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Project Summary

Respiratory Carcinogenicity of Diesel Fuel Emissions

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A large-scale experiment utilizing 3000 hamsters was conducted to compare the carcinogenicity of diesel exhaust particles (administered by 15 weekly intratracheal instillations) to that of organic extracts of diesel particles, coke oven emissions, roofing tar volatiles and cigarette smoke condensate. Appropriate solvent controls, untreated controls and positive controls were included in the design of the experiment.

The overall incidence of respiratory tract tumors in any of the treatment groups was not significantly higher than in control hamsters. Treatment with benzo(a)pyrene resulted in significantly higher tumor incidences, primarily evidenced as polyps in the trachea, lung, and larynx. Similarly, there were no significant differences in the survival rates of hamsters treated with test materials from those of their respective controls. Hamsters treated with test materials generally showed significantly lower mean body weights than control animals. This effect began during the 15-week treatment period and frequently lasted into the post-treatment holding period. Treated hamsters generally showed a delay in time to reach maximum body weight when compared to hamsters in control groups.

Treatment of hamsters with test materials induced a variety of hyperplastic, proliferative and inflammatory lesions of the respiratory tract. The highest incidence rates and greatest severity of the lesions were induced by diesel exhaust particles and coke oven emissions. Diesel exhaust extract and benzo(a)pyrene were less reactive, and cigarette smoke condensate and roofing tar volatiles produced the lowest incidence of respiratory tract lesions.

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 and Summary

The purpose of this study was to assess the carcinogenic potential of diesel engine exhaust particles and organic solvent extracts and to compare their carcinogenicity to that of other combustion products of known epidemiologic significance. The comparison materials included roofing tar, coke oven emissions, and cigarette smoke condensate. All materials and the proper controls were given to hamsters by intratracheal instillation (Saffiotti technique) for 15 weeks. Animals were sacrificed at 110 weeks of age (98-99 weeks on study) and gross and histopathological examinations were made.

In general, there were no significant differences in the survival rates, organ weights, and clinical pathology parameters related to the test compound or test compound concentration within each replicate experiment. However, the survival rates and presence of skin lesions differed significantly between the two replicate trials. These differences were ascribed to the initial housing condition used in the two replicate experiments. In the first replicate the hamsters were housed in groups of three during the first five months of the experiment and individually thereafter. A considerable degree of fighting was seen among the hamsters during the period of group housing, resulting in skin lesions, severe secondary

infections and death. During the second replicate experiment, hamsters were group housed for only one month before the individual housing was established.

Hamsters treated with test materials frequently showed significantly lower mean body weights than their respective solvent controls. This effect was usually seen during the 15-week treatment period and in many cases continued during the post-treatment holding period. Inspection of the computer-drawn body weight curves shows that the body weight change difference frequently was cuased by a shifting (delay) in weight gain in test-material treated hamsters rather than by a reduction in the maximum body weight achieved.

Treatment of hamsters for 15 weeks by once weekly intratracheal instillation of the various test articles induced gross and microscopic lesions of the respiratory tract at response rates that were related to specific test substances.

Diesel emission particles administered alone (DPOO) or in combination with ferric oxide (DPFO) and coke oven emissions (COFO) were the most reactive of the substances tested and produced a variety of pathologic lesions in the respiratory tract at incidence rates significantly higher than their respective controls.

Diesel extract (DEFO) and benzo(a)-pyrene (BPFO) induced less respiratory tract pathology than did diesel particles or coke oven emissions but did produce significant pathologic changes. Treatment with BPFO produced granulomas and mineralization in thoracic and mandibular lymph nodes to a significant degree, an effect not seen with other test materials.

Cigarette smoke condensate (CSFO) and roofing tar volatiles (RTFO) induced minimal changes above those produced by the solvent-ferric oxide control treatments. The incidence of respiratory tract lesions induced by these two substances was significantly lower than seen in hamsters treated with the other test materials.

A Chi Square statistic was used to compare paired samples of pathologic effects in order to address particular questions of interest. Three such analyses are shown in Table 1 and indicate that:

1. Diesel particles alone at a dose level of 5 mg per week induce significantly more pathology than does a 5 mg per week treatment consisting of 2.5 mg of diesel particles admixed with 2.5 mg of ferric oxide. That is, it is the specific properties of diesel particles rather than the total particle load that is significant.
2. A consistent dose response relationship is seen when the effects produced by 5.0 mg per week treatments with diesel particles are compared to those induced by 2.5 mg per week exposures.
3. The addition of an equal quantity of ferric oxide to the 2.5 mg dose of diesel particles generally increased the response rates to a modest degree but statistically significant changes were only seen for one class of lesions. That is, in the case of diesel particles admixed with ferric oxide the induced pathology can be attributed to the diesel particles rather than to an experimentally produced dose particle.

Conclusions

The results of these studies indicate that diesel particles with or without ferric oxide and diesel extract with ferric oxide were not carcinogenic for hamsters when given intratracheally at 5.0, 2.5 and 1.25 mg doses once a week for 15 weeks. Similarly, coke oven extract, cigarette smoke condensate or roofing tar extracts in mixture with ferric oxide were not carcinogenic at 5.0, 2.5 and 1.25 mg doses given over the same time period. Benzo(a)pyrene plus ferric oxide caused numerous polyps in the trachea and a few carcinomas in the lung and trachea, especially in the second replicate experiment.

The presence of diesel particles in the lung, pleuro, and trachea did cause a high incidence of lesions in these organs. The lesions were generally mild to moderate in severity and were dose related. The lesions included inflammatory reactions, granuloma formation, fibrosis, adenomatous and papillary hyperplasia, squamous metaplasia, and particle accumulation in the lung. Particle accumulation and inflammatory reaction were seen in the trachea, larynx and pleura in addition to fibrosis and proliferation in the pleura. The presence of these lesions in the lung, pleura, and trachea did not appear to be life threatening over the time course of this experiment since there were no significant differences between the survival rates of the hamsters given the test articles and their respective controls.

Recommendations

Concern over a postulated increase in the proportion and numbers of light-duty diesel engine powered vehicles in the total automotive fleet has provided the impetus to studies of potential health effects of an increase in emissions of carbonaceous particulate matter which might result. The U.S. Environmental Protection Agency has responded by sponsoring a number of studies to assess such impacts of which this program carried out at IIT Research Institute is one.

The design of this current study incorporated not only the evaluation of diesel emission particles as a potential respiratory carcinogen but also the ability to

Table 1. *Comparison of Pathological Effects of Diesel Particle (DPOO) and Diesel Particle Plus Ferric Oxide (DPFO) Test Materials*

	Treatment				Treatment			
	DPOO - 5 mg		DPFO - 2.5 mg[c]		DPOO - 2.5 mg		DPFO - 2.5 mg	
Lesions	*Pres*	*ABS*	*Pres*	*ABS*	*Pres*	*ABS*	*Pres*	*ABS*
Lungs								
Inflam. Reaction	76	14	55	39[a]	47	43[a]	55	39
Granuloma	71	19	40	54[a]	23	67[a]	40	54[b]
Fibrosis	29	61	14	80[a]	8	82[a]	14	80
Hyperplasia, Adem.	71	19	54	40[a]	42	48[a]	54	40
Hyperplasia, Papil.	27	63	7	87[a]	4	86[a]	7	87
Metaplasia, Cil.	38	52	6	88[a]	8	82[a]	6	88
Metaplasia, Squa.	8	82	4	90	1	89[a]	4	90
Proliferation, Fibro.	11	79	7	87	3	87[a]	7	87
Pleura								
Inflam. Reaction	33	51	10	81[a]	7	78[a]	10	81
Fibrosis	62	22	27	64[a]	21	64[a]	27	64
Proliferation	40	44	38	53	28	57[a]	38	53
Mononucl. Cell Infiltrate	14	70	4	87[a]	5	80[a]	4	87

[a] *Significantly different from DPOO 5 mg.*
[b] *Significantly different from DPOO 2.5 mg.*
[c] *DPFO - 2.5 mg = 2.5 mg diesel particles + 2.5 mg ferric oxide.*

compare any effects produced to those of an organic solvent extract of the diesel particles and to other combustion products of known epidemiologic significance. These other materials included for comparability and ranking of relative health risks were coke oven emissions, roofing tar volatiles, and cigarette smoke condensate.

Under the conditions of this experiment (once weekly intratracheal administration of test materials for 15 weeks and termination at two years) none of the test materials were carcinogenic. The positive control compound, benzo(a)pyrene, induced tumors, primarily polyps, in a significant proportion of treated hamsters using the same treatment regimen.

However, the absence of carcinogenic response to diesel particles and the other test substances under the conditions of this experiment and in the test species utilized does not permit definite conclusions about their lack of carcinogenicity to human beings. The limitations on the total dose that can be effectively administered, the limited life span of the hamsters as test species, total number of animals treated, and possible species differences in sensitivity all mitigate against definitive conclusions. The lack of reported tumor induction in long-term experiments in which test animals have been exposed to diesel exhaust particles by the inhalation route is in agreement with the results of this study.

The instillation of diesel exhaust particles, with or without admixture of Fe_2O_3 carrier particles, produced inflammatory and proliferative responses in various portions of the respiratory tract. These responses were similar in degree to those induced by exposure to coke oven emissions and more severe than those induced by diesel particle extracts, roofing tar volatiles, and cigarette smoke condensate.

While the induced respiratory tract pathology did not affect survival rates in otherwise unstressed hamsters, the effects of diesel particle exposure on the ability of animals to respond to other stressors should be evaluated. Intratracheal administration with diesel particles provides a useful method for loading the respiratory tract of test animals with known and reproducible burdens. Measures of effect which could have significance for public health include:

- Respiratory function under work or exercise stress.
- Reduction in immunocompetence.
- Additive effects in individuals with reduced respiratory function.
- Long-term consequences of the deposition of diesel particles for which longer lived test species would be necessary.
- Cocarcinogenic properties of diesel particles.

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***Judith A. Graham** is the EPA Project Officer (see below).*
The complete report, entitled "Respiratory Carcinogenicity of Diesel Fuel Emissions," (Order No. PB 85-228 120/AS; Cost: $25.00, subject to change) will be available only from:

National Technical Information Service
5285 Port Royal Road
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Telephone: 703-487-4650

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