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Supplemental Material

Toxicological and ecotoxicological potencies of biofuels used for the transport sector – a literature review

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Supplemental Material, Table 1 Overview of reviewed publications arranged according to endpoints investigated

Endpoints and biotest	Tester strain, cell culture or test organism	Exposure phase	Fuel tested	Reference	Findings regarding (eco)toxicological effects
Mutagenicity/ Genotoxicity					
Ames assay	S. typhimurium TA98 and TA100	extracts of PM and gas phase	RSO, RME, GTL, DF	**Bünger et al. (2007)**[1]	Biofuels can strongly increase the mutagenic effects of diesel engine exhaust (RSO) or have similar mutagenicity (RME, TA98+S9 and TA100-S9) compared to GTL and DF.
Ames assay	S. typhimurium TA98 and TA100	extracts of PM	RME, SME, DF (370 ppm), LS-DF (1 ppm)	**Bünger et al. (2000)**[2]	RME, SME and LS-DF decrease mutagenicity of exhaust particle extracts compared to DF. However, mutagenic effects were also found for the biofuels in one tester strain at two load mode and these results partly indicate comparable or higher numbers of revertants for the biofuels compared to LS-DF(when displayed as revertants per hour of engine running time).
Ames assay	S. typhimurium TA98 and TA100	extracts of PM	RME, DF	**Bünger et al. (2000)**[3]	RME and DF extracts were mutagenic in both tester strains. DF extracts induced fourfold (TA98) and twofold (TA100) higher mutagenic effects than RME extracts.
Ames assay	S. typhimurium TA97a, TA98, TA100 and TA102	extracts of PM	RME, DF	**Bünger et al. (1998)**[4]	Mutagenic effects were found for both fuels by using the tester strains TA98 and TA100. RME induced lower numbers of revertants compared to DF.
1. Ames assay 2. Micronucleus assay 3. Chromosomal aberration 4. SCE	1. S. typhimurium TA98, TA98NR and TA100 2.-4. rat hepatocytes	extracts of PM and volatile fraction	RME, DF	**Eckl et al. (1998)**[5]	1. Significanlyt higher mutagenic potentials were found for diesel exhaust extracts or no differences between the extracts. 2. No dose response of micronucleus induction or significant differences(micronucleus induction corrected for the proliferation rate). 3. Diesel and RME exhaust can induce the level of chromosomal aberrations; at idle mode: diesel exhaust particle extract significantly elevated levels of chromosomal aberrations in comparison to RME exhaust particle extract. 4. No differences between diesel and RME exhausts. 2-4. indications that RME and diesel exhaust have high mutagenic potentials.
Ames assay	S. typhimurium TA98, TA100, TA1535, TA1537 and TA98/ 1,8DNP$_6$	extracts of PM and semi-volatile fraction	SME	**Finch et al. (2002)**[6]	The extracts of both PM and the semi-volatile fraction induced mutagenic effects. A major part of the observed mutagenicity could be caused by nitro-aromatics.

Endpoints and biotest	Tester strain, cell culture or test organism	Exposure phase	Fuel tested	Reference	Findings regarding (eco)toxicological effects
Ames assay	S. typhimurium TA98	extracts of PM	diesel blended with SME, RME and biodiesel derived from animal fats); ULSD	**Gagnon and White (2008)**[7]	The mutagenic activity can be reduced with increasing biodiesel content.
Comet assay	Mouse RAW264.7 macrophages	extracts of PM	RME, hydrotreated fresh vegetable oil, DF (EN590)	**Jalava (2010)**[8]	Dose-dependent increase of DNA damage found for all samples. No significant differences between the PM extracts of the fuels at a concentration of 150 g/mL. Relative genotoxic responses were slightly stronger for diesel fuel and HVO compared to RME.
Ames assay	S. typhimurium TA98	extracts of PM	RME, SME, pork lard methyl ester, beef tallow methyl ester, yellow grease methyl ester, DF	**Kado and Kuzmicky (2003)**[9]	Higher mutagenic potencies found for biodiesel fuels compared to DF (regarding the activity per particle with regard to the activity per particle mass. The mutagen emission rates reffering to revertants per BHP-HR were predominantly lower for the biofuels compared to DF but they can be in the same range, depending on the biofuel.
Ames assay	S. typhimurium TA98 and YG1024	extracts of PM and combustion aerosols	biodiesel (EN14214), blends (B20, B10, B5), RSO, DF (sulfur 10 mg/kg)	**Kooter (2011)**[10]	Biodiesel blends, biodiesel and pure plant oil indicate a potential to increase mutagenic effects in the test strains.
Ames assay	S. typhimurium TA98 and TA100	extracts of PM and condensates	RSO, RME, GTL, DF (< 1 ppm)	**Krahl et al. (2009)**[11]	RME, DF and GTL induced weaker effects than RSO. RME particle extracts revealed significantly stronger mutagenic effects when testing TA98 with metabolic activation and TA100 without metabolic activation compared to DF.
Ames assay	S. typhimurium TA98 and TA100	extracts of PM	RME, RSO, GTL, DF and a premium diesel fuel: blend with 60 % DF, 20 % RME, 20 % GTL and additive	**Krahl et al. (2007)**[12]	1. RME and DF with nearly the same mutagenic effects, results of GTL indicate a lower mutagenic potency; PDF indicate higher mutagenicity than each of its components; RSO with higher mutagenic potential than RME and DF but no indication for a worse combustion for RSO; 2. all PM extracts with low mutagenic potentials; no significant differences of mutagenicity between extracts of different fuels; indication for an increase of mutations per plate due to catalyst aging
1. Ames assay 2. Comet assay	1. S. typhimurium TA98 and TA100 2. rat fibrocytes L929 cells	extracts of PM	DF blended with ethanol (5 %, 10 %, 15 % and 20 %) and DF	**Song et al. (2007)**[13]	1. E20 indicate the highest number of brake specific revertants. 2. Results indicate that DF and E20 have a higher genotoxic potential than the other fuel blends.

Endpoints and biotest	Tester strain, cell culture or test organism	Exposure phase	Fuel tested	Reference	Findings regarding (eco)toxicological effects
Ames assay	S. typhimurium TA98, TA100 and TA98/ 1,8DNP$_6$	extracts of PM and semi-volatile compounds	B20 blend (20 % RME) and DF (sulfur < 300 ppm)	**Turrio-Baldassarri et el. (2004)**[14]	No differences found for the mutagenic potential of biodiesel blend and DF emissions. Mutagenic activity was lower in the nitropyrene resistant strain -> presence of genotoxic nitro-aromatics suggested.
1. Ames assay 2. Comet assay 3. Micronucleus assay	1. S. typhimurium TA98 and TA100 2.+3. human lung adenocarcinoma A549 cell line	extracts of PM, condensates and semi-volantile organic compounds	gasoline and methanol	**Zhang et al. (2007)**[15]	No adverse effects measured for methanol engine exhaust, while gasoline engine exhaust induced DNA damage, the formation of micronucleus as well as a significant increase of revertants using the tester strain TA98.
Biochemical parameter					
Inhalation toxicity (EROD and PROD activity and analysis of urinary parameters)	male and female Sprague-Dawley rats	fuel vapor	gasoline, ethanol and ethanol-gasoline mixture (E-85)	**Chu et al. (2005)**[16]	Significantly elevated hepatic microsomal EROD activity was found in male rats treated with gasoline. Uterinary parameters were altered in rats after treatment with gasoline or the mixture.
DR-CALUX	n.a.	extracts of PM	biodiesel fuel blended with SME, RME and biodiesel derived from animal fats; ULSD	**Gagnon and White (2008)**[7]	Dioxin-like activity can increase with increasing biodiesel fuel content.
Oral toxicity (analyses of biochemical parameters)	male Sprague-Dawley rats	fuels themselves	RME, SME, FraME, ULSD	**Poon et al. (2009)**[17]	ULSD significantly increased BROD,EROD, PROD and GST enzyme activity and urinary parameters. AfME significant increase GST enzyme activity. All biodiesels and ULSD: increased acyl-CoA oxidase activity and RME increased urinary albumin.
Oral toxicity (analyses of biochemical parameters)	male Sprague-Dawley rats	fuels themselves	RME, SME, fish oil methyl ester, LS-DF (317 ppm)	**Poon et al. (2007)**[18]	SME and LS-DF significantly increased phase I xenobiotic metabolizing enzymes activities (BROD,EROD,PROD) and phase II enzyme activity (GST) and they significantly affected some urinary analytes. LS-DF also increased the phase II enzyme (UDP-glucuronosyl-transferase) and palmitoyl CoA oxidase activity.

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Inflammatory toxicity					
Cytokine analysis with ELISA kits (TNF-α and MIP-2)	Mouse RAW264.7 macrophages	extracts of PM	RME, hydrotreated fresh vegetable oil, DF (EN590)	**Jalava (2010)**[8]	All fuels increased the chemokine MIP-2 responses at higher doses. Statistically significant differences between the emission particles of the fuels were not found at the higher concentrations applied. All samples induced dose-dependent cytokine TNF-alpha production. Relative responses were stronger for diesel fuel and HVO compared to RME.
Cytokine assays CXCL8/IL-8 and IL-6	BEAS-2B cell line	extracts of PM	SEE, SME, DF	**Swanson et al. (2009)**[19]	Indication for a more potent inflammatory stimulation of treatments with biodiesel PM extracts compared to diesel PM extracts.
whole organism					
Inhalation toxicity (acute cardiovascular and inflammatory toxicity)	male Balb/c mice	whole exhaust	SEE, biodiesel blend (50 % SEE and 50 % diesel), DF (500 ppm, 3 % biodiesel)	**Brito et al. (2010)**[20]	Biodiesel (B100 and B50) and diesel stimulated alterations in the cardiovascular system and inflammation. They concluded from their findings that biodiesel presents equal and/or more toxic effects compared with diesel fuel.
inhalation toxicity (1. body weight/organ weight, 2. hematological parameters ; 3. neurochemical analysis; 4. histological/ morphological changes; 5.serum analysis	male and female Sprague-Dawley rats	fuel vapor	gasoline, ethanol and ethanol-gasoline mixture (E85)	**Chu et al. (2005)**[16]	1. Growth rate of female rats was affected by gasoline-ethanol mixture. Significant changes in the relative weight of some organs were found for all three fuel vapors. 2. Hematological effects were found in male and female rats of gasoline and mixture treatment groups. 3. Neurochemical effects were found after gasoline and ethanol treatment. 4. Morphological changes were mainly found after treatment with E85. 5. Serum phosphate was altered by gasoline treatment (male rats) and glucose concentration altered by gasoline and ethanol treatment (female rats). Most effects were induced by gasoline but indications for additive and synergistic effects were found for co -exposure of gasoline and ethanol. Most of the effects observed were reversible.
Inhalation toxicity	F344 rats	whole emission	SME	**Finch et al. (2002)**[6]	Tests revealed no significant effects on the mortality rate, feed consumption, hematology, neurohistology, micronuclei in bone marrow, sister chromatid exchanges in peripheral blood lymphocytes, fertility or teratology.
1. Acute oral toxicity test 2. Ocular irritation assay 3. Acute dermal irritation assay	1. rats 2. + 3. male New Zealand Albino Rabbits	fuels themselves	1. RME, eRME, SuME HoSuME, DF 2. RME, SuME	**Gateau et al. (2005)**[21]	1. No differences between the fuels (LD_{50} greater than 5,000 mg/kg) 2. + 3. They report that RME and SuME were not irritating for the skin or the eyes and were also not corrosive for the eyes.

Endpoints and biotest	Tester strain, cell culture or test organism	Exposure phase	Fuel tested	Reference	Findings regarding (eco)toxicological effects
Oral toxicity (body weight/organ weight, hematological parameters, histopathology, serum analysis)	male Sprague-Dawley rats	fuels themselves	RME, SME, FraME, ULSD	**Poon et al. (2009)[17]**	ULSD induced more effects than the biodiesels.
Oral toxicity (body weight/organ weight, hematological parameters, histopathology, serum analysis)	male Sprague-Dawley rats	fuels themselves	RME, SME, fish oil methyl ester, LS-DF (317 ppm)	**Poon et al. (2007)[18]**	The overall treatment-related effects were mild (for DF and all biodiesels). Ranking of treatment effects (LS-DF > SME> RME> FME)
1. Acute oral toxicity test 2. Acute dermal toxicity test	1. male and female albino rats 2. male and female albino rabbits	fuels themselves	1. RME, REE, biodiesel blends (RME (50 %), REE (50 %), RME (20 %), REE (20 %), DF (low sulfur); 2. RME, REE, DF	**Reece et al. (1996)[22]**	1. LD_{50} was found to be higher than 5000 mg/kg; the number of clinical observations for the tested fuels increased with higher contents of diesel fuel. RME appeared to induce lesser effects than REE. 2. LD_{50} values were found to be greater than 2000 mg/kg for all fuels. For REE least severe effects were observed.
Acute oral toxicity test	male and female albino rats	fuels themselves	REE	**Varsho et al. (1996)[23]**	The concentration applied caused no mortality (LD_{50} > 5000 mg/kg), remarkable changes in body weights or changes regarding the major organ systems of the cranial, thoracic and abdominal cavities. Wet yellow urogenital staining was noted for three animals only at the first day after dosing. Further clinical observations were not reported.

Endpoints and biotest	Tester strain, cell culture or test organism	Exposure phase	Fuel tested	Reference	Findings regarding (eco)toxicological effects
Cytotoxicity					
Neutral red assay	L929 mouse fibroblast cell line	extracts of PM	RME, DF	**Bünger et al. (2000)**[3]	An increase in cytotoxic effects was found for RME compared to DF. Differences between RME and DF more pronounced at "idling" than at "rated" power.
Neutral red assay	L929 mouse fibroblast cell line	extracts of PM	RME, DF	**Bünger et al. (1998)**[4]	No significant differences in cytotoxicity between RME and DF exhaust extracts.
MTT assay	Mouse RAW264.7 macrophages	extracts of PM	RME, hydrotreated fresh vegetable oil, DF (EN590)	**Jalava (2010)**[8]	All samples decreased cell viability in a dose-response relation. No significant differences were found between the fuels at a concentration of 150 μg/mL. Relative responses were slightly stronger for diesel fuel and HVO compared to RME.
LDH cytotoxicity assay	Mouse macrophage cell line RAW264.7	extracts of PM and combustion aerosols	biodiesel (EN14214), blends (B20, B10, B5), RSO, DF (sulfur 10 mg/kg)	**Kooter (2011)**[10]	B100 significantly increased cytotoxicity. The emission extract of pure plant oil and the blends B5 and B10 significantly reduced the relative cytotoxicity compared to DF (without the application of a diesel particulate filter).
Respiration and enzyme activity of soil dehydrogenases	soil microorganisms	fuels themselves	biodiesel (E DIN 51606), DF (LST EN 590)	**Lapinskiene et al. (2006)**[24]	Respiration of microorganisms and activity of dehydrogenases in soil increased with increasing biodiesel concentrations. A decrease in respiration and activity of dehydrogenases in soil was found for DF concentrations higher than 3 % w/w.
MTT assay	human bronchial epithelium cells (BEAS-2B)	gaseous extracts	B10 (10 % palm fatty acid methyl ester), DF (sulfur content 30 ppmw)	**Liu et al. (2009)**[25]	Indications for a stronger induction of cytotoxic effects by B10 compared to DF.
MTT assay	human bronchial epithelium cells (BEAS-2B)	extracts of PM and semi-volatile fraction	palm oil methyl ester, DF (sulfur 30 ppm) and biodiesel-diesel blends (10, 30, 50 and 75 % v/v)	**Liu et al. (2008)**[26]	Semi-volatile extracts of the diesel blends induced higher toxicity than the particle extracts. Particulate extracts were not considered to be cytotoxic. Semi-volatile extracts of B50 induced strongest inhibitory effects.
MTT assay	human lung adenocarcinoma A549 cell line	extracts of condensates, PM and semi-volatile compounds	gasoline and methanol (100 %)	**Zhang et al. (2007)**[15]	Gasoline engine exhaust induced stronger cytotoxic potential compared to methanol engine exhaust

Endpoints and biotest	Tester strain, cell culture or test organism	Exposure phase	Fuel tested	Reference	Findings regarding (eco)toxicological effects
Acute aquatic toxicity					
Algae toxicity	*Selenastrum capricornutum*	WSFs	RME, eRME, SuMe HoSuME, DF	**Gateau et al. (2005)**[21]	The lowest EC_{50} (interpolated) was determined for DF.
Bacteria toxicity	*Pseudomonas putida*	WAFs	RME, eRME, SuMe, HoSuME, DF	**Gateau et al. (2005)**[21]	Tthe lowest EC_{50} (interpolated) was determined for DF.
Daphnid acute toxicity test	*Daphnia magna*	WSFs	RME, eRME, SuMe, HoSuME, DF	**Gateau et al. (2005)**[21]	The lowest EC_{50} (interpolated) was determined for DF.
Daphnid acute toxicity test	*Daphnia magna*	WAFs and oil in water dispersion	Biodiesel derived from rapeseed oil, soy oil or waste fry oil composed of animal fat; LSD, ULSD (sulfur content < 15 ppm)	**Hollebone et al. (2008)**[27]	**OWD**: Biodiesels (derived from rapeseed oil and waste fry oil) were less toxic than the petro diesels. Results of petro diesels and biodiesel derived from soy oil did not differ. **WSF:** Ranking of the toxicity with regard to LC_{50} values: ULSD > biodiesel derived from waste fry oil and soy oil > biodiesel derived from rapeseed oil and LSD. The confidence intervals of ULSD, biodiesel derived from waste fry oil and biodiesel derived from soy oil were overlapping.
Daphnid acute toxicity test	*Daphnia magna*	oil in water dispersion	biodiesel derived from recycled cooking oils, B20 (Topia), DF	**Khan et al. (2007)**[28]	Lowest LC_{50} value was found for DF, followed by B5, B50, B20 and B100 but the 95 % confidence intervals overlapped. LC_{50} values determined for B20 and Topia B20 indicate a higher toxicity for B20, however, the LC_{50} values are in the range of the 95 % confidence interval of the other biofuel blend.
Daphnid acute toxicity test	*Daphnia magna*	fuels themselves (+/- dispersion)	RME, REE, SME and DF	**Reece et al. (1996)**[22]	Biodiesels revealed higher LC_{50} values than DF with RME beeing more toxic than REE and SME. However, some of the mortality may have been caused by physical nature of the fuels.
Fish acute toxicity	*Brachydanio rerio*	WAFs	RME, eRME, SuMe, HoSuME, DF	**Gateau et al. (2005)**[21]	The lowest LC_{50} (interpolated) was determined for DF.
Fish acute toxicity	*Oncorhynchus mykiss*	oil in water dispersions	Biodiesel derived from rapeseed oil, soy oil or waste fry oil composed of animal fat; LS-DF, ULSD (sulfur content < 15 ppm)	**Hollebone et al. (2008)**[27]	All biodiesels were less toxic than the petrodiesels (statistically significant). Ranking for the toxicity of the biodiesels: Soy biodiesel > waste fry oil composed of animal fat biodiesel > rapeseed oil biodiesel

Endpoints and biotest	Tester strain, cell culture or test organism	Exposure phase	Fuel tested	Reference	Findings regarding (eco)toxicological effects
Fish acute toxicity	*Oncorhynchus mykiss*	fuel stirred into water	biodiesel derived from recycled cooking oils and blends with 5, 20 and 50 % biodiesel content, B20 (Topia), DF	**Khan et al. (2007)[28]**	Diesel and biodiesel blends induced similar effects on trout fry. LC_{50} values calculated for B100 were higher than those determined for DF indicating a lower acute toxicity to trout fry. LC_{50} values determined for B20 and Topia B20 indicate a higher toxicity for B20, however, the LC50 values were in the range of the 95 % confidence interval of the other biofuel blend.
Fish acute toxicity	*Rainbow trout*	fuels themselves (+/- dispersion)	RME, REE, SME, diesel blends [RME (50 %), REE (50 %), RME (20 %) and REE (20 %)], DF	**Reece et al. (1996)[22]**	LC_{50} values could not be determined at the concentrations and test conditions used.
Fish acute toxicity	Bluegill	diesel engine exhaust laden water	SME, DF	**Womac et al. (1996)[29]**	Results indicated higher effects at highest concentration but no dose related response. Low mortality rates were observed for both fuels at lower concentrations. Highest test concentrations revealed a high mortality but these concentrations were considered to be higher than they would occur from a marine vessel.
Microtox test	*Vibrio fischeri*	oil in water dispersions	biodiesel derived from rapeseed, soy or waste fry oil composed of animal fat; LS-DF, ULSD (sulfur: < 15 ppm)	**Hollebone et al. (2008)[27]**	All biodiesels were less toxic than the petro diesels (statistically significant). Amoung the biodiesels, the most toxic fuel was the biodiesel derived from soy oil.
Microtox test	*Vibrio fischeri*	extracts of PM and semi-volatile fraction	palm oil methyl ester, DF (sulfur content 30 ppm) and biodiesel-diesel blends (10, 30,50 and 75 % v/v)	**Liu et al. (2008)[26]**	Semi-volatile extracts of the biodiesel and biodiesel blends indicate higher TUVs (toxicity unit per liter exhaust sampled) compared to the particulate extracts (in contrast to diesel exhaust extracts). Results (data after 5 min) of the semi-volatile extracts indicated higher toxicity for biodiesel and biodiesel blend extracts compared to diesel extracts. Diesel PM with higher or equal acute toxicity compared to palm oil methyl ester or diesel-biodiesel blend PMs (according to TUV_5). Higher acute toxicity of diesel PM compared to all other biodiesel or blends (according to TUW, toxicity unit per µg soluble organic fraction (SOF) of particulate).

Endpoints and biotest	Tester strain, cell culture or test organism	Exposure phase	Fuel tested	Reference	Findings regarding (eco)toxicological effects
Microtox test	*Vibrio fischeri*	gaseous extracts	B10 (10 % palm fatty acid methyl ester), DF (sulfur:30 ppmw)	**Liu et al. (2009)**[25]	Results indicated a higher acute toxicity of B10 compared to diesel. The toxicity was affected by the loading mode.

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