Arch Toxicol (1981) 48: 11–20

Archives of
TOXICOLOGY

Comparative Study of the Sensitivity of Male and Female Rats to Methylmercury

Laszlo Magos[1], George C. Peristianis[2], Thomas W. Clarkson[3], Alwyn Brown[1], Stanley Preston[1], and Roger T. Snowden[1]

[1] Medical Research Council Laboratories, Toxicology Unit, Carshalton, Surrey, SM5 4EF
[2] Shell Toxicology Laboratory (Tunstall), Sittingbourne Research Centre, Sittingbourne, Kent, ME9 8AG, Great Britain
[3] Environmental Health Sciences Center, Department of Radiation Biology and Biophysics, Rochester, New York, 14642, USA

Abstract. Male and female rats were dosed daily by gastric gavage four or five times with 8.0 mg/kg Hg as methylmercury. Treatment lowered the body weight in relation to the body weight of untreated rats to the same extent in male and female rats but when body weight was related to the initial body weight, the effect of methylmercury was more pronounced in females than in males. The importance of differences in growth or loss of body weight is that in spite of the similar whole body clearance mercury concentrations were higher in females than in males. After identical doses the brains of females always contained more mercury than those of males and in both sexes the brain concentration of mercury showed a disproportionate elevation when the number of doses was increased from four to five. However, weight change alone does not explain the sex related difference in the brain concentration of mercury as this was evident even 72 h after a single dose. In agreement with the brain concentration of mercury, female rats developed more intensive co-ordination disorders and after five doses they had more extensive damage in the granular layer of the cerebellum than males.

Key words: Methylmercury – Neurotoxicity – Sex difference – Rat – Cerebellar damage – Coordination disorders

Introduction

At the time of the Iraqi methylmercury epidemic autopsy samples from non-hospitalized cases of suspected methylmercury intoxication were sent to a central laboratory for mercury analysis. The records of 51 randomly selected samples indicated a sex distribution of 3 : 1 in favour of females (Magos et al. 1976). The higher sensitivity of females to the toxic effect of methylmercury compared with males was not supported by either hospital admission or hospital fatality records (Bakir et al. 1973). However, these data are based on a relatively

Send offprint requests to: L. Magos at the above address

0340-5761/81/0048/0011/$ 02.00

small number of people and might not be representative of the general population. An interesting observation made by Greenwood (1975) seems to support this view. He collected general mortality data from most of the affected districts for the 3 months period in which the epidemics culiminated and compared the numbers with earlier mortality data from identical periods. He has found that the number of registered deaths for the two sexes was equal only at the time of the epidemics, while in every previous year substantially more male than female deaths were reported. This means that many female births remain unnotified and therefore their deaths could not be notified either. Thus in the social set-up of rural Iraq the equal number of notified male and female deaths which occurred during the epidemics could be explained only with higher female mortality indicating a sex-dependent difference in sensitivity to methylmercury. The increase in mortality compared with the previous years showed similar sex-distribution pattern as the randomly selected autopsy samples. Thus the possibility that females are more sensitive to the toxic effects of methylmercury remained open and prompted the investigations reported in this paper on the sensitivity of female and male rats to methylmercury.

Materials and Methods

Porton-Wistar rats were given by gastric gavage daily doses of 8.0 mg/kg Hg as methylmercury chloride (K and K Laboratories, Plainview, NY, USA) dissolved in saline to a concentration of 2 mg Hg/ml. The solution was labelled with $Me^{203}HgCl$ (Radiochemical Centre, Amersham, Bucks., GB) to the extent of 0.5 μCi/8 mg Hg in the multiple dose experiments and 0.9 μCi or 26 μCi/8 mg Hg in the single dose experiments. The higher activity was used when mercury concentrations were estimated in distinct brain areas. In the multiple dose experiments, differences in mean body weights between sexes were not more than 10 g and animals were given either four or five daily doses of MeHgCl. The approximate age was for males 7.5 weeks (four doses) and 7 weeks (five doses) and for females 11 weeks. In the single dose experiments 8 weeks old males (261 ± 8.4 g body weight) and 9 weeks old females (209 ± 6.1 g body weight) were used. Body weight was estimated from the first treatment day, and body burden from the last treatment day onward five times per week in the multiple dose experiments. Flailing reflex and hind leg crossing was scored from the last treatment day. Animals were killed by intracardial perfusion with 4% (w/v) glutaraldehyde solution under chloral hydrate (7%, w/v) anaesthesia 1–13 days after the last treatment in the four times dosed groups, 11 and 12 days after the last treatment in the five times dosed groups and 72 h after a single dose. Brain, liver, kidneys, and in the four times dosed groups, dorsal root ganglia from the cervical and lumbar regions were also removed. Blood was taken before perfusion from the heart. The brains of five male and five female rats in the single treatment group were dissected into five distinct areas: (a) olfactory bulbs including the olfactory peduncle; (b) neocortex; (c) thalamus region including the hippocampus, hypothalamus and striatum; (d) medulla corresponding to the medulla oblongata, pons and midbrain; (e) cerebellum.

Methods of radioactive estimation in the whole animals and organs, scoring of co-ordination disorders, perfusion (Magos et al. 1978), the preparation of sagittal and parasagittal cerebellar sections (Magos et al. 1980a), dorsal root ganglia sections (Magos et al. 1980b) and the scoring of the granular layer necrosis (Magos et al. 1978) were described in detail previously. The cerebrum was cut into coronal slices (1–1.5 mm thick) and each slice was divided into left and right hemispheres. The scoring of histological damage in the dorsal root ganglia was based on the following criteria: the size of areas cleared of Nissl substance, the frequency of eccentric nuclei, chromatolysis, the proliferation of satellite cells, Nageotte body formation and dorsal root fibre degeneration. For example when several ganglion cells showed advanced stages of chromatolysis, the proliferation of satellite cells formed several Nageotte bodies and some dorsal root fibres showed marked degeneration, the damage was scored as severe.

Results

Increase in the number of treatment days with methylmercury from four to five had a significant effect on the weights of both female and male rats (see Fig. 1). In females treated four times with methylmercury the maximum decline below the initial body weight was only 2.5% and at 11th post treatment day body weight actually increased above the initial level. Five daily doses resulted in the loss of 17.5% of the initial body weight with no change between the 7th and 11th post treatment days. Contrary to females, male rats lost no body weight, and at least in the four times dosed group body weight actually increased after the last treatment day. However, when weights of treated female or male rats are compared with the weights of non-treated rats, the difference between sexes disappear. Thus 8 days after the last of four treatments the body weights of females lagged 7.0% behind the expected weight and those of the males by 9%. After five treatments with methylmercury the corresponding values were 22.0% for females and 24.0% for males.

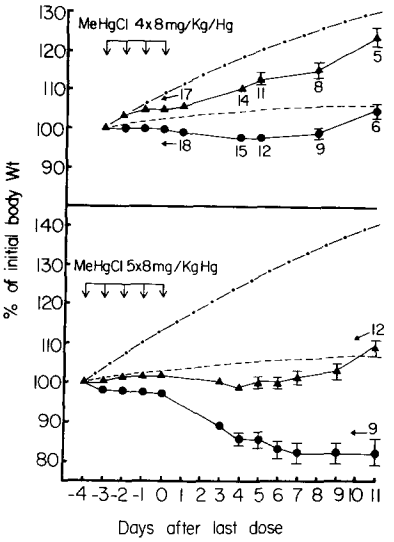

Fig. 1. Changes of body weight caused by treatment with daily doses of methylmercury in male (▲) and female (●) rats. Weight at the first treatment day is taken as 100%. The dotted line shows the average weight of age matched males and the dashed line shows the same for females. The vertical bars give SEM when it is larger than the symbol. The numbers beside the points give the number of animals

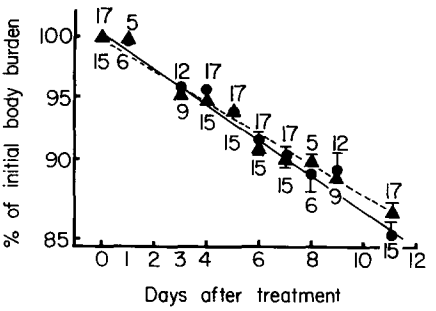

Fig. 2. Changes in the body burden of mercury from the last of four or five treatments (100%) onward in male and female rats. Symbols are the same as in Fig. 1. Half times are: 49.6 days for males and 45 days for females

The whole body clearance of mercury after four daily doses of 8.0 mg Hg/kg did not differ between males and females (Fig. 2). The slope of the lines in Fig. 2 were 0.0061 ± 0.0002 in males and 0.0067 ± 0.0004 ($days^{-1}$) in females and correspond to 49.6 and 45.0 days half times respectively. In previous experiments in the first 11 days after a similar dosing schedule, decline in the

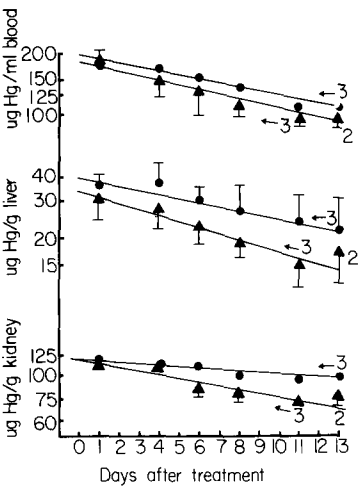

Fig. 3. Changes in the concentrations of mercury in blood, liver, and kidneys after four doses of methylmercury. Symbols are the same as in Fig. 1. Half times in blood were 12.2 days for males, 15.0 days for females; in liver 11.0 days for males and 15.0 days for females; in kidneys 16.8 days for males and 37.0 days for females

Table 1. The effects of four or five daily treatments with 8.0 mg/kg Hg as MeHgCl on body weight, relative organ weights and relative organ mercury contents of male and female rats. Animals were killed 11–13 days after treatment. (The numbers are means ± SEM)

		four doses group		five doses group	
		Male $N = 5$	Female $N = 5$	Male $N = 8$	Female $N = 8$
Final body weight (in g)		279 ± 3.7	233 ± 3.7	250 ± 3.8	180 ± 7.2
Final body weight (in % of initial body weight)		116 ± 2.3	102 ± 2.0	110 ± 2.1	83 ± 3.8
Relative organ weights (in % of body weight)	Brain	0.75 ± 0.025	0.86 ± 0.016*	0.84 ± 0.017**	1.12 ± 0.06* **
	Liver	4.93 ± 0.24	3.86 ± 0.06*	4.06 ± 0.17	4.38 ± 0.027
	Kidneys	1.05 ± 0.09	0.99 ± 0.03	1.11 ± 0.03	1.17 ± 0.05**
Relative organ Hg content (in % of body burden)	Brain	0.20 ± 0.015	0.27 ± 0.021*	0.29 ± 0.010**	0.49 ± 0.32* **
	Liver	3.48 ± 0.50	3.56 ± 0.26	4.32 ± 0.34	5.25 ± 039**
	Kidneys	3.38 ± 0.14	3.94 ± 0.30	3.29 ± 0.12	3.79 ± 0.08*

* Significantly different ($P < 0.05$) from males
** Significantly different ($P < 0.05$) from the four doses group
Statistical differences were calculated with the two directional Student t-test

Table 2. The brain concentration of mercury in male and female rats after daily treatments with 8.0 mg/kg Hg as methylmercury. (The numbers are means ± SEM)

No. of doses	Days after last dose	No. of animals	Brain conc. in μg Hg/g tissue		Brain conc. / Whole body conc.		Brain conc. / Blood conc.	
			Female	Male	Female	Male	Female	Male
4	1	3	7.40 ± 0.40	6.63 ± 0.42	0.237 ± 0.012	0.224 ± 0.014	0.041 ± 0.002	0.037 ± 0.004
	4	3	9.63 ± 0.37	7.73 ± 0.24	0.301 ± 0.010	0.269 ± 0.005	0.055 ± 0.002	0.054 ± 0.003
	6–8	6	9.40 ± 0.28	6.93 ± 0.14	0.341 ± 0.008	0.277 ± 0.007	0.065 ± 0.002	0.057 ± 0.003
	11–13	5	8.08 ± 0.44	6.14 ± 0.26	0.332 ± 0.017	0.269 ± 0.008	0.070 ± 0.014	0.064 ± 0.002
			Difference between pairs					
			1.92 ± 0.25 $N = 17$ $t = 7.64$ $P < 0.001$		0.048 ± 0.009 $N = 17$ $t = 5.36$ $P < 0.001$		0.006 ± 0.002 $N = 17$ $t = 3.12$ $P < 0.01$	
5	11 + 12	8	16.0 ± 1.36	9.6 ± 0.40	0.431 ± 0.021	0.342 ± 0.009		
			Difference between pairs					
			6.34 ± 1.39 $t = 4.55$ $P < 0.001$		0.09 ± 0.027 $t = 3.34$ $P < 0.01$			

whole body burden of mercury indicated the same half time for male (Magos et al. 1978) and approximately 10% shorter half time for female rats (Magos et al. 1980b). The significantly shorter half times in blood, kidney and liver (see Fig. 3) can be explained by the transfer of methylmercury into skin and fur (Magos and Butler 1976). Figure 3 shows that elimination rates were faster for males than females, but the differences were only significant for kidneys (16 days in males and 37 days in females). As the brain mercury concentration started to decline only 6–8 days after the last treatment, elimination half times were not calculated for this organ.

The sex dependent difference in body weight changes influenced the concentration of mercury in the whole body resulting in lower tissue concentrations in males than in females. Table 1 compares organ weights and mercury contents relative to body weight and to mercury body burden at 11–13 days after the last treatment. It can be seen that in the five times dosed group the relative weight and relative mercury content of brain were higher than in the four times dosed groups. The same did not apply to liver or kidneys on which the extension of treatment days had no systematic effect. Table 1 also shows that there was a significant difference in the relative brain weight and the relative mercury content of female and male brains. The brains of females were always significantly larger in relation to body weight and their brain contained a higher proportion of the body burden than the brains of males. Table 2 compares the brain concentration of mercury in absolute values and in relation to concentrations in the whole body and in blood. It can be seen that the brain of female rats accumulated mercury in a higher concentration both in absolute and in relative terms.

Both the brain concentrations and contents of mercury and the relative brain weights were higher in females than in males 72 h after the administration of a single dose of 8.0 mg/kg Hg as MeHgCl. Results shown on Table 3 indicate that the higher relative brain weights and brain mercury levels of females compared with males in the multiple dosed groups were not the sole consequence of differences in weight changes. However as Table 4 shows the regional

Table 3. The brain uptake and concentration of mercury 72 h after a single dose of 8.0 mg/kg Hg as MeHgCl. (The numbers are means ± SEM)

Sex	No. of animals	μg Hg/g brain	μg Hg in whole brain per 100 g body weight	Relative brain weight (in g per 100 g body weight)
Male	16	1.91 ± 0.07	1.41 ± 0.12	0.74 ± 0.03
Female	16	2.38 ± 0.08	2.09 ± 0.16	0.88 ± 0.03
		Differences between pairs		
		0.45 ± 0.06	0.71 ± 0.06	0.14 ± 0.02
		$t = 7.69$	$t = 10.76$	$t = 6.92$
		$P < 0.001$	$P < 0.001$	$P < 0.001$

[a] Body weight on the treatment day was used in the calculation

Table 4. The regional distribution of mercury in the brain 72 h after a single dose of 8 mg/kg Hg as MeHgCl. (Every number is the mean ± SEM of five animals)

Brain region	Weight in g[a]		Total Hg in μg[a]		μg Hg/g Tissue	
	Male	Female	Male	Female	Male	Female
Whole brain	2.06 ± 0.04 (100%)	1.86 ± 0.04 (100%)	3.38 ± 0.08 (100%)	4.12 ± 0.10** (100%)	1.64 ± 0.06	2.21 ± 0.06**
Olfactory bulbs	0.09 ± 0.01 (4.4%)	0.08 ± 0.01 (4.3%)	0.12 ± 0.02 (3.5%)	0.18 ± 0.01* (4.3%)	1.37 ± 0.11	2.19 ± 0.14*
Neocortex	0.77 ± 0.01 (37.4%)	0.71 ± 0.01 (38.2%)	1.36 ± 0.05 (40.2%)	1.72 ± 0.02** (41.7%)	1.77 ± 0.08	2.41 ± 0.05**
Thalamus hypothalamus hippocampus striatum	0.49 ± 0.01 (23.8%)	0.45 ± 0.02 (24.2%)	0.80 ± 0.02 (23.7%)	1.02 ± 0.05* (24.7%)	1.63 ± 0.07	2.36 ± 0.08**
Medulla oblongata pons midbrain	0.42 ± 0.01 (20.4%)	0.39 ± 0.01 (21.0%)	0.60 ± 0.02 (17.7%)	0.76 ± 0.01** (18.4%)	1.43 ± 0.05	1.95 ± 0.04**
Cerebellum	0.31 ± 0.05 (15.0%)	0.27 ± 0.01 (14.5%)	0.57 ± 0.03 (16.8%)	0.70 ± 0.02** (17.0%)	1.84 ± 0.09	2.57 ± 0.12**
Sum of regions	2.08 ± 0.02 (101%)	1.91 ± 0.04 (102%)	3.45 ± 0.12 (101%)	4.38 ± 0.10 (106%)		

[a] Numbers in parenthesis are expressed in % of whole brain values

* Significantly different ($P < 0.05$) from males

** Significantly different ($P < 0.001$) from males. Significant differences were calculated with the two directional Student t-test

distribution of mercury within the brain was the same in males and females.

Co-ordination disorders were more pronounced in the female rats than in the males in both the four and five times dosed groups (see Fig. 4). Dorsal root ganglia from the lumbar region of 15 male and 15 female rats were examined at 1, 4, 6, 8, and 11 days after four doses. The progression of damage was similar in both sexes, though at 11 days only one of three males but all three females had severe damage in the dorsal root ganglia. Neither males or females showed signs of cerebral or cerebellar damage after four doses, but after five treatments both sexes developed cerebellar damage (the cerebrum was not checked) and the damage was significantly more pronounced in females than in males (see Table 5).

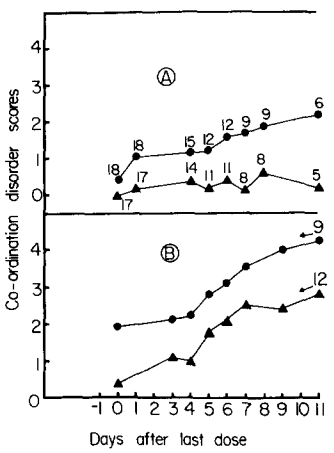

Fig. 4A and B. Co-ordination disorders after treatment with methylmercury in male and female rats. **A** the effect of four, or **B** five daily doses of 8.0 mg/kg Hg as MeHgCl. Symbols are the same as in Fig. 1. Co-ordination disorders shown are the sum of scores for failing reflex and hind leg crossing tests (range of possible scores was 0–6). For further details, see Methods

Table 5. Effects of 5 daily doses of 8.0 mg/kg Hg as methylmercury on the cerebellar granular layer in male and female rats[a]

	No of animals	Histology scores[b, c]				
		0	1	2	3	4
Males	9	–	6	2	1	–
Females	9	–	1	1	5	2

[a] Animals were killed 11–12 days after the last dose
[b] According to Magos et al. 1978
[c] Differences in the distribution of histology scores calculated with the χ^2 test after grouping data separated by the broken line into a 2 × 2 contingency table give $P < 0.001$

Discussion

After identical methylmercury treatment the whole body concentration of mercury may differ within a species due to variation in clearance half time, as in lactating versus non lactating female rats (Magos et al. 1980b) or because of different body weight changes during or after treatment, as in pregnant versus virgin female rats (Magos et al. 1980a). In these physiological conditions the brain concentration of mercury was not affected by subsequent differences in the body concentration of mercury caused by weight loss or increased clearance. Difference in the brain mercury concentration of male and female rats found in the present work could not be explained by dissimilar whole body concentrations caused either by higher weight loss or by slower whole body and blood clearance of mercury in females. Firstly the brain Hg to whole body Hg concentration ratio or the brain Hg to blood Hg concentration ratio was significantly higher in females than in males. Secondly the brain concentration of mercury was significantly higher not only in multiple dose experiments but also 72 h after a single dose of 8.0 mg/kg Hg as methylmercury. The difference in the brain accumulation of mercury was even larger when mercury contents in the whole brain were compared, as females have larger brains in relation to body weight than males.

However, the loss of body weight (or decline in weight gain), which is an early sign of methylmercury intoxication in rats, may accelerate the development of neurotoxicity through the acceleration of methylmercury uptake by the nervous system. Data presented on the brain contents of mercury in Table 1 and on the brain concentrations of mercury in Table 2 show striking differences not only between males and females, but also between four times and five times treated groups. An increase in daily doses from four to five caused a 45% increase in the ratio of brain content to body content of mercury in males and 70% in females 11–13 days after the last treatment day. The corresponding increases in the brain concentration of mercury were 56% in males and nearly 100% in females. A similar rise in the brain concentration of methylmercury with increasing number of doses was demonstrated by Berlin et al. (1975) in squirrel monkeys: the brain Hg to blood Hg ratio showed a sudden increase when the blood mercury concentration increased above 1 μg Hg/ml blood. In rats the increased brain uptake of methylmercury in females, noticeable even after a single treatment, may trigger earlier a redistribution process which favours a further increase in the brain uptake of methylmercury. Moreover as the daily weight gain of untreated female rats is less than that of males, identical anorexic effects (= identical % difference between the expected and actual body weight) are more likely to produce a negative caloric balance in females than in males.

The difference in the brain uptake of mercury between males and females or between four and five times treated rats was reflected by differences in the severity of co-ordination disorders (see Fig. 4). The relationship between co-ordination disorders and morphological damage in the selected parts of the nervous system was not so evident. Similar lack of correlation between cerebellar or dorsal root ganglion damage and co-ordination disorder has been

shown when the toxicity of methylmercury was compared in lactating and non-lactating female rats (Magos et al. 1980b). Thus it seems that in the aetiology of methylmercury induced co-ordination disorders light microscopic cerebellar or dorsal root ganglion damage cannot be a sole factor. One possibility is that before the development of visible light microscopic cerebellar granular layer damage there is some functional disorder in the brain which disturbs co-ordination. Alternatively ganglion damage alone (see males treated four times) cannot produce co-ordination disorders without some additional factor. This factor, which interacts either with a functional disorder in the cerebellum or with dorsal root ganglion damage, may be the loss of muscle tone when the calorie balance of methylmercury treated rats approaches zero or becomes negative.

In summary the brain of methylmercury treated female rats accumulated more mercury than the brain of their male counterparts, they developed co-ordination disorders after 4 days exposure and finally, after 5 days exposure their cerebellar granular layer showed more extensive damage. Whether this sex dependent sensitivity to the neurotoxic effects of methylmercury is present in other species requires further research.

Acknowledgement. The authors wish to thank Dr. Loretta Goolvard-Delaney for dissecting the rat brains for Table 4.

References

Bakir F, Damluji SF, Amin-Zaki L, Murtadha M, Khalidi A, Al-Rawi NY, Tikriti S, Dhahir HI, Clarkson TW, Smith JC, Doherty RA (1973) Methylmercury poisoning in Iraq. Science 181: 230–241

Berlin M, Grant CA, Hellberg J, Hellström J, Schutz A (1975) Neurotoxicity of methylmercury in squirrel monkeys. Arch Environ Health 30: 340–348

Greenwood MR (1975) Methylmercury poisoning in Iraq, an epidemiological assessment of the extent of the 1971–1972 outbreak. Thesis, University of Rochester, Rochester, New York

Magos L, Butler WH (1976) The kinetics of methylmercury administered repeatedly to rats. Arch Toxicol 35: 25–39

Magos L, Bakir F, Clarkson TW, Al-Jawad AM, Al-Soffi MH (1976) Tissue levels of mercury in autopsy specimens of liver and kidney. Bull WHO [Suppl] 53: 93–96

Magos L, Peristianis GC, Snowden RT (1978) Postexposure preventive treatment of methylmercury intoxication in rats with dimercaptosuccinic acid. Toxicol Appl Pharmacol 45: 463–475

Magos L, Peristianis GC, Clarkson TW, Snowden RT, Majeed MA (1980a) Comparative study of the sensitivity of virgin and pregnant rats to methylmercury. Arch Toxicol 43: 283–291

Magos L, Peristianis GC, Clarkson TW, Snowden RT (1980b) The effect of lactation on methylmercury intoxication. Arch Toxicol 45: 143–148

Received December 15, 1980