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Secondary Effects of Catalytic Diesel Particulate Filters: Reduced Aryl Hydrocarbon Receptor-Mediated Activity of the Exhaust

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Diesel exhaust contains numerous toxic substances that show different modes of action such as triggering aryl hydrocarbon receptor (AhR)-mediated pathways. We investigated AhR-mediated activity of exhaust generated by a heavy-duty diesel engine operated with or without iron- or copper/iron-catalyzed diesel particulate filters (DPFs). AhR agonists were quantified using the DR-CALUX reporter gene assay (exposure of cells for 24 h). We found 54–60 ng 2,3,7,8-tetrachlorodibenzo-*p*-dioxin CALUX equivalents (TCDD-CEQs) per m^3 of exhaust in unfiltered samples and 6–16 ng TCDD-CEQ m^{-3} in DPF-treated samples. DPF applications decreased TCDD-CEQ concentrations by almost 90%. Concentrations of known AhR agonists were determined with GC/HRMS and converted to TCDD-CEQ concentrations using compound-specific relative potency values. The analyzed nine polycyclic aromatic hydrocarbons (PAHs) and the 17 2,3,7,8-chlorinated dibenzodioxins/furans (2,3,7,8-PCDD/Fs) contributed only marginally (0.6–1.6%) to the total agonist concentration. However, both DPFs also decreased concentrations of individual PAHs by 70–80%. Variation of the assay exposure time (8, 24, 48, 72, and 96 h) revealed that AhR-mediated activity decreased over time and reached a plateau after 72 h, which was most likely due to biotransformation of AhR agonists by the exposed H4IIE cells. At the plateau, we measured 1–2 ng TCDD-CEQ m^{-3} in both an unfiltered and a filtered exhaust sample. Our findings show that DPFs are a promising technology to detoxify diesel exhaust regarding compounds with AhR-mediated activity.

Introduction

Emissions from diesel engines are complex mixtures of carbonaceous soot particles, hundreds of other combustion products, and unburned fuel and lubrication oil ingredients (*1–3*). Compounds found in diesel exhaust exhibit various chemical structures, physicochemical properties, toxicities, and physiological effects. In our study, we focused on exhaust constituents with aryl hydrocarbon receptor (AhR)-mediated activity. AhR agonists are toxicologically relevant compounds. The AhR is involved in detoxification pathways such as the metabolic degradation of polycyclic aromatic hydrocarbons (PAHs) and other exogenous compounds (*4*). On the other hand, AhR-dependent transformation of certain xenobiotics (e.g., benzo[*a*]pyrene) leads to mutagenic or carcinogenic metabolites (*5*). Furthermore, the long-time activation of the AhR is a key issue for the toxicity of polychlorinated dibenzodioxins/furans (PCDD/Fs) and related persistent organic pollutants (*6, 7*). PCDD/Fs substituted with chlorine at the 2, 3, 7, and 8 position (2,3,7,8-PCDD/Fs) are the most potent AhR agonists known. It has been reported that AhR agonists exhibit antiestrogenic (*8*) and antiandrogenic effects (*9*). Thus, AhR-mediated pathways may be involved in those endocrine-disrupting effects that were observed in both the male and the female reproductive system of mice and rats, which had been exposed to diesel exhaust or diesel particles (*10, 11*).

AhR-mediated activity of single fractions of diesel exhaust (i.e., either particles or semivolatile compounds) has been reported in four in vitro studies, which used various AhR-based end points and different bioassays (*3, 12–14*). In contrast to these previous investigations, in the study presented herein, integral samples, including particle-bound and semivolatile compounds, were analyzed and the overall AhR-mediated activity was quantified as equivalents of the model AhR agonist 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD). We used an in vitro reporter gene assay, the dioxin responsive-chemically activated luciferase expression (DR-CALUX) assay (*15*), to detect AhR agonists in diesel exhaust and to investigate biotransformation of AhR agonists by rat hepatoma H4IIE cells. Assay responses were expressed as 2,3,7,8-TCDD CALUX equivalents per m^3 of exhaust (TCDD-CEQ m^{-3}). Additionally, we used gas chromatography/high resolution mass spectrometry (GC/HRMS) to quantify known AhR agonists. We measured nine toxicologically relevant PAHs (fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, and benzo[*ghi*]perylene) and the 17 2,3,7,8-PCDD/F congeners. To estimate the contribution of these known agonists to the total AhR-mediated activity, GC/HRMS data were converted into TCDD-CEQ concentrations by applying relative potency (REP) values form literature (*16, 17*).

Diesel-powered engines contribute appreciable numbers of fine (<2.5 μm aerodynamic diameter) and ultrafine (<0.1 μm) particles to the air in urban and heavy traffic areas (*1, 18*) and in work places such as mines and construction sites (*19*). These respirable particles pose a health threat to the urban population and to occupationally exposed persons as they are associated with airway inflammation, asthma, allergies, cardiovascular diseases (*20, 21*), and probably also with lung cancer (*22*). To counteract adverse health effects, diesel particulate filters (DPFs) were developed. State-of-the-art DPF-technology lowers the number of diesel particles by more than 95% (*19, 23*). DPFs are increasingly used on- and off-roads due to more and more stringent regulations

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for particulate emissions. Besides the primary effect on particles, DPFs also affect emissions of other exhaust constituents (*23*). These secondary effects may be favorable, when emissions of particle-bound compounds are lowered, or unfavorable. Unfavorable secondary effects include increased emissions of pollutants formed in the DPF by unwanted side-reactions, for example, by chlorination or nitration of primary exhaust constituents (*19*). DPFs provide conditions such as elevated temperatures (Supporting Information (SI), Figure S1), accumulation of reactants (e.g., organic compounds, catalytically active metals from fuel additives or other sources (*19*)), and elongated residence time (e.g., due to sorption to accumulated particles), which may, in principle, also promote the formation of additional AhR agonists. Indeed, it has been found that certain catalytic DPFs induce the formation of PCDD/Fs (*19*).

Primary and secondary effects of DPFs alter the composition of diesel exhaust. Consequently, the application of DPFs may influence AhR-mediated activity of the exhaust as well. We hypothesized that exhaust treatment by DPF would (i) result in diesel exhaust with decreased AhR-mediated activity if most AhR agonists are sorbed to particles, that it would (ii) result in exhaust with no distinct change in activity if most AhR agonists are volatile enough to penetrate the DPF, and that it would (iii) result in exhaust with increased AhR-mediated activity if additional AhR agonists are formed in the DPF. Three major classes of DPFs can be distinguished depending on the strategy used for filtering and combustion of diesel particles: (i) filters built of porous or fibrous substrates coated with catalysts, typically noble metals, (ii) filters consisting of uncoated substrates, which accumulate fuel-borne catalysts, typically transition metal oxides, and (iii) uncoated filters, which use active regeneration, for example burners (*23*). In the present study, we evaluated two commercial, uncoated, cordierite-based, wall-flow DPFs, for which soot combustion was either catalyzed with an iron- or a copper/iron-based fuel additive. We investigated effects of both DPF systems on AhR agonist emissions, using either chlorine-free or chlorine-enriched diesel fuel. Experimental conditions with chlorine-enriched fuel represent a worst case scenario with respect to PCDD/F formation, as previously discussed elsewhere (*19*).

Experimental Section

Diesel Engine Operation and Sampling. Exhaust was generated with a heavy-duty diesel engine with direct fuel injection (Liebherr, type D914T, 6.11 L, 4 cylinders, 105 kW, Bulle, Switzerland) and was either treated by DPF or not filtered. The engine was run with commercial diesel fuel (class D, SN 181190–1:2000), subsequently called reference fuel, and operated according to the eight-stage ISO 8178/4 C1 test cycle valid for construction site engines (SI, Figure S1) (*19*). Emission factors of the engine for CO_2 and regulated pollutants are listed in the Supporting Information (Table S1) and are discussed elsewhere (*19*). Sampling was performed according to the filter/condenser method described in European standard EN-1948–1 (*24*). All-glass sampling devices (SI, Figure S2), consisting of a sampling probe, a quartz fiber filter, a cooler, a condensate separator, and a two-stage adsorber unit (XAD-2), were used to obtain samples that included particle-bound and semivolatile compounds. Samples were collected in individual sampling devices, which all had been cleaned and heated (450 °C) to prevent against possible chemical contamination. Aliquots of undiluted exhaust, which were proportional to the actual mass flow, were taken during two consecutive runs (200 min) of the test cycle, yielding 4.4–6.7 m^3 of exhaust per sample. Consequently, the obtained samples represent a mixed full- and partial-engine load operation, including load transitions, which corresponds to operation modes typical for construction machinery. Table 1 describes the collected samples.

TABLE 1. Experimental Conditions during Generation of Diesel Exhaust Samples

sample code	chlorine (μg g^{-1})[a]	fuel additive (μg g^{-1})[b]	DPF[c]
ref[d]	none (<2)	none (<0.1/<0.1)	none
Fe	none (<2)	Fe (4.5)	none
FeF	none (<2)	Fe (4.5)	F
Cu	none (<2)	Cu (9)/Fe (7.5)	none
CuF	none (<2)	Cu (9)/Fe (7.5)	F
Cl	Cl (14)	none (<0.1/<0.1)	none
ClFe	Cl (14)	Fe (4.5)	none
ClFeF	Cl (14)	Fe (4.5)	F
ClCu	nc	nc	nc
ClCuF	Cl (14)	Cu (9)/Fe (7.5)	F
xCl	nc	nc	nc
xClFe	nc	nc	nc
xClFeF	nc	nc	nc
xClCu	nc	nc	nc
xClCuF	xCl (110)	Cu (9)/Fe (7.5)	F

[a] Chlorine content determined by WD-XRF analysis (*19*). [b] Iron and copper content determined by ICP-OES (*19*). [c] Two new diesel particulate filters (DPFs; Greentop) were used in combination with an iron- or a copper/iron-based fuel additive. [d] A commercial, low-sulfur diesel fuel was used as reference and for the production of chlorine- and/or additive-containing fuels (density: 824.3 kg m^{-3}, cetane number: 56.0, sulfur: 16 μg g^{-1}). ref: reference fuel; Fe: reference fuel with iron additive; Cu: reference fuel with copper/iron additive; Cl, xCl: reference fuel with chlorine; F: exhaust treatment by diesel particulate filter; nc: not collected.

Diesel Particulate Filters and Fuel Additives. Two new, uncoated, cordierite-based, monolithic, wall-flow DPFs (100 cpsi, 22.8 L, Greentop, Grävenwiesbach, Germany) were tested in combination with an iron- or a copper/iron-based fuel additive (ITN, Krakow, Poland). Both additives were mixed with reference fuel to yield two blends with final iron- and copper/iron-concentrations of 4.5 and 9.0/7.5 μg g^{-1}, respectively (*19*). The influence of these two DPF systems on AhR agonist emissions was studied under standard condition with chlorine-free fuel (<2 μg Cl g^{-1} (*19*)) and under worst case condition with chlorine-enriched fuel (Table 1). Additional chlorine in the form of 1,6-dichlorohexane (Fluka, Buchs, Switzerland) was added to reference fuel, resulting in chlorine levels of 14 and 110 μg Cl g^{-1}, respectively (*19*). This simulated a worst case scenario for secondary formation of chlorinated AhR agonists such as PCDD/Fs (*19*). PCDD/Fs are the most potent AhR agonists known and DPFs have a considerable potential to support their formation. PCDD/Fs are easily formed at temperatures between 270 and 430 °C (SI, Figure S1) if sufficient amounts of chlorine are present (*19*).

Sample Extraction and Preparation. All solvents were pro analysis quality or better and were purchased from Merck (Darmstadt, Germany) or Biosolve (Valkenswaard, The Netherlands). After sample collection, each sampling device was rinsed with acetone, toluene, and dichloromethane. The condensate was extracted with dichloromethane and hexane. The rinsing solvents and the extract of the condensate were combined, concentrated, and diluted with toluene. The toluene-diluted extract was used to extract XAD-2 adsorbents and filter materials for 24 h using a Soxhlet apparatus. The Soxhlet extract was filtered, dried with Na_2SO_4, and concentrated to the final volume. Five percent (w/w) of each extract were set aside for bioassay analysis, without performing any further cleanup, and were adjusted to a volume of 20 mL. Out of this, 0.1–1.0 mL were transferred to glass vials and solvent was evaporated (50 °C, N_2). At the point of

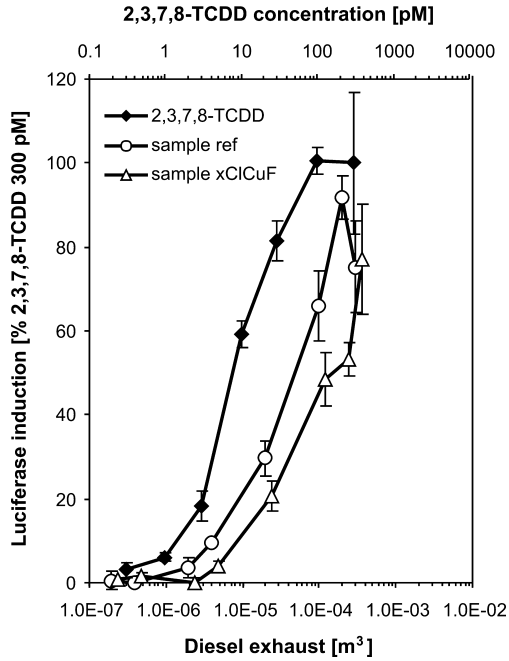

FIGURE 1. Dose–response curves (DR-CALUX, 24-h exposure) of the reference compound 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD, rhombs), a sample of unfiltered exhaust (ref; circles), and a sample of exhaust treated by diesel particulate filter (xClCuF; triangles). Data represent the mean ± standard deviation of triplicate determinations and are expressed as luciferase induction in percent of the highest test concentration of 2,3,7,8-TCDD (300 pM).

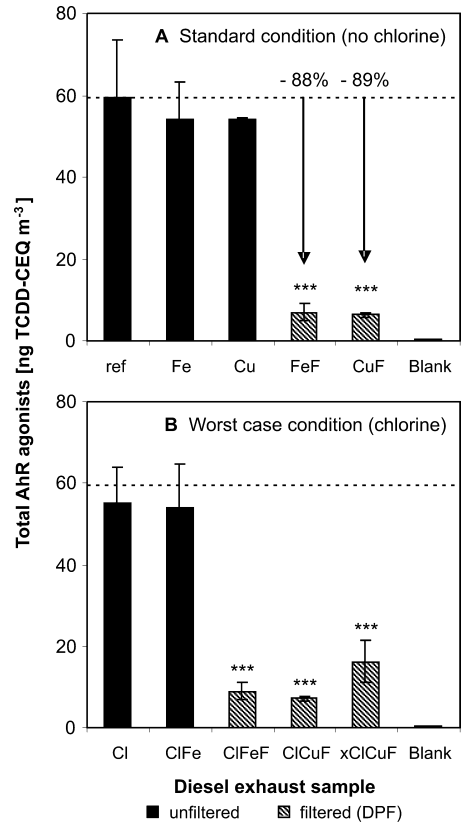

FIGURE 2. Concentrations of aryl hydrocarbon receptor (AhR) agonists in unfiltered (filled bars) and filtered exhaust (hatched bars) collected either under standard (A; chlorine-free fuel) or worst case condition (B; chlorine-enriched fuel). Values are expressed as 2,3,7,8-TCDD CALUX equivalent (TCDD-CEQ) concentrations and represent the mean ± standard deviation of averaged triplicate determinations of two to three exposure experiments (DR-CALUX, 24-h exposure). See Table 1 for a description of the collected samples, including sample abbreviations. (DPF, diesel particulate filter; ***, $p \leq 0.001$, compared with ref).

dryness, 1.0–1.2 mL of dimethylsulfoxide (DMSO, extra pure, Merck, Darmstadt, Germany) was added. Samples in DMSO were stored in the dark at 4 °C. The remaining 95% (w/w) of the Soxhlet extracts were used for GC/HRMS analysis.

Blank Control and Sampling Spike. A sampling device, which was not spiked and not used for sample collection, was extracted and prepared for assay analysis as described above. This yielded a blank control of the sampling device and the extraction and sample preparation procedures. The average sample volume (5.8 m^3) was used to calculate the assay response of the blank control per m^3 of exhaust. We determined 0.48 ± 0.01 ng TCDD-CEQ m^{-3}, which was 13−124 times below the concentrations detected for the collected samples (Figure 2).

Prior to sampling, quartz swab that contained a sampling spike, consisting of $^{13}C_{12}$-labeled 1,2,3,4,6,7-hexachlorodibenzodioxin (HxCDD), $^{13}C_6$-naphthalene, $^{13}C_6$-phenanthrene, and $^{13}C_3$-pyrene (CIL, Andover, MA), was placed into the condensate separator of each sampling device (SI, Figure S2). This allowed an estimation of recoveries by GC/HRMS. Mean overall recoveries of 26 ± 9% for naphthalene, 34 ± 5% for phenanthrene, 59 ± 5% for pyrene, and 57 ± 11% for HxCDD were obtained, covering sampling, extraction, and analysis. As expected, recoveries for naphthalene, the most volatile PAH, and phenanthrene, a 3-ring PAH, were low. Recoveries for pyrene, a semivolatile 4-ring PAH, and HxCDD were comparable and in the expected range of ∼60%. Luciferase induction of the sampling spike was tested separately, using the DR-CALUX assay. The final DMSO solution of the sampling spike did not exhibit any detectable AhR-mediated activity.

Reporter Gene Assay. The DR-CALUX assay is a reporter gene assay used to detect AhR-mediated activity (*15*). The assay is based on rat hepatoma H4IIE cells that are stably transfected with the AhR-controlled luciferase reporter gene construct pGudluc1.1. AhR agonists induce luciferase gene expression in a time- and dose-dependent manner and are indirectly detected via luciferase activity.

Cell culture and assay analysis were performed following the protocols of BioDetection Systems (BDS, Amsterdam, The Netherlands), as described in details elsewhere (*25*). Cells were exposed in triplicate to extracted samples, solvent controls, and serial dilutions of 2,3,7,8-TCDD (0.3–300 pM in DMSO, BDS) with a final DMSO concentration of 0.8% (v/v). To quantify AhR-mediated activity, cells were incubated for 24 h. Triplicate determination of each sample was repeated in a total of two to three exposure experiments. To assess the influence of time and dose on AhR-mediated activity, cells were exposed for 8, 24, 48, 72, and 96 h with a dilution series of samples ref and xClCuF (Table 1).

After cell exposure, luciferase activity was measured as relative light units (RLU) on a microplate luminometer (MLX, Dynex, Chantilly, VA). All RLUs values were corrected for background activity detected in the presence of DMSO alone. Data obtained from the 2,3,7,8-TCDD dilution series was fitted to a sigmoidal curve ($y = a_0/[1 + (x/a_1)^{a_2}]$); y, measured activity in RLUs; x, 2,3,7,8-TCDD concentration in well; a_0, maximum activity in RLUs; a_1, EC_{50}; a_2, slope factor; user-defined curve fit in Microsoft Excel 2003). Luciferase activity induced by samples was converted to TCDD-CEQ concentrations by using the inverse function of the fitted 2,3,7,8-TCDD curve ($x = a_1([a_0/y - 1]^{1/a_2})$). Further details on cell culture, assay analysis, and quality assurance/quality control are given in the Supporting Information.

GC/HRMS Analysis. Known AhR agonists were quantified by gas chromatography/high resolution mass spectrometry (GC/HRMS). Concentrations of nine 4- to 6-ring PAHs (fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, and benzo[*ghi*]perylene), most of them rated as carcinogenic by IARC, were measured as described in the Supporting Information, and concentrations of the 17 2,3,7,8-PCDD/F congeners were measured as described elsewhere (*19*).

To convert GC/HRMS data into TCDD-CEQ concentrations, we used compound-specific relative potency (REP) values that have been determined in the DR-CALUX assay, using an exposure time of 24 h (*16, 17*). REP values express the activity of a single substance relative to a reference compound, in our case 2,3,7,8-TCDD. REPs based on the 5% effect concentration (EC_5) of the dose–response curve of 2,3,7,8-TCDD were applied, as determined by Behnisch et al. (*16*). For compounds, of which EC_5-based REPs were not available, EC_{25}-based REPs reported by Machala et al. were used (*17*) (SI, Table S4).

Statistics. Single-factor ANOVA with post hoc testing (Tukey) was performed for multiple comparisons of samples (Systat 10). *P*-values are mentioned in the Results and Discussion section.

Results and Discussion

AhR Agonists in Diesel Exhaust. The DR-CALUX assay was used to detect and quantify exhaust constituents that activate AhR-mediated pathways. Figure 1 shows the dose–response curve of 2,3,7,8-TCDD and two samples (ref, xClCuF) in a 24 h exposure experiment. The extracts of the exhaust samples induced a dose-dependent response similar to the 2,3,7,8-TCDD curve allowing a quantification of the AhR-mediated activity as TCDD-CEQ concentrations. Unfiltered exhaust (ref) exhibited a maximum luciferase induction of about 90% relative to 2,3,7,8-TCDD. The maximum induction of DPF-treated exhaust (xClCuF) could not be determined. Precipitation during sample concentration did not allow achieving a full dose–response curve. Nevertheless, quantitative analysis was possible throughout the quantifiable range between the limit of quantification (LOQ) and the 50% effect concentration (EC_{50}) of the 2,3,7,8-TCDD curve.

As shown in Figure 2, AhR agonists were found in all examined samples. Concentrations varied from 54 to 60 and from 6 to 16 ng TCDD-CEQ m^{-3} for unfiltered and DPF-treated exhaust, respectively (SI, Table S2). Our data confirm results of earlier in vitro studies (*3, 12–14*); diesel exhaust contains compounds that bind to the AhR and induce AhR-mediated gene expression in mammalian cells.

Effects of Diesel Particulate Filters on AhR Agonist Emissions. To assess effects of catalytic DPFs on AhR agonist emissions, samples of unfiltered exhaust and of DPF-treated exhaust were tested using the DR-CALUX assay. Two sets of samples were analyzed, those collected under standard condition (Figure 2A) and those generated with chlorine-enriched diesel fuel (Figure 2B). Chlorine was added to simulate a worst case scenario by supporting a potential formation of chlorinated AhR agonists such as PCDD/Fs (*19*). Figure 2 illustrates that both DPFs substantially lowered AhR agonist concentrations, whereas fuel additives as well as chlorine had no large effects. AhR-mediated activity of samples of unfiltered exhaust, generated with additive- or chlorine-containing fuels (Fe, Cu, Cl, ClFe), was similar ($p \geq 0.995$) to the activity of the reference sample (ref) of 60 ± 14 ng TCDD-CEQ m^{-3}. Mean values of 54 ± 9, 54 ± 0.4, 55 ± 9, and 54 ± 11 ng TCDD-CEQ m^{-3} were obtained for these samples of unfiltered exhaust (SI, Table S2). AhR-mediated activity of DPF-treated samples (FeF, CuF, ClFeF, ClCuF, xClCuF) was significantly decreased ($p \leq 0.001$) compared to the reference sample (ref). Mean AhR agonist concentrations of 7 ± 2, 6 ± 1, 9 ± 2, and 7 ± 1 ng TCDD-CEQ m^{-3} were found. A higher concentration of 16 ± 5 ng TCDD-CEQ m^{-3} was determined for sample xClCuF. Because fuel additives did not influence AhR-mediated activity in the absence of a DPF, we conclude that exhaust treatment by DPF lowered AhR-mediated activity of the exhaust.

Under standard condition (Figure 2A), that is, when using chlorine-free diesel fuel, the DPFs tested did not lead to different AhR-mediated activities (FeF, CuF). Compared to the reference point (ref), the iron-catalyzed DPF lowered TCDD-CEQ concentrations by 88% and the copper/iron-catalyzed DPF by 89%. This substantial decline in AhR agonist concentration is a beneficial secondary effect of both catalytic DPFs. Consequently, exhaust treatment by both DPFs should also reduce the AhR-mediated toxicity and/or the endocrine-disrupting potential of diesel exhaust. Furthermore, these findings indicate that particles trapped in DPFs must act as carriers for most AhR agonists or AhR agonists with high REPs. Those AhR agonists released from the DPFs were either not sorbed to trapped particles or emitted at higher operating temperatures or formed within the DPFs.

Under conditions favoring the formation of PCDD/Fs (Figure 2B), that is, when using chlorine-enriched diesel fuel, both DPF systems (ClFeF, ClCuF) generated similar AhR-mediated activities as under standard condition without additional chlorine (FeF, CuF). Heeb et al. (*19*) reported an intense formation of PCDD/Fs when the copper/iron-catalyzed DPF was operated in combination with chlorine-enriched fuel. Emissions of 2,3,7,8-PCDD/Fs (I-TEQ) increased by a factor of 4 (ClCuF) to 250 (xClCuF), compared to the unfiltered reference sample (ref) (SI, Table S3). However, DR-CALUX analysis did not indicate such an increase but showed a substantial reduction of the AhR-mediated activity (ClCuF, xClCuF) with respect to sample ref (Figure 2). These findings indicate that the AhR-mediated activity of the PCDD/Fs was strongly masked by the activity of other AhR agonists such as the PAHs, which were effectively filtered by both DPFs (Figure 3).

Contribution of PAHs and PCDD/Fs to the Observed Bioassay Activity. Nine PAHs, which represent only a small proportion of all the PAHs present in diesel exhaust, and all the toxicologically relevant 17 2,3,7,8-PCDD/F congeners were analyzed by GC/HRMS (SI, Table S3). To compare GC/HRMS and bioassay data, concentrations of all compounds measured were converted to TCDD-CEQs by applying REP values (*16, 17*). We assumed that weighted concentrations of individual compounds were additive and no synergistic effects occurred.

Figure 3 reveals that the efficiency of both DPFs in reducing the TCDD-CEQ concentration of the nine summed PAHs is comparable to their efficiency in reducing the overall amount of AhR agonists present in diesel exhaust. About 70–80% of the nine PAHs expressed as TCDD-CEQs were retained by both DPFs under standard (Figure 3A) and worst case conditions (Figure 3B), indicating that most of the measured PAHs were sorbed to trapped particles or degraded in the DPFs. The nine PAHs contributed 0.6–1.2% to the total agonist concentration in samples without exhaust treatment (ref, Fe, Cu, Cl, ClFe) and 0.5–1.6% in DPF-treated samples (FeF, CuF, ClFeF, ClCuF, xClCuF). In all samples, approximately one-third of the summed activity of the nine PAHs was due to benzo[*b*]fluoranthene. Depending on the experimental conditions, other major contributors were benzo[*k*]fluoranthene, pyrene, and indeno[1,2,3-*cd*]pyrene (SI, Table S4). Benzo[*ghi*]perylene did not contribute to the AhR-mediated activity because it does not induce luciferase gene expression in the DR-CALUX assay (*17*).

As shown in Figure 3, the concentration of the summed 2,3,7,8-PCDD/Fs amounted to 0.004–0.01 ng TCDD-CEQ m^{-3}

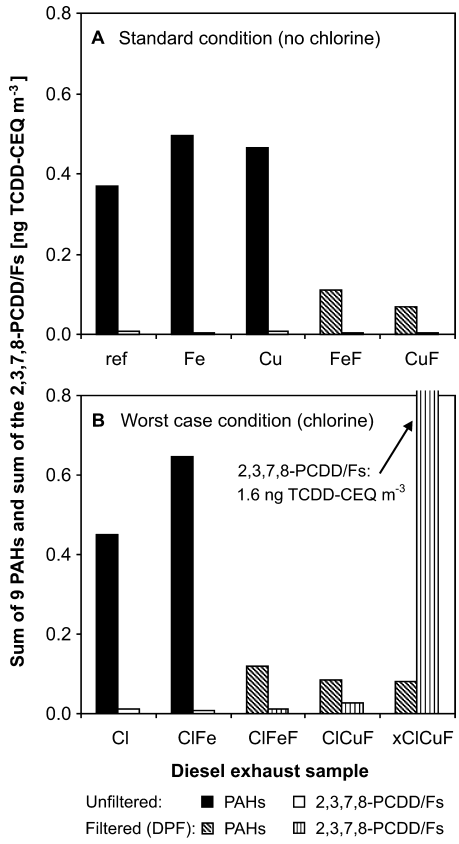

FIGURE 3. Concentrations of nine PAHs (sum of fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*] fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, and benzo[*ghi*] perylene) and concentrations of the 17 2,3,7,8-PCDD/F congeners (sum) measured by GC/HRMS and expressed as 2,3,7,8-TCDD CALUX equivalent (TCDD-CEQ) concentrations based on compound-specific relative potencies (*16, 17*). See Table 1 for a description of the collected samples (DPF, diesel particulate filter).

in samples without exhaust treatment (ref, Fe, Cu, Cl, ClFe). Thus, the 17 2,3,7,8-PCDD/F congeners contributed only very little to the total AhR-mediated activity of unfiltered exhaust, for which a mean value of 55 ng TCDD-CEQ m^{-3} was obtained (Figure 2). Under standard condition (Figure 3A), both DPF systems did not change the concentration of 2,3,7,8-PCDD/Fs, which remained at 0.002–0.003 ng TCDD-CEQ m^{-3}. In case of chlorine-enriched fuels (Figure 3B), DPFs increased emissions of 2,3,7,8-PCDD/Fs to about 0.01–0.03 ng TCDD-CEQ m^{-3} (ClFeF, ClCuF), but the contribution to the total AhR-mediated activity remained small (0.1–0.4%). An exception was sample xClCuF (110 μg Cl g^{-1}), where the calculated assay response of the 2,3,7,8-PCDD/Fs was 1.6 ng TCDD-CEQ m^{-3}, which was 60–660 times higher than in all other samples (Figure 3). Thus, the 2,3,7,8-PCDD/Fs were responsible for 10% of the total AhR-mediated response in sample xClCuF. This partially explains the increased AhR-mediated activity compared to all other DPF-treated samples. According to GC/HRMS analysis, PCDD/F emissions were strongly increased by the copper/iron-catalyzed DPF when operated in combination with diesel fuel of high chlorine content (110 μg Cl g^{-1}). The copper-catalyzed formation of PCDD/Fs in this particular DPF is described in detail elsewhere (*19*).

In summary, the nine selected PAHs and the 17 2,3,7,8-PCDD/Fs accounted for 0.6–1.2% of the total agonist concentrations determined in the DR-CALUX assay for samples without exhaust treatment. Excluding sample xClCuF, these 26 compounds explained 1.1–1.6% of the TCDD-CEQ concentrations found in DPF-treated samples. The analyzed PAHs clearly exceeded the contribution of the

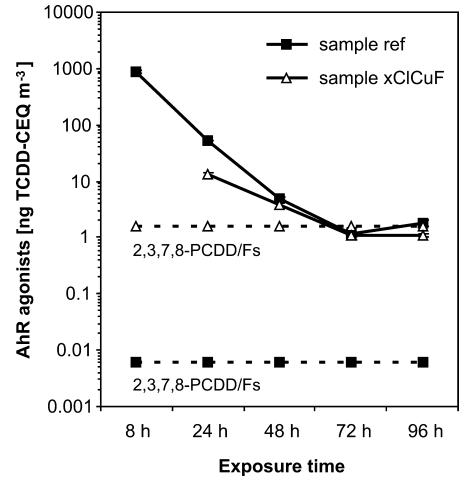

FIGURE 4. Time-dependent concentrations of aryl hydrocarbon receptor (AhR) agonists (solid lines) in a sample of unfiltered (ref; squares) and of filtered diesel exhaust (xClCuF; triangles). After 72 h of cell exposure (DR-CALUX assay), concentrations remained stable at 1.1–1.8 ng 2,3,7,8-TCDD CALUX equivalents per m^3 of exhaust (TCDD-CEQ m^{-3}). Estimated contributions of the 2,3,7,8-PCDD/Fs (dashed lines) were 0.006 (ref; squares) and 1.6 ng TCDD-CEQ m^{-3} (xClCuF; triangles).

2,3,7,8-PCDD/Fs (Figure 3), except for sample xClCuF (SI, Table S4). Furthermore, we conclude that the majority of the AhR agonists present in unfiltered and filtered diesel exhaust are not identified, yet.

Time-Dependent Activity of AhR Agonists in Diesel Exhaust. We investigated the biotransformation of exhaust constituents with AhR-mediated mode of action. H4IIE cells were exposed to an unfiltered sample (ref) and to a DPF-treated sample (xClCuF) for various exposure times of 8, 24, 48, 72, and 96 h. Longer lasting exposure experiments were not conducted, because ideal conditions for cell culture could not be maintained due to overgrowth and acidification of the medium. Both samples showed a fast and substantial decrease of AhR-mediated activity over time, as illustrated in Figure 4. A plateau of 1.1–1.8 ng TCDD-CEQ m^{-3} was reached in both samples after 72 h. At this plateau, TCDD-CEQ concentrations have decreased by 98% (ref) and 93% (xClCuF) compared to the 24 h concentrations. This indicates that most of the AhR agonists present in the analyzed extracts were transformed into nonactivating compounds by the exposed rat hepatoma H4IIE cells. Possible candidates for metabolizable AhR agonists are PAHs and their derivatives (e.g., nitro- and oxy-PAHs). Li et al. (*26*) reported that most of the AhR binding affinity of an extract of diesel particles was due to PAHs and nitro-PAHs. Mason (*12*) showed that AhR binding activity was highest in the nitro-PAH fraction of an extracted sample of diesel particles. In own experiments, we measured the highest AhR-mediated activity in the PAH fraction of a sample of unfiltered exhaust (data not shown).

We suppose that nonmetabolizable AhR agonists are responsible for the remaining activity of 1.1–1.8 ng TCDD-CEQ m^{-3} after 72 h. The contribution of the 2,3,7,8-PCDD/Fs, which accounted for 0.006 and 1.6 ng TCDD-CEQ m^{-3} in samples ref and xClCuF (SI, Table S4), respectively, was assumed to be constant over the examined exposure times due to their persistency (Figure 4). Based on this assumption, the 2,3,7,8-PCDD/Fs were responsible for 0.5% of the plateau concentration in case of unfiltered exhaust (ref). Thus, 99.5% of the TCDD-CEQ concentration measured after 96 h was due to unidentified AhR agonists, which were not transformed into nonactivating compounds within the tested 96 h. In case of sample xClCuF, the plateau concentration was fully explained by the 17 2,3,7,8-PCDD/F congeners (Figure 4). As

reported, an intense PCDD/F formation was noticed when operating the copper-catalyzed DPF in presence of chlorine (*19*).

Environmental Perspective. Depending on the exposure time, the total TCDD-CEQ concentrations in all measured exhaust samples were 11–8800-fold higher than the German guideline for 2,3,7,8-PCDD/F concentration in municipal waste incinerator exhaust (0.1 ng I-TEQ Nm^{3-}). However, our in vitro data are based on total AhR agonists present in diesel exhaust and do not account for the different uptake- and elimination-rates or the physiological effects of AhR agonists in vivo. Therefore, our in vitro data cannot directly be compared with guidelines based on the TEQ-concept. Nevertheless, it becomes clear that further efforts are needed to identify the yet unknown AhR agonists in diesel exhaust.

In vitro studies have shown that AhR agonists are present in airborne particulate matter (APM) (*12, 27*) and are distributed throughout the atmosphere (*27*). Hamers et al. used the DR-CALUX assay to investigate AhR-mediated activity of a series of APM samples (*27*). They detected particle-bound AhR agonists in concentrations corresponding to 0.6–11 (6 h of cell exposure) and <0.3–0.8 pg TCDD-CEQ m^{-3} of sampled air (48 h of cell exposure). Using the same reporter gene assay, we found 880 and 5 ng TCDD-CEQ m^{-3} of unfiltered diesel exhaust after 8 and 48 h of cell exposure, respectively (Figure 4, ref). These values are 4−6 orders of magnitude higher than the concentrations reported by Hamers et al. (*27*). Hence, diesel engines when operated without DPF are a relevant source for atmospheric AhR agonists.

The results presented herein show that both DPFs decreased AhR agonist concentrations by almost 90% and, thereby, reduced emissions of compounds with potential toxic and/or endocrine-disrupting properties (*5–9*). Nevertheless, the copper/iron-catalyzed DPF is not recommended for exhaust treatment because of an intense secondary formation of PCDD/Fs (*19*). DPF applications may particularly be of importance for persons that work or live at places exposed to diesel exhaust, which are, for example, underground workplaces with diesel-powered equipment (e.g., mines, tunnels) and heavy traffic areas with impaired exchange of air (e.g., urban canyons). Indeed, two studies indicate that vehicle emissions locally increase the concentration of AhR agonists in ambient air (*12, 27*).

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Supporting Information Available

ISO 8178/4 C1 test cycle (Figure S1), sampling device (Figure S2), cell culture, assay analysis, quality assurance/quality control for assay analysis, emission factors for CO_2 and regulated pollutants (Table S1), AhR-mediated activity of diesel exhaust (Table S2), PAH analysis, GC/HRMS data (Table S3), REP values and calculated TCDD-CEQ concentrations (Table S4). This material is available free of charge via the Internet at http://pubs.acs.org.

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