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META 4. Prediction of the metabolism of polycyclic aromatic hydrocarbons*

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Abstract. Polycyclic aromatic hydrocarbons (PAHs) are widely distributed in the environment and are often implicated as potential carcinogens. It is generally believed that the carcinogenic potential of polycyclic aromatic hydrocarbons is linked to the formation of ultimate carcinogens generated by metabolic biotransformations. In this paper we propose a methodology that consists of using both quantum chemical properties and structural features of the reaction sites to predict PAH metabolism. Two essential questions have been answered: at which sites will the reaction take place and does the transformation consist of epoxidation or hydroxylation? This methodology has been successfully implemented into an expert system, META, for the evaluation of metabolic transformations of new chemicals.

Key words: Polycyclic aromatic hydrocarbons – Metabolism – P450 – Epoxidation – Hydroxylation – Genetic algorithm

1 Introduction

META[1] is an expert system for the evaluation of metabolic transformations of new chemicals. Basically, META operates from a dictionary of transforms, each consisting of pairs of structural fragments: a ''target'' fragment and a ''transform'' fragment, known to describe a metabolic transformation. The program seeks the presence of ''target'' fragments and replaces them one by one in the molecule by the corresponding ''transform'' fragment, thus generating a number of primary metabolites. The program monitors and evaluates the thermodynamic stability of all the molecules generated by the process by consulting a dictionary of spontaneous reaction that lists unstable structural moieties. Whenever a molecule is found to contain such an unstable moiety, it is transformed into a stable product via an appropriate spontaneous reaction transform contained in the spontaneous reaction dictionary. Upon demand the primary metabolites will be processed further so that a complete metabolic tree can be obtained. A detailed description of the META algorithm has been published [1–3].

While META already has the capability to evaluate a large variety of molecules, one of the important problems remaining to be solved was the prediction of the reaction sites and the nature of products produced by metabolic transformations of polycyclic aromatic hydrocarbons (PAHs).

PAHs are widely distributed in the environment and are often implicated as potential carcinogens [4–6]. It has been proposed that those PAHs which exhibit high carcinogenic potency possess a bay region [7], as in benzo[*a*]pyrene [8–9] (Fig. 1). However, benzo[*e*]pyrene, the isomer of benzo[*a*]pyrene, is practically inactive even though it possesses two such bay regions [10–11].

It is generally believed that the carcinogenic potential of PAHs is linked to the formation of ultimate carcinogens generated by their metabolic biotransformations. Therefore, the carcinogenic potential of PAH is almost certainly structure dependent.

PAHs are known to undergo a wide variety of metabolic transformations as illustrated for benzo[*a*]pyrene [12] in Fig. 2. Most of the transformations are believed to be catalyzed by the cytochrome P450 monooxygenases found predominantly in the endoplasmic reticulum and in nuclear membranes. Aromatic molecules are oxidized to phenols and dihydrodiols sometimes via epoxide intermediates. Evidence suggests that the formation of 7,8- and 9,10-epoxides is the crucial step in the carcinogenic activity of benzo[*a*]pyrene. Further metabolism of 7,8-dihydrodiol and 9,10-dihydrodiol by mixed function oxidases to the diol-epoxide is necessary for the production of the ultimate carcinogen believed to form a nucleic acid adduct. Hence, the ability to predict the possible metabolism sites of a PAH will greatly help us understand its carcinogenic potential.

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[1] META is distributed by MULTICASE Inc., P.O. Box 22157, Cleveland, OH 44122, USA

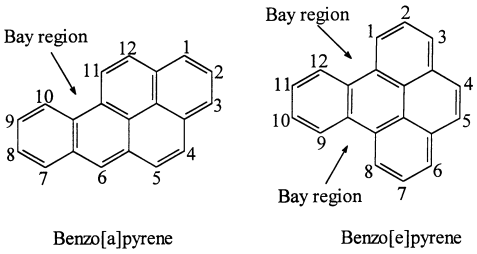

Fig. 1. Bay region in [*a*] and [*e*] benzopyrene

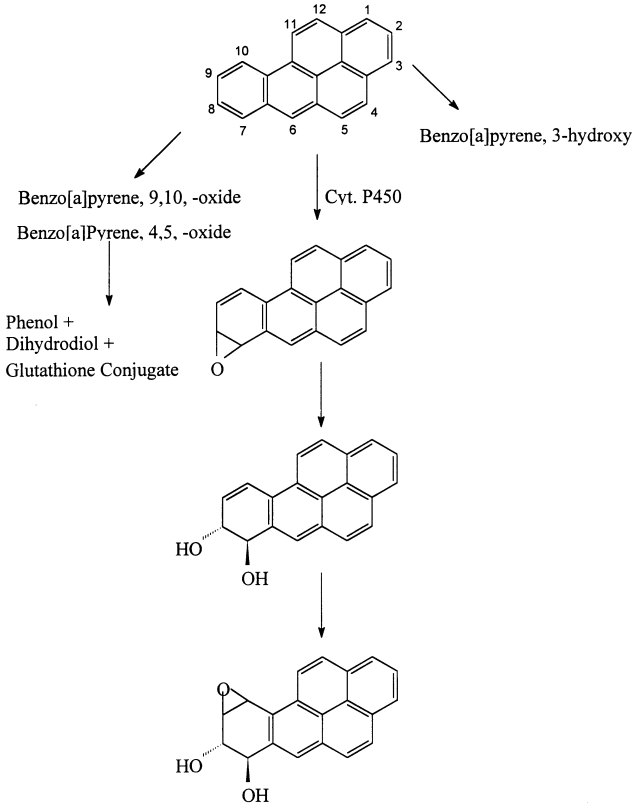

Fig. 2. Part of the metabolism of benzo[*a*]pyrene

Probably the first attempt to quantify the carcinogenic potential of PAHs was made by Pullman [13], who tried to apply Hückel-type quantum chemical calculations to correlate physicochemical properties of PAHs with their carcinogenic potency. Other similar methods were reported later [14, 15]. In the earlier version of the META program, we tried to solve the problem with simple Hückel-type calculations using Van-Catledge's parameters [16]. However, this, like all previous methods, attempted to relate the locations of the metabolism sites to the charge densities and other quantum mechanically derived quantities at these sites. Overall the success was poor because the observed metabolism sites are often different from both the calculated most reactive sites and the observed products formed by reaction with non-biological electrophiles and free radicals. We therefore concluded that the limited success achieved with quantum mechanics might be due to the omission of some other important property. We believe that the missing link is structural and related to the shape of acceptable targets for the cytochromes.

We report here a new approach that consists of using both quantum chemical properties and structural features of the reaction sites to predict targets for metabolism. We attempt to answer two essential questions: at which sites will the reaction take place and does the transformation consist of epoxidation or hydroxylation? We postulate that for a metabolic process catalyzed by an enzyme to take place at a certain site, two criteria have to be met: sufficient reactivity and ready accessibility. If the reactivity of a site is not high enough, there will obviously be no reaction. However, even if the reactivity is high, the reaction will not occur if the accessibility is too low (high steric hindrance). Adequate reactivity is an essential condition, but not a sufficient one for the enzymatic catalyzed reaction to take place.

The reactivity of a site (atom or bond) in a target molecule can be expressed by its quantum chemical properties. In our approach, we use a quantum index (QI) which is derived from the atomic coefficients of the occupied molecular orbital calculated from a simple Hückel method. Accessibility, on the other hand, is related to the structural features of the molecule and is expressed by a graph index (GI), designed to represent encumbrance around the various possible sites.

2 Methods

2.1 QI Evaluation

Oxidation of a double bond in aromatic molecules under catalysis by P450 was treated as an electrophilic reaction in which the LUMO orbital of an electrophilic oxygen atom interacts with the HOMO orbital of the aromatic compound [17, 18] as shown in Fig. 3. The carbon atoms of the double bond are denoted as atoms *A* and *B*, and O^+ indicates the P450's electrophilic oxygen end. Three scenarios are possible when the oxygen atom of P450 approaches the double bond: 1) a bond is formed between *A* and O; (2) a bond is formed between *B* and O; (3) an epoxide adduct is formed.

We hypothesized that the metabolites predominantly formed will be those whose energies are the most favorable, taking into account both the reactivity and accessibility of the reaction sites.

We have used general perturbation theory [19] to evaluate the relative reactivity of the conjugated atoms using the following expression eg. 1. The perturbation energy, QI_A, can be viewed as the total change in energy due to the partial formation of a bond between an atom A of PAH and the oxygen end of P450.

$$\mathrm{Q\,I}_A = \sum_i \frac{(C_{Ai} C_o^{LUMO} \beta)^2}{E_i \beta - E_{P450} \beta} \quad , \tag{1}$$

where C_{Ai} is the coefficient of the occupied atomic orbital *i* of atom *A*. C_o^{LUMO} (assumed to be equal to 1.0) is the coefficient of the

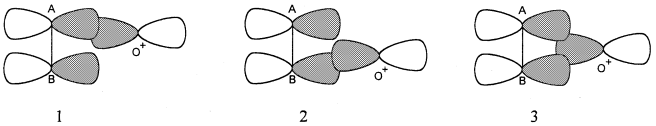

Fig. 3. The three scenarios of oxidation of a double bond: **1** bond *AO*, **2** bond *BO* or **3** epoxide

oxygen atom in the LUMO orbital of P450. E_i is the energy of the occupied molecular orbital i of the PAH in β units. E_{P450} (assumed to 0.1)[2] is the energy of the LUMO orbital of P450 in β units. The energy and coefficients are evaluated by a simple Hückel theory using Van-Catledge's parameters.

Equation (1) can be simplified to:

$$QI_A = \sum_i \frac{(C_{Ai})^2}{E_i - 0.1}\beta, \tag{2}$$

Similarly, the expression for simultaneous attack on the two atoms of the double bond can be expressed as:

$$QI_{AB} = \left[\sum_i \frac{(C_{Ai} + C_{Bi})^2}{E_i - 0.1}\right]\beta \tag{3}$$

An example of such a calculation for attack on benzo[*a*]pyrene is given in Table 1.

Table 1. Calculated quantum index (QI) of different sites for benzo[*a*]pyrene

Double bond atom A – Atom B	QI_A	QI_B	QI_{AB}
1–2	5.811	3.685	3.542
2–3	3.685	5.655	3.186
4–5	5.131	5.132	4.734
7–8	5.151	3.883	3.760
8–9	3.883	4.526	2.749
9–10	4.526	4.473	3.821
11–12	4.463	5.296	4.281

From a quantum mechanical standpoint, the active bonds of benzo[*a*]pyrene are 4–5 and 11–12. The active atoms are 1, 3, 4, 5, 7 and 12. While these results represent well the observed location of electrophilic attack on benzo[*a*]pyrene, they are not in agreement with experimental metabolism data, where the major epoxide adducts are observed at positions 7–8 and 9–10 while the major hydroxylated products are at 3, 7 and 9 [20–22]. However, the predicted inactive bonds 2–3 and 8–9 and the inactive atoms 2, 8, 10 and 11 are indeed found experimentally to be inert toward metabolic oxidation.

The quantum mechanical calculations thus provide some useful information about the lack of reactivity of certain sites of a molecule, but fail to identify the actual metabolites. Thus the methodology helps us to exclude the inactive bonds and atoms, hence allowing us to limit the number of choices. However, as mentioned above, while quantum mechanical calculations give us information about reactivity or lack thereof, they fail to account for accessibility. In order to obtain more accurate predictions, we will also have to consider the structural features of the target site and the impact of the presence of the surrounding atoms.

2.2 Graph index

GIs were introduced with the development of chemical graph theory [23]. These indices are seen to represent the molecular topological shape and were abundantly used to study structure-property relationships. In the META program, a GI is used to characterize the specific arrangement around an atom or a bond in an aromatic molecule. For a given atom, the GI is defined as

$$G = \sum_i \frac{n_i}{r_i^a} \ , \tag{4}$$

where n_i is the number of atoms at a distance r_i from the target atom and a is a constant.

The graph index G can be seen as representing the encumbrance around a given atom in a molecule. This encumbrance decreases rapidly as the distance increases, and this is implemented by using a constant a whose value is set to be greater than 1. In our case, a is given a value of 3.

For a double bond $A = B$, the GI is defined as

$$G_b = \frac{G_A^2 + G_B^2}{2\sqrt{G_A G_B}} \ , \tag{5}$$

where G_A and G_B are atom GIs for atom A and atom B respectively.

The GIs calculated for the relevant atoms of benzo[*a*]pyrene are shown in Table 2. It can be seen from this table that the most accessible bonds of benzo[*a*]pyrene are 8–9, 7–8, and 1–2, while the most accessible atoms are 2, 8 and 9.

2.3 Effects of a PAH's lipophilicity on its reactivity

When we look at the mechanism of oxidation of PAHs by P450 (Fig. 4), one of the most important steps is the approach of the PAH to the lipophilic region of P450. Hence, we hypothesized that the reactivity of the PAH will also be affected by its lipophilicity. The higher the PAH's lipophilicity, the easier it will be oxidized. In our methodology, the PAH's lipophilicity was expressed as the logarithm of its octanol/water partition coefficient (log$P$).

2.4 The final reactivity evaluation

From a chemical standpoint, the reactivity of a site (atom or bond) increases proportionally to the QI. However, such change is not linear in biological reactions. When QI is very small, its exact value is irrelevant because the reaction will not take place at the inactive sites. Similarly, when QI is above a certain threshold, the biological reaction will take place rapidly and probably at the same rate, irrespective of the magnitude of QI. If this is indeed the case, then we can expect to have an S type curve to represent the relationship between metabolic activity and QI. A similar situation exists for the relationship with GI and with the PAH's lipophilicity. The relationship between reactivity and QI and GI is shown in Fig. 5.

The general equation for such an S curve is:

$$f(x) = X_1 - \frac{X_2}{1 + e^{(a - b\times)}} \ , \tag{6}$$

where X_1 and X_2 define the maximal and the minimal value of $f(x)$, while a and b are two variables that affect the location of the center of the curve.

In the META program, we use an index, called P_{value}, to determine the reactivity (or priority) of a transformation reaction. P_{value} is an integer whose value ranges from 0 to 9. Reactions with great reactivity have a low P_{value}. Hence, P_{value} is inversely proportional to the reactivity. In our program, a spontaneous reaction

Table 2. Graph indices for benzo[*a*]pyrene

Atom	G	Bond	G_b
1	2.574	1–2	2.512
2	2.444	2–3	2.512
3	2.574	4–5	2.630
4	2.622	7–8	2.494
5	2.638	8–9	2.412
6	2.776	9–10	2.521
7	2.574	11–12	2.658
8	2.404		
9	2.420		
10	2.611		
11	2.678		
12	2.638		

[2] β is the value of the resonance integral between the π orbitals of two conjugated carbon atoms. We have tried the number −0.1, 0, 0.1, 0.3 and 0.9 β units as the LUMO energy of the approaching atomic site of P450; 0.1 best explains the experimental observations

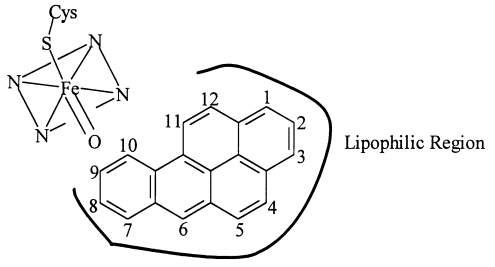

Fig. 4. The addition of a PAH to the lipophilic region of P450

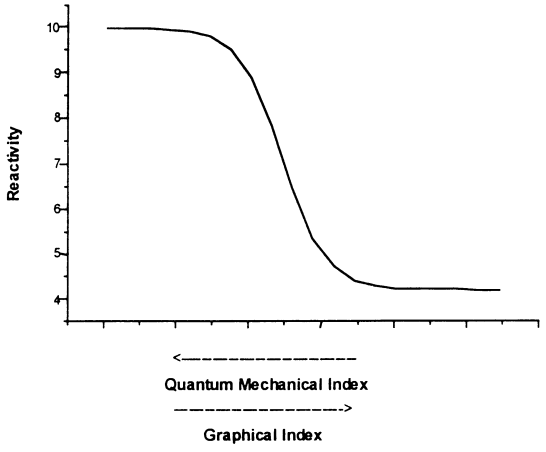

Fig. 5. The relationship of reactivity to QI and GI

will have a P_{value} of 0 and other reactions will range from 1 (fastest) to 9 (slowest). If several metabolic pathways exist for a single molecule, the program chooses the reaction with the highest reactivity (lowest P_{value} value) as the major biotransformation. A P_{value} larger than 9 indicates that the reaction will never take place. If the P_{value} difference between two possible metabolic pathways is larger than 2, only the high reactivity (lowest P_{value}) pathway will be displayed in META.

In order to implement our methodology into META, our task was to evaluate the P_{value} for each reaction site of a series of PAHs from the QI and GI and the partition coefficient. Hence, we define:

$$P_{\text{value}} = \frac{f(\text{QI})}{f(\text{GI})} - f(\log P) \tag{7}$$

where

$$f(\text{QI}) = 20.0 - \frac{4.0}{1 + e^{(a - bQI)}} \tag{8}$$

$$f(GI) = 1.0 - \frac{0.2}{1 + e^{(c - dGI)}} \tag{9}$$

$$f(\log P) = \frac{2.0}{1 + e^{(e - f\log P)}} \quad , \tag{10}$$

where a, b, c, d, e and f are values to be determined. The ideal values for these variables are values that would lead to the best fit between calculated and experimental observed reaction sites. Since there is no explicit mathematical expression to represent such a function, we used a simple genetic algorithm to optimize the relationship.

2.5 Genetic algorithm

The framework of the genetic algorithm (GA) was first introduced by Holland [24]. The main concept of GA is based upon Darwinian principles of natural selection. As an optimization method, GA has recently been widely used for conformation searches. In a typical implementation, a population of random bit strings is used as the starting population of solution trials. Each trial is encoded according to the particular application's representation. The quality of each solution generated by the algorithm is judged by a fitness value, which is used to select certain parents for succeeding generations**.** The fitness value of a possible solution indicates how well it represents the date. In our case, the fitness value of an individual trial is expressed as:

$$\text{Fitness} = \sum \text{predicted} - \sum \text{missed} - \sum \text{over produced} \ ,$$

where the "predicted" term indicates the number of products predicted correctly, the "missed" term indicates the number of products that are missed and the "over produced" term indicates the number of products that are predicted to occur but are not observed experimentally.

The better the solution, as compared with the competitors in the population, the higher the probability that the corresponding bit string is selected for survival and for recombination. Further, a low mutational frequency is introduced when offspring strings are copied from parent strings. Succeeding generations should thus encode increasingly better solutions ("fitter population"), achieved through selection, mutation and recombination. The process is repeated until a few populations dominate. The surviving individuals should represent a near-optimal solution.

We used a simple GA [25], because there are relatively few variables (a, b, c, d, e and f). The specific parameters for the algorithm are:

1. Population size: 200
2. Gene (binary string) length: 16
3. Selection method: tournament selection
4. Tournament selection number: 5
5. Crossover method: single point crossover
6. Crossover probability: 0.60
7. Mutation probability: 0.05.

The convergence criterion is that the standard deviation for two neighboring populations is less than 0.0001 or the generation number is less than 1000.

2.6 Database

To find a general rule to predict the reactivity of different bonds and atoms inside a PAH, a database of 42 conjugated chemicals has been assembled (Table 3). Initial evaluation of the P_{value} of attack at all possible sites of the 42 chemicals was calculated and compared with experimental data [26].

3 Results and discussion

3.1 Epoxidation

There is a total of 300 double bonds in the 42 chemicals and 80 of them have been observed to be epoxidized metabolically. The difference between calculated activity and experimentally observed data was minimized using the GA. The best correlation with experimental values for epoxidation was obtained when the relation between the calculated location of epoxidation sites and the reactivity indices was as shown in Eq. (11).

$$P_{\text{value}} = \frac{20.0 - 14.0/(1.0 + \exp(46.248 - 14.37QI))}{1.0 - 0.9/(1.0 + \exp(48.875 - 18.258GI))} - \frac{2.0}{1.0 + \exp(48.423 - 15.723\log P)} \tag{11}$$

After implementing the above methodology into META, we tested the 42 compounds of the learning set.

Table 3. Conjugated chemicals calculated[a]

Number	Structure	Number	Structure
1	Benzene	22	4-Aminobiphenyl
2	Naphthalene	23	6-Fluorobenzo[*c*]phenanthrene
3	Benzo[*c*]phenanthrene	24	Phenol
4	12-Methylbenz[*a*]anthracene	25	Aniline
5	7,12-Dimethylbenz[*a*]anthracene	26	Benzo[*a*]pyrene
6	7-Ethylbenz[*a*]anthracene	27	Benzo[*f*]quinoline
7	5-Methylchrysene	28	Chlorobenzene
8	6-Methylchrysene	29	4-Chlorobiphenyl
9	6-Nitro-5-methylchrysene	30	Benzo[*k*]fluoranthrene
10	Benzo[*j*]fluoranthene	31	3-Nitro fluoranthrene
11	Benzo[*b*]fluoranthene	32	6-Nitrochrysene
12	Benzo[*f*]quinoline	33	Toluene
13	3-Methylchlosanthrene	34	Cyclopenteno[*c,d*]pyrene
14	1-Nitropyrene	35	4-Vinylcyclohexane
15	Isoprene	36	Fluoranthene
16	Styrene	37	Benzo[*b*]naphtho[2,1,d]thiophene
17	Triphenylene	38	Bromobenzene
18	Benzo[*a*]chrysene	39	*p*-Chloranitrobenzene
19	7-Methyl-benz[*a*]anthracene	40	2-Acetylaminofluorene
20	Dibenz[*a,h*]acridine	41	Benzidine
21	Dibenz[*a,j*]acridine	42	3-Hydroxypyridine

[a] The experimental data were obtained from rat metabolism, see Ref. [26]

Of the 300 possible reaction sites, META predicted the formation of a total of 101 epoxides which included 72 epoxides observed experimentally. Thus, the program missed eight of the epoxides observed experimentally and overpredicted 29.

3.2 Hydroxylation

There is a total of 385 candidate sites for hydroxylation for the 42 chemicals. Of these, 49 are observed experimentally.

Following a procedure similar to that used for epoxidation, the GA led to the following equation for the metabolic reactivity of a position characterized by the indices QI, GI and the lipophilicity ($\log P$):

$$P_{Value} = \frac{20.0 - 17.0/(1.0 + \exp(4.086 - 1.25QI))}{1.0 - 0.9/(1.0 + \exp(38.98 - 15.123GI))} - \frac{2.0}{1.0 + \exp(38.437 - 12.862 \log P)} \quad (12)$$

When implemented in the program, META predicted 30 possible hydroxylation sites for the 42 compounds of the learning set. Of these, 22 were indeed observed experimentally. However, META missed 27 hydroxylation products that are observed experimentally. The prediction of the hydroxylation sites is therefore not as good as that of the epoxidation sites.

3.3 Results for benzo[a]pyrene and benzo[e]pyrene

Using the methodology mentioned above, we calculated the P_{value} for all the atoms and bonds in benzo[*a*]pyrene (Table 4) and benzo[*e*]pyrene (Table 5).

Table 4. Calculated P_{value} for benzo[*a*]pyrene

Atom	P_{value}	Bond	P_{value}
1	9	1–2	4
2	12	2–3	10
3	9	4–5	6
4	13	7–8	4
5	15	8–9	18
7	10	9–10	4
8	11	11–12	8
9	8		
10	16		
11	25		
12	14		

Table 5. Calculated P_{value} for benzo[*e*]pyrene

Atom	P_{value}	Bond	P_{value}
1	10	1–2	19
2	13	2–3	6
3	9	4–5	4
4	10	6–7	6
5	10	7–8	19
6	9	9–10	8
7	13	10–11	12
8	10	11–12	8
9	10		
10	9		
11	9		
12	10		

From Table 4 it can be seen that the major epoxides found in the metabolism of benzo[*a*]pyrene are, as predicted by META, at the 7–8, 9–10 and 1–2 positions. The 4–5 epoxide is a minor one. The 11–12 epoxide will be masked because of its low reactivity (high P_{value}). It can also be seen that the hydroxylated products are the 1, 3 and 9-hydroxy benzo[*a*]pyrene. However, all these

hydroxylated products are minor compared to the epoxide products. The overall results for benzo[*a*]pyrene are therefore in close agreement with the experimental observations [12]. The major products, 7–8 and 9–10 epoxides, are believed to be related to benzo[*a*]pyrene's carcinogenic activity.

The major metabolism product for benzo[*e*]pyrene predicted by META is the 4–5 epoxide (P_{value} of 5). Epoxidation at 2–3 and 6–7 as well as all the hydroxylation reactions are predicted to be minor. This result is also in good agreement with experimental observations [27]. No epoxides are formed in the vicinity of the bay regions (9–10 and 11–12), which is probably the reason why benzo[*e*]pyrene is not carcinogenic.

4 Conclusion

We propose a methodology to predict the metabolic epoxidation and hydroxylation of PAHs and demonstrate how this method can help us understand the carcinogenic potential of some PAHs. The method works very well for the prediction of possible epoxidation sites and reasonably well for the prediction of possible hydroxylation sites. Since the QI and GI calculations used in this study are rather elementary, more sophisticated quantum mechanical calculations and more adequate GIs may improve even further the accuracy of the prediction of the metabolic hydroxylation of aromatic compounds.

Acknowledgement. This paper is submitted in memory of Prof. Kenichi Fukui, whose interest in the chemistry of polycyclic aromatic hydrocarbons led him to the pinnacle of his profession. G.K. remembers with fondness this great scientist and friend

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