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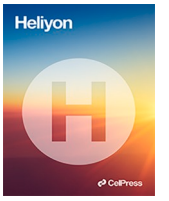

Research article

Co-detection of azoxystrobin and thiabendazole fungicides in mold and mildew resistant wallboards and in children

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ABSTRACT

The study measured the levels of azoxystrobin (AZ) and thiabendazole (TBZ) in wallboards and metabolite levels of these fungicides in children. The paper covering of wallboard samples contained a higher concentration of AZ and TBZ than the gypsum core, and similar amounts (w/w) of these two fungicides were present in the samples. These data suggest that commercial products containing a 1:1 (w/w) amount of AZ and TBZ, such as Sporgard® WB or Azo Tech™, were applied to the wallboard paper. This is the first detection of TBZ in mold-and-mildew resistant wallboards. The TBZ metabolite, 5OH-TBZ, was detected in 48% of urine samples collected from children aged 40–84 months, and was co-detected with AZ-acid, a common AZ metabolite, in 37.5% of the urine samples. The detection frequency of 5OH-TBZ was positively associated with the detection frequency of AZ-acid. These findings suggest that certain types of wallboards used in homes and commercial buildings may be a potential source of co-exposure to AZ and TBZ in humans.

1. Introduction

Azoxystrobin (AZ) is a broad-spectrum fungicide that is frequently used to protect agricultural products from fungal infections. AZ residue is widely detected on crops, fruits, and vegetables across the world [1]. The utilization of AZ is on the rise, and it was estimated that approximately 1000 tons of AZ were used in the United States in 2019. In vitro and in vivo studies suggest that AZ exhibits developmental toxicity, neurotoxicity, and disrupts metabolism [2–7]. Since around 2009, some brands of mold- and mildew-resistant wallboards incorporated AZ to prevent fungal growth. AZ is classified as an emerging environmental exposure and a major frontline target chemical for biomonitoring in the US [8].

While dietary exposure was thought to be the major exposure pathway for AZ because of its use on food crops, exposure assessments suggest that children may also be exposed to AZ through non-agricultural exposure pathways [3,9]. Research on human exposure to AZ is limited. In a study by Chang et al., AZ was found in the blood of 3% of adult participants in China [10]. Gallo et al. detected AZ in the

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urine of 10% of healthy adult participants [11]. In our previous research, we detected an AZ metabolite (AZ-acid) in human urine samples, with a detection rate of 70% in children and 100% in pregnant women. Our study highlighted the need to further investigate wallboards as a potential source of exposure because AZ-containing wallboards are increasingly being used in new construction and renovations in the US and elsewhere in the world [12]. While not explicitly listed in the wallboard material safety data sheets (MSDS), certain commercial products, such as Sporgard WB, and AzoTech have been approved by the US EPA for use in mold and mildew resistant wallboards, paper, and paperboard products to limit fungal growth. The major ingredients in these commercial products are AZ and another fungicide called thiabendazole (TBZ), which are present in a ratio of ~1:1 (w/w). TBZ is presently in widespread use as a pesticide for disease control and as a fungicide to safeguard fruits and vegetables from spoilage, mold, and blight during storage or transportation, and approximately 10 tons of TBZ were used on crops in the United States in 2019 [13,14]. The metabolite of TBZ has been widely detected in human urine samples across the globe [15–18].

Here, we confirmed that some wallboards that are used in new construction in the United States contain ~1:1 (w/w) amounts of AZ and TBZ. The implication of this observation is that co-detection of AZ and TBZ metabolites in human biosamples could indicate exposure to AZ and TBZ from wallboards. To explore this possibility, we measured metabolite levels of TBZ in children's urine samples that we had previously used to measure AZ-acid [3], a metabolite of AZ. Our study further supports the classification of AZas an emerging environmental exposure and a major frontline chemical for biomonitoring in the US [8].

2. Materials and methods

2.1. Chemicals

AZ, d4-AZ, 5-hydroxythiabendazole (5OH-TBZ), and β-glucuronidase/arylsulfatase from Helix pomatia were purchased from Sigma-Aldrich, TBZ was purchased from Fisher Scientific, 13C6-TBZ was purchased from Cambridge Isotope Lab.

2.2. Sample preparation and analysis

Convenience samples of wallboard (n = 8) were collected, the paper covering of wallboard samples was removed from the gypsum layer and both components were analyzed separately. Samples were prepared as described previously with some modifications [19]. In brief, approximately 100 mg of wallboard sample was weighed and spiked with mixed internal standards (10 ng for d4-AZ and 13C6-TBZ). After adding 6 mL of acetonitrile, the mixture was sonicated for 30 min and then vortexed for 5 min. The samples were centrifuged at 4000 rpm for 5 min, and the resulting supernatant was transferred to a clean tube. The acetonitrile extraction step was repeated, and the supernatants were combined and purified by passing through a preconditioned solid-phase extraction cartridge (ENVI-Florisil, 6 mL, 500 mg bed). The cartridge was further eluted with 3 mL of acetonitrile containing 1% formic acid. The elution was collected and dried for subsequent analysis.

Information about children urine samples used in this study was described previously [3] and the characteristics of the population are listed in Table S1. Urine samples were collected between July 2017 and February 2020. Urine samples (0.2 mL) were prepared as described previously [16]. In brief, urine samples were initially spiked with 0.5 ng of C13-TBZ. Subsequently, the samples were adjusted to a pH of 6.5 by adding sodium acetate buffer. β-glucuronidase/arylsulfatase enzymes were introduced, and the samples were incubated at 37 °C for 2 h. Following this, a 1 mL sodium acetate buffer was used to dilute the samples. The ISOLUTE-ENV solid phase extraction cartridge was prepared by prewashing it with 2 mL acetonitrile containing 5% NH3 and 2 mL sodium acetate buffer. The urine samples were then loaded onto the column and subsequently washed with 1 mL water, 1 mL 40% methanol with 1% acetic acid, and 1 mL acetonitrile. To elute 5OH-TBZ, a manually added solution of 3 mL acetonitrile containing 5% NH_3 was used. The resulting extracts were collected and evaporated to dryness, then dissolved in 20% acetonitrile in water. Matrix spiked recovery was estimated by spiking in C13-TBZ to the analytical procedure at a concentration of 1 ng/mL. The matrix effect is 97.6%. The recoveries were procedural blank subtracted. In urine samples, recovery for C13-TBZ is 83.2%. Limit of quantification for 5OH-TBZ is 0.01 ng/mL.

The specific gravity (SG) of urine was used to account for urinary dilution. Analysis of AZ, TBZ, and 5OH-TBZ was performed using a Vanquish UHPLC system coupled with Q Exactive Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific). Conditions for AZ analysis were reported previously [3]. TBZ and 5OH-TBZ were detected by parallel reaction monitoring for the following conditions, detailed information can be found in Supplementary Text S1 and Table S2.

Procedural blank samples were prepared, AZ, TBZ and 5OH-TBZ were not detected from the procedural blank samples (n = 6 for wallboard samples, and n = 6 for urine samples). Calibration curves of AZ, TBZ and 5OH-TBZ were calculated with a concentration series of 0.01, 0.05, 0.1, 0.5, 1, 5, and 10 ng/mL, and the calibration curves had an R^2 of ≥0.99.

2.3. Ethical and consent

This study was approved by the UNC institutional review board (16–1943). We obtained written informed consent from a parent prior to engaging in any research activities. Assent from minors was not obtained, as it is only required for children older than 84 months old and thus was not applicable to our study.

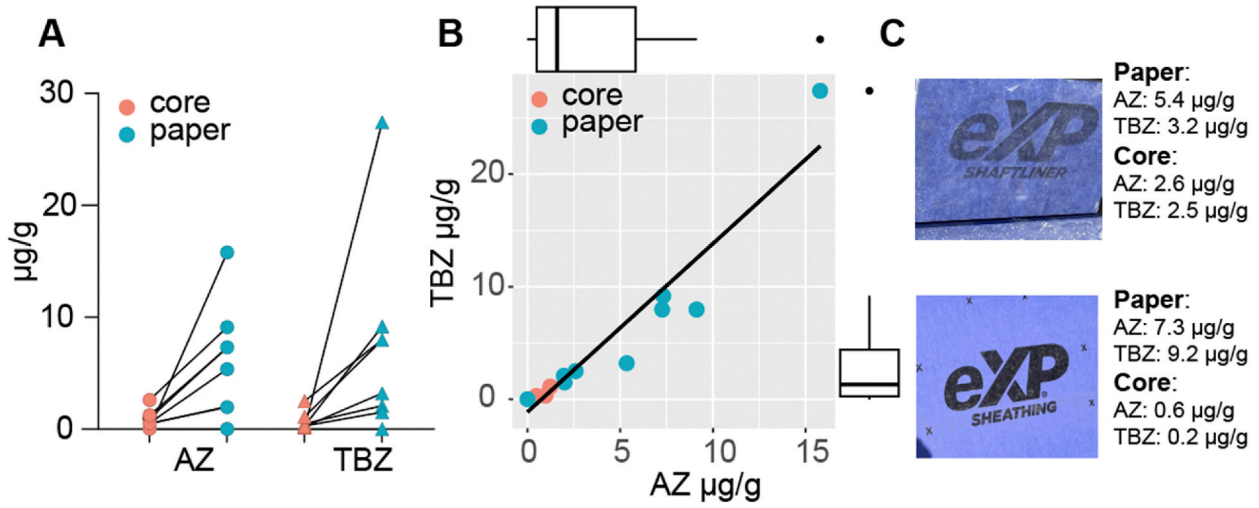

Fig. 1. (A) Concentration of AZ and TBZ in wallboard core and paper samples. (B) Linear regression of concentration of AZ and TBZ in wallboard samples (n = 8 wallboard samples). Correlation coefficient: R squared = 0.9, $p < 0.0001$. (C) Representative pictures of the tested wallboard samples.

2.4. Statistical analysis

All results are presented as mean ± SD. The association between the detection frequency of AZ-acid and 5OH-TBZ in urine samples was evaluated using the chi square test.

3. Results and discussion

3.1. Detection of AZ and TBZ in wallboards

To investigate alternate sources of human exposure to AZ, we evaluated the level of AZ and TBZ from wallboards commonly used in new construction. There are two parts of the wallboard samples: the paper cover and the gypsum core. We separated the paper from the core and quantified the level of AZ and TBZ in each part. AZ and TBZ were detected from 7 of 8 samples, with the paper containing higher amounts of AZ and TBZ than the core (Fig. 1A). The concentration of AZ in the paper ranged from <LOD–15.8 μg/g and averaging 3.4 μg/g. The concentration of TBZ in the paper ranged from LOD–27.4 μg/g and averaging 4.0 μg/g. In the wallboard core, the concentration of AZ ranged from LOD to 1.2 μg/g, and the concentration of TBZ ranged from <LOD to 2.5 μg/g (Fig. 1B and C). From each sample, AZ and TBZ amounts were similar within samples, as might be expected given the ~1:1 (w/w) ratio used in Sporgard® WB and Azo Tech™ products. In support of this assertion, there was a positive correlation between the level of AZ and TBZ in the wallboard samples (R squared = 0.9, $p < 0.0001$).

While certain commercial products, such as Sporgard WB and Azo Tech, were approved by the US EPA for use in wallboard products to prevent fungal growth, these chemicals are not explicitly listed in the MSDS of the wallboard brands that we tested (Gold Bond® eXP® Shaftliner, and Gold Bond® eXP® Sheathing, see Supplementary Table S3 for a complete list of ingredients listed in the MSDSs for these wallboard products). We are the first to detect co-occurrence of AZ and TBZ in wallboards, highlighting an emerging exposure source for both chemicals. Since AZ and TBZ are unlikely to form covalent conjugates with the construction material, these chemicals have the potential to be released from wallboards and distributed into the surrounding environment, particularly indoor environments, such as indoor air and indoor dust samples [12]. The presence of these compounds in wallboard samples suggests that, in addition to dietary ingestion from treated produce, the indoor environment is also a potential source of co-exposure to AZ and TBZ.

3.2. Detection of 5OH-TBZ in children's urine

We previously measured the urinary levels of the AZ metabolite AZ-acid in children aged 40–84 months [3]. Previous research found that TBZ can be rapidly metabolized in vivo and the oxidized product 5OH-TBZ was used as a biomarker for evaluating human TBZ exposure in urine samples collected throughout the world [15,17,18,20]. Thus, we next quantified the levels of 5OH-TBZ in the same urine samples that we previously used to quantify AZ-acid levels [3].

5OH-TBZ was detected in 48% of the children's urine samples (46 out of 96). The SG-adjusted mean concentration of 5OH-TBZ was 0.11 ± 0.02 ng/mL, with a range from <LOQ to 1.27 ng/mL. In comparison, >90% of urine samples collected from adolescents in Sweden between 2000 and 2017 contained detectable amounts of 5OH-TBZ. The SG corrected median concentration of 5OH-TBZ in these samples ranged from 0.01 to 0.05 ng/mL across different years of sample collection [20]. In a study conducted in France, 5OH-TBZ was detected in 3–5% of the 300 adult urine samples collected. Urinary levels of 5OH-TBZ in these samples were 0.26 ± 0.84

Table 1
Number of urine samples in which AZ-acid and/or 5OH-TBZ was detected.

	5OH-TBZ		
AZ-acid	Undetected	Detected	Total
Undetected	20	9	29
Detected	31	36	67
Total	51	45	96

versus 0.16 ± 0.22 μg/g (5OH-TBZ/creatinine) in individuals who reported consuming organic versus conventional food, respectively [15]. In a study conducted in Costa Rica, urine samples from pregnant women were collected from 2010 to 2022. Their SG-corrected urinary levels of 5OH-TBZ were measured. 5OH-TBZ was detected in 76% of the samples with a median concentration of 0.11 ng/mL, ranging from below the limit of detection to 339 ng/mL [18]. The levels of 5OH-TBZ observed in our study population were higher than those reported in Sweden and France, but similar to the levels detected in Costa Rica.

Considering that both AZ and TBZ were co-detected in some wallboard samples, we next performed a correlation analysis to investigate the potential relationship between urinary levels of AZ-acid and 5OH-TBZ. Although individual differences presumably exist in exposure, metabolism, and toxicokinetics, there was a statistically significant association between the detection frequency of AZ-acid and 5OH-TBZ in urine samples (chi square test, $p < 0.05$). The detection frequency suggests that there may be shared sources of exposure for AZ and TBZ, but there could also be some sources of exposure that are specific to each chemical, based on the detect/non-detect frequency (Table 1).

Our results should be interpreted in the context of several limitations: Our study involved a relatively small number of participants. Future research should encompass nationally representative populations for broader insights. In this study, the wallboard and urine samples were not paired. Subsequent investigations should focus on collecting paired samples to enhance our understanding of AZ and TBZ exposure originating from wallboard.

4. Conclusions

Although AZ has been listed as a priority target for biomonitoring in children in the US, empirical data on potential sources, pathways, and routes of exposure to AZ are currently lacking [8]. Our study addresses this knowledge gap by analyzing wallboard samples used in new construction for the presence of AZ and TBZ. Our findings suggest that wallboard may represent a potential exposure pathway for both AZ and TBZ, and that co-detection may serve as a "fingerprint" of wallboard exposure. We also observed a significant positive association between the detection frequency of AZand TBZ metabolites in children's urine samples collected from 2017 to 2020, further supporting the notion that wallboard usage may contribute to exposure to these chemicals. Given that we previously found higher levels of AZ exposure in individuals than expected from food sources alone, further research is needed to evaluate the relationship between wallboard usage and human exposure to AZ and TBZ. Lastly, co-detection of AZ and TBZ metabolites at a fixed ratio could provide further evidence for wallboard as a persistent exposure source.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Wenxin Hu: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Yun-Chung Hsiao:** Methodology. **Nikolas Morrison-Welch:** Resources. **Sophia Lamberti:** Resources. **Chih-Wei Liu:** Methodology. **Weili Lin:** Resources. **Stephanie M. Engel:** Methodology, Formal analysis. **Kun Lu:** Methodology. **Mark J. Zylka:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27980.

References

[1] D.W. Bartlett, J.M. Clough, J.R. Godwin, A.A. Hall, M. Hamer, B. Parr-Dobrzanski, The strobilurin fungicides, Pest Manag. Sci.: formerly Pesticide Science 58 (7) (2002) 649–662.
[2] Z. Meng, Z. Yan, W. Sun, et al., Azoxystrobin disrupts colonic barrier function in mice via metabolic disorders mediated by gut microbiota, J. Agric. Food Chem. 71 (1) (2023) 789–801.
[3] W. Hu, C.-W. Liu, J.A. Jiménez, et al., Detection of azoxystrobin fungicide and metabolite azoxystrobin-acid in pregnant women and children, estimation of daily intake, and evaluation of placental and lactational transfer in mice, Environ. Health Perspect. 130 (2) (2022) 027013.
[4] B.L. Pearson, J.M. Simon, E.S. McCoy, G. Salazar, G. Fragola, M.J. Zylka, Identification of chemicals that mimic transcriptional changes associated with autism, brain aging and neurodegeneration, Nat. Commun. 7 (1) (2016) 11173.
[5] J.M. Simon, S.R. Paranjape, J.M. Wolter, G. Salazar, M.J. Zylka, High-throughput screening and classification of chemicals and their effects on neuronal gene expression using RASL-seq, Sci. Rep. 9 (1) (2019) 1–9.
[6] F. Cao, P. Wu, L. Huang, et al., Short-term developmental effects and potential mechanisms of azoxystrobin in larval and adult zebrafish (Danio rerio), Aquat. Toxicol. 198 (2018) 129–140.
[7] P. Gao, X. Shen, X. Zhang, et al., Precision environmental health monitoring by longitudinal exposome and multi-omics profiling, Genome Res. 32 (6) (2022) 1199–1214.
[8] E.D. Pellizzari, T.J. Woodruff, R.R. Boyles, et al., Identifying and prioritizing chemicals with uncertain burden of exposure: opportunities for biomonitoring and health-related research, Environ. Health Perspect. 127 (12) (2019) 126001.
[9] C.K. Winter, Chronic dietary exposure to pesticide residues in the United States, Int. J. Flow Control 2 (2015) 1–12.
[10] C. Chang, M. Chen, J. Gao, et al., Current pesticide profiles in blood serum of adults in Jiangsu Province of China and a comparison with other countries, Environ. Int. 102 (2017) 213–222.
[11] V. Gallo, P. Tomai, M. Gherardi, et al., Dispersive liquid-liquid microextraction using a low transition temperature mixture and liquid chromatography-mass spectrometry analysis of pesticides in urine samples, J. Chromatogr. A 1642 (2021) 462036.
[12] E.M. Cooper, R. Rushing, K. Hoffman, et al., Strobilurin fungicides in house dust: is wallboard a source? J. Expo. Sci. Environ. Epidemiol. 30 (2) (2020) 247–252.
[13] C.E. Handford, C.T. Elliott, K. Campbell, A review of the global pesticide legislation and the scale of challenge in reaching the global harmonization of food safety standards, Integrated Environ. Assess. Manag. 11 (4) (2015) 525–536.
[14] I.C. Yadav, N.L. Devi, J.H. Syed, et al., Current status of persistent organic pesticides residues in air, water, and soil, and their possible effect on neighboring countries: a comprehensive review of India, Sci. Total Environ. 511 (2015) 123–137.
[15] J. Baudry, L. Debrauwer, G. Durand, et al., Urinary pesticide concentrations in French adults with low and high organic food consumption: results from the general population-based NutriNet-Santé, J. Expo. Sci. Environ. Epidemiol. 29 (3) (2019) 366–378.
[16] E. Ekman, M.H. Faniband, M. Littorin, M. Maxe, B.A. Jönsson, C.H. Lindh, Determination of 5-hydroxythiabendazole in human urine as a biomarker of exposure to thiabendazole using LC/MS/MS, J. Chromatogr. B 973 (2014) 61–67.
[17] C. Hyland, A. Bradman, R. Gerona, et al., Organic diet intervention significantly reduces urinary pesticide levels in US children and adults, Environ. Res. 171 (2019) 568–575.
[18] A.M. Mora, J.A. Hoppin, L. Córdoba, et al., Prenatal pesticide exposure and respiratory health outcomes in the first year of life: results from the infants' Environmental Health (ISA) study, Int. J. Hyg Environ. Health 225 (2020) 113474.
[19] J. Liu, Y. Wan, Y. Jiang, W. Xia, Z. He, S. Xu, Occurrence of azole and strobilurin fungicides in indoor dust from three cities of China, Environ. Pollut. 304 (2022) 119168.
[20] E. Norén, C. Lindh, L. Rylander, et al., Concentrations and temporal trends in pesticide biomarkers in urine of Swedish adolescents, 2000–2017, J. Expo. Sci. Environ. Epidemiol. 30 (4) (2020) 756–767.