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PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Isoxaben, Rat and Mouse Study - Qualitative Risk Assessment of Combined Toxicity and Oncogenicity Study. Caswell #419F

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Summary:

In this two-year chronic oral study of male and female Fisher 344 rats and male and female B6C3F1 mice, a significant increasing trend in mortality with dose was found for the male rats. No survival disparities were found in the mice or female rats. That is there were no trends, no heterogeneity, and no pairwise differences with controls. Since most of the lesions in male rats occurred late, there was no need to adjust for mortality, since the table would collapse to one or two intervals.

For the rats, there was a significant increasing trend with dose for male pheochromocytomas, and both male and females progressive glomerulo nephrosis (PGN). The 0.125% and 1.25% male dose groups were significantly higher than controls as were the females at the 1.25% dose group.

For the mice, there was a significant increasing trend with dose for both male and female hepatocellular adenoma only and pooled hepatocellular adenoma and/or carcinoma. The high dose (125000) males and females had significantly more adenomas than the controls. The high dose (125000) females had significantly more adenomas and/or carcinomas than the controls. There were no trend or pairwise differences between dosed animals and control animals with hepatocellular carcinoma of either sex.

Background:

This study was conducted at Lilly Research Laboratories on male and female Fischer 344 rats and on male and female B6C3F1 mice. The study was done in two replicates, but were so close in time that the two studies were pooled. Technical Isoxaben was administered to 60 rats of both sexes at 0.0125%, 0.125%, and 1.25% in the diet (Study Numbers RO1583 and RO1683). Technical Isoxaben was administered to 60 mice of both sexes at 100ppm, 1000ppm, and 12500ppm in the diet (Study Numbers MO0883 and MO0983). There was also a concurrent control group of 60 animals for both sexes of both species.

Mortality Analysis:

The Thomas, Breslow, Gart Procedure (1977) was used to analyze the survival data. There was a significant trend ($p = .05$) for male rats with mortality increasing with increasing doses of Isoxaben (Table 1) using Cox's test (1972) for life table data. But there was no departure from trend. There were no survival disparities in female rats. There were no significant pairwise comparisons for either sex between the control and any treated group. The pairwise test of control versus high dose was nearly significant for male rats ($p = .09$ by Cox's test).

There were no survival disparities in male or female mice with increasing doses of Isoxaben (Table 2). The same procedure was used to analyze the mouse survival data as was used in the rat data. There were no significant pairwise comparisons for either sex between the control and any treated group.

Table 1. Isoxaben - Rat Study, Mortality Rates[+] and Cox or Generalized K/W Test Results

A. Males

Dose (%)	WEEKS 0-26	27-52	53-78	79-105[a]	TOTALS
0	0/59	0/59	3/59	17/56	20/59 (34)*
.0125	0/60	1/60	0/59	25/59	26/60 (43)
.125	0/60	1/60	4/59	19/55	24/60 (40)
1.25	0/60	0/60	7/60	24/53	31/60 (52)

B. Females

Dose (%)	WEEKS 0-26	27-52	53-78	79-105[a]	TOTALS
0	0/61	0/61	0/61	14/61	14/61 (23)
.0125	0/60	0/60	4/60	16/56	20/60 (33)
.125	0/60	0/60	3/60	11/57	14/60 (23)
1.25	0/60	0/60	0/60	18/60	18/60 (30)

\+ Number of Animals Died/Number of Live Animals at the beginning of the interval.
() Percent
a Final sacrifice was at 104 or 105 weeks.

Note - The above survival tables are broken into aggregate time intervals for display purpose only.
Significance of Trend Analysis denoted at Control.
Significance of pairwise comparison with control denoted at Dose level. (* $p < .05$ ** $p < .01$)

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Table 2. Isoxaben - Mouse Study, Mortality Rates[+] and Cox or Generalized K/W Test Results

A. Males

Dose (ppm)	WEEKS 0-26	27-52	53-78	79-105[a]	TOTALS
0	0/60	0/60	2/60	15/58	17/60 (28)
100	7/60	0/53	2/53	9/51	18/60 (30)
1000	2/60	1/58	1/57	9/56	13/60 (22)
12500	2/60	0/58	2/58	10/56	14/60 (23)

B. Females

Dose (ppm)	WEEKS 0-26	27-52	53-78	79-105[a]	TOTALS
0	0/60	1/60	0/59	7/59	8/60 (13)
100	1/60	1/59	1/58	5/57	8/60 (13)
1000	2/59	1/57	2/56	9/54	14/59 (24)
12500	0/60	0/60	2/60	6/58	8/60 (13)

\+ Number of Animals Died/Number of Live Animals at the beginning of the interval.
() Percent
a Final sacrifice was at 104 or 105 weeks.

Note - The above survival tables are broken into aggregate time intervals for display purpose only.
Significance of Trend Analysis denoted at Control.
Significance of pairwise comparison with control denoted at Dose level. (* $p < .05$ ** $p < .01$)

Tumor Analysis:

Pheochromocytoma, Lymphosarcoma, and Progressive Glomerulo Nephrosis (PGN) was analyzed for the rat studies (Table 3, 4, and 5 respectively). Although there was a survival trend in the male rat, most of the lesions occurred late. Since there were no pairwise differences between control and dosed male rats, a time-adjusted analysis was not necessary. There were no survival disparities for the female rat or either sex of the mice. Therefor the Fisher's Exact Test was used for pairwise comparisons and the Cochran-Armitage Test was used to test for trends. There was a significant trend ($p = .014$) for Pheochromocytoma among male rats but no heterogeneity, and no pairwise differences were detected. There was no significant trend, heterogeneity, or pairwise comparisons for Pheochromocytoma among female rats. There were no significant trend, heterogeneity, or pairwise comparisons with control for Lymphosarcoma for either sex. There was a highly significant trend for both sexes ($p < .001$) for PGN. The heterogeneity Chi-square for males was significant ($p = .03$) but was not significant for females. There was significantly more PGN at the high dose (1.25%) than the controls (males $p = .001$, females $p = .007$). Also the mid dose (0.125%) males were significantly higher ($p = .005$) than controls.

Hepatocellular carcinoma, adenoma, and adenoma/carcinoma was analyzed for the mouse studies (Table 6, 7, and 8 respectively). There were no survival disparities in the mouse studies, hence the same tests were used in these analyses as those used in the rat study. There were no significant differences found for hepatocellular carcinoma for either sex. There was a significant trend for hepatocellular adenoma for both sexes (males $p < .001$, females $p = .004$). The high dose (12500ppm) mice had significantly more adenomas than the controls for both sexes (males $p = .005$, females $p = .006$). There was a significant trend for adenomas and carcinomas combined (males $p = .01$, females $p = .001$). The high dose (12500) female mice had significantly more combined tumors than the controls ($p = .001$) but the high dose males were not significant ($p = .18$).

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Table 3. ISOXABEN - Rat Study, Adrenal Cortex Tumor (Pheochromocytoma) Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results

Dose (%)	0	0.0125	0.125	1.25
Males	11/59 (19)*	10/59 (17)	9/59 (15)	18/59 (31)
Female	3/49 (6)	2/45 (4)	4/49 (8)	1/47 (2)

First male tumor observed at 66 weeks in 1.25% dose group.
First female tumor observed at 102 weeks in 0.125% dose group.

Table 4. ISOXABEN - Rat Study, Lymphosarcoma Rates[+] and Cochran-Armitage Test and Fisher's Exact Test Results

Dose (%)	0	0.0125	0.125	1.25
Males	1/59 (2)	2/60 (3)	4/60 (7)	3/60 (5)
Female	2/47 (4)	0/40 (0)	1/46 (2)	3/42 (7)

First male tumor observed at 46 weeks in 0.125% dose group.
First female tumor observed at sacrifice.

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Table 5. ISOXABEN - Rat Study, Progressive Glomerulo Nephrosis Rates+, Cochran-Armitage Trend Test, and Fisher's Exact Test Results

Dose (%)	0	0.0125	0.125	1.25
Males	26/58 (47)**	33/59 (56)	40/57 (70)**	51/58 (88)**
Female	34/61 (56)**	27/59 (46)	35/60 (58)	47/60 (78)**

\+ Tumor Bearing Animals/ Animals at Risk
First male tumor observed at 70 weeks in 1.25% dose group.
First female tumor observed at week 70 in 0.125% dose group.

Note - Significance of Trend Analysis denoted at Control. Significance of pairwise comparison with control denoted at Dose level. (* $p < .05$, ** $p < .01$)

Table 6. ISOXABEN - Mouse Study, Hepatocellular Carcinoma Rates+, Cochran-Armitage Trend test, and Fisher's Exact Test Results

Dose (ppm)	0	100	1000	12500
Males	9/56 (16)	5/49 (10)	5/55 (9)	3/55 (5)
Female	0/52 (0)	1/52 (2)	0/46 (0)	2/52 (4)

First male tumor observed at 82 weeks in control group.
First female tumor observed at 104 weeks in 100ppm dose group.

Table 7. ISOXABEN - Mouse Study, Hepatocellular Adenoma Rates+, Cochran-Armitage Trend test, and Fisher's Exact Test Results

Dose (ppm)	0	100	1000	12500
Males	3/44 (7)**	1/41 (2)	3/47 (6)	14/48 (29)**
Female	0/52 (0)**	3/52 (6)	2/46 (4)	7/52 (13)**

First male tumor observed at 103 weeks in 12500 ppm group.
First female tumor observed at sacrifice.

Table 8. ISOXABEN - Mouse Study, Hepatocellular Adenoma and/or Carcinoma Rates+, Cochran-Armitage Trend test, and Fisher's Exact Test Results

Dose (ppm)	0	100	1000	12500
Males	12/56 (21)**	6/49 (12)	8/55 (15)	17/55 (31)
Female	0/52 (0)**	4/52 (8)	2/46 (4)	9/52 (17)**

+ Tumor Bearing Animals/ Animals at Risk.
First male tumor observed at 82 weeks in control group.
First female tumor observed at 104 weeks in 100ppm dose groups.

Note - Significance of Trend Analysis denoted at Control.
Significance of pairwise comparison with control denoted at Dose level. (* $p < .05$, ** $p < .01$)

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