Cytotoxicity of layered metal phosphorus chalcogenides (MPXy) nanoflakes; FePS₃, CoPS₃, NiPS₃

Latiff, Naziah M.; Mayorga-Martinez, Carmen C.; Khezri, Bahareh; Szokolova, Katerina; Sofer, Zdeněk; Fisher, Adrian C.; Pumera, Martin

2018

Latiff, N. M., Mayorga-Martinez, C. C., Khezri, B., Szokolova, K., Sofer, Z., Fisher, A. C., & Pumera, M. (2018). Cytotoxicity of layered metal phosphorus chalcogenides (MPXy) nanoflakes; FePS₃, CoPS₃, NiPS₃. *FlatChem*, 12, 1-9. doi:10.1016/j.flatc.2018.11.003

<https://hdl.handle.net/10356/137070>

<https://doi.org/10.1016/j.flatc.2018.11.003>

© 2018 Elsevier B. V. All rights reserved. This paper was published in *FlatChem* and is made available with permission of Elsevier B. V.

Downloaded on 26 Apr 2023 08:29:36 SGT

Cytotoxicity of layered metal phosphorus chalcogenides (MPX_Y) nanoflakes; FePS_3 , CoPS_3 , NiPS_3

Naziah M. Latiff,[†] Carmen C. Mayorga-Martinez,^{†‡} Bahareh Khezri,^{†‡} Katerina Szokolova,[‡] Zdenek Sofer,[‡] Adrian C. Fisher,^ϕ Martin Pumera^{‡}*

[[†]] Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, 21 Nanyang Link, Singapore 637371, Singapore

[[‡]] Department of Inorganic Chemistry, University of Chemistry and Technology Prague, Technická 5, 166 28 Prague 6, Czech Republic

[^ϕ] Department of Chemical Engineering and Biotechnology, University of Cambridge, New Museums Site, Pembroke Street, Cambridge CB2 3RA, UK

KEYWORDS: metal thiophosphite, 2D materials, cell viability assay

Abstract

Metal phosphorus chalcogenides is a new class of 2D materials that have shown interesting semiconducting, electronic, anisotropic, magnetic, dielectric, structural and optical properties. Recently, NiPS₃, FePS₃ and CoPS₃ in particular have been highlighted for promising potential in important energy applications such as electrocatalysts for water splitting and hydrogen storage. However, little is known about the toxicological hazards that they may pose on humans. Here, we investigated the cytotoxicity of NiPS₃, FePS₃ and CoPS₃ on human lung carcinoma cells (A549) and normal human bronchial cells (BEAS-2B) to address this concern. Upon treatment of these cells with different MPX_Y materials, we determined the remaining cell viability using water soluble tetrazolium salt (WST-8) assay. We found that CoPS₃ was most toxic followed by FePS₃ with intermediate toxicity while NiPS₃ showed the lowest toxicity among the three materials tested for both cell lines. When viewed with other layered materials, these MPX_Y samples present comparable toxicities with transition metal dichalcogenides and black phosphorus at low concentrations.

Introduction

Metal phosphorus chalcogenides have a general formula MPX_Y where M is a metal (e.g. Mn, Fe, Co, Ni, Cd), X is a chalcogen (i.e. S or Se) and Y is the number of chalcogen atoms typically either 3 or 4). Many studies have reported interesting semiconducting, electronic, anisotropic, magnetic, dielectric, structural, and optical properties of these materials when they are exfoliated into few layers.¹⁻¹⁰ Similar to other layered materials such as graphite, transition metal

dichalcogenides and black phosphorus, the layers in MPX_Y are held together by weak van der Waals forces and can easily be exfoliated into fewer sheets.¹¹⁻¹⁵ Their structures are also similar whereby within each layer, the metal and phosphorus atoms are sandwiched in between the chalcogen atoms.¹¹ Previously, these MPX_Y materials have been well researched for lithium battery applications dating back to the 1980s.¹⁶⁻²¹ More recently, this class of materials has witnessed a renewed interest as new discoveries unveil a wider scope of their possibilities. Over the past three years, research on $NiPS_3$, $FePS_3$, $CoPS_3$ and their derivatives in particular have been highlighted. For example, our group had reported that $CoPS_3$ and $NiPS_3$ exhibit promising electrocatalytic activity for water splitting (i.e. hydrogen evolution reaction, and oxygen evolution reaction).²² This finding was also further supported by studies from Sampath, Xu, Schuhmann and co-workers for $NiPS_3$ and $FePS_3$ with good stabilities of the materials reported.²³⁻²⁶ Additionally, $NiPS_3$ also demonstrated good potential for hydrogen energy storage²⁷ while $FePS_3$ displayed robust intrinsic magnetism for numerous applications^{22,28}.

With increasing research on these materials for technological advances, it is highly possible that they may be commercialized in the future; especially for promising candidates $NiPS_3$, $FePS_3$ and $CoPS_3$. However, with limited knowledge pertaining to their toxicological effects thus far, it is important to fill in this research gap for us to be aware of the hazards that they may pose. Herein, we attempt to address this concern by investigating the cytotoxicity of MPX_Y ; particularly $FePS_3$, $CoPS_3$ and $NiPS_3$ on human lung carcinoma epithelial cells (A549) and normal human bronchial cells (BEAS-2B). To the best of our knowledge, this is the first cytotoxicity study reported for this class of materials. These cell lines were chosen as the primary route of entry for nanomaterials into our body is likely to be the respiratory tract. Upon exposure of the cells to different concentrations of MPX_Y materials for 24 h, the viability of remaining cells was analysed using water soluble

tetrazolium salt (WST-8) assay. The measurements were normalized with respect to a negative control where cells were not treated to any test materials.

Results and discussion

Prior to cytotoxicity investigation, it is important to properly characterize the nanomaterials under study to verify the material synthesized and understand the physicochemical properties of the materials due to a wide range of toxicological behaviours reported for different variations of the same type of nanomaterial studied.²⁹ This can be achieved by scanning electron microscopy (SEM), scanning transmission electron microscopy (STEM), energy dispersive X-ray spectroscopy (EDS), high resolution transmission electron microscopy (HR-TEM), dynamic light scattering (DLS), Raman spectroscopy, X-ray powder diffraction (XRD) and X-ray photoelectron spectroscopy (XPS).

SEM images in Figure 1 confirm the layered structures of solid FePS₃, CoPS₃ and NiPS₃ samples. NiPS₃ showed a lower degree of exfoliation between layers in comparison with the other two samples. To produce well dispersed solutions of the solids, they were suspended in ultrapure water and sonicated for 3 hours. Thereafter, the solutions were characterized by STEM to probe into the morphology and size of the prepared dispersions. This is important as the cytotoxicity assessment would be performed for the materials prepared in solution. From Figure 1, we can observe that NiPS₃ generally show thicker structures amongst the three MPX_Y materials studied. This could possibly be due to the smaller interlayer distance in its solid form as seen from the SEM images earlier. In the case of FePS₃ and CoPS₃, few layered sheets can be seen from the STEM images. Besides comparing the degree of exfoliation, we are also able to observe a large spectrum of lateral dimensions of the materials ranging from 1 to 5 μm .

Figure 1. Characterization of solid FePS_3 , CoPS_3 , NiPS_3 by SEM (left) and their liquid dispersions by STEM (right). The liquid suspensions were prepared by dispersing the solid samples in ultrapure water followed by ultrasonication for 3 hours.

Another characterization tool, EDS, was conducted to ascertain the composition and formula of the materials (refer to Figure 2). EDS results showed the ratios of P/metal and S/metal are close to the expected ratio of 1:1 and 3:1 (see Table 1). Additionally, we have also performed TEM-EDS which show similar results with regards to size and elements present (see Figure S1).

Figure 2. Characterization of metal phosphorus chalcogenides under study (FePS_3 , CoPS_3 , NiPS_3) by EDS.

Table 1. Results from EDS analysis of the metal phosphorus chalcogenides MPX_Y (FePS_3 , CoPS_3 , NiPS_3) samples. Data below are reported in terms of atomic percentage. P to metal (P/M) and S to metal (S/M) ratios are also calculated.

	Metal (M)	P	S	O	C	P/M	S/M
FePS_3	13.64	13.27	34.83	6.34	31.92	0.97	2.55
CoPS_3	10.86	14.06	34.75	14.11	26.23	1.29	3.20
NiPS_3	10.35	11.93	33.44	2.56	41.72	1.15	3.23

Besides SEM, STEM and DLS, we also characterized the materials using zeta-potential measurement for their surface charges. This is important as surface charge has been reported as a parameter that can influence the toxicity of nanomaterials.³⁰ The surface charges of the MPX_Y

nanomaterials suspended in cell culture media are as shown in Table 2, and revealed near zero values.

Table 2. Results from surface charge analysis of the metal phosphorus chalcogenides MPX_Y ($FePS_3$, $CoPS_3$, $NiPS_3$) samples.

	Surface charge (mV)
$FePS_3$	0.04 ± 0.08
$CoPS_3$	0.00 ± 0.03
$NiPS_3$	0.03 ± 0.04

Furthermore, we also conducted HRTEM on the MPX_Y ($FePS_3$, $CoPS_3$, $NiPS_3$) samples (Figure 3). The samples consisting from individual sheets with sizes up to few microns and typically few layer thickness. The SAED images in inserts shows the hexagonal symmetry of MPS_3 compounds lattice.²² The HR-TEM images in the right column shows the hexagonal arrangement of atoms within MPS_3 structure.

Figure 3. The TEM images of FePS₃, CoPS₃, NiPS₃ with SAED in the image insert and corresponding HR-TEM images in right column. The scale bar in HR-TEM images are 5nm.

Apart from the above characterizations, Raman spectroscopy was also performed (Figure 4). The Raman spectra corresponds to the typical spectra of MPS₃ compounds with C₂ symmetry consisting from P₂S₆ units.²² In the spectra can be fund A_{1g} modes (590, 387, 255 cm⁻¹) as well as E_g modes (560, 283, 235, 180 and 150 cm⁻¹). The cation vibration frequencies can be found at low wavenumbers below 150 cm⁻¹.

Figure 4. Raman spectroscopy for metal phosphorus chalcogenides under study (FePS₃, CoPS₃, NiPS₃).

Following that, the MPX_Y materials were further characterized using XRD (Figure 5). The X-ray diffraction shows presence of single phase corresponding to FePS₃, CoPS₃ and NiPS₃ phases.²²

All samples show high preferential orientation towards (001) reflections due to its layered character.

Figure 5. XRD characterization of metal phosphorus chalcogenides under study (FePS₃, CoPS₃, NiPS₃).

The bonding state and the chemical composition of MPS_x (M = Fe, Co, Ni) were studied by analysis of XPS spectra. The survey spectrums are available in Figure S2. The high-resolution XPS spectra of metals (Fe 2p, Co 2p, Ni 2p), P 2p, and S 2p of these materials are evaluated (see Figure 6). The positions of the respective signals are summarized in Table S1. The high-resolution XPS spectra of metals for FePS₃, CoPS₃, and NiPS₃ show both metallic phase of Fe 2p_(3/2;1/2), Co 2p_(3/2;1/2), Ni 2p_(3/2;1/2) and oxidized phase of FeO (Fe 2p_{3/2}), CoO (Co 2p_{3/2}), and NiO (Ni 2p_{3/2}, Ni 2p_{1/2}).²² The peak fitting of the P 2p high-resolution XPS spectrum could be divided into three major peak components: 131.2 ± 0.2 eV and 131.9 ± 0.1 eV for P 2p_{3/2} and P 2p_{1/2} attributed to P-S bonding^{31,32} and 134.1 ± 0.5 eV for P 2p_{3/2} attributed to P₄O₁₀.³³ Two deconvoluted peaks appear in the S 2p region of all MPX_Y. They can be attributed to S 2p_{3/2} and S 2p_{1/2} with average positions of 161.7 ± 0.2 and 162.9 ± 0.1 eV, respectively, it can be assumed that they confirm the presence of polysulfide.³¹ However, the third peak at position of 164.1 eV can be found in NiPS₃, this peak corresponds to sulphur.³¹

Figure 6. High resolution XPS deconvolution of MPX_Y materials for metal (A), phosphorus (B) and sulphur (C).

For the cytotoxicity assessment of MPX_Y nanomaterials ($FePS_3$, $CoPS_3$ and $NiPS_3$), we considered using two commonly used cell viability assays; methylthiazolyldiphenyl tetrazoliumbromide (MTT) and water soluble tetrazolium salt (WST-8) assays.³⁴⁻³⁶ These assays are made of active coloured tetrazolium reagents that can receive electrons in the presence of viable cells thereby producing a different coloured formazan dye which can be easily measured by absorbance spectroscopy.³⁴⁻³⁶ However, due to reports of possible interferences between nanomaterials with these cell viability assay markers, it is important for us to perform control experiments to ensure reliability of cytotoxicity results.³⁷⁻⁴⁰ These control experiments were

conducted by incubating different concentrations of MPX_Y with the MTT or WST-8 assay reagents in the absence of cells. Data was normalized to the absorbance reading of a blank control which contains no nanomaterials.

In the case of MTT (see Figure S3), more than 800% of normalized formazan was produced at the highest concentration studied (i.e. 100 $\mu\text{g}/\text{ml}$) for FePS_3 and CoPS_3 , whereas for WST-8 (see Figure 7), the normalized formazan produced did not exceed 200% throughout the range of concentration tested (0-100 mg/ml). This indicates that between the two assays, WST-8 assay proves to produce less interference with the MPX_Y materials for the range of concentration studied. As such, we proceeded to perform the cytotoxicity assessment using WST-8 assay for this study.

Figure 7. Control experiments: Normalized formazan produced upon incubation with varying concentrations of MPX_Y materials for WST-8 assay. The results shown are the averaged values with $\pm$ standard deviations obtained from at least three repeat experiments.

Previously, our group had performed two times washing steps with phosphate buffer solution (PBS, pH 7.2) to remove nanomaterials prior to cell viability measurements.^{38,41-47} However, for these samples, we observed that the washing steps were not able to remove traces of nanomaterials efficiently, particularly at the highest concentration of MPX_Y materials tested (i.e. 100µg/ml). Trace metal analysis conducted by inductively coupled plasma mass spectrometry (ICP-MS) of the solutions removed from wells exposed to 100µg/ml MPX_Y suspensions revealed that less than 30% of the MPX_Y samples were removed through the washing steps at the highest concentration tested (refer to Table S2). This could be due to adsorption of the MPX_Y materials to A549 cells hindering their complete removal by washing with PBS.

Due to presence of strong nanomaterial-induced interference effects, we decided to explore a slightly different approach in conducting the cytotoxicity assessment in search for a more accurate analysis. For this study, the absorbance readings from control experiments (in the absence of cells) were used as background subtraction from the values obtained with cells and nanomaterials. In this way, any interference caused by the nanomaterials which affects the absorbance readings can be subtracted away from the measurement.

Using this new approach, we found the cell viabilities determined using WST-8 assay upon exposure to different concentrations of MPX_Y samples with A549 cells for 24h to be as shown in Figure 8. All three MPX_Y materials studied (FePS₃, CoPS₃, NiPS₃) showed high cell viabilities (>75%) at low concentrations tested (0-25µg/ml). This suggests that these MPX_Y samples are safe to use at such low concentration range. However, at higher concentrations (≥50µg/ml), we start to see the toxic effects of the materials. The toxicological profile revealed CoPS₃ to be the most toxic among the three, followed by FePS₃ while NiPS₃ was found to be the least toxic. This can be

clearly seen after incubation of A549 cells with 100 μ g/ml of the MPX_Y samples, where cell viabilities reduce to 43%, 26% and 70% for FePS₃, CoPS₃ and NiPS₃ respectively.

Figure 8. Cytotoxicity assessment of MPX_Y (FePS₃, CoPS₃, NiPS₃) samples with A549 cells analyzed by WST-8 cell viability assay. The results shown are the averaged values with $\pm$ standard deviations obtained from at least three repeat experiments.

To confirm the cellular uptake of these nanomaterials, MPX_Y materials penetrating inside A549 cells after 24 hours exposure with the materials through microscope imaging (see Figure 9) was evaluated. In the cells indicated with red arrow and circles, nanomaterials can be identified to be present inside these cells. Previous reports have shown that cells can engulf nanomaterials through different pathways such as clathrin-mediated endocytosis, caveolae-dependent endocytosis, pinocytosis/macropinocytosis and phagocytosis.⁴⁸ Further studies are required to provide a deeper understanding for this observation.

Figure 9. Microscope images of A549 cells after 24 hours incubation with the MPX_Y materials showing penetration of the materials into the cells. The scale bar represents 10 μm .

Even though A549 is a commonly used cell line for nanotoxicology studies, it is a cancerous cell line and may not provide a good representation for the toxicity towards normal cells. To have a better representation of the cytotoxicity of these MPX_Y materials, we also conducted a cytotoxicity assessment of the materials on BEAS-2B human bronchial epithelial cells. The result from this experiment can be seen in Figure 10.

Here, we also see a similar trend in toxicity; CoPS_3 (most toxic) > FePS_3 > NiPS_3 (least toxic). In comparison between the two cell lines investigated, we observe that BEAS-2B cells are generally more sensitive towards FePS_3 and CoPS_3 as the cell viability values decrease at a much faster rate compared to that seen for A549 cells (Figure 8). Interestingly, for NiPS_3 , we found that the cell viability values remain above 75% throughout the range of concentration tested (0-100 $\mu\text{g/ml}$). This implies that NiPS_3 can be a potentially safe and biocompatible material.

Figure 10. Cytotoxicity assessment of MPX_Y (FePS_3 , CoPS_3 , NiPS_3) samples with BEAS-2B cells analyzed by WST-8 cell viability assay. The results shown are the averaged values with $\pm$ standard deviations obtained from at least three repeat experiments.

Production of reactive oxygen species (ROS) is a common mechanism of cell death induced by nanomaterials by causing oxidative stress and damaging cell components such as proteins, lipids, and nucleic acids.⁴⁹ A nanomaterial's inherently high oxidizing property may result in a higher

generation of ROS in the exposed cells, thereby increasing its oxidative stress to lead to higher toxicity. In a previous study, the inherent electrochemistry of these materials have been investigated.²² It revealed that there were strong innate oxidation signals by CoPS₃ while mild oxidation peaks was exhibited by FePS₃ in their first scans. On the other hand, NiPS₃ showed no inherent oxidation peak in its first scan. This suggest that inherently CoPS₃ is more easily oxidized compared to the other two MPX_Y samples. This is followed by FePS₃ while NiPS₃ is the least oxidizing. We find that the inherent oxidizing behavior of these MPX_Y material matches well with our toxicity trend and ROS production is likely to be a mechanism for inducing cell death.

Previously, there have been reports of thicker layers of 2D nanomaterials exhibiting lower toxicity.^{43,50} In this work, we found that NiPS₃, which show a lower degree of exfoliation compared to CoPS₃ and FePS₃ (as seen from STEM characterization) to be the least toxic among the three MPX_Y materials tested. This difference could contribute to NiPS₃ exhibiting a lower toxicity relative to the other two MPX_Y materials studied. Another possible factor that can contribute to the differences in toxicity of nanomaterials is the level of endotoxin contamination in the samples.⁵¹ We had not considered this factor in this study. Future work has to be done to investigate this effect on MPX_Y materials.

When we compare these results with our previous work on other 2D materials tested under similar conditions (see Table 3), we observe that the MPX_Y samples (i.e. FePS₃, CoPS₃, NiPS₃) display similar toxicities with transition metal dichalcogenide (TMD, e.g. MoS₂) and black phosphorus (BP) while showing lower toxicity compared to graphene oxide. With many recent reports of TMD and BP in the field of biomedicine, this preliminary result suggests good potential of MPX_Y expanding into such areas too.^{52,53} For future work, it would be interesting to study the

toxicity effects of more inorganic metal chalcogenides such as GeSb_2Te_4 , $\text{Rb}_2\text{Mn}_3\text{Se}_4$, and $\text{Cs}_2\text{Mn}_3\text{Se}_4$ which have yet been investigated.⁵⁴⁻⁵⁶

Table 3. Comparison of normalized percentages as determined by WST-8 assay upon treatment of A549 cells for 24 h with 25 $\mu\text{g}/\text{ml}$ of FePS_3 , CoPS_3 , NiPS_3 , MoS_2 , black phosphorus (BP) and 20 $\mu\text{g}/\text{ml}$ of graphene oxide prepared by Hummers oxidation method (GO).

	Cell viability (%)	Reference
FePS₃	92	Our work
CoPS₃	81	Our work
NiPS₃	79	Our work
MoS₂	78	43
BP	81	44
GO	60	41

Conclusion

As more and more nanomaterials progress from research laboratories into our daily lives, it becomes increasingly important for us to keep up with their toxicity studies to safeguard the public and scientific community. Here, we investigated the cytotoxicity of metal phosphorus chalcogenides MPX_Y (NiPS_3 , FePS_3 , CoPS_3) nanoflakes on human lung carcinoma A549 cells and normal human bronchial BEAS-2B cells. For both cell lines, we found that CoPS_3 shows toxic behaviour followed by FePS_3 with mild toxicity while NiPS_3 showed the lowest toxicity amongst them. In comparison with other layered materials, MPX_Y samples showed similar toxicities with transition metal dichalcogenides and black phosphorus at low concentrations. Further toxicity assessments need to be conducted to further analyze the impacts of MPX_Y materials to humans and the environment, especially in the areas of understanding their toxicity mechanism and cellular uptake as well as the role of endotoxin contamination in the samples. Nevertheless, this preliminary study is an important first step in shedding light on the toxicity of this class of 2D materials.

Experimental section

Synthesis of metal phosphorus chalcogenides MPX_Y

The reagents and synthesis procedures for metal phosphorus chalcogenides MPX_Y (M= Fe, Co, Ni) are as reported in reference [22].

Characterizations

The MPX_Y samples were characterized by SEM-EDS and STEM using JOEL 7600F. SEM images were obtained using gentle-beam mode (GB-H) at 2kV accelerating voltage and working distance of 4.7mm whereas EDS images were collected using 15kV accelerating voltage and working distance of 14.8mm. For STEM, the samples were prepared by drop casting 1 mg/mL MPX_Y suspensions in water on STEM Cu grids followed by drying under lamp overnight. ICP-MS was performed using an Agilent 7700x system from Japan. The samples were digested by concentrated nitric acid in a microwave digestion system (Milestone Ethos, Italy) before analysis. X-ray powder diffraction data were collected at room temperature on Bruker D8 Discoverer (Bruker, Germany) powder diffractometer with parafocusing Bragg–Brentano geometry using CuK α radiation ($\lambda = 0.15418$ nm, U = 40 kV, I = 40 mA). Data were scanned over the angular range 10–80° (2 θ) with a step size of 0.015° (2 θ). Data evaluation was performed in the software package EVA. inVia Raman microscope (Renishaw, England) in backscattering geometry with CCD detector was used for Raman spectroscopy. DPSS laser (532 nm, 50 mW) with applied power of 5 mW and 50x magnification objective was used for the measurement. Instrument calibration was achieved with a silicon reference which gives a peak position at 520 cm⁻¹ and a resolution of

less than 1 cm⁻¹. The samples were suspended in deionized water (1 mg/ml) and ultrasonicated for 10 min. The suspension was deposited on a small piece of silicon wafer and dried. High resolution transmission electron microscopy (HR-TEM) was performed using an EFTEM Jeol 2200 FS microscope (Jeol, Japan). A 200 keV acceleration voltage was used for measurement. Elemental maps and EDS spectra were acquired with SDD detector X-MaxN 80 TS from Oxford Instruments (England). Sample preparation was attained by drop casting the suspension (1 mg mL⁻¹ in water) on a TEM grid (Cu; 200 mesh; Formvar/carbon) and dried at 60 °C for 12 h. High resolution X-ray photoelectron spectroscopy (XPS) was performed with ESCAProbeP spectrometer (Omicron Nanotechnology Ltd, Germany) with a monochromatic aluminum X-ray radiation source (1486.7 eV). Wide scan surveys of all elements were performed with subsequent high-resolution scans of the Fe 2p, Co 2p, Ni 2p, P 2p, and S 2p. The samples were placed in a conductive carrier made from a high purity silver bar.

MTT control experiment

Cell-free MTT control experiments were conducted in a similar manner as that for WST-8. However, in this case, 1mg/ml MTT assay was used, the reaction mixtures were incubation for 3h and the absorbance readings were measured at 570nm.

WST-8 control experiment

For cell-free WST-8 control experiments, different concentrations of MPX_Y suspensions were incubated with 10% WST-8 assay in cell culture media for 1h. Thereafter, the absorbance reading was measured at 450nm. The results were normalized with a blank control which was not subjected to any test materials.

Cell culture

A549 human lung carcinoma epithelial cells (PAA laboratories) and BEAS-2B human bronchial epithelial cells (ATCC) were cultured in DMEM (Dulbecco's modified eagle medium, Life Technologies) supplemented with 10% FBS (foetal bovine serum, Life Technologies) and 1% penicillin/streptomycin (Capricorn). The cells were incubated at 37°C under 5% CO₂ atmosphere.

Cell exposure to MPX_Y

Cells were seeded on 96 well plates (5000 cells/well) for 24h. Following that, the cell culture media was removed and replaced with different concentrations of MPX_Y suspensions in cell culture media (0, 3.125, 6.25, 12.5, 50, 100µg/mL; 100µl/well). The MPX_Y suspensions in cell culture media were prepared by dilution from 1 mg/mL MPX_Y suspensions in ultrapure water upon sonication for 3h. A set of positive control containing 10% DMSO (dimethyl sulfoxide, Tedia) was prepared for every set of experiment to check on the vitality of the cells used.

WST-8 Cell viability assay

Following 24h exposure of the A549 cells to varying concentrations of MPX_Y suspensions, WST-8 cell viability assay were conducted. 10µl of WST-8 assay was added directly to each well after the exposure with test materials. Following addition of WST-8 assay, the plates were wrapped in Aluminium foil and incubated at 37°C and 5% CO₂ for 1h before absorbance measurement at 450nm. Absorbance readings obtained from a corresponding set of control experiments were subtracted away from the absorbance readings obtained from wells with cells. Results from these readings were then normalization with the negative control which was not subjected to any test materials.

ASSOCIATED CONTENT

Supporting Information

The following files are available free of charge. Result from MTT control experiment. ICP-MS analysis from washing steps performed on wells exposed to 100 μ g/ml of MPX_Y suspensions.

AUTHOR INFORMATION

Corresponding Author

*E-mail: pumera@ntu.edu.sg (M. Pumera)

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

M.P., A.C.F. and N.M.L were funded by Singapore's National Research Foundation (NRF) under its Campus for Research Excellence and Technological Enterprise (CREATE) programme. Z.S. acknowledges the Czech Science Foundation (GACR No.17-11456S). BEAS-2B cells were kindly provided by Prof. Loo Say Chye Joachim, NTU. M.P. thanks to Tier 1 99/13 grant (Ministry of Education, Singapore) for funding. This work was created with the financial support of the Neuron Foundation for science support. This work was supported by the project Advanced Functional Nanorobots (reg. No. CZ.02.1.01/0.0/0.0/15_003/0000444 financed by the EFRR).

ABBREVIATIONS

MPX_Y, metal phosphorus chalcogenides; WST-8, water soluble tetrazolium salt; MTT, methylthiazolyldiphenyl tetrazoliumbromide; TMD, transition metal dichalcogenides

REFERENCES

- [1] Kuch, W.; Schäfer, R.; Fischer, P.; Hillebrecht, F. U. *Magnetic Microscopy of Layered Structures*, in *Springer Series in Surface Sciences* [Online]; Springer: Heidelberg, 2015; vol. 57, pp 1–9. <http://www.springer.com/gp/book/9783662445310> (assessed Sep 18, 2017).
- [2] Lagadic, I.; Lacroix, P. G.; Clément, R. Layered MPS₃ (M = Mn, Cd) Thin Films as Host Matrixes for Nonlinear Optical Material Processing. *Chem. Mater.* **1997**, *9*, 2004–2012.
- [3] Ouvrard, G.; Brec, R.; Rouxel, J. Structural Determination of some MPS Layered Phases (M = Mn, Fe, Co, Ni and Cd). *Mat. Res. Bull.* **1985**, *20*, 1181–1189.
- [4] Brec, R.; Ouvrard, G.; Rouxel, J. Relationship between Structure Parameters and Chemical Properties in some MPS₃ Layered Phases. *Mat. Res. Bull.* **1985**, *20*, 1257–1263.
- [5] Taylor, B. E.; Steger, J.; Wold, A. Preparation and Properties of some Transition metal Phosphorus Trisulfide compounds. *J. Solid State Chem.* **1973**, *7*, 461–467.
- [6] Joy, P. A.; Vasudevan, S. Magnetism in the Layered Transition-metal Thiophosphates MPS₃ (M = Mn, Fe, and Ni). *Phys. Rev. B* **1992**, *46*, 5425–5433.
- [7] Zhang, X.; Zhao, X.; Wu, D.; Jing, Y.; Zhou, Z. MnPSe₃ Monolayer: A Promising 2D Visible-Light Photohydrolytic Catalyst with High Carrier Mobility. *Adv. Sci.* **2016**, *3*, 1600062.
- [8] Li, X.; Wu, X.; Yang, J. Half-Metallicity in MnPSe₃ Exfoliated Nanosheet with Carrier Doping. *J. Am. Chem. Soc.* **2014**, *136*, 11065–11069.
- [9] Chittari, B. L.; Park, Y.; Lee, D.; Han, M.; MacDonald, A. H.; Hwang, E.; Jung, J. Electronic and Magnetic Properties of single-layer MPX₃ Metal Phosphorous Trichalcogenides. *Phys. Rev. B* **2016**, *94*, 184428.
- [10] Clement, R.; Lomas, L.; Leaustic, A.; Codjovi, E.; Audiere, J. P.; Read, J. A.; Francis, A. H. Molecular Composites of the MPS₃ Layered Inorganic Polymers: Chemistry, Magnetic and Dielectric Properties. In *Inorganic and Organometallic Polymers with Special Properties* [Online]; Laine, R. M., Ed.; Springer: Netherlands, 1992; pp. 115-129. <http://www.springer.com/us/book/9789401051545> (assessed Sep 18, 2017).
- [11] Du, K.; Wang, X.; Liu, Y.; Hu, P.; Utama, M. I. B.; Gan, C. K.; Xiong, Q.; Kloc, C. Weak Van der Waals Stacking, Wide-Range Band Gap, and Raman Study on Ultrathin Layers of Metal Phosphorus Trichalcogenides. *ACS Nano* **2016**, *10*, 1738–1743.
- [12] Jariwala, D.; Sangwan, V. K.; Lauhon, L. J.; Marks, T. J.; Hersam, M. C. Emerging Device Applications for Semiconducting Two-Dimensional Transition Metal Dichalcogenides. *ACS Nano* **2014**, *8*, 1102–1120.

- [13] Chia, X.; Eng, A. Y. S.; Ambrosi, A.; Tan, S. M.; Pumera, M. Electrochemistry of Nanostructured Layered Transition-Metal Dichalcogenides. *Chem. Rev.* **2015**, *115*, 11941–11966.
- [14] Ambrosi, A.; Chua, C. K.; Latiff, N. M.; Loo, A. H.; Wong, C. H. A.; Eng, A. Y. S.; Bonanni, A.; Pumera, M. Graphene and its Electrochemistry – an update. *Chem. Soc. Rev.* **2016**, *45*, 2458–2493.
- [15] Gusmão, R.; Sofer, Z.; Pumera, M. Black Phosphorus Rediscovered: From Bulk Material to Monolayers. *Angew. Chem. Int. Ed.* **2017**, *56*, 8052–8072.
- [16] Foot, P. J. S.; Suradi, J.; Lee, P. A. Optical and Electronic Properties of the Layered Semiconductors NiPS₃ and FePS₃. *Mat. Res. Bull.* **1980**, *15*, 189–193.
- [17] Saikh, I.; Julien, C.; Balkanski, M. The Role of NiPS₃ as a Cathode in Secondary Lithium Solid State Batteries. In *Solid State Microbatteries* [Online]; Akridge, J. R., Balkanski, M., Eds.; NATO ASI Series (Series B: Physics); Springer: United States, 1990; pp. 297–300. <http://www.springer.com/gp/book/9780306435058> (assessed Sep 18, 2017).
- [18] Foot, P. J. S.; Katz, T.; Patel, S. N.; Nevett, B. A.; Piecy, A. R.; Balchin, A. A. The Structures and Conduction Mechanisms of Lithium-intercalated and Lithium-substituted Nickel Phosphorus Trisulphide (NiPS₃), and the use of the material as a Secondary Battery Electrode. *Phys. Stat. Sol. A* **1987**, *100*, 11–29.
- [19] Thompson, A. H.; Whittingham, M. S. Transition metal phosphorus trisulfides as battery cathodes. *Mater. Res. Bull.* **1977**, *12*, 741–744.
- [20] Foot, P. J. S.; Suradi, J.; Lee, P. A. *Mat. Res. Bull.* **1980**, *15*, 189–193.
- [21] Raymond, B. R. E. C. Review on Structural and Chemical Properties of Transition Metal Phosphorus Trisulfides MPS₃. *Solid State Ionics* **1986**, *22*, 3–30.
- [22] Mayorga-Martinez, C. C.; Sofer, Z.; Sedmidubský, D.; Huber, Š.; Eng, A. Y. S.; Pumera, M. Layered Metal Thiophosphite Materials: Magnetic, Electrochemical, and Electronic Properties. *ACS Appl. Mater. Interfaces* **2017**, *9*, 12563–12573.
- [23] Jenjeti, R. N.; Austeria, M. P.; Sampath, S. Alternate to Molybdenum Disulfide: A 2D, Few-Layer Transition-Metal Thiophosphate and Its Hydrogen Evolution Reaction Activity over a Wide pH Range. *ChemElectroChem* **2016**, *3*, 1392–1399.
- [24] Li, K.; Rakov, D.; Zhang, W.; Xu, P. Improving the intrinsic electrocatalytic hydrogen evolution activity of few-layer NiPS₃ by cobalt doping. *Chem. Commun.* **2017**, *53*, 8199–8202.
- [25] Konkena, B.; Masa, J.; Botz, A. J. R.; Sinev, I.; Xia, W.; Koßmann, J.; Drautz, R.; Muhler, M.; Schuhmann, W. Metallic NiPS₃@NiOOH Core–Shell Heterostructures as Highly Efficient and Stable Electrocatalyst for the Oxygen Evolution Reaction. *ACS Catal.* **2017**, *7*, 229–237.
- [26] Mukherjee, D.; Austeria, P. M.; Sampath, S. Two-Dimensional, Few-Layer Phosphochalcogenide, FePS₃: A New Catalyst for Electrochemical Hydrogen Evolution over Wide pH Range. *ACS Energy Lett.* **2016**, *1*, 367–372.
- [27] Ismail, N.; Madian, M.; El-Meligi, A. A. Synthesis of NiPS₃ and CoPS and its Hydrogen Storage Capacity. *J. Alloys Compd.* **2014**, *588*, 573–577.
- [28] Lee, J.; Lee, S.; Ryoo, J. H.; Kang, S.; Kim, T. Y.; Kim, P.; Park, C.-H.; Park, J.-G.; Cheong, H. Ising-Type Magnetic Ordering in Atomically Thin FePS₃. *Nano Lett.* **2016**, *16*, 7433–7438.
- [29] Fojtů, M.; Teo, W. Z.; Pumera, M. Environmental Impact and Potential Health Risks of 2D Nanomaterials. *Environ. Sci.: Nano* **2017**, DOI: 10.1039/c7en00401j.
- [30] Sharifi, S.; Behzadi, S.; Laurent, S.; Forrest, M. L.; Stroeve, P.; Mahmoudi, M. Toxicity of nanomaterials. *Chem Soc Rev.* **2012**, *41*, 2323–2343.

- [31] Lau, W. M.; Jin, S.; Wu, X.-W. In Situ X-Ray Photoelectron Spectroscopic Study of Remote Plasma Enhanced Chemical Vapor Deposition of Silicon Nitride on Sulfide Passivated InP. *J. Vac. Sci. Technol., B: Microelectron. Process. Phenom.* **1990**, *8*, 848–855.
- [32] Zhang, J.; et al. A nanohybrid consisting of NiPS₃ nanoparticles coupled with defective graphene as a pH-universal electrocatalyst for efficient hydrogen evolution. *Journal of Materials Chemistry A* **2017**, *5*, 23536–23542.
- [33] Qi, Z.; Lee, W. XPS Study of CMP Mechanisms of NiP Coating for Hard Disk Drive Substrates. *Tribol. Int.* **2010**, *43*, 810–814.
- [34] Riss, T. L.; Moravec, R. A.; Niles, A. L.; Duellman, S.; Benink, H. A.; Worzella, T. J.; Minor, L. Cell Viability Assays. In *Assay Guidance Manual* [Online]; Sittampalam, G. S., Coussens, N. P., Brimacombe, K., et al., Eds.; Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences, 2004. <https://www.ncbi.nlm.nih.gov/books/NBK92019/> (assessed Sep 18, 2017).
- [35] Präbst, K.; Engelhardt, H.; Ringgeler, S.; Hübner, H. Basic Colorimetric Proliferation Assays: MTT, WST, and Resazurin. In *Cell Viability Assays* [Online]; Gilbert, D. F., Friedrich, O., Eds.; Springer Nature: New York, 2017; pp. 1–17. https://link.springer.com/protocol/10.1007%2F978-1-4939-6960-9_1 (assessed Sep 18, 2017).
- [36] Tominaga, H.; Ishiyama, M.; Ohseto, F.; Sasamoto, K.; Hamamoto, T.; Suzuki, K.; Watanabe, M. A Water-soluble Tetrazolium Salt useful for Colorimetric Cell Viability Assay. *Anal. Commun.* **1999**, *36*, 47–50.
- [37] Wörle-Knirsch, J. M.; Pulskamp, K.; Krug, H. F. Oops They Did It Again! Carbon Nanotubes Hoax Scientists in Viability Assays. *Nano Lett.* **2006**, *6*, 1261–1268.
- [38] Latiff, N.; Teo, W. Z.; Sofer, Z.; Huber, Š.; Fisher, A. C.; Pumera, M. Toxicity of Layered Semiconductor Chalcogenides: Beware of Interferences. *RSC Adv.* **2015**, *5*, 67485–67492.
- [39] Dhawan, A.; Sharma, V. Toxicity Assessment of Nanomaterials: Methods and Challenges. *Anal. Bioanal. Chem.* **2010**, *398*, 589–605.
- [40] Liang, L.; Cui, M.; Zhang, M.; Zheng, P.; Deng, Z.; Gao, S.; Wang, X.; Zhang, X.; Wang, C.; Liu, Y.; Xie, L. Nanoparticles' interference in the evaluation of in vitro toxicity of silver nanoparticles. *RSC Adv.* **2015**, *5*, 67327–67334.
- [41] Chng, E. L. K.; Pumera, M. The Toxicity of Graphene Oxides: Dependence on the Oxidative Methods Used. *Chem. Eur. J.* **2013**, *19*, 8227–8235.
- [42] Latiff, N. M.; Sofer, Z.; Fisher, A. C.; Pumera, M. Cytotoxicity of Exfoliated Layered Vanadium Dichalcogenides. *Chem. Eur. J.* **2017**, *23*, 684–690.
- [43] Chng, E. L. K.; Sofer, Z.; Pumera, M. MoS₂ Exhibits Stronger Toxicity with Increased Exfoliation. *Nanoscale* **2014**, *6*, 14412–14418.
- [44] Latiff, N. M.; Teo, W. Z.; Sofer, Z.; Fisher, A. C.; Pumera, M. The Cytotoxicity of Layered Black Phosphorus. *Chem. Eur. J.* **2015**, *21*, 13991–13995.
- [45] Teo, W. Z.; Chng, E. L. K.; Sofer, Z.; Pumera, M. Cytotoxicity of Exfoliated Transition-Metal Dichalcogenides (MoS₂, WS₂, and WSe₂) is Lower Than That of Graphene and its Analogues, *Chem. Eur. J.* **2014**, *20*, 9627–9632.
- [46] Teo, W. Z.; Chng, E. L. K.; Sofer, Z.; Pumera, M. Cytotoxicity of halogenated graphenes, *Nanoscale* **2014**, *6*, 1173–1180.

- [47] Chng, E. L. K.; Zhao, G.; Pumera, M. Towards biocompatible nano/microscale machines: self-propelled catalytic nanomotors not exhibiting acute toxicity. *Nanoscale* **2014**, *6*, 2119–2124.
- [48] Zhao, F.; Zhao, Y.; Liu, Y.; Chang, X.; Chen, C.; Zhao, Y. Cellular Uptake, Intracellular Trafficking, and Cytotoxicity of Nanomaterials. *Small* **2011**, *7*, 1322–1337.
- [49] Fu, P. P.; Xia, Q.; Hwang, H.-M.; Ray, P. C.; Yu, H. Mechanisms of nanotoxicity: Generation of reactive oxygen species. *J. Food Drug Anal.* **2014**, *22*, 64–75.
- [50] Rasulev, B.; Gajewicz, A.; Puzyn, T.; Leszczynska, D.; Leszczynski, J. Nano QSAR: Advances and Challenges. In *Towards Efficient Designing of Safe Nanomaterials: Innovative Merge of Computational Approaches and Experimental Techniques* [Online]; Leszczynski, J., Puzyn, T., Eds.; RSC Publishing: United Kingdom, 2012; pp. 220–256. <http://pubs.rsc.org/en/content/chapter/bk9781849734530-00220/978-1-84973-453-0> (assessed Sep 18, 2017).
- [51] Li, Y.; Boraschi, D. Endotoxin contamination: a key element in the interpretation of nanosafety studies. *Nanomedicine (Lond.)* **2016**, *11*, 269–287.
- [52] Kalantar-zadeh, K.; Ou, J. Z.; Daeneke, T.; Strano, M. S.; Pumera, M.; Gras, S. L. Two-Dimensional Transition Metal Dichalcogenides in Biosystems. *Adv. Funct. Mater.* **2015**, *25*, 5086–5099.
- [53] Fojtů, M.; Chia, X.; Sofer, Z.; Masařík, M.; Pumera, M. Black Phosphorus Nanoparticles Potentiate the Anticancer Effect of Oxaliplatin in Ovarian Cancer Cell Line. *Adv. Funct. Mater.* **2017**, *27*, 1701955.
- [54] Hardtdegen, H.; Rieß, S.; Schuck, M.; Keller, K.; Jost, P.; Du, H.; Bornhöfft, M.; Schwedt, A.; Mussler, G.; Ahe, M. v.; Mayer, J.; Roth, G.; Grützmacher, D.; Mikulics, M. A model structure for interfacial phase change memories: Epitaxial trigonal Ge₁Sb₂Te₄. *J. Alloys Compd.* **2016**, *679*, 285–292.
- [55] Hardtdegen, H.; Mikulics, M.; Rieß, S.; Schuck, M.; Saltzmann, T.; Simon, U.; Longo, M. Modern chemical synthesis methods towards low-dimensional phase change structures in the Ge–Sb–Te material system. *Prog. Cryst. Growth Charact. Mater.* **2015**, *61*, 27–45.
- [56] Bronger, W.; Hardtdegen, H.; Kanert, M.; Müller, P.; Schmitz, D. Alkalimanganselenide und -telluride A₂Mn₃X₄ – Synthese, Kristall- und Spinstruktur. *Zeitschrift für Anorg. und Allg. Chemie* **1996**, *622*, 313–318.

Supporting Information

Cytotoxicity of metal phosphorus chalcogenides (MPX_Y) nanoflakes; FePS₃, CoPS₃, NiPS₃

*Naziah M. Latiff,¹ Carmen C. Mayorga-Martinez,¹ Bahareh Khezri,¹ Katerina Szokolova,²
Zdenek Sofer,² Adrian C. Fisher,³ Martin Pumera^{*1}*

¹ Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, 21 Nanyang Link, Singapore 637371, Singapore

² Department of Inorganic Chemistry, University of Chemistry and Technology Prague, Technická 5, 166 28 Prague 6, Czech Republic

³ Department of Chemical Engineering and Biotechnology, University of Cambridge, New Museums Site, Pembroke Street, Cambridge CB2 3RA, United Kingdom

^{*}pumera.research@gmail.com (M. Pumera).

Figure S1. TEM-EDS images of MPX_Y materials confirming presence of metal, sulfur and phosphorus and their identical homogeneous distribution.

Figure S2. XPS survey spectra of MPX_Y materials.

Table S1. Summary of peak positions for XPS deconvolution of high resolution scans of M 2p, P 2p and S 2p.

MPS _x	Position of M 2p peaks (eV)	Positions of P 2p peaks (eV)	Positions of S 2p peaks (eV)
FePS ₃	Fe 2p _{3/2} $\approx$ 708.2 Fe 2p _{3/2} $\approx$ 712.4 (FeO) Fe 2p _{1/2} $\approx$ 721.4	P 2p _{3/2} $\approx$ 131.0 P 2p _{3/2} $\approx$ 133.6 (P ₄ O ₁₀) P 2p _{1/2} $\approx$ 131.8	S 2p _{3/2} $\approx$ 161.5 S 2p _{1/2} $\approx$ 162.7
CoPS ₃	Co 2p _{3/2} $\approx$ 779.3 Co 2p _{3/2} $\approx$ 783.3 (CoO) Co 2p _{1/2} $\approx$ 794.7	P 2p _{3/2} $\approx$ 131.1 P 2p _{3/2} $\approx$ 133.6 (P ₄ O ₁₀) P 2p _{1/2} $\approx$ 131.8	S 2p _{3/2} $\approx$ 161.6 S 2p _{1/2} $\approx$ 162.8
NiPS ₃	Ni 2p _{3/2} $\approx$ 854.4 Ni 2p _{3/2} $\approx$ 858.4 (NiO) Ni 2p _{3/2} $\approx$ 864.5 Ni 2p _{1/2} $\approx$ 871.9 Ni 2p _{1/2} $\approx$ 876.1 (NiO) Ni 2p _{1/2} $\approx$ 881.8	P 2p _{3/2} $\approx$ 131.4 P 2p _{3/2} $\approx$ 134.5 (P ₄ O ₁₀) P 2p _{1/2} $\approx$ 132.0	S 2p _{3/2} $\approx$ 161.9 S 2p _{3/2} $\approx$ 164.1 (sulphur) S 2p _{1/2} $\approx$ 162.9

Figure S3. Control experiments: Normalized formazan produced upon incubation with varying concentrations of MPX_Y materials for MTT assay. The results shown are the averaged values with $\pm$ standard deviations obtained from at least three repeat experiments.

Table S2. Amount of Fe, Ni and Co removed through removal of media and two times washing steps with phosphate buffer solution analysed by ICP-MS from the wells exposed to 100µg/ml of MPX_Y suspensions.

		Fe (ppm)	Co (ppm)	Ni (ppm)
Amount of metal removed by	First removal of media	12.77	24.68	6.42
	First washing step	5.17	2.27	0.41
	Second washing step	2.33	2.03	0.21
Total amount of metal removed through two times washing steps		20.27	28.98	7.04