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Project Summary

Evaluation of Mutagenic Effects of Diesel Emissions: I. Tests for Heritable and Germ-Cell Effects in the Mouse

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In order to assess potential risk from heritable effects in human populations, mice were exposed by inhalation to whole diesel exhaust, and a number of genetic endpoints were studied. Exposure times in different groups varied from 5 to 10 weeks. In the maximally-exposed group, the total intake of diesel exhaust per mouse was about 85 times the 30-year (generation length) intake by a person in an average U.S. environment.

The battery of assays was chosen to detect several types of genetic endpoints, namely, point mutations in males (specific-locus test), chromosome damage in males (dominant-lethal and heritable-translocation tests), and chromosome damage in females (dominant-lethal tests). Ancillary studies were carried out to look for direct reproductive damage in both sexes; thus, various parameters were used to assess reproductive performance in females, and histological analyses of germ-cell survival were done in males.

The results of all genetic assays in both sexes were negative. In the ancillary tests, small but unequivocal effects on the reproductive performance of females of one strain could be observed, consisting of a decrease in the number of ovulations and an increase in the interval between mating opportunity and copulation. There was no detectable effect of diesel exposure on the number and distribution of cell types in the testis.

The absence of genetic effects could indicate either that no active metabolites reached the gonads, or that the germcells have an efficient repair system against induction of mutational events by such metabolites. Thus, transmitted genetic effects appear not to be a major hazard from exposure to diesel exhaust. The findings reported must, however, not be used to draw any conclusions concerning possible risks to the exposed individual him/herself.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Although non-mammalian and *in vitro* assays can provide evidence on presence or absence of mutagenicity, they have limitations in addressing the complexities associated with reproductive-cell targets in intact organisms The assessment of risk from heritable damage must therefore utilize *in vivo*

mammalian germline mutagenicity tests.

The effectiveness of inhaled whole diesel exhaust in inducing heritable effects in mammals was studied by a battery of tests in the mouse. The assays chosen were designed to detect a number of genetic endpoints, namely chromosome breakage, chromosome interchange, and point mutations. Ancillary studies involved germcell survival and reproductive performance. Effects were looked for in both sexes and in a variety of germcell stages. Several of the test systems had been developed at Oak Ridge, and all experiments are carried out there. Exposure to the diesel exhaust took place at the EPA laboratory at Cincinnati. In the maximum-exposure group, the total intake of exhaust per mouse was about 85 times the 30-year (generation length) intake by a person in an average U.S environment.

Overall Conclusion

The results of all genetic tests in both sexes were negative Small but unequivocal effects on the reproductive performance of females of one strain could be observed, consisting of a decrease in the number of ovulations and an increase in the interval between mating opportunity and copulation. It is no known whether these effects were the result of damage directly to the ovary or to some other endocrine organ (e.g., pituitary). There was no detectable effect of diesel exposure on the number and distribution of cell types in the testis.

The absence of genetic effects could indicate either that no active metabolites reached the gonads, or that the germcells have an efficient repair system against induction of mutational events by such metabolites In experiments with chemical agents, one does not expect—and, in fact, does not find—good correlation between transmitted damage induced in mammalian germcells *in vivo* and results from other test systems. It is the former result that is pertinent to transmission of genetic lesions to future generations of human beings; and the work summarized in this report thus indicates that transmitted genetic effects are not a major hazard from exposure to diesel exhaust. The findings reported here must, however, not be used to draw any conclusions concerning possible risks to the exposed individual himself.

General Procedure

All mice for the experiment were bred at Oak Ridge, shipped by air-conditioned van to the EPA laboratory at Cincinnati for exposure to diesel exhaust air, and returned to Oak Ridge for the subsequent genetic experiments. The exposure dates are listed separately for the individual projects, but all fell within the period of March 21 to June 4, 1979.

Because of the danger of introducing pathological conditions into the valuable Oak Ridge Mammalian Genetics facility, mice returning from Cincinnati were placed into a quarantine facility in which initial matings were carried out for some of the projects, and the entire procedure took place for others. Animals were monitored for key diseases before their departure to Cincinnati and, again, after their return to the Oak Ridge quarantine building. The first group to return to Oak Ridge was found to carry three viruses not present before they were shipped to Cincinnati. We therefore had to utilize a building separate from the Mammalian Genetics facility (as well as from the quarantine building) in which to complete all experiments.

At Cincinnati, mice were housed 3, 4, or 12 animals to a cage and cages were placed into 100 cf exposure chambers, having a horizontal cross section of 5 x 5 feet. One of these chambers received CBR-filtered and -conditioned air, while the other chamber was connected by piping to an automobile diesel engine exhaust dilution system. The six-cylinder Nissan engine was operated under load on the Federal Short Cycle, and the exhaust diluted with filtered and conditioned air at the ratio of 1.18. The diesel particulate concentration in the chamber averaged 6/mg/m^3 during the exposure period of eight hours per day and seven days of the week. All engine operations, aerometry measurements, and animal care were performed by the EPA staff at Cincinnati. [A few animals were lost during the exposure period (presumably in the process of transfer to clean cages), and a few others during the overnight storage between final removal from the chambers and return shipment to Oak Ridge (when some of the cardboard shipping boxes were chewed through)].

We calculate that, during 10-week exposure under these conditions, the total intake of exhaust per mouse was about 85 times the 30-year (generation-length) intake by a person in an average U.S. environment (urban-rural) This calculation is based on particulate concentration (mouse, 6 mg/m^3 vs. man 0.3 μg/m^3), length of exposure (mouse, 10 x 7 x 8/24 = 23.3 days vs man 30 years = 10,957 days), and pulmonary ventilation rate (mouse 2x man).

Tests were carried out for several types of genetic effects, namely, point mutations in males (specific-locus test), chromosome damage in males (dominant-lethal and heritable translocation test), and chromosome damage in females (dominant-lethal test). In addition, we looked for reproductive effects in females, and for changes in testis histology. The scope of the experiment did not include chemical determinations of what, if any, substances reached the gonads. If active material from diesel exhaust fails to get into mammalian gonads, this would lead to negative findings in the experiments reported, and presumably also to an absence of risk from genetic lesions transmitted to future generations.

Reports of Individual Experiments

Project No. 1

Test for Heritable Point Mutations in Male Mice

Objectives and relation to other projects

The objective of this project was to test for the induction of transmitted point mutations (intragenic changes and small deficiencies) by means of the specific-locus method. In planning the experiment, the number of offspring to be scored was calculated so as to be sufficient either for showing a significantly positive effect, or for ruling out, with a high degree of confidence, that the induced mutation rate, at the level of human exposure (see calculation under Results), could be higher than a small fraction of the spontaneous rate In order to meet the objectives of the latter alternative, mice had to be exposed to a quantity of diesel exhaust that was high multiple of that accumulated by the average American in one human generation.

For assessments of human risk, the stage of prime importance in the male is the spermatogonial stem cell, which can harbor (and transmit) mutations for the lifetime of the individual. The bulk of

our data were therefore derived from diesel exposures to that stage. However, we also obtained enough specific-locus data for the (transitory) postspermatogonial stages to rule out the possibility of a greatly elevated mutational sensitivity during that period.

Since a very large (> 800,000) and reliable historical control exists for spontaneous mutations in specific-locus experiments, all our available resources were used for the study of experimental groups only. A large sample could therefore be accumulated.

Only four exceptional animals were found, and none of these turned out to be due to a mutation at one of the seven loci

Conclusion

There is no evidence for the induction of point mutations in spermatogonia or in meiotic and postmeiotic stages. For spermatogonia, the male stage of major importance to risk assessment, the experimentally observed zero frequency rules out, with 97.5% confidence, that, at the level of exposure encountered by man, the induced mutation rate could be higher than 0.01 times the spontaneous rate.

Project No. 2

Test for Induction of Dominant Lethals in Male Mice

Objectives and relationship to other projects

The effectiveness of diesel exhaust in producing chromosomal aberration effects in germ cells of the male mice was studied using two procedures: the dominant-lethal test (this project) and the heritable translocation tests (Project No. 3). Since males were exposed to diesel exhaust for a prolonged period and mated immediately afterwards (see below), all spermatogenic cell stages that are known to be sensitive to dominant-lethal induction were presumably exposed. Dominant-lethal effects were evaluated by analyzing uterine content of unexposed females mated to the exposed or control males When a sperm carrying a lethal mutation is used in fertilization, the resulting embryo dies before or shortly after implantation.

Conclusions

Results of the dominant-lethal test indicate that the exposure of male mice to diesel exhaust did not induce detectable chromosomal aberration effects in germ cells.

Project No. 3

Test for Induction of Heritable Translocations in Male Mice

Objectives and relationship to other projects

The effectiveness of diesel exhaust in producing chromosomal aberration effects in germ cells of male mice was studied using two procedures: the heritable-translocation test (this project) and the dominant-lethal test (Project No. 2).

The heritable-translocation method is a sensitive and reliable procedure for measuring the frequency of chromosome breakage and rearrangement (exchange of parts) that is transmitted to the next generation. When a sperm carrying chromosome interchange(s) is used in fertilization, the resulting progency is heterozygous for the translocation and produces two types of gametes, balanced and unbalanced, in approximately equal proportions. Both types of gametes are capable of fertilization, but the unbalanced gametes result in embryonic lethality. For this reason, most translocation heterozygotes are only about half as productive as normal mice. Heterozygotes for certain types of translocations are incapable of producing sperm In the heritable-translocation procedure, progeny of treated parents are therefore tested for sterility and "partial sterility" Confirmed sterile and partially sterile progeny are then verified cytologically for presence of a translocation Thus, the heritable-translocation procedure generates the most meaningful information for evaluating hazards from induced chromosome aberration to human population, because it measures *transmissible* genetic damage.

Conclusions

Results of the heritable-translocation study indicate that the exposure of male mice to diesel exhaust, as described above, did not induce transmissible chromosome exchange in male germ cells. This result is consistent with that of the dominant-lethal test (Project No. 2), which indicated that there was no induction of chromosomal breakage in male germ cells. Together, the two tests fail to provide evidence of chromosomal aberration effect induced in male mice by the diesel exposure.

Project No. 4

Test for Induction of Genetic Effects and Oocyte Killing in Females

Objectives and relationship to other projects

The effectiveness of diesel exhaust in producing chromosomal and cytotoxic damages to oocytes was studied by means of total reproductive capacity. The mouse ovary is known to be sensitive to even relatively slight insults from known mutagens. Genetic and/or cytotoxic effects on oocytes in different stages of follicular development were measured by simply counting the offspring produced by exposed and control females over a period of time

Conclusions

The similarity in long-term reproductive performance (pregnancy rate and littersize) between control females and females exposed to diesel exhaust indicates that the exposure to diesel described above did not induce detectable chromosomal or cytotoxic effects in oocytes of the strain of mice employed in this study. Comparisons with short-term effects on female reproduction, studied in another strain, are discussed under Project No. 5

Project No. 5

Test for Dominant-Lethal Induction in Female Mice

Objectives and relationship to other projects

The primary objective of this experiment was to test for induction of chromosomal damages in mature and maturing oocytes. Since the accessibility of the ovary to diesel metabolites may be very different from that of the testis, this project constituted an important companion to Projects No. 1, 2, and 3, which assayed for genetic damage in males. An additional objective of this experiment was to test for any short-term effects of diesel exhaust on reproductive physiology, long-term effects were tested in Project No 4.

Conclusions

A seven-week exposure to exhaust fumes from a Nissan diesel engine produced no transmissible chromosomal damage, as measured in a dominant-lethal experiment, in mature and maturing oocytes. However, significant, though slight, effects were produced on female reproductive functions, as expressed in a decreased number of ovulations (14% overall) and a lengthening (by about 1.5 days) of the time interval between mating opportunity and copulation.

The results of this study, which concerns matings made mostly within a week after the end of exposure, may be compared with first-litter results for Project No. 4 (births 18-24 days after end of treatment). The females used in these two projects, though of the same age, were genetically different (SEC x C57BL)F_1 in Project 4 and (101 x C3H)F_1 in Project No. 5. For the first-week period, only 75.6% of mated control females produced litters in Project No 4, but 100% of controls got pregnant in Project No. 5. It is therefore difficult to predict whether a 1.5-day (average) diesel-induced lengthening in time interval between mating opportunity and copulation (shift in mean from 2.9 to 4 4 days), such as was determined through day-by-day analysis in Project No. 5, would have been detectable in Project No. 4. It seems probable that the method of Project No. 4 was not sensitive enough to detect such a shift, had it been induced in the (SEC x C57BL)F_1 females Project No 4 should, however, have detected a 16% reduction in average littersize, such as appears to have been induced in Project No. 5. The conclusion was reached that there is a genetic difference in sensitivity of females to the physiological damage(s) responsible for reducing number of ovulated eggs.

Project No. 6

Test for Effects of Spermatogonial Survival in the Mouse

Objective

Differentiating spermatogonia of the mouse are extremely sensitive to cytotoxic agents such as radiation and chemicals, and often show a positive response even when no genetic damage is observed. In the latter case, they provide a sensitive test system to determine if test materials reach the gonads. The response observed, however, is a somatic one (i.e. cell death) and is not necessarily associated with heritable effects. Killing of spermatogonia can, of course, have an effect on fertility.

Conclusions

Exposure to diesel exhaust had no effects on the number of spermatogonia or preleptotene spermatocytes of mice after 5 or 10 weeks' exposure Likewise, no irregularities in the normal dynamics of the seminiferous epithelium were observed; cellular associations were the same in controls, and division of the different spermatogonial types occurred at the normal times in the cycle of the seminiferous epithelium.

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The complete report, entitled "Evaluation of Mutagenic Effects of Diesel Emissions: I Tests for Heritable and Germ-Cell Effects in the Mouse," (Order No. PB 81-235 814; Cost: $6.50, subject to change) will be available only from:

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