

# Tumorigenicity of Bay-Region Diol-Epoxides and Other Benzo-Ring Derivatives of Dibenzo(a,h)pyrene and Dibenzo(a,i)pyrene on Mouse Skin and in Newborn Mice

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## ABSTRACT

Dibenzo(a,h)pyrene, [DB(a,h)P], dibenzo(a,i)pyrene [DB(a,i)P], and seven of their benzo-ring derivatives were tested for tumorigenic activity on mouse skin and in newborn mice. In the tumor studies on mouse skin, a single topical application of 50, 200, or 600 nmol of compound was followed 7 days later by twice-weekly applications of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate for 16 or 24 weeks. With the exception of 2,10-difluorodibenzo(a,i)pyrene, all of the compounds had significant tumor-initiating activity at all doses tested. *trans*-1,2-Dihydroxy-1,2-dihydrodibenzo(a,h)pyrene and *trans*-3,4-dihydroxy-3,4-dihydrodibenzo(a,i)pyrene, the metabolic precursors of bay-region diol-epoxides, had tumor-initiating activity that was equivalent to their parent hydrocarbons. Saturation of the double bond in the benzo-ring of these dihydrodiols resulted in the formation of tetrahydrodiols whose tumor-initiating activity was not significantly different from that observed with the corresponding dihydrodiols at the 50-nmol dose. The bay-region diol-epoxides of DB(a,h)P and DB(a,i)P, in which the benzylic hydroxyl group and the oxirane oxygen are *trans* (Isomer 2), induced significantly fewer tumors per mouse than did their dihydrodiol and parent hydrocarbon precursors.

In the tumorigenicity study in newborn mice, a total dose of 87.5 nmol of the hydrocarbon divided into three i.p. injections was administered on the first, eighth, and 15th day of life, and tumorigenic activity was determined when the mice were 49 to 54 weeks old. *trans*-1,2-Dihydroxy-1,2-dihydrodibenzo(a,h)pyrene and *trans*-3,4-dihydroxy-3,4-dihydrodibenzo(a,i)pyrene induced 3- to 8-fold more pulmonary tumors per mouse and 4- to 5-fold more hepatic tumors per male mouse than the respective parent hydrocarbons. The corresponding tetrahydrodiols had no more than one-eighth of the pulmonary tumorigenic activity of the corresponding dihydrodiol. The bay-region diol-epoxide (Isomer 2) of DB(a,h)P had tumorigenic activity equal to the parent hydrocarbon but significantly less than its dihydrodiol precursor. The bay-region diol-epoxide (Isomer 2) of DB(a,i)P was highly toxic, and only 19% of the mice survived to termination of the study. This diol-epoxide had significantly less tumorigenic activity towards the lung than did either its dihydrodiol precursor or the parent hydrocarbon. Notably, 20% of the surviving mice treated with the diol-epoxide of DB(a,i)P had leukemia at the termination of the study. 2,10-Difluorodi-

benzo(a,i)pyrene had no tumorigenic activity in newborn mice at the single dose tested. These results are discussed in relationship to the bay-region theory of polycyclic aromatic hydrocarbon carcinogenicity.

## INTRODUCTION

PAHs<sup>3</sup> are a large class of environmental contaminants (2). They are metabolized to reactive intermediates which bind covalently to critical cellular constituents such as DNA, RNA, and protein, causing mutations and initiating other cellular changes that result in the development of cancer (18, 19). Recent studies have shown that bay-region diol-epoxides of a number of different PAHs are ultimate carcinogens in mice (3, 8, 20, 27). The pathway for the metabolic formation of these bay-region diol-epoxides involves the following sequence of steps: oxidation of a terminal angular benzo-ring of the PAH to form an arene oxide, hydration of the arene oxide to form a *trans* dihydrodiol, and subsequent epoxidation of the bay-region double bond of the dihydrodiol.

DB(a,h)P and DB(a,i)P, 2 hexacyclic aromatic hydrocarbons, are environmental pollutants (10, 16) which have particularly high tumorigenic activity in mice (1, 5, 6, 11, 25). Quantum mechanical calculations designed to predict the ease of triol carbonium ion formation from diol-epoxides indicated that the bay-region diol-epoxides of DB(a,h)P and DB(a,i)P should have very high chemical reactivity (7). Studies of their kinetics of solvolysis have confirmed these predictions (26). Recent studies on the mutagenicity of several benzo-ring derivatives of DB(a,h)P and DB(a,i)P showed that DB(a,h)P 1,2-dihydrodiol and DB(a,i)P 3,4-dihydrodiol, the expected dihydrodiol precursors of the bay-region diol-epoxides, were metabolized by the cytochrome P-450-dependent monooxygenase system to products which were more mutagenic to strains TA98 and TA100 of *Salmonella typhimurium* than the metabolic products formed from their respective parent hydrocarbons (26). Synthetic bay-region diol-epoxides of DB(a,h)P and DB(a,i)P, in which the benzylic hydroxyl group and the oxirane oxygen are *trans* (Isomer 2), had high inherent mutagenic activity in *S. typhimurium* and in Chinese hamster V79 cells (26). In the

<sup>3</sup> The abbreviations used are: PAH, polycyclic aromatic hydrocarbon; DB(a,h)P, dibenzo(a,h)pyrene; DB(a,i)P, dibenzo(a,i)pyrene; DB(a,h)P 1,2-dihydrodiol, *trans*-1,2-dihydroxy-1,2-dihydrodibenzo(a,h)pyrene; DB(a,i)P 3,4-dihydrodiol, *trans*-3,4-dihydroxy-3,4-dihydrodibenzo(a,i)pyrene; 2,10-DFDB(a,i)P, 2,10-difluorodibenzo(a,i)pyrene; DB(a,h)P H<sub>4</sub>-1,2-diol, *trans*-1,2-dihydroxy-1,2,3,4-tetrahydrodibenzo(a,h)pyrene; DB(a,i)P H<sub>4</sub>-3,4-diol, *trans*-3,4-dihydroxy-1,2,3,4-tetrahydrodibenzo(a,i)pyrene; DMSO, dimethyl sulfoxide; TPA, 12-O-tetradecanoylphorbol-13-acetate. All compounds are racemic mixtures where enantiomers are possible.

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present report, we have examined the tumorigenicity of DB(a,h)P and DB(a,i)P and several of their benzo-ring derivatives on mouse skin as well as in newborn mice.

## MATERIALS AND METHODS

DB(a,i)P, DB(a,h)P, and 2,10-DFDB(a,i)P were obtained and purified as described previously (26). All 3 compounds were judged to be essentially pure based on chromatographic, mass spectral, and nuclear magnetic resonance analysis. DB(a,h)P 1,2-dihydrodiol, DB(a,h)P H<sub>4</sub>-1,2-diol, (±)-1β,2α-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydrodibenzo(a,h)pyrene, DB(a,i)P 3,4-dihydrodiol, DB(a,i)P H<sub>4</sub>-3,4-diol, and (±)-3α,4β-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydrodibenzo(a,i)pyrene were synthesized as described previously (12, 26). The structures of the dibenzopyrene derivatives used in this study are shown in Chart 1. DMSO was distilled from calcium hydride under reduced pressure and stored under an argon atmosphere in amber bottles. TPA was purchased from Chemical Carcinogenesis, Inc., Eden Prairie, Minn.

**Tumorigenicity Studies on Mouse Skin.** Female CD-1 mice (7 to 8 weeks old) were purchased from Charles River Breeding Laboratories, North Wilmington, Mass. The mice were shaved on the dorsal surface with electric clippers. Two days later, 30 mice in each treatment group were given a single topical application of DB(a,h)P, DB(a,i)P, or their derivatives in 200 μl of 10% DMSO in tetrahydrofuran. Control mice were treated with solvent. Beginning 7 days after application of the initiator or solvent, all mice received twice-weekly applications of 16 nmol of TPA in 200 μl acetone. Development of skin tumors was recorded once every 2 weeks, and papillomas greater than 2 mm in diameter were included in the cumulative total when they persisted for 2 weeks or longer. Statistical significance of skin tumor data was

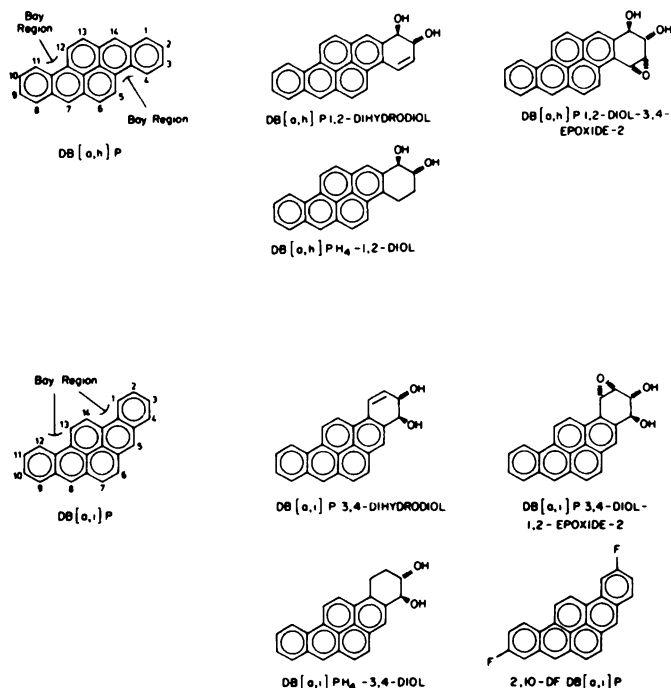


Chart 1. Structures of DB(a,h)P, DB(a,i)P, and their benzo-ring derivatives which were used in this study. Both parent hydrocarbons are symmetric molecules, and the 2 bay-regions of each compound are identical. Diol-epoxides exist as diastereomeric pairs in which the epoxide oxygen and the benzylic hydroxyl group are either *cis* (Isomer 1) or *trans* (Isomer 2). Stereochemistry is relative. DB(a,h)P 1,2-diol-3,4-epoxide-2, (±)-1β,2α-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydrodibenzo(a,h)pyrene; DB(a,i)P 3,4-diol-1,2-epoxide-2, (±)-3α,4β-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydrodibenzo(a,i)pyrene.

determined by the method of Mainland and Murray (17) and by Student's *t* test.

**Newborn Mouse Experiments.** Pregnant Swiss-Webster mice [BLU: Ha(ICR)] were obtained from Blue Spruce Farms, Altamont, N. Y., and were housed in plastic cages on corn cob bedding. They delivered their litters from 2 to 6 days after arrival. Within 24 hr of birth, 10 pups of each litter were given i.p. injections of the first dose of compound. Additional injections were given on the eighth and 15th days of life. A total dose of 87.5 nmol of compound was divided into 3 injections of 12.5, 25, and 50 nmol in 5, 10, and 20 μl DMSO, respectively. Control mice were given injections of DMSO alone. The mice were weaned at 25 days of age, and the experiment was terminated by killing the animals when they were 49 to 54 weeks old. At necropsy, the major organs of each animal were examined grossly, tumors were counted, and tissues were fixed in 10% buffered formalin. A representative number of pulmonary tumors and all hepatic tumors were examined histologically. Pathology of the lung tumors was the same as has been described previously (21). Most of the hepatic tumors were type A or neoplastic nodules (23, 24). In addition, the other major organs from both control mice and mice given hydrocarbons or hydrocarbon derivatives were examined histologically. Statistical significance of the newborn mouse tumor data was evaluated by the Fisher 2 × 2 exact test and the Mann-Whitney *U* test.

## RESULTS

**Tumor-initiating Activity of Dibenzopyrene Benzo-Ring Derivatives on Mouse Skin.** The results of 2 separate experiments assessing the tumor-initiating activity on mouse skin of DB(a,h)P, DB(a,i)P, and several of their benzo-ring derivatives are shown in Table 1. In the initial experiment, 200- and 600-nmol initiating doses of the compounds were used followed by twice-weekly applications of the tumor promoter TPA. By 16 weeks of promotion, it was clear that these doses of the dibenzopyrene compounds were at or near the top of the dose-response curves for most of the compounds. Only 2,10-DFDB(a,i)P was totally inactive as a tumor initiator at the 200- and 600-nmol doses. In the second experiment, the initiating dose of the hydrocarbons and hydrocarbon derivatives was reduced to 50 nmol, and the mice received twice-weekly applications of TPA for 24 weeks. Although DB(a,h)P induced a higher number of tumors per mouse at 24 weeks of promotion than did DB(a,i)P, the 1.9-fold difference was not statistically significant (Table 1). DB(a,h)P 1,2-dihydrodiol and DB(a,i)P 3,4-dihydrodiol, the metabolic precursors of bay-region diol-epoxides, had tumor-initiating activity that was equal to their corresponding parent hydrocarbons at all doses tested. The bay-region tetrahydrodiols, DB(a,h)P H<sub>4</sub>-1,2-diol and DB(a,i)P H<sub>4</sub>-3,4-diol, which cannot be metabolized to bay-region diol-epoxides at the 3,4- and 1,2-positions, respectively, had 55 to 75% of the tumor-initiating activity (average number of tumors per mouse) of their corresponding dihydrodiols and parent hydrocarbons at the 50-nmol dose, but the differences were not statistically significant. At the 200- and 600-nmol doses, DB(a,i)P H<sub>4</sub>-3,4-diol had only 30 to 60% of the tumor-initiating activity (average number of tumors per mouse) of the corresponding dihydrodiol and parent hydrocarbon, but the tumor incidence (percentage of mice with tumors) was not significantly different.

The bay-region diol-epoxides of DB(a,h)P and DB(a,i)P, in which the benzylic hydroxyl group and the epoxide oxygen are *trans* (Isomer 2), had significant tumor-initiating activity at all doses tested, but the average number of tumors per mouse

Table 1

Tumorigenicity of DB(a,h)P, DB(a,i)P, and several of their benzo-ring derivatives on mouse skin after 16 and 24 weeks of promotion with TPA

The indicated dose of compound was applied once, and commencing 7 days later, 16 nmol of TPA was administered twice weekly for 16 or 24 weeks as described in "Materials and Methods." Data for the 50-nmol initiating dose of compounds are italicized to indicate that these results were obtained in a separate experiment. Each treatment group contained 30 mice.

Initiator	16 weeks of promotion			24 weeks of promotion	
	Dose (nmol)	% of mice with tumors	Tumors/mouse	% of mice with tumors	Tumors/mouse
DMSO		0	0	0	0
DB(a, h)P		10	0.10	0	0
	<i>50</i>	55	1.41	72	3.97 ± 0.85 <sup>a</sup>
	200	79	4.72		
DB(a,h)P 1,2-dihydrodiol	600	72	5.52		
	<i>50</i>	39	0.96	79	2.96 ± 0.62
	200	57	2.73		
DB(a,h)P H <sub>4</sub> -1,2-diol	600	80	4.40		
	<i>50</i>	37	0.87	67	2.27 ± 0.49
	200	55	3.21		
DB(a,h)P 1,2-diol-3,4-epoxide 2 <sup>b</sup>	600	67	3.60		
	<i>50</i>	33	0.43	60	1.00 ± 0.24
	200	70	1.87		
DB(a,i)P	600	50	1.83		
	<i>50</i>	28	0.52	69	2.07 ± 0.44
	200	67	5.33		
DB(a,i)P 3,4-dihydrodiol	600	79	5.25		
	<i>50</i>	37	0.60	60	2.14 ± 0.57
	200	66	3.03		
DB(a,i)P H <sub>4</sub> -3,4-diol	600	81	5.00		
	<i>50</i>	27	0.37	55	1.17 ± 0.29
	200	50	1.83		
DB(a,i)P 3,4-diol-1,2-epoxide 2	600	61	1.64		
	<i>50</i>	20	0.20	53	0.90 ± 0.20
	200	43	0.90		
2,10-DFDB(a,i)P	600	67	2.03		
	<i>200</i>	7	0.07		
	600	0	0		

<sup>a</sup> Mean ± S.E.

<sup>b</sup> DB(a,h)P 1,2-diol-3,4-epoxide 2, (±)-1β,2α-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydrodibenzo(a,h)-pyrene; DB(a,i)P 3,4-diol-1,2-epoxide 2, (±)-3α,4β-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydrodibenzo(a,i)pyrene.

was significantly fewer than that observed with their dihydrodiol precursors and respective parent hydrocarbons (Table 1).

**Tumorigenicity of Dibenzopyrene Derivatives in Newborn Mice.** The tumorigenic activity of DB(a,h)P, DB(a,i)P, and 7 of their derivatives in newborn mice at a total dose of 87.5 nmol is shown in Table 2. In the control group, 27% of the mice developed pulmonary tumors with an average of 0.61 tumors/mouse. With the exception of 2,10-DFDB(a,i)P, all of the other compounds induced pulmonary tumors in over 80% of the animals. 2,10-DFDB(a,i)P had no significant tumorigenic activity compared to the incidence observed in solvent-treated control mice. As was observed in initiation-promotion experiments on mouse skin, DB(a,h)P and DB(a,i)P had similar tumorigenic activity in newborn mice. The parent hydrocarbons produced an average of 4.4 to 5.1 lung tumors/mouse and 0.8 to 0.9 hepatic tumors/male mouse. DB(a,h)P 1,2-dihydrodiol induced 17 lung tumors/mouse, a value 3 times higher than that observed with the parent hydrocarbon ( $p < 0.001$ ) and 10 times higher than that observed with the tetrahydrodiol derivative. Although DB(a,h)P 1,2-dihydrodiol appeared to be 4 times more active in inducing hepatic tumors in male mice than was DB(a,h)P or the tetrahydrodiol, the difference was not statistically significant. The bay-region diol-epoxide of DB(a,h)P (Isomer 2) had the same pulmonary tumorigenic activity as the parent hydrocarbon, only 32% of the activity of its dihydrodiol precursor ( $p < 0.001$ ), and 3-fold more activity

than the tetrahydrodiol derivative ( $p < 0.001$ ).

DB(a,i)P 3,4-dihydrodiol was the most tumorigenic compound studied in newborn mice, producing an average of 33 pulmonary tumors/mouse and 4.5 hepatic tumors/male mouse. The parent hydrocarbon had only one-eighth ( $p < 0.001$ ) and one-fifth ( $p < 0.001$ ) of the pulmonary and hepatic tumorigenic activity, respectively, of the dihydrodiol. The bay-region diol-epoxide of DB(a,i)P produced significantly fewer lung tumors/animal ( $p < 0.001$ ) than its dihydrodiol precursor and was slightly less active than the parent hydrocarbon ( $p < 0.05$ ). However, this bay-region diol-epoxide was highly toxic to the newborn mice, and only 19% of the treated animals survived until termination of the study. Twenty % of the surviving mice treated with this diol-epoxide had systemic leukemia which was only observed in one other mouse in the entire study.

**DISCUSSION**

The results obtained with DB(a,h)P and DB(a,i)P on mouse skin and in newborn mice indicated that both compounds have high and equivalent tumorigenic activity at the doses tested. As tumor initiators on mouse skin, DB(a,h)P 1,2-dihydrodiol and DB(a,i)P 3,4-dihydrodiol, the immediate metabolic precursors of bay-region diol-epoxides, had activity equal to their respective parent hydrocarbons. In newborn mice, these di-

Table 2

## Tumorigenicity of DB(a,h)P, DB(a,i)P, and several of their benzo-ring derivatives in newborn mice

Eighty Swiss-Webster mice [BLU:Ha(ICR)] were given i.p. injections of DB(a,h)P, DB(a,i)P, or their benzo-ring derivatives in DMSO on the first, eighth, and 15th days of life. The total dose of compound administered was 87.5 nmol. Control animals received injections of DMSO. The animals were killed at 49 to 54 weeks of age. A representative number of pulmonary tumors, all hepatic tumors, and all other tissues with suspected pathology were examined histologically.

Compound	No. of mice alive at weaning (Day 25)	Sex and no. of mice alive at 49 to 54 weeks of age	Pulmonary tumors		Hepatic tumors		Other neoplasms (no. of mice)
			% of mice with tumors	Average no. of tumors/mouse	% of mice with tumors	Average no. of tumors/mouse	
None	73	Female 39 Male 32 Total 71	28 22 27	0.44 0.80 0.61	0 0 0	0 0 0	
DB(a,h)P	73	Female 14 Male 25 Total 39	93 100 97	4.78 5.20 5.07	7 44.0	0.07 0.88	3 <sup>a</sup>
DB(a,h)P 1,2-dihydrodiol	73	Female 28 Male 17 Total 45	96 100 98	15.82 19.00 17.04	7 41	0.07 3.76	
DB(a,h)P H <sub>4</sub> -1,2-diol	66	Female 32 Male 25 Total 57	81 84 82	1.81 1.64 1.74	0 28	0 0.80	
DB(a,h)P 1,2-diol-3,4-epoxide 2 <sup>b</sup>	50	Female 18 Male 19 Total 37	94 95 95	5.72 5.37 5.54	6 26	0.06 1.37	1 <sup>c</sup>
DB(a,i)P	74	Female 21 Male 39 Total 60	100 95 97	5.80 3.64 4.40	0 54	0 0.82	2 <sup>c</sup> 1 <sup>d</sup>
DB(a,i)P 3,4-dihydrodiol	77	Female 30 Male 21 Total 51	100 100 100	32.2 35.0 33.3	6 67	0.10 4.48	1 <sup>e</sup>
DB(a,i)P H <sub>4</sub> -3,4-diol	68	Female 23 Male 33 Total 56	100 91 95	4.60 3.79 4.13	0 88	0 3.30	1 <sup>f</sup>
DB(a,i)P 3,4-diol-1,2-epoxide 2	39	Female 7 Male 8 Total 15	100 62 86	1.57 3.13 2.57	0 25	0 0.25	3 <sup>e</sup>
2,10-DFDB(a,i)P	74	Female 26 Male 35 Total 61	31 20 25	0.58 0.31 0.43	0 3	0 0.03	1 <sup>d</sup>

<sup>a</sup> Two mice had sarcomas of the skin; one mouse had an adenocarcinoma in the small intestine.

<sup>b</sup> DB(a,h)P 1,2-diol-3,4-epoxide 2, ( $\pm$ )-1 $\beta$ ,2 $\alpha$ -dihydroxy-3 $\alpha$ ,4 $\alpha$ -epoxy-1,2,3,4-tetrahydrodibenzo(a,h)pyrene; DB(a,i)P 3,4-diol-1,2-epoxide 2, ( $\pm$ )-3 $\alpha$ ,4 $\beta$ -dihydroxy-1 $\alpha$ ,2 $\alpha$ -epoxy-1,2,3,4-tetrahydrodibenzo(a,i)pyrene.

<sup>c</sup> Hemangioma of the uterus.

<sup>d</sup> Lymphoma of the thymus.

<sup>e</sup> Systemic leukemia.

<sup>f</sup> Squamous-cell carcinoma of the skin.

hydrodiols were clearly the most tumorigenic dibenzopyrene derivatives tested, with activity 3- to 8-fold greater than their parent hydrocarbons in producing pulmonary tumors. The bay-region diol-epoxides of DB(a,h)P and DB(a,i)P, in which the benzylic hydroxyl group and the bay-region epoxide are *trans* (isomer 2), had significantly less tumorigenic activity than their dihydrodiol precursors, both on mouse skin and in the newborn mouse tumor models. These bay-region diol-epoxides were also significantly less active than their parent hydrocarbons on mouse skin, but they had tumorigenic activity equal to or slightly less than their parent hydrocarbons in the newborn mouse.

The tetrahydrodiols DB(a,h)P H<sub>4</sub>-1,2-diol and DB(a,i)P H<sub>4</sub>-3,4-diol, which only differ from the corresponding dihydrodiols in that their adjacent bay-region double bonds are saturated with hydrogen and thus cannot be metabolized to bay-region diol-epoxides at the 3,4- and 1,2-positions, respectively, had significant tumorigenic activity in each of the animal models. Although these tetrahydrodiols had activity which was not significantly different from their corresponding dihydrodiols on mouse skin, they had only 10 to 12% of the pulmonary tumorigenic activity of the dihydrodiols in newborn mice. Since DB(a,h)P and DB(a,i)P are symmetrical molecules with 2 equiv-

alent bay-regions (*cf.* Chart 1), the tumorigenic activity of the tetrahydrodiols could be due to formation of a diol-epoxide in the other bay-region of the molecule. The aromatic nucleus of both DB(a,h)P H<sub>4</sub>-1,2-diol and DB(a,i)P H<sub>4</sub>-3,4-diol is that of benzo(a)pyrene. The presence of the polar tetrahydrodiol group on the dibenzopyrenes might shift metabolism to the distal angular benzo-ring of the molecule to form a bay-region diol-epoxide which would be anticipated to have significant tumorigenic activity since the bay-region diol-epoxide of benzo(a)pyrene (isomer 2) is tumorigenic on mouse skin (22) and in newborn mice (9). Evidence in support of this concept comes from the complete lack of tumorigenic activity of 2,10-DFDB(a,i)P reported here (Tables 1 and 2) and previously (1). The difluoro derivative would be expected to undergo far less metabolism to a bay-region diol-epoxide since both benzo-rings of the molecule are substituted with fluorine (1).

The results of the present study on the tumorigenic activity of DB(a,h)P, DB(a,i)P, and several of their benzo-ring derivatives are in general agreement with the conclusions of our mutagenesis experiments. DB(a,h)P 1,2-dihydrodiol and DB(a,i)P 3,4-dihydrodiol were both metabolically activated to mutagenic products to a greater extent than their respective parent hydrocarbons, and the tetrahydrodiol derivatives were

only poorly activated to mutagenic metabolites in strains TA98 and TA100 *S. typhimurium* (26). In this regard, tumorigenicity studies in newborn mice with these compounds are totally consistent with the mutagenesis data. Although DB(a,h)P 1,2-dihydrodiol and DB(a,i)P 3,4-dihydrodiol had tumor-initiating activity which was no greater than that of the parent hydrocarbon on mouse skin, these results are analogous to data obtained with benzo(a)pyrene and benzo(a)pyrene 7,8-dihydrodiol (22). Data from a number of studies (13–15) have established benzo(a)pyrene 7,8-dihydrodiol as a proximate carcinogen of benzo(a)pyrene on mouse skin.

The tumorigenicity of the bay-region diol-epoxides of DB(a,h)P and DB(a,i)P is lower than anticipated when one considers the predicted chemical reactivity and high mutagenic activity of these bay-region diol-epoxides. High values of the quantum mechanical parameter  $\Delta E_{\text{deloc}}/\beta$  imply facile carbonium ion formation from these epoxides and therefore predict high chemical reactivity (4, 7). The values of  $\Delta E_{\text{deloc}}/\beta$  for the bay-region diol-epoxides of DB(a,h)P and DB(a,i)P (0.845 and 0.885, respectively) are the highest values for any hydrocarbon whose diol-epoxides have been studied to date (7). Indeed, rate constants for hydrolysis of these diol-epoxides in dioxane-water are in good agreement with predictions based on  $\Delta E_{\text{deloc}}/\beta$  (26). Although these bay-region diol-epoxides have significant tumorigenic activity in both tumor models, the activity is lower than might have been expected of an ultimate carcinogen of DB(a,h)P and DB(a,i)P. The high chemical reactivity of these bay-region diol-epoxides may limit the extent to which the intact diol-epoxide penetrates to critical sites within the cell to initiate tumor formation. Nevertheless, the high biological activity of the bay-region diol-epoxides and their dihydrodiol precursors does lend support to the concept that a bay-region diol-epoxide is a prime candidate as an ultimate carcinogenic metabolite of both DB(a,h)P and DB(a,i)P. The total lack of tumorigenic activity of 2,10-DFDB(a,i)P in both tumor models certainly strengthens this concept.

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