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Urinary metabolite of pesticide, 3,5,6-trichloropyridinol, is associated with all-cause but not with cancer mortality

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Abstract

3,5,6-trichloropyridinol (TCPY) is a metabolite of chlorpyrifos and chlorpyrifos-methyl, whose presence in the environment is of potential toxicity to human. So, it is need to monitor and regualte TCPY levels to protect human health. However, it is not known whether TCPY is associated with all-cause and cancer mortality and to which degree its levels contributed to hazard risk. The study enrolled 3951 participants from the National Health and Nutrition Examination Surveys (NHANES). Ultra-high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry was used to measure urinary TCPY. Cox proportional hazards regression analysis was performed to explore the associations between TCPY and all-cause and cancer mortality. The study found that the average level of TCPY in the cohort was 1.79 μg/L and was higher in those who had passed away. Individuals in the highest quartile had a 1.56-fold independent increase in rate for all-cause mortality compared to those in the lowest quartile (hazard ratio [HR] 1.56, 95% confidence interval 1.09–2.24, p = 0.002). However, while the univariate model showed a hazard ratio of 2.37 (1.19–4.71) for the highest quartile in regards to cancer mortality, this association disappeared after adjusting for demographics, lifestyles, and comorbidities. Exposure to urinary 3,5,6-trichloropyridinol, as a result of insecticide exposure, increased the rate of all-cause mortality but was not independently associated with cancer mortality.

Keywords 3,5,6-trichloropyridinol, Cancer mortality, All-cause mortality, NHANES, Pesticides

Introduction

Pesticides, such as pyrethroids, organophosphorus, and phenoxyalkanoic acid insectides, are synthetic compounds commonly used to control insects in agriculture and gardening [5]. 3,5,6-trichloropyridinol (TCPY) is a metabolite of the organophosphate insecticides, chlorpyrifos and chlorpyrifos-methyl formed in the environment, during metabolism in living organisms, or during chemical degradation [12]. Chlorpyrifos and its metabolites have been associated with adverse effects on non-target organisms, including nervous system [17], reproductive system [1], and overall health [13]. Understanding the toxicology of TCPY is crucial for assessing its potential health risks to humans.

While pesticides are essential for crop yield, they have also been linked to adverse health effects, especially in those exposed to them in agricultural settings [8]. Accordingly, measuring urinary organophosphate insecticide metabolites is crucial in estimating human exposure and assessing the potential for health consequences.

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Past research has shown a correlation between urinary TCPY and decreased serum testosterone in males [11] as well as a decrease in estradiol and sex hormone-binding globulin in females [9].

The primary pyrethroid metabolite, 3-phenoxybenzoic acid, has been found to increase the risk of mortality [2]. Besides, agricultural chlorpyrifos exposure was associated with mortality [7]. However, the relationship between urinary TCPY and mortality remains unclear.

Methods

Study population

The study's population consisted of 3951 participants with complete records of urinary TCPY and mortality status, sourced from the National Health and Nutrition Examination Survey during the years 2007–2010, which is a survey of studies designed to assess the health and nutritional status of adults and children in the United States. Approval for the study was granted by the National Center for Health Statistics under Protocol #2005–06.

Exposure and covariates

Urine specimens are processed, stored, and shipped to the Centers for Disease Control and Prevention for analysis. Urinary 3,5,6-trichloropyridinol was extracted and concentrated from the urine matrix using an automated solid phase extraction system. Selective separation of the analytes is achieved using high-performance liquid chromatography with a gradient elution program. Sensitive detection of the analytes is performed by a triple quadrupole mass spectrometer with a heated electrospray ionization source. Isotopically labeled internal standards are used for precise and accurate quantification (https://wwwn.cdc.gov/nchs/data/nhanes/2013-2014/labmethods/UPHOPM-H-MET-508.pdf). The half-life of TCPY in urine samples was short and metabolized rapidly, but it can vary depending on several factors, including individual metabolism, exposure levels, and other physiological factors. Those TCPY values below the limit of detection (LOD) were expressed as LOD/sqrt 2 and 84 participants had TCPY below the LOD.

From the survey questionnaires, sociodemographic variables, lifestyle variables, and comorbidities were collected. Briefly, physical activity, hypertension, diabetes and CVD were self-reported. Poverty-income ratio was the ratio of the number of people (in a given age group) whose income falls below the poverty line and a ratio less than 1 meant that the income was less than the poverty level.

Mortality status was determined through linkage with the National Death Index by 31 December 2015. We classified causes of deaths according to the codes of ICD-10 (international statistical classification of diseases, 10th revision). All-cause mortality included death from all diseases, while cancer mortality is death specifically from cancer (codes C00-C97). Only the underlying cause of death was used for cancer analysis.

Statistical analysis

Statistical analyses consisted of comparisons between the TCPY quartiles using chi-square and student t-tests for categorical and continuous variables, respectively. Multivariable Cox proportional hazards regression models were used to estimate the association between TCPY and mortality rate, while restricted cubic spline models were used to explore nonlinearity. R 3.6.2 was used to perform all statistical analyses, with statistical significance determined at a p-value < 0.05.

Results

After excluding participants with missing records on TCPY (n = 190) and mortality (n = 84), a total of 3,951 participants (age: 18–80 years) were included in our study, with a mean TCPY level of 1.79 μg/L. Table 1 shows the main characteristics of the participants. Deceased individuals were older (68.2 ± 13.7 in non-survivors group vs. 46.7 ± 17.8 in survivors group), more male (57.6% vs. 47.1%), and non-Hispanic white (57.9% vs. 45.7%) (all $p < 0.001$). The level of urinary TCPY was higher in non-survivors group (2.09 ± 2.15 vs. 1.76 ± 1.92 μg/L; $p = 0.003$).

There were 342 death from all causes and 80 death from cancer. Compared to the lowest quartile, the highest quartile of TCPY was associated with increased all-cause mortality in unadjusted Model 1 (hazard ratio [HR] 2.67, 95% confidence interval [CI] 1.88 to 3.79; $p < 0.001$), partly-adjusted Model 2 (HR 1.46, 95% CI 1.02 to 2.08; $p = 0.038$), and fully-adjusted Model 3 (HR 1.56, 95% CI 1.09 to 2.24; $p = 0.015$) (Table 2). The 2rd and 3th quartile also showed a positive relationship with all-cause mortality in Model 1 (for the 2rd quartile, HR 1.44, 95% CI 1.04 to 1.98, $p = 0.027$; or the 3th quartile, HR 1.61, 95% CI 1.14 to 2.28, $p = 0.007$), but it disappeared in adjusted models.

However, in terms of cancer mortality (Table 3), while the highest quartile of TCPY was associated with a 2.37-fold rate (95% CI 1.19, 4.71; $p = 0.014$), this association disappeared after adjusting for urinary creatinine, age, gender, race, education, poverty-income ratio, BMI, drinker, smoker, activity, hypertension, diabetes, and CVD (HR 1.57, 95% CI 0.77 to 3.20; $p = 0.219$). These results suggested that urinary TCPY was not independently associated with cancer mortality.

Table 1 Characteristics of the study population

Variable	Overall (n = 3951)	Survivors (n = 3609)	Non-survivors (n = 342)	*P* value
Age, years	48.6 (18.5)	46.7 (17.8)	68.2 (13.7)	< 0.001
Male, %	1898 (48.0)	1701 (47.1)	197 (57.6)	< 0.001
Race, %				< 0.001
Non-Hispanic white	1847 (46.7)	1649 (45.7)	198 (57.9)	
Non-Hispanic black	761 (19.3)	692 (19.2)	69 (20.2)	
Mexican American	724 (18.3)	687 (19.0)	37 (10.8)	
Others	619 (15.7)	581 (16.1)	38 (11.1)	
Education, %				< 0.001
Less than high school	1176 (29.8)	1043 (28.9)	133 (38.9)	
High school or equivalent	896 (22.7)	812 (22.5)	84 (24.6)	
College or above	1879 (47.6)	1754 (48.6)	125 (36.5)	
Poverty-income ratio, %				< 0.001
< 1	901 (22.8)	815 (22.6)	86 (25.1)	
1 ~ 3	1671 (42.3)	1486 (41.2)	185 (54.1)	
> 3	1379 (34.9)	1308 (36.2)	71 (20.8)	
BMI, kg/m2	28.9 (6.8)	28.9 (6.8)	28.9 (6.9)	0.876
Drinker (%)	2268 (57.4)	2086 (57.8)	182 (53.2)	0.114
Smoker, %				< 0.001
Never	2782 (70.4)	2564 (71.0)	218 (63.7)	
Past	192 (4.9)	183 (5.1)	9 (2.6)	
Current	977 (24.7)	862 (23.9)	115 (33.6)	
Activity, %				0.002
Inactive	1076 (27.2)	966 (26.8)	110 (32.2)	
Moderate	1669 (42.2)	1514 (42.0)	155 (45.3)	
Vigorous	1206 (30.5)	1129 (31.3)	77 (22.5)	
Hypertension, %	722 (18.3)	600 (16.6)	122 (35.7)	< 0.001
Diabetes, %	609 (15.4)	479 (13.3)	130 (38.0)	< 0.001
CVD, %	399 (10.1)	283 (7.8)	116 (33.9)	< 0.001
Creatinine, mg/dL	125.2 (80.1)	126.8 (81.2)	107.9 (64.1)	< 0.001
TCPY, μg/L	1.79 (1.93)	1.76 (1.91)	2.09 (2.15)	0.003

Data are presented as mean (SD), or n (%). *BMI* body mass index, *CVD* cardiovascular diseases, TCPY, 3,5,6-trichloropyridinol

Table 2 The association between TCPY and all-cause mortality

	Event	Model 1		Model 2		Model 3	
		HR	P	HR	P	HR	P
TCPY, μg/L							
< 0.535	70	Ref.	–	Ref.	–	Ref.	–
0.535 ~ 1.220	86	1.44 [1.04, 1.98]	0.027	1.11 [0.80, 1.53]	0.538	1.12 [0.81, 1.54]	0.495
1.220 ~ 2.330	78	1.61 [1.14, 2.28]	0.007	1.11 [0.79, 1.57]	0.538	1.09 [0.77, 1.53]	0.623
> 2.330	108	2.67 [1.88, 3.79]	< 0.001	1.46 [1.02, 2.08]	0.038	1.56 [1.09, 2.24]	0.015
Continuous	342	1.14 [1.09, 1.20]	< 0.001	1.04 [0.98, 1.10]	0.197	1.05 [0.99, 1.11]	0.112

Model 1: adjusted for urinary creatinine

Model 2: adjusted for urinary creatinine, age, gender, race, education, poverty-income ratio, BMI, drinker, smoker and activity

Model 3: adjusted for urinary creatinine, age, gender, race, education, poverty-income ratio, BMI, drinker, smoker, activity, hypertension, diabetes, and CVD

Table 3 The association between TCPY and cancer mortality

	Event	Model 1		Model 2		Model 3	
		HR	P	HR	P	HR	P
TCPY, μg/L							
< 0.535	19	Ref.	–	Ref.	–	Ref.	–
0.535 ~ 1.220	17	1.03 [0.53, 2.00]	0.940	0.87 [0.45, 1.71]	0.690	0.90 [0.46, 1.76]	0.752
1.220 ~ 2.330	16	1.16 [0.57, 2.35]	0.689	0.93 [0.46, 1.89]	0.838	0.90 [0.44, 1.83]	0.772
> 2.330	28	2.37 [1.19, 4.71]	0.014	1.55 [0.76, 3.17]	0.225	1.57 [0.77, 3.20]	0.219
Continuous	80	1.14 [1.03, 1.27]	0.001	1.06 [0.94, 1.19]	0.351	1.05 [0.94, 1.18]	0.399

Model 1: adjusted for urinary creatinine

Model 2: adjusted for urinary creatinine, age, gender, race, education, poverty-income ratio, BMI, drinker, smoker and activity

Model 3: adjusted for urinary creatinine, age, gender, race, education, poverty-income ratio, BMI, drinker, smoker, activity, hypertension, diabetes, and CVD

Furthermore, restricted cubic splines didn't show an nonlinear relationship between urinary TCPY and all-cause mortality with p for nonlinearity = 0.07 (Fig. 1).

Age can bias the estimated effects of risk factors in epidemiologic cohort studies of chronic diseases. Therefore, we have added a sensitivity analysis with age as the time scale and left truncation at enrollment (Table 4). Smoking was an important confounder; therefore, we re-analyzed the data by ruling out the smokers (Table 5). The conclusion was not changed.

Discussion

Our study found that urinary 3,5,6-trichloropyridinol was associated with all-cause mortality, although it was not independently associated with cancer mortality. Therefore, our findings suggested a potential risk of exposure to TCPY for mortality.

Due to widespread exposure to pesticides, their potential adverse health effects should not be ignored. A study found that pyrethroid pesticides increased the risk of all-cause and cardiovascular mortality [2]. Several epidemiological studies reported the association between pesticide exposure and endocrine system alterations. For example, urinary non-persistent pesticide was negatively

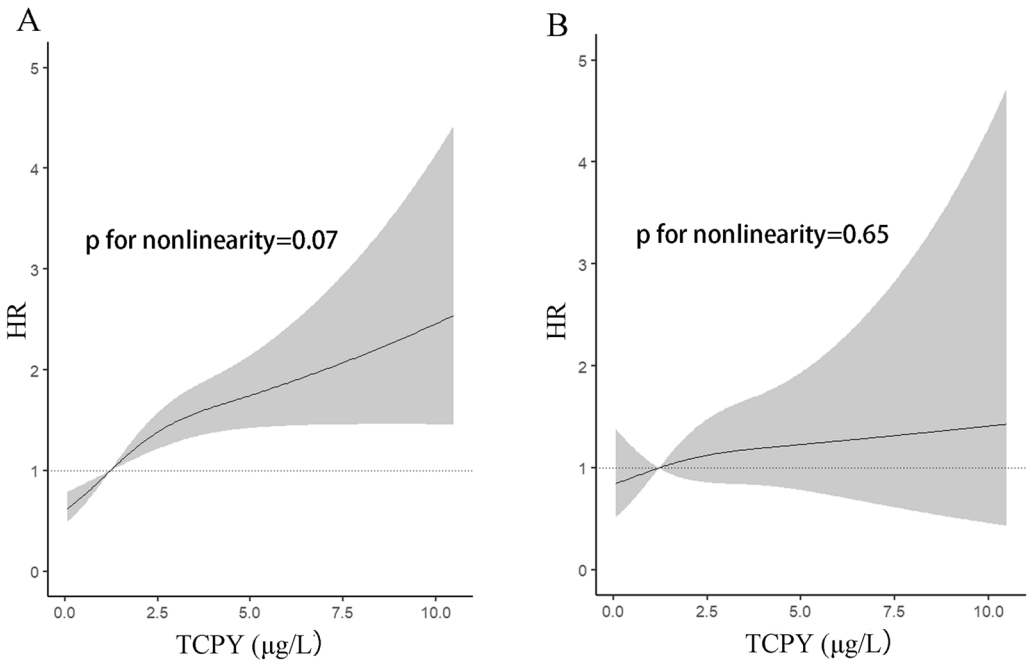

Fig. 1 The dose–response analysis between urinary TCPY and all-cause mortality **A** or cancer mortality **B**

Table 4 A sensitive analysis of association between TCPY and all-cause mortality with age as the time scale

	Model 1		Model 2		Model 3	
	HR	P	HR	P	HR	P
TCPY, μg/L						
< 0.535	Ref.	–	Ref.	–	Ref.	–
0.535 ~ 1.220	1.32 [0.92–1.84]	0.139	0.91 [0.48–1.76]	0.792	0.95 [0.47–1.80]	0.816
1.220 ~ 2.330	1.15 [1.05–2.06]	0.012	1.85 [1.31–2.57]	< 0.001	1.91 [0.99–4.07]	0.056
> 2.330	2.15 [1.05–4.35]	0.038	2.18 [1.08–4.08]	0.031	1.88 [1.31–2.58]	< 0.001

Model 1: adjusted for urinary creatinine

Model 2: adjusted for urinary creatinine, gender, race, education, poverty-income ratio, BMI, drinker, smoker and activity

Model 3: adjusted for urinary creatinine, age, gender, race, education, poverty-income ratio, BMI, drinker, smoker, activity, hypertension, diabetes, and CVD

Table 5 A sensitive analysis of association between TCPY and all-cause mortality in population who never smoked

	Event	Model 1		Model 2		Model 3	
		HR	P	HR	P	HR	P
TCPY, μg/L							
< 0.535	42	Ref.	–	Ref.	–	Ref.	–
0.535 ~ 1.220	54	1.47 [0.97, 2.22]	0.066	1.18 [0.78, 1.78]	0.428	1.20 [0.80, 1.81]	0.382
1.220 ~ 2.330	48	1.63 [1.05, 2.54]	0.031	1.08 [0.70, 1.67]	0.725	1.07 [0.69, 1.66]	0.752
> 2.330	74	3.12 [2.01, 4.84]	< 0.001	1.59 [1.02, 2.48]	0.039	1.64 [1.06, 2.55]	0.027
Continuous	218	1.19 [1.12, 1.26]	< 0.001	1.07 [1.00, 1.15]	0.042	1.07 [1.00, 1.15]	0.038

associated with serum sex hormones [9]. Pyrethroid exposure was related to the alteration of reproductive hormone levels in males [14].

TCPY, the metabolite of chlorpyrifos and chlorpyrifos-methyl, was negatively associated with testosterone and estradiol in female adolescents [9] and adult males [10, 11]. In addition to sex hormones, TCPY has been reported to increase the risk of blood pressure dysregulation [4] thyroid dysfunction [6] and cognitive and behavior deficits [3]. Furthermore, we observed that higher TCPY levels were associated with an increased rate of all-cause mortality, although we did not find an independent association of cancer mortality. Dose–response analysis and adjustments for additional variables revealed that TCPY had a positive association with all-cause mortality.

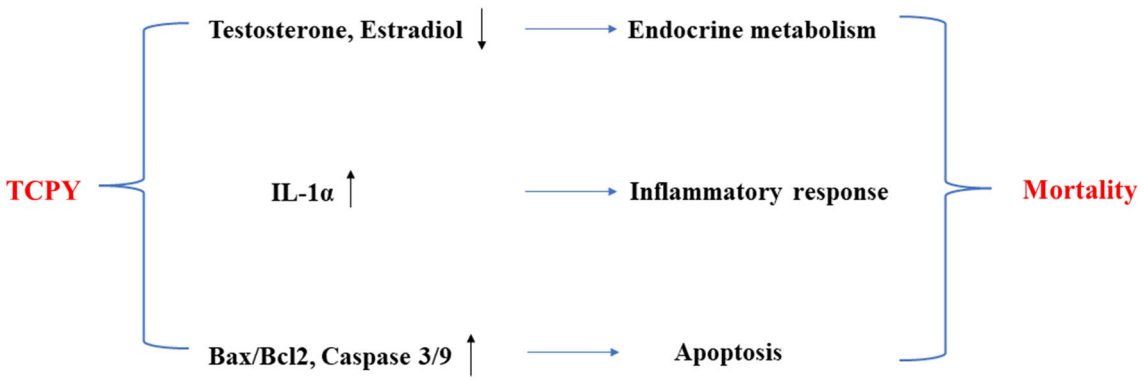

Fig. 2 An illustration graph explaining the physiopathological mechanism between TCPY and mortality. TCPY decreased the levels of testosterone and estradiol, causing endocrine dysfunction. TCPY promoted the expression of IL-1α and Bax /Bcl-2, and caspase 9/3, which upregulated inflammatory responses and cell apoptosis. These results contributed to mortality collectively. *IL-1α* Interleukin-1α, *Bcl2* B-cell lymphoma-2, *Bax* Bcl-2-associated X protein

There may be various underlying mechanisms driving the positive correlation between TCPY and all-cause mortality (Fig. 2). Firstly, urinary TCPY was inversely related to serum testosterone in males [11] and estradiol in females [9]. Besides, TCPY exposure resulted in a strong induction of IL1α, and inflammatory response, being cytotoxic to human embryonic kidney cells [15]. Finally, TCPY up-regulated the expression level of Bax / Bcl-2, and caspase-9/-3 activation, inducing the apoptosis of human hepatocytes [16]. However, more research is necessary to elucidate the biological mechanisms underlying this association.

Several limitations need to be considered when interpreting our findings. Firstly, a one-time point measure may not reflect the long-term burden of TCPY. Secondly, numerous potential variables were not included in our models, such as liver function, and kidney function etc. Third, our findings based on a relatively small sample size and short-term follow-up require cautious interpretation. Finally, more biological evidences should be investigated to explain our findings.

Conclusion

In conclusion, we found that urinary 3,5,6-trichloropyridinol was independently associated with all-cause mortality and was not independently associated with cancer mortality.

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Author contributions
Z F and W L designed the study. Q F and M T performed the statistical analysis. W X wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All the data could be available upon request from the corresponding author.

Declarations

Ethics approval and consent to participate
The study was approved by the institutional review board of National Center for Health Statistics (Protocol #2005-06) and written informed consent was obtained from all subjects.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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