Metabolism of Benzo(a)pyrene by Murine Embryonal Carcinoma Cells

Ron Filler and Sarah Garner-Shinpock

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

ABSTRACT

Murine embryonal carcinoma (EC) cells were characterized with respect to their ability to metabolize the polycyclic aromatic hydrocarbon, benzo(a)pyrene [B(a)P]. The extent of metabolic activation varied more than 100-fold among the teratocarcinoma-derived cell lines examined. This difference in metabolic activity was correlated with an increase in the formation of specific metabolites that were identified by high-pressure liquid chromatography. Maximal in vitro formation of water-soluble products occurred 24 hr after the addition of [3H]B(a)P to the EC cells. Long-term incubation of EC cells with [3H]B(a)P indicated that, within the initial 24 hr, 2.3% of the input had been taken up by the cells. Subcellular analysis of the distribution of radioactivity indicated that 70 to 80% of intracellular radioactivity was associated with isolated nuclei. Therefore, the intranuclear metabolites of B(a)P were also analyzed by high-pressure liquid chromatography.

Embryonal carcinoma cell lines OC15S1 and C86S1 showed significant in vitro toxic effects to B(a)P over a concentration range of 0.05 to 0.3 μg/ml, whereas F9 and PC13 were resistant to concentrations of B(a)P up to 5 μg/ml. Equally resistant to B(a)P was the PYS cell line, a differentiated cell type derived from EC cells. Cytotoxicity was related to the extent of metabolic activation of parent compound.

INTRODUCTION

Previous investigations have used mammalian cell systems in an effort to obtain insight into different aspects of the metabolic activation of polynuclear aromatic hydrocarbons and to elucidate the mechanism(s) involved in chemically induced neoplasia (2, 7, 8, 11, 17, 20, 22, 26, 28, 31). Generally, these in vitro studies have used established cell lines or low-passage cell cultures derived from rodent or primate sources. To date, however, there has been a paucity in the application of early embryonic cells to complement or primar sources. To date, however, there has been a paucity in the application of early embryonic cells to complement these studies. This is due, in part, to the limitations in the amount of embryonic material that is available at early developmental stages.

EC cells offer a convenient alternative to the direct use of mammalian embryos. EC cells, the undifferentiated pluripotent stem cells of teratocarcinomas, are developmentally bivalent in that they have differentiated capabilities (14, 15) and are malignant (24, 33). Consequently, EC cells offer a unique opportunity for studying mammalian regulatory mechanisms governing differentiation (30) as well as for probing the basis of cancer (21). In this report, we have characterized several teratocarcinoma-derived cell lines with respect to their ability to metabolize the polycyclic aromatic hydrocarbon, B(a)P.

MATERIALS AND METHODS

Chemicals. [G-3H]B(a)P (specific activity, 25 to 30 Ci/mmol), obtained from New England Nuclear, Boston, Mass., was purified before use by thin-layer chromatography (silica gel) using hexane:benzene (19:1). The purity of [3H]B(a)P was >98% as determined by HPLC analysis. Unlabeled B(a)P (gold label) was obtained from Aldrich Chemical Co., Milwaukee, Wis., and was determined to be >95% pure by HPLC.

Metabolism of B(a)P. [3H]B(a)P was redissolved in DMSO after the hexane was evaporated under N2 (4°) and was added to the cell culture medium at 1.3 μCi/ml and 0.5% DMSO. This concentration of DMSO did not adversely affect cell growth. Twenty-four hr later, the tissue culture medium was removed and extracted exhaustively with ethyl acetate (27). The extent of B(a)P metabolism was calculated from the amount of radioactive water-soluble metabolites formed after organic solvent extraction (8) and from the amount of B(a)P metabolites present in ethyl acetate as determined by HPLC analysis. The radioactivity recovered between the organic and water phases was >95% of the initial input. All experimental procedures were performed under yellow illumination.

Teratocarcinoma-derived Cells. EC and PYS cells used in this study were kindly sent to us by Drs. M. McBurney (Department of Biology, University of Ottawa, Ottawa, Ontario, Canada), M. Hooper (University of Glasgow, Glasgow, Scotland), D. Solter (Wistar Institute, Philadelphia, Pa), and J. Lehman (University of Colorado Medical Center, Denver, Colo.). Teratocarcinomas were experimentally induced by transferring 6- to 7.5-day embryos to an ectopic site (12, 32). Stem and endodermal cells were isolated from the cultured outgrowths derived from embryoid bodies, which are the ascitic form of the solid tumor (3, 12, 16, 19). PYS-2, F9, PC13, and OC15S1 were derived from the 129 teratocarcinoma, OTT650; C86S1 was derived from the C3H teratocarcinoma, 86. Embryonal carcinoma cells were grown as described previously (10, 19) either in Dulbecco’s modified Eagle’s medium (Grand Island Biological Co.) and 10% fetal bovine serum (Grand Island Biological Co.) or in Dulbecco’s modified Eagle’s Medium supplemented with nonessential amino acids, nucleosides, sodium pyruvate, and 10% fetal bovine serum.

Determination of Cellular Toxicity. EC cells, seeded at 3 × 10^4 cells/sq cm, were treated with various concentrations of B(a)P for 24 hr. The cells were then harvested by mild trypsin treatment and counted in a hemocytometer. Trypan blue exclusion was used for the determination of the toxic effects of B(a)P (23). Cellular incubations were performed in triplicate and were repeated 3 times. Percentage of survival was calculated as that...
fraction of EC cells which were viable after exposure to PAH. Untreated EC cells represented 100% survival.

HPLC. For the chromatographic analysis of B(a)P metabolites, the tissue culture medium was removed from EC cells that had been previously incubated with [3H]B(a)P and subsequently extracted with ethyl acetate. The organic solvent extract was dried over anhydrous MgSO4 and evaporated to dryness under N2, and the metabolites were redissolved in methanol (Spectrograde and glass distilled; Burdick & Jackson Laboratories, Inc., Muskegon, Mich.). The separation of metabolites was performed using a Spectra Physics 3500 high-pressure liquid chromatograph fitted with a DuPont 1-m ODS Permpasph column. Gradient elution was by reverse phase using an initial solvent composition of 30% methanol:70% water to a final solvent composition of 70% methanol:30% water (27). Fractions (0.2 ml) were collected, and 5 ml of Aquasol (New England Nuclear, Nuclear, Boston, Mass.) were added. Radioactivity was then measured in a Beckman LS 8100 liquid scintillation counter. The elution positions of B(a)P and its metabolites were determined by using 14C-metabolite standards.

Isolation of Nuclei and Nuclear Metabolites. Nuclei were isolated from EC cells using the hypotonic citric acid procedure as described by Birnie (4). After EC cells were incubated with [3H]B(a)P for 24 hr, the medium was removed, and the cells were harvested by mild trypsin treatment. The cell pellet was washed twice with phosphate-buffered saline and then resuspended in 20 volumes of 2 mM citric acid. The cell suspension was transferred to a glass Potter-Elvehjem vessel, homogenized 10 times (4°), and then centrifuged at 200 x g for 10 min (4°) in a Beckman TJ-6 centrifuge. The supernatant was carefully removed, and the procedure was repeated twice. After each centrifugation, the isolated nuclei were microscopically monitored by Nomarski differential interference contrast for gross cytoplasmic contamination, nuclear lysis, or distortion. A sample of the final nuclear pellet was also examined by high-resolution electron microscopy to verify the absence of cytoplasmic contaminants and the removal of the outer nuclear membrane. The final nuclear pellet was resuspended in 2 ml phosphate-buffered saline (4°) and centrifuged at 200 x g for 10 min, and 1.5 ml of 6 M guanidine-HCl (Matheson, Coleman & Bell, Norwood, Ohio) containing 10 mM EDTA (pH 7.0) were added. Nuclei were sonicated (Bronwill; Biosonic III) for 10 sec (4°) and then extracted twice with 2.5 volumes of ethyl acetate. The preparation of nuclear metabolites for HPLC analysis was performed as described previously for the tissue culture medium.

RESULTS

Cellular Toxicity to PAH. Teratocarcinoma-derived cell lines showed variable susceptibility to the toxic effects of B(a)P (Chart 1). OC15S1 and C86S1 showed an initial toxicity to B(a)P at a concentration of 0.03 µg/ml and a maximal effect at 0.3 µg/ml (55 and 83%, respectively). F9, PC13, and PYS, however, were resistant to B(a)P up to a concentration of 5 µg/ml. To determine if a cytotoxic effect could be demonstrated, resistant cells were incubated in the presence of B(a)P at 50 µg/ml, subcultured, and then repeatedly exposed to B(a)P (10 µg/ml) for 48-hr intervals over a 2-week period. No toxic effects were observed.5

5 R. Filler and S. Shinpock, unpublished data.

Rate of Water-soluble Metabolite Formation. Since EC cells showed a gradational response to the toxic effects of B(a)P, the capacity of these cells to metabolize the parent PAH was examined. As indicated in Table 1, each EC cell line demonstrated a varying ability to metabolize B(a)P to water-soluble derivatives. Consequently, cell cultures were divided into arbitrary groups based on their efficiency for metabolizing parent compound (7). During a 24-hr incubation period, F9, PC13, and PYS (Group 1) showed minimal activity (6% or less) and OC15S1 and C86S1 (Group 2) converted 30 and 60% of the input B(a)P to water-soluble products. Overall water-soluble product and organic solvent-soluble metabolite formation varied 56- and 250-fold, respectively, among the cell lines examined. Long-term incubations (72 hr) of EC cells with B(a)P indicated that the extent of water-soluble product formation after the first 24 hr did not significantly increase either with Group 1 or with Group 2 cells.6

Nuclear Uptake of B(a)P. Incubation of EC cells with [3H]-B(a)P for 24 hr showed that about 2.3% of the input B(a)P had been taken up by the cells; upon subcellular fractionation, 70 to 80% of the cellular radioactivity was associated with isolated nuclei (Chart 2). As a result of the significant amount of radioactivity associated intracellularly with the nucleus, the rate of nuclear accumulation was determined over 72 hr. Since the

![Chart 1. Cellular toxicity of teratocarcinoma-derived cell lines to B(a)P. Increasing concentrations of B(a)P were added in DMSO (final concentration, 0.5%) to cells growing in 15 ml of medium. Cell cultures were exposed to PAH for 24 hr at 37°C. Toxicity was determined for each B(a)P concentration in 3 replicate cultures and was calculated as that fraction of viable cells surviving input.](chart1.png)

![Table 1. Extracellular B(a)P metabolite formation by teratocarcinoma-derived cell lines. EC cells were incubated for 24 hr with 750 pmol of [3H]-B(a)P. Media was removed and extracted with ethyl acetate, and the distribution of radioactivity was determined between the aqueous and the organic solvent phases. Quantification of organic solvent metabolites was determined directly by HPLC analysis.](table1.png)
Chart 2. Nuclear uptake of [\(^{3}H\)]B(a)P. EC cells were incubated in triplicate for various periods of time with [\(^{3}H\)]B(a)P and harvested, and an aliquot was assayed by direct radioactive measurement for total cellular uptake. Nuclei were then isolated, and an aliquot was assayed for nuclear accumulation (\(\bullet\)). The remaining nuclei were subjected to organic solvent extraction as described in “Materials and Methods.” Nuclear uptake was normalized with respect to a constant cell number. The percentage of nuclear extractable radioactivity (\(\oplus\)) was determined on the basis of that fraction of total nuclear radioactivity that was organic solvent soluble.

Nuclear uptake was significant within the first 8 hr (representing 100% of the cellular radioactivity), decreased slightly at 24 hr, and then reached a constant minimal value (29% of maximal) at 48 to 72 hr (Chart 2). Nuclei were also subjected to ethyl acetate extraction to determine if the observed reduction in radioactivity associated with nuclei was accompanied by a similar reduction in organic solvent extractable material. During the initial 24 hr, there was very little change in extractable radioactivity, but this amount did decrease over the following 48 hr at a rate similar to the rate of nuclear accumulation.

HPLC Analysis of Extracellular and Nuclear Ethyl Acetate-soluble Metabolites. Since the PYS cell line did not significantly metabolize B(a)P, the following comparison was limited to the metabolite profiles from the EC cell lines (Charts 3 to 6). These stem cells showed both qualitative and quantitative differences in the activation of parent compound to B(a)P derivatives which accumulated in the medium and in the nuclei. The overall metabolic activity of EC cells varied approximately 30-fold with F9 and C86S1, converting 3 and 94%, respectively, of the input B(a)P to various metabolic species.

Almost all of the extracellular ethyl acetate-soluble radioactivity from F9 and PC13 incubations consisted of unmetabolized B(a)P (>90%). PC13 was able, nevertheless, to metabolize parent hydrocarbon at a very low level to several B(a)P derivatives resolvable by HPLC. In comparison, OC15S1 and C86S1 showed significant increases in the conversion of B(a)P to all metabolites. Analysis of the extracellular fraction by HPLC indicated that a large class of metabolites from the C86S1 incubation (Chart 6, top) appeared in the initial eluting peak. This region of the HPLC chromatogram, which represented 26% of the total organic solvent-soluble radioactivity (Table 2), has been reported (5) to contain predominantly uncharacter-
ized tetrols and triols along with sulfate ester conjugates of monohydroxybenzo(a)pyrenes.

Of the vicinal glycols, C86S1 had the highest 9,10-dihydriodiol formation followed by OC15S1 and PC13. The K-region diol was significantly higher in OC15S1 incubations than in PC13 or C86S1, and this diol represented 38% of the total metabolites (Table 2). The relative amount of the 7,8-dihydriodiol appeared to increase with the increasing ability of EC cells to activate B(a)P but actually reflected a quantitatively decreasing fraction of the total metabolites that were formed by a given EC cell line (e.g., PC13 > OC15S1 ~ C86S1). The dihydriodols were the most abundant class of metabolites found in the organic solvent-soluble fraction, representing 40 to 55% of the total B(a)P metabolites.

Quinones were also present and represented 10 to 14% of the total metabolites formed by the various EC cell lines.

A striking observation was the ability of EC cells to metabolize preferentially B(a)P to the 3-OH derivative relative to the 9-OH-B(a)P. While a relatively constant amount of 3-OH-B(a)P (17 to 23%) accumulated in the extracellular fraction, the amount of the 9-OH-B(a)P derivative decreased from 11% (Table 2, PC13) to 2.9% (Table 2, C86S1). Consequently, the ratio of 3-OH-B(a)P to 9-OH-B(a)P formation varied approximately 4-fold.

The presence of parent compound and ethyl acetate-soluble metabolites within the nuclei of EC cells are also shown in Charts 3 to 6 (bottom). Although metabolite accumulation was limited in the F9 nuclear fraction, as in the extracellular fraction, a distinct peak was resolved in the region eluting prior to the 9,10-dihydriodiol (Chart 3, bottom). For the other EC cell lines examined, it was apparent that tetrols, triols, 4,5- and 7,8-dihydriodiol, quinones, and phenol derivatives of B(a)P, as well as unmetabolized parent compound, were present in the or

Chart 5. Metabolism of B(a)P to ethyl acetate-soluble metabolites found in the medium (top) and in the nuclei (bottom) from OC15S1 embryonal carcinoma cells. 9,10-diol, 9,10-dihydriodiol; 4,5-diol, 4,5-dihydriodiol; 7,8-diol, 7,8-dihydriodiol; 9-OH, 9-OH-B(a)P; 3-OH, 3-OH-B(a)P.

Chart 6. Metabolism of B(a)P to ethyl acetate-soluble metabolites found in the medium (top) and in the nuclei (bottom) from C86S1 embryonal carcinoma cells. 9,10-diol, 9,10-dihydriodiol; 4,5-diol, 4,5-dihydriodiol; 7,8-diol, 7,8-dihydriodiol; 9-OH, 9-OH-B(a)P; 3-OH, 3-OH-B(a)P.

Table 2

<table>
<thead>
<tr>
<th>EC cells</th>
<th>Tetrols/triols</th>
<th>9,10-Dihydriodiol</th>
<th>4,5-Dihydriodiol</th>
<th>7,8-Dihydriodiol</th>
<th>Quinones</th>
<th>9-OH-B(a)P</th>
<th>3-OH-B(a)P</th>
</tr>
</thead>
<tbody>
<tr>
<td>F9</td>
<td>8.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PC13</td>
<td>9.6</td>
<td>18.5</td>
<td>5.2</td>
<td>13.1</td>
<td>12.6</td>
<td>10.1</td>
<td>20.9</td>
</tr>
<tr>
<td>OC15S1</td>
<td>124.0</td>
<td>97.4</td>
<td>446.9</td>
<td>93.8</td>
<td>168.9</td>
<td>52.8</td>
<td>185.3</td>
</tr>
<tr>
<td>C86S1</td>
<td>657.0</td>
<td>619.7</td>
<td>112.2</td>
<td>209.1</td>
<td>318.8</td>
<td>73.9</td>
<td>553.4</td>
</tr>
</tbody>
</table>

MARCH 1981 1107
The 3 B(a)P-diols, no detectable quantities of the 9,10-dihydro-
(Table 3). The amount of extractable dihydrodiols increased
R. Filler and S. Garner-Shinpock
fetal cells have demonstrated significant cytotoxicity in re
were the most abundant class of nuclear metabolites and
were observed in the HPLC chromatograms of F9, PC13,
amounts of tetrols and triols were extracted from the nuclei
metabolites did accumulate, although at a very low level, in the
medium (Table 2), reduced
organ solvent fraction from EC cell nuclei. In contrast to the
metabolite accumulation in the medium (Table 2), reduced
amounts of tetrois and triols were extracted from the nuclei
(Table 3). The amount of extractable dihydrodiols increased
10-fold with their abundance from individual EC cells ranked in
the following decreasing order: C86S1 > OC15S1 > PC13. Of
the 3 B(a)P-diols, no detectable quantities of the 9,10-dihydro-
diol were observed in the HPLC chromatograms of F9, PC13,
or OC15S1 nuclear extracts. However, C86S1, the EC cell line
which accumulated the largest amount of this metabolite in the
medium (Chart 6, top), also had a significant amount of this
dihydrioil in its nuclei. Lastly, the phenol derivatives of B(a)P
were the most abundant class of nuclear metabolites and
represented 32% of the extractable radioactivity. In contrast to
the extracellular accumulation of 3-OH-B(a)P to 9-OH-B(a)P
derivative formation, phenol accumulation in nuclei was main-
tained at a relatively constant ratio of 1.2.

DISCUSSION

Experiments performed with low-passage hamster or mouse
fetal cells have demonstrated significant cytotoxicity in re-
response to increasing concentrations of PAH (6–8, 11). How-
ever, this effect on cell multiplication was observed after long-
term exposure (3 to 6 days). In contrast, OC15S1 and C86S1
cells showed B(a)P-induced toxic effects within the first 24 hr.
Although no toxic effects were observed with F9, PC13, or
PYS when incubated with B(a)P, organic solvent-soluble me-
etabolites did accumulate, although at a very low level, in the
medium from PC13 cells. This apparent lack of cellular toxicity
may be due to the presence of an efficient detoxification
system, the production of low levels of toxic intermediates, or
the inability to metabolize parent hydrocarbon.

HPLC analysis of the organic solvent-soluble metabolites
produced by the more active EC cell lines clearly demonstrates
the production of both K- and non-K-region dihydrodiols, as
well as quinones and phenolic derivatives. In comparison to the
B(a)P metabolic profiles obtained from mouse and hamster
cells (28), EC cells differ markedly in the production of signifi-
cant quantities of the 4,5-dihydrodiol and show an apparent
reorientation in the enzymatic hydroxylation of B(a)P. Instead
of a 2- to 2.5-fold increase in the ratio of 3-OH-B(a)P to 9-OH-
B(a)P formation, as in the case of mouse or hamster cells (28),
there is a 4-fold greater formation of the 3-hydroxy than the
9-hydroxy species (Table 2). This difference in phenol accumu-
lation may be due to a stereoselective specificity of the hy-
droxylation enzyme system or to a preferential conjugation of
the 9-OH-B(a)P. However, this latter alternative appears un-
likely since EC cells are unable, under the present incubation
conditions, to form glucuronic acid or sulfate ester conjugates.°
The metabolite pattern obtained from EC cells is more similar
to microsomal preparations from rat, mouse, and hamster livers
(28). The relative amount of water-soluble to organic solvent-
soluble product formation indicated that there was a 10-fold
greater increase in nonconjugated product accumulation (Ta-
ble 1). This suggests that detoxifying pathways of the more
active EC cells appears to be limiting in its ability to handle
oxygenated derivatives.

Initial experiments indicated that the kinetics of B(a)P uptake
into each of the EC cell lines was very similar and that intra-
cellular saturation occurred within the first hr of exposure to
the hydrocarbon. Although others have shown that a very small
percentage of the intracellular radioactivity was associated with
the nuclear fraction (17), we observed that nuclei, isolated from
EC cells incubated with high specific activity [3H]B(a)P, accu-
mulated >70% of the cellular radioactivity. This large nuclear-
associated radioactivity may, in part, be due to the striking mor-
phology of EC cells which have a high nucleocytoplasmic ratio
(25). The decrease in nuclear radioactivity during long-
term incubations probably reflects a metabolic equilibrium be-
tween intracellular and extracellular compartments and is sup-
ported by the concomitant decrease in ethyl acetate-extractable
radioactivity from isolated nuclei. HPLC analysis of nuclear
metabolites indicates that unmetabolized parent hydrocarbon,
as well as various reactive intermediates, were present. Of
notable interest was the presence of the 9,10-dihydrodiol. It
has been reported that there appears to be a selectivity in the
cellular distribution of the 9,10- and the 7,8-dihydrodiols with
respect to their preferential accumulation in either extracellular
or intracellular compartments (13, 17). We have also observed
this with respect to their extracellular and nuclear distribution.
Whether this difference is attributable to structural features or
to their interaction with cellular macromolecules is currently
not known.

There is an apparent correlation between the inherent differ-
entiational capability of the various teratocarcinoma-derived
cell lines and their biotransformation of xenobiotics. One of
these cell cultures (i.e., PYS-2) is an endodermal derivative
isolated from the in vitro differentiation of OTT6050 stem cells
(16). Although EC cells have tumorigenic potential when in-
jected into syngeneic mice, they can be further characterized
according to the cell type present in the tumor. EC cells which
give rise to tumors containing only stem cells and have appar-
ently lost their differentiational capacity are designated as
nullipotent. F9 is such an EC cell type which almost exclusively
undergoes stem cell renewal but can, under certain conditions,
form a limited amount of primitive endoderm-like cells (29, 34).

° R. Filler and K. J. Lew, manuscript in preparation.
In contrast, PC13 and OC1S1 are pluripotent EC cells (19, 30). Tumors derived from them contain derivatives of all 3 embryonic germ layers. Although PC13 readily differentiates in vivo, it has a restricted in vitro developmental capacity and requires the presence of retinoic acid for limited differentiation (1). The C86S1 EC cell line has both in vivo and in vitro development capacity (19).

Although we are reporting the metabolic characterization of 4 cell lines derived from the OTT6050 and one cell line derived from the 86 teratocarcinomas, we have also examined another 6050 pluripotent EC cell line, 247 DIP/DES (16), as well as a second nullipotent EC cell, SCC1, derived from the spontaneous teratocarcinoma 402 (18). Analogous results were obtained with these 2 cell types as with OC1S1 and F9. It is notable that the pluripotent state is associated with significant metabolic activity and that either with restriction in developmental potential of these embryo-like cells (i.e., F9) or with their developmental commitment to a differentiated phenotype (i.e., PYS), the presence of an active metabolic character is reduced. This would suggest that certain cell types of the developing embryo may be primary sites of metabolite activation and may serve as a point of origin for producing reactive intermediates that interact with adjacent cell types via metabolic cooperativity (9). This could be of importance in considering the ontological relationship between embryonic susceptibility to transforming species and transplacental carcinogenesis (35). Experiments are in progress to test this hypothesis.

In conclusion, the results reported in this study indicate that the inclusion of EC cells in the examination of biotransformation of PAH's to various reactive intermediates complements and extends other investigations utilizing fetal and adult systems. The formation of toxic and transforming derivatives, which may be involved in carcinogenesis, mutagenesis, or teratogenesis, is modulated by the metabolic balance between activating and detoxifying enzyme systems. This complex biochemical relationship, which may vary with the developmental state of the organism, determines overall susceptibility to chemically induced biological alterations. Thus, the EC cell system with its differential characteristics would serve as a useful probe for the elucidation of regulatory parameters involved in these processes.

ACKNOWLEDGMENTS

We especially thank Dr. Liane B. Russell for her encouragement and support of our work and Dr. James K. Selkirk for his time and patience in unraveling the complexities of HPLC technology and PAH metabolism.

REFERENCES


R. Filler, unpublished data.

Metabolism of Benzo(a)pyrene by Murine Embryonal Carcinoma Cells

Ron Filler and Sarah Garner-Shinpock

Cancer Res 1981;41:1104-1109.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/41/3/1104

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.