Comparison of rat hepatocyte and differentiated hepatoma cell line cultures as bio-indicators of CYP 1A1 inducers in urban air

Alessandro Chiarolini, Maria Teresa Donato, Maria José Gómez-Lechón, Mauro Pala, Federico Valerio and Margherita Ferro

Cytochrome P450 1A1 (CYP1A1) enzymatic activity was evaluated in cultured liver cells, and taken as a biological indicator of the presence of inducers of this isoform in urban airborne particulate matter fraction samples. It is known that CYP1A1 inducers can play an important role in the risk of mutagenesis and carcinogenesis by environmental pollution. Aromatic polycyclic hydrocarbons (PAH) from urban air were collected in the city of Genoa (Italy) at two sites on two different days of the year. The objective of the study was to compare the inducibility of cultured rat hepatocytes with that of MH1C1 and FaO rat hepatoma cell lines after exposure to a PAH mixture and to a standard compound, such as benzo[*b*]fluoranthene (B[*b*]F). Cytotoxic effects of the tested concentrations were evaluated by means of 3-[4,5,-dimenthylthyazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase release (LDH) tests, the potency of inducers by ethoxyresorufin-*O*-deethylase (EROD) assay. The results were in agreement in the three cellular systems: after exposure to the PAH mixture, an induction at low concentrations was observed; whereas no induction, but rather a decrease in activity was shown at higher concentrations; instead, the exposure to pure B[*b*]F showed a dose–response relationship in all cells, even at the highest doses. Such a difference between the toxicity of the complex mixture and that of the pure compound could be ascribed to the presence of drug metabolism inhibitors in the mixture, or to interactions between the original components and their metabolites. The finding that the cell lines responded to the CYP1A1 induction in a very efficient way gives further proof of the applicability of this system to environmental biomonitoring.

Keywords; in vitro *metabolism, air pollution, CYP1A1 inducers, liver cell models, environmental biomonitoring.*

Introduction

High levels of air pollutants are considered to be one of the possible causes of cancer (Friberg and Cederlof 1978, Ford and Bialik 1980) and the urban atmosphere in particular is a source of airborne particulates with both mutagenic and carcinogenic potential (Sasaki *et al.* 1987, Tuominen 1988). Such pollutants are mainly represented by complex mixtures of polycyclic aromatic hydrocarbons (PAH), whose relative concentrations can vary according to different conditions, such as the presence of industries, climate, and traffic (Barale *et al.* 1991, Valerio *et al.* 1992). It is well established that PAH are potent carcinogens requiring metabolic activation before they can interact with DNA, cytochrome P450 1A1 (CYP1A1) being the main specific isoform involved in the bioactivation process (Gonzalez 1989). Moreover, PAH are also potent inducers of CYP1A1, which may thus increase the potential incidence of cancer and/or toxicity (Ioannides and Parke 1993).

Analytical techniques for the detection of PAH are costly and time-consuming, particularly when samples may theoretically contain more than 100 different compounds continuously changing in their relative concentrations. Moreover, it is practically impossible to predict the biological effects of complex mixtures, because the interactions can produce not only additive or synergistic effects but also antagonistic effects (Davis and Safe 1988).

The evaluation of drug metabolism in whole animals or humans is not very practical when detailed knowledge on a particular cytochrome P450 is wanted. As an attempt to reduce complexity and as an alternative to animals, cultured cells such as hepatocytes or differentiated hepatoma cell lines can be employed. The hepatoma cell lines expressing a high degree of differentiation, particularly in drug metabolism, offer several advantages over primary hepatocyte cultures: better experimental reproducibility, long-term evaluation of cyto- and/or genotoxic effects and their reversibility, and avoidance of laboratory animal use.

This simple *in vitro* model has been already proposed for the screening of environmental CYP1A1 inducers (Bradlaw and Casterline 1979, Sawyer and Safe 1982, Casterline *et al.* 1983, Sawyer *et al.* 1984). Induction of CYP1A1 in the rat hepatoma cell line H4IIE has been used as an indicator of dioxin-like compounds in the environment (Tillit *et al.* 1991). The mouse hepatoma cell line Hepa 1 was also found to be suitable for screening the CYP1A1-inducing potency of environmental samples (Kopponen *et al.* 1994).

In this study two well differentiated hepatoma cell lines (FaO and MH1C1), that express cytochrome P450 functions similar to that of rat hepatocytes (Donato *et al.* 1994), have been compared with primary cultured rat hepatocytes to detect the presence of CYP1A1 inducers in samples of urban air of the town of Genoa (Italy).

The aim of this study was to evaluate and compare the sensitivity of the two cellular models (hepatoma and hepatocytes), as well as to assess the toxicity of one sample of airborne particulate, containing a mixture of PAH, in comparison with an individual component of the mixture, namely benzo[*b*]fluoranthene (B[*b*]F). Since ethoxyresorufin-*O*-deethylase (EROD) activity is considered to be an indirect measure of the protein corresponding to the CYP1A1 gene (Nebert and Gonzalez 1987), it was chosen as a parameter for the inducing power of both the mixtu

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compound. All cells were also exposed to 3-methylcholanthrene (MC) as a positive control.

METHODS

Materials

Culture media and sera were obtained from Gibco (Paisley, UK); collagenase, diaphorase, NAD, glutamate-pyruvate-transaminase (GPT), ethyoxyresorufin and β-glucuronidase/arylsulphatase from Boehringer Mannheim (Germany); 3-[4,5-dimethylthyazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) and resorufin were purchased from Sigma Chemical Co. (St Louis, USA), acetonitrile (CH_3CN, HPLC grade) from Scharlau S.S. (Barcelona, Spain), benzo[*b*]fluoranthene (B[*b*]F) from Aldrich-Chemie (Steinheim, Germany) and insulin from Novo Nordisk A/S (Denmark). Dimethylsulphoxide (DMSO), methanol and all other reagents were of the highest grade available from Merck (Darmstadt, Germany).

Samples of airborne particulate

Airborne particulate samples (APS) were collected at two sites (A and B) in Genoa, a town with 700 000 residents, situated in the north of Italy. At site A there is a large steel factory with coke ovens, a blast furnace to produce pig iron together with a furnace to produce ingot and sheet steels. At this site two samples were collected on two different days of the year for the analyses (A 1 and A 2). Site B was characterized by the industrial activity of the petroleum harbour, situated about 700 m south to south-east of the sampling site, and by a road with high traffic density; samples were collected at 20 m above the road surface.

APS were collected using a high volume sampler (Metal Work GM WL 2000): about 2000 m^3 of air were filtered in 24 h using glass fibre filters (Gelman Type A/E 20 × 25 cm), retaining particles > 0.3 μm in diameter with 99.9% efficiency. Filters were pre-treated at 400 °C for 60 min to eliminate organic components. Each filter was extracted with cyclohexane in an ultrasonic bath, the PAH fraction was purified by thin layer chromatography and analyses of PAH were performed by capillary gas liquid chromatography with a flame ionization detector according to the procedure described by Barale *et al.* (1991).

Cell cultures

Hepatocytes were isolated from Sprague–Dawley male rats (200–230 g) by perfusion of the liver with collagenase, as previously described (Gómez-Lechón *et al.* 1992). Cellular viability, as assessed by the trypan blue dye exclusion test, was always more than 85%. The isolated hepatocytes, resuspended in Ham F-12/Leibovitz L-15 (1:1 v/v) medium, supplemented with 0.2% bovine serum albumin, 50 μg ml^{-1} streptomycin and 50 mU ml^{-1} penicillin, 10^{-8} M insulin and 2% newborn calf serum, were seeded on fibronectin-coated dishes, and unattached cells removed by changing the medium after 1 h. After 24 h the medium was renewed daily with serum-free medium containing 10^{-8} M dexamethasone.

The rat hepatoma cell lines MH1C1 (passage 82) and FaO (passage 70) were purchased from the American Type Cell Culture Collection (Rockville, MD, USA).

Cells were routinely cultured in Dulbecco's MEM containing 5% foetal bovine serum, 0.1% non-essential amino acids and antibiotics as described above on uncoated dishes. For subculturing, cells were harvested after trypsin/EDTA (0.05%/0.02%) treatment at 37 °C. Cells were used at the 75% monolayer confluency.

Treatments

The hepatocytes were used 24 h after plating and the cell lines when at the pre-confluency. Cells were seeded on 96-well microplates (Nunclon, Nunc, Denmark) and on plastic dishes (area 9.6 cm^2) for cytotoxicity and biochemical determinations, respectively. Cultures were exposed to serum-free medium containing various concentrations of MC, dried PAH fractions or B[*b*]F. The exposure time was 48 h both for cytotoxicity tests and for EROD assay, whereas the time-course of EROD induction was for 24, 48 and 72 h. MC and the PAH mixture were dissolved in DMSO, B[*b*]F in acetonitrile, whereas control cultures were treated with the same concentration of solvents (0.5%). The three samples of PAH were diluted in different amounts of solvent to obtain appropriate stock solutions; final concentrations corresponding to that of B[*b*]F present in the mixture were obtained by dilution with the serum-free medium.

Cytotoxicity and CYP1A1 induction assays

Cell viability was evaluated by two different well-known end-points of cellular damage, as the tetrazolium MTT test and cellular LDH release. The MTT salt was prepared as a stock solution in PBS (5 mg ml^{-1}), filter-sterilized and stored at 4 °C. Before use, it was diluted 1:10 in complete medium. At the end of the treatments, cells were washed twice with PBS and incubated at 37 °C for 3 h with the MTT solution. Then, after removal of the medium and washing with PBS, the formazan precipitate was resuspended in DMSO and the optical density was measured at 570 nm wavelength (Castell and Gómez-Lechón 1992).

The LDH test was performed according to the method described by Ponsoda *et al.* (1991). The assay solution was prepared before use by mixing tris–lactate–MTT buffer (pH 8), NAD solution, GPT suspension and diaphorase. After treatment with PAH, cells were washed with PBS, resuspended in 0.9% NaCl solution and lysed in an ultrasonic bath. Then, each well received the LDH assay solution at 25 °C, the plate was read at 570 nm and the increase in absorbance with time calculated.

EROD enzymatic activity was measured by a fluorimetric assay (Burke *et al.* 1985), as described elsewhere (Donato *et al.* 1993). Monolayers were washed twice with PBS and the assay was initiated by adding the culture medium containing 10 μM dicumarol and 8 μM ethoxyresorufin. The incubation was performed for 30 min at 37 °C and then the reaction was stopped by separating and freezing the incubation medium. To hydrolyse resorufin conjugates, each sample was incubated with crude β-glucuronidase/arylsuphatase (165 Fishman units per ml and 1330 Roy units per ml, respectively) for 2 h at 37 °C; the reaction was stopped by addition of methanol. After centrifugation at 2000 *g* for 10 min, fluorescence of the supernatant was measured at 530 nm excitation and 585 nm emission. The enzymatic activity, compared with that of a standard solution of resorufin, was expressed as pmol of resorufin formed per minute and per mg of cellular protein. Total proteins were determined in accordance with Lowry's method (Lowry *et al.* 1951).

Statistical analysis

Data were expressed as arithmetical mean (with *n* as indicated in each table or graph) of single values and statistical error indicated as standard error of the mean (SEM). When considering two single data, the median between these and the coefficient of variation were analysed. Significance of values was evaluated by the ANOVA test followed by Dunnett's, considered more powerful when comparing treated with control.

Results

In Table 1 the concentrations in 24-h collections of the principal PAH found in the particulate matter obtained from the sampling sites A and B are shown. At site A two samples (A 1 and A 2) were collected, where the second sample was collected 60 days after the first. Differences in the PAH concentrations, measured at the two sites, were due to the characteristics of the sampling sites. The composition of the two samples collected at site A showed considerable differences in the PAH concentration

Samples	[PAH] (ng m^{-3}) FLU	PY	BNT	CYC	BaA	BbF	BkF	BeP	BaP	IP	DBA	BghiP
A 1	22.2	16.3	1.7	1.6	15.5	52.8	23.2	27.0	26.4	18.0	5.4	13.9
A 2	5.4	4.5	1.8	0.6	6.7	17.1	9.4	10.3	7.7	6.4	2.0	5.5
B	0.1	0.3	0.2	0.1	0.2	0.7	0.3	0.5	0.2	0.5	0.1	0.9

Table 1. Daily concentrations (ng m^{-3}) of main PAH measured in airborne particulate samples collected at site A in two different days of the year and at site B.

FLU: Fluoranthene; PY: pyrene; BNT: benzonaftothiophene; CYC: cyclopenta[*c,d*]pyrene; BaA: benz[*a*]anthracene; BbF: benzo[*b*]fluoranthene; BkF: benzo[*k*]fluoranthene; BeP: benzo[*e*]pyrene; BaP: benzo[*a*]pyrene; IP: indeno[*1,2,3-c,d*]pyrene; DBA: dibenzo[*a,h*]anthracene; BghiP: benzo[*g,h,i*]perylene.

times, which were probably due to the different meteorological conditions. A 1 was collected with prevailing wind direction from the South and in these conditions the high volume sampler retained much of the fall-out of the emission from the coke ovens. A 2 was collected with prevailing wind direction from the East, so the sample was only partially influenced by the coke emissions; site B was influenced principally by the traffic, since the emissions of the petroleum harbour activity were not collected with the high volume samplers, due to their high volatility, as previously described (Valerio *et al.* 1992).

The relative composition of PAH also changes according to the characteristics of emitting sources. For example, the mean [IP]/[BghiP] ratio in automobile emissions was found to be ≈0.4 (Grimmer 1983). This ratio, as calculated in sample B, gave a similar value: 0.5; but those calculated for the A samples gave different values: A 1 = 1.29, A 2 = 1.16.

From the analysis of the PAH fraction purified by thin layer chromatography, we obtained chromatograms with too many peaks to allow for accurate identification. The 12 identified PAH represent 40–70% of the total PAH fraction (data not shown).

Despite a number of endpoints, including mutagenicity in the Ames *Salmonella* bioassay, genotoxicity in prophage and hepatotoxicity *in vivo* (De Marini *et al.* 1987), samples of complex mixtures with very similar chemical characterization may vary greatly in biological activity. The evaluation of cytotoxicity was performed only on sample A 1 with one end-point, due to the low availability of such material. Thus, Table 2 shows the results of the cytotoxicity of the PAH mixture to the three cellular populations, as evaluated by the MTT test. No significant loss of viability was found in the treated cultures with respect to the controls, except for the highest dose tested in the MH1C1 cell line, where there was a reduced viability, down to 82%.

B[*b*]F was chosen as an individual compound to be compared with the mixtures, since it was the major PAH component. The cytotoxicity of B[*b*]F alone (Table 3), as evaluated with MTT and LDH tests, revealed some significant effects on the cell lines only at the highest concentrations (30 and 40 μM), whereas at lower concentrations the percentage of surviving cells was not decreased significantly.

In Table 4 basal EROD activity in all treated cultures is compared with solvent controls and positive induction controls. No significant changes were observed between basal controls and solvent controls, whereas 2 μM MC induced this

Treatment	Hepatocytes	MH1C1	FaO
None	98±2	98±0	106±1
DMSO 0.5% v/v (solvent control)	100	100	100
PAH mixture[a]			
0.07 μM	99±3	88±2	103±1
0.2 μM	92±1	88±2	98±1
0.3 μM	92±2	91±2	99±2
0.7 μM	91±1	104±1	104±2
1.4 μM	100±6	105±4	107±3
2.6 μM	108±5	104±8	109±3
5.6 μM	110±8	82±2[b]	108±4

Table 2. Cytotoxicity (% viability vs control) of the PAH mixture (from sample A 1 airborne particulate) as evaluated by MTT test in cultured rat hepatocytes, MH1C1 and FaO cell lines.

One experiment was performed with at least three to five wells for each dose. The time of exposure was 48 h. Values are expressed as percentage (mean±SEM) of surviving cells with respect to solvent controls. Statistical analysis did not reveal any significant difference among results, except where indicated.

[a] Concentrations are referred to those of B[*b*]F pure component.

[b] $p < 0.01$ versus solvent control.

Treatment	Hepatocytes MTT test	Hepatocytes LDH test[a]	MH1C1 MTT test	MH1C1 LDH test	FaO MTT test	FaO LDH test
None	106±3	105±10	105±2	109±11	104±2	104±2
CH_3CN 0.5% v/v (solvent control)	100	100	100	100	100	100
B[*b*]F						
5 μM	93±0	101±7	97±4	100±4	96±4	97±6
10 μM	93±1	110±5	90±1	95±3	92±1	91±4
20 μM	90±4	94±5	89±2[b]	82±8	90±3	90±2
30 μM	86±4	96±7	89±4[b]	74±2[b]	85±3[c]	84±0[b]
40 μM	88±8	91±11	76±3[c]	61±7[c]	84±2[c]	81±4[c]

Table 3. Cytotoxicity (% viability vs control) of B[*b*]F assayed by MTT and intracellular LDH tests in cultured rat hepatocytes, MH1C1 and FaO cell lines. Values are mean±SEM among percentages of survived cells with respect to solvent controls from three independent experiments, each performed with at least five wells for each dose of B[*b*]F. Exposure time to B[*b*]F was 48 h. Statistical analysis did not reveal any significant difference among results, except where indicated.

[a] Values from a single representative experiment in five wells.

[b] $p < 0.05$

[c] $p < 0.01$ (versus solvent control).

Treatment	Hepatocytes	MH1C1	FaO
None	1.2±0.2 (9)	2.3±0.3 (10)	2.0±0.2 (9)
DMSO 0.5% (v/v)	1.5±0.1 (3)	7.2±0.2 (3)	3.9±1.0 (3)
CH_3CN 0.5% (v/v) (solvent controls)	1.7±0.2 (11)	3.5±0.5 (10)	2.4±0.3 (12)
MC2 μM (positive induction control)	15.2±1.8 (11)	32.2±2.3 (11)	14.5±0.9 (13)

Table 4. EROD activity (pmol mg^{-1} min^{-1}) in cultured rat hepatocytes, MH1C1 and FaO cell lines in basal conditions, solvent controls and after exposure to 3-methylcholanthrene as positive control for monooxygenase induction.

Values are mean±SEM of independent experiments (numbers indicted in parentheses), each performed in two dishes.

Cells were treated for 48 h.

Values obtained from MC-treated samples were statistically significant ($p < 0.01$) versus all other treatments. Solvent controls versus basal controls were not significant.

DMSO: dimethylsulphoxide; MC: 3-methylcholanthrene.

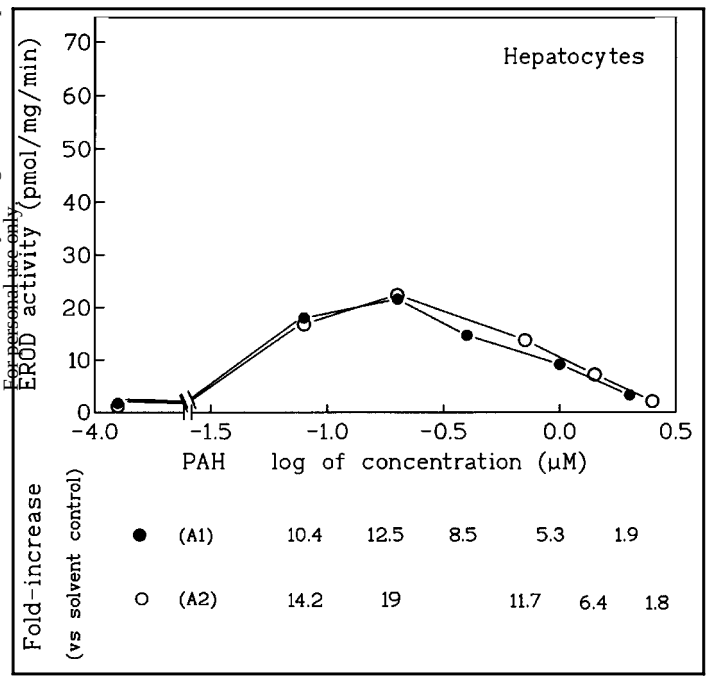

Figure 1. Dose–response curves for EROD induction by PAH mixture in monolayer cultures of rat hepatocytes. Two independent experiments (●): sample A 1; ○: sample A 2) were performed in two dishes. Cells were exposed to an aliquot of the mixture containing a given B[*b*]F concentration as described in Methods. Coefficients of variation for each pair of dishes were always less than 15%. For each experiment, the fold-increases with respect to the solvent control are reported below the *x*-axis.

CYP1A1-dependent activity about 10-, 4.5- and 4-fold in hepatocytes, MH1C1 cells and FaO cells, respectively.

When the PAH mixture extracted from airborne particulate was incubated with hepatocyte cultures for 48 h (Figure 1), an increase of EROD activity was observed with both mixtures at low concentrations with a peak of 12.5-fold (A 1) and of 19-fold (A 2) at 0.2 μM, whereas at higher doses, a sharp decrease in basal activities was observed. It should be pointed out that the concentrations with inhibitory effects were not cytotoxic, as measured by MTT and LDH leakage (Table 2).

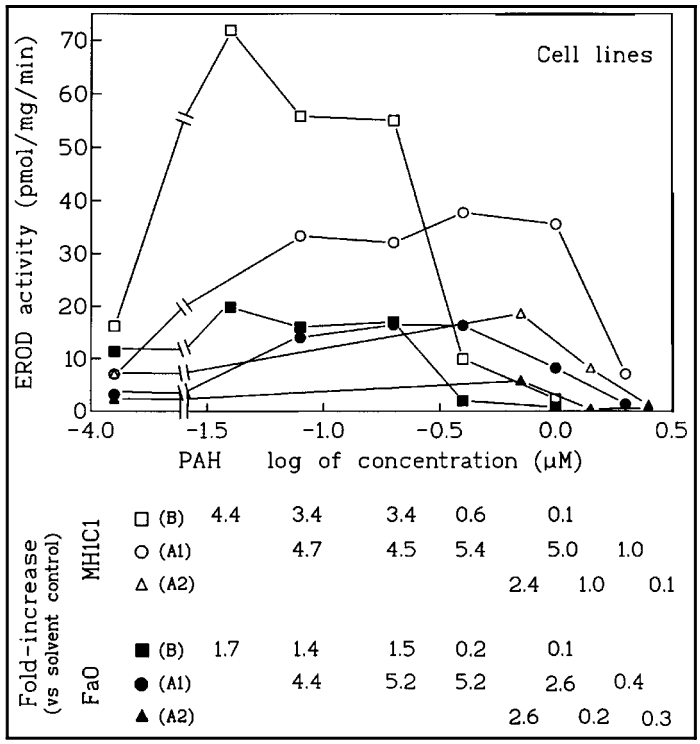

Figure 2. Dose–response curves for EROD induction by PAH mixture in monolayer cultures of MH1C1 and FaO hepatoma cell lines. Three independent experiments with samples from sites B (■), A 1 (●) and A 2 ( ) were performed, each in duplicate. Cells were exposed to an aliquot of the mixture containing a given B[*b*]F concentration as described in Methods. Coefficients of variation for each pair of dishes were always less than 15%. For each experiment, the fold-increases with respect to the solvent control are reported below the *x*-axis.

The dose–response curves for the induction of EROD activity by the PAH mixtures in both MH1C1 and FaO cell lines (Figure 2) were similar to those of hepatocytes, as regards the increase at the lower and the decrease at the higher concentrations. Comparing results at the same concentration, it should be pointed out that the results differ according to the range of concentrations and according to the cell line. In fact, in the low-concentration range site A 1 and B samples were able to induce EROD activity more than site A 2 samples, whereas in the high-concentration range the site A 1 only remained at high levels, with a net drop of activity in site B. Of the two cells lines, MH1C1 was much more sensitive than the FaO line to the components of the mixtures, especially to those contained in samples B and A 1.

When cultures were exposed to the individual chemical, B[*b*]F, the dose–response curves showed no decrease in EROD activity at high concentrations (Figure 3), but a plateau was reached at the maximal induction level of 9.6-fold, 17.4-fold and 9.2-fold for hepatocytes, MH1C1 and FaO cells, respectively. Even if the cytotoxic doses were reached in this case, no decrease in the activity was observed.

The time-course of EROD induction showed a certain difference between hepatocytes and cell lines (Figure 4), in that the latter did not show the same linear increase of activity with time as shown by hepatocytes.

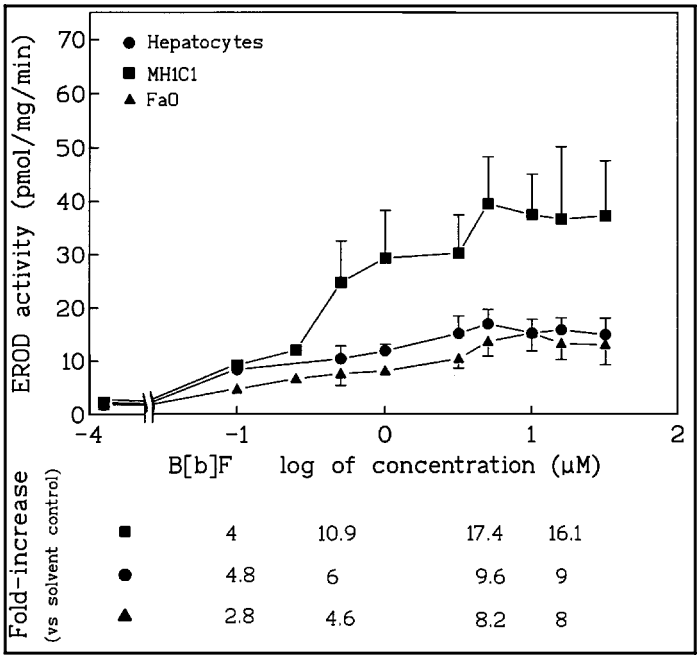

Figure 3. Dose–response curves for EROD induction by pure B[*b*]F in rat hepatocytes, MH1C1 and FaO cell lines. Values are mean±SEM of three independent experiments, set in two dishes for each concentration. Values were significant ($p < 0.01$ for hepatocytes, $p < 0.05$ for the two cell lines) at doses > 3 μM. The fold-increases with respect to the solvent control are reported below the *x*-axis.

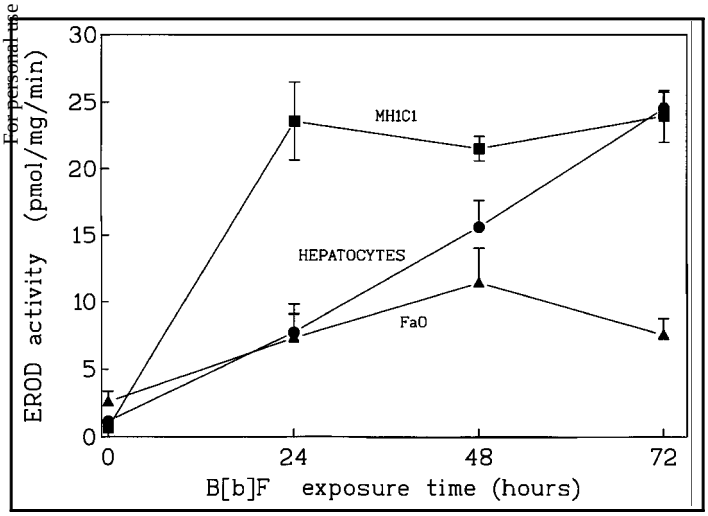

Figure 4. Time course of EROD induction in cultured rat hepatocytes, MH1C1 and FaO cell lines by pure B[*b*]F. Three independent experiments were performed in two dishes exposed to B[*b*]F, 15 μM. Values are mean±SEM.

Discussion

Hepatoma cell lines retaining the expression and inducibility of cytochrome P450 are increasingly used in the biomonitoring of the environment (Sawyer and Safe 1982, Sawyer *et al.* 1984, Kärenlampi *et al.* 1989, Kärenlampi and Törrönen 1990, Tillit *et al.* 1991, Kopponen *et al.* 1994). The results of this study are in agreement with the concept that liver cell cultures can be usefully applied in the assessment of chemical risk. In particular, this model is suitable for the detection of inducers of CYP1A1.

The low availability of the samples did not allow extensive cytotoxicity determinations, but since cytotoxic effects were very low or absent after treatment of the cells with the A 1 sample (Table 2), which contained much more PAH than the two other samples (Table 1), these latter samples should also not be cytotoxic. From our findings it is clear that all the three cellular systems employed are able to respond to the inducers of cytochrome P450, with increased levels of EROD activity. The FaO cell line exhibited an induction pattern rather similar to that displayed by normal hepatocytes, whereas the MH1C1 cell line showed a more marked increase of enzyme activity, especially with the two samples from sites A 1 and B (Figure 2). This cell line was studied in the past for its differentiated phenotype (Thompson *et al.* 1966, Tashjian *et al.* 1970), comprising cytochrome P450 expression (Ferro *et al.* 1984, 1987) and the capability to undergo metabolism of lipid-peroxidation-derived aldehydes (Ferro *et al.* 1988, Canuto *et al.* (1993), and present findings confirm the suitability of this cell line to toxicological applications.

The differences observed between the inducing power of the samples from sites A 1 and A 2, which were shown after exposure of the two cell lines (figure 2), but not by treatment of hepatocytes (Figure 1), might be explained on the basis of the higher concentrations of PAH at site A 1. However, why the same difference was not seen in hepatocytes and why the site B sample, which contained lower concentrations of each PAH (Table 1), so markedly induced EROD activity in MH1C1 cells must still be explained. One explanation for the latter phenomenon could be, speculatively, that one or more component(s) of the mixture is(are) more active at low than at high concentrations. Moreover, it is evident from the results shown in Figures 1 and 2 that the inducibility of EROD activity is not linear with the concentration of the mixture itself. In fact, after an increase of activity, a constant decrease was observed at concentrations (referred to B[*b*]F) higher than 0.3μM. Even if admitting that the lack of data about sites A 2 and B cytotoxicity limits the understanding of the results, the finding that with pure B[*b*]F cytotoxic concentrations were not inhibitory (Figure 3), and with PAH mixture non-cytotoxic concentrations were inhibitory, strongly suggests that the effect cannot be due to cytotoxicity (Figures 1 and 2). The decrease of EROD activity observed at the highest concentrations could be ascribed to the presence in the mixture of inhibitors acting at the higher doses, or to complex interactions between the metabolites of the original component impairing at a certain level the mechanism of induction. This fact should be kept in mind when establishing the ranges of concentration to be tested, since at the highest concentrations the test system may not show a net effect.

However, all three cell cultures responded in a dose-dependent way to the main individual component of the mixture, B[*b*]F, the MH1C1 cell line being again the best 'responder', as in the case of the treatment with PAH mixture (Figure 3).

The time-course of B[*b*]F inducing potency also showed more pronounced effects in MH1C1 cells than in hepatocytes and FaO cells, even if the monooxygenase activity increased during the whole time in normal hep

Therefore, for this reason, at later time points, hepatocytes might be preferable as a screening test to the cell lines. On the other hand, it must be pointed out that hepatocyte primary cultures have generally a shorter durability, as a cell culture system, than cell lines. Alternatively, the hepatocyte cultures may be modified with time, and therefore become more sensitive to metabolic inducers. With respect to the practical applicability of hepatocyte cultures in routine testing, the methods have been recently reviewed, and a number of recommendations listed, to overcome various problems such as the phenotypic instability of this model (Blaauboer *et al.* 1994).

Our findings give further evidence in favour of the applicability of *in vitro* systems and suggest that MH1C1 and FaO hepatoma cell lines are suitable for the screening of CYP1A1 inducers as an alternative model to hepatocytes. It should be pointed out that, although this cytochrome P450 isoform represents only a small part of the whole xenobiotic biotransformation, it plays an important role in toxicity, mutagenicity, teratogenicity and carcinogenicity of environmental chemicals.

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