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Project Summary

Carcinogenic Potential of Arsenic Compounds in Drinking Water

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The primary objective of this research study was to determine whether arsenite (AsIII), arsenate (AsV), dimethylarsinic (DMA) and monomethylarsonic acid (MA) were initiator carcinogens or promoters of DENA-initiated tumors in the rat liver.

The maximum tolerated doses (MTD) of the arsenics to be used in this study were determined by treating male Wistar rats with increasing drinking water concentrations ranging from 10 ppm - 2560 ppm for 7 weeks, depending upon the arsenic studied. The apparent MTD determined were: MA, 800 ppm; AsIII, 160 ppm; AsV, 160 ppm; and DMA, 80 ppm. The calculated LD_{50} were DMA, 98.5 ppm; ASIII, 264.8 ppm; AsV, 294.5 ppm; and MA, 1160.1 ppm.

To determine if those arsenic compounds acted as an initiator or promoter, male Wistar rats were partially hepatectomized, treated with a single dose of diethylnitrosamine (30 mg/kg i.p.); on day 7 treatment began with the MTD of each arsenic for 7, 25, and 43 weeks in the drinking water. This was the promoter protocol. The initiator protocol did not include the diethylnitrosamine treatment. There were no significant numbers of animals with tumors in the liver in any of the arsenic treated animals as compared to their controls at the three time periods of treatment with either the initiation or promotion protocols.

However, in the promotion protocol there was significant increase ($p < 0.05$) in the number of animals exhibiting tumors in the kidney in the AsIII promoted group, 7/10, as compared to the DENA controls, 2/9 when treated for 25 weeks. In both protocols AsIII treated animals showed a significant decrease in thymus gland weights. This was suggested to be a contributory factor in the renal tumorigenesis observed in this study.

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

The final report presents the results of three years of study to determine whether arsenite (AsIII), arsenate (AsV), dimethylarsinic acid (DMA), and monomethylarsonic acid (MA) were initiator carcinogens or promoters of tumors in the rat liver. This work was initiated to gain data that might clarify some of the apparent contradictions between human and animal data and provide the basis for arriving at more realistic estimates of the carcinogenic risk that is associated with the various forms of arsenic found in drinking water. A brief review of literature illustrates the extent of the contradictory data.

Within the past several years many researchers have investigated the relationship between arsenic exposure and development of cancerous lesions in humans; others have sought to corroborate those findings in animal experiments as explained in the next two paragraphs.

The relationship between arsenic exposure and the development of cancerous lesions in humans has been reviewed by

IARC (1973), Yeh (1973), Zaldivar (1974), Bencko (1976), Committee (1977), and Wildenberg (1978). They cite many clinical and epidemiological observations which indicate that when humans are exposed to arsenic, via dietary, medical, environmental or occupational routes, neoplasms of the liver, lung, skin, lymphatic and hematopoietic tissues result. Hueper (1953) referred to arsenic as "after coal soot, the oldest known human carcinogen." Dinman (1974) placed arsenic, along with mustard gas, in a "definitely carcinogenic" category based on epidemiological evidence for "carcinogenic potential."

However, not one of the 46 arsenic exposure experiments listed in the "Survey of Compounds Which Have Been Tested for Carcinogenic Activity," conclusively demonstrated arsenic's carcinogenic potential (Hartwell, 1951; Shubik and Hartwell, 1957). Nor have any recent studies demonstrated arsenic carcinogenesis in animals (Wildenberg, 1978). So, even though epidemiological evidence shows arsenic exposure is related to human cancers, attempts to induce cancer experimentally with arsenic compounds have not been successful. Even in the short-term mutagenicity tests, arsenic compounds (like other metals) have given negative results (Wildenberg, 1978).

Two of the reasons why data from experimental animals have not demonstrated a positive correlation between arsenic exposure and carcinogenesis are:

1. The route of administration was via the diet or by application to the skin. Arsenic, unlike other skin carcinogens, does not exert its action by direct contact. There are no documented cases of human skin cancers following the external application of medicinal arsenicals, nor is there evidence that arsenic in food acts as a human carcinogen. Man's primary routes of arsenical exposure resulting in cancer are from ingestion in aqueous and alcoholic (in wines) solutions and inhalation (Committee, 1977). Schrauzer et al. (1978) have reviewed reports which indicate inorganic arsenic compounds have different toxicities and interactions with selenium when animals drink rather than eat their arsenic.

2. In general, a single concentration of only one arsenic compound was tested and the maximum tolerated dose (MTD) was not determined, leading to poor animal survival. Too few tumors were produced per experimental treatment group to allow a definitive statement concerning arsenic carcinogenesis. Even if the number of animals per test were increased substantially, only those chemicals which cause high tumor incidence would readily be detected as carcinogens. But observations in humans indicate arsenicals are probably weak carcinogens (Wildenberg, 1978).

Therefore, what is needed are bioassays which have greater sensitivity to arsenic than do either the classical long-term feeding studies or the short-term microbiological mutagenicity tests. We propose to explore the use of an assay which uses a two-stage carcinogen treatment to demonstrate experimentally the carcinogenic potential of four different arsenic compounds.

Materials and Methods

The Maximum Tolerated Dose (MTD) and the LD_{50} of Arsenic Compounds in Wistar Rats Exposed to Arsenic Compounds in the Drinking Water for 7 Weeks

Male Wistar rats (60-80 g), obtained from Simonsen Laboratories, Inc. (Gilroy, CA) were randomly divided into groups of five rats each and given access to distilled water containing various parts per million (ppm) arsenic as either sodium arsenite (AsIII); sodium arsenate (AsV); methylarsonic acid, disodium salt (MA); or dimethylarsinic acid, sodium salt (DMA) for seven weeks. Each group was housed in individual gang cages and fed a standard laboratory chow. The weights of all test animals were recorded weekly. Gross observations were made during the test period. At 7 weeks, the animals were sacrificed by guillotine and routine necropsy was performed.

The Stimulation of Gamma-Glutamyltranspeptidase Activity in Rat Liver by Inorganic Arsenics

Five-week old Male Wistar rats obtained from Simonsen Laboratories, Inc. (Gilroy, CA) were randomly divided into groups of five rats each, housed in individual gang cages and given access to standard laboratory rat chow and to distilled water or distilled water containing 160 ppm (elemental arsenic) of either sodium arsenite (AsIII) or sodium arsenate (AsV). The animals were sacrificed at predetermined times by guillotine. The liver tissue was quickly excised and prepared for histochemical and biochemical studies.

The Initiator Potential of Arsenite and Arsenate in Partially Hepatectomized Rats When Promoted by Phenobarbital

Male Wistar rats weighing 70-100 g were 2/3 partially hepatectomized (PH) and 18-24 hours later were exposed to arsenic in their drinking water for a period of 3 days. The AsIII and AsV doses were 160 ppm (MTD) and 320 ppm. The higher dose was given in order that the concentration of As in the plasma and liver would be comparable to doses in animals exposed to 160 ppm As for one week. Previous experiments indicated that 1 week was the minimum period of time necessary for GGTase stimulation at 160 ppm. Animals were then given 0.05% phenobarbital food pellets (Teklad Test Diets) ad libitum 7 days after the pH and continued for 7 weeks. The animals were sacrificed by guillotine; livers were dissected out and processed as previously described in Section 4 of the full report; and routine necropsy was performed.

Promoter Potential of Arsenic Compounds in Partially Hepatectomized DENA-Initiated Rats

Male Wistar rats weighing 70-100 g were partially hepatectomized (PH) and injected i.p. 18-24 hrs later with DENA (Eastman Kodak) at a dose of 30 mg/kg. On day 7 the animals were treated to one of the following: 0.05% phenobarbital (PB) in the diet, arsenite (AsIII) or arsenate (AsV) present in the drinking water at a level of 160 ppm, or organic arsenicals, DMA and MA, present in the drinking water at a concentration of 80 ppm and 800 ppm, respectively. Each group contained a minimum of five animals. At the end of 7 weeks of treatment with PB or the arsenics the animals were sacrificed by guillotine and routine necropsy performed. Liver samples were removed for GGTase assay.

Promoter Potential of Arsenic in Partially Hepatectomized DENA-Initiated Rats

In this study, sham-operated animals were included as control treatment groups for the promotion experiments.

Promoter Potential of Arsenic in Partially Hepatectomized DENA-Initiated Rats

For animals in this study, the water intake for each cage was monitored daily. From this data daily doses for the arsenics were calculated. At the scheduled sacrifice days, both kidneys were removed from each animal, weighed and processed for histochemical, biochemical and histological examinations.

Conclusions and Recommendations

The experimental design for this study which was the broad spectrum initiation-promotion protocol developed at HERL-Cincinnati. It was effective in determining whether a specific arsenic compound had an initiator carcinogen potential or a promoter potential. Although the procedure was designed for testing potential initiators and/or promoters in the liver, the authors believe it can be used to study potential agents in the kidney.

The study also demonstrated that the chronic toxicity of arsenic compounds in drinking water cannot be predicted from acute toxicity studies. An organic arsenic such as dimethylarsinic acid was as toxic as arsenite or arsenate when administered chronically in drinking water. According to acute toxicity studies arsenite and arsenate are methylated to dimethylarsinic acid which is supposed to be an inactive metabolite. There appears to be a discrepancy between the results of the acute toxicity study and the results. The results of this study showed that metabolism would result in an equitoxic metabolite. Thus, it is clear that the toxicity of these compounds have to be reevaluated under chronic exposure conditions in order to determine the effect of metabolism on steady-state levels of the metabolites.

The study demonstrated that arsenite is a promoter in the kidney. Chronic arsenite treatment for 25 weeks promoted DENA-initiated tumors in the kidney. As a result, arsenite may now be used as a known promoter in the kidney to test for potential initiator carcinogens.

We have also demonstrated that arsenite decreases the thymus weight which is suggestive of a decrease in the immune response. Thus, this immunosuppressive effect may be a contributory factor in the carcinogenesis process.

Because these experiments were performed with partial hepatectomy, it must yet be determined that this surgical procedure did not contribute to the promotion effect of arsenite. Experiments should be performed on intact animals under the same conditions of dosing. It is anticipated that the treatment time and the time for appearance of renal tumors will be extended.

Since the arsenite concentration used in this study was the maximum tolerated dose, a dose-respose study should be done to determine at what level arsenite no longer has promoter activity. This would be important for setting safe drinking water standards.

Because of the discrepancies found between the results obtained from chronic dosing in the drinking water as compared to the results found in the literature from acute toxicity studies, the authors recommend that metabolism of the different arsenics be studied *in vivo* chronic dosing in the drinking water. This would also be important in setting safe drinking water standards.

Finally, the immunosuppressive effect of arsenite should be studied further to shed some light on the role of arsenite in the chemical carcinogenesis process.

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The complete report, entitled "Carcinogenic Potential of Arsenic Compounds in Drinking Water," (Order No. PB 86-200 854/AS; Cost: $11.95, subject to change) will be available only from:
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