# THE ROLE OF PEROXIDASES IN THE ACTIVATION OF CHEMICAL CARCINOGENS

[1]Marie Stiborová, [2]Eva Frei, [2]Heinz H. Schmeiser and [3]Pavel Anzenbacher

[1]*Department of Biochemistry, Faculty of Natural Sciences, Charles University, Albertov 2030, 128 40 Prague 2, Czechoslovakia*
[2]*Institute of Toxicology and Chemotherapy, German Cancer Research Center, Im Neuenheimer Feld 280, 6900 Heidelberg, Germany*
[3]*Institute of Physiology, Czechoslovak Academy of Sciences, Vídeňská 1083, 142 20 Prague 4, Czechoslovakia*

## CONTENTS



## SUMMARY

Peroxidases exhibit a wide substrate specificity with respect to various carcinogenic xenobiotics. Peroxidase-mediated activations of several carcinogenic polycyclic aromatic hydrocarbons, aromatic amines, phenols, azo dyes and N-nitrosamines are reviewed, considering their possible involvement in the initiation of chemical carcinogenesis. Activation pathways of these carcinogens examined *in vitro*, in subcellular fractions, cell cultures and *in vivo* are evaluated.

## KEY WORDS

polycyclic aromatic hydrocarbons, aromatic amines, phenol, azo dyes, N-nitrosamines, DNA-adducts

## INTRODUCTION

The induction of chemical carcinogenesis is believed to be initiated by metabolically activated carcinogens which form reactive electrophilic species that bind covalently to nucleophilic macromolecules of the cell, particularly DNA. Several isoenzymes of cytochrome P-450 are generally considered to be responsible for the oxidative metabolic activation of carcinogenic xenobiotics. It has been found, however, that several tissues containing high levels of cytochrome P-450 (e.g., liver, adrenal cortex, testis) are not typical target organs for tumor induction in humans. This indicates that many cytochromes P-450 are also involved in xenobiotics detoxication /1/. In other organs, however, not containing high cytochrome P-450 levels, the process of chemical carcinogenesis is also observed. This fact is difficult to explain. On the one hand, other enzymes are suggested to be involved in the activation of carcinogens in these tissues /1,2/. On the other hand, the carcinogens may be activated by cytochromes P-450 in tissues and/or cells rich in these enzymes (e.g., liver) and then transported (as active metabolites or their conjugates) to target organs and/or cells. Peroxidases have been considered to be another group of enzymes which can play a role in the activation of several carcinogens /1-22/.

The purpose of this paper is to review the available data supporting or proving the role of peroxidases in the activation of several carcinogens *in vitro* and *in vivo*. The latest results of our studies are also presented and discussed.

## PEROXIDASE-MEDIATED ACTIVATION OF XENOBIOTIC CARCINOGENS

Peroxidases are widely distributed in extra-hepatic target tissues, e.g., urinary bladder (prostaglandin synthetase), mammary gland (lactoperoxidase, cytochrome *c*), eosinophils (eosinophil peroxidase), leukocytes (myeloperoxidase), uterus (uterine peroxidase), thyroid (thyroid peroxidase), Zymbal gland (peroxidase) /1/, several cells of lung and kidney (prostaglandin synthetase) /2-5/. Moreover, many carcinogens that do not cause tumors of the liver were found to be substrates for peroxidases and are metabolized to electrophiles binding to macromolecules *in vitro* (e.g, benzidine /7/, 2-naphthylamine /13/, benzo(*a*)pyrene and its derivatives /2,4,5,9/,

2-aminofluorene /14/, 4-aminobiphenyl /2/, benzene metabolites such as phenol, catechol and hydroquinone /15-17/, diethylstilbestrol /2,4,5/, carcinogenic nitrofurans /8/, azo dyes /10-12/, metabolites of phenacetin such as acetaminophen and phenetidine /2,6/, aflatoxin $B_1$ /18/).

These observations have led to the hypothesis that peroxidases are activation enzymes for some carcinogens. Problems, however, arise in establishing the contribution of the peroxidase pathways in intact cells or *in vivo*. The following approaches can be used for such studies. One possibility is the administration of specific peroxidase inhibitors to experimental animals. However, difficulties in interpreting the results of such experiments because of side effects of the inhibitors should be taken into account /1,4/. A more suitable approach is the use of stable biochemical markers or endpoints for the detection of peroxidase metabolism. Such endpoints could be specific metabolites of carcinogens produced by peroxidases. The next approach involves the analysis of DNA adducts specifically derived from peroxidase activation (as opposed to other known enzymatic reactions involving the same substrate) /1/ and comparison with the DNA adducts formed *in vivo*. Such complex studies, involving all the approaches mentioned, have not yet been carried out for the majority of carcinogens. The studies of several carcinogens reported so far, however, shed light on the hypothesis that peroxidases are involved in initiation of chemical carcinogenesis.

**Polycyclic aromatic hydrocarbons**

Benzo(*a*)pyrene, a polycyclic aromatic hydrocarbon, has long been recognized as a lung and skin carcinogen. Activation of benzo(*a*)pyrene catalyzed by cytochrome P-450 is well known /2/. Metabolism of benzo(*a*)pyrene by prostaglandin H peroxidase and horseradish peroxidase leads to the formation of benzo(*a*)pyrene-quinones (1,6-dione, 3,6-dione, 6,12-dione). During this reaction, reactive radicals are formed which bind to DNA /9/. Moreover, peroxidases are also effective in the oxidation of benzo(*a*)pyrene metabolites formed *in vivo* by cytochrome P-450 and epoxide hydrolase to form highly tumorigenic 7,8-dihydroxy-7,8-dihydro-9,10-oxobenzo(*a*)pyrene. The mechanism of this reaction is distinct from that of cytochrome P-450 /19,20/.

Adrianssens *et al.* /21/ reported that the prostaglandin synthetase inhibitors aspirin and indomethacin did not reduce the

level of pulmonary DNA adducts derived from benzo(*a*)pyrene. Pulmonary cytochrome P-450 and not peroxidase is, therefore, mainly responsible for activation of benzo(*a*)pyrene in the lung. On the other hand, indirect studies using inhibitors of peroxidases as well as studies on the induction of skin tumors suggest that peroxidase activation of benzo(*a*)pyrene could occur in the skin. Treatment with specific inhibitors of prostaglandin synthetase considerably reduced the number of tumors observed /5/. In addition, the study of adducts formed by peroxy radical- and cytochrome P-450-dependent activation of (+)7,8-dihydroxy-7,8-dihydrobenzo(*a*)pyrene in mouse skin *in vitro* and *in vivo* supported this view /22/.

Prostaglandin synthetase also catalyzes the epoxidation of the potent carcinogen aflatoxin $B_1$. Rates of this peroxidase-catalyzed epoxidation are very low and, therefore, it seems that peroxidases do not contribute substantially to the tumorigenicity of this carcinogen /18/.

**Aromatic amines and phenols**

The most comprehensive studies on the role of peroxidases in carcinogen activation have been performed with aromatic amines and phenols.

Primary arylamines, such as benzidine and 2-naphthylamine, are urinary bladder carcinogens in humans and dogs /23/. Benzidine is also a hepatocarcinogen in rats, mice and hamsters /7/. Both compounds are activated by both cytochromes P-450 and peroxidases, but the products as well as the catalytic mechanisms differ from each other /1,7,13/.

Benzidine is activated by several peroxidases, such as prostaglandin synthetase, horseradish peroxidase, chloroperoxidase and lactoperoxidase. Benzidine diimine, a two-electron oxidation product of benzidine, is the predominant reactive intermediate for covalent DNA binding mediated by peroxidase /7/. Comparative studies on the structure of adducts formed by peroxidase- and cytochrome P-450-mediated benzidine activation show that (N-deoxyguanosin-8-yl)benzidine and N-(deoxyguanosin-$N^7$-yl)benzidine are formed by both activation pathways. A unique adduct formed only by peroxidase incubations was identified as N,3-(deoxyguanosin-$N^7$,$C^8$-yl)benzidine; it results from the oxidation of DNA that has been modified by benzidine diimine /7/. Intramolecular DNA-benzidine-DNA cross-links also occur as a

consequence of the peroxidative metabolism of benzidine /24/. Adduct profiles obtained from urothelial DNA hydrolysates from dogs administered benzidine *in vivo* are similar to those observed with DNA hydrolysates from peroxidase incubations /7/. The published results, therefore, strongly suggest that peroxidases play a role in benzidine-induced bladder carcinogenesis. It should be noted that DNA adducts derived from cytochrome P-450-mediated reactions are, however, also important for benzidine-dependent carcinogenic processes /1/.

Peroxidase-dependent metabolism of 2-naphthylamine *in vitro* leads to the formation of both ring- and N-oxidation products, 2-aminonaphthol and N-hydroxynaphthylamine, respectively /13/. Both reactions are also catalyzed by cytochrome P-450 monooxygenase. Ring-oxidation is usually considered a detoxicating pathway, while N-oxidation is considered to be primarily responsible for arylamine-induced carcinogenesis. The N-hydroxy arylamine metabolites are then subjected to conjugation in the liver and these conjugates are transported to the urinary bladder lumen /13/. Nitrenium ions or carbenium ions formed by decomposition of these conjugates are the ultimate carcinogens /25/. In the case of 2-naphthylamine, one third of the urothelial DNA adducts found are identical with those formed with bladder peroxidase /13/. The reaction sequence involves a radical mediated C-hydroxylation of the aromatic ring to 2-amino-1-naphthol (which is considered a detoxication product) and its subsequent oxidation to quinone imine which reacts with DNA. The other adducts are formed by N-hydroxy-2-naphthylamine, which is also produced by hepatic cytochrome P-450 monooxygenases /13/.

Administration of 2-aminofluorene to animals results in tumors not only in the liver, but also in the mammary gland, urinary bladder, intestine and the sebaceous gland of the ear duct, i.e., in organs rich in peroxidases /26/. This carcinogen is also an excellent substrate for several peroxidases forming products which bind covalently to macromolecules /1,5,6,26/. Unfortunately, C8-(N2-aminofluorenyl)deoxyguanosine, which is also formed by cytochrome P-450-dependent activation, was the only DNA adduct found in incubations of 2-aminofluorene with DNA and peroxidases from various sources /27/. Therefore, it is difficult to assess the involvement of peroxidases during its formation. This adduct was also detected in uterus DNA of 2-aminofluorene-treated rats, and its formation appears to be dependent on the activity of uterus peroxidase /27/. In addition, it was recently found that this adduct is

also formed in human intact blood neutrophils and that the adduct level correlates with the activity of neutrophil peroxidase /14/.

Although benzene is mainly myelotoxic, its primary metabolism to phenolic products occurs mainly in the liver. Toxicity and carcinogenicity of benzene metabolites such as catechol and hydroquinone (formed by cytochrome P-450 in the liver) is, however, thought to be enhanced by peroxidases in target tissues (e.g., bone marrow, Zymbal gland) /15/. Peroxidases present in these tissues oxidize hydroquinone to highly reactive benzoquinone, which is known to react directly with DNA *in vitro* /16/. Phenol and catechol are oxidized by peroxidases *in vitro* /15,16/ and phenol- and hydroquinone-DNA adducts were found in Zymbal gland cell cultures. Adducts of hydroquinone and *p*-benzoquinone were also detected in intact HL-60 cells (human promyelotic cell line, having significant myeloperoxidase activity) /17/. However, no adducts were detected in the Zymbal gland in *in vivo* experiments where the carcinogens were administered orally /16/.

**Azo dyes**

Among the carcinogenic azo dyes, the N-demethylated product of N,N-dimethylaminoazobenzene (N-methylaminoazobenzene) was studied as a potential substrate of peroxidases. Demethylation of this carcinogen and formation of products binding to macromolecules (proteins) were observed /28/.

We recently extended the study to another azo dye, the non-aminoazo compound, 1-phenylazo-2-hydroxynaphthalene (Sudan I) (a liver and urinary bladder carcinogen) /10-12,29/ and to the hepatocarcinogen N,N-dimethylaminoazo benzene /30/. Both azo dyes are activated efficiently by peroxidases *in vitro*. The levels of DNA- and tRNA-adducts formed by both azo dyes activated by liver cytochrome P-450 or peroxidase differ significantly (Table 1). Four major and two minor adducts were formed from DNA incubated *in vitro* with the ultimate metabolite of N,N-dimethylaminoazobenzene, which is produced by cytochrome P-450 /31/. Two major adducts formed from the carcinogen in these *in vitro* experiments as well as in rat liver DNA *in vivo* were identified as N-(deoxyguanosine-8-yl)- and 3-(deoxyguanosine-$N^2$-yl)-methylaminoazobenzene /31/.

N,N-Dimethylaminoazobenzene activated by peroxidase forms six major and several minor adducts with deoxynucleotides in DNA. The major adduct was with deoxyguanosine and two further

adducts with deoxyadenosine (unpublished results). The structure of these adducts has not yet been established.

Only one major adduct with deoxyguanosine is formed from DNA reacted with Sudan I activated by cytochrome P-450 /32,33; Stiborová *et al.*, manuscript in preparation), but four major and several minor adducts are formed after activation of Sudan I by peroxidase /12/.

In animals exposed to Sudan I, the urine contains only low quantities of the parent compound (Sudan I), but high quantities of its C-hydroxy derivatives resulting from the oxidation of the parent dye in the liver by cytochrome P-450 /34/. Further studies on the major oxidized derivatives of Sudan I (ring-hydroxy derivatives) formed by liver cytochrome P-450 *in vitro* /32/ show that these derivatives are rapidly oxidized by peroxidase forming active metabolites that bind to DNA and tRNA (Table 2). The patterns obtained by the $^{32}$P-postlabeling assay /35/ show the formation of several adducts with deoxynucleotides /36/. Thus the products of detoxication of Sudan I may also be converted to ultimate carinogens by peroxidase inside the urinary bladder *in vivo*. Indeed, our preliminary results obtained from an *in vivo* study on adduct formation in the urinary bladder of rats treated with Sudan I show certain similarities with results obtained *in vitro*.

The results obtained with Sudan I indicate that not only the parent carcinogens should be examined as potential substrates for peroxidase, but also such metabolites that are considered detoxication products. The combination of reactions catalyzed by cytochromes P-450 and peroxidases seems to be crucial for the final activation of carcinogens. This suggestion is also supported by the observation that metabolites of benzo(*a*)pyrene or 2-naphthylamine formed by cytochrome P-450 are activated by peroxidases (see above).

## N-Nitrosamines

N-Nitroso compounds are a unique group of chemicals exerting wide organ- and species-specificity in their carcinogenic effects. Tumors can be obtained in nearly all organs, depending on the route of administration and the structure of the nitrosamine. Some of the target organs are rich in cytochromes P-450 (e.g., liver, nasal cavity, esophagus), some rich in peroxidases (e.g., kidney, lung, urinary bladder) /3,37/.

Although cytochrome P-450 is believed to be the key activating

**TABLE 1**

Binding of Sudan I and N,N-dimethylaminoazobenzene (DAB) activated by cytochrome P-450 and peroxidase to nucleic acids *in vitro*

| Activating system | DNA binding | | tRNA binding | |
|---|---|---|---|---|
| | (nmol/mg) | | | |
| | DAB | Sudan I | DAB | Sudan I |
| Complete cytochrome P-450 system | 0.4 ± 0.01 | 0.36 ± 0.02 | 0.71 ± 0.02 | 0.6 ± 0.02 |
| without cyt P-450 | 0 | 0 | 0 | 0 |
| without NADPH | 0.01 ± 0.001 | 0.01 ± 0.005 | 0.02 ± 0.01 | 0.009 ± 0.001 |
| Complete peroxidase system | 2.3 ± 0.2 | 4.1 ± 0.3 | 14.06 ± 0.07 | 14.0 ± 0.9 |
| without peroxidase | 0 | 0 | 0 | 0 |
| without $H_2O_2$ | 0 | 0 | 0 | 0 |

Incubation mixtures (1.5 ml) contained in the cyt P-450 system: 50 mM potassium phosphate (pH 7.7), 2 mM NADPH, 3.5 mg microsomal proteins, 0.2 mM $^{14}$C-Sudan I or $^{14}$C-DAB, 2 mg of tRNA or DNA; in the peroxidase system: 50 mM Tris-HCl buffer (pH 7.46 for DAB and 8.4 for Sudan I), 0.2 mg of peroxidase (from horseradish), 1.5 mM $H_2O_2$, 0.2 mM $^{14}$C-Sudan I or $^{14}$C-DAB and 2 mg of tRNA or DNA. Incubation time was 120 min.

**TABLE 2**

The binding of 1-(4'-hydroxyphenylazo)-2-hydroxynaphthalene (4'-OH-Sudan I) and 1-phenylazo-2,6-dihydroxynaphthalene (6-OH-Sudan I) activated by peroxidase to DNA and tRNA

| Compound | Binding to (nmol/mg) | | % of converted compound |
|---|---|---|---|
| | DNA | tRNA | |
| 4'-OH-Sudan I | 2.1 ± 0.08 | 1.9 ± 0.1 | 60.1 ± 1.2 |
| 6-OH-Sudan I | 3.3 ± 0.10 | 5.9 ± 0.5 | 93.3 ± 2.0 |

Experimental conditions were as in Table 1 except that C-hydroxy-derivatives of $^{14}$C-Sudan were used.

enzyme system for these carcinogens, other specific activating systems cannot be excluded /3,37,38/.

Prostaglandin synthetase is present at significant concentrations in several types of lung cell (pulmonary neuroendocrine cells, alveolar type II cells and pulmonary endothelia) /3,39/. The involvement of this enzyme in the activation of some N-nitrosamines has been suggested /3/, based on indirect *in vivo* studies with inhibitors of prostaglandin synthetase. Aspirin and indomethacin inhibited efficiently the metabolism of N-nitrosodiethylamine in alveolar type II cells of lungs /39/. Moreover, in hamsters injected with these inhibitors prior to administration of radiolabeled N-nitrosodiethylamine a pronounced decrease in alkylation of macromolecules of pulmonary cells was demonstrated, while inhibitors of cytochrome P-450 did not affect the level of alkylation /3,40/.

We showed that two N-nitrosamines (N-nitrosomethylaniline and N-nitrosomethylbenzylamine) are oxidized by peroxidase *in vitro*. Both compounds cause tumors in esophagus and lung /37,38/.

The same products derived from $\alpha$-C-hydroxylations (aldehydes) were detected as in reactions catalyzed by cytochrome P-450, but in lower amounts (Table 3). During the oxidation of N-nitrosomethylaniline by peroxidase, very reactive metabolites with short half-lives are generated, which bind to nucleic acids, as in the case of carcinogenic azo dyes (Table 4). A mechanism of reaction catalyzed by peroxidase involving radicals, which differs from that catalyzed by cytochrome P-450, was detected /30/.

Several adducts with DNA and tRNA were detected using the $^{32}$P-postlabeling method (unpublished results). The pattern of adducts detected by this method will be compared with the pattern obtained in DNA of animals treated with N-nitrosomethylaniline to strengthen our *in vitro* results.

Although clear evidence for the participation of peroxidases in the initiation of cancer by N-nitrosamines has not yet been established, the activation of N-nitrosomethylaniline by peroxidase described in this paper could play a certain role in N-nitrosomethylaniline disposition, especially in lung cells rich in peroxidases.

**TABLE 3**

Oxidation of N-nitrosomethylaniline (NMA) and N-nitrosomethylbenzylamine (NMBA) by peroxidase

| Concentration of nitrosamines in reaction mixture (mM) | Oxidation products of NMA | NMBA | |
|---|---|---|---|
| | formaldehyde | formaldehyde | benzaldehyde |
| 0 | 0 | 0 | 0 |
| 0.5 | 0.72 | 0.015 | 0.19 |
| 1.0 | 1.10 | 0.13 | 0.29 |
| 1.5 | - | 0.40 | 0.59 |
| 2.0 | 1.30 | 0.65 | 1.14 |
| 3.0 | 1.44 | 0.89 | 1.27 |

Incubation mixtures (3 ml) contained 50 mM potassium phosphate (pH 7.7), 200 $\mu$g of horseradish peroxidase, 1 mM $H_2O_2$ and 0-3 mM nitrosamines. Aldehydes are expressed as nmol aldehyde/min/mg peroxidase.

**TABLE 4**

Dependence of the binding of carcinogens to DNA and tRNA on time of addition of nucleic acids into reaction mixture

| Time of addition of nucleic acids in the reaction medium (min) | | DNA binding (nmol/mg) | tRNA binding (nmol/mg) |
|---|---|---|---|
| N,N-dimethylaminoazobenzene | (0) | 2.30 ± 0.20 | 14.06 ± 0.007 |
| | (10) | 0.10 ± 0.02 | 1.85 ± 0.02 |
| Sudan I | (0) | 4.10 ± 0.30 | 14.00 ± 0.30 |
| | (15) | 0.053 ± 0.008 | 1.41 ± 0.30 |
| N-nitrosomethylaniline | (0) | 0.60 ± 0.10 | 3.01 ± 0.12 |
| | (15) | – | 0.3 ± 0.02 |

Experimental conditions were as in Table 1.

## CONCLUSIONS

Great attention is now focused upon the extra-hepatic transformation of various carcinogens. We can conclude from this review that several peroxidases participate in this transformation. It is evident from the work cited in this paper that peroxidase activation of carcinogens can occur not only with purified peroxidases, but also in subcellular fractions, cell culture systems and *in vivo*. Future detailed *in vitro* studies on the activation of parent carcinogens as well as their metabolites formed by other enzymes (mainly cytochrome P-450 monooxygenases) should be carried out to understand better the peroxidative biotransformation processes in chemical carcinogenesis.

## REFERENCES

1. O'Brien PJ. Radical formation during the peroxidase catalyzed metabolism of carcinogens and xenobiotics: the reactivity of these radicals with GSH, DNA, and unsaturated lipid. Free Radical Biol Med 1988; 4: 169-183.
2. Guengerich FP. Enzymatic oxidation of xenobiotic chemicals. CRC Critical Rev Biochem Molec Biol 1990; 25: 97-153.
3. Schuller HM, Falzon M, McMahon JB. Metabolic activation and biological effects of nitrosamines in the mammalian lung. Pharmac Ther 1990; 46: 95-103.
4. Krauss LJ, Eling TE. Arachidonic acid-dependent cooxidation: a potential pathway for the activation of chemical carcinogens in vivo. Biochem Pharmacol 1984; 33: 3319-3324.
5. Eling TE, Boyd J, Reed G, Mazon R, Sivarajah K. Xenobiotic metabolism by prostaglandin endoperoxide synthetase. Drug Metab Rev 1983; 14: 1023-1053.
6. Meunier B. Horseradish peroxidase: a useful tool for modeling the extra-hepatic biooxidation of exogens. Biochimie 1987; 69: 3-9.
7. Yamazoe Y, Zenser TV, Miller DW, Kadlubar FF. Mechanism of formation and structural characterization of DNA adducts derived from peroxidative activation of benzidine. Carcinogenesis 1988; 9: 1635-1641.
8. Murasaki G, Zenser TV, Davis BB, Cohen SM. Inhibition by aspirin of N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide-induced bladder carcinogenesis and enhancement of forestomach carcinogens. Carcinogenesis 1984; 5: 53-55.
9. Cavalieri EL, Devanesan PD, Rogan EG. Radical cations in the horseradish peroxidase and prostaglandin H synthase mediated metabolism and binding of benzo(*a*)pyrene to deoxyribonucleic acid. Biochem Pharmacol 1988; 37: 2183-2187.
10. Stiborová M, Asfaw B, Anzenbacher P. Activation of carcinogens by peroxidase. Horseradish peroxidase-mediated formation of benzenediazonium ion from a non-aminoazo dye, 1-phenylazo-2-hydroxynaphthalene

(Sudan I) and its binding to DNA. FEBS Lett 1988; 232: 387-390.

11. Stiborová M, Frei E, Klokow K, Wiessler M, Safarík L, Anzenbacher P, Hradec J. Peroxidase-mediated reaction of the carcinogenic non-aminoazo dye 1-phenylazo-2-hydroxynaphthalene with transfer ribonucleic acid. Carcinogenesis 1990; 11: 1789-1794.
12. Stiborová M, Frei E. Schmeiser HH, Wiessler M, Hradec J. Mechanism of formation and $^{32}$P-postlabeling of DNA adducts derived from peroxidative activation of carcinogenic non-aminoazo dye 1-phenylazo-2-hydroxy-naphthalene (Sudan I). Carcinogenesis 1990; 11: 1843-1848.
13. Yamazoe Y, Miller DW, Wies CC, Dooley KL, Zenser TV, Beland FA, Kadlubar FF. DNA adducts formed by ring-oxidation of the carcinogen 2-naphthylamine with prostaglandin H synthase in vitro and in the dog urothelium in vivo. Carcinogenesis 1985; 6: 1379-1387.
14. Shen J-H, Wegenke M, Wolff T. Capability of human blood cells to form the DNA adduct, C8-(N2-aminofluorenyl)-deoxyguanosine-3',5'-diphosphate from 2-aminofluorene. Carcinogenesis 1990; 11: 1441-1444.
15. Sadler A, Subrahmanyam VV, Ross D. Oxidation of catechol by horseradish peroxidase and human leukocyte peroxidase: reactions of *o*-benzoquinone and *o*-benzosemiquinone. Toxicol Appl Pharmacol 1988; 93: 62-71.
16. Reddy MV, Bleicher WT, Blackburn GR, Mackerer CR. DNA adduction by phenol, hydroquinone, or benzoquinone in vitro but not in vivo: nuclease P1-enhanced $^{32}$P-postlabeling of adducts as labeled nucleoside bisphophates, dinucleotides and nucleoside monophosphates. Carcinogenesis 1990; 11: 1349-1357.
17. Levay G, Pongracz K, Bodell WJ. Detection of DNA adducts in HL-60 cells treated with hydroquinone and *p*-benzoquinone by $^{32}$P-postlabeling. Carcinogenesis 1991; 12: 1181-1186.
18. Battista JR, Marnett LJ. Prostaglandin H synthase-dependent epoxidation of aflatoxin $B_1$. Carcinogenesis 1985; 6: 1227-1229.
19. Dix TA, Fontana R, Panthani A, Marnett LJ. Hematin-catalyzed epoxidation of 7,8-dihydroxy-7,8-dihydrobenzo(*a*)pyrene by polyunsaturated fatty acid hydroperoxides. J Biol Chem 1985; 260: 5358-5365.
20. Eling TE, Curtis J, Battista JR, Marnett LJ. Oxidation of (+)-7,8-dihydroxy-7,8-dihydrobenzo(*a*)pyrene by mouse keratinocytes: evidence for peroxyl radical- and monooxygenase-dependent metabolism. Carcinogenesis 1986; 7: 1957-1963.
21. Adrianssens PI, Sivarajah K, Boorman GA, Eling TE, Anderson MW. Effect of aspirin and indomethacin on the formation of benzo(*a*)pyrene-induced pulmonary adenomas and DNA adducts in A/HeJ mice. Cancer Res 1983; 43: 4762-4767.
22. Pruess-Schwartz D, Nimesheim A, Marnett LJ. Peroxy radical- and cytochrome P-450-dependent metabolic activation of (+)7,8-dihydroxy-7,8-dihydrobenzo(*a*)pyrene in mouse skin in vitro and in vivo. Cancer Res 1989; 49: 1732-1737.
23. Garner RC, Martin CN, Clayson DB. Carcinogenic aromatic amines and related compounds. In: Searle CE, ed, Chemical Carcinogens, 2nd edition, Vol, 1, ACS Monograph 182. Washington DC: American Chemical Society, 1984; 175-302.

24. Fourney RM, O'Brien PJ, Davidson WS. Peroxidase catalyzed aggregation of plasmid pBR322 DNA by benzidine metabolites in vitro. Carcinogenesis 1986; 7: 1535-1542.
25. Kadlubar FF. Metabolism and DNA binding of carcinogenic aromatic amines. ISI Atlas of Science: Pharmacology 1987; 1: 129-132.
26. Huang J, Dunford HB. One-electron oxidative activation of 2-aminofluorene by horseradish peroxidase Compound I and II: spectral and kinetic studies. Arch Biochem Biophys 1991; 287: 257-262.
27. Wegenke M, Shen JH, Wolff T. Metabolic activation of 2-aminofluorene in extrahepatic rat tissues: involvement of peroxidase and of cytochrome P-450. Mutat Res 1988; 203: 213.
28. Kadlubar FF, Frederick CB, Weis CC, Zenser TV. Prostaglandin endoperoxide synthetase-mediated metabolism of carcinogenic aromatic amines and their binding to DNA and protein. Biochem Biophys Res Comm 1982; 108: 253-258.
29. Stiborová M, Frei E, Anzenbacher P. Study on oxidation and binding to macromolecules of the carcinogenic non-aminoazo dye 1-phenylazo-2-hydroxynaphthalene catalyzed by horseradish (*Amoracia rusticana* L.) peroxidase. Biochem Physiol Pflanzen 1991; 187: 227-236.
30. Stiborová M, Frei E, Schmeiser HH, Anzenbacher P. Comparison of cytochrome P-450 and peroxidase-mediated activations of chemical carcinogens. In: Proc 7th Int Conf Biochemistry & Biophysics of cytochrome P-450; structure & function, biotechnological & ecological aspects. Moscow 1991, in press.
31. Beland FA, Tullis DL, Kadlubar FF, Straub KM, Evans FE. Characterization of DNA adducts of the carcinogen N-methyl-4-aminoazobenzene in vitro and in vivo. Chem-Biol Interact 1980; 31: 1-17.
32. Stiborová M, Asfaw B, Anzenbacher P, Leseticky L, Hodek P. The first identification of the benzene-diazonium ion formation from a non-aminoazo dye, 1-phenylazo-2-hydroxynaphthalene (Sudan I) by microsomes of rat livers. Cancer Lett 1988; 40: 319-326
33. Stiborová M, Asfaw B, Anzenbacher P, Hodek P. A new way to carcinogenicity of azo dyes: the benzenediazonium ion formed from a non-aminoazo dye, 1-phenylazo-2-hydroxynaphthalene (Sudan I) by microsomal enzymes binds to deoxyguanosine residues of DNA. Cancer Lett 1988; 40: 327-333.
34. Childs JJ, Clayson DS. The metabolism of 1-phenylazo-2-naphthol in the rabbit. Biochem Pharmacol 1966; 15: 1247-1258.
35. Reddy MV, Randerath K. Nuclear P1-mediated enhancement of sensitivity of $^{32}$P-postlabeling test for structurally diverse DNA adducts. Carcinogenesis 1986; 7: 1543-1551.
36. Stiborová M, Frei E, Schmeiser HH, Wiessler M, Hradec J. Products of detoxication of carcinogenic azo dye Sudan I are bound to nucleic acids after activation by peroxidase. Carcinogenesis 1991; submitted for publication.
37. Preussmann R, Wiessler M. The enigma of the organ-specificity of carcinogenic nitrosamines. Trends Pharm Sci 1987; 8: 185-189.
38. Dipple A, Michejda ChJ, Weisburger EK. Metabolism of chemical carcinogens. In: Grunberger D, Goff S, eds, Mechanism of Cell

Transformation by Carcinogenic Agents. New York: Pergamon, 1987; 1-32.
39. Falzon M, McMahon JB, Gazdar AF, Schuller HM. Preferential metabolism of N-nitrosodiethylamine by two cell lines derived from pulmonary adenocarcinomas. Carcinogenesis 1986; 7: 17-22.
40. Hegedus TJ, Falzon M, Margaretten N, Gazdar AF, Schuller HM. Inhibition of N-diethylnitrosamine metabolism by human lung cancer cell lines with features of well differentiated pulmonary endocrine cells. Biochem Pharmacol 1987; 36: 3339-3343.