DATA EVALUATION RECORD

TRICHLORFON

Gene Mutations in Salmonella typhimurium TA98 and TA100

CITATION: Batzinger, RP, Bueding E. 1977. Mutagenic activities in vitro and in vivo of five antischistosomal compounds. J. Pharmacol. Exp. Therap. 200:1-9.

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STUDY TYPE: Gene mutations in Salmonella typhimurium TA98 and TA100.

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ACCESSION NUMBER: Not available.

MRID NUMBER: Not available.

LABORATORY: The Johns Hopkins University, Baltimore.

TEST MATERIAL: Metrifonate, $(CH_3O)_2$:PO-CHOHCCl$_3$, was supplied by Dr. R. Gonnert [purity not stated].

PROTOCOL:

1. Salmonella typhimurium strains TA98 and TA100 were supplied by Drs. B. Ames and P. E. Hartman. For the host-mediated assays, 6- to 8-week old female mixed Swiss-Webster CF1 mice (Charles River) were used. For all assays, metrifonate was initially dissolved in DMSO.

2. The in vitro tests were conducted with and without added S9 fractions from phenobarbital-treated rats according to Ames et al. (1973. Proc. Natl. Acad. Sci. 70:2281-2285). Metrifonate was added to plates at 3.4, 10.2, and 34 µmole/plate. The plates were incubated for 48 hours at 37°C. Negative controls were conducted with DMSO, and positive controls were run with hycanthone.

3. For host-mediated assays, metrifonate was administered to mice [number not stated] by gastric intubation at 50, 100, and 200 mg/kg. Two hours later, the mice were injected intraperitoneally with suspensions of either strain of S. typhimurium. After 6 hours, bacteria were retrieved by injection and removal of 0.85 percent NaCl. The exudates were serially diluted and plated. Plates were counted after 48 hours incubation at 37°C. Negative and positive (hycanthone) controls were included.

4. In another assay, the urine of five treated mice was collected on ice for 24 hours in metabolism cages. The mice were then treated with metrifonate by gastric intubation at 100 and 200 mg/kg. Urine was then collected twice over the two subsequent 24-hour periods. The urine samples were filter-sterilized and assayed for mutagenic

activity (with and without added S9) on both S. typhimurium strains. Additional tests were conducted with urines incubated with β-glucuronidase and arylsulfatase to determine if mutagens were present as inactive glucuronate or sulfate conjugates.

RESULTS:

In vitro Ames tests. The average mutation rates for each strain with metrifonate exceeded control rates in a dose-related manner, but was not influenced by S9 mix (Table 1). It was not stated whether the differences were statistically significant.

Host-mediated assays. In the intraperitoneal system, there was a dose-related mutagenic effect (significant, $p<0.02$) on strain TA100, but not on strain TA98 (Table 1). There was also dose-related mutagenic activity in the urine of treated mice. The effect was greater on strain TA100 than on TA98, and was greater in urine collected during the final 24 hours after treatment (Table 1). It was not stated if the test results with urine were statistically significant. S9 mix had no influence on the mutagenic activity, nor did incubation with β-glucuronidase and arylsulfatase.

CONCLUSIONS:

The results of the in vitro tests demonstrated that metrifonate was mutagenic to both strains. Although statistical analyses were not given, the mean values for each strain, with and without S9 mix, were increased in a dose-related manner. In this system, the mutation rate for strain TA98 (frameshift mutations) was greater than that for strain TA100 (base-pair mutations); e.g., at the high dose, the mutation rate for TA98 was approximately 25-fold greater than the control rate, whereas for TA100 it was approximately 2-fold greater.

In the host mediated assay system, there was a dose-related significant increase in the mutation rate of strain TA100 resulting from metrifonate treatment. In contrast to the in vitro tests, strain TA98 was not affected in the host-mediated system. Therefore, the data from both of these systems indicate different types of mutagenic mechanisms occurred as a result of metrifonate treatment. Although the type of negative control used (i.e., untreated or solvent treated) were not stated, the negative mutagenic response by TA98 serves as a negative control for the purposes of evaluating this study.

There was no increase in the mutagenicity of metrifonate after S9 mix was added. In the urine analyses, S9 mix markedly enhanced the mutagenicity of the positive control (hycanthone) on strain TA98, demonstrating that the S9 mix was active.

The urine analyses for metrifonate cannot be fully evaluated because statistical analyses and control data were not given. Furthermore, urine

was collected prior to treatment, but it was not stated what purpose this served. It is possible that these samples served as controls, as the manner of obtaining control data was not stated.

CORE CLASSIFICATION: Unacceptable.

The following deficiencies were noted:

- The purity of the test material was not stated.
- The number of plates per assay and the number of assays were not stated.
- Phenobarbital was used to treat the rats, rather than Aroclor 1254.
- In the urine assays, statistical analyses were not presented and control data were not given. Furthermore the manner in which control data were obtained are not described.

TABLE 1. Mutagenic Activity of Metrifonate in Salmonella typhimurium TA98 and TA100

Type of test	Metrifonate dosage	Number of revertants in excess of controls[a]			
		TA100		TA98	
		With S9	Without S9	With S9	Without S9
In vitro Ames test	Positive control[b]	1,611	1,609	1,237	1,371
	3.4 µmole/plate	37	49	65	62
	10.2 µmole/plate	142	153	182	189
	34.0 µmole/plate	349	368	539	578
Host-mediated intraperitoneal[c]	Positive control[d]	ND[e]	4.52(±0.30)[f]	ND	7.37(±0.39)
	50 mg/kg	ND	0.29(±0.06)	ND	NS[g]
	100 mg/kg	ND	0.79(±0.09)	ND	NS
	200 mg/kg	ND	1.43(±0.11)	ND	NS
Host-mediated urine 100 mg/kg (0-24 hours)[h]	Positive Control[d]	650	868	3,500	625
	100	37	41	0	0
	200 mg/kg	48	72	8	7
(24-48 hours)[h]	Positive control[d]	205	550	4,600	140
	100 mg/kg	7	5	0	0
	200 mg/kg	13	12	5	3

[a]Control rates: in vitro test, 172 and 23 revertants/plate for TA100 and TA98, respectively.
host-mediated intraperitoneal, 0.68 (SE, ± 0.05)/10^6 and 0.10 (SE, ± 0.03)/10^6 bacteria for TA100 and TA98, respectively.
host-mediated urine, not stated.

[b]Hycanthone at 100 nmol/plate.

[c]Dosed by gastric intubation; bacteria injected intraperitoneally and harvested 2 hours later.

[d]Hycanthone intramuscularly at 100 mg/kg.

[e]ND=Not done.

[f]Numbers in parentheses are standard errors (SE).

[g]NS=Not significant [as reported].

[h]Dosed by gastric intubation; bacteria incubated with urine collected 0-24 and 24-48 hours after dosing.