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KEY WORDS: adducts, initiation, BaP, mixtures

Tumorigenicity of mixtures is often considered to be a sum of the tumorigenicity of individual components of the mixture. We have presented evidence that this may not be so for mixtures derived from the liquefaction of coal (Mahlum et al. 1984). Tumor initiation is considered to be a genetic event in which the DNA is altered by adduction by a carcinogenic chemical or by a physical agent such as ionizing radiation. DiGiovanni et al (1982) have presented evidence that mouse skin tumor initiating activity is closely correlated with the level of DNA adduction. In this paper, we examine the relationship between chemical composition of mixtures and their skin tumor initiating activity. We also examine the effect of mixtures on the skin tumor initiating activity and the adduction of DNA by benzo[a]pyrene (BaP). These studies are directed toward answering the following questions:

1. Can the carcinogenicity of mixtures be predicted from their chemical composition?
2. Is DNA adduct formation a better indicator of tumor initiating activity than is chemical composition?

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MATERIALS AND METHODS

Several mixtures with various boiling ranges obtained from a coal liquefaction process were used as source materials for these studies. The major components of these mixtures ranged from 2- and 3-ring polycyclic aromatic compounds (PAC), their alkylated derivatives, and peri-condensed 4-ring PAC for a distillate boiling between 300 and 700°F to cata-condensed 5- and 6-ring PAC with alkylated homologs for a >850°F distillate. Chemical class fractions were prepared from these distillates by the method of Later et al. (1981). Skin tumor initiating activities of the various test materials were determined by an initiation-promotion assay using female Charles River CD-1 mice. In some experiments, (BaP) was added to the test materials before application to the mouse skin.

To study the relationship between skin tumor initiating activity and DNA adduction, radiolabeled BaP, by itself or in the presence of one of the mixtures, was applied to mouse skin. After 24 hours, DNA was isolated from skin by a modification of the method of Marmur (1961), and the amount of BaP bound to the DNA determined by radioactive counting. Adduct profiles were obtained by digesting the purified DNA using the method of Nishimoto and Varanasi (1985).

RESULTS

The mouse skin tumor initiating activities for two mixtures (boiling ranges of 800-850 and >850°F) and their chemical class fractions are shown in Figure 1. It can be easily seen that the activity of the lower boiling material is only about one-half to one-third that of the higher boiling material whether the parent material or its PAH fraction is considered. When the chemical composition (Figure 2) of the two distillates or their PAH fractions are compared, it appears as if the 800-850°F material should have the higher activity. For example, both materials have similar levels of BaP, a well-known carcinogen; however, the lower boiling material also significant levels of benz[a]anthracene, chrysene, 4-or 6-methylchrysene, and benzo[b+j+k]fluroanthene, all of which have been reported to possess carcinogenic activity.

Because the initiating activity of the organic mixtures appeared to be strongly influenced by the overall composition of the mixture, we decided to directly test the effect of some mixtures on the expression of the initiating activity of BaP. We applied 25 ug of BaP to mouse skin in either methylene chloride (50 ul) or in methylene chloride containing 5 mg of organic mixture. In addition to the mixtures used above, we also used two other mixtures with lower boiling ranges, but which had been shown previously (Mahlum 1984) to be carcinogenic. We also tested a mixture that boiled between 300 and 700°F that had been shown to be inactive as a skin tumor initiator. After two weeks the mice were promoted twice weekly with 5 ug of 12-O-tetradecanoyl-13-acetate (TPA). The greatest number of tumors occurred when BaP was administered alone (Figure 3). Expression of BaP initiating activity was

inhibited by all of the mixtures except for the 300-700°F distillate, providing further evidence that these organic mixtures contain materials that interfere with the activity of its carcinogenic components. Because BaP has to be metabolically transformed before it reacts with DNA, we speculated that the decrease in initiating activity observed in the presence of the mixtures might be due to competition for metabolism by non-carcinogenic PAH. We therefore tested four chemical class fractions from the 750-800°F distillate for their influence on BaP initiating activity; the effect is seen in Figure 4. The neutral PAH and the NPAC fractions had the greatest inhibitory effect, decreasing initiating activity by about 70%; the aliphatic and the hydroxy-PAH fraction were significantly less effective. These results are consistent with the concept that interference with BaP activation is involved in the decreased tumor response seen when BaP is administered in the presence of other PAH. These experiments also further illustrate that the expression of BaP initiating activity can vary widely, depending on the matrix in which it is found.

It has become accepted dogma that tumor initiation is the direct result of the interaction of the ultimate carcinogenic form of a compound with DNA. We therefore undertook studies to determine if the skin tumor initiating activity of BaP in the presence of other organic compounds was better correlated with DNA adduct formation than with the chemical composition of the mixture. In these experiments, we applied radio-labeled BaP to mouse skin, either in a solvent alone or in a solvent containing 5 mg of one of the distillates used in the tumor initiation studies.

All of the distillates used inhibited the binding of BaP to DNA (Figure 5). The 800-850°F and >850°F distillates were the most effective, decreasing

the binding by about seven-fold. Even the 300-700°F material inhibited binding by about 50% even though it did not significantly decrease tumor initiation by BaP.

BaP adduct profiles were prepared from DNA from mouse skin treated with either BaP alone or with BaP in the presence of one of the above distillates (Figure 6). In all cases the amount of radiolabeled adduct eluting from the HPLC column was decreased when BaP was co-administered with one of the organic mixtures, a result consistent with the total amount of labeled BaP bound to DNA. The major adducts in all cases corresponded to anti-BPDE-dGuo and syn-BPDE-dGuo. Interestingly, the ratio of the anti- to syn- isomer was decreased by about 50% (Table 2).

DISCUSSION

Examination of the skin tumor initiation data indicates that several of the complex organic mixtures used in these experiments contained tumor initiating activity. Moreover, the initiating activity tended to increase with increasing boiling ranges of the mixtures. Although chemical analysis indicated that the mixtures contained known carcinogens, the skin tumor initiating activity often did not correlate with measured levels of these carcinogens. The results with the distillates alone suggested that the activity of some or all of their carcinogenic compounds was being masked by other components. Direct examination of the tumor initiating activity of BaP in the presence of the distillates provided support for this conclusion. These data indicate that chemical analysis alone may not adequately predict tumorigenicity of mixtures.

The adduct data obtained in these experiments strongly suggest that there is not a simple relationship between carcinogen binding to DNA and tumor initiation. Although co-administration of mixtures with BaP suppressed both the total binding of BaP to DNA and the number of tumors induced, the effect of the mixtures on binding was substantially greater than their effect on tumor initiation. Moreover, the mixtures decreased the ratio of the anti-BPDE-dGuo to syn-BPDE-dGuo isomer. If the anti-BPDE-dGuo adduct represents the carcinogenic form as generally accepted, our data indicate that mixtures significantly increase the tumorigenic effectiveness of the BaP that becomes bound to DNA, especially as the anti-BPDE-dGuo adduct. These data suggest that measurement of DNA adduction may also not be an adequate predictor of carcinogenicity of materials found in certain mixtures.

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TABLES

Table 1. Chemical class fraction content of complex mixtures with boiling ranges 800-850° and >850°.

Fraction	Percent Composition 800-850°F	>850°F
Aliphatic (A1)	5	2
Neutral PAH (A2)	50	46
Nitrogen-containing (A3) Polycyclic Aromatic Compounds	25	34
Hydroxy-PAH (A4)	18	20

Table 2. Effect of complex organic mixtures on the ratio of anti-benzo[a]pyrene-diol-epoxide (BPDE) to syn-BPDE guanosine adducts from mouse skin DNA after administration of benzo[a]pyrene (BaP).

Test Material	Anti:'Syn Ratio
BaP	12:2
300-700°F + Bap	7.2
700-750F° + BaP	6.4
750-800°F + BaP	6.4
800-850°F + BaP	5.4
>850°F + BaP	5.4

LEGENDS

Figure 1. Mouse skin tumor initiating activity (total tumors per groups of 30 mice) after:

a. Initiation (17 mg) with coal liquids boiling from 800-850°F or >850°F the chemical class fractions prepared from the AH, aliphatics and olefins; PAH, neutral polycylic aromatic hydrocarbons; NPAC, nitrogen-containing polycyclic aromatic compounds; HPAH, hydroxy-PAH. Fractions were applied in the same proportion as they were found in the distillate.

Figure 2. Representative PAH constituents from the 800-850°F and >850°F distillates.

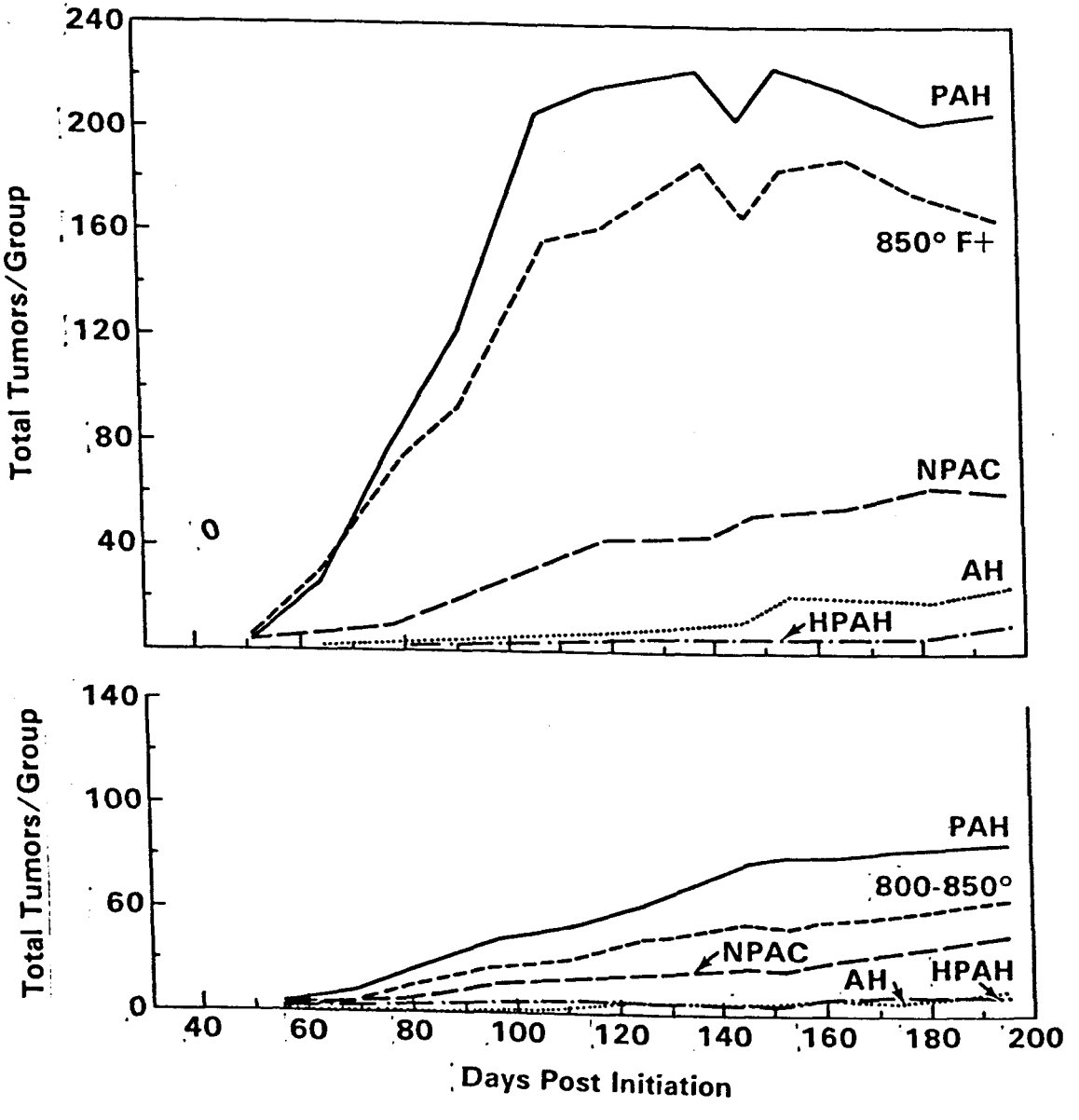
Figure 3. Effect of coal distillates with different boiling ranges on benzo[a]pyrene (BaP) skin tumor initiating activity. Twenty-five ug of BaP was applied to mouse skin in 50 ul methylene chloride or in 50 ul methylene chloride containing 5 mg of coal distillate. Activity is expressed in total number of tumors per group of 30 mice.

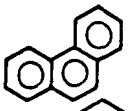
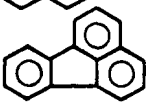
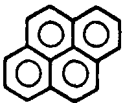
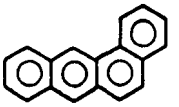
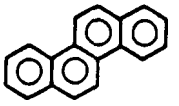
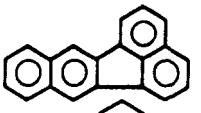
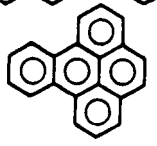
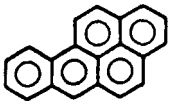
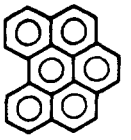
Figure 4. Effect of the 750-800°F coal distillate and its chemical class fractions (see legend form Figure 1a), on benzo[a]pyrene (BaP) skin tumor initiating activity. Twenty-five ug of BaP was applied to mouse skin in 50 ul of methylene chloride or in 50 ul of methylene chloride containing 5 mg of distillate or proportionate amounts of chemical class fractions prepared from 5 mg of distillate.

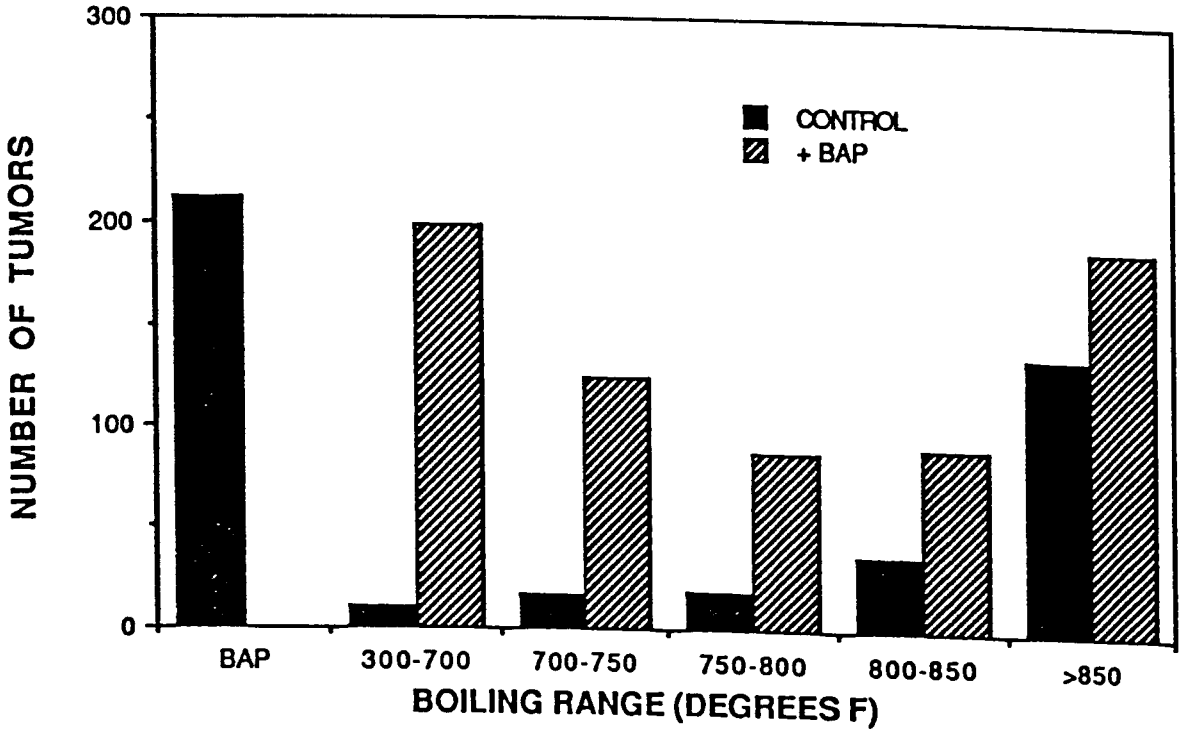
Figure 5. Effect of coal distillates on binding of benzo[a]pyrene (BaP) to mouse skin DNA. ^{3}H-labelled BaP was applied to mouse skin in 100 ul of 1:1 acetone:methylene chloride or in acetone:methylene chloride containing 5 mg of coal distillate. Twenty-four hours later, the skin was removed, digested with proteinase K, and DNA extracted and purified. Binding was estimated from the amount of radioactivity associated with DNA.

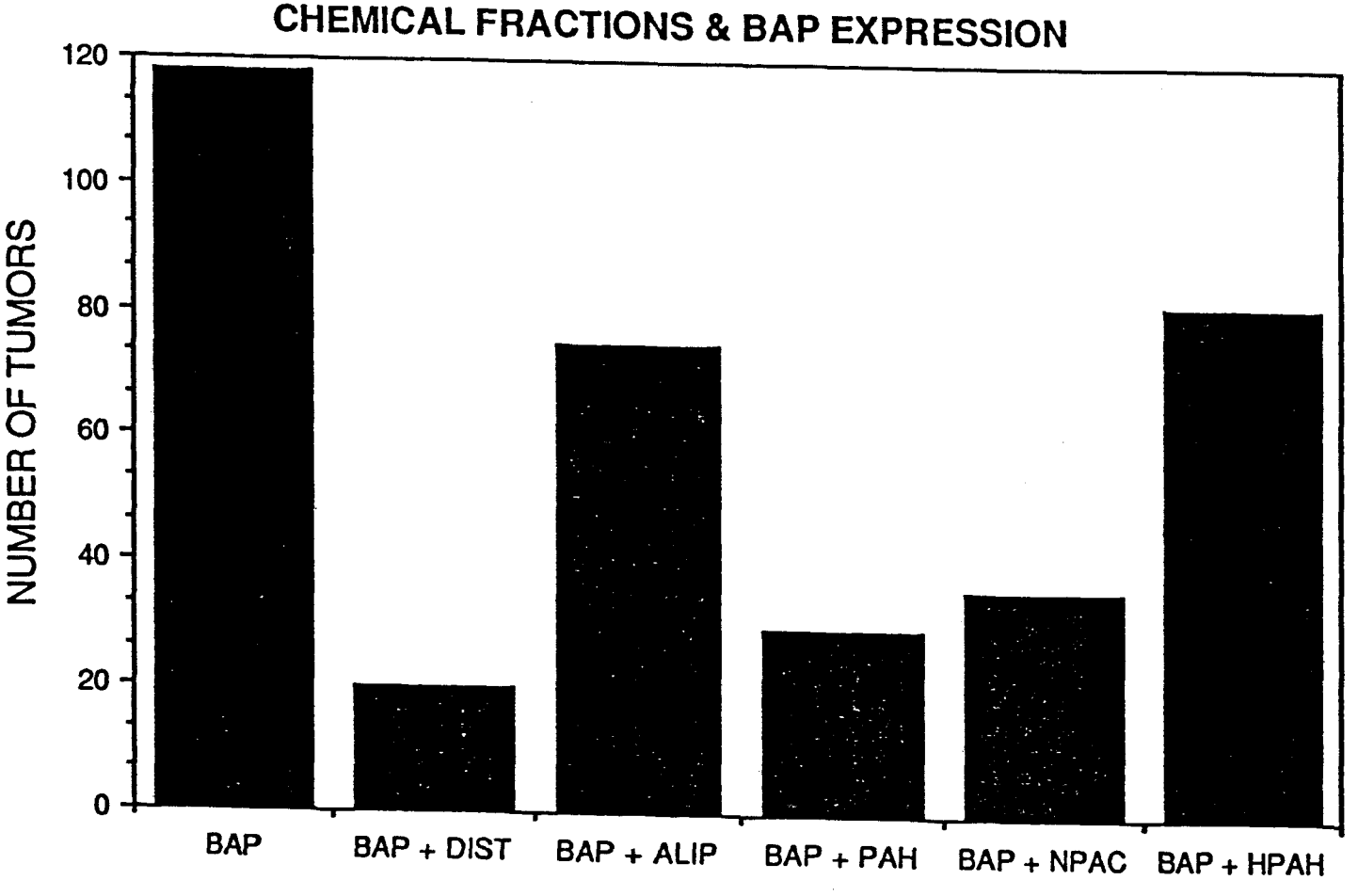
Figure 6. Benzo[a]pyrene (BaP) adducts prepared from mouse skin DNA isolated 24 hours after administration of radiolabeled BaP alone or in the presence of coal distillates with varying boiling ranges. After extensive purification, DNA was hydrolyzed to nucleosides and the adducts separated by high performance liquid chromatography. Profiles were obtained by determining the amount of radioactivity at 0.5 minute intervals.

Tumor Response

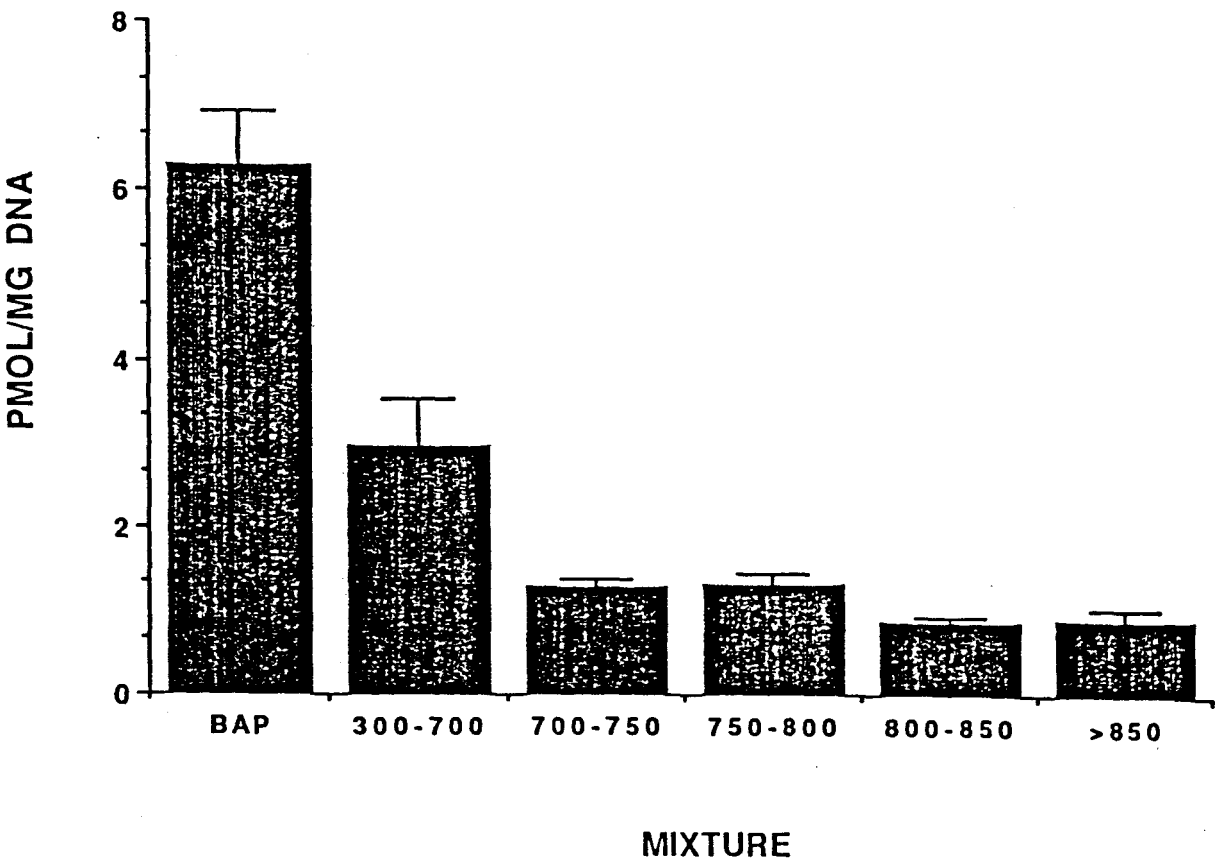


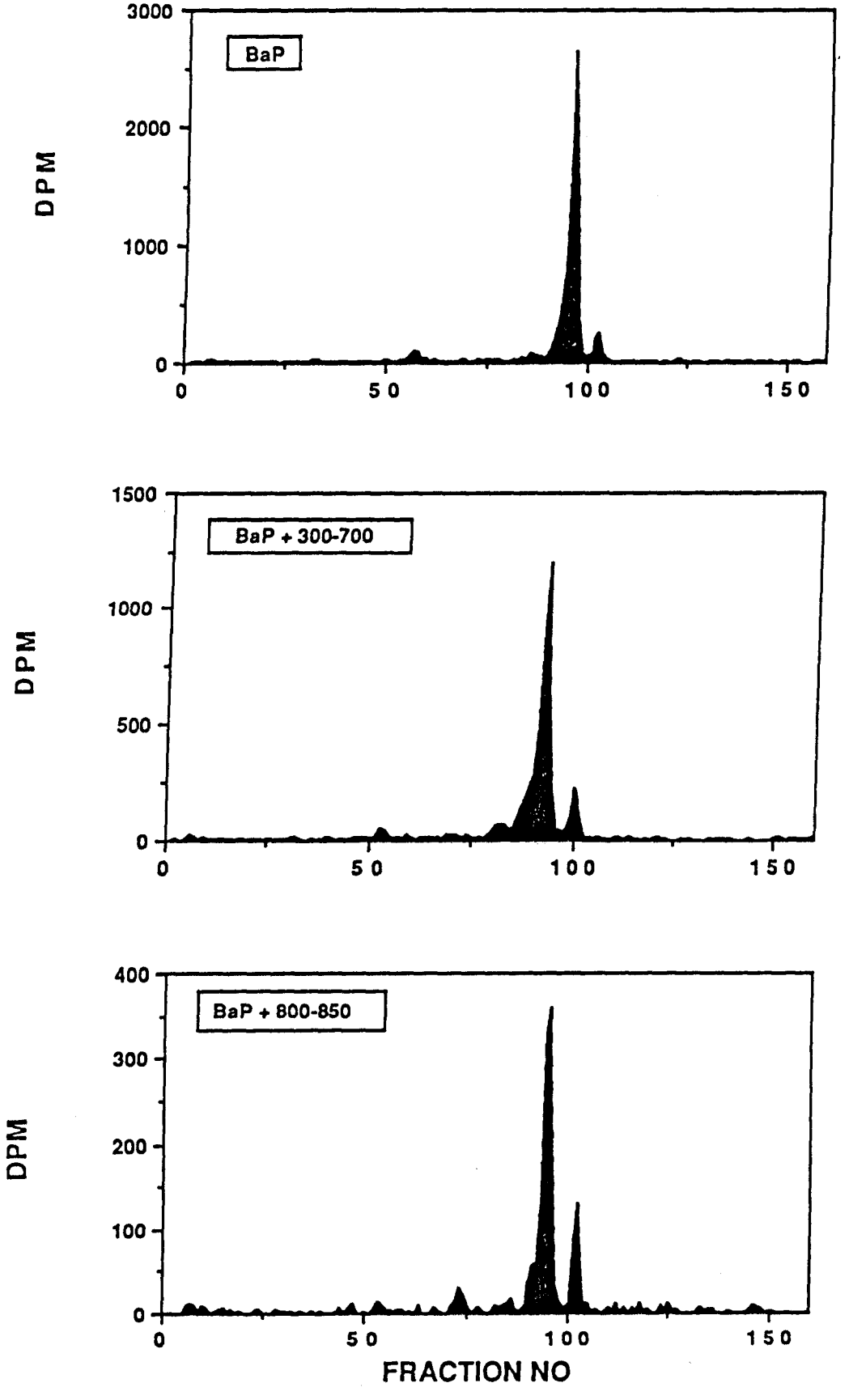
	QUANTITY (μg/g)		
COMPOUND	800-850°	850 +	STRUCTURE
PHENANTHRENE	211	58	
FLUORANTHENE	232	30	
PYRENE	2,375	235	
BENZ(a)ANTHRACENE	2,997	-	
CHRYSENE	7,324	31	
BENZO(k)FLUORANTHENE	3,972	306	
BENZO(e)PYRENE	6,135	5,755	
BENZO(a)PYRENE	3,530	3,637	
BENZO(ghi)PERYLENE		15,311	


300
200
100
0
NUMBER OF TUMORS
CONTROL
+ BAP
BAP
300-700
700-750
750-800
800-850
>850
BOILING RANGE (DEGREES F)


CHEMICAL FRACTIONS & BAP EXPRESSION
NUMBER OF TUMORS
120
100
80
60
40
20
0
BAP
BAP + DIST
BAP + ALIP
BAP + PAH
BAP + NPAC
BAP + HPAH

MIXTURE EFFECTS ON BAP-DNA ADDUCTS




BaP
3000
2000
1000
0
DPM
0
50
100
150
BaP + 300-700
1500
1000
500
0
DPM
0
50
100
150
BaP + 800-850
400
300
200
100
0
DPM
0
50
100
150
FRACTION NO