Health hazards of methylammonium lead iodide based perovskites: cytotoxicity studies†

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New technologies launch novel materials; besides their performances in products, their health hazards must be tested. This applies to the lead halide perovskite CH₃NH₃PbI₃ as well, which offers fulgurate applications in photovoltaic devices. We report the effects of CH₃NH₃PbI₃ photovoltaic perovskites in human lung adenocarcinoma epithelial cells (A549), human dopaminergic neuroblastoma cells (SH-SYSY) and murine primary hippocampal neurons by using multiple assays and electron microscopy studies. In cell culture media the major part of the dissolved CH₃NH₃PbI₃ has a strong cell-type dependent effect. Hippocampal primary neurons and neuroblastoma cells suffer a massive apoptotic cell death, whereas exposure to lung epithelial cells dramatically alters the kinetics of proliferation, metabolic activity and cellular morphology without inducing noticeable cell death. Our findings underscore the critical importance of conducting further studies to investigate the effect of short and long-term exposure to CH₃NH₃PbI₃ on health and environment.

1. Introduction

Solar energy remains one of the most promising alternative sources to supply electricity. However, after decades of research and three generations of solar cell devices, solar power still represents less than 1% in the global energy chart. Nevertheless, the International Energy Agency (IEA) predicts a spectacular increase in the worldwide photovoltaic energy production by mid-century. Therefore, intensive research and investments have been made to develop novel materials that are used in the production of the next generation of photovoltaic cells offering more efficient and cost effective materials with enhanced performance and improved architecture.

The latest breakthrough is due to the organometallic halide perovskite CH₃NH₃PbI₃ (hereafter MAPbI₃ for methylammonium lead iodide). MAPbI₃ represents one such novel material and offers several advantages that have led to its widespread use and application in photovoltaic cells: it is (1) made of inexpensive components; (2) very robust and flexible; (3) used to manufacture a photovoltaic cell easily, and (4) extremely efficient in converting sunlight to electricity. Aside from its remarkable performance in photovoltaic cells, this material seems to be very promising in solar water splitting and optoelectronic devices like LEDs, lasers and photodetectors. It has been estimated that mass manufacturing of this material in industrial facilities could begin as soon as 2018.

The high toxicity of lead combined with the expected extensive use of organometallic halide perovskite CH₃NH₃PbI₃ raises major concerns about the potential health hazards and consequences of short, medium and long-term exposure to this material. Studies on acute and chronic lead exposure of humans, laboratory animals and plants showed that the lowest exposure level resulted in detectable effects, bio-accumulation and poisoning. In the case of MAPbI₃, not only human exposure during device production and handling is a concern, but its release into the environment, soil and waterways, after the failure of large area solar cells (due to a potential thermal shock or damaged encapsulation) also represents major health and environmental risks. In addition to lead toxicity, iodine and methylamine might cause a health hazard, as well. Taken together, these observations emphasize the critical importance of investigating the effect of MAPbI₃ exposure at the cellular and organism levels before its large-scale exploitation.

One of the main access routes of MAPbI₃ to the human body is by inhalation of its volatile nanoparticles. The airborne material can be deposited in the respiratory path, nose, bronchi, and lung getting into contact with epithelial cells. The material can be dissolved in body fluids and through the blood stream or olfactory nerves, can then reach the brain. Thus we were particularly interested in evaluat-
ing the effects of MAPbI₃ in various cellular models including human lung adenocarcinoma epithelial cells (A549), human dopaminergic neuroblastoma cells (SH-SY5Y) and murine primary hippocampal neurons.

Remarkably, the effect of MAPbI₃ appeared to be cell-type dependent. Primary neurons and neuroblastoma cells’ exposure to MAPbI₃ induced massive apoptotic cell death, whereas exposure of the A549 epithelial cells dramatically affected their kinetics of proliferation, their metabolic activity and cellular morphology without inducing noticeable cell death.

Altogether, our findings provide novel insights into the cellular mechanisms by which the potential bioaccumulation of MAPbI₃ could induce health hazards in the long term. Our data emphasize the critical importance of performing further studies (e.g. in living organisms, to apply additional experimental models) and also point-out the need to improve the device architecture by robust confinement of the material.

2. Experimental results

2.1 Preparation and characterization of MAPbI₃ and its solution

MAPbI₃ can be synthesized in various forms: big crystals, nano-crystals in thin films, nanowires, or quantum dots depending on the purpose of their use (Fig. 1a, lower part). All these forms stem from a solution-based process described by Poglitsch and Weber. A macroscopic amount that was prepared for this study is shown in the upper part of Fig. 1a.

As a first step towards assessing the potential toxicity of MAPbI₃, it is important to conduct a quantitative assessment of its volatility and stability to chemical and microbial degradation. It is well known that MAPbI₃ has a hygroscopic character i.e. is sensitive to moisture and decomposes into its water-soluble constituents. Therefore, the solubility of MAPbI₃ polycrystalline powder was determined in deionized water (close to outdoor environmental conditions) as well as in two types of cell culture medium (DMEM and DMEM:F12, mimicking the human body fluid conditions). The perovskite powder was dispersed in these liquids at different concentrations (from 50 to 3000 μg ml⁻¹). These solutions were then filtered and analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES) at various time points (1–30 days). The initial step of the perovskite dissolution was identical in deionized water and in cell culture media. In combination with the liquids, the initially silver-grey colored MAPbI₃ was decomposed into solid, yellow PbI₂ and water-soluble methylammonium iodide derivatives within a few seconds. Importantly, altered dissolution mechanisms were observed on longer soaking times. At the highest dose, a clear and transparent liquid was obtained in deionized water after 4

![Image](https://example.com/image1.png)

**Fig. 1** The MAPbI₃ perovskite and its preparation for the toxicity tests. (a) The different morphological appearances of the material (lower panel) and the typical quantity of microcrystals used for the present study (upper panel; the dice in the background sets the scale); (b) the different steps of the solution preparation in water or in cell culture medium (DMEM). Note that in the last one, the majority of the Pb²⁺ ions precipitate in a yellow/white deposit consisting of lead-phosphate and lead carbonate (see ESI Fig. S2†); (c) concentration levels of lead and iodine in water and in cell culture medium after filtration; (d) chart of the toxicity study of MAPbI₃ on neuronal and epithelial cells with the major techniques and the expected information obtained.
days, indicating that the PbI₂ was completely dissolved. In contrast, in cell culture media, a white precipitate and a reduced amount of water-soluble Pb²⁺ (6%) were observed, even 30 days post-preparation (Fig. 1b and c and ESI Fig. S1†). Elemental analysis confirmed that the white, solid precipitate is composed of a mixture of lead(II) hydroxide, lead(II) carbonate, and lead(II) phosphate compounds (ESI Fig. S2†), which were formed by the reaction of Pb²⁺ with the carbonate, phosphate and hydroxide anions of the cell culture media. These marked differences in the dissolution of MAPbI₃ in water and cell culture media clearly demonstrate that its potential environmental impact can be modulated by different parameters (temperature, pH and chemical environment dependent equilibrium of soluble and solid forms). We targeted concentrations of 50, 100 and 200 µg ml⁻¹ of MAPbI₃, which were administered to cell cultures on time scales extended to a few days. A solution of MAPbI₃ at 100 µg ml⁻¹ has a molecular weight percent of lead, iodine and methylamine of respectively 33.42, 61.41 and 5.17%. As seen in Fig. 1c, the culture media, where MAPbI₃ was added and then filtered, contain more than 90% of the initial iodine and 6% of the initial lead. Methylamine is known to be soluble in a water-rich environment and cannot be quantified using elemental analysis due to the presence of carbon, hydrogen and nitrogen. For simplicity, the nominal MAPbI₃ concentration is used on the histograms and figures.

Fig. 1d provides an overview of our experimental design and methods used to assess MAPbI₃ toxicity. Only the filtered, cell culture medium soluble part of MAPbI₃ was administered to the cell lines A549, SH-SY5Y and the murine primary hippocampal neurons. The effects of MAPbI₃ exposure were measured by flow cytometry, fluorogenic and colorimetric assays, immunocytochemistry (ICC) and transmission electron microscopy (TEM). The combination and complementarity of these assays allowed for assessing the effect of MAPbI₃ on cellular properties, metabolic activity, morphology and viability. Interestingly, the two cell types have radically a different response to MAPbI₃ exposure. The details of these observations are given below.

2.2 MAPbI₃ induces apoptotic cell death in the SH-SY5Y cell line and mice hippocampal primary neurons

We first evaluated the time and concentration dependent effects of MAPbI₃ in neuroblastoma SH-SY5Y cells. MAPbI₃ was administered to cells at concentrations ranging from 50 to 200 µg ml⁻¹ for up to 7 days. We measured cell death by the uptake of Sytox Green (SG), a vital dye that easily penetrates cells with a compromised plasma membrane. The quantification of cell death was performed by flow cytometry in SH-SY5Y cells (Fig. 2 and S3†). The results showed a time-and-concentration-dependent toxicity of MAPbI₃ in this cell line.

Exposure of SH-SY5Y cells to MAPbI₃ at a concentration as low as 50 µg ml⁻¹ was sufficient to trigger significant cell death (from 10 to 30%) after 7 days, whereas at the highest concentration (200 µg ml⁻¹) of MAPbI₃, more than half of the SH-SY5Y cell population was dead 3 days post-treatment, and almost all of them did not resist the treatment after 7 days (Fig. 2a and ESI Fig. S4a†).

To validate our findings we next investigated the effect of MAPbI₃ in the differentiated mice hippocampal primary neurons. These neurons have the advantage of spontaneously differentiating in their growth cell culture medium after two weeks in culture allowing long term toxicity studies (up to four weeks). MAPbI₃ was added to the cell culture medium of the primary hippocampal neurons at various concentrations and up to 25 days. Fig. 3a shows that MAPbI₃ also induced cell death in a time- and concentration-dependent manner in these cells.

Next, we sought to investigate the molecular mechanisms by which MAPbI₃ induced death in these two neuronal models. Using fluorogenic assays combined with flow cytometry, we observed that caspase 3, a key enzyme involved in the execution phase of apoptosis, was significantly activated in SH-SY5Y cells exposed for 5 days to MAPbI₃ (Fig. 2b). It is to be noted that necrotic cell death was not induced under these conditions (ESI Fig. S5†). In order to elucidate the MAPbI₃-mediated apoptotic pathways, we monitored the activation of caspase 8, a key caspase activated by the extrinsic/death receptor pathway, and caspase 9, a key caspase activated by the intrinsic/mitochondrial pathway. Fig. 2c demonstrates a concentration-dependent caspase 9 activation in SH-SY5Y cells treated for 5 days with MAPbI₃. No increase in caspase 8 activity was observed under the same conditions (ESI Fig. S4b†). This finding suggests that apoptosis in SH-SY5Y cells is triggered through the mitochondrial pathway. To confirm this result, we finally quantified the metabolic activity of the SH-SY5Y cells exposed to MAPbI₃ using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide MTT assay normalized to the cell confluence. We found a clear decrease in mitochondrial activity in SH-SY5Y cells exposed to MAPbI₃ for 5 days (Fig. 2d), confirming mitochondrial dysfunction.

The specific activation of caspase 3 (Fig. 3b) and caspase 9 (Fig. 3c) together with the absence of caspase 8 (Fig. 3d) and the decrease in mitochondrial activity (Fig. 3e) also confirmed the initiation of the intrinsic apoptotic pathway in the differentiated hippocampal primary neurons exposed to MAPbI₃ as already monitored in SH-SY5Y cells.

2.3 MAPbI₃ alters the proliferation capacity and mitochondrial activity of A549 cells without inducing cell death

Fig. 4a shows the absence of Sytox Green uptake by A549 cells upon MAPbI₃ exposure, which suggests that the plasma membrane integrity of this cell line is not compromised over the treatment (Fig. 4a and S6†). These findings are consistent with the lack of significant activation of caspases 3, 8 and 9 upon treatment with MAPbI₃ (50–200 µg ml⁻¹) (Fig. 4b).

After 5 days of culture, the non-treated A549 cells reached confluence whereas the density of the cells exposed to MAPbI₃ decreased significantly (4 times less) in a concentration-dependent manner (Fig. 5a). Interestingly, imaging by bright field optical microscopy (Fig. 5b) and immunocytochemistry (Fig. 5c) revealed a heterogeneous population of cells with different morphologies. The non-treated cells exhibited a homogeneous triangle-shape with an average size ranging
from 15 to 20 µm, typical of epithelial cells. After 5 days of exposure to MAPbI3, we observed cell populations of different morphologies (Fig. 5c): the overall size of some cells significantly increased (“giant cells”, size >50 µm) with a round-shaped aspect. Some cells were stretched and acquired an elongated morphology (“elongated cells”, width <7 µm).

One could hypothesize that the decrease in cell density is due to the changes in the cell division cycle of the A549 exposed to MAPbI3. However, Fig. 5c shows that the organization of actin and tubulin network of proteins of the cytoskeleton that is rearranged during cell division) was not affected in A549 cells exposed to MAPbI3 suggesting that there is no major difference in the cell division process between the two conditions. This is confirmed by detailed measurements of the sub-G1, S and G2 cell cycle phases (in simple terms, growth phase after the mitosis, DNA replication and maturation before the cell division, respectively) using flow cytometry. The distribution of cell cycle phases was similar between the non-treated and treated cells with various concentrations of MAPbI3 after 5 days (Fig. 5d).

Since the proportion of A549 cells in the different cell cycle phases remained unchanged after MAPbI3 treatment, we sought to determine if the kinetics of the proliferation were altered by measuring the fluorescence intensity of the Carboxy-Fluorescein Succinimidyl Ester (CFSE) dye by flow cytometry. This dye diffuses passively into cells and its intensity decreases by half after every cell division cycle. The results in Fig. 5e show the fluorescence intensity of the CFSE normalized to non-treated cells after 5 days of exposure for increasing concentrations of MAPbI3. Cells exposed to the highest concentration of MAPbI3 showed a CFSE fluorescence intensity that is 2.3 times higher than the non-treated cells, suggesting that division kinetics have decreased in MAPbI3 treated cells which is the reason for their slower proliferation.

To gain more detailed insight into the molecular and cellular basis of MAPbI3-induced cellular dysfunction, we examined the effect of MAPbI3 on the cellular compartments and architecture of the A549 cells using TEM.

As shown in Fig. 6(a and b), treatment with MAPbI3 resulted in an increased size of cells, multiple nuclei, increased number of lamellar bodies and mitochondrial damage. The multiple nuclei present in the treated cells did not show any ultra-structural defects with well-preserved nuclear membranes and also present multiple nucleoli. Interestingly, in the treated cells, the density of lamellar bodies (LB), secretory organelles that store lung surfactants, markedly amplified.
However, the most striking effect of MAPbI₃ treatment was observed in the mitochondrial compartment. The non-treated cells showed the typical ultrastructure of the mitochondrial cristae organization (Fig. 6c, light green), whereas the cells treated with MAPbI₃ exhibited severely damaged mitochondria (Fig. 6d). The size of mitochondria in these cells increased dramatically and the intra-mitochondrial space became highly dilated with severe damage to the cristae organization (Fig. 6d, dark green). These results suggest that MAPbI₃ induces adverse effects on the mitochondrial function of A549 cells.

To test this hypothesis, we assessed the effect of MAPbI₃ on mitochondrial functions using different assays. We observed a time and MAPbI₃ dose-dependent increase of the mitochondrial activity using the MTT assay (Fig. 7a). We then compared

Fig. 3  MAPbI₃ induces apoptosis in differentiated mice primary hippocampal neurons. Neurons were plated in a 96-well plate. Once differentiated (2 weeks post-plating), primary neurons were treated with increasing concentrations of MAPbI₃ [50, 100 and 200 μg ml⁻¹] and the cellular toxicity was evaluated using complementary cell death assays. (a) Cell death was measured by using a plate reader (487 nm/519 nm) using the fluorescent vital probe Sytox Green (SG), which only penetrates cells with damaged plasma membranes. The cell death level is expressed as the percentage of cells with a compromised cell membrane (SG positive cells) to the total cell number; (b–d) caspase 3, caspase 9 and caspase 8 activities were measured at 20 days of treatment using fluorogenic assays combined with a plate reader (487 nm/519 nm) quantification; (e) the metabolic activity was determined by MTT assay at 3, 10 and 20 days of treatment. Absorbance at 570 nm was quantified by using a plate-reader. These observations corroborate the results obtained on the SH-SY5Y neuron cell line. All the histograms show an average of at least 3 independent repeats (each condition in triplicate). Bars are means ± S.D. One-way ANOVA test followed by a Tukey–Kramer post-hoc test were performed (non-treated vs. MAPbI₃ treated conditions), *p < 0.05, **p < 0.005, ***p < 0.0005.
the mitochondrial membrane potential (ΔΨₘ) in the non-treated and treated cells by measuring the capacity of Mitotracker Red CMXRos to be taken up and retained in the mitochondria. Fig. 7b shows a significant increase of the ΔΨₘ in the cells exposed to MAPbI₃. Finally, we measured the overall mass of the mitochondria, assessed using the Mitotracker green FM and flow cytometry, which was also slightly increased in the treated cells (Fig. 7c).

In summary, MAPbI₃ does not induce the cell death of human lung epithelial cells but heavily affects their phenotype, proliferation kinetics and the mitochondrial activity, potential and mass. The effects of MAPbI₃ on human lung epithelial cells were markedly different compared to the neuronal cells.

3. Discussion

The discovery of a perovskite as a cheap and extremely efficient material to convert sunlight to electricity represents a major breakthrough in the development of photovoltaic cells. Manufacturing companies are already planning to commercialize this novel material in the near future. However, one serious drawback of the large scale exploitation of the perovskite is its lead content that raises major concerns about its potential toxicity during device production, handling and disposal.

In our study we observed that when MAPbI₃ is dispersed in cell culture media a part forms a precipitate, while 6% of its lead and more than 90% of its iodine content stay in solution (see Fig. 1). Methylamine is known to be highly soluble in a water-rich environment.30 In this study, we considered the soluble part of MAPbI₃ only. Herein, we showed that the MAPbI₃ perovskite caused a time-and-dose-dependent toxicity in both the SH-SY5Y neuroblastoma cell line and in differentiated mice primary hippocampal neurons. In both neuronal cell types, MAPbI₃ damaged the plasma membrane and triggered apoptotic cell death via the activation of the mitochondrial/intrinsic pathway signaled by caspase 9. Lead could be the main cause of this effect since it is known to depolarize cell mitochondria resulting in cytochrome c release, caspase activation, and apoptosis.31,32 In humans, a blood lead level >0.1 µg ml⁻¹ is considered to be elevated even though clinical symptoms are rarely seen below 0.6 µg ml⁻¹ in chronic poisoning.12 Moreover, in vivo, the accumulation of lead in the organism amplifies the toxic effect. In our in vitro cellular studies the effect of accumulation was substituted by the increased concentration. It is known that lead is a neurotoxin which is considered to be a major global environmental health hazard. Molecular epidemiology studies showed that chronic exposure to lead was associated with an increased risk of amyotrophic lateral sclerosis,34 Parkinson’s disease35 and late-onset Alzheimer’s disease.36 Although there are many investigations, the mechanisms underlying lead toxicity are still a matter of active research. However, not only lead is released when MAPbI₃ is dissolved in organic fluid, but also iodide and methylamine. Interestingly, methylamine is also known to be toxic since it exerts adverse effects on fetal development and embryo survival in mice.25

Remarkably, in the case of epithelial cells such as A549 human lung cells, exposure to MAPbI₃ did not result in cell death, but the proliferation rate decreased without cell cycle arrest leading to severe morphological changes and significant increases in the mitochondrial mass and activity. Upon exposure to MAPbI₃, A549 cells became giant and poly nucleated; their mitochondria showed an unusual polymorphic arrangement of the cristae. Lead ions are known to enter some cells through calcium channels, then bind to calmodulin (with a higher affinity than calcium) which alters enzyme activities, gene transcription and cell signaling.16,28 On the other hand, methylamines are also known to inhibit protein turnover resulting in a modification of the cells’ DNA and RNA content.25

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**Fig. 4** MAPbI₃ does not induce cell demise in A549 human lung epithelial cells. The cells were treated with increasing concentrations of MAPbI₃ (50, 100 and 200 µg ml⁻¹) and the cellular toxicity was evaluated using flow cytometry (a). A549 cells were harvested after 1, 3, 5 or 7 days post-treatment and stained with SG. The cell death level is expressed as the percentage of cells with a compromised cell membrane (SG positive cells) to the total cell number. (b) A549 cells were harvested 5 days post-treatment and caspase 3, caspase 9 and caspase 8 activities were measured using fluorogenic assays. All the histograms show an average of at least 3 independent repeats (each condition in triplicate). No significance was observed.
The solubility of MAPbI₃ could be at the origin of the poly-nuclearity and the modified mitochondria. Paradoxically, the metabolic activity rate produced by the mitochondria increased significantly in response to MAPbI₃ exposure (Fig. 7), whereas cell proliferation kinetics and confluency decreased. At first sight, this observation seems controversial but as the cells become giants and escape death, they must expand their energy production for survival. Mitochondria are essential regulators of metabolism, stress responses, cell survival and cell death.⁴⁷,⁴⁸

One reason for the different response of the epithelial and neurons to MAPbI₃ exposure might be the presence of lamellar...

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**Fig. 5** Morphological and proliferation changes caused by MAPbI₃ in A549 human lung epithelial cells. Cells were treated over 5 days with increasing concentrations of MAPbI₃ (50, 100 and 200 μg ml⁻¹) and the cellular changes were evaluated using complementary assays. (a) Variation of the cellular density was evaluated using a Neubauer hemocytometer; (b) morphological changes observed by bright field optical microscopy were quantified then normalized to non-treated cells, which have an overall triangle-shaped form and a size between 15 and 20 μm. The size significantly increased (“giant cells”, size >50 μm) in treated cells and became round. Some cells acquired a stretched/elongated morphology (“elongated cells”, width <7 μm). An average of 500 cells were counted for each condition tested; (c) tubulin and actin distribution: A549 cells were immunostained against β-tubulin (green), actin (red) and nuclei using DAPI (blue), then imaged using confocal microscopy. Non-treated condition is shown on the top panel and treated cells on the bottom panel. Scale bars = 10 μm; (d) cell cycle (sub-G₁, S and G2) distribution assessed by flow cytometry; the histograms show the distribution of sorted cells based on their DNA content which did not change even at the highest concentration of MAPbI₃; (e) proliferation capacity assessed using flow cytometry, the fluorescence intensity of CFSE in treated cells is normalized to the non-treated ones. All the histograms show an average of at least 3 independent repeats (each condition in triplicate). Bars are means ± S.D. One-way ANOVA test followed by a Tukey–Kramer post-hoc test were performed (non-treated vs. MAPbI₃ treated conditions), *p < 0.05 and **p < 0.001.
bodies are naturally present in the cytoplasm of mucosal cells; they are part of the cellular protective mechanisms against environmental influences\(^3\) of the lung alveoli. A549 cells exposed to MAPbI\(_3\) show an increase in the number of lamellar bodies in the cytoplasm. A similar increased number of lamellar bodies was observed in epithelial cells exposed to single-walled carbon nanotube exposure.\(^3\) The cell survival strategies involve countless coordinated physiological and genetic changes that serve to escape cell demise.\(^3\) It seems that one of these strategies consists of increasing the number of lamellar bodies which have a protective function. The genome profiling of treated and non-treated cells may provide a better understanding of the cellular mechanisms underlying the differences in cellular toxicity and cellular phenotypes observed in SH-SY5Y cells, differentiated hippocampal primary neurons and A549 cells.

Finally, our findings are alarming and stress the potential health impact of large-scale production and application of hybrid halide perovskites. Here we inherently assumed that the toxic effect of MAPbI\(_3\) comes from the Pb\(^{2+}\) ions. But it is also known that methylamine can be toxic,\(^2\)\(^5\) as well. This suggests that in the search for compositions where lead is replaced by lighter and presumably less toxic elements (e.g. Sn), one should also consider alternatives for the cation other than methylamine.

### 4. Experimental methods

#### 4.1 Antibodies and compounds

The mouse anti-β-tubulin was purchased from Sigma Aldrich (Switzerland). The Alexa Fluor 594 labelled phalloidin (phalloidin\(_{AF594}\)), the secondary antibody donkey anti-rabbit Alexa488, the propidium iodide (PI) dye, the CarboxyFluorescein Succinimidyl Ester (CFSE) proliferation dye, the Sytox Green dead cell dye, the MitoTracker green dye, the MitoTracker CMXRos Red dye, the polyvinyl alcohol mounting medium with DABCO (PV A), the phosphate buffer saline solution (PBS) and all the cell culture media reagents were purchased from Life Technologies (Switzerland). The CaspaTag™ fluorescein caspase 3, 8 or 9 activity kit was purchased from Enzo Life Sciences (Switzerland). Tetrazolium derivative 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma-Aldrich (Switzerland).

#### 4.2 Scanning electron microscopy (SEM) imaging

Scanning electron microscopy images were acquired with a MERLIN Zeiss electron microscope. The elemental composition of the MAPbI\(_3\) crystallites and their solid decomposition byproducts were analyzed by EDS. Energy-dispersive X-ray spectroscopy (EDS) measurements were performed with an X-MAX EDS detector mounted at a 35 degree take-off angle with a SATW window. EDS spectra were taken at a working distance of 8.5 mm with 20 keV accelerating voltage and a current held at 200 pA.

#### 4.3 Inductively coupled plasma atomic emission spectroscopy (ICP-AES)

The solubility of MAPbI\(_3\) polycrystalline powder was determined in deionized water as well as in two types (DMEM and DMEM : F12) of cell culture medium by using Inductively
primary neurons will develop over time axonal and dendritic
weeks after plating): in growth media, the hippocampal
Hippocampal neurons were prepared from P1 mice pups
4.5 Culture of mice primary hippocampal neurons
24 well transparent plates (BD Biosciences, Switzerland) for
respectively plated at a density of 13,000 and 24,000 cells per well in
4.4 Culture of mammalian cell lines
A549 Type II human alveolar basal epithelial cells were cul-
Life Technologies) supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin/Streptomycin (P/S). The human neuroblastoma SH-SY5Y cell line was cultured in a DMEM: F12 (Life Technologies) supplemented with 10% FBS and 1% Penicillin/Streptomycin (P/S). The human neuroblastoma SH-SY5Y cell line was cultured in DMEM medium (Life Technologies) supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin/Streptomycin (P/S). The human neuroblastoma SH-SY5Y cell line was cultured in DMEM : F12), respectively. Aliquots were taken at various time points (1–5 days), and filtered using 0.22 µm pore cellulose filters (Merck Millipore, Switzerland). The lead and iodine concentrations of the clear and transparent solutions were analyzed using inductively coupled plasma optical emission spectroscopy (Shimadzu ICPE-9000). Each sample was analyzed in triplicate.
4.4 Culture of mammalian cell lines
A549 Type II human alveolar basal epithelial cells were cul-
tured in DMEM medium (Life Technologies) supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin/Streptomycin (P/S). The human neuroblastoma SH-SY5Y cell line was cultured in a DMEM: F12 (Life Technologies) supplemented with 10% FBS and 1% P/S. Both the cell lines were cultured under a humidified 5% CO₂ atmosphere at 37 °C and respectively plated at a density of 13,000 and 24,000 cells per well in 24 well transparent plates (BD Biosciences, Switzerland) for further analysis.
4.5 Culture of mice primary hippocampal neurons
Hippocampal neurons were prepared from P1 mice pups (Harlan Laboratories) as previously described by Steiner et al. and were plated at a density of 30,000 cells per well in 96 well plates, coated with poly-L-lysine, in growth medium (minimum Eagle’s medium with 20 mM glucose, 0.5 mM glutamine, 100 units per mL penicillin, 100 µg per mL streptomycin, 10% horse serum). The neurons were used once differentiated (two weeks after plating): in growth media, the hippocampal primary neurons will develop over time axonal and dendritic arbores that can be positively stained respectively with MAP2 and Tau markers. Hippocampal primary neurons are considered as post-differentiated after 2 weeks in culture.
4.6 Preparation of conditioned medium for treatment
5 days before administration to cells, MAPbI₃ was suspended at concentrations of 50, 100 and 200 µg ml⁻¹ in a preheated culture medium. Solutions are prepared at RT and vortexed under a laminar flow. They remained 5 days at RT, then are filtered in a 220 nm pore size filter (Merck Millipore, Switzerland) and then stored at 4 °C.
4.7 Quantification of cell death by dye exclusion method in mammalian cell lines
SH-SY5Y cells and A549 cells were plated in 24 well plates. The following day, the original culture medium was removed from the cells and replaced by a medium spiked with increasing concentrations of MAPbI₃ (50, 100 and 200 µg ml⁻¹). After 1, 3, 5 or 7 days of exposure, cell death was quantified using the vital dye Sytox Green (SG) which only penetrates into cells with a damaged plasma membrane. The supernatant and adherent cells were collected at each indicated time point. After 5 min of centrifugation at 250g, the cells were re-suspended in PBS containing SG at a final concentration of 330 nM. The cells were then analyzed (20,000 events per condition were counted) by flow cytometry using an Accuri C6 (BD Biosciences, Switzerland). SG emission was collected in the FL1 channel and FlowJo software (Treestar, USA) was used for subsequent analysis.
4.8 Quantification of cell death by dye exclusion method in primary hippocampal neurons exposed to MAPbI₃
Hippocampal primary neurons were plated in 96 well plates. After 2 weeks of culture, the original culture medium was
removed from the cells and replaced by a medium spiked with increasing concentrations of MAPbI₃ (of 50, 100 and 200 μg ml⁻¹). Cell death was quantified as previously described by Mahul-Mellier et al. using the vital dye exclusion method using Sytox Green (SG, Life Technologies, Switzerland), a membrane impermeable dye which will enter only into cells with damaged plasma membranes after 3, 10, 17 and 25 days of exposure to MAPbI₃. Briefly neuronal cells were washed 3 times with PBS before being incubated with SG at a final concentration of 330 nM. After 15 minutes of incubation, the cells were washed twice with PBS and the fluorescence was quantified by using a Tecan infinite M200 Pro plate reader (Tecan, Switzerland) with the respective excitation and emission wavelength of 487 nm and 519 nm.

4.9 Quantification of active caspase 3, 8 and 9 in mammalian cell lines and in hippocampal primary neurons

SH-SY5Y cells and A549 cells were plated in 24 well plates. The following day, the original culture medium was removed from the cells and replaced by a medium spiked with increasing concentrations of MAPbI₃ (50, 100 and 200 μg ml⁻¹). Caspase activity in living cells was quantified using the CaspaTag™ fluorogenic kits used specific Fluorochrome-Labeled Inhibitors of Caspases (FLICA). These probes passively enter the cells and bind irreversibly to the active caspases. After 5 days of exposure to MAPbI₃, SH-SY5Y and A549 cells were harvested and incubated for one hour at 37 °C with FAM-DEVD-FMK (caspase 3), FAM-LETD-FMK (caspase 8) or FAM-LEHD-FMK (caspase 9) and with the vital dye Propidium Iodide (PI) in accordance with the supplier’s instructions. Fluorescein emission was analyzed by flow cytometry [Accuri C6 (BD Biosciences)] in the FL1 channel and PI emission was collected in the FL3 channel. FlowJo was used for subsequent analysis.

For hippocampal primary neurons, cells were plated in 96 well plates. After 2 weeks of culture, the original culture medium was removed from the cells and replaced by a medium spiked with increasing concentrations of MAPbI₃ (50, 100 and 200 μg ml⁻¹). Neurons were washed 3 times with PBS and incubated for 30 minutes at 37 °C with FAM-DEVD-FMK (caspase 3), FAM-LETD-fmk (caspase 8) or FAM-LEHD-fmk (caspase 9) in accordance with the supplier’s instructions. Fluorescein emission was quantified by using a Tecan infinite M200 Pro plate reader (Tecan, Switzerland) with the respective excitation and emission wavelength of 487 nm and 519 nm.

4.10 Quantification of the metabolic activity of mammalian cell lines and hippocampal primary neurons

SH-SY5Y and A549 cells were plated in 24-well plates and hippocampal primary neurons in 96 well plates. The following day, the original culture medium was removed from the cells and replaced by a medium spiked with increasing concentrations of MAPbI₃ (50, 100 and 200 μg ml⁻¹). The metabolic activity of the cells was measured using the tetrazolium derivative 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay. This colorimetric assay is based on the capacity of the healthy cells to reduce MTT (Sigma Aldrich, Switzerland) in a purple formazan product. Briefly, after the indicated days of exposure to MAPbI₃, 5 mg ml⁻¹ MTT was added to the different cell cultures. After 3 hours of incubation, the culture medium was aspirated and replaced by 100 μl or 20 μl (respectively in 24 or 96 well plates) of DMSO (Sigma-Aldrich). The levels of formation of formazan products were quantified by using a Tecan infinite M200 Pro plate reader (Tecan). The absorbance of the converted dye was measured at a wavelength of 570 nm with background subtraction at 630-690 nm. Normalization to the number of counted cells had to be performed, in order to take into account both the changes in proliferation and the confluence of A549 and SH cells and thus to fit the measured MTT absorption to the relative mitochondrial activity.

4.11 Counting of the A549 and SH-SY5Y cell density

Cells were plated in 24-well plates. The following day, the original culture medium was removed from the cells and replaced by a medium spiked with increasing concentrations of MAPbI₃ (50, 100 and 200 μg ml⁻¹). After 5 days of exposure, the cell titer was quantified using a Neubauer hemocytometer chamber. The cells were harvested after trypsin treatment (Life Technologies) and 10 μl of the cell suspension was placed in the counting chamber.

4.12 Quantification of the morphological alterations of A549 cells

A549 cells were plated in 24-well plates. The following day, the original culture medium was removed from the cells and replaced by a medium spiked with increasing concentrations of MAPbI₃ (50, 100 and 200 μg ml⁻¹). A549 cells were imaged 5 days after exposure to various concentrations of MAPbI₃ on an EVOS bright field microscope (Life Technologies). The respective sizes and shapes of the cells were analyzed using FIJI software (NIH, Bethesda, MD, USA).

4.13 Quantification of the cell cycle distribution of A549 cells

A549 cells were plated in 24-well plates. The following day, the original culture medium was removed from the cells and replaced with a medium spiked with increasing concentrations of MAPbI₃ (50, 100 and 200 μg ml⁻¹). After 5 days of exposure, the cell cycle was analyzed using propidium iodide (PI) staining (Life Technologies). The cells were harvested using the classical trypsin (Life Technologies) procedure. After 5 minutes of centrifugation at 200g at 4 °C, the cells were suspended in ice-cold buffer, gently mixed then added to an equal volume of cold absolute ethanol and stored at 4 °C for 24 hours. The cells were then centrifuged for 10 minutes at 200g and washed with cold PBS. The pellets were resuspended in cold PBS containing 0.1% (v/v) Triton X-100 (Sigma-Aldrich) and 0.4% (v/v) 5 μg ml⁻¹ PI (which allows the cellular DNA content staining). The cell cycle distribution was analyzed (15,000 events per condition were measured) by flow cytometry using an Accuri C6 (BD Biosciences). PI emission was collected
in the Fl2 channel and FlowJo software (Treestar) was used for subsequent analysis.

4.14 Quantification of cell proliferation of A549 cells
A549 cells were plated in 24-well plates and the medium was replaced by PBS containing CFSE proliferation dye (Life Technologies) at a final concentration of 5 µM and left for 10 minutes at 37 °C then rinsed with PBS and left in complete medium overnight. The following day, the medium was removed from the cells and replaced with a medium spiked with increasing concentrations of MAPbI3 (50, 100 and 200 µg ml⁻¹). After 0, 1, 3 and 5 days of exposure, treated and non-treated A549 cells were harvested using the trypsin procedure and PI was added to the cell suspension. The cell proliferation capacity was then analyzed (15 000 events per condition were measured) by flow cytometry using an Accuri C6 (BD Biosciences). CFSE emission was collected in the Fl1 channel and FlowJo software (Treestar) was used for subsequent analysis.

4.15 Immunocytochemistry (ICC)
A549 cells were seeded onto coverslips coated with poly-l-lysine (Life Technologies) and treated with MAPbI3 at a final concentration of 200 µg ml⁻¹. After the treatment, the cells were washed 3 times with PBS and then fixed in 4% PFA (paraformaldehyde, Sigma-Aldrich) for 20 min at RT. After blocking with 3% BSA (Bovine Serum Albumin) in 0.1% Triton X-100 PBS (Phosphate Buffered Saline) (PBST) for 30 minutes at RT, the cells were incubated with the rabbit anti-β-tubulin primary antibody for two hours at RT. The cells were rinsed five times in PBST and subsequently incubated with the secondary anti-mouse Alexa488 at a dilution of 1/800 in PBST and with the labelled phallolidinAP594 at a dilution of 1/1000 in PBST. The cells were washed five times in PBST and incubated for 30 minutes at RT in DAPI at 2 µg ml⁻¹, before mounting in a polyvinyl alcohol mounting medium with DABCO. The cells were then examined with a confocal laser-scanning microscope (LSM 700, Zeiss) with a 40× objective and analyzed using Zen software (Zeiss).

4.16 Electron microscopy
A549 cells were grown on plastic coverslips (Thermanox) coated with poly-l-lysine. The following day, the original culture medium was removed and replaced by a medium spiked with 100 µg per ml MAPbI3. After 5 days of exposure, the cells were fixed using 4% paraformaldehyde for 10 minutes and washed with PBS. The cells were post-fixed with 1% osmium tetroxide reduced with 1.5% potassium ferrocyanide in 0.1 M sodium cacodylate buffer (pH 7.4) for 40 min, then washed with double-distilled water and en-block stained with 1% uranyl acetate in water. After several steps of dehydration in graded alcohol the cells were gradually infiltrated in Durcupan resin which was polymerized and cured overnight at 65 °C. Ultrathin sections (50–60 nm) of selected cells were cut with a diamond knife (DiATOME, Biel, Switzerland) and collected onto single slot (2 mm) copper grids coated with formvar plastic support films. The sections were contrasted with uranyl acetate and lead citrate and imaged by Tecnai Spirit TEM operating at 80 kV of acceleration voltage and equipped with 4k × 4k Eagle CCD camera controlled with TIA acquisition software (FEI, Netherlands).

4.17 Measurement of the mitochondrial membrane potential changes (∆Ψm) and the overall mass of mitochondria in A549 cells
A549 cells were plated in 24 well plates. The following day, the original culture medium was removed from the cells and replaced by a medium spiked with 100 µg ml⁻¹ of MAPbI3. After 5 days of exposure, the ∆Ψm was analyzed using the fluorochrome MitoTracker Red (CMXRos) dye, and the overall mass of mitochondria was quantified using the fluorochrome MitoTracker Green. In short, CMXRos is a dye which passively enters into living cells and that is selectively taken up and retained in mitochondria depending on ∆Ψm, whereas MitoTracker Green can passively diffuse through the plasma membrane and once inside the cells, this dye selectively binds to the lipids of the mitochondria and fluoresces regardless of the mitochondrial membrane potential. Therefore MitoTracker Green allows the overall measurement of the mitochondrial mass.

After 5 days of exposure to MAPbI3, the cells were resuspended and incubated for 20 minutes at 37 °C with CMXRos and MitoTracker Green in accordance with the supplier’s instructions. MitoTracker emission was analyzed by flow cytometry [Accuri C6 (BD Biosciences)] in the Fl2 channel for the CMXRos and in the Fl1 channel for the MitoTracker Green, 30 000 events per condition were measured. FlowJo (Treestar) was used for subsequent analysis.

4.18 Statistical analysis
The experiments were independently repeated at least three times in triplicate, yielding the same pattern of results. Statistical analysis was performed using one-way ANOVA followed by a Tukey-Kramer post-hoc test. The data were regarded as statistically significant at p < 0.05.

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