Environ Geochem Health
https://doi.org/10.1007/s10653-021-00924-z

SHORT COMMUNICATION

Chronic exposure to volcanic gaseous elemental mercury: using wild Mus musculus to unveil its uptake and fate

R. Camarinho · A. Navarro-Sempere · P. V. Garcia · M. García · Y. Segovia · A. S. Rodrigues

Received: 21 November 2020 / Accepted: 1 April 2021

Abstract Volcanoes are a natural source of gaseous elemental mercury (GEM) (Hg^0). Monitoring GEM releases of volcanic origin has been widely studied; however, few studies have been performed about the biomonitoring of species exposed to GEM, rendering an unknown risk to the worldwide populations living in the vicinity of an active volcano. In this pilot study, we used *Mus musculus* as a bioindicator species to understand to what extent lungs are the main route of mercury uptake in populations chronically exposed to active volcanic environments. Autometallographic silver protocol was used to detect mercury deposits in the histological lung slides. Abundant mercury deposits were found in the lungs of specimens captured at the site with volcanic activity (Furnas Village, S. Miguel Island—Azores). The presence of mercury in the lungs could represent not only hazardous effects to the lung itself but also to other tissues and organs, such as brain and kidneys. This study confirms that the main uptake route for GEM is the lungs and that, even at very low concentrations in the environment, a chronic exposure to Hg^0 results in its bioaccumulation in the lung tissue. These results reinforce that biomonitoring studies should be

R. Camarinho · P. V. Garcia · A. S. Rodrigues (✉)
Faculdade de Ciências E Tecnologia, Universidade Dos Açores, Rua da Mãe de Deus, Apartado 1422, 9501-801 Ponta Delgada, Açores, Portugal
e-mail: Armindo.S.Rodrigues@uac.pt

R. Camarinho
e-mail: Ricardo.AD.Camarinho@uac.pt

P. V. Garcia
e-mail: Patricia.V.Garcia@uac.pt

R. Camarinho · A. S. Rodrigues
University of the Azores, 9501-801 Ponta Delgada, Portugal

A. Navarro-Sempere · M. García · Y. Segovia
Department of Biotechnology, University of Alicante, Apart 99, 03080 Alicante, Spain
e-mail: alicia.navarro@ua.es

M. García
e-mail: m.garcia@ua.es

Y. Segovia
e-mail: yolanda.segovia@ua.es

P. V. Garcia
Centre for Ecology, Evolution and Environmental Changes/Azorean Biodiversity Group, University of the Azores, 9501-801 Ponta Delgada, Azores, Portugal

Published online: 16 April 2021

combined with monitoring classical approaches in order to better characterize the risks of exposure to Hg^0 in volcanic environments.

Keywords Autometallography · *Mus musculus* · Non-eruptive volcanism · Hydrothermal emissions · GEM · Hg^0 · Biomonitoring

Introduction

Mercury (Hg) is a toxic volatile metal mainly emitted from volcanic activity and geothermal systems, mostly as a gaseous elemental form (GEM = gaseous elemental mercury; Hg^0), and it represents $\approx$98% of the global atmospheric mercury (Bagnato et al., 2007; Schroeder & Munthe, 1998). GEM possesses $\approx$0.5–2 years' time in the atmosphere (Ariya et al., 2008) and due to this factor the Hg dispersion and contamination of surface waters occurs through wet and dry deposition (Morel et al., 1998), which then tends to accumulate in the ecosystems and enters the food chain (Zillioux et al., 1993).

Many studies have been performed regarding the monitoring of GEM releases of volcanic origin (Ariya et al., 2008; Bagnato et al., 2007, 2018; Schiavo et al., 2020; Schroeder & Munthe, 1998; Science for Environment Policy, 2017), and most of them did not reveal values superior to the safety thresholds recommended by the World Health Organization (WHO, 2000). However, few animal studies have been performed in the biomonitoring of GEM rendering these concentration values an unknown risk to the worldwide populations living near an active volcano (Beckers & Rinklebe, 2017). The Furnas volcano, located in S. Miguel island (Azores, Portugal), is responsible for the release into the environment of approximately 1000 t d^{-1} of carbon dioxide (CO_2) and other gases (Viveiros et al., 2010), as well of several metals (e.g., Hg, cadmium (Cd), copper (Cu), lead (Pb), rubidium (Rb) and Zinc (Zn)) (Delmelle & Stix, 2000). In 2018, Bagnato et al. monitored the GEM emissions from the Furnas Volcano, revealing that the total (fumarolic + diffusive) GEM output for a study area of 0,04 km^2 inside the volcano crater was 9,6 $\times$ 10^{-5} t d^{-1}; these authors considered that such values probably do not represent a hazard to the health of the exposed populations. Nevertheless, the recent study carried by Navarro-Sempere et al. (2020), working with two wild mice populations, revealed that the individuals living inside the crater of Furnas volcano (chronically exposed to active volcanic environment) presented much more Hg^0 deposits in the brain than the ones living far from this environment. The authors pinpoint the hazardous potential of such deposits in the development of neurological impairments. However, how occurs the uptake of the Hg^0 that reaches the central nervous system remains unclear. Therefore, the main objective of this short communication is to understand to what extent the lungs can be an important route of entry and fate of the volcanic GEM.

Material and methods

For this pilot study, *Mus musculus* was chosen as a bioindicator species. *Mus musculus* have proven to be a good bioindicator species in previous studies regarding chronic exposure to volcanogenic air pollution (Amaral et al., 2007; Camarinho et al., 2019; Navarro-Sempere et al., 2020).

Two mice were live captured in Furnas Village (site with exposure to hydrothermal emissions), and three mice were live captured in Rabo de Peixe Village (reference site, with no active or historical hydrothermal manifestations). The study location, volcanic characteristics and main hydrothermal emissions have been previously described in detail by Camarinho et al. (2019). The mice were euthanized with isoflurane, and the lungs were then removed *en-bloc* and processed for paraffin embedding and histology. Autometallography (AMG) procedure was executed according to the protocol of Danscher and Møller-Madsen (1985). Briefly, sections were deparaffined and rehydrated and then pre-treated with 1% potassium cyanide solution for 2 h (this will avoid unspecific bindings for the rest of the protocol), followed by a developer solution composed by 50% Arabic gum, citrate buffer, hydroquinone and silver nitrate for 60 min at 26° C in the dark. After several washes, the excess silver was removed by immersing the sections in Farner's Solution at 1% for 2 min. Finally, the slides were contrasted in hematoxylin, dehydrated, cleared and mounted with DPX. The slides were then screened for black silver deposits, representative of the silver precipitates of mercury deposits enhanced by the executed protocol.

Photomicrographs were taken with a Luminera Infinity Microscope Camera (Microsen, SCU, Madrid – Spain).

Results and discussion

A large abundance of Hg deposits was observed in the lungs of mice captured at Furnas Village, while no presence of Hg was observed in mice captured at Rabo de Peixe (Fig. 1). Furthermore, it is possible to observe an increase of alveolar space, typical of pulmonary emphysema and increased mononuclear leukocyte cells. Similar signs of lung injury have been previously reported by Camarinho et al. (2019) in mice from the hydrothermal environment.

Approximately 80% of inhaled GEM is absorbed via the lungs and retained in the body. Elemental mercury is poorly absorbed in the gastrointestinal tract (less than 0.01% in rats), although increased blood levels of mercury have been measured in humans following accidental ingestion of several grams of metallic mercury (Elinder et al., 1986). Skin absorption is insignificant in relation to human exposure to GEM (Hursh et al., 1989). Also, the biological half-life of inhaled mercury is about 60 days, and it is estimated to remain in the brain for 20 years (Rice et al., 2014; Rooney, 2014). The conspicuous presence of mercury deposits in the lung tissue of individuals living inside the crater of the active volcano confirms the inhalation as the main route for the entrance of GEM, as well as the lungs as target organs. A few minutes after inhalation, GEM enters the blood stream and is oxidized to mercuric mercury (Hg^{2+}) in the erythrocytes, which is considered to be the toxic form (Clarkson & Magos, 2006). Since that this mercuric form is not able to pass through the plasma membrane and leave the cell, it is reduced again to GEM, which can travel from cell to cell. Also, this oxidation process generates other mercury complexes (like methylmercury) that are dispersed thought the body (Shiavo, et al., 2020), with particular hazardous impacts on the liver, kidneys and brain (Azevedo, et al., 2012). Recent studies show that GEM can cross the blood–brain barrier and placental barrier (Clarkson & Magos, 2006; Pamphlett et al., 2019; Solan & Lindow, 2014). Furthermore, several studies have shown that mercury exposure is related with several neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, among others (Cariccio et al., 2019; Gray & Woulfe, 2015; Rahman et al., 2020). Thus, it is plausible that, as it was stated by Park and Zheng (2012) and Shiavo (2020), the inhaled GEM bioaccumulates in the lung and then enters the bloodstream causing toxicity to other organs such as kidney and brain. Regarding the later, Navarro-Sempere (2020) found much more mercury deposits in the brain blood vessels, white matter (of both brain cortex and cerebellum medulla) and some cells of the hippocampus dentate gyrus of mice chronically

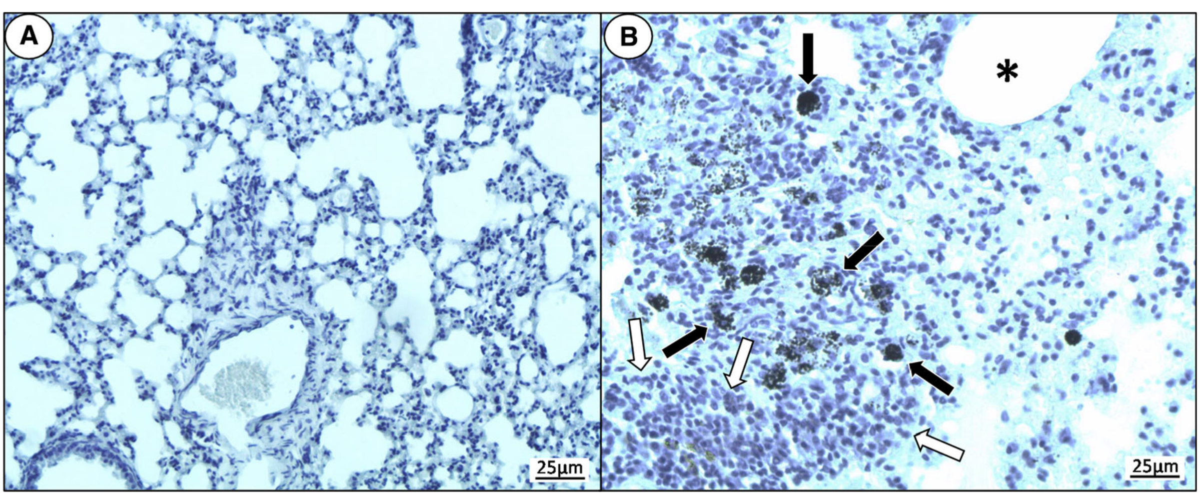

Fig. 1 Autometallography of lung histological sections of mice captured at Rabo de Peixe (**a**) and Furnas (**b**) processed according to the protocol of Danscher and Møller-Madsen (1985). The black arrows point to mercury deposits, white arrows point to mononuclear leukocyte infiltrate, black asterisk represents the increased alveolar space. Scale bar = 25 μm

exposed to the hydrothermal emissions of Furnas volcano than in the individuals not exposed. While the monitoring studies by Bagnato et al., (2018) and by Schiavo et al., (2020) consider that GEM values in hydrothermal areas are below the WHO's threshold (in Furnas the GEM output was 9,6 $\times$ 10^{-5} t d^{-1} for a study area of 0,04 km^2) and thus do not seem to represent an health hazard to the human population, the work of Navarro-Sempere et al. (2020) and the results here presented prove that mercury is bioavailable in the environment and that it bioaccumulates in the lungs and central nervous system, with completely unknown consequences to human health that need to be studied in the near future.

Conclusion

The results of this preliminary study confirm the inhalation as the main route of entry of GEM into the body and the lungs as important target organs for mercury bioaccumulation. Considering the hazardous effects of Hg in the lungs and the fact that Hg can remain in the brain for 20 years causing neuropathologies, this study draws attention to the need of biomonitoring the worldwide populations that live in the vicinity of active volcanoes.

Acknowledgements The authors would like to thank Paulo Melo for the field assistance in the capture of *Mus musculus.* Ricardo Camarinho is currently supported by a PhD fellowship grant (M3.1.a/F/048/2015) from Fundo Regional da Ciência (Regional Government of the Azores).

Data availability and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflicts of interest The authors declare that they have no conflicts of interest.

Ethical statement All procedures were carried out in strict accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS 123: directive 2010/63/EU) and the Portuguese Law Decree (DL 113/2012). This study was approved by the University of Azores ethics committee (10/2020).

References

Amaral, A., Cabral, C., Guedes, C., & Rodrigues, A. (2007). Apoptosis, metallothionein, and bioavailable metals in domestic mice (Mus musculus L.) from a human-inhabited volcanic area. *Ecotoxicology, 16*, 475–482. https://doi.org/10.1007/s10646-007-0156-y.

Ariya, P. A., Skov, H., Grage, M. M. L., & Goodsite, M. E. (2008). Gaseous elemental mercury in the ambient atmosphere: review of the application of theoretical calculations and experimental studies for determination of reaction coefficients and mechanisms with halogens and other reactants. *Advances in Quantum Chemistry, 55*, 43–55.

Azevedo, B. F., Furieri, L. B., Peçanha, F. M., Wiggers, G. A., Vassalo, P. V., Simões, M. R., et al. (2012). Toxic effects of mercury on the cardiovascolar and central nervous systems. *Journal of Biomedicine and Biotechnology*. https://doi.org/10.1155/2012/949048.

Bagnato, E., Aiuppa, A., Parello, F., Calabrese, S., Dalessandro, W., Mather, T. A., et al. (2007). Degassing of gaseous (elemental and reactive) and particulate mercury from Mount Etna volcano (Southern Italy). *Atmospheric Environment, 41*, 7377–7388. https://doi.org/10.1016/j.atmosenv.2007.05.060.

Bagnato, E., Viveiros, F., Pacheco, J. E., D'Agostino, F., Silva, C., & Zanon, V. (2018). Hg and CO2 emissions from soil diffuse degassing and fumaroles at Furnas Volcano (São Miguel Island, Azores): Gas flux and thermal energy output. *Journal of Geochemical Exploration, 190*, 39–57.

Beckers, F., & Rinklebe, J. (2017). Cycling of mercury in the environment: Sources, fate, and human health implications: A review. *Critical Reviews in Environmental Science and Technology, 47*(9), 693–794. https://doi.org/10.1080/10643389.2017.1326277.

Camarinho, R., Garcia, P. V., Choi, H., & Rodrigues, A. S. (2019). Overproduction of TNF-α and lung structural remodelling due to chronic exposure to volcanogenic air pollution. *Chemosphere, 222*, 227–234. https://doi.org/10.1016/j.chemosphere.2019.01.138.

Cariccio, V. L., Samà, A., Bramanti, P., & Mazzon, E. (2019). Mercury involvemente in neuronal damage and neurodegenerative diseases. *Biological Trace Element Research, 187*, 341–356.

Clarkson, T. W., & Magos, L. (2006). The toxicology of mercury and its chemical compounds. *Criticial Reviews in Toxicology, 36*(8), 609–662.

Danscher, G., & Møller-Madsen, B. (1985). Silver amplification of mercury sulfide and selenide: a histochemical method for light and electron microscopic localization of mercury in tissue. *Journal of Histochemistry and Cytochemistry, 33*(3), 219–228.

Delmelle, P., & Stix, J. (2000). Volcanic gases. In H. Sigurdsson, B. F. Houghton, S. R. McNutt, H. Rymer, & J. Stix (Eds.), *Encyclopedia of volcanoes.* (pp. 803–816). Academic Press.

Elinder, C. G., Gerhardsson, L., & Oberdoerster, G. (1986). Biological Monitoring of Toxic Metals—Overview. In T. W. Clarkson, L. Friberg, G. F. Nordberg, & P. R. Sager (Eds.), *Biological monitoring of toxic metals Rochester Series on Environmental Toxicity.* Springer.

Gray, M. T., & Woulfe, J. M. (2015). Striatal blood-brain barrier permeability in Parkinson's disease. *Journal of Cerebral Blood Flow and Metabolism, 35*(5), 747–750.

Hursh, J. B., Clarkson, T. W., Miles, E. F., & Goldsmith, L. A. (1989). Percutaneous absorportion of mercury vapour by Man. *Archives of Environmental Health, 44*, 120–127.

Morel, F. M. M., Kraepiel, A. M. L., & Amyot, M. (1998). The chemical cycle and bioaccumulation of mercury. *Annual Review of Ecology Evolution and Systematics, 29*, 543–566.

Navarro-Sempere, A., Segovia, Y., Rodrigues, A. S., Garcia, P. V., Camarinho, R., & García, M. (2020). First record on mercury accumulation in mice brain living in active volcanic environments: a cytochemical approach. *Environmental Geochemistry and Health*. https://doi.org/10.1007/s10653-020-00690-4.

Pamphlett, R., Kum-Jew, S., & Cherepanoff, S. (2019). Mercury in the retina and optic nerve following prenatal exposure to mercury vapor. *PLoS ONE, 14*(8), 1–16.

Park, J. D., & Zheng, W. (2012). Human exposure and health effects of inorganic and elemental mercury. *Journal of Preventive Medicine and Public Health Yebang Uihakhoe Chi, 45*(6), 344–352. https://doi.org/10.3961/jpmph.2012.45.6.344.

Rahman, A., Rahman, S., Uddin, J., Mamum-or-Rashid, A. N. M., Pang, M., & Rhim, H. (2020). Emerging risk of environmental factors: insight mechanisms of Alzheimer's diseases. *Environmental Science and Pollution Research, 27*, 44659–44672.

Rice, K. M., Walker, E. M., Jr., Wu, M., Gillette, C., & Blough, E. R. (2014). Environmental mercury and its toxic effects. *Journal of Preventive Medicine and Public Health Yebang Uihakhoe chi, 47*(2), 74–83. https://doi.org/10.3961/jpmph.2014.47.2.74.

Rooney, J. P. K. (2014). The retention time of inorganic mercury in the brain – a systematic review of the evidence. *Toxicology and Applied Pharmacology, 274*(3), 425–435.

Schroeder, W. H., & Munthe, J. (1998). Atmospheric mercury-an overview. *Atmospheric Environment, 32*, 809–822.

Science for Environment Policy, 2017. Tackling mercury pollution in the EU and worldwide. In-depth Report 15 produced for the European Commission, DG Environment by the Science Communication Unit, UWE, Bristol. Available at: http://ec.europa.eu/science-environment-policy

Schiavo, B., Morton-Bermea, O., Salgado-Martinez, E., & Hernández-Álvarez, E. (2020). Evaluation of possible impact on human health of atmospheric mercury emanations from the Popocatépetl volcano. *Environmental Geochemistry and Health, 42*, 3717–3729.

Solan, T. D., & Lindow, S. W. (2014). Mercury exposure in pregnancy: a review. *Journal of Perinatal Medicine, 42*(6), 725–729.

Viveiros, F., Cardellini, C., Ferreira, T., Caliro, S., Chiodini, G., & Silva, C. (2010). Soil CO2 emissions at Furnas volcano, São Miguel Island, Azores archipelago: volcano monitoring perspectives, geomorphologic studies and land use planning application. *Journal of Geophysical Research, 115*(B12208), 1–17. https://doi.org/10.1029/2010JB007555.

WHO. 2000. Air quality guidelines for Europe (2nd ed., Vol. 91, pp. 157–162)., European Series Geneva: WHO Regional Publications.

Zillioux, W. J., Porcella, D. B., & Benoit, J. M. (1993). Mercury cycling and effects in freshwater wetland ecosystems. *Environmental Toxicology and Chemistry, 12*, 2245–2264.