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3-CHLORO-*p*-TOLUIDINE HYDROCHLORIDE: IN VITRO MUTAGENICITY STUDIES FOR HUMAN HEALTH HAZARDS DETERMINATIONS

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*3-Chloro-p-toluidine hydrochloride (CPT-HCl) is an aniline derivative used in the manufacture of the dye palatine fast yellow; it is also registered as a selective, low-volume-use (<45 kg/yr) avicide. Three in vitro mutagenicity tests of CPT-HCl were performed according to methods recommended by the U.S. Environmental Protection Agency (EPA): the Ames/*Salmonella *assay, the Chinese hamster ovary/hypoxanthine-guanine phosphoribosyltransferase (CHO/HPRT) mammalian cell forward gene mutation assay, and the CHO chromosome aberration assay. CPT-HCl did not display mutagenic activity using the Ames/*Salmonella *or CHO/HPRT assays. However, CPT-HCl induced statistically significant, concentration-dependent, metabolically activated increases in the proportion of aberrant cells and aberrations/cell in cultured CHO cells. Results are suggestive of minimal mutagenicity effects associated with exposure to anilines and their derivatives.*

Aromatic amines are an important group of industrial chemicals. Most are used as intermediate synthetics for the production of polymers, polyurethanes, dyes, and herbicides/insecticides (Northcott, 1985). Aniline and its derivatives (e.g., toluidines, xylidines, *N*-allyl compounds) represent the simplest aromatic amines.

The aniline derivative 3-chloro-*p*-toluidine hydrochloride (CPT-HCl; Figure 1) has two main industrial applications: quantities are used as an intermediate compound in the production of palatine fast yellow 6GN dye (Society of Dyers and Colourists, 1956), and it is registered as a selective, low-volume-use (<45 kg/yr) avicide (Besser et al., 1967; DeCino et al., 1966; Schafer, 1981).

The aromatic amines and anilines (plus derivatives) pose several potential occupational/human health hazards concerns. Specific chemicals in this class have been linked with dermatitis and bronchial

Received 9 April 1996; sent for revision 7 June 1996; accepted 4 July 1996.

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The authors thank Patricia E. Giglio, Nancy Gongliewski, Susan M. Lucenti, Nira Madison, Kathryn F. McLaren, Diane M. Messina, and Teressa A. Polinsky for their expert technical assistance. Dr. Dawn Mitchell provided a helpful critique of the manuscript.

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Journal of Toxicology and Environmental Health, 50:451–462, 1997
0098-4108/97 $12.00 + .00

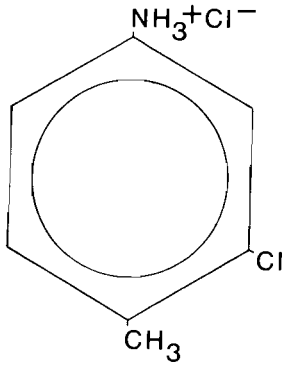

FIGURE 1. Schematic of the chemical structure for CPT-HCl.

asthma (*p*-phenylenediamine, *p*-aminophenol), liver damage (toluenediamine), and bladder cancer (benzidine, 4-aminobiphenyl) (Scott et al., 1989). Nevertheless, carcinogenic effects may be amine or aniline specific. Some derivatives are definitely carcinogens, others are not, and still others lack a sufficient database to make such a determination at this time (Clayson & Garner, 1976; Scott et al., 1989).

This article describes the results of three short-term, in vitro mutagenicity tests conducted with CPT-HCl: (1) the *Salmonella*/microsome reverse mutation assay, (2) the Chinese hamster ovary/hypoxanthine-guanine phosphoribosyltransferase (CHO/HPRT) forward gene mutation assay in CHO cells, and (3) the chromosome aberrations assay in Chinese hamster ovary (CHO) cells. These tests represent hazard evaluations sought by the U.S. Environmental Protection Agency (EPA) for reregistration of pesticides under the 1988 Amendments to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA-88; see Sterner et al., 1992). Test guidelines are described under Subdivision F of the Code of Federal Regulations (CFR) 40, Parts 150–189 (U.S. EPA, 1990).

MATERIALS AND METHODS[1]

CPT-HCl

The technical product ($C_7H_9NCl_2$; CAS 7745-89-3) was purchased (lot 892149) from Pocatello Supply Depot, Pocatello, ID. The product was assayed using a validated gas chromatographic procedure to have

[1]With the exception that the test and control article dosing solutions were not analyzed for stability, homogeneity, or accuracy of preparation, this research was conducted in compliance with the Good Laboratory Practice Regulations for nonclinical laboratory studies as published by the U.S. Food and Drug Administration (FDA, 1990) (21 CFR, Part 58), the Organisation for Economic Co-operation and Development (OECD, 1981) Guidelines for Testing Chemicals (ISBN 92-64-12221-4), and the U.S. EPA (1990) (40 CFR, Part 160). References to trade names and commercial products do not constitute endorsement by the federal government.

a mean (±SD) concentration of 97.1 (±0.5)% active ingredient (A.I.). CPT-HCl is stable in dry air and in the absence of ultra violet light.

Ames Assay

Salmonella typhimurium tester strains TA1535, TA1537, TA1538, TA98, and TA100 were obtained from Dr. Bruce N. Ames (University of California, Berkeley). All tester strains were checked for the presence of the appropriate genetic markers on approximately a monthly basis according to recommended procedures (Maron & Ames, 1983). Fresh cultures for mutagenesis testing were prepared from frozen working stock cultures that were stored at approximately −135°C. Overnight cultures in late exponential or early stationary phase (approximately $1-2 \times 10^9$ cells/ml) were utilized for this study.

The test and control articles were evaluated in triplicate cultures in all five tester strains; evaluations were performed in the presence and absence of an exogenous metabolic activation system (S9). Cultures, untreated and treated with solvent (dimethyl sulfoxide, DMSO; Fisher Scientific, Fairlawn, NJ), served as negative controls; positive controls were known chemical mutagens. CPT-HCl concentrations were selected based upon the results of a preliminary toxicity screen (50, 167, 500, 1670, and 5000 μg/plate) performed in duplicate cultures in strains TA1538 and TA100 in the absence of S9 (not shown); results of this prescreen revealed that concentrations <5000 μg/plate were free of cytotoxic effects.

Tester strains were treated using the plate incorporation method recommended by Ames et al. (1975) and Maron and Ames (1983). Briefly, 2 ml of histidine/biotin-supplemented top agar, 0.1 ml tester strain, and 0.1 ml of the appropriate concentration of the test or control article were combined in prewarmed (~40°C) glass tubes. Cultures treated in the presence of S9 also contained 0.5 ml of the S9 mixture. The S9 mixture contained 8 m*M* $MgCl_2$, 33 m*M* KCl, 4 m*M* NADP, 5 m*M* glucose 6-phosphate, 100 m*M* Na_2HPO_4 (pH 7.4), and 6% (v/v) liver homogenate from Aroclor 1254-induced male Sprague-Dawley rats. The tubes were mixed, poured onto minimal glucose plates, and allowed to solidify. Within an hour the plates were inverted and incubated in the dark at 37°C for 48 h.

Revertant colonies were enumerated on an Artek electronic colony counter interfaced with an IBM PC/AT computer for data acquisition. Background lawns were scored using a stereomicroscope for normal, inhibited, or no growth. Inhibited growth was characterized by the absence of a confluent bacterial lawn and/or the presence of pindot colonies.

A positive result was defined as a statistically significant and concentration-dependent increase in the number of histidine-independent revertants, with at least one concentration inducing a revertant frequency

that was twofold higher than the spontaneous solvent control value. If the test article did not induce a statistically significant or concentration-dependent or twofold higher increase in revertant frequency, the result was considered negative. Statistical analyses were not performed because the maximum increase in revertant frequency, relative to the concurrent negative controls, was below the 50% "trigger" used in this laboratory. This value is based upon the normal, spontaneous variation observed among replicate negative control cultures, as well as the spontaneous fluctuation observed among groups of cultures treated with a variety of test articles judged to be negative in this assay.

CHO/HPRT Assay

CHO-K1-BH4 cells were obtained from Dr. Abraham W. Hsie (Oak Ridge National Laboratory, Oak Ridge, TN). Cultures were maintained in monolayer at 37°C and ≥90% humidity in 5% CO_2 in air in Ham's F12 medium supplemented with 5% heat-inactivated bovine calf serum plus 100 U/ml penicillin and 100 μg/ml streptomycin (F12FCM5).

Cytotoxicity and mutation to TG^r were determined as described by O'Neill et al. (1977a, 1977b). The concentrations of CPT-HCl evaluated were based upon the results of a preliminary toxicity screen performed in the presence and absence of S9 (not shown). Briefly, duplicate cultures of ~1 × 10^6 cells per 25-cm^2 flask were treated (d 0) for 5 h at 37°C in Ham's F12 in the presence (2% final, v/v) and absence of S9. The S9 mixture contained 10 m*M* $MgCl_2$, 10 m*M* $CaCl_2$, 30 m*M* KCl, 5 m*M* glucose 6-phosphate, 4 m*M* NADP, 50 m*M* Na_2HPO_4 (pH 7.4), and 10% (v/v) Aroclor 1254-induced rat liver homogenate (S9). Untreated, solvent (DMSO), and positive controls were evaluated concurrently. To determine cloning efficiency, ~24 h after treatment (d 1) an aliquot of 200 cells was plated in triplicate using 60-mm dishes in 5 ml F12FCM5 for each culture. These cells were incubated for 7–10 d and the surviving colonies were counted. The multiplicative products of these cloning efficiencies and the day 1 cell densities were used to determine total viable cells/culture and to calculate relative survival.

For phenotypic expression, 10^6 cells (or all remaining if $<10^6$) were subcultured on d 1 in F12FCM5 using 100-mm dishes. Approximately 10^6 cells were again subcultured in F12FCM5 in 100-mm dishes at 2- to 3-d intervals, if necessary, to allow expression of the TG^r phenotype. On d 8, 10^6 cells (at a density of 2 × 10^5 per 100-mm dish, or all remaining) were selected in hypoxanthine-free (HX^-) F12FCM5 containing 10 μ*M* TG. Cells plated for selection and cloning efficiency (200 cells in triplicate in HX^-F12FCM5 in 60-mm dishes) were incubated for 7–10 d, the surviving colonies were counted, and the mutant frequency was calculated as TG^r mutants/10^6 clonable cells, corrected for cloning efficiency.

The average mutant frequency observed at each concentration of test article was compared to that for the pooled concurrent negative controls. Data were analyzed by simple linear regression (Hsie et al., 1975), as well as following data transformation (Snee & Irr, 1981). A test article was considered to be positive if it (1) exhibited a statistically significant, concentration-dependent increase in average mutant frequencies ($p \leq .05$) and (2) exhibited at least one concentration that produced an increase in average mutant frequency that was at least threefold the average mutant frequency of the pooled concurrent negative controls and (3) represented a net increase of ≥20 TG^r mutants/ 10^6 clonable cells. A test article exhibiting only one of these responses was considered to be equivocal. A negative test article exhibited none of these responses.

Chromosome Aberration Assay

Treatment of CHO cells for detection of chromosome aberrations was performed according to standard methods (Krahn, 1983). Concentrations were selected based upon the results of a preliminary cytotoxicity screen examining cell proliferation kinetics (not shown). Log phase cultures of approximately 1.6×10^6 cells per 80-cm^2 flask were treated in 10 ml F12CM5 in duplicate under conditions identical to those used for the CHO/HPRT assay. After the 5-h treatment, the cultures were refed with 10 ml F12FCM5 and incubated for an additional 18 h, colcemid (2×10^{-7} *M* final) was added to each culture for the last 2 or 3 h of incubation.

Metaphase cells were collected by "mitotic shake-off," sedimented by centrifugation, resuspended in 0.075 *M* KCl, and incubated at 37°C for 5 min. The cells were sedimented and resuspended (three times) in methanol:acetic acid fixative (3:1, v/v). Following a final centrifugation and resuspension in a small volume of fixative, the cells were dropped onto microscope slides. Slides were stained with 3% Giemsa in phosphate buffer (pH 6.8), air dried, and mounted using Permount. Slides were coded randomly; 100 well-spread metaphases per concentration (50 per duplicate culture) were scored for the presence of chromosome aberrations. Cytogenetic abnormalities were classified according to chromosome or chromatid aberrations, as well as type of aberration, according to the nomenclature of Buckton and Evans (1973) and Savage (1975).

Data Analyses

Data were analyzed by Chi-square analysis (proportion of aberrant cells) and one-tailed *t*-tests (aberrations/cell), comparing each concentration to its concurrent solvent control (Kirk, 1990). In each case, data from the duplicate cultures were pooled, and comparisons were made to the concurrent solvent control. A test article was considered to be

positive if it exhibited both a statistically significant and concentration-dependent increase in aberrations/cell or percent aberrant metaphases ($p \leq .05$). A test article exhibiting only one of these responses was considered to be equivocal. A test article exhibiting neither of these increases was considered to be negative.

RESULTS

Ames Assay

Based upon the results of a preliminary toxicity screen, CPT-HCl was evaluated in triplicate cultures in 5 tester strains in the presence and absence of S9 at concentrations of 50, 167, 500, 1000, 1670, and 2500 μg/plate. The S9 mixture included 6% (v/v) Aroclor 1254-induced male Sprague-Dawley rat liver homogenate with the appropriate buffer and cofactors. Inhibited growth was observed in all tester

TABLE 1. Results of Ames reverse mutation assay [mean (SD) revertants/plate]

		Average revertants/plate				
	S9	TA1535	TA1537	TA1538	TA98	TA100
Solvent controls						
DMSO (100 μl)	(–)	11 (3)	9 (3)	10 (2)	21 (5)	134 (15)
DMSO (100 μl)	(+)	11 (1)	9 (5)	21 (3)	35 (9)	133 (21)
Positive controls (μg/plate)						
Sodium azide, 10.0	(–)	1669 (73)[a]	— (—)	— (—)	— (—)	1701 (80)[a]
9-Aminocridine, 150.0	(–)	— (—)	1058 (138)[a]	— (—)	— (—)	— (—)
2-Nitrofluorine, 5.0	(–)	— (—)	— (—)	1188 (149)[a]	750 (114)[a]	— (—)
2-Anthramine, 2.5	(+)	232 (43)[a]	626 (58)[a]	2436 (68)[a]	2741 (98)[a]	2215 (194)[a]
3-Chloro-*p*-toluidine HCl concentration, (μg/plate)						
50	(–)	12 (3)	6 (1)	8 (4)	21 (5)	112 (22)
167	(–)	13 (4)	6 (4)	8 (1)	21 (6)	106 (17)
500	(–)	14 (2)	9 (5)	7 (2)	19 (5)	112 (8)
1000	(–)	15 (3)	6 (2)	6 (4)	16 (1)	45 (3)[c]
1670	(–)	12 (5)[b]	5 (4)[b]	2 (2)	14 (5)[b,c]	20 (7)[d]
2500	(–)	8 (8)[c,d]	1 (1)[c,d]	4 (3)[d]	8 (7)[c,d]	0 (0)[d]
50	(+)	11 (9)	9 (3)	17 (2)	29 (6)	124 (4)
167	(+)	5 (3)	7 (2)	12 (4)	24 (3)	97 (7)
500	(+)	8 (1)	6 (3)	17 (8)	31 (6)	111 (3)
1000	(+)	10 (3)	5 (3)	13 (6)[b,c]	29 (1)	115 (21)
1670	(+)	3 (3)[c,d]	4 (1)[c,d]	1 (2)[d]	14 (6)	22 (16)[c,d]
2500	(+)	0 (0)[d]	0 (0)[d]	0 (0)[d]	0 (0)[d]	0 (0)[d]

[a]Positive response: ≥2× solvent (TA1535, TA1537, TA1538, TA98, TA100).
[b]Slight toxicity.
[c]Moderate toxicity.
[d]Severe toxicity.

TABLE 2. Results of CHO/HPRT mutation assay using control cultures ±S9 [relative survival (%) and mutant frequencies (based on 10^6 clonable cells)]

Compound	μg/ml	S9 (±)	Relative survival (%)[a]	Mutant frequency (per 10^6 clonable cells)[b] Replicate	Average
Untreated	0	–	95.71	31.17	
Untreated	0	–	109.09	12.24	21.71
Untreated	0	+	120.20	<1.29	
Untreated	0	+	109.09	3.29	<2.29
DMSO	10.0	–	79.29	14.67	
DMSO	10.0	–	92.68	17.72	16.20
DMSO	10.0	+	85.10	<1.44	
DMSO	10.0	+	109.84	11.81	<6.63
ICR 191	0.500	–	88.13	27.63	
ICR 191	0.500	–	86.87	90.00	58.82[c]
ICR 191	1.00	–	81.31	227.12	
ICR 191	1.00	–	57.07	115.18	171.15[d]
DMN	100	+	41.67	178.99	
DMN	100	+	44.19	234.44	206.71[d]

[a]Viable cells per culture = 3.97×10^6.
[b]Corrected for any cultures with 0 total mutants according to 1/cloning efficiency.
[c]Significant increase ($p < .05$; Snee & Irr, 1981).
[d]Significant increase ($p < .01$; Snee & Irr, 1981).

strains at concentrations of 1000, 1670, and 2500 μg/plate with and/or without S9. Revertant frequencies for all concentrations of CPT-HCl in all strains approximated or were less than those observed in the concurrent negative control cultures (Table 1).

CHO/HPRT Assay

Based upon the results of a preliminary cytotoxicity screen, CPT-HCl was evaluated in duplicate cultures in the mutation assay at concentrations of 5, 10, 50, 100, 150, 200, 250, 300, 350, and 400 μg/ml with S9, and 5, 10, 50, 100, 500, 600, 700, 800, 900, and 1000 μg/ml without S9. Those cultures treated at concentrations ≥600 μg/ml without S9 were discarded on the day after treatment, or during subculture for phenotypic expression, due to extreme cytotoxicity. The average mutant frequencies of the negative control cultures ranged from <2.29 to 21.71 TG^r mutants/10^6 clonable cells [$\bar{x}$ = 11.36 ± 10.47 (1 SD); n = 8], while those of the remaining cultures treated with CPT-HCl ranged from <1.83 to 17.41 TG^r mutants/10^6 clonable cells (Tables 2–4). There were no statistically significant or concentration-dependent increases among the cultures treated with CPT-HCl.

TABLE 3. Results of CHO/HPRT mutation assay using CPT-HCl-treated cultures and –S9 mix [relative survival (%) and mutant frequencies (based on 10^6 clonable cells)]

CPT-HCl (μg/ml)	Relative survival (%)[a]	Mutant frequency (per 10^6 clonable cells)[b]	
		Replicate	Average
5	88.64	16.27	
5	90.66	<1.67	<8.97
10	98.99	5.37	
10	96.72	11.97	8.67
50	92.93	4.19	
50	82.83	2.70	3.44
100	82.07	7.45	
100	101.01	16.56	12.01
500	26.77	2.17	
500	6.46	<1.48	<1.83
600	0.18	ND[c]	
600	<0.10	ND	
700	ND		
700	ND		
800	ND		
800	ND		
900	ND		
900	ND		
1000	ND		
1000	ND		

[a]Viable cells per culture = 3.97×10^6.
[b]Corrected for any cultures with 0 total mutants according to 1/cloning efficiency.
[c]Not determined; discarded due to extreme cytotoxicity.

Chromosome Aberration Assay

Based upon the results of a preliminary cytotoxicity screen (examining cell proliferation kinetics; not shown), CPT-HCl was evaluated in duplicate cultures at concentrations of 25, 125, 250, and 350 μg/ml with and without S9. There were no statistically significant or concentration-dependent increases in aberrations/cell or percent aberrant metaphases observed in any of the cultures treated with CPT-HCl in the absence of S9 (Table 5). In contrast, statistically significant, concentration-dependent increases in aberrations/cell and the percent aberrant metaphases were observed in the two highest concentration cultures (250 and 350 μg/ml) treated in the presence of S9.

DISCUSSION

Data obtained with the Ames/*Salmonella* plate incorporation assay and the CHO/HPRT mammalian cell forward gene mutation assay indi-

cate that CPT-HCl was not associated with mutagenic effects under the conditions/criteria of the test protocols. Similarly, CPT-HCl produced no increased aberrations in the in vitro chromosomal aberration assay in CHO cells without metabolic activation. In contrast, CPT-HCl caused increased aberrations at concentrations ≥250 μg/ml in the presence of S9. This pattern of results suggests that CPT-HCl is not really mutagenic, but that a metabolite may be capable of inducing chromosomal aberrations. The nature of this metabolite is difficult to predict, especially in the absence of additional diverse assays.

In previous bioassays, the nonacidic form of the compound (3-chloro-*p*-toluidine, CPT) was fed in diets to Fischer 344 rats and B6C3F1 mice for 78 wk, followed by 25- or 12-wk postingestion (control diet) observation periods, respectively (Anonymous, 1978). Rats were fed time-weighted average concentrations of 3269 or 1635 ppm CPT, whereas 2 separate test groups of male and female mice were fed 1200 or 600 ppm CPT, and 600 or 300 ppm CPT, respectively. Although the pattern of results was complex, with gender and tumor

TABLE 4. Results of CHO/HPRT mutation assay using CPT-HCl-treated cultures and +S9 mix [relative survival (%) and mutant frequencies (based on 10^6 clonable cells]

CPT-HCl (μg/ml)	Relative survival (%)[a]	Mutant frequency (per 10^6 clonable cells)[b]	
		Replicate	Average
5	109.09	7.76	
5	85.35	6.80	7.28
10	95.96	9.33	
10	112.12	8.32	8.82
50	118.68	11.95	
50	81.13	<1.16	<6.56
100	106.81	3.76	
100	115.90	5.83	4.79
150	87.88	5.14	
150	119.69	9.68	7.41
200	88.13	29.27	
200	78.54	5.54	17.41
250	78.28	24.38	
250	57.83	1.67	13.02
300	57.58	5.49	
300	49.49	11.70	8.60
350	33.84	2.45	
350	48.23	<2.36	<2.41
400	7.70	10.00	
400	2.05	5.04	7.52

[a]Viable cells per culture = 3.97×10^6.

[b]Corrected for any cultures with 0 total mutants according to 1/cloning efficiency.

TABLE 5. Results of in vitro chromosome aberration assay [counts of specific types of aberrations per 100 metaphases, percent aberrant cells, and mean (SD) aberrations/cell]

Compound	μg/ml	S9 (±)	Gaps	Chromatid aberration exchanges				Chromosome aberrations				Percent aberrant cells	Mean (SD) aberrations per cell
				Del	Interch	Intrach	Trir	Del	Ring	Dic	Misc		
di-H_2O	0	–	3	3	0	2	0	1	0	0	0	5	0.060 (0.278)
CPT-HCl	25	–	2	5	0	0	1	0	0	0	0	6	0.060 (0.239)
CPT-HCl	125	–	4	6	0	1	1	4	1	0	1[a]	11	0.140 (0.450)
CPT-HCl	250	–	2	6	1	0	0	2	0	0	1[a]	10	0.100 (0.302)
CPT-HCl	350	–	1	1	0	0	1	0	0	0	0	2	0.020 (0.141)
MNNG	1.5	–	8	75	29	16	27	4	3	4	1	71[c]	1.590 (1.609)[c]
di-H_2O	0	+	2	4	1	0	0	1	0	0	0	5	0.060 (0.278)
CPT-HCl	25	+	3	4	0	0	0	1	0	0	0	5	0.050 (0.219)
CPT-HCl	125	+	2	9	0	0	1	0	0	0	2[a,b]	12	0.120 (0.327)
CPT-HCl	250	+	6	22	7	6	1	3	0	0	0	31[c]	0.390 (0.680)[c]
CPT-HCl	350	+	8	35	14	6	4	2	0	1	1[b]	36[c]	0.630 (1.070)[c]
DMN	1000	+	4	38	11	6	11	4	1	0	0	50[c]	0.710 (0.880)[c]

Note. Gaps = achromatic lesions (not considered true aberrations); Del = deletions or severance of the chromosome or chromatid; Interch = interchange; Intrach = intrachanges from lesions within same chromosome; Trir = triradial, that is, isochromatid/chromatid breaks that involve two chromosomes, or intra, which involves both arms of same chromosome; Ring = ring-shaped chromosomes; Dic = dicentric or asymmetrical interchange aberrations due to breakage in each of ≥2 chromosomes with aberrant rejoining; Misc = includes pericentric inversions, reciprocal translocations, and Robertsonian translocations and fissions.

[a]Refers to double minute.

[b]Refers to Robertsonian fission.

[c]Significant increase ($p < .05$) in percent aberrant cells (Chi-square) or in mean aberrations/cell (*t*-test).

type examined for risk relative to control groups, it was concluded that neither significantly increased mortality nor incidence of carcinogenesis in either species.

Regarding human health and safety, the "precautionary statements" for CPT-HCl contained in the upper left portion of EPA-registered, "pesticide-use" labels currently read:

> Harmful if swallowed, inhaled, or absorbed through the skin. Avoid contact with eyes, skin, or clothing. Handle only with protective gloves, clothing, and face mask, or respirator. Wash hands with soap and water after handling.

These statements reflect sound precautions for the prevention of human health hazards from CPT-HCl (Sterner et al., 1992). The association of CPT-HCl exposure with mutagenic effects based on current prokaryotic and eukaryotic cell tests has not been unequivocally demonstrated. Thus, this warning is conservative where formulators/applicators of dyes and avicides should be duly protected by adherence to label guides.

Current evidence is weighted against the likelihood of a specific gene mutation induced by CPT-HCl exposure. However, the in vitro mutagenetic tests are ill suited for the detection of clastogenic agents (DeMarini et al., 1989). Additional replications of the CHO chromosome aberration assay, as well as the conducting of other tests (in vivo cytogenetic assays), are needed to clarify any possible clastogenic effects.

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