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Why Toxic Equivalency Factors Are Not Suitable for Perfluoroalkyl Chemicals

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Abstract

The pervasive nature of perfluoroalkyl chemicals in the environment has generated considerable interest for developing new strategies for risk assessment. In experimental animal models, exposure to perfluoroalkyl chemicals can cause developmental toxicity and hepatotoxicity. Peroxisome proliferator-activated receptor-α (PPARα) is required to mediate some but not all of these effects. Since PPARα has a role in mediating some of these effects, and there is some overlap in the type of toxicities elicited by perfluoroalkyl chemicals, it has been suggested that a scaling system analogous to the toxic equivalency factor (TEF) system used for polychlorinated dibenzo-*p*-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), and polychlorinated biphenyls of species differences in the response mediated by different receptors as well as qualitative differences in toxicities elicited by perfluoroalkyl chemicals. These differences and other data gaps preclude the development of a TEF approach for perfluoroalkyl chemicals.

Graphical Abstract

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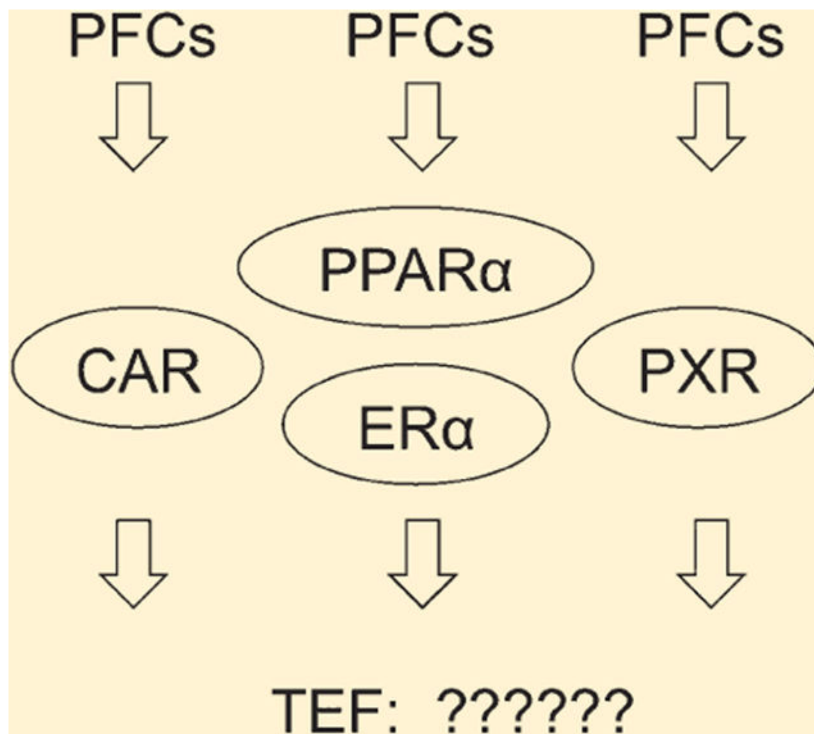

INTRODUCTION

Perfluoroalkyl chemicals exhibit unique surfactant properties that led to their extensive use for many consumer applications including fire-fighting foam, additives in self-shine floor polishes, cement, lubricants, paint, gasoline, and paper, textile and leather treatments, waterproofing of clothing and carpets, and oil repellants in food packages.[1] Two classes of perfluoroalkyl chemicals that are commonly measured in environmental samples are the perfluorinated carboxylic (PFCAs) and sulfonic acids (PFSAs), which have a fatty acid-like structure with a carbon backbone and covalently linked fluorine atoms and a carboxylic acid or sulfonic acid group at one end (Figure 1). It is important to note that perfluoroalkyl chemicals include both PFCAs and PFSAs that contain different acidic groups (Figure 1). The major commercial perfluorinated chemicals are perfluorinated sulfonamide polymers, which are used for stain protection in carpets and textiles, and perfluorinated sulfon-amide-based phosphate fluorosurfactants, which are used as leveling and wetting agents and to greaseproof paper food packaging.[2] Because of their prevalent use and persistent nature, it is not surprising that perfluoroalkyl chemicals have been detected in human serum.[3] Since administration of perfluoroalkyl chemicals can cause toxicity in experimental animal models, including hepatic effects, tumorigenesis, developmental toxicity, and immunotoxicity,[4–6] there remains considerable interest in determining whether exposure to this class of chemicals represents a health risk in humans.

Risk assessment involves both quantitative and qualitative examinations of hazards, including those due to the presence of chemical carcinogens, toxicants, or microbes. There are a number of approaches used to assess risk of different chemicals. Understanding the

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mode of action for any chemical can be very useful for risk assessment because it provides a strong rationale for establishing a causal relationship between chemical exposure and a toxic effect. For example, exposure to *d*-limonene causes renal tumors in male rats through an interaction with $\alpha 2$ μ-globulin leading to hyaline droplets and sustained renal cell proliferation thought to drive tumor formation.[7] However, since humans do not express $\alpha 2$ μ-globulin or an $\alpha 2$ μ-globulin homologue, *d*-limonene-induced renal cancer is unlikely to occur in humans because the mode of action is not functional in the absence of $\alpha 2$ μ-globulin expression.[7] In addition to illustrating the importance of understanding the mode of action for examining relative risk for a chemical, this example also shows that species differences should be considered when this information is available. In recent years, experiments conducted with null mouse models have shown that some chemicals mediate their toxic effects through receptor-based modes of action. For example, the aryl hydrocarbon receptor (AHR) is required to mediate acute toxicity induced by tetracholordibenzo-*p*-dioxin (TCDD) as well as TCDD-induced developmental toxicity,[8,9] the constitutive androstane receptor (CAR) is required to mediate hepatotoxicity induced by phenobarbital,[10] and PPARα is required for hepatocarcinogenesis caused by chronic exposure to the PPARα agonists such as Wy-14,643 and bezafibrate.[11,12] Knowing that a class of chemicals can bind to and activate the AHR and that this class of chemicals elicited similar toxicity in many species contributed to the development of the toxic equivalency factors (TEF) as a tool to help assess the risk associated with exposure to AHR ligands.[13,14]

The concept of TEF currently used for polychlorinated dibenzo-*p*-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), and polychlorinated biphenyls (PCB) is based on the prerequisite that the mechanism of action is understood. For PCDDs, PCDFs, and PCBs, it was recognized that the mode of action for the toxicities associated with these chemicals was mediated by binding to and activation of the AHR.[15–17] It is implied that all molecular events in the mode of action following activation of the AHR are dependent on AHR activities. This allowed for the development of a model whereby the relative toxicity of each chemical was based on its relative ability to bind to and activate the AHR, by comparison with the standard AHR agonist TCDD.[13,14] Thus, the determination of relative risk due to exposures to various mixtures of PCDDs, PCDFs, and/or PCBs was aided by allowing for an estimation of AHR activity based on the relative ability of the chemical to bind to and activate the AHR, which in turn modulates molecular events leading to toxicity. This model relies on several assumptions to justify this approach: (1) the mechanism of toxicity is mediated by one receptor, the AHR; (2) the class of chemicals used for TEF has to cause toxic responses similar to those of TCDD; and (3) there is sufficient evidence demonstrating that the class of chemicals produces toxic effects that are additive.[13,14]

While the TEF provides a common approach for aiding risk assessment for exposure to PCDDs, PCDFs, and PCBs, this approach also has inherent weaknesses.[18,19] For example, additivity is not always observed for all end points for all PCDDs, PCDFs, and PCBs.[19] Moreover, the relative bioavailability and pharmacokinetics of PCDDs, PCDFs, and PCBs is not taken into account, there are known AHR-independent toxic effects induced by some chemicals that activate the AHR, and the molecular mechanisms by which AHR-mediated changes in gene expression cause toxicity remain unclear.[18]

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Recently, Scialli and colleagues performed analysis to determine whether a TEF system could be developed and applied for perfluoroalkyl chemicals.[20] Their analysis focused on data from rat studies examining the toxic effect of four perfluoroalkyl chemicals, PFOA, PFDA, PFOS, and PFBS. While this analysis indicated that combining exposures of perfluoroalkyl chemicals using a TEF approach was not feasible due primarily to discordance in the available data sets, it was suggested that future analysis with new data could provide the basis for developing TEFs for perfluoroalkyl chemicals. Since the time of the Scialli publication,[20] additional data have become available that have increased our knowledge, and these data likely preclude the use of TEFs for perfluoroalkyl chemicals. The main factors that limit the TEF approach are as follows: (1) lack of conclusive evidence demonstrating that a single receptor is required to mediate the toxicities of perfluoroalkyl chemicals; (2) the potential influence of species differences in the response to PPARα ligands that would significantly limit this approach; (3) inconsistent toxicities observed with different perfluoroalkyl chemicals; and (4) a limited toxicological database for a number of perfluoroalkyl chemicals (e.g., perfluorinated sulfonamide polymers and perfluorinated sulfonamide-based phosphate fluorosurfactants). The following sections summarize recent advances to illustrate these points.

IS THERE A SINGLE RECEPTOR-MEDIATED MECHANISM FOR PERFLUOROALKYL CHEMICAL-INDUCED TOXICITIES?

Perfluoroalkyl chemicals can cause hepatotoxicity, tumorigenesis, developmental toxicity, and immunotoxicity.[4–6] The hypothesis that these effects are mediated by a single receptor (PPARα) is based primarily on four lines of evidence: (1) the changes observed in the liver in response to perfluoroalkyl chemicals (e.g., hepatomegaly, increased expression of lipid metabolizing enzymes, etc.) are similar to those observed with PPARα agonists;[4,21–23] (2) perfluoroalkyl chemicals can activate PPARα based on reporter assays and increased expression of PPARα target genes;[24–30] (3) PPARα is required for some perfluoroalkyl chemical-induced hepatic effects or developmental toxicity[22,31–34] (Tables 1 and 2); and (4) it is known that many effects, in particular hepatocarcinogenesis, induced by PPARα agonists are mediated through a mechanism that requires PPARα.[35,36] The evidence supporting the hypothesis that perfluoroalkyl chemicals cause toxicities exclusively through a mechanism that is mediated by PPARα alone is not strong.

Chemicals that activate PPARα typically contain a hydrophobic region that binds hydrophobic amino acids in the ligand binding domain of the receptor and an acidic group that forms hydrogen bonds with tyrosine residues in the ligand binding pocket of the receptor.[37,38] Thus, it is not surprising that PFCAs and PFSAs activate PPARα because both types of perfluoroalkyl chemicals have hydrophobic and acidic regions (Figure 1). The structure—activity relationship between perfluoroalkyl chemicals with increasing carbon length and PPARα activity is of interest, but the dose—response curves do not exhibit good linearity.[24,30] This shows that the relationship between the structure and the ability to activate PPARα is complicated and could be influenced by indirect effects such as the release of endogenous PPARα ligands due in part to the surfactant properties of perfluoroalkyl chemicals, which could influence receptor activity. The complexity of this

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relationship is also nicely illustrated by the observations that whereas PFOA can activate PPARα at lower concentrations as compared to PFOS,[28,29] the effect of developmental exposure to PFOS can be greater as compared to PFOA.[20] This also suggests that PPARα-independent mechanisms could mediate the developmental toxicity associated with perfluoroalkyl chemical exposure in experimental animal models. Indeed, this is supported by the studies showing that while the postnatal lethality observed in response to gestational exposure to PFOA or PFNA requires PPARα since postnatal lethality is only found in wild-type and not Pparα-null mice.[39,40] In contrast, postnatal lethality observed following gestational exposure to PFOS occurs independently of PPARα because postnatal lethality is found in both wild-type and *Ppara*-null mice[41] (Table 1). Whether these differences in phenotype are due in part to structural differences between PFOA/PFNA, which are PFCAs, and PFOS, which is a PFSA, remains unclear. Moreover, while the postnatal lethality observed in response to gestational exposure to PFOA is mediated by PPARα, PFOA-induced early full litter resorptions does not require PPARα39 (Table 1). Administration of PFOA has also been shown to stimulate mammary gland development in both wild-type and *Ppara*-null mice, indicating that mechanisms independent of PPARα are required for this effect.[42] In addition to PPARα-independent effects observed following exposure to perfluoroalkyl chemicals, there is also evidence showing that perfluoroalkyl chemicals can elicit changes in liver that are not mediated by PPARα (Table 2). Relatively low dose exposure to PFOA (0.1—0.3 mg/kg) that results in serum PFOA concentrations similar to those found in humans, increases liver weight, and this effect is not found in mice that do not express PPARα.[31] This is similar to the PPARα-dependent hepatomegaly observed in response to PFBA.[22] In contrast, higher concentrations of PFOA cause hepatomegaly through a mechanism that does not require PPARα,[32,33] an effect that is similar to that found following administration of PFOS.[34] These observations suggest that activating PPARα and the subsequent pleiotropic effects that result is not the only mechanism that mediates the effects of perfluoroalkyl chemicals in the liver. Whether the observed differences in the susceptibility to the developmental and/or liver-specific effects of perfluoroalkyl chemicals are due in part to differences in pharma-cokinetics has not been examined extensively to date. The hypothesis that other mechanisms, independent of PPARα, modulate the effects induced by perfluoroalkyl chemicals is supported by microarray analysis and reporter gene assays, which revealed that in addition to PPARα, perfluoroalkyl chemicals also activate other transcription factors including PPARβ/δ, PPARγ, CAR, and PXR[24–29] (Table 3). Indeed, increased expression of PPARα, CAR, and PXR target genes are found in rat liver following treatment with PFOA, and these changes are associated with hepatomegaly and hepatocyte hypertrophy and hyperplasia.[21] More recently, it was shown that perfluoroalkyl chemicals also weakly interfere with human estrogen receptor-α (ERα)[43] (Table 3), although another study indicates that PFOA and PFOS do not activate ERα.[44] Combined, these observations strongly suggest that the mechanism of toxic action for perfluoroalkyl chemicals is considerably more complicated than the hypothesis that they induce their effects exclusively by activating PPARα. The relative contribution of receptors other than PPARα to the mechanisms underlying perfluoroalkyl chemical-induced effects has not been examined extensively to date.

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DO SPECIES DIFFERENCES IN PPARα ACTIVITIES CONFOUND PREDICTION OF THE MODE OF ACTION OF PERFLUOROALKYL CHEMICAL-INDUCED TOXICITIES?

Many studies show that there are significant species differences in PPARα activities. PFBA, PFHA, PFOA, PFNA, PFDA, PFBS, PFHS, and PFOS can all activate PPARα based on reporter assays.[24,28–30] However, in general, the concentration of perfluoroalkyl chemicals required to minimally activate mouse PPARα is considerably lower as compared to the concentration of perfluoroalkyl chemicals required to activate human PPARα.[30] Additionally, the magnitude of the response to perfluoroalkyl chemicals is generally greater for mouse PPARα as compared to human PPARα.[30] These differences found in reporter assays are similar to multiple studies showing that the hepatic responses (e.g., induction of PPARα target genes, hyperplasia, peroxisome proliferation, etc) induced by PPARα ligands in rodent liver, hepatocytes, and/or liver cell lines are typically greater as compared to human cells (reviewed in refs 36 and 45). For example, PFOA induces expression of PPARα target genes in primary rat hepatocytes, but these changes are markedly attenuated in primary human hepatocytes or HepG2 cells cultured in medium containing PFOA.[46] PPARα-humanized mice respond to PPARα ligand by increasing the expression of lipid catabolizing enzymes, an effect also found in wild-type mice, but they are resistant to PPARα ligand-induced hepatic effects including hyperplasia and hepatocarcinogenesis.[47,48], Studies with mice expressing the human PPARα have also provided evidence that a species difference in the response to perfluoroalkyl chemicals exists. Whereas PFBA increases liver weight, causes hepatocyte hypertrophy, and upregulates PPARα target genes in both wild-type and PPARα-humanized mice, hepatocyte focal necrosis with inflammatory cell infiltrate is only found in wild-type mice but not in *Ppara*-null or PPARα-humanized mice treated with PFBA.[22] These observations show that while PFBA can activate both mouse and human PPARα, there is a species difference in the hepatic effects mediated by mouse and human PPARα. Wild-type mice exposed to PFOA at doses (0.1 and 0.3 mg/kg) that cause increases in serum PFOA concentration comparable to the concentration of PFOA found in humans exhibit increased liver weight and increased expression of PPARα target genes in liver.[31] Since these changes are not found in similarly treated *Ppara*-null mice or PPARα-humanized mice, this demonstrates that relatively low dose exposure of PFOA differentially activates mouse and human PPARα in a mouse model. In contrast, PFOA administered at higher doses (5.0 mg/kg) increases liver weight in wild-type, *Ppara*-null, and PPARα-humanized mice.[32] This effect was found in all three genotypes, thus suggesting that the effects of PFOA administered at higher doses are not mediated exclusively by PPARα. Administration of 5 mg/kg PFOA activated both mouse and human PPARα as shown by the increased expression of fatty acid catabolizing enzymes, but evidence that the effects of PFOA in the liver were differentially affected by either the mouse or human PPARα was also observed. For example, increased presence of lobular inflammatory cells and macrovesicular steatosis in liver was not found in either PFOA-treated wild-type or PPARα-humanized mice but was noted in PFOA-treated *Ppara*-null mice.[32] An increased incidence of hepatic microvesicular steatosis was only found in PPARα-humanized mice administered PFOA, but this effect was not observed in either wild-type or *Ppara*-null mice

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exposed to PFOA.[32] Moreover, hydropic degeneration was observed in *Ppara*-null mice and PPARα-humanized mice treated with PFOA but not in wild-type mice.[32] Combined, exposure to relatively low doses of PFOA can elicit some hepatic effects in wild-type but not *Ppara*-null or PPARα-humanized mice, thus supporting the notion that there are differences in the effects modulated by mouse or human PPARα. However, the diversity in phenotypes observed in response to higher dose exposure to PFOA also suggests that the presence of mouse or human PPARα can each differentially modulate the hepatic effects induced by PFOA in mice. Indeed, in response to PFOA administration, relatively unique phenotypes were found in mice expressing endogenous PPARα, mice lacking expression of endogenous PPARα, and PPARα-humanized mice that lack expression of mouse PPARα but express the human PPARα. This demonstrates the complexity in the mode of action for at least one perfluoroalkyl chemical, PFOA, and suggests that the ability to predict the risk of PFOA-dependent toxicity in humans using data from animal studies is not likely feasible if it is assumed that both mouse and human PPARα mediate similar biological outcomes.

QUALITATIVE DIFFERENCES IN TOXICITIES INDICATE MULTIPLE MODES OF ACTION FOR PERFLUOROALKYL CHEMICAL-INDUCED TOXICITIES

Despite evidence indicating that perfluoroalkyl chemicals elicit toxicity, at least in part, through activation of PPARα, there is also qualitative evidence indicating that other modes of action are likely involved in mediating perfluoroalkyl chemical-induced toxicities (Table 4). For example, chronic administration of PFOA causes hepatocellular adenomas, pancreatic acinar cell adenomas, and Leydig cell adenomas in rats, a phenomenon termed the “tumor triad”.[49] The tumor triad is also found with other PPARα agonists, thus supporting the notion that PPARα could mediate these effects.[45] However, while an increase in the incidence of hepatocellular adenomas is observed following chronic administration of another perfluoroalkyl chemical, PFOS, no significant dose-related changes in the incidence of Leydig cell tumors or pancreatic acinar cell tumors were found.[50] Qualitative differences in toxicity following exposure to perfluoroalkyl chemicals have also been observed for other end points. Whereas developmental exposure to PFBA in mice does not cause neonatal lethality, developmental exposure to PFOA, PFOS, or PFNA causes neonatal lethality in mice (Table 4). Similarly, while administration of PFBS to rats can cause alterations in the kidney, this pathology is not found in rats administered PFBA or PFHA (Table 4). Administration of PFOS causes a marked decrease in serum cholesterol concentration in nonhuman primates, but these changes are not found in this species following administration of PFOA (Table 4). In addition to qualitative differences in the type of toxicities observed in response to exposure to different perfluoroalkyl chemicals, qualitative differences in some end points can also be highly variable in different strains of rodents within species when administered the same perfluoroalkyl chemical. For example, PFOA inhibits mammary gland development in CD1, Balb/c, and C57BL/6 mice but enhances mammary gland development in C57BL/6 mice at lower doses (Table 4). Qualitative differences in the response to perfluoroalkyl chemicals have also been noted with in vitro studies.[51] While secretion of estradiolor progesterone by human adrenocortical H295R carcinoma cells is increased following exposure to PFOS, secretion of estradiol and progesterone by H295R cells is unchanged following treatment with either PFOA or PFNA (Table 4). Further,

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secretion of testosterone by H295R cells is increased in response to PFOS and PFOA, whereas secretion of testosterone by H295R cells is decreased in response to PFNA (Table 4). Combined, while some of these qualitative differences could be influenced by structural differences between PFCAs and PFSAs, or differences in pharmacokinetics, it is clear that there is marked diversity in the types of alterations induced by individual perfluoroalkyl chemicals.

LIMITED TOXICOLOGICAL DATABASE OF COMMERCIALLY USED PERFLUOROALKYL CHEMICALS

The predominant commercially used perfluoroalkyl chemicals are perfluorinated sulfonamide polymers used for stain protection in carpets and textiles and perfluorinated sulfonamide-based phosphate fluorosurfactants used as leveling and wetting agents and to greaseproof paper food packaging.[2] Recent evidence suggests that both direct and indirect exposure to perfluorinated sulfonamide polymers and/or perfluorinated sulfonamide-based phosphate fluorosurfactants represent significant sources of human contamination.[2] However, while these compounds are an important exposure source, it is currently unknown whether perfluorinated sulfonamide polymers and/or perfluorinated sulfonamide-based phosphate fluorosurfactants are capable of activating PPARα or other nuclear receptors. Moreover, while there are relatively large toxicological databases for many PFCAs and PFSAs, comparative studies examining relative exposure and the effects of perfluorinated sulfonamide polymers and/or perfluorinated sulfonamide-based phosphate fluorosurfactants is lacking. Thus, even if a TEF approach could be developed for PFCAs and PFSAs, the lack of comparative databases for perfluorinated sulfonamide polymers and/or perfluorinated sulfonamide-based phosphate fluorosurfactants limits the suitability of TEFs for the broader class of perfluoroalkyl chemicals.

DISCUSSION

There are three assumptions used to help justify the use of TEFs for risk assessment: (1) the mechanism of toxicity is mediated by one receptor; (2) the class of chemicals used for TEF has to cause toxic responses similar to those of a model compound of this class; and (3) there is sufficient evidence demonstrating that the class of chemicals produces toxic effects that are additive.[13,14] While the TEF approach has been used for risk assessment for exposure to PCDDs, PCDFs, and PCBs, there are inherent deficiencies in interpretation.[18,19] Previous analysis by others demonstrated that TEFs were not suitable for risk assessment of four perfluoroalkyl chemicals, PFOA, PFDA, PFOS, and PFBS, due primarily to discordance in the available data sets.[20] However, it was also suggested that future analysis with new data could provide the basis for developing TEFs for perfluoroalkyl chemicals.[20] Since this time, it has become increasingly clear that TEFs are not likely suitable for the risk assessment of perfluoroalkyl chemicals for a number of critical reasons (Figure 2).

There is now compelling evidence that the toxicities induced by perfluoroalkyl chemicals are not mediated by a single receptor. While the PPARα modulates the gene expression profiles and the observed hepatic changes in response to some perfluoroalkyl chemicals, there are other receptors in the liver that can also be activated by these agents including CAR

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and PXR that could influence the resulting effects. In extra-hepatic tissues, the evidence that PPARα is the sole receptor that mediates toxicological events induced by perfluoroalkyl chemicals is not convincing. In fact, enhanced mammary gland development caused by PFOA does not require PPARα.[42] More studies are needed using other null mouse models including compound null mouse models to more conclusively determine the relative role of multiple receptors in the mode of action for perfluoroalkyl chemical toxicities. However, given the likelihood that more than one receptor is involved in mediating the effects of perfluoroalkyl chemicals in different tissues, this caveat significantly limits the suitability of TEFs for risk assessment purposes. This is also complicated by the fact that there can be significant species differences in receptor biology including potential tissue differences in coactivators recruited and/or corepressors dissociated from the receptor complexes following ligand activation or potential differences in target genes for any given nuclear receptor. This is particularly true for PPARα and CAR where it is recognized that significant species difference in receptor function exists, in ligand binding and biological effects. If one or more nuclear receptors were found to have essential roles in modulating effects induced by perfluoroalkyl chemicals, additional studies would still be necessary to determine whether there is a species difference in receptor biology. For this reason, the use of humanized mice and/or complementary analysis of rodent versus human cells types would be of great benefit.

The second limiting factor that precludes developing TEFs for perfluoroalkyl chemical risk assessment is the discordance in toxicities resulting from exposure. For example, PFOA can activate PPARα at lower concentrations as compared to PFOS,[28,29] but the effect of developmental exposure to PFOS can be greater as compared to PFOA.[20] Additionally, there are many examples where one perfluoroalkyl chemical causes a specific effect, while other perfluoroalkyl chemicals do not (Table 4). Moreover, in some cases the same perfluoroalkyl chemical can cause different effects depending on the species, strain, or substrain used. For example, PFOA can either inhibit or enhance mammary gland development in C57BL/6 mice.[52] Some of these differences could be related to marked differences in the pharmacokinetics of perfluoroalkyl chemicals.[53–57] The discordant nature of effects elicited by perfluoroalkyl chemicals further confounds the identification of a model perfluoroalkyl chemical to form the basis of an equivalency factor to establish TEFs. For the AHR, TCDD is the chemical of choice because it causes toxicity at lower concentrations and with greater efficacy than other chemicals in the class of AHR activators. For perfluoroalkyl chemicals, the identity of a chemical that could be used for this purpose has yet to be determined. Given the discordance in effects resulting from perfluoroalkyl chemical exposure, this may not be feasible.

The third limiting factor that precludes the development of TEFs for perfluoroalkyl chemical risk assessment is the lack of evidence demonstrating additivity of the effects induced by this class of chemicals. One major limitation in this data gap is the fact that the effects of perfluoroalkyl chemicals are unlikely to be mediated by a single receptor. Since the TEF model is based on the notion that only one receptor mediates the effects of a class of chemical, and it is clear that the biological effects of perfluoroalkyl chemicals are mediated by more than one receptor, additivity may be extremely difficult, if not impossible, to establish due to the complicated mode(s) of action. For example, while low dose exposure to perfluoroalkyl chemicals induces changes that appear to be mediated by PPARα, higher

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doses of perfluoroalkyl chemicals can also activate other receptors such as CAR and PXR. Since the activity of CAR can significantly inhibit PPARα activity,[58] this type of interaction could make it very difficult to establish an additive effect for perfluoroalkyl chemicals.

The fourth and final limiting factor that precludes the development of TEFs for perfluoroalkyl chemical risk assessment is the lack of a comprehensive toxicological database on exposure and effects of perfluorinated sulfonamide polymers and/or perfluorinated sulfonamide-based phosphate fluorosurfactants. These perfluorinated chemicals may represent a significant source of human exposure, and as compared to PFCAs or PFSAs, considerably less is known about the toxicities induced by these chemicals. Thus, it would be difficult to establish similarities in toxicities, a prerequisite for establishing TEFs. Further, whether these chemicals or the metabolites derived from these chemicals can activate PPARα or other nuclear receptors is also unclear. Moreover, whether a single receptor mediates the toxicities induced by perfluorinated sulfonamide polymers and/or perfluorinated sulfonamide-based phosphate fluorosurfactants has not been examined to date.

Combined, there are at least four facts that diminish the feasibility of the development of TEFs for perfluoroalkyl chemical risk assessment including the following: (1) the effects of perfluoroalkyl chemicals are modulated by more than one receptor, (2) the discordant nature of the effects induced by perfluoroalkyl chemicals, (3) the lack of data demonstrating additivity of effects by this class of chemicals, and (4) the lack of a strong toxicological database for commonly used commercial perfluoroalkyl chemicals. There are also inherent limitations for TEFs that exist including the following: (1) they ignore the issue of bioavailability/pharmacokinetics, (2) there is an incomplete understanding of the target genes that mediate toxicity, (3) the potential influence of species differences that could be modulated by differences in expression patterns of receptor coactivators/corepressors, receptor function, (4) the potential for nonadditive effects, and (5) the uncertain influence of endogenous chemicals that may interact with receptors that mediate the effects for this class of chemicals.[18,19] While a TEF approach might theoretically be developed for application with a smaller, select group of perfluoroalkyl chemicals, this would still require identification of a single receptor that mediates the relevant changes to the most sensitive biological end point, and all of the aforementioned limitations would have to be controlled for. This is currently not feasible and would not be a trivial avenue of investigation to pursue. Given these major limitations pertaining to both perfluoroalkyl chemicals and/or TEFs, the development of TEFs for the broad class of perfluoroalkyl chemicals is likely unsuitable.

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ABBREVIATIONS

AHR	aryl hydrocarbon receptor
CAR	constitutive androstane receptor

ER	estrogen receptor
PCB	polychlorinated biphenyls
PCDD	polychlorinated dibenzo-*p*-dioxins
PCDF	polychlorinated dibenzofurans
PFBA	perfluorobutanoic acid
PFBS	perfluorobutane sulfonic acid
PFDA	perfluorodecanoic acid
PFHA	perfluorohexanoic acid
PFHS	perfluorohexane sulfonic acid
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonic acid
PPAR	peroxisome proliferator-activated receptor-α
PXR	pregnane X receptor
TCDD	tetracholordibenzo-*p*-dioxin
TEF	toxic equivalency factor

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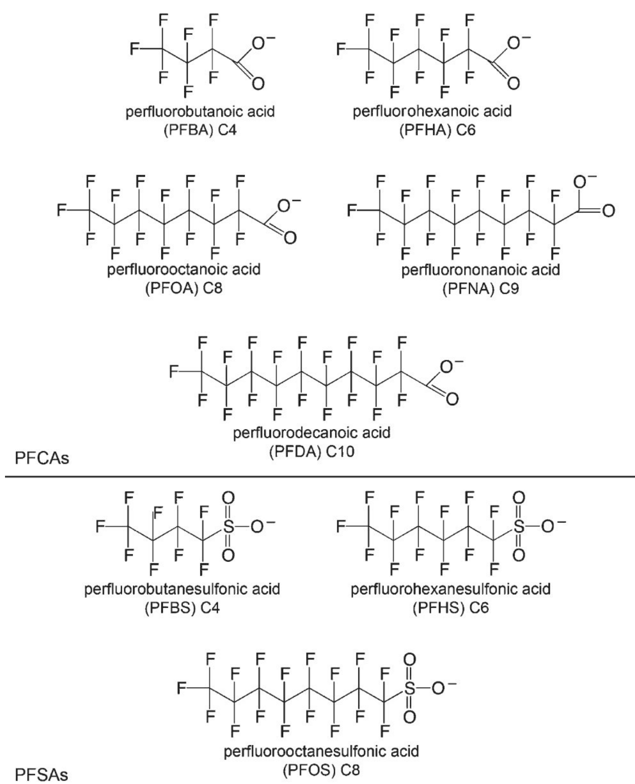

Figure 1.

Chemical structures of representative perfluorinated carboxylic and sulfonic acids. Anionic forms are shown to reflect physiological speciation.

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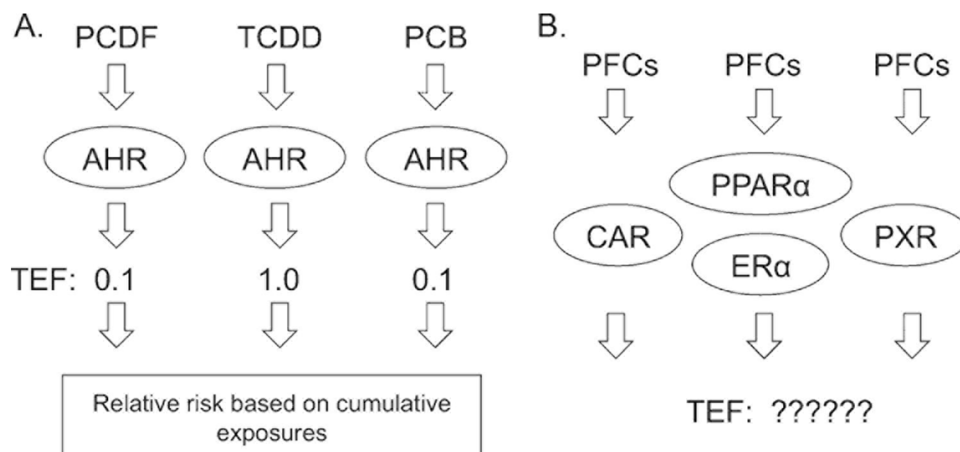

Figure 2.

Contrasting the logic of TEFs for PCDDs, PCDFs, and PCBs versus TEFs for perfluoroalkyl chemicals. (A) PCDDs, PCDFs, and PCBs all bind to and activate the AHR, which is required to mediate the primary toxic effects induced by these classes of chemicals. The relative toxic equivalency factor for each chemical is calculated relative to the reference chemical, TCDD, and used to estimate relative risk because of assumed additivity in effects. The TEFs listed are simply examples, except that for TCDD, which is always 1. (B) Perfluoroalkyl chemicals (PFCs) can activate PPARα, but they also interact with other nuclear receptors that may or may not mediate effects induced by this class of chemicals (e.g., CAR, PXR, PPARβ/δ, PPARγ, ERα, etc.). Because more than one receptor could mediate the effects induced by PFCs, development of a TEF approach does not appear suitable. Moreover, because of discordance in the available data, it is not feasible to establish a reference chemical to estimate a toxic equivalency factors.

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Table 1.

Role of PPARα in Modulating Developmental Effects Induced by Perfluoroalkyl Acids in Mice

compd	dose (mg/kg)	PPARα-dependent effect?	PPARα-independent effect?	refs
PFOA (C8)	0.1–20.0	yes (neonatal lethality)	yes (full litter resorption)	39
PFNA (C9)	0.83–2.0	yes (neonatal lethality)	no	40
PFOS (C8)	4.5–10.5	no	yes (neonatal lethality)	41

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Table 2.

Role of PPARα in Modulating Hepatic Effects Induced by Perfluoroalkyl Acids in Mice

Compd	dose (mg/kg)	PPARα-dependent?	refs
PFBA (C4)	35–350	yes	22
PFOA (C8)	0.1–0.3	yes	31
PFOA (C8)	1.0–5.0	no	32
PFOA (C8)	1.0–10.0	no	33
PFOS (C8)	10–40	no	34

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Table 3.

Perfluoroalkyl Acids Interact with/Activate Mouse or Human Nuclear Receptors[a]

Compd	pparα	PPARβ/δ	PPARγ	CAR	PXR	ERα	refs
PFBA (C4)	yes	ND	ND	ND	ND	ND	30
PFHA (C6)	yes	ND	ND	ND	ND	ND	30
PFOA (C8)	yes	yes	yes	yes	yes	yes	24–30,43
PFNA (C9)	yes	ND	ND	ND	ND	yes	30,43
PFDA (C10)	yes	ND	ND	yes	ND	yes	25,30,43
PFBS (C4)	yes	ND	ND	ND	ND	ND	30
PFHS (C6)	yes	ND	ND	ND	ND	ND	30
PFOS (C8)	yes	yes	yes	yes	yes	yes	28–30,43,59

[a]On the basis of the analysis using reporter assays, gene expression profiles, confirmation in null mouse models and/or in *silica* modeling. yes = evidence of interaction/activation; ND = not determined to date.

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Table 4.

Qualitative Differences in Responses to Perfluoroalkyl Acids

compd	Species	dose range (mg/kg/day)	duration	response	references
PFOA	Rat	30[a]	104 weeks	hepatocellular tumors, pancreatic acinar cell tumors, leydig cells tumors	49
PFOS	rat	1.0 ± 0.4	104 weeks	hepatocellular tumors	50
PFBA	mouse	35–350	GD1-GD17	no postnatal lethality	60
PFOA	mouse	0.6–20	GD1-GD18	postnatal lethality	39
PFOS	mouse	1–20	GD1-GD18	postnatal lethality	61
PFNA	mouse	0.83–2	GD1-GD18	postnatal lethality	40
PFBA	rat	1.2–30	90 days	no change in kidney pathology	62
PFBS	rat	60–600	90 days	hyperplasia of epithelial cells of the medullary and papillary tubules and ducts in the inner medullary regions	63
PFHA	rat	10–200	90 days	no change in kidney pathology	64
PFOA	monkey	3–10	180 days	no change in serum cholesterol concentration	65
PFOS	monkey	0.03–0.75	180 days	decreased serum cholesterol concentration	66
PFOA	mouse (cd1)	5	GD1-GD17	delayed mammary gland development	67
PFOA	mouse (c57bl/6)	1–10	4 weeks (peripubertal)	enhanced and delayed mammary gland development	52
PFOA	mouse (balb/c)	1–10	4 weeks (peripubertal)	delayed mammary gland development	52
PFOS	human adrenocortical h295r cells	0.006–600 μM[b]	48 h of culture	increased estradiol, progesterone and testosterone secretion	51
PFOA	human adrenocortical h295r cells	0.006–600 μM	48 h of culture	no change in estradiol, progesterone secretion; increased testosterone secretion	51
PFNA	human adrenocortical h295r cells	0.006–600 μM	48 h of culture	no change in estradiol, progesterone secretion; decreased testosterone secretion	51

[a] Calculated based on average food intake of 10 g/100 g body weight/day[68] with weights estimated from Figure 1 in Biegel et al.[49]

[b] Concentration in culture medium since this was not an in vivo study.