nmol
Benzo[a]pyrene diol epoxide	38	0.023	460
1-Phenyloxirane	46	0.027	540
9-Methyl-10-anthryloxirane	21	0.10	110
6-Chrysenyloxirane	141	0.22	70
9-Anthryloxirane	172	0.24	53
9-Phenanthryloxirane	140	0.9	25
2-Benzanthryloxirane	115	1.1	20
2-Naphthyloxirane	103	>2	3.0
Styrene oxide	340	>3	0.001

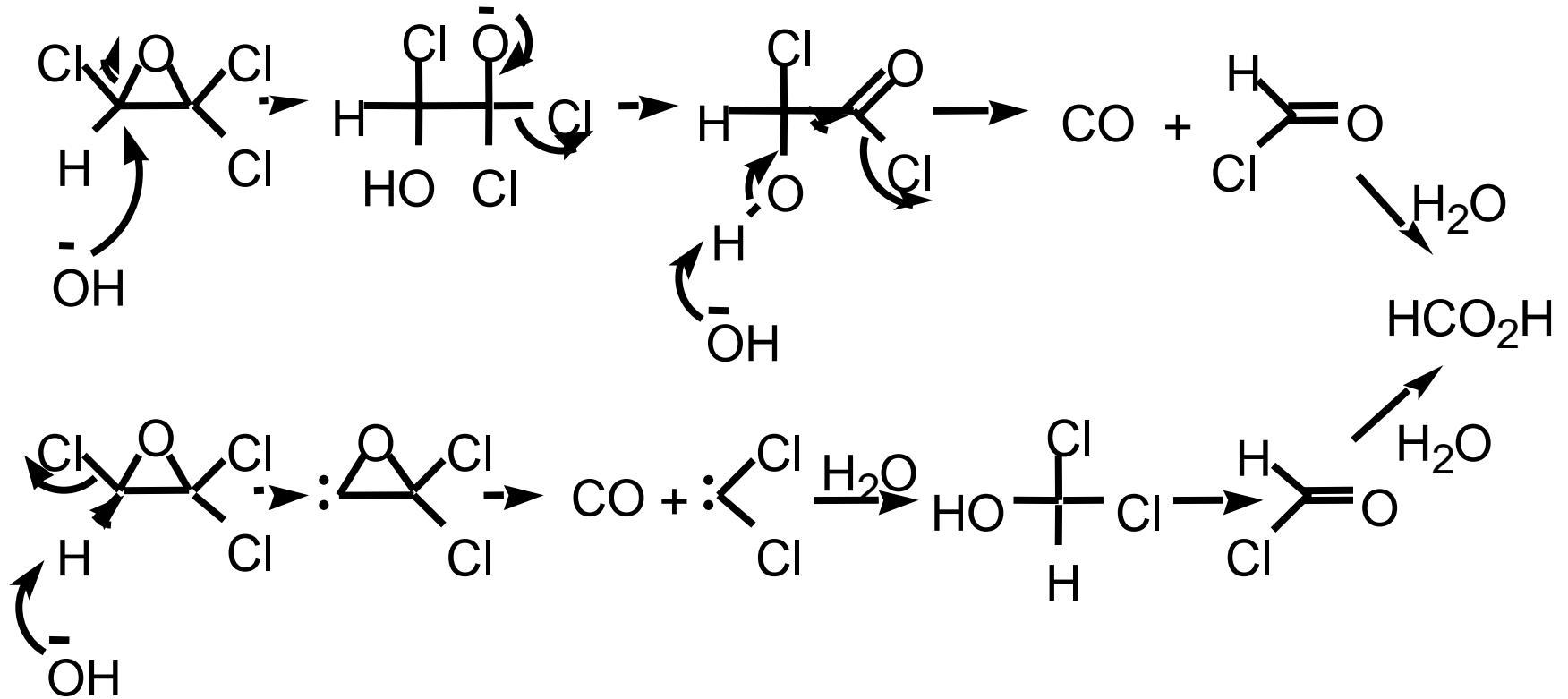
# Stabilities of some epoxides of relevance in toxicology & cancer

		<u><math>t_{1/2}</math>, s (23° C)</u>
		7200
		90
		40
		12
		~2
		60
Aflatoxin B <sub>1</sub>	Aflatoxin B <sub>1</sub> 8,9oxide ( <i>exo</i> )	1

# Oxidation of trichloroethylene (TCE) by P450

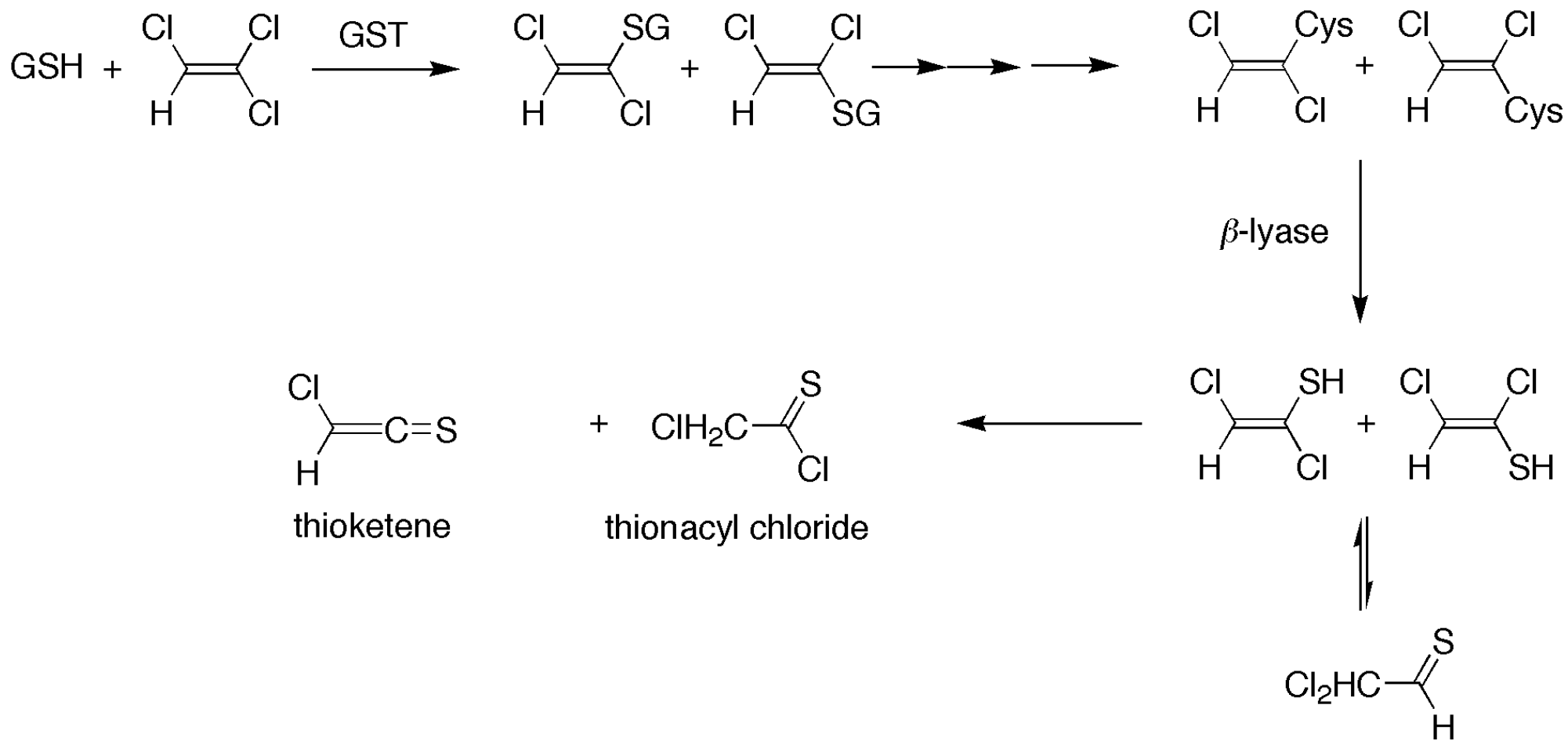


## Some possible mechanisms for scission of TCE oxide





## Activation of TCE by GSH conjugation



## Carcinogenic Substrates for Human P-450 Enzymes

### P-450 1A1

B(a)P

### P-450 1A2

2-NH<sub>2</sub> anthracene  
2-AAF  
2-NH<sub>2</sub> fluorene  
4-NH<sub>2</sub> biphenyl  
2-Naphthylamine  
Glu P-1  
Glu P-2  
IQ  
MeIQ  
MeIQx  
PhIP  
Trp P-2  
NNK

### P-450 2E1

Me<sub>2</sub>N-N=O  
Et<sub>2</sub>N-N=O  
Me, propyl N-N=O  
Me, benzyl N-N=O  
Vinyl Cl  
Vinyl Br  
Acrylonitrile  
Vinyl carbamate  
Urethan  
Styrene  
Benzene  
CCl<sub>4</sub>  
CHCl<sub>3</sub>  
Trichloroethylene

### P-450 3A4

Aflatoxin B<sub>1</sub>  
Aflatoxin G<sub>1</sub>  
Sterigmatocystin  
B(a)P-7,8-diol  
BA-3,4-diol  
DMBA-3,4-diol  
BFA-9,10-diol  
tris(2,3-Br<sub>2</sub> propyl)PO<sub>4</sub>  
6-NH<sub>2</sub> chrysene  
MOCA  
Senecionine

# Variability in human enzymes (expression level/activity)

- P450 2E1 & GST T1-1 (CH<sub>2</sub>Cl<sub>2</sub> liver & lung cancers)
  - Kirman et al. (1999) *Toxicologist* **48**, 83 & Rish et al. (1999) *Toxicologist* **48**, 143
  - Issues: Which in vitro parameters to use ( $V_{max}$ ,  $K_m$ ,  $V_{max}/K_m$ )?
    - Lack on information regarding extent of variation in extrahepatic human tissues (e.g. lung, brain)

More recent drug studies (using *in vivo* clearance)

Doren et al. (2002) *Food Chem. Toxicol.* **40**, 1633-1656

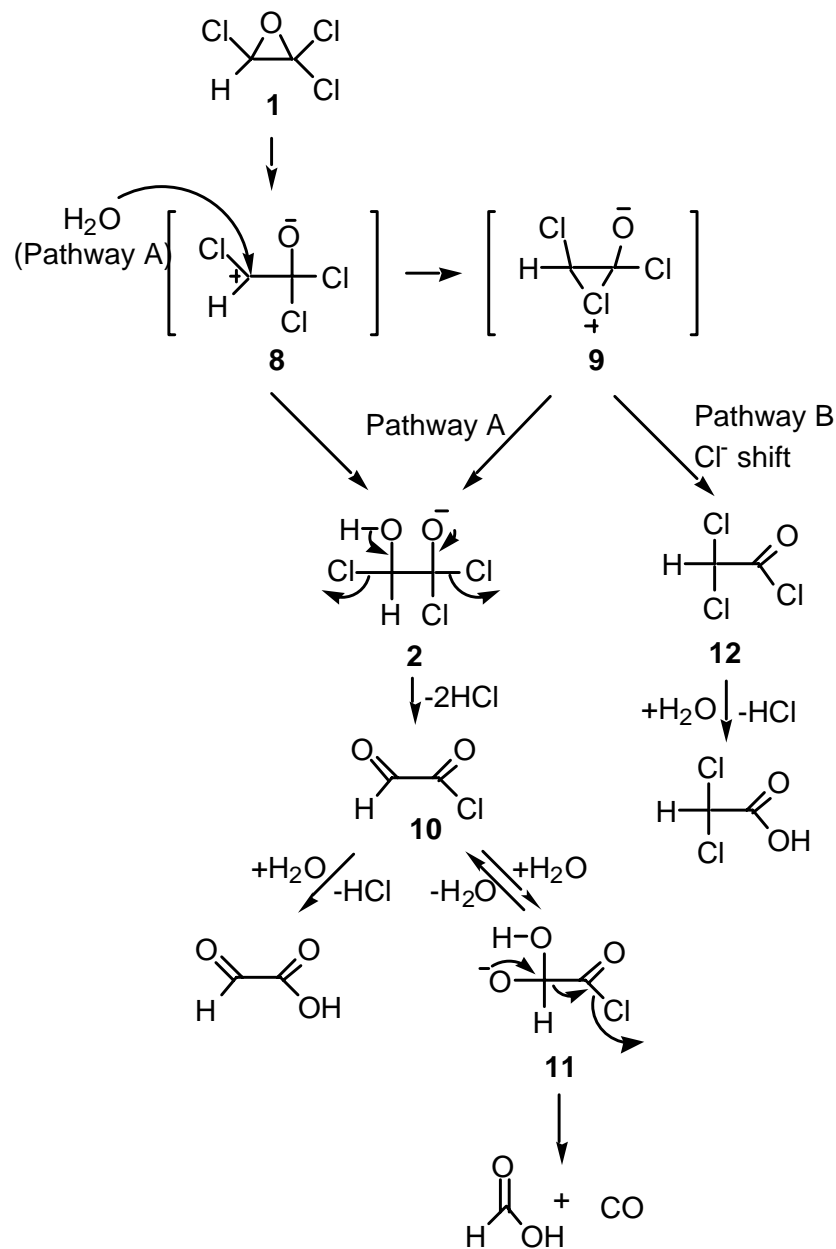
	<u>Uncertainty factor</u>
P450 2D6: non-phenotyped adults, EMs	2.7-4.1
PMs	15-18
Children	22-45

Doren et al. (2003) *Food Chem. Toxicol.* **41**, 201-224

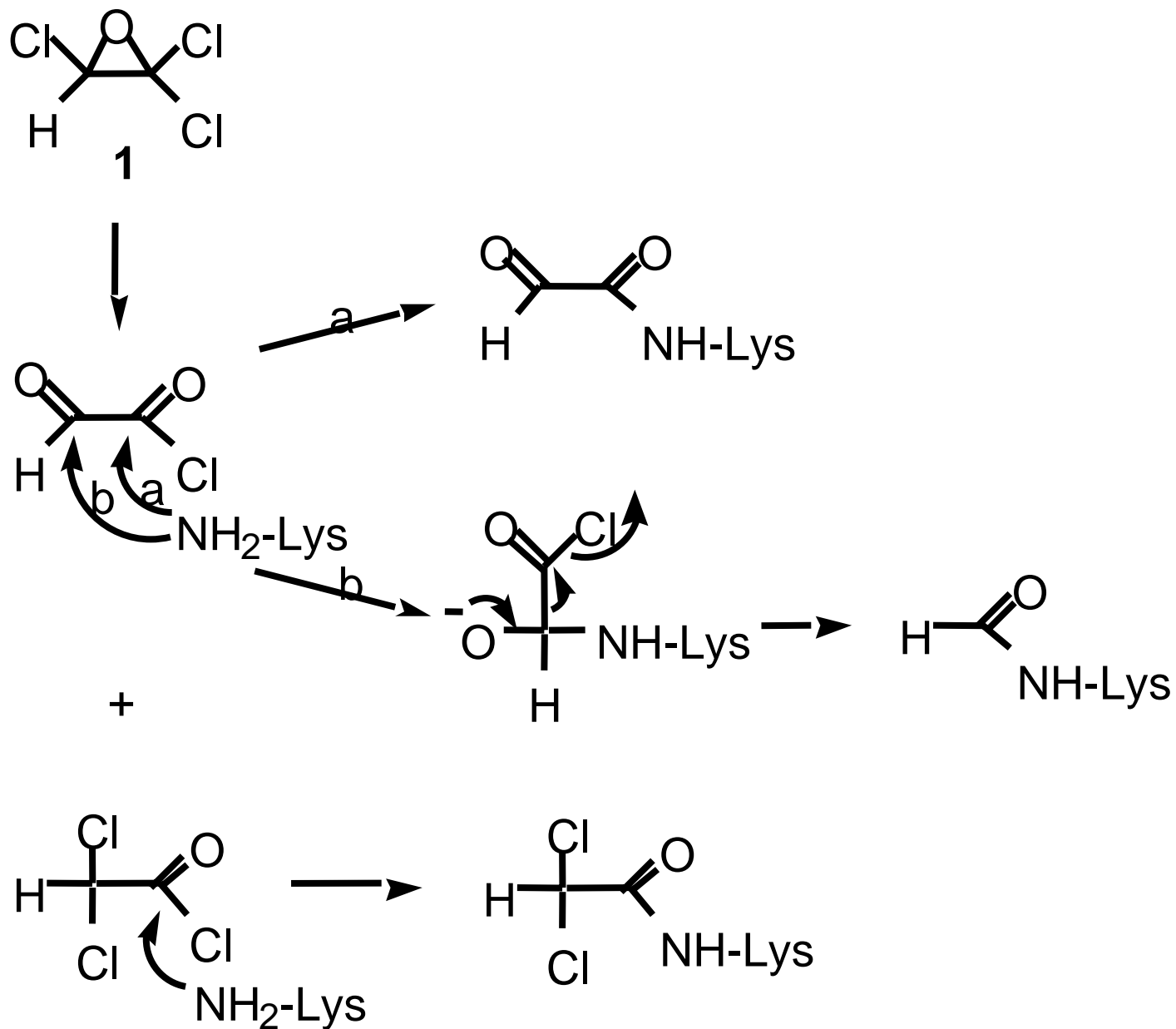
P450 3A4:	Adults	3.2
	Children	12

Factors increase with increase in fraction of metabolism due to a single enzyme (exponential relationship,  $r^2 = 0.8$ )

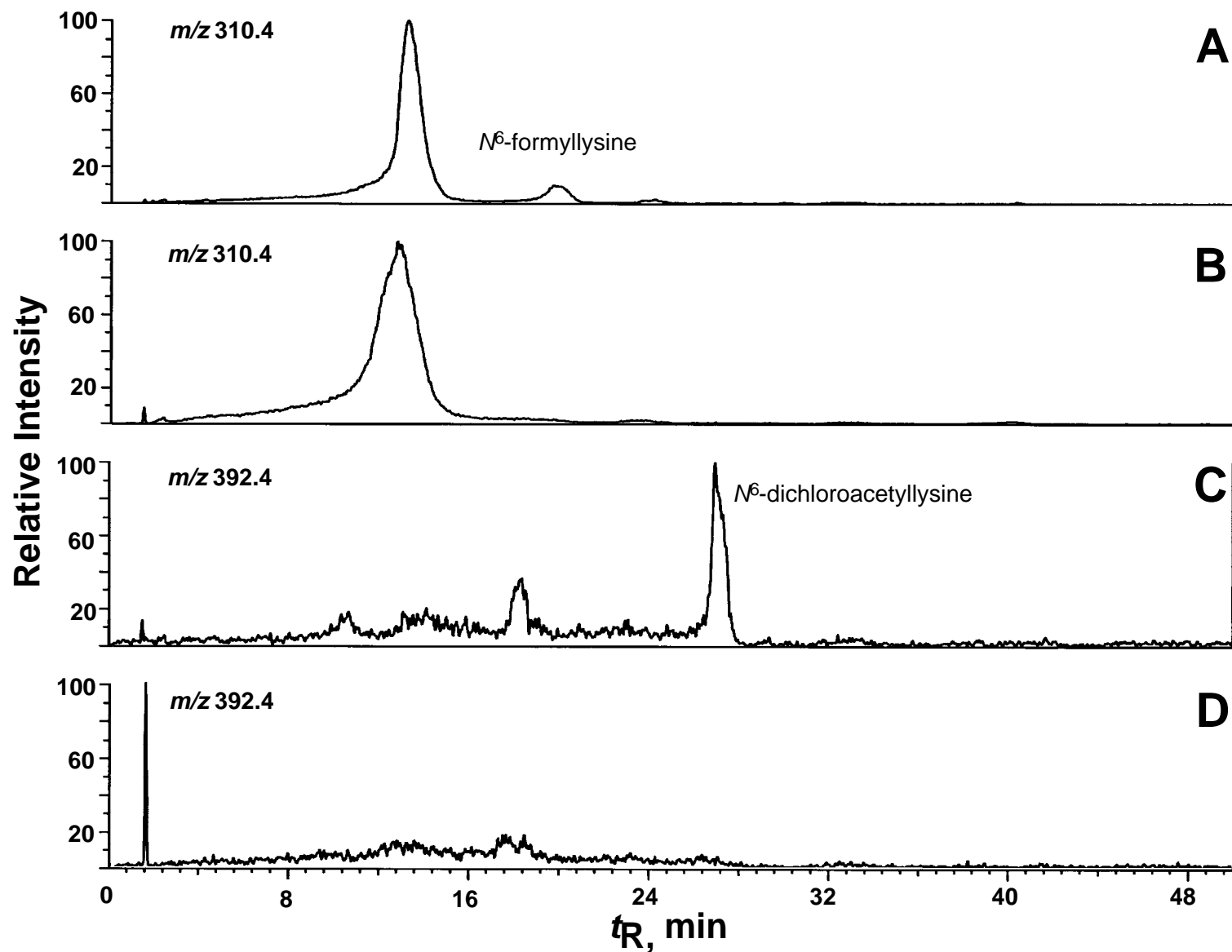
# Mechanisms of hydrolytic decomposition of TCE oxide (determined by $^{18}\text{O}$ & $^2\text{H}$ labeling & mass spectrometry)



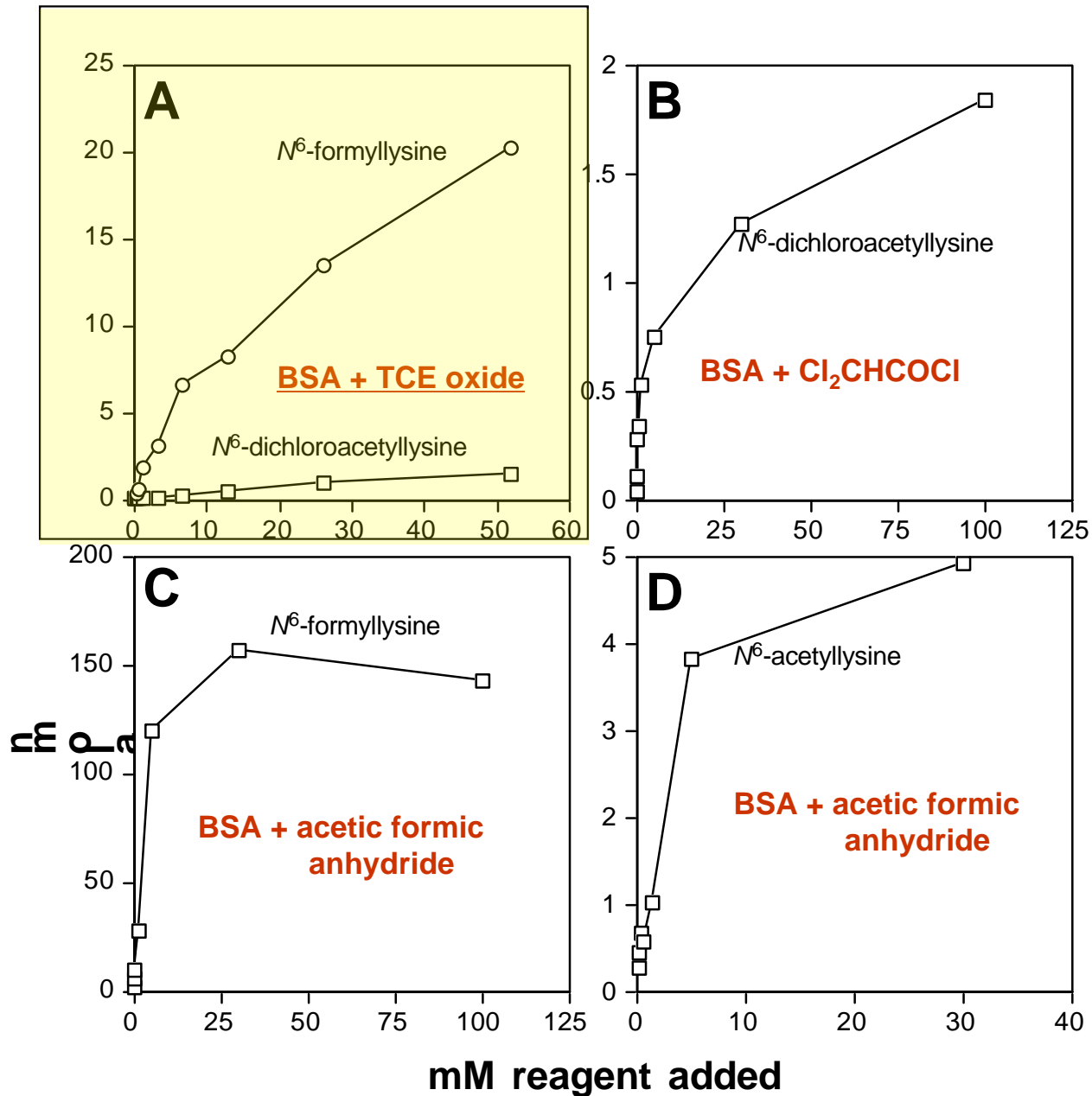
# Mechanisms of reaction of TCE oxide with lysine (determined by $^{18}\text{O}$ & $^2\text{H}$ labeling & mass spectrometry)



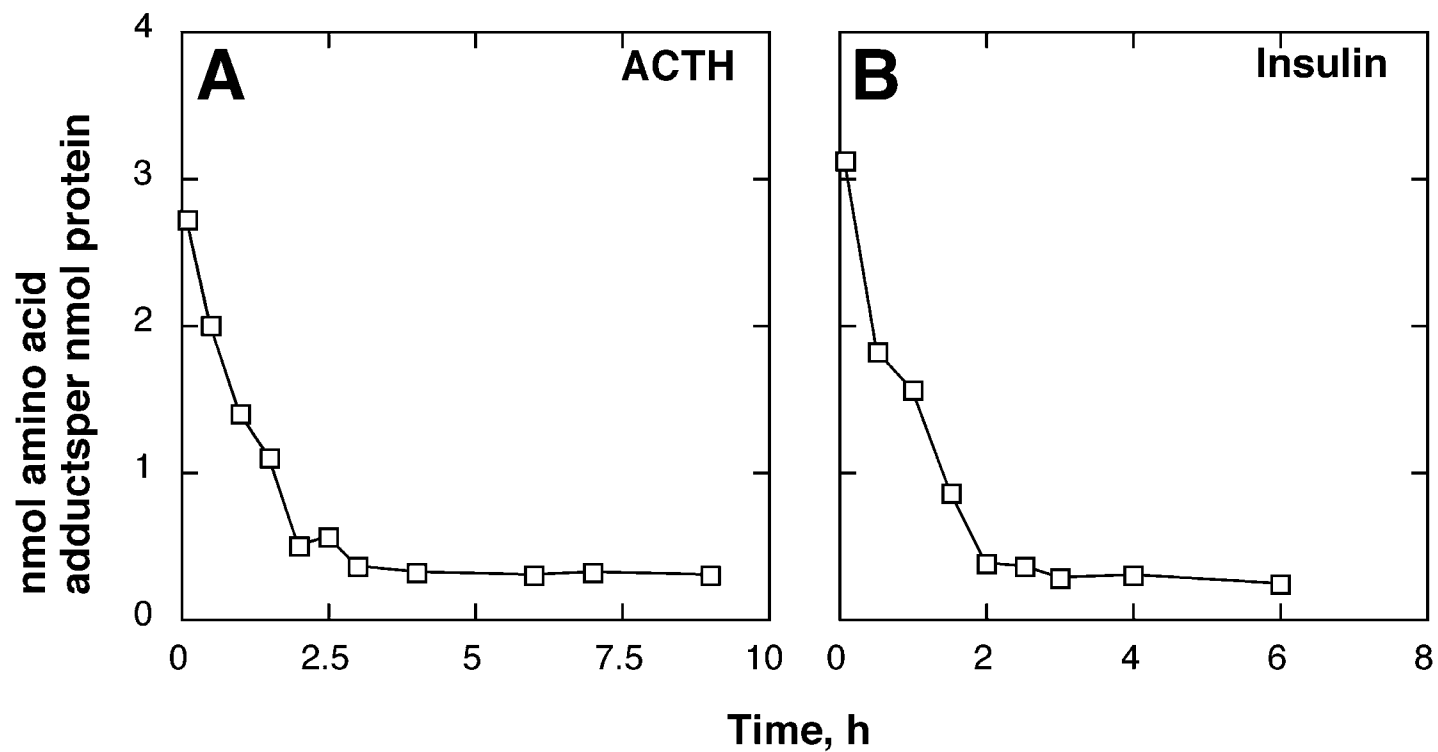
# The products of reaction of TCE oxide with lysine are predominantly formyl adducts



# The products of reaction of TCE oxide with lysine in bovine serum albumin (BSA) are predominantly formyl adducts (part A)



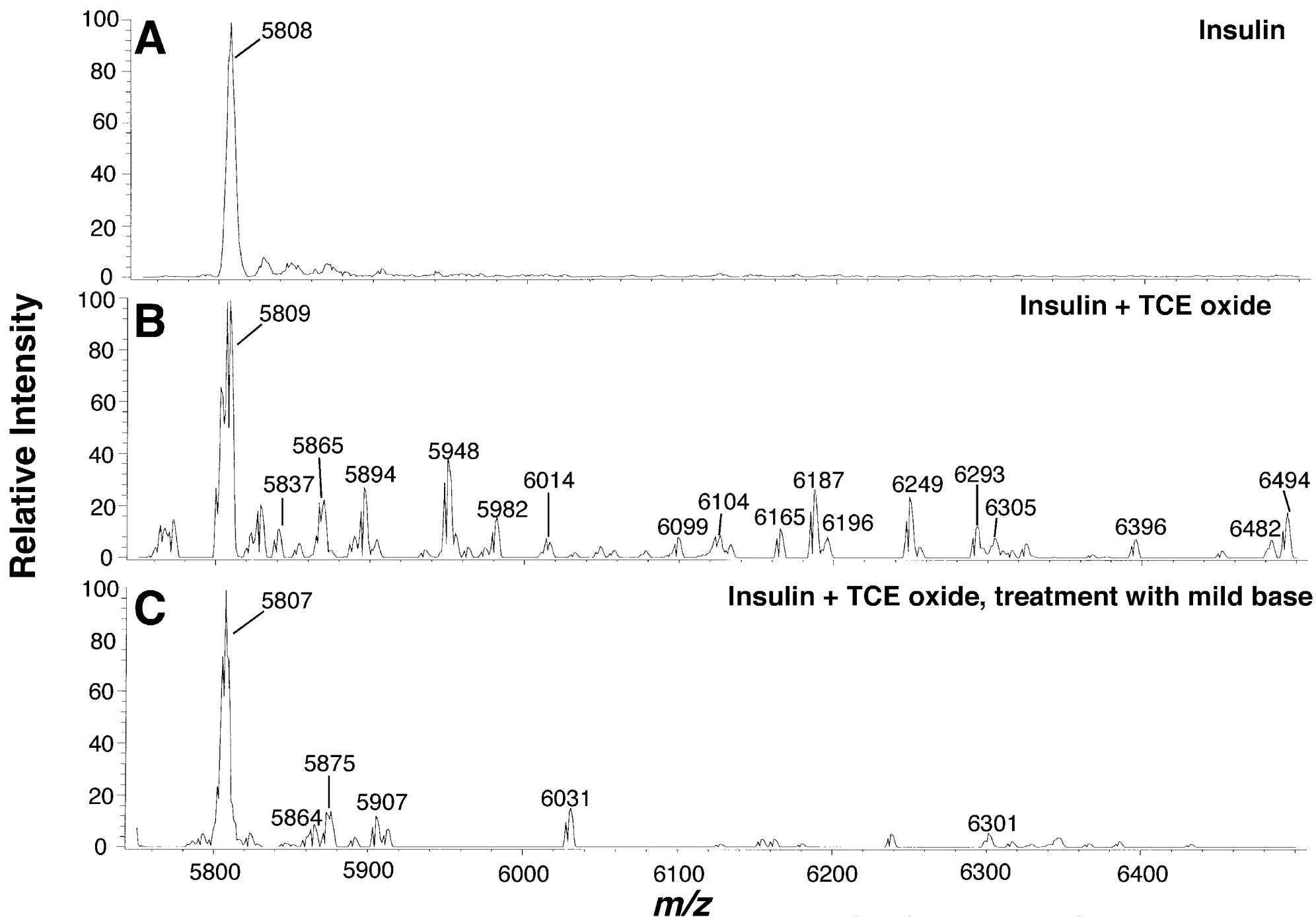
## Loss of Protein Adducts Formed from Reactions of Insulin or ACTH (1-24) with TCE Oxide at pH 7



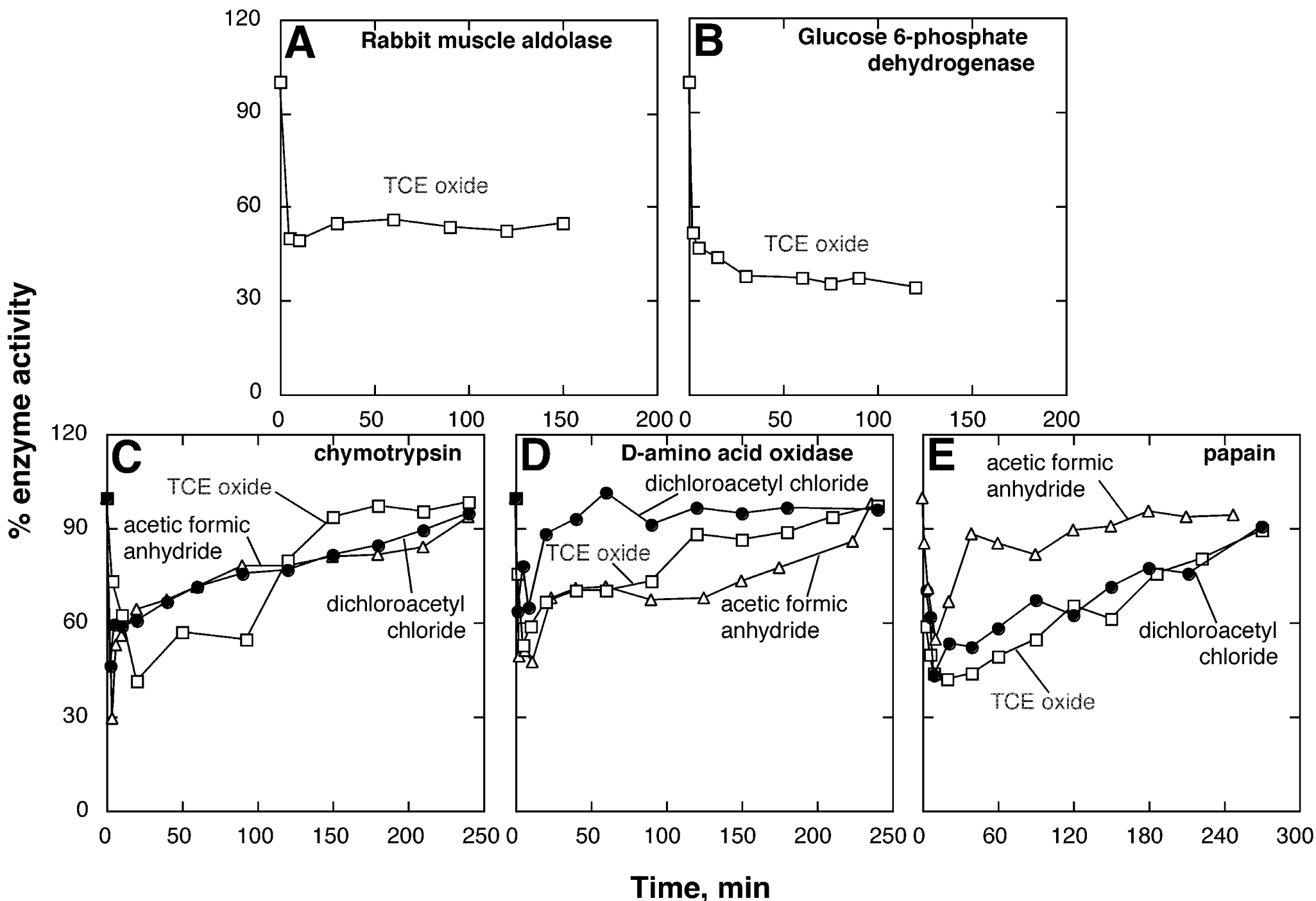
Cai & Guengerich (2001) *Chem. Res. Toxicol.* **14**, 54-61



# Reaction of TCE oxide with small proteins: insulin

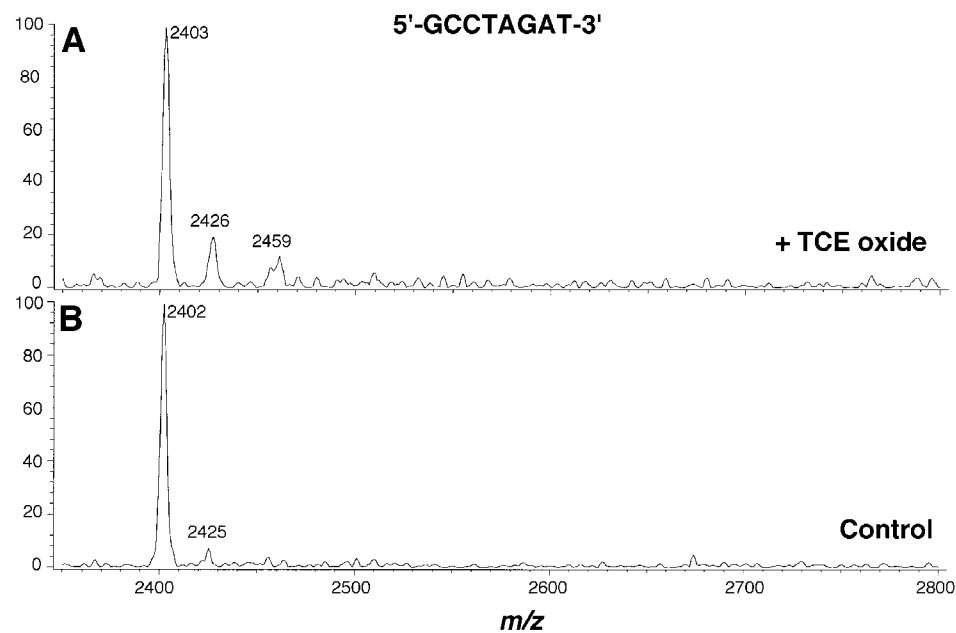


# Time Course of Enzyme Activity of Adducted Proteins

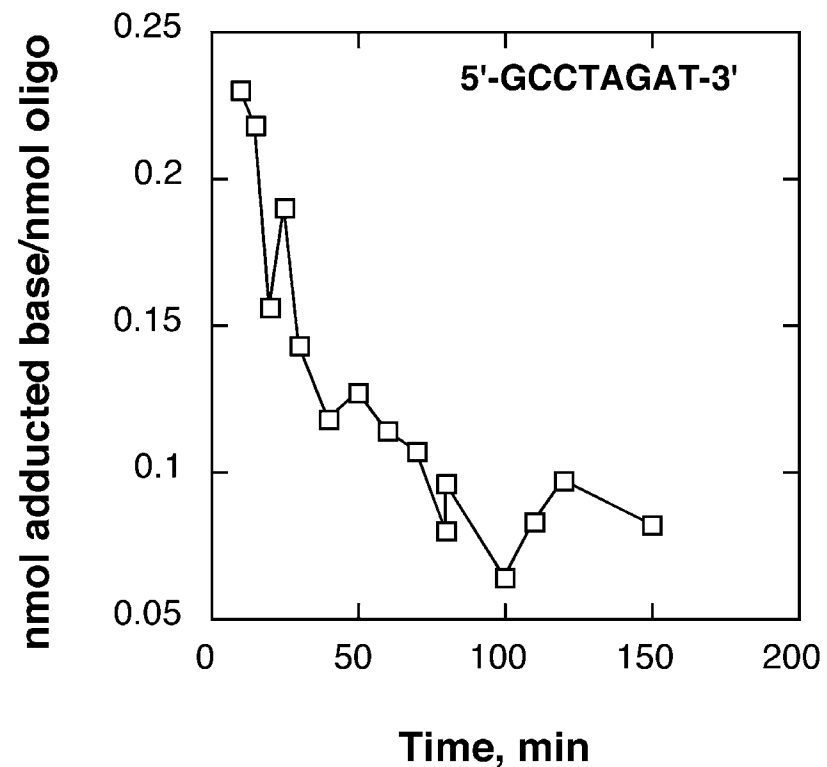


# TCE Oxide-Derived 8-mer Oligonucleotide Adducts

## Reaction

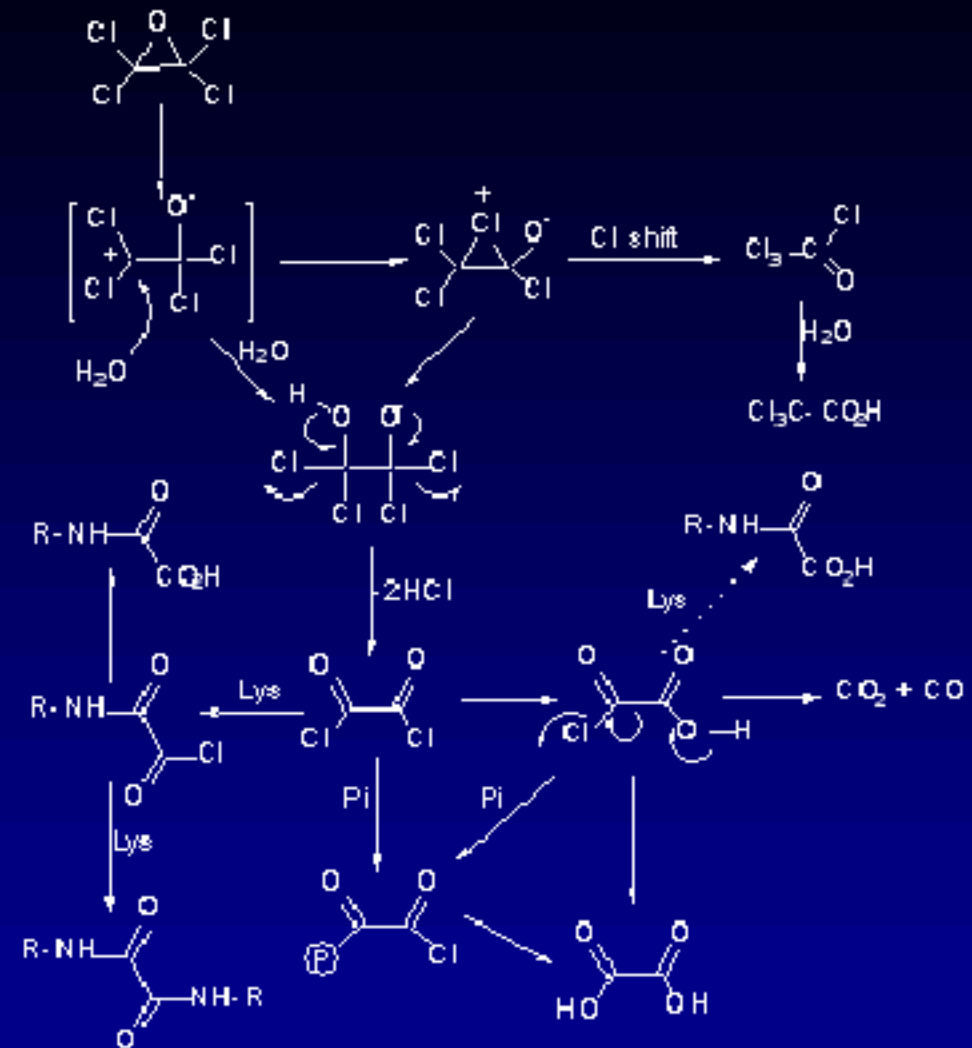
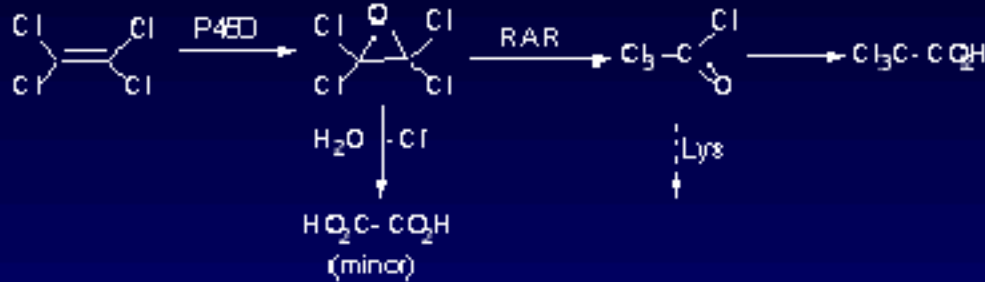


## Stability

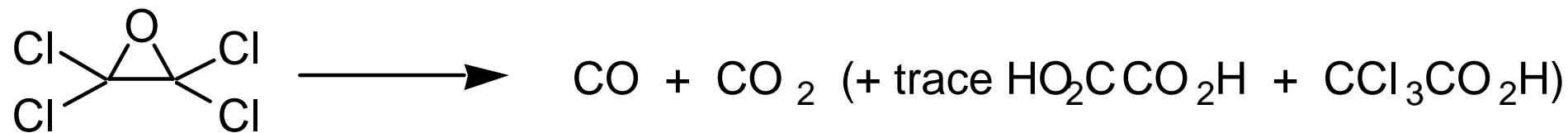
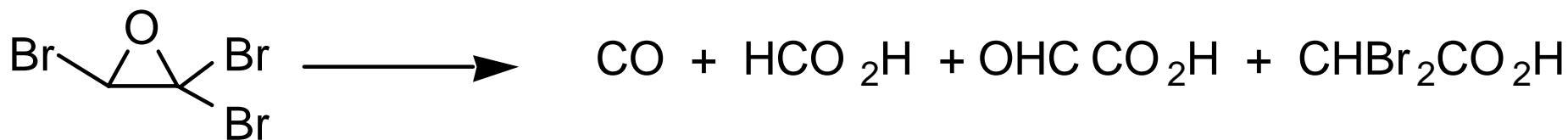
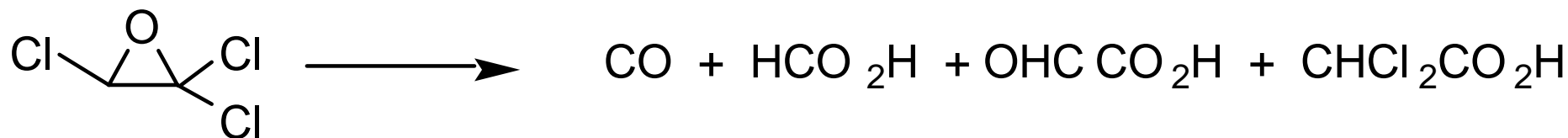
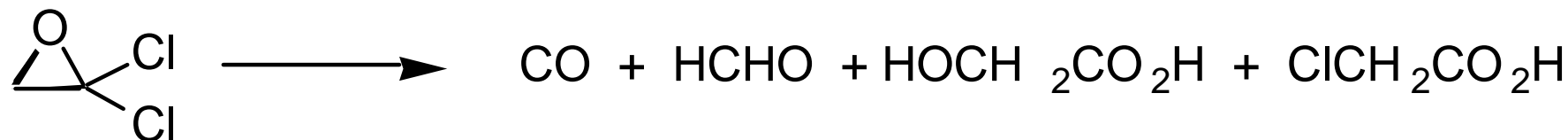
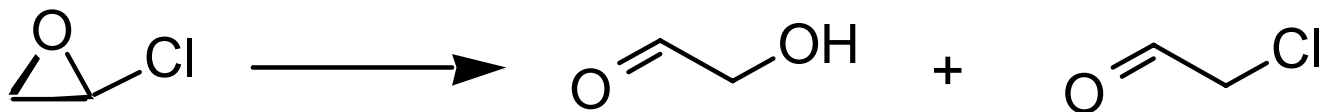


Revised scheme for reactions of perchloroethylene oxide

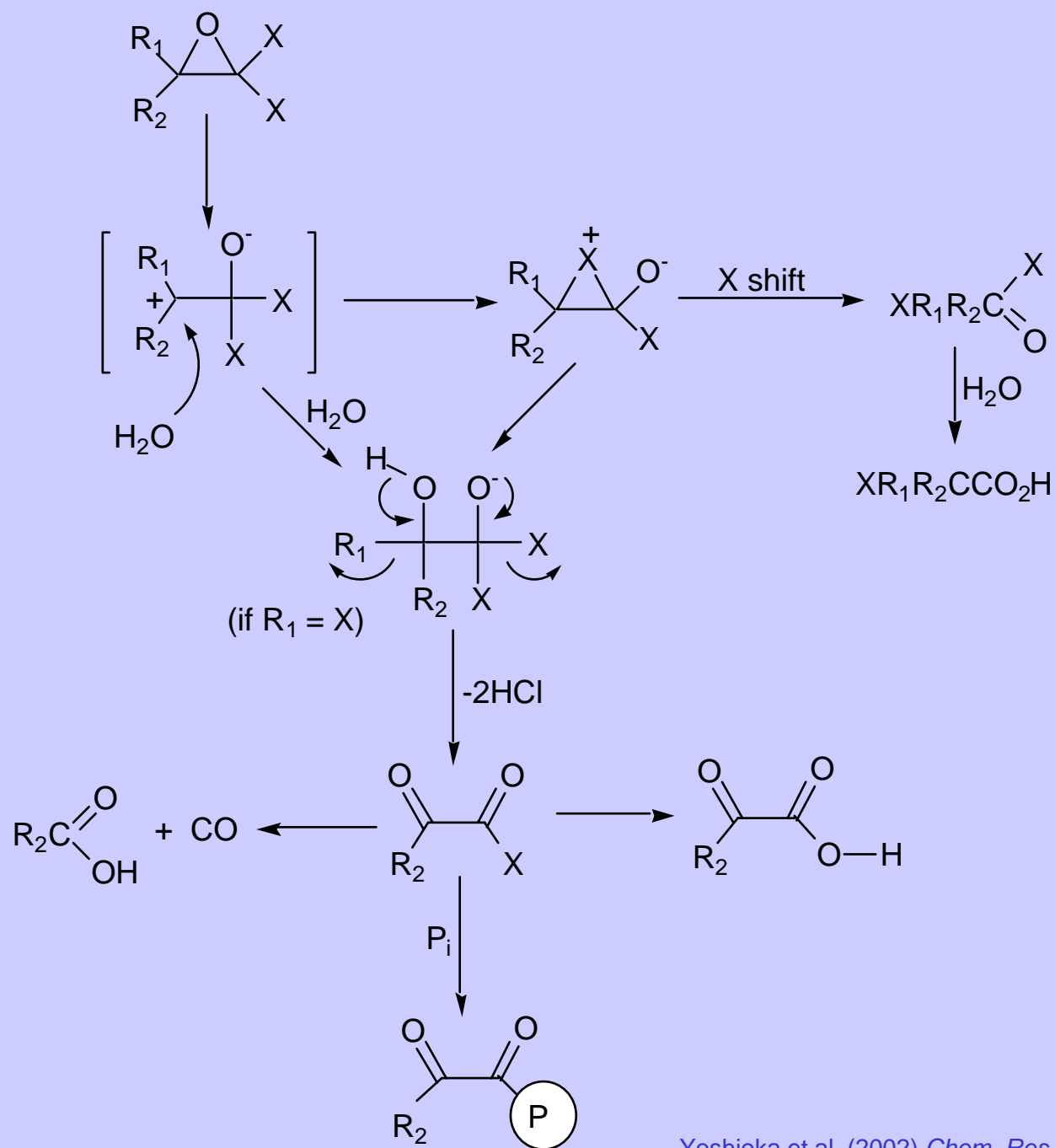
Literature scheme for formation & reactions of tetrachloroethylene (perchloroethylene)oxide  
 e.g., Rebertus (1963) *Mutation Res.* 123, 411-429



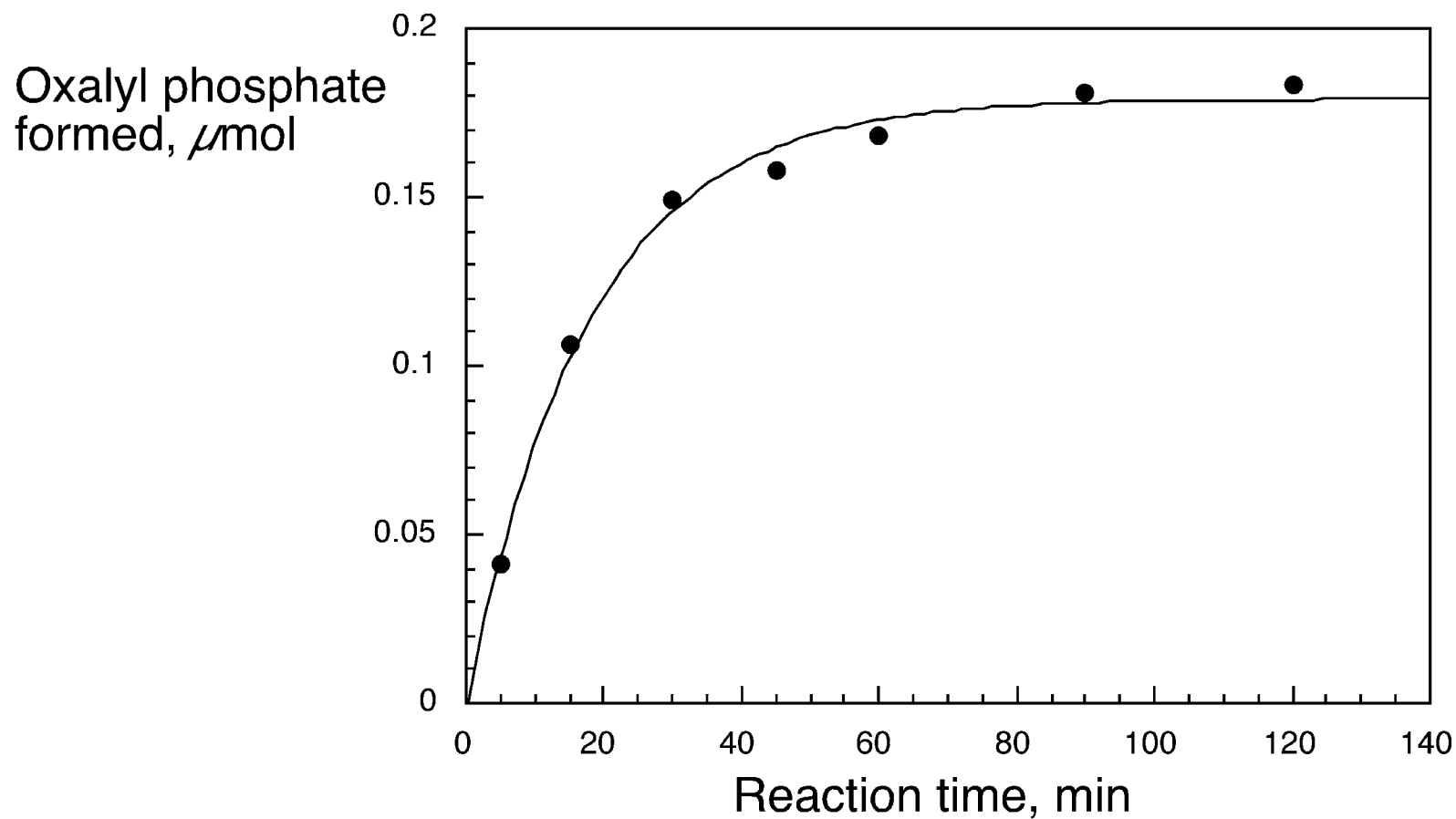
# Generality of Halooxirane Hydrolysis



# Generality of Halooxirane Hydrolysis



## Reaction of Perchloroethylene Oxide with Phosphate Buffer to Form Oxalyl Phosphate



## Summary points

- P450 (2E1) + TCE  $\longrightarrow$  mainly chloral, some epoxide
- TCE epoxide  $\longrightarrow$  acyl halide  $\longrightarrow$  reaction with nucleophiles
- Protein reactions: Lys, -OH (Ser, Thr, Tyr) (& phosphate buffer)
- Issue of semi-stable protein adducts
  - Can affect biological activity transiently
  - Does their existence raise or lower risk?
- PBPK models
  - Should not be based on epoxide  $\longrightarrow$  chloral
  - Consider human variation in activation process



Hongliang Cai



**You didn't just join a lab—it's a fraternity!**

Also thanks to T. Yoshioka for PCE & TBE work  
& to NIH (R35 CA44353, P30 ES00267, R01 ES10546)

