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Biotoxicity of Halide Perovskites in Mice

Guixiang Li, Yong-Tao Liu, Feng Yang, Meng Li, Zuhong Zhang, Jorge Pascual, Zhao-Kui Wang,* Shi-Zhe Wei, Xin-Yuan Zhao, Hai-Rui Liu, Jin-Bo Zhao, Chieh-Ting Lin, Jun-Ming Li, Zhe Li, Antonio Abate,* and Irene Cantone**

Halide perovskites are crystalline semiconductors with exceptional optoelectronic properties, rapidly developing toward large-scale applications. Lead (II) (Pb^{2+}) is the core element used to prepare halide perovskites. Pb^{2+} can displace key 2+ elements, including calcium, zinc and iron, that regulate vital physiological functions. Sn^{2+} can replace Pb^{2+} within the perovskite structure and, if accidentally dispersed in the environment, it readily oxidizes to Sn^{4+}, which is compatible with physiological functions and thus potentially safe. The 3+ salt bismuth (III) (Bi^{3+}) is also potentially safe for the same reason and useful to prepare double perovskites. Here, this work studies the biotoxicity of Pb, Sn, and Bi perovskites in mice for the first time. This work analyses histopathology and growth of mice directly exposed to perovskites and investigate the development of their offspring generation. This study provides the screening of organs and key physiological functions targeted by perovskite exposure to design specific studies in mammalians.

1. Introduction

Halide perovskites are emerging materials with the potential to become semiconductors of widespread use.[1–5] They have an ABX_3 crystalline structure made of monovalent A-site cations, such as methylammonium (MA^+); B-site heavy metal ions, among which lead(II) (Pb^{2+}) is the most primarily used than tin(II) (Sn^{2+}) and bismuth (III) (Bi^{3+}); and X-site halogen anions like iodide (I^-).[6–12] Perovskites have possible large-scale applications in photovoltaics, light-emitting diodes, lasers, sensors, and photodetectors.[13] Particularly in photovoltaics, the power conversion efficiency of Pb-based perovskite solar cells has reached the benchmark of silicon solar cells and has great potential to go beyond it. However, the potential toxicity of perovskite has raised concerns about perovskite mass production. The risk of dispersing heavy metals into the environment is serious since perovskite is relatively water soluble and, thus, bioavailable.[9] Many reports addressed the biotoxicity of different heavy metal compounds[14] with an underline message that biotoxicity depends on the particular compound that contains the potentially hazardous element. Thus, using previous studies to anticipate the biotoxicity of perovskite can lead to wrong conclusions. Perovskite biotoxicity has been

G. Li, M. Li, Z. Zhang, J.-B. Zhao
Key Lab for Special Functional Materials
Ministry of Education
National & Local Joint Engineering Research Center for High-efficiency Display and Lighting Technology
School of Materials Science and Engineering
and Collaborative Innovation Center of Nano Functional Materials and Applications
Henan University
Kaifeng 475004, China
E-mail: mengli@henu.edu.cn

G. Li, M. Li, J. Pascual, J.-M. Li, A. Abate
Helmholtz-Zentrum Berlin für Materialien und Energie
Kekuléstraße 5, 12489 Berlin, Germany
E-mail: antonio.abate@helmholtz-berlin.de

Y.-T. Liu, S.-Z. Wei, X.-Y. Zhao
Department of Life Science and Technology
Xinxiang Medical University
Xinxiang 453002, China

F. Yang, H.-R. Liu
Henan Key Laboratory of Photovoltaic Materials
School of Physics
Henan Normal University
Xinxiang 453007, China

Z.-K. Wang
Institute of Functional Nano & Soft Materials (FUNSOM)
Jiangsu Key Laboratory for Carbon-Based Functional Materials & Devices
Joint International Research Laboratory of Carbon-Based Functional Materials and Devices
Soochow University
Suzhou 215123, China
E-mail: zkwang@suda.edu.cn

C.-T. Lin
Department of Chemical Engineering
National Chung Hsing University
145 Xingda Rd., South Dist., Taichung City 40227, Taiwan

Z. Li
School of Engineering and Materials Science (SEMS)
Queen Mary University of London
London E1 4NS, UK

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/adma.202306860

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directly addressed in non-mammalian model organisms and in vitro.[15,16]

Herein, we study for the first time the biotoxicity of Pb^{2+}, Sn^{2+}, and Bi^{3+}-containing perovskites in mice as mammalian model organisms. We included the analysis for Sn^{4+}, the product resulting from air exposure of Sn^{2+}, as a more representative scenario for Sn perovskite contamination. We monitored the growth and histopathology in mice directly exposed to perovskites and in their offspring. This work aims to provide a large spectrum of qualitative information for determining the target organs and physiological functions upon exposure to perovskites containing different heavy metals.

2. Results and Discussions

Heavy metals can enter the body through ingestion, breathing, and skin contact. Ingesting contaminated water and food is currently one of the most common ways.[17] Heavy metals reach the blood through ingestion and are distributed to the soft tissues, such as the lungs, heart, liver, kidneys, spleen, and brain, and accumulate in bones.[18,19] Heavy metals are known for displacing natural elements in the human body, including calcium, zinc and iron, which regulate essential physiological functions. For example, in the blood, they can interfere with the activity of heme, which is necessary for binding oxygen in the blood.[20] In soft tissues, they can impair the function of enzymes and receptors.[21]

To identify the organs targeted by heavy metals from perovskite, we treated a group of six male and six female mice per each metal (i.e., Pb^{2+}, Sn^{4+}, Sn^{2+}, and Bi^{3+}) for 21 days, and we examined histological changes in lungs, liver, brain, heart, spleen, and kidney. Control groups were created by exposing mice to pure and methylammonium iodide (MAI) containing water (no heavy metals). As shown in Figures S1–S3, Supporting Information, the color and texture of the tissue from the principal organs of the mice treated with pure and MAI-containing water showed normal histology with no evidence of disease.

Mice treated with Pb^{2+}, Sn^{4+}, Sn^{2+}, or Bi^{3+}-containing perovskites showed different degrees of structural abnormalities in the lung (**Figure 1**). Specifically, the lung sections of the Pb^{2+} treatment group have a large number of moderately thickened alveolar walls (e.g., a marked area in Figure S4, Supporting Information), accompanied by a moderate amount of inflammatory cell infiltration (black arrow in Figure 1b). In addition, we also observed local bronchial hemorrhage and small red blood cells (blue arrow, Figure 1b) in the bronchial cavity of mice treated with Pb^{2+} perovskites. The lung tissue sections of the Sn^{2+} treatment group showed a medium-area alveolar wall with heavy thickening, accompanied by abundant infiltrations of inflammatory cells (black arrow, Figure 1d) and focal lymphocytic infiltrations around the local blood vessels (yellow arrow, Figure 1c). We also observed bronchial hemorrhage and a small number of red blood cells (blue arrow, Figure 1d) in the lumen of the bronchus. The lung tissue slices of the Sn^{4+} and Bi^{3+} treatment groups have instead milder lesions. The Sn^{4+} treatment group showed moderate alveolar wall thickening with a small amount of inflammatory cell infiltration (black arrow, Figure 1f), while the bronchus had no apparent abnormalities. The Bi^{3+} treatment group showed many moderately thickened alveolar walls, accompanied by a moderate amount of inflammatory cell infiltration (black arrow, Figure 1h); no apparent abnormalities were observed in the bronchus. Altogether these observations indicate that Pb^{2+} and Sn^{2+} from perovskites have a more severe impact on the lungs compared to Sn^{4+} and Bi^{3+}.

We then analyzed the liver (**Figure 2**). In the Pb^{2+} treatment group, we observed a moderate amount of hepatocyte steatosis in the central vein, around the portal area and in the liver parenchyma. Several circular vacuoles were observed in the cell cytoplasm (black arrow, Figure 2b). No significant inflammatory changes were observed. In the Bi^{3+} treatment group, there were moderate amounts of hepatocyte steatosis in the central vein, around the portal area and in the liver parenchyma, and different numbers of round vacuoles (black arrows, Figure 2h) in the cytoplasm. Focal lymphocytic infiltrates could be seen locally (blue arrows, Figure 2h). There were no alterations in the Sn^{4+} group (Figure 2e,f). While for Sn^{2+}, the lesions were mild, and no apparent abnormalities were observed under the microscope (Figure 2c,d). These results overall suggest low toxicity for the liver.

Figure 3 shows the analysis of the hippocampus and its cornu ammonis 1 (CA1) and dentate gyrus (DG) regions. The Pb^{2+} treatment group showed normal hippocampal pyramidal cells with a neat and dense arrangement, large and round nuclei, apparent nucleoli, rich cytoplasm, clear layers and cell lines (Figure 3a,b). Sn^{2+}, Sn^{4+}, and Bi^{3+} treatment groups showed pyramidal cell nuclei with irregular shapes (black arrow, Figure 3d–h) and no glial cell proliferation in the hippocampus. The images suggest that the four metals did not significantly impact brain tissue structure. The heart, kidney and spleen were also analyzed, but no apparent anomaly was observed, as shown in Figures S5–S7, Supporting Information.

To have a more quantitative measure of perovskite toxicities, we calculated the organ coefficients (**Figure 4**). The organ coefficient is a commonly used index in toxicology experiments that is simple and easy to implement and more sensitive than simple histopathological assessment. It is calculated as the ratio between the weight of an organ in an experimental animal and its body weight. Under normal conditions, the ratio of each organ to body weight is relatively constant. After an animal is poisoned, the weight of the damaged organ can change, so the organ coefficient also changes. We, therefore, collected statistics of organ coefficients, or organ-to-body ratio, for more quantitative analysis, as shown in Figure 4. The metal-free MAI was almost indistinguishable from the control group. The lung and brain coefficient

A. Abate
Department of Chemical
Materials and Production Engineering
University of Naples Federico II
Piazzale Tecchio 80, Naples, Fuorigrotta 80125, Italy

I. Cantone
Department of Molecular Medicine and Medical Biotechnology
University of Naples Federico II
via Pansini 5, Naples 80131, Italy
E-mail: irene.cantone@unina.it

I. Cantone
CNR Istituto di Endocrinologia e Oncologia Sperimentale (IEOS)
Via Pansini, 5, Naples 80131, Italy

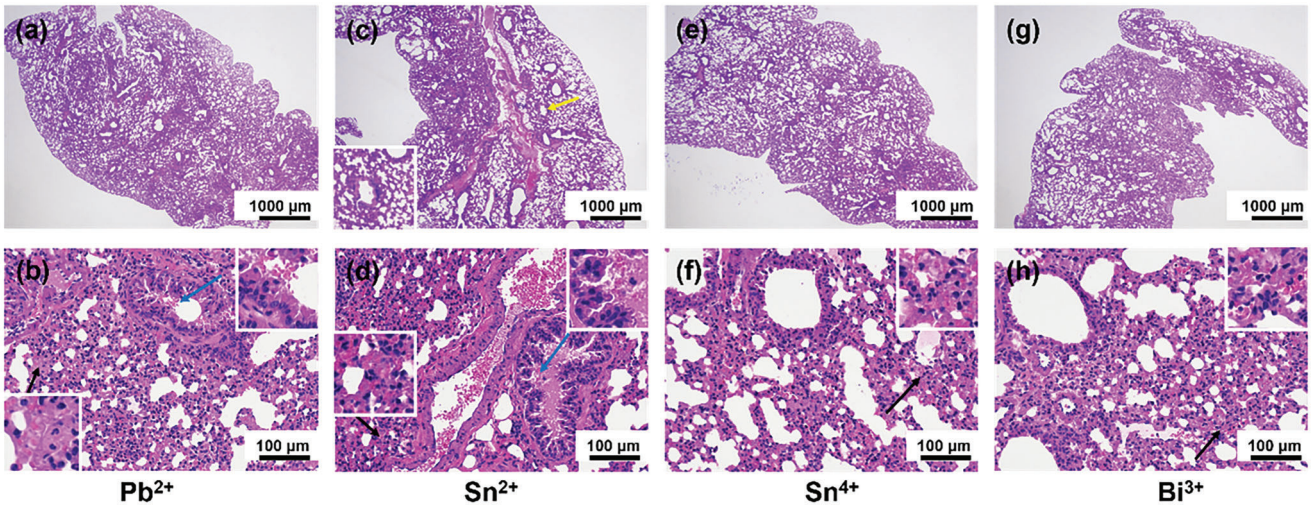

Figure 1. LUNG. Representative images of lungs from a,b) Pb^{2+}, c,d) Sn^{2+}, e,f) Sn^{4+}, and g,h) Bi^{3+} perovskites-treated mice. Yellow arrows indicate lymphocyte infiltrations. Black arrows indicate inflammatory cell infiltration. Blue arrows indicate bronchial hemorrhage and small red blood cell infiltration. Inserts show magnified inlet for the histological changes that are indicated by the arrows.

of Sn^{2+} treated mice were significantly larger than control ($p < 0.05$ for the brain and $p < 0.01$ for the lung, Figure 4a,c), while its oxidated state Sn^{4+} showed no relevant differences from the control, as well as Bi^{3+}. The spleen coefficient of Pb^{2+} was remarkably decreased compared to the control, although not statistically significant (Figure 4e). Altogether this analysis suggests that Sn^{2+} and Pb^{2+} perovskites might impair different organ functionality, whereas Sn^{4+} and Bi^{3+} are relatively safe.

To further assess the effect of metal perovskites on physical development and physical fitness, we recorded the body weight of mice before and after treatment (**Figure 5**). Before perovskite treatment, there was no significant difference in body weight between each treatment group and the control group. After 21 days of intragastric administration, the Pb^{2+} group showed a significant weight loss among all the surviving mice ($p < 0.01$ compared to water control and $p < 0.05$ against MAI control), suggesting a global impairment of fundamental metabolic functions. All the other treatment groups did not show any statistically significant difference from the controls.

Exposing males or females to heavy metals can have an impact on their offspring. Heavy metals might affect fetal development when pregnant females are directly exposed, as they can pass into the fetus through the placenta and cause miscarriage, developmental defects, or mental retardation of the newborn.[22,23] Also, they can promote epigenetic modification in males and females that can be inherited by grandchildren.[24] To investigate the effect of perovskite on the offspring of exposed mice, we treated pregnant female mice with different doses. Specifically, we used 14 (high dose), 7 (medium dose), and 3.5 (low dose) milligrams per week (mg $week^{-1}$) of perovskites.

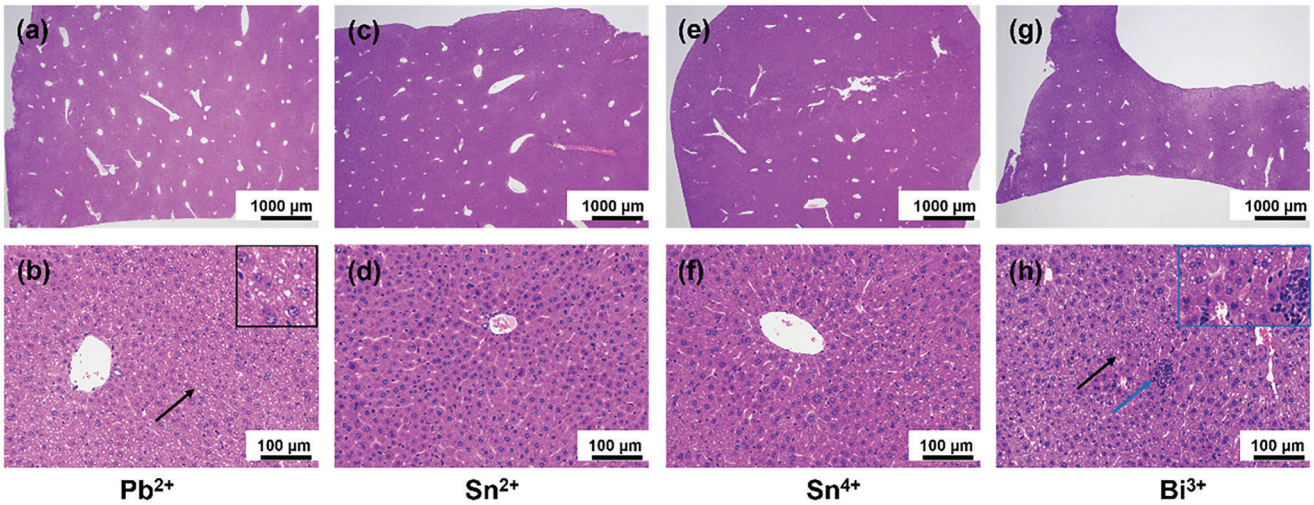

Figure 2. LIVER. Representative images of the liver from a,b) Pb^{2+}, c,d) Sn^{2+}, e,f) Sn^{4+}, and g,h) Bi^{3+} perovskites-treated mice. Black arrows indicate circular vacuoles in the cell cytoplasm. The blue arrow indicates lymphocytic infiltrates. Inserts show magnified inlet for the histological changes that are indicated by the arrows.

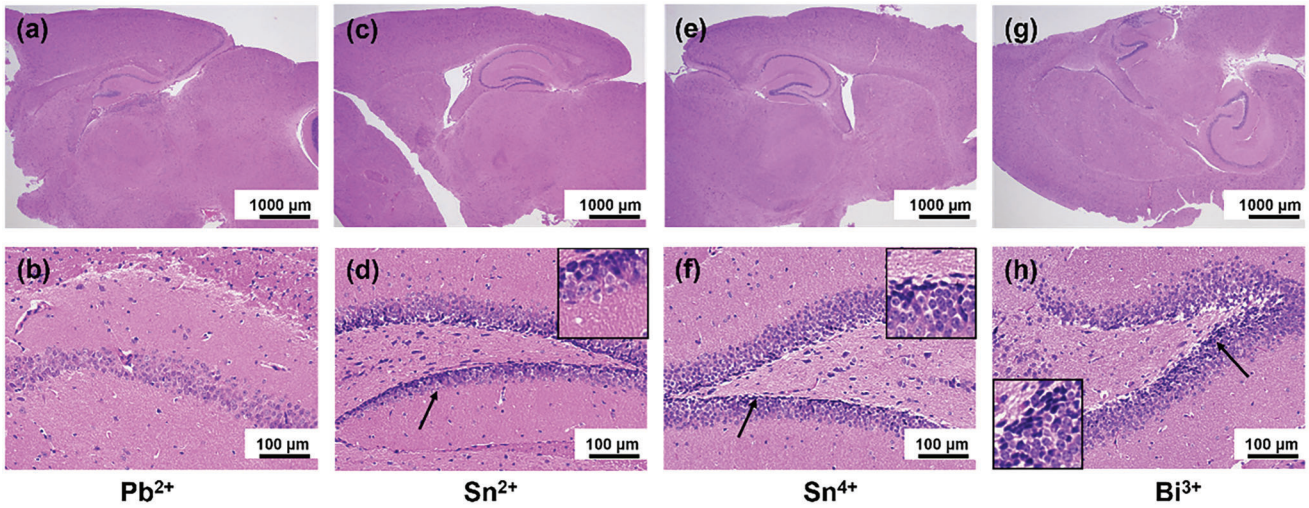

Figure 3. BRAIN. Representative images of the brain from a,b) Pb^{2+}, c,d) Sn^{2+}, e,f) Sn^{4+}, and g,h) Bi^{3+} perovskites-treated mice. Panels (a,c,e,g) show hippocampal sections. Panels (b,d,f,h) show CA1 and DG regions of the hippocampus. Black arrows indicate pyramidal nuclei of irregular shape. Inserts show magnified inlet for the histological changes that are indicated by the arrows.

It is worth noting that almost all the male mice in the Sn^{2+} treatment group gradually died at different times after feeding with Sn^{2+} perovskites (Table S1, Supporting Information). This, however, did not happen in mice treated with perovskites containing Sn^{4+} or other perovskite metals. We believe that the oxidation of Sn^{2+} within the gastrointestinal tract made it impossible for males to absorb nutrients from food, while females were more resistant. Bi^{3+} has not shown any toxicity in histological analyses, and therefore we excluded it from further analyses.

We, indeed, analyzed the weight gain of pregnant females and fetal development (**Figure 6**, Figures S8–S11, Supporting Information) in mice fed with Pb^{2+} and Sn^{4+} perovskites and compared them with the control group (i.e., fed with pure water).

Pregnant mice exposed to the high-dose Sn^{4+} perovskite and Pb^{2+} perovskite after E0.5 (day 0.5 of pregnancy) showed a reduced weight gain at 18 days of gestation compared to the control group ($p < 0.05$). The weight gain of pregnant mice in the high-dose group of Sn^{4+} perovskite and Pb^{2+} perovskite was, in fact,

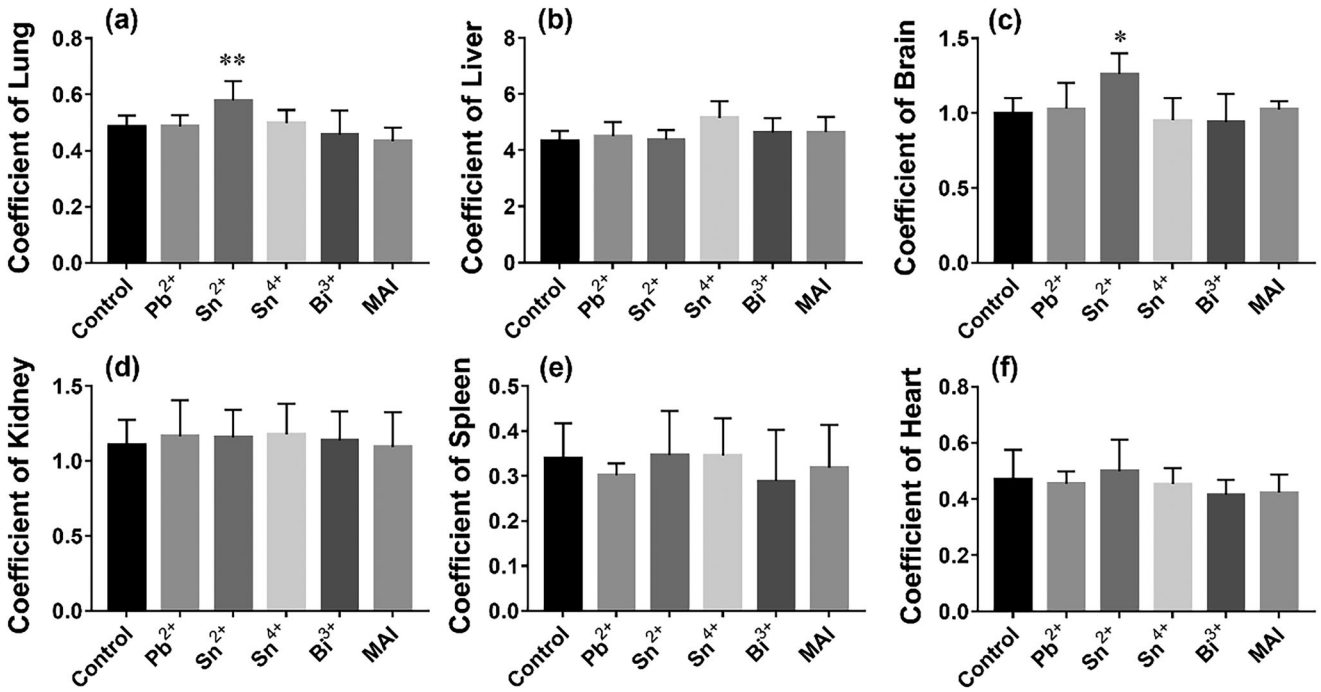

Figure 4. Organ coefficients. Organ coefficient of the a) lung, b) liver, c) brain, d) kidney, e) spleen, and f) heart. * and ** indicate statistical significance at $p < 0.05$ and $p < 0.01$ by ANOVA test, respectively.

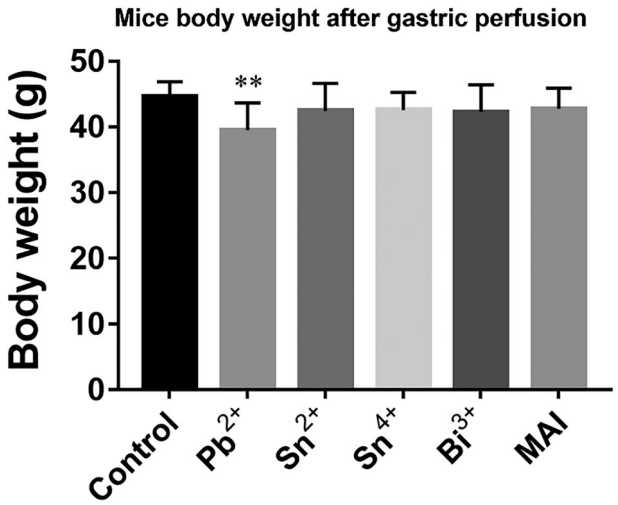

Figure 5. Body weight. ** represents statistical significance at $p < 0.01$. An ANOVA test is a statistical test.

significantly different compared to the control group ($p < 0.05$). Although the absolute value of the weight gain loss of pregnant mice caused by Sn^{4+} perovskite treatment was greater than that of Pb^{2+} perovskite treatment, there was no significant difference between the Pb^{2+} and Sn^{4+} groups ($p = 0.2661 > 0.05$). For the middle-dose treatment group (Figure 6b), we found that the difference between Sn^{4+} and Pb^{2+} perovskite treatment groups was not significant compared to the control group ($p > 0.05$). Nevertheless, in the low-dose treatment (Figure 6c), there is no significant difference between the Sn^{4+} perovskite group and control group ($p > 0.05$), indicating that low-dose Sn^{4+} perovskite has little effect on the weight gain of pregnant mice. On the contrary, the Pb^{2+} perovskite treatment group increases pregnant mice's weight gain. From the above analysis, it can be concluded that increasing the dose rapidly decreased the weight gain of the fetal mice in the Pb^{2+} and Sn^{4+} groups, with consistent harmful effects visible only at high doses (14 mg week^{-1}). Moderate amounts of both Pb^{2+} and Sn^{4+} (below 3.5 mg week^{-1}) will instead be safe.

As shown in **Figure 7**, Figure S10 and Tables S3 and S4, Supporting Information, high-dose Sn^{4+} and Pb^{2+} perovskites treatments resulted in a sharp increase in the number of dead births that significantly differed from the control group ($P_{Sn} = 0.0359 < 0.05$, $P_{Pb} = 0.0166 < 0.05$). This indicates that at the high dose, the two perovskites have a significant impact on the survival of fetuses. However, the one-way analysis of variance was similar between the two groups. For the middle and low dose groups (Figure 7b,c), the Pb^{2+} and Sn^{4+} perovskites treatment groups have no significant difference compared with the control group, indicating they do not hamper fetal survival. We, indeed, conclude that the Pb^{2+} and Sn^{4+} perovskites have a dose-dependent effect on fetal mortality. Consistently, fetal body length was significantly reduced in the Pb^{2+} and Sn^{4+} groups only at high doses (Figure S10, Supporting Information). Interestingly, only the Sn^{4+} treated mice showed a significant reduction in fetal weight both at high and middle doses, suggesting that this heavy metal might have a stronger effect on fetal development, as also shown by weight data on pregnant females.

Altogether these data show that Pb-based and Sn-based perovskites have a significant dose-dependent effect on the development of fetal mice (Figure S11 and Tables S5 and S6, Supporting Information). When the weekly intake is a high dose of 14 mg per week, Sn^{4+} and Pb^{2+} have a similar impact. Additionally, we observed mild effect of perovskite exposure on memory and learning (Figure S12, Supporting Information).

3. Conclusion

Heavy metals are a core component of halide perovskites. Although the biotoxicity of heavy metals has been widely studied in several compounds, little is known about the toxicity of heavy metals in halide perovskites. As perovskites are fast developing for a wide range of applications, we urge a thorough assessment of their health impact. In this work, we investigated the biotoxicity in mice of the main heavy metals used for perovskites.

We found that Pb^{2+}, Sn^{2+}, Sn^{4+}, and Bi^{3+} perovskites have varying degrees of influence on the organs of mice, with histopathological alterations mainly evident in the lungs and liver. We discovered that Pb^{2+} and Sn^{2+} ions seriously damage mice organs,

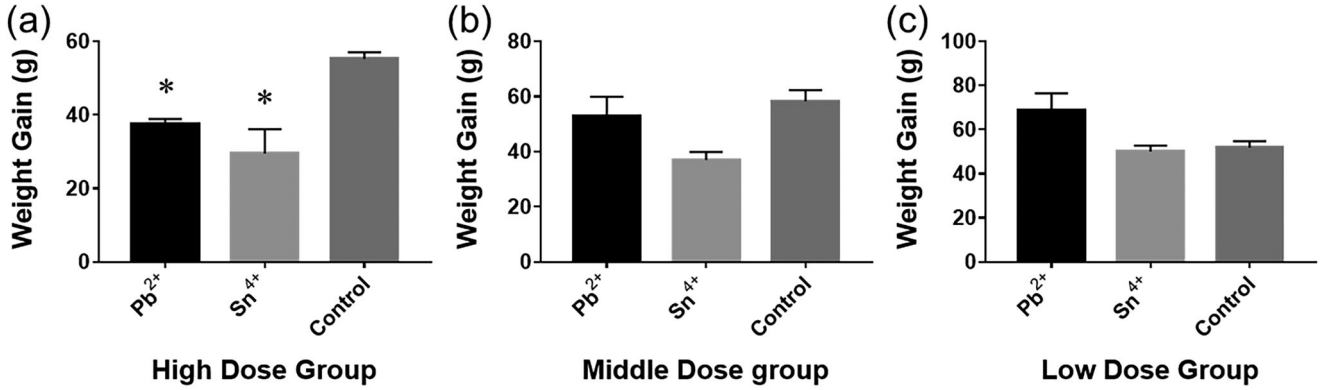

Figure 6. Body weight gain in pregnant mice. a) High dose (14 mg week^{-1}) treatment group, b) medium dose (7 mg week^{-1}) treatment group, c) low dose (3.5 mg week^{-1}) treatment group. * and ** indicate statistical significance compared to control group at $p < 0.05$ and $p < 0.01$ by ANOVA test, respectively.

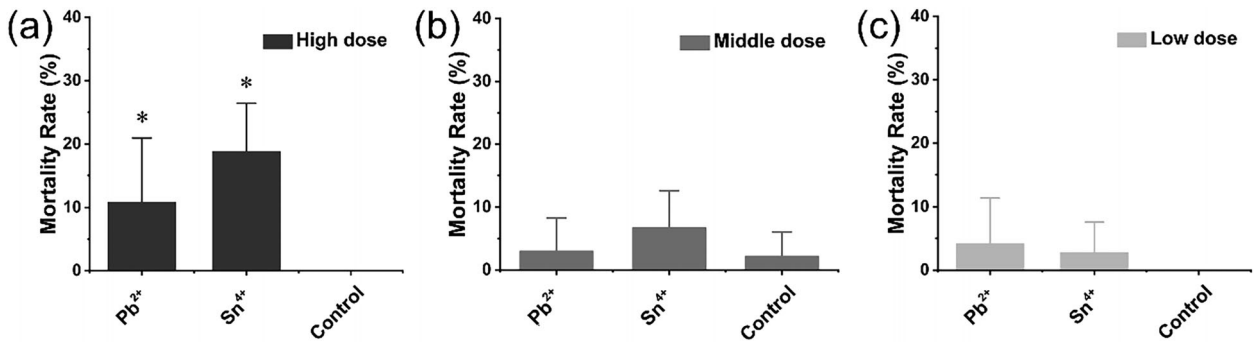

Figure 7. Fetus mortality rate. a) High dose (14 mg week^{-1}) treatment group, b) medium dose (7 mg week^{-1}) treatment group, c) low dose (3.5 mg week^{-1}) treatment group. * and** indicate statistical significance at $p < 0.05$ and $p < 0.01$, respectively. An ANOVA test is a statistical test.

while Bi^{3+} and Sn^{4+} were relatively safe. Pb^{2+} had the most adverse effects leading to a decrease in the mouse's body weight.

For the offspring of exposed mice, we observed a dose-dependent toxicity leading to higher fetal mortality and reduced body weight of both the fetus and the mother only at high doses (14 mg week^{-1}) of both Sn^{4+} than Pb^{2+} perovskites. Such high doses are rather extreme and unlikely to be reached in humans.

This study investigated acute exposure (mg week^{-1}) for screening organs and physiological functions targeted by perovskite exposure in mammalians. Further work is currently ongoing to identify the impact of chronic exposure, which requires monitoring several generations of mice constantly exposed to extremely low doses (< μg week^{-1}) of perovskite. Chronic exposure studies will allow quantitative analysis to determine the safety levels of exposure for each metal in halide perovskite.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Research data are not shared.

Keywords

biotoxicity, health, heavy metals, mice, halide perovskite

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