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Project Summary

Effects of Selected Waterborne Particulates on Cellular and Molecular Parameters

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This project was initiated to determine the effects of particulate samples, some concentrated directly from drinking water supplies, on biological endpoints in cultured mammalian cells and to compare them with those caused by known asbestos materials cultured with the same cell type. A normal human fibroblast cell strain and primary Syrian hamster embryonic cell cultures were used as test systems. The cellular and biochemical parameters examined were: (1) cytotoxicity, (2) virally-directed cellular transformation, (3) cell membrane composition changes, and (4) cyclic nucleotide concentration differences. Three of the particulate samples were collected by filtration from drinking waters from Duluth, Seattle, and San Francisco. The other three samples included taconite tailings, attapulgite clay, and particles from asbestos-cement pipe tapping. All samples were less cytotoxic than Canadian chrysotile. Three of the samples affected the ratio of cyclic nucleotides in a manner similar to what would be expected if the cells had been treated with a chemical carcinogen. The samples had varying effects on glycolipids and glycoproteins. It was apparent that the samples induced differing effects relative to the biological endpoints examined. Some of these endpoints may be useful in predicting potential health hazards of waterborne particulates.

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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

Mineral fibers and particulates are found in air, soil, and water and thus reach man by a number of routes. Many of these materials are relatively inert and, rather than decomposing, recirculate in the environment. Most important, however, their mode of action has not been determined and a method other than epidemiology has not been developed to screen for their potential human health effects.

Cultured mammalian cell-line studies provide biological endpoints that can be used to compare the potential health effects of pollutants. A multidisciplinary approach is necessary to evaluate the effects of biological endpoints such as cytotoxicity, enhancement of virally directed cellular transformation, alteration of cell membrane composition, changes in cyclic nucleotide ratios, modification of chemical carcinogen metabolism, and association with genetic material.

Independent studies have suggested that asbestos induces biochemical alterations in cells in culture and, further, that different types of asbestos induce these alterations to differing extents. Since the extent of the effect induced by various forms of asbestos appears to correlate with the carcinogenic potential of these agents, as determined by epidemiological studies, it was deemed reasonable that a double-blind study be performed to further examine this correlation with unknown samples, which may be found or isolated from drinking water.

Methods and Materials

Samples of particulates, some extracted directly from drinking waters, were referenced only by a code number and sent to the grantee. The testing on the various cell parameters was then done blind.

Samples 1, 2, and 3 were particulates collected by filtration from drinking waters from Duluth, Seattle, and San Francisco, respectively The Duluth sample was taken before the installation of the filtration plant at Duluth. Chrysotile fibers have been identified in the particulate samples from San Francisco and Seattle; amphibole fibers were identified among the particulates in the sample from Duluth. Sample 4 contained the less than 2 micrometer size fraction of taconite tailings which had been prepared by a sedimentation separation procedure. Amphibole fibers were identified among the particulates in sample 4. Sample 5 contained attapulgite clay, a non-asbestos mineral, which consisted of short fibers in the same range as some chrysotile asbestos. Attapulgite fibers have been identified in some water supplies in Georgia and Florida. Sample 6 consisted of particulates collected from water flowing through asbestos cement pipe after the pipe was tapped. Sample 6 contained chrysotile, some crocidolite and cement pieces.

The test systems employed were a normal human fibroblast cell strain established at Columbus and primary Syrian hamster embryonic cell cultures. The cellular and biochemical parameters examined were cytotoxicity, virally-directed cellular transformation, cell membrane composition, and cyclic nucleotide concentrations.

Results

The dose-response curve for each unknown and for Canadian chrysotile was determined over a range of 10^{-8} to 10^{-2} grams at log intervals. At the highest concentration used, all compounds proved toxic; however, at the LD_{50} level, a difference of three orders of magnitude was noted between samples. The effect of each sample on both the total number of colonies formed as well as the size distribution of the colonies as a function of each dose was determined by use of an Artek Size Discriminator. Studies have shown that while all samples tested induced differing shapes and sizes of colonies relative to the control, each class of colony produced a similar dose-survival curve. Relative to potency and due to the large number of plates per point (40 plates counted at least 4 times via an automatic colony counter and size discriminator) it is estimated that under the conditions of these experiments and for this particular normal human fibroblast culture, Canadian chrysotile (control) is more potent than $1 > 6 > 2 > 5 > 4$ based upon their respective LD_{10}'s, LD_{50}'s and LD_{90}'s.

Whereas all six known asbestos samples previously examined increased the frequency of Snyder-Theilen Feline Sarcoma Virus transformation of human cells *in vitro* to one degree or another, the unknown samples did not uniformly do so. Samples 1, 2, 4, 5, and 6 significantly inhibited viral transformation when cells were treated 2 hours prior to infection, whereas substance 3 enhanced transformation at this same time point. Substance 4, at a concentration of 0.1 μg/ml, enhanced transformation when applied 24 hours prior to infection. All substances, with the exception of 6, affected viral-directed transformation in a dose-dependent manner.

Relative to the effect of unknown samples on the cyclic nucleotide response of cells with increasing doses of asbestos, no predictable dose-response relationship was noted; however, there were striking differences in the tissue response of human cells to the different samples examined. These responses could be divided into two (2) groups with samples 1, 2 and 3 having relatively minor effects and samples 4, 5 and 6 causing a consistent decrease in the cyclic nucleotide ratio at all concentrations tested. The most striking effects were seen with sample 4. The only sample studied which exhibited an effect similar to those observed previously with known samples of asbestos was sample 3, which exhibited the biphasic curve observed previously. Interestingly, the striking decrease in ratio observed with certain of these samples was very similar to that obtaine with chemical carcinogens.

Cell membrane monosialoganglioside (GM_1, GM_2) are an index of a simpler ce surface glycolipid pattern which, in prev ious studies with known asbestos sam ples, appeared to change in a directio that was approximately equivalent to th carcinogenic potential of the sample examined.

In the present studies, samples 1 and caused little change in the proportions c surface gangliosides, whereas sample 3, 4, and 5 produced losses in surface GT with the greatest effect being manifeste on the surfaces of cells treated witl samples 4 and 5. The chrysotile-incu bated cells included in the sample 6 stud reacted anomalously to the asbestos making the interpretation of sample changes difficult. When sample 6 treate cells were compared to the control fo samples 3-5, there was a loss in GD_{1A} an a corresponding increase in GM_1. GL-4 i the neutral glycolipids increased witl samples 1 and 2 treatment and decrease with samples 3 and 4 cell incubations Consistent with its actions on ganglio sides, among the test samples, sample 4 caused the greatest reductions in GL-4 Again, sample 6 effects were anomalou due to the fact that distributions o glycolipids in the sample 6 control wer so different from those of the sample 3-5 control.

Very high molecular weight protein increased with cell treatment of sample 1 and 2. The control for samples 3, 4, an 5 predominantly had a high concentratior of a high molecular mass glycoprotein or the cell surfaces. Samples 3-6 allowec the detection of some of this glycoprotein but it was in a lower concentration thar that found in the controls. The loss o such high molecular mass proteins has been correlated with cells undergoing transformation.

Conclusions

Of the unknown samples tested, samples 1, 6, and 2 appeared in that order to be the most toxic. Relative to the effect of these substances on the cyclic nucleotide ratio, samples 1, 2, and 3 had little effect, whereas samples 4, 5, and 6 produced a decrease in the ratio similar to that induced by chemical carcinogens. The effect of these samples on viral-directed transformation was inhibitory, unlike the asbestos fibers previously studied (1) which were stimulatory with the exception of substance 3, which was stimulatory when cells were treated 2 hours before infection.

The order of effectiveness in reduction of surface complex glycolipids was 5 > 6 > 3 > 4 > 2 > 1. The order of effectiveness in reduction of the high molecular mass glycoproteins was 5 > 4 > 3 > 6 > 2 > 1.

Thus, it is apparent that these samples induced differing effects relative to the biological endpoints examined. It is still to be determined which of these endpoints is the most useful in predicting potential health hazards of waterborne particulates.

These studies, taken in combination with previous findings and those of other laboratories, suggest that mineral fibers and particulates may have two effects: (a) cytotoxic and (b) promotional, with their primary role in carcinogenesis being the latter.

Reference

1. Hart, R. W., R. Fertel, H. A. I. Newman, F. B. Daniel, J. R. Blakeslee, 1979. Effects of Selected Asbestos Fibers on Cellular and Molecular Parameters. USEPA-600/1-79-021. 35 p.

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The complete report, entitled "Effects of Selected Waterborne Particulates on Cellular and Molecular Parameters," (Order No. PB 85-127 496; Cost: $10.00, subject to change) will be available only from:

National Technical Information Service
5285 Port Royal Road
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