Environmental Research Section A **87,** 92–98 (2001)
doi:10.1006/enrs.2001.4293, available online at http://www.idealibrary.com on IDEAL®

Declining Minamata Male Birth Ratio Associated with Increased Male Fetal Death Due to Heavy Methylmercury Pollution[1]

Mineshi Sakamoto,*[,2] Atsuhiro Nakano,† and Hirokatsu Akagi*

*Departments of *Epidemiology and †Basic Medical Science, National Institute for Minamata Disease, Minamata City, Kumamoto, Japan*

Received December 20, 2000

The purpose of this retrospective study was to examine the effect of methylmercury pollution on the sex ratio of offspring at birth and of fetuses at stillbirth in Minamata City, Japan, in the 1950s when severe and widespread methylmercury pollution was experienced. In 4 of 5 years from 1955 to 1959 when methylmercury pollution was most severe, lower numbers of male offspring at birth were observed in the city population. The offspring sex distributions from 1950 to 1969 by 5-year period were calculated in the overall population of Minamata City, in the most prevalent area, in fishermen (most heavily exposed occupation group) and among Minamata disease patients using data from birth certificates. We also similarly calculated the sex ratio of stillborn fetuses in the city population using Kumamoto Prefecture's vital statistics on stillbirth. Decreases in male births were observed in offspring in the overall city population, in fishermen, and in maternal Minamata disease patients in the city in 1955–1959, when the methylmercury pollution was most severe. An increase in the proportion of male stillborn fetuses in the city was observed at the time. It is possible that male fetuses were more susceptible to the pollution than their female counterparts, and this could be a cause for the lower numbers of male offspring at birth.

Key Words: **methylmercury pollution; sex ratio; stillbirth; Minamata disease.**

INTRODUCTION

Recently, changes in the offspring sex ratio at birth due to hazardous chemicals have become a matter of international concern. Changes in sex ratios at birth have been reported in some occupational populations and at some sites exposed to chemicals (1–4). However, the mechanism of this skewed sex ratio is still far from being understood. A change in sex ratio has been proposed as a sensitive marker of exposure to toxic chemicals *in utero* (1), and the change in sex ratio caused by dioxin in Seveso, Italy (2, 3), attracted a great deal of public attention. Thus, we suspected that such changes in the sex ratio would also have been caused by the severe and widespread methylmercury pollution in the Minamata area of Kumamoto Prefecture, Japan, in the 1950s. We were able to conduct this study because our institute kept birth certificates in and around Minamata City from 1950 to 1969.

This epidemic called "Minamata disease" is well known as the first instance on record of severe methylmercury poisoning caused by manmade environmental pollution, which occurred mainly among fishermen and their families in and around Minamata City. It originated from the consumption of large amounts of fish and shellfish contaminated with methylmercury discharged from a chemical plant (5). The principal symptoms included neurological disorders such as sensory disorder, cerebellar ataxia, contraction of the visual field, hearing impairments, and disequilibrium (5). The first patient was reported in 1953, and the number of patients rapidly increased after 1955. At that time, the average hair mercury concentration of 9 patients was an astonishing 338.4 ppm (96.8–705.0 ppm) (5). More than 2200 people in the area were subsequently certified as Minamata disease patients in accordance with the medical judgment criteria of the Environment Agency of Japan. Furthermore, many

[1]Part of this work was supported by a grant for Encouragement of Basic Research at the National Research Institute from the Science and Technology Agency, Japan. Birth certificates were made available by the District Legal Affairs Bureau strictly for research purposes under the condition of nondisclosure.

[2]Address correspondence and reprint requests to Mineshi Sakamoto, Ph.D., Department of Epidemiology, National Institute for Minamata Disease, 4058-18 Hama, Minamata City, Kumamoto 867-0008, Japan. Fax: +81-96661-1145. E-mail: sakamoto@nimd.go.jp.

0013-9351/01 $35.00

fetuses were exposed to methylmercury through the placenta of the exposed mother, and 22 cases showed the severe cerebral palsy called typical fetal-type Minamata disease, while their mothers had mild or no manifestations of poisoning (5). Outbreaks of the typical fetal-type Minamata disease occurred from 1955 to 1959 when the mercury pollution appears to have been most severe, judging from the incidence of patients (5) and the mercury concentration in the umbilical cords of inhabitants of the areas (6).

The purpose of this report is threefold. First, we investigated the effect of methylmercury pollution on the offspring sex ratios at birth in the overall Minamata City population, in the most prevalent area, and also among fishermen and Minamata disease patients in the city using birth certificate data. Second, we attempted to determine whether exposure to methylmercury of the mother or father was related to the probable sex ratio of offspring, and that of males in particular. This was done by dividing the offspring into three groups: mother patients, father only patients, and both parents. Third, we calculated the sex ratio of stillborn fetuses in the Minamata City population using Kumamoto Prefecture vital statistics on stillbirths in order to determine the possible cause for the skewed sex ratio at birth.

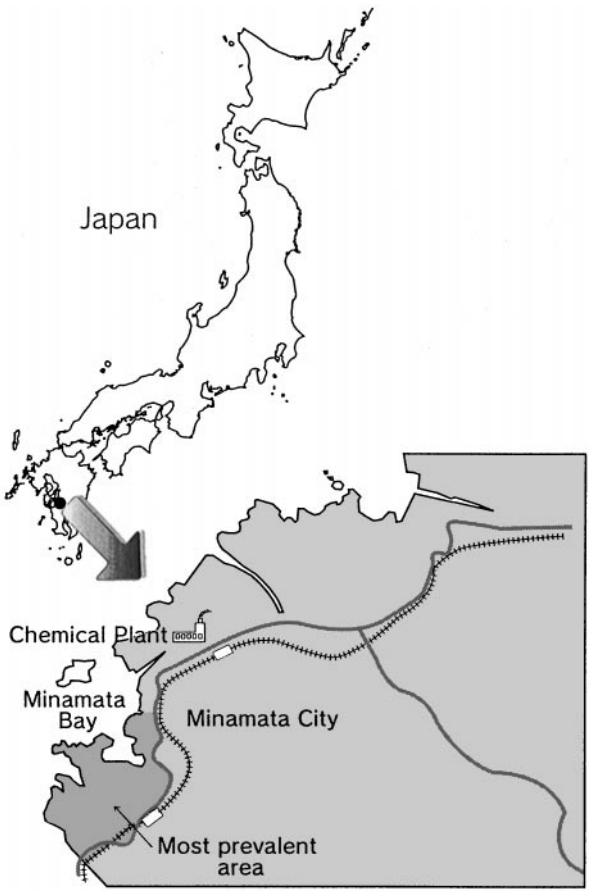

FIG. 1. Map of study areas.

MATERIALS AND METHODS

Subjects, Study Areas, and Groups

The subjects of this study were 20,487 offspring born in Minamata City, Kumamoto Prefecture, Japan, from 1950 to 1969. Kumamoto Prefecture is located in the western part of Japan's southern island of Kyushu. Minamata City is located in the southern part of the prefecture. About 1000 people in the city were certified as Minamata disease patients (about 55% of patients were male). The most prevalent area (552 Minamata disease patients, corresponding to 55.5% of the patients in the city, with an incidence of 14.7% using the population in 1960) was the southern seashore of Minamata Bay in the city. About 50% of the fetal-type Minamata disease occurred in this most prevalent area. The total population in 1960 was 1,856,192 in Kumamoto Prefecture, 48,340 in Minamata City and 3758 in the most prevalent area. Figure 1 shows the geographic locations of Minamata City, the most prevalent area, and a chemical plant. The offspring sex distributions were also studied in mountainous, urban, and seashore areas in Minamata City in order to reflect the amount of fish consumed at that time. Total populations in the areas in 1960 were 11,587, 24,164, and 12,581, respectively. Further, sex distributions at birth in families of fishermen (most heavily, exposed occupation group) and Minamata disease patients in Minamata City were studied. Offspring of families of fishermen were selected according to the father's occupation as stated on birth certificates. Offspring of Minamata disease patients were selected from birth certificates using the database for Minamata disease patients.

Data Analysis

Our institute has kept more than 100,000 birth certificates from in and around Minamata City for the years 1950 to 1969. They were collected from the Regional Legal Affairs Bureaus and regional public health center with official permission under condition of nondisclosure. Of the certificates, about 20,000 were for Minamata City. Information at birth such as name and sex of the offspring, weight at birth, time and date of birth, names of parents, place of birth, address of the offspring, addresses of parents, dates of birth of parents, and occupations of

parents were obtained from the certificates. At first the data for offspring whose address at birth was not in Minamata City were eliminated. The offspring sex distributions by year from 1950 to 1969 were calculated in the overall Minamata City population. Further analysis was conducted from 1950 to 1969 by 5-year period in order to increase the sample size. As mentioned in the Introduction, the second data period (1955–1959) corresponds to that of the most severe pollution. The offspring sex distributions at birth in the overall Minamata City population, in the most prevalent area, and among fishermen (most heavily exposed occupation group) in the city from 1950 to 1969 were calculated by 5-year period. The offspring sex distributions in 1950–1969 were also calculated by 5-year period in mountainous, urban, and seashore areas in order to reflect the amount of fish consumed (the amount of methylmercury exposure) in these areas at the time. The human male proportion at birth is known to be almost stable (about 106 male vs 100 female) (7) in a large population. This yields a sex ratio 0.515 (106 male/206 total birth). The sex ratio is also about 0.515 in Japan or Kumamoto Prefecture population. In the present study, the sex distributions of offspring at birth were tested against an expected sex distribution calculated from the sex ratio 0.515 as a control. The offspring sex distribution at birth among Minamata disease patients was also calculated from 1950 to 1969 by 5-year period. They were grouped into three: mother patients, father only patients, and both parents.

We similarly calculated the sex ratio of stillborn fetuses from 1952 to 1964 by 5-year period (first data period 1952–1954) in the Minamata City population and compared it with Kumamoto Prefecture (excluding data of Minamata City) as a control using the prefecture vital statistics on stillbirth. At this time stillbirth was defined as an intrauterine death at 4 months or later.

Statistical Analysis

The sex distributions were tested against expected sex distributions calculated from the general sex ratio 0.515 (106 males/206 total births) by χ^2 goodness-of-fit test. The sex distributions of stillborn fetuses in the Minamata City population were tested against expected sex distributions calculated from the overall sex ratio in the Kumamoto Prefecture population (excluding data from Minamata City) by the same test.

RESULTS

The sex distributions of offspring at birth in the Minamata City population by year from 1950 to 1969 are shown in Fig. 2. Declines of males at birth in the city's population were observed in 1952, 1955, 1957, 1958, and 1959. In 4 of the 5 years from 1955 to 1959

FIG. 2. Sex distribution at birth in Minamata City from 1950 to 1969 by year period. Dark bars: the fewer males than females were observed: $*P < 0.05$: Tested against an expected sex distribution calculated from a sex ratio of 0.515 (106 male/206 births) by χ^2 goodness-of-fit test (number of offspring in Minamata City was 406 male and 442 female in 1955). Difference between the populations; $\chi^2 = 4.33$, $P = 0.037$ in 1955.

TABLE 1
Sex Distribution of Offspring in Minamata City, the Most Prevalent Area, and Fishermen in Minamata City from 1950 to 1969 by 5-Year Period

	Number of offspring								
	Minamata City			Most prevalent area			Fishermen[a]		
Years	Male	Female	*P*	Male	Female	*P*	Male	Female	*P*
1950–1954	2812	2698	0.53	220	210	0.90	54	38	0.16
1955–1959	2238	2312	0.002**	140	165	0.052	21	34	0.049*
1960–1964	2131	1979	0.61	106	91	0.59	23	24	0.73
1965–1969†	2207	2055	0.46	87	73	0.42	8	9	0.73

Note. In 1955–1959, gender distribution in each area in the city at the time was 463 male/454 female in the mountainous area, 1231 male/1294 female in the urban area, and 214 male/237 female in the seashore area, respectively.

[a]Most heavily exposed occupation group.

[b]Data on occupation are missing for 1967–1969.

*$P < 0.05$, **$P < 0.01$: Tested against an expected sex distribution calculated from the sex ratio of 0.515 (106 male/206 births) in control by χ^2 goodness-of-fit test.

when the methylmercury pollution was most severe, lower numbers of male offspring at birth were observed in the city. The sex distribution at birth in the Minamata City population in 1955 (2238 male/2312 female) was significantly different from the control ($P < 0.05$). Decline in male births in the period before and after 1955–1959 was observed only once in the 15 years from 1950 to 1969. The appearance of the reversed sex distribution in 1955–1959 was significantly different from that in other years by χ^2 test ($P < 0.01$).

The sex distributions of offspring at birth from 1950 to 1969 by 5-year period in the Minamata City population, in the most prevalent area and among fishermen, are shown in Table 1. Occupational data in 1968 and 1969 were not available. There was a decrease in the number of males in the city population in 1955–1959, and the sex distribution was significantly different from the control ($P < 0.01$). In the area, the closer to the seaside, the lower the proportion of male birth was. The male proportion in each area in the city at the time was 0.505 (463 male/454 female) in the mountainous area, > 0.488 (1231 male/1294 female) in the urban area, and > 0.475 (214 male/237 female) in the seashore area, respectively. This tendency was not observed in 1960–1964 nor in 1965–1969. Fewer males were observed in the most prevalent area in 1955–1959; the proportion of males was 0.459, but the sex distribution was not significantly different from the control. This decline of males was also observed among the fishermen's families. The proportion of males among the fishermen's families was 0.382 in 1955–1959, and the sex distribution was significantly different from the control ($P < 0.05$). The sex distribution at birth was no longer significantly different after 1960. These tendencies were not observed among other occupations in Minamata City, nor among fishermen and other occupations in surrounding cities at the time (data not shown).

Figure 3 shows the sex ratios of offspring born from Minamata disease patients from 1955 to 1959. A decline in male births was observed in maternal

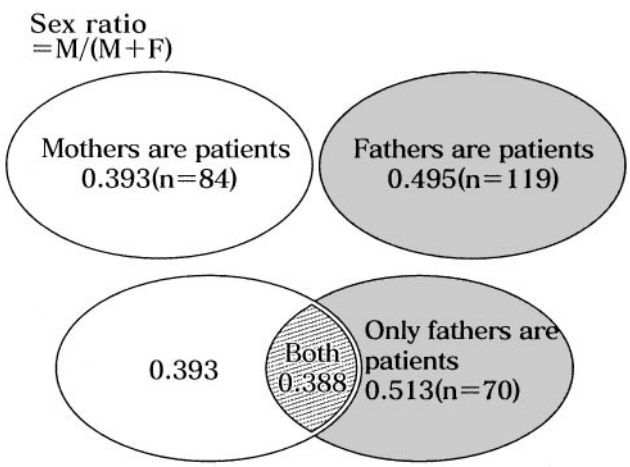

FIG. 3. Sex ratio of offspring at birth in Minamata disease patients in 1950 to 1955. Numbers of offspring born from mother patients: 51 male/36 female* in 1950–1954; 33 male/51 female in 1955–1959; 32 male/25 female in 1960–1964; 23 male/20 female in 1965–1969. *$P < 0.05$: Tested against an expected sex distribution calculated from the sex ratio of 0.515 as a control by χ^2 goodness-of-fit test. Difference between populations: $P = 0.18$ in 1950–1954; $P = 0.026$ in 1955–1959; $P = 0.040$ in 1955–1959; and $P = 0.78$ in 1960–1964.

patients in 1955–1959 when methylmercury pollution was most severe. The sex ratio of offspring born from mother patients at the time was 0.393 (33 male/51 female). The sex distribution from maternal patients was significantly ($P < 0.05$) different from the control. The sex ratio at birth of maternal patients was no longer significant after 1960. The sex ratio of offspring born from mothers whose fathers were patients at the time was 0.459 (54 male/64 female). Here, 49 offspring were born to parents both of whom were patients, and the sex ratio was 0.388 (19 male/30 female). The sex ratio was lowest when both parents were patients, but it was not statistically different from the control, and was similar to that when mothers were patients. The sex ratio of infants born to mothers who were not patients but whose fathers were patients was 0.514 (36 male/34 female). The offspring sex ratio when only the fathers were patients was similar to the control. This tendency was not observed in the other periods.

The proportions of male stillborn fetuses in Minamata City and those of control are shown in Fig. 4. Male stillbirths were about 1.2 times more than female stillbirths in each period in the control; the male proportion was 0.545. The proportions of male stillborn fetuses in Minamata City population were similar to those of the control, and the sex distribution was not significantly different from the control in 1952–1954 and 1960–1964. However, there were 1.7 times more male than female fetuses among stillbirths in 1955–1959 in the city; the male proportion was 0.634, and the sex distribution was significantly different ($P < 0.05$) from the control.

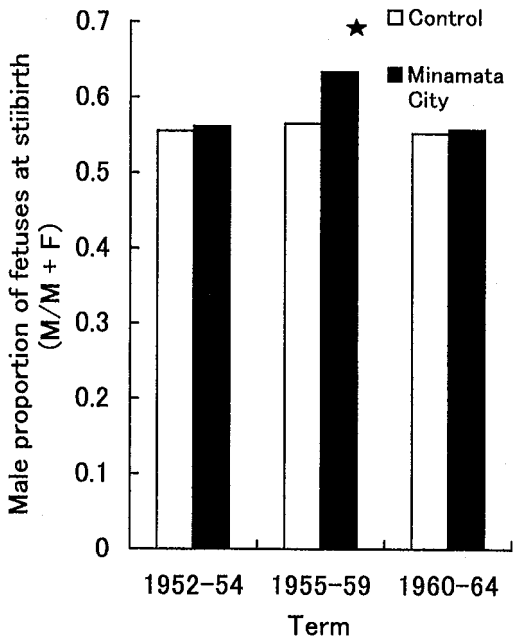

FIG. 4. Sex ratio of stillborn fetuses in Kumamoto Prefecture (excluding Minamata data) and Minamata City from 1952 to 1964 by 5-year period. (Data for 1956 are missing). Number of stillborn fetuses in Minamata City: 148 male/116 female in 1952–1954; 137 male/79 female in 1955–1959; and 161 male/128 female in 1960–1964. *$P < 0.05$: Tested against an expected sex distribution calculated from the male proportion in Kumamoto Prefecture (0.555 in 1953–1954, 0.565 in 1955–1959, and 0.552 in 1960–1964) by χ^2 goodness-of-fit test. Difference between the populations: $P = 0.84$ in 1953–1954; $P = 0.040$ in 1955–1959 and $P = 0.87$ in 1960–1964.

DISCUSSION

This study shows a decrease in the male birth ratio in Minamata City when the methylmercury pollution was most severe. In 4 of the 5 years from 1955 to 1959, fewer males were born in the city. Consuming marine fish contaminated with methylmercury discharged from a chemical plant caused Minamata disease (5). Therefore, the amount of fish consumption reflects the methylmercury intake in the human body. In 1955–1959, the present study showed a dose-dependent decline reflecting the estimated level of methylmercury exposure: 0.515 for the control > 0.492 for Minamata City (0.501 for mountainous area, > 0.488 for urban area, > 0.475 for seashore area), > 0.459 for the most prevalent area, > 0.393 among maternal Minamata disease patients, and 0.382 among families of fishermen. These findings further suggest a strong correlation between methylmercury exposure and the lowered sex ratio at birth. The incidences of the Minamata disease patients were 0.121% for the mountainous area, 0.629% for the urban area, 6.58% for seashore area, and 14.7% for the most prevalent area.

Also, the lower proportion of males at birth seemed to be caused by the exposure of mothers to methylmercury, since fewer males were born to mothers who were patients (0.393) but not to mothers whose fathers only were patients (0.514). Interestingly, this result is in disagreement with the results of dioxin in Seveso. The Seveso study indicated that the father's but not the mother's serum dioxin concentrations were associated with a decrease in the proportion of male births (3). If the origin can be determined as maternal, paternal, or both, much light will be shed on the mechanism itself. In this case, because the exposure of the father did not affect the sex ratio at birth, we can conclude that this phenomenon resulted from the direct effect of methylmercury on fetuses through maternal exposure.

Further, the present study showed the increased ratio of male at stillbirth when the methylmercury

pollution was most severe. The sex distribution at stillbirth in the period before and after 1955–1959 in Minamata City and most of the surrounding cities was approximately 120 to 130 male/100 female (male proportion: 0.55–0.56), suggesting that the male fetus is weaker than the female fetus in fetal life even under ordinary conditions. In addition, the proportion of male stillbirths in the city increased to 173 male/100 female (male proportion: 0.634) when the methylmercury pollution was most severe, indicating that more males were lost in the fetal stage. Methylmercury easily penetrates the placenta and affects the fetus. In the epidemics in Minamata, Japan, and Iraq, many infants were congenitally affected by methylmercury (5, 8). Among cases reported from Minamata, infants had severe cerebral palsy, whereas their mothers had only mild or no manifestation of poisoning (9). This fact demonstrates the far higher vulnerability of fetuses than adults. However, the sex difference in vulnerability with regard to the reproductive toxicity of methylmercury is unknown. The increased proportion of stillborn males will partly explain the lower proportion of males in Minamata City and in the highly exposed groups.

Although the lower proportion of males at birth and an increased ratio of male stillborn fetuses were observed just when the pollution was most severe, the male birth ratio in Minamata City overall, and among fishermen and Minamata disease patients, returned to normal after this period. The proportion males at stillbirth also returned to the normal level thereafter. The methylmercury pollution in Minamata Bay decreased fairly rapidly after 1960 as estimated from the change in mercury concentrations in fish in the bay, even though low levels of methylmercury still existed (10). As mentioned before, cases of typical fetal-type Minamata disease did not occur after 1961. Therefore, the disrupted sex ratios seem to have occurred only under the severe methylmercury pollution during pregnancy. The recovered sex ratios may also suggest that methylmercury did not affect the reproductive organs but fetuses themselves.

Recently, more attention has been focused on the effect of chemicals on the sex ratio at birth. The mechanism of the altered sex ratio at birth remains unexplained. A chemical could cause proportionately fewer males through its estrogen-like effect, resulting in either a reduced fertilizing capacity or lower survival of male spermatocytes, or an increased proportion of aborted or stillborn males. Although further studies are needed to reveal the mechanism for the lower male birth ratio during this epidemic, the increase in female births during severe methylmercury pollution may be explained in part by the weakness of male fetuses as shown by the elevated male fetus ratio among stillbirths in that period. There are few reports dealing with both sex ratios at birth and stillbirths in actual communities. Thus, this may be the first report of a chemical pollution-caused sex disruption among stillbirths. However, there is no evidence from epidemiological studies that methylmercury pollution caused abortion or stillbirth. In animal experiments, litter resorption and stillbirth occur frequently at a comparatively high dose of methylmercury (11, 12). Our present results also provide supporting evidence for lethal effects of severe methylmercury on fetuses in Minamata City.

Stillbirth and infant death are adverse reproductive outcomes with a long tradition as indicators of a society's overall health status. Our results emphasize the importance of studying the sex ratio not only for births but also for stillbirths in populations near hazardous chemical sites. The sex ratio of stillbirths will yield important information concerning changes in the sex ratio at birth. In addition, there is the possibility of decreases in the sex ratio at birth due to various natural or manmade chemicals. Therefore, we propose accurate and long-range accumulation of data concerning the sex ratios both for birth and for stillbirth in communities under ordinary conditions, to compare against unforeseen reproductive hazard in the future.

ACKNOWLEDGMENT

We thank Dr. T. Suzuki, the previous director of National Institute for Environmental Studies, for encouragement in the course of this study.

REFERENCES

1. Davis, D. L., Gottlieb, M. B., and Stampnitzky, J. R. (1988). Reduced ratio of male to female births in several industrial countries: A sentinel health indicator? *JAMA* **279,** 1018–1023.
2. Mocarelli, P., Brambillia, P., Gerthoux, P. M., Patterson, D. G., and Needham, L. L. (1996). Change in sex ratio with exposure to dioxin. *Lancet* **348,** 409.
3. Mocarelli, P., Gerthoux, P. M., and Ferrari, E., *et al*. (2000). Paternal concentrations of dioxine and sex ratio of offspring. *Lancet* **355,** 1858–1863.
4. James, W. H. (1987). The human sex ratio. Part I. A review of the literature. *Human Biol.* **59,** 721–752.
5. Kutsuna, M. (Ed.) (1968). "Minamata Disease." Study Group of Minamata Disease. Kumamoto University, Japan.
6. Nishigaki, S., and Harada, M. (1975). Methylmercury and selenium in umbilical cords of inhabitants of Minamata area. *Nature* **258,** 324–325.

7. James, W. H. (1995). What stabilizes the sex ratio? *Ann. Human Genet.* **59,** 243–249.
8. Amin-zaki, L., Elhassani, S., Majeed, M. A., *et al*. (1976). Perinatal methylmercury poisoning in Iraq. *Am. J. Dis. Child.* **130,** 1070–1076.
9. Harada, M. (1978). Congenital Minamata disease: Intrauterine methylmercury poisoning. *Teratology* **18,** 285–288.
10. Irukayama, K., Fujiki, M., Tajima, S., and Omori, S. (1972). Transition of pollution with mercury of sea food and sediments in Minamata Bay. *Jpn. J. Public. Health.* **19,** 25–32.
11. Khera, K., and Tabacova, S. (1973). Effects of methylmercuric chloride on the progeny of mice and rats treated before or during gestation. *Fed. Cosmet. Toxicol.* **11,** 245–254.
12. Matsumoto, H., Suzuki, A., and Morita, C. (1967). Preventative effect of penicillamine on the brain defect of fetal rat poisoned transplacentally with methylmercury. *Life Sci.* **6,** 2321–2330.