Critical review of recent diesel exhaust exposure health impact research relevant to the underground hardrock mining industry

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Executive Summary

Introduction: Diesel exhaust emissions and exposure of workers in occupational settings such as underground mines are a topic which has attracted increased attention after IARC classification as a group 1 carcinogen[1]. There is ongoing debate over appropriate exposure limits for occupationally exposed workers. This review is intended to consolidate recent research findings relevant to setting appropriate exposure limits, with a specific focus on newer engine and after-treatment technologies.

Method: The medical research database PubMed was searched for studies published since 2005 focussing on the health effects of whole diesel exhaust exposure. Studies were separated into the methodology used (whether they exposed human, animal or tissue) and the type of engine used to generate the exhaust. Engines that used exhaust after-treatment devices including both a diesel oxidation catalyst (DOC) and a diesel particulate filter (DPF) were classified as new technology engines. All other studies were classified as using older technology engines.

Results: Exposure to diesel exhaust from both engine classifications was found to cause negative health impacts on the lungs, heart and brain including increased risk of cancer, increased blood pressure, increased risk of thrombosis, neuroinflammation and increased DNA damage. Subjects with asthma, allergy or respiratory disease were more at risk of negative effects caused by diesel exhaust exposure than healthy subjects. Health impacts were found to occur even in studies using exhaust concentrations below the recommended Australian occupational limit of an 8 hour time weighted average (TWA) of 100 μg/m^3 of elemental carbon.

In addition, the use of exhaust after-treatment devices had little to no impact on the resulting health effects of diesel exhaust exposure, despite exhaust after-treatment devices such as a diesel particulate filter (DPF) being capable of removing over 90% of diesel exhaust particles by mass. Several studies exposed subjects to exhaust both with and without a DPF equipped and found similar health impacts. Thus "new technology" diesel exhaust emissions can meet occupational limits and still cause adverse health effects. DPF's also preferentially remove elemental carbon from diesel exhaust which limits the feasibility of using elemental carbon as an indication of exhaust exposure.

Conclusion: Based on the results of these studies, an 8 hour time weighted average diesel exhaust concentration below 50 μg/m^3 of diesel exhaust particles, 35 μg/m^3 of elemental

carbon, is more appropriate in order to limit health effects. In order to meet occupational limits, many diesel engines will need to be equipped with after-treatment technology such as a DPF. This negates the feasibility of using particle mass based limits, especially ones based on elemental carbon. In order to minimise the negative health effects in the hardrock mining industry, alternative methods of measuring exposure to diesel exhaust should be explored. Suggestions include particle number and nitrogen oxides (NO_x).

Diesel Exhaust

In Australia, over 157 000 miners are occupationally exposed to diesel exhaust every year with Western Australia and Queensland containing the majority of the mining workforce[2]. The lowest levels of occupational exposure are detected in surface workers who are exposed to the exhaust in an open area while the highest levels of occupational mining exposure are detected in underground occupational jobs where the exhaust is generated in an enclosed area. Those who operate the underground heavy diesel equipment have the highest exposures among the various underground mining occupations[3]. In Australia, no occupational diesel exhaust exposure limit has been implemented, although several recommendations have been made[4].

Diesel exhaust has been classified as a class 2a; probable human carcinogen by the International Agency for Research on Cancer (IARC) since 1989. This classification changed to class 1; definitely carcinogenic to humans in 2012 based primarily on a series of studies conducted on occupationally exposed hardrock miners. These studies, now collectively termed the Diesel Exhaust in Miners Study, were conducted by a joint program from the US National Cancer Institute and the US National Institute for Occupational Safety and Health. The studies were retrospectively conducted on a cohort of 12315 non-metal hardrock miners occupationally exposed for a minimum of one year to diesel exhaust between 1947 and 1997. Using nested case control techniques and retrospective cohort mortality analyses, the studies found that the cohort of miners had an increased lung cancer risk. The risk was greatest in surface workers with a standard mortality ratio of 1.33 (1.06-1.66, 95% C.I.) compared to the underground workers slightly lower risk of 1.21 (1.01-1.45, 95% C.I.), despite the underground workers having an average respirable elemental carbon exhaust exposure level that was over 75 times higher[5-6].

Diesel Exhaust Components and Their Health Complications

Diesel exhaust can be separated into two main components; the gaseous phase and the particulate matter (PM) phase. Gaseous components can include carbon monoxide (CO), carbon dioxide (CO_2), Nitrogen Oxides (NO_x) and sulfur dioxide (SO_2) as well as additional gas phase chemical species such as polycyclic aromatic hydrocarbons (PAH) and volatile organic compounds (VOC). The particulate matter is composed of mostly solid elemental carbon particles with potentially toxic chemicals such PAH, VOC, aldehydes, ketones and heavy metals ad/ab-sorbed to the particles[7-11]. Overall diesel exhaust can contain hundreds of different chemical species and concentrations can change significantly depending on engine type, speed, load, whether accelerating or deaccelerating, starting temperature and the usage of exhaust after-treatment devices[7, 12-17].

Carbon monoxide binds to haemoglobin within the blood, causing impaired blood-oxygen transport, and exposure is associated with death at high concentrations with exposure to 1600 ppm causing death within two hours[18-19]. Exposure to lower concentrations, at approximately 61 ppm for two hours, causes neurological impairment including emotional instability, memory dysfunction and difficulty concentrating[18-20]. Exposure to carbon dioxide is associated with cognitive impairment at short term exposure to 1500 ppm (0.15%)[21] and has health impacts on blood pressure and bone formation at 12000 ppm (1.2%)[22-23], which is lower than the carbon dioxide concentrations found within diesel exhaust[24]. Safe work Australia recommends a time weighted 8 hour exposure average of 30 ppm of carbon monoxide, 5000 ppm carbon dioxide[25].

Nitrogen monoxide oxidises within the atmosphere to form nitrogen dioxide[26], which forms nitic acid when interacting with the fluid lining the lungs[27]. Collectively termed nitrogen oxides, acute exposure is associated with coughing, dyspnea and hemotypsis[28]. Lower concentration exposure (<490 ppm for 30 minutes) is associated with increased allergen response in asthmatics[29] and long term exposure to environmental levels (<1 ppm) is associated with decreased lung function, increased respiratory infections and increased risks of stroke[30-31]. Safe work Australia recommends a time weighted 8 hour average of 25 ppm of nitrogen monoxide and three ppm of nitrogen dioxide[25].

Sulfur dioxide reacts with the fluid lining the lungs to produce sulfuric acid which in turn forms sulphites that can enter the cardiovascular system[32-33]. Exposure has been implicated in aggravation of existing cardiovascular and pulmonary conditions as well as short term coughing and increased risk of stroke[30, 34]. Safe work Australia recommends a time weighted 8 hour average of two ppm of sulfur dioxide[25].

Diesel exhaust particles are primarily composed of elemental carbon, with a smaller proportion of organic carbon and toxins (such as PAH and nitro-PAH, aldehydes, ketones and heavy metals) ad/ab-sorbed to the primary (amorphous elemental carbon) particles[7, 9-12]. Many of these components are created through incomplete fuel combustion and unburned engine lubricating oil[8, 35]. Particulate matter PAH's, aldehydes and ketones are implicated as major contributors towards diesel exhausts carcinogenic effects[8, 36-37].

Of most concern however are the ultrafine particles found within diesel exhaust. These particles, at less than 100 nm in size, comprise the majority of diesel exhaust PM with particles smaller than 30 nm comprising over 90% of the total number of particles but only accounting for 10% of the total PM mass[38-39]. Ultrafine particles are capable of penetrating deeper into the lungs than larger sized particles, dispersing over a greater percentage of lung volume and thus causing a greater general respiratory irritant effect[40-41]. In addition, smaller particles have a greater surface area to volume ratio, meaning that a greater amount of potentially toxic substances can adhere to the surface for a given mass of PM[42-43] and thus a greater amount of toxic chemicals are deposited in the lungs as well. Exposure to ultrafine particles is associated with pulmonary inflammation[40] and exacerbation of existing lung diseases including asthma[41, 44]. Ultrafine particles are also capable of bypassing the barrier effect of the lungs to enter the cardiovascular system and cause a range of adverse health effects including increased blood pressure and heart failure[45].

Alone, each individual component of the exhaust can cause its own unique health effects and combined they can interact to cause much more complicated health impacts such as cancer as well as combined effects on the cardiovascular, respiratory and neurological systems[31, 46-51]. This make studying the effects of whole exhaust preferable to those of isolated components, such as PM alone, where the effects of the gas components and their interaction with PM is lost[52-53].

Changes in Engine Technology, Exhaust After-Treatment Devices and Emission Limits

Since 1970, increasing air pollution in major urban cities has been of great concern and steps have been taken globally to mitigate the contribution caused by exhaust pollution[54-57]. Diesel engine technology has steadily increased in complexity with the increasingly stringent pollution emission standards. Engine technology has evolved from older, very basic mechanical fuel injection systems to the modern very high pressure common rail electronic fuel injection systems, which allows both more finely atomised fuel to be injected and fuel injections to be electronically timed with potential for multiple injections per combustion event in order to cause the least exhaust emissions possible[58]. Diesel particulate filters (DPF) and other similar exhaust after-treatment devices such as diesel oxidation catalysts (DOC), exhaust gas recirculation (EGR) and NOx traps and selective catalytic reduction (SCR) for NOx control were introduced to further limit pollution caused by diesel engines[56, 59]. In order for the exhaust after-treatment devices to be used to full capacity, sulfur concentration in diesel fuels had to decrease as high sulfur levels degrade the after-treatment devices[59]. This lead to legislation changes starting in the mid 2000's that introduced ultra-low sulfur diesel (ULSD) into circulation across much of the world, decreasing sulfur levels from above 500 ppm to below 15 ppm[56].

Using a DPF, DOC and other such exhaust after-treatment devices, the components of diesel exhaust change dramatically. A DPF is capable of removing approximately 90% by mass of particulate matter. Elemental carbon (EC) is preferentially removed and ratios of EC to organic carbon reduce from approximately 3 to 0.5[12]. In exhaust without a DPF, EC makes up approximately 75% of PM by weight[60], which reduces to approximately 13% after the use of a DPF. Average particle size also decreased from >40 nm to approximately 25 nm with the use of a DPF and in the ultrafine particle range, larger sized particles closer to 100 nm in size are removed from the exhaust more successfully than smaller sizes[12].

The EURO, US EPA and the US TIER classification systems have been developed as emission standards for light-heavy vehicles on road, heavy duty vehicles on road and off road engine emissions respectively. Most engines classified as EURO IV, US EPA 2007 or US TIER 4 and above require exhaust after-treatment devices, such as a DPF and DOC, for compliance and engines classified as EURO IV and above generally require the latest high pressure common rail electronic fuel injection systems[58]. In a mining setting in Australia, all trucks and cars that can be driven "on road" are required to meet EURO classifications as adopted in Australia (Table 1). All other diesel equipment uses the US TIER "off road" classifications (Table 2). The majority of diesel engines currently used in underground mining in Australia are pre-2007

older technology transitional engines- TIER's 1-3, and thus do not contain exhaust after-treatment devices such as DPF's[4, 61-62].

Table 1: EURO standards for "on road" heavy duty diesel engines[63-64].

			Emission				
Emission Standard	Year of Introduction (Europe)	Year of Introduction (Australia)[a]	CO (g/kWh)	HC (g/kWh)	NO_x (g/kWh)	PM (g/kWh)	Particle Number (1/kWh)
EURO I	1992	1994/1995	4.5	1.1	8.0	0.36	
EURO II	1996	2002/2003	4.0	1.1	7.0	0.25	
EURO II	1998	2002/2003	4.0	1.1	7.0	0.15	
EURO III	2000	2002/2003	2.1	0.66	5.0	0.10	
EURO IV	2005	2007/2008	1.5	0.46	3.5	0.02	
EURO V	2008	2010/2011	1.5	0.46	2.0	0.02	
EURO VI	2013	NA[b]	1.5	0.13	0.40	0.01	$8.0x10^{11}$

a= variable phase-in periods for new vehicle models vs existing models.

b= not applicable as Euro VI has not been introduced for heavy vehicles in Australia.

Table 2: Examples of US TIER standards for "off road" heavy duty engines (engines rating between 450 ≤P < 560 kW)[58, 62].

		Emission (g/kWh)				
Emission Standard	Year of Introduction (US)[a]	CO	HC	$HC+NO_x$	NO_x	PM
TIER 1	1996	11.4	1.3		9.2	0.54
TIER 2	2001	3.5		6.4		0.2
TIER 3	2006	3.5		4		0.2
TIER 4i	2011	3.5	0.19		2	0.02
TIER 4f	2014	3.5	0.19		0.4	0.02

a= The authors could not find any federally mandated emission limits for "off road" diesel engines in Australia.

Methods

The medical research library PubMed was searched using the following term: "Diesel Exhaust" combined with the individual search term "Exposure Health Effect", limiting the search to results published after 2005. From over 600 papers that matched the search criteria, only articles from the search which matched the review criteria, as well as relevant cited references therein, were reviewed. Studies were excluded if they were not written in English, if the results were based on data obtained before 2005, if the diesel fuel used was not classified as ultra-low-sulfur diesel (<15ppm sulfur), if the diesel fuel used exceeded 10% biodiesel concentration, if whole exhaust was not used within the study and finally if the

concentration of the exhaust used within the study or the health outcomes measured were not relevant to occupational mining exposure.

The cut off date of 2005 was selected based on diesel fuel legislation to limit sulfur levels in commercial diesel fuel. The legislation was introduced in multiple countries in the mid 2000's with several years taken to complete the change over[56]. If studies did not specify the amount of sulfur within the diesel fuel used, assumptions were made based on the country the study was performed in and the date that the legislation for ultra-low-sulfur diesel was introduced. If the date of publication fell outside of that range, the study was excluded (Figure 1).

Finally, relevant studies were separated into the research categories occupational exposure studies, acute human exposure studies, *in vivo* exposure studies and *in vitro* exposure studies. Acute human exposure, *in vivo* and *in vitro* studies were further separated into the use of new or older technology engines. Studies that used exhaust from an engine either classified as EURO IV, US EPA 2007 or TIER 4 and above, or as being paired with a DPF and DOC, were classified as using new technology engines. Studies that used exhaust from engines without both after-treatment devices or using an engine at a lower EURO or TIER classification were defined as using older technology.

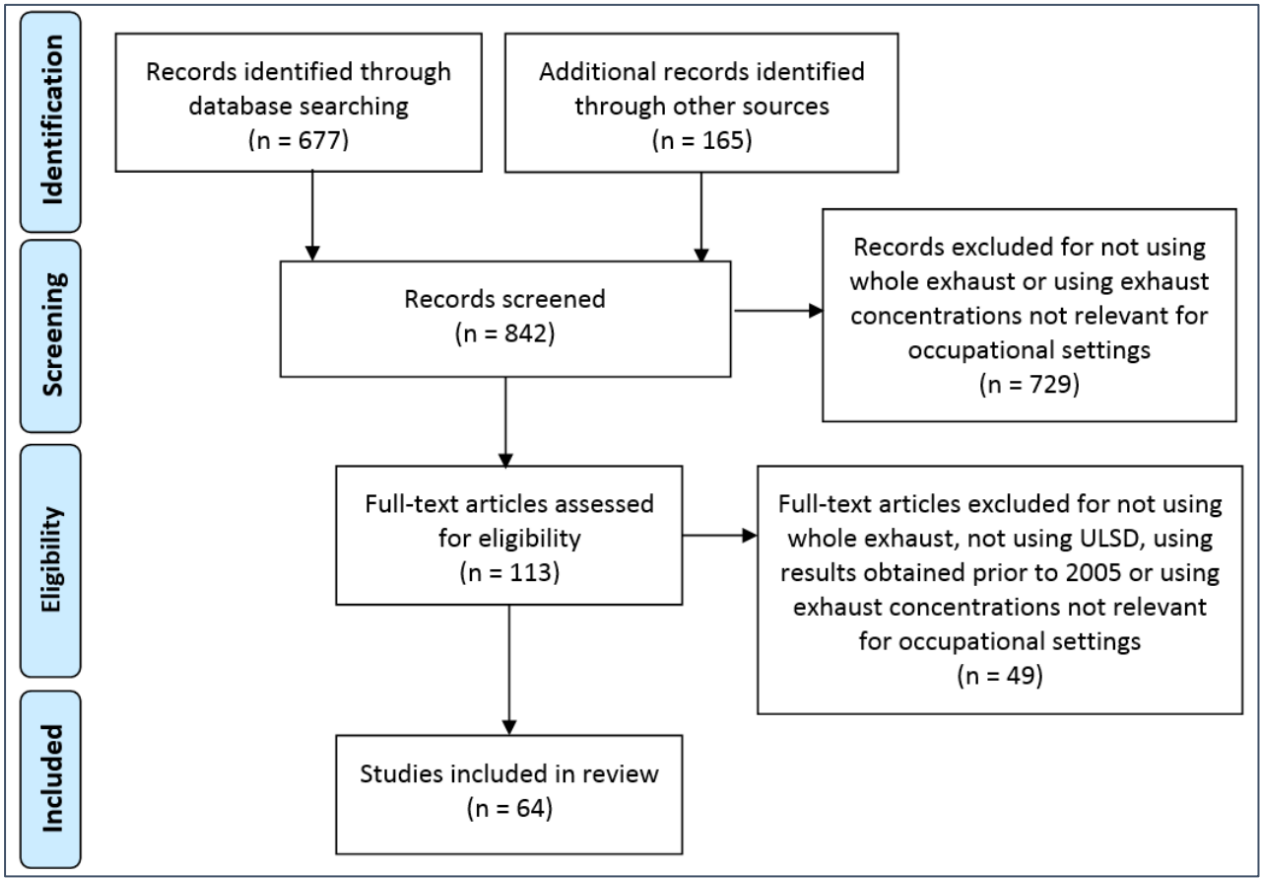

Figure 1: An overview of the methodology used to select appropriate articles for review.

Occupational Exposure Studies

Many occupational exposure studies focus on historical data obtained before 2000, when sulfur levels in fuel were high (>500 ppm and in some cases >5000 ppm)[56] and diesel engines

were not equipped with exhaust after-treatment devices. The Diesel Exhaust in Miners Study is an example of one such analysis, although not the only one[5-6, 65]. The largest issue with occupational exposure studies is that many use population data in order to collect potential health consequences and thus require a longer period of time to complete. In order to measure the effects of a lifetime of occupational exposure to a substance, a lifetime has to have passed, making such studies difficult when the substance being measured (i.e. new technology diesel engine exhaust) is still newly introduced into the workplace.

Thus, compelling evidence has been gathered on the negative health outcomes of occupational exposure to exhaust generated by old technology diesel engines running on high sulfur diesel. Very little has been gathered on the effects of exposure to exhaust generated by new technology engines running on low sulfur diesel. Further, any such evidence is likely to be overshadowed by the health consequences of the arguably more toxic old technology exhaust, especially considering new technology diesel engines have only been in use for a little over a decade, compared to over seven decades of old technology diesel engine use within hardrock mining occupations[5].

A number of studies have been completed over the past five years, focussing on populations that have been more recently exposed to diesel exhaust at occupational concentrations (Table 3). A series of studies on a range of occupational exposure concentrations of diesel exhaust in a cohort of diesel engine testers in China have been published[66-69]. Dai et al. measured inflammation cytokines in blood serum, finding that the greater the levels of exposure, the more the immune response was dysregulated. Surprisingly, the highest level of exposure (greater than 397 μg/m^3) resulted in statistically significant lower serum inflammation than unexposed control subjects. They theorised that this may be one mechanism for increased lung cancer incidence in workers occupationally exposed to diesel exhaust, as the immune system has an important role in eliminating cancerous cells and any immune dysregulation would have a potentially important impact on that role[66]. Wang et al. measured lung function and inflammatory biomarkers in blood serum. They found that lung function, in terms of the commonly used parameters of forced expiratory volume in one second (FEV1) and FEV1/forced vital capacity ratio, decreased significantly with an approximate exposure level of 282 μg/m^3, compared to control subjects, with an approximate exposure level of 92 μg/m^3. Serum markers of local and systemic inflammation were also increased and were associated with the occupational exposure history of the workers, with those working at the testing facility for the longest time periods displaying the greatest effects[67]. Zhang et al. (2015) found increased DNA damage in the peripheral blood lymphocytes of engine testers occupationally exposed to an approximate diesel exhaust level of 268 μg/m^3, in comparison to control subjects with an approximate exposure level of 92 μg/m^3[70]. In the same cohort, Zhang et al. (2016) linked exposure to high levels of diesel engine exhaust to DNA hypomethylation changes that were associated with increased DNA damage. Estimates of the levels of exhaust exposure were made based on urinary biomarkers of exposure. A slight immune dysregulation was also measured in that the diesel exhaust exposed workers exhibited a 12% reduction in the populations of monocytes[71]. Yong Niu et al. measured urinary markers of PAH exposure and cancer biomarkers. They estimated that occupational diesel exhaust exposures that resulted in above 1.08 μg/g urinary creatine, a

marker of PAH exposure, were associated with higher levels of cancer biomarkers such as micronucleus, and thus increased risks of cancer. This corresponds to exposures of approximately 110 μg/m^3 total carbon or 170 μg/m^3 of fine particulate matter according to their measurements[68]. Bassig et al. found immune alterations similar to published lung cancer risk studies in the cohort of workers occupationally exposed to approximately 100 μg/m^3 of diesel exhaust, suggesting a significantly higher risk of lung cancer among the diesel engine testers[69].

León-Mejía et al. found that 120 Colombian mechanics occupationally exposed to diesel exhaust, at an estimated level of 250 μg/m^3, exhibited cytotoxic and genotoxic damage to buccal epithelial cells, found on the inside of the cheek, and peripheral blood lymphocytes (white blood cells). Workers exposed to diesel exhaust for an average period of 11.6 years showed significantly greater levels of cellular and DNA damage than occupationally unexposed workers. In addition, damage to DNA in the form of micronucleation in both cell types was correlated with years of service, suggesting that longer periods of exposure to diesel exhaust resulted in a greater amount of DNA damage[72]. This in turn has concerning implications on lung cancer risk[72-73].

Peters et al. measured EC exposures in Western Australian miners and related this to increased lung cancer risk using previously published risk functions based on the Diesel Exhaust in Miners Study[65]. A lifetime exposure (approximately 45 years) to 14 μg/m^3 of EC was estimated to result in an increase of 5.5 (2.7-9.2, 95% confidence interval) lung cancer deaths per 1000 male workers. An exposure of 44 μg/m^3 of EC was estimated to result in an increase of 38 (19-97, 95% confidence interval) lung cancer deaths per 1000 male workers[3]. Assuming that the majority of exposure occurred from old technology diesel engines, since data was obtained between 2003 and 2011 when new technology engines were still being introduced, then the PM exposure level is an estimated 19 and 59 μg/m^3 respectively.

Rynning et al. measured genotoxic biomarkers in a cohort of 69 Norwegian tunnel finishing workers occupationally exposed to diesel exhaust at approximately 37.8 μg/m^3 of EC, approximately 50 μg/m^3 of particulate matter assuming that the majority of exposure was from old technology diesel exhaust. They found that in comparison to non-exposed control subjects, the tunnel workers had increased levels of DNA damage in their peripheral blood mononuclear cells as well as altered blood plasma profiles. In addition, the expression of several micro RNAs, including some related to carcinogenesis, cell death and oxidative stress, were dysregulated. In other words, DNA damage and markers of stress that leads to future DNA damage and increased cancer risk was found in the blood samples taken from the tunnel finishing workers[74].

Occupational diesel exhaust exposure studies reported effects on lung function and biomarkers that correlated with increased cancer risk[66-69, 72, 74]. All studies reported increased risks of lung cancer in workers occupationally exposed to diesel exhaust. The lower occupational exposures, below 100 μg/m^3 of particulate matter, reported increased DNA damage, immune alterations in a pattern related to increased lung cancer incidents and an estimated risk of 38 lung cancers per 1000 workers exposed to approximately 44 μg/m^3 EC (approximately 59 μg/m^3 particulate matter).

Acute Human Exposure Studies

We found no studies that focussed on the effects of new engine technology exhaust exposure on humans and only one study that focuses on the health effects of acute exposure to diesel exhaust with and without a DPF on humans. This is a gap in knowledge that will hopefully be filled in coming years as the use of exhaust after-treatment devices in research becomes more standard[75]. Thus all studies that involve acute exposure of humans to high levels of diesel exhaust for short time periods have used old technology diesel engines. The majority of studies used exposure chambers and participants were exposed to either diluted whole diesel exhaust at a variety of concentrations and/or air as a control. The measured end point health impact focussed primarily on the effects on the cardiovascular system with fewer studies focusing on the respiratory system. No study exposed participants to diesel exhaust for more than 3 hours. Further information on the exposure methodology can be found in Table 4.

Both minor and major cardiovascular effects were reported with diesel exhaust exposure concentrations between 350-300 μg/m^3 with increased arterial stiffness[76], increased endothelial dysfunction in patients at risk for heart failure[77], reduced vasodilation[78-79], increased thrombus (blood clot) formation[78] and increased blood pressure after two hours of exposure[79-80]. In addition, altered blood plasma profiles were found in healthy individuals and altered blood plasma profiles and altered micro RNA expression in peripheral blood were found in individuals with an allergy or asthma[81-83]. DNA hypomethylation was found in genes associated with oxidative stress and inflammation in asthmatics[84]. Respiratory effects have also been reported, with altered micro RNA and transcription profiles and DNA hypomethylation associated with increased oxidative stress in epithelial cell brushings[85-86] and increased airway hyperactivity and obstruction in individuals with asthma or allergies[81, 87]. Healthy individuals exposed for 30 minutes to 300 μg/m^3 of diesel exhaust also reported significant irritation of the nose, throat and chest after exposure with exercise exacerbating the effects[88]. In comparison, heart rate was not affected[77-78]. Some studies reported no changes in blood pressure after 30-60 minutes of exposure[78, 82, 88] and no changes in markers of inflammation and platelet activation[78, 88], balance was not affected and no changes were found in central nervous system biomarkers[89-90]. Combined, these studies show that exposure to diesel exhaust at concentrations between 350-300 μg/m^3 for periods of less than two hours, can result in negative effects on cardiovascular health and the respiratory system.

Exposures to diesel exhaust at concentrations between 300 and 200 μg/m^3 resulted in similar health effects to exposures between concentrations between 350-300 μg/m^3. Eye irritation was reported by 18 healthy subjects exposed for 75 minutes, and additional nose and throat irritation was diagnosed by a medical professional[91]. Healthy subjects had decreased induced vasodilation[92], increased vasoconstriction[93] and increased blood pressure and inflammation after 2 hours of exposure[48, 80] as well as dose dependant altered gene expression in peripheral blood mononuclear cells, meaning that the negative effects of diesel exhaust exposure are measurable within blood samples taken from the participants[94]. In contrast, 60 minutes of diesel exhaust exposure did not result in any effects on heart rate variability or blood pressure[92]. No indications of increased systemic inflammation were found in healthy volunteers after 60 minutes of exposure[92], no indications of oxidative stress was found in

individuals with metabolic syndrome[95] and no changes in heart rate variability were found after 2 hours of exposure[96].

Studies using exposures below 100 μg/m^3 concentrations of diesel exhaust report minor amounts of vasoconstriction in comparison to 200 μg/m^3 exposures[93], increased airway inflammation in healthy subjects[97], allergic inflammation and viral induced immune responses in allergic individuals[98] and decreased lung function, increased airway acidification and increased respiratory inflammation in asthmatics exposed for 2 hours at exhaust concentrations up to 75 μg/m^3, with more severe asthmatics showing more severe symptoms[99] . No thrombotic effect was found in subjects with metabolic syndrome[100], no impact on heartrate was observed[78, 80, 96], no impact on vasoconstriction was found[78] and there was no evidence of respiratory epithelial cell damage in healthy, allergic or asthmatic individuals following exhaust exposure at 100 μg/m^3 for 2 hours[101].

Only one study has compared the health impact of exposure to diesel exhaust with and without a DPF on 19 healthy volunteers. The use of a DPF decreased PM concentration from 320 to 7.2 μg/m^3. Study participants were exposed for one hour and exposure to whole, unfiltered exhaust resulted in increased thrombotic formation and reduced vasodilation. The use of a particulate filter negated the effects of exposure on vasodilation and decreased the thrombotic effect as well[78].

The majority of acute human exposure studies used exhaust exposure concentrations around 300 μg/m^3, suggesting that this level of exposure is the concentration where an observable response is likely to occur using short exposure periods[80]. At this level of diesel exhaust exposure, health effects are noticeable by the participants themselves with reports of irritation to mucosal surfaces such as the nose and throat after 30 minutes and the eyes after 75 minutes. The majority of studied effects involved the cardiovascular system, likely due to the need for less invasive techniques than would be required for other systems[102]. As exposure levels decreased to 100 μg/m^3, reported health effects lowered in severity and more studies began reporting negative outcomes. Those that reported positive results mostly involved individuals with asthma or allergy, suggesting that they may be an at risk population that requires closer monitoring.

In Vivo (Animal Model) Exposure Studies

In contrast to the acute human exposures studies, the majority of studies involving the use of animal models focus on potential health impacts on the respiratory and central nervous systems. This is likely due to the invasiveness of the procedures used, which makes them inapplicable for human exposure studies. The use of animal models in *in vivo* studies is not without limitations, including the fact that the animal models used display subtle differences in anatomy and physiology and do not mimic human responses perfectly. Despite this, animal *in vivo* studies can help researchers understand the mechanisms of diesel exhaust induced health outcomes by giving an overview and estimation of the affected systems and thus the potential health impacts on humans. An additional strength of these models is that long exposures can be compressed into the relatively short life span of experimental animals, meaning that lifetime exposures can be completed in a much shorter period of time than in

comparative human occupational studies. Thus the majority of *in vivo* studies reviewed expose animals over longer periods of time, which also represent greater proportions of their life expectancy, than human studies. Only a few *in vivo* studies compared the effects of short acute inhalation. Information on animal type and exposure methodology can be found in Tables 5 and 6.

***Older Engine Technology*:** The majority of *in vivo* exposure studies use old technology diesel engines to generate the exhaust. The exhaust exposure concentrations vary greatly, with some studies exposing mice and rats to concentration up to 3000 μg/m^3 and some using below 50 μg/m^3.

Studies that exposed mice to diesel exhaust concentrations between 2000 and 3000 μg/m^3 found a variety of negative respiratory and neurological effects. A 3.2 fold greater mutation frequency was found in the lungs of mice exposed for 12 weeks compared with air exposed controls suggesting greater cancer risk[103], large increases in neuroinflammation were found in the brains of mice exposed for 4 weeks[104] and increased lung inflammation was found in mice exposed for less than a week, with allergic mice exhibiting greater symptoms[105]. In contrast, more recent studies exposing rats for 1 or 4 weeks to approximately 2000 μg/m^3 have found only minor histopathological changes and inflammatory effects on the lungs[106] and minor oxidative stress in the brain[24].

Exposing mice to diesel exhaust between the concentrations of 1000 and 2000 μg/m^3 is reported to have effects on the transcription of stress related genes in the brain at exposure concentrations of 1700 μg/m^3 for 4 weeks[107]. Similar to an exposure concentration of 3000 μg/m^3, a 3.1 fold increase of mutations was found in the lungs of mice exposed to 1000 μg/m^3 for 12 weeks, suggesting greater cancer risk[103].

Studies exposing mice and rats to diesel exhaust concentrations between 500 and 1000 μg/m^3 found oxidative stress and increased inflammation in the lungs of rats exposed to 950 μg/m^3 for less than a week[108], increased neuroinflammation in mice exposed to 650 μg/m^3 for 4 weeks (although still less than that found in 2000 μg/m^3 exposure concentrations)[104], increased flu severity in mice exposed to 500 μg/m^3 for less than 2 weeks[109] and an increased effect of chemically induced arrhythmia in hypertensive rats exposed to 500 μg/m^3 for less than a week[110]. In addition, increased respiratory inflammation was found in allergic mice, but not healthy mice, exposed for less than a week and the effects were lower than that found in mice exposed to 2000 μg/m^3, suggesting dose-response relationships in these particular outcomes[105].

In vivo exposure to diesel exhaust concentrations between 300 μg/m^3 and 100 μg/m^3 has been shown to result in several neurological effects including impaired neurogenesis in male mice exposed for less than a day to 250 μg/m^3 [111], increased neuroinflammation in mice exposed for 4 weeks to 173 and 149 μg/m^3 [112-113] and impact on object recognition ability in mice exposed to 129 μg/m^3 for 12 weeks[114]. No impact was found on spatial learning abilities in mice exposed for 149 μg/m^3 for 4 weeks[113]. Health impacts on other systems included increased respiratory inflammation found in both normal and asthmatic mice exposed to 200 μg/m^3 for 7 weeks or 169 μg/m^3 for 8 weeks respectively[115-116], unfavourable changes in

atherosclerotic plaques (artery blockages) in mice exposed to 200 μg/m^3 for 7 weeks[115], changes in steroidogenesis in male rats exposed for 4 weeks to 149 μg/m^3 [117], an increased effect of chemically induced arrhythmia in hypertensive rats exposed to 150 μg/m^3 for less than a week[110] and increased allergic symptoms in asthmatic mice exposed to 100 μg/m^3 for 12 weeks[118].

Exposure studies that used diesel exhaust concentrations below 100 μg/m^3 found mild increases in the effect of chemically induced arrhythmia in hypertensive rats exposed to 50 μg/m^3 for less than a week (in comparison to exposure to 150 and 500 μg/m^3)[110], minor increases in respiratory inflammation in the lungs of rats exposed to 40 μg/m^3 for less than a week (in comparison to exposure to 950 μg/m^3)[108], minor increases in respiratory inflammation in asthmatic mice exposed for 8 weeks to 39 μg/m^3 (in comparison to exposures to 169 μg/m^3)[116], some impact on steroidogenesis in male rats exposed to 38 μg/m^3 for 8 weeks[117] and no impact on object recognition in mice exposed to 47 μg/m^3 for 12 weeks[114].

New Engine Technology: Very few *in vivo* studies have exposed animals to the exhaust generated from new technology diesel engines. Exhaust concentrations never exceeded 200 μg/m^3 and all studies were published in the past 5 years. Valand et al. exposed rats to 182 μg/m^3 for 1 and 4 weeks and found changes in gene expression of the brain which suggests minor oxidative stress. No histopathological effects were found and the differences compared to rats exposed to old technology exhaust at a concentration of 2000 μg/m^3 were minor[24]. Magnusson et al. found minor respiratory inflammation and oxidative stress in the lungs of rats exposed to approximately 170 μg/m^3 for 1 and 4 weeks. No differences were found when compared to rats exposed to old technology exhaust at a concentration of 2000 μg/m^3 [106]. Douki et al. found only minor indications of accumulated lung DNA damage in rats exposed to less than 100 μg/m^3 for 3 weeks, however effects were found to be worse with new technology exhaust when compared to old, suggesting that toxicity was associated with the ultrafine particulates and the gas phase of the exhaust[119]. A series of studies for the Health Effects Institute, Boston, Massachusetts, exposed rats to 12 μg/m^3 of exhaust for 28-30 months and found only limited effects. Minor histopathological changes associated with exposure to gaseous pollutants were observed and mild increases in inflammatory and thrombotic markers were found in the blood however no damage to DNA was recorded and no increases in tumour development were found[120-123].

In *in vivo* exposure studies using old technology exhaust, exposure concentrations varied greatly with the highest exposures resulting in a range of health impacts to the respiratory, cardiovascular and neurological systems. These symptoms decrease to mild effects between 50-130 μg/m^3 with only mild impact on the cardiovascular system and mild respiratory inflammation. Results are similar for new technology studies, however the small amount of studies available limits the conclusions that can be drawn with only a patchy covering of the different ranges of exhaust exposure concentrations available. Once again, animals with conditions simulating asthma or allergy displayed worse symptoms and the study with the lowest exhaust exposure concentration that still reported exposure health impacts used asthmatic mice as subjects, highlighting potentially susceptible populations. Interestingly, a few studies also reported increased influenza severity in mice exposed to diesel exhaust,

which may help to highlight another susceptible population that wasn't found in the human exposure studies.

In Vitro (Cell Model) Exposure Studies

The majority of *in vitro* studies into the effect of diesel exhaust exposure on cells use particles collected on quartz filters and added directly to the media the cells are grown within[124]. Using this approach to estimate the health effects of exhaust exposure is limited as it ignores the health consequences of the exhaust gases entirely. In addition, the particles collected on the filter agglomerate, sticking together to generate an artificial particle spectrum made of larger particles, often removing the ultrafine particles from the sample and thus from the subsequent analysis of exposure health effects[125]. Studies have found that this approach often underestimates health consequences of exposure and over 16 times higher concentrations of particles are needed to generate the same health consequences as exposure to whole exhaust[126]. All *in vitro* studies included in this review use whole exhaust instead of pre-collected particles and focus on the damage caused to the respiratory epithelium, either using primary human epithelial cells or the alveolar carcinoma cell line A549. All cells are grown in an air-liquid interface in order to expose them directly to the diluted diesel engine exhaust (Tables 7 and 8).

***Older Engine Technology*:** Studies exposing cells to old technology diesel engine exhaust have mostly focussed on cell damage, oxidative stress and inflammatory responses. Okubo et al. exposed A549 cells at air liquid interface to diesel exhaust at a concentration of 1600 μg/m^3, finding inhibited proliferation and increased oxidative stress. The same cells exposed to exhaust after the use of a DPF, at a concentration of 470 μg/m^3, exhibited suppressed immune reactivity in comparison to air exposed controls. Oxidative stress was decreased in comparison to the diesel exhaust exposure concentration of 1600 μg/m^3, however the decreased immune response after exposure was only found in the DPF equipped exhaust[127]. Kooter et al. exposed A549 cells at air-liquid interface to 1300 μg/m^3 and found increased cell death, increased oxidative stress and a decreased inflammatory response[128]. Hawley et al. exposed differentiated primary human bronchial airway epithelium grown at air-liquid interface to diesel exhaust at a concentration of 850 μg/m^3, finding increased oxidative stress and increased PAH adduct formation but no loss of viability[129].

Zarcone et al. have published several studies exposing differentiated primary human airway epithelial cells collected from both healthy volunteers and volunteers with COPD to a range of exhaust concentrations and types[124, 130-131]. All cells were differentiated and grown in an air-liquid interface set up. In the study that used old technology exhaust, Zarcone et al. (2016) found that exposing the cells to approximately 1200 μg/m^3 induced the production of inflammatory markers, oxidative stress, cellular death and increased permeability after 150 minutes of exposure. At 430 μg/m^3 they found increased oxidative stress after 150 minutes and increased permeability after 375 minutes. At 140 μg/m^3 only decreased permeability was recorded, although this has concerning implications on the effect of exposure on the lungs[124].

New Engine Technology: Only three *in vitro* exposure studies were found that include the use of new technology diesel exhaust. Zarcone et al. (2017) found that exposure for 60 minutes

at 1500 μg/m^3 induced oxidative stress and decreased the defence response to infection, although no cellular death occurred[131]. In a separate study, Zarcone et al. (2018) also exposed primary human airway epithelial cells to three different, much lower, exhaust concentrations. They found that exposure to the lowest dose at 34 μg/m^3 had no impact on healthy cells, the second lowest dose at 82 μg/m^3 caused increased oxidative stress in healthy cells and the highest dose, at 206 μg/m^3, caused increased oxidative stress in healthy cells and decreased host defence in the COPD derived cells only[130]. Hawley et al. exposed differentiated primary human airway epithelial cells to 35.3 μg/m^3, finding increased oxidative stress and increased PAH adduct formation. Interestingly, they found no difference in health effects between the new technology exhaust and the old technology exhaust at a concentration of 800 μg/m^3 [129].

Although the *in vitro* studies that used whole exhaust for exposure were small in number, they did show some alarming results. Higher exhaust concentrations in old technology exposures displayed the worst health impacts, as expected, and the study with the lowest concentration at 150 μg/m^3 found increased airway resistance, which has concerning implications for the effect on the lungs. Only three studies exposed cells to new technology exhaust and the lowest concentration used, 35.3 μg/m^3, found health impact in terms of oxidative stress and PAH adduct formation[129].

Table 3: Key experimental data from selected occupational and acute human exposure studies. Diesel exhaust exposure studies use average PM readings to assess PM levels in the work place and thus assume that workers are exposed to the measured level of diesel exhaust for the entirety of their shifts.

Concentration of Diesel Exhaust (µg/m^3)	Cohort Demographic	Health Impacts in Occupational Exposures
19	Personal EC exposure for 8614 Australian Miners collected between 2003 and 2015	Small increase in lung cancer risk: 5.5 extra lung cancer deaths per 1000 workers[3]
50	69 Norwegian tunnel finishing workers and 69 unexposed control subjects working at similar construction sites	Increased DNA damage in peripheral blood mononuclear cells and altered plasma profiles. Micro RNA dysregulation, including several related to carcinogenesis, cell death and oxidative stress[74]
59	Personal EC exposure for 8614 Australian Miners collected between 2003 and 2015	Increased lung cancer risk: 38 extra lung cancer deaths per 1000 workers[3]
100	54 male workers employed at a diesel engine testing facility and 55 unexposed male control workers	Increased levels of inflammatory markers associated with lung cancer[69]
~170	137 male exposed diesel engine tester and 127 male non-exposed workers	Exceeding 1.08 µg/g urinary creatine, approximately 110 µg/m^3 total carbon exposure, was associated with increased cancer biomarkers such as micronucleus, and thus increased risk of cancer[68]
250	120 diesel exhaust exposed Columbian mechanics and 100 unexposed control subjects	Cytotoxic and genotoxic damage to buccal epithelial cells, found on the inside of the cheek, and peripheral blood lymphocytes. Micronucleation correlated with years of service[72]
268	117 male exposed diesel engine tester and 112 male non-exposed control workers	Increased DNA damage in peripheral blood lymphocytes, in comparison to exposures at 92 µg/m^3 [70]
268	117 male exposed diesel engine tester and 112 male	DNA hypomethylation and slight immune dysregulation in comparison to exposures at 92 µg/m^3 [71]

	non-exposed control workers	
282	117 male exposed diesel engine tester and 112 male non-exposed control workers	Lower lung function and increased serum markers of local and systemic inflammation in comparison to exposures at 92 µg/m^3 [67]
<397	41 male exposed diesel engine testers and 46 male unexposed controls	Increased inflammatory cytokine response in blood serum[66]
>397	41 male exposed diesel engine testers and 46 male unexposed controls	Reduced inflammatory cytokine response in blood serum[66]

Table 4: Key experimental data from selected acute human exposure studies.

Concentration of Diesel Exhaust ($\mu g/m^3$)	8 Hour TWA ($\mu g/m^3$)	Exposure Time (hours)	Times Exposed to Diesel Exhaust	Cohort Demographic	Exposure Method	Engine Classification	Health Impacts in Acute Exposures
7.2	0.9	1	2	19 non-smoking healthy males (mean age, 25±3 years)	Exposure chamber	NS*	Exhaust paired with a DPF had no impact on vasoconstriction, mild increased thrombotic effects in comparison to more severe effects at 320 $\mu g/m^3$ [78]
<75	<18.75	2	1	60 non-smoking asthmatics (18-55 years old)	Controlled roadside exposure	Mix	Decreased lung function, increased airway acidification and increased respiratory inflammation in asthmatics, more severe asthmatics displayed greater symptoms[99]
100	25	2	1	22 allergic rhinitics (11 exposed to air, 27.5 ± 8.7 years, 11 exposed to exhaust, 25.6 ± 4.7 years)	Exposure chamber	NS	Increased inflammation and viral effects in subjects with allergy[98]
100	25	2	2	32 asthmatics, 13 rhinitics and 21 healthy controls (18-41 years old)	Exposure chamber	NS[a]	No evidence of epithelial cell damage following exposure[101]
100	25	2	3	6 healthy glutathione-S-transferase-Mu 1 null adults (50-71 years old)	Exposure chamber	NS	No cardiovascular effects, no inflammatory effects[80]
100	25	2	2	16 healthy adults (18-49 years old)	Exposure chamber	NS[b]	No consistent cardiovascular effects[96]
100	25	2	2	10 healthy adults and 17 adults with metabolic syndrome (18-49 years old)	Exposure chamber	NS[b]	Minor amounts of vasoconstriction in comparison to 200 $\mu g/m^3$ [93]
100	25	2	2	16 adults with metabolic syndrome (18-49 years old)	Exposure chamber	NS[b]	No cardiovascular effects in metabolic syndrome patients[100]
100	25	2	2	32 non-smoking asthmatics and 23 non-smoking healthy	Exposure chamber	NS[a]	Increased airway inflammation in healthy subjects but not asthmatics[97]

				controls (18-45 years old)			
200	50	2	3	5 non-smoking healthy adults (20-31 years old)	Exposure chamber	NS[b]	Dose dependant altered genetic profile in peripheral blood mononuclear cells[94]
200	50	2	1	45 healthy non-smokers (18-49 years old)	Exposure chamber	NS[b]	Increased blood pressure, no impact on heart rate[132]
200	50	2	1	10 adults with metabolic syndrome (18-49 years old)	Exposure chamber	NS[b]	No effect on patients with metabolic syndrome[95]
200	50	2	2	16 healthy adults (18-49 years old)	Exposure chamber	NS[b]	No consistent cardiovascular effects[96]
200	50	2	3	6 healthy glutathione-S-transferase-Mu 1 null adults (50-71 years old)	Exposure chamber	NS	Increased inflammation, no cardiovascular effects[80]
200	50	2	2	10 healthy adults and 17 adults with metabolic syndrome (18-49 years old)	Exposure chamber	NS[b]	Increased vasoconstriction[93]
250	31.25	1	1	18 non-smoking healthy males (21-30 years old)	Exposure chamber	NS	Decreased chemically induced vasodilation, no effect on heart rate variability or blood pressure[92]
280	105	3	2	Healthy non-smoking adults (40-66 years old)	Exposure chamber	NS	Irritant effects- eye, throat and nose symptoms[91]
300	18.75	0.5	3	18 non-smoking recreationally active males (24.5 ± 6.2 years)	Exposure chamber	TIER-3[c]	Irritant effects- chest, throat and nose symptoms, no changes in blood pressure[88]
300	18.75	0.5	3	18 non-smoking recreationally active males (24.5 ± 6.2 years)	Exposure chamber	TIER-3[c]	Altered blood plasma profiles. No changes in blood pressure or markers of inflammation[82]
300	37.5	1	1	16 non-smoking asthmatics (20-42 years old)	Exposure chamber	NS	Increased airway hyperactivity and obstruction in individuals with asthma[87]

300	75	2	2	15 non-smoking healthy volunteers with atopy to house dust mite, birch or Pacific grass (19-49 years old)	NS	NS	Altered micro RNA and transcription profiles[85]
300	75	2	1	17 non-smoking healthy adults (20-46 years old)	Exposure chamber	TIER-3[c]	DNA hypomethylation in airway epithelial cells[86]
300	75	2	1	17 non-smoking atopic adults (17-49 years old)	Exposure chamber	TIER-3[c]	Altered genetic and plasma profile and increased airway hyperactivity in subjects with allergies[81]
300	75	2	1	16 non-smoking asthmatics (19-35 years old)	Exposure chamber	TIER-3[c]	DNA hypomethylation in genes associated with oxidative stress and inflammation in asthmatics[84]
300	75	2	3	6 healthy glutathione-S-transferase-Mu 1 null adults (50-71 years old)	Exposure chamber	NS	Increased blood pressure[80]
300	75	2	1	27 non-smoking healthy adults (19-49 years old)	Exposure chamber	TIER-3[c]	No effect on blood Central Nervous System biomarkers[90]
300	75	2	1	28 non-smoking healthy adults (19-49 years old)	Exposure chamber	TIER-3[c]	No effect on balance after exposure[89]
301	75	2	2	13 non-smoking asthmatics (19-35 years old)	Exposure chamber	TIER-3[c]	Changes in micro RNA expression in blood associated with increased oxidative stress in asthmatics[83]
320	40	1	2	19 healthy males (mean age, 25±3 years)	Exposure chamber	NS	Reduced vasodilation and increased thrombus formation. No changes in blood pressure, heart rate, markers of inflammation and platelet activation[78]
325	14	0.35	2	26 adults at risk of heart failure (51±9 years) and 15 healthy controls (45±10 years)	NS	NS	Increased endothelial dysfunction in patients at risk for heart failure. No changes in heart rate[77]
348	75	2	2	16 non-smoking healthy males (18-32 years old)	Exposure chamber	NS	Reduced vasodilation and increased blood pressure[79]

350	43.75	1	1	12 non-smoking healthy males (21-30 years old)	Exposure chamber	NS[a]	Increased arterial stiffness[76]

* = NS - Not Specified

a= Volvo TD45, 4.5L four cylinder 1991 engine model.

b= Turbocharged direct-injection 5.9-L Cummins 2002 B-series diesel engine (model 6BT5.9G6) and a 100-kW generator.

c= EPA Tier 3-compliant, 6.0 kW Coliseum GY6000 generator, with 406 cc Yanmar L 100 EE 4-stroke diesel generator

Table 5: Key experimental data from selected *in vivo* animal exposure studies using old technology exhaust.

Concentration of Diesel Exhaust (μg/m^3)	8 Hour TWA (μg/m^3)	Exposure Period	Animal	Engine Classification	Health Impacts in Older Technology Exhaust Exposures
38	23.8	5 h/day, 5 days/week, 1, 2 or 3 months	Rat	NS*	Some effects on steroidogenesis in male rats[117]
39	24.4	5 h/day, 5 day/week, 8 weeks	Mouse	NS	Minor increases in respiratory inflammation in asthmatic mice[116]
40	30	6 h/day, 1-7 days	Rat	NS	Minor increases in respiratory inflammation[108]
47	29.4	5 h/day, 5 day/week, 3 months	Mouse	NS	No impact on object recognition[114]
50	25	4 h/day, 1 or 5 days	Rat	NS	Mild increased effect of chemically induced arrhythmia (in comparison to 150 and 500 μg/m^3)[110]
82	41	4 h/day, 1 and 3 days.	Rat	NS	Inflammation and increased oxidative stress in lungs. Negative cardiovascular effects. Greater effects than higher exhaust concentration without DPF usage[133]
100	87.5	7h/day, 5 days/week, 12 weeks	Mouse	NS	Increased allergic symptoms in asthmatic mice[118]
129	80.6	5 h/day, 5 day/week, 3 months	Mouse	NS	Impact on object recognition[114]

149	93.1	5 h/day, 5 days/week, 4 weeks	Mouse	NS	Increased neuroinflammation but no impact on spatial learning[113]
149	93.1	5 h/day, 5 days/week, 1, 2 or 3 months	Rat	NS	Effects on steroidogenesis in male rats[117]
150	75	4 h/day, 1 or 5 days	Rat	NS	Increased effect of chemically induced arrhythmia[110]
169	105.6	5 h/day, 5 day/week, 8 weeks	Mouse	NS	Increased respiratory inflammation in asthmatic mice[116]
173	129.7	6 h/day, 5 days/week, 4 weeks	Mouse	NS	Increased neuroinflammation[112]
200	150	6 h/day, 5 days/week, 7 weeks	Mouse	NS	Unfavourable changes in atherosclerotic plaques[115]
250	187.5	6 hour	Mouse	NS	Impaired neurogenesis in male mice[111]
277	138.5	4 h/day, 1 and 3 days.	Rat	NS	Inflammation and increased oxidative stress in lungs. Negative cardiovascular effects[133]
500	250	4 h/day, 1-14 days	Mouse	NS	Increased flu severity[109]
500	250	4 h/day, 1 or 5 days	Rat	NS	Increased effect of chemically induced arrhythmia[110]
500	250	4 h/day, 4 days	Mouse	NS	Increased respiratory inflammation in allergic mice but not in healthy mice[105]
650	325	4 h/day, 5 days/week, 4 weeks	Mouse	NS	Increased neuroinflammation (less than that found in 2000 $\mu g/m^3$ exposures)[104]
950	712.5	6 h/day, 1-7 days	Rat	NS	Increased oxidative stress and inflammation[108]
1000	1000	12 h/day, 7 days/week, 4, 12 and 24 weeks	Mouse	NS	A 3.1 fold increase in mutation burden in lungs[103]
1700	637.5	3 h/day, 5 days/week, 4 weeks	Mouse	NS	Increased transcription of stress related genes in the brain[107]

2000	1500	6 h/day, 7 days or 6 h/day, 5 day/week, 4 weeks	Rat	EURO V (-DPF)	Minor oxidative stress in brain. No histopathological changes[24]
2000	1500	6 h/day, 7 days or 6 h/day, 5 day/week, 4 weeks	Rat	EURO V (-DPF)	Minor histopathological changes, inflammation and oxidative stress in lungs[106]
2000	1000	4 h/day, 4 days	Mouse	NS	Increased respiratory inflammation, allergic mice display greater symptoms[105]
2000	1000	4 h/day, 5 days/week, 4 weeks	Mouse	NS	Large increase in neuroinflammation[104]
3000	3000	12 h/day, 7 days/week, 4, 12 and 24 weeks	Mouse	NS	A 3.2 fold increase in mutation burden in lungs[103]

*= NS - Not Specified.

Table 6: Key experimental data from selected *in vivo* animal exposure studies using new technology exhaust.

Concentration of Diesel Exhaust (µg/m3)	8 Hour TWA (µg/m^3)	Exposure Period	Animal	Engine Classification	Health Impacts in New Technology Exhaust Exposures
12	12	16 h/day, 5 days/week, 2 years.	Rat	US EPA 2007	No DNA damage[122]
12	12	16 h/day, 5 days/week, 2 years.	Rat	US EPA 2007	No DNA damage[121]
12	12	16 h/day, 5 days/week, 2 years.	Rat	US EPA 2007	No tumour development and mild negative effects on lungs[123]
12	12	16 h/day, 5 days/week, 2 years.	Rat	US EPA 2007	Mild inflammatory and cardiovascular effects[120]
<100	<37.5	3 Hours, 5 days/week, 3 weeks	Rat	EURO IV	Limited accumulation of lung DNA damage[119]

170	127.5	6 h/day, 7 days or 6 h/day, 5 day/week, 4 weeks	Rat	EURO V	Minor inflammation and oxidative stress in lungs[106]
182	136.5	6 h/day, 7 days or 6 h/day, 5 day/week, 4 weeks	Rat	EURO V	Minor oxidative stress in brain. No histopathological changes[24]

*= NS - Not Specified.

Table 7: Key experimental data from selected *in vitro* exposure studies using old technology exhaust. All studies human airway epithelial cells and use air-liquid interface cultures.

Concentration of Diesel Exhaust µg/m^3)	8 Hour TWA (µg/m^3)	Exposure Period (Minutes)	Cohort Demographic	Engine Classification	Old Technology Exhaust
140	17.5-109.38	60-375	Mucociliary differentiated primary bronchial epithelial cells obtained from normal volunteers	NS	Decreased permeability[124]
430	53.75-335.93	60-375	Mucociliary differentiated primary bronchial epithelial cells obtained from normal volunteers	NS	Increased oxidative stress and permeability[124]
470	19.58-117.5	20-120	Alveolar basal epithelial cell line A549	NS	Supressed immune response and increased oxidative stress[127]
850	8.86-106.29	5-60	Mucociliary differentiated primary bronchial epithelial cells obtained from normal volunteers	NS	Increased oxidative stress and increased PAH adduct formation. No loss of viability[129]
1200	150-937.5	60-375	Mucociliary differentiated primary bronchial epithelial cells	NS	Increased inflammation, cell death, permeability and oxidative stress[124]

			obtained from normal volunteers		
1300	975	90	Alveolar basal epithelial cell line A549	EURO III	Increased cell death, increased oxidative stress and decreased inflammatory response[128]
1600	66.7-400	20-120	Mucociliary differentiated primary bronchial epithelial cells obtained from normal volunteers	NS	Inhibited proliferation and increased oxidative stress[127]

*= NS - Not Specified.

Table 8: Key experimental data from selected *in vitro* exposure studies using new technology exhaust. All studies human airway epithelial cells and use air-liquid interface cultures.

Concentration of Diesel Exhaust (μg/m^3)	8 Hour TWA (μg/m^3)	Exposure Period (minutes)	Cohort Demographic	Engine Classification	New Technology Exhaust
34	25.5	360	Mucociliary differentiated primary bronchial epithelial cells obtained from both normal and COPD patients	EURO V	No effect on oxidative stress levels[130]
35	0.37-4.41	5-60	Mucociliary differentiated primary bronchial epithelial cells obtained from normal volunteers	NS*	Increased oxidative stress and increased cellular responses to diesel pollutants (PAHs)[129]
82	61.5	360	Mucociliary differentiated primary bronchial epithelial cells obtained from both normal and COPD patients	EURO V	Increased oxidative stress[130]
206	64.37-154.5	150- 360	Mucociliary differentiated primary bronchial epithelial	EURO V	Increased oxidative stress and decreased defence response to infection in COPD derived cells[130]

			cells obtained from both normal and COPD patients		
1500	187.5	60	Mucociliary differentiated primary bronchial epithelial cells obtained from both normal and COPD patients	TIER 4	Increased oxidative stress and decreased defence response to infection[131]

*= NS - Not Specified.

Occupational Exposure Limits and their Applicability

The Australian Institute of Occupational Hygienists recommends a diesel exhaust occupational exposure limit of 100 µg/m^3 as a time weighted average over 8 hours, measured as elemental carbon[4]. Previous diesel exhaust exposure reviews have recommended an occupational exposure limit of 100 µg/m^3 of diesel particulate matter in total, which is equivalent to approximately 75 µg/m^3 elemental carbon[75]. Using the acute human studies reviewed in this report as the basis for the cross comparison, this limit is accurate for reducing the health effects of short term exposure in healthy workers. However, this limit fails to take the comfort and safety of workers with asthma or allergy into account and is far above the occupational exhaust concentrations where studies found significantly increased lung cancer risk. Previously published reviews have also recommended a lower occupational exposure threshold of 50 µg/m^3 of respirable elemental carbon (approximately 67 µg/m^3 of particulate matter) in order to limit lung cancer risk[134], which is again too high based on the reviewed occupational studies. Based on both the reviewed acute human exposure studies and the occupational exposure studies, a limit below 50 µg/m^3 of particulate matter, approximately 35 µg/m^3 elemental carbon, would be more suitable as it is below the acute exposure concentrations where effects were still found in asthmatics and below the exhaust concentrations that found the highest lung cancer risks. In addition, this limit is supported by *in vivo* exposure studies, where exposure concentrations at 50 µg/m^3 only resulted in mild health effects.

However, it should be noted that exposure limits based on both the mass of elemental carbon, as well as the mass of total particulate matter, are limited in their long term applicability. In order to meet these occupational limits, all diesel equipment would have to be fitted with exhaust after-treatment devices, including a DPF. Diesel particulate filters remove particles from the exhaust, however they preferentially select for elemental carbon above other particle types[12, 129], skewing the exhaust output and eliminating elemental carbon as a predictive measure for overall exhaust exposure, making any occupational limits based on elemental carbon unreliable.

Occupational limits based on particle mass have their own drawbacks. To begin with, evidence is accumulating that it is particle size and particle number that contribute more towards health impact than total particle mass[129, 135-136], making occupational limits based on mass, without accounting for particle size and number, a questionable decision. The latest European Emission Standards take this into account and have set limits for both particle mass and particle number[137].

In addition, multiple studies published in the last decade are reporting little to no change in health impacts after the use of a diesel particulate filter. In in vivo and in vitro exhaust exposure studies that compare exhaust exposure health effects before and after the use of a diesel particulate filter, few to no decreases in health impact are found[24, 106, 119, 127, 129, 133, 138] with only a few adverse cardiovascular events being decreased or prevented in an acute human exposure study[78]. Diesel particulate filters remove more than 90% by mass of particles from the exhaust[24, 78, 106, 129]. However they cannot be 100% efficient given pressure drop constraints of the system, therefore some particles (generally in the smaller size ranges) will

pass through the DPF. Also at the operating temperatures of a DPF, many particles (such as PAHs) are liquid and can migrate through the filter and be resuspended[12, 129, 139]. Indeed PAH can melt as low as 80°C and boil as low as 200°C, both of which are well below typical exhaust temperatures[139]. This suggests that either the exhaust gases are having a greater effect on health than previously thought or that ultrafine particles, and the toxic chemicals potentially adsorbed to their surface, are responsible for the majority of health impacts caused by diesel particulate matter[116, 119, 129, 133]. Thus using occupational limits based on particle mass, an exhaust exposure that was over the limit where negative health consequences occur would read as under with the use of a DPF, and yet the DPF would have little to no impact on decreasing the health impacts on an exposed worker.

In addition to occupational exposure limits based on particle mass, limits on particle number should also be addressed. Studies have also found NO_x to be a reliable indicator of diesel exhaust exposure, so long as the majority of sources contributing to the NO_x concentrations are diesel engines[75, 140]. Equipment that measure NO_x concentrations are also less expensive than the equipment needed for EC measurement[140] and thus an additional occupational limit based on NO_x should not prove to be an expensive burden on the mining industry. Using the reviewed studies that focus on exhaust exposure particulate matter concentrations of roughly 50 $\mu g/m^3$, a rough estimate of 0.4 ppm NO_x[99, 114, 116, 141] should be the expected occupational exposure limit for a similar level of exposure however a more thorough review on the health effects of NO_x and its applicability as a diesel exhaust exposure predictive measurement should be conducted before any sort of limit is put into effect. In future, more research needs to be conducted on the health effects of exposure to new technology diesel engine exhaust and further occupational studies need to be based on the possible health outcomes of the increasing application of new technology engines in the mining industry.

Limitations: This review does contain limitations. The majority of literature was sourced from PubMed using strict search criteria and thus it is possible that relevant studies were missed. Studies were only included if they were written in English and thus relevant studies in other languages were also excluded. This review focussed on studies relevant to hardrock mining in Australia and thus studies that used exhaust concentrations not relevant to occupational exposure conditions were not included.

The studies included in this review use a wide variety of engine types with varying emission classifications and after-treatment devices. Details of engine specifications and settings used during the exposures are limited, if they are listed at all. This, combined with the wide range of exposure outcomes measured, makes firm conclusions difficult for setting occupational diesel exhaust exposure limits. Consistency in experimental designs and strict guidelines for reporting engine specifications and settings in diesel exhaust exposure research would help immensely in solving this issue.

Many of the occupational exposure and acute human exposure studies also use exclusively male subjects and more research needs to be done to verify that occupational diesel exhaust exposure has similar health impacts in both men and women. In addition, very few studies exist that exposed human, animal or tissue to “new technology” exhaust and thus further research is needed to confirm the findings of this review. Future studies in diesel exhaust

exposure effects should concentrate on using newer technology engines and after-treatment devices in order to consolidate the health effects of exposure to "new technology" engine exhaust before it becomes more widely used in an occupational setting.

Conclusion:

In conclusion, an occupational exposure limit of 100 µg/m^3 is too high as it does not take increased lung cancer risk caused by high levels of diesel exhaust exposure into effect. A limit of 50 µg/m^3 is more appropriate if lung cancer risk and the effects of exposure on workers with asthma, allergy and respiratory disease are accounted for. An occupational exposure limit based on elemental carbon is not appropriate as after-treatment devices preferentially remove it from the exhaust, making it an unreliable indicator of exhaust exposure. After-treatment devices also make occupational limits based on particle mass unreliable at best and additional limits, such as ones based on particle number or NO_x concentrations, are needed in order for occupational exhaust exposures to be reliably monitored.

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