The Mutagenic Effects of Crude Oil Fuels on Cell Mutation

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9th Grade
The Question

Do common crude oil fuels have significant mutagenic properties, and if so, which fuel is most carcinogenic?
Crude-Oil Fuels (used in this study)

• Regular Unleadled Gasoline
  • Petroleum derived fuel for internal combustion engines
  • Highest purchased fuel
  • Past studies show scant mutagenicity results

• Premium Unleadled Gasoline
  • Blended mixture of hydrocarbons (paraffins, napthenes, and olefins) with added chemicals
  • Increases gas mileage
  • Usually more expensive

• Diesel
  • A fractional distillate of petroleum
  • No fuel ignition requirement; larger vehicles
Previous Studies

United States Environmental Protection Agency (EPA)
• 1987 study of unleaded gasoline
• Used small animals, such as mice, as a model
• Found that unleaded gasoline did show minimal carcinogenic effects, but when analyzed did not show a significant change

Johns Hopkins University
• 2003 lab study
• Showed that petroleum increased cell reversion and decreased replication
• Sparked debate over crude oil carcinogenicity
The Experimental Model
-Lys Yeast Cells

• Most common cell model

• Reproduction, metabolism, and biochemistry similar to that of other advanced eukaryotic cells

• Normally, wild-type can anabolically synthesize lysine, an essential amino acid

• (-Lys) Yeast Cells have an alteration in a gene coding for one of the enzymes in a lysine anabolic pathway
The Ames Test

• Bruce Ames - tested the mutagenic properties of various chemicals by employing a (-His) bacteria

• Increased reversion rate correlated with mutagenicity

Modified Ames Test

• Number of reverted yeast colonies used to quantify mutagenicity
Ultraviolet Rays

• Waves which radiate from the sun at higher frequencies than visible light

• A well-known and established mutagen

• Standard to compare mutagenicicy results
Experimental Purpose

• To assess the mutagenicity of common crude oil fuels: regular unleaded gasoline, premium unleaded gasoline, and diesel

• Applications include cancer research and the creation of less mutagenic fuels
Hypothesis

Null Hypothesis
Exposure to the crude oil fuels regular unleaded gasoline, premium unleaded gasoline, and diesel will not significantly increase the reversion rate of (−lys) yeast cells.

Alternative Hypothesis
Exposure to the crude oil fuels regular unleaded gasoline, premium unleaded gasoline, and diesel will significantly increase the reversion rate of (−lys) yeast cells.
Materials List

- Com (-Lys) agar plates (Yeast Nitrogen Base 1% dextrose 2%, 1.5% agar, complete amino acid mix (minus lysine) 100mg/L
- UV Light Hood (LD-50 on Yeast in 30 seconds)
- (-) Lysine Saccharomyces cerevisiae (John Wolford Lab, Carnegie Mellon University)
- 100 mL of regular unleaded gasoline (purchased from same brand, station, etc as the other experimental crude-oil fuels)
- 100 mL of premium unleaded gasoline (purchased from same brand, station, etc as the other experimental crude-oil fuels)
- 100 mL of diesel fuel (purchased from same brand, station, etc as the other experimental crude-oil fuels)
- 100 mL of 100% ethanol solution
- 200mL SDF (Sterile Dilution Fluid)
- 2 Pipettes (200 microliter capacity and 1,000 microliter capacity, respectively)
- At least 15 Sterile Pipette tips (operable with the above pipettes)
- 1 Solution Vortex
- 1 Centrifuge
- At least 1 Sidearm Flask
- At least 1 Spreader Bar
- A Micro-burner
- 1 pair of Rubber gloves
- 7 Microtubes (10mL capacity)
- 1 Test tube rack
Experimental Procedure

1. A strain of yeast (-) Lys phenotype was grown for several days in YEPD media
2. A series of washes with SDF were performed on the sterile yeast pellet to remove any residual nutrients
3. The pellet in SDF was resuspended in –lys media twice
4. The pellet was left to sit for two days
5. The following liquid combinations were pipetted into sterile microtubes (12 total tubes one for each fuel type and concentration)
## Table of Liquid Concentrations

<table>
<thead>
<tr>
<th>Contents</th>
<th>0% (Control)</th>
<th>0.001%</th>
<th>0.1%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>100% SDF (Sterile Dilution Fluid)</strong></td>
<td>0.8mL</td>
<td>0.79mL</td>
<td>0.79mL</td>
</tr>
<tr>
<td><strong>Variable (Fuel Type)</strong></td>
<td>0mL</td>
<td>0.01mL (0.01% substock)</td>
<td>0.01mL</td>
</tr>
<tr>
<td><strong>Yeast</strong></td>
<td>0.2mL</td>
<td>0.2mL</td>
<td>0.2mL</td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
<td>1mL</td>
<td>1mL</td>
<td>1mL</td>
</tr>
</tbody>
</table>
Experimental Procedure (cont.)

6. The cells were allowed to sit for 5 minutes
7. 0.1mL aliquots from one of the variable tubes (ex. 0.001 regular unleaded) were spread onto 6 complete (-Lys) plates
8. 0.2mL aliquot from the same variable tube were spread onto 2 complete (-Lys) plates, resulting in 8 total replicates (trials) for this fuel and concentration
9. Repeat steps 6-7 with the other 6 variable tubes (including the control)
Experimental Procedure (cont.)

10. After spreading, cells were allowed to sit for 15 minutes
11. 8 complete (-Lys) plates each were exposed to 0 seconds, 15 seconds, and 30 seconds of UV radiation
12. All plates were allowed to incubate for 4 days at 32°C
13. The colonies were counted and recorded. Each colony was assumed to have risen from one cell (the stem cell/progenitor)
Mutagenic Comparison

\[
\begin{array}{ccccccccc}
& \text{Control} & 0.001\% \text{ Regular} & 0.001\% \text{ Premium} & 0.001\% \text{ Diesel} & 0.1\% \text{ Regular} & 0.1\% \text{ Premium} & 0.1\% \text{ Diesel} \\
\hline
\text{P-value} & 0.006239 \\
\end{array}
\]

Y-axis = number of reverted cell colonies

X-axis = concentration of variable
Statistical Analysis
The Dunnett's Test Explained

- This formula is called the Dunnett's Test

\[ t_d = \frac{M_i - M_c}{\sqrt{\frac{2MSE}{n_h}}} \]

- By substituting findings from the ANOVA analysis, the variable’s effect is determined

- In this experiment, a t-value of above 2.47 correlates with a significant increase in reversion
## Dunnett's Test Analysis

<table>
<thead>
<tr>
<th></th>
<th>Regular Unleaded</th>
<th>Premium Unleaded</th>
<th>Diesel</th>
</tr>
</thead>
</table>
| **0.001%** | • $t = \sim 0.632$  
• *No Significant Effect* | • $t = \sim 1.265$  
• *No Significant Effect* | • $t = \sim 3.794$  
• *Significant Effect* |
| **0.1%**   | • $t = \sim 2.951$  
• *Significant Effect* | • $t = \sim 2.53$  
• *Significant Effect* | • $t = \sim 6.323$  
• *Significant Effect* |

$t > t_{\text{crit}}$ = significant effect  
$t < t_{\text{crit}}$ = no significant effect
Regular Unleaded Gasoline
Mutagenic Comparison

Y-axis = number of reverted cell colonies

X-axis = concentration of variable

Mutagenic Comparison Regular Unleaded v. UV

P-value: 1.1E-07
P-value: 1.34E-06
P-value: 0.334282
P-value: 0.006053
Premium Unleaded Gasoline
Mutagenic Comparison

Y-axis = number of reverted cell colonies

X-axis = concentration of variable

Mutagenic Comparison
Premium Unleaded v. UV

- Control (0%)
- UV 15 sec.
- UV 30 sec.
- Pre. 0.001%
- Pre. 0.1%

P-value: 1.1E-07
P-value: 1.34E-06
P-value: 0.154
P-value: 0.003338
Diesel
Mutagenic Comparison

X-axis = concentration of variable

Y-axis = number of reverted cell colonies

Mutagenic Comparison
Diesel v. UV

- Control (0%)
- UV 15 sec.
- UV 30 sec.
- Diesel 0.001%
- Diesel 0.1%

P-values:
- Control vs. UV 15 sec.: P-value = 1.1E-07
- UV 15 sec. vs. UV 30 sec.: P-value = 1.34E-06
- UV 30 sec. vs. Diesel 0.001%: P-value = 0.000884
- UV 30 sec. vs. Diesel 0.1%: P-value = 1.05E-05
Conclusions

• The results of this experiment provide evidence to refute the null hypothesis and accept the alternative hypothesis
• All three crude-oils, in the concentrations used in this experiment, were not close to the carcinogenic standard set by UV radiation
• Diesel had the highest average reversion rate at both 0.001% and 0.1% and in analysis had a significant effect on cell reversion
• Regular and Premium Unleaded Gasoline at 0.1% concentration also showed a significant effect
• Both unleaded gasolines at 0.01% concentration showed no significant effect
Experimental Limitations

• The concentrations of crude-oil had to be small in order to not eliminate cells prematurely
• Even so, some yeast may have died due to excessive contact with the fuels
• Spread plating limited not only by human error
• The spreading of some plates may have been staggered unequally
• The counting of reverted colonies is subject to human error
Future Research and Experimental Extensions

When conducted again, the following will be integrated:

• Non-crude oil fuels, such as ethanol and biodiesel
• Different concentrations (ex. 0.01%, 1%, etc.)
• Mutagenicity of crude oil fumes could be compared to the liquids
• Increased replications


### Single-Factor ANOVA Analysis

**Summary**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 1</td>
<td>24</td>
<td>43</td>
<td>1.791667</td>
<td>1.737319</td>
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<tr>
<td>Column 2</td>
<td>24</td>
<td>44</td>
<td>1.833333</td>
<td>1.449275</td>
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<tr>
<td>Column 3</td>
<td>24</td>
<td>74</td>
<td>3.083333</td>
<td>3.905797</td>
</tr>
</tbody>
</table>

**ANOVA**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>25.86111</td>
<td>2</td>
<td>12.93056</td>
<td>5.469476</td>
<td>0.006239</td>
<td>3.129644</td>
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<tr>
<td>Within Groups</td>
<td>163.125</td>
<td>69</td>
<td>2.36413</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>188.9861</td>
<td>71</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>