A STUDY OF DIESEL EMISSIONS ON DROSOPHILA

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ABSTRACT

A sex-linked recessive lethal test was performed on male fruit flies of the species *Drosophila melanogaster*, (Oregon-R strain), exposed to an approximate five-fold dilution of exhaust from a diesel engine. The eight hour exposure was achieved by drawing diluted diesel exhaust from a three cubic meter stainless steel exposure chamber housing laboratory animals through a two liter reaction flask modified for use with Drosophila. A preconditioned sampling bag was used to collect the emissions after passing through the exposure chamber containing the flies. Results of analyses performed on the diesel exhaust mixture showed: carbon dioxide - 0.17%, carbon monoxide - 12.2 ppm, hydrocarbons - 11.6 ppm, nitrogen oxide - 3.8 ppm, nitrogen dioxide - 2.9 ppm, sulphur dioxide - 1.0 ppm, and particulates - 2.18 mg/m^3.

Two broods of the F_2 generation were investigated for the occurrence of recessive lethal events. These broods approximated the developing gametogenic stages of mature sperm (P_1 matings on days 2 and 3 post-exposure) and spermatocytes (P_1 matings on days 8 and 9). Additionally, the F_3 generation was evaluated for the occurrence of mosaic recessive lethal events which might escape detection in the F_2 generation. An equal number of F_2 and F_3 flies for both broods served as concurrent controls.

Results indicate that, under the conditions tested, the diesel exhaust did not increase the mutation frequency of the

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exposed flies (F_2 rate = 0.30%, F_3 rate = 0%) when compared to the concurrent controls (F_2 rate = 0.37%, F_3 rate = 0.15%).

INTRODUCTION

Within the past few years energy conservation has become a major issue throughout the world. While new sources of energy are being sought and developed, old sources are being modified for more efficient and greater economy. As a result, new emphasis may be placed on the diesel engine and its role in mass transportation and various industrial applications. Therefore, because of the large population potentially exposed to the emissions of such engines, knowledge of any associated hazards is vital. Of particular importance is knowledge of any mutagenic effects of diesel emissions. The purpose of the study described in this paper was therefore to use a standard bioassay to evaluate the potential mutagenic effects of diesel exhaust. Specifically, the Drosophila sex-linked recessive lethal bioassay (1), an excellent screen for genetic hazards, was used in this investigation. This bioassay is a useful system for detecting most types of genic damage and has been used to study mutagenic effects of a number of gases and aerosols.

At the request of the United States Environmental Protection Agency, this Drosophila bioassay was performed at the E.P.A. facility (Center Hill Laboratories, Cincinnati, Ohio) utilizing the available resources of ongoing diesel exhaust inhalation experiments. This study was an effort to extract additional data from these chronic diesel studies and at the same time, the intent was not to affect the integrity of the studies in progress by altering such variables as exposure time or concentration. Thus the current study was limited to an 8 hour exposure at the concentration being tested.

EXPERIMENTAL PROCEDURE

Exposure Regimen

Approximately 200 two to five day old wild type male fruit flies (Oregon-R) were exposed to an air stream consisting of diesel engine exhaust gases diluted five-fold with filtered ambient air. The diesel engine used in this study was a 6 cylinder Chrysler-Nissan engine (198 cu. in. displacement) with automatic transmission and dynamometer. A complete description of the operational parameters of this engine is found in Hinners _et al_. (2). Cross particulates were removed from the ambient dilution air by an MSA-CBR Filter* unit containing high efficiency (99.9% >0.3 microns) HEPA filters. The flies were held in a specially fabricated stainless steel cage (Wire Cloth Company, Cambridge, Maryland).

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Mating and Scoring

Randomly selected 100 exposed and 100 control flies were individually mated to two 3 to 5 day old virgin Muller-5 (In[1] sc^{S1L} sc^{8R} + S, sc^{S1} sc^{8} w^{a} B) females (3) on the second day post-exposure. All flies, throughout the study, were kept incubated at a constant 25°C within 8-dram (∿30 ml) shell vials containing an instant Drosophila medium, Formula 4-24 without dye (Carolina Biological Supply Co., Burlington, NC, 27215). Carbon dioxide was used to anesthetize the flies. Pure CO_2 gas (99.9%) regulated from a cylinder, passed through a cotton-filled box (3 X 5 X 1 inch) having a platform constructed of fine wire mesh. The flies were allowed to deposit their eggs for the next two days. On the fourth day post-exposure, the females were transferred to new vials containing fresh media and allowed to continue egg deposition. Concurrently, the males were individually placed into fresh vials in order to maintain a record of parental lines. These two "sub-broods" represented brood I, i.e., the sperm used in these matings were mature spermatozoa (post-meiotic) at the time of exposure (4).

The males were again individually mated to fresh virgins on the eighth day post-exposure. As before, the females were permitted to deposit their eggs for the next two days before being transferred to fresh vials. These two "sub-broods" represented brood III, i.e., the sperm used in these matings were in the spermatocyte (pre-meiotic) stage of spermatogenesis at the time of exposure (4).

The parents (P_1) of both second "sub-broods" were removed before emergence of the F_1 generation. Ten F_1 females were selected at random from each P_1 vial (from the original matings) up to 70 vials/brood and individually mated to two of her Muller-5 brothers. Therefore, a total of 700 F_1 matings/Brood/control and exposed groups were performed. The resultant F_2 cultures were scored for a sex-linked recessive lethal event according to standard procedures (1). An F_3 test was performed by selecting one female from every other F_2 culture vial and mating her with two of her Muller-5 brothers. The resultant F_3 cultures were scored in the same manner as the F_2 cultures.

RESULTS

Comparative analyses of the atmospheres of the Drosophila chamber and the 3 cubic meter chamber (Table 1) revealed that the concentration values for most atmospheric components were lower in the Drosophila chamber with the exception of the hydrocarbon fraction. Specifically, the Drosophila chamber showed 54.8% of the CO_2 level of the 3 cubic meter chamber, 55.2% of the CO level, 124.7% of the HC level, 29.5% of the NO level, 76.3% of the NO_2 level, 43.5% of the SO_2 level and 30.1% of the particulate level of the three cubic meter chamber.

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The fly cage (Figure 1) was cylindrical in shape (8½ X 3½ inches) with one end terminating into a cone with a quick connect/disconnect port allowing rapid ingress and egress to and from the cage interior; the opposite end, together with the remainder of the structure, was enclosed with 24 by 24 mesh, 0.04-inch wire diameter, stainless steel screen. The cage was placed within a glass chamber (2000 ml Reaction Flask, Ace Glass Inc.). The fly cage was secured within the chamber by a preformed band of silicone rubber which made an occlusive seal between the glass wall and the screen, thus allowing for all the diesel exhaust mixture to pass through the cage. This air was pulled by vacuum pump through this system at a rate of 1.06 liters per minute. The source of the engine exhaust mixture was a 3 cubic meter stainless steel exposure chamber (Figure 2) that was being used for repeated exposures of laboratory animals to diesel exhaust. These animals were present in the chamber during the 8 hour exposure period. The diesel engine was alternately turned on and off at 15-minute intervals throughout the 8 hour exposure period. An identical exposure system was used for an equal number of male flies. However, these flies served as concurrent negative controls exposed only to the filtered ambient air. Both the control and the exposed flies were randomly collected from the same stock of 2 to 5 day old males.

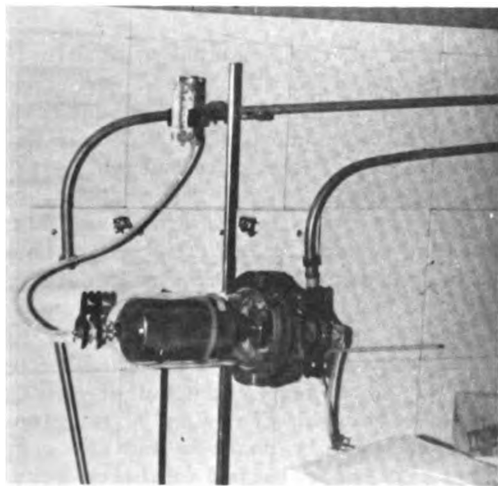

Figure 1
Fly Chamber with Cage

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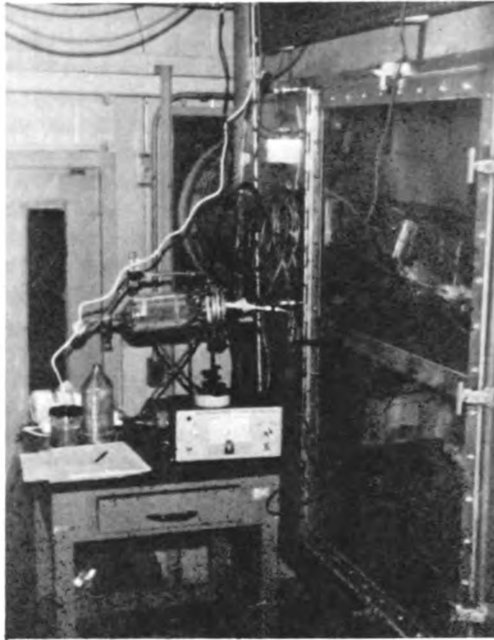

Figure 2
Fly Chamber Connected to 3 Cubic Meter Chamber

Sampling and Analysis

Characterization of the atmosphere within the 3 cubic meter chamber was compared to the atmosphere sampled after passing through the Drosophila chamber (Table 1). Analyses were performed by E.P.A. personnel of the Center Hill Laboratories, with the exception of the gravimetric particulate sample. An integrated air sample of the fly chamber, collected in a preconditioned mylar plastic sampling bag, was taken over the entire exposure period. The CO and CO_2 levels were measured by a Beckman Infrared Analyzer, hydrocarbons by hydrogen flame ionization on a Beckman Total Hydrocarbon Analyzer, SO_2 by pulsed fluorescence on a Thermo-electron SO_2 Analyzer, and NO/NO_2 by a chemiluminescent (ozone reaction) method using a Thermo-electron NO/NO_2 Analyzer. Particulate concentrations were determined gravimetrically using 37 mm glass fiber filters mounted between the fly chamber and the sampling bag. Temperature and percent relative humidity were recorded hourly. Temperature was measured with a thermometer mounted within the Drosophila chamber. Relative humidity of the Drosophila chamber was measured using a Weather Measure Corp. Relative Humidity Indicator, Model HMI-14. Temperature and humidity readings were not available from the 3 cubic meter chamber.

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Scoring of the F_2 cultures resulted in three lethals found in 678 vials scored from brood I in the control group compared with three lethals in 670 vials of the exposed group (see Table 2). Controls from brood III yielded two lethals in 676 vials compared to one lethal in 680 vials of the brood III exposed group. No lethals were found in the F_3 cultures of brood I of both the control (334 vials) and the exposed (also 334 vials) groups; only one lethal was found in the brood III control group (346 vials) while no lethals were found in the exposed group (336 vials).

The combined F_2 (broods I and III) results of five lethals/1354 vials scored for the control group gives a "background" mutation rate of 0.37%. In comparison, the combined F_2 results in the exposed group of four lethals/1350 vials yield a rate of 0.30%. The combined F_3 (broods I and III) results of one lethal/680 vials gives a rate of 0.15% for the control group while the exposed group had no lethals recorded in 670 vials scored.

When the F_2 and F_3 totals are combined, the control group shows a rate of 0.29%, six lethals/2034 vials, versus a rate of 0.20%, four lethals/2020 vials, for the exposed group.

TABLE 1

CHARACTERIZATION OF CHAMBER ATMOSPHERES

Component Measured	3 cu m Chamber	Drosophila Chamber
CO_2	0.31%	0.17%
CO	22.1 ppm	12.2 ppm
HC	9.3 ppm	11.6 ppm
NO	12.9 ppm	3.8 ppm
NO_2	3.8 ppm	2.9 ppm
SO_2	2.3 ppm	1.0 ppm
Particulate	7.3 mg/m^3	2.2 mg/m^3
Relative Humidity	-	29.1% + 1.5
Temperature	-	22.3°C + 0.2

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TABLE 2

RESULTS

SEX-LINKED RECESSIVE LETHAL TEST

F_2 Brood	Control No. lethals/No. scored	Exposed No. lethals/No. scored
I Sub-brood A	0/331	2/332
Sub-brood B	3/347	1/338
III Sub-brood A	1/342	0/346
Sub-brood B	1/334	1/334
Total F_2	5/1354 = 0.37%	4/1350 = 0.30%

F_3 Brood	Control No. lethals/No. scored	Exposed No. lethals/No. scored
I Sub-brood A	0/170	0/171
Sub-brood B	0/164	0/163
III Sub-brood A	1/174	0/167
Sub-brood B	0/172	0/169
Total F_3	1/680 = 0.15%	0/670 = 0%
Total $F_2 + F_3$	6/2034 = 0.29%	4/2020 = 0.20%

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DISCUSSION

The number of recessive lethals found in the F_2 and the F_3 treatment groups of both broods was smaller than in the control groups, indicating a lack of mutagenic activity exhibited by the diesel exhaust under the conditions of this study.

The effluent concentrations of some of the diesel exhaust components of the fly chamber differed substantially from those measured in the 3 cubic meter chamber. Much of the particulate loss may have been a result of adsorption along the network of fine wire mesh of the cage or to the walls of the glass chamber. A set of particulate samples was taken concurrently at a later date to examine this possibility, however, no significant difference in concentrations was found between the sample taken directly from the inhalation chamber and the sample taken immediately after the fly chamber and cage. Other diesel exhaust components may have been adsorbed onto the walls of the chamber or scavenged by the rubber tubing connections before reaching the sampling devices.

Two factors must be considered for the proper interpretation of the results of this study. First, the number of flies used in this test is capable of detecting moderate or stronger mutagens exhibiting 3 to 5 or more times the background or control mutation rate (albeit a low rate of approximately 0.1% to 0.6%-many authors); a weaker activity of twice the background rate or less could have eluded detection. However, there is no evidence in the data that suggests that weak activity might have been present, i.e., more mutations were found in the control groups than in the treated groups. A second, and perhaps more relevant, fact is that the objective of the recessive lethal test is more to reveal qualitative information rather than quantitative or dose-response type information (5). Therefore, a higher concentration should be administered to the flies for a more thorough assessment of the mutagenic potential of the diesel exhaust. Specifically, the concentration level used should result in a significant degree of mortality or sterility in the flies after exposure or, if this is mechanically unachievable, the exposure concentration should represent the maximum level that can be consistently generated and maintained over the duration of the desired exposure period. The duration of exposure can be extended up to approximately 24 hours and still allow for brood analysis with minimal overlapping of the separate stages of spermatogenesis. In addition, more brood types could be investigated to aid in the detection of any stage specificity effect that might otherwise escape detection.

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Furthermore, it has been demonstrated that some of the components of diesel exhaust have been shown to be mutagenic either alone or synergistically in the combined treatment with other substances. Carbon monoxide alone (95% CO, 5% O_2/6 hours) did not increase sex-linked recessive lethals in Drosophila, but when combined with azide or potassium cyanide, a significant increase in recessive lethals was found (6). Azide and potassium cyanide, either alone or in combination, did not show such an increase. Three to four percent nitric oxide (NO) delayed spermatogenesis, increased dominant lethality in Drosophila, and increased the percentage of sex-linked recessive lethals in γ-irradiated Drosophila (7). Sulfur dioxide (SO_2) alone exhibited a significant increase in the mutation rate above spontaneous levels in Tradescantia (8). These facts, coupled with the fact that the hydrocarbon fraction of the exhaust may contain potential carcinogens and/or mutagens, reinforce the need for an additional, more challenging, dose for administration to Drosophila.

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General Discussion

S. SODERHOLM: It is rather off the main point of your talk, but there is a puzzling difference in concentration especially gas concentration between the inhalation chamber and the Drasophila chamber. The fact that it didn't occur a second time suggests that you had a leak in the line somewhere and a small negative pressure in the main chamber. This can occur fairly simply.

R. SCHULER: Yes, one of our disappointments was the inability to pinpoint the exact reasons why we had differences.

R. SCHRECK: Could you tell us the route of entry of the particle into the fly? Do you feel it comes in through their tiny respiratory passageways in the side, and what do you expect the dose is per animal?

R. SCHULER: I don't know the exact dose, however, the route of entry is through the spiracles on the side of the abdomen of the fly. They can selectively open and close these spiracles. However, they only do this when they are challenged with a very irritating type of exposure. Normally, this is demonstrated with erythromycin. However, the fact that they are readily put under anesthesia by carbon dioxide in a fraction of a second indicates that the entry is rapid.

D. BRUSICK: It might be worth noting that the Drosophila can also ingest the particles. It has been well documented mutations can be induced in Drosophila by ingestion. Do you feel that relates in any way to what you observed?

R. SCHULER: Again as I emphasized, I would prefer to be doing a much higher and more challenging dose in terms of concentration and exposure time before I make any definite statements as to why we got these results. We were limited by the conditions of an ongoing experiment.

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