Toxicity of Methylmercury: Effects on Different Ages of Rats*

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It has been reported (SWENSSON and ULFVARSON, 1968; ULFVARSON, 1969) that methylmercury was mainly distributed in the blood stream of rats during the first 24 to 48 hours after dosing. Further CLARKSON *et al* (1973) demonstrated that methylmercury readily equilibrated between the blood and other tissues. They also claimed that reduced levels of methylmercury in the blood led to lower levels of this toxicant in brain and other soft tissues. RISSANEN (1972) reported that, since the ratio of mercury in the whole blood to that of whole body tissues decreased linearly during the first 100 days after single dosing, it was possible to predict the total body burden of mercury from mercury content of blood. However, in chronic methylmercury poisoning, the rate of accumulation of mercury in blood was dependent on the level of dosing and the concentrations of mercury in different tissues at necropsy could not be correlated with the cumulative amount administered (HERIGSTAD *et al*, 1972). Therefore, the assessment of blood mercury level can become an important indication of methylmercury toxicosis.

CASTERLINE and WILLIAMS (1972) observed that the level of mercury in RBC of baby rats, littered from dams orally dosed with methylmercury, showed the same amount as that of the mothers. However, 30 days after birth, the mercury content of RBC in the off-spring was about a tenth of that of mothers indicating the rapid disappearance of mercury from RBC of the baby rats. These findings indicated that the rate of elimination of mercury might be age-dependent. The present experiments provide evidence for the toxicity and the elimination of mercury as age-dependent.

Materials and Methods

Analytical grade methylmercuric chloride was dissolved in corn oil (Mazola) at suitable concentrations (*vide infra*) and each animal was dosed with 1 ml/100 g of its body weight.

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(i) Assessment of LD_{50} of methylmercury chloride in different ages of rats:

Male Sprague-Dawley rats were divided into six groups according to their body weights: 200 g (195-220), 300 g (295-315), 350 g (340-360), 400 g (390-410), 450 g (440-480), and 500 g (500-540) as groups 1-6 respectively.

Each group was further divided into 4 sub-groups and consisted of 20 rats and each sub-group orally received a single dose of a solution of methylmercury chloride. All sub-groups of 1 and 2 were dosed with 25, 30, 35 and 40 mg Hg/kg; sub-groups of 3, 4 and 5 were given 20, 25, 30 and 35 mg Hg/kg; and sub-groups of 6 with 15, 20, 25 and 30 mg Hg/kg. Each rat was caged individually and fed *ad libitum*. Rats were observed for 10 days to determine the LD_{50}, but the groups of 200 g, 350 g and 450 g rats were observed for a further period of 20 days to assess the onset of neurological signs. The LD_{50} was determined by the least square method (BOYD, 1972).

(ii) Determination of mercury levels in blood in different age groups:

From the results of the above experiments, it was decided to select rat groups of body weight 200 g, 350 g and 450 g to evaluate the relationship between the mercury levels in blood and the neurological disorders previously observed in these groups. Two sets of 32 rats of 200 g and 350 g weight groups and 40 rats of 450 g group were given a single oral dose of 25 mg Hg/kg as CH_3HgCl and fed *ad libitum*. The average weight gain, food, and water consumption of 4 rats of each group from the day of dosing till the 30th day were recorded (see Table I). Four animals from each group were sacrificed under ether anesthesia and the blood samples were collected from the carotid artery on the specified time intervals as indicated in Fig. 1. The concentration of mercury in the blood samples was determined by the cold-vapor atomic adsorption technique (MALAIYANDI and BARRETTE, 1970; MALAIYANDI and BARRETTE, 1972; MALAIYANDA and BARRETTE, in press).

Results and Discussions

In the LD_{50} assessment experiment, the relationship between ages and body weights of rats were 8, 10, 12, 15, 19.5 and 24.5 weeks old for body weights of 200 g, 300 g, 350 g, 400 g, 450 g and 500 g respectively. The least square equations employed for the calculation of LD_{50} factor for these groups of rats were: 200 g: $Y = (29.6 + 0.2X) \pm 2.3$ S.E.; 300 g; $Y = (24.6 + 0.17X) + 2.1$ S.E.; 350 g: $Y = (21.8 + 0.17X) + 1.0$ S.E.; 400 g: $Y = (19.6 + 0.15X) \pm 1.0$ S.E.; 450 g: $Y = (16.2 + 0.17X) \pm 1.5$ S.E.; and 500 g: $Y = (15.9 + 0.16X) \pm 1.1$ S.E.; where X represents % mortality and Y denotes the dosage levels. From these equations, the LD_{50} values were obtained by substituting X=50 and calculating the values of Y, and these data are shown in Fig. 2. It can be

Fig. 1 Total mercury content of blood of rats dosed with 25 mg Hg/kg of CH_3HgCl during 0 to 30 days.

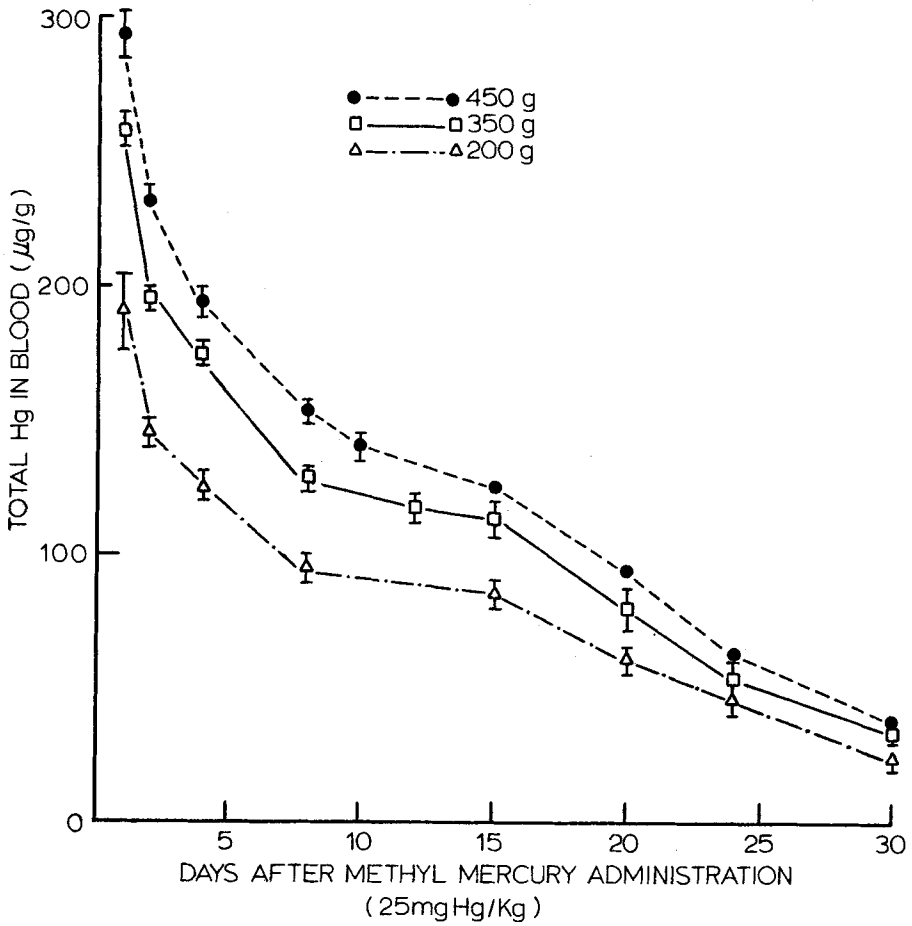
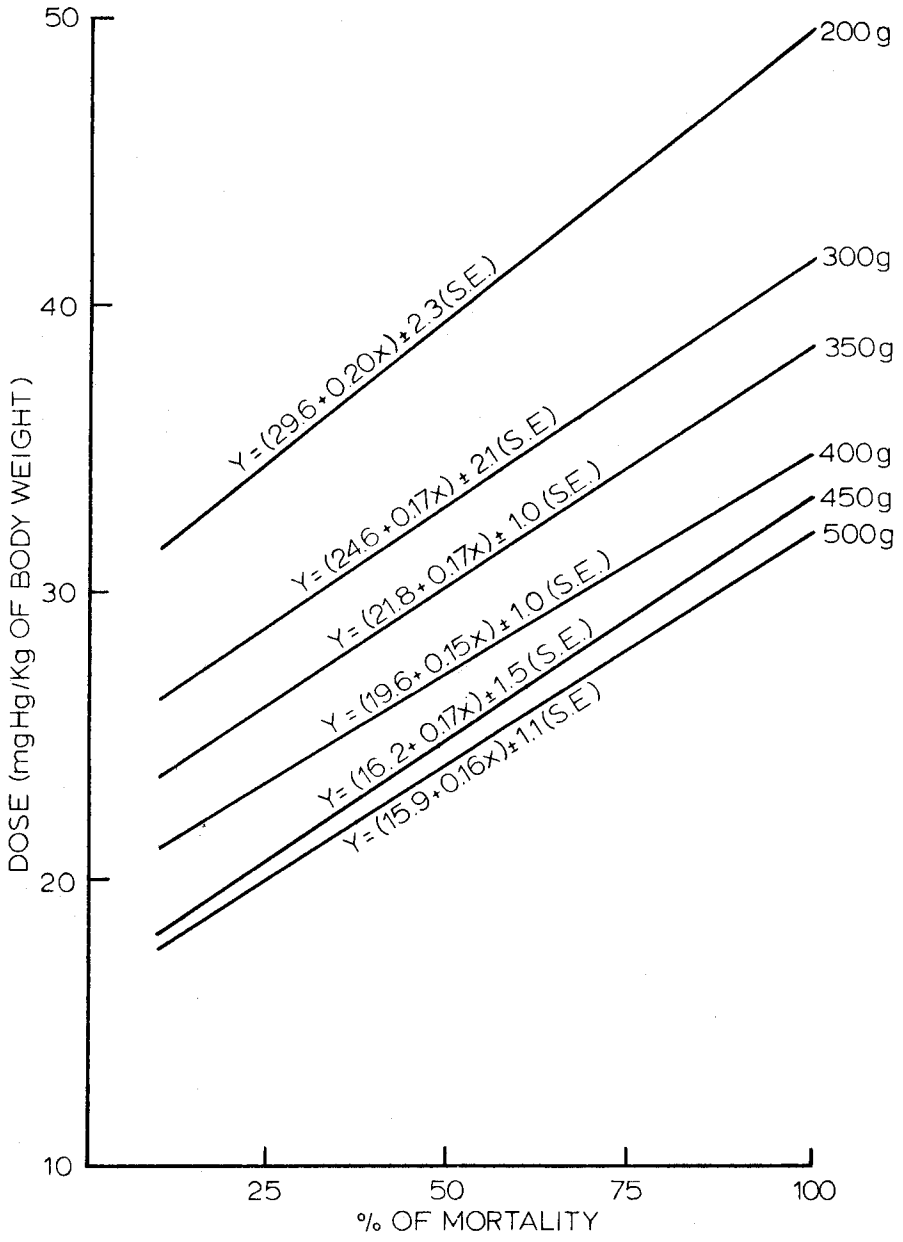


Fig. 2 Evaluation of LD_{50} of CH_3HgCl on different ages (body weights of rats.



seen that the LD_{50} decreases with increase in body weight which in these experiments has a direct relation to the age of animals. For the 8, 10, 12, 15, 19.5 and 24.5 weeks old rats the LD_{50} values were found to be 39.6 ± 2.3, 33.1 ± 2.1, 30.3 ± 1.0, 27.1 ± 1.0, 24.7 ± 1.5 and 23.9 ± 1.1 mg Hg/kg respectively. It is noteworthy that the LD_{50} values of CH_3HgCl for rats weighing 450 and 500 g groups were almost similar. The variation in the LD_{50} values ranging from 39.6 ± 2.3 to 23.9 ± 1.1 mg Hg/kg for animals weighing 200 to 500 g is analogous to the findings of CASTERLINE and WILLIAMS (1972). These authors observed that the baby rats, containing the same amount of CH_3HgCl in the RBC on the sixth day of birth as that of the mothers (59.91 ppm), were able to eliminate at a faster rate than their mothers. At the end of the 30th day, the RBC of the mothers contained about ten times more mercury than those of the baby rats. This rapid clearance rate of CH_3HgCl was possibly due to growth rate. This finding is substantiated in our experiments from the weight gain of the rats weighing 200 g, 350 g and 450 g (Table I). The weight gain of the 8-week old rats (200 g body wt.) during a period of 30 days is about +69% whereas that of the 19.5 week old rats is about -8%, and 12 week old rats, although experienced large weight loss, regained its normal weight during the last 10 days of the 30 day period. Except for the second day the youngest of the batch (8 week olds) continually gained weight with normal consumption of food and water. The oldest batch (19.5 week olds) seemed to have lost appetite during the first week post dosing. In agreement with the findings of HERIGSTAD *et al* (1972) on calves, the 8 week old rats showed a general trend of an increase in body weight.

Rats, after receiving a single dose of methylmercury (25 mg Hg/kg), showed in addition to weight changes, clinical signs of methylmercury toxicosis, such as diarrhea, debility, ruffled coats, loss of appetite and sudden onset of signs of neurological disorders in the central nervous system. The neurological signs were assessed by suspending the animals by their tails, when either of the hind limbs began to show flexion. Acute toxic stages were denoted by crossing of hind limbs and flailing movement during suspension. The 200 g rats after two days of diarrhea initially, did not develop any apparent nervous disorders. The 350 g rats began to exhibit some neurological signs between 8 and 15 days post dosing and the surviving rats of the 450 g group showed acute signs of incoordinated prehension, between 8 and 12 days post dosing. Prior to euthanasia, blood was collected from the carotid arteries for analysis of sercury. Within 15 days post dosing, 50% of the 26 surviving rats of the 350 g group and all of the surviving rats of the 450 g group showed acute neurological signs and 66% of the latter group died of methylmercury toxicosis. At the onset of neurological signs, the mercury levels in the blood of these rats were found to be 113-127 and 125-152.9 ppm for the groups weighing 350 g and 450 g respectively. These observations indicated that it would be of interest to study the retention pattern of CH_3HgCl in the blood of three selected age groups, namely 8, 12 and 19.5 week old rats.

TABLE I

Average weight gain, food and water consumption of groups in 200 g, 350 g, 450 g rats which received 25 mg Hg/kg of CH_3HgCl

Days After Administration:	200 g			350 g *			450 g **		
	Body Wt. (g)	Food (g)	Water (ml)	Body Wt. (g)	Food (g)	Water (ml)	Body Wt. (g)	Food (g)	Water (ml)
0	206	20	30	352	32	22	465	22	31
1	194	5	8	319	0	0	416	0	0
2	198	16	32	315	0	15	404	5	4
4	203	19	43	310	5	25	381	5	10
8	218	18	40	306	10	33	372	8	25
15	270	20	35	309	12	25	385	15	20
20	305	20	31	319	11	29	392	13	27
24	320	17	33	333	14	27	410	13	29
30	342	22	29	360	16	33	428	14	25

* After day 8, only 26 rats survived.

** After day 8, only 20 rats survived; one of the remaining rats died on day 21.

The mercury levels in the blood of these three groups are shown in Table II. One day after dosing, the blood levels of mercury in 8, 12, and 19.5 week old rats were determined to be 192.4 $\pm$ 15.8, 258.2 $\pm$ 6.5 and 294.4 $\pm$ 10.5 ppm respectively although they were all dosed with the same amount of CH_3HgCl (25 mg Hg/kg). Since the rats weighing 450 g had received a dosage level in the proximity of the LD_{50} value (24.7 $\pm$ 1.5 mg Hg/kg; Fig. 2) there were only a few surviving rats for the completion of the experiment with the result that after day 15 post dosing, only one animal in this group was left for final analysis for mercury.

It is obvious that there is less tendency for the blood of young rats (200 g body wt.) to accumulate CH_3HgCl whereas both the 350 g and 450 g groups tend to retain higher percentages of this toxic material than the younger group (Table II)

From Table II, it can be seen that the rate of disappearance of CH_3HgCl in young rats (8 weeks old), one day after administration of the toxicant was almost the same as that of the other two groups. In the case of the 8 week old rats, the mercury level in blood decreased below 100 ppm even on the 8th day post dosing. However, on the same day, the other two groups (350 g and 450 g) had levels

Table II

Days After Administration	200 g	350 g	450 g
	ppm of Total Hg	ppm of Total Hg	ppm of Total mercury
1	192.4 ± 15.8	258.2 ± 6.5 (P<0.01)	294.4 ± 10.5
2	145.2 ± 3.4	195.0 ± 3.2 (P<0.005)	231.8 ± 4.6
4	127.0 ± 3.5	174.9 ± 3.5 (P<0.01)	194.3 ± 5.1
8	95.9 ± 3.5	127.3 ± 3.4 (P<0.005)	152.9 ± 3.6
10			140.8 ± 5.8
12		117.1 ± 3.8	
15	84.4 ± 4.7	113.0 ± 5.5	125.0 (n=2)
20	62.6 ± 6.0	79.8 ± 8.0	94.0 (n=1)
24	46.5 ± 3.3 (P<0.15)	53.7 ± 6.0	63.4 (n=1)
30	23.5 ± 2.6 (P<0.05)	34.8 ± 2.9	37.5 (n=1)

Each figure represents analyses of 4 animals and 1 standard error unless otherwise indicated.

P value represents the comparison between 200 g and 350 g rats; 350 g and 450 g rats.

of 127.3 and 152.9 ppm respectively. Once the blood-mercury level dropped below 100 ppm, very little effect was imposed on the central nervous system indicating the probable critical mercury level in blood.

During these experiments, it was observed that the blood of 8 week old rats dosed with CH_3HgCl was less susceptible to haemolysis than the blood of the other two older groups. It was considered worthwhile to determine the levels of mercury transported through the cell membrane as well as those of plasma.

In our preliminary studies, two sets of 200 g and 450 g rats were dosed with 25 mg Hg/kg as CH_3HgCl. After 24 hrs. the animals were sacrificed as before and the plasma was separated from the heparinized blood by centrifugation. The erythrocytes were haemolyzed, centrifuged, and the supernatant was decanted. The samples of whole blood, plasma and supernatant from haemolysed blood were analysed for mercury content.

The mercury contents of the whole blood were found to be 229 and 280 ppm for 200 g and 450 g respectively indicating the whole blood levels were similar to values obtained in the previous experiment. The mercury levels in plasma and haemolysed fraction were estimated to be 1.98; 19.2 for the 200 g rats and 48.0; 65.5 ppm of mercury for the 450 g rats respectively. This indicates a higher mercury concentration in plasma as well as haemolysed fraction of the older rats. It also shows that the mercury was mainly distributed in the membrane of the erythrocyte, as 92% and 72% and had very large differences in the red blood cells to plasma ratio of mercury concentration, 115:1 and 5:1 for the 200 g and 450 g rats respectively. This increased transport of CH_3HgCl through the erythrocyte membrane might have changed the permeability of the erythrocyte membrane from animals treated with CH_3HgCl might lead to the elucidation of the mechanism of toxicity of methylmercury chloride.

Summary

LD_{50} of methylmercury chloride has been shown to be dependent on the ages of the rats. As the age increases, the LD_{50} decreases, i.e. the younger rats could tolerate higher doses of methylmercury than the older ones. The LD_{50} were 39.6 ± 2.3, 33.1 ± 2.1, 30.3 ± 1.0, 27.1 ± 1.0, 24.7 ± 1.5 and 23.9 ± 1.1 mg Hg/kg for the 200 g, 300 g, 350 g, 400 g, 450 g and 500 g rats respectively.

The elimination of mercury from blood showed little correspondence to age during the 30 days duration. The onset of neurological symptoms after receiving 25 mg Hg/kg of methylmercury chloride occurred between 8 to 15 days post dosing in the surviving rats. Rats unaffected during the latency period did not show neurological signs if their blood-mercury levels decreased to below 100 ppm.

Young and old rats showed marked differences in the distribution of mercury in the blood. In the erythrocyte membrane, the eight week old rats retained a higher concentration of the toxic metal than did the 19.5 week old rats. Also, there were significant differences in the ratios of mercury content in the red blood cells to that of plasma; young rats showing 115:1 and for the old ones being 5:1. The permeability of erythrocyte membrane to mercury might play an important role in the age factors on the suceptibility of methylmercury intoxication.

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References

BOYD, E.M., Predictive Toxicometrics, Scientechnica Ltd., Bristol, England (1972)

CASTERLINE, Jr., J.L. and WILLIAMS, C., Bull. Envir. Cont. Toxicol., 7, 292 (1972)

CLARKSON, T.W., SMALL, H., and NORSETH, T., Arch. Environ. Health, 26, 173 (1973)

HERIGSTAD, R.R., WHITEHAIR, C.K., BEYER, N., MICKELSON, O., and ZABIK, M.J., J. Am. Vet. Med. Assoc., 160, 173 (1972)

MALAIYANDI, M. and BARRETTE, J.P., Anal. Letters, 3, 579 (1970)

MALAIYANDI, M. and BARRETTE, J.P., J. Ass. Off. Anal. Chem., 55, 951 (1972)

MALAIYANDI, M. and BARRETTE, J.P., Arch. Environ. Contam. & Toxicol., (in press)

NORSETH, T., CLARKSON, T.W., Arch. Environ. Health, 21, 170 (1970)

RISSANEN, K., The National Workshop on Mercury in Man, Ottawa, Ontario, Canada, Oct. 16-18 (1972)

SWENSSON, A. and ULFVARSON, U., Acta Pharmacol. et Toxicol., 26, 273 (1968)

ULFVARSON, U., Toxicol. and Appl. Pharmacol., 15, 525 (1969)