Flavonoids as Aryl Hydrocarbon Receptor Agonists/Antagonists: Effects of Structure and Cell Context

Shu Zhang, Chunhua Qin, and Stephen H. Safe

1Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, Texas, USA; 2Institute of Biosciences and Technology, Texas A&M University System Health Science Center, Houston, Texas, USA

Chemoprotective phytochemicals exhibit multiple activities and interact with several cellular receptors, including the aryl hydrocarbon (Ah) receptor (AhR). In this study we investigated the AhR agonist/antagonist activities of the following flavonoids: chrysin, phloretin, kaempferol, galangin, naringenin, genistein, quercetin, myricetin, luteolin, baicalein, daidzein, apigenin, and diosmin. We also investigated the AhR-dependent activities of cantharidin and emodin (in herbal extracts) in Ah-responsive MCF-7 human breast cells, HepG2 human liver cancer cells, and mouse Hepa-1 cells transiently or stably transfected with plasmids expressing a luciferase reporter gene linked to multiple copies of a consensus dioxin-responsive element. The AhR agonist activities of the compounds (1 and 10 μM) were as high as 25% of the maximal response induced by 5 nM 2,3,7,8-tetrachlorodibenzop-dioxin (TCDD), and their potencies were dependent on cell context. Galangin, genistein, daidzein, and diosmin were active only in Hepa-1 cells, and cantharidin induced activity only in human HepG2 and MCF-7 cells. Western blot analysis confirmed that baicalein and emodin also induced CYP1A1 protein in the human cancer cell lines. The AhR antagonist activities of four compounds inactive as agonists in MCF-7 and HepG2 cells (kaempferol, quercetin, myricetin, and luteolin) were also investigated. Luteolin was an AhR antagonist in both cell lines, and the inhibitory effects of the other compound were dependent on cell context. These data suggest that dietary phytochemicals exhibit substantial cell context–dependent AhR agonist as well as antagonist activities. Moreover, because phytochemicals and other AhR-active compounds in food are present in the diet at relatively high concentrations, risk assessment of dietary toxic equivalents of TCDD and related compounds should also take into account AhR agonist/antagonist activities of phytochemicals.

Key words: agonists, Ah receptor, antagonists, flavonoids, interactions, TCDD.

Environmental Health Perspectives 111:1877–1882 (2003), doi:10.1289/ehp.6322 available via http://dx.doi.org/ [Online 22 August 2003]

Received 6 March 2003; accepted 21 August 2003.

Address correspondence to S.H. Safe, Department of Veterinary Physiology and Pharmacology, Texas A&M University, 4466 TAMU, College Station, TX 77843-4466 USA. Telephone: (979) 845-5988. Fax: (979) 862-4929. E-mail: ssafe@cvm.tamu.edu

This study received support from the Research Foundation for Health and Environmental Effects, the National Institutes of Health (grants ES07106 and ES04917), and the Texas Agricultural Experiment Station. The authors declare they have no competing financial interests.

Halogenated aromatic (HA) industrial by-products such as the polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) have been identified as mixtures in the environment, in foods, and in fish, wildlife, and human tissues (Safe 1990). 2,3,7,8-Tetrachlorodibenzop-dioxin (TCDD) is the most toxic HA compound and has been used as a reference standard for hazard and risk assessment of these environmental and dietary contaminants (Ahlborg et al. 1992, 1994; Birnbaum and DeVito 1995; Safe 1990, 1994; Van den Berg et al. 1998). There is good support for the validity of TEFs/TEQs for hazard and risk assessment of PCDDs and PCDFs. However, in mixtures containing PCBs, there is also evidence that some AhR-mediated responses, nonadditive antagonist interactions can be observed (Safe 1998a, 1998b). For example, the antagonistic interactions between many environmentally significant PCBs, including 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB congener 153) interactions with TCDD or 3,3′,4,4′,5-penta-chlorobiphenyl (PCB 126), for several AhR-mediated responses in several in vivo and in vitro models have been reported (Biegel et al. 1989; Davis and Safe 1988, 1989; Morrissey et al. 1992; Tysklind et al. 1995; Zhao et al. 1997a, 1997b). These results are consistent with a receptor-mediated pathway where both agonist and antagonist ligands are routinely identified. However, these results indicate that, among environmentally important HAs, additivity may not be observed for some responses, and this contradicts one of the key assumptions of the TEF/TEQ approach.

TEFs/TEQs have been extensively used for assessing potential dietary TEQ intakes from various foods, and regulatory agencies have used these data to develop guidelines for TEF/TEQ intake. For example, the World Health Organization recently revised their tolerable daily intake value for TEQs from 10 pg/kg/day to 1–4 pg/kg/day (van Leeuwen et al. 2000). These guidelines also assume that TEQs are additive but do not address the increasing evidence that the AhR binds a host of endogenous chemicals, such as bilirubin, biliverdin, 7-ketocholesterol, and structurally diverse phytochemicals (Ashida et al. 2000; Bjeldanes et al. 1991; Casper et al. 1999; Chen et al. 1996; Chun et al. 2001; Ciolino et al. 1998a, 1998b; Ciolino and Yeh 1999; Denison et al. 1998; Gasicewicz et al. 1996; Gradelet et al. 1997; Phelan et al. 1998; Quadri et al. 2000; Savouret et al. 2001; Shertzer et al. 1999; Sinal and Bend 1997; Wang et al. 2001). Many of these phytochemicals, such as flavonoids, resveratrol, carotenoids, indole-3-carbinol, and related compounds, are weak AhR agonists/partial antagonists and are considered to be chemoprotective. This study further investigates a series of phytochemicals and their AhR agonist/antagonist activities; the compounds include the flavonoids chrysin, phloretin, kaempferol, galangin, naringenin, genistein, quercetin, myricetin, luteolin, baicalein, daidzein, apigenin, and diosmin, as well as cantharidin and emodin (in herbal extracts). Some of these compounds exhibit weak AhR agonist and antagonist activities in different cancer cell lines, and the results are interpreted in terms of their potential influence on the validity of the TEF/TEQ approach for risk assessment of HA compounds.

Materials and Methods

Chemicals, biochemicals, and cells. The compounds used in this study were purchased from Sigma-Aldrich (Milwaukee, WI) and include chrysin (purity > 97%), phloretin (95%), kaempferol (> 95%), galangin (95%), naringenin (95%), genistein (98%), quercetin (95%), genistein (98%), and baicalein (> 95%). Carotenoids, indole-3-carbinol, and related phytochemicals, such as flavonoids, resveratrol, and other AhR-active compounds in food are present in the diet at relatively high concentrations, risk assessment of dietary toxic equivalents of TCDD and related compounds should also take into account AhR agonist/antagonist activities of phytochemicals.

Key words: agonists, Ah receptor, antagonists, flavonoids, interactions, TCDD.
Western blot analysis. We extracted whole-cell lysates using 1× Western sampling buffer. Protein samples were heated at 100°C for 5 min, separated on 8% SDS-PAGE, and transferred to polyvinylidene difluoride (PVDF) membrane (Amerham, Piscataway, NJ). The PVDF membrane was blocked for 30 min and incubated with 1:1,000 CYP1A1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 hr at room temperature or with 1:1,000 AhR (Santa Cruz Biotechnology) overnight at 4°C. After vigorous washing for 20 min, 1:3,000 secondary antibody (Santa Cruz Biotechnology) was added, and the membrane was incubated with shaking for 45 min. After washing for 20 min, the membrane was incubated with ECL chemiluminescent substrate (NEN Life Science Products, Inc., Boston, MA) for 1 min, and exposed to Kodak X-Omat AR autoradiography film (Kodak, Rochester, NY). The membrane was reused and probed with the other antibody as indicated.

Statistics. All quantitative data were analyzed by analysis of variance followed by Fisher’s protected least-significant-difference test for significance (p < 0.05). Data from the transfection studies are expressed as mean ± SE (n ≥ 3) for each treatment group.

Results

AhR-mediated induction of CYP1A1 is a sensitive measure of Ah responsiveness. However, many phytochemicals interact with and inhibit CYP1A1 protein catalytic activity (Chen et al. 1996; Shertzer et al. 1999). Therefore, in this study we used a highly sensitive AhR-responsive assay (Denison et al. 1998) in which ligands activate the bacterial luciferase reporter gene activity in cells transfected with constructs containing multiple DRE promoter elements. Figure 1 illustrates structures of the 15 compounds used in this study; these include 12 flavonoids with different hydroxyl substitution patterns, plus the chemicals phloretin (a dihydrochalcone), cantharidin (a lactone), and...
emodin (an herbal laxative). Based on results of preliminary studies, we used 5 nM TCDD as a standard that induced maximal luciferase activity in stably transfected Hepa-1 cells (Figure 2) or in transiently transfected MCF-7 (Figure 3) or HepG2 cells (Figure 4). Results from the stably transfected Hepa-1 cells demonstrate their sensitivity to 5 nM TCDD, with a 124-fold inducibility, whereas lower but significant induction was observed for chrysin, galangin, genistein, baicalein, daidzein, emodin, apigenin, and diosmin. Previous studies have also reported that emodin induced AhR-dependent CYP1A1 in human lung adenocarcinoma CL5 cells (Wang et al. 2001), and diosmin was also an AhR agonist in MCF-7 cells (Ciolino et al. 1998b). In contrast, the reported AhR agonist activity of quercetin in MCF-7 cells (Ciolino et al. 1999) was not observed in stably transfected Hepa-1 cells (Figure 2). Galangin exhibited AhR antagonist activity in BU-11, a murine B cell line (Quadri et al. 2000), but AhR agonist activity was observed in stably transfected Hepa-1 cells (Figure 2), and agonist activity of 60 µM galangin has also been observed in Hepa-1 cells (Wang et al. 2001).

We further investigated the role of cell context in activation of transiently transfected pDRE3 in human MCF-7 and HepG2 cell lines. At concentrations of 1 or 10 µM, only chrysin, cantharidin, baicalein, and emodin activated luciferase activity in MCF-7 cells (Figure 3). With the exception of cantharidin, these compounds were also AhR agonists in stably transfected Hepa-1 cells, and compounds such as galangin, genistein, daidzein, apigenin, and diosmin that were active in Hepa-1 cells did not induce a response in MCF-7 cells. The pattern of induction responses in HepG2 cells was similar to that observed in MCF-7 cells in that chrysin, cantharidin, and baicalein activated gene expression, whereas (10 µM) emodin was not active in this cell line (Figure 4). These data demonstrate that the AhR agonist activities of structurally diverse phytochemicals and cantharidin, which is derived from insect extract, are highly variable among different cell lines, and that their fold inducibility compared with TCDD is also dependent on cell context. The stably transfected Hepa-1 cells are more highly sensitive to the induction of luciferase activity by

Figure 2. AhR-mediated transactivation in stably transfected Hepa-1 cells. Cells were treated with DMSO (control), 5 nM TCDD, or 1 or 10 µM of the flavonoids for 24 hr; luciferase activity was determined as described in “Materials and Methods.” Results are expressed as mean ± SE for three replicate determinations for each treatment group. Compounds #1 through #15 are defined in Figure 1.

*Significant induction, p < 0.05.

Figure 3. AhR-mediated transactivation in MCF-7 cells. Cells were transfected with pDRE3 and treated with DMSO (control), 5 nM TCDD, or 1 or 10 µM of the flavonoids, and luciferase activity was determined as described in “Materials and Methods.” Results are expressed as mean ± SE for each treatment group (three replicate determinations). Compounds #1 through #15 are defined in Figure 1.

*Significant induction, p < 0.05.

Figure 4. AhR-mediated transactivation in HepG2 cells. (A) Compounds #1 through #5. (B) Compounds #10 through #14. (C) Compounds #6 through #9 and #15. Cells were transfected with pDRE3 and treated with DMSO (control), 5 nM TCDD, or 1 or 10 µM of the flavonoids, and luciferase activity was determined as described in “Materials and Methods.” Results are expressed as mean ± SE for three separate determinations for each treatment group. Compounds #1 through #15 are defined in Figure 1.

*Significant induction, p < 0.05.
TCDD (5 nM) than to the other compounds. TCDD at 5 nM induced a 124-fold increase in luciferase activity, whereas only a 14-fold induction response was observed for 10 µM chrysin. In contrast, 5 nM TCDD and 10 µM chrysin, respectively, induced a 20- and 5.5-fold increase in luciferase activity in MCF-7 cells (Figure 3), and the potency of chrysin relative to TCDD was clearly higher in MCF-7 and HepG2 cells compared with stably transfected Hepa-1 cells.

The four compounds that activated luciferase activity in MCF-7 and HepG2 cells (chrysin, cantharidin, baicalein, and emodin) were also investigated as inducers of CYP1A1 protein in these cell lines (Figure 5). The highest nontoxic concentrations of each compound were used in the CYP1A1 protein induction assay because of the decreased sensitivity of this response compared with activation of luciferase activity in the transfected cells. With the exception of cantharidin, higher concentrations could be used because of the short duration (6 hr) of the experiment. Both baicalein and emodin increased CYP1A1 protein at concentrations of 100 µM (MCF-7) or 50 µM (HepG2), whereas chrysin was inactive at the same concentrations (Figure 5).

In contrast, 1 or 10 µM quercetin, kaempferol, and myricetin did not affect induction of luciferase activity by TCDD, whereas luteolin was an AhR antagonist in HepG2 cells (Figure 6B, C). These results demonstrate that AhR antagonist activities of these phytochemicals are also dependent on cell context.

Discussion

Results of this study demonstrate that several structurally diverse phytochemicals and cantharidin activate DRE-dependent luciferase (reporter gene) activity in cancer cell lines derived from mouse and human liver and human breast tumors. There are both similarities and differences in the AhR agonist activities of these compounds that are dependent on both structure and cell context. Our results show that TCDD, chrysin, and baicalein induced luciferase activity in all three cell lines. Cantharidin induced luciferase activity only in the human cells (MCF-7 cells, HepG2 cells), emodin was active in Hepa-1 and MCF-7 cells, and galangin, genistein, daidzein, apigenin, and diosmin were active only in stably transfected Hepa-1 cells. Previous studies have demonstrated that many of these compounds exhibit weak AhR agonist and/or partial antagonist activities in transactivation or receptor transformation assays (Ashida et al. 2000; Chun et al. 2001; Ciolino et al. 1999; Quadri et al. 2000). However, it is apparent that there were some differences between this and other studies on the AhR agonist or antagonist activities of individual phytochemicals. For example, Ciolino et al. (1999) reported that quercetin and kaempferol exhibited AhR agonist and antagonist activities, respectively, in MCF-7 cells, whereas these compounds exhibited minimal AhR agonist activity in our studies in the same cell line (Figure 3).

There could be several explanations for differences in Ah responsiveness of phytochemicals

![Figure 5. CYP1A1 protein induction by chrysin (#1), cantharidin (#8), baicalein (#11), and emodin (#13) in (A) HepG2 cells or (B) MCF-7 cells. Abbreviations: C, control; Conc, concentration. Cells were treated with DMSO (control), 5 nM TCDD, or different concentrations of the flavonoids for 6 hr. Whole-cell lysates were then prepared, and CYP1A1 and AhR proteins were detected by Western blot analysis as described in “Materials and Methods.” These experiments were determined at least two times for each cell line. Comparable results were obtained showing increased CYP1A1 protein after treatment with baicalein (#11; MCF-7/HepG2), emodin (#13; MCF-7/HepG2), and cantharidin (#8; MCF-7). CYP1A1 protein induction was not observed for chrysin (#1).]

![Figure 6. AhR antagonist activities of phytochemicals in MCF-7 and HepG2 cells. (A) MCF-7 cells treated with kaempferol (#3), quercetin (#7), myricetin (#8), or luteolin (#10). (B) HepG2 cells treated with kaempferol (#3) or quercetin (#7). (C) HepG2 cells treated with myricetin (#8) and luteolin (#10). Cells were transfected with pDRE3 and treated with DMSO (control) or with 5 nM TCDD alone or in combination with 1 or 10 µM concentrations of the compounds, and luciferase activities were determined as described in “Materials and Methods.” Results are expressed as mean ± SE for three separate determinations for each treatment group. *Significant inhibition, p < 0.05.]

![Figure 7. Comparison of AhR agonist and antagonist activities of these phytochemicals. (A) AhR agonist activities of compounds in Hepa-1 cells. Cells were transfected with a luciferase reporter gene under the control of the murine DRE-1 region and cotreated with 5 nM TCDD and various concentrations of each compound. Results are expressed as fold induction relative to the control (no TCDD). (B) AhR antagonist activities of compounds in Hepa-1 cells. Cells were transfected with a luciferase reporter gene under the control of the murine DRE-1 region and treated with 5 nM TCDD in the presence of various concentrations of each compound. Results are expressed as fold induction relative to the control (no TCDD). (C) AhR agonist activities of compounds in HepG2 cells. Cells were transfected with a luciferase reporter gene under the control of the human DRE-1 region and cotreated with 5 nM TCDD and various concentrations of each compound. Results are expressed as fold induction relative to the control (no TCDD). (D) AhR antagonist activities of compounds in HepG2 cells. Cells were transfected with a luciferase reporter gene under the control of the human DRE-1 region and treated with 5 nM TCDD in the presence of various concentrations of each compound. Results are expressed as fold induction relative to the control (no TCDD).]
in the Hepa-1, MCF-7, and HepG2 cells. The stably transfected mouse Hepa-1 cell line was more sensitive than the transiently transfected human MCF-7 and HepG2 cells to TCDD and to most of the phytochemicals. This could be due to the stable integration of the construct and the presence of four DREs compared with three DREs in the transiently transfected pDRE, used in the HepG2 and MCF-7 cell studies (Figures 3 and 4). In addition, the mouse AhR expressed in Hepa-1 cells exhibits higher binding affinity for TCDD than does the human AhR (Ema et al. 1994), and structural differences in the mouse and human AhR may also affect the binding and transactivation activities of the phytochemicals. Chrysin (10 µM) was the most consistent inducer in the reporter gene assays in the three cell lines (Figures 2–4). However, at concentrations as high as 100 and 50 µM in MCF-7 and HepG2 cells, respectively, induction of CYP1A1 protein was not observed (Figure 5). This illustrates the high sensitivity of the reporter gene assays for detecting AhR agonists and suggests that relative compound potencies in this assay may be different for other AhR-mediated responses (Figure 5). This has been observed for TCDD and related compounds that also exhibit species- and response-specific potency differences (Safe 1990). Like the nuclear hormone receptors, ligand-induced activation of the AhR is dependent on interactions with nuclear coregulatory proteins (Beischlag et al. 2002; Kumar et al. 1999; Nguyen et al. 1999). Nevertheless, results of this and other studies clearly demonstrate that structurally diverse phytochemicals exhibit AhR agonist activities.

We have also investigated interactions of kaempferol, quercetin, myricetin, and luteolin as AhR antagonists in MCF-7 and HepG2 cells (Figure 6) because these compounds alone at concentrations of 1 or 10 µM did not induce luciferase activity in these cell lines (Figures 3 and 4). The results showed that luteolin blocked TCDD-induced luciferase activity in both cell lines, and these results were comparable with the inhibition of TCDD-induced transformation of the rodent cytosolic AhR as previously reported (Ashida et al. 2000; Thenot et al. 1999). The AhR antagonist activities of kaempferol, quercetin, and myricetin were dependent on the cell context (Figure 6). Myricetin exhibited weak (but not significant) antagonist activity only in MCF-7 cells, and both kaempferol and quercetin were also antagonists in MCF-7 but not HepG2 cells. Because many flavonoids activate the estrogen receptor (ER), it is possible that inhibitory ER–AhR crosstalk that has previously been reported (Jeong and Lee 1998; Ricci et al. 1999) may contribute to AhR antagonist activities observed in MCF-7 cells (Figure 6). It is possible that higher concentrations of compounds 1–15 (Figure 1) may exhibit AhR agonist/antagonist activities. However, higher concentrations were not investigated because of cytotoxicity.

Several studies show that phytochemicals weakly activate the AhR in one or more assays and also act as AhR antagonists. These compounds include kaempferol (Ciolo et al. 1999), resveratrol (Casper et al. 1999; Ciolo and Yeh 1999), galangin (Quadri et al. 2000), rhoontigentin (Chun et al. 2001), indole-3-carbinol (Chen et al. 1996), and diindolylmethane (Chen et al. 1996). Ashida et al. (2000) also showed that ≤ 25 µM concentrations of various phytochemicals block TCDD-induced transformation of rat liver cytosolic AhR, and these include chrysin, baicalein, apigenin, luteolin, tangeretin, galangin, kaempferol, fisetin, morin, quercetin, myricetin, tamarixetin, isorhamnetin, naringenin, eriodictyol, and hesperitin. Total daily intakes of dietary flavonoids may be as high as 1 g (Verdeil and Ryan 1979), and serum levels of some flavonoids such as quercetin and genistein can be in the nanomolar to low micromolar range. The overall serum concentrations of most phytochemicals in humans is unknown. However, levels are probably in the nanomolar to micromolar range and are dependent on the food product and clearance times for individual compounds. 7-Ketocholesterol is also an AhR antagonist with a competitive binding IC₅₀ value (concentration that inhibits 50%) of 500 nM (Saveurat et al. 2001), and plasma concentrations of this compound range from 20 to 200 nM in healthy humans (Dzeletovic et al. 1995). This would suggest that many phytochemicals and endogenous compounds with AhR agonist/antagonist activities are present in human serum.

Risk assessment of HA compounds uses the TEF/TEQ approach. For example, daily TCQ intakes of TCDD and related compounds are 50–200 µg in most countries, and these values have substantially decreased over the past 10 years (van Leeuwen et al. 2000). Serum TEQ values are ≤ 5 ppt (lipid weight) or approximately 0.1 pM for TCDD and related compounds, whereas serum levels of some “natural” AhR agonists are in the nanomolar to low micromolar range. Thus, the serum ratios of flavonoids/TCDD TEQs are 10⁴ to 10⁶, and these ratios are similar to those required for inhibition of TCDD-induced responses by some phytochemicals (Ashida et al. 2000; Chun et al. 2001; Colino et al. 1998b, 1999; Quadri et al. 2000). Results shown in Figure 5 demonstrate that 1 µM luteolin inhibited (> 90%) TCDD-induced transactivation in MCF-7 cells at flavonoid/TCDD ratios as low as 200/1. Moreover, ratios of PCB 153/TCDD TEQs in human tissues are also > 10⁴, which is comparable with ratios required for PCB 153–mediated inhibition of several TCDD-induced biochemical and toxic responses (Safe 1998a, 1998b). It is likely that dietary intakes of most phytochemicals would be below levels required for an AhR agonist response based on results from cell culture studies. The potential chemoprotective effects of the expanding list of AhR-active phytochemicals and related compounds on TCDD-TEQ–mediated adverse responses should be further investigated in vivo models. These results can then be used for development of recommended dietary TCDD-TEQ values that reflect the combined intake of HA compounds plus high levels of “natural/phytochemical” AhR agonists/antagonists.

References

Ahborg UG, Becking GC, Birnbaum LS, Brouwer A, Derks HJJM, Feeley M, et al. 1994. Toxic equivalence factors for dioxin-like PCBs. Chemosphere 28:1049–1067.

Ahborg UG, Brouwer A, Fingeruth MA, Jacobson JL, Jacobson SW, Kennedy SW, et al. 1992. Impact of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls on human and environmental health with special emphasis on application of the toxic equivalence factor concept. Eur J Pharmacol 228:179–199.

Ashida H, Fukuda Y, Yamashita T, Kanazawa K. 2000. Flavonoids and flavonols at dietary levels inhibit a transformation of aryl hydrocarbon receptor induced by dioxin. FEBS Lett 478:213–217.

Beischlag TV, Wang S, Rose DW, Toricha J, Reisz-Porszasz S, Muhammad K, et al. 2002. Recruitment of the NCoR/SMRT/1/180 family of transcriptional coactivators by the aryl hydrocarbon receptor/aryl hydrocarbon receptor nuclear translocator complex. Mol Cell Biol 22:4319–4333.

Biegel L, Harris M, Davis D, Rosengren R, Safe L, Safe S. 1989. 2,3,7,8-tetrachlorodibenzo-p-dioxin antagonist in C3H/HeJ mice. Toxicol Appl Pharmacol 97:561–571.

Birnbaum LS, DeVito MA. 1995. Use of toxic equivalence factors for risk assessment for dioxins and related compounds. Toxicology 109:391–401.

Bjeldanes LF, Kim JY, Grose KR, Bartholomew JC, Bradfield CA. 1991. Aromatic hydrocarbon responsiveness-antagonist agonists generated from indole-3-carbinol in vitro and in vivo—comparisons with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Proc Natl Acad Sci USA 88:9543–9547.

Casper RF, Gusnes M, Rogers IM, Shirato T, Jolivet A, Miletic E, et al. 1999. Resveratrol as an antagonist to the aryl hydrocarbon receptor: implications for prevention of dioxin toxicity. Mol Pharmacol 56:784–790.

Chen I, Safe S, Bjeldanes L. 1998. Indole-3-carbinol and diindolylmethane as aryl hydrocarbon (AhR) agonist and antagonists in T47D human breast cancer cells. Biochem Pharmacol 51:1069–1077.

Chun YJ, Ryu SY, Jeong TC, Kim MY. 2001. Mechanism-based inhibition of human cytochrome P450 1A1 by rhapontigenin. Drug Metab Dispos 29:389–393.

Colino HP, Daschner PJ, Yeh GC. 1998a. Resveratrol inhibits transcription of CYP1A1 in vitro by preventing activation of the aryl hydrocarbon receptor. Cancer Res 58:5307–5312.

——. 1999. Dietary flavonols quercetin and kaempferol are ligands of the aryl hydrocarbon receptor that affect CYP1A1 transcription differentially. Biochem J 340:715–722.

Colino HP, Wang TT, Yeh GC. 1998b. Diosimin and diosmin are agonists of the aryl hydrocarbon receptor that differentially affect cytochrome P450 1A1 activity. Cancer Res 58:2754–2760.

Colino HP, Yeh GC. 1999. Inhibition of aryl hydrocarbon-induced cytochrome P-450 1A1 enzyme activity and CYP1A1 expresion by resveratrol. Mol Pharmacol 56:760–767.

Davarninos NA, Pollenz RS. 1999. Aryl hydrocarbon receptor imported into the nucleus following ligand binding is rapidly degraded via the cytoplasmic proteasome following nuclear export. J Biol Chem 274:28208–28215.
Davis D, Safe S. 1988. Immunosuppressive activities of polychlorinated dibenzofuran congeners: quantitative structure-activity relationships and interactive effects. Toxicol Appl Pharmacol 94:141–149.

———. 1989. Dose-response immunoxicities of commercial polychlorinated biphenyls (PCBs) and their interaction with 2,3,7,8-tetrachlorodibenzo-\(p\)-dioxin. Toxicol Lett 48:35–43.

Denison MS, Seidel SD, Rogers WJ, Ziccardi M, Winter GM, Heath-Pagliuso S. 1996. Natural and synthetic ligands for the Ah receptor. In: Molecular Biology Approaches to Toxicology (Puga A, Kendall RJ, eds). London: Taylor and Francis. 3–33.

Dzeletovic S, Breuer O, Lund E, Diczfalusy U. 1995. Determination of cholesterol oxidation products in human plasma by isotope dilution-mass spectrometry. Anal Biochem 225:73–80.

Ema M, Ohe N, Suzuki M, Ikawa S, et al. 1994. Dioxin binding activities of polymorphic forms of mouse and human aryl hydrocarbon receptors. J Biol Chem 269:27337–27343.

Garrison PM, Tullis K, Aarts JM, Brouwer A, Giesy JP, Denison MS. 1996. Species-specific recombinant cell lines as bioassay systems for the detection of 2,3,7,8-tetrachlorodibenzo-\(p\)-dioxin-like chemicals. Fundam Appl Toxicol 30:194–203.

Gasiewicz TA, Kende AS, Ricci G, Whitney B, Willey JJ. 1996. Analysis of structural requirements for Ah receptor antagonist activity: ellittocines, flavones, and related compounds. Biochem Pharmacol 52:1787–1803.

Gradelet S, Astor P, Pineau T, Canvienc MC, Siess MH, Leclerc J, et al. 1997. Ah receptor-dependent CYP1A induction by two carotenoids, canthaxanthin and 2-apo-8'-carotenal, with no affinity for the TCDD binding site. Biochem Pharmacol 54:307–315.

Jeong HG, Lee SS. 1998. Suppressive effects of estradiol on the ubiquitin–proteasome pathway. Role of the transcription activator and DNA binding of AhR. J Biol Chem 273:8432–8438.

Morrissey RE, Harris MW, Diliberto JJ, Birnbaum LS. 1992. Limited PCB antagonism of TCDD-induced malformations in mice. Toxicol Lett 60:19–25.

Nguyen TA, Haivist D, Lee JE, Safe S. 1999. Interactions of nuclear receptor coactivator/corepressor proteins with the aryl hydrocarbon receptor complex. Arch Biochem Biophys 367:256–257.

Phelan D, Winter GM, Rogers WJ, Lam JC, Denison MS. 1998. Activation of the Ah receptor signal transduction pathway by bilirubin and biliverdin. Arch Biochem Biophys 357:155–163.

Quadri SA, Qadri AN, Hahn ME, Mann KK, Sherr DH. 2000. The biolavonoid galingin blocks aryl hydrocarbon receptor activation and poly cyclic aromatic hydrocarbon-induced pre-B cell apoptosis. Mol Pharmacol 58:515–525.

Ricci MS, Toscano DG, Mattingly CJ, Toscano WA Jr. 1999. Estrogen receptor reduces CYP1A1 induction in cultured human endometrial cells. J Biol Chem 274:3430–3438.

Roberts BJ, Whitelaw ML. 1999. Degradation of the basic helix-loop-helix/Per-ARNT-Sim homology domain dioxin receptor via the ubiquitin/proteasome pathway. J Biol Chem 274:36351–36356.

Safe S. 1989. Polychlorinated biphenyls (PCBs), dibenzo-\(p\)-dioxins (PCDDs), dibenzofurans (PCDFs) and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). CRC Crit Rev Toxicol 21:51–88.

———. 1994. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. CRC Crit Rev Toxicol 24:87–149.

———. 1998a. Development, validation and problems with the TEF approach for risk assessment of dioxins and related compounds. J Anim Sci 76:134–141.

———. 1998b. Limitations of the toxic equivalency factor approach for risk assessment of TCDD and related compounds. Teratogenesis Carcinogen Mutagen 17:285–304.

Savouret JF, Antenas M, Queene M, Xu J, Milgram E, Casper RF. 2001. 7-Ketocholesterol is an endogenous modulator for the aryl hydrocarbon receptor. J Biol Chem 276:2054–2059.

Shertzer HG, Puga A, Chang C, Smith P, Nebert DW, Setchel KD, et al. 1999. Inhibition of CYP1A1 enzyme activity in mouse hepatoma cell culture by soybean isoflavones. Chem Biol Interact 123:31–49.

Sinal CJ, Bend JR. 1997. Aryl hydrocarbon receptor-dependent induction of Cyp1a1 by bilirubin in mouse hepatoma Hepa 1c1c7 cells. Mol Pharmacol 52:590–599.

Thenot S, Chargin M, Bonnet S, Cavailles V. 1999. Estrogen receptor coactivator expression in breast and endometrial human cancer cells. Mol Cell Endocrinol 156:85–93.

Tysklind M, Bosveld ATC, Andersson P, Verhallen E, Simnige T, Seinen W, et al. 1995. Inhibition of ethoxyresorufin-O-deethylase (EROD) activity in mixtures of 2,3,7,8-tetrachlorodibenzo-\(p\)-dioxin and polychlorinated biphenyls. Environ Sci Pollut Res 2:411–216.

Van den Berg M, Birnbaum L, Bosveld ATC, Brunstrom B, Cook P, Feeley M, et al. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect 106:775–792.

van Leeuwen FXR, Feeley M, Scherek D, Larsen JC, Farland VH, Younges M. 2000. Dioxins: WHO’s tolerable daily intake (TDI) revisited. Chemosphere 40:1095–1101.

Verdeal K, Ryan DS. 1979. Naturally-occurring estrogens in plant foodstuffs—a review. J Food Prot 42:577–583.

Wang HW, Chen TL, Yang PC, Ueng TH. 2001. Induction of cytotochrosmes P450 1A1 and 1B1 by emodin in human lung adenocarcinoma cell line CL5. Drug Metab Dispos 29:1229–1235.

Wormke M, Stoner M, Saville B, Safe S. 2000. Crosstalk between estrogen receptor α and the aryl hydrocarbon receptor in breast cancer cells involves unidirectional activation of proteomes. FEBS Lett 478:109–112.

Zhao F, Mayura K, Harper N, Safe S, Phillips TD. 1997a. Inhibition of pentachlorobiphenyl-induced fetal cleft palate and immunotoxicity in CS7BL6 mice by 2,2',4,4',5,5'-hexachlorobiphenyl. Chemosphere 34:1005–1013.

Zhao F, Mayura K, Kocurek N, Edwards JF, Kubena LF, Safe S, et al. 1997b. Inhibition of 3,3',4,4',5,5'-pentachlorobiphenyl-induced chicken embryo toxicity by 2,2',4,4',5,5'-hexachlorobiphenyl. Fundam Appl Toxicol 35:1–8.