

Review of Revised

"Staff Report: Multimedia Evaluation of Biodiesel"
Prepared by the Multimedia Working Group

Tracey Holloway, Ph.D.
Nelson Institute for Environmental Studies
University of Wisconsin--Madison
April 6, 2015

Please find below my review of the revised staff report entitled, "Staff Report: Multimedia Evaluation of Biodiesel," prepared by the Multimedia Working Group (MMWG). This review takes into account the full report and appendices, as well as my earlier review (submitted January 2014), the comments of other reviewers, and the responses of MMWG to these earlier review recommendations.

The new report includes updates to air quality and public health discussions, based on new biodiesel studies and publications, as well as revisions based on the earlier reviewer comments. My expertise relates to air quality and public health impacts of air pollution, which is the requested focus of this review. So, my comments below focus on both air quality and public health.

This review follows the scientific conclusions outlined in Attachment 2 (from the January 21, 2015 letter from Jim M. Aguila to Gerald W. Bowes requesting for supplemental external peer review) "Description of Scientific Conclusions to be Addressed by Peer Reviewers."

1. Air Emissions Evaluation

The conclusion that "with in-use requirements biodiesel does not pose a significant adverse impact on public health or the environment from potential air quality impacts" is supported by the analysis of the Air Resources Board evaluation and discussion in the Biodiesel Staff Report.

This conclusion is based on an analysis of regulated air emissions, toxic air contaminants, greenhouse gas emissions, and ozone precursors. All types of emissions decrease except NO_x, and even then only in heavy-duty vehicles that do not meet newer emissions standards.

Overall, the findings of the air emissions evaluation are well supported, and the revisions to the document have addressed my earlier review concerns. There are still a few points of clarification/correction that would ensure a clear and correct summary of the ARB analysis. These are noted below.

- a. Section 1 has been retitled "Air Emissions" rather than "Criteria Pollutants." This is an improved characterization of the associated content, which has also been strengthened and clarified. However, given that "air emissions" refers to all emissions (criteria, toxic, and greenhouse gas), it seems the breakdown in subsequent sections -"Toxic Air

Contaminants," "Greenhouse Gas Emissions," and "Ozone Precursors" - are all included in the "Air Emissions" topical category. In fact, there exists quite a bit of redundancy among all three sections, confusing an already complex issue.

I appreciate that there are a range of considerations when structuring this type of report, and I offer one possible categorization that reflects the depth and content of material in the report:

- "Health-Relevant Air Emissions" -- everything currently in "Air Emissions" except the paragraphs on CO₂ and fuel consumption + the brief content of "Toxic Air Contaminants"
- "Greenhouse Gas Emissions" (or "Climate-Relevant Air Emissions") -- Same as current Section 2, along with the paragraphs discussing CO₂ emissions and fuel consumption from Section 1.
- "Secondary Air Pollutants" -- Similar to the current Section 4, expanding the discussion on ozone formation, and possibly also noting issues in secondary particulate formation.

Whether these categories or another, I would suggest an overall structure that clarifies the two separate goals of air emission controls: health protection and climate change mitigation. Overall, a clearer separation of health emissions from climate emissions will minimize the risk of confusion on behalf of readers (for example, why an LCA makes sense for CO₂ but less so for NO_x).

- b. Section 1 is greatly improved, noting quantitative changes in emissions as a function of biofuel blend level and engine type. Most results are noted for the 2006 Cummins engine. It would be helpful to know why this is used as the benchmark for most pollutants (it is fine to report results from only one test vehicle, but the rationale for this reporting should be mentioned). The authors note the 2006 Cummins engine in most paragraphs, but omit this detail in paragraph 5 (CO emissions). It could be clearer to include a new paragraph noting that all results are from the 2006 Cummins, and then remove this detail in discussing the pollutants individually.
- c. As noted above, it seems to me that CO₂ and fuel efficiency would fit better in Section 2 than in Section 1. Such an edit would also build consistency with the final paragraph of Section 1 comparing CARB results to the U.S. EPA biodiesel exhaust emissions (for PM, CO, and HC).
- d. Section 2 discusses "Toxic Air Contaminants." As noted above, most of this material would fit better in Section 1. (It is worth noting that currently, paragraph 1 of Section 1 mentions toxics analyses, so if the two sections are not combined, then the mention of toxics should be removed from Section 1).
- e. Section 2, last sentence begins "Genotoxicity assays ..." This sentence does not belong in the air quality section (rather, in belongs in Section C on health). This sentence also appears to be at odds with Section C in terms of relative toxicity.

- f. Section 3 is excellent with no major revisions to suggest. I have two considerations that may further strengthen this discussion: 1) It might be useful to note that chemicals are classified as GHGs because they absorb long-wave radiation and heat up the atmosphere. This is quite different from the health-relevant pollutants, which are reactive and associated with adverse health outcomes. 2) The authors might also note that the GHGs have a long atmospheric residence time -- about 10 years for CH₄, over 100 years for CO₂. The lack of reactivity (i.e. local health impact) and long atmospheric lifetime are fundamental in defining why an LCA methodology is appropriate for GHGs but not for most other pollutants.
- g. As noted, the discussion of CO₂ emissions and fuel efficiency from Section 1 would fit better with the contents of Section 3.
- h. The introductory paragraph for Section 4 "Ozone Precursors" should be rewritten to clarify the health-relevance of ozone control. Currently, the paragraph focuses on the role of ozone as a GHG. However, state controls on these pollutants will have no impact on climate; even global controls on NO_x and THC would have no effect on the climate, given atmospheric chemical processes and interactions among ozone chemistry and methane¹. The importance of ozone, and the discussion of NO_x and THC emissions associated with biodiesel, is due to its impacts on public health and agriculture. As written, the paragraph seems to miss this key point.
- i. As noted above, it makes little sense to focus on ozone as a GHG in Section 4. However, to the degree that this point is included, it should be aligned with the discussion of GHGs in the introduction of Section 3.
- j. Most of the content in Section 4 currently focuses on NO_x emissions. Most of this material has already been presented in Section 1. The value of a stand-alone section on ozone (possibly combined with other secondary pollutants like nitrate PM and secondary organic aerosol) is to discuss how the emission changes in Section 1 impact the abundance of health damaging pollutants in the air.
- k. The authors have included some useful background information on ozone formation. This section could be strengthened if the authors explicitly linked ozone abundance to biodiesel emission changes. For example, where/when in California is ozone production limited by NO_x versus THCs?
- l. If the report does not expand the discussion on expected outcomes for ozone from biodiesel combustion, then I am not sure that a section is needed on this topic. It may be enough to discuss NO_x and THC emissions in the existing Section 1.

2. Public Health Evaluation

¹ See Fiore, et al., "Linking ozone pollution and climate change: The case for controlling methane" *Geophysical Research Letters*, 29, 2002.

The conclusions that "PM from biodiesel combustion emissions is more potent than PM from petroleum diesel combustion emissions ... per mass of PM, [but] less potent ... when the comparison is made on a per mile basis" does not seem to be well supported by the Office of Environmental Health Hazard Assessment (OEHHA) and discussion in the Biodiesel Staff Report.

Overall, the toxicity issue is complex and uncertain. The OEHHA report states "In conclusion, OEHHA cannot determine with certainty whether replacing PD by BD or PD-BD blends for on-road motor vehicle use will reduce adverse health impacts..." To me, this is the heart of the conclusion, and - while restated on p. 13 - is not clear in the "Plain English Summary of the Revised Biodiesel Multimedia Evaluation" (Attachment 1 from the January 21, 2015 letter from Jim M. Aguila to Gerald W. Bowes requesting for supplemental external peer review) nor in the "Public Health Evaluation" conclusions put forward in the "Description of Scientific Conclusions to be Addressed by Peer Reviewers" (Attachment 2 from the January 21, 2015 letter from Jim M. Aguila to Gerald W. Bowes requesting for supplemental external peer review).

I recommend that this section be significantly revised to clarify the uncertainty in toxicity. A few specific recommendations relate to this point:

- a) Paragraph 2 ("A number of studies found...") and paragraph 3 ("The data from recent in vitro and in vivo animal studies indicate...") seem to be saying almost exactly the same thing. Many of these same ideas appear again in paragraph 4 ("The types of published studies evaluating potential toxicity of biodiesel versus petroleum diesel emissions include both in vitro.. and in vivo.. animal exposures."), and paragraph 5 ("Some, but not all, of the more recent studies in 2013 and 2014 raise concerns..."). This whole discussion should be edited for clarity.
- b) Overall, the findings on toxicity do not allow a straightforward conclusion, nor do they support an apples-to-apples comparison with each other. Although the science is inconclusive, the writing about the science should be clear. The report should clarify what is known, what is not known, and where results conflict.
- c) On p. 16, "Conclusions on Public Health Impact," should be much shorter and to-the-point. Currently, these conclusions span five long paragraphs, whereas other sections summarize conclusions in 1-3 short paragraphs or bullets. The authors should identify the main points on public health impact, and state them succinctly.
- d) Content in the conclusions (p. 16-17) should align more closely with the content of this section. At present, the first paragraph mentions CO₂ and air emissions that seem better suited to the Air Emissions Impact section.
- e) The inclusion of CO₂ could be misleading, since this section is focused on chemicals that exert a direct health impact, and CO₂ does not (it does have health implications through climate change, but these would require at least a paragraph to discuss with respect to health). Similarly, the last paragraph in this section (beginning "In summary, ... ") should also omit the reference to greenhouse gas emissions. That sentence could be

misinterpreted to suggest that greenhouse gases impact cancer ("...OEHHA indicates a reduction in cancer risk from the use of biodiesel, and a reduction in greenhouse gas emissions, which ...").

- f) On p. 17, "Conclusions on Public Health Impact," restates the extended (and unclear) discussion on PM toxicity from biodiesel. Given that these are the conclusions, the main point should be put forward clearly - a sentence or two on what is known, a sentence or two on what is not known, and where results disagree.

3. Multimedia Working Group Recommendations

The recommendations of the MMWG are in line with the scientific evidence with respect to air quality and public health.

4. Big Picture

Overall, the staff report is carefully constructed, and makes use of sound science.

- a) "[A]re there any additional scientific issues ... not described above?"

The main issue where additional analysis would strengthen this staff report relates to the air quality impacts of emissions changes, and my suggested changes to Section 4 of the air quality discussion. The report treats emissions very carefully, and this may be sufficient for the context of this report. However, the relationship between emissions and air quality is not straightforward, especially with respect to ozone formation. While both NO_x and hydrocarbons are needed to create ozone, a reduction in one or the other may or may not reduce ozone. In fact, in highly polluted urban areas, a reduction in NO_x can increase ozone. There is tremendous expertise at ARB on the factors controlling ground-level ozone in California. It would be valuable to know how changing the relative emissions of hydrocarbons and NO_x would be expected to affect exposure to ozone across the state.

On a related point, no discussion is provided on the impact of gas-phase vehicle emissions on secondary particulate formation. Even a qualitative discussion on this point would round out the discussion on ozone and provide a more complete framing of air quality impacts.

By extending the discussion of air emissions to ambient concentrations, the report would also strengthen its discussion of health impacts. Currently, health outcomes are linked directly to emissions changes. However, the true health impact depends on where emissions are released, how they are processed in the atmosphere, and what local populations are exposed.

- b) "Taken as a whole, are the conclusions... based upon sound scientific knowledge, methods, and practices?"

Yes, overall the report faithfully represents the state of scientific understanding on the environmental and health impacts of biodiesel.

Gerald Bowes, PhD
Manger, California Environmental Protection Agency
Scientific Peer Review Program
Office of Research, Planning, and Performance

April 3, 2015

Dr. Bowes,

Thank you for the opportunity to re-review the CARB biodiesel diesel report. I am pleased to see that my comments from the first review have been satisfactorily addressed. The following review is based on the new version of the report and specifically addresses only those portions of the report that have substantially changed.

Assessment of specific conclusions

1. Air Emissions Evaluation

Air Resources Board (ARB) staff concludes that with in-use requirements biodiesel does not pose a significant adverse impact on public health or the environment from potential air quality impacts. ARB staff completed a comparative air quality assessment of lower biodiesel blends relative to diesel fuel meeting ARB motor vehicle diesel fuel specifications (CARB). ARB staff updated their evaluation, revised the air quality impact summary, and made conclusions based on their assessment of new emissions test results and air quality data. (Revised Biodiesel Staff Report, Chapters 2 and 3)

I find that this conclusion of the report is based on sound scientific knowledge, methods, and practices. This conclusion is especially true given that newer diesel engines have modifications such as Selective Catalytic Reduction (SCR) which further limit NO_x emissions. The engines used in the emissions studies were older and did not include these engine modifications that have been required by EPA since 2010. These modifications will result in no increase in NO_x emissions for biodiesel versus regular diesel. This is important because NO_x was the only air pollutant to display increased emissions from bio vs regular diesel. This insight needs to be fully integrated into the remaining sections of the report, as noted below. It would be helpful to provide some statistics on the number of new versus old diesel trucks on the road and the replacement rate. The EPA's numbers on this (Fleet Characterization Data for MOBILE6) could be used to estimate how long biodiesel will have an impact on NO_x emissions before the new engines dominate the on-road heavy truck fleet. From what I can gather, this will be about 10-15 years from when the new regulations went into effect in 2010.

2. Public Health Evaluation

After reviewing scientific literature that compares the physical and chemical nature of combustion emissions from diesel engines fueled with biodiesel to the composition of combustion emissions from engines fueled with petroleum diesel, Office of Environmental Health Hazard Assessment (OEHHA) staff concludes that replacing petroleum diesel with an energy-equivalent amount of biodiesel will decrease emissions of particulate matter (PM), benzene, and ethyl benzene but may increase emissions of oxides of nitrogen (NO_x). From studies comparing the biological impacts of biodiesel combustion emissions to those of petroleum diesel combustion emissions, OEHHA staff concludes that PM from biodiesel combustion emissions is more potent than PM from petroleum diesel 2 combustion emissions in eliciting certain responses associated with inflammation and oxidative stress when biological responses per mass of PM are compared. However, in a study carried out at the University of California, Riverside and University of California, Davis, PM from combustion of soy-derived biodiesel is less potent in eliciting the responses associated with inflammation and oxidative stress than is PM in petroleum diesel combustion emissions when the comparison is made on a per mile basis. OEHHA staff reviewed scientific literature that compares the physical and chemical nature of combustion emissions from diesel engines fueled with biodiesel to the composition of combustion emissions from engines fueled with petroleum diesel. OEHHA staff updated their evaluation, revised the public health summary, and made conclusions based on their review of combustion emissions data. (Revised Biodiesel Staff Report, Chapters 2 and 3)

I find that this conclusion of the report is based on sound scientific knowledge, methods, and practices. The new literature review about biological responses to emissions has introduced quite a bit of new information. This has yet to be fully integrated with the rest of the report, as noted in the 'specific comments' section below, but this is a matter of style, not of substance. The report concludes that the increased adverse health effects of particulate matter that are occasionally reported are offset by the decreased PM emissions from biodiesel. I agree. In the report, it would help to quantify this as much as possible. For example, B100 resulted in a 64% decrease in PM emissions, and the biodiesel PM on a mass basis caused an approximate doubling in the health impacts in some studies. Therefore mathematically the reductions in PM emissions entirely offset the increase in adverse health effects.

The phrase here 'may increase emissions of oxides of nitrogen (NO_x)' should be followed by the caveat 'but only for older heavy truck engines without SCR'.

3. Multimedia Working Group Recommendations

The MMWG recommends that the California Environmental Policy Council (CEPC) find that the use of biodiesel, as specified in the biodiesel multimedia evaluation, does not pose a significant adverse impact on public health or the environment. Based on the MMWG's conclusions in Chapter 3 of the revised Biodiesel Staff Report, the MMWG proposes recommendations to the CEPC. (Revised Biodiesel Staff Report, Chapter 4)

I find that this conclusion of the report is based on sound scientific knowledge, methods, and practices. The MMWG has evaluated the air, water, public health, and soil and hazardous waste impacts of biodiesel and has found no adverse impacts compared to CARB diesel. In addition, they have addressed the various other issues raised by the reviewers to my satisfaction. This is a comprehensive review and this conclusion can therefore be stated with a high degree of certainty.

4. Big Picture

As noted above, there are two general issues running through the report. First and most obvious, the new literature review about biological responses to emissions has introduced quite a bit of new information. This has yet to be fully integrated with the rest of the report, as noted in the 'specific comments' section below. Second, there is mention in at least two places that the engines used in the emissions studies were older and did not include the engine modifications that have been required by EPA since 2010. These modifications will result in no increase in NOx emissions for biodiesel versus regular diesel. This is important because NOx was the only air pollutant to display increased emissions from bio vs regular diesel. This insight needs to be fully integrated into the remaining sections of the report, as noted below.

Specific comments

Section 0

On page 8 it is noted that the 2007-2009 model year engine represented the latest technology that was available at the time of testing. Three paragraphs later, the increasing trend in NOx emissions is discussed. It would be helpful to put these increase NOx emissions in context as was done in section C Page 19. Overall, I am left confused. Please clarify. Are the new engines that have become available since 2010 going to be subject to the proposed ADF regulation so that they will produce less NOx or not? Page 16 of the section specifically mentions the proposed ADF regulation, but section C page 19 only discusses newer model cars and doesn't specifically say anything about the ADF regulation. Later, on page 16 under the public health impacts, it is stated that biodiesel may increase NOx emissions. This seems to be a case of the Air Group not communicating with the Public Health Group. All parties should get into agreement on this issue. This point is important because NOx emissions are the only ones that seem to increase with the use of biofuels.

Page 17. The summary of the new review of papers on biological responses to emissions in section E is good. The reviewers seem to have provided a real service here by pointing out some omissions in the literature review. The summary here is excellent and now more completely characterizes the possible adverse health effects of biodiesel and how they are offset by lower PM emissions.

Section C

Page 9 Please describe what changes have been adopted in the new diesel engines and state how they would likely effect emissions.

Page 19 notes that the new SCR systems and light and medium duty trucks do not experience increases in NO_x due to biodiesel. It would be helpful to put this in context. How many vehicles and what fraction of emissions fall under the categories of old trucks vs new trucks vs light and medium duty trucks. My understanding is that all new trucks required selective catalytic reduction as of 2010. Same comment applies to page 25.

Section E

This section significantly updates and expands the literature review on the toxicity of the emissions for diesel engines using regular diesel and biodiesel. The reviewers reach the conclusion that they cannot determine with certainty whether replacing petroleum diesel with biodiesel or blends for on road motor vehicle use will reduce adverse human health impacts attributable to oxidative stress and inflammation from toxic chemicals and diesel engine emissions. This is not the same thing as saying that there will be no adverse increase. In other words they seem to be saying that they cannot determine with certainty that there will be any change. Wording is important here. I think the 'no change' wording is preferable.

Page 17 there appears to be a typo. I believe this is supposed to be a blend of 50% PD and 50% BD.

Section G

Page v. Typo in the spelling of the word "entirely"

Page viii. Misspelling of the word "alleviate"

Page of VI again the issue of NO_x emissions comes up. It is important to fully integrate this information into all sections of the report. It should be stressed that any increase in NO_x emissions was found only for older diesel engines. Again, it is my understanding that diesel engines produced later than 2010 must include selective catalytic reduction. This will cause the NO_x emissions overall be lower or unchanged versus regular diesel.

Page VI bottom bullet. It says that tier 2 air emissions test results show a general trend in *decreasing* emissions of formaldehyde. Later it says "If formaldehyde emission *increases* are real..." Is one of these mistaken? The studies sponsored by CARB showed no change in carbonyl emissions. Only the literature studies sometimes show increases in for formaldehyde. This paragraph is therefore very confusing. Language must be clarified here.

Page VI second bullet. The type of biodiesel feedstock and conventional petroleum diesel can influence these emissions. This paragraph needs to also note that the newer engines with NO_x emissions controls will not have increased NO_x emissions.

Page 5. Second paragraph. "Preliminary tests of biodiesel emissions indicate that... NO_x emissions may increase." I would add the phrase "in older heavy truck engines without selective catalytic reduction."

Page 21. The sentence "it is important to realize that much is unknown about the full implantation [presumably they mean 'implementation'] an emerging transportation fuel system

and will remain uncertain until the fuel system was created." The sentence does not make sense and needs to be rewritten.

Page 23 second paragraph. "NOx emissions may increase for certain biodiesel blends..." I would add the phrase "in older heavy truck engines without selective catalytic reduction."

Same issue later on this page where it says the "increased release of nitrogen oxides during biodiesel combustion for some blends, B20 or higher." I would add the phrase "in older heavy truck engines."

Page 23 fifth paragraph. "Tier II air emissions results show a general trend in *decreasing* emissions in formaldehyde..." Note that "decreased" is misspelled. Later in the same paragraph it says "If formaldehyde emission *increases* are real..." This is the same wording that was used in the previous section and again needs to be corrected. It is not clear whether formaldehyde emissions are increasing or decreasing.

Page 24, the word "additives" in the last sentence of the next-to-last paragraph is misspelled.

Page 25 the first sentence in section 4.2.6 "because materials compatibility issues..." is missing some commas or something it doesn't make sense. Please rewrite.

Page 28 middle of the page "multimedia" is misspelled

Sincerely,



Lisa Rodenburg

Restatement of Objectives –

External peer review of the revised (i.e., March 2015) CalEPA Multimedia Working Group (MMWG) Staff Report *Multimedia Evaluation of Biodiesel* and its associated appendices. This review focusses primarily on the *Public Health Evaluation* of the Office of Environmental Health Hazard Assessment (OEHHA), and Staff Report Appendix E (i.e., OEHHA examination of the potential for oxidant-mediated toxicity of biodiesel exhausts). The review also scrutinised Staff Report Appendices I and J (i.e., peer reviewer comments from February 2014 and MMWG responses).

Brief recap of MMWG's four main conclusions regarding Public Health Impact.

1. Based on the information presented in the evaluation, the substitution of biodiesel appears to reduce the amounts of PM, benzene, ethyl benzene, and PAHs into the atmosphere. However, biodiesel may increase NO_x emissions.
2. Biodiesel may produce higher emissions of some toxic DE constituents such as 1,2-naphthoquinone and acrolein, and increase the proportion of PM emissions that are ultrafine (<100µm).
3. The data presented in recent *in vitro* and *in vivo* studies indicates that biodiesel emissions can induce enhanced oxidative stress and inflammatory responses relative to petroleum diesel emissions (based on comparisons of responses primarily expressed per unit PM mass). This may be offset by lower biodiesel emission rates of PM and PM constituents (e.g., PAHs). Generalisation is complicated by the fact that published studies examined a variety of engines, fuel formulations and test cycles. Further research is warranted to determine whether the increased PM-associated cytotoxicity of biodiesel emissions might outweigh the beneficial reductions of the emission rates of PM and PM-associated toxicants such as PAHs.
4. Switching from petroleum diesel to biodiesel is likely to reduce cancer risks since biodiesel emissions contain significantly lower concentrations of PM, PAHs and benzene. These are well characterised carcinogens and the risk reduction is real. However, the beneficial reduction in the emission rate of carcinogens must be measured against the less certain increase in hazard attributable to PM-induced oxidative and inflammatory stress. In addition, increased NO_x may contribute to adverse respiratory and cardiovascular effects.

This reviewer applauds the MMWG's more careful, judicious consideration of the literature regarding the relative toxicological activity of biodiesel (BD) and petroleum diesel (PD) emissions, and I am pleased to confirm that I support the overall MMWG recommendation that the proposed regulation *does not pose a significant adverse impact on public health or the environment* relative to CARB petroleum-derived diesel. Nevertheless, I do have concerns, comments and criticisms regarding the MMWG's concluding remarks, the revised Staff Report, and the report appendices. For example, I question the MMWG's statement about "real" reductions in cancer risk. I certainly agree that reductions in the emission rates of carcinogens such as PM, PAHs and benzene are well documented, and moreover, that these would presumably translate into reductions in *potential* human hazard. However, risk determination requires knowledge of both exposure and hazard. In essence, we do not have a good handle on either. Actual human hazard will be influenced (i.e., augmented or decreased) by post-emission transformations that will influence the toxicological properties of the emissions (i.e., atmospheric composition). Nevertheless, it is fairly common to simply use the concentrations of noteworthy carcinogens (e.g., PAHs) in complex environmental matrices (e.g., air, soil, etc.), and relative potency factors, to calculate the concentration of a chemical equivalent (e.g., benzo[a]pyrene equivalents) with known carcinogenic potency (e.g., slope factor or unit risk). This can readily be

Name: Paul A. White, PhD

Affiliation: Department of Biology, University of Ottawa, Ottawa, Ontario, Canada

accomplished for diesel exhaust; however, risk determination still requires knowledge about exposure. Actual human exposure will also be influenced by post-emission modifications, as well as the attributes of the receptors (e.g., age, sex, occupation, habits, etc.). In the absence of a detailed risk assessment, or any sort of quantitative risk assessment, it is simply not possible for the MMWG to make statements about human cancer risk, only statements about reductions in the “emission rates of known human carcinogens that would presumably translate into reduced cancer risk”. The difference between this type of statement and “real” risk reductions is important.

Although this review outlines some noteworthy shortcomings of the MMWG’s revised evaluation, it is important to recognise that the revised evaluation contains a far more comprehensive review of available information regarding the comparative toxicological properties of BD and PD emissions (e.g., Appendix E of the Staff Report). Moreover, with respect to effects such as oxidative stress and proinflammatory signalling, the OEHHA Memorandum (i.e., Appendix E) provides a reasonably judicious and balanced description and discussion of the pertinent scientific literature. Nevertheless, this reviewer is obliged to note that the review of the available scientific information is still incomplete. In their response to the first round of reviewer comments (i.e., Appendix J), the MMWG noted that “comprehensive critical review of all studies comparing biodiesel and petroleum diesel emissions would require considerable resources and would be of only limited relevance to California”. I strongly disagree. By my count, there are only about 50 publications that investigated the relative toxicological properties of BD and PD emissions. Of these, only 16 studies investigated the relative ability of BD and PD emissions to elicit changes in markers of oxidative stress and inflammatory signalling in mammalian systems exposed *in vivo* or *in vitro*. With respect to “relevance for the state of California”, although it is true that 15 of the aforementioned 16 studies did not compare BD emissions with CARB-PD emissions (i.e., all but Durbin et al., 2011), almost all the published studies examined ULSD emissions, and the results should be comparable to CARB-PD. As far as this reviewer can tell from the documents provided, CARB diesel is a low sulfur light or middle distillate (i.e., ULSD). In fact, in Appendix J the MMWG notes that the terms “CARB diesel”, “petroleum diesel”, “conventional petroleum diesel”, and “CARB ultralow sulfur diesel” can be used interchangeably. I would be far more concerned about variations in biodiesel feedstocks and the characteristics of the fuel blends examined than differences in the properties of the ULSD. Moreover, it seems paradoxical that only some of the 16 studies that examined oxidative and inflammatory markers would be deemed relevant for the MMWG evaluation (i.e., included in Appendix E). In essence, since the properties of the combustion emissions may be affected by engine type, exhaust aftertreatment, fuel formulation, test cycle, sample collection and handling, and exposure regime, I would expect that the MMWG would want to examine and evaluate all the available information.

In keeping with the obligation to base the MMWG staff report on “sound scientific knowledge”, this reviewer felt obliged to scrutinise all published studies that examined the relative ability of biodiesel and petroleum diesel emission to elicit changes in markers of oxidative stress and inflammation. This was essential to critically assess the strength of the evidence regarding the ability of biodiesel emission samples to elicit stronger oxidative stress and inflammatory responses in experimental animals and/or cultured mammalian cells. Although the OEHHA review of the relevant literature (i.e., Appendix E) constitutes a vast improvement over what was presented in the previous MMWG evaluation, the narrative description of available scientific information fails to provide a scholarly, comparative summary that can readily be interpreted from a public health point of view. In this reviewer’s opinion, it is essential to organise the published information such that the *strength of the evidence* can readily be evaluated and summarised. The results of the 16 aforementioned studies, 11 of which were reviewed by OEHHA, are summarised below in Table 1 (*in vivo* studies) and Table 2 (*in vitro* studies). The 16 studies summarised in Tables 1 and 2 examined the relative ability of BD

Name: Paul A. White, PhD

Affiliation: Department of Biology, University of Ottawa, Ottawa, Ontario, Canada

exhaust (i.e., in comparison with PD exhaust), or samples derived from BD exhaust (i.e., DEP or DEP extract), to augment the levels of oxidative stress and/or inflammatory markers in experimental animals or cultured mammalian cells.

Only three *in vivo* studies examined biodiesel effects on murine inflammatory and/or oxidative stress markers [Yanamala et al., 2013; Fukagawa et al., 2013; Shvedova et al., 2013]. All 3 studies showed increases in inflammatory and oxidative stress markers for BD exhaust in comparison with PD exhaust (i.e., ULSD). Only Shvedova et al. (2013) examined animals exposed to diluted exhaust via inhalation (whole body). The other studies examined animals exposed to DEP via intrapharyngeal instillation. All studies set the doses by DEP mass (i.e., the magnitude of the responses reflect the potency per unit PM mass).

Three studies conducted air-liquid interface exposures to diesel exhaust [Mullins et al., 2014; Hawley et al., 2014; Steiner et al., 2013]. Two of these examined markers of oxidative stress (i.e., [Hawley et al., 2014; Steiner et al., 2013]), and both showed some indication of increased responses for BD exhaust relative to PD, with increased responses for increasing blend percentages in one study (i.e., [Steiner et al., 2013]). Two studies examined inflammatory markers [Steiner et al., 2013; Mullins et al., 2014], and both showed some indication of increased responses for BD compared to PD exhaust, with increased responses for increasing blend percentages.

Eight studies examined cultured cells exposed to DE particulates in suspension [Betha et al., 2012; Bhavaraju et al., 2014; Hemmingsen et al., 2011; Ihalainen et al., 2009; Jalava et al., 2010; Jalava et al., 2012; Fukagawa et al., 2013; Durbin et al., 2011]. Of these, only 4 studies showed increases in inflammatory and/or oxidative stress markers for BD particulates compared to PD particulates [Durbin et al., 2011; Fukagawa et al., 2013; Betha et al., 2012; Bhavaraju et al., 2014]. Betha et al. (2012), Fukagawa et al. (2013) and Durbin et al. (2011) documented increases in oxidative stress in cells exposed to BD particulates. Bhavaraju et al. (2013), Fukagawa et al. (2013), and Durbin et al. (2011) documented increases in inflammatory markers in cells exposed to BD particulates. With the exception of Ihalainen et al. (2009) and Durbin et al. (2011), all comparisons are based on responses expressed per unit mass. Ihalainen et al. (2009) also expressed the responses per unit of engine work (i.e., kW-hr), and Durbin et al. (2011) only expressed responses per engine mile. When expressed per unit of engine work, Ihalainen et al. (2009) noted a reduction in inflammatory marker release for RME compared to PD. Although only based on pooled triplicates, Durbin et al. (2011) noted increases in inflammatory and oxidative stress markers for soy-derived B20 relative to PD when expressed as response per mile equivalent (i.e., macrophages exposed to DEP from 2007 MBE4000, UDDS cycle).

Three studies examined cells exposed to organic extracts of DEP [Swanson et al., 2009; Kooter et al., 2011; Gerlofs-Nijland et al., 2013]. Two of these studies examined inflammatory stress via cytokine release, and both noted significant elevation in inflammatory stress markers for cells exposed to extracts of BD particulates compared to PD particulates [Gerlofs-Nijland et al., 2013; Swanson et al., 2009]. Kooter et al. (2011) did not detect differences in *Ho-1* gene expression in cells exposed to BD particulate extract compared to cells exposed to PD particulate extract.

Despite substantial variability across the various studies with respect to engine type, biodiesel feedstock, fuel blending rates, and engine test cycle, all the *in vivo* and *in vitro* ALI studies documented increases in markers of oxidative and inflammatory signalling for BD emissions compared to PD emissions. However, since most of the studies examined exposures expressed per unit particulate mass, it is not clear whether the well documented reduction in biodiesel PM emission rates would adequately compensate for the observed increases in particulate potency. With respect to the *in vitro* studies, only 3 out of eight studies that examined oxidative stress markers showed elevated

Name: Paul A. White, PhD

Affiliation: Department of Biology, University of Ottawa, Ottawa, Ontario, Canada

responses for BD emissions. Similarly, only 3 out of eight studies that examined inflammatory markers showed elevated responses for BD emissions. Two of the three studies that examined organic extracts of DEP noted elevated markers of inflammation for BD emissions. Therefore, although there is some strong evidence in the scientific literature that BD emissions may indeed have an enhanced ability, relative to PD, to elicit oxidative stress and inflammation, there is considerable room for uncertainty regarding the significance of the published findings with respect to the public health impact associated with the use of biodiesel as an ADF. First, because several well conducted studies failed to show enhanced responses in cells exposed *in vitro*; and second because, as noted by the MMWG, it is not clear whether the well documented reductions in PM emission rates can adequately compensate for the increased PM potency observed in some studies. Nevertheless, the relevance of the Durbin et al. (2011) findings to the state of California cannot be ignored. This study, which examined soy- and animal-based BD emissions relative to CARB diesel, does provide some evidence, albeit limited, that biodiesel emissions can elicit elevated oxidative and inflammatory responses in human macrophages exposed *in vitro* (expressed as response per mile equivalent).

In summary, the information presented in Tables 1 and 2 permitted this reviewer to critically examine the **strength of the evidence** regarding oxidative stress and inflammatory effects. In essence, it appears that the MMWG statement in the revised report are sufficiently judicious and balanced. In other words, although there is some need for concern and further study, *the use of BD as an ADF in the state of California will not contribute to any significant adverse impact on public health relative to PD.*

Although the MMWG's updated review of the literature on the toxicological hazards of BD emissions is more comprehensive than that originally conducted, this reviewer still identified 22 relevant publications (see Appendix A, Tables A1, A2, and A3). I can appreciate the MMWG's point of view with respect to the resources required to review all published information; and moreover, the difficulty of interpreting published results in the context of fuels, feedstocks and engines that are relevant to the state of California. However, when dealing with a highly complex agent such as diesel exhaust, where the composition and toxicological properties can vary widely with engine design, fuel formulation, test cycle, biological test system and endpoints, and exposure regime, it is important to review all available information. As an example, the MMWG is referred to IARC Monograph 105 (Diesel and Gasoline Engine Exhausts and Some Nitroarenes). The complexity of the agents evaluated (i.e., diesel and gasoline exhausts) necessitated detailed, comprehensive review of all available information pertaining to genetic and related effects of the agents in humans and experimental systems (see Monograph 105 pp. 327-398).

Interestingly, the publication by Agarwal et al. (2013) summarised in Table A1 provides an indication that the DE emission rate of BaP equivalents in both primary and secondary aerosols is significantly lower for B20 relative the PD. Although the authors of the study did not examine cancer risk, their calculations provide a clear indication of reduced carcinogenic hazard that is relevant to the MMWG's evaluation [Agarwal et al., 2013].

Table A2 summarises five *in vitro* studies in cultured mammalian cells, three of which have already been discussed above (i.e., [Betha et al., 2012; Kooter et al., 2011; Swanson et al., 2009]). The two additional studies investigated the cytotoxicity of biodiesel emissions (per unit mass of DEP). Ackland et al. (2007) noted reduced apoptosis in cells exposed to BD particulates; all blend levels elicited weaker responses compared to PD [Ackland et al., 2007]. Bunger et al. (1998) did not detect any significant differences between extracts of RME particulates and extracts of PD particulates [Bunger et al., 1998a].

Table A3 summarises the results of 18 studies that examined the relative Salmonella mutagenic

Name: Paul A. White, PhD

Affiliation: Department of Biology, University of Ottawa, Ottawa, Ontario, Canada

potency of BD-derived DEP extracts and PD-derived DEP extracts. The endpoint is highly relevant to the MMWG discussions about potential carcinogenic hazard since mutagenicity has been definitively linked, both empirically and mechanistically, to carcinogenesis. Moreover, the results are highly relevant to the MMWG evaluation since numerous studies base their comparisons on mutagenic activity expressed per unit of engine work (e.g., hph, kW-hr, mile equivalent, etc.). Numerous studies have shown that the Salmonella mutagenic potency of BD-derived DEP extracts, expressed per unit of engine work, are significantly lower than that of PD-derived DEP extracts [Bagley et al., 1998; Bunker et al., 2006; Chase et al., 2000; Kado and Kuzmicky, 2003; Krahl et al., 2003; Krahl et al., 2005; Rantanen et al., 1993; Westphal et al., 2012]. In contrast, a smaller number of studies by Krahl et al. noted increased mutagenic potency, expressed per L of exhaust, of BD-derived DEP extracts in comparison with PD-derived DEP extracts [Krahl et al., 2008; Krahl et al., 2007a; Krahl et al., 2009b]. Some authors have noted that increased cytotoxicity and/or genotoxicity of BD-derived samples, expressed per unit mass of PM or per unit of engine work, is driven by the high levels of extractable organic matter associated with BD-derived particulates [Rantanen et al., 1993; Gerlofs-Nijland et al., 2013]. Nevertheless, the weight of evidence for mutagenic potency expressed per unit of engine work *favours the assertion that the mutagenic potency of BD-derived DEP is lower than that of PD-derived DEP*. Interestingly, some studies noted that the mutagenic potency of BD-derived DEP extracts is higher than that of PD-derived extracts when expressed per mg of particulates. However, significant declines in biodiesel PM emissions rates resulted in lower mutagenic emission rates expressed per unit of engine work [Kado and Kuzmicky, 2003]. I would have expected the MMWG to summarise such studies since the results support the hypothesis that the increased toxicity of BD-derived DEP is outweighed by the significant reductions in PM emission rates. Granted, it is difficult to generalise since the studies summarised in Table A3 examined a wide range of engines, fuel formulations, and test cycles. Interestingly, a limited number of studies also noted that the mutagenic potency of some BD-derived DEP extracts, expressed per unit PM mass, are lower than PD-derived extracts.

Detailed comments about specific sections of the reviewed documents:

Staff Report p. 5 – The MMWG mentions several possible biodiesel feedstocks that are expected to be used in California. It is important to note that very little data exists for some of these feedstocks (e.g., trap grease, safflower oil, yellow grease, corn oil and palm oil).

Staff Report, p. 6 – The MMWG notes that UC researchers used the terms “CARB Diesel”, “CARB Ultralow Sulfur Diesel”, and “Conventional Petroleum Diesel” interchangeably. Without any detailed information about the physical-chemical properties of CARB diesel it is difficult for the reader to know if CARB diesel is similar to the ULSD that would be sold and used in other states/countries. In some sections of the documents the MMWG is arguing that the results presented in some published studies may not be relevant for the state of California since the researchers did not compare BD with CARB diesel. However, it seems unlikely that CARB diesel, which appears to be a “typical” ULSD, would yield different results. It is far more likely that the relevance of published studies is adversely impacted by variability in biodiesel feedstocks.

Staff Report, p. 7 – The MMWG notes that soy- and animal-based feedstocks are representative of typical feedstocks in California. It is unfortunate that very little scientific data exist regarding emission rates for animal-based BD in comparison with PD.

Staff Report, p. 8 – Unless I missed it, the MMWG does not mention the aftertreatment for the vehicles/engines examined in the ARB emissions study. If I understand the ARB compliance requirements summary for trucks and buses, all of the engines examined would have some type of DPF. The 2010 engine would presumably have SCR for NO_x reduction. Please clarify. Update: I

Name: Paul A. White, PhD

Affiliation: Department of Biology, University of Ottawa, Ottawa, Ontario, Canada

checked the Durbin et al. report and it appears that the 206 Cummins ISM and 2007 MBE4000 were both equipped with DPF, and the 2010 Cummins ISX15C was equipped with DPF and SCR. Were any equipped with DOC? Was the Caterpillar C-15 equipped with DPF? Aftertreatment information should be presented in the Staff Report.

Staff Report, p. 8 and Appendix C – The emission values are presumably arithmetic means. The authors present the results of statistical comparisons (i.e., p values), but they do not provide any information about how the values were compared; and moreover, whether the statistical comparison are adjusted for multiple comparisons. Please clarify. Why not provide the mean and standard error of the mean (in brackets), using superscripts to indicate the results of statistical comparisons?

Staff Report, p. 9 – In numerous instances the authors comment on statistical significance, but they do not provide any information about statistical methods or the definition of “significance”.

Staff Report, p. 9 – What is “federal diesel”? Why is federal-diesel relevant to the California evaluation, but ULSD in the literature is viewed with skepticism?

Staff Report, p. 9 – “...PM accounts for 70% of the toxic risk”. Please provide citation for this statement.

Staff Report p. 9 – The statements about the genetic toxicity and cytotoxicity analyses in the Durbin et al., (2011) report are confusing and inaccurate. For example, on p. 215 of the Durbin et al. report the authors state “For the animal biodiesel, there appears to be an increase in the A-20 emissions compared to the CARB sample”. In addition, although the results are based on pooled triplicates (i.e., no statistical comparisons), the report presents evidence of increases in markers of oxidative stress and inflammation in human macrophages exposed to BD-derived DEP from the MBE4000 engine. Lastly, it is important for the authors to appropriately differentiate between genetic toxicity and cytotoxicity.

Staff Report, p. 11 - The ability of SCR to effectively reduce elevated NO_x in BD emission is an important finding.

Staff Report, p. 13 – Note that the *in vivo* studies were conducted on experimental animals (i.e., they are not human studies).

Staff Report, p. 13 – Here and throughout the authors need to make sure statements are accurate and precise. For example, “smaller mass of PM” should presumably be “lower PM emission rate”.

Staff Report, p. 13 – Regarding the statement about volatile constituents likely being involved in the oxidative stress and inflammatory responses. Which studies examined volatiles? Just Shvedova et al. (2013)?

Staff Report, p. 17 – The authors mention that results are “complicated by different types of biodiesel and petroleum diesel, as well as engine and workload protocols”. Aftertreatment can also have a significant effect on DE, and this should be mentioned.

Staff Report, page 17 – Final two paragraphs of the public health impact conclusions are substantially improved in comparison with the previous MMWG report.

Staff Report, p. 18 (2e) – Just wondering who monitors the literature for “available information”.

Appendix C, p. 1 – Again, the specifications of CARB diesel are never provided.

Appendix C, pages 2 to 3 and 9 to 10 – What about aftertreatment? DPF for all? SCR for the 2010 model year?

Name: Paul A. White, PhD

Affiliation: Department of Biology, University of Ottawa, Ottawa, Ontario, Canada

Appendix C, p. 11 – As mentioned earlier, if values are arithmetic averages of six reps, why aren't standard error values provided? Plus, no information about the statistical methods employed.

Appendix C, p. 12 – CO² should be CO₂.

Appendix C, pages 14 to 15 – No point listing p values as 0.000. Simply state <0.0001 or similar. Need to indicate how these p values were obtained. Recommend providing mean ± SEM with p values indicated with superscripted symbols.

Appendix C, page 16 – Statement about genotoxicity results is not correct or precise. First, there were both genotoxicity and cytotoxicity analyses. Second, some of the results showed increased genotoxicity for BD relative to CARB diesel (i.e., MBE4000 engine).

Appendix C, p. 16 – What about the higher molecular weight PAHs that include several known or probable human carcinogens? Perhaps the MMWG could include separate statements about the LMW PAHs and the HMW PAHs?

Appendix C, p. 16 – The authors mention mutagen emissions. Presumably this is emission rate. Please provide unit.

Appendix C, page 16 – regarding the statement “mutagen emissions generally decreased”. The results showed decreases for soy-based BD, but increases for AF-based biodiesel. The Durbin et al report explicitly notes that AF-based biodiesel responses are elevated relative to CARB diesel.

Appendix C, page 20 – Here and throughout the report - when mentioning comparisons between BD and PD, the authors are not consistent with respect to mentioning statistical significance. Sometime the text states “statistically significant” differences, sometimes not. For example, on p. 23 the authors outline differences in fuel consumption values, but for soy-based BD they do not state if they are statistically significant. For animal-based biodiesel, they state that difference are not statistically significant, but fail to define *significance*.

Appendix C, page 22 – Define “latest technology”.

Appendix C, p. 23 – Again, statements about toxicity are not accurate or precise.

Appendix E, p. 2 – Statement indicating that OEHHA “cannot determine with certainty” that biodiesel will reduce the likelihood of effects related to oxidative stress or inflammation, is a definite improvement over the earlier version of the MMWG evaluation.

Appendix E, p. 2 to 3 – Seems highly unlikely that comparisons between CARB diesel and other ULSDs would detect any significant differences. Engine type, fuel formulation and blending ratio, biodiesel source and quality control, test cycle, and aftertreatment likely have the strongest influences on the outcome of BD-PD comparisons.

Appendix A of Appendix E (i.e., review notes) – General comments. Although informative and reasonably comprehensive, the narrative summary of published information appears to have been hastily prepared. There are numerous mistakes and inaccurate statements. In addition, as noted earlier, the review is not complete. Please pay attention to units.

Appendix E, page 5 – Bunger et al. (2001) should be Bunger et al. (2000). The MMWG noted that the reviewed study did not describe emissions controls. The MMWG summary of the ARB emissions study also does not describe emission controls. PM extract mutagenicity in what units? Per unit mass or engine work or both? The response unit is crucial because it relates to the MMWG statements regarding the ability of reduced PM emission rates being able to effectively compensate for increased toxicological activity per unit PM mass.

Name: Paul A. White, PhD

Affiliation: Department of Biology, University of Ottawa, Ottawa, Ontario, Canada

Appendix E, page 7 – Karavalakis et al (2009). Since 2007 EN590 fuels are ULSD, so wouldn't they be analogous to CARB diesel? Important for the MMWG to emphasise that although BD can contribute to increases in the emission rates of toxic aldehydes, the available information indicates that the BD emission rates are highly variable across engine type, fuel formulation and test cycle.

Appendix E, p. 14 – Durbin et al (2011). Important to note that the results are expressed per engine mile. This is important.

Appendix E, p. 15 – The statement about IL-8 release in human macrophages is not correct. Although based on combined triplicates (i.e., statistical comparisons not possible), the results show increased responses for soy-derived BD in comparison with PD.

Appendix E, pages 16 to 17 – Please pay attention to units. Gerlofs-Nijland et al. (2013) – 59% PD should be 50%.

Appendix E, p. 18 – Bakeas and Karavalakis (2013). Presumably the TEFs are RPFs for carcinogenic activity. Please clarify.

Appendix E, pages 19 to 20 – Shvedova et al. (2013). This is a particularly important study since it examined whole body DE exposures and noted distinct increases in markers of oxidative stress and inflammation for BD relative to PD.

Appendix E, p. 22 – Westphal et al. (2014) should be Westphal et al. (2013). What is meant by “many more mutations”? Units please. Are the differences significant?

Appendix E, p. 24 – Hawley et al. (2014). The description of the findings is very confusing. The Hawley et al. results show some increase in HO-1 for BD without DPF at 20 minutes, with median values appearing similar at 60 minutes. Thus, at 60mins there is no evidence that BD responses without DPF are greater than PD. With DPF, the results for BD and PD are very similar at both 20 mins and 60 mins. In their narrative summary of the Hawley et al results, the MMWG appears to be commenting on the relative responses of BD and PD, as well as the BD and PD responses compared to their respective controls. The text is confusing.

Appendix E, pages 26 to 29 – Twenty-two relevant studies were not reviewed. Citation format is erratic and there are numerous mistakes. For example, titles of Bhavaraju et al. and Brito et al. papers are the same. Where are Hemmingsen et al., Yanamala et al., and Fukagawa et al?

Appendix J, p. 9 – Regarding the follow-up ARB study of B5/B10 emission, this is an excellent, highly relevant contribution to existing knowledge.

Appendix J, p. 13 – Regarding statements asserting that the greater potency per unit mass is offset by the reduced PM emission rates for BD, some authors have asserted that declines in PM emission rates for BD may not compensate for increased toxicological activity expressed per unit mass. For example, Gerlofs-Nijland et al. (2013) stated “...PM mass reduction achieved by the use of B50 will not necessarily decrease the hazard of engine emissions”. However, it is important to note that with the exception of the Durbin et al. (2011) study, all studies that examined markers of oxidative stress and inflammation express their results per unit mass of PM.

Appendix J, p. 14 – Agree, although the weight of evidence indicates that the toxicological hazard of BD emissions are likely lower than PD emissions, it is prudent to proceed with caution.

Appendix J, p. 15 – For the reasons already described, this reviewer does not agree that a comprehensive review of the relevant literature would require considerable resources and be of limited relevance to California. There are only about 50 publications on the topic, and most examined

Name: Paul A. White, PhD

Affiliation: Department of Biology, University of Ottawa, Ottawa, Ontario, Canada

ULSD relative to BD and/or BD-ULSD blends. If one person (i.e., this reviewer) could review the available publications, surely the MMWG can do the same.

Appendix J, p. 16 – Pleased that the Shvedova et al. (2013) study is highlighted.

Appendix J, pages 17 and 18 – Thank you for the detailed scrutiny of the Brito et al. (2010) findings.

Appendix J, p. 19 – For reasons already stated, this reviewer has problems with the statement “risk reduction is real”.

Appendix J, p. 32 – As noted earlier, there does not appear to be any basis to expect differences between CARB diesel and other ULSDs. Thus, there is no solid foundation for asserting that studies that did not compare BD emissions to CARB diesel emissions are not relevant to the MMWGs evaluation. Far more difficult to evaluate the utility of studies due to variations in feedstock and biodiesel blending, and BD quality control.

Appendix J, p. 44 – Regrettable that the MMWG did not ask UC researchers to revise the Tier I report. Previously noted shortcoming impact their overall quality and utility.

Table 1. Summary of published *in vivo* studies that examined the relative ability of biodiesel emissions to alter levels of oxidative stress and/or inflammatory markers.

Engine	Fuels Examined	Exposure System	Endpoint(s) Examined	Results Obtained	Reference
Isuzu C240 2.369L with DOC, 4 steady state conditions, high volume DEP sampling system.	ULSD and corn-derived FAME.	C57BL/6 mice exposed to DEP via pharyngeal aspiration, 0, 9 and 18 µg total C per mouse as aqueous suspension, sacrifice 1, 7 and 28 days after exposure.	Pulmonary inflammation (by BAL counts & cytokine levels), oxidative stress (by-products of lipid peroxidation), and morphological changes (by histopathological assessment).	Significant elevation in inflammatory markers for FAME relative to ULSD, evidence of increased tissue damage and oxidative stress for FAME relative to ULSD, significant elevation in inflammatory cytokines, chemokines, growth factors for FAME, histological examination showed impaired clearance and retention of FAME particulates.	[Yanamala et al., 2013]
1.9L light-duty Volkswagen, 9-mode steady state cycle, DEP collected on Teflon-coated GFFs.	ULSD, B20 SME	C56BL/6 mice exposed to B0 (ULSD) or B20 DEP via oropharyngeal aspiration (3 consecutive daily 84µg treatments).	Cell counts in BALF, cytokines in BALF and lung tissues, protein carbonyls and GSH in lung tissues.	For B20 versus B0: no differences in BALF cell counts, elevated BALF levels of G-CSF, IP-10 and IL-6, elevated lung tissue levels of G-CSF, IP-10 and IL-6, slight reduction in GSH, and slight elevations in Nrf2 and GCLC.	[Fukagawa et al., 2013]
Yanmar L70 0.32L single cylinder engine, constant load, diluted exhaust to deliver 50, 150 or 500 µg/m ³ .	Unspecified diesel and soy-derived biodiesel (B100)	BALB/c mice whole body exposures, 4h/d, 5 d/wk for 4 wk. Sacrifice 2hr following final exposure.	Following analyses in lung and liver: total protein, LDH, MPO activity, 4-HNE levels, LMW thiol levels, proinflammatory cytokine levels.	Compared to diesel exhaust, B100 exhaust exposures elicited accumulation of oxidatively modified proteins, increase in 4-HNE, reduction in protein thiols, depletion of GSH, dose-related increase in LDH in lung, and increase in MPO in liver and lung. Lung and liver IL-6 elevated for B100 compared to diesel, lung IL-12p70 elevated for B100, liver MCP-1 elevated for B100.	[Shvedova et al., 2013]

Table 2. Summary of published *in vitro* studies that examined the relative ability of biodiesel emissions to alter levels of oxidative stress and/or inflammatory markers.

Engine	Fuels Examined	Exposure System	Endpoint(s) Examined	Results Obtained	Reference
Isuzu 4BD1-T, 3.9L light-duty engine, constant speed and 20% load, air-liquid interface chamber exposure.	ULSD, B100 and B20 RME, PCO (pure canola oil).	NuLi-1 airway epithelial cell line, 10KT cell line, ALI chamber exposure for 1 h.	Apoptosis and cell viability, inflammatory mediators in culture medium.	Significant reductions in apoptosis and viability for B20, B100 and PCO exhaust compared to DE, in both cell types. Significant increases in IL-6 and IL-8, in both cell types, for B20 and B100 relative to DE. Greatest increase in production of inflammatory mediators, relative to controls, in response to B100.	[Mullins et al., 2014]
John Deere 4.5L 4045H PowerTech engine with DOC and DPF, constant speed and 75% load, air-liquid interface exposure in EAVES chamber (electrostatic PM deposition).	ULSD or unspecified B99.	NHBE cells cultured at ALI for 21 days, exposed to DE for 5, 20 or 60 min.	Gene expression of HO-1, CYP1A1, LDH release.	For B99, some indication of increased expression of HO-1 compared with DE for 20min without DPF; however, B99 levels somewhat lower at 60 mins. With DPF, B99 and DE levels comparable. Some indication of increased expression of CYP1A1 at 60 mins with and without DPF.	[Hawley et al., 2014]
1998 Opel Astra X20DTL (1.995L), continuous flow exposure system (air-liquid interface).	DF, RME (B20 and B100)	In vitro 3D human airway epithelial model, 2 or 6 hr exposures at low and high dilution.	Cytotoxicity as LDH release, oxidative stress as GSH, inflammatory response as TNF- α and IL-8, inflammation, necrosis, apoptosis and oxidative stress by gene expression (<i>HO-1</i> , <i>TNF</i> , <i>IL-8</i> , <i>CASP7</i> , <i>FAS</i>)	Some indication of enhanced cytotoxicity and oxidative stress for B100, pro-inflammatory responses weak relative to air control, some indication of reduced inflammatory response for B20.	[Steiner et al., 2013]
Yanmar single cylinder 296mL diesel generator, steady state at rated speed and 4 loads, DEP collected on Teflon® membranes and quartz filters.	ULSD, B100 and B50 (waste cooking oil).	A549 human alveolar adenocarcinoma cells directly exposed to PM on filters for 48 hr.	Cell viability and cytotoxicity, measured via production of fluorescent products, apoptosis as caspase III/VII, oxidative stress as GSH/GSSG ratio (Promega assays).	Cytotoxicity and oxidative stress higher for B100 relative to DF. Similar for apoptosis response. No significant difference between B100 and DF at lower engine loads, and largest difference at higher engine loads.	[Betha et al., 2012]

Engine	Fuels Examined	Exposure System	Endpoint(s) Examined	Results Obtained	Reference
2002 Cummins 5.9L engine (EPA 2004 certified) with common rail fuel injection, EGR, DOC and DPF, steady state operation. DEP collected by “back-flush” of DPF.	DF and B20 (unspecified)	Freshly isolated rat alveolar macrophages exposed to 100-500 µg PM/mL for 24 hr.	Cytotoxicity (LDH release), inflammatory signalling (<i>Cox-2</i> , <i>Mip-2</i> gene expression), and macrophage activation (PGE ₂ release)	No difference in cytotoxicity between DF and B20. Some increased inflammatory signalling for DF. Some evidence of increased macrophage activation for B20.	[Bhavaraju et al., 2014]
Two light-duty diesel engines representing Euro2 and Euro4 standards. DEP collected on quartz filters.	ULSD, B20 RME, B20 AFME	A549 human alveolar adenocarcinoma cells, HUVEC cells, THP-1 cells exposed to 0.78–100µg PM/mL for 3 h.	DNA strand breaks in A549 cells by comet assay, and fpg-assisted comet assay, ICAM-1 and VCAM-1 expression in HUVEC cells, gene expression of <i>CCL-2</i> and <i>IL-8</i> in THP-1 cells.	All samples elicited concentration-related increases in DNA strand breaks and fpg-sensitive sites. RME B20 response lower than ULSD, AFME similar to diesel. With respect to <i>CCL-2</i> and <i>IL-8</i> expression, biodiesel responses similar or lower than DF. Levels of ICAM-1 and VACM-1 somewhat elevated for DF relative to biodiesel.	[Hemmingsen et al., 2011]
Kubota 1.123L D1105-T diesel engine (EPA Tier I), ISO C1 cycle, with or without DOC/POC, DEP collected using HVCI.	ULSD, HVO and RME	RAW264.7 mouse macrophage cells exposed to DEP suspension for 24 h	Production and release of proinflammatory cytokine TNF- α .	At 150 µg/mL decreased response for RME, relative to DF. HVO similar to DF. When based on per kW-hr exposures, reduced response for RME, especially with DOC/POC. Small reduction for HVO, relative to DF, without aftertreatment only. PM emission rates reduced for RME and HVO, relative to DF. Aftertreatment reduced PM emissions rates by 50-60%.	[Ihalainen et al., 2009]
Kubota 1.123L D1105-T diesel engine (EPA Tier I), ISO C1 cycle, with or without DOC/POC, DEP collected using an HVCI with downstream polyurethane foam (PUF) and Teflon®-coated membrane, ultrasonic extraction with methanol.	ULSD, HVO and RME	RAW264.7 mouse macrophage cells exposed to 5–300µg/mL DEP extract and suspension of insoluble material for 24 h	DNA strand breaks by comet assay, proinflammatory cytokine production (Tnf- α , Mip-2), MTT reduction for cytotoxicity, apoptosis by flow cytometric analysis.	All samples yielded a significant concentration-related increase in cytotoxicity and DNA strand breaks. No difference in cytotoxicity across fuels types and aftertreatment. DOC/POC aftertreatment significantly reduced RME response only. ULSD and HVO elicited larger inflammatory response than RME. DOC/POC increased oxidative potential on a per mass basis; aftertreatment reduced PM emission rates by more than 50%.	[Jalava et al., 2010]

Engine	Fuels Examined	Exposure System	Endpoint(s) Examined	Results Obtained	Reference
2005 Scania 6-cylinder 11.7L Euro 4 engine with EGR, Braunschweig (bus) cycle, with or without DOC/POC (for LSDF and HVO 100 only), DEP collected on Teflon® filter, ultrasonic extraction with methanol.	LSDF, RME (B100 and B30), HVO (B100 and B30)	RAW264.7 mouse macrophage cells exposed to 15–300µg/mL DEP extract and suspension of insoluble material for 24 h	MTT reduction for cytotoxicity, proinflammatory cytokine production (Tnf- α , Mip-2), apoptosis, cell cycle and membrane permeability by flow cytometry. DNA strand breaks by comet assay.	Little differences in cytotoxicity across the fuels and aftertreatment conditions examined. Higher inflammatory response for HVO samples; lowest for RME. Little differences in apoptosis across conditions examined; some indication of higher levels for HVO. DOC/POC greatly reduced PM emission rate and PAH content of PM.	[Jalava et al., 2012]
1.9L light-duty Volkswagen, 9-mode steady state cycle, DEP collected on Teflon-coated GFFs.	ULSD, B20 SME	Differentiated human THP-1 monocytes BEAS-2B cells treated for 24 h with DEP in EtOH.	Levels of cytokines (i.e., G-CSF, IL-8, TNF- α , MCP-1).	For B20, significant elevation in G-CSF in THP-1 cells and IL-8 in BEAS-2B, relative to B0. Also increases in IL-8 and TNF- α for B20. Significant elevation in ROS in THP-1 cells.	[Fukagawa et al., 2013]
2000 Caterpillar C15 six cylinder 14.6L engine, 2007 MBE 4000 six cylinder 12.8L engine with EGR and DOC/DPF combination, chassis dynamometer UDDS and HHDDT, DEP collected on Teflon®-filters, PFE extraction with DCM followed by DCM/Tol, SVOCs on PUF/XAD cartridges, DCM extraction.	CARB DF, SME and AFME blends, renewable (NExBTL HVO)	Human U937 macrophages and NCI-H441 Clara cell line (exposure details not provided)	Expression of oxidative and inflammatory stress markers (CYP1A1, COX-2, IL-8, HO-1, MUC5AC). Details not provided. DNA damage by comet.	For C15, some evidence of declines in oxidative stress and inflammatory responses (per engine mile) for biodiesels relative to DF. Strong declines in oxidative stress for HVO (R100). For MBE 4000 some evidence for increase in oxidative stress and inflammatory signalling (SME only). No appreciable changes in DNA damage (all blends). Nevertheless, some indication of declines for HVO and SME relative to DF, reverse for AFME.	[Durbin et al., 2011]
1997 Caterpillar 3406E 14.6L engine, EPA heavy-duty transient cycle, DEP collected on Teflon®-coated GFFs, DCM extract.	DF, SME, SEE	BEAS-2B bronchial epithelial cells exposed to DMSO solutions of DEP extracts for 24 hr (equiv µg DEP per assay mL).	Cell viability via LDH release and MTT reduction, inflammatory stress via cytokine release (IL-8, IL-6).	No consistent changes in cytotoxicity, induction of cytokine release significant higher for biodiesel, relative to DF (for SOF expressed on per mass DEP).	[Swanson et al., 2009]

Engine	Fuels Examined	Exposure System	Endpoint(s) Examined	Results Obtained	Reference
Six cylinder 12L Euro III truck, no DOC, with or without DPF, 13-mode ESC, DEP collected on Teflon®-coated GFFs, ethanol/DCM (1:1) sonication extract	DF, B100, B5, B10, B20, PPO	RAW264.7 mouse macrophage cells exposed to DEP extract for 24 h	Cytotoxicity via LDH release, oxidative stress as <i>Ho-1</i> gene expression.	Biodiesel blends and PPO elicited less cytotoxicity relative to DF; B100 significantly more cytotoxic (unit unknown). No differences in <i>HO-1</i> expression. Biodiesel associated with reductions in PM (g/kWh), PAHs and oxy-PAHs (µg/kWh).	[Kooter et al., 2011]
Honda Accord (2.2L) 2.2i-CTDi (Euro4) with DOC and de-NOx, Peugeot (2.0L) 407 HDi with DOC and DPF, several composite driving cycles, DEP collected on Teflon®-coated GFFs, sonication MetOH extract.	DF, ULSD, RME	BEAS-2B bronchial epithelial cells exposed to DEP extracts suspended in culture medium, 24 hr, 0-200 µg equiv DEP per assay mL.	Cytotoxicity (necrosis, apoptosis) by flow cytometry, inflammatory stress via cytokine release (IL-6, IL-8).	On per mass basis, B50 significantly increased cytotoxicity and cytokine release. B50 and DPF both contribute to large reductions in PM emission rate. PM emission rate reduction for B50 may not be sufficient to compensate for increased potency per unit mass.	[Gerlofs-Nijland et al., 2013]

APPENDIX A – Summary of BD exhaust toxicity studies that were not examined by the MMWG.**Table A1. Summary of published *in vivo* studies not examined by the MMWG.**

Engine	Fuels Examined	Exposure System	Endpoint(s) Examined	Results Obtained	Reference
Common rail direct injection 3.0L engine (Tata, Safari DICOR), photochemical reaction chamber for secondary aerosols, measurement of PM-bound PAHs.	DF, B20 (unspecified)	Conversion of PAHs to total BaP equivalents in ng/m ³ . Used TEFs from Nisbet and Lagoy (1992) for relative carcinogenicity.	Total BaP equivalents (i.e., total carcinogenic PAH emission rate).	Total BaP equivalents in secondary aerosols higher than primary. B20 lower than DF for both primary and secondary aerosols.	[Agarwal et al., 2013]

Table A2. Summary of published *in vitro* studies in cultured animal cells not examined by the MMWG.

Engine	Fuels Examined	Exposure System	Endpoint(s) Examined	Results Obtained	Reference
1979 1.6L Volkswagen Golf, ECE Euro 2 cycle, DEP collected on “filter papers”.	DF, Biodiesel (unspecified) at B20, B40, B60, B80 B100.	A549 human alveolar adenocarcinoma cells exposed to 25 µg PM/mL for 5 days.	Induction of apoptosis (caspase III protein level, cytokeratin fragmentation)	Semi-quantitative analyses showed stronger induction of apoptosis by petroleum diesel, relative to biodiesel.	[Ackland et al., 2007]
Yanmar single cylinder 296mL diesel generator, steady state at rated speed and 4 loads, DEP collected on Teflon® membranes and quartz filters.	ULSD, B100 and B50 (waste cooking oil).	A549 human alveolar adenocarcinoma cells directly exposed to PM on filters for 48 hr.	Cell viability and cytotoxicity, measured via production of fluorescent products, apoptosis as caspase III/VII, oxidative stress as GSH/GSSG ratio (Promega assays).	Cytotoxicity and oxidative stress higher for B100 relative to DF. Similar for apoptosis response. No significant difference between B100 and DF at lower engine loads, and largest difference at higher engine loads.	[Betha et al., 2012]
Volkswagen Vento 1.9L TDI with DOC, FTP-75, MVEG-A, and modified MVEG-A cycles. DEP collected on Teflon®-coated GFFs, DCM Soxhlet extract	DF and RME	L929 mouse fibroblasts exposed to solvent-exchanged extract (DMSO) in medium, 24 hr.	Cytotoxicity via Neutral Red uptake assay.	No significant difference between cytotoxic potency of RME and DF (based on relative concentration of extracts in culture medium). Slight increase in RME potency for FTP-75 only.	[Bunger et al., 1998b]
Six cylinder 12L Euro III truck, no DOC, with or without DPF, 13-mode ESC, DEP collected on Teflon®-coated GFFs, ethanol/DCM (1:1) sonication extract	DF, B100, B5, B10, B20, PPO	RAW264.7 mouse macrophage cells exposed to DEP extract for 24 h	Cytotoxicity via LDH release, oxidative stress as <i>Ho-1</i> gene expression.	Biodiesel blends and PPO elicited less cytotoxicity relative to DF; B100 significantly more cytotoxic (unit unknown). No differences in <i>HO-1</i> expression. Biodiesel associated with reductions in PM (g/kWh), PAHs and oxy-PAHs (µg/kWh).	[Kooter et al., 2011]
1997 Caterpillar 3406E 14.6Engine, EPA heavy-duty transient cycle, DEP collected on Teflon®-coated GFFs, DCM extract.	DF, SME, SEE	BEAS-2B bronchial epithelial cells exposed to DMSO solutions of DEP extracts for 24 hr (equiv µg DEP per assay mL).	Cell viability via LDH release and MTT reduction, inflammatory stress via cytokine release (IL-8, IL-6).	No consistent changes in cytotoxicity, induction of cytokine release significant higher for biodiesel, relative to DF (for SOF expressed on a per mass DEP basis).	[Swanson et al., 2009]

Table A3. Summary of published Salmonella mutagenicity analyses not examined by the MMWG.

Test Article	Fuels Examined	Salmonella Strains/Test Version	Results Obtained	Reference
DEP and SVOCs from a 1983 Caterpillar 7L heavy-duty engine with DOC, custom 16-mode cycle representing light- and heavy-duty operation. DEP collected on Teflon®-coated GFFs, SVOCs on XAD, DCM Soxhlet extract of DEP and XAD	LSDF and SME	TA98, TA100, TA98NR and TA98/1,8DNP ₆ , microsuspension preincubation version, Aroclor-induced rat liver S9	Mutagenic potency, per kWh, greater for LSDF compared to SME. Potency far greater for DEP extracts than SVOC samples, and DOC resulted in over 50% reduction in mutagenic activity associated with DEP and SVOC. Potency of DEP extract for LSDF dramatically reduced on TA98NR (69–78%) and TA98-DNP (73–83%). SME emissions showed lower TPM, and reduced PAHs and 1NP relative to LSDF.	[Bagley et al., 1998]
DEP from a Fraymann single cylinder engine, 5 load modes (0–85%), with and without DOC. Teflon®-coated GFFs, DCM Soxhlet extract	DF, LSDF, RME, SME	TA98 and TA100, standard plate-incorporation assay, PB/5,6BF-induced rat liver S9	Mutagenic potency (per hr engine operation) generally lower for RME and SME, compared to DF or LSDF. Under partial load DOC generally led to reduced mutagenicity. Under heavy-duty conditions (rated power), DOC frequently led to increases in mutagenic activity. Without DOC, PM emission rate (g per hr) significantly higher for biodiesel relative to diesel (especially LSDF). Authors note this is likely attributable to higher SOF (g per hr) for biodiesel.	[Bunger et al., 2006]
DEP from a Volkswagen Vento 1.9L TDI with DOC, FTP-75, MVEG-A, and modified MVEG-A cycles. Teflon®-coated GFFs, DCM Soxhlet extract	DF and RME	TA98, TA97a, TA102, TA100, standard plate-incorporation assay, PB/5,6BF-induced rat liver S9	Significant positive responses for DF and RME samples on TA98 and TA100, and potency (per mg DEP) generally higher without S9. Potency (per mg DEP) greater for DF compared to RME, particularly on TA98 (1.9- to 5.1-fold). Similar pattern for potency expressed per km. Potency generally higher for cycles that include a cold start (modified MVEG-A).	[Bunger et al., 1998b]
DEP and SVOCs from a 4.6L, 6-cylinder Caterpillar engine, EPA heavy-duty transient test cycle. DEP collected on Teflon®-coated GFF, DCM Soxhlet extract, SVOCs on PUF plugs, supercritical CO ₂ extraction	DF, RME, HySEE HySEE50 blend (HySEE-hydrogenated soy ethyl ester)	TA98 and TA100, microsuspension preincubation version, Aroclor-induced rat liver S9	Mutagenic potency of DEP extract (per hp-hr) higher without S9. HySEE potency lower than 50/50 blend with DF, which was lower than DF alone. SVOC samples from DF about 2-fold more mutagenic than HySEE. HySEE associated with considerable reductions in PM and PAH emission rates (per hp-hr).	[Chase et al., 2000]

Table A3. Summary of published Salmonella mutagenicity analyses not examined by the MMWG.

Test Article	Fuels Examined	Salmonella Strains/Test Version	Results Obtained	Reference
DEP from 3 diesel engines, 1.686L, 4-cylinder light-duty, 10.8L, 6-cylinder heavy-duty with DPF and SCR, 10.52L, 6-cylinder, heavy-duty with DPF, DEP collected on GFF, DCM Soxhlet extract	DF and plant oils (peanut, rapeseed, soy, sunflower)	TA98, TA100, TA Mix, fluctuation assay (Xenometrics)	All samples in the range of the negative control with no evidence of differences in activity between the fuels.	[Dorn and Zahoransky, 2009]
DEP from a 1991 Detroit Diesel DDC Series 60, six cylinder 11.1L engine, heavy-duty transient cycle, DEP collected on Teflon®-coated GFFs, DCM sonication extract	DF, SME, CME, PLME, BTME, YGME (all B100)	TA98, microsuspension preincubation version, Aroclor-induced rat liver S9	All samples elicited a significant positive response. For cold start only DF and CME more potent without S9. For hot start only, DF, SME and CME appreciably greater without S9. All others more potent with S9. For cold start, with S9, potency (per µg PM equiv) of biodiesel samples all higher than DF. Without S9, all samples except SME more potent than DF. For hot start all biodiesel potency values greater than DF. Mutagenicity emission rates (rev per hph) higher for DF compared with any of the biodiesels. PM emission rate for DF almost 4-fold greater than biodiesel rates.	[Kado and Kuzmicky, 2003]
DEP and SVOCs from a Mercedes-Benz, 5.9L, 6-cylinder engine, 13-mode ESC, with and without DOC. DEP collected on Teflon®-coated GFFs, DCM Soxhlet extract, SVOCs from condensates.	2 DFs, B100 RME, B20 RME	TA98 and TA100, standard plate-incorporation assay, PB/5,6BF-induced rat liver S9	Mutagenic potency (unit not provided) uniformly higher without S9. Highest response for DF (reference fuel), with lowest for RME5 and RME. DOC further reduced activity of RME. No significant difference in potencies of SVOCs (per m ³), with complete elimination of activity by DOC.	[Krahl et al., 2009a]
DEP and SVOCs from a Mercedes-Benz, 6.37L, 6-cylinder engine, 13-mode ESC. DEP collected on Teflon®-coated GFFs, DCM Soxhlet extract, and condensates from gas phase collected at 50 °C	DF, RME, GTL, RSO, modified RSO	TA98 and TA100, standard plate-incorporation assay, PB/5,6BF-induced rat liver S9	All samples yielded a positive response, and all potency values (per litre exhaust gas) unchanged or reduced upon addition of S9. DEP extract for RSO yielded the highest potency values (9.7- to 17-fold higher than DF on TA98 and 5.4- to 6.4-fold higher than DF on TA100). Modified RSO potency 2.4- to 3.5-fold higher than RSO. RSO condensate samples also yielded the highest potency values (up to 3-fold DF). Modified RSO 3- to 5-fold higher than RSO. Few differences between DEP extracts for DF, RME and GTL, although RME significantly greater than DF on TA98 with S9 and TA100 without S9.	[Krahl et al., 2007a; Krahl et al., 2009b]

Table A3. Summary of published Salmonella mutagenicity analyses not examined by the MMWG.

Test Article	Fuels Examined	Salmonella Strains/Test Version	Results Obtained	Reference
DEP and SVOCs from 3 heavy-duty diesel engines, Mercedes-Benz, 6.37L, 6-cylinder engine, MAN, 6.87L, 6-cylinder engine, AVL single-cylinder, 1.47L engine, 13-mode ESC, ETC, and rated power. DEP collected on Teflon®-coated GFFs, DCM Soxhlet extract, SVOCs from condensates.	DF, GTL, B100 RME, B20 RME	TA98 and TA100, standard plate-incorporation assay, PB/5,6BF-induced rat liver S9	Mutagenic potency (unit not provided) uniformly higher without S9. For Mercedes engine GTL lowest activity followed by DF. RME similar to DF, but RME20 significantly elevated. For AVL and MAN engines, RME20 significantly elevated relative to DF, but RME lower than DF. For SVOCs from the MAN engine, DF potency greater than RME blends. For the Mercedes and MAN engines, PM emission rates (g/kWh) for RME about half of DF.	[Krahl et al., 2008]
DEP from a Mercedes-Benz 6.37L, 6-cylinder and an IVECO 5.9L, 6-cylinder diesel test engine with SCR, 13-mode ESC. DEP collected on Teflon®-coated GFFs, DCM Soxhlet extract.	DF, RME, RSO, SMDS, B5 RME in SMDS, DF/RME/GTL blend.	TA98 and TA100, standard plate-incorporation assay, PB/5,6BF-induced rat liver S9	Mutagenic potency values uniformly greater without S9. For the Mercedes engine, no significant difference in potency (per L exhaust gas) between DF, RME, SMDS and DF/RME/GTL blend. RO yielded significantly elevated potency (approximately 10-fold), also highest PM output in g/kWh. For the IVECO engine, SCR significantly reduced mutagenic potency, no difference between DF and RME, after 1000hrs SCR less effective. RME associated with reduced PM emissions (g/kWh).	[Krahl et al., 2007b; Krahl et al., 2006]
DEP from a Mercedes-Benz 6.37L, 6-cylinder engine, 13-mode ESC. DEP collected on Teflon®-coated GFFs, DCM Soxhlet extract.	Two DFs, RME, GTL, 4 FAME mixtures from soy, palm and rapeseed	TA98, standard plate-incorporation assay, PB/5,6BF-induced rat liver S9	Mutagenic potency (per m ³) greater without S9 and highest for DF. RME potency less than half of DF potency. DEP emission rates lower (per kWh) for all FAMES.	[Krahl et al., 2005]
DEP from a Mercedes-Benz 4.25L, 4-cylinder engine, 13-mode ESC. DEP collected on Teflon®-coated GFFs, DCM Soxhlet extract.	DF, RME, LSDF, LSDF with high aromatic	TA98 and TA100, standard plate-incorporation assay, PB/5,6BF-induced rat liver S9	Mutagenic potency (per engine hr) lowest for RME. DF 4- to 5-fold higher than RME, LSDF 2- to 3-fold higher. No significant difference with and without S9. DEP emission rates (per kWh) highest for DF.	[Krahl et al., 2003]

Table A3. Summary of published Salmonella mutagenicity analyses not examined by the MMWG.

Test Article	Fuels Examined	Salmonella Strains/Test Version	Results Obtained	Reference
DEP from a 12L 6 cylinder Euro III truck, no DOC, with or without DFP, 13-mode ESC, DEP collected on Teflon®-coated GFFs, ethanol/DCM (1:1) sonication extract	DF, B100, B5, B10, B20, PPO (pure plant oil)	TA98 and YG1024, YG1029. Standard plate incorporation version, Aroclor-induced rat liver S9	No significant response in the presence of S9 for any sample. For TA98, significant response for B20 and PPO only. For YG1024, significant responses for B10, B100 and PPO only. Maximum responses on YG1024 for B100 and PPO (per µg PM). Biodiesel associated with reductions in PM (g/kWh), PAHs and oxy-PAHs (µg/kWh).	[Kooter et al., 2011]
DEPs from four heavy-duty engines (8.5L, 6-cylinder, 7.4L, 6-cylinder and two 9.6L, cylinder), 13-mode ESC. DEP collected on Teflon®-coated GFF, DCM Soxhlet extract	DF, LSDF, 2 reformulated DFs, RME and RME30	TA98, TA98NR, YG1021, standard plate incorporation assay, Aroclor-induced rat liver S9	Mutagenic potency uniformly higher without S9. DF showed the highest mutagenic potency (per µg EOM), followed by LSDF reformulated DFs and RME. When expressed per kWh, RME potency lower than DF, but higher than other fuels (due to high EOM per unit mass). Potency (per µg EOM) reduced on TA98NR and increased on YG1021, compared to TA98. Good correlation between mutagenic potency per kWh and PAH emission per kWh. RME potency higher than predicted by PAH content.	[Rantanen et al., 1993]
DEP from Mercedes-Benz Euro III OM 906 6.37L six cylinder engine, ESC 13-mode test cycle, DEP collected on Teflon®-coated GFF, DCM Soxhlet extract.	DF, RME, LME, SME, PME, CME	TA98, TA100 with and without S9 (details not provided)	Responses higher without S9, and biodiesel responses (unit not provided) lower than DF. TA100 analyses of SME showed similar results relative to DF; B100 somewhat higher response. PM emission rates (g/kW-hr) lower for all biodiesels, relative to DF. PAH emissions for biodiesels far lower, relative to DF (rate not provided).	[Schroder et al., 2012]
DEP and SVOCs from a heavy-duty, 6-cylinder 6.4L Mercedes-Benz OM 906 LA Euro 3-compliant engine, with and without DOC, ESC. DEP collected on Teflon®-coated GFFs, DCM Soxhlet extract, SVOC on chilled surface.	Low-sulphur DF, RME, B5 RME in diesel	TA98, TA100 standard plate incorporation assay, PB/5,6BF-induced rat liver S9	Mutagenic potency of DEP (per m ³ exhaust) modestly higher without S9. Without S9 potency highest for DF, and decreased for RME and 5% v/v RME. DOC contributed to modest reductions in potency without S9, and slight reductions with S9. DOC eliminated the mutagenic activity of SVOC.	[Westphal et al., 2012]

Name: Paul A. White, PhD

Affiliation: Department of Biology, University of Ottawa, Ottawa, Ontario, Canada

REFERENCES

- Ackland ML, Zou L, Freestone D, Van De Waasenburg S, Michalczyk AA. 2007. Diesel exhaust particulate matter induces multinucleate cells and zinc transporter-dependent apoptosis in human airway cells. *Immunol Cell Biol* 85:617-622.
- Agarwal AK, Gupta T, Dixit N, Shukla PC. 2013. Assessment of toxic potential of primary and secondary particulates/ aerosols from biodiesel vis-à-vis mineral diesel fuelled engine. *Inhal Toxicol* 25:325-332.
- Bagley ST, Gratz LD, Johnson JJ, McDonald JF. 1998. Effects of an oxidation catalytic converter and a biodiesel fuel on the chemical, mutagenic, and particle size characteristics of emissions from a diesel engine. *Environ Sci Technol* 32:1183-1191.
- Betha R, Pavagadhi S, Sethu S, Hande MP, Balasubramanian R. 2012. Comparative in vitro cytotoxicity assessment of airborne particulate matter emitted from stationary engine fuelled with diesel and waste cooking oil-derived biodiesel. *Atmos Environ* 61:23-29.
- Bhavaraju L, Shannahan J, William A, McCormick R, McGee J, Kodavanti U, Madden M. 2014. Diesel and biodiesel exhaust particle effects on rat alveolar macrophages with in vitro exposure. *Chemosphere* 104:126-133.
- Bunger J, Krahl J, Franke HU, Munack A, Hallier E. 1998a. Mutagenic and cytotoxic effects of exhaust particulate matter of biodiesel compared to fossil diesel fuel. *Mutat Res* 415:13-23.
- Bunger J, Krahl J, Franke HU, Munack A, Hallier E. 1998b. Mutagenic and cytotoxic effects of exhaust particulate matter of biodiesel compared to fossil diesel fuel. *Mutat Res* 415:13-23.
- Bunger J, Krahl J, Weigel A, Schroder O, Bruning T, Muller M, Hallier E, Westphal G. 2006. Influence of fuel properties, nitrogen oxides, and exhaust treatment by an oxidation catalytic converter on the mutagenicity of diesel engine emissions. *Arch Toxicol* 80:540-546.
- Chase C, Peterson C, Lowe G, Mann P, Smith J, Kado N. 2000. A 322,000 kilometer (200,000 mile) Over the Road Test with HySEE Biodiesel in a Heavy Duty Truck. SAE Technical Paper Series 2000-01-2647. SAE International, Warrendale, PA.
- Dorn B, Zahoransky R. 2009. Non-Esterified Plant Oils as Fuel -Engine Characteristics, Emissions and Mutagenic effects of PM. SAE Technical Paper Series 2009-24-0097. SAE International, Warrendale, PA.
- Durbin TD, Miller JW, Johnson K, Hajbabaie M, Kado NY, Kobayashi R, Vogel CFA, Matsumura F, Wong PS, Cahill T. 2011. CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California. Biodiesel Characterization and NOx Mitigation Study. Final Report. California Air Resources Board, Sacramento, CA.
- Fukagawa NK, Li M, Poynter ME, Palmer BC, Parker E, Kasumba J, Holmén BA. 2013. Soy biodiesel and petrodiesel emissions differ in size, chemical composition and stimulation of inflammatory responses in cells and animals. *Environ Sci Technol* 47:12496-12504.
- Gerlofs-Nijland M, Totlandsdal AI, Tzankiozis T, Leseman DLAC, Samaras Z, Lag M, Schwarze P, Ntziachristos L, Cassee FR. 2013. Cell toxicity and oxidative potential of engine exhaust particles: Impact of using particulate filter or biodiesel fuel blend. *Environ Sci Technol* 47:5931-5938.
- Hawley B, L'Orange C, Olsen DB, Marchese AJ, Volckens J. 2014. Oxidative stress and aromatic hydrocarbon response of human bronchial epithelial cells exposed to Petroor biodiesel exhaust treated with a diesel particulate filter. *Toxicol Sci* 141:505-514.
- Hemmingsen JG, Moller P, Nojgaard JK, Roursgaard M, Loft S. 2011. Oxidative stress, genotoxicity, and vascular cell adhesion molecule expression in cells exposed to particulate matter from combustion of conventional

Name: Paul A. White, PhD

Affiliation: Department of Biology, University of Ottawa, Ottawa, Ontario, Canada

diesel and methyl ester biodiesel blends. *Environ Sci Technol* 45:8545-8551.

- Ihalainen M, Kuuspallo K, Karunen T, Ruusunen P, Willman P, Tapanainen M, Jalava P, Salonen R, Pennanen A, Happonen M, Hirvonen M, Jokiniemi J. 2009. Biobased Diesel Fuels: Particulate Emissions and Their Inflammation Response. 13th ETH Conference on Combustion Generated Nanoparticles, June 22-24, 2009, Zurich, Switzerland.
- Jalava PI, Aakko-Saksa P, Murtonen T, Happonen MS, Markkanen A, Yli-Pirila P, Hakulinen P, Hillamo R, Mäntki-Paakkanen J, Salonen RO, Jokiniemi J, Hirvonen MR. 2012. Toxicological properties of emission particles from heavy duty engines powered by conventional and bio-based diesel fuels and compressed natural gas. *Part Fibre Toxicol* 9.
- Jalava PI, Tapanainen M, Kuuspallo K, Markkanen A, Hakulinen P, Happonen MS, Pennanen AS, Ihalainen M, Yli-Pirila P, Makkonen U, Teinila K, Maki-Paakkanen J, Salonen RO, Jokiniemi J, Hirvonen MR. 2010. Toxicological effects of emission particles from fossil- and biodiesel-fueled diesel engine with and without DOC/POC catalytic converter. *Inhal Toxicol* 22 Suppl 2:48-58.
- Kado N, Kuzmicky P. 2003. Bioassay Analyses of Particulate Matter from a Diesel Bus Engine Using Various Biodiesel Feedstock Fuels. Final Report. Report 3 in a Series of 6. NREL/SR-510-31463. National Renewable Energy Laboratory, Golden, CO.
- Kooter IM, van Vugt, M. A. T. M., Jedynska AD, Tromp PC, Houtzager MMG, Verbeek RP, Kadijk G, Mulderij M, Krul CAM. 2011. Toxicological characterization of diesel engine emissions using biodiesel and a closed soot filter. *Atmos Environ* 45:1574-1580.
- Krahl J, Munack A, Ruschel Y, Schroder O, Bunker J. 2008. Exhaust Gas Emissions and Mutagenic Effects of Diesel Fuel, Biodiesel and Biodiesel Blends. SAE Technical Paper Series 2008-01-2508. SAE International, Warrendale, PA.
- Krahl J, Munack A, Ruschel Y, Schroder O, Bunker J. 2007a. Comparison of Emissions and Mutagenicity from Biodiesel, Vegetable Oil, GTL and Diesel Fuel. SAE Technical Paper Series 2007-01-4042. SAE International, Warrendale, PA.
- Krahl J, Munack A, Ruschel Y, Schroder O, Schwarz S, Hofmann L, Bunker J. 2006. Influence of the Phosphorus Content in Rapeseed Oil Methyl Esters During a 1000 Hours Endurance Test on the Function of a SCR-system Measured by Exhaust Gas Emissions and Health Effects. SAE Technical Paper Series 2006-01-3282. SAE International, Warrendale, PA.
- Krahl J, Munack A, Schröder O, Stein H, Bunker J. 2003. Influence of Biodiesel and Different Designed Diesel Fuels on the Exhaust Gas Emissions and Health Effects. SAE Technical Paper Series 2003-01-3199. SAE International, Warrendale, PA.
- Krahl J, Munack A, Schroder O, Stein H, Herbst L, Kaufmann A, Bunker J. 2005. The Influence of Fuel Design on the Exhaust Gas Emissions and Health Effects. SAE Technical Series 2005-01-3772. SAE International, Warrendale, PA.
- Krahl J, Schroder O, Ruschel Y, Bunker J. 2009a. Ultrafine particles from a heavy duty diesel engine running on rapeseed oil methyl ester. *SAE Int J Fuels Lubr* 2:132-146.
- Krahl J, Knothe G, Munack A, Ruschel Y. 2009b. Comparison of exhaust emissions and their mutagenicity from the combustion of biodiesel, vegetable oil, gas-to-liquid and petrodiesel fuels. *Fuel* 88:1064-1069.
- Krahl J, Munack A, Grope N, Ruschel Y, Schroder O, Bunker J. 2007b. Biodiesel, rapeseed oil, gas-to-liquid, and a premium diesel fuel in heavy duty diesel engines: endurance, emissions and health effects. (Special Issue: Celebrating the 60th birthday of Professor Mufit Bahadir.). *CLEAN - Soil, Air, Water* 35:417-426.

Name: Paul A. White, PhD

Affiliation: Department of Biology, University of Ottawa, Ottawa, Ontario, Canada

- Mullins B, Kicic A, Ling K, Mead-Hunter R. 2014. Biodiesel exhaust–induced cytotoxicity and proinflammatory mediator production in human airway epithelial cells. *Environ Res*, in press.
- Rantanen L, Mikkonen S, Nylund L, Kociba P, Lappi M, Nylund N. 1993. Effect of Fuel on the Regulated, Unregulated and Mutagenic Emissions of DI Diesel Engines. SAE Technical Paper Series 932686. SAE International, Warrendale, PA.
- Schroder O, Munack A, Schaak J, Pabst C, Schmidt L, Bunger J, Krahl J. 2012. Emissions from diesel engines using fatty acid methyl esters from different vegetable oils as blends and pure fuel. 25th International Congress on Condition Monitoring and Diagnostic Engineering (COMADEM). *Journal of Physics: Conference Series* Volume 364. Huddersfield, UK.
- Shvedova AA, Yanamala N, Murray AR, Kisin ER, Khaliullin T, Hatfield MK, Tkach AV, Krantz QT, Nash D, King C, Ian Gilmour M, Gavett SH. 2013. Oxidative stress, inflammatory biomarkers, and toxicity in mouse lung and liver after inhalation exposure to 100% biodiesel or petroleum diesel emissions. *J Toxicol Environ Health A* 76:907-921.
- Steiner S, Czerwinski J, Comte P, Popovicheva O, Kireeva E, Muller L, Heeb N, Mayer A, Fink A, Rothen-Rutishauser B. 2013. Comparison of the toxicity of diesel exhaust produced by bio- and fossil diesel combustion in human lung cells in vitro. *Atmos Environ* 81:380-388.
- Swanson KJ, Kado NY, Funk WE, Pleil JD, Madden MC, Ghio AJ. 2009. Release of the pro-inflammatory markers by BEAS-2B cells following in vitro exposure to biodiesel extracts. *Open Toxicology Journal* 3:8-15.
- Westphal GA, Krahl J, Munack A, Ruschel Y, Schroder O, Hallier E, Bruning T, Bunger J. 2012. Mutagenicity of diesel engine exhaust is eliminated in the gas phase by an oxidation catalyst but only slightly reduced in the particle phase. *Environ Sci Technol* 46:6417-6424.
- Yanamala N, Hatfield MK, Farcas MT, Schwegler-Berry D, Hummer JA, Shurin MR, Birch ME, Gutkin DW, Kisin E, Kagan VE, Bugarski AD, Shvedova AA. 2013. Biodiesel versus diesel exposure: Enhanced pulmonary inflammation, oxidative stress, and differential morphological changes in the mouse lung. *Toxicol Appl Pharmacol* 272:373-383.

Supplemental Review of the Revised
Staff Report: Multimedia Evaluation of Biodiesel

Xiusheng (Harrison) Yang

Department of Natural Resources and the Environment
University of Connecticut
Storrs, CT 06269-4087

April 5, 2015

The Multimedia Working Group (MMWG) has revised its assessment on the biodiesel multimedia evaluation entitled “Staff Report: Multimedia Evaluation of Biodiesel” based on (1) some new studies and publications in the field, and (2) comments and suggestions from external peer reviewers. The revision has led to a number of updates and modifications, especially in the summary and concluding remarks on the impact of biodiesel on air quality and public health. As a consequence, the MMWG recommendations to the Environmental Policy Council of the State of California have been rephrased. Essentially the same as in the previous version, the MMWG concludes that the use of biodiesel in the State does not pose a significant adverse impact on the environment or the public health.

Overall Comments on the Revision

The staff report has been revised by considering many comments and suggestions from the external reviewers, and the excellent work should be complimented. In fact, I often used the MMWG’s response to peer review comments as a guideline to read the revised report. Grouped into 8 topics (e.g. air quality, public health, conclusions on public health impact, water quality, multimedia evaluation, staff report, source report, and proposed regulation, respectively), Appendix J of the revised report summarized the comments from the peer reviewers and MMWG’s responses of that many have been incorporated into the new document.

Overall, the revised Staff Report shows a higher quality than the previous one. In particular, this revision has reflected the newest developments in the field from follow-up experiments, additional data analysis, and more complete literature review. I would conclude that the revised Staff Report is based on sound scientific knowledge, methods, and practices. And consequently, the conclusions of the Staff Report are acceptable.

Comments on specific conclusion statements

1. Air Emission Evaluation

New: Air Resources Board (ARB) staff concludes that with in-use requirements biodiesel does not pose a significant adverse impact on public health or the environment from potential air quality impact.

Previous: Air Resources Board (ARB) staff concludes that the use of biodiesel does not pose a significant adverse impact on public health or the environment from potential air quality impacts.

As I said in the previous review, I generally agree with the findings of the evaluation studies on the direct use of biodiesel. The revised conclusion statements are more accurate and therefore more acceptable. The revised report has taken into consideration the comments from Holloway by providing more background information and reorganizing some materials of the presentation. I would leave that to Dr. Holloway to make her judgement on the related revision. My previous comments included concerns about impact on air quality of feedstock production and processing. According to the responses to my comments, I now understand that this multimedia evaluation is limited to the direct health and environmental impacts from biodiesel, and other life-cycle and indirect impacts are outside the scope of this evaluation. As such, the revised Staff Report is considered up to my expectation.

2. Public Health Evaluation

New: After reviewing scientific literature that compares the physical and chemical nature of combustion emissions from diesel engines fueled with biodiesel to the composition of combustion emissions from engines fueled with petroleum diesel, Office of Environmental Health Hazard Assessment (OEHHA) staff concludes that replacing petroleum diesel with an energy-equivalent amount of biodiesel will decrease emissions of particulate matter (PM), benzene, and ethyl benzene but may increase emissions of oxides of nitrogen (NOx). From studies comparing the biological impacts of biodiesel combustion emissions to those of petroleum diesel combustion emissions is more potent than PM from petroleum diesel combustion emissions in eliciting certain responses associated with inflammation and oxidative stress when biological responses per mass of PM are compared. However, in a study carried out at the University of California, Riverside and University of California, Davis, PM from combustion of soy-derived biodiesel is less potent in eliciting the responses associated with inflammation and oxidative stress than is PM in petroleum diesel combustion emissions when the comparison is made on a per mile basis.

Previous: Office of Environmental Health Hazard Assessment (OEHHA) staff concludes that the substitution of biodiesel for CARB diesel reduces the rate of carbon dioxide to the atmosphere and reduces the amount of particulate matter (PM), benzene, ethyl benzene, and polycyclic aromatic hydrocarbons (PAHs) released into the atmosphere, but may increase the emissions of oxides of nitrogen (NOx) and acrolein for certain blends.

The revision in the conclusions statements regarding the impact on public health has been substantial. Unfortunately, my limited knowledge in this field prevents me from providing more meaningful insights. My understanding is that the previous report was based on limited data and incomplete review of the available literature. In this new revision, OEHHA has revised its conclusion statements based on a more thorough literature study, follow-up tests, and additional data analysis. My previous concern was on the information about the impact on public health of feedstock production, storage, transportation, and processing. I am satisfied with the responses to my comments.