AQUATIC MERCURY POLLUTION: STUDIES OF ITS OCCURRENCE AND PATHOLOGIC EFFECTS ON FISH AND MINK

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in Partial Fulfilment of the Requirements

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in the

Department of Veterinary Pathology

by

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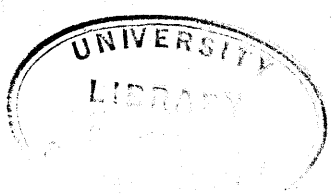

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ABSTRACT

Mercury pollution of water has been documented in Japan and in several Scandinavian countries. No information was available on the status of North American waters with regard to mercury pollution at the time this work was begun.

Muscle tissue from 81 fish from nine locations in the Saskatchewan River contained an average of more than 1.0 ppm of mercury. Fish from two of the sites had much higher concentrations, with individual fish containing up to 11.2 ppm of mercury. These concentrations correspond to values reported for Scandinavian fish collected in areas of industrial pollution.

The acute toxicity of methyl mercury chloride (MeHgCl) for rainbow trout (Salmo gairdneri) fry and fingerlings was measured. The median tolerance limit (TLm) at 24, 48 and 96 hr was 0.084, 0.045 and o.024 mg/litre as mercury respectively for fry; and 0.125, 0.066 and 0.042 mg/litre as mercury respectively for fingerlings. The TLm (24 hr) for mercuric chloride ($HgCl_2$) for fingerlings was 0.90 mg/litre as mercury. Fingerlings exposed to MeHgCl concentrated mercury in their tissues much more rapidly than did those exposed to $HgCl_2$. The acute toxic action of MeHgCl and $HgCl_2$ was exerted on the gills. Mercuric chloride caused severe epithelial necrosis. Poisoning with MeHgCl was characterized by epithelial cell swelling and hyperplasia, a marked increase in the number of epithelial cells

in mitosis and terminally, epithelial desquamation.

Rainbow trout fingerlings were fed rations containing 4, 8, 16 and 24 ppm mercury as MeHgCl over a 105 day period. Fish receiving the 16 and 24 ppm rations had significantly higher blood packed cell volumes than controls. Hyperplasia of the gill epithelium was the only morphologic alteration in these fish. Individual fish accumulated up to 30 ppm mercury in muscle, but no mortality which could be attributed to mercury poisoning occurred. The results suggest that trout can tolerate a large body burden of mercury, if this mercury is acquired over a period of time.

Female and juvenile mink (Mustela vison) were fed rations containing 50 and 75 per cent of fish containing 0.44 ppm mercury over a 145 day period. There was no evidence of intoxication in these animals. Mercury concentrations in tissue appeared to have reached equilibrium at a level below that associated with toxicity.

Adult mink were fed rations containing 1.1, 1.8, 4.8, 8.3 and 15.0 ppm mercury as MeHgCl over a 93 day period. Histologic evidence of injury was present in all groups. Mink fed rations containing 1.8 to 15.0 ppm mercury developed clinical intoxication within the experimental period. The rapidity of onset of intoxication was directly related to the mercury content of the ration. Mercury concentrations in tissues of mink which died were similar, despite differences in the mercury content of the diets and time to death. The average mercury concentration in the brain of mink which died was 11.9 ppm. The disease produced by MeHgCl was primarily related

to neuronal necrosis. Cortical neurons and those in certain subcortical nuclei were most susceptible. In acute intoxication neuronal damage was diffuse throughout the brain. Involvement of granular cells of the cerebellum and peripheral nerve fibres was seen in acutely poisoned animals.

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AUTOBIOGRAPHY

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TABLE OF CONTENTS

		Page
1.0	INTRODUCTION	1
2.0	LITERATURE REVIEW	
	2.1 History of aquatic mercury pollution	3
	2.2 Sources of mercury in the aquatic environment	5
	2.2.1 Japan	6
	2.2.2 Sweden	7
	2.2.3 North America	11
	2.3 Biosynthesis of methyl mercury	12
	2.4 Distribution and concentration in tissues	17
	2.4.1 Fish	17
	2.4.2 Birds	18
	2.4.3 Mammals	22
	2.5 Elimination and biological half-life of methyl mercury	
	2.5.1 Fish	24
	2.5.2 Birds	24
	2.5.3 Mammals	27
	2.6 The pathology of alkyl mercury poisoning in vertebrates	28
	2.6.1 Fish	30
	2.6.2 Birds	33

Page

2.6.3 Mammals 38

2.6.3.1 Prenatal intoxication 38

2.6.3.2 Postnatal intoxication 40

2.6.4 Discussion 51

3.0 OBJECTIVES OF THE STUDIES 56

4.0 EXPERIMENT I. MERCURY CONCENTRATIONS IN THE TISSUES OF FISH FROM THE SASKATCHEWAN RIVER

4.1 Rationale 58

4.2 Materials and methods

4.2.1 Collection of specimens 59

4.2.2 Mercury analysis 61

4.3 Results 62

4.4 Discussion 64

5.0 EXPERIMENT II. THE ACUTE TOXICITY OF METHYL MERCURY CHLORIDE FOR RAINBOW TROUT FRY AND FINGERLINGS

5.1 Rationale 68

5.2 Materials and methods

5.2.1 The fish 68

5.2.2 Experimental design 69

5.2.3 Sampling 72

5.2.4 Mercury analysis 73

5.3 Results

5.3.1 Clinical findings 74

5.3.2 Toxicity of MeHgCl and $HgCl_2$ 75

5.3.3 Accumulation of mercury in tissue 75

Page

5.3.4 Histopathology

5.3.4.1 MeHgCl 78

5.3.4.2 $HgCl_2$ 86

5.4 Discussion 88

6.0 EXPERIMENT III. PROLONGED ORAL ADMINISTRATION OF METHYL MERCURY CHLORIDE TO RAINBOW TROUT FINGERLINGS

6.1 Rationale 95

6.2 Materials and methods

6.2.1 The fish 96

6.2.2 The aquaria 96

6.2.3 The rations 99

6.2.4 Experimental design 100

6.2.5 Sample collection 100

6.2.6 Sample processing 101

6.3 Results .....

6.3.1 Clinical findings 102

6.3.2 Weight gains 103

6.3.3 Hematology 103

6.3.4 Accumulation of mercury in tissues 107

6.3.5 Pathology 107

6.4 Discussion 114

7.0 EXPERIMENT IV. THE USE OF MERCURY CONTAMINATED FISH AS FOOD FOR RANCH MINK

7.1 Rationale 125

7.2 Materials and methods

Page

7.2.1 Experimental design 125

7.2.2 Sampling 127

7.3 Results

7.3.1 Clinical findings 129

7.3.2 Accumulation of mercury in tissue 129

7.3.3 Pathology 134

7.4 Discussion 134

8.0 EXPERIMENT V. EXPERIMENTAL METHYL MERCURY INTOXICATION IN MINK

8.1 Rationale 136

8.2 Materials and methods

8.2.1 Experimental design 136

8.3 Results

8.3.1 Clinical findings 139

8.3.1.1 Group I (control) 141

8.3.1.2 Group II (1.1 ppm Hg in feed) 141

8.3.1.3 Group III (1.8 ppm Hg in feed) 141

8.3.1.4 Group IV (4.8 ppm Hg in feed) 143

8.3.1.5 Group V (8.3 ppm Hg in feed) 143

8.3.1.6 Group VI (15.0 ppm Hg in feed) 144

8.3.2 Weight changes 144

8.3.3 Accumulation of mercury in tissue 146

8.3.4 Gross pathology 146

8.3.5 Histopathology

8.3.5.1 Group VI (15.0 ppm Hg in feed) 148

Page

8.3.5.2 Group V (8.3 ppm Hg in feed) 156

8.3.5.3 Group IV (4.8 ppm Hg in feed) 160

8.3.5.4 Group III (1.8 ppm Hg in feed) 162

8.3.5.5 Group II (1.1 ppm Hg in feed) 162

8.4 Discussion

8.4.1 Clinical manifestations 165

8.4.2 Relation between mercury concentration in tissue and effects 169

8.4.3 Pathology

8.4.3.1 Nervous system 171

8.4.3.2 Other systems 174

9.0 GENERAL DISCUSSION

9.1 Historical perspective 176

9.2 The relationship of tissue mercury concentrations and toxic effects of mercury in fish 177

9.3 Diagnosis of mercury poisoning in mink 180

9.4 Ecological implications of aquatic mercury pollution .. 181

9.5 Long-term prospects for mercury contaminated waters ... 189

10.0 SUMMARY AND CONCLUSIONS 191

11.0 REFERENCES 195

APPENDIX A

Table 1. Common and scientific names of species 216

Table 2. Reports describing the pathology of alkyl mercury poisoning in vertebrates 218

Page

APPENDIX B

Table 1. Median tolerance limits of trout fry and fingerlings for methyl mercury chloride and mercuric chloride 223

APPENDIX C

Tables 1, 2. Weekly weight gains of rainbow trout fed rations containing various amounts of methyl mercury chloride 225, 226

Table 3. Mercury concentration in the muscle of rainbow trout fed rations containing various amounts of methyl mercury chloride 227

APPENDIX D

Tables 1-3. Incidence of histopathologic lesions in mink fed rations containing various amounts of methyl mercury chloride ..... 229 - 231

LIST OF TABLES

Page

1. Biological half-life of alkyl mercury in some poikilothermic aquatic species 25
2. Biological half-life of methyl mercury in various mammals . 29
3. Gross lesions in birds poisoned with alkyl mercurials 35
4. Histologic lesions in organs other than the nervous system of birds poisoned with alkyl mercurials 36
5. Histologic lesions in the nervous system of birds poisoned with alkyl mercurials 37
6. Gross lesions in the muscular, nervous, and respiratory systems of alkyl mercury poisoned mammals 41
7. Gross lesions in the digestive, urinary, lympho-hemopoetic, cardio-vascular and integumentary systems of alkyl mercury poisoned mammals 42
8. Type of histologic lesions reported in the nervous system of alkyl mercury poisoned mammals 44
9. Distribution of histologic lesions in the nervous system of alkyl mercury poisoned mammals 47
10. Mercury concentrations in the tissue of fish from the Saskatchewan River 63
11. Mercury concentration in the muscle, liver, and kidney of 14 fish from the Saskatchewan River 65
12. Characteristics of water used in experiments 71
13. Tolerance limits of rainbow trout fry and fingerlings for methyl mercury chloride and mercuric chloride 76
14. Mercury concentrations in the tissue of rainbow trout fry and fingerlings exposed to methyl mercury chloride and mercuric chloride 77

Page

15. Numbers of cells in mitosis in the epithelium of the gills of rainbow trout exposed to methyl mercury chloride and mercuric chloride 85

16. Histologic lesions in the gills of rainbow trout exposed to methyl mercury chloride and mercuric chloride 89

17. Mean weekly weight gains of rainbow trout fed rations containing methyl mercury chloride over a 15 week period .. 104

18. Mean weekly weight gains of control group and groups III and IV over the final 5 weeks of the experiment 105

19. Packed cell volume and total plasma protein of fish in groups control, I and II 106

20. Packed cell volume and total plasma protein of fish in groups control, III and IV 108

21. Incidence of histologic lesions in the epithelium of the gills of fish fed rations containing methyl mercury chloride 113

22. Body weight at the time of sacrifice of mink fed rations containing mercury contaminated fish 130

23. Mercury (ppm) in the liver of mink fed rations containing various amounts of mercury contaminated fish 131

24. Mercury (ppm) in the kidney of mink fed rations containing various amounts of mercury contaminated fish 132

25. Mercury (ppm) in the brain of mink fed rations containing various amounts of mercury contaminated fish 133

26. Timing of occurrence of clinical signs and death in mink fed rations containing various amounts of methyl mercury chloride 140

27. Weight changes of mink during experimental period 145

28. Mercury concentration in the tissues of mink fed rations containing methyl mercury chloride 147

29. Mercury concentrations in the liver of aquatic birds 187

LIST OF ILLUSTRATIONS

		Page
1.	Map of southern Saskatchewan showing sites at which fish were collected from the Saskatchewan River	60
2.	Mercury (ppm) in tissue of rainbow trout exposed to 0.08 mg Hg/litre for 24 hr and then transferred to mercury-free water	79
3.-5.	Histologic lesions in the gills of rainbow trout exposed to methyl mercury chloride in the water	81
6.-7.	Histologic lesions in the gills of rainbow trout exposed to methyl mercury chloride in the water	84
8.	Numbers of mitotic figures in the gill epithelium of rainbow trout exposed to 0.08 mg Hg/litre for 24 hr and then transferred to mercury-free water	87
9.	Secondary gill lamellae of a rainbow trout exposed to 0.75 mg Hg/litre as mercuric chloride for 24 hr	84
10.	Diagrammatic view of aquarium unit	98
11.	Mercury concentrations in the muscle of rainbow trout fed rations containing varying amounts of methyl mercury chloride	109
12-15.	Secondary gill lamellae of rainbow trout fed rations containing varying amounts of methyl mercury chloride	110,112
16-17.	Posterior kidney of rainbow trout which received 10mg Hg/kg as methyl mercury chloride via intraperitoneal injection	117
18,19.	Posterior kidney of rainbow trout which received 20 mg Hg/kg as methyl mercury chloride via intraperitoneal injection	118
20.	Secondary gill lamellae of rainbow trout which received 20 mg Hg/kg as methyl mercury chloride via intraperitoneal injection	119

		Page
21-22.	Pseudobranchiae of control trout and trout which received 20 mg Hg/kg as methyl mercury chloride via intraperitoneal injection	120
23-24.	Mink showing clinical signs of mercury intoxication....	142
25-29.	Occipital region of cerebral cortex of mink which received a ration containing 15.0 ppm Hg	149,150
30.	Degeneration of cerebellar granular cells, mink which received a ration containing 15.0 ppm Hg	152
31-34.	Cerebellar peduncle, mink which received a ration containing 15.0 ppm Hg	152,153
35,36.	Red nucleus and pons of mink which received a ration containing 15.0 ppm Hg	154
37.	Isolated nerve fibres from sciatic nerves of mink which received a ration containing 15.0 ppm Hg	155
38,39.	Liver and kidney of mink which received a ration containing 15.0 ppm Hg	157
40,41.	Spleen of control mink and mink which received a ration containing 15.0 ppm Hg	148
42.	Laminar necrosis of neurons in the cerebral cortex of a mink which received a ration containing 8.3 ppm Hg ..	159
43.	Neuronal necrosis in the red nucleus of a mink which received a ration containing 8.3 ppm Hg	159
44.	Laminar necrosis of neurons. Occipital cerebral cortex of mink which received a ration containing 4.8 ppm Hg	161
45,46.	Red nucleus and nucleus raphis of mink which received a ration containing 1.8 ppm Hg	163
47.	Neuronal necrosis, dentate nucleus of mink which received a ration containing 1.8 ppm Hg	164
48.	Spleen of mink which received a ration containing 1.8 ppm Hg	164
49.	Neuronal necrosis in the nucleus of the lateral lemniscus of a mink which received a ration containing 1.1 ppm Hg	166
50.	Cerebellar peduncle of a mink which received a ration containing 1.1 ppm Hg	166

1.0 INTRODUCTION

Pollution has been defined as "an undesirable change in the physical, chemical, or biological characteristics of our air, land, and water that may or will harmfully affect human life or that of any other desirable species, or industrial processes, living conditions, or cultural assets; or that may or will waste or deteriorate our raw natural resources".[1] The list of pollutants which may produce some or all of these effects is increasing rapidly. The problems caused by pollution can be viewed in two ways. The first is to consider pollution primarily as a hazard to human health; the other is to recognize that toxicity of pollutants to humans is only one aspect of the total problem, and that the effects on other areas of the ecosystem may be equally important. The metal mercury (Hg) and its compounds have many properties which make them serious environmental contaminants. These compounds are stable in the environment, they are toxic to organisms at many trophic levels, they tend to accumulate within organisms and within biological food chains and they are widely used in industrial processes. Aquatic Hg pollution is of concern primarily because of the hazard to human health, and the effect on other organisms has received little attention.

Pollution problems which occur in one area of the world are likely

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[1] Waste management and control. Committee on pollution, National Academy of Sciences. National Research Council, Washington, D.C., 1966.

to occur in other areas in which similar human activities are taking place. Aquatic Hg pollution had been documented in Japan and some Scandinavian countries, but had not been searched for in North America. This study was undertaken to determine if aquatic Hg pollution occurred in Saskatchewan, and to explore the effects of Hg on fish and a fish-eating mammal.

2.0 LITERATURE REVIEW

2.1 History of aquatic mercury pollution

The metal Hg and its derivatives possess properties which have made these compounds extremely useful for many of man's activities. Mercury is one of the ancient metals and industrial Hg poisoning was described as early as 1524 (Goldwater, 1964). As an example of the interest in mercurial toxicity, Voress and Smelcer (1957) compiled a bibliography containing over 1,600 titles dealing with this subject. Mercury poisoning in the past has largely been due to industrial exposure (Goldwater, 1964); however, within the past 20 years a new problem has emerged which poses a potential threat to a much wider segment of the human population, and to other parts of the ecosystem.

In 1953 an unidentified disease of the central nervous system began to occur in humans living in the vicinity of Minamata Bay, Japan. By 1956 this disease had reached epidemic proportions, and in that year the disease was linked to the consumption of fish and shellfish caught in Minamata Bay. In 1959 it was disclosed that the disease was caused by the ingestion of fish and shellfish contaminated with organic mercurial compounds (Kutsuna, 1968).

This compound was first identified as methyl mercury thio-methyl (MeHg-S-Me) (Uchida, et al., 1961) but subsequently Irukayama et al. (1962) and Kondo (1964) identified the causative agent as methyl

mercury chloride (MeHgCl). Tanaka (1968) felt that this discrepancy was likely due to the methods used for protein hydrolysis in the Hg extraction, and stated that both these compounds were responsible for "Minamata disease".

Irukayama et al. (1962) also identified MeHgCl in sediments from Minamata Bay and in sludge within the reaction tube of an acetaldehyde plant which discharged effluent into the bay. On the basis of toxicity studies using several species of mammals it was determined that the toxic agent was an organomercurial compound having the general formula CH_3Hg+ or CH_3Hg-S+ in which the characteristic toxic group was the CH_3Hg+ radical (Tanaka, 1968). During the period 1953 to 1960 there were 111 reported cases of Minamata disease with 41 fatalities (Nomura, 1968).

At approximately the same time as the Hg problem in Japan was being identified (mid-1950's) it became evident to workers in Sweden that Hg poisoning was widespread in Swedish wildlife, and that this was associated with the use of organic Hg compounds as seed disinfectants (Borg et al., 1969). The widespread use of alkyl Hg seed dressing was also associated with elevated concentrations of Hg in eggs and meat products produced in Sweden (Westoo, 1966a, 1967a; Underdahl, 1968 a,b). As a result of controversy concerning Hg contamination of food products in Sweden, surveys were initiated to determine the Hg content of Swedish fish. These surveys indicated the presence of high concentrations of Hg in fish from many areas of Sweden (summarized by Lofroth, 1969). The Hg in fish was found to exist almost entirely in the MeHg form (Westoo, 1966b, 1967b; Westoo and Noren, 1967). There have been no reports of human poisoning due to the

ingestion of contaminated fish in Sweden. Subsequent investigations (summarized by Lofroth, 1969) revealed that a similar situation existed in other Scandinavian countries.

A second outbreak of Minamata disease occurred near Niigata City, on the Agano River, in Japan, in 1964. A total of 120 persons in the area showed one or more symptoms suggestive of organomercurial poisoning (Lofroth, 1969) and 26 cases with five deaths were confirmed (Irukayama, 1968).

2.2 Sources of mercury in the aquatic environment

Mercury is a rare element comprising less than 30 billionths of the earth's crust (Goldwater, 1971) and having a terrestrial abundance of the order of 50 parts per billion (ppb) (Jonasson and Boyle, 1971). Mercury may occur in water both from natural sources and as a result of man's activities. In general it appears that the natural levels of Hg in water have been little studied. The available data (Wiklander, 1970; Jonasson and Boyle, 1971; Smith et al., 1971; Burton and Leatherland, 1971) indicate that the normal content of soil and river water is of the order of 0.01 to 0.1 ppb. Concentrations of over 0.1 ppb likely represent either natural or industrial contamination (Jonasson and Boyle, 1971). The distribution of Hg is not uniform in the lithosphere and waters in areas of Hg-rich soil or rock may contain much higher levels of Hg, i.e., natural contamination (Jonasson and Boyle, 1971). This natural phenomenon might result in elevated Hg concentrations in fish from waters with no industrial contamination, as has been suggested by Nelson et al. (1971).

Mercury and its compounds are used widely in both industry and agriculture and the background or normal levels of Hg in water are often obscured by additions from these sources. In all cases where Hg pollution has been recognized, certain industries have been incriminated as the source. The type of industry involved has varied in different areas of the world.

2.2.1 Japan

At both Minamata Bay and the Agano River the offending industries were industrial complexes which utilized mercuric chloride ($HgCl_2$) as a catalyst for the production of vinyl chloride and mercuric sulphate ($HgSO_4$) as a catalyst for the production of acetaldehyde (Irukayama et al., 1969). The acetaldehyde plants within these complexes were the most important source of Hg (Irukayama et al., 1969). The waste water from the acetaldehyde plant at Minamata Bay contained ca. 80 parts per million (ppm) of total Hg and ca. 50 ppm of MeHg, while that from the vinyl chloride plant contained ca. 0.5 ppm total Hg and ca. 0.3 ppm MeHg (Irukayama et al., 1969). The total loss of Hg from the complex was estimated to be about 360 kg in 1955 and over 1,000 kg in 1960 (Kurland et al., 1960). Mud samples from various areas of the bay were found to contain 12 to 133 ppm of Hg (Kurland et al., 1960). Mud from the immediate drainage area from the factory contained 2,010 ppm of Hg in 1960 (Kurland et al., 1960) and about 600 to 800 ppm approximately 1 year after equipment for waste treatment was completed in January, 1960 (Irukayama et al., 1962). By October, 1963, the Hg content of the mud in the drainage area of the factory had declined to about 30 ppm (Irukayama, 1968), however, at this same time mud samples

from other areas of the bay still contained up to several hundred ppm of Hg and in some areas this zone of Hg-rich sediment extended to a depth of from 3 to 4 m (Irukayama, 1968).

The unusual feature of the Minamata Bay tragedy was that MeHg in the form of MeHgCl was present in the effluent from the acetaldehyde plant (Irukayama et al., 1962). This situation has not been demonstrated elsewhere.

2.2.2 Sweden

The major sources of Hg pollution in Sweden have been reviewed by several authors (Lofroth, 1969; Larrson, 1970; Hanson, 1971; Nelson et al., 1971). The following sources were described as being of primary importance:

(1) Chlorine-alkali industry: Metallic Hg is used as a cathode for the electrolytic production of chlorine and alkali from saline solutions. The loss of Hg to the environment from this industry in Sweden has been variously estimated at 51 to 86 g Hg (Nelson et al., 1971) to 100 to 200 g Hg (Hansson, 1971) per metric ton of chlorine produced. Of this loss about 50 to 60 per cent was lost to the water, while the majority of the remainder was lost to the atmosphere (Nelson et al., 1971). The total annual loss of Hg to the environment by this industry was estimated to be between 25 and 35 metric tons (Lofroth, 1969). The Hg lost to the water was in the elemental or inorganic form.

(2) Pulp and paper industry: Phenyl Hg compounds were used as slimicides by this industry from 1946 to 1968 (Berglund et al., 1971). The total amount of Hg lost to water by this industry

during this period has been estimated at 145 metric tons, with annual losses varying from about 1 metric ton in 1948 to a maximum of 13 metric tons in 1960 (Halldin, 1969). The Hg lost was in the form of phenyl Hg, primarily phenyl mercuric acetate (PHgA).

(3) Mercury catalysts: One vinyl chloride plant operated in Sweden until 1968, and was reported to have discharged 320 kg Hg during 1967 (Lofroth, 1969). No examination for the presence of MeHg in the effluent was performed.

(4) Mercurial seed dressings: Various Hg compounds have been used as fungicidal seed dressings in Sweden since the 1920's. The use of alkyl mercurials for this purpose began in the 1940's and these compounds replaced other forms until their use was banned in 1966 (Berglund et al., 1971). During the period that the alkyl Hg compounds were used, it was estimated that about 80 metric tons of Hg were applied to soils in Sweden (Berglund et al., 1971). There was little evidence that this use caused serious direct pollution of water. Johnels et al. (1967) could find little or no difference between the average Hg concentrations in tissue of pike[1] collected in remote non-agricultural areas and pike collected in areas where seed dressings had been used. It has been suggested that Hg was lost to the water during the manufacture of these compounds (Berglund et al., 1971) and to the atmosphere during seed dressing operations (Halldin, 1969).

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[1]Common names used by authors are used. Scientific names of species are listed in Appendix A.

(5) Fossil fuels: The contribution of Hg to the environment from this source is largely unknown. Fuel oil in Sweden has been found to contain only about 3 ppb Hg, while pit coal contained 60 to 400 ppb (Larsson, 1970). Secondary water pollution from these sources was considered to be small (Larsson, 1970).

(6) Miscellaneous sources: It has been estimated that Hg is used in at least 3,000 different ways in industry (King, 1957), so that the list of other potential sources is long. All of these sources likely result in a very diffuse distribution from many discharge points, and identification of each source is likely very difficult. The following sources were considered to be the more important among this group:

(a) Urban sewage: The concentration of Hg in sludge from urban sewage treatment plants in Sweden has been found to vary from 0.8 to 120 ppm (dry weight basis) (Lofroth, 1969; Larsson, 1970). Usually no distinct source can be found for this Hg (Hanson, 1971) but the concentrations were highest in highly urban areas with mixed industry (Larsson, 1970).

(b) Processing of raw materials and industries using basic chemicals: Mercury occurs in many areas of bedrock and particularly in sulfide ores in Sweden; and emission of Hg may be important from any industry utilizing these ores, particularly if processing involves heating the ore (Berglund et al., 1971). Hanson (1971) reported that a single smelter (type unspecified) had an annual loss to the

environment of 6,000 kg of Hg. The importance of this type of pollution might be considerable, since the annual emission from this smelter would be roughly six times as great as that of the industrial complex at Minamata Bay before waste treatment was instituted. The industries involved would include: iron and steel, ceramics, lime, glass, fertilizer, and cement manufacturers (Henriques, 1969; Larsson, 1970; Hanson, 1971). Many of the basic chemicals used by industry also contain Hg which might be lost to the environment during processing. For example, the alkali produced in chlorine-alkali plants may contain up to 5 ppm Hg (Nelson et al., 1971).

(c) Mercury containing instruments and equipment: The total usage of metallic Hg for these purposes is large, and the potential for water pollution is present (Hanson, 1971), but the effect of this source does not appear to have been studied. Mercury losses from many of these sources may enter water directly while in other cases the Hg is lost to the atmosphere and might produce secondary water pollution. Johnels et al. (1967) speculated that airborne pollution of unknown origin might be responsible for increased Hg levels in fish from waters with no known direct source of contamination.

Jernelov (1969) estimated that over 500 metric tons of Hg have been deposited in the sediments of Swedish waters as a result of human activity.

2.2.3 North America

Prior to the recognition of the occurrence of widespread Hg pollution of water in North America, Fimreite (1970) reviewed the uses of Hg in Canada and warned of the potential for water pollution. In many ways the situation in North America is analagous to that in Sweden, with the same type of industries being involved. However the use of phenyl mercurial compounds as slimicides in the pulp and paper industry in North America has not been as universal as it was in Sweden. The use of these compounds was suspended in most Canadian plants about 1960, with only nine mills using Hg slimicides in 1969 (Fimreite, 1970). The use of Hg for this purpose in the United States had similarly declined from about 112 metric tons in 1960 to about 14 metric tons in 1969 (National Materials Advisory Board, 1969). In 1968 the consumption of Hg by the pulp and paper industry in the U.S.A. represented only about 0.5 per cent of the total consumption for that year (National Materials Advisory Board, 1969).

The chlorine-alkali industry would appear to be the major source of water contamination in Canada, consuming about 91 metric tons of a total of 136 metric tons of Hg used in Canada in 1969 (Bligh, 1971). Mercury contaminated fish have been found in the vicinity of every Canadian chlorine-alkali plant utilizing Hg electrolytic cells (Bligh, 1971).

The major consumer of Hg in the U.S.A. was the electrical industry which utilized 25.2 per cent of the total Hg used in 1968, compared to 22.9 per cent used by the chlorine-alkali industry in the same year (National Materials Advisory Board, 1969). In the U.S.A., the

estimated total Hg discharge per day in July, 1970, from the 50 industries known to be Hg dischargers was 130 kg (Stroud, 1970). Individual industries were reported to have discharged up to 33 kg of Hg per day (Stroud, 1970).

Kurland et al. (1960) reported Hg contents of up to 12.5 ppm in mud in Galveston Bay, Texas, near vinyl chloride plants but oysters (unspecified) from the bay contained only 0.3 ppm Hg. The method used in the U.S.A. for purifying the vinyl chloride differed from that in Minamata Bay, which might have accounted for the difference (Kurland et al., 1960).

Elevated levels of Hg have been found in fish from Pinchi Lake, British Columbia, near the site of a Hg mining operation (Peterson et al., 1970; Bligh, 1970); however, the relative contributions from the mining operation and from the high Hg content of the soil in the area (Warren et al., 1966) have not been defined. Other potential sources of Hg listed by Fimreite (1970) and Peterson et al. (1970) were essentially the same as those in Sweden.

2.3 Biosynthesis of methyl mercury

Mercury in fish in Sweden and Canada has been found to occur mainly in the form of MeHg (Westoo, 1966b; Bligh, 1970); however, there is some indication that MeHg may have made up only a fraction of the total Hg content of Japanese fish (Jensen, 1969).

When the toxic mercurial compounds present in fish and shellfish from Minamata Bay were identified as MeHg compounds, methylation of Hg in the water was suggested (Fujiki, 1963). This was subsequently largely discounted on the basis of the presence of MeHgCl in sludge

within the acetaldehyde plant (Irukayama et al., 1962), and it was assumed that the MeHg in fish came from this source.

In Sweden the Hg discharged into water as industrial wastes was in the inorganic or aryl form, but the main part of the Hg in fish was present as MeHg (Westoo, 1966b). Recently Yamaguchi et al. (1971) demonstrated the presence of small quantities of "a compound resembling methyl mercury" in the effluent from a caustic soda factory. These authors state that the methylation occurred mainly in a sedimentation pit for effluent water and they do not appear to have considered the possibility of biological methylation. Jensen and Jernelov (1969) demonstrated methylation of Hg by aquarium and lake bottom sediments, and in systems containing decaying fish. Wood et al. (1968) showed that extracts from a methanogenic bacterium, originally isolated from canal mud, were capable of methylating Hg. These authors also described nonenzymatic methylation of Hg by in vitro solutions of methyl cobalamine (a Vitamin B_{12} analog). Chemical methylation of Hg with methyl cobalamine was confirmed by Imura et al. (1971).

Nonenzymatic methylation of Hg by methyl cobalamine has been found to be predominant in aerobic organisms which use methylcorrinoids in their intermediary metabolism (J. M. Wood, personal communication[a]). This

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[a]Dr. Wood supplied a manuscript: Wood, J. M., M. W. Penley and R. E. Desimone. Mechanisms for the methylation of mercury in the environment. This was to appear in: Mercury Handbook (1971). Int. Atom. Energy Comm. This volume has not been published to date.

nonenzymatic reaction did not occur in the presence of the mercuric ion (Hg_2^{++}) or Hg^0 both of which are formed under anaerobic conditions (J. M. Wood, 1970, personal communication). Enzymatic synthesis of MeHg likely also involves methylcorrinoids through several enzyme systems (J. M. Wood, 1970, personal communication). It is interesting that one of these enzyme systems (cobalamine dependent methionine synthetase) is present in many aerobic and facultative anaerobic microorganiams capable of MeHg synthesis, as well as in mammalian liver (J. M. Wood, 1970, personal communication), extracts of which have also been shown to methylate Hg (Westoo, 1968).

Another interesting point is that it was suggested that this enzyme system could produce MeHg-S-Me in addition to MeHg+. MeHg-S-Me was the compound originally identified as the cause of Minamata disease (Uchida et al., 1961), and the evidence for biosynthesis of this compound perhaps supports the contention that biosynthesis of MeHg occurred in Minamata Bay.

Both Wood et al. (1968), and Jensen and Jernelov (1969) found that a mixture of monomethyl Hg and dimethyl Hg were formed during these methylation reactions. Imura et al. (1971) stated that the initial product of the methyl cobalamine reaction was dimethyl Hg, which was then converted to the monomethyl form. Wood et al. (1968) and Imura et al. (1971) found high concentrations of Hg++ in solution favoured the production of the monomethyl form. Water pH is also important for determining the type of alkyl Hg formed. It has been suggested that alkaline waters might favour organisms which produce the dimethyl form (Larsson, 1970), in addition dimethyl Hg is unstable at low pH and decomposes to the monomethyl form in acid environments (Westoo, 1967; Imura et al., 1971).

Dimethyl Hg is a very volatile compound (b.p. 94°C) and in alkaline waters this compound may largely volatize to the atmosphere (Olsson, 1968, cited in Larsson, 1970), while in acid environments this dimethyl Hg would be converted to the less volatile monomethyl form and tend to remain in the water (Larsson, 1970). Johnels (1971) suggested that the reduction of volatilization of Hg from acid waters might account for the occurrence of elevated Hg concentrations in fish from remote acid lakes free of known contamination.

It appears that microbial mechanisms exist for the methylation of Hg under both aerobic and anaerobic conditions (Jernelov, 1969; J. M. Wood, 1970, personal communication). Jernelov (1969) suggested that in anaerobic situations inorganic divalent Hg might be precipitated as very poorly soluble mercuric sulfide and Miettinen (1970) found that in completely anaerobic situations the rate of methylation was reduced. However, since both aerobic and anaerobic methylation systems exist, the rate of synthesis may be dependent upon (1) the populations of microorganisms present, and (2) the concentration of Hg substrate available for methylation.

Jernelov (1969) has presented evidence that the rate of methylation tends to increase with increasing content of inorganic Hg in the substrate, and as stated previously Wood et al. (1968) and Imura et al. (1971) found that increasing content of inorganic Hg favoured the production of the monomethyl form, probably due to direct interaction of dimethyl Hg with the inorganic Hg to form the monomethyl form (Imura et al., 1971). It would thus appear that the combination of sediments containing large quantities of Hg and nutrient rich water

such as occur in many areas contaminated by urban sewage would provide optimum conditions for methylation of Hg.

All forms of Hg likely to be released in industrial effluents can be methylated in natural waters, and microorganisms capable of methylating Hg were present in sediments from all of over 100 lakes and rivers examined in Sweden (Jernelov, 1969). Jernelov (1969) suggested that the arylmercurial, PHgA, used as a slimicide may be more rapidly methylated than other forms. In contrast, Matsumura et al. (1971) found that no MeHg derivative was produced from PHgA under anaerobic conditions by 35 different bacterial isolates from soil and lake sediments. These authors suggest that the methylation of PHgA may be a two-step process. Bacteria have been isolated which will convert phenyl Hg to metallic Hg (Ueda, 1971) and conversion could then proceed to MeHg.

Jernelov (1970, 1972a) has shown that organisms larger than bacteria may also be important in the conversion of inorganic Hg in sediment to MeHg. In aquaria containing no macroorganisms methylation occurred only when inorganic Hg was present at the surface of the sediment, but methylation proceeded when the Hg-rich sediment was covered to a depth of 2 cm and 9 cm by Hg-poor sediments when oligochaete worms (Tubificidae) and a bivalve (Anodonta), respectively, were present. The evidence for biosynthesis of MeHg within larger animals is very fragmentary. Westoo (1967, 1968) has demonstrated that liver homogenates can methylate Hg, and hens fed inorganic, aryl and alkyl-oxyalkyl mercurials have been found to convert a small fraction of these compounds to MeHg which was excreted in the eggs

(Westoo, 1967; Kiwimae et al., 1969).

The presence of chlorinated hydrocarbons in Hg-rich sediments may retard methylation of Hg, probably due to inhibition of methionine synthetase. In this case fish living in the waters accumulate chlorinated hydrocarbons but have only low concentrations of Hg in their tissues (J. M. Wood, 1970, personal communication).

2.4 Distribution and concentration in tissues

2.4.1 Fish

The uptake of MeHg by fish either via absorption through the surface epithelium, or through the digestive tract is rapid (Hannerz, 1968; Backstrom, 1969; Giblin and Massaro, 1973). After absorption the distribution pattern within tissues changes with time. Within a few hours, high concentrations are present in the blood, kidney, liver, spleen, pseudobranchiae, and gills (Backstrom, 1969; Miettinen et al., 1970b; Giblin and Massaro, 1973). In relatively short term experiments Hg concentrations are high in the liver, spleen, kidney, and gills, and low in the brain and skeletal muscles (Giblin and Massaro, 1973).

The concentration of Hg in the brain and skeletal muscle increases with time (Hannerz, 1968; Backstrom, 1969; Miettinen et al., 1970b; Giblin and Massaro, 1973). Giblin and Massaro (1973) found that after a single exposure to MeHg, the maximum concentrations in the skeletal muscle and brain were reached after 34 and 56 days respectively. Maximum levels were attained in most other organs after about 7 days.

Although the general distribution pattern in fish is similar to

that in mammals and birds, certain differences have been noted. The spleen in fish concentrates more Hg than does this organ in mammals or birds (Backstrom, 1969; Miettinen et al., 1970b; Giblin and Massaro, 1973). The most important difference between fish and mammals is in the blood-brain Hg ratio. This ratio in various species of fish has been found to be approximately 10:1 (Hannerz, 1968; Backstrom, 1969; Giblin and Massaro, 1973), while the corresponding ratio in mammals is generally in the range 0.1 to 2.0: 1 (Berglund et al., 1971).

Backstrom (1969) commented on the slow kinetics of MeHg once distributed in the fish body, and Giblin and Massaro (1973) stated that skeletal muscle seemed to act as a reservoir for MeHg, accumulating Hg while concentrations in other organs were decreasing.

The concentration of Hg in the muscle of normal freshwater fish from uncontaminated environments has been reported to range from 0.05 to 0.2 ppm (Johnels et al., 1967; Rucker, 1968; Rucker and Amend, 1969). In contrast, Hg concentrations of more than 20 ppm in muscle have been reported in fish from contaminated environments (Bligh, 1971).

Johnels et al. (1967), Hannerz (1968) and Johnels (1971) have estimated that the concentration factor from water to fish flesh in contaminated waters is of the order of 2,000 to 5,000 or more.

2.4.2 Birds

Much of the information available on MeHg in birds relates to the terrestrial environment and the use of organomercurial seed dressings. This has resulted in direct accumulation of Hg in seed-eating birds

and secondary accumulation in raptores. This information, while not directly related to this review, must be considered in lieu of specific data on fish-eating birds.

The first reference to the accumulation of Hg in birds as a result of ingestion of contaminated fish was from the Minamata Bay area of Japan, where crows and sea birds (unspecified) developed a neurologic disease. The pathologic changes present in these birds were described by Takeuchi (1968a). Since this initial report increased Hg concentrations have been demonstrated in feathers, tissues, or eggs of fish-eating birds in Sweden (Berg et al., 1966); other areas of Japan (Muto and Suzuki, 1967), Finland (Henriksson et al., 1966; Karpannen et al., 1970), Ireland (Eades, 1966), and Canada (Keith and Gruchy, 1971).

Tejning (1965) demonstrated that Hg may accumulate in the feathers of birds. Berg et al. (1966) conducted a survey of the Hg content of the feathers of birds in the collection of the Swedish Museum of Natural History. These birds have been collected from 1829 onwards. The results indicated a dramatic increase in the Hg content in the feathers of birds collected after about 1940. This increase occurred in both terrestrial birds and the white-tailed eagle, whose diet consisted of about 50 per cent fish. During the total period tested the levels in the white-tailed eagle were much higher than those of terrestrial birds collected at about the same time. Subsequent studies of a similar nature revealed that two distinct situations existed in birds in Sweden.

The Hg concentrations in feathers of seed-eating birds and their

avian predators remained relatively constant until about 1940. Then there was a sudden rise in the Hg content of feathers of seed-eating birds and an even greater (up to 20 fold) increase in the concentration in feathers of terrestrial raptores (Johnels et al., 1968; Edelstam et al., 1969; Johnels and Westermark, 1969). This sudden increase in the Hg content of the feathers of terrestrial birds corresponded to the advent of widespread use of alkyl mercurial seed dressing agents (Berg et al., 1966). Borg et al. (1969) reviewed Hg poisoning in Swedish wildlife and concluded that alkyl Hg seed dressings were the primary source. This was later confirmed by the demonstration of declining Hg levels in terrestrial birds after the use of these compounds was suspended in 1966 (Wanntorp et al., 1967).

The situation in aquatic birds was different. Feathers of museum specimens of the osprey and the great crested grebe showed a steady increase in Hg content from about 1900 onward. This increase was considered to be a reflection of general industrial development in Sweden, and Johnels and Westermark (1969) listed many industrial processes, including the chlorine-alkali industry, which began operation in Sweden about the turn of the century. Larsson (1970) stated that osprey and great crested grebes living in Hg polluted areas had Hg concentrations in their tissues three times greater than those in birds from non-contaminated areas. Feathers grown by osprey during their stay in Sweden had a considerably higher Hg content than did feathers grown during the portion of each year that these birds spend in the Mediterranean area and South Africa (Johnels and Westermark, 1969).

There is very little information available on the distribution of Hg within the bodies of fish-eating birds, however the data from other species present a general picture which is likely applicable to these species also. In general the distribution in chickens and terrestrial birds can be summarized as follows: kidney and liver - high and roughly equivalent Hg content; muscle, nervous tissue, heart, lung, ovary - Hg concentration 20 to 50 per cent of that in liver and kidney.

The plumage accumulates substantial quantities of Hg during its growth (Tejning, 1965; Berg et al., 1966). Tejning (1967) suggested that ingested MeHg was primarily deposited in the blood cells and internal organs and then gradually transported to the feathers. The plumage would eventually contain almost all of the Hg not eliminated in excrement or eggs (Tejning, 1967). Berg et al. (1966) suggested that if the differences in water content were taken into consideration the feathers generally contained about seven times as much Hg as did muscle. Since birds moult their plumage at least once each year, the loss of Hg via this route may be an important method of excretion.

It has also been observed that significant amounts of MeHg may be excreted in the eggs of birds fed or injected with MeHg (Smart and Lloyd, 1963; Tejning and Vesterberg, 1964; Tejning, 1967; Backstrom, 1969; Borg et al., 1969; Fimreite, 1971; Wahlberg et al., 1971). The Hg is more concentrated in the white of the eggs (Smart and Lloyd, 1963; Backstrom, 1969; Borg et al., 1969; Wahlberg et al., 1971). This could be associated with high concentrations of Hg in the albuminiferous portion of the oviduct observed by Tejning (1967)

and Backstrom (1969). Backstrom (1969) found that about 50 per cent of an injected dose of MeHg was excreted via the eggs. During incubation of eggs containing MeHg, the Hg was transferred from the albumin to the yolk and subsequently to the embryo (Backstrom, 1969).

There are very few data available on the normal Hg content of the tissues of fish-eating birds, and such information is difficult to obtain at the present time due to the high mobility of birds and the widespread use of Hg in the environment. Data from museum specimens in Sweden would indicate that the normal Hg content of fish-eating birds has always been higher than that of terrestrial birds (Berg et al., 1966). Johnels et al. (1968) estimated the natural level of Hg in feathers of the osprey and great crested grebe to be about 4 ppm. Berg et al. (1966) estimated that the normal value for the feathers of the white-tailed eagle was about 6.6 ppm. Applying the equilibrium factor of feather to muscle levels of seven proposed by Berg et al. (1966), the average Hg content of muscle of these birds would be about 0.5 to 0.9 ppm respectively. Also if the concentration factor from diet to muscle of four to five found in goshawks by Borg et al. (1970) is applicable to fish-eating birds, and if the normal Hg content of fish may be up to 0.2 ppm (Johnels et al., 1968) such tissue levels would appear to be possible in the absence of man-made contamination.

2.4.3 Mammals

The distribution of Hg within the tissues of MeHg poisoned mammals has been reviewed by Berglund et al. (1971). Although the distribution varied somewhat between species, and with the rate and route of administration, the basic pattern was similar in the species studied.

The highest concentrations in the body were usually found in the liver. Kidney levels were similar to those in the liver. Brain usually contained substantially less Hg than did either the liver or kidney. The average ratio of liver to brain Hg concentration calculated from the data reviewed by Berglund et al. (1971) was 4.9:1. This ratio was higher than would occur if data on animals poisoned with fish from Minamata Bay, Japan, were excluded. In some of these animals liver to brain Hg ratios of 15:1 to as high as 26:1 were reported (Kitamaru, 1968). As previously stated, there is some evidence that not all of the Hg in Japanese fish was in the form of MeHg (Jensen, 1969), which might explain this discrepancy.

In all species studied, the Hg present in the blood was primarily bound to the cells, and only small amounts were present in the plasma. The ratio of Hg concentration in the blood to that in the brain was in the range 0.1 to 2.0:1 in most species. In those species in which hair was analyzed, a high concentration of Hg was found to be present (Berglund et al., 1971).

There are few data on the concentration factor for MeHg from the diet to muscle of mammals. Hanko et al. (1970) calculated this concentration factor to be about six for ferrets. Calculation of a concentration factor from diet to muscle of the cats studied by Albanus et al. (1972) gave a factor of 4.5. These concentration factors are similar to those calculated for certain birds (Borg et al., 1970).

2.5 Elimination and the biological half-life of methyl mercury

It appears that the elimination of MeHg from the body is a rather slow process in all species in which this has been studied.

2.5.1 Fish

The routes of elimination of MeHg in fish have not been studied in detail. Giblin and Massaro (1973) suggested that the feces were a main excretion route. Table 1 shows the information available on the elimination rate of alkyl Hg compounds from fish and a few other aquatic organisms. Other authors (Hannerz, 1968; Backstrom, 1969) had insufficient data to arrive at a calculation of biological half-life, but have commented on the slow kinetics of MeHg once distributed in the body. The mechanisms which may account for the slow metabolism of Hg in fish have been discussed by Backstrom (1969). Among the factors considered were lower metabolic rate, high content of Hg binding sites (methionine and cysteine) in fish muscle, and higher content of nitrogen in fish muscle than in mammalian or avian muscle. The reasons for the slow elimination must still be considered unknown; however, water temperature likely plays an important role in determining elimination rate (Jarvenpaa et al., 1970).

The very slow elimination (biological half-life of 1 to 2 years) likely explains the ability of fish to concentrate Hg and the high levels which have been detected in fish in many parts of the world.

2.5.2 Birds

The routes of elimination of MeHg by birds have received some attention. Tejning (1967) found that approximately 11 per cent of

TABLE 1. Reported biological half-life ($TB_{1/2}$) of alkyl mercury in some poikilothermic aquatic species.

Species	$TB_{1/2}$ (days)	Author
Mollusc (Tapes)	480	Miettinen et al. (1970a)
Mussel	>1,000	Miettinen et al. (1970a)
Shore crab	400	Miettinen et al., (1970a)
Northern pike	600	Tillander et al. (1969)
	110 ± 20	Miettinen et al. (1970b)
	640 ± 120	Jarvenpaa et al. (1970)
	750 ± 50	Jarvenpaa et al. (1970)
	780 ± 80	Jarvenpaa et al. (1970)
	ca. 2 years	Lockhart et al. (1972)
Flounder	170 ± 60	Tillander et al. (1969)
	780 ± 120	Jarvenpaa et al. (1970)
	700 ± 50	Jarvenpaa et al. (1970)
	1200 ± 400	Jarvenpaa et al. (1970)
Sea perch	270	Miettinen et al. (1970a)
Perch	170 ± 60	Tillander et al. (1969)
Eel	910 ± 40	Jarvenpaa et al. (1970)
	1030 ± 70	Jarvenpaa et al. (1970)
	1030 ± 80	Jarvenpaa et al. (1970)
Rainbow trout	>200	Giblin and Massaro (1973)

doses of MeHg ingested by domestic fowl was eliminated in the excrement. Whether this represented true elimination or simply passage of nonabsorbed Hg is unclear, since the author stated "Only a minor proportion of the mercury deposited in internal organs was subsequently eliminated in excrement". In one bird maintained for 41 days after the cessation of dosing with MeHg, only 2.5 per cent of the Hg retained in the body was eliminated in the excrement (Tejning, 1967), suggesting that most of Hg passed in the feces was in fact nonabsorbed.

Deposition of Hg in the plumage may represent an important method of clearance in birds. Tejning (1967) felt that MeHg was primarily deposited in blood cells and internal organs and then gradually transported to the feathers; and that eventually the plumage would contain all of the Hg not eliminated by other routes. In one bird killed 41 days after the last administration of MeHg, the plumage contained 73.9 per cent of the ingested Hg, compared to 3.8 per cent in the internal organs. Backstrom (1969) also noted a heavy accumulation of Hg in the feathers of experimentally poisoned Japanese quail, but did not give quantitative data. Muto and Suzucki (1967) found up to 21 ppm of Hg in the feathers of Japanese storks in which chronic Hg poisoning was suspected. Excretion of Hg in eggs has been noted by a number of authors and Backstrom (1969) noted that male Japanese quail eliminated Hg much less rapidly than did females. The elimination of MeHg via the egg was rapid and 50 per cent of the dosage was excreted via the egg, primarily during the first week after injection (Backstrom, 1969). Tejning (1967) found that Hg excretion

via the egg increased with increasing intake, but at a relatively lower rate. In contrast, Backstrom (1969) found that excretion via the egg increased relatively more rapidly than did intake and he theorized that "The egg evidently acted as a safety valve; excreting the excess of mercury not excreted by other organs". More data are needed to clarify this point. Although the methods of elimination have not been precisely defined, it is comparatively rapid in birds and the biological half-life in chickens has been estimated to be of the order of 30 to 35 days (Ulfvarson, 1962; Swensson and Ulfvarson, 1963, 1968a). Tejning (1967) calculated the time for total elimination of Hg from the organs of pheasants to be 5 months.

2.5.3 Mammals

The elimination rate of alkyl Hg varies between species but is rather slow in all mammals studied. The principle routes of excretion are via the feces, urine, and hair.

Elimination via the feces is the most important route, with only a small fraction of the Hg being excreted in the urine (Friberg, 1959; Gage and Swan, 1961; Miller et al., 1961; Ulfvarson, 1962, 1970; Gage, 1964; Swensson and Ulfvarson, 1967; Platonow, 1968; Norseth, 1969). In two humans given a small dose of MeHg, approximately 3 per cent of the dose was excreted in urine and 34 per cent in the feces over a 49 day period (Aberg et al., 1969). Elimination via sequestration of Hg in the hair is likely an important route of elimination in animals. Berglund (1969) found that the hair contained 30 per cent of the total body burden of Hg in rats exposed to MeHg for 7 months. Similarly 50 per cent of the body burden was present in the

skin of squirrel monkeys exposed to MeHg for 12 weeks (Nordberg et al., 1971).

The biologic half-life of MeHg has been reported for a number of species (Table 2); however, many of the determinations were based on the use of radio isotopes and whole body counting and no allowance was made for the Hg content of the hair. Mercury within hair should be regarded as excreted (Berglund et al., 1971), thus the true half-life values may be shorter than shown.

Other methods of expressing elimination rate are as a daily elimination constant or as a percentage of total body burden/day. Using these measures the daily elimination constants were .083 to .108 for mice (Ulfvarson, 1970), 0.03 for rats (Ulfvarson, 1962, 1970), and less than 0.01 for man (Aberg et al., 1969; Miettinen et al., 1971) and the corresponding percentage of body burden/day was 8.3 to 10.8 for mice, 3 for rats and less than 1 per cent for man.

2.6 The pathology of alkyl mercury poisoning in vertebrates

Poisoning of man and animals has occurred by exposure to alkyl Hg by two main routes: (1) exposure to chemically formulated alkyl mercurials, primarily when used as antifungal seed dressings, (2) exposure to biologically methylated Hg formed in an aquatic environment.

The pathology of experimental and naturally occurring cases of alkyl Hg poisoning has been described in a number of species (Appendix A), but no review of the comparative aspects has been made. Published reports of alkyl Hg poisoning have largely been confined to

TABLE 2. Reported biological half-life ($TB_{1/2}$) of methyl mercury in various mammals.

Species	$TB_{1/2}$ (days)	Remarks	Author
Mouse	3.7 - 12.6	Varied with dose	Ostlund (1969)
	7		Ulfvarson (1970)
	6 - 7	Brain	Suzuki (1969)
	7	Blood	Suzuki (1969)
Rat	20	1/2 of dose excreted in 20 days	Ulfvarson (1962)
	20		Norseth (1969)
	16	measured over 9 days	Swensson and Ulfvarson (1968b)
	51[a]	measured over 169 days	Swensson and Ulfvarson (1968b)
Squirrel monkey	50 - 60	Blood	Nordberg et al. (1971)
	150[a]		Nordberg et al. (1971)
Ring seal	20 and 500	two phase exponential	Tillander (1969), Tillander et al. (1970)
Man	70 - 74[a]	whole body	Aberg et al. (1969)
	50 $\pm$ 7	Blood	Miettinen et al. (1971)
	73 $\pm$ 3[a]	Whole body	Miettinen et al. (1971)

[a]- Whole body measurements including mercury content of hair.

those dealing with the short chain alkyl mercurials, primarily MeHg and ethyl (Et) Hg compounds. This review will be confined to this class of alkyl mercurials, and since there seems to be little morphologic distinction in the patterns of damage due to these compounds, the individual chemical compounds used by various authors will be alluded to infrequently.

The review of the published literature dealing with the pathology of Minamata disease in humans in Japan and experimental animal studies in connection with Minamata disease, was made difficult by the multiplicity of reports by different authors, or by different combinations of the same authors describing cases and experiments which appear to be the same. In instances where duplication was evident the report which provided the most complete description was used for tabulating data.

2.6.1 Fish

Only very limited data are available on the pathologic effects of alkyl Hg on fish. Takeuchi (1968a) described changes in fish dying in Minamata Bay, Japan, at the time Minamata disease occurred in humans near the bay. The fish were emaciated and cataracts were present. Degeneration of neurons at unspecified sites in the brain and the loss of granular cells in the cerebellum were also described. The value of these observations is limited because no information on the species or numbers of fish examined, or on the incidence of lesions was presented. Photomicrographs of similar areas from normal fish of the same species for comparison would have aided interpretation.

Amend et al. (1969) described necrosis and desquamation of gill

epithelium in rainbow trout exposed to 0.125 ppm of Et Hg PO_4 for 1 hour in water with 5 ppm dissolved oxygen (DO). At 9 ppm DO there was less necrosis and hypertrophy of gill epithelium was the prominent feature.

Miettinen et al. (1970b) administered MeHg at a dosage range of 9.6 to 17.0 mg/kg via stomach tube to pike and rainbow trout. The Hg was in two forms, $MeHgNO_3$ and protein-bound MeHg. The mean life span of pike after dosing with these compounds was 33 and 18 days, respectively. The corresponding life span for rainbow trout was 94 and 70-plus days, respectively.

The gross lesions observed in pike included swelling of the kidney and pancreas, and the presence of green "fluid and jelly" in the body cavity. The liver of these fish were described as being "dirty brown" in color and three of 10 fish had areas of necrosis in the liver. The gall bladder was enlarged in about half of the fish, and bile staining of the stomach and intestinal mucosa was observed. Serosal vessels on the intestines were engorged. Ruptured vessels were observed in the muscle near the lateral line. The histopathologic lesions observed in northern pike were necrosis of gill lamellae, inflammation of the pseudobranchiae (also present to some degree in control fish), focal hepatic necrosis, "oedema in the glomeruli and the tubules", and tubular degeneration. No histopathologic or gross lesions were detected in the eyes or nervous system of these fish. The only gross lesions observed by Miettinen et al. (1970b) in rainbow trout were dark swollen spleens and dark gall bladders. No histopathologic findings were reported.

Backstrom (1969) reported green discoloration of the flesh and liver of pike exposed to phenyl Hg in a pond (there was a possibility that alkyl-Hg was formed by biological methylation in this system). This discoloration was felt to be due to deposition of bile pigments in the flesh, but no hepatic abnormality other than excess bile in bile ducts was detected on histopathology.

From the limited data available it appears that acute exposure to alkyl Hg may result in damage to the gill epithelium. This may be due to selective accumulation of alkyl Hg in this site. Backstrom (1969) found that a high content of Hg was present in the gills of fish irrespective of route of administration. Miettinen et al. (1970b) produced peracute poisoning (death within 2 hours) in rainbow trout by oral administration of 11.9 mg/kg body weight of MeHg. These fish died in respiratory distress with the mouth open and opercula flared. The Hg concentration in the gills of these fish was more than 10 times the mean concentration in all body tissues.

The pathology of more chronic alkyl Hg poisoning in fish is contradictory. Takeuchi (1968a) described lesions in the eyes and nervous system, but these were not observed by Miettinen et al. (1970b). Conversely, the renal and hepatic lesions described by Miettinen et al. (1970b) were not reported by Takeuchi (1968a). This discrepancy could be due to differences in length, rate and route of exposure to Hg, inter-specific differences, and possibly differences in chemical form of the Hg involved. It seems likely that in both the natural cases from Japan (Kitamura, 1968) and the experimental work from Sweden (Miettinen et al., 1970b) MeHg made up the bulk of the Hg present

in the fish, however the Japanese method of analysis may have indicated concentration values lower than the methods employed in Sweden (Berglund et al., 1971).

The length and rate of exposure to Hg may be the most important factor in producing the differences in lesions observed. The studies of Miettinen et al. (1970b) were of short duration (less than 38 days) while the natural cases reported by Takeuchi (1968a) can be assumed to be of longer duration.

Backstrom (1969), and Giblin and Massaro (1973) have shown that the concentration of Hg rises more slowly in the brain than in the liver and kidney so that fish given high doses of Hg might succumb from gill, liver, or kidney failure before brain lesions were recognizable.

The relationship of Hg concentration in tissue to pathologic effect deserves more study since grossly normal fish with Hg concentrations similar to those reported in poisoned fish have been reported from contaminated waters in many areas of the world (Johnels et al., 1967; Hannerz, 1968; Hasanen and Sjoblom, 1968; Bligh, 1971; Bails, 1972; Greig and Seagran, 1972).

2.6.2 Birds

Widespread mortality of wild birds in association with the consumption of seed grain treated with various seed dressing agents was noted in the mid-1950's in both Britain (Carnaghan and Blaxland, 1957; Murton and Vizoso, 1963), and Sweden (Borg et al., 1969). Carnaghan and Blaxland (1957) concluded that seed dressing agents containing organomercurials, including MeHg dicyandiamide were non-toxic to wood-pigeons and pheasants, and Murton and Vizoso (1963)

also felt that organomercurials were of little significance. In contrast Borg et al. (1969) concluded that organomercurials, principally MeHg dicyandiamide, were important in poisoning of seed-eating birds and in secondary poisoning of raptorial birds. Takeuchi (1968a) briefly described nervous lesions in crows and seabirds (unspecified) thought to be poisoned by ingestion of Hg contaminated fish from Minamata Bay, Japan.

The gross lesions reported in both naturally occurring and experimental alkyl mercurial poisoning of birds appear to be very non-specific (Table 3). The muscle atrophy and emaciation noted in both natural cases (Borg et al., 1969) and experimental poisoning (Borg et al., 1969, 1970; Fimreite and Karstad, 1971) were likely the result of inanition in the affected birds. Degenerative changes in the liver and kidneys have been reported by all authors who examined these organs from alkyl Hg poisoned birds (Table 4); degenerative changes in skeletal muscle were likely secondary to the paralysis (Fimreite and Karstad, 1971). The extent and distribution of lesions in the nervous tissue of birds was highly variable (Table 5). Borg et al. (1969, 1970), and Fimreite and Karstad (1971) stressed the occurrence of degenerative changes in myelinated axons in peripheral nerves and the spinal cord. These lesions were found to be most prominent in the dorsal spinal funiculi by Fimreite and Karstad (1971), and in the ventral horn (Borg et al., 1970). Takeuchi (1968a) did not describe these lesions, but described the loss of granular cells of the cerebellum. Brown and Yoshida (1965) observed degeneration of both granular cells and Purkinje cells in the cerebellum.

TABLE 3. Gross lesions reported in birds poisoned with alkyl mercurials

Lesion										
Catarrhal enteritis	Ulceration of the crop	Hemorrhage into intestine	Ascites	Icterus	Generalized congestion	Anaemia	Muscular atrophy	Atrial hemorrhage	Inflammation and edema of spleen, kidney, liver	Author
+		+								Leedy and Cole (1950)[a]
+	+			+		+		+	+	Palmer (1963)[a]
+					+	+	+			Borg et al. (1969)[a,b]
		+					+			Borg et al, (1970)[a]
			+				+			Fimreite and Karstad (1971)[a]

[a] - Experimental

[b] - Natural cases

TABLE 4. Histopathologic lesions in organs other than the nervous system of birds poisoned with alkyl mercurials

Hepatic degeneration	Hepatic necrosis	Enteritis	Degeneration of skeletal muscle	Myocardial degeneration	Degeneration of smooth muscle of vessels	Tubulonephrosis	Tubulonecrosis	Author
+						+	+	Leedy and Cole (1950)[a]
+	+	+	+	+		+		Borg et al. (1969)[a,b]
+						+		Borg et al. (1970)[a]
+			+	+	+	+		Fimreite and Karstad (1971)[a]

[a] - Experimental

[b] - Natural cases

TABLE 5. Reported incidence and distribution of histopathologic lesions in nervous tissue of birds poisoned with alkyl mercurials

Cerebrum					Cerebellum				Midbrain and Brainstem				Spinal Cord			Spinal Ganglia		Peripheral Nerves		Author
Myelin degeneration	Axonal degeneration	Neuronal degeneration	Gliosis	Perivascular infiltration	Myelin degeneration	Axonal degeneration	Granular cell loss	Purkinje cell degeneration	Myelin degeneration	Axonal degeneration	Neuronal degeneration	Gliosis	Myelin degeneration	Axonal degeneration	Neuronal degeneration (Ventral Horn)	Heterophil infiltration	Neuronal degeneration	Myelin degeneration	Axonal degeneration	
							+	+												Brown and Yoshida (1965)[a,b]
		+					+													Takeuchi (1968a)[b]
				+														+		Borg et al. (1969) (natural cases)
				+	+				+		+	+						+	+	(experimental)
									+		+	+	+		+			+	+	Borg et al. (1970)[b]
+	+		+	+				+	+	+			+	+		+	+	N. E.[c]		Fimreite and Karstad (1971)[b]

[a] - Only cerebellum examined

[b] - Experimental

[c] - N.E. - Not examined.

Reactive gliosis and perivascular accumulation of inflammatory cells were also observed with varying frequency by different authors.

The pathologic lesions described from natural cases of poisoning in birds (Takeuchi 1968a; Borg et al., 1969) may not represent those of "pure" alkyl mercurial poisoning, since other factors, or combinations of factors, may have been involved. For example, seed dressing agents used prior to 1964 in Sweden were often combinations of Aldrin and MeHg compounds (Borg et al., 1969).

2.6.3 Mammals

Alkyl Hg poisoning in man and other mammals has occurred after exposure to chemically formulated alkyl mercurials, primarily in the form of seed dressings, and through ingestion of MeHg contaminated fish. The reports of lesions include both natural and experimental poisoning with a wide range of alkyl Hg compounds in several species.

Alkyl mercurials readily cross the placental barrier in mammals and may produce prenatal poisoning, so that the pathology of prenatal intoxication will be considered separately from that of postnatal intoxication.

2.6.3.1 Prenatal intoxication

Experimental intoxication of pregnant mammals has been attempted by several authors. The results of these experiments are very difficult to interpret, because in many cases few or no control animals were used, and no attempt was made to eliminate other factors which might produce similar lesions. For example, Morikawa (1961a) described cerebellar atrophy (hypoplasia?) in one of eight kittens

produced by three cats given bis EtHg-S during pregnancy. No control animals were used, and similar lesions have been reported due to intrauterine viral infection in cats (Kilham et al., 1967).

Murakami (1971) reviewed the prenatal effects of organic Hg compounds; however, the results covered in this review are also difficult to interpret for the above reasons, and also because the same experiments appear to have been described by more than one author. Nolen et al. (1972 a,b), in a series of more adequately controlled experiments, reported a higher overall incidence of anomalous fetuses in rats exposed to MeHgCl when compared to controls. Lesions reported were hydronephrosis, hydroureter, bladder defects, undescended testes, absence of the fifth sternebrae, incomplete calcification of the skeleton and cleft palate. The fetal form of human alkyl Hg poisoning has been well documented, but it is difficult to tabulate results since the same cases were apparently described in detail by more than one author (for example, Matsumoto et al., 1965, and Takeuchi, 1968a, and it is not clear if the clinical cases reviewed by Murakami, 1971, also included these same cases).

The gross lesions described in two cases by Matsumoto et al. (1965) and Takeuchi (1968a) were: talipes equines deformity of the feet, asymmetry of the head, abnormal dentition, muscular atrophy, hypoplasia of the cerebral cortex, brain stem, spinal cord, and corpus callosum; hypoplasia and atrophy of the cerebellum, thickening of the leptomeninges, underdevelopment of body organs, and hypoplasia of the bone marrow. Murakami (1971) described microcephalus of varying degrees, and dental abnormalities in 7 and 18 respectively

of the 23 clinical cases reviewed.

The histologic lesions described by Takeuchi (1968a) were diffuse disorganization of cortical cellular architecture with disarrangement and malformation of neurons. The cerebellum also showed evidence of atrophy and hypoplasia of all layers, but this change was most severe in the granular layer. Matrix cells were preserved in the periventricular areas. Proliferation of microglia and oligodendroglia was observed in damaged areas of the brain, and slight perivascular infiltration of small round cells was noted in several areas. There was poor myelination of the white matter, particularly of the pyramidal tracts, but no evidence of demyelination. No lesions were reported in peripheral nerves. The bone marrow was hypoplastic.

2.6.3.2 Postnatal intoxication

Gross lesions: Gross lesions have been described in almost all body systems in cases of alkyl Hg poisoning in mammals (Tables 6,7). Gastritis and enteritis were likely associated with ingestion of caustic alkylmercurials, while emaciation and muscular atrophy may have been secondary to inanition and nervous tissue damage.

Grossly visible atrophy of the brain has been a common finding in humans, but has only been reported twice in other mammals. Takeuchi et al. (1962) and Takeuchi (1968a, 1972) did not observe gross atrophy in acute human cases, but the lesion was consistent in more chronic cases. Similarly Tryphonas (1968) found atrophy only in chronic cases of alkyl Hg poisoning in swine. These findings suggest that gross atrophy is a consequence of chronic exposure to alkyl Hg, and as such

TABLE 6. Gross lesions reported in the muscular, nervous, and respiratory systems of alkyl mercury poisoned mammals

	Muscular	C N S								Respiratory		
Species	Atrophy Emaciation	"Venous infarction"	Swelling	Congestion	Atrophy	"Hydrops ex vacuo"	Excessive CSF	Haemorrhage	Thickening of meninges	Edema	Malacia	Catarrhal bronchitis Pulmonary edema	Author
Rat	+ +												Hunter et al. (1940) Diamond and Sleight (1971)
Ferret	+ +												Hanko et al. (1970)
Cat			+							+			Morikawa (1961b)
			+							+			Takeuchi (1968 a,b)
Dog					+								Kahrs (1968)
Pig	+ +				+		+						Tryphonas (1968)
Sheep										+			Palmer (1963)
Ox												+	Fujimoto (1956)
Rhesus monkey		+ +											Hunter et al. (1940)
Man					+								Hunter and Russel (1954)
					+								Brown (1954)
					+			+		+			Kurland et al. (1960)
												+	Okinaka et al. (1964)
					+								Ii (1966)
					+		+						Prick et al. (1967)
													Takeuchi (1968a)
			+							+	+		"acute cases"
	+ +				+	+			+				"chronic cases"
					+								Schmidt and Harzmann (1970)

TABLE 7. Gross lesions reported in the digestive, urinary, lympho-hemopoetic, cardiovascular and integumentary systems of alkyl mercury poisoned mammals

Species	Digestive								Urinary				Lympho-hemopoetic				Cardio-vascular		Skin	Author
	Liver																			
	Necrosis	Atrophy	Hepatomegaly	Fatty degeneration	Gastritis	Enteritis	Ulcerative colitis		Hemorrhage	Pale Kidney	Inflammation of kidney	Congestion	Lymphadenopathy	Malpighian	Corpuscles enlarged	Bone marrow reduced	Hemorrhage	Ventricular dilation	Depiliation	
Mouse																			+	Saito et al. (1961)
Rat					+					+										Hunter et al. (1940)
Ferret					+							+						+		Hanko et al. (1970)
Cat					+	+														Morikawa (1961b)
Pig										+										Jubb and Kennedy (1963)
						+			+								+			Kahrs (1968)
		+							+											Tryphonas (1968)
Sheep					+	+					+									Palmer (1963)
Ox													+		+		+		+	Fujimoto (1956)
	+		+		+	+			+											Jubb and Kennedy (1963)
Rhesus monkey												+								Hunter et al. (1940)
Man												+								Hunter and Russel (1954)
																	+			Ii (1966)
				+		+										+				Takeuchi (1968a)

would not be expected in more acute experimental or natural cases.

The pattern of atrophy described by various authors differs somewhat. Atrophy of both cerebral cortex and cerebellum was reported in human cases by Hunter and Russel (1954), Kurland et al. (1960), Ii (1966), Takeuchi et al. (1962), and Takeuchi (1968a, 1972). Ii (1966) also reported grossly visible atrophy of the thalamus and basal ganglion. Atrophy restricted to the cerebral cortex has been reported in human cases (Brown, 1954; Schmidt and Harzmann, 1970), in the dog (Kahrs, 1968), and in swine (Tryphonas, 1968).

Histopathology: The description of the pathology of alkyl Hg poisoning in rats by Hunter et al. (1940) reported a progressive disease beginning with Wallerian degeneration of peripheral nerves, dorsal spinal roots, and the trigeminal nerve, followed by degeneration in the posterior column of the spinal cord. Later changes were selective degeneration of granular cells of the cerebellum with relative sparing of the Purkinje cells. These authors also described neuronal degeneration in the cerebral cortex together with gliosis and perivascular reaction in an alkyl Hg poisoned rhesus monkey. Subsequent reports of the pathology of alkyl Hg intoxication in man and other mammals have been largely confirmatory.

The type of lesions described in the nervous system of mammals is shown in Table 8. Neuronal degeneration, necrosis and loss were the most consistently observed lesions. Gliosis was common, but in many cases the cell type involved was not specified. Where cell type was described, reactive astrogliosis was most common. Hyaline or

TABLE 8. Type of pathological lesions described in the nervous system of mammals poisoned with alkyl mercurials

Species	Neurons						Vessels								Author
	Degeneration	Necrosis	Neuronophagia	Loss	Mineralization	Gliosis	Edema	Hemorrhage	Advential proliferation	Perivascular cuffing	Hyaline degeneration	Myelin degeneration	Peripheral neuropathy		
Mouse	+	+		+			+								Saito et al. (1961)
	+	+		+		+									Takeuchi (1968b)
						+									Mukai (1972)
Rat	+			+	+	+						+	+		Hunter et al. (1940)
	+					+						+	+		Miyakawa et al.[a]
	+	+			+							+	+		Cavanagh and Chen (1971 a,b)
	+												+		Berglund et al. (1971)
													+		Grant (1971)
	+			+											Chang and Hartmann (1972)
	+	+	+			+					+		+		Diamond and Sleight (1972)
		+					+								Magos and Butler (1972)
Ferret	+			+		+				+		+	+		Hanko et al. (1970)
Cat	+	+	+	+		+		+		+					Takeuchi (1968 a,b)
	+	+								+			+		Grant (1971)
	+	+								+			+		Albanus et al. (1972)
Dog	+		+			+									Yoshino et al. (1966)
	+					+						+			Kahrs (1968)
Pig	+	+		+		+	+				+				Jubb and Kennedy (1963)
										+	+				Kahrs (1968)
	+	+	+	+	+	+			+		+				Tryphonas (1968)
Ox	+					+		+		+					Fujimoto et al. (1956)
	+					+			+		+				Jubb and Kennedy (1963)
	+	+		+			+								Herigstad et al. (1972)
Rhesus monkey	+					+				+			+		Hunter et al. (1940)
Squirrel monkey	+	+				+									Grant (1971)
	+	+													Nordberg et al. (1971)
Man	+			+		+						+			Brown (1954)
	+			+		+									Hunter and Russel (1954)
	+	+	+	+			+	+		+		+	+		Takeuchi et al. (1962)
	+	+		+							+				Hay et al. (1963)
	+		+	+		+					+				Okinaka et al. (1964)
	+	+				+						+			Ii (1966)
	+			+		+					+				Prick et al. (1967)
	+	+	+	+			+	+	+			+	+		Takeuchi (1972)

[a] Results of 1968, 1970a and 1971a combined.

fibrinoid degeneration of vessels has been described in many species, as has degeneration of peripheral nerves. The reported distribution of pathologic lesions in the cerebrospinal axis is shown in Table 9.

Lesions have been described in almost all areas of the nervous system by one or more authors; however, the virtually consistent involvement of the cerebral cortex, cerebellum, and peripheral nerves (when examined) is rather remarkable in view of the number of species and the differences in rate and type of intoxication.

Cerebellar lesions were almost invariably described as a selective loss of granular cells which underwent pyknosis and karyorrhexis. In man the loss of granular cells began directly under the Purkinje cell layer and then spread diffusely (Takeuchi et al., 1962). The change was most severe at the base of the sulci.

Relative sparing of the Purkinje cells has been reported by most authors, but in both man (Takeuchi et al., 1962) and animals (Chang and Hartmann, 1972a) loss of Purkinje cells in severe or chronic cases has been reported, indicating that the refractivity was not absolute.

Lesions in the cerebral cortex were characterized by neuronal degeneration, necrosis and loss, reactive gliosis, and the formation of a status spongiosus. Hunter and Russel (1954) reported a selectivity for damage to the visual cortex in man. This was confirmed by Takeuchi et al. (1962), Okinaka et al. (1964), and Prick et al. (1967). Nordberg et al. (1971) and Grant (1971) described lesions as being most severe in the visual cortex of squirrel monkeys, but other areas were also involved. This high degree of specificity

TABLE 9. Distribution of lesions in the nervous system of mammals with alkyl mercury poisoning

Species	Cerebral cortex	Basil ganglia	Diencephalon	Pons	Cerebellum	Medulla oblongata	Spinal cord	Dorsal root ganglia	Peripheral nerve	Author
Mouse		+			+					Saito et al. (1961)
	+				+					Takeuchi (1968b)
	+		+	+	+	+				Mukai (1972)
Rat							+	+	+	Hunter et al. (1940)
							+	+	+	Miyakawa et al.[a]
	+	+	+	+	+	+	+		+	Takeuchi (1968 a,b)
					+			+	+	Cavanagh and Chen (1971 a,b)
	+				+			+	+	Berglund et al. (1971)
					+			+		Chang and Hartmann (1972)
	+	+			+				+	Diamond and Sleight (1972)
									+	Grant (1971)
					+					Magos and Butler (1972)
Ferret	+			+		+	+		+	Hanko et al. (1970)
Cat	+	+	+	+	+	+	+		+	Takeuchi (1968 a,b)
	+				+			+	+	Albanus et al. (1971)
	+				+					Morikawa (1961)
	+				+				+	Grant (1971)
Dog	+				+					Yoshino et al. (1966)
Pig	+				+		+			Jubb and Kennedy (1963)
	+	+								Kahrs (1968)
	+	+	+	+			+	+		Tryphonas (1968)
Ox	+									Fujimoto et al. (1956)
	+				+		+			Jubb and Kennedy (1963)
	+				+					Herigstad et al. (1972)
Rhesus monkey	+	+				+		+	+	Hunter et al. (1940)
Squirrel monkey	+				+					Nordberg et al. (1971)
	+									Grant (1971)
Man	+						+			Brown (1954)
	+				+					Hunter and Russel (1954)
	+	+	+		+	+	+		+	Takeuchi et al. (1962)
	+				+		+			Hay et al. (1963)
	+				+		+			Okinaka et al. (1964)
	+	+	+	+	+	+	+			Ii (1966)
	+	+			+	+				Prick et al. (1967)
	+	+	+		+	+	+			Schmidt and Harzmann (1970)
	+	+	+		+	+	+		+	Takeuchi (1972)

[a]Results of 1968, 1970a and 1971a combined.

has not been reported in other mammals. Lesions have been described as: uniform in frontal and occipital cortex (Hunter et al., 1940); diffuse but most severe in temporal and/or occipital lobes (Yoshino et al., 1965; Takeuchi, 1968 a,b; Tryphonas, 1968); restricted to sensory cortex (Mukai, 1972); diffuse with no consistent distribution (Hanko et al., 1971; Grant, 1971; Diamond and Sleight, 1972; Herigstad et al., 1972).

Neuronal degeneration in the cerebral cortex was often described to have a laminar pattern, but descriptions of the laminae involved differ. Lesions were described as being most severe in laminae II and III in man (Takeuchi et al., 1962), naturally poisoned cats (Takeuchi, 1968a) and squirrel monkeys (Grant, 1971; Nordberg et al., 1971) and cats (Grant, 1971). Localization of lesions in deeper laminae was reported in man (Prick et al., 1967), experimentally poisoned cats and rats (Takeuchi, 1968b), cattle (Herigstad et al., 1972) and swine (Tryphonas, 1968). Involvement of all laminae was reported in severe cases in man (Takeuchi et al., 1962) and in dogs (Yoshino et al., 1965). Grant (1971) reported that damage first occurred in laminae II and III, and that in more severe cases only laminae I and VI remained intact.

Peripheral nerve degeneration has been reported by a number of authors since originally described by Hunter et al. (1940). The specificity of this lesion for sensory nerves was stressed by Hunter et al. (1940), Miyakawa et al. (1970), and Cavanagh and Chen (1971), all of whom found that dorsal spinal nerve roots were affected while ventral motor roots were not.

The degenerative process was described as being of Wallerian type by Hunter et al. (1940), but few details were given. Hanko et al. (1970) described myelin degeneration and disappearance and axon cylinder disintegration in peripheral nerves of ferrets. Takeuchi et al. (1962) reported only minimal lesions in peripheral nerves of humans with Minamata disease, but on re-investigation described disappearance of nerve fibers with collagen proliferation, demyelination, irregular size and arrangement of nerve fibers and proliferation of Schwann's cells and macrophages in sensory nerves (Takeuchi, 1972). Diamond and Sleight (1972) described severe demyelination, fiber disruption and proliferation of macrophages, fibroblasts and collagen in peripheral nerves of rats.

The sequential development of peripheral nerve lesions has been studied at the light and electron microscope level by Miyakawa et al. (1970) and Cavanagh and Chen (1971). These authors agreed that the degenerative process started at the node of Ranvier, that there was no selective segmental change and that both myelin and axon were damaged. Miyakawa et al. (1970) found that both macrophages and Schwann's cells "digested" degenerative myelin; but could not determine whether the primary damage was to the axon or the myelin sheath. Chang and Hartmann (1972b) agreed that the degenerative process began at the nodes of Ranvier, and that degeneration involved both axon and myelin sheath, but suggested that the degeneration might be segmental, similar to that of lead neuropathy.

Miyakawa et al. (1969) supported the thesis of Hunter et al. (1940) that peripheral sensory nerves were the first site to show injury in

alkyl Hg poisoning. Grant (1971) reported damage limited to peripheral nerves and their dorsal roots in rats, but in contrast cerebral damage without peripheral nerve lesions was observed in squirrel monkeys. Hunter et al. (1940) observed only minimal alterations in neurons of the dorsal root ganglia, despite severe damage to nerve fibres. These findings were confirmed by Miyakawa et al. (1970b, 1971) and Cavanagh and Chen (1971). Cavanagh and Chen (1971) reported that damage to fibres on the proximal side of the ganglion was as severe as on the distal side, and used the term "whole fibre death" to describe the situation where the whole fibre degenerates leaving the cell body intact. These authors produced neuronal necrosis in dorsal root ganglia only with high doses of Hg, while fibre degeneration occurred at lower intakes. Similarly Chang and Hartmann (1972b) found that nerve fibre injury preceded ganglial neuronal injury.

The occurrence of advanced neuronal necrosis and neuronophagia in the dorsal root ganglia without apparent dorsal root fibre lesions as described in swine by Tryphonas (1968) is difficult to explain.

Hyaline degeneration of vessel walls in the nervous system was described by Hay et al. (1963), Prick et al. (1967) and Kahrs (1968); fibrinoid necrosis of vessels by Jubb and Kennedy (1963), Tryphonas (1968), and Diamond and Sleight (1972), and perivascular infiltration of mononuclear cells by Hunter et al. (1940), Fujimoto et al. (1956), Takeuchi et al. (1962), Kahrs (1968), Takeuchi (1968 a,b), Hanko et al. (1970), and Albanus et al. (1972).

Miyakawa and Deshimaru (1969) reported vacuolar changes in vascular endothelial cells. This lesion was present early in the disease and persisted for 150 days. Diamond and Sleight (1972) found

that fibrinoid necrosis of vessels was present in rats which died 72 to 78 hr after a single injection of MeHg, and that the lesions regressed in animals which survived for 3 weeks. The vascular lesions were more severe in animals given weekly injections of MeHg over an extended period. These authors suggested that vascular damage was responsible for the neuronal degeneration noted, citing Steinwall and Olsson (1969) who showed impairment of the blood-brain barrier after injection of inorganic Hg and MeHg. Tryphonas (1968) found that vascular disease only occurred in chronic cases, and suggested that vascular lesions might be secondary to hypertension associated with capillary endothelial proliferation.

Degenerative changes in the epithelium of the proximal convoluted tubules of the kidney were described in almost all cases in which the kidneys were examined. These lesions were usually described as hydropic or vacuolar degeneration, but tubulonecrosis was not uncommon. Hunter et al. (1940) and Magos and Butler (1972) described interstitial nephritis with fibrosis in association with tubulonecrosis. Fowler (1972 a,b) studied renal lesions at the light and electron microscopic level, and described extrusion of cytoplasmic masses composed of smooth endoplasmic reticulum aggregates from proximal tubular cells. He suggested that this was due to conversion of alkyl Hg to inorganic Hg at this site, with enzyme inhibition and removal of non-functional organelles by potocytosis.

Vacuolar degeneration of hepatocytes was also commonly described, and focal hepatic necrosis was reported by Fujimoto et al. (1956), Jubb and Kennedy (1963), and Tryphonas (1968). Diamond and Sleight

(1972) described atrophy of hepatocytes. A variety of lesions in the heart have been described: myocardial degeneration (Hunter et al., 1940; Hanko et al., 1970; Schmidt and Harzmann, 1972); myocarditis (Fujimoto et al., 1956); myocardial necrosis (Schmidt and Harzmann, 1972); and degeneration of the Purkinje network with histiocyte infiltration, fibrosis and calcification (Jubb and Kennedy, 1963).

Hanko et al. (1970) and Diamond and Sleight (1972) reported lymphoid follicle depletion and reticular hyperplasia in the spleen. In contrast, Fujimoto et al. (1956) reported an increase in the size of Malpighian corpuscles. Hypoplasia of the bone marrow was described in both man and animals (Morikawa, 1961b; Takeuchi et al., 1962; Takeuchi, 1968a).

Skeletal muscle degeneration was described by Hanko et al. (1970), Miyakawa et al. (1971b) and Herigstad et al. (1972). Mild inflammation of the intestine was commonly described, but necrosis of the colonic mucosa has only been described in swine (Kahrs, 1968; Tryphonas, 1968). Both of these authors also found hyaline or fibrinoid necrosis of arteries of the submucosa of the colon. Saito et al. (1961) described atrophy of spermatogenesis in mice, and Prick et al. (1967) recorded spermatogenesis as slight, with peritubular fibrosis in a human case.

2.6.4 Discussion

Alkyl Hg compounds as a group have certain characteristics which set them apart from other forms of Hg. They are absorbed readily from the gastro-intestinal tract, are bound to cells in the blood, pass the blood-brain barrier and placental barrier readily, and give high Hg

concentrations in the brain.

Published information indicates that alkyl Hg compounds are neurotoxic for fish, birds, and mammals; and that damage to the nervous system is the primary toxic action of these compounds in birds and mammals. Data on the effects upon fish are too meagre to allow critical evaluation, but the neuropathology described by Takeuchi (1968a) suggests a pattern similar to that in other vertebrates. Damage to the gills of fish is likely a special feature of poisoning of this group.

The distribution of lesions in the nervous system of birds requires further investigation; however, the involvement of peripheral nerves (Borg et al., 1969, 1970); spinal ganglia (Fimreite and Karstad, 1971), dorsal fasciculi of the spinal cord (Fimreite and Karstad, 1971), and cerebrum and cerebellum (Takeuchi, 1968a; Borg et al., 1969; Fimreite and Karstad, 1971) suggests a pattern similar to that in mammals.

The original description of alkyl Hg poisoning in mammals by Hunter et al. (1940) has been largely confirmed by subsequent workers. Thepattern which seems to apply for most mammals is one of selective involvement of sensory peripheral nerves and dorsal fasciculi of the spinal cord, followed by cerebrocortical damage with a tendency for most severe involvement of the visual areas, and varying degrees of involvement of the granular layer of the cerebral cortex. With increasing severity and/or chronicity of intoxication lesions appear to become more diffuse, with involvement of virtually every area of the nervous system having been reported by one or more authors. Degenerative changes in vasculature, and perivascular accumulation of mononuclear

cells were common lesions in many species. Degenerative changes in the proximal tubular epithelium of the kidney, and in the liver were reported in fish and birds, and were virtually consistent in all cases of poisoning in mammals.

It seems probable that many of the discrepancies from this basic pattern resulted from differences in exposure conditions with regard to rate and route of intoxication, length of survival, compounds used, and techniques employed in the various studies. The finding of simultaneous degeneration of Purkinje cells and granular cells by Brown and Yoshida (1965) is somewhat anomalous. However, very young chicks were used in this study, and the results may be analogous to the more diffuse involvement of the brain observed by Takeuchi (1968a) in fetal human poisoning.

The pathogenesis of alkyl Hg poisoning, and the basic mechanisms responsible for cellular damage still require elucidation. Among the mechanisms which have been suggested were: enzyme inhibition (Brown and Kulkarni, 1967); protein precipitation (Clarkson, 1968), interference with protein formation (Yoshino et al., 1966; Cavanagh and Chen, 1971 a,b), effects on regulatory mechanisms governing nucleolar-ribosome-membrane interaction (Brubaker et al., 1971) and a "contact inhibitory" effect on membranes (Chang and Hartmann, 1972 a,b). The role of any one or combinations of the above awaits clarification; however, Hg compounds in general have a high affinity for thiol, amino, carboxyl, and hydroxyl groups found in enzymes (Brown and Kulkarni, 1967) so that interference with enzyme systems seems likely. Inhibition of protein synthesis in nervous tissue by alkyl Hg has been

demonstrated by Yoshino et al. (1966), Cavanagh and Chen (1971), and Paterson et al. (1971), so that this action may also be important.

Steinwall and Olsson (1969), and Chang and Hartmann (1972c) have demonstrated impaired blood-brain barrier function after the injection of MeHg. This occurred with 12 hours after injection of even minute amounts of MeHg (Chang and Hartmann, 1972c). Diamond and Sleight (1972) suggested that neuronal damage was secondary to vascular injury and impaired blood-brain barrier function; however, Miyakawa and Deshimaru (1969) could not correlate the degree of change in cerebellar granular cells to the degree of disturbance of the blood-brain barrier. Tryphonas (1968) suggested that morphologic lesions in the vasculature were secondary to neuronal damage, but that vascular damage could increase neuronal damage through hypoxia or anoxia.

Biotransformation of alkyl Hg, i.e., cleavage of the covalent C-Hg bond, with release of inorganic Hg was suggested as a basis for the pathologic action of alkyl Hg (Norseth and Clarkson, 1970a). Later manuscripts by the same group point out that this is unlikely to be the case in nervous tissue because of the low content of inorganic Hg found in the brain (Norseth and Clarkson, 1970b; Norseth, 1971). Biotransformation is likely responsible for renal tubular damage (Fowler, 1972 a,b).

The apparent selectivity of damage for certain areas of the nervous system remains unexplained. Preferential accumulation of Hg in these sites might account for this susceptibility. Relative concentration of Hg in the grey matter of the cerebellum has been described in birds (Backstrom, 1969), mice (Berlin and Ullberg, 1963), and man

(Falk et al., 1970). Yoshino et al. (1966) found that the visual area of the cortex contained more Hg than did other areas.

Nordberg et al. (1971) found that the distribution pattern of Hg changed with time to correspond generally with the topography of brain damage; however, as these authors pointed out, there was the possibility that Hg could accumulate secondarily in damaged areas. High concentrations of Hg have also been observed in portions of the brain in which damage was not conspicuous (Berlin and Ullberg, 1963; Backstrom, 1969). Chang and Hartmann (1972a) reported that more Hg accumulated in Purkinje cells than in granular cells, although degeneration was more extensive in the granular cells.

Yoshino et al. (1966), Cavanagh and Chen (1971), and Chang and Hartmann (1972a) speculated that differential susceptibility was related to differences in cellular metabolism, and the former two authors suggested protein synthesizing activity as the crucial factor in determining susceptibility. Chang and Hartmann (1972b) suggested that Hg in the nerve cell bodies could be "eliminated" by axonal flow into the axons; and the long axons of ventral horn neurons might thus render these cells more "tolerant" to Hg toxicity than cells with smaller axonal processes. No explanation has been offered for the apparent susceptibility of sensory nerves. It is likely that the susceptibility of various portions of the nervous system are only relative, and that in cases of severe intoxication most areas will be affected.

3.0 OBJECTIVES OF THE STUDIES

Review of the literature indicated that Hg pollution of natural waters had occurred in Japan and the Scandinavian countries, and that human poisoning had occurred in Japan due to the ingestion of Hg contaminated fish. No information was available on the status of North American waters with regard to possible Hg pollution. The primary sources of Hg pollution in Sweden were the chlorine-alkali industry and the pulp and paper industry (Lofroth, 1969). Both types of industry are present in Saskatchewan, and effluent from these plants enter the Saskatchewan River. Fish appear to be reliable indicators of Hg contamination of water (Johnels et al., 1967). Because there seemed to be a possibility of Hg pollution in the Saskatchewan River, fish were collected from various sites, and tissues were analyzed for Hg (experiment I). High levels of Hg were detected in these fish, but little information was available in the literature to indicate what, if any, significance these Hg residues might have for the health of the fish. Experiments II and III were undertaken to study the effects of acute exposure to MeHg in the water; and prolonged exposure to MeHg through the diet, respectively, upon rainbow trout; and to attempt correlation of Hg levels in tissue with effects upon the fish.

Little information was available in the literature on the effects of MeHg upon piscivorous animals. An understanding of the possible

hazards of Hg in fish for piscivorous animals was important because fish from waters potentially polluted with Hg are used as a large portion of the diet of ranch mink in some areas of Canada, and the use of Hg contaminated fish as mink food was being considered as a method of utilization of commercially caught fish containing levels of Hg judged to be unsafe for human consumption. Such information would also be of value in assessing the risk to wild piscivorous animals dependent upon fish from the Saskatchewan River. Experiment IV was designed to study the safety of Hg contaminated fish taken from a polluted water body as a dietary constituent for ranch mink. In experiment V rations, to which MeHgCl had been added, were fed to ranch mink to determine the pathology of MeHg poisoning in mink, and to correlate clinical signs of intoxication and pathologic lesions to Hg intake, length of exposure, and Hg concentrations in tissue. This information was required to aid in the diagnosis of possible field cases of Hg poisoning in mink.

4.0 EXPERIMENT I. MERCURY CONCENTRATIONS IN THE TISSUES OF FISH FROM THE SASKATCHEWAN RIVER[1]

4.1 Rationale

Fish are reliable indicators of Hg contamination of water (Johnels et al., 1967). Fish were collected from various sites in the Saskatchewan River to determine if Hg contamination of this river had occurred. Axial muscle was selected as the most suitable tissue for Hg analysis for several reasons. Since this tissue has been used in other countries as a means of estimating the level of Hg present in the aquatic environment (Johnels et al., 1967) direct comparison would be possible. Although tissue distribution studies suggest that muscle does not have so great an ability to concentrate Hg as some other tissues, muscle from fish in waters with moderate or no detectable contamination has a higher content of Hg than most other tissues (Johnels et al., 1967). Finally, as muscle is the tissue used for human consumption, Hg concentrations in it are of most concern.

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[1]The results of Experiment I have been published: Wobeser, G., N. O. Nielsen, R. H. Dunlop and F. M. Atton. 1970. Mercury concentrations in tissues of fish from the Saskatchewan River. J. Fish. Res. Bd. Canada. 27:830-834. Subsequent reports of Hg pollution in North America are discussed in General Discussion.

4.2 Materials and methods

4.2.1 Collection of specimens

Eighty-one fish of 10 species were obtained by gill-netting, by personnel of the Department of Natural Resources, Province of Saskatchewan, at nine sites in the North and South Saskatchewan Rivers within the province of Saskatchewan from September to November, 1969 (Fig. 1).

The fish were brought to the laboratory as rapidly as possible (usually within 6 to 12 hr) and, where necessary, were chilled with ice during transit. Four fish from sources other than the Saskatchewan River were also analyzed. Two of these were marine fish obtained from a local retail food store. The other two fish were a rainbow trout[1] netted in a reservoir in agricultural land near Saskatoon, and a lake trout obtained from a remote lake in northern Saskatchewan.

Portions of liver, kidney, and muscle (muscle only in the case of fish from sources other than Saskatchewan River) were placed in individual plastic bags and frozen until analyzed for Hg content. The muscle samples were taken from the longitudinal dorsal muscles on the anterior portion of the body. Muscle samples from all of the fish were analyzed for Hg concentrations.

Liver and kidney tissue from 14 of the fish from the Saskatchewan River were also analyzed for Hg content.

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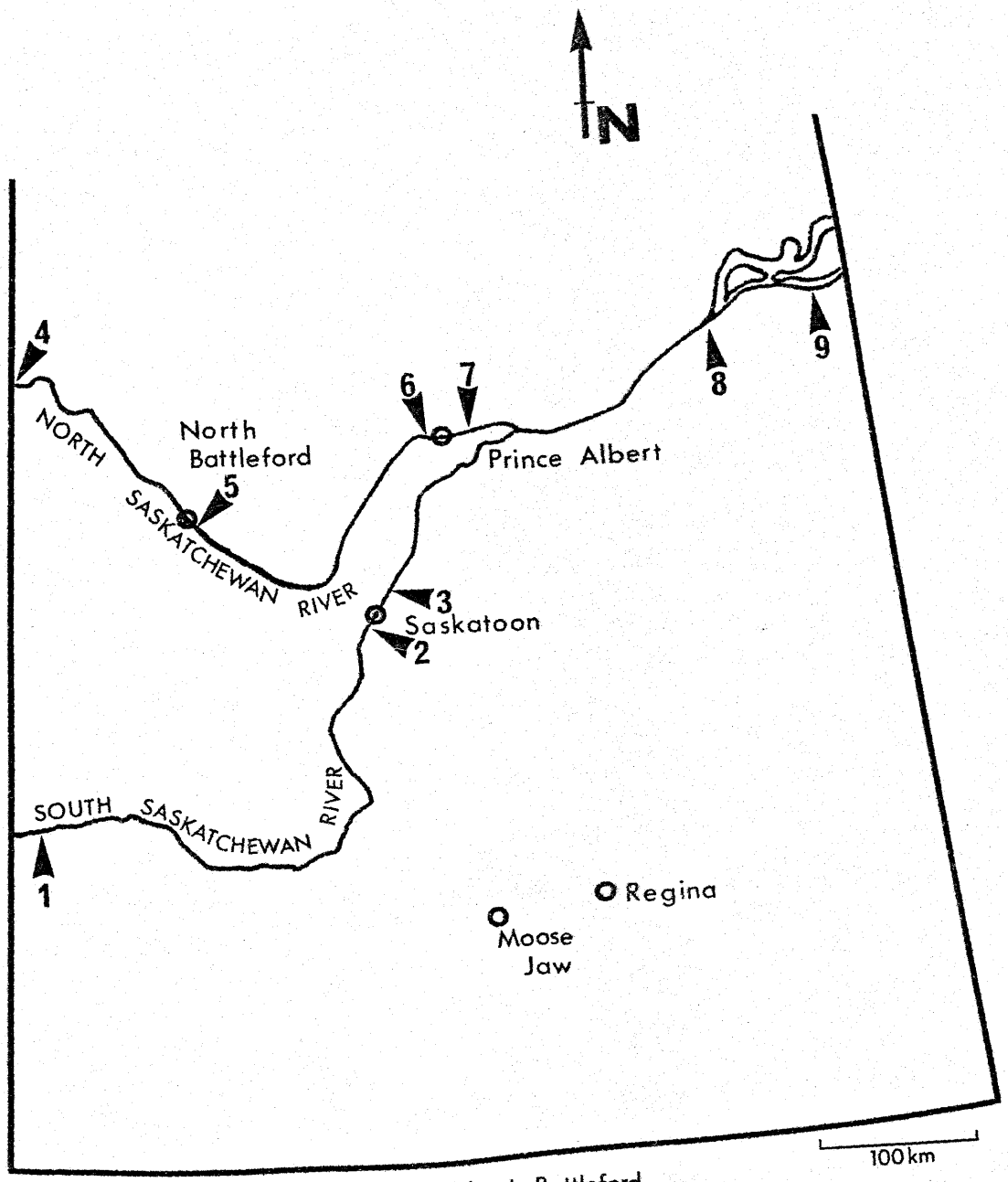
[1] Common names of fish species are those used by the American Fisheries Society (Bailey et al., 1960).

Fig. 1. Map of southern Saskatchewan showing sites on the North and South Saskatchewan Rivers at which fish were collected.



Collection site

1-Leader Ferry

2 - 1 mile above Saskatoon

3- Clarkboro Ferry

4-Alberta-Saskatchewan border

5-North Battleford

6-4 miles above Prince Albert

7- Cecil Ferry

8- Tobin Lake

9- Cumberland House

4.2.2 Mercury Analysis

Mercury analyses were done in the Department of Veterinary Physiology, Western College of Veterinary Medicine, by the method of Jacobs et al. (1960) with the following modifications. For digestion, 3 ml of concentrated sulfuric acid were used rather than 2 ml; after heating, the solution was allowed to cool for 1 hr rather than 15 minutes; after cleaning with hydroxylamine hydrochloride solution the pH was adjusted to 1.0 to 1.5 with ammonium hydroxide. An electric furnace[1] was used instead of a Bunsen burner for distillation of the mercury-dithizone complex. The vapor generated in the furnace was sucked through an optical flow-through 10 x 2-cm cuvette (rather than 23 x 4-cm) in an atomic absorption spectophotometer[2]. The light source used was a Hg element[3]. An electronic recorder[4] was connected to the system to record the absorption curve. In the original method a modified Hg vapor meter was used (Jacobs et al., 1960).

In the analysis, a standard curve was obtained concurrently with each group of samples. The low standard of 0.2μg of Hg/ml was stable and reproducible from day to day. Recovery of Hg added to animal tissues in the form of $HgCl_2$ ranged from 88 to 99 per cent.

Samples of muscle from six of the fish from the Saskatchewan River

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[1] Type 70-T, Multiple Unit Electric Furnance, Heviduty Heating Equipment Co., Watertown, Wisconsin, U.S.A.

[2] Evans, E.E.L. Model 140, Electroselenium Ltd., Halstead, Essex, England.

[3] EEL Type 2847, International Sales Associates, Langhome, Pennsylvania 19047. U.S.A.

[4] Microcord Model 44. Photovolt Corp. New York, N.Y., U.S.A.

were analyzed at the analytical laboratory, Department of Chemistry and Chemical Engineering, University of Saskatchewan, Saskatoon, Saskatchewan, without knowledge of the results obtained in the other laboratory.

The method employed in that laboratory involved hot digestion of the sample with nitric acid, oxidation with perchloric acid, extraction with dithizone, flash decomposition of the mercury-dithizone complex by heating to produce Hg vapor, and estimation of Hg by atomic absorption spectrophotometry.

The concentrations of Hg (ppm) in the six fish as determined by the two laboratories were:

Fish No.	A	B	C	D	E	F
Veterinary Physiology	2.1	0.9	5.5	10.5	5.6	11.2
Chemistry & Chemical Engineering	2.2	0.5	5.7	8.0	6.5	6.8

All Hg concentrations were expressed on a wet-weight basis.

4.3 Results

The concentrations of Hg in muscle tissue of fish from all sites sampled in the Saskatchewan River were higher than those reported elsewhere for normal freshwater fish (Table 10). There were wide variations in the Hg content in fish of the same species from the same site and among fish from various sites. Although the number of fish sampled at each site was small, significant differences occurred between mean Hg concentrations in fish from the various sites. The mean Hg concentrations in fish from the Clarkboro Ferry and Cumberland House sites were significantly higher ($P<0.05$) than the

TABLE 10. Mercury concentrations (means and, in parentheses, ranges) in muscle from various species of fish taken from nine sites on the Saskatchewan River during 1969.

Species	No. fish	Hg (ppm)
North Saskatchewan River		
Alberta-Saskatchewan Border (Oct.19)		
Goldeye	2	1.0(0.9-1.1)
Northern pike	2	1.2(0.7-1.6)
Sauger	2	1.3(1.1-1.5)
Walleye	2	1.0(0.6-1.4)
White sucker	2	1.0(0.7-1.1)
Mean ± SD		1.1 ± 0.35[a]
North Battleford (Oct. 19)		
Goldeye	1	1.0
Longnose sucker	1	0.7
Northern redhorse	1	2.4
Walleye	3	0.9(0.6-1.3)
White sucker	1	1.1
Mean ± SD		1.1 ± 0.62[a]
4 miles above Pr. Albert (Oct.20)		
Goldeye	2	2.5(2.2-2.8)
Northern pike	2	1.1(1.0-1.1)
Mean ± SD		1.8 ± 0.87[c]
Cecil Ferry (Sept. 24)		
Goldeye	4	2.0(1.1-2.4)
Longnose sucker	4	1.0(0.8-1.3)
Sauger	1	1.4
Walleye	2	2.1(1.4-2.8)
White sucker	5	2.2(0.9-5.5)
Mean ± SD		1.8 ± 1.16[c]
South Saskatchewan River		
Leader Ferry (Oct. 29)		
Goldeye	3	1.5(1.3-1.6)
Longnose sucker	2	1.3(0.8-1.7)
Walleye	2	2.6(1.6-3.6)
Northern pike	1	1.0
Northern redhorse	2	1.0
Mean ± SD		1.5 ± 0.81[a,c]
1 mile above Saskatoon (Oct.5-Nov.11)		
Northern pike	1	1.0(0.5-1.7)
Mean ± SD		1.0 ± 0.50[a,c]
Clarkboro Ferry (Oct. 7)		
Burbot	1	5.3
Goldeye	2	4.2(1.2-7.2)
Northern pike	3	9.1(6.1-10.6)
Walleye	2	4.9(4.4-5.3)
White sucker	2	8.3(5.3-11.2)
Mean ± SD		6.7 ± 3.10[b]
Saskatchewan River		
Cumberland House (Nov. 3)		
Goldeye	11	5.0(2.1-11.0)
Mean ± SD		5.0 ± 2.64[b]
Tobin Lake (Nov. 7)		
Burbot	1	1.2
Goldeye	3	1.3(0.9-1.9)
Northern redhorse	1	2.1
Walleye	2	2.7(2.6-2.7)
Yellow perch	1	1.4
Mean ± SD		1.8 ± 0.68[c]

[a,b,c] - Means followed by the same superscript are not significantly different (P>0.05).

mean values for fish from all other sites. Fish from three sites (Tobin Lake, Prince Albert, and Cecil Ferry) had significantly higher mean levels of Hg in their tissue than did fish from the Alberta border, Leader Ferry, and North Battleford sites.

Fish collected above the city of Prince Albert had the same mean Hg content in their muscle as did fish below the city (Cecil Ferry site), even though effluent from a pulp mill enters the river between the two sites. In contrast, fish collected below the city of Saskatoon (Clarkboro Ferry site) had on the average over six times as much Hg in their tissue than did fish collected above Saskatoon.

The Hg concentrations found in muscle, liver, and kidney tissue of 14 fish from various sites in the Saskatchewan River is shown in Table 11. The livers and kidneys of these fish contained an average of 1.9 and 1.7 times as much Hg respectively as did muscle from the same fish. Four fish from sources other than the Saskatchewan River were also found to contain Hg. The Hg concentrations in the muscle of these fish were 0.3 and 0.6 ppm respectively for two marine fish obtained from a retail food store and 1.0 and 0.8 ppm respectively for a rainbow trout from a reservoir near Saskatoon, and a lake trout from a remote lake in Northern Saskatchewan.

4.4 Discussion

The results of this study indicate that abnormal concentrations of Hg occur in fish in the Saskatchewan River within the province of Saskatchewan. The Hg concentrations reported in normal freshwater fish from other areas of the world have ranged from 0.05 ppm (Rucker,

TABLE 11. Mercury concentration in the muscle, liver, and kidney of 14 fish from the Saskatchewan River

Organ	Hg (ppm)													
Muscle	10.4	10.6	6.1	4.4	5.3	1.2	5.3	11.2	5.3	1.1	1.5	0.6	1.0	0.5
Liver	14.3	12.8	13.4	10.4	12.7	1.2	4.9	16.5	8.7	2.5	1.9	2.5	1.6	1.3
Kidney	13.1	21.1	20.5	10.5	9.5	1.2	-	13.4	6.0	1.6	2.3	1.4	1.4	-

1968) to 0.2 ppm (Johnels et al., 1968; Rucker and Amend, 1969), very much lower than those found in any of the fish analyzed in this study. The concentrations found in the present study correspond to those reported in Sweden in fish from waters directly contaminated by industrial effluents (1.21 ppm, Johnels et al., 1967; up to 8 ppm, Berglund and Wretling, 1967), but were lower than those reported in shellfish and fish from Minamata Bay, Japan (10 to 40 ppm wet-weight basis, Rucker, 1968; 33 to 150 ppm dry-weight basis, Saito et al., 1961; 27 to 102 ppm wet-weight basis, Kurland et al., 1960).

Johnels et al. (1967) found significant concentrations of Hg (0.75 to 1.1 ppm) in fish from apparently uncontaminated waters in Sweden. They speculated that these concentrations could be the result of the influence of Hg in the bedrock, or, alternatively, aerial fallout from unidentified industrial sources. The latter was considered the more likely explanation. No information is available on the Hg content of Saskatchewan soils or bedrock. The bedrock is primarily sedimentary (Richards and Fung, 1969), which in general has a higher Hg content than does igneous rock (James, 1962). This might in part account for the rather high Hg content in fish at sites upstream from any sites of industrial activity. The significant difference in Hg content of fish above and below Saskatoon was difficult to explain at the time the study was done. Subsequently Fimreite (1970) surveyed the uses of Hg in Canada and suggested that Hg pollution was likely to occur at this point because of the presence of a chlorine-alkali plant using a Hg electrolytic cell in the area.

The wide variation in Hg concentration within species from the

same site agrees with the observations of Hannerz (1968) who found as much as 10-fold variation in Hg content among fish of the same species given identical exposure to Hg. This author also found that direct accumulation from the environment through the epithelium was the important route of accumulation, which may explain the lack of specific variation, even among species with very different food habits.

The relative distribution of Hg in liver, kidney, and muscle of the 14 fish from which these tissues were analyzed was similar to that reported by Hannerz (1968).

Although this study was limited and the numbers of fish analyzed were small, it did demonstrate for the first time that Hg pollution of water, similar to that reported in Japan and Scandinavian countries, occurred in North America.

5.0 EXPERIMENT II. THE ACUTE TOXICITY OF METHYL MERCURY CHLORIDE FOR RAINBOW TROUT FRY AND FINGERLINGS

5.1 Rationale

The toxicity for fish of some inorganic forms of Hg, notably $HgCl_2$ has been well demonstrated (for a review see Doudoroff and Katz, 1953), and some authors feel that this compound may be infinitely toxic for fish (Boetius, 1960). The toxicity of different Hg compounds for mammals and birds has been tested by a number of methods and has been found to vary greatly. There are few comparable data regarding the relative toxicity of Hg compounds for aquatic organisms.

Because of the paucity of information on this subject, the present study was undertaken to:

(1) measure the acute toxicity of MeHgCl for rainbow trout fry and fingerlings.

(2) to compare the toxicity of MeHgCl and $HgCl_2$.

(3) to measure the rate of accumulation of Hg in tissues of fish exposed to MeHgCl and $HgCl_2$.

(4) to determine the histopathologic effects of acute Hg poisoning.

5.2 Materials and methods

5.2.1 The fish

Fry: Rainbow trout fry hatched in the laboratory from eggs obtained

in one lot from a supplier in Alberta were used for toxicity trials within 2 to 7 days of hatching. The fry were not fed prior to or during the trials.

Fingerlings: Rainbow trout fingerlings ranging in length from 4.0 to 6.0 cm obtained in one lot from a supplier in Montana were used in all trials. Fish were selected for the trials so that the largest fish was no more than 1.5 times the length of the smallest.

The fingerlings were acclimated in the laboratory for a minimum of 10 days prior to the toxicity trials. During the holding period, the fingerlings were fed once daily with a commercial trout food[1] containing less than 0.1 ppm Hg. Feeding was suspended 2 days prior to the beginning of individual toxicity trials and the fish were not fed during the trials. The absence of respiratory and other movements and failure to respond to mild mechanical stimulation were used as criteria to identify dead fish.

5.2.2 Experimental design

A similar experimental design was used for both sizes of fish. Ten fish were used for each trial and trials at each concentration were performed in triplicate. Six test vessels were used in each set of trials. The vessels used for the fry experiments were 1 litre flasks containing 1 litre of test solution. Rectangular glass tanks (battery jars) measuring 17 x 20 x 32 cm and containing 7 litres of test solution were used for the fingerling experiments. These jars were lined with polyethylene bags which were changed between

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[1] Silver Cup Self-Sustaining Trout Feed, Ferguson Feeds Ltd., Drinkwater, Saskatchewan.

trials. Five of the vessels in each set of trials contained various concentrations of mercury solution and one which contained only diluent water served as a control. The trial vessels were partially immersed within a recirculating aquarium[1] which contained approximately 200 litres of water. This water was cooled by the aquarium system and maintained at a temperature of 10 $\pm$ 0.7C.

Test solutions were renewed at 24 hr intervals, oxygenated by air driven by a small pump through regulating valves and bubbling stones in the fingerling experiment, and glass tubes inserted about 3 cm into the solution in the fry experiment. In both cases a very low rate of air flow was maintained, which was found to be adequate to maintain the dissolved oxygen content above 8 mg/litre.

City tap water dechlorinated by passage through an activated charcoal filler was used as both culture and diluent water. The characteristics of this water are shown in Table 12. Stock solutions of MeHgCl[2] and $HgCl_2$[3] containing 50 mg Hg/litre were prepared.

Initial trials were performed to determine suitable dilution ranges for testing. The concentrations of MeHgCl and $HgCl_2$ used were as follows:

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[1] Living Stream Model LS-700, Frigid Units Inc., Toledo, Ohio, U.S.A.

[2] Alpha Inorganics Inc., Beverly, Mass., U.S.A.

[3] The British Drug Houses, Ltd., Poole, England.

TABLE 12. Chemical and physical characteristics of water used for all experiments[a]

pH	8.55
Spec. Conductivity (u mhos/cm at 25°C)	297
	ppm
Carbonates (CO_3)	6
Bicarbonates (HCO_3)	73
Hydroxides (OH)	nil
Sulphates (SO_4)	73
Chloride (Cl)	1
Calcium (Ca)	18.8
Magnesium (Mg)	13.2
Sodium (Na)	19.8
Potassium (K)	2.8
Copper (Cu)	<0.005
Total dissolved solids (110°C)	190
Total hardness ($CaCO_3$)	101
Carbonate (total alkalinity)	70
Non-carbonate	31
Ca	47
Mg	54

[a] - Analysis performed by the Chemical Division, Saskatchewan Research Council, Saskatoon, Saskatchewan.

Fish	Compound	Hg (mg/litre)								
Fingerlings	MeHgCl	.01	.018	.032	.04	.056	.07	.08	.10	.135
Fingerlings	$HgCl_2$							.50	.75	1.0
Fry	MeHgCl	.01	.018	.032		.056			.10	

These concentrations represent a logarithmic series as recommended for bioassay in Standard Methods (American Public Health Association, 1965) with the addition of three intermediate dilutions (.04, .07, .08 mg Hg/litre) in the case of fingerlings exposed to MeHgCl. Survival rate was recorded after 24, 48 and 96 hr exposure to MeHgCl and 24 hr exposure to $HgCl_2$, and median tolerance limits (TLm) were determined by graphical straight line interpolation. Mean TLm and their 95 per cent confidence limits were calculated for each time period and compound.

In an attempt to determine the chronological occurrence of lesions, and the ability of fish to recover after exposure, 20 fingerlings were placed in two test vessels containing a solution of 0.08 mg/litre MeHgCl and two fish were sampled after 3, 6, 12 and 24 hr exposure. After 24 hr exposure the remaining fish were transferred to a 200 litre aquarium equipped with an overflow to which fresh water was added at the rate of 30 litre/hr. Two fish were removed from this tank at each of 3, 6, 12, 24, 48, and 96 hr.

5.2.3 Sampling

Fish selected for histopathologic examination were removed from the test vessels and killed by sectioning the spinal column caudal to the head. The abdomen was opened along the ventral midline and the

fish were immediately placed in Bouin's solution. After fixation for 24 hr, the fish were transferred to a 70 per cent solution of ethyl alcohol and stored until trimmed for histologic processing. The head was sectioned transversely at 3 mm intervals and these sections together with portions of liver, spleen, stomach, and intestine and transverse sections through the trunk at the level of the anterior and posterior kidney were embedded in paraffin, sectioned at 6μ and stained with haematoxylin and eosin (H & E).

5.2.4 Mercury analysis

Fish for analysis were removed from the tanks after specific intervals of exposure. They were placed in plastic bags and frozen immediately. Later the fish were thawed and the head, fins, and all internal organs were removed. The remaining muscle, bone, and skin was ground and refrozen until analyzed. All analyses were performed blind on pooled tissues from several fish collected after identical exposure. Analyses were performed in the Department of Veterinary Physiology, Western College of Veterinary Medicine, using the digestion and extraction procedures of Uhte et al. (1970). The cuvettes, atomic absorption spectrophotometer and light source used were those previously described (experiment I). All determinations were performed in triplicate and the results were expressed in ppm Hg. A standard curve was obtained concurrently with each group of samples. The low standard of 0.1 μg/ml was stable and reproducible.

5.3 Results

5.3.1 Clinical findings

Fingerlings exposed to MeHgCl at 0.01 mg Hg/litre did not show any evidence of intoxication during the first 72 hr of exposure. The earliest change after this time was flaring of the opercula together with an increase in both the frequency and extent of respiratory movements. No other signs were noted during the 96 hr period of the experiment. Fingerlings exposed to concentrations of MeHgCl greater than 0.01 mg Hg/litre showed similar signs initially. The rapidity of the onset of signs was directly proportional to the concentration of Hg in the water. This apparent respiratory distress was followed by a loss of equilibrium so that the fish often swan sluggishly in abnormal positions either on their side or vertically. Terminally the fish became inactive and lay on the bottom of the tanks with flared opercula. No accumulation of mucus or "coagulation film" was observed on any of these fish prior to death; however, a grey film formed within a few hours after death on fish which were not removed from the tanks.

After 24 hr exposure to 0.08 mg Hg/litre as MeHgCl, the fingerlings were sluggish and tended to lie on the bottom of the test jars. When transferred to the large aquarium this behavior persisted for a short time; however, within 30 minutes the fish began to swim actively against the current within the tank. Within 1 hr the behavior of the fish had apparently returned to normal. In this experiment no fish had died after 24 hr exposure and no mortality was observed during the 96 hr period after transfer to MeHg-free water.

Fingerlings exposed to $HgCl_2$ behaved in a similar manner to those exposed to MeHgCl. These fish appeared to produce much more mucus so that the test solutions became turbid and a white sediment collected on the bottom of the vessels.

Fry exposed to MeHgCl became sluggish and tended to rest on the bottom. Immediately prior to death there was dorsal deviation of the head and caudal extremity, and dead fish were typically found in this curved posture.

5.3.2 Toxicity of MeHgCl and $HgCl_2$

No mortality occurred within the 96 hr test period among fry or fingerlings exposed to MeHgCl at 0.01 mg Hg/litre. All concentrations of MeHgCl higher than this were associated with mortality. There was no mortality in any of the control trials. The TLm of fry exposed to MeHgCl were significantly lower than those for fingerling at all lengths of exposure (Table 13). MeHgCl under the test conditions was more than seven times as toxic as $HgCl_2$ for fingerlings with a 24 hr period (Table 13). All fingerlings used in the experiments were of approximately the same age, but length varied by a factor of 1.5 and weight by a factor of 5.1. There were no significant differences between the mean weight or length of fingerlings which died and those fingerlings which survived exposure to concentrations of MeHgCl or $HgCl_2$ which were toxic to some but not all fish.

5.3.3 Accumulation of mercury in tissue

Table 14 shows the concentration of Hg in the tissue (pooled muscle, skin, and bone) of fingerlings after varying lengths of exposure

TABLE 13. Mean median tolerance limits (TLm) in mg Hg/litre and their 95 per cent confidence limits (95% CL) of rainbow trout fry and fingerlings for methyl mercury chloride (MeHgCl) and mercuric chloride ($HgCl_2$)

Exposure (hr)	Fry (MeHgCl)		Fingerlings MeHgCl		Fingerlings $HgCl_2$	
	TLm	95% C L	TLm	95% CL	TLm	95% CL
24	.084[a]	.081-.087	.125[a]	.120-.130	.903[a]	.783-1.023
48	.045[b]	.036-.054	.066	.063-.069	--	--
96	.024[b]	.022-.026	.042	.025-.059	--	--

[a] - Significantly different ($P<0.001$) from other means at 24 hours.

[b] - Significantly different ($P<0.01$) from mean for fingerlings at same exposure.

TABLE 14. Mercury concentrations in samples of pooled muscle, skin, and bone of fingerlings after varying lengths of exposure to different concentrations of methyl mercury chloride (MeHgCl) and mercuric chloride ($HgCl_2$). Each value is for pooled tissue from several fish.

Compound	Exposure (hr)	Number of pools (fish per pool)	Hg (ppm) water[a]	Hg (ppm) tissue, mean (range)	Concentration factor[b], mean (range)
Control	96	3 (5,5,5)	control	0.2 (0.2)	-
MeHgCl	96	1 (5)	0.01	4.8	480
	96	3 (5,5,5)	0.032	6.9 (5.3-9.0)	216.7 (165.9-296.9)
	96	3 (5,5,5)	0.04	7.9 (6.9-9.3)	198.3 (172.5-232.5)
	72	2 (5,9)	0.056	6.0 (5.4-6.5)	106.3 (96.4-116.1)
	48	1 (5)	0.056	5.6	100
	48	3 (4,6,5)	0.07	6.3 (6.2-6.5)	90.5 (88.6-92.9)
	48	3 (10,6,5)	0.08	5.7 (4.8-6.2)	71.3 (60.0-77.5)
	48	2 (10,10)	0.10	7.1 (7.0-7.1)	70.5 (70.0-71.0)
	36	1 (3)	0.10	4.7	47.0
	24	3 (8,8,8)	0.135	4.9 (4.6-5.1)	36.3 (34.1-37.8)
$HgCl_2$	24	3 (5,5,5)	0.56	1.7 (1.1-2.4)	3.0 (2.0-4.3)
	24	3 (5,5,5)	.075	1.2 (1.0-1.5)	1.6 (1.3-2.0)
	24	3 (6,5,7)	1.00	1.7 (1.4-2.2)	1.7 (1.4-2.2)

[a] - Initial concentration.
[b] - Concentration factor = (concentration in pooled tissue/initial concentration in water)

to different concentrations of MeHgCl and $HgCl_2$, as well as the concentration factor (concentration in tissue/initial concentration in water). The accumulation of Hg in the tissues was very rapid with MeHgCl and the highest concentration factor was found in those fish exposed for the longest period (96 hr) to the lowest concentration in the water (.01 mg Hg/litre). By comparison the concentration factors for $HgCl_2$ were very low.

The sequential concentration of Hg in the fish exposed to MeHgCl at 0.08 mg Hg/litre for 24 hr and then transferred to MeHg-free water is shown in Figure 2. The concentration of Hg in tissue continued to rise rapidly even after transfer to MeHg-free water.

5.3.4 Histopathology

5.3.4.1 MeHgCl

Morphologic changes which could be associated with acute poisoning by MeHgCl were restricted to the gills, with the most notable changes occurring in the epithelium of the secondary gill lamellae.

In the control fish the lamellae consisted of a thin central pillar with blood lacunae covered by a layer of flattened epithelial cells (Fig. 3). The changes observed in fish poisoned with MeHgCl were similar at all concentrations tested, with differences only in severity between various concentrations.

The first observed change was swelling of the epithelial cells (Fig. 4). In some cases this swelling was accompanied by cytoplasmic vacuoles, but usually it consisted of an increase of weakly eosinophilic cytoplasm. The nuclei also increased in size. The epithelium then

Fig. 2. Mercury (ppm) in pooled muscle, skin and bone of trout exposed to 0.08 mg Hg/litre as methyl mercury chloride (MeHgCl) for 24 hr, and then transferred to MeHg-free water. Each point is the mean value of two fish.

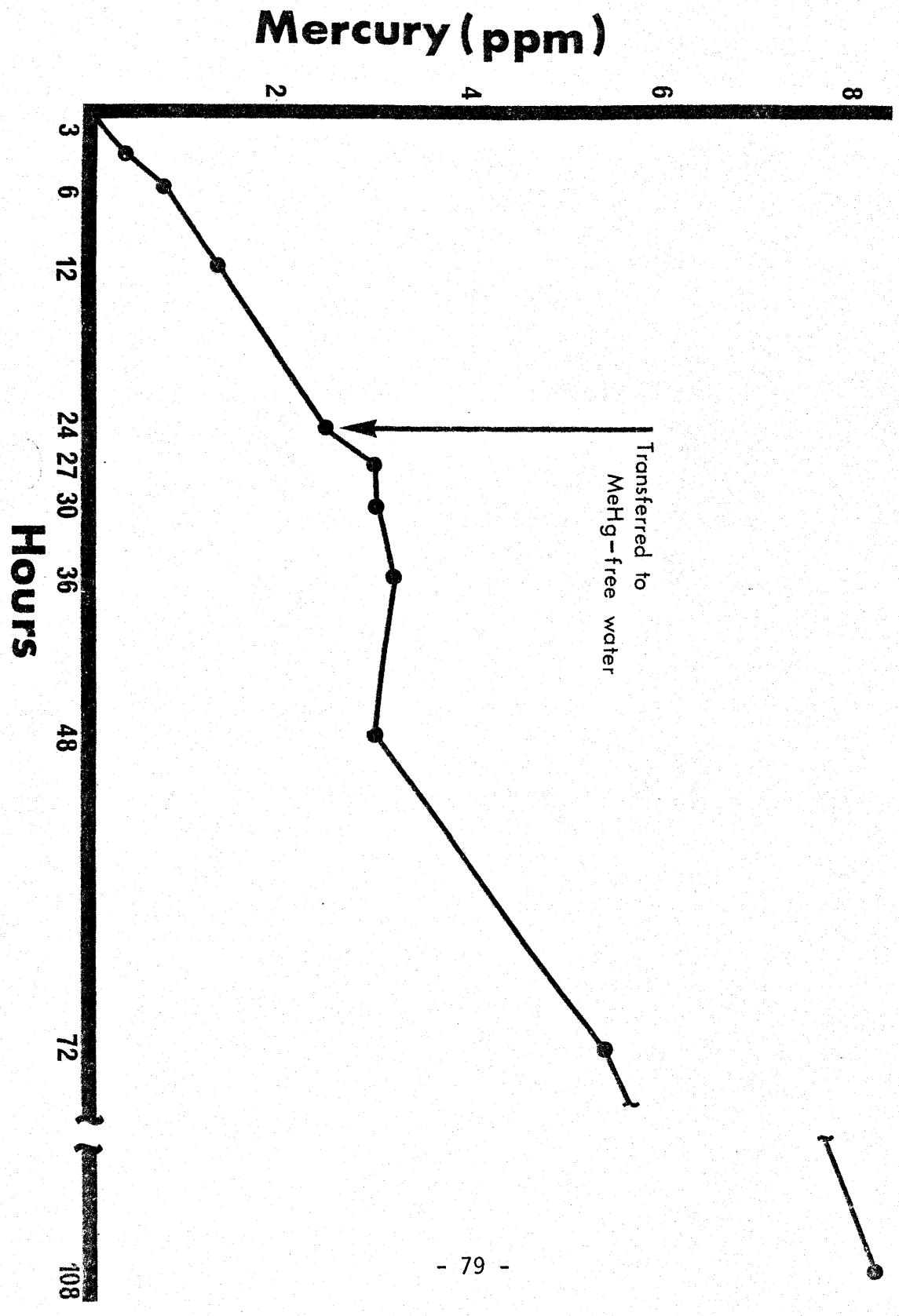
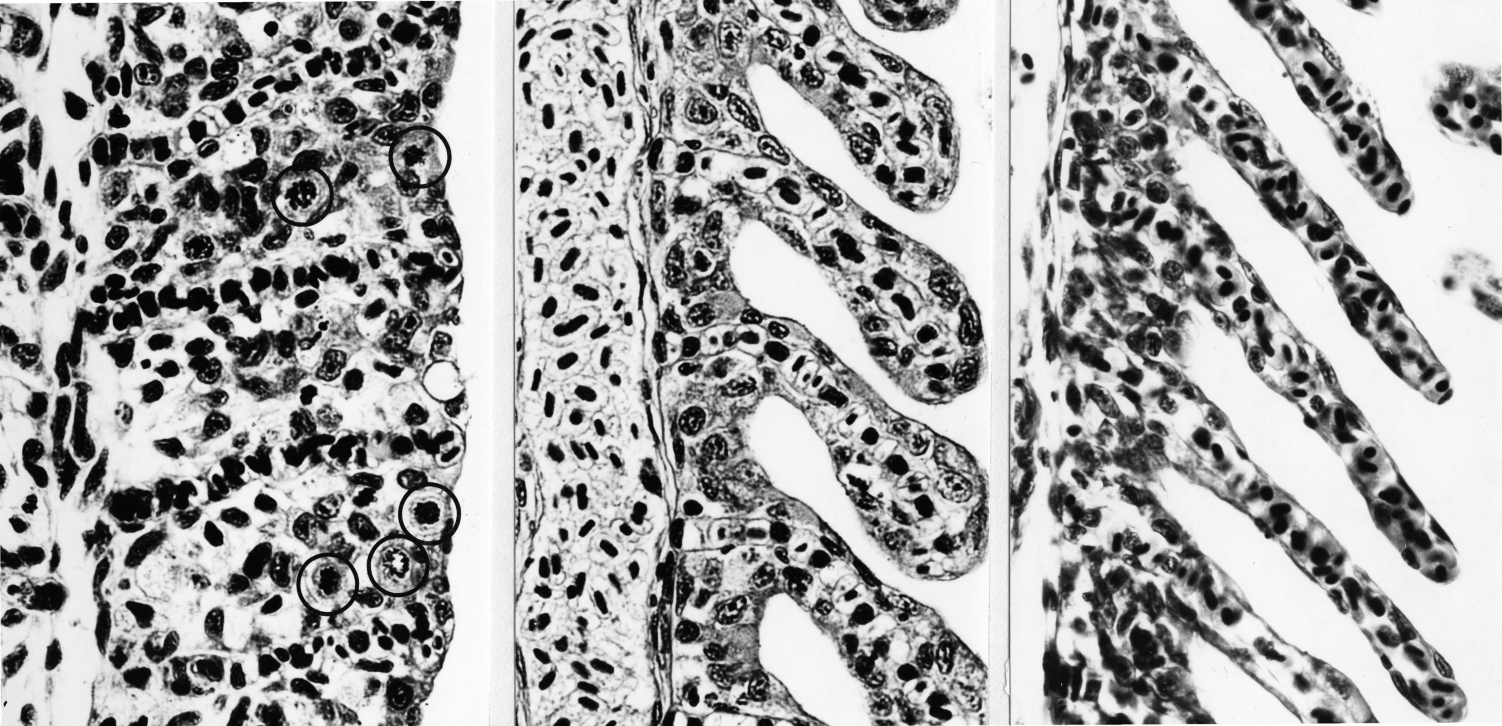


Fig. 3. Control trout, secondary gill lamellae.
Note single layer of flattened epithelial cells.
H & E X 540

Fig. 4. Trout exposed to 0.032 mg Hg/litre as methyl mercury chloride for 96 hr. Secondary gill lamellae. Swelling of epithelial cells.
H & E X 540

Fig 5. Trout exposed to 0.07 mg Hg/litre as methyl mercury chloride for 24 hr. Swelling of epithelial cells, epithelial hyperplasia, and numerous mitotic figures (circled) near tips of lamellae.
H & E X 540



underwent hyperplasia so that the lamellae at first were lined by several layers of epithelial cells (Fig. 5) and later the entire interlamellar spaces became filled with hyperplastic epithelium (Fig. 6). Ballooning of individual epithelial cells was noted at all concentrations tested.

Separation of the epithelium from the lamellae was a late phenomenon. In some cases, faintly eosinophilic material was present within this area of separation suggesting the presence of edema fluid of low protein content. The process of degeneration and separation advanced until the hyperplastic epithelium was desquamated, leaving bare central pillars surrounded by a mat of epithelial cell remnants (Fig. 7).

A striking feature of the gill lamellae of the fish exposed to MeHgCl was the large number of mitotic figures present in the epithelium (Figs. 5,6). In an attempt to quantitate this change, the number of cells in mitosis per 100 interlamellar spaces of 10 fish from each of the various trials were counted. The results shown in Table 15 indicated a great increase in numbers of cells in mitosis in those fish exposed to concentrations of MeHgCl greater than .032 mg Hg/litre. The change in cells in mitosis was of a qualitative as well as quantitative nature. In the control fish and those exposed to $HgCl_2$, the normal progression of mitosis to telophase was observed. In those fish exposed to MeHgCl cells in either anaphase or telophase were very rare. The majority of the mitotic cells had an abnormal appearance and had a disorganized clumping or dispersion of chromosomal material, which in some cases was in the form of a ring around

Fig. 6. Trout exposed to 0.08 mg Hg/litre as methyl mercury chloride for 24 hr. Secondary gill lamellae. Epithelial hyperplasia, ballooning degeneration of epithelial cells and numerous mitotic figures (circled).

H & E X 540

Fig. 7. Trout exposed to 0.10 mg Hg/litre as methyl mercury chloride for 24 hr. Secondary gill lamellae. Total degeneration of the epithelial layer with separation and desquamation.

H & E X 540

Fig. 9. Trout exposed to 0.75 mg Hg/litre as mercuric chloride for 24 hr. Secondary gill lamellae. Mild epithelial hyperplasia, with extensive necrosis and karryorhexis of epithelial cells.

H & E X 540

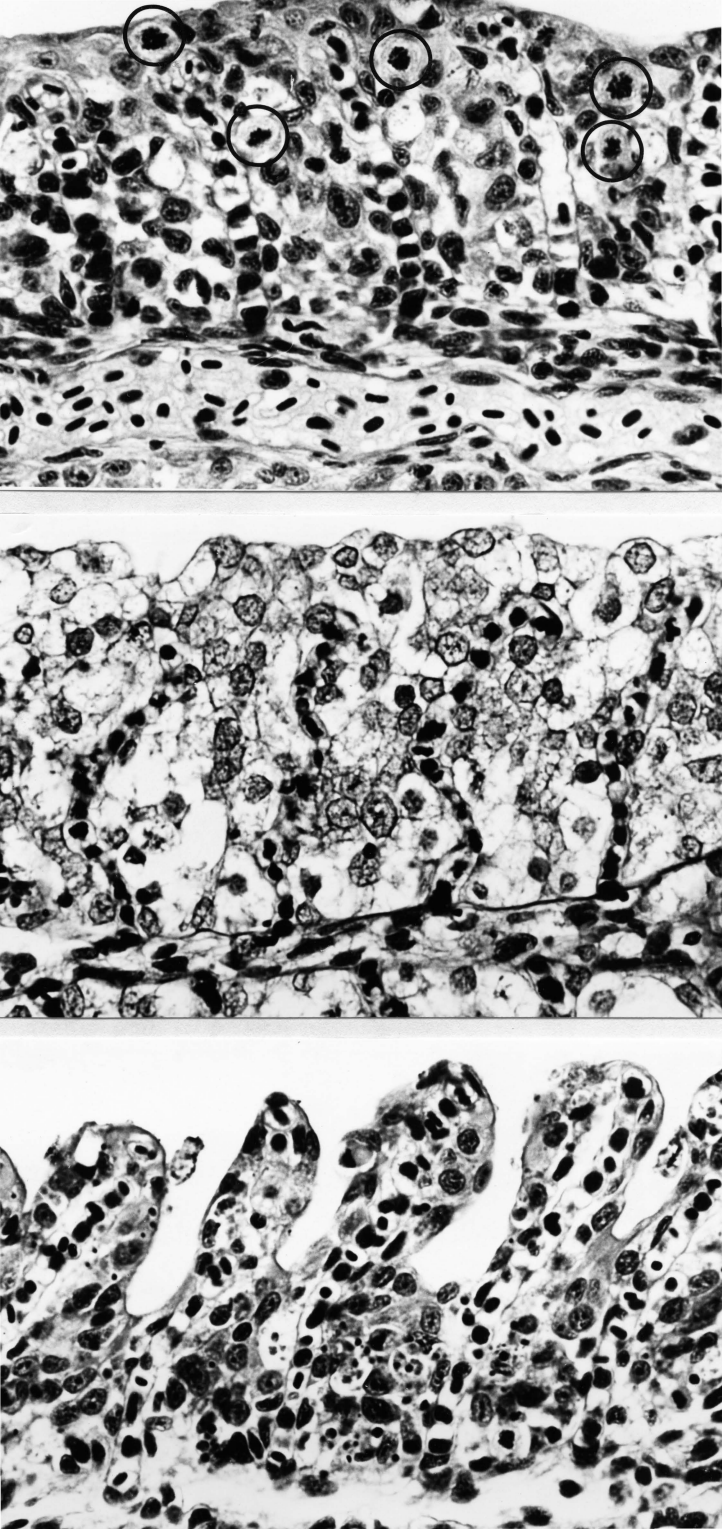


TABLE 15. Numbers of cells in mitoses per 100 interlamellar spaces on the gills of fingerling trout exposed to methyl mercury chloride (MeHgCl) and mercuric chloride ($HgCl_2$). The value at each concentration of mercury represents the mean for 10 fish.

Group	Concentration of Hg in water (mg/litre)	Exposure (hr)	Mean number of mitotic figures	% difference from control	Significance of difference from control
Control	nil	96	6.8 ± 4.2	--	--
MeHgCl	0.135	24	22.5 ± 9.9	+ 230.8	P<.005
	0.07	48	28.8 ± 8.1	+ 323.5	P<.005
	0.056	48	29.6 ± 10.3	+ 335.3	P<.005
	0.04	96	10.1 ± 6.8	+ 48.5	P<.05
	0.032	96	7.1 ± 3.0	+ 5.5	P>.05
$HgCl_2$	1.0	24	6.8 ± 2.3	0	P>.05
	0.75	24	6.6 ± 4.1	- 2.9	P>.05
	0.50	24	6.1 ± 3.8	- 10.3	P>.05

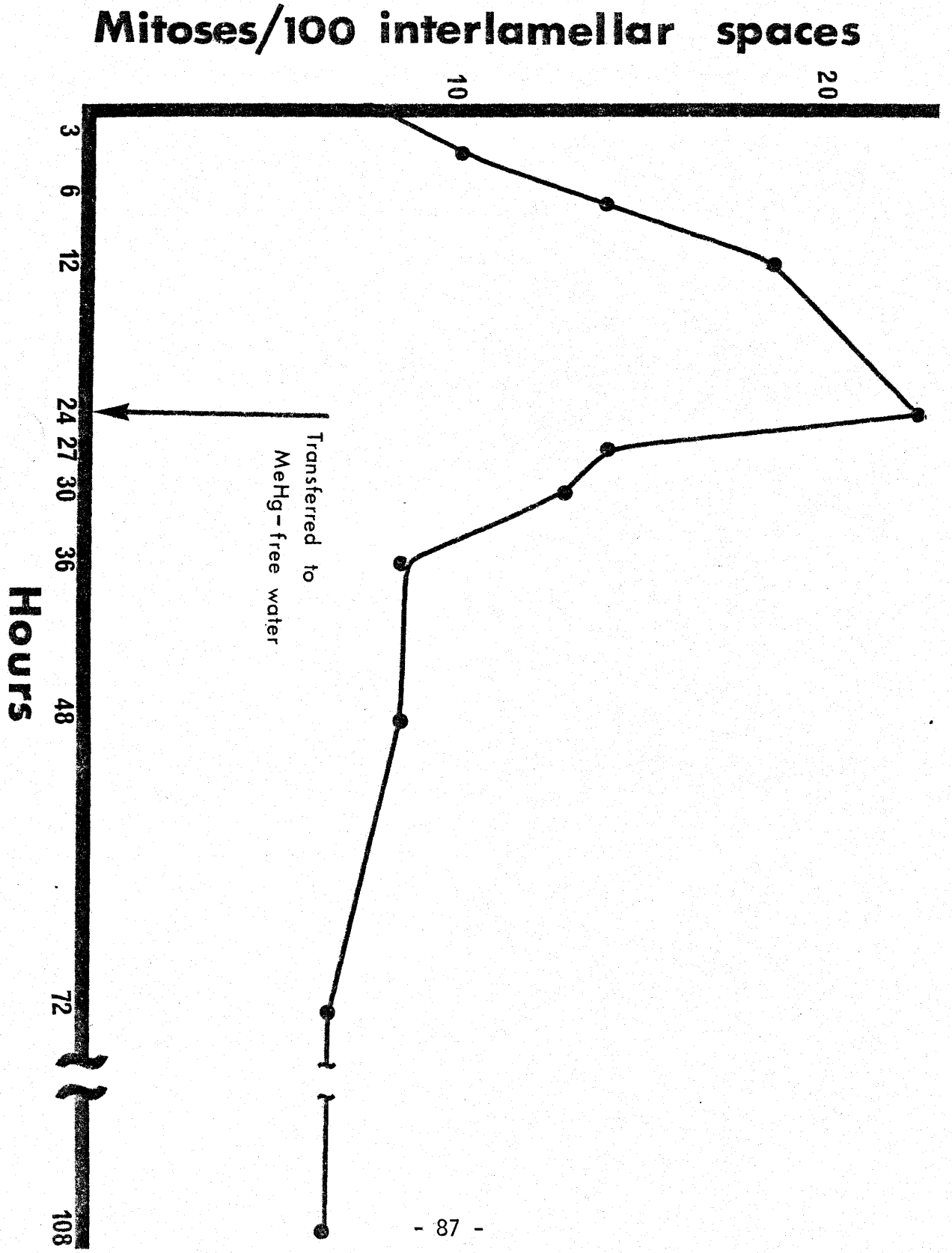
a central empty space or in others irregularly divided into three or four clumps. Mitotic figures were commonly observed near the tips of lamellae in fish exposed to MeHgCl, in contrast to the control fish in which mitotic activity was largely confined to the area near the base of the lamellae.

A film of coagulated mucus was never observed on the gills of fish exposed to MeHgCl. The changes noted chronologically in the fish exposed to 0.08 mg Hg/litre MeHgCl for 24 hr consisted of epithelial cell swelling, epithelial hyperplasia, and an increase in the number of cells in mitosis. Very slight separation of the epithelial layer was observed only after 24 hr exposure. Following transfer to MeHg-free water, it appeared that many of the damaged epithelial cells continued to degenerate and likely were desquamated; however, separation of the epithelial layers did not progress and the numbers of cells in mitosis declined rapidly after removal from the mercury solution (Fig. 8). By 48 hr after exposure to MeHgCl, the gills appeared morphologically normal.

5.3.4.2 $HgCl_2$

The histopathologic appearance of the gill lamellae of fish exposed to $HgCl_2$ differed from those of fish exposed to MeHgCl in several respects. The most obvious difference was the large number of necrotic epithelial cells present with karyorrhectic nuclei (Fig. 9). Karyorrhexis of occasional nuclei was observed in the lamellae of fish exposed to MeHgCl but never to the degree seen in those exposed to $HgCl_2$. There appeared to be some degree of epithelial hyperplasia; however, the numbers of mitotic figures present were not significantly

Fig. 8. Numbers of mitotic figures in the epithelium of the gills of trout exposed to 0.08 mg Hg/litre as methyl mercury chloride for 24 hr and then transferred to MeHg-free water. Each point is the mean value for two fish.



different from those of the controls (Table 15). Separation of epithelial cells from the central pillar was not a prominent feature of acute $HgCl_2$ poisoning; instead, the cells were destroyed *in situ*. The histopathologic lesions observed in both MeHgCl and $HgCl_2$ poisoning are summarized in Table 16.

5.4 Discussion

There appear to be no reports to which these results can be directly compared, with regard to the acute toxicity of alkyl mercurials for fish. Amend *et al*. (1969) studied factors which could influence the acute toxicity of $EtHgPO_4$ for rainbow trout. They exposed fish to 0.125 ppm Hg as $EtHgPO_4$ for 1 hr and found that in hard water (256 ppm as $CaCO_3$) with a low dissolved oxygen content (4 to 6 ppm) mortality could be as high as 40 per cent; at higher dissolved oxygen concentrations there was no mortality. Corner and Sparrow (1957) found that MeHgCl was 15 and 4.7 times as toxic as $HgCl_2$ for the crustaceans *Artemia salina* and *Elminius modestus* respectively. These authors suggested that the differential toxicity was related to lipoid solubility, and in turn to ease of penetration of the test animals. The relative toxicity of the aryl mercury compound, phenyl mercuric chloride, was similar to that of MeHgCl for these species (Corner and Sparrow, 1957). Boetius (1960) found that PHgA was considerably more toxic than $HgCl_2$ for rainbow trout. Akiyama (1970) found that PHgA was approximately seven times more toxic than $HgCl_2$ for *Oryzias latipes*. R. C. MacLeod and E. Pessah (1970, *personal communication*) found that PHgA was about 12 times more toxic than $HgCl_2$ for rainbow

TABLE 16. Histopathologic lesions observed in the gill epithelium of fingerling trout which survived exposure to various concentrations of methyl mercury chloride ($MeHgCl_2$) and mercuric chloride ($HgCl_2$) for varying time intervals.

	Hgmg/litre (hr exposure)										
	MeHgCl								$HgCl_2$		
Lesion	0.01(96)	0.032(96)	0.04(96)	0.056(48)	0.07(48)	0.08(48)	0.10(24)	0.135(24)	0.56(24)	0.75(24)	1.0(24)
Swelling of cells	7/7[a]	8/8	14/14	8/8	15/15	14/14	b	b	11/11	12/12	6/6
Hyperplasia	7/7	8/8	14/14	8/8	15/15	14/14			11/11	12/12	6/6
Increased mitotic activity	0/7	0/8	14/14	8/8	15/15	14/14			2/11	2/12	0/6
Mitotic figures near tip of lamellae	4/7	2/8	14/14	8/8	15/15	14/14			1/11	1/12	0/6
Ballooning degeneration	7/7	8/8	14/14	8/8	15/15	13/14			7/11	7/12	3/6
Karyorrhexis	1/7	1/8	5/14	2/8	11/15	7/14			11/11	12/12	6/6
Desquamation	1/7	0/8	1/14	0/8	4/15	2/14			2/11	2/12	1/6

[a] - Number of fish with lesion/total number of fish examined.

[b] - Total degeneration of epithelium with desquamation.

trout at 10°C. From these reports and the present study, it appears that the relative acute toxicities of alkyl and phenyl mercurial compounds for fish may be rather similar, with both being considerably more toxic than the inorganic compound $HgCl_2$.

Miettenin et al. (1970b) administered MeHg in the ionic form (as methyl mercury nitrate) and protein bound MeHg orally to pike and rainbow trout and found the LD_{50}/30 days to be about 15 ± 3 mg/kg body weight for pike. The LD_{50}/30 days for rainbow trout varied greatly depending on the rate of administration.

In the present study rainbow trout fry appeared to be much more sensitive to MeHgCl than were the fingerlings. Akiyama (1970) found that the resistance of Oryzias latipes to methoxyethyl mercury chloride and phenyl mercuric chloride similarly increased with increasing age of the fish. Boetius (1960) found that the survival time of Tilapia natalensis exposed to $HgCl_2$ increased with rising body weight.

This study confirmed the ability of fish to concentrate Hg rapidly from water, as described by Hannerz (1968), and also confirmed that the chemical form of the Hg is an important factor affecting the concentration rate. The continued rise in the concentration of Hg in tissues after transfer to MeHg-free water suggested that Hg was being transferred from other sites within the body to the skin, muscle, and bone. The accumulation rate of MeHg in different tissues of fish has been found to vary greatly, with the concentration in muscle and bone rising much more slowly than in organs such as the gills, kidney, and liver (Hannerz, 1968; Backstrom, 1969).

Amend et al. (1969) described changes in the gills of rainbow

trout exposed to $EtHgPO_4$ (0.125 ppm as Hg for 1 hr). At low dissolved oxygen concentrations, the changes observed were necrosis and separation of the epithelium from the lamellae. At higher dissolved oxygen concentrations the changes were described as being of a less severe nature with hypertrophy of epithelial cells. The photographs in their article show changes very similar to those observed in the present study, but prominent mitotic activity was not mentioned. Lindahl and Hell (1970) described gill lesions in Leuciscus rutilus exposed to phenyl mercuric hydroxide. The lesions consisted of separation of the intact epithelial layer from the central pillar. No changes were described in the epithelial cells. These authors also described a progressive decrease in blood flow to the gill lamellae, and the occurrence of a mucus layer on the lamellae. No explanation was given as to how the decrease in blood flow to the gills was assessed.

Akiyama (1970) described the formation of a "veil-like film" on the gills of Oryzias latipes exposed to PHgA and methoxyethyl mercury chloride and suggested that "coagulation-film anoxia" was important in the toxicity of these compounds. Unfortunately, histopathologic observations were not presented. Miettinen et al. (1970b) described necrosis of the gills, inflammation of the pseudobranchiae, nephrosis and focal hepatic necrosis in pike to which MeHg was administered via stomach tube. These fish lived an average of 18 days after the administration of the MeHg so the lesions likely cannot be compared to those seen in the present study because of differences in route of administration and length of exposure.

From the literature and the present experiment, it appears that

the acute toxic action of various Hg compounds is exerted upon the gill epithelium, likely resulting in death due to asphyxia. There appear to be differences in the action of the various compounds on the gill epithelium. Mercuric chloride caused severe necrosis while poisoning with MeHgCl at equivalently toxic concentrations was characterized by hyperplasia, greatly increased numbers of mitotic figures, degeneration, and terminal desquamation of the epithelium. There was no evidence to support the coagulation film-like anoxia theory as a feature of the toxicity of either of these compounds. These findings are similar to those which have been observed in rainbow trout poisoned by other heavy metals (Lloyd, 1960; Erickson-Jones, 1964).

The great increase in numbers of cells in mitosis in the gills of fish exposed to MeHgCl does not appear to have been previously described in fish poisoned with mercurial compounds. Gardner and Yevish (1970) described epithelial hyperplasia and increased mitotic activity in the lamellae of Fundulus heteroclitus exposed to 50 ppm cadmium for more than 20 hr. However, these changes were described as being focal in nature and were accompanied by lymphocytic infiltration, both of which differ from the lesions observed in the present study.

The apparent increase in the numbers of cells in mitosis could be a direct response to the MeHgCl or alternatively a response to epithelial damage. The mitotic index in those fish exposed to $HgCl_2$ apparently did not increase, even though epithelial damage was severe. The mitotic index dropped rapidly in fish transferred to "clean water"

after exposure to MeHgCl. In this situation it could be expected that repair would be associated with a high mitotic rate. These considerations suggest that response to epithelial damage was not the primary cause of increased mitotic activity. The alternative hypothesis, that MeHgCl was directly responsible is attractive, particularly in view of the large body of literature documenting the mitosis-disturbing ability of alkyl Hg compounds. The ability to disrupt normal mitotic activity has been observed in plants (Fiskejo, 1969; Ramel, 1969 a,b), Drosphila melanogaster (Ramel and Magnusson, 1969), HeLa cells (Umeda et al., 1969) and human leucocytes (Fiskesjo, 1970) in vitro and bacteria (E. Fiskesjo, 1970, personal communication). Thrasher and Adams (1972) found that MeHg (as well as other Hg compounds) increased the generation time of a ciliate (Tetrahymena pyriformes).

This action of alkyl Hg compounds is believed to involve interference with sulfhydryl groups in spindle fibre proteins, thus effectively blocking mitosis at metaphase (Ramel, 1969a). The highest dose of various alkyl mercurials without c-mitotic effect on Allium root tips was found to fall within the range 0.04 to 0.06 ppm Hg (Ramel, 1969). Thrasher and Adams (1972) found that concentrations of MeHgCl of 0.014 mg/litre or less had little or no effect on the generation time of T. pyriformes, but a concentration of 0.042 mg/litre increased generation time by 25 per cent.

These concentrations are very similar to the Hg concentration in water below which the number of cells in mitosis did not significantly differ from the control in the present experiment.

It would seem unlikely that concentrations of MeHg compounds as high

as used in the present study would occur except in grossly contaminated natural waters. There are relatively few data available on Hg content of natural waters; however, the levels reported generally fall within the range 0.001 - 0.4 ppb, with the mean likely near 0.05 ppb (Westermark et al., 1966; Wiklander, 1970). By contrast the content of MeHg (primarily MeHgCl) in the waste water from a vinyl chloride plant at Minamata, Japan, was reported to be about 0.3 ppm (Irukayama et al., 1969) and the waste water from an acetaldehyde plant in the same location contained approximately 50 ppm of MeHgCl (Nakamura, 1969).

The United States and the Soviet Union have tentatively adopted 5 ppb as the maximum allowable concentration of Hg in drinking water, and Japan has adopted 10 ppb of MeHg as the maximum allowable concentration in industrial waste water (Harris et al., 1970). Concentrations of organic mercurial compounds well below these standards have been found to have detrimental effects on phytoplankton (Harris et al., 1970). The present study indicates that MeHgCl at concentrations (10-135 ppb) only slightly higher than these standards may have toxic effects on fish. If very young fish are the most susceptible group in a population as was suggested by this and earlier studies, mortality due to poisoning would be very difficult to detect. This study also shows that even brief exposure to MeHgCl in water at levels similar to these standards results in the rapid concentration of Hg in fish tissue.

6.0 EXPERIMENT III. PROLONGED ORAL ADMINISTRATION OF METHYL MERCURY CHLORIDE TO RAINBOW TROUT FINGERLINGS

6.1 Rationale

The previous experiment (II) indicated that MeHg was highly toxic for rainbow trout under conditions of acute exposure to Hg in the water, but did not provide a basis for understanding the possible effects of chronic exposure to MeHg, as would occur in a more normal situation in a contaminated environment.

Prolonged administration of Hg through the water did not appear to be feasible because an adequate apparatus for dispensing and metering Hg addition to the partial free-flowing aquarium system available would have been difficult and expensive to construct, and no facilities were available for the routine monitoring of Hg concentrations at the ppb level required for this type of study. For these reasons it was decided to feed trout a diet containing MeHg at concentrations similar to those found in fish from the contaminated areas of the Saskatchewan River, in an attempt to artificially reproduce a situation similar to that encountered by predatory fish in the River.

In the initial set of trials, no mortality occurred due to Hg administration, and the levels of Hg in the fish tissue were similar to those found in fish from the Saskatchewan River. In the second set

of trials, levels of Hg higher than those found in the Saskatchewan River fish were used in the feed in an attempt to produce poisoning in the fish within the experimental period.

6.2 Materials and methods

6.2.1 The fish

The fish utilized in these experiments were rainbow trout fingerlings obtained in one group from a supplier in southern Saskatchewan. These fish had originally been imported from the same source in Montana as the fingerlings used in experiment II. The experimental period extended from 18 May, 1972, to 30 August, 1972, for the first three trials, and from 26 September, 1972, to 9 January, 1973, for the second three trials. The fish used in the first three trials measured from 9.4 cm to 13.3 cm (snout to tail fork) with a mean of 11.7 cm at the beginning of the trial period. These fish weighed from 16.6 g to 33.0 g with a mean of 20.9 g. The fish used in the second three trials were larger, measuring from 11.9 to 16.1 cm, with a mean of 13.8 cm at the beginning of the trial period. These fish weighed from 21.2 to 52.2 g with a mean of 31.7 g. All fish had been held in the laboratory for a minimum of 14 days to allow for acclimation to occur before the beginning of the first trial.

6.2.2 The aquaria

The experimental unit consisted of four aquaria[1] arranged in two groups of two each. Each pair of aquaria was covered by a common

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[1] Living Stream Model LS-700, Frigid Units, Inc., Toledo, Ohio, U.S.A.

sheet aluminum fume hood which exhausted outside the building. The fume hoods were used to remove any possible Hg vapor arising from the aquaria. The aquaria were of fibreglass construction, and each was fitted with a self-contained cooling and recirculation unit. The aquaria measured approximately 200 cm x 58 cm x 52 cm deep, and contained approximately 450 litres of water.

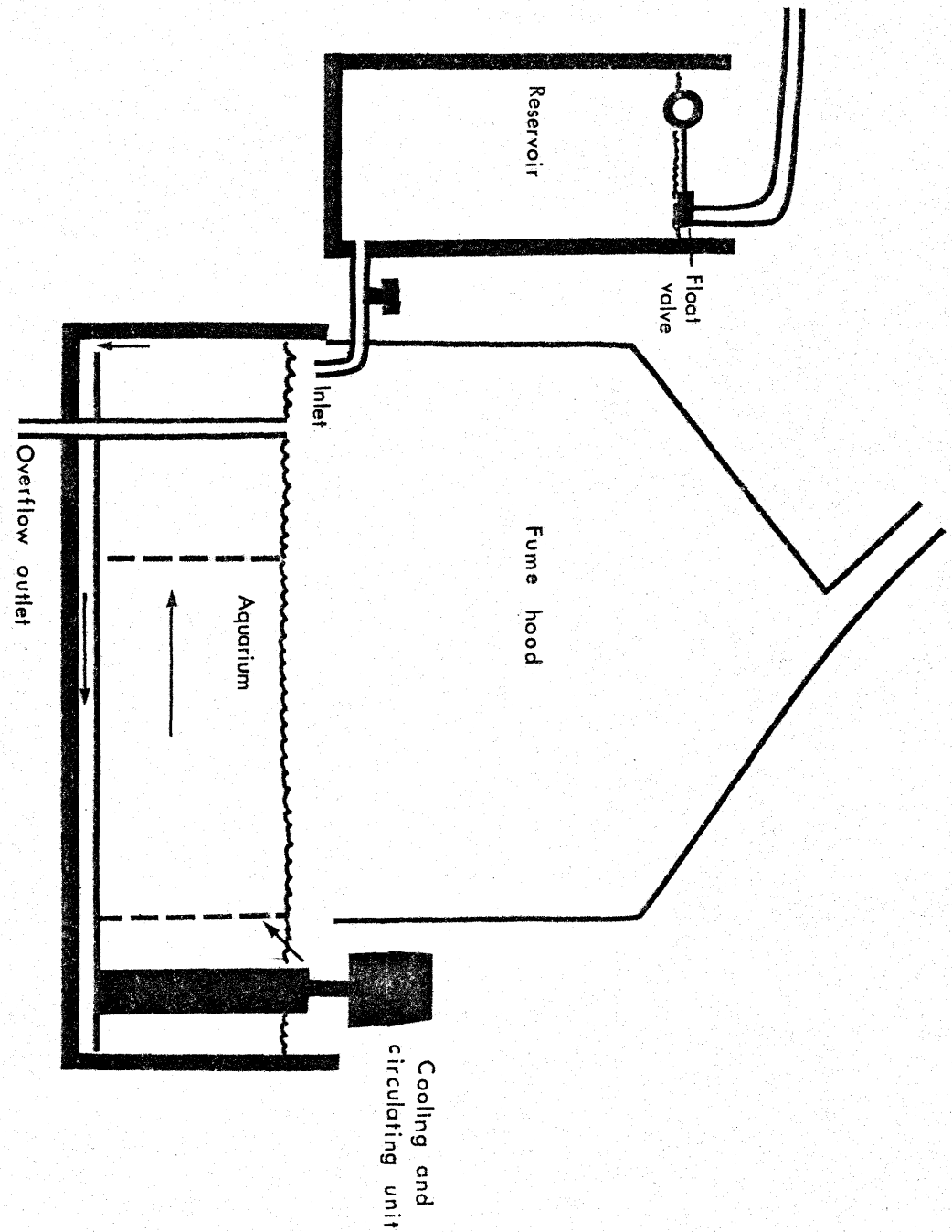
Each aquarium had an overflow drain pipe which maintained the water at a constant depth. City tap water, dechlorinated by passage through an activated charcoal filter, was carried to the aquarium area in plastic piping. At each aquarium water from the piping entered an elevated 100 litre capacity fibre glass reservoir through a float valve. This reservoir maintained a constant head of water. Water left this reservoir through a valve at the bottom and entered the aquarium.

Water flow into each aquarium was controlled at approximately 500 ml/minute. A diagram of one aquarium unit is shown in Fig. 10.

One aquarium was used for each trial, and the fish were confined by a screen to the two-thirds of the aquarium nearest the recirculation unit. Fecal material and uneaten food passed through the screen and collected on the floor of the aquarium behind the screen, from where it was removed daily by a syphon device.

Water entering the aquaria reservoir was checked daily for the presence of chlorine using orthotolidine as an indicator. The water temperature within the aquaria was maintained at 10$\pm$ 0.7C. Dissolved oxygen content of the aquarium water was determined frequently by a modified Miller's method as described by Thomas (1953), and was found to range from 7.3 to 8.3 ppm.

Fig. 10. Diagrammatic view of one aquarium unit.



6.2.3 The rations

Prior to being placed on trial, fish were maintained on a commercially available dry trout food[1]. The ration used for the trials consisted of ground pork liver plus the dry trout food mixed at a ratio of 5:1 by weight. Several samples of this basal ration were analyzed for Hg content and found to contain less than 0.1 ppm Hg. Methyl mercury chloride in the form of an aqueous solution containing 1.02 mg Hg/litre was added in appropriate amounts to this basal ration to provide concentrations of 4 ppm for group I, 8 ppm for group II, 16 ppm for group III and 24 ppm for group IV. Distilled water was added to the control rations and those rations with lower Hg content, so that all rations had a similar water content. After addition of Hg solution and/or water, the rations were mixed thoroughly in electric blenders and the semi-solid food was dispensed into 50 ml plastic syringes which were then sealed in plastic bags and frozen until used. At the time of feeding, one syringe of each ration was thawed, and the appropriate weight of food ejected into each aquarium over a 3 to 4 minute time interval. The semi-solid food formed "worm-like" threads when dispensed from the syringe and was eaten readily by the fish. Feed was supplied at a rate of 3 to 4 per cent of total body weight of fish in the tank once each day. This rate represented some degree of overfeeding and not all of the food supplied was consumed each day.

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[1]Silver Cup Self-Sustaining Trout Feed, Ferguson Feeds, Ltd., Drinkwater, Saskatchewan.

6.2.4 Experimental design

Two sets of three trials were performed. In each set, a control group of 30 fish received only the basal ration, while two groups of 30 fish each received the same ration plus MeHgCl added at various concentrations.

In the first set of trials, group I received a ration containing MeHgCl at 4 ppm as Hg; group II received food containing MeHgCl at 8 ppm as Hg. In the second set of trials, group III received food containing 16 ppm MeHgCl as Hg, and group IV was fed a ration containing 24 ppm MeHgCl as Hg. The total weight of fish in each group was determined at weekly intervals. Two fish from each group were killed weekly, and a blood sample, and tissues for histopathology and mercury analyses were collected.

Fish were anaesthetized for weighing and handling by immersion in a solution of ethyl-M-aminobenzoate methane sulfonate[1] at a concentration sufficient to produce anaesthesia within 30 - 40 seconds (approximately 50 - 100 mg per litre of water).

6.2.5 Sample collection

The two fish sampled from each group each week were deeply anaesthetized and placed in a grooved styrofoam block for handling. The caudal peduncle was severed with a scalpel and a blood sample collected in a heparinized micro-capillary tube[2]. After collection of

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[1]Tricaine methane sulphonate, Fraser Medical Supplies Ltd., Vancouver British Columbia.

[2]Capilets, B4415-2, Canadian Laboratory Supplies Ltd., Toronto, Ontario.

the blood sample the cartilaginous cranium surrounding the dorsal and lateral aspects of the brain was carefully dissected away, leaving the brain exposed in situ. The trunk was then sectioned transversely immediately posterior to the head, and the opercula were removed. The entire head with brain and gills exposed was fixed in approximately 20 volumes of Bouin's fluid. The abdomen was opened by a longitudinal midventral incision and the viscera removed. Portions of liver, spleen, stomach and pyloric caeca, and terminal intestine plus 4 mm thick transverse sections through the trunk taken at the level of the anterior kidney, and at the level of the dorsal fin were fixed in Bouin's fluid.

The dorsal axial muscles from the anterior portion of the trunk were dissected away, placed in a plastic bag and frozen until used for Hg analysis.

6.2.6 Sample processing

Blood collected in micro-capillary tubes was centrifuged[1] for 4 minutes, and the packed cell volume (PCV)[2] and the total plasma protein (TP)[3] content of the serum were determined.

Tissues for histopathology were fixed for 24 hr in Bouin's fluid, and then transferred to a 70 per cent solution of ethyl alcohol until trimmed. The head with brain in situ was sectioned transversely into

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[1] International Micro-capillary Centrifuge, Model MB, International Equipment Co., Needham Hts., Mass., U.S.A.

[2] International Micro-capillary Reader, Model CR, International Equipment Co., Needham Hts., Mass., U.S.A.

[3] AO TS Refractometer, AO Instruments Co., Buffalo, New York, U.S.A.

slices approximately 3 mm thick and all sections together with those from the other tissues collected were embedded in paraffin and sections were cut at a thickness of 6μ and stained with H & E. The following stains were also employed selectively when thought to be of value in interpreting changes in the nervous system: Holzer's stain for astrocytes, Luxolfast blue stain for myelin, and Bielchowsky's stain for axons as modified for paraffin sections (Culling, 1963; Luna, 1968). Muscle tissue was analyzed for Hg by the method described for experiment II.

6.3 Results

6.3.1 Clinical findings

No mortality which could be associated with the feeding of MeHgCl contaminated feed occurred in this experiment. The only mortality which did occur was during the early weeks of the study and was a direct result of handling the fish. In one case three fish died from an anaesthetic overdose. Other losses resulted from the removal of five fish, which had escaped during handling and fallen to the floor, from the experimental group. No abnormalities were detected in groups I, II, or III during the course of the experiment. Fish in group IV developed a slight diffuse darkening of the body, when compared to controls, after approximately 50 days on trial. This darkening of the body persisted for approximately 3 weeks, and then body color returned to normal.

No noticeable difference in appetite or willingness to feed was observed in any of the groups over the period of the study. However, during the last 4 weeks of the trial, fish in groups III and IV tended

to occupy the middle portion of the tank space, rather than maintaining a position near the upstream end of the tank. Control fish usually maintained a position close to the upstream end of the tank. There was no appreciable difference in the ease with which fish from different groups could be captured for weighing. All fish appeared to have normal vision and evaded the net used for capture with equal facility.

6.3.2 Weight gains

The weight gain per week (expressed as a percentage of the total weight at the beginning of the week) varied widely from week to week, and in general intragroup variation from week to week was greater than variations among groups.

The mean weight gains of principal groups over the entire length of the experiment did not differ significantly from those of controls in either set of trials (Table 17). However the mean weight gains of groups III and IV in the final 5 weeks of the experiment were significantly lower than that of the control group during the same period (Table 18).

6.3.3 Hematology

The packed cell volume (PCV) and total plasma protein (TP) of blood were measured on principal and control fish. In the first set of trials the mean PCV values of principals did not differ significantly from that of the controls (Table 19). However, it appeared that the PCV values in group II were rising in the latter weeks of the experiment. The mean TP value for fish in group II was slightly but significantly ($P<0.05$) higher than that of the control fish. The

TABLE 17. Mean weekly weight gains, calculated as a percentage of weight at beginning of the week, of trout fed rations containing <0.1 to 24 ppm Hg as methyl mercury chloride over a 15 week period

Group	Hg in ration (ppm)	Weekly weight gain (%) Mean ± SD
Control	< 0.1	7.4 ± 1.4
I	4.0	7.1 ± 1.1
II	8.0	7.5 ± 1.9
Control	< 0.1	7.4 ± 2.0
III	16.0	6.1 ± 1.4
IV	24.0	6.4 ± 1.2

TABLE 18. Mean weekly weight gain, calculated as a percentage of weight at beginning of the week, of Control group, and groups III and IV over the final 5 weeks of the experiment.

Group	Hg in ration (ppm)	Weekly weight gain (%) Mean ± SD
Control	<0.1	8.3 ± 1.4
III	16.0	5.6 ± 0.9[a]
IV	24.0	6.0 ± 0.8[a]

[a] - Significantly different ($P<0.05$) from mean of Control group.

TABLE 19. Packed cell volume (PCV) and total plasma protein (TP) of fish in groups Control, I, and II.

Week	Group (Hg in ration, ppm)					
	Control (<0.1)		I (4.0)		II (8.0)	
	PCV	TP	PCV	TP	PCV	TP
1	32.0	5.2	33.0	5.7	34.0	5.1
	34.5	5.6	31.5	5.2	37.0	5.2
2	34.0	5.2	39.0	5.6	38.0	5.6
	39.0	6.0	38.5	5.4	33.0	4.8
3	38.0	5.6	39.0	5.7	36.0	5.3
	31.0	5.3	34.5	4.9	39.5	6.0
4	34.5	5.1	34.5	5.3	37.0	5.5
	33.5	4.9	37.0	5.4	33.5	5.1
5	39.5	5.6	39.5	6.1	--	--
	37.0	5.2	39.0	5.7	--	--
6	40.0	6.6	35.0	5.3	34.0	6.1
	37.5	5.8	37.0	6.0	39.0	5.6
7	36.0	5.5	33.5	5.2	34.0	5.2
	34.5	5.3	34.0	4.9	39.5	6.6
8	37.5	5.3	35.5	5.3	39.5	5.8
	32.5	5.3	39.5	6.1	42.5	7.4
9	34.0	5.4	33.0	5.3	38.5	5.7
	33.0	4.9	37.0	5.8	40.5	7.0
10	38.5	5.7	34.0	5.1	36.5	5.6
	34.5	5.3	--	--	39.0	6.0
11	40.5	6.7	38.0	5.9	37.0	5.5
	39.5	5.9	--	--	43.0	7.2
12	37.0	5.6	33.0	5.1	33.0	4.9
	34.5	5.7	39.0	6.1	42.0	6.5
13	38.5	5.2	41.5	7.0	44.0	6.7
	39.0	6.3	34.5	5.7	46.0	6.2
14	38.5	5.5	39.5	6.3	52.0	8.6
	36.5	5.5	40.5	6.3	--	--
Mean ± SD	36.3 ± 2.7	5.5 ± 0.4	36.6 ± 2.8	5.6 ± 0.5	38.7 ± 4.5	6.0 ± 0.9[a]

[a] - Mean significantly different ($P<0.05$) from that of Control group.

mean PCV values for fish in both groups III and IV were significantly higher than the control (Table 20). The mean TP values of these groups were not significantly different from control.

6.3.4 Accumulation of mercury in tissue

Mercury concentrations in the muscle tissue of principal fish are shown graphically in Fig. 11. The muscle tissue of control fish from both groups consistently contained less than 0.2 ppm Hg, and there was no change in the levels over the course of the experiments.

The concentration of Hg in muscle was directly related to intake, with those fish receiving a larger intake accumulating mercury at a faster rate. In groups I, II, and IV approximately 65 days feeding elapsed before Hg concentrations in muscle reached parity with those in the diet. The Hg concentrations in the muscle of fish in group III did not approximate those in the diet until after about 85 days of feeding.

Individual fish in all groups had higher Hg concentrations in their muscle than were present in their diet

6.3.5 Pathology

No gross lesions were detected in any of the fish in this experiment.

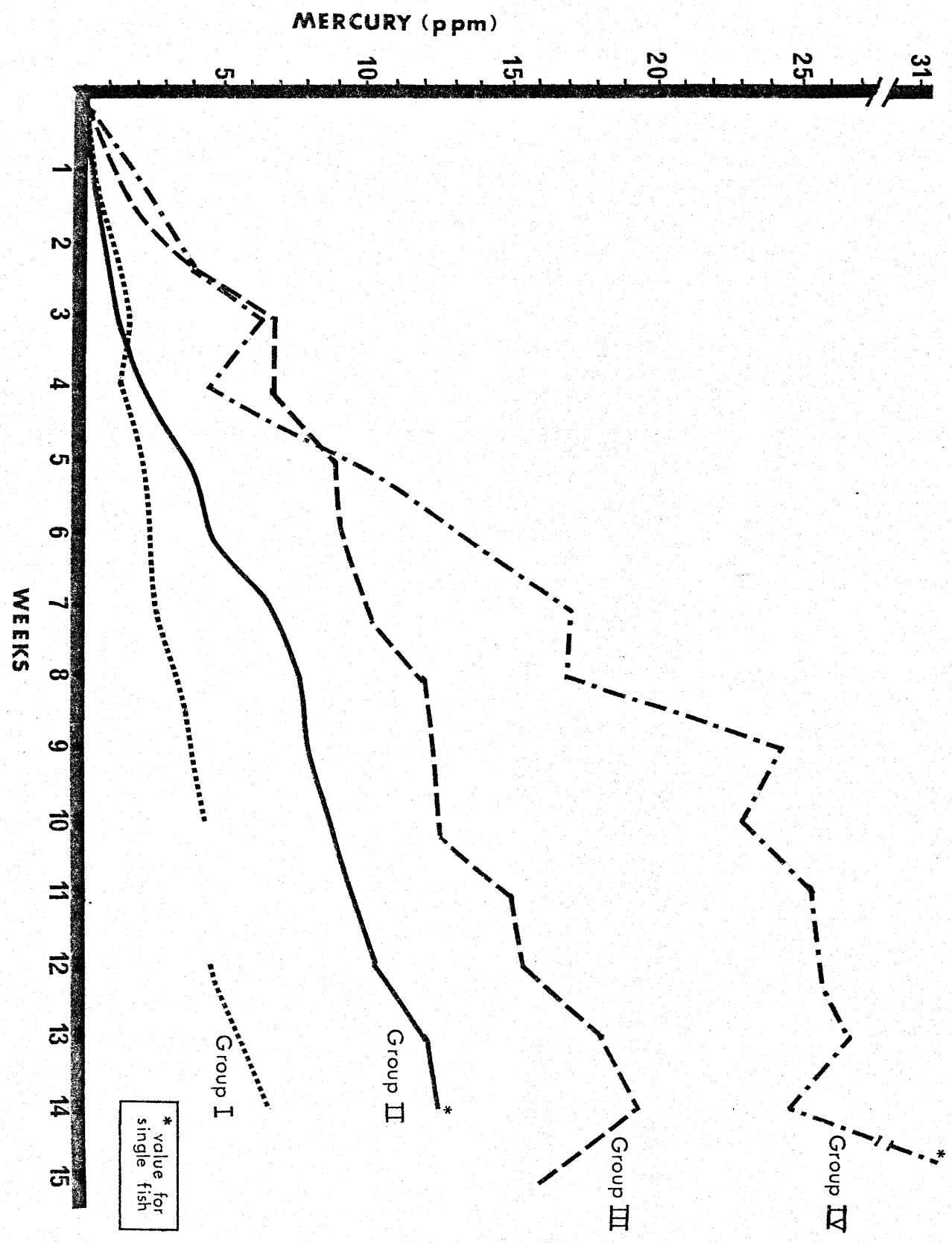
Histopathologic lesions were also very minor and were confined to the gills and posterior kidney. In the control fish from both groups the secondary gill lamellae were covered by a single layer of flattened epithelial cells. The epithelial layer was approximately three cells thick over the interlamellar filament (Fig. 12). Occasional epithelial

TABLE 20. Packed cell volume (PCV) and total plasma protein (TP) of fish in groups Control, III and IV.

Week	Group (Hg in ration, ppm)					
	Control (<0.1)		III (16.0)		IV (24.0)	
	PCV	TP	PCV	TP	PCV	TP
1	38.0	6.0	--	--	41.5	5.5
	32.0	5.2	45.5	5.9	46.0	5.6
2	33.0	4.9	38.0	5.3	37.0	5.9
	38.0	5.6	42.0	6.4	47.5	6.9
3	35.0	6.0	28.0	5.8	34.0	5.9
	32.0	5.0	31.5	5.7	33.0	6.6
4	36.0	6.8	41.5	6.5	40.5	6.5
	35.5	6.0	45.0	7.4	42.0	7.8
5	34.0	5.5	40.5	6.9	30.0	5.6
	39.0	6.4	37.5	7.1	40.0	6.5
6	36.0	6.0	37.0	5.4	36.0	5.1
	39.5	6.1	49.0	7.1	39.5	5.9
7	40.5	5.9	45.5	6.7	45.0	6.5
	41.0	6.9	40.0	6.5	38.5	6.4
8	37.5	5.8	41.0	6.0	40.0	6.1
	--	--	38.0	5.7	37.5	5.9
9	36.5	5.7	40.0	6.0	39.0	6.2
	--	--	34.0	5.5	41.0	6.9
10	33.5	5.4	41.5	6.0	32.5	6.4
	37.0	5.6	39.5	6.3	50.5	7.6
11	38.5	5.4	40.5	5.6	40.0	6.0
	39.5	6.2	35.5	5.5	41.0	5.3
12	33.0	5.2	37.0	6.1	38.5	5.1
	38.0	5.7	41.5	6.0	33.0	5.9
13	36.0	5.6	38.0	5.5	43.0	6.1
	--	--	35.5	5.6	41.0	5.9
14	39.5	6.7	41.0	6.1	40.5	5.6
	42.0	6.7	40.5	6.3	39.0	5.8
15	--	--	43.0	5.6	40.0	5.4
	38.0	5.7	39.5	5.4	--	--
Mean ± SD	36.9 ± 2.8	5.9 ± 0.5	39.6 ± 4.3[a]	6.1 ± 0.6	39.6 ± 4.5[a]	6.1 ± 0.7

[a] - Mean significantly different ($P<0.05$) from mean of Control group.

Fig. 11. Mercury (ppm) in the muscle of trout in groups I, II, III and IV fed rations containing 4, 8, 16 and 24 ppm Hg, respectively. Each weekly value is the mean for two fish.



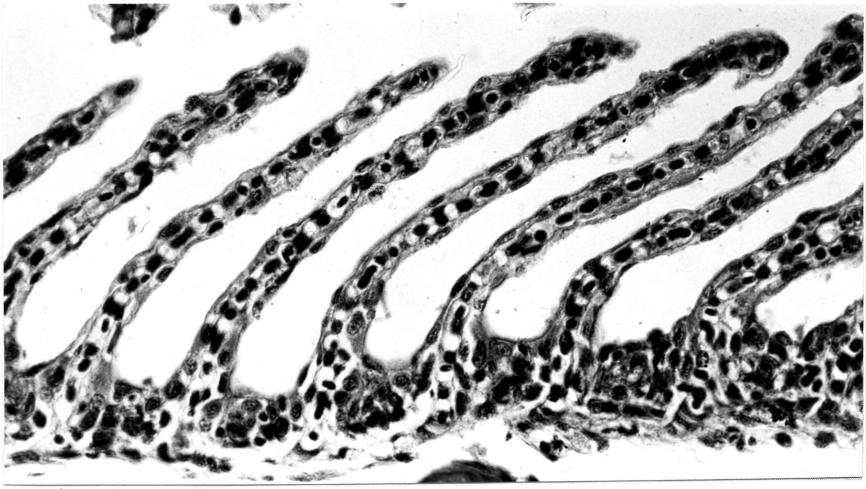


Fig. 12. Secondary lamellae of gill of control fish.
H & E X 375

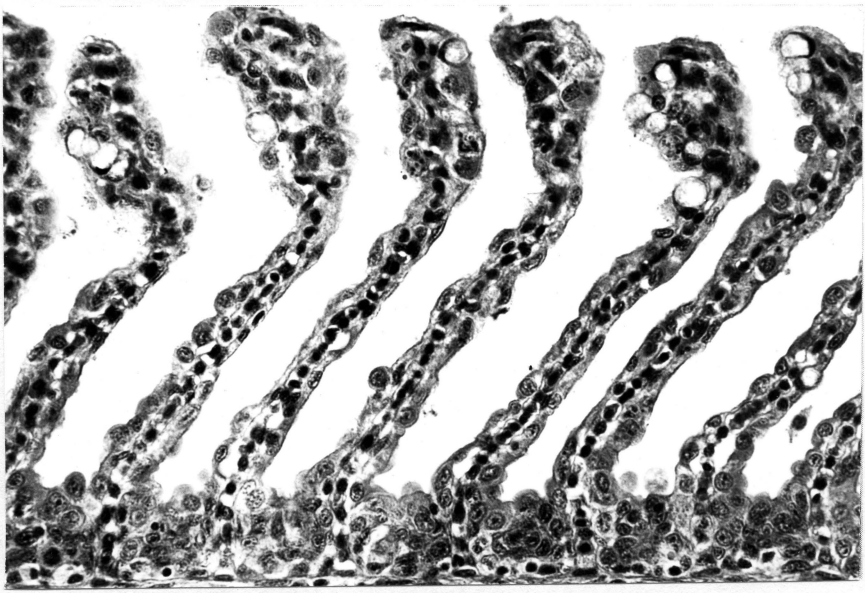


Fig. 13. Secondary lamellae of gill of fish which had received a diet containing 8 ppm Hg as methyl mercury chloride for 70 days. Swelling and hyperplasia of epithelial cells near tips of lamellae.
H & E X 375

cells containing large vacuoles were present at the tips of many lamellae.

The changes occurring in the gills of fish in all principal groups were essentially similar. The initial changes noted were cellular swelling, increase in numbers of vacuolated cells, and slight epithelial hyperplasia at the tips of the lamellae (Fig. 13). This change was termed "clubbing" of the lamellae. (This term is often used by fisheries workers to describe gill changes seen in a wide variety of conditions.) Clubbing began as a localized phenomenon involving only a few adjacent lamellae, and then became more diffuse. Subsequently epithelial cell swelling and hyperplasia became more generalized along the length of the lamellae (Fig. 14), and more diffuse over the entire gill. However, even in the most severely affected fish these hyperplastic changes did not involve the entire gill, and areas showing only very minor clubbing could be found. Fusion of the epithelium of adjacent lamellae was observed occasionally (Fig. 15). Single ectatic dilations of the lacunae of lamellae were noted in one fish from each of groups I, II, and IV. Similar structures have been reported in apparently normal rainbow trout (Finn, 1970) so that the significance of these structures is doubtful.

The incidence of lesions in the gills is shown in Table 21. The only change from controls noted in the kidneys of principals was a slight swelling of the epithelial cells lining Bowman's capsule. This change did not occur consistently among fish of any group, but was more common in fish in groups III and IV late in the experiment. No lesions were detected in the liver, stomach, spleen, anterior

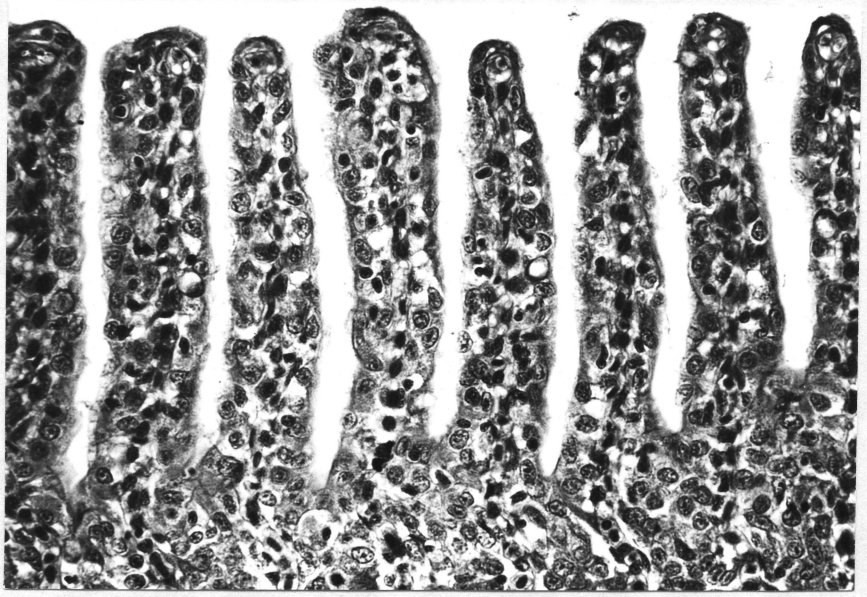


Fig. 14. Secondary lamellae of gill of fish which had received a ration containing 16 ppm Hg as methyl mercury chloride for 70 days. Swelling and hyperplasia of epithelial cells.
H & E X 375

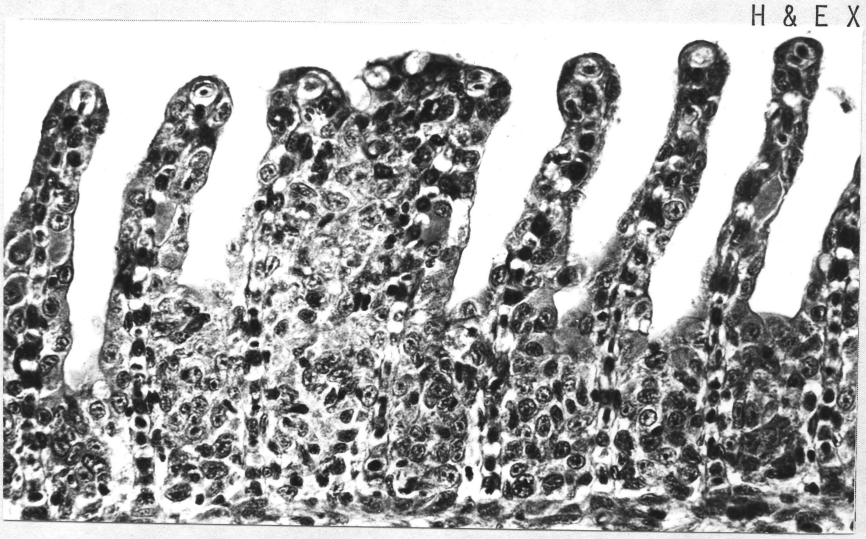


Fig. 15. Secondary lamellae of gill of fish which received a ration containing 24 ppm Hg as methyl mercury chloride for 56 days. Epithelial cell swelling with fusion of epithelial layers of adjacent lamellae.
H & E X 375

TABLE 21. Incidence of lesions in the epithelium of the secondary lamellae of the gills of rainbow trout fed diets containing various amounts of methyl mercury chloride over a 15 week period.

Group (Diet Hg ppm)	Epithelial lesion	Week 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
I (4)	Clubbing[a]			+		+		+		+	+	+	+	+	++	c
	Fusion[b]										+					
	Hyperplasia											+		+	+	
II (8)	Clubbing	+	+	++	+		+	++	+	+	+	++	++	+	+	c
	Fusion															
	Hyperplasia		+					++	+	+	+	+	++		+	
III (16)	Clubbing				+	++	++	+	++	++	++	++	+	+[d]	++	++
	Fusion						+								+	
	Hyperplasia				+	+	++	+	++	++	++	+	+	+	++	++
IV (24)	Clubbing			++	++	++	++	++	++	++	+	++	++	++	+[d]	+[d]
	Fusion							+	+							
	Hyperplasia				++	++	++	++	++	++	+	++	++	++	+	+

[a] - Clubbing - cellular swelling, and hyperplasia restricted to the tips of lamellae.

[b] - Fusion - fusion of epithelial surfaces of adjacent lamellae.

[c] - No fish examined.

[d] - Only one fish examined.

kidney, pancreas, distal intestine, brain, spinal cord, or in cranial or spinal nerve roots and dorsal root ganglia.

6.4 Discussion

Miettinen et al. (1970b) estimated on the basis of peroral administration of MeHg over a 2 day period that with "fast dosing" the LD_{50}/30 days of MeHg for pike and rainbow trout was of the order of 15 mg/kg body weight. However, they stated that the toxicity of MeHg for rainbow trout appeared to vary greatly with the rate of administration, and speculated that the LD_{50}/30 days was less than fish could tolerate over months of chronic intake. The present experiment supports this view.

Miettinen et al. (1970b) felt that their results agreed well with data from Japan (Kitamaru, 1968), which indicated that 10 to 20 ppm Hg in muscle of fish was associated with toxicity for the fish. Kitamaru (1968) reported that the Hg concentration in 14 fish of seven species described as, "enfeebled fishes floating over the surface of seawater;. . ." ranged from 1.09 to 24.1 ppm. The mean Hg content of these fish was 9.8 ppm. It is unclear which tissues were analyzed; however, it was stated that, "within the fish body, most of the mercury was found in the liver, followed by other viscera and meat." This suggests that the values given were for the entire fish, in which case muscle levels alone would likely be even lower. Takeuchi (1968a) stated that Hg poisoning occurred in fish in Minamata Bay, and described the presence of cataracts and neuronal degeneration and loss, particularly in the cerebellum of these fish. Unfortunately

no information was presented on the numbers or species examined, or on the Hg content of these fish.

In direct contrast to the Japanese work which suggested that Hg concentrations from 1.09 to 24.1 ppm were associated with toxicity, is the work of Lockhart et al. (1972) who captured northern pike from a highly contaminated lake, removed muscle biopsies for Hg analysis and then transferred these fish to an uncontaminated lake. The Hg concentration in the muscle of these fish varied from 6.82 to 13.75 ppm at the time of transfer. These fish were recaptured for up to 1 year after transfer, and during this year Hg concentrations had declined only 30 per cent. The fact that these fish survived for a year with Hg concentrations similar to, and in some cases, higher than those in some of the Japanese fish indicated that these concentrations were not critical levels for this species. A further group of northern pike with muscle concentrations of from 6.29 to 16.0 ppm Hg had no macroscopic evidence of disease with the exception of some emaciation, and no histopathologic evidence of disease was seen except "reduced fat storage" in the liver (Lockhart et al., 1972). This evidence plus that of the present experiment suggests that the association of disease with the tissue levels of Hg reported in the Japanese fish may have been spurious.

Because of the discrepancies between the results of the present study and those of Miettinen et al. (1970b) a very limited attempt was made to duplicate their work. A number of rainbow trout were dosed via stomach tube with an aqueous solution of MeHgCl at doses to supply 5, 10, 15, and 20 mg Hg/kg. Food coloring had been added to the

Hg solution to aid in the detection of vomition which had been reported by Miettinen et al. (1970b). This method was discarded because vomition was so consistent that no estimation of Hg retention was possible. In an attempt to produce acute poisoning, rainbow trout measuring between 12 and 17 cm were injected with an amount of a 1 mg Hg/litre solution of MeHgCl to provide 10, 15, and 20 mg Hg/kg body weight. Five fish were used at each level. These fish either died or were sacrificed when moribund between 12 and 22 hr post injection. The histopathologic lesions in gills, posterior kidneys and pseudobranchiae were somewhat similar to those reported by Miettinen et al. (1970b). In the fish receiving 10 mg Hg/kg the renal tubules and Bowman's spaces were widely dilated (Fig. 16). Miettinen et al. (1970b) had described ". . .oedema in the glomeruli and tubules" of pike. Also present in the dilated proximal tubules of these fish were numerous small, circular, eosinophilic, hyaline bodies (Fig. 17), similar to the structures described in the tubules of rats given MeHgCl (Fowler, 1972a).

Fish which received 15 and 20 mg Hg/kg had hydropic degeneration of tubular epithelium (Fig. 18) and scattered foci of tubulonecrosis (Fig. 19). Fish in all groups had severe gill damage with swelling and necrosis of epithelial cells, and separation of the epithelium from the lamellae and filament (Fig. 20). Inflammation of the pseudobranchiae as described by Miettinen et al. (1970b) was not observed, but the epithelial cells were swollen and occasionally showed ballooning degeneration (Figs. 21, 22).

These very limited results indicate that the rate of intake of MeHg has a great influence on the toxicity and morphologic damage

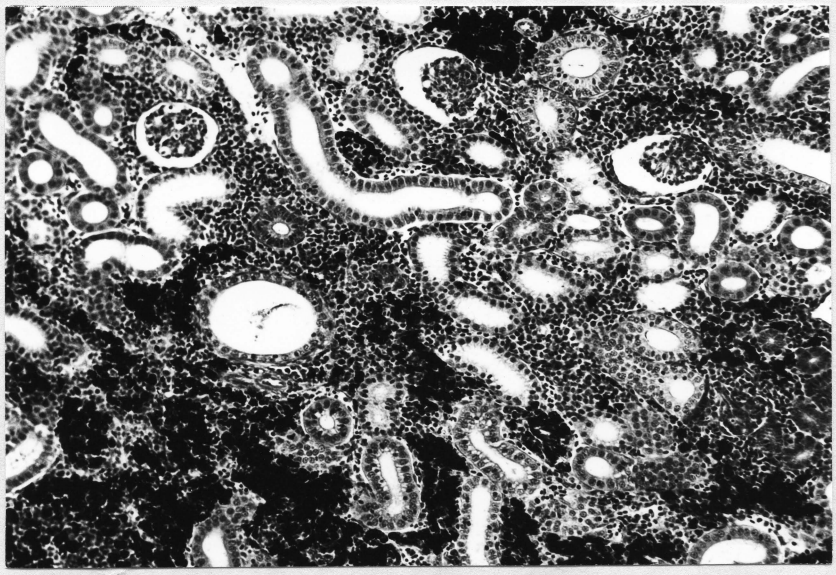


Fig. 16. Posterior kidney of fish which received 10 mg Hg/kg as methyl mercury chloride by intraperitoneal injection. Dilation of tubules and Bowman's space.

H & E X 144

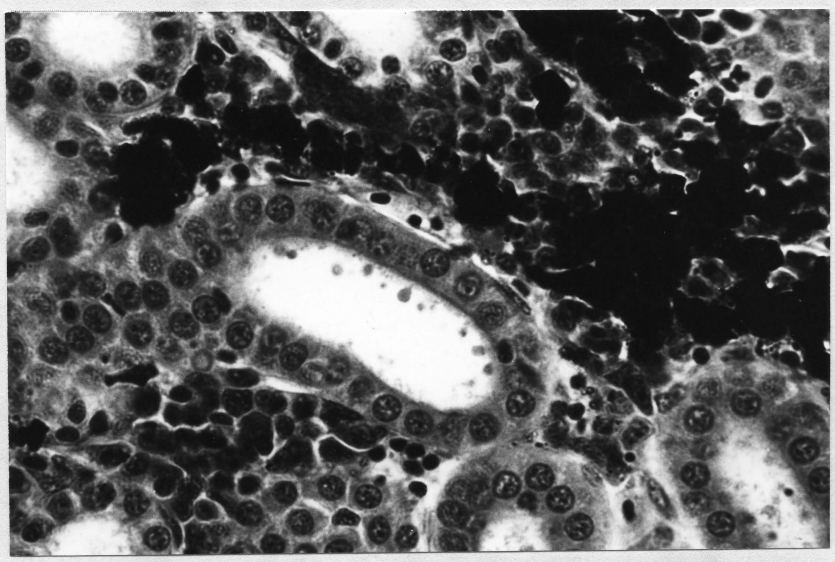


Fig. 17. Posterior kidney of fish which received 10 mg Hg/kg as methyl mercury chloride by intraperitoneal injection. Numerous eosinophilic, hyaline bodies in lumen of proximal convoluted tubule.

H & E X 620

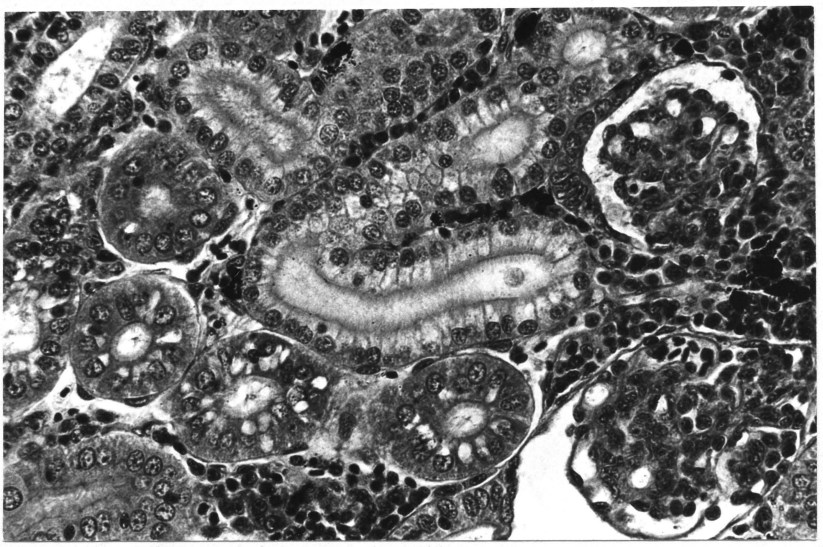


Fig. 18. Posterior kidney of fish which received 20 mg Hg/kg as methyl mercury chloride by intraperitoneal injection. Hydropic degeneration of tubular epithelium.

H & E X 490

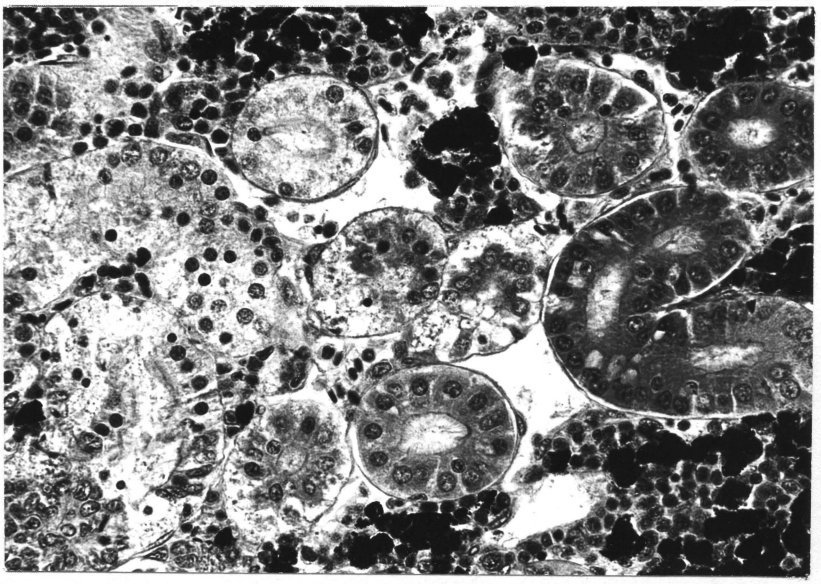


Fig. 19. Posterior kidney of fish which received 20 mg Hg/kg as methyl mercury chloride by intraperitoneal injection. Hydropic degeneration and necrosis of epithelium of proximal convoluted tubules.

H & E X 490

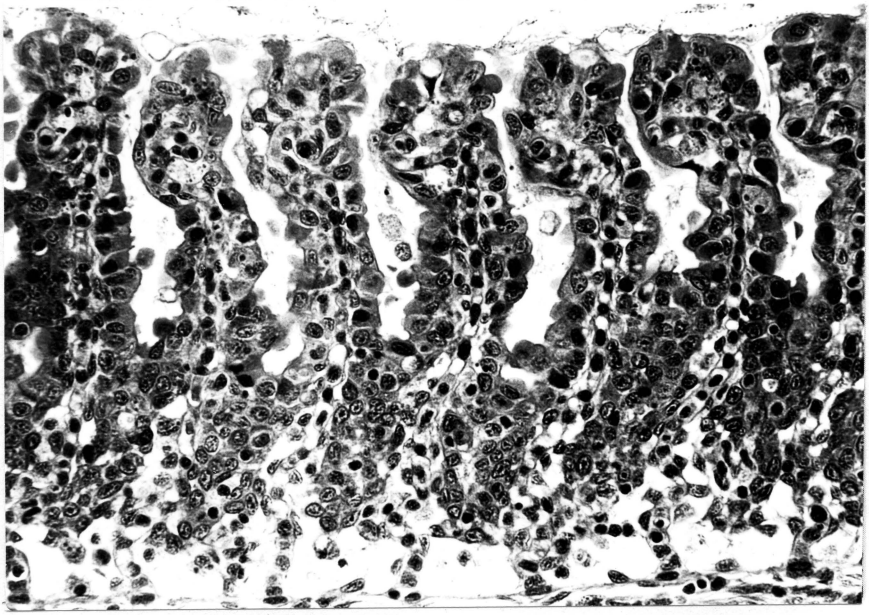


Fig. 20. Secondary lamellae of gill of fish which received 20 mg Hg/kg as methyl mercury chloride by intraperitoneal injection. Swelling and necrosis of epithelial cells with separation of the epithelium from the interlamellar portion of the filament.

H & E X 375

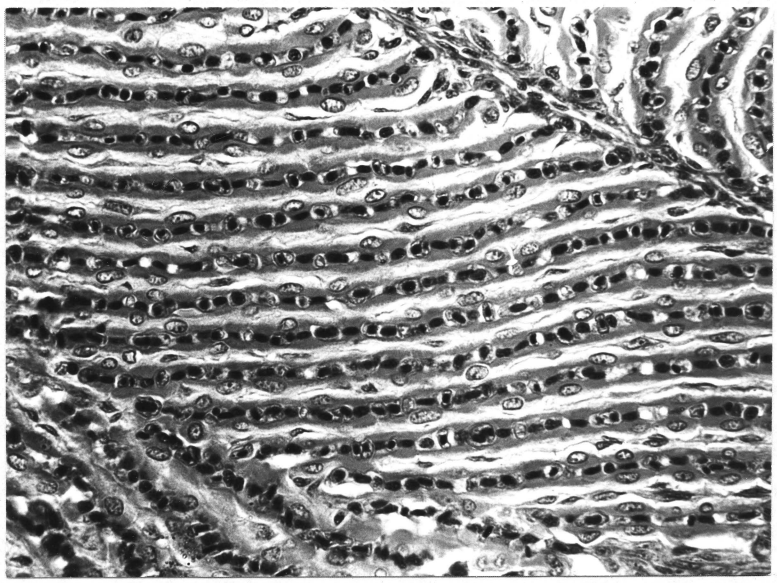


Fig. 21. Pseudobranchial organ of control fish.
H & E X 500

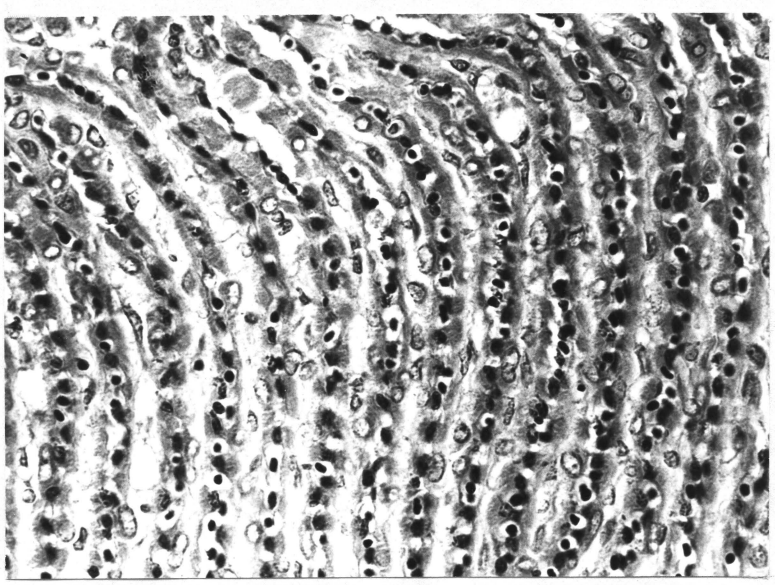


Fig. 22. Pseudobranchial organ of fish which received 20 mg Hg/kg as methyl mercury chloride by intraperitoneal injection. Swelling and disruption of the epithelial layer with ballooning of occasional cells.
H & E X 500

produced by Hg. It is interesting that Smith and Piper (1972) have described almost identical lesions in the pseudobranchiae and kidneys, and similar lesions in the gills of rainbow trout exposed to high concentrations of formalin for 1 hr, indicating that the lesions have little specificity.

The rate of accumulation of Hg in muscle tissue of fish in the present experiment was rather similar to that found by Rucker and Amend (1969). In the present case approximately 65 to 85 days were required to reach parity with the diet. In Rucker and Amend's (1969) study, chinook salmon were fed rainbow trout fingerlings containing, on the average, 3 ppm Hg for 30 days. Muscle of the salmon contained 1.9 ppm Hg (or about 63 per cent of the dietary level) after 30 days.

The highest concentrations found in individual fish in group I were similar to the average concentrations of all fish collected from the most highly contaminated region of the Saskatchewan River in experiment I. The highest mercury concentration in an individual fish in group II (13.0 ppm) was higher than that found in any of the fish from the Saskatchewan River. The Hg concentrations in many of the fish in groups III and IV were much higher than in any from the Saskatchewan River. These levels were not associated with clinical signs of intoxication and only with minor disturbances in blood PCV and histopathologic evidence of hyperplastic gill disease. There are relatively few data available on the hematologic response of fish to various intoxications; however, an increase in PCV has been reported in association with hypoxia, i.e., low oxygen in the environment

(Hall et al., 1926; Hall, 1928; Prosser et al., 1957). Smith and Piper (1972) noted a rise in PCV in rainbow trout poisoned acutely with formalin, and associated this with gill damage and hypoxia. In the present study the PCV values were slightly higher than control in the latter portion of the experiment with group II, and significantly higher than control in both groups III and IV. These were also the fish in which hyperplasia of gill epithelium was most obvious. The mean PCV of fish in groups III and IV were significantly higher than that of the control group, while the mean TP values did not differ significantly, suggesting that dehydration was not a factor.

The absence of neurologic lesions in the fish was unusual in light of the established neurotoxicity of MeHg in other vertebrate groups. Takeuchi (1968a) described lesions in the brains of fish from Minamata Bay, Japan; however, as indicated earlier, the etiology of these lesions seems somewhat doubtful. Miettinen et al. (1970b) did not detect any morphologic injury in the nervous system of MeHg poisoned fish.

The response of the teleostean nervous system to injury has received little attention, and most of the reports available deal with the regenerative ability following mechanical injury. In general, it appears that teleosts have at least some ability to regenerate damaged nervous tissue (Segaar, 1965; Bernstein and Bernstein, 1967) and formation of new neurons has been reported in some species (Segaar, 1965; Bernstein and Sadlach, 1969). The ability for regeneration after injury does not appear to have been investigated in salmonids; however, regenerative capacity is inversely related to

phylogenetic advancement (Bernstein, 1967) and salmonids are considered to be rather primitive fish (Young, 1962), so that one might expect some degree of regenerative capacity to exist. While the ability to regenerate damaged nervous tissue might be of advantage for fish exposed to a neurotoxin, there was no evidence of any injury to the nervous system of fish in the present study. It seems more likely that the Hg concentration in nervous tissue did not reach toxic levels.

Vertebrates at various evolutionary levels may show a marked difference in vulnerability to noxious agents, for example, the LD_{50} of a pesticide may vary by a factor of 10 among birds (Tucker and Haegele, 1971), and species variation in LD_{50} of 1,000-fold are known among vertebrates (Hodge, 1965). Similarly Olsson et al. (1972) found that an elasmobranch (Ginglymostoma cirratum) was unaffected by massive doses of gamma radiation to the brain, while rats given similar exposure died with extensive lesions in the brain.

Other factors which might have influenced the toxicity of MeHg in this case were the water temperature and the DO concentration in the water. Renwoldt et al. (1972) found that the toxicity of Hg for fish was increased as the water temperature rose. Perhaps the cold water temperature (10 $\pm$ 0.7C) used in this experiment had some protective effect. Lloyd (1961) found that low DO concentrations in water increased the toxicity of many poisons, including the metals lead, zinc, and copper for fish. Amend et al. (1969) found that low DO concentrations increased the mortality rate after exposure to $EtHgPO_4$. Low DO content in the water would compound the effects of any toxin which exerts its toxic action on the gill epithelium and interferes with the respiratory function of this organ.

The well aerated water used in this experiment may have had a protective effect, and the results may not be directly applicable to polluted waters which often have low DO concentrations.

7.0 EXPERIMENT IV. THE USE OF MERCURY CONTAMINATED FISH AS FOOD FOR RANCH MINK

7.1 Rationale

Little information was available in the literature dealing with the effects of MeHg on piscivorous animals. This lack of knowledge was of concern because freshwater fish form a large part of the diet of ranch mink in many areas of Canada, and the possibility of Hg poisoning exists. Ranch mink were also considered as a method for the disposal of large quantities of Hg contaminated fish. For these reasons it was considered important to determine the safety of such fish as a dietary constituent for ranch mink. An attempt was made in this experiment to duplicate normal mink ranch management as closely as possible.

7.2 Materials and methods

7.2.1 Experimental design

Twenty-five adult female mink of the pearl color phase and their litters (approximately 1 month of age) were purchased from a local mink rancher. These mink were moved to an unused area of the mink ranch, and the rancher was responsible for watering and caring for these mink in the normal manner used on the ranch. The mink were divided into three groups: group I contained five females and 19 kits,

group II contained 10 females and 34 kits, and group III contained 10 females and 29 kits.

All mink were marked by toe-clipping, using a pattern similar to that proposed by Baumgartner (1940). Each family group was initially maintained in a single cage; later after weaning of the kits the litters were divided, and the young mink were placed either singly or two together in a pen.

The mink in group I served as controls and received the normal ranch ration which was prepared twice weekly. The exact composition of this ration varied somewhat over the period of the study depending upon the availability of ingredients. Its approximate composition per 100 kg was:

Chicken offal	50 kg
Beef tripe and offal	30 kg
Cereal	10 kg
White-tailed jack rabbit carcasses[1]	5 kg
Fish[2]	5 kg

This ration contained 0.5 per cent salt (NaCl). Several samples of this ration were analyzed for Hg content over the experimental period, and the content was found to be consistently less than 0.1 ppm Hg.

The Hg contaminated fish used in this study were freshwater drum (also called sheepshead) from Lake Winnipeg, Manitoba. The fish was supplied in a ground, frozen form by the Freshwater Institute,

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[1]Obtained from a fur buyer and frozen during the winter months, thawed as needed during the remainder of the year.

[2]Locally called "tulibee" (correctly named Cisco), obtained from Last Mountain Lake, Saskatchewan.

Fisheries Research Board of Canada, Winnipeg, Manitoba. Pooled samples of this fish contained 0.44 ± .02 ppm Hg (C.K.C. Tam, 1970, personal communication). This material was maintained frozen until used in the rations for groups II and III. The composition of these rations per 100 kg is shown below:

	Group II	Group III
Fish	50.0 kg	75.0 kg
Cereal	5.0 kg	7.5 kg
Normal ranch ration	44.7 kg	17.1 kg
Salt (NaCl)	0.3 kg	0.4 kg

These rations were prepared fresh weekly and thoroughly mixed with a large electric mixer[1]. Thereafter the rations were divided into amounts required for daily feeding, packaged in heavy plastic bags, and frozen until required for feeding. Food coloring dye was added to the rations to identify the feed.

All mink were fed once daily on the cage wire in slight excess of consumption. Food left on the wire from the previous day was removed prior to the daily feeding. Water was supplied ad libitum. The experimental period extended from 15 June, 1970, to 1 November, 1970.

7.2.2 Sampling

Group I. One female mink and three to six juveniles were sacrificed at 30 day intervals beginning 30 days after being placed on the ration.

Groups II and III. One female and three to six juveniles from each group were sacrificed at 15 day intervals beginning 30 days after

[1]Hobart Manufacturing Co., Ltd., Toronto, Ontario.

being placed on the experimental rations. In one instance in each of groups II and III, two female adults were sacrificed at one time because of lacerations due to fighting. On the final date, 1 November, 1970, the remaining two females in each group were sacrificed.

All mink were euthanitized by the intraperitoneal injection of a saturated solution of sodium pentobarbitone[1]. The mink were weighed and a necropsy was performed. The brain was removed by skinning the head, dissecting away the dorsal musculature, and carefully removing the calvarium with an electric saw[2]. The spinal cord was collected by dissecting away the dorsal musculature, and then carefully removing the dorsal arches of the vertebrae with fine bone forceps.

The brain of the adult and one juvenile mink at each collection date were sectioned mid-sagitally and one-half together with portions of liver and kidney were placed in individual plastic bags, frozen, and retained for Hg analysis. The other half plus the spinal cord and the entire brain and spinal cord from the other mink in each group together with portions of liver, kidney, stomach, colon, spleen, lung, myocardium and mesenteric lymph node, were immersed in 10 per cent neutral buffered formalin.

After approximately 10 days fixation, the brain was sectioned transversely at 5 mm intervals and these slices together with transverse sections of the cervical, thoracic and lumbar spinal cord,

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[1] 453.6 g pentobarbitone sodium (U.S.P.)
1000.0 ml absolute ethyl alcohol
400.0 ml distilled water

[2] Stryker Autopsy Saw, No. 8128, Lipshaw Manufacturing Co., Detroit, Mich., U.S.A.

and sections of the other organs were embedded in paraffin, sectioned at 6μ, and stained with H & E.

Mercury analyses on liver, brain, and kidney tissues were performed as outlined for experiment I.

7.3 Results

7.3.1 Clinical findings

No clinical signs of disease were observed in any of the mink within the experimental period. No mortality occurred which could be associated with the feeding of Hg contaminated fish. One adult female in the control group (group I) died of nursing sickness 7 days after the start of the experiment, and one juvenile male in group III died on day 35. The probable cause of death was heat stroke; however, cannibalism prevented necropsy. One juvenile mink from each of groups I and II escaped and were not recovered.

There appeared to be no impairment of growth of the juvenile mink in the principal groups, and in general, mink receiving the high fish rations were heavier than controls of the same sex and of similar age (Table 22).

7.3.2 Accumulation of mercury in tissue

The Hg concentrations in the tissues of mink in groups II and III were higher than those in group I (Tables 23, 24, 25). The concentrations of Hg in the liver and kidney increased more rapidly than did those in brain tissue. Although the analytic data are limited they suggest that Hg concentrations rose rapidly in liver and kidney during the early portion of the study and then became rather

TABLE 22. Body weight at the time of sacrifice of mink in groups I, II, and III fed rations containing 0, 50, and 75 per cent of Hg contaminated fish respectively.

			Weight (g) Days on trial							
Group	Age	Sex	30	45	60	75	90	105	120	145
I	adult	female	720		750		770		1000	
	juvenile	female			610		800		865	
					700		760		900	
							675		865	
	juvenile	male	790		1020		1070		1200	
			740				1245		1610	
			810						1640	
			750							
II	adult	female	755	670	740	615	710	1020	800	810
							1070			920
	juvenile	female	590	570	750	950	1020	790	850	925
			710	550	825	865	1050	805	940	975
								775		870
	juvenile	male	895	850	1030	1780	1975		1405	1920
			815	825	1265	1220	1520		1750	1505
III	adult	female	725	680	710	730	970	860	780	815
								895		700
	juvenile	female	615	640	750	740	880	1095		880
					730	855	915			810
	juvenile	male	895	940	1425		1600	1210	1950	1740
			990	1070				1720	1905	1535
			825	1035					1450	
									1595	

TABLE 23. Mercury (ppm) in the liver of mink in groups I, II, and III fed rations containing 0, 50, and 75 per cent of Hg contaminated fish respectively.

		Days on trial						
Group	Age	30	45	60	75	90	105	120
I	adult	0.7		--		0.7		0.5
	juvenile	0.2		0.2		0.3		0.2
II	adult	2.1	2.7	3.9	3.9	5.7	2.2	4.1
	juvenile	0.7	1.1	1.8	3.4	4.0	2.8	2.6
III	adult	4.9	4.9	6.6	2.4	6.8	7.8	4.2
	juvenile	1.2	2.2	6.2	4.8	2.9	3.4	2.2

TABLE 24. Mercury (ppm) in the kidney of mink in groups I, II, and III fed rations containing 0, 50, and 75 per cent of Hg contaminated fish respectively.

		Days on trial						
Group	Age	30	45	60	75	90	105	120
I	adult	0.3		--		0.7		1.0
	juvenile	0.2		0.1		0.4		0.3
II	adult	2.0	3.1	3.4	3.3	4.5	2.4	2.6
	juvenile	0.6	1.0	1.4	2.3	2.9	2.5	1.5
III	adult	4.7	4.4	4.5	3.6	6.4	6.5	2.9
	juvenile	0.9	1.8	4.1	3.9	4.4	3.1	2.1

TABLE 25. Mercury (ppm) in the brain of mink in groups I, II, and III fed rations containing 0, 50, and 75 per cent of Hg contaminated fish.

		Days on trial						
Group	Age	30	45	60	75	90	105	120
I	adult	0.1		--		0.1		0.3
	juvenile	--		0.4		0.1		0.1
II	adult	0.6	0.9	3.2	1.0	1.1	0.4	0.5
	juvenile	0.2	0.3	0.4	0.6	0.7	0.5	0.5
III	adult	1.2	1.5	1.4	0.8	1.7	2.5	3.4
	juvenile	0.4	8.3	7.2	1.0	0.9	0.7	2.6

stationary. During the first 60 days adult mink had substantially more Hg in their tissues than did juveniles but the difference became less apparent later in the study.

7.3.3 Pathology

No gross or histologic lesions suggestive for Hg poisoning were detected in any of the mink. One adult female from group I had several granulomatous lesions present in the lungs. These areas contained acid-fast bacilli and a diagnosis of tuberculosis was made.

7.4 Discussion

Diets containing 50 and 75 per cent, respectively, of fish containing 0.44 $\pm$ 0.2 ppm of Hg appeared to have no adverse affects upon mink during the course of this experiment.

The distribution of Hg among liver, kidney, and brain tissue of the mink in the principal groups was similar to that reported in MeHg poisoned ferrets (Hanko et al., 1970). The maximum Hg concentrations found in the tissues of mink were approximately one order of magnitude lower than the values reported in poisoned ferrets (Hanko et al., 1970). Borg et al. (1970) stated that the fatal brain level of MeHg in ferrets was of the order of 30 to 40 ppm. The levels of Hg found in the brain tissue of two animals in group III (7.2 and 8.3 ppm respectively) seem to be anomalous, particularly when compared to the concentrations in liver and kidney of the same animals. The findings suggested sample contamination with Hg prior to analysis, but no explanation for how this might have occurred was found.

The analytic data were limited, and variations between individual mink in the same group were very great, so that only a suggestion can be made that an equilibrium between intake and excretion of Hg was reached during the experimental period. The percentage of total body burden of alkyl Hg which is excreted each day has been calculated for several mammals. The elimination rate ranged from 8.3 to 10.8 per cent/day for mice (Ulfvarson, 1970), to 3 per cent/day for the rat (Ulfvarson, 1962, 1970) and to less than 1 per cent/day for man (Aberg et al., 1969; Miettinen et al., 1971). Although the daily Hg intake of these mink was not known, given the low Hg content of the ration it appears that equilibrium could be achieved.

Adult mink initially accumulated Hg at a more rapid rate than did the juveniles. This was likely the result of higher food intake by the females during the period in which the young were partially dependent upon milk, and also the rapid growth of the young mink would have a "diluting" effect upon Hg concentrations in their tissues.

On the basis of this study it appears likely that fish containing concentrations of Hg similar to those used, could be used as a ration ingredient for ranch mink. The feeding of such fish should probably be confined to relatively short periods of time, possibly during the growth and furring-out of young mink, and not used for extended periods of time without studies of the long term effects of low Hg intake.

8.0 EXPERIMENT V

EXPERIMENTAL METHYL MERCURY INTOXICATION IN MINK

8.1 Rationale

The levels of Hg present in the diets used in experiment IV were insufficient to produce clinical or histopathological evidence of intoxication in mink during the experimental period. A further experiment was required to establish the pathology of MeHg intoxication in mink and to correlate clinical signs and pathologic lesions to dose, length of exposure, and Hg concentrations in tissue.

8.2 Materials and methods

8.2.1 Experimental design

Thirty adult female mink of the pearl color phase were obtained from the same source as those in experiment IV. The mink were randomly assigned to one of six groups of five mink each. All mink were weighed, and placed in individual wire cages which measured 72 x 40 x 45 cm. The mink were fed in slight excess once daily on the cage wire. Water was supplied ad libitum. While it would have been desirable to know the precise feed intake of each mink this was impractical. Only small amounts of feed were consumed at any one time and some feed was lost through the cage floor. In addition, the feed tended to dry on the wire so that the moisture content of

feed remaining at any time varied.

The mink were examined in the cages several times each day, and periodically were removed from the cages individually and allowed to run about the room, so that gait and behavior could be more adequately assessed. Daily food consumption was recorded as normal, less than normal or absent.

A manufactured complete mink food[1] was used as the basal ration for all groups. The control group (group I) received this ration, while the principal groups received the same ration to which MeHgCl had been added at the concentrations shown below:

Group	Hg (ppm)
II	1.1
III	1.8
IV	4.8
V	8.3
VI	15.0

These rations were mixed thoroughly with an electric mixer[2] in a fume hood. The prepared rations were sealed in plastic containers, placed within plastic bags, and frozen until needed.

Two mink from each group were allowed to die of intoxication or were euthanitized at the termination of the experiment (93 days) in the case of groups I and II. These mink were necropsied as shortly

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[1]Pelsifood. Trouw of Canada, Ltd., P.O. Box 370, Seaforth, Ontario.

[2]Model A200D, Hobart Manufacturing Co. Ltd., Toronto, Ontario.

after death as possible and tissues were collected for histopathologic examination as in experiment IV. The brains of these animals were sectioned mid-sagitally and one-half, together with portions of skeletal muscle, liver, kidney, and a quantity of fur from the dorsal aspect of the body, were placed in individual plastic bags and frozen until analyzed for Hg.

The remaining three mink in each group were sacrificed when showing obvious clinical signs (group III to VI), or at the termination of the experiment in the case of groups I and II. These animals were anaesthetized by the intraperitoneal injection of Nembutal (pentobarbitol sodium)[1] and placed in dorsal recumbency. The thorax was opened by a mid-ventral incision, the pericardium was incised, and the heart exposed. A 14 guage, 5 cm hypodermic needle was inserted into the left ventricle and positioned through the aortic valves, so that the tip was present in the ascending aorta. An additional quantity of anaesthetic was then injected through this needle, and an infusion apparatus was connected. Prior to the commencement of infusion the right ventricle was incised to allow exsanguination from the venous sytem. A solution composed of 210 ml of heparinized saline and 40 ml of 10 per cent neutral buffered formalin was infused until fluid leaving the right ventricle contained very little blood. The amount used varied from 120 to 180 ml. Following exsanguination,

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[1]Abbot Laboratories Ltd., Montreal Quebec.

approximately 300 ml of 10 per cent neutral buffered formalin was infused. All solutions were infused from a height of approximately 60 cm. The brain and spinal cord, together with the Gasserian (semi-lunar) ganglia and portions of liver, kidney, spleen, mesenteric lymph node, stomach, duodenum, colon, lung, and the sciatic nerves were immersed in 10 per cent neutral buffered formalin.

Specimens for histopathology were processed as previously described, sectioned at 6μ and stained with H & E. Luxolfast blue stain for myelin, Holzer's stain for astrocytes and Bielchowsky's stain for axons as modified for paraffin sections were also employed selectively.

After fixation, one sciatic nerve from each mink was processed by the method of Walsh (1970). This involved immersion of the nerve in a 1 per cent solution of osmium tetroxide for 24 hr and then washing and maceration in a mixture of two parts glycerol and one part water for 36 hr. The nerve fibers were separated by teasing and small groups of well separated fibers were mounted on slides in Farrant's medium[1].

Specimens for Hg analysis were processed and analyzed by the methods previously described in experiment II.

8.3 Results

8.3.1 Clinical findings

Table 26 shows the onset and clinical course of intoxication in the principal groups. The rapidity of onset of clinical signs was

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[1]The British Drug Houses Ltd., Toronto, Ontario.

TABLE 26. Timing of occurrence of clinical signs and of death in mink fed rations containing various amounts of methyl mercury chloride.

Group	Hg in ration (ppm)	Animal Number	Onset of Clinical Signs (days)		
			Anorexia	Ataxia	Death
II	1.1	1			93[a]
		2			93[b]
		3			93[a]
		4			93[b]
		5			93[a]
III	1.8	1	51	--	59[b]
		2	64	78	91[b]
		3	51	60	93[b]
		4	51	60	85[b]
		5	59	78	79
IV	4.8	1	23	28	29[b]
		2	23	31	36[b]
		3	--	--	26[b]
		4	25	32	36[b]
		5	25	31	36[b]
V	8.3	1	20	21	23[b]
		2	16	18	19
		3	18	20	23[b]
		4	17	21	26[b]
		5	19	20	22[b]
VI	15.0	1	--	--	18
		2	16	17	19[b]
		3	18	18	20[b]
		4	17	18	20[b]
		5	18	18	19[b]

[a] - Sacrificed, clinically normal.

[b] - Sacrificed, clinical signs of intoxication present.

directly related to the Hg content of the diet.

8.3.1.1 Group I (control)

All animals remained clinically normal during the experimental period.

8.3.1.2 Group II (1.1 ppm Hg in feed)

Food consumption in this group remained normal during the experiment. The only clinical sign noted was a slight tendency for two of the animals to move more slowly than normal during the last 3 days of the experiment. No deaths occurred.

8.3.1.3 Group III (1.8 ppm Hg in feed)

Partial anorexia was observed in three of the mink on day 51. After this time, feed intake declined in all of the mink. Animal III-1 was found dead on day 59. No premonitory signs other than partial anorexia had been noted. Slight posterior ataxia was observed in two mink (III-3 and 4) on day 60. By day 74, mink III-4 moved very slowly and when placed on a smooth tile floor the rear legs "splayed" to the side, similar to the posture shown in Figure 23. This mink appeared to be very inquisitive and totally unafraid of the investigator. No clinical signs other than decreased food intake were observed in animals III-2 and 5, until day 78. At that time slight posterior ataxia was observed. Animal III-5 died the following day. The remaining animals (III-4, 2, and 3) were sacrificed on days 85, 91 and 93, respectively, at which time all animals exhibited marked posterior ataxia.

Fig. 23. Mink (group V, day 23) showing "splaying" of the hind legs.

Fig. 24. Mink (group IV, day 35). Animal had fallen to lateral recumbency while walking and was unable to right itself.

8.3.1.4 Group IV (4.8 ppm Hg in feed)

Reduced food intake was observed in two mink on day 23 and one of these mink seemed listless and indifferent on the following day. On day 25 three of the mink showed reduced food consumption and all seemed listless. Animal IV-3 was found dead on day 26 without any premonitory signs having been seen. Mild posterior ataxia, and a stilted "shuffling" gait was first noted in one mink on day 28, and by the following day this animal was markedly ataxic and a clonic convulsion of approximately 1 minute duration occurred when the animal was handled. This animal died on day 29. Mild posterior ataxia and a shuffling gait with the tail raised were noted in two of the remaining animals on day 31 and in the final animal on day 32. The ataxia became more pronounced over the following 3 days, and the animals would often fall to lateral recumbency while walking (Fig. 24). One of these animals showed evidence of dysphonia, the vocalization being higher-pitched, more irregular, and "rougher" than normal. These animals were sacrificed on day 36.

8.3.1.5 Group V (8.3 ppm Hg in feed)

Clinical signs observed were similar to those in group IV, however, the onset was more rapid and the clinical course was shorter.

Clonic convulsions of 1 to 2 minutes' duration accompanied by chewing and salivation occurred in two mink when they were handled. Fine head tremors were observed in two mink. Vomition after eating was noted in one mink on day 20 and dysphonia as previously described was noted in one mink on day 25. Mink showing ataxia exhibited a splayed-leg posture when placed on a smooth tile floor and moved with

a stilted shuffling gait, and often fell into lateral recumbency when placed on surfaces which provided better traction. Vision in these mink was difficult to assess because of the indifference of the mink toward their environment; however, the mink which survived for 26 days appeared to be blind at that time, in not making any attempt to avoid objects placed in its path.

8.3.1.6 Group VI (15.0 ppm Hg in feed)

Clinical signs were essentially as seen in group V. These animals ran an extremely short clinical course, progressing from a normal state to one of lateral recumbency in only 1 to 2 days.

Periodic, spontaneous, clonic convulsions accompanied by chewing and salivation were observed in three of the mink. Fine head tremors, as previously described, and pronounced coarse lateral movements of the head were observed. These animals would often lie in ventral or lateral recumbency and make swimming motions with their limbs, and circling was common in those that could walk.

8.3.2 Weight changes

Mink in group I (controls) weighed virtually the same at the end of the experimental period as at the beginning. This was somewhat unusual since food was supplied in excess; however, the mink were in good condition when purchased and further weight gain might not have been expected. Mink in all of the principal groups lost a considerable amount of weight over the course of the experiment (Table 27).

TABLE 27. Weight of mink at the beginning of trial period and at the time of necropsy.

Group	Hg in ration (ppm)	Animal number	Body weight (g)	
			Initial	Necropsy
I	<0.1	1	1340	1330
		2	990	1020
		3	1080	1090
		4	1090	840
		5	960	960
II	1.1	1	980	840
		2	1020	810
		3	930	780
		4	1200	730
		5	910	790
III	1.8	1	980	870
		2	1080	810
		3	1070	830
		4	1260	840
		5	840	570
IV	4.8	1	840	760
		2	1070	800
		3	900	840
		4	960	840
		5	1020	940
V	8.3	1	960	810
		2	960	800
		3	960	800
		4	1320	1180
		5	900	730
VI	15.0	1	1080	900
		2	960	720
		3	900	720
		4	1020	870
		5	1090	900

8.3.3 Accumulation of mercury in tissue

The Hg concentration in the tissue of the two mink which were allowed to die in groups III, IV, V, and VI; and the two mink sacrificed without being infused with formalin in groups I and II are shown in Table 28. In general, the Hg concentrations in the tissues of mink which died were similar despite differences in the Hg content of the diet and time to death. Mercury concentrations in brain and muscle tissue were rather similar, and lower than those in liver and kidney. The Hg content of the fur of mink in Groups V and VI was similar to the control group. The mink which survived longer (groups II, III, and IV) had somewhat higher levels of Hg in their fur.

The low concentration of Hg in the brain of mink III-1 which died after 59 days is difficult to explain. The level in muscle was similar to that in brain, but the concentrations in liver and kidney were similar to those of mink with much higher concentrations in the brain.

The mean concentration of Hg (ppm) in the tissues of mink which died were: kidney, 23.1; brain, 11.9; muscle, 16.0; liver, 24.3. The Hg concentrations in the tissues of mink in group II which were clinically normal when sacrificed were similar to, and in some instances, higher than those in individual mink which died.

8.3.4 Gross pathology

Mink in the principal groups had less adipose tissue than those in group I; however, all mink had some remaining adipose tissue. The liver of all mink in groups III to VI and of two mink in group II were paler than normal, with a variable yellow color. The digestive tracts

TABLE 28. Mercury concentration in the tissues of mink which received rations containing various amounts of methyl mercury chloride.

Group	Hg in ration (ppm)	Animal Number	Hg (ppm)				
			Liver	Kidney	Brain	Muscle	Fur
I	<0.1	3[a]	0.7	1.0	0.2	0.3	1.1
		5[a]	0.2	0.5	0.1	0.1	0.7
II	1.1	3[b]	30.2	21.2	7.1	7.1	1.8
		5[b]	20.5	23.6	9.3	8.5	1.8
III	1.8	1[c]	21.3	26.7	4.1	4.9	--
		5[c]	21.3	17.9	12.2	--	2.3
IV	4.8	1[c]	23.0	26.7	12.3	20.5	1.7
		3[c]	18.1	17.9	8.6	7.8	1.7
V	8.3	2[c]	37.2	23.2	14.5	20.2	1.2
		3[c]	26.2	19.8	12.1	14.7	1.2
VI	15.0	1[c]	20.3	24.5	15.9	23.0	1.0
		2	27.3	28.3	15.4	21.0	1.4

[a] - Sacrificed, clinically normal.

[b] - Sacrificed, clinical signs of intoxication present.

[c] - Died.

of mink which died, and of most of those showing clinical signs when sacrificed were empty. A small amount of dark material resembling blood was present in the stomachs of one of the mink which died in each of groups V and VI. No source for this was detected.

8.3.5 Histopathology

The incidence of lesions is tabulated in Appendix D. No remarkable lesions were observed in group I.

8.3.5.1 Group VI (15.0 ppm Hg in feed)

Nervous system: The severity of lesions was uniform within this group. Neuronal necrosis was the chief lesion seen and this change was rather generalized throughout the brain. The most disturbed area was the occipital region of the cerebral cortex where there was severe necrosis of neurons in the middle laminae, with only laminae I, II, and VI appearing relatively intact (Fig. 25). Focal areas of diffuse neuronal necrosis of all laminae were also present (Fig. 26). Necrotic neurons were shrunken and angular with condensed eosinophilic cytoplasm and had small pyknotic nuclei (Fig. 28). Contraction of neuronal cytoplasm resulted in the affected areas having a somewhat fenestrated appearance. Proliferation and swelling of capillary endothelial cells, and of glial cells, particularly elongated microglial cells, was prominent in the damaged areas.

Changes were less severe in other areas of the cerebral cortex but necrotic neurons and foci of glial cells could be found in all areas. The most outstanding feature was extensive perivascular accumulations of cells resembling lymphocytes and histocytes about

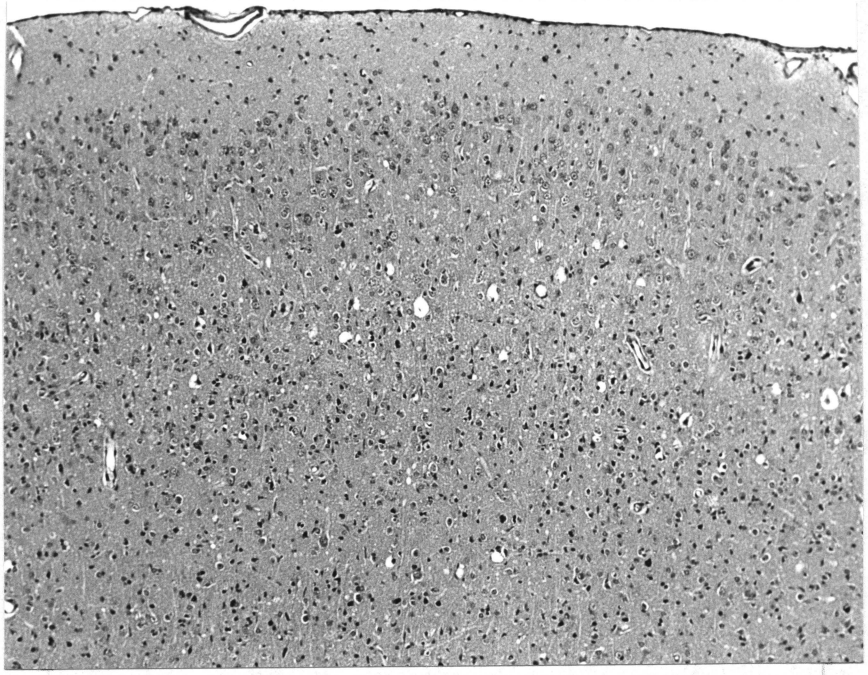


Fig. 25. Mink (group VI-3). Occipital region of cerebral cortex. Laminar necrosis of neurons in laminae III to V.
H & E X 85

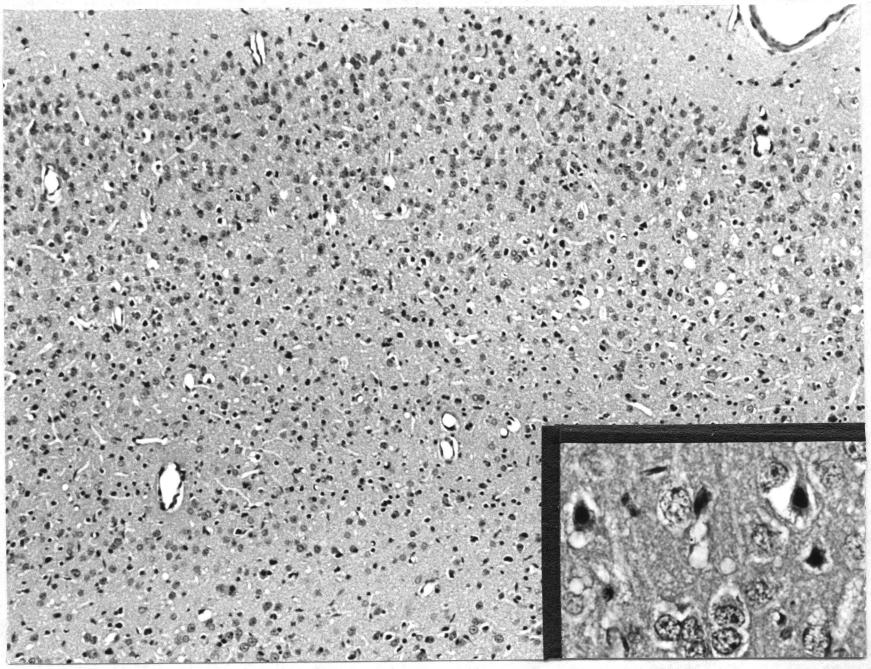


Fig. 26. Mink (group VI-3). Occipital region of cerebral cortex. Necrosis of neurons in all laminae. H & E X 110

Inset. Necrotic neurons with pyknotic nuclei, and swollen astrocytes.
H & E X 570

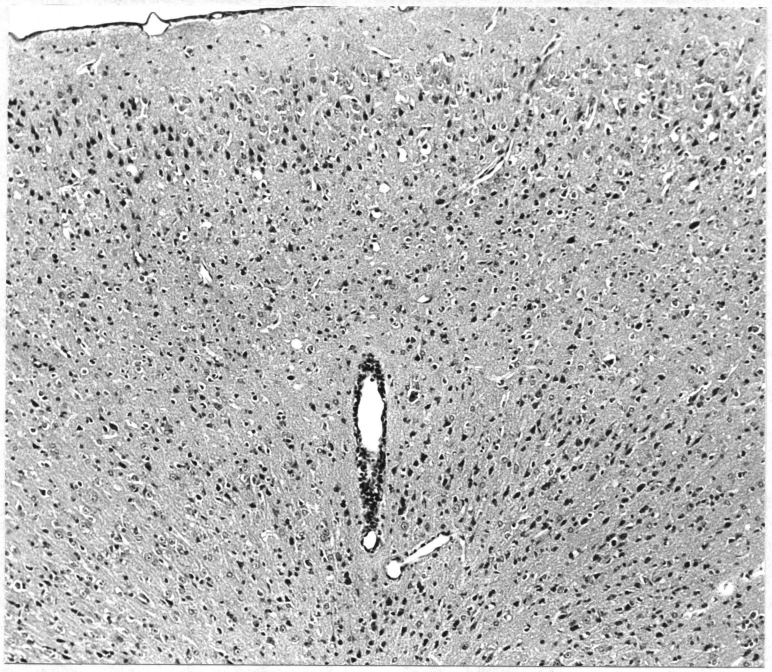


Fig. 27. Mink (group VI-5). Occipital region of cerebral cortex. Laminar necrosis of neurons with perivascular lymphoid cell accumulation.
H & E X 85

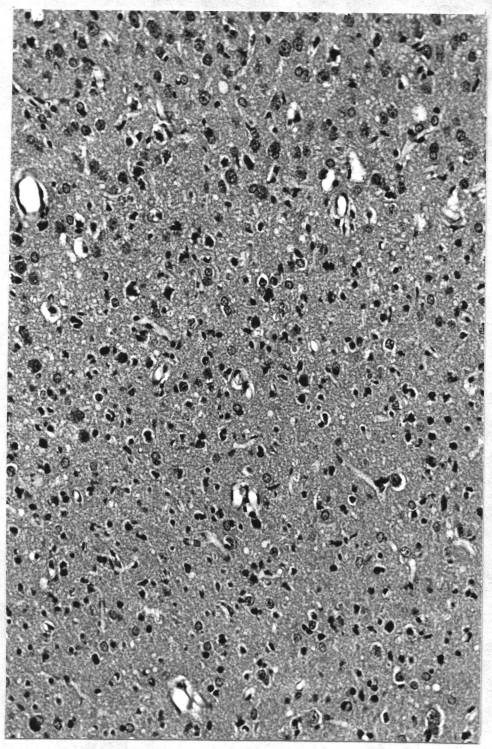


Fig. 28. Mink (group VI-3). Occipital cerebral cortex. Laminar necrosis of neurons.
H & E X 140

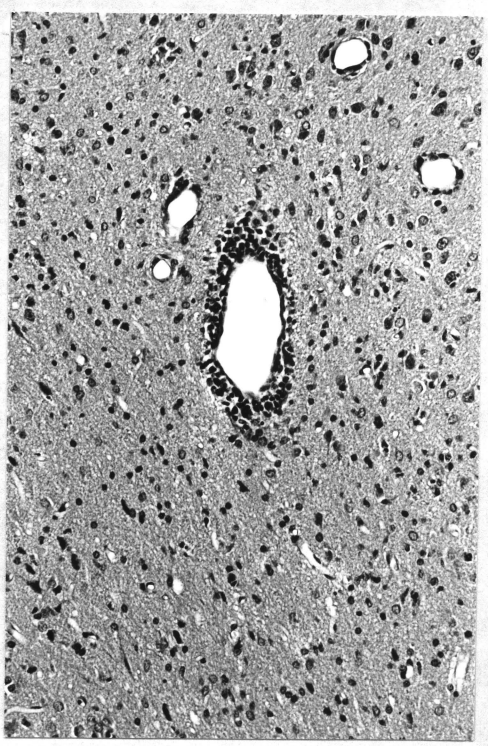


Fig. 29. Mink (group VI-3). Occipital cerebral cortex. Vessel with perivascular lymphoid cell accumulation.
H & E X 145

about larger vessels throughout both the cerebral grey and white matter (Figs. 27, 29).

Neuronal degeneration and necrosis characterized by pyknosis of cells was present in the granular layer of the cerebellum of all animals (Fig. 30). This change was confined to the median and basal areas of the cerebellum, being most promiment in the lingula. All mink in this group had marked porosity of the white matter of the cerebellar peduncles, particularly in the superior and inferior cerebellar peduncles (Figs. 31, 32). This change appeared as swelling and vacuolation of the myelin sheaths. Numerous eosinophilic granular bodies were evident in this area (Fig. 33) and longitudinal sections of axons indicated that these represented swollen degenerating axons (Fig. 34). Perivascular cellular accumulations similar to those in the cerebral cortex were present about many large vessels. Necrosis of neurons, gliosis, eosinophilic granular bodies, and perivascular cellular accumulations were present in nuclei throughout the thalamus, hypothalamus, mid brain, and in the fastigial and dentate nuclei (Figs. 35, 36). The basal ganglia appeared to be relatively unaffected. Neuronal necrosis with neuronophagia was evident in the Gasserian ganglion of one mink.

Changes in the sciatic nerves were difficult to interpret on histological slides of this structure, and were limited to local swelling and vacuolation of myelin. In teased preparations stained with osmium tetroxide individual fibres were found which had disruption and contraction of the myelin sheath at the nodes of Ranvier, and the formation of osmophilic masses (Fig. 37). These changes were

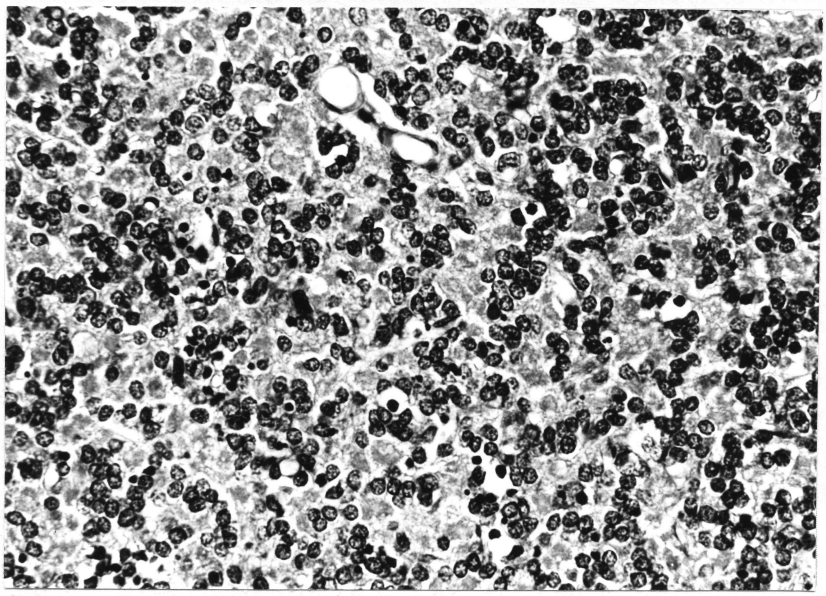
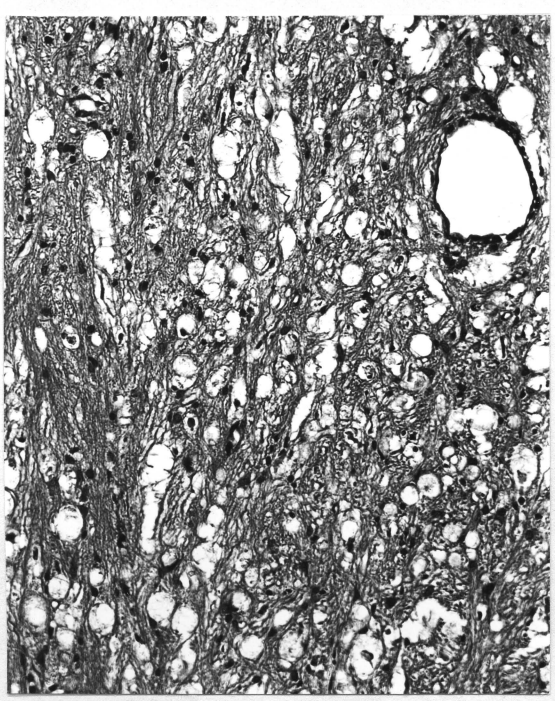


Fig. 30. Mink (group VI-3). Lingula of the cerebellum. Numerous pyknotic nuclei in the granular layer.
H & E X 500



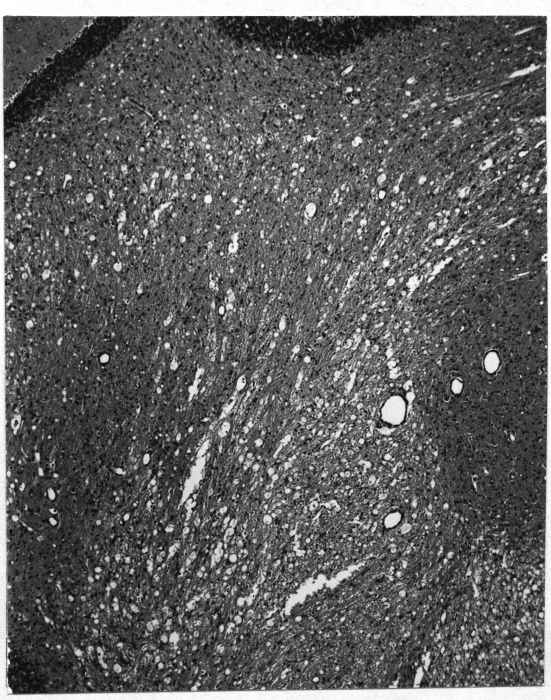


Fig. 31,32. Mink (group VI-3). Cerebellar peduncles. Marked porosity of white matter. Swelling of myelin sheaths and swelling of axons.
H & E X 198 H & E X 52

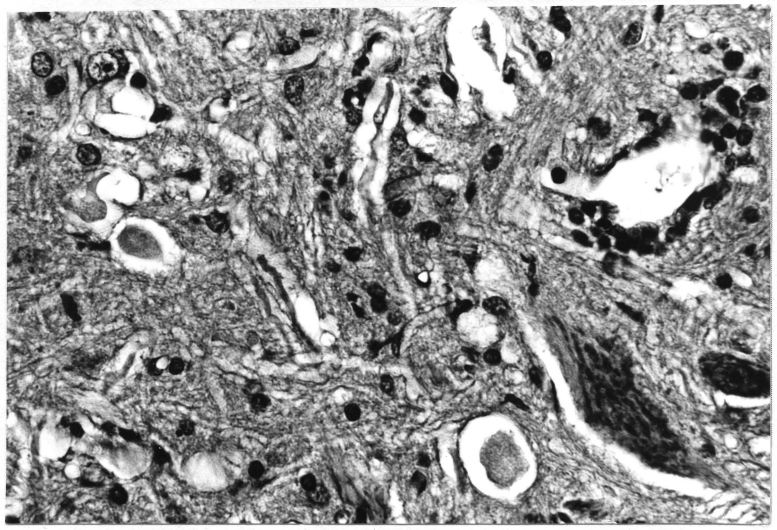


Fig. 33. Mink (group VI-3). Cerebellar peduncle near the dentate nucleus. Eosinophilic granular bodies and perivascular cellular accumulation.

H & E X 500

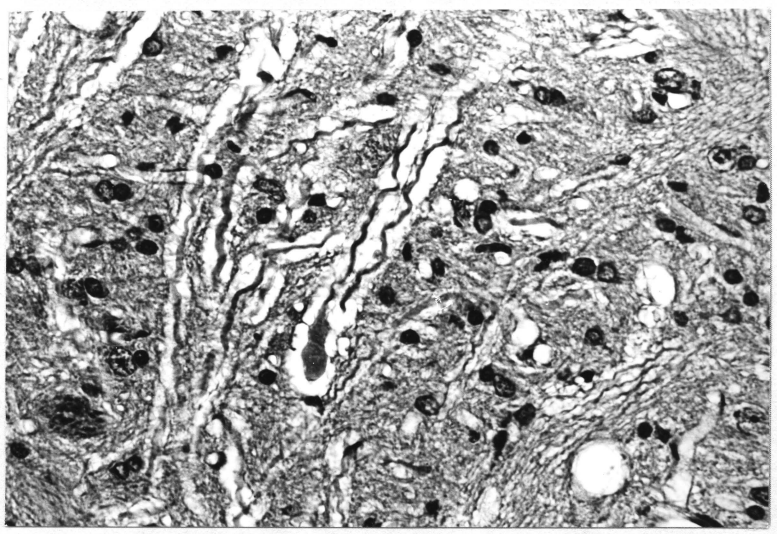


Fig. 34. Mink (group VI-3). Cerebellar peduncle near the dentate nucleus. Axon fibre with granular swelling.

H & E X 500

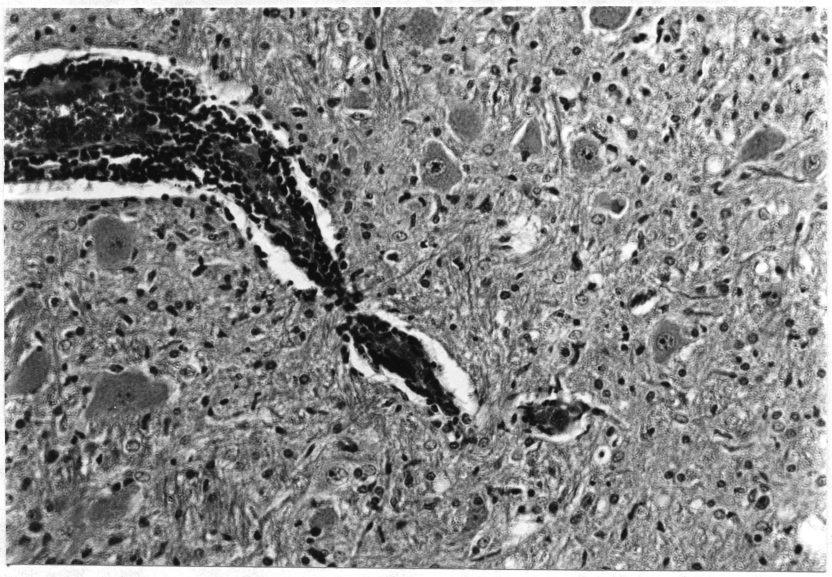


Fig. 35. Mink (group VI-1). Red nucleus. Gliosis and perivascular cellular accumulation.
H & E X 161

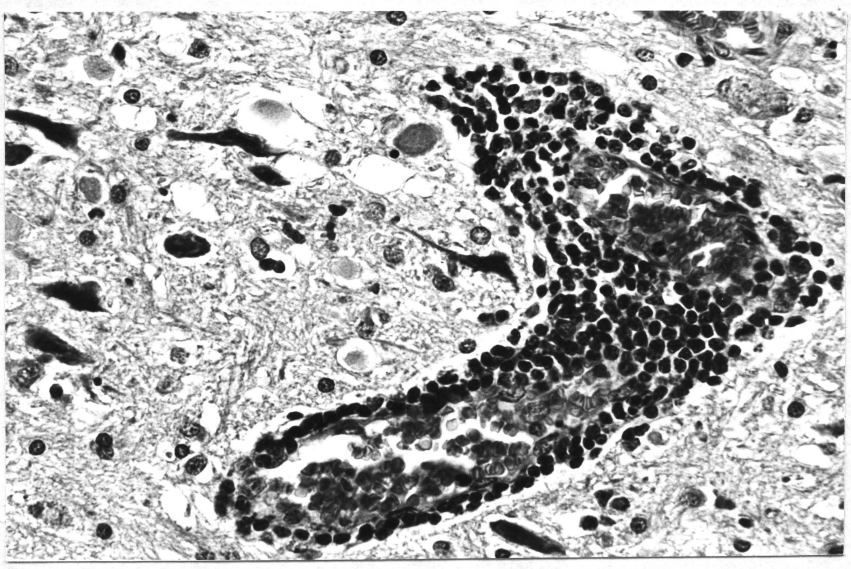


Fig. 36. Mink (group VI-2). Pons. Neuronal necrosis, eosinophilic granular bodies and marked lymphoid cell accumulation about vessel.
H & E X 485

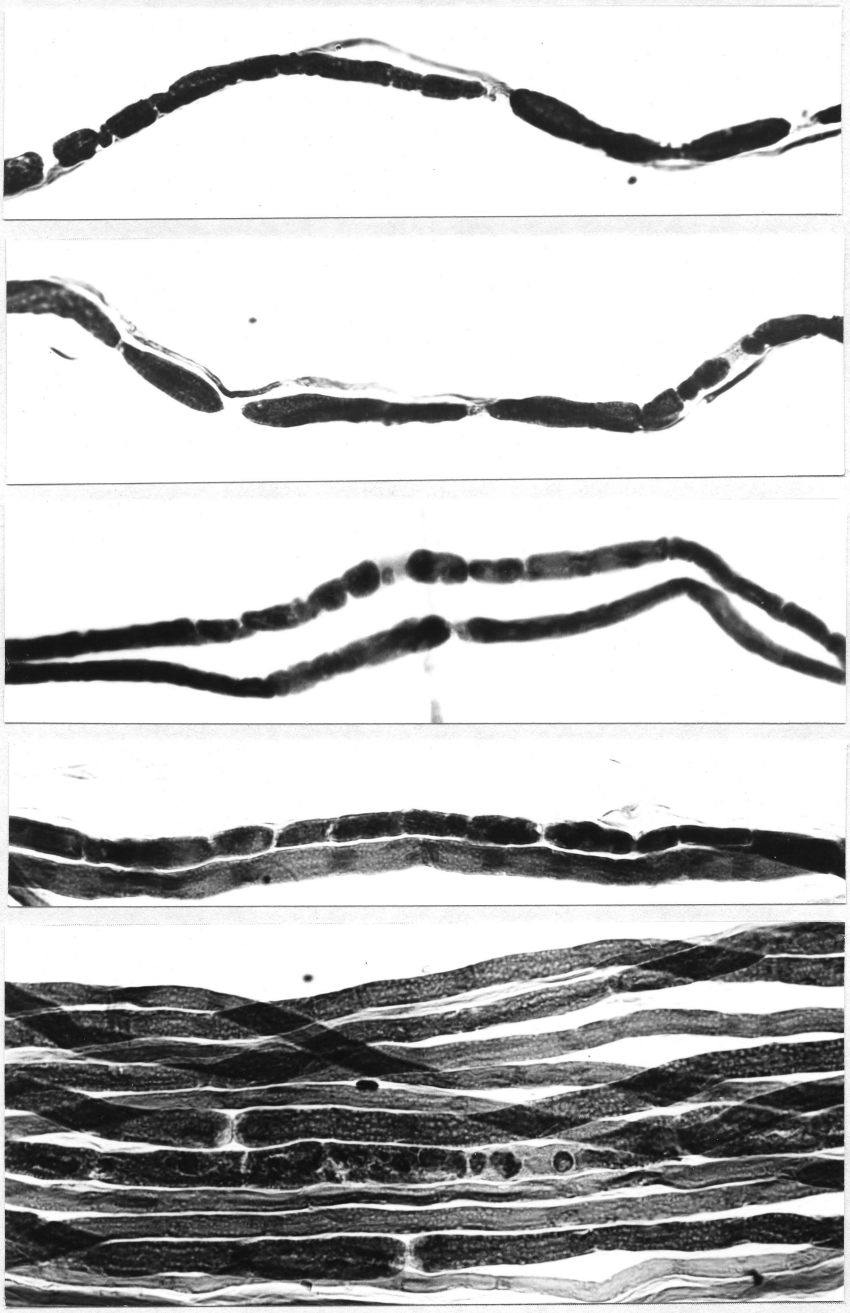


Fig. 37. Mink (group VI). Isolated nerve fibres from the sciatic nerves. Degeneration and retraction of myelin at the nodes of Ranvier, with the formation of circular osmophilic masses. Note that only one fibre in the lower group is so affected.

Osmium tetroxide X 370

consistent along the length of individual fibres, but were present in only a small percentage of nerve fibres. This may explain the difficulty in appreciating changes in nerves sectioned longitudinally.

Vacuolation of myelin was noted in foci in the dorsal columns of the spinal cord and occasional lymphoid cells were present about vessels in these areas.

Other systems: The liver of all animals had extensive vacuolar degeneration (Fig. 38). The epithelium of the proximal convoluted tubules of both control and principal mink in this experiment contained numerous vacuoles. However, this vacuolation was more prominent in the mink in group VI (Fig. 39). Within the spleens of these mink the character of cells in the Malpighian corpuscles differed from that seen in control animals in which the cells within the corpuscles were uniformly small, with dark basophlilic nuclei (Fig. 40). In animals in group VI the cells in the marginal and central areas of the follicles were larger with pale vesicular nuclei. Often a thin layer of normal appearing cells separated the marginal and central areas which contained larger cells (Fig. 41).

8.3.5.2 Group V (8.3 ppm Hg in feed)

Nervous system: The character and distribution of lesions in this group were essentially the same as that seen in animals in group VI. The most severe neuronal necrosis occurred in the occipital cerebral cortex. Promiment perivascular accumulations of lymphoid and histocytic cells were present about vessels in all areas of the cerebral cortex and also in areas of necrosis in the thalamus and hypothalamus (Fig. 42). Degenerative changes in the cerebellar peduncles were

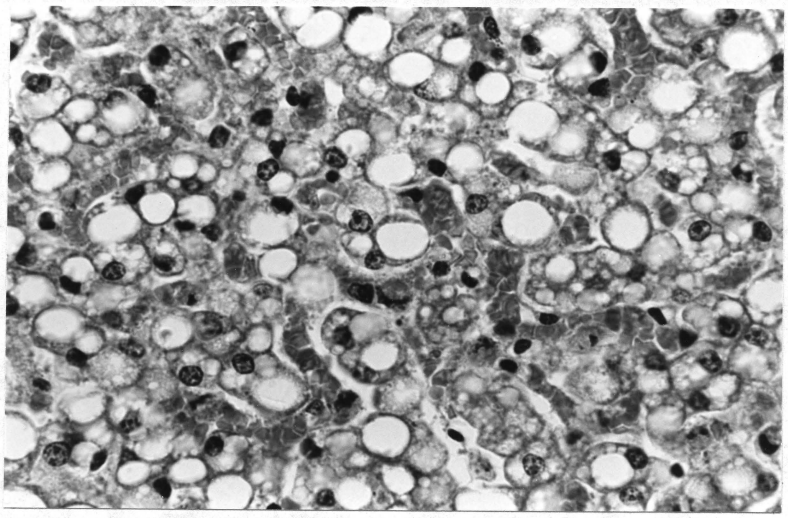


Fig. 38. Mink (group VI-2). Liver. Severe vacuolar degeneration of hepatocytes.

H & E X 560

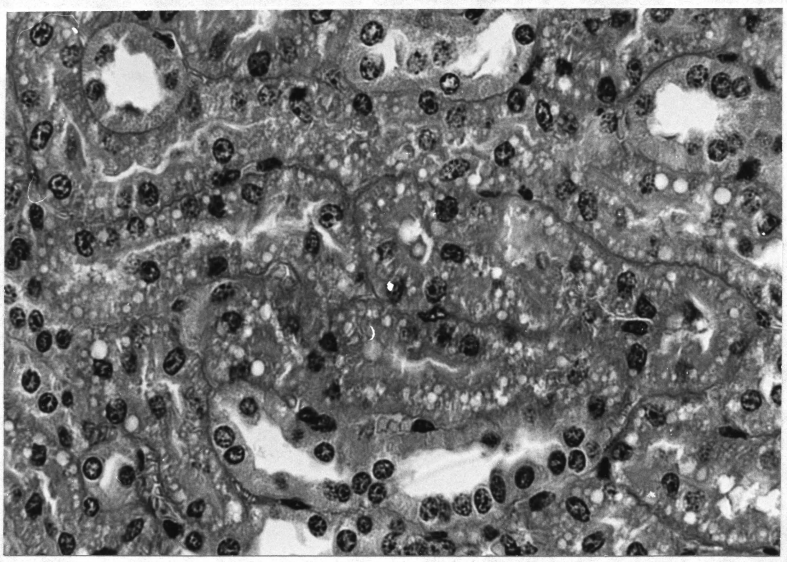


Fig. 39. Mink (group VI-5). Kidney. Hydropic degeneration of epithelium of proximal convoluted tubules.

H & E X 400

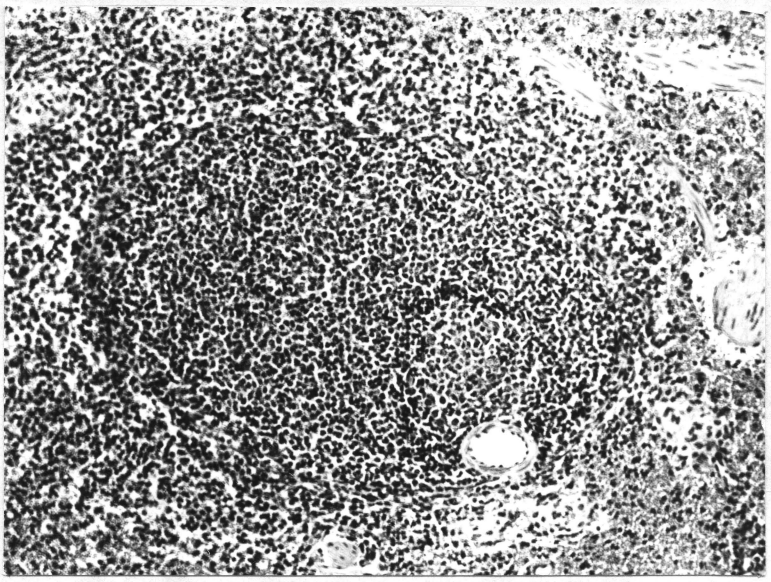


Fig. 40. Mink (group I-2). Spleen. Uniform population of small, basophilic lymphocytes in Malpighian corpuscle.
H & E X 145

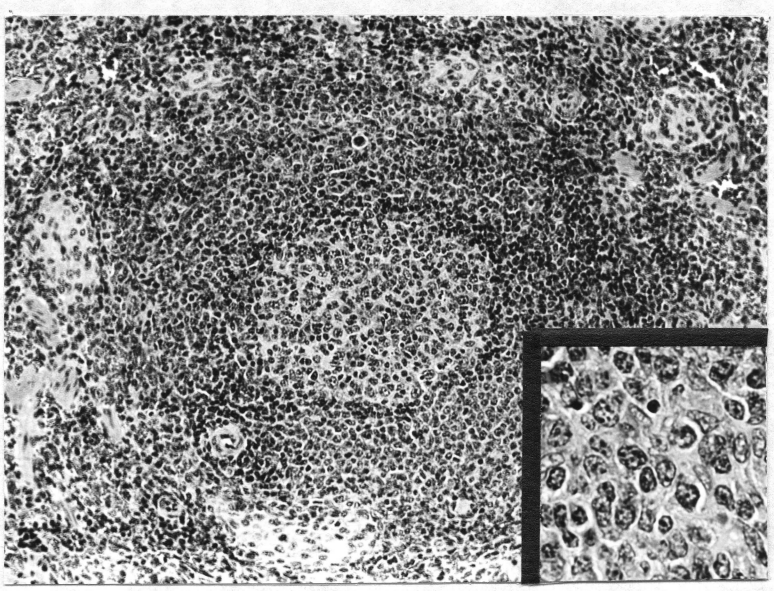


Fig. 41. Mink (group VI-2). Spleen. Central and marginal areas of Malpighian corpuscle composed of large cells with pale, vesicular nuclei. H & E X 145

Inset. Cells from central region. H & E X 528

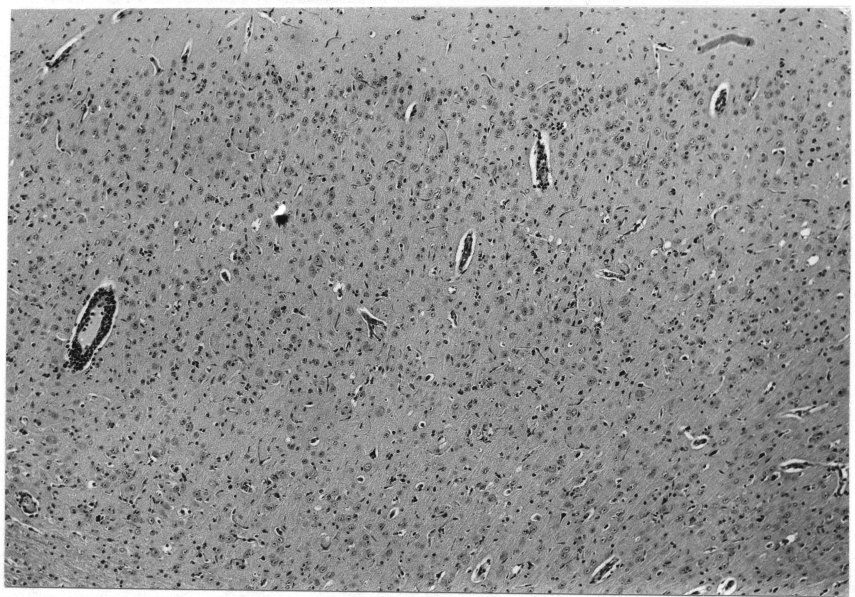


Fig. 42. Mink (group V-3). Parietal cerebral cortex. Laminar necrosis of neurons, perivascular cellular accumulation.

H & E X 62

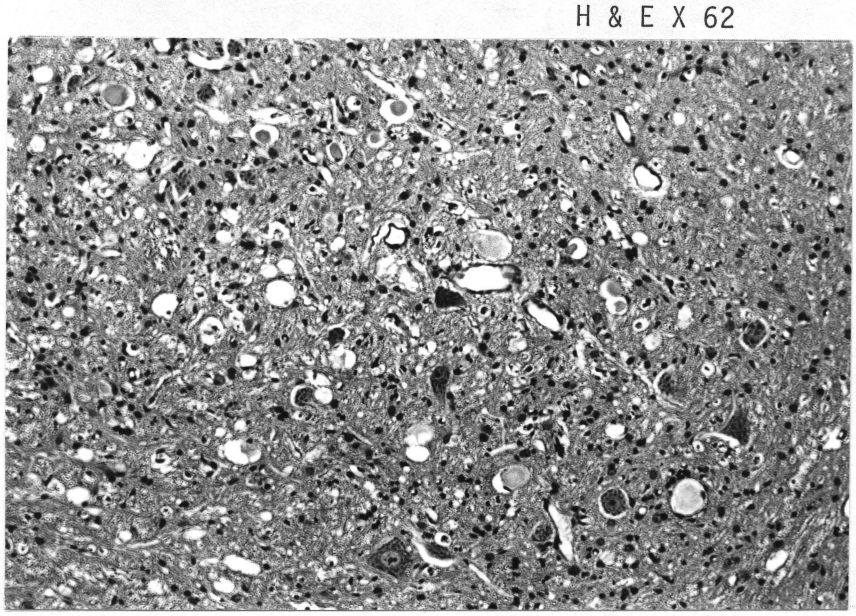


Fig. 43. Mink (group V-4). Red nucleus. Neuronal necrosis, glial cell proliferation and numerous eosinophilic, granular bodies.

H & E X 146

present in all mink as was pyknosis of granular cell nuclei in the basal and median areas of the cerebellum. The red, fastigial and dentate nuclei appeared to be more severely involved than other nuclei (Fig. 43). The sciatic nerves were similarly involved to those of mink in group VI.

Other systems: Changes in liver, spleen, and kidney were similar to those in group VI.

8.3.5.3 Group IV (4.8 ppm Hg in feed)

Nervous system: Lesions in animals in this group were similar in distribution to those in the prior two groups, but were more variable in intensity. Neuronal necrosis and loss was evident in laminae III, IV, and V of the occipital cortex of all mink (Fig. 44). This was accompanied by microgliosis and astrocyte and capillary endothelial proliferation, and the presence of eosinophilic granular bodies. Perivascular cellular accumulations similar to those previously described were present about cerebro-cortical vessels in three mink. Neuronal necrosis was present in other areas of the cerebral cortex but to a much lesser degree than in more acutely poisoned mink, and occurred as scattered foci accompanied by glial cell proliferation. Degeneration of granular cells of the cerebellum was present in one mink in this group; the cerebellar peduncles had lesions similar to, but less severe than those previously reported. Occasional necrotic neurons were detected in subcortical nuclei, and in two mink neuronal necrosis with neuronophagia, gliosis, capillary endothelial proliferation, and axonal degeneration were present in the red nucleus. A similar lesion was present in the nucleus of the lateral lemniscus in one

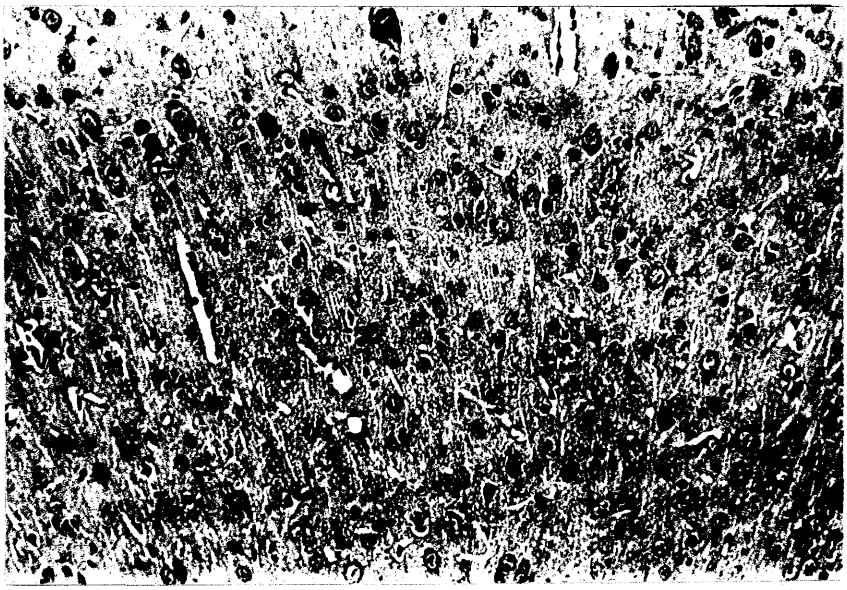


Fig. 44. Mink (group IV-2). Occipital cerebral cortex. Laminar necrosis of neurons.

H & E X 150

mink. In this mink (IV-2) a large vessel in the area of the red nucleus had a prominent perivascular "cuff". Only very occasional fibres in the sciatic nerves of these mink showed changes similar to those seen in groups V and VI.

Other systems: Vacuolar degeneration of the liver was present in two mink. The previously described change in the spleen was present in all mink. The kidneys were similar to those of the control group.

8.3.5.4 Group III (1.8 ppm Hg in feed)

Nervous system: Lesions in this group were similar to those in group IV. Perivascular accumulation of lymphoid and histocytic cells was found around only one vessel in the cerebral cortex of one mink. Scattered pyknotic granular cell nuclei were found in the basal region of the cerebellum of one mink. Occasional necrotic neurons were present in subcortical nuclei; these were most prominent in the red, fastigial , and dentate nuclei, and in the nucleus of the lateral leminiscus (Figs. 45, 46, 47).

Other systems: Vacuolar degeneration of hepatocytes was present in the liver of one mink. The spleen of four of the animals had changes as previously described (Fig. 48). The kidneys were unremarkable.

8.3.5.5 Group II (1.1 ppm Hg in feed)

Nervous system: Lesions in the cerebral cortex were limited to small foci of neuronal necrosis in laminae III and IV of the occipital cortex. A vessel in association with one of these foci in one mink had a single layer of surrounding lymphoid cells. Necrosis of neurons

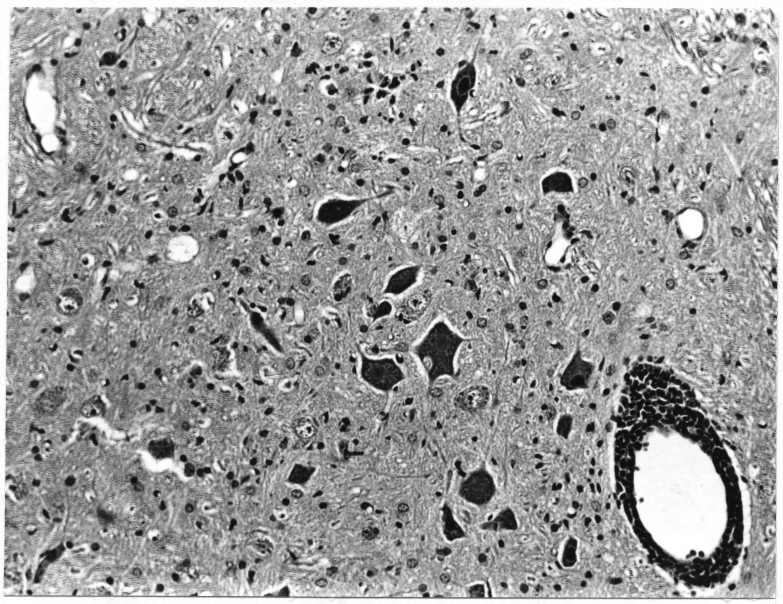


Fig. 45. Mink (group III-2). Red Nucleus. Neuronal necrosis, neuronophagia, glial cell proliferation, and perivascular lymphoid cell accumulation. H & E X 196

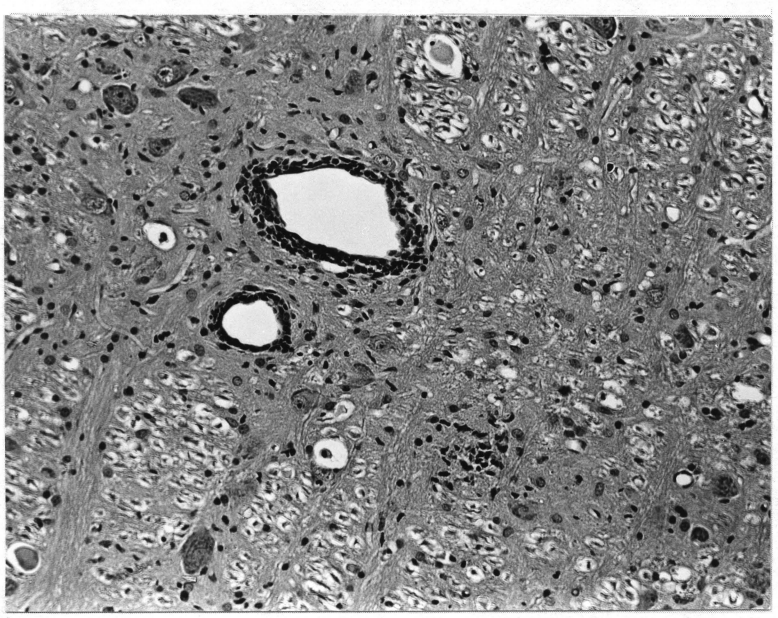


Fig. 46. Mink (group III-2). Nucleus raphis. Neuronal necrosis, neuronophagia, eosinophilic granular bodies and perivascular lymphoid cell accumulation. H & E X 196

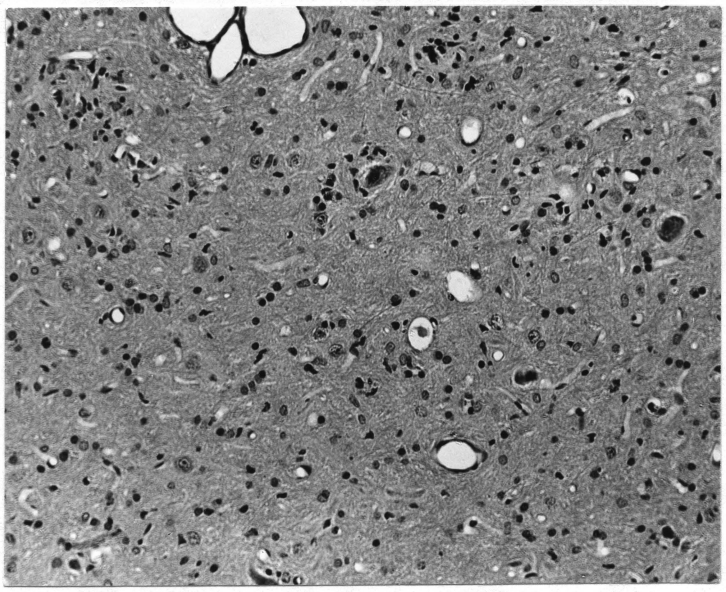


Fig. 47. Mink (group III-2). Dentate nucleus. Neuronal necrosis.

H & E X 146

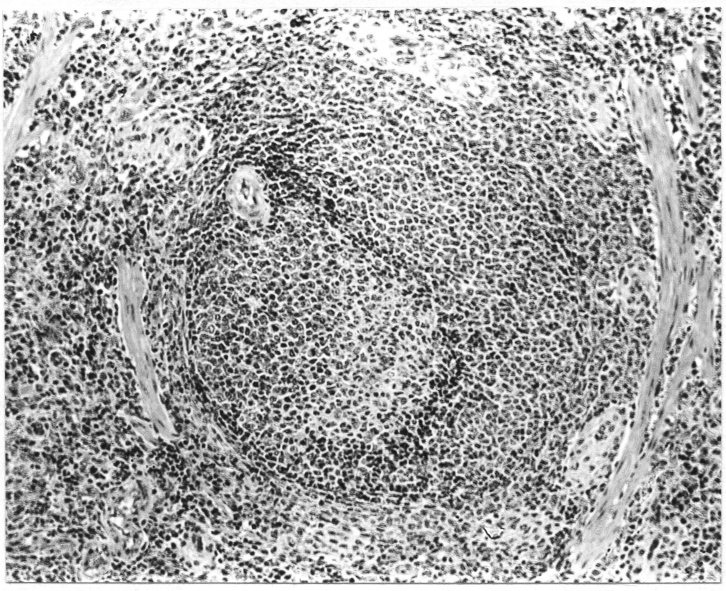


Fig. 48. Mink (group III-2). Spleen. Large cells with pale vesicular nuclei in Malpighian corpuscle.

H & E X 148

and astrocytosis was observed in the nucleus of the lateral leminiscus of two mink (Fig. 49). In one mink a few vacuolated spaces with swollen axons were observed in the superior cerebellar peduncle (Fig. 50) and in another mink necrosis of neurons in the fastigial nucleus was observed. Retraction of myelin from about the axon was not observed in teased specimens of sciatic nerves from these mink, although the myelin occasionally had a granular appearance near the nodes of Ranvier.

Other systems: Liver and kidney from these mink were unremarkable. Three of the five mink showed cellular changes in the Malpighian corpuscles of the spleen, as described in other groups.

8.3 Discussion

Mercury in the form of MeHgCl administered in the diet was found to be toxic for mink. Rations containing from 1.8 to 15.0 ppm Hg produced clinical evidence of toxicosis within the 95-day experimental period. A ration containing 1.1 ppm Hg caused pathological alterations in the nervous system, but did not produce obvious clinical evidence of intoxication within the experimental period.

8.4.1 Clinical manifestations

The clinical signs of anorexia, weight loss, head tremor, ataxia and convulsions observed in mink in the present study were similar to those reported in alkyl Hg intoxication in other carnivores. Hanko et al. (1970) reported loss of appetite, weakness, trembling, and twitching of the head, followed by ataxia, paralysis, apathy, and death

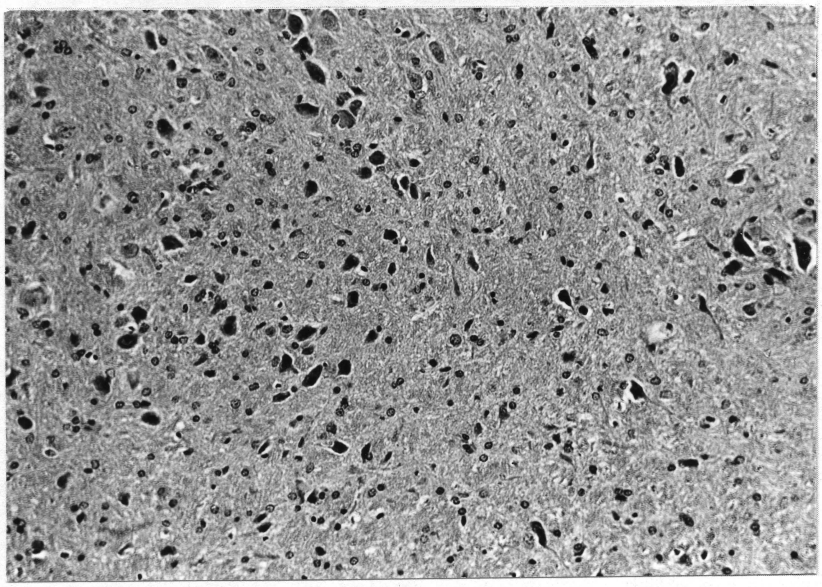


Fig. 49. Mink (group II-4). Nucleus of the lateral lemniscus. Neuronal necrosis.

H & E X 148

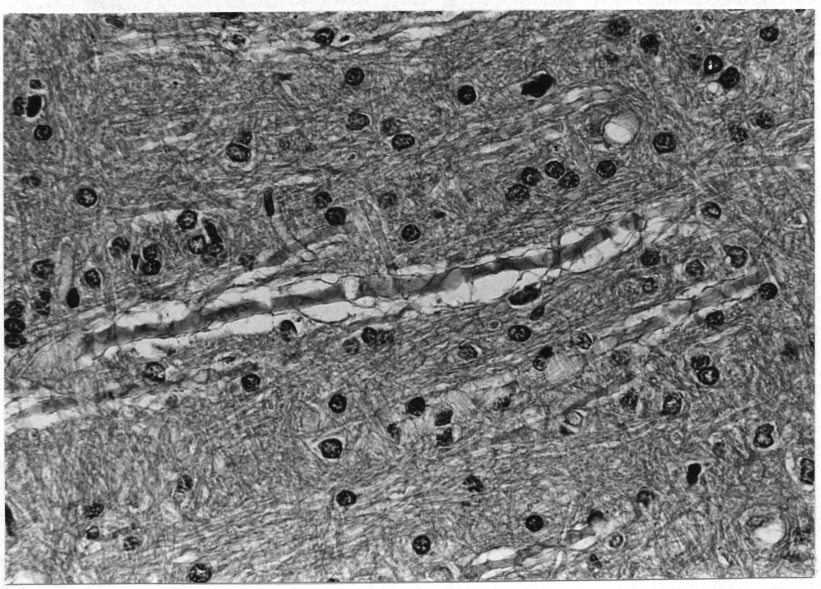


Fig. 50. Mink (group II-4). Swelling of axon and myelin sheath in superior cerebellar peduncle.

H & E X 480

in ferrets fed MeHg contaminated poultry flesh. Excitation with "yelling" and circling was also reported in these animals. Takeuchi (1968 a,b) reported slow movements, ataxic gait, and convulsions in natural and experimental cases of alkyl Hg poisoning in cats. Albanus et al. (1972) described behavioral changes and impaired movement, followed by convulsions in experimentally poisoned cats. The behavioral changes observed in these cats ranged from wariness and aggressive behavior to affectionate behavior (Albanus et al., 1972). The main behavioral changes noted in the present study were listlessness and indifference to the investigator. A "stiff waddling gait" progressing to definite ataxia was also reported in cats (Albanus et al., 1972). No clear assessment of the visual perception of the animals could be made either in the present study or in the studies described above.

The clinical course of poisoning in mink was shorter than that reported in other carnivores receiving rations with similar levels of Hg. Hanko et al. (1970) reported a latent period before the appearance of clinical signs of about 14 and 21 days in ferrets receiving rations containing 7 and 5 ppm Hg, respectively. These latent periods were similar to those of mink in group V which received a ration containing 8.3 ppm Hg, and were shorter than the latent period of mink in group IV which received food containing 4.8 ppm Hg. The survival period of the ferrets after the onset of clinical signs ranged from 21 to 37 days (Hanko et al., 1970), while the longest survival of mink receiving similar levels of Hg in the food was 13 days, and the average survival after onset of clinical signs was only 6.4 days. The mean survival time was influenced by the sacrifice of

animals when showing clinical signs; however, based on the short clinical course of animals which died, it is unlikely that this had a great effect. Albanus et al. (1972) poisoned cats with MeHg contaminated fish, and with fish to which MeHg had been added. The Hg content of both rations was about 6 ppm. Convulsions first occurred in these cats between days 60 and 83, but changes in behavior and movement were noted 4 to 33 days before convulsions were first observed.

Mink in all principal groups lost weight over the experimental period. This was a consistent finding in the earlier studies (Takeuchi, 1968 a,b; Hanko et al., 1970; Albanus et al., 1972) and was likely related to anorexia.

The length of the latent or asymptomatic period which preceded the development of signs of intoxication varied inversely with the Hg content of the diet. The occurrence of similar Hg levels in the tissues of mink from different groups at the time of death suggests that this period represented the time required for accumulation of toxic levels of Hg in tissue. Supporting this view is the fact that Hg concentrations in the tissues of mink in group II, which were beginning to show clinical signs, were similar to those in mink which died or had obvious clinical disease.

In view of the lesions present in the nervous system of animals in group II, it could be expected that they would have developed clinical signs had the experimental period been longer. The occurrence of clinically-silent damage to the nervous system of alkyl Hg poisoned animals has been previously reported by Tryphonas (1968) and Grant (1971). The occurrence of clinically-silent damage to the nervous system

undoubtedly reflects to some degree the difficulty in adequately assessing nervous function in experiment animals (Grant, 1971), but it is also possible that the nervous system of these animals was functioning in a state of compensation for loss of parenchyma (Tryphonas, 1968).

The clinical signs of anorexia, ataxia, and convulsions as observed in these mink would not provide a reliable method for the diagnosis of Hg poisoning in field cases. Mink infected with viral diseases such as distemper and Aujeszky's disease may show similar clinical signs (Budd et al., 1966; Christodoulou et al., 1970), and the clinical signs of thiamine deficiency (Chastek's paralysis) might also resemble those of alkyl Hg intoxication (Budd et al., 1966).

8.4.2 Relation between mercury concentrations in tissue and effects

The Hg concentration in the brains of mammals showing neurologic symptoms of alkyl Hg poisoning has been found to range from 2 to 61 ppm (Berglund et al., 1971). Berglund et al. (1971) concluded that, despite differences in species and widely different doses of MeHg used by authors to produce intoxication, the critical level in brain may be of the order of 10 ppm Hg for most mammals. The average Hg concentration in the brains of mink which died after showing neurologic signs was 11.9 ppm. The level of 4.1 ppm Hg in the brain of one mink which died is anomalous, particularly since a level more than twice as high was found in the brain of a mink which was apparently clinically normal (Table 28). This may represent a difference in individual susceptibility to alkyl Hg poisoning.

The only prior report of MeHg poisoning in a mustelid was that

of Hanko et al. (1970). Total Hg and MeHg concentrations in the brains of the four ferrets used in that study were reported, but the results are difficult to interpret because MeHg content was higher than the total Hg content in three of the four animals. In one ferret the brain was found to contain 14 ppm Hg in the form of MeHg, but only 7 ppm of total Hg. The mean total Hg concentration in the brains of the four ferrets at the time of death was approximately 26.8 ppm (range 7 to 39 ppm). This value was considerably higher than the average value for mink in the present study. This discrepancy may be related to interspecific differences in susceptibility. The ferrets survived an average of 46.9 days (Hanko et al., 1970), compared to an average of 27.5 days for mink in groups IV and V which received diets containing similar levels of mercury. Thus there was ample opportunity for higher accumulation in the ferrets. Differences in the toxicity of the MeHg compounds used in the two studies might be a factor, however, the data reviewed by Berglund et al. (1971) and that of Albanus et al. (1972) suggests that there are no distinct differences in the toxicity of MeHg salts, or between MeHg salts and MeHg in the form found in Hg contaminated fish. Differences in brain Hg content at the onset of clinical signs similar to those between the present study and that of Hanko et al. (1970) have been reported within the same species. The average Hg concentration in the brains of alkyl Hg poisoned cats has varied from 11.8 (Kitamaru, 1968) to 18 ppm (Albanus et al., 1972).

The Hg distribution pattern among organs in the mink was similar to that reported in most other mammals (reviewed by Berglund et al.,

1971) and was very similar to that reported in ferrets (Hanko et al., 1970). The Hg concentration in the fur of mink in the principal groups did not differ widely from that of the controls. This finding is in direct contrast to reports of Hg concentration in the hair of poisoned humans (Kitamaru, 1968) and cats (Kitamaru, 1968; Albanus et al., 1972). Albanus et al. (1972) found that at least 20 per cent of the total body Hg burden was present in the fur of cats poisoned over approximately a 90 day period. The explanation for this discrepancy lies in the seasonal nature of fur growth in mink (Kennedy, 1951). The mink in this study had just become "prime", i.e., had just completed the growth of their winter fur, prior to the beginning of the study, and little or no hair growth would have been expected during the period of the study.

8.4.3 Pathology

8.4.3.1 Nervous system

The nervous system appears to be the "critical organ" in MeHg poisoning of mink as in other mammalian species (Berglund et al., 1971). This term, critical organ, was defined by Berglund et al. (1971) as "the organ in the body whose function is especially affected by a chemical agent." The primary action of MeHg seemed to be neurotoxicity and, in general, the severity of this action was directly related to the Hg concentration in the ration and thus rate of intake. The concentration of Hg in the brain of mink which died in all groups were rather similar; however, the rate of accumulation of this "critical level" seemed to be important in determining the extent and distribution

of neurologic damage. In those groups (V and VI) receiving the highest levels of Hg in the ration neuronal necrosis was evident throughout the cerebral cortex, subcortical nuclei, and in the basal areas of the cerebellum, and advanced degeneration of myelinated tracts in the cerebellar peduncle was evident. Those groups (II, III, and IV) with a longer latent period and lower daily rate of Hg intake had lesions of a more localized nature, particularly involving the occipital region of the cerebral cortex and certain nuclear groups. Involvement of the granular layer of the cerebellum was much less common than in the more acutely poisoned group. It is unlikely that a state of equilibrium between intake and elimination was reached in any of these groups, so that all cases can be regarded as a form of acute or subacute poisoning, in which Hg levels were increasing in tissues until the critical level for that organ was reached.

The occurrence of mononuclear cell accumulations in a perivascular location has been reported in alkyl Hg poisoned rats (Hunter et al., 1940), cattle (Fujimoto et al., 1956), and swine (Kahrs, 1968) and seems to have been a common finding in carnivores; having been reported in cats (Takeuchi et al., 1962; Takeuchi, 1968 a,b; Albanus et al., 1972), and ferrets (Hanko et al., 1970). The lesion would appear to be a response to neuronal damage rather than a primary lesion since it was not evident in some animals in the subacute groups which did have early neuronal degeneration and necrosis, but was consistent in animals more acutely poisoned and showing severe neuronal necrosis. These perivascular cuffs occurred in association with areas of necrosis.

Degenerative arteriopathy has been described in alkyl mercury

poisoning in several species (Hay et al., 1963; Jubb and Kennedy, 1963; Prick et al., 1967; Kahrs, 1968; Tryphonas, 1968 and Diamond and Sleight, 1972). This lesion was not observed in mink and has not been reported in cats (Takeuchi, 1968 a,b; Albanus et al., 1972), or in ferrets (Hanko et al., 1970).

The type and distribution of lesions seen in mink were similar to those reported in ferrets (Hanko et al., 1970). In ferrets focal peripheral neuropathy, "demyelination and vacuolization" in the cerebellar medulla and pons were found (Hanko et al., 1970). Neuronal degeneration was described in the cerebrum, medulla and pons, but the location of these lesions was not described further. No changes were observed in the cerebellum (Hanko et al., 1970). Grant (1971) and Albanus et al. (1972) described the pathology of MeHg poisoning in the same group of cats. The lesions noted were degeneration of granular cells of the basal and median areas of the cerebellum, degeneration of individual fibres in peripheral nerves, and scattered small foci of neuronal necrosis in the cerebral cortex. Perivascular cuffs of lymphocytes were common in or near damaged areas of the cortex.

The degeneration of myelinated tracts within the cerebellar area would seem to be a feature of acute MeHg poisoning of mustelids. It appeared that both the myelin sheath and axons were involved and it is difficult to determine which site received the initial injury.

The changes in the sciatic nerves were restricted to individual fibres. Similar findings have been previously reported (Miyakawa et al., 1970; Albanus et al., 1972) and the literature suggests that this represents selective involvement of sensory fibres within a

nerve containing both sensory and motor fibres (Miyakawa et al., 1970).

8.4.3.2 Other systems

Vacuolation of hepatocytes was a common finding in mink receiving higher levels of Hg in the diet. Frozen sections of liver stained for lipids indicated that this was the result of accumulation of lipid within hepatocytes. The accumulation of lipid in the liver results from an imbalance in the mechanisms which normally control the amount of fat in the liver and in this case may have been due to a combination of increased metabolism of fat mobilized from depots as a result of anorexia, and possibly a direct interference with normal fat metabolism in the liver. Interference with hepatic enzyme systems by MeHg has been demonstrated by Lucier et al. (1971).

Fatty degeneration of the liver was observed in MeHg poisoned ferrets (Hanko et al., 1970) and has been a common finding in MeHg poisoning in many other species.

Hydropic degeneration of proximal tubular epithelium of the kidney has also been a common finding in MeHg poisoning and may be the result of demethylation of MeHg, with the accumulation of inorganic mercury at this site, and enzyme inhibition (Fowler, 1972a).

The changes observed in the Malpighian corpuscles of the spleen in mink do not appear to have been previously described. Hanko et al. (1970) reported "hypoplasia of the lymphatic tissue of the spleen. . ." in ferrets, and Diamond and Sleight (1972) described partial or complete loss of lymphoid follicles with reticulo-endothelial cell hyperplasia in the spleens of sub-chronically poisoned rats. The change in the mink appeared to be a lack of mature small lymphocytes,

and replacement by larger cells with more abundant cytoplasm and a less basophilic nucleus. Lukes (1970) has classified the types of splenic white pulp seen in various states. That seen in the mink does not correspond exactly with any of the preposed classes, but most closely resembles the class described as Type III-A (or activated with basophilic stem cells and large and small lymphocytes). The clinical states associated with this type were graft rejection, infectious mononucleosis, Herpes simplex infection, post-radiation and post bone marrow damage (Lukes, 1970). Bone marrow from the mink was not examined. Bone marrow hypoplasia has been reported in both human and animal MeHg intoxication (Morikawa, 1961b; Takeuchi et al., 1962; Takeuchi, 1968a). The association of the post-radiation clinical state with a change in lymphoid follicles similar to that seen in MeHg poisoning is very interesting. The loss of lymphocytes from the spleen after radiation (Casarett, 1968) appears to be similar to that described by Diamond and Sleight (1972) in MeHg poisoning. MeHg may be somewhat radiomimetic in other ways, for example, both ionizing radiation and MeHg have been reported to selectively injure granular cells in the cerebellum (Herndon, 1968), both may injure the blood-barrier (Steinwall and Olsson, 1969; Brightman et al., 1970), and both are genetically active (Berglund et al., 1971) and may produce an increased incidence of chromosomal breakages (Skerfving et al., 1970). This aspect of the action of MeHg deserves further study.

9.0 GENERAL DISCUSSION

9.1 Historical perspective

The occurrence of aquatic Hg pollution and human poisoning from the consumption of fish from Minamata Bay, Japan, was first reported in 1960 (Kurland et al., 1960), and since that time a great number of reports have been published dealing with Minamata disease. The first report in English literature describing Hg contamination of fish in Sweden appeared in 1967 (Johnels et al., 1967). The demonstration of high Hg concentrations in fish from the Saskatchewan River in the present study was the first evidence of Hg contamination of water in North America. Information about the occurrence of high concentrations of Hg in fish was communicated to appropriate government officials of the Province of Saskatchewan, and of the Department of Fisheries and Forestry, Canada, in November of 1969. The Department of Fisheries and Forestry immediately detained all commercial stocks of fish originating, or suspected of having originated from the entire Saskatchewan River, and began analyzing samples for Hg content. Only fish containing less than 0.5 ppm Hg were released for sale, and during the following 3 to 4 months more than one million lb of fish were destroyed because of Hg concentrations higher than this standard (Bligh, 1971).

A national survey was begun to determine the Hg concentration in

fish from other areas of Canada. The results were summarized by Bligh (1971) and showed that most major water systems in southern Canada were contaminated with Hg to some degree. Major surveys were begun shortly after to determine the Hg concentration in fish in various areas of the United States. As a result of these surveys commercial fisheries were suspended in at least 10 states, and warnings against public consumption of fish from water contaminated with Hg were issued in at least 20 states (Harriss, 1971).

9.2 The relationship of tissue mercury concentrations and toxic effects of mercury in fish

Relatively high concentrations of Hg were found in the tissues of fish from the Saskatchewan River, but these fish appeared to be grossly normal. Rainbow trout fry and fingerlings exposed to MeHg in the water rapidly accumulated concentrations of Hg in their flesh similar to those found in river fish; however, MeHg at the concentrations used was highly toxic for these trout under the test conditions. In contrast, rainbow trout fed diets containing MeHg at levels similar to or higher than those to be expected in the diet of large predatory fish in the Saskatchewan River accumulated Hg in their tissues comparatively slowly, and although the concentrations of Hg in muscle were similar to or higher than those in either river fish or the acutely poisoned fish, these fish showed only slight evidence, either clinically or histopathologically, of disease.

The results indicated that the rate of accumulation of MeHg is extremely important in determining the toxic effect of this mercury

on fish.

The critical organ concept used by Berglund et al. (1971) may be useful in explaining the differences between the experiments. It seems clear that the gills were a critical organ in fish acutely exposed to MeHg in the water. The gills represent not only the respiratory organ of fish, but also the main site for nitrogen excretion (Forster and Goldstein, 1969), and an extrarenal site for electrolyte regulation (Conte, 1969). Thus any interference with gill function is likely to have a profound effect upon fish. The fish in experiment II showed signs of toxicity with levels of Hg in muscle similar to those in fish from the Saskatchewan River and lower than those in fish from experiment III. This indicates that Hg concentration in muscle tissue alone is not a good indicator of the probable toxic effect of MeHg in fish acutely exposed through the water. Hannerz (1968), Backstrom (1969), and Miettinen et al. (1970b) have shown that MeHg rapidly accumulates in the gills. Miettinen et al. (1970b) produced peracute MeHg poisoning (death within 2 hours), in rainbow trout by oral administration of Hg. The Hg concentration in the gills of these fish was more than 10 times that in the whole body. This would indicate that under conditions of acute exposure, regardless of the route of administration, critical levels of Hg are reached in the gills before such levels are reached in other tissues.

The critical organ or organs for more chronic MeHg poisoning in fish have not been established. The results of experiment III suggest that the gills may be this organ. Definite morphological damage was evident in the gills, but not in the nervous system. Similarly

Miettinen et al. (1970b) reported damage to the gills, plus the pseudobranchiae, kidney and liver, but not to the nervous system of pike which survived 10 to 41 days after the administration of MeHg.

The uptake of Hg by the brain and muscle in fish is relatively much slower than that in organs such as the liver, kidney and gills (Hannerz, 1968; Backstrom, 1969; Giblin and Massaro, 1973). Thus it is possible that long-term exposure to low levels of Hg could result in the accumulation of toxic concentrations in the brain. Whether or not these levels could be achieved before damage to other organs, such as the gills, would be limiting is a point which requires clarification.

The concentration of Hg in the brain and muscle tissue of fish are rather similar (Hannerz, 1968; Backstrom, 1969; Miettinen et al., 1970b; Giblin and Massaro, 1973). If this was true in the trout in experiment III, the critical brain level of MeHg for the rainbow trout must be substantially higher than the figure of 10 ppm proposed as an average critical level in the brains of mammals (Berglund et al., 1971).

The results of experiment III indicate that concentrations of Hg similar to those found in fish in the Saskatchewan River are not associated with marked toxic effects upon the fish. Generalizations such as this among the species of fish involved are dangerous. However, this conclusion is supported by the work of Lockhart et al. (1972), which showed that northern pike could survive for almost one year without evidence of disease, despite having muscle Hg concentrations as high as or higher than any found in the fish from the Saskatchewan River. These results contradict earlier work (Kitmaru, 1968; Miettinen

et al., 1970b), which indicated that levels of Hg of this magnitude were associated with toxicity. As stated earlier, the association made between Hg concentrations in tissues and toxicity in the Japanese fish (Kitamaru, 1968) is somewhat tenuous, because the fish were wild, and the possibility of occurrence of other disease producing agents was apparently not eliminated. The method of dosing of fish in the experiments of Miettinen et al. (1970b) was to administer a large quantity of MeHg in split-doses over a 2 day period. The results of this acute administration cannot be compared directly either with those of experiment III or with a natural situation where Hg would accumulate more slowly. Despite the rapid rate of administration, 9 of 12 rainbow trout used by Miettinen et al. (1970b) survived the 94-95 day trial period without showing clinical evidence of disease. The average Hg concentrations in the muscle and brain of these fish at the end of this period were 12.6 and 10.2 ppm respectively.

9.3 Diagnosis of methyl mercury poisoning in mink

One of the objectives of experiment V was to determine the clinical signs and pathology of MeHg poisoning in mink, and to relate these to Hg intake and Hg concentrations in tissue; to provide the basis for the diagnosis of field cases of Hg poisoning in mink. The reasons for requiring this information have been outlined.

The differential diagnosis of Hg poisoning in mink should be based upon clinical evaluation, and pathological and analytical findings. The clinical signs alone could be easily confused with those of a number of neurological diseases. The pathology of MeHg poisoning in

mink is similar to that reported in other mammals. The presence of prominent perivascular cellular accumulations could be a source of confusion, and the lesions might be misinterpreted on casual examination as those of a viral encephalitis. The distribution and severity of lesions are related to the rate of intake of Hg, and so might be rather variable. The most susceptible areas of the brain appear to be the occipital region of the cerebral cortex, the red, fascicular and dentate nuclei, and the nucleus of the lateral lemniscus.

Brain and muscle are the most suitable organs for Hg analysis. Mercury concentrations of approximately 10 ppm or greater in these organs, in association with appropriate clinical and pathological findings, could be used as criteria for the diagnosis of Hg poisoning.

9.4 Ecological implications of aquatic mercury pollution

Mercury has become of concern as an environmental contaminant primarily because of the tragic human poisoning which occurred at Minamata Bay and the Agano River in Japan. Legislative action banning the use of fish from contaminated waters for human food has lessened the liklihood of future occurrences of this type, and a great deal of effort has been directed toward defining the toxicologic and epidemiologic risks of MeHg in fish for humans (Berglund et al., 1971).

The significance of Hg contamination for forms in lower trophic levels, or for fish-eating species other than man, has not received the same degree of attention.

As outlined in the review section, biological methylation of Hg is important for the conversion of relatively insoluble inorganic Hg to a more soluble form. Invertebrates aid this process by exposing Hg

buried in sediments (Jernelov, 1970, 1972a). The affect of MeHg on these invertebrates, and on vegetation is poorly known.

Hannerz (1968) compared the uptake of various Hg compounds by a variety of aquatic organisms. Plants, in general, concentrated Hg less than 100 times the levels found in water. Hannerz (1968) felt that this apparent concentration was largely the result of adsorption of Hg to the surface of the submerged portion of the plants. The invertebrates studied included molluscs, insects, and annelids. The molluscs (Planorbis and Lymnaea sp.) had concentration factors of about 3000. The predacious insect larvae generally had higher concentration factors than did detritus feeders; however, the highest concentration factor recorded (8,470) was found in a filter feeder (Corixa sp.). Hannerz (1968) concluded that successive accumulation in the food chain could not alone explain the differences in concentration factor between species at different trophic levels, and that other factors such as activity and metabolic rate might be important. Copeland (1972) reported average Hg concentrations of 0.44, 0.2, and 0.3 ppm in phytoplankton, zooplankton, and benthos material, respectively, from an "uncontaminated" lake. Fish (unspecified) from the same environment were reported to contain less than 0.01 ppm of mercury. These findings are unusual and would appear to indicate a situation exactly the reverse of accumulation through the food chain. Bligh (1971) reported mean Hg concentrations in broad trophic groupings. The values at these levels were: "algae eaters", 0.05 ppm; "zooplankton eaters", 0.04 ppm; "omnivores", 0.45 ppm, "detritus feeders", 0.54 ppm; and "predators" (which included

insect larvae and adults, and frogs), 0.73 ppm. These suggest some biological magnification. Johnels et al. (1968) analyzed samples of various aquatic organisms collected upstream from, and below a paper mill which used mercurial slimicides. Insects and annelids below the mill contained Hg concentrations approximately 50 to 200 times higher than did the same species collected upstream from the mill. Harriss et al. (1970) have shown that organomercurials at concentrations below 1 ppb in water inhibited photosynthesis by some species of phytoplankton and Harris (1971) speculated that this type of action of alkyl Hg could have a severe impact within an ecosystem, by removing portions of the food chain and thus altering the type and amount of food available for higher trophic levels. Thrasher and Adams (1972) found that MeHg at concentrations of from 40 to 140 ppb caused an increase in the generation time of a ciliate (Tetrahymena pyriformes). Approximately 50 per cent of the cells were killed within 1 hr in concentrations of 210 to 280 ppb MeHg, and 100 per cent death within 1 hr was observed in those exposed to 300 ppb MeHg. These authors also found that MeHg inhibited cilia regeneration in T. pyriformes. The toxicity of MeHg for this species under conditions of more long-term exposure were not investigated; however, an increase in generation time might reduce the population of this species and have an effect similar to that suggested by Harris (1971).

The results of experiment II indicate that MeHg concentrations in the range of 10 to 135 ppb in water are toxic for small fish. There are relatively few data available on the Hg content of contaminated waters; however, concentrations of MeHg of from 300 to 50,000 ppb

have been reported in waste water from factories (Irukayama et al., 1969; Nakamura, 1969). Voege (1971) reported on the analysis of 455 water samples from water bodies across Canada. Mercury concentrations greater than 1, 5, and 10 ppb were found in 28, 18, and 4 of these samples, respectively. The highest concentration found was 500 ppb in a single sample from the St. Clair River (Voege, 1971). These limited data suggest that concentrations of Hg similar to those found to be toxic to phytoplankton, zooplankton, and fish may occur in contaminated waters.

In general, it appears that higher Hg concentrations are found in predatory fish than in fish at lower trophic levels. Jernelov (1972b) determined the Hg concentration in muscle of pike, and of the fish (whitefish[1]) found in the stomachs of the pike, and of the bottom fauna in the stomachs of the whitefish. The average Hg concentration at the three trophic levels was 5.8, 3.1, and 0.3 ppm, respectively. Jernelov (1972b) calculated that the fish could only have retained a maximum of about 20 per cent of the Hg in their food, and that only a portion of the total Hg in the fish could be accounted for by Hg obtained from the diet; the remainder having been absorbed directly from the water. Jernelov (1972b) calculated that pike obtained approximately 50 per cent of their total Hg from the diet, while whitefish (the main prey of pike in the area) obtained only 10 per cent of their Hg content from this source.

Hannerz (1968) stated that accumulation by direct absorption from

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[1]Scientific name of this species not supplied.

the water was more important than that via the diet. The results of experiments II and III confirm that Hg can be accumulated rapidly from the water, and that accumulation via the diet is a somewhat slower process.

It appears that fish can accumulate and maintain large quantities of Hg in their tissues without suffering obvious ill effects. The possibility of more subtle and sub-lethal effects of MeHg on fish has received little attention. Lindahl and Schwanbom (1971) attempted to investigate this point, and found that even very slight exposure to MeHg resulted in a decreased ability of fish to perform in an elaborate testing procedure. The results of this experiment are somewhat questionable, however, since the testing procedure used was a very unnatural one, and a similar decrease in performance was noted in control fish. This was attributed to maintenance in captivity without food.

Kihlstrom et al. (1971) demonstrated that PHgA at concentrations of from 1 to 20 ppb decreased the numbers of eggs laid by zebrafish, and that the frequency of hatching of eggs was diminished in eggs spawned in water containing 0.2 to 1 ppb of the same compound. A similar effect on reproduction has not been tested for with MeHg.

The toxicity of MeHg for piscivorous animals, including man, at the apex of this aquatic food chain has been well established. Cats, crows, and fish-eating sea birds in addition to humans were poisoned at Minamata Bay, Japan (Takeuchi, 1968a). The results of experiment V confirmed that diets containing MeHg at concentrations similar to those found in fish from contaminated waters can result in poisoning.

Albanus et al. (1972) have demonstrated that there are no significant differences in toxicity between the MeHg present in fish from contaminated waters, and the simple MeHg salts used in many toxicologic studies.

Clear cut evidence of MeHg poisoning of fish-eating birds and mammals has not been documented anywhere but in Japan. This is probably the result of inadequate investigation. In general, the occasional death of a wild animal attracts little public attention, and it is only when a large number of individuals die simultaneously that specimens are submitted to a diagnostic laboratory. Because of the chronic nature of MeHg poisoning, and the long latent or subclinical period, massive "die-offs" due to Hg poisoning would not be expected to occur.

Despite the lack of evidence of mortality due to MeHg, there is abundant documentation of the occurrence of high concentrations of Hg in the tissues of aquatic birds (Table 29). The significance of these levels of Hg is unclear; however, Hg levels of about 20 ppm were found in the livers in red-tailed hawks which succumbed to experimental MeHg poisoning (Fimreite and Karstad, 1971). In addition to the data presented in Table 29, Hg poisoning has been suspected in Japanese storks (Muto and Suzuki, 1967) and concentrations of over 100 ppm Hg have been found in the tissues of dead bald eagles from the mid-western United States (Hazeltine, 1971).

A marked increase in the Hg in feathers of the white-tailed eagle, osprey, and great crested grebe has been noted over the period 1840 to 1966 in Sweden (Berg et al., 1966; Johnels et al., 1968). Borg et al.

TABLE 29. Mercury concentrations reported in the liver of aquatic birds.

Bird	Hg (ppm)	Author
Ducks	40.0 - 62.0[a]	Borg et al. (1969)
	.23 - 5.6	Dustman et al. (1972)
	.16 - .94	Fimreite et al. (1971)
	.06 - 1.98	Vermeer & Armstrong (1972)
Gulls and Terns	3.0 - 5.8[a]	Borg et al. (1969)
	.65- 39.0	Dustman et al. (1968)
Cranes	7.0 -17.0[a]	Borg et al. (1969)
Herons	1.75-175.0	Dustman et al.(1971)
	2.0 - 9.5	Faber et al. (1972)
Shore birds	0.2[a]	Borg et al. (1969)
	0.5 - 2.3	Dustman et al. (1971)
Raptores	9.0 -60.0[a]	Borg et al. (1969)
	4.6 -27.1[a]	Henriksson et al. (1966)

[a] - Birds found sick or dead.

(1969) reported residues of 3.5 to 11 ppm in addled eggs from the nests of white-tailed eagles and suggested that the decline of this species could be attributed to poisoning. Goosander eggs in Finland have been found to contain between 0.3 to 3.5 ppm Hg (Wahlberg et al., 1971).

High concentrations of Hg have also been reported in fish-eating mammals including fresh-water seals in Finland (Helminen et al., 1968), otter and mink in Sweden (Vostal, 1972), northern fur seals on the Pribiloff Islands and in Alaska (Anas, 1971), beluga whales in Hudson's Bay (Bligh, 1971) and ring seals from Hudson's Bay (G. Wobeser, unpublished data). No mortality of aquatic mammals due to Hg poisoning has been reported.

The overall assessment of the significance of Hg in the aquatic ecosystem is difficult, because the effects might occur at several levels such as the loss of some portion of the food chain, interference with reproduction, sublethal effects on behaviour or functional performance, or as chronic poisoning resulting in sporadic deaths. The assessment is further complicated by the simultaneous occurrence of several types of environmental contamination. For example, Faber et al. (1972) found significant residues of DDT compounds, polychlorinated biphenyls and Dieldrin as well as Hg in the tissues and eggs of common egrets and great blue herons which were experiencing reproductive problems. The relative importance of the various contaminants in such a situation virtually defies assessment with our present knowledge.

The interaction between various contaminants in the ecosystem may be very important. Corner and Sparrow (1956) have demonstrated a

synergistic effect between sublethal doses of copper and alkyl Hg for Artemia sp. Brown (1968) reviewed data on the toxicity of combinations of poisons for fish and concluded that in complex mixtures such as sewage effluent and those that occur in contaminated waters, the toxicity of the mixture was greater than the sum of the toxicities of the individual components. The evidence that synergism occurs between contaminants means that laboratory results obtained with single toxins cannot be directly extrapolated to the situations occurring in contaminated waters. Various contaminants may also have an antagonistic action, for example, the presence of chlorinated hydrocarbons decreases the methylation rate of Hg in water, thus resulting in lower Hg concentrations in fish in the water (J. M. Wood, 1970, personal communication). It is likely that a wide range of such interactions exist among environmental contaminants.

9.5 Long-term prospects for mercury contaminated waters

It appears that waters which have been contaminated with Hg will continue to yield fish with high Hg concentrations in their tissues for some time after Hg pollution ceases. Irukayama et al. (1969) described a rapid decrease in the Hg content of sediments in the discharge area from an acetaldehyde plant in Minamata Bay, Japan, after waste treatment facilities were installed. However, sediment samples from other areas of the bay were still rich in Hg, and these Hg-rich sediments were several meters thick.

Jernelov (1972) stated that a lake in Sweden which had received no industrial effluent containing Hg for over 45 years still had

high Hg concentrations in sediment and in fish. In other Swedish lakes Hg-rich sediments have been buried over a 25 to 30 year period and fish from these lakes have a relatively low Hg content (Jernelov, 1972). Jernelov (1972) concluded that the recovery of a water body likely depends upon the sedimentation rate, and that oligotrophic lakes will recover much more slowly than will eutrophic lakes.

There is no information available on which to base a prediction of what might occur in rivers, such as the Saskatchewan River. The concentration of Hg in fish from the Saskatchewan River had not changed significantly from 1969 to late 1972 (F. M. Atton, 1972, personal communication), although emission of Hg by the chlorine-alkali plant, felt to be the major source, virtually ceased in early 1970. The emission of Hg by major industries can be controlled relatively effectively, but the emission of Hg from the multitude of other potential sources is much more difficult to control, so that it is likely that pollution from these sources will continue.

10.0 SUMMARY AND CONCLUSIONS

Mercury contamination of the Saskatchewan River within the province of Saskatchewan was demonstrated. The concentrations of Hg in the tissues of fish from some areas of the Saskatchewan River were similar to those reported from industrially contaminated waters in other areas of the world.

Methyl mercury chloride at concentrations of from 10 to 135 ppb in water was toxic for rainbow trout fry and fingerlings within a 96 hr period. Trout exposed to these concentrations of MeHgCl rapidly accumulated large amounts of Hg in their tissues. Methyl mercury at these concentrations caused proliferation and desquamation of the epithelium of the gills. Concentrations of MeHg similar to those found to be toxic for young trout have been reported from Hg polluted waters.

Rainbow trout accumulated high concentrations of Hg in their muscle when fed diets containing MeHgCl. The Hg concentrations in the muscle of many of these fish were two to three times higher than those found in any of the fish sampled from the Saskatchewan River; however, no mortality which could be attributed to Hg intoxication occurred.

Morphologic lesions were restricted to the epithelium of the secondary lamellae of the gills. Rainbow trout given 10 to 20 mg Hg/kg as MeHgCl via intraperitoneal injection died within 24 hr. These fish

had degenerative lesions in the gills, posterior kidney, and pseudobranchiae. The findings suggest that the rate of uptake of MeHg is extremely important in determining the toxicity of the compound for fish, and that rainbow trout can tolerate a large body burden of Hg if this Hg is acquired over a period of time.

Adult and juvenile mink did not develop any clinical or morphologic evidence of Hg intoxication when fed diets composed of 50 and 75 per cent of fish containing 0.44 ppm Hg, for 145 days. Mercury concentrations in the tissues of these mink were substantially lower than those in mink poisoned with MeHgCl. Fish with mercury content similar to those used in the experiment could be used as a dietary constituent for ranch mink over a several month feeding period.

Diets containing from 1.1 to 15.0 ppm Hg as MeHgCl produced histopathologic injury to the nervous system of mink. Mink fed diets containing 1.8 to 15.0 ppm Hg as MeHgCl showed clinical evidence of intoxication within a 93 day experimental period. The rate of onset of clinical intoxication was directly related to the Hg content of the diet. Mercury concentrations in the tissues of mink which died of Hg intoxication were similar, despite differences in Hg content of the diets and survival time. The occurrence of similar Hg levels in tissues of mink from the various groups at the time of death suggests that these levels represent the critical level for MeHg poisoning. The average Hg content of the brain of mink which died was 11.8 ppm. The severity of injury to the nervous system was directly related to the Hg content of the ration. The occurrence of similar levels of Hg in tissue, but differences in the severity of lesions in mink from

different groups suggests that the rate of intake of mercury is important in determining the pathologic effects.

Methyl mercury poisoning in mink is characterized primarily by neuronal necrosis. Neurons in the occipital region of the cerebral cortex, and in the red, dentate, and fascicular nuclei, and in the nucleus of the lateral lemniscus appear to be most susceptible. In acute poisoning damage is more diffuse with variable degrees of involvement of all areas of the cerebral cortex, subcortical nuclei, and granular cells of the cerebellum. Degeneration of the myelinated tracts of the cerebellar peduncles, and of individual nerve fibres in the sciatic nerve also occurred in acutely poisoned animals. Accumulation of mononuclear cells in the perivascular spaces about cortical and subcortical vessels was a prominent feature of the disease. Because of the presence of these perivascular cuffs the lesions of acute methyl mercury poisoning could be mistaken on casual examination for those of a viral encephalitis.

The concentrations of Hg in the diets which produced intoxication in mink are similar to the range of Hg concentrations found in fish from the Saskatchewan River; indicating that Hg poisoning might occur in piscivorous animals dependent on fish from this river.

The overall assessment of the impact of Hg pollution on the aquatic environment will be difficult because the most important effects are likely to be those related to subtle changes in the ecosystem, and chronic toxicity for organisms at all trophic levels. Further research should be directed at the study of truly chronic Hg exposure, which would mean exposure of organisms to levels of Hg

which allow the establishment of an equilibrium between intake and elimination, so that constant body burdens of Hg are maintained over a long period of time. Research is also required to elucidate the interactions of various pollutants which occur as mixtures in polluted environments.

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APPENDIX A

TABLE 1. Common and scientific names of species

Fish and shellfish:	
Northern pike (pike)[a]	Esox lucius (L.)
Flounder	Pleuronectes flesus[b]
Sea perch	Serranus serranus[b]
Perch	Perca fluviatilis[b]
Eel	Anguilla vulgaris[b]
Rainbow trout	Salmo gairdneri (Richardson)
Lake trout	Salvelinus namaycush (Walbaum)
Goldeye	Hiodon alosoides (Rafinesque)
Sauger	Stizostedion canadense (Smith)
Walleye	Stizostedion vitreum vitreum (Mitchill)
White sucker	Catostomus commersoni (Lacepede)
Longnose sucker	Catostomus catostomus (Forster)
Northern redhorse	Moxostoma macrolepidotum (LeSeur)
Burbot	Lota lota (L.)
Yellow perch	Perca flavescens (Mitchill)
Chinook salmon	Onchorhyncus tshawytscha (Walbaum)
Freshwater drum	Aplodinotus grunniens (Rafinesque)
Zebrafish	Brachydanio zerio (Ham.-Buch.)
Cisco	Coregonus artedii (LeSeur)
Shore crab	Carcinus maena[b]
Mussel	Mytilus galloprovincialis[b]

[a]- Northern pike (pike). Same species referred to as pike in Scandinavian literature, northern pike in North American.

[b]- Authority not given for scientific name used by original author.

Birds:

White-tailed eagle	Haliaetus albicilla[b]
Osprey	Pandion haliatus[b]
Common egret	Casmerodius albus (Gmel.)
Great blue heron	Ardea herodias[b]
Great crested grebe	Podiceps cristatus (Lath.)
Japanese stork	Ciconia ciconia boyciana (Swinhoe)
Wood pigeon	Columba palumbus (L.)
Pheasant	Phasianus colchicus[b]
Red-tailed hawk	Buteo jamaicensis[b]
Goosander	Mergus merganser (L.)
Bald eagle	Haliaeetus leucocephalus (L.)
Japanese quail	Coturnix coturnix japonica[b]
Goshawk	Accipiter gentilis (L.)

Mammals:

Northern fur seal	Callorhinus ursinus (Walbaum)
Ring seal	Phoca hispida (Scopoli)
Freshwater seal	Phoca hispida saimensis (Nordq.)
Ferret	Mustela furo X M. putorius
Otter	Lutra lutra (L.)
Mink	Mustela vison (Schreber)
Beluga whale	Delphinapterus leucus (Pallas)
Rhesus monkey	Macaca mulatta (Zimmerman)
Squirrel monkey	Saimiri sciurius (Voigt)
White-tailed jack rabbit	Lepus townsendii (Bachmann)

TABLE 2. Reports describing the pathology of alkyl mercury poisoning in vertebrates

Species	Type of Intoxication (compound)	Author
Fish:		
species not described	Natural (MeHg compound)	Takeuchi (1968a)
Rainbow trout	Experimental, acute ($EtHgPO_4$)	Amend et al. (1969)
	Experimental ($MeHgNO_3$, and "protein bound MeHg")	Miettinen et al. (1970b)
Pike	Experimental ($MeHgNO_3$, and "protein bound MeHg")	Miettinen et al. (1970b)
Birds:		
Pheasant	Experimental ($EtHgPO_4$)	Leedy and Cole (1950)
Domestic fowl	Experimental (EtHg-p-toluene sulfonanilide)	Palmer (1963)
	Experimental (MeHg dicyandiamide)	Smart and Lloyd (1963)
	Experimental (diEtHg and $MeHgNO_3$)	Brown and Yoshida (1965)
	Experimental (MeHg dicyandiamide)	Tejning (1967)
Crows and sea birds	Natural (MeHg contaminated fish)	Takeuchi (1968a)
Many species	Natural (MeHg fungicides) Experimental (MeHg dicyandiamide)	Borg et al. (1969)
Goshawk	Experimental, secondary (MeHg dicyandiamide)	Borg et al. (1970)
Red-tailed hawk	Experimental, secondary (MeHg dicyandiamide)	Fimreite and Karstad, 1971)
Mammals:		
Mouse	Experimental MeHg contaminated fish, several MeHg and EtHg compounds)	Saito et al. (1961)

Species	Type of Intoxication (compound)	Author
	Experimental, prenatal ($EtHgPO_4$)	Oharazawa (1968)
	Experimental (MeHg contaminated fish)	Takeuchi (1968b)
	Experimental (EtHg-S-cysteine)	Mukai (1972)
Rat	Experimental (Na-EtHg thiosalicylate)	Powell and Jamieson (1931)
	Experimental (MeHgI, $MeHgNO_3$)	Hunter et al. (1940)
	Experimental, prenatal (EtHg-S-HgEt)	Matsumoto et al. (1965)
	Experimental, prenatal (MeHg)	Matsumoto et al. (1967)
	Experimental, prenatal (MeHg)	Nakamura and Suzuki (1967)
	Experimental, prenatal (MeHgCl, MeHg-S-Me)	Harada et al. (1968)
	Experimental (MeHg-S-Me)	Miyakawa et al. (1968)
	Experimental, prenatal (MeHgCl, MeHg-S-Me)	Moriyama (1968)
	Experimental (MeHg contaminated fish, and a variety of MeHg and EtHg compounds)	Takeuchi (1968b)
	Experimental, prenatal (MeHg-S-Me)	Deshimura (1969)
	Experimental (MeHg-S-Me)	Miyakawa et al. (1969)
	Experimental, prenatal (MeHg)	Nonaka (1969)
	Experimental (MeHg-S-Me)	Miyakawa et al. (1970)
	Experimental (MeHg dicyandiamide)	Cavanagh and Chen (1971)
	Experimental (MeHgOH)	Grant (1971)
	Experimental (MeHg-S-Me)	Miyakawa et al. (1971 a,b)
	Experimental (MeHgOH)	Nordberg et al. (1971a)

Species	Type of Intoxication (compound)	Author
	Experimental (MeHgCl)	Chang and Hartmann (1972 a,b,c)
	Experimental (MeHg dicyandiamide)	Diamond and Sleight (1972)
	Experimental (MeHg dicyandiamide)	Magos and Butler (1972)
	Experimental, prenatal (MeHgCl)	Nolen et al. (1972 a,b)
Rabbit	Experimental (Na-EtHg thio salicylate)	Powell and Jamieson (1931)
	Experimental (EtHg acetone)	Schmidt and Harzmann (1971)
Ferret	Experimental, secondary (MeHg dicyandiamide)	Hanko et al. (1970)
Cat	Experimental, prenatal (EtHg-S-Et)	Morikawa (1961a)
	Experimental (several EtHg compounds)	Morikawa (1961b)
	Experimental (several MeHg compounds)	Koya (1964)
	Experimental, prenatal (MeHgCl, MeHg-S-Me)	Moriyama (1968)
	Natural (MeHg contaminated fish) and Experimental (MeHg contaminated fish and several MeHg and EtHg compounds)	Takeuchi (1968 a,b)
	Experimental (MeHg contaminated fish and MeHgOH)	Albanus et al. (1971)
	Experimental (MeHg contaminated fish and MeHgOH)	Grant (1971)
Dog	Experimental (MeHg thioacetamide)	Yoshino et al. (1966)
	Natural (MeHg dicyandiamide)	Kahrs (1968)
Pig	Natural ($EtHgPO_4$)	Jubb and Kennedy (1963)
	Natural (MeHg dicyandiamide)	Kahrs (1968)

Species	Type of Intoxication (compound)	Author
	Experimental (MeHg dicyandiamide and EtHgCl)	Tryphonas (1968)
Sheep	Experimental (EtHg-p-toluene sulfonanilide)	Palmer (1963)
Cattle	Natural and Experimental (EtHg-p-toluene sulfonanilide)	Fujimoto et al. (1956)
	Natural (EtHg-p-toluene sulfonanilide)	Jubb and Kennedy (1963)
	Experimental (MeHg-2,3- dehydroxy propyl mercaptide)	Herigstad et al. (1972)
Squirrel monkey	Experimental (MeHgOH)	Grant (1971)
	Experimental (MeHgOH)	Nordberg et al. (1971)
Man	Accidental (EtHg-p-toluene sulfonanilide)	Brown (1954)
	Industrial ($MeHgPO_4$, $MeHgNO_3$)	Hunter and Russel (1954)
	Natural (MeHg contaminated fish)	Kurland et al. (1960)
	Natural (MeHg contaminated fish)	Takeuchi et al. (1962)
	Industrial (EtHgCl)	Hay et al. (1963)
	Iatrogenic (MeHg thioacetamide)	Tsuda et al. (1963)
	Iatrogenic (MeHg thioacetamide)	Okinaka et al. (1964)
	Natural, prenatal (MeHg contaminated fish)	Matsumoto et al. (1965)
	Suspected alkyl Hg (source unknown)	Ii (1966)
	Natural (MeHg contaminated fish)	Hiroshi et al. (1967)
	Industrial (MeHg dicyandiamide)	Prick et al. (1967)
	Natural (MeHg contaminated fish)	Takeuchi (1968a)
	Industrial (EtHg acetone)	Schmidt and Harzmann (1970)
	Natural, prenatal (MeHg contaminated fish)	Murakami (1971)
	Natural (MeHg contaminated fish)	Takeuchi (1972)

APPENDIX B

Median tolerance limits (TLm) of rainbow trout and fingerlings for methyl mercury chloride (MeHgCl) and mercuric chloride ($HgCl_2$)

Exposure (hours)	Trial No.	Fry (MeHgCl)	Fingerlings (MeHgCl)	Fingerlings ($HgCl_2$)
24	1	0.084	0.126	0.94
	2	0.083	0.126	0.91
	3	0.085	0.123	0.86
48	1	0.049	0.065	-
	2	0.046	0.067	-
	3	0.042	0.067	-
96	1	0.024	0.042	-
	2	0.025	0.043	-
	3	0.024	0.042	-

APPENDIX C

TABLE 1. Weekly weight gain (as a percentage of the weight at the beginning of the week) and mean amount of food supplied daily (as a percentage of total body weight), for trout in groups Control, I and II which received rations containing <0.1, 4 and 8 ppm Hg as methyl mercury chloride respectively.

	Control		Group I		Group II	
Week	Weight gain	Food supplied	Weight gain	Food supplied	Weight gain	Food supplied
1	7.1	3.1	5.8	3.1	6.1	3.1
2	6.9	4.0	4.6	3.2	5.9	3.4
3	8.1	3.3	6.3	3.0	5.8	3.5
4	6.3	3.0	6.4	3.3	6.9	3.2
5	4.7	3.3	8.1	3.7	8.2	3.1
6	8.2	3.7	7.2	3.1	7.2	3.7
7	8.1	3.8	8.3	3.3	6.3	3.8
8	10.1	3.9	8.9	3.5	4.8	4.0
9	5.4	3.1	7.9	3.1	11.1	3.0
10	7.3	3.2	6.9	3.8	8.2	3.1
11	8.1	3.6	8.0	3.2	11.4	3.5
12	7.2	3.1	7.1	3.1	8.1	3.2
13	6.0	3.0	7.3	3.4	7.4	3.3
14	9.0	3.6	6.7	3.6	7.6	3.6
15	7.9	3.6	-	-	-	-
Mean ± SD	7.4 ± 1.4		7.1 ± 1.1		7.5 ± 1.9	

TABLE 2. Weekly weight gain (as a percentage of the weight at the beginning of the week) and mean amount of food supplied daily, (as a percentage of total body weight), for trout in groups Control, III and IV which received rations containing <0.1, 16 and 24 ppm Hg as methyl mercury chloride respectively.

	Control		Group III		Group IV	
Weed	Weight gain	Food supplied	Weight gain	Food supplied	Weight gain	Food supplied
1	7.8	3.1	3.5	3.3	6.1	3.1
2	4.1	3.8	8.6	3.1	8.4	3.0
3	4.4	3.4	4.9	3.0	3.9	3.6
4	7.4	3.6	5.3	3.2	5.5	3.1
5	9.4	3.9	8.0	3.1	6.5	3.9
6	8.7	3.6	7.9	3.5	8.5	3.5
7	10.2	3.7	6.9	3.4	6.3	3.7
8	4.4	3.2	5.1	3.2	7.3	3.0
9	7.7	3.3	6.4	3.3	6.4	3.2
10	5.9	3.5	7.2	3.7	7.1	3.1
11	7.0	3.1	6.1	3.6	6.2	3.4
12	8.9	3.7	5.9	3.3	5.4	3.4
13	10.4	3.9	6.7	3.4	7.3	3.6
14	8.0	3.0	5.1	3.6	5.3	3.8
15	7.2	3.3	4.4	3.3	5.8	3.0
Mean ± SD	7.4 ± 2.0		6.1 ± 1.4		6.4 ± 1.2	

TABLE 3. Mercury concentration in the muscle of rainbow trout fed rations containing 4, 8, 16 and 24 ppm Hg as methyl mercury chloride respectively.

Week	Group I	Group II	Group III	Group IV
1	0.2	0.5	-	2.2
	0.5	0.6	1.0	1.3
2	1.2	1.0	2.9	2.3
	1.3	0.8	3.2	2.6
3	1.3	1.4	7.2	6.6
	1.8	1.3	6.0	6.3
4	1.1	1.9	5.9	4.3
	1.6	2.5	7.3	4.6
5	1.9	3.4	7.7	12.8
	2.3	3.9	9.8	7.7
6	1.9	4.2	9.8	14.5
	2.5	4.8	8.2	12.1
7	2.4	6.6	8.4	16.1
	2.4	6.5	11.4	18.2
8	3.6	6.8	13.0	12.7
	3.2	8.1	10.9	20.9
9	3.5	7.4	13.2	19.9
	4.0	8.0	11.3	28.4
10	3.8	8.7	12.9	22.9
	4.7	8.3	11.6	22.4
11	-	8.3	17.5	25.2
	-	10.3	12.3	25.3
12	3.7	10.0	15.3	19.3
	4.6	10.2	15.7	31.0
13	4.9	10.7	20.8	26.1
	5.6	13.0	15.0	26.5
14	5.9	12.3	23.7	28.5
	6.4	-	14.4	20.1
15	-	-	16.1	31.3
	-	-	14.9	-

APPENDIX D

TABLE 1. Type and incidence of lesions in the nervous system of mink which received rations containing 1.1 to 15.0 ppm Hg as methyl mercury chloride.

Group	Hg in ration (ppm)	Animal No.	Neuronal necrosis	Astrogliosis	Microgliosis	Capillary endothelial proliferation	Axonal degeneration	Myelin degeneration	Perivascular cellular accumulation	Peripheral neuropathy
II	1.1	1	+	+	+					
		2	+	+	+					
		3	+							a
		4	+	+	+		+	+	+	
		5	+	+	+					
III	1.8	1	+	+	+	+				+
		2	+	+	+	+	+	+	+	+
		3	+	+	+	+				a
		4	+	+	+	+	+	+		+
		5	+	+	+	+	+	+		
IV	4.8	1	+	+	+	+	+	+	+	+
		2	+	+	+	+	+	+	+	+
		3	+	+	+	+	+			+
		4	+	+	+	+	+	+	+	+
		5	+	+	+	+	+	+	+	+
V	8.3	1	+	+	+	+	+	+	+	+
		2	+	+	+	+	+	+	+	+
		3	+	+	+	+	+	+	+	a
		4	+	+	+	+	+	+	+	+
		5	+	+	+	+	+	+	+	+
VI	15.0	1	+	+	+	+	+	+	+	+
		2	+	+	+	+	+	+	+	+
		3	+	+	+	+	+	+	+	+
		4	+	+	+	+	+	+	+	+
		5	+	+	+	+	+	+	+	+

[a] - Not examined.

TABLE 2. Distribution and incidence of lesions in the nervous system of mink which received rations containing 1.1 to 15.0 ppm Hg as methyl mercury chloride.

Group	Hg in ration (ppm)	Animal No.	Cerebral cortex	Basal ganglia	Thalamus	Hypothalamus	Midbrain	Pontine nuclei	Gasserian ganglia	Cerebellum (granular cells)	Cerebellar nuclei
II	1.1	1	+								
		2	+ +				+				
		3	+ +								+
		4	+ +				+				
		5	+								
III	1.8	1	+								
		2	+ +				+	+		+	+
		3	+ +				+				+
		4	+ +								
		5	+								
IV	4.8	1	+				+				+
		2	+ +		+		+	+		+	+
		3	+ +								
		4	+ +		+		+	+			+
		5	+								
V	8.3	1	+		+	+	+	+		+	+
		2	+ +		+	+	+	+		+	+
		3	+ +		+	+	+	+		+	+
		4	+ +		+	+	+	+		+	+
		5	+		+	+	+	+		+	+
VI	15.0	1	+		+	+	+	+		+	+
		2	+ +		+	+	+	+		+	+
		3	+ +		+	+	+	+		+	+
		4	+ +		+	+	+	+		+	+
		5	+		+	+	+	+	+	+	+

TABLE 3. Incidence of lesions in the liver, kidney, and spleen of mink which received rations containing 1.1 to 15.0 ppm Hg as methyl mercury chloride.

Group	Hg in ration (ppm)	Animal No.	Hepatic vacuolar degeneration	Renal tubulonephrosis	Spleen Activation of white pulp
II	1.1	1			+
		2			
		3			+
		4			+
		5			
III	1.8	1			+
		2	+		+
		3			+
		4			
		5			+
IV	4.8	1			+
		2	+		+
		3			+
		4	+		+
		5			+
V	8.3	1	+	+	+
		2	+	+	+
		3	+		+
		4	+	+	+
		5	+	+	+
VI	15.0	1	+	+	+
		2	+	+	+
		3	+	+	+
		4	+	+	+
		5	+	+	+