Use of Genetic Toxicology Data in U.S. EPA Risk Assessment: The Mercury Study Report as an Example

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Assessment of human health risks of environmental agents has often been limited to consideration of the potential for the agent to cause cancer or general systemic toxicity after long-term exposure. The U.S. Environmental Protection Agency (U.S. EPA) is increasingly moving toward the development of integrated assessments, which consider all potential health endpoints including developmental toxicity, neurotoxicity, immunotoxicity, reproductive effects, and germ cell mutagenicity. The U.S. EPA has a responsibility to assess risks to nonhuman species or ecosystems when appropriate data are available. An example of a recent integrated human health and ecological risk assessment can be found in the U.S. EPA Mercury Study Report to Congress. This report covers the following topics in separate volumes: an inventory of anthropogenic mercury emissions in the United States; an exposure assessment using measured and predicted values and including indirect dietary exposure; an evaluation of human health risks; an assessment of ecologic risk wherein water criteria are presented for several wildlife species; an overall integrated characterization of human and nonhuman risk; and a discussion of risk management considerations. In the evaluation of human health risk, genetic toxicology data were considered for three forms of mercury: elemental, inorganic (divalent), and methylmercury. These data were used in judgments of two types of potential health effects (carcinogenicity and germ cell mutagenicity). In assessment of potential carcinogenicity of inorganic and methylmercury, genetic toxicity data were key. Data for clastogenicity in the absence of mutagenicity supported the characterization of inorganic and methylmercury as materials that produce carcinogenic effects only at high, toxic doses. The evidence for clastogenicity, coupled with information on metabolism and distribution, resulted in a judgment of a moderate degree of concern (or weight of evidence) that inorganic mercury can act as a human germ cell mutagen. For methylmercury, the degree of concern for germ cell mutagenicity is high. — Environ Health Perspect 104(Suppl 3):663–673 (1996)

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Introduction

Risk assessments done by the U.S. Environmental Protection Agency (U.S. EPA) follow the paradigm established by the National Academy of Sciences (1). This entails a series of interconnected steps including hazard identification, dose-response assessment, exposure assessment, and risk characterization, which are briefly described below.

Hazard identification uses available data on biological end points related to a material to determine if that material is likely to pose a hazard to human health. These data are also used to define the type of potential hazard; that is, does the material induce tumor formation, cause developmental effects, act as a kidney toxicant, and so forth.

In the dose–response assessment, data from human and animal studies are used to estimate the amount of material that is expected to produce a given effect in humans. In this step it is generally necessary to apply mathematical models to the data to calculate a quantitative risk estimate usable for low-dose exposure.

The exposure assessment seeks to determine the extent to which a population is exposed to the material. Exposure assessment uses available data relevant to population exposure, such as emission data, measurement of the material in environmental media, and biomarker information. Fate and transport of the material in the environment, routes of exposure, and pharmacokinetics of the material once in the body may be considered in the exposure assessment. Data limitations on the populations of interest often necessitate the use of modeling to provide relevant estimates of exposure.

Risk characterization is the last step of the risk assessment process. This step evaluates assessments of human health and ecological effects, identifies human subpopulations or ecological species potentially at risk, and delineates areas of uncertainty, limitations, and assumptions made in the risk assessment.

The U.S. EPA has published guidelines to provide for consistency of application and communication of risk assessment. These guidelines are for assessment of developmental effects, germ cell mutagenicity, carcinogenic effects, and exposure and effects of chemical mixtures. Guidelines are also in place for assessment of reproductive effects and are in preparation for assessment of effects on other organ systems.

Despite the availability of guidelines on assessment of multiple endpoints, emphasis until recently has been on assessment of carcinogenicity, sometimes to the exclusion of other important types of toxicity. The U.S. EPA has been reversing this trend on the advice of publications such as the recent NRC/NAS report Science and Judgment in Risk Assessment (2). That report emphasizes that the goal of risk characterization is to provide understanding of the type and magnitude of potential adverse effects of an agent under the particular circumstances of its release. This directs the U.S. EPA to consider timing of effects and end points other than cancer. A contemporary risk assessment publication (3) stresses the need for...
risk assessment to evaluate in an integrated fashion all potential hazards posed by an agent.

An example of an integrated assessment is the Mercury Study Report to Congress, which will be released by the U.S. EPA in 1996. This study was done as part of the requirements of the Clean Air Act Amendments of 1990 (4) which specified the following:

The Administrator shall conduct, and transmit to the Congress not later than 4 years after the date of enactment of the Clean Air Act Amendments of 1990, a study of mercury emissions from electric utility steam generating units, municipal waste combustion units, and other sources, including area sources. Such study shall consider the rate and mass of such emissions, the health and environmental effects of such emissions, technologies which are available to control such emissions, and the costs of such technologies.

In response to this mandate, the U.S. EPA prepared a seven-volume Mercury Study Report to Congress, which included the following: an inventory of anthropogenic emissions in the United States, a nationwide exposure assessment, an assessment of human health risks from three forms of mercury, an assessment of wildlife hazard from mercury, and a characterization of risk that compared and integrated potential hazard to human and nonhuman species. The report concludes with an evaluation of control technologies, costs of implementation, and an estimate of some social costs of mercury pollution.

The human health risk assessment made use of the applicable guidelines and standard risk assessment procedures for evaluation of cancer risk, developmental toxicity, germ cell mutagenicity, and general noncancer systemic effects. In this evaluation, data of many sorts were considered: epidemiological and case study data from exposed humans, laboratory animal data for a dozen end points, and in vitro data for genotoxicity. The remainder of this discussion will focus on the use of genotoxicity data in the evaluation of two important health end points: germ cell mutagenicity and carcinogenicity.

Methods

Application of U.S. EPA Guidelines

The U.S. EPA (5) has published guidelines for classification of potential hazard of mutagenic effects in human germ cells. This is a weight-of-evidence process in which all available information on an agent is considered. Evidence from human and animal in vivo and in vitro systems is considered in the judgment as to the level of concern or likelihood that an environmental agent causes heritable damage in germ cells. In general, the hierarchy of preference for data type is the following: a) data on germ cells are preferred to data on somatic cells; b) in vivo tests are preferred to in vitro; and c) data from tests in eukaryotes are preferred to data from prokaryotes.

The weight-of-evidence categories defined in the 1986 guidelines are these, presented in order of decreasing strength of evidence for human germ cell mutagenicity.

1. Positive data derived from human germ cell mutagenicity studies.
2. Valid positive results from studies on heritable mutational events (any kind) in mammalian germ cells.
3. Valid positive results from mammalian germ cell chromosome aberration studies that do not include an intergeneration test.
4. Sufficient evidence for a chemical's interaction with mammalian germ cells, together with valid positive mutagenicity test results from two assay systems, at least one of which is mammalian. The positive results may be both for gene mutations or both for chromosome aberrations; if one is for gene mutations and the other for chromosome aberrations, both must be from mammalian systems.
5. Suggestive evidence for a chemical's interaction with mammalian germ cells, together with valid positive mutagenicity evidence from two assay systems as described under 4.
6. Positive mutagenicity test results of less strength than defined under 4, combined with suggestive evidence for a chemical's interaction with mammalian germ cells.
7. Nonmutagenic. Although definitive proof of nonmutagenicity is not possible, a chemical could be classified operationally as a nonmutagen for human germ cells if it gives valid negative results for all end points of concern.
8. Not classifiable based on inadequate evidence bearing on either mutagenicity or chemical interaction with mammalian germ cells.

To date, no environmental agent has been conclusively demonstrated to have caused mutagenic effects in germ cells in an exposed human population; that is, no increase in mutation frequency in a population has been attributed to a single agent. Thus, there have been no category 1 mutagens identified. In keeping with the trend in risk assessment for informative narrative classifications rather than simple alphanumeric tags, it was decided for the Mercury Study Report to Congress to treat these categories as guidance in assessing a level of concern for an agent's likelihood to be a germ cell mutagen. In the report to Congress, three forms of mercury were discussed in terms of level of concern rather than an assigned numerical category.

According to U.S. EPA (5), a dose–response assessment of an agent's potential for human germ cell mutagenicity can presently be done using only data from in vivo heritable germ cell tests. This will remain the case until such time as other assays are demonstrated to have an equivalent predictability for human effects. The usable tests are limited to morphological–specific locus and biochemical–specific locus assays and heritable translocation tests. Data from such assays are generated from exposures much higher than those expected for humans as a consequence of environmental exposure. Estimation of extent of human risk is done by extrapolating the observed mutation frequency or phenotypic effects downward to the expected human exposure range. Available data and mechanistic considerations are used in the choice of the dose–response model and extrapolation procedure.

The U.S. EPA has generally categorized the carcinogenic potential of a chemical based on the overall weight-of-evidence scheme published in 1986 Guidelines. The categories and their requirements are as follows:

**Group A: Human Carcinogen.** Sufficient evidence exists from epidemiology studies to support a causal association between exposure to the chemical and human cancer.

**Group B: Probable Human Carcinogen.** There is sufficient evidence of carcinogenicity in animals with limited (Group B1) or inadequate (Group B2) evidence in humans.

**Group C: Possible Human Carcinogen.** There is limited evidence of carcinogenicity in animals in the absence of human data.

**Group D: Not Classified as to Human Carcinogenicity.** There is inadequate human and animal evidence of carcinogenicity or no data are available.
Group E: Evidence of Noncarcinogenicity for Humans. There is no evidence of carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiologic and animal studies.

In the 1986 guidelines, the procedure was to evaluate first the existing human and animal data for carcinogenicity of the agent and, based on those data, to assign a provisional categorization. The second step was to consider any additional data, which included an assessment of in vitro and in vivo genetic toxicity data. After this evaluation, the risk assessor could either raise the level of concern (category) or lower it based on the results of the other data. In practical terms, the evaluation of supporting data often had little effect on the outcome of the weight of evidence judgment for carcinogenicity.

The U.S. EPA has been in the process of revising its guidelines for cancer risk assessment. The revised guidelines will implement the use of narrative categorization and will also encourage greater use of mechanistic data, including information that can be gained from genetic toxicology. Data that elucidate the mode of action of an agent will also have a direct impact on the dose-response assessment for carcinogenicity. In the past, a default procedure for dose-response assessment was most often followed—that of linear low-dose extrapolation using an upper bound on the low-dose term of a linearized multistage mathematical model. The revised guidelines dictate that the type of low-dose extrapolation to be used, if any, be guided by information on the carcinogens mode of action. Evidence of genetic toxicity has now become key in making decisions about dose-response assessment, in particular whether it is likely that there is a threshold for effect.

The Mercury Study Report to Congress was prepared before the revised Carcinogen Risk Assessment Guidelines were completed and approved. It was thus necessary to apply the existing guidelines' alphanumeric categories; however, an expanded narrative was done, and the weight of evidence judgment followed closely the revised format for expanded consideration of mechanistic data.

Results

Background on Environmental Mercury

Mercury is a pervasive and persistent chemical in the environment. It is a naturally occurring element that is released from a variety of sources including human activities. Once released into the environment, mercury undergoes a series of complex chemical and physical transformations as it cycles among the atmosphere, land, and water. Humans, plants, and animals are routinely exposed to mercury and accumulate it during this cycle, potentially resulting in a variety of ecological and human health impacts.

Elemental mercury metal is a liquid at typical ambient temperatures and pressures; it partitions strongly to air in the environment. Most of the mercury encountered in the atmosphere is elemental mercury vapor. Mercury can exist in three oxidation states: Hg0 (metallic), Hg2+ (mercurous), and Hg22+ (mercuric). The properties and behavior of mercury depend on the oxidation state. Mercury and mercuric mercury can form numerous inorganic and organic chemical compounds; however, mercurous mercury is rarely stable under ordinary environmental conditions. Most of the mercury encountered in water/soil/sediments/biota (all environmental media except the atmosphere) is in the form of inorganic mercury salts and organomercuric.

Mercury is used throughout the worldwide industrial base because of its diverse properties. It serves an important role as a process or product ingredient in several industrial sectors. In the electrical industry, mercury is used in components such as fluorescent lamps, wiring devices and switches (e.g., thermostats), and mercuric oxide batteries. Mercury also is used in navigational devices, instruments that measure temperature and pressure, and other related uses. It also is a component of dental amalgams used in repairing dental caries (cavities). In addition to specific products, mercury is used in numerous industrial processes. The largest quantity of mercury used in manufacturing in the United States is in the production of chlorine and caustic soda by mercury cell chlor-alkali plants. Other processes include amalgamation, use in nuclear reactors, wood processing (as an antifungal agent), use as a solvent for reactive and precious metals, and use as a catalyst. Mercury compounds are also frequently added as a preservative to many pharmaceutical products.

Three forms of mercury were assessed in the Mercury Study Report to Congress: elemental mercury, inorganic mercury, and methylmercury. They are characterized by somewhat different biological effects relevant to human health risk assessment. Elemental mercury is readily absorbed through the lungs and may be distributed throughout the body. Data in experimental animals are considered sufficient evidence for developmental effects of elemental mercury. Evidence from animal tests and from occupational exposure in humans indicates that neurotoxicity is the adverse effect most likely to occur at lowest exposure levels.

Absorption of ingested inorganic mercury is related to the solubility of the particular salt. Data on developmental effects of inorganic mercury are insufficient for estimation of the likelihood of human effects. Systemic toxic effects (rather than cancer, germ cell mutagenicity, or developmental toxicity) are most likely to occur in humans as a consequence of environmental exposures. The sentinel toxic end point is kidney damage mediated through an autoimmune effect.

Methylmercury is rapidly and extensively absorbed through the gastrointestinal tract; once absorbed, it is widely distributed in the body and across brain and placental barriers. There are ample data in humans and animals to consider methylmercury to be a developmental toxicant. The most important toxic effect of methylmercury is on the nervous system. Neurologic abnormalities have been observed in humans exposed as adults or in utero.

Evaluation of Mercury for Germ Cell Mutagenicity

Elemental Mercury. Results for an association of somatic cell chromosomal effects with occupational exposure to elemental mercury are variable. Popescu et al. (6) and Verschaeye et al. (7) reported increased incidence of aneuploidy. Most recently, Barregard et al. (8) showed a significant correlation between cumulative exposure to elemental mercury and micronuclei induction in T lymphocytes. Negative results were reported by Verschaeye et al. (9) and Mabille et al. (10). No studies of mutagenic effect, in vitro or in animals, have been reported.

Absorbed elemental mercury is widely distributed throughout the body; there are no data, however, on elemental mercury in gonadal tissue. Based on both positive and negative findings for somatic cell chromosomal aberrations in workers, the lack of mutagenicity data, and the lack of evidence that elemental mercury can reach germ cells, it is placed in a group of low confidence for potential as a human germ cell mutagen.
**Inorganic Mercury.** There are no data on inorganic mercury from human germ cell mutagenicity studies or from studies on induction of mutations in animals. Results of tests for mutagenicity have been variable; generally test results in prokaryotes are negative for mutagenicity (but may be positive for DNA damage), and results in eukaryotes are positive for clastogenicity. Anwar and Gabal (11) reported a statistically significant increase by comparison to age-matched controls in both chromosomal aberrations and micronuclei in lymphocytes of workers exposed to mercury fulminate. There was a correlation between frequency of aberrations and exposure duration.

As summarized by the National Toxicology Program (NTP) (12) and U.S. EPA (5), mercuric chloride has produced some positive results for clastogenicity in a variety of in vitro and in vivo genotoxicity assays, but mixed results regarding its mutagenic activity have been reported. Mercuric chloride was negative in gene mutation tests with *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA102 with or without hepatic microsomal preparations (S9) (13–15). Mercuric chloride has shown evidence of DNA damage in the *Bacillus subtilis* rec assay (16) but did not induce lytic phage in a lysogenic *Escherichia coli* strain (17).

Chromosome aberrations were observed in somatic cells of mice exposed by gavage (18) and in Chinese hamster ovary (CHO) cells treated in vitro (12,19). Positive dominant lethal results have been obtained in studies in which rats were administered mercuric chloride orally (20). Suter (21) observed a small but significant increase in the number of nonviable implants when female mice were administered mercuric chloride by intraperitoneal injection; this effect was not observed when males were treated. It was not clear whether the increase in nonviable implants was due to maternal toxicity or to a true dominant lethal effect of the treatment. Sex-linked recessive lethal mutations were not observed as a consequence of exposure to male *Drosophila melanogaster* by either feeding or injection (12).

Inorganic mercury is not as well distributed in the body as elemental mercury; it does not readily pass blood–brain or placental barriers. In one reported study (22), mice treated intraperitoneally were not shown to have an increased incidence of aneuploidy in spermatogonia. Watanabe et al. (23), however, showed that, while hamsters injected subcutaneously with mercuric chloride had no increase in aberrations in metaphase II oocytes, there was detectable mercuric chloride in ovaries and some inhibition of ovulation.

DNA damage (single strand breaks) has also been observed in assays using rat and mouse embryo fibroblasts (20) and CHO cells and human KB cells (24–32). Mercuric chloride also produced chromosome aberrations and sister chromatid exchange (SCE) in CHO cells (19) and SCE in human leukocytes (33). Negative results for chromosomal aberrations were reported for FM3A cells (from a mouse mammary carcinoma) (34) and for two human diploid lines, WI38 and MRC5 (35). Negative results for SCE were reported for don cells (36) and for P388D cells, mouse cells, and CHO cells (37). Evidence of gene mutations (considered weakly positive) was observed in L5178Y mouse lymphoma cells in the presence of microsomal preparations (38).

The NTP (12) reached the following conclusions from their *in vitro* testing of mercuric chloride: mercuric chloride was not mutagenic for *Salmonella typhimurium* in preincubation protocols with and without rat and hamster liver preparations; positive for L5178Y cells without addition of hepatic preparations; negative for SCE in CHO cells without addition of S9, but weakly positive when rat S9 was added; and positive for chromosomal aberrations in CHO cells in the absence but not the presence of liver preparations (it was not clear what role was played by cytotoxicity in the generation of these chromosomal aberrations).

The totality of available data indicates a moderate weight of evidence for germ cell mutagenicity for inorganic mercury: sex-linked recessive and dominant lethal results were compromised, but there are positive results for chromosomal aberrations in multiple systems (including *in vivo* exposure) and evidence that mercuric chloride can reach female gonadal tissue.

**Methylmercury.** Methylmercury appears to be clastogenic but is not a potent mutagen. Table 1 gives results from genetic toxicity testing *in vitro*. Evidence of DNA damage has been observed in the *Bacillus subtilis* rec assay (16). Kanematsu et al. (16) reported negative results for methylmercury in spot tests for mutagenicity in the following bacterial strains: *E. coli* B/WP2 and WP2 and *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100. Jensen and Ramel (46), in a review article, indicated that methylmercury acetate was negative in both micronucleus assays and in mutagenicity tests in *Salmonella*; the authors referred to Heddle and Bruce (45) and provided no experimental details. Weak mutagenic responses for methylmercuric chloride and methoxyethyl mercury chloride were observed in Chinese hamster V79 cells at doses near the cytotoxic threshold (42), and methylmercury produced a slight increase in the frequency of chromosomal nondisjunction in *Saccharomyces cerevisiae* (43). Methylmercury, however, caused neither gene mutations nor recombination in *S. cerevisiae* (43). Methylmercury retarded DNA synthesis and produced single strand breaks in DNA in L5178Y cells (44). Methylmercuric chloride and dimethylmercury were both shown to induce chromosomal aberrations and aneuploidy in primary cultures of human lymphocytes; methylmercuric chloride was the more potent clastogen at equally toxic doses (39). Both methylmercury and mercuric chloride induced a dose-dependent increase in SCE in primary human lymphocytes and muntjac fibroblasts; methylmercury was about five times more effective in this regard (33,40). Impaired growth and development was noted in

| Table 1. *In vitro* genotoxicity of methylmercury. |
|-----------------------------------------------|
| **System** | **Effect** | **References** |
| Primary human lymphocytes | Chromosome aberrations, aneuploidy | (39) |
| Primary human lymphocytes | SCE | (40) |
| Muntjac fibroblasts | SCE | (33) |
| V79, rat glioblastoma cells | DNA strand breaks | (41) |
| *E. coli B/WP2, WP2, Salmonella TA1535, TA1537, TA1538, TA98, TA100* | Negative spot test | (16) |
| V79 cells | Weak mutagenicity at cytotoxic dose | (42) |
| Saccharomyces cerevisiae | Chromosome nondisjunction, negative for gene mutations and recombination | (43) |
| L5178Y cells | Decreased DNA synthesis, single strand breaks | (44) |
| Micronucleus, *Salmonella* mutagenicity | Negative | (45) |
| *Bacillus* rec assay | DNA damage | (16) |
cultured mouse embryonic tissue treated in vitro with methylmercuric chloride, but there was no increase in SCE (47). Costa et al. (41) showed that methylmercuric chloride caused DNA strand breaks in both V79 and rat glioblastoma cells treated in vitro. Methylmercuric chloride produced more strand breaks than did mercuric chloride.

Results of studies in humans are given in Table 2. Studies have reported increased incidence of chromosome aberrations (50,51) or SCE (48) in lymphocytes of humans ingesting mercury-contaminated fish or meat. A finding of increased frequency of chromosome breaks in workers was compromised by other concurrent exposures (6). A study in cats treated in vivo gave inconsistent results for unscheduled DNA synthesis and chromosome breaks in leukocytes (Table 3).

Other in vivo studies are summarized in Table 4. Strain-specific differences exist with respect to the ability of methylmercury to produce dominant lethal effects in mice (21). When (SEC x C57Bl)F1 males were injected with 10 mg/kg methylmercury hydroxide, there was a slight reduction in the total number of implantations and a decrease in the number of viable embryos. This was not observed when (101 x C3H)F1 males were exposed in a similar fashion. When female (101 x C3H)F1 mice were treated with methylmericuric hydroxide, no increase in the incidence of dead implants was observed (unlike the case for mercuric chloride). Changes in chromosome number, but no increase in chromosome aberrations, were observed in oocytes of Syrian hamsters treated with one intraperitoneal (ip) injection of 10 mg/kg methylmercuric chloride (53). Methylmercury was administered subcutaneously (sc) to golden hamsters at doses of 6.4 or 12.8 mg Hg/kg/body weight. Polyploidy and chromosomal aberrations were increased in bone marrow cells, but there was no effect on metaphase II oocytes. There was an inhibitory effect on ovulation, which the authors noted was not as severe as that induced by mercuric chloride in the same study (23). Nondisjunction and sex-linked recessive lethal mutations were seen in Drosophila melanogaster treated with methylmercury in the diet (54).

Methylmercury is widely distributed in the body, breaching both blood–brain and placental barriers in humans. There are data indicating that methylmercury administered ip reaches germ cells and

### Table 2. Genotoxicity of methylmercury in humans.

| Number per sex | Exposure duration | Dose (mg/kg/day) | Effects, limitations, and BML | References |
|----------------|-------------------|------------------|-------------------------------|------------|
| 24–63 (both sexes) | NS | NS | Incidence of SCEs in cultured peripheral lymphocytes correlated with intake of seal meat in an Eskimo population (as a surrogate for mercury intake), p = 0.001. Other factors also correlated with SCEs, but multiple regression analysis found that some of the effect was attributable to mercury. Limitation: limited exposure data. BML not reported | (49) |
| 51 M | Measured as seafood meals per week; range = 2–14. | NS | Incidence of micronuclei positively correlated with blood mercury concentration and with age. No correlation with smoking or number of seafood meals per week. Limitation: no control group. BML: range: 10.08–403.11 µg/g blood | (49) |
| 18 M exposed; 10 M control | 10.5 year (occupational) | 0.15–0.44 (HgCl2) | Increased frequency of chromosomal breaks. Limitations: workers also exposed to mercuric chloride and one worker had history of benzene poisoning; control group was not matched for sex, smoking habits, or sample size. BML: = 890 µg/l in urine (average) | (6) |
| 6M; 3F exposed; 3 M, 1 F control | >5 year, ≥3 x per week | NS | Correlation between blood mercury concentration and chromosome breaks in lymphocytes cultured from people who ate mercury-contaminated fish. Limitations: small sample size, limited exposure data. BML range: 4–650 µg/l in blood | (50) |

**Abbreviations:** M, male; F, female; NS, not specified; BML, blood mercury level.

### Table 3. Genotoxicity of methylmercury in cats.

| Number per sex | Exposure duration | Dose (mg/kg/day) | Effects, limitations, and BML | References |
|----------------|-------------------|------------------|-------------------------------|------------|
| Breed and sex not specified | 39 months, 7 days/week | 0.008, 0.020, 0.046 | No dose-related changes in unscheduled DNA synthesis in cultured lymphocytes or frequency of chromosomal aberrations in bone marrow of cats fed mercury-contaminated fish or a fish diet supplemented with methylmercuric chloride. Limitations: no positive control; no assessment of cytotoxicity. BML range: 500–13,500 µg/l Hg in blood | (52) |

### Table 4. Genotoxicity of methylmercury in animals.

| Species (strain) | Exposure duration | Dose (mg/kg/day) | Effects and limitations | References |
|-----------------|-------------------|------------------|-------------------------|------------|
| Mice, (SEC x C57Bl)F1, M | Once | 10 | Dominant lethal test; slight reduction in implantation | (27) |
| Mice, (101 x C3H)F1, M | Once | 10 | Dominant lethal test, no effect | (27) |
| Mice, (101 x C3H)F1, F | Once | 10 | Dominant lethal test, no effect | (27) |
| Hamster (Syrian) | Once, intraperitoneal | 10 | Changes in chromosome number, no aberrations in oocytes | (53) |
| Hamster (golden) | Once, subcutaneous | 6.4 or 12.8 | Polyplody, chromosomal aberrations in bone marrow, no effect in oocytes; inhibition of ovulation | (23) |
| Drosophila melanogaster | Diet | Sex-linked recessives and nondisjunction | (54) |
may produce adverse effects. Because there are data for mammalian germ cell chromosome aberration and limited data from a heritable mutation study, methylmercury is placed in a group of high concern for potential human germ cell mutagenicity. All that keeps methylmercury from the highest level of concern is the lack of positive results in a heritable mutation assay.

Assessment of Mercury for Carcinogenic Effects

**Elemental Mercury.** Human data regarding the carcinogenicity of inhalation of elemental mercury are insufficient to determine whether such exposures may result in increased cancer incidence. Several studies report statistically significant increases in lung cancer mortality among groups of exposed workers (55-58). The interpretation of these studies is limited by small sample sizes, probable exposure to other known lung carcinogens, failure to consider confounders such as smoking, and failure to observe correlations between estimated exposure and the cancer incidence. A study of dental professionals found a significant increase in the incidence of glioblastomas (59). It is not known whether exposure to mercury, X-rays, or other potential carcinogens in the workplace contributed to the effects observed. No increase in cancer mortality was observed among workers exposed to mercury vapor in a nuclear weapons facility (60), but this study was also limited by the small sample size. No studies were identified that examined cancer incidence in animals exposed chronically to elemental mercury vapor.

The overall findings from genetic toxicity testing (described above) are very limited and point to clastogenicity in workers occupationally exposed to mercury by inhalation. There are no reported tests for gene mutation.

In summary, human epidemiological studies failed to show a correlation between exposure to elemental mercury vapor and increased cancer incidence, but the studies are limited by confounding factors. Only one study in animals is reported (61): tumors were found only at contact sites, and the study is incompletely reported as to controls and statistics. Animal data are also inadequate. Findings from assays for genotoxicity are limited and provide no convincing evidence that mercury exposure has an effect on the number or structure of chromosomes in human somatic cells. The most appropriate category is, thus, Group D, not classifiable as to human carcinogenicity.

**Inorganic Mercury.** There are no data available on the carcinogenic effects of inorganic mercury (mercuric chloride) in humans. In animals, there is equivocal evidence of carcinogenicity in rats and mice (Table 5). In rats gavaged with mercuric chloride for 2 years (62), survival was significantly reduced in males (17 and 8% survival in low- and high-dose males, respectively, versus 43% survival in controls), indicating that the maximally tolerated dose (MTD) was exceeded. There was an increased incidence of forestomach squamous cell papillomas. Papillary hyperplasia of the forestomach was also significantly elevated in both male dose groups and in high-dose females. In addition, the incidence of thyroid follicular cell carcinomas in treated males showed a significantly positive trend. There were, however, no increases in thyroid hyperplasia of adenomas; it is not clear that the increase in thyroid carcinomas is a treatment-related effect. The NTP considered the forestomach tumors to be of limited relevance to humans; there was no evidence that these contact site tumors progressed to malignancy.

In a companion study in mice (62), there was a significantly increased trend for renal tubular cell tumors (adenomas and adenomacarcinomas). This is presented in Table 6. No dose groups were statistically significantly different from the control by pair-wise comparison, although the incidence in the high-dose group was elevated. There was a significant increase in severe nephropathy in treated animals.

In summary, there are no data in humans linking mercuric chloride with carcinogenic effects, and data in animals are limited. Focal hyperplasia and squamous cell papillomas of the forestomach, as well as thyroid follicular adenomas and carcinomas, were observed in male rats exposed to mercuric chloride (62). In the same study, evidence for increased incidence of squamous cell forestomach papillomas in female rats and renal adenomas and carcinomas in male mice were considered equivocal. All increased tumor incidences were observed at what were considered high doses (in excess of the MTD). In this context, the relevance of the thyroid tumor to human health evaluation has been questioned; these tumors are considered to be secondary to the hyperplastic response. Results from *in vitro* and *in vivo* tests for genotoxicity have been mixed; there is no clear indication of a strong mutagenic effect in somatic cells. This lack of a mutagenic effect supports a judgment of limited data for carcinogenicity.

**Methylmercury.** The available human data are inconclusive regarding the carcinogenicity of methylmercury in humans exposed by the oral route. A study of leukemia patients from a rural area in Poland showed a significantly higher mercury content in hair in the leukemia patients than in healthy unrelated patients or healthy relatives (62). The population studied was small, and the study did not adjust for other leukemia risk factors. In addition, two studies of larger populations exposed to methylmercury during the Minamata incident failed to show increases in leukemia or total cancer incidence (63,64).

### Table 5. Incidence<sup>a</sup> of neoplastic lesions in rats in the NTP 2-year gavage study of mercuric chloride.

| Tumor site and type       | Male dose groups (mg Hg/kg/day) | Female dose groups (mg Hg/kg/day) |
|---------------------------|---------------------------------|-----------------------------------|
|                           | 0                               | 1.9                               | 3.7                               | 0                               | 1.9                               | 3.7                               |
| Foregut                    | 0/50                            | 16/50*                            | 35/50*                             | 5/50                            | 5/49                              | 20/50*                            |
| Papillary hyperplasia      | 0/50                            | 3/50                              | 12/50**                            | 0/50                            | 0/49                              | 2/50                              |
| Squamous cell papilloma    | 1/50                            | 4/50                              | 0/50                               | ---                             | ---                               | ---                               |
| Thyroid follicular cellα   | 1/50                            | 2/50                              | 6/50†                              | ---                             | ---                               | ---                               |
| Adenoma or carcinomas      | 2/50                            | 6/50                              | 6/50                               | ---                             | ---                               | ---                               |

Data from the NTP (12). *Overall rate. *Data on thyroid follicular cell lesions were reported for males only. *p<0.01, *p≤0.001; trend test also *p<0.001. **p=0.044, logistic regression.

### Table 6. Incidence<sup>a</sup> of renal tubule tumors in male mice in the NTP 2-year gavage study of mercuric chloride.

| Tumor type                | Dose group (mg Hg/kg/day) |
|---------------------------|---------------------------|
|                           | 0                         | 5                         | 10                         |
| Adenoma                   | 0/50                      | 0/50                      | 2/49                       |
| Carcinoma                 | 0/50                      | 0/50                      | 1/49                       |
| Adenoma or carcinomas     | 0/50                      | 0/50                      | 3/49*                      |

Data from the NTP (12). *Overall rate. *p=0.107; trend test *p=0.032.
Table 7. Carcinogenic effects of methylmercury in animals: oral exposure.

| Species (strain) number per sex | Exposure duration | Dose (mg/kg/day) | Effects, limitations, and BML | References |
|---------------------------------|-------------------|------------------|-------------------------------|------------|
| Rat (strain NS) 25 M, 25 F     | 2 year, *ad libitum* in feed | 0, 0.004, 0.02, 0.1 | Tumors at comparable incidence in all groups. Limitations: small sample size; failure to achieve MTD. BML average: 850 μg/l in blood at 0.004, 6.500 μg/l at 0.02, and 38,000–39,000 μg/l at 0.1 | (65) |
| Rat (Sprague-Dawley) 56 M, 56 F | 130 week *ad libitum* in feed | M: 0.01, 0.05, 0.28; F: 0.014, 0.064, 0.34 | No increase in tumor incidence. | (66,67) |
| Mice (Swiss) 54 M, 54 F         | From weaning until death in drinking water | 0, 0.19, 0.19–0.95 | No increase in gross tumor incidence. Limitation: histological examination not performed. | (68) |
| Mouse (ICR) 60 M, 60 F         | 78 week *ad libitum* in feed | 0, 1.6, 3.1 | Increased incidence of renal adenomas and adenocarcinomas in low-dose males. Limitations: very poor survival in both male dose groups. | (69) |
| Mouse (ICR) 60 M, 60 F         | 104 week *ad libitum* in feed | 0, 0.02, 0.03 0.11, 0.15, 0.6, 0.73 | Incidence of renal epithelial adenocarcinoma significantly increased in males at 0.73; not invasive. Limitations: MTD exceeded (including severe renal damage in high-dose males). | (70) |
| Mouse (B6C3F1) 60 M, 60 F      | 2 year, *ad libitum* in feed | M: 0.03, 0.14, 0.69; F: 0.03, 0.13, 0.6 | Renal epithelial carcinomas and adenomas in males at 0.69. Limitation: MTD exceeded in high-dose males. | (71) |
| Mice (Swiss) NS                 | 15 week *ad libitum* in drinking water | 0, 0.03, 0.07, 0.27 | Number of lung adenomas/mouse and tumor size/mouse increased with dose. | (72) |
| Cat (domestic) 4–5 M, 4–5 F     | 2 year, *ad libitum* in feed | 0, 0.0084, 0.02, 0.046, 0.074, 0.176 | No increase in tumor incidence. Limitations: small group size, short exposure duration, no pathological data for three lowest doses. | (73) |

Abbreviations: NS, not specified, M, male; F, female.

Although one of these studies showed a significant increase in liver cancer incidence, factors other than mercury exposure were likely contributors to the increase.

Animal studies show some evidence of carcinogenicity in two strains of mice, but studies in rats have not shown similar results (Table 7). Male ICR mice given methylmercuric chloride in the diet for up to 2 years had significantly increased incidences of renal epithelial adenomas and adenocarcinomas (69,70). Similarly, male B6C3F1 mice given methylmercuric chloride in the diet for up to 2 years had significantly increased incidences of renal epithelial carcinomas and adenomas (71). In contrast, Sprague-Dawley rats administered methylmercury in the diet for up to 130 weeks exhibited no increase in tumor incidence (66,67). Although the dose was lower in the rats than in the mice, a maximally tolerated dose was achieved in the rat study as evidenced by an approximately 20 to 30% decrease in body weight gain and by significant increases in renal and neuronal toxicity in both male and female rats at the highest dose tested. Other studies also failed to show increases in tumor incidence after chronic exposure to methylmercury (65,68), but these studies were limited by small sample sizes, failure to achieve a maximally tolerated dose, and/or incomplete histopathological examinations.

In summary, data for carcinogenicity from human studies are considered inadequate. Three studies that examined the relationship between methylmercury exposure in humans and increased incidence of cancer were limited by poor study designs or incomplete descriptions of methodology or results. Data from animal studies are considered to provide limited evidence of carcinogenicity.

Male ICR and B6C3F1 mice exposed to methylmercuric chloride in the diet were observed with increased incidence of renal adenomas, adenocarcinomas, and carcinomas. Tumors were observed at a single site in a single species and sex. Renal epithelial cell hyperplasia and tumors were observed only in the presence of profound nephrotoxicity; tumors were suggested to be consequent to reparative changes in the affected organs. Genotoxicity test data suggest that methylmercury is clastogenic rather than mutagenic. The lack of a strong mutagenic response does not raise the overall level of concern and supports the weight-of-evidence judgment of limited data or Group C, possible human carcinogen.

Dose–Response Assessment for Mercury

Data do not support the generation of quantitative estimates for germ cell mutagenicity for any form of mercury. Methylmercury was considered to have a high level of concern for the likelihood that it could be a human germ cell mutagen. The data for methylmercury all point to the probability that it acts as a clastogen and does not cause point mutations. There are not sufficient data–response data for genetic toxicity of methylmercury to support a low-dose extrapolation for human germ cell effects. Moreover, there is a great deal of uncertainty as to the appropriate model to be applied to an agent that acts as a clastogen in the absence of observable point mutation activity.

Elemental mercury is categorized as Group D, unable to be classified as to human carcinogenicity. A quantitative estimate for carcinogenic effect is, thus, inappropriate.

Quantification of the potential carcinogenic effects of mercuric chloride was not done. The data on squamous cell papillomas of the forestomach and thyroid follicular cell carcinomas from the NTP (12) were evaluated for suitability as a basis for low-dose extrapolation. The forestomach tumors in this assay were not used because these particular tumors were probably the result of irritation of the forestomach, cell death, and epithelial proliferation. Because the carcinogenic mechanism may be specific to irritation at the high doses used in the bioassay, use of these tumors as a basis for human health assessment of low doses of inorganic mercury is inappropriate.

Regarding the thyroid carcinomas, a variety of drugs, chemicals, and physiological perturbations result in the development of thyroid follicular tumors in rodents. For a number of chemicals, the mechanism of tumor development appears to be a secondary effect of longstanding hypersecretion of thyroid-stimulating hormone by the pituitary (74,75). In the absence of such long-term stimulatory effects, induction of thyroid follicular cell cancer by such chemicals usually does not occur (76). Use of the incidence of thyroid...
The lack of extrapolation is therefore questionable. The lack of a mutagenic effect likewise calls into question the application of a linear low-dose model. The use of linear low-dose models is based on a hypothesis for carcinogenicity that predicates an irreversable first step in a multistep process. This first step is usually considered to be one involving mutagenicity as an irreversible change at the genome level.

Quantification of the potential carcinogenic effects of methylmercury (classified as Group C, possible human carcinogen) was not done; only renal epithelial tumors in male mice were reported to be increased. The two studies by Mitsuomori et al. (69,71) were limited by high mortality in the high-dose males, the only group to exhibit a statistically significant increase in tumor incidence. The study by Hirano et al. (70) was not limited by survival problems, but the tumors were observed in conjunction with nephrotoxicity and appear to be a high-dose phenomenon that may not be linear at low doses. The tumors appeared to originate from focal hyperplasia of the tubular epithelium induced as a reparative change. The hyperplasia was not observed in tubular epithelium that was undergoing early degenerative changes; thus, the tumors may not occur where degenerative changes do not occur. The appropriateness of deriving a quantitative risk estimate using the assumption of linearity at low doses based on data for which a threshold may exist is questionable. The lack of a mutagenic response in the absence of clastogenicity also argues against linear low-dose extrapolation.

Summary and Discussion

All three species of mercury were assessed for their potential to cause mutations in germ cells using the Guidelines for Mutagenicity Risk Assessment (5). Elemental mercury was placed in a category of low confidence for potential as a human germ cell mutagen. This is based on reports of both positive and negative findings for chromosomal aberrations in somatic cells of exposed workers. There are no reports of assays for mutagenicity of elemental mercury. While absorbed elemental mercury is widely distributed throughout the body, there are no data on the presence of elemental mercury in rodent gonadal tissue.

The somewhat more complete database for inorganic mercury indicates a moderate weight of evidence for germ cell mutagenicity. Sex-linked recessive mutations were not observed in Drosophila melanogaster, and dominant lethal assays in mice were compromised by maternal toxicity. There were, however, positive results for chromosomal aberrations in multiple systems including in vivo exposure in occupationally exposed humans and in somatic cells of gavaged mice. There is evidence that mercuric chloride can reach gonadal tissue (oocytes) but no evidence of damage to that tissue.

Because there are data for mammalian germ cell chromosomal damage and limited data from a heritable mutation study, methylmercury was placed in a group of high concern for potential human germ cell mutagenicity. All that keeps methylmercury from the highest level of concern is lack of positive results in a heritable mutation assay such as the mouse specific locus test. Data do not support quantitative estimates of germ cell mutagenicity. The effects noted have been chromosomal damage and dominant lethal effects at levels which also produced maternal toxicity. There is no evidence of mutagenic effects at less than toxic doses.

Available data on carcinogenic activity and related experimental end points were evaluated for elemental mercury, mercuric mercury, and methylmercury. According to U.S. EPA's Guidelines for Carcinogenic Risk Assessment (77), elemental mercury is categorized as Group D, unable to be classified as to human carcinogenicity. Human epidemiologic studies failed to show a correlation between exposure to elemental mercury vapor and increased cancer incidence, but these studies were limited for interpretation by confounding factors. Only one inadequate study in animals has been reported. Findings from assays for genotoxicity were limited and did not provide convincing evidence that elemental mercury causes mutations or chromosomal effects.

Mercuric mercury (inorganic mercury) has been categorized as Group C, possible human carcinogen, according to the U.S. EPA Guidelines for Carcinogenic Risk Assessment (77). There are no data on the carcinogenic effects of inorganic mercury in humans. In animals there is equivocal evidence of carcinogenicity in rats and mice reported by NTP (12). Focal hyperplasia and squamous cell papillomas of the forestomach, as well as thyroid follicular adenomas and carcinomas, were observed in male rats gavaged with mercuric chloride. The relevance of the thyroid tumors to evaluation of potential human risk has been questioned; these tumors are considered to be secondary to the hyperplastic response. Evidence for increased incidence of squamous cell forestomach papillomas in female rats and renal tubule adenomas and carcinomas in male mice was considered equivocal. All increased tumor incidences were observed at what were considered to be high doses; that is, in excess of the MTD. Results from in vivo and in vitro tests for genotoxicity have been mixed, with no clear indication of production of gene mutations by inorganic mercury administered at less than toxic doses.

Methylmercury has also been categorized as Group C, possible human carcinogen, according to the U.S. EPA Guidelines for Carcinogenic Risk Assessment (77). Data from human studies were considered inadequate. Three studies on the relationship between methylmercury exposure in humans and increased cancer incidence were limited by poor study designs or incomplete descriptions of methodology or results. Data from animal studies were considered to be limited. Male ICR and B6C3F1 mice exposed to methylmercury in the diet had increased incidences of renal adenomas, adenocarcinomas, and carcinomas. These were found only in the presence of profound nephrotoxicity and were suggested to be consequent to reparative changes in the damaged organ. MTDs were exceeded in studies in which increased tumor incidence was observed. Results from genotoxicity tests indicated evidence for clastogenicity, but not for mutagenicity, and did not support raising the level of concern for methylmercury as a low-dose human carcinogen.

The current U.S. EPA cancer guidelines indicate that for agents classified as Group C, possible human carcinogen, a quantitative risk estimate should be considered on a case-by-case basis. No quantitative estimation of low-dose cancer risk from either inorganic mercury or methylmercury (both categorized as Group C, possible human carcinogen) has been done. Modes of action for both forms of mercury were not linked to processes such as gene mutation, for which a model employing low-dose linearity would be plausible. The data are too limited to permit use of a nonlinear low-dose estimation.

It is likely that systemic noncancer effects for both inorganic and methylmercury would be seen at exposures lower than those required for tumor formation. Long-term administration of methylmercury to experimental animals produces either neurotoxic signs or overt neurotoxicity at
daily does an order of magnitude lower than those which induce cancer in mice (Table 8). More sensitive measures applied to humans exposed for a relatively short duration have been the basis of a lowest observed adverse effect level (LOAEL) of 3.0 μg/kg/day methylmercury for paresthesia in adults. A benchmark dose (95% lower confidence limit on 10% risk) of 1.1 μg/kg/day has been calculated for composite neurological end points in offspring of 81 exposed women from the Iraq poisoning incident (80). It is likely that a quantitative assessment such as a reference dose, based either on the human benchmark or LOAEL, would be protective against potential carcinogenic hazard from methylmercury. In summary, it is likely that in the near future genetic toxicity information on environmental agents will play an enhanced role in their risk assessment. This is due to two trends in overall characterization of human risk. The first is the move to a more integrated or holistic approach to human health risk evaluation, which considers end points other than cancer as important to understanding the magnitude and type of potential hazard. The second trend is in the increased emphasis on mechanistic data in the assessment of an agent’s potential to be a human carcinogen. The ability to assess the totality of a material’s genotoxicity database will clearly be an asset in the application of risk assessment principles to the evaluation of hazards relevant to human health.

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