Mutagenicity Testing of 1,3-Butadiene, 1,4-Pentadiene-3-ol, Isoprene, 2,4-Hexadiene, *cis*- and *trans*-Piperlylene

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(Received March 19, 2001; Accepted October 5, 2001)

A mutagenicity test was conducted for 1,4-pentadiene-3-ol, 2,4-hexadiene, isoprene, *cis*- and *trans*-piperlylene in the bacterial mutation assay using *Salmonella typhimurium* (*S. typhimurium*) strain TA 100, TA98, TA1535 and *Escherichia coli* (*E. coli*) WP2uvrA/pKM101 with and without metabolic activation by S9 mix in the preincubation method. The mutagenicity of 1,3-butadiene was tested by the gas exposure method. Mutagenicity was weakly positive for 1,4-pentadiene-3-ol in TA 100 with S9 mix and was pseudopositive for WP2uvrA/pKM101 without S9 mix. Mutagenicity was very clear for 1,3-butadiene in 10 fold concentrated TA1535 with S9 mix. The 2,4-hexadiene, isoprene, *cis*-piperlylene and *trans*-piperlylene were not mutagenic on *S. typhimurium* TA98, TA100, TA1535 or *E. coli* WP2uvrA/pKM101 with or without metabolic activation. It can be concluded from these results, that chemicals, containing double bond carbon (C=C) chemical generally tend to show weak mutagenicity in the presence of the metabolic activation system.

Key words — mutagenicity, metabolic activation, preincubation method, *Salmonella typhimurium*, *Escherichia coli* WP2uvrA/pKM101

INTRODUCTION

Human beings are exposed to many chemicals directly or indirectly in their daily life activity. There are thousands of chemicals in commercial use and the scorecard contains reports on only 6800 of these chemicals (<http://www.scorecard.org/chemical-profile>). A review¹⁾ of chemical hazards was made in one report. Among various chemicals, 1,3-butadiene has been the subject of study, and a number of studies on its toxicity have been reviewed particularly its metabolism, carcinogenic and mutagenic properties.²⁻⁴⁾ Its toxicity on human beings was also studied.⁵⁾

Most of the toxicity studies were conducted on butadiene and isoprene⁶⁾ but not on the chemicals *cis*-piperlylene, *trans*-piperlylene (isomers of 1,3-pentadiene), 2,4-hexadiene or 1,4-pentadiene-3-ol. These chemicals are structurally related to 1,3-butadiene which contains carbon double bond. Basic tests to identify a hazardous feature such as carcinogenicity, chronic toxicity, developmental or reproductive toxicity, ecotoxicity, environmental fate, mutagenicity or neurotoxicity of *cis*-piperlylene, *trans*-piperlylene, 2,4-hexadiene and 1,4-pentadiene-3-ol are not available in the literature.¹⁾ Only one report evaluated 1,3-pentadiene for mutagenicity by *Salmonella/mammalian* assay.⁷⁾

The uses of *cis*-piperlylene, *trans*-piperlylene, 1,3-pentadiene and 1,4-pentadiene-3-ol are not known but their structures are related to 1,3-butadiene (Table 1). The structures of these chemicals show the carbon number distribution as C4-C6. The petroleum gases also consist of C4 to C6 carbon atoms⁸⁾ and when inhaled by human beings have several negative effects on human health. Research reports on environmental contamination and human exposure to *cis*-piperlylene, *trans*-piperlylene, 1,3-pentadiene and 1,4-pentadiene-3-ol are not available. Therefore, considering the above, an attempt was made in the present study to determine the mutagenicity of *cis*-piperlylene, *trans*-piperlylene, isoprene 2,4-hexadiene and 1,4-pentadiene-3-ol by preincubation assays using the bacterial tester strains *Salmonella typhimurium* (*S. typhimurium*) TA98, TA100, TA1535 and *Escherichia coli* (*E. coli*)

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Table 1. Details of the Test Chemicals Used in the Present Study

Chemical	Structure	CAS No.	Boiling point (C)	Toxicity	Reference
<i>cis</i> -piperylene*	$\text{CH}_3\text{CH}=\text{CHCH}=\text{CH}_2$	1574-41-0	44	No mutagenicity	7
<i>trans</i> -piperylene*	$\text{CH}_3\text{CH}=\text{CHCH}=\text{CH}_2$	2004-70-8	42	No mutagenicity	7
1,4-Pentadiene-3-ol	$\text{H}_2\text{C}=\text{CHCH}(\text{OH})=\text{CH}_2$	922-65-6	26	Weakly mutagenic	This study
2,4-Hexadiene	$\text{CH}_3\text{CH}=\text{CHCH}=\text{CHCH}_3$	592-46-1	82	No mutagenicity	This study
Isoprene	$\text{CH}_2=\text{C}(\text{CH}_3)\text{CH}=\text{CH}_2$	78-79-5	34	No mutagenicity, carcinogenic	6
1,3-Butadiene	$\text{CH}_2=\text{CHCH}=\text{CH}_2$	106-99-0	-4	Carcinogenic, mutagenic, genotoxic	5

*These two are isomers of the 1,3-Pentadiene and in the literature mutagenicity test was reported as 1,4-Pentadiene.

WP2*uvrA*/pKM101 in the presence and absence of S9 mix. An assay was also conducted to measure the mutagenicity for 1,3-butadiene by the gas exposure method.

MATERIALS AND METHODS

Chemicals —

Chemical sources: 1,3-Butadiene was from Takachiho-Shoji Co Ltd. (Tokyo, Japan), 1,4-pentadiene-3-ol from Aldrich, 2,4-hexadiene, isoprene, *cis*- and *trans*-piperylene were purchased from Wako Pure Chemical industries, Ltd. (Osaka, Japan). L-Histidine, biotin and agar were also from Wako Pure Chemical Industries, Ltd. Rat liver S9 and cofactor were purchased from Oriental Yeast Corporation (Japan). The nutrient broth was from Oxoid, Ltd. (Basingstoke, Hampshire, England).

Tester Strains — The tester strains used in this study: *S. typhimurium* TA98, TA100, TA1535 and *E. coli* WP2*uvrA*/pKM101 were provided by Dr. B. Ames via Matsushima [Japan Bioassay Research Center (Kanagawa, Japan)]. The culture stocks were stored at -80°C. The tester strain was freshly prepared by pre-culturing for 10 hr in nutrient broth (Oxoid no. 2).

Equipment — Tesmedia plates were obtained from Wako Pure Chemical Industries, Ltd. (Japan) and Tedlar bags (10 L) used in the gas exposure test, were purchased from GL Sciences Inc. (Tokyo, Japan).

Mutagenicity Testing —

For Liquid Chemicals: The mutagenicity experiment was conducted using preincubation assay.⁹⁾ The chemicals used in this study is evaporated easily, so we covered the test tube with parafilm [4 inch × 125 ft. roll, American Can Company (Greenwich, CT,

U.S.A.)] during the preincubation. The S9 mix (enzyme cofactors) was prepared according to the procedure of Ames *et al.*¹⁰⁾ Tester strains were precultured with the nutrient broth and the reaction mixture containing phosphate buffer/S9, cofactor, preculture strain and the test chemical (at concentrations of 500 µg, 1000 µg, 2000 µg or 5000 µg) was then preincubated while shaking at 37°C for 20 min. After shaking the mixture was mixed and immediately after adding top agar (2 ml) containing histidine/biotin, the mixture was poured onto minimal glucose agar plates. The positive control used during -S9 mix was AF2 (2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide) for TA98, TA100, and WP2*uvrA*/pKM101., and ENNG (*N*-ethyl-*N'*-nitro-*N*-nitrosoguanigine) for TA1535 strains. The positive control used during +S9mix was BaP(benzo[*a*]pyrene) for TA100, TA98 and for WP2*uvrA*/pKM101 and TA1535 was 2AA (2-Aminoanthracene). The combined solutions were vortex mixed and poured on minimal glucose agar plates. Plates were incubated at 37°C in the dark for 60 hr and counted using an automatic laser colony counter [Spiral System Instrument Inc., Model 500A (Bethesda, MD, U.S.A.)].

For Gas Exposure Method: The mutagenicity test for 1,3-butadiene was conducted according to Araki *et al.*¹¹⁾ as follows: The tester strains were precultured with the nutrient broth. The reaction mixture contained phosphate buffer, S9, cofactors and the preculture strain, these were mixed and immediately after adding the top agar containing histidine/biotin, the mixture was poured onto glucose agar plates. The bacterial plates were placed upside down without their lids, in a plate holder. The plate holder was placed in a 10-L tedlar bag through an opening on one side of the bag made by scissors. The bag was then closed by folding the opening 2 or 3 times and sealing it with adhesive tape. Air in the

bag was removed and then the gas was put in with a test substance at a fixed amount per plate. The bacterial plates in the bag were kept at 37°C for a fixed period. After termination of the exposure (24 hr), the test substance gas in the bag was removed. The tape was peeled off, the bag was left to stand for 30 min and then covered with a lid and incubated for a fixed time. Then the revertant colonies were counted.

RESULTS AND DISCUSSION

Mutagenicity was conducted by preincubation assay⁹⁾ to test the toxicity of *cis*- and *trans*-piperylene, 1,4-pentadiene-3-ol, 2,4-hexadiene and 1,3-butadiene. The tests in each set were conducted in triplicates three times with four different concentrations of each chemical. The results are discussed for each chemical.

cis- and *trans*-Piperylene

These are isomers of the 1, 3 pentadiene, they were tested for bacterial mutagenicity using the *S. typhimurium* strains TA100, TA98, TA1535 and *E. coli* WP2uvrA/pKM101. The concentrations of the two isomers used was 500 µg, 1000 µg, 2000 µg and 5000 µg per plate (Fig. 1A and 1B). *Cis*- and *trans*-piperylene did not show any dose related marginal increase of revertant colonies in the presence or absence of the metabolic activation by S9 mix. From these results it is judged that none of the tester strains is sensitive to the isomers either in the presence or absence of metabolic activation.

1,4-Pentadiene-3-ol

1,4-Pentadiene-3-ol was tested for bacterial mutagenicity using the *S. typhimurium* strains TA100, TA98, TA 1535 and *E. coli* WP2uvrA/pKM101. Concentrations of 1,4-pentadiene-3-ol used were 500 µg, 1000 µg, 2000 µg and 5000 µg per plate. The mutagenicity was weakly positive for 1,4-pentadiene-3-ol in *S. typhimurium* TA 100 with S9 mix and was pseudopositive in WP2uvrA/pKM101 without S9 mix. The results are shown in Fig. 1C. In other strains no mutagenicity was observed in either the presence or absence of S9 mix. There are no other reports on this chemical in the literature to compare or discuss. These results indicate that tester strain TA 100 is more sensitive to 1,4-pentadiene (in the presence of S9 mix) than the other strains used in this study.

2,4-Hexadiene

2,4-Hexadiene was tested for bacterial mutagenicity using the *S. typhimurium* strains TA100, TA98, TA1535 and *E. coli* WP2uvrA/pKM101. Concentrations used were 500 µg, 1000 µg, 2000 µg and 5000 µg per plate. The 2,4-hexadiene was not mutagenic and no dose related marginal increase of revertant colonies was observed in the tester strains used; the results are shown in the Fig. 1D. There are no other reports on this chemical in the literature. The result shows that the none of the tester strains used is sensitive to 2,4-hexadiene.

Isoprene

Isoprene was tested for bacterial mutagenicity using the *S. typhimurium* strains TA 100, TA 98, TA 1535 and *E. coli* WP2uvrA/pKM101. Concentrations used were 500 µg, 1000 µg, 2000 µg and 5000 µg per plate. The isoprene was not mutagenic and no dose related marginal increase of revertant colonies was observed in the tester strains, the results are shown in the Fig. 1E. One earlier report also found that isoprene was not mutagenic in *S. typhimurium* and did not induce sister chromatid exchanges⁶⁾ in mice. The present results confirmed this, none of the tester strains used was sensitive to the chemical.

1,3-Butadiene

1,3-Butadiene was tested in the bacterial mutation assay using *S. typhimurium* TA 1535 with and without metabolic activation by S9 mix and showed a positive response. The response was not marginal in the absence of metabolic activation (S9). The revertant colonies increased in TA 1535 with increasing the concentration of 1,3-butadiene (Fig. 2). Different concentrations of 1,3-butadiene in the tedlar bag were used as control (only air), 10%, 25% and 50%. The mutagenicity test was conducted with and without concentrated tester strain culture. The positive results were more clear with the 10 fold concentrated tester strain than with the one not concentrated. The S9 mix at different volume (100 µl and 200 µl per plate) did not show much difference in the revertant colonies. From these results, it is judged that the concentrated tester strain TA1535 is more sensitive to 1,3-butadiene than are TA100, TA98 and WP2uvrA/pKM101 (results not shown). The revertant colonies increased at 25% and decreased with 50% of 1,3-butadiene. This shows that the 50% gas is toxic to bacterial strain TA1535, and the metabolic activation decreased at higher concentration of 1,3-butadiene. Similarly, other studies also

Fig. 1. Dose Response Curves of the *trans*-Piperylene, 2,4-Hexadiene, 1,4-Pentadiene-3-ol, *cis*-Piperylene and Isoprene on *S. typhimurium* TA 98 (—■—), TA 100 (—●—) and TA 1535 (—×—) and *E. Coli* WP2uvrA/pKM101 (—▲—)

A) *trans*-piperylene B) *cis*-piperylene C) 1,4-pentadiene-3-ol D) 2,4-hexadiene and E) isoprene.

Fig. 2. Results of the Mutagenicity of 1,3-Butadiene Assayed by Plate Exposure Method on *S. typhimurium* in TA 1535

(▨ 50 µl S9/plate; □ 100 µl S9/plate; ■ 200 µl S9/plate). A: Bacteria culture solution not concentrated. B: 10 times concentrated bacteria culture solution.

showed TA1535 is sensitive to butadiene in the presence of S9 mix.^{2,11)} It is therefore concluded that the concentrated bacterial strain can be used for the gas exposure method.

Toxicity of 1,3-butadiene was studied on rat, mouse, hamster and human beings and a review is available in the literature.⁵⁾ Toxicity data on other chemicals which are structurally related chemicals to 1,3-butadiene: 1,4-pentadiene-3-ol, 2,4-hexadiene, isoprene, *cis*- and *trans*-piperlylene is scarce in the literature. Detailed studies on these chemicals relating to carcinogenicity, reproductive toxicity, chronic toxicity, neurotoxicity, ecotoxicity and their environmental impact on different species including human beings has to be explored in future.

In conclusion, the mutagenic test was weakly positive for 1,4-pentadiene-3-ol in TA100 with S9 mix and was pseudopositive in WP2uvrA/pKM101 without S9 mix. The test was very clear for 1,3-butadiene in TA1535 (in 10 fold concentration) with metabolic activation. The results were negative for 2,4-hexadiene, isoprene, *cis*- and *trans*-piperlylene. Negative results in this study do not prove, however, that these compounds lack toxicity in intact mammals including human beings. No published reports are available on the hazards of *cis*- and *trans*-piperlylene, 1,4-pentadiene-3-ol, 2,4-hexadiene to compare or discuss with the results of the present study. Further detailed toxicological investigations are necessary on *cis*- and *trans*-piperlylene, isoprene, 1,4-pentadiene-3-ol and 2,4-hexadiene.

Acknowledgements The Science and Technology

Agency and the Ministry of Health and Welfare of Japan supported this study.

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