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EFFECTS OF DIETARY MERCURY ON MINK[1]

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A study was conducted to ascertain the effects of dietary mercury on mink. Five parts per million of dietary methylmercury was lethal to adult mink in about one month. Ten parts per million of mercuric chloride in the diet for five months did not produce adverse effects. Clinical signs of methyl mercurialism were anorexia, loss of weight, incoordination, tremors, and paroxysmal convulsions. The latency period was about 24 days; survival time averaged 33 days. Pathological changes were evident in the tissues of the mink that died of mercury poisoning. Tissue mercury residue analyses showed the highest concentration of mercury in tissues from mink fed methylmercury.

Recent accounts of mercury contamination in Lake Erie and other lakes and rivers of the United States and Canada (Bails 1970, Dustman *et al.* 1970, Eyl *et al.* 1970, Fimreite 1970b, Fimreite *et al.* 1971, Montague and Montague 1971, Takeuchi 1970) have aroused suspicion among mink ranchers concerning the possibility of secondary mercury poisoning in mink from the use of mercury-contaminated fish in mink rations. Since the mid-1960s, reproductive complications and mortality have been associated with the feeding of Great Lakes fish to mink (Aulerich *et al.* 1971, Hartsough 1965). Mercury was a prime suspect in these problems because most of the mercury in fish tissue is in the methylated form (Westoo 1966), which is highly toxic to man (Kutsuna 1968) and animals (Borg *et al.* 1966, Fimreite 1970a, Fimreite 1971, Fimreite *et al.* 1970, Hanko *et al.* 1970, Piper *et al.* 1971, Swensson and Ulfarson 1969, Takeuchi 1968).

Documented reports of mercurialism in mink are lacking, except for a brief account by Kirk (1971), who stated that " . . . mink can be raised on a daily diet with up to 0.5 ppm mercury content-fish source. A level of 1.0 ppm wet-weight basis can prove fatal in approximately two months' time."

The objectives of this study were to (1) determine the effects of dietary mercury on mink reproduction and neonate viability, (2) compare the toxicity and effects of organic and inorganic forms of mercury on mink, and (3) ascertain the clinical signs, lesions, and tissue residues associated with mercurialism in mink.

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Procedure

The experiment was conducted from December 27, 1970, to June 1, 1971. Proven breeder mink consisting of 3 males and 12 females per group were fed a basal diet (Table I) supplemented as follows:

Group I	Unsupplemented basal diet (control)
Group II	5 ppm supplemental methylmercury[2]
Group III	10 ppm supplemental mercuric chloride

Each mercury compound was dissolved in water and incorporated into the experimental diet by blending the solution with a ground commercial mink cereal to form a cereal-mercury mixture. This premix was then mixed with the other ingredients of the diet to yield a ration that contained the desired amount of mercury.

Table I. *Composition of basal diet.*

Ingredient	Percent of diet
Supplemented cereal	15.0
Tripe	20.0
Beef liver	5.0
Horsemeat	15.0
Chicken	15.0
Mixture of cod, haddock, and flounder	30.0

Standard ranch procedures were followed in feeding, breeding, and caring for the animals. The mink were immunized against canine distemper, botulism, and virus enteritis prior to the start of the study.

The mink were weighed individually at the start of the experiment and at approximately monthly intervals thereafter, except during the gestation period. The females were checked daily for young during the whelping period. Kits were counted and weighed on the day of birth and at four weeks of age.

Mink that died during the experiment were necropsied, their organ weights recorded, and selected tissues collected for histopathologic examination and mercury residue analysis. Routine histologic procedures were employed in preparing the tissues for histopathologic examination. Tissue samples collected for mercury residue analysis were stored in glass vials at 0°C until prepared for analysis as follows: A one-g sample homogen-

[2]As contained in Ceresan-L concentrate (methylmercury 2, 3-di-hydroxy propyl mercaptide 2.89 percent, methylmercury acetate 0.62 percent), mercurial seed disinfectant, I. E. Dupont, Wilmington, Del.

ate of each tissue was blended with 4.5 ml of 10 percent trichloroacetic acid for 10 minutes. The mixture was then centrifuged at 2,000 × G for 15 minutes and the supernatant separated from the precipitate, and both fractions saved. Five ml concentrated sulfuric acid was added to the precipitate, which was placed in an ice bath and stirred. The mixture was then allowed to digest at room temperature for 24 hours. One-half ml of 30 percent hydrogen peroxide was added to the acid digest (cooled in an ice bath) and the solution was allowed to stand at room temperature for 24 hours. The solution was then combined with the supernatant (cooled in an ice bath), and the sample was analyzed for mercury content on a Coleman (model MAS-50) mercury analyzer using the procedure described by Hatch and Ott (1968).

Results

No adverse effects attributed to the diets were observed in the mink during the first three weeks of the experiment. Starting on the 25th day of feeding, the mink in Group II (5 ppm methylmercury) showed signs of incoordination, loss of balance, anorexia, and loss of weight. These clinical signs grew progressively more severe, and within approximately four days after the initial symptoms were noted, the affected animals exhibited ataxia, paralysis and tremors accompanied by paroxysmal convulsions and high-pitched vocalizations. When suspended by the tail, the mink showed the typical limb-crossing phenomena described by Takeuchi (1970) and Klein *et al.* (1972) in rats. Death usually occurred within 24 to 48 hours after the onset of the tremors.

In an attempt to arrest the condition, the diet of these mink was changed to the control ration on the 29th day. On the 31st day the eight least severely affected mink in Group II were randomly divided into two groups of four mink each. One group was treated every other day with subcutaneous injections of 100 mg of ethylenediamine tetra-acetate (EDTA) in an attempt to chelate mercury within the body; the other group received daily injections of 300 mg of methionine in an effort to reverse the effects of the mercurialism in these animals. Mercury is known to interfere with sulfation (Seven 1960), which may lead to a deficiency of sulfur-containing amino acids. Neither the change in diet nor the treatments, however, alleviated the condition, as all 15 mink in Group II died between the 30th and 37th day.

Necropsy of the mink that died from methylmercury poisoning showed the following common changes and lesions: hemorrhagic and congested lungs; enlarged heart; pale liver and kidneys; local, superficial, hemorrhagic gastric ulcers (primarily in the cardiac region of the stomach); and occasionally an enlarged and mottled spleen. Hyperemia, fatty and hydropic degeneration of the liver and kidneys, splenic and glomerular amyloidosis, and an abundance of splenic giant cells were observed in the tissues from these animals.

The mink in Groups I (control) or III (10 ppm mercuric chloride) showed no clinical signs that could be attributed to methylmercury poisoning during the study. One female in Group III died three days after whelping but the cause of death was not attributed to mercury poisoning.

The average initial body weights and average weight gains (from December 1970 t February 1971) of the mink on the various dietary treatments are shown in Table I Supplemental methylmercury significantly depressed body weight.

Table II. *Body weight gain and loss of mink fed a basal control ration and those fed supplemental mercury.*

Dietary treatment	No. mink	Av. initial wt. (g ± S. E.)	Av. wt. gain or loss (g ± S. E.)	
			In 1 month	In 2 month
Control (Group I)				
Males	3	1880 ± 98.7	-7 ± 24.0	-317 ± 79.
Females	12	1067 ± 42.3	-9 ± 10.0	-145 ± 24.
5 ppm methyl-mercury (Group II)				
Males	3	1803 ± 42.1	-323 ± 96.8[a]	—[c]
Females	12	1038 ± 51.2	-190 ± 39.8[b]	—[c]
10 ppm mercuric chloride (Group III)				
Males	3	2130 ± 153.1	-47 ± 13.3	-307 ± 43.
Females	12	972 ± 40.8	-21 ± 18.6	-123 ± 23.

[a]Significantly different ($P \leq 0.05$) from controls by t-test.
[b]Significantly different ($P \leq 0.01$) from controls by t-test.
[c]All mink died before two months were up.

Although all the mink in Group II died before the breeding season and no studies the effects of methylmercury on reproduction could be carried out, the reproducti performance and mortality of the females in Groups I and III are shown in Table III. the table indicates, the mercuric chloride treatment did not adversely affect the reprodu tive performance of the females in Group III. No teratism was noted.

Table III. *Reproductive performance and mortality of control mink and those fed supplemental mercuric chloride.*

Dietary treatment	No. females mated	No. females whelped	No. kits born		No. kit alive at 4 week
			Alive	Dead	
Basal diet (Group I)	12	11	35	19	29
10 ppm mercuric chloride (Group III)	12	11	41	10	29

Table IV shows the average weight of the kits at birth and at four weeks of age (weaning). The reduction in kit weight at birth from mink fed mercuric chloride is without explanation at this time. The number of kits alive at four weeks of age and the average body weight at four weeks were comparable in the control and the mercuric chloride-treated groups.

Table IV. *Body weight at birth and four weeks of age of kits whelped by females fed a basal diet and those fed supplemental mercuric chloride.*

Dietary treatment	At birth		At age 4 weeks	
	No. live kits	Wt. (g ± S. E.)	No. live kits	Wt. (g ± S. E.)
Control (Group I)	35	10.2 ± 0.37	29	187.1 ± 6.91
10 ppm mercuric chloride (Group III)	41	9.3 ± 0.21[a]	29	183.4 ± 4.59

[a]Significantly different ($P \leq 0.05$) from controls by t-test.

In Table V the mean organ weights (heart, liver, kidneys, and spleen) of the female mink in Group II are shown with equivalent data for seven control animals. Statistical analysis of these data indicated that the hearts and kidneys of the mink from Group II weighed significantly more ($P \leq 0.05$) than those of the controls.

The average mercury residue in the tissues from the mink that died during the experiment in Groups II and III and from sacrificed control animals is shown in Table VI. Tissues from the mink in Group II contained the highest mercury residues, although these animals received the mercury supplemented diet for by far the shortest period of time. The concentration of mercury in all tissues except hair of the mink in Group II was significantly greater ($P \geq 0.01$) than in the controls. Tissues from only one mink in Group III were analyzed. Mercury residue levels in this animal were similar to those of the control mink, except for the liver and kidneys, which contained notably higher mercury residues.

Table V. *Mean organ weights of 7 female control mink and 5 female mink fed diets containing supplemental methylmercury.*

Organ	Organ wt. (g/100 g brain wt. ± S. E.)	
	Group I (control)	Group II (5 ppm methylmercury)
Heart	75.8 ± 4.76	109.9 ± 13.87[a]
Liver	399.5 ± 48.79	349.6 ± 27.36
Kidney	65.3 ± 3.10	80.3 ± 5.75[a]
Spleen	35.5 ± 5.94	48.2 ± 8.61

[a]Significantly different ($P \leq 0.05$) from controls by t-test.

Table VI. *Mean mercury content of tissues from mink fed a control diet and those fed experimental rations containing supplemental mercury.*

Tissue	Mercury content (ppm)		
	Group I (control)	Group II (methylmercury)	Group III (mercuric chloride)
Liver	0.28 ± 0.06 (9)[a]	55.6 ± 4.11 (15)[b]	3.16 (1)
Kidney	0.68 ± 0.14 (9)	37.7 ± 4.19 (15)[b]	32.8 (1)
Skeletal muscle (thigh)	0.05 ± 0.03 (7)	25.2 ± 4.93 (15)[b]	0.12 (1)
Spleen	0.24 ± 0.22 (7)	24.8 ± 2.02 (7)[b]	0.35 (1)
Brain	0.22 ± 0.14 (9)	19.9 ± 4.55 (15)[b]	0.24 (1)
Lung	0.02 ± 0.01 (7)	17.1 ± 1.05 (6)[b]	0.19 (1)
Heart (myocardium)	0.12 ± 0.09 (7)	16.7 ± 0.96 (13)[b]	0.15 (1)
Hair	1.13 ± 0.08 (4)	1.22 ± 0.12 (13)	1.23 (1)
	Average No. of days on experiment		
	155	32	135

[a]Number in parentheses represent the number of animals analyzed.
[b]Significantly different ($P \leq 0.01$) from controls by t-test.

Discussion

The results show that mink are quite sensitive to dietary methylmercury but comparatively tolerant of mercury in an inorganic form, such as mercuric chloride. The inorganic form's being more readily excreted from the body and the organic form's easy penetration into the brain (Eyl *et al.* 1970 and Kurland *et al.* 1960), probably account for the difference in toxicity.

The 24-day latency period for methyl mercurialism observed in mink was comparable to the 24-day latency period reported by Hanko *et al.* (1970) in ferrets fed 5 ppm methylmercury. The average survival time of 33 days for mink, however, was much shorter than the 58 days noted for ferrets.

If, as is assumed, adult female mink consume 150 g of feed per day, the total intake of methylmercury per female in Group II was about 18 mg. Applying the same assumption to Kirk's (1971) statement, "A level of 1.0 ppm wet-weight basis can prove fatal in approximately two months' time," consumption of only 9 mg of mercury (fish source) could be lethal to mink. One-half ppm mercury (fish source), however, did not produce signs of mercury poisoning in female mink when fed for three months (Kirk 1971). The estimated intake of 18 mg of methylmercury by the female mink that died in this study compares favorably with the results of an experiment by Hanko *et al.* (1970) in which 20 mg of mercury were consumed by female ferrets that died while receiving a ration that contained 5 ppm methylmercury. Takeuchi (1968) reported that a dietary intake of

from 20 to 60 mg of mercury (as contained in shellfish) produced mercurialism in cats. As shown by data presented by Hanko *et al.* (1970), there appears to be a time-dose relationship associated with methylmercurialism, which may account for the variation in the response of the mink in our study and Kirk's (1971).

Mink in Group III (10 ppm mercuric chloride) consumed approximately 233 mg mercury per female during the experimental period without adverse effects. Whether continued consumption of the mercuric chloride-supplemented diet beyond five months would eventually produce adverse effects was not ascertained.

The clinical signs and lesions of methylmercury poisoning observed in this study were similar to those reported for rats and cats (Takeuchi 1970), pigs (Piper *et al.* 1971), and ferrets (Hanko *et al.* 1970). The loss in body weight of the mink in Groups I and III during the late winter months (Table II) was probably a result of the restricted feeding procedures initiated to bring the mink into optimum breeding condition, a common practice on most commercial mink ranches.

Although no adverse effects on reproduction were noted in the mink fed mercuric chloride (Group III), Khera (1973) reported a dose-related reduction in litter size in rats where the male parents were treated with methylmercury chloride.

The concentration and distribution of mercury in the tissues of mink fed methylmercury (Table VI) differed considerably from that of the mink that received mercuric chloride. Since organic mercury compounds tend to accumulate more readily in the brain than inorganic mercury compounds, the ratio of mercury in the brain to mercury in the liver and kidneys is higher in organic mercury poisoning than in inorganic mercury poisoning (Kurland *et al.* 1960). In this experiment the mink fed methylmercury had ratios of 1:2.8 for liver and 1:1.9 for kidneys which are indicative of acute methylmercurialism. Those fed mercuric chloride (Group III) had ratios of 1:13.2 for liver and 1:136.7 for kidneys.

The similar low concentrations of mercury in the hair of mink from the various dietary treatments may be the result of the absence of hair growth during most of the experimental period, coupled with the very slow rate of mercury accumulation in hair, although it may become highly concentrated with prolonged exposure (Takeuchi 1970).

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