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Translocation and Fluxes of Mercury in Neonatal and Maternal Rats Treated with Methyl Mercuric Chloride During Gestation[1] (38315)

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Although the placenta constitutes a strong barrier for mercurial transmission (1–4), mercury can still be transferred to the young (2, 5, 6). Embryolethal and teratogenic manifestations were seen in fetuses of experimental animals after pregnant mothers received organomercurial compounds (7–9). In humans, neurological symptoms developed in children whose mothers were exposed to mercury during pregnancy (10).

Previous findings indicated a threefold increase of mercury in the brain of fetal rats within 5 days after feeding methyl mercury to the mother (6). Similarly, within 5 days, brains of nursing rats contained mercury 2.3 times tnat found in the brain of lactating rats fed methyl mercury (10). Thus, indications are that fetal and neonatal rats can accumulate higher concentrations of mercury in their brains than their mothers'. This work was undertaken to study the mercury deposition in various brain parts and tissues of neonatal and maternal rats after the mothers were force-fed ^{203}Hg as methyl mercuric chloride during gestation. Furthermore, the pool sizes, turnover rates and times and half-lives of mercury in various organs were determined in both mothers and pups.

Procedures. Sprague–Dawley female rats averaging 275 g and mature males of the same strain were cohabitated and allowed to mate. Vaginal smears were examined daily to establish the presence of sperms. The day sperms were found in the smear was considered as day zero of pregnancy. Pregnant rats were kept individually in cages with pine-chip beddings. A nutritonally adequate grain diet and water were fed *ad libitum*.

Pregnant rats during the 16th day of gestation were force-fed by stomach tubing 4 μCi of ^{203}Hg as CH_3 ^{203}HgCl[3] dissolved in corn oil so that 1 g contained the predetermined radioactivity. The specific activity of CH_3 ^{203}HgCl was 2.5 mCi/mg Hg.

At birth, 7, 14, and 21 days after parturition equivalent to 5, 12, 19, and 26 days after the administration of the mercurial compound four dams and 12 pups (3 progenies/dam) were killed by decapitation. An additional six pups representing two pups each from three mothers were sacrificed one week after weaning or 33 days after the administration of the methyl mercury chloride. Because of the number of pups needed, only litter sizes of 10 or more were used.

The following tissues from both the pup and mother were excised and counted for ^{203}Hg radioactivities: cerebrum, cerebellum, pons + midbrain, medulla, hypothalamus, pituitary, olfactory bulbs, kidneys, liver, and gastrocnemius muscle[4]. ^{203}Hg activities were also measured in the blood collected by heart puncture, except in the newborn rats, under ether anesthesia. Dam hair samples and pup hair samples starting on the 14th day after birth were plucked along the back, rinsed with acetone and air dried. All the tissues were digested at 50° with hyamine hydroxide. Blood, liver, and kidney samples were decolorized with benzoyl peroxide. The volume of the samples were made to 15 ml with Aquasol.[5]

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[3] CH_3 ^{203}Hg was puchased from New England Nuclear, Boston, MA.

[4] Medial head according to Greene, E. C., Anatomy of the Rat, Hafner Publishing Co., NY (1968).

[5] Aquasol is a xylene-based scintillation solution containing all the necessary fluors. It was purchased from New England Nuclear, Boston, MA.

^{203}Hg radioactivity were counted with a Nuclear Chicago Scintillation Counter according to the statistical procedure of Loevinger and Berman (12) to an accuracy of ± 2%. The ^{203}Hg radioactivities and standards were corrected for both background activities and physical decay.

In another experiment, foster dams were used to rear pups delivered by dams force-fed 4 μCi of ^{203}Hg as methyl mercuric chloride during the 16th day of gestation. Foster mothers were provided each with 10 pup siblings from mothers treated with the mecurial compound. The foster dams used were those untreated control mothers that delivered pups on the same day as the ^{203}Hg treated dams. On the 7th and 21st day of foster rearing, three foster dams and 12 pups (4 littermates/foster dam) were killed by decapitation. An additional six pups (2 pups/litter) were also sacrificed one week after weaning or at 28 days after birth. Tissues collected, sample preparation techniques and radioactive counting methods were similar to those of the first experiment.

Results and Discussion. No clinical symptom was anticipated throughout the experiment in either mothers or pups since only 0.0058 mg Hg/kg body wt were force-fed to each pregnant rat. This is equivalent to 0.03% of LD_{50} for an adult rat (13). The LD_{50} for pregnant rats is not known.

The concentration (% of dose/g tissue) of ^{203}Hg radioactivities in all tissues except hair of dams (Table I) and pups (Table II) decreased as the time after the administration of methyl mercury chloride increased. In the dams as well as in the pups, the highest concentration of ^{203}Hg among the different brain parts at any particular time period was found in the pituitary. Among the other tissues in the dams, kidney had the highest concentration, and in the pup, blood had the highest concentration at birth and hair the highest from 14 days of age. The higher concentration of radioactivities of the kidneys as compared to the blood of the mother of the present work is in accord to that found earlier in adult rats (14). A comparison of the blood concentrations between mothers and pups revealed that at birth the pup had approximately 27% higher concentration (Table I vs Table II). Furthermore, the mean concentration of ^{203}Hg in all other tissues except in the kidneys of the pups at birth was higher than in the corresponding tissues of the dams. Interestingly, the ^{203}Hg concentration in the hair of the pups was as much as 38 times that in hair from mothers (Table I vs Table II).

Among brain parts in both the dams (Table I) and pups (Table II), cerebrum contained the highest absolute quantity of ^{203}Hg (expressed as % of dose/organ) and pituitary the lowest at each time period. A similar observation was reported in earlier studies (6, 11). In the mothers as well as in the pups, the liver because of its greater weight contained a higher absolute quantity of ^{203}Hg than the kidneys at all time periods except at the last period. The kidney tripled in weight between 21 and 28 days of age in the pup and this was the reason for the 2.4 times increase in ^{203}Hg found in this tissue (Table II).

Since it is known that there are differential toxic effects of mercury on various organs and parts of the brain as well as between young and old of the same species, we also determined whether there are differential pool sizes and several parameters of fluxes of the metal in the various tissues used in these studies (Table III).

Thus, pool size, turnover rate, turnover time and half-life of mercury based on data in Tables I and II and calculated according to the procedures described by Cook (15) are presented (Table III). The values in Table III for the pups should be considered in a different light from those for the dams since the pups had influx of mercury throughout the nursing period whereas the dams received mercury only once. We feel that the continuous influx of ^{203}Hg from mothers to pups provided a novel way of studying fluxes. It is novel in that we considered the pups just as we considered a mother's organ liver, for example, as part of the mother. In this example, the pup like the liver continuously accumulated ^{203}Hg, each at its own rate (fluxes), both depended on the mothers. In contrast to the classical way of providing a single pulse in determining fluxes, the continuous influx of ^{203}Hg via the milk will necessarily be slower in turnover time, turnover rate, and half-life values. The recycling of mercury from pups to mothers via the ingestion of excreta from the pup is minimal and is discussed in a later section. The recycling of excreta ^{203}Hg is in a way a reverse phenomenon of the cycling from mother's milk to the pup.

Among the various brain parts in both the mother and pups, cerebrum contained the highest mercury pool size. In both mothers and pups, the liver pool size was larger than the kidney

TABLE I. Concentrations and Quantities of ^{203}Hg at Parturition, 7, 14, and 21 Days After Parturition in Tissues of Dams Force-Fed CH_3 $^{203}HgCl$ During the 16th Day of Gestation.

Tissues	% of dose/g × 10^{-2}				% of dose/organ × 10^{-2}			
	Parturition	7 days	14 days	21 days	Parturition	7 days	14 days	21 days
Brain[b]	10.17 ± 0.28[a]	6.63 ± 0.60	4.38 ± 0.21	2.54 ± 0.41	18.07 ± 0.27	12.14 ± 1.08	7.92 ± 0.44	4.66 ± 0.73
Cerebrum	10.74 ± 0.45	7.57 ± 0.71	4.73 ± 0.22	2.73 ± 0.54	12.38 ± 0.24	8.82 ± 0.81	5.55 ± 0.26	3.13 ± 0.59
Cerebellum	10.99 ± 0.61	7.37 ± 0.88	4.21 ± 0.46	2.68 ± 0.34	2.93 ± 0.15	1.93 ± 0.38	1.16 ± 0.14	0.76 ± 0.09
Pons + midbrain	7.26 ± 0.23	4.84 ± 0.35	3.09 ± 0.16	1.89 ± 0.21	1.09 ± 0.13	0.90 ± 0.07	0.46 ± 0.05	0.31 ± 0.05
Medulla	7.38 ± 0.40	4.08 ± 0.30	2.79 ± 0.18	1.07 ± 0.18	0.72 ± 0.11	0.43 ± 0.05	0.27 ± 0.02	0.18 ± 0.03
Hypothalamus	11.47 ± 1.25	7.86 ± 0.67	6.45 ± 0.80	3.96 ± 0.55	0.26 ± 0.02	0.20 ± 0.03	0.13 ± 0.02	0.07 ± 0.01
Pituitary	20.38 ± 1.30	20.47 ± 8.82	6.36 ± 0.96	4.48 ± 1.02	0.23 ± 0.02	0.23 ± 0.09	0.07 ± 0.01	0.04 ± 0.01
Olfactory bulbs	8.96 ± 0.37	6.06 ± 0.72	3.57 ± 0.06	1.97 ± 0.18	0.68 ± 0.11	0.37 ± 0.04	0.28 ± 0.02	0.16 ± 0.02
Kidneys	132.95 ± 14.98	120.59 ± 8.56	93.91 ± 3.58	59.95 ± 9.20	105.67 ± 9.51	109.83 ± 6.04	98.51 ± 3.78	60.92 ± 8.29
Liver	20.64 ± 1.92	16.20 ± 2.56	7.17 ± 0.46	3.17 ± 0.42	236.27 ± 20.00	219.48 ± 35.22	116.05 ± 6.56	52.07 ± 6.89
Gastrocnemius	21.08 ± 1.83	12.33 ± 0.66	6.18 ± 0.32	3.18 ± 0.51	15.18 ± 1.84	8.66 ± 1.13	4.16 ± 0.43	1.76 ± 0.40
Blood	57.76 ± 2.63	31.51 ± 2.37	25.04 ± 1.52	11.73 ± 1.44				
Hair	4.18 ± 1.02	5.97 ± 0.37	9.69 ± 3.24	3.16 ± 0.66				

[a] Each value represents the average of four observations ± SE.

[b] Brain values calculated from weighted values of the individual brain parts.

TABLE II. Concentrations and Quantities of ^{203}Hg in Tissues of Pups at Birth, 7, 14, 21, and 28 Days of Age. The Mothers of Pups were Exposed to CH_3 $^{203}HgCl$ During the 16th Day of Gestation.

Tissues	% of dose/g × 10^{-2}					% of dose/organ × 10^{-2}				
	Birth	7 days	14 days	21 days	28 days	Birth	7 days	14 days	21 days	28 days
Brain[c]	15.74 ± 0.57[a]	5.43 ± 0.13[a]	1.84 ± 0.06[a]	0.88 ± 0.05[a]	0.43 ± 0.06[b]	3.78 ± 0.09	3.82 ± 0.08	2.13 ± 0.06	1.19 ± 0.07	0.60 ± 0.06
Cerebrum	14.68 ± 0.43	5.18 ± 0.16	1.87 ± 0.06	0.89 ± 0.06	0.42 ± 0.06	2.48 ± 0.06	2.52 ± 0.07	1.50 ± 0.05	0.87 ± 0.06	0.40 ± 0.05
Cerebellum	17.02 ± 1.21	4.81 ± 0.17	1.47 ± 0.05	0.70 ± 0.04	0.32 ± 0.02	0.24 ± 0.02	0.22 ± 0.01	0.20 ± 0.01	0.12 ± 0.01	0.06 ± 0.01
Pons + midbrain	16.99 ± 0.70	6.28 ± 0.19	2.09 ± 0.06	0.78 ± 0.05	0.50 ± 0.03	0.38 ± 0.02	0.29 ± 0.01	0.22 ± 0.01	0.07 ± 0.01	0.05 ± 0.01
Medulla	17.74 ± 0.77	5.76 ± 0.17	1.72 ± 0.12	0.82 ± 0.05	0.48 ± 0.05	0.40 ± 0.03	0.22 ± 0.01	0.09 ± 0.00	0.05 ± 0.00	0.04 ± 0.00
Hypothalamus	20.05 ± 1.77	7.70 ± 0.35	3.00 ± 0.17	1.82 ± 0.20	1.31 ± 0.16	0.17 ± 0.01	0.12 ± 0.01	0.05 ± 0.00	0.03 ± 0.00	0.02 ± 0.00
Pituitary		55.62 ± 15.55	5.48 ± 1.14	5.18 ± 1.26	3.48 ± 1.05		0.07 ± 0.02	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Olfactory bulbs	24.28 ± 3.09	5.39 ± 0.30	1.74 ± 0.09	0.94 ± 0.11	0.66 ± 0.13	0.08 ± 0.01	0.11 ± 0.01	0.07 ± 0.00	0.04 ± 0.00	0.03 ± 0.01
Kidneys	33.55 ± 0.98	22.55 ± 0.53	11.96 ± 0.30	7.86 ± 0.62	5.99 ± 0.37	1.07 ± 0.05	1.98 ± 0.06	1.79 ± 0.07	1.62 ± 0.12	3.93 ± 0.25
Liver	29.85 ± 1.80	14.00 ± 0.57	5.05 ± 0.33	2.28 ± 0.21	0.64 ± 0.10	7.69 ± 0.56	6.91 ± 0.25	4.74 ± 0.36	3.53 ± 0.24	1.78 ± 0.24
Gastrocnemius		8.37 ± 0.29	2.41 ± 0.09	1.14 ± 0.11	0.50 ± 0.03		0.20 ± 0.03	0.07 ± 0.01	0.06 ± 0.00	0.06 ± 0.01
Blood	79.57 ± 4.02	22.37 ± 0.88	6.01 ± 0.22	2.50 ± 0.16	1.52 ± 0.19					
Hair			165.44 ± 5.51	121.23 ± 14.91	121.93 ± 6.30					

[a] Each value represents the average of 12 observations ± SE.

[b] Each value represents the average of six observations ± SE.

[c] Brain values calculated from weighted values of the individual brain parts.

TABLE III. Pool Size, Turnover Rate, Turnover Time and Half-Life of ^{203}Hg in Tissues of Maternal and Neonatal Rats Exposed to $CH_3\,^{203}HgCl$ During the 16th Day of Gestation.

Tissues	Pool size (mg Hg × 10^{-5})		Turnover rate (mg Hg/day × 10^{-6})		Turnover time (days)		Half-life (days)		Correlation coefficient (r)[a]	
	Dam	Pup	Dam	Pup	Dam	Pup	Dam	Pup	Dam	Pup
Brain[b]	31.13	8.40	8.69	2.53	35.84	33.22	10.79	10.00	−0.998	−0.966
Cerebrum	22.15	5.53	6.32	1.62	35.09	34.25	10.56	10.31	−0.994	−0.957
Cerebellum	4.98	0.47	1.41	0.10	35.34	47.62	10.64	14.33	−0.999	−0.923
Pons + midbrain	1.99	1.16	0.55	0.45	36.23	26.04	10.91	7.84	−0.981	−0.918
Medulla	1.19	0.70	0.35	0.27	34.84	26.46	10.49	7.96	−0.999	−0.983
Hypothalamus	0.48	0.33	0.13	0.12	36.90	28.41	11.11	8.55	−0.984	−0.990
Pituitary	0.56	0.14	0.23	0.05	25.06	27.62	7.54	8.31	−0.946	−0.775
Olfactory bulbs	1.11	0.16	0.32	0.03	34.84	54.35	10.49	16.36	−0.991	−0.889
Kidneys	163.77	1.21	17.85	0.18	91.74	67.11	27.61	20.20	−0.830	+0.806
Liver	508.43	14.07	163.21	3.14	31.15	44.84	9.38	13.50	−0.951	−0.966
Gastrocnemius	33.29	0.34	14.88	0.08	22.37	42.74	6.73	12.86	−0.996	−0.837
Blood[c]	99.60	157.58	30.98	98.82	32.15	15.95	9.68	4.80	−0.982	−0.986

[a] All correlation coefficients are highly significant at $P < 0.05$ when concentrations were regressed against time periods using data presented in Tables I and II.

[b] Brain values calculated from weighted values of the individual brain parts.

[c] Values expressed in terms of a unit (1 g) of blood.

pool size. Both the liver and cerebrum pool sizes reflected to a large degree the size of the organ. The pool sizes of the muscle mass as represented by gastrocnemius, and blood must be very large since they represent a larger proportion of the body than any of the other organs studied.

With few exceptions, turnover rates (mg Hg/day) found in the different organs and brain parts of the pups were faster and probably reflect the faster growth of the pups as compared to the mothers. The half-lives of mercury in brain, kidneys, liver and gastrocnemius of mother rats were 10.79, 27.61, 9.38, and 6.73 days, respectively. In the pups, the corresponding half-lives were 10.00, 20.20, 13.50, and 12.86 days.

Correlation coefficients (r values) calculated by regressing concentrations against time indicate that the two factors are significantly related and the signs of the correlations indicate the direction of the relationship. All correlations were negative except for pup kidneys (Table III). The positive correlation coefficient obtained for kidneys of the pups suggest that the mercury lost from other tissues was deposited in the kidneys. This finding also implies the increasing affinity of growing kidneys for mercury.

The deposition of ^{203}Hg in the different brain parts and organs of untreated foster dams while nursing the ^{203}Hg contaminated pups demonstrates that the pups' excreta were ingested by the mothers. This phenomenon is known to occur in rats when the young are cleaned, groomed and induced to urinate by their mothers (16). The pattern of deposition and concentration of ^{203}Hg in brain parts and tissues of the foster dams, although much lower in magnitude, were similar to those of the dams directly exposed to mercury by gavage during gestation (Table I vs Table IV). The concentration of ^{203}Hg in the blood, kidneys, gastrocnemius, pons + midbrain, medulla, and olfactory bulbs increased with foster rearing time. Generally, the deposition of ^{203}Hg in the foster mothers ranged from 0.0001 to 0.067% of the dose originally given to the pregnant rats.

In the foster reared pups, just like in the pups of the first experiment that were exposed to mercury during fetal development and nursing period, the deposition and concentration of mercury in the various organs and brain parts decreased with time (Table II vs Table V). Starting at 7 days after birth, the concentration and absolute quantity of ^{203}Hg in the tissues of foster reared pups were lower than corresponding tissues of the pups reared by their natural mothers. Lower values were expected in foster-reared pups since they were essentially exposed to mercury only during fetal development and not during the nursing period.

Summary. Pregnant rats, during the 16th day of gestation, were force-fed a tracer dose of ^{203}Hg as methyl mercuric chloride. At parturi-

TABLE IV. Concentrations and Quantities of ^{203}Hg at 7 and 21 Days in Tissues of Foster Dams After Rearing Pups Delivered by Mothers Force-Fed CH_3 ^{203}HgCl During the 16th Day of Gestation.

Tissues	% of dose/g × 10^{-2}		% of dose/organ × 10^{-2}	
	7 days	21 days	7 days	21 days
Brain[b]	0.44 ± 0.26[a]	0.33 ± 0.06	0.79 ± 0.44	0.64 ± 0.08
Cerebrum	0.47 ± 0.29	0.36 ± 0.05	0.54 ± 0.32	0.44 ± 0.06
Cerebellum	0.55 ± 0.34	0.27 ± 0.06	0.15 ± 0.09	0.08 ± 0.02
Pons + midbrain	0.09 ± 0.02	0.21 ± 0.04	0.01 ± 0.00	0.04 ± 0.00
Medulla	0.16 ± 0.02	0.28 ± 0.08	0.01 ± 0.00	0.03 ± 0.00
Hypothalamus	1.31 ± 0.81	0.66 ± 0.36	0.02 ± 0.01	0.01 ± 0.00
Pituitary	1.79 ± 0.86	1.41 ± 0.43	0.02 ± 0.00	0.01 ± 0.00
Olfactory Bulbs	0.20 ± 0.13	0.23 ± 0.04	0.01 ± 0.00	0.01 ± 0.00
Kidneys	0.72 ± 0.43	1.72 ± 0.33	0.76 ± 0.50	2.15 ± 0.35
Liver	0.47 ± 0.03	0.36 ± 0.09	6.69 ± 0.73	6.67 ± 2.09
Gastrocnemius	0.21 ± 0.19	0.47 ± 0.05	0.13 ± 0.11	0.32 ± 0.08
Blood	0.35 ± 0.13	0.76 ± 0.10		
Hair	2.24 ± 1.04	1.51 ± 0.64		

[a] Each value represents the average of three observations ± SE.

[b] Brain values calculated from weighted values of the individual brain parts.

TABLE V. Concentrations and Quantities of ^{203}Hg at 7, 21, and 28 Days in Tissues of Foster Reared Pups Exposed to CH_3 $^{203}HgCl$ During the 16th Day of Embryonation.

Tissues	% of dose/g × 10^{-2}			% of dose/organ × 10^{-2}		
	7 days	21 days	28 days	7 days	21 days	28 days
Brain[c]	4.82 ± 0.28[a]	0.71 ± 0.05[a]	0.41 ± 0.07[b]	3.53 ± 0.17	0.92 ± 0.06	0.55 ± 0.09
Cerebrum	4.57 ± 0.25	0.68 ± 0.05	0.42 ± 0.08	2.34 ± 0.12	0.61 ± 0.04	0.40 ±0.08
Cerebellum	4.50 ± 0.27	0.66 ± 0.05	0.25 ± 0.02	0.21 ± 0.01	0.10 ± 0.01	0.04 ± 0.00
Pons + midbrain	5.59 ± 0.40	0.71 ± 0.03	0.34 ± 0.03	0.55 ± 0.03	0.07 ± 0.01	0.03 ± 0.00
Medulla	5.30 ± 0.40	0.76 ± 0.07	0.33 ± 0.05	0.17 ± 0.01	0.05 ± 0.01	0.02 ± 0.00
Hypothalamus	7.35 ± 0.44	1.79 ± 0.20	0.87 ± 0.16	0.11 ± 0.01	0.03 ± 0.00	0.01 ± 0.00
Pituitary	30.05 ± 8.62	8.93 ± 1.33	2.09 ± 1.37	0.03 ± 0.01	0.02 ± 0.00	0.01 ± 0.00
Olfactory bulbs	5.28 ± 0.50	0.88 ± 0.09	0.45 ± 0.06	0.12 ± 0.01	0.04 ± 0.00	0.02 ± 0.00
Kidneys	19.62 ± 0.54	6.74 ± 0.34	5.05 ± 0.31	1.97 ± 0.09	1.60 ± 0.10	3.98 ± 0.19
Liver	12.01 ± 0.71	1.98 ± 0.13	0.41.± 0.03	7.09 ± 0.26	3.02 ± 0.19	1.39 ± 0.08
Gastrocnemius	7.69 ± 0.63	0.95 ± 0.09	0.26 ± 0.03	0.14 ± 0.02	0.06 ± 0.00	0.04 ± 0.00
Blood	18.18 ± 1.31	2.02 ± 0.10	0.83 ± 0.04			
Hair		106.38 ± 11.95	75.75 ± 1.76			

[a] Each value represents the average of 12 observations ± SE.
[b] Each value represents the average of six observations ± SE.
[c] Brain values calculated from weighted values of the individual brain parts.

tion, 7, 14, 21, and 28 days after parturition, the ^{203}Hg radioactivities in the different brain parts and organs of maternal and neonatal rats were determined. The concentration of ^{203}Hg in the brain, brain parts, and organs of mothers and pups decreased with time after force-feeding. At all time periods, cerebrum contained the greatest quantity of ^{203}Hg and pituitary the lowest in both mothers and pups due primarily to the size of the tissue.

The blood concentration of ^{203}Hg in the pups at birth was 27% higher than that of the dams. Similarly, at birth, the concentration of ^{203}Hg in the different brain parts and organs of the pups, except in the kidney, was higher than corresponding tissues in the dams.

Mercury pool size in the brain and brain parts of maternal and neonatal rats also primarily reflected the size of the tissues studied. In general, a faster turnover of mercury in the pups was observed. The half-life of mercury in the maternal brain, kidneys, liver, and gastrocnemius were 10.79, 27.61, 9.38, and 6.73 days, respectively. In the pups, corresponding half-life values were 10.00, 20.20, 13.50, and 12.86 days.

In another study, foster rearing of pups originally contaminated in utero with the radioactive mercury demonstrated that mercury excreted by pups recirculated to the foster dams. The uptake of ^{203}Hg in the brain, brain parts and organs of foster dams ranged from 0.0001 to 0.067% of the original dose administered to the mothers of the contaminated pups.

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