1971

Mercury Contamination In Canada And Its Effects On Wildlife

Norvald Fimreite

MERCURY CONTAMINATION

IN

CANADA AND ITS EFFECTS ON WILDLIFE

by

Norvald Fimreite

Department of Zoology

Submitted in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

/

Faculty of Graduate Studies

The University of Western Ontario

London, Canada

December 1970

ABSTRACT

The Canadian consumption of mercury has increased sharply in the last decade. The major proportion of this increase can be accounted for by the chlor-alkali industry from which nearly 200,000 lbs. of mercury are released into the environment each year. The use of mercury compounds for slime control in the pulp industry is decreasing. Also decreasing, but still considerable, is the use of mercurials for control of soil and seed-borne diseases in cereals.

Alkyl mercury derivatives, especially those containing methyl mercury, are most common in seed-dressings. There is also evidence that inorganic mercury, which is the form of mercury that is released from the chlor-alkali industry, is changed to methyl mercury under anaerobic conditions.

An experiment conducted on ring-necked pheasants indicated that dietary methyl mercury has a strong adverse effect on reproduction, and increases the frequency of shell-less eggs. In another experiment red-tailed hawks were fed chickens raised on a diet containing methyl mercury, the mercury levels in the liver of the chickens varying between 3.9 and 10 ppm. Mortality or severe damage to the nervous system such as extensive demyelination was observed in the hawks, except for those on the least contaminated diet, after an exposure period of one month or more.

Elevated mercury levels were found frequently in seed-eating birds, rodents, and predators collected in Alberta and Saskatchewan. The source of contamination must be mercury-containing seed-dressings as seed-

eaters shot on treated fields had significantly higher mercury levels than those shot on untreated fields. Furthermore, it was found that both seed-eaters and predators from Alberta, a province with widespread use of mercury seed-dressings, had significantly higher residues when compared with those from Saskatchewan, where seed-treatment is less common.

Almost 100% of the fish collected in the Great Lakes, Baie de Chaleur, Ottawa River, and from Pinchi Lake in north-central British Columbia showed elevated mercury levels. Of these, the highest levels were recorded in those from Pinchi Lake, the St. Clair River, and Lake St. Clair with maximum concentrations in the muscle of 10.5, 7.09 and 5.01 ppm respectively. Fish-eating birds from Baie de Chaleur and Pinchi Lake carried similarily high mercury residues both in eggs and liver tissue. The high mercury concentrations in the fish and fish-eating birds are probably a result of contamination from chlor-alkali plants and pulp mills and in one case (Pinchi Lake) mercury mining.

The significance of mercury concentrations is discussed and it is concluded that reproduction may be affected in birds such as prairie falcons, merlins, and fish-eating birds as eggs of these species carried mercury levels that experimentally were shown to reduce hatchability in pheasants. Consumption of heavily contaminated fish may represent a health hazard to humans.

ACKNOWLEDGEMENTS

I wish to acknowledge the guidance and supervision provided by Dr. W. N. Holsworth during the course of this study and by Dr. J. A. George who stepped in as my supervisor while Dr. Holsworth was on leave of absence.

The topic of this thesis was suggested by Mr. J. A. Keith who also provided me with welcome guidance and constructive criticism throughout the study and during preparation of the thesis. I am grateful to Dr. L. Karstad and Mr. R. Fyfe for performing pathological examinations of test birds, and collecting predatory birds respectively, and to both of them for assistance with preparation of parts of the thesis.

I would like to thank Professor J. P. Walker for providing mercury-contaminated chicks for the experiment with red-tailed hawks and Dr. N. Platonow for conducting the mercury analysis. Thanks are directed to the following persons who assisted in collecting fish and bird specimens: Dr. S. U. Qadri, Mr. P. A. Pearce, Mr. D. V. Turner, Mr. D. Osmond, and Mrs. Iola Gruchy.

I express my thanks to Mrs. Elizabeth Kipp for assistance with the statistical analysis and to Mrs. Heather Redick for typing the thesis and for her many valuable suggestions as to the necessary improvements of the English.

To my wife, Solveig, who made some of the drawings in the thesis and assisted me on field trips, I express my sincere gratitude.

Finally, I wish to thank the Ontario Waterfowl Research Foundation

for making facilities available for the experiments at their Niska Waterfowl Research Station.

The study was financed by a contract with The Canadian Wildlife Service. The experimental work was also supported by a grant to Dr. W. N. Holsworth from The Canadian National Sportsmen's Show.

TABLE OF CONTENTS

Page

Page

LIST OF ILLUSTRATIONS

LIST OF TABLES

TABLE Page

APPENDIX II

GENERAL INTRODUCTION

Mercury is a heavy metal which occurs in trace amounts everywhere in nature, and according to King (1957) was one of the first to be discovered by the human race. The knowledge of the toxic properties of mercury and its compounds also has a long history. Both the toxic and therapeutic effects of the metal were known by the earliest medical authors and mercury compounds are said to have been used as drugs in Greece by Hippocrates in about 400 B.C. Some mercury preparations are used extensively in modern medicine and have become important for treatment of several diseases such as congestive heart failure and syphilis. Others have played an important role as diuretics, but because of the narrow margin between the effective therapeutic and toxic doses these have been replaced by other less toxic therapeutic agents (Bidstrup 1964). Much attention has further been paid to mercury as an occupational hazard in certain industries and mining operations. Mercury or its compounds are estimated to be used in at least 80 different industries and in more than 3,000 different ways, many of these acting as serious sources of exposure to factory workers and miners and thus giving rise to poisoning (King 1957).

However, it is less than two decades ago since it was realized that certain uses of mercury or mercury compounds may lead to general contamination of our biosphere with most serious consequences not only for man but also for wildlife.

The effects of mercury contamination on wildlife have been fairly well investigated in the Scandinavian countries, especially in Sweden where mercury contamination has produced more wildlife problems than has, for example, organo-chlorine pesticides. This contamination is ascribed to the industrial and agricultural use of mercury and mercury compounds.

Canada uses mercury compounds as does Sweden, for the same purposes, and frequently in larger quantities. In addition, Canada still uses alkyl mercury compounds which for some years have been banned in Scandinavia because of their hazards to man and animal life.

A comprehensive report on the high mercury content in wildlife in Sweden was published some five years ago (Borg et al. 1965, 1969). This report was based on the analysis of 1,500 specimens and was conducted at the State Veterinary Institute in Stockholm. In addition to animals shot, or trapped, a large number of specimens found dead on the countryside were investigated. A very high proportion of these were shown to be mercury poisoned, especially seed-eating and predatory birds. Mercury poisoning occurred also in red fox (Vulpes vulpes), marten (Martes martes), and polecat (Mustela putorius), but not in roe deer (Capreolus capreolus) and hares (Lepus spp). High level residues were most frequent in the predatory birds. In some localities, a low rate of reproduction was reported in the white-tailed eagle (Haliaeetus albicilla) and their eggs carried 3.5 ppm to 11 ppm of mercury. Experimentally, these levels were shown to effect reproduction in pheasants (Borg et al. 1965, 1969 and Tejning 1967a).

The mercury in wildlife was mainly ascribed to seed-dressings,

because of their widespread use in Sweden at that time, and also because the highest levels of mercury were found during and shortly after the seeding season. However, the mercury in the white-tailed eagle must have originated from another source, since it is a fish-eating bird and it is suggested that industry was a major source of mercury contamination in wildlife.

Elevated mercury levels in seed-eating birds in Sweden, especially pheasants, are also reported by Ulfvarson (1965) and Tejning (1967). Wanntorp et al. (1967) investigated the mercury levels in wood pigeon (Columba palumbus) before and after alkyl mercury compounds were banned as seed-dressings in Sweden, 1964 and 1966 respectively, and found that the mercury levels in this species had dropped considerably after the ban was carried through. Lihnell and Stenmark (1967) investigated mercury residues in rodents and found in these much lower levels than in seed-eating birds.

Berg et al. (1966) investigated the mercury content in the feathers of museum specimens of eleven different bird species collected over the last 100 years. Included were both seed-eating birds and predators. They found that for all species the mercury level was roughly constant in specimens from the period 1840 to 1940. In the 1940's concentration increased 10 to 20 times above the previous levels. At that time mercury seed-dressings and mercurial slimicides in the pulp and paper industry were introduced in Sweden, and the chlor-alkali industry began using mercury cathodes in their electrolytical processes. The highest mercury levels were found in white-tailed eagle and the lowest in pheasants and partridges.

Hasselrot (1967) kept young salmons in net cages upstream and downstream from pulp mills and chlor-alkali factories, and found up to 20 times as much mercury in the liver of fish kept downstream as compared with those kept upstream.

Hannerz (1967) conducted experimental investigations of the accumulation of different mercury compounds in water organisms. He found the accumulation rate in fish was fast, while the elimination rate was slow, especially for methyl mercury, for which concentration factors of 2,000 and 9,000 were found for water to muscles and water to kidneys respectively. When phenylmercury acetate is used Hannerz concluded that the biological half-life of mercury in muscles of fish (pike) is in order of 65 to 70 days.

Mercury occurrences in fish have also been studied by Johnels et al. (1967), Westöö (1967), Noren and Westöö (1967), Westöö and Noren (1967), and Westöö and Rydälv (1969). In many cases their findings could be connected with the sources of contamination; for example, pulp and paper mills and/or chlor-alkali factories. The reported concentrations were for the most part in the range of 0.2 to 1.0 ppm (in muscle tissue). About 1% of the total Swedish water areas is inhabited by fish with more than 1 ppm (Löfroth 1969). However, all waters have not yet been surveyed. It is now prohibited to sell or give away fish from waters where more than 1 ppm has been found. This limit has been widely criticized as being too high and the FAO/WHO suggests a tolerance level of 0.05 ppm in food.

Almost 100% of the mercury in Sweden's fish seems to be in the form of methyl mercury, even in cases where other forms of mercury have

been released into the water (Westöö 1967, Noren and Westöö 1967). Jensen and Jernelöf (1967) were able to show that unidentified microorganisms can methylate inorganic mercury. These findings were later verified by Wood et al. (1968) who also isolated a methanorganic bacterium (Methanobacterium omelianskii) capable of methylating mercury and found that methylation of inorganic mercury is a normal biological process which can occur in anaerobic ecosystems. This is important from an ecological viewpoint since methyl mercury is known to be highly toxic and stable in the body (Brown and Kulkarni 1965, Friberg 1959), and anaerobic or nearly anaerobic ecosystems frequently are found in heavily industrialized areas where mercury contamination most likely occurs.

Only few reports from outside Sweden on mercury levels in wildlife are available. Henriksson et al. (1966) found high levels of mercury in six white-tailed eagles from Finland (in liver from 12.2 to 27.1 ppm). These were brought in dead and the authors assumed that mercury had caused their death. They also connected the recent decline in the white-tailed eagle population with mercury contamination. There are also reports from Finland on high mercury levels in several species of fish (Häsänen and Sjöblom 1967) and in ringed seal (Pusa hispida) (Helminen 1968). The seals from Lake Saimen contained extremely high mercury levels of up to 200 ppm in the kidney. The contamination probably originates from Finland's considerable pulp and paper industry. Ash (1962) investigated seed-dressing residues in pheasant eggs from Britain, but only traces of mercury were found. Recent studies by Warren and Delavault (1969) revealed unexpectedly high concentrations of mercury of up to 5 and even 15 ppm in British garden soils; the

authors however, concluded that these must be of geological origin.

Perhaps the most serious consequences of mercury contamination have been reported from Japan (Kurland et al. 1960, Irukayama 1966). Releases of mercury from a factory using methyl mercury chloride as a catalyst in the production of acetaldehyde led to high amounts of mercury in fish and shell-fish, which after being eaten caused the death of 41 persons in the city of Minamata. Even congenital neurological injuries were described indicating that mercury penetrates the placenta barrier, later confirmed by Tejning (1968). A similar case was later reported from Niigata in Japan (Irukayama 1966). Not only human beings but also cats and rats feeding on shell-fish developed symptoms of mercury poisoning.

Westöö and Rydälv (1969) analyzed fish and crustaceans exported from several countries in Europe, South and North America, and Asia, to Sweden. Among these were three specimens of crayfish from Canada which had levels as high as those in crayfish from Sweden's "black-listed" lakes, about 0.5 ppm. This was not the case with crayfish or fish from other countries outside Sweden. Unfortunately, the location in Canada was not given.

With regard to mercury poisoning in man as a result of occupational exposure or therapeutic uses, the literature is reviewed by Bidstrup (1964). An excellent review of the metabolism of mercury and its compounds is further given by Brown et al. (1967).

The aim of the present study was to investigate the mercury levels in Canada's wildlife from selected areas where mercury contamination was expected and by experiments contribute to the understanding of the bio-

logical effects of mercury. The possible sources of contamination should be investigated.

The study includes a survey on mercury uses and releases into the environment. This survey was undertaken to provide information necessary to select relevant study areas and to decide which species should be investigated.

Experiments were conducted on ring-necked pheasants to study the effects of mercury seed-dressings on reproduction. Another experiment was conducted on red-tailed hawks to evaluate the effects of mercury found in raptorial birds.

On the basis of our knowledge on the distribution of mercury contamination wildlife specimens likely to be contaminated were collected, and analyzed for mercury. A small number of specimens from presumably uncontaminated areas were also investigated for comparison. As mercury seed-dresings and releases of mercury into streams and lakes from certain industries were shown to be the chief contaminators, attention was especially given to seed-eating birds and their predators, in addition to fish and bird species, which are known to prey on fish or other water living animals.

Parts of the thesis have been accepted for publication in various journals while other parts have been submitted for that purpose. The thesis has been prepared by presenting the various sections as they were submitted.

COMMENTS ON THE PROCEDURE

The study was financed by a contract with the Canadian Wildlife Service. According to this contract the wildlife specimens should be analyzed at Gulf General Atomic Incorporated, San Diego, California, by the neutron activation technique (see page 111).

It was unfortunate that due to financial limitations only 400 wildlife specimens from my collection of 630 could be analyzed, and so some sacrifice of the depth in the final results was unavoidable. Also it was not always possible to collect the desired number of specimens of selected species because of restrictions on the collecting permit, especially for some birds of prey.

In the experiment with pheasants and red-tailed hawks it was desirable to analyze tissue samples and eggs for mercury. I had the choice of either analyzing a restricted number of samples (about 50) by the neutron activation technique or five times as many by a dithizone technique (Oliver and Funnell 1958). The last alternative was chosen because this method had been successfully applied for determination of total mercury in animal tissue and its sensivity (0.2 ppm) was satisfactory for the purpose. The recovery of mercury added to liver tissue (2 - 50 mg/gram) was 97.5 - 99.0 percent (Appendix III). With the neutron activation technique no losses can occur since the sample is sealed in quartz (page 111). For animal tissues therefore these two methods should be agreeable for levels above 0.2 ppm.

MERCURY USES IN CANADA AND THEIR POSSIBLE HAZARDS AS SOURCES OF MERCURY CONTAMINATION

INTRODUCTION

Mercury has been known as a toxic agent since the time of the earliest medical authors, and as an occupational hazard, in certain industries, it has received much attention. On the other hand, the general contamination of the biosphere has only recently been recognized by ecologists.

The consumption of mercury and its compounds has since the last world war shown a strong upward trend, especially in such uses that empirically lead to considerable losses of mercury to the environment. A large percentage of these losses include highly toxic and persistent mercury compounds.

Mercury contamination has been fairly well investigated in the Scandinavian countries where mercury has produced more wildlife problems than have DDT and other organo-chlorine pesticides.

This study reviews the mercury uses in Canada for the purpose of evaluating their possible hazards as sources of environmental contamination, with special reference to effect on wildlife.

METHODS AND MATERIALS

Most of the information included in this report was gathered by mail surveys during 1968. Questionnaires were sent to industries likely to use mercury or mercury compounds in their processing including all pulp

and paper mills and chlor-alkali factories in Canada. Questionnaires were sent also to all importers of mercury-containing pesticides and to the Provincial Agricultural departments. The information obtained together with the statistics compiled by the Dominion Bureau of Statistics (DBS) and other available literature, provided the basic data for this study.

Answers were received from 80% of the pulp mills and 100% of the chlor-alkali plants and the Agricultural departments. About 80% of the importers were willing to give only limited information as to the quantities of mercurial pesticides imported. However, regarding mercury pesticides, the DBS pesticide statistics are informative and comprehensive as they are based upon reports from firms that are estimated to account for at least 95% of the total sales (D.B.S. Cat. 46 - 212).

CANADIAN CONSUMPTION OF MERCURY

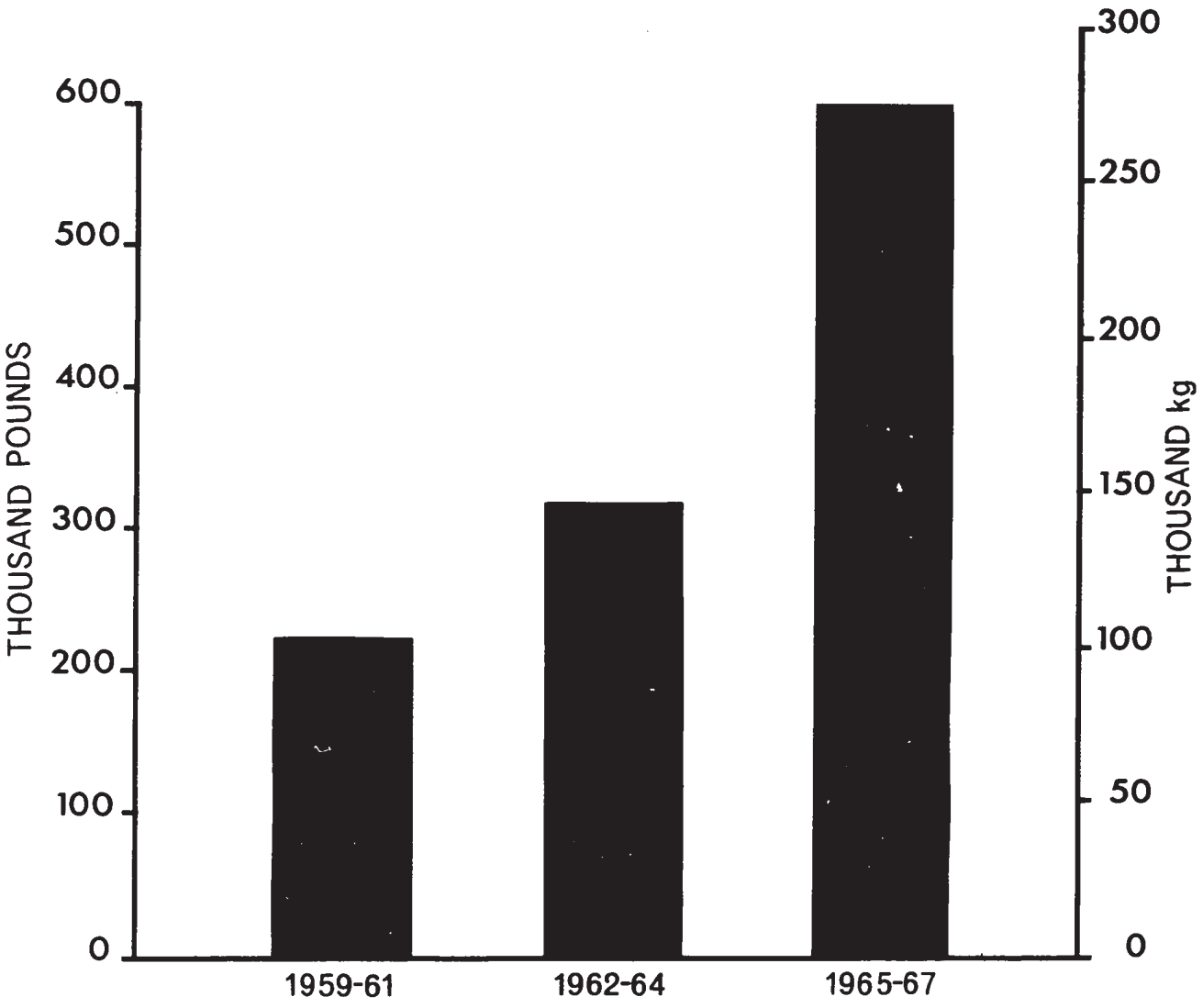
Canadian imports of mercury for the years 1959 - 1967 are shown in Fig. 1. The imports during these years showed a strong upward trend with 1965 being a boom year. That year more than one million pounds (450,000 kg) were imported largely to meet inventory requirements of new or expanded chlor-alkali plants (Anonymous 1968).

The consumption of mercury for the aforesaid period probably corresponds to the quantities imported as Canada had practically no mercury production of her own and only negligible quantities were re-exported, according to DBS mineral statistics.

The increase in mercury consumption in Canada has been considerable

Fig. 1. Mercury imported by Canada, 1959 - 1967. Each column represents the average for the period indicated.

Source: DBS import statistics.


600
500
400
300
200
100
0
THOUSAND POUNDS
300
250
200
150
100
50
0
THOUSAND kg
1959-61
1962-64
1965-67

when compared with the free world's corresponding growth which had averaged only 3% annually during the last decade (Anonymous 1968a).

In 1968 only 168,600 pounds (76,700 kg) of mercury were imported but in September of that year Cominco's old mercury mine at Pinchi Lake in the north-central part of British Columbia was brought back into operation. The prospective annual production at this mine is 1 - 1 1/2 million pounds (450,000 - 700,000 kg) of mercury when in full operation (Anonymous 1968).

Chlor-alkali industry

The most important consumer of mercury in Canada is undoubtedly the chlor-alkali industry. Production of chlorine and caustic soda is an electrolytical process, and until recently most plants used the so-called diaphragm cells which require no mercury. However, changing economics has favoured mercury cells, which employ a flowing cathode requiring large inventories of mercury; from 75,000 - 150,000 lbs. (35,000 - 70,000 kg) for a plant with a capacity of 100 tons of chlorine a day. The exact amounts depend upon cell design and operating conditions (Sommers 1968). Theoretically, no mercury is consumed in the production, but in any plant there are some losses, usually 0.5 lb. (0.2 kg) per ton of chlorine produced (Anonymous 1966, Murozumi 1967).

At the present time Canadian chlor-alkali plants probably hold almost two million pounds (900,000 kg) of inventory mercury and require about 200,000 lbs.(90,000 kg) annually as make-up; e.g.,to replace mercury losses (Anonymous 1968). Most of the lost mercury finds its way to streams and lakes. Traces of mercury are also carried to the atmosphere with hydrogen gas and ventilation system (20-40 mg/ton chlorine), while some mercury is

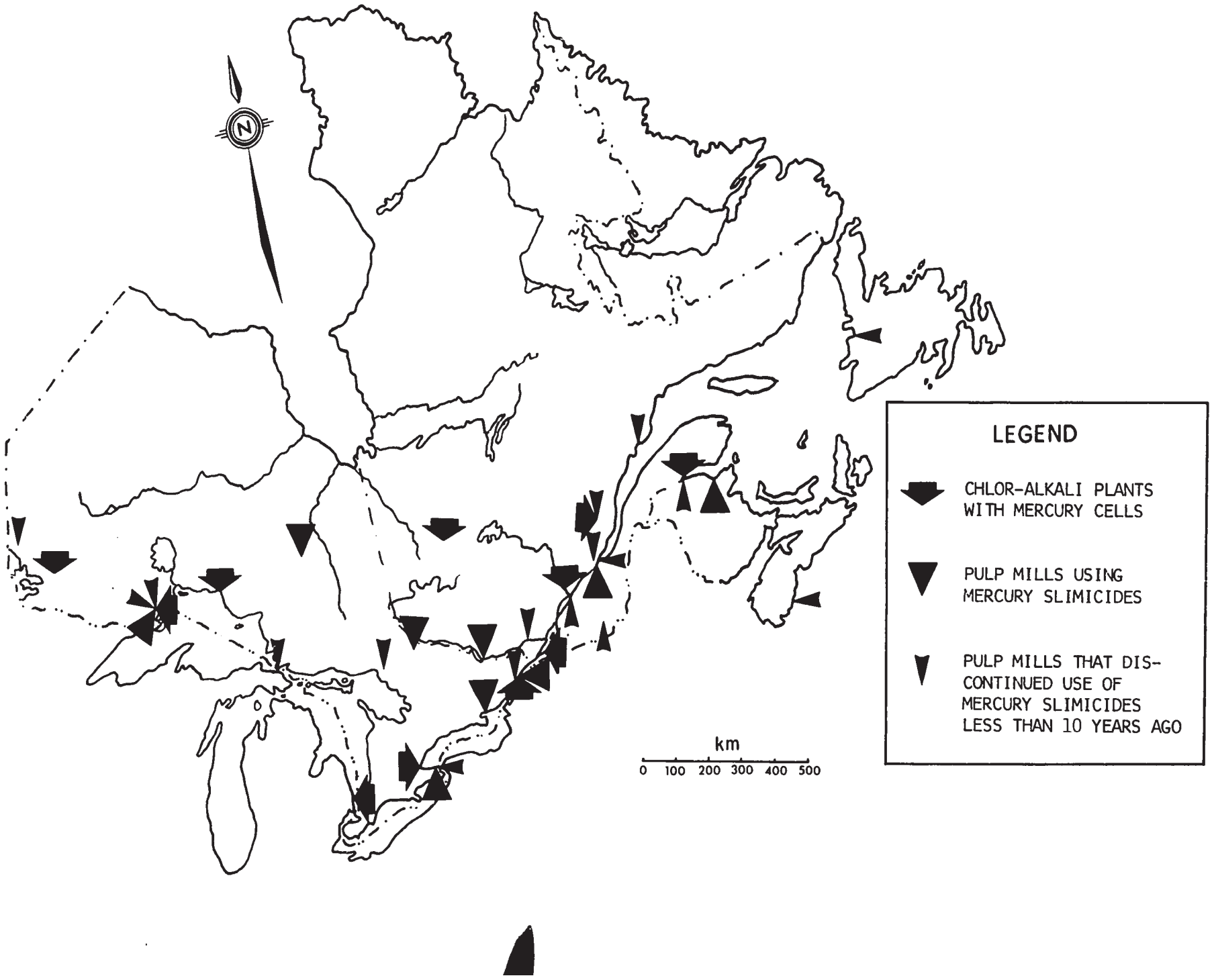
retained by the caustic soda usually less than 5 ppm (Bouveng & Ullman 1969 and Henry 1968).

Chlorine production has increased sharply in Canada in the 1960's and mercury cells have had a steadily increasing share of new capacity. More than 50% of the chlorine and caustic soda is now produced in mercury cell plants (Anonymous 1968), most of which started up during the last decade .

The price of mercury has increased sharply. For example, in the period 1964 - 1967 it rose from $250 - $510 per flask (34.5 kg). Due to the high cost of mercury, some chlorine producers have hesitated in building mercury cell plants. However, now that Cominco has up-dated their old mercury mine at Pinchi Lake, Canada need no longer depend upon European suppliers. This may result in a continued growth, perhaps at an accelerating tempo, of mercury-dependent plants. The switch to mercury cells is also encouraged by experts, the advantage being production of high quality caustic soda without costly additional equipment (Anonymous 1966).

On the maps (Figs. 2 - 3) one can see the distribution of chlor-alkali plants using mercury cells, and accordingly, we must expect mercury contamination to be more prevalent in the St. Lawrence River system. The abundant quantities of water will of course have a high diluting effect and only analysis of aquatic organisms and their predators can tell us to what extent we already have a serious mercury pollution problem. However, investigation by Hasselrot (1967) in Sweden revealed significantly increased mercury content in fish exposed downstream from a chlor-alkali plant, which had an estimated loss of 1,400 kg mercury into the water annually.

Fig. 2. Distribution of important sources of mercury contamination of water in Canada's eastern provinces.

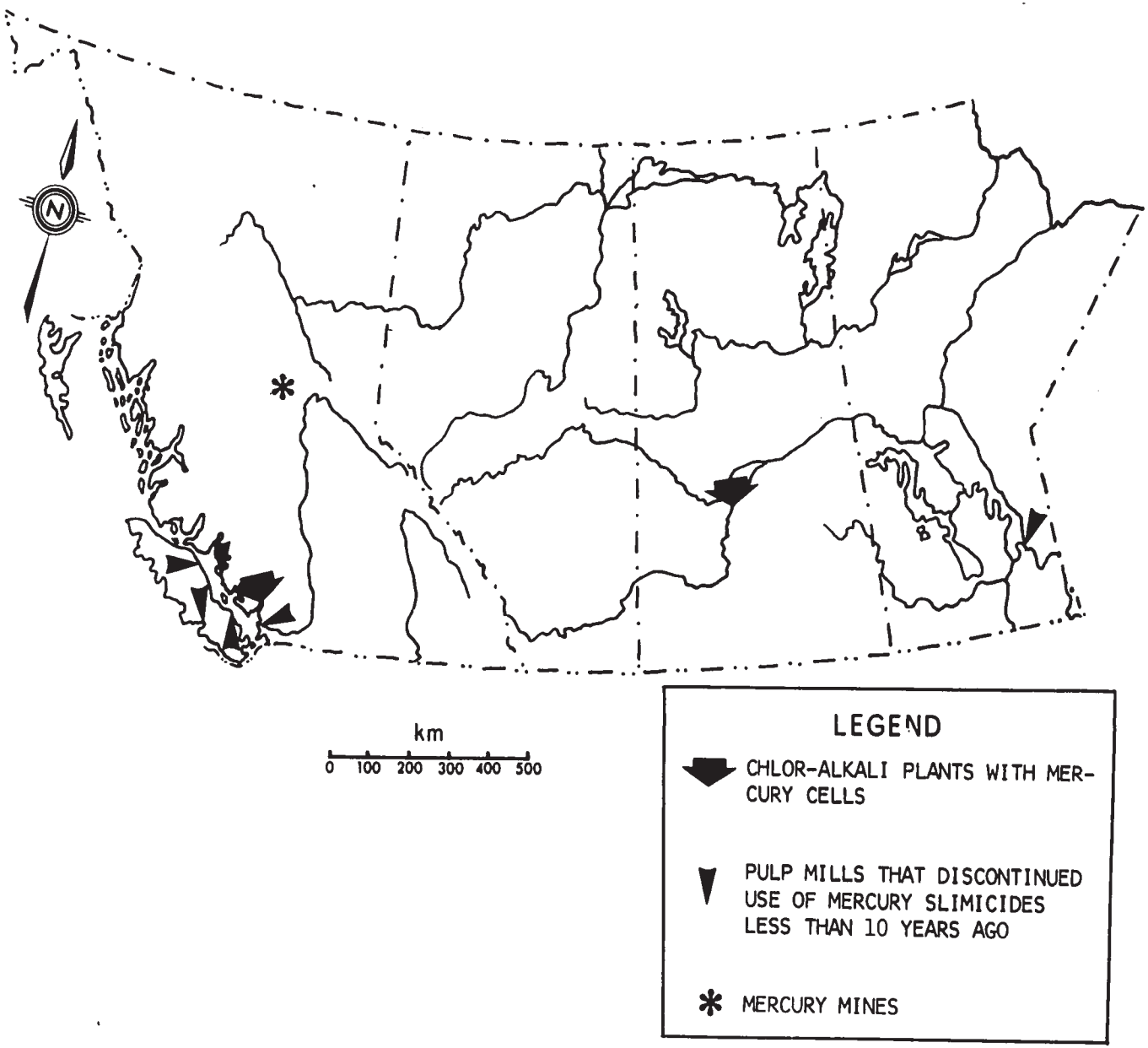

N
LEGEND
CHLOR-ALKALI PLANTS WITH MERCURY CELLS
PULP MILLS USING MERCURY SLIMICIDES
PULP MILLS THAT DISCONTINUED USE OF MERCURY SLIMICIDES LESS THAN 10 YEARS AGO
km
0 100 200 300 400 500

Only metallic mercury is used in mercury cells, but in waste waters mercury also occurs as chlorides. Metallic mercury can also be changed to the inorganic form under the influence of the electric tenstion difference that exists between the oxygen-rich surface water and the oxygen-poor layer near the bottom in the lakes. Interesting in this connection are the findings of Jensen and Jernelöf (1967), who showed that micro-organisms in the bottom mud are able to change inorganic mercury to methyl mercury. These findings have been verified by Wood et al. (1968) who also isolated a methanogenic bacterium capable of methylating mercury and found that methylation of inorganic mercury is a normal biological process which can occur in anaerobic ecosystems. This is important from an ecological viewpoint since methyl mercury is known to be highly toxic and stable in biological systems (Brown et al. 1967, Friberg 1959) and anaerobic or nearly anaerobic ecosystems frequently are found in heavily industrialized areas where mercury contamination most likely occurs.

Electrical instruments and apparatus

Mercury is considered important in the production of electrical instruments and apparatus, and in the U.S., for example, this industry was the largest consumer of mercury until recently (Anonymous 1966). In Canada, however, this industry has a more restricted capacity and the annual use of mercury for the purpose did not exceed 25,000 lbs. (11,000 kg) in the 1960's, according to DBS mineral statistics. We must also expect that only a minor percentage of this mercury would be released into the environment and thus, hardly be a serious source of contamination; although occasionally, it happens that this industrial use of mercury also causes problems (Landell 1968).

Fig. 3. Distribution of important sources of mercury contamination of water in Canada's western provinces.


N
km
0 100 200 300 400 500
LEGEND
CHLOR-ALKALI PLANTS WITH MERCURY CELLS
PULP MILLS THAT DISCONTINUED USE OF MERCURY SLIMICIDES LESS THAN 10 YEARS AGO
MERCURY MINES

Pulp and paper industry

Organic mercury compounds, particularly phenyl mercury acetate, have for a long time, been the predominating slimicides in the manufacture of pulp and paper. However, in order to conform with the Food and Drug Act of the U. S., where a large percentage of the Canadian pulp and paper products are being sold, most of the Canadian companies have discontinued the use of mercury-containing slimicides during the past ten years. Our mail survey indicated that only nine mills are now (1969) using slimicides, the active ingredient being phenyl mercury acetate, (Figs. 2 - 3). According to their information, a total of less than 30,000 lbs. (13,500 kg) of slimicides containing 10% phenyl mercury acetate are used by these companies annually. Only a minor part of the mercury (5% - 20% depending on mill operation) would reach the watercourse with the waste water. The rest will remain in the products (Bouveng 1967).

Since the pulp and paper industry is the chief consumer of caustic soda, which may contain as much as 5 ppm of mercury if produced in a chlor-alkali plant with mercury cells (Henry 1968), it might well be a significant contaminator even though no mercury slimicides are used. Further investigations, however, are needed before anything definite can be said.

There are several reports from Sweden of occurrences of high concentrations of mercury in fish and other aquatic organisms due to contamination from the pulp and paper industry. Hasselrot (1967) kept young slamon in net cages in a stream receiving discharge from pulp factories and found up to twenty times as much mercury in the liver of fish kept downstream as in those kept upstream after one month of exposure. High mercury concentrations were found in fish from many lakes and streams in the southern and central parts of Sweden (Johnels et al.

1967). Values as high as 9 mg/kg in the muscles were reported in cases where the chief source of contamination appeared to be mercury in waste water from pulp factories.

Since phenyl mercury acetate is the active ingredient in these slimicides, it is of interest to note that recent investigations in Sweden indicate that a change from phenyl mercury to methyl mercury takes place in water (Noren & Westöö 1967, Westöö & Noren 1967). High concentrations of methyl mercury in fish were reported where no methyl mercury had been released into the water, but where industrial wastes containing phenyl mercury were known to have been discharged. These changed are probably associated with micro-biological activities.

Seed protection

Seed treatment with organic mercury compounds for the control of soil and seed-borne diseases is widespread in Canada. Both cereals and flax seed are treated. Edgington (1967) listed the following diseases to be controlled by organic mercurials; common bunt in wheat, loose and covered smut in oats, covered smut in barley, and seed rot, and seedling blights in wheat, oats, barley and flax.

Mercury seed dressings are either used separately or in combination with an insecticide, usually aldrin or heptachlor, for the additional control of wireworms. They are formulated as liquids or powders, and recently drillboxes have come into use.

DBS compiles statistics of the annual sales of seed treatments. The sales figures for the period 1963 - 1968, given in TABLE 1, are based upon reports from firms that are estimated to account for at least 95% of the total sales.

TABLE 1.

Annual sale of mercury seed treatments in Canada.

Compounds \ Year	1963	1964	1965	1966	1967	1968
Organic mercurials						
Powders, lbs.	81,192	376,670	167,844	50,360	55,380	17,579
Drillbox, lbs.				310,393	170,760	174,018
Liquids, Imp.gal.	167,620	132,014	147,641	111,050	119,312	104,289
Organic mercurials and insecticides						
Powders, lbs.	131,670	96,505	179,678	28,504	25,394	10,632
Drillbox, lbs.				107,897	142,109	77,841
Liquids, Imp.gal.	41,183	25,251	62,251	39,131	20,107	30,172

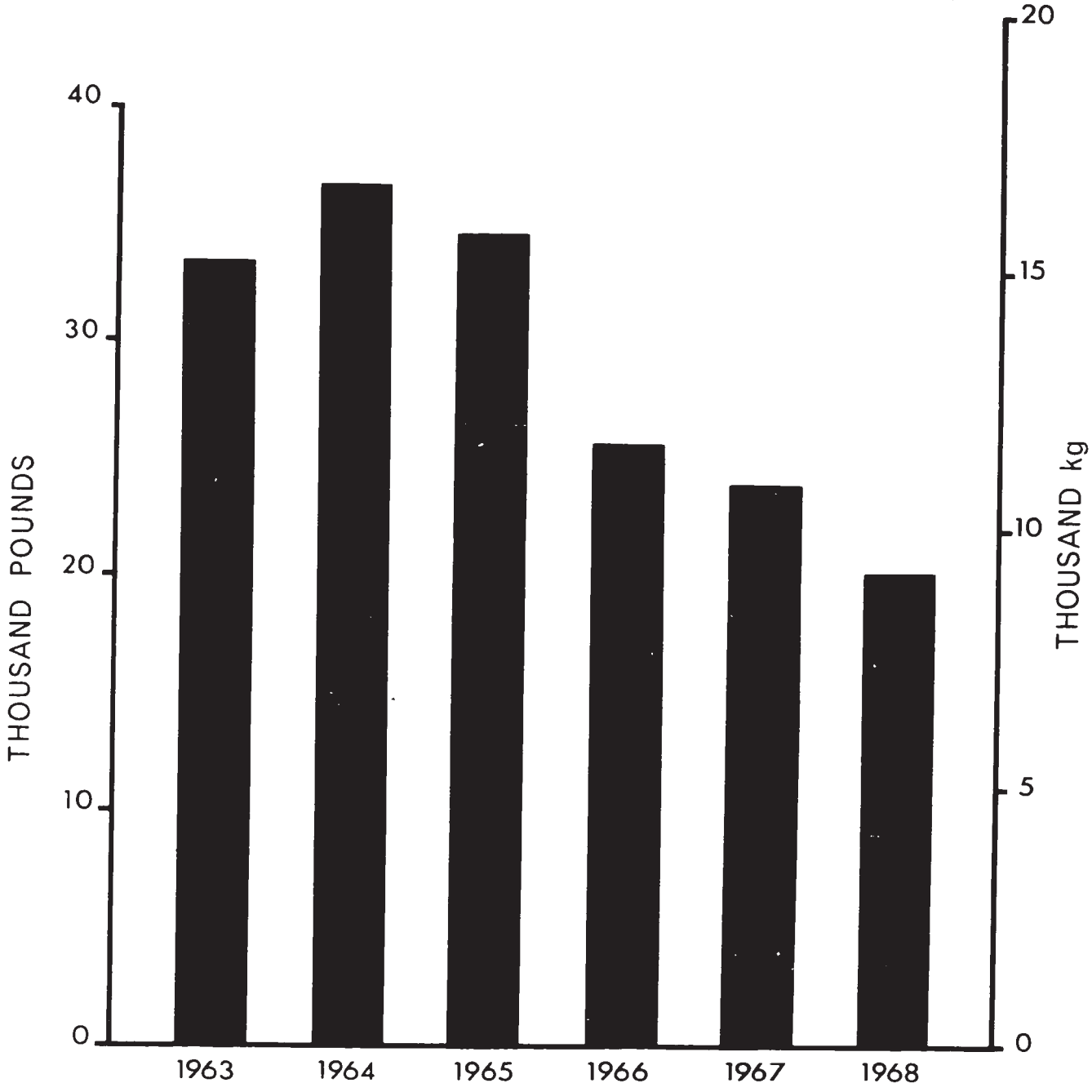
Source: DBS (Cat. no. 46 - 212)

There is a considerable variation in the concentration for mercury in the seed dressings (Smart 1968) and fig. 4, which shows the calculated amounts of mercury equivalent in seed dressings, may therefore give a better picture of the trend in mercury uses for this purpose. The calculations are based upon the percentage of mercury in the most common product within each formulation group and therefore are not exact, but since we do not know the quantities of all products sold, this may be the best way to determine the total mercury used in seed dressings.

Fig. 4 shows that use of mercury in seed treatments have had a downward trend, but the quantities used are still considerable. On the basis of the abovementioned sales figures, seeding rates, and recommended rates of treatment, the author has estimated that nearly 70% of all grain seed in Canada was treated with mercury compounds in 1967. This is equivalent to 30 million seeded acres.

Since wet or humid conditions favour growth of seed disease organisms, seed treatment seems to be more common in areas where we have such conditions. In Ontario it is estimated that almost 100% of the grain seed was treated in 1967 - 1968 (Fallis 1968), while in Saskatchewan, 40%, and in Alberta, 75% (Gurba 1968). The 1968 - 1969 figures for the Prairie Provinces indicate that the amount of seed treatment was 20% less than in the previous year (Vaartnou 1969). Irrigation is far more widespread in Alberta than in Saskatchewan, which may explain the difference between the two Prairie Provinces in this regard. Our mail survey conducted with the Provincial Departments of Agriculture, indicates that the most frequently used mercurial treat-

Fig. 4. Canadian consumption of mercury in seed-dressings, 1963 - 1968, estimated on the basis of DBS pesticide sale statistics and the concentration of mercury in commonly used seed-dressings.


THOUSAND POUNDS
40
30
20
10
0
THOUSAND kg
20
15
10
5
0
1963
1964
1965
1966
1967
1968

ments contain alkyl mercury; e.g., methyl mercury dicyandiamide (MMD) in Panogen. From a wildlife viewpoint this is an undesirable situation. Students in this field have shown that alkyl mercury, in addition to being more acutely poisonous than other organo mercury compounds, also are more slowly eliminated from the animal body (Bidstrup 1964, Friberg 1959). Kimura and Miller (1964) who studied degradation of different organo mercury fungicides in soils, found MMD to be the most persistent.

Alkyl mercury compounds are now banned as seed dressings in the Scandinavian countries, and in their place, the less persistent alkoxyalkyl mercury compounds have come into use. Wanntorp et al. (1967) investigated the mercury levels in wood pigeons before and after alkyl mercury compounds were banned as seed dressings in Sweden, 1964 and 1966 respectively, and found that the mercury levels in this species had dropped considerably after the ban was carried through. High amounts of mercury in seed-eating birds in Sweden before 1966 were also reported by Borg et al. (1965), Tejning (1967), and Ulfvarson (1965). There was a considerable variation in concentration of mercury with the time of year. Mercury was found more frequently and in higher concentrations during and after the seeding period.

Tejning (1967) maintains that the mercury in seed-eating birds such as pheasants originates from uncovered seeds picked up by the birds. Möller (1965) studied the sowing on 44 randomly chosen farms in Sweden with respect to the quantities of seed grain left on the surface, and found an average of 1.3 kg/hectare or 3.3 kernels/m^2 On the headlands, which were generally 10 - 15 m broad, he found

0.65 kg or uncovered grain per 100m, with the total quantity of uncovered grain seed averaging 0.9% of that seeded. I have seen no similar investigations from Canada, but because of minimal precipitation in the Prairie Provinces during the seeding season, the seed would probably be sown deeper here than in Sweden, resulting in a smaller quantity of uncovered seed. Another factor which draws in the same direction is that Canadian fields are larger and thus, the headlands form a smaller percentage of the total area. This, together with comparatively small seed rates and about 50% of the land on fallow each year, are all factors which should contribute to a smaller intake of treated grain seed by seed-eating animals. On the other hand, the author detected considerable amounts of spilled treated seed on many farms in Western Canada during the summer of 1968. This spillage was usually seen in or near the farmyards.

Horticulture

Mercury compounds are used both as foliar sprays and as aerosols in greenhouses. Examples of fungicidal diseases controlled by mercurials are scab in apples and pears, late blight in potatoes, and leaf mould in tomatoes. An excellent survey regarding the different mercury compounds used for the control of various plant diseases is given by Smart (1968). Our mail surveys directed to the Provincial Departments of Agriculture and the suppliers of pesticides, revealed that the quantities of mercury containing fungicides used for this purpose are rather limited in Canada. The use is almost entirely restricted to fruit growing districts in Ontario, British Columbia, and the Atlantic Provinces. Canadian studies (Ross and Stewart 1962, 1964, Stewart and Ross 1967) indicate that consider-

able residues of mercury accumulate in leaves, fruits, potato tubers and soils following application of this type of mercurial fungicide.

A review of the residues of mercury in fruits, vegetables, and other foodstuffs is given by Smart (1968). It appears that use of mercurial sprays frequently leads to residues that exceed the WHO/FAO proposed maximum acceptable residue level of 0.05 ppm.

Turf fungicides

DBS pesticide statistics do not give any information regarding mercurial turf fungicides, but in our mail survey pesticide suppliers indicated that the consumption is considerable. Thus, two firms in Ontario and Quebec reported selling 102,179 lbs. (46,000 kg) of mercury turf fungicides in 1967, containing a calculated amount of more than 6,000 lbs. (2,800 kg) of mercury (Hg). Golf courses are the predominant consumers of these fungicides, bowling greens and home lawns are others. The active ingredients are mercurous chloride, mercuric chloride, or phenyl mercury acetate. The mercury content varies between 6% and 73% in these highly concentrated products.

Frequent use of these turf fungicides may lead to considerable accumulation of mercury in soils since as much as 15 lbs. of mercury may be added to each acre following an application (Booer 1944).

Paints

Mercury compounds are widely used as anti-fouling and mildew-proofing additives in paints, and smaller quantities are often added as preservatives against bacterial attack during storage. The mercury-

containing paints are sold for both industrial and domestic purposes, although no statistics are available to show the quantities used for this purpose in Canada. However, in the U. S. the quantity exceeds the amount of mercury used in mercurial fungicides in agriculture (U. S. Bureau of Mines Statistics 1967). The mercury content of a commercial paint is about 0.05% and the compounds are more or less volatile and may cause air pollution (Taylor 1965). Several cases of poisoning in man due to this use of mercury compounds have been reported (Brown and Kulkarni 1967).

Other possible sources of contamination

The use of mercurials in the production of pharmaceuticals and dental preparations is important even though the consumption of mercury for this purpose in Canada comes to only a few thousand pounds annually.

The quantity of mercury used in amalgamation for the recovery of gold and silver is estimated by DBS to be about 3,000 lbs. (1,400 kg) annually.

Mercury is further known as an excellent catalyst in many chemical reactions; e.g., in the production of acetaldehyde and vinylchloride. The aquatic mercury contamination that caused the death of 41 persons in the Japanese city of Minamata was due to this use of mercury (Kurland et al. 1960). Because of the rapid growth of the plastics industry the use of mercury for this purpose should be surveyed and steps taken to restrict possible contamination. Minor quantities of mercury are vital for general laboratory use, in the manufacture of thermometers, in atomic reactors, and many other uses too numerous to mention here.

Mercury may also be set free and enter the biosphere by the burning of coal and other fuels known to contain traces of mercury (Stock and Cucuel 1934). Even sewage sludge may contain considerable amounts of mercury (Anderson 1967a), and if repeatedly applied as fertilizers or otherwise spread into the environment it may well lead to enrichment of mercury in soils and fauna.

THE NATURAL OCCURRENCE OF MERCURY IN CANADA

In an attempt to study the man-made mercury contamination, the natural occurrence or background level of mercury in the biosphere should be considered. Canadian studies have revealed high concentrations of mercury in soils and plants in British Columbia (Stevenson 1940, Warren et al. 1966). Soil containing 0.1 ppm of mercury may be considered normal (Erikson 1967, Stock and Cucuel 1934) but as much as 1 - 10 ppm has frequently been found in soils around the cinnabar deposits in British Columbia (Warren et al. 1966) where mercury occurs as sulphide, HgS (cinnabar). Considerable cinnabar deposits are found at Pinchi Lake, Kamloops Lake, Yalakon River, Bridge River, and Alberni Canal (Stevenson 1940). In the vicinity of gold, molybdenum, and base-metal deposits, examined by Warren et al. (1966), soils were found to contain 0.05 - 0.25 ppm and in a few instances as much as 2 ppm.

The amount of mercury in plants was related to that found in soils, although much variation was shown both between species and between different organs of the plants. Few, if any, similar analyses have been conducted on soils and plants in other provinces in Canada.

But, smaller amounts of mercury have been produced at some gold and molybdenum mines in Ontario. Many kinds of ores besides simple mercury ores may be highly enriched with mercury. Mercury may occur in many minerals of geochemically related metals (Goldsmith 1954). Of these, the highest concentrations are reported from argentiferous lead-zinc deposits (Hawkes & Williston 1962). There are several lead and zinc mining districts in Canada, with south-eastern British Columbia being the most important; others are found in Manitoba, Ontario, Quebec, New Brunswick, and Newfoundland. The limited information available thus indicates that the natural background levels of mercury in rocks and soils is subject to much variation. However, to what extent this variation is reflected in wildlife still remains to be investigated.

The probable occurrence of mercury contamination in air and water in the surroundings of Cominco's mercury mine at Pinchi Lake (Fig. 3) warrants analysis of fish and other organisms regularly, as this is a popular lake for sport-fishing and heavy contamination of the water may therefore have serious consequences.

CONCLUSION

It is evident that by far the largest quantities of mercury and mercury compounds are used for industrial purposes. The industrial and urban areas in Ontario and Quebec must account for at least two-thirds of all mercury used in Canada. In this area we must anticipate mercury contamination. The aquatic ecosystems are most likely to be contaminated, with the highest amounts of mercury appearing at the tops

of foodchains, in animals such as large predaceous fish, fish-eating birds and mammals. In the St. Lawrence River drainage we must also consider the releases of mercury from the U. S. side, which may be considerable because of highly industrialized and urbanized localities bordering the Great Lakes.

Lesser, but still considerable amounts of mercury are used in seed dressings. On the other hand, the mercury in these products occurs primarily in its most toxic and stable form, alkyl mercury. If sufficient quantities of the treated seeds are picked up by seed-eaters, a considerable wildlife hazard may develop. The accumulation of mercury in soils caused by the use of seed dressings is slow, with less than one gram of mercury being added to each acre of wheat field annually. This is comparable to the amount of mercury added to soils in precipitation (Anderson 1967) while the natural occurrence of mercury amounts to approximately 0.5 kg (1.1 lbs.) per acre (Erikson 1967). The accumulation of mercury following industrial uses may be more serious not only because of the higher amounts of mercury and mercury compounds being used and released but also because these relatively large amounts of mercury will enter a more distinct part of the biosphere. The resulting total mercury concentration will therefore be pronounced where such contamination occurs. The aquatic ecosystems exposed include multistep foodchains through which mercury accumulates (Hannerz 1967) and often reaches hazardous levels.

As pointed out earlier in this report, some mercury uses will lead to air pollution and through the air, mercury-containing gases may be spread even to remote areas.

SUMMARY

During the past ten years mercury consumption has shown a strong upward trend in Canada. The major proportion of this increase can be accounted for by the chlor-alkali industry, from which nearly 200,000 lbs. (90,000 kg) of mercury are released into the environment each year. Most of this mercury finds its way to watercourses exposing aquatic ecosystems where mercury is known to accumulate. The use of mercury compounds for slime control in the Canadian pulp industry, formerly an important consumer of mercury, is decreasing. Also decreasing is the use of seed-dressings containing mercury, although this use of mercurials is still considerable, and in lieu of findings in other countries elevated mercury levels in seed-eating birds and their predators, from districts with intensive grain farming, must be expected. The significance of other potential sources of mercury contamination including natural occurrences of mercury in soils and rocks is discussed.

EFFECTS OF DIETARY METHYL MERCURY ON RING-NECKED PHEASANTS, WITH SPECIAL REFERENCE TO REPRODUCTION

INTRODUCTION

Organic mercury derivatives are commonly used as seed treatments for the control of seed-borne cereal diseases such as smut and bunt in wheat, barley, and oats. Since these compounds appeared on the market about 50 years ago variations have been introduced (Sharvelle 1962). Among these are alkyl mercury derivatives which combine a strong, all-round effect against various types of seed-borne pathogens with a comparatively low phytotoxicity. Due to these advantages, the alkyl-mercury compounds primarily those containing the methyl homologue, have become extensively used for seed-treatment during the last two decades. From a wildlife viewpoint this is an undesireable development as these mercury compounds have shown to be highly toxic (Grolleau 1965), and stable in the body (Friberg 1959). Seed-eating birds and mammals pick up mercury from uncovered treated grain seed, and in turn, pass the mercury on to their predators. Borg et al. (1969) reported several cases of hazardous mercury levels in Sweden's wildlife ascribed to the use of seed-dressings containing alkyl mercury. High levels of mercury have recently been found in pheasants and partridges collected in the grain growing districts of Alberta. These findings will be reported in another paper (Fimreite et al. 1970).

In order to evaluate the significance of the residues of mercury found in wildlife, basic information, obtained in controlled experiments

is needed to understand the biological effects of mercury contamination.

One important aspect, in which little research has been done, is the effect of mercury on reproduction. Borg et al. (1969), using only one level of mercury treated grain, reported reduced hatchability in pheasant eggs after the hens received treated grain for only nine days. They also reported that in pheasant eggs collected from typical agricultural districts where there was widespread use of mercury seed-dressings, low hatchability occurred when incubated artificially. In chickens a more comprehensive study has been conducted by Tejning (1967a).

The ring-necked pheasant (Phasianus colchicus) was chosen for this study for several reasons: (i) it is a typical seed-eating species widely common to grain growing districts, (ii) a large percentage of its diet consists of grain, (iii) elevated mercury levels are frequently found in wild specimens, and (iv) it is an important game bird.

The aim of this study was to investigate the effects on reproduction of methyl mercury treated grain, added to the diet at specific rates and at certain intervals in the egg-laying season. The influence on egg production, shell formation, hatchability, embryonic and chick mortality will be discussed. General health observations and effects on food consumption are also reported, as such effects indirectly may have an influence on reproduction.

MATERIALS AND METHODS

Test Birds

The pheasants tested in this experiment were obtained as yearlings

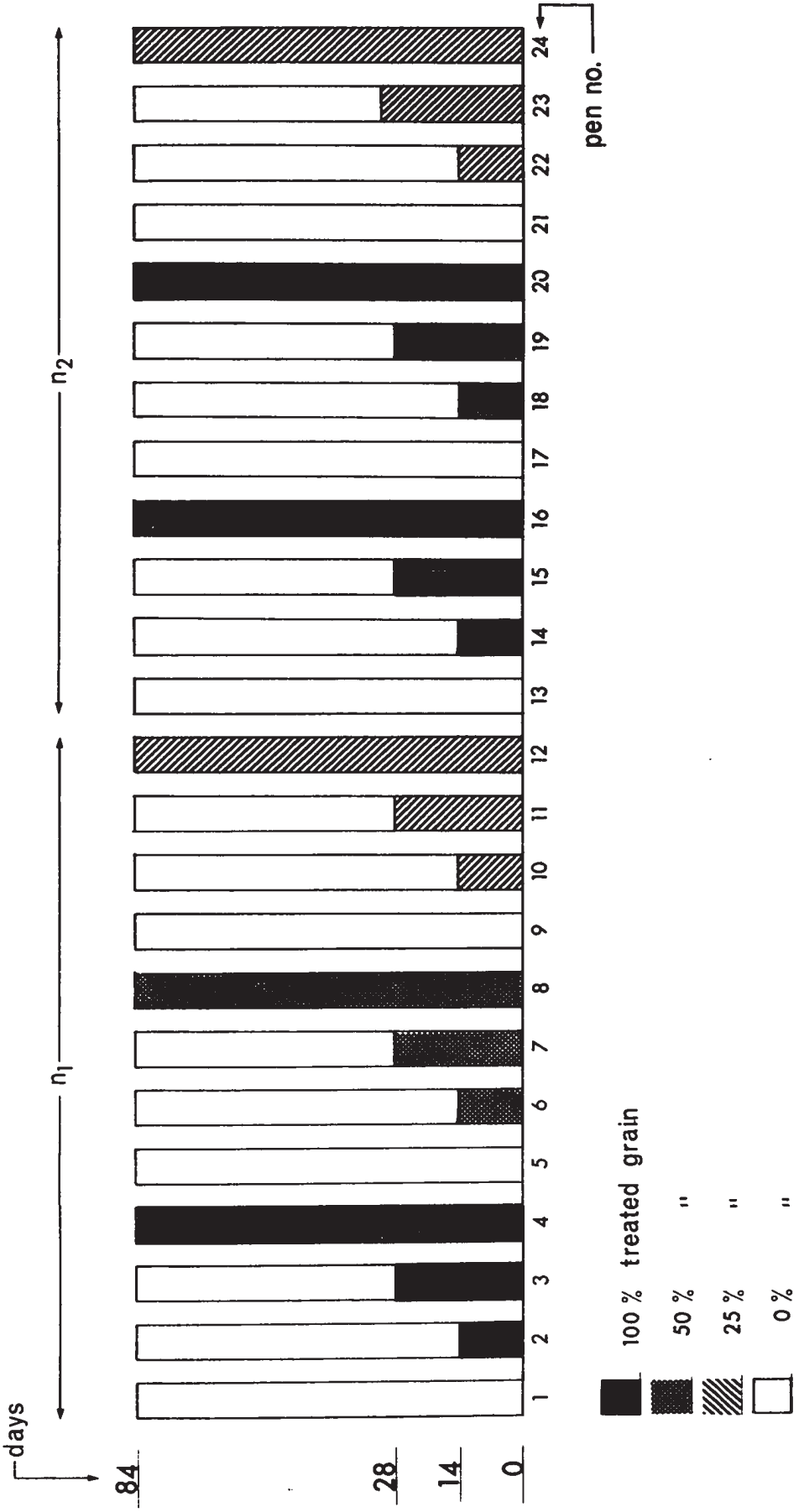
(hatched in June 1968) from Al Straibs Pheasantry, Aylmer, Ontario and taken to Niska Waterfowl Research Station, Guelph, Ontario, in January 1969, where the study was conducted. The pheasants were confined to two large pens, each 1,000 sq. ft., until they were grouped and put into their respective experimental pens. Until two weeks prior to the experiment the food consisted of 1/2 grain mixture (wheat and barley) and 1/2 CO-OP duck breeder ration (17% protein). During the final two weeks they were fed pure breeder ration. Both feed and water were given to the pheasants ad libitum until the experiment commenced.

The birds were weighed three times; shortly before the experiment, four weeks from the start, and at the time they were killed, 10 or 12 weeks from the start. Birds that died during the experiment were examined at the Department of Pathology, University of Guelph.

Design of the experiment

The experiment was designed as shown in Fig. 5. The variables involved were: (a) three different mercury levels; 100%, 50%, and 25% mercury treated grain in diet and, (b) three experimental feeding periods; 2, 4, and 12 weeks. Each of the nine experimental groups as well as the three control groups consisted of two sub-groups (pens) bringing the number of pens to 24. To each pen there were seven hens and one cock. In future reference the groups will be identified by proportion of treated grain in the diet (per cent) and the length of the period (weeks) fed treated grain. For example, the group that was fed 100% treated grain throughout the experiment (pen nos. 4 & 16) will be referred to as 100/12.

Fig. 5. Plan of experiment to show effects of dietary methyl mercury on ring-necked pheasants. Each group consisted of two subgroups (pens, housing seven hens and one cock) one from n_1 and one from n_2. Mercury levels in diet (% treated grain) and length of experimental feeding periods are shown.


days
84
28
14
0
n_1
n_2
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
pen no.
100 % treated grain
50 % "
25 % "
0 % "

The experiment lasted 12 weeks, April 4 to June 27, covering most of the egg-laying period.

The groups were weighed, and banded and placed in their assigned pens about two weeks before the experiment started in order to give them time to adapt to the new environment, and avoid disturbances when egg-laying commenced. Unfortunately, pens 21 - 24 could not be ready until 3 to 4 days prior to start of the experiment.

Each pen was 22 m^2 (2.2 x 10 or 3.3 x 6.6 m) divided by wire netting and roofed at one end to provide shelter.

Pens no. 1 - 15 had been used for pheasants prior to the experiment. Red-tailed hawks had been kept in pens no. 16 - 21, and pens no. 22 - 24 were new. All of the used pens were cleaned out before the experiment started.

Diet and feeding regime

The diet consisted of one-third pelleted pheasant breeder ration (18% protein) formulated by United CO-OPerative of Ontario, and two-thirds grain mixture (equal parts of wheat and barley). Fig. 5 shows the percentage and duration of treated grain in the diet. The daily ration per bird was 90 grams, weighed and portioned out each day. Every third day the feed left over was collected, and weighed in order to determine the food consumption. The feed was given in relatively deep, open feeders placed on trays (24" x 36"), enabling spilled feed to be picked up easily. Grit, oyster-shells and water were freely available.

The mercury compound

The seed-dressing was Panogen 15 containing 2.5% methyl mercury dicyandiamide, as the active ingredient (structural formula given in Fig. 6). It is a liquid treatment, applied to the seed by a machine process and probably the most commonly used in Canada. The recommended rate of treatment is three-quarters of an ounce per bushel; this corresponds to 12 mg mercury per kg of wheat and 15 mg mercury per kg of barley. The seed is usually treated in the late winter season, and because of the volatile characteristics of Panogen some mercury may be lost prior to seeding. The machine treated seed used in this experiment was bought from a grain elevator on March 25, and kept in sealed glass containers throughout the experimental period.

Collection and incubation of eggs

Eggs were collected at a fixed time (7 p.m.) each day, except on sunny, hot days when collection was done more frequently to avoid damage to the eggs. Frequent collection of eggs was also done in pens where egg-eating was observed. One average size egg from each pen was taken weekly for analysis. All other eggs, except the shell-less and broken ones, were incubated, weighed soon after collection, and stored at 15° to 18° C until incubation.

Two Jamesway 2940 incubators were used for both incubation, and hatching. The eggs were turned four times a day and the temperature was maintained between 37.1° and 37.4° C. Eggs were set every second or third day.

Fig. 6. The structural formula of methyl mercury dicyandiamide, the active ingredient in Panogen.

Chicks and unhatched eggs

The chicks were tagged with small numbered wing-tags shortly after hatching. One day after hatching the chicks were removed from the incubators and placed in brooders where the temperature was maintained between 27° and 30° C. All unhatched eggs were opened and the age of the dead embryos determined, according to Labisky and Opsahl (1958). Chick mortality was recorded for the first two weeks after hatching.

Determination of mercury

The mercury content in samples of eggs and liver was determined by Dr. N. Platonow at the Laboratory ot Toxicology, University of Guelph according to a method developed by Oliver and Funnell (1958) (Appendix III).

Statistical procedures

The variance analysis were performed according to Bone (1965). All percentages were arc sine transformed before being used as the basis for analysis. The significance of difference observed in egg weight was determined by t-tests.

RESULTS

General health observations

The mortality of the test birds is shown in TABLE 2. The results indicate that the mercury levels used in this experiment cause no increase in adult mortality. On the contrary no mortality was observed in groups that received 100% to 50% treated grain throughout the exper-

iment. In only one case could the diagnosis be mercury poisoning, a hen which had been fed 100% treated grain for four weeks. Egg peritonitis was registered in several cases. Other significant mortality factors were staphylococcal infections and pneumonia. Some of the birds that had been fed contaminated diet had extensive demyelination of the spinal cord.

The birds were infected by lice (Menacanthus stramineus), and in one case three pairs of gape worms (Syngamus trachea) were found. In all but two cases signs of sickness were observed only a few days prior to death. The exceptions were a hen from pen 1 and a hen from pen 22, in which weakness of the extremities, progressing slowly into ataxia, was observed for many weeks.

All of the cocks survived and remained healthy during the experiment with no apparent reduction in their capacity to copulate.

Most birds lost weight during the experiment (TABLE 2) with the average weight loss being less than 10%. In general, weight losses were most pronounced in the birds which were the heaviest at the start of the experiment. Some of the lightest birds gained weight during the experiment. There seemed to be no relationship between weight losses or gains and amount of mercury ingested.

Food consumption and intake of mercury

Although the statistical variance analysis (TABLE 7) revealed highly significant differences in food consumption, both with regard to dietary mercury levels and time of exposure, they are rather small in magnitude as indicated in TABLE 3 and Fig. 7. Group 100/12 was the

TABLE 2. Initial weight, mortality, and weight changes of pheasants fed methyl mercury treated grain.

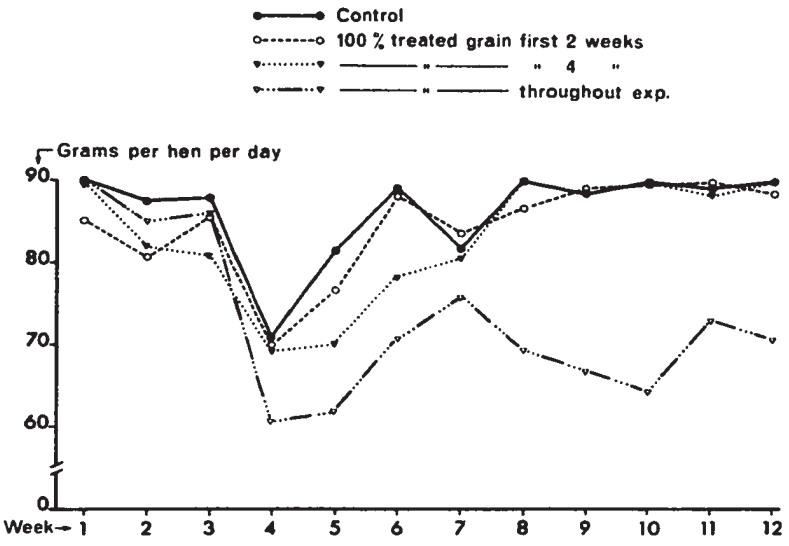
Per cent treated grain of total grain ration	Weeks fed Hg treated grain	Initial weight of birds (Grams) Mean	Initial weight of birds (Grams) Range	Mortality during experiment # of birds	Weight lost (-) or gained (+) during experiment (% of initial weight) Mean	Weight lost (-) or gained (+) during experiment (% of initial weight) Range
100%	0 (Control 1)	1414	1170 - 1815	3	-6.5	-19.3 - +11.1
	2	1337	1140 - 1745	2	-3.9	-16.3 - +18.4
	4	1386	1150 - 1820	2	-5.0	-18.9 - +13.9
	12	1462	1340 - 1780	0	-4.4	-12.9 - +13.9
50%	0 (Control 2)	1352	1018 - 1720	1	-6.1	-18.3 - +16.5
	2	1351	1090 - 1840	2	-7.1	-16.7 - + 9.2
	4	1334	1090 - 1740	0	-3.8	-21.2 - +18.6
	12	1468	1085 - 1950	0	-10.0	-20.3 - + 3.3
25%	0 (Control 3)	1364	1085 - 1920	0	-5.0	-24.5 - +11.7
	2	1381	1050 - 1950	2	-7.0	-23.3 - +17.6
	4	1318	1050 - 1670	1	-6.1	-18.7 - + 7.8
	12	1288	1060 - 1755	1	-4.2	-18.0 - +16.2

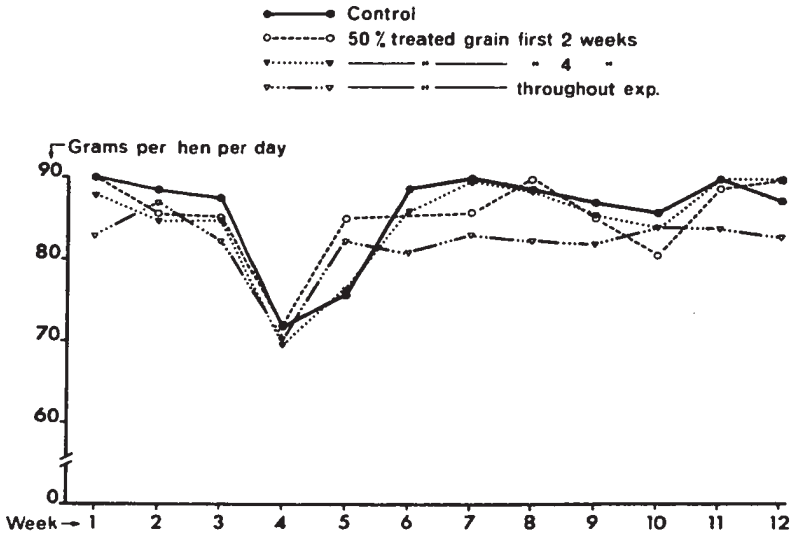
TABLE 3. Total food consumption, intake of mercury, and mercury residues in liver of pheasants fed methyl mercury treated grain

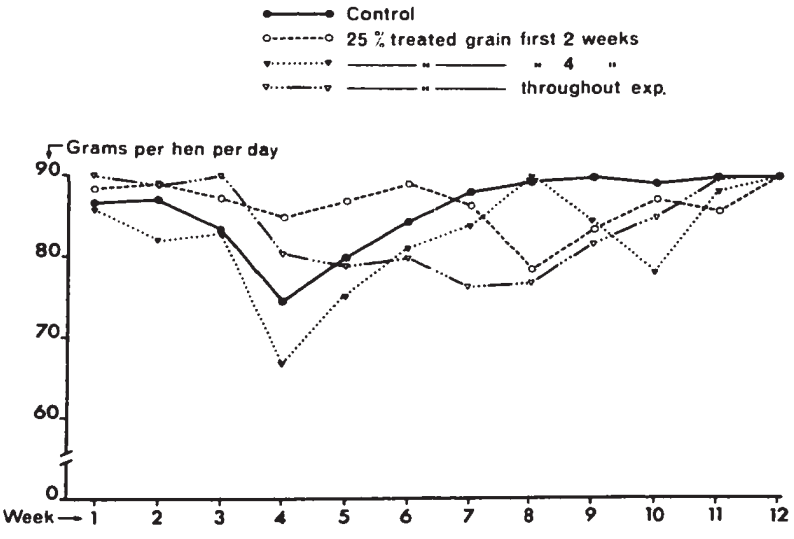
Hg treated grain in diet	Weeks fed Hg treated grain	Food consumption kgs/hen	Estimated consumption of mercury mg/hen	Mercury in liver* ppm Mean	Range
100% of grain ration	2	7.10	6.25	0.98	0,37 - 2,28
	4	6.98	12.38	4.44	2.20 - 6.80
	12	6.13	33.66	7.83	4.03 -13.70
50% of grain ration	2	7.18	3.38	0.59	0.34 - 1.11
	4	7.12	6.29	1.87	0.93 - 2.97
	12	6.88	18.91	4.47	3.36 - 7.62
25% of grain ration	2	7.26	1.71	0.68	0.00 - 1.55
	4	6.92	3.05	1.15	0.39 - 3.26
	12	6.43	9.69	1.86	0.59 - 5.08
Controls	--	7.23	--	0.09	0.00 - 0.66

* Based upon 5 specimens from each group (killed 10 weeks from start of the experiment).

Fig. 7. Weekly food consumption of pheasants receiving diets in which 100%, 50%, or 25% of the grain portion had been treated with MMD.


Control
100 % treated grain first 2 weeks
" " " " 4 "
" " " " throughout exp.
Grams per hen per day
90
80
70
60
0
Week→ 1 2 3 4 5 6 7 8 9 10 11 12


Control
50 % treated grain first 2 weeks
" " " " 4 "
" " " " throughout exp.
Grams per hen per day
90
80
70
60
0
Week→ 1 2 3 4 5 6 7 8 9 10 11 12


Control
25 % treated grain first 2 weeks
" " " " 4 "
" " " " throughout exp.
Grams per hen per day
90
80
70
60
0
Week→ 1 2 3 4 5 6 7 8 9 10 11 12

only one that differed significantly ($P < 0.01$) from controls (TABLE 8). The other groups consumed practically all the daily ration of 90 grams/bird, except during weeks 4 and 5 when food consumption declined in all groups, including controls.

Of the feed that remained in the feeders, there was more barley than wheat and usually very little of the breeder pellets. There was no detectable difference in consumption of treated versus untreated grain.

The total consumption of mercury of each group of pheasants is given in TABLE 3, based on the consumption of treated grain assuming that untreated grain, treated grain, and pellets were consumed in the same proportion as in the diet. As already indicated, this is not completely true, but since in most cases only a negligible amount of feed was not consumed, the figures should be accurate. It was impractical to select out the pellets and weigh grain and pellets separately.

Mercury residues in eggs and birds

Five hens from each group were killed ten weeks from the start of the experiment and the mercury level in the liver determined (TABLE 3). The levels although showing much variation, correlated well with the estimated mercury consumption.

The mercury levels in the eggs showed a great deal of variation and were relatively low. Even in the groups that received the largest amounts of mercury the mercury content in the eggs did not exceed 1.5 ppm. The mercury levels in the eggs from samples with a significantly decreased hatchability ranged from 0.5 to 1.5 ppm. The mercury levels in eggs seemed to reach a maximum after a feeding period of 4 - 7 weeks with

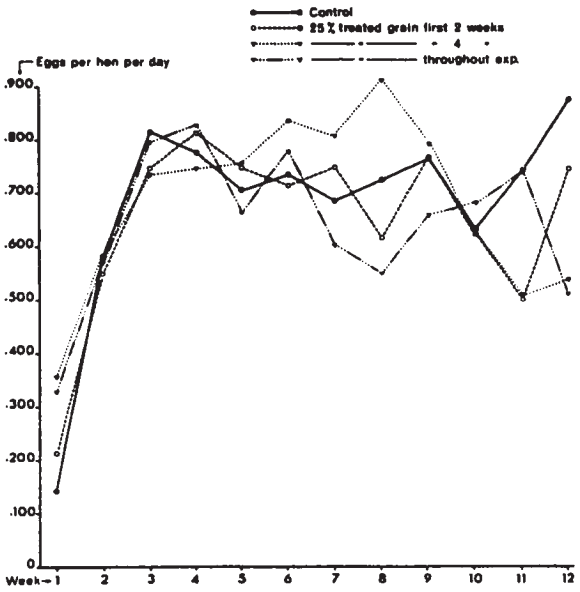
treated grain; the shorter the time period, the higher the dietary levels. After 4 - 7 weeks the levels decreased even though mercury feeding was continued. The hens that received treated grain only during the first two weeks of the experiment seldom showed detectable levels of mercury in the eggs during the last 2 or 3 weeks. The mercury levels in the eggs largely reflected those in the diet, although this relationship was not always clear.

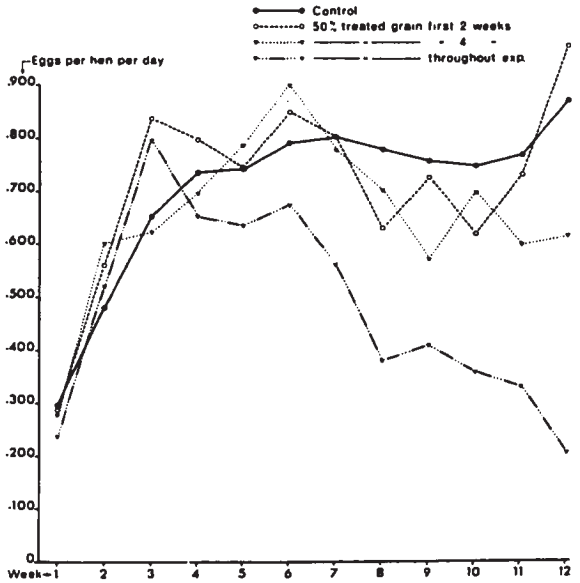
Egg production

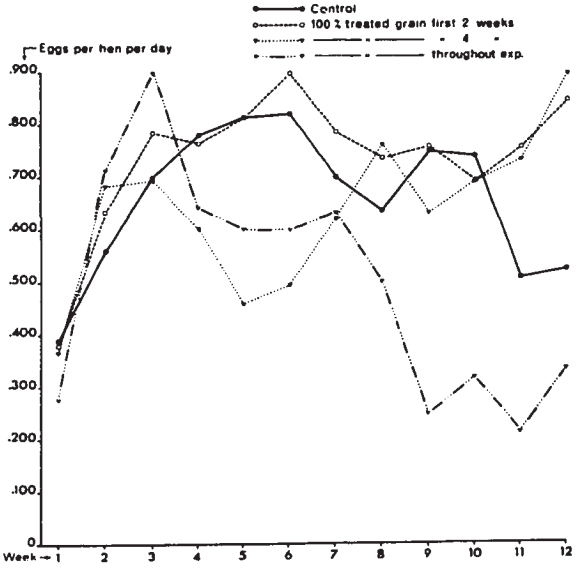
The total number of eggs laid in the different pens and groups is given in TABLE 6, and the weekly egg production during the experimental period in Fig. 8. The results are expressed as eggs per hen and eggs per hen per day respectively.

The over-all analysis of variance (TABLE 7) shows that there were statistical, significant ($P < 0.01$) differences in egg production due to the length of periods given contaminated grain, but not to mercury levels in the diet. However, there is a significant interaction ($P < 0.05$) between these factors. The length of time mercury was fed, and the weeks into the experiment showed significant ($P < 0.05$) interaction, but there are no significant second-order interactions. Comparisons with the controls (TABLE 8) indicate that only groups 100/12 and 50/12 are significantly different from the control groups ($P < 0.01$). Group 100/4 had a temporary decline in egg production, lasting for about four weeks (Fig. 8). After this time it gradually recovered and during the last two weeks even exceeded in egg production when compared to the control group. The observed decline in egg production was most pronounced

Fig. 8. Weekly egg production of pheasants receiving diets in which 100%, 50%, or 25% of the grain portion has been treated with MMD.


Control
25 % treated grain first 2 weeks
throughout exp.
Eggs per hen per day
.900
.800
.700
.600
.500
.400
.300
.200
.100
0
Week→ 1 2 3 4 5 6 7 8 9 10 11 12


Control
50 % treated grain first 2 weeks
throughout exp.
Eggs per hen per day
.900
.800
.700
.600
.500
.400
.300
.200
.100
0
Week→ 1 2 3 4 5 6 7 8 9 10 11 12


Control
100 % treated grain first 2 weeks
throughout exp.
Eggs per hen per day
.900
.800
.700
.600
.500
.400
.300
.200
.100
0
Week→ 1 2 3 4 5 6 7 8 9 10 11 12

one to two weeks after feedings with mercury contaminated grain were discontinued. When considering the low egg production in pen 22 it should be taken into account the factors mentioned previously; one hen remained sick for a long time until it died, and this together with pens 21, 23, and 24, were not ready until three days before the experiment started. All of these had a lower egg production than the other pens within the respective groups (pens 9, 10, 11, and 12).

In cases where egg breaking and subsequent egg eating were observed, collection of eggs was carried out more frequently. However, it was still impossible to prevent some eggs from being broken. The remaining shells made it possible to record these eggs, and they, together with the ones broken accidentally, are listed as broken eggs in TABLE 6. Egg eating was observed most frequently in pens 4, 18, and 24, and occasionally in pens 12, 20, and 23. Shell-less eggs had probably been eaten in pens 4 and 24 and very few shell-less eggs and a lower egg production were found in these pens when compared with pens 16 and 12 respectively. As there was no way to record the number of shell-less eggs eaten, this may account for lower recorded production of eggs within these groups.

Group 100/2 produced 11.4% more eggs than did the control group indicating that this level of dietary methyl mercury for a short period of time may stimulate egg production.

Egg weight and egg abnormalities

a. Weight

The mean egg weight of all except shell-less and broken eggs is

shown in TABLE 4. Student's t-test analysis detected highly significant differences between the most contaminated groups and their controls from week five and thereafter.

The influence of mercury was more pronounced in the groups receiving 100% treated grain, but during the last three to four weeks a significant reduction was quite common for the lower levels. The effect was shown after a latent period for the groups that received a contaminated diet during the first two or four weeks, producing eggs with reduced egg weight late in the experimental period.

b. Colour

Abnormal colour was found in a large proportion of the eggs from group 100/12, and to a lesser degree in groups 100/4 and 50/12. The colour appeared lighter (usually light-greenish or almost white) than a normal pheasant egg which is olive-buff. This phenomenon occurred after week 4. Fig. 9 shows eggs from pen 4 (most heavily contaminated group, 100/4) and pen (control) collected on May 7, compared to illustrate the differences in colour and size.

c. Shell-less eggs

A significantly increased number of shell-less eggs were laid by several groups on contaminated diet (TABLE 8) but, as shown in Fig. 10, groups 100/12, 50/12, and to some extent 25/12 were different from the others by producing an abnormally high number of shell-less eggs during the last four weeks of the experimental period. This indicates, and is confirmed by variance analysis (TABLE 7) that the duration of the exper-

TABLE 4. Mean weight ± standard error (grams) of eggs laid by pheasants fed methyl mercury treated grain.

	Mercury treated grain in diet (% of grain ration)									
	100%			50%			25%			
	Weeks fed methyl mercury treated grain									
Weeks into experiment	2	4	12	2	4	12	2	4	12	Controls
1	32.61 ± .447	31.78 ± .645	32.78 ± 1.002	30.82 ± .851	31.56 ± .543	32.00 ± .510	31.22 ± .411	32.51 ± .566	31.48 ± .540	31.96 ± .390
2	32.56 ± .434	32.57 ± .398	32.57 ± .435	30.89 ± .501*	31.85 ± .368	33.10 ± .431	31.36 ± .269	32.84 ± .403	31.94 ± .563	32.36 ± .320
3	32.24 ± .468	31.74 ± .339	32.25 ± .287	31.15 ± .395*	32.58 ± .575	33.71 ± .406**	32.66 ± .483	32.92 ± .332	31.87 ± .375	32.48 ± .331
4	31.69 ± .570	31.04 ± .422	31.45 ± .419	31.49 ± .356	31.63 ± .500	33.66 ± .362**	32.69 ± .384	32.56 ± .443	31.85 ± .314	31.81 ± .331
5	31.67 ± .569	30.33 ± .513**	30.50 ± .345**	32.03 ± .418	31.83 ± .524	32.79 ± .601	32.38 ± .280	33.18 ± .470	31.25 ± .351**	32.80 ± .297
6	31.96 ± .262**	30.49 ± .740**	30.10 ± .534**	32.86 ± .235	31.28 ± .315**	32.07 ± .477*	32.65 ± .453	32.51 ± .430	32.08 ± .303**	33.15 ± .235
7	31.87 ± .424**	30.73 ± .597**	29.94 ± .379**	32.31 ± .494	31.24 ± .294**	31.38 ± .677**	32.43 ± .476	32.60 ± .406	31.75 ± .485**	33.30 ± .241
8	31.62 ± .535**	30.15 ± .672**	29.75 ± .648**	32.70 ± .718	30.72 ± .377**	31.57 ± .738	32.38 ± .482	32.13 ± .382**	30.70 ± .395**	33.26 ± .278
9	31.41 ± .353**	28.63 ± .424**	30.27 ± 1.102	31.96 ± .380**	29.89 ± .380**	31.17 ± .760**	31.92 ± .545**	32.52 ± .537*	31.20 ± .320**	33.56 ± .286
10	32.43 ± .436*	28.73 ± .307**	29.13 ± 1.115**	31.56 ± .384**	30.12 ± .420**	30.53 ± .985*	32.21 ± .343**	32.85 ± .507*	31.00 ± .465**	33.56 ± .238
11	32.93 ± .340	28.66 ± .400**	29.00 ± 1.683**	32.14 ± .481**	30.09 ± .454**	32.57 ± 1.122	32.36 ± .769	33.31 ± .665	31.14 ± .347**	33.84 ± .294
12	32.38 ± .291*	29.18 ± .404**	28.00(1 egg only)	31.97 ± .506*	30.60 ± .502**	27.00(2 eggs only)	33.04 ± .766	33.35 ± .417	30.53 ± .425**	33.51 ± .397

* Significantly different from controls ($P < 0.05$)
** Significantly different from controls ($P < 0.01$)

Fig. 9. Effects of dietary methyl mercury on the size and colour of pheasants' eggs. The eggs marked 4 were laid by a group fed 100% treated grain through four weeks, whereas, those marked 5 were laid by control hens.

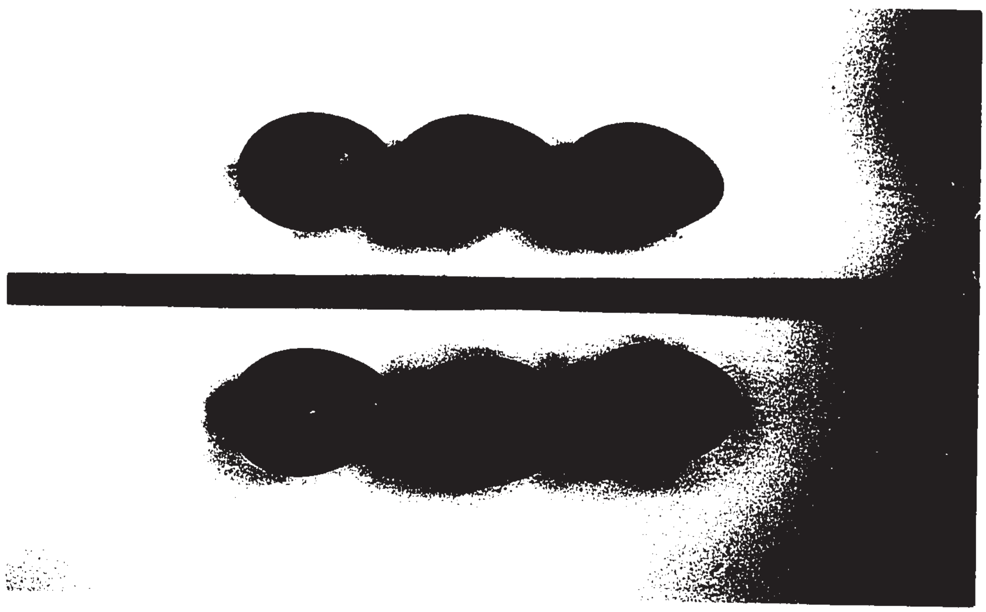
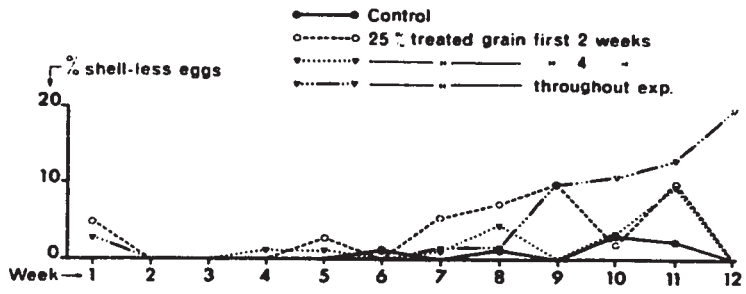
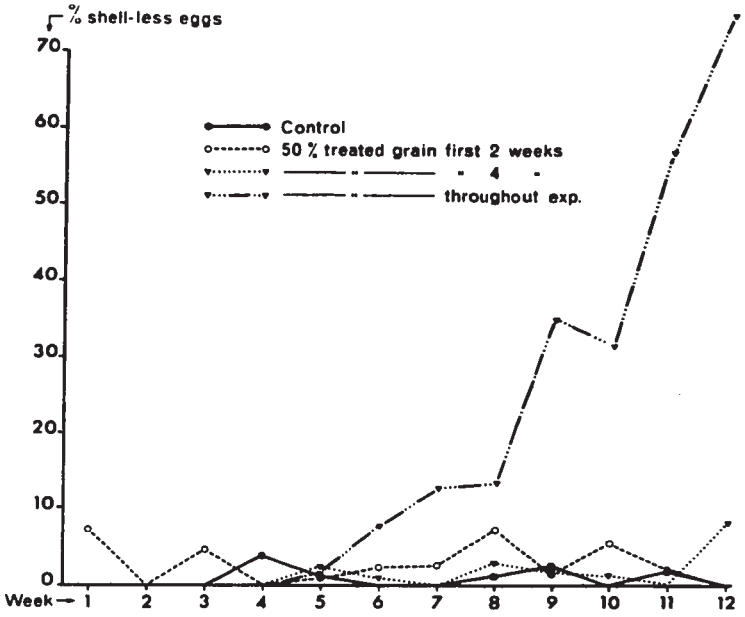
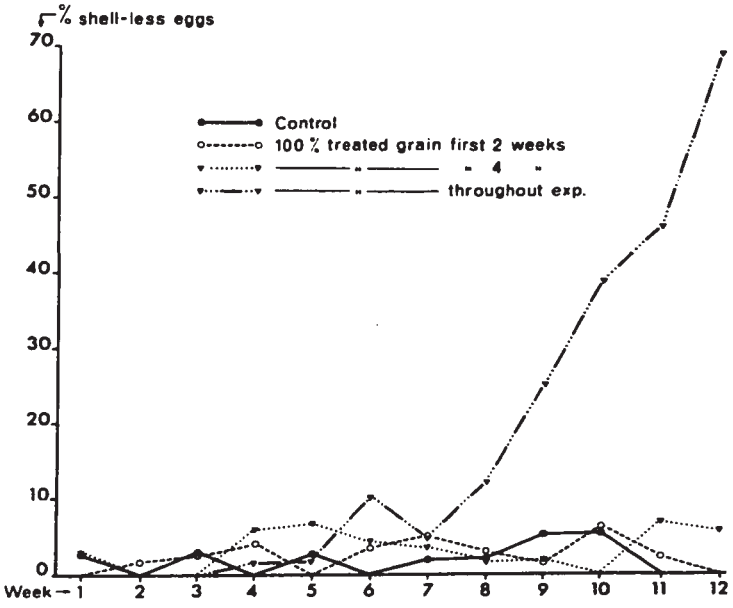


Fig. [illegible]. Effects of dietary methyl mercury on the size and colour of pheasant eggs. The eggs marked 4 were laid by a group [illegible] treated again through four weeks, whereas, those [illegible] were laid by [illegible].

Fig. 10. Frequency of shell-less eggs laid by pheasants receiving diets in which 100%, 50%, or 25% of the grain portion had been treated with MMD.


Control
25 % treated grain first 2 weeks
" " " 4 "
throughout exp.
% shell-less eggs
20
10
0
Week — 1 2 3 4 5 6 7 8 9 10 11 12


% shell-less eggs
70
60
50
40
30
20
10
0
Control
50 % treated grain first 2 weeks
" " " 4 "
throughout exp.
Week — 1 2 3 4 5 6 7 8 9 10 11 12


% shell-less eggs
70
60
50
40
30
20
10
0
Control
100 % treated grain first 2 weeks
" " " 4 "
throughout exp.
Week — 1 2 3 4 5 6 7 8 9 10 11 12

imental feeding period is the most important in order to introduce this effect.

It was deduced that shell-less eggs were eaten in pens 4 and 24 on a comparatively large scale and according to this the production of shell-less eggs would be more pronounced than the results given in TABLE 5, and Fig. 10 indicate.

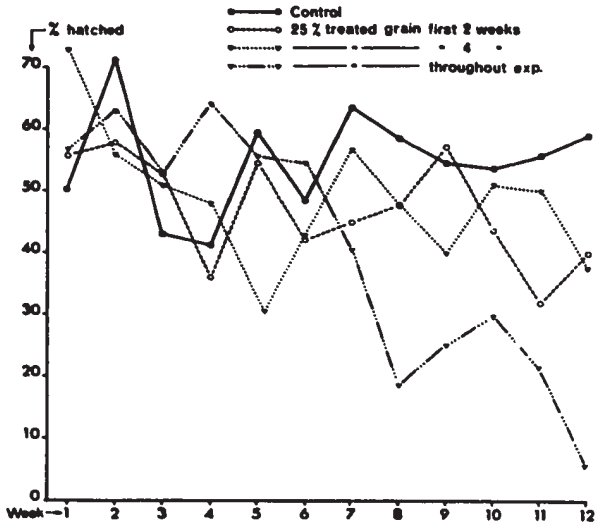
Hatchability

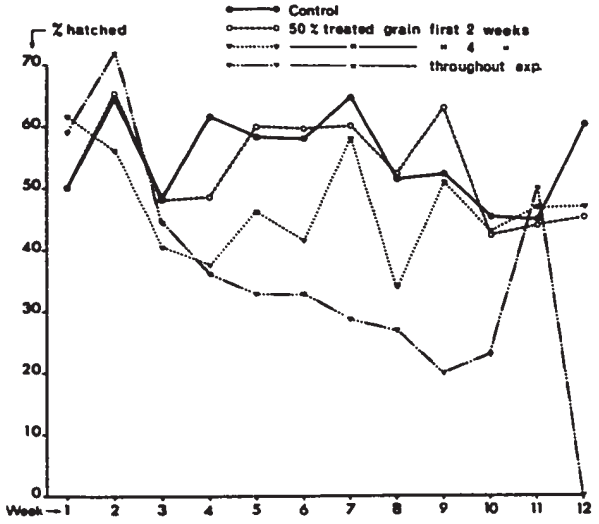
Analysis of variance (TABLE 7) of the data presented in TABLE 6 and Fig. 11 indicated a difference in hatchability both among mercury levels and duration of time fed mercury treated grain ($P < 0.01$), and as shown in TABLE 8 all groups fed mercury throughout the experiment plus group 100/4, differed significantly from the control ($P < 0.05$). Group 100/4 had a very pronounced, temporary decline in hatchability, maximized 1 - 2 weeks after mercury feeding had been discontinued. Temporary decline in hatchability was also seen in groups 100/2 and 50/4, but this decline was not significant at the 5% probability level.

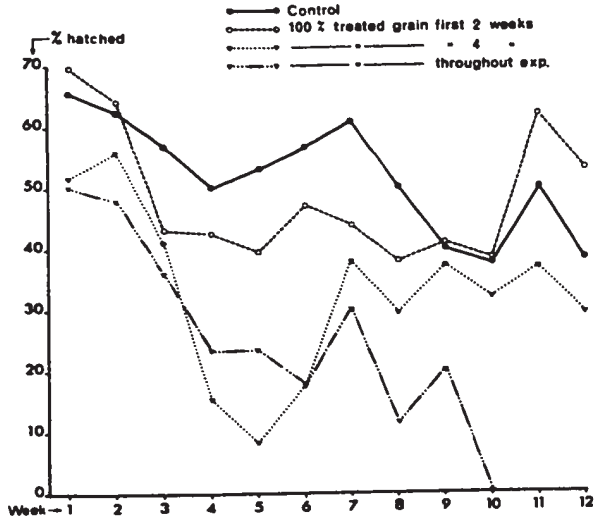
The over-all hatching percentages in the control groups vary between 52 and 55, which may seem to be low; however, in this study hatchability is expressed as per cent of incubated eggs, not of fertilized eggs, as is common in commercial practices and all small eggs were incubated.

The incubation period averaged 24 days, with a variation of 23 - 25 days. There was no difference between the control groups and the mercury groups, with respect to the length of the incubation period.

Fig. 11. Hatchability (per cent of incubated eggs) in groups receiving diets in which 100%, 50%, or 25% of the grain portion had been treated with MMD.


Control
25 % treated grain first 2 weeks
throughout exp.
% hatched
70
60
50
40
30
20
10
0
Week — 1 2 3 4 5 6 7 8 9 10 11 12


Control
50 % treated grain first 2 weeks
throughout exp.
% hatched
70
60
50
40
30
20
10
0
Week — 1 2 3 4 5 6 7 8 9 10 11 12


Control
100 % treated grain first 2 weeks
throughout exp.
% hatched
70
60
50
40
30
20
10
0
Week — 1 2 3 4 5 6 7 8 9 10 11 12

Chick production

Chick production, as expressed as the number of chicks hatched per hen is affected by egg production, hatchability and proportion of shell-less eggs. Of these, the effects on hatchability appeared to be the most important (TABLE 5). But the other factors also largely draw in the same direction and the analysis of variance (TABLE 7) indicated highly significant effects both with regard to mercury levels and duration of mercury feeding periods ($P < 0.01$). All groups on contaminated diets except 100/2, 50/2, and 25/4 produced significantly fewer chicks than the control groups (TABLE 8).

The results as they appear in TABLE 6 and Fig. 12 have been adjusted for broken eggs and eggs taken for analysis, assuming that the hatchability would be the same for these eggs as for the other eggs in the respective groups.

Embryonic mortality

All unhatched eggs were opened and the stage of development at death was recorded. The following classification was used: (1) 0 - 8th day, (2) 9th - 16th day and, (3) 17th - 24th day. The over-all results are given in TABLE 6, whereas Fig. 13 shows the variation in embryonic mortality during the experiment.

The statistical analysis (TABLE 7) indicates that an increased embryonic mortality during the first stage of the incubation period is highly significant and correlated both with the dietary mercury level and the length of the feeding period. Since unfertilized eggs are also included and only few eggs had detectable embryos, it may be concluded

TABLE 5. Comparisons between the influence of dietary methyl mercury on egg production and hatchability in pheasants fed methyl mercury treated grain.

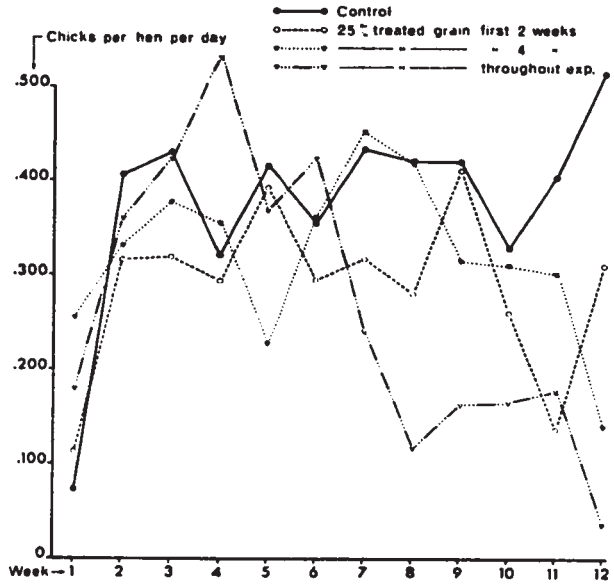
Comparisons [1]	Per cent reduction (-) or increase (+) in: Egg production	Hatchability [2]
100/2 vs control	+11.37	-10.37
100/4 vs control	- 3.96	-36.00
100/12 vs control	-24.82	-46.11
50/2 vs control	+ 1.52	- 1.27
50/4 vs control	- 6.94	-16.52
50/12 vs control	-38.81	-28.49
25/2 vs control	- 4.88	-16.79
25/4 vs control	+ 0.02	-13.54
25/12 vs control	- 5.93	-17.33

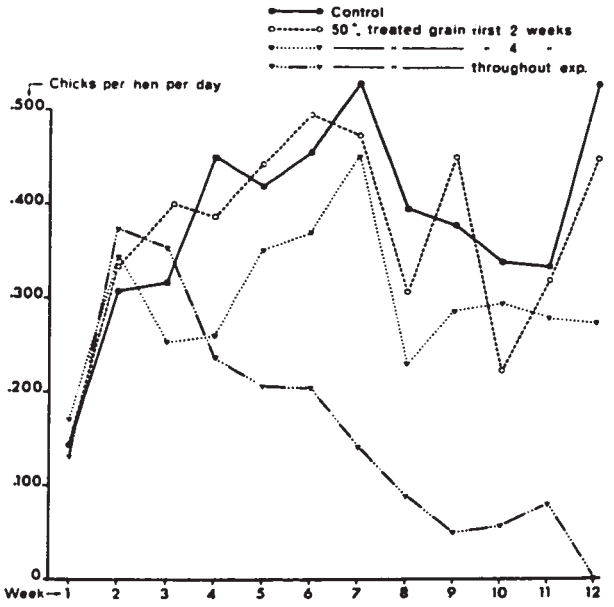
[1] The groups on contaminated diets are referred to according to per cent (100,50,25) treated grain of total grain ration and weeks (2,4,12) fed treated grain.

[2] Calculated as:

$$\frac{\text{Per cent hatched of comparing groups - per cent hatched of control}}{\text{per cent hatched of control}}$$

Fig. 12. Chick production in groups receiving diets in which 100%, 50%, or 25% of the grain portion had been treated with MMD.


Control
25 % treated grain first 2 weeks
" " 4 "
" " throughout exp.
Chicks per hen per day
.500
.400
.300
.200
.100
0
Week — 1 2 3 4 5 6 7 8 9 10 11 12


Control
50 % treated grain first 2 weeks
" " 4 "
" " throughout exp.
Chicks per hen per day
.500
.400
.300
.200
.100
0
Week — 1 2 3 4 5 6 7 8 9 10 11 12

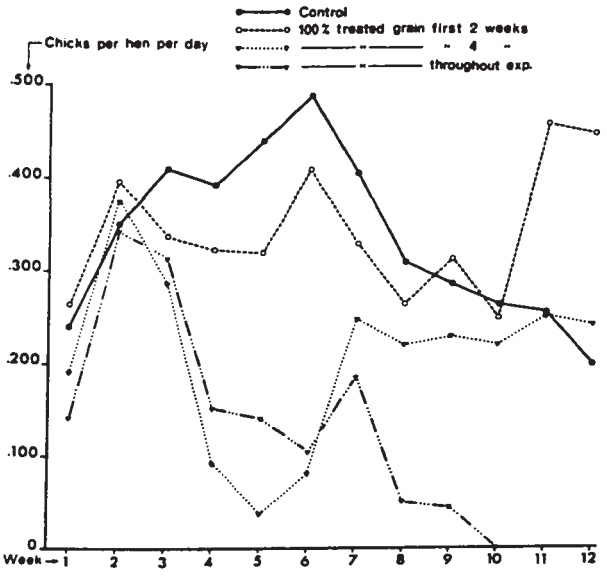
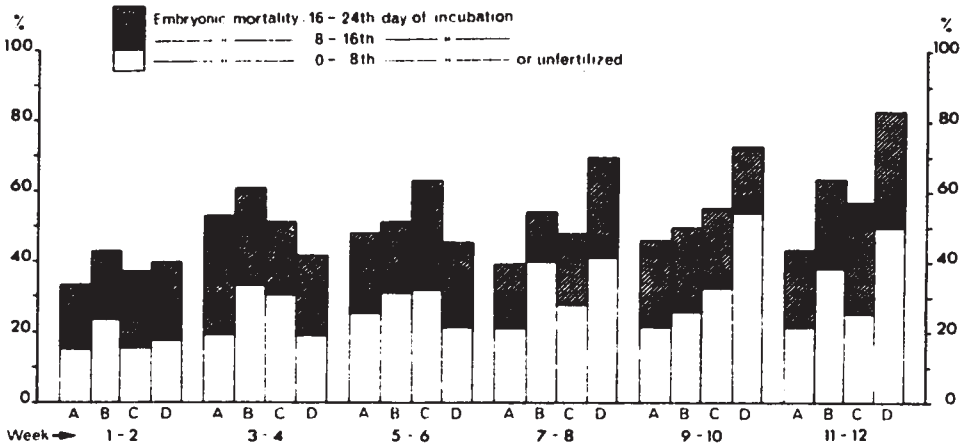
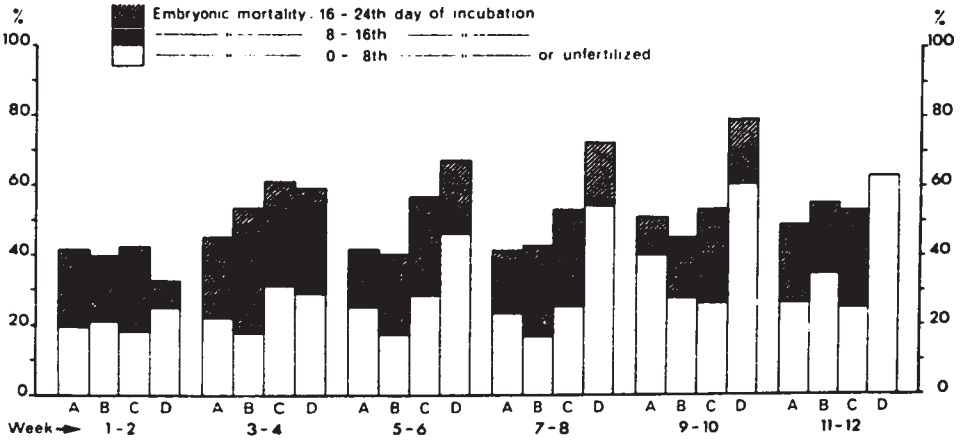

Control
100 % treated grain first 2 weeks
" " 4 "
" " throughout exp.
Chicks per hen per day
.500
.400
.300
.200
.100
0
Week — 1 2 3 4 5 6 7 8 9 10 11 12

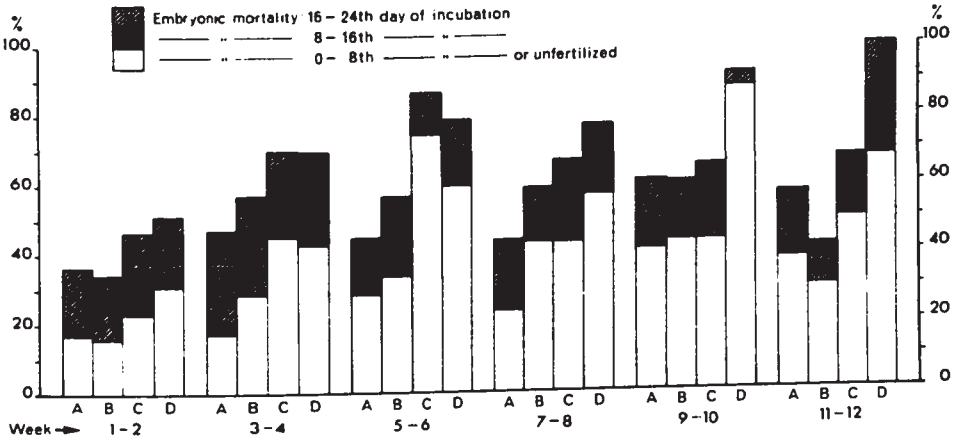
Fig. 13. Embryonic mortality (per cent of incubated eggs) in groups receiving diets in which 100%, 50%, or 25% of the grain portion had been treated with MMD.



A : Control
B : 25 % treated grain first 2 weeks
C : " " " 4 "
D " " throughout exp



A : Control
B : 50 % treated grain first 2 weeks
C : " " " 4 "
D : " " throughout exp.



A : Control
B : 100 % treated grain first 2 weeks
C : " " " 4 "
D : " " throughout exp.

that the effect on fertilization was highly significant. The comparatively high mortality in group 50/2 could not be interpreted.

Striking points from Fig. 13 are that virtually no embryonic mortality occurred between days 9 and 16, and that with low rate of mortality, mortality was evenly divided between early and late periods. With high high rate of mortality, the mortality was concentrated in the early period, and as confirmed by the statistical analysis this was most pronounced in the groups on the heaviest contaminated diets.

Chick mortality

The total chick mortality the first two weeks after hatching is given in TABLE 6. The over-all analysis of variance (TABLE 9) detected no significant difference either between mercury levels or between feeding periods, and paired comparison with the controls indicated only one group had significantly higher mortality than the controls (TABLE 10).

The mortality was particularly high in the beginning of the experiment. At least part of this was caused accidently. Later, on two occasions, a heat lamp in the incubator blew out during the night with a resulting increase in death rate. A number of chicks became crippled the first week because brooders with perforated bottoms were used instead of wire. This was of course changed as soon as the cause was detected.

Most of the chicks that died did so one to three days after hatching, and there seemed to be no difference between the groups in this regard.

TABLE 6. The overall reproductive success in pheasants fed methyl-mercury treated grain

Hg treated grain in diet	Weeks fed Hg treated grain	Pen No.	Eggs per hen	Per cent of laid eggs				Embryo mortality (per cent)					
				Shell-less	Broken	For analysis	Incubated	Days of incubation			Hatchability Per cent of incubated eggs	Chicks hatched per hen	Chick mortality first two weeks Per cent
								0 - 8	9 - 16	17 - 24			
100% of grain ration	0	1	54.3	2.7	7.7	3.6	86.0	28.1	1.4	17.7	52.8	27.4	13.8
		13	57.9	1.3	4.5	3.2	91.0	25.5	2.1	20.8	51.6	29.4	14.4
		Mean	55.6	2.0	6.2	3.4	88.4	26.9	1.7	19.2	52.2	28.4	14.1
	2	2	62.1	1.8	7.3	2.8	88.1	30.3	0.9	20.9	47.9	30.3	23.3
		14	62.3	3.6	5.4	2.6	88.4	36.3	0.3	17.8	45.6	27.6	12.2
		Mean	62.2	2.7	6.4	2.7	88.2	33.3	0.6	19.3	46.8	28.8	17.9
	4	3	52.2	3.1	4.7	4.0	88.2	46.0	0.7	22.6	30.7	15.6	12.6
		15	54.7	2.6	4.2	3.2	90.0	44.6	1.8	17.5	36.1	19.0	21.8
		Mean	53.4	2.9	4.5	3.7	88.9	45.3	1.2	20.1	33.4	17.3	17.6
	12	4	35.8	0.8	7.8	3.7	87.7	57.5	1.4	21.0	20.1	6.9	25.6
		16	48.6	15.2	3.2	2.8	78.8	42.6	2.8	18.5	36.1	13.7	23.3
		Mean	41.8	8.9	5.2	3.2	82.7	49.5	2.2	19.6	28.7	10.3	24.1
50% of grain ration	0	5	67.3	0.7	3.8	3.1	92.4	24.1	0.5	17.6	57.8	39.0	13.7
		17	50.0	1.7	6.8	3.8	87.7	13.1	1.6	16.3	51.0	25.5	19.9
		Mean	59.1	1.1	5.0	3.4	90.5	26.9	0.9	17.1	55.1	32.2	16.0
	2	6	60.7	0.9	4.5	3.1	91.6	15.4	1.8	28.8	54.0	31.2	19.9
		18	59.2	4.7	12.5	3.3	79.4	27.3	1.4	16.4	54.9	30.9	12.1
		Mean	60.0	2.8	8.6	3.2	86.7	20.9	1.6	23.1	54.4	31.3	16.2
	4	7	50.2	2.4	2.8	3.0	91.8	25.9	2.0	20.3	51.8	25.2	17.7
		19	60.1	0.3	4.7	3.2	91.8	26.9	0.9	31.2	41.0	24.7	18.2
		Mean	55.0	1.3	3.8	3.1	91.8	26.4	1.4	26.2	46.0	24.9	17.9
	12	8	42.8	11.5	9.1	3.5	75.9	42.4	1.8	15.2	40.6	14.7	22.7
		20	37.6	11.0	9.8	2.8	76.4	37.6	2.1	22.2	38.1	12.3	13.5
		Mean	40.3	11.3	9.5	3.1	76.1	40.2	1.9	18.5	39.4	13.5	18.5
25% of grain ration	0	9	61.0	0.5	6.0	2.9	90.6	23.3	1.7	21.4	53.6	33.1	11.9
		21	53.5	0.9	4.2	3.2	91.7	18.5	1.3	22.7	57.5	29.0	19.2
		Mean	57.3	0.7	5.1	3.0	91.2	21.0	1.5	22.1	55.4	31.6	15.5
	2	10	63.4	3.8	7.1	3.1	86.0	37.1	0.9	19.9	42.1	25.8	18.3
		22	44.4	2.9	10.0	4.7	82.4	23.9	1.5	21.8	52.8	22.3	17.3
		Mean	54.5	3.5	8.3	3.6	84.6	32.2	1.1	20.6	46.1	24.1	17.9
	4	11	64.2	2.4	5.5	2.8	89.3	23.2	1.3	25.6	49.9	31.0	20.9
		23	50.0	0.7	9.1	2.8	87.4	35.3	2.9	16.7	45.1	22.5	18.4
		Mean	57.4	1.6	7.1	2.8	88.5	28.4	2.0	21.7	47.9	26.8	19.9
	12	12	61.9	6.9	4.2	2.9	86.0	37.1	2.8	23.0	37.1	19.9	23.9
		24	47.6	0.0	11.0	3.6	85.4	22.4	2.3	18.9	56.4	23.9	14.7
		Mean	53.9	3.8	7.2	3.2	85.8	30.5	2.5	21.2	45.8	21.9	18.9

TABLE. 7. Analysis of variance for food consumption, egg production, frequency of shell-less eggs, hatchability embryonic mortality, and chick production of pheasants fed methyl mercury treated grain.

		Food consumption grams/hen/day		Egg production eggs/hen/day		Shell-less eggs per cent of eggs laid		Hatchability per cent of eggs incubated		Chick production chicks/hen/day		Embryonic mortality, per cent of eggs incubated					
												0 - 8 days		Days in incubation period 9 - 16 days		17 - 24 days	
Source of variation	df	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
A (Hg levels)	2	278.310	10.83**	0.022	0.97	0.036	4.26*	0.379	13.47**	0.091	8.77**	0.419	19.21**	0.001	1.56	0.010	1.34
B (Length of feeding period)	3	605.733	23.58**	0.350	15.08**	0.234	27.21**	0.557	19.79**	0.474	45.24**	0.480	22.02**	0.001	1.36	0.017	2.23
A X B (Interaction)	6	212.050	8.25**	0.089	3.83**	0.038	4.45**	0.063	2.26*	0.057	5.52**	0.084	3.86**	0.000	1.07	0.006	0.80
C (Weeks in experiment)	11	508.955	19.81**	0.375	16.16**	0.055	6.48**	0.199	7.08**	0.081	7.74**	0.207	9.49**	0.001	1.85	0.030	3.75**
A X C (Interaction)	22	29.555	1.15	0.013	0.59	0.006	0.70	0.015	0.54	0.010	0.96	0.026	1.21	0.001	1.68	0.008	1.02
B X C (Interaction)	33	47.970	1.86	0.039	1.69	0.044	5.15**	0.073	2.61	0.029	2.81**	0.064	2.94**	0.001	1.62*	0.004	0.60
A X B X C (Interaction)	66	27.783	1.08	0.023	1.02	0.006	0.79	0.029	1.05	0.010	1.02	0.014	0.65	0.000	0.88	0.007	0.92
Error	144	25.684	0.02	0.023	0.00	0.008		0.028		0.010		0.021		0.000		0.008	
Total	287																

* P < 0.05
** P < 0.01

TABLE 8. Comparisons of groups of pheasants fed methyl mercury treated grain with the appropriate controls as to food consumption, egg production, frequency of shell-less eggs, hatchability, embryonic mortality and chick production. All comparisons were F-tests with 1,46 d.f.

	Food consumption grams/hen/day	Egg production eggs/hen/day	Shell-less eggs per cent of eggs laid	Hatchability per cent of eggs incubated	Chick production chicks/hen/day	Embryonic mortality, per cent of eggs incubated		
						Days in incubation period 0 - 8 days	9 - 16 days	17 - 24 days
	F	F	F	F	F	F	F	F
Comparisons [a]								
100/2 vs control	0.86	2.23	0.83	0.09	0.08	1.15	3.70	0.00
100/4 vs control	2.68	0.36	1.38	7.45**	13.83**	11.61**	0.40	0.03
100/12 vs control	32.27**	7.23**	5.41*	33.03**	34.23**	11.31**	0.07	0.11
50/2 vs control	0.04	0.07	4.90*	0.02	0.02	0.12	0.04	1.04
50/4 vs control	0.34	0.50	0.48	1.32	5.81*	0.10	0.30	3.52
50/12 vs control	3.95	12.99**	12.24**	4.22*	31.69**	12.54**	0.19	1.32
25/2 vs control	0.09	0.46	5.43*	2.59	6.81*	5.61*	0.55	0.02
25/4 vs control	3.01	0.00	1.80	0.83	2.71	1.26	0.10	0.74
25/12 vs control	1.14	0.28	5.05*	7.05*	7.25**	5.04*	3.65	0.03

* Significantly different from controls ($P < 0.05$)

** Significantly different from controls ($P < 0.01$)

a The groups are referred to according to per cent (100,50,25) treated grain of total grain ration and weeks(2,4,12) fed treated grain.

TABLE 9. Analysis of variance for mortality in chicks hatched from eggs laid by pheasants fed methyl mercury treated grain.

Source of variation	df	MS	F
A (Hg levels)	2	1.369	< 1 ns
B (Length of period fed Hg treated grain)	3	14.863	1.136 ns
A X B (Interaction)	6	5.439	< 1 ns
Error	12	13.077	
Total	23		

ns - Not significant

TABLE 10. Comparisons of groups of pheasants fed mehtyl mercury treated grain with the appropriate controls as to mortality of their chicks. All comparisons were F-tests with 1,2 d.f.

Comparisons[a]	Chich production chicks/hen/day
	F
100/2 vs control	0.38
100/4 vs control	0.41
100/12 vs control	37.32**
50/2 vs control	0.05
50/4 vs control	0.16
50/12 vs control	0.03
25/2 vs control	0.42
25/4 vs control	1.12
25/12 vs control	0.41

** Highly significantly different from controls ($P < 0.01$)

a The groups are referred to according to per cent (100, 50, 25) treated grain ration and weeks (2, 4, 12) fed treated grain.

DISCUSSION

As pointed out by Romanoff and Romanoff (1949) reproduction in birds is influenced by a number of environmental, physiological and inherent factors. In the present study this was manifested by a relatively high variance within the groups in spite of the efforts that were made to keep the error down to a minimum. Aware that these phenomena could occur, the experiment was designed with three control groups each with two replications (sub-groups) as was the case also for the groups on contaminated diets. With such a design the natural variations under the test conditions could be established, which in turn would increase the significance of the valuation of the effects due to the factor concerned; dietary methyl mercury.

Health and Weight

The condition of the hens was apparently little affected by the mercury. The over-all mortality rate was low and no hens died in the group that received the heaviest treated diet throughout the experiment. It seems obvious that the mercury levels in no case reached the mortality level. The highest level found in the liver was 13.7 ppm while 30 ppm has been reported as a significant mortality level in adult pheasants (Borg et al. 1969). The relatively low mercury levels in the pheasants reflect similar content found in the treated grain (5.5 ppm). Surprisingly as it may seem, no hens died in the groups that received the largest amounts of mercury (100/12 and 50/12). In these groups, however, egg production was significantly reduced and as losses are

more likely to occur in high egg production flocks (Romanoff and Romanoff 1949), the sub-lethal mercury levels might well have reduced this stress or even served as a curative agent to the pathogens concerned. The therapeutic properties of mercury compounds are well known (Bidstrup 1964). According to Mellanby (1967) mercury treated grain has in fact, been suggested as advantageous to health. This suggestion coincides with the findings in this experiment but has proved not to be the case with more elevated mercury levels in the diet (Borg 1969). Also Tejning (1967 I) in an experiment with Panogen-treated grain to chickens found that hens on a diet of 18.4 ppm of mercury displayed typical signs of neurological disturbances with difficulties in standing and walking. However, he did not report any effects of this type for hens given 9.2 or 4.4 ppm. The latter coincides fairly well with the highest mercury levels in the present experiment. For those hens that received the largest amounts of mercury, histological examinations revealed pathological changes to the nervous system, particularily demyelination. This is probably a result of the affinity of alkyl mercury to the nervous system (Friberg 1967) and confirms the experimental findings of Borg et al. (1969).

Most birds lost weight during the experiment and the contaminated groups did not differ from the controls. This was the case even for group 100/12 that had a significantly lower food consumption than the other groups. Why a much lower food consumption or calorie intake is not reflected in body weight changes can only be ascribed to the fact that this group also had a highly significant reduced egg production. These results are not surprising as it has been found that even more elevated dietary alkyl mercury levels have had no decided effects on

body weight in adult chickens (Heuser 1956, Tejning 1967aI). Again we must turn to the already mentioned therapeutic properties of mercury for an explanation. The pheasants, as is the case with most gallinaceous birds, commonly suffer from a number of parasites and diseases. Within certain levels the therapeutic properties of mercury make up for its possible toxic effects on growth and health. In order to prove such a hypothesis, healthy control birds completely free of parasites are needed which would be hard to obtain without introducing another therapeutic agent.

Reproduction

Egg production was only moderately affected by the mercury in the present experiment. The total production was reduced significantly only in those groups that received 100% or 50% treated grain throughout the experiment. For group 100/12 this effect could be ascribed to a lower calorie intake, but as no significant effect on food consumption was observed in group 50/12 the effect of mercury is more likely direct than indirect by way of reduced food intake.

These findings can best be compared with those of Smart and Lloid (1963). They fed grain treated with methyl mercury dicyandiamide (6 ppm of Hg) to chickens for eight weeks and found no effects in either direction on egg production in spite of a somewhat higher mercury consumption. This may indicate that there could be a species difference between pheasants and chickens in tolerance to mercury. Borg et al. (1969) fed heavily treated grain (21 ppm) to pheasants for three, six, and nine days with no significant effect on egg production. Because

of much shorter feeding periods their results can not be compared with those from the present experiment.

It is interesting to note that group 100/4 had a strong temporary decline in egg production but recovered completely three weeks after feeding of treated grain was discontinued. This probably indicates that the decreasing egg production is not due to the degenerative effects of methyl mercury, such as heavy demyelination and cell degeneration in the central nervous system, which the histopathological examinations revealed, and which also appeared in group 100/4.

Egg weight was strongly reduced in the heaviest contaminated groups. This effect, not as pronounced but still significant, was further extended to most of the contaminated groups the last weeks of the experimental period, even in those groups that had been on mercury free diet for several weeks. The relatively long latent period possibly indicates that a metabolite rather than the mercury compound itself is acting. Previous writers did not discuss the effect of mercury on egg weight in their experiments, possibly because such an effect was not expected and therefore not looked for.

In the present experiment a large percentage of eggs with abnormal colour was produced but only by hens on the most heavily contaminated diets. This effect was very conspicuous and it is surprising that it is not reported by other students who have been investigating the reproductive effect of methyl mercury in pheasants. However, it might be that these experiments have not covered a period long enough to have this effect introduced.

The frequency of shell-less eggs was comparatively high in those

groups that received treated grain throughout the experiment. The effect was not significant until about eight weeks after the experiment commenced, but then the frequency of shell-less eggs rose sharply and reached about 70% in groups 100/12 and 50/12 during the last experimental week. These findings indicate that a relatively long experimental feeding period is needed in order to produce a significant number of shell-less eggs and they also indicate that in this regard the length of the feeding period is even more important than the dietary mercury levels. There is a coincidence between these findings and those of Tejning (1967aII). The percentage of shell-less eggs in Tejnings experiment were somewhat higher, but here we have to take into account that the number of shell-less eggs in the present experiment may be underestimated because of possible egg eating.

Tejning (1967aI) suggested that probable causes are accelerated passage of the egg through the shell-forming portion of the oviduct or inhibition of some enzyme necessary for shell formation.

Hatchability was strongly affected by the mercury compound as all contaminated groups showed a lower hatching frequency than their control, though not significant in all cases. These findings, apparently do not coincide with those of Borg et al. (1969) in their previously mentioned experiment with pheasants. They found that the shortest experimental feeding periods, three and six days, significantly increased the hatching per cent, whereas nine days feeding has significant effect in the opposite direction. The amount of mercury ingested by the hens in the experiment of Borg et al. was in all cases well above the smallest doses in the present study. The apparent discrepancies between the

results of these studies may show that the length of the experimental feeding period is of as much importance as the amounts of mercury ingested, so that a certain amount of dietary mercury may have one effect when ingested over six days or less, and another when ingested for 14 days or more. The mercury levels in eggs from groups where significant reduction in hatchability was found, varied between 0.5 - 1.5 ppm. Although these levels to a certain degree reflect those in the diet, no clear relationship between mercury levels in eggs and hatchability could be found. Similar findings are reported by Tejning (1967a VIII). His explanation is that the hatching prospects may depend upon the duration of mercury feeding as well as upon the mercury amounts in the eggs, which in his case, far exceeded those found in the present study.

Increased embryonic mortality in the contaminated groups was caused primarily by an increase in the number of unfertilized eggs, including eggs with no detectable embryos. Tejning (1967aIV, V) and Bäckström (1969) reported a very pronounced accumulation of mercury in the albumen secreting part of the oviduct and in the albumen and Tejning (1967aVIII) concluded that possible damage to the spermatozoa by the mercural secretion could occur. It is possible that the findings in the present experiment could be explained on this basis. The spermatozoa could also be damaged before leaving the cock, as the cock had access to treated grain as well as the hens, but this is unlikely as mercury accumulates to a much lesser extent in the testes than in the oviduct (Tejning 1967aXII, Bäckström 1969). No difference in the copulation rate was observed between the control groups and any of those

that received treated grain.

The mercury compound seems to have had comparatively little effect on the chick survival which in the present study was recorded the first two weeks after hatching. Only the chicks from the most heavily contaminated group had a significantly higher mortality than the control. According to Tejning (1967aII, III), about fifty per cent of the mercury is found in the yolk sac at the time of hatching. In the present experiment only in group 100/12 has mercury accumulated to such levels that it counts as a mortality factor.

CONCLUSION

The present study indicates that methyl mercury treated grain may have gross adverse effects on reproduction in pheasants even in cases where no symtoms of mercury poisoning can be observed in the laying hens.

A reduced hatchability was the most important single factor with reduced egg production and effect on shell formation being others of high significance. Chick mortality on the other hand seems to be of minor importance.

An important question is how and to what extent can these findings be related to wild bird populations.

Studies from Sweden have shown that mercury levels similar to those found in the most contaminated groups in the present experiment frequently occur in pheasants and other seed-eating birds and their predators (Borg et al. 1969). Elevated mercury levels in seed-eating birds and predators are also reported from Canada (Fimreite et al. 1970).

Since the highest levels were found during and shortly after the seeding season the source of contamination is believed to be seed-dressings. This suspicion was further confirmed by Wanntorp et al. (1967) who found a considerable drop in the mercury levels of wood pigeons after the ban on alkyl mercury seed-dressings was carried through in Sweden in 1966.

Thus we must believe that at least hatchability can be affected among wild birds where they have access to treated grain, either occurring as uncovered seeded grain or as spillage.

We can not draw any conclusion about egg production, because the large number of eggs laid by penned pheasant hardly occur under wild conditions. The same could probably be said about the effect on shell formation as this effect was significant first after about eight weeks of mercury feeding. Decolouration of the eggs as it occurred in the present experiment could be a significant factor of ecological importance if such eggs would be easier to detect by predators. However, as this phenomenon was observed only in eggs from the most contaminated groups, they would probably not hatch in any case. Finally, it should be mentioned that nesting behaviour, an important aspect of reproduction, could not be studied in the present experiment.

SUMMARY

The effects of methyl mercury treated grain (methyl mercury dicyandiamide) on penned pheasants (Phasianus colchicus) were studied in a two-way factorial experiment, the factors involved being; mercury levels (100%, 50%, and 25% treated grain of the grain ration) and the length of the experimental feeding period (2, 4, and 12 weeks). An additional three groups served as controls.

Apparently no weight reduction in the adult birds could be ascribed to the mercury compound. Compared to the controls, mortality was lower than average in the gorups that received mercury contaminated diet throughout the experiment, suggesting a possible therapeutic effect of mercury. Food consumption was affected only in the group that received the largest amounts of mercury.

Strong adverse effects on reproduction were found, the most important indication being reduced hatchability, followed by a reduced egg production, and lastly, a large number of shell-less eggs. Chick survival was comparatively less affected. Egg weight was reduced significantly in most of the experimental groups, especially during the last weeks of the experiment, and the highest mercury levels produced a large number of eggs with an abnormal colour.

The relevance of these findings to mercury poisoning among wild bird populations is discussed, and it is concluded that at least hatchability might be adversely affected to an extent that is significant where mercury seed-dressings are used extensively.

EFFECTS OF DIETARY METHYL MERCURY ON RED-TAILED HAWKS

INTRODUCTION

The highly toxic and persistent alkyl mercury compounds are widely used in seed-dressing to protect grain seed against seed-borne diseases such as smut and bunt. Dangerous levels of mercury often concentrate in predators when preying on seed-eating birds and rodents that pick up uncovered treated seed during the seeding season. This was demonstrated in Sweden (Borg *et al.* 1969) where, in 1966, alkyl mercury compounds were banned for use in seed-dressings.

In Canada, however, these alkyl mercury compounds are still used in seed-dressing and the quantities of mercury utilized for this purpose are considerable (Fimreite 1970). It has been further demonstrated that seed-eating birds such as horned larks (*Eremophila alpestris*), partridges (*Perdix perdix*), pheasants (*Phasianus colchicus*), and predators such as prairie falcon (*Falco mexicanus*), collected in the grain growing districts of Alberta and Saskatchewan, carry elevated levels of mercury (Fimreite *et al.* 1970).

The present study was undertaken to evaluate possible hazards of mercury contaminated birds as food for predators. This was accomplished by raising chicks on a mercury contaminated diet, establishing in them levels similar to those found in wild birds. These chicks were then used as experimental food for red-tailed hawks.

MATERIALS AND METHODS

The test birds were 24 healthy red-tailed hawks (Buteo jamaicensis) obtained as nestlings in June, 1968 and kept at Niska Waterfowl Research Station, Guelph, Ontario, where the experiment was conducted in 1969. The birds were fed chickens, freely available throughout the pre-experimental period.

The groups of cockerels serving as experimental food had been raised on diets with added Panogen 15, a commercial seed-treatment containing 2.5% of its active ingredient, methyl mercury dicyandiamide (MMD).

The cockerels were killed at five weeks old, after being fed MMD contaminated diet during their last three weeks at rates of 6, 12, and 18 ppm of MMD respectively. A fourth group of cockerels, raised on untreated diet, served as food for the controls.

The hawks were divided into eight groups of three birds each and housed in wire pens (6 x 3 x 3 m) roofed in the middle to provide shelter. Two of the groups served as controls and two as testbirds for each of the mercury levels. One group within each level, however, was put on a mercury free diet after four weeks to study possible recovery from poisoning (TABLE 11). The hawks were offered one chick (weighed to the nearest gram) each per day. Residues that were left over were collected at intervals of 3 - 4 days in order to determine food consumption.

All hawks were banded, weighed on the day the experiment started (April 20), when it was completed (July 14), or when they died.

Table 11. Plan of an experiment to show effects of dietary methyl mercury on red-tailed hawks.

Group[a]	Hg in Livers of Chicks Fed to Hawks[b] (mg/kg)	Length of Experimental Feeding Period (weeks)
I	3.9	4
II	3.9	12
III	7.2	4
IV	7.2	12
V	10.0	4
VI	10.0	12
Control 1	0.03	--
Control 2	0.03	--

[a] There were three hawks in each group.

[b] Mercury residues in the chicks were determined by the neutron activation technique by the Gulf General Atomic Inc., San Diego, California. Two average chicks within each group were analyzed.

Daily observations were made in an attempt to detect abnormal behaviour or any other effects that might be ascribed to the mercury compound. Hawks that died were brought immediately to the pathology laboratory for necropsy. Pieces of the following organs were fixed in buffered formalin, embedded in paraffin, sectioned and stained with hemotoxylin and eosin and, in nervous tissues, also with luxol fast blue for myelin: lung, heart, liver, spleen, kidney, brain, cervical spinal cord. The histopathologic investigations were conducted by Dr. L. Karstad, Department of Pathology, University of Guelph.

Determination of mercury content in liver, breast muscle, and brain was conducted at the Laboratory of Toxicology, University of Guelph, by Dr. N. Platonow according to a method described by Oliver and Funnell (1958) (Appendix III).

The average daily intake of mercury for the chicks used as experimental food was, based on their food consumption and the amounts of mercury added to their diet, calculated to be approximately 0.08, 0.16, and 0.24 mg per chick per day for the three groups respectively.

The amount of mercury retained in their bodies at the end of the 21 day period was calculated using the following formula developed by Ulfvarson (1962, 1965):

$$X = \delta (1 - e^{-ht})/h$$

where; t = length of the period fed methyl mercury

X = concentration of mercury in organism at time "t"

h = relationship between the elimated mercury per unit of time and the total amount of mercury in the organism

δ = the amount of mercury added per unit of time divided by the body weight.

Ulfvarson (1962, 1965) has calculated "h" to be approximately 3% per day for methyl mercury. The mercury intake by hawks (TABLE 12) was calculated on the basis of their consumption of chicks, and the amount of mercury found to be retained in the chicks according to the above formula.

According to Tejning (1967aXII) a considerable amount of ingested mercury may be transported to the plumage during its development. Since feathers are undigestable this mercury has probably no biological effects when ingested. This must be taken into account when considering the calculated data on mercury intake as it appears in TABLE 12. Furthermore, there may be several sources of error involved in the method applied for calculation of mercury retained in the chicks, as pointed out by its developer (Ulfvarson 1962, 1965).

RESULTS

Food consumption

From TABLE 12 it appears that the mercury compound has only a slight effect on food consumption except for the groups on the most heavily contaminated diets, where mortality occurred. Birds that showed symptoms of being poisoned seemed to maintain their appetite, at least until a very advanced stage of poisoning had been reached, but they obviously had difficulties in dismembering the chicks, which may partly account for a reduced food intake and feather impaction in one case.

TABLE 12. Food consumption (kg/hawk) and calculated intake of mercury for red-tailed hawks fed methyl mercury contaminated chicks.

Group	Weeks in experiment			Total intake of mercury (mg/hawk)[a]
	1 - 4	5 - 8	9 - 12	
I	6.6	6.0	6.8	17.1
II	6.7	5.3	6.6	48.3
III	6.5	6.3	7.1	33.8
IV	6.5	5.4	6.2[b]	94.1[b]
V	6.1	5.9	7.1[b]	47.5[b]
VI	5.7	4.9	5.1[b]	122.4[b]
Control 1	7.0	6.3	7.2	0.0
Control 2	6.6	6.3	7.2	0.0

[a] Adjusted for hawks that died

[b] On the basis of the calculated Hg content of the chicks

Mercury residues

The mercury residues found in liver, breast muscle, and brain are presented in TABLE 13. Unfortunately, because of an accident a great number of the samples could not be analyzed, which of course reduced the significance of the comparison between levels of different tissues. However, in cases where we are able to make such comparisons, the highest levels were found in the liver. Hawks that died had 17 - 20 ppm in the liver, indicating that the lethal levels in these hawks are close to 20 ppm. The lowest level (17 ppm) was found in a hawk that had been on mercury free diet for about five weeks prior to death, and we must assume that some mercury had been lost through this period.

The mercury residues in tissues are largely in accordance with those found in the diet. The residues are low in hawks from the groups in which feeding with mercury contaminated diet was discontinued eight weeks prior to analysis, and in group I, fed chicks with the lowest level of mercury for only four weeks, practically no detectable residues were found.

Furthermore, the results in TABLE 13 indicate that there are smaller differences between the mercury levels in brain and liver in five hawks fed on the lowest amount of mercury (groups I and II) than there were in two hawks fed higher amount of mercury (groups III and IV).

Body weight

The effect on weight was not uniform, but in general the control birds, as well as those on the lowest mercury level, gained weight during the experiment (TABLE 13). Most birds on the two most heavily

TABLE 13. Weight changes and mercury residues in red-tailed hawks fed methyl mercury contaminated diet.

Group	Hawk #	Weight in grams 20 April	14 July or at death	Weight gained (+) or lost (-) per cent	Mercury residues (ppm)* Liver	Breast Muscle	Brain
I	2861	1010	1200	+18.8	0.90	--	--
	2869	1260	1440	+14.3	--	--	--
	2894	900	950	+ 5.6	--	--	--
II	2862	1220	1420	+16.4	1.36	0.91	1.52
	2874	1240	1290	+ 4.0	2.19	0.41	1.32
	2876	1160	1410	+21.6	N.A.	1.43	1.33
III	2858	1180	1200	+ 1.7	3.00	--	1.16
	2870	930	920	- 1.1	0.66	--	--
	2879	830	880	+ 6.0	N.A.	0.80	1.17
IV	2867	1170	1290	+10.2	N.A.	N.A.	2.63
	2895	890	710(died June 19)	-20.2	18.76	10.50	N.A.
	2899	1370	1320	- 3.6	N.A.	N.A.	2.10
V	2871	1330	1285	- 3.4	3.89	0.83	0.93
	2878	970	1000	+ 3.1	N.A.	N.A.	0.91
	2098	950	625(died June 23)	-34.2	16.74	4.30	N.A.
VI	2859	1160	1250	+ 7.7	N.A.	N.A.	3.11
	2866	980	750(died June 4)	-23.5	N.A.	N.A.	N.A.
	2893	1120	715(died June 30)	-36.2	19.98	11.36	N.A.
Control 1	2872	1250	1350	+ 8.0	N.A.	N.A.	--
	2875	1150	1080	- 6.7	N.A.	--	--
	2877	1260	1320	+ 4.8	--	N.A.	--
Control 2	2863	1110	1190	+ 7.2	N.A.	N.A.	--
	2868	1100	1210	+10.0	N.A.	N.A.	--
	2873	1230	1410	+14.6	--	--	--

* N.A. = Not analyzed
A dash indicates less than 0.2 ppm

contaminated diets lost weight or showed only minor changes in body weight.

Birds that died during the experiment had body weights reduced by 20% to 36%.

Mortality and clinical manifestations

Mortality occurred only in the most heavily contaminated groups (TABLE 13). The four hawks that died were also the only ones that showed obvious signs of being poisoned. The first hawk to display such signs was no. 2098 from group V, observed first May 15, or less than four weeks from start of experiment. The signs were obviously neurological, consisting in the beginning of weakness in extremities, which rapidly increased. Within 3 - 4 days it lost all ability to co-ordinate its muscle movements, and attempts to fly or walk only resulted in uncontrolled motions. The hawk seemed to retain an appetite but had difficulty eating. Three days after the first signs of poisoning had been observed the hawk was put on a mercury free diet, but without recovery; on the contrary its condition gradually weakened and it died on June 23.

Hawks no. 2866 and no. 2893 from group VI died on June 4 and June 30 respectively. The clinical signs resembled those described for hawk no. 2098 but the period of time between the first signs of poisoning and occurrence of death was shorter, 2 - 3 weeks versus 5 weeks, probably due to the hawks being fed contaminated chicks continously.

Hawk no. 2895 from group IV which died on June 19 displayed similar neurological disturbance prior to death, but the signs developed more

gradually. A reduced spontaneous activity was observed from June 6. However, it was still able to eat, apparently without difficulties and by supporting itself with its tail, it was able to continue to move around the cage for about a week. The final signs however, could not be distinguished from those of the other affected hawks, and the slower development of the toxicological manifestation could be ascribed to the lower mercury levels in the chicks consumed.

The hawks that died were among the lightest in weight within their groups (TABLE 13).

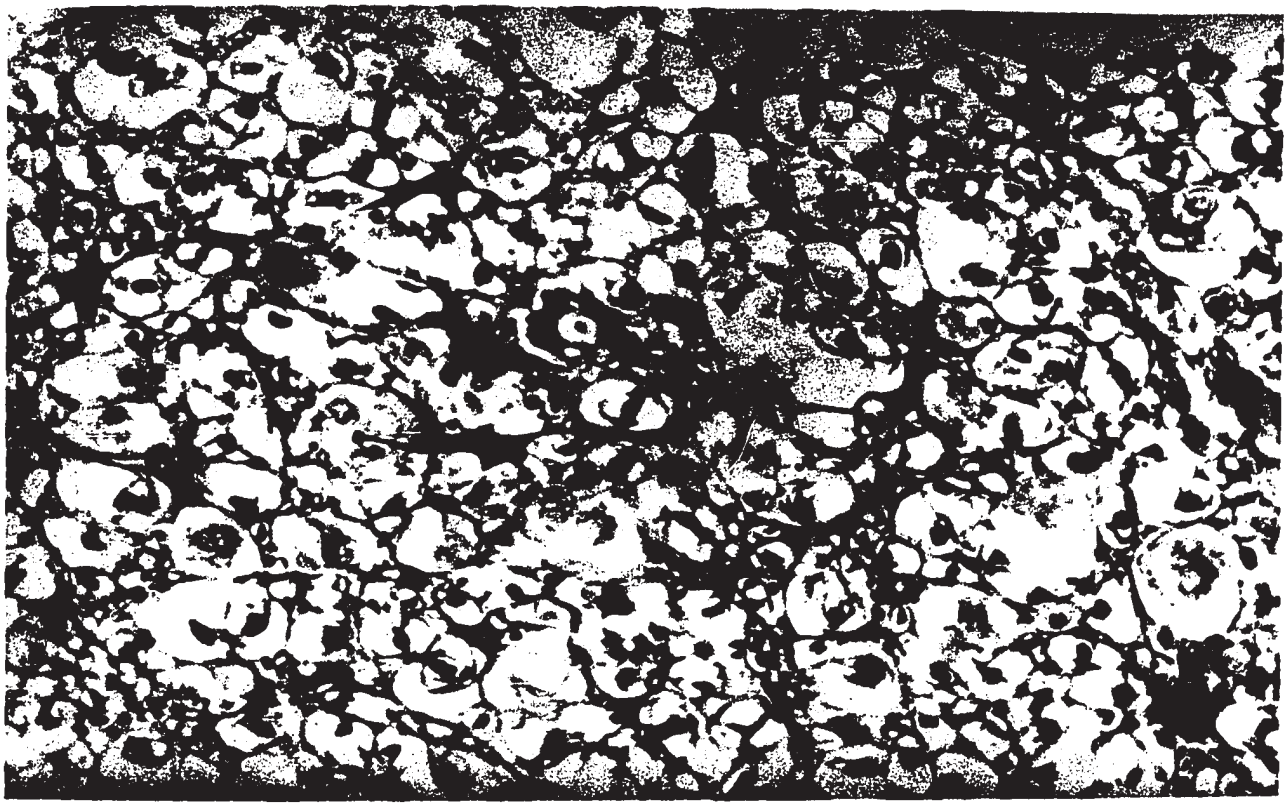
Pathological findings

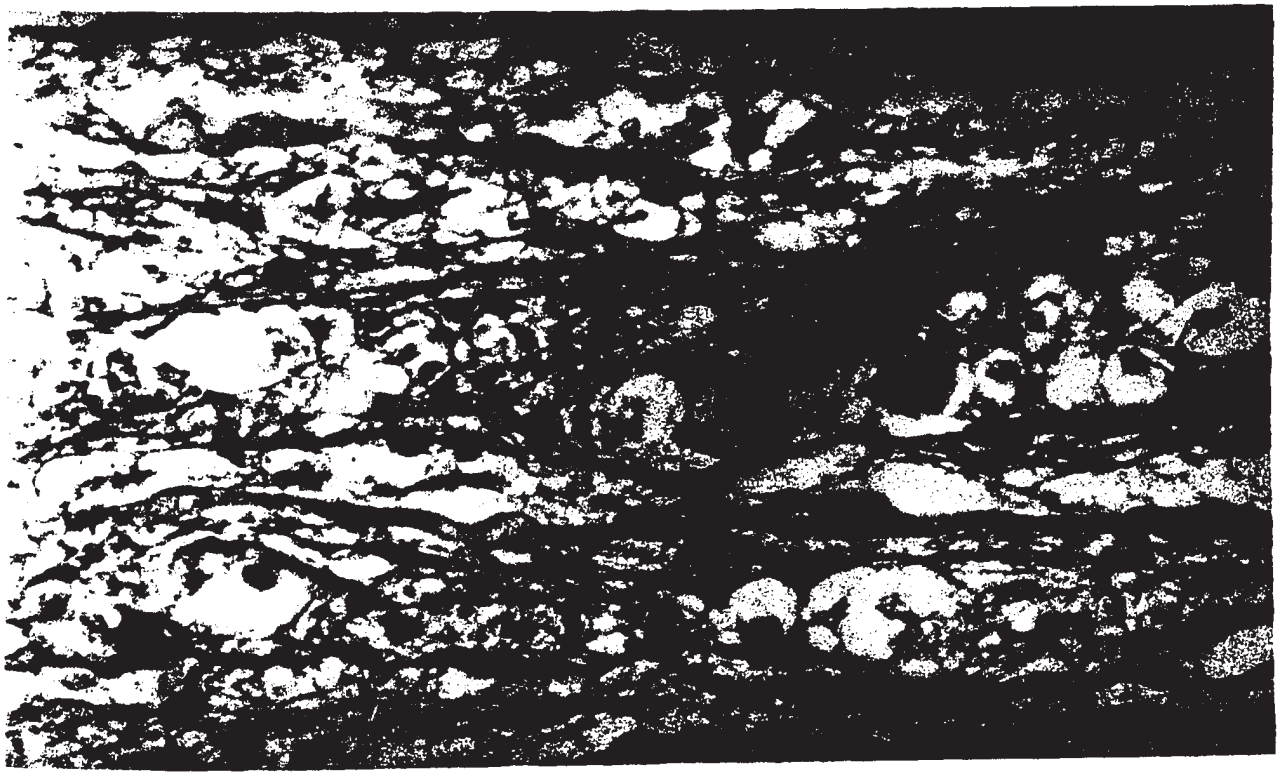
Gross lesions were minimal and limited to emaciation in the hawks on the higher levels of mercury, as indicated by the body weights given in TABLE 13. In addition, at the time of death, hawk no. 2098 had a large bolus of feathers which was packed into the lower half of the esophagus and extended into the proventriculus and the gizzard. Another hawk which died had ascites (no. 2893).

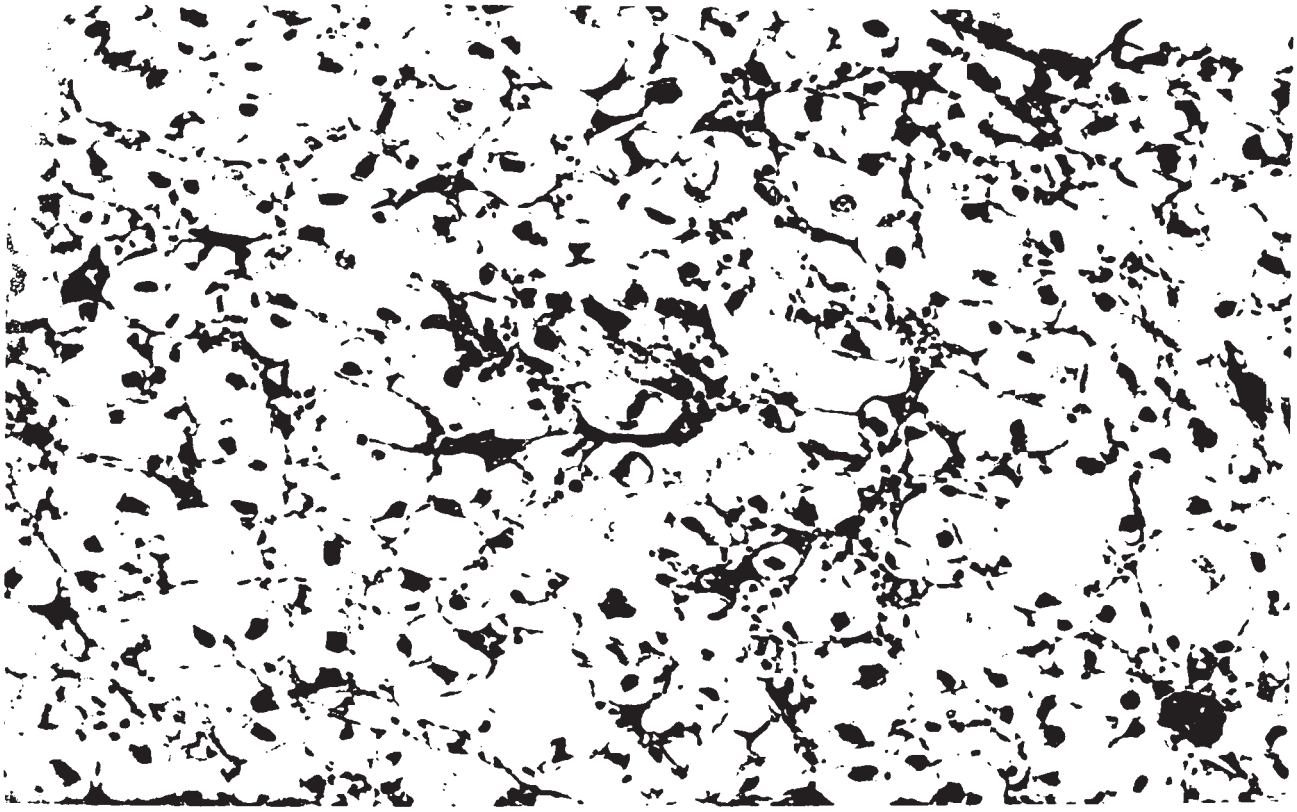
The most consistent microscopic changes were swelling of axons of myelinated nerves in the spinal cord with dilatation of myelin sheaths and loss of myelin. These changes were most pronounced in the dorsal funiculi but were seen also in lateral and ventral funiculi of the cord (Fig. 14). In the most severe cases, similar lesions were found in some axons in the brain stem, mid brain, cerebellum, and cerebrum. In individual birds, lesions were most pronounced in the cord and were progressively reduced cerebrally. The most severely affected axons were swollen, fragmented, sometimes contracted or

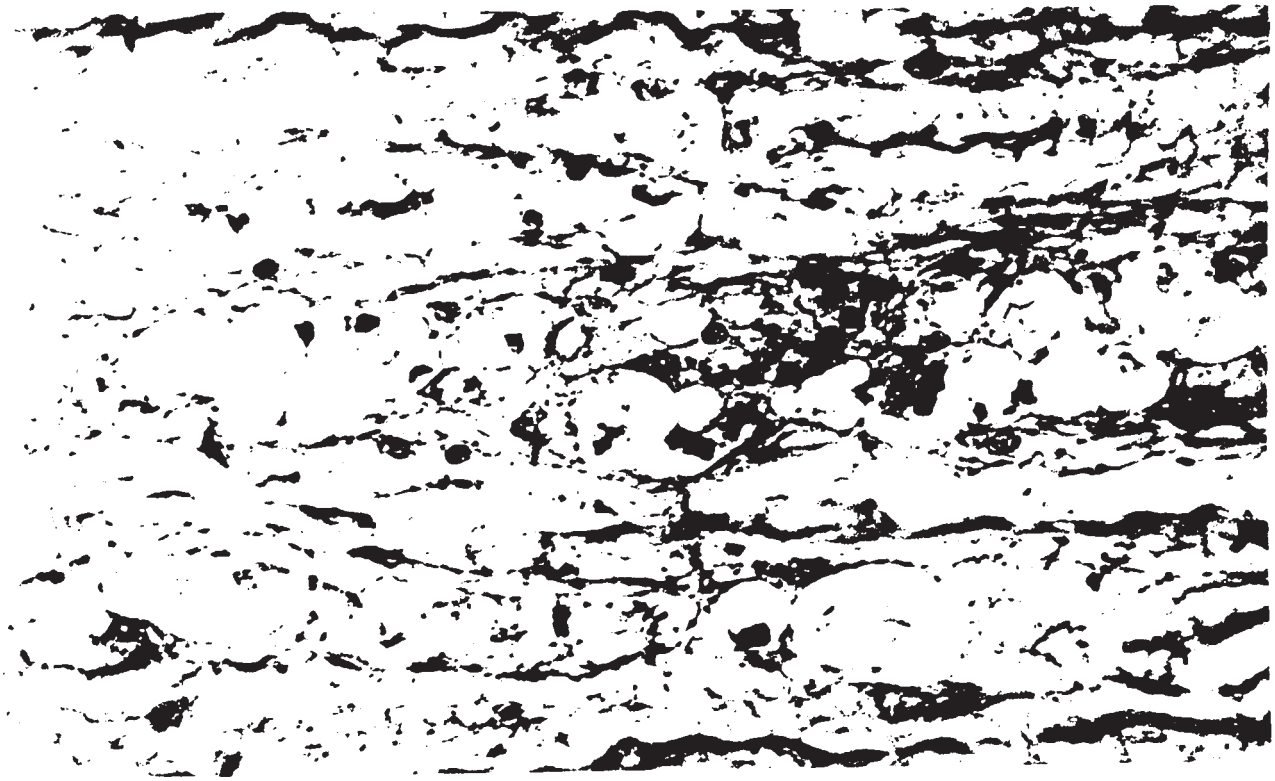
Fig. 14. Ventral funiculus of spinal cord, hawk no. 2871. There is swelling and disintegration of axons and dilatation of myelin sheaths. All figures are from hematoxylin and eosin stained sections X 600.

Fig. 15. Longitudinal section of the spinal cord illustrated in Fig. 14. Note irregularly swollen axons and myelin sheaths; also focal proliferation of supporting cells X 600.









curled and undergoing lysis in digestion chambers (Fig.15). Gitter cells were not abundant but there were increased numbers of other glial cells. Lesions of axons and myelin sheaths were seen in all of the hawks in groups V and VI (10 mgHg/kg), in all of the hawks in group IV (7.2 mg/kg for 12 weeks) and in two of the three hawks in group III (7.2 mg/kg for 4 weeks). Such lesions were not seen in hawks given lesser amounts of mercury nor in the controls.

A second type of nerve lesion was seen in spinal nerve roots in all nine of the hawks in which sections of spinal cord included nerve roots. This consisted of infiltration of the nerve root and dorsal root ganglion with heterophils (Fig.16). These heterophil infiltrations were found in hawks with the axon lesions described above; also in two of three hawks in group I which received the lowest dose of mercury and in two of the six control hawks. The significance of this lesion is unknown.

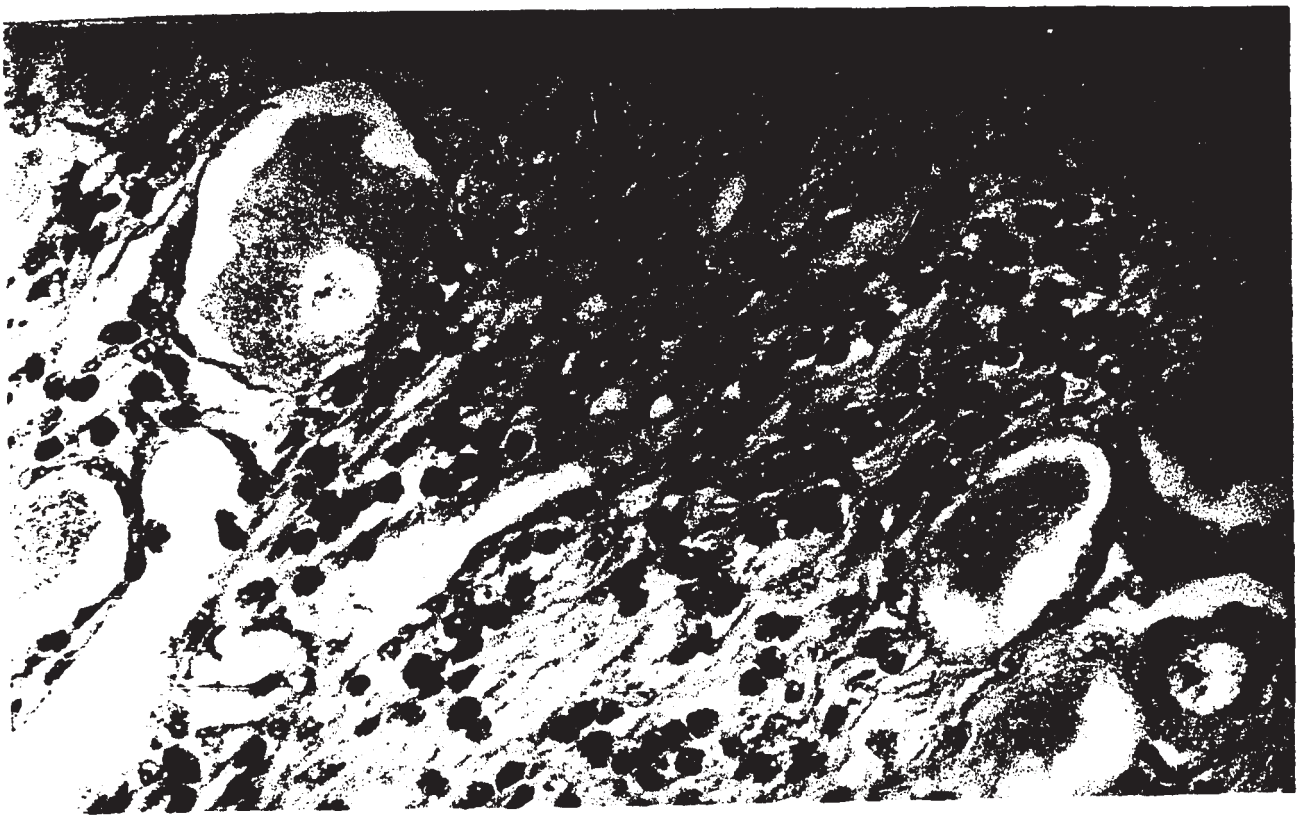
With exception of the spinal nerve lesions, inflammatory changes were minimal in the brains and spinal cords of the hawks. Perivascular infiltration with lymphocytes and histiocyticmononuclear cells were seen in only two birds, hawks no. 2859 and 2871 in the optic lobes (Fig.17). Glial proliferation was seen in some cases.

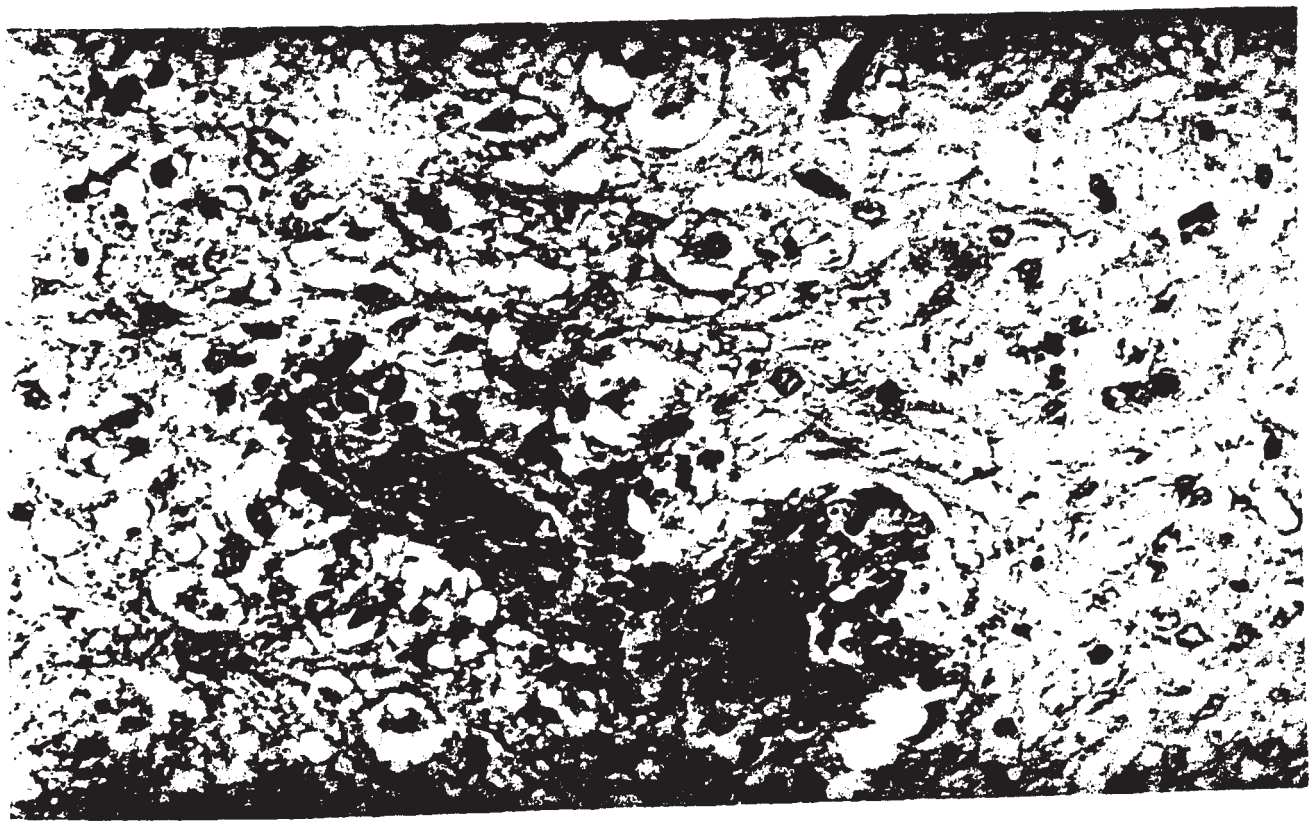
Some hawks on the highest doses of mercury had degenerative changes in Purkinje cells, in spinal ganglion cells and in some neurons in the ventral horns of the spinal cord. These consisted of central chromatolysis, vacuolation, and nuclear swelling.

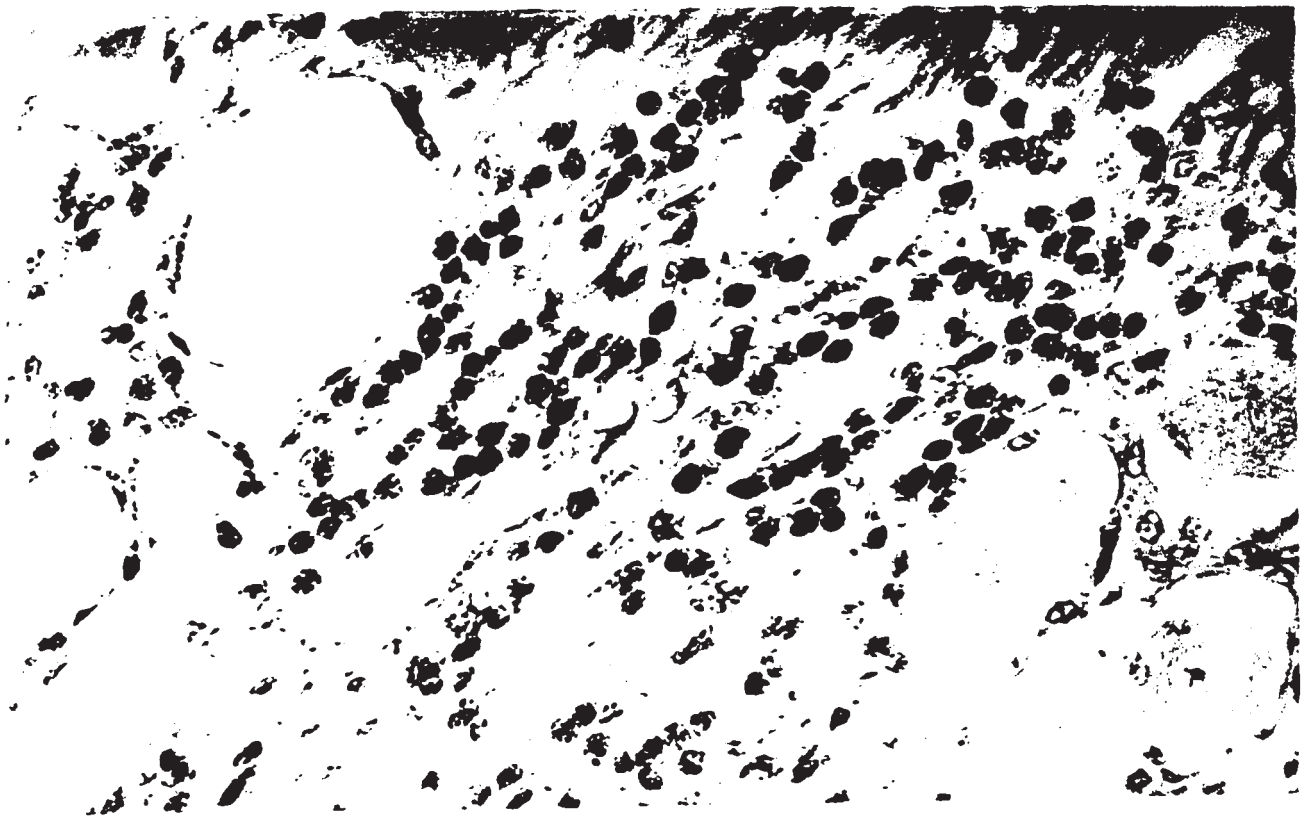
Hawks which died and one of the others on the highest doses of mercury, had granular changes in the cytoplasm of myofibres in the

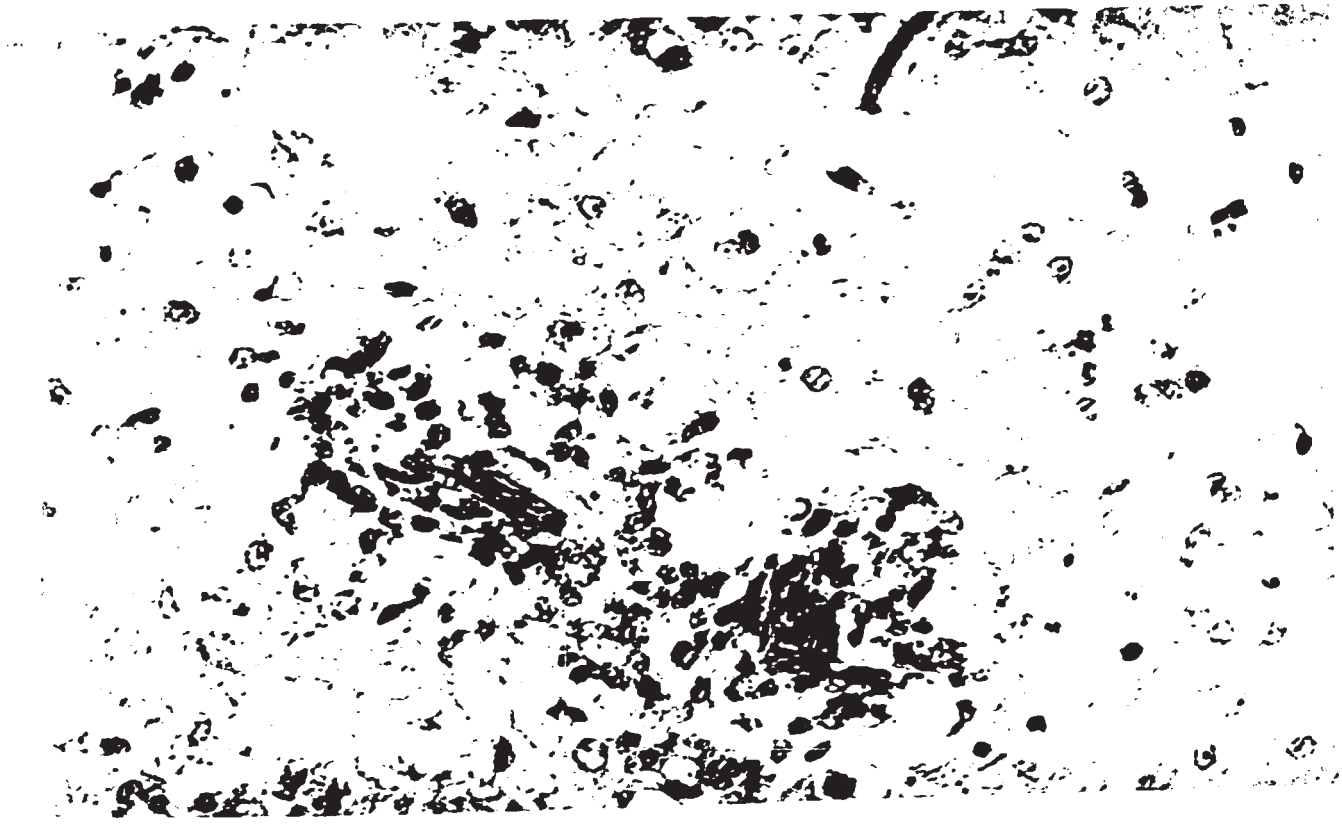
Fig. 16. Dorsal root ganglion, hawk no. 2878. Nerve fibres are separated by infiltrating leukocytes, mostly heterophils X 600.

Fig. 17. Optic lobe of hawk no. 2871. Note perivascular cuff composed of lymphocytes and histiocytic cells and vacuolation of the cytoplasm of some nerve cells X 600.









heart (Fig.18), in smooth muscle cells of some blood vessels and in parenchymal cells of the liver and kidneys. The liver of hawk no. 2878 had some dilatation of bile ducts and peribiliary infiltration with lymphocytes and heterophiles.

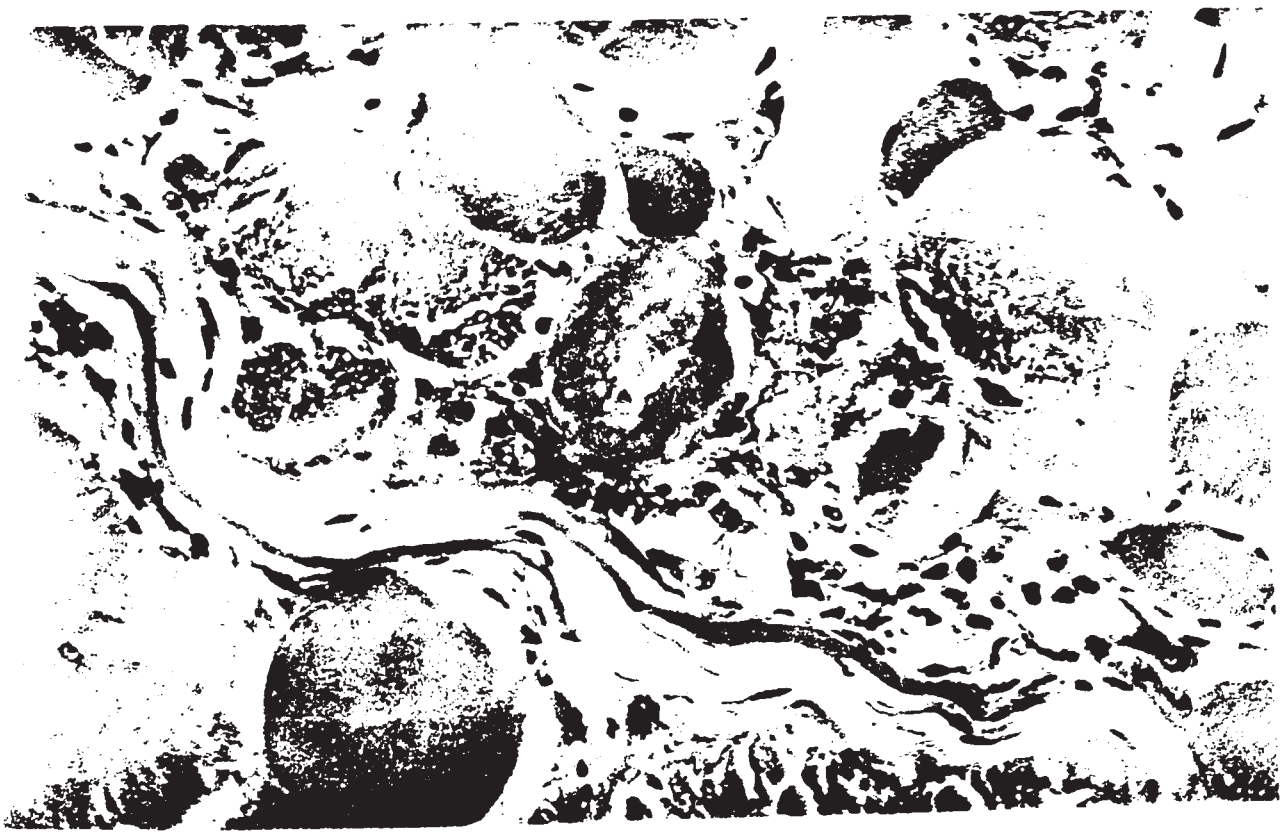
Striated muscles were examined in only one case, hawk no. 2893, which died during the tenth week of feeding mercury at the highest level. Since this bird had shown leg weakness, the femoral muscles were sectioned. There was swelling and necrosis of individual muscle fibres, with mineralization of some of the necrotic fibres (Fig.19). There was proliferation of sarcolemma cells and fibrous connective tissue cells between muscle bundles. The femoral nerve was edematous but otherwise normal in appearance.

DISCUSSION

The present study indicates that a steady diet of chicks containing 7 - 10 ppm of mercury in the liver may have lethal effects on hawks, the signs of poisoning prior to death being essentially neurological, consisting of weakness in the extremities, difficulty in walking and standing, and inability in controlling muscle movements. One of the hawks that died of mercury poisoning was put on a mercury free diet three days after the symptoms developed but did not recover. The hawks that survived did not display signs of being poisoned, but a reduced weight was observed in birds on the heaviest contaminated diet, in contrast to the birds on the lowest mercury level in which no effect on food consumption was observed. These findings can be explained on

Fig. 18. Myocardium, hawk no. 2893. Note degenerative changes consisting of nuclear pyknosis and granular swelling of sarcoplasm X 600.

Fig. 19. Femoral muscle, hawk no. 2893. Myofibrils are irregularly swollen and some are transformed to a homogeneous eosinophilic mass. There is some proliferation of sarcolemma and fibrous connective tissue X 600.



the basis of the affinity of alkyl mercury to the nervous system. In rats, Friberg (1959) found concentrations of mercury to be ten times higher in the brain after injection of methyl mercury, when compared with mercuric chloride. Our findings are largely consistent with those of Borg et al. (1969) who experimentally studied the effects of a methyl mercury contaminated diet on pheasants, and also those of Tejning (1967a) who conducted similar experiments with chickens.

Borg et al. (1969) found axon swelling and demyelination in the cerebellum, the pons and in peripheral nerves but no lesions in the spinal cords of experimentally poisoned pheasants. In our hawks, lesions were most pronounced in the spinal cord. It is unfortunate that we did not examine peripheral nerves. Borg et al. (1969) listed fatty changes in liver and kidneys and muscular atrophy among the changes most consistently found in naturally poisoned birds and in experimentally poisoned pheasants. In our experimentally poisoned hawks, fatty changes were not consistent finding but granular cytoplasmic degeneration was seen in cardiac and smooth muscles and in at least one case, pronounced degenerative changes occurred in skeletal muscles of the legs. It is possible that these latter lesions were caused, at least in part, by paralysis referable to nerve lesions, rather than direct effects of mercury on striated muscle.

There is, however, a discrepancy with respect to what was found to be lethal levels of mercury. In the present study 17 - 20 ppm were found in the livers of hawks that died, while in the aforementioned studies, 30 ppm or more were reported as lethal levels. These findings therefore indicate that the tolerance level to mercury could be lower

in hawks than in pheasants and chickens.

From the present study we can deduce that chicks containing less than 50 mg of mercury (orginating from ingested methyl mercury) may kill a hawk when ingested over a period of four weeks, while on the other hand a hawk survived after being fed 124 mg of mercury through a 12 week period. This indicates a considerable variance of tolerance to mercury, the smallest individuals, however, being those that first succumbed. The sex of the hawks was not recorded, but the smallest individuals were likely males as male red-tailed hawks are markedly lighter than females (Craighead and Craighead 1956). Other sex-associated differences in response to experimental conditions were probably minimal as only yearling hawks were used. Bäckström (1969) reported that hen quail (Coturnix coturnix) excreted methyl mercury more rapidly than cocks, but this pehnomenon was apparently due to the affinity of methyl mercury to the oviduct and albumen which resulted in accumulation of mercury in the eggs..

The sudden appearance and rapid development of the above mentioned signs in the affected hawks, together with the fact that they did not recover after being put on a mercury free diet, supports Löfroth's (1969) theory that as to the gross clinical symptoms, a threshold mechanism operates; the clinical effects do not show up until a certain number of brain cells have been damaged.

An important question is how and to what extent can these findings be related to wild bird populations. Fimreite et al. (1970) reported up to 10 ppm of mercury in livers of seed-eating birds from Alberta. Thus we must anticipate that predators that largely prey upon seed-eating

birds may be subject to mercury poisoning where mercury seed-dressings are used on a large scale.

SUMMARY

Six groups of one year old red-tailed hawks (Buteo jamaicensis) of three birds each were fed chicks contaminated with methyl mercury at three different levels (3.9, 7.2, and 10.0 ppm of mercury were found in their livers respectively). The experimental feeding periods were 4 or 12 weeks. An additional two groups served as controls. Mortality occurred in the groups on the most contaminated diets after an exposure period of one month or more, the signs of poisoning prior to death being essentially neurological. The mercury levels in the liver of hawks that succumbed by mercury poisoning were about 20 ppm. These hawks as well as those that survived on the two heaviest contaminated diets showed pathological changes of which the most consistent were swelling of axons of myelinated nerves in the spinal cord with dilatation of myelin sheaths and loss of myelin.

MERCURY CONTAMINATION OF CANADIAN PRAIRIE SEED-EATERS AND THEIR AVIAN PREDATORS

INTRODUCTION

Alkyl mercury derivatives have successively become the predominant disinfectants for control of seed-borne diseases in cereals and flax. This is due to their strong fungicidal effect, comparatively low phytotoxity and, from an agricultural point of view, favourable physical properties. Unfortunately, however, the alkyl homologues are also the most toxic and persistent form of mercury. By entering the food chain through seed-eating birds that pick up uncovered treated grain, alkyl mercury may have the most serious consequences for wildlife. This has been demonstrated in Sweden by Borg et al. (1965, 1969) who reported hazardous levels of mercury both in seed-eating birds and their predators. As a result the use of alkyl mercury-containing seed-dressings was banned in Sweden in 1966 and replaced by the less persistent alkoxy-alkyl mercury compounds. This change, together with a general reduction in the use of seed-dressings, has apparently eliminated the use of mercury seed-dressings as a hazard to wildlife in Sweden (Wanntorp et al. 1967).

In Canada, however, alkyl mercury derivatives are the predominant fungicides used in seed-dresings, and are used extensively (Fimreite 1970). The present study was undertaken with the aim of finding out whether Canadian seed-eating animals and their predators were contaminated by mercury from seed-treatment of prairie grain.

STUDY AREAS, METHODS, AND MATERIALS

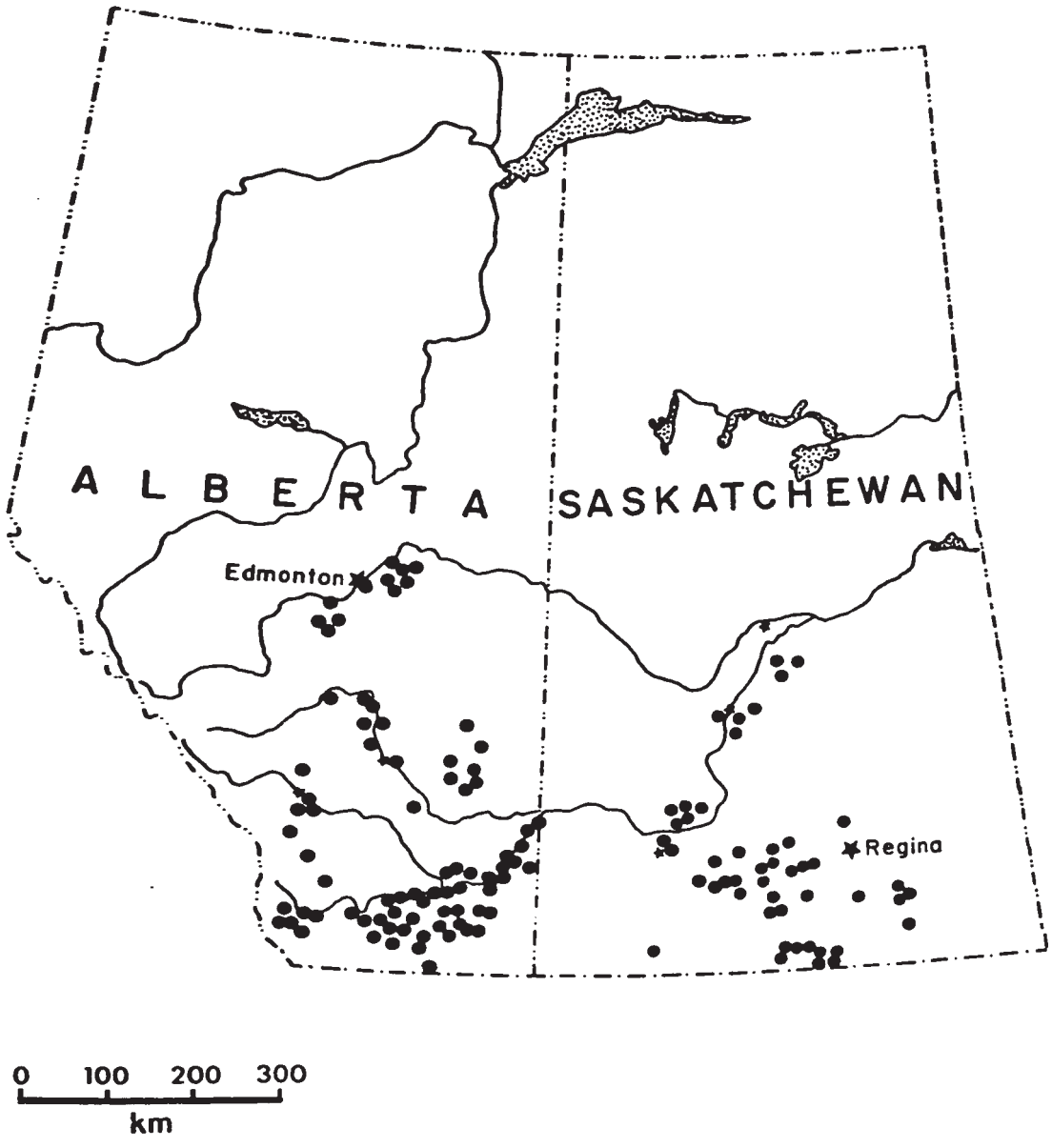
Following research into the current uses of mercury in Canada (Fimreite 1970), and based on the Swedish experience (Borg et al. 1969), samples were collected of those forms of wildlife believed most likely to be contaminated.

The material for this study was collected in the spring and early summer of 1968 and 1969, and the major collection areas were southern Alberta and southern Saskatchewan (Fig. 20). In southern Alberta, irrigation is widespread and mercury seed-dressings are used extensively while in contrast, southern Saskatchewan is an entirely dry-farming district where seed-treatment is less common. A comparison between these areas was judged valuable in order to test the significance of seed-dressings as a source of mercury contamination of terrestrial wildlife.

The sample material included seed-eating birds and rodents, predatory birds, and a few predatory mammals. Among seed-eating birds, the emphasis was laid on important upland game birds (pheasants and partridges) and certain small seed-eating passerine birds known to be important as food for bird-eating birds of prey. In 1968, attention was also paid to collecting seed-eating species specifically from areas where grain seed was known to have been treated as well as from areas where treated seed was not readily available.

In sharp contrast to the Swedish studies, which included many samples found dead (Borg et al. 1969), practically all of my samples of birds and mammals were shot in the wild. Raptor egg samples were collected at every opportunity and include fresh, partly incubated, and addled eggs.

Fig. 20. Outline of map of Alberta and Saskatchewan showing sampling locations.


ALBERTA
SASKATCHEWAN
Edmonton
Regina
0 100 200 300
km

Liver was chosen as the most suitable tissue to work with for residue comparisons in samples showing a wide range in size. This organ was chosen because it is known to concentrate and hold mercury entering the body, is readily accessable through dissection, and is sufficiently large that enough tissue is available from even the smaller birds and mammals.

For birds of prey, eggs were sampled instead of livers, since mercury has been shown experimentally to have strong adverse effects on reproduction (Fimreite 1970a), and because it was undersirable to collect a large number of adults. Egg samples were analyzed for both mercury and organochlorine residues. Prior to sending the egg contents for analysis, the outer dimensions of the intact eggs were measured and recorded. The contents were then removed and the shells were washed and allowed to dry at room temperatures. Once dry the shells were weighed to 0.01 gm. and Ratcliffe's (1967) shell-thickness index calculated (Weight/Length x Width).

All samples were freeze-dried by L. M. Reynolds, Ontario Research Foundation, and analyzed for mercury using the neutron activation technique by Gulf General Atomic Incorporated, San Diego, California with the following analytical procedure:

> "Weighed portions of each sample, and a comparator mercury standard, were sealed in clean quartz ampoules, numbered, weighed, and then irradiated for 67 hours in a nuclear reactor at a thermal-neutron flux of 2×10^{12} n/cm^2-sec.

After allowing the samples to decay for two days, in order to reduce the amount of ^{24}Na activity present, the samples were subjected to radiochemical separation by the prodecure of Sjöstrand (Anal. Chem, 36 (1964) 814). In this procedure, the irradiated sample is dissolved, in a nitric and sulfuric acid mixture (containing 20 mg of non-radioactive mercury carrier) under reflux conditions. After the sample is dissolved, mercury is distilled and finally electroplated onto a preweighed gold foil. The gold foil is weighed, to obtain the net weight of recovered mercury carrier, and the sample is counted with a 2-inch by 2-inch well-type NaI(Tl) detector, coupled to a multichannel pulse-height analyzer. Comparison of the integrated x-ray/γ -ray photopeaks at 68-77 keV from 65-hour Hg^{197} in the samples with that of the standard, followed by correction for the carrier recovery, provides for the quantitation of Hg."

Mercury residue values are given in parts per million (ppm) on a wet (fresh) weight basis.

A number of eggs of predators were also analyzed for organochlorine pesticides by Ontario Research Foundation. The analytical procedure is described in Appendix IV.

RESULTS

All samples analyzed contained mercury residues, however, considerable variation was found between species and within samples of the same species. The mercury levels for each species sampled are summarized by province in TABLES 14, 15, and 16.

Among seed-eating birds (TABLE 14), the most elevated mean residue levels were found in ring-necked pheasant (Phasianus colchicus) and gray partridge (Perdix perdix) the two species of resident upland game birds most frequently associated with farmyards, roadsides and cultivated fields, while in sharp-tailed grouse (Pedioecetes phasianellus) which prefers habitats such as wild grasslands, only moderate or low levels occurred. Horned lark (Eremophila alpestris) an early spring migrant that also frequents roadsides and cultivated fields feeding on weed seeds and grain carried mercury levels much similar to those found in pheasants and partridges. Slightly lower levels were found in later migrants including red-winged blackbirds (Agelaius phoeniceus), Brewers blackbirds (Euphagus cyanocephalus) and western meadowlarks (Sturnella neglecta) all collected near farm buildings or in cultivated fields. The lowest residues were in vesper sparrows (Pooecetes gramineus) and chestnut-collared longspurs (Calcarius ornatus) which were collected in areas of open grassland.

In 1968, 55 samples of seed-eating birds and rodents were specifically collected from treated and untreated areas to demonstrate any possible relationship between tissue mercury levels and the use of mercurial fungicides for seed-treatment. A comparison of the mean residue levels

TABLE 14. Mercury residues in livers of seed-eating animals (ppm).

	ALBERTA			SASKATCHEWAN		
GROUP/SPECIES	$\bar{x} \pm$ SE	(n)	Range	$\bar{x} \pm$ SE	(n)	Range
UPLAND GAME BIRDS						
Phasianus colchicus	2.835 ± 0.583	(10)	0.484 - 5.92			
Perdix perdix	1.116 ± 0.406	(7)	0.447 - 2.71	0.549 ± 0.506	(9)	0.019 - 4.60
Pedioecetes phasianellus				0.027 ± 0.151	(7)	0.021 - 1.11
PIGEONS & DOVES						
Columba livia	0.705 ± 0.364	(3)	0.015 - 3.16	0.304	(1)	
Zenaidura macroura	0.239 ± 0.053	(8)	0.139 - 0.319	0.415	(1)	
WATERFOWL						
Anas platyrhynchos	0.316 ± 0.053	(2)	0.215 - 0.417			
SONGBIRDS						
Eremophila alpestris	1.573 ± 1.004	(10)	0.020 - 10.2	0.454 ± 0.142	(8)	0.019 - 0.943
Agelaius phoeniceus	0.884	(1)				
Euphagus cyanocephalus	0.489 ± 0.115	(4)	0.304 - 0.817			
Sturnella neglecta	0.284 ± 0.181	(3)	0.077 - 0.64			
Molothrus ater	0.274	(1)				
Pooecetes gramineus	0.162	(2)	0.113 - 0.210			
Calcarius ornatus	0.098	(2)	0.066 - 0.129			
RODENTS						
Spermophilus richardsonii	1.051 ± 0.571	(7)	0.018 - 3.47	0.102 ± 0.020	(2)	0.082 - 0.122
Peromyscus spp.				0.231 ± 0.203	(4)	0.017 - 0.-38

(TABLE 17) shows considerably higher mercury content in each group of samples from treated areas than in the groups from untreated areas. The difference between the total sample of seed-eating animals from treated and untreated areas is significant ($P < 0.01$).

A similar comparison of residue levels in seed-eating species from Alberta, where mercurial fungicides are used extensively, with those from Saskatchewan, where mercurial seed-treatment is much less extensive, is shown in TABLE 18. The difference in mercury residue levels in the samples from the two areas is also significant ($P < 0.05$).

Only a limited number of adult predators was examined for mercury residues in liver. Among these were three short horned owls (Asio flammeus) of which two showed high mercury levels, the highest (11.3 ppm) being recorded in a specimen found dead (TABLE 15).

Elevated mercury levels were found in the egg contents of the majority of the predatory birds sampled (TABLE 16). However, as with the seed-eaters, and with the exception of those forms most specific in their food preferences, a wide variability in the mercury content was evident in the eggs of each species. The range between residue levels was most pronounced in the eggs of the great horned owl (Bubo virginianus) and red-tailed hawk (Buteo jamaicensis) two species which frequently utilize a wide variety of both birds and mammals as prey (Rusch et al. 1970, Luttich et al. 1970).

The eggs collected in Alberta contained significantly ($P < 0.05$) higher mercury levels than those from Saskatchewan (TABLE 18).

The highest mean levels with the least variability were found in the egg contents of the Richardson's merlin (Falco columbarius

TABLE 15. Mercury residues in livers of predators of seed-eating animals (ppm).

GROUP/SPECIES	ALBERTA			SASKATCHEWAN		
	$\bar{X} \pm$ SE	(n)	Range	$\bar{X} \pm$ SE	(n)	Range
OWLS						
Asio flammeus	6.837 ± 3.289	(3)	0.0420 - 11.30			
Speotyto cunicularia	3.735	(2)	1.23 - 6.24	0.729	(1)	
FALCONS						
Falco mexicanus	1.260	(1)				
Falco sparverius	0.755	(1)				
BUTEOS						
Buteo swainsoni	0.762 ± 0.239	(5)	0.230 - 1.48	0.451 ± 0.081	(8)	0.219 - 0.949
Buteo jamaicensis	0.483	(1)				
HARRIERS						
Circus cyaneus				0.069 ± 0.030	(3)	0.028 - 0.127

TABLE 16. Mercury residues in eggs of predators of seed-eating animals (ppm).

GROUP/SPECIES	ALBERTA $\bar{X} \pm SE$	(n)	Range	SASKATCHEWAN $\bar{X} \pm SE$	(n)	Range
OWLS						
Bubo virginianus	1.940	(1)		0.076 ± 0.017	(6)	0.014 - 0.121
Speotyto cunicularia				0.112	(1)	
FALCONS						
Falco c. richardsonii	0.283 ± 0.045	(10)	0.153 - 0.543			
Falco mexicanus	0.240 ± 0.038	(46)	0.019 - 1.71	0.169 ± 0.036	(12)	0.033 - 0.427
ACCIPITERS						
Accipiter striatus				0.119	(1)	
Accipiter cooperii				0.085	(2)	0.044 - 0.126
BUTEOS						
Buteo jamaicensis	0.342 ± 0.165	(10)	0.035 - 1.62	0.052 ± 0.013	(4)	0.023 - 0.080
Buteo swainsoni	0.120 ± 0.054	(7)	0.030 - 0.417	0.283	(1)	
Buteo regalis	0.066 ± 0.031	(7)	0.011 - 0.249	0.034 ± 0.009	(4)	0.023 - 0.061
EAGLES						
Aquila chrysaetos	0.100 ± 0.059	(3)	0.024 - 0.218	0.025	(2)	0.-17 - 0.032
HARRIERS						
Circus cyaneus	0.031 ± 0.008	(5)	0.018 - 0.060	0.014	(1)	

TABLE 17. Comparison of mercury levels (ppm) in livers of predominantly seed-eating birds and rodents from treated and untreated areas.

	Hg Residues from Treated Areas		Hg Residues from Untreated Areas		Significance of the difference between the groups
	(n)	$\bar{X} \pm SE$	(n)	$\bar{X} \pm SE$	
Rodents	(6)	1.248 ± 0.683	(5)	0.180 ± 0.149	
Songbirds	(10)	1.632 ± 0.997	(3)	0.034 ± 0.012	
Upland Game Birds	(19)	1.880 ± 0.444	(12)	0.348 ± 0.223	
All Seed Eaters	(35)	1.701 ± 0.379	(20)	0.259 ± 0.139	$P < 0.01$

TABLE 18. Comparison of mercury levels (ppm) in eggs of predators and in livers of seed-eating prey from Alberta and Saskatchewan.

	Alberta		Saskatchewan		Significance of the difference between the groups
	(n)	$\bar{\chi} \pm$ SE	(n)	$\bar{\chi} \pm$ SE	
Predatory Birds (egg)	(89)	0.236 ± 0.035	(34)	0.103 ± 0.017	$P < 0.05$
Seed-eating Prey (liver)	(61)	1.160 ± 0.231	(32)	0.370 ± 0.148	$P < 0.05$

richardsonii), which feeds primarily on small birds, and the prairie falcon (Falco mexicanus) which primarily, though not exclusively, feeds on passerine birds. Similarily uniform and equally elevated mercury residues (0.315 - 0.568 ppm) were present in four eggs of peregrine falcon (Falco peregrinus) from North West Territories. The peregrine falcon is also known to prey heavily on small birds throughout the year.

In contrast to the high residue levels found in the predominately bird-eating falcons, there are low mercury levels in the eggs of the most specific mammalian feeders. Consistent low levels are most evident in the eggs of the ferruginous hawk (Buteo regalis), which in Western Canada feeds almost exclusively on Richardson's ground squirrels (Spermophilus richardsonii); the golden eagle (Aquila chrysaetos), which in this study area feed primarily on the white-tailed jackrabbit (Lepus townsendii); and the marsh hawk (Circus cyaneus), which preys heavily on Microtus and Peromyscus spp.

A comparison of the mercury residues in the eggs of the bird-eating falcons and accipiters with those in the eggs of the mammal-eating buteos, harriers, and eagles (TABLE 19) shows a differerence which is significant ($P < 0.05$).

DISCUSSION

Seed-eaters

Elevated mercury levels were found in the majority of the seed-eating birds. However, there was a significant difference between birds collected from treated areas and those from untreated areas, the

TABLE 19. Mercury levels (ppm) in eggs of buteos, harriers and eagles compared with accipiters.

	Buteos, Harriers and Eagles		Falcons and Accipiters		Significance of the difference between the groups
	(n)	$\bar{X} \pm SE$	(n)	$\bar{X} \pm SE$	
Mercury Residues in eggs	(44)	0.133 ± 0.042	(71)	0.228 ± 0.027	$P < 0.05$

former having the highest mercury levels. This suggests that mercury seed-dressing is the primary source of contamination, an indication that is also supported by the fact that the mercury levels in the Alberta specimens significantly exceeded those from Saskatchewan where seed-treatment is far less common.

However, since there may be inter-specific differences and the proportion of specimens from treated and untreated fields and from Alberta and Saskatchewan are not the same for all species included, the data are not exactly comparable.

Within seed-eating birds the highest mean mercury levels were found in the two uplans game bird species, pheasants and partridges. This is not surprising as grain figures prominently in the diet of these birds (Trippensee 1948), in contrast to sharp-tailed grouse, a typical grassland species, which carried only low or moderate mercury levels. Among songbirds there seems to be more variation, the horned lark being the best represented and showing a range of 0.02 ppm to 10 ppm. The variation between the mercury levels from treated versus non-treated fields was also more pronounced for small seed-eating birds and rodents than for game birds. This is in part explained by the fact that large birds such as pheasants and partridges have a larger feeding territory than do smaller birds and rodents (Schoener 1968) and thus the chance for having their feeding territory restricted to a specific field would be much less.

Our findings can be best compared with those of Borg et al. (1969) who found that of 298 seed-eating birds shot for investigation in Sweden before alkyl mercury compounds were banned there, 41% carried liver

mercury levels of above 2 ppm. The percentages of seed-eating birds in our investigation carrying similarly high levels were 23% and 4% for Alberta and Saskatchewan respectively. This suggests that mercury contamination of Canadian game and other seed-eating birds is less pronounced than was the case in Sweden where Borg et al. (1969) reported that a large number of birds found dead obviously had been poisoned by mercury, and that lethal effects in pheasants were associated with liver levels on the order of 30 ppm or more. My specimens did not reveal mercury concentration approaching such levels, suggesting that direct mortality of seed-eating birds may not have been associated with mercury seed-treatment on the Canadian prairies.

In experiments involving 192 penned pheasants Fimreite (1970b) found that hens with mercury liver levels of 3 - 13 ppm laid eggs with concentrations of 0.5 to 1.5 ppm which had significantly lowered hatchability than the controls. Five of 10 wild pheasants taken in Alberta had liver levels above 3 ppm so that some reduced hatchability of Alberta pheasants due to mercury is a distinct possibility. The penned pheasants as well as their eggs were analyzed by another method (Oliver and Funnell 1958) than the neutron activation technique applied in the present study, and reservations must therefore be taken as to possible discrepencies between these two analytical techniques.

For popular game birds we also have to take into account the possibility of human hazard. Although the levels in muscles would be lower by a factor of 2 to 4 than those found in liver (Westermark 1967) it is obvious that that the levels in the Alberta pheasant and partridge muscle samples would be expected to exceed the FAO/WHO Codex Alimentarius

proposed concentration in foodstuff of 0.05 ppm.

It is probable that the most serious wildlife problem will arise at higher trophic levels. As food for raptors, the contaminated seed-eating birds can cause ecological problems since alkyl mercury is persistent in the animal body and concentrates in food chains. This suggestion has been supported experimentally by a demonstration of increased mortality or severe damage of the nervous system in red-tailed hawks fed a diet of chicks containing 7 to 10 ppm of mercury in the liver (Fimreite and Karstad 1970). These concentrations are comparable to the highest levels found in seed-eating birds in our samples.

Predators

It should be noted that since liver tissue was analyzed from the seed-eating bird samples no direct comparison of levels can be made with the residues in the egg contents of the predatory birds. However, experimental data on residues in livers of hen pheasants and their eggs (Fimreite 1970a) and the residue levels in the liver of one adult prairie falcon and her egg suggest mercury residues in the livers of female birds to be 5 to 9 times those in the eggs.

Because of the large hunting territories of the birds of prey it was not possible to determine with centainty whether the prey of a specific pair was restricted to either treated or untreated areas. However, since raptor egg samples collected in southern Alberta (where mercurial seed-dressings are used extensively) are widely separated geographically from those collected in southern Saskatchewan (where these seed-dressings are used infrequently) the comparison of residues found in samples from

these areas is essentially a comparison of samples from a treated and an untreated area.

The differences by area shown in these comparisons is significant and points to seed-dressings as the primary source of mercury contamination in these species. These results also strongly suggest seed-eating prey as the major source of mercury contamination of these predatory birds.

The wide variation in the mercury content of the eggs of those species frequently utilizing both birds and mammals for food suggests a correspondingly wide variation in the levels of contamination of their prey. Similarly it is apparent that the uniformly low residue levels in the eggs of those species feeding mainly on mammals show a wide separation from the relatively high yet equally uniform levels in the eggs of species preying primarily on birds. A comparison of the mercury residues found in the predominately bird-eating falcons and accipiters with the residues in the predominately mammal eating buteos, harriers and eagles shows the difference between the two groups to be significant, and these findings closely parrallel those reported by (Borg et al. 1969). These data clearly point to the seed-eating birds as the major carrier of mercury contamination and at the same time indicate mammals are much less of a mercury hazard to predatory birds.

Unfortunately the sample of small mammals is not representative and is too small to provide any indication of general levels, however, the comprehensive study of Lihnell and Stenmark (1967) shows Swedish small mammals were carrying much lower levels of mercury than were the seed-eating birds. On the other hand, my mammal sample does indicate

that we cannot entirely ignore contamination in these prey since the ground squirrels collected in treated areas did show elevated mercury levels.

The potential ecological mercury hazard created by the presence of high residues in seed-eating birds is demonstrated by the occurrence of high levels in the horned lark and in the eggs of both the Richardson's merlin and the prairie falcon which both prey heavily on the horned lark (Enderson 1964, Fox 1964). A similar relationship appears in our finding pheasant remains at the nest site of the pair of prairie falcons whose eggs showed the highest mercury content for this species. In 1968 only one of five eggs in the nest hatched, the remaining four eggs contained mercury levels ranging from .9 to 1.7 ppm. In 1969 only the female of the pair was observed at the site, and no nesting occurred.

As already indicated experiments with pheasants indicate that alkyl mercury may have strong adverse effects on reproduction (Fimreite 1970a). Since the mercury concentrations in the eggs of falcons frequently are in the same range, effects must be expected in wild falcons unless falcons should be much less sensitive to mercury than pheasants This coincides also with the recently documented declines in prairie falcon (Fyfe et al. 1969) and Richardson's merlin (Fyfe 1970) populations in Western Canada and is reinforeced by the fact that several of the eggs collected for this study were addled and contained high mercury concentrations. Reproductive failures in the white-tailed eagle in Finland and Sweden have been caused by mercury contamination according to Henriksson et al. (1966) and Borg et al. (1969). The source in this

case, however, was mercury-containing slimicides in the pulp industry.

Samples of seed-eating birds in western Canada have also shown relatively high DDE residues in their tissues (Fyfe 1970), therefore it is not surprising that we found a positive correlation between mercury and DDE levels in eggs of prairie falcons which in some areas feed heavily on passerine birds. However, comparison between eggshell thickness (using the Ratcliffe index) and DDE content in prairie falcon eggs showed a highly significant inverse correlation ($P < 0.01$) while a similar comparison of mercury contant and shell thickness in 59 prairie falcon eggs failed to show any correlation.

It has been shown that mercury may interfere with detoxification enzyme chains in the vertebrate liver (Arrhenius 1967). Possible synergism between mercury and DDE and between mercury and heptachlor should therefore be taken into account as considerable DDE and heptachlor concentrations were found in many of the eggs and were positively correlated with those of mercury. Such a synergism would also in all probability be most serious for the bird-eating falcons which are most exposed to both organochlorine pesticides and mercury contamination through seed-eating birds.

SUMMARY

The investigation revealed that mercury contamination is rather widespread in Canadian prairie wildlife. Seed-eaters, showed a difference in the mercury levels in specimens shot on field seeded with treated grains as compared with those collected from untreated fields thereby

indicating mercury seed-dressings as the most probable source of contamination. Corroborating evidence is further provided in the fact that the mercury concentrations both with regard to seed-eaters and their predators were significantly higher in specimens from Alberta, a province with widespread use of mercury seed-dressings as compared with Saskatchewan where seed-treatment is less common.

There was a considerable variance in the mercury levels both between and within species. Among seed-eating birds the highest residue levels (average 2.8 ppm in liver) were found in pheasants. Lesser, but still considerable mercury concentrations were shown to be present in partridges and small seed-eating passerine birds such as horned larks. Among the rodents ground squirrels were shown to have high mercury levels when inhabited treated fields.

In eggs of falcons as well as of accipiters (birds-eating birds of prey) were frequently found with elevated mercury levels while the levels in eggs of those eagles, buteos and harriers which prey largely on rodents were low. It appears that this difference can be explained satisfactorily on the basis of their food habits alone as birds were shown to carry higher mercury levels than rodents.

As to the significance of the reported mercury levels it is concluded that falcons and accipiters may be adversly affected as eggs of these species frequently carried mercury levels which experimentally have been shown to reduce hatchability in pheasant eggs. The current findings coinside with the recent declines reported in prairie falcon, merlin and peregrine falcon.

MERCURY CONTAMINATION OF CANADIAN FISH AND FISH-EATING BIRDS

INTRODUCTION

Elevated mercury levels in freshwater fish in Sweden and Finland have been reported by several authors (Johnels et al. 1967, Noren and Westöö 1967, Westöö 1967a, Westöö and Noren 1967, Häsänen and Sjöblom 1968, and Westöö and Rydälv 1969). According to Löfroth (1969) about one per cent of the total Swedish water areas are inhabited by fish with more than 1 ppm in muscle tissue.

Borg et al. (1969), in a comprehensive study on the occurrence of mercury in Swedish wildlife, found elevated mercury levels in tissues of a number of fish-eating birds such as gulls (Larus sp), crane (Grus grus), and white-tailed eagle (Haliaeetus albicilla). They reported mercury residues of 3.5 to 11 ppm in six white-tailed eagles' eggs from five different nests and suggested that the decrease in the reproduction of this species could be ascribed to mercury poisoning. A corresponding decline in the white-tailed eagle population in Finland is likewise associated with mercury contamination (Henriksson et al. 1966).

The loss of mercurials used for slime control in the pulp industry was considered the major source of contamination, but other industrial releases of mercury, primarily the chlor-alkali industry, are also important. Young salmon kept in cages downstream from pulp mills and chlor-alkali plants showed elevated mercury levels after one to two months exposure (Hasselrot 1967).

In Japan, water contaminated by mercury in effluent from plastic factories resulted in high mercury levels in fish, and consumption of the contaminated fish caused either death or severe neurologic disorders in humans (Kurland et al. 1960 and Irukayama 1966).

Fimreite (1970) has shown that Canadian pulp mills, chlor-alkali plants, and other industries also use considerable quantities of mercury, and therefore mercury contamination of Canadian wildlife species was expected.

This paper reports the data collected on the mercury content of selected fish and wildlife species from ecosystems where mercury contamination was anticipated, and discusses the results in relation to the effects of mercury on fish, wildlife and humans.

MATERIALS AND METHODS

Specimens analyzed included 113 fish, 30 birds and 6 bird eggs collected specifically for this study. Walleye (Stizostedion vitreum) was chosen as the chief test species from the Great Lakes as it is a typical predaceous fish, and because of the accumulating properties of mercury walleye were likely to have high concentrations where contamination occurs. Elsewhere when possible, other species also likely to be near the top of the food chain, were selected. In most cases samples were taken in the vicinity of chlor-alkali plants or pulp mills from which mercury was known to have been released. "Control" samples were taken where possible; e. g., upstream from pulp mills situated on a river. The specimens from Pinchi Lake, B.C., were taken within a mile

from Cominco's old mercury mine, partly before and partly after the mine was reopened in 1968. Most of the avian material consisted of adult or immature fish-eating birds. The eggs included were of red-breasted merganser (Mergus serrator) and common tern (Sterna hirundo). The specimens from the Great Lakes, the St. Maurice River, Quebec, Baie de Chaleur, N.B., and some from Pinchi Lake were collected in the autumn of 1969 and all others during the summer of 1968.

Both the fish and bird specimens were frozen immediately after being collected. Samples taken from the lateral muscle of fish, liver of birds and the homogenized content of eggs were freeze-dried by L.M. Reynolds, Ontario Research Foundation and analyzed for mercury mercury using the neutron activation method by Gulf General Atomic Incorporated, San Diego, California, analytical procedure is quoted on page 111.

Mercury residue values are given in parts per million (ppm) on a wet (fresh) weight basis.

Five fish specimens from the Great Lakes were also analyzed for methyl mercury by the National Institute of Public Health, Stockholm, Sweden, using a combined gaschromatographic and thin-layer chromatographic technique described by Westöö (1966, 1967).

RESULTS

Elevated mercury concentrations were found in all freshwater fish (TABLE 20), the levels being highest in fish from Pinchi Lake, the St. Clair River, and Lake St. Clair, where the maximum concentrations in muscles were 10.5, 7.09, and 5.01 ppm respectively. The lowest levels were found in fish taken upstream from the town of Shawinigan Falls and the chlor-alkali plant on St.Maurice River. This was the only sample of

freshwater fish that did not exceed the Canadian maximum acceptable level of 0.5 ppm of mercury as set by the Food and Drug Directorate for human consumption. Fish from coastal waters showed moderate or low levels of mercury (TABLE 21). The lowest were recorded in the Atlantic herring (Clupea harengus) (less than 0.6 ppm). Levels were also low in alewife (Alosa pseudoharengus) and in Atlantic tomcod (Microgadus tomcod). The American eel (Aquilla rostrata) had a somewhat higher mean level, 0.32 ppm, while in a single specimen of copper rockfish (Sebastodes caurinus), was found as much as 0.88 ppm. The statistical analysis revealed that the freshwater species had significantly ($P < 0.01$) higher mercury concentrations than the marine species.

Most samples showed a positive correlation between mercury levels and weight of the fish specimens, indicating that larger fish contain relatively more mercury per weight unit than smaller fish (Fig.21). The exceptional samples, which showed a negative correlation between weight and mercury level had only three specimens in each sample.

The mercury levels in birds (TABLE 22) were highest in red-necked greb (Podiceps grisegena) from Pinchi Lake where one specimen contained 17.4 ppm in the liver. Lower, but still considerable were the mercury levels found in the pelagic cormorant (Phalacrocorax pelagicus) from the Nanaimo area, and the double-creasted cormorant (Phalacrocorax auritus), great blue heron (Ardea herodias), and common tern from the Baie de Chaleur. Eggs of common tern and red-breasted merganser from the Baie de Chaleur contained an average of 0.582 and 0.812 ppm of mercury respectively. The bird specimens from the Ottawa River, shot about 20 miles downstream from the city of Ottawa, represent four species with different feeding habits, the percentage of animal food in their diet ranging from 10 to 100. As indicated in TABLE 23, there seems to be a close relationship between the species' mercury residue levels and proportion of animal matter in their diet.

Possible sources of mercury water pollution are shown in Fig. 2 and 3. A chlor-alkali plant in Sarnia is thought to be largely responsible for the high levels in the Lake St. Clair and the St. Clair River as the levels in fish there are significantly higher ($P < 0.1$) than in those from above this plant in Lake Huron. The fish taken downstream from the chlor-alkali plant in Shawinigan Falls (Maurice River) had also significantly ($P < 0.1$) higher mercury levels than those upstream from the plant. Even more obviously, the high levels in saugers (Stizostedion canadense) downstream from a pulp mill in Ottawa result from that mill's use of mercury slimicides. The latter carried significantly ($P < 0.01$) higher mercury concentrations than those upstream from the mill. Herrings and eels from Baie de Chaleur were taken close to a pulp mill that used mercury slimicides. The Bay also received the effluent from a chlor-alkali plant, but the small number of specimens made it difficult to compare levels in birds shot in the vicinity of Bathurst (Pulp mill) with those from the Dalhousie (Chlor-alakli plant) area.

Mercury slimicides may also be responsible for the elevated mercury levels in copper rockfish and pelagic cormorant from British Columbia specimens taken in the vicinity of pulp mills that discontinued use of mercury slimicides less than 10 years ago (Fig. 3, TABLE 21). The highest mercury level in the fish from Pinchi Lake was in a specimen collected after the mercury mine reopened mining operations in 1968, whereas in contrast the most elevated level in birds occurred in specimens collected before the mine was updated.

Five specimens of walleye from the Great Lakes were analyzed for the form in which mercury occurred. Ninety-six (range: 88 - 100) per cent

TABLE 20. Mercury residues in lateral muscle of fish from Canadian inland waters where mercury contamination was suspected.

Locality/Species	n	Body weight (grams) $\bar{x}$	Body weight (grams) Range	Mercury residues $\bar{x}$	Mercury residues Range	Correlation (r) between body weight and Hg residues in muscle tissue
PINCHI LAKE, B.C.						
Salvelinus namaycush	2	1700	1700 - 1700	5.78	1.07 - 10.5	
Hybopsis plumbea	1	50		0.841		
Prosopium williamsoni	4	307	230 - 429	0.654	0.295- 1.50	0.96*
Salmo gairdneri	4	243	161 - 322	0.381	0.248- 0.681	0.86
LAKE HURON, SOUTH END						
Stizostedion vitreum	8	807	725 - 984	1.08	0.581- 2.74	0.40
ST. CLAIR RIVER						
Ambloplites rupestris	6	646	55 - 368	2.30	0.548- 4.64	0.29
Lepomis gibbosus	3	64	46 - 95	2.64	0.255- 7.09	-0.56
Roccus chrysops	1	75		1.62		
Stizostedion vitreum	6	646	370 - 1018	1.60	0.887- 2.43	0.90*
Esox lucius	1	2265		1.00		
LAKE ST. CLAIR						
Stizostedion vitreum	8	819	363 - 1928	2.88	1.29 - 5.01	0.37
LAKE ERIE, WEST END						
Stizostedion vitreum	8	595	462 - 907	0.709	0.580- 0.901	0.32
OTTAWA RIVER, Downstream from pulp mill						
Stizostedion canadense	10	144	23 - 389	1.48	0.471- 2.73	0.90**
OTTAWA RIVER, Upstream from pulp mill						
Stizostedion canadense	10	165	117 - 217	0.719	0.423- 1.00	0.18
ST. MAURICE RIVER, Que. Downstream from chlorine plant						
Catostomus commersonii	3	5.7	3.8 - 9.2	0.66	0.519- 0.912	-0.38
Perca flavescens	3	2.2	2.0 - 2.4	0.53	0.260- 0.714	-0.99
ST. MAURICE RIVER, Que. upstream from chlorine plant						
Perca flavescens	2	1.6	1.4 - 1.8	0.195	0.194- 0.196	
Culaea inconstans	2	1.0	1.0 - 1.0	0.192	0.185- 0.199	

* Significant correlation ($P < 0.05$)
** Significant correlation ($P < 0.01$)

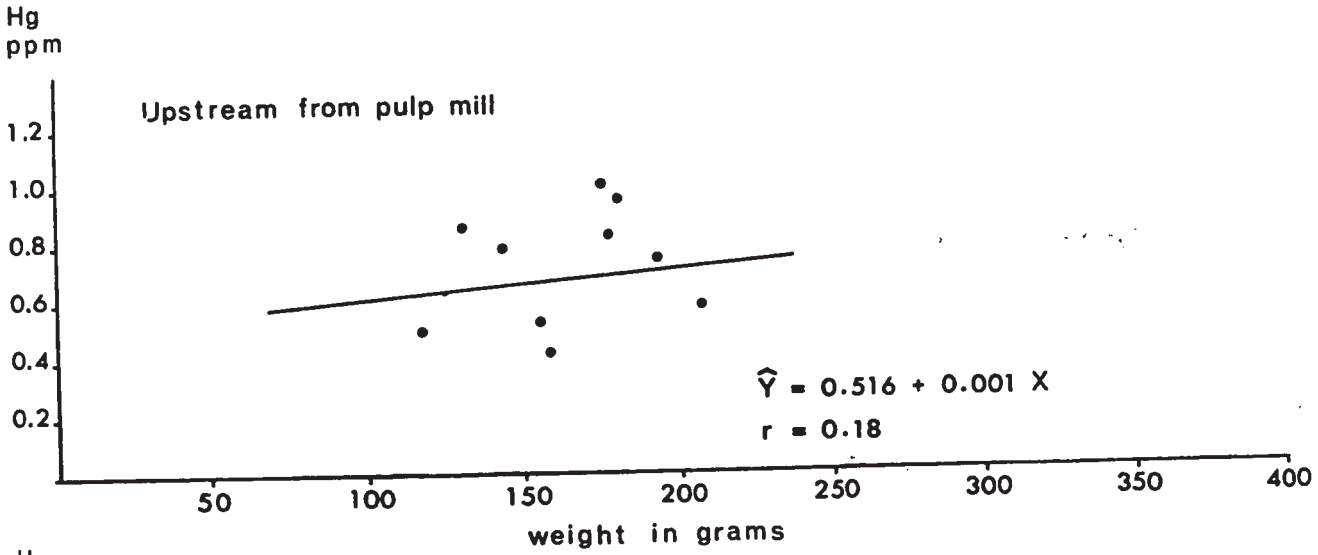
TABLE 2 Mercury residues in lateral muscle of fish from Canadian coastal waters.

	n	Mercury residues (ppm) $\bar{X}$	Range	Body weight (grams) $\bar{X}$	Range	Correlation (r) between body weight and Hg residues in muscle tissue
PORT ALBERNI, B.C.						
Sebastes caurinus	1	0.876		870		
NANAIMO, B.C.						
Sebastes caurinus	1	0.478		1065		
HORSESHOE BAY, B.C.						
Sebastes caurinus	1	0.176		353		
BAIE DE CHALEUR (Bathurst, N.B.)						
Pseudopleuronectes americanus	2[a]	1.10	0.862 - 1.33	215		
Anguilla rostrata	4	0.315	0.283 - 0.382	205	129 - 324	0.41
Microgadus tomcod	1[a]	0.180		100		
Alosa pseudoharengus	2[a]	0.100	0.096 - 0.104	81		
BAIE DE CHALEUR (Dalhousie, N.B.						
Clupea harengus	4	0.043	0.025 - 0.057	236	186 - 288	0.89

[a] *P. americanus* and *A. pseudoharengus* - two analyses of two pooled samples each containing four fish.
M. tomcod - one analysis of a pooled sample of four fish.

Fig.21. Relationship between body weight and mercury concentrations in lateral muscle of Sauger (Stizostedion canadense) from the Ottawa River, collected upstream and downstream from a pulp mill which used mercury-containing slimicides.

** Correlation significant ($P < 0.01$)


Hg
ppm
Upstream from pulp mill
1.2
1.0
0.8
0.6
0.4
0.2
$\hat{Y}$ = 0.516 + 0.001 X
r = 0.18
50
100
150
200
250
300
350
400
weight in grams

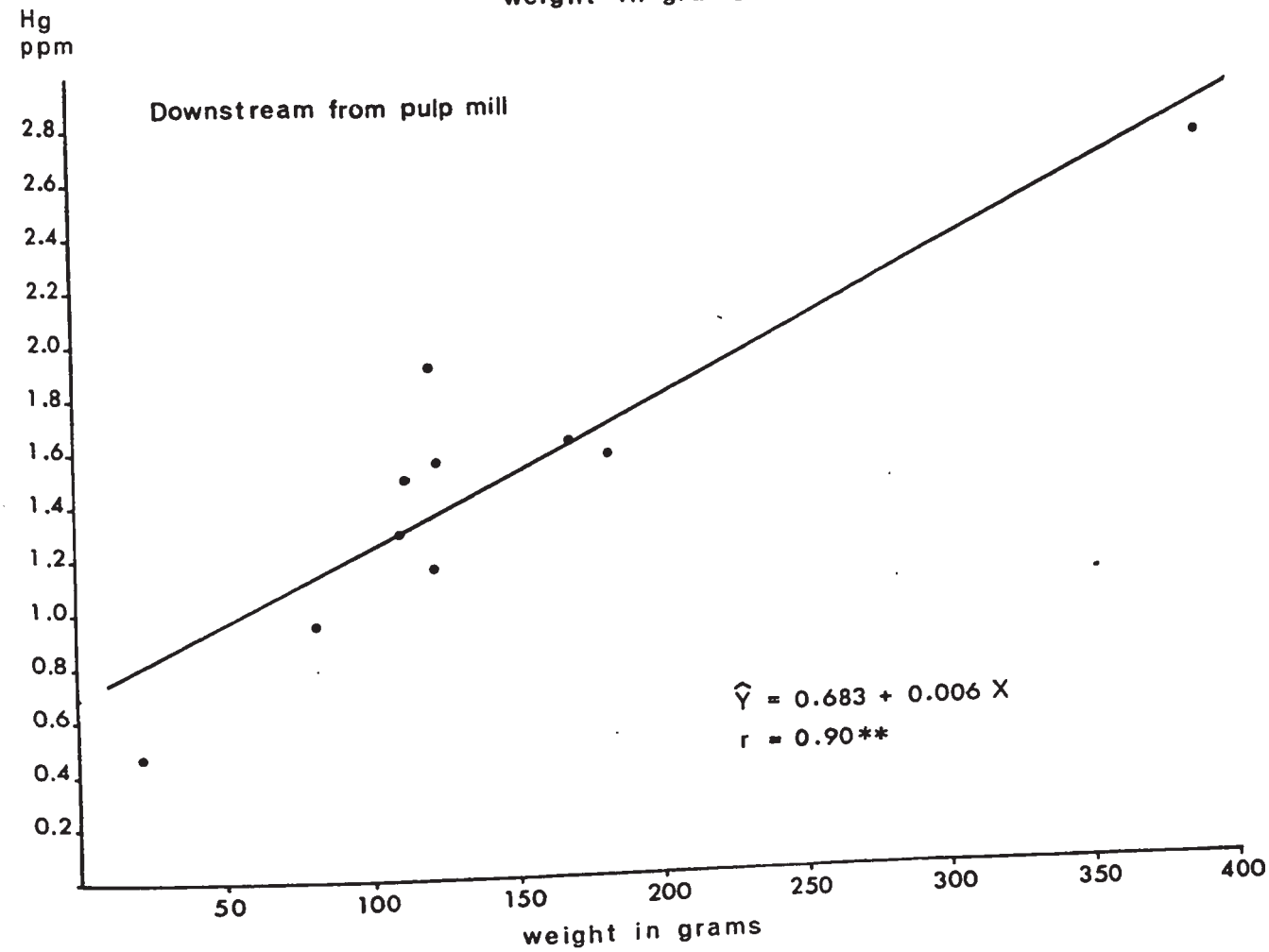

Hg
ppm
Downstream from pulp mill
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
$\hat{Y}$ = 0.683 + 0.006 X
r = 0.90**
50
100
150
200
250
300
350
400
weight in grams

TABLE 22. Mercury residues in birds collected where mercury contamination was suspected.

Locality/Species	Age Class	n	Tissue	Mercury residues (ppm) $\bar{X}$	Range
INLAND WATERS					
Pinchi Lake, B.C.					
Podiceps grisegena	Adult	3	Liver	10.32	0.448 - 17.4
Ottawa River (Baie Noire)					
Megaceryle alcyon	Adult	1	Liver	0.935	
Bucephala clangula	Adult	1	Liver	0.632	
Anas rubripes	Adult	5	Liver	0.381	0.021 - 0.771
Aix sponsa	Adult	3	Liver	0.158	0.101 - 0.212
COASTAL WATERS					
Nanaimo, B.C.					
Phalacrocorax pelagicus	Adult	5	Liver	2.06	1.22 - 3.68
Larus occidentalis	Adult	3	Liver	0.250	0.120 - 0.369
Baie de Chaleur (Bathurst, N.B.)					
Phalacrocorax auritus	Adult	1	Liver	11.3	
Ardea herodias	Immature	1	Liver	4.53	
Sterna hirundo	Adult	4	Liver	2.50	2.28 - 2.70
Mergus serrator		2	Eggs	0.812	0.453 - 1.17
Sterna hirundo		4	Eggs	0.582	0.182 - 1.42
Baie de Chaleur (Dalhousie, N.B.)					
Phalacrocorax auritus	Immature	3	Liver	3.51	3.08 - 4.01

TABLE 23. Relationship between mercury levels in liver of four species of birds and the percentage of animal food in their diet. The specimens were all collected from the same site, on the Ottawa River about 20 miles downstream from the city of Ottawa.

Species	n	Percentage animal food in their diet[a]	Mean mercury residues in liver ppm
Megaceryle alcyon	1	100	0.935
Bucephala clangula	1	74	0.632
Anas rubripes	5	24	0.381
Aix sponsa	3	10	0.158

[a] The duck animal food proportions are averages of a great many birds, and are taken from Kortright (1943): Ducks, Geese, and Swans of North-America.

of the mercury occurred as a methyl mercury compound (TABLE 24).

DISCUSSION

The material in this study was selected to determine the levels of mercury in Canada's fish and fish-eating birds in waterways contaminated by industries releasing mercury-containing effluent (Fimreite 1970). In this preliminary study, the results are representative only for areas where mercury contamination was expected. Uncontaminated areas were not sampled extensively due to financial limitations on the number of specimens that could be analyzed.

Our results indicate considerable contamination in several waterways in Canada, inlcuding the Great Lakes where walleye in Lake St. Claire averaged 2.9 ppm of mercury. The maximum concentrations however, were recorded in specimens from Pinchi Lake, 10.5 and 17.4 ppm in muscles of lake trout (Salvelinus namaycush) and liver of red-necked grebe respectively. Coastal waters also seem to be contaminated. In particular, Baie de Chaleur, where the contamination is best reflected in fish-eating birds such as cormorants, herons, terns, and mergansers. In eels from the Bay were also recorded somewhat elevated mercury levels while the pelagic herrings, on the contrary carried very low mercury concentrations.

The sources of contamination were in most cases proved to chlor-alkali plants with mercury cells, and current or previous use of mercury slimicides in the pulp industry. This could be confirmed statistically by comparing the levels in samples taken above and below the potential source.

TABLE 24. Total mercury and methyl mercury in five specimens of walleye (*Stizostedion vitreum*) from the Great Lakes.[a]

Locality	Specimen no.	Weight grams	Total mercury ppm	Methyl mercury ppm	Methyl mercury as percent of total mercury
Lake Huron South end	1	984	0.581	0.565	97.25
Lake St. Clair	2	363	1.29	1.22	94.57
Lake St. Clair	3	1021	2.06	1.82	88.35
Lake St. Clair	4	1928	3.42	3.40	99.42
Lake Erie West end	5	907	0.708	0.710	100.28
Average					95.97

[a] The total mercury was determined by Gulf General Atomic Incorporated, San Diego, California (neutron activation) and the methyl mercury by the National Institute of Public Health, Stockholm, Sweden (gaschromatography and thin-layer chromatography).

Recent studies by Wobesser et al. (1970) and Bligh (1970) revealed severe mercury contamination of fish from Saskatchewan River and Wabigoon-English River system. Concentrations above 10 ppm were recorded in some samples of predaceous fish and apparently in both cases mercury releases from chlor-alkali plants are the chief contaminators.

Sprague and Carson (1970) who investigated marine fishes from the Gulf of St. Lawrence found only moderate or low mercury levels, the most elevated (0.4 ppm) appearing in cod (Gadus morhua). Even in a remote area such as La Verendrye Provincial Park in Quebec, elevated mercury levels (1.4 ppm) have been reported in pike (Keith and Reynolds 1969). This must be due to air-borne pollutants, or an extraordinarily high natural background level. Studies elsewhere suggest that levels below 0.2 ppm in fish muscles do not indicate industrial contamination (Löfroth 1969). However, high background levels may be expected in British Columbia where elevated mercury levels in soils and plants have been reported from several areas (Warren et al. 1966). This has to be considered when evaluating the high mercury levels in fish and grebes from Pinchi Lake, but since specimens from Pinchi Lake collected before the mercury mine reopened contained abnormally high mercury concentrations it may well be that mercury released during mining operations nearly 25 years ago still is an important source of contamination. Fish and cormorants collected in the vicinity of pulp mills which discontinued use of mercury slimicides 5 - 10 years ago also contained elevated mercury levels. These findings support Löfroth's (1969) hypothesis that unless mercury is made biologically unavailable effects of mercury pollution may last for decades.

A wide variation in mercury concentrations is common both within and

among species, and also among specimens from the same catch-site. Such variation was also demonstrated experimentally by Hannerz (1968) in fish exposed to identical amounts of mercury. An accumulation of mercury through the food chain is indicated by the fact that the highest mercury levels generally were found in species at the higher trophic levels, while the pelagic and planktivorous Atlantic herring are little affected by mercury pollution, having less than 0.06 ppm residue levels. Furthermore, in bird species taken from the same area a close relationship between mercury and proportion of animal food in their diet was demonstrated, the highest being found in kingfisher (Megaceryle alcyon) which is a fish-eating bird, the lowest in wood duck (Aix sponsa), which diet consists only on 10% animal matter (Kortright 1943).

In fish, however, the variation in the mercury levels is partly connected with variation in the body weight. Johnels et al. (1967) found a positive correlation between weight and mercury concentrations in muscles. With some deviations from the rule, the same was demonstrated in the present study. Seasonal fluctuations in the mercury levels may also occur which are dependent on the rate of metabolism of the fish and the rate of food intake. Experimentally this has been demonstrated by Hasselrot (1967) who exposed salmon to mercury contaminated water and recorded higher levels in the summer than in the winter.

The levels found in Canadian fish-eating birds are comparable to those from Sweden (Borg et al. 1969), if the birds found dead are

excluded from their data. The very high levels of mercury in Finnish white-tailed eagle reported by Henriksson et al. (1966) likewise refer to specimens found dead, presumably poisoned by mercury.

In five walleyes from the Great Lakes 96% (88 - 100) of the mercury occurred as a methyl mercury compound. In Sweden it has been demonstrated based on a large number of analyses that about 90% of the mercury in fish is in the form of methyl mercury (Noren and Westöö 1967, Westöö and Noren 1967), in spite of the fact that mercury is released into the environment frequently in the form of other organic mercury compounds or even in a metallic or inorganic form. This is probably due to the ability of certain aquatic organisms, especially Methanobacterium omelianskii, to methylate inorganic mercury, a process which seems to be normal in anaerobic ecosystems (Jensen and Jernelöv 1967, 1969 and Wood et al. 1968). Since the conditions for such transformation of mercury seem to be present where inorganic mercury is released into the environment and on the basis of our fragmentary data we can conclude that the mercury in Canada's fish is also in the form of methyl mercury.

Relatively little is known about the toxicity of methyl mercury in fish. Symptoms of poisoning associated with degeneration of nerve cells in different parts of the brain were reported in methyl mercury contaminated fish from Japan (Kurland et al. 1960). Miettinen et al. (1969) reported severe damage to liver, kidneys and gills in pike (Esox lucius) exposed experimentally to methyl mercury, and that the lethal dose of methyl mercury for pike was 20 - 25 mg/kg fresh weight when administered orally within interval of a few days between doses. In Japan it was concluded that severely poisoned or dead fish (Hemibarbus sp) carried

more than 20 ppm of mercury (Berlin et al. 1968). We are therefore probably safe in concluding that the levels found in the present study are generally well below lethal levels. However, no conclusions can be drawn as to the probable effect of sub-lethal levels.

With regard to fish-eating birds the red-necked grebes from Pinchi Lake had accumulated very high amounts, up to 17.4 ppm in the liver, which is close to the level that Fimreite and Karstad (1970) found to be lethal in red-tailed hawks. Tern and merganser eggs from Baie de Chaleur averaged 0.66 ppm of mercury and their reproduction may be affected since it has been shown experimentally that similar levels significantly reduce hatchability in pheasants (Fimreite 1970a). Possible interspecific differences, however, must be taken into account. Adult terns from the same area carried an average 2.5 ppm of mercury in liver. These levels were exceeded by those found in specimens of red-necked grebe, double-crested cormorant and great blue heron. Adverse effects on reproduction may therefore occur among fish-eating birds inhabiting mercury contaminated areas.

If contaminated fish are consumed regularly the possible effects on humans have to be considered. On the basis of data collected in Niigata, Japan it was concluded that daily consumption of fish containing 5 to 6 ppm of mercury may be lethal (Birke et al. 1967). The highest levels of mercury in fish from the St. Clair River, Lake St. Clair, and Pinchi Lake are therefore in the range where prolonged daily consumption could be lethal. Elsewhere, with few exceptions, mercury levels in fish exceeded the suggested safety level of 0.5 ppm.

Mercury in fish in a methylated form may produce serious sub-lethal

effects. Its affinity to the nervous system may lead to destruction of brain cells with subsequent neurologic disorders (Hook et al. 1954, Kurland et al. 1960 and Irukayama 1966). Furthermore, methyl mercury acts as a mitotic disturbing agent (Ramel 1969), and the ease with which it penetrates the placenta barrier may lead to accumulation of mercury in the unborn child (Tejning 1968), which in turn may result in congenital neurologic disorders even when the mother appears unaffected (Kurland et al. 1960 and Irukayama 1966).

SUMMARY

Levels of mercury were determined in fish muscle and liver and eggs of fish-eating birds collected from selected sites across Canada; most of the specimens, however, were taken from areas where mercury contamination was suspected. Elevated mercury levels were found in practically all samples of freshwater fish. Especially high were the levels in walleye and pumpkinseed from Lake St. Clair, the St. Clair River, Ontario, and Pinchi Lake, British Columbia, where maximum levels of mercury in muscles reached 5.01, 7.1, and 10.5 respectively. Almost all fish from Lake Huron, Lake Eire, Ottawa River, and Pinchi Lake, contained mercury levels that exceeded 0.5 ppm. Somewhat elevated levels were found in fish from coastal waters. There was in most areas a positive correlation between the body weight of the fishes and the mercury levels.

In birds the highest level, 17.5 ppm in liver, was found in red-necked grebe from Pinchi Lake. Liver levels between 2.5 and 10 ppm occurred in double crested cormorants, common terns and great blue

herons from the Baie de Chaleur, New Brunswick where the levels in eggs of common terns and mergansers from Baie de Chaleur average about 0.7 ppm.

The chief sources of contamination appear to be chlor-alkali plants with mercury type electrolytic cells, pulp mills where mercurials are used for slime control, and in one case mercury mining. It is concluded that the mercury in the fish occurs as a methyl mercury compound. The possible hazards to human health and to reproduction in fish-eating by consumption of such contaminated fish are discussed.

GENERAL DISCUSSION

This study revealed that a considerable environmental contamination with mercury has taken place for many years in Canada and has increased rapidly in recent years. The chief contaminator appears to be the chlor-alkali industry which uses mercury in the electrolytical preparation of chlorine and caustic soda. Approximately 200,000 lbs. of mercury are released annually by this industry, most of which reaches watercourses with the waste-water. Considerable amounts of mercury are retained in the caustic soda and it may also eventually enter water bodies, since it is mostly used for bleaching purposes in the pulp and paper industry. The pulp industry until recently also used considerable quantities of mercurials for slime control but this practise has now been discontinued by most mills in order to conform with the food and drug regulations.

There has been a slight decrease in the use of mercury in seed-dressings during the last five years, but still more than 50% of Canada's grain fields are seeded with mercury treated seed. The amount of mercury added to each acre annually by use of seed-dressings is less than one gram and therefore, no significant accumulation or run-off to water systems can be expected, but seed-eating animals may pick up uncovered treated seed and pass the mercury on to predators.

These uses of mercury are undoubtedly the predominant sources of mercury contamination and those to which special attention was paid in

the present study. This picture may change somewhat in the future. The growing electrical industry is steadily increasing its share of the total consumption of mercury, and according to DBS statistics now uses about 25,000 lbs. annually, as compared with less than 5,000 lbs. only five years ago. Other potential sources to be considered are various laboratory uses of mercury and application of mercury containing turf fungicides, the latter being applied heavily on golf courses. In Ontario 95% of the golf courses use mercurial fungicides, some of these are sprayed at 10 day intervals throughout the summer season (Frank 1970).

The mercury in seed-dressings is for the most part in the form of methyl mercury while the active ingredient in slimicides is phenyl mercury acetate. From the chlor-alkali plants metallic or inorganic mercury (mercury chlorides) is released. Studies undertaken by Jensen and Jernelöv (1968, 1969) and Wood et al. (1968) showed that certain micro-organisms convert inorganic mercury to methyl mercury, particularly under anaerobic conditions which frequently occur where industrial wastes are discharged. Phenyl mercury compounds are rapidly degraded to inorganic mercury (Friberg 1969), and thus they too may be subject to methylation. Findings in Sweden (Noren and Westöö 1967, Westöö and Noren 1967) as well as our own fragmentary data also indicate that methyl mercury is the predominant form in which mercury occurs in fish and other aquatic animals, therefore there is reason to believe that mercury, in the contaminated Canadian environment, is in the form of methyl mercury.

In our experiment with pheasants dietary methyl mercury provided through treated grain seed was shown to have a forcible adverse effect on reproduction. Reduced hatching success associated with mercury levels

in eggs of 0.5 - 1.5 ppm was the most consistent finding while a significant decline in egg production was introduced only in the groups on the heaviest contaminated diets. A large number of shell-less eggs were laid by hens fed treated grain for eight weeks or more. The eggs laid by the contaminated groups frequently had a reduced egg weight compared with the controls and often an abnormal colour.

These findings are consistent with those of Borg et al. (1969) and Tejning (1967) who carried out similar experiments with pheasants and chickens respectively. Neither of them however, reported any reduced egg weight or abnormal colour due to mercury, but they did record severe symptoms of neurologic origin. Such effects were not observed in our pheasant experiment, probably because our experimental grain food contained only 5.5 ppm of mercury as compared with about 20 ppm in the grain used in their experiments.

In the groups of red-tailed hawks fed methyl mercury contaminated diet mortality or severe damage to the nervous system was associated with mercury levels of 7.2 - 10.0 ppm in the liver of chicks provided as experimental food. Signs of poisoning were observed after one month of exposure and were essentially of neurologic origin. Because no recovery occurred in affected hawks when put on a mercury free diet, it can be concluded that the damage is permanent. Histologic investigations revealed severe pathologic changes in the nervous system, particularily in the spinal cord but also in the brain. The most consistent finding was swelling of axons of myelinated nerves with dilatation of myelin sheaths and loss of myelin. In severe cases the axons were fragmented or contracted and undergoing lysis in digestion chambers.

Neurotoxic effects like those described seem to be characteristic for methyl mercury poisoning in birds as similar findings also were reported by Tejning (1967a) and Borg et al. (1969). The latter also examined naturally mercury poisoned game birds and reported pathologic changes consistent with those introduced experimentally.

In pheasants Borg et al. (1969) reported liver levels of 30 ppm or more at death whereas in our experiment about 20 ppm were found in hawks that died. This may indicate a species difference to the tolerance of mercury.

Wildlife specimens collected for analysis consisted for the most part of seed-eating birds and their avian predators as well as fish and fish-eating birds. Most specimens were collected in areas where mercury contamination was anticipated, although some material from presumably uncontaminated areas was included for comparison. Accordingly, our findings do not reflect the over-all distribution of mercury in Canada's avian and piscine fauna, either geographically or ecologically.

These findings nevertheless, indicate that mercury contamination is widespread and prove that the current uses of mercury in seed-dressings, pulp mills, and chlor-alkali industry, and even mercury mining, lead to elevated mercury levels in wildlife. This was confirmed statistically by comparing bird specimens from contaminated with those from uncontaminated grain fields and by similar comparisons in fish taken upstream and downstream from pulp mills or chlor-alkali factories. Corroborating evidence is the findings of Wobeser et al. (1970), and Bligh (1970) in their recent studies on mercury occurrences in Canadian freshwater fish.

Borg et al. (1969) in a comprehensive study on the occurrence of

mercury in Swedish fauna reported that a large number of animals found dead or dying in the countryside was intoxicated by mercury and carried mercury levels significantly higher than those shot. Unfortunately, only a few dead specimens were included in the present study. The ecological significance of our findings can therefore best be evaluated by comparing the levels of mercury found in collected specimens with those shown experimentally to have detrimental biological effects. When doing so we must conclude that the present mercury contamination may interfer with reproduction, especially in avian predators and birds feeding on fish and other aquatic animals since eggs of those species frequently carried mercury levels in the range (0.5 - 1.5 ppm) that considerably reduced hatchability in penned pheasants. Supporting evidence is that the Canadian population of prairie falcon has been declining apparently due to hatching failure (Fyfe et al. 1969). The eggs of this species contained generally high mercury levels, particularily eggs that failed to hatch.

In eggs of prairie falcon and Richardson's merlin a positive correlation between the concentrations of DDE and mercury and also between heptachlor and mercury was found, but the significance of the synergetic effects, if any, is not known.

An increased adult mortality might also occur in the most exposed species either as a result of accumulating lethal levels of mercury or indirectly by severely damaging the nervous system and thus making the birds more vulnerable to predators or disease. The highest mercury levels found in seed-eating birds resembled those experimentally established in chicks which applied as experimental food introduced lethal

or severe sub-lethal effects in red-tailed hawks. Lethal effects however, most likely occur in fish-eating birds since the levels in fish exceeded those found in seed-eating animals. Red-necked grebes, a typical fish-eating species, carried mercury levels up to 17.4 ppm which is close to those found to be lethal in red-tailed hawks.

Since many of the contaminated fish and game bird species form portions of the human diet, food hygienic risks should be considered. Birke et al. (1968) concluded that regular consumption of fish containing mercury levels in muscles of 5 - 6 ppm may be lethal and the highest levels found in walleye from the Great Lakes were in this range while a lake trout from Pinchi Lake contained as much as 10.5 ppm. Wobeser et al. (1970) and Bligh (1970) also reported maximum levels above 10 ppm in pike from the Saskatchewan River and Clay Lake respectively. Even irregular consumption of such contaminated fish may have serious consequences, assuming that the mercury is in the methylated form. According to experimental and clinical findings (Ramel 1969, Kurland et al. 1960, Friberg 1969) probable sub-lethal effects will be of genetic, neurologic and/or teratogenic nature.

With regard to the persistence of mercury contamination Löfroth (1969) maintains that the effects of mercury pollution may last for decades unless the mercury somehow is made biologically unavailable. The present study adds supporting evidence to this hypothesis, as fish and birds collected from Pinchi Lake carried clearly elevated mercury levels 25 years after the probable source of contamination, a mercury mine, had been closed.

In this connection the natural occurrence of mercury has to be

considered, particularily in British Columbia where many areas, including the vicinity of Pinchi Lake, carry high background levels of mercury (Warren et al. 1966). Although the major portion of this mercury occurs as cinnabar, a very insoluble mineral, one can not completely ignore its possible contribution to the elevated mercury levels found in living organisms. If this contribution is significant it means less clearance for any man-made contamination. Because of interference of air pollution, for example caused by chlor-alkali plants, it may be hard to prove the exact background level in living organisms, except in very remote areas.

CONCLUSION AND SUGGESTIONS

The present study has indicated that environmental mercury contamination in Canada has reached considerable proportions. Through industrial use of mercury, in particular the chlor-alkali industry, large quantities of mercury are released into streams and lakes including Canada's major water bodies, the Great Lakes. In certain fish species from contaminated waters mercury has accumulated to an extent that they are unacceptable for human consumption, and abnormally high concentrations were found in some fish-eating birds. The current use of mercury seed-dressings is apparently responsible for elevated mercury levels in seed-eating birds and their avian predators and the recent decline in the prairie falcon populations is probably connected with mercury contamination.

Considering the proportions of the present mercury pollution, and in order to take steps to limit its baleful effects, further research is needed. Investigations should be extended to wildlife species not included in the present study but which might be affected, such as fish-eating mammals. Other possible sources of contamination than those now being considered most harmful should also be subject to investigation because of an apparently rapid development of some other mercury demanding activities. This is for example, the case with use of mercurials as turf fungicides by which mercury, through soil organisms, may enter food chains other than those investigated.

The pronounced qualitative differences in the behaviour of various mercury compounds and their biological effects, warrant determination of the forms in which mercury occurs in exposed species. It is of special interest to know more about the occurrences of methyl mercury in living organisms and the mechanism and efficiency of mercury methylation in the environment.

Information of the significance of the background level of mercury is desirable as well as on the possible synergistic effects that might exist between mercury and other environmental pollutants, primarily DDT and it metabolites. The latter should be evaluated experimentally and preferably with species most likely to be naturally exposed.

REFERENCES

ABERG, B., L. EKMAN, R. FALK, U. GREITZ, G. PERSSON, and JAN-OLOF SNIHS. 1969. Metabolism of Methyl Mercury (^{203}Hg) Compounds in Man. Arch Environ. Health 19: 478-484.

AMEND, D. F., W. T. YASUTAKE, and R. MORGAN. 1969. Some Factors Influencing Suceptibility of Rainbow Trout to the Acute Toxicity of an Ethyl Mercury Phosphate Formulation (Timsan). Trans. Amer. Fish. Soc. 98(3): 420-425.

ANDERSSON, A. 1967. Kvicksilvret i marken. Grundförbättring 20(3-4): 95-105.

ANDERSSON, A. 1967a. Kvicksilver i rötslam. Grundförbättring 20(3-4): 149-150.

ANONYMOUS. 1966. Demand for mercury increases shraply as chlorine production rises. Chem. & Eng. News 73(24): 30-32.

ANONYMOUS. 1968. Comminco launches mercury project. Can. Chem. Proc. 52(5): 77-79.

ANONYMOUS. 1968a. Canada's only mercury mine is gearing up for production early in 1969. Eng. & Mining J. 169(2): 146.

ARRHENIUS, E. 1967. Effects of Organic Mercury Compounds on the Detoxification Mechanism of Liver Cells in Vitro. Oikos suppl. 9: 32-35.

ASH, J. S. 1962. Residues of seed-dressings in pheasants' eggs. British Birds 55: 478-480.

BÄCKSTRÖM, J. 1969. Distribution Studies of Mercuric Pesticides in

Quail and Some Fresh-Water Fishes. Acta Pharm. Toxicol. 27 (suppl.3): 1-103.

BERG, W., A. JOHNELS, B. SJÖSTRAND, and T. WESTERMARK. 1966. Mercury content in feathers of Swedish birds from the past 100 years. Oikos 17(1): 71-83.

BERLIN, M., C. RAMEL, and A. SWENSSON. 1968. Forgiftning genom konsumtion av fisk innehållande metylkvicksilverforening. Unpublished report from a study tour to Japan.

BIDSTRUP, P. L. 1964. Toxicity of mercury and its compounds. Elsvier, London, 112 pp.

BIRKE, G., A JOHNELS, L. O. PLANTIN, B. SJÖSTRAND, and T. WESTERMARK. 1967. Metylkvicksilverforgifning genom fortaring av fisk. Svenska Lakartidn 64: 3628-3637.

BLIGH, E. G. 1970. Mercury and the contamination of freshwater fish. Fisheries Research Board of Canada. Manuscript report no. 1088. 27 pp.

BOËTIUS, J. 1960. Lethal Action of Mercuric Chloride and Phenylmercuric Acetate on Fishes. Meddelelser fra Danmarks Fiskeri- og Havundersökelser 3(4): 93-115.

BONE, G. B. 1965. Analysis of Variance/Covariance Processor; designed for Brigham Young University Computer Research Center. 39 pp.

BOOER, J. R. 1944. The behaviour of mercury compounds in soil. Ann. Appl. Biol. 31: 340-359.

BORG, K., H. WANNTORP, K. ERNE, and E. HANKO. 1965. Kvicksilverforgiftningar bland vilt i Sverige. Report from State Vet. Med. Inst., Stockholm, 50 pp. + tables.

BORG, K., W. WANNTORP, K. ERNE, and E. HANKO. 1969. Alkyl Mercury Poisoning in Terrestrial Swedish Wildlife. Viltrevy 6(4): 301-379.

BOUVENG, H. O. 1967. Organo-mercurials in pulp and paper industry. Oikos suppl. 9: 18.

BOUVENG, H. O., and P. ULLMAN. 1969. Reduction of mercury in waste waters from chlorine plants. Report from Swedish Water and Air Pollution Research Laboratory, Stockholm. 17 pp.

BROWN, J. B., and M. V. KULKARNI. 1967. A review of the Toxicity and Metabolism of Mercury and Its Compounds. Med. Services J. Can. XXIII(5): 786-808.

CRAIGHEAD, J. J., and F. C. CRAIGHEAD. 1956. Hawks, Owls and Wildlife. The Stackpole Comp., Pensylvania and Wildlife Management Inst., Washington, D.C. 443 pp.

EDGINGTON, L. V. 1967. Chemicals for seed treatment of small grains. Publ. 252, Ont. Dept. of Agr. & Food, 2 pp.

ENDERSON, J. H. 1964. A study of the prairie falcon in the central Rocky Mountain region. Auk 81: 332-352.

EKMAN, L., U. GREITZ, G. PERSSON, and B. ABERG. 1968. Omsättning av metylkvicksilver hos människa. Nordisk Medisin 79(14): 450-456.

ERIKSON, E. 1967. Mercury in nature. Oikos suppl. 9: 13.

FALLIS, K. E. 1968. Personal communication.

FIMREITE, N. 1970. Mercury Uses In Canada and Their Possible Hazards as Sources of Mercury Contamination. Environmental Pollution 1(2).

FIMREITE, N. 1970a. Effects of Dietary Methyl Mercury on Ring-necked Pheasants, with Special Reference to Reproduction. Accepted for publication (Can. Wildl. Service, Occasional papers).

FIMREITE, N., R. W. FYFE, and J. A. KEITH. 1970. Mercury Contamination of Canadian Prairie Seed-eaters and Their Avian Predators. Accepted for publication in Can. Field Nat.

FIMREITE, N., and L. KARSTAD. 1970. Effects of Dietary Methyl Mercury on Red-tailed Hawks. Accepted for publication in J. Wildl. Mgmt. 35(2).

FOX, G. A. 1964. Notes on a western race of the pigeon hawk. Blue Jay 22: 140-147.

FRANK, R. 1970. Mercury Uses in Ontario Agriculture. Report from Ont. Dept. Agr. and Food. 10 pp.

FRIBERG, L. 1959. Studies on the Metabolism of Mercuric Chloride and Methyl Mercury Dicyandiamide. A.M.A. Arch Industr. Health 20: 42-49.

FRIBERG, L. T. (Chairman). 1969. Maximum Allowable Concentrations of Mercury Compounds. Report of an Int. Committee. Arch. Environ. Health 19: 891-905.

FYFE, R. W. 1969. Regional population declines and organochlorine Insecticides in Canadian prairie falcons. Can. Field Nat. 83: 191-200.

FYFE, R. W. 1970. Personal communication.

GOLDSMITH, V. M. 1954. Geochemistry. Oxford Univ. Press, 730 pp.

GROLLEAU, G. 1965. Toxicité des Produits de Traitement des Semences a L'Egard des Gallinaces-Gibier. Ann. Epiphyties 16(2): 129-143.

GURBA, J. B. 1968. Personal communication.

HANNERZ, L. 1967. Experimental Investigations on the Accumulation of Mercury in Water Organisms. Inst. of Freshwater Research, Sweden, Report 48: 120-176.

HASSELROT, T. B. 1967. Report on Current Field Investigations Concerning The Mercury Content in Fish, Bottom Sediment and Water, Inst. Freshwater Research, Sweden, Report 48: 102-111.

HÄSÄNEN, E., and V. SJÖBLOM. 1968. Kaljoen elohopeapitoisuus Suomessa vuonna. Suomen Kalatalous 36: 1-24. (With a summary in English).

HAWKES, H.E., and S. H. WILLISTON. 1962. Mercury Vapor as a Guide to Lead-Zinc-Silver Deposits. Mining Congress J. 48: 30-32.

HELMINEN, M., E. KARPPANEN, and J. I. KOIVISTO. 1968. Saimaan norpan Elohopeopitoissuudesta, 1967. Suomen Elainlaakarilehti. 120: 87-89.

HENRIKSSON, K., E. HARPPANEN, and M. HELMINEN. 1966. High residue of mercury in Finnish white-tailed eagle. Ornis Fennica 43: 38-45.

HENRY, L. G. 1968. Personal communication.

HEUSER, G. F. 1956. Feeding Chemically Treated Seed Grains to Hens. Poultry Science 35: 160-162.

HOOK, K., D. LUNDGREN, and A. SWENSSON. 1954. On Alkyl Mercury Poisoning. Acta Med. Scand. 150(2): 131-137.

IRUKAYAMA, K. 1966. The Pollution of Minamata Bay and Minamata Disease. Paper given at Third Intern. Conf. on Water Pollution Research, Munich, Germany. 13 pp.

JENSEN, S., and A. JERNELÖV. 1967. Biosyntes av metylkvicksilver i slam. Biocidinformation 10: 4.

JENSEN, S., and A. JERNELÖV. 1969. Biological methylation of mercury in aquatic organisms. Nature 223(5207): 753-754.

JOHNELS, A. G., T. WESTERMARK, W. BERG, P. I. PERSSON, and B. SJÖSTRAND. 1967. Pike (Esox lucius L.) and some other aquatic organisms in Sweden as indicators of mercury contamination in the environment. Oikos 18(2): 323-333.

KEITH, J. A., and L. M. REYNOLDS. 1969. Mercury Residues in Wildlife Addendum to Canadian report. O.E.C.D. Study 1967/68. 4 pp.

KUMURA, Y., and V. L. MILLER. 1964. The degradation of Organomercury Fungicides in Soil. Agr. & Food Chem. 12(3): 253-257.

KING, C.V. 1957. Mercury: Its scientific history and its role in physical chemistry and electrochemistry. Ann. N.Y. Acad. Sci. 65: 360-368.

KORTRIGHT, F. H. 1943. The Ducks, Geese and Swans of North-America. The Am. Wildl. Inst. Washington, D.C. 476 pp.

KURLAND, L. T., S. M, FARO, and H. SIEDELER. 1960. Minamata Disease. World Neurol. 1: 370-395.

LABISKY, R. F., and J. F. OPSAHL. 1958. A Guide to Aging of Pheasant Embryos. Biological Notes No. 39, Dept. Reg., and Educ. State of Illinois, 4 pp.

LANDELL, N. E. 1968. Fogeldöd, Fiskhot, Kvicksilver. Aldus/bonniers, Stockholm, 133 pp.

LIHNELL, D., and A. STENMARK. 1967. Bidrag til kännedomen om forekomsten av kvicksilver i smågnagare. The Swedish Nat. Inst. for Plant Protection, Contrib. 13(110): 361-379.

LÖFROTH, G. 1969. Methyl mercury. A review of the health hazards and side effect associated with the emission of mercury compounds into natural systems. Bulletin no. 4, Ecological Research Committee of the Swedish Natural Science Research Council, 38 pp.

LUTTICH, S., D. H. RUSCH, E. C. MESLOW, and L. B. KEITH. 1970. Ecology of red-tailed hawk predation in Alberta. Ecology 51(2): 190-203.

MCCULLY, K. A., and W. P.MCKINLEY. 1964. Determination of chlorinated pesticide residues in fat by electron capture gas chromatography. J. Ass. Ott. Anal. Chem. 47: 652-659.

MELLANBY, K. 1967. Pesticides and Pollution. Collins, London, 221 pp.

MIETTINEN, V., Y. OHMOMO, M. VALTONEN, E. BLANKENSTEIN, K. RISSANEN, M. TILLANDER, and J. K. MIETTINEN. 1969. Preliminary notes on the distributuion and effects of two chemical forms of methylmercury on pike. Fifth R.I.S. Symp., Helsinki.

MÖLLER, N. M. 1965. In: Kvicksilverfrågan i Sverige: 182-185, Stockholm.

MUROZUMI, M. 1967. A New Type of Mercury Chlorine Cell. Electrochem. Technol. 5(5-6): 236-238.

NOREN, K., and G. WESTÖÖ. 1967. Metylkvicksilver i fisk. Vår Föda 19(1): 1-24.

OLIVER, W. T., and H. S. FUNNELL. 1958. The Quantitative Determination of Mercury in Animal Tissues. Am. J. Vet. Res. 19: 999-1000.

RAMEL, G. 1969. Methylmercury as a mitosis disturbing agent. J. of the Japan Med. Ass. 61: 1072-1076.

RATCLIFFE, D. A. 1967. Decrease in egg shell weight in certain birds of prey. Nature 215: 208-210.

REYNOLDS, L. M. 1969. Polychlorobiphenyls (PLB's) and their interference with pesticide residue analysis. Bull. Environ. Contam. and Toxicol. 4: 128-143.

ROMANOFF, A. L., and A. H. ROMANOFF. 1949. The Avian Egg. John Wiley and Sons, New York, 918 pp.

ROSS, R. G., and D. K. R. STEWART. 1962. Movement and accumulation of mercury in apple trees and soil. Can. J. Plant Sci. 47: 169-174.

ROSS, R. G., and D. K. R. STEWART. 1964. Mercury residues in potatoes in relation to foliar sprays of phenyl mercury chloride. Can. J. Plant Sci. 44: 123-125.

RUSCH, D. H., E. C. MESLOW, P. D. DOERR, and L. B. KEITH. 1969. Response of great horned owl populations to changing prey densities (in press).

SCHOENER, T. W. 1968. Sizes of Feeding Territories Among Birds. Ecology 49(1): 123-141.

SHARVELLE, E. G. 1962. The Nature and Uses of Modern Fungicides. Burgess Publ. Co., Minneapolis, 308 pp.

SJÖSTRAND, B. 1964. Simultaneous Determination of Mercury and Arsenic in Biological and Organic Materials by Activation Analysis. Anal. Chem. 36(4): 814-819.

SMART, N. A. 1968. Use and residues of mercury compounds in agriculture. Residue Reviews 23: 1-36.

SMART, N. A., and M. K. LLOID. 1963. Mercury Residues in Egg, Flesh and Livers of Hens Fed on Wheat Treated With Methylmercury Dicyandiamide. J. Sci. Fd. Agric. 14: 734-740.

SOMMERS, H. A. 1968. Mercury Cell Anode and Anode Stem Design and Other Factors Relating to Lowered Power Consumption and Operating Cost. Electrochem. Technol. 6(3-4): 124-132.

SPRAGUE, J. B., and W. G. CARSON. 1970. Spot-checks of Mercury Residues in Some Fishes from the Canadian Atlantic Coast. Fisheries Research Board of Canada. Manuscript report no. 1085, 16 pp.

STEVENSON, J. S. 1940. Mercury Deposits of British Columbia. Bull. 5, British Columbia Dept. of Mines, 93 pp.

STEWART, D. K. R., and R. G. ROSS. 1967. Mercury residues in apples in relation to spray date, variety and chemical composition of fungicide. Can. J. Plant Sci. 47: 169-174.

STOCK, A., and F. CUCUEL. 1934. Die Verbreitung des Quecksilbers. Die Naturwissenschaften 23: 390-393.

TAYLOR, C. G. 1965. The Loss of Mercury From Fungicidal Paints. J. Appl. Chem. 15: 232-236.

TEJNING, S. 1967. Mercury in Pheasants (Phasianus colchicus) deriving from seed grain dressed with methyl and ethyl mercury compounds. Oikos 18(2): 334-344.

TEJNING, S. 1967a. (I - VII). Biological effects of methyl mercury dicyandiamide-treated grain in the domestic fowl Gallus gallus L. Oikos suppl. 8: 1-116.

TEJNING, S. 1968. Kvicksilverhalterna i blodkroppar och i blodplasma hos "normala" mödrar och deras nyfödda barn. Report 68 02 20 from Dept. of Occup. Med., University Hospital, Lund, Sweden, 5 pp.

TRIPPENSEE, R. E. 1948. Wildlife Management 1. McGraw-Hill, New York, 439 pp.

ULFVARSON, U. 1962. Distribution and excretion of some mercury compounds after long term exposure. Int. Arch. für Gewerbepathol. und Gewerbehyg. 19: 412-422.

ULFVARSON, U. 1965. Kvicksilver, aldrin och dieldrin i fasaner. Svensk Kemisk Tidssdrift 77(5): 235-246.

VAARTNOU, H. 1969. Personal communication.

WANNTORP, H., K. BORG, and K. ERNE. 1967. Mercury Residues in Wood-Pigeons (Columba p. palumbus) in 1964 and 1966. Nord. Vet. Med. 19: 474-477.

WARREN, H. V., R. E. DELAVAULT, and J. BARAKSO. 1966. Some observations on the geochemistry of mercury as applied to prospecting. Economic

Geology 61(6): 1010-1028.

WARREN, H. V., and R. E. DELAVAULT. 1969. Mercury content of some British soils. Oikos 20(2): 537-539.

WESTERMARK, T. 1967. Mercury content in feathers of Swedish birds from the past 100 years. Oikos suppl. 9: 39-40.

WESTÖÖ, G. 1966. Determination of Methylmercury Compounds in Foodstuff. I. Methylmercury Compounds in Fish, Identification and Determination. Acta. Chem. Scand. 20(8): 2131-2137.

WESTÖÖ, G. 1967. Determination of Methylmercury Compounds in Foodstuff. II. Determination of Methylmercury in Fish, Egg, Meat, and Liver. Acta. Chem. Scand. 21(7): 1790-1800.

WESTÖÖ, G. 1967a. Kvicksilver i fisk. Vår Föda 19(1): 1-11.

WESTÖÖ, G., and K. NOREN. 1967. Kvicksilver och metylkvicksilver i fisk. Vår Föda 19(10): 136-178.

WESTÖÖ, G., and M. RYDÄLV. 1969. Kvicksilver och metylkvicksilver i fisk och kraftor. Vår Föda 21(3): 18-111.

WOOD, J. M., F. S. KENNEDY, and C.G. ROSEN. 1968. Synthesis of Methyl-mercury Compounds by Extracts of a Methanoganic Bacterium. Nature 220: 174.

WOBESSER, G., N. O. NIELSEN, R. H. DUNLOP, and F. M. ATTON. 1970. Mercury concentrations is tissues of fish from the Saskatchewan River. J. Fish R. Bd. Canada 27(4): 830-835.

APPENDIX I

Pheasant experiment - Tables

TABLE 1. Weight and weight changes of test birds

Hg treated grain in diet	Weeks fed Hg treated grain	Pen	Bird #	Weight in grams: Before start of exp. (March 26 - April 2)	Weight in grams: May 2	Weight in grams: June 13* or June 27	Weight gained (+) or lost (-) during the experimental period (surviving birds): Grams	Weight gained (+) or lost (-) during the experimental period (surviving birds): Per cent
100% of grain ration	0	1	1542 ♂	1765	1700	1620	-145	- 8.2
			43	1540	1500	Died on May 17		
			44	1305	1250	--		
			45	1525	1260	1230	-295	-19.3
			46	1425	1380	1340*	- 85	- 6.0
			47	1455	1300	1450*	- 5	- 0.3
			48	1455	1180	1270	-185	-12.7
			49	1350	1240	1280	- 70	- 5.2
			Mean	1477	1351	1365	-130	- 8.6
		13	2041 ♂	1815	1850	1760	- 55	- 3.0
			42	1170	1020	940	-230	-19.6
			43	1495	Died on April 19	--		
			44	1210	1060	Died on May 6		
			45	1190	1320	--		
			46	1375	1530	1390*	+ 15	+ 1.1
			47	1170	1420	1300	+130	+11.1
			48	1395	1380	1300	- 95	- 6.8
			Mean	1352	1577	1338	- 47	- 3.4
	2	2	1550 ♂	1530	1520	1460	- 70	- 4.6
			51	1250	1240	1100*	-150	-12.0
			52	1305	1160	--		
			53	1240	1120	1140	-100	- 8.1
			54	1505	1220	--		
			55	1445	1150	1210	-235	-16.3
			56	1490	1380	1400*	- 90	- 6.0
			57	1260	1110	1270*	+ 10	+ 0.8
			Mean	1378	1237	1263	-105	- 7.7
		14	2049 ♂	1745	1750	1640	-105	- 6.0
			50	1305	1230	Died on June 17		
			51	1140	1390	1350*	+210	+18.4
			52	1430	1400	1330	-100	- 7.0
			53	1260	1290	1320*	+ 60	+ 4.7
			54	1090	1380	Died on May 27		
			55	1240	1210	--		
			56	1160	1090	1070	- 90	- 7.6
			Mean	1296	1342	1342	- 5	+ 0.5

Table 1 (continued)

Weight and weight changes of test birds

Hg treated grain in diet	Weeks fed Hg treated grain	Pen	Bird #	Weight in grams			Weight gained (+) or lost (-) during the experimental period	
				Before start of exp. (March 26 - April 2	May 2	June 13* or June 27	Grams	Per cent
100% of grain ration	4	3	1558♂	1820	1550	1580	-240	-13.2
			59	1660	1440	--		
			60	1330	1290	1300*	- 30	- 2.3
			61	1310	1230	1200	-110	- 8.3
			62	1420	1170	1220	-200	-14.1
			63	1440	1490	1540*	+100	+ 6.9
			64	1640	1370	1330	-310	-18.9
			65	1370	1240	Died on May 18		
			Mean	1498	1347	1361	-13.2	- 8.3
		15	2057♂	1540	1500	1560	+ 20	+ 1.3
			58	1150	1350	1230	+ 80	+ 7.0
			59	1285	1080	1130	-155	-12.1
			60	1320	1390	1320*	0	0.0
			61	1260	1290	1240*	- 20	- 1.6
			62	1215	1200	Died on May 12		
			63	1225	1170	--		
			64	1200	1220	--		
			Mean	1274	1275	1296	- 15	- 1.1
	12	4	1566♂	1780	1810	1610	-170	- 9.5
			67	1360	1180	--		
			68	1340	1370	1450*	+110	+ 8.2
			69	1390	1090	1210	-180	-12.9
			70	1340	1290	1330*	- 10	- 0.7
			71	1370	1280	1380	+ 10	+ 0.7
			72	1690	1440	1500	-190	-11.2
			73	1430	1350	1280*	-150	-10.5
			Mean	1462	1351	1394	- 83	- 5.1
		16	2065♂	1740	1700	1630	-110	- 6.3
			66	1370	1290	1280*	- 90	- 6.6
			67	1405	1560	1600*	+195	+13.9
			68	1380	1290	1300	- 80	- 5.8
			69	1420	1360	--		
			70	1360	1110	1240	-120	- 8.8
			71	1480	1420	--		
			72	1555	1580	1440*	-115	- 7.4
			Mean	1463	1413	1415	- 53	- 3.5

Table 1 (continued)

Weight and weight changes of test birds

Treated grain	Weeks fed treated grain	Pen	Bird #	Weight in grams			Weight gained (+) or lost (-) during the experimental period	
				Before start of exp. (March 26 - April 2)	May 2	June 13* of June 27	Grams	Per cent
50% of grain ration	0	5	1574♂	1660	1600	1500	-160	- 9.6
			75	1490	1340	1280	-210	-14.1
			76	1720	1560	--		
			77	1450	1240	1330	-120	- 8.3
			78	1460	1150	1220*	-240	-16.4
			79	1420	1250	1160*	-260	-18.3
			80	1240	1200	--		
			81	1510	1230	1290	-220	-14.6
			Mean	1493	1321	1296	-201	-13.5
		17	1273♂	1575	1680	1500	- 75	- 4.8
			74	1250	1500	1340	+ 90	+ 7.2
			75	1145	1220	1100	- 45	- 3.9
			76	1200	1160	1110*	- 90	- 7.5
			77	1230	1340	1240*	+ 10	+ 0.8
			78	1030	1330	1200	+170	+16.5
			79	1015	1060	--		
			80	1245	730	Died on May 7		
			Mean	1211	1252	1248	+ 10	+ 1.4
	2	6	1582♂	1700	1700	1530	-170	-10.0
			83	1090	1000	Died on May 11		
			84	1130	1070	1180*	+ 50	+ 4.4
			85	1240	1040	1070	-170	-13.7
			86	1420	1280	1270*	-150	-10.6
			87	1240	1300	--		
			88	1380	1380	--		
			89	1240	1090	1050	-190	-15.3
			Mean	1380	1232	1220	-126	- 9.0
		18	2090♂	1840	1760	1600	-240	-13.0
			2082	1175	1140	1030*	- 45	- 3.8
			83	1405	1460	1390	- 15	- 1.1
			84	1095	1260	--		
			85	1425	1270	Died on May 23		
			87	1090	1300	1190	+100	+ 9.2
			88	1200	1080	1000*	-200	-16.7
			89	1350	1380	1250	-100	- 7.4
			Mean	1322	1331	1243	- 83	- 5.4

Table 1 (continued)

Weight and weight changes of test birds

Treated grain	Weeks fed treated grain	Pen	Bird #	Weight in grams			Weight gained (+) or lost (-)during the experimental period	
				Before start of exp. (March 26 - April 2)	May 2	June 13* or June 27	Grams	Per cent
50% of grain ration	4	7	1590♂	1740	1740	1670	- 70	- 4.0
			91	1210	1180	970	-240	-19.8
			92	1320	1410	1220	-100	- 7.6
			93	1180	1220	930	-250	-21.2
			94	1280	1420	1380*	+100	+ 7.8
			95	1280	1170	1200*	- 80	- 6.3
			96	1090	1230	1260*	+170	+15.6
			97	1350	1190	--		
			Mean	1309	1319	1232	- 67	- 5.1
		19	2860♂	1613	1560	1510	-103	- 6.4
			2091	1135	1100	--		
			92	1390	1410	--		
			93	1300	1270	1300*	0.0	0.0
			94	1340	1300	1190	-150	-11.2
			95	1450	1510	1400	- 50	- 3.4
			96	1155	1460	1370*	+215	+18.6
			97	1495	1310	1320*	-175	-11.7
			Mean	1359	1365	1348	- 44	- 1.3
	12	8	2001♂	1460	1510	1350	-110	- 7.5
			2	1550	1540	1250	-300	-19.4
			3	1580	1480	1360*	-220	-13.9
			4	1350	1360	1360*	+ 10	+ 0.7
			5	1610	1530	1500*	-110	- 6.8
			6	1430	1130	1140	-290	-20.3
			7	1950	1830	--		
			8	1680	1280	1340	-340	-20.2
			Mean	1576	1457	1328	-194	-12.4
		20	2816♂	1805	1720	1550	-355	-19.7
			17	1085	1240	--		
			18	1395	1320	1320*	- 75	- 5.4
			19	1325	1350	1370*	+ 45	+ 3.3
			20	1150	1250	1020	-130	-11.3
			21	1325	1400	--		
			22	1270	1220	1210*	- 60	- 4.7
			23	1180	1280	1100	- 80	- 6.8
			Mean	1316	1347	1261	-109	- 7.4

Table 1 (continued)

Weight and weight changes of test birds

Treated grain	Weeks fed treated grain	Pen	Bird #	Weight in grams			Weight gained (+) or lost (-) during the experimental period	
				Before start of exp. (March 26 - April 2)	May 2	June 13* or June 27	Grams	Per cent
25% of grain ration	0	9	2009♂	1920	1900	1790	-130	- 6.8
			10	1650	1610	1490	-160	- 9.7
			11	1415	1360	--		
			12	1370	1190	--		
			13	1130	1100	1100	- 30	- 2.6
			14	1520	1470	1380*	-140	- 9.2
			15	1240	1230	1190*	- 50	- 4.0
			16	1195	1120	900	-295	-24.7
			Mean	1430	1372	1308	-134	- 9.5
		21	2824♂	1790	1660	1740	- 50	- 2.8
			25	1085	1210	1210*	+125	+11.5
			26	1270	1320	--		
			27	1175	1100	1060	-115	- 9.7
			28	1190	1310	--		
			29	1215	1320	1110	-135	-11.1
			30	1275	1300	1370*	+ 95	+ 7.5
			31	1390	1440	1550*	+160	+11.5
			Mean	1299	1335	1340	+13,3	+ 1.2
	2	10	2017♂	1950	1900	1760	-190	- 9.7
			18	1440	1420	1330	-110	- 7.6
			1919	1265	1270	970*	-295	-23.3
			20	1420	1320	1300*	-120	- 8.5
			21	1170	1250	--		
			22	1460	1400	1170	-290	-19.9
			23	1500	1460	Died on May 26		
			24	1530	1520	--		
			Mean	1466	1442	1306	-201	-13.8
		22	2832♂	1685	1550	1510	-175	-10.4
			33	1390	1390	1380	- 10	- 0.7
			34	1050	1200	--		
			35	1410	1240	1100	-310	-22.0
			36	escaped	--			
			38	1090	1270	Died on May 30		
			39	1250	1540	1470	+220	+17.6
			40	1250	1440	1430*	+180	+14.4
			Mean	1296	1375	1378	- 19	- 0.4

Table 1 (continued)

Weight and weight changes of test birds

Treated grain	Weeks fed treated grain	Pen	Bird #	Weight in grams			Weight gained (+) or lost (-) during the experimental period	
				Before start of exp. (March 26 - April 2)	May 2	June 13* or June 27	Grams	Per cent
25% of grain ration	4	11	2025♂	1605	1520	1460	-145	- 9.0
			26	1300	1280	--		--
			27	1290	1410	1190*	-100	- 7.6
			28	1295	1340	1200	- 95	- 7.3
			29	1340	1260	1090	-250	-18.7
			30	1300	1300	1190*	-110	- 8.5
			31	1420	1350	1320	-100	- 7.0
			32	1050	1090	1020*	- 30	- 2.9
			Mean	1325	1318	1210	-118	- 8.7
		23	2841♂	1670	1570	1630	- 40	- 2.6
			42	1245	1500	--		
			43	1295	1140	--		
			44	1345	1400	1400*	+ 55	+ 4.1
			45	1400	1120	Died on May 25		
			46	1050	1130	--		
			47	1270	1110	1080	-190	-15.0
			48	1215	1220	1310	+ 95	+ 7.8
			Mean	1311	1273	1355	- 20	- 1.4
	12	12	2033♂	1755	1740	1900	+145	+ 8.2
			34	1175	1080	1060	-115	- 9.7
			35	1060	1340	1220	+160	+15.0
			36	1260	1300	Died on May 30		
			37	1235	1140	1090*	-145	-11.7
			38	1290	1220	1190*	-100	- 7.8
			39	1205	1140	--		
			40	1170	1350	--		
			Mean	1268	1288	1292	- 29	- 1.2
		24	2849♂	1720	1580	1470	-250	-14.5
			50	1200	1350	1360*	+160	+13.3
			51	1125	1200	1080	- 45	- 4.0
			52	1285	1110	1060	-215	-16.7
			53	1190	1220	--		
			54	1110	1190	1290	+180	+16.2
			55	1410	1390	1240	-170	-12.1
			2837	1280	1150	1050	-230	-18.0
			Mean	1308	1273	1221	- 81	- 5.1

TABLE 2. Ingredients in CO-OP 18% pheasant breeder pellets.

Ingredients
Wheat and/or Oats and/or Corn and/or Barley. Dehydrated Alfalfa. Shorts. Bran. Soybean Meal and/or Corn Gluten Meal. Meat Meal. Fish Meal. Lignosol Feed Binder. Whey. Fat. Calcium Phosphate. Limestone. Salt. Antibiotic Feed Supplement. Choline Chloride. Calcium Pantothenate. Methionine. Folic Acid. Riboflavin. Niacin. Vitamin A. Vitamin B-12. Vitamin D. Vitamin E. Vitamin K. Iodine. Cobalt. Copper. Iron Manganese. Zinc. Ethoxyquin.

Guaranteed Analysis	
Crude Protein (min.)	18%
Crude Fat (min.)	3%
Crude Fibre (max.)	7.5%
Salt (actual)	0.5%
Calcium (actual)	2.2%
Phosphorus (actual)	0.7%
Added Zinc (actual)	0.010%
Vitamin A (min.)	4000 IU/lb.
Vitamin D-3 (min.)	950 IU/lb.

TABLE 3. Ingredients in CO-OP 28% pheasant starter krums.

Ingredients
Wheat and/or Oats and/or Corn and/or Barley. Dehydrated Alfalfa. Shorts. Soybean Meal and/or Corn Gluten Meal. Meat Meal. Fish Meal. Lignosol Feed Binder. Whey. Fat. Calcium Phosphate. Limestone. Salt. Antibiotic Feed Supplement.
Choline Chloride. Calcium Pantothenate. Methionine. Folic Acid. Riboflavin. Niacin. Vitamin A. Vitamin B-12. Vitamin D. Vitamin E. Vitamin K. Iodine. Cobalt. Copper. Iron Manganese. Zinc. Ethoxyquin.

Guaranteed Analysis	
Crude Protein (min.)	28%
Crude Fat (min.)	3%
Crude Fibre (max.)	6%
Salt (actual)	0.25%
Calcium (actual)	1.1%
Phosphorus (actual)	1.0%
Added Zinc (actual)	0.010%
Vitamin A (min.)	3000 IU/lb.
Vitamin D-3 (min.)	750 IU/lb.

TABLE 4. Analysis of variance between the control groups for food consumption, egg production, frequency of shell-less eggs, hatchability, embryonic mortality, and chick production.

		Food consumption grams/hen/day		Egg production eggs/hen/day		Shell-less eggs per cent of eggs laid		Hatchability per cent of eggs incubated		Chick production chicks hatched/hen/day		Embryonic mortality, per cent of eggs incubated					
												Days in incubation period					
												0 - 8 days		9 - 16 days		17 - 24 days	
Source of variation	df	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Groups	2	1.0108	0.028 ns	0.0053	0.130 ns	0.0001	0.547 ns	0.0258	1.251 ns	0.0157	0.885 ns	0.0157	1.699 ns	0.0000	0.026 ns	0.0012	0.186 ns
Error	69	36.0709		0.0410		0.0002		0.0206		0.0178		0.0092		0.0007		0.0064	
Total	71																

ns - not significant

TABLE 5. Analysis of variance

between control groups for mortality in chicks.

Source of variation	df	MS	F
Groups	2	2.332	<1 ns
Error	3	9.456	
Total	5		

ns - Not significant

APPENDIX II

Egg shell thickness, mercury, DDE, and heptachlor residues in eggs of Prairie falcon (Falco mexicanus) and Richardsons merlin (Falco columbarius richardsonii) from Alberta and Saskatchewan.

	Specimen No.	Eggshell thickness Ratcliffe index[a]	Mercury ppm	DDE ppm	Heptachlor ppm
ALBERTA					
Prairie falcon	1	1.61	1.23[b]	5.93	-
Prairie falcon	2	1.25	0.691	13.2	
Prairie falcon	3	2.06	0.457	.895	-
Prairie falcon	4	1.43	.729	9.81	-
Prairie falcon	5	1.48	.276	5.77	-
Prairie falcon	6	1.65	.209	6.96	-
Prairie falcon	7	1.88	.371	4.36	-
Prairie falcon	8	2.08	.140	2.46	-
Prairie falcon	9	1.63	.112	3.52	.430
Prairie falcon	10	1.69	.102	.819	.212

	Specimen No.	Eggshell thickness Ratcliffe index[a]	Mercury ppm	DDE ppm	Heptachlor ppm
Prairie falcon	11	1.68	.242	.968	.401
Prairie falcon	12	1.66	.109	1.39	.115
Prairie falcon	13	1.70	.522	4.25	.808
Prairie falcon	14	1.59	.052	.761	.758
Prairie falcon	15	1.88	.107	.659	.357
Prairie falcon	16	1.74	.140	.275	.189
Prairie falcon	17	1.87	.019	3.22	.295
Prairie falcon	18	1.65	.245	2.34	.243
Prairie falcon	19	1.87	.157	2.04	.313
Prairie falcon	20	1.70	.184	1.82	.222
Prairie falcon	21	1.61	.189	1.60	.980
Prairie falcon	22	1.98	.047	1.50	.100
Prairie falcon	23	1.68	.124	6.00	.889
Prairie falcon	24	1.83	.126	1.92	.050
Prairie falcon	25	1.49	.236	3.52	.251
Prairie falcon	26	1.66	.116	2.22	.432
Prairie falcon	27	1.37	.238	5.81	.537

	Specimen No.	Eggshell thickness Ratcliffe index[a]	Mercury ppm	DDE ppm	Heptachlor ppm
Prairie falcon	28	1.60	.142	2.88	.432
Prairie falcon	29	1.43	.119	4.17	.723
Prairie falcon	30	1.85	.247	3.10	.160
Prairie falcon	31	1.56	.111	1.67	.759
Prairie falcon	32	1.61	.951	1.64	1.68
Prairie falcon	33	1.84	.951	.526	.752
Prairie falcon	34	1.82	.063	.371	.137
Prairie falcon	35	1.55	.087	1.77	.132
Prairie falcon	36	1.57	.042	.915	.134
Prairie falcon	37	1.85	.252	1.15	.343
Prairie falcon	38	1.72	.081	.922	.601
Prairie falcon	39	1.63	.035	1.26	.224
Prairie falcon	40	1.39	.075	6.86	.352
Prairie falcon	41	1.42	.144	9.01	2.33
Prairie falcon	42	1.61	.060	3.96	.447
Prairie falcon	43	1.64	.088	3.53	.933
Prairie falcon	44	-	.131	3.62	.817

	Specimen No.	Eggshell thickness Ratcliffe index[a]	Mercury ppm	DDE ppm	Heptachlor ppm
Richardsons merlin	45	1.31	.194	-	-
Richardsons merlin	46	1.15	.543	-	-
Richardsons merlin	47	.92	.352	-	-
Richardsons merlin	48	1.04	.277	-	-
Richardsons merlin	49	.93	.153	-	-
Richardsons merlin	50	1.10	.221	-	-
Richardsons merlin	51	1.05	.156	-	-
Richardsons merlin	52	1.09	.219	-	-
Richardsons merlin	53	1.02	.517	-	-
Richardsons merlin	54	1.06	.514	-	-
SASKATCHEWAN					
Prairie falcon	55	1.69	.194	1.79	-
Prairie falcon	56	1.83	.427	4.85	-
Prairie falcon	57	1.47	.106	8.21	-
Prairie falcon	58	1.73	.142	8.68	-
Prairie falcon	59	1.83	.095	4.86	-
Prairie falcon	60	1.48	.391	7.39	-

	Specimen No.	Eggshell thickness Ratcliffe index[a]	Mercury ppm	DDE ppm	Heptachlor ppm
Prairie falcon	61	1.67	.091	1.33	.467
Prairie falcon	62	1.80	.117	.904	.107
Prairie falcon	63	1.82	.247	2.72	.186
Prairie falcon	64	1.75	.105	3.09	.191
Prairie falcon	65	1.49	.033	2.09	.390
Prairie falcon	66	1.74	.091	2.35	.139

[a] Calculated according to Ratcliffe (1967). Decrease in eggshell weight in certain birds of prey. Nature 215: 208 - 210.

[b] Average of four eggs from the same nest.

APPENDIX III

The Quantitative Determination of Mercury in Animal Tissues. (Quoted from American Journal of Veterinary Research 19: 999-1000, 1958)

The Quantitative Determination of Mercury in Animal Tissues

W. T. OLIVER, Ph.D., and H. S. FUNNELL, B.A.

Guelph, Ontario

CERTAIN mercurial preparations are used for the treatment and prevention of fungus infection in cereal grain and, in this way, are not uncommon sources of poisoning in animals. Since many mercurial salts are volatile, any digestion technique requiring heat used in testing the mercurial content of tissues may cause significant loss of the metal. In the method reported here, this source of error is overcome by the relatively low temperature digestion permitted by the use of a combined condenser-cold finger apparatus.

MATERIALS AND METHODS

Apparatus.—The apparatus (fig. 1) consisted of a Pyrex water-cooled condenser sealed at one end to form a vessel with a volume of 10 ml. The top of the condenser had a standard taper 24/40 joint to accommodate a cold finger which extended to just above the bottom of the water jacket. A 2-mm. hole between the standard taper joint and the top of the water jacket acted as a vent for gas generated during digestion and as an opening for the addition of the oxidizing (permanganate) solution.

Reagents.—The reagents used were reagent-grade sulfuric acid (concentrated), reagent-grade potassium permanganate (saturated solution), hydroxylamine hydrochloride (5% solution), approximately 0.25 N hydrochloric acid (21 ml. concentrated/liter of solution), potassium bromide (40% solution), and fractionated chloroform (59.5–60.5 C. fraction collected).

Buffer.—The buffer consisted of 150 Gm. of disodium phosphate dodecahydrate and 38 Gm. of anhydrous potassium carbonate in 1 liter of solution. The solution was purified by extracting with dithizone and washing with chloroform, and was stored in a polyethylene or Pyrex bottle.

Dithizone Solution A (Stock).—This solution consisted of 100 mg. of pure dithizone dissolved in 800 ml. of redistilled chloroform. Dithizone solution B (weak) is prepared by diluting 50 ml. of solution A to 500 ml. with chloroform. Weaker solutions may be made by further dilutions if required.

Stock Solution of Mercury.—Mercuric chloride (6.767 Gm.) was dissolved in water and diluted to 1 liter. The standard solution was prepared by diluting 10 ml. of the stock to 1 liter with water.

Procedure.—The tissue to be analyzed was macerated in a blendor and a 1-Gm. aliquot was passed through the standard taper joint at the top of the condenser into the digestion vessel. Contamination of the inner sides of the apparatus was prevented by weighing the tissue into a gelatin capsule before passing it into the vessel. Connections were joined between the cold water line outlet and the condenser-cold finger inlets. Water (2 ml.) and sulfuric acid (3 ml.) were added to the tissue in the vessel and the cold finger fitted into the condenser.

The apparatus was placed on a water bath, with the digestion vessel submerged in the boiling water and heated with frequent agitation for 45 minutes after the tissue has been liquefied. Potassium permanganate solution (1 ml.) was added through the vent and mixed with the digest by agitation. When the foaming had stopped, another milliliter of permanganate was added and the contents were heated until no further color change was evident. Increments of potassium permanganate were added, with heating, until the contents became clear and had a light amber color.

The apparatus was taken from the water bath and the contents cooled under running water. The cold finger was raised and rinsed with 5 ml. of hydroxylamine hydrochloride solution, followed by distilled water. The digest was transferred from the condenser to a 250-ml. separatory funnel, the condenser rinsed several times with distilled water, and the rinsings added to the separatory funnel. The cold finger and condenser were rinsed with dithizone solution B and the rinsings added to the separatory funnel. The final volume of dithizone should be about 50 ml.

The funnel was shaken for two minutes, the layers allowed to separate, and the dithizone layer transferred to a second separatory funnel. The aqueous phase was washed with three 5-ml. portions of redistilled chloroform and the washings were transferred to the second separatory funnel. The dithizone layer was washed with 25 ml. of 0.25 N HCl and transferred to a third funnel. The HCl was washed with three 5-ml. portions of chloroform and the washings added to the third separatory funnel. The HCl solution was discarded. Fifty milliliters of 0.25 N HCl and 5 ml. of potassium bromide solution were added to the dithizone, and the solutions were shaken to transfer the mercury from the organic to the aqueous phase.

The dithizone layer was discarded and the aqueous layer washed with three 5-ml. portions of chloroform. The washings were discarded. The aqueous phase was adjusted to approximately pH 6 by the addition of 11 ml. of buffer and titrated with dithizone solution B. This was carried out by adding the solution in increments of 0.5 to 1.0 ml. and shaking after each addition.

From the Division of Pharmacology, Ontario Veterinary College, Guelph.

TABLE 1—Recovery of Mercury from Liver Tissue

Mercuric chloride added (μg./Gm.)	Recovery (%)				Average recovery (%)
0.0	0	0	0	0	0.0
5.0	94	94	94	110	98.0
12.5	96	96	100	100	98.0
25.0	96	96	100	98	97.5
50.0	96	98	102	100	99.0

If mercury is present, the organic phase takes on the salmon-orange color of mercury dithizonate. Any other color change indicates the end point. Estimation also may be made by the mixed color method.

The dithizone solution was standardized by titration with an aliquot of a standard solution of mercury in 50 ml. of 0.25 N HCl, 5 ml. of potassium bromide, and 11 ml. of buffer.

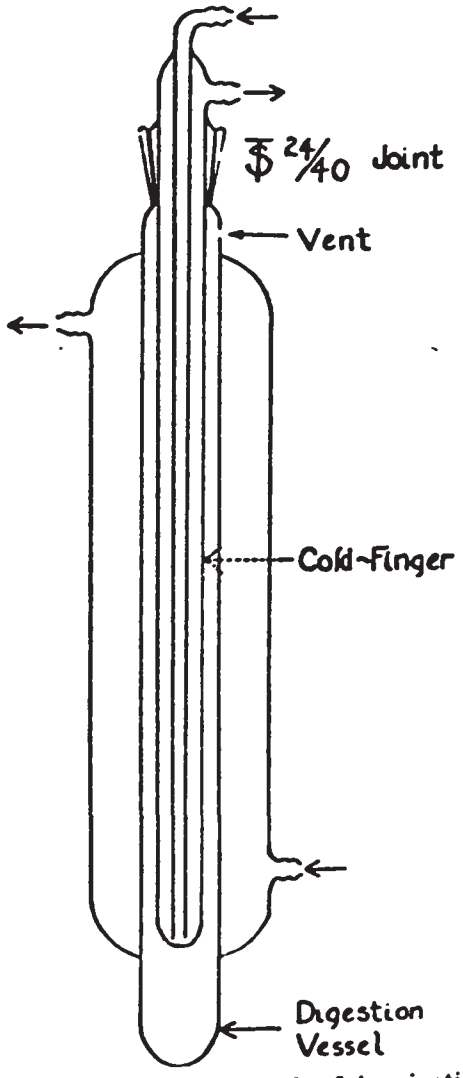


Fig. 1—Apparatus used for the determination of mercury in tissues.

The amount of mercury in the unknown is calculated by: The number of milliliters required for titration, multiplied by the value of dithizone solution, is equal to the micrograms of mercury per gram.

RESULTS

The results (table 1) were obtained by adding mercuric chloride to 1 Gm. of liver and then treating by the method described. The method also has been used successfully with brain, muscle, and kidney tissue.

DISCUSSION

The digestion flask is designed to have a minimum glass area[2] and a continuity of surface. The use of a similar cold finger adapted by a standard taper joint to a 50-ml. digestion flask resulted in an average recovery in three trials of only 55 per cent of the mercury added.

After digestion, the excess permanganate is reduced with hydroxylamine and the mercury is transferred from the acid aqueous to the organic phase as mercury dithizonate. Since silver dithizonate is unstable in the presence of dilute halogen,[1] the silver is separated from the mercury by washing the dithizone with dilute hydrochloric acid solution. The organic layer is shaken with an acid solution of potassium bromide, which destroys the mercury dithizonate, and the mercury passes to the aqueous phase as potassium mercuric bromide, leaving the copper in the chloroform layer.[1] Under the conditions described, (*i.e.*, extraction with dilute dithizone solution), bismuth will not interfere even if present at 100 times the concentration of mercury.[1] Finally, the solution is adjusted to pH 6 to permit the mercury to react with dithizone for estimation as mercury dithizonate. Gold and palladium may interfere, but neither is a common cause of poisoning in domestic or wild animals.

SUMMARY

A method is described for the recovery of mercury from and the quantitative determination of mercury in tissue. An average of 98 per cent recovery has been effected, within the range of 0 to 50 μg.

References

[1] Laug, E. P., and Nelson, K. W.: The Determination of Mercury in Foods and Biological Materials. J. A. Off. Agric. Chem., 25, (1942): 399–403.

[2] Sandell, E. B.: Colorimetric Determination of Traces of Metals. Interscience Publishers, Inc., New York, N.Y. (1944): 321.

APPENDIX IV

Analytical procedure for organochlorine pesticides at the Ontario Research Foundation (quoted): "For extraction of the organochlorine residues, the frozen egg sample was thawed out and homogenized in a Waring Blendor.

An aliquot (2-5 gm.) of the blend was weighed into a 50 ml. beaker to the nearest milligram and dried in a vacuum oven at 45°C with slight vacuum to constant weight (approximately 36 hours needed). The per cent moisture was then calculated from the difference in weights.

After constant weight was obtained, the dried sample was broken up by adding 5-10 gm. anhydrous Na_2SO_4 and grinding with a flattened glass rod. The dried material was removed from the sides and bottom of the beakers by grinding and scraping. The mixture was poured into a Soxhlet thimble and the beaker rinsed several times with ether: n-hexane (1:1). A glass wool plug was used to cover the sample in the thimble which was then extracted in a Soxhlet apparatus for 2 hours, using about 150 ml. of 1:1 ether-hexane mixture at a rate of 10 siphonings per hour.

After extraction, the solvent was removed in a flash evaporator and dried flask was weighed. The per cent fat was calculated for the difference in weights.

The fat residue was dissolved in 150 ml. of 5% benzene in acetone and the solution was cleaned-up by cold precipitation, essentially according to the method of McCully and McKinley (1964). The solution was chilled to -70°C and stirred for 35 minutes in a dry ice-methanol

cold bath. The mixture was then concentrated and made to a volume of 5 ml. with hexane. Additional cleanup was effected by use of a Florisil column and the cleaned-up extract was analysed for pesticide residues by gas liquid chromatography-electron capture (GLC-EC) technique with parameters as described by Reynolds (1969).

Screening was carried out for all the common organochlorine pesticides including α-, β-, and ɣ-BHC, heptachlor, heptachlor epoxide (HE), aldrin, Kelthane, DDE, dieldrin, DDD(TDE), o,p'-DDT, p,p'-DDT, methoxychlor and endrin.

No corrections were made for pesticide losses during the extraction and cleanup processes, although recovery studies for 8 of the more common pesticides showed an average loss of about 10%. Confirmation of specific residues was made by use of more polar phase GLC columns, derivatization and use of characteristic GLC retention times of the derivatives, and by thin layer chromatography where possible."