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POLYCYCLIC AROMATIC HYDROCARBON CARCINOGENESIS: STRUCTURE-ACTIVITY RELATIONSHIPS

Volume I

Shen K. Yang
B. D. Silverman

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Polycyclic Aromatic Hydrocarbon Carcinogenesis: Structure-Activity Relationships

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PREFACE

The induction of carcinogenesis by polycyclic aromatic hydrocarbons (PAH) is currently understood to be a complex multistep process that is dependent upon many factors. One intriguing aspect has been the wide range of chemical potency that depends upon PAH molecular geometry. This variation in potency is found to depend, not only on different salient structural features of the parent PAH, but also on the different detailed stereochemical geometries of potentially reactive metabolites and their precursors. Investigations over the past decade have led to a clearer appreciation of factors that are important during the ''initiation stage'' of PAH induced carcinogenesis. Such factors include the metabolic fate of parent PAHs, the extent of formation and reactivity of electrophilic species and subsequent enzymatic repair of PAH-DNA adducts formed from such species. All of these processes depend sensitively upon molecular geometry. The present volume is, therefore, devoted to investigations aimed at elucidating the role played by PAH structure in the initiation of tumorigenesis. Hopefully, an understanding of the structural factors resulting in different carcinogenic potencies of PAHs and their metabolites can assist practical considerations of cancer prevention.

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Chapter 1

CARCINOGENICITY AND MUTAGENICITY OF PROXIMATE AND ULTIMATE CARCINOGENS OF POLYCYCLIC AROMATIC HYDROCARBONS

Peter G. Wislocki and Anthony Y. H. Lu

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I. INTRODUCTION

Kennaway and Hieger[1] and Cook et al.[2] isolated polycyclic aromatic hydrocarbons as the first pure chemicals which could cause cancer in animals. This induction of tumors by a pure chemical was a landmark in the field of chemical carcinogenesis. It afforded cancer researchers a tool by which they could examine the carcinogenic process. Since those first steps, much progress has been made in our understanding of how chemicals cause cancer. It is of interest that although the polycyclic aromatic hydrocarbons were the first pure chemical carcinogens that were identified, our understanding of the steps involved in the carcinogenicity of polycyclic aromatic hydrocarbons came later than our understanding of the steps involved in the carcinogenicity of other chemical carcinogens such as aromatic amines and amides, and nitrosamines. Drs. James and Elizabeth Miller[3] were the first to formulate the theory that the key step involved in chemical carcinogenesis was the interaction of the electrophilic chemical carcinogen or electrophilic metabolite of a chemical carcinogen with cellular nucleophiles to initiate the carcinogenic process. The idea that certain chemical carcinogens required metabolic activation in order to act as carcinogens led to the concept of proximate and ultimate carcinogens. An ultimate carcinogen is defined as that metabolite of the parent carcinogen which reacts with the critical cellular nucleophile of the cell to initiate the carcinogenic process. It is reactive of itself and requires no further metabolic activation in order to react with cellular components. The proximate carcinogen is an intermediate metabolite of the parent carcinogen which must undergo further metabolism to the ultimate carcinogen. There could be several proximate carcinogenic metabolites which are on the pathway of metabolism from the parent carcinogen to the ultimate carcinogen.

Based on these definitions of proximate and ultimate carcinogen, they should have several characteristics. The proximate carcinogenic metabolite should be more carcinogenic than its parent compound since it is closer to the ultimate carcinogen and is exposed to fewer inactivation pathways than the parent compound. In a series of proximate carcinogens, those which are closer to the ultimate carcinogenic metabolite should in theory be more carcinogenic than those which are closer to the parent compound. The ultimate carcinogenic metabolite should be more carcinogenic than both the parent compound and its proximate carcinogenic metabolites. Although this indeed should be the case, care should be exercised in the use of these criteria in the characterization of metabolites as proximate and especially ultimate carcinogens. Since ultimate carcinogens are chemically reactive, they may not be stable enough to be administered to animals by the same route as used for the parent or proximate carcinogens. Therefore, selection of the method for carcinogenicity testing of the proposed ultimate carcinogens is of critical importance.

Besides comparing the carcinogenicity of the proposed ultimate carcinogen with that of the parent compound and proximate carcinogenic metabolites, several other methods can be used to determine what the ultimate carcinogenic metabolite of a carcinogen is. Classically it was determined whether the proposed ultimate carcinogen or a closely related model compound would react with typical cellular nucleophiles to form covalently bound adducts. The presence of such covalently bound adducts, especially those bound to DNA, was sought in the tissues of animals treated with the parent compound in vivo. It was also determined whether the parent compound and its proximate carcinogenic metabolites could undergo metabolic activation using in vitro enzyme systems. More recently, with the advent of short-term in vitro tests, such as the Ames test, to detect the interaction of chemicals with DNA to cause mutations, these methods have been used to determine the ability of metabolites to cause mutations in the presence and absence of metabolic activation systems. By this means, the potential of metabolites to be proximate or ultimate carcinogens has been assessed. These in vitro tests have also been used successfully to determine what enzymes are involved in the metabolic activation of carcinogens. Ultimately, however, only carcinogenicity tests can define the carcinogenicity of a compound.

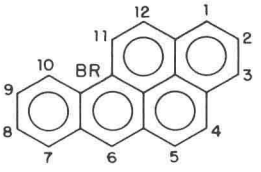

FIGURE 1. Benzo(a)pyrene with bay region (BR) indicated.

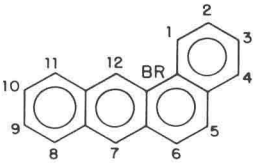

FIGURE 2. Benz(a)anthracene with bay region (BR) indicated.

In this chapter, the results of the mutagenicity and carcinogenicity testing of the proximate and ultimate carcinogenic metabolites of polycyclic aromatic hydrocarbons will be discussed. The advantages and disadvantages of these methods for determining the pathways for the metabolic activation of polycyclic aromatic hydrocarbons should become evident. Other chapters in this volume will deal with the aspects of metabolism and DNA binding of both polycyclic aromatic hydrocarbons and other types of carcinogens and therefore these subjects will not be discussed in detail in this chapter. Another chapter will also discuss in detail the methylchrysenes.

II. HISTORICAL PERSPECTIVE

In the early 1970s an understanding of the metabolic activation of several of the classes of chemical carcinogens had been obtained. This was not the case with polycyclic aromatic hydrocarbons. It was still not clear whether these compounds which induced cancer at the site of administration required metabolic activation for their carcinogenic activity. Boyland[4] had proposed earlier that epoxides of polycyclic aromatic hydrocarbons were responsible for their carcinogenic activity. Such epoxides would be the ultimate carcinogens of polycyclic aromatic hydrocarbons. For many years the K-region (that area at the "bend" of the molecule: the 4,5 carbons of benzo(a)pyrene, Figure 1; and the 5,6 carbons of benz(a)anthracene, Figure 2) was proposed to be the key area for metabolic activation of polycyclic aromatic hydrocarbons.[5] Indeed, in a review by Sims et al.[6] in 1973, just prior to the explosive growth of this area of chemical carcinogenesis, the major thrust of the review was concerned with the feasibility of the K-region oxide as the ultimate carcinogen of polycyclic aromatic hydrocarbons. Yet in this same review, in retrospect it was evident that other regions of the polycyclic aromatic hydrocarbons might be important in the biological activity of this class of compounds. It was reported that the 9,10-epoxide of 7,8,9,10-tetrahydrobenzo(a)pyrene was extremely toxic in the Ames test and showed strong alkylating ability. Its parent compound, the 7,8-dihydrobenzo(a)pyrene, was strongly carcinogenic. This region of the molecule later became known as the bay region. The first direct evidence of the importance of this region of the benzo(a)pyrene molecule also came from the laboratory of Sims and Grover. In 1974, Sims et al.[7] reported the metabolic formation of benzo(a)pyrene 7,8-diol-9,10-epoxide and its binding to the DNA of cultured cells. Earlier, Borgen et al.[8] showed that benzo(a)pyrene 7,8-dihydrodiol can be metabolically activated to a species which showed great ability to bind to DNA. These findings initiated more than a decade of studies on the

metabolic activation of polycyclic aromatic hydrocarbons to their ultimate carcinogens. These studies were first concentrated on benzo(a)pyrene and later on other polycyclic aromatic hydrocarbons. The results of studies on the mutagenicity and carcinogenicity of the proximate and ultimate carcinogenic metabolites of many of the polycyclic aromatic hydrocarbons will be reported in this chapter.

The findings of Borgen et al. and Sims et al. led to a veritable "land rush" of research among several laboratories. Much of the competition between laboratories resulted in the collaboration between two or more laboratories with the capability of synthesizing the chemicals, and those which could test the chemicals for biological activity. The laboratories of Conney at Hoffmann-La Roche and Jerina at the National Institute of Arthritis, Metabolism, and Digestive Diseases had begun collaborating in 1973 in order to synthesize and test as many of the metabolites of benzo(a)pyrene as possible. By this means it was hoped that the proximate and ultimate carcinogenic metabolites could be determined. The laboratory of Gelboin and Yang at the National Cancer Institute, NCI, collaborated with Huberman and with Sugimura. The laboratory of Sims and Grover collaborated with Marquardt and with Malaveille and Bartsch at the International Agency for Research on Cancer, IARC, and with Chouroulinkov. Other laboratories such as Slaga's at Oak Ridge and Harvey's in Chicago also made significant contributions in the area of polycyclic aromatic hydrocarbons carcinogenesis with respect to the testing or synthesis of proximate or ultimate carcinogens. These collaborations proved very fruitful and the competition between laboratories enhanced both the quality and quantity of research performed.

III. METHODS

A. Mutagenicity

The main in vitro method used to determine potential proximate and ultimate carcinogenic metabolites of polycyclic aromatic hydrocarbons was the Ames test.[9] In brief, this test involves the incubation of bacteria with the chemical to be tested in the presence or absence of enzymes capable of metabolically activating the chemical to a mutagenic species. The source of the enzymes could be the microsome and cytosol containing S9 fraction isolated from the liver of rats which had been treated with inducers of microsomal enzymes (such as phenobarbital, 3-methylcholanthrene, or the polychlorinated biphenyl mixture Aroclor® 1254) or could be the purified microsomal enzymes (cytochromes P-450) themselves. The interaction of the mutagenic species with the bacteria causes the bacteria to revert from one requiring histidine for growth to one which can grow in the absence of histidine to form a colony which can be tabulated. Several Ames strains of Salmonella were used in the studies reported. As will be indicated below, they varied in their relative sensitivities to the compounds tested.

The ability of a chemical to mutate V79 cells was another indication of mutagenic potency.[10] After treatment of the Chinese hamster lung cells with the chemical, the cells were treated with 8-azaguanine or ouabain. Those cells which had mutated and had become resistant to the toxic effects of these selection agents were counted. Since V79 cells have limited capacity to metabolically activate chemicals, they were sometimes supplemented with other cell types which could metabolically activate the various polycyclic aromatic hydrocarbon metabolites, such as golden hamster embryo cells.[11] Transformation assays in vitro also were used to a small extent in these studies.

B. Carcinogenicity

Several carcinogenicity models were used to assess the oncogenic potential of the various derivatives of the polycyclic aromatic hydrocarbons. The two-stage initiation and promotion model was used extensively.[12] In this model, one applies the chemical once to the shaved

backs of mice to initiate the tumorigenic process and cause the development of papillomas. This treatment was then followed with a promoter such as 12-O-tetradecanoylphorbol-13-acetate (TPA) to complete the carcinogenic process. The degree of tumorigenicity of the compounds was related to the percentage of mice with tumors and more specifically to the number of tumors/tumor-bearing mouse.

A second method of assessing the carcinogenicity of the compounds is to apply the chemical chronically by topical administration to the back of the mouse.[13] This method enables one to determine whether the compound under test is a complete carcinogen and not just an initiator. The disadvantage is that this method requires a significantly greater amount of compound than the initiation-promotion experiment. The development of carcinomas on the backs of the mice was the end point used for the assessment of the carcinogenicity of the compound.

The method which proved to be the best method for determining the carcinogenicity of the polycyclic aromatic hydrocarbon metabolites was the newborn mouse model.[14-17] This model involved the administration of small quantities of the chemical to mice at 1, 8, and 15 days of age by intraperitoneal injection in DMSO. The mice developed lung adenomas, liver tumors, and malignant lymphomas over a period of 18 to 40 weeks. The length of time required for tumor formation was dependent on the dose and the carcinogenic potential of the compound. This method used small amounts of compound, was only labor intensive at the beginning of the study, and proved to give the most meaningful results.

Other methods such as the induction of subcutaneous tumors by subcutaneous injection of the compound into the nape of the neck or i.v. administration of the compound to female rats to cause mammary tumors did not prove as successful as the methods discussed.

As indicated earlier, care must be taken in the interpretation of results since the reactivity of ultimate and in some cases proximate carcinogens could lead to unexpected results. The results with some benzo(a)pyrene metabolites can be used as an example of the care which must be taken in the interpretation of results. Based on the finding that a 7,8-diol-9,10-epoxide was the ultimate carcinogen of benzo(a)pyrene, benzo(a)pyrene 7,8-oxide, and benzo(a)pyrene 7,8-dihydrodiol were, therefore, proximate carcinogenic metabolites. However, when tested for biological activity in the various systems, they did not always behave as expected. Wislocki et al.[18] found that benzo(a)pyrene 7,8-oxide was not strongly mutagenic to V79 cells. This was probably due to the absence of activating enzymes. In the presence of purified activating enzymes, Wood et al.[19] reported that benzo(a)pyrene 7,8-oxide and benzo(a)pyrene 7,8-dihydrodiol were metabolically activated to mutagenic species to a greater extent than benzo(a)pyrene. These data indicated that further metabolism was required to form the mutagenic species. Slaga et al.[20] had found that the presumed ultimate carcinogens of several polycyclic aromatic hydrocarbons were less active than the parent compound in the initiation-promotion model. Yet these same compounds were active in the newborn mouse model. Therefore, the results from several different systems should be considered before the final conclusions of what metabolites might be the proximate or ultimate carcinogens are drawn.

IV. BENZO(a)PYRENE

The first study on the mutagenicity of benzo(a)pyrene 7,8-dihydrodiol and the 7,8-diol-9,10-epoxide was reported by Malaveille et al.[21] The 7,8-dihydrodiol of benzo(a)pyrene was more mutagenic towards *Salmonella typhimurium* strain TA100 than was benzo(a)pyrene. The diol-epoxide also displayed mutagenic activity although it was not remarkably stronger than benzo(a)pyrene 4,5-oxide. The strong mutagenic activity of a benzo(a)pyrene 7,8-diol-9,10-epoxide in both the Ames *S. typhimurium* strains and in cultured Chinese hamster V79 cells was demonstrated by Wislocki et al.[22] Since the epoxide oxygen can be either cis or

trans to the 7-hydroxyl group, a pair of enantiomers exists for each diol-epoxide. In the early stage of the study of the diol-epoxides of benzo(a)pyrene, only the geometric relationship of the 7-hydroxy group and the epoxide oxygen was considered. The diol-epoxide studied by Wislocki et al.[22] had the 7-hydroxy group on the same side of the plane of the molecule as the epoxide oxygen. Based on the nomenclature of Jerina, it was designated as diol-epoxide-1. The diol-epoxide which earlier workers had studied[21] had the 7-hydroxy group on the opposite side of the plane of the ring relative to the epoxide oxygen. It was designated as diol-epoxide-2. (In Gelboin's laboratory, these epoxides were known as diol-epoxides (II) and (I), respectively.) Diol-epoxide-2 was a racemic mixture of a pair of enantiomers. It was several-fold more mutagenic than benzo(a)pyrene-4,5-oxide in the Ames strains TA98 and TA100. Likewise in V79 cells, this diol-epoxide was approximately 40 times more mutagenic than the 4,5-oxide of benzo(a)pyrene. Earlier, Huberman et al.[11] had found that both diol-epoxides-1 and -2 were more mutagenic to V79 cells than the 4,5-oxide. Diol-epoxide-2 was 20 times more active than diol-epoxide-1. The stronger activity of diol-epoxide-2 compared to diol-epoxide-1 was later confirmed by Wood et al.[23] who found that diol-epoxide-2 was 2 times more mutagenic in V79 cells than was diol-epoxide-1. Newbold and Brookes[24] also described the higher mutagenicity of the diol-epoxide-2 when compared to diol-epoxide-1 in V79 cells. Although in their studies diol-epoxide-2 was severalfold more mutagenic than diol-epoxide-1, this result was confirmed by Marquardt and Baker[25] who found a tenfold difference in mutagenicity between the two diol-epoxides. In studies of the mutagenicity of the diol-epoxides in the Ames strains, findings of high mutagenicity for both diol-epoxides were also being reported. Significantly in the Ames strains, diol-epoxide-1 was more mutagenic than diol-epoxide-2.[23,26] The difference in results obtained with the diol-epoxides in V79 cells and the Ames test led to the question of which test system best predicts the carcinogenicity of the diol-epoxides. In parallel to the mutagenicity testing of the proposed proximate and ultimate carcinogens of benzo(a)pyrene, carcinogenicity testing of the compounds was also being performed.

Wood et al.[13] in 1976 showed that when benzo(a)pyrene 7,8-oxide, a proposed proximate carcinogen of benzo(a)pyrene, was chronically applied to the backs of mice, it was a complete carcinogen. Although weaker than benzo(a)pyrene at low doses, it caused a substantial number of tumors at high doses (equal in potency to benzo(a)pyrene). The finding of insignificant tumorigenic activity of the 4,5-oxide or 9,10-oxide served to confirm the importance of activation at the 7, 8, 9, 10 positions. Chouroulinkov et al.[27] found that of the 4,5-, 7,8-, and 9,10-dihydrodiols, only the benzo(a)pyrene 7,8-dihydrodiol possessed tumor-initiating activity similar to that of benzo(a)pyrene. Likewise, at the same time, Slaga et al.[28] had determined that benzo(a)pyrene 7,8-oxide was one third as active as benzo(a)pyrene, and that benzo(a)pyrene 7,8-dihydrodiol was equally potent as benzo(a)pyrene. Levin et al.[29] reported that benzo(a)pyrene 7,8-dihydrodiol was equally potent as benzo(a)pyrene as a complete carcinogen after repeated topical administration of the compound to mouse skin. These data further confirmed the theory that benzo(a)pyrene 7,8-dihydrodiol was a proximate carcinogen of benzo(a)pyrene in the tumor-initiation system. However, Slaga in the first test of its carcinogenic potential[28] had also tested benzo(a)pyrene diol-epoxide-2 and surprisingly this compound showed only weak tumor-initiating activity. It was proposed that this may have been due to its high reactivity. The lack of greater carcinogenic activity by the diol-epoxides of benzo(a)pyrene on mouse skin was confirmed in several studies,[30-32] as was the lack of significantly greater carcinogenic activity of the 7,8-dihydrodiol of benzo(a)pyrene compared to that of benzo(a)pyrene.[32] These studies[31,32] did, however, indicate that diol-epoxide-2, although not as strong a carcinogen as benzo(a)pyrene, was more carcinogenic than diol-epoxide-1.

It was not until the newborn mouse model was used to test for the carcinogenicity of the proposed proximate and ultimate carcinogens of benzo(a)pyrene, that results consistent with

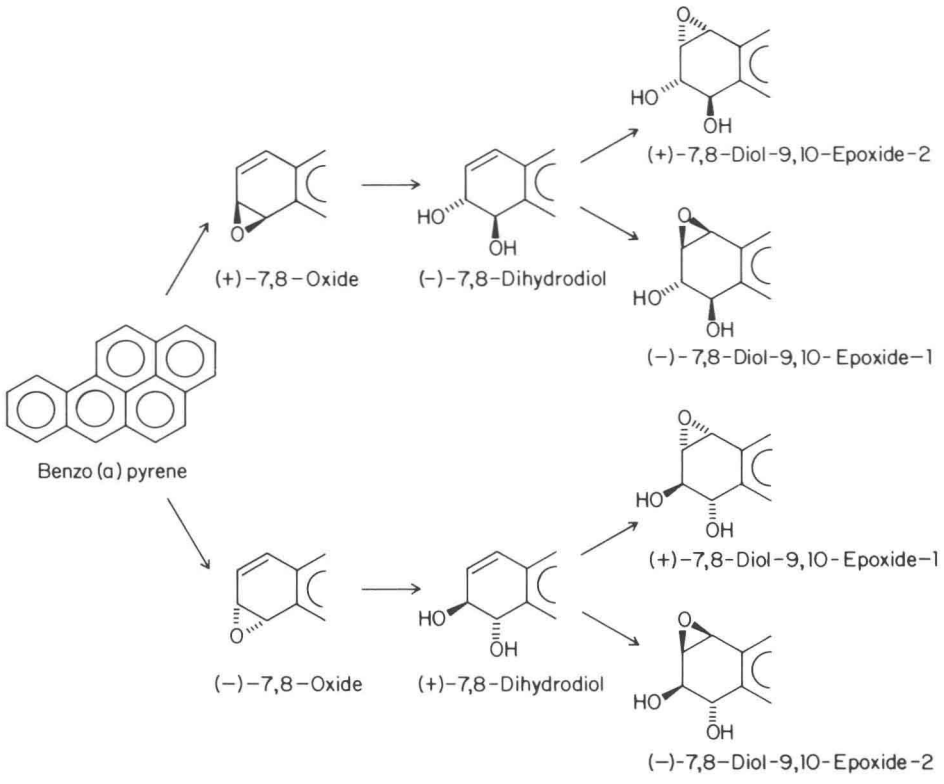

FIGURE 3. The metabolic activation of benzo(a)pyrene to optically active proximate and ultimate carcinogens.

their proposed importance were found. Kapitulnik et al.[17] reported that benzo(a)pyrene 7,8-dihydrodiol was about 12 times more carcinogenic than benzo(a)pyrene in the induction of lung adenomas and induced a high incidence of malignant lymphomas which were not observed in the benzo(a)pyrene-treated group. Benzo(a)pyrene diol-epoxide-2 could only be tested at a level of $^{1}/_{50}$ of the dose at which benzo(a)pyrene was tested. At this level diol-epoxide-2 was equally carcinogenic to benzo(a)pyrene. Therefore, the 7,8-dihydrodiol and diol-epoxide-2 appeared to be proximate and ultimate carcinogens of benzo(a)pyrene, respectively, in the newborn mouse. Diol-epoxide-1 proved to be too toxic and a sufficient number of mice were not available for tumor incidence determination. In a definitive study, Kapitulnik et al.[33] demonstrated that diol-epoxide-1 was not tumorigenic in the newborn mouse. Diol-epoxide-2 was approximately 40 times more carcinogenic than benzo(a)pyrene and greater than two times more carcinogenic than benzo(a)pyrene 7,8-dihydrodiol. Based on these studies, there was a better correlation between the mutagenicity of the diol-epoxides in V79 cells compared to their mutagenicity in the Ames bacterial strains.

It was known that benzo(a)pyrene 7,8-oxide, 7,8-dihydrodiol, and 7,8-diol-9,10-epoxides were mixtures of enantiomers (optical isomers). When metabolism studies[11,34-36] indicated that benzo(a)pyrene was metabolized stereoselectively to (−)-7,8-dihydrodiol, and furthermore that the (−)-7,8-dihydrodiol was metabolized stereoselectively to (+)-diol-epoxide-2 (Figure 3), it became essential to determine the biological activity of the enantiomers of the proximate and ultimate carcinogenic metabolites of benzo(a)pyrene. When the (+)- and (−)-enantiomers of benzo(a)pyrene *trans*-7,8-dihydrodiol were tested for mutagenicity in the Ames strain,[37] the (+)-enantiomer showed greater activity, while in the V79 cells,[38] the (−)-enantiomer showed greater activity. These data raised intriguing questions concerning the role of metabolism in the carcinogenicity of the dihydrodiols and the diol-epoxides.

Enantiomers of diol-epoxides-1 and -2 could be the metabolic products of the (+)- and (−)-7,8-dihydrodiol. It therefore remained a question as to what the carcinogenicities of the different optical isomers of the proposed proximate and ultimate carcinogenic metabolites of benzo(a)pyrene were. No carcinogenicity studies had been done previously on the enantiomers of any carcinogens, especially ultimate carcinogens. Therefore, the (+)- and (−)-enantiomers of benzo(a)pyrene 7,8-dihydrodiol were tested for carcinogenicity in both the initiation-promotion model on mouse skin and in the newborn mouse. Levin et al.[39,40] found that the (−)-7,8-dihydrodiol of benzo(a)pyrene was approximately tenfold more tumorigenic as an initiator than the (+)-isomer. It was also significantly more active then benzo(a)pyrene. These data were the first indication that the 7,8-dihydrodiol of benzo(a)pyrene could be more active than its parent compound.

This greater carcinogenicity of the (−)-isomer was also found in the newborn mouse model. In this model, the difference in carcinogenicity between the two isomers was even more dramatic. The (−)-7,8-dihydrodiol was 15 times more active in causing lung adenomas than (+)-7,8-dihydrodiol. The (−)-isomer also caused a much higher incidence of malignant lymphomas compared to the (+)-isomers. When the four diastereomers of the 7,8-diol-9,10-epoxides of benzo(a)pyrene were tested for carcinogenicity, it was found that only one of the isomers possessed significant tumor-initiating or carcinogenic activity. Buening et al.[41] showed that (+)-7,8-diol-9,10-epoxide-2 was greater than 30 times more active than any of the other three optically active diol-epoxides and approximately 100 times more carcinogenic than benzo(a)pyrene in the newborn mouse model. This was the first study which demonstrated that enantiomers of an ultimate carcinogen possessed different carcinogenic activity.

In the initiation-promotion model, Slaga et al.[42] also showed that (+)-7,8-diol-9,10-epoxide-2 was the only isomer which initiated a significant number of papillomas. However, this isomer still had lower tumor-initiating potential than benzo(a)pyrene.

When these carcinogenicity results were compared to the mutagenicity results obtained in V79 cells, and in the Ames tester strains TA100 and TA98, it was clear that the results from studies using V79 cells were a much better predictor of the carcinogenicity of these compounds than were the results with the Ames strains. Wood et al.[43] reported that in the Ames strain TA98, the (−)- and (+)-enantiomers of diol-epoxide-1 were equally mutagenic, while in the strain TA100, (−)- and (+)-7,8-diol-9,10-epoxide-1 and the (+)-isomer of benzo(a)pyrene 7,8-diol-9,10-epoxide-2 were equally mutagenic. This is in stark contrast to the results obtained in the carcinogenicity experiments. However, in V79 cells, (+)-benzo(a)pyrene 7,8-diol-9,10-epoxide-2 was the most mutagenic species. It was five- to tenfold more mutagenic than the other diol-epoxides. These latter results were in agreement with the tumorigenicity studies.

With regard to the tumorigenicity of the proximate carcinogenic metabolite, benzo(a)pyrene 7,8-oxide, studies on its optical isomers were also done. Levin et al.[44] demonstrated that (+)-benzo(a)pyrene 7,8-oxide, which is the isomer predominantly formed by the mixed function oxidase system, was 2 to 10 times more tumorigenic than the (−)-isomer in the tumor-initiation and newborn mouse models. Of interest was the finding that the racemic mixture of the (+)- and (−)-isomers possessed more tumorigenic activity than either of the enantiomers. This synergistic tumorigenic effect of the enantiomers was due, at least in part, to the differences in affinity to epoxide hydrolase and differences in the rates of hydration.

Table 1 gives the relative tumorigenic activities of the 7,8,9,10-benzo ring derivatives of benzo(a)pyrene. These data serve to indicate the differences observed between the models used to test the compounds and the optical activity of the compounds themselves.

As pointed out by Levin et al.,[44] the metabolism of benzo(a)pyrene at each point (7,8-oxide formation, hydration of this oxide, and oxidation of the 7,8-dihydrodiol) led to the formation of the proximate or ultimate carcinogenic metabolite in preference to less active