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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

February 26, 2002

TXR#: 050498

MEMORANDUM

SUBJECT: ***Thiabendazole*** (P.C. Code 060101)-Evaluation of Covance Statistical Analysis of Rat Tumor Data

DP Barcode: D280353 MRID 45591801

TO: Lorilyn Montford/Susan Lewis-PM 51
Special Review Reregistration Division (7508C)

FROM: Sanjivani Diwan, Toxicologist
Reregistration Branch 4
Health Effects Division (7509C)

THROUGH: Susan Hummel, Branch Senior Scientist
Reregistration Branch 4
Health Effects Division (7509C)

On behalf of Syngenta Crop Protection, Inc., Greensboro, NC, Covance Laboratory submitted the statistical analyses of survival and thyroid tumor data for the combined chronic toxicity and carcinogenicity study in Sprague-Dawley rats (MRID 45591801). HED concluded that although Covance performed some statistical tests not performed by EPA, the methods and results are similar. The HED stands by the results of its original analyses (Refer to the attached memorandum by Lori Brunsman for detailed response).

Attachment

cc: Ray Kent

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MEMORANDUM

January 30, 2002

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: **Thiabendazole :** Comments to Covance Submission of 1/9/2002

RE: Statistical Analyses of Survival and Thyroid Tumor Data

P.C. Code 060101

TO: Ray Kent, Branch Chief
Reregistration Branch 4
Health Effects Division (7509C)

FROM: Lori L. Brunsman, Statistician
Science Information Management Branch
Health Effects Division (7509C)

THROUGH: Jess Rowland, Branch Chief
Science Information Management Branch
Health Effects Division (7509C)

Background

In a combined chronic toxicity and oncogenicity study in Sprague Dawley rats with Thiabendazole, two separate control groups of 50 animals each per sex were run concurrently [1]. There were no statistically significant differences between the two control groups in either mortality or tumor incidence, so they were combined into a single control group of 100 animals per sex for the EPA analyses [3] .

In an addendum provided by Covance [2], analyses of survival and tumor data which conflict with that of EPA were presented. This memo is in response to the Covance analysis.

EPA Response

In the EPA analysis, male rats showed no significant incremental changes in mortality with increasing doses of Thiabendazole. The statistical evaluation of mortality indicated a significant decreasing trend with increasing doses of Thiabendazole in female rats. As stated above, in all of the EPA analyses the two control groups were combined.

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In the Covance analysis, the two control groups were combined for the mortality analyses and they agreed with the EPA finding that female rats "showed a significant negative trend in mortality". However, for tumor analysis, the treated groups were compared to the control groups, both individually and combined. Based on this, Covance concluded that the differences in male rat thyroid follicular cell adenoma rates between treated groups and either control group are due to background variation and that "it is unlikely that the slight differences between the treated and control groups in this study are attributed to treatment with thiabendazole."

For the EPA analysis, the incidence of thyroid adenomas was statistically significant in both males and females, compared to the *combined* control group. In males there were significant differences from control at both the mid- and high doses at $p<0.01$ and a trend also at $p<0.01$. In females there was a significant difference from control at the high dose at $p<0.05$ and a trend also at $p<0.05$. EPA disagrees with the Covance assessment and considers the thyroid tumors in males to be treatment-related.

Covance also maintains that there was a thyroid follicular cell adenoma incidence of 3/50 in the male control group 1, while control group 2 had an incidence of 0/50.

EPA disagrees, since there was no difference in the thyroid follicular cell adenoma rates of the control groups; neither the individual animal data the company submitted to EPA for analysis, nor appendix 1 of their addendum containing the individual animal tumor incidence indicates <u>any</u> thyroid follicular cell adenomas in <u>either</u> male control group.

The Covance addendum calls the increases in male rat thyroid follicular cell adenoma rates "slight". As stated above, the EPA analyses indicate a significance level of $p<0.01$ for the male rat thyroid follicular cell adenoma trend and in the pair-wise comparisons of the mid- and high dose groups. A significance level of $p<0.01$ is not "slight", but rather highly significant.

Covance and EPA agreed on the analyses of the thyroid follicular cell adenoma incidence in female rats, though they called the trend "marginal" and the EPA finding indicated a trend of $p = 0.013$, or very significant. The addendum states that there were only "slight" differences between the treated and control groups, and that the tumor incidence in the treated groups was "low". However, the male rat thyroid follicular cell adenoma rates were 0/97 (0%) in the combined control and 5/46 (11%) at the high dose, resulting in a trend and pair-wise significance of $p = 0.003$.

<u>Conclusion</u>

EPA believes that the Covance analyses which compare the treated groups with the individual control groups account for the differences in the evaluations, and that there are no statistical or biological reasons to keep them separate. Though Covance performed some statistical tests not performed by EPA, the methods and results are similar. EPA stands by the results of their original analyses.

References

1. Combined chronic toxicity and oncogenicity study in Sprague-Dawley Crl:CD BR rats was conducted by Hazleton Washington, Inc., Vienna, Virginia, for Merck Research Laboratories, West Point, Pennsylvania, and completed September 29, 1993 (HWA Study No. 284-172; Merck Project No. 618-67/TT #90-9009; MRID No. 435932-01).

2. Addendum to above study (1) conducted by Covance Laboratories, Inc., Vienna, Virginia, for Syngenta Crop Protection, Inc., Greensboro, North Carolina, entitled Statistical Analyses of Survival and Thyroid Tumor Data and completed January 9, 2002.

3. REVISED Thiabendazole Qualitative Risk Assessment Based on Sprague-Dawley Crl:CD BR Rat Dietary Study, L. Brunsman to P. Gaunt, 8/19/1999 (EPA).

Attachment: REVISED Thiabendazole Qualitative Risk Assessment Based on Sprague-Dawley Crl:CD BR Rat Dietary Study, L. Brunsman to P. Gaunt, 8/19/1999.

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

013684

MEMORANDUM August 19, 1999

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: REVISED Thiabendazole Qualitative Risk Assessment Based On Sprague-Dawley Crl:CD BR Rat Dietary Study

P.C. Code 060101

TO: Patricia Gaunt, Toxicologist
Reregistration Branch 4
Health Effects Division (7509C)

FROM: Lori L. Brunsman, Statistician
Science Analysis Branch
Health Effects Division (7509C)

THROUGH: William L. Burnam, Branch Chief
Science Analysis Branch
Health Effects Division (7509C)

Background

A combined chronic toxicity and oncogenicity study in Sprague-Dawley Crl:CD BR rats was conducted by Hazleton Washington, Inc., Vienna, Virginia, for Merck Research Laboratories, West Point, Pennsylvania, and completed September 29, 1993 (HWA Study No. 284-172; Merck Project No. 618-67/TT #90-9009; MRID No. 435932-01).

The study design allocated groups of 50 rats per sex to dose levels of 0, 10, 30, or 90 mg/kg/day of Thiabendazole for 105 weeks. Two separate control groups of 50 animals each per sex were run concurrently in this study. There were no statistically significant differences between the two control groups, so they have been combined into a single control group of 100 animals per sex for these analyses.

Survival Analyses

Male rats showed no significant incremental changes in mortality with increasing doses of Thiabendazole. The statistical evaluation of mortality indicated a significant decreasing trend with increasing doses of Thiabendazole in female rats. See Tables 1 and 2 for mortality test results.

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The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program.

Tumor Analyses

Male rats had significant increasing trends, and significant differences in the pair-wise comparisons of the 90 mg/kg/day dose group with the controls, for thyroid follicular cell adenomas and adenomas and/or carcinomas combined, all at $p < 0.01$. There were also significant differences in the pair-wise comparisons of the 30 mg/kg/day dose group with the controls at $p < 0.01$ for thyroid follicular cell adenomas and at $p < 0.05$ for thyroid follicular cell adenomas and/or carcinomas combined.

Female rats had significant increasing trends in thyroid follicular cell adenomas and adenomas and/or carcinomas combined, both at $p < 0.05$. There was a significant difference in the pair-wise comparison of the 90 mg/kg/day dose group with the controls for thyroid follicular cell adenomas at $p < 0.05$.

The statistical analysis of the male rats were based upon the Exact trend test and the Fisher's Exact test for pair-wise comparisons. The statistical analyses of the female rats were based upon Peto's Prevalence Test since there was statistically significant differential mortality with increasing doses of Thiabendazole. See Tables 3 and 4 for tumor analysis results.

Table 1. Thiabendazole - Sprague-Dawley Crl:CD BR Rat Study

Male Mortality Rates[†] and Cox or Generalized K/W Test Results

Dose (mg/kg/day)	Weeks 1-26	27-52	53-78	79-106[f]	Total
0	0/100	3/100	6/97	28/91	37/100 (37)
10	2/50	2/48	3/46	13/43	20/50 (40)
30	1/50	1/48[a]	7/47	17/40	26/49 (53)
90	1/50	1/48[b]	2/47	9/44[c]	13/48 (27)

[†]Number of animals that died during interval/Number of animals live at the beginning of the interval.

[f]Final sacrifice at week 105.

[a]One accidental death at week 43, dose 30 mg/kg/day.

[b]One accidental death at week 37, dose 90 mg/kg/day.

[b]One accidental death at week 79, dose 90 mg/kg/day.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 2. Thiabendazole - Sprague-Dawley Crl:CD BR Rat Study

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (mg/kg/day)	Weeks 1-26	27-52	53-78	79-106[f]	Total
0	0/100	3/100	15/97	44/82	62/100 (62)
10	0/50	2/50	9/48	21/37[a]	32/48 (67)
30	0/50	0/50	6/50	21/43[b]	27/49 (55)
90	1/50	1/48[c]	3/47	20/44	25/49 (51)

[+]Number of animals that died during interval/Number of animals live at the beginning of the interval.

[f]Final sacrifice at week 105.

[a]One accidental death at week 79, dose 10 mg/kg/day.

[b]One accidental death at week 79, dose 30 mg/kg/day.

[b]One accidental death at week 52, dose 90 mg/kg/day.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 3. Thiabendazole - Sprague-Dawley Crl:CD BR Rat Study

Male Thyroid Follicular Cell Tumor Rates[+] and
Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	10	30	90
Adenomas (%)	0/97 (0)	1/46 (2)	5[a]/47 (11)	5/46 (11)
p =	0.003**	0.322	0.003**	0.003**
Carcinomas (%)	1[b]/97 (1)	0/46 (0)	0/47 (0)	1/46 (2)
p =	0.353	0.678[n]	0.674[n]	0.541
Combined (%)	1/97 (1)	1/46 (2)	5/47 (11)	6/46 (13)
p =	0.003**	0.541	0.014*	0.005**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.

[a]First adenoma not in an accidental kill animal observed at week 92, dose 30 mg/kg/day.

[b]First carcinoma observed at week 105, dose 0 mg/kg/day.

[n]Negative change from control.

Note: Accidental kill animals are not included in this analysis. One accidental kill animal in the 90 mg/kg/day dose group had an adenoma.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 4. Thiabendazole - Sprague-Dawley Crl:CD BR Rat Study

Female Thyroid Follicular Cell Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (ppm)			
	0	10	30	90
Adenomas (%)	3[a]/82 (4)	0/36 (0)	1/43 (2)	5/44 (11)
p =	0.013*	-	-	0.047*
Carcinomas (%)	1[b]/38 (3)	0/16 (0)	0/22 (0)	0/24 (0)
Combined (%)	4/82 (5)	0/36 (0)	1/43 (2)	5/44 (11)
p =	0.032*	-	-	0.096

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First adenoma observed at week 81, dose 0 mg/kg/day.

[b]First carcinoma observed at week 105, dose 0 mg/kg/day.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

References

Cox, D.R. (1972) Regression Models and Life Tables (with discussion). J. Royal Stat. Soc. Ser. B. 34, 187-220.

Gart, J.J., D. Krewski, P.N. Lee, R.E. Tarone, and J. Wahrendorf (1986) The Design and Analysis of Long-Term Animal Experiments. In: Statistical Methods in Cancer Research, Volume III. IARC Scientific Publications No. 79. Lyon, France: International Agency for Research on Cancer, p. 18.

Peto, R., M. Pike, N. Day, R. Gray, P. Lee, S. Parish, J. Peto, S. Richard, and J. Wahrendorf (1980) Guidelines for Simple, Sensitive, Significant Tests for Carcinogenic Effects in Long-Term Animal Experiments. In: Monographs on the long-term and short-term screening assays for carcinogens: a critical appraisal. IARC Monographs, Supplement 2. Lyon, France: International Agency for Research on Cancer, pp. 311-426.

Thomas, D.G., N. Breslow, and J.J. Gart (1977) Trend and Homogeneity Analyses of Proportions and Life Table Data. Computers and Biomedical Research 10, 373-381.