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The significance of polycyclic aromatic hydrocarbons as environmental carcinogens

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Abstract: In vivo and in vitro experiments have shown that various polycyclic aromatic hydrocarbons are potent mutagens and/or carcinogens. Occupational and epidemiological studies suggest that they also have to be considered as hazards for humans. Due to their formation during all kind of incomplete combustions they are widespread to the environment.
Balancing the carcinogenic effect of environmentally polluted matrices, after separation into PAH-free and PAH-containing fractions, by animal test systems (skin painting, lung implantation) evidence has been obtained that PAH containing more than 3 rings are by far the most relevant carcinogenic constituents in these matrices. The relative potencies of PAH in comparison to benzo[a]pyrene allow their ranking and result in efficiency equivalents generally applicable for risk assessment.
Since PAH require biochemical activation prior to exhibiting carcinogenic effects the balance of various enzymes plays a key role in biological endpoints in vivo. Complex patterns of metabolites are formed in the mammalian organism the composition of which were found to be species-dependent. Hence, extrapolations from one species to another are limited and evaluations on experimental rodent data have to be reconsidered for their validity.

Polycyclic aromatic hydrocarbons are a well-known class of environmental pollutants with carcinogenic properties which are permanently formed by all sorts of incomplete combustion and hence may be considered to be ubiquitous. Accordingly, human or environmental exposure to them is practically unavoidable. Many individual PAH have been tested for carcinogenicity in various animals by different routes of application (oral, intraperitoneal, subcutaneous, epicutaneous, intratracheal and intrapulmonary) and resulted in both benign and malign tumors. In in vitro experiments with tissue and cell cultures as well as with subcellular fractions, but also in microbial test systems cell-transforming properties of PAH have been clearly demonstrated indicating mutagenic and carcinogenic potentials. Moreover, a carcinogenic risk potential for humans by PAH may be deduced from a variety of occupational studies (e.g. coke plant workers, people employed at industries working with coal tar and its derivatives, carbon black, mineral oil, in aluminium smelters, or working places with high exposure to engine exhaust, but also chimney sweeps, etc.) leading back to as early observations as those made by Sir Percival Pott (1) who reported the formation of the comparatively rare scrotum cancer in chimney sweeps some 200 years ago. The disease is supposed to be caused by a permanent exposure to coal tar and soot. More recent studies by Gustavsson (2) have confirmed these findings and evidenced high incidences of esophagus, bladder and lung tumors in chimney sweeps.
Apart from carbocyclic parent compounds also methyl-substituted PAH and heterocyclic isosters may display high mutagenic and/or carcinogenic activities. Several potent carcinogens are found among the sulfur-analogues (3) (thiaarenes) and nitrogen-analogues (4) (azaarenes) of PAH. There are also highly active compounds found among the amino- and nitro-derivatives of PAH (5,6). Especially aromatic amines (such as 2-aminonaphthalene and 4-aminobiphenyl) seem to play an important role as occupational carcinogens. Some typical structures are presented in Fig. 1.

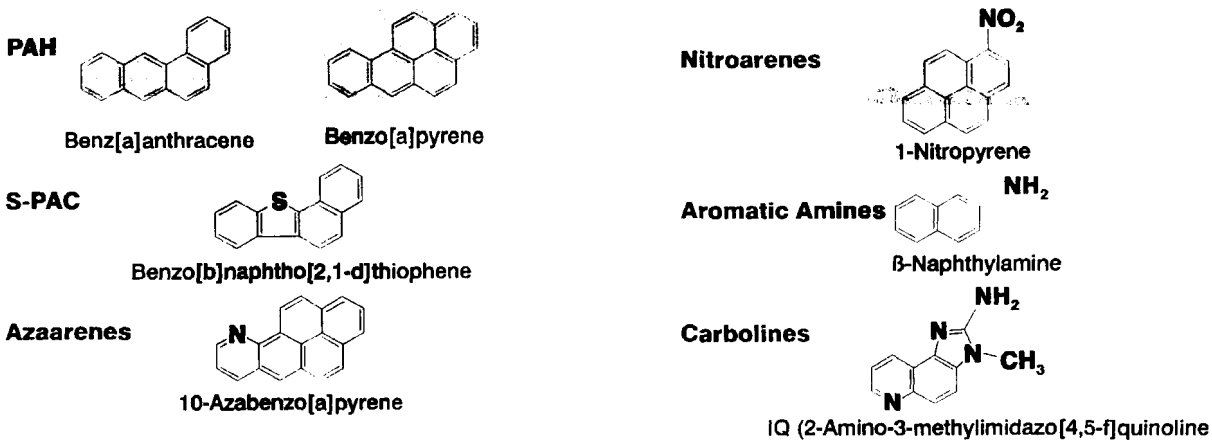

Fig. 1 Structures of PAH and PAH-derivatives exhibiting mutagenic and/or carcinogenic potentials.

Do PAH actually play a role in environmentally caused cancer? The attribution of the carcinogenic potential of mixtures such as ambient air, exhaust, used motor oil, etc. to a certain class of compounds may be achieved by a chemical fractionation in combination with a carcinogen-specific biological detector (animal experiment or in vitro test). Experiments with vehicle exhaust, used motor oil and hard-coal combustion effluents clearly have shown that PAH almost exclusively cause the carcinogenic effect in these matrices (for review see ref. 7). A fractionation of hard-coal combustion exhaust condensate is presented in Fig. 2.

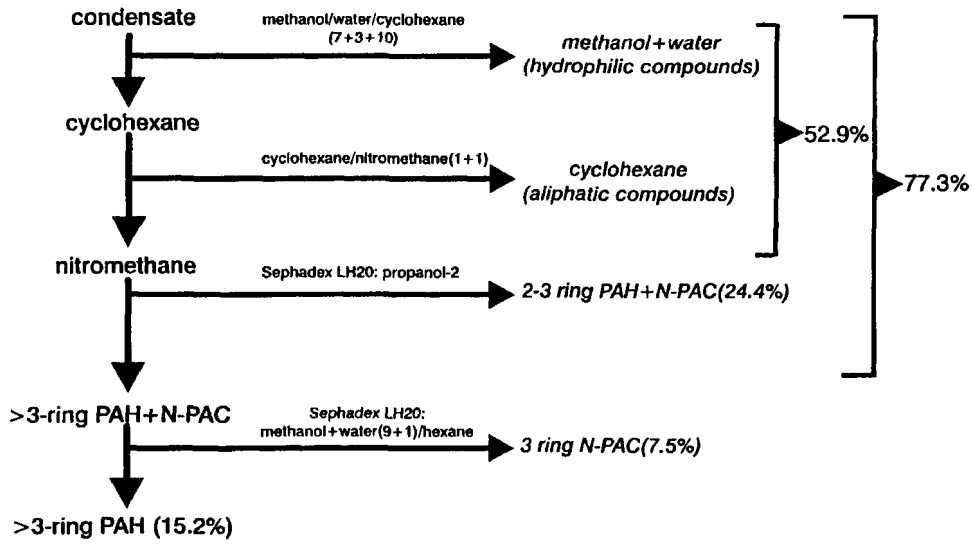

Fig. 2 Fractionation of hard-coal combustion exhaust.

When tested by epicutaneous application to mice the carcinogenic effect of the original condensate is almost exclusively found in the fraction containing PAH with more than 3 rings (Fig. 3).

From these experiments it is obvious that PAH represent the predominant part of the biologically active compounds in the above matrices. Benzo[a]pyrene (BaP) itself, however, represents only a small part of the total carcinogenic potential and is associated with many other PAH some of which are even more potent than BaP, not to speak of any synergistic effects which may arise from the induction of monooxygenases caused by PAH. Table 1 shows that BaP seldom contributes by more than 10 % to the total carcinogenic potential of environmental matrices.

Apart from BaP there are many other PAH with carcinogenic potential as shown in Table 2.

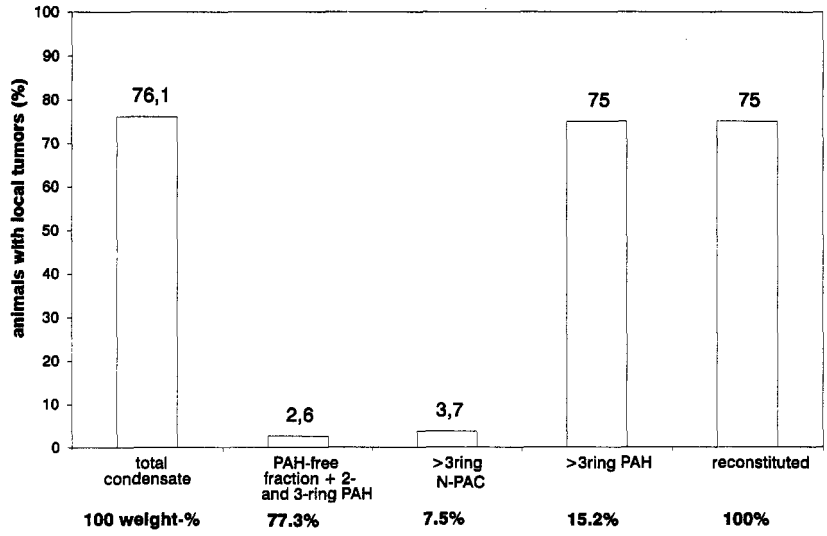

Fig. 3 Comparison of the carcinogenic potential of hard-coal combustion condensate and fractions thereof. Dose: 0.411 mg twice weekly (epicutaneous; fractions were dosed proportionally). Similar results were obtained with used motor oil as shown in Fig. 4.

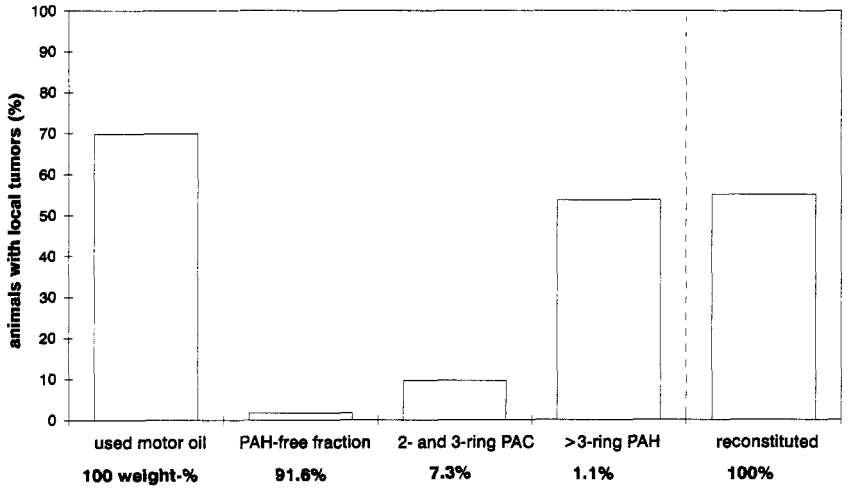

Fig. 4 Comparison of the carcinogenic potential of used motor oil and fractions thereof. Dose: 5.625 mg twice weekly (epicutaneous; fractions were dosed proportionally).

TABLE 1. Percentage contribution of PAH and benzo[a]pyrene to the carcinogenic potency of various matrices using two biological test models.

	Percentage of effect (%)			
	mouse, epicutaneous		rat, intrapulmonary	
	PAH**	B[a]P	PAH**	B[a]P
used motor oil	70	18		
weight-%*	1,1	0,02		
vehicle exhaust (Otto)	85	6	81	2,4
weight-%	3,5	0,04	2,8	0,05
flue gas of coal-fired furnaces	90	11	>90	1,4
weight-%	22,7	0,1	29	0,11
diesel exhaust condensate (organic extract)			80	4
weight-%			0,9	0,01
cigarette smoke, sidestream			75	0,17
weight-%			3,5	0,0004

* weight-% = percentage of PAH- and B[a]P by weight related to the original material
** PAH with 4 and more rings

TABLE 2. Carcinogenic potencies of various PAH relative to benzo[a]pyrene (= 1,00) [$CP_{rel.\ B[a]P}$].

	$CP_{rel.\ B[a]P}$*
Benzo[a]pyrene	1,00
Dibenzo[a,l]pyrene	>>2.00
Dibenz[a,h]anthracene	1,91
Anthanthrene	0,19
Cyclopenta[cd]pyrene	0,15
Benzo[b]fluoranthene	0,11
Indeno[1,2,3-cd]pyrene	0,08
Benzo[k]fluoranthene	0,03
Benzo[j]fluoranthene	0,03
Chrysene	0,03
Benzo[b]naphtho[2,1-d]thiophene	0,02
Benz[a]anthracene	0,01
Fluoranthene	0,00

*Test-model: Intrapulmonary injection into female Osborne-Mendel rat

How important high-boiling PAH may be as environmental carcinogens can be deduced from the following experiment: the biologically active PAH-fraction of hard-coal combustion exhaust condensate has been further separated into more and less volatile PAH by vacuum sublimation. Both fractions are about equal by weight and surprisingly exhibit also equal carcinogenic potentials. The volatile fraction contains most of the well-known PAH-carcinogens as can be seen from the GC-chromatogram (Fig. 5) whereas practically none of them are found in the residue. In other terms, potent carcinogens are still hidden in this fraction which are definitely different from the PAH commonly determined. Their identification appears to be urgently needed.

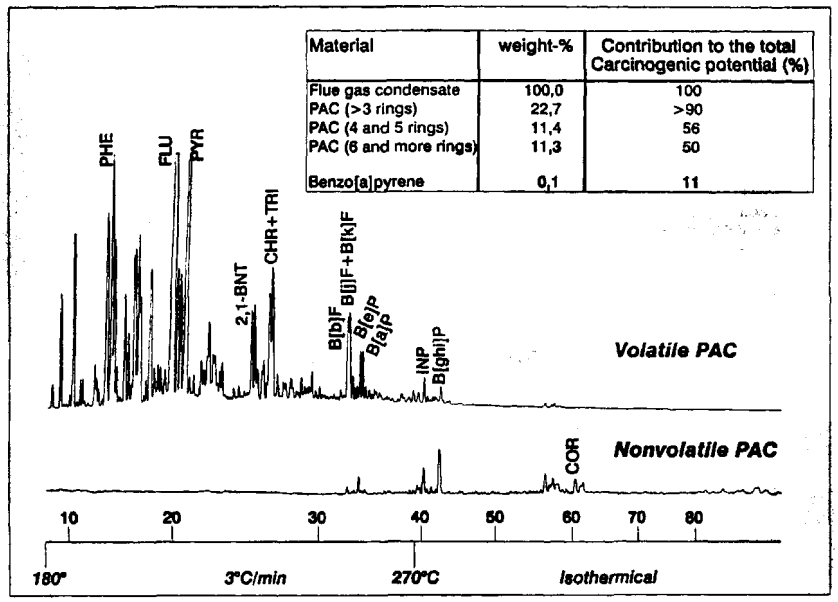

Fig. 5
Carcinogenicity of hard-coal combustion exhaust condensate and fractions thereof (epicutaneous application, mouse).

PAH are non-toxic by themselves and require metabolic activation to exert carcinogenic or mutagenic effects. In a primary step they are activated to arene oxides by more or less regiospecifically operating cytochrome P450-dependent monooxygenases. Arene oxides may be either spontaneously isomerized to phenols or converted enzymatically to *trans*-dihydrodiols by epoxide hydrase(s). A further oxidation of the latter to diolepoxides plays an important role in the formation of ultimate carcinogens which may possibly react along with other metabolites with DNA and thus initiate the malign cell transformation. The various sequences of activation and detoxification are demonstrated for BaP in Fig. 6 and Fig. 7.

Many attempts have been undertaken to explain the carcinogenic potential of certain PAH and the ineffectiveness of others on a theoretical basis. It was initially thought that a reactive site comparable to the 9,10-position in phenanthrene, which is the most reactive one in this molecule, is a prerequisite for the carcinogenic potential of PAH (8-10). Accordingly the 9,10-position surrounded by 2 quarternary C-atoms was designated as k-region. Later on it became obvious that the very opposite is true since the metabolic activation at the k-region mainly results in non-toxic and non-carcinogenic metabolites and hence rather plays a role in detoxification.
Various hypotheses and theories on PAH-induced carcinogenicity have been added to the above one such as bay-region-, di-region-, -C1-transfer-, one electron oxidation-activation, etc. to all of which there are more or less many exceptions so that none of them describes the situation perfectly. Nevertheless, these theories have stimulated the scientific work tremendously. Especially the bay-region activation has been favoured to explain the carcinogenic potency of PAH. The high reactivity of the epoxide ring on one hand, and the fact that due to its olefinic structure dihydrodiolepoxides do not spontaneously isomerize to a phenol configuration on the other hand may explain their reactivity with nucleophilic macromolecules such as DNA. Among the different dihydrodiolepoxides of various PAH the bay-region derived ones exhibit the most pronounced tendency to form carbonium ions and turned out to be most reactive. Quantum mechanical model calculations have been undertaken to predict reactivities for various dihydrodiolepoxides.

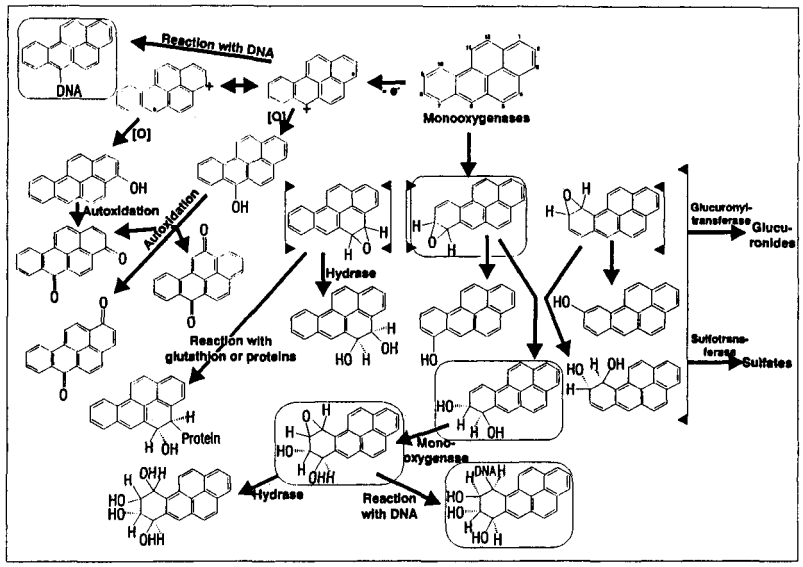

Fig. 6 Metabolic activation of benzo[a]pyrene.

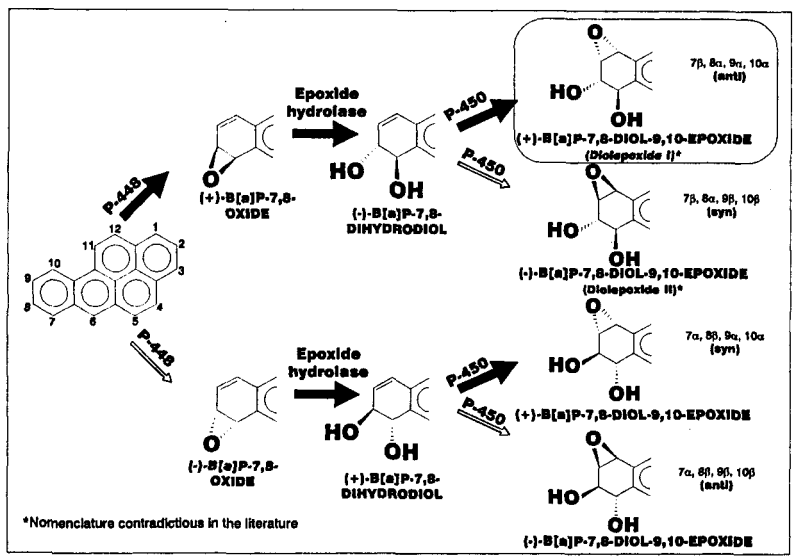

Fig. 7 Metabolic formation of 9,10-epoxy-7,8-dihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene.

The lack of a carcinogenic potential for phenanthrene and benzo[e]pyrene (with even 2 bay-regions) and the carcinogenicity of e.g. cyclopenta[cd]pyrene or anthanthrene (see Fig. 8) cannot easily be explained by the bay-region theory, however.

Apart from the bay-region activation, further metabolic pathways apparently contribute to the formation of carcinogenic reactants.

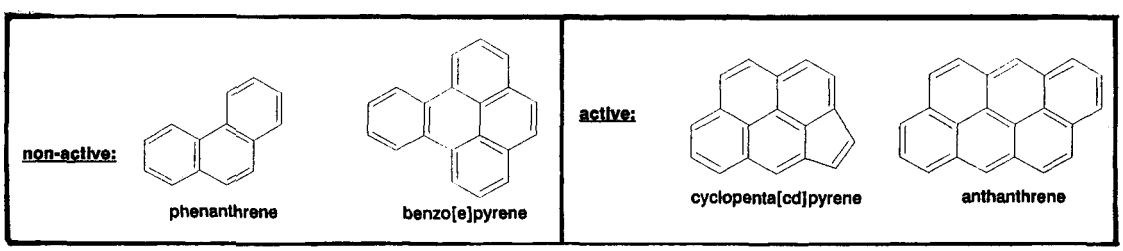

Fig. 8 Exception to the Bay-Region Theory.

TABLE 3. Cytochrome P450 families in mammals*

P450	No. of subfamilies	No. of species	reactions
CYP 1	1	2	metabolism of xenobiotics
CYP 2	8	57	metabolism of xenobiotics and steroids
CYP 3	2	10	metabolism of xenobiotics and steroids
CYP 4	2	10	hydroxylation of fatty acids
CYP 7	1	1	cholesterol 7α-hydroxylase
CYP11	2	3	steroid 11β-hydroxylase
CYP17	1	1	steroid 17α-hydroxylase
CYP19	1	1	aromatase
CYP21	1	1	steroid 21-hydroxylase
CYP27	1	1	cholesterol 27-hydroxylase

*(ref. 11)

There are many isoforms of CYP450-dependent monooxygenases (a total of about 220 and more than 80 even present in mammals) (Table 3).

Only the first three families of CYPs are relevant for the activation of PAH. The great difficulties of isolating pure CYP forms from tissues have been overcome by expressing heterologous cDNAs in suitable immortalized cell systems. By this technique a permanent supply of various CYPs became available and this initiated systematic studies on the regiospecific oxidation of various PAH by a series of CYPs. Unfortunately, the regio-specificity of human isocytochromes has been found not to be excitingly great. Most of the individual forms tested exhibited overlapping activities.

The hope that the metabolism of carcinogenic PAH could be directed to the formation of non-toxic metabolites by induction of specific CYPs has not been realized so far. However, the inductions of monooxygenase activity by various xenobiotics such as chlorinated biphenyls, various drugs, but also PAH themselves may play an important role to indirectly enhance the carcinogenic potential of human carcinogens such as benz[a]anthracene. For instance, benzo[k]fluoranthene which is almost inactive as a carcinogen drastically induces the metabolism of benz[a]anthracene and other PAH when orally or intraperitoneally applied to rats (12) (Table 4).

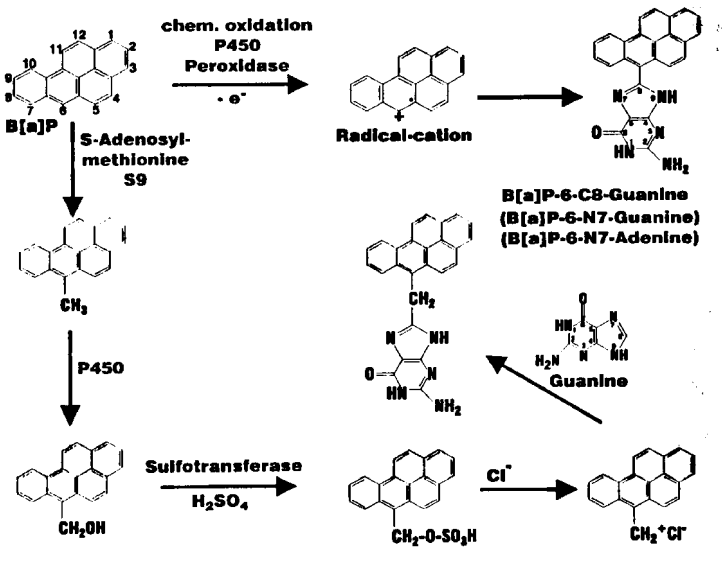

Fig. 9 Formation of proximate and ultimate carcinogens others than dihydrodiolepoxides.

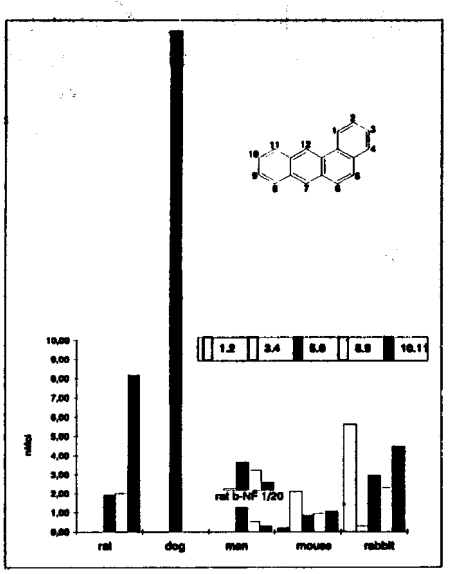

Fig. 10 Species-specific equipment of liver monooxygenase isozymes measured by the oxida tion products of a test substrate (benz[a]anthracene; liver microsomes). Pre-treatment of rats with β-naphthoflavone results in a metabolite profile similar as in man.

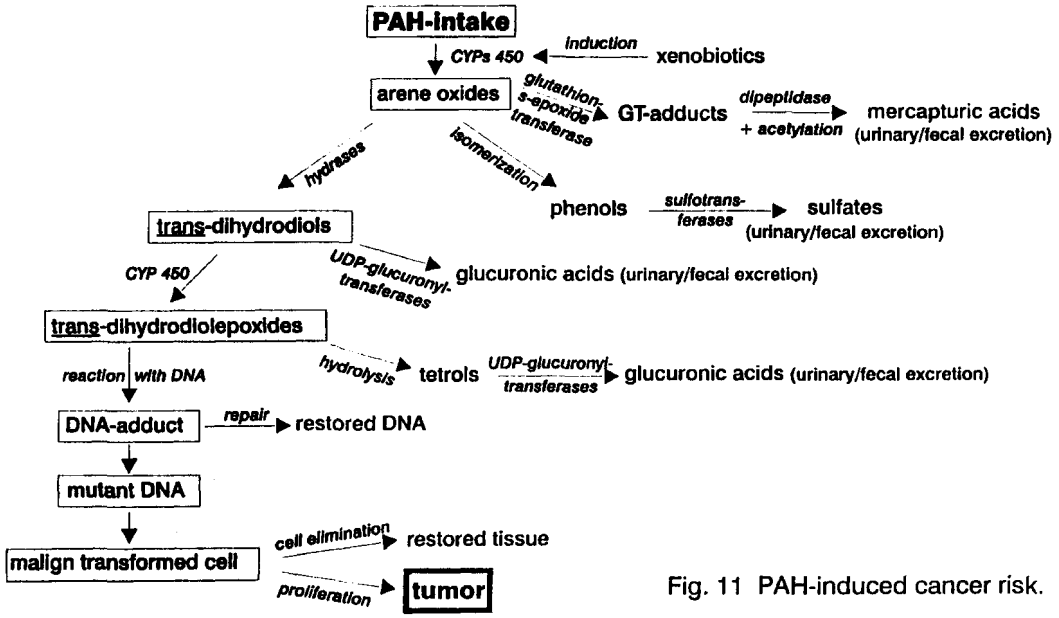

Fig. 11 PAH-induced cancer risk.

TABLE 4. Monooxygenase-inducing potencies of various xenobiotics following administration to rats (40 mg/kg).

inducer(pretreatment)	Induction factors for the rat liver microsomal metabolism of benz[a]anthracene	chrysene	benzo[e]pyrene	pyrene
5,6-benzoflavone	4,7	7,8	9,8	1,9
polychlorinated biphenyl	6,2	6,8	14,6	2,4
3,3', 4,4'-tetrachlorobiphenyl	4,8	9,3	10,8	2,2
phenobarbital	3,8	3,7	8,7	1,8
benz[a]anthracene	4,4	n.d.	7,2	1,5
chrysene	6,4	n.d.	11,2	1,8
benzo[a]pyrene	5,9	8,1	11,7	2,2
benzo[e]pyrene	1,6	3,0	1,0	0,8
benzo[b]fluoranthene	6,4	11,6	n.d.	2,9
benzo[j]fluoranthene	6,4	13,0	13,7	1,4
benzo[k]fluoranthene	6,3	n.d.	13,0	2,2

In other terms, even non-carcinogenic PAH may operate synergistically by enhancing the formation of ultimate carcinogenic metabolites of carcinogenic PAH via monooxygenase induction. Though there is no doubt that PAH are carcinogens for mammals, extrapolation from animal experiments to the human situation is problematic. Out of the various studies on the species-specific metabolism of PAH, two results shall be discussed:

1. When the primary (or phase I-) metabolism of benz[a]anthracene with liver microsomes from various species is studied, significantly different metabolite profiles are obtained for rat, dog, man, mouse and the rabbit (13) (Fig. 10).

A non-induced Wistar rat converts most of the substrate into the 10,11-dihydrodiol, whereas the dog exclusively oxidizes benz[a]anthracene at the 5,6-position. Interestingly, the mouse prefers the 3,4-position; the rabbit, on the other hand, preferentially forms the 1,2-dihydrodiol. Actually there is no metabolite pattern in any of these species which resembles that formed with human microsomes, though all these species are commonly used for carcinogenicity studies. The only model - at least for the liver - which comes very close to the human situation is a Wistar rat which had been moderately induced with β-naphthoflavone or other similarly operating inducers. The liver is not the target organ for PAH-induced tumor formation, but significant species-specific differences in the metabolite profiles of a number of PAH have also been found in epithelial lung cells which finally undergo malign transformation.

2. Even on the enzyme level, structure-dependent differences are found with regard to the end products formed (Table 5).

TABLE 5. Species-specificity of the regiospecific oxidation of phenanthrene in man, mouse and rat CYP 1A1 and CYP 1A2.

	oxidation of phenanthrene at position*			
	1,2-	3,4-	9,10-	ref.
human CYP 1A1	40	3	57	[14]
mouse CYP 1A1	24	7	69	[15]
rat CYP 1A1	9	-	91	[14]
human CYP 1A2	41	38	21	[14]
mouse CYP 1A2	18	35	47	[15]
rat CYP 1A2	6	11	84	[14]

* in % of total metabolites formed

For instance, the CYP 1A1 and 1A2 from human, mouse and rat form different metabolite pattern from phenanthrene. In case of CYP 1A1 the ratio of k-region- to non-k-region-oxidation is 1.3 in man, 2.2 in mouse, and 10.1 in rat and similarly for 1A2 where ratios of 0.3, 0.9 and 3.4 are found. Actually 21% of the amino acid sequences of human and rat CYP 1A1 are different and it may be expected that other isoforms also do not exhibit a greater homology. The evolutionary homology of monooxygenases for various species appears to be fairly limited, yielding markedly different catalytic activities.

These findings to which further could be added already now, suggest that extrapolations from one species to another are limited and evaluations on experimental rodent data have to be reconsidered for their validity.

Figure 11 summarizes the various mechanisms involved in the PAH-induced cancer risk. Framed events and metabolites contribute to the tumor formation, others are detoxifying actions. It is likely, however, that this scheme will still undergo many corrections in the future.

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