

Fuel/Fuel Additives Rule and of Testing of Biodiesel

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Overview

- Brief description of 211 Fuel/Fuel Additive Rule, promulgated on May 27, 1994
- Design and results of testing of biodiesel

Fuels/Fuel Additives Rule (40 CFR Part 79)

- Legislative mandate
- Clean Air Act section 211(b and e) gives EPA authority to **require F/FA manufacturers** “to conduct tests to determine potential public health effects of such fuel or fuel additive”
- Objectives:
 - “Establish registration requirements which will provide information for identifying and evaluating the potential adverse effects of designated F/FA emissions...”
 - Test for effects of **inhalation** to F/FA **combustion** and **evaporative** emission mixtures

Structure of 211 Tests

- Tier 1:
 - Literature search for existing health effects data
 - Emission characterization
 - Production/use data
- Tier 2: toxicity screening for specified endpoints (combustion and/or evaporative emissions)
- Alternative Tier 2: more extensive testing in lieu of, or in addition to, standard Tier 2
- Tier 3: more extensive follow-up testing as needed

Tier 2 Standard Requirements

90-day inhalation study for toxicity screening of combustion and/or evaporative emissions for

Overall health:

food consumption, body weight, ophthalmology, hematology, urinalysis

Pulmonary toxicity:

lung histology and pathology

Neurotoxicity:

morphologic changes in CNS, level of glial fibrillary acidic protein (GFAP) in CNS regions (associated with astrocytic hypertrophy)

Fertility/teratogenicity:

gonadal function, fertility, conception, fetal abnormalities, histopathology of reproductive organs

Tier 2 Standard Requirements

(cont.)

90-day inhalation study for toxicity screening of combustion and/or evaporative emissions for

Mutagenicity/carcinogenicity

- Micronucleus (MN): measures frequency of micronucleated bone marrow cells
- Sister chromatid exchange (SCE): measures exchange of DNA between two sister chromatids in lymphocytes



In vitro mutagenicity:
Salmonella Thyphimurium
assay measuring histidine reversions,
which are caused by DNA mutations

F/FA Tested under 211 Rule

Standard Tier 2

- Biodiesel
- Diesel with water emulsion (Puri NOx)

Alternative Tier 2

- Gasoline
- Gasoline containing MTBE, ETBE, TAME, Ethanol, DIPE, tert-butanol
- Manganese compounds (MMT)

Exposure System for Tier 2 Testing of Biodiesel

- 1998 Cummins heavy-duty engine operated following the EPA HD transient burning biodiesel (soybean-based)
- Chamber exposure adjusted to match target NO_x concentrations of 5, 25, and 50 ppm
- Exhaust dilution ratio 5:1 or greater

Summary of Chamber Exposure

	Control	Low Exp	Med Exp	High Exp
NO_x (ppm)	1 ± <1	5 ± 1	26 ± 3	51 ± 5
NO₂ (ppm)	1 ± 4	1 ± <1	1 ± <1	2 ± 1
PM (μg/m³)	17 ± 22	40 ± 30	200 ± 100	500 ± 100
CO (ppm)	0.5 ± 0.5	2.2 ± 1.2	15.2 ± 0.5	36.8 ± 10.2
SO ₂ (ppm)	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.1
HC (ppm)	0.1 ± 0.2	0.1 ± 0.2	0.3 ± 1.4	0.5 ± 1.0

Study Protocol for Tier 2 Testing of Biodiesel

- 90-day exposure, 6 hrs/day, 5 days/week,
- 3 concentration levels plus clean air
- 120 males (♂) and 220 females (♀) F344 rats
- 10 ♂ + 10 ♀ positive controls (acrylamide and dimethylbenzanthracene)
- Tests conducted at Lovelace Respiratory Research Institute

Animals Assigned to Endpoints

	Control	Low Exp	Inter Exp	High Exp
General Histology	15♂+15♀ (all organs)	15♂+15♀ (lungs only)	15♂+15♀ (lungs only)	15♂+15♀ (all organs)
Special Histology	5♂ + 5♀ (all organs)	5♂ + 5♀ (lung, brain, repro organs)	5♂ + 5♀ (lung, brain, repro organs)	5♂ + 5♀ (all organs)
GFAP, MN, SCE	5♂ + 5♀	5♂ + 5♀	5♂ + 5♀	5♂ + 5♀
Fertility, Repro	25♀	25♀	25♀	25♀
Recovery				10♂+10♀

Biodiesel Tier 2 Testing

Reported results

- **No significant exposure-related effects on**
 - Food consumption, clinical condition
 - Mortality
 - Ophthalmology
 - Micronucleus, sister chromatid exchange
 - Neuropathology: brain weight and histopathology, GFAP (↓ weight in LE)
 - Reproduction (fertility, reproductive organs' histopathology, teratology)

Biodiesel Tier 2 Testing

Reported results (cont.)

- **Statistically significant effects on**

Lungs:

- dose-related increase in macrophages containing particles
- minimal-to-mild alveolar macrophage hyperplasia
- higher relative lung weights in high level females
- alveolar bronchiolarization in high level females (4/30)
- alveolar histiocytosis in high level females (4/30)

Biodiesel Tier 2 Testing

Reported results (cont.)

- **Statistically significant effects on**

Other organs:

- lower liver weights, higher testis weights at high level
- various clinical chemistry and hematology changes without related pathology

Salmonella assay: positive mutagenicity with particulate and semi-volatile extracts

- **Other findings**

- particles found nearly exclusively in cytoplasm of alveolar macrophages

Conclusions

- EPA has accepted the study as being "complete" and therefore satisfying the Tier 2 requirements, but has not yet completed a review of the reported results.
- Reports of Biodiesel tests can be found at <http://biodiesलगear.com/documentation/>