Mutagenicity Studies with Toxaphene Congeners

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Introduction

Toxaphene (CAS No. 8001-35-2; chlorinated camphene) is a mixture of chlorinated terpenes. In the United States toxaphene was manufactured, for the most part, by chlorinating camphene to produce a product containing 67-69% chlorine. Manufacture of toxaphene ceased in the United States during the 1980's as registrations for its use as a pesticide were cancelled. Manufacture and use of toxaphene-like materials have been reported to still occur in Eastern European and Latin American countries. (1).

Toxaphene is composed of approximately 670 individual compounds, including chlorobornanes (1,2). Some of these chlorobornanes have been found in the environment, e.g., in soil and tissues of fish, and in laboratory mammals dosed with toxaphene (3,4,5). There is little toxicity information available for the chlorobornanes. Due to the potential for exposure of humans and ecologic species to these chlororobornanes, there is interest in determining their toxicities. A difficulty in obtaining toxicity data for these toxaphene components has been the lack of sufficient supplies of individual purified congeners.

Toxaphene is reported to be mutagenic to *Salmonella typhimurium* strains TA98 and TA100, but not to other histidine requiring *S. typhimurium* strains used in mutagenicity assays. Mutagenic activity is lost in the presence of the S9 metabolic system (6,7). In the study described here four toxaphene congeners, which are available as purified individual compounds, and toxaphene were tested for mutagenic activity in *S. typhimurium* strains TA98 and TA100. Due to the limited quantities of the congeners available, a validated microsuspension procedure was used to perform the assays instead of the usual plate incorporation procedure.

Material and Methods

The chemicals tested were toxaphene; USC 601 (Parlar 26; 2-exo,3-endo,5-exo,6-endo,8b,8c,10a,10b-Octachlorobornane); USC 602 (Parlar 50; 2-exo,3-endo,5-exo,6-endo,8b,8c,9c,10a,10b-Nonachlorobornane); USC 603 (Parlar 62; 2,2,5,5,8b,8c,9c,10a,10b-Nonachlorobornane); and USC 604 (Parlar 32; 2,2,5-endo,6-exo,8b,9c.10a-Heptachlorobornane). Toxaphene was supplied by Hercules Incorporated, Wilmington,

Delaware, U.S.A. USC 601, USC 602, USC 603 and USC 604, all of 98% purity, were purchased from Promochem GmbH, Wesel, Germany. The *Salmonella typhimurium* tester strains, TA98 and TA100, were obtained from the UK NCTC.

A microsuspension modified Ames test, which has demonstrated that mutagenicity can be detected with much lower concentrations of test material than the standard plate incorporation assay (8,9,10), was used due to the limited amounts of the congeners available. The congeners were dissolved in 1% methyl cellulose (MC) in dimethylsulfoxide (DMSO) with vortex mixing, sonication and heating to 37°C. This procedure resulted in solutions/suspensions with 12.5, 25, 50 or 100 mg/mL which eventually resulted in final test concentrations of 312.5, 625, 1250 and 2500 μg/mL of each congener. In further experiments all congeners and toxaphene were dissolved in or suspended in 1% MC in DMSO with heating to 70°C. Concentrations of the solutions/suspensions prepared in this manner were 6.25, 12.50, 25.0, 50.0, 100.0, 200.0 and 400.0 mg/mL which resulted in final test concentrations of 156.25, 312.5, 625, 1250, 2500, 5000 and 10000 μg/mL. Toxaphene was also assayed in the standard plate incorporation assay (11) to confirm its mutagenic activity. Given the differences in treatment procedures in the two assays, final test concentrations of toxaphene in the plate incorporation assay were as comparable as possible to those used in the microsuspension assay. The final test concentrations of toxaphene in the plate incorporation assay were 156.25, 312.5, 625, 1250, 2500, 5000 and 10000 μg/plate. 2-Nitrofluorene and sodium azide were used as positive control materials for strains TA98 and TA100, respectively. The solvent, 1% MC in DMSO, served as the negative control material. All assays were performed in triplicate.

Pre-incubation mixes were prepared by placing 100 μL bacterial culture, 5 μL test solution or suspension or negative control material, and 95 μL buffer solution in a treatment well (in a 96 well plate) followed by gentle mixing while incubating at 37°C for 90 minutes. The mixes were added to 2 mL molten agar at 46°C, rapidly mixed, and poured onto Minimal Davis agar plates. The plates were inverted and incubated at 37°C in the dark for 3 days. They were then examined for toxicity and revertant colonies. Colonies were counted both electronically and manually to ensure accurate scoring in those cases when preciptation of the test article was seen.

Statistical analysis was by Dunnett's test. The materials were considered to be mutagenic if Dunnett's test gave a significant response ($p \leq 0.01$), the data showed a significant dose correlation, and the results were reproducible.

Results and Discussion

No toxicity was seen at any concentration of toxaphene in either the microsuspension/pre-incubation or plate incorporation assays. No toxicity was seen at any of the concentrations of the congeners. Precipitation of USC 601, USC 602, and USC 604 was seen at the 10000 μg/mL concentration; no precipitation of USC 603 was seen at any concentration.

In the plate incorporation assay, toxaphene was mutagenic to strain TA98 at a concentration of 10000 μg/plate and to strain TA100 at all concentrations tested. In the microsuspension/pre-incubation assay, toxaphene was not mutagenic to strain TA98 at any concentration tested, but was mutagenic to strain TA100 at concentrations of 2500, 5000, and 10000 μg/mL.

Studies with USC 601, USC 602, USC 603 and USC 604 at final test concentrations of 312.5, 625, 1250 and 2500 μg/mL resulted in no mutagenic activity in either strain TA98 or TA100.

In other experiments, due to the lack of mutagenicity of toxaphene to strain TA98 in the microsuspension/pre-incubation assay, only strain TA100 was used. In the experiment in which toxaphene and all congeners were tested simultaneously in strain TA100, toxaphene was mutagenic at a concentration of 10000 μg/mL, but USC 601, USC 602, USC 603 and USC 604 were not mutagenic to strain TA100 at any concentration tested. The data obtained from experiments with strain TA100 are summarized in the following table.

Summary of Assays with Toxaphene, USC 601, USC 602, USC 603 and USC 604 with *S. typhimurium* strain TA100

Test Concentration μg/mL	Toxaphene Revertants Mean ± SD	USC 601 Revertants Mean ± SD	USC 602 Revertants Mean ± SD	USC 603 Revertants Mean ± SD	USC 604 Revertants Mean ± SD
156.25	228 ± 11[a] 164 ± 16[b]	196 ± 18[a] 159 ± 3[c]	194 ± 26[a] 191 ± 13[c***]	186 ± 25[a] 139 ± 22[c]	191 ± 19[a] 122 ± 9[c]
312.5	233 ± 100[a] 169 ± 10[b]	184 ± 10[a] 152 ± 21[c]	196 ± 15[a] 131 ± 4[c]	194 ± 15[a] 151 ± 30[c]	189 ± 15[a] 111 ± 13[c]
625	218 ± 42[a] 157 ± 9[b]	192 ± 16[a] 168 ± 3[c]	200 ± 5[a] 148 ± 7[c]	175 ± 21[a] 139 ± 7[c]	192 ± 15[a] 96 ± 14[c]
1250	174 ± 20[a] 157 ± 10[b]	183 ± 15[a] 150 ± 2[c]	173 ± 4[a] 153 ± 6[c]	184 ± 28[a] 159 ± 12[c]	186 ± 12[a] 132 ± 3[c]
2500	209 ± 42[a] 211 ± 43[b***]	172 ± 23[a] 135 ± 20[c]	174 ± 17[a] 154 ± 11[c]	183 ± 20[a] 168 ± 14[c]	176 ± 20[a] 126 ± 35[c]
5000	214 ± 29[a] 263 ± 41[b***]	189 ± 13[a] 147 ± 9[c]	205 ± 24[a*] 177 ± 15[c*]	176 ± 30[a] 154 ± 14[c]	202 ± 34[a] 116 ± 11[c]
10000	463 ± 171[a***] 362 ± 54[b***]	202 ± 10[a*] 154 ± 18[b]	200 ± 30[a] 157 ± 14[b]	198 ± 6[a] 158 ± 13[b]	199 ± 10[a] 141 ± 16[b]

Negative Controls: 1% MC in DMSO at 5μl:
Revertants (Mean ± SD): [a]168 ± 20 ; [b]125 ± 11; [c]148 ± 18
Positive control: (Sodium azide) tested at10 μg/mL:
Revertants (Mean ± SD): [a]888 ± 138; [b]613 ± 7; [c]661 ± 54
* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.005$

It is concluded, based on these studies that, while toxaphene itself is mutagenic, the congeners tested, i.e., a heptachlorobornane, an octachlorobornane and two nonachlorobornanes, are not mutagenic. These findings are important with regard to risk evaluations based on analytes found in environmental samples, such as soil and fish. These

findings demonstrate that toxicity data developed for the parent toxaphene are not relevant for the congeners and that toxaphene congeners should not be evaluated using toxicity data developed for toxaphene. Consideration should also be given to the fact, when evaluating risk from exposure to the congeners, that metabolism of toxaphene results in loss of mutagenic activity. The lack of mutagenic activity, while not an indication of the lack of other toxicities, does indicate that if there is human exposure to the congeners, the exposure is to chemical substances of lower mutagenic potential than toxaphene.

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