

The Role of Depurinating DNA Adducts in the Initiation of Cancer by Estrogens

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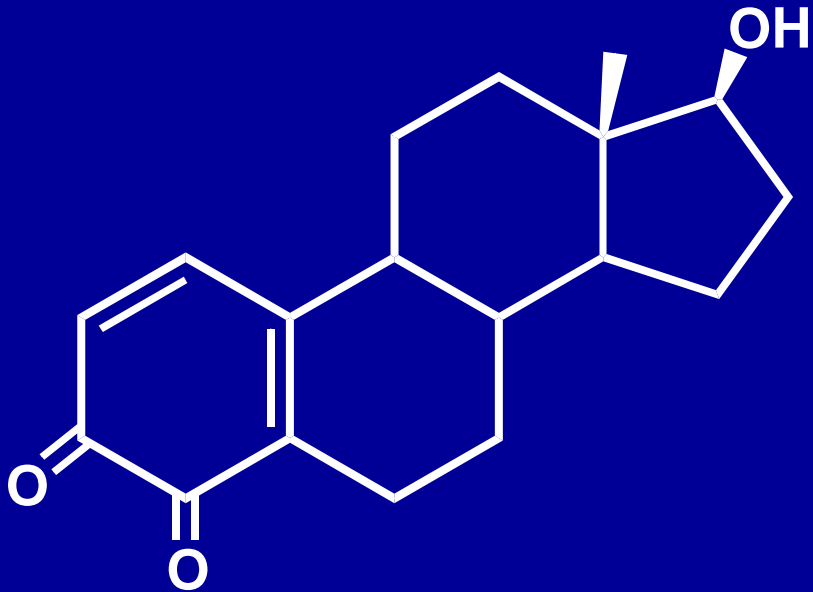
Naphthalene State-of-the-Science Symposium
Monterey, California – October 9-12, 2006

“The conversion of a normal cell to a cancer cell is the result of a mutation, a permanent genetic change in that cell”.

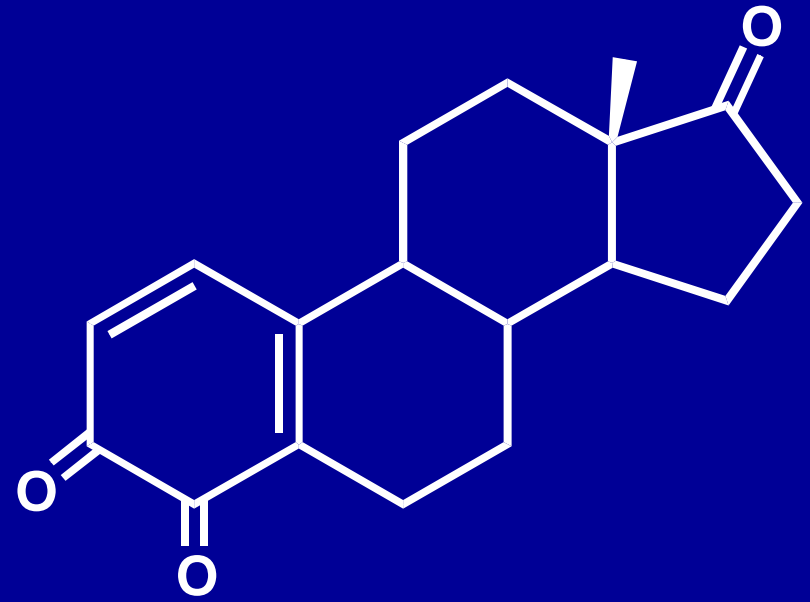
**D.M. Prescott, A.S. Flexer
CANCER, The Misguided Cell**

The central question is...

“Where do these cancer-causing mutations come from?”



Estradiol-3,4-Quinone



Estrone-3,4-Quinone

Paradigm 1: Through receptor-mediated processes, estrogens increase cell proliferation, favoring mutations that lead to cancer.

Paradigm 2: Specific oxidative metabolites of estrogen, i.e., catechol estrogen quinones, can be endogenous carcinogens that cause the mutations leading to cancer initiation.

Understanding the mechanism of the origin of these mutations opens the door to strategies for controlling and preventing cancer.

Outline

Hypothesis

Depurinating DNA adducts

Metabolism, DNA adducts and carcinogenicity of estrogens

Reactivity of estrogen quinones with DNA

Mutations induced by 4-OHE₂ and E₂-3,4-Q

Biomarkers of susceptibility to breast and prostate cancer

Conclusions

Outline

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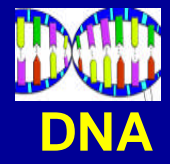
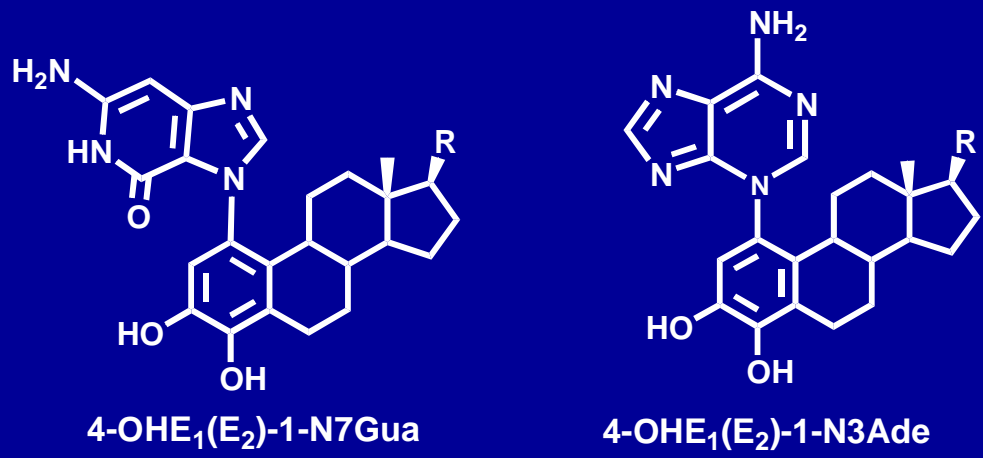
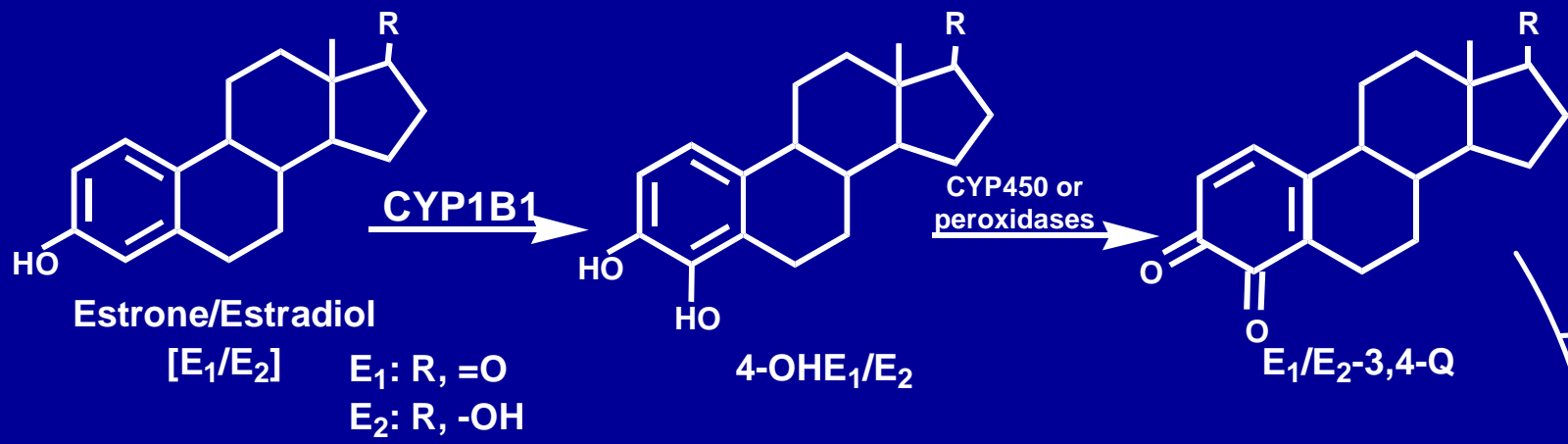
Mutations induced by 4-OHE₂ and E₂-3,4-Q

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Endogenous estrogens are metabolized to catechol estrogen-3,4-quinones that can react with DNA to generate the mutations that initiate breast, prostate and other cancers.



Depurinating adducts

Apurinic sites ← **Error-prone base excision repair**

Mutations → → → **Cancer**

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➤ **Depurinating DNA adducts**

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Biomarkers of susceptibility to breast and prostate cancer

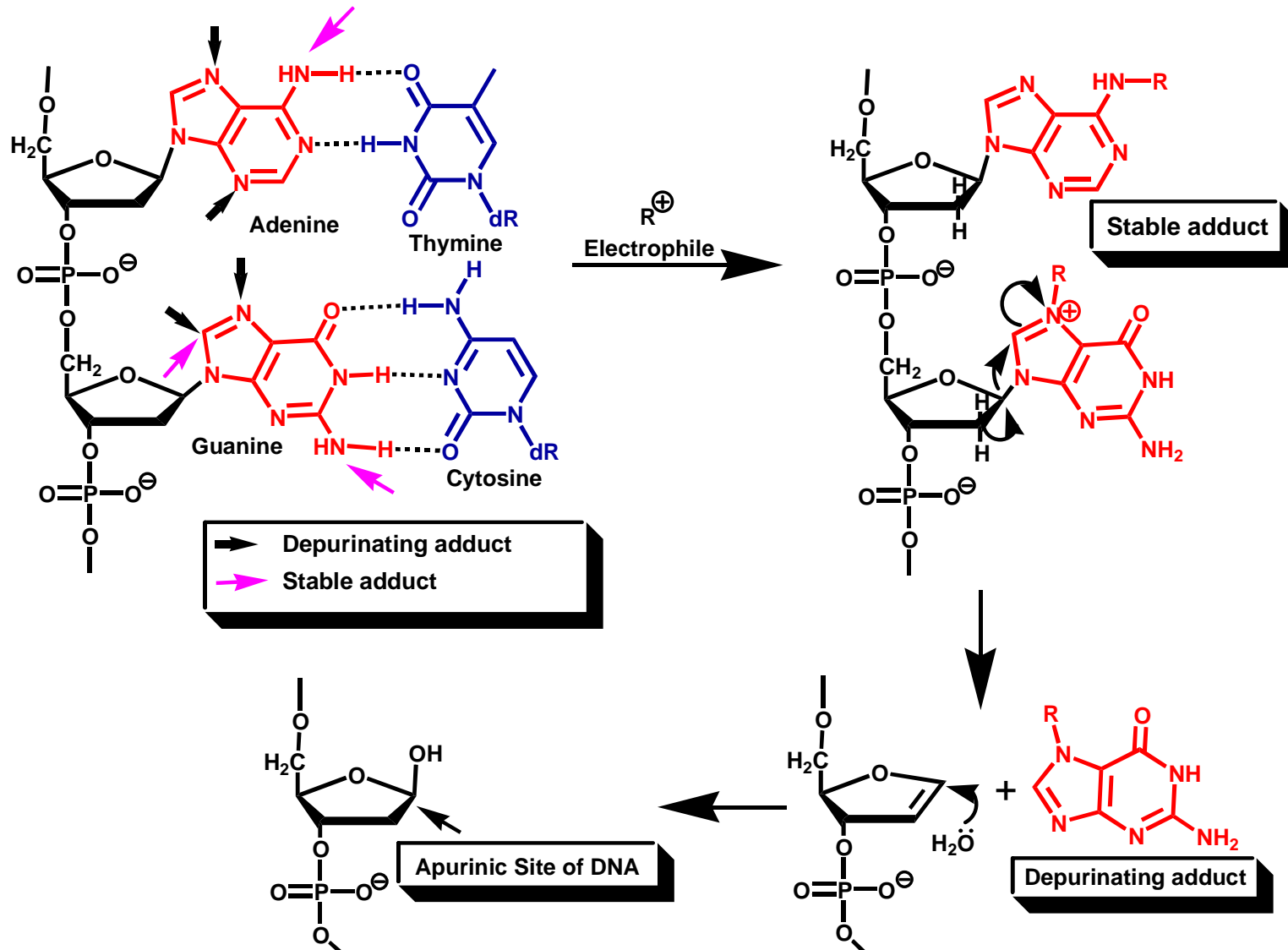
Conclusions

Carcinogens + DNA →

Stable Adducts and

Depurinating Adducts

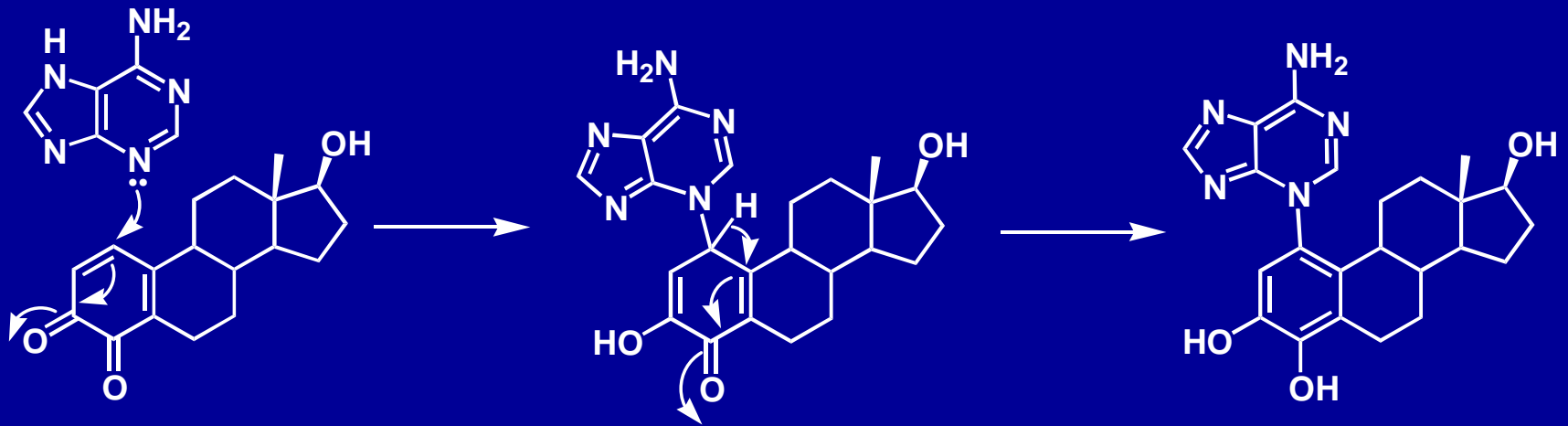
Formation of Stable & Depurinating DNA Adducts & Generation of Apurinic Sites



Depurinating adducts play the major role in the mutations that lead to cancer.

1,4-Michael Addition

Adenine



Estradiol-3,4-quinone

4-Hydroxyestradiol
-1-N3Adenine

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Depurinating DNA adducts

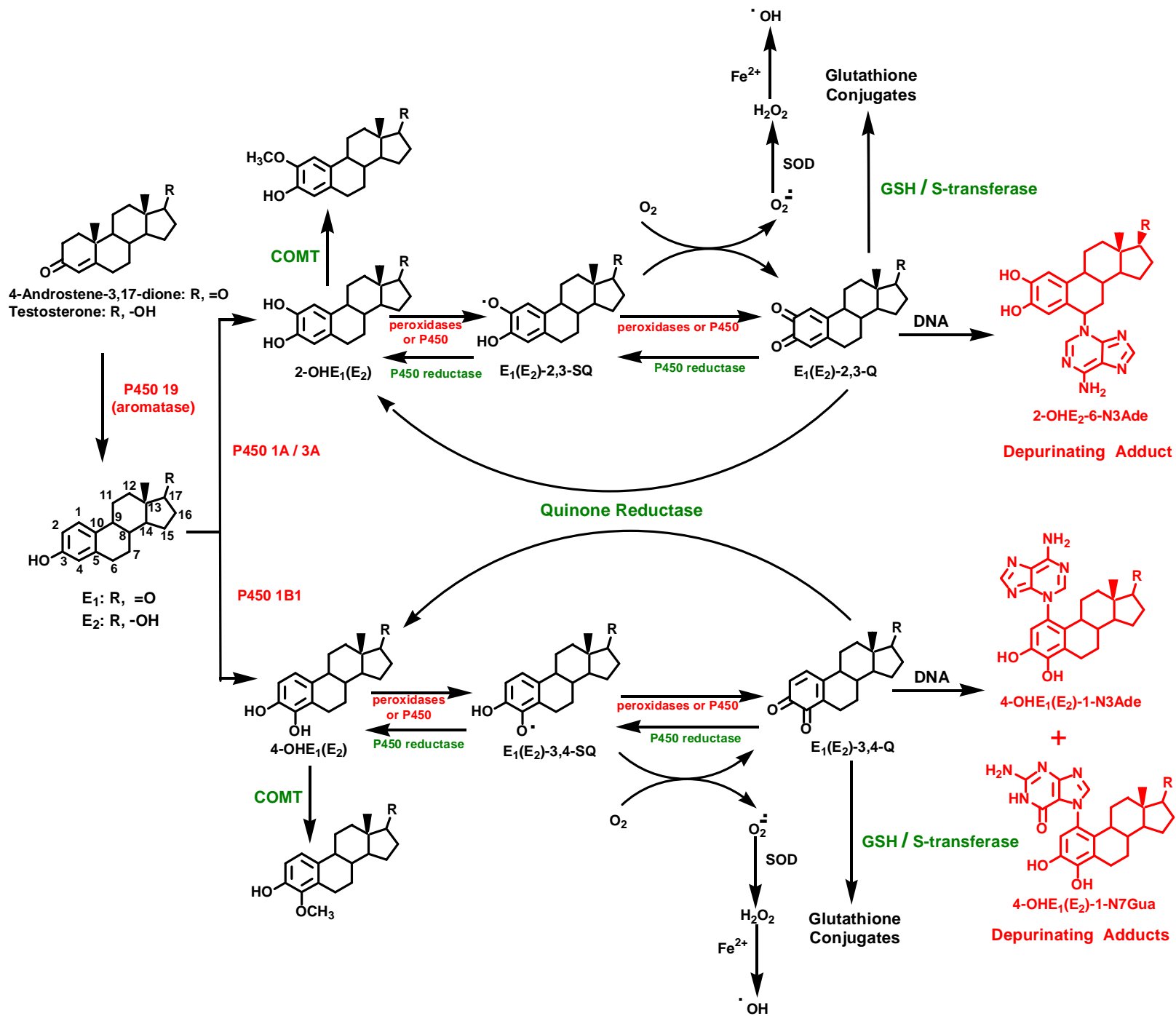
➤ **Metabolism, DNA adducts and carcinogenicity of estrogens**

Reactivity of estrogen quinones with DNA

Mutations induced by 4-OHE₂ and E₂-3,4-Q

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4-Catechol Estrogens

- **Are carcinogenic in the kidney of Syrian golden hamsters**

Liehr, et al., J. Steroid Biochem., 24, 353, 1986.

Li and Li, Fed. Proc., 46, 858, 1987.

- **Are carcinogenic in the uterus of CD-1 mice**

Newbold and Liehr, Cancer Res., 60, 235, 2000.

2-Catechol Estrogens

- ❑ **Are not carcinogenic in the kidney of Syrian golden hamsters**

Liehr, et al., *J. Steroid Biochem.*, 24, 353, 1986.

Li and Li, *Fed. Proc.*, 46, 858, 1987.

- ❑ **Are borderline carcinogenic in the uterus of CD-1 mice**

Newbold and Liehr, *Cancer Res.*, 60, 235, 2000.

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Metabolism, DNA adducts and carcinogenicity of estrogens

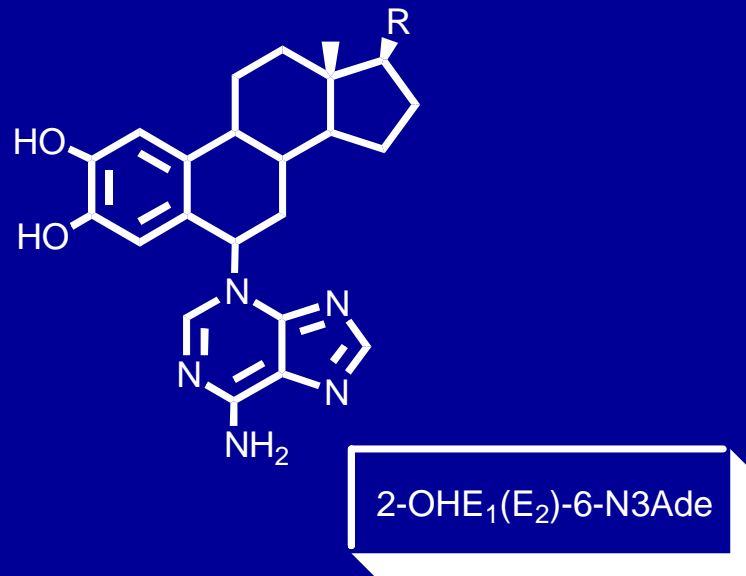
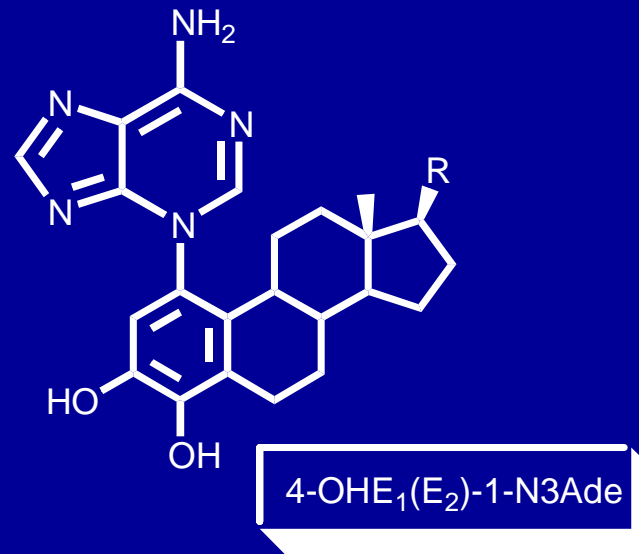
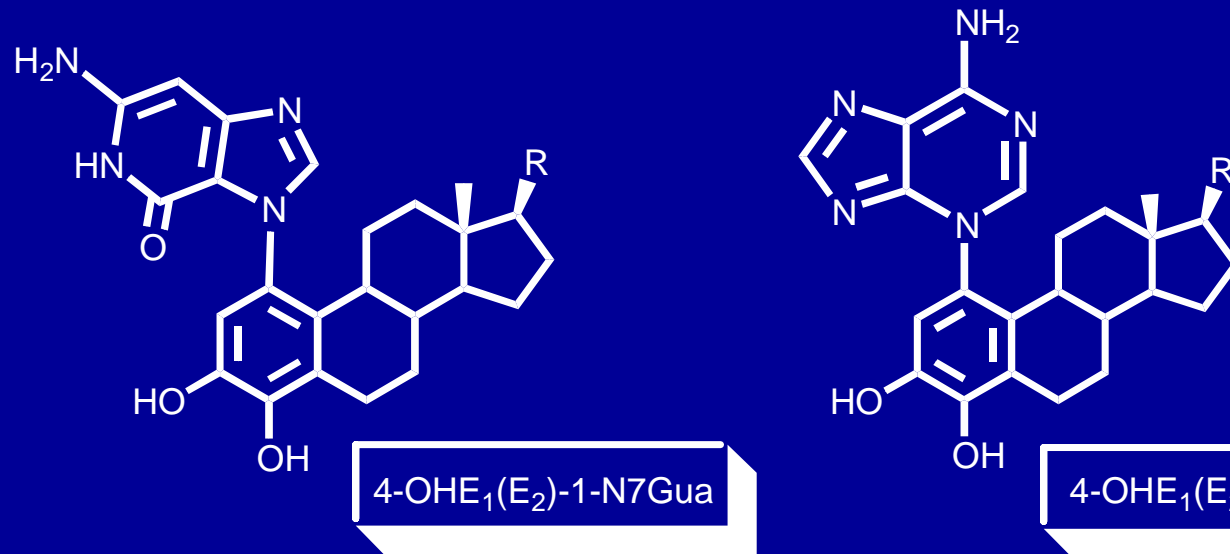
➤ **Reactivity of estrogen quinones with DNA**

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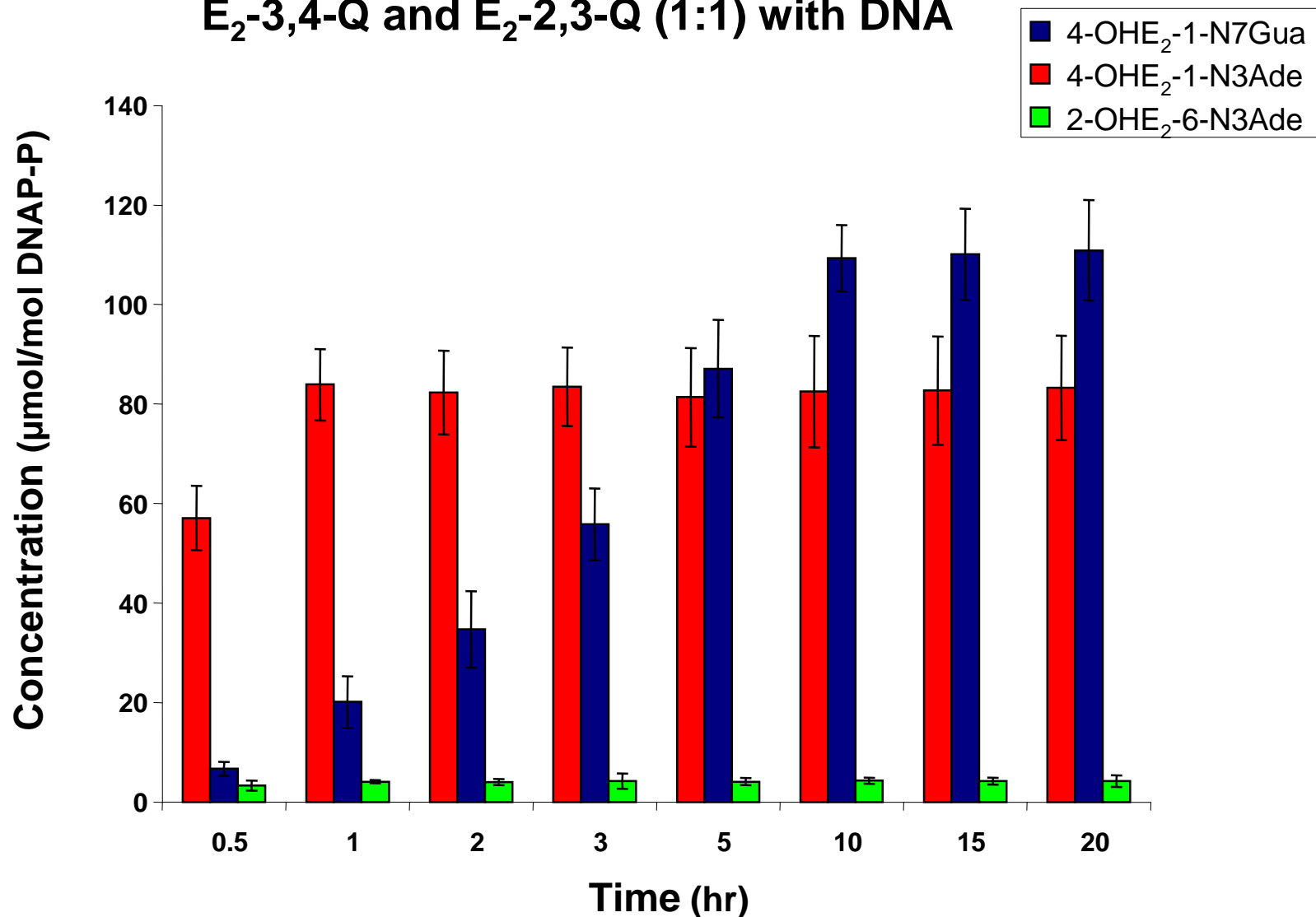
Conclusions

Depurinating Adducts



E₁: R, =O
E₂: R, -OH

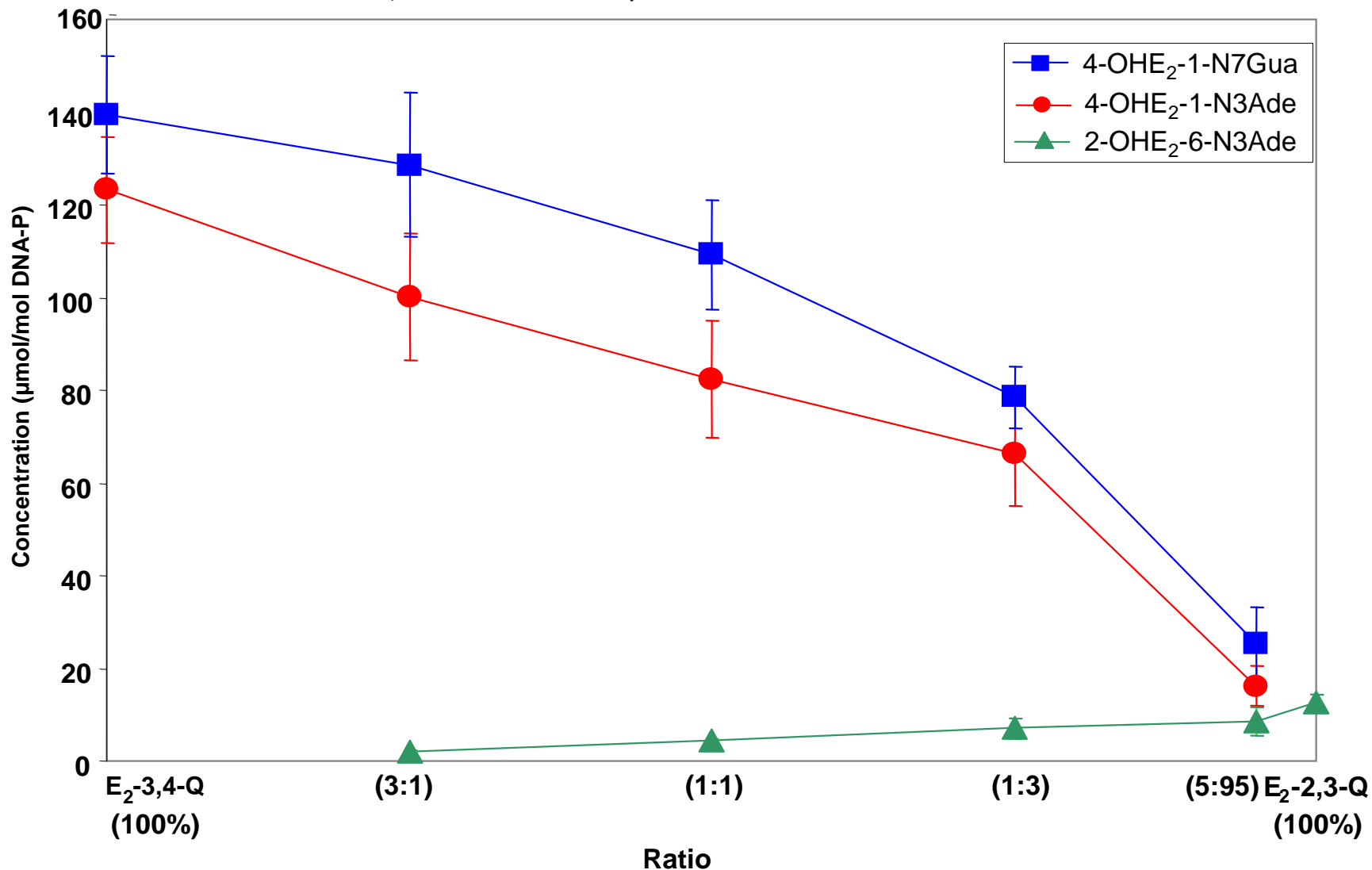
Depurinating adducts formed after the reaction of E₂-3,4-Q and E₂-2,3-Q (1:1) with DNA



After 10 h, the level of stable adducts was < 1.0 µmol/mol DNA-P, < 0.5% of total adducts.

Zahid, M., et al., *Chem. Res. Toxicol.*, 19: 164, 2006.

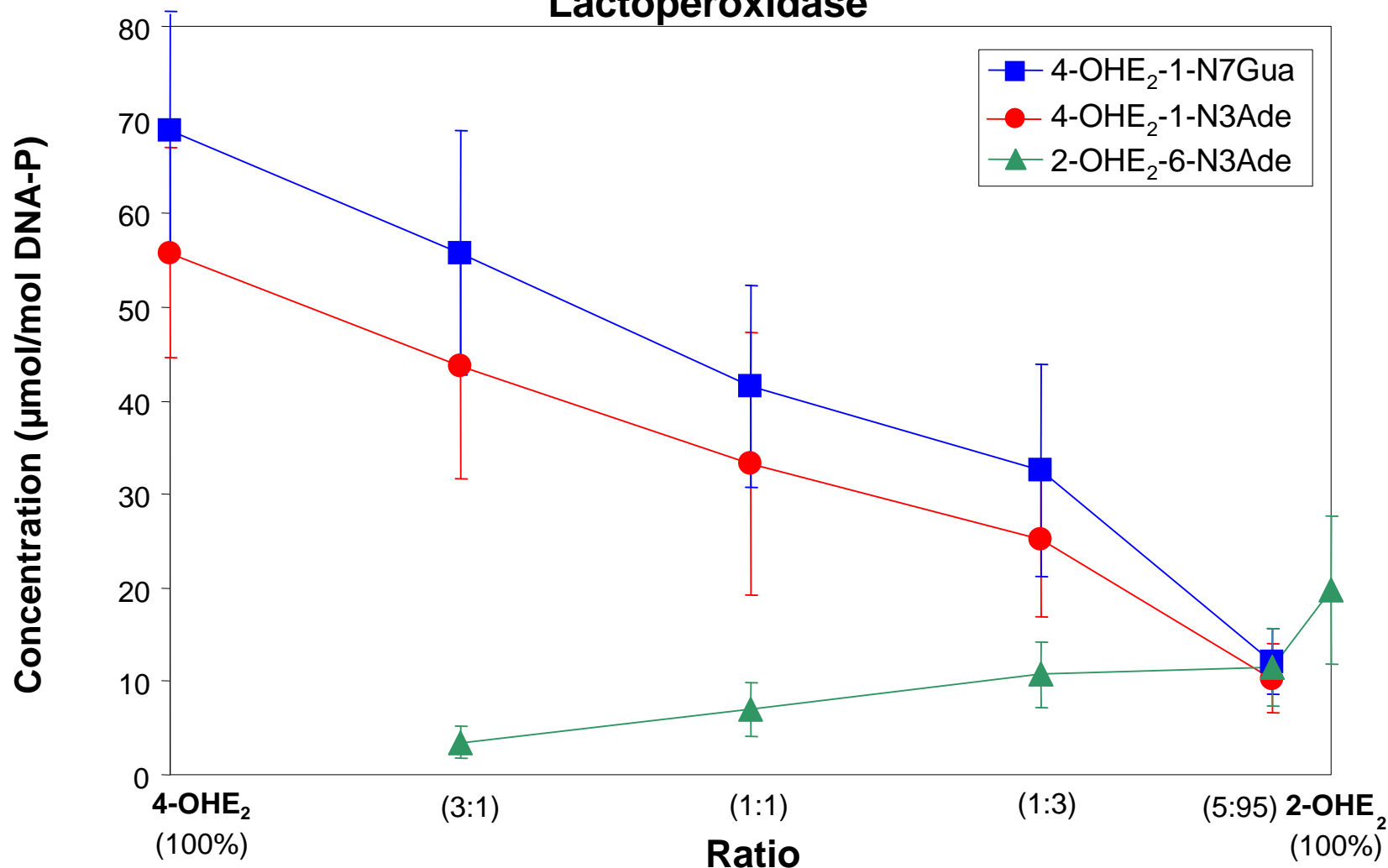
Depurinating adducts formed after 10 h by mixtures of E_2 -3,4-Q and E_2 -2,3-Q reacted with DNA



The level of stable adducts formed in the mixtures ranged from 0.1% to 1% of total adducts.

Zahid, M., *et al.*, *Chem. Res. Toxicol.*, 19: 164, 2006.

Depurinating adducts formed after 10 h by mixtures of 4-OHE₂ and 2-OHE₂ with DNA in the presence of Lactoperoxidase



The level of stable adducts formed in the mixtures ranged from 0.2% to 0.3% of total adducts.

Zahid, M., et al., *Chem. Res. Toxicol.*, 19: 164, 2006.

Summary

1. The reactivity of E₂-3,4-Q with DNA is far greater than that of E₂-2,3-Q.
2. The 4-OHE₂-1-N3Ade adduct depurinates instantaneously, whereas the 4-OHE₂-1-N7Gua adduct depurinates slowly, with a half-life of approximately 3 h.

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Mutagenesis by E₂-3,4-Quinone

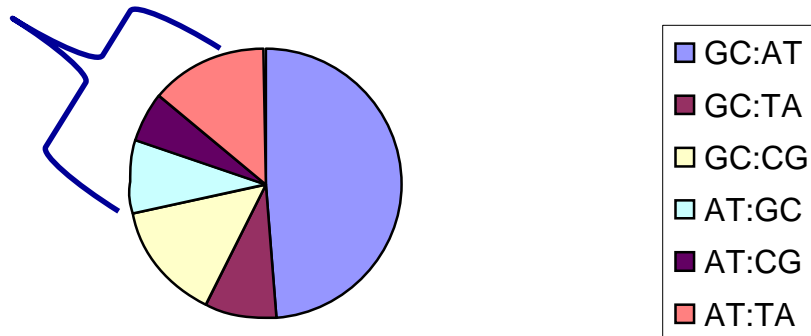
Tissue	Depurinating Adducts μmole/mol DNA-P		Stable Adducts μmole/mol DNA-P	H-ras mutations	
	4-OHE ₂ -1- N3Ade	4-OHE ₂ -1- N7Gua		A → G Total clones	Other Total clones
	SENCAR mouse skin ^a	12.5	12.1	0.004	
6 h				5/29	2/29
12 h				4/30	2/30
1 d				7/50	4/50
3 d				3/40	1/40
ACI rat mammary gland ^b	81	90	0.017		
6 h				16/29	3/29
12 h				14/34	6/34

^aChakravarti, D., *et al.*, *Oncogene*, 20: 7945-53, 2001.

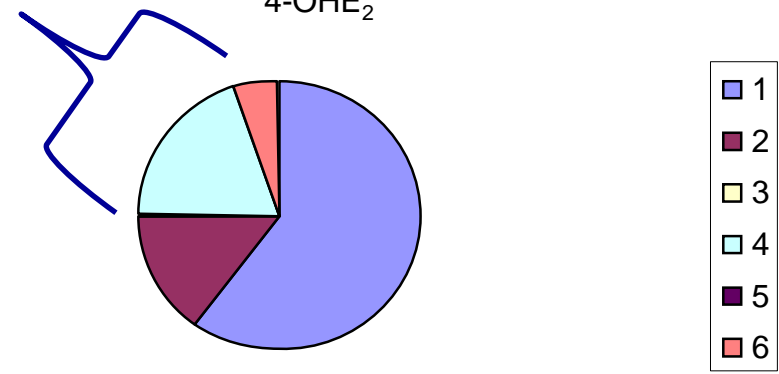
^bMailander, P.C., *et al.*, *J. Steroid Biochem. Mol. Biol.*, in press, 2006.

Mutational specificities of E₂, 4-OHE₂, 4-OHE₂ + E₂, and background mutations in Big Blue rats

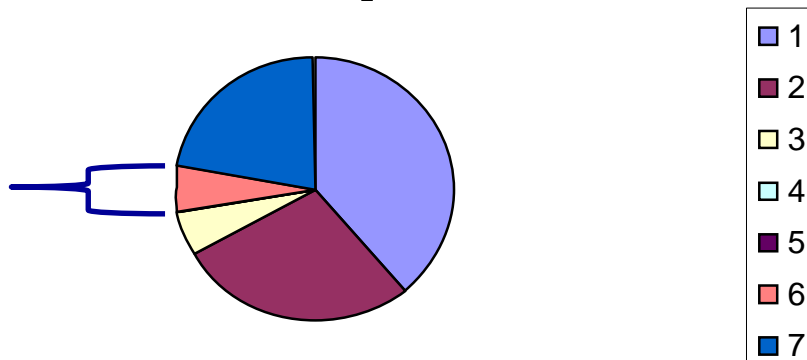
4-OHE₂ + E₂



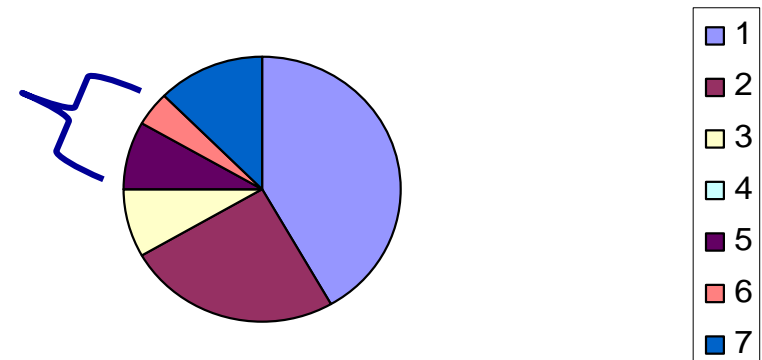
4-OHE₂



E₂



untreated



■ 7, small deletions were only found in the E₂ and untreated groups

Brackets indicate mutations at A:T base pairs

Higher levels of mutations at A:T base pairs, particularly AT:GC transitions were observed in groups that received 4-OHE₂

Summary

- E_2 -3,4-Q induces primarily depurinating adducts in mouse skin and rat mammary gland.
- The fast depurinating N3Ade adducts induce the important lesions for mutagenesis.
- A majority of mutations in the reporter Harvey-*ras* gene are A.T to G.C transitions. These mutations appear as early as 6 and 12 h, before mutagenesis during replication could occur.
- Similar A.T to G.C mutations are observed in the *lacI* reporter gene in the mammary gland of Big Blue rats implanted with 4-OHE₂.

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➤ **Biomarkers of susceptibility to breast and prostate cancer**

Conclusions

Biomarkers in Urine from Men

human urine

Pass over an immunoaffinity column containing MAb for 4-OHE₁(E₂)-1-N3Ade

analyte bound to MAb and released

phosphorescence
spectroscopy
and
CE-FASS

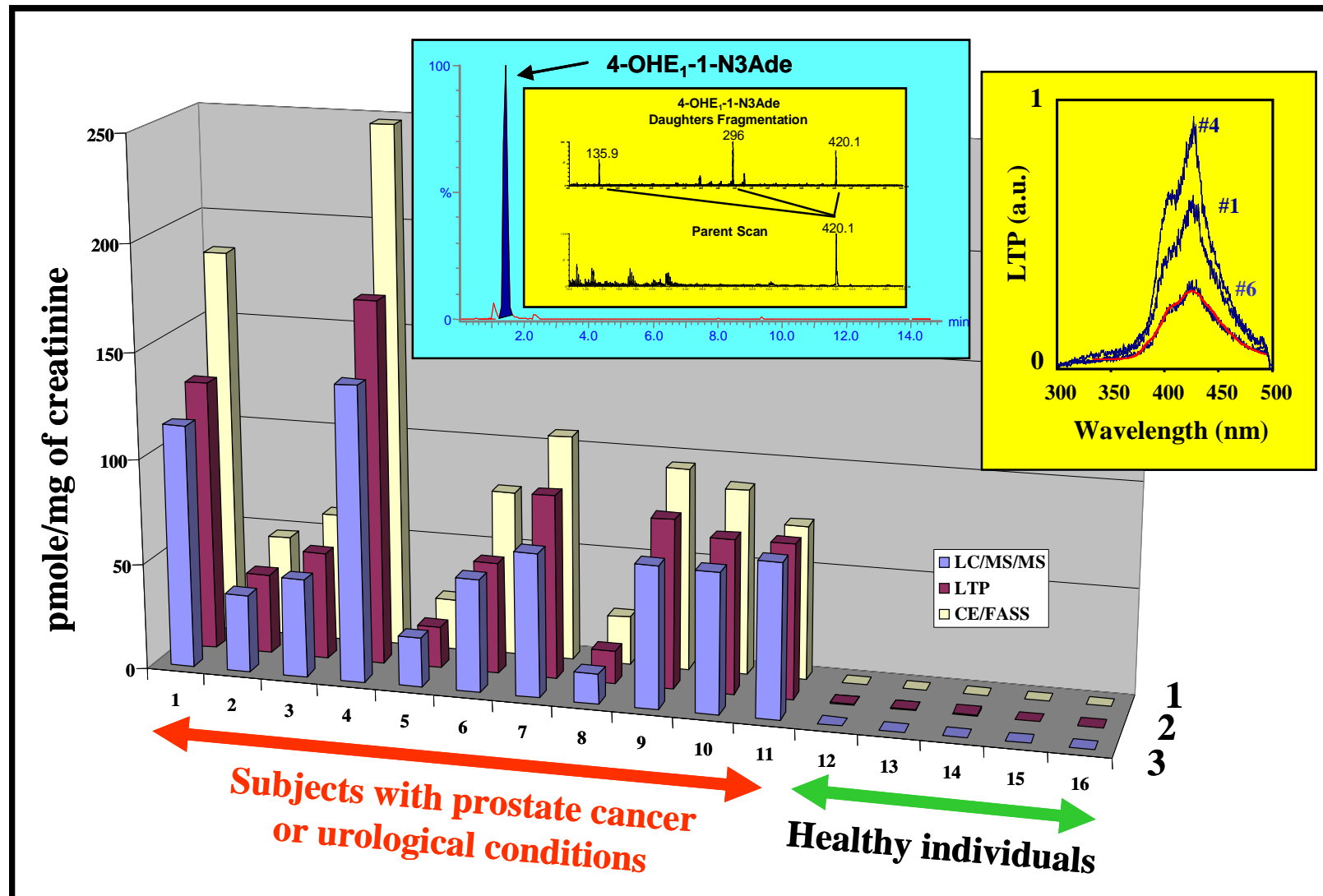


Biomarker: 4-OHE₁(E₂)-1-N3Ade

E₁: R, =O
E₂: R, -OH

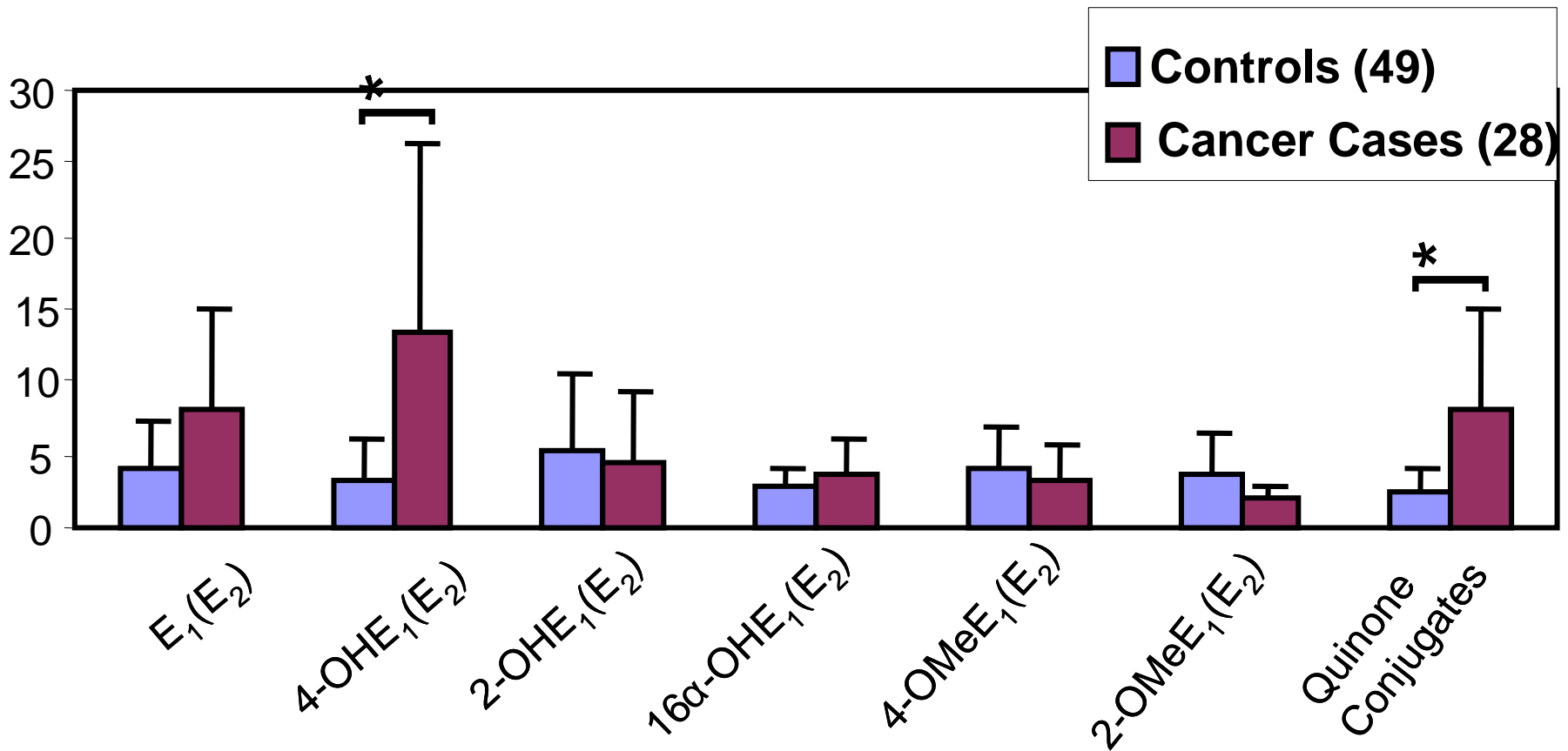
LC/MS/MS

Identification of the 4-OHE₁-1-N3Ade adduct in human urine samples



Markushin, et al., *Prostate*, 66: 1565, 2006.

Analysis of estrogen metabolites in human breast tissue from women with and without breast cancer



Controls are benign fatty breast tissue and benign fibrocystic changes. Quinone conjugates are 4-OHE₁(E₂)-2-NACys, 4-OHE₁(E₂)-2-Cys, 2-OHE₁(E₂)-(1+4)-NACys, and 2-OHE₁(E₂)-(1+4)-Cys. *Statistically significant differences were determined using the Wilcoxon rank sum test: 4-OHE₁(E₂), $p < 0.01$ and quinone conjugates, $p < 0.003$.

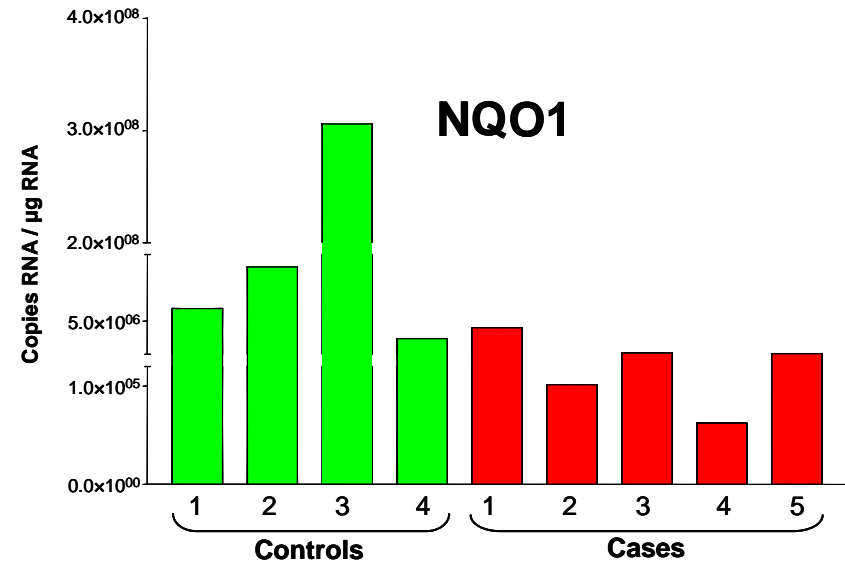
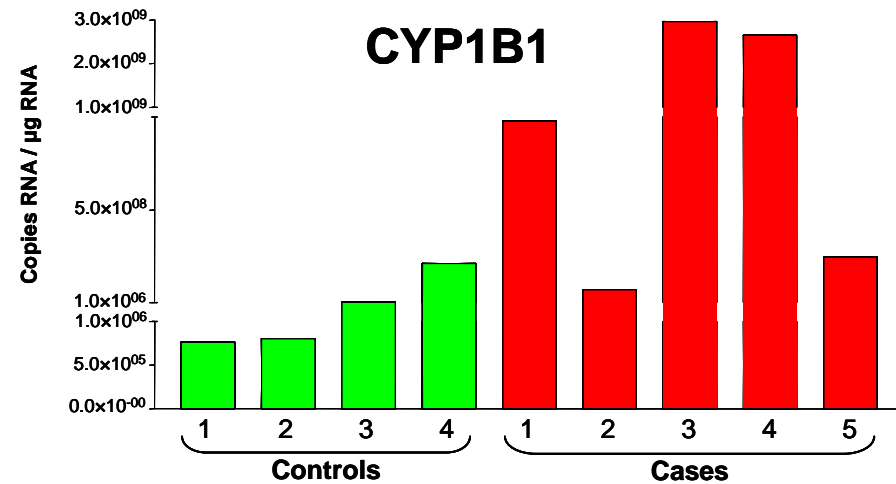
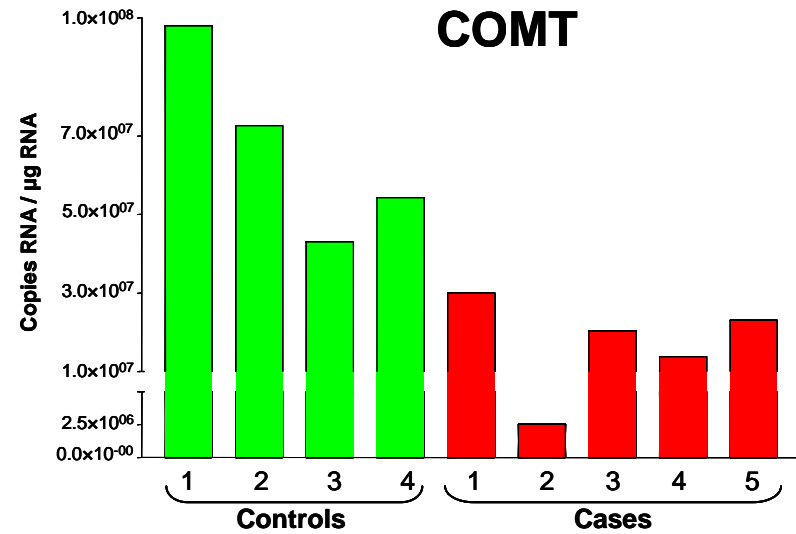
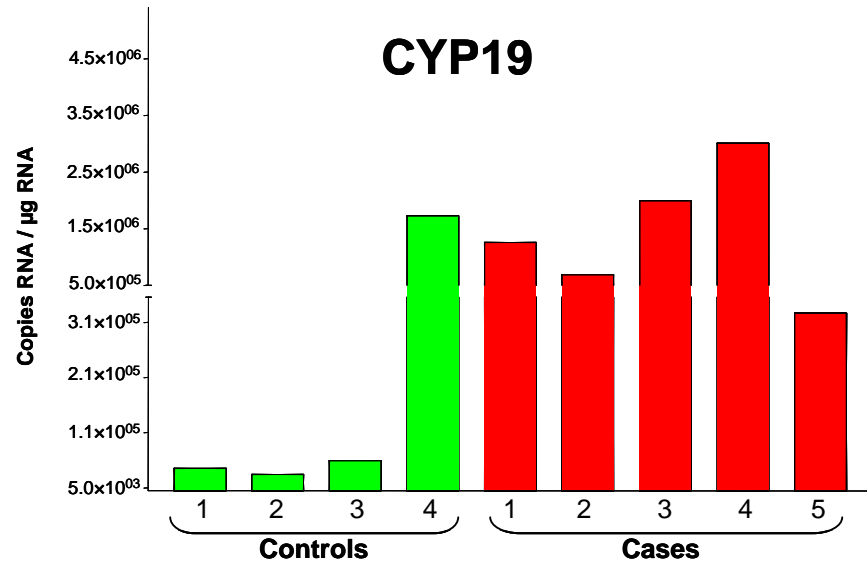
Rogan, E., et al., *Carcinogenesis*, 24: 697-702, 2003.

Summary

- 1. Formation of 4-OHE₁(E₂) was significantly higher in breast tissue from women with breast cancer compared to women without breast cancer.**
- 2. Significantly higher amounts of catechol estrogen quinone conjugates with GSH were formed in breast tissue from women with breast cancer compared to women without breast cancer.**

Expression of estrogen-metabolizing enzymes in the human breast

■ = Controls
■ = Cases



Summary

- 1. Expression of estrogen activating enzymes is higher in breast tissue from women with breast cancer, whereas expression of protective enzymes is higher in controls.**
- 2. These results are consistent with the hypothesis that breast cancer is initiated by reaction of catechol estrogen-3,4-quinones with DNA to generate cancer-causing mutations.**

Sample Preparation

Urine or serum

Nipple aspirate fluid

Solid phase
extraction

Sample for
UPLC/MS/MS

analysis of estrogens

catechol estrogen metabolites

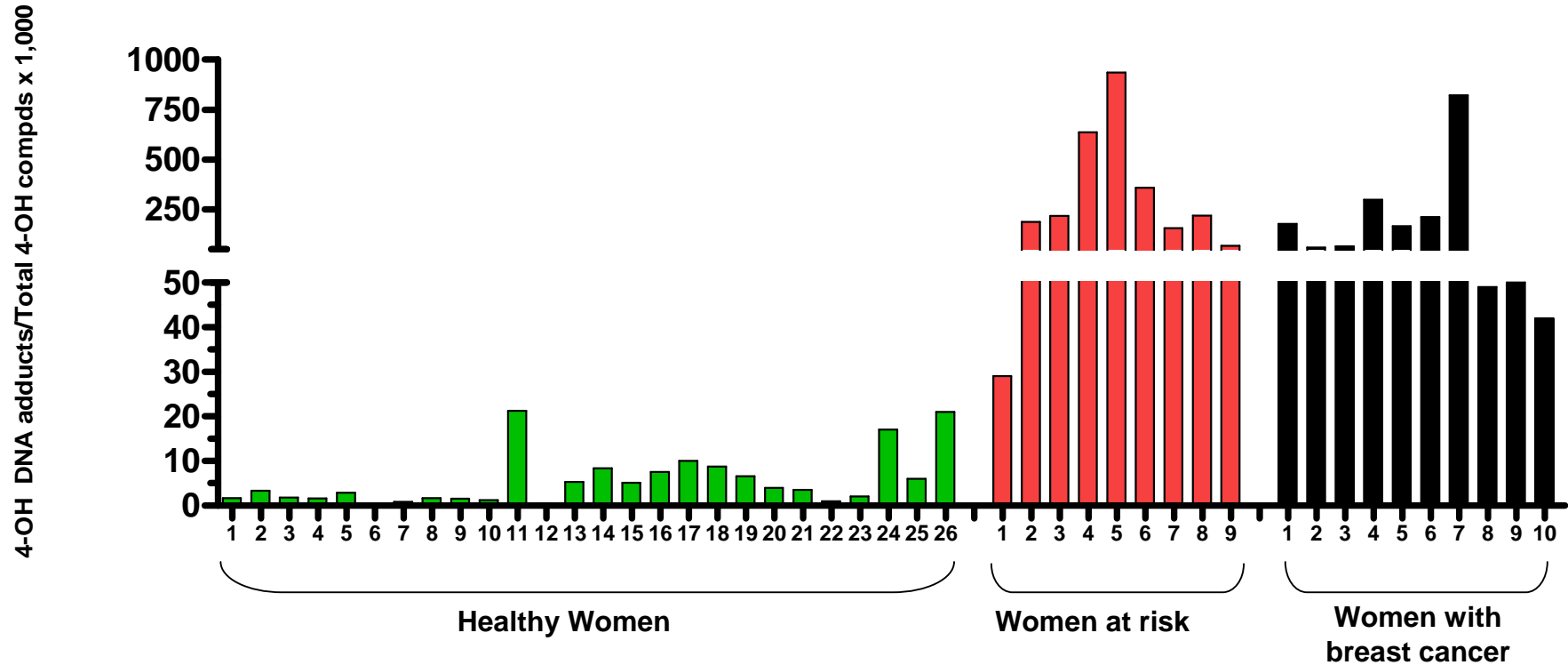
catechol estrogen-GSH conjugates

depurinating catechol estrogen-DNA adducts

Women with breast cancer

Breast Cancer_McN						
N0.	Compound	pm/ mg Cr mean, n=2	Standard Deviation ±	Total pmole/mg creatinine	adduct/ Tot 4-Cat. Deriv.	Total of 4-Ade & 4-Gua Ratio
1	AD	NA				
2	Test	NA				
3	E2	39	6	54		
4	E1	15	1			
5	E1-Sulfate	NA				
6	2-OHE2	0	0	0		
7	2-OHE1	0	0			
8	4-OHE2	1	0	1		
9	4-OHE1	0	0			
10	16a-OHE2	3	1	10		
11	16a-OHE1	7	2			
12	2-OCH3E2	5	1	6		
13	2-OCH3E1	1	0			
14	4-OCH3E2	6	1	7		
15	4-OCH3E1	1	0			
16	2-OH-OCH3E2	NA				
17	2-OH-OCH3E1	NA				
18	2-OHE2-1-SG	0.00	0.00			
19	2-OHE2-4-SG	0.00	0.00			
20	2-OHE1-1-SG	0.01	0.00			
21	2-OHE1-4-SG	0.01	0.00			
22	2-OHE2-1+4-Cys	0.02	0.00			
23	2-OHE1-1-Cys	1.01	0.01	2.16		
24	2-OHE1-4-Cys	0.99	0.03			
25	2-OHE2-1-NAcCys	0.04	0.01			
26	2-OHE2-4-NAcCys	0.03	0.02			
27	2-OHE1-1-NAcCys	0.02	0.00			
28	2-OHE1-4-NAcCys	0.04	0.00			
29	4-OHE2-2-SG	0.01	0.00			
30	4-OHE1-2-SG	0.00	0.00			
31	4-OHE2-2-Cys	0.36	0.02	0.46		
32	4-OHE1-2-Cys	0.02	0.00			
33	4-OHE2-2-NAcCys	0.03	0.03			
34	4-OHE1-2-NAcCys	0.04	0.00			
35	4-OHE2-1-N7Gua	5.22	0.33	5.41	116	
36	4-OHE1-1-N7Gua	0.19	0.11			822
37	4-OHE2-1-N3Ade	32.81	2.08	32.85	706	
38	4-OHE1-1-N3Ade	0.03	0.01			
39	2-OHE2-6-N3Ade	0.00	0.00	0.01	1.6000	
40	2-OHE1-6-N3Ade	0.01	0.00			

Urinary Estrogen-DNA Adducts in Women



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Mutations induced by 4-OHE₂ and E₂-3,4-Q

Biomarkers of susceptibility to breast and prostate cancer

➤ **Conclusions**

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1. We have acquired evidence for a unifying mechanism of initiation of breast, prostate and other human cancers.
2. This mechanism entails formation of endogenous estrogen-3,4-quinones that can react with DNA and produce depurinating adducts. Specific mutations generated by apurinic sites can initiate abnormal cell proliferation leading to cancer.
3. Formation of depurinating adducts such as 4-OHE₁(E₂)-1-N³Ade leads to a biomarker of susceptibility that can potentially be used to monitor the risk of breast or prostate cancer long before a tumor appears.

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