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HEALTH EFFECTS OF METHYLMERCURY

Technical Report

Prepared by:
MONITORING AND ASSESSMENT RESEARCH CENTRE
Chelsea College, University of London

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Health effects of methylmercury

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Abstract

This study critically reviews recent data relating to the health effects of methylmercury in man and the attendant dose-response relationships. New data obtained from animal studies are also examined, including pre- and post-natal exposure. The consumption of fish represents the major source of methylmercury exposure in the general population. For this reason the mercury levels in fish throughout the world have been assessed, with particular attention being paid to the situation in the Mediterranean Sea. Here, there is limited knowledge of methylmercury intake in critically exposed populations such as fishermen, employees of the fish industries and their families.

The measurement of mercury in hair is now regarded as the most useful indicator of exposure but more experimental data are required to increase the value of this index.

The threshold levels of methylmercury in blood, hair and for dietary intake, as estimated by the World Health Organization, have been largely endorsed but new information from Japan and Canada suggests a latency period for some effects which are inversely related to the duration of exposure. Incorporation of such findings should therefore lead to the designation of lower threshold values than presently recognized. Pregnant women and the foetus have been identified as groups which may be at special risk. Blood mercury levels in the foetus are approximately twice those of the mother and this should be taken into account when assigning threshold concentrations for the protection of the foetus.

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FOREWORD

This report was originally prepared by MARC as a background document for a WHO consultation in Geneva in April 1980, to re-examine the WHO Environmental Health Criteria Document on Mercury. Following this meeting, the report was completed by the addition of chapter 5 (selenium) and the other chapters were revised in the light of newly acquired data.

The second draft was subsequently used as a consultation document at a UNEP/FAO/WHO meeting of experts on "Environmental Quality Criteria for Mercury in Mediterranean Seafood" (Geneva 3–6 November 1980).

With some exceptions, the report covers data in the literature from the period 1975–1979, together with some reports which were available early in 1980. For relevant references covering the period prior to 1975, the reader is referred to the Environmental Health Criteria Document, 1: Mercury, WHO Geneva (1976b).

The authors wish to acknowledge assistance obtained from the Global Environmental Monitoring System (GEMS) and Regional Seas Programme Activity Centre (RS/PAC) of the United Nations Environment Programme (UNEP). Throughout the project, co-operation was maintained with the Division of Environmental Health, World Health Organization, Geneva, and the International Register of Potentially Toxic Chemicals (IRPTC) of UNEP.

The authors are greatly indebted to Dr J. Parizek (WHO, Geneva) for his initiative and continued interest, and to Professor T. W. Clarkson (University of Rochester, New York) for arranging a study tour in the U.S.A. and Canada for Prof. Piotrowski, as well as for discussion of the problem. Drs D. O. Marsh and B. Weiss (University of Rochester, New York), N. K. Mottet (University of Washington, Seattle), W. A. Reynolds (Ohio State University, Columbus), D. J. Thomas (University of North Carolina School of Medicine, Chapel Hill, North Carolina), J. M. Spyker (University of Arkansas for Medical Sciences, Little Rock), C. T. Miller, S. M. Charbonneau and B. Wheatley (Health and Welfare, Ottawa, Canada), all made this report possible, not only through discussion of the most recent developments in the field but in many instances by

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1 Methylmercury in fish: the source of human exposure

1.0 Summary of information contained in the Health Criteria Document [HCD]

The mercury level in the muscle tissue of most freshwater and marine fish is less than 100 to 200 $\mu g\ kg^{-1}$ wet weight*, but higher levels of 200 to 5,000 $\mu g\ kg^{-1}$ may be found in fish from contaminated freshwater, reaching 20,000 $\mu g\ kg^{-1}$ in badly polluted waters. The highest levels are usually found in the large carnivorous fish at the top of the food chain, such as the oceanic tuna and swordfish, where levels of up to 1,000 $\mu g\ kg^{-1}$ or more may be found. Mercury levels in tuna from the Mediterranean sea appear to be elevated in comparison with levels found in tuna from other areas. The geographical location of the fish and particularly the age (or weight) seem to be important factors governing the mercury level in the fish species. Virtually all the mercury in fish muscle is in the form of methylmercury and analyses of museum specimens seem to indicate that there may have been little change in mercury levels of oceanic species since the turn of the century.

1.1 General comments

Levels of mercury in fish vary widely depending on the position of the various fish species in the food chain, with the higher levels usually occurring in the large secondary carnivores and the lowest in the small herbivores and omnivores. Increasing mercury concentration with increasing size or age has often been demonstrated in predatory species but is less apparent in other fish.

The majority of the commercially important food fishes throughout the world have mercury concentrations in their muscle tissue of less than 200–300 $\mu g\ kg^{-1}$. Higher levels found in predatory species—often 500–1,000 $\mu g\ kg^{-1}$—may occur even in non-contaminated waters and include such fish as tuna, swordfish, marlin and members of the shark family in the marine environment, and pike, largemouth bass and walleye from freshwater rivers and lakes. Levels above 500 $\mu g\ kg^{-1}$ have also been found in other fish species from polluted areas and from many parts of the Mediterranean sea; in the latter case, some of the areas correspond to regions of known geochemical mercury anomalies.

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* Wet weight values are used throughout.

1.2 Freshwater fish

The natural or "background" mercury concentrations for most freshwater species appear to range from 50 to 200 $\mu g\ kg^{-1}$ and may be less than 10 $\mu g\ kg^{-1}$ in short-lived herbivorous species (Johnels, Tyler and Westermark 1979, Suckcharoen, Nuorteva and Häsänen 1978). In predatory species such as pike, "natural" background levels of about 200 $\mu g\ kg^{-1}$ for a 1.0 kg fish are suggested (Johnels *et al.* 1979) and for larger fish, levels of up to 1,000 $\mu g\ kg^{-1}$ are not uncommon. Average levels of 250 to 300 $\mu g\ kg^{-1}$ have been found in pike from lakes and rivers in Hungary (Gergely, Soos, Erdelyi and Cieleszky 1977) and from the coast of Finland (Linko and Terho 1977).

In a few areas of the world, levels in freshwater fish have been considerably higher. In Niigata Prefecture, Japan, 45 per cent of edible fish analysed from the Agano River in 1965 had mercury levels of between 1,000 and 20,000 $\mu g\ kg^{-1}$. The two major fish species consumed, *Hemibarbus barbus* and *Triblodon hakonensis*, had mean mercury levels of 6,600 and 2,600 $\mu g\ kg^{-1}$ respectively (Tsubaki and Irukayama 1977(a)).

In Canada, in the late sixties and early seventies, considerably elevated values were reported for pike, walleye and burbot from the severely polluted Wabigoon–English–Winnipeg river system, with average levels from 9,000 to 22,000 $\mu g\ kg^{-1}$ in one area. In 1975, a large survey of fish from both "contaminated" and "uncontaminated" lakes in northwest Ontario, Canada, revealed levels of 400 to 1,300 $\mu g\ kg^{-1}$ in walleye* and pike* and 40 to 280 $\mu g\ kg^{-1}$ in whitefish* (Bishop and Neary 1976). A more recent review of reported data from "contaminated" and "uncontaminated" waters in Quebec, Canada, gives average levels of 600 to 900 $\mu g\ kg^{-1}$ with upper levels of about 5,000 $\mu g\ kg^{-1}$ (Sherbin 1979). In waters where industrial contamination has ceased or been reduced, the mercury levels in fish have tended to decline year by year, but fish in many of the polluted river systems in Canada and the U.S.A. are still often high in mercury.

New reports of elevated mercury concentrations in freshwater fish species are still being published and an example of recent concern may

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* Since the demonstrated correlation between the methylmercury level and the size of fish, some authors give "normalized" levels for given sizes of fish, in this case 20″ for walleye and whitefish, and 25″ for pike. This allows comparison of fish of widely varying sizes and weights, from one lake to another and from one year to the next.

be the high levels—up to 2,000 $\mu g\ kg^{-1}$—in a commercially fished species, *Ophiocephalus striatus*, from freshwaters near a chlor-alkali plant in Samut Prakarn Province, Thailand (Suckcharoen *et al.* 1978).

Despite the concern over the danger of high mercury levels, the available data for freshwater fish are limited to a few areas of the world only, such as the U.S.A., Canada, Japan and Scandinavia. For other highly industrialized areas (including the rest of Europe), relatively few data are available.

1.3 Marine fish

The situation regarding mercury concentrations in marine fish has not altered appreciably since assessments were carried out in the mid-seventies. Levels in the majority of commercially important species are less than 500 $\mu g\ kg^{-1}$ with average levels usually 100 to 200 $\mu g\ kg^{-1}$, except for the larger predatory species such as tuna, shark and swordfish, and also for large slow-growing fish such as halibut.

In the U.S.A., a survey of 205 species of fish, molluscs and crustaceans (representing 93 per cent of the American commercial catch) revealed mean mercury levels exceeding 500 $\mu g\ kg^{-1}$ in less than 2 per cent of cases, with average levels below 300 $\mu g\ kg^{-1}$ in most of the "finfish" species (Hall *et al.* 1976). In another survey of 32 edible fish and shellfish species, an overall mean concentration of 130 $\mu g\ kg^{-1}$ was found with an average level in shellfish and crustaceans of 50 $\mu g\ kg^{-1}$ (Zook *et al.* 1976).

In 1971, a comprehensive survey of mercury levels in commercially important Canadian species, including cod, haddock, redfish, herring and mackerel, showed average values ranging from 20 to 230 $\mu g\ kg^{-1}$, with most below 100 $\mu g\ kg^{-1}$, although higher levels from 180 to 1,000 $\mu g\ kg^{-1}$ were reported for tuna, swordfish, lingcod and halibut (Bligh and Armstrong 1971). In waters off Quebec, Canada, mean levels of 270 $\mu g\ kg^{-1}$ were reported for 9 species of marine fish. Mean values for salmon, herring, anchovy and tuna samples were all below 500 $\mu g\ kg^{-1}$ in samples from British Columbia (Sherbin 1979).

Elsewhere, low levels have been found for cod—20 to 40 $\mu g\ kg^{-1}$—in catches off Greenland and in the Norwegian and Barents seas and slightly higher values of 20 to 320 $\mu g\ kg^{-1}$ were reported for cod from the North Atlantic (ICES 1977). In the North Sea, levels of 70 to 200 $\mu g\ kg^{-1}$ were reported for fish including herring, whiting, plaice and sole

(Baeyens, Decadt and Elskens 1979). Higher concentrations have been found in fish from areas nearer industrially contaminated coasts, with a range of 100 to 600 $\mu g\,kg^{-1}$ in parts of the west Irish Sea (Gardner 1978). In a dietary study undertaken in the United Kingdom, the average mercury concentration in the fish consumed was about 200 $\mu g\,kg^{-1}$ (Haxton *et al.* 1979). In several studies of levels in crabs, shrimps and mussels, the range tended to be 20 to 200 $\mu g\,kg^{-1}$, with most levels below 100 $\mu g\,kg^{-1}$, except for industrial coastlines where levels were slightly higher (De Wolf 1975).

In other parts of the world, levels in the more important food fishes are generally low, with some reported values of 160 $\mu g\,kg^{-1}$ average in the Persian Gulf (Parvaneh 1979), 90 $\mu g\,kg^{-1}$ in western Malaysia (Babji, Embong and Woon 1979), usually less than 100 $\mu g\,kg^{-1}$ in fish from the Pacific Coast and Gulf of Mexico (Reimer and Reimer 1975), and very low levels, from 5 to 40 $\mu g\,kg^{-1}$, in fish from waters around the Indian subcontinent (Ramamurthy 1979). Mean levels of less than 100 $\mu g\,kg^{-1}$ were found in herbivorous, omnivorous and (primary) carnivorous fish species in Hawaiian waters (Klemmer, Unninayer and Okubo 1976).

Recent concern over the high levels of mercury in certain fish species from Mediterranean waters has prompted considerable research, and published data have been comprehensively collected (IRPTC 1980). In general, the abundant data reveal the following: for many species, including tuna, anchovy, sardine and mackerel, and using combined data from several sources, significantly elevated mercury levels have been shown when compared with levels in the same species from the Atlantic Ocean (Bernhard and Renzoni 1977; Bernhard 1978). For the majority of commercially important fish species harvested from the Mediterranean and Black Sea—anchovy, pilchard, mackerel, sardinella, red mullet and bogue (Charbonnier 1977)—average mercury levels usually range from 100 to 300 $\mu g\,kg^{-1}$, similar to the values for the economically less important hake, picarel, grey mullet and goby species (IRPTC 1980 and other sources). A regional comparison of levels found in the red mullet has been made (Bernhard and Renzoni 1977 and Bernhard 1978), and in most areas of the Mediterranean sea mercury levels are about 300 $\mu g\,kg^{-1}$ (see also IRPTC 1980) with higher levels of 1,000 to 3,700 $\mu g\,kg^{-1}$ occurring in an area of the Tuscany coast of Italy which receives drainage water from an area where mercury ore is actively being mined. Bonito and horse mackerel represented about 15 per cent of the

total commercial fish catch in 1969 (Charbonnier 1977). Limited data available for these two species pointed to higher levels of about 600 $\mu g\,kg^{-1}$ (see IRPTC 1980). Evidence for enhanced mercury exposure of predatory species in the Mediterranean is given by the identification of two separate populations of tuna fish, one of which spends only four to five months of the year there in the summer and the rest of the year in the Atlantic Ocean. This population has a lower mercury level to body weight ratio with tissue mercury concentrations below 1,000 $\mu g\,kg^{-1}$ whereas the resident tuna population has a mercury to body weight ratio three to five times as high and tissue levels up to 4,000 $\mu g\,kg^{-1}$ (Bernhard and Renzoni 1977, and Renzoni, Bernhard, Sara and Stoeppler 1978). With regard to the more important invertebrate catches in the Mediterranean, mercury levels in mussels, cuttlefish, shrimps and octopus range from 160 to 340 $\mu g\,kg^{-1}$, with some levels occasionally being above 500 $\mu g\,kg^{-1}$, usually near areas of industrially contaminated inshore waters (see IRPTC 1980).

Intercomparisons between areas are made difficult when the sizes of fish analysed are not given, as high mercury content may indicate greater size (age) of an organism rather than an elevated environmental exposure (Stoeppler, Bernhard, Backhaus and Schulte 1979). Nevertheless, the data from the Mediterranean appear to point to levels higher than in other areas. Tuna, in particular the bluefin variety, have often been found with mercury levels above 1,000 $\mu g\,kg^{-1}$ (Stoeppler *et al.* 1979, Cumont *et al.* 1975, Aubert 1975, Bo 1977) and mean levels appear to be about 1,200 $\mu g\,kg^{-1}$ (IRPTC 1980, see Table 1). A survey of fish on sale in Modena, Italy, revealed a mean level of 1,000 $\mu g\,kg^{-1}$ in tuna and 4,000 $\mu g\,kg^{-1}$ in shark meat, with one sample of the latter containing almost 10,000 $\mu g\,kg^{-1}$ (Vecchi, Bergomi and Vivoli 1977). Fifty-seven per cent and 100 per cent respectively of these fish species had levels above 700 $\mu g\,kg^{-1}$, as did 54 per cent of fish samples analysed from the coast near Bari, south-east Italy (Signorile 1979). Levels here averaged 1,800 $\mu g\,kg^{-1}$ for ray and dogfish species and 4,300 $\mu g\,kg^{-1}$ for anglerfish, one sample of which contained 13,000 $\mu g\,kg^{-1}$.

Outside the Mediterranean, tuna fish species also have elevated mercury levels with 100 to 700 $\mu g\,kg^{-1}$ being reported for fish from the Pacific, Atlantic and Indian Oceans (Greig and Krzynowek 1979, Cumont, Gilles and Feinberg 1977, IRPTC 1978 and Ramamurthy 1979). In the literature, the highest levels reported have been for black marlin (weighing up to 500 kg) which have mean mercury levels of about

7,000 $\mu g\ kg^{-1}$ up to a maximum of 16,500 $\mu g\ kg^{-1}$ in oceanic waters off north-east Australia (Mackay, Kazacos, Williams and Leedow 1975). In nearby waters, commercial shark species contained 1,000 to 3,000 $\mu g\ kg^{-1}$ in their muscle tissue with the grey nurse shark having an upper level of 7,000 $\mu g\ kg^{-1}$ (Caputi, Edmonds and Heald 1979). In swordfish and blue marlin, levels of up to 4,000 and 8,000 $\mu g\ kg^{-1}$ have been reported for specimens from the Atlantic and Pacific Oceans respectively (Freeman, Shum and Uthe 1978 and Shultz *et al.* 1976) although the mean level for these fish is usually 1,000 to 1,500 $\mu g\ kg^{-1}$. It is thought that these high levels in large oceanic fish are "natural" in origin and are due to their predatory feeding habits and long life.

1.4 Estimate of effective human exposure to methylmercury from dietary fish consumption (see also Table 2)

The average daily consumption of fish produce varies widely from country to country depending on dietary habits. In countries bordering the Mediterranean sea, the average per capita intake ranges from about 4 g to 60 g per day (see Bernhard and Renzoni 1977). A figure of 20 g per day is used to represent an average daily intake (N.A.S. 1978 and F.A.O. 1977) and corresponds approximately to the consumption of one fish meal per week.

Some countries in Europe such as Sweden, Denmark and Spain have a daily intake two to three times as high as this, and in Japan the average consumption is about 100 g per day.

Figures as high as 200 to 300 g per day have been given as either typical or the upper limits for selected populations in the U.S.A., the U.K. and Sardinia (N.A.S. 1978, Haxton *et al.* 1979 and Bernhard and Renzoni 1977). On the other hand, values as high as 300 g per day were reported from Minamata and Niigata in Japan (Tsubaki and Irukayama 1977(a)) and 450 g per day for some of the native Canadian Indians (Barbeau, Nantel and Dorlot 1976).

The very highest fish consumers, for whom the daily intake of 1,000 g may apply, are mainly fishermen, fish retailers and tourist guides in sports fishing areas. In western Europe, the highest estimated intake is 800 g per day (Bernhard and Renzoni 1977) which compares with estimated upper limits of about 1,300 g per day in Canadian Indians during the high fishing season (Barbeau *et al.* 1976) and 800–1,500 g

Table 1 Approximate values* for average levels of mercury ($\mu g\ kg^{-1}$ wet weight) in muscle tissue of various fish species

Fish species \ Ocean/Sea	Atlantic	Pacific	Indian	Mediterranean§
Mackerel	70–200[a]	160–250[b]	5[c]	240[d]
Sardine	30–60[e]	30[f]	6[g]	150[h]
Unspecified no. of edible (non-predatory) spp.	80–270[i]	70–90[i]	20–160[k]	100–300[j]
Predatory				
Tuna spp.	300–800[m]	300[n]	65–400[p]	1200[q]
Swordfish	800–1300[r]	1600[s]	—	1800[t]
Shark, Dogfish, Ray spp.	1000[u]	700–1100[v]	40–1500[w]	1800[x]

References:
a 17, 89, 244
b 6, 58
c 208
d 108 (average of mean values in many reports)
e 17, 244
f 6
g 209
h 108
i 83, 96, 271
j 11, 124, 251
k 201, 208
l 108
m 23, 60
n 91
p 91, 107, 208
q 24, 108 (main source), 235, 273
r 23, 74
s 6 (one value only)
t 24, 108
u 89
v 23, 176
w 33, 208
x 24, 108 (main source), 235, 273

* The range of mean values in most cases.
§ Majority of information comes from IRPTC (1980).

per day in poisoned inhabitants from Minamata and Niigata, Japan (Tsubaki and Irukayama 1977(a), Takeuchi and Eto 1975).

The health consequences arising from the consumption of contaminated fish depend on the dose of methylmercury absorbed over long periods of time. As seen in Table 2, the effective amount of methylmercury absorbed via fish consumption depends on two factors: the concentration in the fish and the amount of fish consumed. Even at levels as low as 200 $\mu g\ kg^{-1}$, selected members of the fishing community who

Table 2 Estimate of effective human exposure to methylmercury from fish consumption

Human consumption of fish (g day^{-1}) / Concentration of mercury in fish (μg kg^{-1})		Intake of methylmercury from fish (μg day^{-1}) with different fish mercury concentrations:				
		200	500	1,000	2,000	5,000
Average	20	4	10	20	40	100
Elevated	100	20	50	100	200	500
High	300	60	150	300	600	1,000
Very high	1,000	200	500	1,000	2,000	5,000

The calculation given in Table 2 is aimed at estimating human exposure from fish, assuming levels encountered in practice and food consumption thought typical for selected groups of populations. A similar range of consumption levels has been used by Kitamura, Sumino, Hayakawa and Shibata (1975b). Note:

a) The threshold body burden would be reached with a daily consumption of about 200–500 μg methylmercury per day.

b) The allowable intake, according to the Joint FAO/WHO Expert Committee on Food Additives, 1972 (200 μg per week), is equivalent to a daily intake of about 30 μg.

depend mainly on fish for their food source may receive levels of methylmercury approaching toxic concentrations. On the other hand, even with an average level in fish of 1,000 μg kg^{-1}, the threshold is unlikely to be achieved if fish consumption is reduced to only one meal per day.

2 Metabolism of methylmercury

2.0 Summary of information contained in the Health Criteria Document

The uptake from the gastro-intestinal tract is high, about 95 per cent in humans, irrespective of whether methylmercury is administered as a salt or in a protein-bound form. Within the body, methylmercury is distributed relatively uniformly. The blood : brain ratio varies from 10–20 in the rat to 0.1 in the primates. The hair : blood ratio in humans is about 250.

Methylmercury is converted slowly to inorganic mercury. Elimination occurs mainly in the faeces; the enterohepatic circulation of methylmercury (but not of inorganic mercury) is largely responsible for slow elimination.

Clearance of methylmercury in man is a single exponential first order process occurring with a half-time of about 70 days with wide individual variations. Evidence exists for a bimodal distribution of half-lives, with mean values calculated from hair analysis around 65 and 119 days.

At steady state, a linear relationship exists between the intake of methylmercury and blood level and the equation $y = ax + b$ can be applied. For daily intake (x) expressed in $\mu g\ day^{-1}$ (70 kg body wt) and blood concentration (y) expressed in $ng\ m\ell^{-1}$ the coefficient "a" amounted, from various reports, to between 0.3 and 0.8, and from tracer studies (single dose) it was 1.0.

Methylmercury crosses the placental barrier and the levels found in the foetus are in excess of those in the mother. Methylmercury also penetrates maternal milk where concentrations achieve about 5 per cent of those in the blood.

2.1 The distribution of mercury in the body

Although the intestines may methylate inorganic mercury, this source may be considered negligible in humans (Rowland, Davies and Grasso 1977).

The exact nature of methylmercury complexes occurring in the tissues is not known. In the rabbit and human erythrocytes, methylmercury is bound mainly to a low molecular weight substance, possibly glutathione. In rat erythrocytes, however, binding to haemoglobin prevails (Naganuma and Imura 1979). In the tissues (liver, kidney) of rats, methylmercury is contained in the form of a high molecular weight complex, possibly also combined with haemoglobin (Mengel and Karlog 1980a). In the brain of rats, apart from macromolecular complexes, a low molecular weight complex was also found and considered likely to be methylmercury glutathione (Thomas and Smith 1979a). (See also 2.2).

The biodegradation of methylmercury results in the presence of both methylmercury and inorganic mercury in various tissues and in excreta. In squirrel monkeys exposed to methylmercury over a period of approximately four months, the percentage of inorganic mercury in the liver was inversely correlated with the concentration, in the limits of 10–35

per cent. Kidneys contained 30–75 per cent, bile 25–85 per cent, faeces 30–50 per cent and urine 30–80 per cent of inorganic mercury. The brain, however, contained only traces of inorganic mercury, between 1.6 and 4 per cent* in all but one case (Berlin, Carlson and Norseth 1975). Similar observations were made on rats (Omata, Sato, Sakimura and Sugano 1980). In humans exposed through the consumption of fish (Canadian Indians), inorganic mercury accounted for only 5 per cent in blood and about 20 per cent in hair (Phelps, Clarkson, Kershaw and Wheatley 1980). In contrast to the above, the proportion of inorganic mercury, as found in various tissues of the Japanese people ("unexposed population"), was almost 90 per cent and even in the brain over 80 per cent was inorganic (Kitamura, Sumino, Hayakawa and Shibata 1975a, 1976a).

The relative distribution of methylmercury in various organs and tissues has been studied in squirrel monkeys. At blood concentrations of 60–700 ng $m\ell^{-1}$ the ratio of concentrations tissue : blood was in the range of kidney, 5–7.5; liver, 6–10; brain 2.2–3.3. In the brain in particular, the above ratio seemed to be dose-dependent. It was about 3 for concentrations in the blood up to 900 ng $m\ell^{-1}$, but about 4.5 for blood concentrations in the range 1,000–3,000 ng $m\ell^{-1}$ (Berlin *et al.* 1975). In the macaque monkeys, at blood concentrations of 2,400–3,400 ng $m\ell^{-1}$ the ratios tissue : blood were: kidney, 21–28; liver, 12–13; brain, 1.6–3.8 (the latter depending on the region of the brain) (Evans, Garman and Weiss 1977). From experiments on *m. fasicularis* the average ratio brain : blood was about 5 for blood concentrations 8–9 $\mu g\ g^{-1}$ (Willes, Truelove and Nera 1978).

In humans, the distribution of both total mercury and organic mercury (methylmercury) has been reported from autopsy studies on the Japanese population (Kitamura *et al.* 1975a, 1976a). With "total mercury" in the body amounting to 3.3 mg, the individual organs and tissues contained: muscle, 44 per cent; liver, 22 per cent; kidneys, 9 per cent; blood, 9 per cent; skin, 8 per cent; and brain, 4 per cent. Methylmercury, which represented slightly over 10 per cent of the total mercury, was contained in muscle, 54 per cent; liver, 19 per cent; blood, 15 per cent; brain, 7 per cent; and intestines, 3 per cent. From the study of Canadian Indians,

* It has been hypothesized that even such small break-down to inorganic mercury in the brain may become an insult of importance to the muscarinic receptors of the brain (von Burg, Northington and Shamoo 1980).

the average brain content of mercury was found to be 3.4 per cent of body burden with wide variation (0.7–10 per cent) (Ontario Ministry of Labour 1977).

When discussing the accumulation and retention of methylmercury in the brain it should be realized that the ultimate form in which mercury is contained in this organ over long periods of time, has not been definitely identified. More recent re-analysis of the autopsy samples from the Minamata victims, using electron microscopic X-ray micro-analysis, points to mercury "particles" 10 nm or less in size, located in the cytoplasm of nerve cells, astrocytes and endothelial cells, and in the extracellular spaces of the grey and white matter. In these particles mercury was detected together with selenium and sulphur (Shirabe, Eto and Takeuchi 1979).

No clear-cut picture is possible regarding the age dependence of mercury levels in humans. From the study of Kitamura *et al.* (1975a) total mercury showed a decreasing trend with age in kidneys, but an opposite trend seems possible with the major compartment, muscle, as well as the trachea and intestines. In the case of methylmercury, the highest levels were found in the middle age groups (30–50 years) but this seems inconclusive. Higher tissue levels of total mercury were found in the age group 30–60 years by Mottet and Body (1974). A rising tendency with age has also been reported for total mercury in the blood of young women (Pitkin, Bahns, Filer and Reynolds 1976) as well as in the blood of members of selected "exposure groups" in Italy (Paccagnella, Prati and Bigoni 1973) and in Sweden (Skerfving 1974). Since the relatively short half-life does not allow for such accumulation with age, the basic trends require confirmation. Such an effect could possibly be due to a higher intake of mercury in the middle age groups; however, internal redistribution with age cannot be excluded. An increase in half-life with age, as an alternative explanation, seems less likely.

2.2 Elimination of methylmercury

Faecal elimination plays the dominant role. In the liver of rats, methylmercury contained in the cytosol is present partly as a complex with glutathione (GSH) (Omata, Sakimura, Ishii and Sugano 1978a) and is found in the same form in the bile (Refsvik and Norseth 1975). Although the availability of GSH seems a prerequisite for a biliary excretion

(Refsvik 1978) it is not thought to represent the limiting step in the elimination rate (Magos, Clarkson and Allen 1978b). The latter is supposed to be associated with ligandin, a carrier protein of the liver, probably capable of forming complexes with GSH-bound methylmercury (Magos, Clarkson, Allen and Snowden 1979a).

The Health Criteria Document (see also Clarkson 1977a) reported research aiming at mobilizing methylmercury from the tissues, and two different concepts were referred to. First of them assumed enhanced renal excretion through formation of soluble methylmercury complexes and the penicillamines were reported to be most promising. Penicillamines as well as dimercapto propansulphonate have been further explored experimentally with positive results (for review see Magos and Webb 1979). More recently, a derivative of British Anti-Lewisite (BAL) was proposed, the mesodimercapto succinic acid (DMSA) (Friedheim and Corvi 1975) and was found effective with methylmercury (Magos, 1976, Magos, Peristianis and Snowden 1978a). The other concept assumed enhanced faecal excretion through interruption of the enterohepatic circulation. In addition to polystyrene resins containing fixed sulphhydryl groups (Clarkson, Small and Norseth 1973) other macromolecular polymers have been tested with positive results, such as mercapto-starch (Aaseth and Norseth 1977) and a polymer obtained from terephthal-dicarboxaldehyde and mercaptoethyl sulphide (Harbison *et al.* 1977). In order to improve the effectiveness of such procedures, especially with regard to reduction of methylmercury level in the brain, combinations of low and high molecular weight thiols were also tested in animals with some success (Magos and Clarkson 1976, Aaseth and Norseth 1977). A report on application of three complexing agents, D-penicillamine, N-acetyl-DL-penicillamine and dimercaptopropansulphonate, as well as a complexing resin, in Iraqi patients has become available (Clarkson *et al.* 1980). Another chemical capable of enhancing biliary excretion of methylmercury, which was first suggested in 1973 by Magos and Clarkson, is phenobarbitone which increases bile secretion. Both phenobarbitone alone (Ohsawa and Fukuda 1976) and in combination with a polythiol resin (Magos and Clarkson 1976) were found effective.

In this connexion it should be noted that phenobarbital may exert a protective effect against methylmercury nephrotoxicity through entirely different mechanisms which are, however, poorly understood (Fowler and Lucier 1975).

2.3 Kinetics of methylmercury

In the generally accepted single-compartment model, the basic rôle is played by the estimates of the biological half-life in blood. Data obtained from analysis of hair are suggestive of a bimodal distribution (Al-Shahristani and Shihab 1974, Al-Shahristani, Shihab and Al-Haddad 1976, Nordberg and Strangert 1978). This could be due to some unknown genetic factors, since great differences in the half-life of mercury have been found in rats of different strains. Whether other factors are also involved does not seem to be known. Data presented by Al-Shahristani and Shihab (1974) based on hair analysis and those of Greenwood *et al.* (1978) are not suggestive of a difference in half-life in males and (non-lactating) women, although sex-related differences were found in rats (Thomas, Mushak and Hall 1980).

The half-life of methylmercury is shorter in lactating women (42 days) than in non-lactating women (about 70 days) (Greenwood *et al.* 1978). In the rat, clearance half-life is dependent on the diet (Landry, Doherty and Gates 1979). Whether there is a dose-dependence of the half-life is not known with certainty. In rats, Verschuuren *et al.* (1976) have found increasing half-life with dose, as based on accumulation curves. In monkeys, however, (based on post-exposure clearance) a decrease of half-life with dose was found, prompting the authors to accept higher clearance efficiency with high doses (Luschei, Mottet and Shaw 1977, Finocchio, Luschei, Mottet and Body 1980). Available data do not point to age dependence of half-life in adults, at least not to age-dependent increases of the values (Greenwood *et al.* 1978). In children (2–16 years) the half-life in hair has been reported to be only 56 days (Amin-Zaki, Majeed, Clarkson and Greenwood 1978). However, data contained in other reports (Al-Shahristani and Shihab 1974, Greenwood *et al.* 1978) do not suggest a difference in half-life between children and adults. It seems uncertain which value applies to infants early after delivery. In new-born rats and mice, there is an initial lag phase of blood levels (for almost three weeks in rats) before the clearance starts (Doherty, Gates and Landry 1977, Thomas *et al.* 1980), and in this respect methylmercury resembles inorganic mercury (Thomas and Smith 1979b). In infant monkeys, clearance from the blood seems to follow closely that of maternal blood (Mottet, personal communication), although other data, now available only in the form of original protocols, do not necessarily exclude a lag time (Reynolds 1979). Available observations in

humans were too limited to allow any conclusions (Amin-Zaki *et al.* 1976).

Not all data on methylmercury half-life in humans is consistent with the concept of a single compartment model: (a) In observations on humans whose long-term exposure has been interrupted and blood or hair levels monitored, the average estimates of half-life remain around 70 days (Al-Shahristani and Shihab 1974, Greenwood *et al.* 1978) and this value is usually accepted for risk assessment. On the other hand, data from a single exposure (whether tracer or not) give a much shorter estimate of about 50 days (Kershaw, Clarkson and Dhahir 1980). (b) The estimates of half-life in the whole body are in excess of those for blood: in the squirrel monkey it is 134 days compared with 49 days (Berlin *et al.* 1975). The available data for humans, including those recently published, point to 70 days compared with 50 days (single exposure). Such disparity is partly as a result of measuring the whole body burden including skin and hair: in the case of the squirrel monkey, the fur contained about 50 per cent of the body burden. In the cat, the estimates of whole body half-life were 117 days compared with only 76 days, if corrected for mercury contained in the fur (Hollins *et al.* 1975). This, however, is not necessarily the only reason. In the cat, the blood half-life is much shorter (39 days) (Charbonneau *et al.* 1974) than it is for the whole body after correction (76 days) (Hollins *et al.* 1975). A multicompartment model would generally have resulted in the differences mentioned in (a) and (b) above, if described using single half-life values.

Such a hypothesis seems especially attractive if one assumes that the brain is the compartment of slow turnover. More recent experiments on rats gave conflicting results (Omata, Sakimura and Sugano 1975, Kitamura, Sumino, Hayakawa and Shibata 1975b). Regarding the human brain, the half-life for both total mercury and methylmercury was suggested to be very long, about 240 days (Takeuchi, Eto, Sakai and Kojima 1974; Takeuchi and Eto 1975). A serious overestimation of the value seems likely: it is difficult to estimate the half-life from autopsy data and, in addition, the background levels have not been subtracted in calculating the half-life. Moreover, if the above holds true, one would expect time-trends of the ratio brain : blood and, in its absence, of brain : kidney or brain : liver, in data from humans with a varying history of exposure. Such trends seem to be absent in the data of Okabe and Takeuchi (1980). Evidence obtained in monkeys does not support

the view of a much slower brain-clearance as compared with blood-clearance of methylmercury except, possibly, for brain areas of highest concentrations (Evans *et al.* 1977). Nevertheless, it seems likely that the human brain retains methylmercury somewhat longer than do other tissues, but the difference may not be so great as to be easily detected. It seems unlikely that the brain as a reservoir of methylmercury could alone be responsible for the inconsistency in the kinetic data discussed above: in the cat, long-term deposits seem to exist primarily in the liver, gall-bladder and kidneys (Hollins *et al.* 1975).

The concept of a single-compartment model has been used for the modelling of continuous exposure, using the simple formula recommended in the Health Criteria Document (Gerstner and Huff 1977a, Ontario Ministry of Labour 1977, Willes 1979, Butler 1979). A more generally accepted procedure is to transform the simple kinetic equation into a form which assumes "saturation" with continuous exposure. Because of the practical importance of the estimates, however, it requires experimental support. Some idea regarding the reliability of such transformation can be obtained from experiments on primates. The earlier data (Luschei *et al.* 1977) obtained on monkeys dosed with 0.05–0.1 mg kg^{-1} day^{-1} methylmercury have shown significant deviations from the expected shape. This, however, was not evident from the most recent data from the same laboratory (Mottet, personal communication). No attempt has been made so far to assess the reliability of the calculated "saturation functions" in a quantitative way. From studies on rats' blood, methylmercury levels increase more than proportionately to the methylmercury intake (Verschuuren *et al.* 1976).

2.4 Estimates of practical importance (blood, hair, intake, body burden)

For the assessment of exposure to methylmercury, the estimates of blood mercury levels still play the dominant role. In populations considered to be under exposure (from fish), it is of subordinate importance whether "total" or "organic" mercury is being determined, since the latter represents about 95 per cent of the total (Phelps *et al.* 1980). In blood the cells : plasma ratio is high, about 20 in both humans and squirrel monkeys (Kershaw *et al.* 1980, Berlin *et al.* 1975). Therefore both the whole blood and blood cells may be used for analysis, provided the hematocrit is known.

Some drawbacks of using blood mercury levels solely as an index of exposure or body burden have been recognized: (a) the reconstitution of past exposure is difficult; (b) blood mercury levels are subject to transient increases following intake of a single, relatively high dose, thus possibly overestimating the overall exposure or body burden (Kershaw *et al.* 1980). Both of the above shortcomings can be eliminated through hair analysis, since the hair : blood ratio seems constant. This ratio only seems reasonably reproducible if blood samples are compared with properly time-matched segments of hair (Clarkson, Amin-Zaki and Al-Tikriti 1976). Otherwise, values may be obtained which are at variance with the above estimate (for examples see Bernstein 1976, Galster 1976, Igata, Niina, Hamada and Ohkatsu 1975, Methylmercury Study Group 1980). The ratio hair : blood for new-born infants seems lower than in others (Nishima *et al.* 1977). Some data became available recently which allow for the evaluation of the scatter of the hair : blood values (Kershaw *et al.* 1980): the 95 per cent confidence limit of the ratio was found to be 210–476 (from five individuals) thus indicating the error range of $-\frac{1}{3}$ and $+\frac{2}{3}$. Hair analysis is recently gaining the value of an indicator independently linked with health effects (Marsh *et al.* 1979).

The relation between blood mercury level (y) and daily intake (x) may be obtained in two ways: (a) from empirical data in a steady-state situation and (b) from single dose experiments in which one applies two assumptions (i) that one litre of blood contains roughly one per cent of body burden of mercury in a 70 kg adult and (ii) that steady-state level can be calculated from the half-life alone. No new empirical estimates of the type (a) seem available for humans. Of the "kinetic" type (b), one new experiment relates directly to humans (Kershaw *et al.* 1980): when using the half-life in blood of 52 days, the estimate of y/x equals 0.9, close to the one obtained earlier with a tracer dose −1.0. (However, if applying a half-life of 70 days, the y/x ratio would be about 1.2). Empirical data allowing for estimates of the type (a) were recently obtained in the macaque monkey (Evans *et al.* 1977, Finocchio *et al.* 1980, Mottet, personal communication). These cannot be compared directly with human data because of the differences in body weight and clearance half-life (in the rhesus the half-life of mercury in blood has been reported to be approximately 22–30 days). Should such comparison be undertaken on the basis of simple recalculation for body weight and ratio of half-lives (22, 30 and 70 days), the respective coefficient would

be within the range of 0.5 and 1.2 (to be compared with human empirical data for y/x ranging from 0.3 to 0.8). (Authors' approximate calculation).

2.5 Mercury in infants from exposed mothers

The mechanisms of transplacental transfer of methylmercury are now better understood (Reynolds and Pitkin 1975, Olson and Massaro, 1977b, Kelman and Sasser, 1977).

In the rat, neo-natal levels are higher than the maternal levels in blood (ratio 1.7) and brain (ratio 1.1), but lower in other tissues such as liver, kidneys and muscles. The ratio infant : mother (blood values) was also considerably higher in the monkey (rhesus) (Reynolds 1979). In the macaque monkey with maternal blood concentrations up to 1,000 ng $m\ell^{-1}$ the above ratio was close to 2.0 (Mottet, personal communication, Mottet 1979). Estimates of the ratio infant : mother for blood mercury concentrations in humans have recently been reported to be in the range of 1.0–2.0. Lower estimates seem typical for the general population not under considerable exposure: the estimates are 1.0–1.5 (1.3 in the hair) (Fujita and Takabatake 1977, Pitkin *et al.* 1976, Reynolds 1979). In a population which was under low exposure through fish consumption, the above ratio infant : mother seemed dependent upon the level in the mother, and with maternal blood levels from 9 to 50 ng $m\ell^{-1}$, the ratio was between 1.2–1.9 (the authors' estimate is 1.8 overall) (Galster 1976, see also Nishima *et al.* 1977, Amin-Zaki *et al.* 1976).

Transfer of maternal mercury to the breast-fed infant has been confirmed, with a ratio of concentrations milk : blood of 0.14 (Fujita and Takabatake 1977), or slightly over 0.08 (Amin-Zaki *et al.* 1976).

3 Experimental studies on the effects of methylmercury in adult animals

3.0 Summary of information contained in the Health Criteria Document

The LD_{50} for methylmercury is of the same range as for other mercury compounds, 10–40 mg kg^{-1} (b.wt). Although the main effect is the action on the central nervous system (CNS), other effects may occur earlier. Thus, in the liver, methylmercury causes a decrease in the activity of the mixed function oxidase, increased degradation of cytochrome P-450

and changes in the endoplasmic reticulum. Early morphological and functional damage occurs in the kidneys; such effects have been ascribed to inorganic mercury split from methylmercury.

Neurotoxic effects, which were given major attention, were defined as irreversible. In general, effects known from human studies were also reproducible in experimental animals. These included: visual disturbances, manifest by gradual constriction of the visual field; impaired motor co-ordination and (possibly) sensory disturbances. Such effects were produced irrespective of whether methylmercury was administered as a salt or in protein-bound form (mercury-contaminated fish). The damage to the peripheral sensory nerves was found to be the most sensitive effect. The primary site of the effect is the cell bodies of the dorsal root ganglia, which also contain the highest concentrations of mercury. Prior to the onset of overt symptoms or signs of neurotoxicity, morphological, electrophysiological and biochemical changes in the nervous system can be manifest (silent damage). Of the biochemical changes, those relating to protein synthesis in the brain have been emphasized. Damage to the blood-brain barrier was also noted.

Studies on primates have only recently been initiated but reflect the above views. The dose-rate and the period of dosing influence the pattern of damage. High exposure of short duration results in abrupt visual change leading to blindness. Long-term, low-level exposure leads to more generalized damage to the cortex and is accompanied by gradual onset of visual changes and other signs of CNS involvement, such as ataxia.

3.1. General signs of toxicity and effects other than on the nervous system

The LD_{50} (24 hour) values for the rat, hamster and squirrel monkey were about 12–22 mg kg^{-1} (b.wt) but at observation periods prolonged to 30 days, the LD_{50} for the monkey decreased to only 5–6 mg kg^{-1} (Hoskins and Hupp 1978).

In rats subject to chronic exposure, the non-specific general effect, decreased gain of body weight, was detected with dose-level of 0.25 mg kg^{-1} day^{-1} (Verschuuren *et al.* 1976, Munro *et al.* 1980). At the same dose level and time, haematological changes were observed (decreased haematocrit and haemoglobin). Kidney injury was found in both sexes at the same level of exposure, mainly in the cortical layer

(proximal tubules). At a lower level (0.05 mg kg^{-1} day^{-1}) some change was seen at the glomeruli but only in males. In the liver, at the same dose level some decreased basophilia in the hepatocytes was seen (Munro *et al.* 1980). In mice fed with vitamin-enriched milk, decreased food intake and body weight were detected after 22 days with a dose of 1.0 mg kg^{-1} day^{-1}. Weakness of the limbs was apparent at a dose level of 0.25 mg kg^{-1} day^{-1} (Berthoud, Garman and Weiss 1976). Loss of appetite and diminished activity were also apparent indications of intoxication in monkeys (Joiner and Hupp 1978). From life-term experiments on mice which were administered methylmercury in drinking water, decreased growth was noted with a concentration in water of 5 mg ℓ^{-1}, whereas at 1 mg ℓ^{-1} an opposite effect was apparent (Schroeder and Mitchener 1975).

In the rhesus monkey, with exposure levels of 500–1,000 μg kg^{-1} day^{-1}, subtle lesions were found in the heart (myofibrilar degeneration) (Mottet, personal communication). Morphological and functional effects on the cardiac tissue have also been found *in vitro* at concentrations of 0.5–2.0 μg $m\ell^{-1}$ (Su and Chen 1979). At blood levels above 2,000 ng $m\ell^{-1}$ in the macaque monkey specific lesions of the intestinal epithelial cells were found. These were confined to the Paneth cells, the possible site of intestinal elimination of heavy metals (Mottet and Body 1976).

In the rat, changes in serum enzyme activity have been detected under low-level exposure in respect of two enzymes: malate dehydrogenase (MDH) (Chmielnicka, Komsta-Szumska and Gajda 1975) and glucose phosphate isomerase (PHI) (Chmielnicka, Balcerska, Pietruszcsak and Brzénicka 1977). Such changes are probably due to subtle liver and kidney damage caused by inorganic mercury accumulated with time in these organs (Brzeźnicka and Chmielnicka 1979). The sensitivity of MDH changes in particular has been reported to be very high and changes have been found at 2 μg kg^{-1} day^{-1} (Chmielnicka and Balcerska 1975, Brzeźnicka 1977). This phenomenon is not limited to methylmercury; other forms of mercury also induce similar changes. Whether this effect also occurs in species other than the rat is not at present known.

In vitro studies of the supernatants from rat liver homogenates have shown marked inhibition of the glutathione peroxidase activity at concentrations of methylmercury exceeding 5×10^{-6} (about 1 μg $m\ell^{-1}$) (Hirota, Yamaguchi, Shimojoh and Sano 1980).

In rabbits, methylmercury was found to suppress the humoral immune system at relatively low levels in the blood (600 ng $m\ell^{-1}$) and brain (1,600 ng g^{-1}), at which levels no other symptoms of toxicity were observed (Koller, Exon and Arbogast 1977a). Similar observations were made in mice whose exposure resulted in mercury renal concentration of about 40 μg g^{-1} (Koller, Exon and Branner 1977b). In this species, both inorganic and organic mercury were found to cause these effects (Miller *et al.* 1980a), and response similar to methylmercury was also obtained for tetraethyllead and sodium arsenite (Blakley *et al.* 1980). From other studies on mice, however, it has been reported that the said effects were apparent with acute type of exposure (5×34 mg kg^{-1}) rather than with low, chronic exposure (5 mg kg^{-1} in the diet for a few weeks) (Ohi *et al.* 1976a). In the cat, immune suppression has been studied with positive results at an extremely low dose, 3 μg kg^{-1} day^{-1} (Miller, personal communication). *In vitro*, 50 per cent suppression of the secondary antibody response of mouse spleen cell culture to sheep red blood cells has been noted at a concentration of methylmercury of 3×10^{-7} M (about 60 ng $m\ell^{-1}$) (Seto, Akiyama and Mizogushi 1977, Seto and Akiyama 1978).

More recently, Miller and Miller (1979) have offered a hypothesis which attempts to identify the primary site of damage as interference with the DNA synthesis and/or repair. The effect has been studied through unscheduled DNA synthesis (UDS), provoked by an insult of methylmethanesulphonate (MMS). So far there is only limited evidence in support of this hypothesis, pointing to either decreased or increased UDS, apparently depending on the conditions of the experiment (Miller, Zawidzka, Nagy and Charbonneau 1979, Miller, Krewski and Tryphonas 1980b). Increased repair could be expected at a relatively low level of damage and be followed by a complete failure of the repair mechanism as the damage progresses: thus polymodal functions have been suggested (Miller 1980). The effect of methylmercury exposure on the DNA repair (UDS) can be studied using leucocytes, with a high sensitivity: depending on the conditions of the experiment, increased UDS was detected in cat leucocytes at blood mercury levels in the range 60–500 ng $m\ell^{-1}$ (Miller 1980).

3.2 Effects on the nervous system found in animal experiments

An extensive survey of the neurotoxic effects of mercury, covering literature up to the early seventies, was published by Chang (1977). In

a more recent short survey, the following effects on the nervous system of experimental animals were mentioned (Willes 1979): ataxia, abnormalities in gait and limb reflexes, depressed peripheral sensation, general lethargy are signs common to most species. Further, blindness due to lesions of the visual cortex has been found in swine, dogs, cats and monkeys. Peripheral nerve damage occurred in rats, guinea pigs, swine and dogs. In rats, reduced neuromuscular function has been observed. Because of the unusually emphasized effects on the peripheral nervous system it has been suggested that the data on methylmercury neurotoxicity in the rat may not be applicable to other species, especially humans.

The information given below is confined to chronic effects found in non-human primates on which much new data have become available. Using the techniques of behavioural studies the following effects are usually observed: decrement of food intake, loss of fine control of distal musculature, signs of paresthesia and changes in the sensory nerves, decreased movement and loss of voluntary control of movement, ataxia due (probably) to disturbances in vision, blindness (signs quoted in sequence of their appearance after Finocchio *et al.* 1980). Hypesthesia (impaired sense of touch) and motor disturbances have been reported from earlier studies (Evans, Laties and Weiss 1975). Another earlier study gave the following sequence of effects: hypesthesia, loss of activity, tremor of the trunk, muscular weakness, ataxic gait, anorexia, tremor of extremities, visual and hearing impairments, general tonic and clonic seizures. These signs did not show in animals having blood mercury levels below 1,200 ng $m\ell^{-1}$ (Sato and Ikuta 1975).

An effect studied in much detail in monkeys is the constriction of the visual field, known from human pathology. Apart from visual constriction, impaired brightness discrimination was reported, which could point to lesions occurring also in the central vision cortical neurons (Evans *et al.* 1975). The vision impairment becomes apparent with mercury blood concentrations of 2,500–3,000 ng $m\ell^{-1}$, after 20–30 weeks of exposure (Evans *et al.* 1975). Prevailing opinion suggests that the impairment of vision does not represent the earliest effect in the monkey and is preceded by a number of other signs (Luschei *et al.* 1977, Finocchio *et al.* 1980, Sato and Ikuta 1975). It has been suggested that the scotopic vision becomes affected much earlier (Evans and Garman 1980).

The appearance of toxic symptoms depends on both the blood level of methylmercury and the duration of such levels. The latent period preceding neural signs is inversely related to blood concentration (Evans *et al.* 1977). Similar conclusions were drawn from another study where a post-exposure treatment with DMSA resulted in decreased body burden (Magos *et al.* 1978a).

Although there seem to be differences between various monkey species, more recent histopathological studies allow some conclusions to be drawn.

Inconsistent with the early data of Hunter, degeneration of the peripheral nerves has not been confirmed in monkeys. Within the brain, there is no major damage to the cerebellum, and the primary target is the cerebral cortex. In this respect the experiments on non-human primates revealed dissimilarities with the observations made in Minamata disease. Within the cerebral cortex, the occipital cortex was constantly and preferentially involved (Shaw, Mottet and Chen 1980). Histopathological examination reveals atrophy of the calcarine cortex with neuronal changes and proliferation of astrocytes. The degeneration of the nerve cells, seen by electron microscopy, consists of loss of mitochondria and endoplasmic reticulum, and breakdown of nuclear membrane. Necrotic cells are surrounded by astrocytes and macrophages (Sato and Ikuta 1975). In the absence of symptoms of toxicity, ultrastructural changes in the calcarine cortex were seen in monkeys dosed with 0.03 mg kg^{-1} d^{-1} for over 300 days, where the mercury blood level was as low as 460 ng $m\ell^{-1}$ (Sato and Ikuta 1975). From another early study the threshold level of methylmercury in blood connected with neuropathological findings was reported to be about 1,000 ng $m\ell^{-1}$ (Garman, Weiss and Evans 1975).

Autoradiographic studies failed to reveal close relation between the areas of highest mercury concentration and those of greatest damage (Garman *et al.* 1975, Shaw, Mottet, Body and Luschei 1975 and Shaw *et al.* 1980). Therefore, a mechanism of damage involving an indirect factor seems not to be excluded. Cerebrovascular lesions were found in the brain of rhesus monkeys, which resembled those found occasionally in human cases (Shaw, Mottet, Luschei and Finocchio 1979, see also Mottet personal communication). This is probably a secondary effect, connected with cerebral cortical degeneration.

The neurotoxic effects of methylmercury have been classified in the HCD as irreversible. More recent evidence obtained in monkeys,

however, shows that after several weeks of exposure when blood concentrations fall below 600 ng $m\ell^{-1}$, some reversal is seen in water intake and mastication (Finocchio *et al.* 1980).

Although various biochemical effects may be found *in vitro*, and also in the brain in the toxic phase of methylmercury exposure, some can probably be ruled out as primary mechanisms of neurotoxic action. This is the case with those effects which lack sensitivity and/or appear only after other symptoms become apparent. The inhibition of the neurotransmitter high-affinity transport occurs *in vitro* only at a high methylmercury concentration of about 10,000 ng $m\ell^{-1}$ (Bondy, Anderson, Harrington and Prasad 1979). Synaptosomal respiration becomes affected *in vitro* at a low concentration of about 1,000 ng $m\ell^{-1}$, but *in vivo* becomes apparent only after other signs of poisoning have become manifest (Verity, Brown and Cheung 1975).

Inhibition of protein synthesis in the brain has been reported earlier (HCD). From more recent *in vitro* studies inhibition has been found at a very high concentration, about 100 μg $m\ell^{-1}$ (Sugano, Omata and Tsubaki 1975). *In vivo* experiments on the rat, where brain and blood concentrations were respectively about 30 and 300 μg g^{-1} have shown inhibition of protein synthesis in the brain slightly ahead of overt signs of intoxication (Omata, Sakimura, Tsubaki and Sugano 1978b). It appears that detection of methylmercury influence upon protein synthesis in the brain is dependent upon the conditions of the experiment. Thus, Verity, Brown, Cheung and Czer (1977) were able to detect this effect *in vitro* at concentrations of methylmercury of only 2 μg $m\ell^{-1}$. *In vivo*, however, the inhibition was seen in the rat synaptosomes and brain slices only at a dosage level of 10 mg kg^{-1} (Verity *et al.* 1977). Much higher sensitivity has been reported by Syversen (1977), in isolated neurons where changes were seen at a level of mercury as low as 2 μg g^{-1}, a concentration known not to produce symptoms of overt neurotoxicity. These changes, however, have consisted of initial inhibition and subsequent increase of synthesis, the latter only in the cerebrum and in the Purkinje cells of the cerebellum. As quoted by Syversen (1977) increased biosynthesis of protein in the brain had been reported earlier by Brubaker *et al.* (1973) and Richardson and Murphy (1974). It should be mentioned in this connexion that, in general, deleterious effects can be linked with both inhibition and stimulation of protein synthesis, and that the same stimulus may result in opposite effects in various tissues of the same affected animal (Sidransky 1977).

As mentioned under 3.1, Miller and Miller (1979) have put forward a hypothesis that explains neurological damage by the effects of methylmercury on DNA synthesis as the primary cause. In cats fed 176 $\mu g\ kg^{-1}$ (b.wt) d^{-1} of mercury for 12 weeks (clinical signs developed at mercury levels in the cerebrum of 12 $\mu g\ g^{-1}$), the unscheduled DNA synthesis of cerebrum and cerebellum was elevated. The effect was most pronounced in those cell types prone to neurodegeneration (Purkinje cells, the granular layer of cerebellum and the large neurons in the cerebrum). There were indications of the involvement of the mitochondrial DNA (Miller *et al.* 1980b). Whether this effect could be detected ahead of clinical signs is unknown at present.

As well as the signs of general toxicity, the neurotoxic effects of methylmercury are also accelerated or enhanced by a protein deficiency in the diet (Friedman, Eaton and Bailey 1978a).

4 Development toxicity (effects of pre-natal and early post-natal exposure) in animals

4.0 Summary of information contained in the Health Criteria Document

The general view was expressed that much more damage is inflicted on the foetus than on the exposed mother. Some performance deficits in mice exposed pre-natally were noted. Virtually no information was available on the morphological and biochemical factors related to pre-natal damage. Teratogenic effects, in particular, were not discussed.

4.1 General symptoms and signs of toxicity in pre-natally and post-natally exposed animals

Several reports point to abortions and embryolethal effects of methylmercury. These may be found under such conditions of exposure where the mothers are not seriously affected (Spyker and Smithberg 1972, Su and Okita 1976b, Hughes and Annau 1976, Spyker and Spyker 1977, Nobunaga, Satoh and Suzuki, 1979, Lee, Chan, Sairenji and Nickumi 1979, Tsubaki and Irukayama 1977(b)). The limits of dose-levels, in which embryolethality is obtained in the absence of overt toxicity to mothers, are probably narrow. For instance, in monkeys dosed weekly with 1 $mg\ kg^{-1}$, lethal effects were obtained in both mothers and foetuses,

and with 0.5 mg kg^{-1} 50 per cent of the foetuses were affected while the mothers were unaffected (Reynolds 1979). In rats, first embryotoxic effects were found with mercury levels in maternal liver of 15 μg g^{-1}, and the severity progressed with increasing concentrations up to about 75 μg g^{-1} in the maternal liver where maternal toxicity became apparent (Mottet 1974a). Both embryotoxic and teratogenic effects (these will be discussed under 4.2) appear specially with exposure late in organogenesis: in mice, a cut-off for resistance to these effects was day 8–9 of gestation (Spyker and Smithberg 1972).

With an appropriately lower level of exposure, where embryolethality may be absent, a reduction of foetal weight is almost constantly reported. This effect has been noted in mice with single methylmercury doses of between 2 and 5 mg kg^{-1} (Spyker and Smithberg 1972, Hughes and Annau 1976, Lee *et al.* 1979), and with multiple exposures of 6× 4 mg kg^{-1} or 0.8–1.6 mg kg^{-1} d^{-1} for a longer time (Su and Okita 1976a, Nobunaga *et al.* 1979). In the rat, this effect was noted when total methylmercury in the foetus was 15 μg g^{-1} (Chen, Body and Mottet 1979). However, this has not been found in another study where the mercury content of the foetal liver exceeded 100 μg g^{-1} (Tsubaki and Irukayama 1977(b)). In the pre-natally exposed animals, reduced survival and/or retardation of growth (weight gain) to weaning was also observed (Chen *et al.* 1979, Spyker and Spyker 1977, Hughes and Annau 1976). The above effect (smaller body weight) disappears at maturity (Hughes and Annau 1976). It has been found, however, that in this later period the pre-natally exposed animals may exhibit neuromuscular and immunological deficiencies (Spyker and Spyker 1977).

The retardation of foetal growth is reflected biochemically in decreased DNA content, protein content and biosynthesis. The latter may be due to an inhibition of the placental/foetal amino acid transport (Olson and Massaro, 1977a).

There are reports on the biochemical damage found in the liver, such as diminished metabolism of corticosterone (Grady, Kitay, Spyker and Avery 1978) or depressed activity of the cytochrome P-450-dependent mono-oxygenase system (Robbins, Hughes, Sparber and Mannering 1978). The former is probably due to loss of liver weight rather than any more specific mechanisms. The latter was only seen in males reaching maturity.

Other biochemical effects relating to pre-natally exposed animals are discussed under 4.3.

Data seem scarce with regard to the early exposure of infant animals. According to Willes, Truelove and Nera (1978), the general signs of methylmercury toxicity, the tissue distribution and histopathology observed in infant monkeys (exposed post-natally to 500 $\mu g\,kg^{-1}\,d^{-1}$) were similar to those reported for the adults.

4.2 Teratogenic and mutagenic effects

Only changes classified as "gross malformations" are discussed under this section. Other data pertinent to this field are discussed under 4.3.

In the introduction to their report, Nobunaga *et al.* (1979) quote a number of earlier reports on this issue starting in the late sixties. The most frequently quoted teratogenic effect is the cleft palate produced in mice. The incidence of the malformation increases with the dose of methylmercury provided that the exposure is properly timed relative to the organogenesis (Lee *et al.* 1979, Nobunaga *et al.* 1979, Su and Okita 1976b). Apart from the cleft palate, other malformations and changes are often reported such as micrognathia, encephaly, exencephaly, anophthalmia, facial deformities, loss of hind limbs (Su and Okita 1976b), hypoplasia of the cerebellum, hydrocephalus, vaulted cranium, rib fusion, club foot and others (cited after Mottet 1974a). The malformations are obtained at roughly the same dose level as required for other embryotoxic effects, but apparently some species or even strains are much more vulnerable than others, as seen in mice (Spyker and Smithberg 1972, Spyker and Spyker 1977). On the other hand, certain laboratories have failed to discover teratogenic effects in rats (Mottet 1974a, Chen *et al.* 1979). There seem to be no reports on teratogenic effects (gross malformations) in monkeys.

To what extent the presence or absence of teratogenic effects depends on the susceptibility of the species (or strain) on the one hand, and on the regime of the exposure on the other, seems unclear. Su and Okita (1976b) have observed in mice that higher doses are likely to produce embryocidal effects, while low doses produce teratogenic effects. Mottet (1974a), however, links the lack of malformations in rats with the chronic relatively low exposure where high peak levels in blood have been avoided. This is also likely to be a factor in the absence of malformations in monkeys exposed in chronic experiments (Mottet, personal communication).

There are no reports on mutagenic activity of methylmercury. Hughes and Annau (1976) have observed that, under certain experimental

conditions, deviations from the "normal" found in animals exposed pre-natally were not seen in the next generation.

4.3 Neurotoxic effects resulting from pre-natal exposure

Pre-natal exposure to methylmercury may result in some behavioural deficits, such as spontaneous locomotor activity or exploratory behaviour (Hughes and Annau 1976, Su and Okita 1976a). Some authors express the opinion that these reflect learning deficits rather than motor impairments (Hughes and Annau 1976). Learning deficits have been noted in female (but not in male) offspring even at such low exposure levels as 4×0.05 mg kg^{-1}, in the absence of effects on general motility and motor co-ordination (Musch, Bornhausen, Kriegel and Greim 1978). Behavioural deficits, however, have not been noted in other experiments (Mottet 1974a). Such effects may require time to develop (Musch *et al.* 1978) and may also diminish gradually as the animal grows older (Su and Okita 1976a).

In a recent review, Reuhl and Chang (1979) noted that the neuropathological lesions in animals resulting from pre-natal exposure to methylmercury were first studied by the Japanese workers Moriyama (1967), Tatetsu, Takagi and Miyakawa (1968) and Nanaka (1969) and were first reviewed by Murakami (1972). Other early works are those of Khera and Nera (1971), Khera (1973) and Khera and Tabacova (1973) (all quoted after Reuhl and Chang 1979).

Histopathological changes observed by light microscopy include: loss of neurons and cyto-architectural changes in the cerebrum, cystic changes and small haemorrhages in the cortex and white matter, white matter destruction in the temporal lobes (all in rats), delayed migration of the external granular cell layer (mice), hypoplastic and atrophic neurons and incomplete granular cell layer formation.

The same review (Reuhl and Chang 1979) discusses those biochemical changes which may underlie the congenital effects on the CNS. There seems to be a variety of findings but the amount of data on any given issue may be insufficient to find out the limiting step in the process of biochemical damage in the brain. Much of the existing knowledge may be explained by decreased protein synthesis, linked with a decrease in the DNA level (Olson and Massaro 1977c, cited after Reuhl and Chang 1979). As stated by the same authors, this could be linked with reduced transport of amino acids across the placenta. Another process which is

likely to disturb brain development is the alteration in carbohydrate metabolism, which is reflected in hypoglycemia (Snell, Ashby and Barton 1977).

Other forms of biochemical damage may contribute to overall neurotoxicity but they may either not be pronounced enough or be only transient. In this category belong alterations in the lipid metabolism, changes in the biogenic amines, including tryptophan metabolism and various biochemical changes related to the activity of mitochondria (quoted after Reuhl and Chang 1979).

5 The influence of selenium on the toxicity of methylmercury

5.0 Summary of information contained in the Health Criteria Document

Selenite salts can protect experimental animals against toxic effects of inorganic mercury and methylmercury. The elevated selenium concentrations in tuna fish may be sufficiently high to give similar protection.

5.1 Protective effects of selenite-selenium *in vivo*

In continuing investigations into the rôle of selenium in combating the toxic effects of methylmercury, the majority of workers have demonstrated protective action in studies with rats, mice, guinea pigs, pigs and poultry, in terms of mortality and survival times, growth-rate and neurotoxicity. No level seems to provide absolute protection; a delay in the occurrence of signs of toxicity is the more usual observation. There were also indications of a protective rôle played by vitamin E, when applied alone or in combination with selenium, and with regard to this issue the reader is referred to the review of Ganther (1978).

Protection by selenium occurs over a limited range of methylmercury doses, and selenium cannot prevent death if the total administered dose is much above the lethal range for methylmercury alone. With a total dose of 80 mg kg^{-1} (b.wt) administered over eight days, 100 per cent mortality occurred nine days after the last dose in rats given no, or very low, selenium supplements, but with higher doses of selenite (0.5 and 1.0 mg kg^{-1} (b.wt)), a 2–3 day delay in death occurred (Ohi, Seki, Maeda and Yagyu 1975a). However, with total dose of methylmercury amounting to 60 mg kg^{-1} (b.wt), which is still in excess of the LD_{50}, selenite administration (6×1.0 mg kg^{-1} (b.wt)) showed marked protection with 50 per cent of the animals surviving the three-month observation period.

The greatest protection was achieved in this experiment with the highest dose of selenite (1 mg kg^{-1} (b.wt)) at which the molar ratio of selenium : methylmercury was still only about 1 : 3. Even with a lower selenium : methylmercury ratio of less than 1 : 10 selenite has extended the survival time of rats (Stillings, Lagally, Bauersfeld and Soares 1974); at these low molar ratios, the protective effect of selenium could be augmented by cystine addition to the diet.

From earlier work, the optimum molar ratio was supposed to be about 1 : 1 (see Ohi *et al.* 1975b) but more recently, authors have avoided this 1 : 1 ratio in order to prevent the toxic effects of selenium itself; sodium selenite is more toxic than methylmercury: a dose of 5–10 mg kg^{-1} (b.wt) results in lethal effects in animals within a few days (see Skerfving 1978).

On the other hand, in experiments on poultry, higher selenium doses have still been used which kept the selenium: methylmercury molar ratio at about 1 : 1. Protection, as manifest in reduced mortality, was especially evident if a pre-treatment period with selenium was applied and if the administration of selenium was continued over the whole period of methylmercury administration (Stoewsand, Bache and Lisk 1974, Stoewsand, Anderson, Gutenmann and Lisk 1977, Welsh and Soares, 1976, El Begearmi, Sunde and Ganther 1977 and Sell and Horani 1976).

Selenium was also shown to protect against decreased growth-rate caused by methylmercury in rats (Johnson and Pond 1974, Stillings *et al.* 1974, Ohi *et al.* 1975a, 1975b, 1976b), and quail (Welsh and Soares, 1976). Augmented protection was provided by dietary cystine (Stillings *et al.* 1974) or vitamin E (Welsh and Soares 1976). Protection against a decreased growth rate was not, however, observed by Sell and Horani (1976) in chicks. Indeed, both elements, when fed independently, caused a decrease in the growth rate and, when given together, the effect was even more pronounced.

Magos and Webb (1979) have pointed out that the observation most commonly used in ascertaining protective action of selenium, the restoration of body weight gain, does not apply to humans (for whom anorexia or inhibition of body weight gain has not been reported). They also question the validity of such observations, as well as those on survival time, if used as the only criteria for the protective action of selenium against the toxicity of methylmercury, because such findings could be influenced by other factors. They suggest that delay in the occurrence of sensory disorders, as well as histological and other evidence of the absence of damage, should be considered sounder evidence of protection.

Protective action of selenium against neurotoxicity of methylmercury has been demonstrated for rats (Ohi *et al.* 1975b, 1976b) and for poultry (Sell and Horani, 1976, Stoewsand *et al.* 1977, Welsh and Soares, 1976 and El-Begearmi *et al.* 1977). In male wistar rats fed methylmercury in their diet (20 $\mu g\, g^{-1}$) with or without selenite (3 $\mu g\, g^{-1}$), after 6 weeks, 36 per cent of the group fed methylmercury alone had developed paralysis or crossing of the hind-legs whilst the methylmercury plus selenite group remained unaffected (Ohi *et al.* 1975b). During a 3–4 week experiment with young chicks and quail fed methylmercury (20 $\mu g\, g^{-1}$) in their diet, a concurrent supplement of dietary selenium (8 $\mu g\, g^{-1}$) prevented morbidity (tremors and loss of balance) in both species (Sell and Horani, 1976). Selenite-selenium supplementation to the diet of pigs subsequently given a single dose of methylmercury (7 $mg\, kg^{-1}$ (b.wt)) was found to decrease toxicity compared to control animals not given selenium, as determined by clinical signs and histopathological examination (Froseth, Piper and Carlson 1974). There was also evidence suggesting that methylmercury aggravated selenium deficiency in pigs fed methylmercury with a low selenium diet.

From the above evidence, it appears that the majority of authors were able to confirm some protective effects of selenium during the post-natal life in a variety of experimental animals, as indicated by decreased mortality, extended survival time, alleviation of growth-rate inhibition and reduced neurotoxicity.

The effects of sodium selenite on methylmercury-induced embryotoxicity and teratogenicity have been investigated in mice. Selenium was not found to act in a uniformly antagonistic manner (Nobunaga *et al.* 1979, Lee *et al.* 1979). Overall detrimental effects on the foetus were either increased or decreased by selenite, depending on the combination of doses of methylmercury and selenium and other parameters involved (Nobunaga *et al.* 1979). Thus, with mice fed 4 $\mu g\, g^{-1}$ methylmercury in food, a low dietary selenite supplement decreased the incidence of cleft palate. However, with a higher methylmercury intake, equivalent to 8–25 $\mu g\, g^{-1}$ in the diet, a high supplement of selenium increased the incidence of cleft palate (Nobunaga *et al.* 1979). On the other hand, high selenite supplement decreased the occurrence of resorptions and implantation sites, and also partly reduced the occurrence of dead embryos and foetuses, caused by high doses of methylmercury administered to the dams (Nobunaga *et al.* 1979). In experiments with joint administrations of methylmercury and selenium where the level of

selenium alone was close to the lethal level, an additive toxicity was observed; mice given methylmercury and selenium (5 and 3.5 mg kg^{-1} (b.wt) respectively) on days 7 through 12 of pregnancy had a reduced survival rate compared to animals given each chemical alone (Lee *et al.* 1979).

In conclusion, there is only limited evidence for protection by selenium against embryotoxicity and fetotoxicity induced by methylmercury.

5.2 Protective effects of marine fish diets containing selenium

Marine fish have often been used as a natural source of selenium in animal studies of the antagonistic action of selenium against methylmercury toxicity, for the following reasons: (a) relatively high natural levels of selenium are found in the muscle tissue of marine fish, usually up to about 1 μg g^{-1} in tuna and about 2 μg g^{-1} in swordfish (Friedman, Eaton and Carter 1978b and Freeman *et al.* 1978); and (b) fish is the main source of methylmercury in the human diet and therefore consumption of marine fish with high methylmercury levels usually involves intake of correspondingly high levels of selenium. (The molar ratio selenium: mercury in marine fish is usually at least 1:1 (Friedman *et al.* 1978b)). In nearly all marine fish examined by Luten *et al.* (1980) the stoichiometric Se: Hg ratio was greater than one.

However, there are several reservations concerning the use of tuna and other marine fish in animal experiments as the source of selenium. First, the active protective agent in fish muscle, if selenium, has not yet been isolated and other protective agents may exist. For example, arsenic, as well as selenium, has been implicated as being able to modify toxicity of methylmercury (El-Begearmi, Ganther and Sunde 1974 and 1975; see also Ganther 1978). High levels of arsenic have often been reported in marine fish (Hill, 1975) and in some species, concentrations up to several hundred μg g^{-1} have been found (NRCC 1978). Second, difficulties may arise in providing true control diets. Some of the toxic effects of methylmercury can be partly alleviated by the addition to the diet of cystine, casein or proteins, including proteins from fish not necessarily containing high levels of selenium. For example, Stillings *et al.* (1974) observed that a 20 per cent content of fish protein in the diet of weanling rats exposed to methylmercury reduced and delayed signs of toxicity to a greater extent than did a 10 per cent protein level. However, the fish protein was originally prepared from marine fish and its selenium

content (perhaps the protective factor) was not reported. Thus difficulties may arise in ascribing the protective effects to the selenium or to non-selenium component(s) of the diet.

Difficulties also exist in preparing appropriate control groups if the fish tissue used contains selenium levels likely to be protective. For example, Friedman *et al.* (1978b) found that a 15 per cent swordfish supplement (containing about 2 $\mu g\ g^{-1}$ selenium) was effective in delaying mortality compared with 'control' rats fed a 15 per cent casein supplemented diet. Casein itself, however, has been shown to have a protective effect against the toxicity of methylmercury: Ohi *et al.* (1976b) demonstrated that in experiments with rats, 10–30 per cent casein supplements (in a diet containing methylmercury) alleviated decreases in growth rate and delayed the expression of neurological signs of toxicity, when replacing corn oil in the diet.

Ohi *et al.* (1976b) have found both selenite-selenium and "tuna selenium" to be about equally effective in preventing growth-rate inhibition of methylmercury-fed rats. The "tuna selenium" was only about half as effective, however, in protecting against neurological manifestations. Stillings *et al.* (1974) also mention findings where sodium selenite was slightly more effective than seleno-methionine in reducing signs of neurotoxicity. Working with quail, Stoewsand *et al.* (1977) found selenite or selenium from natural wheat (known to be primarily selenomethionine (Olsen, Novacek, Whitehead and Palmer 1970) to have about the same effectiveness against toxic signs and death caused by acute dosage of methylmercury. It is likely that the "biological availability" of selenium present in tuna and other marine fish is relatively low (Cantor, Scott and Noguchi 1975, Skerfving 1978) and this may explain the greater effectiveness of selenite-selenium evidenced in some cases. On the other hand, although a lower percentage of selenium provided in organic form may be absorbed compared to administered selenite-selenium, the retention time in the body may be longer than with selenite, as reported by Stoewsand *et al.* (1977) in studies with quail. Many different forms of selenium are known to exist in plants and animal tissues including amino acids such as selenomethionine, cystathionine and methylselenocysteine (Carty and Malone, 1979; see also NAS 1976).

When discussing the possible protective rôle of selenium contained in marine fish, use is also made of observations made on cats poisoned in the epidemic areas. In both the Minamata and Niigata outbreaks, cats had been observed to exhibit signs of poisoning prior to symptoms

occurring in humans. Also in Canada, around areas known to contain lakes and rivers with mercury-contaminated fish, cats have died and others have shown similar neurological signs of methylmercury poisoning to those from the Japanese areas (see Section 6). Takeuchi *et al.* (1977) have revealed that elevated mercury levels existed in these cats similar to those from Minamata and others experimentally dosed with methylmercury. This would speak against a major protective rôle of selenium in these intoxications. Experiments on cats, however, gave equivocal results: Ganther (1978) has reported that some degree of protection could be demonstrated in cats fed tuna compared to cats fed pike and that this protection correlated with the higher levels of selenium in the marine fish. On the other hand, similar levels of methylmercury in both marine fish and freshwater fish have been reported elsewhere to cause signs of toxicity; these occurred in the experiments of Chang and Yamaguchi (1974) and Chang, Yamaguchi and Dudley (1974) after a 7–11 month period when cats were fed tuna* containing 0.5 $\mu g\, g^{-1}$ methylmercury; Charbonneau (1979, personal communication) observed signs after about 14 months with cats fed pike* containing about 0.8 $\mu g\, g^{-1}$ methylmercury.

From the overall survey it seems likely that the high level of selenium usually present in marine fish can provide some protection against methylmercury toxicity. Thus, some authors (for example Friedman *et al.* 1978b) have suggested that a reconsideration should be made of the health risk of methylmercury from the consumption of marine fish containing high selenium levels.

Obviously, however, the significance of findings referred to above with regard to human health is limited. Both serious Japanese epidemics have involved poisoning due to the consumption of large quantities of fish contaminated by methylmercury. In the Minamata area, selenium levels in the fish were elevated and, at one point, selenium itself was suggested as a possible causative agent in the disease§ (see Ganther and Sunde 1974).

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* In the study of Chang and Yamaguchi (1974) the selenium levels were not reported but Skerfving (1978) has suggested that a level of about 1.0 $\mu g\, g^{-1}$ could reasonably be assumed. In the study of Charbonneau (1979) a low level of selenium existed in the diet (0.13 $\mu g\, g^{-1}$) which would not generally be expected to provide much protection.

§ Dietary selenium levels of 4–5 $\mu g\, g^{-1}$ may inhibit growth in test animals, and histopathological observations suggest that lower levels, down to 1.0 $\mu g\, g^{-1}$ and below, may also be toxic, depending on the criteria used to determine the no-effect level (NAS 1976).

In the Niigata epidemic, which took a much milder course, the source of methylmercury was from freshwater fish where the selenium levels would be expected to be relatively low*; whether dietary selenium could have prevented mild, subclinical forms of methylmercury toxicity in humans in this area if the exposure were from marine fish instead, is not known. It may be concluded, therefore, that the existing evidence regarding the protective rôle of selenium contained in marine fish against toxic effects of methylmercury in humans is not conclusive enough to call for a re-evaluation of the health risk.

5.3 *In vitro* studies on the protective effects of selenium

No unequivocal positive influence of selenium on methylmercury effects emerges from the *in vitro* studies. Several authors have used the technique of growth of cell cultures. Growth may be inhibited by either selenite or methylmercury, when added alone. If added jointly the spectrum of results points to a variety of possible effects.

In conditions favouring selenite toxicity, Potter and Matrone (1977) have shown protective effects of both inorganic mercury and methylmercury on the growth of Chang's liver cells and mouse fibroblast (3 TC cells). However, no protection by selenite of growth inhibition induced by mercury compounds could be demonstrated. No unequivocal protective effect on methylmercury induced growth suppression was found with human fibroblasts in culture (Alexander, Høstmark, Førre and von Kraemer Bryn 1979). Indeed, there were indications of additive toxicity in experiments with rat cerebellar tissue in culture (Kasuya 1976). Negative results were also obtained with mice bone marrow cells (Strom, Johnson and Uyeki 1979). On the other hand, protective effects of selenite were shown for rat hepatoma cells (Alexander *et al.* 1979) and primary cell cultures of rat cerebellar tissue (Kasuya 1976) at certain concentrations below the critical toxic level of selenite.

Methylmercury alone is known to depress antibody formation (see Section 3), but in the presence of selenite a synergistic enhancement of antibody formation was found in mice (Koller *et al.* 1979).

No overall conclusions can be drawn from the observations referred to above. As pointed out by Alexander *et al.* (1979), it seems likely that

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* The levels of selenium observed in pike from Sweden (Jernalov, Johansson, Sorensen and Svenson 1976) and Canada (Charbonneau *et al.* 1976) were generally about 0.1 $\mu g\ g^{-1}$.

the methylmercury/selenium antagonism does not involve a direct chemical reaction but is rather mediated through specific cell dependent processes.

5.4 The influence of selenium on the metabolism of methylmercury

In the case of inorganic mercury, administration of selenite results in a major shift of the metal from the kidney to the liver, where mercury is deposited mainly in the nuclear and mitochondrial fractions (Komsta-Szumska and Chmielnicka 1977). In the case of methylmercury, levels in tissues have not been found to be drastically changed by selenium administration (Ohi *et al.* 1975b and 1976b), and experimental data do not suggest a uniform response; apparently the pattern of redistribution depends on many factors, including the dose size and administration of both methylmercury and selenium, the time space lapse from administration to the tissue analysis, and the test animals used. Most authors found that there was no enhancement of elimination following selenium treatment.

Levels of mercury in the kidney after selenium treatment have been found to be either increased (Ohi *et al.* 1975b, Ohi *et al.* 1976b, Koller *et al.* 1979) or decreased (Stillings *et al.* 1974, Brzeźnicka and Chmielnicka 1980, and Brzeźnicka, Chmielnicka and Wachocka 1977, Mengel and Karlog 1980b, Chen, Lacy and Whanger 1975, Alexander and Norseth 1979).

Little evidence exists for an increased rate of demethylation in rats dosed with methylmercury and selenium. Most authors found no increase* in the percentage of inorganic mercury in the kidney (Brzeźnicka *et al.* 1977, Nishigaki *et al.* 1977, Ohi *et al.* 1976b).

Perhaps the most intriguing finding is that an elevation in brain mercury concentration often occurs in animals treated with selenite compared with those treated with methylmercury only. This has been reported in short-term experiments with rats (Magos and Webb 1977, Chen *et al.* 1975, Alexander and Norseth, 1979) and mice (Iijima, Tohyama, Lu and Matsumoto 1978) and also in longer-term experiments (Brzeźnicka and Chmielnicka 1980, Minowa, Seki, Konno and Ohi 1978, and Mengel and Karlog 1980b). In male quail which had been administered both methylmercury and selenium, brain mercury con-

* Findings at variance with this have been reported by Tamura *et al.* (1977).

centrations up to 40 $\mu g\ g^{-1}$ were found with no overt signs of neurotoxicity, whereas high mortality occurred in birds fed methylmercury alone, with much lower brain mercury levels (Stoewsand *et al.* 1974). Ohi *et al.* (1976b) suggested that the critical "threshold concentration" of mercury in the brain above which signs of toxicity occur (see Section 8) may be raised by selenium. Rats dosed with methylmercury and selenium contained methylmercury levels in the brain in excess of those normally associated with symptoms of toxicity (about 10 $\mu g\ g^{-1}$), yet the animals were apparently unaffected.

Tamura *et al.* (1977) found that the foetal/maternal ratio of methylmercury in blood was reduced from 1.15 to 0.8 when pregnant rats fed with methylmercury were given selenium supplement (as tuna) in their diet. As a result, the foetal brain mercury level was reduced by about half. This suggests that selenium may play a rôle in reducing the risk of methylmercury toxicity to the foetus, which is considered to be at special risk when maternal blood levels of methylmercury are elevated.

Considerable interest has centred on the ability of marine mammals to accumulate high levels of inorganic mercury and selenium in their kidneys and livers. In many studies on wild seals, the concentration of mercury in the liver was shown to be several hundreds of $\mu g\ g^{-1}$ with correspondingly high levels of selenium, usually in a 1 : 1 molar ratio (Koeman *et al.* 1973, Koeman *et al.* 1975, Smith and Armstrong 1975, and Martin *et al.* 1976). With fish containing methylmercury as the primary food source, this suggests the existence of an efficient de-methylating capacity and the rôle of selenium in this process seems possible. Although no evidence was found for a biochemical de-methylation in *in vitro* studies of seal liver homogenates (van de Ven, Koeman and Svenson 1979), this could be due to the relatively slow rate of the process in question. In favour of such an assumption would be the preliminary findings made in experiments on seals (van de Ven *et al.* 1979). Animals fed methylmercury (0.2 $mg\ kg^{-1}$ (b.wt) per day) for 1 to 6 months revealed a low percentage of inorganic mercury compared to levels in wild seals, even in the kidney and liver (about 40 *vs* 70 per cent and 40 *vs* 80 per cent respectively).

Although there is no dramatic influence of selenium on the metabolism of methylmercury, the reverse does not apply. Thus, there seems to be evidence that in many tissues, the retention of selenium increases in relation to increases in methylmercury. In particular, many authors have found increases in brain selenium of between 2 and 10 times in animals

dosed with both selenium and methylmercury (Johnson and Pond 1974, Ohi *et al.* 1975b, Fang 1977, Minowa *et al.* 1978 and Iijima *et al.* 1978). In the rat brain, methylmercury administration results in a shift of selenium from the cytosol fraction to the mitochondria (Prohaska and Ganther 1977). In the kidney, levels of selenium were markedly increased in rats dosed with methlmercury and selenium (Ohi *et al.* 1975b, Nishigaki *et al.* 1977, and Minowa *et al.* 1978) and, interestingly, it was observed that selenium concentrations in the kidneys also increased when methylmercury was given alone with no added selenium (Nishigaki *et al.* 1977, Tamura *et al.* 1977 and Ohi *et al.* 1975b). Iijima *et al.* (1978) also found increased selenium concentrations in the foetuses of pregnant mice previously given simultaneous injections of selenium and methylmercury.

In contrast to these observations, Nishigaki and Harada (1975) did not find increased selenium levels in the umbilical cords of inhabitants of the Minamata area, as a result of exposure to methylmercury.

High levels of selenium had previously been reported in the organs of animals and patients who died of Minamata disease (see Shirabe *et al.* 1979). Electron probe X-ray micro-analysis of the brains of Minamata disease victims has shown that selenium and sulphur were associated with mercury in particles present in the cell cytoplasm and extracellular spaces (Shirabe *et al.* 1979).

The exact nature of the methylmercury complexes occurring in animal tissues is still unclear (see Section 2). However, the high affinity of methylmercury with sulphydryl groups is well known and it has been found that even stronger affinity exists with selenohydryl groups (Sugiura, Hogo, Tamai and Tanaka 1976). The order of binding affinity for various donor groups towards methylmercury was reported as $SeH > SH > Se\text{–}Se > NH_2 > S\text{–}S, SeCH_3$ (Sugiura, Tamai and Tanaka 1978). Rabenstein and Evans (1978) have underlined the difficulty in identifying the complexes present in *in vitro* studies; procedures which involve disruption of the cellular membranes result in the exposure of a multitude of sulphydryl-containing bio-molecules to methylmercury compounds with the possibility of redistribution among the ligands.

Selenite can release methylmercury from protein-bound forms in blood and other tissues, enabling changes in tissue distribution to occur. Whether such release involves formation of selenium complexes is not clear at present: Magos, Webb and Hudson (1979b) reported an unstable complex with a methylmercury : selenium ratio of (2 : 1 (possibly

bis-methylmercury selenide), but this is at variance with the results of an earlier study (Sumino, Yamamoto and Kitamura 1977).

From the overall review it is apparent that the present-day recognition of selenium's influence on the metabolism of methylmercury is of limited use in the understanding of mechanisms involved in the selenium : methylmercury interactions.

6 Toxicity of methylmercury in adult humans

6.0 Summary of information contained in the Health Criteria Document

It was recognized that the most common signs and symptoms of chronic methylmercury intoxication were: paraesthesia, loss of sensation in the extremities and around the mouth, ataxia, constriction of the visual fields, and impairment of hearing. Damage to the brain is found both in the cerebrum and in the cerebellum. In the former, destruction of the neurons in the cortex occurs, mainly in the visual areas of the occipital cortex, while in the latter there is damage to the granular layer. The threshold concentration of methylmercury in the brain was supposed to be consistent with the data from animal experiments, and a figure of $5\ \mu g\ g^{-1}$ was proposed. Basically no reliable pathological information was available regarding the peripheral nervous system.

Reversibility of the neurological effects was a controversial issue. Information from Iraq was inconsistent with Japanese data and pointed to the possibility of some reversal in motor disturbances and paraesthesia.

Of the other possible health effects, renal damage due to methylmercury was seen as rare. Dermatitis was recognized in some cases. Data on cytogenetic effects were controversial.

The Health Criteria Document gave a detailed review of various groups under exposure from both contaminated grain and fish and discussed at length data pertinent to the calculation of the dose-response relationships and thresholds in humans.

6.1 Studies of human populations exposed to methylmercury

In a follow-up of the known epidemics referred to in the Health Criteria Document, more information became available from Iraq and Japan (Minamata and Niigata areas). A new area of concern was discovered

in the Canadian Indian communities. Attention was also directed to the situation in the Mediterranean area, Italy in particular.

Before discussing new reports from the epidemic areas it may be of interest to recall the number of cases of methylmercury poisoning worldwide. According to Clarkson (1977b), these are as follows: Iraq (1956), approximately 100; Iraq (1960), approximately 1,000; Iraq (1971), approximately 6,000; Pakistan (1969), approximately 100; Guatemala (1963–65), approximately 45; and Ghana (1967), approximately 144. The poisonings from contaminated fish in Minamata, Japan (1953–60), were approximately 120, and in Niigata (1964–65), approximately 48. Thus, the total number of cases has been in excess of 8,000. There is, however, a huge discrepancy in the number of poisonings ascribed to both Japanese areas. The original Japanese reports quote much higher figures compared with those of Clarkson. More recently, Tsubaki *et al.* (1978) mentioned about 1,000 identified patients in the Minamata area and 646 in the Agano area (Niigata).

6.2 The Iraqi epidemic

At the time of preparation of the Health Criteria Document the epidemic was already over and most of the relevant data were known to the expert group. The basic report had been published before the Health Criteria Document (Bakir *et al.* 1973). The epidemic was reviewed at a special conference in Baghdad (WHO 1976a). Other reports on the epidemic were published simultaneously with the Health Criteria Document (Clarkson *et al.* 1976) or shortly thereafter (e.g. Gerstner and Huff 1977b). Not included in the Health Criteria Document was the report of Rustam and Hamdi (1974) which was confined to the neurological study of 53 patients with a one-year follow-up. Pertinent to the objectives of the present report is the observation that the mildly and moderately affected patients improved remarkably, with or without specific treatment. Improvement was seen in cerebellar function, superficial sensation and visual acuity.

The question of involvement of peripheral neuropathy in neurological damage has not been unequivocally resolved (Rustam and Hamdi 1974). Some electrophysiological evidence obtained long after the onset of poisoning was suggestive of damage to the lower brain stem, and of high incidence of interference with myoneural transmission (von Burg and Rustam 1974).

6.3 Minamata disease in the Minamata area

More systematic reports on Minamata disease in this area became available in 1975 (Takeuchi and Eto 1975), revealing on the whole and up to 1974 about 800 cases* of poisoning and 3,000 suspects. In the Minamata area, the occurrence of chronic methylmercury poisoning was assumed in residents whose hair contained over 10 $\mu g\, g^{-1}$ of mercury (Takeuchi and Eto 1975). Such low levels of mercury in hair of subjects with diagnosed Minamata disease have been confirmed also in a more recent comprehensive report (Tsubaki and Irukayama 1977(c)). Such an unexpectedly low figure (corresponding to only 30 ng $m\ell^{-1}$ in the blood) may have resulted from the long time lapse between the onset of symptoms and actual monitoring. The authors, however, do not exclude that the threshold may actually be that low in subjects with an extended period of exposure.

Theoretically, there may be several reasons possibly justifying such an attitude: (1) a low threshold for blood or hair applicable to very long exposure duration could be connected with the hypothetical long half-life of methylmercury in the brain, allowing for long-term accumulation (see Section 2.3). Such a hypothesis has been in fact put forward by Rose (1979) in an attempt to elucidate the origin of neurological symptoms in Canadian Indians at a relatively low level of exposure; (2) independently of (1), the time component of the exposure estimate could contribute to the development of symptoms at relatively low levels of methylmercury in the body (see Evans *et al.* 1977); and (3) the threshold could be lowered by compounding factors, such as malnutrition.

It should be stressed, however, that the time lapse between the onset of symptoms and the monitoring of hair inherent in the Minamata cases, probably offers a sufficient explanation. This may explain why the Research Committee on Minamata Disease (Kumamoto University, 1975) has accepted that a minimal toxic dose of methylmercury is 5 $\mu g\, kg^{-1}\, d^{-1}$. (Quoted after NAS 1978.)

When describing the symptomatology, Takeuchi and Eto (1975) have distinguished groups of patients differing in the severity of the disease. (a) The most severely affected ended with death or permanent disability distinguished by apallic syndrome and idiotic disorders. (A detailed pathological study of 64 prolonged cases of Minamata disease is now

* A more recent figure is about 1,000 (Tsubaki *et al.* 1978).

available (Eto and Takeuchi 1978)). (b) Advanced cases of moderate poisoning showed tremor, disturbance of sensation, ataxia, dysarthria, constriction of visual fields, and difficulty in hearing. Patients of this group were likely to become disabled. (c) Less advanced chronic cases had ataxia, dysarthria, constriction of visual fields, disturbance of eye ball movements*, and ocular dysmetria (cerebellar origin). (d) The mildest cases had mainly unspecific symptoms such as fatigue, impairment of memory, slight mental disorders, headaches, slow movements and numbness, and tremor of the lips and fingers. The causal relation of these unspecific symptoms to methylmercury exposure is not certain (Tsubaki, personal communication).

With regard to the latent period, it has been observed that this could be very prolonged, over 10 years or more (Takeuchi and Eto 1975) and a number of cases were observed where clinical symptoms had worsened with time, despite reduced or discontinued exposure (Igata *et al.* 1975).

The neuropathology of the severe cases, as described by Takeuchi, was consistent with knowledge gained elsewhere. It pointed to the involvement of the cerebral and cerebellar cortices; only secondary degeneration and slight changes of neurons were noted in the diencephalon, brain stem and spinal cord (Takeuchi and Eto 1975). In adults, usually only limited brain atrophy (5–16 per cent) was found, in contrast to the childhood poisoning cases (Takeuchi and Eto 1975). Some evidence was reported indicating involvement of the peripheral nervous system, as seen in the sural nerve (Nagaki 1975, Tsubaki and Irukayama 1977(d)). Apparently it is not known whether this is a primary or secondary effect (Takeuchi and Eto 1975). Other pathological findings detected in the cases discussed above comprised only slight changes in the liver, kidney, bone marrow and duodenum. In addition, the Langerhans islets in the pancreas were disturbed (Takeuchi and Eto 1975).

6.4 Minamata disease in the Niigata area

This epidemic was already well recognized at the time of preparing the Health Criteria Document. A systematic review, however, has become available more recently (Tsubaki and Irukayama 1977).

The poisonings in this area had originally been discovered in 1964/65 and thereafter a more systematic epidemiological study of the area was

* See also Tsutsui (1980).

undertaken by Japenese authorities. As at the end of 1974, a total of 520 cases of poisoning had been identified and the number was still increasing* (Tsubaki and Irukayama 1977(a)). Since most of the late cases were discovered not by the spontaneous reporting of sickness but by active monitoring and search, this area may provide insight into the development of Minamata disease with a relatively lower level of long-term exposure.

The first 26 patients discovered early in 1964/65 had hair levels typical of high exposure (approximately 60–600 $\mu g\, g^{-1}$, indicative of blood levels in the range 200–2,000 ng $m\ell^{-1}$); the actual blood levels, however, were in the range of only 60–900 ng $m\ell^{-1}$.

The survey also revealed a number of asymptomatic subjects with unusually high hair levels, in excess of 150 $\mu g\, g^{-1}$, of which most developed symptoms later. On the whole, the survey discovered about 4 per cent of inhabitants with hair levels in excess of 100 $\mu g\, g^{-1}$, and about 28 per cent with levels in the limits of 20–100 $\mu g\, g^{-1}$.

Overall, the poisonings are thought to represent a less severe stage of damage compared with the original Minamata epidemic. Even in the initial group of patients discovered, this was demonstrated by a lower frequency of occurrence of individual symptoms and signs (corresponding data from the original Minamata epidemics in parenthesis): superficial sensory disturbances 92 per cent (100), hearing impairment 69 per cent (85), finger-nose, knee-heel tests 41 per cent (80), deep sensory disturbances 38 per cent (100), speech impairments 35 per cent (88), constriction of visual fields 33 per cent (100), gait disturbances 31 per cent (82), tremor at rest 26 per cent (76) and mental disturbance 16 per cent (70) (Tsubaki and Irukayama 1977(d)).

In seven patients with hair mercury levels in excess of 200 $\mu g\, g^{-1}$, the following sequence of symptoms was found to be developed in a five-year period (first examination, 1965): (a) sensory disturbance of extremities started earliest and became prevalent after three years; (b) sensory disturbance of the perioral area started after one year and became prevalent after three to five years; (c) ataxia started after one year and became prevalent after four to five years; (d) constriction of the visual fields started after a delay of four years and became prevalent a year later (Tsubaki and Irukayama 1977(d)). This pattern, however, was also typical of another small group of patients having much lower levels of

* A more recent figure is 646 cases (Tsubaki *et al.* 1978).

mercury in hair (50–100 $\mu g\ g^{-1}$, equivalent to blood concentrations of 150–300 ng $m\ell^{-1}$).

The Niigata study provides evidence that pathological changes, once initiated, can progress in time (years) even if exposure has ceased (as evidenced by a dramatic decrease of mercury in the hair) (Tsubaki and Irukayama 1977(d)). The evidence presented suggests that unspecific mild symptoms, such as numbness of extremities, tiredness, headaches, dizziness, memory impairment, as well as sensory disturbance in the extremities could be initiated by mercury levels reflected in hair concentrations below 100 $\mu g\ g^{-1}$, with an average of about 30–40 $\mu g\ g^{-1}$ (equivalent to blood concentrations of 100 ng $m\ell^{-1}$). The more advanced symptoms, such as sensory disturbance of the perioral area and loss of co-ordination, could be initiated by levels around 150 $\mu g\ g^{-1}$, equivalent to roughly 500 ng $m\ell^{-1}$ in the blood (Tsubaki and Irukayama 1977(d)).

In a more recent review Tsubaki *et al.* (1978) assume that "the lowest mercury concentration (in hair) associated with clinical effects was 52 $\mu g\ g^{-1}$". The same report gives data on re-examination of the hair samples preserved from the time of onset of the symptoms. The new results, obtained by atomic absorption, tended to be higher than the original ones, obtained by dithizone method. For the single patient who had clinical symptoms at the concentration of mercury in hair of 50 $\mu g\ g^{-1}$, new results pointed to 96 $\mu g\ g^{-1}$ with maximum value (preceding the onset of symptoms) around 200 $\mu g\ g^{-1}$. The re-evaluated data, based on only 10 patients, are presented in Table 3. Here, patients are divided into two groups characterized by "early onset" and "delayed onset".

It has to be noted that the estimates of blood levels associated with clinical effects refer to either the time of onset (former interpretation)

Table 3 Mercury concentration in hair and blood associated with clinical effects (after Tsubaki *et al.* 1978)

	Total number studied	Mercury concentration	
		Hair, $\mu g\ g^{-1}$ (determined)	Blood ng g^{-1} * (estimated)
Patients of early onset	6	100–500	400–2,000
Patients of delayed onset	4	50–200	200–800

* The original paper erroneously gives the blood concentration in $\mu g\ g^{-1}$.

or the maximum value preceding the onset. In none of the assessments has there been any attempt to introduce the integral of concentrations over time as the measure of health risk (see Evans *et al.* 1977).

The pattern of deterioration found in the Niigata cases did not exclude individual cases of improvement. The overall tendency, however, is at variance with the prevalent trend of no change/improvement in the Iraqi epidemic (Kazantzis *et al.* 1976, Rustam and Hamdi 1974).

6.5 Methylmercury exposure in Canadian Indians

The problem was first discovered in 1970 in two communities of Indians in the White Dog and Grassy Narrows reserves. The population of each of these tribes is about 500–600 members and both tribes had been uprooted within the past 25 years and moved to new locations, about 60 miles north of Kenora, Ontario.

The mean levels of mercury in blood, as determined in 1970, were about 77 ng $m\ell^{-1}$ (up to 385 ng $m\ell^{-1}$) in White Dog, and 46 ng $m\ell^{-1}$ (up to 160 ng $m\ell^{-1}$) in Grassy Narrows (Health and Welfare Canada 1973). Further, more systematic surveys revealed a similar range of values, but attention was paid to the cyclic occurrence of high mercury levels in the summer/autumn periods due to high fish catches in these seasons. The highest likely blood concentration, recalculated from hair analysis, extended to 500 ng $m\ell^{-1}$ (Clarkson 1975).

With the estimates of exposure largely consistent over the reported time period, the medical assessment gave conflicting results. The early report of Barbeau *et al.* (1976) claimed a high incidence of various symptoms, such as sensitivity problems, constriction of the visual fields, co-ordination disturbance and tremors. The frequency of symptoms resembled that of Minamata patients and was contrasted by a low incidence observed in the white population of the same area. These results have been confirmed by an independent investigation performed by a Japanese team (Harada, Fujino, Akagi and Nishigaki 1976). A health survey conducted on 89 inhabitants revealed a high incidence of signs and symptoms typical of Minamata disease, such as impaired hearing, sensory disorders, tremor, hyperreflexia, disturbance of eye movements, constriction of the visual fields, (in decreasing frequency of 40 to 16 cases) and, with lesser frequency, ataxia and dysarthria (8 and 5 cases). The authors' conclusion was that "the present situation of Canada is exactly like that of Minamata before the mass outbreak of

the disease". The results of the above reports, however, have been questioned because (a) the clinical symptoms could not be interpreted in terms of dose-response reasoning, (b) the overall level of exposure has been too low to justify a high incidence of intoxication in the light of accepted dose-response data, and (c) there are specific conditions under which the Indians live, and other factors such as deficient nourishment and alcoholism could have largely contributed to the health status of the population (Wheatley, personal communication; for discussion see also Shephard 1976, Charlebois 1978, Wheatley, Barbeau, Clarkson and Lapham 1979).

Recent evidence (Health and Welfare, Canada 1979) has revealed that from over 35,000 results obtained in 350 communities, over two-thirds of the blood levels were within "normal" limits of 20 ng $m\ell^{-1}$, but 2.5 per cent (over 900 individuals) were "at risk" having blood levels in excess of 100 ng $m\ell^{-1}$. The highest levels detected were 660 ng $m\ell^{-1}$. Only five persons had blood levels in excess of 500 ng $m\ell^{-1}$. Detailed clinical examination has been reported for 84 subjects of the "at risk" group. Although positive findings were reported in 38 persons, 27 of them were not related to methylmercury. Still, a group of 11 persons remained in which methylmercury intoxication could not be excluded, but also could not be positively stated. A follow-up of the early studies shows that none of the subjects could have been classified with any certainty as suffering from methylmercury intoxication. It is understood, however, that of the total group "at risk", which currently accounts for over 500 people, only a few have ever had blood mercury concentrations in the assumed area of threshold (200–500 ng $m\ell^{-1}$), assumed in the Health Criteria Document (WHO 1976b).

The most recent study of the McGill University (Methylmercury Study Group 1980) has concentrated on the Cree Indians of North-western Quebec: one coastal community, Great Whale, and two inland communities, Mistassini and Waswanipi. Only native Cree Indians were included, over 30 years of age, and the number of people for whom both medical examination and data on exposure were available amounted to 541. The approximate range of mercury concentrations in blood was 10–260 ng $m\ell^{-1}$, and in the hair 1–50 $\mu g\, g^{-1}$. The most frequent mercury concentrations in hair were in the range 10–20 $\mu g\, g^{-1}$ (241 subjects). Above this range, 109 subjects had concentrations 20–30 $\mu g\, g^{-1}$ and 63 subjects in excess of 30 $\mu g\, g^{-1}$. Data referred to above represent the maximum level from determinations made on 1 cm seg-

ments of hair from each individual. Apart from data collected in 1978, past data from 1975/76 were also available and pointed to a similar range of exposure levels.

The health effects which could possibly be ascribed to methylmercury exposure were assessed by neurologists, different for individual areas under study. The most frequent abnormalities found in this study were: tremor, abnormalities in co-ordination, movements and reflexes, and eye movements. The frequency of occurrence of these abnormalities differed slightly between the tribes. The severity of deviations was in general mild, which contributed to the difficulties in the diagnosis.

Confounding factors greatly influenced the outcome of this study. The frequency of these mild and unspecific symptoms tended to increase with age and could also have been influenced by alcohol and caffeine, as well as nutritional deficiences. Also, the scoring by individual neurologists showed appreciable differences and made intercomparisons difficult. Due to the above, the findings were not suitable for a study in the conventional dose-response arrangement, and more sophisticated, indirect statistical methods had to be used for the assessment of the causal relation between effects and exposure.

The results were assessed using the approach of a case-control study, and complemented by a study of observer-variation. In the case-control study, after adjustment for disturbing variables, an increased risk of being a case was found for each increment of the exposure indices equal to 20 $\mu g\, g^{-1}$ of mercury in hair (relative risk = 2.4 for all communities and sexes together). Although this association was statistically significant ($p = 0.01$) for both men and women, as based on the 1978 exposure indices, some uncertainty remains: before the correction for confounding factors was introduced, the association was higher by one order of magnitude. Thus, if statistical corrections could have reduced the significance of the original findings to such a great degree, the impression of uncertainty remains regarding the validity of the residual values. Besides, the relative risk (2.4) cannot be interpreted in terms of a dose-response relationship to yield figures compatible with those known from populations at higher risk.

In the complementing observer-variation study the number of cases included for assessment was reduced, for various technical reasons, to only 137. This study revealed that a highly positive association between effects and exposure indices was found by only 4 "observers" out of 7, and after adjustment for age, has pointed to a relative risk of being a

case with increased exposure, ranging from 2.4 to 8.3. The other three "observers" while having found association between abnormalities and age, failed to find association with exposure.

Thus, the above study can be interpreted as having yielded equivocal results. It seems likely that there is a slightly increased risk of acquiring mild, unspecific neurological symptoms with a long-term exposure to methylmercury in fish, equivalent to maximum levels of mercury in hair of 20 $\mu g\ g^{-1}$ (which corresponds to maximum levels in blood of about 80 $ng\ m\ell^{-1}$). The "increased risk", however, can hardly be interpreted in terms of absolute increase in frequency, suitable for reasoning in terms of dose-response relationships.

The authors of the quoted study refrained from suggesting a threshold for the long-term exposure to methylmercury. The main reason was that effects observed in this study could, in fact, have been brought about by past exposure to higher levels prior to 1975. This reservation, however, could be challenged on the grounds that such past exposure is likely to have influenced all groups under this study independently of the actual level of exposure.

6.6 Other populations with a large intake of fish

Other populations which may be relevant to the objective of this report have been studied previously and the available data were reported in the Health Criteria Document.

The other new area, already considered in the Health Criteria Document, has been the Mediterranean—Italy in particular.

The average "normal" levels of mercury in the blood and hair of Italian people were 20 $ng\ m\ell^{-1}$ and about 1.6 $\mu g\ g^{-1}$ respectively (Paccagnella and Prati 1974). However, levels much higher have been found in the coastal area of Carloforte, Cagliari in Sardinia (Paccagnella *et al.* 1973). Other coastal areas (Reggio Calabria, and Piacenza Provinces) also have fish-eating populations with elevated mercury levels: the average blood levels of the inhabitants under study were 60 and 70 $ng\ m\ell^{-1}$ respectively (Riolfatti 1977). Another area (Vada, Leghorn) has been studied by Bacci *et al.* (1976). The highest levels of mercury in the erythrocytes were within the range of 200–250 $ng\ m\ell^{-1}$ (the corresponding whole blood levels are lower by a factor of 2) and in the hair, 10–80 $\mu g\ g^{-1}$. One individual was reported to have an erythrocyte level of over 900 $ng\ m\ell^{-1}$ and hair concentration of 109 $\mu g\ g^{-1}$. This

report only contains suggestions regarding the health effects in the population under study. The study failed to discover symptoms of "acute intoxication" but subclinical damage could not be excluded. Further epidemiological studies in Italy are under way (Paccagnella, on-going project; personal communication).

Similar studies performed in fishing communities around the north-eastern Irish Sea failed to reveal individuals with levels of mercury likely to cause any health effects (Haxton *et al.* 1979).

7 The developmental toxicology of methylmercury in humans (pre-natal, infant, and childhood exposure)

7.0 Summary of information contained in the Health Criteria Document

Data were already available from Minamata disease in Japan as well as preliminary data from the Iraqi epidemics. One major difference was apparent between the Japanese and Iraqi cases; in Japan, cases were reported of severe cerebral involvement in infants, whereas mothers were basically asymptomatic. In Iraq, in most cases where infants were affected, mothers were also symptomatic and this discrepancy was ascribed to the relatively short duration of high exposure. Belief was expressed that pre-natal life was more vulnerable to methylmercury damage, but no quantitative assessment was possible at that time. Evidence was quoted pointing to the lack of chromosomal aberrations in pre-natally exposed children. No specific data were available to evaluate the vulnerability of post-natally exposed infants and children.

7.1 Effects of pre-natal exposure

A more careful investigation of the Japanese cases revealed that at least some of the mothers of affected infants were also symptomatic. Thus, there is no major discrepancy between the reports from Japan (Tsubaki and Irukayama 1977(e)) and the Iraqi cases (Amin-Zaki *et al.* 1979, as well as Gerstner and Huff 1977b), although the severity of the effects in the Japanese mothers might have been less compared with those from Iraq. This may possibly be attributed to the longer periods of low-level exposure in Japan compared with the limited period of high intake in Iraq. This is also reflected in the reported mercury concentrations in maternal hair: a range of 10–200 $\mu g\, g^{-1}$ in Japan (Tsubaki and Irukayama 1977(e)) and 40–700 $\mu g\, g^{-1}$ in Iraq (Amin-Zaki *et al.* 1979).

However, part of the above difference has to be ascribed to the fact that the Iraqi data (Amin-Zaki *et al.* 1979) refers to time-selected peak concentrations; the Japanese evidence, on the other hand, has been obtained long after delivery (one to four years), with unknown history of exposure.

Any attempt to reconstruct the mother's exposure during pregnancy in terms of blood mercury concentrations is difficult. Recalculation from the hair concentrations (see 2.4) would yield maternal average blood concentrations in the range of 30–600 ng $m\ell^{-1}$ (with undetermined relation to the period of gestation) in the Japanese cases and about 100 to over 2,000 ng $m\ell^{-1}$ in peak periods of the Iraqi epidemics. Apart from the implications regarding infants, these figures, if accepted, would influence judgements regarding the sensitivity of adults, pregnant women in particular (for more discussion see Section 8). In general, the blood levels in infants at delivery would be expected to be twice as high (see 2.5). Data collected in varying periods after delivery in the Iraqi series point to concentrations mostly in the range of 100–1,000 ng $m\ell^{-1}$ (with extremes between 40 and 4,000 ng $m\ell^{-1}$). These values should be regarded as underestimates, since the values which existed at the time of delivery must have been higher. On the other hand, however, cases may have been included which were not related causally to mercury exposure, thus depressing the range unjustly. Another series of investigations, carried out as a follow-up to the epidemic in Iraq, included milder cases which have been discovered through the hair-monitoring programme of women who were pregnant during the epidemics. A significantly increased rate for several symptoms was found in infants whose mothers had peak concentrations in hair of 100–400 $\mu g\, g^{-1}$, indicating blood levels of 300–1,200 ng $m\ell^{-1}$ (Marsh *et al.* 1980). From the data it is apparent, however, that this range was already well above the possible threshold for health effects in infants.

In contrast to animal observations, the human data do not point to miscarriages or stillbirths. In most cases the course of pregnancy was normal, and mothers have delivered, in full time, live infants. Also, in most cases no major deficits in the weight of the infant were noted (Tsubaki and Irukayama 1977(e)).

Apart from the smaller and slightly deformed head (microcephalus) no major or minor congenital malformations were reported in the children irrespective of the origin of the report (Tsubaki and Irukayama 1977(e), Amin-Zaki *et al.* 1979, Gerstner and Huff 1977b). No change

has been noted in the chromosomes or in the hormonal function in the infants (Tsubaki and Irukayama 1977(e)).

Clinical symptoms in the infants comprised: delay of disappearance of primitive reflexes, mental disturbances, retardation of physical development, retardation of the emergence of behaviour, disturbance in mastication and swallowing, disturbance in motility and impairment of voluntary movements and co-ordination (ataxia), involuntary movements, constriction of the visual field, and abnormal movements of the eye balls (Tsubaki and Irukayama 1977(e)). Not all the symptoms and signs necessarily manifest themselves at the same time. In the Iraqi series, a broader spectrum of severity was observed. Thus, cases were divided into two classes: (a) with early abnormal clinical signs, and (b) without early symptoms. The first group (a) representing more severe damage, was characterized by hyperreflexia, Babinski sign +, motor and speech delay and, in most cases, cerebral palsy.

Those of group (b) had no cerebral palsy, showed no hyperreflexia at early examination, but in most cases developed it later in childhood. Babinski sign + had developed later together with delay in motor activity and speech. In this group, cases with evident microcephalus were less frequent (Amin-Zaki *et al.* 1979). Both severe cases described by Gerstner and Huff (1977b) were blind.

Whereas data on infants and children from mothers severely exposed during pregnancy point to the involvement of the central nervous system, little is known on the possible effects of mild exposure. The only available study is that by McGill University (Methylmercury Study Group 1980) and refers to infants from mothers whose maximum hair mercury level during pregnancy was between 10 and 35 $\mu g\, g^{-1}$. The association with exposure, found only in boys, was apparent for muscle tone and reflexes. Whether this reflects a qualitative change in the type of effects with lower exposure level is not certain at present.

In the Iraqi observations, cerebral palsy was associated with highest maternal hair mercury concentrations (in the range 400–500 $\mu g\, g^{-1}$, indicating peak blood levels in mothers of the order of 1,500 ng $m\ell^{-1}$). The group showing milder damage was associated with maternal hair mercury levels in the range 100–400 $\mu g\, g^{-1}$ (indicating possible maternal blood levels in the range of 300–1,200 ng $m\ell^{-1}$) (Amin-Zaki *et al.* 1979, see also Marsh *et al.* 1980).

Several children died either in early infancy or in childhood. The post-natal lethality was associated with maternal hair mercury levels in

the range 200–600 $\mu g\, g^{-1}$ (corresponding to blood mercury levels of 700–2,000 ng $m\ell^{-1}$) (Amin-Zaki *et al.* 1979, Choi, Lapham, Amin-Zaki and Saleem 1978). The blood levels in infants, detected at delivery, were in the range 500–1,600 ng $m\ell^{-1}$ (Choi *et al.* 1978).

In general, there seems to be no sharp cut-off in the maternal exposure levels which lead to injuries of varying degrees of severity. This could indicate that, as with other biological phenomena, there is a wide range of sensitivity to various effects and the ranges for different effects largely overlap (Amin-Zaki *et al.* 1979).

Histological examinations of infant brains were performed in the most severe cases which died either in infancy, or in childhood and even adolescence. Those reported by Gerstner and Huff (1977b) were characterized by microcephaly (only two-thirds of normal), reduced cerebral and cerebellar white matter, disorganization of cellular architecture, a sharp decline in the number of nerve cells (replaced by proliferating astrocytes), degeneration of the nerve cells, destruction of the granular cell layer, and reduction of the Purkinje cell layer. The most relevant feature of the damage, however, has been described by Choi *et al.* (1978). The major effect, the report says, is not confined to destructive focal neuronal damage (as is the case with adults) but to faulty development of brain structure. This is caused by abnormal migration of neurons to the cerebellar and cerebral cortices. The effect is accompanied by diffuse astrocytosis.

The abnormal migration of the neurons has been confirmed *in vitro* with human foetal cerebral cells, in concentrations of methylmercury of about 4 and 40 $\mu g\, m\ell^{-1}$ (Choi, Cho and Lapham 1979). Changes in astrocytic membranes were observed in similar experiments in concentrations of only 2 $\mu g\, m\ell^{-1}$, and could be prevented by dimercapto succinic acid (complexing agent for methylmercury) (Choi and Lapham 1980).

The damage to the brain of the pre-natally exposed infant is basically irreversible (Amin-Zaki *et al.* 1979). Nevertheless it has been reported (Tsubaki and Irukayama 1977(e)) that some improvement in vision has been noted with age.

7.2 Effects of perinatal exposure of infants and children

Observations have been made of Iraqi children aged 2–16 years. Those admitted to hospital were occasionally asymptomatic (with blood mer-

cury levels in the range of 1,000–2,000 ng $m\ell^{-1}$), but mainly symptomatic (with blood levels in the range of 1,500–4,000 ng $m\ell^{-1}$). Levels were higher in those children who had been admitted earliest (February 1972) (Amin-Zaki *et al.* 1978).

The following prevalence of symptoms and signs, listed in order of frequency, was noted: hyperreflexia, weakness, dysarthria, visual disturbances, involuntary movements, hearing disturbances and ataxia (Amin-Zaki *et al.* 1978).

Contrary to children exposed pre-natally, a rather high rate of improvement or even recovery was noted. The recovery was inversely related to the severity of symptoms. All children with only mild symptoms recovered within one year. With more advanced damage, recovery was still possible but required a longer time (two years). However, no improvement was found in those having very severe impairments (Amin-Zaki *et al.* 1978). This is why the final evaluation may differ, depending on the severity of cases under observation: Takeuchi *et al.* (1979) still regard the damage to the children's brain as essentially irreversible.

From the autopsy samples of brain from five children exposed post-natally at the age of 3–5 years and having survived for a period ranging from a few months to over 10 years, the following observations seem characteristic (Takeuchi *et al.* 1979): (a) the changes in the brain were widespread and the whole cortex was affected including the frontal lobe; (b) a reduction of brain weight by 26–55 per cent was seen; and (c) heavy loss of neurons was observed, in some cases exceeding 50 per cent. In the latter case, a decortication syndrome developed.

Views on the susceptibility of the developing child's brain to methylmercury action differ considerably, depending probably on the severity of cases under observation: Takeuchi *et al.* (1979) express the view that the brain in early childhood is more susceptible than the adult brain. Amin-Zaki *et al.* (1978) are of the opinion that children are no more susceptible than adults and, moreover, that their capacity to improve or even recover is greater.

8 Dose-response relationships and thresholds

8.0 Summary of information contained in the Health Criteria Document

Some of the questions discussed in the Health Criteria Document were as follows:

It was understood that the onset of neurological symptoms was associated with an average brain mercury level of approximately 5 $\mu g\, g^{-1}$, both in experimental animals and in humans. From experiments on animals it has been recognized that the morphological, electrophysiological and biochemical changes (silent damage) may occur prior to the onset of overt signs of poisoning, but no specific levels of methylmercury in brain or blood were mentioned in this connexion.

The dose-response relationships and thresholds for adult humans have been derived from a variety of epidemiological studies in Iraq and Japan and emphasis was given to the proper reconstitution of exposure levels at the time of the onset of symptoms. The basic dose-response data were those of Bakir *et al.* (1973), although other data as well as different approaches have been examined, all yielding a consistent picture. The threshold concentrations for paraesthesia were estimated to be in the range of 200–500 $ng\, m\ell^{-1}$ in blood, 50–125 $\mu g\, g^{-1}$ in hair, and 3–7 $\mu g\, kg^{-1}\, d^{-1}$ for daily intake.

Pre-natal exposure was thought to be more dangerous but no specific threshold for the foetus has been proposed.

8.1 The threshold concentration of methylmercury producing selected effects in experimental animals and in *in vitro* model studies

The lowest effective levels found in chronic experiments on mice differed depending on the source. For functional tests, brain levels of 10 $\mu g\, g^{-1}$ (Suzuki and Shishido 1976) and much lower, 2.0 $\mu g\, g^{-1}$ (0.7–4.5) (Berthoud *et al.* 1976) have been reported, but general symptoms such as inhibition of growth have been detected at lower levels (Munro *et al.* 1980).

In the rat, disturbances in motor functions have been found at brain levels of 6–8 $\mu g\, g^{-1}$ (Hoskins and Hupp 1978), but kidney injury has been found at a level of exposure fivefold lower (Munro *et al.* 1980).

In cats, the lowest effective brain levels were 6–7 $\mu g\, g^{-1}$ (Charbonneau *et al.* 1976, Takeuchi *et al.* 1977).

For monkeys, the susceptibility depends on the species. For the squirrel monkey the lowest effective levels in the brain were 2–4 $\mu g\, g^{-1}$, and for macaque monkeys 5–10 $\mu g\, g^{-1}$. For the former, the corresponding blood levels were 500–1,000 $ng\, m\ell^{-1}$ (Evans *et al.* 1977). For squirrel monkeys, other sources give higher critical brain levels: neurological degeneration was associated with concentrations in excess of 8 $\mu g\, g^{-1}$

(Hoskins and Hupp 1978). In the rhesus monkey subtle lesions (not limited to neurological symptoms) were found with blood levels above 1,000 ng $m\ell^{-1}$ (Mottet 1974b). In another study, effects on the brain, assessed by light microscopy, were apparent in monkeys having brain levels 2–5 $\mu g\ g^{-1}$ (Garman *et al.* 1975).

Theoretically, the lowest level producing damage can only be proved experimentally above the possible threshold. The threshold can hardly be estimated directly in experiments on small groups of animals. Therefore, Munro *et al.* (1980) are of the opinion that the threshold for neurological effects, both in animals and in man, is in the same range of brain concentrations, namely 1–2 $\mu g\ g^{-1}$. This is consistent with the view of Sato and Ikuta (1975) who proposed a threshold for the neurological symptoms in monkeys of 1.5 $\mu g\ g^{-1}$ in the brain with corresponding other indices: blood 460 ng $m\ell^{-1}$, hair 60 $\mu g\ g^{-1}$, daily intake 30 $\mu g\ kg^{-1}\ d^{-1}$. At the subclinical level, effects on protein synthesis in the brain were found at a brain mercury level of 1 $\mu g\ g^{-1}$ (Syversen 1977).

Whether a brain level of 1–2 $\mu g\ g^{-1}$ represents the real threshold for neurological damage cannot yet be ascertained. In various *in vitro* experiments, much lower concentrations have been found to cause effects. The viability of HeLa S3 cells in culture is affected at a level of 200 ng $m\ell^{-1}$ (Gruenwedel and Fordan 1978). Inhibition of growth of glioma cells (brain tumour) has been noted at only 40 ng $m\ell^{-1}$ (Prasad, Nobles and Ramanujam 1979) while a change in sensitivity of adenylate cyclase occurred in the above system at only 20 ng $m\ell^{-1}$ (Spuhler and Prasad 1980). The same level affects the permeability of the liposome membrane (Nakada, Inoue, Nojima and Imura 1978). The most sensitive response *in vitro* found so far is a decrease of immuno-response (mouse spleen cells against SRBC) detected at a concentration of only 6 ng $m\ell^{-1}$ (25 per cent inhibition) (Seto *et al.* 1977). It seems unlikely that any of the corresponding effects could occur with similar sensitivity *in vivo* because of the variety of metabolic processes which limit the availability of the active compound to sensitive sites in the living organism. Nevertheless, some attention should be paid to the immunological effects which have been shown by some authors to occur *in vivo* at relatively low concentrations (for discussion see Section 3.1).

8.2 The threshold for adults (excluding pregnant women)

The dose-response relationships for methylmercury were subject to discussion at international level shortly after the Health Criteria

Document was prepared. In their review, Clarkson and Marsh (1976) supported the views contained in the Health Criteria Document, and incorporated more detailed data on Korean fishermen and Samoan shoreworkers. The evidence presented suggested that the time period over which exposure occurs may not be of great importance in methylmercury poisoning. This statement, if valid, adds to the relevance of the Iraqi studies. This point nevertheless deserves further discussion in the light of several studies and/or opinions expressed by experts (see Takeuchi and Eto 1975, Rose 1979, Evans *et al.* 1977). When modelling the exposure-effect relationship, two questions should be taken into account: (a) whether the brain clearance is sufficiently slow to result in greater and more prolonged accumulation of methylmercury in this tissue compared with blood (see 2.3), and (b) to what extent the latent period influences the onset of symptoms assuming constant brain concentrations (Evans *et al.* 1977).

Nordberg and Strangert (1976 and 1978) have calculated the change in the risk estimates resulting from the bimodal distribution of half-life of methylmercury. According to their calculations the daily dose of 200 μg is connected with an 8 per cent risk of effects, and with 100 μg d^{-1} the risk is reduced to about 2 per cent. Thus, if the risk is to be limited to below 5 per cent, the threshold should be set at about 150 μg d^{-1}.

The "threshold" body burden (or intake) proposed by Kitamura *et al.* (1976b, 1975b) does not apply here since it is based on animal data as the primary source. Having introduced a safety factor, to account for the variability in susceptibility, they arrived at a safe body burden of 10 mg. As mentioned in 6.3, the collective assessment of a Japanese group (quoted after Takeuchi and Eto 1975) has suggested a threshold dose of 5 μg kg^{-1} d^{-1}, equivalent to a blood level of 350 ng $m\ell^{-1}$. Takeuchi and Eto proposed the following threshold values for humans: brain 1.0 μg g^{-1}, blood 100 ng $m\ell^{-1}$, hair 40 μg g^{-1} (Takeuchi and Eto 1975). This implies a brain : blood ratio of 10 (for discussion see 2.1).

In more recent articles, the dose-response study of Bakir *et al.* (1973) has been criticized because of the pre-selection of subjects already showing symptoms (hospitalized patients) (Whitehead 1980). The same author suggests that the confidence limits of values from this study were extremely wide. Rose (1979) has criticized the same study of Bakir *et al.* for applying a mathematical model which neglects the S-shape of the dose-response curve. As regards the value of the threshold, the two reports mentioned above lead to contradictory conclusions.

A comprehensive assessment by NAS (1978) did not suggest any change of approach or new proposals and it endorsed the estimates introduced in the Health Criteria Document regarding adults. From an overall survey of reports, however, there seems to be some expectation that the threshold for adults will have to be slightly lowered.

The only set of data which is likely to influence the already established view on the threshold for methylmercury in non-pregnant adults, is that of the McGill study (Methylmercury Study Group 1980). This study, referred to in Section 6, is based on exposure estimates (mercury in hair) and neurological symptoms in over 500 adults of both sexes, whose exposure to methylmercury was extended over their life-time. Thus, if the duration of exposure were to play a significant rôle in the likelihood of health impairments at levels of exposure below those currently recognized (200–500 ng $m\ell^{-1}$ in blood, 50–120 $\mu g\ g^{-1}$ in hair) they could show in this population where hair mercury levels in excess of 20 $\mu g\ g^{-1}$ were found in about 170 subjects. As a result of this study, an increased risk has been associated with an increment of hair mercury level of 20 $\mu g\ g^{-1}$. Although this result is by no means definite and, also, we cannot interpret it in terms of response rate attributed to methylmercury exposure, the fact remains that a positive association is being discussed at a level which corresponds to mercury level in blood of only 80 ng $m\ell^{-1}$. Whether such a low threshold will gain the credibility of the international scientific community remains to be seen.

It seems likely, however, that the above question will never be definitely resolved if based solely on direct observation. As with other problems characteristic of the "grey area", we shall have to rely partly on the limited direct evidence and partly on more hypothetic modelling. The two aspects of the modelling, (i.e. accumulation in the brain and influence of the duration of exposure), if introduced, are likely to contribute to a consistent view on the above question.

8.3 The threshold for pregnant women

Although it has never been explicitly stated, a higher sensitivity for women during pregnancy seems likely. Two reports in particular give support to a lower threshold for pregnant women: (a) In the 1977 publication on Minamata disease (Tsubaki and Irukayama 1977(e)) out of 22 mother/infant pairs, five women suffered during pregnancy from "numbness of extremities and neurological symptoms"; these symptoms were transient. The concentrations in hair, determined one to four years

after delivery, were in the range of 10–200 $\mu g\, g^{-1}$; (b) Marsh *et al.* (1979) have constructed a dose-response curve for pregnant women which indicates that the onset of paraesthesia corresponds with levels of between 13–37 $\mu g\, g^{-1}$ in the hair. The Japanese data above may be questioned because of the time lapse between delivery and hair analysis. Nevertheless, both reports gave surprisingly consistent ranges of values. In common with observations from the two above-mentioned sources, the symptoms in pregnant women have been referred to as "transient during pregnancy" (Marsh *et al.* 1980). Although a formal statistical analysis performed on a narrower group of mother/infant pairs failed to prove a significant increase of symptoms in mothers with hair concentrations below 100 $\mu g\, g^{-1}$ (Marsh *et al.* 1980), reasoning in terms of dose-response functions, as well as the transient character of such symptoms, suggest this is highly likely. It seems justified, therefore, to propose that the threshold for pregnant women should be in the region of 30 $\mu g\, g^{-1}$ in hair and 100 ng $m\ell^{-1}$ in blood. It may also be anticipated that, if applying the criterion of low probability (say 5 per cent incidence over the background level), such a threshold is likely to be further lowered.

8.4 The threshold for the foetus

New data highly relevant to this subject have been offered by Marsh *et al.* (1977 and 1979). In the latter, more complete report, these authors have constructed a dose-effect curve based on observations of 84 mother/infant pairs, divided into six groups depending on maternal hair mercury levels. Concentrations were used which were likely to correspond to the period of gestation. As a measure of effects in a given group, a total score was calculated based on symptoms of motor, speech and mental retardation. These data suffer from a high "background level" which makes subtle distinctions difficult. Nevertheless, a clearly increased score of 16 symptoms (per 14 infants) was noted in infants whose mothers' hair levels averaged 37 $\mu g\, g^{-1}$ (range 18–68 $\mu g\, g^{-1}$). A provisional threshold for such severe neurological symptoms could be proposed, therefore, at a maternal hair mercury level of about 30–40 $\mu g\ g^{-1}$, corresponding to a maternal blood mercury level of about 100 ng $m\ell^{-1}$.

Whether symptoms occur in the pre-natally exposed infants which are milder than those examined by Marsh *et al.* (1979) and possibly transient in character is apparently not known at present. In this respect, of interest

may be the McGill study (Methylmercury Study Group 1980). As regards pre-natal exposure, this study contains data on over 200 children aged 12–30 months of both sexes whose mothers during pregnancy had hair mercury levels up to 35 $\mu g\, g^{-1}$. This study has revealed an association with maternal exposure, in boys only, with a mild deviation in muscle tone or reflexes. Association with exposure was seen with an increment of maternal hair concentration of 10 $\mu g\, g^{-1}$ (corresponding to 40 ng $m\ell^{-1}$ in maternal blood). In the absence of a similar response in the girls, the results are difficult to interpret. Nevertheless, they indicate that the "grey area" of threshold for pre-natal exposure may be very low. Whatever the doubt, there is no basis for anticipating that the foetal brain is less vulnerable than that of the mother during pregnancy. In the latter, the threshold blood concentration for the mild symptom, paraesthesia, is likely to be 100 ng $m\ell^{-1}$. The same blood concentration occurs in the infant at a maternal blood level of only about 50 ng $m\ell^{-1}$. It is proposed, therefore, that for congenital exposure, as measured by maternal blood or hair mercury concentrations, the "assumed threshold" should be accepted as 50 ng $m\ell^{-1}$, and 15–20 $\mu g\, g^{-1}$, respectively.

9 Summary evaluation

1. Except for contaminated areas, most edible fish from freshwaters have relatively low mercury levels, between 50 and 200 $\mu g\, kg^{-1}$. Elevated levels are found in predatory species, but natural levels only occasionally approach 1,000 $\mu g\, kg^{-1}$. Much higher levels are found in contaminated areas, especially in predatory fish, and values may reach 5,000 $\mu g\, kg^{-1}$ or more.

Most marine fish also have levels below 300 $\mu g\, kg^{-1}$, except for several predatory long-lived species, like tuna, swordfish, and fish of the shark family, where levels around 1,000 $\mu g\, kg^{-1}$ are typical. Predatory fish from the Mediterranean sea generally contain mercury levels of between 1,000 and 2,000 $\mu g\, kg^{-1}$, and values in excess of these are often reported.

2. An average consumption, typical of Europe as a whole, of 20 g fish per day (one meal of fish per week) is unlikely to result in any health effects, irrespective of the level of mercury in fish. In groups where fish consumption is elevated (100 g per day, one meal daily) high concentrations of mercury may be of some concern. Exceptionally high consumption, found in fishermen, tourist guides, shoreworkers, etc.

where amounts of the order of 1,000 g per day may be consumed, can create some health risk even when mercury levels do not exceed 500 $\mu g\ kg^{-1}$.

3. It is uncertain whether the steady-state levels of methylmercury in humans are age-dependent. Some reports suggest higher levels in the age groups 30–50 years, but the reasons for this phenomenon are obscure.

The brain : blood ratio of concentrations in non-human primates is around 3 and shows a tendency to increase slightly with the blood mercury level, up to a value of about 5. Such values are also likely to apply for humans. There is no firm indication of age and sex-dependent differences in half-life of methylmercury in humans, and 70 days remains the best estimate of the average value.

4. Existing evidence suggests that the single-compartment kinetic model used so far for methylmercury represents an oversimplification, and that a two-compartment model with slightly differing clearance coefficients would possibly be more adequate. One contentious issue in this regard is the differing views held on the clearance half-life from the brain. Depending on the standpoint, far-reaching differences in toxicological forecasts are possible.

The modelling of continuous exposure, so far based on the single-compartment model, requires more detailed support in experimental data.

5. In risk evaluation, hair analysis is gaining in importance. More data are required to assess the error estimates connected with the use of hair analysis *vs* blood analysis, and attempts should be continued to increase the value of hair analysis as an indicator directly correlated with body burden and health effects.

6. In infants from exposed mothers, the concentrations of mercury in blood are higher by a factor of 1.0–2.0, the higher ratio being typical for elevated maternal blood levels.

7. Increased evidence has recently become available regarding chronic effects of methylmercury in non-human primates. Apart from neurological damage, other effects have been found (at relatively low exposure levels), such as loss of appetite and diminished activity, lesions in the heart and intestines. Neurotoxic effects are evidenced by a loss of control of distal musculature, signs of paraesthesia, changes in sensory nerves, ataxia, disturbances in vision and hearing, and seizures. So far, no symptoms have been reported from monkeys in which the blood mercury

concentration was below 1,000 ng $m\ell^{-1}$. Above this level, the appearance of symptoms depends on both blood mercury level and the duration of exposure to such levels. The latent period is inversely related to blood concentration.

Histopathological studies of non-human primates have failed to confirm degeneration of the peripheral nerves. Within the brain, damage is primarily concentrated in the cerebral cortex, with no major involvement of the cerebellum. The threshold for neuropathological changes seems to correspond to a blood level of approximately 1,000 ng $m\ell^{-1}$, but ultrastructural changes can be seen at blood levels of about 500 ng $m\ell^{-1}$. Some reversal of symptoms can occasionally be seen, once the mercury blood concentration falls below 600 ng $m\ell^{-1}$.

8. Recent evidence obtained in experimental animals as well as from *in vitro* studies suggests that there are a variety of subtle biochemical changes which can be, or are likely to be, detected at levels of exposure below those referred to above. Their relevance, however, is not yet clear. Such effects include increased activity of certain enzymes in blood serum (MDH, PHI), suppression of the immune response, effects on DNA synthesis, and effects on protein synthesis. Only the last effect has gained sufficient recognition, and it seems to be useful in studying effects in the brain at threshold levels of exposure.

9. Increased evidence has become available on the embryotoxicity of methylmercury. Embryotoxic effects can occur in the absence of maternal toxicity; however, the range of such selective effects is narrow. At lower exposure levels, where embryolethal effects may be absent, a decrease of foetal weight is usually observed. Following congenital exposure, retardation of growth up to weaning, and reduced survival have been observed.

No evidence was obtained regarding higher susceptibility of young animals exposed post-natally.

10. A number of teratogenic effects were observed in various animal species at about the same level of exposure which produces other embryotoxic effects. Thus, depending on the animal species or even a strain within a species, either teratogenic or other embryotoxic effects may prevail. It is not clear to what extent the level of exposure and its timing influence the appearance of malformations.

Teratogenic effects have not so far been reported from experiments on non-human primates.

Apparently there is no evidence for carcinogenic or mutagenic effects of methylmercury in mammals: congenitally exposed animals yield normal offspring.

11. The most outstanding effects of congenital exposure are seen in the nervous system, particularly in the brain. Congenitally exposed animals show abnormal behaviour which may reflect their impaired learning ability. Histopathology of the brain reveals a number of changes, of which loss and atrophy of neurons, cyto-architectural changes in the cerebrum, and delayed migration of the external granular cell layer appear most eminent.

12. Limited protection against methylmercury poisoning in experimental animals can be achieved by administering selenite-selenium. This is evidenced by decreased mortality, extended survival time, restored growth rate and alleviation of neurotoxic effects.

13. The influence of selenium on methylmercury toxicity is not reflected in any uniform way in the changes of methylmercury metabolism. Selenium does not enhance methylmercury excretion. Brain accumulation of methylmercury increases under the influence of selenium, but is not paralleled by increased neurotoxic effects.

The only case where significant changes in the metabolism are ascribed to selenium is in the sea mammals. These changes are manifest in an increased accumulation of inorganic mercury in the organs which suggests an enhanced demethylation process.

The mechanism through which selenite-selenium influences methylmercury toxicity remains obscure.

14. Limited protection against methylmercury toxicity was also achieved through feeding animals with marine fish protein, containing high natural levels of selenium. Evidence in support of this, however, is less strong because of many compounding factors inherent in such experiments.

There is no direct evidence of such protective effects in humans. It is suggested that, at the present stage of knowledge, there is no ground for modifying views on the health risk of methylmercury contained in selenium-rich marine fish.

15. With regard to toxic effects in adult humans, more evidence has become available from the Minamata and Niigata areas in Japan, although most of the findings were originally made prior to 1975. The following observations deserve attention:

(a) Clinical diagnosis of the "Minamata disease" often refers in Japanese cases to concentrations of mercury in hair which are well below the present estimates of threshold. In most cases, this can be explained by a long interval of time from the onset of symptoms to the measurements. Some of the Japanese authors, however, emphasize the possibility of effects even at such low levels because of the assumed importance of long duration of exposure. Such explanation can now be supported to some extent by experimental data which show that the latent period is inversely related to the exposure level. One aspect of this line of reasoning is the possible long-term accumulation of methylmercury in the brain, an assumption that further increases the possibility of obtaining toxic effects at long-term low-level exposure.

(b) Another question raised in the Japanese reports is the length of the latent period. This has been observed to be of several years duration (up to 10 years) even if exposure has ceased. Also, in diagnosed cases, the worsening of clinical symptoms with time has been reported as typical. This pattern seems to be at variance with the findings made in the Iraqi epidemic.

16. Symptoms reported from the Niigata area, where the exposure was less dramatic than in the Minamata area, appeared in the following sequence: sensory disturbances of extremities, sensory disturbance of the perioral area, ataxia, constriction of the visual fields. The latent period for the more advanced signs extended up to five years.

17. So far findings from the studies of Canadian Indians are equivocal. At this moment, about 900 people have been identified with blood mercury levels in excess of 100 ng $m\ell^{-1}$ ("at risk" group). Of these, however, only a few have concentrations in the range of the presently recognized threshold (200–500 ng $m\ell^{-1}$). Clinical findings made in the past were highly positive; these, however, have been challenged because of the possible interference of other causes. The most recent clinical and toxicological investigation has failed to discover well defined cases of poisoning when based on individual diagnoses. This does not exclude, however, effects which can be proved using an epidemiological approach. Most recently, a case-control study performed on a group of over 500 persons has revealed that an increased risk of being a "case" is associated with an increment of exposure indices equal to 20 $\mu g\, g^{-1}$ of mercury in the hair (corresponding to 80 ng $m\ell^{-1}$ in blood).

18. Apart from Canada,, the only new area of concern seems to be the Mediterranean. More data, however, are necessary to make any

assessment of the health risk in this area, where the level of exposure in critical groups of the population is likely to be below that of the Canadian Indians.

19. Effects of congenital exposure can be manifest in humans in the absence of toxic effects in mothers. This seems to be especially true if the level of exposure is relatively low, as evidenced in the Japanese studies.

There have been no reports of miscarriages or stillbirths associated with methylmercury exposure in humans. Also, data from humans do not point to any reduction in the weight of infants at birth. The only malformation observed refers to cases of a small and slightly deformed head (microcephalus) in the infant.

20. Clinical symptoms in congenitally exposed infants are usually observed only after some time delay. In severe cases, however, cerebral palsy may be recognized early on. Other signs include disturbed mastication and swallowing. Later, other signs become apparent such as retarded development of various psycho-motor functions, occasionally accompanied by impaired vision and hearing. In general, the effects of pre-natal exposure are irreversible.

21. The dominant feature of brain damage resulting from congenital exposure to methylmercury is deficient and defective neuronal structure, caused by inhibited migration of neurons during brain development.

22. The following symptoms and signs have been noted in infants and children exposed perinatally: hyperreflexia, weakness, dysarthria, visual disturbances, involuntary movements, hearing disturbance, and ataxia. If damage is very severe, no improvement with time is noted. In milder cases, however, improvement or even recovery may be obtained within one to two years.

The brain pathology of perinatally exposed children shows changes which are widespread over the whole cortex: reduction of brain weight may reach 55 per cent with heavy loss of neurons, and a decortication syndrome may develop.

There is some controversy regarding the sensitivity of infants and children to methylmercury poisoning. Some authors regard this group as particularly vulnerable, while others consider that children are not especially susceptible, and that their capacity to improve or even recover is greater than in adults.

23. Data from more recent reports indicate that the minimum level of mercury in the brain capable of producing toxic effects is lower than

previously believed. The value of 1–2 $\mu g\, g^{-1}$ in the brain seems close to the threshold, in both man and experimental animals. To date, this value has also been the lower limit of sensitivity of biochemical effects studied *in vivo*, such as disturbed protein synthesis in the brain and immunosuppression.

24. With regard to the threshold levels of methylmercury in blood, hair, and daily intake, the estimates given in the Health Criteria Document have been in general accepted and endorsed. Nevertheless, details of the procedure leading to the calculation of the threshold were criticized from various points of view and new facts have shed some doubts as regards the correctness of the former estimates.

25. The following view emerges from the review of the recent data as regards the threshold level of methylmercury in non-pregnant adults:

(a) The threshold estimates made in the HCD were mainly based on the observations from Iraq, where exposure duration was short.

(b) Evidence exists that at low level of exposure, the onset of symptoms is delayed and that the latency period is inversely related to the level of exposure. Thus, one would expect that a threshold estimated under (a) above may represent an overestimation if applied to conditions of long-term exposure.

(c) There seems to be evidence from epidemiological studies of populations exposed through fish consumption over the life-time that some risk exists of acquiring mild unspecific neurological symptoms if the hair mercury concentration is of the range of 20 $\mu g\, g^{-1}$ (equivalent to only 80 ng $m\ell^{-1}$ in the blood).

(d) The exposure estimates leading to "threshold" values should not, therefore, be based solely on mercury concentrations, but on a value which also incorporates a measure of the exposure duration. Assuming life-long exposures, this would lead to lower threshold values as compared with those presently recognized.

26. Pregnant women may represent a group at special risk. The evidence suggests that the mild transient symptoms of methylmercury intoxication may appear in pregnant women at a lower level of mercury in hair when compared with the threshold for adults in general. A threshold value of about 30–40 $\mu g\, g^{-1}$ in hair, corresponding to 100 ng $m\ell^{-1}$ in blood, seems justified for this group.

27. An approximate calculation of threshold for pre-natally exposed infants from the only existing set of dose-effect data, points to the same value as given above for pregnant women (30–40 $\mu g\, g^{-1}$ in maternal

hair). The signs, however, on which this dose-effect function was based, are associated with severe irreversible damage. In another study, milder and unspecific symptoms have been associated, in boys only, with an increment of maternal hair concentration of only 10 $\mu g\, g^{-1}$ (equivalent to only 40 $ng\, m\ell^{-1}$ in blood). It seems prudent to anticipate that the brain of the foetus has a sensitivity similar to that of the mother. Since the blood mercury level in the infant may exceed that of the maternal blood by a factor of two, the threshold concentration of mercury in maternal blood may be anticipated to be about 50 $ng\, m\ell^{-1}$, with corresponding maternal hair level of 15–20 $\mu g\, g^{-1}$.

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