From Aromatic Hydrocarbons to Estrogens and then Back to Benzene and Naphthalene

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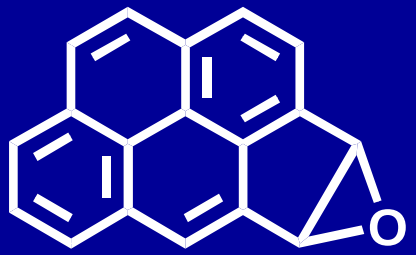
Naphthalene State-of-the-Science Symposium
Monterey, California – October 9-12, 2006

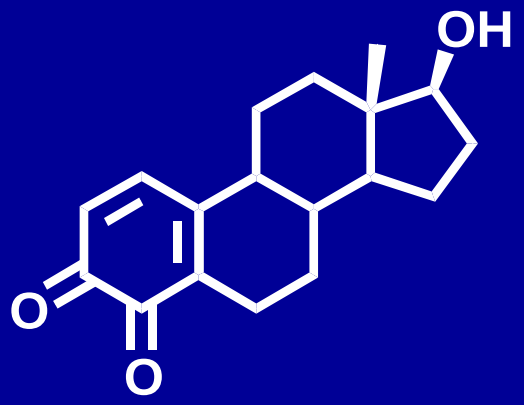
Ultimate Carcinogenic Metabolites

Benzo[*a*]pyrene radical cation

Benzo[*a*]pyrene diol epoxide



Cyclopenta[*c,d*]pyrene epoxide



Estradiol-3,4-quinone

Outline

Stable and depurinating DNA adducts

Mechanisms of metabolic activation of PAH to form DNA adducts

Metabolic activation of natural and synthetic estrogens, benzene and dopamine

Mechanisms of metabolic activation of naphthalene to form DNA adducts

DNA adducts formed in mouse skin by naphthalene and some metabolites

Conclusions

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- **Stable and depurinating DNA adducts**

  Mechanisms of metabolic activation of PAH to form DNA adducts

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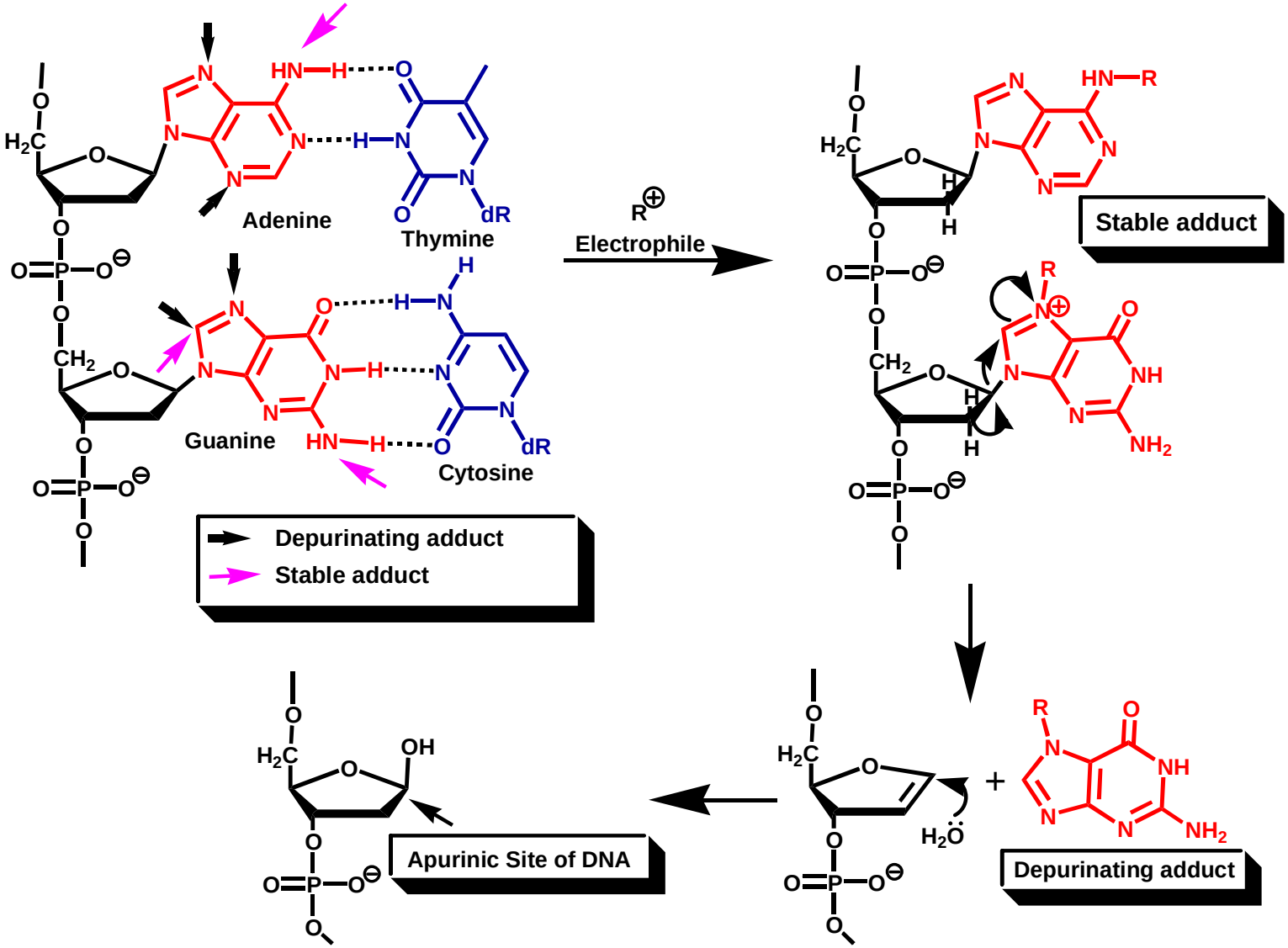
  Conclusions

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.

Outline

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CRTOEC

Chemical Research in Toxicology®

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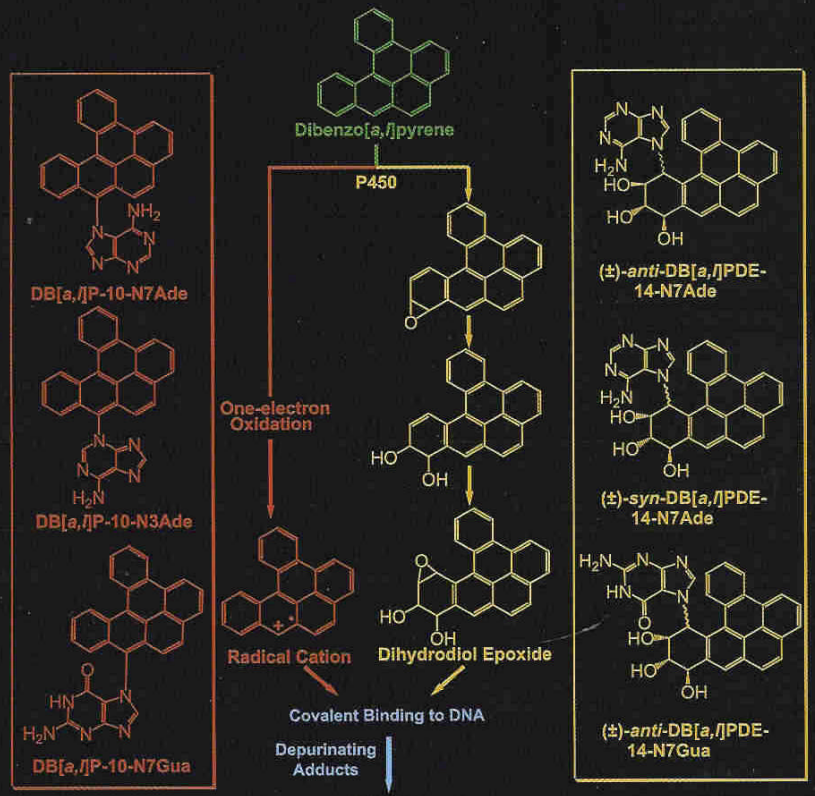

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Outline

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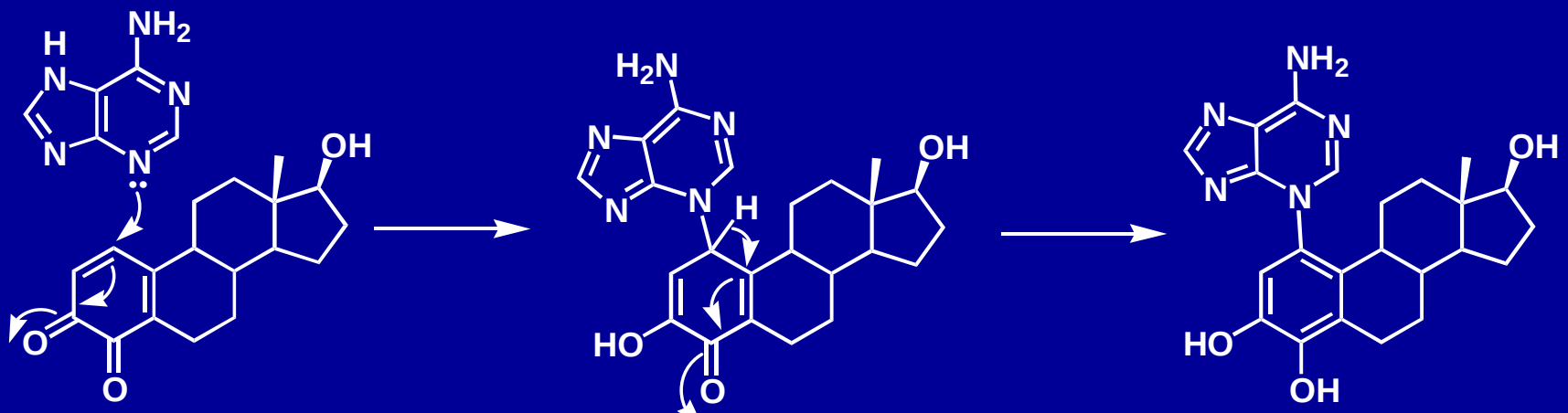
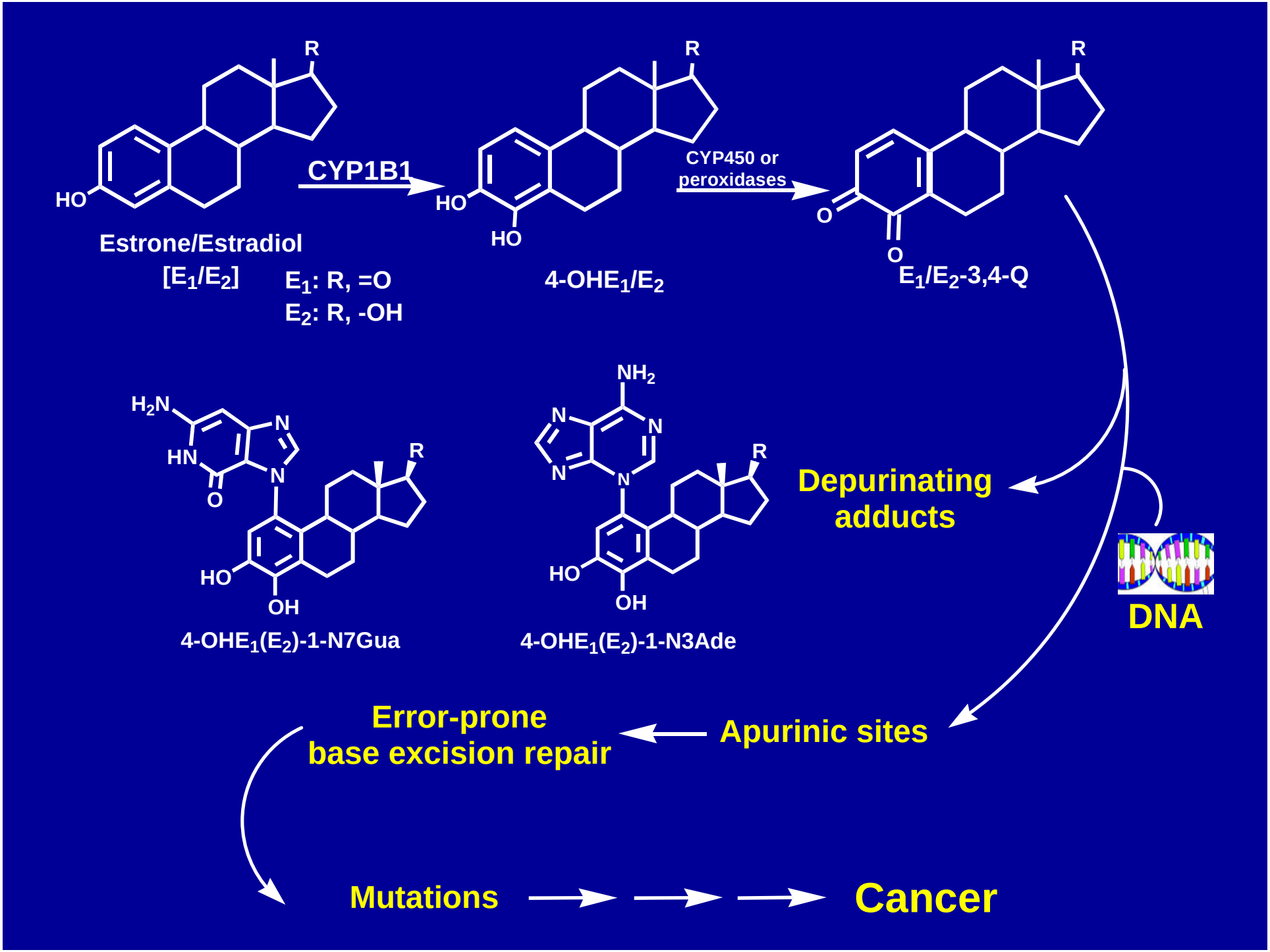
1,4-Michael Addition

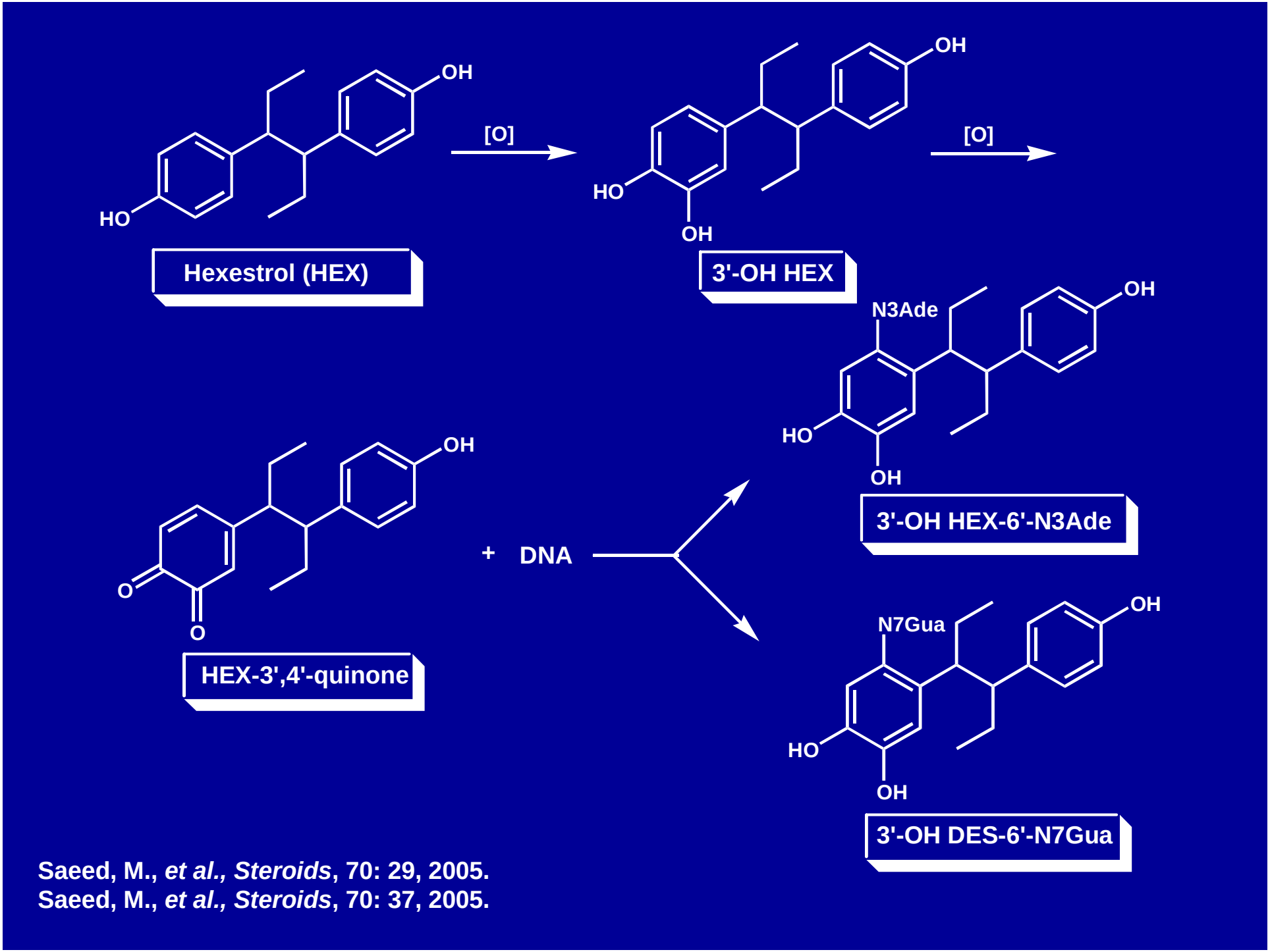
Adenine



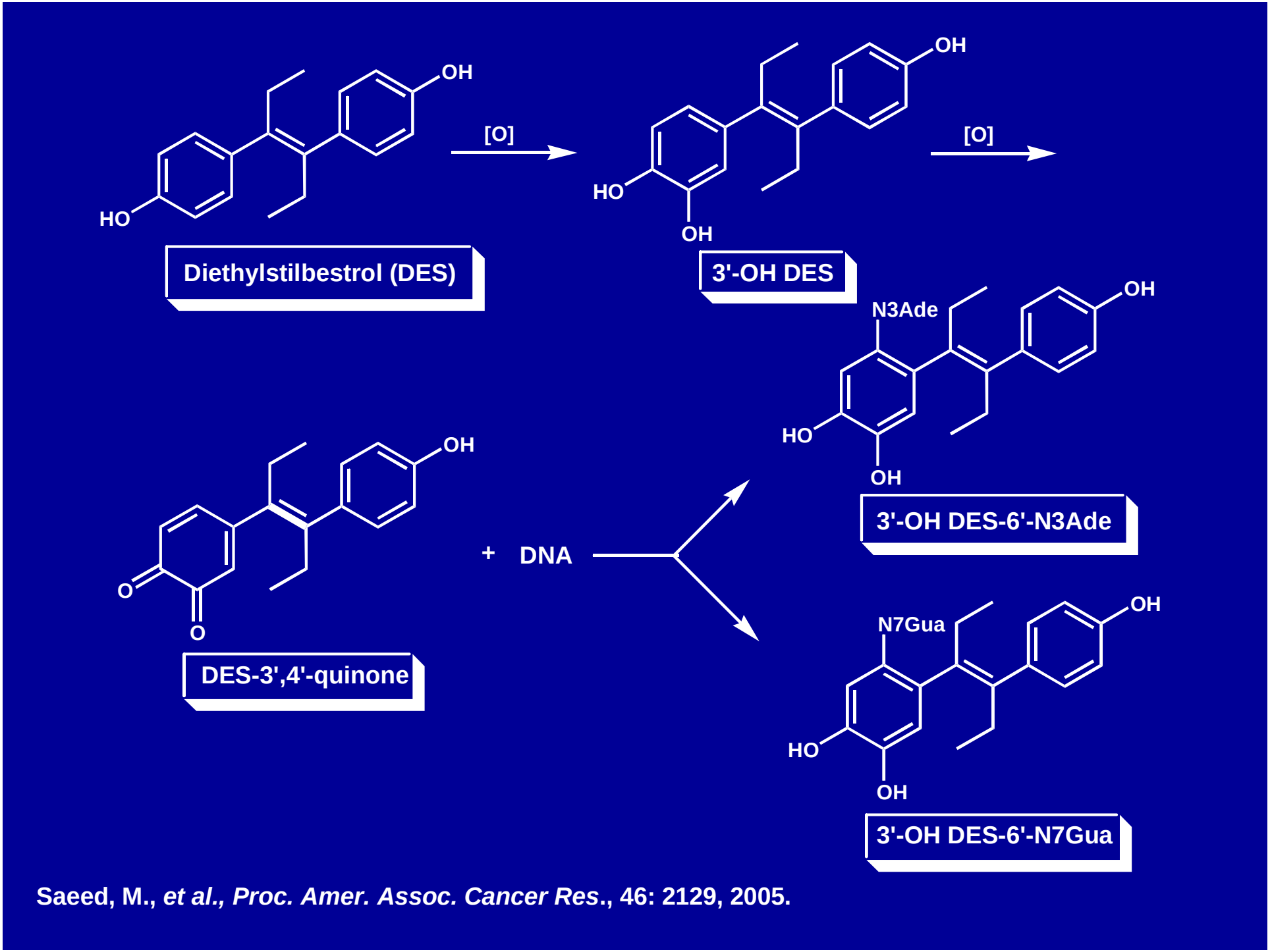
Estradiol-3,4-quinone

4-Hydroxyestradiol
-1-N3Adenine

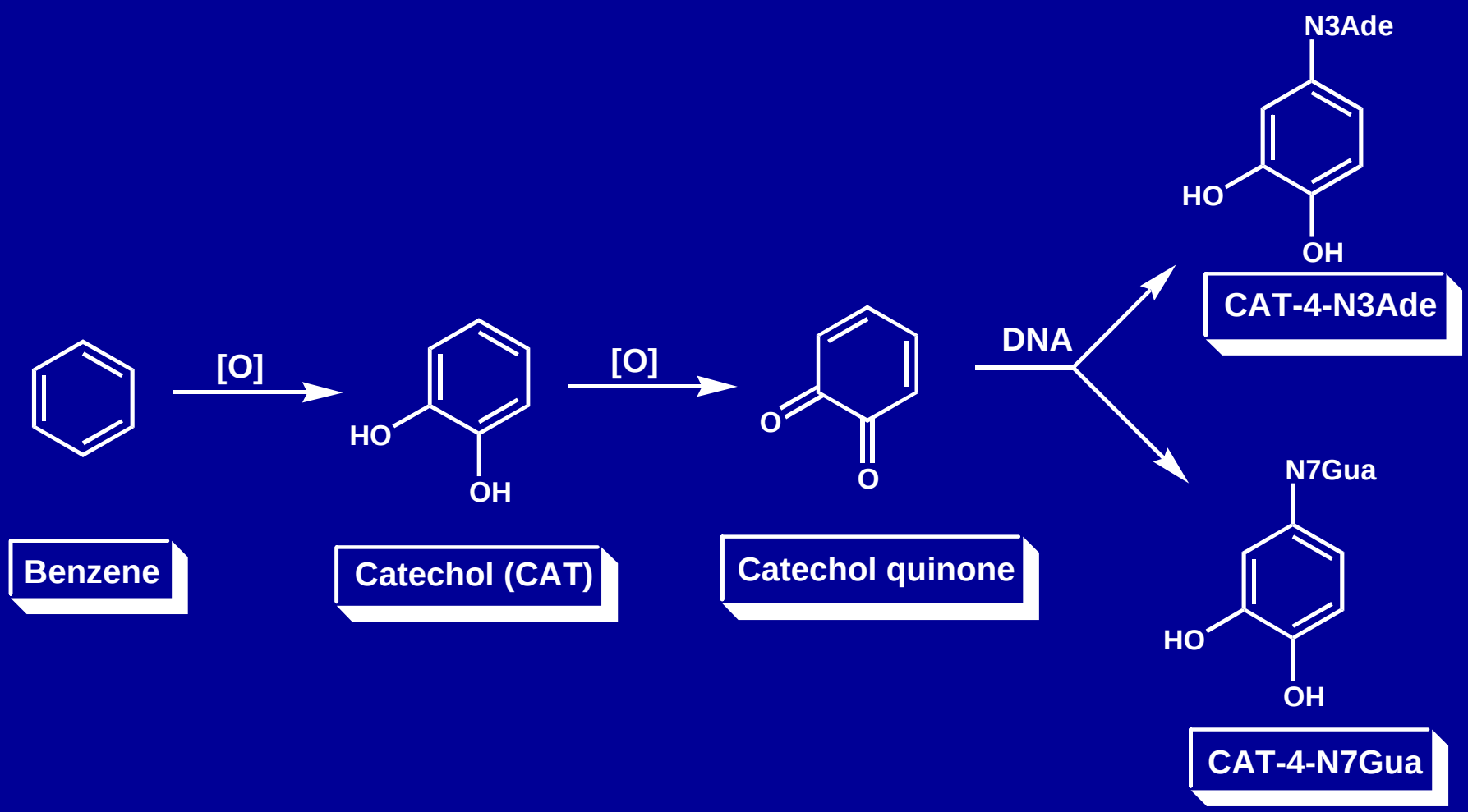

R
HO
CYP1B1
HO
HO
CYP450 or
peroxidases
O
O
R
R
Estrone/Estradiol
[E1/E2]
E1: R, =O
E2: R, -OH
4-OHE1/E2
E1/E2-3,4-Q
H2N
HN
N
N
O
R
HO
OH
NH2
N
N
N
N
R
HO
OH
4-OHE1(E2)-1-N7Gua
4-OHE1(E2)-1-N3Ade
Depurinating
adducts
DNA
Error-prone
base excision repair
Apurinic sites
Mutations
Cancer



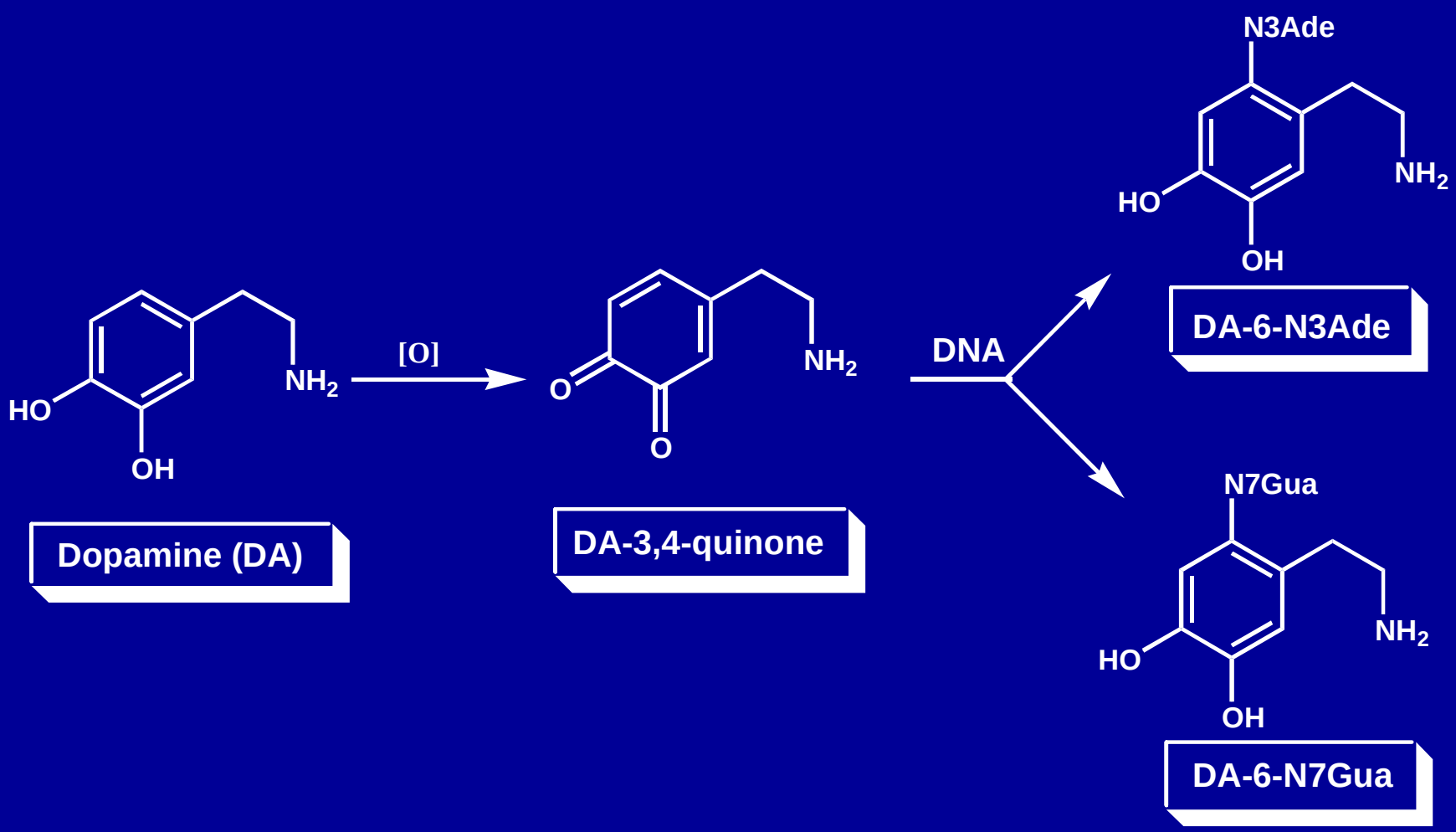
Saeed, M., *et al., Steroids*, 70: 29, 2005.
Saeed, M., *et al., Steroids*, 70: 37, 2005.



Saeed, M., *et al., Proc. Amer. Assoc. Cancer Res*., 46: 2129, 2005.



Cavalieri, E., *et al., Carcinogenesis,* 23: 1071, 2002.



Dopamine (DA)

DA-3,4-quinone

DA-6-N3Ade

DA-6-N7Gua

Cavalieri, E., *et al., Carcinogenesis,* 23: 1071, 2002.

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- **Mechanisms of metabolic activation of naphthalene to form DNA adducts**

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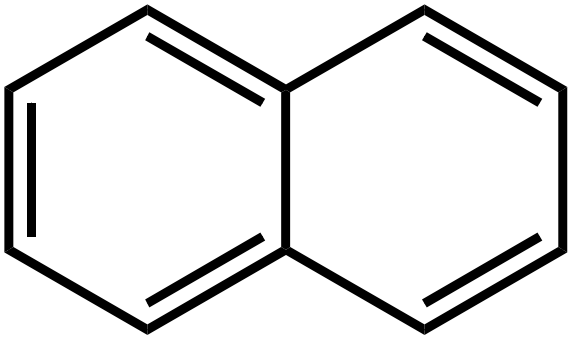
F344/N Rats

Chronic Exposure

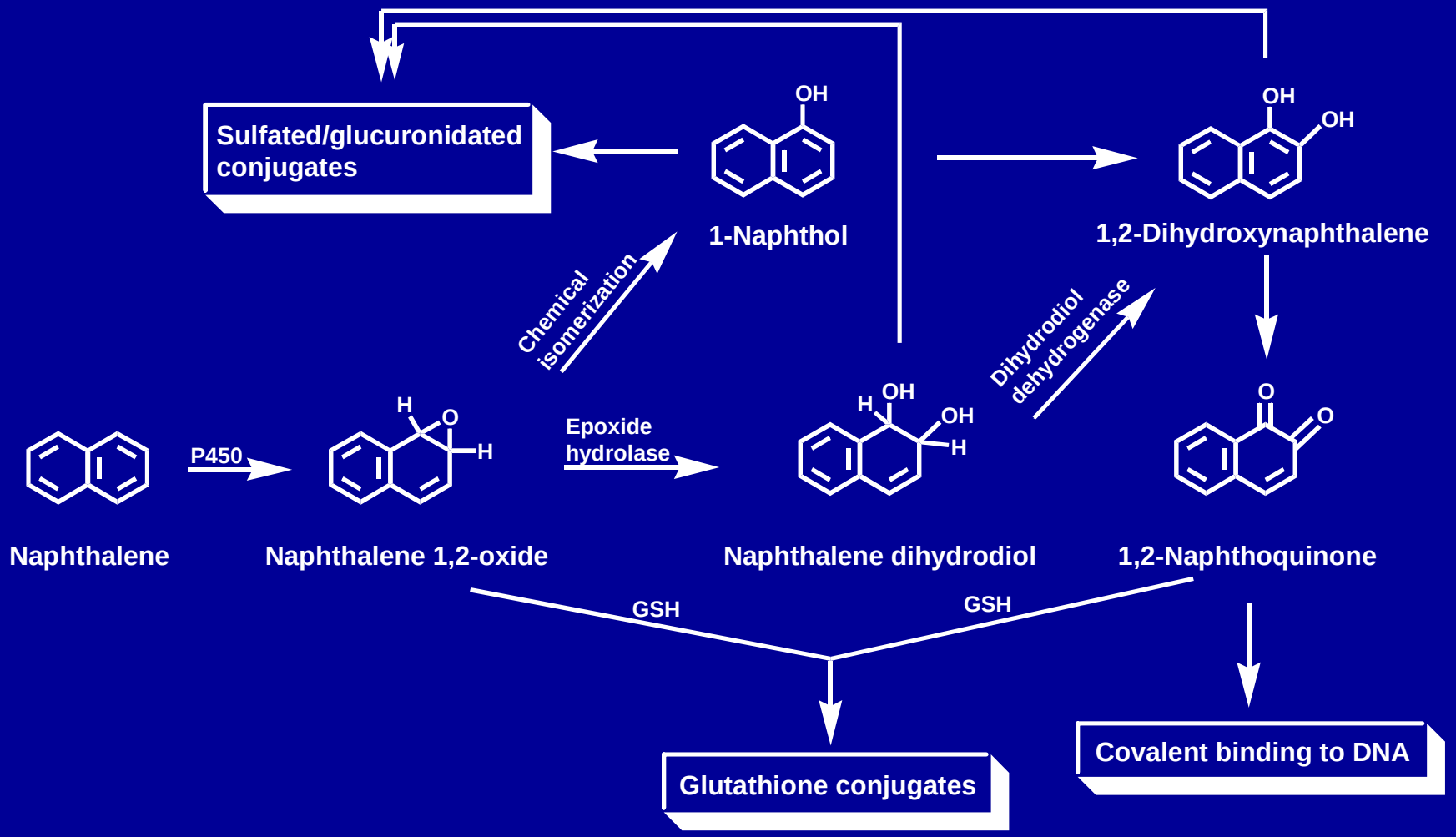
Neuroblastomas of the olfactory epithelium and adenomas of the nasal respiratory epithelium

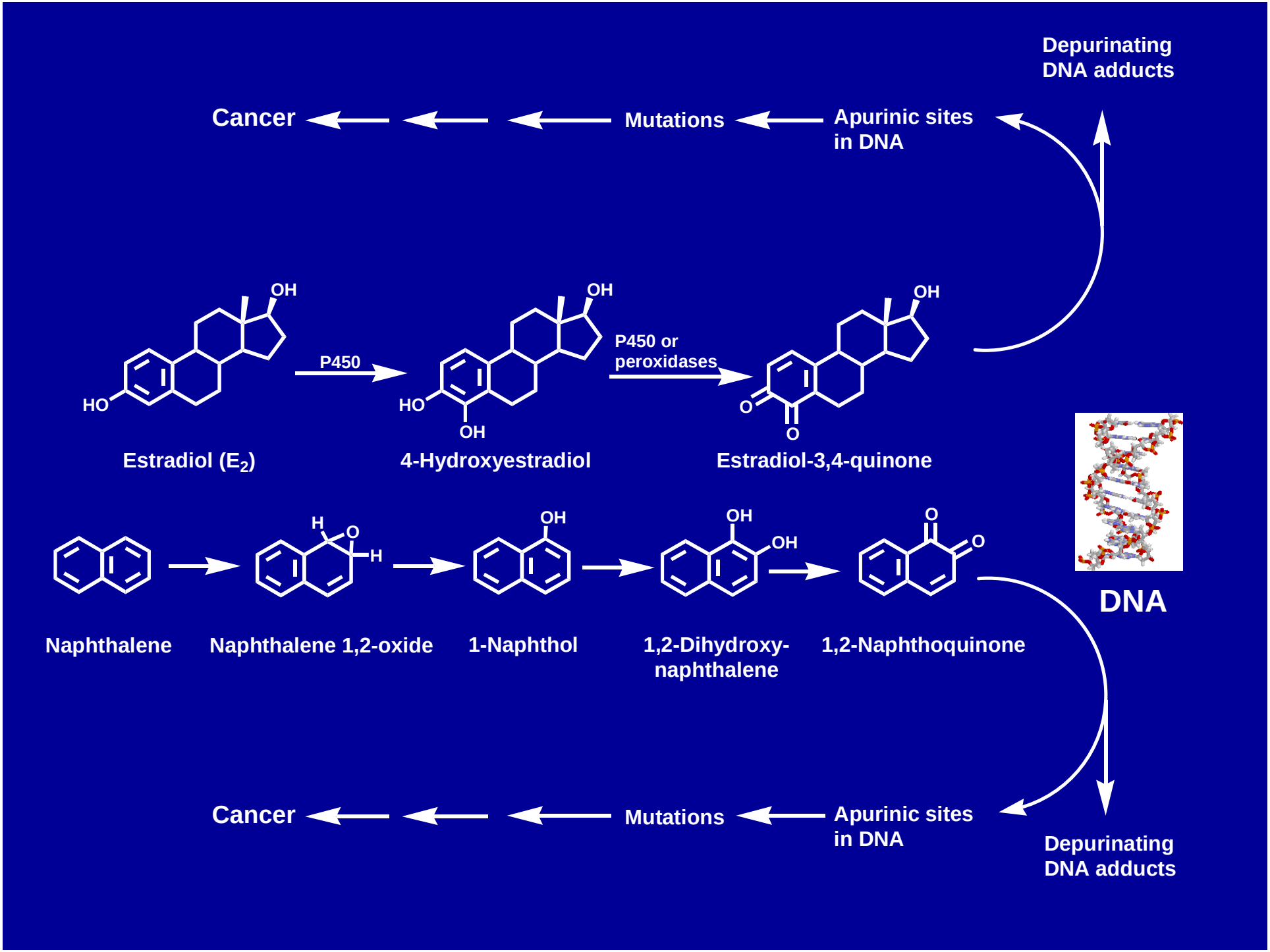


Naphthalene Vapors

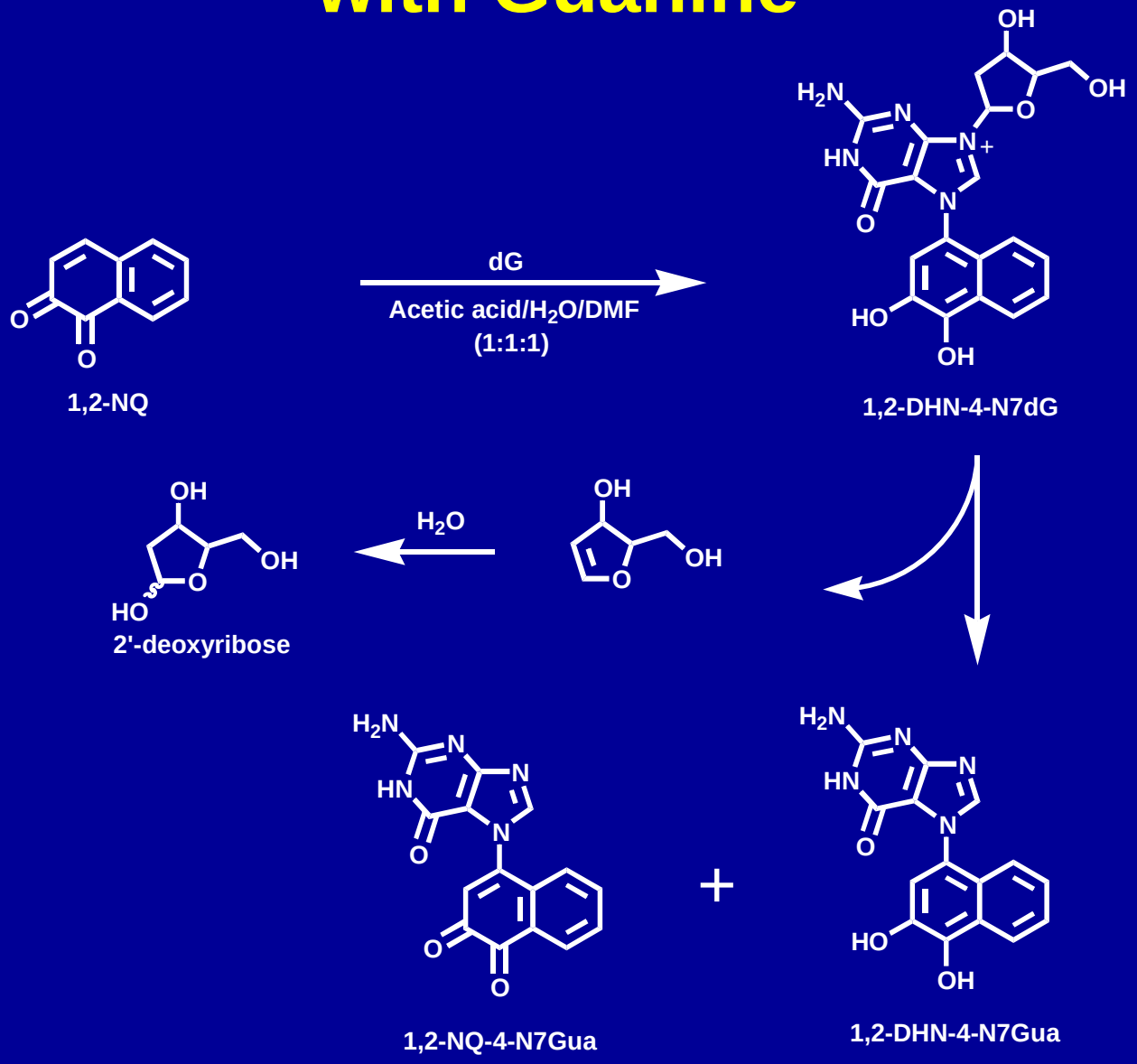
National Toxicology Program (2000) Toxicology and carcinogenesis studies of naphthalene in Fischer 344/N rats (Inhalation studies). NTP Technical report No. 500.

Metabolic Activation of Naphthalene

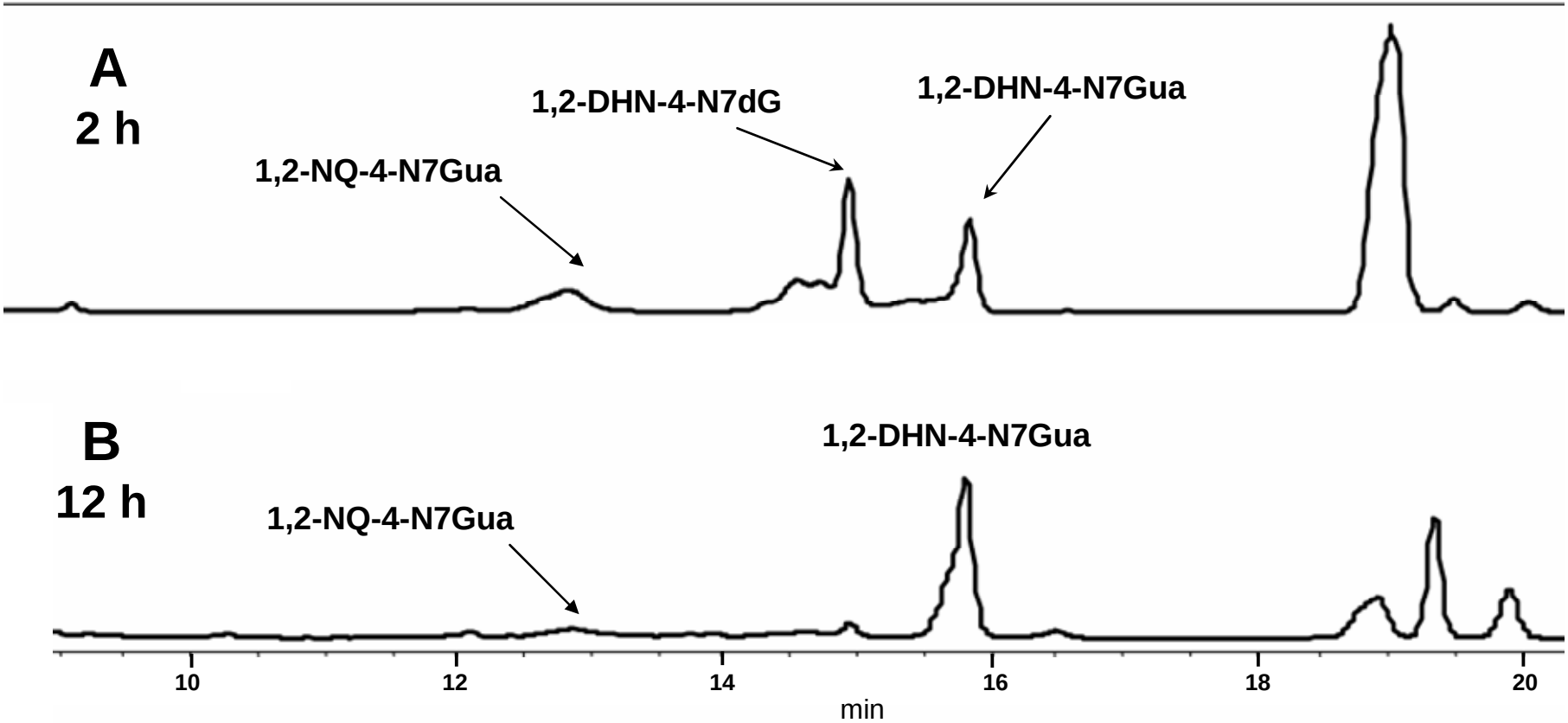



Depurinating
DNA adducts
Cancer
Mutations
Apurinic sites
in DNA
OH
HO
P450
OH
HO
OH
P450 or
peroxidases
OH
O
O
Estradiol (E_2)
4-Hydroxyestradiol
Estradiol-3,4-quinone
DNA
H
O
H
OH
OH
OH
O
O
Naphthalene
Naphthalene 1,2-oxide
1-Naphthol
1,2-Dihydroxy-
naphthalene
1,2-Naphthoquinone
Cancer
Mutations
Apurinic sites
in DNA
Depurinating
DNA adducts

Synthesis of Naphthalene Adducts with Guanine

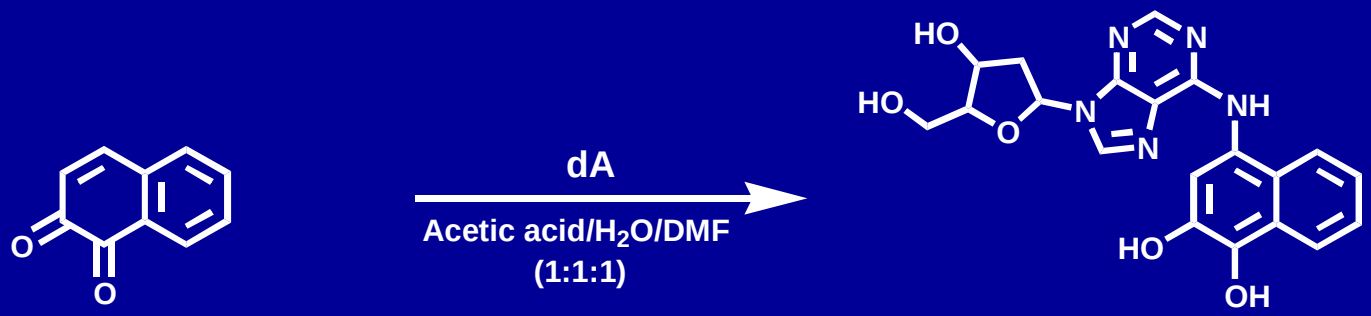


Slow Loss of Deoxyribose from 1,2-DHN-4-N7dG



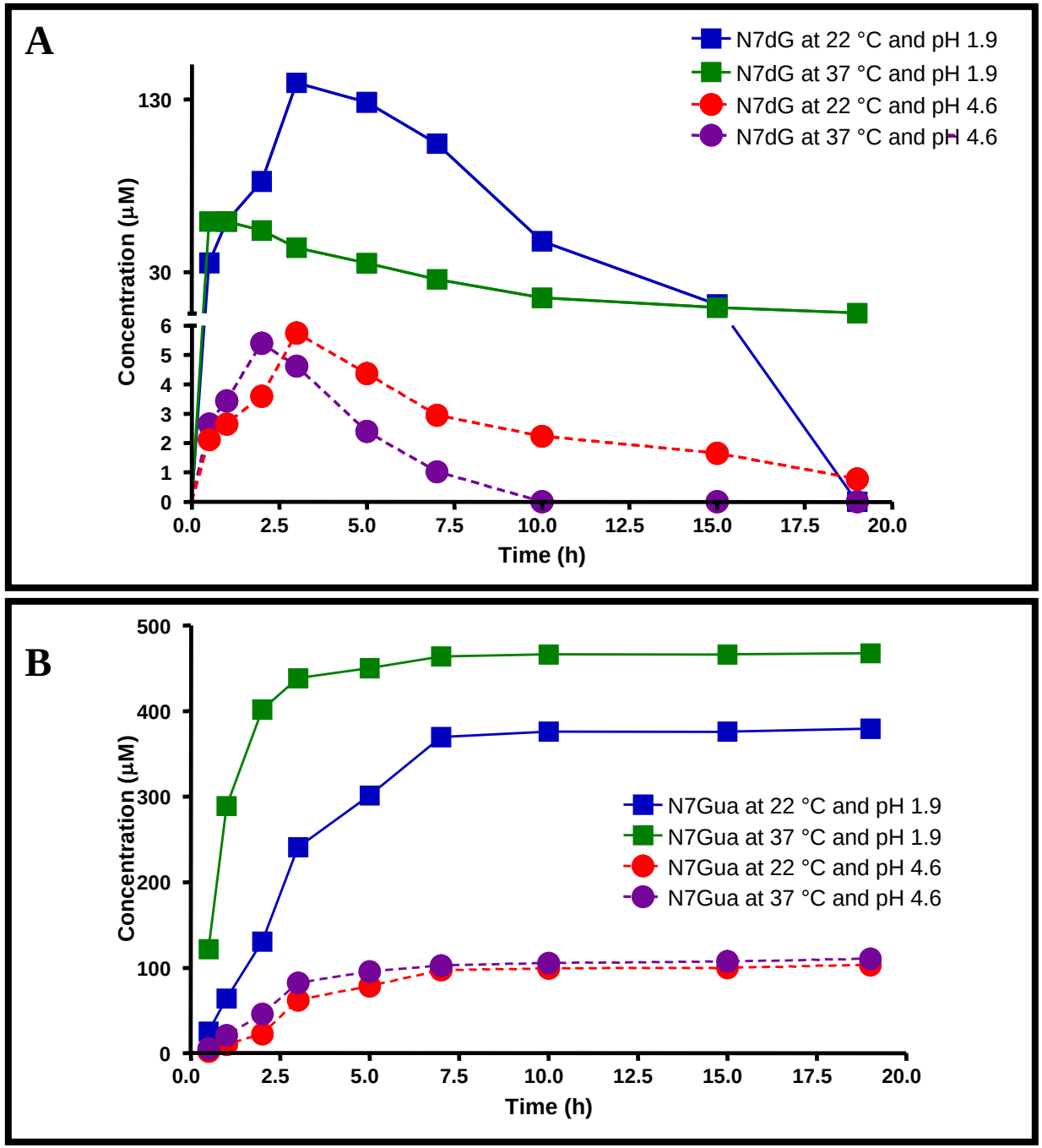
Synthesis of Naphthalene Adducts with Adenine

1,2-NQ | 1,2-DHN-4-N1Ade | 1,2-DHN-4-N^{6}Ade | 1,2-DHN-4-N3Ade



1,2-NQ | 1,2-DHN-4-N^{6}dA

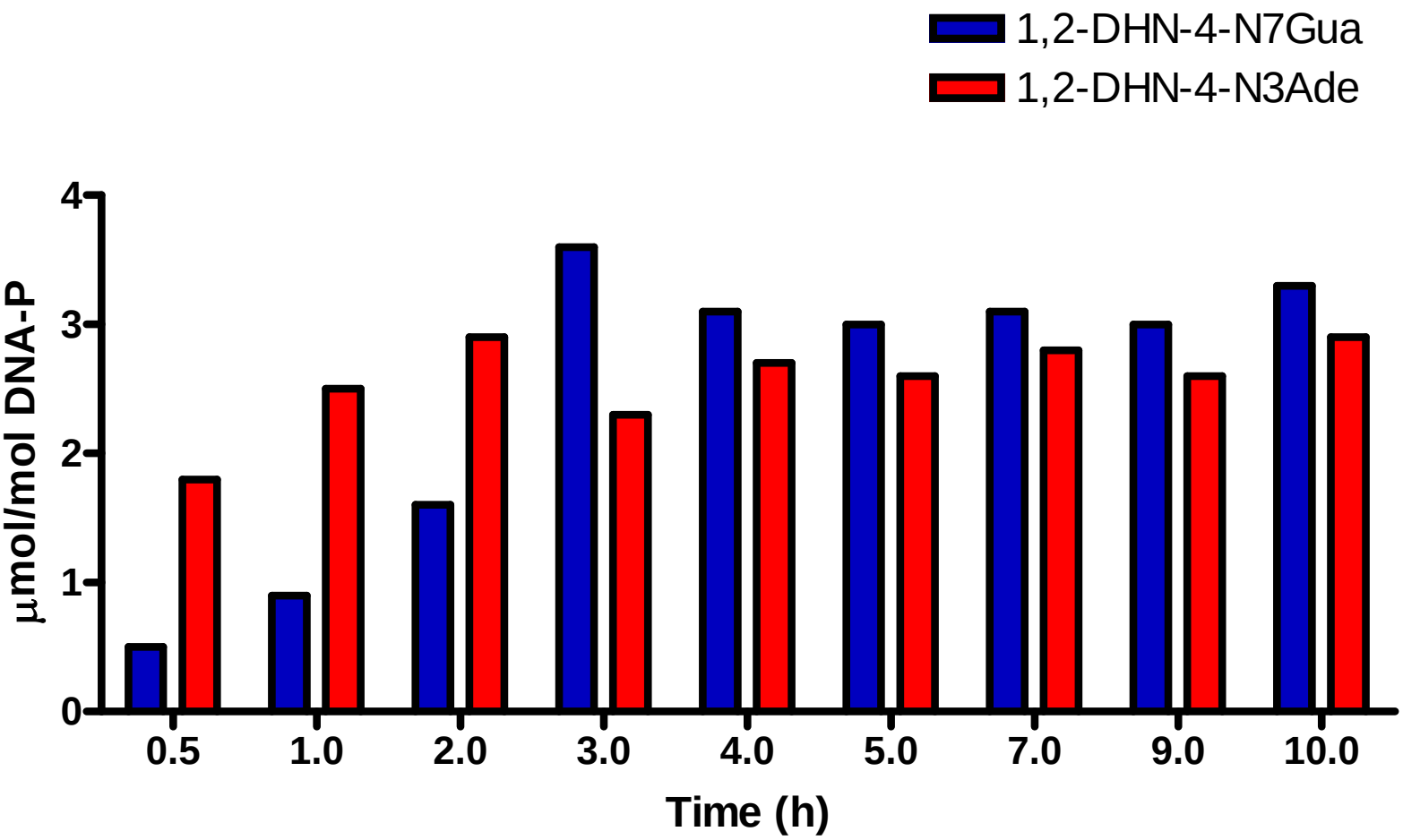
Formation of 1,2-DHN-4-N7Gua from 1,2-DHN-4-dG at various temperatures and pH



Reaction of 1,2-NQ or Enzyme-Activated 1,2-DHN with DNA

- 1,2-NQ (3.2 mM) was reacted with 3 mM calf thymus DNA in 0.067 M Na-K phosphate, pH 7.0, in 10-mL mixtures at 37 °C for 10 h. The DNA was precipitated with 2 vol. ethanol and purified for ^{32}P-postlabeling analysis of stable adducts. The ethanol-buffer supernatant was analyzed for depurinating adducts.
- 1,2-DHN (1.6 mM) was reacted with 3 mM DNA in the presence of tyrosinase, prostaglandin H synthase or MC-induced rat liver microsomes with the appropriate cofactor(s) under the same conditions.

Rate of Depurination of Adducts from DNA



Formation of adducts after reaction of 1,2-NQ with DNA[a]

Compound	Depurinating adducts µmol/mol DNA-P		Stable adducts µmol/mol DNA-P
	1,2-DHN-4-N3Ade	1,2-DHN-4-N7Gua	
1,2-NQ	2.5 ± 0.9	3.1 ± 0.7	1.6 ± 0.9
1,2-DHN			
Air	0.5 ± 0.1	0.4 ± 0.1	ND[b]
Tyrosinase	25.6 ± 2.1	17.5 ± 1.0	2.5 ± 0.3
Prostaglandin H Synthase	3.7 ± 0.3	6.4 ± 0.1	4.0 ± 0.3
MC-induced rat liver microsomes	0.8 ± 0.3	1.0 ± 0.4	3.1 ± 0.8

[a]These results are the average of three reactions.

[b]Not determined.

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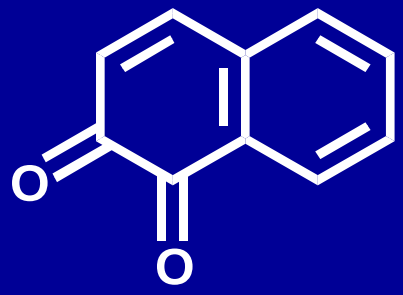
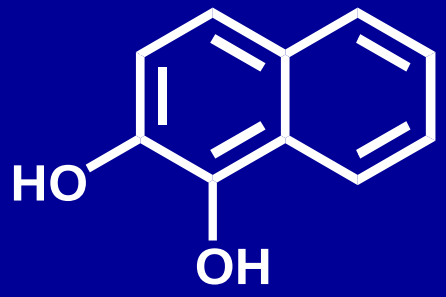
- Conclusions

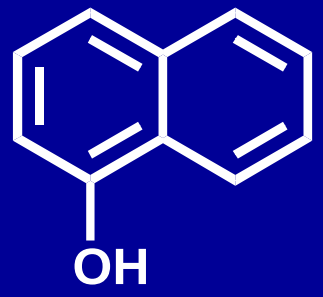
Structures of Naphthalene and Some Metabolites



1,2-Naphthoquinone
(1,2-NQ)

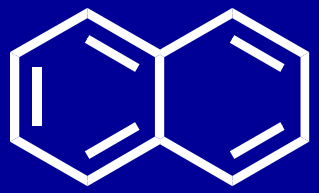


1,2-Dihydroxynaphthalene
(1,2-DHN)



1-Naphthol
(1-Nap)

Naphthalene-1,2-dihydrodiol
(1,2-DDN)

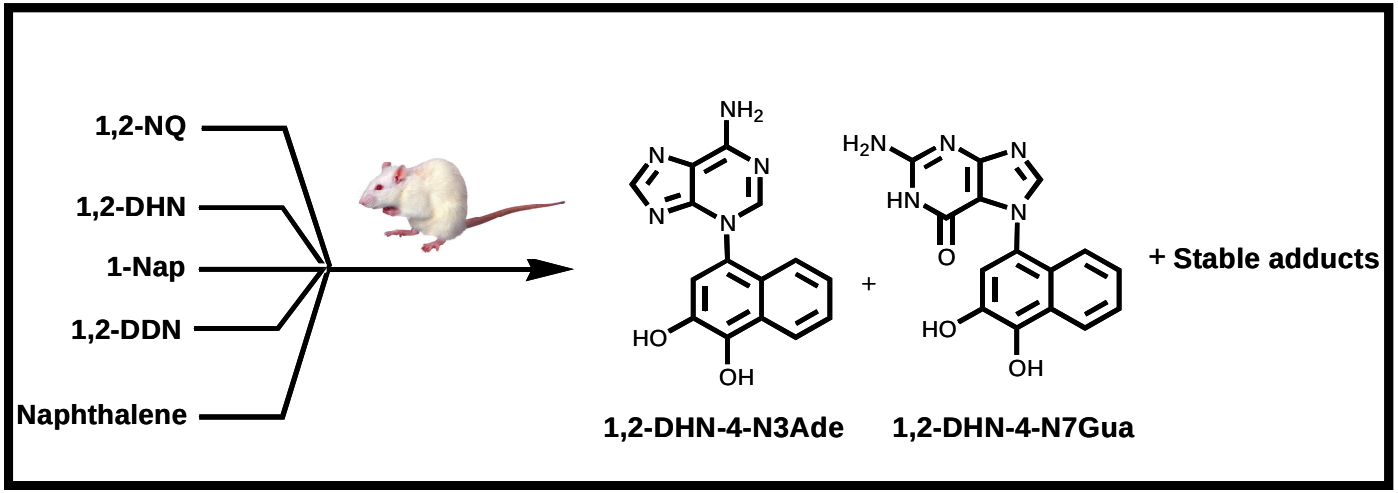


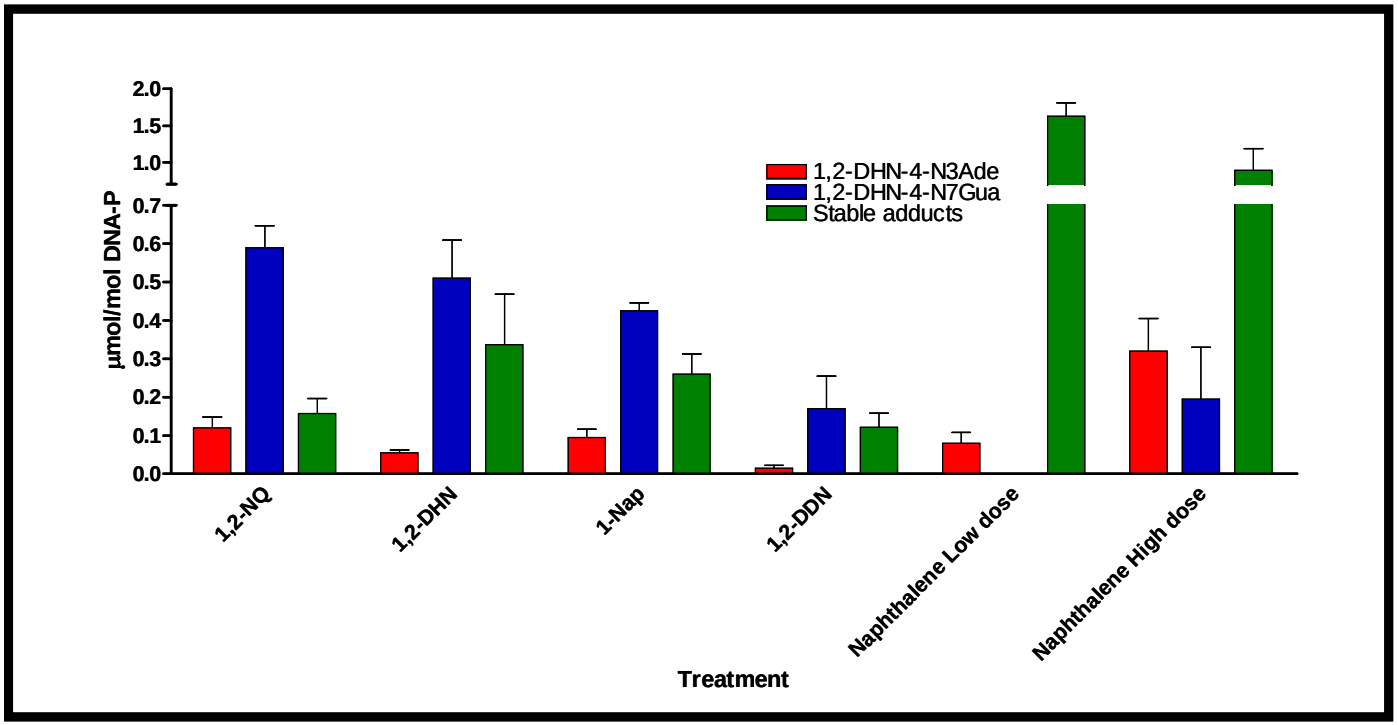
Naphthalene

Treatment of Mouse Skin with Naphthalene or Some Metabolites

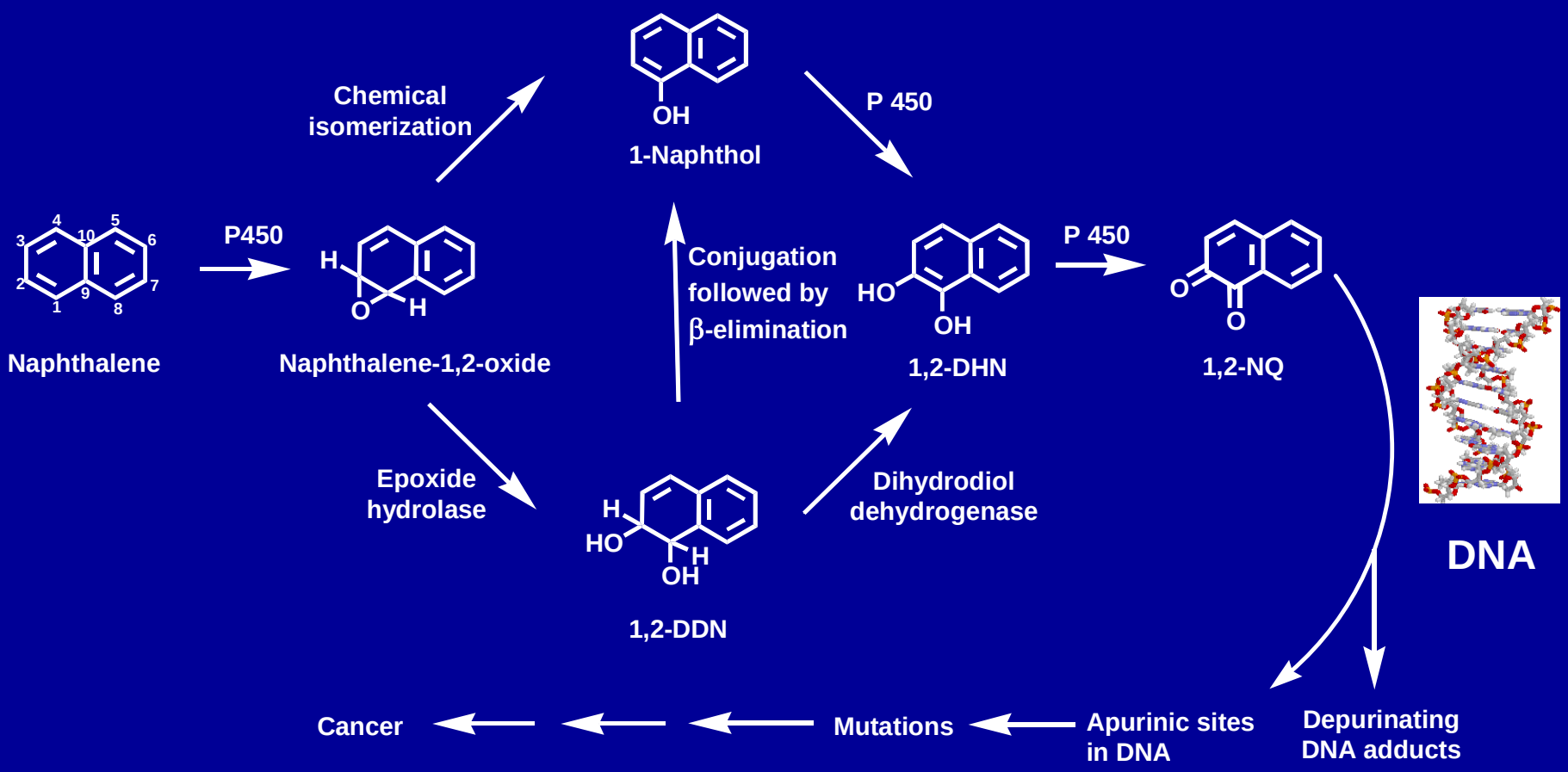
- A shaved area of dorsal skin of 8-week-old female SENCAR mice was treated with 500 nmol of naphthalene, naphthalene 1,2-dihydrodiol, 1-naphthol, 1,2-dihydroxynaphthalene or 1,2-naphthoquinone in 50 µL acetone. Naphthalene was also administered at 1,200 nmol.
- After 4 h the mice were sacrificed and the treated area of skin was excised. The epidermis was isolated, ground in liquid nitrogen and divided into 2 aliquots. One aliquot was used to purify DNA for ^{32}P-postlabeling analysis of stable adducts. The other aliquot was extracted with buffer, dried, resuspended in methanol/water (1:1), and analyzed by UPLC/MS/MS for depurinating adducts.

Depurinating and Stable DNA Adducts from Naphthalene and Some Metabolites





Mechanism of Metabolic Activation of Naphthalene to Initiate Cancer



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Conclusions

1. Naphthalene is metabolized to 1,2-NQ via two pathways. One is the conversion of 1-naphthol to 1,2-DHN, which is further enzymatically oxidized to 1,2-NQ. The other occurs via conversion of 1,2-dihydrodiol to 1,2-DHN catalyzed by dihydrodiol dehydrogenase, followed by oxidation of 1,2-DHN to 1,2-NQ.
2. Reaction of 1,2-NQ with DNA generates the depurinating N3Ade and N7Gua adducts.

Conclusions continued

3. When mouse skin is treated with naphthalene, 1,2-dihydrodiol naphthalene, 1-naphthol, 1,2-dihydroxynaphthalene or 1,2-NQ, both N7Gua and N3Ade depurinating adducts are obtained.

4. Analogously to the natural and synthetic catechol estrogen quinones, as well as the catechol quinones of benzene and dopamine, the 1,2-quinone of naphthalene reacts with DNA to form the depurinating N3Ade and N7Gua adducts.

Conclusions continued

5. Depurination of the N3Ade adduct occurs instantaneously, whereas the N7Gua adduct depurinates slowly with a half life of 1.5 h. This phenomenon is analogous to the depurination of N3Ade and N7Gua adducts of natural and synthetic estrogens, benzene and dopamine.

6. Because naphthalene cannot be metabolically activated via formation of a radical cation or a bay-region diol epoxide, the metabolic activation reported here accounts for the extremely weak carcinogenicity of this compound.

Acknowledgements

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Muhammad Saeed
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