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

JUN 14 1985

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Chlordimeform Risk Assessment.

FROM: Bertram Litt, Leader, Statistical Team
Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: Albin B. Kocialski, Ph.D.
Section Head, Section VII
Toxicology Branch/HED (TS-769)

THRU: Reto Engler, Chief
Mission Support Staff
Toxicology Branch/HED (TS-769)

Three life-time feeding studies have been performed on Chlordimeform HCl and two of its metabolites (N-formyl-4-chloro-o-toluidene and 4-Chloro-o-toluidene HCL). The design of these studies are discussed on page four of the attached "Special Report, Risk Assessment of Chlordimeform and Two Metabolites" by Brion Cook and William McLellan of Dynamac, EPA 68-01-6561, Task: 35, April 1985. The tumor findings of interest -- benign hemangioma of the liver blood vessels and malignant hemangioendothelioma, of the same tissue, are summarized in Table 4-6 of the Dynamac report: chlordimeform (Study 1) in Table 4, N-formyl-4-chloro-otoluidene (Study 2) in Table 5, and 4-chloro-o-toluidene (Study 3) in Table 6. The "crude totals" shown in these three Dynamac tables are the number of animal examined while the "effective total" refers to the number of examined animals who had survived until at least six weeks before the first blood vessel tumor was diagnosed. The "crude total" is used in survival analyses and time-to-death-with-tumor risk extrapolation (Rank 81). The "effective totals" are used as a time adjustment to obtain an appropriate number of animals at risk for extrapolation models which do not include time as a factor in calculating the low-dose/risk estimates (e.g., multistage, probit, 2 parameter Weibull etc.).

A risk assessment has been performed by the State of California which considered only the Chlordimeform (Study 1) data but did not include consideration of the data for the metabolites. ("Cancer Risk Assessment for Persons Involved in Application of Chlordimeform as a Pesticide to Cotton Fields in California" by Cheng L. Liao, HS-1150 Revised 10/3/84.)

The Dynamac report presents the p-values resulting from their statistical analyses of survival and incidence of hemangioma and/or hemangioendothelioma for these studies in their Tables 1-3. These tables demonstrate that regardless of whether or how the incidence of hemonioma/hemangioandothelioma is weighted by time of death, there is a statistically significant, $p < .001$ dose-related life-shortening trend (particularly for 20 ppm and higher dosed groups) for all three chemicals among both male and female animals. In addition, the tables indicate that Exact test results and other paired comparisons of individual dose-responses with control rates show statistically significant elevation for each chemical tested at $p < .001$ for 100 ppm or 500 ppm exposures and $p < .02$ for 4-chloro-o-toluidene HCl at 20 ppm.

The statistical significance associated with survival presents a source of bias which complicates the interpretation of carcinogenicity. In Table 1 below, the date of final kill for each sex/dose/study group is listed as "date of last death." The animals in the control group were allowed to continue living far beyond (i.e. 100-265 days) the time when the higher dosed animals had died or were sacrificed (Part A). Moreover, the date of the first tumor in each group (Part B) shows a dose related pattern. The data have therefore been abstracted from the Dynamac survival printouts to illustrate the number of animals remaining alive at the beginning of each three months study interval (denominators of tables 2 and 3) and the number dying with-the-tumor-of-interest (numerators) during each three month interval. These tables show that appropriate low-dose extrapolation should take time-to-death-with-tumor into account. For this reason the Crump-Rank 81 program has been used to incorporate time into the multi-stage model.

The Dynamac report indicates on page 13 and their Table 8 and 9, the adjustments used to convert animal dose in ppm to mg/kg/day in human equivalents. The maximum likelihood estimator (MLE) for fitting five models (probit, logit, Weibull, Multi-hit, Multi-stage) to each dose and the related lower confidence limit on the dose associated with cancer risks of 10^{-4} and 10^{-6} are shown in Dynamac Tables 11 to 19. Except for study 2 males, the Chlordimeform data shows a poor

TABLE 1. End of Study and Date of First Hemangioma/ Hemangioendothelioma by Study Group

A) Date of Last of Death

Dose in ppm	0	2	20	100	500
FEMALE					
97% Chlordimeform HCL	925	-	954	793	715
97% N-Formyl-4 Chloro-o-Toluidene	927	-	900	748	567
97% 4-Chloro-o-Toluidene HCL	932	866	849	793	504
MALES					
97% Chlordimeform HCL	975	-	975	863	686
97% N-Formyl-4-Chloro-o-Toluidene	849	-	898	793	550
97% 4-Chloro-o-Toluidene HCL	963	884	884	814	575

B) Date of First Tumor

Dose in ppm	0	2	20	100	500
FEMALE					
97% Chlordimeform HCL	782	-	593	501	376
97% N-Formyl-4 Chloro-o-Toluidene	393	-	739	481	291
97% 4-Chloro-o-Toluidene HCL	725	709	671	356	281
MALES					
97% Chlordimeform HCL	526	-	537	508	231
97% N-Formyl-4-Chloro-o-Toluidene	533	-	748	483	311
97% 4-Chloro-o-Toluidene HCL	534	880	613	488	296

TABLE 2. Survival and Incidence of Hemangioma/Hemangioendothelioma in Females

Time	Dose	97% Chlordimeform HCL				97% N-Formyl-4 Chloro-o-Toluidene				97% 4-Chloro-o-Toluidene HCL			
Days 0-182	0 ppm	0/43				0/46				0/45			
Weeks 0-26	2		-				-				0/45		
	20			0/46				0/47				0/47	
	100				0/47				0/43				0/48
	500					0/49				0/48			
Days 183-273	0 ppm	0/42				0/45				0/44			
Weeks 27-39	2		-				-				0/45		
	20			0/44				0/45				0/46	
	100				0/46				0/43				0/47
	500					0/49				0/47			
Days 274-365	0 ppm	0/41				0/41				0/39			
Weeks 40-50	2		-				-				0/38		
	20			0/42				0/44				0/44	
	100				0/43				0/40				1/43
	500					0/44				8/45			
Days 366-456	0 ppm	0/36				1/40				0/36			
Weeks 53-65	2		-				-				0/35		
	20			0/32				0/38				0/41	
	100				0/37				0/37				3/36
	500					11/41				19/33			
Days 457-548	0 ppm	0/33				0/37				0/31			
Weeks 66-78	2		-				-				0/30		
	20			0/30				0/34				0/33	
	100				2/32				4/33				7/31
	500					17/25				9/12			
Days 549-639	0 ppm	0/28				1/32				0/24			
Weeks 79-91	2		-				-				0/27		
	20			1/28				0/23				0/26	
	100				8/28				15/26				14/21
	500					6/7				2/2			
Days 640-730	0 ppm	0/22				1/26				1/21			
Weeks 92-104	2		-				-				1/24		
	20			0/24				0/22				4/23	
	100				10/19				5/10				5/6
	500					1/1				-			
Days 731-821	0 ppm	1/19				1/20				0/17			
Weeks 105-117	2		-				-				0/14		
	20			0/18				2/15				3/15	
	100				4/7				1/1				1/1
	500					-				-			
Days 822-913	0 ppm	3/15				2/12				2/14			
Weeks 118-130	2		-				-				0/9		
	20			2/13				2/9				4/8	
	100				-				-				-
	500					-				-			
Days 914-1004	0 ppm	0/6				2/6				0/7			
Weeks 131-143	2		-				-				-		
	20			2/6				-				-	
	100				-	-			-				-
	500									-			

Numerators = Number dying with or due to tumor of interest.
Denominators = Number of live animals entering an interval with later pathology exam.

TABLE 3. Survival and Incidence of Hemangioma/Hemangioendothelioma in Males

Time	Dose	97% Chlordimeform HCL				97% N-Formyl-4 Chloro-o-Toluidene				97% 4-Chloro-o-Toluidene HCL			
Days 0-182	0 ppm	0/44				0/42				0/50			
Weeks 0-26	2		-				-				0/47		
	20			0/44				0/48				0/47	
	100				0/49				0/46				0/47
	500					0/48				0/47			
Days 183-273	0 ppm	0/41				0/42				0/50			
Weeks 27-39	2		-				-				0/47		
	20			0/43				0/45				0/47	
	100				0/48				0/44				0/47
	500					1/47				0/46			
Days 274-365	0 ppm	0/41				0/41				0/49			
Weeks 40-50	2		-				-				0/46		
	20			0/41				0/44				0/46	
	100				0/47				0/42				1/47
	500					1/45				7/45			
Days 366-456	0 ppm	0/39				0/40				0/46			
Weeks 53-65	2		-				-				0/44		
	20			0/38				0/44				0/44	
	100				0/44				0/40				0/45
	500					5/42				24/36			
Days 457-548	0 ppm	1/34				1/36				1/44			
Weeks 66-78	2		-				-				0/44		
	20			1/36				0/40				0/40	
	100				2/38				3/36				4/44
	500					11/36				8/10			
Days 549-639	0 ppm	0/26				0/34				0/38			
Weeks 79-91	2		-				-				0/40		
	20			0/31				0/33				1/36	
	100				2/31				5/21				8/36
	500					12/22				1/1			
Days 640-730	0 ppm	0/24				1/28				0/32			
Weeks 92-104	2		-				-				0/31		
	20			0/29				0/29				3/35	
	100				3/28				7/14				10/26
	500					9/9				-			
Days 731-821	0 ppm	1/23				2/17				0/24			
Weeks 105-117	2		-				-				0/20		
	20			0/25				3/19				3/22	
	100				4/20				3/5				9/12
	500					-				-			
Days 822-913	0 ppm	0/14				0/10				1/19			
Weeks 118-130	2		-				-				1/12		
	20			0/21				4/10				5/14	
	100				6/8				-				-
	500					-				-			
Days 914-1004	0 ppm	0/9				-				1/11			
Weeks 131-143	2		-				-				-		
	20			0/10				-				-	
	100				-				-		1		-
	500					-				-			

Numerators = Number dying with or due to tumor of interest.
Denominators = Number of live animals entering an interval with later pathology

fit (Dynamac Table 10) to all models in both males and femals as well as combined data for all dose levels. This is because the data dose not fit the monotonicity assumption of these models. The data for each sex/chemical sub-study show a reduction in the increment of added response rate (flattening of the curve) when the 20 ppm - control increment is compared to the 100 ppm - 20 ppm increment which is then compared to the 500-100 ppm increment. Moreover all but 2 data sets (study 2 males and study 1 females) have control rates which exceed the low-dose rate.

The problem of lack of monotonicity is resolved by the use of the time-to-death-with-tumor approach. The monotonicity of dose (time-to-death) response is illustrated for all six data sets in tables 2 and 3 above.

The potency estimates assocated with the multi-stage model and its time adjusted analog are show is Table 4, below. The cancer potency Q_1* increases with the length time on study. Agency policy is to utilize the data set with the highest potency for cancer. In this set of three studies, the Q_1* estimates of potency for chlordineform, shown in Table 4 are ordered in progression from the pesticide chemical, chlordimeform HCL, which has a potency in females of Q_1* = 6.5 to the proximal metabolite, N-formyl-4-chloro-o-toluidene, which has a potency in males of Q_1* = 12.9 to the terminal metabolite, 4-chloro-o-toluidene HCL, which as a potency in females of Q_1* = 19.2. Thus, Q_1* = 6.5 to 19.2 depending upon the proportion of chlordimeform which breaks down to these metabolites (if any).

To characterize the risk of cancer associated with human exposure to chlordimeform, multiply specific estimates of average daily lifetime exposure by the value of Q_1* (e.g., $\frac{\text{TMRC}}{\text{60 kg bw}}$ x Q_1* = upper 95% bound on the dietary risk).

TABLE 4. Estimates of Chlordimeform Carcinogenicity Potency (Q_1*)

	97% Chlordimeform HCL		97% N-Formyl-4 Chloro-o-Toluidene		97% 4-Chloro-o-Toluidene HCL	
	Females	Males	Females	Males	Females	Males
All Doses[1] Sexes Combined	0.83	0.48	0.52	0.62	0.70	0.76
	(0.58)		(0.53)		(0.68)	
Omitting High Dose Sexes Combined	1.2	0.36	0.44	0.79	2.1	1.8
	(0.48)		(0.51)		(1.8)	
Time Adjusted Risks[2] (All Doses)						
0 - 365 Days	-	.017	≠	.0064	.047	0.043
0 - 457 Days	.090	.050	≠	.047	.30	0.20
0 - 548 Days	0.33	0.16	≠	0.14	0.75	0.45
0 - 634 Days	0.76	0.34	≠	0.38	1.8	0.88
0 - 720 Days	1.4	0.63	≠	0.94	3.5	1.2
0 - 862 Days	-	1.5	≠	12.9	19.2	12.0
0 - 953 or last day	6.5	-	≠	-	-	-

1 Estimates computed by Global 83 extra risk and global options.

2 Estimates computed by Rank 81 with extra risk and global options.

≠ Did not compute.