Summary of Inhalation Carcinogenicity Study of Tetrachloroethylene in F344 Rats

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Japan Bioassay Laboratory

Japan Industrial Safety and Health Association

PREFACE

The tests were contracted and supported by the Ministry of Labour of Japan. The tests were conducted by Japan Bioassay Laboratory (JBL) and the report was prepared by JBL and peer reviewed by outside expert pathologist. Complete report was submitted to Ministry of Labour of Japan on March 31, 1993.

This English Summary was translated by JBL from Japanese complete report.

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Purpose, materials and methods

Tetrachloroethylene (CAS No. 127-18-4) is a colorless liquid with a boiling point of 121.2°C. It is insoluble in water and soluble ethanol, ether, chloroform and benzene.

The carcinogenicity and chronic toxicity of tetrachloroethylene (purity : 99.0%) were examined by inhalation exposure using F344/DuCrj (Fischer) rats. Groups of test animals were exposed to tetrachloroethylene vapors at target concentrations of 0 (clean air), 50, 200 or 600 ppm (v/v) for 6 hours/day, 5 days/week for 2 years (104 weeks). Each group of test animals consisted of either 50 male or 50 female rats. Both sexes were exposed to each concentration of tetrachloroethylene vapor. The highest dose level was chosen so as not to exceed the maximum tolerated dose (MTD), based on both growth rate and toxicity in a previous 13-week toxicity study. The identity of the tetrachloroethylene used in these experiments was confirmed by both infrared spectrometry and mass spectrometry. The chemical was analyzed by both infrared spectrometry and gas chromatography before and after use to affirm its stability. Stainless-steel inhalation exposure chambers (volume: 7.6 m^3) were used throughout the 2-year exposure period. Tetrachloroethylene vapor-air mixtures were generated by bubbling clean air through tetrachloroethylene liquid and the mixtures delivered to the inhalation exposure chambers. Air concentrations of the tetrachloroethylene in the inhalation exposure chambers were monitored at 15 min intervals by gas chromatography. The animals were observed daily for clinical signs and mortality. Body weight and food consumption were measured once a week for the first 14 weeks and every 2 weeks thereafter. All animals, including those found dead or in a moribund state as well as those surviving to the end of the 2-year exposure period, underwent complete necropsy. Urinalysis was performed near the end of the exposure period. Hematology and blood biochemistry analysis were performed at the terminal necropsy: surviving animals were fasted overnight and bled under anesthesia. Organs and tissues were removed, weighed and examined for macroscopic lesions at necropsy. The organs and tissues were then fixed and embedded in paraffin. Three μm thick tissue sections were prepared and stained with hematoxylin and eosin and examined microscopically. Incidences of neoplastic lesions were statistically analyzed by Fisher's exact test. Any positive dose-response trends of tetrachloroethylene induction of neoplastic lesions were analyzed by Peto's test. Incidences of non-neoplastic lesions and urinalysis were analyzed by the Chi-square test. Changes in body weight, food consumption, hematological and blood biochemical parameters, and organ weights were analyzed by Dunnett's test. The present studies were conducted with reference to the Organisation for

Economic Co-operation and Development (OECD) Good Laboratory Practice and the OECD Guideline for Testing of Chemicals 451 “Carcinogenicity Studies”.

Results

Survival rates of tetrachloroethylen-exposed groups of both sexes tended to be decreased as compared with the control groups in a dose-related manner. The decreased survival rates in both sexes were related to the the increased incidences of mononuclear cell leukemia in the spleen. Body weights of 600 ppm-exposed male rats were significantly suppressed from 7th week to 100th week. Body weights of 200 and 600 ppm-exposed female rats were significantly suppressed 32rd week and afterward, as compared with the respective controls. Slight decrease in food consumption were found in most weeks of all tetrachloroethylen exposed groups. Mean corpuscular hemoglobin was increased in 600 ppm-exposed females. Triglyceride was significantly decreased in the 600 ppm-exposed females. ALT were significantly increased in the 600 ppm-exposed males and 200 and 600ppm-exposed females. Urea nitrogen were significantly increased in the 200 and 600ppm-exposed females.

The incidences of selected neoplastic lesions in male and female rats are presented in the tables below. A dose-dependent increase in the incidences of mononuclear cell leukemia in the spleen as indicated by a significant positive trend by Peto’s test in males and females was observed. The incidences of mononuclear cell leukemia in the spleen in the 600 ppm-exposed males was significantly increased.

Nuclear enlargement in the proximal tubule and atypical tubular dilation in the proximal tubule were increased in the 600 ppm-exposed males and females. Nuclear enlargement in the proximal tubule was also increased in the 200 ppm-exposed males. In the liver, significant increase in the incidence of spongiosis hepatis and hyperplasia was observed in the 600 ppm-exposed males.

Conclusions

In rats, there was some evidence of carcinogenic activity of tetrachloroethylene in males and females based on the dose-dependent increase in the incidences of mononuclear cell leukemia.

Incidences of selected neoplastic lesions of male rats in the 2-year inhalation carcinogenicity study of tetrachloroethylene

Dose (ppm)		0	50	200	600	Peto test	Cochran-Armitage test
Number of examined animals		50	50	50	50		
benign tumor							
subcutis	fibroma	1	5	3	5		
liver	hepatocellular adenoma	3	0	0	2		
pituitary	adenoma	16	16	18	15		
thyroid	C-cell adenoma	6	10 [1)]	7	3		
pancreatic islet	adenoma	3	4	1	3		
adrenal	pheochromocytoma	8	5	3	3		
testis	interstitial cell tumor	47	46	45	48		
malignant tumor							
spleen	mononuclear cell leukemia	11	14	22	27 *	↑↑	↑↑
thyroid	C-cell carcinoma	1	1 [1)]	3	0		

Incidences of selected neoplastic lesions of female rats in the 2-year inhalation carcinogenicity study of tetrachloroethylene

Dose (ppm)		0	50	200	600	Peto test	Cochran-Armitage test
Number of examined animals		50	50	50	50		
benign tumor							
pituitary	adenoma	12	16 [1)]	16	11		
thyroid	C-cell adenoma	4	2	1	3 [1)]		
pancreatic islet	adenoma	3	4	1	3		
uterus	endometrial stromal polyp	8	3	2	3		
mammary gland	adenoma	4	0	1	0		
	fibroadenoma	3	13 *	1	0		↓↓
malignant tumor							
spleen	mononuclear cell leukemia	10	17	16	19	↑	
thyroid	C-cell carcinoma	1	3	0	1 [1)]		

Tumors occurred more than 5% of examined animals at least in one group were presented.

1): Number of examined animals is 49.

Significant difference

*: p≦0.05		**: p≦0.01		(Fisher test)
↑: p≦0.05	increase	↑↑: p≦0.01	increase	(Peto, Cochran-Armitage test)
↓: p≦0.05	decrease	↓↓: p≦0.01	decrease	(Cochran-Armitage test)

SELECTED TABLES

(Study No. 0104, 0105)

TABLE 10 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES IN MALE RAT

(TWO-YEAR STUDIES)

Week on Study	Control Av.Wt.	Control No.of Surviv. <50>	50 ppm Av.Wt.	50 ppm % of cont. <50>	50 ppm No.of Surviv.	200 ppm Av.Wt.	200 ppm % of cont. <50>	200 ppm No.of Surviv.	600 ppm Av.Wt.	600 ppm % of cont. <50>	600 ppm No.of Surviv.
0	127 (50)	50/50	127 (50)	100	50/50	127 (50)	100	50/50	127 (50)	100	50/50
1	160 (50)	50/50	160 (50)	100	50/50	159 (50)	99	50/50	158 (50)	99	50/50
2	196 (50)	50/50	193 (50)	98	50/50	193 (50)	98	50/50	193 (50)	98	50/50
3	223 (50)	50/50	221 (50)	99	50/50	219 (50)	98	50/50	220 (50)	99	50/50
4	247 (50)	50/50	244 (50)	99	50/50	242 (50)	98	50/50	243 (50)	98	50/50
5	266 (50)	50/50	263 (50)	99	50/50	261 (50)	98	50/50	262 (50)	98	50/50
6	282 (50)	50/50	279 (50)	99	50/50	276 (50)	98	50/50	277 (50)	98	50/50
7	299 (50)	50/50	296 (50)	99	50/50	293 (50)	98	50/50	292 (50)	98	50/50
8	314 (50)	50/50	311 (50)	99	50/50	307 (50)	98	50/50	305 (50)	97	50/50
9	328 (50)	50/50	325 (50)	99	50/50	318 (50)	97	50/50	318 (50)	97	50/50
10	338 (50)	50/50	335 (50)	99	50/50	329 (50)	97	50/50	328 (50)	97	50/50
11	346 (50)	50/50	344 (50)	99	50/50	337 (50)	97	50/50	338 (50)	98	50/50
12	355 (50)	50/50	353 (50)	99	50/50	348 (50)	98	50/50	347 (50)	98	50/50
13	364 (50)	50/50	362 (50)	99	50/50	357 (50)	98	50/50	355 (50)	98	50/50
14	371 (50)	50/50	368 (50)	99	50/50	364 (50)	98	50/50	361 (50)	97	50/50
16	382 (50)	50/50	381 (50)	100	50/50	379 (50)	99	50/50	375 (50)	98	50/50
18	393 (50)	50/50	393 (50)	100	50/50	390 (50)	99	50/50	386 (50)	98	50/50
20	405 (50)	50/50	404 (50)	100	50/50	400 (50)	99	50/50	392 (50)	97	50/50
22	417 (50)	50/50	414 (50)	99	50/50	411 (50)	99	50/50	403 (50)	97	50/50
24	425 (50)	50/50	422 (50)	99	50/50	419 (50)	99	50/50	413 (50)	97	50/50
26	432 (50)	50/50	431 (50)	100	50/50	426 (50)	99	50/50	418 (50)	97	50/50
28	437 (50)	50/50	437 (50)	100	50/50	431 (50)	99	50/50	420 (50)	96	50/50
30	447 (50)	50/50	445 (50)	100	50/50	438 (50)	98	50/50	427 (50)	96	50/50
32	455 (50)	50/50	455 (50)	100	50/50	447 (50)	98	50/50	437 (50)	96	50/50
34	460 (50)	50/50	461 (50)	100	50/50	452 (50)	98	50/50	442 (50)	96	50/50
36	465 (50)	50/50	466 (50)	100	50/50	458 (50)	98	50/50	449 (50)	97	50/50
38	471 (50)	50/50	472 (50)	100	50/50	461 (50)	98	50/50	454 (50)	96	50/50
40	475 (50)	50/50	477 (50)	100	50/50	465 (50)	98	50/50	457 (50)	96	50/50
42	480 (50)	50/50	480 (50)	100	50/50	469 (50)	98	50/50	462 (50)	96	50/50
44	488 (50)	50/50	485 (50)	99	50/50	475 (50)	97	50/50	467 (50)	96	50/50
46	487 (50)	50/50	486 (50)	100	50/50	476 (50)	98	50/50	467 (50)	96	50/50
48	484 (50)	50/50	484 (50)	100	50/50	477 (50)	99	50/50	468 (50)	97	50/50
50	490 (50)	50/50	489 (50)	100	50/50	480 (50)	98	50/50	471 (50)	96	50/50
52	490 (50)	50/50	487 (50)	99	50/50	479 (50)	98	50/50	471 (50)	96	50/50
54	491 (50)	50/50	487 (50)	99	50/50	481 (50)	98	50/50	475 (50)	97	50/50
56	496 (50)	50/50	492 (50)	99	50/50	485 (50)	98	50/50	476 (50)	96	50/50
58	500 (50)	50/50	495 (50)	99	50/50	488 (50)	98	50/50	478 (50)	96	50/50
60	503 (50)	50/50	499 (50)	99	50/50	490 (50)	97	50/50	479 (50)	95	50/50
62	505 (50)	50/50	503 (50)	100	50/50	493 (50)	98	50/50	480 (50)	95	50/50
64	507 (50)	50/50	502 (50)	99	50/50	494 (50)	97	50/50	481 (49)	95	49/50
66	509 (50)	50/50	505 (49)	99	49/50	496 (50)	97	50/50	482 (49)	95	49/50
68	509 (50)	50/50	507 (48)	100	48/50	497 (50)	98	50/50	482 (49)	95	49/50
70	510 (50)	50/50	507 (48)	99	48/50	499 (49)	98	49/50	484 (49)	95	49/50
72	509 (50)	50/50	507 (48)	100	48/50	498 (49)	98	49/50	484 (49)	95	49/50
74	508 (50)	50/50	506 (48)	100	48/50	501 (48)	99	48/50	483 (49)	95	49/50
76	507 (50)	50/50	507 (48)	100	48/50	506 (48)	100	48/50	486 (49)	96	49/50
78	499 (50)	50/50	501 (48)	100	48/50	488 (47)	98	47/50	476 (49)	95	49/50
80	495 (50)	50/50	497 (48)	100	48/50	487 (46)	98	46/50	469 (49)	95	49/50
82	491 (50)	50/50	491 (47)	100	47/50	484 (46)	99	46/50	467 (46)	95	46/50
84	494 (48)	48/50	488 (47)	99	47/50	481 (46)	97	46/50	467 (45)	95	45/50
86	493 (47)	47/50	485 (47)	98	47/50	479 (45)	97	45/50	461 (45)	94	44/50
88	489 (46)	46/50	483 (47)	99	47/50	479 (45)	98	45/50	462 (44)	94	44/50
90	487 (45)	45/50	482 (46)	99	46/50	472 (45)	97	45/50	461 (43)	95	43/50
92	479 (44)	43/50	480 (44)	100	44/50	467 (44)	97	44/50	459 (42)	96	42/50
94	481 (41)	41/50	477 (44)	99	44/50	457 (44)	95	44/50	446 (42)	93	42/50
96	477 (41)	41/50	474 (44)	99	44/50	453 (42)	95	41/50	443 (39)	93	39/50
98	478 (40)	40/50	468 (43)	98	43/50	453 (40)	95	40/50	438 (37)	92	36/50
100	474 (40)	40/50	461 (42)	97	41/50	445 (38)	94	37/50	434 (34)	92	33/50
102	467 (40)	40/50	454 (37)	97	37/50	456 (33)	98	33/50	433 (32)	93	32/50
104	459 (38)	37/50	445 (35)	97	34/50	454 (32)	99	30/50	436 (28)	95	28/50

< >:No.of effective animals, ( ):No.of measured animals Av.Wt.: g

(Study No. 0104, 0105)

TABLE 11 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES IN FEMALE RAT

(TWO-YEAR STUDIES)

Week on Study	Control Av.Wt.	Control No.of Surviv. <50>	50 ppm Av.Wt.	50 ppm % of cont. <50>	50 ppm No.of Surviv.	200 ppm Av.Wt.	200 ppm % of cont. <50>	200 ppm No.of Surviv.	600 ppm Av.Wt.	600 ppm % of cont. <50>	600 ppm No.of Surviv.
0	101 (50)	50/50	101 (50)	100	50/50	101 (50)	100	50/50	101 (50)	100	50/50
1	116 (50)	50/50	116 (50)	100	50/50	115 (50)	99	50/50	114 (50)	98	50/50
2	131 (50)	50/50	130 (50)	99	50/50	130 (50)	99	50/50	129 (50)	98	50/50
3	143 (50)	50/50	142 (50)	99	50/50	140 (50)	98	50/50	140 (50)	98	50/50
4	153 (50)	50/50	150 (50)	98	50/50	148 (50)	97	50/50	149 (50)	97	50/50
5	161 (50)	50/50	158 (50)	98	50/50	157 (50)	98	50/50	158 (50)	98	50/50
6	168 (50)	50/50	165 (50)	98	50/50	163 (50)	97	50/50	164 (50)	98	50/50
7	174 (50)	50/50	172 (50)	99	50/50	169 (50)	97	50/50	171 (50)	98	50/50
8	179 (50)	50/50	177 (50)	99	50/50	174 (50)	97	50/50	176 (50)	98	50/50
9	184 (50)	50/50	184 (50)	100	50/50	179 (50)	97	50/50	181 (50)	98	50/50
10	189 (50)	50/50	188 (50)	99	50/50	184 (50)	97	50/50	185 (50)	98	50/50
11	192 (50)	50/50	191 (50)	99	50/50	186 (50)	97	50/50	189 (50)	98	50/50
12	196 (50)	50/50	196 (50)	100	50/50	192 (50)	98	50/50	193 (50)	98	50/50
13	199 (50)	50/50	200 (50)	101	50/50	195 (50)	98	50/50	196 (50)	98	50/50
14	202 (50)	50/50	202 (50)	100	50/50	198 (50)	98	50/50	198 (50)	98	50/50
16	207 (50)	50/50	207 (50)	100	50/50	203 (50)	98	50/50	204 (50)	99	50/50
18	211 (50)	50/50	212 (50)	100	50/50	207 (50)	98	50/50	210 (50)	100	50/50
20	216 (50)	50/50	218 (50)	101	50/50	212 (50)	98	50/50	212 (50)	98	50/50
22	222 (50)	50/50	224 (50)	101	50/50	218 (50)	98	50/50	218 (50)	98	50/50
24	226 (50)	50/50	228 (50)	101	50/50	222 (50)	98	50/50	225 (50)	100	50/50
26	230 (50)	50/50	233 (50)	101	50/50	225 (50)	98	50/50	226 (50)	98	50/50
28	232 (50)	50/50	233 (50)	100	50/50	226 (50)	97	50/50	227 (50)	98	50/50
30	236 (50)	50/50	237 (50)	100	50/50	230 (50)	97	50/50	231 (50)	98	50/50
32	241 (50)	50/50	241 (50)	100	50/50	233 (50)	97	50/50	235 (50)	98	50/50
34	245 (50)	50/50	246 (50)	100	50/50	237 (50)	97	50/50	237 (50)	97	50/50
36	249 (50)	50/50	250 (50)	100	50/50	240 (50)	96	50/50	241 (50)	97	50/50
38	252 (50)	50/50	254 (50)	101	50/50	243 (50)	96	50/50	244 (50)	97	50/50
40	255 (50)	50/50	256 (50)	100	50/50	245 (50)	96	50/50	246 (50)	96	50/50
42	257 (50)	50/50	259 (50)	101	50/50	246 (50)	96	50/50	248 (50)	96	50/50
44	261 (50)	50/50	262 (50)	100	50/50	249 (50)	95	50/50	252 (50)	97	50/50
46	263 (50)	50/50	265 (50)	101	50/50	252 (50)	96	50/50	253 (50)	96	50/50
48	261 (50)	50/50	263 (50)	101	50/50	252 (50)	97	50/50	254 (50)	97	50/50
50	267 (50)	50/50	268 (50)	100	50/50	255 (50)	96	50/50	255 (50)	96	50/50
52	271 (50)	50/50	272 (50)	100	50/50	259 (50)	96	50/50	259 (50)	96	50/50
54	274 (50)	50/50	273 (50)	100	50/50	263 (50)	96	50/50	262 (50)	96	50/50
56	279 (50)	50/50	278 (50)	100	50/50	266 (50)	95	50/50	264 (50)	95	50/50
58	282 (50)	50/50	283 (50)	100	50/50	270 (50)	96	50/50	267 (50)	95	50/50
60	286 (50)	50/50	285 (50)	100	50/50	271 (50)	95	50/50	269 (50)	94	50/50
62	289 (50)	50/50	289 (50)	100	50/50	274 (50)	95	50/50	271 (50)	94	50/50
64	291 (50)	50/50	289 (50)	99	50/50	275 (50)	95	50/50	273 (50)	94	50/50
66	295 (50)	50/50	295 (49)	100	49/50	279 (50)	95	50/50	277 (50)	94	50/50
68	298 (50)	50/50	298 (49)	100	49/50	281 (50)	94	50/50	278 (50)	93	50/50
70	303 (50)	50/50	302 (48)	100	48/50	285 (48)	94	48/50	283 (49)	93	49/50
72	304 (50)	50/50	304 (48)	100	48/50	285 (48)	94	48/50	285 (49)	94	49/50
74	307 (50)	50/50	306 (47)	100	47/50	289 (47)	94	47/50	284 (49)	93	49/50
76	308 (50)	50/50	307 (47)	100	47/50	286 (47)	93	47/50	287 (49)	93	49/50
78	303 (50)	50/50	302 (47)	100	47/50	281 (47)	93	47/50	277 (49)	91	49/50
80	301 (50)	50/50	304 (46)	101	46/50	276 (46)	92	46/50	274 (48)	91	48/50
82	299 (49)	49/50	300 (46)	100	46/50	276 (46)	92	46/50	272 (47)	91	47/50
84	299 (49)	49/50	300 (46)	100	46/50	275 (45)	92	45/50	273 (46)	91	46/50
86	301 (49)	49/50	298 (46)	99	46/50	277 (45)	92	45/50	272 (46)	90	45/50
88	311 (49)	49/50	303 (45)	97	45/50	283 (45)	91	45/50	288 (45)	93	45/50
90	317 (49)	49/50	314 (42)	99	42/50	292 (42)	92	42/50	295 (45)	93	45/50
92	319 (49)	49/50	318 (42)	100	42/50	294 (41)	92	40/50	298 (44)	93	44/50
94	322 (48)	48/50	319 (41)	99	41/50	296 (40)	92	40/50	291 (42)	90	42/50
96	323 (48)	48/50	317 (41)	98	40/50	298 (39)	92	38/50	290 (42)	90	42/50
98	322 (48)	48/50	319 (37)	99	37/50	301 (38)	93	38/50	288 (41)	89	41/50
100	328 (46)	46/50	317 (37)	97	37/50	302 (37)	92	38/50	294 (38)	90	38/50
102	328 (45)	45/50	314 (37)	96	37/50	305 (36)	93	36/50	296 (36)	90	36/50
104	326 (43)	42/50	321 (34)	98	34/50	303 (35)	93	34/50	299 (34)	92	34/50

< >:No.of effective animals, ( ):No.of measured animals Av.Wt.: g

TABLE 12 INCIDENCE AND TIME OF MASS OCCURRENCE(CLINICAL OBSERVATION) :RAT :MALE

Time of mass occurrence		Dosing week 0～13	14～26	27～39	40～52	53～65	66～78	79～91	92～104	0～104
The kind of mass		No. of animals with mass (No. of dead and moribund animals with mass)								
Internal mass										
	Control	0	0	0	0	0	0	0	3	3 (1)
	50 ppm	0	0	0	0	0	0	3	2	5 (4)
	200 ppm	0	0	0	0	1	0	1	6	7 (6)
	600 ppm	0	0	0	0	0	1	4	10	13(11)
External mass	Control	0	0	0	0	10	13	11	13	27 (8)
	50 ppm	0	0	0	0	7	10	12	17	30(15)
	200 ppm	0	0	0	0	14	23	18	20	36(13)
	600 ppm	0	0	0	0	4	9	19	23	29(11)

TABLE 13 INCIDENCE AND TIME OF MASS OCCURRENCE(CLINICAL OBSERVATION) :RAT :FEMALE

Time of mass occurrence		Dosing week 0～13	14～26	27～39	40～52	53～65	66～78	79～91	92～104	0～104
The kind of mass		No. of animals with mass (No. of dead and moribund animals with mass)								
Internal mass										
	Control	0	0	0	0	0	0	1	3	4(3)
	50 ppm	0	0	0	0	1	0	5	4	10(8)
	200 ppm	0	0	0	0	0	1	4	5	8(6)
	600 ppm	0	0	0	0	0	1	2	6	8(7)
External mass	Control	0	0	0	0	0	0	3	5	5(1)
	50 ppm	0	0	0	0	1	4	8	12	14(5)
	200 ppm	0	0	0	0	2	1	5	6	8(2)
	600 ppm	0	0	0	0	2	5	4	2	8(5)

TABLE 14 FOOD CONSUMPTION IN MALE RAT (TWO-YEAR STUDIES)

Week on Study	Control Av.FC.	Control No.of Surviv. <50>	50 ppm Av.FC.	50 ppm % of cont. <50>	50 ppm No.of Surviv.	200 ppm Av.FC.	200 ppm % of cont. <50>	200 ppm No.of Surviv.	600 ppm Av.FC.	600 ppm % of cont. <50>	600 ppm No.of Surviv.
1	15.1 (50)	50/50	14.8 (50)	98	50/50	14.5 (50)	96	50/50	14.3 (50)	95	50/50
2	17.3 (50)	50/50	16.9 (50)	98	50/50	16.6 (50)	96	50/50	16.9 (49)	98	50/50
3	18.1 (50)	50/50	17.8 (50)	98	50/50	17.1 (50)	94	50/50	17.4 (50)	96	50/50
4	18.5 (50)	50/50	18.4 (50)	99	50/50	17.7 (50)	96	50/50	18.3 (50)	99	50/50
5	18.9 (50)	50/50	18.6 (50)	98	50/50	18.1 (50)	96	50/50	18.6 (50)	98	50/50
6	18.5 (50)	50/50	18.3 (50)	99	50/50	18.0 (50)	97	50/50	18.4 (50)	99	50/50
7	18.9 (50)	50/50	18.6 (50)	98	50/50	18.0 (50)	95	50/50	18.3 (50)	97	50/50
8	18.9 (50)	50/50	18.4 (50)	97	50/50	18.1 (50)	96	50/50	18.5 (50)	98	50/50
9	19.0 (50)	50/50	18.5 (50)	97	50/50	18.0 (50)	95	50/50	18.2 (50)	96	50/50
10	18.8 (50)	50/50	18.5 (50)	98	50/50	17.9 (50)	95	50/50	18.5 (50)	98	50/50
11	18.4 (50)	50/50	17.9 (50)	97	50/50	17.9 (50)	97	50/50	18.5 (50)	101	50/50
12	18.4 (50)	50/50	18.3 (50)	99	50/50	18.2 (50)	99	50/50	18.7 (50)	102	50/50
13	18.8 (50)	50/50	18.2 (50)	97	50/50	18.1 (50)	96	50/50	18.2 (50)	97	50/50
14	17.9 (50)	50/50	17.6 (50)	98	50/50	17.7 (50)	99	50/50	17.9 (50)	100	50/50
18	18.6 (50)	50/50	18.2 (50)	98	50/50	18.0 (50)	97	50/50	18.5 (50)	99	50/50
22	19.1 (50)	50/50	18.6 (50)	97	50/50	18.2 (50)	95	50/50	18.3 (50)	96	50/50
26	19.0 (50)	50/50	18.8 (50)	99	50/50	18.6 (50)	98	50/50	18.6 (50)	98	50/50
30	19.4 (50)	50/50	19.1 (50)	98	50/50	19.0 (50)	98	50/50	18.7 (50)	96	50/50
34	19.1 (50)	50/50	18.8 (50)	98	50/50	19.0 (50)	99	50/50	19.0 (50)	99	50/50
38	18.7 (50)	50/50	18.6 (50)	99	50/50	18.0 (50)	96	50/50	18.5 (50)	99	50/50
42	19.3 (50)	50/50	18.8 (50)	97	50/50	18.8 (50)	97	50/50	19.0 (50)	98	50/50
46	18.1 (50)	50/50	18.0 (50)	99	50/50	18.1 (50)	100	50/50	18.1 (50)	100	50/50
50	18.4 (50)	50/50	18.5 (50)	101	50/50	18.3 (50)	99	50/50	18.0 (50)	98	50/50
52	17.9 (50)	50/50	17.5 (50)	98	50/50	17.9 (50)	100	50/50	18.0 (50)	101	50/50
54	17.8 (50)	50/50	17.6 (50)	99	50/50	18.0 (50)	101	50/50	18.1 (50)	102	50/50
58	18.5 (50)	50/50	18.5 (50)	100	50/50	18.5 (50)	100	50/50	18.0 (50)	97	50/50
62	19.0 (50)	50/50	18.8 (50)	99	50/50	18.7 (50)	98	50/50	18.2 (50)	96	50/50
66	19.0 (50)	50/50	18.8 (49)	99	49/50	18.7 (50)	98	50/50	18.3 (49)	96	49/50
70	18.7 (50)	50/50	18.7 (48)	100	48/50	18.4 (49)	98	49/50	18.5 (49)	99	49/50
74	18.7 (50)	50/50	18.5 (48)	99	48/50	18.6 (48)	99	48/50	18.8 (49)	101	49/50
78	17.8 (50)	50/50	17.6 (48)	99	48/50	17.7 (46)	99	47/50	17.6 (49)	99	49/50
82	17.5 (50)	50/50	17.7 (47)	101	47/50	17.9 (46)	102	46/50	16.9 (48)	97	46/50
86	17.4 (48)	47/50	17.5 (47)	101	47/50	17.7 (46)	102	45/50	17.4 (45)	100	44/50
90	18.6 (46)	45/50	18.3 (46)	98	46/50	17.8 (45)	96	45/50	18.2 (43)	98	43/50
94	16.9 (42)	41/50	18.0 (44)	107	44/50	17.0 (44)	101	44/50	17.2 (42)	102	42/50
98	17.8 (40)	40/50	16.8 (43)	94	43/50	17.3 (40)	97	40/50	17.1 (37)	96	36/50
102	17.2 (40)	40/50	16.7 (39)	97	37/50	17.5 (33)	102	33/50	17.4 (32)	101	32/50
104	16.4 (39)	37/50	17.5 (35)	107	34/50	17.7 (32)	108	30/50	17.4 (29)	106	28/50

< >:No.of effective animals,( ):No.of measured animals Av.FC.: g

TABLE 15 FOOD CONSUMPTION IN FEMALE RAT (TWO-YEAR STUDIES)

Week on Study	Control Av.FC.	Control No.of Surviv. <50>	50 ppm Av.FC.	50 ppm % of cont. <50>	50 ppm No.of Surviv.	200 ppm Av.FC.	200 ppm % of cont. <50>	200 ppm No.of Surviv.	600 ppm Av.FC.	600 ppm % of cont. <50>	600 ppm No.of Surviv.
1	11.3 (50)	50/50	11.0 (50)	97	50/50	10.9 (50)	96	50/50	10.7 (50)	95	50/50
2	11.7 (50)	50/50	11.5 (50)	98	50/50	11.3 (50)	97	50/50	11.5 (50)	98	50/50
3	11.7 (50)	50/50	11.5 (50)	98	50/50	11.2 (50)	96	50/50	11.3 (50)	97	50/50
4	11.9 (50)	50/50	11.6 (50)	97	50/50	11.4 (50)	96	50/50	11.9 (50)	100	50/50
5	11.8 (49)	50/50	11.7 (50)	99	50/50	11.5 (49)	97	50/50	11.7 (50)	99	50/50
6	12.0 (50)	50/50	11.5 (50)	96	50/50	11.6 (50)	97	50/50	11.6 (50)	97	50/50
7	12.0 (50)	50/50	11.8 (50)	98	50/50	11.4 (50)	95	50/50	11.6 (49)	97	50/50
8	11.8 (50)	50/50	11.6 (50)	98	50/50	11.4 (50)	97	50/50	11.7 (50)	99	50/50
9	11.8 (50)	50/50	12.2 (50)	103	50/50	11.6 (50)	98	50/50	11.8 (50)	100	50/50
10	11.9 (50)	50/50	11.9 (50)	100	50/50	11.4 (49)	96	50/50	11.7 (50)	98	50/50
11	11.2 (50)	50/50	11.3 (50)	101	50/50	11.0 (50)	98	50/50	11.3 (50)	101	50/50
12	12.0 (50)	50/50	12.3 (50)	103	50/50	11.8 (50)	98	50/50	12.2 (50)	102	50/50
13	12.0 (50)	50/50	12.3 (50)	103	50/50	12.1 (50)	101	50/50	11.9 (50)	99	50/50
14	12.0 (50)	50/50	11.6 (50)	97	50/50	11.7 (50)	98	50/50	11.6 (50)	97	50/50
18	12.0 (50)	50/50	12.3 (50)	103	50/50	12.0 (50)	100	50/50	12.3 (50)	103	50/50
22	12.5 (50)	50/50	12.6 (50)	101	50/50	12.1 (50)	97	50/50	12.4 (50)	99	50/50
26	12.8 (50)	50/50	13.2 (50)	103	50/50	12.2 (50)	95	50/50	12.5 (50)	98	50/50
30	12.5 (50)	50/50	12.6 (50)	101	50/50	12.5 (50)	100	50/50	12.4 (50)	99	50/50
34	12.8 (50)	50/50	13.0 (50)	102	50/50	12.9 (50)	101	50/50	12.4 (50)	97	50/50
38	12.7 (50)	50/50	13.0 (50)	102	50/50	12.2 (49)	96	50/50	12.3 (50)	97	50/50
42	12.5 (50)	50/50	12.5 (50)	100	50/50	12.0 (50)	96	50/50	12.6 (50)	101	50/50
46	12.5 (50)	50/50	12.7 (49)	102	50/50	12.3 (50)	98	50/50	12.2 (50)	98	50/50
50	13.0 (50)	50/50	13.5 (50)	104	50/50	12.8 (50)	98	50/50	12.3 (50)	95	50/50
52	12.6 (50)	50/50	12.6 (50)	100	50/50	12.4 (50)	98	50/50	12.5 (50)	99	50/50
54	12.1 (50)	50/50	12.2 (50)	101	50/50	12.4 (50)	102	50/50	12.4 (49)	102	50/50
58	12.6 (50)	50/50	12.9 (50)	102	50/50	12.9 (50)	102	50/50	12.7 (50)	101	50/50
62	13.0 (50)	50/50	13.3 (50)	102	50/50	13.0 (50)	100	50/50	13.0 (50)	100	50/50
66	13.2 (50)	50/50	13.2 (50)	100	49/50	13.2 (50)	100	50/50	13.4 (50)	102	50/50
70	13.7 (50)	50/50	13.6 (49)	99	48/50	12.9 (49)	94	48/50	13.6 (50)	99	49/50
74	13.3 (49)	50/50	13.5 (47)	102	47/50	13.5 (47)	102	47/50	13.4 (49)	101	49/50
78	11.9 (50)	50/50	11.9 (46)	100	47/50	11.7 (47)	98	47/50	11.5 (49)	97	49/50
82	12.4 (49)	49/50	12.8 (46)	103	46/50	12.4 (46)	100	46/50	12.5 (43)	101	47/50
86	12.9 (49)	49/50	12.5 (46)	97	46/50	12.7 (44)	98	45/50	12.7 (46)	98	45/50
90	14.8 (49)	49/50	14.5 (43)	98	42/50	14.0 (43)	95	42/50	14.8 (45)	100	45/50
94	14.3 (48)	48/50	14.5 (40)	101	41/50	14.0 (40)	98	40/50	14.4 (42)	101	42/50
98	13.6 (48)	48/50	14.5 (36)	107	37/50	14.2 (38)	104	38/50	13.7 (41)	101	41/50
102	13.3 (46)	45/50	13.7 (36)	103	37/50	13.9 (36)	105	36/50	13.5 (37)	102	36/50
104	12.9 (44)	42/50	14.6 (35)	113	34/50	14.3 (35)	111	34/50	14.2 (33)	110	34/50

< >:No.of effective animals,( ):No.of measured animals Av.FC.: g

TABLE 16 NEOPLASTIC LESIONS (SPLEEN) INCIDENCE AND STATISTICAL ANALYSIS : RAT : MALE

Group Name	Control	50 ppm	200 ppm	600 ppm
SITE : spleen				
TUMOUR : mononuclear cell leukemia				
Overall Rates(a)	11/50 (22.0)	14/50 (28.0)	22/50 (44.0)	27/50 (54.0)
Adjusted Rates(b)	24.32	17.65	40.00	42.86
Terminal Rates(c)	9/37 (24.3)	6/34 (17.6)	12/30 (40.0)	12/28 (42.9)
Standard Rates(d)	P=0.0022**			
Prevalence Rates(d)	P=0.0104*			
Combind analysis(d)	P=0.0001**			
Cochran-Armitage Test(e)	P=0.0005**			
Fisher Exact Test(e)		P=0.3777	P=0.0707	P=0.0201*

TABLE 17 NEOPLASTIC LESIONS (SPLEEN) INCIDENCE AND STATISTICAL ANALYSIS : RAT : FEMALE

Group Name	Control	50 ppm	200 ppm	600 ppm
SITE : spleen				
TUMOUR : mononuclear cell leukemia				
Overall Rates(a)	10/50 (20.0)	17/50 (34.0)	16/50 (32.0)	19/50 (38.0)
Adjusted Rates(b)	14.29	20.59	22.50	20.59
Terminal Rates(c)	6/42 (14.3)	7/34 (20.6)	7/34 (20.6)	7/34 (20.6)
Standard Rates(d)	P=0.0486*			
Prevalence Rates(d)	P=0.3153			
Combind analysis(d)	P=0.0571			
Cochran-Armitage Test(e)	P=0.1397			
Fisher Exact Test(e)		P=0.1636	P=0.2039	P=0.1027

(a):Number of tumor-bearing animals/number of animals examined at the site.
(b):Kaplan-Meire estimate tumor incidence at the end of study after adjusting for intercurrent mortality.
(c):Observed tumor incidence at terminal kill.
(d):Beneth the control incidence are the P-values associated with the trend test.
Standard method : Death analysis
Prevalence method : Incidental tumor test
Combind analysis : Death analysis + Incidental tumor test
(e):The Cochran-Armitage and Fisher's exact test compare directly the overall incidence rates.
? :The conditional probabilities of the largest and smallest possible out comes can not estimated or this P-value beyond the estimated P-value.
------:There is no data which should be statistic analysis.

TABLE 18 NUMBER OF RAT WITH SELECTED LIVER LESIONS

	Male				Female			
Group	Control	50ppm	200ppm	600ppm	Control	50ppm	200ppm	600ppm
Number of examined	50	50	50	50	50	50	50	50
Spongiosis hepatis	5	4	10	16	0	0	0	0
Hyperplasia	4	2	5	13	2	1	5	1
Clear cell focus	3	1	0	1	1	0	0	1
Acidphilic cell focus	1	0	1	0	0	0	0	0
Basophilic cell focus	4	3	0	4	1	1	2	0
Vacuolic cell focus	0	2	1	1	0	0	0	2
Mixed cell focus	4	3	1	1	0	0	0	2
Hepatocellular adenoma	3	0	0	2	0	0	1	0
Cholangiocellular adenoma	1	0	0	0	1	0	0	0
Cholangiocellular carcinoma	1	0	1	0	0	0	0	0

TABLE 19 NUMBER OF RAT WITH SELECTED KIDNEY LESIONS

	Male				Female			
Group	Control	50ppm	200ppm	600ppm	Control	50ppm	200ppm	600ppm
Number of examined	50	50	50	50	50	50	50	50
Nuclear enlargement: proximal tubule	0	0	23	48	0	0	1	18
Atypical tubular dilation: proximal tubule	0	0	0	24	0	0	0	6
Liposarcoma	0	0	0	1	0	0	0	0
Renal cell adenoma	1	2	1	2	1	0	0	0
Renal cell carcinima	0	0	0	0	0	0	0	1

TABLE 20 CAUSE OF DEATH :RAT

Group	Male				Female			
	Control	50ppm	200ppm	600ppm	Control	50ppm	200ppm	600ppm
Number of dead/moriboud animal	13	16	20	22	8	16	16	16
Renal lesion	1	0	2	0	0	0	1	0
Chronic nephropathy	0	0	0	1	0	0	0	0
Tumor death : leukemia	3	8	9	14	4	10	7	12
: subcutis	0	1	2	1	0	0	0	1
: spleen	0	0	0	0	1	0	0	0
: small intestine	0	0	0	1	0	0	1	0
: kidney	0	0	0	2	0	0	0	0
: pituitary gland	4	2	4	1	3	1	2	3
: thyroid	0	0	0	0	0	1	0	0
: adrenal	1	1	0	1	0	1	0	0
: uterus	0	0	0	0	0	1	3	0
: mammary gland	1	0	0	0	0	1	0	0
: prep./cli. gland	0	0	1	0	0	1	1	0
: brain	1	0	0	0	0	0	1	0
: Zymbal gland	0	1	1	0	8	8	8	9
: muscle	1	0	0	0	0	0	1	0
: bone	0	1	1	1	5	2	2	2
: pleura	1	0	0	0	1	0	0	0
: mediastinum	0	1	0	0	1	2	2	1
: peritoneum	0	1	0	0	0	0	0	0

SELECTED FIGURES

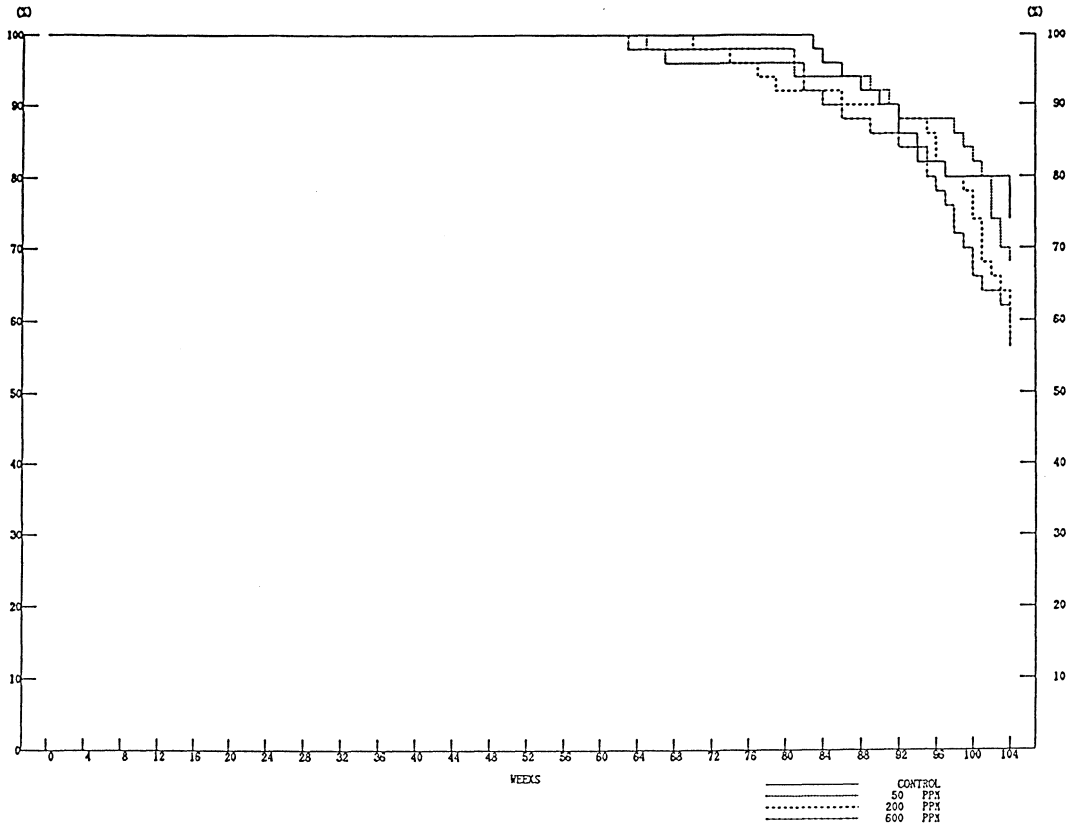


FIGURE 3 SURVIVAL ANIMAL RATE : RAT:MALE(TWO-YEAR STUDIES)

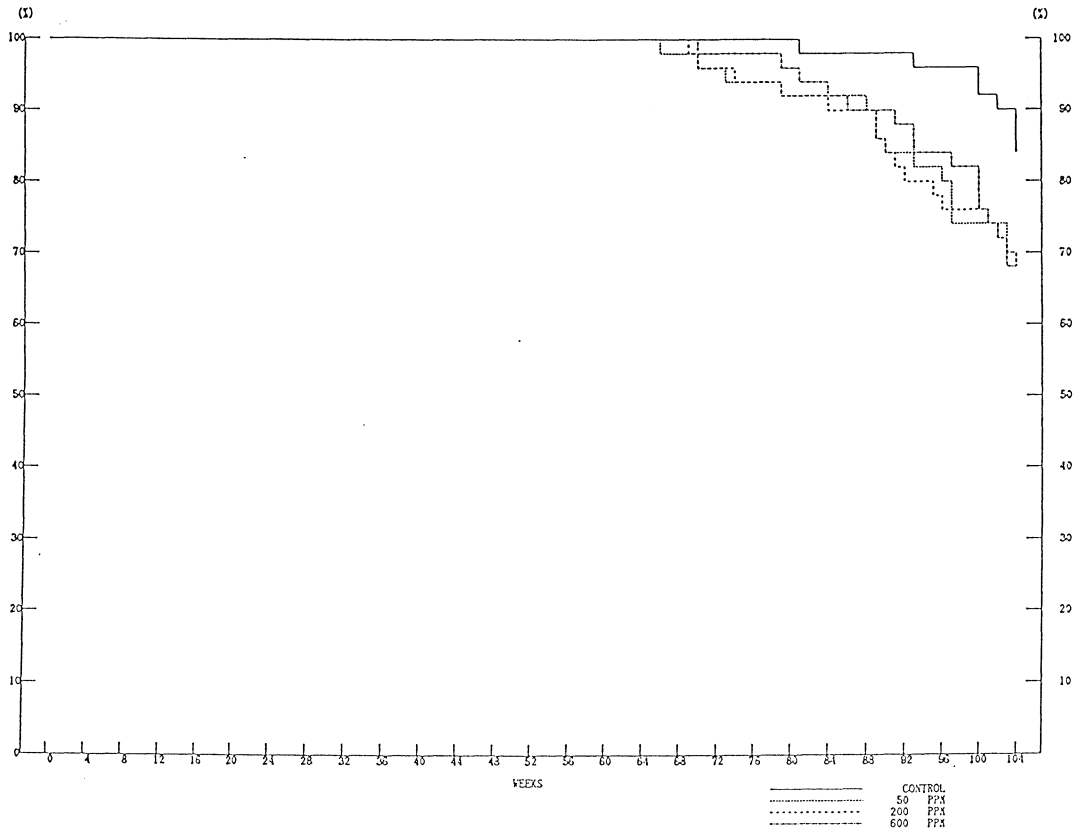


FIGURE 4 SURVIVAL ANIMAL RATE : RAT:FEMALE(TWO-YEAR STUDIES)

(Study No. 0104, 0105)

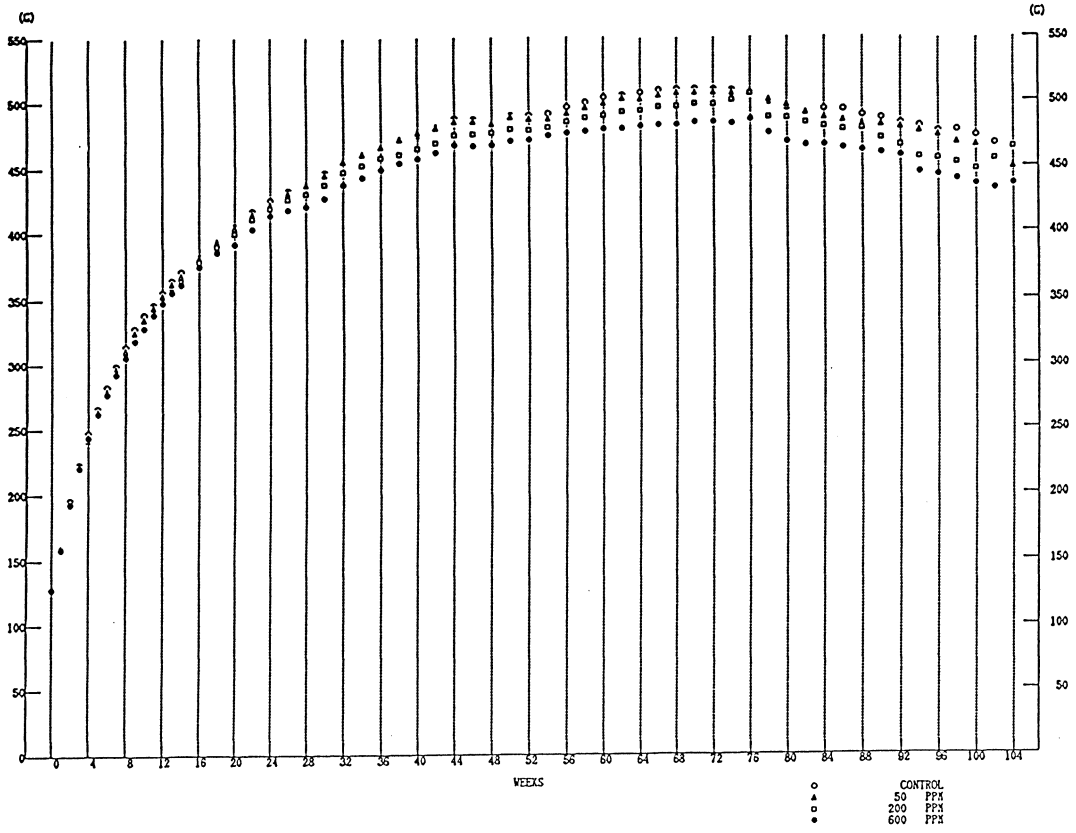


FIGURE 5 BODY WEIGHT CHANGES : RAT:MALE(TWO-YEAR STUDIES)

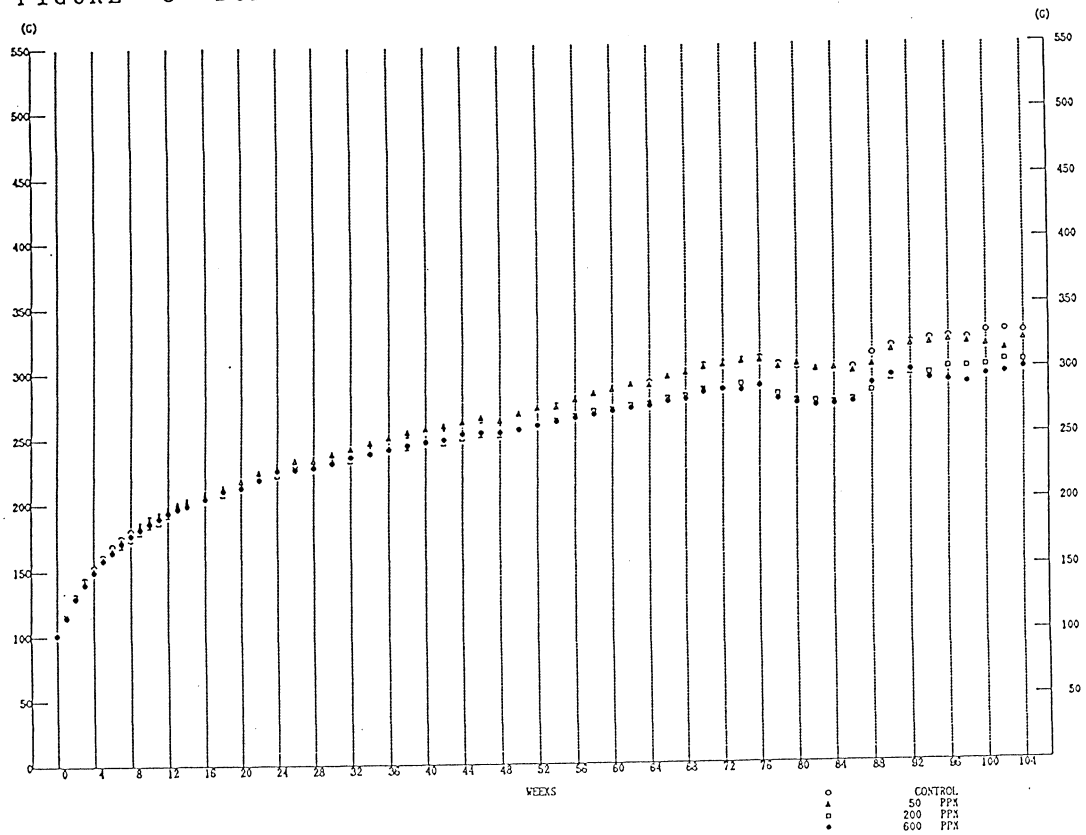


FIGURE 6 BODY WEIGHT CHANGES : RAT:FEMALE(TWO-YEAR STUDIES)

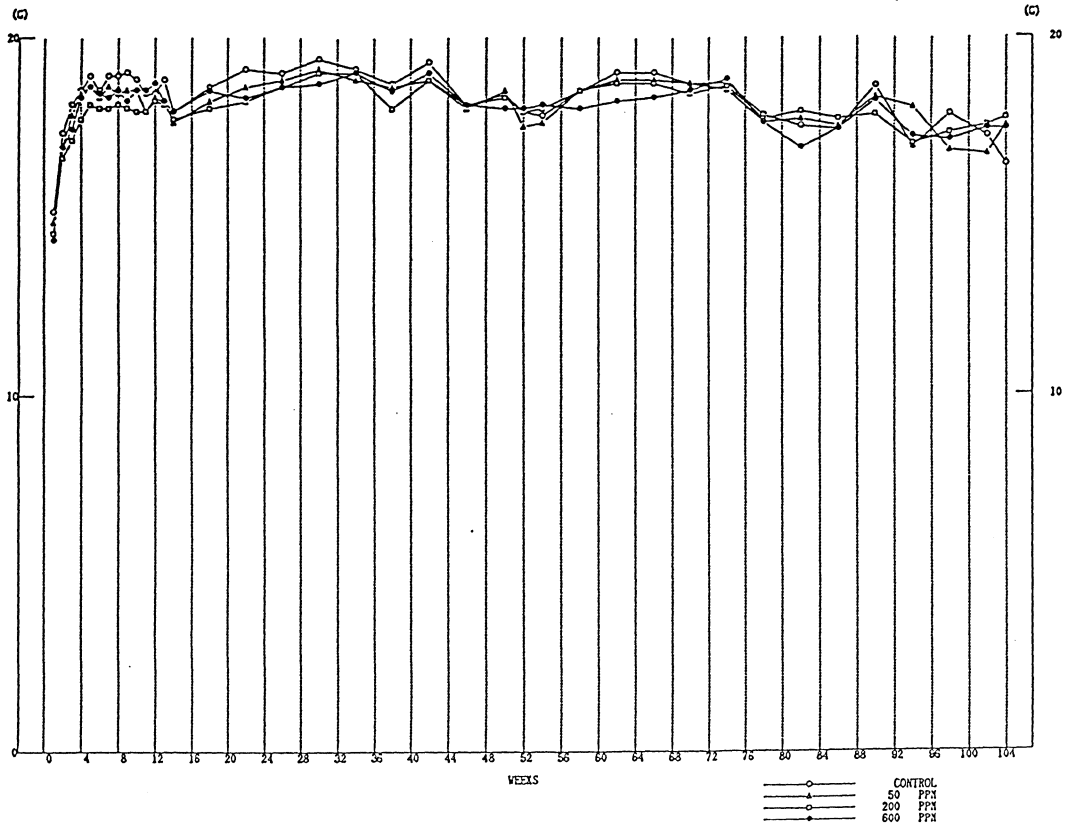


FIGURE 7 FOOD CONSUMPTION : RAT:MALE(TWO-YEAR STUDIES)

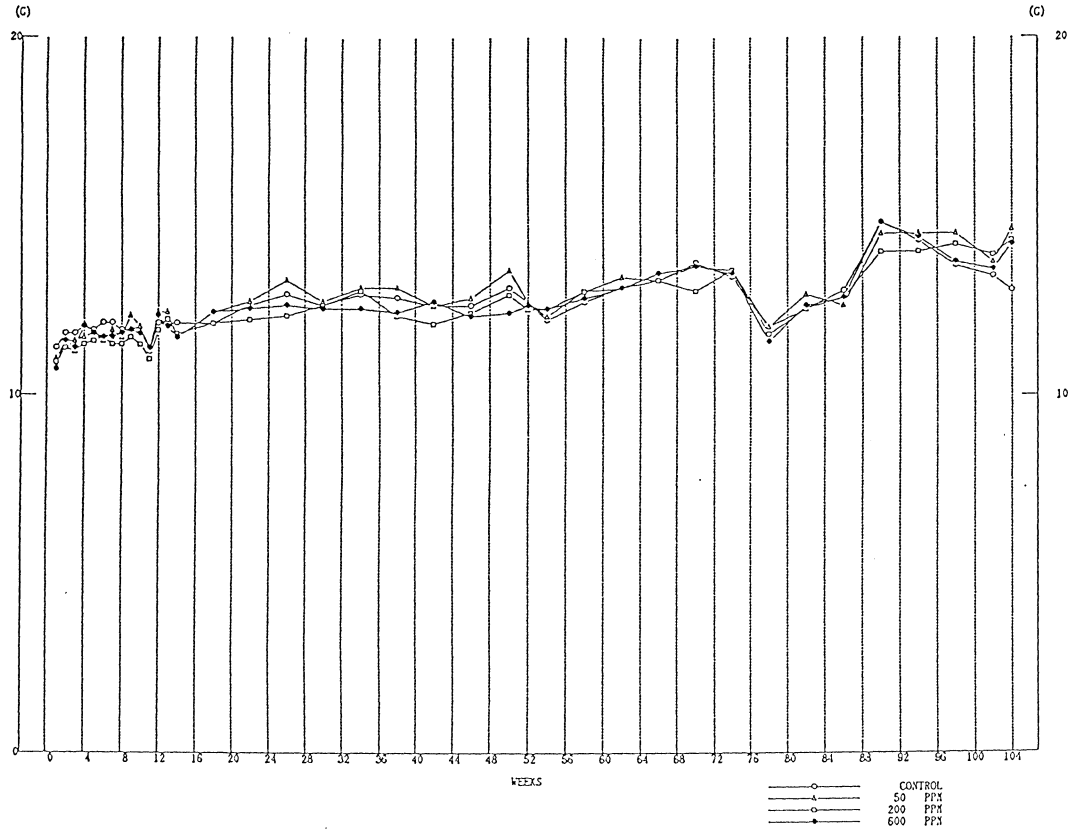


FIGURE 8 FOOD CONSUMPTION : RAT:FEMALE(TWO-YEAR STUDIES)

PHOTOGRAPHS

PHOTOGRAPH 1
SPLEEN, MONONUCLEAR CELL LEUKEMIA
TWO-YEAR STUDY, RAT, MALE, 600 ppm, ANIMAL NO.0104-1302
(H. E., X152)

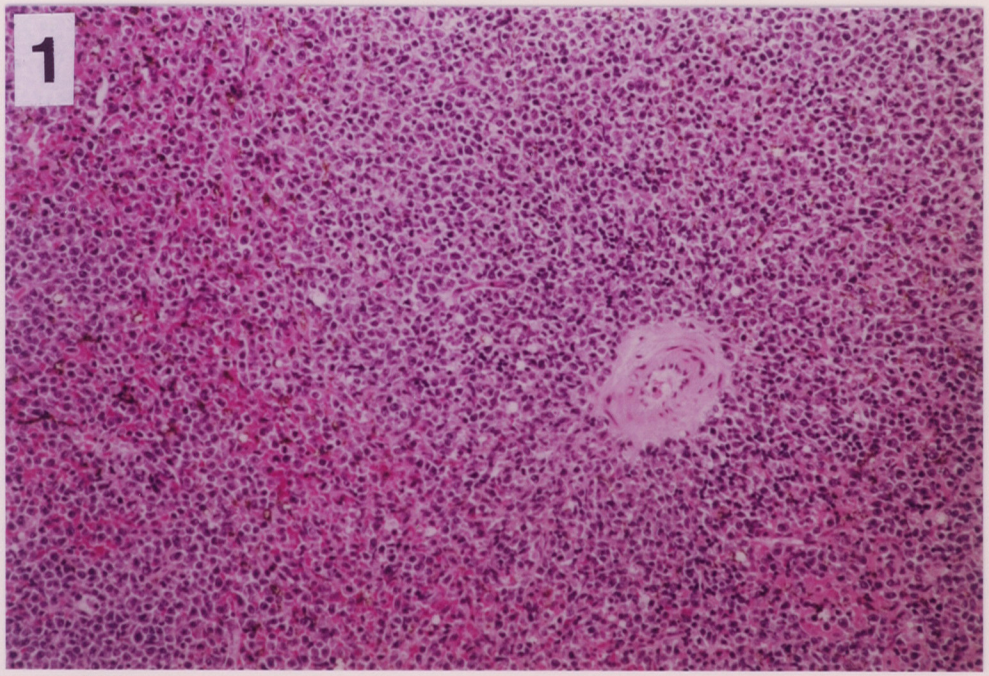
PHOTOGRAPH 2
HARDERIAN GLAND, ADENOMA :A
TWO-YEAR STUDY, RAT, MALE, 600 ppm, ANIMAL NO. 0104-1327
(H. E., X60)

PHOTOGRAPH 3
LIVER, SPONGIOSIS HEPATIS :A
TWO-YEAR STUDY, RAT, MALE, 600 ppm, ANIMAL NO. 0104-1305
(H. E., X60)

PHOTOGRAPH 4
LIVER, HYPERPLASIA : A
TWO-YEAR STUDY, RAT, MALE, 600 ppm, ANIMAL NO. 0104-1307
(H.E., X60)

PHOTOGRAPH 5
KIDNEY, NUCLEAR ENLARGMENT:PROXIMAL TUBULE :A
ATYPICAL TUBULAR DILATATION:PROXIMAL TUBULE :B
TWO-YEAR STUDY, RAT, MALE, 600 ppm, ANIMAL NO. 0104-1333
(H. E., X304)

PHOTOGRAPH 6
KIDNEY, CHRONIC NEPHROPATHY
TWO-YEAR STUDY, RAT, MALE, 600 ppm, ANIMAL NO. 0104-1334
(H.E., X60)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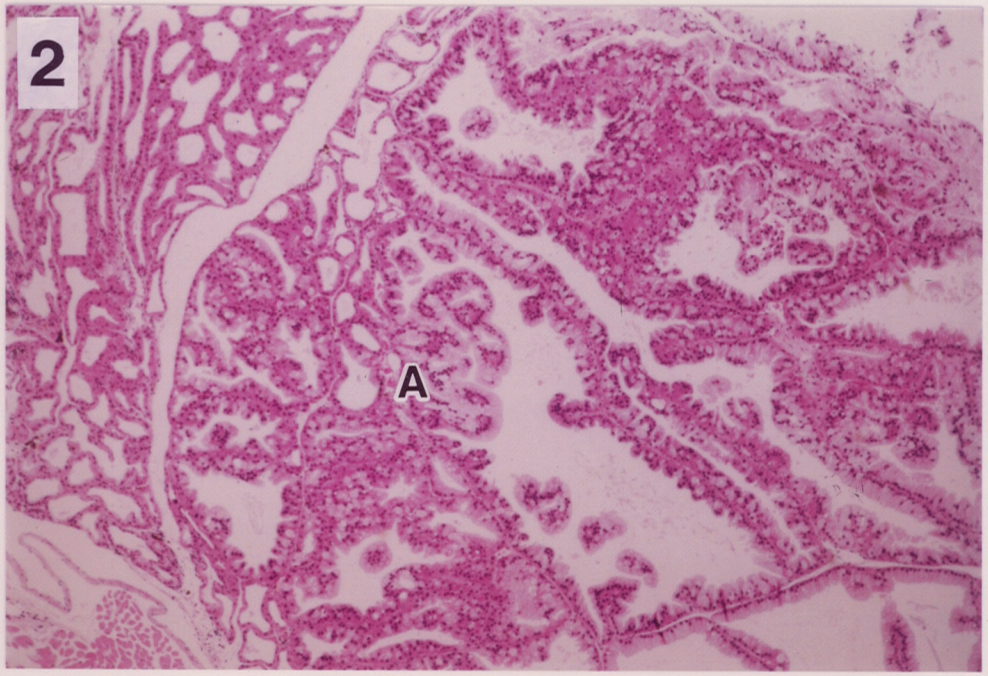
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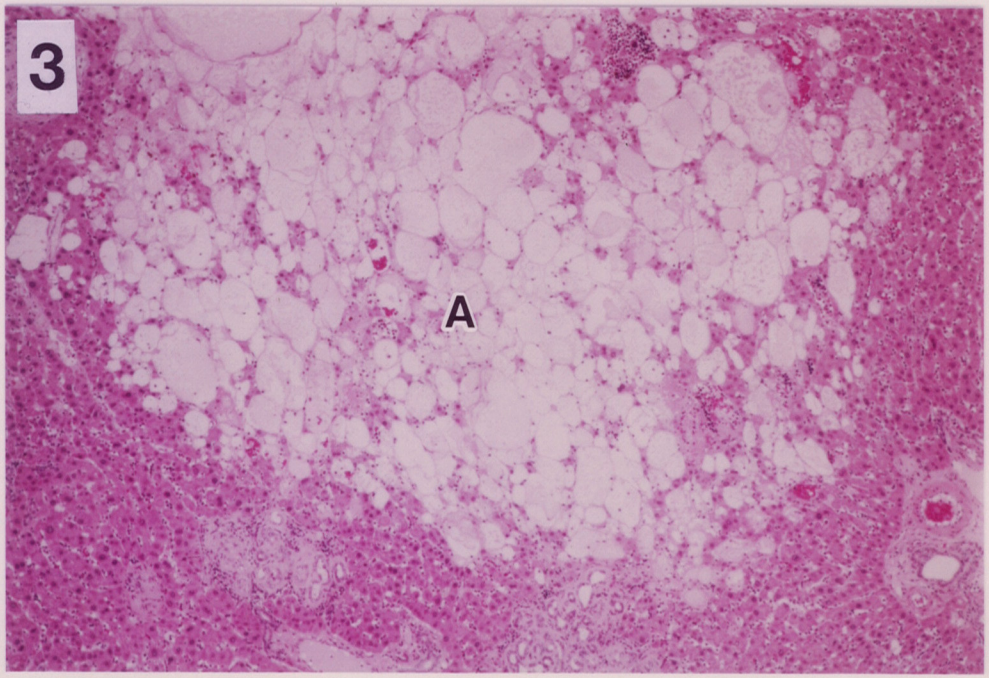
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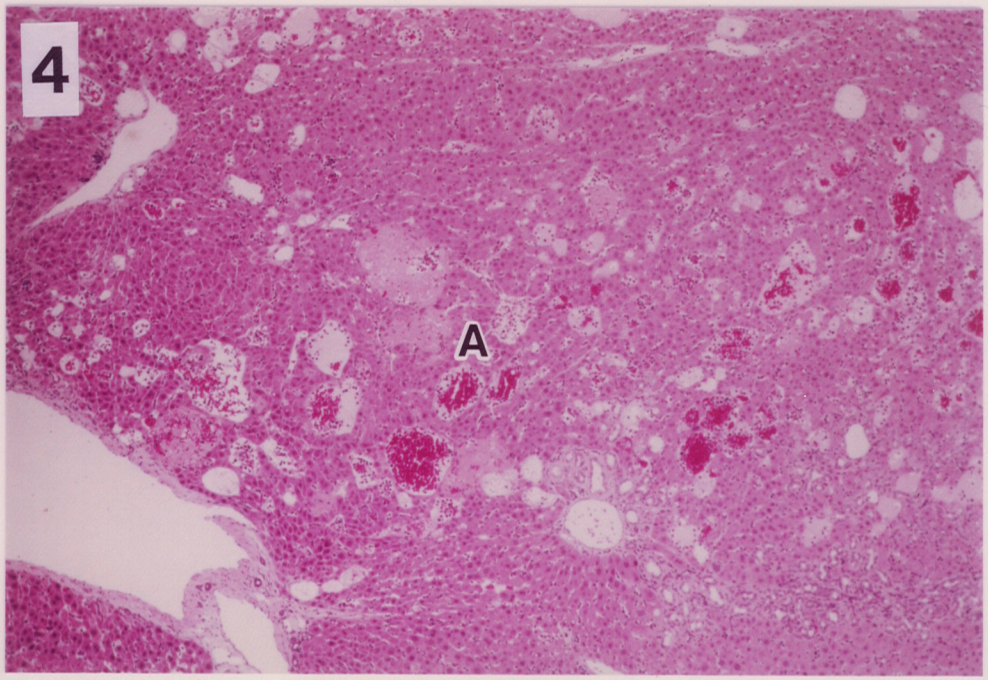
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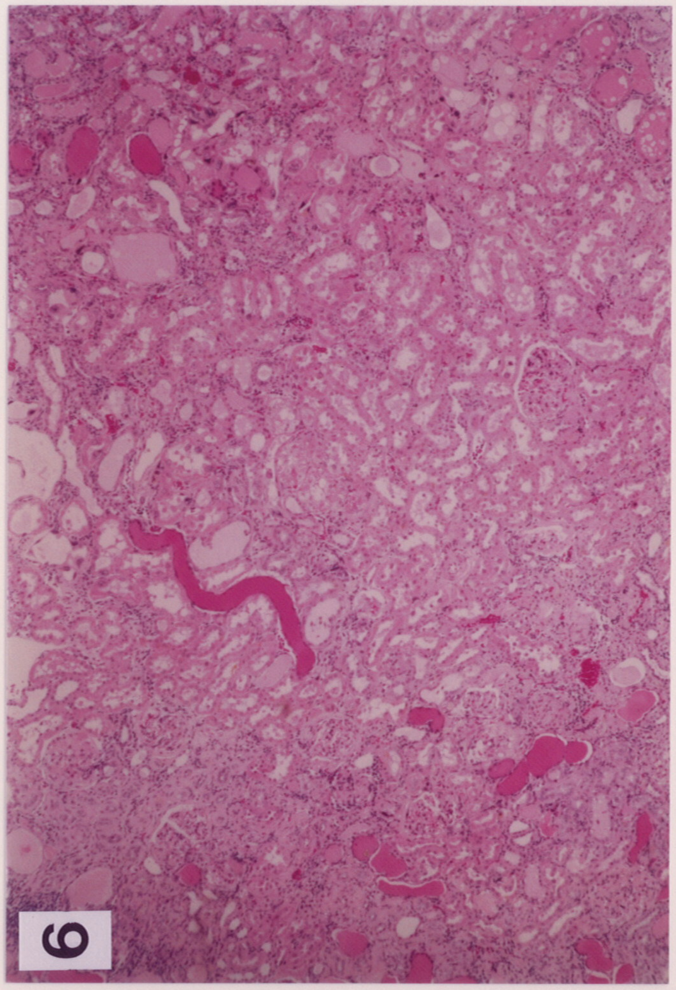
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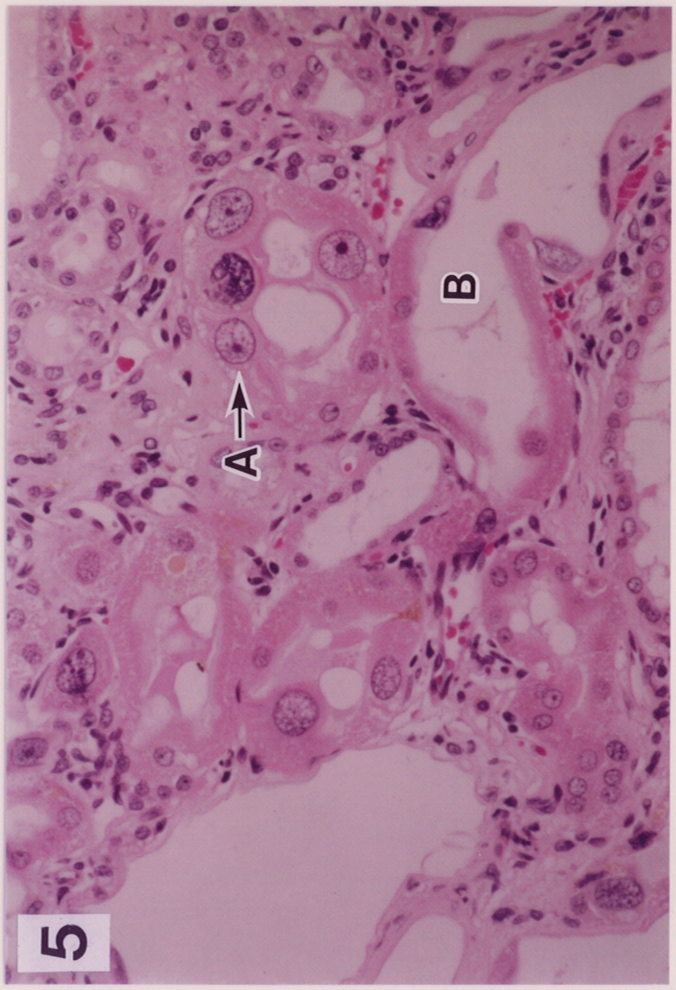
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