Comparative toxicity of crude and refined coal liquids and analogous petroleum products. I. Chronic dermal toxicity in mice.

H. P. Witschi, L. H. Smith, M. E. Goad, M. R. Guerin, W. H. Griest, and C-h. Ho

Biology Division and Analytical Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831

Introduction

Human exposure to crude and refined coal liquids is most likely to occur via skin contact. In order to estimate eventual risks to human health as a consequence of incidental and prolonged skin contact it is necessary to obtain some information on the potential of coal-derived liquids to elicit skin cancer. This can be done with animals in experimental studies which mimic anticipated human exposure. In addition it also must be established whether prolonged dermal exposure will produce signs of toxicity not only on the skin, but internal organs. This paper reports data obtained in a life-long skin painting study with mice designed to explore these questions.

Materials and Methods

The following materials were tested: Raw H-coal blend, containing 5700 ppm N; H-coal blend after low hydrotreatment (2650 ppm N); H-coal blend after high hydrotreatment (0.2 ppm N); H-coal "home heating oil", a devolatilized version of the high-hydrotreatment H-coal blend; and an H-coal reformed naphtha. Two petroleum-derived reference samples were obtained from the American Petroleum Institute: Petroleum No. 2 fuel oil and high catalytically cracked naphtha (gasoline). Benzo(a)pyrene (99% pure; Sigma Chemical Company) was used as reference substance.

Experimental animals were male and female C3H mice, bred and maintained in the barrier facility of the Biology Division, Oak Ridge National Laboratory. The test agents were applied three times a week onto the shaved skin of the back. The following concentrations were used: 50 ul of undiluted material, 50 ul of a 1:1 or 50 ul of a 1:3 dilution with acetone. Positive controls received 50 ug, 25 ug, or 12.5 ug of benzo(a)pyrene in 50 ul of acetone per application (three times a week) and negative controls were painted with acetone alone or not painted at all. Twenty-five animals of each sex were used per dose level.

All animals had free access to food and water and were kept 5 to a cage on hardwood shavings for the duration of the study. They were weighed periodically. The animals were treated on Mondays, Wednesdays and Fridays. Whenever a skin abnormality became visible at the site of test agent application, painting continued for another two weeks. When the skin mass was found to persist, the animal was killed, and the skin lesion excised and fixed in neutral buffered formalin. A complete necropsy was performed, all observations were noted and selected tissues were fixed in neutral buffered formalin. All skin lesions were examined on paraffin sections under the light microscope. Animals not showing any skin lesions were continuously painted until found moribund or dead. A complete necropsy was performed on all animals killed or found dead.

Results and Discussion

Data on overall tumor incidence are shown in Table 1 and can be summarized as follows: In animals treated with 50 ug of benzo(a)pyrene per application, the first skin lesions began to appear 14 weeks later. In animals given the lowest dose of benzo(a)pyrene (12.5 ug per application) the first tumors appeared after 19 weeks. Tumor incidence then rose dramatically in all animals exposed to benzo(a)pyrene within 5-10 weeks after appearance of the first skin lesion. All animals exposed

eventually developed skin tumors. Histologically, the majority of tumors, 70%, were squamous cell carcinomas. On the other hand, in animals painted with acetone alone or not painted at all, very few skin tumors were observed which were benign papillomas.

Among the coal liquids only the raw H-coal blend produced an almost 100% incidence of skin tumors at all 3 concentrations tested. The first tumors were seen 10 weeks after the first application of 50 ul of the undiluted material (highest dose group). With the lower concentrations, the first tumors were seen after 20 and 33 weeks, respectively. Once the first tumor was discovered in any group, it took approximately one year until most animals in a given group had developed skin tumors. Of these tumors 46% were malignant squamous cell carcinomas.

Hydrotreatment dramatically reduced the carcinogenic potential. The low hydrotreatment preparation, painted undiluted onto the skin, produced the first tumors after 58 weeks, and 92 weeks after beginning of the experiment, tumor incidence was only 14%. In animals exposed to the high hydrotreated preparation, tumors began to appear usually after 70 to 80 weeks and the final tumor incidence in all 3 dose groups was between 16% and 44%. Only 2 tumors were found in the 150 animals exposed to H-coal reformed naphtha. However, in animals exposed to H-coal "home heating oil", the devolatilized version of the highly hydrotreated H-coal blend, tumors began to appear around 40 to 50 weeks of exposure and final tumor incidence in animals exposed to the undiluted material was 30%.

The two petroleum-derived samples had practically no carcinogenic potential; only 3 animals with tumors overall were found in animals painted with gasoline and only a total of 13 animals had tumors 95 weeks after beginning of the experiment. An evaluation of the gross necropsy findings showed that many animals suffered from lesions usually expected to develop in a certain percentage of aging animals, such as myocardial calcification, renal failure, liver tumors, ovarian tumors, and lymphomas, among others. However, the gross lesions were not associated with any particular treatment regimen and were also seen in the control and untreated animals.

It is concluded that the carcinogenic potential of a raw H-coal blend can be mostly abolished by hydrotreatment; however some carcinogenic potential remains associated with a devolatilized version of a severely hydrotreated sample. Petroleum derived products have considerably less carcinogenic activity, an observation compatible with earlier findings. Finally we did not find gross signs of toxicity in organs other than the skin, and the compounds treated seemed not to act as systemic carcinogens.

Acknowledgement

We wish to thank the American Petroleum Institute (API) for having made the two samples available to us.

Research sponsored by the Office of Health and Environmental Research, U. S. Department of Energy under contract DE-AC05-840R21400 with the Martin Marietta Energy Systems, Inc.

Table 1: Skin Tumor Incidence

Compound	Dose per[1] application	No. of animals with skin tumors/ No. of animals exposed	Median time to tumor (days)[2]
931 Raw H-coal blend	50	41/50	45
	25	45/50	52
	12.5	44/50	62
934 Low hydrotreated (2650 ppm N)	50	7/50	112
	25	6/50	117
	12.5	1/50	149
935 High hydrotreated (0.2 ppm N)	50	10/50	100
	25	17/50	111
	12.5	8/50	119
978 H-coal "home heating oil"	50	14/50	97
	25	12/50	108
	12.5	8/50	112
936 H-coal reformed naphtha	50	0/50	-
	25	0/50	-
	12.5	2/50	155
975 API No. 2 fuel oil	50	5/50	120
	25	6/50	131
	12.5	2/50	144
976 API gasoline	50	3/50	125
	25	4/50	130
	12.5	6/50	143
Benzo(a)pyrene[3]	0.1	25/25	21
	0.05	25/25	24
	0.025	25/25	31

[1]High dose was 50 ul of undiluted material; 50 ul lower doses obtained by dilution with acetone; all doses applied 3 times weekly.

[2]Animals killed 2 weeks after appearance of skin tumors; all tumors confirmed by histological diagnosis.

[3]Doses are in percent (w/v) benzo(a)pyrene, 50 uL per mouse.