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Mercury-Contaminated Sediments Affect Amphipod Feeding

Mirco Bundschuh · Jochen P. Zubrod · Frank Seitz · Michael C. Newman · Ralf Schulz

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Abstract A 125-mile reach of the South River, Virginia, was contaminated with mercury during the first half of the 20th century. As increased concentrations of mercury have persisted, researchers have carefully studied its distribution in the river biota and estimated associated risks. The present study evaluated the influence of mercury on feeding rate and uptake by the amphipod *Hyalella azteca*. The test organisms were exposed for 7 days with leaf discs to reference and contaminated field sediment during the preliminary experiment and additionally to Sedimite (a commercial mercury-sequestering agent) amended sediments during the final experiment. The preliminary experiment demonstrated a decreased feeding rate (approximately 35%) of *H. azteca* in sediment from a contaminated site relative to sediment from a reference site. The test design of the final experiment took advantage of the knowledge gained in the preliminary experiment by increasing the number of replicates, which decreased the type II error rate. First, the results of the final experiment confirmed the results of the preliminary experiment by again demonstrating differences in the feeding rate of approximately 35% between reference and contaminated sediment. Second, the results indicated a lower feeding rate in reference sediment in the presence of Sedimite. Third, an opposite tendency, although not significant, was apparent for Sedimite-amended contaminated sediment. Thus, Sedimite appears to decrease sediment quality, whereas this conclusion is based on the feeding rate of *H. azteca*. However, Sedimite and its value as a mercury-sequestering agent requires further evaluation.

M. Bundschuh (✉) · J. P. Zubrod · F. Seitz · R. Schulz
Institute for Environmental Sciences, University of Koblenz-Landau, 76829 Landau, Germany
e-mail: bundschuh@uni-landau.de

M. C. Newman
Virginia Institute of Marine Science, College of William & Mary, Gloucester Point, VA 23062, USA

The mercury contamination of sediments in the South River, below Waynesboro, Virginia, can be attributed to industrial activities during the first half of the 20th century (Carter 1977). Although its release was stopped decades ago, mercury sediment concentrations remain high (Bergeron et al. 2007, Table 1). Due to mercury's persistence and its capacity to cause serious harm to wildlife (Mergler et al. 2007) as well as humans (Scheuhammer et al. 2007), research on the South River basin has mainly focused on mercury bioaccumulation and biomagnification. Fish that prey on benthic invertebrates downstream of the former point source in Waynesboro show increased concentrations of mercury (Tom et al. 2010). Mercury concentrations in turtle blood were recently linked to their respective feeding ecology (Bergeron et al. 2007), with those feeding on invertebrates and fish at contaminated sites showing substantially higher mercury body burdens. Moreover, models predict substantial biomagnification of mercury in aquatic biota at six locations at the most contaminated reach of South River (Tom et al. 2010).

Remediation technologies have been developed to decrease the bioavailability and concentrations of toxicants, such as mercury, in sediments. Dredging has been shown to decrease the concentration of polychlorinated biphenyls in harbour sediments, although sublethal effects on *Hyalella azteca* were still observable (Kemble et al. 2000). Acid neutralization with limestone alone and in combination with phosphorus was shown to decrease toxicity of sediments from an acid mine pit lake (Neil et al. 2009). Remediation of sediment with Sedimite was recently developed as an in situ technique to decrease the bioavailability of polyaromatic

Table 1 Mean loss of weight on ingestion ($n = 3$) and median mercury concentrations ($n = 5$) in treatments of the preliminary experiment[a]

Proportion of reference sediment (%)	Mean ±95% CI loss of weight on ignition (%)	Median ±95% CI Hg concentration (mg/kg dry weight)
100	10.61 ± 0.85	0.009 ± 0.007
75	NA	5.9 ± 1.4
50	NA	10.3 ± 1.7
25	NA	13.5 ± 5.1
0	10.64 ± 0.73	30.1 ± 26.9

NA not analysed

[a] The 100% and 0% treatments were also used for the final experiment

hydrocarbons, polychlorinated biphenyls, and mercury by forming agglomerates (Menzie 2010).

However, knowledge concerning non-bioaccumulative-related ecotoxicologic implications of both mercury sediment contaminations in the South River basin and sediment remediation techniques, such as Sedimite addition, is limited. The present study aimed to assess the mercury toxicity of sediments from the South River as well as the potential impact of Sedimite added to these sediments. The amphipod species *H. azteca*, which is a widely distributed leaf-shredding invertebrate (Hargrave 1970) in North America (Bousfield 1958), and frequently used in sediment toxicity testing (Borgmann and Norwood 1997b), was chosen as the test organism. Compared with the majority of studies published on sediment toxicity for *H. azteca* that measured bioaccumulation and mortality (Borgmann and Norwood 1997a, b), we investigated feeding rate on leaf material. This end point is known to be sensitive, robust, and ecologically relevant with regard to the feeding rate of the amphipod genus *Gammarus* (Maltby et al. 2002). Current shortcomings of hypothesis testing were avoided by employing confidence interval (CI) testing, as recently recommended by various investigators (e.g., DiStefano 2004; Nakagawa and Cuthill 2007; Newman 2008). This level of testing allows examination of the following questions: (1) Is there any observable difference in feeding behavior between reference and contaminated sediments? (2) Does exposure to Sedimite change feeding behavior? (3) Does the addition of Sedimite to contaminated sediment decrease toxicity measured as feeding rate of *H. azteca*?

Materials and Methods

Sediments

Sediments, more precisely fine settled particulates, were gathered as composite samples in September 2009 using a pipette from the upper 1 to 2 cm of the sediment layer at 10 randomly chosen spots within approximately 10 m^2 of the bank-side riverbed within the South River, VA. The reference site was located at North Oak Road, a site upstream of the historic Waynesboro discharge site. The mercury-contaminated sediment was collected downstream at the Dooms, VA, city park. Although freezing may affect the physicochemical and ecotoxicologic properties of sediments, both field sediments were freeze dried for approximately 3 days using a Freezone 4.5 freeze-dryer (Labconco, Kansas, MO) to ensure a well-defined exposure in terms of the amount of sediment and the sediment particle size. The sediments were then pulverized, and only particles passing a 0.67-mm mesh Nytex screen were used. Sedimite was also crushed and passed through the same mesh screen before being introduced into the sediments for the final experiment.

Both the reference and contaminated sediments were additionally analysed for their organic matter content. Therefore, mean loss of weight was measured after ignition at 550°C for 4 h (Table 1).

Leaf Discs and Test Organisms

The leaf discs were prepared as described in detail in Bundschuh et al. (submitted). Briefly, black alder leaves (*Alnus glutinosa* L. Gaertn.) were collected shortly before leaf fall in October 2008 from a group of trees near Landau, Germany (49°11′N; 8°05′E) that had no history of mercury contamination and stored frozen at −20°C until further use. After thawing, discs (2.0-cm diameter) were cut from each leaf with a cork borer. To establish a microbial community on the leaf discs, discs were conditioned in a nutrient medium together with alder leaves previously exposed in the Rodenbach, Germany (49°33′N, 8°02′E). After a conditioning period of 10 days, the discs were dried at 60°C to constant weight (approximately 24 h) and weighed to the nearest 0.01 mg. Determination of leaf disc dry weight ensured accurate measurement of the amphipods' feeding rate (*cp*. Maltby et al. 2002). After being soaked in tap water for 24 h, the leaf discs were assigned randomly to the vessels of the respective experiment.

Adult *H. azteca* with a mean (±95%CI) dry weight of 0.36 (±0.02) mg were obtained from a commercial supplier (Chesapeake Cultures, Hayes, VA) shortly before the start of each experiment. The test organisms were also randomly allocated to the test vessels of the respective experiment.

Feeding Trials

Both the preliminary and the final feeding trials were conducted in plastic 12-well plates that were placed in total darkness at an average temperature of 23°C (±1°C) in an

incubator. The different treatments and replicates were arranged randomly to the wells. Each well (area = 4.15 cm^2) contained 3 ml aged tap water, one leaf disc, one amphipod, and 150 mg sediment, resulting in a layer thickness of approximately 0.66 mm, and was treated as one replicate. Before the introduction of the leaf discs and *H. azteca*, the test system was given 24 h to facilitate the sedimentation of suspended particles. Five additional wells per treatment without any *H. azteca* were set up to account for any abiotic or microbial loss in dry weight of leaf discs during the feeding trial. After the study duration of 7 days, the test organisms were transferred into fresh aged tap water for another 24 h to facilitate gut clearance, which was necessary for accurate determination of mercury body burden (Neumann et al. 1999). Afterward, the leaf discs and *H. azteca* were dried and weighed as described previously.

For the preliminary experiment, *H. azteca* were exposed to different mixtures of sediments sampled from the contaminated and reference sites, resulting in five treatments containing 0%, 25%, 50%, 75%, and 100% of reference sediment, respectively. After passing the sediments through a 0.67-mm mesh screen, these treatments were achieved by mixing the sediments in the respective proportions according to dry weights (w/w%) for 10 min using a vortexer. Each treatment was replicated 20 times. Considering the feeding rate and its SD measured in the preliminary experiment, a power analysis for the final experiment was conducted based on type I and II error rates of 0.05. This analysis lead to a set up with 30 replicates per treatment for the final experiment, which was slightly different from the preliminary one because *H. azteca* were exposed to either 100% reference sediment or 100% contaminated sediment. Two additional treatments contained one of the two sediments plus Sedimite. If Sedimite addition was required, 16.42 mg were added onto the sediment surface. This application rate was based on the recommendations of the producer (Exponent Inc., Alexandria, VA), who suggested a field application rate of 2 to 3 kg/m^2. However, because Sedimite is mixed into the sediment by biological activity (Menzie 2010), the recommended application rate was adjusted for both the area (4.15 cm^2) and the thickness of the sediment layer in each well (0.66 mm). The latter was calculated based on the assumption that a biologically active sediment layer of 50 mm is representative for the South River. At the end of the final experiment, groups of 8 to 10 *H. azteca* were randomly pooled, leading to three replicate measurements of mercury body burden per treatment.

Mercury Analysis

Total mercury content in pooled sediment samples ($n = 5$) and test organisms ($n = 3$) was analyzed using a direct mercury analyzer (DMA-80; Milestone, Shelton, CT). Briefly, samples were introduced into the DMA and dried in an oxygen stream before combustion. Mercury vapor was collected on a gold amalgamation trap and quantified using atomic absorption spectrometry at a wavelength of 254 nm. Standard curves were created before analysis with standards purchased from the National Research Council Institute for National Measurement Standards (Montreal, Canada). Standard material Dorm-3 was used to generate calibration curves for amphipod body burdens and calibrations were checked with the standard material Tort-2. Standard reference material 2702 (National Institute of Standards and Technology, Gaithersburg, MD) was used to generate a calibration curve for analyzes of sediment mercury concentrations.

Data Analysis

The feeding rate was expressed as consumed leaf mass per mg dry mass per day of the test species (C) and was calculated as follows (Maltby et al. 2000):

$$C = \frac{L_b * (k) - L_e}{g * t}, \quad (1)$$

where L_b = initial dry mass of the leaf discs, L_e = final dry mass of the leaf discs, g = dry mass of *H. azteca*, t = feeding time in days, nd k = leaf change correction factor given by the following equation:

$$k = \frac{\sum \frac{L_{ob} - L_{oe}}{L_{ob}}}{n}, \quad (2)$$

where L_{ob} = initial dry mass of the leaf discs, L_{oe} = final dry mass of the leaf discs both measured in replicates without any *H. azteca* present, and n = number of replicates.

The statistical analyses were based on CI testing as described by Altman et al. (2000). Parametric CIs were used to judge significance in both experiments after underlying assumptions were met. Analysis was based on $\ln(x + 1)$ transformed values because the data of the preliminary experiment were log-normally distributed. After statistical analysis, values were back-transformed to display data on the original scale (Altman et al. 2000). If a normal distribution of data was not achievable using common transformations, nonparametric CIs were calculated to assess differences between medians. To display mercury concentrations in sediments, medians and their respective 95% CIs were derived by the Kaplan–Meier estimator because it can accommodate censored data (Helsel 2005). This method is described in detail by Altman et al. (2000). The statistics program R Version 2.10 (Lee 2009; Lemon et al. 2009; R Development Core Team 2009) was used to generate all statistical analyses and figures.

Results

The median sediment mercury concentrations measured in the preliminary experiment increased with a decreasing proportion of reference sediment (Table 1). These concentrations are also appropriate for the final experiment because the same sediment field samples were used. At the end of both the preliminary and final experiment, not all *H. azteca* were still recoverable from their respective wells, most likely due to mortality and subsequent decomposition, resulting in a final number of replicates of at least 16 in the preliminary and 27 in the final experiment, respectively. During the preliminary experiment, the test organisms had more than a 35% decrease of mean feeding rate in treatments containing ≤50% reference sediment compared with the treatment containing 100% reference sediment (Fig. 1). However, these differences were only statistically significant for treatments containing 50% (difference between geometric means 0.29 mg/mg animal/d [95% CI 0.06 to 0.58]) and 25% (difference between geometric means 0.23 mg/mg animal/d [95% CI 0.02 to 0.49]) reference sediment, respectively. *H. azteca* exposed to 0% reference and thus 100% contaminated sediment showed a 37.5% (but not a statistically significant) difference (difference between geometric means 0.21 mg/mg animal/d [95% CI −0.01 to 0.49]). Based on the results of the preliminary experiment, a power analysis (type I and II errors = 0.05; effect size = 35%) suggested 30 replicates for the final experiment regarding the feeding rate of *H. azteca*. The decreased feeding of 35% of individuals in sediments taken from the contaminated site compared with sediments from the reference site was significant (difference between medians 0.193 mg/mg animal/d [95% CI 0.059 to 0.332]; Fig. 2). Mercury body burden was significantly increased (difference between means 157.20 µg/kg dry weight [95% CI 112.75 to 201.65]) in sediment from the contaminated site (Fig. 3). The addition of Sedimite to the reference sediment resulted in a decreased feeding rate relative to the unamended reference sediment (difference between medians 0.264 mg/mg animal/d [95% CI 0.091 to 0.450]). Moreover, the feeding rate was even lower than the level of those organisms exposed to sediment taken from the contaminated site (Fig. 2). In addition, the mercury body burden of *H. azteca* was significantly increased (difference between means 115.14 µg/kg [95% CI 43.24 to 187.05]) in reference sediment treated with Sedimite (Fig. 3). A slight but not statistically significant increase in median feeding rate was apparent (difference between medians 0.047 mg/mg animal/d [95% CI −0.270 to 0.190]) if contaminated sediment was compared with the same sediment with Sedimite added (Fig. 2). In contrast, the mercury body

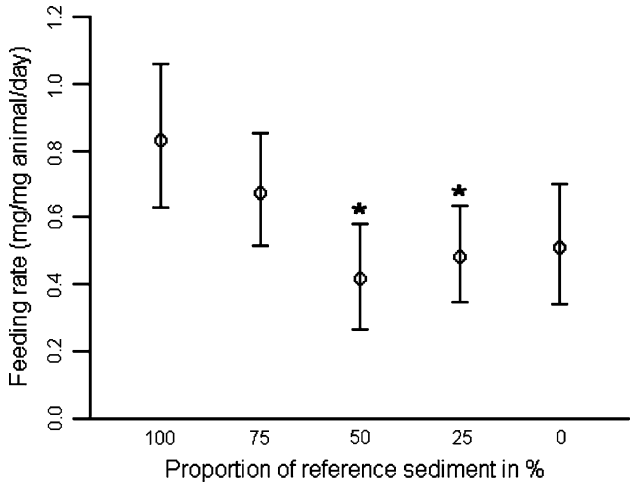

Fig. 1 Mean (with 95% CI) feeding rate of *H. azteca* exposed during the preliminary experiment to sediment mixtures containing different ratios of sediments taken from the reference and contaminated sites. * Significant differences compared with the treatment containing 100% reference sediment as assessed by using CI testing on log-transformed data ($n \geq 16$)

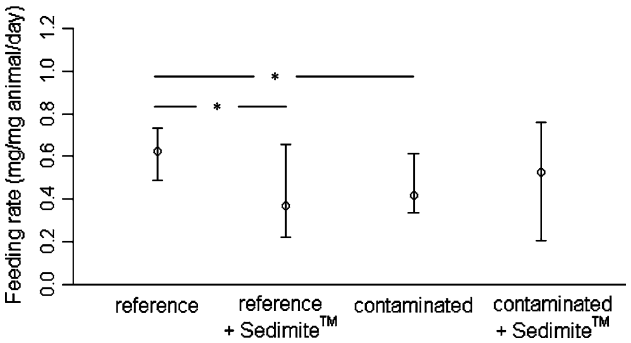

Fig. 2 Median (with 95% CI) feeding rate of *H. azteca* exposed to reference and contaminated sediments with or without Sedimite addition. * Significant differences between treatments as assessed using CI testing ($n \geq 27$)

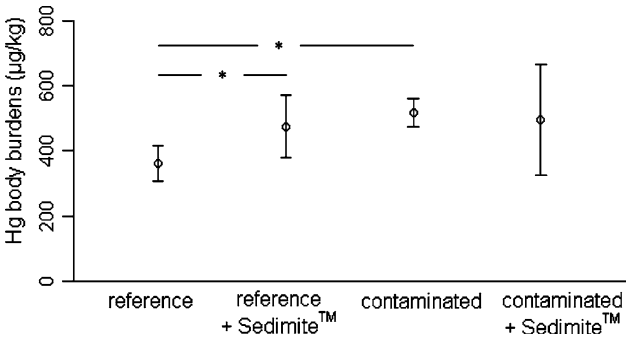

Fig. 3 Mean (±95% CI) mercury body burden of *H. azteca* exposed to reference and contaminated sediments with or without Sedimite addition. * Significant differences between treatments as assessed using CI testing ($n = 3$)

burden decreased slightly (difference between means 22.92 µg/kg [95% CI −90.18 to 136.01]; Fig. 3).

Discussion

The results of the preliminary experiment provide direct evidence of ecotoxicologic effects of mercury-contaminated sediment sampled from the South River downstream of Waynesboro. The feeding rate of *H. azteca* in sediment mixtures containing ≤50% reference sediment, which is equivalent to a median (±95% CI) mercury concentration of 10.3 ± 1.7 mg/kg sediment or higher (Table 1), decreased materially. A statistically significant difference, however, was not detected in sediment containing 0% reference sediment, although the feeding rate was meaningfully decreased by approximately 35%. This lack of statistical significance might be driven mainly by the stringent adjustment of the type I error in consequence of the number of statistical comparisons conducted, which increases the likelihood of type II error (Perneger 1998). This problem was addressed, as described previously, by a higher number of replicates used for the final experiment, which decreased the likelihood of type II error. Despite these limitations, the results of the preliminary experiment are consistent with an earlier publication of Winger et al. (1993), who investigated sediments from Brunswick estuary, Georgia. They proved a significant decrease in the feeding rate of *H. azteca* exposed to sediments containing 17.8 and 24.7 mg mercury/kg sediment, respectively. However, the sediments used by Winger et al. (1993) also contained nonnegligible concentrations of other heavy metals as well as polycyclic aromatic hydrocarbons and polychlorinated biphenyls. Because, compared with the publication by Winger et al. (1993), the sediments used in the present study seem to be primarily contaminated by mercury (unpublished data, summer 2005), a direct link between the sediment mercury concentration and the effects displayed can be assumed. However, one might argue that a higher content of organic material in the sediment from the contaminated site and not the mercury was the driving factor for the effects observed by making the sediment itself an appropriate food source for the test organisms. However, differences in mean loss of weight after ignition was negligible (Table 1).

To our knowledge, the final experiment is the first to investigate sublethal ecotoxicologic effects of Sedimite addition to (un)contaminated sediments. Three questions were addressed by the test system applied. First, the final experiment confirmed the results of the preliminary experiment by again displaying an average decrease in feeding rate (= effect size) of approximately 35% with exposure to sediment from the contaminated site; however, the decrease was statistically significant for the final experiment. This demonstrated that the applied bioassay delivers reproducible and robust results with regard to the effects sizes displayed. Second, the addition of Sedimite to the reference sediment resulted in a decreased feeding rate. This might have been caused either by a direct impact of Sedimite on the test organisms or due to the ingestion of Sedimite instead of the leaf material. That the amphipod *H. azteca* also feeds on Sedimite was confirmed by another experiment where the gut exhibited a deep black color when amphipods were exposed to Sedimite in clean aged tap water (Fig. 4a; starved amphipods exposed to aged tap water solely showed clear guts [Fig. 4c]). However, in some replicates, Sedimite also partly covered the amphipods' integument (Fig. 4b). Thus, *H. azteca* exposed to sediments treated with Sedimite might have undergone additional stress because adhesion of particles may have physical implications, such as the loss of mobility (Baun et al. 2008a), which finally was expressed as decreased feeding rate. Because this was not observed in amphipods during the feeding trial of the final experiment, direct effects on feeding were assumed to be negligible. Third, Sedimite addition to the mercury-contaminated sediment increased the median feeding rate of *H. azteca* from 0.416 to 0.525 mg/mg animal/d, which is not statistically significant. Mercury body burdens also provided insight, given that the mercury body burden of *H. azteca* exposed to the reference sediment in the present study is of the same

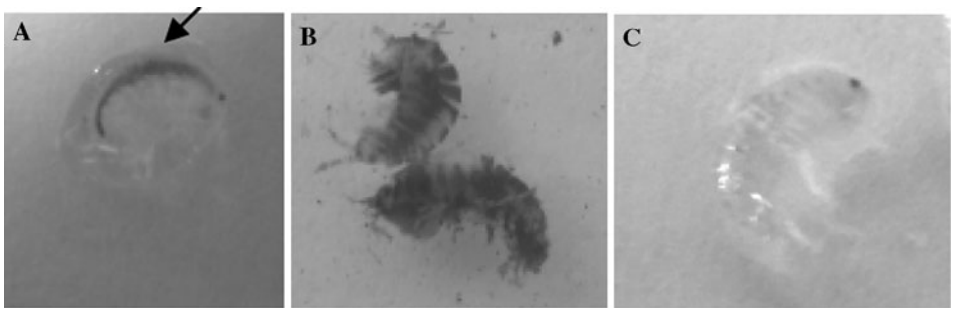

Fig. 4 *H. azteca* fed Sedimite showing a black gut as indicated by the arrow (**a**) and covered with it (**b**). Starved *H. azteca* in contrast did not show any black discoloration (**c**)

order of magnitude as those reported in other crustacean species sampled from a reference lake (Gorski et al. 2003). First, the mercury body burden of the amphipods was >40% higher in sediment taken from the contaminated site. Such an increase can be expected because mercury has a strong affinity for organic matter, such as leaf material (Lawrence and Mason 2001), which was ingested by the test organisms. However, Borgmann et al. (1993) observed a >200-fold increase in *H. azteca* body burden after 10 weeks of exposure to water-dissolved mercury. This is in agreement with the present study's results. The deviation regarding the effect sizes, however, is most likely driven both by the longer study duration and by the differing exposure routes. Second, the higher content of mercury in the amphipods exposed to Sedimite-amended reference sediment compared with the unamended one seems to be caused by a shift in feeding strategy. As *Daphnia magna* ingests fullerenes (Baun et al. 2008b) comparable effects can be assumed in the present study where *H. azteca*, as deposit-feeding (Hargrave 1972) and leaf-shredding organism, has possibly ingested both leaf material and Sedimite (Fig. 4a). This shift might have resulted in an increased mercury body burden of the test organisms that assimilated mercury from Sedimite, which therefore might have agglomerated mercury from the sediment. Third, *H. azteca* exposed to Sedimite-amended contaminated sediment did not exhibit a shift in mean mercury body burden, which is in accordance with the insignificant differences between both treatments regarding feeding rate (Fig. 2).

In conclusion, the present study demonstrates that relative impairment (= effect size) in the feeding rate of leaf-shredding amphipods, i.e. *H. azteca*, is a reproducible end point by which to assess the ecotoxicologic effects of (mercury) contaminated field sediment (see also Maltby et al. 2002). Although the addition of Sedimite produced inconsistent results, its potential to decrease bioaccumulation and toxicity of mercury from contaminated sediments could not be supported; rather, increased toxicity was indicated. Therefore, further experiments are necessary to assess the influence of Sedimite treatment on both the fate and toxicity of mercury to invertebrates. In addition, adsorption of mercury onto Sedimite and leaf material, followed by an evaluation of toxicokinetics and -dynamics within test organisms, must be assessed. Furthermore, experiments similar to those described in the present study should be used to assess the influence of Sedimite on both feeding rate and mercury body burden after different periods of aging within sediments by also taking field-relevant exposure scenarios into account.

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