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TUMOR INITIATING ACTIVITIES OF VARIOUS DERIVATIVES OF BENZ(A)ANTHRACENE AND 7,12-DIMETHYL-BENZ(A)ANTHRACENE IN MOUSE SKIN.[1]

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DISCLAIMER

Running Title: DMBA AND BA TUMORIGENICITY

Abbreviations:

PAH, polycyclic aromatic hydrocarbons;

BP, benzo(a)pyrene;

BP-7,8-diol, (+)trans-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene;

BP-7β,8α-diol-9α,10α-epoxide, (+)trans-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene;

BA, benz(a)anthracene;

BA 1,2-diol, (+)trans-1,2-dihydroxy-1,2-dihydrobenz(a)anthracene;

BA 3,4-diol, (+)trans-3,4-dihydroxy-3,4-dihydrobenz(a)anthracene;

BA 5,6-diol, (+)trans-5,6-dihydroxy-5,6-dihydrobenz(a)anthracene;

BA 8,9-diol, (+)trans-8,9-dihydroxy-8,9-dihydrobenz(a)anthracene;

BA 10,11-diol, (+)trans-10,11-dihydroxy-10,11-dihydrobenz(a)anthracene;

BA 3,4-diol-1,2-epoxide I, (+)trans-3α,4β-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydrobenz(a)anthracene;

BA 1,2-diol-3,4-epoxide I, (+)trans-1β,2α-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydrobenz(a)anthracene;

BA 8,9-diol-10,11-epoxide I, (+)trans-8β,9α-dihydroxy-10α,11α-epoxy-8,9,10,11-tetrahydrobenz(a)anthracene;

BA 8,9-diol-10,11-epoxide II, (+)trans-8β,9α-dihydroxy-10β,11β-epoxy-8,9,10,11-tetrahydrobenz(a)anthracene;

DMBA, 7,12-dimethylbenz(a)anthracene;

7-OHM-12-MBA, 7-hydroxymethyl-12-methylbenz[a]anthracene; 7-OHM-12-MBA-5,6-oxide, 7-hydroxymethyl-12-methylbenz[a]anthracene-5,6-oxide; 7-CHO-12-MBA, 7-formyl-12-methylbenz[a]anthracene; 12-CHO-7-MBA, 12-formyl-7-methylbenz[a]anthracene; 7,12-diCHOBA, 7,12-diformylbenz[a]-anthracene; 7-BRME-12-MBA, 7-bromomethyl-12-methylbenz[a]anthracene; 12-BRME-7-MBA, 12-bromomethyl-7-methylbenz[a]anthracene;

Abbreviations (continued):

1-, 2-, 3-, 4-, 5-, 9- and 10-PHDMBA, various phenols of DMBA; DMBA 5,6-diol, (+)trans-5,6-dihydroxy-5,6-dihydro-DMBA; DMBA 8,9-diol, (+)trans 8,9-dihydroxy-8,9-dihydro-DMBA; DMBA 8,9-diol-10,11-epoxide (+)trans-8β,9α-dihydroxy-10α,10α-epoxy-8,9,10,11-tetrahydro-DMBA; 1-CH_3-, 2-CH_3-, 2-CH_3, and 5-CH_3-DMBA, either 1-, 2 or 5, 7,12-trimethylbenz(a)anthracene; 1-, 2-, 5-, and 11-fl-DMBA, 1-, 2-, 5- and 11-fluoro-DMBA; TPA, 12-O-tetradecanoyl-phorbol-13-acetate.

Introduction

Current information indicates that polycyclic aromatic hydrocarbons (PAH) exert their toxic, mutagenic and carcinogenic activities after they have been metabolically activated by target cells to reactive epoxides (1, 11, 14, 23, 30). The results obtained from *in vivo* and *in vitro* binding (3, 6, 17, 22, 24, 27, 40), mutagenicity (12, 26, 41, 42), metabolism (30, 38, 39, 49), and carcinogenicity (4, 18-20, 32-34) studies have led to the conclusion that BP-7,8-diol is a proximate carcinogenic metabolite of BP, and the BP-diol-epoxide is an ultimate carcinogenic metabolite of BP. Recent results concerning the strong carcinogenicity of BP-7β,8α-diol-9α,10α-epoxide in newborn mice (16) and in mouse skin (36) strongly indicate that it is the ultimate carcinogenic metabolite of BP.

Since diol-epoxides may be responsible for the carcinogenicity of PAH other than BP, we have undertaken the testing of diols and diol-epoxides as well as other derivatives of PAH for skin tumor-initiation in a two-stage system of tumorigenesis. In addition, since activation of methylated PAH may involve the side-chain methyl group, the skin tumor-initiating activity of various side-chain derivatives of methylated PAH are being determined. Specifically, in this report we are comparing the skin tumor initiation of various derivatives of a nonmethylated PAH, BA as well as a methylated PAH, DMBA. The data suggests that "bay region" diol-epoxides may be important in BA and DMBA carcinogenicity in mice which is supportive of the theory proposed by Jerina and co-workers which predicts that diol-epoxides in the "bay region" are the major determinants of PAH carcinogenicity (13, 15). A "bay region" occurs in a PAH when an angularly fused benzo ring is present (13). The "bay

regions" of BP, BA and DMBA are shown in Figure 1.

Materials and Methods

Chemicals. BA was purchased from Aldrich Chemical Co., Milwaukee, Wisconsin and DMBA from Sigma Chemical Co., St. Louis, Missouri, with both being more than 99% pure. TPA was obtained from Dr. Peter Borchert, University of Minnesota, Minneapolis, Minnesota. The DMBA and BA diols and diol-epoxides were synthesized and purified as previously described (8, 10). The DMBA and BA diol-epoxides were applied topically in tetrahydrofuran (distilled over $LiAlH_4$ and stored over sodium wire). The following compounds were synthesized by the method described in the cited reference: 7-OHM-12MBA (2), 7-OHM-12-MBA-5,6-oxide (31), 7-CHO-12-MBA (29), 12-CHO-7-MBA (29), 7,12-diCHOBA (29), 7-BrME-12-MBA (2), and 12-BrMe-7-MBA (29). The 1-, 2-, 5-, and 11-fluoro DMBA, the 1-, 2-, and 5-CH_3DMBA and the 1-, 2-, 3-, 4-, 5-, 9-, and 10-OHDMBA were generous gifts from Professor M. Newman, Ohio State University, Columbus, Ohio. The above compounds plus DMBA and BA were applied topically in spectro-quality acetone. In some cases other reactive compounds such as 7-OHM-12-MBA-5, 6-oxide and the Bromo derivatives of DMBA were applied in tetrahydrofuran. All hydrocarbons were consistently prepared under yellow light immediately before use. The mice were treated topically with the above compounds under subdued light with the time lapse between preparation of the above solutions and animal treated being less than one-half hour. TPA was prepared in stock solutions and kept in a freezer until use. Mice received twice weekly applications of 10 μg of TPA 1 week after treatment.

Tumor Experiments. Female CD1 mice were purchased from Charles River Farms, North Wilmington, Massachusetts. Mice, 7 to 9 weeks old, were shaved with surgical clippers 2 days before treatment and only those in the resting phase of the hair cycle were used. In the tumor experiments groups of 30 animals received a single topical application of the test compound, followed 1 week later by twice weekly applications of TPA. The incidence of both papillomas and carcinomas was recorded weekly and papillomas and carcinomas were removed at random for histological verification.

Results and Discussion

Carcinogenicity of BA Derivatives. Shown in Table 1 are the skin-tumor-initiating activities of BA and the 1,2-, 3,4-, 5,6-, 8,9- and 10,11-diols of BA. It is quite evident that BA 3,4-diol was the most active tumor initiator when compared with BA and its other diols. After 26 weeks of promotion with TPA, BA 3,4-diol had induced tumors in 85% of the animals, with an average of 4.7 papillomas per mouse, whereas BA induced tumors in 57% of the animals, with approximately one papilloma per mouse. The 1,2- and 8,9-diols of BA had weak tumor-initiating activity, whereas the other diols were extremely weak. Although BA 3,4-diol at a dose level of 2 μmoles is a good tumor initiator when compared with BA, its tumor-initiating activity is less impressive when compared with BP at a dose of 0.2 μmole (Table 1).

The incidence of skin tumors in female CD1 mice after a single topical application of BA, BA 3,4-diol-1,2-epoxide I, BA 1,2-diol-3,4-epoxide I, BA 10,11-diol-8,9-epoxide I, BA 8,9-diol-10,11-epoxide I, or BA 8,9-diol-10,11-epoxide II, followed by twice weekly applications of TPA, is shown in Table 2. BA 3,4-diol-1,2-epoxide I was found to be approximately five times more active

than BA. This should be noted as a first example of a reactive intermediate of a PAH being more active as a tumor initiator on mouse skin than the parent hydrocarbon. BA 8,9-diol-10,11-epoxide I had weak tumor-initiating activity in mouse skin, whereas the other diol epoxides were extremely weak in this regard.

Wood et al. recently reported that BA 3,4-diol-1,2-epoxides, in which the epoxide forms part of the "bay region" were more mutagenic to bacterial cells than were the other diol epoxides (44, 46). Wood et al. also reported that BA-3,4-diol was more tumorigenic toward mice than were BA or the four other possible trans diols of BA (45). In our studies we found that both the 3,4-dihydrodiol and the 3,4-diol-1,2-epoxide of BA are much stronger skin tumor initiators than BA or the other possible diols and diol epoxides of BA (37). In addition we reported that BA 3,4-diol was a potent mutagen in mammalian cells (37).

It should be emphasized that in most in vivo animal tumorigenic models, as well as in in vitro cell transformation systems, BA is generally considered to be noncarcinogenic (35). Previously we reported that, although not a complete skin carcinogen, BA is a weak tumor initiator in a two-stage tumorigenic system (35).

Carcinogenicity of DMBA Derivatives. The skin tumor-initiating activities of various substituted derivatives of DMBA are shown in Table 3. Both 7-OHM-12MBA and 12-OHM-7MBA were found to be strong tumor-initiators but less active than DMBA. 7BrME-12-MBA, 12BrMe-7-MBA, and 7CHO-12-MBA were also strong tumor-initiators but once again less so than DMBA. 12-CHO-7-MBA and 7,12-diCHO-BA were less active than 7-CHO-12-MBA, whereas 7-OHM-12-MBA-5,6-oxide was found to be essentially inactive (Table 3). It is of interest to point out that

7-CHO-12-MBA was found to be nearly as potent as DMBA in producing cancer in the rat whereas, as stated above, 7-CHO-12-MBA was found to have strong skin tumor-initiating activity although of a lesser degree than DMBA. In addition, Pataki and Huggins (28) stated that when two or three methyl groups were added to BA, in positions 6, 7, 8 and 12 (any combination), the resulting products were equally carcinogenic in the rat. However, we found that 6,8-dimethyl BA as well as 3,9-dimethyl BA and 5,7-dimethyl BA had very weak skin tumor initiating activity when compared to DMBA (Table 4).

Direct evidence for determining the importance of the "bay region" diol-epoxide of DMBA is difficult because of the hindrance of the side chain methyl groups during synthesis. However, the influence on carcinogenicity of the addition of methyl and fluoro groups in the bay region would provide indirect evidence. Table 4 shows that either a methyl or fluoro addition to the 1,2 and 5 positions almost completely destroyed the skin tumor-initiating activity whereas a fluoro addition to position 9 and 11 did not. Harvey and Dunne (9) recently reported that DMBA fluorinated at positions 1 and 2 also drastically reduced the sarcomagenic activity of DMBA when injected into rats although fluorination at position 11 did not. These results suggest that, indeed, the bay region of DMBA is involved in the metabolic activation of DMBA into a carcinogen. In addition, our data, implicate the 5 position in such an activation. Moschel et al. (25) have recently presented fluorescence data which implicates the bay region diol-epoxide of DMBA in the interaction of DMBA with cellular DNA. In addition, binding of DMBA to cells in culture analyzed by LH20 chromatography also suggests that a bay region diol epoxide is involved in DMBA binding (7).

Table 5 shows the relative skin tumor-initiating activities of the

various derivatives of DMBA. The 1-, 2-, 3-, 4- and 5-OH DMBA were found not to have skin tumor-initiating activity whereas the 9 and 10-OH DMBA had weak activity. The 5,6- and 8,9-diols of DMBA were also found to be inactive and the (±)DMBA 8β,9α-diol-10α,11α-epoxide, a non-bay region diol-epoxide, had only weak skin tumor-initiating activity. The difficult synthesis of the bay region diol of DMBA has been recently achieved (unpublished results). In the early stages of an ongoing tumor experiment, DMBA 3,4-diol has approximately 10 times the skin tumor-initiating activity as DMBA (Table 5). Lack of tumorigenicity of DMBA 8,9-diol and DMBA 8β,9α-diol-10α,11α-epoxide, the results concerning the fluoro and methyl substitution of DMBA, the extreme potency of DMBA 3,4-diol in initiating skin tumors plus studies on binding of DMBA to cellular DNA after metabolism (7, 25) strongly implicates the "bay region" diol-epoxide of DMBA in its carcinogenic activity. The results in this report plus those recently published (4, 5, 12, 13, 15, 16, 18-21, 26, 32-34, 36, 37, 43-46, 48) provide evidence that "bay region" diol-epoxides are involved in BP, BA chrysene, 7-methylbenz(a)anthracene, dibenz(a,h)anthracene and DMBA carcinogenicity and mutagenicity. Again, this data supports the theory proposed by Jerina and co-workers, which predicts that diol-epoxides in the "bay region" are the principle determinants of PAH carcinogenicity (13, 15).

Acknowledgements

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Table 1

Skin-tumor-initiating activities of BP, BA, and BA dihydrodiols after TPA tumor promotion

All the BA compounds were applied at a dose of 2 μmoles, whereas BP was given at 0.2 μmole. Each of these treatments was followed 1 week later by twice weekly applications of 10 μg of TPA.

Initiator	No. of mice[a]	Papillomas/mouse at:[b]		Mice with tumors (%) at:[c]	
		15 wk	26 wk	15 wk	26 wk
BP	29	2.0	5.3	60	92
BA	30	0.7	1.2	37	57
BA 1,2-dihydrodiol	30	0.2	0.4	13	33
BA 3,4-dihydrodiol	28	3.2	4.7	80	85
BA 5,6-dihydrodiol	29	0.1	0.2	10	14
BA 8,9-dihydrodiol	30	0.2	0.8	18	46
BA 10,11-dihydrodiol	29	0.1	0.2	10	14
BA control[d]	30	0	0	0	0
TPA control[e]	29	0	0.1	0	6

[a] Surviving at the 26th week after promotion.

[b] Total number of papillomas divided by the total number of surviving mice.

[c] Percentage of surviving mice with papillomas.

[d] Treated once with 2 μmoles of BA, not followed by promotion.

[e] Treated with TPA only, twice weekly for 26 weeks.

Table 2

Skin-tumor-initiating activities of BA and BA diol epoxides after TPA tumor promotion

All the compounds were applied at a dose of 2 μmoles and were followed 1 week later by twice weekly applications of 10 μg of TPA.

Initiator	No. of mice[a]	Papillomas/mouse at:[b]		Mice with tumors (%) at:[c]	
		15 wk	26 wk	15 wk	26 wk
BA	30	0.5	1.0	38	52
BA 1,2-diol-3,4-epoxide I	28	0	0.1	0	10
BA 3,4-diol-1,2-epoxide I	27	4.2	5.0	100	100
BA 8,9-diol-10,11-epoxide I	29	0.3	0.6	25	40
BA 8,9-diol-10,11-epoxide II	28	0	0.2	0	20
BA 10,11-diol-8,9-epoxide I	29	0.1	0.2	7	17

[a] Surviving at the 26th week after promotion.

[b] Total number of papillomas divided by the total number of surviving mice.

[c] Percentage of surviving mice with papillomas.

Table 3

Tumor-initiating ability of various 7- and 12-substituted derivatives of DMBA[a]

Hydrocarbon	Dose[b]	No. of mice[c]	Time of 1st tumor[d]	Papillomas/ mouse[e]	Mice with Tumors (%)[f]
DMBA	10	29	8	4.50	90
7-OHM-12-MBA-5,6-oxide	200	31	24	0.10	10
7-OHM-12-MBA-5,6-oxide	400	30	15	0.17	17
7-CHO-12-MBA	740	28	9	6.10	84
12-CHO-7-MBA	740	28	11	0.75	54
7,12-diCHOBA	704	30	15	0.75	52
7-BRME-12-MBA	600	30	7	6.80	96
12-BRME-7-MBA	600	29	7	7.20	87
7-OHM-12-MBA	400	29	12	1.80	48
12-OHM-7-MBA	200	29	9	1.73	73

[a]Thirty mice were used per experiment and all mice were promoted one week after initiation with twice weekly applications of 10 µg TPA for 30 weeks.

[b]Doses of all hydrocarbons are expressed in nmoles.

[c]Number of mice surviving at the 30th week.

[d]Time to appearance of first tumor in weeks.

[e]Total number of papillomas divided by total number of surviving mice.

[f]Percent of surviving mice with papillomas.

Table 4

Skin tumor-initiating activities of various fluoro and methyl substituted DMBA after TPA promotion[a]

DMBA and Derivative	No. of mice[b]	Papillomas per mouse[c]	Mice with tumors (%)[d]
Control (only DMBA initiation)[e]	30	0	0
Control (only TPA promotion)[f]	29	0.1	6
DMBA	28	9.1	100
1-CH_3DMBA	30	0.03	3
2-CH_3DMBA	30	0.10	7
5-CH_3DMBA	29	0.40	34
1-fl DMBA	30	0.03	3
2-fl DMBA	29	0.10	10
5-fl DMBA	28	0.20	15
11-fl DMBA	29	8.2	100
6,8-dimethyl BA	29	0.7	40
3,9-dimethyl BA	29	0.07	3
5,7-dimethyl BA	28	0.03	3

[a]DMBA and DMBA derivatives were applied at a dose of 200 nmoles and were followed one week later by twice weekly applications of 10 µg of TPA.

[b]Surviving at the 30th week after promotion.

[c]Total number of papillomas divided by total number of surviving mice.

[d]Percentage of surviving mice with tumors.

[e]These mice were only initiated with 200 nmoles of DMBA.

[f]These mice were only promoted twice weekly with 10 µg of TPA for 30 weeks.

Table 5

Skin tumor-initiating activities of various derivatives of DMBA after TPA promotion[a]

DMBA and Derivatives	No. of mice[b]	Papillomas per mouse[c]	Mice with tumors (%)[d]
Control (only DMBA initiation)[e]	30	0	0
Control (only TPA promotion)[f]	29	0.1	6
DMBA	28	8.86	100
1-OH	29	0.10	10
2-OH	30	0.07	7
3-OH	30	0.14	14
4-OH	28	0.17	14
5-OH	29	0.10	10
9-OH	30	0.40	24
10-OH	30	0.47	30
3,4-diol[g]	30	3.3	80
5,6-diol	29	0.10	10
8,9-diol	29	0.21	18
8,9-diol-10,11-epoxide	28	0.30	20

[a]The DMBA and DMBA derivatives were applied topically at a dose of 200 nmoles and were followed one week later by twice weekly applications of 10 µg of TPA.

[b]Surviving at the 20th week after promotion.

[c]Total number of papillomas divided by total number of surviving mice.

[d]Percentage of surviving mice with tumors.

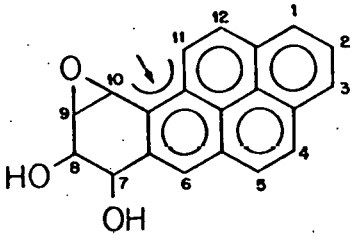
[e]These mice were only initiated with 200 nmoles of DMBA.

[f]These mice were only promoted twice weekly with 10 µg of TPA for 30 weeks.

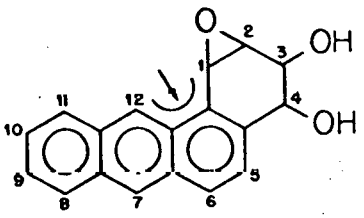
[g]Tumor response after 9 weeks of promotion for 100 nmoles of DMBA-3,4-diol.

BAY REGION DIOL-EPOXIDES

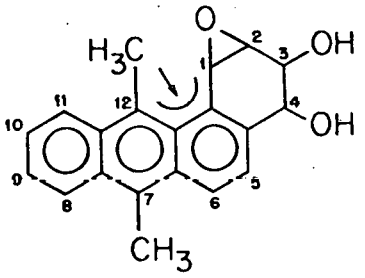
BP



BA



DMBA



References

1. Ames, B. N., Dursten, W. E., Yamasaki, E., and Lee, F. D. (1973): Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. Proc. Natl. Acad. Sci. 70: 2281-2885.

2. Boyland, E. and Sims, P. (1965): Metabolism of polycyclic compounds. The metabolism of 7,12-dimethylbenz[a]anthracene by rat-liver homogenates. Biochem. J. 95: 780-787.

3. Borgen, A., Darvey, H., Castagnoli, N., Crocker, T. T., Rasmussen, R. E., and Wang, I. Y. (1973): Metabolic conversion of benzo(a)pyrene by syrian hamster liver microsomes and binding of metabolites to DNA. J. Med. Chem. 16: 502-506.

4. Chouroulinkov, I., Gentil, A., Grover, P. L., and Sims, P. (1976): Tumor-initiating activities on mouse skin of dihydrodiols derived from benzo(a)pyrene. Br. J. Cancer 34: 523-532.

5. Chouroulinkov, I., Gentil, A., Tierney, B., Grover, P., and Sims, P. (1977): The metabolic activation of 7-methylbenz(a)anthracene in mouse skin: High tumor-initiating activity of the 3,4-dihydrodiol. Cancer Letters 3: 247-253.

6. Dandel, P., Duquesne, M., Vigny, P., Grover, P. L., and Sims, P. (1975); Fluorescence spectral evidence that benzo(a)pyrene-DNA products in mouse skin arise from diol-epoxides. FEBS Lett. 57: 250-253.

7. Dipple, A., and Nebzydoski, J. A. (1978): Evidence for the involvement of a diol-epoxide in the binding of 7,12-dimethylbenz(a)anthracene to DNA in cells in culture. Chem.-Biol. Interactions 20: 17-26.

8. Fu, P. P., and Harvey, R. G. (1977): Synthesis of the Diols and Diol Epoxides of Carcinogenic Hydrocarbons. Tetrahedron Lett. 2059-2062.

9. Harvey, R. G., and Dunne, F. A. (1978): Multiple regions of metabolic activation of carcinogenic hydrocarbons. Nature 273: 566-567.

10. Harvey, R. G., and Sukumoran, K. B. (1977): Synthesis of the A-Ring Diols and Diol Epoxides of BA. Tetrahedron Lett. 2387-2390.

11. Heidelberger, C. (1975): Chemical carcinogenesis. Ann. Rev. Biochem. 44: 79-121.

12. Huberman, E., Sachs, L., Yang, S. D., and Gelboin, H. V. (1976): Identification of mutagenic metabolites of benzo(a)pyrene in mammalian cells. Proc. Natl. Acad. Sci. 73: 607-611.

13. Jerina, D. M., and Daly, J. W. (1977): Oxidation at Carbon. In: D. V. Parke and R. L. Smith (eds.), Drug Metabolism, pp. 15-33. London: Taylor & Francis, Ltd.

14. Jerina, D. M., and Daly, J. W. (1974): Arene oxides. A new aspect of drug metabolism. Sci. 185: 573-582.

15. Jerina, D. M., Lehr, R. E., Yagi, H., Hernandez, O., Dansette, P. M., Wislocki, P. G., Wood, A. W., Chang, R. L., Levin, W., and Conney, A. H. (1977): Mutagenicity of Benzo(a)pyrene Derivatives and the Description of a Quantum Mechanical Model Which Predicts the Ease of Carbonium Ion Formation from Diol Epoxides. In: F. J. de Serres, J. R. Fouts, J. R. Bend, and R. M. Philpot (eds.), In Vitro Metabolic Activation and Mutagenesis Testing, pp. 159-177. Amsterdam: Elsevier/North Holland Biomedical Press.

16. Kapitulnik, J., Levin, W., Conney, A. H., Yagi, H., and Jerina, D. M. (1977): Benzo(a)pyrene 7,8-dihydrodiol is more carcinogenic than benzo(a)pyrene in newborn mice. Nature 266: 378-380.

17. King, H. W. S., Osborne, M. R., Beland, F. A., Harvey, R. G. and Brooks, P. (1976): (±)-7α, 8β-dihydroxy-9β, 10β-epoxy-7,8,9,10-tetrahydrobenzo(a)-pyrene is an intermediate in the metabolism and binding to DNA of benzo(a)-pyrene. Proc. Natl. Acad. Sci. U.S. 73: 2679-2681.

18. Levin, W., Wood, A. W., Yagi, H., Jerina, D. M., and Conney, A. H. (1976): (±)-trans-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene: A potent skin carcinogen when applied topically to mice. Proc. Natl. Acad. Sci. U.S., 73: 3867-3871.

19. Levin, W., Wood, A. W., Chang, R. L., Slaga, T. J., Yagi, H., Jerina, D. M. and Conney, A. H. (1977): Marked differences in the tumor-initiating activity of optically pure (+)- and (-)-trans-7,8-dihydroxy-7,8-dihydro-benzo(a)pyrene on mouse skin. Cancer Res. 37: 2721-2725.

20. Levin, W., Wood, A. W., Yagi, H., Dansette, P. M., Jerina, D. M. and Conney, A. H. (1976): Carcinogenicity of benzo(a)pyrene 4,5-, 7,8- and 9,10-oxides on mouse skin. Proc. Natl. Acad. Sci. U.S. 73: 243-247.

21. Malaveille, C., Bartsch, H., Grover, P. L., and Sims, P. (1975): Mutagenicity of non-K-region diols and diol epoxides of benzo(a)anthracene and benzo(a)pyrene in S. typhimurium TA100. Biochem. Biophys. Res. Commun. 66: 693-700.

22. Meehan, T., Straub, K. and Calvin, M. (1976): Elucidation of hydrocarbon structure in an enzyme catalyzed benzo(a)pyrene-poly (G) covalent complex. Proc. Natl. Acad. Sci. U.S. 73: 1437-1441.

23. Miller, E. C., and Miller, J. A. (1974): Biochemical mechanisms of chemical carcinogenesis. In: Busch (ed.). Molecular Biology of Cancer, pp. 377-402. New York, Acad. Press, Inc.

24. Moore, P. D., Koreeda, M., Wislocki, P. G., Levin, W., Conney, A. H., Yagi, H. and Jerina, D. M. (1977): In vitro reactions of the diastereomeric 9,10-epoxides of (+)- and (-)-trans-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene with polyguanylic acid and evidence for formation of an enantiomer of each diastereomeric 9,10-epoxide from benzo(a)pyrene in mouse skin. In: D. M. Jerina (ed.), Concepts in Drug Metabolism No. 44, pp. 127-154, Washington, D. C.

25. Moschel, R. C., Baird, W. M., and Dipple, A. (1977): Metabolic activation of the carcinogen 7,12-dimethylbenz(a)anthracene for DNA binding. Biochem. Biophys. Res. Commun. 76: 1092-1098.

26. Newbold, R. F., and Brooks, P. (1976): Exceptional mutagenicity of a benzo(a)pyrene diol epoxide in cultured mammalian cells. Nature 261: 52-54.

27. Osborne, M. R., Thompson, M. H., Tarmy, E. M., Beland, F. A., Harvey, R. G. and Brooks, P. (1976): The reaction of 7,8-dihydro-7,8-dihydroxybenzo(a)-pyrene-9,10-oxide with DNA in relation to the benzo(a)pyrene DNA products isolated from cells. Chem.-Biol. Interactions 13: 343-348.

28. Pataki, J. and Huggins, C. B. (1969): Molecular site of substituents of benzo(a)anthracene related to carcinogenicity. Cancer Res. 29: 506-509.

29. Pataki, J., Wlos, R. and Cho, Y-J. (1971): Adrenocorticolytic derivatives of benz(a)anthracene. J. Med. Chem. 11: 1083-1086.

30. Sims, P. and Grover, P. L. (1974): Epoxides in polycyclic aromatic hydrocarbon metabolism and carcinogenesis. Advan. Cancer Res. 20: 165-274.

31. Sims, P. (1973): Epoxy derivatives of aromatic polycyclic hydrocarbons. The preparation and metabolism of epoxides related to 7,12-dimethylbenz(a)anthracene. Biochem. J. 131: 405-413.

32. Slaga, T. J., Bracken, W. M., Viaje, A., Levin, W., Yagi, H., Jerina, D. M. and Conney, A. H. (1977): Comparison of the tumor-initiating activities of benzo(a)pyrene arene oxides and diol-epoxides. Cancer Res. (in press).

33. Slaga, T. J., Viaje, A., Berry, D. L., Bracken, W., Buty, S. G. and Scribner, J. D. (1976): Skin tumor initiating ability of benzo(a)pyrene 4,5-, 7,8-, and 7,8-diol-9,10-epoxides and 7,8-diol. Cancer Letters 2: 115-122.

34. Slaga, T. J., Viaje, A., Bracken, W. M., Berry, D. L., Fischer, S. M., Miller, D. R., and LeClerc, S. M. (1977): Skin tumor initiating ability of benzo(a)pyrene-7,8-diol-9,10-epoxide (anti) when applied topically in tetrahydrofuran. Cancer Letters 3: 23-30.

35. Slaga, T. J., Bowden, G. T., Scribner, J. D., and Boutwell, R. K. (1974): Dose-response studies on the ability of 7,12-dimethylbenz(a)anthracene and benz(a)anthracene to initiate skin tumors. J. Natl. Cancer Inst. 53: 1337-1340.

36. Slaga, T. J., Bracken, W. J., Gleason, G., Levin, W., Yagi, H., Jerina, D. M., and Conney, A. H. (1978): Marked differences in the skin tumor-initiating activities of the optical enantiomers of the diastereomeric benzo(a)pyrene 7,8-diol-9,10-epoxides. Cancer Res. (in press).

37. Slaga, T. J., Huberman, E., Selkirk, J. K., Harvey, R. G., and Bracken, W. M. (1978): Carcinogenicity and mutagenicity of benz(a)anthracene diols and diol-epoxides. Cancer Res. 38: 1699-1704.

38. Thakker, D. R., Yagi, H., Akagi, H., Koreeda, M., Lu, A. Y. H., Levin, W., Wood, A. W., Conney, A. H., and Jerina, D. M. (1977): Metabolism of benzo(a)pyrene. VI. Stereoselective metabolism of benzo(a)pyrene and benzo(a)pyrene 7,8-dihydrodiol to diol epoxides. Chem.-Biol. Interact. 16: 281-300.

39. Thakker, D. R., Yagi, H., Lu, A. Y. H., Levin, W., Conney, A. H. and Jerina, D. M. (1976): Metabolism of benzo(a)pyrene: Conversion of (+)-trans-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene to highly mutagenic 7,8-diol-9,10-epoxides. Proc. Natl. Acad. Sci. U.S. 73: 3381-3385.

40. Weinstein, I. B., Jeffrey, A. M., Jennette, K. W., Blobstein, S. H., Harvey, R. G., Harris, C., Autrup, H., Kasai, H. and Nakanishi, K. (1976): Benzo(a)pyrene diol epoxides as intermediates in nucleic acid binding in vitro and in vivo. Science 193: 592-595.

41. Wislocki, P. G., Wood, A. W., Chang, R. L., Levin, W., Yagi, H., Hernandez, O., Dansette, P. M., Jerina, D. M., and Conney, A. H. (1976): Mutagenicity and cytotoxicity of benzo(a)pyrene oxides, phenols, quinones and dihydrodiols in bacterial and mammalian cells. Cancer Res. 36: 3350-3357.

42. Wislocki, P. G., Wood, A. W., Chang, R. L., Levin, W., Yagi, H., Hernandez, O., Jerina, D. M. and Conney, A. H. (1976): High mutagenicity and toxicity of a diol epoxide derived from benzo(a)pyrene. Biochem. Biophys. Res. Commun. 68: 1006-1012.

43. Wislocki, P. G., Wood, A. W., Chang, R. L., Levin, W., Yagi, H., Hernandez, O., Jerina, D. M., and Conney, A. H. (1976): High mutagenicity and toxicity of a diol epoxide derived from benzo(a)pyrene. Biochem. Biophys. Res. Commun. 68: 1006-1012.

44. Wood, A. W., Chang, R. L., Levin, W., Lehr, R. E., Schoefer-Redder, M., Karle, J. M., Jerina, D. M., and Conney, A. H. (1977): Mutagenicity and Cytotoxicity of benz(a)anthracene diol epoxides and tetrahydroepoxides: Exceptional activity of the bay region 1,2-epoxides. Proc. Natl. Acad. Sci. U.S. 74: 2746-2750.

45. Wood, A. W., Levin, W., Chang, R. L., Lehr, R. E., Schaefer-Redder, M., Karle, J. M., Jerina, D. M., and Conney, A. H. (1977): Tumorigenicity of five dihydrodiols of benz(a)anthracene on mouse skin: Exceptional activity of benz(a)anthracene-3,4-diol. Proc. Natl. Acad. Sci. U.S. 74: 3176-3179.

46. Wood, A. W., Levin, W., Lu, A. Y. H., Ryan, D., West, S. B., Lehr, R. E., Schaefer-Redder, M., Jerina, D. M., and Conney, A. H. (1976): Mutagenicity of metabolically activated benz(a)anthracene-3,4-dihydrodiol. Evidence for Bay Region Activation of Carcinogenic Polycyclic Hydrocarbons. Biochem. Biophys. Res. Commun. 72: 680-686.

47. Wood, A. W., Levin, W., Ryan, D., Thomas, P. E., Yagi, H., Moh, H. D., Thakker, D. R., Jerina, D. M., and Conney, A. H. (1977): High mutagenicity of metabolically activated chrysene-1,2-dihydrodiol. Evidence for bay region activation of chrysene. Biochem. Biophys. Res. Commun. 78: 847-854.

48. Wood, A. W., Levin, W., Thomas, P. E., Ryan, D., Karle, J. M., Yagi, H., Jerina, D. M. and Conney, A. H. (1978): Metabolic activation of dibenzo(a,h)anthracene and its dihydrodiols to bacterial mutagens. Cancer Res. 38: 1967-1973.

49. Yang, S. K., McCourt, D. W., Roller, P. P., and Gelboin, H. V. (1976): Enzymatic conversion of benzo(a)pyrene leading predominantly to the diol-epoxide r-7, t-8-dihydroxy-t-9,10-oxy-7,8,9,10-tetrahydrobenzo(a)-pyrene through a single enantiomer of r-7, t-8-dihydroxy-7,8-dihydrobenzo(a)-pyrene. Proc. Natl. Acad. Sci. U.S. 73: 2594-2598.