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Cell-mediated Mutagenicity in Chinese Hamster V79 Cells of Dibenzopyrenes and Their Bay-Region Fluorine-substituted Derivatives[1]

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ABSTRACT

The polycyclic aromatic hydrocarbons dibenzo(a,i)pyrene and dibenzo(a,h)pyrene, each of which possesses two bay regions, and their bay-region difluorinated derivatives were tested for mutagenicity for ouabain and 6-thioguanine resistance in Chinese hamster V79 cells. Since V79 cells do not metabolize polycyclic aromatic hydrocarbons, mutagenesis was tested in both the presence and the absence of golden hamster embryo cells capable of metabolizing polycyclic aromatic hydrocarbons. Neither of the dibenzopyrenes nor their fluorinated derivatives were mutagenic in the absence of the golden hamster embryo cells. In the presence of these cells (cell-mediated assay), both dibenzopyrenes were mutagenic, whereas the difluorinated derivatives, 2,10-difluorodibenzo(a,i)pyrene and 3,10-difluorodibenzo(a,h)pyrene, either were inactive or exhibited (on a dose basis) a weak response. However, the mutagenicity of the dibenzopyrenes was eliminated when they were coincubated with 7,8-benzoflavone, a mixed-function oxidase inhibitor. The results suggest that metabolic oxidation of these polycyclic aromatic hydrocarbons at the bay region (presumably to diol-epoxides) is required for a mutagenic response in the cell-mediated assay.

INTRODUCTION

DBPs[4] are in the environment as constituents of coal tar (29), polluted urban air (15, 33), exhaust fumes from gasoline engines (25), and cigarette smoke condensate (26). Two of these DBPs, DB(a,i)P and DB(a,h)P, are potent carcinogens; the sarcomagenic effect of DB(a,i)P exceeds that of B(a)P (11, 12, 14, 16, 36). A number of studies with bacterial mutagenesis assays in which activation was carried out in Aroclor-induced rat liver homogenates have shown DBPs to be mutagenic (2, 6, 32).

In this study, we used Chinese hamster V79 cells to assay for the mutagenicity of DBPs and their fluorinated derivatives to further establish the relationship between mutagenesis of PAHs in the cell-mediated assay and their ability to induce tumors in experimental animals; we further sought to determine the role of the bay region in the activation of DBPs to mutagens for mammalian cells. Resistance to OUA and TG served as the genetic markers. Metabolism of the PAHs in the cell-mediated assay was supplied by hamster embryo fibroblasts.

MATERIALS AND METHODS

Chemicals. DB(a,h)P was a gift from Dr. Pierre Jacquignon; DB(a,i)P was obtained from Koch-Light Laboratories, Toyle, England. 2,10-Difluoro-DB(a,i)P was synthesized by the method described by Boger *et al.* (5). 3,10-Difluoro-DB(a,h)P was prepared by a base-catalyzed double cyclization procedure described recently (27). The 3-fluoro- and 2-fluoro-DB(a,i)P derivatives were synthesized according to the methods described by Boger *et al.* (5) and Sardella *et al.* (28).

Cell Culture. Chinese hamster V79 cells, originating from subclone V79-4 (Professor E. Y. Chu, University of Michigan, Ann Arbor, Mich.), were used as target cells for mutagenesis, and secondary and tertiary cultures derived from whole embryos of golden hamsters served as the PAH-metabolizing cells in the cell-mediated mutagenesis assay (18–20). Both cell types were propagated in Dulbecco's modified Eagle's medium with 10% fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.). The cultures were maintained in a humidified incubator at 37° and were supplied with a constant amount (10%) of CO_2 in air.

For the cell-mediated assay, 2×10^6 hamster embryo cells (X-ray irradiated with 5000 R) were seeded in 3 ml of medium in 60-mm Petri dishes and incubated overnight. Their plating efficiency ranged from about 50 to 70%. On the following day, the irradiated cells were overlaid with 1 ml of medium containing 3×10^5 V79 cells/dish. The hydrocarbons in 1 ml of medium were added 4 to 5 hr later. Cultures were then incubated for 48 hr, after which the medium was removed. The cells were dissociated with EDTA-trypsin and counted in a hemacytometer. The V79 cells could be easily distinguished from the larger and granulated irradiated hamster embryo cells. For determination of cloning efficiency and consequently of cytotoxicity of the tested hydrocarbons, the cells were seeded in 8 dishes in 4 ml of medium at 200 cells/dish. The number of colonies was determined 7 days later after Giemsa staining. For determination of the number of OUA^R mutants, 16 to 32 dishes were seeded with 5×10^4 V79 cells/dish. OUA (1 mM final concentration) was added 2 days after seeding. The number of mutant colonies was determined 12 to 14 days later, after Giemsa staining. The number of TG^R mutants was determined after a 7-day expression time, which we have found previously to be optimal. After cultivation, the cultures were dissociated, and a total of 2×10^6 V79 cells were seeded into 100-mm Petri dishes in 10 ml of medium without TG. After a total of 7 days (a period which included one subculture to prevent confluency), the cells were dissociated, and the V79 cells were seeded at 200 cells/dish (8 dishes) for determination of the cloning efficiency and at 2×10^4 cells/dish (16 to 32 dishes) for selection of TG resistance. TG was added at a final concentration of 30 μM. Cloning efficiency was determined 7 days after seeding, and TG resistance

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[4] The abbreviations used are: DBP, dibenzopyrene; DB(*a,i*)P, dibenzo(*a,i*)pyrene; DB(*a,h*)P, dibenzo(*a,h*)pyrene; B(*a*)P, benzo(*a*)pyrene; PAH, polycyclic aromatic hydrocarbon; OUA, ouabain; TG, 6-thioguanine; 2,10-difluoro-DB(*a,i*)P, 2,10-difluorodibenzo(*a,i*)pyrene; 3,10-difluoro-DB(a,h)P, 3,10-difluorodibenzo(*a,h*)pyrene; 3-fluoro-DP(a,i)P, 3-fluorodibenzo(*a,i*)pyrene; 2-fluoro-DB(a,i)P, 2-fluorodibenzo(*a,i*)pyrene; OUA^R, ouabain-resistant; TG^R, 6-thioguanineresistant; BF, 7,8-benzoflavone; 3,10-difluoro-DB(a,h)P, 3,10-difluorodibenzo(*a,h*)pyrene.

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was determined after 11 days. The mutation frequencies for OUA and TG resistance were determined per 10^6 colony-forming cells based on cloning efficiency and the number of cells seeded for mutant selection. Maintenance of the drug-resistant phenotype was established by tests on a series of isolated mutant cells after they were subcultured for 2 to 6 weeks in the absence of a selecting agent.

RESULTS

Mutagenicity of DB(a,i)P and DB(a,h)P. In the absence of PAH-metabolizing cells, DB(a,i)P, DB(a,h)P, and their fluorinated derivatives displayed no cytotoxicity and no enhanced mutagenicity in V79 cells over the concentration range 0.03 to 0.3 μg/ml (data not shown). However, in the presence of the PAH-metabolizing hamster embryo cells, DB(a,i)P and DB(a,h)P induced in a dose-dependent manner both OUA^R and TG^R mutations in V79 target cells (Table 1). For comparison, we tested the mutagenicity of B(a)P under the same conditions. Treatment with 0.3 μg of B(a)P per ml resulted in about 60 OUA^R and about 270 TG^R colonies/10^6 colony-forming cells (Table 1). This corresponds to about 50 and 20% of the mutagenicity of DB(a,i)P and about 70 and 40% of the mutagenicity of DB(a,h)P for OUA and TG resistance, respectively.

The involvement of microsomal oxidation in the metabolism of these DBPs to mutagens was tested by using BF, a known inhibitor of mixed-function oxidases (9, 35). BF at concentrations of 3 μg/ml reduced the mutagenicity of DB(a,i)P and DB(a,h)P by more than 95%. In the presence of BF, the mutagenicity of DB(a,i)P at 0.1 and 0.3 μg/ml was reduced in the case of OUA resistance from about 50 and 120 mutants/10^6 colony-forming cells, respectively, to control level. The frequency of TG^R mutants at DB(a,h)P concentrations of 0.1 and 0.3 μg/ml was reduced from 290 and 1200 to 5 and 26 mutants/10^6 colony-forming cells, respectively. Similarly, the mutagenicity of DB(a,h)P at 0.3 μg/ml was reduced from about 80 and 670 to 2 and 8 OUA^R and TG^R mutants/10^6 colony-forming cells, respectively. In addition to the reduction or elimination of mutagenicity, BF also caused elimination of the cytotoxic effect of these DBPs. In the presence of BF, the surviving fraction of cells treated with each of the DBPs at 0.3 μg/ml was increased from 0.4 to that of control values (Table 1). These results indicate that the metabolism of the DBPs into mutagenic and cytotoxic derivatives requires microsomal oxidation in the cell-mediated assay.

Mutagenicity of Bay-Region Fluorinated Derivatives of DB(a,i)P and DB(a,h)P. The presence of a fluorine atom in one of the bay regions of DB(a,i)P caused the mutation frequency for OUA and TG resistance to decrease by 50% or greater. When both bay regions were fluorinated, the mutagenicity dropped essentially to background levels for both the OUA^R and TG^R markers (Table 1). A similar response was obtained for DB(a,h)P. At 0.3 μg/ml, this DBP induced in V79 cells about 80 and 670 OUA^R and TG^R cells, respectively; whereas 3,10-difluoro-DB(a,h)P at a dose as high as 3 μg/ml yielded only 4 and 10 such mutants per 10^6 colony-forming cells, respectively.

DISCUSSION

Our results show that DB(a,h)P and DB(a,i)P induce mutations in V79 cells in the presence but not in the absence of

Table 1

Cell-mediated mutagenesis with DBPs and their bay-region fluorine derivatives

PAH	Concentration (μg/ml)	Surviving fraction[a]	No. of OUA^R mutants/10^6 colony-forming cells[b]	No. of TG^R mutants/10^6 colony-forming cells[c]
DB(a,i)P	0.03	0.9	21 ± 5[d]	124 ± 9
	0.1	0.7	48 ± 7	289 ± 12
	0.3	0.4	117 ± 9	1211 ± 22
DB(a,i)P + BF[e]	0.1	0.7	2 ± 1	5 ± 1
	0.3	1.1	2 ± 1	26 ± 3
2-Fluoro-DB(a,i)P	0.1	1.1	23 ± 4	32 ± 4
	0.3	1.1	52 ± 7	42 ± 6
3-Fluoro-DB(a,i)P	0.1	1.1	11 ± 4	20 ± 3
	0.3	1.2	57 ± 5	126 ± 9
2,10-Difluoro-DB(a,i)P	0.3	0.5	3 ± 1	6 ± 2
	1.0	1.2	4 ± 2	5 ± 2
	3.0	1.0	3 ± 1	6 ± 2
DB(a,h)P	0.03	0.8	8 ± 3	110 ± 9
	0.1	0.6	18 ± 3	264 ± 12
	0.3	0.4	84 ± 8	668 ± 17
DB(a,h)P + BF[e]	0.3	1.1	2 ± 1	8 ± 1
3,10-Difluoro-DB(a,h)P	1.0	0.9	2 ± 1	3 ± 1
	3.0	0.9	4 ± 1	10 ± 2
B(a)P	0.3	1.0	61 ± 8	267 ± 13
	1.0	0.7	97 ± 9	293 ± 8

[a] Surviving fraction is given relative to the untreated control, which is taken as 100%.
[b] Solvent-treated control yielded 2 ± 1.
[c] Solvent-treated control yielded 4 ± 1 TG^R mutants/10^6 colony-forming cells.
[d] Mean ± S.E.
[e] Concentration of BF in culture media is 3 μg/ml.

hamster embryo fibroblasts, which are known to metabolize PAH (18). In this cell-mediated assay, both DB(a,h)P and DB(a,i)P were mutagenic at doses as low as 0.3 μg/ml. Furthermore, at similar doses, they yielded mutation frequencies greater than that for B(a)P. Responding in this way, the hamster embryo fibroblast-mediated mutagenesis assay is in agreement with tumorigenesis data that classify the DBPs as potent tumor inducers. DB(a,i)P is a potent sarcomagenic agent when administered by s.c. injection (24); when compared with B(a)P, it induced more tumor-bearing mice by a factor of about 7 (27). In addition, both DB(a,i)P and DB(a,h)P are potent tumor initiators in mouse skin (11, 27); when compared to B(a)P DB(a,h)P was found to be more active than B(a)P.

Fluorine substitution of a PAH ring carbon reduces the electron density of the ring carbon atom with minimal steric effects by the fluorine that has replaced the hydrogen atom. A reduction in the mutagenic and carcinogenic activity of a fluorinated compound compared with its parent implies the involvement of that particular fluorinated carbon atom(s) in the metabolic activation of the parent compound. The fluorine substitution technique has been used in studies of carcinogenesis (11–13, 17, 21, 30), mutagenesis (21, 30), and DNA repair (7, 8).

Fluorine substitution at both bay regions of DB(a,i)P [2,10-difluoro-DB(a,i)P] essentially inactivates the hydrocarbon in terms of tumors produced by s.c. injection and reduces to 5% its initiating response in mouse skin (11, 27). A similar type of response was reported with 3,10-difluoro-DB(a,h)P (12). The fibroblast-mediated mutagenesis assay responded in the same manner. At the tested doses, both parent DBPs exhibited frequencies more than 2 orders of magnitude higher than control values. Fluorination at the 2,10- and the 3,10-positions of DB(a,i)P and DB(a,h)P, which are the bay regions of the DBPs, reduced the mutagenic response of the parent compounds essentially to that of the control. These results, coupled with the data which indicate that BF, a mixed function oxidase inhibitor, reduced the conversion of these DBPs into mutagenic metabolites (21–23) strongly implicate bay-region oxidative metabolic products of the parent compound in the tumorigenic and mutagenic processes. Hecht *et al.* (11) and Wood *et al.* (38) have related diol-epoxides of DBPs to tumorigenesis and mutagenesis.

While the cell-mediated V79 assay correlated with mouse tumorigenesis with both DBP isomers and their bay-region difluorinated analogs (Table 1), the results of the *Salmonella*-microsome assay (1) with DB(a,i)P and 2,10-difluoro-DB(a,i)P (38) indicate that the *Salmonella* assay does not necessarily parallel the negative carcinogenic response of the difluorinated compound. Twenty-five nmol of the unhalogenated DB(a,i)P produce 115 and 410 net *his* revertants/plate in strains TA100 and TA98, respectively. The noncarcinogenic 2,10-difluoro-DB(a,i)P, which produced little or no response in the cell-mediated assay, produces a mutagenic response about 75% of that of the parent compound in the *Salmonella*-microsome assay, 90 and 305 net *his* revertants/plate for TA100 and TA98, respectively. These results are summarized in Table 2. The negative response of cell-mediated mutagenesis when both bay regions of DBPs are blocked with fluorine atoms *vis-à-vis* the strong positive mutagenic response in the presence of microsome-mediated metabolism strongly suggests that an alternative pathway not present in intact cells is provided by microsome-mediated metabolism. This alternative metabolic product may be the K-region epoxide known to occur in microsome-mediated metabolism of other PAHs (10).

Table 2

Comparison of DBP tumor data and mutagenicity data determined in the fibroblast-mediated mammalian cell assay and in the Salmonella-microsome assay

		Mutagenesis			
		Fibroblast-mediated V79 assay[b]		*Salmonella*-microsome assay[c]	
	Tumorigenesis (skin painting and/or s.c. injection)[a]	OUA^R	TG^R	TA 100	TA98
DB(a,i)P	++++	117	1211	115	410
DB(a,h)P	++++	84	668	40	45
2,10-Difluoro-DB(a,i)P	±	3	6	90	305

[a] Based on data of Hecht *et al.* (11) and Sardella *et al.* (27).
[b] See Table 1.
[c] Based on data of Wood *et al.* (37); values represent units of revertants per plate at DBP concentrations of 25 nmol.

The lack of correlation between the skin tumor data and the results from the *Salmonella* assay may be due not to the fact that *Salmonella* is the target cell but rather to the method of activation. Studies with 7,12-dimethylbenz(a)anthracene (3, 4) have demonstrated that liver microsomes *in vitro* can render a metabolic product yield that is quite different from that derived from *in vivo* activation.

On the basis of the results showing the ability of the V79 cell-mediated mutagenesis assay to activate DBPs in a way that produces mutagenesis values that parallel the mouse sarcoma and skin tumor data and also on the basis of the evidence that the *Salmonella*-microsome test may predict false-positive results in fluorinated PAH compounds (Table 2), we suggest that the V79 cell-mediated assay may be more appropriate in detecting potential carcinogenic hazards of PAHs.

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REFERENCES

1. Ames, B. N., McCann, J., and Yamasaki, E. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian microsome mutagenicity test. Mutat. Res., *31:* 347–364, 1975.
2. Andrews, A. W., Thibault, L. H., and Lijinsky, W. Relationship between carcinogenicity and mutagenicity of some polynuclear hydrocarbons. Mutat. Res., *51:* 311–318, 1978.
3. Bigger, C. A. H., Tomaszewski, J. E., and Dipple, A. Differences between products of binding of 7,12-dimethylbenz(a)anthracene to DNA in mouse skin and in a rat liver microsomal system. Biochem. Biophys. Res. Commun., *80:* 229–235, 1978.
4. Bigger, C. A. H., Tomaszewski, J. E., and Dipple, A. Variation in route of microsomal activation of 7,12-dimethylbenz(a)anthracene with substrate concentration. Carcinogenesis, *1:* 15–20, 1980.
5. Boger, E., O'Malley, R. F., and Sardella, D. J. Active sites in dibenzopyrenes: synthesis and studies of 3-fluoro- and 2,10-difluorbenzo(*rst*)pentaphene. J. Fluorine Chem., *8:* 513–525, 1976.
6. Brown, J. P., and Brown, R. J. Mutagenesis by 9,10-anthraquinone derivatives and related compounds in *Salmonella typhimurium*. Mutat. Res., *40:* 203–224, 1957.
7. D'Ambrosio, S. M., Daniel, F. B., Hart, R. W., Cazer, F. D., and Witiak, D. T. DNA repair in Syrian hamster embryo cells treated with 7,12-dimethylbenz(a)anthracene and its weakly carcinogenic 5-fluoro analog. Cancer Lett., *6:* 255–261, 1979.
8. Daniel, F. B., Cazer, F. D. D'Ambrosio, S. M., Hart, R. W., Kim, W. H., and Witiak, D. T. Comparative metabolism and DNA binding of 7,12-dimethylbenz(a)anthracene and its weakly carcinogenic 5-fluoro analog. Cancer Lett., *6:* 263–272, 1979.

9. Diamond, L., and Gelboin, H. V. Alpha-Naphthaflavone: an inhibitor of hydrocarbon cytotoxicity and microsomal hydrase. Science (Wash. D. C.), *166:* 1023–1025, 1969.
10. Harvey, R. G., and Dunne, F. G. Multiple lesions of metabolic activation of carcinogenic hydrocarbons. Nature (Lond.), *273:* 566–568, 1978.
11. Hecht, S. S., LaVoie, D. J., Bedenko, V., Hoffmann, D., Sardella, D. J., Boger, E., and Lehr, R. E. On the metabolic activation of dibenzo[*a,i*]pyrene and dibenzo[*a,h*]pyrene. *In:* M. Cooke and A. J. Dennis (eds.), Polynuclear Aromatic Hydrocarbons: Chemical Analysis and Biological Fate, pp. 43–54. Columbus, Ohio: Battelle Press, 1981.
12. Hecht, S. S., LaVoie, E. J., Bedenko, V., Pingaro, L., Katayama, S., Hoffman, D., Sardella, D. J., Boger, E., and Lehr, R. E. Reduction of tumorigenicity and of dihydrodiol formation by fluorine substitution in the angular rings of dibenzo(*a,i*)pyrene. Cancer Res., *41:* 4341–4345, 1981.
13. Hecht, S. S., LaVoie, E. J., Mazzarese, R., Hirota, N., Ohmori, T., and Hoffman, D. Comparative mutagenicity, tumor-initiating activity, carcinogenicity, and *in vitro* metabolism of fluorinated 5-methylchrysenes. J. Natl. Cancer Inst., *63:* 855–861, 1979.
14. Hoffman, D., and Wynder, E. L. Beitrag zur Carcinogenen Wirkung von Dibenzopyrenen. Z. Krebsforsch., *68:* 137–149, 1966.
15. Hoffman, D., and Wynder, E. L. Organic particulate pollutants—chemical analysis and bioassays for carcinogenicity. *In:* A. C. Stern (ed.), Air Pollution, pp. 361–455. New York: Academic Press, Inc., 1977.
16. Homburger, F., and Tregier, A. Modifying factors in carcinogenesis. Prog. Exp. Tumor Res., *1:* 311–328, 1960.
17. Huberman, E., and Jones, C. A. The use of liver cell cultures in mutagenesis studies. Ann. N. Y. Acad. Sci., *349:* 264–272, 1980.
18. Huberman, E., and Sachs, L. Cell-mediated mutagenesis of mammalian cells with chemical carcinogens. Int. J. Cancer, *13:* 326–333, 1974.
19. Huberman, E., and Sachs, L. Mutability of different genetic loci in mammalian cells by metabolically activated polycyclic hydrocarbons. Proc. Natl. Acad. Sci. U. S. A., *73:* 88–92, 1976.
20. Huberman, E., Sachs, L., Yang, S. K., and Gelboin, H. V. Identification of mutagenic metabolites of benzo(*a*)pyrene in mammalian cells. Proc. Natl. Acad. Sci. U. S. A., *73:* 607–611, 1976.
21. Huberman, E., and Slaga, T. J. Mutagenic and tumor-initiating activity of fluorinated derivatives of 7,12-dimethylbenz(*a*)anthracene. Cancer Res., *39:* 411–414, 1979.
22. Huberman, E., Yang, S. K., McCourt, D. W., and Gelboin, H. V. Mutagenicity to mammalian cells in culture by (+) and (−) *trans*-7,8-dihydroxy-7,8-dihydrobenzo(*a*)pyrenes and the hydrolysis and reduction products of two stereoisomeric benzo(*a*)pyrene 7,8-diol-9,10-epoxides. Cancer Lett., *4:* 35–43, 1977.
23. Jones, C. A., and Huberman, E. A sensitive hepatocyte-mediated assay for the metabolism of nitrosamines to mutagens for mammalian cells. Cancer Res., *40:* 406–411, 1980.
24. Kelley, T. F. Fate of subcutaneously injected benzo(*rst*)pentaphene in C57BL/6 mice. Proc. Soc. Exp. Biol. Med., *133:* 1402–1404, 1970.
25. Lyons, M. J., and Johnson, H. Aromatic hydrocarbons from vehicular exhausts. Br. J. Cancer, *11:* 60–66, 1957.
26. Lyons, M. J., and Johnston, H. Chemical investigation of the neutral fraction of cigarette smoke tar. Br. J. Cancer, *11:* 544–562, 1957.
27. Sardella, D. J., Boger, E., and Ghoshal, P. K. Active sites in hexacyclic carcinogens probed by the fluorine substitution methodology. *In:* M. Cooke and A. J. Dennis (eds.), Polycyclic Aromatic Hydrocarbons: Chemical Analysis and Biological Fate, pp. 529–538. Columbus, Ohio: Battelle Press, 1981.
28. Sardella, D. J., Mariani, H. A., Mahathalang, P., and Boger, E. Synthesis of 2-fluorobenzo(*rst*)pentaphene and 2-fluorobenzo(*rst*)-pentaphene-5-^{13}C. J. Labeled Compd. Radiopharm. *16:* 633–643, 1978.
29. Schoental, R. Isolation of 3,4,9,10-dibenzopyrene from coal tar. Nature (Lond.), *180:* 606, 1957.
30. Slaga, T. J., Huberman, E., DiGiovanni, J. and Gleason, G. The importance of the "bay-region" diol epoxide in 7,12-dimethylbenz(*a*)anthracene skin tumor initiation and mutagenesis. Cancer Lett., *6:* 213–220, 1979.
31. Slaga, T. J., Iyer, R. P., Lyga, W., Secrist, A., Daub, G. H., and Harvey, R. G. Comparison of skin tumor-initiating activities of dihydrodiols, diol-epoxides and methylated derivative of various polycyclic aromatic hydrocarbons. *In:* A. Bjirseth and A. J. Dennis (eds.), Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects, pp. 753–769. Columbus, Ohio: Battelle Press, 1980.
32. Teranishi, K., Hamada, K., and Watanabe, H. Quantitative relationship between carcinogenicity and mutagenicity of polyaromatic hydrocarbons in *Salmonella typhimurium* mutants. Mutat. Res., *31:* 97–102, 1975.
33. Tokiwa, H., Morita, K., Takeyoshi, H., Takahashi, K., and Ohnishi, Y. Detection of mutagenic activity in particulate air pollutants. Mutat. Res., *48:* 237–248, 1977.
34. Waterfall, J. F., and Sims, P. Metabolism of dibenzo(*a,h*)pyrene and dibenzo(*a,i*)pyrene and related compounds by liver preparations and their enzyme-induced binding to cellular macromolecules. Biochem. Pharmacol., *22:* 2469–2483, 1973.
35. Wiebel, F. J., Leutz, J. C., Diamond, L., and Gelboin, H. V. Aryl hydrocarbon [benzo(*a*)pyrene] hydroxylase in microsomes from rat tissues: differential inhibition and stimulation by benzoflavones and organic solvents. Arch. Biochem. Biophys., *144:* 78–86, 1971.
36. Wodinsky, I., Helinski, A., and Kensler, C. J. Experimental tumorigenesis in the hamster cheek pouch. Nature (Lond.), *207:* 770–772, 1965.
37. Wood, A. W., Chang, R. L., Levin, W., Lehr, R. E., Schoefer-Redder, M., Karle, J. M., Jerina, D. M., and Conney, A. H. Mutagenicity and cytotoxicity of benzo(*a*)anthracene diol epoxides and tetrahydroperoxides: exceptional activity of the bay-region 1,2-epoxides. Proc. Natl. Acad. Sci. U. S. A., *74:* 2746–2750, 1977.
38. Wood, A. W., Chang, R. L., Levin, W., Ryan, D. E., Thomas, P. E., Lehr, R. E., Kumar, S., Sardella, D. J., Boger, E., Yagi, H., Sayer, J. M., Jerina, D. M., and Conney, A. H. Mutagenicity of the bay-region diol-epoxides and other benzo-ring derivatives of dibenzo(*a,h*)pyrene and dibenzo(*a,i*)pyrene. Cancer Res., *41:* 2589–2597, 1981.
39. Wood, A. W., Levin, W., Ryan, D., Thomas, P. E., Yagi, H., Mah, H. D., Thakker, D. R., Jerina, D. M., and Conney, A. H. High mutagenicity of metabolically activated chrysene 1,2-dihydrodiol: evidence for bay-region activation of chrysene. Biochem. Biophys. Res. Commun., *78:* 847–854, 1977.

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