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TRP channels and traffic-related environmental pollution-induced pulmonary disease

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Abstract

Environmental pollutant exposures are major risk factors for adverse health outcomes, with increased morbidity and mortality in humans. Diesel exhaust (DE) is one of the major harmful components of traffic-related air pollution. Exposure to DE affects several physiological systems, including the airways, and pulmonary diseases are increased in highly populated urban areas. Hence, there are urgent needs to (1) create newer and lesser polluting fuels, (2) improve exhaust aftertreatments and reduce emissions, and (3) understand mechanisms of actions for toxic effects of both conventional and cleaner diesel fuels on the lungs. These steps could aid the development of diagnostics and interventions to prevent the negative impact of traffic-related air pollution on the pulmonary system. Exhaust from conventional, and to a lesser extent, clean fuels, contains particulate matter (PM) and more than 400 additional chemical constituents. The major toxic constituents are nitrogen oxides (NOx) and polycyclic aromatic hydrocarbons (PAHs). PM and PAHs could potentially act via transient receptor potential (TRP) channels. In this review, we will first discuss the associations between DE from conventional as well as clean fuel technologies and acute and chronic airway inflammation. We will then review possible activation and/or potentiation of TRP vanilloid type 1 (TRPV1) and ankyrin 1 (TRPA1) channels by PM and PAHs. Finally, we will discuss and summarize recent findings on the mechanisms whereby TRPs could control the link between DE and airway inflammation, which is a primary determinant leading to pulmonary disease.

Keywords

TRPV1; TRPA1; Asthma; Acute lung injury; Diesel exhaust particles (DEP); Polycyclic aromatic hydrocarbons (PAHs); Clean diesel

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Introduction

Epidemiologic investigations have linked human morbidity and mortality to elevated levels of traffic-related air pollution and ambient particulate matter (PM) [1–3]. Ambient and synthetic PM is capable of inducing airway inflammation [4]. Besides PM, engine emissions contain more than 400 species of harmful chemicals, many of which are incorporated into ambient PM. Key components of chemicals in traffic-related air pollutants are polycyclic aromatic hydrocarbons (PAHs), which also have strong apoptotic and pro-inflammatory actions on airways [5, 6]. Some of the main producers of PMs and PAHs in traffic-related air pollutants are diesel engines. Diesel-related PAHs may represent a major source of gaseous hydrocarbons in urban environments [7]. In this review, we will discuss data on how diesel exhaust (DE) and two major toxic constituents, PM and PAHs, could involve transient receptor potential channels (TRPs) to regulate multiple cellular pathways and produce airway inflammation, which is considered a critical event in the mechanistic pathway leading to human pulmonary disease.

TRPs and conventional diesel-induced airway inflammation

Diesel exhaust particles (DEP) are available from several commercial and “in-house” generated sources. DEP are often used to model health effects in controlled exposures in animals and humans [8]. For research purposes, the source for DEP is vital and can strongly influence the outcome of experiments. Generally, data obtained with in-house-generated DEP is the most reliable and reproducible [9, 10].

In humans, moderate (>300 μg/m^3) but not lower doses of DEP from older-generation engines (referred to as *conventional DEP*) induce acute but reversible airway inflammation and impair pulmonary function without causing persistent airway hyperreactivity (AHR) [4, 11–13]. The acute adverse effects of conventional DEP have been associated with the production of inflammatory mediators from airway macrophages and epithelial cells [4, 12–14]. Aside from acute inflammation of airways, in-house-generated conventional DEP samples can act as an adjuvant, leading to chronic airway inflammation by enhancing allergic sensitization via amplification of allergic responses [15]. In this context, conventional DEP synergize with allergens to create allergic asthma phenotypes [10], which are characterized by the promotion of T-helper type 2 (Th2) immune responses that are associated with IL-4 and allergen-specific IgE production in animals and humans and AHR in animal models of allergic asthma [15–19].

Mechanisms underlying the generation of acute, and especially chronic, airway inflammation and dysfunction by conventional DEP are still not completely clear. Nevertheless, several theories have emerged. One line of investigation provided solid evidence that the electrophilic components of conventional DEP induce Ca^{2+} influx and activate inward currents in airway epithelial cell lines via TRP vanilloid type 1 (TRPV1) [20–22] (Fig. 1). The use of DEP that do not generate chronic allergic inflammation [10], however, also exhibited small responses in TRPA1 overexpressing cell lines [23] (Fig. 1). Activation of airway neurons and primary and acutely isolated non-neuronal lung cells, including epithelial cells, by conventional DEP has not been reported.

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The originally suggested mechanism of DEP actions on airways postulates that oxy- and nitro-PAHs, components of conventional DEP, are metabolized in macrophages and epithelial cells by several isoforms of cytochrome P450s (CYPs) into quinones. Quinones generate excess reactive oxygen species (ROS) which, in turn, induce inflammatory mediator production in macrophages and airway epithelial cells [8]. Very high, non-physiological concentrations (>100 μM) of quinones as well as ROS are capable activating cell lines overexpressing TRPV1, and to a lesser extent, TRPA1 [23–25] (Fig. 1). Activation of airway neurons and lung non-neuronal cells via TRPV1 and TRPA1 channels with physiological concentrations of quinones and ROS has not been studied. Moreover, there are alternative cellular pathways for ROS action that show an increase in the activation of nociceptive pulmonary vagal C fibers via a non-TRPA1 and non-TRPV1 protein kinase C pathway [26]. Nonetheless, activation and/or potentiation of TRPV1, and to a lesser extent, TRPA1 by DEP, are putative mechanisms leading to the induction of airway inflammation. It is now well accepted that TRPV1 and TRPA1 can be gated by two classes of ligands, electrophilic or lipophilic, and both can be derived from exogenous or endogenous sources. In this regard, ROS and pH represent endogenous electrophilic compounds [27], while capsaicin and PAHs are exogenous lipophilic compounds. During the last decade, we and others have discovered several physiologically important endogenous lipophilic TRPV1- or TRPA1-activating ligands, including endocannabinoids, vanilloids, and arachidonic and linoleic acid metabolites [28–31]. Interestingly, PAHs activate CYP enzymes [8], which are capable of catalyzing the production of TRPV1-and TRPA1-activating endogenous lipids [28, 29, 32, 33]. Further, TRPV1 and TRPA1 are Ca^{2+}-permeable ion channels; hence, their activation could lead to an intracellular Ca^{2+} ($[Ca^{2+}]_i$) rise in airway cells, which can result in induction of NF-κB and NFAT with subsequent pro-inflammatory mediator production and release [34–37] (Fig. 1).

The above pathways have been used in experiments designed to explain the role of conventional DEP in acute airway inflammation in animals and humans. However, these two pathways, and especially, the role of TRPA1 or TRPV1 in DEP-induced allergic asthma, have not been demonstrated as of yet. It is an important and relevant topic of research, since TRPA1 [38] and, according to some reports, TRPV1 [39, 40], could be involved in the development of allergic asthma in a basic ovalbumin (OVA) murine asthma model. Interestingly, specific ablation of TRPV1-expressing pulmonary C fibers blocked OVA-induced AHR but not airway inflammation [40], suggesting that TRPV1 may control bronchial smooth muscle contraction while TRPA1 could be more important for allergic inflammation.

TRPs and clean diesel-induced airway inflammation

In response to the toxicities of conventional DE, *clean diesel* technologies have been developed to reduce emissions of nitrogen oxides (NOx), PM, and certain toxic PAHs [41, 42]. There are two primary approaches to clean diesel technologies: (1) development of “cleaner” fuels such as ultra-low sulfur diesel and biodiesel or (2) the use of post-combustion aftertreatments, such as *diesel particulate filters* (DPF) and *catalytic conversion-based modifications* (Fig. 2). The most widely implemented post-combustion catalytic systems include diesel oxidation catalyst (DOC), urea selective catalytic reduction (SCR),

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and diesel particulate NOx reduction (DPNR), which are typically used in combination with a DPF (Fig. 2). Hence, unlike conventional DEP, all clean diesel aftertreatments incorporate a DPF, which dramatically reduces PM mass in clean DE. Besides reduction of PM mass (30–50-fold), these technologies substantially decrease the size of emitted PM and reduce NOx and certain PAHs, particularly the most toxic oxy- and nitro-PAHs (Tables 1 and 2). Despite these sophisticated aftertreatment systems for DE, it has recently been reported that emissions from ultra-low sulfur diesel, as well as DPF and SCR-treated DE, may still cause *acute* lung inflammation similar to conventional DE [43–46]. These reports have shown that clean diesel can still utilize the PAH oxidization pathway and ROS generation in cells [8] to cause acute lung inflammation [45, 46]. Thus, it was suggested that ultra-fine PM in clean DE could be as harmful, if not more, than the PM from conventional DE [44].

The contribution of clean diesel to allergic asthma is grossly understudied, and to our knowledge there are no publications on this topic. Additionally, epidemiological studies on clean diesel health effects would be difficult to conduct, since the current truck fleets consist of a variety of vehicles with and without clean diesel systems. Also, the effects of clean DE on TRPs have not been studied. Thus, there is a critical gap in knowledge, since the development and adoption of novel clean diesel technologies is a rapidly evolving process that urgently requires additional information on the potential health hazards versus benefits of clean diesel.

TRP activation by PM and PAH in the induction of airway inflammation

The formulation of novel clean diesel technologies can considerably be aided by understanding the relative health hazards/benefits of conventional/clean diesel components. There is agreement that among the >400 different toxic constituents in DE, there are three dominant components: PM, NOx, and PAHs. Activation of TRPs by the larger particle-sized PM from conventional DE but not the ultra-fine PM present in clean DE has been studied [20–22]. The current view is that PM activates TRPV1 and, perhaps to a lesser extent, TRPA1. Synthetic particles of different sizes that can be detected in conventional DE have been shown to generate depolarizing currents and increase Ca^{2+} influx in capsaicin- and acid-sensitive sensory neurons and in TRPV1-expressing HEK 293 cells [21]. PM activation of epithelial cell lines, which express TRPV1, can trigger apoptosis [21]. Interestingly, environmental PM generated from coal and oil fly ash and ash from Mount St. Helens also activates TRPV1 [20, 47]. In contrast, some reports indicate that PM from conventional DEP activates TRPA1 in overexpressing cells and dorsal root ganglion neurons [23]. One of the DEP (NIST 2975) utilized in these studies also generated chronic inflammation but not AHR in an allergic asthma model [9]. In addition, Ca^{2+} influx assays have not been performed at the single cell level; hence, Ca^{2+} influx in cultured DRG cells could be attributed to damage of DRG cells by DEP or by activation of non-neuronal cells, which are present in DRG cultures [23]. Moreover, the concentrations of DEP required for the activation of TRP channels are also a vital parameter. Thus, the use of >80 μg/ml DEP for TRPA1 activation in an overexpressing system will unlikely model true exposure response, since that amount of DEP in DE would not exist even in highly polluted areas [23].

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There are more than 50 different types of PAHs in DE. They can be divided into five major categories: unsubstituted, oxy-, nitro-, benzo- and methyl-PAHs (Tables 1 and 2). Tables 1 and 2 show that different types of aftertreatments or combinations of aftertreatments not only reduce PM in exhaust but also substantially diminish PAHs. Importantly, some aftertreatment systems, such as SCR and DPNR, almost completely remove nitro- and oxy-PAHs and some unsubstituted PAHs from DE. To investigate the activation of TRPs by PAHs, realistic concentrations have to be selected. Thus, Table 1 represents the calculation of PAHs produced by a diesel engine that was driven for 1 km [48–50]. Activation of TRPs by realistic concentrations of different classes of PAHs is unknown as yet.

It is well documented that NOx and PM can cause acute lung inflammation; these actions may involve TRP channels [20–23, 25, 51]. PAHs also produce acute inflammation in the pulmonary system via generation of quinones and ROS (Fig. 1) [8]. While it is clear that unfractionated DEP and NOx promote allergic asthma, it is as yet unknown which of the individual components of DEP, i.e., the carbon core of PM, electrophilic components of PM, individual constituent PAHs, or a combination of these, are the primary adjuvants that promote allergic asthma. Overall, many questions need to be addressed in this research field to fully understand the adverse impact on the pulmonary system by conventional and especially clean DE and their predominant chemical components.

Types of pulmonary cells targeted by conventional and clean DE

Considering that conventional and clean DE could modulate TRP channels and potentially lead to acute and chronic airway inflammation, what types of cells could mediate these actions? Several TRP mRNAs and proteins have been identified in non-neuronal airway cells. Thus, using RT-PCR and in situ hybridization, low level of TRPV1 mRNA was seen in non-neuronal lung cells [52, 53]. Pathological conditions may or may not affect TRPV1 expression in non-neuronal lung cells [54, 55]. Other reports indicate that TRPA1, but not TRPV1, is expressed in mouse and human lung epithelial and smooth muscle cells [56]. Moreover, several human lung epithelial and smooth muscle cell lines respond to specific TRPA1 activation [56]. However, it is still not clear whether TRPV1- or TRPA1-gated currents could be recorded from acutely isolated non-neuronal lung cells that did not undergo in vitro culture and immortalization procedures. This is a principal and critical question, since it has been speculated that acute and chronic inflammation in the pulmonary system could be mainly neurogenic in nature [38]. The concept of neurogenic inflammatory mechanisms in chronic pulmonary disease has been proposed for many years as a part of the axon reflex theory [57]. This process is thought to involve the release of sensory neuropeptides, such as substance P (SP), neurokinin A (NKA), and calcitonin gene-related peptide (CGRP) [58–60]. Expression of SP and NKA can be seen in back-traced airway jugular and nodose ganglia [61]. In models of allergic asthma, SP and NKA expressed in the terminals of C fibers can be locally released upon stimulation of these terminals and produce potent effects on airway smooth muscle tone, mucus secretion, and edema and on immune cells that impact neurogenic inflammation. These neuropeptides act via specialized NK1, NK2, or CGRP receptors, which are present on non-neuronal lung cells [62–64]. Blocking NK1 and NK2 attenuates the allergic inflammatory response. Despite this wealth of information, there is still no direct proof that sensory neuropeptides are key controllers of

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chronic airway inflammation. Expression of these neuropeptides in certain non-neuronal lung cells further complicates the overall picture. Moreover, direct application of CGRP into airways reduced AHR without altering the overall inflammatory response, suggesting a potentially protective modulatory role for CGRP [65]. Additionally, NKA inhibition has had a modest impact on human asthma, and apparent differences between animal and human studies could be explained by the relatively sparse release of tachykinins from human tissue [66, 67].

Nevertheless, the involvement of airway nerves and neuronal TRPs in the development of allergic asthma and possibly COPD has plenty of *indirect* support. TRPA1 was identified as the receptor for some of the principal components of cigarette smoke causing neurogenic inflammation [68]. However, this response is heavily influenced by TRPV1 [69]. Allergen exposure potentiates activities of nodose ganglion neurons and TRPV1 channels on vagal C fibers [70]. Allergen-induced airway inflammation increased capsaicin sensitivity in myelinated pulmonary afferents with an increased expression of TRPV1 in nodose ganglia [71]. Hydrogen sulfide has also been shown to sensitize TRPV1 channels [72]. TRPV1-expressing fibers induce a counter-regulatory mechanism to suppress endotoxin-induced airway inflammation via the release of somatostatin [73].

A number of important studies have been conducted in rodent models of asthma using OVA as the allergen, with intraperitoneal sensitizing injections followed by OVA inhalational challenge, a model heavily dependent on immune mechanisms. TRPA1 pharmacological inhibition attenuated the late asthmatic response in rats after OVA challenge [74]. TRPA1, but not TRPV1, global ablation resulted in substantial reduction in Th2 cytokines, airway inflammation, and methacholine (Mth)- provoked AHR [38]. On the other hand, other reports showed that suppression of TRPV1 channels with siRNA attenuated OVA- or IL-13-induced airway inflammation and Mth-provoked AHR [39]. TRPV1 inhibitors also attenuated histamine-provoked AHR in OVA-sensitized guinea pigs [75]. Moreover, depletion of TRPV1-expressing cells (not only TRPV1-positive sensory neurons) blocked allergen-induced AHR but not airway inflammation [40]. These results could be interpreted to suggest that TRPV1 may influence primary control of bronchial smooth muscle contractility and that TRPA1 could be more important for allergic inflammation.

Overall, despite a substantial amount of information on the roles of TRPA1 and TRPV1 in acute and chronic airway inflammation and AHR, there is yet no consensus. The mixed findings could be due to methodological or strain differences. The models used in the allergen sensitization studies described above employed sensitization by intraperitoneal exposure to allergen (i.e., OVA) followed by inhalational challenge with OVA, which may bypass pulmonary sensitization mechanisms, particularly those that involve neural sensitization and a critical role for pulmonary mast cells. Functional expression of TRPs in the lung is also not fully established. Moreover, TRPV1 and TRPA1 have significant interactions and synergy [76–78]. One possible mechanism is that modulation of these channels occurs within a complex containing associated TRPA1 and TRPV1 channels and adapter protein Tmem100 [79, 80]. In this respect, conventional and clean DE, PM, and PAHs could act via several sites and activate (or potentiate) TRP channels on several lung

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cell types—non-neuronal and neuronal. The roles of these activation sites by DEP, PM, and PAHs are yet to be fully investigated.

Conclusions

Rapid growth of the world's population, development of major urban centers, and increasing use of internal combustion vehicles make it imperative to study the adverse effects of traffic-related air pollution on human health. These requirements are especially demanding with the development of new fuel technologies, which are already widely used throughout the world. Recent studies imply that TRP channels could play critical roles in mediating adverse effects of air pollutants, but there are still many unanswered questions, which have been discussed in this review. In this respect, involvement of TRP channels in mediating possible adverse effects of clean diesel and its key components, ultra-fine PM and PAHs, remains a priority. These studies will provide invaluable knowledge for evaluating and understanding the adverse effects of new fuels as well as formulating novel and safer DE aftertreatment technologies.

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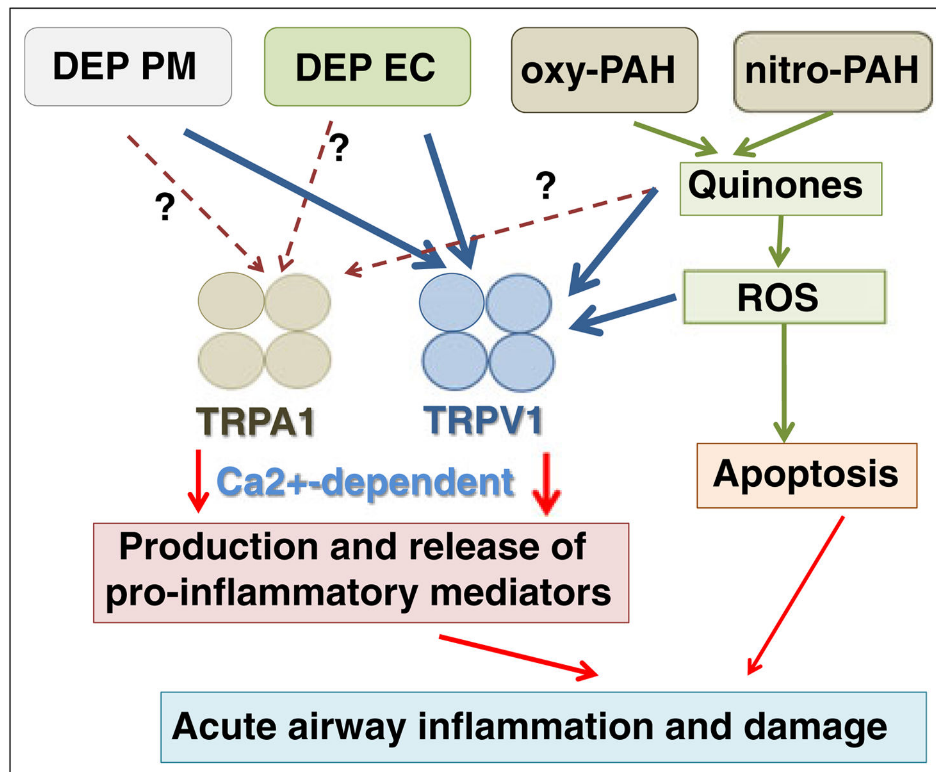

Fig. 1.

Schematic for Ca^{2+}-dependent and Ca^{2+}-independent mechanisms underlying conventional DEP-induced acute airway inflammation and lung damage. Particulate matter (PM) and oxy- and nitro-substituted subclasses of polycyclic aromatic hydrocarbons (PAH) could activate TRPV1 and TRPA1 channels. This could induce Ca^{2+}-dependent production and release of pro-inflammatory mediators with subsequent airway inflammation. PAHs could also induce reactive oxygen species (ROS) formation in lung cells and Ca^{2+}-independent generation of pro-inflammatory mediators. DEP PM refers to the particular matter component of diesel exhaust particles (DEP). *DEP* diesel exhaust particles, *ECs* electrophilic components. *Dashed lines* and *question marks* indicate putative TRPV1 and/or TRPA1 activation pathways

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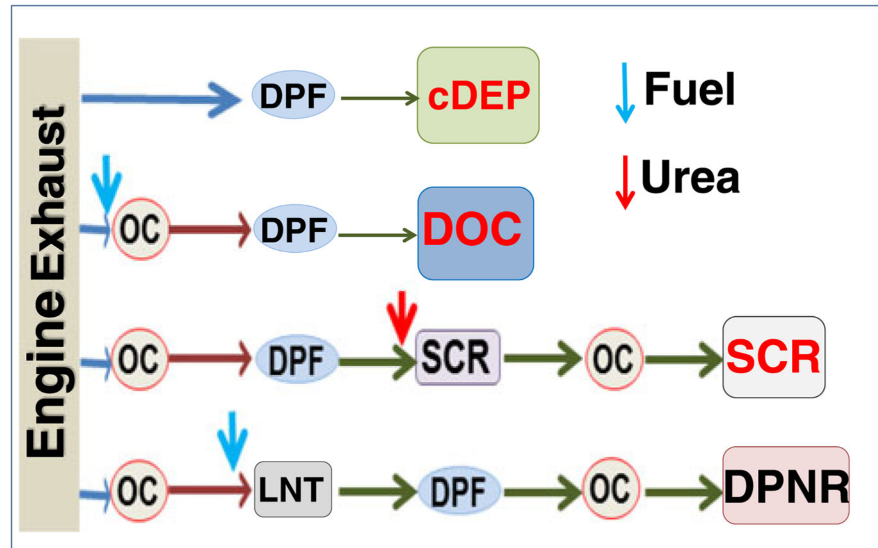

Fig. 2.

Generation of clean DE. Schematic represents different post-combustion aftertreatments. *DPF* diesel particulate filter, *OC* oxidation catalyst, *LNT* lean NOx trap, *cDEP* control diesel exhaust particles, *DOC* diesel oxidation catalyst, *SCR* selective catalytic reduction, *DPNR* diesel particulate and NOx reduction; *Arrows* indicate the injection of fuel or urea for SCR

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Table 1

Composition of clean DEP with different aftertreatments

PM and PAHs	DOC	DPNR	SCR	fDEP
Particulate mass	32	11	35	38
Phenanthrene	0.9	0.51	1.1	1.1
Anthracene	0.19	0.068	0.11	0.2
Fluoranthene	0.89	0.18	0.49	0.96
Pyrene	1.6	0.53	0.52	1.3
Chrysene	1.3	0.19	0.27	3.2
9-Nitroanthracene	1.4	0.022	UD	2.7
2/3-Nitrofluoranthene	0.015	UD	UD	UD
4-Nitropyrene	0.06	UD	UD	UD
1-Nitropyrene	1.3	UD	UD	2.4
2-Nitropyrene	UD	UD	UD	UD
Oxy-PAHs	26.73	2.621	1.119	35.36
Methyl-PAHs	1.096	1.148	1.016	1.232

Compositional analysis of DEP from three diesel vehicles with different aftertreatment systems, diesel oxidation catalyst (DOC), DPF and NOx reduction system (DNPF), and a urea-based selective catalytic reduction system (SCR). Particulate mass is measured in mg/km and polycyclic aromatic hydrocarbons (PAH) in μg/km. fDEP represents control filtered DEP with reduced particulate mass, but without additional aftertreatment. DOC, diesel particulate NOx reduction (DPNR), and SCR represent different aftertreatments of DE. The amounts of oxy- and methyl-PAHs are the combined amounts of all subtypes of these PAHs. Significant changes are underlined

UD, undetectable

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Table 2

Effect of aftertreatment on reduction of DEP PAHs using combined aftertreatments

Compounds, ng/filter	Engine out	Aftertreatment out	% Reduction
2-Methylnaphthalene	5578	139	97.5 %
1-Methylnaphthalene	2141	48	97.8 %
Naphthalene	4973	791	84.1 %
Biphenyl	1249	37	97.0 %
Acenaphthylene	1171	<1.0	–
Acenaphthene	147	22	84.9 %
Fluorene	1141	11	99.0 %
Phenanthrene	3355	78	97.7 %
Anthracene	362	3	99.2 %
Fluoranthene	411	12	97.2 %
Pyrene	1000	8	99.2 %
Perylene	7	<1.0	–
Chrysene	82	<1.0	–
Coronene	28	<1.0	–
Benzo(b,j)fluoranthene	16	<1.0	–
Benzo(k)fluoranthene	7	<1.0	–
Benzo(e)pyrene	38	<1.0	–
Benzo(a)pyrene	33	<1.0	–
Benzo(a)anthracene	49	<1.0	–
Indeno(123-cd)pyrene	9	<1.0	–
Dibenz(ah)anthracene	2	<1.0	–
Benzo(b)chrysene	2	<1.0	–
Benzo(ghi)perylene	77	<1.0	–

DEP were collected from a 2012 6.7L Ford engine equipped with DOC, DPF, and SCR aftertreatments using the Federal Test Procedure (FTP) heavy-duty transient cycle. Organic extracts were analyzed by gas chromatography/ionized mass spectrometry according to California Environmental Protection Agency method 429