Caswell file

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: DIMILIN (p-Chloroaniline) - Interim Quantitative Risk Assessment from an NTP Rat and Mouse Oncogenicity Study. (preliminary data) Caswel #~~364A~~ 346A

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Summary:

In a 2-year oncogenicity study, male and female rats and mice were fed p-Chloroaniline, a metabolite of dimilin. A review of preliminary summary tables from the study indicated survival problems. Because individual animal data were not provided, the Crump multistage procedure was used to obtain Q_1^*'s using the effective proportions as an attempt to account for survival disparities.

For the male rats, spleen osteosarcomas incidence resulted in an estimated Q_1^* for extra risk of 3.9×10^{-2} $[mg/kg/dy]^{-1}$ in human equivalents.

For the male mice, hepatocellular carcinoma incidence resulted in an estimated Q_1^* for extra risk of 2.6×10^{-1} $[mg/kg/dy]^{-1}$ in human equivalents.

Background:

p-Chloroaniline, a metabolite of dimilin, was fed to male and female Fischer 344 rats at doses of 0, 2, 6, and 18 mg/kg/day for 105 weeks. The same compound was fed to male and female B6C3F1 mice for 104 weeks. There were 50 animals of each sex, species-strain, dose combination. The Study was conducted by the Battelle Laboratory for the National Toxicology Program. The studies are identified as Experiment 05020-01 and 05020-02. No individual animal data were provided, only summary tables of neoplastic and non-neoplastic lesions.

Without the individual animal data we were unable to follow our normal analysis procedure. Only a very preliminary mortality adjustment is attempted by eliminating the animals that died before the appearance of the first tumor.

Some statistics in this memo are quoted directly from the summary tables provided with this request. Analyses were preformed on data from the same tables.

The chemical p-Chloroaniline, a metabolite of dimilin, produces many tumors in two rodent species in this study. Individual animal data is not available, therefore combining of tumors is not possible. For each species, rat and mouse, one tumor was selected for analysis. Tumors were chosen for the following reasons:

a) A significant positive linear trend with dose.
b) At least one pairwise significant comparison between a dose group and the control group.
c) Tumor site or malignancy.

In summary this is a preliminary analysis and 2 Q_1^* are provided.

Survival:

For male rats, there was significantly less mortality in the low (2 mg/kg) and mid (6 mg/kg) dose groups than the controls. The linear trend statistic was not significant (Table 1).

For male mice, there was significantly higher mortality in the mid (10/mg/kg) dose group than the controls. The trend statistic was not significant (Table 2).

QUANTITATIVE ANALYSIS:

Since it appears that their is a survival problem with both species, the Crump multistage procedure was used on the effective proportions. Effective proportions are defined as those animals that died or were sacrificed on or after the date of the observation of the first tumor of interest. Normally we would use the multistage procedure with a time factor for adjusting for survival disparities. Since the individual animal data were not

available, we used effective proportions as a crude adjustment for possible mortality differences.

For the male rats, spleen osteosarcomas incidence resulted in an estimated Q_1^* for extra risk of 3.9×10^{-2} $[mg/kg/dy]^{-1}$ in human equivalents. The rat Q_1^* for extra risk was 7.3×10^{-3} $[mg/kg/dy]^{-1}$. This was converted to human equivalents by the interspecies surface area adjustment as recommended by the EPA Cancer Guidelines. We assumed an average human weighs 60 kg and an average rat weighs 400 gm (Table 3).

For the male mice, hepatocellular carcinoma incidence resulted in an estimated Q_{1*} for extra risk of 2.6×10^{-1} $[mg/kg/dy]^{-2}$ in human equivalents. The mouse Q_1^* for extra risk was 2.0×10^{-2} $[mg/kg/dy]^{-1}$. This was converted to human equivalents by the interspecies surface area adjustment as recommended by the EPA Cancer Guidelines. We assumed an average human weighs 60 kg and an average mouse weighs 25 gm (Table 4).

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TABLE 1. PARA-CHLOROANILINE

STATISTICAL ANALYSIS OF SURVIVAL DATA
MALE RATS
FIRST TERMINAL SACRIFICE AT 729 DAYS

SURVIVAL SUMMARY STATISTICS

	DOSE			
	VEHICLE	2 MG/KG	6 MG/KG	18 MG/KG
SURVIVAL AT END OF STUDY (KAPLAN-MEIER)	36.7%	64.0%	66.8%	42.9%
SIGNIFICANCE (B) (LIFE TABLE)	P=0.793	P=0.007N	P=0.005N	P=0.367N
MEAN DAY OF NATURAL DEATHS (C) (STANDARD ERROR)	590.5 (26.2)	645.1 (14.9)	592.9 (31.3)	645.8 (15.1)
MEAN LIFE SPAN (D) (STANDARD ERROR)	641.4 (19.1)	698.8 (7.8)	675.3 (14.5)	677.3 (11.0)

(A) FIRST TERMINAL SACRIFICE
(B) THE FIRST ENTRY IS THE TREND TEST (TARONE, 1975) RESULT. SUBSEQUENT ENTRIES ARE THE RESULTS OF PAIRWISE TESTS (COX,1972). NEGATIVE TRENDS ARE INDICATED BY "N".
(C) MEAN OF ALL UNCENSORED DEATHS PRIOR TO TERMINAL SACRIFICE
(D) MEAN OF ALL DEATHS (UNCENSORED, CENSORED, TERMINAL SACRIFICE)

TABLE 2. PARA-CHLOROANILINE

STATISTICAL ANALYSIS OF SURVIVAL DATA
MALE MICE
FIRST TERMINAL SACRIFICE AT 728 DAYS

SURVIVAL SUMMARY STATISTICS

	DOSE			
	VEHICLE	3 MG/KG	10 MG/KG	30 MG/KG
SURVIVAL AT END OF STUDY (KAPLAN-MEIER)	86.0%	73.5%	58.0%	70.0%
SIGNIFICANCE (B) (LIFE TABLE)	P=0.295	P=0.211	P=0.005	P=0.110
MEAN DAY OF NATURAL DEATHS (C) (STANDARD ERROR)	589.0 (29.2)	590.7 (42.7)	614.1 (22.3)	623.7 (25.5)
MEAN LIFE SPAN (D) (STANDARD ERROR)	708.5 (7.9)	680.4 (17.7)	680.2 (12.2)	696.7 (10.1)

(A) FIRST TERMINAL SACRIFICE
(B) THE FIRST ENTRY IS THE TREND TEST (TARONE, 1975) RESULT. SUBSEQUENT ENTRIES ARE THE RESULTS OF PAIRWISE TESTS (COX,1972). NEGATIVE TRENDS ARE INDICATED BY "N".
(C) MEAN OF ALL UNCENSORED DEATHS PRIOR TO TERMINAL SACRIFICE
(D) MEAN OF ALL DEATHS (UNCENSORED, CENSORED, TERMINAL SACRIFICE)

Table 3. Spleen Osteosarcoma in Fischer 344 Rats fed Para-Chloroaniline (Terminal Sacrifice 105 weeks)

DOSE	MALE VEHICLE	2 MG/KG	6 MG/KG	18 MG/KG
TUMOR RATES				
OVERALL (a)	0/49 (0%)	0/50 (0%)	1/50 (2%)	19/50 (38%)
ADJUSTED (b)	0.0%	0.0%	3.1%	62.8%
TERMINAL (c)	0/18 (0%)	0/32 (0%)	1/32 (3%)	11/21 (52%)
FIRST INCIDENCE (DAYS)	---	---	729 (T)	494
STATISTICAL TESTS (d)				
LIFE TABLE	P<0.001 **	(e)	P=0.615	P<0.001 **
INCIDENTAL TUMOR	P<0.001 **	(e)	P=0.615	P<0.001 **
LOGISTIC REGRESSION	P<0.001 **	(e)	P=0.615	P<0.001 **
COCHRAN-ARMITAGE	P<0.001 **			
FISHER EXACT		(e)	P=0.505	P<0.001 **

Table 4. Hepatocellular Carcinoma in B6C3F1 Mice fed Para-Chloroaniline (terminal Sacrifice 104 weeks)

DOSE	MALE VEHICLE	3 MG/KG	10 MG/KG	30 MG/KG
HEPATOCELLULAR CARCINOMA				
TUMOR RATES				
OVERALL (a)	3/50 (6%)	7/49 (14%)	11/50 (22%)	17/50 (34%)
ADJUSTED (b)	7.0%	16.3%	25.9%	37.9%
TERMINAL (c)	3/43 (7%)	2/36 (6%)	1/29 (3%)	8/35 (23%)
FIRST INCIDENCE (DAYS)	728 (T)	637	514	490
STATISTICAL TESTS (d)				
LIFE TABLE	P<0.001 **	P=0.127	P=0.011 *	P<0.001 **
INCIDENTAL TUMOR	P=0.001 **	P=0.282	P=0.245	P=0.002 **
LOGISTIC REGRESSION	P<0.001 **	P=0.149	P=0.034 *	P<0.001 **
COCHRAN-ARMITAGE	P<0.001 **			
FISHER EXACT		P=0.151	P=0.020 *	P<0.001 **

Table 5. DIMILIN - Male Rat Spleen Osteosarcoma Rates[+] and Cochran-Armitage Trend or Fisher's Exact Test Results.

Dose (mg/kg/dy)	0	2	6	18[a]
Spleen Osteosarcoma	0/44** (0)	0/49 (0)	1/46 (2)	19/48** (40)

+ Number of lesion bearing animals/number of animals at risk. (Animals are excluded that died before the first tumor [effective proportions].)

( ) Percent.

a) First Osteosarcoma occurred at week 71 (day 494) in dose 30 mg/kg/dy.

Note: Significance of trend analysis denoted at Control. Significance of pairwise comparison between control and dosed groups denoted at Dose level. ** = $p < .01$ and * = $p < .05$.

Table 6. DIMILIN - Male Mice Hepatocellular Carcinoma Rates[+] and Cochran-Armitage Trend or Fisher's Exact Test Results.

Dose (mg/kg/dy)	0	3	10	30[a]
Hepatocellular Carcinomas	3/50** (6)	7/45 (16)	11/47 (23)	17/49** (35)

+ Number of lesion bearing animals/number of animals at risk. (Animals are excluded that died before the first tumor [effective proportions].)

( ) Percent

a) First Carcinoma occurred at week 70 (day 490) in dose 30 mg/kg/dy.

Note: Significance of trend analysis denoted at Control. Significance of pairwise comparison between control and dosed groups denoted at Dose level. ** = $p < .01$ and * = $p < .05$.

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