11 22 (39)

*Environmental Health Criteria 1*

# MERCURY

Published under the joint sponsorship of
the United Nations Environment Programme
and the World Health Organization





World Health Organization
Geneva, 1976

# CONTENTS

4

## ORIGIN AND OBJECTIVES OF THE PROGRAMME

During the last two decades, evaluation of the health hazards from chemical and other environmental agents has received considerable attention in several WHO programmes. High priority was given to drinking water quality (*1*), food additives (*2*), and pesticide residues (*3*), to occupational exposure (*4*), air quality in urban areas (*5*), and, more recently, to the carcinogenic risk of chemicals to man (*6*).

In most instances, man's *total* exposure to a given agent, from different media or conditions (air, water, food, work, home), was not considered. The inadequacy of this approach is obvious for pollutants that may reach man by several pathways, as is the case with lead, cadmium, and some other metals, and certain persistent organic compounds. In response to a number of World Health Assembly resolutions (WHA23.60, WHA24.47, WHA25.58, WHA26.68) and taking into consideration the relevant recommendations of the United Nations Conference on the Human Environment (*7*) held at Stockholm in 1972, and of the Governing Council of the United Nations Environment Programme (UNEP) (*8*), an integrated and expanded programme on the assessment of health effects of environmental conditions was initiated in 1973 under the title of: WHO Environmental Health Criteria Programme, with the following objectives:

(i) to assess existing information on the relationship between exposure to environmental pollutants (or other physical and chemical factors) and man's health, and to provide guidelines for setting exposure limits consistent with health protection, i.e., to compile environmental health criteria documents;

(ii) to identify new or potential pollutants by preparing preliminary reviews on the health effects of agents likely to be increasingly used in industry, agriculture, in the home or elsewhere.

(iii) to identify gaps in knowledge concerning the health effects of recognized or potential pollutants or other environmental factors, to stimulate and promote research in areas where information is inadequate, and

---

[a] Prepared by the WHO Secretariat. References are listed on page 14.

(iv) to promote the harmonization of toxicological and epidemiological methods in order to obtain research results that are internationally comparable.

The general framework of the Environmental Health Criteria Programme was formulated by a WHO meeting held in November 1972 (9), and further elaborated by a WHO Scientific Group that met in April 1973 (10).

## DEFINITIONS, TERMINOLOGY, AND UNITS

### Terminology

In the framework of the WHO Environmental Health Criteria Programme, it is understood that the term "criteria" designates the relationship between exposure to a pollutant or other factor and the risk or magnitude of undesirable effects under specified circumstances defined by environmental and target variables (9). This corresponds to the definition proposed by the Preparatory Committee for the United Nations Conference on the Human Environment (11). Other Preparatory Committee definitions of immediate interest to the criteria programme are:

— "*exposure*: the amount of a particular physical or chemical agent that reaches the target";
— "*target* (or *receptor*): the organism, population, or resource to be protected from specific risks";
— "*risk*: the expected frequency of undesirable effects arising from a given exposure to a pollutant".

The WHO Scientific Group on Environmental Health Criteria (10) accepted these definitions for the purposes of its discussions, but felt that they were not altogether satisfactory, and recommended that WHO, in collaboration with other international organizations, should reconsider them, along with other necessary definitions, at an appropriate international meeting. In accordance with this recommendation, the WHO Secretariat is preparing a list of basic terms to be used in the Environmental Health Criteria Programme that will be submitted to the national institutions and other international organizations for discussion.

The Scientific Group (10) found the definition of "exposure" particularly inadequate and considered that it should be expanded to include the concepts of concentration and length of exposure in addition to the amount of the agent.

The WHO Secretariat considers it useful to attach specific meanings to the terms "effect", "response" and "dose" as was done by the Sub-committee on the Toxicology of Metals of the Permanent Commission and International Association on Occupational Health at the Tokyo meeting (12). These terms will be used in the following sense unless indicated differently in specific criteria documents:

— "*effect*: a biological change caused by (or associated with)[a] an exposure";

— "*response*: the proportion of a population that demonstrates a specific effect";

— "*dose*: the amount or concentration of a given chemical at the site of the effect".

The concept of "response" as defined above is generally accepted but the terminology used to describe this concept varies widely. Many toxicologists use the terms "effect" and "response" interchangeably to denote a specific biological change associated with exposure, whereas different terms are used to indicate the proportion of a population affected (e.g., incidence, cumulative response frequency, response rate, etc.).

There is no general agreement as to the use of the term "dose" for chemical agents. Its common usage is to express the amount of substance administered, for instance, to an experimental animal (e.g., oral dose, injected dose, etc.). In most cases, the amount or concentration of a given agent at the site where its presence induces a given effect cannot be determined by direct measurement and has to be estimated from experimental, occupational, or general environmental exposure, or from measurements in biological indicator media such as blood, urine, faeces, sweat, or hair (12). To avoid misunderstanding, it is, therefore, necessary in each case to make as clear as possible the way in which the "dose" is measured or estimated, including the units used.

Because of the existing differences in the use of terms, no attempt has been made at this stage to impose a uniform terminology in all criteria documents. Until an internationally agreed terminology becomes available, the task groups on specific criteria documents are given freedom to choose their terminology, provided the terms are defined and used consistently throughout the document under consideration.

---

[a] Added by the WHO Secretariat.

## Units

An attempt has been made to express all numerical values in a uniform fashion, for instance, the concentrations are always expressed as mass concentrations in units acceptable to the SI system (e.g. mg/litre or mg/kg) (*13*). Some departures from this are made where the introduction of new units would cause confusion, e.g., lead in blood is expressed in μg/ 100 ml and not in μg/litre.

## Priorities

Considering the large number of environmental agents and factors that may adversely influence human health, a practical programme for the preparation of criteria documents must be based on clearly defined priorities. The list of priorities has been established by a WHO Scientific Group (*10*), and is based on the following considerations:

— "*Severity and frequency of observed or suspected adverse effects on human health*. Of importance are irreversible or chronic effects, such as genetic, neurotoxic, carcinogenic, and embryotoxic effects including teratogenicity. Continuous or repeated exposures generally merit a higher priority than isolated or accidental exposures.

— *Ubiquity and abundance of the agent in man's environment*. Of special concern are inadvertently produced agents, the levels of which may be expected to increase rapidly, and agents that add to a natural hazard.

— *Persistence in the environment*. Pollutants that resist environmental degradation and accumulate, in man, in the environment, or in food chains, deserve attention.

— *Environmental transformations or metabolic alterations*. Since these alterations may lead to the production of chemicals that have greater toxic potential, it may be more important to ascertain the distribution of the derivatives than that of the original pollutant.

— *Population exposed*. Attention should be paid to exposures involving a large portion of the general population, or occupational groups, and to selective exposures of highly vulnerable groups represented by pregnant women, the newborn, children, the infirm or the aged."

The full list contains some 70 chemicals and physical hazards, and it will be periodically reviewed. In preparing this list, it was realized that each country must assess environmental health problems in the light of its own national situation and establish its own priorities, which may not have been covered by this list.

8

## SCOPE AND CONTENT OF ENVIRONMENTAL HEALTH CRITERIA DOCUMENTS

### Scope

As stated on page 5, the purpose of the criteria documents is to compile, review, and evaluate available information on the biological effects of pollutants and other environmental factors that may influence man's health, and to provide a scientific basis for decisions aimed at protecting man from the adverse consequences of exposure to such environmental factors, both in the occupational and general environment. Although attainment of this objective entails consideration of a wide range of data, no attempt is made to include in the documents an exhaustive review of all published information on the environmental and health aspects of specific agents. In the process of collecting the required information, the available literature has been carefully evaluated and selected as to its validity and its relevance to the assessment of human exposure, to the understanding of the mechanism of biological effects, and to the establishment of dose–effect and dose–response relationships. Environmental considerations are limited to information that can help in understanding the pathways leading from the natural and man-made sources of pollutants to man. Non-human targets (e.g., plants, animals) are not considered unless the effects of their contamination are judged to be of direct relevance to human health. For similar reasons much of the published information on the effects of chemicals on experimental animals has been omitted.

### Content

The criteria documents consist of three parts:

(i) A summary, which highlights the major issues, followed by recommendations for research to fill existing gaps in knowledge;

(ii) The bulk of the report, which contains the findings on which the evaluation of the health risks is based. This part has a similar structure in all the criteria documents on chemical agents and contains the following chapters: chemical and physical properties and analytical methods; sources of environmental pollution; environmental transport, distribution and transformation; metabolism; experimental studies of effects; and epidemiological and clinical studies of the effects. The subdivision of these chapters differs from document to document.

(iii) Evaluation of health risks to man from exposure to the specific agent. This part of the criteria document states the considered

opinion of the task group, which examined the findings contained in the second part (see (ii) above), and typically contains the following sections: relative contributions to the total dose from air, food, water, and other exposures; dose-effect relationships; dose-response relationships and, whenever possible, guidelines on exposure or dose limits.

### Chemical and physical data

The chemical and physical data included in the criteria documents are limited to the properties that are considered relevant to the assessment of exposure and to the understanding of the effects. Where applicable, the impurities that may occur in commercial products are examined. Analytical techniques are discussed only to the extent needed to understand and evaluate data on levels in the environment and biological samples. The methods described should not be considered as recommended procedures. Where feasible, information is included on the applicability of a given method for the analysis of different types of sample, on detection limits, precision, and accuracy. The detection limit represents the smallest total amount the method is able to determine. In most cases, the amount of sample is limited so that it is useful in practice to express the smallest concentration that can be determined by that method. Precision of a method is defined in terms of the standard deviation or the coefficient of variation of a number of analyses made on the sample. Accuracy denotes systematic deviation of the measured values from the true value. It is impossible to ascertain the accuracy with absolute certainty; the evidence for the accuracy of a method is often circumstantial and is based either on inter-laboratory data-quality control studies or on the agreement of results obtained with procedures using different approaches. The results of one "accurate" procedure should agree with those of another "accurate" procedure for a given set of samples.

### Production, use, and environmental levels

Data on the production, use, and levels in the environment of pollutants are reported only to illustrate the magnitude and extent of the problem and are not meant to represent an exhaustive and critical review. It is hoped that, in the future, better data will be available and that closer collaboration will be established with other governmental and non-governmental organizations qualified to supply such information.

**Biological data**

Although every effort is made to review the whole literature, it is possible that some publications have been overlooked. Some studies have purposely been omitted because the information contained therein was not considered valid or relevant to the scope of the criteria documents, or because they only confirmed findings already described. In general, the information is summarized as given by the author; however, certain shortcomings of reporting or of experimental design are also pointed out. The data on carcinogenicity have been examined and evaluated in consultation with the International Agency for Research on Cancer.

Whenever possible, the dose-effect and the dose-response relationships reported in the criteria documents are based on epidemiological and other human studies, and animal data are used, in general, as supporting evidence.


## ARRANGEMENTS FOR THE PREPARATION OF CRITERIA DOCUMENTS

In order to obtain balanced and unbiased information, the collection and evaluation of information is done in close collaboration with national scientific and health institutions. About 20 Member States of WHO have designated national focal points for collaboration in the WHO Environmental Health Criteria Programme. Without this collaboration no progress could have been made in its implementation.

In addition, a number of WHO collaborating centres on environmental health effects have been designated to extend and complement the expertise available in the WHO Secretariat.

Two procedures have been used in preparing the criteria documents. One is based on the consolidation of national contributions and the other on a draft criteria document prepared by consultants or the collaborating centres in association with the Secretariat.


### Procedure based on national contributions

Criteria documents are prepared in four stages: (1) the preparation of national contributions by focal points in the Member States reviewing all relevant research results obtained in these countries; (2) consolidation of the national contributions into a draft document, which is done on a contractual basis with individual experts or WHO collaborating centres; (3) the draft criteria documents are circulated to the national focal points

for comments and additions, based on which a second draft is prepared, and (4) the second draft document is reviewed and the information assessed at a meeting of internationally recognized experts (the task group meetings).

National contributions to the criteria documents consist of a review of data on health effects of environmental agents, as revealed by experimental, clinical, and epidemiological studies, and of other relevant information on research carried out in each country and published in scientific journals or official publications. In order to facilitate the integration of national contributions into draft criteria documents, detailed outlines are prepared for each environmental agent considered, and the national focal points are requested to follow these outlines as closely as possible and to attach all publications referred to in the review in the form of reprints or microfiches.

## Procedure for drafts prepared by the Secretariat

With the exception of steps 1 and 2 (which are replaced by the preparation of a draft criteria document by individual experts or WHO collaborating centres), the procedure is the same as described above. This procedure is applied in cases where much preparatory work has been done in Member States and where criteria-like documents (WHO or national) already exist.

## Task group meetings

The task group meetings that are convened to complete the criteria documents have the following terms of reference:

(i) to verify, as far as possible, that all available data have been collected and examined;

(ii) to select those data relevant to the criteria documents;

(iii) to determine whether the data, as summarized in the draft criteria document, will enable the reader to make his own judgement concerning the adequacy of an experimental, epidemiological, or clinical study;

(iv) to judge the health significance of the information contained in the draft criteria document, and

(v) to make an evaluation of the dose–effect, dose- response relationships and of the health risks from exposure to the environmental agents under examination.

12

Members of task groups serve in a personal capacity, as experts and not as representatives of their governments or of any organization with which they are affiliated. In addition to the first and second draft criteria documents, the members of the task group are requested to refer to the original publications whenever they deem that necessary, and to review national and other comments on the first draft criteria document to make sure that no significant information is omitted and that the final document properly reflects the work done in different countries.

## Collaboration with the United Nations Environment Programme (UNEP) and other international organizations

The WHO Environmental Health Criteria Programme has received substantial financial assistance from UNEP which is acknowledged with appreciation. In addition, the programme has been planned from the out-set in consultation with the UNEP Secretariat. The UNEP Secretariat receives all the drafts of criteria documents and their comments are care-fully considered in the preparation of the final documents. UNEP is regularly invited to be represented at the task group meetings.

The United Nations, their subsidiary bodies and specialized agencies, and the IAEA are as a rule invited to provide comments on the draft criteria documents and to participate in the task group meetings. The same applies to selected nongovernmental organizations in official relationship with WHO.

## Note to readers of the criteria documents

While every effort has been made to present information in the criteria documents as accurately as possible without unduly delaying their publication, mistakes might have occurred and are likely to occur in the future. In the interest of all users of the environmental health criteria documents, readers are kindly requested to communicate any errors found to the Division of Environmental Health, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda which will appear in subsequent volumes.

In addition, experts in any particular field dealt with in the criteria documents are kindly requested to make available to the WHO Secretariat any important published information that may have inadvertently been omitted and which may change the evaluation of health risks from expo-sure to the environmental agent under examination, so that the informa-tion may be considered in the event of updating and re-evaluation of the conclusions contained in the criteria documents.

13

1. *International Standards for Drinking Water,* third edition. Geneva, World Health Organization, 1971.
2. WHO Technical Report Series. Nos: 129 (1957), 228 (1962), 281 (1964), 309 (1965), 339 (1966), 373 (1967), 383 (1968), 430 (1969), 445 (1970), 462 (1971), 488 (1972), 505 (1972), 539 (1974).
3. WHO Technical Report Series, Nos: 370 (1967), 391 (1968), 417 (1969), 458 (1970), 474 (1971), 502 (1972), 525 (1973), 545 (1974), 574 (1975), 592 (1976).
4. WHO Technical Report Series, No.: 415 (1969).
5. WHO Technical Report Series, No.: 506 (1972).
6. INTERNATIONAL AGENCY FOR RESEARCH ON CANCER. *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man,* Vol. 1–11 (1972–76).
7. UNITED NATIONS GENERAL ASSEMBLY. *Report of the United Nations Conference on the Human Environment held at Stockholm,* 5–16 June 1972 A/CONF.48/14. 3 July 1972.
8. UNITED NATIONS ENVIRONMENT PROGRAMME. *Report of the Governing Council of the United Nations Environment Programme (First session)* UNEP/GC/10, 3 July 1973.
9. *The WHO Environmental Health Criteria Programme* (unpublished WHO document EP/73.1).
10. *Environmental Health Criteria.* Report of a WHO Scientific Group (unpublished WHO document EP/73.2).
11. UNITED NATIONS GENERAL ASSEMBLY. *Report of the Preparatory Committee for the United Nations Conference on the Human Environment on its Third Session.* United Nations document A/CONF.48/PC/13, 30 September 1971.
12. NORDBERG, G. F., ed. *Effects and dose–response relationships of toxic metals, Proceedings from an international meeting organized by the Sub-committee on the Toxicology of Metals of the Permanent Commission and International Associations on Occupational Health, Tokyo, 18–23 November 1974.* Amsterdam, Oxford, New York, Elsevier Scientific Publishing Company, 1976.
13. LOWE, D. A. *A guide to international recommendations on names and symbols for quantities and on units of measurement.* Geneva, World Health Organization, 1975. 314 pp. (*Progress in Standardization No. 2.*)

## WHO TASK GROUP ON ENVIRONMENTAL HEALTH CRITERIA FOR MERCURY

*Geneva 4–10 February 1975*

## Participants:

*Members*

Professor T. Beritić. Institute for Medical Research and Occupational Medicine. Zagreb, Yugoslavia

Dr H. Blumenthal, Division of Toxicology, Bureau of Foods. Food and Drug Administration, Department of Health. Education and Welfare, Washington. DC, USA (*Rapporteur*)

Dr J. Bouquiaux, Department of the Environment, Institute of Hygiene and Epidemiology. Brussels, Belgium

Dr G. J. van Esch, Laboratory for Toxicology. National Institute of Public Health, Bilthoven, Netherlands

Professor L. Friberg. Department of Environmental Hygiene, Karolinska Institute, Stockholm, Sweden (*Chairman*)

Professor G. L. Gatti. Istituto Superio di Sanità. Rome. Italy

Dr L. Magos, Toxicology Research Unit, Medical Research Council Laboratories, Carshalton, Surrey, England

Dr J. Parizek. Institute of Physiology. Czechoslovak Academy of Sciences. Prague, Czechoslovakia

Dr J. K. Piotrowski. Department of Biochemistry, Institute of Environmental Research, Medical Academy in Łódź. Łódź, Poland (*Vice-Chairman*)

Dr E. Samuel, Health Protection Branch. Department of National Health and Welfare. Ottawa, Ontario, Canada

Dr S. Skerfving. Department of Internal Medicine, University Hospital. Lund, Sweden

Dr T. Tsubaki. Brain Research Insitiute. Niigata University, Niigata. Japan

Professor H. Valentin, Institute for Occupational and Social Medicine. Erlangen, Federal Republic of Germany

*Representatives from other organizations*

Dr A. Berlin. Health Protection Directorate. Commission of the European Communities, Luxembourg

15

Dr D. Djordjević, Occupational Health and Safety Branch, ILO, Geneva, Switzerland

Dr W. J. Hunter, Commission of the European Communities, Luxembourg

Dr G. D. Kapsiotis, Senior Officer, Food Policy and Nutrition Division. FAO. Rome, Italy

Dr E. Mastromatteo, Chief, Occupational Health and Safety Branch, ILO, Geneva, Switzerland

*Secretariat*

Dr T. Clarkson, University Center in Environmental Health Sciences. The University of Rochester. School of Medicine and Dentistry, Rochester NY, USA (*Temporary Adviser*)

Dr F. C. Lu, Chief, Food Additives. WHO, Geneva, Switzerland (*Secretary*)

Dr B. Marschall, Medical Officer, Occupational Health. WHO, Geneva, Switzerland

# ENVIRONMENTAL HEALTH CRITERIA FOR MERCURY

A WHO Task Group on Environmental Health Criteria for Mercury met in Geneva from 4-10 February 1975. Dr B. H. Dietrich, Director, Division of Environmental Health, opened the meeting on behalf of the Director-General. The Task Group reviewed and amended the second draft criteria document and made an evaluation of health risks from exposure to mercury and its compounds. The revised draft was sent for comments to all members of the Task Group.

A group of WHO temporary advisers (Dr T. Clarkson, Dr L. Friberg, Dr A. Jernelöv,[a] Dr L. Magos, and Dr G. Nordberg[b]) assisted the Secretariat in the final scientific editing of the document. They met in Geneva on 13 and 14 November 1975.

The first and second draft criteria documents were prepared by Dr T. Clarkson, Environmental Health Sciences Centre, the University of Rochester School of Medicine and Dentistry, Rochester, New York, USA. The comments on which the second draft was based were received from the national focal points for the WHO Environmental Health Criteria Programme in Bulgaria, Czechoslovakia, the Federal Republic of Germany, Italy, Japan, the Netherlands, New Zealand, Poland, Sweden, the USA, and the USSR; and from the United Nations Industrial Development Organization (UNIDO), Vienna, and the United Nations Scientific, Educational and Cultural Organization (UNESCO), Paris. Comments from the International Labour Organisation, Geneva, the United Nations Food and Agriculture Organization, Rome, and the Commission of the European Communities Health Protection Directorate, Luxembourg, were submitted at the task group meeting.

Comments were also received, at the request of the Secretariat, from Dr L. Amin-Zaki, Iraq, Dr G. J. van Esch, Netherlands, Dr K. Kojima, Japan, and Dr S. I. Shibko, USA.

The collaboration of these national institutions, international organizations, WHO collaborating centres and individual experts is gratefully acknowledged. Without their assistance the document could not have been completed. The Secretariat wishes to thank in particular Dr T. Clarkson for his help in all phases of the preparation of the document.

---

[a] Institute for Water and Air Pollution Research, Stockholm, Sweden.
[b] Department of Environmental Hygiene, Karolinska Institute, Stockholm, Sweden.

This document is based primarily on original publications listed in the reference section. However, several recent publications broadly reviewing health aspects of mercury and its compounds have also been used. These include reviews by the Swedish Expert Group (1971)., Hartung & Dinman (1972), IAEA (1972), and Wallace et al. (1971). Reviews devoted primarily to the biological effects of mercury have been published by Clarkson (1972a, 1972b) and Miller & Clarkson (1973). Furthermore, several recent symposia have provided extensive reviews of the environmental aspects of mercury (Bouquiaux, 1974; D'Itri, 1972; Krenkel, 1975). A systematic review of various environmental health aspects of mercury, including a broad review of the accessible literature up to 1971, has been presented by Friberg & Vostal (1972).

## 1. SUMMARY AND RECOMMENDATIONS FOR FURTHER RESEARCH

### 1.1   Some definitions

In order to clarify the meaning of certain terms used in the document, some definitions are given below. However, it should be noted that these definitions have not been formally adopted by WHO.

The terms *critical effects*, *critical organ*, and *critical organ concentration* have recently been defined by the Sub-Committee on Toxicology of Metals of the Permanent Commission and International Association of Occupational Health (Nordberg, 1976). The term "critical" as defined by the Committee differs from its usual meaning in clinical medicine, where it refers to a situation in which the patient's condition may deteriorate suddenly and dramatically. It also differs in meaning from that used in the field of radiation protection, where the "critical" organ is defined as the organ of the body whose damage by radiation results in the greatest injury to the individual. In this document, the term "critical" does not refer to a life-threatening situation, but to a key decision point for taking preventive action. For example, at some point in the dose–effect relationship, a critical effect can be identified. The appearance of an effect in an individual signals the point at which measures should be taken to reduce or prevent further exposure.

### 1.2   Summary

#### 1.2.1   Analytical methods

The method of choice for determining total mercury in environmental and biological samples is flameless atomic absorption. The technique is

rapid and sensitive and the procedure is technically simple. Neutron activation is now principally used as a reference method against which the accuracy of atomic absorption procedures may be checked. Gas–liquid chromatography combined with an electron-capture detector is the most widely used method for identifying methylmercury in the presence of other compounds of mercury.

The methods of sampling require careful consideration of the type of exposure to be monitored and the material to be analysed. Errors arising in collection, storage, and transportation of samples may be as important as instrument errors in contributing to the total error in the measurement of mercury in the sample. These include contamination of the sample, and the loss of mercury by adsorption on the walls of the container, and by volatilization. In estimating human exposure, special care should be taken to see that the sample is truly representative, e.g. the mercury vapour concentration in the breathing zone and the concentration of methylmercury in the daily diet.

### 1.2.2 Sources of environmental pollution

The major source of mercury is the natural degassing of the earth's crust and amounts to between 25 000 and 125 000 tonnes per year. Anthropogenic sources are probably less than natural sources. World production of mercury by mining and smelting was estimated at 10 000 tonnes per year in 1973 and has been increasing by an annual rate of about 2%. The chloralkali, electrical equipment, and paint industries are the largest consumers of mercury, accounting for about 55% of the total consumption. Mercury has a wide variety of other uses in industry, agriculture, military applications, medicine, and dentistry.

Several of man's activities not directly related to mercury account for substantial releases into the environment. These include the burning of fossil fuel, the production of steel, cement, and phosphate, and the smelting of metals from their sulfide ores. It was extimated that the total anthropogenic release of mercury would amount to 20 000 tonnes per year in 1975.

### 1.2.3 Environmental distribution and transport

Two cycles are believed to be involved in the environmental transport and distribution of mercury. One is global in scope and involves the atmospheric circulation of elemental mercury vapour from sources on land to the oceans. However, the mercury content of the oceans is so large, at least seventy million tonnes, that the yearly increases in concentration due to deposition from the global cycle are not detectable.

The other cycle is local in scope and depends upon the methylation of inorganic mercury mainly from anthropogenic sources. Many steps in this cycle are still poorly understood but it is believed to involve the atmospheric circulation of dimethylmercury formed by bacterial action.

The methylation of inorganic mercury in the sediment of lakes, rivers, and other waterways and in the oceans is a key step in the transport of mercury in aquatic food chains leading eventually to human consumption. Methylmercury accumulates in aquatic organisms according to the trophic level, the highest concentrations being found in the large carnivorous fish.

Alkylmercury fungicides used as seed dressings are important original sources of mercury in terrestrial food chains. Mercury is passed first to seed eating rodents and birds and subsequently to carnivorous birds.

Accumulation of methylmercury in aquatic and terrestrial food chains represents a potential hazard to man by consumption of certain species of oceanic fish, of fish or shellfish from contaminated waters, and of game birds in areas where methylmercury fungicides are used.

### 1.2.4 Environmental exposure levels

The concentration of mercury in the atmosphere is usually below 50 $ng/m^3$ and averages approximately 20 $ng/m^3$. A concentration of 50 $ng/m^3$ would lead to a daily intake of about 1 μg. "Hot spots" near mines, smelting works, and refineries require further investigation but could lead to daily intakes as high as 30 μg. Daily intakes would be higher for occupational exposures to mercury vapour. An average mercury concentration in air of 0.05 $mg/m^3$ would lead to an average daily intake via inhalation of about 480 μg. The highest occupational exposures usually occur in mining operations but over 50 specific occupations or trades involve frequent exposure to mercury vapour.

Mercury in drinking water would contribute less than 0.4 μg to the total daily intake. Bodies of fresh water for which there is no independent evidence of contamination contain mercury at less than 200 ng/litre. Oceanic mercury is usually less than 300 ng/litre.

Food is the main source of mercury in nonoccupationally exposed populations, and fish and fish products account for most of the methylmercury in food. Mercury in food other than fish is usually present at concentrations below 60 μg/kg. Mercury is present in freshwater fish from uncontaminated waters at concentrations of between 100 and 200 μg/kg wet weight. In contaminated areas of freshwater, mercury levels between 500 and 700 μg/kg wet weight are often described and in some cases, concentrations are even higher. Most species of oceanic fish have mercury levels of about 150 μg/kg. However, the large carnivorous

species (e.g. swordfish and tuna) usually fall in the range of 200–1500 µg/kg. With few exceptions methylmercury accounts for virtually all the mercury in both freshwater and marine fish.

Intake of mercury from food is difficult to estimate with precision. Daily intake from food other than fish is estimated as 5 µg but the chemical form of mercury is not known. Most of the methylmercury in diet probably comes from fish and fish products. The median daily intake of methylmercury in Sweden has been estimated as 5 µg. In most countries the daily intake is less than 20 µg but in subgroups in certain countries where there is an unusually high fish intake (dieters) the daily intake may rise to 75 µg and may even be as high as 200–300 µg (in coastal villages dependent on large oceanic fish as the main source of protein). In areas of high local pollution, daily intakes could be well in excess of 300 µg and these levels have led to two recorded outbreaks of methylmercury poisoning.

### 1.2.5  Metabolism of mercury

Approximately 80% of inhaled mercury vapour is retained. Information on pulmonary retention of other forms of mercury in man is lacking. Absorption of inorganic mercury compounds from foods is about 7% of the ingested dose. In contrast, gastrointestinal absorption of methyl-mercury is practically complete. Little information is available on skin absorption although it is suspected that most forms of mercury can penetrate the skin to some extent. In the case of methylmercury, poisoning has resulted from skin application.

Animal data indicate that the kidneys accumulate the highest tissue concentrations no matter what form of mercury is administered. The distribution of mercury between red cells and plasma depends upon the form of mercury. The red cell to plasma ratio is highest for methylmercury (approximately 10) and lowest for inorganic mercury (approximately 1) in man.

The hair is a useful indicator medium for people exposed to methyl-mercury. The concentration of mercury in hair is proportional to the concentration in the blood at the time of formation of the hair. The relationship between hair and blood concentrations is not known for other forms of mercury.

Most forms of mercury are predominantly eliminated with urine and faeces. In workers exposed over a long period to mercury vapour, urinary excretion slightly exceeds faecal elimination. On a group basis, mercury excretion in urine is proportional to the time-weighted average air concentration. Large individual fluctuations are common in daily

mercury excretion in urine in people under the same exposure conditions.

Faecal elimination accounted for approximately 90% of total mercury elimination in volunteers given a single dose of methylmercury. Urinary concentrations of total mercury do not correlate with blood levels after exposure to methylmercury.

Animal data indicate that elemental mercury vapour rapidly crosses the placenta. The transplacental transfer of methylmercury compounds is well documented in man. The mercury concentrations in plasma in the mother and the newborn infant are similar but the concentration in the fetal red blood cells is approximately 30% higher than in those of the mother.

Details on transmission into breast milk are available only for methylmercury. The concentration of mercury in breast milk is approximately 5% of the simultaneous mercury level in blood in the mother, and infants can accumulate dangerously high blood concentrations by suckling if their mothers are heavily exposed.

Tracer studies in volunteers and in exposed populations have established the main features of the metabolic model for methylmercury in man. Clearance half-times from the whole body and from blood are about 70 days. Daily intakes of methylmercury will lead to a steady-state balance in about one year, when the body burden will be approximately one hundred times the daily intake. In steady-state, the numerical value of the concentration of mercury in whole blood in µg/litre is virtually equal to the numerical value of the daily intake in µg/day/70 kg body weight. Considerable individual variation around these average values has been noted, which must be taken into account in the estimation of risk in exposed populations.

The metabolic models for other forms of mercury are less well developed.

### 1.2.6 Experimental studies on the effects of mercury

Reversible and irreversible toxic effects may be caused by mercury and its compounds, depending upon the dose and duration of exposure. Reversible behavioural changes may be produced in animals by exposure to mercury vapour.

Methylmercury compounds produce irreversible neurological damage in animals. Many of the neurological signs seen in man have been reproduced in animals. Methylmercury is equally toxic to animals whether it is given in the pure chemical state or in fish where it has accumulated naturally. A latent period lasting weeks or months is observed between cessation of exposure and onset of poisoning. Morphological changes

have been seen in the brain before onset of signs. This phenomenon has been referred to as "silent damage". Animal data support epidemiological evidence from Japan, that the fetus is more sensitive than the adult.

Little is known about the physical and chemical factors affecting the toxicity of mercury. Selenium is believed to be protective against inorganic and methylmercury compounds.

### 1.2.7 Epidemiological and clinical studies

The classic symptoms of poisoning by mercury vapour are erethism (irritability, excitability, loss of memory, insomnia), intentional tremor, and gingivitis. Most effects of mercury vapour are reversible on cessation of exposure, although complete recovery from the psychological effects is difficult to determine. Recovery may be accelerated by treatment with penicillamine and unithiol (2,3,dimercaptopropansulfonate).

Studies of occupational exposure to mercury vapour reveal that the classic symptoms of mercurialism do not occur below a time-weighted average mercury concentration in air of 0.1 mg/m$^3$. Symptoms such as loss of appetite and psychological disturbance have been reported to occur at mercury levels below 0.1 mg/m$^3$.

The most common signs and symptoms of methylmercury poisoning are paraesthesia, constriction of the visual fields, impairment of hearing, and ataxia. The effects are usually irreversible but some improvement in motor coordination may occur. Complexing and chelating agents may be useful in prevention if given early enough after exposure but BAL is contraindicated in cases of methylmercury poisoning as it leads to increased brain levels of mercury.

Epidemiological investigations have been made on populations in whom the intensity and duration of exposure to methylmercury through diet differs, for example, a population in Iraq having high daily mercury intakes (as high as 200 µg/kg/day) for a brief period (about 2 months), populations in Japan having lower daily intakes with exposure for several months or years, and several fish-eating populations having daily intakes of mercury usually below 5 µg/kg but with exposure lasting for the lifetime of the individual. The results of these studies indicate that the effects of methylmercury in adults become detectable in the most sensitive individuals at blood levels of mercury of 20–50 µg/100 ml, hair levels from 50–120 mg/kg, and body burdens between about 0.5 and 0.8 mg/kg body weight.

Observations on the Minamata outbreak in Japan indicate that the fetus is more sensitive to methylmercury than the adult but the difference in degree of sensitivity has not yet been established.

### 1.2.8 Evaluation of health risks to man from exposure to mercury and its compounds

Adverse health effects have not yet been identified in workers occupationally exposed to a time-weighted average air concentration of mercury of $0.05$ mg/m$^3$. This air concentration is equivalent to an average mercury concentration in blood of 3.5 μg/100 ml and an average mercury concentration in urine of 150 μg/litre on a group basis. The corresponding ambient air concentration of mercury for exposure of the general population would be $0.015$ mg/m$^3$.

It is estimated that the first effects associated with long-term daily intake of methylmercury should occur at intake levels between 3 and 7 μg/kg/day. The probability of an effect (paraesthesia) at this intake level is about 5% or less in the general population. These figures apply only to adults. Prenatal life may be the most sensitive stage of the life cycle to methylmercury. Furthermore experiments on animals indicate a potential for genetic damage by methylmercury.

### 1.3 Recommendations for Further Research

#### 1.3.1 Environmental sources and pathways of mercury intake

More information is needed on the physical and chemical forms of mercury in air, food, and water. With the exception of fish tissue, little is known of the proportion of total mercury in the diet that is in the form of methylmercury.

The concentration of mercury in the air in "hot spots" near points of industrial release is not yet adequately documented. The few reports reviewed in this criteria document indicate that people living near points of emission may receive substantial exposure to airborne mercury. Levels of mercury in the oceans are still inadequately documented. The pathways of methylation of mercury in the ocean and its uptake by fish of different trophic levels are poorly understood.

Studies are needed to estimate quantitatively the dietary intake of methylmercury in populations dependent on fish for their main source of protein. Average dietary intakes for the populations of several industrialized countries have been reported. However, of much greater importance are the identification of those subgroups of the population having unusually high dietary intakes of methylmercury and the careful quantitative estimation of average daily intake in these groups.

24

### 1.3.2 Metabolic models in man

The kinetic parameters of uptake, distribution, and excretion of methylmercury in man are documented in much more detail than for other forms of mercury. However, questions still remain on the linearity of this metabolic model at high toxic doses of methylmercury. Specifically, the applicability of the metabolic model derived from human tracer-dose studies should be verified at higher dose levels. Information on this point would greatly facilitate the interpretation of results of epidemiological studies on heavily exposed populations.

Recent findings of large individual variations in clearance half-times of methylmercury from blood are of considerable importance in the estimation of risks from long-term dietary intake. Further studies are needed to establish the statistical parameters of the distribution of individual clearance half-times, and on the biological mechanisms underlying these differences.

A more complete metabolic model for inhaled mercury vapour in man is urgently needed. Despite the continuous occupational exposure of thousands of workers annually and the long history of man's exposure to this form of mercury, we still do not have sufficient information to relate mercury concentrations in air to accumulated body burdens and to identify the most appropriate indicator media for levels of mercury vapour in the target organ (the brain). Animal experiments have indicated the ability of the inhaled vapour to cross the placenta; no information is available on human subjects concerning this important question.

### 1.3.3 Epidemiological studies

Several types of epidemiological study are needed. Long-term studies on adults should concentrate on those areas of the dose–response relationship where the effects of methylmercury become just detectable. There are still uncertainties concerning the concentrations of total mercury in indicator media and the equivalent long-term daily intake of mercury as methylmercury associated with the earliest effects in the most sensitive group in the adult population.

So far, dose–response relationships in human populations have been based on outbreaks of poisoning in which daily exposure was high and limited to months or a few years at the most. To extrapolate these relationships to the general population, more information is needed on the potential influence of long-term exposure.

In addition to continuing studies on mature adults, groups of the population specially sensitive to methylmercury should be identified.

Special studies should be made on the relationship between the dose received by the expectant mother and the effect on her infant including the development and growth of the child.

Further epidemiological studies are needed on groups occupationally exposed to mercury vapour. Whenever possible, collaborative studies should be carried out in which cohorts should be followed in time and different groups related to each other.

### 1.3.4 Interaction of mercury with other environmental factors

The extrapolation to the general population of epidemiological data from outbreaks of methylmercury poisoning that have occurred in certain parts of the world is fraught with uncertainties, unless the possible inter-action of local environmental factors can be taken into account. For example, the conditions under which selenium exerts antagonistic and synergistic effects and its mode of action should be studied. Alcohol influences the metabolism of mercury and may affect the toxicity of inhaled vapour in man. Genetic factors should also be considered. Acatalasaemic individuals may metabolize inhaled mercury vapour differently from normal individuals.

Mercury, along with other heavy metals, has the potential to alter the activity of drug metabolizing enzymes. Studies should be made on these potential effects with special emphasis on those individuals carrying high body burdens of mercury.

### 1.3.5 Biochemical and physiological mechanisms of toxicity

Long-term investigations of the mode of toxic action of mercury and its compounds are needed to give an insight into the causes of individual differences in sensitivity to mercury and into differences in metabolism such as clearance half-times. Methylmercury is known to produce "silent damage" in that morphological changes can be seen in the brains of experimental animals before functional disturbances are detectable. Biochemical disturbances such as inhibition of protein synthesis precede overt signs of damage. There is a great need to develop sensitive biochemical and physiological tests, especially in the case of methylmercury poisoning.

A deeper understanding of the toxic action of mercury should lead to the development of more effective means of treatment. Present methods depend mainly on prevention, using complexing and chelating agents to remove the metal from the body before serious damage has occurred.

26

# 2. PROPERTIES AND ANALYTICAL METHODS

## 2.1 Chemical and Physical Properties

Mercury can exist in a wide variety of physical and chemical states. This property presents special problems to those interested in assessing the possible risk to public health. The different chemical and physical forms of this element all have their intrinsic toxic properties and different applications in industry, agriculture, and medicine, and require a separate assessment of risk.

The chemistry of mercury and its compounds has been outlined in several standard chemistry texts (Rochow et al., 1957; Gould, 1962; Cotton & Wilkinson, 1972). Mercury, along with cadmium and zinc, falls into Group IIb of the Periodic Table. In addition to its elemental state, mercury exists in the $+1$ (mercury(I)) and $+2$ (mercury(II)) states in which the mercury atom has lost one and two electrons, respectively. The chemical compounds of mercury(II) are much more numerous than those of mercury(I).

In addition to simple salts, such as chloride, nitrate, and sulfate, mercury(II) forms an important class of organometallic compounds. These are characterized by the attachment of mercury to either one or two carbon atoms to form compounds of the type RHgX and RHgR′ where R and R′ represent the organic moiety. The most numerous are those of the type RHgX. X may be one of a variety of anions. The carbon–mercury bond is chemically stable. It is not split in water nor by weak acids or bases. The stability is not due to the high strength of the carbon–mercury bond (only 15–20 cal/mol and actually weaker than zinc and cadmium bonds) but to the very low affinity of mercury for oxygen. The organic moiety, R, takes a variety of forms, some of the most common being the alkyl, the phenyl, and the methoxyethyl radicals. If the anion X is nitrate or sulfate, the compound tends to be "salt like" having appreciable solubility in water; however, the chlorides are covalent non-polar compounds that are more soluble in organic solvents than in water. From the toxicological standpoint, the most important of these organometallic compounds is the subclass of short-chain alkylmercurials in which mercury is attached to the carbon atom of a methyl, ethyl, or propyl group.

An expert committee, considering occupational hazards of mercury compounds, distinguished two major classes of mercury compounds— "organic" and "inorganic" (MAC Committee, 1969). Inorganic mercury compounds included the metallic form, the salts of mercury(I) and

mercury(II) ions, and those complexes in which mercury(II) was reversibly bound to such tissue ligands as thiol groups and protein. Compounds in which mercury was directly linked to a carbon atom by a covalent bond were classified as organic mercury compounds. This distinction is of limited value because the toxic properties of elemental mercury vapour differ from those of the inorganic salts and, furthermore, the short-chain alkylmercurials differ dramatically from other mercurials that fall within the definition of organic mercury. From the standpoint of risk to human health, the most important forms of mercury are elemental mercury vapour and the short-chain alkylmercurials.

Mercury in its metallic form is a liquid at room temperature. Its vapour pressure is sufficiently high to yield hazardous concentrations of vapour at temperatures normally encountered both indoors and outdoors under most climatic conditions. For example, at 24°C, a saturated atmosphere of mercury vapour would contain approximately 18 mg/m$^3$— a level of mercury 360 times greater than the average permissible concentration of 0.05 mg/m$^3$ recommended for occupational exposure by the National Institutes of Safety and Health, USA (NIOSH, 1973). Apart from the noble gases, mercury is the only element having a vapour which is monatomic at room temperature. However, little is known about the chemical and physical states of mercury found in the ambient air and in the air where occupational exposure occurs.

Elemental mercury vapour is generally regarded as insoluble. Nevertheless, small amounts dissolved in water and other solvents are important from the toxicological point of view. At room temperatures, in air-free water, its solubility is approximately 20 μg/litre. In the presence of oxygen, metallic mercury is rapidly oxidized to the ionic form—mercury(II) — and may attain concentrations in water as high as 40 μg/litre.

Calomel or mercury(I) chloride ($Hg_2Cl_2$) is the best known mercury(I) salt. Widely used in the first half of this century in teething powders and in anthelmintic preparations, the low toxicity of this compound is due principally to its very low solubility in water. Mercury(I) forms few complexes with biological molecules. However, in the presence of protein and other molecules containing SH groups, it gives one atom of metallic mercury and one mercury(II) ion. In general, an equilibrium is established between $Hg^0$, $Hg_2^{++}$ and $Hg^{++}$ in aqueous solution. The distribution of mercury between the three oxidation states is determined by the redox (oxidation–reduction) potential of the solution and the concentration of halide, thiol, and other groups that form complexes with $Hg^{++}$. The dissociation of mercury(I) chloride by thiol groups should be understood in this context. Extra halide and thiol compounds, added to solution, form complexes with mercury(II) ions and the mercury(I) chloride splits to

28

restore the equilibrium between $Hg^0$, $Hg_2^{++}$ and $Hg^{++}$. The split results in the formation of one atom of mercury for every mercury(I) chloride molecule dissociated.

The mercury(II) ion, $Hg^{++}$, is able to form many stable complexes with biologically important molecules. Mercury(II) chloride (corrosive sublimate), a highly reactive compound, readily denatures proteins and was extensively used in the past century as a disinfectant. It is soluble in water and, in solution, forms four different complexes with chloride, $HgCl^+$, $HgCl_2$, $HgCl_3^-$ and $HgCl_4^=$. It has been suggested that the negatively charged chlorine complexes are present in sea water (see section 5).

Phenylmercury compounds have a low volatility. However, the halide salts of methyl-, ethyl-, and methoxyethylmercury can give rise, at 20°C, to saturated mercury vapour concentrations of the order of 90, 8, and 26 mg/m$^3$, respectively (Swensson & Ulfvarsson, 1968). In the case of methylmercury this saturated vapour concentration is several orders of magnitude greater than the maximum allowable concentration in the working atmosphere. This hazardous property of the halide salts of the short-chain alkylmercurials is not always fully appreciated in industrial and agricultural use and even in research laboratories (Klein & Hermen, 1971). In contrast, methylmercury dicyandiamide, previously widely used as a gungicide, has a much lower vapour pressure, being 340 times less volatile than the chloride salt.

Although the carbon–mercury bond is chemically stable, in the living animal, the bond is subject to cleavage (for review, see Clarkson, 1972a). The nature of the R radical is all important. If R is a phenyl or methoxy-alkyl group, rapid breakdown occurs in animal tissues so that most of the organic compound has disappeared within a few days. Enzymes that break the carbon-mercury bond have been discovered and isolated (Tonomura et al., 1968a, 1968b, 1968c). The short-chain alkylmercurials undergo the slowest breakdown *in vivo* with methylmercury being the most stable. Differences in the stability of the carbon–mercury bond play an important role in determining the toxicity and mode of action in man. The rapid breakdown of phenyl- and methoxymercury results in toxic effects similar to those of inorganic mercury salts. The relative stability of the alkylmercurials is one important factor in their unique position with regard to toxicity and risks to human health.

The organic and inorganic cations of mercury, in common with other heavy metal cations, will react reversibly with a variety of organic ligands[a] found in biologically important molecules. The chemical affinity of

---

[a] Ligands are chemical groups within a molecule that are capable of donating electrons to a metal cation to form a chemical bond. Examples of biologically important ligands are the carboxyl, and especially with regard to heavy metals, the sulfhydryl (SH) groups.

mercury(II) and of its monovalent alkylmercury cations for a variety of biologically occurring ligands is so great that free mercury would be present *in vivo* at concentrations so low as to be undetectable by present methods.

## 2.2  Purity of Compounds

Impurities in mercury and its compounds are not important in assessing the hazards to man. Those compounds of mercury used in industry and agriculture have impurities of less than 10%. Bakir et al. (1973) reported that a methylmercury fungicide responsible for an epidemic of poisoning in Iraq contained 10% or less of ethylmercury as an impurity. Inorganic mercury usually amounts to no more than 1% of the total mercury in organomercurial preparations and rarely exceeds 5%.

Impurities are of importance in the preparation of standard solutions for analytical procedures and in experimental research in animals where impurities in radioactive mercury may give misleading results. Preparations of methylmercury labelled with the isotope $^{203}$Hg are subject to radiolytic breakdown to inorganic compounds depending on the pH. This instability must be taken into account in the interpretation of some original reports in which the purity of the radioisotope was not checked properly.

## 2.3  Sampling and Analysis

Before reviewing various aspects of sample collection and analysis it may be worth taking an overview of the various sources of error in the determination of mercury content. Not only are there errors in the instrumental determination of mercury and in the laboratory procedures, but significant and often major errors occur during the collection, transportation, and storage of the samples. The accuracy of the determination of mercury in environmental samples should be assessed from this broad point of view. The error will be the sum of the errors in collection, storage, transportation and, in the instrumental determination. It is of the greatest importance to determine the greatest source of error in each particular case. This, in itself, may lead to considerable improvement in the overall accuracy of the determination. For example, the introduction of a new and more sensitive instrumental technique may allow the collection of smaller samples and thus facilitate storage and transport. On the other

30

hand, there is little value in proceeding further with improvements in instrumental measurements if major errors remain at the collection, storage, or transport stages.

### 2.3.1  Sample collection

Methods of sample collection for the determination of mercury in air have recently been reviewed (NIOSH, 1973). A recommended method for the determination of total mercury in air is presented. Essentially the method consists of using two bubblers in series, containing sulfuric acid and potassium permanganate. The mercury in these traps is subsequently determined by atomic absorption procedures. Problems of the determination of mercury in air are critically evaluated. Included in these problems is the fact that numerous chemical and physical forms of mercury may exist in air and that these are subject to interconversion. The volatility of mercury and its compounds is a special problem in the determination of mercury bound to particles. The separation of particulates from air, such as by filtration, may result in the loss of mercury by volatilization from the particulate. Published methods of sample collection consist of removal of mercury from the air by passing it through scrubbing devices, or direct collection of the air sample, for example in a plastic bag or syringe. The scrubbing device may take the form of bubblers, filters, absorbants, or amalgam collectors. Unfortunately many of the published procedures do not report collection efficiency. Attention is drawn to the importance of the use of standard dust chambers to check the efficiency of absorption.

The procedure recommended by NIOSH (1973) has a collection efficiency for total mercury of more than 90%, when mercury is in the form of elemental vapour or inorganic salts. Organomercurials in air are collected with an efficiency of more than 80%, except in the case of the short-chain alkylmercurials. Bramen (1974) has described a procedure for separating and measuring different physical and chemical forms of mercury in air. Previous reports distinguishing between mercury vapour and particle-bound mercury have not reported the efficiency of collection.

An early method (Polešajev, 1936) for the determination of mercury in air involved absorption in iodine and subsequent determination of the coloured complex in the sediment. This method is still widely used in the Soviet Union and some countries of eastern Europe.

Commercially available portable monitoring devices are used to determine mercury directly in air. The air is pumped through an optical cell that measures the absorption of light emitted from a mercury vapour lamp. These units, although convenient, measure only elemental mercury vapour and are subject to a wide variety of interferences and interfering

substances many of which are likely to be present in the working environment. These units should be calibrated each time before use. The commercial units also suffer from the deficiency that they sample only small volumes of air that may not give a representative picture of the working environment. Research should be directed towards the development of personal monitoring devices. These devices should be small and portable so that they can be carried by workmen throughout the working day and thereby give a cumulative picture of the exposure of each individual. In most cases it would be necessary only to devise systems for collecting total mercury.

The method of Wolf et al. (1974) allows the direct detection of mercury using reactive tubes (Draeger tubes) providing a simple screening method for determining mercury in working places at sporadic intervals.

The collection of samples for the determination of mercury in water must take into account the following factors; (a) the low concentration of mercury in water, normally of the order of 10 ng/litre; (b) the tendency of mercury to adsorb on to the surface of the collection vessel at these low concentrations; (c) the possibility, if not likelihood, of volatilization of mercury from the sample (Toribara et al., 1970) and (d) the type of collection vessel. Greenwood & Clarkson (1970) have reported on the rates of loss of mercury from containers made from ten different materials and suggested that Pyrex, polycarbonate, and Teflon are the best materials for storing and handling mercury. Further studies of possible losses of organomercurials through the walls of some plastic containers should, however, be studied. Losses due to volatilization may be reduced by the addition of oxidizing substances such as potassium permanganate (Toribara et al., 1970). Lamm & Ruzika (1972) have recommended that radioactive-tracer mercury be added to the sample to check the losses discussed above. They note that this procedure has rarely been adopted to date.

For the collection and storage of food samples, acceptable procedures are usually followed. The most important food items for determination of mercury are those containing fish and fish products. Mercury levels in other foodstuffs usually do not amount to a significant fraction of daily exposure unless the food has accidently been contaminated, such as by the use of pesticides. In the collection and storage of food samples prior to analysis, care should be taken to avoid bacterial growth leading either to the breakdown of organic mercury compounds or to the volatilization of mercury (Magos et al., 1964).

Samples of blood, hair, and urine have been used to monitor the exposure of human beings to mercury. The methods of collecting and storing these samples are of great importance. With respect to blood samples, care should be exercised to avoid any clot formation. If this does

32

occur, the sample should be homogenized thoroughly before analysis. It is useful, in certain situations, to determine mercury in the red cells and plasma and it is thus important to avoid any haemolysis of the blood sample. The nature of the anticoagulants used does not affect the mercury determinations, of either the total mercury in whole blood or the distribution of mercury between plasma and red blood cells. "Vacutainers"[a] are convenient for blood collection and allow storage of the blood samples in Pyrex tubing under aseptic conditions. Blood samples that have been contaminated by microorganisms and stored in the refrigerator at 4°C for a month or more may give misleading results due to the breakdown of methylmercury and other organic mercury compounds (Clarkson, personal communication, 1974). The storage of blood samples in the frozen state or freeze-dried is suitable providing that mercury is determined only for whole blood. Significant losses of mercury do not occur during freeze-drying procedures (Albanus et al., 1972).

Measurement of mercury in urine samples has been used as a measure of exposure to mercury under industrial conditions. The popularity of this approach in early studies was mainly due to the ease of digestion of the urine sample. However, there are serious problems in the collection and storage of urine samples that may seriously influence the results. The following factors have been recognized; (a) the time of day of urine collection (Piotrowski et al., 1975), (b) bacterial contamination, which might give rise to significant losses of mercury by volatilization (Magos et al., 1964), (c) the nature of the container (Greenwood & Clarkson, 1970), (d) contamination from mercury in workers' clothing and from the collection of urine samples under working conditions. It should be noted that urine samples do not give a reliable indication of exposure to methylmercury (Bakir et al., 1973).

Hair samples are becoming the samples of choice in determining exposure to methylmercury through diet. Depending upon the length of the hair sample, it is possible to recapitulate exposure to methylmercury for several years[b]. The concentration of mercury in hair when formed is directly proportional to the concentration of mercury in the blood, the concentration in hair being about 250 times the concentration in blood. The ratios are well established for exposure to methylmercury but only limited information is available for inorganic mercury. Attention has been drawn to the errors introduced during the collection and transportation of hair samples (Giovanoli & Berg, 1974). Usually

---

[a] Trade name of heparinized test-tube manufactured by Becton & Dickinson, USA. and used for collection of blood samples.

[b] The average rate of growth of hair is approximately 1 cm per month (Giovanoli et al., 1974; Shahristani & Shihab, 1974).

50- 100 strands of hair are needed for analysis. Differential rates of growth for each strand and lateral displacement of the samples during cutting and transportation of the hair will affect the longitudinal profiles of mercury in the hair sample. Giovanoli & Berg (1974) have described a computerized procedure for the correction of these artifacts.

### 2.3.2  Analytical methods

Methods of analysis are usually classified according to the type of instrument used in the final measurement. This convenient classification will be used here. However this approach tends to belittle the role of the skill and experience of the analyst. In fact a poor method in the hands of a highly skilled analyst is more likely to yield accurate results than a good method in the hands of a poor analyst. In recent years it has become a practice to test methods by a "round robin" distribution of a standard sample. Comparison of results from the participating laboratories is more likely to give information on the competence of the analysts in the laboratory than it is to give a critical evaluation of the method itself.

Measurement of the very low levels of mercury found in the non-contaminated environment makes special demands both on the skills of the analyst and the resources of the method employed. No matter how frequently used, a method for the determination of mercury in nanogram quantities cannot be regarded as a routine procedure. Continued vigilance over the results is an absolute requirement. Furthermore, where conditions allow, it is highly desirable that the results with one method and from one laboratory be checked against those with a different method from another laboratory. One useful combination of different procedures is the analysis of total and inorganic mercury by selective atomic absorption and the selective analysis of organic mercury compounds (usually methylmercury and other short-chain mercurials) by gas chromatography (Giovanoli et al., 1974).

The literature is full of papers concerning methods of determining mercury. Several recent reviews have appeared (D'Itri, 1972; NIOSH, 1973; Burrows, 1975, Swedish Expert Group, 1971; Wallace et al., 1971; CEC Working Group of Experts, 1974). The most frequently used methods for measurements of total mercury are colorimetric (dithizone), flameless atomic absorption, and neutron activation. The flameless atomic absorption method has become the "work-horse" for measurement of environmental samples. Difficulties might arise in the measurement of mercury owing to the fact that it is strongly bound to the organic materials in most samples. Many procedures require the destruction of organic materials by wet oxidation or by high temperatures. Loss of mercury by

34

volatilization may occur. If the wet oxidation is too mild the result will be inadequate recovery. A high reagent blank may be introduced by the chemicals used for oxidation. In certain procedures involving atomic absorption or neutron activation the digestion of the sample or heating of the sample is not necessary. These procedures have the advantage of having a low blank but problems of variable recovery or interference may arise.

The determination of mercury by colorimetric measurement of a mercury dithizonate complex has been the basis of most of the methods in the 1950s and in the 1960s. Other related methods using dithizone for measuring mercury in environmental samples have been described by Kudsk (1964) and Smart et al. (1969). The above procedures all make use of wet oxidation of the sample followed by extraction of mercury in an organic solvent as a dithizonate complex and finally the colorimetric determination of the complex itself.[a] Selectivity for mercury is obtained by adjusting the conditions of extraction. Copper is the metal most likely to interfere with mercury measurement by dithizone.

The dithizone procedure has an absolute sensitivity of about 0.5 μg of mercury. A sample size of 10 g is suitable for most digestion procedures so that mercury can be determined at the 0.05 mg/kg level in most foodstuffs and tissues.

Kudsk (1964) has described a dithizone procedure for measuring mercury in air that will measure as little as 0.05 μg of mercury. With the usual sample size of 0.1 m$^3$, the detection limit would be 0.5 μg/m$^3$. This is more than adequate sensitivity for monitoring air in the working environment with the MAC levels in force. The quoted recovery rates from foodstuffs and tissues are in the range of 85–99% and the reproducibility can yield a coefficient of variation of as low as 2%. On account of its long history of use, the dithizone procedure has been used to measure mercury in virtually all types of environmental samples including air, water, food, tissues, and soils. It suffers from the disadvantage that it is time consuming and its sensitivity is not high when compared with atomic absorption procedures.

The latest developments in atomic absorption procedures have recently been reviewed by Burrows (1975). The most commonly used method in the USA is that of Hatch & Ott (1968) as modified by Uthe et al. (1970). The procedure involves oxidative digestion ("wet ashing"), followed by reduction, aeration, and measurement of mercury vapour absorption at 253.7 nm. The detection limit is approximately 1–5 ng of mercury. The wide popularity of cold vapour atomic absorption has

---

[a] The organic material may also be destroyed by combustion in an oxygen flask (Gutenmann & Lisk, 1960; White & Lisk, 1970; and Fujita et al., 1968). This allows all biological materials to be treated alike but has the disadvantage of requiring dried material.

35

resulted in a large number of publications dealing with various applications of this procedure to the measurement of mercury in sediments, soils, and biological samples (including foodstuffs). Of the 16 publications reviewed by Burrows (1975), 13 reported recoveries of 90% or more. The relative standard deviation was 10% or less in half of the published procedures, and was less than 20% in more than 90% of these procedures.

The measurement of very low levels of mercury in water samples requires some preconcentration. This may be achieved by dithizone extraction (Chau & Saiton, 1970; Thomson & McComas, 1973), by electro-deposition (Doherty & Dorsett, 1971) and by an amalgamation on silver wire (Hinkle & Learned, 1969; Fishman, 1970), in each case permitting detection limits of 1 ng/litre–10 ng/litre. Winter & Clements (1972) have described a procedure that will measure mercury in water in the range of 200 ng/litre and does not require preconcentration.

Magos (1971) has described a reduction technique that selectively determines total and inorganic mercury in biological samples without digestion of the material. This technique has been modified by Magos & Clarkson (1972) to permit determination of mercury in blood samples at the low levels found in unexposed populations (0.1–1.0 μg/100 ml). The technique has a sensitivity of approximately 0.5 ng of mercury. Recently it has been successfully applied to the measurement of total and inorganic mercury in hair samples (Giovanoli et al., 1974). The relative standard deviation was 2% and the recovery rates were quoted as being close to 100%. The technique has the advantage of high speed— each determination taking less than 2 minutes—high sensitivity, and the apparatus involved is light, portable, and suitable for field applications. Its widest application to date has been in the measurement of mercury in biological samples in the large Iraq outbreak (Bakir et al., 1973). Since the procedure does not require digestion of the biological sample, internal standards are used in each determination. The rates in this procedure must be checked for each new biological matrix.

The atomic absorption techniques referred to above are subject to interference. The most common interfering substances are benzene and other aromatic hydrocarbons that absorb strongly in the 253.7 nm region. Interference from a variety of organic solvents has been reported by Kopp et al. (1972).

The combustion–amalgamation method has undergone a series of developments to avoid difficulties due to interfering substances. Reference may be made to the work of Lidmus & Ulfvarson (1968), Okuno e⁺ (1972), and Willford (1973) who developed techniques for oxidation* biological sample, and the trapping of mercury vapour on silver or g. followed by its release into an atomic absorption measuring device. All

these methods have sensitivities down to the 1 µg/litre level and avoid the risk of interference from other substances. However, as pointed out by Burrows (1975), care must be taken in the design and operation of the combustion tube to avoid losses of volatile mercury derivatives.

In summary, a wide variety of applications of atomic absorption procedures have now been published. The technique is rapid and sensitive and the procedure is technically simple. Procedures are available for avoiding difficulties due to interfering substances. Most procedures have a detection limit in the range of 0.5–5 ng of mercury and a relative standard deviation of about 10% or less. Recovery rates are usually of the order of 95–100% depending on the technique used in the preparation of the biological sample and the rate of release of mercury from it.

Procedures for neutron activation analysis of total mercury have recently been reviewed by Wallace et al. (1971), Swedish Expert Group (1971), Westermark & Ljunggren (1972), and Burrows (1975). The method is based on the principle that when natural mercury (a mixture of stable isotopes) is exposed to a high flux of thermal (slow) neutrons, it is converted to a mixture of radioactive isotopes, principally $^{197}$Hg and $^{203}$Hg, which have decay half-lives of 65 hours and 47 days, respectively. The Sjostrand (1964) technique has been used most in the measurement of environmental samples. After the sample has been irradiated with neutrons, a precise weight of carrier mercury is added and the sample subjected to digestion and organic destruction. On completion of digestion, mercury is isolated by electrodeposition on a gold foil and the radioactivity is determined with a gamma counter. The use of carrier mercury corrects for any losses of mercury during the digestion, extraction, and isolation procedures. The limit of detection is 0.1–0.3 ng of mercury. The sample size is 0.3 g giving a concentration limit of 0.3–1 µg/kg in most biological samples. The relative standard deviation in samples of kale, fish, minerals, oil, blood, and water is less than 10%. Samuel (unpublished data) decomposed biological material irradiated with neutrons using fuming sulfuric acid and hydrogen peroxide and after the addition of hydrogen bromide, distilled the mercury as bromide together with other trace elements. This method, which is suitable for series analysis, is characterized by high recovery (96%) and good reproducibility. Trace mercury in biological and environmental materials can also be rapidly and satisfactorily determined through isolation as mercury(II) oxide or mercury(II) sulfide after digestion and clean-up procedures following neutron activation (Pillay et al., 1971; Samuel, unpublished data).

In general, the analyst is faced with three major options in the use of neutron activation procedures; (a) destruction or non-destruction of the sample, (destruction and isolation of the mercury is usually required in

samples containing less than 1 μg of mercury); (b) the choice of isotope $^{197}$Hg (if the longer-lived isotope, $^{203}$Hg, is used the sample may be allowed to stand to avoid interference from short-lived elements activated along with the mercury—however, $^{203}$Hg requires a more intense neutron flux or a longer irradiation time to achieve the same activity as the $^{197}$Hg); (c) the choice of detector (the sodium iodide (thallium) detector does not have as high a resolution as the germanium (lithium) detector, although its sensitivity is significantly higher).

Interference may come from the following elements, produced at the same time as the radioactive mercury isotopes, $^{24}$Na, $^{82}$Br, $^{32}$P, and $^{75}$Se. Interference from these isotopes may be avoided, as in the Sjostrand (1964) procedure, by chemical isolation of the radioactive isotope. However, $^{75}$Se may not be completely removed by the isolation procedures and might interfere if the sodium iodide (thallium) detector is used. The better resolution of the germanium (lithium) detector allows correction for $^{75}$Se interference through use of other lines in the $^{75}$Se spectrum. For samples containing more than 1 μg of mercury, the required selectivity can be achieved without destruction of the sample, i.e., by instrumental analysis only. One procedure is to measure the $^{203}$Hg isotope, after allowing the sample to stand for approximately one month to eliminate interference due to sodium, phosphorous, and bromine. Another procedure is to make use of the discriminating germanium (lithium) detector when the gamma irradiation from the radioactive isotope may be determined to the exclusion of most of the interfering radioactivity.

A recent non-destructive procedure for measuring mercury in coal makes use of a low-energy photon detector to estimate levels at the 100 μg/kg level with a precision of 10% (Weaver, 1973).

Burrows (1975) has recently reviewed 11 publications describing the application of neutron activation to a variety of environmental samples. Non-destructive (instrumental) determination was used in only two of these publications. In 9 of these publications the $^{197}$Hg isotope was determined. Mercury levels were reported in lake water (4 μg/litre, relative standard deviation 23%), in glacial ice (0.2 μg/kg, relative standard deviation 90%), in coal (100 μg/kg, relative standard deviation 10%), in whole blood (0.7 μg/100 ml,[a] relative standard deviation 10%), in fish (1–3 mg/kg, relative standard deviation less than 10%). Many environmental samples were measured by neutron activation, especially in Sweden, before the introduction of the atomic absorption technique (Westermark & Ljunggren, 1972).

---

[a] In this document the concentration of mercury in blood is expressed in μg/100 ml although in some original papers the values are given in μg/100 g. For practical purposes the difference of about 5% can be neglected.

Compared with other methods reviewed here, the neutron activation procedure has the following advantages: (1) high sensitivity (approximately 0.5 µg/kg); (2) no reagent blank; (3) independence from the chemical form of the element; and (4) non-destructive instrumental methods applicable to samples containing 1 µg of mercury or more. It has the disadvantages that it cannot be adapted to field use and, that if there are large numbers of samples, special radiation facilities and data processing are required. It is generally agreed that the neutron activation procedure finds its most important use as a reference method against which other procedures can be checked.

A variety of other instrumental techniques. such as X-ray fluorescence, mass spectrometry, and atomic fluorescence, for the measurement of total mercury have been reviewed by Lamm & Ruzicka (1972) and by Burrows (1975). In general, some of these methods may have a potentially higher sensitivity or selectivity for mercury. The fact is that, at the time of writing, these procedures have not yet found useful application in the measurement of mercury in environmental samples.

To summarize the present methods for the determination of total mercury in environmental samples, it would appear that the method of choice is that of flameless atomic absorption. No single procedure is appropriate, however, in all circumstances. The methods of sample handling depend upon the particular biological matrix to be analysed. Neutron activation is principally of use as a reference method against which atomic absorption methods may be checked.

### 2.3.3 Analysis of alkylmercury compounds in the presence of inorganic mercury

Techniques for the identification and measurement of alkylmercury compounds in the presence of other compounds of mercury have been reviewed recently (Swedish Export Group, 1971; Tatton, 1972; Sumino. 1975; Westöö, 1973). In general, three methods are available for the identification of alkylmercury compounds. These include (a) paper chromatography (Kanazawa & Sato, 1959; Sera et al., 1962), (b) thin layer chromatography (Johnson & Vickery, 1970; Westöö, 1966, 1967; Tatton & Wagstaffe, 1969), (c) gas–liquid chromatography (Westöö, 1966, 1967; Sumino, 1968; Tatton & Wagstaff, 1969). The paper chromatographic techniques have given way to thin-layer chromatography (TLC) for qualitative identification of the organomercurial compounds. Most quantitative work is now carried out using TLC techniques, and also gas-liquid chromatography (Westöö, 1966, 1967; Sumino. 1968; Tatton & Wagstaffe, 1969; Solomon & Uthe, 1971). However, the method of

Magos & Clarkson (1972) that selectively determines organic mercury by cold vapour atomic absorption is frequently applicable to the determination of methylmercury at levels occurring in fish and blood. Methylmercury is the only organic form of mercury present in fish. Blood samples from people exposed to methylmercury contain only inorganic mercury and methylmercury compounds. Thus the determination of organic mercury by this procedure is an accurate measure of methylmercury in these situations.

The basic procedures for samples of food, soil, and biological materials are first, homogenization of the sample, acidification by a hydrogen halide acid followed by extraction with an organic solvent, usually benzene, a clean-up step involving the conversion of the organomercurial compound to a water soluble compound usually the hydroxide or sulfate or a cysteine complex, and re-extraction with benzene. The benzene layer is now ready for analysis by thin-layer chromatography for qualitative purposes or by gas–liquid chromatography if quantitative measurements are required. A recent variant by Rivers et al. (1972) converts the organic into inorganic mercury and then makes use of cold vapour atomic absorption for final determination.

The gas–liquid chromatographic system is the one most commonly used. Problems may be encountered both in the pre-treatment of the sample and in the gas chromatographic determination itself. All these techniques involve non-destructive extraction of mercury from the sample. Thus recovery rates have to be checked for every different type of sample matrix. The efficiency of extraction of mercury is determined by both the nature of the sample matrix and the extraction procedures themselves. Von Burg et al. (1974) introduced the idea of adding a tracer amount of radioactively labelled methylmercury to the homogenate and counting the final benzene extract to check variations in the efficiency of extraction. This procedure is well worth consideration for routine use as it is most difficult to check extraction recovery rates.

Acidification of the homogenate is usually achieved by the addition of a hydrogen halide acid (usually HCl). At this point mercury(II) chloride may be added to either the homogenate or the benzene to tie up excess sulfur compounds and prevent recombination of methylmercury with sulfur. Westöö (1968) has shown that this approach may give high recovery rates but cannot be used with liver as there is a danger of methylation of the inorganic mercury. Clean-up of the first benzene extract is usually achieved by using solutions of cysteine. However, this complexing agent is subject to oxidation, particularly by substances in muds. A more suitable system in the presence of oxidizing agents is the ammonium hydroxide–sodium sulfate solution described by Westöö. No problems

are usually encountered in the reextraction of methylmercury from cysteine to benzene using 3 mol/litre hydrochloric acid. However, in the extraction procedures, volumetric errors may arise especially when the concentration of hydrochloric acid is low (1 mol/litre) and when small amounts of methylmercury are extracted from large volumes (Westöö, 1973).

In gas chromatography, the main object is to produce sharp peaks and attain high sensitivity. Tatton (1972) has noted that most commercial preparations of alkylmercury salts are not pure enough to use as standards. Sumino (1973) prepares pure methylmercury from the combination of inorganic mercury with tetramethyl lead salts. The peak is identified by electron-capture detectors using tritium or nickel as the source of beta particles. These detectors are subject to overloading and not more than 100 ng of mercury should be determined at one time (Tatton, 1972). Absolute confirmation of the identity of the peak should be made by mass fragmentation methods (Sumino, 1975).

The detection limit in the Westöö procedure is approximately 1–5 µg per kilogram of sample using a 10 g sample. The precision is 3% at the 0.05 mg/kg level for fish samples. Recovery rates are generally above 90% but do vary with the sample matrix. Solomon & Uthe (1971) developed a semimicro-method for the rapid determination of methylmercury in fish tissues. Samples of about 2 g were used. A precision of 2% was reported with recovery rates of about 99%. Samples such as blood, liver, and kidney are much more difficult to extract than fish tissues.

Thin-layer chromatography usually requires, for optimum spot size, 2 µg of mercury for each type of compound.

## 3. SOURCES OF ENVIRONMENTAL POLLUTION

The sources of mercury leading to environmental pollution have been the subject of several recent reviews (Wallace et al., 1971; D'Itri et al., 1972; Joint FAO/WHO Expert Committee on Food Additives, 1972; Heindryckx et al., 1974; Korringa & Hagel, 1974). Estimates of both natural and anthropogenic sources of mercury are subject to considerable error. In the first place the levels of mercury in environmental samples such as ice from Greenland are extremely low and close to the limit of sensitivity of the analytical methods. These low values are then converted by large multiplication factors (annual total global rainfall, $5.2 \times 10^5$ km$^3$) so as to obtain values for the global sources and turnover of mercury. Enormous fluctuations may be seen in samples such as coal and oil, which are believed to be an important anthropogenic source of mercury. Values

quoted by D'Itri (1972) indicate ranges of concentrations of mercury in crude oil varying by a factor of 1000 and ranges in coal even greater than this. Estimates of industrial production and consumption of mercury are subject to the vagaries of the economic market and in recent years to government regulation because of concern over mercury pollution. Nevertheless, despite all the assumptions and approximations in these procedures, the general picture that emerges from a variety of independent calculations is that the natural sources of mercury are at least as great as, and may substantially outweigh, the anthropogenic sources. However, man-made sources may be of considerable importance in terms of local contamination of the environment. For example, Korringa & Hagel (1974) have calculated that the man-made release of mercury in the Netherlands is 100 times greater than the release of mercury by natural degassing processes.

### 3.1   Natural Occurrence

A recent review by the Joint FAO/WHO Expert Committee on Food Additives (1972) quotes the major source of mercury as the natural degassing of the earth's crust and quotes figures in the range of 25 000–150 000 tonnes of mercury per year. These figures originate from a paper by Weiss et al. (1971) on concentrations of mercury in Greenland ice that was deposited prior to 1900. The most recent calculations on natural sources of mercury have been published by Korringa & Hagel (1974). These authors also made use of the figures of Weiss et al. (1971) to calculate the annual amount of mercury reaching the earth's surface due to precipitation of rainfall and arrived at a figure of approximately 30 000 tonnes. It was admitted that the sources of this atmospheric mercury are not yet clearly established but that volcanic gases and evaporation from the oceans are probably significant sources. It was also calculated by these authors that the run-off of mercury from rivers having a "natural mercury" content of less than 200 ng/litre would account for approximately 5000 tonnes of mercury per year. Measurements of the concentrations of mercury in air attached to aerosols (Heindryckx et al., 1974) indicate that soil dispersion to the atmosphere is not an important source of mercury.

Significant local contamination may result from natural sources of mercury. For example, Wershaw (1970) has shown that water sources located near mercury ore deposits may contain up to 80 μg/litre as compared with the levels of 0.1 μg/litre in non-contaminated sources.

## 3.2  Industrial Production

According to a recent review by Korringa & Hagel (1974), world production averaged about 4000 tonnes per year over the period 1900–1940. Production in 1968 was 8000 tonnes per year and, in 1973, attained 10 000 tonnes per year. Although considerable yearly fluctuations were noted, the average rate of increase since 1950 has been about 2% per year. Recent concern over environmental problems related to the use of mercury seems to have stabilized production rates and to have led to a dramatic fall in the price of mercury. For example, according to figures quoted by Korringa & Hagel (1974), the 1966 price was $452 per flask (a flask is 34.5 kg), the 1969 price had risen to $510.00 but by 1972 it had fallen dramatically to $202 per flask.

It is difficult to estimate the amount of mercury released into the environment as a result of the mining and smelting of this metal. High levels of mercury in lake and stream waters have been attributed to the dumping of materials and tailings (for review, see Wallace et al., 1971). It has been estimated that stack losses during smelting operations should not exceed 2–3%. Thus, based on a production figure for mercury of 10 000 tonnes in 1973, one might expect to find losses to the atmosphere of the order of 300 tonnes per year.

## 3.3  Uses of Mercury

Wallace et al. (1971) have attempted to give a picture of the use of mercury in the USA. They note that 26% of the mercury mined is not reusable. They point out, however, that at least from the theoretical point of view most of the remaining mercury (i.e. 74% of the mercury mined) is reusable. To what extent these theoretical possibilities are attained is debatable at the present moment.

Rauhut & Wild (1973) reported on the consumption and fate of mercury in the Federal Republic of Germany in 1971. Flewelling (1975) noted that the chloralkali industry, one of the largest users of mercury, has been able to cut losses in water effluent by at least 99% in the last two or three years; consequently losses from chloralkali plants now occur predominantly by emission into the atmosphere. Losses by volatilization into the atmosphere have been reduced (approximately 50%) by the introduction of cooling systems for effluent gases. Korringa & Hagel (1974) take a more pessimistic point of view and conclude that there is every reason to assume that by about 1975 all the 10 000 to 11 000 tonnes of

mercury produced per year due to mining operations will finally find its way into the environment, predominantly via the atmosphere.

Average consumption patterns for industrialized countries have been summarized by Korringa & Hagel (1974) as follows: chloralkali plants, 25%; electrical equipment, 20%; paints, 15%; measurements and control systems, such as thermometers and blood pressure meters, 10%; agriculture, 5%; dental, 3%; laboratory, 2%; and other uses including military uses as detonators, 20%. This pattern of consumption in industrialized countries is similar to that published by D'Itri (1972) for the consumption in the USA in 1968. Included in "other uses" are mercury compounds in catalysts, preservatives in paper pulp industries, pharmaceutical and cosmetic preparations, and in amalgamation processes. The use of mercury in the paper pulp industries is dramatically declining and it was banned in Sweden in 1966 (Swedish Expert Group, 1971). Hasanen (1974) has reported that no mercury compounds have been used in the paper pulp industry in Sweden and Finland since 1968.

### 3.4 Contamination by Fossil Fuels, Waste Disposal, and Miscellaneous Industries

Industrial activities not directly related to mercury can give rise to substantial releases of this metal into the environment. The most significant source is probably the burning of fossil fuels. Heindryckx et al. (1974) calculated the following approximate figures based on reports published in 1971 and 1972 (Joensuu, 1971; Cardozo, 1972): the combustion of coal and lignite, 3000 tonnes per year; the refining and combustion of petroleum and natural gas, 400 tonnes per year; the production of steel, cement, and phosphate, 500 tonnes per year. Korringa & Hagel (1974) made similar calculations from published material (Joensuu, 1971; Filby et al., 1970; Cardozo, 1972; Weiss et al., 1971). They estimated for the year 1970, an annual release of 3000 tonnes of mercury from coal burning, 1250 tonnes from mineral oil, and 250 tonnes from the consumption of natural gas. They expected that, by 1975, a total of 5000 tonnes of mercury would be emitted from burning fossil fuels.

Smelting of metals from their sulfate ores should contribute some 2000 tonnes annually and the making of cement and phosphate and other processes involving heating should have contributed another 5000 tonnes per year by 1975.

D'Itri (1972) points out that the disposal of sewage might be an important source of environmental mercury. Calculations from data in the literature indicate that somewhere between 200 and 400 kg of mercury per

million population may be released from sewage disposal units. This would amount to approximately 40–80 tonnes per year for the entire population of the USA. He further points out that sewage sludge can retain high amounts of mercury according to published studies from Sweden (6–20 mg/kg). This sludge is sometimes used as a fertilizer resulting in widespread dispersal of mercury or is sometimes heated in multiple hearth furnaces when most of the mercury would probably be released into the atmosphere. If the United States production is taken as being roughly 30% of world consumption, one might extrapolate the sewage release figure for the United States to indicate that something of the order of 1000 tonnes of mercury may be released frow sewage systems on a global scale.

The anthropogenic release of mercury has been well summarized in a recent article by Korringa & Hagel (1974) and will be briefly stated here. The total global release of mercury is taken as the sum of the global production (following their pessimistic view that all will be released into the environment) plus the release from fossil fuels and natural gas and release from non-mercury related industries.

It was calculated that by 1975 the total anthropogenic release of mercury on a global scale would be about 20 000 tonnes per year. These figures should be compared with a minimum estimated release of 25 000 to 30 000 tonnes per year from natural sources. The latter figure may, in fact, be as high as 150 000 tonnes per year, given the uncertainties in calculations on the natural global release of mercury.


## 4. ENVIRONMENTAL TRANSPORT, DISTRIBUTION, AND TRANSFORMATION

Jenson & Jernelov (1972) have suggested different types of cycle for the distribution of mercury. One cycle is global in scope and depends upon the atmospheric circulation of elemental mercury vapour. The other cycle is local and is based on an assumed circulation of volatile dimethylmercury compounds. In the global cycle most of the mercury is derived from natural sources whereas the local cycle is predominantly concerned with man-made release.


### 4.1 Distribution between Media—the Global Mercury Cycle

Recent calculations on the global circulation of mercury have been reported by Korringa & Hagel (1974). Their calculations are based

principally on data giving mercury levels in ice samples collected in Greenland and in the Antarctic as reported by Weiss et al. (1971). The circulation of mercury from natural sources was calculated using a figure of 0.06 µg of mercury per kilogram of Greenland ice samples collected prior to the year 1900. Using a reported figure for the global precipitation of water as $5.2 \times 10^5$ km³ per year, they estimated that minimum transport from the atmosphere to the earth should have been about 30 000 tonnes annually, prior to 1900. The contribution by dust particles was regarded as insignificant, an assumption now supported by the findings of Heindryckx et al. (1974). Based on a published figure of $4.1 \times 10^5$ km³ for annual precipitation over the oceans, these authors estimated the annual delivery of mercury to the oceans as 25 000 tonnes.

Korringa & Hagel (1974) also calculated the contribution of the man-made release of mercury to the atmospheric transport cycle. They assumed that 16 000 tonnes of mercury is now released per year to the atmosphere from man-made sources and that the mercury is returned to the continental land surfaces and would soon re-evaporate to the atmosphere. The 16 000 tonnes per year would eventually find its way into the oceans and thus the annual delivery to the oceans from both natural and man-made sources would be 25 000 plus 16 000 tonnes which on a proportional basis should increase the background level from the 0.06 µg/kg observed prior to the 1900s in Greenland ice to a predicted level of 0.1 µg/kg. However, they point out that since most of the man-made release is probably in the northern hemisphere, the present level in Greenland ice should be somewhat higher than 0.1 µg/kg. They note that this estimate agrees well with the observations of Weiss et al. (1971) who found present levels in Greenland ice to range from 0.09 to 0.23 µg/kg with an average of 0.125 µg/kg. Thus, from these rough estimates, it would appear that present day "background" levels in rainwater, and presumably in the atmosphere, have a substantial component related to man-made release (approximately one-third).

Observations on "background" mercury levels in the atmosphere tend to confirm the quantitative features of this global picture (Heindryckx et al., 1974). These authors assume that 50 000 tonnes are released each year from the continental land masses, that the mercury mixes up to a height of 1 km and that, in effect, the 50 000 tonnes are located over the continental land masses that account for 30% of the earth's surface.[a] The assumption of the location of this mercury over the land masses is not in contradiction with the calculations of Korringa & Hagel (1974). It assumes only that the atmosphere above the land masses is in steady state.

[a] Recent studies in Sweden cast some doubt on the validity of this assumption.

and receives 50 000 tonnes of mercury a year as evaporation and loses 50 000 tonnes per year to the atmosphere over the oceans. Their figure of 50 000 tonnes per year comes from the publication of Bertini & Goldberg (1971) and agrees well with the figure of 41 000 tonnes per year as indicated above. With these assumptions, Heindryckx et al. (1974) concluded that the background continental levels of mercury vapour plus aerosols should be 10 ng/m$^3$. The assumed mixing height of 1 km is probably the maximum level and they suggest that the actual level of mercury in air would lie between 1 and 10 ng/m$^3$. These figures are in good agreement with the published air levels as indicated in section 5.1.

Korringa & Hagel (1974) estimate the amount of mercury transported by rivers to the oceans to be 5000 tonnes per year based on quoted figures of 37 000 km$^3$ of water flow via the rivers and a natural mercury content of less than 0.2 µg/litre in river water. They note that this figure does not change substantially if one takes into account the fact that most of the mercury in river water is adsorbed to suspended matter with a mercury content of 200 500 µg/kg and that some $10^{10}$ $10^{11}$ tonnes of sediment are carried each year to the oceans. In fact river transport of mercury to the oceans may be less than 5000 tonnes per year. Heindryckx et al. (1974) noted that the concentrations of mercury in the North Sea and in the coastal areas around the North Sea were far less than would be predicted if all the mercury in the rivers entering this area were, in fact, delivered into the oceans. Presumably a considerable amount of mercury observed in river water is retained in sediments in the rivers and estuaries and does not reach the ocean by normal flow of the river. Thus it would appear that the major pathway of global transport of mercury is metallic mercury transported in the atmosphere.

An important conclusion from these calculations on the global cycle of mercury is that the concentration of mercury in the oceans should not change substantially in the foreseeable future, and that the mercury concentration in the oceans has not changed significantly since the beginning of the industrial era. The amount of mercury in the oceans has been calculated as 70 million tonnes using a figure for total ocean volume of $1.37 \times 10^9$ km$^3$ and taking the average mercury content of ocean water as 50 ng/litre. Thus contrary to what has been observed for the mercury content of the atmosphere, it will be a long time before the mercury content in sea water is significantly increased. Since water is thought to remain in the surface layers of the ocean for 10 50 years, these authors concluded that the mercury resulting from man-made activities should be well distributed in the water of all the oceans and therefore should not lead to high local concentrations.

This conclusion is consistent with the findings reported in section 5.1

that mercury levels in swordfish and tuna fish caught at the beginning of the century fall within the same range as mercury levels reported in recent catches.

The origin of mercury released by natural processes is not well established. Volcanic emissions are a possible source in view of the high concentrations of mercury vapour reported in the vicinity of volcanoes (for review, see Jonasson & Boyle, 1971). The general "degassing" of the earth's surface is probably a major source (Weiss et al., 1971). Levels of metallic mercury vapour in the atmosphere over soils rich in mercury (the humus layers of topsoil) have been reported in the range of 20–200 $ng/m^3$ as compared to background levels of 5 $ng/m^3$ according to a report by Barber et al. (quoted by Vostal, 1972). Korringa & Hagel (1974) have raised the possibility that evaporation from the oceans may make a contribution to the mercury present in the atmosphere in view of the substantial quantities of water vapour that evaporate ($4.48 \times 10^5$ $km^3$). However, it seems unlikely that mercury would evaporate at the same rate as water in view of the fact that it is believed to be in a complex form in the oceans (see section 5.1). Furthermore, the observations of Williston (1968) (referred to in section 5.1) indicate that the mercury content of the atmosphere over the oceans is considerably lower than that over land (industrialized and rural areas).

The mechanisms of volatilization of mercury from the land masses are not well understood. Presumably release of mercury from volcanoes is due to the high temperatures associated with volcanic activity. Vostal (1972) has suggested two major mechanisms, firstly the reduction of mercury in soils by a chemical process depending on the local redox potential, and secondly reduction by the activity of microorganisms. The quantitative importance of these two processes is not known. Mercury-volatilizing microorganisms are known to exist and have been identified (Magos et al., 1964; Furukawa et al., 1969; Tonomura & Kanzaki, 1969).

## 4.2    Environmental Transformation—the Local Mercury Cycle

Mercury is present naturally in the environment and released from manmade sources in a variety of chemical and physical states. The principal mercury ore is cinnabar, which is mercury sulfide. Andersson (1967) has shown that mercury in soils is complexed to the organic (humus) content. Metallic mercury may be discharged into the environment from natural sources as discussed above and also from man-made sources such as chloralkali plants. A variety of organomercurial compounds are also

discharged into the environment as a result of human activities (see section 3). Both the inorganic forms of mercury (such as metallic mercury vapour and cinnabar) and the organic forms of mercury are subject to conversion in the environment.

Jensen & Jernelov (1972) have summarized the major pathways of transformation. The inorganic forms of mercury ($Hg^0$ and HgS) undergo transformations in the environment mainly by oxidation–reduction reactions. Mercury vapour is oxidized to ionic divalent mercury ($Hg^{++}$) in water in the presence of oxygen. Concentrations as high as 40 g/litre have been attained when water saturated with oxygen was exposed to mercury vapour (Wallace et al., 1971). As pointed out by Jensen & Jernelov (1972) the oxidation of metallic mercury to inorganic divalent mercury is greatly favoured when organic substances are present in the aquatic environment.

Ionic mercury, once present in water, is capable of forming a wide variety of complexes and chelates with organic materials. Of considerable importance is its reaction with the sulfide ($S^{--}$) ion to form highly insoluble mercury(II) sulfide. This reaction is likely to occur in anaerobic aquatic environments owing to the presence of hydrogen sulfide gas. This sulfide complex of mercury is highly stable and will not normally become involved in transformation under anaerobic conditions. However, in the presence of oxygen, the insoluble mercury(II) sulfide can become oxidized to the soluble sulfite and sulfate salts of mercury which allow the metal to ionize and enter subsequent chemical reactions.

In addition to the oxidation of metallic vapour, inorganic mercury ($Hg^{++}$) can be formed by the breakdown of a variety of organic mercury compounds. The alkoxyalkylmercury compounds are very unstable in acid conditions and it has been reported (see Jensen & Jernelov, 1972) that, in humid soil (pH = 5), methoxyethylmercury has a half-life of only 3 days. Aryl- and alkylmercury compounds can all be degraded in the environment by chemical and physical processes and by biologically mediated processes.

Divalent inorganic mercury ($Hg^{++}$) can undergo two important reactions in the environment. The first is the reduction to metallic mercury vapour, a reaction that will occur in nature under appropriate reducing conditions. As mentioned above, certain bacteria, particularly of the genus *Pseudomonas*, can convert divalent mercury into metallic mercury (Magos et al., 1964; Furukawa et al., 1969). The formation of inorganic divalent mercury in nature and its reduction to metallic mercury vapour are probably key processes in the global cycle of mercury. The reduction to metallic mercury vapour must be the key step in the release of mercury because of degassing of the earth's surface. The oxidation of metallic

mercury vapour to divalent ionic mercury must be the critical step in the uptake of mercury vapour in rainwater and in the oceans. Unfortunately, other than these crude generalizations, little is known of the details of the kinetics of these processes in nature.

The second important reaction that ionic divalent mercury ($Hg^{++}$) undergoes in nature is its conversion to methylmercury and dimethyl-mercury compounds and the interconversions between these compounds. These reactions play a critical role in the so called "local cycle" of mercury and are worth further discussion. Some countries, particularly those in Scandinavia, that used methylmercury fungicides extensively, experienced a general rise in the mercury content of their agricultural products. High levels were also noted in some species of birds. The increase corresponded with the onset of the use of methylmercury fungicides. However, it was discovered that mercury levels in fish were also high and that these fish were obtained in areas where methylmercury compounds were not used (Jensen & Jernelov, 1969). It was subsequently discovered that methyl-mercury was the predominant form of mercury in fish regardless of the nature of the mercury pollutant. This was the first evidence that trans-formations of mercury compounds must occur in the environment and that, indeed, they must be of great significance. It has now been demon-strated that biological methylation of mercury occurs in the organic sedi-ments of aquaria and in sediments from freshwater and coastal waters of Sweden (Jensen & Jernelov, 1967, 1969; Jernelov, 1968).

Two biochemical pathways of methylation of mercury have been identified, one anaerobic the other aerobic. The anaerobic pathway involves the methylation of inorganic mercury by methylcobalamine compounds produced by methanogenic bacteria in a mildly reducing environment (Wood et al., 1968). The process is non-enzymic and is strictly anaerobic. The aerobic pathway has been described by Landner (1971) in studies of *Neurospora crassa*. His findings indicate that methyl-mercury bound to homocysteine becomes methylated by those processes in the cell normally responsible for the formation of methionine. In other words, the methylmercury-homocysteine complex is methylated by "mis-take".

Despite the fact that an anaerobic pathway for methylmercury produc-tion is well known, it seems unlikely that significant amounts of methyl-mercury are formed in the aquatic environment under anaerobic condi-tions. The chief reason for this, as pointed out by Jensen & Jernelov (1972), is that, in natural water when oxygen is exhausted, hydrogen sulfide is formed and divalent mercury becomes bound up as mercury(II) sulfide. In this sulfide form, mercury is not available for methylation under anaerobic conditions (Jernelov, 1968; Rissanen, quoted by Jensen &

50

Jernelov, 1972), and methylation is slow even under aerobic conditions (Fagerstrom & Jernelov, 1971).

In an aquatic environment under aerobic conditions, it must be borne in mind that the upper sedimentary layers and sedimentary particles suspended in the water may be both aerobic and anaerobic, the exterior being well oxygenated and the interior deficient in oxygen. Thus both pathways, aerobic and anaerobic, are possible routes of methylation in water that is oxygenated.

The ability to methylate mercury is not confined to a limited number of species of microorganism. Thus, conditions that promote bacterial growth in general, will lead to enhanced methylation of mercury. The highest rates of methylation in the aquatic environment are, therefore, seen in the uppermost part of the organic sediments and on suspended organic material in water (Jernelov, 1973).

The formation of dimethylmercury from monomethylmercury compounds has been shown to occur in decomposing fish (Jensen & Jernelov, 1968), and from (originally) inorganic mercury in sediments. The anaerobic pathway using methylcobalamines is one means by which dimethylmercury can be synthesized. The reaction is greatly favoured by high pH whereas the formation of monomethylmercury is favoured by a low pH environment.

The ability to methylate mercury at a high rate correlates with the resistance of the microorganism to concentrations of inorganic mercury (for review, see Jernelov, 1973).

The observations, reviewed above, of the interconversion of the various mercury compounds in nature have led to a hypothesis for a local cycle (Jensen & Jernelov, 1972). Inorganic divalent mercury is formed either by the oxidation of metallic mercury vapour by physico-chemical processes or by the cleavage of the carbon–mercury bond in organomercurial compounds either chemically or enzymatically. The divalent ionic mercury becomes attached to sediments either suspended in the water or in the sedimentary layers. The upper sedimentary layers are biologically active but it is postulated that, with the passage of time, large quantities of inorganic mercury will penetrate down to the inorganic mineral layers of the sediments where the mercury should remain inactive. In the surface layers of the sediment, part of the inorganic mercury becomes methylated. Methylation significantly increases the ability of mercury to cross biological membranes. This is why aquatic organisms contain mainly methylmercury.

If conditions of pH are appropriate, dimethylmercury will be formed. Dimethylmercury is water insoluble, possesses a very high volatility, and is postulated to diffuse from the aquatic environment into the atmosphere.

Once in the atmosphere, it is subject to removal by rainfall. If the rainwater is acidic, the dimethylmercury is converted to monomethylmercury compounds and is thereby returned to the aquatic environment completing the cycle. In the presence of mercury(II), dimethylmercury is converted to two methylmercury molecules (Jensen & Jernelov, 1969).

Key parts of this local cycle remain conjectural. It is known that dimethylmercury compounds can be formed and that the conditions for their formation can exist in an aquatic environment. Unfortunately analytical data are sparse but Bramen & Johnson (1974) have identified both mono- and dimethyl compounds in the atmosphere both outdoors and indoors in the USA. Evidence is still lacking for methylmercury compounds in rainwater. The analytical difficulties are considerable. Nevertheless the present weight of evidence supports the existence of a local cycle for the transport of mercury involving dimethylmercury as the key intermediary for the atmospheric turnover in this cycle. The observations available today on this cycle refer to local bodies of water such as lakes and rivers and the cycle itself would represent the best available explanation for the presence of methylmercury compounds in freshwater fish.

The origin of methylmercury compounds in oceanic fish has not been well described. Inorganic mercury is available in unlimited quantities in the oceans, as has been indicated in the calculations reported in section 4.1. The site of methylation of this mercury is not known. Sediment suspended in oceanic water would seem to be a prime suspect. Methylation of mercury is also known to occur in the slime covering fish but it does not occur in the fish tissues themselves (Jensen & Jernelov, 1972). It would seem an important research priority to describe the methylation pathways in ocean waters. Only then will it be possible to state whether the rate of formation of methylmercury in ocean waters and uptake in oceanic fish is related to the total deposit of mercury in the oceans (70 million tonnes) or whether it is related to a very small sub-fraction of the mercury in the oceans that may respond to man's activities more dramatically than the total ocean pool.

### 4.3 Interaction with Physical or Chemical Factors

The interaction of mercury with physical or chemical factors has been referred to frequently in the previous section, so that only a brief summary will be given here. In terms of the global distribution of mercury, such physicochemical factors as temperature, pH, redox potential, and chemical affinities for the organic materials in soil will interact to determine the degree of volatility of mercury under specific local conditions

and the rate of release of mercury from the earth's crust as elemental mercury vapour. The interplay between these factors is so complex that studies of mercury volatilization from soil and from the earth's crust, in general, do not lend themselves easily to experimental work. Once in the atmosphere, metallic mercury is liable to both physical and chemical interactions. Physically it may be adsorbed on to particulate materials in air but evidence reviewed in section 5.1 indicates that the aerosol fraction of mercury is 5% or less of the total mercury in air. Metallic mercury vapour should distribute more or less evenly between air and water providing it remains in the unoxidized metallic state (Hughes, 1957). However, the reported levels in rainwater (see section 5.1) are higher than the background level by a factor of at least 2 or 3. This is no doubt a consequence of the oxidation of metallic mercury to ionic mercury in the water in the presence of oxygen. Once deposited in the ocean from rainwater, any remaining metallic mercury should be liable to oxidation to ionic mercury whereupon it will undergo rapid chemical combination with various chemical compounds in ocean water. Sillen (1963) has estimated that the mercury may be present as negative chloride complexes (section 5.1). However, it seems probable that, because of its affinity for sulfhydryl groups, mercury will also bind strongly to living organisms in ocean waters.

Another aspect that should be considered is the relationship between mercury and selenium. Recent data indicate that selenium compounds known to detoxify mercury, increase mercury retention in some organisms changing the tissue distribution (Parizek et al., 1971). High mercury concentrations were accompanied by high selenium concentrations in tissues of several animal species (Ganther et al., 1972; Koeman et al., 1972, 1973) and also in man (Kosta et al., 1975; Byrne & Kosta, 1974). This relationship is further discussed in section 7 of this document.

In the local cycle of mercury, the same physico–chemical factors will be operative. Oxygen tension in the aquatic environment will determine the degree of formation of insoluble mercury(II) sulfide that will limit the rate of methylation. The pH of the aquatic environment and also of the rainwater will determine the distribution of the methylated forms of mercury between dimethyl and monomethyl compounds.

### 4.4  Bioconcentration

The short-chain alkylmercurials, especially methylmercury compounds, have a strong tendency to bioaccumulation since they possess a group of properties that makes them unique among the mercury com-

53

pounds. Methylmercury is very efficiently absorbed through biological membranes. In mammals, absorption of methylmercury from food is virtually complete. Methylmercury is degraded much more slowly into inorganic mercury than are the other classes of organomercurial compounds. It is excreted from living organisms much more slowly than other mercury compounds. It possesses a very high chemical affinity for the sulfhydryl group. Since this group occurs mainly in proteins in living organisms, methylmercury, once it has entered the organism, is soon converted to a non-diffusible protein-bound form. However, even though most of the methylmercury is bound to protein, a small fraction remains in a diffusible form. Methylmercury rapidly equilibrates between diffusible and non-diffusible binding sites and thus retains its mobility within animal tissues.

In view of its ability to accumulate in living organisms, one would, in general, expect to see higher concentrations of methylmercury at higher trophic levels in natural food chains. Qualitatively, this generalization appears to be true but quantitative predictions are not possible because of the complex interplay of a host of factors that influence the accumulation and movement of mercury in food chains. For example, remarkably large species differences exist in biological half-times which vary from approximately 7 days in the mouse, to 70 days in the monkey and man, 500 days in seals, and over 1000 days in some species of fish (for review, see Clarkson, 1972a).

The origin of methylmercury in terrestrial food chains is predominantly the use of mercury fungicides in the treatment of seed grain (D'Itri, 1972). The seeds are consumed by grain-eating birds or rodents and the rodents themselves become victims of the large carnivorous birds. The dramatic increase in the concentration of mercury in feathers of carnivorous birds in Sweden was associated with the introduction of methylmercury fungicides in 1940 (for review, see Swedish Expert Group, 1971). High concentrations of mercury in pheasants and other game birds are also a result of this terrestrial food chain and have led to restrictions on hunting in certain areas of North America. The replacement of methylmercury by the alkoxyalkylmercury compounds in Sweden led to a diminished level in this terrestrial food chain. Generally speaking, alkoxyalkyl- and phenylmercury compounds are either less well absorbed or more easily degraded to inorganic mercury and more rapidly excreted.

The accumulation of methylmercury compounds in aquatic food chains has been the subject of a recent review (Fagerstrom & Larsson, unpublished report). This chain or group of chains is considerably more complex than the terrestrial ones. Nevertheless, several tentative generalizations seem plausible at this time. Once methylmercury is formed in the

upper sedimentary layers or in suspended sediments in water, it readily leaves the sedimentary particle (Gavis & Ferguson, 1973). The reason for this is not fully established but Fagerstrom & Larsson suggest that it may be due to the pathway of synthesis of methylmercury compounds. For example, if methylmercury is formed by the pathway proposed by Landner (1971), it will be in the form of a diffusible complex with homocysteine. In contrast, inorganic mercury in the sediment is probably bound to large macromolecules. Once methylmercury has diffused from the sedimentary particle into the water, it must be rapidly accumulated by living organisms. This accumulation is so efficient that methylmercury has never been detected in filtered water. Fagerstrom & Larsson, in reviewing recent experimental work on methylmercury accumulation, noted that this form of mercury accumulates in all species, whether plant or animal, that possess membranes for gas exchange with their aquatic environment.

The accumulation of methylmercury in food chains in freshwater systems has been proposed as a three-step process by Fagerstrom & Larsson. The first step is an accumulation by bottom fauna that are in closest proximity to the active sedimentary layers where the methylmercury is formed. Accumulation in the bottom fauna, including plankton, would be followed by accumulation in species such as the roach and finally in the large carnivorous fish such as the northern pike. The authors point out that the relative importance of uptake of methylmercury directly from water through the gill membranes, as opposed to intake from food, should depend upon the trophic level of the fish. The higher the trophic level the more important the intake from food. However, for the overall food chain, uptake through the gills is the key process. If for some reason there is a dramatic change in the environmental layers of methylmercury, the authors predict that it would take from 10–15 years for the levels in the top predators to readjust to the new environment.

These generalizations on freshwater species should be expected to apply to oceanic fish. The remarkably high levels of methylmercury seen in swordfish and tuna fish are due to a variety of factors. First these species are large carnivorous fish at the end of a food chain. They live for a relatively long time compared with other species of fish and it is well established that methylmercury levels show a positive correlation with age (and or weight) of the fish. They are highly active fish having insatiable appetites. Because of their activity, large quantities of oceanic water pass through the gill membranes each day. Thus it is possible that tuna fish, swordfish and related species have a high intake of methylmercury both from their food supply and from the surrounding water.

Accumulation of mercury in the terrestrial and aquatic food chains

(Fagerstrom & Larsson) results in risks for man mainly through the consumption of: game birds in areas where methylmercury fungicides are in use; fish from contaminated waters, especially predator species, tuna fish, swordfish and other large oceanic fish even if caught considerably off shore; other seafoods including muscles and crayfish; fish-eating birds and mammals; and eggs of fish-eating birds.

Space does not permit a full discussion of the important questions concerning the chain of mercury transport from soil to plant to domestic animals and ultimately to man. Important parameters in this transport include absorption and availability in the soil, intake and distribution in the plant, toxic effects on the plant, and intake by domestic animals and by man. The maximum amounts tolerated in the soil may be key factors in determining the possible enrichment in food chains and the ultimate hazards to man (Koronowski, 1973; Kloka, 1974).


## 5. ENVIRONMENTAL LEVELS AND EXPOSURES

The levels of mercury in the environment have been reviewed either partially or completely by: Swedish Expert Group (1971), Joint FAO/ WHO Expert Committee on Food Additives (1972), Holden (1972), D'Itri (1972), Petersen et al. (1973), Bouquiaux (1974), and CEC (1974). The principal findings may be summarized as follows. The concentration of mercury vapour in the atmosphere is so low that it does not contribute significantly to human intake of mercury. A few "hot spots" may exist but these require further investigation. Concentrations of mercury in water, particularly drinking water, are also sufficiently low as not to contribute significantly to human exposure. The industrial release of methylmercury compounds into a sheltered ocean bay (Minamata Bay) and into a river (the Agano River) in Japan have led to extremely high concentrations of methylmercury in fish (up to 20 000 μg/kg wet weight) and resulted in human poisonings and fatalities. The industrial release of a variety of chemical and physical forms of mercury into inland waters has led to local pollution, to mercury levels in fish occasionally over 10 000 μg/ kg but usually less than 5000 μg/kg, and to the restriction of fishing for sport and commercial fishing in these areas. The mercury level in most freshwater and oceanic fish is below 200 μg/kg. However, in large carnivorous fish such as tuna, swordfish, halibut, and shark, levels are usually above 200 μg/kg and can be as high as 5000 μg/kg wet weight. The general population face no significant hazards from the consumption of methylmercury in the diet. However, certain sub-populations, either those

eating locally contaminated fish or those with an unusually high consumption of large carnivorous oceanic fish eventually develop blood levels of mercury in the range of the lowest levels associated with signs and symptoms of poisoning in the Japanese outbreak. It is estimated that the average daily intake of the general population is less than 20 μg of mercury per day in the diet. An appreciable amount of this would be methylmercury. However, individuals in certain sub-populations having unusually high exposure may ingest daily amounts of mercury of up to 200 μg, mainly as methylmercury compounds.

## 5.1   Levels in Air, Water, and Food

*Air*

The average concentration of mercury in the general atmosphere was reported by Stock & Cucuel (1934) to be 20 ng/m$^3$. These results were confirmed by Eriksson (1967) in Sweden. Sergeev (1967) noted concentrations of 10 ng/m$^3$ in the USSR. Fujimura (1964) reported concentrations of 0–14 ng/m$^3$ in non-industrialized regions of Japan. The lowest reported levels are those reported by McCarthy (1968) in Denver, USA, of 2–5 ng/m$^3$. Williston (1968) reported mercury levels in the vicinity of San Francisco, USA, of 0.5–50 ng/m$^3$, the level depending greatly on the direction of the wind. Williston's method would have detected only mercury vapour.

Levels of particle-bound mercury have also been reported. Goldwater (1964) noted that airborne dust in New York City contained from 1 to 41 ng/m$^3$ and that outdoors the concentration was from 0 to 14 ng/m$^3$. Brar et al. (1969) noted that particle-bound mercury in air above Chicago ranged from 3 to 39 ng/m$^3$. Heindryckx et al. (1974), in the most recent study, found that aerosol mercury levels corresponding to remote background levels in Norway and Switzerland were as low as 0.02 ng/m$^3$. In a heavily industrialized area of Belgium, near Liège, the aerosol mercury levels noted were as high as 7.9 ng/m$^3$. Other sampling stations in Belgium reported values roughly an order of magnitude below this. Unfortunately it is not known to what extent particle-bound mercury contributes to total mercury levels in the atmosphere. An indirect reference to Jervis by Heindryckx et al. (1974) indicates that aerosol mercury accounts for only 5% of total mercury in the atmosphere. All the particle-bound mercury reported by Heindryckx et al. (1974) had a particle size of less than 0.4 μm.

"Hot spots" of mercury concentration have been reported in atmospheres close to industrial emissions or above areas where mercury fungicides have been used extensively. Fujimura (1964) reported air levels up

to 10 000 ng/m$^3$ near rice fields where mercury fungicides had been used and values of up to 18 000 ng/m$^3$ near a busy super highway in Japan. McCarthy et al. (1970) noted air values of up to 600 and 1500 ng/m$^3$ near mercury mines and refineries. Fernandez et al. (1966) reported maximum values of 800 000 ng/m$^3$ in a village close to a large mercury mine in Spain. The remarkably high mercury vapour levels reported by these authors indicate the need for further studies into localized high concentrations of mercury in the atmosphere.

*Water*

Limited data are available for concentrations of mercury in rainwater and snow. First reported values were 50–500 ng/litre (Stock & Cucuel, 1934). Eriksson (1967) found values from 0 to 200 ng/litre. Brune (1969) noted values of approximately 300 ng/litre in rainwater in Sweden. Values for mercury in snow have been reported by Johnels et al. (1967) as 70 ng/kg and by Byrne & Kosta (quoted by Holden, 1972) as 1000–3000 ng/kg in centrifuged melted snow. It is probable that mercury levels in snow depend greatly on the collection conditions and upon how long the snow has laid on the ground. For example, Straby[a] noted values of 80 ng/kg in fresh snow but 400 500 ng/kg in snow that may have partly melted or evaporated over the winter. Analysis of ice deposited in Greenland prior to the 1900s (Weiss et al., 1971) indicates values of 60 ng/kg.

Bodies of freshwater for which there is not independent evidence for mercury contamination, contain levels of mercury of less than 200 ng/litre. Stock & Cucuel (1934) reported 10–50 ng/litre in well-water and 100 ng/litre in the River Rhine. Dall'Aglio (1968) in measurements of 300 samples from natural water in Italy found values in the range of 10–50 ng/litre. Voege (1971) reported levels up to 40 ng/litre for uncontaminated Canadian waters. Durum et al. (1971) have reported data on the concentration of mercury in surface waters of the USA. In areas where mercury mineralization was present, values of up to 200 ng/litre were seen. The results of the CEC International Symposium, reviewed by Bouquiaux (1974), indicate that the purest surface water (drinking quality) contains less than 30 ng/litre based on over 700 samples collected from drinking reservoirs in the Federal Republic of Germany. Rivers believed to have low contamination, such as the Danube, and bodies of water such as the Boden See, have values close to 150 ng/litre based on the analysis of 152 samples. The rivers in the lowland countries of Western Europe that flow

[a] STRABY, A. (1968) *Analysis of snow and water.* In: Westermark, T. & Ljunggeren, K., ed. *Development of analytical methods for mercury and studies of its dissemination from industrial sources.* Stockholm, Swedish Technical Research Council, mimeographed documents.

into the North Sea have mercury values in the range of 400–700 ng/litre no doubt reflecting the high industrialization of this area (Schramel et al., 1973). Reports by Hasselrot[a], Fonds (1971), and Smith et al. (1971a), indicate that mercury is predominantly particle-bound in contaminated water-ways. In the Federal Republic of Germany the mercury concentration measured was around 400 ng/litre in inland waters, between 100 ng and 1800 ng/litre in rivers, and 600 ng/litre in a sample of potable water. (Reichert, 1973; Schramel et al., 1973.)

Data for mercury concentrations in ocean waters are not as extensive as those reported for freshwater. Findings of Stock & Cucuel (1934) giving a mean value of 30 ng/litre were confirmed by Sillen (1963). Sillen, on the basis of physico-chemical arguments, suggested that most of the mercury in seawater would be present as negatively charged halide complexes. Hosohara (1961) noted the following levels in the Pacific Ocean: at the surface, 80–150 ng/litre; at a depth of 500 metres, 60–240 ng/litre and at a depth of 3000 metres, 150–270 ng/litre. Levels reported at the CEC International Symposium (reviewed by Bouquiaux, 1974) were 20 ng/litre in 14 samples from the English Channel but were as high as 150 ng/litre in samples taken from the Belgian shoreline and the Waddenzee in the Netherlands. Other references such as Burton & Leatherland (1971) and Leatherland et al. (1971) also support the general rule that oceanic levels are below 300 ng/litre. Higher concentrations have been produced as a result of local contamination such as in Minamata Bay where Hosohara et al. (1961) have reported values up to 600 ng/litre.

In view of questions, discussed earlier, on the total mercury content of the ocean, the stability of mercury levels in the ocean over the past 50 years, and on the high mercury levels in species of oceanic fish, the paucity of data on oceanic levels of mercury is remarkable. This would seem to be one area for future studies of environmental levels of mercury. These efforts should include attempts to analyse the different physical (particulate, or soluble) and chemical (inorganic, or methyl) forms of mercury.

*Food* (except fish)

Smart (1968) has reviewed data concerning mercury concentrations in foods and the most recent data from Europe have been summarized by Bouquiaux (1974). Mercury levels in milk products (81 samples from the Federal Republic of Germany and the United Kingdom) ranged from 0 to 40 μg/kg with a median value of 6 μg/kg. Levels in eggs (440 samples, taken from Denmark, the Federal Republic of Germany and the United

---

[a] HASSELROT, T. (1971) Mercury in fish, water, and bottomless sediments. Investigations at the research laboratories of the National Swedish Environment Protection Board (mimeographed document).

Kingdom, ranged from 0 to 100 µg/kg with most of the values between 10 and 20 µg/kg. Levels in meat, meat products, and prepared meat products (318 samples from the United Kingdom) ranged from 0 to 50 µg/kg with most values lying between 10 and 20 µg/kg. Various kinds of cereal and flour (2133 samples, taken from the Federal Republic of Germany and the United Kingdom) ranged from 0 to 20 µg/kg with most values being close to 3 µg/kg. Mercury levels in cereal products from the same countries (52 samples) ranged up to 50 µg/kg with most values close to 20 µg/kg. Vegetables and fruits (288 samples) from Belgium, the Federal Republic of Germany, and the United Kingdom had mercury levels up to 50 µg/kg with most values close to 7 µg/kg. The analysis of nearly 1400 foods, excluding fish, in Canada during 1970 showed mercury residues to be less than 60 µg/kg in bread, flour, grains, and eggs and less than 40 µg/kg in meats and vegetables (Somers, 1971).

A Swedish Expert Group (1971) has reviewed Swedish experience on the effects of widespread use of methylmercury fungicides on food levels of mercury. As a result of a ban on the use of methylmercury fungicides, food levels fell by a factor of three. For example, the mercury levels in Swedish hen eggs (whole) averaged 29 µg/kg prior to April 1966. Between October 1967 and September 1969, following the ban on methylmercury fungicides instituted in 1966, the level in Swedish hen eggs fell to 9 µg/kg.

The chemical form of mercury in foodstuffs other than fish has not been well identified. The reason is that the levels are, in general, so low as to preclude gas chromatographic identification. However, Westöö (quoted by a Swedish Expert Group, 1971) has noted that methylmercury accounts for over half the total mercury in samples of pork chop and liver, filet of beef, and egg white. Inorganic mercury can account for more than half the total mercury in pig kidney, pig brain, ox liver, and egg yolk.

*Fish*

The earliest reported mercury levels for freshwater fish are those of Stock & Cucuel (1934) and Raeder & Snekvik (1949) and range from 30 to 180 µg/kg wet weight. Upper limits for mercury levels have been quoted as, 200 µg/kg wet weight (Lofroth, 1969) for Sweden, 150 µg/kg (Sprague & Carson, 1970) for Canada, and 100 µg/kg (Ui, 1967) for Japan. These are probably to be regarded as normal levels, i.e. for fish in uncontaminated water. The WHO Regional Office for Europe (1973) has summarized references indicating that fish caught in contaminated freshwater areas may have values of 200–5000 µg/kg and, where the water is heavily polluted, values may be as high as 20 000 µg/kg.

The CEC International Symposium (Bouquiaux, 1974) quote levels in freshwater fish caught in Western Europe as ranging from 0 to 1000 µg/kg

with most values being between 200 and 400 µg/kg wet weight. Canned fish, excluding tuna taken from several Western European countries (597 samples), had values up to 500 µg/kg with an average close to 50 µg/kg wet weight. Canned tuna from the same areas (1798 samples) had values ranging up to 4000 µg/kg with most values falling into the range of 200–500 µg/kg. Salmon appears to have remarkably low levels of mercury. Measurements of some 260 samples of Atlantic Ocean, Canadian, and Baltic Sea salmon had mercury levels ranging up to 150 µg/kg with most values being close to 50 µg/kg. On the other hand, pike caught in contaminated rivers in Denmark had average mercury values of 5000 µg/kg, results which are in agreement with experiences summarized by a Swedish Expert Group (1971) in contaminated freshwater areas in Sweden and Finland. The concentration of mercury in marine fish showed marked variations. Not all the factors responsible for these variations are understood but it is generally realized that the species of fish, the geographical location, and the age and/or weight of the fish are important. The highest values of mercury are usually seen in those fish at the end of a long foodchain such as the large carnivorous species.

The concentration of mercury in marine fish has been the subject of intense study in recent years. The first measurements reported by Stock & Cucuel (1934) and Raeder & Snekvik (1941) are in agreement indicating levels from 44 to 150 µg/kg wet weight. The most recent reports (Peterson et al., 1973; Bouquiaux, 1974) indicate that mercury levels in most species of oceanic fish fall in the range of 0–500 µg/kg wet weight with most values close to 150 µg/kg wet weight (more than 1600 samples). The most important exceptions to this rule are swordfish, tuna fish, and halibut, whose values usually range from 200 to 1500 µg/kg (reviewed by the Joint FAO/WHO Expert Committee on Food Additives, 1972). Skipjack, white tuna, and yellowfin tuna (911 samples) ranged from 0 to 1000 µg/kg with most values ranging from 200 to 300 µg/kg. These samples were caught in the Atlantic, Pacific, and Indian Oceans. Bluefin tuna from the Bay of Biscay (285 samples) ranged from 200 to 800 µg/kg with most values close to 500 µg/kg. The same species caught in the Mediterranean Sea (136 samples) ranged from 500 to 2500 µg/kg with most values close to 1100 µg/kg. Big-eye tuna (20 samples from various origins) had mercury values ranging from 400 to 1000 µg/kg. Over 5200 samples of tuna, variety not specified but originating from Italy, had levels in the range of 0–1750 µg/kg with most values ranging from 300 to 500 µg/kg wet weight.

Swordfish caught in the western Atlantic (210 samples) had mercury values ranging from 50 to 4900 µg/kg with a mean value of 1150 µg/kg. 40 samples of swordfish, originating near Italy, had values ranging from 650 to 1750 µg/kg with most values close to 1100 µg/kg wet weight.

The geographical location appears to be important. This is illustrated by mercury analysis of cod (Dalgaard-Mikkelsen, 1969). Samples recovered from the strait between Denmark and Sweden, which is heavily contaminated, had values up to 1290 µg/kg; cod caught in the area of Greenland had values of 12–36 µg/kg whereas North Sea cod had values in the range of 150-195 µg/kg wet weight. Peterson et al. (1973) quote evidence that halibut caught in the southern areas of the Northern Pacific had higher mercury levels than those caught in the North. Beckett & Freeman (quoted by Peterson et al., 1973) in a study of 210 swordfish from six areas extending from the Caribbean Sea to the Grand Banks noted significant variations from one area to another in average mercury levels.

Metabolic differences may also affect mercury levels. For example, Barber et al. (1972) noted differences in mercury content in different species of benthopelagic fish despite the fact that they had identical feeding habits and ecological requirements and were exposed to mercury in the same area for the same length of time.

The age (or weight) of the fish appears to be an important determinant of mercury levels. A positive correlation between mercury concentrations and the weight of the fish has been demonstrated by Beckett & Freeman (quoted by Peterson et al., 1973) for swordfish, halibut, benthopelagic morid (Barber et al., 1972), spiny dogfish (Forrester et al., 1972), blue marlin (Rivers et al., 1972), and tuna (quoted by Peterson et al., 1973). In the last study, mercury levels were measured in 88 yellowfin tuna whose sizes ranged up to 100 kg. Tuna having weights below 25 kg had mercury levels not exceeding 250 µg/kg; tuna having body weights below 50 kg had mercury levels not exceeding 500 µg/kg. Tuna with body weights above 60 kg had values ranging up to 1000 µg/kg. However, large variations in mercury content were noted in tuna with body weights in the range of 60–100 kg. A relationship between mercury content and body weight has previously been noted for freshwater fish (Johnels, 1967; Kleinart, 1972; Bache et al., 1971).

Mercury content may also differ with the sex of the fish. For example, Forrester et al. (1972), in studies of spiny dogfish on the coast of British Columbia, noted that males had a higher mercury content than females for a given body weight. These authors suggested that this difference may be due to the fact that the males grow more slowly than the females.

Mercury in fish appears to be predominantly in the form of methylmercury. Swedish measurements of freshwater fish, summarized by a Swedish Expert Group (1971), indicated that virtually all of the mercury is present in the form of methylmercury compounds. Smith et al. (1971b) confirmed these findings for fish on the North American continent and for swordfish and tuna fish. Exceptions to this rule are Pacific marlin

caught off the coast of Hawaii where methylmercury accounts for only a small fraction of the total mercury (Rivers et al., 1972) and also lake trout where methylmercury seems to account for only 21–35% of total mercury (Bache et al., 1971).

Interpretation of the results of observations on museum specimens of tuna fish and swordfish caught at the turn of the century (Miller et al., 1972) indicates that mercury levels in these species of fish have not changed significantly throughout the twentieth century. Specimens from preserved fish of this age are necessarily limited. In seven samples of tuna reported by Miller et al. (1972), the mercury concentrations ranged from 180 to 640 μg/kg. These compare with present values in tuna ranging roughly from 200 μg/kg to over 1000 μg/kg wet weight. Given this variation, it is true to say that there is no statistically significant difference between samples caught in 1900 and those caught in 1970. However, because of the wide range of values, the data at present available do not preclude the possibility that some change may have taken place and that the change might be quite substantial.

## 5.2  Occupational Exposures (See also section 8.1.1)

Occupational exposure to elemental mercury vapour is still the principal hazard to human health when mercury is considered. More than 50 specific occupations or trades involving frequent exposure to mercury have been described by Gafafer (1966). Diseases caused by mercury or its toxic compounds are classical occupational diseases and in most countries are notifiable and qualify for compensation. Reporting of occupational poisoning by mercury has been inadequate, as is the case with all other occupational diseases, particularly in developing countries where there is evidence that large numbers of workers are exposed to high concentrations of mercury leading to poisoning. Occurrence of occupational mercury poisoning in a wide variety of industries in different parts of the world has been reported. In accordance with the information available, most people exposed to elemental mercury vapour appear to be employed in the mining industry, or in chloralkali plants (McGill et al., 1964; Ladd et al., 1966; West & Lim, 1968; Smith et al., 1970) and in the manufacturing of instruments where mercury finds application. These publications, all appearing within the last ten years, indicate that mercury levels in air may attain values as high as 5 mg/m$^3$. The highest mercury concentrations in air are reported in papers on exposure in mining operations. The concentration of mercury in urine may attain levels as high as 2175 μg/litre.

In mining for metals other than mercury (e.g. copper), mercury ore may be present in the mine and give rise to occupational exposure. Donovan (1974) has reported levels of mercury in urine samples (91 samples, number of workers not stated) ranging from 30 to 700 µg/litre in a non-mercury related mining operation. In the two years (1972–73), seven urine samples were found with mercury levels in excess of 250 µg/litre and some of the miners were admitted to hospital.

Ladd et al. (1964) have reported on occupational exposure to phenylmercury compounds. Air mercury concentrations ranged up to 0.1 mg/m³ and urinary mercury levels ranged from 1 to 788 µg/litre. A total of 67 workers were involved in these studies. Phenylmercury compounds continue to be used as fungicides in the paint industry (for review, see Goldwater, 1973) so that occupational exposure to phenylmercury compounds is still significant.

The Swedish Expert Group (1971) have summarized reports on occupational exposures to methyl- and ethylmercury compounds. All these reports were published within the period 1940–60 except for the reports on laboratory personnel published by Edwards in 1865 and 1866. Restrictions on the agricultural application of ethyl- and methylmercury compounds by various industrialized countries probably accounts for the lack of recent reports on occupational exposure.

### 5.3   Estimate of Effective Human Exposure

The daily intake of elemental mercury vapour by the general population may be calculated from the published data on ambient air levels discussed above and on the assumption that 80% of the inhaled mercury vapour is retained and that the daily ventilation in the average person is 20 m³ of air. The ambient air level, except in polluted areas, appears to be of the order of 20 ng/m³ and appears not to exceed 50 ng/m³ (see section 5.1). Assuming an ambient air level of 50 ng/m³, the average daily intake of metallic mercury vapour would amount to 1 µg/day due to inhalation. The average daily intake of those sub-groups of the general population living in specially polluted areas is difficult to estimate with any accuracy. If we use the figures of McCarthy (1970), it is possible to find mercury levels as high as 0.0015 mg/m³ close to points of emission. Individuals living continuously in these areas would have intakes of 30 µg/day. Daily intake from occupational exposure is almost impossible to estimate because of the wide variation in exposure conditions in industry (see section 5.2). Assuming that, generally, the time-weighted average threshold limit value of 0.05 mg/m³ (ACGOH, 1976) is being followed,

average occupational exposure would lead to an average daily intake of 300 µg of mercury or less, assuming a ventilation of 10 m³/day at work and 225 working days per year. The published reports are insufficient to estimate occupational daily intake from other forms of mercury. The proposed guideline of 0.1 mg/m³ for phenylmercury (MAC Committee, 1969) should lead to an intake in workers exposed to phenylmercury compounds of 500 µg/day or less.

The intake of mercury from drinking water by the general population is more difficult to estimate but it is probably very low in comparison with intake from diet. The major problem is that the chemical form of mercury in water has not always been identified and the efficiency of absorption from the gastrointestinal tract depends greatly on the form of mercury. Methylmercury compounds are absorbed almost completely whereas absorption of inorganic mercury may be 15% or less. In making the following calculations the worst case will be assumed, namely that all mercury in drinking water is methylmercury. It will also be assumed that the daily intake of water in adults is 2 litres/day (Joint FAO/WHO Expert Committee on Food Additives, 1972). Published reports indicate that pure well-water and drinking water from reservoirs have mercury levels not exceeding 50 ng/litre (see section 5.1). Thus the daily intake of mercury from drinking water would not normally exceed 0.1 µg/day. However, drinking water in certain areas may derive either from natural waters such as those reported in Italy that have levels as high as 300 ng/litre because of exposure to mineralized mercury deposits, or from rivers in heavily industrialized areas reported to have values up to 700 ng/litre (see section 5.1). Taking the highest reported figure and assuming that mercury is not removed during purification of the water, the highest daily intake would be close to 1.4 µg/day. The advised upper limit for mercury in drinking water is 1 µg/litre (World Health Organization, 1971) which would allow intakes of up to 2 µg/day from this source.

The intake of mercury from food is the most difficult of all to estimate because of the different levels of mercury in different classes of foodstuffs and different dietary habits of individuals in the general population. The one important generalization that emerges is that the intake of mercury as methylmercury is related to fish intake. Thus normal levels for intake of mercury cannot be stated in general without some reference to the fish intake of the population in question.

Over the past forty years, various estimates have been made on the intake of mercury by the general population assuming that fish intake is close to the average values for that population. These reports have been reviewed by a Joint FAO/WHO Expert Committee on Food Additives (1972) and indicate that the range of daily intake of mercury in the general

population is from 1 to 20 µg/day. The most complete reviews of dietary intake published to date are those of a Swedish Expert Group (1971) and Jonsson et al. (1972). The reports refer specifically to the Swedish population. It was noted that the intake of mercury in the diet from sources other than fish in Sweden is about 5 µg/day and that the methylmercury content is not known precisely. The median supply of methylmercury from fish is stated to be 5 µg/day or less. As fish consumption exceeds the median value for Sweden, the daily intake of methylmercury will increase in proportion. It was noted that the average daily intake of fish flesh was 30 g, that 10% of the adult men might consume between 80 and 100 g and that a few individuals may consume as much as 500 g/day.

Epidemiological studies summarized by a Swedish Expert Group (1971) indicate that in fishermen and their families, daily intakes of methylmercury can rise to values of 200 µg/day and that one individual had an unusually high intake of 800 µg/day. Another example of a Swedish fish eater with very heavy methylmercury exposure has now been published (Skerfving, 1974b).

Dietary intake of mercury in other countries is not as well documented as that in Sweden. Recent studies reported in a CEC Symposium (Bouquiaux, 1974) indicate that average dietary intake in the United Kingdom, based on total diet samples, is less than 20 µg/day. Observations on fish eating groups, such as fishermen based in American Samoa, indicate that blood mercury levels of up to 20 µg/100 ml can be obtained through fish intake (Clarkson et al., 1975). Such blood levels would be equivalent to a daily intake of between 200 and 300 µg/day of methylmercury in fish. McDuffie (1973) has reported on intakes of mercury in dieters in the United States who consume substantial amounts of tuna and swordfish. He estimated that in the 40 dieters, who had the highest daily intake of fish, 25% consumed 9–16 µg/day, that the second quartile consumed 17–26 µg/day, the third quartile consumed 27–38 µg/day and that the highest quartile consumed 40–75 µg/day. On the basis of radiochemical measurements, Diehl & Schellenz (1974) estimate the total intake of mercury with food in the Federal Republic of Germany to be between 57 and 192 µg per person per week.

Some industrial countries appear to have an average daily intake of less than 20 µg/day but sub-groups in these countries with unusually high fish intakes (dieters, fishermen's families) may have intakes rising to 75 µg/day (dieters) and even to 800 µg/day (an extremely heavy fish-eater in Sweden).

In countries depending greatly on fish as the major source of dietary protein, there is a great need for dietary studies including the measurement of mercury in the diet of these populations. Initial studies from a South

American country indicate that coastal villages have populations that are comparable to the Swedish fishermens' families in terms of daily intake of methylmercury (Turner et al., 1974).

# 6. METABOLISM OF MERCURY

## 6.1 Uptake

### 6.1.1 Uptake by Inhalation

Inhalation is the most important route of uptake for elemental mercury vapour. From what is known of the general principles governing pulmonary retention of vapours, the high diffusibility and appreciable lipid solubility of metallic mercury vapour should ensure a high rate of absorption in the alveolar regions of the lung (Task Group on Metal Accumulation, 1973). Calculations made by Nordberg & Skerfving (1972) indicate that mercury vapour should be distributed between air and body tissues in the proportion of 20 to 1 in favour of tissue deposition. Experiments on animals confirm that the major site of absorption is alveolar tissue where virtually complete absorption of the vapour takes place (Magos, 1967; Berlin et al., 1969; Hayes & Rothstein, 1962). If mercury vapour is completely absorbed across the alveolar membranes, one would expect that, owing to the physiological dead space, 80% of the inhaled vapour would be retained. This has been confirmed by observations in man where retention of the inhaled vapour was in the range of 75–85%, at mercury concentrations between 50 and 350 $\mu g/m^3$. (Teisinger & Fierova-Bergerova, 1965; Kudsk, 1965a). The retention of mercury vapour in man can be reduced by moderate amounts of alcohol (Kudsk, 1965b). Magos et al. (1973) have shown that the action of alcohol is due to the inhibition of oxidation of the vapour in the red blood cells and other tissues. More recently Magos et al. (1974) have shown that the herbicide, aminotriazole, has a similar action to that of alcohol.

No specific data are available on the monoalkylmercury compounds. However, it is generally believed that absorption is high, of the order of 80% of the inhaled amount (Task Group on Metal Accumulation, 1973). Ostlund (1969a, 1969b) reported a high retention of inhaled dimethylmercury in mice. The inorganic and organic compounds of mercury may also exist in the atmosphere in particulate form (see section 5). No detailed studies have been reported on pulmonary retention and clearance of mercury aerosols. In general, one would expect that aerosols of mercury

should follow the general physical laws governing deposition in the respiratory system.

Particulates with a high probability of deposition in the upper respiratory tract should be cleared quickly. For particulates deposited in the lower respiratory tree. longer retention will be expected, the length of which will depend on solubility, among other factors (Task Group on Lung Dynamics, 1966). Approximately 45% of a mercury(II) oxide aerosol having a mean diameter of 0.16 μm was cleared in less than 24 hours and the remainder cleared with a half-time of 33 days according to experiments on dogs by Morrow et al. (1964). Information on pulmonary retention of aerosols of the organomercurials is lacking. Pulmonary absorption of monoalkylmercury must be significant to judge from the incidents of poisoning resulting from occupational exposures to dusts and vapours of the alkylmercury fungicides. It should be noted that the gastrointestinal route may include those particulates of mercury compounds that have been cleared from the lung in the bronchociliary tract.

### 6.1.2   Uptake by ingestion

The general principles underlying the gastrointestinal absorption of mercury and its compounds are not clearly understood. Probably the formation of soluble salts and complexes is a prerequisite for absorption of metals ingested from food.

Liquid metallic mercury has long been considered to be poorly absorbed from the gastrointestinal tract. Based on the data of Bornmann et al. (1970), in animals given gram quantities by mouth, Friberg & Nordberg (1973) have calculated that less than 0.01% of an administered dose of metallic mercury was absorbed. Persons who had accidently ingested several grams of metallic mercury showed increased blood levels of mercury (Suzuki & Tanaka, 1971).

The efficiency of absorption from food depends greatly upon the type of mercury compound (Clarkson, 1972a). Studies on mice revealed that the absorption of inorganic salts of mercury from food was 15% or less in contrast with 80% or more in the case of phenyl- or methylmercury compounds. Observations on volunteers given tracer doses of inorganic mercury revealed that the efficiency of absorption was the same with both free and protein-bound mercury. The absorption from food in these volunteers was an average of about 7% (Rahola et al., 1973).

Aberg et al. (1969) and Miettinen (1973) have reported on the absorption of radioactive methylmercury compounds in volunteers given oral doses. The absorption of the administered dose was 95% irrespective of whether the methylmercury was administered as a salt dissolved in water

or in a protein-bound form. Information on the absorption in humans of other organic compounds of mercury including the other short-chain alkylmercurials is not available. As episodes of accidental poisoning due to ingestion of food contaminated with ethylmercury compounds have occurred, absorption must be significant.

The Task Group on Metal Accumulation (1973) considered the possibility that the gastrointestinal absorption of one metal may be influenced by the presence of another. Studies on animals and animal tissues (Sahagain et al., 1966, 1967) suggest the possibility that some interaction may occur between zinc, manganese, cadmium, and inorganic mercury.


### 6.1.3   Absorption through skin

Debate has persisted throughout most of the present century about the importance of skin as a route for entry of metallic mercury into the body. Early studies on man (Juliusberg, 1901) and animals (Schamberg et al., 1918), where inhalation of mercury vapour was prevented, indicated that appreciable skin absorption of metallic mercury took place. It would appear that metallic mercury can cross the skin barrier but to what extent is not known.

Studies on experimental animals reveal that inorganic salts of mercury, principally mercury(II) chloride, may be absorbed in significant amounts through skin. For example, Friberg et al. (1961) and Skog & Wahlberg (1964) indicate that 5% of mercury in a 2% water solution of mercury(II) chloride was absorbed through intact skin of guinea-pig over a 5-hour period. Such a penetration rate, if applicable to man, could result in absorption of substantial amounts of mercury under conditions of high exposure.

Friberg et al. (1961) and Wahlberg (1965) have demonstrated in guinea-pigs that methylmercury dicyandiamide was absorbed from a water solution through intact skin, the rate was more or less the same as that for mercury(II) chloride reported above. No information is available on animals with respect to ethyl- or other alkylmercury compounds.

No quantitative data are available for skin absorption of the short-chain alkylmercurials in man. People have been poisoned by administration of methylmercury compounds locally to the skin such as methylmercury thioacetamide (Tsuda et al., 1963; Ukita et al., 1963; Okinaka et al., 1964; Suzuki & Yoshino, 1969; Suzuki et al., 1970). The methylmercury compound was absorbed in sufficient amounts to cause severe poisoning although the possibility of some inhalation exposure cannot be excluded.

69

## 6.2   Distribution in the Organism

Details on the organ distribution of mercury have been recently reviewed (Clarkson, 1972a; Nordberg & Skerfving, 1972). New publications since that time have not substantially changed the general picture. Methylmercury and its homologous short-chain alkylmercurials, which are much more uniformly distributed throughout the body than are the other organomercurials, and inhaled elemental mercury vapour are distinguished from other types of mercury compound in their ability to cross the blood–brain barrier and placenta rapidly.

Organ distribution is not only affected by the type of mercury compound ingested or inhaled but also changes with time after exposure. For example, the phenylmercurials are subject to rapid conversion in the body to inorganic mercury so that the distribution of mercury following administration of these compounds and related organomercurials approaches that of inorganic mercury with increasing time after exposure (for details, see Clarkson, 1972b).

The distribution between cells and plasma (the red cell/plasma ratio) depends upon the form of mercury to which the subject is exposed. Studies on fish-eating populations reported by Birke et al. (1972) and on a heavily exposed population in Iraq (Bakir et al. 1973) indicate that the cell to plasma ratio for methylmercury is approximately 10, as was found in human volunteers given tracer doses of radioactive methylmercury (Aberg et al., 1969; Miettinen, 1973). The red cell to plasma ratio in human volunteers given radioactive inorganic mercury salts was 0.4 (Miettinen, 1973).

The distribution of mercury between hair and blood tends to follow a constant ratio in people exposed to methylmercury (Table 1). In various populations having a broad range of dietary methylmercury intake from fish, the concentration of total mercury in hair is proportional to the concentration in whole blood. The ratio of hair to blood concentration is about 250 as determined by linear regression analysis. The data in Table 1 are from populations of individuals, most of whom probably have a steady concentration of methylmercury in hair and blood. In the Iraq epidemic, hair and blood concentrations underwent rapid changes. Two cases have been reported in Iraq in which blood and hair concentrations were measured when both were declining following cessation of heavy exposure. (Amin-Zaki et al., in press.) The ratios of hair to blood concentrations were constant and the value of the ratio was close to 250. However it should be noted that, when hair and blood concentrations are changing, it is important to choose the segment of hair for analysis that corresponds to the blood sample. Depending on the length of hair seg-

ment used for analysis and the rate of growth of hair, there is a delay of about 2–4 weeks between the time of sampling the blood, and the emergence of the appropriate segment of hair above the scalp (Amin-Zaki et al., in press).

Table 1. Relationship between concentrations of mercury in samples of blood and hair in people having long-term exposure to methylmercury from fish

| No. of subjects | Whole blood $(x)$ (mg/kg) | Hair $(y)$ (mg/kg) | Linear regression | References |
|---|---|---|---|---|
| 12 | 0.004–0.65 | 1–180 | $y = 280x - 1.3$ | Birke et al. (1972) |
| 51 | 0.004–0.11 | 1–30 | $y = 230x + 0.6$ | Swedish Expert Group (1971) |
| 50 | 0.005–0.27 | 1–56 | $y = 140x + 1.5$ | Swedish Expert Group (1971) |
| 45 | 0.002–0.80 | 20–325 | $y = 260x + 0$ | Tsubaki (1971) |
| 60 | 0.044–5.5 | 1–142 | $y = 230x - 3.6$ | Skerfving (1974b) |

In people occupationally exposed to metallic mercury vapour, the red cell to plasma ratio may be as high as 2 (Lundgren et al., 1967; Suzuki et al., 1970; Einarsson et al., 1974). Work on experimental animals has shown that the ratio was higher in animals given radioactive vapour compared with those given salts of inorganic mercury.

Studies on a variety of experimental animals indicate that the kidney is the chief depository of mercury after the administration of inorganic salts and exposure to elemental mercury vapour. Over 50% of the body burden of mercury can be found in the kidneys of rats exposed to mercuric salts and metallic mercury vapour a few days after receiving the dose. This percentage may rise to 90% or more as the length of time after exposure increases (Rothstein & Hayes, 1960; Hayes & Rothstein, 1962; Trojanovska, 1966). However, it should be noted that in experimental animals, the brain levels of mercury following exposure to elemental mercury vapour were ten times higher than brain levels after equal doses of inorganic salts (Berlin et al., 1966; Magos, 1967; Nordberg & Serenius, 1969). A more uniform distribution of methylmercury throughout the body also results in much higher brain levels for a given body burden of mercury as compared with inorganic salts.

Little information is available on the distribution of mercury in human organs following exposure to elemental mercury vapour. Takahata et al. (1970) and Watanabe (1971) have reported mercury levels in the brain several times higher than those in the liver and other organs (except the kidney) of miners with long-term exposure to high concentrations of mercury vapour. These concentration ratios were maintained even several years after cessation of exposure. High mercury concentrations in the thyroid and pituitary glands in persons connected with mercury mining

have been reported (Kosta et al., 1975). It should be noted that organ distribution of mercury after inhalation of elemental mercury vapour can be dramatically affected by moderate intakes of alcohol and small doses of the herbicide aminotriazole, as shown in animals (Magos et al., 1973, 1974). These agents reduce levels in the lung and increase levels in the liver several-fold.

The percentage of the body burden of methylmercury found in the brain is much higher in primates than in other animal species (Swedish Expert Group, 1971). Observations on human volunteers given tracer doses of radioactive methylmercury (Aberg et al., 1969) indicate that 10% of the radioactivity in the whole body is located in the posterior part of the head. Probably not all of this represents methylmercury in the brain but would include methylmercury attached to the hair. Studies by Miettinen's group (quoted by a Swedish Expert Group, 1971), on volunteers given tracer doses of radioactive methylmercury, indicate that an initial rapid distribution throughout the body is followed by a further slow redistribution of methylmercury to the brain.


### 6.3 Elimination in Urine and Faeces

Urine and faeces are the principal routes of elimination of mercury from the body. The contribution of each pathway to total elimination depends upon the type of mercury compound and the time that elapses after exposure. Experiments in animals indicate that elimination of inorganic mercury by the gastrointestinal tract depends on the size of the dose and the time after exposure. The faecal route is dominant soon after exposure. The urinary route is favoured when high doses are given (Prickett et al., 1950; Friberg, 1956; Rothstein & Hayes, 1960; Ulfvarson, 1962; Cember, 1962; Phillips & Cember, 1969; Nordberg & Skerfving, 1972).

Data obtained on rats subjected to a single exposure of labelled $^{203}$Hg vapour indicated that about 4 times more mercury was eliminated in the faeces than in the urine (Hayes & Rothstein, 1962). In prolonged exposure of rats, the proportion changed in favour of urinary excretion (Gage, 1961). In workers exposed to mercury vapour, the output of mercury in urine slightly exceeded that in the faeces (Tejning & Ohman, 1966). High individual variation and great fluctuation from day to day were the principal features of urinary excretion in workers under similar exposure conditions (Goldwater et al., 1963; Jacobs et al., 1964). There is evidence that, on a group basis, urinary excretion is roughly proportional to exposure (air concentration) to elemental vapour (MAC Committee, 1969). Occupational exposure of at least 6 months, 5 days per week at average air con-

centrations of mercury of 0.05 mg/m$^3$, should lead to average urinary concentrations of mercury of about 150 µg/litre.

Piotrowski et al. (1975) have reported changes in urinary rates of excretion in workmen following exposure to elemental mercury vapour. They noted that urinary excretion could be described by a two-term exponential equation with rate constants equivalent to half-times of 2 and 70 days. The short half-time compartment accounted for about 20–30% of the excretion rate under conditions of steady-state excretion. Piotrowski et al. (1975) suggested that there is variation in urinary mercury excretion in individuals and that this can be greatly reduced by collecting the urine samples at the same time in the morning.

Mercury exhalation found in animals after exposure to the elemental vapour (Clarkson & Rothstein, 1964) has also been confirmed in man (Hursh et al., 1975). This pathway of excretion accounted for about 7% of the total excretion of mercury in volunteers following inhalation of a tracer dose. Recent observations indicate that the concentration of mercury in sweat may be sufficiently high to be taken into account in the overall mercury balance in workers exposed to elemental mercury vapour (Lovejoy et al., 1974).

The faecal route is most important in the elimination of mercury after acute or chronic dosing with methylmercury. Studies on human volunteers (Aberg et al., 1969; Miettinen, 1973) indicate that approximately 90% of the elimination takes place via the faeces. This proportion does not change with time after exposure. Concentrations of total mercury in urine showed no correlation with blood mercury in people heavily exposed to methylmercury (Bakir et al., 1973).

## 6.4 Transplacental Transfer and Secretion in Milk

The transplacental movement of mercury in women exposed to elemental mercury vapour has not been studied thoroughly.

Experiments on animals reveal that after brief (approximately 20 minutes) exposure to radioactive elemental mercury vapour, the radioactive mercury easily penetrates the placental barrier (Clarkson et al., 1972). These authors report that, after equal exposure of pregnant rats, the fetal uptake was 10–40 times higher after exposure to elemental mercury vapour than to inorganic salts. In contrast, the placental content of mercury after exposure to elemental mercury vapour was only about 40% of that after exposure to inorganic salts of mercury.

The alkylmercuric compounds have been known for some time to penetrate the placenta readily as indicated from studies on experimental animals (for review, see Swedish Expert Group, 1971). In a recent study,

73

Childs (1973) noted that the level of methylmercury in the fetus may be twice that in the maternal tissues when low levels of methylmercury are fed to rats in a tuna fish matrix. At higher dose levels, the ratio between fetal and maternal tissues becomes close to unity. Transplacental movement of methylmercury in women has been sufficient to cause several cases of prenatal poisonings in various countries (Engleson & Herner, 1952; Harada, 1968; Bakulina, 1968; Snyder, 1971; Bakir et al., 1973; Amin-Zaki et al., 1974a). Tejning (1970) has reported methylmercury levels in fetal blood cells to be 30% higher than in maternal cells in studies on women having normal pregnancies and a low to moderate fish intake. The relatively higher concentrations in fetal blood have been confirmed in a study by Suzuki et al. (1971). It was noted that the plasma levels in both types of blood were similar and that differences arose only in terms of concentrations in the red blood cells.

Information on the transplacental movement of other compounds of mercury in women is lacking. Animal experiments indicate that those compounds rapidly converted to inorganic mercury in the body, such as phenylmercury compounds, behave in this respect like inorganic mercury (for review, see Clarkson, 1972b).

Mercury has been reported in breast milk in women exposed to methylmercury from fish (Harada, 1968; Skerfving, 1974a) and from bread contaminated with methylmercury fungicides in the 1971–72 outbreak in Iraq (Bakir et al., 1973). In Iraq, it was noted that levels of total mercury in milk correlated closely with levels in whole blood and averaged 5% of simultaneous concentrations in maternal blood. The total mercury in milk consisted of two fractions identified as inorganic mercury (40%) and methylmercury (60%). Skerfving's (1973) observations on 15 lactating mothers exposed to methylmercury in fish are in general agreement with the findings in Iraq except that methylmercury accounted for only 20% of the total mercury in milk.

Despite the relatively low concentration in milk as compared with maternal blood, the suckling infants accumulated high concentrations of mercury in their blood if their mothers were heavily exposed (Amin-Zaki et al., 1974b). Some Iraqi infants, exposed only through maternal milk, had blood levels in excess of 100 µg/100 ml. In prenatally exposed infants, intake of methylmercury by suckling is one factor responsible for the slower decline in blood levels as compared with the mother (Amin-Zaki et al., 1974a).

## 6.5  Metabolic Transformation and Rate of Elimination

The most dramatic example of metabolic transformation is the conversion of metallic mercury to divalent ionic mercury in the body. This

oxidation reaction has been shown to take place *in vitro* in the red cells (Clarkson et al., 1961). More recent studies indicate that it probably takes place in most other tissues (for details, see Kudsk, 1973). The process is enzyme mediated and the catalase complex is the most likely site of biochemical oxidation (Kudsk, 1973; Magos et al., 1974).

Studies on the biotransformation of elemental mercury make it possible to develop a picture of the role of the oxidation process in the accumulation of mercury vapour in the body and its transport to the site of action (for details, see Clarkson, 1972a). Elemental mercury vapour, after inhalation, is absorbed into the blood stream. Despite the rapid oxidation that has been shown to take place in the red blood cells, some elemental mercury remains dissolved in the blood long enough for it to be carried to the blood–brain barrier and to the placenta. Its lipid solubility and high diffusibility allow rapid transit across these barriers. Tissue oxidation of the mercury vapour in brain and fetal tissues converts it to the ionic form which is much less likely to cross the blood brain and placental barriers. Thus oxidation in these tissues serves as a trap to hold the mercury and leads to accumulation in brain and fetal tissues.

Most studies on the metabolic transformation of organomercury compounds have concentrated on measurements of the rate of cleavage of the carbon–mercury bond. There is no evidence in the literature supporting the possibility of the synthesis of organomercury compounds in human or mammalian tissues.

The absolute rates of cleavage of the carbon–mercury bond in man or experimental animals is not known. The relative rates of cleavage of different mercury compounds have been estimated by measurements of the amounts of inorganic mercury deposited in tissues following single doses of organomercury compounds. In general, these studies reveal that the phenyl-(aryl) and the methoxyethyl compounds are converted rapidly to inorganic mercury in the body (for reviews, see Gage, 1974; Clarkson, 1972a). The short-chain alkylmercurials are converted more slowly to inorganic mercury with the methylmercury compounds being converted the most slowly of all. The phenyl- and methoxyethylmercurials are probably converted to inorganic mercury more or less completely within a few days whereas methylmercury can be detected in human tissue months after exposure has stopped (Bakir et al., 1973). Suzuki et al. (1973) have reported the only case in which the metabolic conversion of ethylmercury has been studied in man. Proportional values of inorganic mercury to total mercury ranging from 12 to 69% were detected in red cells, plasma, brain, spleen, liver, and kidney in a patient exposed for about 3 months to ethylmercurythiosalicylate.

The role of biotransformation in determining the toxicity of organo-

mercurials is not well understood (for discussion, see Clarkson, 1972b). The rapid conversion of phenylmercury to inorganic mercury probably accounts for the fact that, in chronic studies on animals, the effects of this organomercury compound on kidneys were similar to those of inorganic mercury (Fitzhugh et al., 1950).

The conversion of organic to inorganic mercury may increase or decrease the total rate of excretion of mercury from the body. If the intact molecule of an organomercurial is more rapidly excreted than inorganic mercury, biotransformation will decrease the overall excretion rate. This has been demonstrated in the case of the diuretic, chlormerodrin, where the intact molecule is almost completely excreted within 24 hours, but inorganic mercury remains in the animal for much longer periods (Clarkson et al., 1965). The phenyl- and methoxyethylmercury compounds are excreted at a rate similar to that of inorganic mercury according to studies on experimental animals. In the case of methylmercury, biotransformation may play an important part in determining the rate of excretion of total mercury from the body (Swensson & Ulvarson, 1968, 1969). Inorganic mercury accounts for approximately 50% of the total mercury in faeces, the principal pathway of excretion following single or chronic doses of methylmercury compounds. Methylmercury undergoes extensive enterohepatic recirculation in rats but inorganic mercury does not (Norseth & Clarkson, 1971). Thus a small rate of metabolic transformation in the liver leading to biliary excretion of inorganic mercury could make an important contribution to the faecal elimination of mercury.

### 6.6   Accumulation of Mercury and Biological Half-time ("Metabolic Model")

The body accumulates a metal when uptake exceeds elimination. At a certain stage a steady state may be reached when uptake and elimination are equal. A common way to express the accumulation is in terms of biological half-time. The biological half-time for mercury would be the time taken for the amount of mercury in the body to fall by one-half. The concept of biological half-time is meaningful, however, only if the elimination can be approximated to a single exponential first-order function. This will be true if the distribution and turnover of a metal in different tissues of the body are faster than the elimination from the body as a whole. If elimination from one organ is slow compared with that from other organs then the calculation of a biological half-time for the whole body may be completely misleading from the toxicological point of view (Task Group on Metal Accumulation, 1973; Nordberg, 1976).

Studies on experimental animals and volunteers indicate that, for methylmercury compounds, the elimination can be approximated to a single exponential first-order function (Miettinen, 1973; Aberg et al., 1969; for reviews, see Clarkson, 1972b; Swedish Expert Group, 1971; Task Group on Metal Accumulation, 1973). Observations on experimental animals indicate that the elimination of mercury after exposure to mercury vapour, inorganic mercury salts, and the phenyl and methoxyethyl compounds does not follow such a pattern and thus the accumulation and elimination of mercury ("the metabolic model") is much more complex. The pattern of elimination of these mercury compounds, when administered to animals, is dose- and time-dependent (Rothstein & Hayes, 1960; Ulfvarson, 1962; Piotrowski et al., 1969).

In cases where the elimination of a metal such as methylmercury follows a single exponential first order function, the concentration in an organ at any time can be expressed by the following equation;

$$C = C_o . e^{-b \cdot t} \tag{1}$$

$C$ = concentration in the organ at time $t$
$C_o$ = concentration in the organ at time $o$
$b$ = elimination constant
$t$ = time

The relation between the elimination constant and the biological half-time is the following:

$$T = \ln 2/b$$

$T$ = biological half-time
$\ln 2$ (natural logarithm of 2) = 0.693

If data on exposure and absorption of the metal are known, then it will be possible to predict the body burden of the metal at constant exposure over different time periods. If a constant fraction of the intake is taken up by a certain organ, the accumulated amount in that organ can also be calculated. The following expression gives the accumulated amount of metal in the total body (or organ):

$$A = (a/b)(1 - \exp(-b \cdot t)) \tag{2}$$

$A$ = accumulated amount
$a$ = amount taken up by the body (or organ) daily

At steady state the following applies:

$$A = a/b \tag{3}$$

In other words, the steady state amount in the body (or organ) $A$ is proportional to the average daily intake and inversely proportional to the elimination rate. The latter point will be taken up later (section 9) in discussing hazards to man, as large individual variations in elimination rates imply large individual variation in steady state body burden, even in people having the same average daily intake.

Equations (1), (2), and (3) are illustrated graphically in Fig. 1. During
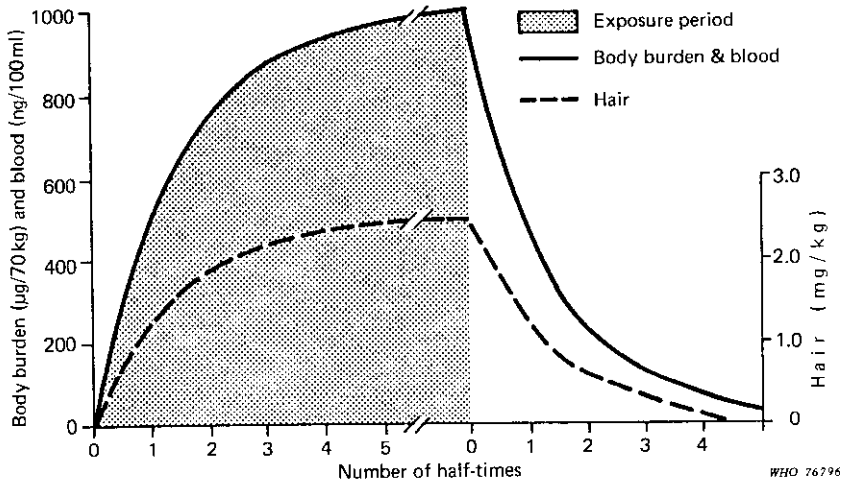


Fig. 1. The changes in the body burden and hair and blood concentrations of mercury during constant daily exposure (shaded area) and after exposure. This calculation was based on a daily intake of 10 μg of methylmercury during the exposure period, an elimination half-time of 69 days, and a hair to blood concentration ratio of 250.

the period of steady daily intake (assumed to be 10 μg/70 kg body weight), the amount in the body rises rapidly at first, reaching half its maximum (steady state) value in a time equivalent to one elimination half-time (assumed to be 69 days for methylmercury in man). After an exposure period equivalent to 5 elimination half-times (approximately one year for methylmercury), the body burden is within 3% of its final steady state value. The steady state value is one hundred times the average daily intake assuming an elimination half-time of 69 days. On cessation of exposure, the body burden will immediately begin to fall following an exponential curve that is an inverse image of the accumulation curve. Thus the body burden will have returned to within 3% of pre-exposure values in 5 half-times.

In this example, it is assumed that the hair to blood ratio is constant and equal to 250 and that 1% of the body burden is found in 1 litre of blood in a 70-kg man.

78

That this model provides a reasonable approximation to the accumulation of methylmercury in man over a wide range of daily intakes is indicated by the data in Tables 2 and 3. Data on elimination rates for the whole body reported by Aberg et al. (1969) on 5, and by Miettinen (1973) on 15 volunteers were in good agreement indicating average values close to the value of 69 days used in Fig. 1. An average value of $50 \pm 7$ days for clearance half-time from blood was reported by Miettinen (1973) in volunteers receiving a single tracer dose. Blood clearance values are difficult to measure accurately with tracer doses owing to the low counting rates in the blood samples. Skerfving (1974b) reported clearance from whole blood ranging from 58 to 87 days in 4 people having high intake (up to 5 µg/kg body weight) of methylmercury from fish, one individual had a clearance half-time of 164 days. Bakir et al. (1973) reported that patients having very high blood levels (over 100 µg/100 ml) in Iraq, had clearance half-times in the same range (45–105 days, mean 65 days). "Clearance" from hair is estimated by analysis of consecutive short (0.2–1 cm) segments of hair samples and plotting the mercury concentrations against the distance from the scalp on semilogarithmic paper (for details, see Birke et al., 1972). A straight line is usually obtained, the slope of which is equivalent to a biological half-time if the growth rate of the hair is known. "Clearance" half-times from hair are assumed to reflect clearance half-times for blood. Data from a fish eating population (daily intake up to 5 µg/kg) and on a highly exposed population in Iraq (daily intake up to 50 µg/kg) are compatible with this assumption given the wide range of individual variations (Table 2).

The relationship between steady state body burden $(A)$ and average daily intake is given by equation (3); using data derived from tracer observations on volunteers (Aberg et al., 1969; Miettinen, 1973), one

Table 2. Mercury intake and clearance

| No. of subjects | Hg intake (µg/kg/day) | Clearance half-times (days) | | | References |
|---|---|---|---|---|---|
| | | Body | Blood | Hair | |
| 5 | tracer | 70 | — | — | Aberg et al. (1969) |
| 15 | tracer | 76 (52–93) | 50 | — | Miettinen (1973) |
| 5 | up to 5 | — | — | (33–120) | Birke et al. (1972) |
| 5 | up to 5 | — | see [a] (58–164) | — | Skerfving (1974) |
| 16 | up to 50 | — | 65 (45–105) | — | Bakir et al. (1973) |
| 48 | up to 50 | — | — | 72[b] (35–189) | Shahristani & Shihab (1974) |

[a] One person had a biological half-time of 164 days. The other four were in the range of 58–87 days.

[b] The data were distributed bimodally. One group accounting for 89% of the samples had a mean value of 65 days and the other group had a mean value of 119 days.

would predict that the steady state blood level ($y$ ng/ml) is numerically equal to the average daily intake ($x$ μg/day/70 kg body weight) as indicated in Table 3. This calculation assumes a 69-day elimination half-time from the whole body, that 1% of the body burden is found in 1 litre of blood in a 70-kg "standard man". Observed steady state relationships between blood

Table 3. Relationship of steady state blood concentrations to daily intake of methyl mercury[a]

| No. of subjects | Time of exposure | Ave. Hg intake (μg/day/70 kg B.W.) | Steady blood concentration (ng/ml) | References |
|---|---|---|---|---|
| | | ($x$) | ($y$) | |
| 6 + 26[b] | years | 0–800 | $y = 0.7x + 1$ | Birke et al. (1967) |
| 139 + 26[b] | years | 0–400 | $y = 0.3x + 5$ | Tejning (1967, 1969a, 1969b, 1969c) |
| 6 + 14[b] | years | 0–800 | $y = 0.8x \cdot 1$ | Birke et al. (1972) |
| 725[c] | years | 0–800 | $y = 0.5x - 4$ | Estimated from Kojima & Araki (unpublished data) |
| 22 | years | 0–800 | $y = 0.5x \cdot 10$ | Skerfving (1974b) |
| 30[d] | 1–2 months | 0–2340 | $y = 0.8x$ | Estimated from Shahristani & Shihab (1974) and Shahristani et al. (1976) |
| 15 | single tracer dose | | $y = 1.0x$ | Estimated from Miettinen (1973) |

[a] For details of these calculations, see text.

[b] None or low fish consumers.

[c] Estimated from data on hair concentrations and daily intake. The hair to blood concentration ratio was assumed to be 250 and the average body weight of the population under study to be 60 kg.

[d] Estimated from data on hair concentrations and daily intake. The hair to blood concentration ratio was assumed to be 250.

concentration ($y$) and daily intake ($x$) are given in Table 3 for several populations. These populations consist of fishermen and their families who had had a high dietary intake of fish for many years. The range of intake between different individuals is high—up to 800 μg/day. The relationship between blood concentrations and average daily intake was found to be linear for each population studied. Linear regression analysis reveals that the observed relationship between $y$ and $x$ is less than that predicted by tracer studies. The coefficient of $x$ lies between 0.5 and 0.8 in the fish eating populations as compared with the predicted value of unity from tracer studies.

Given the difficulties in the accurate measurement of dietary intake and the uncertainty in tracer studies based on counting blood samples, it is likely that differences between the observed and the tracer values are not real. This conclusion is supported by the fact that the Iraqi populations (Table 3, Shahristani et al., 1974), having an extremely high dietary intake yielded a factor of 0.8 suggesting that the relationship between $y$ and $x$ is not substantially changed at high doses.

In summary, a considerable body of evidence exists to support the

linearity of the metabolic model for methylmercury in man. No definitive evidence is yet available that refutes this conclusion. However, we cannot exclude the possibility that the mean values of the parameters of the metabolic model could change by about a factor of two over a wide dose range. We have, however, taken the predicted value from the tracer data, since this approach would offer a greater margin of safety in estimates of hazards to human health.

Biological half-times for other mercury compounds are not well established and this is particularly true for those organs that are of toxicological importance. It seems, however, that the biological half-time for the greatest part of retained salts of inorganic mercury has an average value of about 40 days (Miettinen, 1973). In five female volunteers the average half-time was 37 days and in a similar number of males the average half-time was 48 days.

The biological half-time of both methyl and inorganic salts of mercury does not appear to be affected whether the compound is administered in an ionic form or bound to protein (Miettinen, 1973).

Limited information is available on biological half-times of mercury in the body following exposure to elemental vapour. Five volunteers inhaled radioactive mercury vapour for 10–15 minutes and were subjected to whole body counting for up to 43 days after exposure (Hursh et al., 1975). Elimination from the body followed a single exponential process having a biological half-time of 58 days with a range of individual values of 35–90 days.

As noted above, whole body elimination half-times may not be a reliable guide to accumulation in specific organs. For example, the fact that Takahata et al. (1970) and Watanabe (1971) found high mercury concentrations in the brain in an individual 10 years after cessation of exposure indicates that accumulation in the brain does not follow the same kinetics as seen by whole-body counting. Observations on experimental animals also indicate that the half-time in brain is longer than in other organs (Task Group on Metal Accumulation, 1973).


### 6.7   Individual Variations—Strain and Species Comparisons

As a general rule, the processes of absorption by inhalation or ingestion do not appear to be subject to large species and strain differences (for detailed review, see Clarkson, 1972b). Elemental mercury vapour and methylmercury compounds are well absorbed across the pulmonary epithelium and the gastrointestinal tract, respectively, in a variety of animal species. Distribution in the body tissues is subject to species

differences. A very pronounced example is the case of the red cell to plasma ratios of methylmercury where the ratio can be as high as 300 in the rat and as low as 10 in primates. Differences in red cell to plasma ratio may account for species differences in blood to brain ratios (Vostal, 1972). The blood to brain ratio has been reported to be approximately 10–20 for the rat, approximately unity for the cat, 0.5 for the dog and pig, and 0.1 for primates (from data reviewed by a Swedish Expert Group, 1971). Careful and accurate quantitative comparison of species differences and distribution is not possible because of the different experimental conditions in these studies.

The oxidation of elemental mercury vapour to ionic mercury and the cleavage of the carbon–mercury bond in a variety of mercurials has been described for several different species of animal. However the observations in this field are not adequate to allow quantitative comparison of metabolic rates of breakdown in different species.

The rate of elimination of mercury from the body is subject to wide species variation (for detailed review, see Clarkson, 1972a). In general, animals of small body weight tend to excrete mercury more rapidly than larger animals and the cold blooded species, particularly fish, appear to retain mercury for an extremely long time.

Species differences in the elimination of methylmercury have been reported. The mouse and the rat have half-times between 8 and 16 days as compared with 70 days in primates. The seal is reported to have a half-time of 500 days and fish and crustaceans appear to have half-times ranging from 400 to over 1000 days. These species differences indicate that we cannot extrapolate parameters describing the metabolic fate of mercury in animals to that in man. Furthermore, since these parameters determine the amount of mercury accumulated in the body, it would appear that quantitative information on toxicities cannot be directly extrapolated from animals to man.

The half-time of clearance of mercury from blood and hair varies considerably between individuals exposed to methylmercury (Birke et al., 1972; Skerfving, 1974). In the outbreak in Iraq the half-time of clearance from blood ranged from 40–105 days in 16 subjects (Bakir et al., 1973) and from hair the range was from 35–189 days (Shahristani & Shihab, 1974). The Iraqi data on blood samples (Bakir et al., 1973) were obtained about 1–2 months after the termination of exposure.

Average biological half-times for groups of at least 15 individuals seem to be remarkably constant. The average half-time in blood was 65 days (Bakir et al., 1973), in hair 72 days (Shahristani & Shihab, 1974), and in the whole body in 15 subjects given a tracer dose, 76 days (Miettinen, 1973).

## 7. EXPERIMENTAL STUDIES ON THE EFFECTS OF MERCURY

### 7.1 Experimental Animal Studies

#### 7.1.1 Acute studies

Little information is available on the acute toxicity of elemental mercury vapour to animals. Ashe et al. (1953) reported evidence of damage to brain, kidney, heart, and lungs in rabbits exposed to mercury vapour at a mercury concentration of 29 mg/m$^3$ of air. This concentration under the circumstances of their experiments would represent an atmosphere saturated with mercury vapour. The first effects were seen within 1 hour of exposure and subsequent severe changes resulted after longer exposures.

Information on the $LD_{50}$ (the dose of mercury that kills half the test population) has been reviewed by a Swedish Expert Group (1971). The results reported for different mercury compounds are not easily comparable since different animal species and different routes of administration have been used for the test. Nevertheless, despite all these differences, the $LD_{50}$ lies between approximately 10 and 40 mg/kg body weight for all compounds tested to date including inorganic mercury, arylmercury, alkoxyalkyl- and alkylmercury compounds. The remarkable similarity in $LD_{50}$s of these various types of mercury compound is probably due to the fact that when given in acute massive doses, mercury in whatever chemical form will denature proteins, inactivate enzymes, and cause severe disruption of any tissue with which it comes into contact in sufficient concentration. The symptoms of acute toxicity usually consist of shock, cardiovascular collapse, acute renal failure and severe gastrointestinal damage. A variety of complexing and chelating agents, all of which contain sulfhydryl groups can modify the $LD_{50}$s of mercury and its compounds (for review, see Clarkson, 1972a). These agents are most effective when given either prior to the mercury dose or in the few hours following a single dose of mercury. The importance of time of administration is to be expected since the effects of these agents are to reduce the reactivity of mercury in the body and to do so before irreversible damage has been inflicted on the tissue (see also, section 9.2).

Irreversible damage is used in this document to define cellular or organ damage that is not repaired even after the cessation of exposure; some improvement in function or in the condition of the poisoned tissue may occur but recovery is never complete. In the case of reversible damage, regeneration of cells and restoration of function takes place after the cessation of exposure.

### 7.1.2 Subacute and chronic studies

#### 7.1.2.1 *Reversible damage*

This section deals with toxic effects known to be reversible, at least up to a certain dose and/or duration of exposure. It should be noted, however, that at higher doses or longer duration of exposure, the damage can surpass the stage of reversibility.

Studies by Trahtenberg (1969) (reviewed by Friberg & Nordberg, 1973) indicate that exposure of rats to concentrations of elemental mercury vapour in the range of 0.1–0.3 mg/m$^3$ for over 100 days increased uptake of radioactive iodine by the thyroid. Friberg & Nordberg (1973) also refer to unpublished observations by Aveckaja which, under different conditions including prolonged exposure, indicate the opposite effect. Kournossov reported in 1962 (reviewed by Friberg & Nordberg, 1973) effects on the behaviour of rats at mercury concentrations in air as low as 0.005 mg/m$^3$. Studies by Armstrong et al. (1963) on pigeons showed irreversible behavioural changes only at vapour levels well in excess of recommended maximum allowable concentrations. It is clear that many more studies need to be carried out on behavioural and other subtle changes resulting from exposure to mercury vapour at these low concentrations.

Fitzhugh et al. (1950) studied the toxicity of mercury(II) chloride and phenylmercury acetate when added to the diet of rats for periods of up to 2 years. Morphological changes were induced in kidney tissue at approximately the same mercury levels for both compounds. The similarity in the effects of the two compounds on the kidney is probably due to the fact that phenylmercury compounds are rapidly converted to inorganic mercury in animal tissues.

The main effect of alkylmercurials is the irreversible action on the central nervous system. However, Lucier et al. (1971, 1972, 1973) have reported that subacute doses (with no neurological signs) caused a marked decrease in rat liver mixed-function oxidase activity. This effect was shown to be due to an increased degradation rate of cytochrome P-450 *in vivo*. Methylmercury also depressed the activity of enzymes dependent upon cytochrome P-450. Ultrastructural changes involving the endoplasmic reticulum in liver have been reported by Chang & Yamaguchi (1974) and these effects were reversible.

In rats, administration of methylmercury can produce kidney damage manifested by tubular degeneration in the distal convoluted tubules after daily doses of 10 mg/kg for 7 days (Klein et al., 1972). With lower doses of methylmercury, morphological and functional damage is produced in kidney tissue in the absence of any signs of neurological dysfunction

(Fowler, 1972a, 1972b; Magos & Butler, 1972; Klein et al., 1973). Ultra-structural studies by Fowler (1972a) showed that female rats given 2 mg/kg methylmercury in their diet were more sensitive to methylmercury than males. The primary lesion was characterized by extrusion of cyto-plasmic masses from proximal tubular cells. It has been suggested that the nephrotoxic effect is due to inorganic mercury split from methyl-mercury *in vivo* (Klein et al., 1973).

### 7.1.2.2 *Irreversible damage*

With the exception of massive doses of inorganic compounds or prolonged exposure to extremely high concentrations of elemental mercury vapour, as in the experiments of Ashe et al. referred to below, the effects of inorganic mercury on tissues are generally reversible.

Ashe et al. (1953) reported microscopically detectable changes in the organs of dogs, rabbits, and rats exposed to concentrations of elemental mercury vapour ranging from 0.1 to 30 mg/m$^3$ for different periods of time. Severe damage was noted in kidneys and brains at mercury levels in air of 0.9 mg/m$^3$ after an exposure period of about 12 weeks. After longer periods of exposure to 0.1 mg/m$^3$, no microscopically detectable effects could be seen.

The short-chain alkylmercurials are primarily neurotoxic in man. After a single dose there is a latency period of days or weeks before signs of poisoning occur. Many of the signs of methylmercury poisoning observed in man can be reproduced in animals under appropriate con-ditions. For example, Berlin et al. (1973) noted that a sudden visual disturbance occurred in monkeys given subacute doses of methylmercury. Prolonged exposure to methylmercury resulted in a gradual constriction of the visual field and impaired motor coordination and possibly sensory disturbances. Neurological signs of damage have also been produced in the mouse, rat, ferret, cat, and dog by feeding them methylmercury com-pounds (Chang et al., 1974; for review, see Swedish Expert Group, 1971). The toxicity of methylmercury to animals does not appear to be affected whether it is given to them as a pure chemical, e.g. methylmercury chloride, or whether it has accumulated naturally as in fish such as the Northern Pike (Swedish Expert Group, 1971; Albanus et al., 1972).

Grant (1973), in his studies of primates experimentally poisoned with methylmercury, reported findings confirming those of Hunter & Russell (1954). Neuronal damage and destruction was observed in the visual cortex, and the granular layer of the cerebellum. The dose-rate of methylmercury, the period of dosing, and the animal species all influence the pattern of pathological damage. For example, in monkeys after short periods of exposure to high doses, there is an abrupt visual change over

two days leading to blindness. This is accompanied by damage to the neurons in the visual cortex. Longer exposure to lower daily doses of methylmercury leads to more generalized damage to the cortex and is accompanied by gradual onset of visual changes and other signs of central nervous involvement such as ataxia.

Recent studies on rats reviewed by Somjen et al. (1973a) have confirmed the findings of Hunter et al. (1940) that the earliest neurological effects in these animals is damage to the peripheral sensory nerves. Later the disease affects other parts of the central and peripheral nervous systems. There is now evidence that the primary site of the disease is the cell bodies in the dorsal root ganglia with secondary deterioration in their fibres (Chang & Hartman, 1972a, 1972b). Consistent with this interpretation, Somjen et al. (1973b) found that the spinal dorsal root ganglia contained the highest concentrations of mercury. Electrophysiological investigations confirmed the findings drawn from morphological evidence that the cell bodies in the spinal ganglia are the primary sites of action (Somjen et al., 1973a).

Morphological, electrophysiological and biochemical changes have been demonstrated in animals prior to the onset of overt signs of poisoning. These phenomena, especially with respect to morphological changes, have been referred to as "silent damage". For example, Nordberg et al. (1971) and Grant (1973) noted that morphological damage was present in certain of the test monkeys before signs of visual impairment could be detected. Somjen et al. (1973a) reported electrophysiological manifestations of methylmercury intoxication in rats preceding clinical signs. Yoshino et al. (1966) noted a decreased uptake of amino acids in brain slices taken from animals given high doses of methylmercury at a time before signs of poisoning had appeared. Cavanagh & Chen (1971) reported that incorporation of amino acids into protein was impeded in spinal root ganglia of rats, treated with methylmercury before signs of poisoning were present. Chang & Hartman (1972c) noted damage to the blood–brain barrier as early as 12 hours after a dose of 1 mg/kg of methylmercury chloride to rats. If these observations on experimental animals may be extrapolated to man, the possibility must be considered that significant damage to the central and peripheral nervous systems may take place prior to the onset of clinical signs and symptoms.

Methylmercury compounds have been demonstrated to disturb mitosis in the plant cell, in human leucocytes treated *in vivo*, and in human cells in tissue culture. The short-chain alkylmercurials cause chromosome breakage in plant cells and point mutations in *Drosophila* (for detailed reviews, see Swedish Expert Group, 1971; Ramel, 1972).

Clegg (1971) has given a detailed review of the embryotoxicity of the

short-chain alkylmercurials. In general, animal experiments confirm the idea derived from epidemiological observations in the Minamata epidemic that much more damage was inflicted on the fetus than on the mother. Spyker et al. (1972) have recently reported on performance deficits in mice treated prenatally with methylmercury. The alkylmercury compounds may also damage the gametes prior to fertilization (Khera, 1973). Virtually no information is available on the morphological and biochemical factors related to prenatal damage in experimental animals. The results, however, do point to incipient hazards to human fetuses exposed before birth.

In discussing the biological effects of methylmercury compounds, species differences should be considered. Although man, monkeys, and pigs may become blind at high exposures, similar visual disturbances in cats were not detected (Albanus et al., 1972; Charbonneau et al., 1974). Pronounced morphological changes are seen in the peripheral nervous system of rats (Somjen et al., 1973a), but again, were not detected in cats (Albanus et al., 1972; Charbonneau, 1974). However, one cannot exclude the possibility that qualitative differences, reported in studies of different species including man, may reflect differences in degree and intensity of exposure in man and in experimental conditions in animal studies.

### 7.1.2.3  *Interactions with physical and chemical factors*

Parizek & Ostadalova (1967) reported that selenite salts could protect experimental animals against the toxic effects of inorganic mercury. Selenium also depressed the passage of inorganic mercury into fetuses and its secretion into milk (for review, see Parizek et al., 1969, 1971, 1974). Parallelism between tissue concentrations of mercury and selenium has been reported in human subjects exposed to elemental mercury vapour (Kosta et al., 1975). Several recent publications have claimed that selenite added to the diet protects experimental animals against methylmercury compounds (Ganther et al., 1972; El Bergerami et al., 1973; Potter & Metrone, 1973). Ganther & Sunde (1974) have reviewed evidence indicating that the content of selenium in tuna fish is sufficiently high to provide substantial protection against methylmercury. However, the protective factor in tuna fish, whether selenium or some other substance, has yet to be isolated.

Toxicological interactions have been reported between methylmercury and the chlorinated hydrocarbon pesticide, dieldrin. Rats dosed with both dieldrin and methylmercury, showed less morphological damage of the pars recta tubule than animals given only methylmercury. However, there was degeneration in proximal tubular cells (Fowler, 1972b). It has been demonstrated recently that phenobarbital administration increases

the biliary excretion of methylmercury compounds (Magos & Clarkson, 1973).

Estrogenic hormones (Lehotzky, 1972) and spironolactone (Selye, 1970) protect the kidney from methoxyethylmercury salts and mercury(II) chloride, respectively. The mechanism of these actions is unknown.

The question of the interaction of the physical and chemical factors on the toxicity of methylmercury is important and should be a major priority in future research studies. Extrapolation of epidemiological and toxicological data from populations in Japan and in Iraq suffering from methylmercury poisoning is fraught with difficulties when the possible interactions of local factors are not taken into account.

### 7.1.3   Biochemical and physiological mechanisms of toxicity

A physiological basis for the action of mercury and other heavy metals had already been propounded prior to 1967 (for reviews, see Hughes, 1957; Passow et al., 1961; Peters, 1963; Webb, 1966). Two general concepts on the mechanisms of action of mercury and other heavy metals are discussed in these reviews. The first dates back to the 1940s and is attributed to Peters, 1963. The toxic sequelae of heavy metal action on tissues result from a primary "biochemical lesion" whereby a critical enzyme or metabolic process is inhibited. Unfortunately, despite a considerable amount of research work (see Webb, 1966), it has not been possible to locate the biochemical lesion associated with the toxic actions of mercury.

An alternative general concept mainly proposed by Passow et al. (1961) is that the cell membrane is the first site of attack by heavy metals. Topographically, this would seem reasonable. Furthermore, the membrane is known to contain sulfhydryl groups that are essential to the normal permeability and transport properties of the cell membrane. These same sulfhydryl groups are known to have a very high affinity for mercury and its compounds. Passow et al. (1961) summarized a great many experimental studies that support this general idea. However, it must be admitted that most experimental work testing this idea is based on *in vitro* studies on isolated cells and tissues so that the role of membrane damage in the pathogenesis of heavy metal poisoning remains to be established. The effect of mercury in intercellular membranes is also of some interest.

The affinity of mercury for thiol groups in proteins and other biological molecules is far in excess of its affinity for other biologically occurring ligands (Clarkson, 1972b). As pointed out by Rothstein (1973), the affinity of mercury(II) cations for the sulfhydryl groups of proteins

creates a "severe logistics problem" for those interested in elucidating the mechanisms of action of mercurials. "Although mercurials are highly specific for sulfhydryl groups, they are highly unspecific in terms of proteins. Almost all proteins contain sulfhydryl groups that are metal-reactive. Furthermore, because most sulfhydryl groups are important in most protein functions, mercurials can disturb almost all functions in which proteins are involved. Thus almost every protein in the body is a potential target". In other words, the mercurials are potent but non-specific enzyme poisons. Mercury will inflict cellular damage wherever it accumulates in sufficient concentrations. This reasoning has given rise to the idea that the selective toxicity of mercury is related to its selective distribution. In general, there seems to be some truth in this. Inorganic mercury compounds are avidly accumulated by the kidney which is the target organ for this compound.

Studies by Somjen et al. (1973b) on the microdistribution of mercury in the nervous system also lend credence to the importance of distribution of methylmercury in that the spinal root ganglia, the site of peripheral nerve damage, are also the area of highest accumulation of methylmercury in rats.

However, it seems that distribution factors alone cannot give a complete explanation for the toxicity of methylmercury. The kidney is always the site of the highest accumulation of mercury irrespective of the form of the mercury compound involved. For example, kidney levels of methylmercury are much higher than brain levels and yet kidney damage, except in the rat, is much less than that seen in the central nervous system.

In recent years, interest has arisen in the biocomplexes of mercury in the body. The toxicity of any mercury compound will be determined by its chemical activity close to its site of action. For example the chloride salts of mercury compounds when added *in vitro* to tissue preparations are highly toxic, whereas when mercury is added in the presence of sulfhydryl compounds the toxicity is very much less (for review of this subject, see Clarkson & Vostal, 1973). Thus the chemical state of combination of mercury in plasma and other body fluids may be of primary importance in determining the particular site of action of the mercurial.

Mercury accumulated in the kidney is contained there partly in form of a metallothioneine-like complex (Jakubowski et al., 1970; Wisniewska, et al., 1970). In the rat, binding by this protein is especially effective in repeated exposure to mercury(II) chloride, owing to the induction of higher levels of the metallothioneine-like protein by mercury (Piotrowski et al., 1974a, 1974b). This form of binding probably also occurs in the case of exposure to elemental mercury vapour since this exposure results in enhancement of the metallothioneine level in the kidneys of rats (Sapota

et al., 1974). Binding of inorganic mercury in other organs may also involve storage of a similar form, as found in the liver (Wisniewska et al., 1972) and brain of the rat (Sapota et al., 1974).

The binding of mercury by metallothioneine-like protein of the kidneys is enhanced by the presence of cadmium (Shaikh et al., 1973) and therefore may play an important role in man whose kidneys accumulate, in normal conditions, considerable amounts of cadmium (Piscator & Lind, 1972). The above applies also to organic mercurials, which are rapidly converted into inorganic mercury, as in the case of phenylmercury acetate (Piotrowski & Bolanowska, 1970).

The primary biochemical lesions associated with mercury poisoning have not yet been established. Virtually nothing is known of the biochemical disturbances associated with exposure to metallic mercury vapour. Studies referred to in section 7.1.2.2 by Cavanagh & Chen (1971) and Yoshino et al. (1966) suggest that protein synthesis may undergo an early biochemical change preceding clinical signs and symptoms of methylmercury poisoning. This may give an explanation for the latent period associated with this form of mercury poisoning.

# 8. EFFECTS OF MERCURY ON MAN— EPIDEMIOLOGICAL AND CLINICAL STUDIES

## 8.1 Epidemiological Studies

### 8.1.1 Occupational exposure to mercury vapour, alkylmercury vapour and other compounds

Occupational exposures to elemental mercury vapour have been the subject of recent reviews by Friberg & Nordberg (1972, 1973) and NIOSH (1973). Many studies dating back to the 1930s have related the frequency of signs and symptoms of mercury poisoning to exposure. These studies, involving observations of more than one thousand individuals, indicate that the classical signs and symptoms of elemental mercury vapour poisoning (objective tremors, mental disturbances, and gingivitis) may be expected to appear after chronic exposure of workers to air concentrations of mercury above 0.1 mg/m$^3$ (Neal et al., 1937; Smith & Moskowitz, 1948; Smith et al., 1949; Friberg, 1951; Bidstrup et al., 1951; Vouk et al., 1950; Kesić & Heusler, 1951; Baldi et al., 1953; Seifert & Neudert, 1954; McGill et al., 1964; Ladd et al., 1966; Copplestone & McArthur, 1967; Smith et

al., 1970). The industries involved included the chloralkali industry, the manufacture of thermometers and graduated scientific glassware, the repair of DC electrical meters, the mining and milling of mercury, the manufacture of artificial jewellery, the felt hat industry and others (NIOSH, 1973). Most of the publications referred to above do not report time-weighted average exposures and few give information as to the physical and chemical forms of mercury in the atmosphere. Different methods of measurement of mercury in air were employed some of which measured only mercury vapour, while others attempted to include particulate forms of mercury. Most of the studies, if not all, assumed that exposure occurred only during the working day. However, evidence has now come to light that, in certain industries, metallic mercury may be entrapped in the clothing and contaminate the home, particularly in those industries actually handling liquid metallic mercury (West & Lim, 1968; Danzinger & Possick, 1973).

Effects of elemental mercury vapour, other than those designated as classical mercurialism, have been reported (Smith et al., 1970; Trahtenberg, reviewed by Friberg & Nordberg, 1973). The study of Smith and co-workers involved observations on 567 workers exposed to mercury in chloralkali plants. The air concentrations of mercury (measured by a mercury vapour meter) ranged from less than 0.01 to 0.27 mg/m$^3$ and time-weighted averages were calculated for each worker. A significant increase in the frequency of objective tremors was noted at mercury levels in air above 0.1 mg/m$^3$ in agreement with previous reports on occupational exposure. However, a significant increase was observed at mercury concentrations in air of 0.06–0.1 mg/m$^3$ in such non-specific signs and symptoms as loss of appetite, weight loss, and shyness.

Studies related to assessment of the occurrence of a so-called "asthenic–vegetative syndrome" or "micromercurialism" have been reported by Trahtenberg (1969). This syndrome may occur in persons with or without mercury exposure. For a diagnosis of mercury-induced asthenic vegetative syndrome Trahtenberg (1969) (reviewed by Friberg & Nordberg, 1972) required that not only neurasthenic symptoms should be present but as supporting evidence three or more of the following clinical findings; tremor, enlargement of the thyroid, increased uptake of radio-iodine in the thyroid, labile pulse, tachycardia, dermographism, gingivitis, haemotological changes, and excretion of mercury in the urine which was above normal or increased 8-fold after medication with unithiol.

Trahtenberg, in her monograph (1969) considered that this syndrome would be more frequently found in persons exposed to mercury concentrations between 0.004–12 mg/m$^3$ but, upon detailed scrutiny of her data (see review by Friberg & Nordberg, 1972), there does not seem to be any

difference between exposed and control groups that can be related to mercury exposure.

Studies on the prevalence of a similar syndrome defined as a combination of insomnia, sweating, and emotional lability have been reported by Trahtenberg, et al., quoted by Trahtenberg (1969), in workers exposed to mercury levels of 0.006–0.01 mg/m$^3$ and temperatures of 40–42°C in the summer and 28–38°C in the winter. They found 28–50% prevalence of this syndrome in this exposed group and only 13% of the same syndrome in a control group exposed to only 38–42°C temperatures. Details about the selection and evaluation of these workers are not known.

The studies of Bidstrup et al. (1951) and Turrian et al. (1956) also indicate that psychological disturbances may be seen at air concentrations of mercury below 0.10 mg/m$^3$. Thus it is impossible, at this time, to establish a lower exposure limit at which no effects occur. There is a continuous need for research studies on the effects of exposure of people to mercury vapour concentrations below 0.1 mg/m$^3$.

Short-chain alkyl compounds have been the subject of recent reviews by a Swedish Expert Group (1971) and Kurland (1973), but no new information has appeared in the literature on occupational exposures to methyl-mercury or other short-chain alkylmercury compounds. Following the description of the first two cases of occupational exposure to diethyl-mercury compounds in 1865 by Edwards (1866), occupational exposures have been infrequent and usually limited to a few individuals. For example, exposure has occurred in laboratory personnel, workers, and farmers involved in either the production of alkylmercury fungicides or their application to cereal seeds, in people in seed testing institutes, and in workers in pulp mills and saw mills. Exposure is presumed to be mainly by inhalation of the vapour or dust but it is possible that, in some cases, absorption of the liquid preparation of the fungicide may have occurred through the skin.

Occupational exposures to alkylmercury compounds, although not important numerically or epidemiologically, have been the occasion for the description of the signs and symptoms of poisoning. For example, four workmen exposed to methylmercury fungicide were the basis of the now classic reports of Hunter et al. (1940) and Hunter & Russell (1954) which gave the detailed pathology of methylmercury poisoning in man.

Occupational exposures to the phenyl- and methoxyethylmercury compounds have been the subject of a recent review (Goldwater, 1973). The hazards from industrial exposure to these compounds appear to be very low. Ladd et al. (1964), in a study of 67 workers occupationally exposed to phenylmercury compounds, found no evidence of adverse health effects. Mercury levels in air in this study were mainly below

0.1 mg/m$^3$. Comparison of readings with a mercury vapour detector and estimates of total mercury in the atmosphere revealed that elemental mercury vapour was the principal form of mercury. Goldwater (1964, 1973) makes reference to seven workers who had spent approximately six weeks preparing and packaging a batch of material containing methoxyethylmercury chloride. Four weeks after they had completed this task their whole-blood mercury levels were in the range of 34–109 µg/100 ml with an average of 65 µg/100 ml. At no time did any toxic sign or symptom appear. These workers may also have had a limited exposure to phenylmercury compounds.

At this point it is worth while to quote from the Task Group on Metal Accumulation (1973) indicating the need for carefully controlled studies of occupationally exposed groups. "There is a need in industrially exposed populations for standardized, wherever possible collaborative, epidemiological studies, where cohorts can be followed in time and where groups can be related to each other. With some occupational exposures to the less common metals, only small groups may be available for study in any one country, so that international collaboration in epidemiological studies would again be of value".

### 8.1.2   General population

Epidemics of poisoning in the general population due to exposure to phenyl- and methoxyethylmercury compounds have not been reported. Two outbreaks of poisoning due to elemental mercury vapour occurred in the 19th century, one due to a fire in the mercury mines in Idria, the other being caused by spillage of metallic mercury in a British warship in the early 1800s (Bidstrup, 1964). Fernandez et al. (1966) have reported that, in the village of Almaden, the site of the large mercury mines in Spain, air levels exceeded 0.1 mg/m$^3$. However, there are no reports as yet about the health status of the population in the village.

Methyl- and ethylmercury compounds have been the cause of several major epidemics of poisoning in the general population due either to the consumption of contaminated fish or to eating bread prepared from cereals treated with alkylmercury fungicide. The two major epidemics of methylmercury poisoning in Japan in Minamata Bay (Katsuna, 1968) and in Niigata (Niigata Report, 1967) were caused by the industrial release of methyl- and other mercury compounds into Minamata Bay and into the Agano River followed by accumulation of the mercury by edible fish. The median level of total mercury in fish caught in Minamata Bay at the time of the epidemic has been estimated as 11 mg/kg fresh weight and in

the Agano river in Niigata as less than 10 mg/kg fresh weight (Swedish Expert Group, 1971).

A recent report by Tsubaki (1971) indicates that follow-up observations on exposed people in Niigata revealed a much larger number having mild signs and symptoms than the original 46 that had been reported. These milder cases may only have had paraesthesia. By 1971 a total of 269 cases of methylmercury poisoning had been reported in Minamata and Niigata, of which 55 proved fatal. By 1974, more than 700 cases of methylmercury poisoning had been identified in Minamata and more than 500 cases had been identified in Niigata (personal communication, Tsubaki, 1975).[a] The two Japanese epidemics have been the subject of intensive studies on the effects of methylmercury on man and have resulted in important conclusions concerning dose–response relationships (Swedish Expert Group, 1971).

Epidemics resulting in the largest number of cases of poisoning and of fatalities have been caused by the ingestion of contaminated bread prepared from wheat and other cereals treated with alkyl- (methyl- or ethyl-) mercury fungicides. The largest recorded epidemic took place in the winter of 1971–72 in Iraq resulting in the admission of over 6000 patients to hospital and over 500 deaths in hospital (Bakir et al., 1973). Previous epidemics have occurred in Iraq (Jalili & Abbasi, 1961), in Pakistan (Haq, 1963), in Guatemala (Ordonez et al., 1966), and on a limited scale in other countries (Snyder, 1971). Reports on these epidemics have resulted in interesting clinical findings but quantitative studies relating exposure to effects have been reported only on the recent epidemic in Iraq (Bakir et al., 1973; Kazantzis et al., 1976; Mufti et al., 1976; Shahristani et al., 1976).

In the Iraqi outbreak, the mean methylmercury content of the wheat was 7.9 mg/kg with most samples falling between 3.7 and 14.9 mg/kg. The mean methylmercury content of wheat flour samples was 9.1 mg/kg with a range of 4.8–14.6 mg/kg in 19 samples (Bakir et al., 1973). The average weight of the home-made loaves was about 200 g with a moisture content of about 30% of the fresh weight (Damluji, 1962). The range of daily intake of bread varied widely. In an epidemiological survey of a heavily affected village, Mufti et al. (1976) reported that the average total ingested dose of a group of 426 people was about 150 mg of mercury but some people may have consumed as much as 600 mg. The average daily intake of contaminated loaves was 3.2 loaves although individual variation was large, some people eating up to 10 loaves per day. The daily intake of

<hr>

[a] It has not been possible for this group to review data on the new cases reported since the publication of the Niigata and Minamata Reports.

methylmercury would vary greatly. The average daily intake of mercury in this village would be 80 µg/kg assuming a body weight 50 kg for the population, with extremes of daily intake attaining 250 µg/kg. In the most severely affected group, reported by Bakir et al. (1974), the highest daily intake of mercury was about 130 µg/kg body weight. The average period of consumption for groups of patients reported by Bakir et al. (1973) ranged from 43–68 days. Mufti et al. (1976) reported mean consumption periods in villages to be about 32 days but some people continued for up to 3 months. Birke et al. (1972) and Skerfving (1974b) have reported on
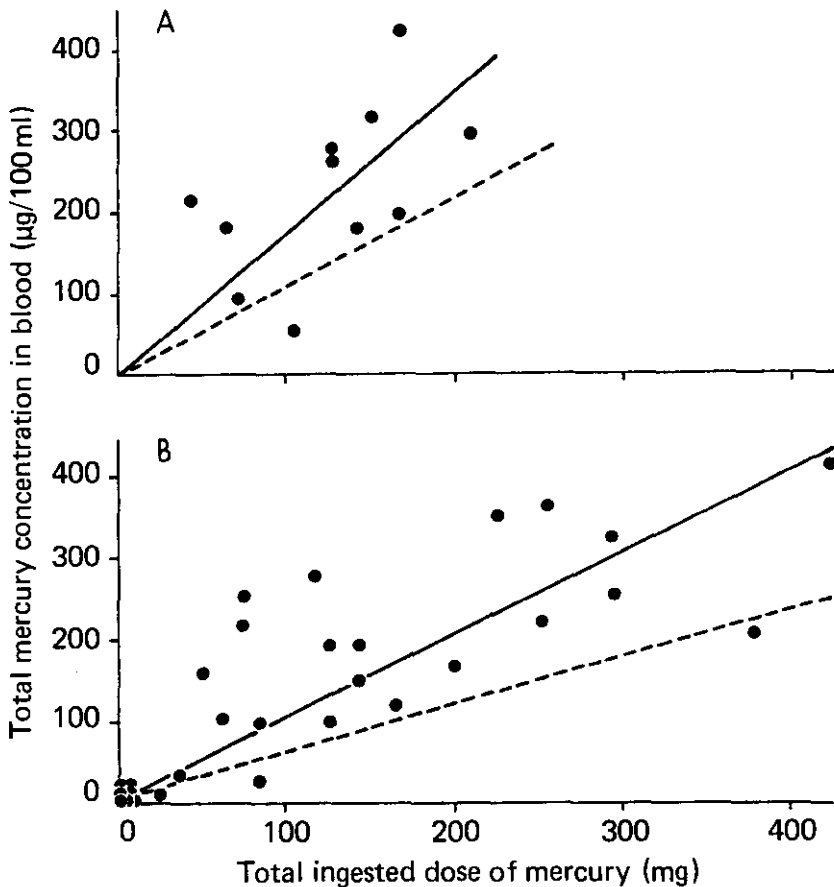


Fig. 2. The relationship between the concentration of the total amount of mercury in the blood and the estimated total amount of mercury ingested from contaminated bread. The solid line was drawn from linear regression analysis. The dotted line is the predicted concentration in the blood estimated from published data by Miettinen (1973) on 15 volunteers given a single oral dose of labelled methylmercury. (A) Patients aged 10–15 years. (B) Patients over 18 years of age. From *Mercury, Mercurials and Mercaptans, 1973*. Courtesy of Charles C. Thomas, Publisher, Springfield, Illinois.

families in Sweden consuming fish containing mercury levels of 0.3–7 mg/kg. Daily intake ranged up to approximately 5 µg/kg body weight. In two cases, intake was as high as 10–20 µg/kg. The highest recorded blood level of mercury was 1.2 µg/g of red cells or approximately 60 µg/100 ml of whole blood. A total of 188 people were referred to in these studies. No signs or symptoms of poisoning attributable to methylmercury were noted.

Clarkson et al. (1975) and Marsh et al. (1974) have offered preliminary information on 163 fishermen based in American Samoa who ingested unusually high amounts of fish containing methylmercury. Data on daily intake of mercury were not reported but the mercury levels in blood in this population ranged as high as 28 µg/100 ml. No signs or symptoms of poisoning could be ascribed to methylmercury.

Turner et al. (1974) have reported on neurological examinations of 186 persons living in two fishing villages in northern Peru. Concentrations
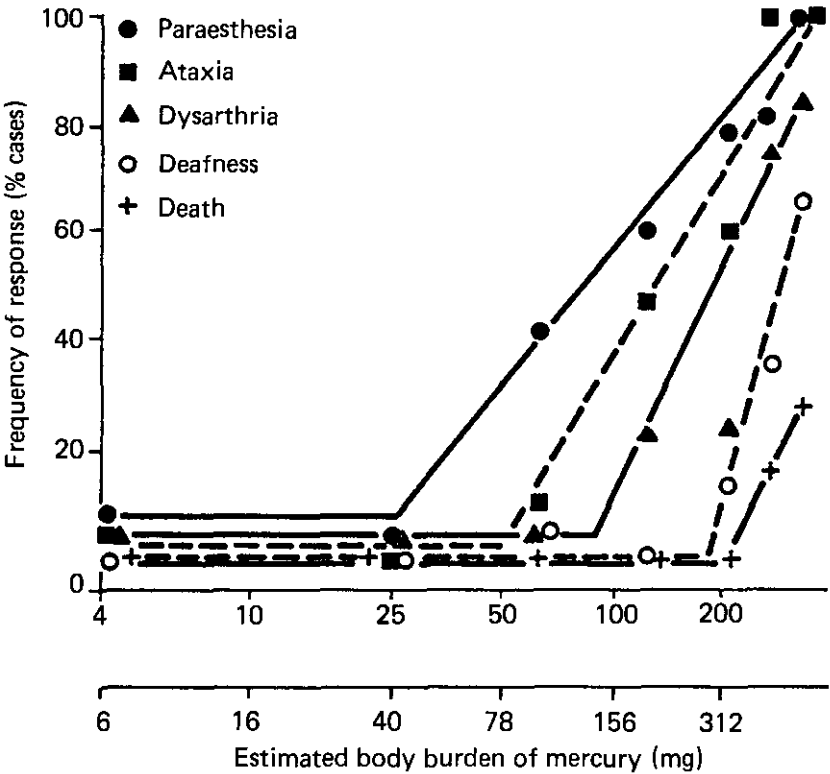


Fig. 3. The relationship between frequency of signs and symptoms and the estimated body burden of methylmercury as reported by Bakir et al. (1973). Both scales of the abscissa refer to body burdens of methylmercury (mg) at the cessation of exposure. The two scales represented different methods of calculating the body burden as discussed in the text (section 8). Copyright 1973 by the American Association for the Advancement of Science.

96

of total mercury, methyl- and inorganic mercury were measured in blood samples from 141 of these villagers. The concentration range of methylmercury in blood was from 1.1 to 27.5 $\mu g$/100 ml with a mean of 8.9 $\mu g$/100 ml. The mean intake of fish was approximately 10 kg per family per week and the average family size was six.

Fifty-one persons from a "control" village were also examined. The mean intake of fish was 1.0 kg per family per week and the mean family size was 6.4. Methylmercury concentrations in blood averaged 0.99 $\mu g$/100 ml with a range of 0.33–2.5 $\mu g$/100 ml. No correlation was observed between blood levels of methylmercury and the frequency of signs and symptoms usually associated with methylmercury poisoning (paraesthesia, ataxia of gait and limbs, impaired vision, and deafness).

Paccagnella et al. (1974) have reported blood concentrations of mercury in a community in the Mediterranean island of S. Peitro (Cagliari). The average dietary weekly intake of fish was 300 g. Tuna fish, with an average total content of mercury of 1.23 mg/kg and a methylmercury content of 0.92 mg/kg was an important dietary item. Other fish in their diet
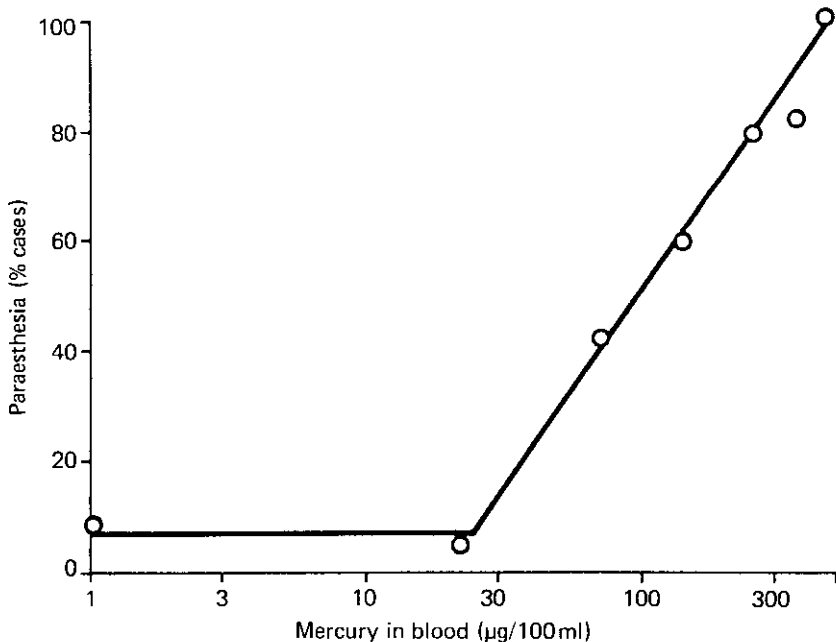


Fig. 4. The frequency of paraesthesia as a function of the concentration of mercury in blood, 65 days after cessation of exposure. The graph uses data from Table 4 of Bakir et al. (1973). The mean blood concentrations are computed as the logarithmic means for each cohort in their table. The line connecting the first two points was assumed to be horizontal. The line connecting the other points was computed by least squares linear regression analysis. Copyright 1973 by the American Association for the Advancement of Science.

had an average total mercury concentration of 0.33 mg/kg. The concentration of total mercury in blood was measured in 115 people aged from 10 years upwards. The mean mercury concentration in blood in the males was approximately 8 μg/100 ml and the maximum recorded concentration was 23 μg/100 ml. The females had average mercury levels in blood of 6 μg/100 ml with a maximum level of 24 μg/100 ml. Three individuals in the sample population of 115 had blood levels higher than 20 μg/100 ml.

Epidemiological studies show that these exposed populations may be classified into three categories distinguished by intensity and duration of exposure to the short-chain alkylmercurials. Populations consuming contaminated grain had a high daily intake of mercury (reaching over 200 μg/kg for brief periods averaging 1 2 months). The outbreaks in Japan fall into the second category where daily mercury intakes ranged from 5 to 100 μg/kg with a median of 30 μg/kg/day with exposure times lasting from several months to years, in Niigata, although doubts on the accuracy
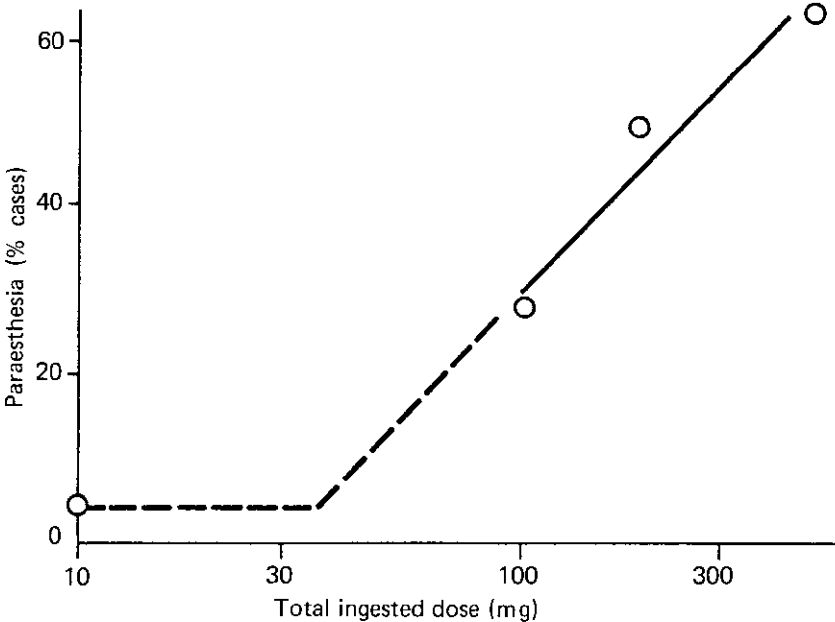


Fig. 5. The relationship between frequency of paraesthesia and the total ingested dose of methyl-mercury-data from Mufti et al. (1976). Each loaf contained 1.4 mg of mercury as methylmercury according to Bakir et al. (1973). The broken lines are extrapolations. The horizontal line is taken to represent background frequency of paraesthesia—using the figure of 4% as given in Mufti et al. (1976)—for the group receiving a total of 1–49 loaves. The line connecting points at higher ingested doses was drawn by least squares linear regression analysis and was extrapolated to intersect the horizontal line.

of those figures have been expressed by a Swedish Expert Group (1971). The third category includes populations having unusually high fish intakes for years if not for most of their lives, such as in parts of Sweden (Skerfving, 1974) the Samoan fishermen (Marsh et al., 1974), and in Peru (Turner et al., 1974). A small proportion of those in this category may attain mercury levels up to 5 μg/kg/day or even higher. Quantitative studies relating frequency of signs and symptoms to various indices of exposure have been reported for all three categories so that it is now possible to compare the effects of differences in intensity and duration.

Before discussing these studies, it would be well to point out some of the attendant difficulties. Methylmercury and the other short-chain alkyl-mercurials produce effects unique among the mercury compounds. However, some, if not all, of these effects can be caused by agents other than mercury or by certain disease states. Thus, in examining the population for neurological changes, it must be borne in mind, that there could be, at least in theory, many causes of the observed neurological effects other than methylmercury itself. Dose–response relationships derived from these epidemiological studies imply a cause–effect relationship. In fact, the only proof we have that methylmercury caused certain effects in these populations is that (1) these effects coincide in time with exposure to methylmercury, (2) the frequency of these effects in a given population increases with increasing exposure to methylmercury, and (3) the major
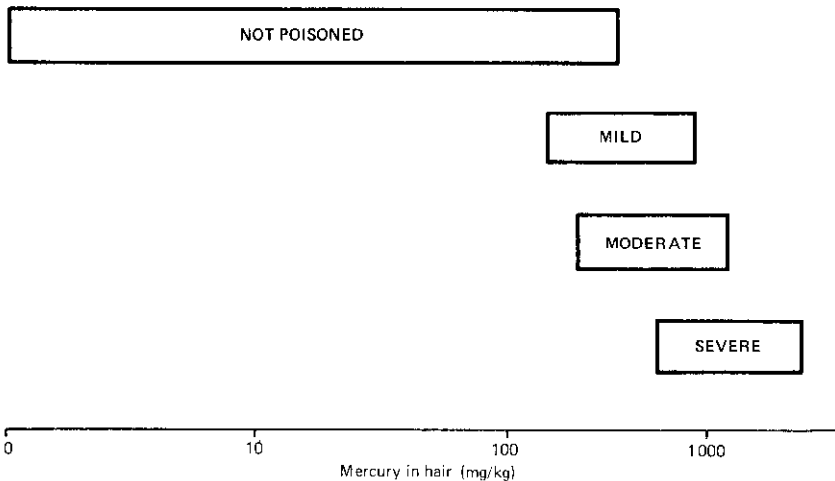


Fig. 6. This diagram is based on data reported by Shahristani et al. (1976) on an Iraqi population that consumed contaminated bread. Hair samples were divided into 1 cm segments and the concentration of mercury measured in each segment by neutron activation analysis. The mercury concentration in the segment with the highest concentration is compared to the severity of signs and symptoms of methylmercury poisoning. The length of each box is the range of maximum mercury concentrations in hair.

signs have been reproduced in some animal models. One of the key problems in these studies is to distinguish between the background frequency of a sign or symptom and the increase in that frequency due to increased exposure to methylmercury.

The studies in the Iraqi population (typical of category one) were made 2–3 months after cessation of exposure and in most cases, sometime after the onset of signs and symptoms in the patients. Thus the investigators were faced with the problem of recapitulation of exposure and of determining the dose received by the individual.



Fig. 7. Concentration of mercury in samples of blood collected from patients suffering from methylmercury poisoning in the Niigata outbreak. Samples from the same patients are connected by a straight line. The arrow indicates the estimated time of onset of symptoms. Data is taken from a report of a Swedish Expert Group (1971).

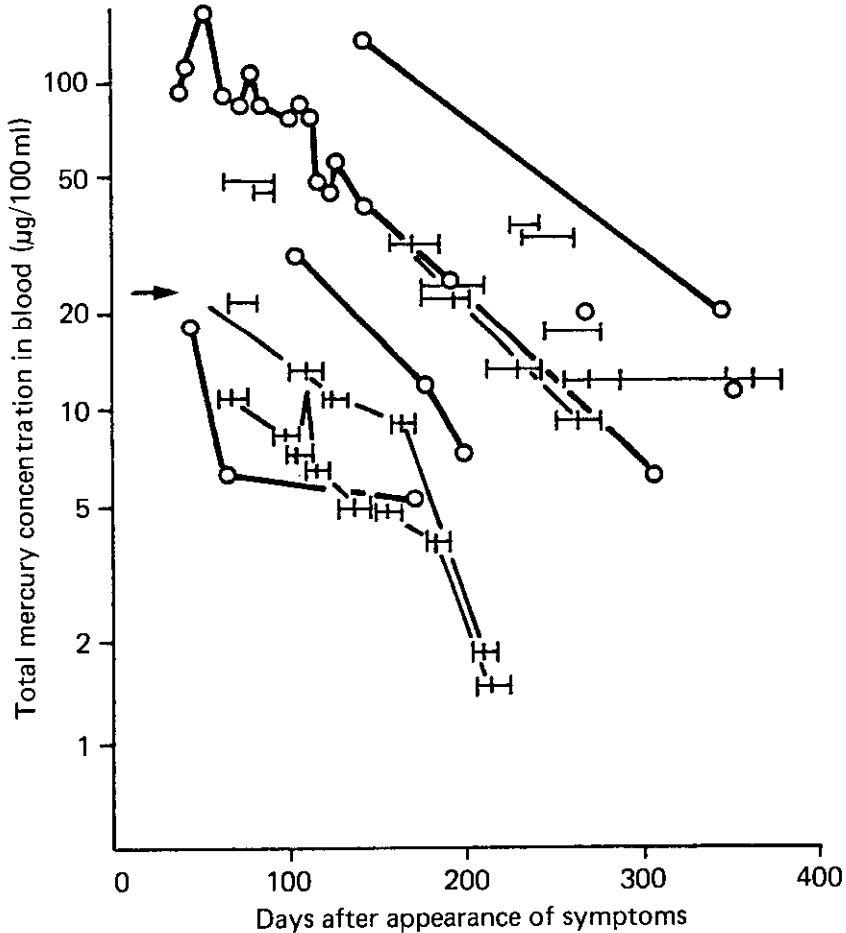Bakir et al. (1973) classified the population into cohorts according to blood levels of mercury. The first blood samples were collected at various times after cessation of exposure. They were corrected to an average point in time corresponding to 65 days after cessation of exposure using a clearance half-time of 65 days. Back extrapolation to times earlier than 65 days was not attempted because the pattern of mercury clearance from blood was not established for this period. In fact it was noted that in 11 cases, blood mercury concentrations did not exhibit statistically significant decline during the first 20 days of March 1972. These individuals had stopped consumption of bread 45 days before collection of the first blood sample.

Thus, instead of attempting to back extrapolate the blood samples to the time of onset of symptoms (for example in the Niigata studies to be discussed below), a different approach was made to recapitulate exposure. It was noted that 58 individuals (approximately half the population studied by Bakir et al., 1973) gave sufficient information on their consumption of contaminated bread to allow estimation of the ingested dose of mercury. When the blood levels in these individuals, corrected to the time point of 65 days after exposure, were plotted against the ingested dose as reported by the patients, the relationship between blood levels and estimated dose was linear for both adults and children, the results for the children giving a steeper slope consistent with a smaller volume of distribution of methylmercury. The correlation coefficient for people over 18 years of age was 0.85 and for people of 10 15 years it was 0.89 (Fig. 2). This empirical correlation between blood mercury and ingested dose was used to estimate ingested dose from observed blood levels (corrected to 65 days after exposure).

Bakir et al. (1973) proceeded to estimate the average amount of methylmercury for each group of the people by use of the exponential equation (equation 1) discussed in section 6. They assumed complete absorption of methylmercury from the diet and that the average elimination half-time was 70 days. This estimated body burden, plotted on a logarithmic scale, was related to the frequency of signs and symptoms in each group of the population (see Fig. 3). The signs and symptoms were paraesthesia, ataxia, visual changes, dysarthria, hearing defects, and death. It was noted that there was a background frequency of signs or symptoms that were not related to the mercury level, and that the frequency increased in relation to the mercury levels, at high doses of mercury. The threshold[a]

---

[a] The phrase "threshold body burden" is meant to indicate the value of the body burden at which effects due to methylmercury become detectable above the background frequency. It is not intended to mean that methylmercury does not produce effects in some individuals below this level.

body burden for this mercury-related increase in frequency was estimated for each sign of symptom. Paraesthesia had the lowest threshold body burden. From the dose–response curve, the onset of symptoms was estimated to occur at approximately 25 mg of mercury or 0.5 mg/kg body weight.

Bakir et al. (1973) noted that the empirical relationship between blood level and ingested dose did not conform with the relationship expected from Miettinen's tracer studies on volunteers. Bakir et al. (1973) pointed out that this difference may be due "either to differences in conditions of exposure between the Iraqi patients and the volunteers given labelled methylmercury, or to underestimations of dose in Iraq, or to both causes". They noted that, in 14 patients, the average exposure period reported by the patients was 48 days as compared with 66 days calculated from hair analysis of the same patients. Thus, an alternative dose–response relationship was plotted by increasing the estimates of ingested dose by a factor of about 1.6, which made their empirically observed blood-ingested dose relationship identical to that calculated from Miettinen. This yielded a threshold body burden for paraesthesia of 40 mg of mercury or 0.8 mg/kg body weight.

An alternative calculation may be made directly from the blood level, to try to estimate the minimum concentration of mercury in blood at which paraesthesia became detectable. If the mean values (estimated as geometric means) of the blood mercury for each cohort, as reported by Bakir et al., are plotted on a logarithmic scale against the frequency of paraesthesia, the relationship has basically the same pattern as observed when body burden was used (Fig. 4). The horizontal portion of the line relates to the background frequency at a mean blood mercury level of between 1.0 and 22 µg/100 ml. The next points lie significantly above the background frequency level. A least-squares linear regression line through these points intercepts the background level at a mercury level of approximately 24 µg/100 ml. However, the estimated threshold value is for mercury concentrations in the blood 65 days after cessation of exposure. If correction is made using a 65 day clearance half-time, the actual threshold level could be twice as high, i.e. 48 µg/100 ml. Evidence noted above suggested that blood mercury may have been cleared at longer half-times than 65 days. Thus the actual threshold mercury value for blood probably lies between 24 and 48 µg/100 ml.

Mufti et al. (1976) and Kazantzis et al. (1976) have reported the results of a survey of 956 persons in a heavily affected village in Iraq, on an additional 207 persons living nearby, and on 1014 persons in a control village that did not receive the treated grain. Mufti et al. (1976) reported on 427 persons who had eaten contaminated bread. They were divided

into groups according to the total consumption of contaminated bread (loaves per day × period of consumption) and the frequency of parasthesia was reported for each group. The total quantity of mercury consumed can be estimated from the mercury content of each load.

Figure 5 is a plot of the frequency of paraesthesia in this population against the log of the mean total ingested dose for each group plotted on a logarithmic scale. A horizontal line is drawn through the point corresponding to 4% paraesthesia assuming this to be the background frequency in this population. The points at higher ingested doses lie significantly above this line. A linear regression line drawn through these points intercepts the background frequency at a total infested dose of 37 mg of mercury. Assuming 50 kg to be the average body weight for this population (Mufti et al., 1976), this threshold dose would be 0.7 mg/kg. However, during the period of consumption (average 32 days), some excretion of methylmercury took place, so that the maximum body burden must have been less than the total ingested. The difference would be small in view of the short period of consumption.

Thus the study of this large population, carried out approximately 6 months after the study by Bakir et al. (1973), would also be compatible with a body burden of mercury in the range of 0.5–0.8 mg/kg body weight.

Shaharistani et al. (1976) have reported on 184 persons in rural Iraq, 143 of whom consumed the contaminated bread.[a] The signs and symptoms were classified as mild, moderate, and severe. People classified as mild cases complained of numbness of the extremities and had slight tremors and mild ataxia. Moderate cases had difficulty of hearing, tunnel vision, and partial paralysis. The severe cases generally suffered from a combination of the following; complete paralysis, loss of vision, loss of hearing, loss of speech, and coma. The dose was expressed as the peak hair concentration of mercury determined by neutron activation of consecutive 1-cm segments of the hair sample, and graphical determination of the peak concentration as described by Giovanoli & Berg (1974).

Shahristani et al. (1976) did not formulate the usual dose–response relationships. Instead the population was classified according to the signs and symptoms of poisoning into four groups (no symptoms, mild, moderate, and severe symptoms). Figure 6 is redrawn from a similar figure presented by Shahristani et al. to indicate the range of peak mercury concentrations in hair for each group. The group having no signs and symptoms attributable to mercury poisoning had hair values in the range 1–300 mg/kg, the mild group in the range of 120–600 mg/kg, the moderate

---

[a] The clinical observations were made by a local physician in the rural district and by a resident physician of the hospital where the hair samples were collected (Shahristani, personal communication).

in the range 200–600 mg/kg and the severe in the range 400–1600 mg/kg. Unfortunately, insufficient information is given in the paper to allow formulation of the usual dose–response relationship in which the population is classified according to dose, and so these results cannot be compared quantitatively with the results of Bakir et al. (1973) and Mufti et al. (1976). However, they do indicate that mild cases have been reported with peak hair levels as low as 120 mg/kg.

Shahristani et al. gave sufficient information on the 30 cases in the group they studied to allow estimation of their daily intake of methylmercury from contaminated bread. It was possible to compare the mercury concentrations in hair, as they increased during the ingestion period, with the daily intake of mercury. It was found that the concentration of mercury in hair was related to daily intake by an exponential equation similar to that described in section 6 relating body burden to daily intake. Thus they were able to calculate the ratio of mercury concentration in hair (mg/kg) to average concentration of mercury in the body (mg/kg). The average ratio was found to be 137 with a range of 82–268 in the 30 individuals. Using this average ratio, the hair level of 120 mg/kg at which mild symptoms were first observed would be equivalent to a body burden of mercury of 0.8 mg/kg body weight.

Observations on the Niigata outbreak of methylmercury poisoning included figures on concentrations of mercury in samples of blood and hair as well as detailed clinical reports. A Swedish Expert Group (1971) estimated the blood levels in patients at the time of onset of symptoms. This involved a graphical procedure in which the concentration of mercury in samples of blood, collected from the patient at various times after admission to hospital, were plotted against the time of collection on semilogarithmic paper (Fig. 7). The decline in blood levels of mercury corresponded to a clearance half-time of 70 days, although there was considerable scatter of the data. Back extrapolation of time of onset of symptoms revealed that the lowest group (about 3 patients) had blood mercury levels in the range of 20–40 µg/100 ml.

Data on hair concentrations in the Niigata outbreak were analysed and discussed by the Swedish Expert Group (1971). One hair sample, collected close to the time of onset of symptoms, contained mercury at 52 mg/kg. Unfortunately, no corresponding blood sample was available. Accurate comparison of hair to blood concentrations in the Niigata samples was not possible because the hair specimens were not representative of the current blood levels. Tsubaki (1971) has presented data relating fish consumption to hair values in the Niigata outbreak which also identified cases of poisoning (also published by the Swedish Expert Group, 1971). The hair concentrations cannot be related to the signs and symp-

that slight symptoms in the mothers might have been overlooked (Harada, 1971). It should also be noted that the infants were examined some years after birth and that no mercury levels are available either for the mothers or for the affected infants. A case of prenatal methylmercury poisoning has been reported for a family that consumed meat from pigs that ate grain treated with methylmercury fungicide (Pierce et al., 1972). The mother was exposed in early pregnancy and had a hair mercury level of 186 mg/kg. It was stated that the mother had no symptoms other than a slight slur of speech which occurred during two weeks in early pregnancy. The child, which was delivered normally after an uneventful pregnancy, exhibited tremulous movement of the extremities in the first few days of life and subsequently developed myclonic convulsions (Snyder, 1971). At one year of age the infant exhibited normal physical growth but could not sit up and was blind.

Cases of prenatal poisoning have been referred to in a preliminary report on the Iraqi epidemic where it was noted that blood levels in the infants at birth and in the first few months after birth could be considerably higher than those of the mother (Bakir et al., 1973). Quantitative data on the prenatal exposure of the infants is not yet available. These observations, however, have led to the belief that prenatal life in man is more sensitive than adult life but the difference in the degree of sensitivity has not yet been quantitatively established.

Amin-Zaki et al. (1974a) have reported clinical examinations of 15 infant–mother pairs in which the infant was exposed prenatally to methylmercury. Clinical manifestations were evident in 6 of the mothers and in at least 6 of the infants. Five of the infants were severely affected having gross impairment of motor and mental development. However, in only 1 infant–mother pair was the infant affected and the mother free of signs and symptoms.

These observations from the 1971–72 Iraq outbreak must be regarded as preliminary. It may take time for some of the consequences of prenatal exposure to manifest themselves. Harada (1971) scrutinized the chromosomes of 7 victims with congenital Minamata disease and 1 infant victim with severe noncongenital Minamata disease, and reported that the chromosome patterns were within normal range.

## 8.2   Clinical Studies of Effects of Mercury-binding Compounds

Attempts to treat mercury poisoning have generally involved the use of antidotes that reduce the amount of mercury in the target tissue, either by forming an inactive complex with mercury, or by enhancing its removal

from the tissue. Such antidotes are of course used in conjunction with general supportive therapy. Ideally the antidote should have a sufficiently high affinity for mercury so that nontoxic doses are able to remove mercury from tissue binding sites. The mercury chelate so formed should be less toxic than mercury and preferably should be rapidly excreted. The agent should be metabolically stable so that dosing should not be too frequent and preferably the agent should be given by mouth. These antidotes are most effective when given early after exposure to mercury. Clearly the removal of mercury is without much advantage if irreversible damage has already occurred.

The first effective antidote, 2,3-dimercaptopropanol (British Anti-lewisite—BAL), is a sulfur-containing compound (a dithiol molecule, possessing a remarkably high affinity for divalent ionic mercury) and was developed on the basis that mercury and other heavy metals combined with sulfur groups in the body (for detailed review, see Levine, 1970). This compound is life saving in cases of acute mercury(II) chloride poisoning, alleviates symptoms from overdoses of mercury diuretics, and dramatically relieves certain symptoms of acrodynia. For alkylmercury poisoning BAL is contraindicated since it increases brain mercury levels (Berlin & Rylander, 1964; Magos, 1968). It also does not alleviate neurological disorders caused by mercury vapour exposure (Hay et al., 1963; Glomme & Gustavson, 1959). Unithiol (2,3, dimercaptopropansulfonate) is a water soluble derivative of BAL that is apparently more effective in mobilizing mercury (Trojanowska & Azendzikowski, in press; Dutkiewicz & Oginski, 1967). Furthermore unithiol does not produce redistribution to the brain, as has been observed after BAL treatment. Unithiol is effective in the treatment of occupational mercurialism (Fesenko, 1969), but there are no reports on its effects on alkylmercury poisoning.

The penicillamines (D-penicillamine and N-acetyl-DL-penicillamine) are effective in increasing the excretion of mercury after exposure to mercury vapour and in relieving the symptoms of chronic mercury vapour poisoning (Smith & Miller, 1961; Parameshvera, 1967). Fatal brain damage can be prevented in the offspring of rats treated with methylmercury, by D-penicillamine according to the experiments of Matsumoto et al. (1967). Recent literature reports (Bakir et al., 1973; Suzuki & Yoshino, 1969) indicate that the penicillamines are capable of mobilizing mercury from tissues and increasing the excretion of mercury in cases of methylmercury poisoning in man. Thus it appears that the penicillamines offer advantages over BAL in that they are orally effective, less toxic, and effective in treating mercury vapour poisoning and probably alkylmercury poisoning, when administered immediately following exposure.

A slight increase in the urinary excretion of mercury has been noted in

methylmercury poisoned patients with "Minamata disease" (Katsuna, 1968), after administration of EDTA.

Thioacetamide increases urinary excretion of mercury in animals dosed with mercury(II) chloride but probably one of the major causes of this effect is kidney damage caused by the combination of the toxic effects of thioacetamide and mercury leading to an increased exfoliation of renal tubular cells (Trojanowska et al., 1971).

Some interesting new ideas in the realm of antidotes to mercury are worth noting. Aaseth (1973) described the application of large molecular weight mercaptodextran in the successful treatment of mercury(II) chloride poisoning in animals. This agent does not enter the intracellular spaces and achieves removal of mercury from the body without redistribution. However, its effectiveness is limited by the time of administration. For example, if given more than 2 hours after exposure to mercury, this compound is totally ineffective whereas BAL is still useful.

A second approach is to give a nonabsorbable mercury-binding compound in the diet, in order to trap the mercury that is secreted in the bile, to prevent its reabsorption, and to greatly increase the faecal elimination (Takahashi & Hirayama, 1971; Clarkson et al., 1973a). A polystyrene resin containing fixed sulfhydryl groups has been shown to increase mercury excretion in experimental animals given methylmercury, and to reduce blood mercury levels in the victims of methylmercury poisoning in Iraq (Clarkson et al., 1973a; Bakir et al., 1973). There is, however, variation in response among patients. More recently it has been demonstrated that phenobarbital can increase the biliary excretion of methylmercury compounds (Magos et al., 1974).

A new technique for the removal of methylmercury directly from blood has been proposed for use in methylmercury poisoning (Kostyniak et al., 1975). The simultaneous combination of extracorporeal regional complexation of methylmercury with haemodialysis has been effective in producing a rapid removal of mercury in both experimental animals and man (Kostyniak et al., 1974; Al-Abbasi et al., 1974, unpublished reports).


### 8.3 Pathological Findings and Progression of Disease

The signs and symptoms of acute toxicity, severe gastrointestinal damage, shock, cardiovascular collapse, and acute renal failure, after large doses of divalent mercury and pulmonary irritation after inhalation of massive doses of the vapour, reflect the fact that all mercury compounds are chemically reactive, can denature proteins, inactivate enzymes, and disrupt cell membranes, leading to cellular death and the destruction of

any tissue with which they come into contact in sufficient concentration. The pathological effects following long-term exposure to lower doses of mercury compounds are more subtle and depend upon the type of mercury compound to which the subjects are exposed. The remainder of this section will be concerned with long-term exposure, as this type of exposure is most important in helping to assess the hazards to man of the presence of mercury in food and air.

Pathological findings on human subjects exposed to mercury and its compounds have been reviewed by a Swedish Expert Group (1971) and by Friberg & Vostal (1972).

Pathological findings demonstrate that methyl- and ethylmercury compounds are primarily neurotoxic and produce similar types of lesion in man. The main pathological features consist of the destruction of neurological cells in the cortex particularly in the visual areas of the occipital cortex and various degrees of damage to the granular layer in the cerebellum. Damage to the peripheral nerves may occur as indicated by clinical signs but no definitive pathological observations are available for man. Takeuchi (1970) has reported on changes in the diameter of the peripheral nerves in patients suffering from heavy exposure to methylmercury in the Minamata Bay epidemic. However, Von Burg & Rustam (quoted by Bakir et al., 1973) could not find any changes in conduction velocities in the patients in Iraq who received very high exposure to methylmercury.

Brain concentrations of mercury associated with the onset of pathological changes following exposure to the vapour or to doses of the alkylmercury compounds are not fully known. Takahata et al. (1970) demonstrated mean mercury levels of 11 mg/kg wet weight in the brains of two workers, poisoned by exposure in a mercury mine, who had had no known exposure to mercury for 10 years prior to death. Studies on occupationally poisoned individuals (Swedish Expert Group, 1971), and the patients who died in the Minamata epidemic indicate that the onset point of signs and symptoms corresponds to an average brain level of approximately 5 mg/kg wet weight. These findings are in general agreement with threshold methylmercury concentrations in studies on experimentally poisoned animals.

### 8.3.1 Psychiatric and neurological disturbances

Trahtenberg (as reviewed by Friberg & Nordberg, 1972) reported minor psychiatric disturbances such as insomnia, shyness, nervousness, and dizziness in workers exposed to elemental mercury vapour concentra-

toms because they were not extrapolated back to the time of onset of symptoms.

Several fish-eating populations (category 3) have been examined for signs and symptoms of poisoning, and determinations made of concentration of mercury in samples of blood and hair. A Swedish population of fish eaters had blood levels of mercury up to 56 µg/100 ml. Skerfving (1972) reported a dose–response curve calculated from data for this population, which had no cases of mercury poisoning, and for the Niigata cases reviewed above. The frequency of signs and symptoms was related to concentrations of mercury in the hair. The apparent threshold effect corresponded to 50–90 mg/kg in the hair and thus to a blood mercury level of 20–36 µg/100 ml.

The studies by Turner et al. (1974) and Marsh et al. (1974) on populations of fish eaters in Peru and Samoa are consistent with the relationship published by Skerfving (1972). Thus Turner et al. noted that 8 people had mercury blood levels between 20 and 30 µg/100 ml and Marsh et al. also found 2 people having blood levels in the same range.

Paccagnella et al. (1974) could not find any connexion between the prevalence of neurological defects and concentrations of mercury in samples of blood and hair in the high fish consumers on S. Pietro Island. No neurological deficits were reported in the three individuals having blood mercury levels between 20 and 24 µg/100 ml. However the dose–response data reviewed above indicate that the risk of poisoning at these levels is small.

Table 4 records the results obtained in studies on the populations discussed above. The studies on the Niigata population and the report of Shahristani et al. on the Iraqi population identify cases of methylmercury poisoning. The quoted levels in hair and blood are those seen in the most sensitive individuals in that population. The results (Table 4) indicate that such "sensitive" individuals may exhibit symptoms of methylmercury

Table 4. Summary of concentrations of mercury in samples of blood and hair and the body burden of mercury associated with effects (usually paraesthesia) in the most sensitive group in the population[a]

| Population | Total No. studied | Mercury concentration | | Mercury in the body (mg/kg) | References |
| | | Blood (µg/100 ml) | Hair (mg/kg) | | |
| --- | --- | --- | --- | --- | --- |
| Niigata | 17 | 20–40 | 52 | — | Swedish Expert Group (1971) |
| Iraq | 184 | — | 120 | 0.8 | Shahristani et al. (1976) |
| Iraq | 125 | 24–48 | — | 0.5–0.8 | Bakir et al. (1973) |
| Iraq | 427 | — | — | 0.7 | Mufti et al. (1976) |

[a] The numbers quoted in this table should not be considered independently of the accompanying test.

poisoning at blood mercury levels in the range of 20–40 µg/100 ml and at hair levels of 50–60 mg/kg. Unfortunately these studies do not indicate the percentage of the general population that is sensitive. Studies on high fish consumers in Sweden, Peru, Samoa, and Italy, suggest a low probability of symptoms at mercury levels of 20–40 µg/100 ml blood. At least 15 persons having blood levels in this range did not exhibit symptoms of poisoning.

The studies by Bakir et al. (1973) and Mufti et al. (1976) on the Iraqi population followed a different approach. Individual cases of methylmercury poisoning were not reported. Instead, the population was divided into groups according to observed levels of mercury in the blood and according to estimated dose, and the frequency of specific signs and symptoms was reported for each group. Using this approach, it is necessary to distinguish between the background frequency of signs and symptoms and the increase of frequency due to methylmercury. The figures quoted in Table 4 represent a graphical estimate (see Fig. 3, 4, and 5) of the blood level, a body burden or dose of methylmercury where the frequency of paraesthesia emerges above the background level. Variation in observed background frequencies ranged from 4% (Fig. 5) to 9.5% (Fig. 3), thus setting a practical limit to the accuracy of such graphical estimates. Thus the numbers quoted in Table 4 are compatible with frequencies of paraesthesia due to methylmercury of 5% or less.

The data in Table 4 apply only to neurological signs and symptoms in adults. They do not apply to infants exposed either prenatally or in the early postnatal period.

Skerfving et al. (1974) have reported on the cytogenetic effects of methylmercury in 23 people exposed through intakes of various amounts of fish containing methylmercury (0.5–7 mg/kg) and in 16 people with a low or moderate intake of mainly oceanic fish. The mercury levels in blood in the "exposed" subjects ranged from 1.4 to 11.6 µg/100 ml and in the "non-exposed" from 0.3 to 1.8 µg/100 ml. A statistical relationship was found between frequency of chromosome breaks and blood mercury levels. In a study carried out in Iraq (Firman, unpublished report), no statistically significant difference was noted in an exposed group compared with a control population with regard to chromosome damage.

### 8.1.3 Children and infants with *in utero* exposure

Of the cases of poisoning reported from Minamata (Harada, 1968), 23 were due to prenatal exposure to methylmercury. These infants had severe cerebral involvement (palsy and retardation) whereas their mothers had mild or no manifestations of poisoning. However, there is a possibility

tions of the order of 0.1 mg/m$^3$. The classical literature (for review, see Friberg & Nordberg, 1973) contains detailed accounts of the consequences of long exposure to higher concentrations of elemental mercury vapour where the full syndrome of erethism is seen. Individual variation in exposed people is the rule but the most commonly reported syndrome includes loss of memory, insomnia, lack of self-control, irritability and excitability, anxiety, loss of self-confidence, drowsiness, and depression. In the most severe cases delirium with hallucinations, suicidal melancholia, or even manic-depressive psychoses have been described.

The presence of tremor is one of the most characteristic features of mercurialism and usually follows the minor psychological disturbances referred to above. With continuing exposure to elemental mercury vapour, the tremor develops gradually in the form of fine trembling of the muscles interrupted by coarse shaking movements every few minutes. It may be seen in the fingers, but also on the closed eyelids, lips, and on the protruding tongue. The frequency is of the order of 5–8 cycles per second. It is intentional and stops during sleep. On cessation of exposure, the tremor gradually disappears. Dramatic alterations in the steadiness of the handwriting may be seen in persons suffering from mercurial tremor.

The most common signs and symptoms in cases of poisoning due to methyl- or ethylmercury compounds are paraesthesia, loss of sensation in the extremities and around the mouth, ataxia, constriction of the visual fields, and impairment of hearing. In the Japanese experience the effects of alkylmercury poisoning are usually irreversible but the coordination may improve after rehabilitation (Kitagawa, 1968). On the other hand, in Iraq, improvement in motor disturbances was often spontaneous. In addition, paraesthesia was often reversible in Iraq but a persistent symptom in Japan (Damluji, 1974; Tsubaki, 1971).

### 8.3.2  Eye and visual effects

Occupational exposure to elemental mercury vapour causes the appearance of a greyish-brown or yellow haze on the anterior surface of the lens of the eye (Atkinson, 1943). It appears usually after long-term exposure and the depth of colour depends upon the length of time and the air concentration of mercury to which the worker has been exposed. The presence of this coloured reflex may or may not be associated with signs and symptoms of poisoning.

The narrowing of the visual fields is a classic sign of poisoning due to short-chain alkylmercurial compounds (Hunter et al., 1940). In cases of severe exposure, the constriction may proceed to complete blindness.

### 8.3.3  Kidney damage

Kazantzis et al. (1962) reported four cases of proteinuria in two groups of workmen exposed to elemental mercury vapour.  Exposure conditions were not specified, but all four cases were excreting mercury in urine in excess of 1000 µg/litre at the time of the first examination.  The proteinuria disappeared after the workers were removed from exposure. Joselow & Goldwater (1967) noted that the mean urinary protein concentration (90 mg/litre) in a group of workers exposed to elemental mercury vapour was significantly higher than the mean protein concentrations (53 mg/litre) in a nonexposed group.  The urinary protein correlated with urinary mercury levels.

Renal involvement after exposure to methylmercury compounds is very rare (Bakir et al., 1973).  Cases of renal damage have been reported in an outbreak of ethylmercury poisoning (Jalili & Al-Abbasi, 1961).

### 8.3.4  Skin and mucous membrane changes

Dermatitis has been reported after occupational exposure to phenyl-mercurials (for review, see Goldwater, 1973).  However dermatitis may result from exposure to inorganic mercury (Hunter, 1969).  Sensitivity to metallic mercury in tooth fillings has resulted in facial and intra-oral rashes.  Skin reactions due to sensitivity to phenylmercury have also been reported (for review, see Clarkson, 1972b).

Dermatitis has been reported after skin contact with the alkylmercurials (for review, see Swedish Expert Group, 1971).  Oral ingestion of methyl- and ethylmercury compounds may also result in this condition, as observed in the Iraqi epidemics (Jalili & Al-Abbasi, 1961; Damluji et al., 1976).

# 9. EVALUATION OF HEALTH RISKS TO MAN FROM EXPOSURE TO MERCURY AND ITS COMPOUNDS

## 9.1  General Considerations

In order to control risks to human health from the presence of mercury in the environment, it is necessary to attempt to define the degree of risk in any given environmental situation.  The health risk evaluation depends in part on a knowledge of dose–effect, dose–response relationships.  It

112

also depends on a knowledge of the variation in exposure (or intake) in any given situation. A general review of sampling and analytical techniques (section 2), and of environmental sources and exposure levels (sections 3 and 5) has been presented in this document. However, specific assessment of exposure or intake must be made by the public health authorities responsible for any given population or group. This section of the report is concerned, therefore, primarily with a summary of those dose–effect, dose–response relationships that are relevant to human populations in both occupational and general environmental exposure to mercury and its compounds.

Species differences in the metabolism and toxicity of mercury and its compounds are so great that this health risk evaluation is based primarily on data for man. Animal data have been included or considered only when data for man are lacking, and have generally been used in a qualitative way. Thus animal data may in certain cases indicate the potential for genetic effects, or that a certain stage of the life cycle, for example, the fetus appears to be the most sensitive stage. General patterns of deposition of mercury in the body, as seen from animal experiments, have been considered to be broadly applicable to man in a qualitative sense.

Quantitative estimates of the dose–response relationship in man are fraught with many difficulties. Observations of occupationally-exposed persons should offer the best possibilities for well controlled studies. However, the exposure range and population sizes are limited and difficulties are usually encountered in obtaining accurate estimates of time-weighted average exposure to airborne mercury. The most difficult situation for controlled study arises in the case of methylmercury where our knowledge derives primarily from observations on the unexpected outbreaks of poisoning from contaminated food in Japan and Iraq. Generally the studies commenced some time after the start of the outbreak or after the end of the exposure. Attempts had to be made either to recapitulate exposure by the back-extrapolation of blood levels, or to estimate the ingested dose from the patient's ability to remember. Doubts have also been expressed on the reliability of analytical methods in the earlier Japanese outbreaks.

Estimates will be made of minimum effect exposures or concentrations in indicator media since these numbers may be of value to authorities in setting safety standards. These minimum effect figures (e.g. Tables 5 and 6) should be viewed in terms of the overall dose response relationship, namely, that as the dose is decreased, so also is the probability of poisoning, and that the minimum effect level is that dose (expressed as exposure level, daily intake, concentration in indicator

113

media) that is associated with the first detectable effect in the population under study. The effect will be present at some specified frequency in the population. The value of the minimum effect dose is the dose derived from the observed dose–response relationship. (It will be subject to more statistical uncertainty than, for example, the dose giving 50% frequency of the effect.) Its value will depend on the size of the population under study; the larger the population, the more likely it will be that effects will become evident in the more sensitive individuals.

### 9.1.1 Elemental mercury vapour

The central nervous system is the critical organ for the toxic effects of inhaled elemental mercury. Effects on the kidney such as proteinuria have been reported but only at doses higher than those associated with the onset of signs and symptoms from the central nervous system. No information is available with regard to mercury levels in the central nervous system at the time of onset of signs and symptoms or at death in man. Furthermore, we do not have indicator media such as urine or blood the mercury levels of which would reflect those in the brain. Observations on animals exposed to elemental mercury vapour reveal large regional differences in distribution within the brain so that average brain concentrations, even if they were available for man, might not be of much value.

Our evaluation of the health risks from exposure to elemental mercury vapour in man has therefore to be based on an empirical relationship between air levels (exposure) and the frequency of signs and symptoms in exposed populations. Concentrations of mercury in urine and blood are related, on a group basis, to average air concentrations.

A complete metabolic model relating air levels to absorption, accumulation, and excretion of mercury in man following exposure to elemental mercury vapour is not available. However, some useful generalizations are beginning to emerge from recent observations. Most of the inhaled vapour (approximately 80%) is retained in the lung. Once absorbed into the blood stream, it is rapidly oxidized to ionic mercury. The limited information on biological half-times in man suggests that a workman exposed to a constant average concentration of mercury vapour in his working environment would not reach a state of balance (steady state) until after one year of exposure. Consequently one would expect the concentrations of mercury in blood or urine to exhibit a consistent relationship to air levels after the worker had been exposed for at least one year. Unfortunately, most of the publications in the literature do not indicate

114

the period of employment of the worker. However, general experience in occupational health studies (for review, see MAC Committee, 1969) reveals that exposure of workers to an average air concentration of mercury of 0.05 mg/m$^3$ are associated, on a group basis, with blood levels of approximately 3.5 µg/100 ml, and with urinary concentrations of 150 µg/litre. Linear relationship has been observed between urinary and blood concentrations of mercury in people occupationally exposed to elemental mercury vapour. Thus, measurements of mercury concentrations in the working atmosphere and in samples of blood and urine from the workmen may be used as an index of exposure. These values in turn may be compared with the frequency of clinical signs and symptoms in workers experiencing different degrees of exposure. Such studies, reported in detail in section 8 and briefly reviewed below, form the basis for establishing "threshold limit values" or maximal allowable air concentrations of mercury in occupational exposure.

Early studies dating back to the 1930s indicate that cases of poisoning occurred at atmospheric mercury levels above 0.1 mg/m$^3$ (for details, see section 8.1.1). Recent data also demonstrate that there was an increase in complaints of appetite-loss and insomnia in a group of workers exposed to time-weighted average air concentrations of mercury between 0.06 and 0.1 mg/m$^3$ as compared with two lower exposure groups (0.01 and 0.05 mg/m$^3$). These findings are in agreement with data published in the 1940s and 1950s indicating that mercury intoxication occurred in workers exposed to mercury in air concentration less than 0.2 mg/m$^3$, but no data were given on the lower exposure limits.

At this time it is difficult, if not impossible, to establish a lower limit at which no effects occur. Studies reviewed in section 8.1.1 indicate that mental disturbances may be seen at extremely low mercury concentrations in air. However, the problem in the interpretation of these reports is that, as the air concentration of mercury decreases, it becomes more difficult to correlate effects with exposure with any degree of confidence. For example, it would appear that effects of mercury levels below 0.05 mg/m$^3$ have not been unequivocally established.

Concentrations of mercury in blood and urine equivalent to average mercury concentrations in air of 0.05 and 0.1 mg/m$^3$ are given in Table 5. In general these relationships are not seen in indivduals but only when averaged over a substantial number of workers.

An occupational limit for mercury in air concentration of 0.05 mg/m$^3$ would be equivalent to an ambient air level for the general population of approximately 0.015 mg/m$^3$. This calculation is based on the assumptions of a daily ventilation of 10 m$^3$ during working hours and 20 m$^3$ for a 24-hour day and that there are 225 working days in the year. The data of

Smith et al. (1970) would indicate that the probability of seeing adverse effects at air levels of, 0.05 mg/m³ for occupational exposure, and 0.015 mg/m³ for continuous environmental exposure, is low for such symptoms as loss of appetite, weight loss, and shyness. However, this calculation does not take into account the sensitive groups in the general population. It should be noted that concentrations found in ambient air are far below these levels (see section 5).

Table 5. The time-weighted average air concentrations associated with the earliest effects in the most sensitive adults following long-term exposure to elemental mercury vapour. The table also lists the equivalent blood and urine concentrations[a]

| Air (mg/m³) | Blood (µg/100 ml) | Urine (µg/litre) | Earliest effects |
|---|---|---|---|
| 0.05 | 3.5 | 150 | non-specific symptoms |
| 0.1–0.2 | 30 | 600 | tremor |

[a] Blood and urine values may be used only on a group basis owing to gross individual variations. Furthermore, these average values reflect exposure only after exposure for a year or more. After shorter periods of exposure, air concentrations would be associated with lower concentrations in blood and urine.

### 9.1.2 Methylmercury compounds

The estimate of risks to human health from methylmercury compounds is important for several reasons. First, many thousands of people have been poisoned following accidental consumption of food contaminated with methylmercury fungicides or the consumption of fish contaminated by industrial release of methylmercury. Second, methylmercury probably accounts for a significant part of mercury in the human diet and is especially important in fish and fish products. Third, the risk–benefit calculations with regard to methylmercury in fish are of critical importance in those countries and areas of the world where fish is an important dietary source of protein, or where the fish industry is of economic importance.

This evaluation of risk from exposure to methylmercury compounds is based primarily upon data in man. The data consist of observations on the frequency of signs and symptoms in populations exposed to a wide range of mercury intake, observations on the concentrations of mercury in hair and blood samples, and on estimates of dietary intake (section 8).

In estimates of risks to human health, the custom has been followed of attempting to determine the lowest concentration in indicator media or the lowest daily intake associated with the onset of toxic signs and symptoms in man, along with information on maximal intakes which produce no effects. This procedure has been followed in estimating the data presented in Table 6. The levels in blood and hair, and the amount

116

of the body burden of mercury associated with the onset of signs and symptoms are taken from Table 4.

The reports on the Minamata outbreak to the effect that infants were born having cerebral palsy due to methylmercury whereas their mothers lacked or had only slight symptoms led to the belief that the fetus was the stage of the life cycle that was most sensitive to methylmercury. Studies on animals, exposed during the gestation period to methylmercury led to qualitative confirmation of this conclusion.

Table 6 lists our conclusions on concentrations of total mercury in indicator media associated with the earliest effects of methylmercury in the most sensitive group in the adult population. As discussed below, the prevalence of the earliest effects would be expected to be approximately 5%. The equivalent long-term daily intake quoted in Table 6 was calculated on the most conservative relationship (quoted in Table 3) i.e. that the steady state blood concentration (ng/ml) is numerically equal to the average daily intake (μg/day/70 kg body weight).

However, Nordberg & Strangert (1976) have proposed an alternative approach to estimating risks of poisoning on long-term exposure. The relationship between long-term daily intake and steady state blood levels (and hair levels and body burden) depends, *inter alia*, on the biological half-time of methylmercury in man. Biological half-time times are subject to individual variation as discussed in section 6. Thus an individual having a long biological half-time (a slow excretor of methylmercury) would accumulate higher steady state levels than one having a short biological half-time. Thus the statistical distribution of biological half-times should be taken into account in estimates of risk of poisoning.

Variations also occur in threshold concentrations for the appearance of signs and symptoms in individuals in the population. These may be estimated from empirical relationships relating frequency of signs and symptoms to concentrations of methylmercury in indicator media (blood and hair) or body burdens (e.g. Fig. 3, 4, and 5). On the assumptions that the distribution of biological half-times was normal, that the distribution of individual threshold values for paraesthesia was log-normal, and that these distributions were independent of each other, Nordberg & Strangert (1974) estimated the overall probability of an individual developing symptoms of paraesthesia. Their calculations were based on data of Shahristani & Shihab (1974) and gave the distribution of biological half-times estimated from analysis of hair samples after the Iraqi outbreak, and the statistical distribution of individual threshold values estimated from the data of Bakir et al. (1973) on the relationship of frequency of paraesthesia to body burdens. Their results indicated that with a long-term daily intake of 4 μg/kg would yield the risk of paraesthesia of about

8%. Subsequent calculations based on the data by Mufti et al. (1976) would indicate a risk of between 3% and 4% for the same daily intake.[a]

The figures estimated by Nordberg & Strangert may somewhat overestimate the risk of poisoning. The errors to be expected in both field and laboratory observations would tend to decrease the slope in dose-response relationships. This would lead to an overestimate of the variance of threshold values in the general population. Nevertheless, the estimate of risk by Nordberg & Strangert is in reasonable agreement with the more empirical estimates discussed in section 8 and indicates that, with a long-term daily intake as listed in Table 6, the prevalence of the earliest effects could be expected to be 5% or less.

Table 6. The concentrations of total mercury in indicator media and the equivalent long-term daily intake of mercury as methylmercury associated with the earliest effects in the most sensitive group in the adult population[a,b]

| Concentrations in indicator media | | |
|---|---|---|
| Blood ($\mu g/100$ ml) | Hair ($\mu g/g$) | Equivalent long-term daily intake[c] ($\mu g/kg$ body weight) |
| 20–50 | 50–125 | 3–7 |

[a] The prevalence of the earliest effects could be expected to be approximately 5%.

[b] The WHO Task Group specifically urged that this table should not be considered independently of the text in section 8.

[c] A Japanese group has recently concluded that a daily intake of mercury of 5 $\mu g/kg$ is the "minimal toxic dose", following a ten-year follow-up study of the Minamata outbreak (Research Committee on Minamata Disease, 1975).

Occupational hazards have arisen mainly from airborne concentrations of alkylmercurials. Skin contact has also been noted, but the quantitative importance of skin contact and percutaneous absorption cannot be estimated. Hazards from occupational exposures can be estimated only by reference to data already discussed above with respect to dietary intake of methylmercury compounds. The data summarized in Table 6 indicate that the first effect (paraesthesia) associated with long-term intake of methylmercury arises at an intake level of approximately 5 $\mu g/kg$ body weight per day. Assuming a daily ventilation at work of 10 m³ of air, 80% retention of the inhaled mercurial, 225 working days to the year, the average time-weighted air concentrations that would give rise to this intake would be 0.07 mg/m³. Consideration of occupational risks should also take into account the possibility of a high but brief exposure to methylmercury compounds.

The estimates in Table 6 apply only to adults. As discussed previously,

[a] Nordberg & Strangert, personal communication.

118

prenatal life may be the stage of the life-cycle most sensitive to methylmercury. It would be prudent therefore to follow the advice of the MAC Committee (MAC, 1969), not to expose females of child bearing age occupationally to methylmercury compounds.

Studies by Skerfving et al. (section 8) have indicated that chromosome breaks may be associated with exposure to methylmercury. There are, however, other studies in which no such relationship was found. Furthermore the health significance of chromosome breakage is not known. However, as reviewed elsewhere in this criteria document, experiments on animals and other forms of life do indicate the potential for genetic damage by methylmercury.

### 9.1.3  Ethylmercury compounds and other short-chain alkylmercurials

Insufficient information is available to allow risk calculations based on data from human exposure to ethyl- or higher short-chain alkylmercurial compounds. Suzuki et al. (1973) have reported observations on five patients poisoned with ethylmercury compounds. The picture of distribution between plasma and red cells and observations on autopsy tissue indicate that the metabolism and patterns of distribution of ethylmercury are generally similar to those of methylmercury compounds. However, in one individual the clearance-time from blood was only 10 days. Evidence reviewed in section 6.6 indicates that ethylmercury compounds may be more rapidly converted to inorganic mercury in the body than methylmercury compounds. Thus, these limited observations suggest that ethylmercury compounds are probably less hazardous than the methylmercury compounds in so much as they remain in the body for a shorter time because of transformation to inorganic mercury and more rapid excretion. Thus, standards set for methylmercury compounds will probably be sufficient to control the hazards from other short-chain alkylmercurial compounds.

### 9.1.4  Inorganic mercury, aryl- and alkoxyalkylmercurials

The risk to human health from long-term ingestion of inorganic, aryl-, and alkoxyalkyl- compounds of mercury in the diet is difficult to estimate because there are not any recorded cases of human poisoning under these circumstances. Data from animals cannot be used for exact quantitative extrapolation because of species differences in the metabolism and toxicity of these compounds. However, certain qualitative conclusions based on animals can probably be extrapolated to man. For example, the arylmercurials are rapidly converted to inorganic mercury in mammals. The

penetration of mercury across the blood–brain and placental barriers is less after doses of inorganic and aryl compounds than after equivalent doses of elemental mercury vapour and short-chain alkylmercurials. Animal studies indicate that the kidney is the critical target organ for exposure to inorganic, alkyl-, and alkoxy-alkylmercurials. Kidney involvement appears to be minimal in workers exposed to concentrations of mercury vapour ($0.05$–$0.1$ mg/m$^3$) that elicit the first signs and symptoms of damage to the central nervous system. Thus guidelines for health protection, set for long-term exposure to elemental mercury vapour, should offer an even greater safety margin for equivalent exposures to inorganic, alkyl, and alkoxyalkyl compounds. In fact, recognizing the lower toxic potential of these forms of mercury, the MAC Committee (1969) advised a maximum allowable concentration for occupational exposure of $0.1$ mg/m$^3$—twice as high as that for elemental mercury vapour.

In the discussion of guidelines for exposure to elemental mercury vapour, it was concluded that long-term exposure of the general population to $0.015$ mg/m$^3$ was equivalent, in terms of average daily mercury intake, to the occupational limit of $0.05$ mg/m$^3$. Assuming an average pulmonary retention of 80%, the average daily amount entering the blood stream is 280 μg based on a daily ventilation of 20 m$^3$.

The equivalent daily intake in diet of phenylmercury compounds would also be about 240 μg, as animal studies indicate virtually complete absorption in the gastrointestinal tract. The alkoxyalkyl and aryl compounds are probably absorbed equally well from food. Tracer studies on volunteers indicate that approximately 10% of inorganic mercury compounds are absorbed from the diet, so that dietary intakes approximately ten times greater than those of phenylmercury compounds would offer no greater risk of poisoning.

A daily intake of mercury of 240 μg is in the same range as the daily intake listed for methylmercury compounds in Table 6. The biological half-time for inorganic mercury, based on tracer studies in man, appears to be less than that for tracer doses of methylmercury. Animal data suggest that aryl and alkoxyaryl compounds have biological half-times lower than that for methylmercury and similar to that for inorganic mercury. Thus the long-term ingestion of inorganic, aryl, and alkoxyaryl compounds should offer no greater hazards and probably substantially less than the hazards from ingestion of methylmercury compounds.

## 9.2   Summary and Guidelines

In the case of elemental mercury and alkylmercury the Task Group was able to construct tables (Tables 5 and 6) that related exposure

to symptoms as well as to concentrations in indicator media in the human body. In the case of inorganic mercury, arylmercurials, and alkoxyalkyl mercurials, this could not be done because of the inconsistency in the animal data available as well as a paucity of data in man.

The tables for elemental mercury and alkylmercury are given above. In constructing these tables, the Task Group evaluated the results of studies summarized in this document, and drawing on their experience and judgement, identified the concentration and amounts of mercury associated with certain observed effects. There is insufficient information available to permit precise quantification of this risk. Usually, the proportion that may be expected to be affected is small.

The expected health effects for elemental mercury at a level in air of $0.05 \text{ mg/m}^3$ have been quoted only for occupational exposure assuming 8 hours per day and 225 working days a year. The equivalent environmental mercury levels in air for continuous exposure would be approximately $0.015 \text{ mg/m}^3$ to give the same degree of risk. The urine and blood values, of course, would be the same as those quoted in Table 5. Even though the figures do not only take into account specific sensitive groups, it is highly unlikely that the concentration in the general environment approaches levels of toxicological significance.

The ranges of minimum effect values quoted in Table 6 for methylmercury reflect the uncertainty in estimations.

Although it was not possible to identify even approximate minimum effect values for inorganic, aryl-, and alkoxyalkylmercurials, the Task Group concluded that the limited experience for occupational exposure suggested that these forms of mercury were probably less hazardous than either elemental mercury vapour or methylmercury compounds. Thus the figures for occupational exposure to elemental mercury vapour given in Table 5 would serve as conservative figures for occupational exposure to these forms of mercury and those in Table 6 would offer conservative figures for dietary intake.

A Joint FAO/WHO Expert Committee on Food Additives (1972) established a provisional tolerable weekly intake of 0.3 mg of total mercury per person of which no more than 0.2 mg should be present as methylmercury (expressed as mercury); these amounts are equivalent to 5 μg and 3.3 μg, respectively, per kg of body weight. Where the total mercury intake in the diet is found to exceed 0.3 mg per week, the level of methylmercury compounds should also be investigated. If the excessive intake is attributable entirely to inorganic mercury, the above provisional limit for total mercury no longer applies and will need to be reassessed in the light of all prevailing circumstances.

121

AASETH, J. (1973) *Acta Pharm. Toxicol.*, **32**: 430.

ABERG, B. ET AL. (1969) *Arch. environ. Health*, **19**: 478.

ACGIH (1976) *Threshold limit values for chemical substances and physical agents in the work-room environment with intended changes for 1976.* Cincinnati, American Conference of governmental industrial hygienists, pp. 94.

ALBANUS, L. ET AL. (1972) *Environ. Res.*, **5**: 425.

AMIN-ZAKI, L. ET AL. (1974a) *Pediatrics*, **54**: 587.

AMIN-ZAKI, L. ET AL. (1974b) *J. Pediatr.*, **85**: 81.

AMIN-ZAKI, L. ET AL. (in press) *Am. J. Dis. Children.*

ANDERSSON, A. (1967) *Grundforbattring*, **20**: 95.

ARMSTRONG, R. D. ET AL. (1963) *J. Am. ind. Hyg. Assoc.*, **24**: 366.

ASHE, W. F. ET AL. (1953) *Arch. ind. Hyg. occup. Med.*, 7: 19.

ATKINSON, W. (1943) *Am. J. Ophthalmol.*, **26**: 685.

BACHE, C. A. ET AL. (1971) *Science*, **172**: 951.

BAKIR, F. ET AL. (1973) *Science*, **181**: 230.

BAKULINA, A. V. (1968) *Sov. Med.*, **31**: 60.

BALDI, G. ET AL. (1953) *Med. Lav.*, **44**: 161.

BARBER, R. T. ET AL. (1972) *Science*, **178**, 636.

BERLIN, M. & RYLANDER, R. (1964) *J. Pharm. exp. Ther.*, **146**: 236.

BERLIN, M. ET AL. (1966) *Arch. environ. Health*, **12**: 33.

BERLIN, M. ET AL. (1969) *Arch. environ. Health*, **18**: 42.

BERLIN, M. ET AL. (1973) *The uptake and distribution of methyl mercury in the brain of* Saimiri sciureus *in relation to behavioral and morphological changes.* In: Miller, M. W. & Clarkson, T. W. ed. *Mercury, mercurials and mercaptans.* Springfield, C. C. Thomas. p. 187.

BERTINI, K. K. & GOLDBERG, F. D. (1971) *Norw. Hyg. Tidschr.*, Suppl. 4, p. 65.

BIDSTRUP, P. L. (1964) *Toxicity of mercury and its compounds,* Amsterdam, Elsevier Publishing Co., p. 34.

BIDSTRUP, P. L. ET AL. (1951) *Lancet*, **2**: 856.

BIRKE, G. ET AL. (1972) *Arch. environ. Health*, **25**: 77.

BORNMANN, G. ET AL. (1970) *Arch. Toxicol.*, **26**: 203.

BOUQUIAUX, J. (1974) In: *Proceedings of the International Symposium on the problems of contamination of man and his environment by mercury and cadmium, Luxembourg, 3 5 July, 1973,* CEC Luxembourg, p. 23.

BRAMEN, R. S. & JOHNSON, D. L. (1974) In: *XIth Annual NSF-RANN Trace Contaminants Conference, California, 29–31 August, 1974.* National Technical Information Service, US Department of Commerce, Springfield, Virginia, p. 75.

BRAR, S. S. ET AL. (1969) *Thermal neutron activation analysis of airborne particulate matter in Chicago metropolitan area,* Washington DC, National Bureau of Standards, p. 43.

BRUNE, D. (1969) *An. Chem. Acta.*, **44**: 15.

BURROWS, W. D. (1975) In: Krenkel, D. A., ed. *Heavy metals in the aquatic environment,* Oxford, Pergamon.

BURTON, J. D. & LEATHERLAND, T. M. (1971) *Nature (Lond.)*, **231**: 440.

BYRNE, A. R. & KOSTA, L. (1974) *Talanta*, **21**: 1083.

CARDOZO, R. L. (1972) *Chem. Weekbl.*, **68**: 9.

CAVANAGH, J. B. & CHEN, F. C. K. (1971) *Acta Neuropathol.*, **19**: 216.

CEC, Working Group of Experts (1974) *Non-organic Micropollutants of the Environment. Methods of Analysis. Report.* Luxembourg, Commission of the European Communities. VF/1966/74é.

CEMBER, H. (1962) *Am. ind. Hyg. Assoc. J.*, **23**: 304.

CHANG, L. W. & HARTMANN, H. A. (1972a) *Acta Neuropathol.*, **20**: 122.

CHANG, L. W. & HARTMANN, H. A. (1972b) *Acta Neuropathol.*, **20**: 316.

CHANG, L. W. & HARTMANN, H. A. (1972c) *Acta Neuropathol.*. **21**: 179.

CHANG, L. W. & YAMAGUCHI, S. (1974) *Environ. Res.*. 7: 133.

CHANG, L. W. ET AL. (1974) *Acta Neuropathol.*, **27**: 171.

CHARBONNEAU, S. M. ET AL. (1974) *Toxicol. appl. Pharmacol.*, **27**: 569.

CHAU, Y. K. & SAITOH, H. (1970) *Environ. Sci. Technol.*, **4**: 839.

CHILDS, E. A. (1973) *Arch. Environ. Health*, **27**: 77.

CLARKSON, T. W. (1972a) *Recent advances in toxicology of mercury with emphasis on the alkyl mercurials.* In: Goldberg, L. ed. *Critical reviews in toxicology*, Vol. 1, Cleveland, The Chemical Rubber Co., issue 2, p. 203.

CLARKSON, T. W. (1972b) *Ann. Rev. Pharmacol.*, **12**: 375.

CLARKSON, T. W. ET AL. (1961) *Studies on the equilibration of mercury vapor with blood,* Rochester, University of Rochester Atomic Energy Project, U.R. 582, p. 64.

CLARKSON, T. W. & ROTHSTEIN, A. (1964) *Health Phys.*, **10**: 1115.

CLARKSON, T. W. & VOSTAL, J. (1973) *Mercurials, mercuric ion and sodium transport,* In: Lant, A. F. & Wilson, G. M., ed. *Modern diuretic therapy in the treatment of cardiovascular disease*, Amsterdam, Excerpta Medica, p. 229.

CLARKSON, T. W. ET AL. (1965) *Br. J. Pharmacol.*, **24**: 1.

CLARKSON, T. W. ET AL. (1972) *Biol. Neonat.*, *(Basel)*, **21**: 239.

CLARKSON, T. W. ET AL. (1973a) *Arch. environ. Health*, **26**: 173.

CLARKSON, T. W. ET AL. (1975) In: Krenkel, D. A., ed. *Heavy metals in the environment,* Oxford, Pergamon.

CLEGG, D. J. (1971) In: *Proceedings of the Symposium on Mercury in Man's Environment, Ottawa, 15–16 February 1971,* Ottawa, Royal Society of Canada, p. 141.

COPPLESTONE, J. F. & McARTHUR, D. A. (1967) *Br. J. ind. Med.*, **24**: 77.

COTTON, F. A. & WILKINSON, L. (1972) *Advanced inorganic chemistry—a comprehensive text,* New York, Wiley Inter-Science Publications. Third edition.

DALGAARD-MIKKELSEN, S. (1969) *Norw. Hyg. Tidschr.*, **50**: 34.

DALL'AGLIO, M. (1968) *The abundance of mercury in 300 natural water samples from Tuscany and Altium.* In: Ahrens, L. H., ed. *Origin and distribution of the elements,* New York, Pergamon Press, p. 1065.

DAMLUJI, S. (1962) *J. Fac. Med. Baghdad,* 4: 83.

DAMLUJI, S. ET AL. (1976) In: *World Health Organization Conference on Intoxication due to Alkyl mercury Treated Seed, Baghdad, 9–13 November 1974,* Geneva, World Health Organization, p. 11 (Suppl. to *Bull. World Health Organ.*, Vol. 53).

DANZINGER, S. J. & POSSICK, P. A. (1973) *J. occup. Med.*, **15**: 15.

DDR Standards (1968) TGL 22310. Berlin, German Democratic Republic.

DIEHL, J. F. & SCHELLENZ, R. (1974) In: *Proceedings of the International Symposium on the problems of contamination of man and his environment by mercury and cadmium, Luxembourg, 3–5 July 1973,* CEC, Luxembourg, p. 401.

D'ITRI, F. M. (1972) *The environmental mercury problem.* Cleveland, Chemical Rubber Co., p. 69.

DOHERTY, P. E. & DORSETT, R. S. (1971) *Anal. Chem.*, **43**: 1887.

DONOVAN, P. P. (1974) In: *Proceedings of the International Symposium on the problems of contamination of man and his environment by mercury and cadmium, Luxembourg, 3 5 July 1973,* CEC, Luxembourg, p. 573.

DURUM, W. H. ET AL. (1971) *Reconnaissance of minor elements in surface waters of the United States,* Washington, DC, p. 49 (US Geological Survey Circular 643).

DUTKIEWICZ, T. & OGINSKI, M. (1967) *Int. Arch. Gewebepath. Gewebehygiene,* **23**: 197.

EINARSSON, K. ET AL. (1974) *Acta med. scand.*, **195**, 527.

EL-BERGEAMI, M. M. ET AL. (1973) *Fed. Proc.*, **32**: 886.

ENGLESON, G. & HERNER, T. (1952) *Acta pediatr. scand.*, **41**: 289.

ERIKSSON, E. (1967) *Oikos,* Suppl. 9, p. 13.

FAGERSTROM, R. & JERNELOV, A. (1971) *Water Res.*, **5**: 121.

FERNANDEZ, M. DE N. ET AL. (1966) *Rev. de Sanid Hyg. Publica, Madrid,* **40**: 1.

FESENKO, S. (1969) *Vrac. Delo*, **19**: 85.

FILBY, R. H. ET AL. (1970) *Mikrochim. Acta.*, **6**: 1130.

FISHMAN, M. J. (1970) *Anal. Chem.*, **42**: 1462.

FITZHUGH, O. G. ET AL. (1950) *Arch. ind. Hyg. occup. Med.*, **2**: 433.

FLEWELLING, F. J. (1975) In: Krenkel, D. A., ed. *Heavy metals in the aquatic environment.* Oxford, Pergamon.

FONDS, A. W. (1971) *TNO-Nieuws.*, **26**: 375.

FORRESTER, C. R. ET AL. (1972) *J. Fish. Res. Board Can.*, **29**: 1487.

FOWLER, B. A. (1972a) *Science*, **175**: 780.

FOWLER, B. A. (1972b) *Am. J. Pathol.*, **69**: 163.

FRIBERG, L. (1951) *Norw. Hyg. Tidschr.*, **32**: 240.

FRIBERG, L. (1956) *Acta pharmacol. toxicol.*, **12**: 411.

FRIBERG, L. & VOSTAL, J. ed. (1972) *Mercury in the environment—a toxicological and epidemiological appraisal*, Cleveland, The Chemical Rubber Co.

FRIBERG, L. & NORDBERG, G. F. (1972) *Inorganic mercury— relation between exposure and effects.* In: Friberg, L. & Vostal, J. ed. *Mercury in the environment— a toxicological and epidemiological appraisal*, Cleveland, Chemical Rubber Co., p. 7.

FRIBERG, L. & NORDBERG, G. F. (1973) *Inorganic mercury  -a toxicological and epidemiological appraisal.* In: Miller, M. W. & Clarkson, T. W., ed. *Mercury, mercurials and mercaptans*, Springfield, C. C. Thomas, p. 5.

FRIBERG, L. ET AL. (1961) *Acta derm. venereol.*, **41**: 4952.

FUJIMURA, Y. (1964) *Jpn. J. Hyg.*, **18**: 10.

FUJITA, M. ET AL. (1968) *Anal. Chem.*, **40**: 2042.

FURUKAWA, K. ET AL. (1969) *Agric. biol. Chem.*, **33**: 128.

GAFAFER, W. M. ed. (1966) *Occupational diseases  -a guide to their recognition.* US Department of Health, Education and Welfare, Public Health Service, pp. 175–176 (Public Health Service Publication 1097).

GAGE, J. C. (1961) *Br. J. ind. Med.*, **18**: 287.

GAGE, J. C. (1964) *Br. J. ind. Med.*, **21**: 197.

GAGE, J. C. (1974) *The metabolism of methoxyethyl mercury and phenyl mercury in the rat.* In: Miller, M. W. & Clarkson, T. W., ed. *Mercury, mercurials and mercaptans.* Springfield, C. C. Thomas. p. 346.

GANTHER, H. E. & SUNDE, M. L. (1974) *J. Food Sci.*, **39**: 1.

GANTHER, H. E. ET AL. (1972) *Science*, **175**: 1122.

GAVIS, J. & FERGUSON, J. F. (1973) *Water Res.*, **6**: 989.

GIOVANOLI, T. & BERG, G. G. (1974) *Arch environ Health*, **28**: 139.

GIOVANOLI, T. ET AL. (1974) *Clin. Chem.*, **30** (2): 222.

GLOMME, J. & GUSTAVSON, K. H. (1959) *Acta med. scand.*, **164**: 175.

GOLDWATER, L. J. (1964) *J. R. Inst. publ. health Hyg.*, **27**: 270.

GOLDWATER, L. J. (1973) *Aryl and alkoxyalloyl mercurials.* In: Miller, M. W. & Clarkson, T. W., ed. *Mercury, mercurials and mercaptans.* Springfield, C. C. Thomas, p. 56.

GOLDWATER, L. J. ET AL. (1963) *Arch. environ. Health*, 7: 586.

GOULD, E. S. (1962) *Inorganic reactions and structures*, New York, Holt, Rinehart and Winston.

GRANT, C. A. (1973) *Pathology of experimental methyl mercury intoxication.* In: Miller. M. W. & Clarkson, T. W., ed. *Mercury, mercurials and mercaptans*, Springfield, C. C. Thomas, p. 294.

GREENWOOD, M. R. & CLARKSON, T. W. (1970) *Am. ind. Hyg. Assoc.*, **31**: 250.

GUTENMANN, W. H. & LISK, D. J. (1960) *J. Agric. Food Chem.*, **8**: 306.

HAQ, I. U. (1963) *Br. med. J.*, **5335**: 1579.

HARADA, Y. (1968) *Congenital (or fetal) Minamata Disease.* In: Katsuna, M., ed. *Minamata Disease*, Kumamoto University, p. 93.

HARADA, Y. ET AL. (1971) *Clinical Study (Rinsho to Kenkyu)*, **48**: 1431.

HASANEN, E. (1974) In: *Proceedings of the International Symposium on the problems of contamination of man and his environment by mercury and cadmium, Luxembourg, 3 5 July 1973.* CEC. Luxembourg, p. 109.

124

HATCH, W. R. & OTT, W. L. (1968) *Anal. Chem.*, **40**: 2085.

HAY, W. J. ET AL. (1963) *J. Neurol. Neurosurg. Psychiatr.*, **26**: 199.

HAYES, A. L. & ROTHSTEIN, A. (1962) *J. Pharmacol. exp. Ther.*, **138**: 1.

HEINDRYCKX, R. ET AL. (1974) In: *Proceedings of the International Symposium on the problems of contamination of man and his environment by mercury and cadmium, Luxembourg, 3 -5 July 1973*, CEC, Luxembourg, p. 135.

HINKLE, M. E. & LEARNED, R. E. (1969) *U.S. Geol. Survey Prof. Paper*, No. 650, p. 1251.

HOLDEN, A. B. (1972) *Present levels of mercury in man and his environment*. In: *Mercury contamination in man and his environment*, Vienna, IAEA, p. 143.

HOSOHARA, K. (1961) *J. chem. Soc. Jpn.*, **82**: 1107.

HUGHES, W. L. (1957) *Ann. N.Y. Acad. Sci.*, **65**: 454.

HUNTER, D. (1969) *Diseases of occupations*, London, Little, Brown and Co., p. 306.

HUNTER, D. ET AL. (1940) *Quart. J. Med.*, **9**: 193.

HUNTER, D. & RUSSELL, D. S. (1954) *J. Neurol. Neurosurg. Psychiat.*, **17**: 235.

HURSH, J. ET AL. (1976) *Arch. environ. Health* (in press).

INTERNATIONAL ATOMIC ENERGY AGENCY (1972) *Mercury contamination in man and his environment*, Vienna, International Atomic Energy Agency, Technical Report Series No. 137.

INTERNATIONAL LABOUR ORGANISATION (1970) *Permissible levels of toxic substances in the working environment*, Geneva, International Labour Organisation, Occupational Safety and Health Series 20.

JACOBS, M. B. ET AL. (1964) *Arch. environ. Health*, **9**: 454.

JAKUBOWSKI, M. ET AL. (1970) *Toxicol. appl. Pharmacol.*, **16**: 743.

JALILI, M. A. & ABBASI, A. H. (1961) *Br. J. ind. Med.*, **18**: 303.

JENSEN, S. & JERNELOV, A. (1967) *Biocidinformazion*, **10**: 4.

JENSEN, S. & JERNELOV, A. (1968) *Biocidinformazion*, **16**: 3.

JENSEN, S. & JERNELOV, A. (1969) *Nature (Lond.)*, **223**: 753.

JENSEN, S. & JERNELOV, A. (1972) *Behavior of mercury in the environment*. In: *Mercury contamination in man and his environment*, Vienna, International Atomic Energy Agency, Technical Report Series No. 137, p. 43.

JERNELÖV, A. (1968) *Fatten*, **24**: 456.

JERNELÖV, A. (1973) *A new biochemical pathway for the methylation of mercury and some ecological considerations*. In: Miller, M. W. & Clarkson, T. W., ed. *Mercury, mercurials and mercaptans*, Springfield, C. C. Thomas, p. 315.

JOENSUU, O. L. (1971) *Science*, **172**: 1027.

JOHNELS, A. G. (1971) *Observed levels and their dynamics in the environment, results from Sweden*. In: *Proceedings of the Symposium on Mercury in Man's Environment, Ottawa, 15–16 February 1971*, Ottawa, Royal Society of Canada, p. 66.

JOHNELS, A. G. ET AL. (1967) *Oikos*, **18**: 323.

JOHNSON, W. C. & VICKARY, C. (1970) *Analyst.*, **95**: 357.

JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (1972) *Evaluation of mercury, lead, cadmium and the food additives amaranth, diethylpyrocarbamate and octyl gallate*, WHO Food Additives Series, No. 4, FAO Nutrition Meeting Report Series No. 51a.

JOINT FAO/WHO MEETING (1974) *The use of mercury and alternative compounds as seed dressings*, WHO Technical Report Series, No. 555, 29 pp.

JONASSON, I. R. & BOYLE, R. W. (1971) In: *Proceedings of the Symposium on Mercury in Man's Environment, Ottawa 15–16 February 1971*, Ottawa, Royal Society of Canada, p. 5.

JONSSON, E. ET AL. (1972) *Var Foda*, **24**: 59.

JOSELOW, M. M. & GOLDWATER, L. J. (1967) *Arch. environ. Health*, **15**: 155.

JULIUSBERG, F. (1901) *Arch. Derm. Syph.*, **56**: 5.

KANAZAWA, J. & SATO, R. (1959) *Jpn. Analyst.* **8**: 322.

KATSUNA, M. ed. (1968) *Minamata disease*, Kumamoto University, Japan.

KAZANTZIS, G. ET AL. (1962) *Quart. J. Med.*, **31**: 403.

KAZANTZIS, G. ET AL. (1976) In: *World Health Organization Conference on Intoxication due to Alkyl mercury Treated Seed, Baghdad, 9–13 November 1974*, Geneva, World Health Organization, pp. 37, 49 (Suppl. to *Bull. World Health Organ.*, Vol. 53).

KESIC, B. & HAEUSLER, V. (1951) *Ind. Med. Surg.*, **20**: 485.

KHERA, K. S. (1973) *Toxicol. appl. Pharmacol.*, **24**: 167.

KITAGAWA, T. (1968) *Clinical investigations in Minamata Disease. Rehabilitation in Minamata Disease.* In: Katsuna, M., ed. *Minamata Disease*, Kumamoto University, p. 127.

KLEINERT, S. J. (1972) *Mercury levels in Wisconsin fish*, In: Hartung, R. & Dinman, E. D., ed. *Environmental mercury contamination*, Ann Arbor, Ann Arbor Science Publishers, p. 58.

KLEIN, R. H. & HERMAN, S. P. (1971) *Science*, **172**: 872.

KLEIN, R. ET AL. (1972) *Arch. Pathol.*, **93**: 408.

KLEIN, R. ET AL. (1973) *Arch. Pathol.*, **96**: 83.

KLOKE, A. (1974) In: *Proceedings of the International Symposium on the problems of contamination of man and his environment by mercury and cadmium, Luxembourg, 3–5 July 1973*, CEC Luxembourg, p. 83.

KOEMAN, J. H. ET AL. (1972) *TNO Nieuws*, **27**: 570.

KOEMAN, J. H. ET AL. (1973) *Nature Lond.*, **245**: 385.

KOPP, J. F. ET AL. (1972) *J. Am. Water Works Assoc.*, p. 40.

KORONOWSKI, P. (1973) *Nebenwirkungen von Quecksilberverbindungen auf Mensch und Tier.* Mitt. d. Biolog. Bundesanstalf f. Land-und Forstwirtschaft, No. 153, Berlin.

KORRINGA, P. & HAGEL, P. (1974) In: *Proceedings of the International Symposium on the problems of contamination of man and his environment by mercury and cadmium, Luxembourg 3–5 July 1973*, CEC, Luxembourg, p. 279.

KOSTA, L. ET AL. (1975) In: *Recent advances in the assessment of the health effects of pollution, WHO/EPA/CEC Symposium, Paris 8–11 June 1974*, Paris, p. 245.

KOSTYNIAK, P. J. ET AL. (1975) *J. Pharmacol. exp. Ther.*, **192**: 260.

KRENKEL, D. A., ed. (1975) *Heavy metals in the aquatic environment*, Oxford, Pergamon, pp. 352.

KUDSK, F. N. (1964) *Scand. J. clin. Lav. Invest.*, **16**: 575.

KUDSK, F. N. (1965a) *Acta Pharmacol.*, **23**: 250.

KUDSK, F. N. (1965b) *Acta Pharmacol.*, **23**: 263.

KUDSK, F. N. (1973) *Biological oxidation of elemental mercury*, In: Miller, M. W. and Clarkson, T. W., ed. *Mercury, mercurials and mercaptans*, Springfield, C. C. Thomas, p. 355.

KURLAND, L. T. (1973) *An appraisal of the epidemiology and toxicology of alkyl mercury compounds.* In: Miller, M. W. & Clarkson, T. W., ed. *Mercury, mercurials and mercaptans*, Springfield, C. C. Thomas, p. 23.

LADD, A. C. ET AL. (1964) *Arch. environ. Health*, **9**: 43.

LADD, A. C. ET AL. (1966) *J. occup. Med.*, **8**: 127.

LAMM, C. G. & RUZICKA, J. (1972) *The determination of traces of mercury by spectrophotometry, atomic absorption, radioisotope dilution and other methods.* In: *Mercury contamination in man and his environment.* Vienna, International Atomic Energy Agency. Technical Report Series No. 137, p. 111.

LANDNER, L. (1971) *Nature (Lond.)*, **230**: 452.

LEATHERLAND, T. M. ET AL. (1971) *Nature (Lond.)*, 232: 112.

LEHOTZKY, K. (1972) *Int. Arch. Arbeitsmed.*, **30**: 193.

LEVINE, W. G. (1970) *Heavy metal antagonists.* In: Goodman, L. S. & Gilman, A., ed. *The pharmacological basis of therapeutics*, 4th ed., London, Macmillan, p. 944.

LIDMUS, V. & ULFVARSON, U. (1968) *Acta chem. scand.*, **22**: 2150.

LOFROTH, G. (1969) *Methyl mercury. A review of health hazards and side effects associated with the emission of mercury compounds into natural systems*, Stockholm, Swedish Natural Research Council, Ecol. Research Bull., No. 4.

LOVEJOY, H. B. ET AL. (1974) *J. occup. Med.*, **15**: 590.

LUCIER, G. ET AL. (1971) *Chem. biol. Interactions*, **4**: 265.

LUCIER, G. ET AL. (1972) *Pestic. Biochem. Physiol.*, **2**: 244.

LUCIER, G. ET AL. (1973) *Molecular Pharmacol.*, **9**, 237.

LUNDGREN, K. ET AL. (1967) *Scand. J. clin. Lab. Invest.*, **20**: 164.

MCCARTHY, J. H. (1968) *Mining Eng.*, **20**: 46.

MCCARTHY, J. H. (1970) *Mercury in the atmosphere.* In: *Mercury in the environment*, Washington, DC, US Government Printing Office, US Geological Survey, Professional Paper No. 713, p. 37.

MCDUFFIE, B. R. (1973) *Discussion.* In: Miller, M. W. & Clarkson, T. W., ed. *Mercury, mercurials and mercaptans*, Springfield, C. C. Thomas, p. 50.

MCGILL, C. M. ET AL. (1964) *J. occup. Med.*, **6**: 335.

MAC COMMITTEE (1969) *Arch. environ. Health*, **19**: 891.

MAGOS, L. (1967) *Environ. Res.*, **1**: 323.

MAGOS, L. (1968) *Br. J. ind. Med.*, **25**: 152.

MAGOS, L. (1971) *Analyst.*, **96**: 847.

MAGOS, L. & BUTLER, W. H. (1972) *Food, Cosmet. Toxicol.*, **10**: 513.

MAGOS, L. & CLARKSON, T. W. (1972) *J. Assoc. Offic. Anal. Chem.*, **56**: 966.

MAGOS, L. ET AL. (1964) *Br. J. ind. Med.*, **21**: 294.

MAGOS, L. ET AL. (1973) *Toxicol. appl. Pharmacol.*, **26**: 180.

MAGOS, L. ET AL. (1974) *Toxicol. appl. Pharmacol.*, **28**: 367.

MARSH, D. O. ET AL. (1974) In: *Proceedings of the 1st International Congress on Mercury, Barcelona 6–10 May 1974*, Barcelona, p. 235.

MATSUMOTO, H. ET AL. (1967) *Life Sci.*, **6**: 2321.

MIETTINEN, J. K. (1973) *Absorption and elimination of dietary mercury ($Hg^{++}$) and methyl mercury in man.* In: Miller, M. W. & Clarkson, T. W., ed. *Mercury, mercurials and mercaptans*, Springfield, C. C. Thomas, p. 233.

MILLER, G. E. ET AL. (1972) *Science*, **175**: 1121.

MILLER, M. W. & Clarkson, T. W. (1973) *Mercury, mercurials and mercaptans*, Springfield, C. C. Thomas.

MORROW, P. ET AL. (1964) *Health Phys.*, **10**: 543.

MUFTI, A. W. ET AL. (1976) In: *World Health Organization Conference on Intoxication due to Alkylmercury Treated Seed, Baghdad 9–13 November 1974*, Geneva, World Health Organization p. 23 (Suppl. to *Bull. World Health Organ.*, Vol. 53).

NEAL, P. A. ET AL. (1937) *Study of chronic mercurialism in the hatters, furcutting industry.* Washington, DC, USPHS, Public Health Bulletin 234.

NIIGATA REPORT (1967) *Report on the cases of mercury poisoning in Niigata*, Tokyo, Ministry of Health and Welfare.

NIOSH (1973) *Criteria for a recommended standard. Occupational exposure to inorganic mercury.* Washington, Public Health Service, US Department of Health, Education and Welfare.

NORDBERG, G. F., ed. (1976) Effects and dose-response relationships of toxic metals. Proceedings from an international meeting organized by the Sub-committee on the Toxicology of Metals of the Permanent Commission and International Associations on Occupational Health, Tokyo, 18–23 November 1974. Amsterdam, Elsevier.

NORDBERG, G. & SERENIUS, F. (1969) *Acta Pharmacol. Toxicol.*, **27**: 269.

NORDBERG, G. & SKERFVING, S. (1972) *Metabolism.* In: Friberg, L. & Vostal, J., ed. *Mercury in the environment*, Cleveland, Chemical Rubber Co., p. 29.

NORDBERG, G. & STRANGERT, P. (1976) In: *Effects and dose-response relationships of toxic metals. A report from an International Meeting arranged by the Sub-committee on the Toxicity of Metals, Tokyo, 18–23 November 1974*, Tokyo, p. 273.

NORDBERG, G. ET AL. (1971) *Proceedings of the International Congress of Occupational Health*, **16**: 234.

NORSETH, T. & CLARKSON, T. W. (1971) *Arch. environ. Health*, **22**: 568.

OKINAKA, S. ET AL. (1964) *Neurology*, **14**: 69.

OKUNO, I. ET AL. (1972) *J. Assoc. Offic. Anal. Chem.*, **55**: 96.

ORDONEZ, J. V. ET AL. (1966) *Bull. Offic. Sanit. Panamer.*, **60**: 510.

OSTLUND, K. (1969a) *Norw. Hyg. Tidschr.*, **50**: 82.

OSTLUND, K. (1969b) *Acta Pharmacol., Suppl.* **1**: 1.

PACCAGNELLA, R. ET AL. (1974) In: *Proceedings of the International Symposium on the problems of contamination of man and his environment by mercury and cadmium, Luxembourg 3–5 July 1973*, CEC, Luxembourg, p. 463.

PARAMESHVERA, V. (1967) *Br. J. ind. Med.*, **24**: 73.

PARIZEK, J. & OSTADALOVA, J. (1967) *Experientia*, **23**: 142.

PARIZEK, J. ET AL. (1969) In: Barltrop, D. & Burland, W. L., ed. *Mineral metabolism in paediatrics*, Oxford and Edinburgh, Blackwell Scientific Publications, p. 117.

PARIZEK, J. ET AL. (1971) In: Mertz, W. & Cornatzer, W. E., ed. *Newer trace elements in nutrition*, NY, M. Dekker Inc., pp. 85-122.

PARIZEK, J. ET AL. (1974) In: Hoekstra, W. G., Ganther, H. E. & Mertz, W., ed. *Trace element metabolism in animals*, University Park Press, p. 119.

PASSOW, H. ET AL. (1961) *Pharmacol. Rev.* **13**: 185.

PETERS, R. A. (1963) *Biochemical lesions and lethal synthesis*, New York, Macmillan.

PETERSON, C. L. ET AL. (1973) *Fisheries Bull.*, **71**: 603.

PHILLIPS, R. & CEMBER, H. (1969) *J. occup. Med.*, **11**: 170.

PIERCE, P. E. ET AL. (1972) *J. Am. med. Assoc.*, **220**: 1439.

PILLAY, K. K. ET AL. (1971) *Anal. Chem.*, **43**: 1419.

PIOTROWSKI, J. & BOLANOWSKA, W. (1970) *Med. Pracy.*, **21**: 338.

PIOTROWSKI, J. ET AL. (1969) *Med. Pracy.*, **20**: 589.

PIOTROWSKI, J. ET AL. (1974a) *Arch. Toxicol.*, **32**: 351.

PIOTROWSKI, J. ET AL. (1974b) *Toxicol. appl. Pharmacol.*, **27**: 11.

PIOTROWSKI, J. ET AL. (1975) *Int. Arch. Arbeits. Med.* (in press).

PISCATOR, M. & LIND, B. (1972) *Arch. environ. Health*, **24**: 426.

POLEŠAJEV, N. (1936) *N. Gigena truda i technicka bezopastnos i*, No. 6, p. 86.

POTTER, S. D. & MATRONE, G. (1973) *Fed. Proc.*, **32**: 929.

PRICKETT, C. S. ET AL. (1950) *Proc. Soc. exp. biol. Med.*, **73**: 585.

RAEDER, M. G. & SNEKVIK, E. (1940) *Kgl. Norske Videnskab. Selskabs Forhandl.*, **13**: 169.

RAEDER, M. G. & SNEKVIK, E. (1948) *Kgl. Norske Videnskab. Selskabs Forhandl.*, **21**: 102.

RAHOLA, T. ET AL. (1973) *Ann. clin. Res.*, **5**: 214.

RAMEL, C. (1972) *Genetic effects*. In: Friberg, L. & Vostal, J., ed. *Mercury in the environment —a toxicological and epidemiological appraisal*, Cleveland, Chemical Rubber Co., p. 9.

RAUHUT, A. & WILD, L. (1973) *Metallurgy*, **27**: 933.

REICHERT, J. K. (1973) *Z. Gewasserschultz, Wasser, Abwasser*, **10**: 277.

RESEARCH COMMITTEE ON MINAMATA DISEASE (1975) *Pathological, clinical and epidemiological research about Minamata Disease, 10 years after*, Kumamoto, Kumamoto University.

RIVERS, J. B. ET AL. (1972) *Bull. environ. Contamin. Toxicol.*, **8**: 257.

ROCHOW, E. L. ET AL. (1957) *The chemistry of organometallic compounds*, New York, John Wiley & Sons.

ROTHSTEIN, A. (1973) *Mercaptans, the biological targets for mercurials*. In: Miller, M. W. & Clarkson, T. W., ed. *Mercury, mercurials and mercaptans*, Springfield, C. C. Thomas, p. 68.

ROTHSTEIN, A. & HAYES, A. (1960) *J. Pharmacol. exp. Ther.*, **130**: 166.

SAHAGAIN, B. M. ET AL. (1966) *J. Nutr.*, **90**, 259.

SAHAGAIN, B. M. ET AL. (1967) *J. Nutr.*, **93**: 291.

SAPOTA, A. ET AL. (1974) *Med. Pracy*, **25**: 129.

SCHAMBERG, J. ET AL. (1918) *J. Am. med. Assoc.*, **70**: 142.

SCHRAMEL, P. ET AL. (1973) *Intern. J. environ. Stud.*, **5**: 37.

SEIFERT, P. & NEUDERT, H. (1954) *Zbl. Arbeitsmed.*, **4**: 129.

SELYE, H. (1970) *Science*, **168**: 775.

SERA, K. ET AL. (1962) *Kumamoto med. J.*, **15**: 38.

SERGEEV, YE. A. (1967) *Methodology of mercuriometric investigations*. In: Kransnikov, V. I., ed. *Proceedings of the first all-union conference on geochemical methods of prospecting for ore deposits*. Moscow.

SHAHRISTANI, H. & SHIHAB, K. (1974) *Arch. environ. Health*, **28**: 342.

SHAHRISTANI, H. ET AL. (1976) In: *World Health Organization Conference on Intoxication due to Alkyl mercury Treated Seed, Baghdad 9-13 November 1974*, Geneva, World Health Organization, p. 105 (Suppl. to *Bull. World Health Organ.*, Vol. 53).

SHAIKH, Z. A. ET AL. (1973) *Sequestration of mercury by cadmium-induced metallothioneine.* In: Hemphill, D. D., ed. *Trace Substances in Environmental Health,* Columbia, University of Missouri, Vol. 7, p. 12.

SILLEN, L. G. (1963) *Svensk. Kem. Tidskr.,* **75**: 161.

SJOSTRAND, B. (1964) *Anal. Chem.,* **36**: 814.

SKERFVING, S. (1974a) *Conference on Environmental Effects of Mercury, Brussels, November 1973.* In: Publicaties om het Institut voor Weltenschappen om het haefmilieu, p. 103, Brussels, Vrije Universiteit.

SKERFVING, S. (1974b) *Toxicol.,* **2**: 3.

SKERFVING, S. ET AL. (1974) *Environ. Res.,* **7**: 83.

SKOG, E. & WAHLBERG, J. E. (1964) *J. Invest. Derm.,* **43**: 187.

SMART, N. A. (1968) *Residue Rev.,* **23**: 1.

SMART, N. A. ET AL. (1969) *Analyst.,* **94**: 143.

SMITH, A. D. M. & MILLER, J. W. (1961) *Lancet,* **1**: 640.

SMITH, A. R. & MOSKOWITCH, S. (1948) *Monthly Rev. N.Y. State Dept. Labor, Div. Ind. Hyg. Safety Stand.,* **27**: 45.

SMITH, A. R. ET AL. (1949) *Monthly Rev. N.Y. State Dept. Labor, Div. Ind. Hyg. Safety Stand.,* **28**: 17.

SMITH, R. G. ET AL. (1970) *Am. ind. Hyg. Assoc. J.,* **31**: 687.

SMITH, J. C. ET AL. (1971a) *Nature (Lond.),* **232**: 393.

SMITH, J. C. ET AL. (1971b) *Fed. Proc.,* **30**: 221.

SNYDER, R. D. (1971) *New Eng. J. Med.,* **284**: 1014.

SOLOMON, J. & UTHE, J. F. (1971) In: Buhler, D. R., ed. *Mercury in the western environment,* Corvallis, Continuing Education Publications, p. 265.

SOMERS, E. (1971) In: *Proceedings of the Symposium on Mercury in Man's Environment, Ottawa 15–16 February 1971,* Ottawa, Royal Society of Canada, p. 99.

SOMJEN, G. G. ET AL. (1973a) *J. Pharmacol. exp. Ther.,* **186**: 579.

SOMJEN, G. G. ET AL. (1973b) *J. Pharmacol. exp. Ther.,* **187**: 602.

SPRAGUE, J. B. & CARSON, W. G. (1970) *Spot-checks of mercury residues in some fishes from the Canadian Atlantic coast,* Ottawa, Fisheries Research Board, Canada, Manuscript Rep. No. 1085, p. 16.

SPYKER, J. M. ET AL. (1972) *Science,* **177**: 621.

STOCK, A. & CUCUEL, F. (1934) *Naturwissenschaften,* **22**: 319.

SUMINO, K. (1968) *Kobe J. med. Sci.,* **14**: 115.

SUMINO, K. (1975) In: Krenkel, D. A., ed. *Heavy metals in the aquatic environment,* Oxford. Pergamon.

SUZUKI, T. & YOSHINO, K. (1969) *Ind. Med.,* **11**: 21.

SUZUKI, T. & TANAKA, A. (1971) *Ind. Med.,* **13**: 52.

SUZUKI, T. ET AL. (1979) *Ind. Health,* **8**: 39.

SUZUKI, T. ET AL. (1971) *Bull. environ. Contam. Toxicol.,* **5**: 502.

SUZUKI, T. ET AL. (1973) In: Miller, M. W. & Clarkson, T. W., ed. *Mercury, mercurials and mercaptans,* Springfield, C. C. Thomas, p. 209.

SWEDISH EXPERT GROUP (1971) *Norw. Hyg. Tidschr.,* Suppl. 4, p. 65.

SWENSSON, A. & ULFVARSON, U. (1968) *Acta Pharmacol. Toxicol.,* **26**: 273.

SWENSSON, A. & ULFVARSON, U. (1969) *Poult. Sci.,* **48**: 1567.

TAKAHATA, N. ET AL. (1970) *Folio Psychiat. Neurol. Jpn.* **24**: 59.

TAKEUCHI, T. (1970) In: *International Conference on Environmental Mercury Contamination, Ann Arbor 3–7 September, 1970,* Ann Arbor, p. 50.

TAKAHASHI, H. & HIRAYAMA, K. (1971) *Nature (Lond.),* **232**: 201.

TASK GROUP ON LUNG DYNAMICS (1966) *Health Phys.,* **12**: 173.

TASK GROUP ON METAL ACCUMULATION (1973) *Environ. Physiol. Biochem.,* **3**: 65.

TATTON, J. O. (1972) *Identification of mercurial compounds.* In: *Mercury contamination in man and his environment,* Vienna, International Atomic Energy Agency, Technical Report Series 137, p. 131.

TATTON, J. O. & WAGSTAFFE, P. J. (1969) *J. Chromatog.,* **44**: 284.

TEISINGER, J. & FÍEROVA-BERGEROVA, V. (1965) *Ind. Med. Surg.*, **34**: 580.

TEJNING, S. (1970) *Mercury contents in blood corpuscles and in blood plasma in non-fisheaters.* Department of Occupational Medicine, University Hospital, Lund, Report No. 700406.

TEJNING, S. & OHMAN, H. (1966) In: *Proceedings of the XVth International Congress of Occupational Health, Vienna 1966*, Vienna, p. 239.

THOMPSON, J. A. J. & McCOMAS, F. T. (1973) *Environ. Lett.*, **5**: 189.

TONOMURA, K. & KANZAKI, E. (1969) *Biochem. Biophys. Acta*, **184**: 227.

TONOMURA, K. ET AL. (1968a) *J. Ferment. Technol.*, **46**: 685.

TONOMURA, K. ET AL. (1968b) *J. Ferment. Technol.*, **46**; 506.

TORIBARA, T. Y. ET AL. (1970) *Talanta*, **17**: 1025.

TRAHTENBERG, I. M. (1969) *Zdorov'e Kiev*, p. 292 (in Russian, German translation).

TROJANOWSKA, B. (1966) *Med. Pracy.*, **17**: 535.

TROJANOWSKA, B. & AZENDZIKOWSKI, S. (in press) *Toxicol. appl. Pharmacol.*

TROJANOWSKA, B. ET AL. (1971) *Toxicol. appl. Pharmacol.*, **18**: 374.

TSUBAKI, T. (1971) In: *Special Symposium on Mercury in Man's Environment, Ottawa 15-16 February 1971*, Ottawa, p. 131.

TSUDA, M. ET AL. (1963) *Yokohama med. Bull.*, **14**: 287.

TURNER, M. D. ET AL. (1974) In: *Proceedings of the 1st International Congress on Mercury, Barcelona 5–10 May 1974*, Barcelona (in press).

UI, J. (1967) *Norw. Hyg. Tidschr.*, **50**, 139.

UKITA, T. ET AL. (1963) *J. Hyg. Chem.*, **9**: 138.

ULFVARSON, U. (1962) *Int. Arch. Gewerbepath.*, **19**: 412.

UTHE, J. P. ET AL. (1970) *J. Fish. Res. Board, Canada*, **27**: 805.

VOEGE, F. A. (1971) In: *Proceedings of the Symposium on Mercury in Man's Environment, Ottawa 15–16 February 1971*, Ottawa, Royal Society of Canada, p. 107.

VON BURG, R. ET AL. (1974) *J. Chromatogr.*, **97**: 65.

VOSTAL, J. (1972) *Transport and transformation of mercury in nature and possible routes of exposure.* In: Friberg, L. & Vostal, J. ed. *Mercury in the environment*, Cleveland, Chemical Rubber Co., p. 3.

VOSTAL, J. (1972) In: *Proceedings of the XVIIth International Congress on Occupational Health, Buenos Aires, October 1972*, Buenos Aires.

VOUK, V. B. ET AL. (1950) *Br. J. Med.*, **7**: 168.

WALLACE, R. A. ET AL. (1971) *Mercury in the environment, the human element.* Oak Ridge, Oak Ridge National Laboratory, ORNL NSF EP-1.

WATANABE, S. (1971) In: *Proceedings of the XVIth International Congress on Occupational Health, Tokyo, 22 27 September 1969*, Tokyo, p. 553.

WAHLBERG, J. E. (1965) *Arch. environ. Health*, **11**: 201.

WEAVER, J. N. (1973) *Anal. Chem.*, **45**: 1950.

WEBB, J. L. (1966) *Enzyme and metabolic inhibitors*, New York, Academic Press, Vol. 2.

WEISS, H. V. ET AL. (1971) *Science*, **174**: 692.

WERSHAW, R. L. (1970) *Sources and behavior of mercury in surface waters.* In: US Geological Survey, *Mercury in the environment*, Washington, DC, US Government Printing Office, Professional paper No. 713.

WEST, I. & LIM, J. (1968) *J. occup. Med.*, **10**: 697.

WESTERMARK, T. & LJUNGGREN, K. (1972) *The determination of mercury and its compounds by destructive neutron activation analysis.* In: *Mercury contamination in man and his environment*, Vienna, IAEA, p. 99.

WESTÖÖ, G. (1966) *Acta chem. scand.*, **20**: 2131.

WESTÖÖ, G. (1967) *Acta chem. scand.*, **21**: 1790.

WESTÖÖ, G. (1968) *Acta chem. scand.*, **22**: 2277.

WESTÖÖ, G. (1973) *Heavy metals in the aquatic environment, Nashville 4–7 December 1973.* Nashville (in press).

WHITE, M. N. & LISK, D. J. (1970) *J. Assoc. Offic. Anal. Chem.*, **53**: 530.

WHO REGIONAL OFFICE FOR EUROPE (1973) *The hazards to health of persistent substances in water.* Copenhagen, p. 147.

WILLFORD, W. A. ET AL. (1973) *J. Assoc. Offic. Anal. Chem.*, **56**: 1006.

130

WILLISTON, S. H. (1968) *J. geophys. Res.*, **73**: 7051.

WILLISTON, S. H. (1971) *J. geophys. Res.*, **28**: 1285.

WINTER, D. ET AL. (1968) *Arch. environ. Health*, **16**: 626.

WINTER, J. A. & CLEMENTS, H. A. (1972) *Analyses of mercury in water—a preliminary study of methods*. Cincinnati, US Environmental Protection Agency, EPA-R4-72-303, p. 56.

WISNIEWSKA, J. M. ET AL. (1970) *Toxicol. appl. Pharmacol.*, **16**: 754.

WISNIEWSKA, J. M. ET AL. (1972) *Acta Biochim. Pol.*, **19**: 11.

WOLF, D. ET AL. (1974) *Quecksilbermessungen am Arbeitsplatz bei der Herstellung von Leuchtstoffrohren*. STF report 1–74 Staubforschungsinstitut der gewerblichen Berufsgenossenschaften, Bonn 1974.

WOOD, K. ET AL. (1968) *Nature (Lond.)*, **220**: 173.

WORLD HEALTH ORGANIZATION (1971) *International standards for drinking water*, 3rd ed., Geneva

YOSHINO, Y. ET AL. (1966) *J. Neurochem.*, **13**: 1223.

# ERRATA

Page 29, line 21:

> *For* "gungicide" read "fungicide"

Page 41, line 28–29:

> *Delete* D'Itri et al., 1972
> *Insert* D'Itri, 1972

Page 55, line 28:

> *Delete* environmental layers
> *Insert* environmental levels