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FINAL

DATA EVALUATION REPORT

PROPICONAZOLE (CGA 64 250)

Study Type: Mutagenicity: Salmonella typhimurium/Host Mediated Assay in Mice

Prepared for:

Health Effects Division
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GUIDELINE § 84: MUTAGENICITY
SALMONELLA

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DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Salmonella typhimurium/host mediated assay in mice

EPA IDENTIFICATION Numbers:

Tox Chem. Number: 323 EE

MRID Number: 420505-04

TEST MATERIAL: CGA 64 250

SYNONYMS: Propiconazole

SPONSOR: Ciba-Geigy, Corporation, Greensboro, NC

STUDY NUMBER: 830120

TESTING FACILITY: Ciba-Geigy Ltd., Basle, Switzerland

TITLE OF REPORT: Intrasanguine Host-Mediated Assay with S. typhimurium

AUTHOR: B. Ogorek

REPORT ISSUED: May 10, 1983

CONCLUSIONS--EXECUTIVE SUMMARY: Two host-mediated assays were conducted with CGA 64 250 using either groups of noninduced male mice (6/group, orally gavaged with 350, 700, or 1400 mg/kg) or groups of induced male mice (6/group, orally administered 320 mg/kg CGA 64 250 once a day for 7 days followed by oral gavage dosing with 250, 700, or 1400 mg/kg). The indicator microorganisms, Salmonella typhimurium strains TA1535, TA100, and TA98, were exposed via tail vein injection to the in vivo metabolites of the test material. A separate group of 10 noninduced (treated with the vehicle, 2% carboxymethyl cellulose) and 10 induced (treated with 320 mg/kg CGA 64 250) mice were included to determine whether CGA 64 250 could cause enzyme induction.

Based on the marked increase in absolute and relative liver weights in animals receiving the test material in the separate study, it was concluded that CGA 64 250 induced enzymatic activity. No deaths or clinical signs of compound toxicity were reported for the host-mediated assay phase of testing. Results further indicated that the selected doses of CGA 64 250 administered to noninduced or induced mice were not cytotoxic to any S. typhimurium tester strain. However, 2-fold increases in the mutation frequency (MF) of all three

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strains were observed in noninduced animals receiving 1400 mg/kg; a similar increase in the MF of strain TA98 was seen in noninduced animals of the 700-mg/kg group. There was no evidence of a mutagenic response in the surviving population of any strain recovered from induced animals in the three dose groups. However, owing to the lack of primary data or means and standard deviations for mutant and survivor colony counts, a full evaluation of the findings was not possible. In addition to the limitations posed by the inadequate reporting of the findings, the following technical deficiencies compromised the study:

1. The highest dose evaluated (1400 mg/kg) was neither toxic to the animals nor cytotoxic to the tester strains; therefore, the maximum tolerated dose was not achieved.

2. A positive control was not included.

3. Actual inoculum size for the three strains and the percentage recovery of inoculated organisms were not provided.

4. There was no information on bacterial strain maintenance or data verifying the relevant genetic markers.

5. Pooled samples for each group were used to determine organism survival.

Based on the above considerations, we conclude that the study is unacceptable. However, the results demonstrating that CGA 64 250 is an inducer in conjunction with the absence of a mutagenic response in the induced animals would appear to imply that if either the parent compound is mutagenic or if mutagenic metabolites were formed, as suggested by the findings from the high-dose noninduced animals, they may have been detoxified by CGA 64 250-stimulated enzymatic systems. The validity of this assumption can only be established by repeating the assay in a manner that addresses the issues that have been raised in this review.

STUDY CLASSIFICATION: Unacceptable. The study cannot be used to satisfy Guideline requirements (§84.2) for genetic effects Category I, Gene Mutations.

A. MATERIALS:

1. Test Material: CGA 64 250

   Description:
   Identification number: Batch number: OP. 103119
   Purity: 90.7%
   Receipt date: Not reported
   Stability: Reported to be ensured by the sponsor
   Contaminants: None listed
   Vehicle used: 2% Carboxymethyl cellulose (CMA)
   Other provided information: Neither the storage condition for the test material nor the frequency of dosing solution preparation were reported.

2. Control Materials:

Negative/route of administration: Untreated

Vehicle/final concentration/route of administration: 2% CMA was administered by oral gavage; the dosing volume was not reported.

Positive/final concentration/route of administration: None

3. Test Compound:

Route of administration: Oral gavage

Dose levels used:

- Noninduced animals: 350, 700, or 1400 mg/kg (single administration)
- Induced animals: 320 mg/kg administered once daily for 7 consecutive days (day 0-6) followed by a single administration of 350, 700, or 1400 mg/kg on day 7.

NOTE: In an addendum to the report (see CBI, p. 21) it was stated that 1400 mg/kg was selected as the high dose for the host-mediated assay based on the LD_{50} of CGA 64 250 (1490 mg/kg) in mice.

4. Test Animals:

(a) Species: mouse Strain: Tif: MAGf (SPF), NMRI Age: Not reported
Weight range: (at initiation of dosing): 20-28 g Sex: Male
Source: Ciba-Geigy, Tierfarm, Sisseln

(b) Number of animals used per dose: 6/group/bacterial strain in both the noninduction and induction phases of the study.

NOTE: Additional groups of 10 animals were administered either the vehicle control or 320 mg/kg of the test material for an unspecified number of days and were used for the comparative analysis of liver and body weights.

(c) Properly maintained? No information on animal maintenance or the environmental conditions were provided. The report indicated, however, that animals were fed a standard diet (NAFAG no. 890), and water ad libitum.

5. Test Organism Used: S. typhimurium strains

____	TA97	x	TA98	x	TA100	____	TA102	____	TA104
x	TA1535	____	TA1537	____	TA1538				

list any others:

Test organisms were properly maintained: Storage conditions were not reported.
Checked for appropriate genetic markers (rfa mutation, R factor): Not reported.

B. TEST PERFORMANCE:

1. Bacterial Strain Preparation: Cultures of each strain were incubated overnight in nutrient broth, washed, and adjusted to cell densities of $0.6\text{-}2.5\text{x}10^{10}$ organisms/mL. Incubation conditions used to generate the cultures were not reported.

2. Compound Administration: Group of 6 noninduced and 6 induced (7 daily oral gavage administrations of 320 mg/kg CGA 64 250) mice received single oral gavage doses of 350, 700, or 1400 mg/kg of the test material. Animals in the vehicle control groups were orally administered the vehicle control (2% CMC) once either on the day of treatment for the noninduced animals, or on day 7 postdosing for the induced animals.

3. Host-Mediated Assay: Following administration of the selected test material levels or vehicle control, 0.3 mL of the prepared suspension of each bacterial strain was injected into the lateral tail vein of each mouse. Additional groups of untreated mice (6/group), injected with the appropriate bacterial suspension, served as the "absolute" negative control. Animals were sacrificed by cervical dislocation 2.5 hours postinjection of the bacteria; livers were removed and homogenized. Liver homogenates were centrifuged and undiluted samples were plated (5 minimal agar plates containing biotin) to determine mutants. The remaining portion of the liver homogenates from each group were pooled and diluted; the 10^{-4}, 10^{-5}, and 10^{-6} dilutions were inoculated onto nutrient broth agar (the number of replicates was not specified) to determine the total population of surviving cells. Incubation conditions were not reported for mutants or survivors.

4. Statistical Analysis: Data were evaluated for statistical significance at $p<0.01$ using nonparametric multiple comparisons, Jonckheere's trend test, or the nonparametric Williams' test.

5. Evaluation Criteria: The test material was considered positive if the multiple comparison of the control group with the dose groups revealed a reproducible significant result ($p<0.01$) or if there was an increasing trend and the Williams' test showed a significant effect.

6. Protocol: A protocol was not provided.

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C. REPORTED RESULTS

1. Animal Observations: Summarized results from the separate experiment conducted with groups of 10 mice treated either with the vehicle control or the induction dose of CGA 64 250 (320 mg/kg) indicated that the absolute mean liver weight for induced animals (2.73±0.21 g; mean body weight: 28.3±1.5 g) was increased compared to noninduced liver weights (mean liver weight = 1.52±0.26 g; mean body weight = 27.2±2.0 g). The marked increase in relative liver to body weight, calculated by our reviewers, for treated (9.65%) versus control (5.59%) animals tends to support the study author's conclusion that CGA 64 250 caused enzyme induction. The study author also stated that "two animals died after the second application of the test compound." However, no clinical signs or deaths were reported in noninduced mice administered 350, 700, or 1400 mg/kg, or mice induced with 350 mg/kg CGA 64 250 for 7 days and subsequently administered comparable treatment doses of the test material.

2. Host-Mediated Assay: Neither the actual number of organisms of each bacterial strain injected into the mice nor the total number of bacteria recovered per animal were reported. We are, therefore, unable to determine the percentage of the inoculated population that was recovered from the test animals or the solvent-treated animals. Although reduced survival was seen in the treatment groups, the reductions were sporatic and not dose related (Table 1). In general, relative survival in the treatment groups was either comparable to or higher than that of the control groups. Overall, the results suggest that administration of the selected doses of the CGA 64 250 to noninduced or induced mice had no cytotoxic effect on the indicator organisms.

   The study author stated that there were no significant increases in mutant colonies of any strain in any group of noninduced or induced animals compared to the corresponding vehicle control group. This result is not surprising considering the wide variation in background MFs, particularly for strains TA100 and TA98. For example, the MF for strain TA100 in the untreated animals was 0.88×10^{-8} as compared to MFs of 16 and 5.4×10^{-8} for the vehicle control group. The high variability of background MFs is not unexpected in the host-mediated assay and can be somewhat overcome by increasing the number of animals. Since the animals in the vehicle control groups were treated in a similar manner (i.e., single administration of 2% CMA immediately before organism inoculation), our reviewers combined the MFs for each strain and compared the MFs for noninduced and induced groups to these average values. As the results presented in Table 1 show, MFs for bacterial populations recovered from animals induced with the test material and subsequently dosed with 350, 700, or 1400 mg/kg were not increased compared to the respective average vehicle control value. However, ≥2-fold increases in the MF of all tester strains were noted for noninduced animals in the high-dose group. A similar increase in the MF of strain TA98 was seen in noninduced mid-dose animals. Trend analyses performed by the study author did not reveal a significant

TABLE 1: Representative Results of the Host-Mediated Assays in Mice with CGA 64 250

Substance	Dose (mg/kg)	No. of Animals	S. typhimurium Tester Strain											
			TA1535				TA100				TA98			
			Survivors x10^8	Relative Percent Survival[a]	Mutants	Mutation Frequency[b] x10^{-8}	Survivors x10^8	Relative Percent Survival[a]	Mutants	Mutation Frequency[b] x10^{-8}	Survivors x10^8	Relative Percent Survival[a]	Mutants	Mutation Frequency[b] x10^{-8}
Negative Control														
Untreated animals	--	6	0.34	100	1.33	3.9	0.76	100	0.67	0.88	2.0	100	3.50	1.75
Vehicle Control														
2% Carboxymethyl-cellulose	--	6[c]	1.30	100	7.17	5.5	0.24	100	3.83	16.0	0.45	100	7.50	16.7
	--	6[d]	0.51	100	1.33	2.6 (4.1)[e]	0.31	100	1.67	5.4 (10.7)[e]	0.65	100	0.67	1.0 (8.9)[e]
Test Material														
CGA 64 250	350	6NI[f]	1.60	123	11.50	7.2	0.27	113	3.33	12.3	0.63	140	9.33	14.8
	700	6NI	1.30	100	8.50	6.5	0.38	158	5.17	13.6	0.34	76	7.83	23.0[g]
	1400	6NI	1.10	85	8.83	8.0[g]	0.29	121	6.50	22.4[g]	0.49	109	10.83	22.1[g]
	350	6I[f]	0.50	98	1.50	3.0	0.67	216	1.33	2.0	1.00	154	3.50	3.5
	700	6I	0.39	76	1.00	2.6	1.40	452	4.58	3.3	0.11	17	1.33	12.1
	1400	6I	0.47	92	2.17	4.6	0.51	165	4.00	7.8	0.84	129	5.33	6.3

[a]Percent Survival = $\frac{\text{Survivors in Test Group}}{\text{Survivors in Vehicle Control Group}}$ x 100; calculated by our reviewers.

[b]Mutation Frequency (MF) = $\frac{\text{Mutants}}{\text{Survivors x } 10^8}$; calculated by our reviewers.

[c]Vehicle control for noninduced animals
[d]Vehicle control for induced animals
[e]MF for both vehicle control groups averaged by our reviewers.
[f]NI = noninduced; I = induced
[g]MFs were ~≥2-fold higher than the average MF of the vehicle control group.

dose response; however, the effect with strain TA100 was borderline significant (p=0.0526).

Without the primary data or means and standard deviations for survivors and mutants, our reviewers were unable to perform independent statistical evaluations; therefore, the relevance, if any, of the increased MFs cannot be ascertained. The study author concluded, however, that CGA 64 250 was not mutagenic in the intrasanguine host-mediated assay with S. typhimurium strains TA1535, TA100, or TA98.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the increased MFs in cell populations of each tester strain recovered from noninduced animals receiving the high dose (1400 mg/kg) of CGA 64 250 suggested a possible mutagenic effect. As previously stated, however, reporting average values without some indication of data variability (i.e., means and standard deviations), which is an unacceptable practice, precluded a full evaluation of the findings. Similarly, the pooling of liver homogenates for each group and presenting a single group value for survivors is not a scientifically sound approach. In addition, the lack of overt toxic effects in high-dose animals indicates that a level >1400 mg/kg could be used to determine if the suspected mutagenic response is dose dependent.

There was, however, convincing evidence from the separate experiment conducted with noninduced and induced mice to support the study author's claim that CGA 64 250 caused enzymatic induction. The results demonstrating that CGA 64 250 is an inducer, in conjunction with the absence of a mutagenic response in the induction phase of the host-mediated assay, would tend to imply that if the parent compound was mutagenic or if mutagenic metabolites were formed, the enzymatic system provoked by CGA 64 250 stimulated their detoxification. Nevertheless, the validity of this assumption is not fully supported because the available data were insufficient to draw definitive conclusions.

The following additional technical deficiencies further compromised the study:

1. A positive control was not included.

2. The number of organisms inoculated and percent recovery of inoculated organisms were not reported.

3. No information on strain maintenance or confirmation of the genetic markers was provided.

Although Guidelines do not exist for the host-mediated assay, we conclude based on the above considerations, that the study is unacceptable and cannot be used to satisfy Guideline requirements (§84.2) for genetic effects, Category I, Gene Mutations.

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E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? A signed statement dated September 21, 1988, indicated that the study was completed prior to the enactment of FIFRA GLPs; however, a quality assurance statement signed and dated May 5, 1983 was present.

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 8-10.

CORE CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84.2) for genetic effects, Category I, Gene Mutations.

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APPENDIX A

MATERIALS AND METHODS
CBI pp. 8-10

RIN 1067-98

Propiconazole (Tilt) Tox Review

Page_____ is not included in this copy.

Pages 11 through 13 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

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