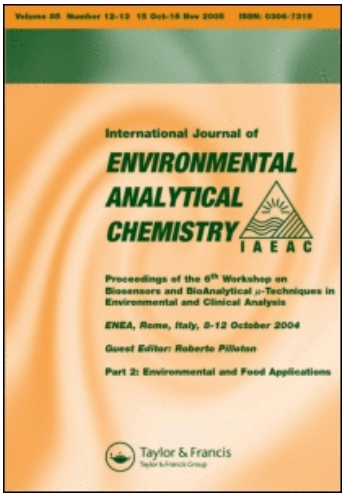

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METABOLISM OF PYRENE AND CHRYSENE IN EPITHELIAL HUMAN BRONCHIAL AND HAMSTER LUNG CELLS

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The metabolism of pyrene and chrysene in epithelial human bronchial and in hamster lung cells has been studied and found to be very similar in both systems, although it differs from that observed in rat lung microsomes. Metabolite profiles have been analyzed by means of capillary GC and by GC/MS.

KEY WORDS: Metabolism of pyrene, metabolism of chrysene, human lung cell cultures, hamster lung cell cultures, PAHs.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) have been shown to be carcinogenic in animal test systems after metabolic activation (for review see refs. 1 and 2). To minimize animal experiments, various *in vitro* systems have been established.[3,4] As a contribution to the latter, epithelial human bronchial and hamster lung cells have been used in this investigation to study the metabolism of pyrene and chrysene, two representative environmental PAH of the peri- and cata-condensed type, respectively. The pyrene and chrysene metabolism with rat lung microsomes has previously been investigated[5,16] and allows now to compare the metabolic activation of these PAH in three different species. According to previous studies[6,7,8] the metabolites were recorded by means of capillary GC and by GC/MS.

MATERIALS AND METHODS

Chrysene and pyrene were obtained from the Commission of the European Community, Brussels (BCR) in a purity >99.0% and metabolite standards were supplied by NCI Chemical Repository, USA. Preparation of fetal Syrian hamster

Table 1 Relative GC retention times (RRT) and main mass spectral fragments of some pyrene and chrysene metabolites (as OTMS-ethers)

Compound	*RRT*[a]	*Relative intensities of MS fragments* (%)							
		M	*M-15*	*M-31*	*m/z 73*				
1-OTMS-pyrene	1.343	100	29	14	72				
1-OTMS-chrysene	1.192	100	42	17	43				
2-OTMS-chrysene	1.211	100	52	22	90				
3-OTMS-chrysene	1.188	100	40	15	55				
4-OTMS-chrysene	1.133	100	50	46	55				
		M	*M-15*	*M-90*	*M-103*	*M-178*	*m/z 191*	*m/z 147*	*m/z 73*
t-1,2-di-OTMS-1,2-dihydrochrysene	1.261	27	9	21	30	27	100	60	75
t-3,4-di-OTMS-3,4-dihydrochrysene	1.108	16	9	16	28	14	100	27	100
1,2,3-tri-OTMS-1,2-dihydrochrysene	1.381	3	2	1	–	2	61	12	100

[a]Related to the parent PAH (pyrene and chrysene, resp.).

lung cells (FSHL), a permanent clonal line of fetal Syrian hamster lung epithelial cells (M3E3/C3) and the fetal human bronchial epithelial cell line (FHBE) used in this study as well as the culture techniques applied have been previously described.[9,10,11,12]

After the incubation periods (1, 2, 3, 6 and 8 days) and subsequent treatment with sulfatase/glucuronidase in order to cleave sulfate and glucuronic acid conjugates cells and media were extracted with ethyl acetate. Extracts were purified by LH 20 Sephadex chromatography. Metabolites were converted into their TMS-ethers and analysed by capillary GC (SE 54; 25 m × 0.32 mm) and by MS (Nermag R10-10; 70 eV) (for detail see ref. 13).

RESULTS

Pyrene

The only metabolite detectable after pyrene incubation was 1-hydroxypyrene in all cell systems as indicated by comparison of the mass spectra with that of an authentic sample (for data see Table 1).

However, the total recovery after 8 days of incubation decreased to 54% (FSHL), 70% (M3E3/C3) and 80% (FHBE), respectively, indicating that water-soluble metabolites (glutathione conjugates) were also formed. They may be calculated from the difference between original substrate concentration minus (pyrene + 1-hydroxypyrene).

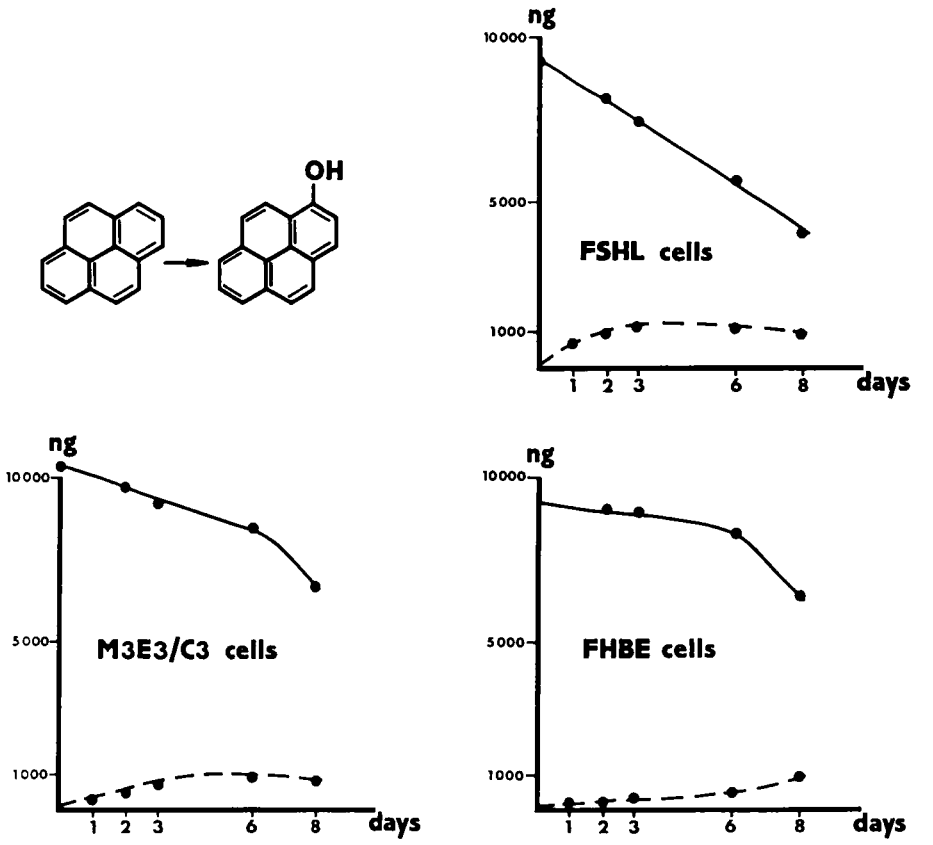

Figure 1 Time-dependent pyrene metabolism in fetal Syrian hamster lung (FSHL) and epithelial cells (MSE3/C3) and in fetal bronchial human cells (FHBE). Solid line: pyrene; dotted line: 1-hydroxypyrene.

Chrysene

Chrysene was oxidized at the 1,2- and the 3,4-position in all 3 cell systems resulting in the formation of dihydrodiols (1,2-dihydroxy-1,2-dihydrochrysene and 3,4-dihydroxy-3,4-dihydrochrysene) and phenols (1-, 2-, 3-, and 4-hydroxychrysene) but no K-region oxidation could be observed (Figure 2).

In Figure 3 the metabolite profile as recorded by capillary GC is presented.

Mass spectral and GC-retention time data are listed in Table 1.

The kinetics for the chrysene metabolism in the various cell systems are given in Figures 4–6, indicating that the 1,2-oxidation (formation of the proximate carcinogen) is more pronounced in the hamster than in human lung cells. However, the overall formation of dihydrodiols predominates in the latter system and is about ten times higher than the phenol formation, whereas higher phenol than dihydrodiol formation rates are found in hamster lung cells. The hamster lung epithelial cells form metabolite patterns which resemble more to those formed with human

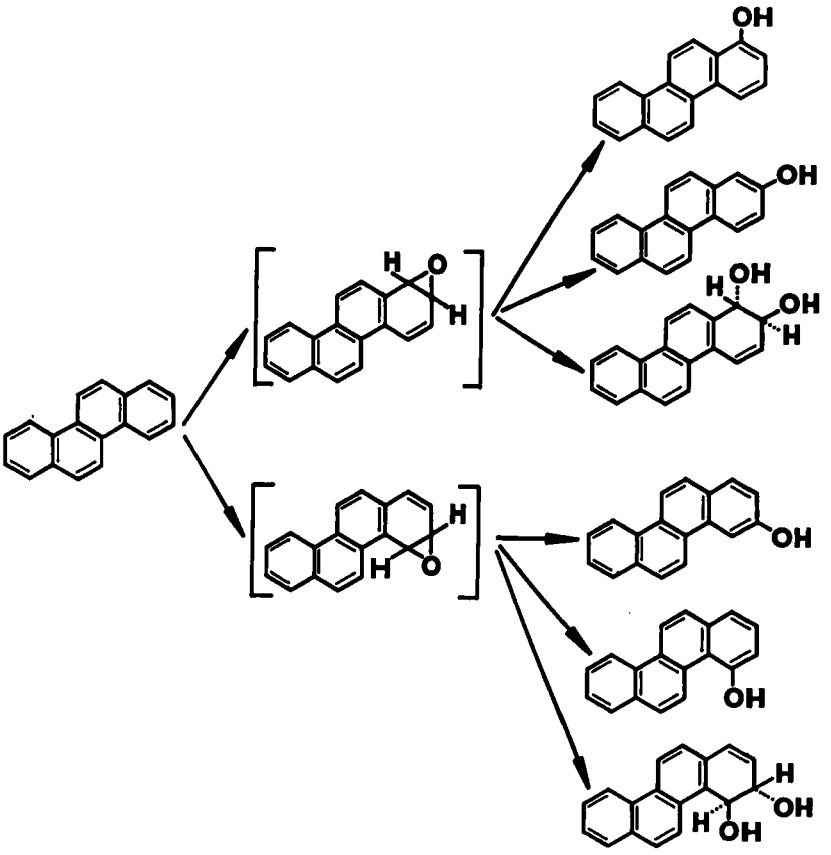

Figure 2 Metabolic activation of chrysene in the cell systems used.

lung cells. As in case of pyrene indications for the formation of water-soluble metabolites can be obtained from the recoveries found 47% (M3E3/C3) 55% (FSHL) and 97% (FHBE), respectively.

DISCUSSION

The metabolic activation of pyrene and chrysene results in qualitatively similar metabolite patterns in hamster lung and human bronchial cells. Both cell systems exclusively form 1-hydroxypyrene. In contrast, rat lung microsomes convert pyrene into both, 1-hydroxypyrene and 4,5-dihydroxy-4,5-dihydropyrene (K-region diol) and furthermore into higher oxidized derivatives (triols) when the experimental animals had been pretreated with inducers of the P-448-monooxygenase system such as polychlorinated biphenyls or β-naphthoflavone.[5] The 1-hydroxylation also predominates in rat liver microsomes[7] and in rat and human hepatocytes (Paul and Jacob, unpublished results).

In case of chrysene also quantitatively similar metabolite profiles were obtained with the cell systems tested and the epithelial cell line M3E3/C3 resembled most the

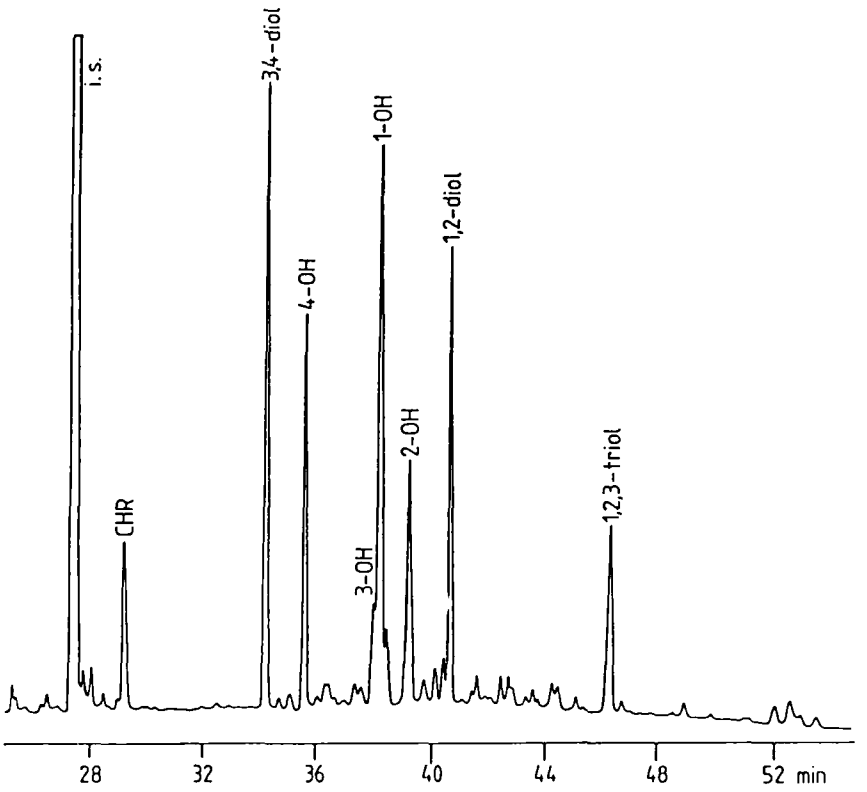

Figure 3 Metabolite profile of chrysene after 8 days incubation with M3E3/C3 cells as recorded by capillary GC after conversion into TMS-ethers.

human situation. In all these cell systems the ultimate carcinogen of chrysene (1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydrochrysene) which has been claimed to be responsible for the carcinogenic potential of this PAH,[14,15] has been detected. These results again demonstrate the marked difference of hamster and lung cell systems versus rat lung microsomes for which only the 3,4-oxidation has been observed.[16] From this it appears that the rat is less suitable than the hamster to simulate the chrysene metabolism (if not PAH-metabolism at all) in the human lung. Care must be taken in attempts to extrapolate animal experiments to the human situation, since e.g. PAH-metabolism in the liver may be very different in species commonly used in *in vivo* carcinogenicity tests. This is demonstrated in Figure 7 with benz(a)anthracene as PAH-substrate. This PAH unfortunately has not yet been investigated in the cell systems used here. Further studies are required to prove that these species-specificities exist also for lung microsomes and complete lung cell systems with regard to other PAH-substrates.

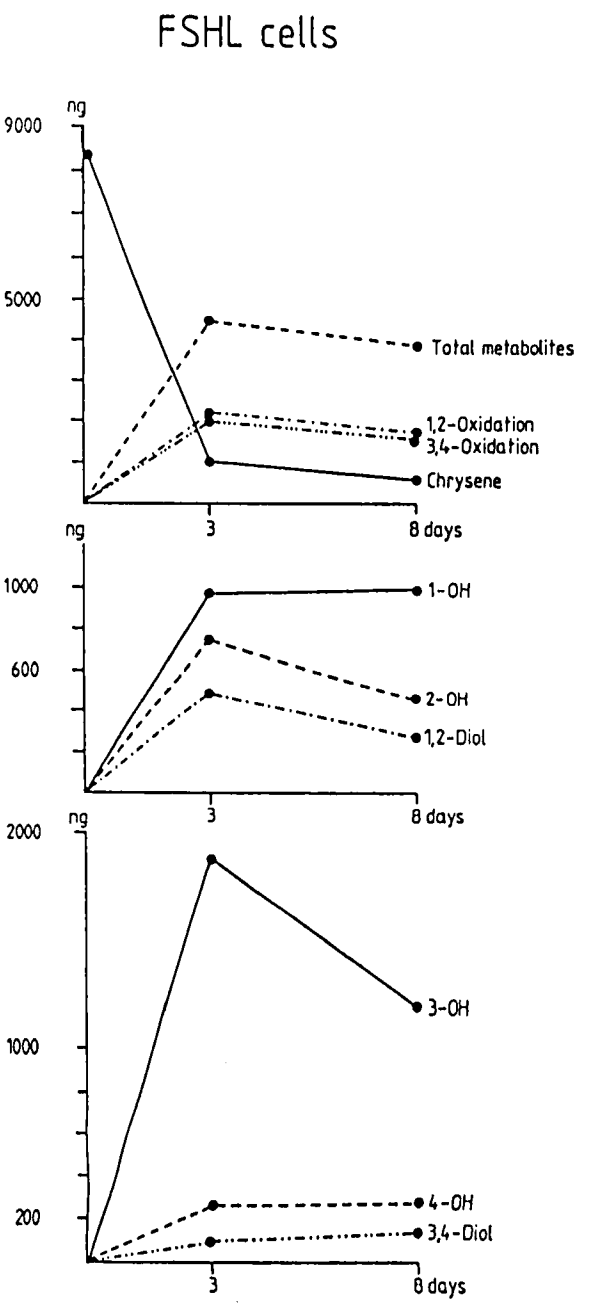

Figure 4 Time-dependent chrysene metabolism in fetal Syrian hamster lung cells (FSHL).

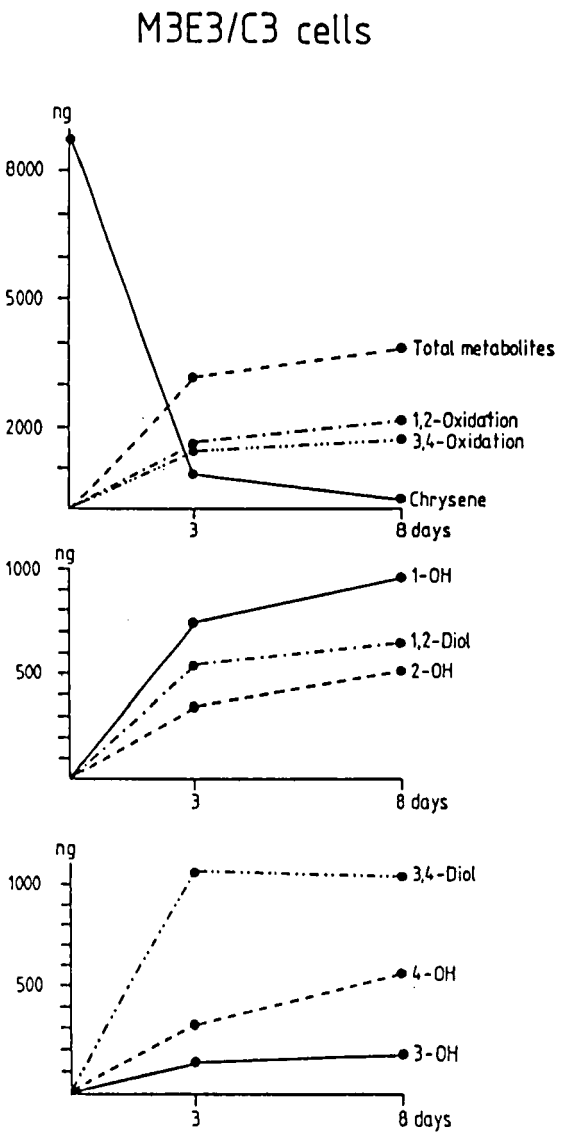

Figure 5 Time-dependent chrysene metabolism in fetal Syrian hamster lung epithelial cells (M3E3/C3).

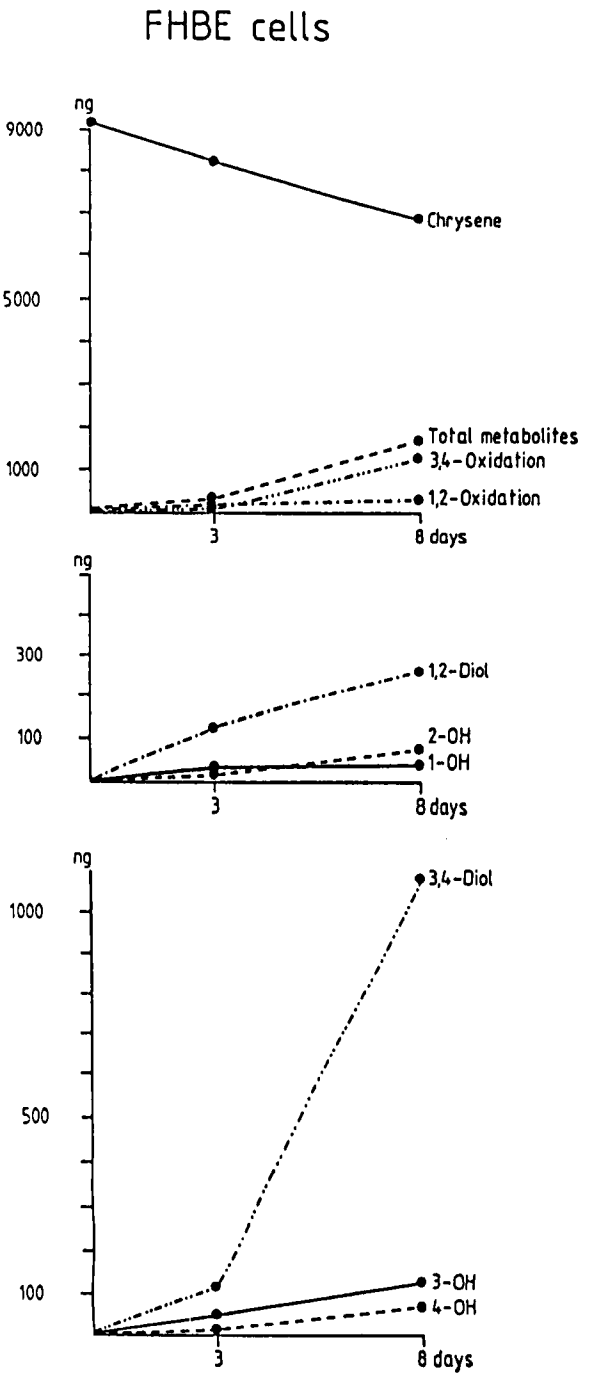

Figure 6 Time-dependent chrysene metabolism in fetal human bronchial cells (FHBE).

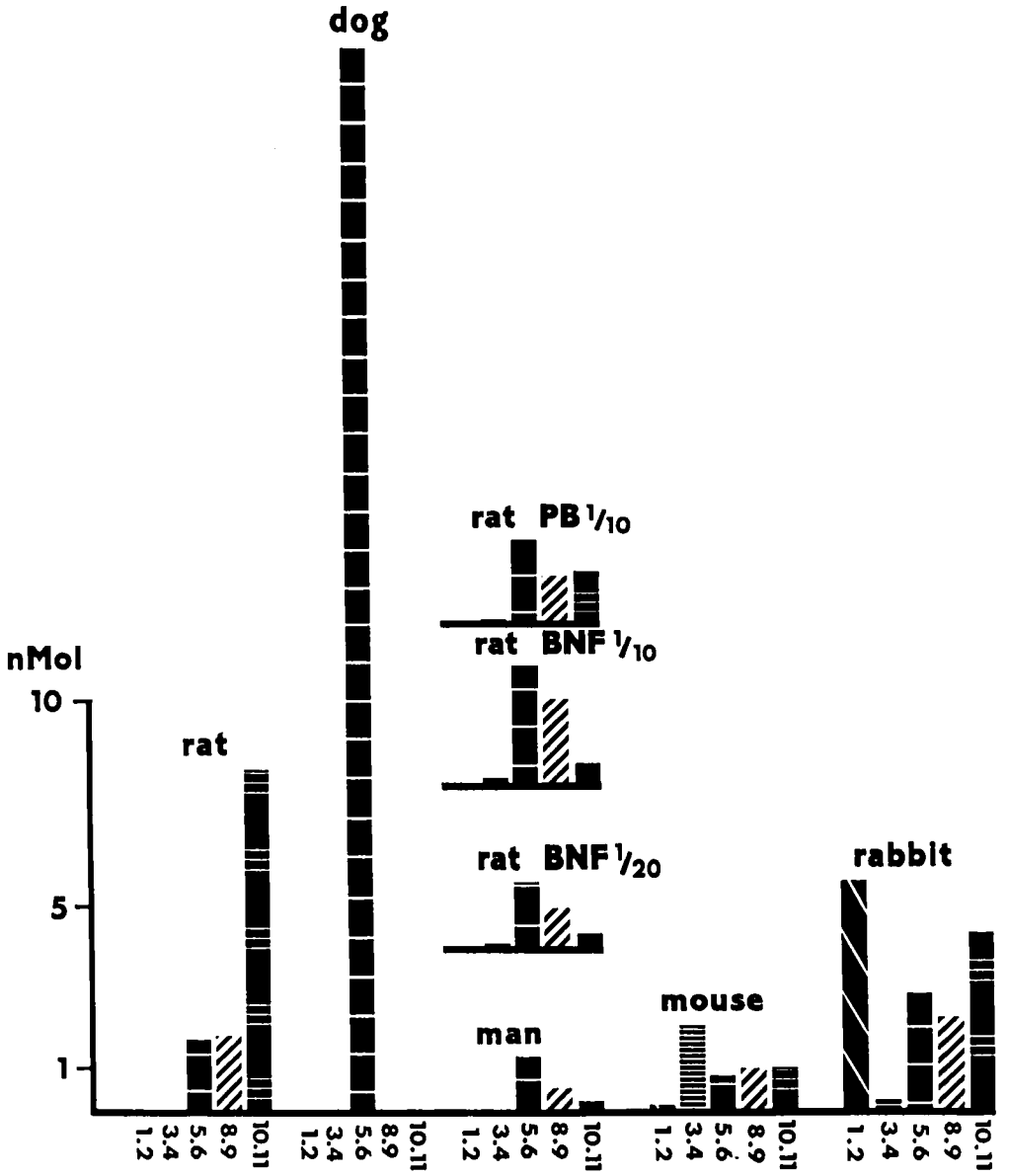

Figure 7 Metabolite profile of benz(a)anthracene obtained with liver microsomes of various mammalian species including man.

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