HEI

December 2015

The Advanced Collaborative Emissions Study (ACES)

EXECUTIVE SUMMARY

Health Effects Institute

AB86-COMM-19-19

ABOUT HEI

The Health Effects Institute is a nonprofit corporation chartered in 1980 as an independent research organization to provide high-quality, impartial, and relevant science on the effects of air pollution on health. To accomplish its mission, the institute

- Identifies the highest-priority areas for health effects research;
- Competitively funds and oversees research projects;
- Provides intensive independent review of HEI-supported studies and related research;
- Integrates HEI's research results with those of other institutions into broader evaluations; and
- Communicates the results of HEI's research and analyses to public and private decision makers.

HEI typically receives balanced funding from the U.S. Environmental Protection Agency and the worldwide motor vehicle industry. Frequently, other public and private organizations in the United States and around the world also support major projects or research programs. HEI has funded more than 330 research projects in North America, Europe, Asia, and Latin America, the results of which have informed decisions regarding carbon monoxide, air toxics, nitrogen oxides, diesel exhaust, ozone, particulate matter, and other pollutants. These results have appeared in more than 260 comprehensive reports published by HEI, as well as in more than 1000 articles in the peer-reviewed literature.

HEI's independent Board of Directors consists of leaders in science and policy who are committed to fostering the public–private partnership that is central to the organization. The Health Research Committee solicits input from HEI sponsors and other stakeholders and works with scientific staff to develop a Five-Year Strategic Plan, select research projects for funding, and oversee their conduct. The Health Review Committee, which has no role in selecting or overseeing studies, works with staff to evaluate and interpret the results of funded studies and related research. For the ACES studies, a special ACES Review Panel — comprising Health Review Committee members and outside experts — fulfilled this role.

All project results and accompanying comments by the Health Review Committee are widely disseminated through HEI's Web site (*www.healtheffects.org*), printed reports, newsletters and other publications, annual conferences, and presentations to legislative bodies and public agencies.

CONTENTS

EXECUTIVE SUMMARY

The Advanced Collaborative Emissions Study (ACES)

In 2005 the Health Effects Institute and its partners and sponsors launched the Advanced Collaborative Emissions Study (ACES*). The goals of ACES were the detailed characterization of emissions (termed *new-technology diesel exhaust* [NTDE]) from engines compliant with U.S. Environmental Protection Agency (EPA) rules for model-year (MY) 2007 and 2010 heavy-duty diesel engines and the testing and evaluation of any health effects using a 2007 engine. The results of these studies have previously been presented in detailed reports and peer-reviewed publications (ACES 2015; Khalek et al. 2009, 2013; Mauderly and McDonald 2012). Here we summarize key features of the program, its major findings, and some of its implications for clean air and public health.

INTRODUCTION

Diesel engines are a key part of the world's transportation and industrial infrastructure, especially in heavy-duty applications such as trucks, buses, construction and farm equipment, locomotives, and ships. Diesel engines are more efficient than gasoline engines: they emit less carbon dioxide (CO_2, a greenhouse gas) and carbon monoxide (CO, a toxic gas). They are also more durable than gasoline engines and provide greater power output. Despite these advantages, there have been concerns about the impact of exhaust from older diesel engines on the environment and human health: traditional-technology diesel exhaust (TDE) contains higher levels of soot (or black smoke), consisting of particulate matter (PM) and toxic compounds, per vehicle mile traveled than emissions from gasoline engines. These particulates are of special concern regarding health effects because many of them are small enough to be readily respirable and deposited in the lung, and they have many chemicals adsorbed to their surfaces, including known or suspected mutagens and carcinogens. TDE also contains higher levels of nitrogen oxides (NO_x), a mixture mainly of

What This Study Adds

- ACES set out to evaluate emissions and health effects from new-technology (MY 2007 and 2010) heavy-duty, on-road diesel engines.
- The results show that the aftertreatment technologies used in such modern diesel engines are highly effective and that they meet — and exceed — the reductions mandated by U.S. regulations. The study reports the effectiveness of diesel particulate filters in greatly reducing PM emissions and of selective catalytic reduction systems in reducing NO_x emissions; similarly, emissions of more than 300 other compounds — some with known carcinogenic and toxic properties — measured in the exhaust were also reduced relative to exhaust from traditional-technology diesel engines.
- Exposure to new-technology diesel exhaust (NTDE) from a 2007 engine tested in Phase 3 of ACES was not carcinogenic in the rat, unlike traditional-technology diesel exhaust (TDE) from older engines, which is known to cause lung tumors under similar conditions. A few NTDE-associated effects in rat lungs in ACES were observed; however, these effects were consistent with exposure to NO_2, a pollutant present in 2007 engine emissions that was further reduced in exhaust from MY 2010 engines, which deployed a selective catalytic reduction system.
- ACES results demonstrate, even after considering some inherent limitations in any such study, that diesel particulate filters greatly reduce the amount of PM from modern diesel engines and that the overall toxicity of exhaust from modern diesel engines is significantly decreased compared with the toxicity of emissions from traditional-technology diesel engines.

This Executive Summary is a description of Phases 1, 2, and 3 of ACES, including a summary of the results of all phases of the study and of the HEI ACES Review Panel Commentary.

Although this document was produced with partial funding by the United States Environmental Protection Agency under Assistance Award CR–83467701 to the Health Effects Institute, it has not been subjected to the Agency's peer and administrative review and therefore may not necessarily reflect the views of the Agency, and no official endorsement by it should be inferred. Although specific components of this study were funded by the United States Department of Energy under Assistance Award DE-AC26-05NT42429 to the Health Effects Institute, it has not been subjected to the Department's peer and administrative review and therefore may not necessarily reflect the views of the Department, and no official endorsement by it should be inferred. The contents of this document also have not been reviewed by private party institutions, including those that support the Health Effects Institute; therefore, it may not reflect the views or policies of these parties, and no endorsement by them should be inferred.

* A list of abbreviations and other terms appears at the end of this Executive Summary.

nitrogen dioxide (NO_2) and nitric oxide (NO), which can have toxic effects and are also ozone precursors.

Concerns about the potential health effects from human exposure to diesel emissions began with studies in in vitro systems and animal models during the 1970s and 1980s; these studies provided evidence that whole TDE, PM extracts, and some PM constituents were mutagenic and carcinogenic. Some epidemiological studies also showed an elevation in lung cancer among occupationally exposed workers. In 1988, a panel convened by the International Agency for Research on Cancer (IARC) classified diesel exhaust as "probably carcinogenic to humans" (Group 2A, IARC 1989). In 2012, in light of newer epidemiological data, an IARC panel concluded that "[d]iesel engine exhaust is *carcinogenic to humans*" (Group 1, IARC 2012).

Because of these health concerns, regulatory agencies in the United States and in other countries adopted progressively more stringent regulations to reduce emissions from diesel engines (Figure 1). These regulations stimulated the manufacturers to develop engine controls and exhaust aftertreatment technologies to curb the emissions of concern, while minimizing the impact on fuel efficiency. As such systems developed in the late 1990s and during the first decade of the new century, emissions of PM, NO_x, hydrocarbons (HCs), and other toxic compounds in diesel exhaust were expected to be substantially lowered.

In light of new, stringent emission standards that were to become effective starting with MY 2007 heavy-duty diesel engines, HEI's sponsors in industry and the EPA, as well as other private interests and public agencies, requested that HEI undertake research to characterize emissions from engines that incorporated the new technologies needed to meet those standards and to evaluate any resulting health effects (see related text box "HEI's Work on the Health Effects of Diesel Emissions"). In response, HEI launched ACES, a comprehensive testing of the emissions of the new engines and their health effects.

REGULATORY CHALLENGES AND SOLUTIONS

The major concern with TDE has been the high levels of soot (or black smoke), NO_x, and other chemicals. Beginning with the "smoke standards" promulgated in 1968 — before passage of the Clean Air Act in 1970 — numerous regulations to control diesel emissions have been put in place by the U.S. EPA. As knowledge of the potential health effects of diesel emissions increased, the emissions standards were gradually tightened during the subsequent years, culminating in a steep acceleration in regulatory actions during the 1990s (see Figure 1; see EPA 2013 for a summary). In 1997, the EPA established new emission limits for MY

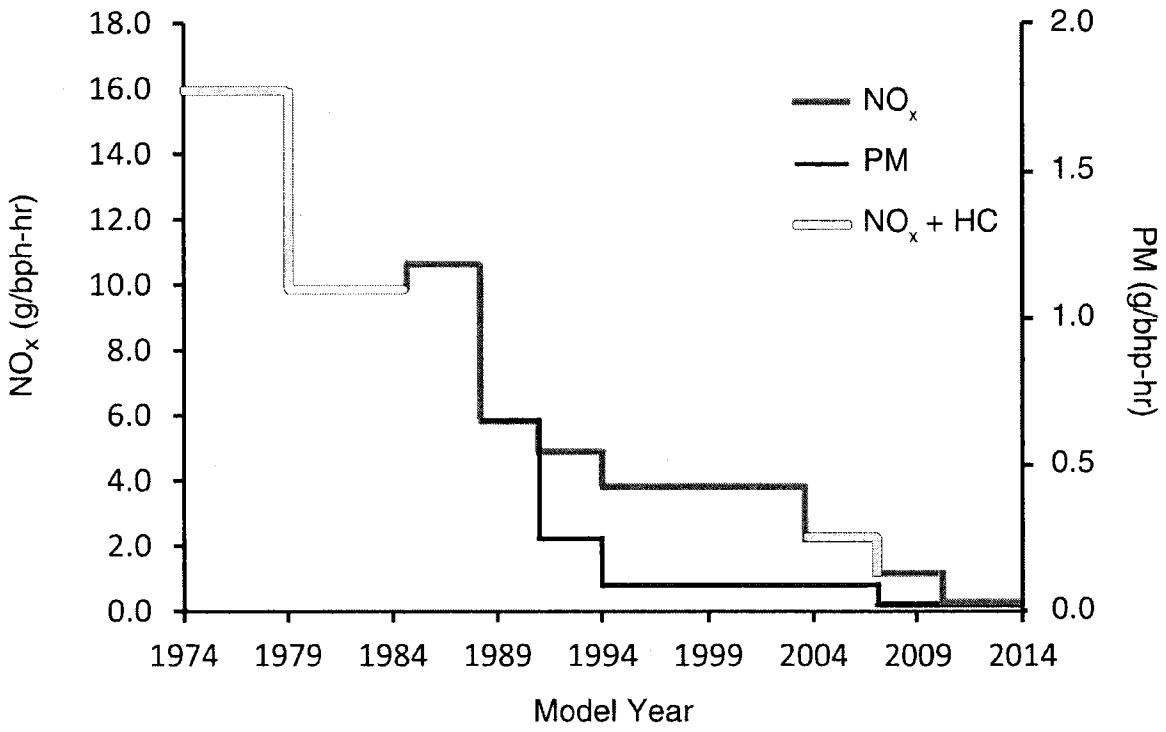


Figure 1. U.S. EPA PM and NO_x emissions standards since 1974 for heavy-duty diesel engines. (Source: U.S. EPA 2013.)

HEI's Work on the Health Effects of Diesel Emissions

Since its inception in 1980, HEI has played a very active role in evaluating the potential health effects from exposure to diesel exhaust. Some of HEI's earliest research focused on toxic compounds — particularly nitropyrenes — found on diesel particulates. HEI also funded some of the early human epidemiology studies in the field and a study on the potential impact of diesel particulate filters on PM toxicity. Starting in 1995, HEI has conducted and published several key reviews on the epidemiology of diesel exposure and human health. In addition to the ACES program, which evaluated the emissions and health effects of new-technology diesel engines and whose results are reported here, the latest example of HEI's work in this area is a new review of recently published occupational diesel-exposure epidemiology studies, titled *Diesel Emissions and Lung Cancer: An Evaluation of Recent Epidemiological Evidence for Quantitative Risk Assessment* (HEI Diesel Epidemiology Panel 2015).

2004 heavy-duty engines, seeking to limit NO_x and non-methane hydrocarbon (NMHC) emissions to a level of 2.4 grams per brake horsepower-hour (g/bhp-hr, a measure of output of heavy-duty diesel engines) (EPA 1997). The PM standard — set at 0.10 g/bhp-hr for MY 1994 engines — remained unchanged.

Most engine manufacturers met these limits by employing air-cooled exhaust gas recirculation (EGR) and diesel oxidation catalysts (DOCs). The EGR system reduces NO_x formation by reintroducing a part of the engine exhaust back into the combustion chambers, thereby diluting and cooling the air–fuel mix. A DOC, similar to a three-way catalyst used in gasoline engines, oxidizes NO, CO, and NMHC and other volatile compounds. It does not oxidize PM because the temperature of the exhaust is not high enough.

During the first decade of the 21st century, the PM and NO_x emission standards for diesel engines have been made far more stringent, with the issuance by the EPA of the Heavy Duty Highway Diesel Emissions Rule for MY 2007 and for 2010 engines (EPA 2001). These new rules were preceded by the lowering of the sulfur content limit for diesel fuel to <15 ppm, effective in 2006. Such fuel was designated *ultra-low-sulfur diesel* (ULSD) fuel and is essential for performance of the catalysts used in aftertreatment devices, which can be easily poisoned with sulfur.

In 2007, a lower standard for PM emissions of 0.01 g/bhp-hr went into effect. To meet this standard, which is 10 times lower than the earlier limit, engine manufacturers employed diesel particulate filters (DPFs), often in conjunction with a DOC. DPFs, which are generally made from a honeycomb-like ceramic structure in which alternate channels have been blocked (to force the exhaust through the filter walls) and are coated with precious metal catalysts (HEI 2011), are extremely effective at removing diesel PM (see Figure 2 for a schematic of the aftertreatment system). During use, DPFs can become clogged with soot particles, and so the filters must be regenerated by removal of the accumulated particles. There are two types of regeneration processes: *passive* (in which the oxidation temperature for soot is lowered with the aid of an appropriately formulated catalyst) and *active* (in which heat is added, by electric heating or combustion of a small amount of fuel injected in the exhaust, in order to reach temperatures sufficient for soot combustion).

NO_x emissions from diesel engines have also been a concern. To reduce the formation of PM, diesel engines operate under a high air-to-fuel ratio. Under these conditions, however, the combustion process leads to substantial NO_x formation, whose reduction is particularly challenging. The DOC and DPF reduce PM levels but increase the amount of NO_2 in tailpipe emissions, because both oxidize NO to NO_2. Regulations beginning in 2007 also saw the start of the phase-in of a lower standard for NO_x, which was to be brought down to 0.2 g/bhp-hr by MY 2010. The PM standard for MY 2010 engines remained at the same level as that for MY 2007 engines.

To meet the 2010 limit for NO_x emissions, selective catalytic reduction (SCR) devices — originally developed for stationary sources — were introduced and placed downstream of the DPF (see Figure 2) (HEI 2011). The SCR injects a reductant — generally a solution of urea — into the exhaust stream. Urea decomposes to ammonia (NH_3), which then reduces NO_2 to nitrogen (N_2) in the SCR device. If NH_3 is not fully consumed in the catalytic process, it is emitted in the exhaust. An ammonia oxidation

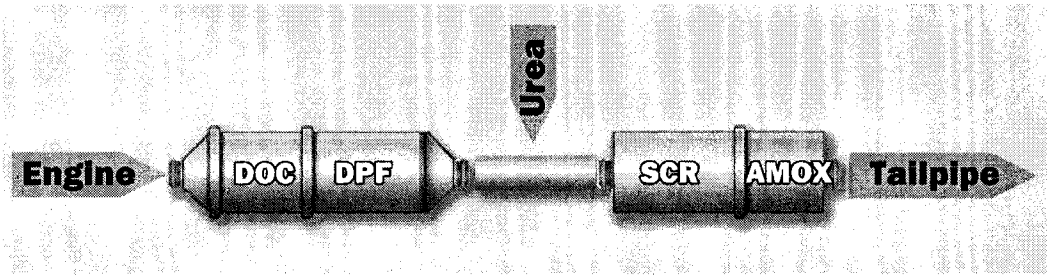


Figure 2. Schematic of diesel engine aftertreatment technologies. (AMOX = ammonia oxidation catalyst; DOC = diesel oxidation catalyst; DPF = diesel [exhaust] particulate filter; SCR = selective catalytic reduction [device].)

catalyst (AMOX) is commonly used to convert any remaining NH_3 also to N_2. However, AMOX can lead to the formation of nitrous oxide (N_2O) in small quantities, a gas that contributes to climate change.

In addition to these emission standards, the U.S. EPA also established requirements both for durability of the aftertreatment technologies and for compliance with the standards during the life of the engines. Altogether, these rules became the most stringent effort in the world to control diesel emissions.

The U.S. EPA, in addition to the heavy-duty engine rules, also required new, more stringent standards for light-duty diesel vehicles beginning in 2006 when the new ULSD fuel became available. At the same time, regulatory agencies in Europe, Japan, and many other industrialized countries also adopted more stringent rules to reduce emissions that require many of the same technologies, and regulatory actions are also being taken in many emerging markets (Kodjak 2015).

THE ACES PROGRAM

These regulatory and technological developments motivated HEI's sponsors in industry and the EPA, as well as other private interests and public agencies, to request that HEI develop and implement research to characterize emissions from the new-technology engines and test for health effects (see related text box "Testing of NTDE in ACES"). NTDE is defined as emissions from MY 2007 diesel engines or later models, which incorporate a variety of engine improvements, including electronic fuel injection and combustion controls, exhaust gas recirculation, use of ULSD fuel, and employment of aftertreatment devices such as a DOC and DPF (engines older than MY 2007 may also be included if they employ retrofit diesel filters); post 2010 engines also include SCR systems, which generally utilize urea. In addition to the evaluation, and possible confirmation, of highly reduced emissions from the newly developed engines, this research was intended to evaluate the presence of any new compounds with possible adverse health effects that might result from the introduction of the technologies, even though overall pollutant levels were greatly diminished.

Testing of NTDE in ACES

Background

The U.S. EPA and environmental agencies in other countries regulate emissions from mobile sources by setting standards for specific pollutants emitted from particular engines or vehicle categories. Tail-pipe emissions of the following pollutants are currently regulated in the United States: PM, NO_x, CO, and NMHCs. Vehicles in the United States are also subject to other regulations, such as fuel economy, which affects CO_2 emissions.

To ensure compliance with U. S. emission standards, manufacturers must follow test procedures specified in the *Code of Federal Regulations*. Unlike the requirements for light-duty diesel and gasoline vehicles, U.S. EPA regulations require certification of the heavy-duty diesel engine on a dynamometer, a device that simulates engine operation, rather than certification of the complete vehicle as it would be operated on the road. The EPA-required certification test for on-road heavy heavy-duty diesel (HHDD) engines is the Federal Test Procedure (FTP) transient cycle, described below. The HHDD standards in the United States are expressed in grams per brake horsepower-hour (g/bhp-hr, a measure of power output), while those for light-duty diesel and gasoline engines are expressed in grams per mile. In Europe and Asia, the power output of diesel engines is expressed in grams per kilowatt hour.

Test Cycles Used in ACES

Two main cycles were used in ACES: the FTP and the 16-hour cycles. The FTP cycle is specified by the U.S. EPA in the standard as a sequence of speed and torque commands from the dynamometer. It was developed to simulate a variety of heavy-duty truck and bus driving patterns in American cities, including traffic in and around the cities on roads and expressways. As a result, the normalized speed and torque vary with time during FTP testing. The FTP cycle comprises a cold start (after parking the engine overnight), followed by idling and acceleration and deceleration phases, with a variety of speed and torque modes. This is followed by three repetitions of the same test with the warmed-up engine with 20-minute intervals between each repetition. The emission rates are averaged over the four test cycles using a weighing factor of 1/7 and 6/7 for the cold- and hot-start measurements, respectively (DieselNet 1999). During ACES, the FTP was also run as three repeats of the hot-start test cycle using a warmed-up engine.

The 16-hour engine test cycle used in ACES was developed by researchers, led by Nigel Clark at West Virginia University, in order to more closely represent the real-world operations of modern diesel engines than the test cycles used in older long-term animal studies of TDE (Clark et al. 2007). This cycle consisted of four repeats of a 4-hour segment. Each segment was composed of three FTP cycles and four steady-state modes (creep, transient, cruise, and high-speed cruise); these modes were parts of a test cycle developed by the California Air Resources Board based on real-world data from 84 heavy-duty trucks operating in the state of California (Khalek et al. 2009).

After planning workshops with multiple stakeholders in 2003 and 2004, HEI, in collaboration with the Coordinating Research Council (CRC), a nonprofit organization with extensive expertise in emissions characterization, launched ACES in 2005. The overall goals of ACES were (1) detailed characterization of NTDE from heavy heavy-duty diesel (HHDD) engines (Class 8 engines with gross vehicle weight [weight of vehicle, cargo, and passengers] rating of over 33,000 pounds), which used aftertreatment systems and ULSD fuels to comply with U.S. EPA 2007 and 2010 regulations; and (2) evaluation of any health effects in rodents exposed to 2007-compliant NTDE produced by an HHDD engine.

ACES was conducted in three phases:

Phase 1: Characterization of emissions from four HHDD engines equipped with control systems and designed to be compliant with 2007 U.S. EPA standards for reduced PM.

Phase 2: Characterization of emissions from a group of HHDD engine and control systems that met the more stringent 2010 U.S. EPA standards (including more advanced NO_x controls).

Phase 3: Assessment of health effects in rodents — through a chronic study assessing cancer and non-cancer effects in rats and a shorter-term study in mice — from inhalation of NTDE from a 2007-compliant HHDD engine system chosen from among the four engines tested in Phase 1.

See the text box "Sponsorship, Planning, Oversight, and Review of the ACES Program" for a description of the sponsors of ACES, along with the processes that HEI and CRC used in the planning, oversight, and review of the program.

Sponsorship, Planning, Oversight, and Review of the ACES Program

ACES was funded by the U.S. Department of Energy (DOE), Truck and Engine Manufacturers Association (EMA), U.S. Environmental Protection Agency (EPA), California Air Resources Board (CARB), and the American Petroleum Institute (API). Specific engine manufacturers also provided their engines for testing in all phases of ACES.

The development and implementation of the ACES program were carried out jointly by the Coordinating Research Council (CRC) and HEI, with input from a wide range of experts and stakeholders serving on the ACES Steering Committee, the CRC–ACES Technical Panel, the ACES Oversight Committee, and the ACES Health Advisory Committee (all described below).

The ACES Steering Committee consisted of high-level representatives of the DOE, EPA, CARB, engine manufacturers, emissions control manufacturers, the petroleum industry, and the Natural Resources Defense Council. This committee guided the initial efforts to secure support for the study from the stakeholders.

During Phases 1 and 2, the CRC staff and the CRC–ACES Technical Panel were responsible for the solicitation and selection of an emissions characterization facility, the design and oversight of the emissions characterization studies, and review of the final reports. The CRC–ACES Technical Panel comprised representatives of engine manufacturers and emissions control manufacturers, the petroleum industry, government agencies (EPA, DOE, and CARB), HEI, and other stakeholders.

Near the end of Phase 1, HEI set up an Engine Selection Process Group that comprised a subset of members of the CRC–ACES Panel and the HEI ACES Oversight Committee in order to guide HEI in the process of engine selection for the health study (Phase 3; see below). The HEI ACES Oversight Committee comprised members of HEI's Research Committee — a body of experts unaffiliated with sponsors, which selects and oversees research funded by HEI — augmented by independent experts.

In Phase 3, the HEI ACES Oversight Committee, assisted by HEI staff, was responsible for the solicitation and selection of the investigator teams, as well as for oversight of the design and construction of the emissions-generation and exposure facility, the development of the protocol for the chronic bioassay and additional endpoints, and the overall implementation of the health effects research. The ACES Oversight Committee had frequent meetings with the Health Advisory Committee comprised of experts from among ACES stakeholders, who had the opportunity to provide comments and advice.

Near the end of Phase 3B, HEI set up an ACES Review Panel to review reports submitted by each investigator team at interim time points and at the completion of the health effects studies. The Panel comprised members of HEI's Review Committee — a body of experts unaffiliated with sponsors, which reviews final reports submitted by HEI investigators — supplemented by experts in the fields of cardiovascular effects, pathology, genotoxicity, and biostatistics.

In addition, HEI convened a panel of expert pathologists, the Pathology Working Group, to evaluate the histopathology data collected in the Phase 3B core study at Lovelace Respiratory Research Institute. The Pathology Working Group also evaluated the histopathology findings from ACES side by side with findings from prior long-term studies of exposure to TDE and oxidant gases; this provided a context in which to compare and contrast the ACES histology findings with those of other relevant long-term studies of air pollutant effects.

To review the results of the exposure chamber characterization during the course of the exposures and to assess the impact of the presence of animals on the exposure atmosphere, HEI set up a separate review panel, the ACES Exposure Characterization Review Panel.

PHASES 1 AND 2: EMISSIONS CHARACTERIZATION

To ensure the most rigorous characterization of engine emissions, the general strategy in ACES was to use representative models of the major manufacturers' largest on-road diesel engines (i.e., Class 8 HHDD engines) meeting U.S. 2007 or 2010 standards; subject them to detailed testing using a specially developed, strenuous engine-operating cycle that was a better representation of real-world operating conditions for trucks than the Federal Test Procedure (FTP, the federally specified engine operation cycle) (see the text box "Testing of NTDE in ACES"); and characterize a large number of compounds in the exhaust. Through a competitive process, CRC selected a team led by Imad Khalek at the Southwest Research Institute (SwRI) to carry out the ACES emissions characterization investigations.

ENGINES TESTED

In Phase 1, four MY 2007 diesel engines, provided by Caterpillar, Cummins, Detroit Diesel Corporation, and Volvo, were tested on an engine dynamometer (Khalek et al. 2009). They were randomly assigned the identification letters A, B, C, and D and were equipped with EGR and DOC technology, followed by a catalyzed DPF. In Phase 2, three MY 2011 diesel engines, provided by Cummins, Detroit Diesel Corporation, and Mack (Volvo Powertrain), were tested (Khalek et al. 2013). The engines were randomly assigned the identification letters X, Y, and Z. In addition to use of the particle filtration system, these engines controlled NO_x emissions by employing a urea-based SCR device and AMOX, placed downstream of the particulate emission controls.

Experimental Set Up

Test Cycles The engines tested were all brand new, except for 125 hours of a manufacturer-run break-in operation. They were tested on a dynamometer at SwRI on the following test cycles (see also the text box "Testing of NTDE in ACES"), each repeated three times:

- FTP, certification,
- FTP with hot-start phase only, and
- a special 16-hour test cycle, developed for ACES.

Fuel — a typical commercial ULSD fuel (4.5–6.5 ppm) that conformed to U.S. EPA fuel specifications — was provided by CRC through a commercial fuel supplier. Lube oil was provided by Lubrizol.

Engine Testing Protocol The experimental setup and emissions characterization in Phase 1 and 2 were the same with a couple of exceptions, noted below (Khalek et al. 2009, 2013).

The tailpipe exhaust was diluted with filtered air using a full-flow constant volume sampler (CVS) at an average dilution ratio of 20:1 in Phase 1 and between 5:1 and 8:1 in Phase 2 (depending on the engine used). The lower dilution ratio in Phase 2 was chosen to enhance the detection limit of the emissions measurements.

In both Phases 1 and 2, a forced regeneration was performed before the start of the three repeated FTP and hot-start FTP cycles in order to condition the exhaust and the CVS system and to clean the DPF to a baseline level. In Phase 1, a forced, active DPF regeneration was also performed before each of the three repeated 16-hour cycles that had at least one active regeneration for each engine during emissions testing (Khalek et al. 2009). However, in Phase 2, the forced regeneration was performed only before the start of the first 16-hour cycle (Khalek et al. 2013). The reason for the change in protocol was to increase the probability of capturing an active DPF regeneration during one of the three 16-hour repeat tests of the 2010-technology engines, totaling 48 hours; due to improvements in engine and aftertreatment emissions controls, the manufacturers reported that the 2010 engines trigger active DPF regenerations much less frequently compared with 2007-technology engines, and so it was not clear that active DPF regeneration would take place in a 16-hour test period. While the elimination of the active regenerations between the 16-hour cycles did not guarantee the occurrence of an active regeneration event, it increased the chances of regeneration occurring during testing.

EMISSIONS CHARACTERIZATION

The regulated emissions measured included PM, NO_x, CO, and NMHCs. PM was collected on a filter for gravimetric analysis from both the CVS and from an empty rodent exposure chamber to simulate the conditions that would be encountered during the rodent exposures in the Phase 3B chronic bioassay (see the section below, "Phase 3: Health Effects Testing"). Exhaust routed to the exposure chamber from the CVS was diluted by a factor of 2. For measuring NO_x, CO, and NMHC, the investigators sampled emissions from the CVS.

Unregulated emissions included particle number concentration and size distribution, total hydrocarbons, methane (CH_4), CO_2, NO, NO_2, N_2O, NH_3, organic carbon (OC), elemental carbon (EC), metals and elements, inorganic ions, polycyclic aromatic hydrocarbons (PAHs), nitro-PAHs, hopanes, steranes, aldehydes and ketones, dioxins and furans, and many other compounds. In Phase 2, with the advent of the urea-based SCR for NO_x control, special attention was also paid to urea and five of its particle-phase decomposition products, namely, melamine, cyanuric acid, ammelide, ammeline, and biuret. All of these species were measured in samples collected in the CVS with the exception of EC, OC, particle mass, and the number and size distribution, which were determined in an exposure chamber. Emission rates from the ACES engines were compared with 1998- and 2004-technology engines previously tested at SwRI.

Regulated Emissions of PM, CO, NO_x, and NMHCs

As the results summarized in Table 1 show, the 2007 and 2010 engines tested in ACES exceeded the reductions mandated by the U.S. EPA standards. In Phase 1, emissions of PM and NO_x were 86% and 9% lower than the 2007 EPA standard, respectively. In Phase 2, NO_x emissions were 93% below the 2007 average emissions and 60% below the 2010 NO_x standard. In addition, even though the PM standard did not change between 2007 and 2010, emissions of PM in Phase 2 were lower than those from the 2007 engines tested in Phase 1. Emissions of CO and NMHC were also much lower than their standards and TDE.

Table 1. U.S. EPA Emissions Standards and Average Emission Levels Measured in ACES for Regulated Pollutants (g/bhp-hr, FTP cycle)

	MY 1998	MY 2004	MY 2007 ACES Phase 1		MY 2010 ACES Phase 2	
	Standard	Standard	Standard	Measured[a]	Standard	Measured[b]
PM	0.1	0.1	0.01	0.0014	0.01	0.0008
CO	15.5	15.5	15.5	0.48	15.5	0.5
NMHC[c]	1.3	—	0.14	0.015	0.14	0
NO_x	4.0	2.4[d]	1.2[e]	1.09	0.2	0.08

[a] Khalek et al. 2011, Table 4.

[b] Khalek et al. 2015, Table 4.

[c] NMHC is reported as the difference between measured total hydrocarbons and methane.

[d] Combined NO_x + NMHC (or 2.5 g/bhp-hr for NO_x, with a limit of 0.5 g/bhp-hr for NMHC).

[e] Average value between 2007 and 2009, with full enforcement in 2010 at 0.20 g/bhp-hr.

Abbreviations: CO = carbon monoxide; FTP = Federal Test Procedure; g/bhp-hr = grams per brake horsepower-hour; MY = model year; NMHC = non-methane hydrocarbon; NO_x = nitrogen oxides; PM = particulate matter.

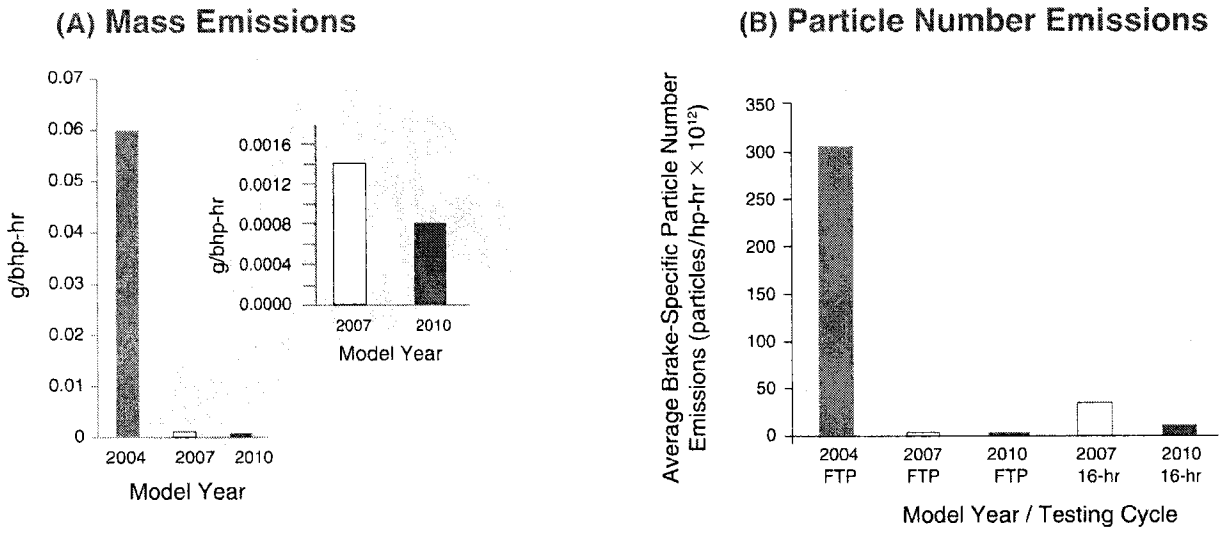
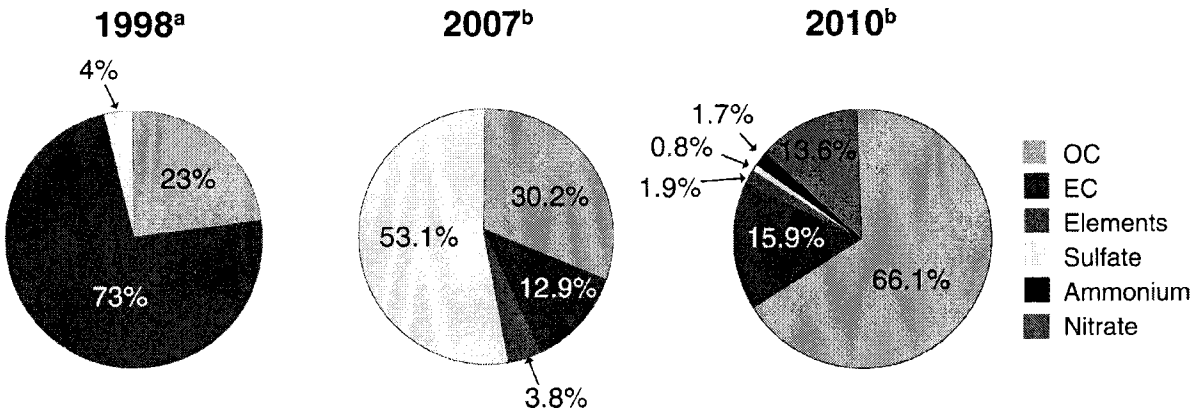


Figure 3. Comparison of PM emissions from old- and new-technology diesel engines: (A) particle mass (measured using the FTP cycle, which is too short to induce active regeneration) and **(B)** particle number emissions (*2004 engines*: without DOC or DPF, no regeneration; *2007 engines*: with DOC and DPF, no regeneration with the FTP cycle, 2 or 3 regenerations with the 16-hour cycle; and *2010 engines*: with DOC and DPF, no regeneration during the FTP or 16-hour cycles). (Data from Khalek et al. 2009, 2015.)

Particle Mass and Composition

In ACES, many features of the PM emissions were investigated. Particle mass was substantially (approximately 90%) below the levels specified under the regulations. Particle mass varied depending on whether the PM was collected during the FTP, the hot-start FTP, or the 16-hour cycle due to differences in the filter temperature, the residence time, or both. Regardless of the cycle or the sampling location, emissions of PM were lower in 2010-compliant engines than in 2007-compliant engines, as shown in Figure 3A and Table 1.

The composition of the PM also differed as diesel engines modernized (Figure 4). In PM in exhaust from MY 1998 engines, EC was the major component (about 70%), while the percentage of EC in 2007- and 2010-engine exhaust was much lower (approximately 13% and 16%, respectively). Also, about 50% of PM from 2007 engines consisted of sulfate, but sulfate composed less than 1% of PM from MY 2010 exhaust; OC constituted about 30% of PM from 2007-engine exhaust, but about 66% of PM from 2010 engines.



[a] Personal communication, Imad Khalek, 22 March 2012.
[b] Adapted from Khalek et al. 2015.

Figure 4. PM composition for the 1998-, 2007-, and 2010-technology engines. (For illustration of the significant differences between PM mass and number emissions between the engines, see Figure 3.)

Particle Number

Particle number measurements were taken exclusively inside the empty animal exposure chamber during both the hot-start FTP and 16-hour cycles. Figure 3B shows a reduction of 2 orders of magnitude in particle number emissions in NTDE relative to TDE during the FTP cycle (regeneration events did not take place during the FTP cycle). There also was a 41% reduction in the particle number in the exhaust from 2010 engines as compared with that from 2007 engines, even though the regulatory requirement did not change.

The particle number in 2007-engine exhaust was higher during the 16-hour cycle than during the FTP cycle due to the 1 to 3 regeneration events observed during the 16-hour cycle. However, the particle number emissions were still about 10-fold lower than those observed from TDE. The peaks in particle number during the regeneration events, each lasting 30 to 45 minutes, were roughly 10 to 100 times higher than the PM emissions when regeneration was not taking place (Khalek et al. 2009). As mentioned earlier, the 2010 engines, on the other hand, did not trigger any active regeneration events during three repeated 16-hour cycles.

Unregulated Inorganic Gases

Findings comparing emissions of key gases are summarized in Table 2.

NO_2 Under the oxidizing conditions prevalent for PM removal in the DOC and DPF, over 60% of the NO was converted to NO_2. As a result, while total emissions of NO_x were lower in Phase 1 than what the 2004 standard called for (Table 1), emissions of NO_2 were 33% higher and the NO_2/NO_x ratio was about 5-fold higher (see Table 5 in Khalek et al. 2011). In Phase 2, NO_2 emissions were 94% lower than in Phase 1 (Table 2 and Figure 5), as a result of the use of a SCR.

SO_2 An unexpected finding in the Phase 2 testing was that emissions of SO_2 were about 70% lower than those measured in Phase 1. SO_2 is a combustion by-product of the small amounts of sulfur in the ULSD fuel and lube oil; the sulfur content in the fuel and lube oil was comparable in the two phases. Khalek and colleagues (2015) attributed this observation to storage of sulfur in the aftertreatment system in MY 2010 engines.

NH_3 and Other Urea-Decomposition Compounds Introduction of urea as the reducing agent in SCR gave rise to the possibility of emissions of NH_3 (ammonia, the major product of urea decomposition, needed to reduce NO_x) or urea decomposition by-products or other nitrogen-containing compounds that may be formed in the SCR or AMOX. Therefore, characterization of such emissions was important. MY 2010 engines deploy the AMOX downstream of the SCR device, which oxidizes any unreacted NH_3. Overall, NH_3 emissions were higher in Phase 2 than in Phase 1 (Table 2). Still, the level of NH_3 (0.0025 g/bhp-hr) was much lower than the proposed European limit (10–15 ppm). (There is no NH_3 emission standard in the United States.) Out of the six urea-related compounds measured in the particle phase, only urea and cyanuric acid were detected during the 16-hour cycle (0.87 ± 0.75 and 9.0 ± 9.0 µg/bhp-hr, respectively). This reflects a very low mass concentration (9 ppb) of cyanuric acid in diesel engine exhaust (Khalek et al. 2015).

N_2O A product of reactions of NH_3 in the SCR and AMOX, N_2O is of concern because of its impact on climate. Emissions of N_2O during the 16-hour cycle measured in Phase 2 (0.073 g/bhp-hr) were lower than the forthcoming 2014 regulatory standard of 0.1 g/bhp-hr.

CO_2 CO_2 emission is a good indicator of fuel economy. In view of the role played by CO_2 as a major greenhouse gas, there was an interest in measuring these emissions in ACES. The first CO_2 emission standard took effect in 2014 (567 g/bhp-hr), so ACES engines were not designed to meet a specific standard. However, a comparison of emission rates measured in ACES Phase 1 and 2 showed that they slightly exceeded or were very close to the 2014 standard.

Table 2. Emissions of Selected Gases in NTDE from 2007- and 2010-Technology Engines (g/bhp-hr, 16-hour cycle)[a]

Pollutant	2007-Technology Engines	2010-Technology Engines	% Change in 2010- Relative to 2007-Technology Engines
NO_2	0.73 ± 0.20	0.046 ± 0.029	−94
SO_2	0.00112 ± 0.00025	0.00033 ± 0.00016	−71
NH_3	< 0.0001	0.0025 ± 0.0014	> 2400
N_2O	0.010 ± 0.003	0.073 ± 0.030	730
CO_2	590.2 ± 22.7	571.3 ± 41.4	[b]

[a] Adapted from Khalek et al. 2015.

[b] No discernible change within the measurement uncertainties.

Abbreviations: g/bhp-hr = grams per brake horsepower-hour; CO_2 = carbon dioxide; N_2O = nitrous oxide; NH_3 = ammonia; NO_2 = nitrogen dioxide; NTDE = new-technology diesel exhaust; SO_2 = sulfur dioxide.

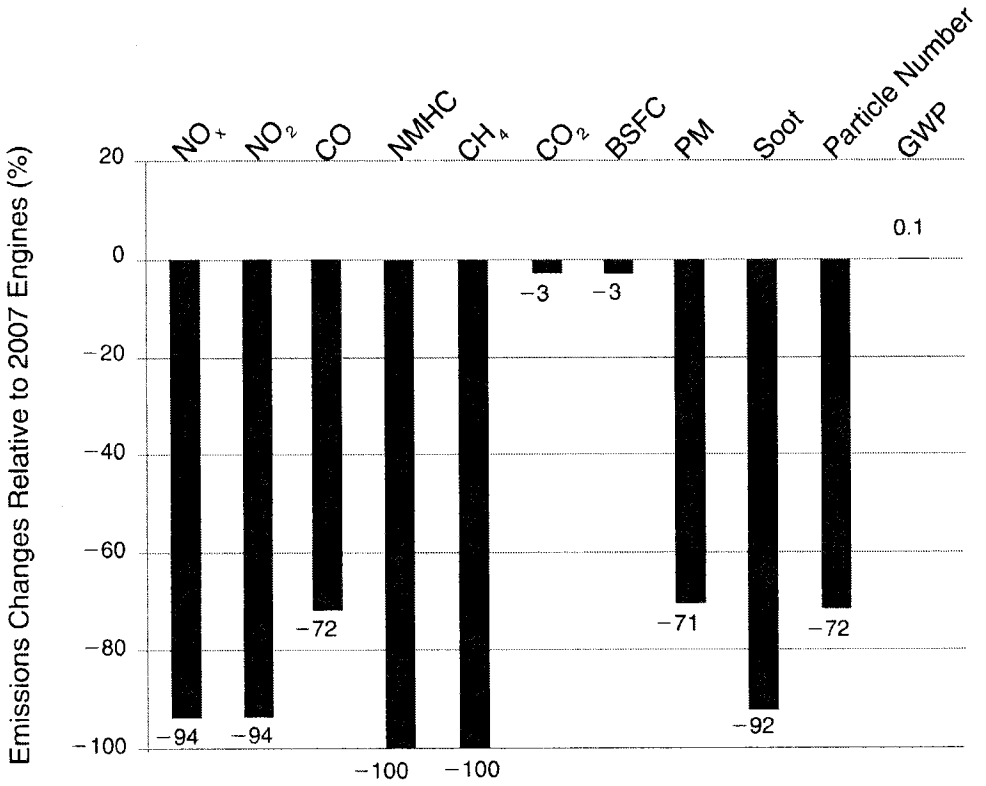


Figure 5. Percent change in emissions from 2010 engines relative to 2007-engine emissions. (BSFC = brake-specific fuel consumption; GWP = global-warming potential.) (From Khalek et al. 2012, Figure ES-2.)

Other Unregulated Emissions

Average emissions of unregulated pollutants and air toxic contaminants from MY 2007 engines showed a significant reduction, compared with emissions in previous studies testing 2004 engines. These included particle components (EC, OC, metals and other elements, and inorganic ions), and gas- and particle-phase volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs), including PAHs and nitro-PAHs (Table 3, Figure 5). Their emissions from the MY 2010 engines showed a 90% to 99% reduction compared with 2004 engines.

PHASE 1 AND 2 CONCLUSIONS

PM emissions from all new-technology diesel engines tested in ACES were substantially below (90%) the stringent 2007

Table 3. Summary for Unregulated Emissions for Groups of Compounds in the Constant Volume Sampler from 2004-, 2007-, and 2010-Technology Engines[a]

	2004 Engines (Avg ± SD, mg/hr)	2007 Engines (Avg ± SD, mg/hr)	2010 Engines (Avg ± SD, mg/hr)	2010 Engines (Avg % Reduction Relative to 2004 Engines)	2010 Engines (Avg % Reduction Relative to 2007 Engines)
Single-ring aromatics	405.0 ± 148.5	71.6 ± 32.97	40.63 ± 49.04	90	[b]
PAHs	325.0 ± 106.1	69.7 ± 23.55	2.4 ± 1.0	> 99	97
Hopanes and steranes	8.2 ± 6.9	0.1 ± 0.12	0.010 ± 0.007	> 99	90
Nitro-PAHs	0.3 ± 0.0	0.1 ± 0.0	0.0011 ± 0.0005	> 99	99
Carbonyls	12500.0 ± 3535.5	255.3 ± 95.2	57.4 ± 39.1	> 99	78
Metals and elements	400.0 ± 141.4	6.7 ± 3.0	1.4 ± 1.0	> 99	79
OC	1180.0 ± 70.7	52.8 ± 47.1	39.2 ± 33.6	97	[b]
EC	3445.0 ± 1110.2	22.6 ± 4.7	12.2 ± 6.2	> 99	46
Dioxins/furans	N/A	6.2×10^{-5} $\pm 5.2 \times 10^{-5}$	8.5×10^{-9} $\pm 1.1 \times 10^{-8}$	> 99[c]	> 99

[a] Adapted from Khalek et al. 2015. The g/bhp-hour emission rates for the 2007 and 2010 engines were converted to mg/hr for comparisons with the reported emission rates of the 2004 engines.

[b] No discernible change within the measurement uncertainties.

[c] Relative to 1998-technology engines.

Abbreviations: EC = elemental carbon; OC = organic carbon; N/A = not available; PAHs = polycyclic aromatic hydrocarbons.

PM standard. Similarly, average NO_x levels in NTDE from the three 2010 engines were below the 2010 NO_x standard. Highly reduced levels of CO, NMHC, and unregulated toxic air contaminants (including metals and gas- and particle-phase VOCs and SVOCs) were observed in NTDE compared with TDE. Though the PM standard in 2010 was the same as that in 2007, PM emissions in 2010-engine exhaust were lower than those in the 2007-engine exhaust. Only NO_2 emissions showed a slight deviation from this pattern: Though the level of NO_x in MY 2007 engine exhaust was below the 2004 standard, the level of NO_2 was higher due to oxidation of NO in the DOC and DPF. The introduction of SCR, beginning with the MY 2010 engines, resolved this issue, as noted above.

There were several notable differences in PM composition between TDE and NTDE and between Phase 1 (2007-engine) and Phase 2 (2010-engine) NTDE (Figure 4). One was the large reduction in sulfate in PM from 2010 engines compared with 2007. In addition, EC, the major component of PM in TDE (about 70% of the total mass of diesel PM) was substantially reduced in both the 2007 and 2010 NTDE (approximately 13% and 16%, respectively,). Also, whereas sulfate was the dominant component (53%) in PM emitted by 2007 engines, OC was the dominant component (66%) in the PM from 2010 engines.

Improved engine controls and an aftertreatment system (incorporating DOC, DPF, SCR, and AMOX technology) in the 2010 engines tested in Phase 2 were largely responsible for these emission reductions, either by direct reduction in the amount of pollutants coming out of the engine or by reductions within the aftertreatment system. The improvements and engine calibration were such that active DPF regeneration events were not triggered during the three consecutive 16-hour cycles in Phase 2. The lack of regeneration events was thought to result in reduced emissions of SO_2 and sulfate, as well as PM mass and number — although it is very likely that the average PM emissions during any test cycle with or without regeneration would be below the standard, as was the case in Phase 1 testing.

Under normal operations, sulfur compounds are adsorbed on the DPF and are released due to the high temperatures that occur during active regeneration. The retention of sulfate, which can serve as a precursor for nanoparticles, on the DPF in 2010 engines may be one of the reasons for the lower particle number emission rate in Phase 2. The absence of regeneration in Phase 2 was thought to be associated with a higher filtration efficiency of the loaded DPF and consequently with the reduction in EC, metal compounds, and particle number. Future research on the emission contribution of regeneration in 2010-compliant engines will be useful.

SELECTION OF A 2007-TECHNOLOGY ENGINE FOR PHASE 3

One of the goals of Phase 1 was to select one engine, from among the four 2007 engines tested, for generating the exhaust for the rodent inhalation exposures in the chronic animal study (Phase 3). The engine selection process was guided by a group comprising a subset of members of the CRC–ACES Panel and the HEI ACES Oversight Committee. After a review of the emissions indicated similar emissions of gaseous and particulate-phase compounds from all four engines, engine B was randomly selected

for the health study. Subsequently, the manufacturer of engine B (Detroit Diesel, a subsidiary of Daimler Corp.) provided a backup engine (referred to as B′) to SwRI. Engine B′ had the same technical specifications and was equipped with the same emission controls as engine B, but was MY 2008. Engine B′ underwent testing at SwRI for overall performance and for characterization of regulated emissions. At the end of the testing, both engines were shipped to Lovelace Respiratory Research Institute (LRRI), where they underwent further testing before the start of the rodent exposures.

PHASE 3: HEALTH EFFECTS TESTING

Through competitive processes, HEI funded several investigator teams in Phase 3, which was conducted in two subphases:

- Phase 3A: the establishment of an exposure facility, as well as the characterization and optimization of emission exposure conditions before the start of the inhalation study; and
- Phase 3B: the evaluation of health outcomes in rats exposed via inhalation to different concentrations of NTDE (low, medium, or high) or to filtered air for up to 30 months and in mice exposed for up to 3 months.

Exposure levels in Phase 3B were set based on NO_2 rather than PM levels — which had been used in previous studies of TDE — because the PM level in NTDE was greatly reduced (ACES 2015). NO_2 was present in relatively high concentrations in the exhaust of 2007 engines and was known to be associated with non-cancer effects. In addition there were studies of exposure to NO_2 alone that could be used to determine the target NO_2 concentrations for ACES.

In keeping with the approach normally applied by the National Toxicology Program (NTP) to set the highest exposure at a level that was expected to result in observable effects during the study, the highest NO_2 exposure concentration, 4.2 ppm, was chosen to provide a comparison with the same cumulative exposure (the product of concentration and exposure duration) as in a prior HEI-funded long-term inhalation study in rats exposed to NO_2 alone conducted by Mauderly and colleagues (1989). In that study, minor biological changes — but no cancer or precancerous changes — were observed in the respiratory tract. The intermediate concentration was lower by a factor of five (i.e., 0.8 ppm). The lowest concentration selected in ACES was 0.1 ppm, in order to provide a likely *no observed adverse effect level*; this concentration is only twice the ambient NO_2 standard of 0.053 ppm (annual average) mandated under the National Ambient Air Quality Standards (NAAQS).

PHASE 3A

Phase 3A was conducted by Drs. Joe L. Mauderly and Jacob McDonald at LRRI. At the time ACES was launched, no existing facility was available to conduct a lifetime exposure of rodents to emissions from a HHDD engine. A special facility was constructed for housing the dynamometer and the 2007-compliant engine — which was considerably larger than diesel engines previously tested at LRRI — and for delivering the exhaust to the animal exposure chambers (Mauderly and McDonald 2012).

The main goals of Phase 3A were (1) to confirm proper performance of the engine and dynamometer, (2) to fine-tune the dilution settings for generating the three target pollutant concentrations and characterize the test atmospheres in animal exposure chambers, and (3) to verify proper performance of the engine and the dynamometer over a period of 15 to 30 days.

In order to ensure that all operating and control systems were functioning correctly, the LRRI investigators used a so-called mule engine (a third engine, of the same make and model as engine B) in order to avoid the unlikely possibility that the test engines B or B′ would be damaged during this process. Subsequently, the investigators characterized the emissions of engine B in the dilution tunnel using the FTP cycle as well as emissions from both engines B and B′ in the chambers using the 16-hour cycle. Overall, the results of Phase 3A showed that both engines performed according to the manufacturer's specification and were stable over time and that the target NO_2 concentrations could be achieved within 10% of the targets (Mauderly and MacDonald 2012).

PHASE 3B

This phase comprised several studies, each selected after solicitation of applications and peer review (ACES 2012, 2015). The "core" study, conducted at LRRI evaluated more than 100 health endpoints in rats exposed to NTDE via inhalation for up to 30 months and in mice for up to 3 months. The core study was led initially by Dr. Joe L. Mauderly and then by Dr. Jacob McDonald after Dr. Mauderly retired.

To maximize the information obtained from exposed animals in Phase 3B, HEI funded "ancillary" studies to measure additional endpoints that were not normally part of long-term rodent exposure studies. The ancillary studies focused on markers of genotoxicity and on markers of inflammation and damage in the airways and other organs. The LRRI team sent samples from animals exposed there to the ACES ancillary teams. These ancillary studies were led by Drs. Jeffrey C. Bemis of Litron Laboratories, Rochester, New York; Lance M. Hallberg of the University of Texas Medical Branch, Galveston, Texas; and Daniel J. Conklin and Maiying Kong of the University of Louisville, Louisville, Kentucky.

Originally, the mouse study was also planned as a lifetime study, but the length of exposure in mice was reduced for two main reasons: first, rats were the rodent species in which cancer and other responses to lifetime exposure had been observed in previous diesel exhaust studies and, second, the cost of Phase 3 was already substantial. Therefore, the ACES Oversight Committee determined that it would be sufficient to reduce the duration of the mouse exposures to 3 months, with the goal of exploring any short-term effects in both species.

Hypothesis

The (null) hypothesis for the study was that NTDE would not cause an increase in tumor formation or have substantial toxic health effects in rats or mice, although some biological effects might occur.

Approach

To test the hypothesis, the ACES health experiments were designed to be the most rigorous possible testing of NTDE — significantly more rigorous than protocols recommended in lifetime animal testing for most compounds by the NTP. This increased rigor included a strenuous engine operations cycle; exposure for 16 hours each day, 5 days a week, over the animals' approximately 30-month lifetimes (as compared with 6 hours a day for 24 months, typically used by NTP); a large number of rats in each group (140 in ACES vs. generally 50 by NTP); and a very large number (> 100) of biological endpoints.

Exposures McDonald and colleagues generated exhaust from two similar 2007-compliant heavy-duty diesel engines, termed B and B′, as described earlier (for details, see Mc Donald et al. 2015). Engine B′ was used at the start (February 2010–September 2011) of the animal exposures because this slightly newer model better represented the engines in the market at that time. Because of engine maintenance requirements, this engine was replaced with engine B, which continued in operation until May 2012. Engine B′ was reinstalled in June 2012 and operated until the end of the study (December 2012). Both engines were fuelled with ULSD fuel meeting current on-road specifications and were operated with a dynamometer. The engines and associated systems were maintained as recommended by the engine manufacturer. The engine was run on the 16-hour cycle developed for ACES (see related text box "Testing of NTDE in ACES"). The emissions were characterized as they entered the animal exposure chambers, as well as inside the chambers, allowing an assessment of the impact of the animals' presence on the composition of the exposure atmospheres.

The investigators exposed male and female 8-week-old Wistar Han rats (140 animals of each sex per exposure level) and male and female 8-week-old C57BL/6 mice (66 animals of each sex per exposure level) to NTDE at one of the three target concentrations — 4.2 (high), 0.8 (mid), or 0.1 (low) ppm NO_2 — or to filtered air as a control. Exposures were conducted for 16 hours per day for 5 days per week. The 3-month mouse study was completed before the rat study commenced, providing an additional opportunity to test the robustness of the exposure system before starting the longer-term study.

Choice of Rat and Mouse Strains The Wistar Han rat was selected for ACES for several reasons: the strain's longevity, its previous use in chronic inhalation studies of TDE (particularly in the studies of Heinrich and colleagues [1986, 1995]), the existence of historical information on cancer incidence in this strain, the relatively low rate of spontaneous background tumors compared with some other species, and an acceptable maximum body weight expected to be reached by males during the study (affecting housing in inhalation chambers). Some questions were raised during design of ACES about the sensitivity of the Wistar Han rat strain to exhibit certain toxic effects. The ACES Oversight Committee determined that, although a proportion of these rats are known to have a mutation in the aryl hydrocarbon receptor (AhR) gene, conferring it resistance to dioxin's lethality, the strain is still responsive to other dioxin-mediated effects (Okey et al. 2005). Moreover the Wistar Han strain is known to develop lung tumors after long-term exposure to TDE and other particles (Karagianes et al. 1981; Heinrich et al. 1986, 1995). The C57BL/6 mouse strain was chosen because of low incidence of lung tumors and its history of use in diesel-exposure animal bioassays.

Core Study Evaluations Groups of male and female rats were euthanized at LRRI after 1, 3, 12, and 24 months of exposure, as well as at the terminal sacrifice. Based on pre-established survival criteria, the final sacrifice of all surviving male rats was conducted after 28 months of exposure and of all surviving female rats after 30 months of exposure. Mice were euthanized after exposures of 1 or 3 months.

The LRRI investigators harvested blood and tissues at the various time points (10 animals of each sex per exposure group at 1, 3, 12, and 24 months, and 100 rats of each sex per exposure group for the terminal sacrifice) and evaluated animals histologically at each point for the presence of tumors and other lesions in the airways and in multiple tissues. In addition, they examined a vast array of biological endpoints: hematologic (multiple cell types and coagulation); serum chemistry (including triglyceride and protein components); in lung lavage (including numbers of cells and levels of cytokines, markers of oxidative stress, and tissue injury); and, in rats only, pulmonary function.

Ancillary Study Evaluations The LRRI investigators sent aliquots of blood and tissue samples from 5 to 10 animals of each sex per exposure group to the ancillary studies investigators. As a marker of genotoxicity, Bemis and colleagues measured the number of reticulocytes — immature red blood cells — containing micronuclei in peripheral blood. Hallberg and colleagues assessed other markers of genotoxicity, in particular, oxidative damage to cell components, which is believed to be involved in the induction of carcinogenesis. To detect damage to DNA, the Hallberg team used the comet assay on lung cells and measured 8-hydroxy-deoxyguanosine (8-OHdG) levels in blood. As a measure of damage to lipids, they also assessed levels of thiobarbituric acid reactive substances (TBARs) in brain tissue. Conklin and Kong measured more than 20 plasma markers of inflammation and thrombosis — including lipids, cytokines, and other soluble factors — and assessed the histopathological effects of chronic exposure on cardiac fibrosis and remodeling of the aorta.

RESULTS OF HEALTH STUDIES

Emissions Characterization with Animals in Exposure Chambers

With animals in the chambers, the most abundant pollutants by mass were CO_2, CO, NO, and NO_2, consistent with the findings in Phase 1 (McDonald et al. 2015). Concentrations of sulfur dioxide (SO_2), VOCs, and SVOCs were very low. High-molecular-weight alkanes and polar compounds (derivatives of benzoic acid) were the major SVOCs, with lower levels of PAHs.

As expected, NTDE particle concentrations generated by the engine were very low over the course of the study, ranging from 2.5 to approximately 8 $\mu g/m^3$ for the three exposure levels; particle number concentrations ranged from 2 to 8×10^5 particle/cm^3. Continuous measurements of particle number indicated that most

of the particles were generated during regeneration of the DPF, which occurred once or twice in a 16-hour exposure period, consistent with the Phase 1 findings (Khalek et al. 2009). These combustion-derived particles were in the ultrafine range — median particle size was approximately 20 nm (based on number) and 40 nm (based on mass).

In the exposure chambers, rats generated particles — from dander and feed — ranging from 3.5 to 4 µm in diameter, which were major contributors to the total particle mass. Additionally, the animals contributed to VOCs and also produced nitrate and ammonium ions (not detected in Phase 1), which formed inorganic salts. The analysis of particle composition in the chambers showed that approximately 50% of the particle mass was EC, similar to findings in Phase 1. However the EC to OC ratio was found to have decreased over the course of the study (from 0.45 to 0.04); the investigators speculated this might have been due to improved efficiency of the DPF in capturing EC and potential artifacts in OC results due to adsorption of VOC to the filter. Most of the remainder of the PM mass consisted of nitrate, ammonium, sulfate, and the elements zinc, manganese, copper, and iron.

Table 4 compares the average concentrations of PM, NO_2, and NO_x in ACES with data from two much earlier bioassays with TDE. The percentage of NO_2 relative to NO_x was much lower in the chamber atmosphere from the older engines (11% and 16%) than in the chamber atmosphere from the 2007 engine (36%) at the highest exposure level. At the same time, the NO_x concentration to which animals were exposed was higher for the older engines (approximately 23 to 33 ppm) than for the 2007 engine (approximately 12 ppm), showing the progress made in reducing NO_x emissions in the 2007 engines. The changes in NO_2 and PM levels in the different engine emissions were reflected in changed NO_2 to PM ratios: high in NTDE (140) and low in TDE (approximately 0.6).

Core Study: Key Biological Findings

Histopathology in Rats after Lifetime Exposure Chronic exposure to NTDE did not induce tumors or precancerous changes in the rat lung (Figure 6) and did not increase tumor incidence in any tissue outside the lungs. Some mild histological changes were found in the lung — periacinar epithelial hyperplasia, bronchiolization, accumulation of macrophages, and periacinar interstitial fibrosis — that were confined to a small region, the centriacinus, which is involved in gas exchange. Histological changes were detected as early as 3 months after the start of exposure in rats exposed to high-level NTDE. The effects of NTDE in the lung over the entire exposure duration closely resembled changes noted after long-term exposures to gaseous oxidant pollutants, in particular NO_2 (Figure 7 and Mauderly et al. 1989; Kubota et al. 1987).

These findings are in marked contrast to the effects of chronic exposure to TDE in previous rat studies in which lung tumors were detected (at 30 months in Heinrich et al. 1986, shown in Figure 8; and at 24 months in Mauderly et al. 1994), and the deposition of soot, as well as precancerous changes such as the presence of a chronic inflammatory response, was observed in the lung.

Other Endpoints in Rats after Lifetime Exposure Of more than 100 different biological endpoints evaluated for up to 28 months in males and 30 months in females in lung tissue, bronchoalveolar lavage fluid (BALF), and blood, only a few showed NTDE-associated changes. These included small increases in levels of heme oxygenase, interleukin-6, keratinocyte-derived chemokine, micro–total protein, total white blood cells, and macrophages in the lung, consistent with mild pulmonary inflammation and oxidative stress.

There were small decreases in some measures of respiratory function over the course of the study, in particular those

Table 4. Comparison of Exhaust Characteristics for Diesel Emissions Used for Chronic Bioassays

Study	Exposure level; dilution	PM mass gravimetric (mg/m3)	NO_2 (ppm)	NO_x (ppm)	NO_2/PM (ratio)	NO_2/NO_x (%)
ACES Phase 3B (NTDE, 2007 heavy-duty diesel engine and ultra-low-sulfur fuel, rats)	High exposure; 25:1 dilution	0.030	4.2	11.7	140	36
Mauderly et al. 1994 (TDE, 1988 6.2-L light-duty diesel engine and high-sulfur fuel, rats)	High exposure; dilution ratio NR	6.33	3.8	23.5	0.6	16
Heinrich et al. 1995 (TDE,1.6-L light-duty diesel engine[a] and high-sulfur fuel, rats)	High exposure; 9:1 dilution	6.98	3.8	33.1	0.54	11

[a] Age of engine not specified.

Abbreviations: NO_2 = nitrogen dioxide; NO_x = nitrogen oxides; NR = not reported; NTDE = new-technology diesel exhaust; PM = particulate matter; TDE = traditional-technology diesel exhaust.

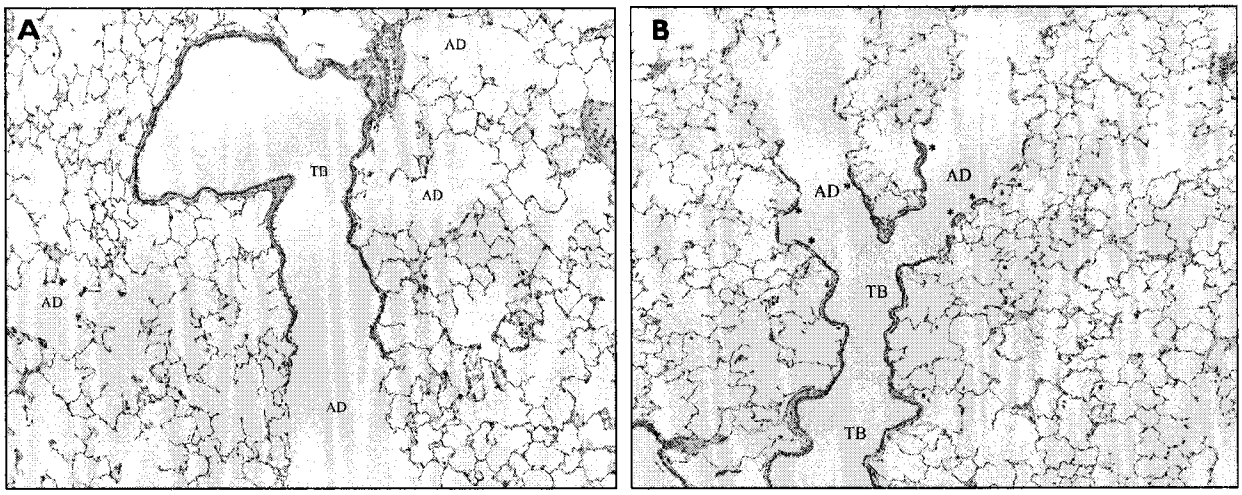


Figure 6. Few effects seen in lungs of female Wistar Han rats exposed for 30 months to 2007-technology diesel exhaust in ACES Phase 3B: (A) Control exposure (to filtered air), showing a normal rat lung with a terminal bronchiole (TB) opening downward and ending with an alveolar duct (AD) opening into alveolar spaces. Other alveolar ducts and alveoli surround the larger airway. In contrast to Figure 6B, there is no thickening of the alveolar duct interstitium or alveolar walls, nor are they lined by an increase in cuboidal epithelial cells; **(B)** High-level exposure to NTDE (4.2 ppm NO_2), showing a terminal bronchiole (TB) dividing into two alveolar ducts (AD) that exhibit a very minimal increase in thickening of the walls of the ducts, which are lined with a minimal increase in cuboidal non-ciliated epithelial cells where the walls are thickened by increased collagen (*). These changes were found at only the highest NTDE level. (Original magnification 100×; photographs by Rod Miller, Experimental Pathology Laboratories [EPL].)

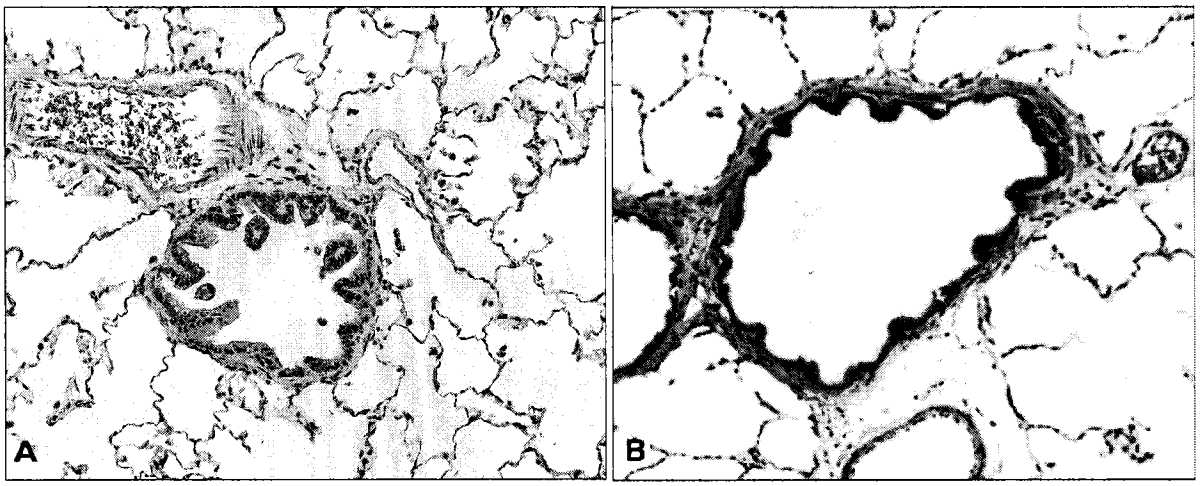


Figure 7. Long-term exposures of rats to NTDE and NO_2 show similar effects in the lungs: (A) Exposure to 2007-technology high-level NTDE (4.2 ppm NO_2) in male Wistar Han rats, for 28 months in ACES Phase 3B (original magnification 200×). Preterminal bronchiole showing epithelial hyperplasia accompanied by papillary projections into the bronchiole that was found in some rats exposed only to the highest level of diesel exhaust (photograph by Rod Miller, EPL.); **(B)** High-level NO_2 (9.5 ppm NO_2) in male F344 rats, exposed for 24 months, from Mauderly et al. 1989 (original magnification 200×). Preterminal bronchiole showing epithelial cell crowding, similar to what is shown in Figure 7A, but with less inward projection of the epithelium. These changes were uncommon in the NO_2 study of Mauderly and colleagues in F344 rats, but were seen more often in the 1987 study by Kubota and colleagues in which Wistar Han rats were exposed to 4 ppm NO_2 for 27 months. (Lung slide provided by Andrew Gigliotti, LRRI; photograph by Rod Miller, EPL.)

concerned with expiratory flow. They occurred predominantly at the highest exposure level and more in females than males. Decreases in some measures of flow — including peak expiratory flow, forced expiratory flow between 25% and 50% of forced vital capacity, and maximal mid-expiratory flow — were detected in female rats in the high-level NTDE exposure group after 3 months. The diffusing capacity of CO (a measure of alveolar–capillary gas exchange) showed a small decrease in male and female rats as a result of exposure to NTDE.

Histopathology in Mice after 1- and 3-Month Exposures No significant exposure-related changes were detected histopathologically in the lungs of male and female mice after exposure to NTDE for 1 or 3 months. Of the many lung cell and biochemical endpoints examined, small changes were reported in just a few, and these were found only in BALF. Respiratory function was not evaluated.

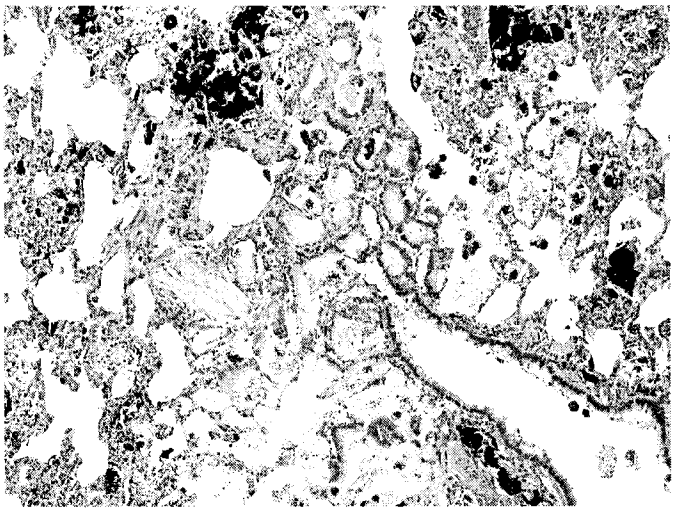


Figure 8. Effects of long-term exposure to TDE (4.2 mg/m^3 PM) for 30 months in female Wistar Han rats (Heinrich et al. 1986). Prominent black diesel soot particulates are present free in alveoli, in the numerous pulmonary alveolar macrophages, and in interstitial tissues. Marked centriacinar epithelial hyperplasia and bronchiolization occurred. Marked chronic inflammation also occurred, characterized by a mixed inflammatory cell infiltrate, fibrosis, and some sterol cleft formation. The entire lung was involved, and this picture is dramatically different from what was found in ACES Phase 3B. (Original magnification 100×; lung slide provided by Heinrich Ernst, Fraunhofer Institute, Hannover, Germany; photograph by Rod Miller, EPL.)

Ancillary Studies: Key Results

Lack of Genotoxic Effects Exposure of rats for up to 24 months or mice for up to 3 months to NTDE did not increase the frequency of micronuclei-containing reticulocytes in peripheral blood (Bemis et al. 2015) and did not cause DNA damage (Hallberg et al. 2015). A few small and scattered effects were noted on DNA damage after 1- and 3-month exposures in rats, but no effects were seen at 12 and 24 months. The 24-month exposure in rats did not induce oxidative damage (as measured by 8-OHdG levels in serum) or lipid peroxidation in the hippocampus (as measured by TBARs). A few small and scattered effects were noted in 8-OHdG levels in some NTDE-exposed groups at 12 months in rats, but these were not exposure-dependent and were not seen at other exposure times.

Lack of Vascular and Cardiac Effects In the Conklin and Kong study (2015) exposure to NTDE at any concentration for up to 24 months in rats showed few effects on the more than 20 markers measured. Some scattered changes were detected in one or more NTDE-exposed groups; however, most of these changes were found at only one exposure time point, predominantly in females, and so were of uncertain pathophysiological significance. NTDE had no effect on cardiac fibrosis or aortic remodeling after up to 24 months of exposure.

In mice, exposure to NTDE at all concentrations for 1 or 3 months had no effect on most of the blood markers measured, apart from some scattered changes in one or more NTDE-exposed groups.

PHASE 3 CONCLUSIONS

In its independent review of the core Phase 3B report by McDonald and colleagues (2015), the HEI ACES Review Panel concluded that this study is the first to conduct a careful, comprehensive, and well-executed evaluation in rats of lifetime inhalation exposure to NTDE from a 2007-compliant engine. The findings confirmed that the concentrations of components of NTDE differ strikingly from those of older engines, in which the concentrations of PM, as well as volatile and PM-associated organic species, are much higher.

The investigators confirmed the study's hypothesis, namely, that exposure to NTDE would not cause an increase in tumor formation or substantial toxic health effects in rats, although some biological effects might occur. The overall conclusion was that chronic exposure of rats to NTDE did not produce tumors in the lung; these observations are in marked contrast to the effects of chronic exposure to TDE observed in multiple previous rat studies, in which lung tumors, as well as inflammation and the deposition of soot in the lung, were observed. The Pathology Working Group (see description in text box "Sponsorship, Planning, Oversight, and Review of the ACES Program") noted that the histological effects of NTDE in the lung more closely resembled changes noted after long-term exposures to gaseous oxidant pollutants, in particular NO_2, or to TDE from which particles had been filtered out. It is possible that components of NTDE other than NO_2 may have contributed to the effects reported, but the low levels of other components suggested that they were not likely to be primarily responsible.

The small decreases in some respiratory endpoints that were found, in particular those concerned with expiratory flow, were predominantly at the highest exposure level and more in females than males. These were consistent with the histopathological findings of mild changes in the gas-exchange regions of the lung, indicating that the histological changes might have produced functional effects. In addition, a few small changes in a few markers of oxidative stress and inflammation were detected in lung tissue, BALF, and blood, but the ACES Review Panel agreed that these were not of clear health significance.

The Review Panel concluded that ancillary studies by Bemis and colleagues, Hallberg and colleagues, and Conklin and Kong were valuable extensions to the ACES core investigation. These generally well implemented studies took advantage of samples collected by McDonald and colleagues at several exposure time points up to 24 months to assess multiple endpoints that are not normally part of chronic inhalation bioassays. No genotoxic effects associated with exposure for up to 24 months to NTDE were detected. However, the ACES Review Panel noted that the assays measured relatively short-term effects (lasting 1 month or less), which somewhat reduced confidence in the utility of these negative findings. In Conklin and Kong's study (2015), NTDE had no effect on cardiac fibrosis or aortic remodeling and few effects — predominantly in females and of uncertain pathophysiological significance — on the inflammatory and thrombotic pathway endpoints measured in plasma over 24 months of exposure.

Overall, these results indicate that rats exposed to one of three levels of NTDE from a 2007-compliant engine for up to 30 months, for 16 hours per day, 5 days a week, with use of a

strenuous operating cycle that more accurately reflected the real-world operation of a modern engine than cycles used in previous studies, produced no lung tumors and showed few other exposure-related biological effects. In contrast to the findings in rats chronically exposed to TDE, there was no induction of tumors or precancerous changes in the lung and no increase in tumors that were considered to be related to NTDE exposure in any other tissue. The effects that were observed with NTDE exposure were limited to the respiratory tract and were mild and generally seen only at the highest exposure level. The histological changes in the lungs were consistent with previous findings in rats after long-term exposure to NO_2 — a major component of the exposure atmosphere, which was substantially further reduced in 2010-compliant engines.

DISCUSSION AND CONCLUSIONS

The results of ACES Phase 1 and 2 showed that the aftertreatment technologies introduced to control emissions from diesel engines in 2007 and 2010 were highly effective and met — and exceeded — the reductions mandated by U.S. regulations. These regulations, along with the durability requirements for the aftertreatment devices and in-use testing to detect any malfunction of the devices, are the most stringent in the world. The 2007-compliant new-technology diesel engines tested in ACES — which were equipped with EGR and other sophisticated engine controls, along with DOC and DPF aftertreatment technologies, and operated using ULSD fuel — produced significantly reduced levels of PM (≥ 90%) and VOCs and SVOCs (>90%) compared with emissions from old, or traditional-technology, engines. The NO_x emissions from the 2007 engines were reduced, but the full extent of NO_x controls was required only in 2010, with the introduction of urea-SCR (and AMOX to capture any excess NH_3). In the 2010 engines, NO_x levels were reduced by more than 90% compared with 2007 engines; PM emissions were also further reduced. The ACES engine testing was performed using the usual certification (FTP) test cycle, as well as a more demanding 16-hour cycle, developed especially for the ACES program, that better represents real-world driving conditions and gives greater confidence in the relevance of the emissions testing results.

Emissions from a 2007 new-technology engine, when tested in the ACES Phase 3 health study, were found to be not carcinogenic in the rat. The exposure conditions in ACES were optimized to deliver as high a dose as practical: animals were exposed 16 hours a day, 5 days a week over their lifetime (up to 30 months) to one of three levels of emissions. The key observation was that these emissions did not increase lung tumors or have substantial toxic effects; this is in marked contrast to the effects of chronic exposure to TDE observed in multiple previous studies in rats in which lung tumors, as well as inflammation and accumulation of copious amounts of soot in the lungs, were observed. A few NTDE-associated effects in rat lungs in ACES were observed; however, these effects more closely resembled changes seen in earlier studies after long-term exposures to gaseous oxidant pollutants, in particular NO_2, and to TDE from which PM was removed. It is possible that components of NTDE other than NO_2 may have contributed to the effects reported, but the low levels of all other components suggest that they are unlikely to be primarily responsible. A large number of genetic, physiological, and biochemical endpoints studied in Phase 3B also did not show any consistent exposure-related effects.

In interpreting the results of the ACES program, it is useful to consider them in the context of human hazard and risk assessment. *Hazard assessment* is the determination of whether a substance poses a health risk at any level of exposure. *Risk assessment,* the next step, attempts to determine whether and how risk from that substance occurs at specific levels of exposure, generally those found in today's ambient or occupational environment.

The ACES health investigation, like every previous chronic study, was performed in rodents and not in humans. Given the impossibility of conducting a chronic, controlled exposure study of diesel emissions — or any other substance — in humans, assessment in rodents for carcinogenicity is routinely performed and, when the results are positive, provides suggestive evidence for human carcinogenicity. In the case of TDE, studies in rats have provided evidence for carcinogenicity, though whether such carcinogenicity is due to overload of lungs with particles has been a subject of debate because exposure to other types of particles at high levels can also induce cancer in rats. Additionally, epidemiology studies have found an association between occupational exposure to TDE and lung cancer. The studies of TDE exposure in rats, as well as human epidemiology studies, led IARC to update its hazard assessment of diesel engine emissions, classifying diesel emissions as Category 1 or "known human carcinogen," even while noting the substantially reduced emissions from new-technology engines (IARC 2012). ACES now provides data showing greatly reduced amounts of PM and other toxic compounds in NTDE, as well as evidence for a lack of carcinogenicity in rodents.

In conducting any risk assessment for NTDE, two key elements should be considered: the toxicity and composition of the emissions, and the contribution of the pollutants to ambient exposure.

ACES provided useful and detailed information about the toxicity and composition of PM from the new engines. Consistent with regulatory requirements, the mass of PM contained in NTDE was much lower than that in TDE (Table 1, Figure 3). In terms of composition, compared with PM in TDE, PM in NTDE had a substantially lower proportion of EC, a different ratio of OC:EC, and a much larger proportion of sulfate (Figure 4). Importantly, PM in NTDE had a significantly lower level of known carcinogens, such as the PAHs benzo[*a*]pyrene and benzo[*e*]pyrene and nitro-PAHs such as nitro- and dinitropyrenes (Table 3).

In conducting the rat study in Phase 3, ACES investigators took steps to ensure that the cumulative dose of PM to which animals were exposed was maximized. While ACES was not designed to address the specific question of whether the toxicity-per-unit mass of the PM emitted from the 2007-compliant engines was different from the PM emitted from the older engines, the findings of significant reductions in carcinogenic components, as well as the much reduced mass of PM in NTDE, strongly suggest that the overall toxicity of PM in NTDE is reduced relative to PM

in TDE. Although the precise role played by each component of PM in causing effects is not known, it is highly unlikely that the changes in PM in NTDE relative to PM in TDE would significantly increase the overall toxicity of PM in NTDE.

As with any study with negative results, a question may still be raised whether ACES had sufficient statistical power to detect a carcinogenic effect in view of the very low levels of PM in NTDE. However, since the reduction in PM mass is of the order of 100-fold, the per-unit-mass toxicity of either the PM or one of its constituents in NTDE would need to be substantially higher to observe carcinogenicity that is comparable to that of TDE. There is no evidence for such a phenomenon. Therefore, the most straightforward interpretation of the observations of the ACES health investigation is that the overall toxicity of NTDE is significantly decreased compared with the toxicity of TDE.

Given the changes in emission rates, the contribution of diesel engines to ambient levels of air pollution is declining in the United States. During the years since the new diesel emissions regulations took effect, there has been a steady turnover in the diesel fleet, and older, more polluting engines are gradually being replaced with modern, clean engines, with a resulting substantial decrease in emissions and ambient pollutant levels. One estimate suggests that more than a third of the truck and bus fleet in the United States conforms with 2007 or later regulations, though there are variations among geographic areas in the United States, as well as among the different fleet operators (Diesel Technology Forum 2014). Additional regulations to reduce emissions from older engines — such as retrofit aftertreatment devices and school bus anti-idling programs — have also been mandated in various jurisdictions, and substantial federal and state funding has been made available to subsidize such replacements. Several studies examining emissions under real-world conditions from individual vehicles have reported reductions in PM emissions as the proportion of new or retrofitted diesel engines increases, showing the effectiveness of in-use DPFs (Bishop et al. 2013; Preble et al. 2015). The net effect of these measures can now be seen from data on ambient $PM_{2.5}$ (particulate matter $\leq$ 2.5 μm in aerodynamic diameter) levels in urban areas — of which diesel emissions are an important part — which are showing a steady decline (Propper et al. 2015; South Coast Air Quality Management District 2015).

As with the introduction of any new technology on a large scale in the marketplace, some challenges with the use of the new aftertreatment technologies have been observed. First, though DPFs are very effective, some recent reports suggest that, under real-world conditions, a small proportion of new-technology engines still produce higher emissions of PM (Envirotest Canada 2013; Bishop et al. 2015). More stringent in-use inspection programs are currently being considered to identify such vehicles and rectify their higher-than-expected emissions.

Second, elevated NO_2 emissions have been noted when the SCR device does not operate effectively, for example, during cold-start, low-load conditions and during stop-and-go driving, when the exhaust is not sufficiently hot to allow optimum SCR function (Franco et al. 2014; Misra et al. 2013). This limitation of the SCR device is being observed at a time when there is a strong interest in even greater NO_2 controls in some areas in the United States that are out of compliance with the ozone or NO_2 ambient standard or both. Engine and aftertreatment technology manufacturers are developing new and supplemental approaches to address this problem.

Third, the recent discovery that Volkswagen (VW) in the United States used software to disable NO_x controls when their light-duty vehicles were operating under real-world conditions (i.e., when not being tested for emissions compliance) (Thompson et al. 2014) might raise questions about the likely real-world performance of the new-technology heavy-duty diesel engines tested in ACES. Under the 2007 and 2010 heavy-duty rules, however, in-use compliance by EPA requires on-road testing using the Portable Emissions Monitoring System (PEMS) (U.S. EPA 2010) — the system used to uncover the problematic VW software — providing assurance that the on-road engine performance does not violate the standards. It is also useful to note that even in the case of VW, the PM emissions from the engines in the tests that brought to light the NO_x problem in the United States were compliant with regulatory requirements (Thompson et al. 2014). In the aftermath of the VW incident, the U.S. EPA has begun to test light-duty vehicles for emissions during their normal operations and use (Hakim and Mouawad 2015).

Above and beyond the continuing need for refinements of the new-technology diesel engines, one additional issue has arisen, particularly in Europe, regarding NO_2 emissions from earlier-generation DPF-equipped engines (i.e., engines meeting the EURO 5/V standards). As noted earlier, engines equipped with DPF alone — such as the 2007 engine tested in ACES — emit relatively high amounts of NO_2 (Table 2). Consequently, the use of new-technology engines without SCR devices can result in higher emissions and therefore higher ambient levels of NO_2, even while the emissions of PM are very low. In the European Union, with a large number of light-duty diesel vehicles on the road, implementation of stringent NO_x standards has lagged behind the United States; consequently, high levels of NO_2 in urban areas with high volumes of traffic have been observed in several studies (for example, see Carslaw and Rhys-Tyler 2013). The implementation of EURO 6/VI regulations, requiring lower NO_x emissions and incorporating more realistic test cycles and in-use testing, is expected to address this situation.

One additional trend in the area of new-technology engines should be noted. For non-road applications — for construction and farm equipment, for example — the U.S. EPA has adopted new regulations that reduce emissions substantially, but that are not as stringent as those for on-road engines. As a result, some manufacturers now market non-road engines in the United States that produce lower amounts of PM than is emitted by traditional-technology engines, but without the need to install a DPF. Although the reduction of non-road emissions of PM is an improvement, it seems clear that the benefits documented in ACES apply only to engines with a DPF and its comprehensive control of PM.

In sum, the ACES results demonstrate the effectiveness of modern aftertreatment technologies used in the modern diesel engines: they greatly reduce the emissions of PM, NO_x, and NO_2, and the levels of other toxic components of NTDE, when tested in the laboratory using FTP and more stringent testing cycles.

After a lifetime of exposure, NTDE does not produce tumors in rats, unlike TDE. Thus, the ACES results demonstrate the effectiveness of DPFs, not only in greatly diminishing the amount of PM from new-technology engines, but also in reducing the toxicity of NTDE significantly as compared with TDE.

The ambient levels of PM have gone down, especially in areas where aggressive approaches to reducing diesel emissions have been enforced, such as the Los Angeles basin. Thus, the regulations in the United States to control and reduce diesel engine emissions — and similar efforts in other industrialized countries — are already producing likely public health benefits, and this trend can be projected to continue as fleets change over and as refinements further enhance engine and aftertreatment technologies. The ACES results also hold promise for developing countries, though a lack of resources and non-availability of low-sulfur fuel have hampered implementation of diesel emissions regulations. Many countries, including Mexico, China, and India, are now taking steps to implement new fuel standards that will enable them to reduce diesel emissions by adopting new-technology diesel engines. It is hoped that other countries will follow with similar actions and the adverse health effects of exposure to diesel emissions will begin to be reduced worldwide.

ACKNOWLEDGMENTS

This Executive Summary was prepared by members of the HEI staff: Director of Science Rashid Shaikh, Principal Scientist Maria Costantini, and Senior Scientist Geoffrey Sunshine. The data summarized in this document are taken from previous CRC and HEI publications for the ACES program (Khalek et al. 2009, 2011, 2013, 2015; Mauderly and McDonald 2012; ACES 2015). The publication team included HEI staff members Hilary Selby Polk and Hope Green, as well as consultants Fred Howe and Ruth Shaw.

REFERENCES

Advanced Collaborative Emissions Study (ACES). 2015. Advanced Collaborative Emissions Study (ACES): Lifetime Cancer and Non-Cancer Assessment in Rats Exposed to New-Technology Diesel Exhaust. Research Report 184. Boston, MA:Health Effects Institute.

Advanced Collaborative Emissions Study (ACES). 2012. Advanced Collaborative Emissions Study (ACES) Subchronic Exposure Results: Biologic Responses in Rats and Mice and Assessment of Genotoxicity. Research Report 166. Boston, MA:Health Effects Institute.

Bemis JC, Torous DK, Dertinger SD. 2015. Part 2. Assessment of micronucleus formation in rats after chronic exposure to new-technology diesel exhaust in the ACES bioassay. In: Advanced Collaborative Emissions Study (ACES): Lifetime Cancer and Non-Cancer Assessment in Rats Exposed to New-Technology Diesel Exhaust. Research Report 184. Boston, MA:Health Effects Institute.

Bishop GA, Hottor-Raguindin R, Stedman DH, McClintock P, Theobald E, Johnson JD, et al. 2015. On-road heavy-duty vehicle emissions monitoring system. Environ Sci Technol 49:1639–1645.

Bishop GA, Schuchmann BG, Stedman DH. 2013. Heavy-duty truck emissions in the South Coast Air Basin of California. Environ Sci Technol 47:9523–9529.

Carslaw DC, Rhys-Tyler G. 2013. New insights from comprehensive on-road measurements of NO_x, NO_2 and NH_3 from vehicle emission remote sensing in London, UK. Atmos Environ 81:339–347.

Clark NN, Zhen F, Bedick C, Gautam M, Wayne W, Thompson G, et al. 2007. Creation of the 16-hour engine test schedule from the heavy heavy-duty diesel engine test schedule. CRC Report No. ACES-1a. Alpharetta, GA:Coordinating Research Council. Available at http://www.crcao.org/reports/recentstudies2007/ACES-1a/CRC%20ACES-1a%20Final%20Report%2007-11-2007.pdf [accessed 28 September 2015].

Conklin DJ, Kong M. 2015. Part 4. Assessment of plasma markers and cardiovascular responses in rats after chronic exposure to new-technology diesel exhaust in the ACES bioassay. In: Advanced Collaborative Emissions Study (ACES): Lifetime Cancer and Non-Cancer Assessment in Rats Exposed to New-Technology Diesel Exhaust. Research Report 184. Boston, MA:Health Effects Institute.

DieselNet. 1999. Emission Test Cycles. Heavy-Duty FTP Transient Cycle. Available: http://www.dieselnet.com/standards/cycles/ftp_trans.php [accessed 24 September 2015].

DieselNet. 2015. Emission Standards/ United States/ Heavy-Duty Onroad Engines. ECOpoint, Inc. Available: www.dieselnet.com/standards/us/hd.php [accessed 24 September 2015].

Diesel Technology Forum. 2014. New technology clean diesel trucks with near zero emissions now make up 33% of all trucks on U.S. highways [press release, 2 July 2014]. Available: www.dieselforum.org/news/new-technology-clean-diesel-trucks-with-near-zero-emissions-now-make-up-33-of-all-trucks-on-u-s-highways [accessed 13 Nov 2015].

Envirotest Canada. 2013. Greater Vancouver Regional District: remote sensing device trial for monitoring heavy-duty vehicle emissions. Burnaby, British Columbia. Available at www.metrovancouver.org/services/air-quality/AirQualityPublications/2013_RSD_HDV_Study.pdf [accessed on 25 November 2015].

Franco V, Sánchez FP, German J, Mock P. 2014. Real-World Exhaust Emissions from Modern Diesel Cars: A Meta-Analysis of PEMS Emissions Data from EU (Euro 6) and US (Tier 2 Bin 5/ULEV II) Diesel Passenger Cars. White Paper. San Francisco, CA:International Council on Clean Transportation.

Hallberg LM, Ward JB, Hernandez C, Ameredes BT, Wickliffe JK. 2015. Part 3. Assessment of genotoxicity and oxidative damage in rats after chronic exposure to new-technology diesel exhaust in the ACES bioassay. In: Advanced Collaborative Emissions Study (ACES): Lifetime Cancer and Non-Cancer Assessment in Rats Exposed to New-Technology Diesel Exhaust. Research Report 184. Boston, MA:Health Effects Institute.

Health Effects Institute. 2011. The Future of Vehicle Fuels and Technologies: Anticipating Health Benefits and Challenges. Communication 16. Boston, MA:Health Effects Institute.

HEI Diesel Epidemiology Panel. 2015. Diesel Emissions and Lung Cancer: An Evaluation of Recent Epidemiological Evidence for Quantitative Risk Assessment. HEI Special Report 19. Boston, MA:Health Effects Institute.

Hakim D, Mouawad J. 2015. Galvanized by VW scandal, E.P.A. expands on-road emissions testing. New York Times 8 November.

Heinrich U, Fuhst R, Rittinghausen S, Creutzenberg O, Bellmann B, Koch W, et al. 1995. Chronic inhalation exposure of Wistar rats and two different strains of mice to diesel engine exhaust, carbon black, and titanium dioxide. Inhal Toxicol 7:533–556.

Heinrich U, Muhle H, Takenaka S, Ernst H, Fuhst R, Mohr U, et al. 1986. Chronic effects on the respiratory tract of hamsters, mice and rats after long-term inhalation of high concentrations of filtered and unfiltered diesel engine emissions. J Appl Toxicol 6:383–395.

IARC (International Agency for Research on Cancer). 1989. Diesel and gasoline engine exhausts and some nitroarenes. IARC Monogr Eval Carcinog Risk Hum 46:1–458.

IARC (International Agency for Research on Cancer). 2012. Diesel and gasoline engine exhausts and some nitroarenes. IARC Monogr Eval Carcinog Risk Hum vol 105.

Karagianes MT, Palmer RF, Busch RH. 1981. Effects of inhaled diesel emissions and coal dust in rats. Am Ind Hyg Assoc J 42:382–391.

Khalek IA, Blanks MG, Merritt PM. 2013. Phase 2 of the Advanced Collaborative Emissions Study. CRC Report: ACES Phase 2. Alpharetta, GA:Coordinating Research Council. Available: www.crcao.org/publications/emissions/index.html [accessed 9 October 2015].

Khalek IA, Blanks MG, Merritt PM, Zielinska B. 2015. Regulated and unregulated emissions from modern 2010 emissions-compliant heavy-duty on-highway diesel engines. J Air Waste Manag Assoc 65(8):987–1001.

Khalek IA, Bougher TL, Merritt PM. 2009. Phase 1 of the Advanced Collaborative Emissions Study. CRC Report: ACES Phase 1. Alpharetta, GA:Coordinating Research Council. Available: www.crcao.org/publications/emissions/index.html [accessed 25 September 2015].

Khalek IA, Bougher TL, Merritt PM, Zielinska B. 2011. Regulated and unregulated emissions from highway heavy-duty diesel engines complying with U.S. Environmental Protection Agency 2007 emissions standards. J Air Waste Manag Assoc 61:427–442.

Kodjak D. 2015. Policies to Reduce Fuel Consumption, Air Pollution, and Carbon Emissions from Vehicles in G20 Countries. Washington, DC:International Council on Clean Transportation.

Kubota K, Murakami M, Takenaka S, Kawai K, Kyono H. 1987. Effects of long-term nitrogen dioxide exposure on rat lung: morphological observations. Environ Health Perspect 73:157–169.

Mauderly JL, Bice DE, Cheng YS, Gillett NA, Henderson RF, Pickrell JA, et al. 1989. Influence of Experimental Pulmonary Emphysema on the Toxicological Effects from Inhaled Nitrogen Dioxide and Diesel Exhaust. Research Report 30. Cambridge, MA:Health Effects Institute.

Mauderly JL, McDonald JD. 2012. Advanced Collaborative Emissions Study (ACES). Phase 3A: Characterization of U.S. 2007-Compliant Diesel Engines and Exposure System Operation. Communication 17. Boston, MA:Health Effects Institute.

Mauderly JL, Snipes MB, Barr EB, Belinsky SA, Bond JA, Brooks AL, et al. 1994. Part I: Neoplastic and nonneoplastic lung lesions. In: Pulmonary Toxicity of Inhaled Diesel Exhaust and Carbon Black in Chronically Exposed Rats. Research Report 68. Cambridge MA:Health Effects Institute.

McDonald JD, Doyle-Eisele M, Seagrave J, Gigliotti AP, Chow J, Zielinska B, et al. 2015. Part 1. Assessment of carcinogenicity and biologic responses in rats after lifetime inhalation of new-technology diesel exhaust in the ACES bioassay. In: Advanced Collaborative Emissions Study (ACES) Lifetime Cancer and Non-Cancer Assessment in Rats Exposed to New-Technology Diesel Exhaust. Research Report 184. Boston, MA:Health Effects Institute.

Misra C, Collins JF, Herner JD, Sax T, Krishnamurthy M, Sobieralski W, et al. 2013. In-use NO_x emissions from model year 2010 and 2011 heavy-duty diesel engines equipped with aftertreatment devices. Environ Sci Technol 47:7892–7898.

Okey AB, Franc MA, Moffat ID, Tijet N, Boutros PC, Korkalainen M, et al. 2005. Toxicological implications of polymorphisms in receptors for xenobiotic chemicals: the case of the aryl hydrocarbon receptor. Toxicol Appl Pharmacol 207 (2 Suppl):43–51.

Preble CV, Dallmann TR, Kreisberg NM, Hering SV, Harley RA, Kirchstetter TW. 2015. Effects of particle filters and selective catalytic reduction on heavy-duty diesel drayage truck emissions at the Port of Oakland. Environ Sci Technol 49:8864-8871

Propper R, Wong P, Bui S, Austin J, Vance W, Alvarado Á, et al. 2015. Ambient and emission trends of toxic air contaminants in California. Environ Sci Technol 49:11329–11339.

South Coast Air Quality Management District (SCAQMD). 2015. MATES IV. Final Report. Multiple Air Toxics Exposure Study in the South Coast Air Basin. Diamond Bar, CA:SCAQMD. Available at www.aqmd.gov/docs/default-source/air-quality/air-toxic-studies/mates-iv/mates-iv-final-draft-report-4-1-15.pdf?sfvrsn=7 [accessed 25 September 2015].

Thompson GJ, Carder DK, Besch MC, Thiruvengadam A, Kappanna HK. 2014. In-Use Emissions Testing of Light-Duty Diesel Vehicles in the United States. Final Report. Morgantown, WV:Center for Alternative Fuels, Engines and Emissions, West Virginia University. Available: www.theicct.org/sites/default/files/publications/WVU_LDDV_in-use_ICCT_Report_Final_may2014.pdf [accessed 24 September 2015].

U.S. Environmental Protection Agency. 2001. Control of air pollution from new motor vehicles: heavy-duty engine and vehicle standards and highway diesel fuel sulfur control requirements; final rule. Fed Reg 66:5002–5193.

U.S. Environmental Protection Agency. 1997. Control of emissions of air pollution from highway heavy-duty engines; final rule. 40 CFR, parts 9 and 86. Fed Reg 62:54694–54730.

U.S. Environmental Protection Agency. 2010. Direct Final Rule: revisions to in-use testing for heavy duty diesel engines and vehicles; emissions measurement and instrumentation; not-to-exceed emission standards; and technical amendments for off-highway engines. Federal Register 75:68448–68467.

U.S. Environmental Protection Agency. 2013. Heavy-Duty Highway Compression–Ignition Engines and Urban Buses — Exhaust Emission Standards. Available: www.epa.gov/oms/standards/heavy-duty/hdci-exhaust.htm [accessed 27 August 2015].

ABBREVIATIONS AND OTHER TERMS

8-OHdG	8-hydroxy-deoxyguanosine
ACES	Advanced Collaborative Emissions Study
AhR	aryl hydrocarbon receptor
AMOX	ammonia oxidation catalyst
API	American Petroleum Industry
BALF	bronchoalveolar lavage fluid
BSFC	brake-specific fuel consumption
CARB	California Air Resources Board
CH_4	methane
CO	carbon monoxide
CO_2	carbon dioxide
CRC	Coordinating Research Council
CVS	constant volume sampler
DOE	U.S. Department of Energy
DOC	diesel oxidation catalyst
DPF	diesel (exhaust) particulate filter
EC	elemental carbon
EGR	exhaust gas recirculation
EMA	Engine and Truck Manufacturers Association
EPA	U.S. Environmental Protection Agency
FTP	Federal Test Procedure
g/bhp-hr	grams per brake horsepower-hour
GWP	global-warming potential
HC	hydrocarbon
HHDD	heavy heavy-duty diesel (engines)
IARC	International Agency for Research on Cancer
LRRI	Lovelace Respiratory Research Institute
MY	model year
NAAQS	National Ambient Air Quality Standards
N_2	nitrogen
NH_3	ammonia
NMHC	non-methane hydrocarbon
NO	nitric oxide
NO_2	nitrogen dioxide
NO_x	nitrogen oxides
N_2O	nitrous oxide
NRDC	Natural Resources Defense Council
NTDE	new-technology diesel exhaust
OC	organic carbon
PAHs	polycyclic aromatic hydrocarbons
PM	particulate matter
$PM_{2.5}$	particulate matter $\leq$ 2.5 μm in aerodynamic diameter
SCR	selective catalytic reduction
SO_2	sulfur dioxide
SVOC	semivolatile organic compound
SwRI	Southwest Research Institute
TBARs	thiobarbituric acid reactive substances
TDE	traditional-technology diesel exhaust
ULSD	ultra-low-sulfur diesel
VOC	volatile organic compound

Publishing history: This document was posted at *www.healtheffects.org* in December 2015.

Citation:

Health Effects Institute. 2015. Executive Summary. The Advanced Collaborative Emissions Study (ACES). Boston, MA:Health Effects Institute.

Health Effects Institute
101 Federal Street, Suite 500
Boston, MA 02110, USA

Phone: +1-617-488-2300. Fax: +1-617-488-2335. *www.healtheffects.org*.