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Comparison of the Skin Tumor-Initiating Activities of Dihydrodiols, Diol-epoxides, and Methylated Derivatives of Various Polycyclic Aromatic Hydrocarbons[1]

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Running Title: Tumorigenicity of Various PAH Derivatives

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FOOTNOTES

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[3]The abbreviations used are: PAH, polycyclic aromatic hydrocarbon; B(a)P, benzo(a)pyrene; 1-12MB(a)P, 1 through 12 methylbenzo(a)pyrene; B(a)A, benz(a)anthracene; 7-MB(a)A, 7-methylbenz(a)anthracene; 12-MB(a)A, 12-methylbenz(a)anthracene; DMB(a)A, 7,12-dimethylbenz(a)anthracene; 5,7,12-TMB(a)A, 5,7,12-trimethylbenz(a)anthracene; DB(a,h)A, dibenz(a,h)-anthracene; B(e)P, benzo(e)pyrene; 3-MC, 3-methylcholanthrene; 3,11-DMC, 3,11-dimethylcholanthrene; 5-F,12-MB(a)A, 5-flouro-12-methylbenz(a)anthracene; 5-F,7-MB(a)A, 5-flouro-7-methylbenz(a)anthracene; 5,7-DMBA(a)A, 5,7-dimethylbenz(a)anthracene; 7-MB(a)A 3,4-dihydrodiol, (±)trans-3,4-dihydroxy 3,4-dihydro-7-MB(a)A; DB(a,h)A 3,4-dihydrodiol, (±)trans-3,4-dihydroxy-3,4-dihydro-DB(a,h)A; chrysene 1,2-dihydrodiol, (±)trans-3,4-dihydroxy-3,4-dihydrochrysene; B(e)P 9,10-dihydrodiol, (±)trans-9,10-dihydroxy 9,10-dihydrobenzo(e)pyrene; DB(a,c)A 10,11-dihydrodiol, (±)trans-10,11-dihydroxy-10,11-dihydro-DB(a,c)A; 7-MB(a)A 3,4-diol-1,2-epoxide, trans-3,4-dihydroxy-anti-1,2-epoxy-1,2,3,4-tetrahydro-7-MB(a)A; DB(a,h)A 3,4-diol-1,2-epoxide, trans-3,4-dihydroxy anti-1,2-epoxy-1,2,3,4-tetrahydro-DB-(a,h)A; chrysene 1,2-diol-3,4-epoxide, trans-1,2-dihydroxy anti-3,4-epoxy-1,2,3,4-tetrahydro chrysene; chrysene 3,4-diol-1,2-epoxide, trans3,4-dihydroxy-anti-1,2-epoxy-1,2,3,4-tetrahydrochrysene; B(e)P 9,10-diol-11,12-epoxide, trans-9,10-dihydroxy-anti-11,12-epoxy-9,10,11,12-tetrahydro-

B(e)P; DB(a,c)A 10,11-diol-12,13-epoxide, trans-10,11-dihydroxy-anti-12,13-epoxy-10,11,12,13-tetrahydro-DB(a,c)A; TPA, 12-O-tetradecanoylphorbol-13-acetate.

ABSTRACT

The abilities of dihydrodiols and diol-epoxides of diben(a,h)-anthracene [DB(a,h)A], dibenz(a,c)anthracene [DB(a,c)A], chrysene, 7-methylbenz(a)anthracene [7-MB(a)A], benzo(e)pyrene [B(e)P] and various methylated derivatives of benzo(a)pyrene [B(a)P], benz(a)anthracne [B(a)A], and 3-methylcholanthrene (3-MC) to initiate skin tumors in mice were determined by using a two-stage system of tumorigenesis. The trans-3,4-dihydrodiol of 7-MB(a)A, trans 9,10-dihydrodiol of B(e)P and the trans 1,2-dihydrodiol of chrysene were found to be more active than their corresponding parent hydrocarbon when applied topically to Sencar mice, followed by twice weekly applications of 12-O-tetradecanoylphorbol-13-acetate. The trans 3,4-dihydrodiol of DB(a,h)A was found to have one-half of the tumor initiating ability of DB(a,h)A. The trans 3,4-dihydrodiol of chrysene and trans 10-11-dihydrodiol of DB(a,c)A were essentially inactive as skin tumor initiators. B(e)P-9,10-diol-11,12-epoxide, DB(a,h)A 3,4-diol-1,2-epoxide, DB(a,c)A 10,11-diol-12,13-epoxide and 7-MB(a)A, 3,4-diol-1,2-epoxide were also found to be inactive as tumor initiators. Chrysene 1,2-diol-3,4-epoxide had 25% of the activity as chrysene. Results show that the dihydrodiols of 7-MB(a)A, DB(a,h)A and chrysene, which are the immediate metabolic precursors of bay-region diol-epoxides, have moderate to high initiating activity, whereas the dihydrodiol B(e)P (an extremely weak initiator) was inactive. The bay region diol-epoxide of chrysene was the only diol-epoxide tested that had significant activity when compared to the corresponding parent hydrocarbon. Substitution of a methyl group in the "peri" position of

7-MB(a)A, 12-MB(a)A, DMB(a)A, 3-MC and B(a)P almost completely blocked the skin tumor initiating activity. Likewise, substitution of a methyl group in the bay region of B(a)P and DMB(a)A was very effective in decreasing their tumor initiating activity.

INTRODUCTION

Current information indicates that at least some of the PAH are metabolized by microsomal monooxygenase systems to reactive arene oxides (16,19,32). Jerina and coworkers (20,21) have proposed a theory which predicts that "bay region" diol-epoxides of PAH are important in their carcinogenic activity. A bay region occurs in a PAH when an angularly fused benzo ring is present (Figure 1). There is now direct evidence from tumorigenicity studies that bay region diol-epoxides of B(a)P (22,23,33,34) and BA (28,38,39) are ultimate carcinogenic metabolites. In addition, recent studies have shown that certain benzo ring dihydrodiols (immediate precursors of bay region diol-epoxides) of the carcinogens B(a)P, B(a)A, 7-MB(a)A, DMB(a)A, DB(a,h)A, chrysene, and 3-MC are tumorigenic in mice (5-7,22,23,27-30,33-35,38,39).

Recent studies have revealed that a substitution of a methyl or fluoro group not only in the 1 and 2 positions of DMB(a)A which are part of the bay region but also in the 5 position almost completely blocked the skin tumor initiating and V79 mutagenic activities of DMB(a)A (17,37). The 5 position is part of the K-region and also has been called the "peri" position which is adjacent to an angular benzene ring (Figure 1). Hecht and coworkers (14,15) reported that a methyl or fluoro substitution in the peri position of 5-methylchrysene (12 position) was also less active as a skin tumor initiator and a mutagen than 5-methylchrysene. In addition, they reported that a fluoro substitution in the 1 and 3 positions which are part of the bay region of 5-methylchrysene are also less active as a tumor initiator and a mutagen (14,15).

In this report we compare the skin tumor initiating activities in mice of dihydrodiols and diol-epoxides of 7-MB(a)A, DB(a,h)A, DB(a,c)A, chrysene, B(e)P, and methylated and fluoro derivatives of various PAH in order to better understand the role of the bay region and peri position in PAH carcinogenesis. Our results show that the 3,4-dihydrodiol of 7-MB(a)A, 1,2-dihydrodiol of chrysene and the 9,10-dihydrodiol of B(e)P are more potent than the corresponding parent hydrocarbon whereas the 3,4-dihydrodiol of DB(a,h)A is less active. In general, the bay region diol-epoxides of the above hydrocarbons were very weak skin tumor initiators. Substitution of a methyl or fluoro group in the peri position as well as the bay region is very effective in blocking the skin tumor initiating activities of B(a)P, DMB(a)A, 7-MB(a)A, and 12-MB(a)A.

MATERIALS AND METHODS

Chemicals. TPA was obtained from Dr. P. Borchert, University of Minnesota, Minneapolis, MN. DMB(a)A and 3-MC were purchased from Sigma Chemical Co., St. Louis, MO; DB(a,h)A and DB(a,c)A from Eastman Kodak, Rochester, NY, and chrysene, B(e)P and B(a)P from Aldrich Chemical Co., Milwaukee, WI. 3,11-DMC, 5-F,7-MB(a)A, 5-F,12-MB(a)A, 5-F-DMB(a)A, 5,7-DMB(a)A and 5,7,12-TMB(a)A were a generous gift from Dr. M. Newman, Ohio State University, Columbus, OH.

The various methyl derivatives of B(a)P were synthesized and purified as described in the literature (1-4,8,9,31). The 7,8-diol-9-methyl B(a)P was synthesized starting from 9 methyl-7-keto-7,8,9,10-tetrachydrobenzo(a)pyrene as recently described (18). 7-methylbenz(a)anthracene was synthesized from 7-bromo-B(a)A through reaction with phenyllithium to afford

7-lithio-B(a)A followed by methylation with methyl iodide (25). The dihydrodiols and diol epoxides of chrysene (10,11), B(e)P (13), DB(a,h)A (26), DB(a,c)A (12), and 7-MB(a)A (25), were synthesized by the methods described. The diol-epoxides were the racemic anti isomers (i.e., the epoxide oxygen and the benzylic hydroxyl group are located on opposite faces of the ring system) in all cases. All of the PAH were consistently prepared under yellow light immediately before use.

Tumor Experiments. Female Sencar mice were originally obtained from Dr. R. K. Boutwell, Madison, Wisconsin, and are presently raised in Oak Ridge, TN. 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 papillomas was recorded weekly and they were removed at random for histological verification. DMBA, 12-MB(a)A, 3-MC, 7-MB(a)A, DB(a,h)A, DB(a,c)A, chrysene and B(e)P and their dihydrodiols and methyl derivatives were applied topically in acetone. The diol-epoxides of the above PAH were applied topically in tetrahydrofuran (distilled over $LiAlH_4$ and stored over sodium wire). The dose levels of the various parent hydrocarbons used in this study were based on previously determined dose-response studies (manuscript in preparation).

RESULTS AND DISCUSSION

Carcinogenicity of PAH Dihydrodiols and Diol-epoxides

A comparison of the skin tumor initiating activities of the 3,4-dihydrodiol of 7-MB(a)A and DB(a,h)A, their diol-epoxides and the corresponding parent hydrocarbons are shown in Table 1. After 15 weeks of TPA promotion 7-MB(a)A 3,4-dihydrodiol at a dose of 400 nmoles had induced tumors in all the mice with an average of 6.8 papillomas per mouse, whereas 400 nmoles of 7-MB(a)A induced tumors in only 83% of the mice with an average of 3.8 papillomas per mouse. While the 3,4-dihydrodiol of 7-MB(a)A was approximately twice as potent as 7-MB(a)A, the 3,4-dihydrodiol of DB(a,h)A was found to be about half as potent as DB(a,h)A (Table 1). The bay region diol-epoxides of 7-MB(a)A and DB(a,h)A were found to be very weak when tested as skin tumor initiators. Chouroulinkov et al. (7) recently reported that the 3,4-dihydrodiol of 7-MB(a)A was more active as a skin tumor initiator than 7-MB(a)A but was less active than that reported in the present study. The 3,4-dihydrodiol of DB(a,h)A was approximately half as active as a skin tumor initiator as was DB(a,h)A. Buening et al. (5) also reported that the 3,4-dihydrodiol of DB(a,h)A was less active as a skin tumor initiator than DB(a,h)A.

Table 2 shows the skin tumor initiating activities of the weak carcinogenic PAH chrysene and some of its dihydrodiols and diol-epoxides. At a dose of 2 umoles chrysene 1,2-dihydrodiol induced papillomas in 88% of the mice with an average of 2.8 papillomas per mouse whereas the parent hydrocarbon initiated tumors in 73% with an average of 1.6 papillomas per mouse. The 3,4-dihydrodiol of chrysene was essentially inactive as a skin tumor initiator since the TPA only controls induced the same level of

tumor response (0.1 papillomas per mouse). Similar results to ours were also reported by Levin et al. (27) for the tumorigenicity of chrysene 1,2- and 3,4-dihydrodiol. The bay region diol-epoxide (chrysene 1,2-diol-3,4-epoxide) induced tumors in 38% of the mice with an average of 0.5 papillomas per mouse whereas chrysene 3,4-diol-1,2-epoxide initiated only 0.2 papillomas per mouse.

The skin tumor initiating activities of two other weak carcinogenic PAH and some of their dihydrodiols and diol-epoxides are shown in Table 3. The B(e)P 9,10-dihydrodiol which is the immediate precursor to the bay region diol-epoxide induced a significant number of tumors (0.5 papillomas per mouse at a dose of 2 umoles whereas the parent hydrocarbon did not (Table 3). It is of interest to point out that the bay region diol-epoxide of B(e)P was also ineffective as a tumor initiator. The 10,11-dihydrodiol of DB(a,c)A and the corresponding diol-epoxide (non-bay region) were found to be essentially inactive as skin tumor initiators.

It is of interest to compare the experimentally determined activities of the various dihydrodiols in Tables 1-3 with the MO theoretically predicted relative activities of the corresponding diol-epoxide metabolites. According to the "bay region theory" (20,21), the bay region diol-epoxides of carcinogenic PAH are characterized by exceptional reactivity predictable by ΔE_{deloc}, the calculated difference in β-delocalization energies between the diol-epoxide structure and the related ring-open benzylic carbocation structure. In Table 4 are listed the values of ΔE_{deloc} of the diol-epoxides considered to be the metabolically activated forms of the dihydrodiols in this table. In order to compare the carcinogenic activities of the dihydrodiols which were assayed at different dosages, the data is

expressed in terms of an arbitrary "tumorigenic index" = (papillomas per mouse) X % mice with tumors/dose (umoles). Correlation between the tumorigenic index and ΔE_{deloc} is generally good, the sole exception being the lower activity of B(e)P 9,10-dihydrodiol in relation to chrysene 3,4-dihydrodiol. The weak activity of the former may be in part a consequence of its existence in the diaxial conformation (13,40), whereas all the remaining less sterically restricted dihydrodiols in Table 4 are shown by NMR evidence (10-12,25,26) to exist predominantly in the diequatorial conformation. Although the biological consequences of this conformational difference are unknown, it is conceivable that the diaxial hydroxyl groups of B(e)P 9,10-dihydrodiol sterically interfere with its enzymatic epoxidation and subsequent reaction of the resulting diol-epoxide with DNA. This suggests that the steric as well as the electronic properties of the active dihydrodiol and diol-epoxide metabolites should be taken into account in any theory of carcinogenesis.

Although the dihydrodiols of the various PAH used in this study which are the immediate metabolic precursors of the bay region diol-epoxides were active as tumor initiators, the various bay region anti-diol-epoxides tested were extremely weak as tumor initiators except for the bay region anti-diol-epoxide of chrysene which had approximately one-third the activity as the parent hydrocarbon. It is of interest to point out that the only bay region diol-epoxide tested so far that had more activity than the parent hydrocarbon is related to the weak carcinogen B(a)A. The bay region (±)-anti-diol-epoxide of B(a)A was found to have approximately 6 times the activity as the parent hydrocarbon (28,38). The bay region (±)-anti-diol-epoxide of B(a)P was found to have about one-third the

activity as B(a)P (34). When the (+) and (-) enantiomeric forms of the anti-diol-epoxide of B(a)P were tested as skin tumor initiators, the (+)-anti-diol-epoxide of B(a)P was approximately as active as B(a)P whereas the (-)-anti-diol-epoxide was essentially inactive (33). The reasons for the very weak activities of the various diol-epoxides tested in the study in relation to the corresponding dihydrodiols are presently unknown. However, it is very likely that it is primarily a consequence of the indiscriminant interaction of these relatively reactive molecules with water, proteins, and other nucleophiles before they can reach the critical cellular target (presumably DNA).

Carcinogenicity of Monomethylated Derivatives of B(a)P

A comparison of the skin tumor initiating activities of various monomethylated derivatives of B(a)P is shown in Table 5. It is quite evident that methyl substitution at 7, 8, 9 and 10 positions of B(a)P eliminates tumor-initiating activity. Likewise, 9-methyl B(a)P 7,8-dihydrodiol was found to be inactive. This is consistent with the concept of "bay region" activation of B(a)P at these positions and in contrast to the observation by Harvey et al., who found 7-MB(a)P to have moderate tumorigenic activity when injected subcutaneously into rats. Methyl or fluoro substitution in the bay region of DMB(a)A also effectively eliminates its carcinogenic activity (17,37). 3-MB(a)P has weak tumor-initiating activity under our test conditions in contrast to the high sarcomogenic activity observed by Lacassagne et al. (24) of this derivative. The carcinogenicity of 3-M and 4-MB(a)P derivatives can be explained in terms of interference with detoxification mechanisms at these two positions. By a similar argument a steric inhibition

of detoxification at 3 position and inhibition of activation at 7,8 positions by methyl substitution at 1 and 5 positions respectively, cannot be ruled out to explain the tumor-initiating activity of these two derivatives.

However, the most striking observation of all is the fact that 11-MB(a)P has about three times the tumor-initiating activity of B(a)P. It should be noted that in case of the 11-methyl derivative the methyl group is attached to the carbon which forms part of a "bay region", but occupying a position distal to the site of activation. Similar situation arises in case of B(a)A, where substitution of methyl group at the distal carbon of the "bay region" makes the compound highly tumorigenic (e.g. 12-methyl B(a)A or 7,12-dimethyl B(a)A. Likewise, 5-methylchrysene is more tumorigenic than chrysene (14). Although not tested, one would expect 3,6-dimethylcholanthrene to be more tumorigenic than 3-MC and 7 or 14 methyl DB(a,h)A to be more tumorigenic than DB(a,h)A. One explanation which might seem tangible at least in the B(a)P series is that the substitution at 11-position directs the enzymatic epoxidation stereospecifically to the highly tumorigenic anti-diol-epoxide, by steric inhibition of the formation of the less carcinogenic syn-isomer. It has been shown for example, that enzymatic epoxidation of 9,10 double bond in B(a)P is not entirely stereospecific but rather stereoselective (20). The non-carcinogenicity, in mouse skin, of the syn isomer has been related to its high reactivity and consequent inability to reach a target site. If this explanation is taken for granted, then it is probable that by steric inhibition of the formation of a more reactive ultimate carcinogen through substitution (for example at 11-position) a more carcinogenic isomer of the ultimate carcinogen is generated.

Importance of an Unhindered Peri Position in PAH Carcinogenicity

A comparison of the skin tumor initiating activities of 3-MC, DMB(a)A, 7-MB(a)A, 12-MB(a)A and their activities with substitution of a methyl or fluoro group in their peri position are shown in Table 6. It is quite evident that a hindered peri position in the above PAH (positions 11, 5, 5 and 5, respectively of 3-MC, DMB(a)A, 7-MB(a)A and 12-MB(a)A) drastically decreases their skin tumor initiating activities. Likewise, Table 5 shows that 6-MB(a)P (peri position) is much less active as a skin tumor initiator than B(a)P. From these results, as well as published results, we suggest that an unhindered peri position adjacent to an angular benzene ring is necessary for carcinogenic activities (14, 17,36,37). We previously reported that substitution of a methyl or fluoro group in the bay region as well as peri position of DMB(a)A almost completely counteracted the skin tumor initiating activities of DMB(a)A, whereas substitution of a fluoro in the 9 and 11 position had no effect on the activity of DMB(a)A (37). Hecht and coworkers (14) also found that a methyl or fluoro substitution in the peri position of 5-methylchryrene (position 12) drastically decreased the skin tumor initiating activity of 5-methylchrysene. What influence a hindered peri position has on the formation of a bay region diol-epoxide is presently not known but it is quite clear that an unhindered peri position is a necessary prerequisite for metabolic activation in bay region of five different methylated PAH carcinogens and one nonmethylated PAH carcinogen.

CONCLUSION

The data presented and discussed in this report allows us to suggest three important generalizations about PAH carcinogenicity in mouse skin. First, that bay region dihydrodiols which are the immediate precursors of bay region diol-epoxides are the principle determinants of PAH carcinogenicity as proposed by Jerina and coworkers (20,21). This data is supported by the data which shows that substitution of a methyl or fluoro group into the positions involved in a bay region diol-epoxide drastically decreases its carcinogenicity (Figure 1). Second, that an unhindered peri position adjacent to an angular benzene ring is necessary for carcinogenic activity. Third, that a methyl group attached at the distal carbon of the bay region makes the compound more tumorigenic than the parent PAH.

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Table 1

A comparison of the skin tumor-initiating activities of some dihydrodiols and diol-epoxides of 7-MB(a)A and DB(a,h)A

7-MB(a)A and derivatives and DB(a,h)A and derivatives were applied at a dose of 400 nmoles and 100 nmoles, respectively, and followed one week later by twice weekly applications of 2 ug of TPA.

Initiator	No. of mice at 15 weeks	Papillomas per mouse at 15 weeks	Mice with tumor (%) at 15 weeks
7-MB(a)A	29	3.8	83
7-MB(a)A 3,4-dihydrodiol	28	6.8	100
7-MB(a)A 3,4-diol-1,2-epoxide	29	0.3	22
DB(a,h)A	29	1.4	50
DB(a,h)A 3,4-dihydrodiol	29	0.7	37
DB(a,h)A 3,4-diol-1,2-epoxide	29	0.1	7
Control (only TPA promotion)	30	0.1	6

Table 2

Skin tumor-initiating activities of several dihydrodiols and diol-epoxides of the weak carcinogen chrysene

Chrysene and the various dihydrodiols and diol-epoxides were applied at a dose of 2 umoles and followed one week later by twice weekly applications of 2 ug of TPA.

Initiator	No. of mice at 15 weeks	Papillomas per mouse at 15 weeks	Mice with tumors (%) at 15 weeks
Chrysene	29	1.6	73
Chrysene 1,2-dihydrodiol	28	2.8	83
Chrysene 1,2-diol-3,4-epoxide	29	0.6	38
Chrysene 3,4-dihydrodiol	30	0.1	11
Chrysene 3,4-diol-1,2-epoxide	29	0.2	7
Control (only TPA promotion)	30	0.2	10

Table 3

Skin tumor-initiating activities of some dihydrodiols and diol-epoxides of the weak carcinogens B(e)P and DB(a,c)A

B(e)P and DB(a,c)A and various dihydrodiols and diol-epoxides were applied at a dose of 2 umoles and followed one week later by twice weekly applications of 2 ug of TPA.

Initiator	No. of mice at 15 weeks	Papillomas per mouse at 15 weeks	Mice with tumors (%) at 15 weeks
B(e)P	29	0.2	17
B(e)P 9,10-dihydrodiol	28	0.6	32
B(e)P 9,10-diol-11,12-epoxide	29	0.1	6
DB(a,c)A	28	0.5	27
DB(a,c)A 10,11-dihydrodiol	29	0.1	10
DB(a,c)A 10,11-diol-12,13-epoxide	29	0.1	10
Control (only TPA promotion)	30	0.1	10

Table 4

Comparison between the observed tumorigenic activities of PAH dihydrodiols and the MO theoretically predicted reactivities of the corresponding diol-epoxide metabolites

Dihydrodiol	Tumorigenic index[a]	ΔE_{deloc}
Chrysene-1,2	0.5	0.526
DB(a,c)A-10,11	0.5	0.544
B(e)P-9,10	10	0.714
Chrysene-3,4	116	0.640
DB(a,h)A-3,4	259	0.738
7-MB(a)A-3,4	1700	0.766[b]

[a]Tumorigenic index = (papillomas per mouse) X (% mice with tumors)/dose (umoles) under the experimental conditions described.

[b]This value of ΔE_{deloc} is calculated for B(a)A-3,4-dihydrodiol, since the effect of methyl substitution cannot be accurately estimated by simple perturbation theory.

Table 5

Skin tumor-initiating activities of various mono-methylated derivatives of B(a)P after TPA promotion.

B(a)P and its mono-methylated derivatives were applied topically at a dose of 200 nmol and were followed 1 week later by twice-weekly applications of 2 ug of TPA.

Initiator	No. of mice at 15 weeks	Papillomas per mouse at 15 weeks	Mice with tumors (%) at 15 weeks
Control (only TPA promotion)	29	0.1	6
B(a)P	30	2.0	67
1-MB(a)P	29	2.62	70
2-MB(a)P	29	0.55	38
3-MB(a)P	29	2.62	76
4-MB(a)P	30	2.03	67
5-MB(a)P	30	0.37	27
6-MB(a)P	29	0.35	24
7-MB(a)P	27	0	0
8-MB(a)P	28	0.03	3
9-MB(a)P	29	0	0
10-MB(a)P	29	0	0
11-MB(a)P	30	4.6	80
12-MB(a)P	29	1.7	69
9-MB(a)P 7,8-dihydrodiol	28	0.07	7
B(a)P 7,8-dihydrodiol	30	2.53	77

Table 6

The Effects of a Hindered Peri Position on the

Skin Tumor Initiating Activities of

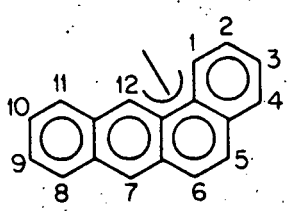
3-MC, DMB(a)A, 7-MB(a)A and 12-MB(a)A

The PAH were applied at the doses shown and followed one week later by twice weekly applications of 2 µg of TPA.

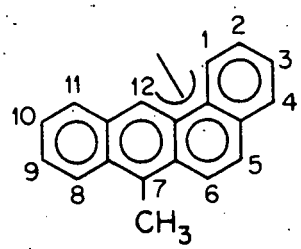
Initiator	Dose (nmoles)	No. of mice at 16 weeks	Papillomas per mouse at 16 weeks	Mice with papillomas (%) at 16 weeks
3-MC	100	29	4.5	92
3,11-DMC	100	30	0.2	10
DMB(a)A	10	28	8.6	100
5-F DMB(a)A	10	29	0.2	15
5,7,12-TMB(a)A	10	28	0.4	34
7-MB(a)A	400	30	3.1	86
5-F,7-MB(a)A	400	29	0.4	33
5,7-DMB(a)A	400	29	0.6	41
12-MB(a)A	400	29	1.6	73
5-F,12-MB(a)A	400	29	0.2	10
Control (TPA only)		29	0.1	6
Control (3-MC only)		30	0	0

FIGURE LEGEND

Figure 1 - The structures of B(a)A, 7-MB(a)A, DMB(a)A, 3-MC, chrysene, B(a)P, B(e)P, DB(a,h)A and DB(a,c)A. The arrows give the location of the bay regions of the various PAH.



B(a)A



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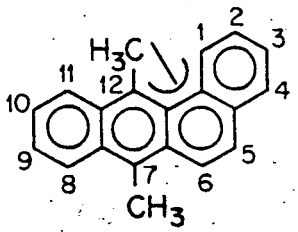

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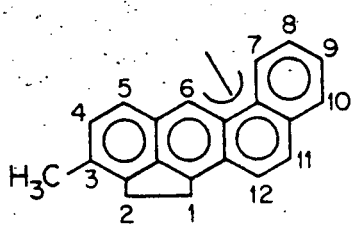

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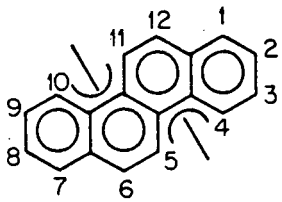
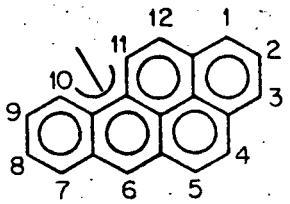
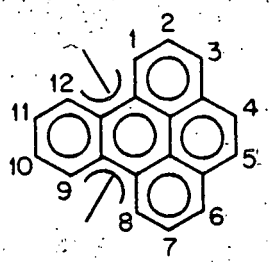


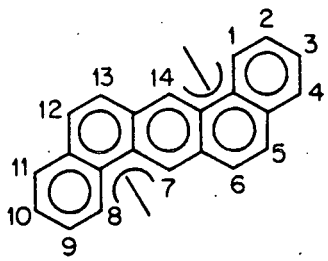
Chrysene



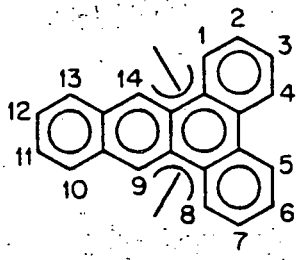
B(a)P



B(e)P



DB(a,h)A



DB(a,c)A