National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Impact of increasing the blend ratio of biodiesel on engine emission associated toxicity

A quick scan by RIVM and TNO

RIVM Letter Report 240007001/2014

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Er zijn geen sterke aanwijzingen dat het bijmengen van FAME (“Fatty Acid Methyl Ester”) biodiesel tot 7 vol%[1] en HVO (“Hydrotreated Vegetable Oil”) biodiesel tot 30 vol% zal leiden tot significante toename van schadelijke uitstoot van het wegverkeer. Deze conclusie is gebaseerd op de beperkte toxicologische gegevens die suggereren dat er slechts kleine of verwaarloosbare veranderingen optreden in het toxiciteitsprofiel als gevolg van het bijmengen van biodiesel.

Het gebruik van biodiesel/petroleum dieselmengsels kunnen worden toegepast om de Nederlandse doelstelling voor duurzame energiebronnen te halen. De gevolgen voor de gezondheid door veranderingen in de samenstelling van de motoruitstoot is onzeker. Om het potentiële effect van het verhogen van de mengverhouding van FAME en HVO biodiesel op de menselijke gezondheid te onderzoeken, is in opdracht van het Ministerie van Infrastructuur en Milieu onderzocht wat de veranderingen van de toxiciteit van de uitstoot zal zijn. De beoordeling is gebaseerd op literatuurstudies van zowel gemeten schadelijke componenten als toxicologische studies.

Vanwege technische beperkingen van personenvoertuigen met een roetfilter zal het percentage FAME biodiesel zeer waarschijnlijk niet meer dan 7 vol% kunnen worden. Hogere percentages biodiesel tot 30 vol % kunnen met HVO biodiesel worden bereikt.

De beperkte emissie- en toxicologische gegevens leveren geen sterke argumenten om een aanzienlijke verhoging van de toxiciteit van de uitstoot bij een mengsel tot 7 vol% FAME biodiesel te verwachten. Onderzoeken met HVO zijn zeer schaars. De verwachting is dat HVO mengsels tot 30 vol% minder toxische uitstoot veroorzaken vanwege de structurele overeenkomsten in de koolwaterstoffen en de hogere zuiverheid ten opzichte van op aardolie gebaseerde diesel. Deze aanname moet worden bevestigd door toxicologische studies. Bovendien zijn de gevolgen voor de toxiciteit van uitstoot van voertuigen bij hogere biodiesel blend percentages (> 30 vol%) momenteel niet duidelijk, noch kan een kritisch percentage worden geïdentificeerd waarbij de giftigheid van de uitstoot zijn maximum bereikt.

Daarnaast wordt voorspeld dat de motoruitstoot de komende jaren fors zal afnemen als gevolg van de meest recente emissiewetgeving. Slechts een klein percentage van de daling zal het resultaat van de vervanging van aardolie diesel voor biobrandstof zijn. Al met al, suggereren de beschikbare gegevens dat het niet waarschijnlijk is dat de hoeveelheid schadelijke emissies aanzienlijk zal toenemen door het mengen van lage percentages van deze twee soorten biodiesel, hoewel dit wordt omgeven door onzekerheid vanwege ontbrekende gegevens.

[1] Vol% = volumeprocent, de hoeveelheid van een bepaalde stof in een mengsel

Abstract

Impact of increasing the blend ratio of biodiesel on engine emission associated toxicity
A quick scan by RIVM and TNO

There are no strong indications that mixing FAME (Fatty Acid Methyl Ester) biodiesel up to 7 vol%[2] and HVO (Hydrotreated Vegetable Oil) biodiesel up to 30 vol% will result in significant increase in harmful emissions by road traffic. This conclusion is based on the limited toxicological data suggesting low or negligible changes in the hazard profiles upon blending biodiesel.

The usage of biodiesel/petroleum diesel blends can be applied to achieve the Dutch targets for the use of renewable energy. However, the implications for human health due to changes in the composition of the engine exhaust is uncertain. To examine the potential impact of increasing the blend ratio of FAME and HVO biodiesel on human health, the engine emission associated toxicity has been examined by order of the Dutch Ministry of Infrastructure and the Environment. The assessment was based on literature of both measured harmful components as well as on toxicological studies.

Due to technical limitations of light duty vehicles with diesel particulate filter FAME biodiesel will most likely not exceed 7 vol%. The residual amount of biodiesel percentage up to 30 vol% will be HVO biodiesel.

Based on the limited available emission and toxicological data there are no strong arguments to expect a substantial increase of the toxicity of the exhaust using a blend of 7 vol% FAME biodiesel. Studies with HVO are very scarce. HVO blends up to 30 vol% are expected to produce a less toxic exhaust due to the structural similarities in the hydrocarbons and the higher purity compared to fossil fuel. This assumption needs to be confirmed by toxicological studies. Furthermore, the implications for the hazard of engine exhaust from vehicles at higher biodiesel blend percentages (>30 vol%) is at present not clear nor can a critical percentage be identified at which the toxicity of the exhaust reaches its maximum.

In addition, it is predicted that engine emissions will substantially decrease in upcoming years due to the latest emission legislation. Only a small percentage of the decrease will be the result of the replacement of petroleum diesel for biofuel blends. Altogether, the available evidence suggest that it is not likely that the amount of harmful emissions will increase substantially by blending low percentages of these two types of biodiesel, although this is surrounded by uncertainty due to a data gap.

[2] Vol% = volume percentage, the quantity of a given substance in a mixture

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Summary

Available information on adverse health effects of traffic-related emissions using biodiesel as a fuel is limited and often contradictory. In light of the uncertainties, the implication of increasing the blend ratio of biodiesel on engine emissions associated toxicity has been examined in a quick scan based on existing knowledge. This has been initiated by the Dutch Ministry of Infrastructure and the Environment (I&M), involved in the development of a vision for future renewable biofuel usage. The following questions were asked by I&M:

1. How would RIVM and TNO assess the human health hazard from being exposed to (diluted) engine emissions in light of the increasing percentages of biodiesel mixed into pure fossil petroleum oil up to at least 30%?
2. Is there a critical percentage at which the toxicity of the exhaust reaches its maximum?

The **objective** of this quick scan is to present the implications of increasing the blend ratio of biodiesel (up to at least 30%) as used for light (LD[3]) and heavy-duty (HD) road vehicles on exhaust emissions and its toxicity (hazard).

What is the impact of increasing percentages of biodiesel blends on engine emissions?

Due to technical limitations of LD vehicles with diesel particulate filter and catalysts technology, FAME (Fatty Acid Methyl Ester) biodiesel will most likely not exceed 7 vol% until 2030 and the residual amount of biodiesel percentage up to 30% will be HVO (Hydrotreated Vegetable Oil) biodiesel. It is expected, due to logistical reasons, that one diesel fuel will be applied for LD and HD vehicles. However, in exceptional cases a different fuel with higher FAME blends might be used for HD. In light of this, as well as the latest emission legislation and the implementation of new technologies, engine emissions will decrease. The decrease is mainly due to technology and only a small percentage is caused by the replacement of petroleum diesel for biofuel blends.

Regulated emission components

The emission of carbon monoxide (CO), total hydrocarbons (THC), particulate matter (PM_{10}) and nitrogen oxides (NOx, NO_2) are regulated. Blending biodiesel (7-30 vol% composed of maximal 7% FAME and residual % HVO) will result in decreasing carbon monoxide, total hydrocarbons and particulate matter emissions. Nitrogen oxides emissions, however, will not change significantly due to biodiesel blending. In case the application of higher HVO blends (> 30 vol%) will be used for some specific HD vehicle fleets, it is expected to result in decreased emissions including NOx at all blend percentages.

Non-regulated emission components

Engine exhaust emissions also contain components that are not regulated such as polyaromatic hydrocarbons (PAH), nitro- and oxy-PAH and carbonyls. The quick scan revealed that most non-regulated emission components show a decrease for the application of 100 vol% FAME (B100). Low FAME biodiesel blends (B7-20) show, however, no consistent emission trends mainly due to the large variety of tested biodiesel feedstocks, test protocols and different vehicle

[3] LD vehicles category includes passenger cars and light commercial vehicles

technologies in the different research programs. However, nitro- and oxy-PAH and carbonyl containing components seem to increase most likely with low biodiesel blend rates and therefore require further attention. Very few studies addressed the influence of HVO biodiesel. It is assumed that there is no particular concern of increasing non-regulated emission by using HVO blends due to the pure paraffinic hydrocarbon structure of HVO without aromatics and oxygen groups. However, this needs to be confirmed with experimental data.

What is the impact of increasing percentages of biodiesel blends on toxicity?

Two approaches have been applied: a) assessing the hazardous properties of engine exhaust based on data on measured emission components and b) a literature review on toxicological studies in which engine exhaust or fractions thereof have been tested in biological systems (cells, animals, and volunteers) for their toxic potential.

Hazard assessment based on emission studies

The first approach resulted in a very scattered picture with large gaps in the available data for hazard assessments. The hazard indication of engine emissions from LD vehicles fuelled with FAME biodiesel blends up to B7 is expected to be similar or slightly less than pure petroleum diesel based on non-regulated emission components. There are no data on chemical composition changes using LD vehicles and HVO. The limited data on HD fuelled with blends of FAME and HVO up to 30 vol% minimally indicate that a strong increase is unlikely. The scarcity of emission data in particular for HVO is, however, an important limitation for the hazard assessment described in this report.

Hazard assessment based on toxicity studies

Several reviews on the toxicity of engine exhaust have concluded that there is a general lack in data on biodiesel blends. Based on the data on oxidative potential, cytotoxicity, inflammation, mutagenicity measured using in general particles or organic extracts from (diluted) engine exhaust from LD and HD vehicles fuelled with FAME (rapeseed) blends as assessed in this quick scan, there are no strong arguments to expect a substantial increase of the toxicity of the exhaust.

Conclusions quick scan

- Up to and beyond 2030 an ongoing decrease in the absolute level of regulated and most likely non-regulated emissions is expected, which is mainly based on technology improvement and tighter emission standards and just slightly caused by biofuel blending.
- **FAME** For LD and HD vehicles it is most likely that no more than 7 vol% FAME will be used in biodiesel blends towards 2030 due to technical limitations related to the current particle filter technology of LD vehicles and the expectation that one diesel fuel will be used for both LD and HD vehicles. However, specific HD fleets may go as high as 30 vol% FAME. Some non-regulated components may not show proportional decreases with the regulated emission components. Further investigation on the non-regulated carbonyl emissions, PAH and nitro- and oxy-PAH using low biodiesel blends is required given their toxic nature and the scarcity of data at present in order to improve hazard assessment.
- **HVO** The use of HVO can easily reach 30 vol% within the specifications of the current EN590 standard. HVO blends up to 30 vol% are expected to

produce a less toxic exhaust due to the pure paraffinic structure and the absence of aromatics and oxygen groups. Emission studies for HVO are very scarce however. Performance of such studies would provide more certainty as to how regulated and unregulated emissions as well as the hazard of the emission components will develop.

- The lack of standardization of engine emission generation and characterisation and toxicological tests is a major impediment when comparing the various study results. Based on the limited useful emission and toxicological data and on the expected decrease in the absolute emission levels applying new technology there are no strong indications that mixing FAME up to 7 vol% and HVO up to 30 vol% will result in significant increase in harmful emission.
- The hazard assessment based on emission components and toxicological tests did not show a consistent view to support conclusions on a critical blend percentage.

It is important to note that the published information on the physicochemical and toxicological characteristics is of a fragmented nature only and results are often not comparable between studies due to differences in design. Blend comparison could only be made within a single study. Due to these limitations as to available data the conclusions as drawn in this quick scan are indicative only. This is inevitable given the incompleteness of the current information.

Recommendations

The lack of standardization of engine emission generation and characterization and toxicological tests is a major impediment when comparing the various study results. A limited set of chemicals have been used in this quick scan, whereas exhaust contains a lot more, also hazardous substances. Therefore, it is recommended that a more systematic evaluation will be commissioned in which engine, test conditions and measures for physicochemical composition and the toxicological profile of diluted engine exhaust will be assessed to allow proper comparison among various tests and studies and to reduce the uncertainties of the conclusions drawn in this quick scan.

1 Introduction

Draining fossil fuel reserves, increases in petroleum prices as well as concern over vehicle emission pollutants and global warming have raised awareness towards development of alternative fuel resources. There has been a focus on biofuels, because of their putative reducing CO_2 (greenhouse-gas) potential and their drive to diminish fossil fuel use. However, stimulation of biofuel use will lead to changes in engine exhaust emissions caused by differences in physicochemical composition compared to fossil fuels. It will therefore be important to assess the impact on human health and not just only consider emission reduction and sustainability aspects of introducing biofuels.

Available information on adverse health effects by traffic-related emissions when biodiesel is used in automobile fuels is limited and often contradictory. Effects related to exposure to biodiesel engine exhaust on health related parameters show increased, equal, or decreased toxicity with biodiesel usage (Bünger et al., 2012).

In light of the uncertainties and the fact that the Dutch Government intends to stimulate the use of biodiesel for road transport the impact of increasing the blend ratio of biodiesel on engine emissions associated toxicity has been assessed in a quick scan. By order of the Ministry of Infrastructure and the Environment the following questions are addressed in this quick scan:

1. How would RIVM and TNO assess the human health hazard from being exposed to (diluted) engine emissions in light of the increasing percentages of biodiesel mixed into pure fossil petroleum oil up to at least 30%?
2. Is there a critical percentage at which the toxicity of the exhaust reaches its maximum?

The assessment was focussed on road transportation and the most commonly used or foreseen in use biodiesels. Two different types of biodiesel have been distinguished: FAME (Fatty Acid Methyl Ester) and HVO (Hydrotreated Vegetable Oil[4]). Both types of biodiesel can be produced with the same feedstock but are treated in two different ways. These two types of biodiesel have very different properties and this may result in constraints in vehicle applications.

This quick scan of biodiesel blends is based on the definition of EU goals for biofuel blending and is focussed on the changes in the composition of the emissions of blending biodiesel up to 30 vol% into petroleum diesel. In addition, an assessment is made of the expected trends of the hazard of the engine exhaust specifically for particulates, polycyclic aromatic hydrocarbons and carbonyl group(s) containing compounds. The toxicity is furthermore evaluated based on published toxicological studies in which emission mixtures were examined in in vitro or in vivo test systems. The in this quick scan described hazard assessment is limited to a maximum of 7 vol% FAME usage for light duty vehicles due to the current technical limitations applying diesel particulate filter technology.

[4] Despite the name, this type of oil also originates from animal fat

2 Methods

2.1 Regulated and non-regulated emission components

The data on emission studies in which different biodiesel types and blend rates were used, have been retrieved via a literature search (see annex 1). In addition, the expected blend ratios of biodiesel in 2030 were determined based on European and Dutch fuel policies, state of the art knowledge on fuels and engine technologies and the technical limitations of these engines.

2.2 Toxicology

The data on studies in which toxicological effects of whole engine exhaust or fractions were tested in biological test systems were retrieved via a literature search (see annex 2). Additional information was obtained from researchers of the University of Umea, University of Edinburgh, Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (IPA), Ruhr-University Bochum, Thünen Institute of Agricultural Technology, Coburg University of Applied Sciences and Arts, Fuels Joint Research Group (www.fuels-jrg.de), University of Ottawa, Health Canada, and U.S. Environmental Protection Agency. Based on this information an assessment was made of the likelihood that an increase in biofuel % could result in more hazardous engine exhaust mixtures.

In addition, as a parallel approach, based on available data on emission the likelihood that an increase in biofuel % would result in increased emissions of individual regulated and non-regulated chemicals is determined (see sections 3.4 and 3.5) and this is followed by a chemical-specific hazard assessment for these possibly higher emissions (section 3.6).

3 Results

3.1 Biodiesel types

A short overview of the current status of biofuel types on the European and Dutch market is given in this paragraph based on Kooter et al. (2014).

Two types of biodiesel need to be distinguished on the market because of their different chemical composition:

- FAME, which is a methyl ester and consequently has oxygen groups within its molecules;
- Paraffinic based biodiesel such as HVO (Hydrotreated Vegetable Oil) and BTL (Biomass to Liquid). These are plain hydrocarbons without oxygen groups or aromatics.

Up to 2014, FAME has been the most applied biodiesel in Europe because it is relatively cheap and production plants are available. Currently in the Netherlands, FAME is mainly produced from tallow, animal fats and used cooking oil, while in the rest of Europe rapeseed is the most important source. At present pure fossil diesel fuel (B0; 0 vol% biodiesel), specified by the EN590-2009 standard may already contain up to 7 vol% FAME biodiesel. This might mean that pure fossil diesel is not really B0 but B7 (blend with 7% biodiesel) instead. In line with this it could well be that B0 as defined in the studies reviewed for this quick scan contain ≤7 vol% FAME and are nevertheless defined as pure fossil diesel.

HVO biodiesel is more expensive than FAME biodiesel as the treatment of the feedstock and the processing of the fuel requires a dedicated production plant. Blending percentages up to 30 vol% HVO biodiesel within the EN590-2009 fuel specifications are possible even without registration of the amount that has been added to B0.

3.2 Biodiesel blends and trends

3.2.1 Biodiesel blends on the European and Dutch market

This paragraph gives a short overview of the current status and the expected situation in 2030 of biofuel blends on the European and Dutch market and it makes a prediction of the share of biofuels in the year up to 2030 (Kooter et al., 2014).

At present, the EN590 diesel fuel standard specifies a maximum FAME content of 7 vol%. Diesel fuels with higher FAME blends (> 7 vol%) cause severe engine oil contamination, especially in light duty vehicles with diesel particulate filter. Due to post injection of fuel, necessary for diesel particulate filter regeneration, FAME components can enter the oil sump. This can lead to problems such as oil dilution and polymer formation. Many heavy duty vehicles (i.e. city buses) can operate with FAME blends up to 30 vol% or higher in some cases.

Large scale deployment of fuel with 7 vol% biodiesel is possible in Europe and the Netherlands, irrespective the use of FAME or HVO. The deployment up to 30 vol% is possible on a relatively large scale provided that HVO is used or in case of FAME, engines are adapted.

For the period up to 2030, it is expected that:

- FAME type biodiesel is blended with regular diesel up to some 5-7 vol %. However, higher blend percentages (such as 30 vol%) for certain market segments or HD niche markets cannot be fully excluded.
- HVO type biodiesel blend will likely be used to further top up the blend percentage above 7 vol%. For HD niche markets, a higher blend percentage of up to 30 vol% or even pure HVO might be used.

The uncertainty of the future biodiesel blends is largely due to the absence of European targets for the period of after 2020. The target for 2020 is the use of 10% biofuel based on energy content.

Regarding biodiesel blends for the period 2014 – 2017, the following is concluded:
• B7 (7 vol% FAME) will generally be the maximum blend for both LD and HD vehicles.
• Blends with 30 vol% (B30) or 100 vol% (B100) FAME can be deployed on a small scale for heavy duty vehicles (EURO I-V).
• The expected deployment of HVO is relatively small for this time period. HVO will be used primarily as a 30 vol% blend or pure (100 vol%) for applications (Euro V buses and older, mobile machinery) where emissions are major contributors to local air pollutant concentrations (urban areas).
• 30 vol% HVO blend can be added to neat fossil diesel (B0) as well as to B7.

With respect to biodiesel blends for the period 2018 – 2020, the following is expected:
• It is still uncertain with which biofuel blends in the Netherlands and in Europe the European RED[5] blending target of 10 e% will be achieved by 2020. This mainly depends on the double-counting[6] capabilities and the actual availability of this double-counting fuel. The current minimum required biofuel scheme for road transport fuel is:

- 2011 4.25 e%
- 2012 4.50 e%
- 2013 5.00 e%
- 2014 5.50 e%
- 2020 10.0 e%

• 10 vol% FAME for trucks is possible and is one of the most cost-effective options to contribute to the 2020 RED target.
• 30 vol% for trucks is also possible and certainly not excluded.

The following conclusions are made regarding the development of biodiesel blends for the Netherlands up to 2030:
- It is expected that the FAME content will generally not exceed the maximum 7 vol% according to the current fuel specification.

[5] RED: Renewable Energy Directive. Target for 2020 is 10% on energy basis (e%)
[6] Double counting can be applied to biodiesel made from residues or from non-food crops. If these feedstocks will be applied for biodiesel production on a large scale, the 10 e% biofuel target can be achieved with up to 7 vol% FAME

- The pressure to use more than 7 vol% biodiesel (FAME + HVO) will be low, since the 2020 RED target of 10e% can primarily be achieved by the generous use of 'double counting' biodiesel and due to the absence of European targets for road transport for the period after 2020.
- It is expected, that for niche markets higher blends (up to 30 vol%) of HVO will be used.

3.2.2 Summary biodiesel blends and trends

Due to the EN590 specifications of diesel fuel which allows a maximum FAME content of 7 vol% and the possibility to apply the ' FAME double counting' option to achieve the 2020 RED target, it is expected that in 2020 diesel fuel will contain no more than 7 vol% biodiesel (probably FAME).

3.3 Effect of biodiesel on regulated emission components

3.3.1 Regulated emission components

The blending of HVO and FAME will most likely result in differences in the physicochemical composition of the engine exhaust. This paragraph gives a short overview of the impact of FAME and HVO biodiesel on regulated emission components based on Kooter et al., 2014. The following components are regulated: carbon monoxide (CO), total hydrocarbons (THC), nitrogen oxides (NOx), nitrogen dioxide (NO_2) and particulate matter (PM_{10}) and recently particle number.

During the past decade, the emission legislation for both LD and HD vehicles has become more stringent due to the steps in emissions legislation from Euro 3 around the year 2000 to Euro 6 in 2013/2014. This has led to the introduction of (improved) emission control systems for all regulated components. In the coming years up to 2030, it is expected that most Euro 5 and older vehicles will be replaced for Euro 6 vehicles. This will lead to a steady reduction of regulated emission components of the total vehicle fleet.

In Figure 1, the regulated emissions trends (g/km) are shown for the LD and HD vehicle fleet from 2015 to 2030. Emission reduction as shown in Figure 1 is mainly due to the increased uptake of 'clean' vehicle technologies in the fleet and only slightly by blending up to 30 vol% biodiesel (7 vol% FAME + 23 vol% HVO).

It is concluded, that between 2015 and 2030, there will be a steady reduction in the emission of regulated components due to the renewal of the vehicle fleet. The expected use of biodiesel blends will likely lead to a further small reduction in regulated emissions or have an insignificant effect.

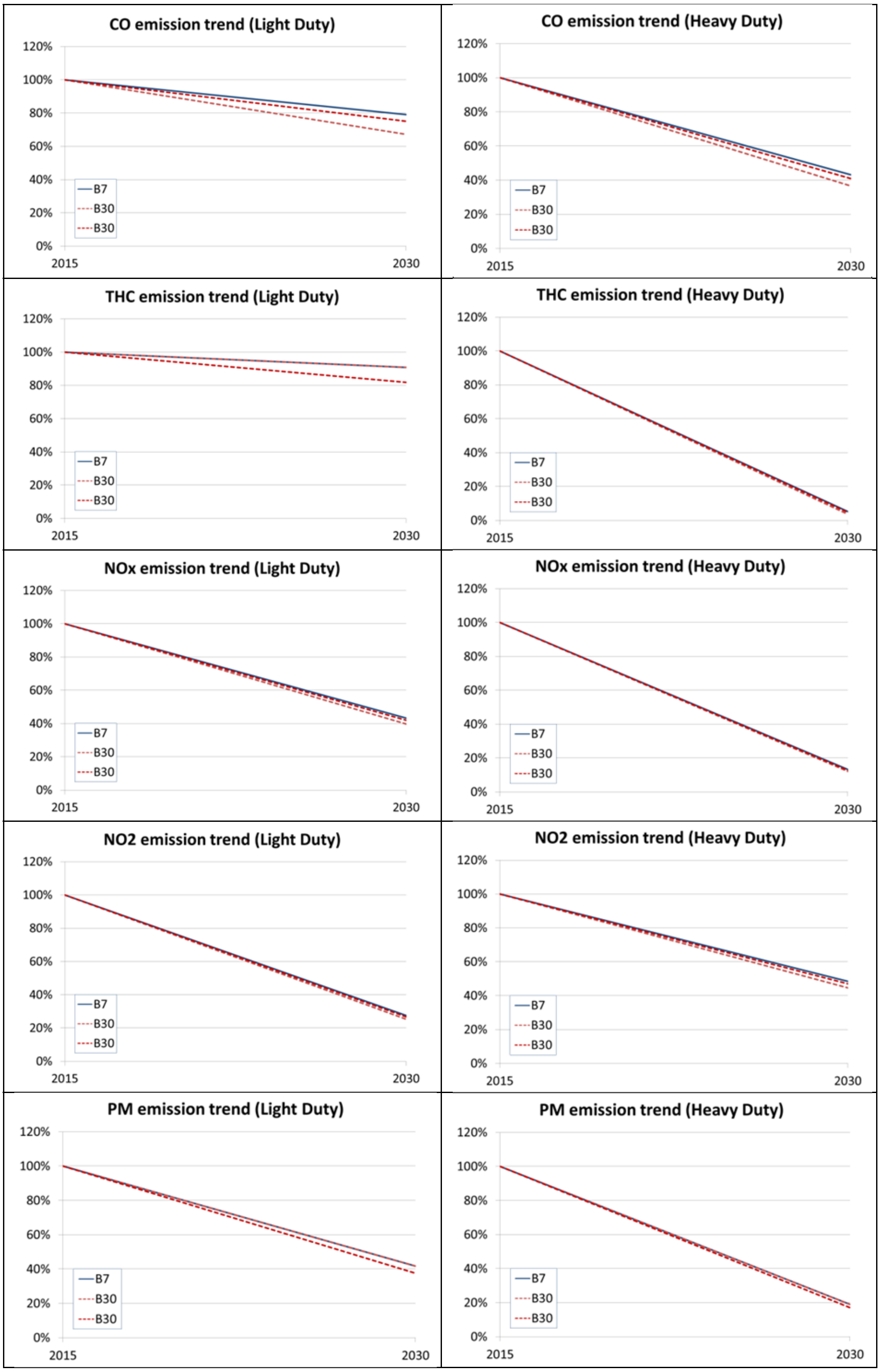

Figure 1: Expected emission trends 2015-2030 – LD and HD vehicle fleet (g/km). The black lines show the effect with a constant fuel composition with a maximum biodiesel content of 7 vol% (FAME + HVO). The red lines show the bandwidth if the HVO content would be gradually increased to 30 vol% (7 vol% FAME + 23 vol% HVO).

3.3.2 *Summary regulated emission components*

Up to 2030, due to removal of old vehicles which are replaced by vehicles with modern exhaust aftertreatment technology, vehicle emissions will decrease (substantially). This will happen irrespective of the fuel composition. An increase of the use of biofuel up to 30 vol% (7 vol% FAME, supplemented with 23 vol% HVO) will result in a relatively small additional decrease of regulated emissions.

3.4 Effect of biofuels on non-regulated emission components

The following overview on the impact of FAME and HVO biodiesel on the non-regulated emission components is based on Kooter et al., 2014.

3.4.1 *Non-regulated emission components -FAME*

3.4.1.1 *General observations*

- *PAH:* No consistence trend of increases or decreases of PAH levels has been identified for low FAME biodiesel blends B7 – B20. Some studies show a decrease of light PAH with increasing blend rate and an increase of heavy PAH with increasing blend rate. PAH content generally decreases when using B100. Unsaturated biodiesel (e.g. soybean) shows less decrease compared to saturated biodiesel (e.g. coconut) but neither will be a dominant source for biodiesel on the European market.
- *Nitro and oxy-PAH*: The very few studies found addressing nitro- and oxy-PAH, showed a decrease of these PAH types with B100.
- *Benzene:* Data are not consistent and no conclusion can be drawn
- *Carbonyl containing components*: often appear to increase with biodiesel blend rates, although one study showed a decrease of heavy carbonyl as a function of biodiesel blend rate. Based on expert judgement an increase in carbonyl containing is to be expected due to the poorer combustion at higher FAME percentages. Control technologies may prevent the emission of these substances, but data is lacking to support this statement.
- *Elementary carbon*: generally decreases substantially as a function of the biodiesel blend rate for both FAME and HVO type biodiesel (percentage with low blends t.b.d).
- *Organic carbon*: can vary significantly and does not show a clear pattern with biodiesel blends.

3.4.1.2 *Light duty vehicles*

- *PAH:* In general, PAH emissions decrease when B100 FAME biodiesel is applied. No consistence response with low blends B7 – B20 are identified. Some studies show an increase of light PAH with increasing blend rate and a decrease of heavy PAH with increasing blend rate.
- *Nitro- and oxy-PAHs*: Only few studies addressed analyses of nitro- and oxy-PAHs, which showed in general a decrease in the total amounts of these compounds with application of biodiesel. However, individual compounds might increase.
- *Carbonyl containing components:* A clear increase of these components is observed with increasing FAME blend percentages.
- *Elemental carbon and organic carbon*: A clear decrease of these components is observed with increasing FAME blend percentages.

3.4.1.3 *Heavy duty vehicles*

- *PAH:* For HD vehicles PAH generally decreases when using B100. Unsaturated biodiesel (e.g. soy bean) shows less decrease compared to saturated biodiesel (e.g. coconut). No consistence response with low blends B7 – B20 are identified, and both decreases and increases are reported. As well can light and heavy PAH have an opposite response, showing a decrease of light PAH and an increase of heavy PAH with addition of biodiesel.

- *Nitro- and oxy-PAH:* seem to increase most likely at the low blends, but a reduction is observed for B100.
- *Carbonyl containing components*: An increase at low blends. Has been observed. A study investigating different blends showed an optimum in increase at B5 and a decrease in carbonyls from B10 onwards.
- *Elementary carbon:* In general, a decrease of these components is observed with increasing FAME blend percentages, but this is not consistent.
- *Organic carbon:* The effect of increased percentages FAME on organic carbon emissions can vary significantly and does not show a clear pattern with biodiesel blends.

3.4.2 Non-regulated emission components - HVO

Very few studies addressed the influence of blending petroleum and HVO biodiesel on the emissions of the engines. No studies have been reported investigating HVO for LD vehicles. A single HD Euro IV study in which HVO application (B20) is examined showed a 27% decrease in PAH (compared to 22% for rapeseed-derived FAME (RME; Rapeseed Methyl Ester)).

3.4.3 Summary non-regulated emission components

In general, no consistent trend is observed for non-regulated emissions of FAME application. However, carbonyl and nitro- and oxy-PAH often appear to increase with low biodiesel blend rates and therefore require future attention. For HVO only one HD study was found, which showed similar PAH emission values compared to RME (no other non-regulated emissions were investigated).

3.5 Hazard assessment based on available emission studies

3.5.1 General

The feedstock that has been used in studies regarding the emissions using different percentages of FAME is mainly rapeseed or soybean. However, the quick scan revealed that currently and possibly also in the future most FAME actually used in practice in the Netherlands derives from animal fat. For FAME derived from animal fat no emission data are available for low biodiesel blends up to 30 vol%. This is an important data gap for the hazard assessment for FAME as presented in this report. Data for HVO are limited to a single study on PAH emission for a HD engine. Given the structural similarities in the hydrocarbons and the higher purity of HVO compared to petroleum diesel the impact of HVO up to 30 vol% may be envisaged to be insignificant. However, this conclusion is uncertain because of the scarcity of relevant studies for HVO. Both of these data gaps, i.e. the lack of emission studies for animal fat-derived FAME and the scarcity of emission data for HVO are important limitations for the hazard assessment in this report.

3.5.2 Light duty vehicles - FAME

The hazard assement is made based on the expectation that a blend percentage up to 30 vol% can be reached for the period up to the year 2030. For FAME biodiesel a maximum of 7 vol% is taken into account. HVO is assumed to be the major biodiesel used for reaching the level of 30 vol%.

3.5.2.1 PAH

PAH are linked with the initiation and development of cancer. The emission data for PAH refer to a number of individual PAH. For hazard assessment only the emission levels for benzo(a)pyrene (B[a]P) were taken into account as this is considered to be the most harmful PAH of the generally measured PAH. This is the 'indicator approach' as used in risk assessment for PAH as a group. In the present case this approach was chosen in preference to the alternative approach for PAH, the 'potency factor' approach.

The limited amount of data as available for vegetable oil-derived FAME products, suggests that application of up to B7 might result in a slight reduction in total PAH compared to B0. The overall impact of this small decrease on air PAH levels around areas of intense traffic is expected to be slight to negligible. In line with this, the expected health impact in terms of the PAH-induced cancer risk is expected to be slight to negligible based on available data. Albeit that this quick scan does not assess the influence on the actual exposure levels for the population: a relative decrease for a specific vehicle can still result in an increased exposure if the fleet mileage will increase.

3.5.2.2 Nitro- and oxy-PAH

For nitro-PAH and oxy-PAH for FAME data are even more limited than for PAH. Data for a EURO-4 type vehicle indicate decreased emission of nitro- and oxy-PAH for FAME blends up to B30. For a EURO-2 type vehicle, however, a somewhat higher emission of nitro-PAH was seen at B50 while markedly increased oxy-PAH emission was found with high blends. In view of the data for PAH a slight decrease in nitro- and oxy-PAH emission for FAME B7 seems a reasonable expectation. This might mean a slight reduction of the cancer risk due to nitro- and oxy-PAH but it should be noted that quantitative cancer risk assessment is not available for this group of compounds. Overall, given the scarcity of data a firm conclusion cannot be drawn for the hazard of the emissions based on nitro- and oxy-PAH emissions in case of use of FAME biodiesel at B7.

3.5.2.3 Carbonyl containing components

Based on the available data for FAME for vegetable oil-derived products, application of up to B7 could result in a slight increase in carbonyl emissions compared to B0. The overall impact on air carbonyl levels around areas of intense traffic is expected to be slight. Various carbonyl compounds are known to produce sensory irritation and local damage to the airways. At high concentrations these effects can result in a carcinogenic response. This has been observed for formaldehyde in particular. Formaldehyde at high concentrations is also linked to respiratory sensitization. It is uncertain if increased emissions of carbonyls due to use of biofuels could lead to increased risks for these effects among the population. In case of use of FAME up to B7, however, any increases in carbonyl emissions can reasonably be expected to be low only and thus a priori an increased hazard of the emissions seems unlikely.

3.5.3 *Heavy duty vehicles - FAME*

A substitution rate of up to 30 vol% is assumed for the period up to the year 2030. For both FAME and HVO maximum use percentages of B30 are taken into account.

3.5.3.1 *PAH*

The limited amount of available data for FAME from vegetable oil-derived products is conflicting in part. Both increases and decreases have been reported. Especially at lower percentages up to B20 slight increases were found. On the whole, however, the results, including those for B100, seem to suggest a decrease, also given the results for LD vehicles. In conclusion, for FAME up to B30 the effect on PAH emissions is highly uncertain based on available data. Minimally the data indicate that a strong increase is unlikely. This makes a large impact on air PAH levels around areas of intense traffic also not likely. Thus, any expected impact on the hazard of the emissions in terms of the PAH-induced cancer risk would be limited. This conclusion, however, is uncertain because of the limited and conflicting available data.

3.5.3.2 *Nitro- and oxy-PAH*

For nitro- and oxy-PAH for FAME data are even more limited than for PAH. Only one study for a EURO III engine was identified. The result showed a slight reduction for nitro-PAH (up to -7%) and an increase for oxy-PAH (up to +30%) at B10 and B20 whereas decreases were observed for both nitro- and oxy-PAH at B100 (respectively -60% and -80%). Further data are lacking. A slight decrease in nitro-PAH emission with FAME up to 30% would mean also a slight reduction of the cancer risk posed by nitro-PAH. On the other hand, increased emission of oxy-PAH could lead to higher exposures via ambient air, which in turn could produce an increased cancer risk due to oxy-PAH. It should however be noted that quantitative cancer risk assessment is not available for these groups of chemicals. Given the scarcity of data a firm conclusion cannot be drawn for nitro- and oxy-PAH emissions in case of use of FAME biodiesel at B30.

3.5.3.3 *Carbonyl containing components*

Three studies are available, showing conflicting results. Two studies found increases for B20 and B100 (in agreement with data for LD vehicles) but the third found clear decreases for B10, B20 and B100. These are studies for FAME derived from vegetable oil. Based on available data it is unclear if application of FAME as biodiesel up to B30 in HD vehicles would lead to increased carbonyl emissions. Data for LD vehicles indicate increased emissions. It seems plausible that a similar effect would be present for HD vehicles but the available data do not bear this out. Thus no conclusion can be drawn concerning emissions of carbonyl compounds when FAME biofuel is used at levels up to B30.

3.5.4 *Heavy duty vehicles - HVO*

For HVO a single study is available showing a decrease in PAH for a EURO IV engine but a relative increase when an SCR (Selective Catalytic Reduction) step was included (lower absolute PAH levels after SCR). Thus, for HVO up to B30 a decrease in PAH is expected. The expected health impact of this would be a proportionally lower PAH-induced cancer risk.

3.5.5 *Ultrafine particles*

Within the complex mixture of particulate matter, ultrafine particles get and will get more attention because of the expected impact on human health. Ultrafine particles have not only a higher likelihood of penetrating deep into the lung; they can also cross biological barriers and thereby reaching other organs like heart, brain and liver. Despite the fact the recent developments in emission control technologies have resulted in a substantial decrease in ultrafine particle emissions (measured as the number of particles per cc), very little data are published on the implications on the road, both in terms of primary emitted particles as well as the so called secondary particles, particles formed from gases. As we do not have relevant data on ultrafine particle emissions, a hazard assessment is not possible.

3.5.6 *Summary hazard assessment based on emission studies*

For FAME, carbonyl-containing compounds such as aldehydes may increase in concentration in the engine exhaust. Other components discussed tend to be reduced with increasing FAME percentages up to 7 vol%. Given the lack of data on a range of biodiesel blends, no firm statement on the optimal percentage of FAME can be provided at which the overall toxicity/hazard of the emitted mixture from either LD or HD is lower compared to that of petroleum diesel. In general, there is no particular concern of increasing hazard of emissions from engines fuelled with HVO blends.

3.6 Hazard assessment based on available toxicity studies

There is a wide variety of tests available to assess the toxicity of chemical substances. The following section addresses toxicity tests in mammalian cell test systems (in vitro), systems using bacteria to assess the mutagenicity as well as studies performed in experimental animals (in vivo). Below, we have differentiated the studies in which only the particulates of the engine exhaust were tested from those in which the whole engine exhaust was tested. For the toxicological evaluation, especially biodiesel blends from FAME based on rapeseed (RME) were included in this quick scan as the data available on FAME based on other feedstock or HVO are too limited.

3.6.1 *In vitro tests using only particulate matter*

The toxicological effects of different biodiesel fuels and blends have been assessed in cell cultures using as toxicity endpoints; cytotoxicity, apoptosis, oxidative stress, or inflammation-triggering responses.

3.6.1.1 *Cytotoxicity*

Cytotoxicity, as the indication of cell viability, seems to vary greatly with fuel type and blending specification. Several rapeseed-based FAME biodiesel EN14214 blends (RME blends; B0, B5, B10, B20, B100) were tested in a Euro III heavy duty engine and the collected particles were analysed for their cytotoxic potential effects (Kooter et al., 2011). Particles from engines using low biodiesel blends (5-10 vol%) elicited less cytotoxicity than petroleum diesel. However, 100% RME (B100) induced significantly higher cytotoxicity whereas B20 was more or less comparable with B0. On the other hand, increased cytotoxicity upon exposure to collected particles from a light duty vehicle has been shown recently for a blend of 50% RME (Gerlofs–Nijland et al., 2013).

3.6.1.2 *Apoptosis*

Apoptosis is the process of programmed cell death initiated by biochemical events like exposure to engine exhaust emission. Non-significant differences in apoptosis were seen in macrophages upon exposure to engine exhaust from a heavy duty diesel engine generator using conventional diesel fuel (EN590), RME or HVO biodiesel both at 100 vol% or a 30 vol% blend (Jalava et al., 2012).

3.6.1.3 *Oxidative potential*

Oxidative stress results when the generation of reactive oxygen species (ROS) or free radicals exceeds the available antioxidant defences. Disturbances in the normal redox state of cells can cause toxic effects. The oxidative potential of particles is a measure that reflects the capacity of particles to generate ROS or free radicals and gives an indication for possible development of oxidative stress. Particles collected from engine exhaust of a heavy duty vehicle using a 5 vol% FAME based biodiesel blend (B5) showed increased oxidative potential (30% increase) compared to petroleum diesel, while a significant decrease (90%) was observed with B100 (Kooter et al., 2011). Besides, the oxidative potential of particles collected using a light duty vehicle running on a 50% rapeseed-based FAME blend (B50) was similar or reduced compared to particles from B0 (Gerlofs-Nijland et al., 2013).

3.6.1.4 *Inflammation*

The inflammatory response, as an indication for airway infection, can be assessed by measuring in vitro the response of pro-inflammatory markers upon exposure. Jalava et al. (2012) exposed macrophages to particles collected from a heavy duty engine fuelled with either petroleum diesel (B0), RME (B30, B100) or HVO (30 vol% and 100 vol%) to assess the inflammatory response. A dose-dependent response was observed for all fuels tested and there were no significant differences between the fuels. On the other hand, a study by Gerlofs-Nijland et al (2013) using particles collected from a light duty vehicle running on 50 vol% RME, showed an increased release of pro-inflammatory markers compared to particulate matter derived from diesel-fuelled vehicles, irrespective of the dose metric (per mass or per kilometre driven).

3.6.2 *In vitro tests using whole engine exhaust*

Steiner et al (2013) provided the only published information on the toxicity of whole engine exhaust. They investigated the effect of petroleum diesel (B0) and rapeseed-based FAME blends (B20, B100) on human epithelial cells by exposing the cells directly to diluted engine exhaust of a light duty vehicle. Compared to B0 exhaust, B20 exhaust decreased oxidative stress and pro-inflammatory responses, whereas B100 exhaust, depending on exposure duration, decreased oxidative stress but increased pro-inflammatory responses. The lower percentage blend was classified as less toxic.

3.6.3 *Assessment of the mutagenicity using particles and exhaust condensates*

The majority of studies assessing the toxic potential of biodiesel have focused on mutagenicity of components absorbed on emitted particles. A substance or mixture is described as mutagenic if it causes changes in the genetic information (DNA) of an organism above background level. Some mutations can be lethal, or cause serious disease, such as cancer. Others have only minor or no effect. Not every change in the DNA caused by mutagenic substances will eventually result in the promotion of cancer or in the reinforcement of its propagation. There is sufficient evidence that exposure to diesel engine exhaust is associated with increased lung cancer risk and therefore diesel engine exhaust is classified as carcinogenic to humans[7]. Burning biodiesel may produce similar carcinogenic substances at equal, higher or lower levels than petroleum diesel. In that respect, biodiesel might be an interesting alternative to reduce the health impact of petroleum diesel.

Bünger et al. (2012) concluded in their review that older studies demonstrated that exhaust from engines fuelled with biodiesel had a lower content of PAH, which was mirrored by a weaker mutagenicity compared to regular diesel engine exhaust. At the same time, however, the mutagenicity of regular diesel engine exhaust also showed a decreasing trend, most likely due to removal of sulfur and the use of new technology diesel engines (Figure 2). The first studies with HVO point towards a reduced mutagenicity of engine exhaust particles using HVO compared to FAME. Kooter et al (2011) noted that rapeseed-based FAME up to 20% in a Euro III heavy duty engine did not cause any difference compared to B0 with respect to mutagenicity of the emitted particles and only the use of 100% RME increased the mutagenic potency of the emitted particles. More recently, Schröder et al. (2013) observed an increase of the mutagenic potential of exhaust condensates for rapeseed-based FAME biodiesel blends with the maximum observed for B20. They concluded that B20 must be considered as a critical blend when petroleum diesel/B0 and FAME biodiesel are used as binary mixtures. The considered the fact that there is a non-linear relationship reason for caution.

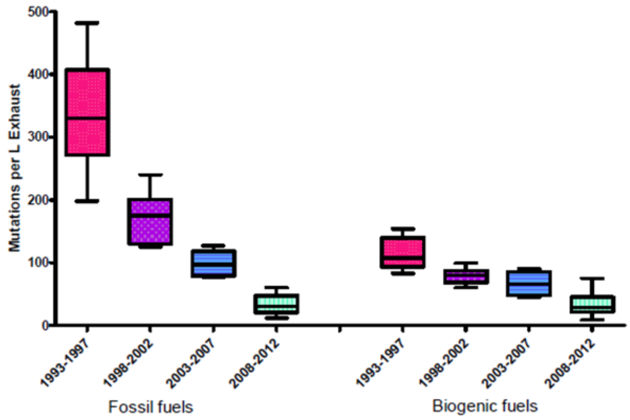

Figure 2 – Development of mutagenicity of engine exhaust using fossil or biogenic fuels from 1993-2012 (Courtesy Bünger et al.)

3.6.4 *In vivo tests using only particulate matter*

There are no in vivo particle studies of blends or of pure FAME rapeseed-based biodiesel. Fukagawa et al. (2013) studied the adverse effects of a 20% soybased FAME biodiesel blend (B20) in mice by instillation of particles collected from a light duty engine. A 20-30% increase in pro-inflammatory markers was

[7] http://www.iarc.fr/en/media-centre/pr/2012/pdfs/pr213_E.pdf

observed after exposure to B20 compared to pure diesel fuel. Another study by Yanamala et al. (2013) described the in vivo response of pro-inflammatory and oxidative stress markers as well as tissue damage of diesel engine exhaust particles (B0) and 100% corn-based FAME particles (B100). This study indicates that pure FAME corn-based biodiesel exerts more pronounced adverse effects than does petroleum diesel.

3.6.5 *In vivo test using whole exhaust*

Brito et al. (2010) exposed mice for 1 hour to diluted engine exhaust from a stationary diesel generator fuelled with metropolitan diesel (3% biodiesel) or FAME biodiesel from soybean (B50 and B100). No differences in inflammatory response were seen between diesel and soy-based FAME biodiesel. However, there is some indication that cardiovascular irregularities increased with biodiesel relative to metropolitan diesel. Gilmour et al. (unpublished) have performed a comparative toxicity and mutagenicity study on FAME soy-based biodiesel blends. The preliminary data suggest that soy-based FAME biodiesel (B20 and B100) are not more toxic than petroleum diesel.

3.6.6 *Summary hazard assessment based on toxicity studies*

Conflicting outcomes complicate the toxicity assessment of engine exhaust emitted using new technologies or fuels. In addition to the limited amount of data that is available, interpretation of the results is hampered by confounding factors such as the engine used in exposures and other study conditions like the animal model used and duration of exposure. The toxicological effects of biodiesel RME and HVO blends strongly depend upon the biological endpoint that is measured, the blends used and the engine that is tested. Some studies indicate that FAME biodiesel percentages around 20% may be associated with an increased hazard of the exhaust, primarily related to carcinogenicity.

4 Key messages

Up to and beyond 2030 an ongoing decrease in the absolute level of regulated and non-regulated emissions is expected, which is mainly based on technology improvement and tighter emission standards and just slightly caused by biofuel blending.

For light and heavy-duty vehicles it is most likely that no more than 7 vol% FAME will be used in biodiesel blends towards 2030 due to technical limitations related to the current particle filter technology of light duty vehicles and the expectation that one diesel fuel will be used for both light and heavy-duty vehicles. However, specific heavy duty fleets may go as high as 30 vol% FAME. Some non-regulated components may not show proportional decreases with the regulated emission components. Further investigation on the non-regulated carbonyl emissions, PAH and nitro- and oxy-PAH using low blend biodiesel is required given their toxic nature and the scarcity of data at present in order to improve hazard assessment.

The use of HVO can easily reach 30 vol% within the specifications of the current EN590 standard. HVO blends up to 30 vol% are expected to produce a less toxic exhaust due to the pure paraffinic structure and the absence of aromatics and oxygen groups. Emission studies for HVO are very scarce however. Performance of such studies would provide more certainty as to how regulated and unregulated emissions as well as the hazard of the emission components will develop.

The lack of standardization of engine emission generation and characterisation and toxicological tests is a major impediment when comparing the various study results. Based on the limited useful emission and toxicological data and on the expected decrease in the absolute emission levels applying new technology there are no strong indications that mixing FAME up to 7 vol% and HVO up to 30 vol% will result in significant increase in harmful emission.

The hazard assessment based on emission components and toxicological tests did not show a consistent view to support conclusions on a critical blend percentage.

5 Limitations

In this quick scan, we have focussed on the biodiesels that are likely to be used within the next couple of years in the Netherlands. Thus, information was collected on FAME and HVO and we did not extensively reviewed other types of biodiesel. Due to the limited toxicological data the hazard assessment based on toxicity studies is mainly based on rapeseed-derived FAME biodiesel and some soy studies as data on FAME from other feedstock were not available. Currently, fossil diesel fuel is already blended with biodiesel. For FAME-blends the technical fuel specification (EN590) specifies a maximum FAME content of 7 vol% since 2009 mainly due to the technical limitations for light duty vehicles applying diesel particulate filter technology. Therefore, the in this quick scan described hazard assessment is limited to a maximum of 7 vol% FAME usage for light duty vehicles. In practice, the 7 vol% restriction does not apply for heavy duty vehicles up to Euro V. For Euro VI an extended type approval for the higher blend would be necessary. HVO can generally be blended up to some 30 vol% within the fuel specification due to its composition and its hazard has been assessed in this quick scan for blends up to 30 vol%.

The published information on the physicochemical and toxicological characteristics is of a fragmented nature only and results are often not comparable between studies due to differences in design. Very few studies have measured the physicochemical properties of whole exhausts of an engine fuelled with various percentages of a particular biodiesel blend. As to toxicological data even less studies are available. Blend comparison could only be made within a single study. Due to these limitations as to available data the conclusions as drawn in this quick scan are indicative only. This is inevitable given the incompleteness of the current information.

The lack of emission studies for animal fat-derived FAME and the scarcity of emission data for HVO are important limitations for the hazard assessment as described in this report.

This quick scan does not provide estimates concerning the increase or decrease in health and environmental risks since the impact on (outdoor) concentration of air pollution has not been considered.

6 Recommendations

Based on the pure paraffinic hydrocarbon structure of HVO without aromatics or oxygen groups HVO is expected to result in cleaner engine emissions and might be preferred above FAME. However, there are insufficient studies to support this expectation and definitely insufficient evidence to make a similar statement on the hazard of the exhaust.

There is a clear need for data on the emission of primary and the formation of secondary ultrafine particles as those are generally believed to be a higher threat for human health than the larger sized (PM_{10}) particles).

Likewise we do not know to what extent biological contamination from micro-organisms may influence the toxicity of the exhaust; there are indications that bacterial growth in biodiesel can result in highly toxic endotoxin production which is still detectable in engine exhaust.

It is virtually impossible to analyse all chemical emissions from an engine in order to estimate the hazard of the mixture of biodiesel blends and petroleum diesel. In addition, to clarify the benefits of biodiesel usage, hazard assessment of biodiesel blends and pure fossil fuel needs to be performed in studies in which blends of biodiesel and pure fossil fuel are compared. However, the lack of standardization of engine emission generation and characterisation and toxicological tests is a major impediment when comparing the various study results. Therefore, it is recommended that a more systematic evaluation will be commissioned in which engine, test conditions and measures for physicochemical composition and the toxicological profile of diluted engine exhaust will be assessed to allow proper comparison among various tests and studies. This includes toxicological test using diluted engine exhaust and not only the often used particulates or even the chemicals extracted from the particulates. Particular attention should be paid to unregulated constituents such as polycyclic aromatic hydrocarbons and carbonyl-containing chemicals (aldehydes) as well as ultrafine particles and substances from microbial origin such as endotoxins. In addition, tests have to be performed with both light and heavy-duty vehicle engines under conditions that are representative for normal daily operations as well as for locations where populations will be exposed e.g. urban areas.

Although the general profile suggests that the use of biodiesel (primarily FAME from rapeseed up to 7 vol% and HVO up to 30 vol%) in the Netherlands in combination with tighter emission standards will result in lowering of (harmful) emissions, it is recommended that the results of this quick scan will be extended by exposure modelling to allow a risk characterization (increase of decrease) for the population.

7 References

Brito JM, Belotti L, Toledo AC, Antonangelo L, Silva FS, Alvim DS, et al. Acute cardiovascular and inflammatory toxicity induced by inhalation of diesel and biodiesel exhaust particles. Toxicol Sci., 2010;116(1):67-78.

Bunger J, Krahl J, Schroder O, Schmidt L, Westphal GA. Potential hazards associated with combustion of bio-derived versus petroleum-derived diesel fuel. Crit Rev Toxicol., 2012;42(9):732-50.

Fukagawa NK, Li M, Poynter ME, Palmer BC, Parker E, Kasumba J, et al. Soy biodiesel and petrodiesel emissions differ in size, chemical composition and stimulation of inflammatory responses in cells and animals. Environ Sci Technol., 2013;47(21):12496-504.

Gerlofs-Nijland ME, Totlandsdal AI, Tzamkiozis T, Leseman DLAC, Samaras Z, Lag M, et al. Cell toxicity and oxidative potential of engine exhaust particles: impact of using particulate filter or biodiesel fuel blend. Environ Sci Technol., 2013;47(11):5931-8.

Jalava PI, Aakko-Saksa P, Murtonen T, Happo MS, Markkanen A, Yli-Pirilä P, et al. Toxicological properties of emission particles from heavy duty engines powered by conventional and bio-based diesel fuels and compressed natural gas. Particle and Fibre Toxicol., 2012:37.

Kooter IM, van Vugt MATM, Jedynska AD, Tromp PC, Houtzager MMG, Verbeek RP, et al. Toxicological characterization of diesel engine emissions using biodiesel and a closed soot filter. Atmos Environ., 2011;45(8):1574-80.

Kooter IM, Jedynska AD, Kadijk G, van Zyl PS, Verbeek RP, Koornneef GP. Projected impact of biodiesel on road transport emissions up to 2030 Background report for the RIVM-TNO report "Impact of increasing the blend ratio of biodiesel on engine emission associated toxicity – a quick scan by RIVM and TNO". TNO report, 2014:R10715 1-47

Schröder O, Bünger J, Munack A, Knothe G, Krahl J. Exhaust emissions and mutagenic effects of diesel fuel, biodiesel and biodiesel blends. Fuel., 2013;103:414-20.

Steiner S, Czerwinski J, Comte P, Popovicheva O, Kireeva E, Müller L, et al. Comparison of the toxicity of diesel exhaust produced by bio- and fossil diesel combustion in human lung cells invitro. Atmos Environ., 2013;81:380-8.

Yanamala N, Hatfield MK, Farcas MT, Schwegler-Berry D, Hummer JA, Shurin MR, et al. Biodiesel versus diesel exposure: Enhanced pulmonary inflammation, oxidative stress, and differential morphological changes in the mouse lung. Toxicol Appl Pharmacol., 2013;272(2):373-83.

Annexes

Annex 1: Search strategy for physicochemical data

A literature search was performed in ScienceDirect using keywords ‘non-regulated’ or ‘unregulated’ in combination with ‘biodiesel’, ‘emissions’, and individual components like: ECOC, CO, BTEX, NO_2, 1-3 butadiene, dioxin, metal, svoc, furna, PAH, nitro-PAH, oxy-PAH, aldehydes, carbonyls in combination with ‘biodiesel’ and ‘emission’. In addition, for regulated components the extensive TNO literature database was checked for references and the NEA, EEA and VTT websites were visited. More information in Kooter et al (2014)

Annex 2: Search strategy for hazard assessment based on toxicological studies

Database: MEDLINE
1 (biofuel* or biodiesel* or bio-diesel or bio diesel or alternative fuel* or soybean oil* or rape* oil* or plant oil* or vegetable oil* or hvo or fame? or fatty methyl ester?).tw,ot,kw. (13361)
2 exp Biofuels/ (3339)
3 exp Plant Oils/ (23913)
4 1 or 2 or 3 (35622)
5 ((emission* or exhaust*) adj3 (biodiesel* or diesel*)).tw,ot,kw. (2585)
6 exp Vehicle Emissions/ (6847)
7 4 and (5 or 6) (195)
8 *transportation/ or exp motor vehicles/ or automobiles/ or ships/ (21991)
9 (vehicle* or diesel engine* or automobile* or automotive or road or traffic).tw,ot,kw. (133222)
10 (emission* or exhaust*).tw,ot,kw. (160177)
11 4 and (8 or 9) and 10 (196)
12 Inhalation Exposure/ (6270)
13 (lung? or pulmonar* or respir* or inhal* or carcinogen* or cancer* or genotox* or mutagen* or neurotox* or inflammation or oxidative stress or cytotox* or mortal* or lethal* or letal* or morbid* or injur* or cardiovascular* or epidemiolog* or toxic* or expos* or health* or hazard* or safety or risk?).mp. (7831652)
14 (7 or 11) and 13 (101)

Number of hits are indicated between ()

Database: Scopus
(TITLE(biofuel* OR biodiesel* OR (bio W/2 diesel*) OR alternative-fuel* OR soybean-oil* OR rape*-oil* OR plant-oil* OR vegetable-oil* OR hvo OR fame? OR fatty-methyl-ester?)) AND (TITLE-ABS-KEY(emission* OR exhaust*)) AND (TITLE-ABS-KEY(vehicle OR diesel-engine OR automobile OR road OR traffic OR transport*)) AND ((TITLE-ABS-KEY(lung OR pulmonar* OR respir* OR inhal* OR carcinogen* OR cancer* OR genotox* OR mutagen* OR neurotox* OR inflammation OR oxidative-stress OR cytotox* OR cardiovascular* OR epidemiolog*) OR TITLE(toxic* OR expos* OR health* OR hazard* OR safety OR risk?)))
Number of hits are indicated ref’s
TITLE(lung OR pulmonar* OR respir* OR inhal* OR carcinogen* OR cancer* OR genotox* OR mutagen* OR neurotox* OR inflammation OR oxidative-stress OR cytotox* OR cardiovascular* OR epidemiolog*)
Number of hits are indicated 40

RIVM database: biofuels
Biodiesel and health or toxicity or toxicology or cytotoxicity or genotoxicity or mutagenicity or inflammation or oxidative stress or in vitro

References retrieved from the different databases were combined (75 hits) and references dealing with emissions only, non-rapeseed feedstock (for in vitro studies only), ecotoxicology or references that did not include blends were excluded (15 hits).

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