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S 140 Managing Biotransformation: The Metabolic, Genomic, and Detoxification Balance Points
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INTRODUCTION

Over the past forty years, concern has grown that some of the 80,000 chemicals used commercially could be exerting adverse effects on children's health. Many of these chemicals were synthesized for the first time within recent decades, suggesting that the body's detoxification mechanisms, the results of thousands of years of evolution, might not be effective in limiting their impact. The potential for exposure is substantial, as the US Environmental Protection Agency (US EPA) estimates that 2.5 billion pounds of chemicals are emitted yearly by large industrial facilities. At the same time, it is remarkable how limited are the data on the toxicities associated with most of these chemicals. The US EPA maintains the Integrated Risk Information System (IRIS), which serves as the repository of the consensus scientific opinions on chemical toxicity. Yet IRIS lists only 550 chemicals (www.epa.gov/iriswebp/iris/stand-al.htm), indicating significant lacunae in the knowledge needed to estimate and manage the risks associated with current exposures.

For many chemicals, most of the available data pertains to occupational exposures. The amount of data available regarding the potential effects of chemicals on children's brain development is much more limited. It was not until the 1990s that the US EPA published guidelines for registrants with regard to testing in animal models of the developmental neurotoxicity of certain chemicals, primarily organophosphate pesticides, for application in human risk assessments (US EPA OPPTS Health Effects Test Guideline 870.6300; www.epa.gov/EPA-TOX/1998/May/Day-14/t12303.htm).

At present, for many of the chemical exposures of current concern with regard to children, little or no data are available on either the extent of exposures or the neurological effects. This is true for exposures associated with living in proximity to hazardous waste sites, emissions from municipal waste incinerators, solvents, groundwater pollutants such as arsenic and manganese, and widely used materials such as phthalates (plasticizers) and polybrominated

diphenyl ethers (flame retardants). More information is available about population exposures to potential neurotoxicants such as pesticides, dioxins, elemental mercury, and fluoride, but detailed data are lacking on potential effects of such exposures. The data available can be characterized as “considerable” only for the so-called “big three”: inorganic lead, methylmercury, and polychlorinated biphenyls. Fortunately, recent initiatives undertaken by the US Centers for Disease Control (US CDC) are addressing these issues, issuing a periodic National Report on Human Exposure to Environmental Chemicals, based on the National Health and Nutrition Examination Survey (NHANES). The Second Report, issued in 2003, provided data on 116 chemicals, 89 of which had never before been measured in a nationally representative sample of the US population, including many that would be expected to affect brain function. In the Third Report, issued in July 2005 (http://www.cdc.gov/exposurereport/), data were provided on 148 chemicals. This effort, while important, represents only half of the challenge. The other half involves the difficult task of determining the dose-response relationships associated with these chemicals, since the mere presence of a chemical in blood or urine does not mean that it is affecting health.

METHYLMERCURY

Mercury is a heavy metal that is present in the environment as a result of both natural processes and human activities (referred to as anthropogenic sources). The natural sources include volcano emissions and the weathering of rock containing mercury ore. The primary anthropogenic sources are the combustion of fossil carbon fuels, particularly from coal-fired utility boilers; other such sources include municipal, medical, and hazardous waste incineration.1 Mercury can travel long distances in the atmosphere and contaminate sites far from its point of release. Furthermore, the complex biogeochemistry of mercury fate and transport creates uncertainty in efforts to apportion the relative contributions of these processes to global mercury pollution. The US EPA estimated that 50 to 75% of the total yearly input of mercury into the environment is anthropogenic2; the United Nations suggests that it accounts for more than half of the inputs (http://www.chem.unep.ch/mercury/Report/GMA-report-TOC.htm).

Mercury exists in the environment in several different forms, including metallic, inorganic, and organic; interconversion between forms can occur. The form of mercury of greatest concern with regard to seafood consumption is methylmercury (MeHg). Methylmercury results when mercury in other forms is deposited in water bodies and biotransformed through the process of methylation by microorganisms. It bioaccumulates up the aquatic trophic food chain as smaller organisms are consumed by larger organisms. Because methylmercury is persistent, this biomagnification process results in the highest concentrations in large long-lived predatory species, such as shark,

swordfish, and tuna. Methylmercury levels can also be high in marine mammals such as whales and in animals that feed on marine life, such as polar bears and sea birds. Consumption of marine life is the major route of human exposure to methylmercury.

The devastating effects that high-dose exposure to methylmercury can have on neurological development were first recognized following a decades-long poisoning episode that occurred in the region of Minamata Bay in southern Japan as the result of industrial discharge of mercury salts. Women who consumed methylmercury-contaminated fish from the area gave birth to children with what came to be called Congenital Minamata Disease (CMD), which includes growth disturbances, primitive reflexes, movement and coordination disorders (cerebellar ataxia, chorea, athetosis, dysarthria), sensory impairments, cerebral palsy, and mental retardation. Because of the delay in identifying methylmercury as the cause, it was not possible to determine the critical dose required to produce CMD. It was noted, however, that the mothers of some children with CMD appeared to be asymptomatic or to suffer only mild, transient paresthesias. Another episode of mass poisoning occurred in Iraq in the 1970s, when, rather than being planted, mercury-treated seed grain was ground into flour and consumed.

In this episode, as well as in Minamata, it was apparent that the critical doses necessary to produce severe, debilitating neurological outcomes in the fetus were far lower than those necessary to produce effects in adults, resulting in the recognition that the pregnant woman is the critical population subgroup. Autopsy studies of the brains of affected individuals revealed a striking age-dependence in the distribution of methylmercury-associated lesions. In individuals exposed only in adulthood, the distribution was highly focal, primarily in the cerebellum, calcarine fissure of the occipital cortex, and post-central gyrus, as might be expected given the specific clinical signs of adult methylmercury poisoning. In the individual exposed prenatally, however, lesions were diffusely distributed throughout the brain.3 This is most likely because methylmercury arrests mitotic cells in metaphase, thus disrupting cell proliferation and migration in the brain. The abnormalities

observed include reduced cell densities, islands of heterotopic neurons, glial proliferation, incomplete myelination, and disturbances in brain cytoarchitecture.

Recognizing the devastating effects of high-dose exposure to methylmercury, investigators were led, beginning in the 1980s, to ask whether milder neurological effects are associated with the lower-dose in utero exposures to MeHg that are more typical within the general population of seafood consumers. Based on the Iraqi study, the WHO identified maternal hair levels of 10 to 20 micrograms/gram (or parts per million, ppm) as the range within which the risk of adverse neurodevelopmental outcomes such as delayed walking and talking began to rise.4 Several longitudinal prospective studies involving the recruitment of birth cohorts were undertaken to evaluate this conclusion, most importantly in New Zealand,5 the Faroe Islands (located in the Northern Atlantic Ocean),6 and the Seychelles Islands (located in the eastern Indian Ocean).7 These populations were selected for study because of the prominence of seafood in the diet. For example, the women who enrolled in the Seychelles Islands study reported eating an average of 12 fish meals per week. In addition to frequent fish consumption, the Faroese also periodically consume pilot whale, which contains high levels of methylmercury. The New Zealand and Faroe Islands studies have generally been interpreted as demonstrating inverse associations between prenatal exposure to methylmercury and children's neurodevelopment, while the Seychelles Islands study has not. In the Faroe Islands study, cord-blood mercury level was inversely associated with children's scores on tests of attention, language, and memory. In follow-up evaluations at age 14 years, children's hair mercury levels were positively associated with delayed responses on brainstem auditory evoked potentials.8 Inverse associations between children's outcomes and maternal hair mercury levels, which averaged between 4 and 5 ppm, were also observed. In the New Zealand study, maternal hair mercury levels greater than 10 ppm were associated with a doubling of the risk of IQ scores below 70.

The apparent inconsistencies in study findings have posed a challenge to risk assessors attempting to establish intake guidelines for methylmercury. Some risk assessors have chosen the Seychelles Islands study9 or used an integrative strategy that took into account the results of all three studies.10 Adopting a precautionary approach, the US EPA elected to base its derivation of the Reference Dose (RfD) for methylmercury on the Faroe Islands study. [The RfD is defined as "…an estimate of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime."] One consideration motivating this choice was that it would result in a guideline that is more protective of the population than would a guideline based on the Seychelles Islands study.11 Using the benchmark dose method to determine a

point of departure and incorporating uncertainty factors, a critical dose of 5.8 micrograms/liter of cord blood (equivalent to a maternal hair mercury level of 1.2 ppm) was identified. By making a variety of toxicokinetic assumptions, the US EPA established an RfD of 0.1 micrograms/kilogram bodyweight/day as the methylmercury intake that, over a lifetime, should not produce adverse effects.

In 2004, the US Food and Drug Administration and the US EPA offered a joint advisory regarding fish consumption by pregnant women, women considering becoming pregnant, and young children (http://www.cfsan.fda.gov/~dms/admehg3.html). This advisory recommended the avoidance of four types of fish that, on average, have the highest levels of mercury: shark, tile fish, king mackerel, and swordfish. Furthermore, it suggested that these population subgroups can eat up to 12 oz (two average meals) a week of a variety of fish and shellfish that are lower in MeHg (e.g., shrimp, canned light tuna, salmon, pollock, catfish). It noted that because albacore or "white" tuna tends to have more MeHg than canned light tuna, up to six ounces of albacore tuna can be consumed per week. Finally, those who consume fish from local lakes, rivers, and coastal areas were encouraged to check local advisories for guidance and, if none were available, to eat ≤ 6 oz per week of such fish and to avoid eating any other fish that week.

With regard to the distribution of mercury burdens within the US population, in the NHANES 1999 survey, women of child-bearing age (16-49 years) had a median hair mercury level of 0.2 ppm, although 8 to 10% of women had levels that were consistent with mercury intake above the RfD (1.2 ppm).12 Moreover, the strong influence of fish consumption on mercury burden was evident. More than 25% of women who reported consuming 9 or more fish meals per month had a burden that indicated mercury intake above the RfD, as did 10 to 25% of women who reported consuming 5 to 8 fish meals per month.13

Overall, the consensus view of "how much mercury is too much" has declined steadily since 1970 and has been accompanied by concomitant changes in the regulatory standards. It can be expected that this process will continue as additional research, using more sensitive methods of exposure and outcome assessment, is conducted.

LEAD

Lead, a useful metal that has been mined and smelted by humans for millennia, has been recognized as a potent toxicant for nearly as long. Interestingly, recognition of children as the subgroup of the population that is at greatest risk from excess exposure occurred only a little more than a century ago. Voluminous research conducted over the past half century has catalogued a wide array of processes by which lead produces neurotoxicity, including apoptosis, excitotoxicity, impaired cellular energy metabolism, impaired heme

synthesis, oxidative stress, lipid peroxidation, impaired first and second messenger systems, and many others.14 The relative importance of specific mechanisms of neurotoxicity is likely to be dose-dependent. At the lower doses characteristic of community-level exposures, it is thought that lead's disruption of the role of neurotransmitter systems in the sculpting of the brain is important. Specifically, by increasing the slow tonic (normal basal) release of neurotransmitter and inhibiting the release evoked by depolarization, the presence of lead in the neuronal environment increases the level of background "noise" in excitatory synapses, disrupting activity-dependent plasticity at developing synapses, including the process by which neuronal connections are selectively pruned (eg, organization of "whisker-tobarrel" sensory pathway in rodents).15

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As was the case with methylmercury, the view of "how much lead is too much" has declined dramatically since the 1960s, when pediatric textbooks identified a blood lead level in a child of 60 micrograms/deciliter (µg/dL) as the upper limit of "normal." In retrospect, this seems remarkable, given that the risk of encephalopathy is increased at 100 µg/dL and death occurs at 150 µg/dL. It is somewhat less surprising, however, in light of the high prevalence, at that time, of broad lead levels of 40 µg/dL or more among poor children living in inner cities.16 Based on a steady accretion of epidemiological studies documenting adverse effects at lower and lower levels, the value used to define "elevated" was decreased to 40 in 1971, 30 in 1975, 25 in 1985, and 10 in 1991. Fortunately, recognition that levels formerly regarded as safe were, in fact, associated with increased risk of adverse effects resulted in governmental initiatives that produced rapid and substantial declines in children's exposures to lead. The most important of these were a ban on the amount of lead used in residential paints and the elimination of the use of lead as a gasoline additive. Whereas the median blood lead level of US preschool children was 15 µg/dL in the late 1970s, with 88% having a level of 10 µg/dL or greater, this level now stands at under 2 µg/dL, with 2% having a level greater than 10. This still represents an unacceptably large number of children with exposure to a toxicant that is known to reduce cognitive function. Moreover, in many US cities, the prevalence of levels greater than 10 µg/dL still exceeds 10%, primarily among poor minority children, reflecting the continued socioeconomic bias in the occurrence of this disease.

Many public health advocates are urging the US CDC to reduce once again the definition of an elevated blood lead level. Much of the

impetus for this is provided by the results of analyses that pooled the data from a set of 7 prospective studies conducted in four countries.17 These analyses indicated that the inverse association between children's blood lead levels and their IQ scores holds even at levels below 10 μg/dL. Moreover, it appears that the slope of the inverse association is even steeper below 10 μg/dL than it is above 10 μg/dL. In these pooled data, over the range of 1 to 30 μg/dL, children's IQ scores declined 9.2 points, but as much as 6.2 points of this decline occurred in the range of 1 to 10 μg/dL.

The importance of the magnitude of the changes in children's cognitive function observed in association with exposures such as lead is frequently questioned. How important is, for example, a shift of several points in IQ, a change that would likely not be readily discerned. In part, this perspective reflects a failure to acknowledge the distinction between individual and population risk. Whereas a loss of 5 points in an individual's IQ might be inconsequential, a shift of 5 points in the mean IQ score within an entire population (eg, from 100 to 95) would have large implications. If the other characteristics of the IQ distribution remain constant, such a mean shift implies a doubling of the number of individuals with scores 2 or more standard deviations below the mean and a halving of the number with scores 2 or more standard deviations above the mean.18

Even for chemicals as well-studied as lead, detailed answers are lacking to many important questions of toxicological as well as public health importance. Among the unresolved issues are the functional form of the dose-effect relationship, particularly whether it is linear or supralinear at levels below 10 μg/dL, the critical window(s) of vulnerability (prenatal, early postnatal, concurrent, cumulative exposure), the factors that influence prognosis of lead-associated injuries, characteristics of the "behavioral signature" injury and its dependence of dose, timing, and chronicity, and a unified understanding of neurobiological mechanisms of injury.

An issue that has stimulated particular concern is chemical exposures that might disrupt endocrine-mediated processes by mimicking or antagonizing natural hormones, so-called "endocrine disrupting chemicals." It is known, for example, that gonadal hormones are important in producing sex-specific regional differentiation in the brain and the expression of sexually-dimorphic reproductive and nonreproductive behaviors. Exposure to some environmental chemicals interferes with these modulatory effects of sex hormones on brain development and behavior. Some pesticides and phthalates (plasticizers) are anti-androgenic, with developmental exposure of male rats producing a feminization of social behavior (play).19 Bisphenol A, a chemical used in the food industry and dentistry, is estrogenic, with developmental exposure of female rats producing masculinization of play and sociosexual exploration.20

It is a consistent observation that, at chemical burdens typical of children's environmental exposures, there is substantial interindividual variability in the response of individuals at a given level of exposure. In order to make risk assessments as accurate as possible, it is important to understand all the sources of this variability. It could result from imprecision (ie, misclassification) in the measurement of the exposure biomarker or in the extent to which it characterizes the dose at the critical target organ, or represent the most appropriate exposure averaging time for the health endpoint of interest (i.e., concurrent, age-specific, cumulative). For example, in the case of lead's neurotoxicity, we are most interested in the amount of lead in the brain, the critical target organ. Because this cannot be measured in humans, the exposure biomarker most often used is blood lead, yet only about 5% of an individual's total body burden is in the blood compartment. Moreover, most of the lead in blood is tightly bound to erythrocytes, whereas the most important toxicologic fraction of the blood compartment is the lead in plasma, due to its access to soft tissues such as the brain. Thus, using blood lead as an index of exposure is likely to result in a considerable, but unknowable, amount of exposure misclassification, and thus likely underestimation of lead's neurotoxicity. Similarly, with respect to methylmercury, the exposure biomarker most commonly measured is hair mercury, a compartment that is a considerable toxicokinetic distance from the brain, which is the compartment of greatest interest. Another component of variability is likely to be true variability in response, reflecting biological processes that are not captured by the terms included in our statistical models.

Some of the apparent inter-individual variability in response almost certainly reflects factors that systematically render some more vulnerable and others less vulnerable to toxicant exposures. One class of such factors is genetic polymorphisms that modify the association between external dose and internal biomarkers (toxicokinetic variability) or between the biomarkers and health outcomes (toxicodynamic variability). Few such polymorphisms have been identified, however. In the case of lead, studies have shown that individuals with a variant allele of the heme pathway enzyme, amino levulinic acid dehydratase, have higher blood lead, but lower bone lead levels, and, at a given lead level, have reduced renal function and an increased risk of amyotrophic lateral sclerosis. Individuals with a variant allele of the vitamin D receptor have higher blood lead levels and increased blood pressure. In children who carry this allele, the slope of the association between floor dust lead and blood lead is steeper than it is among children with the wild-type allele. The E4 allele of apolipoprotein has been shown to increase the neurobehavioral toxicity of lead in adults.21 Other alleles that have been investigated are nitric oxide synthase and the HFE protein (hemochromatosis).

Non-genetic factors that appear likely to influence response to toxicant exposures include nutritional status and social characteristics. Again, most of the work exploring these issues has been conducted on lead. Calcium and iron are known to influence lead absorption and might influence toxicity as well. Animal and human studies suggest that being reared in an environment that provides less cognitive stimulation increases the toxicity of lead. In one study, rats were lead-exposed during gestation and lactation, and their spatial learning was assessed using a water maze at 50 days of age. Some of the rats were raised in groups in cages that contained objects to explore ("enriched"). Others were raised alone in empty cages ("isolated"). The performance of the enriched, lead-exposed rats was indistinguishable from that of the enriched, non-exposed rats, but the isolated, non-exposed rats learned more slowly than either of these groups. The isolated, lead-exposed rats did not show any improvement in performance over the learning trials. The better performance of the enriched lead-exposed rats was accompanied by changes in brain biochemistry; increased induction of BDNF mRNA expression in the hippocampus was observed, as well as recovery of deficits in gene expression of the NR1 subunit of NMDAR (N-methyl d-aspartate receptor) in the hippocampus (CA1-CA4) and granule cell layer of dentate gyrus.22 In children, observational studies have shown that the magnitude of neurobehavioral deficits evident at a given blood lead level is greater among children who are socioeconomically disadvantaged,23 and that the extent of recovery from early deficits is greater among more socioeconomically advantaged children. 24 Some evidence suggests that the effect of a chemical exposure on brain plasticity might provide a sensitive index of toxicity. For instance, in rats, prenatal exposure to methylazoxymethanol acetate reduced the magnitude of their response to an enriched postnatal environment, operationalized as the change in the thickness of the occipital cortex. The dose needed to produce the same reduction in cortical thickness directly was >10 mg/kg, but a dose of 1 mg/kg was sufficient to observe the same reduction in the capacity for experience-dependent cortical plasticity.25

Achieving success in characterizing the extent and the bases of inter-individual variability in susceptibility to toxicants will permit significant implications for the risk assessments of the toxicants. It will allow for a quantitative rather than qualitative evidence-based characterization of relative subgroup susceptibility, which will enable risk assessors to move beyond the practice of setting exposure standards

by dividing a “no observed effect level” by ad hoc “one size fits all” uncertainty factors (e.g., 10, 100) in order to provide a margin of safety for susceptible subgroups.

ENVIRONMENTAL CHEMICAL EXPOSURES AND PSYCHIATRIC MORBIDITY

In recent years, investigators have begun to expand the scope of the health endpoints evaluated as potential consequences of toxicant exposures in children to include non-cognitive brain-based disorders. The psychiatric sequelae of high-dose, usually occupational, exposure of adults to various metals have long been recognized. The syndrome of erethism, resulting from exposure to inorganic mercury and the origin of the phrase, “mad as a hatter,” is characterized by irritability, excitability, emotional lability, extreme shyness and avoidance of strangers, sudden anger, fatigue, memory loss, insomnia, and, in severe cases, to depression, manic depression, hallucinations, delusions, and suicidality. Manganese exposure is associated with mania, insomnia, hallucinations, aggression, incoherent speech, inappropriate affect, and emotional lability, while trimethyl tin exposure is associated with alternating bouts of rage and depression, sleep disturbance, fatigue, memory loss, and apathy.

Most of the epidemiological work on toxicants and psychiatric morbidity has focused on lead. In adults, case studies have suggested associations between high-dose exposure and depression, and also affective or schizophreniform psychosis. A facility in which tetraethyl lead was manufactured was known as the “House of Butterflies” because of the hallucinations suffered by workers. In occupational studies, greater depression, irritability, interpersonal conflict, fatigue, anger, tension, and decreased libido have been noted in lead workers, compared to controls. Environmental or pharmacologic interventions that reduce workers’ blood lead levels have sometimes been found to reduce the severity of their mood disturbances. Finally, some reports suggested improvements in the clinical status of psychiatric patients following chelation therapy.26-28

A recent case-control study suggested that higher levels of in utero lead exposure, reflected in higher levels of amino levulinic acid in archived samples of maternal serum from the 2nd trimester of pregnancy, were associated with an increased risk of schizophrenia.29

It is on the association between lead exposure and behavior disorders in children that the greatest data are available. Many studies have demonstrated that higher exposures are associated with increased distractibility, impulsivity, poor organization skills, inability to follow directions, low frustration tolerance, and a lack of persistence.30-32 Recently, several studies have shown that adolescents with higher exposures are at higher risk of increased aggression and juvenile delinquency, 33-35 with some speculating an association between lead and homicide.36,37 Experimental studies with animal models support the

plausibility of this association. The threshold eliciting predatory attack behavior in cats decreased following a lead challenge, increased during a washout period, and decreased in response to a second lead challenge.38 In another animal study, lead-exposed rhesus monkeys engaged in less play, particularly social play, than controls, and in more self-stimulation and fear grimacing.39 These impaired social interactions persisted after cessation of exposure.

TREATMENT FOR CHEMICAL-INDUCED MORBIDITIES

Given the accumulating evidence that environmental chemical exposures are contributing to neurodevelopmental morbidities in children, the issue of whether these morbidities are amenable to treatment has become of paramount importance. Chelating agents have been administered to lead-poisoned children since the 1950s in spite of little published evidence that such interventions were effective. It was only in 2001 that the results of the first randomized trial of chelation were published, and the results were disappointing. This was the Treatment of Lead-Exposed Children (TLC) trial, which enrolled 780 12 to 33 month olds with a baseline blood lead level of 20 to 44 μg/dL. Children were randomized to receive either a placebo or the oral chelator succimer. Although blood lead level declined significantly faster in the succimer group following initiation of treatment, after one year the mean blood lead levels in the two groups were equivalent. Moreover, the scores of the two groups on a large number of neurodevelopmental tests were not significantly different three years following treatment40 or at 7 years of age.41 The findings from observational studies of children with lower exposures to lead are consistent with those of the TLC trial in suggesting that the neurodevelopmental morbidities are persistent and possibly permanent.42-44 The clear implication is that a primary prevention strategy is necessary if lead-associated morbidity is to be reduced. Waiting to identify and treat children who have been overexposed will not be effective.

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SUMMARY

The potential exists for developmental exposure of children to myriad chemicals, many of which are known to be neurotoxic. Some, such as the organophosphate pesticides, are specifically designed to attack the central nervous system. Despite the known and suspected risks associated with such exposures, critical aspects of the doseresponse relationships are unknown or, at best, poorly characterized for the overwhelming majority of chemicals. Among the major knowledge gaps for most chemicals are the critical window(s) of vulnerability, the threshold or “no observed adverse effect level,” and the

host/environmental characteristics that modify individual vulnerability. Investigation of the role of genetic polymorphisms in determining vulnerability has barely begun. In the real-world, children are not exposed to a single chemical at a time but to complex mixtures of chemicals, and we have only a minimal understanding of the way in which exposures might interact with one another. Effective medical/environmental treatments for the adverse effects associated with chemical exposures are largely unknown, rendering primary prevention of exposure the most effective strategy for protecting children.

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