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EFFECTS OF METHYL MERCURY ON THE HARP SEAL

by

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The present experiment reports the results of controlled exposure of captive animals to methyl mercury chloride.

Four seal pups (73-2, 4, 7 and 8) 2-3 weeks of age, captured on the ice of the Gulf of St. Lawrence in March of 1973, and two lactating adults, one (71-7) 16 years old, weighing 111 kg, and another (D7) 11 years old, weighing 89.3 kg with young were brought to Guelph from the Gulf of St. Lawrence on March 20, 1970.

Hand feeding of the pups commenced on day 1 and by day 67 all were eating normally. The normal adult food intake averaged 3.5 kg/day before the experiment. All seals received supplements according to their food intake and weight (Ronald et al. 1970).

Pre-feeding electrocardiograms were obtained every month preceeding the start of methyl mercury exposure and weekly thereafter. The electrocardiograms were compared to:

1. normal cardiograms of 40 seals, previously examined,
2. with the animal's own previous record as the experiment progressed.

The eyes of the seals were examined for pupil response before and during the experiment (Lavigne and Ronald 1975). Changes in normal patterns should indicate changes in neurological responses possibly associated with mercury toxicity.

The methyl mercury was in the powdered chloride form. It was placed in a gelatin capsule and weighed to an accuracy of

0.01 mg. Supplements, fish, tank water and personnel were examined for mercury and selenium levels, none was significant. The flow rate in the holding tanks was adequate to change the water every 4 h.

The seals were paired according to dose levels namely 73-2 and 8 (25 mg/kg) 71-7 and 73-7 (0.25) 73-4 and D7 (0.0).

Description of animals and experimental conditions

The animals varied in temperament before and during the experiment. All harp seals in the Guelph colony are supervised for diseases and other changes and also offered continuous medical supervision during the experimental period.

High dose

Both seals lost weight (19.8% and 17.2%). The animals became listless within days (2 and 3) of methyl mercury exposure. Both animals vomited after feeding but retained the mercury enclosed in the gelatin capsule. Both seals showed decreased appetites but remained responsive to noise and movement up to death. One seal (73-8) often exhibited the "possum" reaction (Ronald et al 1969), this and the lack of quantity of blood in the hind flipper indicated that the peripheral stunt was in play. 73-8 also retained the pupillary response (Lavigne and Ronald 1975) indicating the presence of a corneal reflex, up to a few hours before death. Tremors occurred, especially in the hind limbs. The seals died 25 and 26 days after exposure.

Low dose

There was no definite change in 71-7 during the feeding of methyl mercury which began on day 289 (January 24) and continued for 61 days. Her weight varied from 184 kg on day 289 to 158 kg on day 343. CH_3HgCl was fed 73-7 on day 289 and continued for 93 days. He was given 0.25 mg/kg daily and his weight was 66 kg on day 289, he began losing weight on day 264 and weighed 60 kg at death on day 382. His appetite remained unaffected throughout. From day 351 the seal became much quieter but more erratic.

Controls

The controls seemed healthy and in good condition throughout the experiment. They maintained their weight throughout the exposure period.

Food Consumption

In the high dose animals 73-2 regurgitated almost every day until her death and 73-8 became increasingly more lethargic. The food consumption of the lower dose animals did not appear to change with the intake of CH_3HgCl . The controls ate well and were healthy throughout the entire experiment except that their appetites declined on days they were handled.

Weight changes

The pups 73-2, 4, 7 and 8 gained weight slowly for the first 196 days. Weight losses resulted when the seals were being

moved, after some injections, and during the moult (as in nature). After the early doses of CH_3HgCl were administered the high dose animals began to lose weight due to 73-2's regurgitation and 73-8's lethargy. The adults appeared to keep a fairly steady weight pattern throughout the experiment.

Mercury levels

Methyl mercury levels in seal blood remained constant before mercury exposure.

1. Blood - the two high dose seals showed a sharp increase in their blood mercury during exposure to methyl mercury. The two low dose seals showed a steady increase in total and methyl mercury levels in the blood. The control group remained relatively constant throughout the experiment.

2. Hair - during the first week of feeding of methyl mercury the high dose animals showed an immediate increase of mercury levels in their hair, which increased steadily up to death. The two low dose seals showed no change relative to the appropriate control. Higher levels of total mercury were found in the hair of the older seals.

As there have been reports of interaction between the selenium and mercury levels (Koeman et al 1974) selenium levels were monitored in the food of the seals, as well as in their blood. No significant changes occurred in the amount of selenium in the blood (mean 1.5, range 1.36 to 1.84 ppm). The levels in the food were normal.

After exposure the average total mercury levels (ppm) in the harp seal tissue were as Table 1.

The liver was the prime deposition site in exposure to low dose over relatively long time periods. The kidney seemed to be the only organ that accumulated higher residues of total mercury under low than high dose exposures. Muscle deposition is somewhat similar at either exposure level. Blubber is not a preferential site of deposition (Tables 2, 3).

The high dose animals most likely died of gastro-intestinal involvement as they exhibited surface sloughing of much of the gastro-intestinal tract.

The low deposition rate in the intestine of lower dose animals is interesting, in fact, if the animal does not die of gastro-intestinal failure, then there is a chance for total mercury to be deposited at higher rates in the liver, kidney and muscle.

Electrocardiograms

The seals were examined for heart rate and signal strength, there was a marked QRS shift in 71-7 and 73-4. This had been noted in other harp seals during moult (deKleer 1972). Although there was some evidence of changes in the action of the right ventricle there was no evidence of myocardopathy. Sinus arrhythmia was seen but this has been reported from the normal animal (Casson and Ronald 1975).

Behavioural Changes

Apart from the marked spasmodic activity at the time of

the high dose seals' death there was little other behavioural evidence in the cinephotographic records of the experiment. The only behaviour change was a general lethargy in all the seals exposed to mercury. The technique however was inadequate to allow a more significant analysis to be made.

Blood values - physical

Erythrocyte

The erythrocyte value in the adult control was much lower than that of the other experimental animals. On day 267 there was a rise in value for 73-2 and 73-7, possibly as a result of a local infection. When CH_3HgCl was first fed the blood values were lowered and fluctuated until death.

Leucocyte

The numbers of leucocytes in the blood varied considerably, high peaks indicated seals with low grade infections. No constant pattern was observed after feeding CH_3HgCl .

Differential counts varied intraspecifically but no significant change other than a slight elevation in neutrophils occurred.

Mean corpuscular volume

The mean corpuscular volume appeared to be higher for adults than for the pups, otherwise there was no significant change.

Haematocrit

From day 175 until the beginning of the experiment on day 289 the pups showed a constant haematocrit pattern, No change was noticed in the two adults from day 200 until the end of the exposure. Changes in haematocrit were observed in the two high dose seals from day 300 until death, and 73-7, a low dose animal showed a significant drop during the last two weeks of life.

Haemoglobin

A levelling off of haemoglobin in the blood occurred for the pups at day 150 and continued until 300. From this point on a sharp drop in haemoglobin was noticed in the high dose seals. They were both very debilitated during this period due to CH_3HgCl exposure. 73-7, a low dose seal and 73-4, a control, followed a similar pattern until approximately the 340th day, 73-7 showing a haemoglobin depression from day 364 until death.

The level for the low dose adult rose after moult and remained constant up to day 308 when it began to drop until euthanasia on day 335. D7 continued in apparent good health until euthanasia at day 349. The haemoglobin level however dropped after day 322 in a pattern comparable to that of 71-7. No external signs of moulting were seen in D7 during this later period, but it is presumed the moult was underway physiologically.

Mean corpuscular haemoglobin

The levels for the adult seals were slightly higher than those of the pups, otherwise there were no significant differences. On day 267 the MCH levels were lower for 73-2 and 73-7, associated with a skin infection. At the time of death there was a drop in MCH in the high dose animals.

Mean corpuscular haemoglobin concentration

No significant changes in MCHC resulted from feeding of CH_3HgCl to the seals. In 73-4, a control, the MCHC was down on day 196 when she started to moult.

Blood values - chemical

Ionic balance

Sodium - seal 73-8 showed a steady decrease in plasma sodium levels until death. This seal received vitamin and salt tablets daily except on the day she died. The plasma sodium level of 71-7 dropped on day 80 although everything else appeared normal.

Chloride - no significant changes in plasma chloride levels occurred.

Potassium - the plasma potassium levels of the pups and adults remained relatively constant up to the beginning of exposure. At day 50, 73-4 reached a peak of 5.9 mEq which was the upper limit of the normal range. Seal 73-2, a high dose animal, showed a great deal of variation during the experiment, moving

from 4.9 mEq to 2.5 mEq and up to 7.1 mEq at time of death, all in a three week period. The other high dose animal, 73-8, reached a final level of 6.2 mEq on the day of her death. No significant changes were noticed for the low dose or control animals.

Blood urea nitrogen - there was a standard pattern of the seals blood urea levels up to day 200. After CH_3HgCl feeding the only change in blood urea nitrogen levels was observed in the two high dose animals whose BUN levels were elevated during the last week of exposure. It is possible that the high dose group was suffering some renal failure due to the impairment of the kidney from the accumulation of methyl mercury (Table 3).

Lactic dehydrogenase - as the seals began to ingest CH_3HgCl a significant LDH increase was noticed in the high dose animals. LDH is the enzyme which catalyses the reversible oxidation of lactic acid to pyruvic acid. A very essential reaction in a diving mammal. Serum LDH can be elevated however by many clinical and physiological conditions.

Transaminase

1. Glutamic pyruvic - all seals showed a peak between 14 and 168 days which might be an indication of a circannual rhythm. From day 175 on the serum glutamic pyruvic transaminase in all seals remained relatively unchanged except for the high dose animals from day 289. Seal 73-8 showed a marked increase on the day she died. Seal 73-2 also showed an increase on the day she died but this was assayed on jugular blood.

2. Glutamic oxalacetic - apart from the high dose animal 73-7 at 238 days (when she had cloudiness of the cornea) and at death there was no marked change in serum glutamic oxalacetic transaminase levels. SGOT has been used to diagnose myocardial infraction, the early detection of liver disease and becomes elevated in cases of muscular dystrophy and renal infarction.

Serum cholesterol - all pups showed abnormally high mean (520 m%)(for human) cholesterol values when they first arrived. These values dropped and remained at a normal pattern for harp seals (350 mg%) until the start of the experiment at which time the cholesterol levels went up in both the high and low dose seals (427 mg%). While most tissues synthesize cholesterol, the liver is the predominant place for such production.

Serum protein - the adult seals' serum protein levels were generally much higher (mean 7.95 g%) in range than those of the pups (6.5 g%).

Serum alkaline phosphate - serum alkaline phosphate is elevated in a variety of conditions in which there is an increase in osteoblastic activity, an impairment of liver function or an obstruction in bile flow. There was no significant change in alkaline phosphate content.

Bilirubin - only in the two high dose seals were significant changes seen. These elevations were possibly due to liver

damage. The low dose animal 73-7 showed a sudden increase to a level of 0.9 mg% bilirubin at 373 days but returned to the usual level a week later. This may have been a sign of a short term change in liver function but as it returned to approximately a normal level there may have been no incidental effects.

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TABLE 1

Tissue	Control	DOSE	
		Low (0.25 ppm)	High (25 ppm)
Intestine	0.4	17.2	1695.0
Hair	5.2	7.9	378.0
Liver	13.8	73.3	130.0
Spleen	0.6	20.2	85.2
Claws	3.7	22.1	55.9
Kidney	3.5	60.1	55.4
Heart	0.3	14.4	48.5
Muscle	0.7	35.2	40.9
Brain	0.4	18.3	33.4
Adrenal	N.D.	19.6	30.9
Gonad	N.D.	13.0	30.0
Blood	0.1	11.5	29.6
Lung	0.2	14.4	27.8
Blubber	N.D.	0.2	1.3

Average concentrations of total mercury in 3 groups of seals exposed to various levels of methyl mercury. Tissues are in order of concentration for the high dose animals.

TABLE 2

Levels of total mercury mg/kg body weight in harp seals						
Seal Number	73-4	D7	71-7	73-7	73-2	73-8
Dose	0.00	0.00	0.25	0.25	25.0	25.0
<u>Tissue</u>						
Brain	*0.36	*0.45	*14.8	*21.8	*23.8	42.9
Muscle	0.54	0.78	27.6	42.7	125	96.7
Blubber	N.D.	N.D.	0.28	0.20	0.937	1.71
Liver	1.72	25.8	64.0	82.5	142	134
Hair	1.26	9.17	8.21	1.66	434	322
Spleen	0.33	9.92	20.4	20.0	68.6	81.8
Claws	2.36	5.10	21.9	22.3	38.8	72.9
Intestine (small)	0.37	0.44	17.2	*17.1	*1730	1660
Heart	0.38	0.25	12.9	15.9	50.9	46.3
Kidney	0.92	5.98	69.5	50.6	*62.5	*48.3
Lung	*0.26	0.12	10.6	*18.2	*30.8	*24.8
Adrenal	N.D.	-	14.2	25	*34.9	*26.8
Gonad	-	N.D.	13	-	*33.0	*27.0
Blood	0.1	0.12	9.93	13.1	30.3	28.8

*Tissues held in 10% formalin buffered saline for a period of time before total HG assay. Other tissues assayed while fresh or frozen.
- indicates no tissue.

TABLE 3

Levels of methyl mercury mg/kg body weight in harp seals						
Seal Number	73-4	D7	71-7	73-7	73-2	73-8
Dose	0.00	0.00	0.25	0.25	25.0	25.0
<u>Tissue</u>						
Muscle	0.5	0.56	35.2	40.2	115	91.5
Blubber	0.0	0.05	0.58	0.55	1.0	0.59
Liver	0.2	0.16	18.48	75.7	125	127
Hair	1.5	1.27	7.3	4.1	350	428
Spleen	0.0	0.63	-	14.5	51.5	60.3
Intestine	0.0 (out)	0.09 (out)	0.5 (out)	0.5 (out)	*1367	-
	8.5 (in)	10.3 (in)	18.95 (in)	21.5 (in)		
Heart	0.0	-	11.8(Peri) - 18.04(Endo) 20.93(Mid)		41.5	40.2
Kidney	0.2	0.27	51.6	10.4	*20.5	*11.7
Lung	0.0	-	-	10.6	*5.7	*7.3
Adrenal	0.0	-	10.75	21.7	*10.4	*7.5
Gonad	-	N.D.	10.08	11.0	*29.1	*21.5
Blood	0.1	0.1	8.85	12.5	23.7	19.0

*Tissues held in 10% formalin buffered saline for a period of time before Hg test. Other tissues were fresh frozen.

- indicates no tissue