Photooxidized tryptophan (TRP) in tissue culture medium elicits a transient cytochrome P450 (CYP1) induction response in cultured cells. We show here that exposure of TRP to window sunlight (aTRP) greatly increased the potency, efficacy, and duration of CYP1A induction by TRP in primary chick embryo hepatocytes and in vivo. Aqueous TRP exposed to sunlight for 7 days exhibited a 100-fold or greater increase in potency over TRP in medium. The induction response was sustained for at least 48 h and was comparable in efficacy to 2,3,7,8-tetrachlorodibenzo-p-dioxin. In hepatocytes, increases in mRNAs for CYP1A4 and CYP1A5, chick orthologs of mammalian CYP1A1 and 1A2, preceded increases in CYP1A proteins and enzyme activities, 7-ethoxyresorufin deethylation (EROD) for CYP1A4 and arachidonic acid epoxidation for CYP1A5, consistent with a transcriptional mechanism. Aryl hydrocarbon receptor (AhR) dependence was evidenced by aTRP induction of EROD in wild-type Hepa1c1c7 cells but not in AhR-defective (c35) mutants. Preparations of aTRP were stable for many months at 4°C and were relatively resistant to metabolism by hepatocytes or liver microsomes. Fractionation of aTRP by HPLC analysis coupled with EROD assays showed that aTRP contained multiple photoproducts and CYP1A inducing components, which varied in sensitivity to metabolism by hepatocytes. The previously identified TRP photoproduct, 6-formylindolo[3,2-b]carbazole (FICZ), was one component, but FICZ was not required for CYP1A induction by the aTRP mixture. These findings identify the indoor environment, and window sunlight in particular, as a new source of CYP1A inducers. Further, the evidence that biologically active metabolites of an endogenous substrate, arachidonic acid, are formed by aTRP-induced CYP1A provides a pathway by which TRP photoproducts, like toxic xenobiotics such as TCDD (Poland and Knutson, 1982), could have significant physiologic effects.

Key Words: tryptophan photooxidation; chick embryo; CYP1A4; CYP1A5; dioxin; arachidonic acid epoxidation; sunlight.

The focus of scientific interest in the function and regulation of the aryl hydrocarbon receptor (AhR) and cytochrome P450 (CYP1A) induction has shifted from the toxin 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin, TCDD) to more common AhR ligands including indole-based compounds, related to the amino acid tryptophan (TRP) (Denison and Nagy, 2003). This shift reflects increasing evidence that the AhR has physiologic roles (Fernandez-Salgueiro et al., 1995; Lahvis et al., 2000; Vasquez et al., 2003) in addition to mediating the toxicity of xenobiotics such as TCDD (Poland and Knutson, 1982).

TRP-related compounds have received particular attention because of their ubiquity in the environment. They include dietary compounds, metabolites or spontaneous breakdown products, derivatives found in vivo (Adachi et al., 2001; Bittinger et al., 2003; Bjeldanes et al., 1991), or photoproducts (Helferich and Denison, 1991; Rannug et al., 1987). Many TRP-derived products have been shown to bind to the AhR and to induce CYP1A (Adachi et al., 2004; Gillner et al., 1993; Heath-Pagliuolo et al., 1998; Helferich and Denison, 1991; Jellinck et al., 1993; Lorenzen et al., 1993; Rannug et al., 1987; Sindhu et al., 2000; Spink et al., 2003).

Solutions of aqueous TRP or of TRP in peptide linkages are readily photooxidized by ultraviolet (UV) light (Tassin and Borkman, 1980). Early studies (Paine, 1976, 1977) showed that UV irradiation of tissue culture medium produced an inducer of CYP1A enzyme activity in the medium. CYP1A induction in human keratinocytes and lymphocytes by UV light has been attributed to oxidation of TRP in the medium (Wei et al., 1999), as has the induction of CYP1A by medium change in diverse cell types (Kocarek et al., 1993; Oberg et al., 2005; Segner et al., 2000; Sindhu et al., 1996, 2000; Spink et al., 2003).

There are several reports of TRP photoproducts binding to the AhR (Denison and Nagy, 2003; Heath-Pagliuolo et al., 1998; Helferich and Denison, 1991; Rannug et al., 1987). Identified products include kynurenine (Denison and Nagy, 2003) and tryptamine, which are photoproducts as well as metabolites of TRP (Creed, 1984), and two photoproducts with extremely high AhR binding affinity, 6-formylindolo[3,2-b]carbazole (FICZ) and 6,12-di-formylindolo[3,2-b]carbazole (dFICZ) (Rannug et al., 1995).

Notwithstanding the high binding affinity of some TRP photoproducts, CYP1A induction responses to TRP photooxidized by UV light in human cells (Wei et al., 1998) or in response to medium change in various other cells have been reported to be transient, with maximal effects seen soon after...
exposure and induction no longer detectable after 24 h (Kocarek et al., 1993; Oberg et al., 2005; Segner et al., 2000; Sindhu et al., 1996, 2000; Spink et al., 2003).

We report here that exposure of TRP to ordinary sunlight passing through window glass greatly increases the inducing potency of TRP. TRP exposed to sunlight for 7 days, for example, was more potent than TRP in culture medium by two to three orders of magnitude. The induction response was comparable to TCDD in efficacy and sustained for at least 48 h in cultured hepatocytes and in vivo. Sunlight-exposed TRP was found to contain a mixture of CYP1A inducers, varying in sensitivity to metabolism, of which FITC was only one component. The induction response included enhanced formation of CYP-dependent arachidonic acid epoxides, indicating that TRP photoproducts can alter formation of biologically active endogenous substances. The results show that light in the indoor environment and, in particular, sunlight passing through window glass is a new potential source of potent and effective CYP1A inducers.

MATERIALS AND METHODS

Reagents. Unless otherwise indicated, chemicals were from Sigma-Aldrich (St. Louis, MO), and tissue culture reagents from GIBCO BRL Life Technologies (Gaithersburg, MD). High pressure liquid chromatography (HPLC) reagents were HPLC grade. Fertilized chicken eggs, White Leghorn strain, were obtained from Burr Farm (Hampton, CT), and FITC from Biozol (Plymouth Meeting, PA).

Cell Cultures

Primary hepatocytes. Livers were removed from 15- or 16-day-old chick embryos after the hearts were perfused with 5 ml of Hank’s balanced salt solution (140 mM NaCl, 5.5 mM D-glucose, 5.4 mM KCl, and 0.4 mM of KH₂PO₄, pH 7.3) and minced and disaggregated for 20 min in 10 ml of warm Hank’s solution with 0.05% collagenase. Cell suspensions were centrifuged three times at 200 × g at 4°C, each time for 7 min. The combined pellets were suspended in standard Ham’s medium (Std. Ham’s) with 2% fetal bovine serum (FBS). Hepatocytes were plated in Costar 24-well cell culture clusters in standard Ham’s medium (Std. Ham’s) with 2% fetal bovine serum (BD Falcon, Franklin Lakes, NJ). The term “cultured hepatocytes” refers to experiments, as indicated below, cells were plated in 6- or 10-cm culture dishes 3 times at 200 3 10⁶ cells per well in 1 ml of medium or in Costar 96-well assay plates (white-walled clear bottomed) (FBS). Hepatocytes were plated in Costar 24-well cell culture cluster flat-bottomed plates (Corning Inc, Corning, NY) at 0.5 × 10⁶ cells per well in 1 ml of medium or in Costar 96-well assay plates (white-walled clear bottomed) (Corning Inc.) at 80,000 cells per well in 0.16 ml of medium and maintained in 5% CO₂ in air for 48 h before medium change and further treatment. In some of medium or in Costar 96-well assay plates (white-walled clear bottomed) (FBS). Cells at semiconfluence were treated with light-exposed TRP and assayed for 7-ethoxyresorufin deethylase (EROD) (see below) after 24 h.

Hepa1c1c7 and AhR mutant (c35) cells. Mouse hepatoma Hepa1c1c7 wild type and c35 mutant cells from ATCC (Manassas, VA), were plated in 24 well dishes at 0.25 × 10⁶ cells per well in dMEM (minimum essential medium) with 10% FBS. Cells at semiconfluence were treated with light-exposed TRP and assayed for 7-ethoxyresorufin deethylase (EROD) (see below) after 24 h.

Tissue culture media used for primary hepatocytes. (1) Std. Ham’s—9.18 g of Basal Medium Eagle (BME) (Cellgro, Mediatech Herndon, VA) and 2.2 g of NaHCO₃ were dissolved in 900 ml of distilled water. Additions were: 20 ml of 50× MEM essential amino acid solution containing 36 mM L-arginine, 10 mM L-cystine, 13.5 mM L-histidine, 20 mM L-isoleucine, 20 mM L-leucine, 25 mM L-lysine, 5 mM L-methionine, 10 mM L-phenylalanine, 20 mM L-threonine, 10 mM L-tyrosine, and 20 mM L-valine. 2.5 mM L-tryptophan, 200,000 U/penicillin-streptomycin, 20 ml of 100× MEM vitamin solution, 5 ml of 100× MEM nonessential amino acids, and 0.1 ml (10 mg/ml) of d-Biotin. pH was adjusted to 7.3–7.5, FBS added to a final concentration of 2%, and the total volume brought to 1 l with distilled water. (2) TRP-free medium—TRP-free Basal Medium Eagle (BME) was custom-prepared by Specialty Media (Phillipsburg, NJ). Additions were the same as for Std. Ham’s, except that TRP was excluded from the MEM essential amino acid solution.

Photoactivation of TRP. Aqueous TRP at 100 μM, unless otherwise indicated (1 × is defined as the concentration of TRP in Std. Ham’s (14.2 μg per ml or 69 μM)), was dissolved in 50 ml of distilled water and placed on the indoor sill of a large east-facing window in a 50-ml polypropylene tube, unless otherwise indicated. Light-exposed TRP (aTRP) was wrapped in aluminum foil and kept at 4°C before dilution in TRP-free medium for treatment of cultured hepatocytes or further analysis. TRP kept at the window wrapped in foil was a regular control.

For the dose of UVA (320 to 400 nm) received by TRP exposed to window sunlight for 7 days, an approximate dose range from 37.5 to 225 mJ/cm² per week was calculated as follows: the average daily UV dose to the earth’s surface in New York (0.63 kJ/m²) (for the 3 winter months) to 3.78 kJ/m² (for the 3 summer months) (http://temis.nl) was multiplied by 0.17 (percent of UV A in sunlight), by 0.5 for the average transmission of light over the UVA range through the 1-in clear insulating window glass (Viracon, Owatonna, MN), and by 7 (days per week). For visible light (400 to 700 nm) an approximate dose range from 0.3 to 0.93 kJ/Cm² per week was calculated as follows: the daily dose, from 0.04 to 0.17 kJ/cm² (Freitas, 1999), times 0.78 (percent of visible light transmitted through the glass), and multiplied by 7. The window glass screens UVB (280–315 nm) virtually entirely.

Treatment of hepatocytes. β-Naphthoflavone (β-NF) in dimethylsulfoxide (DMSO) (10 mM) was diluted in TRP-free medium to 1 or 10 μM, the concentrations used in culture. TCDD (1.5 mM) in dioxane (J.T. Baker, Phillipsburg, NJ) was diluted in dioxane to 1.5 μM and further diluted in TRP-free medium to 1 mM, a maximal CYP1A-inducing concentration, for addition to hepatocytes. Equivalent amounts of DMSO or dioxane were used as controls (final concentrations <0.1%). For treatment of hepatocytes, compounds were diluted and added to cells in TRP-free medium. Triplicate wells were used for each treatment.

Treatment of chick embryos in ovo. Test compounds were administered to 15-day-old embryos via injection into the fluids surrounding the embryos through a hole in the shell. After 24 or 48 h, livers were removed, and microsomes prepared (Rifkind et al., 1994).

Enzyme Assays

Assays were performed under conditions where product formation was a linear function of enzyme concentration and incubation time and substrate was not limiting. Microsomes were incubated at 37°C in a shaking water bath in air.

EROD. (1) In cultured hepatocytes—24-well plates—Medium was removed, cells washed with phosphate buffered saline (PBS; Cellgro by Mediatech), and 0.5 ml of the EROD reaction mixture added to each well (4 μM 7-ethoxyresorufin (7-ER) and 10 μM dicumarol in Std. Ham’s). After 30 min at 37°C, two 0.2-ml aliquots were removed per well, 0.25 ml of cold acetone was added to each followed by centrifugation at 1400 3 g for 15 min. Resorufin fluorescence was measured in a Perkin Elmer MFP 3 spectrophotofluorimeter (Excitation (Ex) and Emission (Em) λ, 558 nm and 595 nm, respectively), using a quinine sulfate standard previously calibrated against resorufin. Emission spectra were obtained for selected samples to confirm the presence of a resorufin peak at 590 nm. Results for 24-well plates are given as pmol resorufin/well. 96-well plates—Medium was removed, cells washed with PBS, and 0.08 ml of the EROD reaction mixture described above added to each well. After 30 min at 37°C, resorufin fluorescence was read on a SpectraFluor fluorescence plate reader (Tecan, Durham, NC) at Ex and Em λ, 535 nm and 595 nm, respectively. A standard curve for resorufin (4.8 nM to 4.8 μM) added to wells of nontreated
cultured hepatocytes immediately before reading was included in each experiment. Results for 96-well plates are given as pmol resorufin/ml. (2) In liver microsomes and hepatocyte homogenates—Reaction mixtures (0.24 ml) contained 15 to 30 µg chick embryo liver microsomal protein or 200 µg of hepatocyte homogenate protein in 0.039 M Tris-phosphate, pH 8.3, with 1.25 mM EDTA and 1 mM MgCl₂, 7-ER (4 µM unless otherwise indicated), and for hepatocyte homogenates, 10 µM dicumarol. After preincubation at 37°C for 1 min, reactions were started with 1 mM NADPH and incubated for 5 min. After adding 0.25 ml of cold acetone and centrifugation at 3,000 rpm for 15 min, resorufin was measured as above using the spectrophotofluorometer (Rifkind et al., 1994).

Arachidonic Acid (AA) metabolism. CYP1A5-dependent AA metabolism was measured in homogenates of chick embryo hepatocytes cultured in 10 cm BD Falcon polystyrene dishes (BD Biosciences, San Jose, CA) at 7 × 10⁶ cells per dish or in liver microsomes (Capdevila et al., 1990; Rifkind et al., 1994), using duplicate dishes for each treatment. For each determination in hepatocytes, cells from one dish were scraped into 0.3 ml of Hank’s balanced salt solution and homogenized. Reaction mixtures for hepatocytes (0.5 ml) contained 300 µg homogenate protein and for microsomes (0.25 ml), 50 µg liver microsomal protein. Both contained 30 µM 1-µC-arachidonic acid (48 µCi/µmol) (Perkin Elmer, Torrance, CA). After 2 min of preincubation at 37°C, reactions were started with 1 mM NADPH, 10 mM isocitric acid, 0.2 U isocitric dehydrogenase/ml, and 10 mM MgCl₂ and incubated for 10 min at 37°C. Addition of acetic acid (0.1 or 0.2 ml for microsomes and hepatocyte homogenates, respectively) was followed by two extractions with 3 ml of ethyl acetate containing 0.005% butylated hydroxytoluene. The organic phases were pooled, dried under N₂, and resuspended in 0.11 ml of 50% acetonitrile in water and 0.1% acetic acid. Products in 0.05 ml were resolved by reverse phase high pressure liquid chromatography (HPLC) using a Vydac C₁₈ (Vydac, Hesperia, CA) column (90 Å, 5 µm particle size, 4.6 × 250 mm) and a linear gradient from 50 to 100% acetonitrile in water containing 0.1% acetic acid, at 1 ml per min for 40 min. Radioactivity was measured using a Flu-O-Three Beta Model S radioactivity flow monitor (Packard Instrument Company, Downers Grove, IL). Products were identified by HPLC retention times of pure standards (Rifkind et al., 1994).

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) of CYP1A4 and CYP1A5. Total RNA was extracted from cultured chick hepatocytes (4 × 10⁶ cells per 10-cm dish) using RNA STAT-60 (Tel-Test ''B,'' Friendswood, TX). First-strand cDNA was synthesized from the total RNA using Moloney Murine Leukemia virus reverse transcriptase (Roche, Indianapolis, IN) as directed by the manufacturer: 4 µl of 5X incubation buffer, 2 µg of total RNA, 2 µl of 10X concentrated hexamers, 1 µl of 10 mM dNTPs, 0.5 µl of 40U/µl RNase inhibitor, 1 µl of 20 U/µl of the reverse transcriptase, and DEPC-water up to 20 µl, were incubated for 2 hr at 37°C; all components from Roche. PCR amplification was performed using primers from chicken CYP1A4, CYP1A5, CYP2H1, or GAPDH (glyceraldehyde-3-phosphate dehydrogenase) as an internal standard (respective Gene Bank Accession Nos.: X99453, X99454, M13454, and AF047874). Primers used (Invitrogen (Carlsbad, CA)) were: for CYP1A4, 5'-GGACGCGGCTGCAAGGTG-3' and 5'-GTGCAAAACGCTGAGGGATTCT-3' (Fig. 1), and 5'-GTCGAGCATGGTGTGAG-3' instead of the latter in Figure 7; for CYP1A5, 5'-CTTCGTCCCTCCTACCATCCT-3' and 5'-CACCGGAAATGATCAC-3' instead of the latter in Figure 7; for CYP2H1, 5'-GTCGTCCCTTCTGTGTG-3' and 5'-GGCTCTCAAGTTGGCGGT-3' for GAPDH, 5'-GGGTCTTATGACCACTGTCC-3' and 5'-GATGAGCTCCCTATTCGCT-3'. Reaction mixtures contained 2 µl of single-strand cDNA, 2 µl of the relevant sense and antisense CYP primers (final concentration in the reaction mixture, 200 nM), and 45 µl of Platinum Supermix (Invitrogen), which contains all the other components needed for the PCR reactions.

Amplification conditions were for CYP1A4, 94°C × 5 min for 1 cycle, 94°C × 1 min, 59°C × 30 s, 72°C × 40 s for 30 cycles, 72°C × 10 min for 1 cycle; for CYP1A5, 94°C × 5 min for 1 cycle, 94°C × 1 min, 56°C × 30 s, 72°C × 30 s for 28 cycles, 72°C × 10 min for 1 cycle; for CYP2H1, 94°C × 5 min for 1 cycle, 94°C × 1 min, 58°C × 30 s, 72°C × 30 s for 29 cycles, 72°C × 10 min for 1 cycle. For GAPDH 1 µl of the sense and antisense GAPDH primers were added to the reaction mixture for each CYP and amplified for 23 cycles. All PCR products were obtained at cycle number below plateau levels. PCR products were separated on a 1.5% agarose gel and visualized with ethidium bromide using a UV transilluminator. Expected sizes of the PCR products were: CYP1A4, 404 bp (Fig. 1), 109 bp (Fig. 7); CYP1A5, 219 bp; CYP2H1, 286 bp; GAPDH, 170 bp. Band intensities were measured by densitometry using AlphaEaseFC software (Alpha Innotech, San Leandro, CA).

SDS-polyacrylamide gel electrophoresis/Western blotting. Hepatocytes from triplicate wells with the same treatment on 24-well plates were scraped together, lysed in 0.15 ml of 2X sample buffer (125 mM Tris–HCl, pH 6.8, 4% SDS, 16% glycerol, 10% β-mercaptoethanol, 0.002% bromphenol blue) and boiled for 10 min. Protein concentrations were determined (Lowry et al., 1951) on a duplicate set of three wells for each treatment, separately for each well. Identical SDS gels were prepared in each experiment and stained with Coomassie blue to confirm equal loading of lanes. Electrophoresis was at 15 mA and 30 mA through the stacking and running gels, respectively, followed by Western blotting (Kanetoshi et al., 1992; Rifkind et al., 1994). Primary antibodies were immunopurified antisera recognizing both CYP1A4 or CYP1A5 at 1:400 dilution or nonimmunopurified antisera recognizing both CYP1A4 and CYP1A5 at 1:3000 dilution; the secondary antibody was peroxidase-conjugated goat anti-rabbit at 1:1000 dilution. Detection was by chemiluminescence using “Western blotting detection reagent” (Amersham, Buckinghamshire, UK). Band intensities were measured by densitometry as described above.

Hydrogen peroxide (H₂O₂). H₂O₂ was measured using an Amplex® Red H₂O₂/peroxidase assay kit (Molecular Probes, Eugene OR) on hepatocytes in 96-well plates (1.6 × 10⁶ cells per well) by the manufacturer’s directions.

HPLC fractionation of aTRP and TRP. One ml of a 100X solution of aTRP exposed to sunlight for 7 days, unless otherwise indicated, or TRP not exposed to light was dried under N₂ and resuspended in 0.010 ml of DMSO followed by 1 ml of 35% acetonitrile in water containing 0.1% trifluoroacetic acid (TFA). 0.225 ml (equivalent to 22.5X) were injected onto a Denali C₁₈ monomeric, 100A˚, 5 µm particle size reverse-phase column (4.6 mm × 250 mm) (Vydac) and resolved by HPLC on a linear gradient of 35% to 85% acetonitrile in water containing 0.1% TFA at 1 ml/min for 40 min. Detection was at 254 nm and in replicate samples at 386 nm using a Waters 486 UV detector (Waters Corporation, Milford, MA). The signal was transmitted from the UV detector through the Flo-One Beta Model S radioactivity flow monitor (Packard Instrument Company, Downers Grove, IL). Products were identified by HPLC retention times of pure standards (Rifkind et al., 1994).

RESULTS

TRP-Dependent CYP1A Induction by Medium Change in Chick Embryo Hepatocytes

In preliminary experiments (data not shown) medium change with Std. Ham’s medium selectively induced mRNAs for CYP1A4 and CYP1A5 but not the AhR-independent CYP2H1 (Gilday et al., 1996; Hobbs et al., 1986) in primary chick embryo hepatocytes, indicating selective CYP1A

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induction by medium change. The induction by medium change was transient, with a maximum effect at 5 h returning to basal levels by 24 h. EROD activity, which is selectively catalyzed by chick CYP1A4 (Kanetoshi et al., 1992; Rifkind et al., 1994) was also transiently increased. The induction response was entirely attributable to tryptophan (TRP) in the medium. CYP1A was not induced by spent medium or by changing serum concentrations of the medium or by TRP-free medium. The transient effect has been shown for diverse cell types including rat hepatocytes, trout liver cells, human keratinocytes, mouse hepatoma Hepa1c1c7 cells, and rat hepatoma MH1C1 cells (Kocarek et al., 1993; Oberg et al., 2005; Segner et al., 2000; Sindhu et al., 1996, 2000; Spink et al., 2003). Our preliminary findings underscore the ubiquity of the CYP1A induction response to medium change and show that it occurs in a new cell type, primary chick embryo hepatocytes.

Enhancement of CYP1A Induction by Exposure of TRP to Sunlight; AhR Dependence of Induction by Light-Activated TRP

Aqueous solutions of TRP were exposed to window sunlight as described in “Materials and Methods” for 1 to 7 days and used to treat cultured hepatocytes at a concentration equivalent to 1× TRP, the concentration of TRP in Std. Ham’s (14.2 μg/ml or 69 μM). Henceforth, unless otherwise indicated, the term “activated TRP (aTRP)” will refer to TRP exposed to sunlight for 7 days. TRP exposed to light for 1 day increased CYP1A mRNAs (Fig. 1a) and EROD (Fig. 1b) more than Std. Ham’s, but the induction was still transient (lower at 24 h than 5 h). The induction response for TRP exposed to light for 7 days was sustained at 24 h, and its efficacy was comparable to TCDD. Figure 1b shows that the sustained response was achieved after 3 days of TRP exposure and that the levels were maximum

FIG. 1. Sunlight exposure increases the CYP1A-inducing capacity of TRP (a) RT-PCR. RT-PCR analysis of CYP1A4 and CYP1A5 mRNAs at 5 and 24 h after medium change (m.c.) with standard Ham’s medium (Std. Ham’s) or with 1× TRP previously exposed to window sunlight for 1 or 7 days (aTRP) added in TRP-free medium (1× is defined as the equivalent of the amount of TRP in Std. Ham’s (14.2 μg/ml or 69 μM)) or with 1 nM TCDD. GAPDH, RT-PCR control. Results are shown for two replicate 10-cm dishes, each assayed independently. Amplification product sizes were as expected: for CYP1A4, CYP1A5, and GAPDH, 404, 219, 117 base pairs (bp), respectively. bp markers, bottom to top: 154, 234/22, 298, 394, 453, 517, 653 1033, 1230, 1766, 2176. (b) Enhancement of EROD induction by aTRP. Cultured hepatocytes in 96-well plates were subjected to medium change with TRP-free medium supplemented with TRP not exposed to sunlight (0 days), or exposed for 1, 3, 5, or 7 days at

1×. TCDD (1 nM), positive control. EROD was measured after 5 h (clear bars) or 24 h (black bars). Means ± SE are shown for three replicate wells per treatment group. (c) Western blots for CYP1A4 and 1A5 proteins. Western blotting with immunopurified antisera against CYP1A4 (upper panels) and CYP1A5 (lower panels). Left panels: Lysates of cultured hepatocytes prepared as described in “Materials and Methods” before medium change (0 h) or at 5, 24, or 48 h after medium change with 1× or 10× aTRP. Results shown are for one of two duplicate blots. Lanes 1–2, 5 μg of liver microsomal protein from chick embryos treated for 24 h with 0.1 ml DMSO or 6.7 mg β-NF per egg; lanes 3–9, 70 μg of total hepatocyte protein per lane. Right panels: Western blots of purified CYP1A4 and 1A5 to show specificity of the antisera. (d) Enhancement of CYP1A5 dependent arachidonic acid (AA) metabolism by aTRP. Hepatocyte homogenates were assayed for CYP-dependent AA metabolism at 5 or 24 h after medium change with 1× aTRP or no medium change (m.c.), as described in “Materials and Methods.” Mean values for duplicate culture dishes are shown for AA epoxygenase products, EETs and EET-diols, left panel, and HETEs, right panel; dots, individual values. (e) AhR dependence of CYP1A induction by aTRP. EROD was assayed in mouse Hepa1c1c7 cells and c35 mutant Hepa1c1c7 cells defective in AhR/XRE binding 24 h after treatment with aTRP at 0.1× or 1×. Mean values for resorufin production ± SE for three replicate wells from a 24-well plate are shown.
starting at 5 days. In most experiments, aTRP was added to cells during medium change, but induction effects were essentially the same when aTRP was added without changing the medium. Further, photoproducts produced by exposure of TRP to sunlight at 10× had the same effects as those produced by exposure at 100× (data not shown).

Figure 1c shows that aTRP increased CYP1A4 and CYP1A5 proteins. The protein levels were elevated for at least 48 h, with the 48-h levels at 90% of the 24-h levels. After 5 h of treatment, 10× aTRP increased CYP1A protein 20% more than 1× aTRP, whereas after 24 h or 48 h of treatment, 1× and 10× were comparable in their CYP1A protein induction effects.

To determine whether CYP1A5 enzymatic activity was increased by aTRP, CYP1A5-mediated arachidonic acid (AA) metabolism was measured (Kanetoshi et al., 1992; Rifkind et al., 1994) (Fig. 1d). AA epoxygenase products, epoxyeicosatrienoic acids (EETs) and EET-diols, were increased 1.6-fold after 5 h and 4.9-fold after 24 h, and monohydroxylated products, monohydroxyeicosatetraenoic acids (HETEs), were increased by 1.7-fold after 5 h and 2.8-fold after 24 h.

Figure 1e shows that the CYP1A induction by aTRP is AhR dependent. aTRP induced EROD in wild type mouse hepatoma Hepa1c1c7 cells but not in c35 cells, a mutant line derived from the wild type and expressing an AhR defective in XRE binding (Sun et al., 1997).

The inducing effect of exposure to sunlight under the conditions described above was compared to other environmental conditions in the laboratory (Table 1). TRP was activated slightly more when exposed to sunlight in polypropylene than in glass tubes. TRP wrapped in tin foil was not activated by sunlight whether kept at 4°C or at the window, indicating that sunlight was acting via a photoactivation rather than a heat effect. Exposure to UV light from a germicidal bulb and 34W T12 fluorescent bulb, a 34W T12 fluorescent lamp (Sylvania, Danvers, MA); each was at a distance of 24 inches. Note. Aqueous TRP (1.42 mg/ml, 100×), subjected to the conditions described, was used to treat cultured hepatocytes (at 1×) in TRP-free medium. EROD was measured after 24 h. Exposures were in polypropylene tubes except where indicated, with EROD values for 7-day exposure to window sunlight in polypropylene taken as 100% in each experiment. EROD values for aTRP exposed to window sunlight in Experiment 1 and 2 (100%) levels were 240 ± 0.7 and 138 ± 2.7 pmol resorufin/well ± SE, respectively. SE are derived from three replicate wells for each treatment. The percent of maximal induction ± SE for hepatocytes not subjected to medium change or treated with Std. Ham’s were, in Experiment 1, 2 ± 0.1 and 4 ± 0.1 and, in Experiment 2, 3 ± 0.2 and 8 ± 0.1, respectively. The UV lamp was a G36T8 germicidal sterile lamp (Nuaire, Plymouth, MN), and the fluorescent bulb, a 34W T12 fluorescent lamp (Sylvania, Danvers, MA); each was at a distance of 24 inches. "p < 0.001 for increase over Std. Ham’s. b "p < 0.001 for less than exposure to window sunlight.

with aTRP. Treatment with aTRP for 48 h produced a mean increase for the two concentrations tested of 7.3-fold for CYP1A protein (Fig. 2a) and of 11- and 5-fold for CYP1A4 and 1A5 enzyme activities, EROD and AA epoxygenation, respectively, compared to equivalent amount of TRP not exposed to sunlight (Fig. 2b). Effects of aTRP in vivo were dose related. The results show that the inducing compounds in aTRP were sufficiently stable in the physiologic environment to produce a substantial and sustained induction in vivo even after 48 h.

The small increase in CYP1A induction effects at the higher dose of unexposed TRP seen in Figure 2 is probably attributable to the presence of small amounts of photoactivated TRP or other contaminants in the commercial preparations of TRP as also found by others (Heath-Pagliuso et al., 1998; Kocarek et al., 1993; Rannug et al., 1987).

Characterization of Dose-Response Relationships for EROD Induction by aTRP in Hepatocytes

The effect of aTRP on EROD 5 and 24 h after treatment was examined at half-log interval dilutions from 10× to below the induction threshold (about 0.001× for the preparation of aTRP shown (Fig. 3)). The results shown are representative of at least

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### Table 1

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<thead>
<tr>
<th>Source of light</th>
<th>Number of days exposed</th>
<th>% of maximal induction ± SE</th>
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<tbody>
<tr>
<td>Window</td>
<td>7</td>
<td>100 ± 0.3a</td>
</tr>
<tr>
<td>Window, wrapped in foil</td>
<td>14</td>
<td>4 ± 0.1b</td>
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<td>UV lamp</td>
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<td>78 ± 1.6a</td>
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<td>Fluorescent bulb</td>
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<td>13 ± 0.6a</td>
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<tr>
<td>Ambient room light</td>
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<td>6 ± 0.1a</td>
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Note. Aqueous TRP (1.42 mg/ml, 100×), subjected to the conditions described, was used to treat cultured hepatocytes (at 1×) in TRP-free medium. EROD was measured after 24 h. Exposures were in polypropylene tubes except where indicated, with EROD values for 7-day exposure to window sunlight in polypropylene taken as 100% in each experiment. EROD values for aTRP exposed to window sunlight in Experiment 1 and 2 (100%) levels were 240 ± 0.7 and 138 ± 2.7 pmol resorufin/well ± SE, respectively. SE are derived from three replicate wells for each treatment. The percent of maximal induction ± SE for hepatocytes not subjected to medium change or treated with Std. Ham’s were, in Experiment 1, 2 ± 0.1 and 4 ± 0.1 and, in Experiment 2, 3 ± 0.2 and 8 ± 0.1, respectively. The UV lamp was a G36T8 germicidal sterile lamp (Nuaire, Plymouth, MN), and the fluorescent bulb, a 34W T12 fluorescent lamp (Sylvania, Danvers, MA); each was at a distance of 24 inches. "p < 0.001 for increase over Std. Ham’s. b "p < 0.001 for less than exposure to window sunlight.

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### Induction of CYP1A4 and 1A5 by aTRP In Vivo

To determine if aTRP retained its inducing effect in a physiologic environment, we treated chick embryos in ovo...
10 other preparations of aTRP, with some variations in potency. There were four noteworthy findings: (1) Sunlight exposure greatly increased the inducing potency of TRP over the effect of Std. Ham’s medium (about 330-fold for the preparation shown, based on the extrapolation that a dose halfway between 0.001× and 0.01× would have produced induction equivalent to Std. Ham’s at 5 h). (2) The response to aTRP was biphasic, with 10× aTRP producing lower hepatocyte EROD levels than 1× aTRP, notwithstanding similar CYP1A4 protein levels at 1× and 10× aTRP (see Fig. 1c). (3) EROD induction was transient (lost at 24 h) for aTRP below 0.01× and became sustained (greater at 24 h than 5 h) for aTRP at 0.01× and

FIG. 2. Induction of CYP1A by aTRP in vivo. Chick embryos (15 days old) were treated for 48 h with TRP or aTRP, at doses equivalent to 0.36 or 0.72 mg of TRP in 0.25 or 0.5 ml H2O, respectively, or with 6.7 mg β-NF in 0.1 ml of DMSO per egg as a positive control. A negative control group without treatment (UT) was included. For each treatment, three to four livers were pooled, and microsomes prepared. (a) Western blot. 15 μg of liver microsomal protein per lane. Immunoblotting was performed as described in “Materials and Methods” using an antibody recognizing both CYP1A4 and 1A5 (Rifkind et al., 1994). (b) EROD (CYP1A4), left panel, and arachidonic acid epoxygenation (CYP1A5), right panel, in the same liver microsomes. Assays were as described in “Materials and Methods.” Clear bars, UT; light gray bars, TRP not exposed to light; dark gray bars, aTRP; black bars, β-NF.

FIG. 3. Dose-response relationships for EROD induction by aTRP. Hepatocytes cultured in 96-well plates were subjected to medium change with aTRP at log or half-log interval dilutions from 10× to 3 times 10−3×, Std. Ham’s or 1 nM TCDD. Cells not subjected to medium change (no m.c.) were also included. Hepatocyte EROD was measured after 5 h (clear bars) or 24 h (black bars). Mean values for resorufin produced per well ± SE are shown for three replicate wells for each treatment.
higher. (4) aTRP produced highly effective induction. The maximum response after 24 h was about 93% of the maximum response for TCDD.

**Differences in Rates of Metabolism of Std. Ham’s or aTRP**

We investigated whether the loss of induction at 24 h by Std. Ham’s and the more sustained effect at 24 h of aTRP were due to differences in metabolism of the inducers in each. Std. Ham’s and aTRP were exposed to cultured hepatocytes for 5 or 24 h. The hepatocytes were subjected to medium change with the removed preincubated Std. Ham’s or aTRP or with Std. Ham’s or aTRP that had not been preincubated with hepatocytes (0 h preincubation). Some hepatocytes in the second set were not subjected to medium change (No m.c.). Hepatocyte EROD in the second set was measured after 5 h (clear bars) or 24 h (black bars); *p < 0.05 versus Std. Ham’s. **p < 0.002 versus aTRP (not preexposed). In separate experiment (inset), the effect of exposure of Std. Hams’ to hepatocytes for 30 min to 5 h on its EROD-inducing capacity was examined. $r^2 = 0.92$, for the correlation between loss inducing activity by Std. Ham’s and duration of preexposure to hepatocytes; $t_\text{13} = 1.6 h$. (b) Effect on EROD induction capacities of Std. Ham’s or aTRP of preincubated with microsomes. Std. Ham’s or aTRP were incubated for 5 min with liver microsomes (60 or 240 μg microsomal protein per reaction mixture (RM)) from 17-day-old chick embryos previously treated for 24 h with β-NF (6.7 mg/egg) or 0.1 ml DMSO. Reaction mixtures (3.5 ml) also contained 0.5 mM of NADPH and 10 U/ml isocitrate dehydrogenase, 10 mM MgCl$_2$, and 10 mM isocitric acid, and aTRP (1×) or Std. Ham’s, 0.5 ml of the preincubated aTRP or Std. Ham’s reaction mixture or equivalent amounts of nonpreincubated aTRP or Std. Ham’s (bars labeled “0”) were added to cultured hepatocytes. Hepatocyte EROD was examined 5 h later. Mean values for pmol resorufin per well ± SE are shown for three replicate wells for each experimental group. *p < 0.02 versus treatment with no incubation with microsomes.

**FIG. 4.** CYP1A inducers in Std. Ham’s are more readily metabolized than the inducers in aTRP. (a) EROD induction by Std. Ham’s or aTRP preexposed to hepatocytes. Std. Ham’s or aTRP were incubated with a first set of cultured hepatocytes for 5 or 24 h. The medium was removed. A second set of cultured hepatocytes was subjected to medium change with the removed preincubated Std. Ham’s or aTRP or with Std. Ham’s or aTRP that had not been preincubated with hepatocytes (0 h preincubation). Some hepatocytes in the second set were not subjected to medium change (No m.c.). Hepatocyte EROD in the second set was measured after 5 h (clear bars) or 24 h (black bars); *p < 0.05 versus Std. Ham’s. **p < 0.002 versus aTRP (not preexposed). In separate experiment (inset), the effect of exposure of Std. Hams’ to hepatocytes for 30 min to 5 h on its EROD-inducing capacity was examined. $r^2 = 0.92$, for the correlation between loss inducing activity by Std. Ham’s and duration of preexposure to hepatocytes; $t_\text{13} = 1.6 h$. (b) Effect on EROD induction capacities of Std. Ham’s or aTRP of preincubated with microsomes. Std. Ham’s or aTRP were incubated for 5 min with liver microsomes (60 or 240 μg microsomal protein per reaction mixture (RM)) from 17-day-old chick embryos previously treated for 24 h with β-NF (6.7 mg/egg) or 0.1 ml DMSO. Reaction mixtures (3.5 ml) also contained 0.5 mM of NADPH and 10 U/ml isocitrate dehydrogenase, 10 mM MgCl$_2$, and 10 mM isocitric acid, and aTRP (1×) or Std. Ham’s, 0.5 ml of the preincubated aTRP or Std. Ham’s reaction mixture or equivalent amounts of nonpreincubated aTRP or Std. Ham’s (bars labeled “0”) were added to cultured hepatocytes. Hepatocyte EROD was examined 5 h later. Mean values for pmol resorufin per well ± SE are shown for three replicate wells for each experimental group. *p < 0.02 versus treatment with no incubation with microsomes.
aTRP to elicit a sustained response at 24 h was unaffected by the partial loss of its capacity to induce at 5 h. The inset in Figure 4a shows the rapid loss of the inducing capacity of Std. Ham’s by exposure to hepatocytes ($t_{1/2}$ of about 1.6 h).

Similar results using a different approach are shown in Figure 4b. The inducing effect of Std. Ham’s was completely lost after Std. Ham’s was incubated with liver microsomes from DMSO- or β-NF-treated chick embryos at 240 μg per reaction mixture. Microsomes from β-NF- treated chick embryos at 60 μg per reaction mixture caused more inactivation than those from the DMSO-treated, suggesting that increased CYP1A enhanced the rate of metabolism of inducers in Std. Ham’s. aTRP incubated with the same microsomes lost only about 35% of its inducing capacity. There were no additional losses when incubations were continued for up to 30 min. In preliminary experiments (not shown) heat-inactivated microsomes did not affect EROD induction by Std. Ham’s or aTRP, and inclusion of the NADPH regenerating system was required for the metabolism.

**HPLC Fractionation of aTRP**

To determine whether the induction by aTRP was due to one or more components in aTRP, aTRP and TRP were subjected to reverse phase HPLC (Fig. 5a). Multiple 254 nm absorbing peaks are evident in aTRP that were missing in TRP. Detection
Fractions were incubated at 1 to separate into metabolism-sensitive or resistant groups. The 14 fractions isolated from aTRP by HPLC could be and to be a mixture of inducers (Fig. 5), we examined whether Inducers in HPLC Fractions of aTRP Are Differently Resistant to Metabolism.

Dose-Response Relationships for EROD Induction by HPLC-Separated Fractions of aTRP

We examined whether the aTRP fractions, which had an induction effect more than double that of the DMSO control (Fractions 1–5, 7–9, and 11), had dose effects differing from those of the unfractinated mixture (Fig. 6). The results show that the aTRP mixture contains inducing components capable of their own induction equivalent to that in the mixture. The sum of the EROD responses for the individual fractions approximated the induction for the mixture at 0.33 x 3 and 0.1 x 3 dilutions, respectively, while the sum approximated the induction for the mixture at 0.33 x and 0.01 x.

Inducers in HPLC Fractions of aTRP Are Differently Resistant to Metabolism

As aTRP was shown to be resistant to metabolism (Fig. 4) and to be a mixture of inducers (Fig. 5), we examined whether the 14 fractions isolated from aTRP by HPLC could be separated into metabolism-sensitive or resistant groups. The 14 fractions were incubated at 1 x with cultured hepatocytes for 24 h, and the medium was then transferred to a second set of cultured hepatocytes. Other hepatocytes in the second set were treated with fractions of aTRP that had not been preexposed to hepatocytes. After 24 h total RNA was extracted from the second set of hepatocytes and used to measure CYP1A4 mRNA induction by PCR. The results (Fig. 7) showed that the fractions varied in their sensitivity to metabolism. Induction by Fractions 4 and 5, the most effective inducing fractions (induction equivalent to aTRP), were not affected by preincubation with hepatocytes, indicating that the main inducers in those fractions are resistant to metabolism. Fractions 3, 6, 8, and 9, less effective inducers, were also metabolism resistant. On the other hand, Fractions 1, 2, 7, and 11–14 were sensitive to metabolism. Fraction 7 showed the greatest loss of inducing capacity after exposure to hepatocytes (44% loss); losses were 18% and 27% for Fractions 1 and 2. Fractions 11, 12, 13, and 14 essentially lost their inducing capacities after the preincubation.

Further Resolution of the More Polar HPLC Fractions of aTRP

To resolve further the components in the more polar fractions in Figure 5, which contained most of the inducing activity, Fractions 1 to 5 (F1–F5) were subjected to HPLC using a gradient of 5% to 85% acidified acetonitrile (see “Materials and Methods”) (Fig. 8). UV detection at 254 nm revealed that each of those aTRP fractions contained multiple peaks. Groups of peaks or single peaks were collected and assayed for EROD (Fig. 8, right panels). The EROD-inducing capacity varied for the subfractions. Almost all of the subfractions induced EROD over the baseline (TRP-free medium). Exceptions were: subfraction b and h in F1, a and h in F3, and a, b, f, g, d in F2, a and h in F3, and a, b, f, g, and h in F5). The capacity to induce was not correlated with peak size. For F1, for example, most of the inducing activity was in the subfractions eluting after the TRP peak (i.e. fractions n–q). Subfraction q in F1, which had only some small peaks, was the most effective inducer for F1, containing about half of the inducing capacity of the unfractinated F1. For F4, the main inducing capacity resided in the smaller of the two main peaks in the fraction.

For all of the fractions except F1, the sum of the EROD values for the subfractions approximated the induction by the unfractionated fraction. For F1, the sum of the EROD values for the subfractions exceeded that of the unfractionated F1, suggesting a possible inhibitory effect among the components.

FICZ in aTRP

As FICZ has been identified as a photoproduct of TRP (Ranug et al., 1995), we investigated whether FICZ was present in the aTRP mixture (Fig. 9). Figure 9a shows that FICZ (Biomol) eluted as a single peak at about 30–32 min (the 35–85% octanitritle gradient), corresponding to aTRP Fraction
11 (see Fig. 5a) using the same HPLC system. There were no additional breakdown products for FICZ exposed to sunlight for 7 days (not shown). As the peak for Fraction 11 was very small even at 22.5 μM aTRP (Fig. 5), a larger amount of aTRP equivalent to 600 μM was separated on HPLC. Two peaks were evident for the elution time of Fraction 11 (Fig. 8b insert). The second peak, Fraction 11b, induced EROD (Fig. 8b), but Fraction 11a did not. Fraction 11b exhibited the same emission spectra reported for FICZ at Ex 310, 390, and 460 (Rannug et al., 1987), shown in Figure 8c for Ex 390, while Fraction 11a did not have a FICZ emission spectrum. The concentration of FICZ in Fraction 11b was measured by reference to a standard curve for the fluorescence of Biomol FICZ. The amount of FICZ in 1× aTRP was calculated to be 72 pM. To determine if FICZ was needed for the inducing effect of aTRP, the effects on EROD of combined aTRP eluates from HPLC with and without Fraction 11 included were compared (Fig. 9d). The inducing effect of aTRP was unaffected by fractionation on HPLC or by exclusion of the fraction containing FICZ from the mixture. Thus, FICZ is present in aTRP, but it was not required for the induction effect of the aTRP mixture.

**DISCUSSION**

It has been known for a long time that medium change can elicit transient CYP1A induction. Induction by medium change is of low intensity and no longer evident by 24 h. It has been attributed to photooxidized TRP in medium and was shown by Kocarek (1993) to be elicited simply by a brief (1 h) exposure of TRP-containing medium to an ordinary 15-W fluorescent bulb. CYP1A induction by medium change is often overlooked as a confounding factor in studies of constitutive AhR expression in cultured cells, but a recent report by Oberg (2005), published while these studies were under completion, renewed attention to the phenomenon. Oberg et al. (2005) showed that medium exposed to fluorescent light in a standard culture hood for 24 h or even to a 40-W incandescent bulb...
produced transient CYP1A1 induction in rat hepatoma MH1C1 cells. The previously identified TRP photoproduct FICZ was detected in the light-exposed medium, although its contribution to the observed induction response was not determined. Here we report the new observation that aqueous TRP can be oxidized by sunlight to multiple photoproducts that elicit high efficacy CYP1A induction, sustained for at least 48 h in hepatocytes and in vivo and comparable in intensity to the induction by TCDD. The activation occurs simply by exposure to sunlight passing through ordinary window glass without inclusion of any additional photosensitizers such as riboflavin. Our findings show that the indoor environment is a potential source of generation of highly effective CYP1A inducers from TRP.

After 7 days of exposure to sunlight, the induction potency of TRP was increased by 100-fold or more. The photoproducts and inducing capacity of the sunlight activated TRP mixture (aTRP) were stable for months at 4°C. HPLC analysis in combination with EROD assays showed that aTRP contained over 100 photoxidation products and over

FIG. 8. Further resolution of EROD-inducing components in Fractions 1–5 of aTRP. The more polar aTRP fractions, which had the most photoproducts and highest EROD induction (F1 to F5, Fig. 5), were further resolved on HPLC using a gradient of 5% to 85% acetonitrile over 80 min at a flow rate of 1 ml/min (chromatograms in left panels). The insets show the original fraction as seen in Figure 5a, highlighted in black, from which each set of subfractions was derived. CTS, instrument readout for UV absorbance at 254 nm; min, peak retention time. The largest peak among the subfractions from F1 (Fig. 8a, subfraction “1”) is TRP. Subfractions collected separately were used to analyze light and dark gray. Each subfraction was used to treat hepatocytes (1×); EROD induction was assayed after 24 h (right panels). Mean EROD values ± SE with baseline levels for TRP-free medium subtracted, are shown for three replicate wells per treatment group or dots for duplicates. EROD induction for unfractionated Fractions F1–F5, are shown at three dilutions, 0.1, 0.33, and 1×, white, gray, and black, respectively. Mean EROD value ± SE for TRP-free medium, 66 ± 3.8 nmol resorufin per ml.

FIG. 9. Contribution of FICZ to aTRP. (a) HPLC of reference FICZ. 6.6 nmoles of FICZ (Biomol) were dissolved in 250 μl of 35% acetonitrile in water with 0.1% TFA and resolved by HPLC using a gradient of 35% to 85% acetonitrile in water as described in “Materials and Methods” with detection at 254 nm. A single peak eluted at 30–32 min. CTS, instrument readout for UV absorbance. (b) Presence of two peaks in F11. aTRP (600×) was dried under N2 and resolved on HPLC using the same gradient as in (a). Two peaks eluted between 30–32 min (a and b, in the insert). Both were collected, dried, and used to treat cultured hepatocytes at dilutions of 1×, 0.33×, and 0.1×. Hepatocyte EROD was assayed after 24 h. Mean values ± SE are shown for three replicate wells per treatment group. (c) Fluorescence emission spectra. F11b (solid line) had the same emission spectrum as FICZ (dotted line) (emission at 522 nm for excitation at 390 nm), not seen for F11a (dashed line). All spectra were read in the same solvent (74% acetonitrile in water). Reference FICZ at 0.1 μM. (d) EROD induction by aTRP with and without FICZ-containing F11. Aliquots of the same preparation of aTRP were subjected twice to HPLC elution at 22.5×, using the 35–85% acetonitrile gradient. The first time, the entire eluate was collected and dried under N2. The second time, the eluates containing Fractions 12–14 were collected and combined, but Fraction 11 was excluded. The eluates were dried under N2, resuspended in 0.1 ml of DMSO and 0.9 ml of H2O, diluted 1:22.5 in TRP-free medium, and used to treat cultured hepatocytes. EROD was assayed after 24 h. The preparation of aTRP from which the fractions were derived was also tested. Means ± SE of three replicates per treatment group are shown.
30 inducing compounds, one of which was identified as FICZ. The evidence that FICZ was confined to only one of 14 HPLC-separated fractions of aTRP, and that its subtraction from the aTRP mixture did not affect CYP1A induction by the mixture (see Fig. 9), shows that FICZ is not critical to the inducing capacity of the aTRP mixture. The data in Figure 7 showing that F11 was metabolism sensitive are in agreement with reports that FICZ is rapidly metabolized (Wei et al., 2000) and further indicate that FICZ is not among the metabolism-resistant compounds in aTRP. The data show that aTRP contains high-efficiency inducers in addition to FICZ that remain to be identified. As shown in Figure 8, several components in aTRP, in particular subfractions q in F1, d in F4, and d in F5, were found to be particularly effective inducers, and they are leading candidates for further investigation.

As aTRP contained only small amounts of FICZ (calculated at 72 pM at 1X, for the sample in Fig. 5) which could entirely account for the induction by Fraction 11 of aTRP, the results indicate the high potency and efficacy of FICZ as a CYP1A inducer in the chick hepatocyte model. The estimate of 72 pM FICZ in 1X aTRP is reasonably consistent with the finding of Oberg et al. (2005) that 8 pM of FICZ was generated in tissue culture medium after 24 h of exposure to incandescent light, considering the differences in exposures and the difficulties in measuring the small amounts of FICZ produced.

Characterization of the inducing components in the aTRP mixture will be useful to identify the high-efficiency CYP1A-inducing photoproducts in aTRP and to understand their contribution to the induction effects of the mixture, but the mixture may in fact be more relevant than its components to human exposures, as light produces mixtures of TRP photoproducts, and human effects are likely to reflect exposures to such mixtures. The amount of TRP exposed at the window may seem high, but exposure at 10X, 1/10 of the standard levels used here (100X) produced the same effects and the same HPLC photoproducts as 100X (data not shown). Moreover, 100X TRP (6.9 mM TRP) is close to concentrations that might be expected for food products that could potentially be exposed to high-intensity light (dairy products contain up to 3.5 mM TRP (http://www.nationaldairycouncil.org)).

A biphasic CYP1A induction response to aTRP, with inhibition at 10X, shown in Figure 3, was also found for high concentrations of other indole-related inducers, indole-3-acetate and indirubin (Adachi et al., 2004; Bittinger et al., 2003). Inhibition of CYP1A activity at high concentrations of indole-related inducers could represent a means by which induction effects of such inducers could be limited in vivo. 10X aTRP did not appear to be cytotoxic, in so far as the appearance of the cells and protein measurements were unchanged and CYP levels were not depressed. Further, H2O2 levels in hepatocytes were not increased by 10X aTRP (data not shown). The evidence that the sum of EROD levels found for the components of the mixture exceeded the EROD activity of the mixture when tested at concentrations equivalent to 0.1X and higher (see Fig. 6) is compatible with competition among inducing components in the aTRP mixture for binding to the AhR or at CYP1A catalytic sites.

The rapid metabolism of the inducers in Std. Ham’s by hepatocytes or liver microsomes seen in Figure 4 is consistent with the transience of the induction response to medium change with Std. Ham’s. The greater resistance to metabolism of aTRP could contribute to its more sustained effects. As shown in Figure 7, the aTRP mixture contains inducers which are sensitive to metabolism, as well as inducers which are resistant. The metabolism resistance could reflect intrinsic insensitivity to metabolism, but could also be due to the amounts of the inducers in aTRP exceeding the metabolic capacity of the chick embryo hepatocytes or to the presence of inhibitors of metabolism in the aTRP fractions. Further studies will be needed to discriminate among those possibilities.

The increase in CYP-dependent AA metabolism by aTRP is significant because epoxygenase metabolites of AA have many biologic effects, including the capacity to increase intracellular calcium and alter cell proliferation and vascular function (Capdevila et al., 2000). While the ability of CYP1B1 to metabolize endogenous substrates such as estrogens is well recognized (Murray et al., 2001), CYP1A is commonly held to be involved in metabolism or activation of toxic xenobiotics. The findings show that CYP1A activated by AhR ligands, to which people are likely to be more commonly exposed than to toxic xenobiotics like TCDD, can alter production of endogenous AA metabolites.

As UVC is screened by the atmosphere, and UVB by window glass, only UVA and visible light (400–700 nm) are likely to contribute to the photoactivation by sunlight. Fluorescent bulbs emit mainly visible light with very small amounts of UVA. Even if visible light does not activate TRP directly, it could participate in the effects seen by further oxidizing TRP photoproducts initially generated by UVA. Davies and Truscott, 2001). Identification of the components in sunlight producing the photoactivation described here will require further investigation.

We show here that sunlight, even when screened by window glass, is sufficiently powerful to produce TRP photoproducts causing high-intensity CYP1A induction. Exposures for periods of weeks to such conditions can occur in the work environment. The toxicological or physiologic consequences of the generation of CYP1A inducers of TRP by light have yet to be determined. Wei et al. (1999) suggested that light may be a transducer of chemical signals involving the AhR. The AhR activation in response to such signals could be involved in regulation of normal homeostatic processes. The possibility that ingestion of food or dietary supplements containing TRP exposed to light either during or after manufacturing could lead to CYP1A induction in vivo gains credence from the evidence that aTRP was able to induce CYP1A in vivo.

Sunlight might oxidize TRP in proteins as well as in aqueous solutions, as TRP in proteins is also readily oxidized (Creed,
1984). The ability of UVA to penetrate through the epidermis and dermis to subdermal blood vessels (Moan, 2001) raises the further possibility that exposure to sunlight could lead to CYP1A induction in diverse organs via photoactivation of TRP in skin or by other routes, such as the eye (Davies and Truscott, 2001), or via the circulation. Experimental exposure to UV light can photoactivate TRP in skin (Brancaleon et al., 1999) and induce CYP1A in skin and liver in humans and animals (Goerz et al., 1996; Katiyar et al., 2000). UVA as well as UVB have been implicated in skin cancer and aging (He et al., 2004).

Seasonal changes in TRP activation by sunlight may also explain the long-known but still unexplained activation of aryl hydrocarbon hydroxylase in human lymphocytes and its seasonal variation (Paigen et al., 1981). In fact, we found that the ability of different preparations of aTRP to induce CYP1A varied seasonally (Supplemental Data, item 2). While the findings here focus on CYP1A induction effects of aTRP, a wide range of systemic effects involving TRP or its metabolites are known to be affected by sunlight, including mood and immune system function (see Allegrì et al., 2003, for a comprehensive overview). These also warrant consideration as potentially significant biological consequences of TRP activation by sunlight.

SUPPLEMENTARY DATA
Supplementary data are available online at www.toxsci.oxfordjournals.org.

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