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TOXICITY OF METHYLMERCURY CHLORIDE IN RATS II. REPRODUCTION STUDY

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SUMMARY

A reproduction study over 3 generations of rats was carried out in which groups of 20 female and 10 male rats received in the diet 0, 0.1, 0.5 and 2.5 ppm MeHgCl. The parameters studied included growth, food intake, haematology, serum and urinalysis, organ weights and reproductive performance.

No effect was exerted on fertility index, lactation index or on the 21-day body weights of pups but the viability index was impaired at 2.5 ppm in the F1 and F2 generations. Weight gain reductions observed at 12 weeks for the 2.5 ppm level were not accompanied by reductions in food intake. At 6 months, F1a females on 2.5 ppm showed a reduced leucocyte count whilst P males on 0.5 and 2.5 ppm showed an increase in neutrophils and a decrease in lymphocytes.

The relative weights of the kidneys, heart, spleen, brain and thyroid were increased at 2.5 ppm and in some cases the increases of kidney weights were inconsistently seen at the 0.1 and 0.5 ppm levels. No significant histological changes were seen at any level. In a special 7-week study involving the F3a generation, weanling rats obtained from the four different F2a groups, each comprising 20 females and 10 males, were all transferred to diets containing 25 ppm MeHgCl. Toxicity signs were evident at 7 weeks. No evidence was obtained of increased susceptibility to the toxicity of MeHgCl in successive generations.

INTRODUCTION

An introduction to the toxicity of organic mercury compounds, especially methylmercury, was presented in the first of the three papers in this series [1].

Abbreviations: AH, aniline hydroxylase; Alk.Pase, alkaline phosphatase; APDM, aminopyrine demethylase; GPT, glutamic-pyruvic transaminase.



In addition to studying the effect of MeHgCl on breeding performance, the current reproduction study examined the possibility of increased susceptibility to MeHgCl toxicity in successive generations.

EXPERIMENTAL

The material tested, animals and diets used, analytical methods and enzyme assays and statistical analysis conducted are as described in the first paper in this series [1] except that 10% cellulose was added to the standard diet in order to reduce the nutritive value.

Experimental design and conduct

Four groups, each of 20 female and 10 male weanling rats on 0, 0.1, 0.5 and 2.5 ppm MeHgCl were taken from the long-term study [2] and used for the production of two litters and then returned to complete the long-term test. Each male was allowed to mate with 2 females for one week to produce the first litter (F1a) and this was repeated after 6 weeks to produce the second litter (F1b). The same procedure was followed for each generation. The animals of F1a and F2a generations were used to produce two nests of the following generations F2a + F2b and F3a + F3b, respectively. The F3a generation was used in a special 7-week experiment. The F1b, F2b and F3b litters were killed at weaning. Offspring were counted on days 1, 5 and 21 and the nest weights were recorded at days 5 and 21. All pups were observed for macroscopic changes.

The F1a and F2a generations were observed for weight gain, food intake, behaviour and mortality. Haematological examination was carried out on 10 female and 10 male rats of each group after 6 months for haemoglobin concentration, packed cell volume, total erythrocyte and total and differential leucocyte counts. After 6 months, serum levels of Alk.Pase, GPT and urea and hepatic levels of AH, APDM and glycogen were determined in 4–10 animals per group.

Urinalysis was carried out in 10 female and 10 male animals per group for pH, the presence of protein, glucose, ketone bodies, bilirubin and blood and for osmolality. The F1a and F2a generations were killed after 6–7 months and the heart, brain, liver, kidneys, spleen, adrenals, thyroid, pituitary, uterus, ovaries, testes and prostate were weighed. Histopathological examination was performed on brain, liver, kidneys, eye, spinal cord and Nervus ischiadicus of rats of all groups.

In the special 7-week study with the F3a generation, weanling rats obtained from the four different F2a groups, each comprising 20 females and 10 males on 0, 0.1, 0.5 and 2.5 ppm were all transferred to diets containing 25 ppm. A fifth group of 20 females and 10 males on 0 ppm remained on the basal diet.

Food intake was recorded at weeks 1, 2 and 5 and body weights weekly. After 7 weeks total mercury or MeHgCl concentration was determined in blood, kidneys, brain and liver of 5 males per group. Urinalysis (Bililabstix®)

was carried out on 10 females and 10 males per group. The experiment was terminated after 7 weeks. Liver, kidneys and brain were weighed. These organs and the spinal cord, peripheral nerves and eyes were examined microscopically.

RESULTS

Significant reductions in weight gain were seen in P females on 0.1 ppm at 6 and 12 weeks, increased weights were seen in F1a females on 0.1 and 2.5 ppm at 6 weeks and in F1a males on 0.1 ppm at 6 weeks, decreased weights in F2a females on 0.1, 0.5 and 2.5 ppm at 12 weeks and in F2a males on 2.5 ppm at 12 weeks (Table I). Growth retardation was not accompanied by a corresponding reduction in food intake and in several cases food intake was significantly increased.

Alterations in the head profile and the regularity of the teeth as described in the long-term test were observed in the F1a generation (2 females and 1 male on 0 ppm, 1 male on 0.1 ppm and 3 females on 0.5 ppm) and in the

TABLE I
WEIGHT GAIN (g) OVER 6 AND 12 WEEKS IN THREE GENERATIONS OF RATS

Generation	Week	Number of animals	Initial weight (g) and weight gain (g)			
			0 ppm	0.1 ppm	0.5 ppm	2.5 ppm
<i>Females</i>						
P	0	25	47	48	47	46
	6	25	112	102 ^{xx}	110	108
	12	25	149	141 ^{xx}	146	145
F1a	0	20	65	58	70	60
	6	20	88	97 ^x	91	95 ^x
	12	20	117	125	123	122
F2a	0	10	48	47	53	54
	6	10	113	108	109	105
	12	10	153	142 ^x	140 ^x	134 ^x
<i>Males</i>						
P	0	35	50	50	50	50
	6	35	183	180	189	178
	12	34	271	271	276	260
F1a	0	10	62	67	73	69
	6	10	173	194 ^x	192	186
	12	10	250	267	260	250
F2a	0	10	55	54	63	56
	6	10	198	209	213	191
	12	10	283	295	294	258 ^x

x, $P < 0.05$; xx, $P < 0.01$.

F2a generation (2 females on 0 ppm, 1 female on 0.1 ppm and 1 male on 2.5 ppm). It is unlikely that this phenomenon is caused by MeHgCl as it also occurs in control animals.

Haematological examination of the P, F1a and F2a generations after 6 months revealed a reduction in the leucocyte count in F1a females on 2.5 ppm and an increase in neutrophils and a decrease in lymphocytes in P males on 0.5 and 2.5 ppm (Table II). The opposite effect, namely lowered neutrophil and raised lymphocyte count was seen in P females on 0.1 ppm and in F2a males on 0.5 ppm.

Serum levels at 6 months of GPT, Alk.Pase and urea were normal in all groups of all generations, except for a decreased urea content in F2a females on 2.5 ppm.

Urinalysis of the F1a and F2a generations gave normal values for all groups at 6 months. Liver microsomal enzyme assays showed the activity of AH to be decreased non-significantly in F1a males on 0.5 and 2.5 ppm.

Liver glycogen was decreased non-significantly in F1a males and females on 2.5 ppm.

The most notable changes in relative organ weights (Table III) were the increases in the relative kidney weight at 2.5 ppm in the P, F1a and F2a generations, at 0.5 ppm in F2a males and females and at 0.1 ppm in F2a females and F1a males. Significant increases were also seen in the relative heart weight in F1a and F2a males on 2.5 ppm (F2a females on 2.5 ppm showed a decrease), in the relative brain weight of F2a females on 0.5 and 2.5 ppm, in the relative spleen weight in F1a males on 2.5 ppm and in the relative thyroid weight of F2a females and males on 2.5 ppm and in F1a males on 2.5 ppm.

The results obtained on reproductive performance showed no effect on the fertility index, lactation index and body weight of pups at 21 days. The viability index was decreased at 2.5 ppm in the F1 and F2 generations (Table IV).

No significant macroscopic changes nor histological changes were seen in the F1a and F2a generations at any level, except for a slight degeneration of the granular cells in the brain of 1 male on 2.5 ppm.

In the special study involving the F3a generation, all groups which received 25 ppm MeHgCl showed a similar degree of growth retardation and decreased food intake and no differences in relation to the original dose levels were observed (Table V).

In some animals fractures in the Os nasale or loose incisors were observed. The animals recovered from this discomfort except in one case. From week 7 onwards rats receiving 25 ppm MeHgCl started to show signs of paralysis and for this reason the experiment was terminated. Although the first signs of clinical intoxication were observed somewhat earlier than in the short-term toxicity study, this was not regarded as significant.

Urinalysis revealed higher protein contents in all groups given 25 ppm and also the presence of ketone bodies in males (Table VI).

In all rats which had received 25 ppm MeHgCl, the weights of the kidneys

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TABLE II
HAEMATOLOGICAL EXAMINATION IN 3 GENERATIONS AFTER 6 MONTHS

	MeHgCl (ppm)	Females			Males		
		P	F1a	F2a	P	F1a	F2a
Haemoglobin (mmoles/l)	0	9.6	10.3	9.4	9.5	9.6	9.0
	0.1	9.4	9.8 ^x	9.5	9.4	9.6	9.3
	0.5	9.6	9.7	9.8	9.6	9.4	9.3
	2.5	9.6	9.9	9.9	9.3	9.6	9.1
Packed cell volume (%)	0	47	51	46	46	47	45
	0.1	44	48 ^{xx}	46	45	47	46
	0.5	46	48	47	46	46	45
	2.5	46	48 ^x	48	45	47	44
Erythrocytes (10 ⁶ / mm ³)	0	7.5	8.1	7.7	7.8	8.3	8.0
	0.1	7.2	8.1	8.0	7.7	8.3	8.3
	0.5	7.4	8.1	8.0	7.7	8.2	8.3
	2.5	7.3	8.0	8.4 ^x	7.5	8.1	8.3
Total leucocytes (10 ³ /mm ³)	0	8.7	7.9	9.9	10.2	9.8	12.5
	0.1	9.0	7.4	9.2	10.4	8.9	11.7
	0.5	8.8	7.3	8.9	9.7	8.9	11.0
	2.5	8.7	6.5 ^{xx}	8.6	9.5	8.6	10.7
<i>Differential leucocyte count (%)</i>							
Basophils	0	0.4	0.3	0.4	0.2	0.2	0.3
	0.1	0.4	0.1	0.4	0.5	0.4	0.5
	0.5	0.8	0.4	0.1 ^x	0.3	0.5	0.3
	2.5	0.5	0.2	0.2	0.5	0.2	0.2
Eosinophils	0	2.4	1.6	1.9	1.2	1.5	1.5
	0.1	2.0	1.4	1.5	2.2	1.3	1.9
	0.5	2.6	1.6	1.9	1.7	1.5	1.4
	2.5	2.2	1.5	2.1	2.6	1.7	2.5
Neutrophils	0	15.1	12.0	10.6	11.1	12.0	14.0
	0.1	9.9 ^x	14.2	11.5	13.3	14.1	9.9
	0.5	17.8	16.6	15.5	17.3 ^x	13.4	9.7 ^x
	2.5	13.3	14.4	13.1	16.4 ^{xx}	12.7	11.0
Lymphocytes	0	79.7	84.0	83.5	85.4	84.1	79.8
	0.1	85.1	81.7	82.6	81.9	81.4	83.6
	0.5	76.6	79.1	77.6	78.7 ^x	82.6	85.5 ^x
	2.5	81.6	82.4	81.1	78.8 ^{xx}	83.2	82.4
Monocytes	0	2.5	2.2	3.6	2.2	2.4	4.4
	0.1	2.7	2.8	4.0	2.3	2.8	4.1
	0.5	2.4	2.5	4.9	2.1	2.1	3.1
	2.5	2.5	1.6	3.5	1.8	2.2	3.9
Atypical cells	0	2.5	3.3	5.1	2.3	2.6	6.5
	0.1	2.2	3.4	7.2	2.2	3.4	6.1
	0.5	3.1	3.5	5.7	2.8	3.1	6.1
	2.5	2.7	3.3	13.1 ^x	2.1	2.5	6.6

Values are for groups of 10 males and 10 females.
x, $P < 0.05$; xx, $P < 0.01$.

TABLE III
RELATIVE ORGAN WEIGHTS (%)

	Females				Males			
	0 ppm	0.1 ppm	0.5 ppm	2.5 ppm	0 ppm	0.1 ppm	0.5 ppm	2.5 ppm
Number of animals	10	10	10	9	9	10	10	10
<i>P-generation</i> ^b								
Heart	0.404	0.417	0.396	0.461	0.318	0.306	0.309	0.345
Brain	0.667	0.656	0.650	0.668	0.442	0.434	0.444	0.452
Liver	3.31	3.09	3.36	3.55	3.06	2.87	3.20	3.26
Kidneys	0.797	0.780	0.866	1.086 ^{xx}	0.717	0.698	0.842	0.929 ^{xx}
Spleen	0.203	0.182	0.186	0.219	0.205	0.169	0.200	0.198
Adrenals	0.019	0.017	0.018	0.020	0.009	0.008	0.009	0.010
Thyroid	—	—	—	—	—	—	—	—
Pituitary	0.005	0.005	0.005	0.006	0.003	0.003	0.003	0.003
Uterus/prostate	0.225	0.188	0.224	0.209	0.720	0.667	0.647	0.679
Ovaries/testes	0.023	0.026	0.028	0.026	0.101	0.110	0.103	0.102
Number of animals	10	10	10	10	10	10	10	10
<i>F1a-generation</i> ^a								
Heart	0.365	0.342 ^x	0.328 ^x	0.352	0.264	0.293 ^{xx}	0.260	0.282 ^x
Brain	0.767	0.726	0.749	0.817	0.482	0.481	0.480	0.501
Liver	4.44	4.14	2.77 ^{xx}	3.91 ^x	4.14	4.45	4.09	4.31
Kidneys	0.919	0.841	0.929	1.003	0.716	0.802 ^{xx}	0.770	0.856 ^{xxx}
Spleen	0.196	0.167 ^x	0.159 ^x	0.190	0.134	0.140	0.135	0.172 ^{xxx}
Adrenals	0.022	0.018 ^x	0.019	0.019	0.010	0.011	0.010	0.009
Thyroid	0.006	0.006	0.005	0.006	0.004	0.005	0.004	0.005 ^{xx}
Pituitary	0.006	0.005	0.005	0.006	0.003	0.003	0.003	0.003
Ovaries/testes	0.189	0.188	0.168	0.231	0.817	0.825	0.704 ^x	0.783
Uterus/prostate	0.036	0.029 ^x	0.032	0.031	0.094	0.077	0.083	0.087
Number of animals	13	17	20	8	8	7	10	5
<i>F2a-generation</i> ^a								
Heart	0.438	0.356	0.364	0.377 ^{xx}	0.270	0.269	0.267	0.298 ^x
Brain	0.736	0.760	0.786 ^{xx}	0.822 ^x	0.511	0.467 ^x	0.501	0.559
Liver	4.06	3.82	3.67 ^{xx}	3.80	4.04	3.83	3.62 ^x	3.77
Kidneys	0.834	0.927 ^{xx}	0.947 ^{xx}	1.105 ^{xx}	0.756	0.741	0.811 ^x	0.893 ^{xx}
Spleen	0.174	0.179	0.179	0.189	0.148	0.148	0.131	0.149
Adrenals	0.021	0.020	0.021	0.019 ^x	0.010	0.009	0.009	0.009
Thyroid	0.004	0.005 ^x	0.005	0.005 ^{xx}	0.004	0.004	0.004	0.005 ^x
Pituitary	0.006	0.006	0.005	0.006	0.003	0.002	0.003	0.003
Ovaries/testes	0.175	0.178	0.191	0.179	0.867	0.773 ^x	0.716 ^{xx}	0.734
Uterus/prostate	0.035	0.036	0.035	0.030	0.081	0.070	0.093	0.075

x, $P < 0.05$; xx, $P < 0.01$; xxx, $P < 0.001$.

^a After 6 months on test.

^b After 2 years on test.

TABLE IV
REPRODUCTION PERFORMANCE

Generation	0 ppm	0.1 ppm	0.5 ppm	2.5 ppm				
Fertility index ^a								
P	78	81	68	75				
F1	75	72	74	70				
F2	70	79	80	89				
Viability index ^b								
P	86	83	86	85				
F1	66	66	59	28				
F2	64	74	70	52				
Lactation index ^c								
P	68	57	77	66				
F1	69	70	86	88				
F2	94	65	88	92				
Mean body weight at day 21 (g)								
P	30	31	34	33				
F1	34	32	34	36				
F2	34	36	34	38				
Number of rats in each group								
	F	M	F	M	F	M	F	M
P	20	10	16	8	16	8	18	9
F1	20	10	18	9	18	9	20	10
F2	15	8	17	9	20	10	9	5

^a Number of pregnancies × 100 divided by the number of matings

^b Number of pups alive at day 5 × 100 divided by the number of pups born

^c Number of pups alive at day 21 × 100 divided by the number of pups alive at day 5

TABLE V
WEIGHT GAIN IN SPECIAL F3a GENERATION STUDY AT 6 WEEKS

Parameter	0 ppm	0 + 25 ppm	0.1 + 25 ppm	0.5 + 25 ppm	2.5 + 25 ppm
<i>Females</i>					
Initial weight (g)	87	89	93	87	76
Weight gain (g) at week 6	75	56 xxx	60 xxx	55 xxx	59 xx
<i>Males</i>					
Initial weight (g)	109	105	113	104	90
Weight gain (g) at week 6	159	137 x	132 x	120 xxx	127 xx

x, $P < 0.05$; xx, $P < 0.01$; xxx, $P < 0.001$.

Significant differences are those between experimental groups, as compared with 0 ppm group. No significant difference exists between experimental groups and 0 + 25 ppm group.

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reproduction

TABLE VI
URINALYSIS IN SPECIAL F3a GENERATION STUDY AT 6 WEEKS

Parameter		0 ppm	0 + 25 ppm	0.1 + 25 ppm	0.5 + 25 ppm	2.5 + 25 ppm
<i>Females</i>						
Protein content	± ^a	1	0	0	0	0
	+	8	1	2	2	1
	++	1	7	7	7	7
	+++	0	2	1	1	2
Ketones	—	10	10	10	9	10
	+	0	0	0	1	0
<i>Males</i>						
Protein content	+	2	1	0	0	0
	++	8	7	8	8	3
	+++	0	2	2	2	7
Ketones	—	10	1	1	3	2
	±	0	3	4	1	3
	+	0	5	3	6	3
	++	0	1	2	0	1
	+++	0	0	0	0	1

^a —, none; ±, trace; +, some; ++, moderate; +++, much.

The pH was nearly always 6.0, in a few cases 6.5. Glucose, bilirubin and blood were not detected.

TABLE VII
RELATIVE ORGAN WEIGHTS (%) IN SPECIAL F3a GENERATION STUDY AT 6 WEEKS

Organ	0 ppm	0 + 25 ppm	0.1 + 25 ppm	0.5 + 25 ppm	2.5 + 25 ppm
<i>Females</i>					
Number of animals	20	19	20	20	17
Liver	3.92	4.39	4.21	4.38	4.50
Kidneys	0.915	1.319	1.385	1.458 ^x	1.438 ^{xx}
Brain	0.998	1.142	1.098	1.217 ^x	1.261 ^{xx}
<i>Males</i>					
Number of animals	10	9	8	7	6
Liver	4.51	4.29	4.57	4.20	4.18
Kidneys	0.860	1.309	1.490 ^{xx}	1.394	1.443 ^x
Brain	0.647	0.752	0.763	0.790	0.815

x, $P < 0.05$; xx, $P < 0.01$.

Significance was calculated in comparison with the 0 + 25 ppm group. The relative weights of kidneys and brain of the four experimental groups were significantly increased in both sexes in comparison with the 0 ppm group ($P < 0.01$ or 0.001) as did the relative liver weight in females.

TABLE VIII

TISSUE LEVELS OF Hg AFTER 7 WEEKS IN SPECIAL F3a GENERATION STUDY

Tissue	0 ppm	0 + 25 ppm	0.1 + 25 ppm
Blood (total Hg)	0.18 ± 0.034	146 ± 18.1	227 ± 21.5 ^{xx}
Kidneys (total Hg)	0.47 ± 0.15	86.8 ± 8.75	92.8 ± 17.5
Liver (MeHgCl)	—	67.9 ± 2.2	84.8 ± 6.5 ^{xx}
Brain (MeHgCl)	—	10.6 ± 0.9	12.9 ± 1.3 ^x

Tissue	0.5 + 25 ppm	2.5 + 25 ppm
Blood (total Hg)	227 ± 37.0 ^{xx}	151 ± 26.1
Kidneys (total Hg)	79.2 ± 13.1	81.3 ± 15.2
Liver (MeHgCl)	103 ± 24 ^{xx}	87.6 ± 3.6 ^{xx}
Brain (MeHgCl)	11.0 ± 1.3	11.2 ± 1.5

Values are for group of 5 males.

x, $P < 0.05$; xx, $P < 0.01$.

Significance is calculated compared with the 0 + 25 ppm group.

and the brain were significantly increased when compared with the 0 ppm control group (Table VII). The relative liver weight of females was increased non-significantly in all 25 ppm groups. When the individual groups were compared to the 0 + 25 ppm group, increased relative weights were seen in the kidneys and brain of females on 0.5 + 25 ppm and on 2.5 + 25 ppm and in the kidneys of males on 0.1 + 25 ppm and on 2.5 + 25 ppm.

The characteristic lesions of the nervous system and kidneys seen histologically in the short-term study were found in all groups which received 25 ppm. No differences between the treated groups could be observed. Lesions in the cerebellum were only observed in treated females. MeHgCl administration to the parent generation did not profoundly affect tissue concentrations in the F3a generation and except for blood the concentrations obtained at various dose levels were comparable (Table VIII).

DISCUSSION

For ease of comparison, the results obtained in the reproduction study have been discussed in the light of those obtained in the short- and long-term studies. The discussion appears in the report on the long-term study.

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