

RESEARCH ARTICLE

Reference values of urinary metabolites of organophosphate in healthy Iranian adults

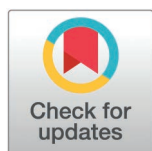
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Abstract

Organophosphorus pesticides are widely used in agriculture in Iran; we evaluated exposure to these pesticides among Iranian adults. Pesticide-specific urinary metabolites were used as biomarkers for exposure to various pesticides, including organophosphorus insecticides. The aim of the study was to estimate reference values (RV95) and their relationships with the measured factors. We used the 95th percentile as the basis for deriving these reference values. The analysis included descriptive statistics and multiple linear regression, conducted using Python software. We measured metabolites for Chlorpyrifos (TCP: 2-isopropyl-4-methyl-6-hydroxypyrimidine), Diazinon (IMPY: 2-isopropyl-4-methyl-6-hydroxypyrimidine), and Malathion (Malathion dicarboxylic acid) in 490 healthy Iranian adults. Additionally, we recorded age, gender, wealth index, and body composition parameters including body fat, muscle mass, visceral fat, and BMI. Fasting urine sampling, along with body composition and demographic measurements, were conducted. Urine samples were subsequently analyzed. The Chlorpyrifos, Diazinon, and Malathion Reference Value (RV95) levels ranged from ND-24.9 µg/L (RV95: 2.8 µg/L, 2.9 µg/gcrt), ND-64.36 µg/L (RV95: 8.6 µg/L, 9.3 µg/gcrt), and ND-47.69 µg/L (RV95: 9.8 µg/L, 8.2 µg/gcrt), respectively. Diazinon (IMPY) and Malathion (Malathion dicarboxylic acid) showed no significant relationship between their urinary levels and demographic features. However, visceral

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fat percentage had a significant inverse correlation with urinary levels of Chlorpyrifos (TCP) ($P=0.038$). Other factors such as age, sex, visceral fat, BMI, and wealth index showed no significant relationship with urinary levels ($P>0.05$). Non-zero levels were found in 98.8% of adults' urine samples for this metabolite. The reference value of this pesticide metabolite in urine could be helpful for policymakers in assessing the level of exposure among Iranians.

Introduction

Over the past century, population growth, urbanization, and industrial and agricultural advancements have increased exposure to heavy metals, pesticides, and polycyclic aromatic hydrocarbons [1]. Organophosphate pesticides are highly toxic chemicals that inhibit the enzyme acetylcholinesterase (AChE), leading to an accumulation of acetylcholine in the nervous system. This accumulation can cause severe health issues, including respiratory failure, seizures, and long-term neurological impairments, such as memory loss and mood disorders [1–3].

In Iran, the annual use of pesticides in agriculture has continued to rise, largely due to the expansion of agricultural activities across urban and rural areas [2–5]. Vulnerable groups, particularly agricultural workers and children, face heightened risks due to occupational exposure and environmental contamination [6,7]. As pesticide use in agriculture has risen, so has human exposure to these chemicals, which occurs not only through food consumption but also through the inhalation of contaminated dust and air [8–10]. The ecological effects are also alarming, as these pesticides can leach into ecosystems, harming non-target species like aquatic life and disrupting food webs [11].

There is growing concern about the overuse of pesticides in Iran, which has been linked to several public health issues in recent years [4]. Diazinon, in particular, has been identified as a significant pesticide residue in fruits and vegetables, often exceeding safety limits in the country. Chlorpyrifos, while approved for agricultural use, is closely monitored due to its toxicity. Regulatory efforts are underway to manage pesticide use more safely, but gaps remain in comprehensive data on pesticide residues in food crops, highlighting the need for improved monitoring and regulation to protect public health and the environment [5–7,9].

Human biomonitoring (HBM) involves collecting biological samples, such as blood, urine, and breast milk, to analyze pollutant concentrations and their metabolites [12,13]. This process determines human exposure to chemicals at both occupational and environmental levels, enabling corrective actions if necessary, and reveals differences in chemical distribution across countries and regions [14–16]. Urine is the most utilized matrix in biomonitoring studies due to its abundance, ease of collection, and ability to improve analytical quantification levels for chemicals with a low response factor in chromatography [17,18].

HBM data from nationally representative surveys are considered the best source for calculating reference values (RV95s) for environmental chemicals in human

biomaterials [13]. An RV95 represents the upper limit of the general population's background exposure to a substance at a specific period [19].

Although researchers commonly utilize reported RVs to interpret data, country-specific variables such as life-style factors, food consumption patterns, and levels of ambient pollution can result in significant differences in RVs among populations. Therefore, RV95s must be established at national or regional levels to accurately reflect local conditions.

The German HBM Commission defines the reference value, RV95, as the 95th percentile of the substance of interest at a specific time point, rounded off within its 95% confidence interval (CI) [20], which aligns with the International Union of Pure and Applied Chemistry (IUPAC) guidelines [21]. The selection of the 95th percentile and 95% CI is motivated by the convention in hypothesis testing, where the 5% most extreme sample values indicate unusual values [20].

Several countries, including Canada, Germany, and Spain, have successfully conducted HBM studies to monitor exposure to chemical compounds and calculate RV95s [20,22–24]. However, in Iran, despite a sharp increase in pesticide use over the past decade—particularly the use of organophosphorus pesticides in agriculture—there is a notable absence of national biomonitoring data and no reported RV95 values specific to the Iranian population. This gap raises significant concerns about the toxicological and health impacts of pesticide exposure in Iran.

This study aims to address this critical gap by calculating RV95s for pesticide metabolites using data from an HBM study that analyzed urine samples and examined key demographic factors in Iran.

Materials and methods

Sampling

In this survey, the sample size and sampling locations were based on the protocol study that we have widely discussed in protocol article [25]. Sampling was done from 1/5/2019–15/3/2021 on Iranian adults aged 25 and above. Overall, 660 households participated in six provinces (Fig 1) [25], after data cleaning, 490 samples were used for statistical analysis in this research. Data collection was done in confidentially [25].

The sampling design was developed based on a calculated sample size of 383, assuming a 50% baseline prevalence for urinary toxic metal(loid) levels, as no prior national surveys had assessed these parameters. Using Cochran's formula for large populations [26] and accounting for a 1.5 design effect [27] and an 85% response rate [28], the sample size was adjusted to 660 across 132 clusters. Clusters were chosen via model-based clustering [29,30], allowing us to organize Iranian provinces into six super-clusters (SCs) based on covariates such as particulate matter (PM10), pesticide use, and rates of cancer and chronic respiratory diseases. The capitals of these provinces—Ardabil, Kermanshah, Mashhad, Zahedan, Tehran, and Yazd—served as essential sampling units, with cluster coordinates within urban zones generated for systematic sampling [31]. Table 1 and S1 Table provides details on each SC, cluster, and assigned sample size and inclusion and exclusion criteria.

After Recruitment people addresses, randomly selected according to the sampling method in protocol article, two copies of the consent form were filled out and signed by each participant, (One copy was given to the participant and one archived). Then two questionnaires (Demographic, Household Assets) all valid and reliable filled, the demographic questionnaire collected information about age, gender, education, and career. In addition, Anthropometry gathered the anthropometric indices and body composition that measured by portable body composition scale (Omron, BF 511) with 100-g precision. The mobile body composition scale is an 8-sensor bioelectrical impedance analyzer that can report body fat (in %), skeletal muscle (in %), and visceral fat. The gender, age, and height of the participants entered manually. We measure of metabolite malathion, diazinon and chlorpyrifos Because these three compounds are used more in Iran, we chose them [25]. This research with this code of ethics IR.NIMAD.REC.1397.310 is in accordance with the ethical principles and national norms and standards of conducting medical research in Iran.

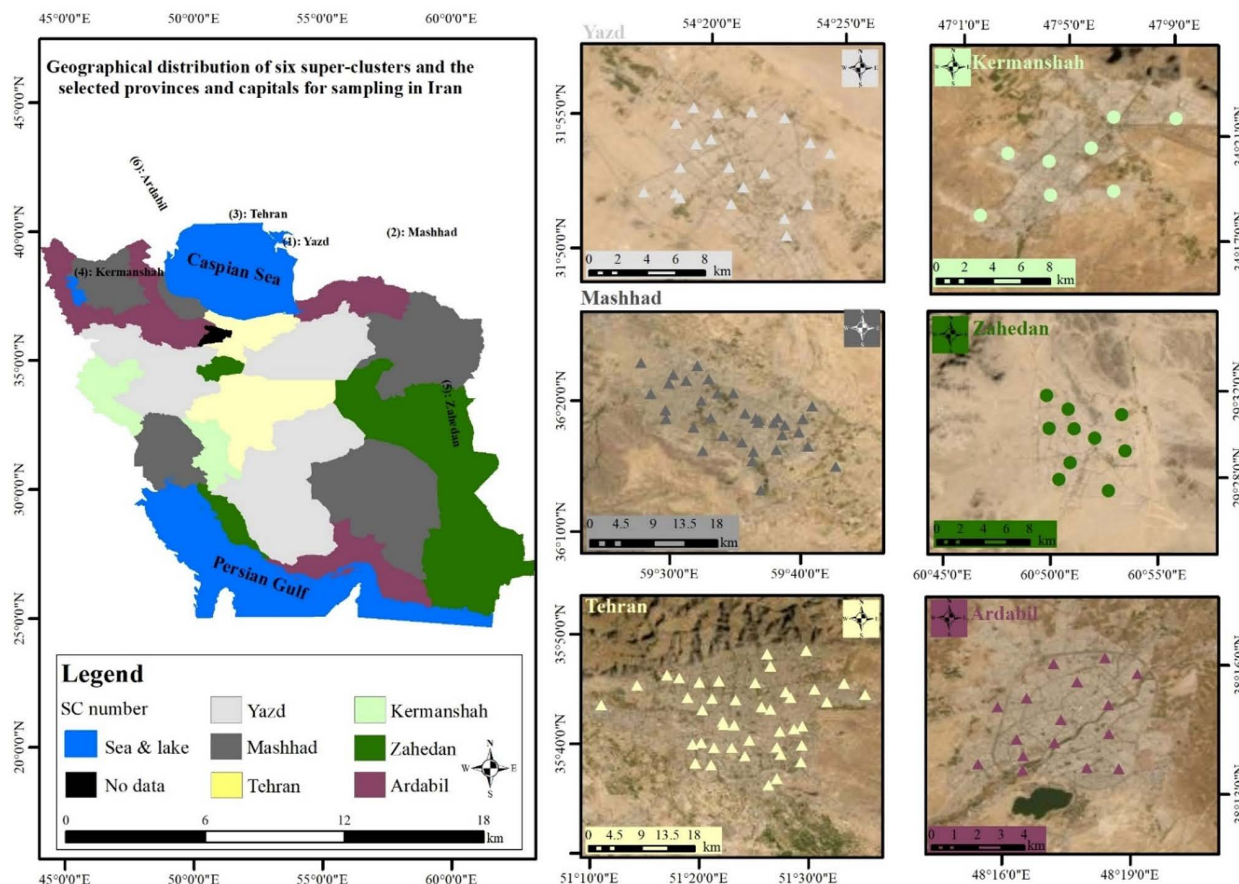


Fig 1. Geographical distribution of the six super-clusters (SCs) and the selected provinces and capitals for sampling in Iran [25].

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Table 1. Inclusion and exclusion criteria in this study [25].

Inclusion	Exclusion	Explain
Age 25 or over	Pregnant	—
Not smoker and contact with smokers	contact with smoker in home and work place	—
Individuals should be apparently healthy.	Gets sick	—
Individuals should not take any medication within the last month.	Gets sick	—
Individuals should not have a history of any chemotherapy or radiotherapy throughout their lifetime.	Gets sick	—
Occupational exposure is not allowed	Have an occupational exposure	Their occupation should not include the following items; Casting, Metallurgy, Welding, and Soldering Rubber, asphalt, and coke industries Pesticide manufacturing industries Agriculture and Greenhouse Cooking (in large restaurants)

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Sampling urine

For sampling and measurement of pollutants in urine, we measured metabolized pesticides in urine samples. The urine sample of 300 ml fasting urine was collected from participants. The samples were stored at 4°C in cooling boxes during transport to ensure preservation until laboratory processing. The samples were then separated into aliquots and stored at -20°C for a few days before being transferred to the laboratory or stored in -80°C units for long-term storage. Participants received explicit instructions in the form of a leaflet on how to collect the urine sample according to the study's Standard Operating Procedure. Female participants were given specific instructions on washing before sampling during menstruation [32].

Sample preparation and analysis

The target pesticides were analyzed in urine samples following enzymatic deconjugation and solid-phase extraction, similar to the method described by the CDC [33]. Briefly, 0.5 mL of urine was transferred into a 15-mL polypropylene (PP) tube, and 1 ng of each labeled internal standard mixture was added. The urine samples were mixed with 400 µL of 0.2 M sodium acetate buffer containing 745 units/mL of β-glucuronidase and 56 units/mL of sulfatase, then incubated at 37°C for at least 6 hours. The samples were subsequently passed through Oasis® HLB 3 mL solid-phase extraction cartridges that had been conditioned with 2 mL of acetone and 2 mL of 1% acetic acid in water. After loading the samples, the cartridges were washed with acetic acid/methanol/water (1:5:94, v/v/v) and vacuum dried for 5 minutes. Analytes were recovered by elution with 3 mL of acetone followed by 3 mL of hexane. The combined eluates were evaporated to dryness under nitrogen, re-dissolved in 100 µL of acetonitrile in water (1:1, v/v), and transferred into glass vial inserts for instrumental analysis [34].

Quality Assurance and Quality Control (QA & QC)

Standard solutions were prepared using Pesticide Mix 1510 (Sigma Aldrich Company) at a concentration of 50 ppm, which included the pesticides Diazinon, Malathion, and Chlorpyrifos. From this mix, standards at concentrations of 20, 50, 100, and 200 ppb were prepared and analyzed in the LC-MS device in the same volume as the samples. Isotopically labeled Dialkyl Phosphates, including Diethyl Phosphate (DEP)-d10 and Dimethyl Phosphate (DMP)-d6, were used as internal standards [35,36].

This study employed a rigorous methodology to validate calibration curves and ensure reliable results. Calibration standards were created at 20, 50, 100, and 200 ppb, and these were analyzed using the LC-MS device. The calibration curve was validated through linear regression analysis, targeting a correlation coefficient (*r*) greater than 0.995. Acceptance criteria required back-calculated concentrations to be within ±15% of nominal values, with the exception of the lower limit of quantification (LLOQ), which was allowed a deviation of ±20% (Table 2).

Additionally, QC samples were prepared by spiking a blank matrix to continuously monitor method performance. Accuracy and precision were assessed according to the same stringent criteria applied to the calibration standards. These comprehensive QA & QC measures ensured the robustness and reliability of the analytical method throughout the study.

Table 2. LOD and LOQ for the compounds in the pesticides.

Compound	LOD (ppb)	LOQ (ppb)	RSD	R2	Recovery (%)
Chlorpyrifos (TCP)	0.6	0.96	6.78	0.997	79
Diazinon (IPMP)	0.5	1.65	9.7	0.998	82
Malathion (MALOXONE)	1	3.3	10.9	0.998	80

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Statistical analysis

All analyses were conducted using Python, version 3.7.8 (64-bit). Data were tested for normality prior to analysis using the Kolmogorov–Smirnov test. Descriptive analysis was performed to calculate the mean, standard deviation, and confidence interval of the mean for the pesticide levels. These results were analyzed by station and by sex. Pesticide values below the limit of detection (LOD) were replaced by $\text{LOD}/\sqrt{2}$ [37], a method that has shown desirable results in pesticide bio-marker analysis [38].

A descriptive table of the wealth index based on stations was then created. Physical measurements of the participants, including sex, visceral fat, muscle percentage, body fat, and body mass index, were analyzed across different age groups. Pesticide levels are presented both in $\mu\text{g/L}$ and $\mu\text{g/g}$ creatinine (converted to per gram of creatinine from micrograms per liter using the creatinine concentration measured in each participant's urine).

Using data from asset questionnaires, participants were classified into five distinct wealth groups based on their wealth index. This categorization was achieved by applying principal component analysis to the assets of all households in the study, resulting in five wealth groups based on the derived index. The detailed methodology for determining the wealth index for each family is thoroughly described in Hjelm et al. (2017) [39].

Potential relationships between physical measurements and pesticide levels in urine were analyzed using multivariate linear regression analysis. Before performing the regression, the distribution of each pesticide was tested for normality. The Kolmogorov–Smirnov test was applied to each variable to assess normality. If the data did not follow a normal distribution, a log transformation was considered for the regression. Multivariate linear regression analysis was then performed for each pesticide, with variables including the physical characteristics of the participants: sex, age, visceral fat, muscle percentage, body fat, and body mass index.

Results

Baseline characteristics

Of the 490 participants from six stations (Mashhad, Yazd, Ardebil, Kermanshah, Zahedan, and Tehran), Kermanshah had the lowest and Mashhad the highest population in this study. Table 3 shows the study population divided into five quintiles: the Q1 quintile represents those at the lower end of the income/wealth spectrum, and the Q5 quintile represents those at the top. While participants in Tehran were predominantly in the Q5 quintile, Ardebil had the highest population in the Q1 quintile. The largest proportion of participants in Mashhad fell into the Q3 quintile.

Table 4 presents the characteristics of the study participants. The majority were female (58.09%); however, in the age group of 55–65, the gender distribution was equal. Most participants were younger than 45 years old, with the 35–45 age group being the largest.

Table 3. Economic status of people participating in the study.

Station	Q1	Q2	Q3	Q4	Q5
Mashhad	11	25	34	30	25
Yazd	19	13	10	17	18
Ardebil	41	29	11	7	3
Kermanshah	7	7	13	16	4
Zahedan	12	16	16	2	4
Tehran	5	8	14	28	45
Total	95	98	98	100	99

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Table 4. Baseline demographics of participants based on different age groups.

Age group	25–35		35–45		45–55		55–65		>65		Total	
	Mean±SD	P_value	Mean±SD	P_value	Mean±SD	P_value	Mean±SD	P_value	Mean±SD	P_value	Mean±SD	P_value
Height (cm)	160.89±6.21	<0.001	174.43±6.79	<0.001	174.99±6.52	<0.001	170.58±7.78	<0.001	170.94±6.21	<0.001	159.43±6.50	<0.001
Weight (kg)	66.2±11.76	<0.001	78.07±15.22	<0.001	83.92±14.31	<0.001	75.67±13.85	<0.001	81.91±12.29	<0.001	70.11±12.72	<0.001
Body mass index (kg/m ²)	25.6±4.7	<0.001	26.83±4.74	<0.001	27.81±4.17	<0.001	26.33±3.42	<0.001	28.73±4.73	<0.001	27.61±4.78	<0.001
Body fat (%)	37.99±7.75	<0.001	26.75±8.58	<0.001	28.22±7.15	<0.001	25.47±5.61	<0.001	29.48±8.50	<0.001	40.36±7.02	<0.001
Body muscle (%)	25.27±2.44	<0.001	31.80±5.25	<0.001	32.16±4.02	<0.001	33.11±3.41	<0.001	30.7±4.5	<0.001	24.97±2.68	<0.001
Visceral Fat (%)	5.76±1.78	<0.001	7.96±3.76	<0.001	9.68±3.98	<0.001	9.67±3.16	<0.001	10.94±3.61	<0.001	7.38±2.45	<0.001
Resting Metabolism (Cal)	1365.31±132.20	<0.001	1671.17±34.72	<0.001	1709.76±02.57	<0.001	1646.25±84.13	<0.001	1666.65±36.69	<0.001	1395.31±150.22	<0.001
P_value	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	

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Anthropometric indices of men and women

Men were generally taller and weighed more than women, while women had higher BMI and body fat percentages. Detailed statistics are provided in Table 4. Body muscle percentage was higher in men (32.00 ± 4.41 vs. 24.97 ± 2.68), and men also had a higher visceral fat percentage compared to women (9.17 ± 3.79 vs. 7.38 ± 2.45). In addition, men showed a higher resting metabolic rate (1669.18 ± 210.64 Cal vs. 1395.31 ± 150.22 Cal). For height, weight, body fat percentage, and resting metabolism, there are significant differences between males and females across most age groups, as indicated by p-values less than 0.001. This suggests that these physical measurements are consistently different between genders. However, for body mass index (BMI), significant differences are only observed in the 45–55 age group, with p-values indicating no significant differences in other age groups. Visceral fat percentage shows significant gender differences in some age groups but not others. The only instance where no significant gender difference is observed for height is in the >65 age group (p-value = 0.09), and for weight, there is no significant difference in the 55–65 age group (p-value = 0.84).

Urine pesticide levels in the Iranian population

The adjusted and unadjusted urine levels of Chlorpyrifos (TCP), Diazinon (IMPY), and Malathion (Malathion dicarboxylic acid), categorized by participants' sex, are available in Tables 5 and 6. The mean and RV95 urine concentration of Chlorpyrifos (TCP) were similar between men and women, with mean values of 0.768 ± 0.173 $\mu\text{g/L}$ for women and 0.718 ± 0.295 $\mu\text{g/L}$ for men, and RV95 values of 3.0 $\mu\text{g/L}$ for women and 2.6 $\mu\text{g/L}$ for men (Table 5). However, the creatinine-corrected concentration was higher in men than in women. Adjusted values increased in men (0.888 ± 0.555 $\mu\text{g/gcr}$) and decreased in women (0.754 ± 0.181 $\mu\text{g/gcr}$), widening the difference between sexes; RV95 increased in women (RV95: 3 $\mu\text{g/gcr}$) and decreased in men (RV95: 2.1 $\mu\text{g/gcr}$) (Table 5). Among super-clusters, Yazd had the lowest unadjusted (0.05 ± 0.02 $\mu\text{g/L}$) and adjusted (0.06 ± 0.03 $\mu\text{g/gcr}$) Chlorpyrifos (TPC) levels, while Kermanshah had the highest

Table 5. Level of pesticides in urine of the Iranian population.

Asset	Type	Population	Mean $\pm$ SE	P_value	95% CI	P5	P10	P50	P90	P95	RV95
Chlorpyrifos (TCP)	$\mu\text{g/L}$	Female	0.768 ± 0.173	0.65	0.428–1.11	0.004	0.004	0.115	1.18	3.00	3.0
		Male	0.718 ± 0.295		0.130–1.30	0.004	0.004	0.085	0.996	2.57	2.6
		Both	0.75 ± 0.15		0.46–1.05	0.004	0.004	0.106	1.13	2.82	2.8
	$\mu\text{g/g crt}$	Female	0.754 ± 0.181	0.98	0.398–1.11	0.002	0.003	0.114	1.18	3.28	3.3
		Male	0.888 ± 0.555		–0.216–1.99	0.002	0.002	0.055	1.09	2.06	2.1
		Both	0.79 ± 0.20		0.39–1.19	0.002	0.002	0.092	1.16	2.88	2.9
Diazinon (IPMP)	$\mu\text{g/L}$	Female	2.67 ± 0.231	0.07	2.22–3.13	0.148	0.382	1.80	5.68	7.78	7.8
		Male	3.77 ± 0.774		2.23–5.31	0.131	0.393	2.18	7.40	9.38	9.4
		Both	2.98 ± 0.27		2.44–3.51	0.137	0.387	1.92	5.93	8.60	8.6
	$\mu\text{g/g crt}$	Female	2.83 ± 0.283	0.94	2.27–3.39	0.195	0.378	1.55	6.71	9.77	9.8
		Male	2.79 ± 0.560		1.67–3.90	0.194	0.259	1.50	6.29	8.51	8.5
		Both	2.82 ± 0.26		2.31–3.32	0.194	0.328	1.52	6.70	9.28	9.3
Malathion	$\mu\text{g/L}$	Female	2.57 ± 0.232	0.03	2.11–3.03	0.007	0.189	1.53	5.84	9.16	9.2
		Male	3.84 ± 0.682		2.49–5.20	0.121	0.448	1.92	8.07	14.4	14
		Both	2.92 ± 0.25		2.42–3.42	0.007	0.242	1.62	6.61	9.84	9.8
	$\mu\text{g/g crt}$	Female	2.33 ± 0.209	0.3	1.92–2.75	0.014	0.236	1.43	5.11	8.03	8.0
		Male	2.82 ± 0.525		1.78–3.87	0.072	0.314	1.27	5.34	10.1	10.1
		Both	2.47 ± 0.21		2.06–2.89	0.016	0.260	1.36	5.19	8.19	8.2

Data are presented as mean $\pm$ SE. Abbreviations: SE, standard Error; CI, confidence interval.

<https://doi.org/10.1371/journal.pone.0325037.t005>

Table 6. Concentrations of pesticides in different super-clusters.

Station	Unite	Gender	Chlorpyrifos (TCP)	P_value	Diazinon (IPMP)	P_value	Malathion	P_value
			Mean $\pm$ SE		Mean $\pm$ SE		Mean $\pm$ SE	
Ardebil	$\mu\text{g/L}$	Female	0.285 $\pm$ 0.180	0.33	3.02 $\pm$ 0.716	0.72	2.63 $\pm$ 0.691	0.69
		Male	0.608 $\pm$ 0.334		2.68 $\pm$ 0.598		2.25 $\pm$ 0.634	
		Both	0.43 $\pm$ 0.18		2.87 $\pm$ 0.47		2.45 $\pm$ 0.47	
	$\mu\text{g/g crt}$	Female	0.116 $\pm$ 0.072	0.17	2.09 $\pm$ 0.491	0.98	1.62 $\pm$ 0.293	0.46
		Male						
		Both	0.33 $\pm$ 0.18		2.09 $\pm$ 0.37		1.48 $\pm$ 0.20	
Tehran	$\mu\text{g/L}$	Female	0.269 $\pm$ 0.215	0.47	0.962 $\pm$ 0.183	<0.001	1.64 $\pm$ 0.586	0.1
		Male	0.023 $\pm$ 0.007		3.59 $\pm$ 0.846		3.78 $\pm$ 1.34	
		Both	0.18 $\pm$ 0.14		1.93 $\pm$ 0.37		2.43 $\pm$ 0.63	
	$\mu\text{g/g crt}$	Female	0.162 $\pm$ 0.112	0.4	0.772 $\pm$ 0.104	<0.001	1.20 $\pm$ 0.328	0.06
		Male						
		Both	0.11 $\pm$ 0.07		1.69 $\pm$ 0.41		1.78 $\pm$ 0.37	
Zahedan	$\mu\text{g/L}$	Female	2.14 $\pm$ 0.927	0.15	2.73 $\pm$ 0.665	0.74	3.53 $\pm$ 0.802	0.23
		Male	0.540 $\pm$ 0.467		3.21 $\pm$ 1.56		5.54 $\pm$ 1.60	
		Both	1.72 $\pm$ 0.70		2.86 $\pm$ 0.63		4.06 $\pm$ 0.73	
	$\mu\text{g/g crt}$	Female	1.77 $\pm$ 0.876	0.18	2.86 $\pm$ 0.798	0.45	2.61 $\pm$ 0.453	0.26
		Male						
		Both	1.37 $\pm$ 0.65		2.58 $\pm$ 0.61		2.89 $\pm$ 0.40	
Mashhad	$\mu\text{g/L}$	Female	0.384 $\pm$ 0.031	0.19	2.73 $\pm$ 0.248	0.43	2.39 $\pm$ 0.270	0.91
		Male	0.478 $\pm$ 0.074		2.37 $\pm$ 0.132		2.33 $\pm$ 0.492	
		Both	0.41 $\pm$ 0.03		2.64 $\pm$ 0.19		2.38 $\pm$ 0.24	
	$\mu\text{g/g crt}$	Female	0.441 $\pm$ 0.045	0.71	3.21 $\pm$ 0.314	0.03	2.68 $\pm$ 0.322	0.72
		Male						
		Both	0.43 $\pm$ 0.04		2.91 $\pm$ 0.25		2.62 $\pm$ 0.33	
Kermanshah	$\mu\text{g/L}$	Female	1.69 $\pm$ 0.639	0.61	4.52 $\pm$ 1.24	0.09	3.27 $\pm$ 0.856	0.08
		Male	5.33 $\pm$ 3.94		13.5 $\pm$ 10.2		10.1 $\pm$ 7.62	
		Both	2.32 $\pm$ 0.85		6.07 $\pm$ 2.00		4.44 $\pm$ 1.47	
	$\mu\text{g/g crt}$	Female	1.67 $\pm$ 0.702	0.11	3.80 $\pm$ 1.25	0.15	2.71 $\pm$ 0.868	0.15
		Male						
		Both	2.81 $\pm$ 1.39		4.81 $\pm$ 1.53		3.44 $\pm$ 1.10	
Yazd	$\mu\text{g/L}$	Female	0.028 $\pm$ 0.014	0.02	2.16 $\pm$ 0.549	0.87	2.22 $\pm$ 0.843	0.07
		Male	0.138 $\pm$ 0.082		5.70 $\pm$ 1.43		5.68 $\pm$ 1.68	
		Both	0.05 $\pm$ 0.02		2.96 $\pm$ 0.61		3.01 $\pm$ 0.80	
	$\mu\text{g/g crt}$	Female	0.066 $\pm$ 0.039	0.14	3.88 $\pm$ 3.28	0.07	1.21 $\pm$ 0.623	0.08
		Male						
		Both	0.06 $\pm$ 0.03		3.63 $\pm$ 2.26		1.85 $\pm$ 0.55	
Iran	$\mu\text{g/L}$	Both	0.75 $\pm$ 0.15	0.98	2.98 $\pm$ 0.27	0.07	2.92 $\pm$ 0.25	0.03
	$\mu\text{g/g crt}$	Both	0.79 $\pm$ 0.20	0.65	2.82 $\pm$ 0.26	0.94	2.47 $\pm$ 0.21	0.3

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unadjusted (2.32 $\pm$ 0.85 $\mu\text{g/L}$) and adjusted (2.81 $\pm$ 1.39 $\mu\text{g/gcrt}$) levels (Table 6). The Concentrations of pesticides in different super-clusters are shown in Table 6. For Chlorpyrifos (TCP), most p-values are not statistically significant, indicating that the observed differences between genders are likely due to random chance. However, in Yazd, the p-value is 0.02 for females in $\mu\text{g/L}$, suggesting a statistically significant difference. For Diazinon (IPMP), the p-value is less than

0.001 in Tehran, indicating a significant difference between genders. Malathion shows significant differences in Iran overall (p -value=0.03) and in Yazd (p -value=0.07), suggesting that gender differences in Malathion concentrations are unlikely to occur by chance in these areas (Table 6). Overall, these p -values help assess whether observed differences in pesticide exposure are due to random variation or reflect real gender differences.

For Diazinon (IMPy), mean and RV95 urine concentrations were higher in men than women (3.77 ± 0.774 $\mu\text{g/L}$ [RV95: 9.4 $\mu\text{g/L}$] vs. 2.67 ± 0.231 $\mu\text{g/L}$ [RV95: 7.8 $\mu\text{g/L}$]) (Table 5). However, creatinine-corrected levels and RV95 values showed slightly different trends, with women exhibiting higher concentrations (2.83 ± 0.283 $\mu\text{g/gcr}$ [RV95: 9.8 $\mu\text{g/gcr}$] vs. 2.79 ± 0.560 $\mu\text{g/gcr}$ [RV95: 8.5 $\mu\text{g/gcr}$]). In men, adjusted Diazinon levels were lower than unadjusted levels (Table 5). Concentrations of Diazinon in the male population of Kermanshah were sparse, resulting in high SE values (unadjusted: 13.5 ± 10.2 $\mu\text{g/L}$; adjusted: 9.70 ± 6.69 $\mu\text{g/L}$). The lowest urine levels of Diazinon in the total population were found in Tehran (unadjusted: 1.93 ± 0.37 $\mu\text{g/L}$; adjusted: 1.69 ± 0.41 $\mu\text{g/gcr}$) (Table 6).

For Malathion (Malathion dicarboxylic acid), men had higher mean and RV95 urine levels than women (3.84 ± 0.682 $\mu\text{g/L}$ [RV95: 14 $\mu\text{g/L}$] vs. 2.57 ± 0.232 $\mu\text{g/L}$ [RV95: 9.2 $\mu\text{g/L}$]). However, after adjusting for creatinine, the difference between men and women was greatly reduced (women: 2.33 ± 0.209 $\mu\text{g/gcr}$ [RV95: 8.0 $\mu\text{g/gcr}$]; men: 2.82 ± 0.525 $\mu\text{g/gcr}$ [RV95: 10.1 $\mu\text{g/gcr}$]) (Table 5). Overall, as with Diazinon, adjusted Malathion levels decreased after creatinine adjustment (from 2.92 ± 0.25 $\mu\text{g/L}$ to 2.47 ± 0.21 $\mu\text{g/gcr}$). Among the total population, the urine concentration of Malathion (2.92 ± 0.25 $\mu\text{g/L}$) was higher than that of Chlorpyrifos (TPC) (0.75 ± 0.15 $\mu\text{g/L}$) but lower than that of Diazinon (2.98 ± 0.27 $\mu\text{g/L}$), as shown in Table 6 and Fig 2.

For Chlorpyrifos (TCP), the p -values are generally high (e.g., 0.65 for females in $\mu\text{g/L}$), indicating that the observed differences between genders are not statistically significant, suggesting that the data are consistent with the null hypothesis of no difference (Table 5). For Diazinon (IPMP), p -values are also high (e.g., 0.07 for females in $\mu\text{g/L}$) (Table 5), but this value is closer to the typical significance threshold of 0.05, suggesting some evidence against the null hypothesis, though not strong enough to reject it conclusively. Malathion shows a statistically significant difference between genders in $\mu\text{g/L}$ (p -value=0.03), indicating that the observed differences are unlikely to occur by chance, providing evidence against the null hypothesis (Table 5).

The relationship between pesticide levels and demographic features

The relationship between creatinine-adjusted urinary levels of Chlorpyrifos (TCP), Diazinon (IMPy), and Malathion with demographic features is summarized in Table 7. Although no significant association was found between Diazinon or Malathion levels and demographic features, visceral fat percentage showed a significant negative correlation with Chlorpyrifos (TCP) levels ($\beta = 0.927$ [95% CI 0.862 to 0.996]; $P = 0.038$).

Discussion

Key findings and applications

The current study examined urinary pesticide concentrations, including creatinine-corrected levels, in relation to gender and geographical superclusters. The findings revealed that Kermanshah had the highest corrected urine levels of Chlorpyrifos, while Tehran had the lowest. Similarly, Kermanshah recorded the highest Malathion concentration among the six regions. In the case of Diazinon, the highest levels were observed in the Yazd cluster, with a corrected concentration of 5.19 ± 8.19 $\mu\text{g/g}$ creatinine. The study also examined associations between demographic features and pesticide concentrations, with significant correlations found only between Chlorpyrifos levels, body fat, and BMI. The underlying mechanism for this association might relate to observations from animal studies, where Chlorpyrifos exposure was linked to dyslipidemia and obesity [40,41]. The biological cause may involve Chlorpyrifos promoting adipogenesis in 3T3-L1 adipocytes and fat accumulation, alongside inhibition of AMPK phosphorylation, thus encouraging expression of key adaptogenic regulators [42]. The lack of association between sex and age and pesticide levels suggests that the entire population is at risk of exposure

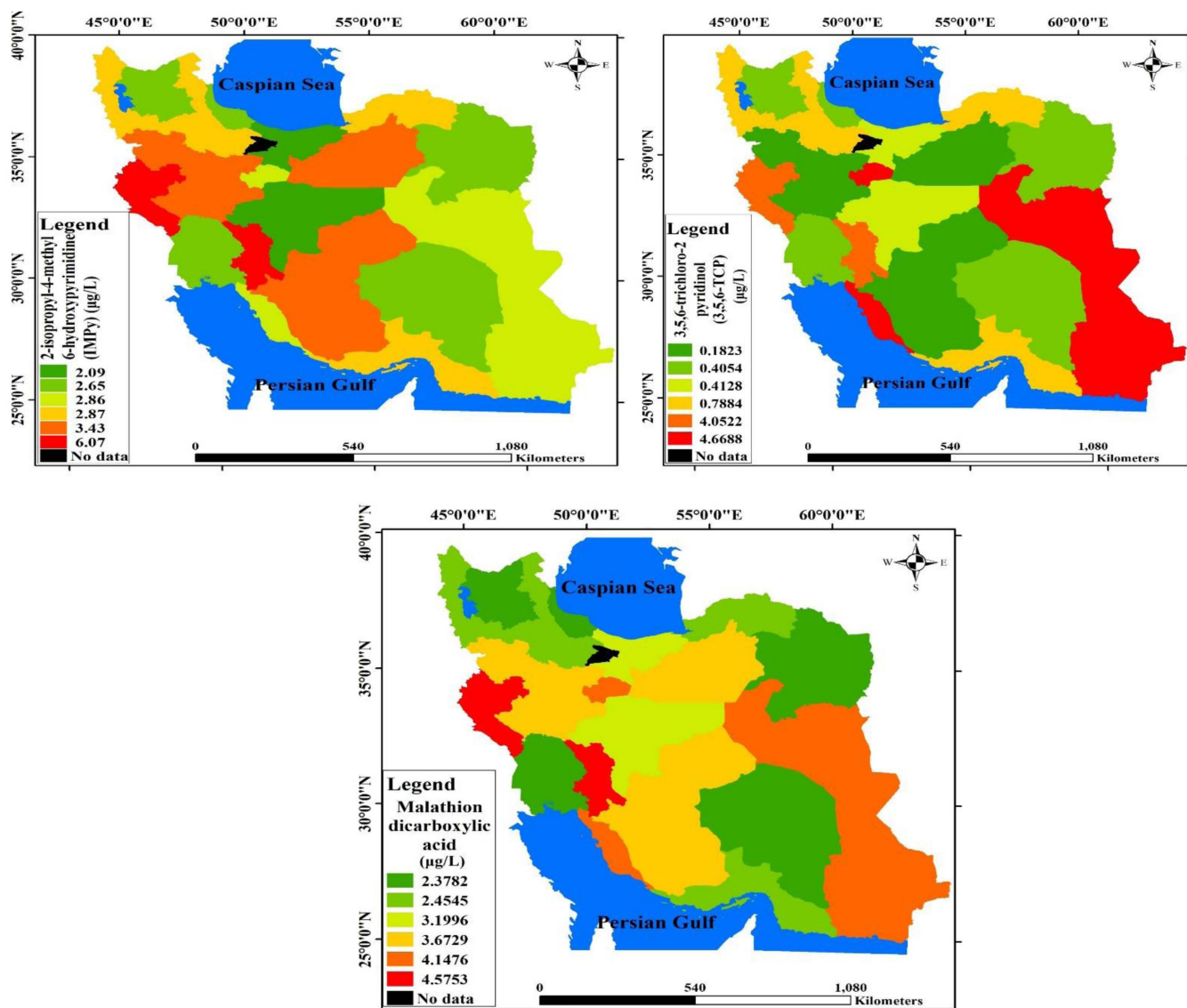


Fig 2. Map of the state of pesticide metabolite concentrations measured urine in Iranian adults.

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to these chemicals, underscoring the need for inclusive policies in Iran. Furthermore, unlike many exposures, no correlation was found between wealth index and urine levels of Chlorpyrifos, Diazinon, and Malathion. Consequently, interventions should not be limited by socioeconomic status but should target the general population [43,44].

Chlorpyrifos

Chlorpyrifos is a broad-spectrum organophosphate insecticide widely used globally [45], with neurotoxicity linked to inhibition of acetylcholinesterase [46]. This substance has been extensively discussed in the literature; Table 8 provides an expanded summary of studies reporting urinary chlorpyrifos metabolite levels across diverse populations and regions. Earlier research was concentrated in the U.S. during the 1990s and 2000s, while more recent studies are from Europe and Asia [47–59]. These newer studies report mean values ranging from 1.69 µg/L in Swedish adolescents to 7.35 µg/L in

Table 7. Relationship between pesticide concentrations in urine and demographic features.

Variables	Chlorpyrifos (TCP)			Diazinon (IPMP)			Malathion		
	β	95% CI	P-value	β	95% CI	P-value	β	95% CI	P-value
Constant	0.037	0.005–0.288	0.002	0.861	0.257–2.883	0.808	0.934	0.236–3.699	0.922
Sex	0.810	0.486–1.351	0.418	1.068	0.792–1.441	0.663	1.008	0.717–1.416	0.965
Age	0.998	0.984–1.013	0.823	1.000	0.992–1.009	0.931	0.992	0.982–1.002	0.107
Visceral fat	0.927	0.862–0.996	0.038	0.996	0.955–1.039	0.841	1.019	0.971–1.069	0.436
Body muscle	1.042	0.988–1.100	0.125	1.001	0.970–1.033	0.959	1.008	0.973–1.044	0.655
Body fat	1.022	0.988–1.057	0.202	1.001	0.982–1.021	0.885	0.997	0.975–1.019	0.786
Body mass index	1.026	0.973–1.083	0.337	1.007	0.976–1.040	0.642	1.010	0.974–1.046	0.595
Wealth index	1.043	0.934–1.164	0.449	0.985	0.924–1.050	0.648	0.971	0.903–1.045	0.433

CI, confidence interval.

In addition, a non-significant negative association was observed between Chlorpyrifos levels and sex ($\beta=0.810$ [95% CI 0.486 to 1.351]; $P=0.418$) and age ($\beta=0.998$ [95% CI 0.984 to 1.013]; $P=0.823$). Conversely, a non-significant positive relationship was noted between Chlorpyrifos levels and other demographic factors, including body muscle ($\beta=1.042$ [95% CI 0.988 to 1.100]; $P=0.125$), body fat ($\beta=1.022$ [95% CI 0.988 to 1.057]; $P=0.202$), BMI ($\beta=1.026$ [95% CI 0.973 to 1.083]; $P=0.337$), and wealth index ($\beta=1.043$ [95% CI 0.934 to 1.164]; $P=0.449$).

No significant associations were found between creatinine-adjusted urinary levels of Diazinon (IPMPy) and demographic factors. However, a non-significant positive association was noted with sex ($\beta=1.068$ [95% CI 0.792 to 1.441]; $P=0.663$), body muscle ($\beta=1.001$ [95% CI 0.970 to 1.033]; $P=0.959$), body fat ($\beta=1.001$ [95% CI 0.982 to 1.021]; $P=0.885$), and BMI ($\beta=1.007$ [95% CI 0.976 to 1.040]; $P=0.642$). The relationships with age ($\beta=1.000$ [95% CI 0.992 to 1.009]; $P=0.931$), visceral fat ($\beta=0.996$ [95% CI 0.955 to 1.039]; $P=0.841$), and wealth index ($\beta=0.985$ [95% CI 0.924 to 1.050]; $P=0.648$) were negative but not significant.

Lastly, for adjusted Malathion levels, all associations with demographic features were non-significant. Positive relationships were noted with sex ($\beta=1.008$ [95% CI 0.717 to 1.416]; $P=0.965$), visceral fat ($\beta=1.019$ [95% CI 0.971 to 1.069]; $P=0.436$), body muscle ($\beta=1.008$ [95% CI 0.973 to 1.044]; $P=0.655$), and BMI ($\beta=1.010$ [95% CI 0.974 to 1.046]; $P=0.595$). Negative associations were found with age ($\beta=0.992$ [95% CI 0.982 to 1.002]; $P=0.107$), body fat ($\beta=0.997$ [95% CI 0.975 to 1.019]; $P=0.786$), and wealth index ($\beta=0.971$ [95% CI 0.903 to 1.045]; $P=0.433$).

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Portuguese adults. Compared to these, our current study's mean adjusted value of 0.79 $\mu\text{g/g}$ creatinine in Iranian adults is among the lowest reported. This contrast may reflect differences in pesticide regulation, dietary patterns, and environmental exposure. Notably, the Iranian values are also lower than the 95th percentile levels reported in recent HBM4EU-aligned studies, underscoring the value of establishing localized reference values to inform regional risk assessments and policy decisions [41,44].

Our results are also comparable to those from the European Human Biomonitoring Initiative (HBM4EU), which prioritizes pesticides for exposure monitoring [60]. According to HBM4EU data, Cyprus showed a high median (P50) TCPy urine level among children, while this was Israel for adults [61]. However, there has been a significant decline in pesticide exposure in European countries since 2016 [61]. Interestingly, HBM4EU studies found a positive correlation between higher education levels, lower BMI, and TCPy, contrary to our findings [49,62]. This difference may reflect Iran's limited dietary and exposure variance across educational levels. Additionally, the TCPy levels observed in our Iranian sample were lower than the mean and 95th percentile (P95) levels set by HBM4EU-aligned studies [23]. Iran could benefit from a similar national database to better understand exposure across various populations and regions.

Several other studies assessed chlorpyrifos exposure by urinary levels in general populations. Berkowitz et al. and Meeker et al. evaluated chlorpyrifos levels in mothers and men, respectively [56,57], reporting higher concentrations than in our study, likely due to methodological and environmental differences. A notable study from Iran, in Rasht (part of the Mashhad supercluster), measured chlorpyrifos levels in maternal urine and breast milk, showing infant exposure risks [58]. Although minor, variations between this study and ours (1.3 $\mu\text{g/L}$ in Rasht vs. 1.13 and 0.41 $\mu\text{g/L}$ nationally and in Mashhad, respectively) may arise from differences in measurement techniques and populations.

Table 8. Comparing the average concentration of Chlorpyrifos metabolite in the urine of Iranian adults with other similar studies.

Pesticide	Study	Country	Study Period	N	Population	Mean (RV95)	Adjusted Mean (Rv95)
Chlorpyrifos	Current study	Iran	2019-2020	490	General population	0.75 (2.8) µg/L	0.79 (2.9) µg/g
Chlorpyrifos	Dalsager et al. (2019) [47]	Denmark	2010-2012	948	Pregnant women	2.08 (8.49) µg/L	—
Chlorpyrifos	Pirard et al. (2020) [48]	Belgium	2016	229	Children, 9–12 y	4.92 (12.12) µg/L	—
Chlorpyrifos	Fernández et al. (2020) [49]	Spain (Valencia Region)	2016	568	Children, 5–12 y	2.96 (11.08) µg/L	—
Chlorpyrifos	Namorado et al. (2020) [50]	Portugal	2019-2020	296	General population (Adults)	7.35 µg/L	—
Chlorpyrifos	Probst-Hensch et al. (2020) [51]	Switzerland	2020	299	General population (Adults)	3.64 µg/L	—
Chlorpyrifos	Weber et al. (2020) [52]	Germany	2015-2020	180	General population (Adults)	2.87 µg/L	—
Chlorpyrifos	Eiríksdóttir et al. (2021) [53]	Iceland	2019-2021	182	General population (Adults)	2.07 µg/L	
Chlorpyrifos	Govarts (2020) [54]	Belgium	2019-2020	133	General population (Children)	3.24 µg/L	
Chlorpyrifos	Zock (2020) [55]	Netherlands	2020	102	General population (Children)	3.49 µg/L	
Chlorpyrifos	Berkowitz et al. (2004) [56]	United States	1998-2002	404	General population (mothers and infants)		11.5 µg/g
Chlorpyrifos	Meeker et al. (2008) [57]	United States	2000-2003	N/A	General Population (men)	2.59 µg/L	
Chlorpyrifos	Brahmand et al. (2019) [58]	Iran (Rasht)	2017	61	Mothers	1.3 µg/L	
Chlorpyrifos	Galea et al. (2015) [59]	United Kingdom	2011-2012	440	Adults and children living near agricultural land		3.0 (9.6) µg/g

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The elevated chlorpyrifos levels in the Kermanshah supercluster have policy implications, emphasizing that targeted policies, rather than nationwide mandates, may be more effective. Regional dietary habits and agricultural practices likely influence these variations, as high dietary fat has been shown to increase chlorpyrifos bioavailability [63]. Chlorpyrifos has been banned in multiple countries, including parts of Europe and America [64], and Iran could consider similar restrictions. Additionally, the association between chlorpyrifos and visceral fat highlights the need for caution when assessing risk in populations potentially facing higher exposure. Nonetheless, further research is necessary to confirm these findings.

Diazinon and malathion

Diazinon and Malathion, two other pesticides assessed in this study, have been less researched compared to Chlorpyrifos. Diazinon is a moderately hazardous organophosphorus insecticide (class II, World Health Organization system) [65]. Limited studies on Diazinon exist; Garfitt et al., in a small study of five subjects, reported that urinary dialkyl phosphate levels peaked 2–12 hours after Diazinon exposure, with 60% of oral and 1% of dermal doses excreted as urinary DAP metabolites [66]. Malathion, another organophosphate, is used in mosquito-borne disease control and as a treatment for head lice [67,68]. The differences in urine levels of Diazinon and Malathion across the six Iranian superclusters indicate the need for region-specific policies on insecticide usage.

Differences between men and women

This study examined the reported pesticides separately in men and women. For chlorpyrifos, men generally had higher corrected urinary levels; however, in Tehran, Zahedan, Mashhad, and Yazd, females exhibited higher levels. Malathion was slightly higher in men, while Diazinon was slightly lower. Among other demographic factors, significant associations were found between body muscle, body fat, and chlorpyrifos levels. A rat study linked chlorpyrifos exposure with weight gain [69], and the U.S. NHANES reported higher urinary concentrations of the pesticide 2,5-dichlorophenol in obese individuals [70].

Strengths and limitations

This study provides valuable data on pesticide exposure in six representative regions in Iran, marking the first nationwide assessment of organophosphate pesticide exposure [25]. The study utilized the clustering model by Banfield et al. and Scrucca et al., producing a representative sample [29,30]. However, several limitations should be acknowledged. First, the urine levels reflect recent exposure, whereas long-term exposure may be more relevant for policy decisions. Second, comparing mean and RV95 urine pesticide levels across regions requires caution due to potential population differences. Third, the RV95 is a statistical model and not intended for health effect predictions, unlike human biomonitoring guidance values (HBM-GVs), which are essential for assessing population chemical exposure in health risk assessments [71]. This study did not establish HBM-GVs for the pesticides studied; future research should aim to determine these values. Additionally, lifestyle factors, diet, and other influential factors were not assessed, underscoring the need for further research. Despite clustering and modeling, the sample may not represent all populations across Iran's 31 provinces. The study's cross-sectional design, along with potential selection, information, and confounding biases, as well as self-reported data, may affect the generalizability of findings and prevent causal conclusions.

Conclusion

In the current study, three main pesticides were measured in the urine of a nationwide sample, revealing several notable differences. Body visceral fat was found to influence urine pesticide metabolite levels. Additionally, non-zero levels were detected in 96.8%, 93%, and 65% of urine samples for Chlorpyrifos, Diazinon, and Malathion metabolites, respectively. Detectable amounts of these metabolites were present in the urine samples analyzed. The highest levels of Chlorpyrifos and Malathion were found in Kermanshah. Our findings offer a clear picture of pesticide exposure in Iran, based on a clustering method that represents the entire Iranian population. Given the extensive use of pesticides in Iran and the limited literature on the reported levels of these three main pesticides—Chlorpyrifos, Diazinon, and Malathion—our study provides regional reference levels and cutoffs that can help policymakers design programs to reduce pesticide exposure. Future research should focus on assessing additional pesticides and providing a more comprehensive evaluation of these organophosphates, with a larger sample size and the calculation of health-based guidance values [HBM-GVs].

Supporting information

S1 Table. Descriptive statistics on super-clusters (SCs), clusters and the assigned number of samples.
(DOCX)

S1 File. Urine pest.
(RAR)

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References

1. Karthick Rajan D, Mohan K, Rajarajeswaran J, Divya D, Thanigaivel S, Zhang S. Toxic effects of organophosphate pesticide monocrotophos in aquatic organisms: a review of challenges, regulations and future perspectives. *Environ Res*. 2024;244:117947. <https://doi.org/10.1016/j.envres.2023.117947> PMID: 38109962
2. Firozjaei SAA, Latifi AM, Khodi S, Abolmaali S, Choopani A. A review on biodegradation of toxic organophosphate compounds. *J Appl Biotechnol Reports*. 2015;2(2):215–24.
3. Vale A, Lotti M. Organophosphorus and carbamate insecticide poisoning. *Handb Clin Neurol*. 2015;131:149–68. <https://doi.org/10.1016/B978-0-444-62627-1.00010-X> PMID: 26563788
4. Toorang F, Sasanfar B, Pouraram H, Eskandari S. Pesticide residue in Iranian fruits and vegetables: a systematic review. *J Nutrition Food Security*. 2024;9(1):132–43.
5. Mahdavi V, Eslami Z, Molaee-Aghaee E, Peivasteh-Roudsari L, Sadighara P, Thai VN, et al. Evaluation of pesticide residues and risk assessment in apple and grape from western Azerbaijan Province of Iran. *Environ Res*. 2022;203:111882. <https://doi.org/10.1016/j.envres.2021.111882> PMID: 34390713
6. Khademi N, Rajabi S, Fararouei M, Rafiee A, Azhdarpoor A, Hoseini M. Environmental exposure to organophosphate pesticides and effects on cognitive functions in elementary school children in a Middle Eastern area. *Environ Sci Pollution Res*. 2023;30(51):111076–91.
7. Abdollahdokht D, Asadikaram G, Abolhassani M, Pourghadamyari H, Abbasi-Jorjandi M, Faramarz S, et al. Pesticide exposure and related health problems among farmworkers' children: a case-control study in southeast Iran. *Environ Sci Pollut Res Int*. 2021;28(40):57216–31. <https://doi.org/10.1007/s11356-021-14319-1> PMID: 34086178
8. GhorbanzadehZafarani G, Karbalaee S, Al-Attar WM, Golshani R, Tayefeh FH, Ashrafizadeh A. Baseline occurrence of organochlorine and organophosphate pesticides in water, sediment, and fish in the Miankaleh wetland, Iran. *Mar Pollut Bull*. 2023;192:115097. <https://doi.org/10.1016/j.marpolbul.2023.115097> PMID: 37267870
9. Azh Baragoori B, Karimzadegan H, Mohammadi Galangash M, Shariati Feizabadi F, Ooshaksaraei L. Evaluating concentration, distribution, and risk assessment of organophosphate pesticides in paddy fields of Sepidrud River watersheds, Rudbar county, Guilan Province, Iran. *Caspian J Environ Sci*. 2024;22(3):621–6.
10. Rezaei F, Nejati R, Sayadi M, Nematollahi A. Diazinon reduction in apple juice using probiotic bacteria during fermentation and storage under refrigeration. *Environ Sci Pollut Res Int*. 2021.
11. Forman J, Silverstein J, Committee on Nutrition, Council on Environmental Health, American Academy of Pediatrics. Organic foods: health and environmental advantages and disadvantages. *Pediatrics*. 2012;130(5):e1406–15. <https://doi.org/10.1542/peds.2012-2579> PMID: 23090335

12. Garí M, González-Quintero Y, Bravo N, Grimalt JO. Analysis of metabolites of organophosphate and pyrethroid pesticides in human urine from urban and agricultural populations (Catalonia and Galicia). *Sci Total Environ*. 2018;622–623:526–33. <https://doi.org/10.1016/j.scitotenv.2017.11.355> PMID: [29220776](#)
13. Ewers U, Krause C, Schulz C, Wilhelm M. Reference values and human biological monitoring values for environmental toxins. Report on the work and recommendations of the Commission on Human Biological Monitoring of the German Federal Environmental Agency. *Int Arch Occup Environ Health*. 1999;72(4):255–60. <https://doi.org/10.1007/s004200050369> PMID: [10491780](#)
14. Najafabadi MM, Mirzaei A, Laskookalayeh SS, Azarm H. An investigation of the relationship among economic growth, agricultural expansion and chemical pollution in Iran through decoupling index analysis. *Environ Sci Pollut Res Int*. 2022;29(50):76101–18. <https://doi.org/10.1007/s11356-022-21004-4> PMID: [35666413](#)
15. Heudorf U, Butte W, Schulz C, Angerer J. Reference values for metabolites of pyrethroid and organophosphorous insecticides in urine for human biomonitoring in environmental medicine. *Int J Hyg Environ Health*. 2006;209(3):293–9. <https://doi.org/10.1016/j.ijheh.2006.01.001> PMID: [16459145](#)
16. Bevan R, Jones K, Cocker J, Assem FL, Levy LS. Reference ranges for key biomarkers of chemical exposure within the UK population. *Int J Hyg Environ Health*. 2013;216(2):170–4. <https://doi.org/10.1016/j.ijheh.2012.03.005> PMID: [22494935](#)
17. Mostafalou S, Karami-Mohajeri S, Abdollahi M. Environmental and population studies concerning exposure to pesticides in Iran: a comprehensive review. *Iran Red Crescent Med J*. 2013;15(12):e13896.
18. Jallow MFA, Awadh DG, Albaho MS, Devi VY, Ahmad N. Monitoring of pesticide residues in commonly used fruits and vegetables in Kuwait. *Int J Environ Res Public Health*. 2017;14(8):833. <https://doi.org/10.3390/ijerph14080833> PMID: [28757570](#)
19. Schulz C, Angerer J, Ewers U, Kolossa-Gehring M. The German human biomonitoring commission. *Int J Hyg Environ Health*. 2007;210(3–4):373–82.
20. Vogel N, Conrad A, Apel P, Rucic E, Kolossa-Gehring M. Human biomonitoring reference values: differences and similarities between approaches for identifying unusually high exposure of pollutants in humans. *Int J Hyg Environ Health*. 2019;222(1):30–3. <https://doi.org/10.1016/j.ijheh.2018.08.002> PMID: [30146177](#)
21. Poulson OM, Holst E, Christensen JM. Calculation and application of coverage intervals for biological reference values (Technical Report). *Pure Appl Chemistry*. 1997;69(7):1601–12. <https://doi.org/10.1351/pac199769071601>
22. Haines DA, Saravanabhavan G, Werry K, Khoury C. An overview of human biomonitoring of environmental chemicals in the Canadian Health Measures Survey: 2007–2019. *Int J Hyg Environ Health*. 2017;220(2 Pt A):13–28. <https://doi.org/10.1016/j.ijheh.2016.08.002> PMID: [27601095](#)
23. Govarts E, Gilles L, Rodriguez Martin L, Santonen T, Apel P, Alvim P. Harmonized human biomonitoring in European children, teenagers and adults: EU-wide exposure data of 11 chemical substance groups from the HBM4EU Aligned Studies (2014–2021). *Int J Hygiene Environ Health*. 2023;249:114119.
24. Peña-Fernández A, González-Muñoz MJ, Lobo-Bedmar MC. “Reference values” of trace elements in the hair of a sample group of Spanish children (aged 6–9 years) - are urban topsoils a source of contamination? *Environ Toxicol Pharmacol*. 2014;38(1):141–52. <https://doi.org/10.1016/j.etap.2014.05.011> PMID: [24930126](#)
25. Soleimani Z, Haghshenas R, Masinaei M, Naddafi K, Yunesian M, Nabizadeh Nodehi R, et al. Iranian population exposures to heavy metals, PAHs, and pesticides and their intake routes: a study protocol of a national population health survey. *Environ Sci Pollut Res Int*. 2021;28(13):16744–53. <https://doi.org/10.1007/s11356-020-12004-3> PMID: [33398731](#)
26. Cochran WG. Sampling techniques. John Wiley & Sons; 2007.
27. Salganik MJ. Variance estimation, design effects, and sample size calculations for respondent-driven sampling. *J Urban Health*. 2006;83(6 Suppl):i98–112. <https://doi.org/10.1007/s11524-006-9106-x> PMID: [16937083](#)
28. Dey EL. Working with low survey response rates: The efficacy of weighting adjustments. *Res Higher Education*. 1997;38(2):215–27.
29. Banfield JD, Raftery AE. Model-based Gaussian and non-Gaussian clustering. *Biometrics*. 1993;:803–21.
30. Scrucca L, Fop M, Murphy TB, Raftery AE. mclust 5: clustering, classification and density estimation using gaussian finite mixture models. *R J*. 2016;8(1):289–317. PMID: [27818791](#)
31. Bivand R, Rundel C, Pebesma E, Stuetz R, Hufthammer KO, Giraudoux P. Package ‘rgeos’. 2019.
32. Chang C, Obeid W, Thiessen-Philbrook H, Parikh CR. Sample processing and stability for urine biomarker studies. *J Appl Lab Med*. 2021;6(6):1628–34. <https://doi.org/10.1093/jalm/jfab082> PMID: [34414423](#)
33. CDC. Centres for Disease Control and Prevention (CDC). Urinary pyrethroids, herbicides, and OP metabolites in urine NHANES 2009–2010. 2013. https://www.cdc.gov/nchs/data/nhanes/public/2007/labmethods/uphophm_e_met.pdf
34. Li AJ, Kannan K. Urinary concentrations and profiles of organophosphate and pyrethroid pesticide metabolites and phenoxyacid herbicides in populations in eight countries. *Environ Int*. 2018;121(Pt 2):1148–54. <https://doi.org/10.1016/j.envint.2018.10.033> PMID: [30808487](#)
35. Kumar D, Sinha SN, Vasudev K. Development and validation of a new UFLC-MS/MS method for the detection of organophosphate pesticide metabolites in urine. *Molecules*. 2023;28(15).
36. Sinha SN, Reddy BV, Vasudev K, Rao MVV, Ahmed MN, Ashu S. Analysis of dialkyl urine metabolites of organophosphate pesticides by a liquid chromatography mass spectrometry technique. *Analytical Methods*. 2014;6(6):1825–34.

37. Hornung RW, Reed LD. Estimation of average concentration in the presence of nondetectable values. *Appl Occupational Environ Hygiene*. 1990;5(1):46–51.
38. Curl CL, Meierotto L, Castellano RLS, Spivak MR, Kannan K. Measurement of urinary pesticide biomarkers among Latina farmworkers in south-western Idaho. *J Expo Sci Environ Epidemiol*. 2021;31(3):538–48. <https://doi.org/10.1038/s41370-020-00285-2> PMID: [33473152](#)
39. Hjelm LAM, Miller D, Wadhwa A. Creation of a wealth index: VAM guidance paper. World Food Programme; 2017.
40. Lassiter TL, Brimijoin S. Rats gain excess weight after developmental exposure to the organophosphorothionate pesticide, chlorpyrifos. *Neurotoxicol Teratol*. 2008;30(2):125–30. <https://doi.org/10.1016/j.ntt.2007.10.004> PMID: [18166376](#)
41. Peris-Sampedro F, Cabré M, Basaure P, Reverte I, Domingo JL, Teresa Colomina M. Adulthood dietary exposure to a common pesticide leads to an obese-like phenotype and a diabetic profile in apoE3 mice. *Environ Res*. 2015;142:169–76. <https://doi.org/10.1016/j.envres.2015.06.036> PMID: [26162960](#)
42. Jiang F, Peng Y, Sun Q. Pesticides exposure induced obesity and its associated diseases: recent progress and challenges. *J Future Foods*. 2022;2(2):119–24.
43. Burke RD, Todd SW, Lumsden E, Mullins RJ, Mamczarz J, Fawcett WP, et al. Developmental neurotoxicity of the organophosphorus insecticide chlorpyrifos: from clinical findings to preclinical models and potential mechanisms. *J Neurochem*. 2017;142 Suppl 2(Suppl 2):162–77. <https://doi.org/10.1111/jnc.14077> PMID: [28791702](#)
44. Wolejko E, Łozowicka B, Jabłońska-Trypuć A, Pietruszyńska M, Wydro U. Chlorpyrifos occurrence and toxicological risk assessment: a review. *Int J Environ Res Public Health*. 2022;19(19):12209. <https://doi.org/10.3390/ijerph191912209> PMID: [36231509](#)
45. John EM, Shaik JM. Chlorpyrifos: pollution and remediation. *Environ Chem Lett*. 2015;13(3):269–91. <https://doi.org/10.1007/s10311-015-0513-7>
46. Das GP, Jamil K, Rahman MF. Effect of four organophosphorus compounds on human blood acetylcholinesterase: in vitro studies. *Toxicol Mech Methods*. 2006;16(8):455–9. <https://doi.org/10.1080/15376520600719281> PMID: [20021020](#)
47. Dalsager L, Fage-Larsen B, Bilenberg N, Jensen TK, Nielsen F, Kyhl HB, et al. Maternal urinary concentrations of pyrethroid and chlorpyrifos metabolites and attention deficit hyperactivity disorder (ADHD) symptoms in 2–4-year-old children from the Odense Child Cohort. *Environ Res*. 2019;176:108533. <https://doi.org/10.1016/j.envres.2019.108533> PMID: [31229776](#)
48. Pirard C, Remy S, Giusti A, Champon L, Charlier C. Assessment of children's exposure to currently used pesticides in Wallonia, Belgium. *Toxicol Lett*. 2020;329:1–11. <https://doi.org/10.1016/j.toxlet.2020.04.020> PMID: [32371136](#)
49. Fernández SF, Pardo O, Adam-Cervera I, Montesinos L, Corpas-Burgos F, Roca M, et al. Biomonitoring of non-persistent pesticides in urine from lactating mothers: Exposure and risk assessment. *Sci Total Environ*. 2020;699:134385. <https://doi.org/10.1016/j.scitotenv.2019.134385> PMID: [31678881](#)
50. Namorado S. Insef-expoquim - exposure of the Portuguese population to environmental chemicals: a study nested in insef. 2020. <https://ipchem.jrc.ec.europa.eu/#showmetadata/INSEFEXPOQUIM>
51. Probst-Hensch PN. HBM4EU-study Switzerland - Human Biomonitoring for Europe Program for Switzerland. 2020. <https://ipchem.jrc.ec.europa.eu/#showmetadata/HBM4EUSTUDYINSWITZERLAND>
52. Weber T. ESB - Environmental Specimen Bank. 2019. <https://ipchem.jrc.ec.europa.eu/index.html#showmetadata/ESB>
53. Eiríksdóttir ÁV. Diet_HBM - National nutrition survey and biomonitoring 2021. <https://ipchem.jrc.ec.europa.eu/#showmetadata/DIETHBM>
54. Govarts E. 3xG - 3xG study. 2015. <https://ipchem.jrc.ec.europa.eu/index.html#showmetadata/3XG>
55. Zock JP. SPECIMEn-NL - Dutch arm of the HBM4EU pesticide study SPECIMEn. 2020. <https://ipchem.jrc.ec.europa.eu/#showmetadata/SPECIMENNL>
56. Berkowitz GS, Wetmur JG, Birman-Deych E, Obel J, Lapinski RH, Godbold JH, et al. In utero pesticide exposure, maternal paraoxonase activity, and head circumference. *Environ Health Perspect*. 2004;112(3):388–91. <https://doi.org/10.1289/ehp.6414> PMID: [14998758](#)
57. Meeker JD, Ravi SR, Barr DB, Hauser R. Circulating estradiol in men is inversely related to urinary metabolites of nonpersistent insecticides. *Reprod Toxicol*. 2008;25(2):184–91. <https://doi.org/10.1016/j.reprotox.2007.12.005> PMID: [18249523](#)
58. Brahmand MB, Yunesian M, Nabizadeh R, Nasser S, Alimohammadi M, Rastkari N. Evaluation of chlorpyrifos residue in breast milk and its metabolite in urine of mothers and their infants feeding exclusively by breast milk in north of Iran. *J Environ Health Sci Eng*. 2019;17(2):817–25. <https://doi.org/10.1007/s40201-019-00398-3> PMID: [32030155](#)
59. Galea KS, MacCalman L, Jones K, Cocker J, Teedon P, Cherrie JW, et al. Urinary biomarker concentrations of captan, chlormequat, chlorpyrifos and cypermethrin in UK adults and children living near agricultural land. *J Expo Sci Environ Epidemiol*. 2015;25(6):623–31. <https://doi.org/10.1038/jes.2015.54> PMID: [26374656](#)
60. Ougier E, Ganzleben C, Lecoq P, Bessems J, David M, Schoeters G, et al. Chemical prioritisation strategy in the European Human Biomonitoring Initiative (HBM4EU) - Development and results. *Int J Hyg Environ Health*. 2021;236:113778. <https://doi.org/10.1016/j.ijheh.2021.113778> PMID: [34089975](#)
61. Tarazona JV, González-Caballero MDC, Alba-Gonzalez M de, Pedraza-Díaz S, Cañas A, Domínguez-Morueco N, et al. Improving the risk assessment of pesticides through the integration of human biomonitoring and food monitoring data: a case study for chlorpyrifos. *Toxics*. 2022;10(6):313. <https://doi.org/10.3390/toxics10060313> PMID: [35736921](#)

62. Bravo N, Grimalt JO, Mazej D, Tratnik JS, Sarigiannis DA, Horvat M. Mother/child organophosphate and pyrethroid distributions. *Environ Int*. 2020;134:105264. <https://doi.org/10.1016/j.envint.2019.105264> PMID: [31706197](#)
63. Chen AS, Liu DH, Hou HN, Yao JN, Xiao SC, Ma XR, et al. Dietary pattern interfered with the impacts of pesticide exposure by regulating the bio-availability and gut microbiota. *Sci Total Environ*. 2023;858(Pt 2):159936. <https://doi.org/10.1016/j.scitotenv.2022.159936> PMID: [36336046](#)
64. Shen D, Yu K, Hu J, Zhong J, Shen G, Ye Q, et al. Reducing environmental risks of chlorpyrifos application in typical soils by adding appropriate exogenous organic matter: evidence from a simulated paddy field experiment. *Environ Pollut*. 2022;293:118513. <https://doi.org/10.1016/j.envpol.2021.118513> PMID: [34793913](#)
65. World Health O, International Programme on Chemical S. The WHO recommended classification of pesticides by hazard and guidelines to classification 2009. Geneva: World Health Organization; 2010.
66. Garfitt SJ, Jones K, Mason HJ, Cocker J. Exposure to the organophosphate diazinon: data from a human volunteer study with oral and dermal doses. *Toxicol Lett*. 2002;134(1–3):105–13. [https://doi.org/10.1016/s0378-4274\(02\)00178-9](https://doi.org/10.1016/s0378-4274(02)00178-9) PMID: [12191867](#)
67. Barr DB, Angerer J. Potential uses of biomonitoring data: a case study using the organophosphorus pesticides chlorpyrifos and malathion. *Environ Health Perspect*. 2006;114(11):1763–9. <https://doi.org/10.1289/ehp.9062> PMID: [17107865](#)
68. Roberts RJ. Clinical practice. Head lice. *N Engl J Med*. 2002;346(21):1645–50. <https://doi.org/10.1056/NEJMcp012640> PMID: [12023998](#)
69. Meggs WJ, Brewer KL. Weight gain associated with chronic exposure to chlorpyrifos in rats. *J Med Toxicol*. 2007;3(3):89–93. <https://doi.org/10.1007/BF03160916> PMID: [18072142](#)
70. Wei Y, Zhu J, Nguyen A. Urinary concentrations of dichlorophenol pesticides and obesity among adult participants in the U.S. National Health and Nutrition Examination Survey (NHANES) 2005–2008. *Int J Hyg Environ Health*. 2014;217(2–3):294–9.
71. Angerer J, Aylward LL, Hays SM, Heinzow B, Wilhelm M. Human biomonitoring assessment values: approaches and data requirements. *Int J Hyg Environ Health*. 2011;214(5):348–60. <https://doi.org/10.1016/j.ijheh.2011.06.002> PMID: [21764371](#)