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# PRODUCTION OF A NOVEL RECOMBINANT CELL LINE FOR USE AS A BIOASSAY SYSTEM FOR DETECTION OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN-LIKE CHEMICALS

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**Abstract**—Exposure to specific halogenated aromatic hydrocarbons (HAHs), such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), can produce a wide variety of species- and tissue-specific toxic and biological effects. The presence of HAHs in environmental samples as complex mixtures has made it difficult to predict the biological and toxic potency of these chemicals. We have used aspects of the molecular mechanism of action of these chemicals to develop a species-specific bioassay system for detection of bioactive HAHs in complex mixtures. Here we describe construction and utilization of a recombinant expression vector that responds to these HAHs with the induction of an easily measurable gene product, heat-stable human placental alkaline phosphatase (PAP). This vector contains the PAP gene under TCDD-inducible control of four dioxin-responsive DNA enhancer elements. HAH-inducible expression of PAP from the recombinant vector occurs in a dose- and Ah-receptor- (AhR-) dependent manner. Stable transfection of this vector into mouse hepatoma cells has produced a novel cell line in which AhR-dependent induction of gene expression can easily be measured. This transfected cell line can readily be used for detection and relative quantitation of AhR agonists in complex mixtures of environmental and biological samples and for identification and characterization of novel AhR agonists.

**Keywords**—2,3,7,8-Tetrachlorodibenzo-*p*-dioxin TCDD Dioxin Ah receptor Halogenated hydrocarbons

## INTRODUCTION

Halogenated aromatic hydrocarbons (HAHs), such as polychlorinated dibenzo-*p*-dioxins, biphenyls, and dibenzofurans, represent a group of compounds that cause toxic effects at concentrations that can occur in the environment [1–5]. HAHs have been identified worldwide in a variety of wildlife, domestic, and human tissues as well as in food, water, and soil samples [1,6–8]. Because of their ubiquitous distribution, resistance to biological and chemical degradation, toxicity, and potential for bioaccumulation in the food chain, HAHs have generated considerable concern. Because exposure to and bioaccumulation of HAHs have been observed to produce a wide variety of species- and tissue-specific toxic and biological effects, such as birth defects, liver toxicity, impaired immune function, and cancer [9,10], the development of techniques for the identification and quantitation of these compounds in various samples is of importance. However, in the environment HAHs are present as complex mixtures rather than individual congeners. This has complicated their detection and made accurately predicting the biological and toxic potency of these mixtures difficult in animals at risk [2].

Instrumental techniques exist for the detection of minute quantities of HAHs; however, these procedures can be costly and time-consuming, particularly when samples may theoretically contain up to 209 different PCB, 135 different polychlorinated dibenzofuran (PCDF), and 75 different polychlorinated dibenzo-*p*-dioxin (PCDD) isomers and congeners [2]. Furthermore, for a number of biologically active compounds neither routine methods nor authentic standards are available. Even for the routinely measured compounds, a number of congeners are not completely resolved by routine analysis. In addition, even if reliable congener-specific concentrations of HAHs in tissues can be determined, it is nearly impossible to predict their biological effects because the toxicities of PCB, PCDF, and PCDD congeners vary tremendously, and interactions among various HAHs have exhibited nonadditivity, including potentiation or antagonism, depending on the compounds present and their relative concentrations [2,11,12].

Numerous studies of the mechanism of action of HAH congeners have revealed that most of the critical and sensitive toxic and biological responses are mediated by a soluble intracellular protein, the Ah receptor (AhR), to which these chemicals bind with high affinity [2,9,10]. After ligand binding, the HAH:AhR complex undergoes transformation into its DNA-binding form and translocates into the nucleus [13,14]. The transformed HAH:AhR complex associates with a specific DNA sequence, the dioxin-responsive enhancer (DRE), resulting in transcriptional activation of adjacent responsive genes [13,15–17]. As the mechanism of action of HAHs is mediated via the AhR, calculation of the relative biological/toxicological potency of complex mixtures

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of HAHs can be conducted by measuring the ability of the mixture to induce a particular AhR-dependent response. The induction of cytochrome P450-IA1-dependent ethoxyresorufin-*O*-deethylase (EROD) activity in rat hepatoma (H4IIE) cells in culture is one such response that has been examined in detail [2,4,18,19]. The results of these studies have revealed a strong correlation between the induction of EROD activity, in this cell line in vitro, and the toxicity of various HAH congeners and HAH mixtures in rats in vivo [1,2,10,20]. Consequently, this technique has been used as a bioassay to evaluate the relative biological/toxicological potencies of complex mixtures of HAHs [1,2,18–20].

Because several recent studies have demonstrated significant species differences in the AhR relative to its ligand-binding affinity, specificity, and physicochemical properties [21–26], the rat H4IIE bioassay may not be an appropriate model system for assessment of the relative biological potency of HAHs in all species. Our recent studies have revealed that although the AhR may vary among species, the specific DNA recognition site of the AhR (the DRE) as well as the nucleotide-specific DNA-binding of the HAH:AhR complex is highly conserved [27,28]. Here we report the construction and use of a recombinant plasmid vector that contains a reporter gene (human placental alkaline phosphatase [PAP]) whose expression is inducible by AhR ligands and whose enzymatic activity can be rapidly and inexpensively measured in transfected cells. This vector can be stably transfected into a variety of AhR-containing cell lines to produce a variety of species- and tissue-specific bioassay systems for use in the detection and relative quantitation of bioactive HAHs.

## MATERIALS AND METHODS

### *Materials*

Molecular biological enzymes were purchased from New England Biolabs (Beverly, MA) or Bethesda Research Laboratory (Gaithersburg, MD). Polybrene, benzo[*a*]pyrene, β-naphthoflavone, and 3-methylcholanthrene were from Aldrich Chemical Co. (Milwaukee, WI); dexamethasone from Sigma Chemical Co. (St. Louis, MO); and the antibiotic G418 from Gibco/BRL (Grand Island, NY). TCDD and 2,3,7,8-tetrachlorodibenzofuran were obtained from S. Safe (Texas A&M University, College Station, TX), and 3,3′,4,4′-tetrachlorobiphenyl, 3,3′,4,4′,5-pentachlorobiphenyl, and 2,2′,4,4′,5,5′-hexachlorobiphenyl were from Accustandard (New Haven, CT). These compounds are extremely toxic substances and were handled with special precautions, including the use of disposable benchtop paper, gloves, plasticware, and glassware. The plasmids pMcat5.9 and pMcat5.0 were obtained from J.P. Whitlock, Jr. (Stanford University, Stanford, CA) and pSVoApap from T. Kadesch (University of Pennsylvania, Philadelphia, PA).

### *Expression vector construction*

To construct the HAH-inducible expression vector pMpap1.1 (Fig. 1), a 1,810-base pair (-bp) HindIII fragment was isolated from the plasmid pMcat5.9 [29] and ligated into the HindIII site of the plasmid pSVoApap [30], immediately upstream of the PAP gene. This HindIII fragment contains a modified mouse mammary tumor virus

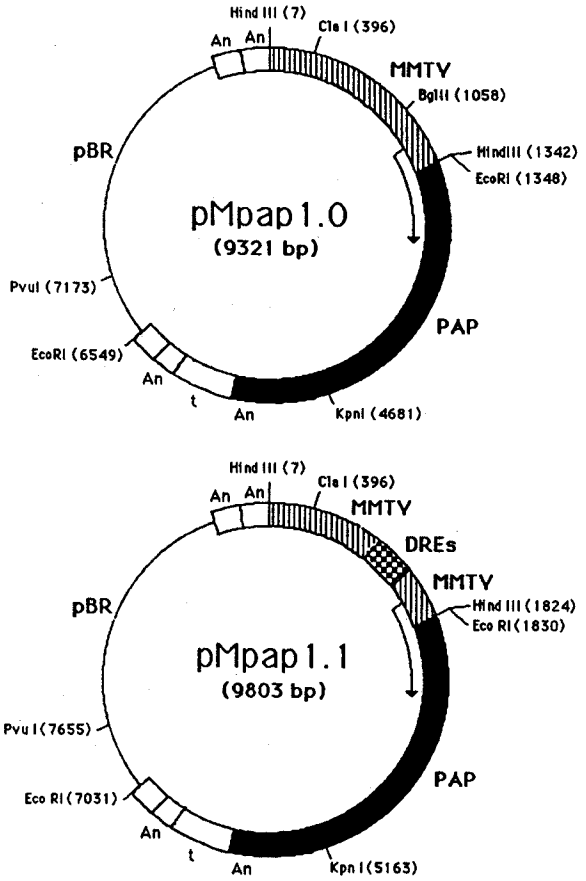


Fig. 1. Structure of the expression vectors pMpap1.0 and pMpap1.1. The vectors contain the human placental alkaline phosphatase (PAP) gene (filled black) under control of the MMTV (hatched) viral promoter. The DREs (checkered) directly upstream of the promoter confer HAH responsiveness on the viral promoter and PAP gene of pMpap1.1. The pBR322 portion of the plasmids is denoted by a line and the transcription start site by an arrow. *An* and *t* (unfilled) indicate the position of poly (A) terminator signals and the SV40 small tumor antigen intron, respectively.

(MMTV) long terminal repeat and viral promoter but lacks the endogenous glucocorticoid-responsive elements [31]. In addition, a 482-bp fragment containing four functional DREs, isolated from the 5′- flanking region of the murine CYP IA1 gene, was subcloned directly upstream of the MMTV viral promoter [29]. The resultant vector, pMpap1.1, contains the MMTV viral promoter and PAP gene under HAH-inducible control of four DREs. The control vector, pMpap1.0, is identical to pMpap1.1, except that it lacks the 482-bp DRE-containing DNA fragment (Fig. 1) and is thus not HAH-inducible. Recombinants were isolated as ampicillin-resistant colonies in *Escherichia coli* DH5 and the orientation of the insert confirmed by restriction analysis.

### *Cell cultures, transfection, chemical treatment, and cell harvest*

Wild-type and variant mouse hepatoma (Hepalc1c7) cells (Hepal) were obtained from J.P. Whitlock, Jr., and grown

as previously described [32]. Confluent plates of cells were transfected using polybrene as previously described [33]. In transient transfection experiments, cells were transfected with the indicated vector (15 μg) and allowed to grow for 48 h, followed by the addition of DMSO (1 μl/ml) or TCDD (1 nM) in DMSO. After further incubation for 24 h, the cells were harvested and assayed for PAP activity. In stable transfection experiments, cells were cotransfected with the vectors pMpap1.1 and $pSV_2neo$ (15 μg of each) as described above. Following 24 h of growth in nonselective medium, the transfected cells were split one to 10 and replated in selective medium containing the antibiotic G418 (1 mg/ml). After about two weeks of growth in selective medium, individual cell colonies were identified and cloned. Stable cell clones were exposed to TCDD (1 nM) for 24 h, transferred onto ice, the media removed, and the plates rinsed twice with cold TBS buffer (50 mM Tris-HCl, pH 7.5, 154 mM NaCl, and 1 mM $MgCl_2$). Cells were harvested by scraping, collected in 1 ml of TBS, transferred into a microfuge tube, and pelleted at 1,500 *g* for 10 min. The supernatant was removed, and the cell pellet was assayed for PAP activity immediately or stored frozen at −80°C until use.

*PAP bioassay*

Pelleted cells were assayed for PAP activity as described by Henthorn et al. [30]. The cell pellet was resuspended in 300 μl TBS and sonicated (25 pulses using a Heat Systems-Ultrasonics sonicator [Farmingdale, NY, model W-225R set at 60% duty cycle and output control of seven]), followed by heating at 65°C for 30 min (to inactivate endogenous alkaline phosphatase [AP] activity). After centrifugation for 10 min in a microfuge, aliquots (30 μl) of the heated cell sonicate supernatant were added to 1 ml reaction mixture followed by incubation at 37°C for the desired time. The reaction mixture consisted of a fresh solution of substrate (5 mM *p*-nitrophenyl phosphate [PNPP]) in diethanolamine (DEA) buffer (1 M DEA, pH 9.85, 0.28 M NaCl, and 0.5 mM $MgCl_2$). After the incubation period, the samples were pelleted at 4°C and the amount of *p*-nitrophenol formed in the supernatant determined by measurement of the absorbance at 405 nm relative to reaction mixture alone. In the standard assay, PAP activity was expressed as the number of nanomoles of *p*-nitrophenol formed per hour per milligram protein, based on an extinction coefficient of 18.5 absorbance units per millimole per milliliter at 405 nm. Protein concentrations of the cell sonicates were measured by the method of Bradford [34] using BSA as the standard.

## RESULTS

*Transient transfection experiments*

Previous studies have demonstrated that DRE sequences can confer HAH responsiveness on a heterologous promoter and gene [15,33,35]. Transient transfection of the reporter constructs pMpap1.0 or pMpap1.1 into wild-type and variant Hepal cells [32,33,36]; the results of subsequent exposure to TCDD are shown in Table 1. TCDD treatment of wild-type Hepal cells transiently transfected with pMpap1.0 (which lacks DREs) failed to induce PAP activity, whereas a significant induction of PAP activity (34-fold) was observed in wild-type cells transfected with the DRE-containing vector pMpap1.1. These results demonstrate the TCDD-dependent nature of the induction response. In addition, because TCDD treatment failed to induce PAP activity in pMpap1.1-transfected variant Hepal cells containing a defective AhR that failed to bind to the DRE with high affinity [13,36], induction of the PAP gene by TCDD is AhR dependent. The above results, combined with the ability of TCDD to induce PAP activity (from pMpap1.1) in several other cell lines (MCF7, HepG2, H4IIE, and GPC16 [Table 2]), demonstrate the utility of this reporter construct in cell lines from other species and tissues (human breast, human liver, rat liver, and guinea pig intestine, respectively).

Table 1. Effect of TCDD on PAP activity in wild-type and variant mouse hepatoma (Hepa1c1c7) cells transiently transfected with the expression vectors

| Cell type | Vector | Treatment | PAP activity (nmol/h/mg protein) |
|---|---|---|---|
| Wild-type cells | pMpap1.0 | DMSO | 0.07 ± 0.11[a] |
| | | TCDD | 0.16 ± 0.10 |
| | pMpap1.1 | DMSO | 0.21 ± 0.23 |
| | | TCDD | 7.19 ± 0.32[b] |
| Variant cells | pMpap1.1 | DMSO | 0.11 ± 0.05 |
| | | TCDD | 0.22 ± 0.16 |

[a]Values represent the mean ± SD of triplicate transfections.
[b]Value is significantly different from DMSO-treated cells at $p < 0.01$, as determined by Student's *t* test.

*Stable transfection experiments*

Because Hepal cells transiently transfected with pMpap1.1 can respond to TCDD with the induction of PAP activity, we prepared Hepal cells stably transfected with this vector for further studies. Cells were cotransfected with pMpap1.1 and $pSV_2neo$ and, after two to three weeks of growth in selection media (G418), at least 50 single-cell clones were identified, 20 of which were isolated for further characterization. Because resistance of the isolated clones to the antibiotic (G418) indicates only that each contains the neomycin (neo) gene, the presence of functional (inducible) pMpap1.1 DNA was confirmed by measurement of TCDD-inducible PAP activity in each clone. Isolated clones were grown and conflu-

Table 2. TCDD-inducible PAP activity in several cell lines transiently transfected with the expression vector pMpap1.1

| Cell line | PAP activity (nmol/h/mg protein) | |
|---|---|---|
| | DMSO | TCDD |
| HepG2 | 0.07 ± 0.05[a] | 3.9 ± 0.79[b] |
| MCF7 | 0.12 ± 0.10 | 2.3 ± 1.2[b] |
| H4IIE | 0.26 ± 0.19 | 4.1 ± 0.63[b] |
| GPC16 | 0.15 ± 0.11 | 1.3 ± 0.32[b] |

[a]Values represent the mean ± SD of triplicate transfections.
[b]Value is significantly different from DMSO-treated cells at $p < 0.01$, as determined by Student's *t* test.

ent plates of cells subsequently incubated in the absence or presence of TCDD for 24 h. After the incubation, cells were harvested and assayed for PAP activity. Of the 20 isolated clones, 14 exhibited TCDD-inducible PAP activity. Only one (T13) exhibited TCDD-inducible responsiveness for an extended period in culture (over one year), whereas the remaining clones lost inducible activity throughout this period (data not shown). All subsequent studies were carried out with the Hepal T13 clone.

*Characterization of the TCDD-responsive cell clone*

The dose-dependent nature of PAP induction in T13 cells was determined by incubation of the cells with several concentrations of TCDD and measurement of PAP activity after 24 h (Fig. 2). Induction of PAP activity in the T13 cells was dose dependent, with a maximal induction at 1.0 nM TCDD (50-fold), and ED50 of about 0.35 nM and a minimal detection limit (at a 95% C.I.) of 0.1 nM (0.03 ppb) TCDD (Fig. 2B). To determine the time course of induction, cells were treated with 1 nM TCDD and PAP activity was determined at various times up to 48 h postexposure (Fig. 3). TCDD-inducible PAP activity in T13 cells was detectable at the earliest examined time point (2 h) and increased linearly over 48 h.

Under the conditions of the assay, PAP substrate was saturating, and a linear correlation between PAP activity and protein concentration was observed (Fig. 4). In addition, unlike some reporter enzymes, PAP is relatively stable. Its stability is demonstrated by its resistance to thermal inactivation and by the consistent PAP activity observed upon continual incubation with substrate for up to 7 d at 37°C (Fig. 5), as well as at 20 and 4°C (data not shown).

Because the T13 recombinant cell line was to be used for detection of various AhR ligands, we examined the ability of various compounds to induce PAP activity in T13 cells (Fig. 6). Between 55- and 100-fold induction of PAP was observed in T13 cells incubated with TCDD, 2,3,7,8-tetrachlorodibenzofuran, 3-methylcholanthrene, benzo[*a*]pyrene, 3,3′,4,4′-tetrachlorobiphenyl, and 3,3′,4,4′,5-pentachlorobiphenyl but not in those exposed to dexamethasone or 2,2′,4,4′,5,5′-hexachlorobiphenyl. These results are consistent with previously published results on the ability of each of these chemicals to bind to the AhR and induce gene expression [2,9,10,21].

## DISCUSSION

Studies of structure–activity relationships have suggested that the AhR is involved in many of the toxic and biological actions of TCDD and related HAHs, in addition to its role in mediating the induction of cytochrome P450 1A1 [2,9,10]. As a result, induction of P450-1A1-dependent EROD monooxygenase activity, particularly in rat H4IIE cells, has been used to provide a relative measure of the concentration and biological potency of dioxin-like chemicals in complex mixtures in a given sample extract [1,2,4,5,18,20]. Although the H4IIE bioassay system appears to be suitable for detection of these chemicals in sample extracts, its appropriateness as a model system for HAH responsiveness in all species remains to be determined. Recent studies have reported differences among species and tissues in ligand-binding affinity, ligant specificity, and physicochemical properties of the AhR [21–26], as well as significant differences in responsiveness to TCDD and related HAHs [9,10]. The availability of HAH-inducible bioassay systems in cell lines from other species and tissues would allow detailed examination of these differences. Consequently, using recombinant techniques, we have constructed an HAH-inducible reporter plasmid, pMpap1.1, which can be stably transfected into AhR-containing cells to produce a variety of sensitive species- and tissue-specific bioassay systems. The HAH-inducible reporter

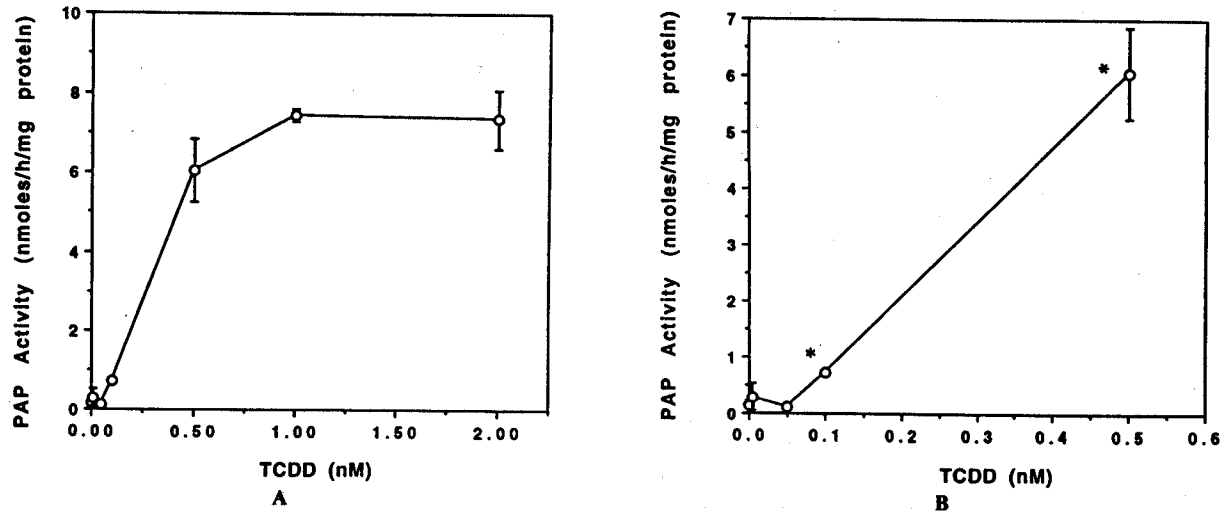


Fig. 2. Dose–response curve for induction of PAP activity in clone T13 by TCDD. (**A**) Confluent plates of T13 cells were incubated with the indicated concentration of TCDD for 24 h, and PAP activity was determined in cell sonicates as described in "Materials and Methods." The values represent the mean ± SE ($n = 4$). (**B**) Enlargement of the low end of the dose–response curve from part A. The asterisks indicate a significant difference ($P \leq 0.05$, as determined by Student's $t$ test) from control (noninduced) cells.

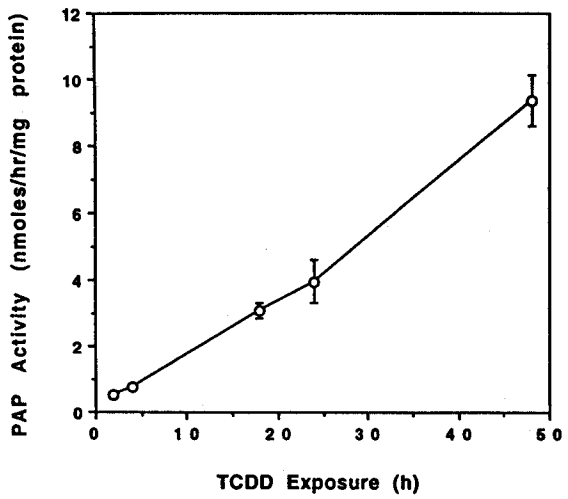


Fig. 3. Time course for induction of PAP activity by TCDD. Confluent plates of cells (80% confluence) were incubated with TCDD (1 nM) for indicated times, after which PAP activity in cell sonicates was determined. Values represent the mean ± SE ($n = 4$).

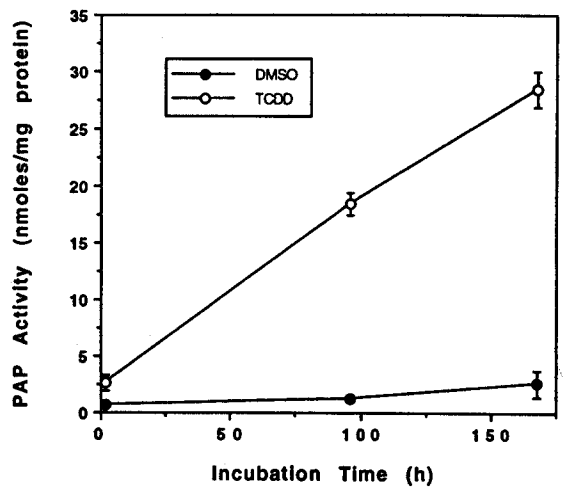


Fig. 5. Stability of PAP enzymatic activity. Confluent plates of cells were incubated with DMSO or TCDD (1 nM) for 24 h and cell sonicate prepared. The cell sonicates were incubated with the PAP assay reagent at 37°C, and overall PAP activity was determined at the indicated time points as described in "Materials and Methods." Values represent the mean ± SE ($n = 4$).

plasmid described here contains the human heat-stable PAP gene and the MMTV viral promoter under HAH-inducible control of four DREs.

The PAP expression plasmid vector was selected for these studies because this vector/gene has several distinct advantages: (a) The presence of terminator sequences upstream of the promoter and responsive elements eliminates "read-through" transcription of the reporter gene and minimizes background PAP expression; (b) PAP activity can be measured in both cell extracts and intact cells (because of the permeability of cells to some PAP substrates); and (c) the stability of the PAP enzyme allows samples containing low levels of induced PAP activity to be incubated for long periods to obtain a more accurate measure of PAP activity without concern for enzyme inactivation. Although it might appear that a major disadvantage of using AP as a reporter would be that many cells contain endogenous AP activity (which would complicate detection of the transfected PAP gene), several properties of the human PAP enzyme are distinctly different from other forms of AP and make PAP very useful as a reporter gene. Specifically, PAP is normally ex-

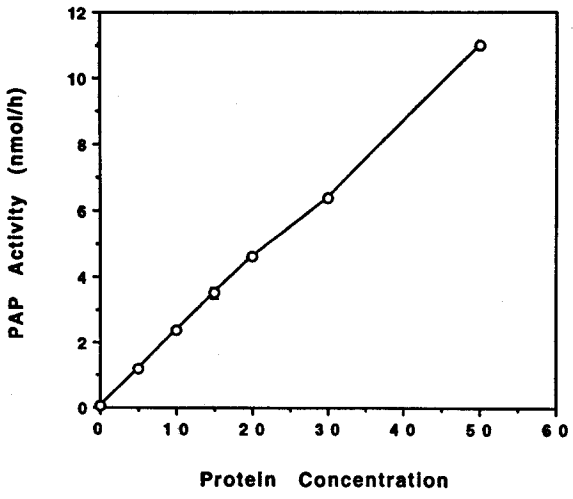


Fig. 4. Linearity of PAP activity with protein concentration. Confluent plates of cells were incubated with TCDD (1 nM) for 24 h, and PAP activities of increasing amounts of the cell sonicate were determined as described in "Materials and Methods." Values represent the mean ± SE ($n = 4$).

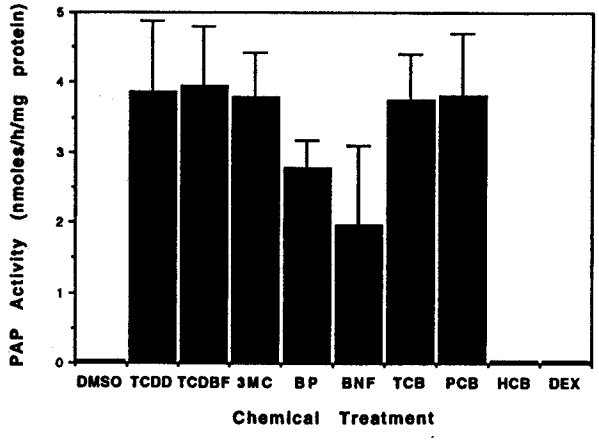


Fig. 6. Effects of various chemicals on PAP activity in T13 cells. Cells were incubated with DMSO, 1 nM TCDD, 10 nM 2,3,7,8-tetrachlorodibenzofuran (TCDBF), 1 μM 3-methylcholanthrene (3MC), 1 μM benzo[*a*]pyrene (BP), 1 μM β-napthoflavone (BNF), 1 μM 3,3′,4,4′-tetrachlorobiphenyl (TCB), 1 μM 3,3′,4,4′,5-pentachlorobiphenyl (PCB), 1 μM 2,2′,4,4′,5,5′-hexachlorobiphenyl (HCB), or 1 μM dexamethasone (DEX) for 24 h, and PAP activity in cell sonicates was determined as described in "Materials and Methods." Values represent the mean ± SE ($n = 6$–8).

pressed only by cells derived from the placenta of higher primates and by some transformed primate cell lines, and it is unaffected by the presence of 10 mM homoarginine, an effective inhibitor of other AP isozymes [37,38]. In addition, PAP is extremely resistant to heat denaturation at 65°C, with heat-labile forms of AP exhibiting a half-life of about 1 min at this temperature [30]. However, although most endogenous AP activity can readily be inactivated by these treatments, a new cell line used in transfection studies should be examined for the presence of heat-stabile phosphatase activity. During initial screening of cell lines for TCDD responsiveness, extremely high levels of heat-stable phosphatase activity were found in two human cell lines, A431 and LS180, whereas low or nondetectable levels were found in most other examined cells (data not shown).

Recently, Postlund et al. [39] have described production of a recombinant human HepG2 cell line (101L) that was stably transfected with a reporter construct, containing the 5′- flanking region of the human CYP IA1 gene (inclusive of three DRE sequences) immediately upstream of the firefly luciferase gene. Similar to our results, exposure of 101L cells to TCDD and related chemicals resulted in high levels of expression of the reporter gene. Comparison of the time course, relative responsiveness, and sensitivity of induction of the mouse T13 PAP gene with that of the human 101L luciferase gene and rat H4IIE CYP IA1 gene revealed that although the time course of induction and estimated ED50s for TCDD were comparable (0.34, 0.35, and 0.1 nM, respectively), the TCDD detection limit in T13 cells (0.1 nM) was not as low as that in the H4IIE (about 0.01 nM) or 101L cells (0.001 nM). The similarities in response are surprising, especially given that each of these bioassay systems contains either the entire upstream regulatory region from the CYP IA1 gene (H4IIE and 101L cells) or a 480-bp DRE-containing DNA fragment from the CYP IA1 gene (T13 cells). The significant difference in detection sensitivity between these cell lines likely results from inherent differences in the analytical methodology used for measurement of the respective enzyme activity rather than significant differences in their inducibility. Nominal detection limits for spectrophotometric, fluorescent, and luminescent analytical assays are in the approximate ranges of 1 to $30 \times 10^8$, 1 to $5 \times 10^6$, and 1 to $2 \times 10^5$ molecules [40]. Although the spectrophotometric PAP assay used in our studies is not especially sensitive, a single-step chemiluminescent AP assay using a phenylphosphate-substituted dioxetanes substrate reportedly can detect as few as $1 \times 10^3$ molecules of phosphatase [41]. However, this specific detection level was obtained using purified enzyme under ideal conditions, and the effective sensitivity of this reaction in the T13 cell bioassay system remains to be determined. Use of the chemiluminescent phosphatase substrate could increase the sensitivity of the PAP assay up to 100-fold greater than the bioluminescent-based luciferase assay, up to 1,000-fold greater than fluorometric assays, and between $1 \times 10^6$- and $3.2 \times 10^6$-fold than the spectrophotometric PAP assay.

The HAH-responsive vector described here can be transfected into a variety of different AhR-containing cell lines. Mouse Hepal cells were selected for our initial studies not only because they exhibit a high degree of HAH responsiveness and have been extensively characterized, but also due to the availability of a variant Hepal cell line that allowed initial characterization of the role of the AhR in the inducible response [15,33,36]. Induction of PAP activity in the selected Hepal stable transfectant T13 was relatively rapid and occurred in a ligand-, AhR-, and DRE-dependent manner, similar to that previously reported for the endogenous CYP IA1 gene [16,36], demonstrating the utility of this bioassay system for detection of TCDD-like chemicals. We have used this expression vector in transient transfection experiments to demonstrate formation of dioxin-like compounds by UV irradiation of tryptophan [33].

The approach described here for production of a bioassay based on the ability of a ligand- (HAH-) dependent transcriptional factor (the AhR) to bind to a specific DNA recognition site (the DRE) on the reporter plasmid and thus activate transcription of an adjacent reporter gene, has much broader applications than detection of dioxin-like chemicals. Ligand-dependent induction of gene expression has been demonstrated for other toxicologically and pharmacologically important chemicals such as heavy metals [42,43], peroxisome proliferators [44,45], and estrogens [46,47]. Activation of gene expression by each of these classes of compounds is similar to that described for TCDD, in that induction involves the interaction of a chemical-dependent transcription factor with a specific DNA regulatory element adjacent to the responsive gene. Stable transfection of recombinant expression vectors that contain metal-, peroxisome-proliferator-, or estrogen-responsive elements upstream of a reporter gene into an appropriate cell line (that contains the necessary chemical-dependent trans- acting factors) could produce a cell bioassay for specific classes of chemicals. Thus, any chemical or class of chemicals that stimulates gene expression via a mechanism similar to that described here could be detected with a bioassay system analogous to ours.

Overall, this sensitive and inexpensive bioassay could be used for a variety of screening purposes, such as providing a relative measure of levels of toxic/bioactive HAHs in food, biological, or environmental samples, and the effectiveness of remediation procedures designed to decrease levels of toxic HAHs. We are developing a series of species- and tissue-specific cell bioassay systems for use in environmental screening and in studies examining species differences in the molecular mechanism of HAH responsiveness.

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