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Pollution evaluation of organochlorine pesticides and heavy metals from cheese samples in Kirklareli, Turkey

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Abstract

The purpose of this article is to develop an easy analytical method to determine organochlorine pesticide and metals pollution using kashar cheese and white cheese in Kirklareli, Turkey, by gas chromatography-mass spectrometry (GC-MS) and flame atomic absorption spectrometry (FAAS). Kirklareli was selected as the study area because of its intense industry and agriculture. Organochlorine pesticides in samples were prepared for analysis using the silica with solid-phase extraction (SPE) method. Concentrations of hexachlorocyclohexane (ΣHCH), aldrin, heptachlor, dieldrin, endosulfan, methoxychlor and dichlorodiphenyltrichloroethane (ΣDDT), Cd, Pb, Ni, Cu, Zn, Fe and Mn were determined in cheese samples collected from different points in Kirklareli. Concentrations of Pb, Ni, Cd, Heptachlor-endo-epoxide, alpha-Endosulfan and Endrin-aldehyde were not determined in cheese species. Zn>Fe>Mn>Cu concentrations were found in cheese samples.

Key words: White cheese, kashar cheese, pesticides, pollution, GC-MS, FAAS, trace metals, solid phase extraction.

Introduction

Kirklareli, one of the Black Sea provinces in northwestern (Thrace region) Turkey, has a catchment area of 6550 km^2 [1]. The use of pesticides in agricultural areas can increase levels of toxicity and pollution in the ecological environment. The pesticides are potential chemical pollutants extensively used for agricultural purposes due to lower cost and higher effectiveness. They may accumulate in living creatures as decomposition, which can cause degradation in the environment. Among the most important sources of heavy metals, such as Cd, Ni, Pb, Cu, Zn, Mn and Fe, and pesticides originating from agricultural applications and industrial pollutants of soil, can be counted inorganic and organic fertilizers, fungicides, liming, sewage sludge and irrigation water and pesticides [2,3]. About half of all chemicals (whether natural or synthetic) tested in animal cancer tests at the maximum tolerated dose (MTD) are carcinogens [4].

Organochlorine pesticides in cheese may have accumulated in the environment in various ways such as industry, fossil fuels, agriculture, and other human activities. The increasing agricultural and anthropogenic activities of humans have intensified the emission of various pollutants, including toxic metals and pesticides, into the environment [5]. Pollution by heavy metals and pesticides is a problem of increasing significance for ecological, evolutionary, nutritional and human health reasons, and the natural cycle is shown in Fig.1. The pollutants (metals and pesticides) are transferred to animals either from the surrounding environment or their diets. At the same time, the lipid content of animal foods influences the bioaccumulation process [6-9].

The Turkish positive list for Maximum Residual Limits (MRL) of agricultural chemicals in food was made effective in 2011. MRL of concentrations in foods of the organochlorine pesticides are listed in Table 1.

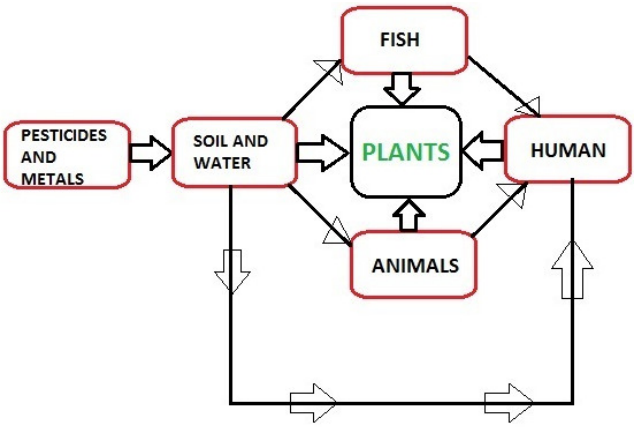

Figure 1. Cycle in nature of pesticides and metals.

Table 1. The maximum residues limits in foods of organochlorine pesticides.

Pesticide	μg kg^{-1}
HCH (hexachlorocyclohexane) (α, β, γ, δ)	10
Heptachlor	10
Heptachlor-endo-epoxide	10
Aldrin (HHDN)	10
Alpha-Endosulfan	50
4,4'-DDE, 4,4'-DDD, 4,4'-DDT	50
Dieldrin	10
Endrin	10
beta-Endosulfan	50
Endrin-aldehyde	10
Endosulfan-sulfate	50
Endrin-ketone	10
Methoxychlor	5

The extensively used high sensitivity analytical techniques for the determination of heavy metals at low concentrations in food samples are electrothermal atomic absorption spectrometry (ETAAS), inductively coupled plasma-atomic emission spectrometry (ICP-AES), and inductively coupled plasma-mass spectrometry (ICP-MS). The ETAAS is a more sensitive technique than flame atomic absorption spectrometry (FAAS). However, FAAS can be considered the preferred method because it is faster, less expensive, and does not require expert operators if its sensitivity is sufficient [10-14]. The extensively used high sensitivity analytical techniques for determination of these pesticides at low concentrations in food samples are GC-MS, HPLC-MS, HPLC-DAD and GC-ECD [15-18]. The function of GC-MS is identification, quantification and analysis of a compound. Name, molecular structure, molecular weight and fragmentation pattern can also be detected by GC-MS [19].

The aim of this study was to determine the concentrations of organochlorine pesticides and metals in white cheese and kashar cheese in the province of Kirklareli (Turkey) by gas chromatography-mass spectrometry (GC-MS) (pesticides including ΣBHC (alpha-BHC, beta-BHC, gamma-BHC, delta-BHC), ΣDDT (4,4'-DDD, 4,4'-DDE, 4,4'-DDT), alpha-Endosulfan, beta-Endosulfan, Endosulfan sulfate, Heptachlor, Heptachlor-endo-epoxide, aldrin, dieldrin, Endrin aldehyde, Endrin ketone, Endrin and Methoxychlor) and flame atomic absorption spectrometry (FAAS) (trace metals including Cd, Ni, Pb, Zn, Fe, Mn and Cu). Kirklareli was selected as the study area because of its intensive industry and agriculture.

Experimental

Instrumentation: The organochlorine pesticides were analyzed by GC-MS, using an Agilent 5975C MSD system and Agilent 7890A model at Kirklareli University. HP-5 MS IU column (30 m x 250 μm x 0.25 μm) was used with helium as the carrier gas. Injector temperature, split flow and injection volume were 250°C, 1 mL/min and 1 μL. The GC oven temperature was kept constant at 110°C for 5 min to 320°C at a rate of 8°C min^{-1}. MS fragmentation voltage was taken at 70 eV. Component identification was carried out using spectrometric electronic libraries (NIST).

The Cd, Ni, Pb, Cu, Zn, Fe and Mn concentrations were determined using an Agilent 240 Duo FAAS. Samples were prepared with microwave digestion by Mars 6 (CEM Corporation). The applied instrumental conditions are listed in Table 2.

Standards and reagents: The standard stock solutions of Organochlorine Pesticide Mix 2, 2000 ng/μL in toluene, (including ΣBHC (alpha-BHC, beta-BHC, gamma-BHC, delta-BHC), ΣDDT (4,4'-DDD, 4,4'-DDE, 4,4'-DDT), alpha-Endosulfan, beta-Endosulfan, Endosulfan sulfate, Heptachlor, Heptachlor-endo-epoxide, aldrin, dieldrin, Endrin aldehyde, Endrin ketone, endrin and Methoxychlor) were purchased from Dr. Ehrenstorfer GmbH (a standard mixture solution containing all 18).

The standard solutions were prepared from dilution of their stock standard solutions at concentrations of 1000, 500, 100, 50, 25, 10 and 5 μg/L and redistilled in toluene. Standard identification, quantification and analysis of the compound were done by GC-MS. For organochlorine pesticides the detection used was retention time (Table 3).

The Cd, Ni, Pb, Zn, Mn, Fe, Cr and Cu solutions (1000 mg/L) were prepared from Merck stocks (Darmstadt, Germany). Afterwards, calibration standards of each metal were prepared by appropriate dilution (with 2.5% HNO_3) of the stock solution. For digestion of the samples, nitric acid (65%, Merck) and hydrogen peroxide (35%, Merck) were used. All chemicals used were of analytical reagent grade. Double-distilled water obtained with a water purification system (ELGA) was used for all preparations. When not in use, all Pyrex glassware and vessels were kept permanently full of 2.5% HNO_3. All Teflon digestion vessels were previously cleaned in a bath of 15% (v/v) nitric acid solution for 48 h to avoid cross contamination.

Sample preparation: Kashar cheese and white cheese were utilized as samples in this study. About 500 g of the cheese species were collected from a public bazaar in Kirklareli. All samples were homogeneously mixed and put into dry plastic bags (washed with acid (1% NHO_3) and distilled water).

Table 2. Instrumental operating conditions for FAAS.

Parameter	Cd	Ni	Pb	Cu	Zn	Mn	Fe
Wavelength (nm)	228.8	352.5	283.3	324.8	213.9	279.5	372.0
HCL current (mA)	4.0	4.0	10.0	4.0	5.0	5.0	5.0
Acetylene flow rate (L/min)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Air flow rate (L/min)	13.5	13.5	13.5	13.5	13.5	13.5	13.5
Slit width (nm)	0.5	0.2	0.5	0.5	1.0	0.2	0.2

Table 3. Retention times of organochlorine pesticide standard in GC-MS.

Pesticide	Retention time
HCH (α)	7.816
HCH (β)	8.501
HCH (γ)	8.666
HCH (δ)	9.282
Heptachlor	10.578
Aldrin (HHDN)	11.501
Heptachlor-endo-epoxide	12.674
Alpha-Endosulfan	13.516
4,4'-DDE	14.146
Dieldrin	14.198
Endrin	14.765
beta-Endosulfan	15.006
4,4'-DDD	15.231
Endrin-aldehyde	15.502
Endosulfan-sulfate	16.112
4,4'-DDT	16.192
Endrin-ketone	17.302
Methoxychlor	17.666

Sample preparation for analysis of organochlorine pesticides: QuEChERS analytical methods for pesticide residues in foodstuffs were followed. Of cheese samples 100 g was chopped and homogenized. Approximately 10 g of the sample was stirred in a vortex for one min with 10 mL of dichloromethane. The samples were centrifuged at 5°C and 5 min at 7000 rpm to obtain the two phases. Na-acetate 1.5 g and anhydrous magnesium sulfate 6.0 g were added to remove moisture and further stirred for 3 min using the vortex. The sample was then centrifuged again at 5°C and 5 min at 7000 rpm. The supernatant extraction was followed by a clean-up step using solid-phase extraction with 400 mg PSA, 1200 mg $MgSO_4$ (extracted using an SPE cartridge) [20]. Pesticides in the sample extract were concentrated as dryness by using a gentle nitrogen stream. This was dissolved in 1.5 mL of hexane and filtered through a syringe filter of 0.45 µm. Afterwards, the extracts (1µL injection volume) were quantified by gas chromatograph with mass detector (GC-MS).

Sample preparation for analysis of trace-metals: The cheese samples were homogeneously mixed. The homogenized samples were stored carefully in dry, clean polythene bags. Of each sample 0.5 g was digested by adding a mixture of 6 ml nitric acid (HNO_3) and 3 ml hydrogen peroxide (H_2O_2) and microwaved in a 75 ml express vessel in a microwave (Mars 6) using a program in two stages: step 1 (time 15 min, T1 120°C, power 600 W); step 2 (time 15 min, T1 120°C, power 800 W); step 3 (time 15 min, T1 120°C, power 600 W). The vessel was cooled and 3 ml of 2.5% HNO_3 was added. The resulting solutions were analyzed by FAAS.

Quantitation: Linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ) and recovery parameters were determined for the pesticides. Seven level (5-1000 µg L^{-1}) calibration series with three analyses at each concentration level were determined for linearity and the calibration curve was plotted automatically. LOD and LOQ had a signal to noise ratio of 3 and 10, respectively.

The methods of AAS and GC-MS were validated by the method of standard addition [21, 22]. In the calculation of limit of detection (LOD) and LOQ values, the lowest concentration in the calibration plot of each element was analyzed ten times. Linear ranges for four elements were also determined.

Results and Discussion

Kirklareli is one of the Black Sea provinces in the northwestern Thrace region of Turkey. Industrial facilities and agriculture are among the main activities there. Trace-level residues of organochlorine pesticides and metals found in cheese have become a subject of many studies that have gained international attention.

In this study, 18 organochlorine pesticides were chosen for analysis and determination in white cheese and kashar cheese. When doing SCAN for 500 µg L^{-1} standard was used with NIST library in GC-MS SPE, which allows very fast extraction and low solvent volumes. The data were compared with each other. After elution-cleaning with SPE cartridge for pesticides in cheese samples has been developed as a simultaneous method for analysis by GC-MS. The main advantage of this method is that extraction and clean-up are performed in less time with a low volume of solvent. Additionally, the proposed method is a sensitive, reproducible and reliable alternative to the normally used methods; moreover it is inexpensive, easy and rapid (QuEChERS method). This study demonstrated that the method using QuEChERS and GC-MS is very effective in analyzing the organochlorine pesticides in the cheese.

Calibration graphs for pesticides were established through the range of 5-1000 µg L^{-1} with correlation of coefficients from 0.9855 to 0.9978 for all analytes. The correlation of coefficients (R^2), LODs and LOQs are shown in Table 4. Limits of detection ranged from 0.37 to 8.90 µg kg^{-1} for the eighteen pesticides. Limits of quantification ranged from 1.23 to 26.8 µg kg^{-1} for the eighteen pesticides

Our study clearly shows that there are contamination and toxicity traces of pesticides in the cheese but there is not such an accumulation of heavy metals. Endrin-aldehyde, heptachlor-endo-epoxide, alpha-endosulfan, Cd, Pb and Ni contamination were not found below the detection limit in the cheese samples (Tables 5 and 6).

Organochlorine pesticide residues have a relation with the lipid content of cheese. Pesticides that are organic toxic compounds may enter the cheese through the initial stage of the food chain.

Table 4. LOD, LOQ and correlation coefficients in pesticide standard solution range different levels.

Pesticide	LOD ($\mu g\ L^{-1}$)	LOQ ($\mu g\ L^{-1}$)	Correlation (R^2)
HCH (α)	0.42	1.40	0.9944
HCH (β)	6.54	21.8	0.9949
HCH (γ)	8.05	26.8	0.9927
HCH (δ)	1.90	6.35	0.9925
Heptachlor	12.2	40.6	0.9923
Aldrin (HHDN)	1.02	3.38	0.9958
Heptachlor-endo-epoxide	-	-	0.9943
Alpha-Endosulfan	-	-	0.9937
4,4'-DDE	0.37	1.23	0.9932
Dieldrin	3.87	12.9	0.9932
Endrin	4.68	15.6	0.9955
beta-Endosulfan	1.58	5.27	0.9937
4,4'-DDD	0.56	1.87	0.9855
Endrin-aldehyde	-	-	0.9901
Endosulfan-sulfate	0.59	1.96	0.9885
4,4'-DDT	0.57	1.91	0.9978
Endrin-ketone	0.70	2.32	0.9909
Methoxychlor	0.52	1.73	0.9964

Therefore, it is important to determine the pesticide residue in cheese.

Organochlorine pesticides that accumulate in fatty tissue due to the strong lipophilic properties were the first environmental contaminants detected in milk [23]. Their levels have declined in recent years in many countries but many of these chemicals, called persistent organic pollutants, persist in our environment.

It was found that the samples tested did not contain any Cd, Pb or Ni contamination. Lopez *et al.* reported an analysis of Fe, Zn and Cu in some foods consumed in Mexico including vegetables, legumes, fruits, cereals and animal foods [24]. Metal concentrations were determined in the kashar and white cheese, as shown in Table 6. Fe, Zn, Mn and Cu content of trace metals of kashar and white cheese samples in Kirklareli are shown in Figs 2 and 3, respectively.

Concentrations of Pb, Ni, Cd, Heptachlor-endo-epoxide, alpha-Endosulfan and Endrin-aldehyde were not determined in cheese species. Zn in kashar cheese was 50 times greater than in white cheese. The amount of Heptachlor in kashar cheese1 was 2 to 7 times more than in the others. In addition, the amount of beta-

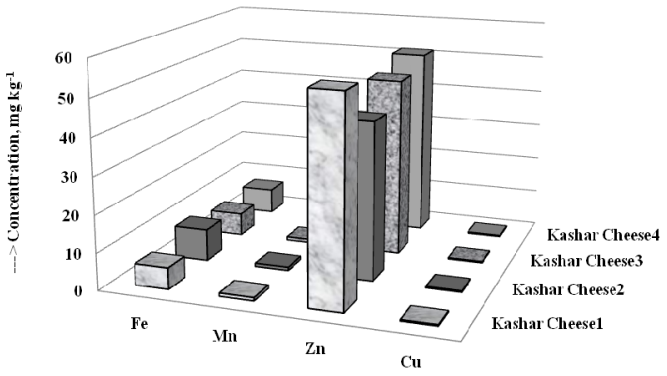

Figure 2. Fe, Mn, Zn and Cu concentrations in kashar cheese.

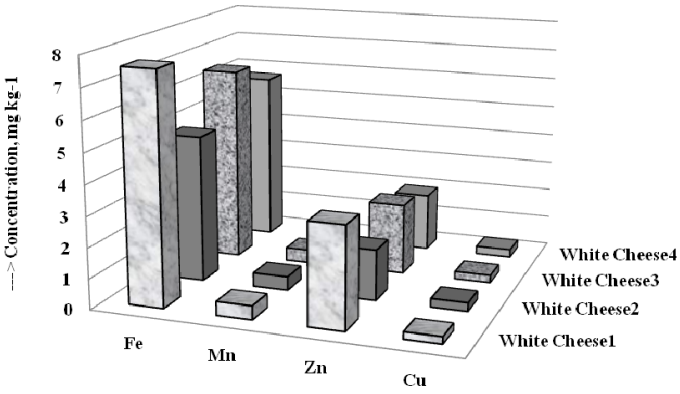

Figure 3. Fe, Mn, Zn and Cu concentrations in white cheese.

HCH, gamma-BHC, Dieldrin, Endrin, and Methoxychlor in the same example was over the MRL border value. In addition to this, the amount of Methoxychlor in all samples was 1.1 to 2.4 times greater than the MRL value. The value of Endrin was 1.5 to 2.7 times greater and 2.1 to 18.8 times greater in white cheese1-2 and kashar cheese2-1, respectively, than the MRL value. Dieldrin was also detected at rates changing from 1.2 to 2.5 times. The concentrations of alpha-HCH, beta-HCH, beta-Endosulfan, Endrin-ketone, Endosulfan-sulfate, and DDT and its derivatives were below the MRL values.

This could possibly have been the result of industrial pollution, as trace elements and pesticides could easily have been absorbed in plant leaves from the air or soil. Alternatively, it could have been the result of animals eating the leaves.

Conclusions

In the article we studied the content of trace metals and organochlorine pesticides in kashar and white cheese. Organochlorine pesticides are of global concern because of their widespread occurrence, bioaccumulation, persistence and toxicity to humans. So for the improvement of the world and food safety the sources of pollution should be identified and eliminated. Such information is of value to health care professionals, researchers and food manufacturers in preparing nutritious products. Levels of metals and pesticides in some products might also be unexpected and hence informative and may lead to further analyses and research.

Acknowledgements

The author thanks the Kirklareli University Research Foundation for their support.

Table 5. Concentrations ppb (μg kg^{-1}) of organochlorine pesticides in cheese samples.

Pesticide	White cheese1	White cheese2	White cheese3	White cheese4	Kashar cheese1	Kashar cheese2	Kashar cheese3	Kashar cheese4
HCH (α)	7.47 ± 0.14	6.63 ± 0.11	6.72 ± 0.12	5.45 ± 0.09	7.72 ± 0.22	3.70 ± 0.11	5.65 ± 0.15	4.78 ± 0.12
HCH (β)	8.86 ± 1.62	5.58 ± 0.81	2.45 ± 0.33	6.82 ± 0.61	15.2 ± 4.2	4.62 ± 0.19	6.58 ± 0.21	4.32 ± 0.12
HCH (γ)	7.60 ± 1.66	7.97 ± 0.83	7.52 ± 0.68	6.75 ± 0.63	15.71 ± 1.49	10.65 ± 1.11	8.75 ± 0.89	3.97 ± 0.25
HCH (δ)	7.77 ± 0.42	3.78 ± 0.07	3.74 ± 0.12	3.82 ± 0.17	9.92 ± 0.78	3.86 ± 1.55	6.41 ± 0.58	5.63 ± 0.55
Heptachlor	12.7 ± 1.1	7.17 ± 0.57	8.33 ± 0.71	7.09 ± 0.55	23.5 ± 9.1	3.86 ± 1.55	8.05 ± 0.73	4.68 ± 0.45
Aldrin (HHDN)	6.90 ± 0.15	3.56 ± 0.08	5.82 ± 0.18	2.48 ± 0.09	9.48 ± 0.75	4.21 ± 0.38	5.84 ± 0.55	4.61 ± 0.43
Heptachlor-endo-epoxide	nd	nd	nd	nd	nd	nd	nd	nd
Alpha-Endosulfan	nd	nd	nd	nd	nd	nd	nd	nd
4,4'-DDE	7.11 ± 0.16	3.67 ± 0.08	2.18 ± 0.11	4.63 ± 0.28	15.6 ± 5.8	3.68 ± 2.90	5.62 ± 0.48	4.78 ± 0.39
Dieldrin	16.2 ± 1.9	21.4 ± 2.1	10.3 ± 0.9	14.6 ± 1.1	16.2 ± 1.9	21.4 ± 2.1	25.4 ± 1.7	11.5 ± 0.9
Endrin	26.9 ± 2.1	14.9 ± 1.0	16.6 ± 1.6	15.8 ± 1.5	26.9 ± 2.1	14.9 ± 1.0	18.8 ± 1.5	21.2 ± 1.9
beta-Endosulfan	7.49 ± 0.08	3.80 ± 0.04	5.65 ± 0.48	3.88 ± 0.14	7.49 ± 0.08	3.80 ± 0.04	9.82 ± 0.33	4.97 ± 0.06
4,4'-DDD	8.64 ± 0.15	4.21 ± 0.07	6.64 ± 0.21	2.32 ± 0.09	8.64 ± 0.15	4.21 ± 0.07	9.10 ± 0.35	4.30 ± 0.17
Endrin-aldehyde	nd	nd	nd	nd	nd	nd	nd	nd
Endosulfan-sulfate	9.45 ± 0.18	4.60 ± 0.09	3.60 ± 0.09	5.45 ± 0.11	9.45 ± 0.18	4.60 ± 0.09	nd	nd
4,4'-DDT	10.6 ± 0.2	5.03 ± 0.11	nd	nd	10.6 ± 0.2	5.03 ± 0.11	nd	nd
Endrin-ketone	8.47 ± 0.18	4.11 ±0.09	3.35 ± 0.11	6.14 ± 0.19	8.47 ± 0.18	4.11 ±0.09	9.08 ± 0.44	4.85 ± 0.22
Methoxychlor	11.5 ± 0.3	5.58 ± 0.12	1.52 ± 0.09	6.34 ± 0.15	11.5 ± 0.3	5.58 ± 0.12	7.91 ± 0.22	5.80 ± 0.11

Table 6. Concentrations ppm (mg kg^{-1} of heavy metals in cheese samples.

Samples	Cd	Fe	Ni	Pb	Mn	Zn	Cu
Kashar Cheese1	nd	5.8 ± 0.48	nd	nd	0.88 ± 0.04	55.1 ± 2.1	0.32 ± 0.01
Kashar Cheese2	nd	8.61 ± 0.86	nd	nd	0.90 ± 0.03	42.5 ± 1.2	0.35 ± 0.02
Kashar Cheese3	nd	6.18 ± 0.59	nd	nd	0.91 ± 0.02	48.3 ± 1.1	0.33 ± 0.01
Kashar Cheese4	nd	6.92 ± 0.58	nd	nd	0.89 ± 0.03	51.3 ± 2.2	0.32 ± 0.02
White Cheese1	nd	7.56 ± 0.64	nd	nd	0.45 ± 0.03	3.32 ± 0.21	0.23 ± 0.01
White Cheese2	nd	4.84 ± 0.44	nd	nd	0.45 ± 0.03	1.67 ± 0.17	0.28 ± 0.01
White Cheese3	nd	6.45 ± 0.52	nd	nd	0.44 ± 0.03	2.35 ± 0.18	0.25 ± 0.01
White Cheese4	nd	5.69 ± 0.48	nd	nd	0.45 ± 0.03	1.93 ± 0.18	0.26 ± 0.01

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