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Mutagenicity of Drinking Well Water

Fung-Jou Lu,[1] Ching-Lin Hong,[2] Ming-Fen Lu,[1] and Hidesuke Shimizu[3]

[1]Department of Biochemistry, College of Medicine, National Taiwan University, Republic of China; [2]Department of Public Health, Taipei Medical College, Republic of China; and [3]Department of Public Health, Jikei University, School of Medicine, Tokyo, Japan

Residents in the southwest coast of Taiwan suffer from high levels of skin, liver, lung and bladder cancers. The high content of arsenic in the drinking well water may be associated with the high incidence of cancers in the area (Chen et al. 1985). This paper reports that mutagens (or carcinogens) other than arsenic are present in the drinking well water of that area.

MATERIALS AND METHODS

Water was obtained from an artesian well located in Tainan County on the southwest coast of Taiwan. The water was concentrated, acidified, and extracted with alcohol to obtain a crude extract of fluorescent humic substances about 300 mg/L (Lu et al. 1988). Ten grams of the crude extract were put into Soxhlet apparatus, and extracted with 100 mL of n-hexane, followed by benzene, ethylacetate, ethanol and methanol. The time of each extraction was one hour. The ethylacetate fraction (EA fraction) was further extracted with freshly prepared 2% $NaHCO_3$ solution. The water extract was called EA_1 fraction, the remaining ethylacetate extract was called EA_2 fraction. All extracts and residue were examined for their ability to cause mutation in Salmonella typhimurium strain TA 100. A dose response study was carried out using EA and EA_2 fractions and TA 98 and TA 100 strains with and without S9 mix (Maron and Ames 1983). Positive controls consisted of AF-2 (0.01 ug/0.1 mL/plate for TA 100 strain and 0.05 ug/0.1 mL/plate for TA 98 strain), and B[a]P (5 ug/0.1 mL/plate for both strains). Each test at one dose level was performed in triplicate. Positive controls and blanks were followed for each trial of the tests.

S9 was prepared from the liver of male Sprague Dawley rats (about 200 g of body weight) induced with PCB

Send reprint requests to Prof. Fung-Jou Lu at the above address.

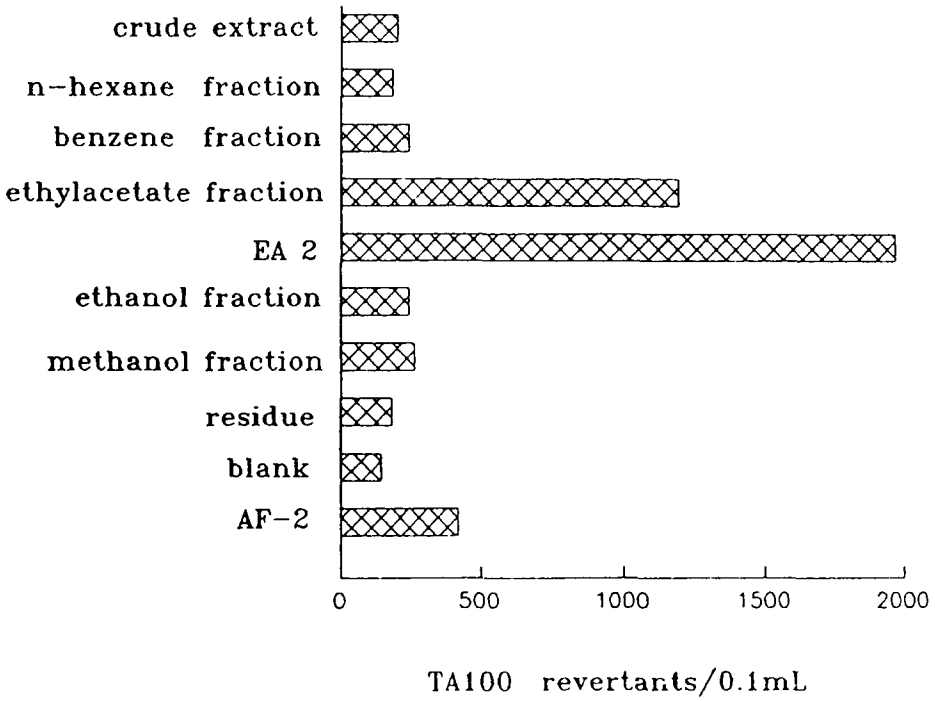

Figure 1. Comparison among the mutagenicities of different fractions of fluorescent humic substances in well water on TA 100 at the same dose level of 1,000 ug/0.1 mL/plate.
AF-2: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide

(Aroclor 1254). The homogenate (3 mL of 0.15 M KCl/g liver) was centrifuged as described by Ames et al. (1975). S9 mix(10%) (rat liver microsomal enzyme systems) contained S9(5 mL), 0.012% $MgCl_2$-0.08% KCl salts(1.0 mL), 1 M glucose-6-phosphate(0.25 mL), 0.1 M NADP(2.0 mL), 0.2 M phosphate buffer, pH 7.4(25 mL), and distilled water(16.75 mL).

RESULTS AND DISCUSSION

The EA fraction and its purified layer (EA_2 fraction) produced the larger number of TA 100 revertants whereas other fractions produced smaller number of revertants (Figure 1).

EA fraction induced twice as many TA 98 and TA 100 revertants as spontaneously produced by the blank at doses of 50 and 100 ug/0.1 mL/plate without S9 mix, and at doses of 250 and 500 ug/0.1 mL/plate with S9 mix respectively. Dose-response curves became linear as shown in Figures 2 and 3.

EA_2 fraction induced much more His^+ revertants of both

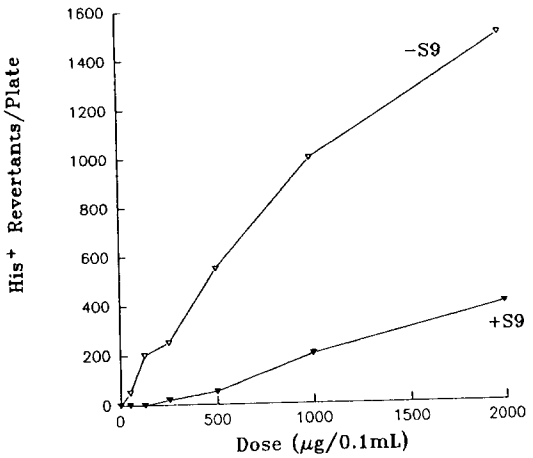

Figure 2. The dose response curve of EA fraction on TA 98 strain. Each point was three sample means. Spontaneous revertant was deduced from each point.

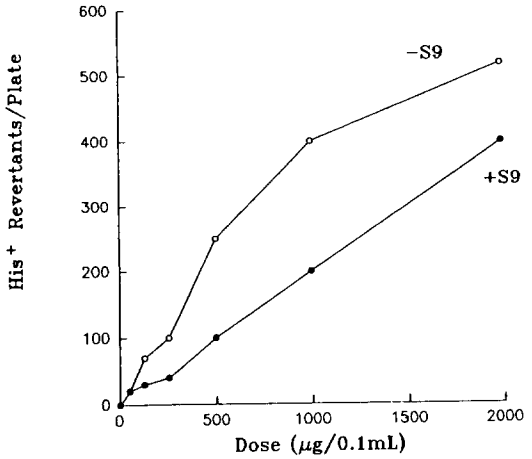

Figure 3. The dose response curve of EA fraction on TA 100 strain. Each point was three sample means. Spontaneous revertant was deduced from each point.

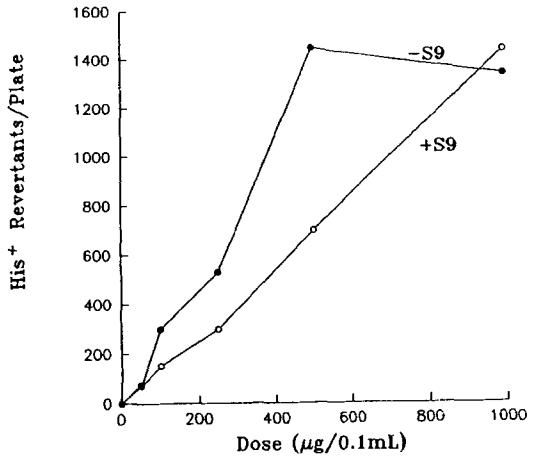

Figure 4. The dose response curve of EA_2 fraction on TA 98 strain. Each point was three sample means. Spontaneous revertant was deduced from each point.

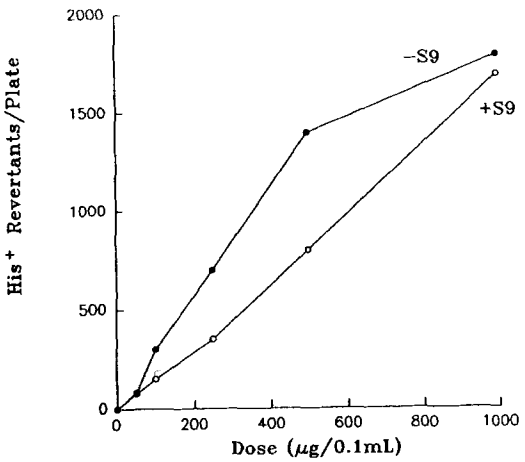

Figure 5. The dose response curve of EA_2 fraction on TA 100 strain. Each point was three sample means. Spontaneous revertant was deduced from each point.

tester strains. Doses of 10 and 50 ug/0.1 mL/plate increased significantly number of revertants of TA 98 and TA 100 respectively without S9 mix. Mutagenicity potencies were 2.72 and 2.74 net revertants/ug for TA 98 and TA 100. However, when S9 mix was added, effective doses elevated to 50 and 100 ug/0.1 mL/plate respectively, and dose-response curves became more straight, and mutagenicity potencies were about 1.42 and 1.72 net revertants/ug for TA 98 and TA 100 respectively (Figures 4 and 5). Owing to the cytotoxic killing effect, the dose-response curve of TA 98 without S9 mix slowed when the dose reached 1,000 ug/0.1 mL/plate.

This report showed that drinking well water used by the residents in the southwest coast of Taiwan contains some mutagens. The ethylacetate extracted fraction (EA or EA_2) of the well water fluorescent humic substances showed mutagenicity to Salmonella typhimurium, no matter whether or not rat liver microsomal enzyme systems(S9 mix) were added. However, if S9 mix was added, the mutagenicity was lower than that without S9 mix. At a higher dose , the dose-response curve tended to level off when S9 mix was not added; nevertheless, when S9 mix was added, the dose-response curve kept increasing. Therefore, two kinds of mutagenic actions might be involved in the fluorescent humic substances of well water. One was direct action, but with some cytotoxicity at high dose. These mutagens could directly cause bacterial mutation. The other kind, indirect action, allowed mutagens to induce bacterial mutation when S9 mix was added.

Drinking well water, used by residents of the southwest coast of Taiwan, contained a high concentration of arsenic. It has been pointed out epidemiologically that arsenic might be the causal factor of various cancers prevailing in that area (Chen et al. 1985), but arsenic was negative in Ames' test (Flora S D). This paper proved that there were some substances in the well water reacting positively to Ames' test. The evidence showed there may be at least two kinds of carcinogens in the well water. The first are substances which are Ames' test-negative, such as arsenic. Others are Ames' test-positive, such as the ethylacetate extracts of the fluorescent humic substances of the well water reported here. What are their compositions, and what quantity of mutagens composes that fraction? Study is in progress.

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