Summary of Drinking Water Carcinogenicity Study of 2-Hydroxyethyl Acrylate in F344 Rats

September 2003

Japan Bioassay Research Center

Japan Industrial Safety and Health Association

PREFACE

The tests were contracted and supported by the Ministry of Health, Labour and Welfare of Japan. The tests were conducted by Japan Bioassay Research Center (JBRC) and the report was prepared by JBRC and peer reviewed by outside expert pathologist. Complete report was submitted to Ministry of Health, Labour and Welfare of Japan on September 24, 2003.

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

Summary of Drinking Water Carcinogenicity Study of 2-Hydroxyethyl Acrylate in F344 Rats

Purpose, materials and methods

2-Hydroxyethyl acrylate (HEA, CAS No. 818-61-1) is a transparent liquid with a boiling point of 82°C and is soluble in water.

The carcinogenicity and chronic toxicity of HEA were examined in groups of 50 F344/DuCrj (Fischer) rats of both sexes administered HEA in drinking water for 2 years (104 weeks). The drinking water concentration of HEA was 0, 320, 800 or 2000 ppm (w/w). The highest dose level was chosen so as not to exceed the maximum tolerated dose (MTD), based on both growth rate and toxicity in the previous 13-week toxicity study. HEA was analyzed for purity and stability by both infrared spectrometry and gas chromatography before and after its use. The concentrations of HEA in drinking water were determined by gas chromatography at the time of preparation, and on the 4th, 8th and 11th days after preparation, while stored at room temperature. The animals were observed daily for clinical signs and mortality. Body weight, water consumption and food consumption were measured once a week for the first 14 weeks and every 4 weeks thereafter. Animals found dead, in a moribund state, or surviving to the end of the 2-year administration period underwent complete necropsy. Urinalysis was performed near the end of the 2-year administration period. For hematology and blood biochemistry, the surviving animals were bled under ether anesthesia, after they were fasted overnight, at the terminal necropsy. Organs and tissues were removed, weighed and examined for macroscopic lesions at necropsy. The organs and tissues were fixed and embedded in paraffin. Tissue sections of 5 μm thick were prepared and stained with hematoxylin and eosin and examined for histopathology. Incidences of neoplastic lesions were statistically analyzed by Fisher's exact test. A positive trend of the dose-response relationship for the neoplastic incidence was analyzed by Peto's test. Incidences of non-neoplastic lesions and urinalysis were analyzed by Chi-square test. Changes in body weight, water consumption, food consumption, hematological and blood biochemical parameters, and organ weights were analyzed by Dunnett's test. The present studies were conducted in accordance with the Organisation for Economic Co-operation and Development (OECD) Good Laboratory Practice and with reference to the OECD Guideline for Testing of Chemicals 451 "Carcinogenicity Studies".

Results

There was no significant difference in survival rate between any HEA-administered group of either sex and the respective control. Body weight, water consumption and food consumption were decreased dose-dependently in all the HEA-administered groups as compared with the respective controls.

The incidence of hepatocellular adenomas was increased in the 320 ppm-administered males, and the significantly increased incidence of hepatocellular adenomas was noted in the 2000 ppm-administered males. As a pre-neoplastic lesion, the incidence of basophilic cell foci in the liver was significantly increased in the males administered 800 ppm and above. In the HEA-administered females, the incidence of hepatocellular adenomas tended to increase, but not significantly at the high dose level. In addition, no increase in the incidence of pre-neoplastic lesions was noted. As non-neoplastic lesions, the incidences of papillary necrosis and chronic progressive nephropathy (chronic nephropathy, CPN) in the kidney were increased in the males and females administered 320 ppm and above. The incidences of forestomach squamous cell hyperplasia and basal cell hyperplasia were slightly increased in the HEA-administered females, suggesting positive effects of the HEA administration. In blood biochemistry, plasma levels of γ-GTP, T-cholesterol, phospholipid and urea nitrogen were increased dose-dependently in the HEA-administered males and females.

A lowest-observed-adverse-effect-level (LOAEL) for the renal endpoint of papillary necrosis was estimated at 320 ppm (equivalent to 0.013 to 0.032 g/kg/day for males and 0.016 to 0.038 g/kg/day for females). A lower confidence limit of the benchmark dose yielding a response with 10% extra risk ($BMDL_{10}$) for the same renal endpoint was determined at 148 ppm for males and at 112 ppm for females.

Conclusions

In rats, there was some evidence of carcinogenic activity of HEA in males, based on the increased incidences of hepatocellular adenomas and hepatic basophilic cell foci as the pre-neoplastic lesion. There was equivocal evidence of carcinogenic activity of HEA in females, based on the slightly increased incidence of hepatocellular adenomas. As non-neoplastic lesions, the increased incidences of papillary necrosis and CPN were noted in the HEA -administered males and females, suggesting that HEA exerts adverse effects on the kidney.

TABLES

TABLES (Continued)

TABLES (Continued)

TABLE 1 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE

Group	Control		320 ppm			800 ppm			2000 ppm		
	<50>		<50>			<49>			<50>		
Week on Study	Survival No.	BW g	Survival No.	BW g	%	Survival No.	BW g	%	Survival No.	BW g	%
0	50	123 (50)	50	123 (50)	100	49	123 (49)	100	50	123 (50)	100
1	50	156 (50)	50	153 (50)	98	49	151 (49)	97 **	50	141 (50)	90 **
2	50	185 (50)	50	182 (50)	98	49	179 (49)	97 **	50	167 (50)	90 **
3	50	207 (50)	50	204 (50)	99	49	201 (49)	97 **	50	189 (50)	91 **
4	50	224 (50)	50	222 (50)	99	49	218 (49)	97 **	50	205 (50)	92 **
5	50	239 (50)	50	236 (50)	99	49	232 (49)	97 **	50	220 (50)	92 **
6	50	251 (50)	50	248 (50)	99	49	242 (49)	96 **	50	230 (50)	92 **
7	50	262 (50)	50	258 (50)	98	49	253 (49)	97 **	50	239 (50)	91 **
8	50	270 (50)	50	267 (50)	99	49	261 (49)	97 **	50	246 (50)	91 **
9	50	279 (50)	50	274 (50)	98	49	268 (49)	96 **	50	253 (50)	91 **
10	50	285 (50)	50	280 (50)	98	49	275 (49)	96 **	50	259 (50)	91 **
11	50	291 (50)	50	285 (50)	98	49	280 (49)	96 **	50	264 (50)	91 **
12	50	296 (50)	50	291 (50)	98	49	285 (49)	96 **	50	269 (50)	91 **
13	50	302 (50)	50	295 (50)	98 *	49	290 (49)	96 **	50	273 (50)	90 **
14	50	307 (50)	50	299 (50)	97 *	49	294 (49)	96 **	50	277 (50)	90 **
18	50	322 (50)	50	314 (50)	98 **	49	308 (49)	96 **	50	292 (50)	91 **
22	50	335 (50)	50	326 (50)	97 **	49	321 (49)	96 **	50	305 (50)	91 **
26	50	348 (50)	50	336 (50)	97 **	49	332 (49)	95 **	50	317 (50)	91 **
30	50	355 (50)	50	343 (50)	97 **	49	340 (49)	96 **	50	324 (50)	91 **
34	50	364 (50)	50	350 (50)	96 **	49	345 (49)	95 **	50	331 (50)	91 **
38	50	374 (50)	50	357 (50)	95 **	49	353 (49)	94 **	50	338 (50)	90 **
42	50	382 (50)	50	363 (50)	95 **	49	359 (49)	94 **	49	343 (49)	90 **
46	50	388 (50)	50	369 (50)	95 **	49	365 (49)	94 **	49	348 (49)	90 **
50	50	392 (50)	50	373 (50)	95 **	49	369 (49)	94 **	49	351 (49)	90 **
54	50	398 (50)	50	379 (50)	95 **	49	374 (49)	94 **	49	355 (49)	89 **
58	50	403 (50)	50	383 (50)	95 **	49	377 (49)	94 **	49	357 (49)	89 **
62	50	406 (50)	50	387 (50)	95 **	48	381 (48)	94 **	49	356 (49)	88 **
66	50	409 (50)	50	392 (50)	96 **	48	385 (48)	94 **	47	357 (47)	87 **
70	49	406 (49)	49	393 (49)	97 *	47	385 (47)	95 **	46	355 (46)	87 **
74	49	416 (49)	48	400 (48)	96 **	47	387 (47)	93 **	46	355 (46)	85 **
78	49	419 (49)	47	402 (47)	96 *	46	388 (46)	93 **	45	356 (45)	85 **
82	48	428 (48)	47	407 (47)	95 **	46	388 (46)	91 **	45	357 (45)	83 **
86	48	425 (48)	47	405 (47)	95 **	44	385 (44)	91 **	44	348 (44)	82 **
90	47	423 (47)	47	404 (47)	96 **	42	382 (42)	90 **	43	341 (43)	81 **
94	45	419 (45)	47	399 (47)	95 **	41	376 (41)	90 **	41	332 (41)	79 **
98	41	419 (41)	47	393 (47)	94 **	41	367 (41)	88 **	39	325 (39)	78 **
102	40	412 (40)	44	388 (44)	94 **	39	357 (39)	87 **	36	318 (36)	77 **
104	40	408 (40)	44	383 (44)	94 **	37	353 (37)	87 **	35	316 (35)	77 **

< > : No.of effective animals, ( ) : No.of measured animals % : % of control group
Significant Difference, * : p≦0.05, ** : p≦0.01, Test of Dunnett

TABLE 2 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE

Group	Control		320 ppm			800 ppm			2000 ppm		
	<50>		<50>			<50>			<50>		
Week on Study	Survival No.	BW g	Survival No.	BW g	%	Survival No.	BW g	%	Survival No.	BW g	%
0	50	96 (50)	50	96 (50)	100	50	96 (50)	100	50	96 (50)	100
1	50	115 (50)	50	113 (50)	98	50	112 (50)	97 **	50	108 (50)	94 **
2	50	126 (50)	50	124 (50)	98	50	123 (50)	98 *	50	119 (50)	94 **
3	50	134 (50)	50	132 (50)	99	50	132 (50)	99	50	127 (50)	95 **
4	50	140 (50)	50	138 (50)	99	50	138 (50)	99	50	132 (50)	94 **
5	50	147 (50)	50	146 (50)	99	50	144 (50)	98	50	137 (50)	93 **
6	50	151 (50)	50	149 (50)	99	50	149 (50)	99	50	141 (50)	93 **
7	50	154 (50)	50	153 (50)	99	50	152 (50)	99	50	143 (50)	93 **
8	50	157 (50)	50	156 (50)	99	50	155 (50)	99	50	146 (50)	93 **
9	50	160 (50)	50	160 (50)	100	50	158 (50)	99	50	149 (50)	93 **
10	50	163 (50)	50	163 (50)	100	50	161 (50)	99	50	150 (50)	92 **
11	50	166 (50)	50	165 (50)	99	50	164 (50)	99	50	154 (50)	93 **
12	50	168 (50)	50	168 (50)	100	50	167 (50)	99	50	155 (50)	92 **
13	50	170 (50)	50	171 (50)	101	50	169 (50)	99	50	158 (50)	93 **
14	50	171 (50)	50	173 (50)	101	50	171 (50)	100	50	159 (50)	93 **
18	50	179 (50)	50	180 (50)	101	50	177 (50)	99	50	165 (50)	92 **
22	50	185 (50)	50	187 (50)	101	50	184 (50)	99	50	171 (50)	92 **
26	50	189 (50)	50	191 (50)	101	50	188 (50)	99	50	175 (50)	93 **
30	50	194 (50)	50	197 (50)	102	50	193 (50)	99	50	179 (50)	92 **
34	50	198 (50)	50	200 (50)	101	50	195 (50)	98	50	181 (50)	91 **
38	50	200 (50)	50	204 (50)	102	50	197 (50)	99	50	183 (50)	92 **
42	50	205 (50)	50	208 (50)	101	50	201 (50)	98	50	186 (50)	91 **
46	50	210 (50)	50	212 (50)	101	50	205 (50)	98	50	188 (50)	90 **
50	49	213 (49)	50	215 (50)	101	49	208 (49)	98	50	191 (50)	90 **
54	49	217 (49)	50	219 (50)	101	49	212 (49)	98	50	193 (50)	89 **
58	49	220 (49)	50	223 (50)	101	49	215 (49)	98	50	194 (50)	88 **
62	49	224 (49)	49	228 (49)	102	48	219 (48)	98	50	195 (50)	87 **
66	49	230 (49)	49	233 (49)	101	48	223 (48)	97	50	198 (50)	86 **
70	48	234 (48)	49	234 (49)	100	48	225 (48)	96	50	199 (50)	85 **
74	47	241 (47)	48	241 (48)	100	48	229 (48)	95	50	199 (50)	83 **
78	47	247 (47)	48	247 (48)	100	48	233 (48)	94 *	50	202 (50)	82 **
82	46	253 (46)	48	254 (48)	100	47	240 (47)	95 *	49	206 (49)	81 **
86	44	257 (44)	48	255 (48)	99	47	242 (47)	94 *	47	210 (47)	82 **
90	42	262 (42)	47	257 (47)	98	47	242 (47)	92 **	46	211 (46)	81 **
94	42	262 (42)	46	257 (46)	98	46	243 (46)	93 **	46	211 (46)	81 **
98	40	265 (40)	45	257 (45)	97	44	242 (44)	91 **	46	212 (46)	80 **
102	37	268 (37)	40	264 (40)	99	42	242 (42)	90 **	46	212 (46)	79 **
104	37	266 (37)	40	261 (40)	98	42	240 (42)	90 **	42	208 (42)	78 **

< > : No.of effective animals, ( ) : No.of measured animals % : % of control group
Significant Difference, * : p≦0.05, ** : p≦0.01, Test of Dunnett

TABLE 3 WATER CONSUMPTION CHANGES OF MALE RATS
IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE

Group	Control		320 ppm			800 ppm			2000 ppm		
Week on Study	<50> Survival No.	WC g	<50> Survival No.	WC g	%	<49> Survival No.	WC g	%	<50> Survival No.	WC g	%
1	50	18.0 (50)	50	15.6 (50)	87 **	49	14.2 (49)	79 **	50	11.7 (50)	65 **
2	50	18.8 (50)	50	16.8 (50)	89 **	49	15.1 (48)	80 **	50	12.1 (50)	64 **
3	50	20.3 (50)	50	16.9 (50)	83 **	49	15.4 (49)	76 **	50	13.3 (50)	66 **
4	50	19.9 (50)	50	17.3 (50)	87 **	49	15.5 (48)	78 **	50	13.4 (50)	67 **
5	50	18.9 (50)	50	16.4 (50)	87 **	49	14.9 (49)	79 **	50	12.8 (50)	68 **
6	50	18.6 (50)	50	16.1 (50)	87 **	49	14.6 (49)	78 **	50	12.2 (50)	66 **
7	50	18.5 (50)	50	16.9 (50)	91 **	49	14.5 (49)	78 **	50	12.2 (50)	66 **
8	50	18.2 (50)	50	16.4 (50)	90 **	49	13.9 (49)	76 **	50	11.6 (50)	64 **
9	50	17.9 (50)	50	15.8 (50)	88 **	49	13.9 (49)	78 **	50	11.9 (50)	66 **
10	50	17.8 (50)	50	16.0 (50)	90 **	49	14.6 (49)	82 **	50	12.1 (50)	68 **
11	50	17.1 (50)	50	15.2 (50)	89 **	49	13.5 (49)	79 **	50	11.6 (50)	68 **
12	50	16.9 (50)	50	15.0 (50)	89 **	49	13.6 (49)	80 **	50	11.5 (50)	68 **
13	50	17.7 (50)	50	16.0 (50)	90 **	49	14.2 (49)	80 **	50	11.8 (50)	67 **
14	50	17.5 (50)	50	15.3 (50)	87 **	49	13.9 (49)	79 **	50	11.7 (50)	67 **
18	50	16.5 (50)	50	14.8 (50)	90 **	49	13.8 (49)	84 **	50	11.7 (50)	71 **
22	50	16.2 (50)	50	14.9 (50)	92 **	49	13.1 (49)	81 **	50	11.4 (50)	70 **
26	50	16.7 (50)	50	14.6 (50)	87 **	49	13.3 (49)	80 **	50	11.6 (49)	69 **
30	50	16.5 (50)	50	15.0 (50)	91 **	49	13.5 (49)	82 **	50	11.6 (50)	70 **
34	50	16.5 (50)	50	14.4 (50)	87 **	49	13.3 (49)	81 **	50	11.7 (50)	71 **
38	50	16.4 (50)	50	14.4 (50)	88 **	49	13.6 (49)	83 **	50	12.3 (50)	75 **
42	50	16.3 (50)	50	14.4 (50)	88 **	49	13.7 (49)	84 **	49	12.0 (49)	74 **
46	50	16.4 (50)	50	14.7 (50)	90 **	49	13.7 (49)	84 **	49	12.6 (49)	77 **
50	50	16.5 (50)	50	15.4 (50)	93 **	49	14.1 (49)	85 **	49	12.6 (49)	76 **
54	50	17.5 (50)	50	15.8 (50)	90 **	49	14.9 (49)	85 **	49	13.1 (49)	75 **
58	50	16.8 (50)	50	15.3 (50)	91 **	49	14.5 (49)	86 **	49	12.8 (49)	76 **
62	50	17.5 (50)	50	15.9 (50)	91 **	48	15.4 (48)	88 **	49	13.5 (49)	77 **
66	50	17.0 (50)	50	15.7 (50)	92 **	48	15.0 (48)	88 **	47	13.3 (47)	78 **
70	49	18.6 (49)	49	16.6 (49)	89 **	47	15.7 (47)	84 **	46	13.7 (46)	74 **
74	49	18.3 (49)	48	16.4 (48)	90 **	47	15.5 (47)	85 **	46	13.7 (46)	75 **
78	49	18.3 (49)	47	16.4 (47)	90 **	46	15.3 (45)	84 **	45	13.7 (45)	75 **
82	48	17.8 (48)	47	16.4 (47)	92 **	46	15.3 (46)	86 **	45	13.6 (45)	76 **
86	48	18.0 (48)	47	16.2 (47)	90 **	44	15.1 (43)	84 **	44	13.7 (43)	76 **
90	47	18.4 (47)	47	16.7 (47)	91 **	42	16.4 (42)	89 **	43	14.1 (43)	77 **
94	45	18.9 (45)	47	17.0 (47)	90 **	41	16.5 (41)	87 **	41	14.1 (41)	75 **
98	41	19.7 (41)	47	17.2 (47)	87 **	41	17.4 (41)	88 **	39	15.3 (39)	78 **
102	40	20.4 (40)	44	17.7 (44)	87 **	39	17.6 (38)	86 **	36	15.8 (36)	77 **
104	40	20.4 (40)	44	17.5 (44)	86 **	37	17.7 (37)	87 **	35	15.8 (35)	77 **

< > : No.of effective animals, ( ) : No.of measured animals % : % of control group
Significant Difference, ** : p≦0.01, Test of Dunnett

TABLE 4 WATER CONSUMPTION CHANGES OF FEMALE RATS
IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE

Group	Control		320 ppm				800 ppm				2000 ppm			
Week on Study	<50> Survival No.	WC g	<50> Survival No.	WC g	%		<50> Survival No.	WC g	%		<50> Survival No.	WC g	%	
1	50	15.5 (50)	50	13.3 (50)	86	**	50	12.2 (50)	79	**	50	10.0 (50)	65	**
2	50	16.5 (49)	50	14.0 (50)	85	**	50	11.8 (50)	72	**	50	9.6 (50)	58	**
3	50	16.9 (47)	50	14.8 (50)	88	**	50	12.1 (49)	72	**	50	9.9 (50)	59	**
4	50	17.7 (49)	50	15.8 (48)	89	*	50	12.2 (50)	69	**	50	9.8 (50)	55	**
5	50	16.3 (48)	50	14.9 (50)	91	*	50	11.9 (50)	73	**	50	10.0 (50)	61	**
6	50	16.8 (48)	50	15.0 (49)	89	*	50	12.3 (49)	73	**	50	10.3 (50)	61	**
7	50	15.9 (46)	50	14.3 (50)	90	*	50	11.8 (50)	74	**	50	8.8 (50)	55	**
8	50	15.9 (46)	50	14.3 (48)	90	*	50	11.5 (50)	72	**	50	8.6 (50)	54	**
9	50	16.5 (48)	50	14.8 (46)	90		50	11.4 (47)	69	**	50	9.2 (50)	56	**
10	50	16.7 (48)	50	14.7 (47)	88	*	50	11.4 (48)	68	**	50	8.5 (50)	51	**
11	50	15.2 (48)	50	14.1 (48)	93		50	11.7 (49)	77	**	50	8.9 (50)	59	**
12	50	16.5 (48)	50	14.5 (48)	88	*	50	12.1 (48)	73	**	50	9.2 (50)	56	**
13	50	17.1 (49)	50	15.1 (46)	88		50	11.6 (49)	68	**	50	8.8 (50)	51	**
14	50	17.4 (50)	50	15.1 (46)	87	*	50	11.4 (48)	66	**	50	8.8 (50)	51	**
18	50	17.5 (46)	50	15.3 (47)	87		50	11.8 (48)	67	**	50	8.8 (50)	50	**
22	50	16.6 (47)	50	14.4 (44)	87	*	50	12.3 (49)	74	**	50	9.0 (50)	54	**
26	50	16.1 (49)	50	14.8 (49)	92		50	12.0 (50)	75	**	50	8.9 (50)	55	**
30	50	17.1 (49)	50	15.3 (47)	89		50	12.9 (50)	75	**	50	9.0 (50)	53	**
34	50	16.6 (50)	50	14.7 (49)	89		50	11.2 (49)	67	**	50	8.8 (50)	53	**
38	50	16.1 (49)	50	15.2 (50)	94		50	11.3 (49)	70	**	50	9.0 (48)	56	**
42	50	16.8 (50)	50	16.0 (50)	95		50	12.4 (50)	74	**	50	9.0 (50)	54	**
46	50	15.5 (49)	50	15.3 (50)	99		50	12.0 (50)	77	**	50	9.3 (50)	60	**
50	49	15.3 (49)	50	13.9 (50)	91		49	11.3 (49)	74	**	50	9.2 (50)	60	**
54	49	16.6 (49)	50	14.8 (50)	89		49	12.1 (49)	73	**	50	9.6 (50)	58	**
58	49	14.4 (49)	50	13.6 (50)	94		49	10.6 (49)	74	**	50	9.4 (50)	65	**
62	49	16.5 (49)	49	14.8 (49)	90		48	11.8 (48)	72	**	50	10.2 (50)	62	**
66	49	15.8 (47)	49	15.1 (49)	96		48	12.3 (48)	78	**	50	10.4 (50)	66	**
70	48	16.0 (48)	49	14.0 (49)	88	*	48	12.0 (48)	75	**	50	10.8 (50)	68	**
74	47	15.7 (47)	48	14.1 (48)	90		48	11.7 (48)	75	**	50	10.8 (50)	69	**
78	47	15.1 (47)	48	13.2 (47)	87	*	48	11.8 (48)	78	**	50	11.1 (50)	74	**
82	46	15.7 (46)	48	13.0 (48)	83	**	47	11.5 (47)	73	**	49	11.0 (49)	70	**
86	44	15.4 (43)	48	13.8 (48)	90	*	47	11.5 (47)	75	**	47	11.3 (47)	73	**
90	42	15.9 (40)	47	14.2 (47)	89	*	47	12.6 (47)	79	**	46	12.0 (46)	75	**
94	42	16.5 (42)	46	14.7 (46)	89	**	46	12.0 (46)	73	**	46	12.8 (46)	78	**
98	40	17.4 (40)	45	15.3 (45)	88	**	44	12.4 (44)	71	**	46	13.5 (46)	78	**
102	37	18.1 (36)	40	16.5 (40)	91	**	42	13.0 (42)	72	**	46	13.7 (46)	76	**
104	37	17.4 (37)	40	14.5 (40)	83	**	42	12.7 (42)	73	**	42	13.2 (42)	76	**

< > : No.of effective animals, ( ) : No.of measured animals % : % of control group
Significant Difference, * : $p \leqq 0.05$, ** : $p \leqq 0.01$, Test of Dunnett

TABLE 5 FOOD CONSUMPTION CHANGES OF MALE RATS
IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE

Group	Control		320 ppm			800 ppm			2000 ppm		
	<50>		<50>			<49>			<50>		
Week on Study	Survival No.	FC g	Survival No.	FC g	%	Survival No.	FC g	%	Survival No.	FC g	%
1	50	14.3 (50)	50	13.6 (50)	100 *	49	13.7 (49)	96 *	50	11.9 (50)	83 **
2	50	15.4 (50)	50	15.1 (50)	98	49	14.6 (49)	95 **	50	13.4 (50)	87 **
3	50	15.9 (49)	50	15.5 (48)	97 *	49	15.2 (49)	96 **	50	14.2 (50)	89 **
4	50	15.8 (50)	50	15.5 (50)	98	49	15.2 (49)	96 **	50	14.3 (50)	91 **
5	50	15.6 (50)	50	15.2 (50)	97 *	49	14.9 (49)	96 **	50	14.3 (50)	92 **
6	50	15.1 (50)	50	14.7 (50)	97 *	49	14.4 (49)	95 **	50	13.8 (50)	91 **
7	50	15.5 (50)	50	15.0 (50)	97 *	49	14.7 (49)	95 **	50	14.0 (50)	90 **
8	50	14.9 (50)	50	14.4 (50)	97 **	49	14.1 (49)	95 **	50	13.5 (50)	91 **
9	50	14.9 (50)	50	14.4 (50)	97 *	49	14.1 (49)	95 **	50	13.6 (50)	91 **
10	50	14.7 (49)	50	14.2 (50)	97 *	49	13.9 (49)	95 **	50	13.1 (50)	89 **
11	50	14.5 (50)	50	14.1 (50)	97 *	49	13.7 (49)	94 **	50	13.3 (50)	92 **
12	50	14.2 (50)	50	13.8 (50)	97 *	49	13.5 (49)	95 **	50	13.3 (50)	94 **
13	50	14.4 (50)	50	13.8 (50)	96 **	49	13.8 (49)	96 **	50	13.3 (50)	92 **
14	50	14.0 (50)	50	13.4 (50)	96 **	49	13.5 (49)	96 **	50	12.9 (49)	92 **
18	50	14.6 (50)	50	14.2 (50)	97 *	49	13.9 (49)	95 **	50	13.5 (50)	92 **
22	50	14.6 (50)	50	14.0 (50)	96 **	49	14.0 (49)	96 **	50	13.6 (50)	93 **
26	50	15.4 (50)	50	14.7 (50)	95 **	49	14.5 (49)	94 **	50	14.2 (50)	92 **
30	50	14.9 (50)	50	14.4 (50)	97 **	49	14.4 (49)	97 *	50	14.1 (50)	95 **
34	50	15.4 (50)	50	14.7 (50)	95 **	49	14.7 (49)	95 **	50	14.7 (50)	95 **
38	50	15.6 (50)	50	14.6 (50)	94 **	49	14.8 (49)	95 **	50	14.9 (50)	96 **
42	50	15.6 (50)	50	14.7 (50)	94 **	49	15.2 (49)	97	49	14.9 (49)	96 *
46	50	15.4 (50)	50	14.8 (50)	96 **	49	15.0 (49)	97	49	15.1 (49)	98
50	50	15.5 (50)	50	14.9 (50)	96	49	15.1 (49)	97	49	15.2 (49)	98
54	50	15.9 (50)	50	15.1 (50)	95 **	49	15.3 (49)	96	49	15.2 (49)	96 **
58	50	15.8 (50)	50	15.1 (50)	96 **	49	15.5 (49)	98	49	15.0 (49)	95 **
62	50	15.8 (50)	50	15.2 (50)	96 **	48	15.4 (48)	97	49	14.8 (49)	94 **
66	50	15.5 (50)	50	15.2 (50)	98	48	15.4 (48)	99	47	14.9 (47)	96 **
70	49	16.2 (49)	49	15.5 (49)	96 **	47	15.8 (47)	98	46	15.3 (46)	94 **
74	49	16.2 (49)	48	15.7 (48)	97	47	15.6 (47)	96 *	46	15.2 (46)	94 **
78	49	15.9 (49)	47	15.2 (47)	96 **	46	15.5 (46)	97	45	15.0 (45)	94 **
82	48	15.9 (48)	47	15.4 (47)	97	46	15.5 (46)	97	45	15.1 (45)	95 **
86	48	15.8 (48)	47	15.4 (47)	97	44	15.6 (44)	99	44	15.0 (44)	95 **
90	47	15.7 (47)	47	15.4 (47)	98	42	15.2 (42)	97	43	14.8 (43)	94 **
94	45	15.9 (45)	47	15.7 (47)	99	41	15.7 (41)	99	41	15.0 (41)	94 **
98	41	16.2 (41)	47	15.4 (47)	95	41	15.1 (41)	93 *	39	14.5 (39)	90 **
102	40	16.4 (40)	44	15.6 (44)	95	39	15.2 (39)	93 *	36	14.9 (36)	91 **
104	40	15.7 (40)	44	15.2 (44)	97	37	15.1 (37)	96	35	14.7 (35)	94

< > : No.of effective animals, ( ) : No.of measured animals % : % of control group
Significant Difference, * : $p \leqq 0.05$, ** : $p \leqq 0.01$, Test of Dunnett

TABLE 6 FOOD CONSUMPTION CHANGES OF FEMALE RATS
IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE

Group	Control		320 ppm			800 ppm			2000 ppm		
	<50>		<50>			<50>			<50>		
Week on Study	Survival No.	FC g	Survival No.	FC g	%	Survival No.	FC g	%	Survival No.	FC g	%
1	50	10.6 (50)	50	10.4 (50)	98	50	10.1 (50)	95 **	50	9.2 (50)	87 **
2	50	10.5 (50)	50	10.6 (50)	101	50	10.1 (50)	96 **	50	9.7 (50)	92 **
3	50	10.8 (50)	50	10.6 (50)	98	50	10.5 (50)	97	50	10.0 (50)	93 **
4	50	10.9 (50)	50	10.7 (50)	98	50	10.4 (50)	95 **	50	10.1 (50)	93 **
5	50	10.7 (50)	50	10.7 (50)	100	50	10.3 (50)	96 *	50	9.9 (50)	93 **
6	50	10.3 (50)	50	10.3 (50)	100	50	10.0 (50)	97	50	9.4 (50)	91 **
7	50	10.2 (50)	50	10.2 (50)	100	50	10.0 (50)	98	50	9.3 (50)	91 **
8	50	9.9 (50)	50	9.8 (50)	99	50	9.6 (50)	97	50	9.1 (50)	92 **
9	50	10.1 (50)	50	10.0 (50)	99	50	9.5 (50)	94 **	50	9.0 (50)	89 **
10	50	9.8 (50)	50	9.8 (50)	100	50	9.5 (50)	97	50	8.7 (50)	89 **
11	50	9.8 (50)	50	9.8 (50)	100	50	9.5 (50)	97	50	8.7 (50)	89 **
12	50	9.8 (50)	50	9.8 (50)	100	50	9.6 (50)	98	50	8.8 (50)	90 **
13	50	9.7 (50)	50	10.0 (50)	103	50	9.6 (50)	99	50	8.8 (50)	91 **
14	50	9.7 (50)	50	9.9 (50)	102	50	9.6 (50)	99	50	9.0 (50)	93 **
18	50	10.3 (49)	50	10.2 (50)	99	50	9.9 (50)	96 *	50	9.1 (50)	88 **
22	50	10.1 (50)	50	10.3 (50)	102	50	10.0 (50)	99	50	9.3 (50)	92 **
26	50	10.2 (50)	50	10.3 (50)	101	50	10.1 (50)	99	50	9.5 (50)	93 **
30	50	10.6 (50)	50	10.8 (50)	102	50	10.4 (50)	98	50	9.6 (50)	91 **
34	50	10.5 (49)	50	10.7 (50)	102	50	10.2 (50)	97	50	9.6 (50)	91 **
38	50	10.6 (50)	50	10.9 (50)	103	50	10.2 (50)	96	50	9.8 (48)	92 **
42	50	10.7 (49)	50	10.9 (50)	102	50	10.5 (50)	98	50	10.0 (50)	93 **
46	50	10.8 (50)	50	11.0 (50)	102	50	10.8 (50)	100	50	10.0 (50)	93 **
50	49	10.7 (49)	50	10.9 (50)	102	49	10.4 (49)	97	50	9.9 (50)	93 **
54	49	11.2 (49)	50	11.4 (50)	102	49	11.0 (49)	98	50	10.3 (50)	92 **
58	49	10.9 (49)	50	11.0 (50)	101	49	10.6 (49)	97	50	9.9 (50)	91 **
62	49	11.3 (49)	49	11.4 (49)	101	48	11.0 (48)	97	50	10.3 (50)	91 **
66	49	11.5 (49)	49	11.8 (49)	103	48	11.0 (48)	96	50	10.4 (50)	90 **
70	48	11.4 (48)	49	11.4 (49)	100	48	11.1 (48)	97	50	10.4 (50)	91 **
74	47	11.7 (47)	48	11.9 (48)	102	48	11.1 (48)	95 *	50	10.3 (50)	88 **
78	47	11.9 (47)	48	11.7 (48)	98	48	11.1 (48)	93 **	50	10.1 (50)	85 **
82	46	11.6 (46)	48	11.9 (48)	103	47	11.4 (47)	98	49	10.6 (49)	91 **
86	44	11.7 (44)	48	11.8 (48)	101	47	11.2 (47)	96 **	47	10.8 (47)	92 **
90	42	11.8 (42)	47	11.7 (47)	99	47	11.1 (47)	94 **	46	10.4 (46)	88 **
94	42	11.8 (42)	46	11.9 (46)	101	46	11.4 (46)	97	46	10.7 (46)	91 **
98	40	12.0 (40)	45	11.6 (45)	97	44	11.0 (44)	92 **	46	10.9 (46)	91 **
102	37	12.1 (37)	40	12.0 (40)	99	42	11.3 (42)	93 *	46	10.6 (46)	88 **
104	37	11.6 (37)	40	11.5 (40)	99	42	11.0 (42)	95	42	10.6 (42)	91 *

< > : No.of effective animals, ( ) : No.of measured animals % : % of control group
Significant Difference, * : $p \leqq 0.05$, ** : $p \leqq 0.01$, Test of Dunnett

TABLE 7 INCIDENCE AND TIME OF MASS OCCURRENCE IN CLINICAL OBSERVATION OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE

Time of mass occurrence (week)	0-13	14-26	27-39	40-52	53-65	66-78	79-91	92-104	0-104
External mass									
Control	0/50	0/50	0/50	0/50	2/50	1/50	6/49	16/47	17/50 (6/10)
320 ppm	0/50	0/50	0/50	0/50	1/50	1/50	6/47	14/47	14/50 (1/ 6)
800 ppm	0/49	0/49	0/49	1/49	2/49	2/48	6/47	10/42	13/49 (5/12)
2000 ppm	0/50	0/50	0/50	2/50	2/49	1/47	3/45	7/42	9/50 (2/15)
Internal mass									
Control	0/50	0/50	0/50	0/50	0/50	0/50	0/49	2/47	2/50 (1/10)
320 ppm	0/50	0/50	0/50	0/50	0/50	0/50	0/47	1/47	1/50 (0/ 6)
800 ppm	0/49	0/49	0/49	0/49	0/49	0/48	0/47	0/42	0/49 (0/12)
2000 ppm	0/50	0/50	0/50	0/50	0/49	0/47	0/45	0/42	0/50 (0/15)

No. of animals with mass / No. of survival animals at first week on each period.
(No. of dead and moribund animals with mass / No. of dead and moribund animals)

TABLE 8 INCIDENCE AND TIME OF MASS OCCURRENCE IN CLINICAL OBSERVATION OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE

Time of mass occurrence (week)	0-13	14-26	27-39	40-52	53-65	66-78	79-91	92-104	0-104
External mass									
Control	0/50	0/50	0/50	1/50	1/49	2/49	3/46	4/42	6/50 (3/13)
320 ppm	0/50	0/50	0/50	0/50	0/50	1/49	2/48	3/47	3/50 (1/10)
800 ppm	0/50	0/50	0/50	0/50	0/49	2/48	6/48	9/46	10/50 (1/ 8)
2000 ppm	0/50	0/50	0/50	0/50	0/50	1/50	4/50	7/46	7/50 (1/ 8)
Internal mass									
Control	0/50	0/50	0/50	0/50	0/49	1/49	1/46	1/42	3/50 (3/13)
320 ppm	0/50	0/50	0/50	0/50	0/50	0/49	0/48	1/47	1/50 (1/10)
800 ppm	0/50	0/50	0/50	0/50	0/49	0/48	0/48	0/46	0/50 (0/ 8)
2000 ppm	0/50	0/50	0/50	0/50	0/50	0/50	0/50	0/46	0/50 (0/ 8)

No. of animals with mass / No. of survival animals at first week on each period.
(No. of dead and moribund animals with mass / No. of dead and moribund animals)

TABLE 9 HEMATOLOGY OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE (SELECTED)

Group	Control	320 ppm	800 ppm	2000 ppm
No. of animals examined	40	44	37	34
Red blood cell ($10^6/\mu$ L)	8.00 ± 1.60	8.50 ± 1.26	8.36 ± 1.38	8.47 ± 1.60
Hemoglobin (g/dL)	13.5 ± 2.8	14.1 ± 1.8	13.7 ± 2.1	13.4 ± 2.4
Hematocrit (%)	41.2 ± 7.2	42.9 ± 4.7	42.0 ± 5.3	41.8 ± 6.6
MCV (fL)	52.2 ± 6.5	51.0 ± 5.7 *	50.9 ± 5.2 **	49.9 ± 3.9 **
MCH (pg)	17.0 ± 1.6	16.8 ± 1.6	16.5 ± 1.1 *	15.9 ± 1.0 **
MCHC (g/dL)	32.6 ± 1.7	32.9 ± 1.1	32.5 ± 1.6	32.0 ± 1.2 **
Platelet ($10^3/\mu$ L)	891 ± 277	861 ± 228	932 ± 148	856 ± 163
WBC ($10^3/\mu$ L)	8.19 ± 11.57	7.02 ± 2.63	6.96 ± 2.68	11.15 ± 23.07

Data represent means ± S.D.
Significant difference, * : $p \leqq 0.05$, ** : $p \leqq 0.01$, Test of Dunnett

TABLE 10 HEMATOLOGY OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE (SELECTED)

Group	Control	320 ppm	800 ppm	2000 ppm
No. of animals examined	37	37	41	42
Red blood cell ($10^6/\mu$ L)	8.11 ± 0.74	8.00 ± 0.85	7.14 ± 1.92 **	7.09 ± 1.05 **
Hemoglobin (g/dL)	14.8 ± 1.3	14.6 ± 1.3	13.1 ± 3.3 **	13.1 ± 1.5 **
Hematocrit (%)	43.6 ± 2.9	43.1 ± 3.3	39.2 ± 8.5 **	39.5 ± 4.0 **
MCV (fL)	53.9 ± 2.4	54.2 ± 3.2	57.3 ± 10.6	56.4 ± 4.6 **
MCH (pg)	18.3 ± 0.5	18.3 ± 0.8	18.7 ± 2.5	18.7 ± 1.2
MCHC (g/dL)	34.0 ± 0.9	33.9 ± 0.7	33.0 ± 2.3 **	33.2 ± 0.8 **
Platelet ($10^3/\mu$ L)	618 ± 103	645 ± 93	641 ± 169 *	718 ± 77 **
WBC ($10^3/\mu$ L)	13.51 ± 59.34	4.04 ± 5.69	5.03 ± 11.32	4.79 ± 13.31

Data represent means ± S.D.
Significant difference, * : $p \leqq 0.05$, ** : $p \leqq 0.01$, Test of Dunnett

TABLE 11 BIOCHEMISTRY OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE

Group	Control	320 ppm		800 ppm		2000 ppm	
No. of animals examined	40	44		37		34	
Total protein (g/dL)	6.5 ± 0.5	6.7 ± 0.4	**	6.6 ± 0.4		6.5 ± 0.3	
Albumin (g/dL)	3.3 ± 0.4	3.4 ± 0.3		3.3 ± 0.3		3.4 ± 0.2	
A/G ratio	1.1 ± 0.1	1.1 ± 0.2		1.0 ± 0.1		1.1 ± 0.1	
T-Bilirubin (mg/dL)	0.22 ± 0.44	0.66 ± 3.18	*	0.22 ± 0.15	**	0.33 ± 0.35	**
Glucose (mg/dL)	153 ± 18	155 ± 19		142 ± 24	*	146 ± 17	
T-Cholesterol (mg/dL)	164 ± 42	218 ± 50	**	252 ± 72	**	285 ± 43	**
Triglyceride (mg/dL)	73 ± 51	108 ± 83	*	115 ± 82	**	195 ± 148	**
Phospholipid (mg/dL)	232 ± 71	301 ± 70	**	341 ± 87	**	409 ± 67	**
GOT (IU/L)	95 ± 62	106 ± 87		104 ± 24	*	171 ± 69	**
GPT (IU/L)	41 ± 16	52 ± 50		47 ± 15		66 ± 19	**
LDH (IU/L)	208 ± 58	196 ± 39		177 ± 42	**	193 ± 134	**
ALP (IU/L)	231 ± 115	283 ± 95	*	335 ± 116	**	484 ± 171	**
γ -GTP (IU/L)	12 ± 8	24 ± 11	**	46 ± 29	**	111 ± 33	**
CPK (IU/L)	106 ± 73	94 ± 14		94 ± 21		107 ± 71	
Urea nitrogen (mg/L)	19.2 ± 6.9	18.7 ± 3.1		21.4 ± 4.0	**	23.0 ± 4.0	**
Creatinine (mg/dL)	0.5 ± 0.1	0.5 ± 0.1		0.6 ± 0.1		0.5 ± 0.1	
Sodium (mEq/L)	142 ± 2	141 ± 2		141 ± 1	**	140 ± 1	**
Potassium (mEq/L)	3.7 ± 0.4	3.8 ± 0.5		3.8 ± 0.4		4.0 ± 0.4	*
Chloride (mEq/L)	107 ± 2	105 ± 2	**	105 ± 2	**	105 ± 2	*
Calcium (mg/dL)	10.2 ± 0.4	10.2 ± 0.9		10.3 ± 0.3	*	10.4 ± 0.3	**
Inorganic phosphorus (mg/dL)	4.3 ± 0.7	4.3 ± 1.3		4.4 ± 0.5		4.2 ± 0.5	

Data represent means ± S.D.
Significant difference, * : $p \leqq 0.05$, ** : $p \leqq 0.01$, Test of Dunnett

TABLE 12 BIOCHEMISTRY OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE

Group	Control	320 ppm	800 ppm	2000 ppm
No. of animals examined	37	37	41	42
Total protein (g/dL)	6.9 ± 0.5	6.8 ± 0.4	6.7 ± 0.5	6.3 ± 0.4 **
Albumin (g/dL)	3.9 ± 0.3	3.9 ± 0.2	3.9 ± 0.3	3.8 ± 0.2 **
A/G ratio	1.3 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.5 ± 0.2 **
T-Bilirubin (mg/dL)	0.17 ± 0.11	0.16 ± 0.2	0.47 ± 1.42	0.17 ± 0.11
Glucose (mg/dL)	145 ± 13	150 ± 14	140 ± 22	144 ± 16
T-Cholesterol (mg/dL)	130 ± 26	140 ± 29	154 ± 36 **	155 ± 23 **
Triglyceride (mg/dL)	64 ± 54	62 ± 44	92 ± 98	75 ± 116
Phospholipid (mg/dL)	231 ± 48	235 ± 46	263 ± 63 *	261 ± 43 *
GOT (IU/L)	159 ± 111	110 ± 39	172 ± 199	120 ± 79 *
GPT (IU/L)	63 ± 35	45 ± 18 *	52 ± 41 *	42 ± 17 **
LDH (IU/L)	330 ± 225	263 ± 78	379 ± 378	261 ± 104
ALP (IU/L)	125 ± 75	115 ± 34	144 ± 101	154 ± 58 **
γ -GTP (IU/L)	5 ± 4	5 ± 2	8 ± 6 *	12 ± 8 **
CPK (IU/L)	150 ± 290	96 ± 21	158 ± 320	110 ± 47
Urea nitrogen (mg/L)	17.3 ± 1.7	17.4 ± 5.8	17.5 ± 3.1	20.3 ± 3.2 **
Creatinine (mg/dL)	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Sodium (mEq/L)	140 ± 1	140 ± 2	140 ± 2	140 ± 2
Potassium (mEq/L)	3.8 ± 0.5	3.7 ± 0.4	3.9 ± 0.4	4.0 ± 0.5 *
Chloride (mEq/L)	105 ± 2	105 ± 2	105 ± 3	106 ± 2
Calcium (mg/dL)	10.2 ± 0.3	10.1 ± 0.4	10.2 ± 0.4	10.1 ± 0.3
Inorganic phosphorus (mg/dL)	4.0 ± 0.8	3.9 ± 0.8	4.2 ± 0.6	4.4 ± 0.5 *

Data represent means ± S.D.

Significant difference, * : $p \leqq 0.05$, ** : $p \leqq 0.01$, Test of Dunnett

TABLE 13 URINALYSIS OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE (SELECTED)

Group		Control	320 ppm	800 ppm	2000 ppm
No. of animals examined		40	44	38	35
pH	6.0	1	0 **	2	2
	6.5	3	0	3	0
	7.0	12	5	7	6
	7.5	20	22	14	16
	8.0	3	16	12	11
	8.5	1	1	0	0
	(Grade)				
Protein	−	0	0	0 **	0
	±	0	0	0	0
	+	0	0	0	0
	2+	2	2	0	0
	3+	27	21	12	18
	4+	11	21	26	17
Occult blood	−	39	42	37	27 *
	±	1	0	0	2
	+	0	0	0	0
	2+	0	1	1	3
	3+	0	1	0	3

Significant difference, * : $p \leqq 0.05$, ** : $p \leqq 0.01$ Chi square test

TABLE 14 URINALYSIS OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE (SELECTED)

Group		Control	320 ppm	800 ppm	2000 ppm
No. of animals examined		37	40	42	45
pH	6.0	0	0	0	1
	6.5	3	2	5	12
	7.0	11	11	12	8
	7.5	12	13	13	10
	8.0	9	13	10	13
	8.5	2	1	2	1
	(Grade)				
Protein	−	0	0 **	0 **	0 **
	±	2	0	0	0
	+	10	1	0	1
	2+	16	16	9	6
	3+	6	11	21	27
	4+	3	12	12	11
Occult blood	−	35	37	30 *	9 **
	±	1	1	4	2
	+	0	0	0	0
	2+	1	1	0	0
	3+	0	1	8	34

Significant difference, * : $p \leqq 0.05$, ** : $p \leqq 0.01$ Chi square test

TABLE 15 ORGAN WEIGHTS OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE

Group		Control	320 ppm	800 ppm	2000 ppm
No. of animals examined		<40>	<44>	<37>	<35>
Adrenal	(g)	0.070 ± 0.015	0.081 ± 0.066	0.083 ± 0.121	0.090 ± 0.170 *
	(%)	0.018 ± 0.003	0.023 ± 0.019	0.027 ± 0.048	0.030 ± 0.054 **
Testis	(g)	2.665 ± 1.057	2.599 ± 1.004	2.597 ± 0.877	2.787 ± 0.748
	(%)	0.706 ± 0.295	0.726 ± 0.275	0.782 ± 0.231	0.937 ± 0.213 **
Heart	(g)	1.178 ± 0.118	1.127 ± 0.105	1.103 ± 0.091 **	1.023 ± 0.089 **
	(%)	0.309 ± 0.035	0.316 ± 0.032	0.338 ± 0.051 **	0.350 ± 0.049 **
Lung	(g)	1.442 ± 0.373	1.370 ± 0.155	1.347 ± 0.168	1.377 ± 0.400 **
	(%)	0.380 ± 0.125	0.385 ± 0.057	0.415 ± 0.094 **	0.486 ± 0.261 **
Kidney	(g)	2.515 ± 0.197	2.666 ± 0.276 **	2.764 ± 0.210 **	2.791 ± 0.217 **
	(%)	0.660 ± 0.071	0.750 ± 0.117 **	0.846 ± 0.108 **	0.956 ± 0.150 **
Spleen	(g)	1.570 ± 3.764	1.566 ± 3.674	1.035 ± 0.762	1.380 ± 2.353
	(%)	0.442 ± 1.186	0.456 ± 1.151	0.314 ± 0.232 *	0.469 ± 0.805 **
Liver	(g)	10.574 ± 2.260	11.038 ± 1.625	11.425 ± 1.535 **	11.545 ± 2.291 **
	(%)	2.770 ± 0.669	3.091 ± 0.485 **	3.473 ± 0.403 **	3.922 ± 0.757 **
Brain	(g)	2.017 ± 0.063	2.020 ± 0.050	2.023 ± 0.053	2.019 ± 0.054
	(%)	0.530 ± 0.048	0.568 ± 0.051 *	0.619 ± 0.065 **	0.693 ± 0.105 **

Data represent means ± S.D.

Significant difference, * : $p \leqq 0.05$, ** : $p \leqq 0.01$, Test of Dunnett

TABLE 16 ORGAN WEIGHTS OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE

Group		Control	320 ppm	800 ppm	2000 ppm
No. of animals examined		<37>	<40>	<42>	<42>
Adrenal	(g)	0.067 ± 0.011	0.067 ± 0.009	0.065 ± 0.010	0.058 ± 0.013 **
	(%)	0.027 ± 0.004	0.027 ± 0.004	0.029 ± 0.005	0.030 ± 0.010
Ovaries	(g)	0.129 ± 0.019	0.135 ± 0.033	0.142 ± 0.102	0.122 ± 0.027
	(%)	0.052 ± 0.009	0.056 ± 0.016	0.063 ± 0.040	0.063 ± 0.015 **
Heart	(g)	0.842 ± 0.100	0.837 ± 0.077	0.818 ± 0.092	0.745 ± 0.050 **
	(%)	0.340 ± 0.040	0.344 ± 0.027	0.370 ± 0.074 *	0.385 ± 0.035 **
Lung	(g)	1.066 ± 0.186	1.046 ± 0.191	1.030 ± 0.157	0.949 ± 0.115 **
	(%)	0.431 ± 0.079	0.434 ± 0.109	0.466 ± 0.104	0.492 ± 0.077 *
Kidney	(g)	1.666 ± 0.103	1.897 ± 0.300 **	1.902 ± 0.230 **	1.786 ± 0.128 **
	(%)	0.672 ± 0.046	0.784 ± 0.157 **	0.854 ± 0.116 **	0.924 ± 0.086 **
Spleen	(g)	0.854 ± 1.247	0.743 ± 0.869	1.313 ± 2.631	0.599 ± 0.406
	(%)	0.346 ± 0.488	0.308 ± 0.374	0.619 ± 1.269	0.315 ± 0.230 *
Liver	(g)	6.493 ± 1.190	6.592 ± 1.002	6.702 ± 1.160	6.347 ± 0.981
	(%)	2.606 ± 0.388	2.697 ± 0.283	2.996 ± 0.503 **	3.277 ± 0.512 **
Brain	(g)	1.861 ± 0.047	1.848 ± 0.042	1.835 ± 0.046 *	1.835 ± 0.039 *
	(%)	0.754 ± 0.078	0.766 ± 0.097	0.830 ± 0.118 **	0.953 ± 0.104 **

Data represent means ± S.D.

Significant difference, * : $p \leqq 0.05$, ** : $p \leqq 0.01$, Test of Dunnett

TABLE 17 NEOPLASTIC LESIONS OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE (SELECTED)

Group No. of animals examined	Control <50>	320 ppm <50>	800 ppm <49>	2000 ppm <50>		
Skin						
Keratoacanthoma	3 (6%) [a)]	2 (4%)	1 (2%)	2 (4%)		
Squamous cell papilloma	1 (2%)	2 (4%)	1 (2%)	1 (2%)		
Subcutis						
Fibroma	7 (14%)	2 (4%)	4 (8%)	3 (6%)		
Lung						
Bronchiolar-alveolar adenoma	1 (2%)	3 (6%)	2 (4%)	1 (2%)		
Spleen						
Mononuclear cell leukemia	6 (12%)	4 (8%)	6 (12%)	5 (10%)		
Stomach						
Squamous cell papilloma	1 (2%)	1 (2%)	0 (0%)	0 (0%)		
Squamous cell carcinoma	0 (0%)	0 (0%)	0 (0%)	1 (2%)		
Liver						
Hepatocellular adenoma	1 (2%)	4 (8%)	4 (8%)	10 (20%) **	⬆⬆	⇧⇧
Pituitary						
Adenoma	21 (42%)	22 (44%)	14 (29%)	13 (26%)		⇩
Thyroid						
C-cell adenoma	7 (14%)	10 (20%)	6 (12%)	11 (22%)		
Follicular adenoma	0 (0%)	0 (0%)	1 (2%)	2 (4%)		
Follicular adenocarcinoma	0 (0%)	1 (2%)	0 (0%)	1 (2%)		
Follicular adenoma / adenocarcinoma	0 (0%)	1 (2%)	1 (2%)	3 (6%)	⬆	
Testis						
Interstitial cell tumor	28 (56%)	31 (62%)	35 (71%)	35 (70%)	⬆	

[a)] : No. of animals with bearing tumor (incidence ; %)

** : Statistically differenct from control group at $p \leqq 0.01$ by Fisher exact test

⬆ and ⬆⬆ : The trend of treated groups statistically different from control group at $p \leqq 0.05$ and $p \leqq 0.01$ by Peto test, respectively.

⇩ and ⇧⇧ : The trend of treated groups statistically different from control group at $p \leqq 0.05$ and $p \leqq 0.01$ by Cochran-Armitage test, respectively.

TABLE 18 NEOPLASTIC LESIONS OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE (SELECTED)

Group No. of animals examined	Control <50>	320 ppm <50>	800 ppm <50>	2000 ppm <50>		
Spleen						
Mononuclear cell leukemia	7 (14%) [a)]	6 (12%)	6 (12%)	8 (16%)		
Stomach						
Squamous cell papilloma	0 (0%)	1 (2%)	0 (0%)	0 (0%)		
Liver						
Hepatocellular adenoma	0 (0%)	1 (2%)	0 (0%)	3 (6%)	⬆	⇧
Pituitary						
Adenoma	19 (38%)	15 (30%)	16 (32%)	15 (30%)		
Thyroid						
C-cell adenoma	6 (12%)	4 (8%)	5 (10%)	8 (16%)		
Follicular adenoma	1 (2%)	2 (4%)	0 (0%)	0 (0%)		
Uterus						
Endometrial stromal polyp	7 (14%)	8 (16%)	11 (22%)	7 (14%)		
Endometrial stromal sarcoma	4 (8%)	1 (2%)	0 (0%)	0 (0%)		⇩
Mammary gland						
Fibroadenoma	3 (6%)	3 (6%)	6 (12%)	7 (14%)		

a) : No. of animals with bearing tumor (incidence ; %)

⬆ : The trend of treated groups statistically different from control group at $p \leqq 0.05$ by Peto test

⇧ ⇩ : The trend of treated groups statistically different from control group at $p \leqq 0.05$ by Cochran-Armitage test

TABLE 19 NON-NEOPLASTIC LESIONS OF MALE AND FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE (SELECTED)

		Male				Female			
Group		Control	320 ppm	800 ppm	2000 ppm	Control	320 ppm	800 ppm	2000 ppm
No. of animals examined		<50>	<50>	<49>	<50>	<50>	<50>	<50>	<50>
Liver	Grade								
Basophilic cell focus	+	9	8	15	16	3	3	4	6
	2+	0	0	7	7	1	1	0	0
	3+	0	0	0	0	0	0	1	0
Kidney									
Chronic nephropathy	+	10	3	4	3	15	18	16	31
	2+	25	13	15	7	2	6	13	6
	3+	13	28	26	36	2	7	6	0
	4+	0	2	3	2	0	0	0	0
Papillary necrosis	+	1	12	14	20	0	7	23	7
	2+	0	0	1	4	0	0	0	19
	3+	0	0	0	0	0	0	0	2
Mineralization : papilla	+	3	2	11	19	3	3	6	22
	2+	0	0	0	0	0	0	1	1
Urothelial hyperplasia : pelvis	+	16	18	25	25	9	9	9	27
	2+	0	0	0	1	0	0	0	0
Stomach (Forestomach)									
Squamous cell hyperplasia	+	3	0	0	5	1	3	3	4
Basal cell hyperplasia	+	0	0	0	0	0	0	0	3

Grade + : Slight 2+ : Moderate 3+ : Marked 4+ : Severe

TABLE 20 CAUSE OF DEATH OF MALE AND FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE

Group	Male				Female			
	Control	320 ppm	800 ppm	2000 ppm	Control	320 ppm	800 ppm	2000 ppm
No. of dead/moribund animals	<10>	<6>	<12>	<15>	<13>	<10>	<8>	<8>
Chronic nephropathy	0	0	0	3	0	0	0	0
Urinary retention	0	0	1	0	0	0	0	0
Thrombosis	0	0	0	1	0	0	0	0
Deglutition disorder	0	0	0	0	0	0	2	0
Tumor death :								
leukemia	2	2	5	3	1	3	2	4
skin/appendage	1	0	0	1	0	0	0	0
subcutis	2	0	0	0	1	0	0	0
tongue	0	0	0	1	0	0	0	0
salivary gland	0	0	2	0	0	0	0	0
pancreas	0	0	0	0	0	0	1	0
pituitary	1	2	1	4	4	5	1	2
adrenal	1	0	1	0	0	0	0	0
uterus	-	-	-	-	5	0	0	0
mammary gland	0	0	0	0	0	0	0	1
clitoral gland	-	-	-	-	1	0	0	0
brain	0	0	0	0	0	0	1	0
spinal cord	0	0	0	0	0	1	0	0
Zymbal gland	0	0	0	1	0	0	0	0
bone	1	0	1	0	0	0	1	0
vertebrae	0	0	1	0	0	0	0	0
peritoneum	1	0	0	0	0	0	0	0
No microscopical confirmation	1	2	0	1	1	1	0	1

TABLE 21 HISTORICAL CONTROL DATA OF SELECTED NEOPLASTIC LESIONS IN JAPAN BIOASSAY RESEARCH CENTER : F344/DuCrj MALE RATS

Organs / Tumors	No. of animals examined	No. of animals with bearing tumor	Incidence (%)	Min. - Max. (%)
Skin	<1248>			
Keratoacanthoma		39	3.1	0 - 8
Squamous cell papilloma		14	1.1	0 - 4
Subcutis	<1249>			
Fibroma		90	7.2	2 - 14
Lung	<1249>			
Bronchiolar-alveolar adenoma		37	3.0	0 - 8
Spleen	<1249>			
Mononuclear cell leukemia		152	12.2	4 - 22
Stomach	<1248>			
Squamous cell papillpma		2	0.2	0 - 2
Squamous cell carcinoma		0	0	0
Liver	<1249>			
Hepatocellular adenoma		20	1.6	0 - 6
Pituitary	<1244>			
Adenoma		439	35.3	18 - 66
Thyroid	<1243>			
C-cell adenoma		155	12.5	4 - 26
Follicular adenoma		12	1.0	0 - 4
Follicular adenocarcinoma		27	2.2	0 - 8
Testis	<1249>			
Interstitial cell tumor		1099	88.0	74 - 98

25 carcinogenicity studies examined in Japan Bioassay Research Center were used.
Study No. : 0043, 0059, 0061, 0063, 0065, 0067, 0095, 0104, 0115, 0130, 0141, 0158, 0162, 0189, 0205, 0210, 0224, 0242, 0267, 0269
0284, 0288, 0294, 0296, 0318

TABLE 22 HISTORICAL CONTROL DATA OF SELECTED NEOPLASTIC LESIONS IN JAPAN BIOASSAY RESEARCH CENTER : F344/DuCrj FEMALE RATS

Organs Tumors	No. of animals examined	No. of animals with bearing tumor	Incidence (%)	Min. - Max. (%)
Spleen	<1197>			
Mononuclear cell leukemia		160	13.4	2 - 26
Stomach	<1197>			
Squamous cell papilloma		2	0.2	0 - 2
Liver	<1197>			
Hepatocellular adenoma		16	1.3	0 - 6
Pituitary	<1195>			
Adenoma		493	41.3	16 - 71
Thyroid	<1191>			
C-cell adenoma		115	9.7	0 - 16
Follicular adenoma		12	1.0	0 - 4
Uterus	<1197>			
Endometrial stromal polyp		172	14.4	2 - 28
Endometrial stromal sarcoma		7	0.6	0 - 2
Mammary gland	<1197>			
Fibroadenoma		130	10.9	0 - 20

24 carcinogenicity studies examined in Japan Bioassay Research Center were used.
Study No. : 0043, 0059, 0061, 0063, 0065, 0067, 0095, 0104, 0115, 0130, 0141, 0158, 0162, 0189, 0205, 0210, 0224, 0242, 0267, 0269
0284, 0296, 0303, 0318

FIGURES

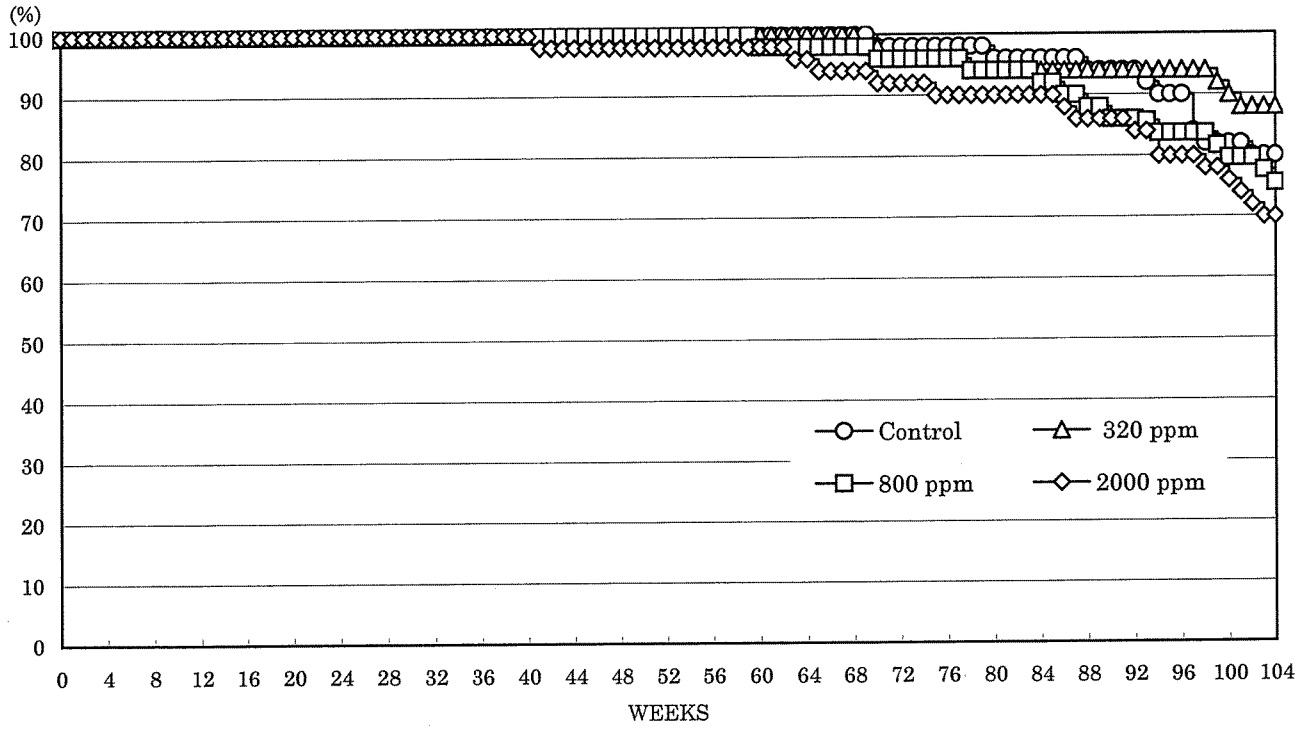


FIGURE 1 SURVIVAL RATE OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE

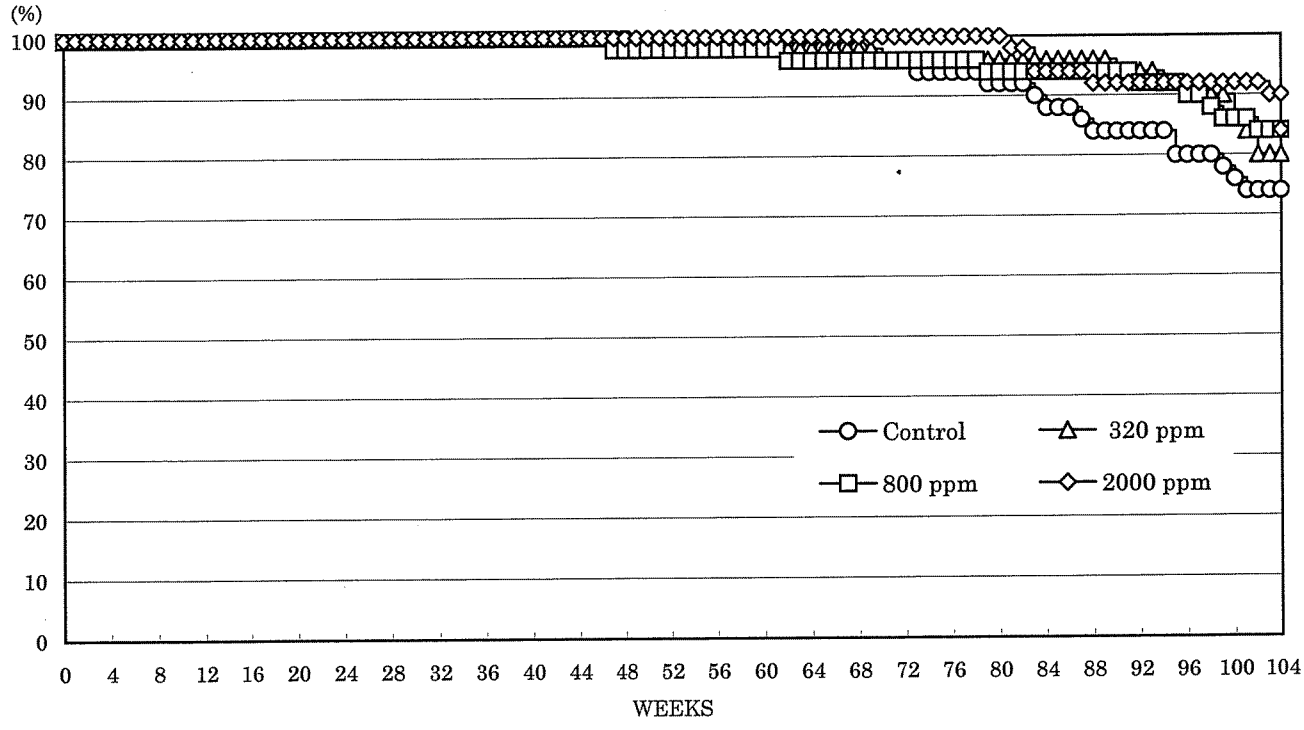


FIGURE 2 SURVIVAL RATE OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE

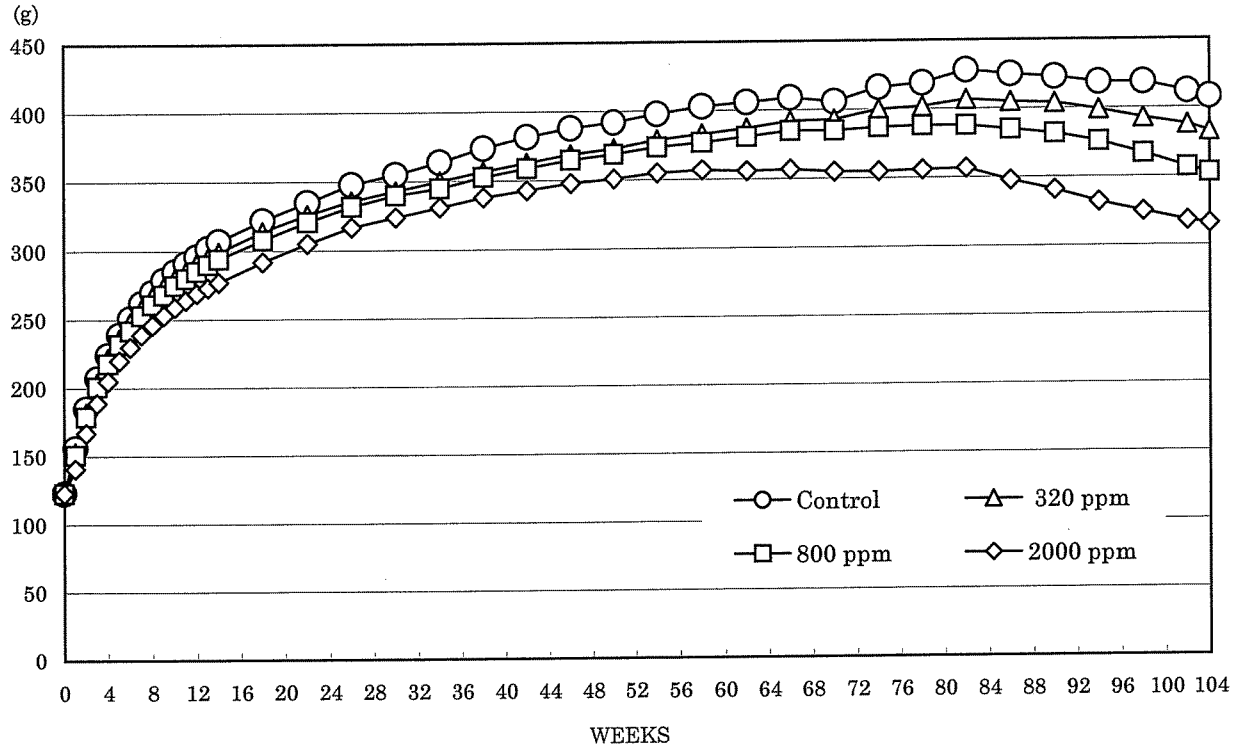


FIGURE 3 BODY WEIGHT CHANGES OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE

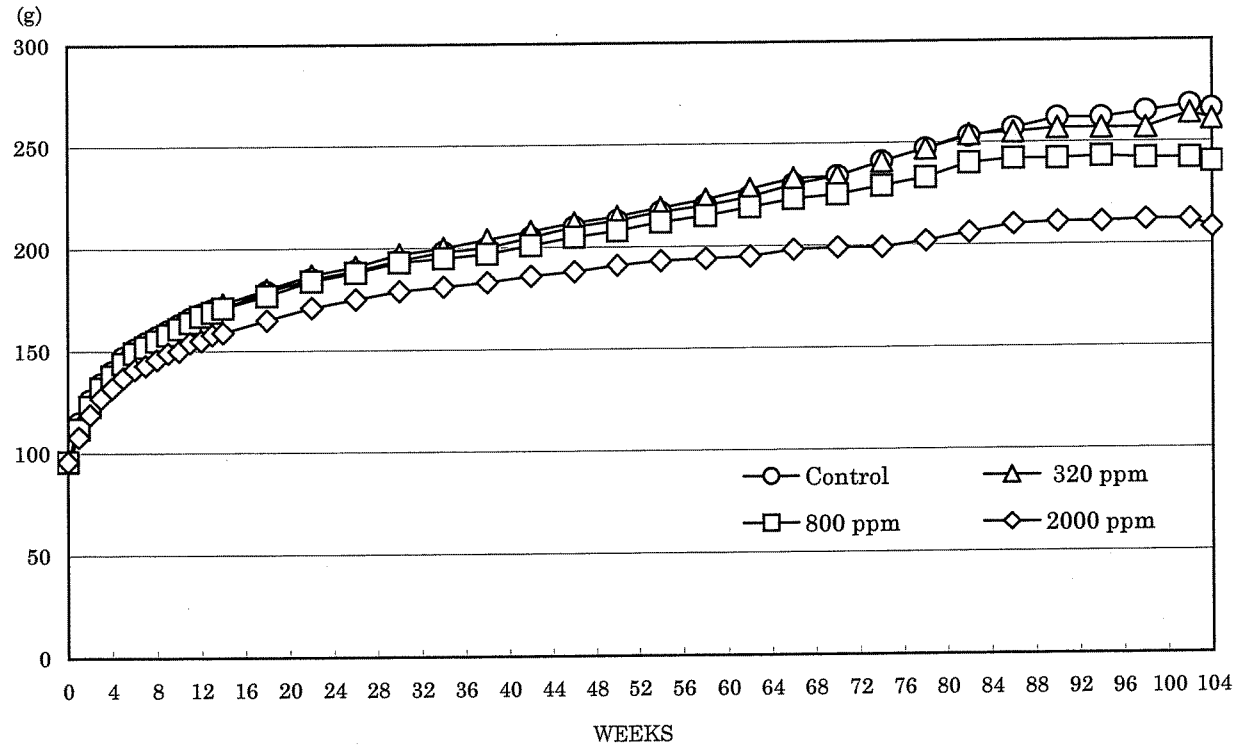


FIGURE 4 BODY WEIGHT CHANGES OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE

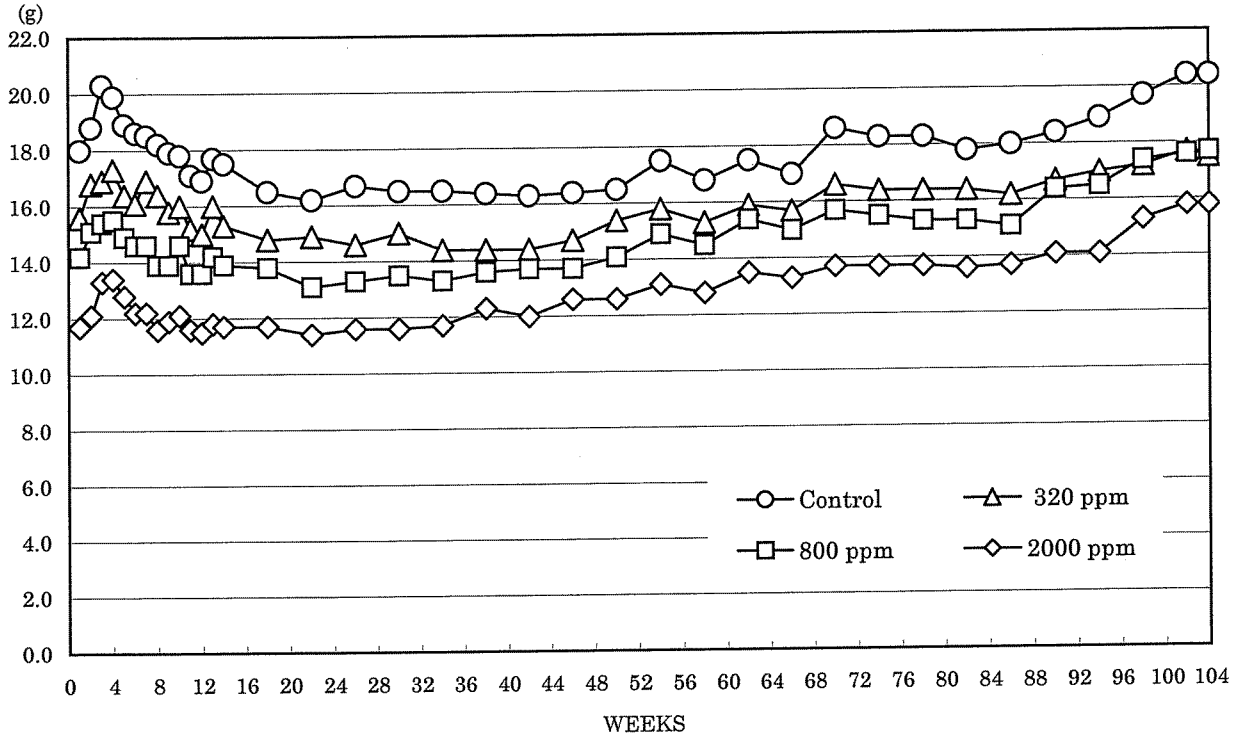


FIGURE 5 WATER CONSUMPTION CHANGES OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE

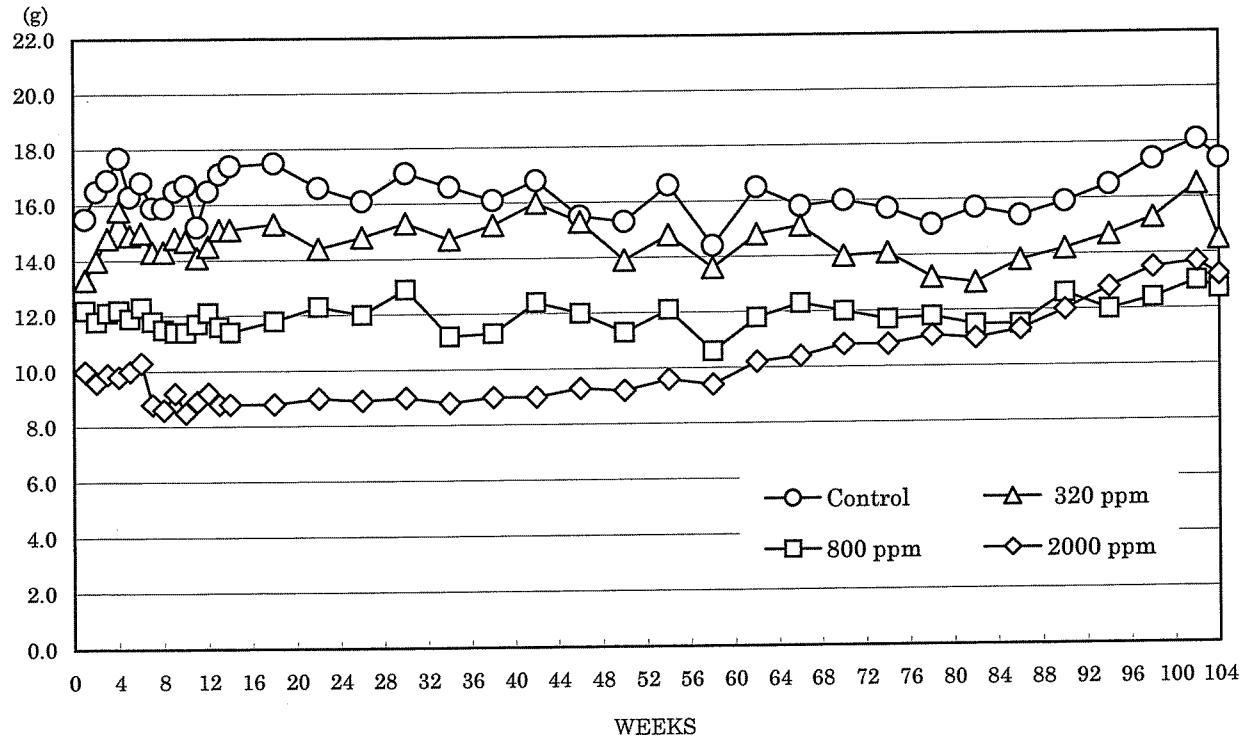


FIGURE 6 WATER CONSUMPTION CHANGES OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE

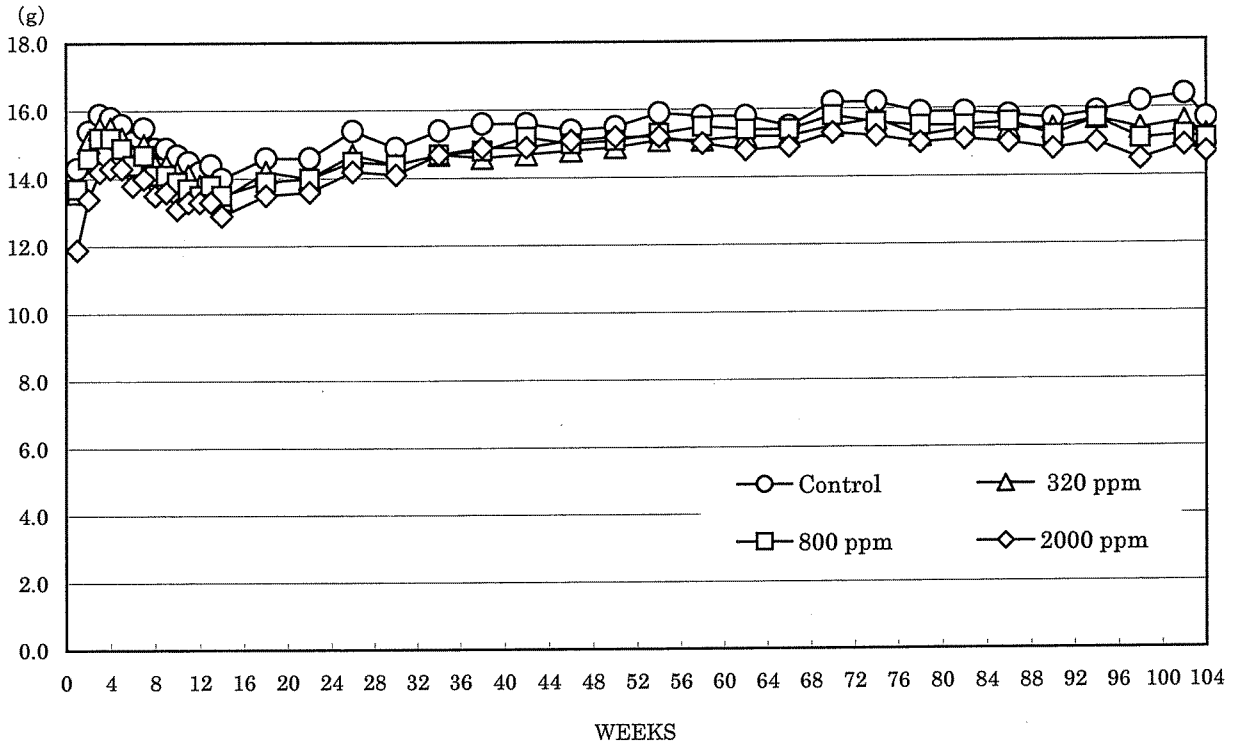


FIGURE 7 FOOD CONSUMPTION CHANGES OF MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE

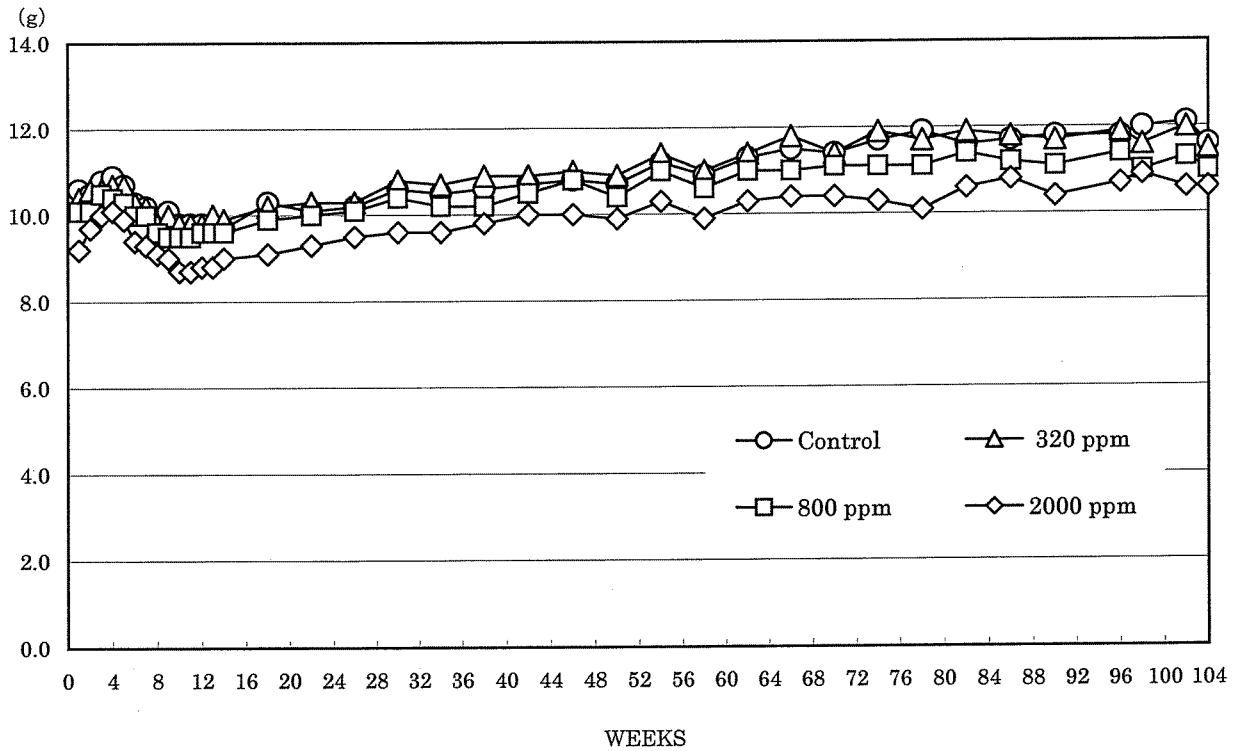


FIGURE 8 FOOD CONSUMPTION CHANGES OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF 2-HYDROXYETHYL ACRYLATE

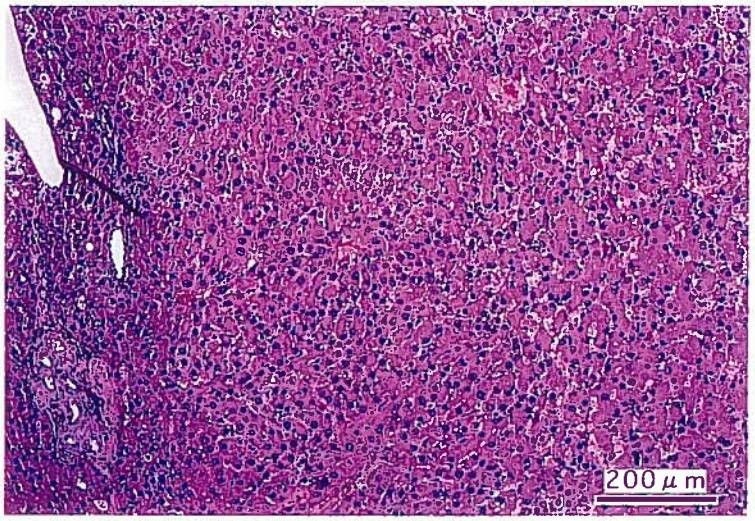
PHOTOGRAPHS

PHOTOGRAPH 1 LIVER : HEPATOCELLULAR ADENOMA,
RAT, MALE, 2000 ppm, ANIMAL NO. 0347-1341 (H&E)

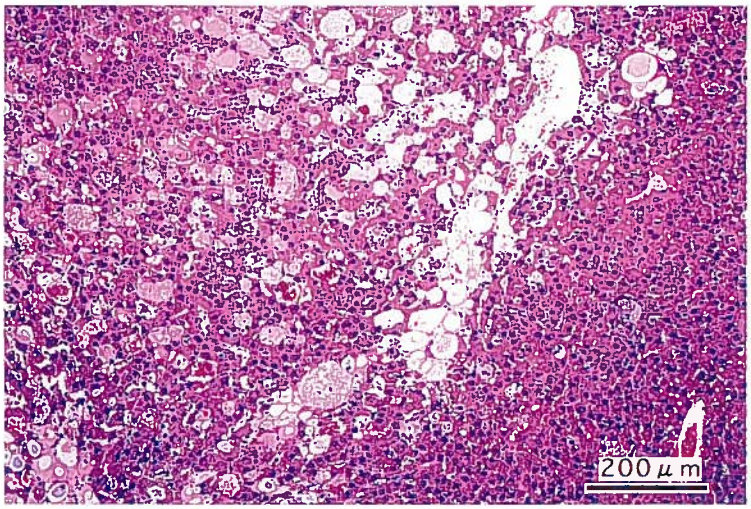
PHOTOGRAPH 2 LIVER : BASOPHILIC CELL FOCUS
RAT, MALE, 2000 ppm, ANIMAL NO. 0347-1336 (H&E)

PHOTOGRAPH 3 KIDNEY : CHRONIC NEPHROPATHY,
RAT, MALE, 2000 ppm, ANIMAL NO. 0347-1319 (H&E)

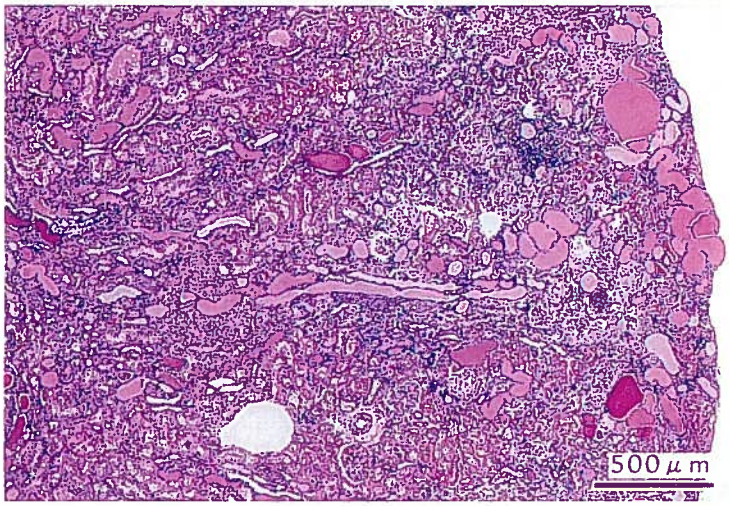
PHOTOGRAPH 4 KIDNEY : (A) PAPILLARY NECROSIS
(B) MINERALIZATION : PAPILLA
(C) UROTHELIAL HYPERPLASIA : PELVIS
RAT, FEMALE, 2000 ppm, ANIMAL NO. 0347-2319 (H&E)



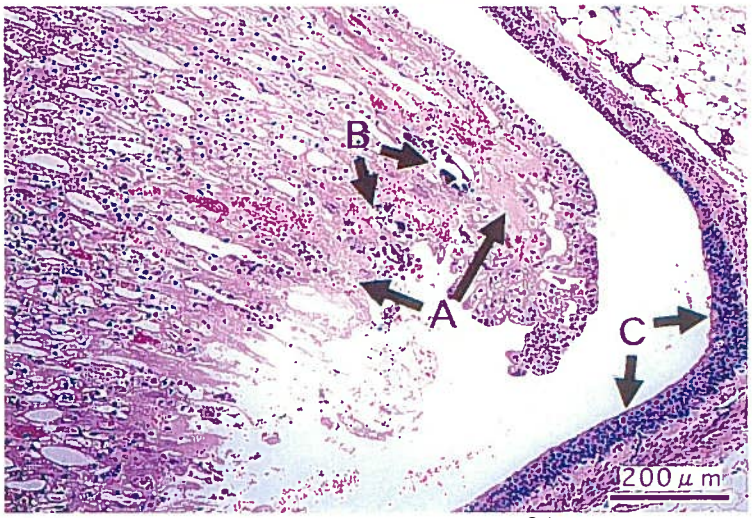
PHOTOGRAPH 1



PHOTOGRAPH 2



PHOTOGRAPH 3



PHOTOGRAPH 4