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Determination of elemental impurity levels in protein powder supplements: A potential consumer health risk assessment study

Protein tozu takviyelerindeki elementel impürite düzeylerinin belirlenmesi: Tüketici sağlığı açısından olası risk değerlendirme çalışması

Özge Nur TÜRKERİ[1], Fadime CANBOLAT[1]

[1]Çanakkale Onsekiz Mart University, Health Services Vocational School, Pharmacy Services Department, 17020, Çanakkale-Turkey

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Abstract

Aim: The study aims to investigate the potential hazards of risk elements such as Cadmium (Cd), Lead (Pb), Arsenic (As) and Mercury (Hg) in protein supplements on human health.

Materials and Methods: The levels of risk elements in 10 different supplements were determined using ICP-MS and both the hazard index (HI) and total carcinogenic risk (TCR) values were calculated based on the results obtained.

Results: Cd (4.41 - 44.17 µg/kg), Pb (39.01 - 122.89 µg/kg), As (4.27 - 37.26 µg/kg) and Hg (7.25 - 95.36 µg/kg) were found in the supplements. HI< 1 and TCR values (1×10^{-4} - 1×10^{-6}) were found for 1 and 3 serving scenarios.

Conclusion: The HI and TCR values of potential toxic elements in the supplements examined are within acceptable limits, but dosage and brand differences may affect risk levels.

Keywords: Protein powder supplement; Elemental impurity; Risk analysis; Human health.

Öz

Amaç: Çalışma protein takviyelerindeki Kadmiyum (Cd), Kurşun (Pb), Arsenik (As) ve Civa (Hg) gibi risk elementlerinin insan sağlığı üzerindeki potansiyel tehlikelerini incelemeyi amaçlamaktadır.

Gereç ve Yöntem: 10 farklı takviyenin risk element düzeyleri ICP-MS ile belirlenmiş, elde edilen sonuçlar üzerinden hem tehlike indeksi (HI) hem de toplam kanserojenik risk (TCR) değerleri hesaplanmıştır.

Bulgular: Takviyelerde Cd (4,41 - 44,17 µg/kg), Pb (39,01 - 122,89 µg/kg), As (4,27 - 37,26 µg/kg) ve Hg (7,25 - 95,36 µg/kg) olarak bulunmuştur. 1 ve 3 porsiyonluk senaryolar için HI< 1 ve TCR değerleri (1×10^{-4} - 1×10^{-6}) bulunmuştur.

Sonuç: İncelenen takviyelerdeki potansiyel toksik elementlerin HI ve TCR değerlerinin kabul edilebilir sınırlar içinde olduğu ancak dozaj ve marka farklılıklarının risk düzeylerini etkileyebileceği gösterilmektedir.

Anahtar Kelimeler: Protein tozu takviyesi; Elementel safsızlık; Risk analizi; İnsan sağlığı.

Yazışma Adresi/Address for Correspondence: Özge Nur TÜRKERİ, Çanakkale Onsekiz Mart University, Health Services Vocational School, Pharmacy Services Department, 17020, Çanakkale-Turkey, E-mail: ozgenur.turkeri@comu.edu.tr

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Introduction

The use of dietary supplements is increasing significantly every day. The Dietary Supplement Health and Education Act of 1994 defines dietary supplements as products that support nutrition contain vitamins, minerals, proteins, amino acids and biologically active substances.[1] Among these products 'protein powders' attract attention due to their widespread use. Protein powders are dietary supplements in powder form derived from dairy-based sources such as casein and whey as well as plant-based raw materials such as egg whites, soy, rice, peas and pumpkin seeds.[2]

The recommended daily protein intake is approximately 0.83 g per kilogram of body weight. In high-protein diets (HPD), this ratio is generally defined as 1.2 - 2.0 g/kg/day.[3,4] Athletes and individuals involved in bodybuilding use protein supplements more frequently to support muscle protein synthesis and enhance performance. It has been reported that protein supplement usage rates among young athletes and bodybuilders are significantly higher than in the general population.[5,6] Studies conducted in European countries also show that protein powders are widely consumed among individuals who regularly attend gyms.[7–9]

The content of protein supplements is not limited to protein alone due to the production process, raw materials used and environmental factors, they may potentially be contaminated with toxic elements. Among these elements, cadmium (Cd), lead (Pb), arsenic (As) and mercury (Hg) are the most prominent.[10,11] Various studies conducted in the US have reported that significant amounts of these elements were found above safe limits in protein supplements. Data from the Clean Label Project and Consumer Reports support growing concerns about the safety of protein powders.[3,12,13] Chronic exposure to these elements is known to be associated with adverse effects on carcinogenicity, neurotoxicity, nephrotoxicity, and reproductive health.[14–20] Therefore assessing element exposure through protein supplements is important for public health. The daily exposure amount (PDE) to the elements in these supplements is reported in the United States Pharmacopeia (USP) guidelines.[21] Toxicity symptoms may occur when exposure exceeds the specified limits. The examination of potential hazards arising from an individual' s exposure to a chemical is defined as risk assessment and risks to human health are quantified in terms of both carcinogenic and non-carcinogenic effects.

In risk assessments related to human health, reference dose (RfD) values defined by the United States Environmental Protection Agency (USEPA) are used and non-carcinogenic risks are quantified using the target hazard quotient (THQ). A THQ value of 1 or above indicates a potential health risk. The hazard index (HI) is calculated in the assessment of multiple element exposure, and an HI ≥ 1 value is significant in terms of adverse health effects.[22–24] The lifetime cancer risk (CR) associated with the use of protein supplements reveals the potential carcinogenic effects of long-term exposure to risk elements found in these products. The detection of elements such as Cd, Pb, As, and Hg in these supplements is associated with biochemical toxicity and cellular damage mechanisms.[25] Therefore, it is important to conduct regular assessment studies to determine elemental exposure in sports supplements and reduce risks.

The reliable determination of trace metals requires analytical methods with high sensitivity and accuracy. Inductively coupled plasma mass spectrometry (ICP-MS) is a widely used technique for ultra-trace level metal analysis.[23,24,26] This study aimed to determine the levels of Cd, Pb, As and Hg in 10 commonly consumed animal-derived protein powder supplements using ICP-MS and to perform non-carcinogenic and carcinogenic risk assessments based on the data obtained. Thus, the potential effects of elemental impurities in protein supplements on human health were evaluated in light of scientific data.

Materials and Methods

Sample collection and contents

Ten popular protein powder supplements originating from Turkey were sourced from a nationally operating online platform in single-serving packages between February 20 and June 20, 2025. Consistently best-selling protein supplements, unaffected by short-term fluctuations and campaigns during the four month sampling period, were included in the study.

The supplements were in ready-to-consume powder packets (to be mixed with 200-300 mL of water or milk). The portion and content information for the products is shown in Table 1. In this analysis, the portion size information reported on the product label was used to determine consumption rates (1 serving/day and 3 servings/day). 1 serving per day represents average consumer intake, while three servings per day was considered the upper exposure limit scenario. The selection of the 3-serving scenario is supported by manufacturer instructions on product labels indicating that more than one serving per day may be consumed during periods of intense physical activity. Furthermore, in human health risk assessment studies on protein powder supplements conducted by Bandara et al.[27], daily multiple-serving intake scenarios were used to represent high levels of consumer exposure.

Table 1. Protein powder supplements analyzed and their ingredients.

Additional	1 porsiyon (g)	1 serving (g)	Flavor	Sweetener
S1	32	Whey protein concentrate, acidity regulator (citric acid), thickener, vitamin B6	Chocolate flavoring	Acesulfame- K., aspartame
S2	36	Whey protein concentrate, creatine monohydrate, anti-caking agents	Reduced fat cocoa powder, chocolate flavoring	Acesulfame-K, Sucralose
S3	33	Blend of whey concentrate and whey isolate, creatine monohydrate, vitamin B6	Chocolate flavor, cocoa powder	Sucralose
S4	34	Whey protein concentrate, sweetener, anti-caking agent, enzyme blend (protease, papain)	Cocoa powder, chocolate flavoring	Acesulfame-K, Sucralose
S5	30	Egg white powder, thickener, anti-caking agent	Chocolate flavor, cocoa	Sucralose
S6	23	Whey concentrate and whey isolate, thickener, anti-caking agent	Chocolate flavor, cocoa	Sucralose
S7	22	Whey protein concentrate, cocoa, taurine, L-glutamine, L-Alanine, L-Arginine, BCAA (L-Leucine, L-Isoleucine, L-Valine), anti-caking agent	Chocolate flavor,	Acesulfame-K, Sucralose
S8	35	Whey protein concentrate and isolate, vitamin B6, thickener	Chocolate flavor, cocoa powder	Sucralose
S9	30	Whey concentrate, inulin, emulsifier	Cocoa powder, chocolate flavoring, natural flavoring (vanilla)	Sucralose
S10	30	Whey protein concentrate, amino acid blend (leucine, isoleucine, valine), creatine monohydrate, anti-caking agent, soy lecithin (anti-caking agent)	reduced-fat cocoa powder, flavoring (chocolate)	Sucralose

Preparation and analysis of samples

Risk element contents were determined using an ICP-MS device, Model Agilent 7700x (Agilent, Santa Clara, CA, USA) at the Dicle University Science and Technology Application and Research Center (Diyarbakır, Turkey) Laboratory. Metal levels were given in micrograms per kilogram (µg/kg or ppb). Samples were analyzed in 'gasless' mode for Cd, Pb and Hg while Helium (He) gas was used for As. Prior to analysis samples were subjected to microwave digestion using Mars Xpress (CEM, Matthews, NC, USA). Approximately 0.5 g of each sample was weighed and placed into polytetrafluoroethylene (PTFE) tubes. 3 mL of hydrogen peroxide (H_2O_2, 30 %) and 2 mL of concentrated nitric acid (HNO_3, 65 %) were added and microwaved. After digestion the resulting solutions were diluted with ultrapure water and the final volume was adjusted to 15 mL. Each protein supplement was analyzed in triplicate. In this analysis portion size information reported for each product was used for 1 and 3 portions taking into account similar studies in the literature to characterize consumption rates.[27]

Risk assessment for human health

To determine the noncarcinogenic and carcinogenic effects of risk elements present in protein powders, assessments were conducted according to the metrics specified in the guidelines of the USEPA and the European Medicines Agency (EMA).[28,29] The HI value was considered for noncarcinogenic assessment while the CR and cumulative cancer risk (TCR) values were considered for carcinogenic assessment.[24, 26, 30- 33]

Non-carcinogenic risk assessment

Estimated Daily Intake (EDI)

When assessing the risks of a contaminant a commonly used measure is the EDI which is the highest amount of a contaminant that a person can safely consume every day throughout their lifetime without experiencing adverse effects. Excessive consumption of this amount can lead to toxicity. The EDI represents the average oral exposure of a population to a contaminant and is expressed in mg/kg body weight/day.[33]The EDI for the risk elements Cd, Pb, As and Hg was calculated using Equation (1) below.

$$\text{EDI (mg/kg/day)}=((C \times IR \times ED \times EF)/(BW \times AT)) \times 0.001 \qquad \text{Equation (1)}$$

C; concentration of risk elements detected in the supplement (µg/kg), IR; calculated based on the protein supplement dosage recommended by the manufacturer (kg/day per capita) (Table 2). BW; body weight was taken as 70 kg.[30] According to the USEPA recommendation, EF (exposure frequency) and ED (exposure duration) were taken as 260 days/year and 30 years. Additionally 365 days/year × 30 years and 365 days/year × 66.4 years were determined as AT noncarcinogenic and AT carcinogenic respectively.[33]

Table 2. Elemental impurity levels in protein powder supplements and risk assessment.

Sample	Element	MC (µg/kg) mean±SD	EDI (mg/kg/day)	THQ	HI	CR	TCR
One serving							
S1	Cd	4.42 ± 1.08	0.14×10^{-5}	0.001	0.05	$4,09 \times 10^{-6}$	5.09×10^{-6}
	Pb	46.01±3.21	1.49×10^{-5}	0.004		$5,75 \times 10^{-8}$	
	As	4.27±1.54	0.14×10^{-5}	0.005		$9,42 \times 10^{-7}$	
	Hg	36.39±2.23	1.19×10^{-5}	0.039		NC	
S2	Cd	10.86±2.63	0.39×10^{-5}	0.004	0.17	1.13×10^{-5}	1.85×10^{-5}
	Pb	122.90±8.03	4.50×10^{-5}	0.013		1.73×10^{-7}	
	As	28.22±4.37	1.03×10^{-5}	0.034		7.01×10^{-6}	
	Hg	95.36±7.21	3.49×10^{-5}	0.116		NC	
S3	Cd	5.40±1.15	0.18×10^{-5}	0.002	0.05	5.16×10^{-6}	9.46×10^{-6}
	Pb	58.24±3.42	1.96×10^{-5}	0.006		7.51×10^{-8}	
	As	18.56±2.24	0.62×10^{-5}	0.021		4.22×10^{-6}	
	Hg	16.59±1.12	0.56×10^{-5}	0.019		NC	
S4	Cd	11.36±3.48	0.39×10^{-5}	0.004	0.04	1.12×10^{-5}	1.35×10^{-5}
	Pb	48.37±5.51	1.67×10^{-5}	0.005		6.43×10^{-8}	
	As	9.51±1.17	0.33×10^{-5}	0.011		2.23×10^{-6}	
	Hg	17.67±2.68	0.61×10^{-5}	0.020		NC	
S5	Cd	36.68±3.21	1.12×10^{-5}	0.011	0.09	3.19×10^{-5}	3.96×10^{-5}
	Pb	49.84±6.53	1.52×10^{-5}	0.004		5.84×10^{-8}	
	As	37.27±2.24	1.14×10^{-5}	0.038		7.71×10^{-6}	
	Hg	36.51±3.41	1.11×10^{-5}	0.037		NC	
S6	Cd	9.32±3.37	0.22×10^{-5}	0.002	0.05	6.21×10^{-6}	7.03×10^{-6}
	Pb	60.23±8.04	1.41×10^{-5}	0.004		5.41×10^{-8}	
	As	4.83±1.07	0.11×10^{-5}	0.004		7.67×10^{-7}	
	Hg	46.28±4.62	1.08×10^{-5}	0.036		NC	

S7	Cd	44.02±1.08	0.99 × 10^{-5}	0.009	0.05	2.81 ×10^{-5}	3.06 × 10^{-5}
	Pb	42.97±5.13	0.96 × 10^{-5}	0.003		3.69 ×10^{-8}	
	As	16.31±2.42	0.37 × 10^{-5}	0.012		2.47 ×10^{-6}	
	Hg	29.66±2.08	0.66 × 10^{-5}	0.022		NC	
S8	Cd	11.68±1.01	0.42 × 10^{-5}	0.004	0.09	1.18 ×10^{-5}	1.71 × 10^{-5}
	Pb	72.99±5.17	2.59 × 10^{-5}	0.007		9.98 ×10^{-8}	
	As	21.51±1.13	0.77 × 10^{-5}	0.026		5.19 ×10^{-6}	
	Hg	51.58±3.41	1.84 × 10^{-5}	0.061		NC	
S9	Cd	44.17±3.21	1.35 × 10^{-5}	0.013	0.05	3.84 ×10^{-5}	3.95 × 10^{-5}
	Pb	57.38±3.18	1.75 × 10^{-5}	0.005		6.73 ×10^{-8}	
	As	5.11±1.03	0.16 × 10^{-5}	0.005		1.06 ×10^{-6}	
	Hg	22.33±1.72	0.68 × 10^{-5}	0.023		NC	
S10	Cd	16.73±2.26	0.51 × 10^{-5}	0.005	0.03	1.45 × 10^{-5}	1.73 × 10^{-5}
	Pb	39.01±1.16	1.19 × 10^{-5}	0.003		4.57 × 10^{-8}	
	As	12,89±2.13	0.39 × 10^{-5}	0.013		2.67 × 10^{-6}	
	Hg	7.25±1.34	0.22 × 10^{-5}	0.007		NC	
Three servings							
S1	Cd	4.42±1.08	0.43 × 10^{-5}	0.004	0.15	1.23 × 10^{-5}	1,53 × 10^{-5}
	Pb	46.01±3.21	4.50 × 10^{-5}	0.013		1.73 × 10^{-7}	
	As	4.27±1.54	0.42 × 10^{-5}	0.014		2.83 × 10^{-6}	
	Hg	36.39±2.23	3.56 × 10^{-5}	0.119		NC	
S2	Cd	10.86±2.63	1.19 × 10^{-5}	0.012	0.50	3.39 ×10^{-5}	5.55 × 10^{-5}
	Pb	122.90±8.03	13.51 × 10^{-5}	0.03859073		5.19 ×10^{-7}	
	As	28.22±4.37	3.10 × 10^{-5}	0.10339228		2.10 ×10^{-5}	
	Hg	95.36±7.21	10.48 × 10^{-5}	0.34934964		NC	
S3	Cd	5.40±1.15	0.54 ×10^{-5}	0.005	0.14	1.55 ×10^{-5}	2.84 × 10^{-5}
	Pb	58.24±3.42	5.87 × 10^{-5}	0.017		2.25 ×10^{-7}	
	As	18.56±2.24	1.87 × 10^{-5}	0.062		1.27 ×10^{-5}	
	Hg	16.59±1.12	1.67 × 10^{-5}	0.056		NC	
S4	Cd	11.36±3.48	1.18 × 10^{-5}	0.012	0.12	3.36 ×10^{-5}	4.04 × 10^{-5}
	Pb	48.37±5.51	5.02 × 10^{-5}	0.014		1.93 ×10^{-7}	
	As	9.51±1.17	0.99 ×10^{-5}	0.033		6.69 × 10^{-6}	
	Hg	17.67±2.68	1.83 × 10^{-5}	0.061		NC	
S5	Cd	36.68±3.21	3.36 × 10^{-5}	0.034	0.27	9.56 × 10^{-5}	1.19 × 10^{-4}
	Pb	49.84±6.53	4.56 × 10^{-5}	0.013		1.75 × 10^{-7}	
	As	37.27±2.24	3.41 × 10^{-5}	0.114		2.31 × 10^{-5}	
	Hg	36.51±3.41	3.34 × 10^{-5}	0.111		NC	
S6	Cd	9.32±3.37	0.65 × 10^{-5}	0.007	0.14	1.86 × 10^{-5}	2.11 × 10^{-5}
	Pb	60.23±8.04	4.23 × 10^{-5}	0.012		1.62 × 10^{-7}	
	As	4.83±1.07	0.34 × 10^{-5}	0.011		2.30 × 10^{-6}	
	Hg	46.28±4.62	3.25 × 10^{-5}	0.108		NC	
S7	Cd	44.02±1.08	2.96 × 10^{-5}	0.029	0.14	8.42 × 10^{-5}	9.17 × 10^{-5}
	Pb	42.97±5.13	2.89 × 10^{-5}	0.008		1.11 × 10^{-7}	
	As	16.31±2.42	1.09 × 10^{-5}	0.037		7.42 ×10^{-6}	
	Hg	29.66±2.08	1.99 × 10^{-5}	0.066		NC	
S8	Cd	11.68±1.01	1.25 × 10^{-5}	0.012	0.29	3.55 ×10^{-5}	5.14 × 10^{-5}
	Pb	72.99±5.17	7.79 × 10^{-5}	0.022		2.99 ×10^{-7}	
	As	21.51±1.13	2.29 × 10^{-5}	0.077		1.56 ×10^{-5}	
	Hg	51.58±3.41	5.51 × 10^{-5}	0.184			
S9	Cd	44.17±3.21	4.05 × 10^{-5}	0.040	0.14	1.15 ×10^{-4}	1.19 × 10^{-4}
	Pb	57.38±3.18	5.25 × 10^{-5}	0.015		2.02 ×10^{-7}	
	As	5.11±1.03	0.47 × 10^{-5}	0.016		3.17 ×10^{-6}	
	Hg	22.33±1.72	2.04 × 10^{-5}	0.068		NC	
S10	Cd	16.73±2.26	1.53 × 10^{-5}	0.015	0.09	4.36 ×10^{-5}	5.18 × 10^{-5}
	Pb	39.01±1.16	3.57 × 10^{-5}	0.010		1.37 ×10^{-7}	
	As	12.89±2.13	1.18 × 10^{-5}	0.039		7.99 ×10^{-6}	
	Hg	7.25±1.34	0.66 × 10^{-5}	0.022		NC	

*NC: not calculated.

Target hazard quotient (THQ) and hazard index (HI)

THQ is a comparative measure that evaluates potential exposure to a substance relative to a threshold at which no adverse effects are expected. It is commonly used to assess potential non-carcinogenic risks associated with metals in protein supplements and is calculated using Equation (2).

THQ= EDI/RfD Equation (2)

RfD is the oral reference dose of the risk element in mg/kg/day. The RfD values for As, Cd, Hg and Pb are 3×10^{-4}, 1×10^{-3}, 3×10^{-4} and 3.5×10^{-3} mg/kg/day respectively.

HI was calculated cumulatively for each risk element present in each sample using Equation (3); An HI value greater than 1 indicates a non-carcinogenic health risk. [24, 26, 33-35]

$HI = THQ_{Cd} + THQ_{Pb} + THQ_{Hg} + THQ_{As}$ Equation (3)

Carcinogenic risk and cumulative cancer risk

Cd, Pb and As are risk elements that are extremely toxic and classified as probable carcinogens by the IARC.[30] For Hg, CSF data were not avail able. Nevertheless, mercury's cancer slope factor (6.177 mg kg−1 per day) was determined for the first time using the human cancer equivalent dose (HED) and the animal lower bound benchmark dosage due to the metal's significant toxicities.[31] The lifetime CR associated with the use of protein supplements contaminated with risk elements refers to the likelihood of developing cancer over a lifetime due to prolonged exposure to the elements present in these products. This risk was calculated for Cd, Pb, As and Hg using Equation (4).

CR= EDI X CSF Equation (4)

CSF is an oral dose factor for a carcinogen and is 1.5 mg/kg/day for As, 0.0085 mg/kg/day for Pb, 6.3 mg/kg/day for Cd and 6.177 mg/kg/day for Hg. Any risk above a CR threshold of 1×10^{-4} is considered unacceptable. Similarly CR values of 1×10^{-3} and higher are serious and require urgent, high-priority care. A CR value below 1×10^{-6} generally does not pose a significant health risk. CR values in the range of 1×10^{-6} to 1×10^{-4} are generally considered to represent a tolerable level of risk. [26,31, 33-36]

When multiple carcinogenic metals are present the individual risks from each metal are summed to obtain the TCR.[32] This is important because people are often exposed to multiple pollutants simultaneously which can increase the total risk. TCR can be calculated using the following formula (5).

TCR= CRCd + CRPb + CRAs Equation (5)

Statistical Analysis

Statistical analyses were performed using the SPSS 23.0 statistical software program (SPSS; Chicago, IL, USA). The Post-Hoc Test was used to detect statistical differences between the protein powder supplement samples (S1-S10) groups. A $p<0.05$ value was considered statistically significant.

Results

Heavy metal concentrations

ICP-MS analysis revealed the presence of Cd, Pb, As and Hg metals in all samples examined. The concentrations of the identified elements are presented in Figure 1. Among the measured metals, Pb was detected at the highest levels in most samples, followed by Hg. In contrast, Cd and As were found at lower levels (Figure 1).

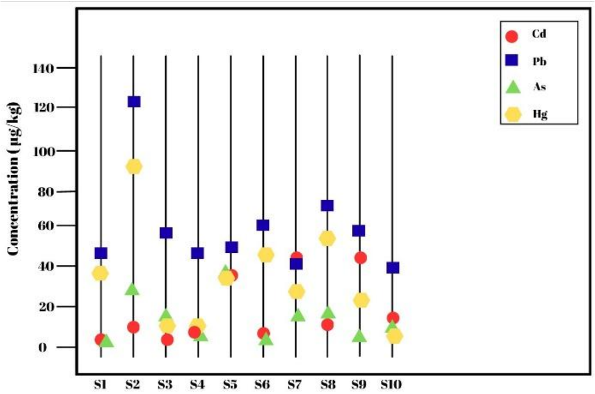

Figure 1. Concentration levels of risk elements in samples analyzed by ICP-MS.

In the heat map shown in Figure 2, purple and blue tones represent low levels while areas with concentrated green and yellow tones indicate medium and high element accumulation. Accordingly it is noteworthy that the S2 protein powder sample in particular has high Hg and Pb concentrations compared to other samples (Figure 2).

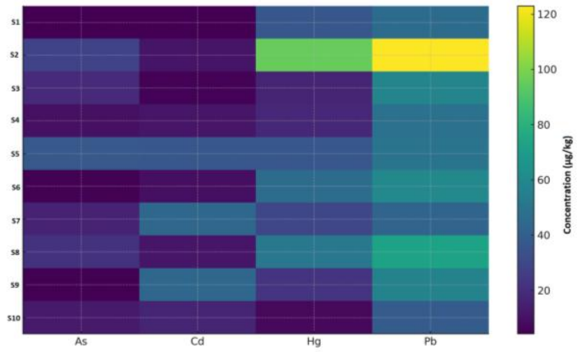

Figure 2. Heat map of the concentrations of risk elements in samples analyzed by ICP-MS.

To better explain the relationship between the identified risk factors and to track possible sources of contamination, protein powder samples were compared using a Post Hoc Test and presented as a bar graph in Figure 3. Statistically significant differences were found between the groups ($p<0.05$) (Figure 3).

All values are presented as mean ± SD. Different letters indicate statistically significant differences between groups according to Tukey's post-hoc test ($p<0.05$).

Figure 3-A shows that Cd was detected in all samples, ranging from 4.41 to 44.17 µg/kg. S7 and S9 had the highest Cd concentrations (44.02 µg/kg; 44.17 µg/kg; *p>0.05*), while S1 and S3 showed the lowest values (4.41 µg/kg; 5.3 µg/kg $p>0.05$). There was no statistically significant difference between these two groups ($p>0.05$). Pb was detected in all samples, ranging from 39.01 to 122.89 µg/kg. The relationship between Pb levels is shown in Figure 3-B. Accordingly, the S2 group protein powders had the highest Pb level and were found to be statistically significantly higher than the other samples ($p<0.05$). Figure 3-C As was detected in all samples, ranging from 4.27 to 37.26 µg/kg. As levels were highest in the S5 sample and were statistically significantly different from the other supplements ($p<0.05$). In contrast, the lowest levels were detected in samples S1, S6 and S9 (4.2 µg/kg, 4.8 µg/kg and 5.1 µg/kg respectively), no significant difference was found between these three samples ($p>0.05$), but they showed a statistically significant difference compared to the other groups ($p<0.05$). Figure 3-D shows that Hg was detected in all samples, ranging from 7.25 to 95.36 µg/kg. The highest Hg value was observed in S2, while S10 (7.25 µg/kg) showed the lowest values. Both samples were found to be statistically significantly different when compared to the other samples ($p<0.05$).

The conclusions drawn from the tables indicate that the homogeneity between products is low and that exposure levels can vary significantly depending on the supplement chosen by the consumer.

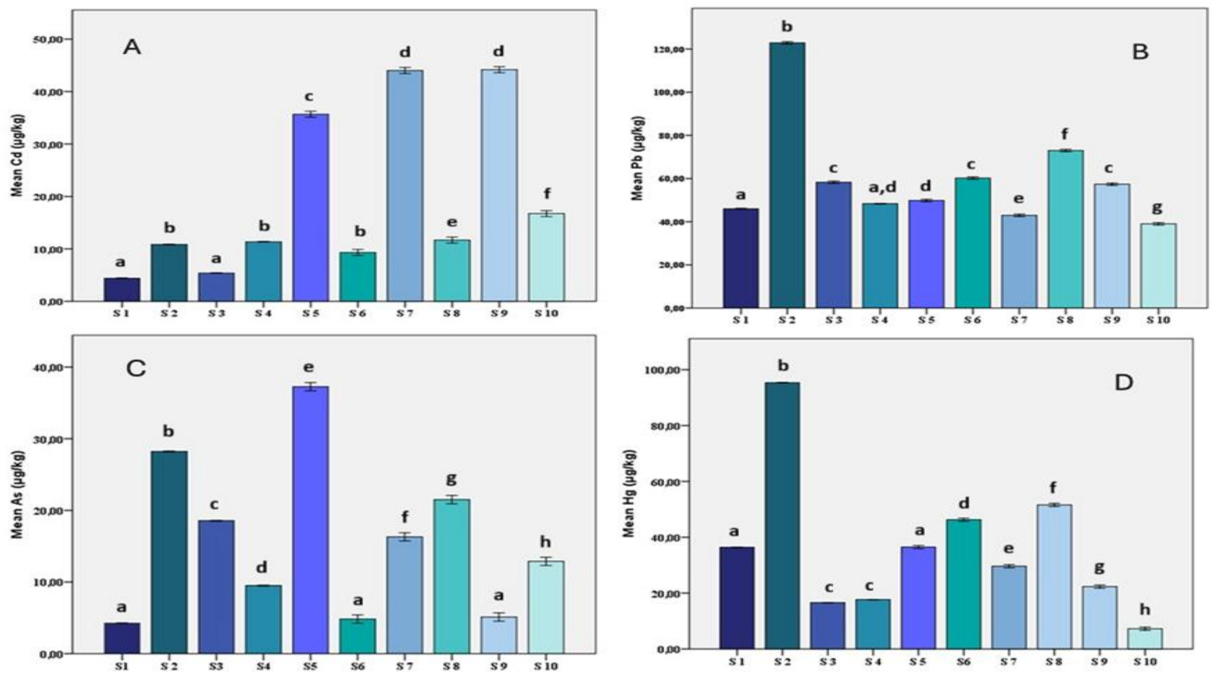

Figure 3. Comparison of Cd, Pb, As, and Hg levels in protein powder supplements between groups.

Health risk assessment

The mean ± standard deviation (mean ± SD) values (MC) of the risk elements in the protein powder supplements used in this study, based on portion weights, the EDI amount for 1 and 3 portions per day for an adult weighing 70 kg, the THQ and HI values for non-carcinogenic effects, and the CR and TCR values for carcinogenic effects were considered to determine the health risk (Table 2).

The non-carcinogenic effects of elemental impurities Cd, Pb, As and Hg on human health through protein supplement consumption are shown in Table 2. The THQ value for each risk element in the samples was found to be less than one. Additionally, the HI values for the cumulative non-carcinogenic risk assessment of the risk elements detected in each sample were also found to be less than one. When the CR values in Table 2 are examined in detail for the carcinogenic risk assessment of Cd, Pb and As elements detected in protein supplements, the CR value from the Cd level in a single serving of samples S1 and S6 is within the tolerable risk range of 1×10^{-4} - 1×10^{-6}, while the CR values for Pb and As levels were found to be less than 10^{-6}, posing no carcinogenic health risk. However, the CR values for Cd and As levels in the remaining samples were found to be within the tolerable risk range of 1×10^{-4} - 1×10^{-6} for Cd and As levels. Additionally, in the cumulative carcinogenic risk assessment, the TCR values are found to be within the tolerable risk level of 1×10^{-4} to 1×10^{-6} range.

When examining the effects of carcinogenic risk elements in 3-portion consumption, the CR values derived from Cd and As levels in all samples were within the tolerable risk range of 1×10^{-4} to 1×10^{-6}, while the CR value derived from Pb levels was found to be less than 10^{-6}, thus not posing a carcinogenic health risk. Consequently, when evaluating both non-carcinogenic risk (HI<1) and carcinogenic risk (TCR 1×10^{-4} - 1×10^{-6}) that may arise from consuming one or three servings of all samples, it is predicted that no health risk will arise from exposure to the four risk elements in the consumption of allsamples. Additionally, the HI and TCR values for 1 and 3 serving usage scenarios of protein powder supplements are compared in Figures 4 and 5 (Figure 4, Figure 5).

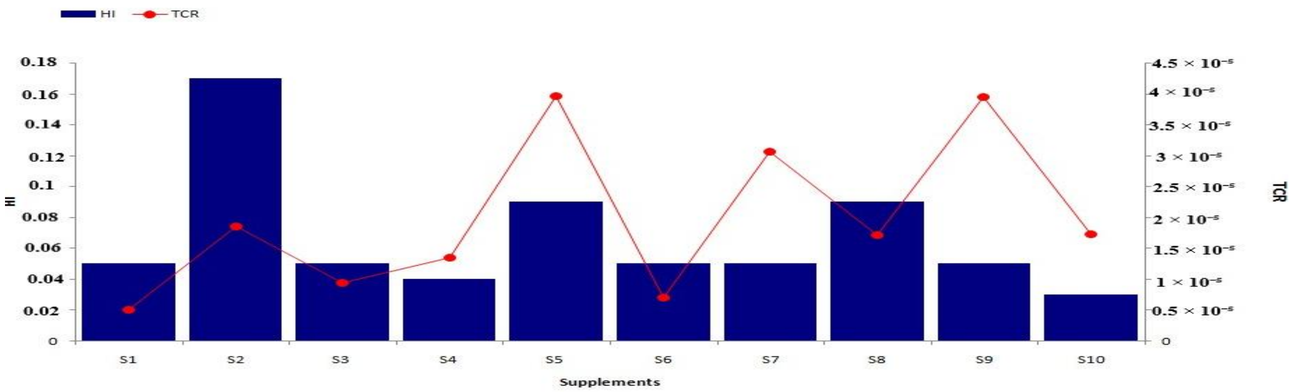

Figure 4. HI and TCR values for a single serving of protein powder supplements.

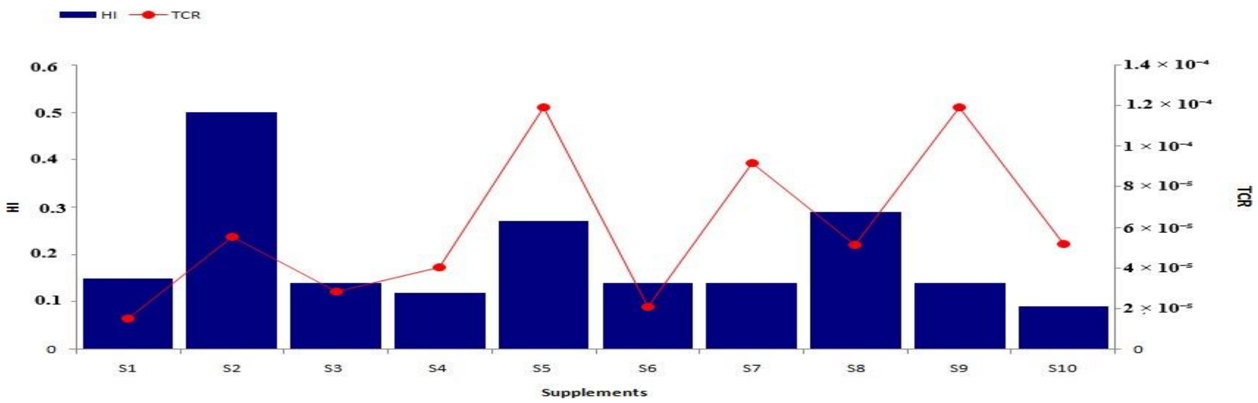

Figure 5. HI and TCR values for 3 servings of protein powder supplements.

When examining the elemental impurity levels detected in 10 samples and the risk assessment results of these levels on human health, it was shown that the HI value, which is an important metric in evaluating the non-carcinogenic effects of all supplements when consumed in 1 and 3 servings, was less than 1. It has been determined that the samples analyzed within the scope of the consumption scenarios examined do not pose a measurable health risk in terms of the calculated risk indicators. Additionally when examining the TCR values, an important parameter in assessing the carcinogenic effects of Cd, Pb and As elements, it was determined that the TCR values of all protein supplements were in the range of 1×10^{-4} to 1×10^{-6} when consumed in both 1 and 3 servings. This range corresponds to a tolerable level of carcinogenic risk.

Discussion

The use of protein, vitamin and mineral supplements is increasing due to the influence of social media. Individuals involved in sports are particularly turning to protein powder for purposes such as muscle building, weight control or meal replacement.[37] However, most users believe these products are safe. For example, a study by Whitehouse et al. reported that protein supplement users believe that using these products does not pose any health risks.[38]

However, protein supplements can be contaminated with risk elements depending on the raw materials, additives, production process and cultivation conditions used in their content and may pose potential health risks.[24,39] Plant and milk-based protein sources primarily carry these elements; plants absorb elements from soil, water, and air, while milk can also be contaminated as a result of environmental pollution.[27,40] Whey protein, in particular, is a byproduct of dairy products, so contamination of the raw milk is the main factor determining the element content of the product. Additionally, formula components such as cocoa and flavoring additives can increase the element load.[41]

In this study, 10 animal-derived protein powders originating from Turkey were examined and cocoa-containing products were selected as the carrier in all cases (Table 1). The analyses were performed using a closed-system microwave sample preparation method on a high-precision ICP-MS device in accordance with USP and EMA guidelines.[3,24,26,27,42]

In the present study Cd, Pb, As and Hg were detected in all analyzed protein powder samples and the average concentrations of these elements are presented in Table 2. This finding indicates that protein supplements should be evaluated not only for their nutritional components but also for potential risk elements. In particular the detection of Hg in all samples indicates that this element should not be overlooked in exposure assessments.

Although Hg is not consistently classified as carcinogenic in all regulatory frameworks in terms of oral exposure, recent ecotoxicological and human health risk assessment studies report that it has been included in carcinogenic risk calculations to reflect long-term exposure scenarios. In this study, the carcinogenic risk associated with Hg was estimated to represent long-term exposure using slope factors suggested in the literature, without making a universal oral carcinogenicity assumption.[27,35,36]

The Cd and Pb levels obtained were found to be lower than the values reported in a study conducted on protein supplements obtained from different pharmacies and supermarkets in Pakistan.[33] This may be due to differences in the market where the products were obtained, the source of raw materials and the production processes. Similarly, in a study conducted in Egypt, Cd and Pb levels differed from those in the present study and Hg was not detected in that study.[12] In contrast, the detection of Hg in all samples in our study suggests that differences in product content are not limited to heavy metal levels but may also vary in terms of element diversity.

Another study conducted with whey protein powder samples representing the European Union market showed that Cd and As concentrations largely overlapped with the ranges obtained in the present study but Pb and

As levels were higher in our samples. These findings reveal that elemental impurities in protein supplements can vary significantly depending on the geographical region, raw material sourcing, and production standards. Therefore, it is important to consider regional data and continue regular monitoring studies when assessing the safety of protein supplements.

All these data reveal that, despite having the same origin and similar contents, the supplements examined did not show a homogeneous distribution and that there were significant reliability differences between products on the market in terms of the content of elements in the risk group. Therefore, the level of exposure to risk elements can vary significantly depending on the brand or product type preferred by the consumer. In this context, the identified statistical differences are considered a critical finding in terms of consumer health.

The EDI, THQ, HI, CR and TCR values calculated for protein supplements in this study are presented in Table 2. The carcinogenic risk assessment was performed based on Cd, Pb and As elements and the obtained TCR values were interpreted according to risk thresholds widely accepted in the literature. Accordingly CR values below 1×10^{-6} represent a negligible risk level, while values in the range of 1×10^{-6}–1×10^{-4} represent a tolerable risk level.[31,33–35]

In the present study, the total TCR values calculated for both the 1-serving and 3-serving consumption scenarios were found to remain within the tolerable risk range. This indicates that the protein supplements examined are within acceptable limits in terms of carcinogenic risk at the consumption levels considered. When evaluated on an element-by-element basis, it was observed that Pb-related CR values remained at negligible levels in all samples, while risks from Cd and As approached the tolerable risk range in some samples but did not reach an unacceptable risk level.

Although an expected upward trend in TCR values was observed with increasing consumption levels the fact that risk levels remained within acceptable limits even in the 3-serving scenario highlights that serving size is a critical determinant in assessing the long-term effects of exposure. This finding emphasizes that the safety of protein supplements depends not only on their content but also on consumption habits.

Compared to the literature, Irshad et al. reported that the TCR values calculated for various elements, including Cd and Pb, in protein powder supplements used by athletes were generally at the 10^{-4} level.[33] Ring et al.[30] noted that in a 3-serving consumption scenario, TCR values reached 10^{-3} levels in many products, which could potentially pose a health risk. In the present study, although an increase in TCR values was observed, particularly in 3-serving consumption, it was determined that these values did not reach the high risk levels reported in the literature and remained within tolerable limits. These differences can be explained by variations in product formulations, element contents and consumption scenarios.

Similarly, HI values calculated under the same consumption scenarios were also found to be below 1 in all samples, thus demonstrating that the elements are also safe from a non-carcinogenic perspective. The studies by Bandara et al. and Horváth et al. also reported that HI values in protein powder samples were below 1 and did not pose a non-carcinogenic health risk.[3,27] However, the results in the literature are not always consistent. Both Ring et al. and the Clean Label Project reported that protein powder supplements could pose a non-carcinogenic health risk when consumed in three servings, as the HI value exceeded 1.[30,44]

Although the HI, CR and THQ values calculated in our study do not pose a risk, the risk elements As, Cd, Hg and Pb in Class 1 are toxic elements whose use in pharmaceutical production should be restricted or prohibited. They have also been associated with various health problems in long-term exposure. Cohort studies have shown that even consumption below the temporarily tolerable weekly intake (PTWI) of these supplements may pose a health.[45]

Cd is recognized as a carcinogenic risk factor. Cd exposure can lead to kidney damage, cardiovascular disease and cancer risk.[45,46] Long-term exposure has been associated with skin, lung, bladder, kidney and possibly liver and prostate cancer.[47] Hg and Pb can also adversely affect the nervous system, kidneys, cardiovascular system and cognitive functions.[48,49]

In conclusion, our current findings indicate that the 10 different popular protein powder samples selected do not pose a non-carcinogenic or carcinogenic health risk when consumed in 1 and 3 servings. Furthermore since different dosage and brand choices may alter the risk level, it is critical for consumer health that protein supplements are regularly monitored for risk factors.

Ethics Committee Approval

This study does not require ethics committee permission or any special permission.

Author Contributions

Author contributions were determined based on the "Author Contribution to the Research Form." All authors contributed to the drafting of the study or to significant intellectual/critical review of the content, and fulfilled at least three of the types of contributions specified in the study.

Conflict of Interest

The authors have no conflict of interest.

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Peer-review

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