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Evaluation of the Genotoxic Potential of Zinc Pyrithione in the *Salmonella* Mutagenicity (Ames) Assay, CHO/HGPRT Gene Mutation Assay and Mouse Micronucleus Assay

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The mutagenic potential of zinc pyrithione (Znpt) was evaluated *in vitro* in the *Salmonella*/mammalian microsome plate incorporation mutagenicity (Ames) assay and the CHO/HGPRT gene mutation assay. The clastogenic potential of Znpt was evaluated *in vivo* using the mouse micronucleus test. Znpt was negative in the Ames test in five tester strains in the presence and absence of rat liver microsomal enzymes when assayed at concentrations ranging between 10 and 333 μg per plate and between 0.03 and 33 μg per plate, respectively. Znpt also produced negative results in the CHO/HGPRT assay. No significant increases in mutant frequencies were seen in the presence and absence of rat liver microsomal enzymes. In each case, the highest concentrations reduced cellular viability by 83% and 85%, respectively. Znpt also did not induce increased frequencies of micronuclei in mouse bone marrow cells when tested at the maximally tolerated dose (MTD) (44 mg kg^{-1}). These data support the conclusion that Znpt lacks genotoxic activity under the conditions of these tests.

INTRODUCTION

The water-insoluble chemical zinc pyridine-2-thiol-1-oxide, commonly known as zinc pyrithione (Znpt), is a broad-spectrum antimicrobial and antifungal agent that has been utilized as an active ingredient in antidandruff shampoo formulations since 1962. The toxicity of Znpt by various routes of exposure has been studied extensively in several species of animals and has been described previously.[1–12]

While much work has been carried out to determine the acute, chronic and reproductive toxicity of Znpt, less work has been completed to assess its mutagenic potential. Znpt was tested in the *in vivo* dominant lethal assay and failed to reveal significant differences between treated groups and control animals.[13] Notwithstanding this *in vivo* assay, the available data to define the mutagenic potential of Znpt is considered insufficient to evaluate this aspect of its toxicological profile.

A series of *in vitro* and *in vivo* assays was conducted to provide a package of data to characterize the mutagenicity of Znpt and to support the reregistration of this biocide with the United States EPA.

MATERIAL AND METHODS

The mutagenicity studies were conducted at Microbiological Associates, Inc. of Rockville, Maryland, during the period from 7 May 1990 through 28 September 1990. All assays were carried out in accordance with EPA/FIFRA guidelines and governmental standards for Good Laboratory Practice (GLP).

Test material

A sample of zinc pyrithione (lot number 9RC-290-109, ZP purity 96%) in a 48% aqueous dispersion was obtained from the Olin Research Center in Cheshire, CT.

Salmonella/mammalian microsome plate incorporation mutagenicity assay (Ames test)

The experimental materials, methods and procedures are based on those described by Ames *et al.*[14] and Maron and Ames.[15]

The vehicle used to deliver Znpt to the test system was 100% ethanol (EtOH) (CAS no. 64-17-5), purchased from Pharmco Products Incorporated, Bayonne, NJ.

For assays conducted with external metabolic activation, Aroclor-induced rat liver microsomal enzyme (S-9) was added to the test system in 0.5-ml aliquots.

The S-9 homogenate was characterized for its ability to metabolize the promutagens 7,12-dimethylbenzanthracene and 2-aminoanthracene to mutagens, as described by de Serres and Shelby.[16]

The tester strains used were the *Salmonella typhimurium* histidine auxotrophs TA98, TA100, TA1535, TA1537 and TA1538 as described by Ames *et al.*[14] and were received directly from Dr Bruce Ames,

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Experimental design. The assay was performed in three phases. The first phase was used to establish the dose range over which the test article would be assayed. The range-finding study was conducted using ten dose levels: 10, 33, 67, 100, 33, 667, 1000, 3333, 6667 and 10,000 μg of test article per plate. The second and third phases consisted of an initial and confirmatory mutagenicity assay.

The test article was serially diluted immediately before its use. In the absence of S-9 mix, 100 μl of tester strain and 50 μl of vehicle or test article were added to 2.5 ml of molten selective top agar at 45 ± 2°C. When S-9 mix was required, 0.5 ml of S-9 mix, 100 μl of tester strain and 50 μl of vehicle or test article were added to 2.0 ml of molten selective top agar at 45 ± 2°C. When plating the positive controls, the test article aliquot was replaced by a 50-μl aliquot of appropriate positive control. After vortexing, the mixture was overlaid onto the surface of 25 ml of minimal bottom agar. After the overlay had solidified, the plates were inverted and incubated for ca. 48 h at 37 ± 2°C. Plates that were not counted immediately following the 48-h incubation period were stored at 4 ± 2°C until colony counting could be conducted.

The dose range-finding study included ten dose levels of test article using TA100 only, both in the presence and absence of S-9.

In the initial mutagenicity assay, Znpt was tested at five dose levels along with appropriate vehicle and positive controls on tester strains TA98, TA100, TA1535, TA1537 and TA1538 in the presence and absence of rat liver microsomal enzymes. The results of the dose range-finding study in the presence and absence of microsomal enzymes indicate that, owing to the toxicity to the test system, the maximum dose to be plated in the mutagenicity assay would be 333 μg per plate in the presence of microsomal enzymes and 3.3 μg per plate in the absence of microsomal enzyme. Doses tested were 0.0, 0.03, 0.1, 0.3, 1.0 and 3.3 μg per plate in the absence of S-9 and 0.0, 10, 33, 50, 100 and 333 μg per plate in the presence of S-9, along with vehicle and positive controls. Concentrations tested in the confirmatory assay were 0.0, 0.03, 0.1, 0.3, 1.0, 2.0, 3.3, 5.0 and 10 μg per plate in the absence of S-9 and 0.0, 10, 33, 50, 100 and 333 μg per plate in the presence of S-9. All dose levels of test article vehicle controls and positive controls were plated in triplicate. After the data generated in the initial mutagenicity assay were evaluated, the confirmatory mutagenicity assay was conducted.

Criteria for evaluation of test results. For a test article to be scored as positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain with a minimum of two increasing concentrations of test article with maximum increases specified as follows: for tester strains TA1535, TA1537 and TA1538, data sets are judged as positive if the increase in mean revertants at the peak of the dose response is equal to or greater than three times the mean vehicle control value. For tester strains TA98 and TA100, data sets are judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than twice the mean vehicle control value.

CHO/HGPRT gene mutation assay

Mammalian cells. The methods are similar to those described by O'Neill *et al.*[17] in the conduct of the assay using the CHO-K_1-BH_4 cell line.

The CHO-K_1-BH_4 cells were obtained directly from Dr Abraham Hsie, Oak Ridge National Laboratories, Oak Ridge, TN. The freeze lot of cells was tested and found to be free of mycoplasma using both the agar culture and Hoechst staining procedures.[18] For assays conducted with external metabolic activation, Aroclor-induced rat liver S-9 was added to the treatment medium. Ethyl methanesulfate (0.2 μl ml^{-1}) was the positive control in assays without metabolic activation. Benzo[*a*]pyrene (4 μg/ml^{-1}) was the positive control in assays with metabolic activation. Also, the solvent vehicle for the test article was used as the solvent control.

Chemicals used in the CHO/HGPRT assay were solvent for test article (ethanol), solvent for positive controls (DMSO, CAS no. 67-68-5), ethyl methanesulfonate (EMS, CAS no. 62-50-0) and benzo[*a*]pyrene (B(a)P, CAS no. 50-32-8).

The optimal dose levels for the mutation assay were selected following a preliminary toxicity test based on the colony-forming efficiency. CHO cells were exposed to solvent alone and to nine concentrations of test article, ranging from 0.03 to 3.6 μg ml^{-1} for 5 h at 37 ± 1°C. The final solvent concentration in the culture medium was 1% by volume. The following day, the treated cells were trypsinized and reseeded at a density of 100 cells per 60-mm dish. The cloning efficiency was determined 7–10 days later. The cell survival of the test-article-treated groups are expressed relative to the solvent control (relative cloning efficiency).

Experimental design. The mutation assay was performed according to a protocol developed from published methodologies.[17, 19] The time of initiation of chemical treatment was designated as day 0. Cells were exposed, in duplicate, to five concentrations of the test article for 5 h at 37 ± 1°C. The final solvent concentration in the cultured medium was 1% by volume. Dose levels for the CHO/HGPRT mutation assay were selected following preliminary toxicity tests. Toxicity was based upon cloning efficiency of the cells after test article treatment relative to solvent control. CHO cells were exposed to solvent alone and to nine concentrations, ranging from 3.6–0.03 μg ml^{-1} in the absence of microsomal enzymes and from 57.6 to 0.45 μg ml^{-1} in the presence of microsomal enzymes. Based on the results of the preliminary toxicity test in the presence and absence of microsomal enzymes, the maximal dose chosen was 2.0 μg ml^{-1} in the absence of microsomal enzymes and 30 μg ml^{-1} in the presence of microsomal enzymes. The initial assay treatments in the absence of microsomal enzymes were 0.25, 0.5, 0.75, 1.0 and 2.0 μg ml^{-1} test article, 0.2 μg ml^{-1} EMS, untreated control and solvent control. In the presence of microsomal enzymes, the following treat-

Table 1. Initial *Salmonella* mutagenicity assay[a] with tester strains TA98, TA100, TA1535, TA1537 and TA1538

Concentration[b] (μg per plate)	TA98	TA100	TA1535	TA1537	TA1538
Without S-9					
Solvent control[c]	24 ± 6	170 ± 26	11 ± 5	8 ± 4	9 ± 2
0.03	22 ± 9	156 ± 14	7 ± 7	6 ± 3	8 ± 3
0.1	28 ± 3	174 ± 10	10 ± 4	7 ± 1	9 ± 2
0.3	29 ± 5	183 ± 12	9 ± 1	11 ± 5	7 ± 2
1.0	34 ± 7	171 ± 12	7 ± 3	10 ± 5	6 ± 4
3.3	33 ± 8	254 ± 15	9 ± 3	7 ± 5	4 ± 1
Positive control[d]	238 ± 19	619 ± 47	217 ± 26	194 ± 21	384 ± 30
With S-9					
Solvent control[c]	29 ± 1	152 ± 13	30 ± 6	11 ± 3	18 ± 2
10	31 ± 7	213 ± 18	28 ± 6	12 ± 3	13 ± 6
33	41 ± 7	222 ± 16	28 ± 4	6 ± 3	10 ± 3
50	47 ± 6	227 ± 9	26 ± 6	6 ± 1	9 ± 3
100	40 ± 11	196 ± 57	23 ± 5	5 ± 2	5 ± 3
333	14 ± 3	0 ± 0	11 ± 4	1 ± 1	1 ± 1
Positive control[d]	580 ± 18	783 ± 42	93 ± 13	91 ± 11	721 ± 47
Without S-9[e]					
Solvent control[c]	39 ± 10		8 ± 4	10 ± 1	
0.1	32 ± 2		9 ± 3	4 ± 1	
0.3	30 ± 5		8 ± 4	7 ± 1	
1.0	27 ± 5		10 ± 4	8 ± 2	
3.3	32 ± 2		7 ± 3	5 ± 1	
10	20 ± 5		2 ± 2	0 ± 1	
33	19 ± 2		1 ± 1	0 ± 0	
Positive control[d]	248 ± 16		366 ± 22	190 ± 20	

[a] Data are average revertants per plate ± standard deviation.
[b] Each concentration was tested in triplicate
[c] Solvent control (100% ethanol, plating aliquot of 50 μgl).
[d] Positive controls without S-9: TA98 and TA1538 (2-nitrofluorene, 1.0 μg per plate), TA100 and TA1535 (sodium azide, 1.0 μg per plate), TA1537 (ICR-191, 2.0 μg per plate). Positive controls with S-9: all tester strains (2-aminoanthracene, 0.5 μg per plate).
[e] Supplemental testing owing to lack of toxicity in the initial assay.

ments were administered: 5.0, 7.5, 10, 20 and 30 μg ml^{-1} test article, 4.0 μg ml^{-1} B(a)P, untreated control and solvent control. A confirmatory assay was performed utilizing the following treatments in the absence of microsomal enzymes: 0.5, 0.6, 0.75, 1.2, 1.5 and 1.8 μg ml^{-1} test article, 0.2 μg ml^{-1} EMS, untreated control and solvent control. In the presence of microsomal enzymes, the following treatments were used: 2.5, 5.0, 7.5, 10, 20 and 30 μg ml^{-1} test article, 4.0 μg ml^{-1}B(a)P, untreated control and solvent control. At this time, the cells were subcultured to assess cytotoxicity and to initiate the phenotypic expression period.

Historical values for the mutant frequencies observed in this laboratory for the untreated, solvent and positive control cultures over the past several years have been calculated. The current confidence interval at $P < 0.01$ is 9.8 mutants per 10^6 clonable cells.

Evaluation of test results. An assay is considered positive when a dose-dependent increase in mutant frequency is observed and one or more of the five concentrations tested for mutation has induced a significant increase in mutant frequency. A significant increase would be one that was > 40 mutants per 10^6 clonable cells. An assay would be considered suspect if there was no dose response but one or more doses induced a significant increase in the mutant frequency. The assay would be considered negative if none of the doses tested induced a mutant frequency that was considered to be significant.

Micronucleus assay in mice

Materials Animals used for this assay were ICR Mice, 6–8 weeks old, with males being 24–31 g of body weight and females being 18–28 g of body weight at the time of dose administration. Animals were received from Harlan Sprague Dawley, Inc., Frederick, MD.

Biological reagents used were fetal bovine serum (FBS), May–Gruenwald stain, Giemsa stain, methanol and Permount. Chemicals used in the assay were distilled water, carrier vehicle for the test article and triethylenemelamine (TEM), which is the positive control.

Animals received for testing were quarantined for no less than 5 days after receipt. Mice were then observed each working day for signs of illness, unusual food and water consumption and other conditions of poor health. The animals were judged to be healthy prior to utilization in the assay.

Table 2. Confirmatory *Salmonella* mutagenicity assay[a] with tester strains TA98, TA100, TA1535, TA1537 and TA1538

Concentration[b] (μg per plate)	TA98	TA100	TA1535	TA1537	TA1538
Without S-9					
Solvent control[c]	15 ± 5	127 ± 7	10 ± 3	7 ± 1	5 ± 5
0.03	[e]	137 ± 15	[e]	[e]	6 ± 2
0.1	16 ± 2	143 ± 13	5 ± 4	7 ± 2	8 ± 2
0.3	11 ± 2	143 ± 18	10 ± 6	5 ± 1	4 ± 1
1.0	13 ± 3	141 ± 5	6 ± 2	8 ± 2	5 ± 3
2.0	[e]	139 ± 17	[e]	[e]	[e]
3.3	8 ± 2	146 ± 10	6 ± 4	4 ± 4	5 ± 3
5.0	[e]	119 ± 24	[e]	[e]	[e]
10	5 ± 2	[e]	4 ± 2	3 ± 2	[e]
Positive control[d]	237 ± 86	464 ± 43	442 ± 13	74 ± 8	344 ± 16
With S-9					
Solvent control[c]	25 ± 5	157 ± 14	14 ± 2	16 ± 2	24 ± 6
10	25 ± 6	172 ± 21	13 ± 3	16 ± 5	21 ± 5
33	22 ± 2	151 ± 6	16 ± 2	15 ± 5	21 ± 6
50	13 ± 3	165 ± 11	13 ± 1	13 ± 2	15 ± 1
100	11 ± 4	74 ± 41	9 ± 2	11 ± 3	8 ± 3
333	3 ± 1	18 ± 4	3 ± 2	1 ± 2	2 ± 1
Positive control[d]	1912 ± 43	7210 ± 45	178 ± 16	282 ± 15	1619 ± 230

[a] Data are average revertants per plate ± standard deviation.
[b] Each concentration was tested in triplicate.
[c] Solvent control (100% ethanol, plating aliquot of 50 μl).
[d] Positive controls as specified in Table 1.
[e] Not tested.

The animals were housed in an AAALAC-accredited facility with a controlled environment of 74 ± 6°F, 50 ± 20% relative humidity, and a 12-h light/dark cycle. Mice were group-housed up to five per cage in plastic autoclavable cages with filter tops. Hardwood chips were used for bedding. Animals had free access to tap-water and to certified laboratory rodent chow that had been analyzed for environmental contaminants.

Experimental design. For the micronucleus assay, the animals were assigned to thirteen experimental groups of five males and five females each, based on a computer-generated randomization program. Each animal was given a sequential number and identified by ear tag. Dose range-finding experiments conducted prior to the initiation of the micronucleus assay determined a maximally tolerated dose (high dose) to be 44 mg kg^{-1} of body weight by intraperitoneal (i.p) administration (80% of i.p. LD_{50}). (Data not presented). The protocol outline was as follows:

	Animals per sex to be sacrificed after dose administration		
	24 h	48 h	72 h
Vehicle control	5	5	5
Low test dose (11 mg kg^{-1})	5	5	5
Mid test dose (22 mg kg^{-1})	5	5	5
High test dose (44 mg kg^{-1})	5	5	5
TEM (0.25 mg kg^{-1})	5	–	–

The test article–vehicle mixture or the vehicle alone was administered by i.p. injection at a constant volume of 10 ml kg^{-1} body wt. The positive control, TEM, was injected i.p. at a dose level of 0.25 mg kg^{-1}. All mice in the experimental and control groups were weighed immediately prior to dose administration and the dose volume was based on individual body weights. Animals were observed within 4 h after dose administration and each day thereafter for clinical signs of chemical effect.

At the scheduled sacrifice time, five mice per sex were sacrificed by CO_2 asphyxiation. Immediately following sacrifice, the femurs were exposed, cut just above the knee, and the bone marrow was aspirated into a syringe containing a small amount of FBS. The bone marrow cells were transferred to a capped centrifuge tube containing ca. 1 ml of FBS. The bone marrow cells were pelleted by centrifugation. The cells were resuspended by aspiration with a capillary pipet and a small drop of bone marrow suspension was spread onto a clean glass slide. Two to four slides were prepared from each animal.

Evaluation of test results. The incidence of micronucleated polychromatic erythrocytes per 100 polychromatic erythrocytes was determined from each animal and treatment group. Statistical significance was determined using the Kastenbaum–Bowman tables, which are based on the binomial distribution.

In order to quantify the proliferation state of the bone marrow as an indicator of bone marrow toxicity, the proportion of polychromatic erythrocytes to total erythrocytes was determined for each animal and treatment group.

The test article is considered to induce a positive response if a treatment-related increase in micronucle-

Table 3. Results of the initial CHO/HGPRT assay

Concentration[a] (μg ml^{-1} Znpt)	Cloning efficiency[b]	Relative cloning efficiency[c] (%)	Total mutant colonies	Mutant frequency[d]
Without S-9				
Untreated control	1.41	129	0	<0.9[g]
Solvent control	1.09	100	0	<0.9[g]
0.25	1.08	99	0	<1.0[g]
0.5	1.32	121	9	8.0
0.75	1.15	106	0	<0.9[g]
1.0	1.12	103	9	7.6
2.0	0.18	17	24	29.0
EMS[e]	0.94	87	199	178.7
With S-9				
Untreated control	1.08	100	11	9.0
Solvent control	1.09	100	1	0.7
5.0	1.28	117	4	3.8
7.5	1.09	101	6	6.0
10	1.16	106	7	5.9
20	0.71	65	0	<0.8[g]
30	0.17	15	0	<1.35[g]
B(a)P[f]	0.11	10	186	206.7

[a] Cells were exposed to the test article for 5 h at 37 ± 1°C.
[b] Cloning efficiency following treatment = total colonies counted/100 cells × number of replicates.
[c] Relative cloning efficiency
$$= \frac{\text{Cloning efficiency of treatment group}}{\text{Cloning efficiency of study group}} \times 100.$$
[d] Mutant frequency
$$= \frac{\text{Total mutant colonies}}{\text{Number selection dishes} \times \text{cloning efficiency} \times 2 \times 10^5 \text{ cells} \times 10^6}.$$
[e] EMS, 0.2 μg ml^{-1} ethyl methanesulfonate.
[f] B(a)P, 4.0 μg ml^{-1} benzo[*a*]pyrene
[g] Calculated on the basis of < 1 mutant colony observed in a total of five dishes.

Table 4. Results of the confirmatory CHO/HGPRT assay

Concentration[a] (μg ml^{-1} Znpt)	Cloning efficiency[b]	Relative cloning efficiency[c] (%)	Total mutant colonies	Mutant frequency[d]
Without S-9				
Untreated control	0.98	108	16	20.1
Solvent control	0.91	100	5	6.3
0.5	0.87	96	1	1.2
0.6	0.79	87	6	7.9
0.75	0.57	63	0	<1.3[g]
1.2	0.10	11	0	<1.5[g]
1.5	0.05	5	0	<1.7[g]
1.8	0.01	1	0	18.8
EMS[e]	0.77	85	224	314.0
With S-9				
Untreated control	0.95	94	8	9.3
Solvent control	1.01	100	10	9.6
2.5	0.98	97	27	24.7
5.0	1.03	102	18	18.2
7.5	1.00	98	3	2.6
10	0.99	98	10	10.4
20	0.25	25	18	26.9
30	0.08	8	0	<1.6[g]
B(a)[f]	0.18	17	145	203.3

[a] Cells were exposed to the test article for 5 h at 37 ± 1°C.
[b] Cloning efficiency = total colonies counted/100 cells × number of replicates.
[c] Relative cloning efficiency
$$= \frac{\text{Cloning efficiency of treatment group}}{\text{Cloning efficiency of study group}} \times 100.$$
[d] Mutant frequency
$$= \frac{\text{Total mutant colonies}}{\text{Number selection dishes} \times \text{cloning efficiency} \times 2 \times 10^5 \text{ cells} \times 10^6}$$
[e] EMS, 0.2 μg ml^{-1} ethyl methanesulfonate
[f] B(a)P, 4.0 μg ml^{-1} benzo[*a*]pyrene
[g] Calculated on the basis of < 1 mutant colonies observed in a total of five dishes.

ated polychromatic erythrocytes is observed relative to the vehicle control ($P < 0.05$, Kastenbaum–Bowman Tables). The positive response must be dose-dependent or must be reproducibly positive for at least one of the test points.

Criteria for determination of a valid test. The mean incidence of micronucleated polychromatic erythrocytes must not exceed 5/1000 polychromatic erythrocytes (0.5%) in the negative (vehicle) control. The incidence of micronucleated polychromatic erythrocytes in the positive control group must be significantly increased relative to the negative control ($P < 0.05$, Kastenbaum–Bowman Tables).

RESULTS

Salmonella/mammalian microsome plate incorporation mutagenicity assay

The results of the *Salmonella*/mammalian microsome plate incorporation mutagenicity assay are presented in Tables 1 and 2.

No positive responses were observed with any of the tester strains in the initial assay. Owing to the

Table 5. Results of the micronucleus test

Dose[a]	Sex[b]	Polychromic erythrocyte/Total erythrocyte ratio 24 h	48 h	72 h
0	M	0.46	0.60	0.60
	F	0.58	0.63	0.46
	M,F	0.52	0.62	0.53
11	M	0.56	0.60	0.56
	F	0.60	0.59	0.59
	M,F	0.58	0.60	0.58
22	M	0.50	0.63	0.61
	F	0.59	0.51	0.67
	M,F	0.55	0.57	0.64
44	M	0.50	0.58	0.55
	F	0.65	0.58	0.60
	M,F	0.58	0.58	0.58
TEM[c]	M	0.56	[d]	[d]
	F	0.56	[d]	[d]
	M,F	0.56	[d]	[d]

[a] Dose in mg kg^{-1}.
[b] Five of each sex per dose.
[c] TEM, triethylenemelamine, positive control.
[d] Not tested.

Table 6. Results of the micronucleus test[a]

Dose (mg kg^{-1})	Sex (5)[b]	24-h Sacrifice MPCE[c]	24-h Sacrifice MPCE/PCE[d]	48-h Sacrifice MPCE	48-h Sacrifice MPCE/PCE	72-h Sacrifice MPCE	72-h Sacrifice MPCE/PCE
0	M	0.4 ± 0.55	2/5000	1.4 ± 0.89	7/5000	0.0 ± 0.00	0/5000
	F	0.8 ± 0.84	4/5000	0.8 ± 0.45	4/5000	0.2 ± 0.45	1/5000
	M,F	0.6 ± 0.70	6/10000	1.1 ± 0.67	11/10000	0.1 ± 0.23	1/10000
11	M	0.6 ± 0.55	3/5000	1.6 ± 0.89	8/5000	0.4 ± 0.55	2/5000
	F	0.2 ± 0.45	1/5000	0.6 ± 0.89	3/5000	0.2 ± 0.45	1/5000
	M,F	0.4 ± 0.50	4/10000	1.1 ± 0.89	11/10000	0.3 ± 0.50	3/10000
22	M	1.0 ± 0.00	5/5000	1.2 ± 1.30	6/5000	0.4 ± 0.55	2/5000
	F	0.2 ± 0.45	1/5000	1.0 ± 0.71	5/5000	0.2 ± 0.45	1/5000
	M,F	0.6 ± 0.23	6/10000	1.1 ± 1.01	11/10000	0.3 ± 0.50	3/10000
44	M	1.6 ± 0.55	8/5000	0.4 ± 0.55	2/5000	0.0 ± 0.00	0/5000
	F	1.2 ± 0.84	6/5000	0.6 ± 0.89	3/5000	0.2 ± 0.45	1/5000
	M,F	1.4 ± 0.70	14/10000	0.5 ± 0.72	5/10000	0.1 ± 0.23	1/10000
TEM[e] 0.25	M	14.6 ± 4.22	73/5000[f]	–	–	–	–
	F	18.2 ± 7.66	91/5000[f]	–	–	–	–
	M,F	16.4 ± 5.94	164/10000	–	–	–	–

[a] Each value represents the mean ± standard deviation.
[b] Number of animals of each sex per dose.
[c] MPCE, micronucleated polychromatic erythrocytes per 1000 PCE, mean ± SD.
[d] MPCE/PCE, number of MPCE per PCE scored.
[e] TEM (triethylenemelamine) positive control.
[f] $P \leq 0.05$ (Kastenbaum–Bowman tables).

lack of toxicity, supplementary testing with tester strains TA98, TA1535 and TA1537 in the absence of microsomal enzymes was performed at the following doses: 0.1, 0.3, 1.0, 3.3, 10 and 33 μg per plate (Table 1). No positive responses were noted in the supplemental assay.

The results from the confirmatory assay are presented in Table 2. No positive responses were noted at any dose level tested with any tester strain in the presence or absence of microsomal enzymes.

CHO/HGPRT mutation assay with confirmation

Results of the CHO/HGPRT assay are presented in Tables 3 and 4. The test article did not induce a positive response, regardless of treatment concentration or the presence or absence of microsomal enzymes. Three slightly elevated mutant frequencies were noted, one in the initial study at 2.0 μg ml^{-1} (29.0 mutants per 10^6 cells) and two in the confirmatory study at 20 μg ml^{-1} and 2.5 μg ml^{-1} (26.9 and 24.7 mutants per 10^6 cells, respectively). The positive controls, EMS and B(a)P, induced mutant frequencies of 178.7 and 206.7 mutants per 10^6 clonable cells.

Micronucleus cytogenetic assay in mice

The ratio of polychromatic erythrocytes to total erythrocytes is presented in Table 5. The ratios for treated groups fell within those observed for the vehicle control group, indicating no chemical-induced bone marrow toxicity.

Results of the micronucleus assay are presented in Table 6. The number of micronucleated polychromatic erythrocytes (MPCE) per 1000 polychromatic erythrocytes was not statistically increased in males or females, regardless of dose level or bone marrow collection time ($P > 0.05$, Kastenbaum–Bowman Tables). Vehicle control group mean MPCE/animal ranged from 0.1 to 1.0 in males and females combined, while test-article-treated groups were within the range 0.1–1.4 for males and females combined; TEM induced a significant increase in micronucleate polychromatic erythrocytes in male and female mice relative to the vehicle control ($P < 0.05$, Kastenbaum–Bowman Tables).

DISCUSSION

The present battery of assays has provided data on the mutagenic potential of Znpt. Two mechanisms were examined, i.e. point mutations to bacteria and mammalian cells *in vitro* and chromosomal aberrations *in vivo* (micronuclei). This latter end-point can occur via structural breakage and/or malfunction of the spindle apparatus. The results from this investigation have demonstrated that Znpt lacks the potential to produce genetic damage via point mutation or chromosomal aberration in these assays at levels that produce clear evidence of cellular or systemic toxicity.

The results achieved in these studies are consistent with mutagenicity data generated previously for sodium pyrithione.[21–24] Sodium pyrithione (Napt) did not produce evidence of either point mutations to DNA or chromosomal aberrations. The congruity of mutagenicity data for Znpt and Napt is not unexpected since the overall toxicological profile for each compound is similar.

The results presented in this paper demonstrate the lack of genotoxicity of Znpt. These data, combined with the supporting evidence associated with Napt, indicate an absence of mutagenic potential for Znpt.

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* Unpublished reports can be made available upon request.