

In Vitro Genotoxicity
of Particulate
and Semi-Volatile Organic Compound
Exhaust Materials from a Set of
Gasoline and a Set of Diesel Engine
Vehicles Operated at 30⁰ F

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The Analysis of Genotoxic Activities of Exhaust Emissions from Mobile Natural Gas, Diesel, and Spark-Ignition Engines

An Interagency Agreement Study by

the US Centers for Disease Control and Prevention –
National Institute for Occupational Safety and Health

and

the US Department of Energy –
Office of Freedom Car and Vehicle Technologies

Engine Exhaust Samples

Gasoline or diesel engine autos and light-duty trucks

chassis dynamometer run at 30⁰F ambient,
California Unified Driving Cycle (SwRI)

- Exhaust particulate was filter collected, acetone wash recovered
- SVOC was sorbent resin-collected, acetone extraction recovered (DOE-NREL, LRRI, DRI)
- Standard comparison = NIST standard diesel exhaust particulate 1650a
(1980's technology automotive diesel particulate)

In vitro genotoxicity assays

Gene mutation -

Salmonella typhimurium reverse mutation

Chromosomal damage -

micronucleus induction in V79 mammalian cells

DNA damage -

single-cell gel electrophoresis for single and double DNA strand breaks in V79 cells

Mutagenicity Assay: “Ames” *Salmonella typhimurium* histidine reversion test

- Test for reverse-mutation to histidine independence
- Tester strains YG 1024 and YG 1029 for frameshift and for base-pair substitution mutations
- +/- S9 microsomal enzyme activation of the test materials
- Acetone extracts of materials assayed as suspensions in Tween 80

Salmonella test protocol

Experiments for each sample:

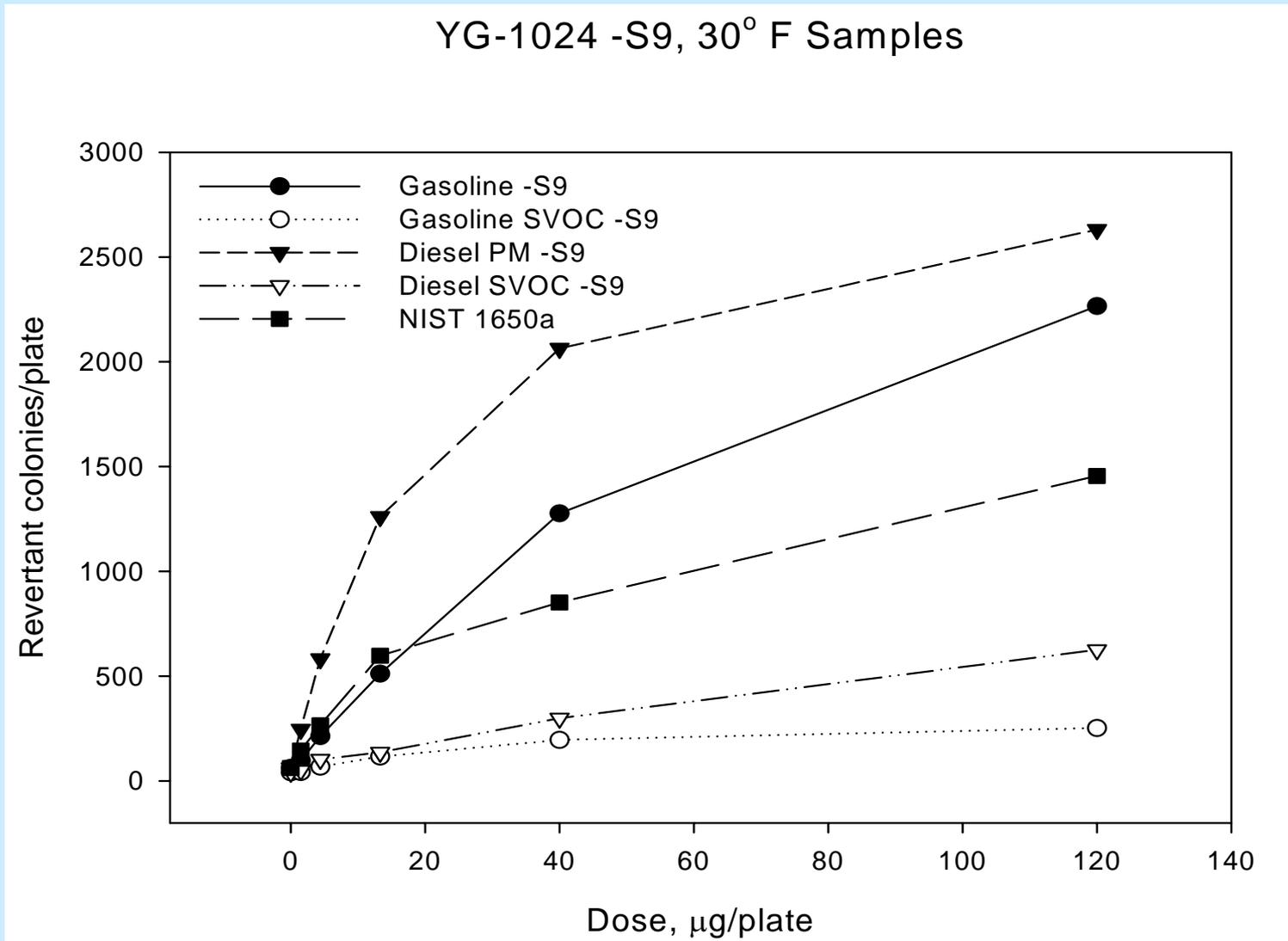
- attempt 5 doses for each sample
- doses spanned range to toxicity
- 2 plates read for each sample/dose
- 3 colony counter readings for each plate, 1 recorded
- 2 replicate experiments

Mutagenicity (Gene Mutation) of Particulate Fractions 30⁰F operation^a

| Sample Concentration (µg/plate) | | YG1024 | | YG1029 | |
|------------------------------------|-------|----------|-----------|----------|----------|
| | | -S9 | +S9 | -S9 | +S9 |
| Tween 80 | 700 | 62±16 | 66±11 | 99±6 | 97±24 |
| 2-AA | 0.05 | 143±100 | 1863±1746 | 109±8 | 2656±127 |
| 1-NP | 0.5 | 1382±322 | 2519±1152 | 835±113 | 2214±206 |
| D6 | 1.48 | 97±9 | 132±35 | 110±9 | 140±6 |
| D6 | 4.44 | 214±64 | 240±116 | 159±15 | 247±8 |
| D6 | 13.3 | 512±138 | 592±229 | 295±27 | 499±49 |
| D6 | 40.0 | 1277±188 | 1166±424 | 570±7 | 903±55 |
| D6 | 120.0 | 2266±143 | 1890±516 | 1149±119 | 1255±29 |
| D7 | 1.48 | 246±19 | 248±107 | 131±22 | 247±17 |
| D7 | 4.44 | 582±86 | 680±337 | 229±10 | 637±64 |
| D7 | 13.3 | 1261±319 | 1558±751 | 491±74 | 1365±171 |
| D7 | 40.0 | 2063±740 | 2248±1321 | 1349±206 | 2219±77 |
| D7 | 120.0 | 2631±951 | 2488±1323 | 2143±328 | 2543±290 |

^a Average number of revertant colonies per plate

Mutagenicity (Gene Mutation) of diesel and gasoline exhaust



Mutagenicity (Gene Mutation) slope estimates
(revertants/ug extract)
30°F

| | YG1024 | | YG1029 | |
|----------------|--------|------|--------|------|
| | -S9 | +S9 | -S9 | +S9 |
| D6 Gas PM | 33.8 | 39.5 | 14.7 | 30 |
| D6 Gas SVOC | 5.6 | 2.5 | 6.8 | 2.8 |
| D7 Diesel PM | 90.1 | 164 | 29.5 | 95 |
| D7 Diesel SVOC | 7.1 | 9.2 | 8.5 | 13.8 |
| NIST | 54 | 48.1 | 22.3 | 51.5 |

Mutagenicity (Gene Mutation) slope estimates:
 (revertants/ug) or (revertants/mile)
 and
 ug-to-miles normalization factors

| | YG1024-S9 | | YG 1024+S9 | | YG1029-S9 | | YG1029+S9 | | Nor |
|-----------|-----------|-----------|------------|-----------|-----------|-----------|-----------|-----------|------|
| | Rev/ug | kRev/mile | Rev/ug | kRev/mile | Rev/ug | kRev/mile | Rev/ug | kRev/mile | |
| Gas PM | 33.8 | 574.6 | 39.5 | 671.5 | 14.7 | 250 | 30 | 510 | 17 |
| Gas SVOC | 5.6 | 37 | 2.5 | 16.5 | 6.8 | 44.9 | 2.8 | 18.5 | 6.6 |
| Diesel PM | 90.1 | 14506 | 164 | 26404 | 29.5 | 4750 | 95 | 15295 | 161 |
| Dsl. SVOC | 7.1 | 339.7 | 9.2 | 440.2 | 8.5 | 406.7 | 13.8 | 660.3 | 47.9 |
| NIST | 54 | NA | 48.1 | NA | 22.3 | NA | 51.5 | NA | NA |

Results of Mutagenicity (Gene Mutation) Assays

All samples, D6, D7, SVOC6, SVOC7, NIST, were positive for both tester strains, +/- S9 activation

- Particle extract mutagenic activities, on a mass basis:
D7 (diesel) > NIST > D6 (gasoline)
D7 approximately 2X to 3X the activity of D6
- Particle extract mutagenic activities, on a mileage basis:
D7 (diesel) were on the order of 10- to 30-fold greater than for D6 (gasoline)

Particle extract activities generally were an order of magnitude greater than SVOC activities

- On a mass basis, SVOC 7 activity was about 1X to 3X that of SVOC 6
- On a mileage basis, SVOC 7 was on the order 10X to 30X greater than SVOC 6

Pro-mutagens

- YG1024 → frameshift mutations
- YG1029 → base pair substitutions and frameshift mutations

- D6 / YG1024 → little change in activity with +S9 activation
- D6 / YG1029 → ca. 50% increase +S9
- D7 / YG1024 → little change +S9
- D7 / YG1029 → ca. 100% increase +S9

- Indicates pro-mutagens in D6, D7 for base-pair substitution mutations

- SVOC7 / YG1029 → small increase +S9

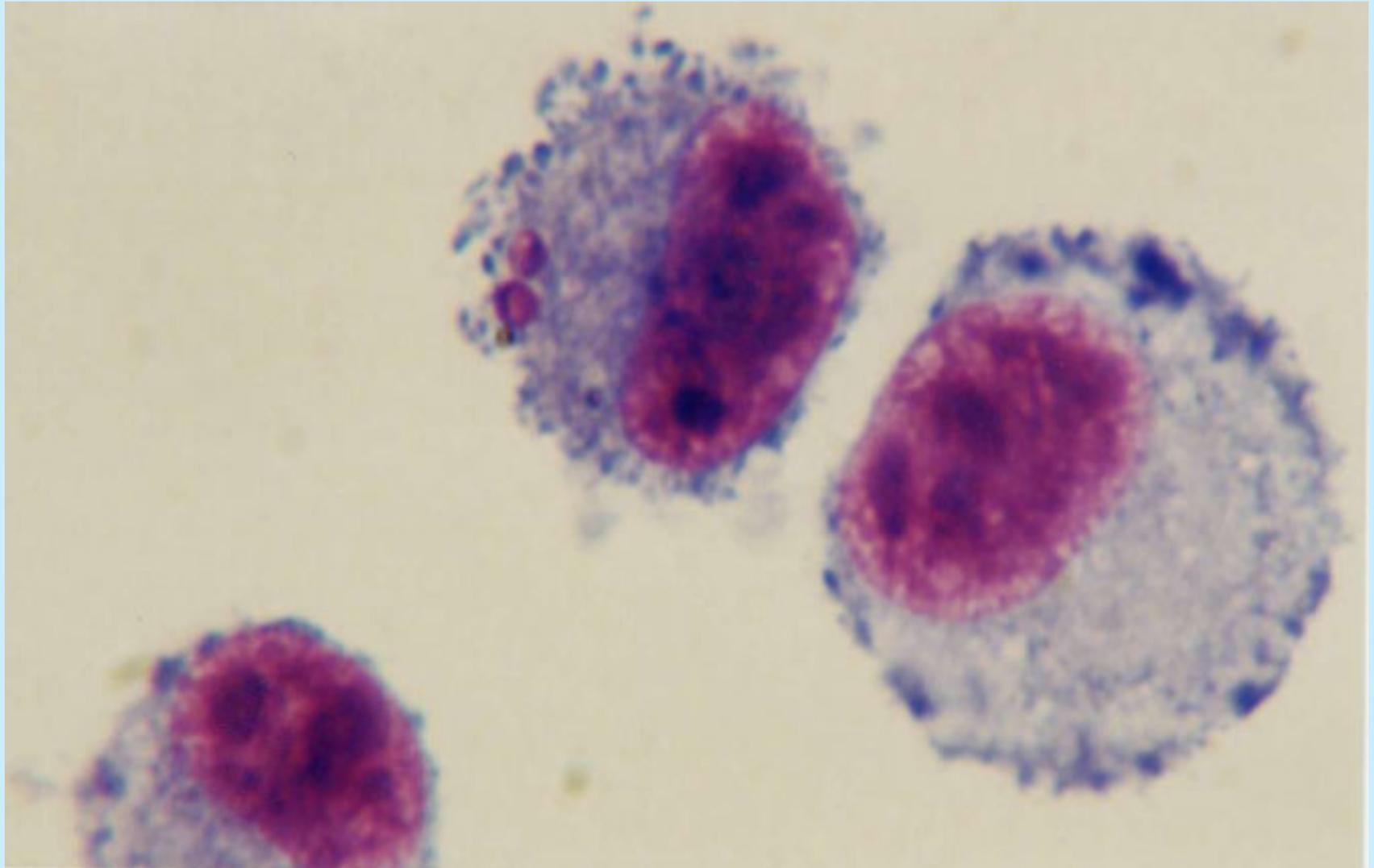
- NIST / YG1029 → ca. 75% increase +S9

Chromosomal Damage Assay

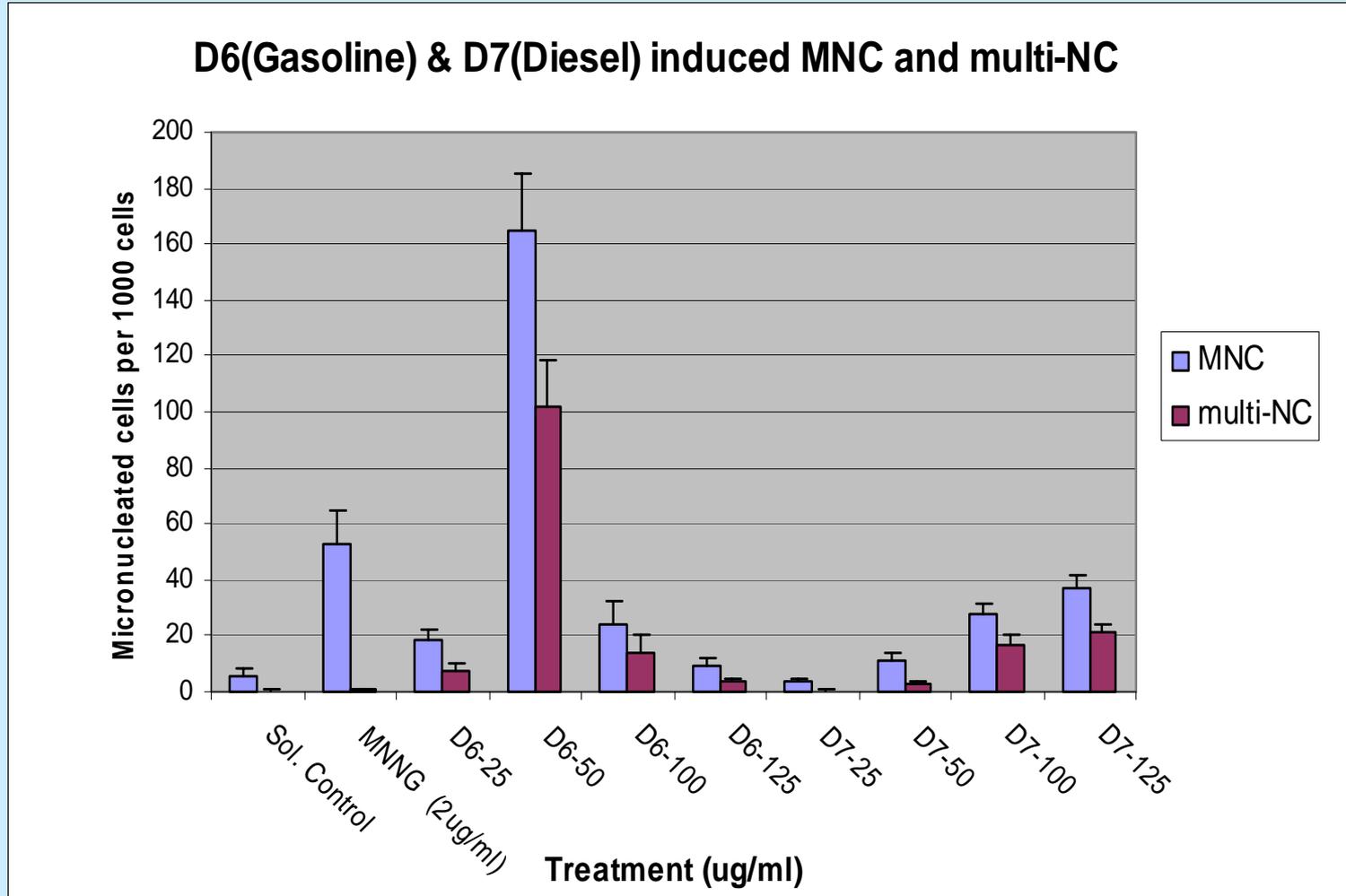
Induction of Micronucleated V79 mammalian cells *in vitro*

- Cells incubated in complete medium
- Cells challenged 24h
- Cells challenged at 4 or more sample concentrations, up to evident cellular toxicity
- Cells harvested, fixed, prepared by cytopsin on microscope slides, stained
- 4 slides read for each concentration
- Experiment repeated
- 3000 cells total scored for each concentration

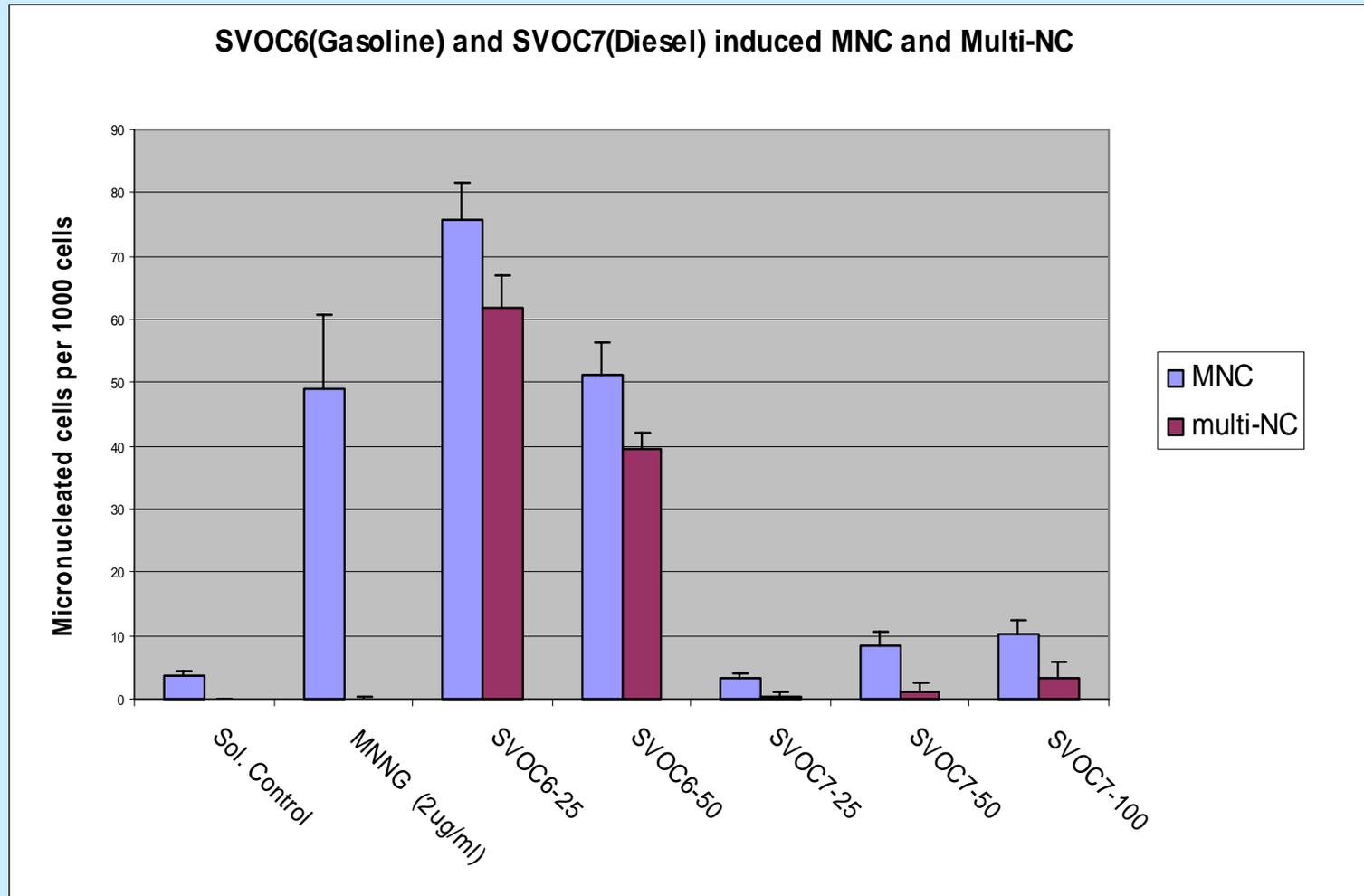
Micronucleus induction in V79 cells



Micronucleus Induction - Chromosomal damage



Micronucleus Induction - Chromosomal damage



Chromosomal damage assay micronucleus induction in V79 Cells

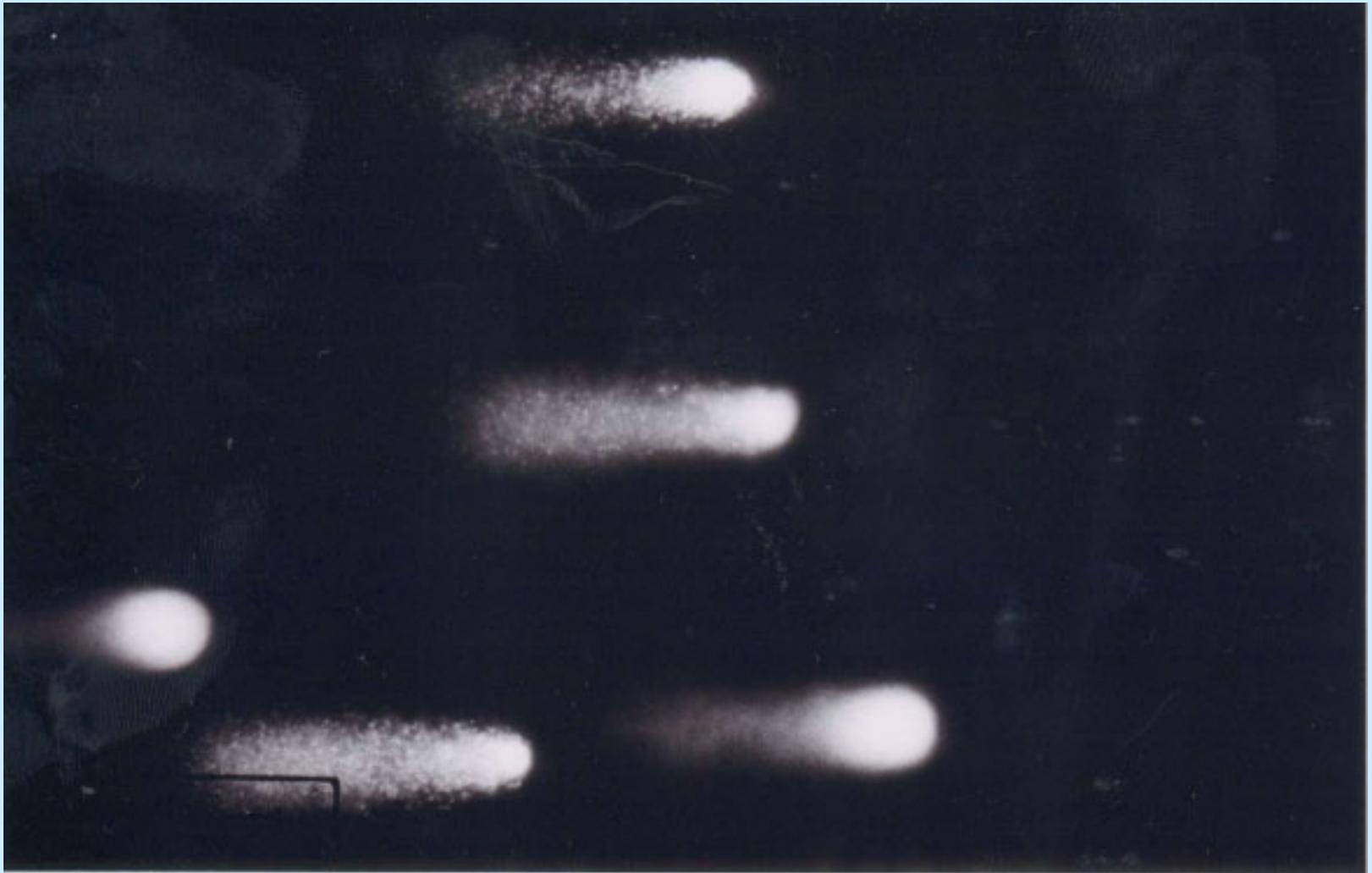
D6 PM and SVOC 6 strongly active for micronucleus induction at intermediate doses; toxicity interference at high doses,
indicative of chromosomal breaks and/or spindle damage

D7 PM and SVOC 7 relatively weakly active at higher doses

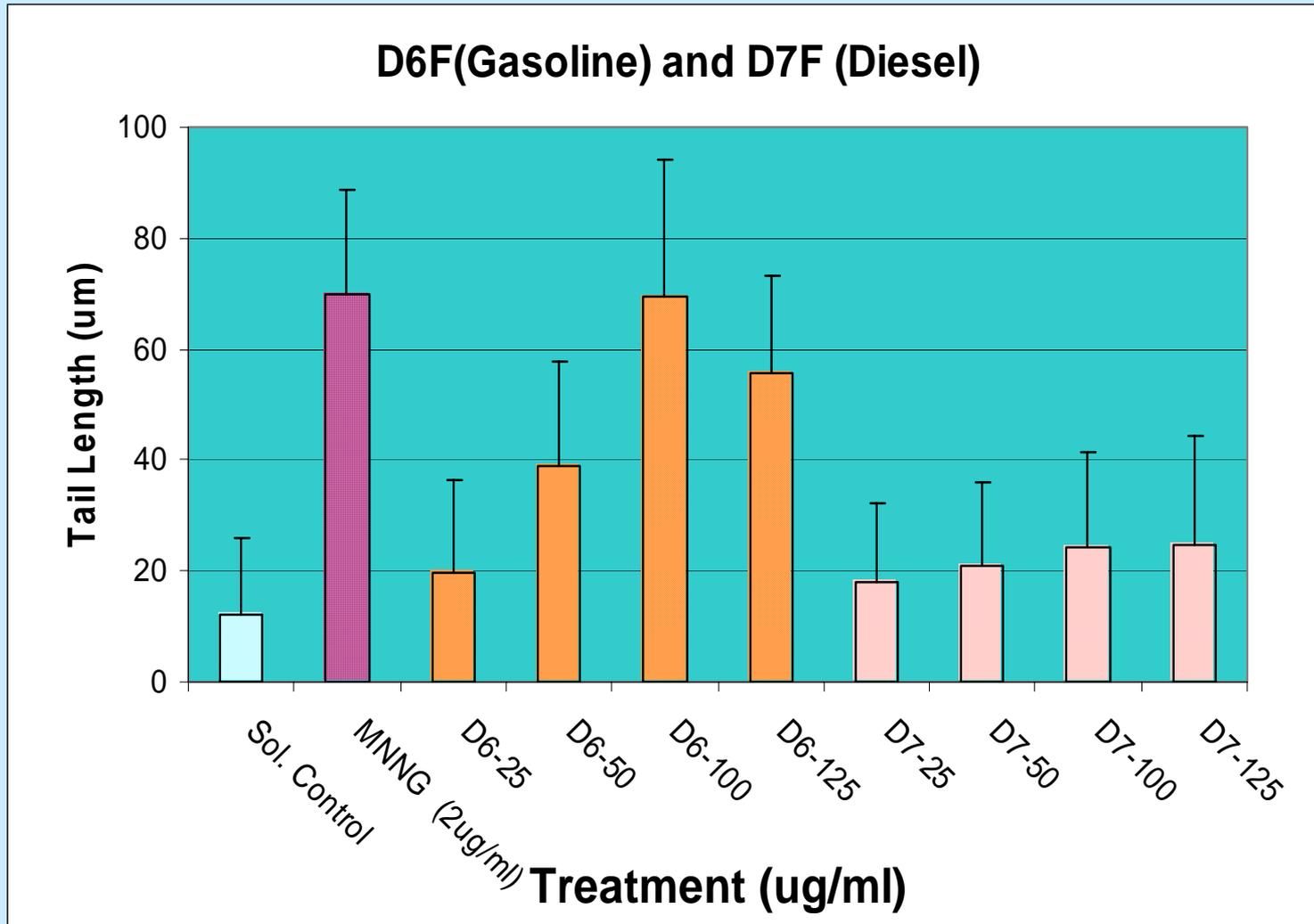
DNA Damage Assay

- Single-cell gel electrophoresis (SCGE) assay (the “Comet” assay)
- Assay for single-and double-stranded DNA breaks in V79 cells:
electrophoretic migration patterns for DNA fragments
- Results expressed as electrophoretic pattern tail length
- 2 Experiments
- 100 cells total read at each concentration

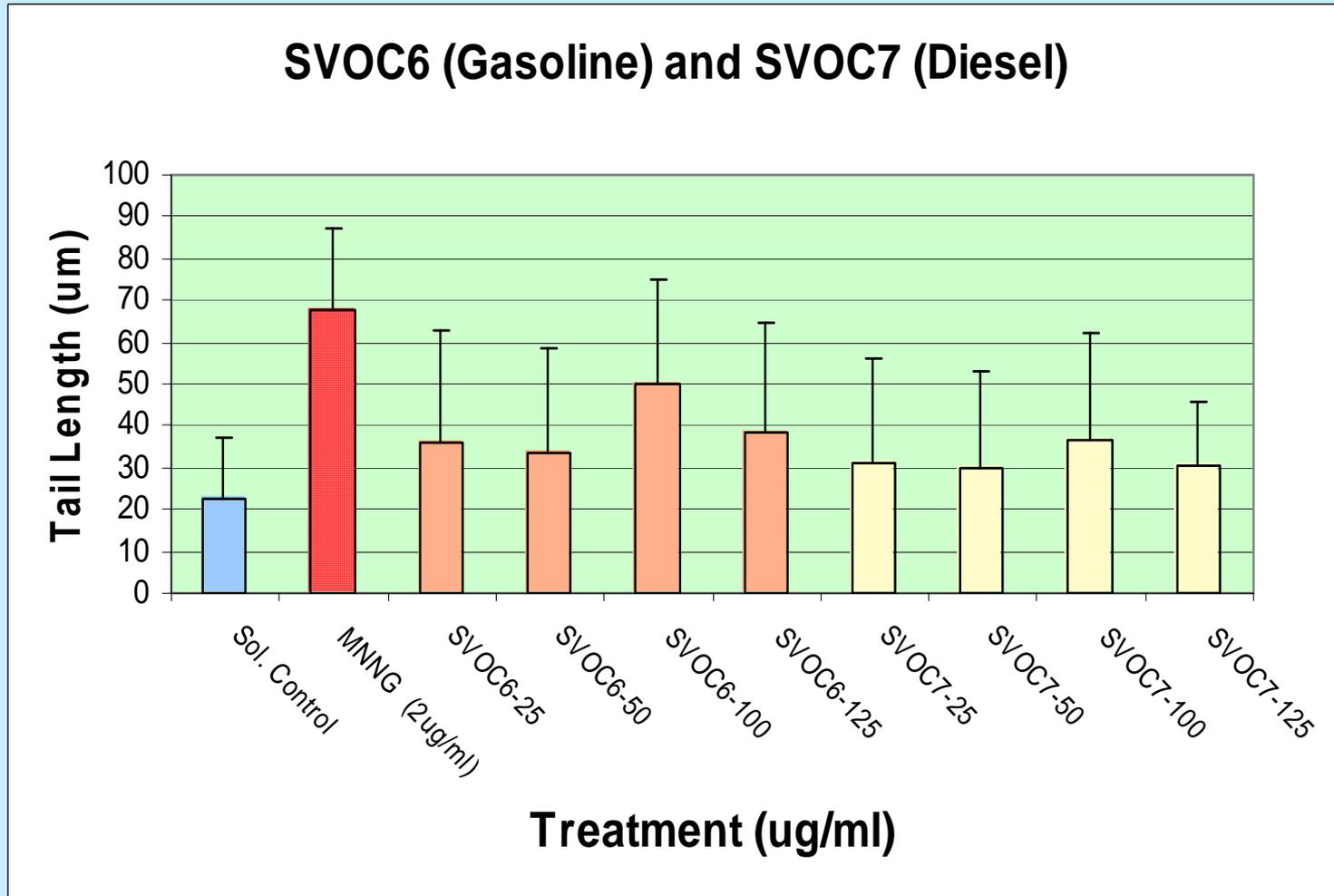
Single cell gel electrophoresis (*Comet*) assay for DNA damage in V79 cells



DNA Damage by Single Cell Gel Electrophoresis



DNA Damage by Single Cell Gel Electrophoresis



Results of DNA damage assays

- D6 PM extract positive for DNA damage at all but the lowest dose (Single-cell gel electrophoresis assay) with some toxicity effect at highest dose
- SVOC 6 weakly active
- D7 PM weakly active at highest doses, inactive at lowest doses
- SVOC 7 weakly active

Assay Summary/Interpretation

30⁰F operation

Gene Mutation:

Diesel 2X to 3X > gasoline/ug extract

Diesel > NIST > gasoline

Diesel 10X to 30X > gasoline/mile

Assay Summary/Interpretation

30⁰F operation

Mammalian cell genotoxicity:

- Gasoline PM **strongly active for DNA damage and for chromosomal damage**
- Diesel PM weakly active for DNA damage and for chromosomal damage
- Gasoline SVOC weakly active for DNA damage; **strongly active for chromosomal damage**
- Diesel SVOC inactive or weakly active for DNA damage or chromosomal damage

Qualitative Summary

30⁰F operation

| Sample | Gene mutation | DNA Damage | Chromosomal Damage |
|---------------|---------------|------------|--------------------|
| Diesel PM | + | weak | weak |
| Gasoline PM | + | + | + |
| Diesel SVOC | weak | weak | weak |
| Gasoline SVOC | weak | weak | + |

Qualitative Summary

72°F operation

| Sample | Gene mutation | DNA Damage | Chromosomal Damage |
|---------------|---------------|------------|--------------------|
| Diesel PM | + | (+) toxic | - |
| Gasoline PM | + | + | + |
| Diesel SVOC | weak | - | - |
| Gasoline SVOC | weak | (+) toxic | + |

Engine Operating Parameters Affect the *In Vitro* Genotoxicity of Diesel Exhaust Particulate Extract

Some NIOSH / DOE collaborative studies

- US Dept. of Energy/METC-90/6110 DE90000480 (1990)
- U.S.Dept. Energy/METC-91/6122; DE91002091 (1991)
- McMillian MH,et al. Society of Automotive Engineers Technical Paper 2002-01-1699, pp. 1-18. (2002).

Quadratic response surface:

Mutagenicity (gene mutation) vs. engine speed and load

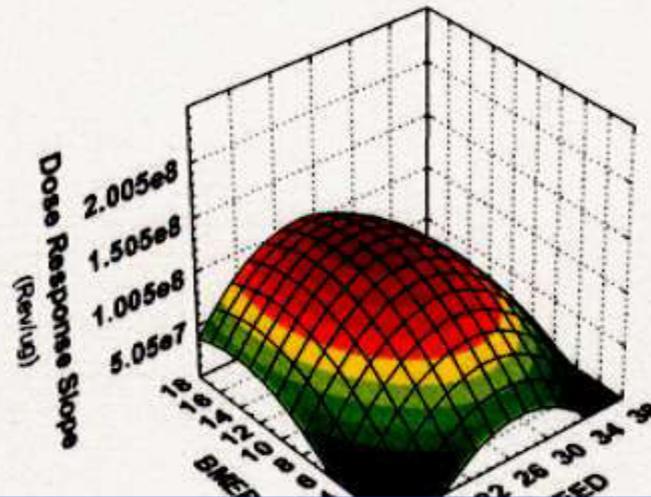
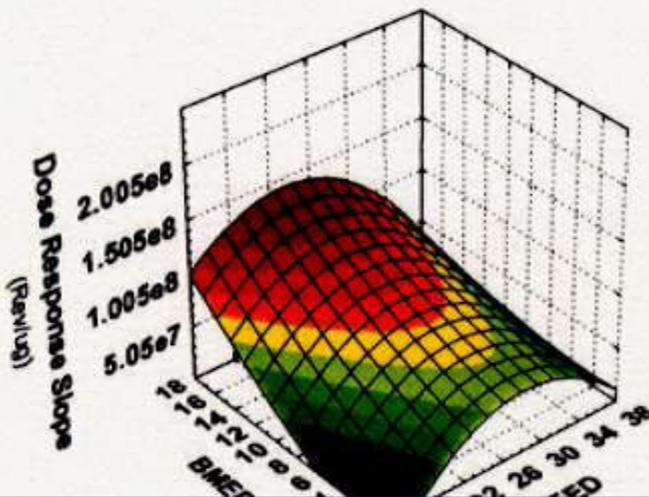
McMillian MH, et al. Society of Automotive Engineers Technical Paper 2002-01-1699, pp. 1-18.
(2002).

FUEL: DF2 $z = -3.212e8 + 2.692e7 \cdot x + 9.162e6 \cdot y - 4.783e5 \cdot x^2 - 2.544e5 \cdot x \cdot y + 1.186e5 \cdot y^2$

FUEL: FT $z = -4.321e8 + 3.487e7 \cdot x + 1.937e7 \cdot y - 6.486e5 \cdot x^2 - 1.844e5 \cdot x \cdot y - 5.983e5 \cdot y^2$

X-axis: Speed

Y-axis: BMEP



Are data on the genotoxic activities of organic solvent extracts of exhaust particulate physiologically meaningful?

Background Finding by DOE, EPA, Industry

- *Organic solvent extracts* of Diesel Exhaust Particulate cause in vitro damage to genetic material.
- *Lung lining fluid – pulmonary surfactant- extracts* of Diesel Exhaust Particulate do not cause significant damage in vitro.
- Question:
is the organic genotoxicant material in particulate exhaust soot biologically-available in the lung?

NIOSH Findings: bioavailability of soot genotoxicants in lung fluids

Some diesel exhaust whole particulate material dispersed in lung surfactants induce in vitro genetic damage:

Mutation in bacterial cells

J. Tox. Env. Health, 21:163-171 (1987)

Environmental Hygiene II, pp. 7-10; Springer-Verlag, Berlin (1990), ISBN 0-387-52725-4

J. Environ. Sci. Health, A28:505-523 (1993)

ref. in : IARC Monograph 46 (1989)

DNA or Chromosomal damage in mammalian cells

Mutation Res. 260:233-238 (1991)

Mutation Res. 279:55-60 (1992)

Ann. Occ. Hyg. 38:345-349 (1994)

Air. Poll. Health Eff. Lab., Report 99-01, U. Cal. Irvine, pp. 611-616 (1999)

Lung surfactant-mediated genotoxicity of gasoline or advanced fuel engine exhausts has not yet been measured

Conclusions and directions

- Diesel and Gasoline engine exhaust particulate can contain genotoxic compounds
- DEP genotoxicant content is affected by fuel, engine operating condition
- DEP can express genotoxic activity *in vitro* under conditions modeling soot deposition in the lung

Conclusions and directions

- Biologically-available genotoxicant activity of engine exhaust particulate can be assayed in short-term tests in a physiologically-plausible manner
- Correlation can be sought with fuel, engine design and operation parameters
- To help evaluate and guide the development of fuels, engine design, and emission controls

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