Environmental Health Perspectives
Vol. 74, pp. 103-107, 1987

The Role of Biomarkers in Reproductive and Developmental Toxicology

by Thomas W. Clarkson*

A massive outbreak of methylmercury poisoning took place in the winter of 1971–1972 due to the consumption of homemade bread contaminated with a methylmercury fungicide. The longitudinal analysis of the mother's head hair, collected after delivery of the baby, provided a means of recapitulating exposure to methylmercury during pregnancy. Methylmercury is incorporated into newly formed hair at a concentration that is proportional to the simultaneous concentration in blood. Since hair grows at a rate of approximately 1 cm/month, longitudinal analysis of the hair strand, centimeter by centimeter, will give a month by month recapitulation of blood levels. Depending on the length of the hair strand, it is possible to recapitulate several years of exposure.

Using longitudinal hair analysis, it was possible to compare the methylmercury levels in the mother during pregnancy with the severity and frequency of effects in her offspring. As in the previous incidents, high levels of prenatal exposure led to severe brain damage. However, it was also possible to identify milder effects of methylmercury as manifested by delayed development. It was possible to demonstrate a dose-effect and dose-response relationship between the maximum concentration of methylmercury in maternal hair during pregnancy and evidence of delayed development and mild neurological abnormalities in the offspring. These relationships provided quantitative evidence that the developing nervous system is more susceptible to damage than the mature brain.

This paper discusses biological markers as they relate to measuring the dose. The example I will discuss is human exposure to methylmercury. The biological marker is the concentration of mercury in maternal head hair and the dose to be estimated is the dose to the fetus.

Methylmercury is perhaps the only well-documented human teratogen that occurs in the environment. The outbreak of methylmercury poisoning in Minamata, Japan, in the midfifties revealed that 21 children suffered from cerebral palsy, whereas their mothers experienced only mild symptoms during pregnancy. Exposure of the children had been during the prenatal period (*1*). Subsequently, animal models confirmed that the prenatal period was the most susceptible stage of the life cycle to methylmercury (*2*).

Methylmercury readily crosses the human placenta. The concentration in fetal blood closely parallels the simultaneous concentration in maternal blood (*3*). The concentration of methylmercury in hair can recapitulate the concentration in maternal blood for months or years, depending on the length of the hair sample (*4*). This paper will discuss first the relationship between methylmercury concentration in hair and blood; second, the application of hair analysis to develop dose-response relations for adult exposure; and third, dose-response relations for prenatal exposure. Finally, mechanisms of damage to the developing central nervous system will be discussed.

*Environmental Health Sciences Center, University of Rochester School of Medicine, Rochester, NY 14642.

Methylmercury in Hair

Figure 1 indicates the relationship between hair and blood concentrations of methylmercury. Volunteers consumed fish containing methylmercury for a period of 100 days in an experiment conducted by Hislop and co-workers (*5*). Blood and hair concentrations of methylmercury were measured during exposure (the rising

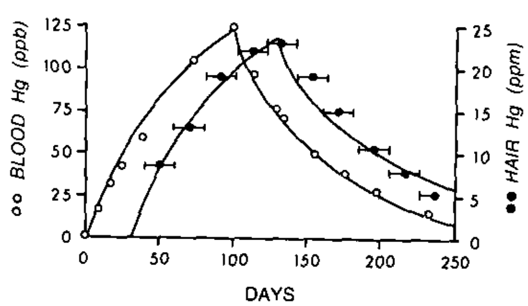

FIGURE 1. The concentration of mercury in samples of blood and hair collected from a volunteer during and after consumption of fish (halibut) containing naturally elevated methylmercury [adapted from (*5*)]. The consumption period was 100 days. Samples of hair were cut close to the scalp. The concentrations are for 8-mm segments of hair plotted according to the growth period. The horizontal line represents the 8-mm growth period, assuming a growth rate of 11.8 mm/month.

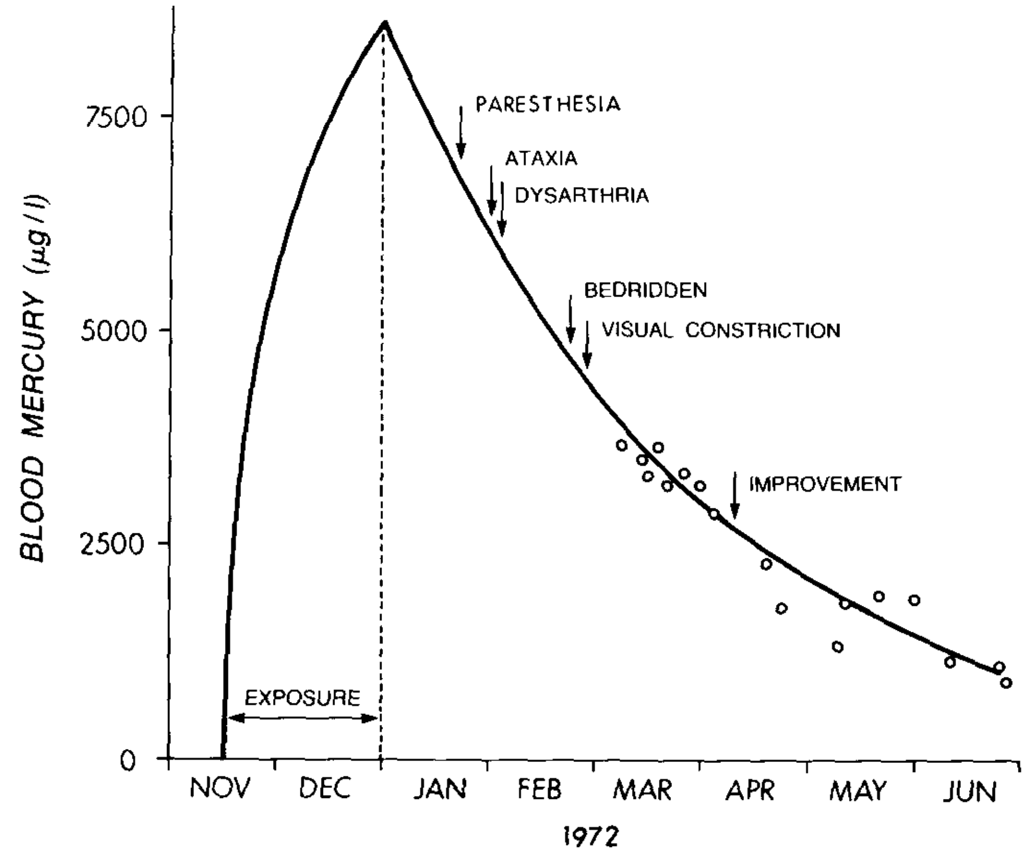

FIGURE 2. The sequence of appearance of signs and symptoms of methylmercury poisoning in a patient in the Iraq outbreak (6). The patient was admitted to the hospital in March 1972 when measurements of mercury in blood (○) began. The continuous line prior to March is a recapitulation of blood levels based on the patient's history of exposure, bread intake, and the biological half-time in blood and confirmed by hair analysis. Adapted from (7).

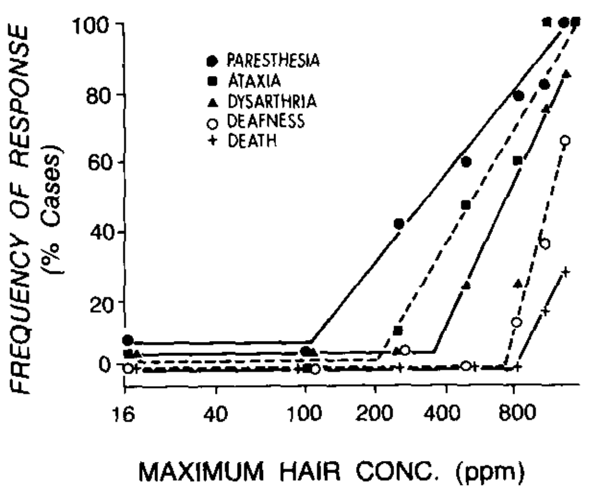

FIGURE 3. The frequency of signs and symptoms of methylmercury poisoning in adult victims in Iraq plotted according to the estimated maximum hair concentration. Hair samples were not available for all patients and are estimated based on the patient's history of exposure, observed blood levels, and blood half-times and a hair to blood concentration ratio of 250 to 1 (4). The figure is adapted from (6).

phase) and following exposure (the falling phase). The hair samples were about ½ cm in length. Hair grows at a rate of about 1 cm/month. The close parallel between blood and hair concentration is apparent. There is about a 20-day lag in the hair values. This represents the time taken for methylmercury to enter the hair follicle and to appear in the hair above the scalp.

Adult Dose-Response Relations

Hair analysis has been useful in studies of exposure in adults. Figure 2 records blood levels in a victim of methylmercury poisoning in an outbreak in Iraq in 1971–1972. The exposure was due to consumption of bread contaminated with a methylmercury fungicide. The continuous line records the blood levels estimated from a pharmacokinetic model. Hair analysis was useful in confirming the predictions of this model. No signs or symptoms appeared during the exposure period and indeed the first neurological symptom, paresthesia, did not appear until about 1 month after the end of exposure. Subsequently, more serious signs of damage to the brain appeared such as ataxia and visual constriction.

Since CH_3Hg produces irreversible damage due to destruction of certain groups of neuronal cells, it was

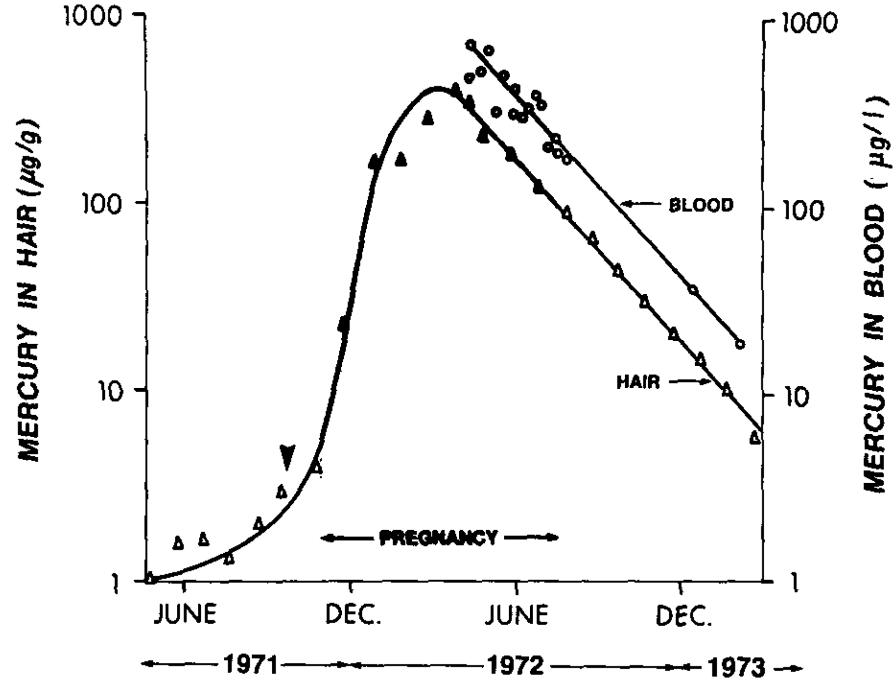

FIGURE 4. The longitudinal analysis of a sample of head hair, and the comparison with analysis of methylmercury in blood [adapted from (4)]. The hair sample was collected in early 1973 by selecting a bunch of the longest strands and cutting close to the scalp. Consecutive 1-cm segments were analyzed, and the results were plotted according to the date of formation of the segment, assuming a growth rate of 1 cm/month. Blood samples were collected after the patient was admitted to hospital.

decided to use the peak concentration as a measure of the dose and as a predictor of adverse effects. A number of neurological effects are plotted in Figure 3. The data are plotted as a threshold model. The frequency of paresthesia rises above background levels above an apparent threshold of 100 ppm in hair. The more serious signs of damage follow a similar type of relation but with the thresholds at higher values.

Prenatal Dose-Response Relations

In the outbreak in Iraq, women of childbearing age were also exposed. The question arose whether hair analysis could be used to recapitulate blood levels during pregnancy and thereby relate maternal blood levels to effects in the offspring due to prenatal exposure.

Figure 4 illustrates how the longitudinal analysis of a hair sample recapitulates blood levels during pregnancy. A hair sample was collected in January of 1973 and measured centimeter by centimeter from the scalp. It is clear that it is possible to recapitulate mercury levels as far back as 1971, prior to the actual exposure. The entire period of pregnancy is covered. This includes the rising phase, the peak hair values, and part of the falling phase. Exposure stopped when the patient was admitted to the hospital and blood samples could be collected.

The blood values closely paralleled the methylmercury concentration in hair. Thus the stage was set to see if hair concentrations during pregnancy were predictors of adverse effects in the offspring. An extensive sampling program was started to collect hair samples from mothers who had been exposed to the contaminated bread during pregnancy. It was also possible to collect hair samples from mothers who had not been exposed and lived in the same rural village as the exposed mothers. The children were subsequently examined by a team of neurologists and pediatricians. Since exposed and nonexposed mothers lived in the same area, it was possible to conduct the examinations blind to the exposure status. Lists of names were given to the examining team that contained control and exposed infant-mother pairs.

Severe cerebral palsy was found in the heavily exposed, as previously reported in the Minamata outbreak in Japan. However, in the lower exposed group, milder effects were seen, taking the form of developmental delays. These milder effects are illustrated in Figure 5, taken from early data on the study on 29 infant-mother pairs. Historical data from the mother gave evidence of developmental delay and neurological and mental dis-

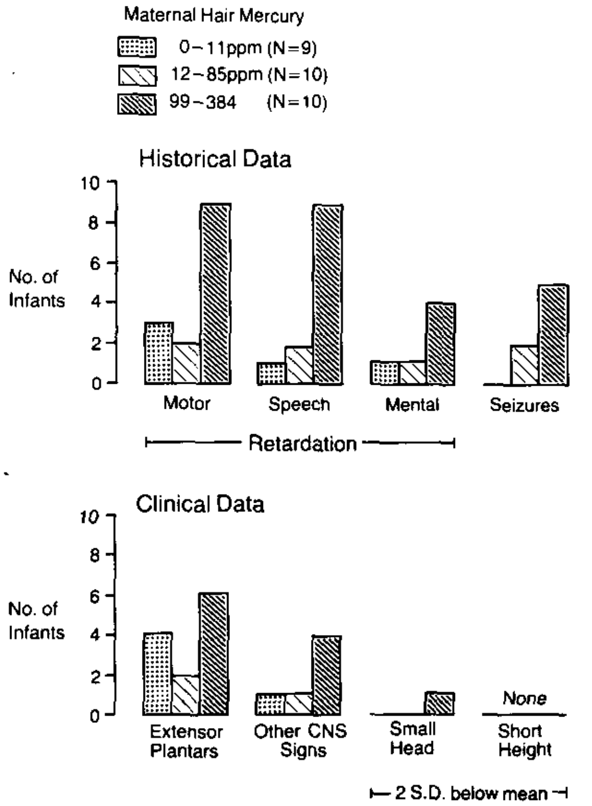

FIGURE 5. Signs and symptoms in prenatally exposed children according to the maximum maternal hair concentration during pregnancy. Adapted from (*8*).

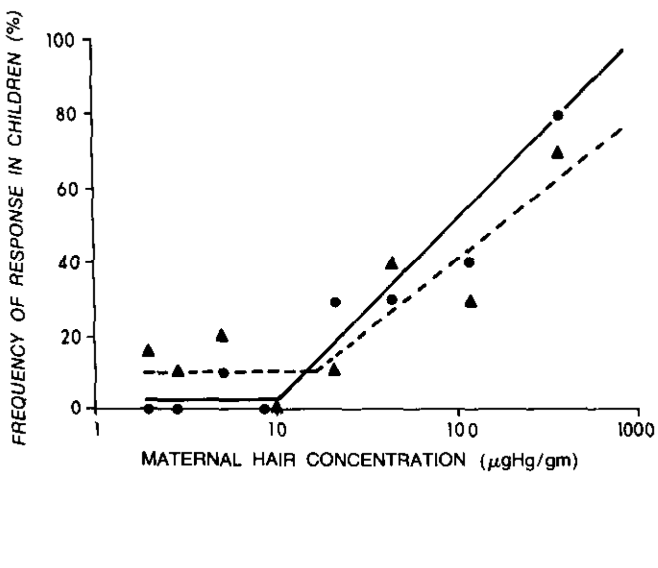

FIGURE 6. The frequency of motor retardation (●) and abnormal central nervous system (CNS) signs (▲) in prenatally exposed children versus the estimated maximum maternal hair concentration during pregnancy. The abnormal CNS signs were defined according to a scoring system ranging from unity to ten (most severe). Children with scores greater than three were defined as having CNS signs. Adapted from (*9*).

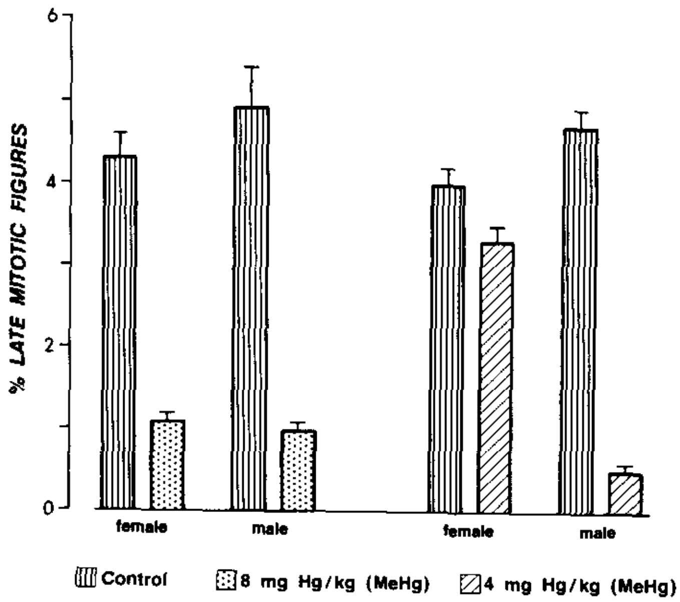

FIGURE 7. The effect of methylmercury treatment on cell division in mice [adapted from (*14*)]. Two-day-old mice were dosed orally with 4 and 8 mg Hg/kg as methylmercury and sacrificed 24 hr later. The total number of mitotic figures in the external granule layer of the cerebellum in matched sections were recorded and classified as early or late. Data are reported as late mitotic figures, bars are SE.

turbances. Examination by the clinical team confirmed the neurological effects. After the study group was enlarged to 82 infant-mother pairs, it was possible to demonstrate dose-response relations for a number of these effects. Figure 6 describes the frequency of motor retardation or signs of neurological damage (CNS signs) as a function of the maximum maternal hair concentration during pregnancy. The data are plotted with a threshold model. The results suggest an apparent threshold in the range of 10 to 20 ppm, which is lower than the threshold seen for the most sensitive effects in adults (Fig. 3). Despite the statistical uncertainty in the estimates of these thresholds, these data confirm the suspicion from the Minamata outbreak that the developing nervous system is more susceptible to damage than the mature human nervous system.

More recently, epidemiological studies of Cree Indians in Canada found mild or questionable effects at hair levels just above 10 ppm in mothers consuming methylmercury in fish (*10*). A study just reported for New Zealand, also in a fish-eating population, claims to find evidence of developmental delays in the region of 10 ppm (*11*). In all these studies, the dose was based on hair concentration of methylmercury during pregnancy.

Mechanisms

The susceptibility of the developing nervous system to methylmercury has prompted a number of studies on mechanisms. Examination of brain at autopsy from two full-term, newborn infants prenatally poisoned in the

Iraq outbreak revealed substantial derangement of the cytoarchitecture (*12*), confirming reports from the Minamata outbreak (*1*). Specifically, examination revealed evidence of incomplete abnormal migration of neurons of the cerebellar and cerebral cortices and deranged cortical organization of the cerebrum. The major defect appeared to be faulty development, not destructive focal neuronal damage as seen in adult poisonings.

Another mechanism of methylmercury poisoning is the inhibition of cell division, as indicated by animal experiments (*13*). This is illustrated in Figure 7. Neonatal rodents were given a single dose of methylmercury (8 mg/kg and 4 mg/kg). The higher dose produced drastic reductions in the percentage of late mitotic figures in both female and male animals. The lower dose affected only the males. It is of interest that the study on Cree Indians in Canada found effects only in males. More recent studies by Howard and Mottet (*15*) confirmed that methylmercury affected neuronal proliferation in the cerebellum in rats given repeated doses. In short, methylmercury affects the two processes most important to the developing central nervous system—cell migration and cell division.

The reason these processes may be sensitive to CH_3Hg may be the ability of CH_3Hg to depolymerize microtubules—structures of the cytoskeleton essential for cell division and migration (*16*). Abe et al. (*17*) reported that methylmercury caused depolymerization of reassembled microtubules *in vitro*. Miura et al. (*18*), in studies of the effects of methylmercury on cell division of mouse glioma cells, concluded that the primary effect was on the microtubules. Sager et al. (*19*) noted the complete disappearance of microtubules from cultured cells on addition of methylmercury. Methylmercury cation probably reacts with the sulfhydryl group of the tubulin monomers (*20*).

Conclusion

In conclusion, methylmercury concentration in hair is an excellent biomarker for fetal and adult absorbed doses. Of special importance is the ability of the hair sample to recapitulate blood levels during pregnancy. To what extent the hair sample will serve as a biomarker for other xenobiotics remains to be investigated.

This work has been supported in part by a NIEHS Center Grant ES01247 and in part by a NIEHS Program Project Grant ES01248.

REFERENCES

1. Harada, Y. C. Clinical investigations on Minamata Disease. In: Minamata Disease, Study Group of Minamata Disease (M. Katsuna, Ed.), Kumamoto University, Kumamoto, Japan, 1968, pp. 73–92.
2. Spyker, J. M. Behavioral teratology and toxicology. In: Behavioral Toxicology (B. Weiss and V. G. Laties, Eds.), Plenum Press, New York, 1975, pp. 311–344.
3. Skerfving, S. Methylmercury exposure, mercury levels in blood and hair, and health status in Swedes consuming contaminated fish. Toxicology 2: 3–23 (1974).
4. Amin-Zaki, L., Elhassani, S., Majeed, M. A., Clarkson, T. W., Doherty, R. A., and Greenwood, M. Perinatal methylmercury poisoning in Iraq. Am. J. Dis. Child. 130: 1070–1076 (1976).
5. Hislop, J. S., Collier, T. R., White, G. F., Khathing, T., and French, E. The use of keratinised tissues to monitor the detailed exposure of man to methylmercury from fish. In: Chemical Toxicology and Clinical Chemistry of Metals (S. S. Brown and J. Savory, Eds.), Academic Press, New York, 1983, pp. 145–148.
6. Bakir, F., Damluji, S. F., Amin-Zaki, L., Murthada, M., Khalidi, A., Al-Rawi, N. Y., Al-tikriti, S., Dhahir, H. I., Clarkson, T. W., Smith, J. C., and Doherty, R. A. Methylmercury poisoning in Iraq. Science 181: 230–241.
7. Clarkson, T. W. Methylmercury toxicity to the mature and developing nervous system: possible mechanisms. In: Biological Aspects of Metals and Metal Related Diseases (B. Sakar, Ed.), Raven Press, New York, 1983, pp. 183–197.
8. Marsh, D. O., Myers, G. J., Clarkson, T. W., Amin-Zaki, L., Al-tikriti, S., and Majeed, M. A. Fetal methylmercury poisoning: clinical and toxicological data in 29 cases. Ann. Neurol. 7: 348–355 (1980).
9. Clarkson, T. W., Cox, C., Marsh, D. O., Myers, G. J., Al-tikriti, S. K., Amin-Zaki, L., and Dabbagh, A. R. Dose-response relationships for adult and prenatal exposures to methylmercury. In: Measurement of Risks (G. G. Berg and H. D. Maillie, Eds.), Plenum Press, New York, 1981, pp. 111–126.
10. McKeown-Eyssen, G. E., Reudy, J., and Neims, A. Methylmercury exposure in Northern Quebec. II. Neurological findings in children. Am. J. Epidemiol. 118: 470–479 (1983).
11. Kjellstrom, T., Kennedy, P., Wallis, S., and Mantell, C. Physical and Mental Development of Children with Pre-Natal Exposure to Mercury from Fish. National Swedish Environment Protection Board, Box 1302, S-171 25 Solna, Sweden, 1986.
12. Choi, B. H., Lapham, L. W., Amin-Zaki, L., and Saleem, T. Abnormal neuronal migration, deranged cerebral cortical organization and diffuse white matter astrocytosis of human fetal brain: a major effect of methylmercury poisoning *in utero*. J. Neuropathol. Exp. Neurol. 38: 719–733 (1978).
13. Sager, P. R., Aschner, M., and Rodier, P. M. Persistent differential alterations in developing cerebellar cortex of male and female mice after methylmercury exposure. Dev. Brain. Res. 12: 1–11 (1984).
14. Clarkson, T. W., Nordberg, G. F., and Sager, P. R. Reproductive and developmental toxicity of metals. Scand. J. Work Environ. Health 11: 145–154 (1985).
15. Howard, J. D., and Mottet, N. K. Effects of methylmercury on the morphogenesis of the rat cerebellum. Teratology 34: 89–95 (1986).
16. Sager, P. R., Syversen, T. L. M., Clarkson, T. W., Cavanagh, J. B., Elgsaeter, A., Guldberg, H. C., Lee, S. D., Lichtman, M. A., Mottet, N. K., and Olmsted, J. B. Structure and function of the cytoskeleton. In: The Cytoskeleton, A Target for Toxic Agents (T. W. Clarkson, P. R. Sager, and T. L. M. Syversen, Eds.), Plenum Press, New York, 1986, pp. 3–21.
17. Abe, T., Haga, T., and Kurokawa, M. Blockage of axoplasmic transport and depolymerization of assembled microtubules by methylmercury. Brain Res. 86: 504–508 (1975).
18. Miura, K., Suzuki, K., and Imura, N. Effects of methylmercury on mitotic mouse glioma cells. Environ. Res. 17: 453–471 (1978).
19. Sager, P. R., Doherty, R. A., and Olmsted, J. Interaction of methylmercury with microtubles in cultured cells and *in vitro*. Exp. Cell Res. 146: 127–137 (1983).
20. Sager, P. R., and Syversen, T. L. M. Disruption of microtubules by methylmercury. In: The Cytoskeleton, A Target for Toxic Agents (T. W. Clarkson, P. R. Sager, and T. L. M. Syversen, Eds.), Plenum Press, New York, 1986, pp. 97–116.