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THE PREVALENCE OF CAESIUM137 RADIONUCLIDE ACTIVITY IN THE MEAT OF WILD BOARS, THAT WERE HUNTED IN ROMANIA: RESULTS FROM A STUDY USING GAMMA SPECTROMETRY WITH HPGE DETECTOR

PREVALENȚA ACTIVITĂȚII RADIONUCLIDULUI CS137 ÎN CARNEA DE LA MISTREȚI VÂNAȚI ÎN ROMÂNIA: REZULTATE DINTR-UN STUDIU UTILIZÂND SPECTROMETRIA GAMA CU DETECTOR DE HPGE

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ABSTRACT | REZUMAT

Identifying the presence of radionuclides ^{134}Cs and ^{137}Cs through laboratory tests represents one of the forms of assessing the radioactive contamination of water, food products of animal and non-animal origin, and animal feed. Conducting tests through high-resolution gamma spectrometry with HPGe (hyperpure germanium) detector on 189 samples of wild boar meat hunted in Romania and identifying the presence of radionuclide ^{137}Cs in 24 samples, with specific activity values ranging from 2.18 Bq/kg to 333 Bq/kg (the highest value recorded in the study), revealed a prevalence of 12.69% for this radionuclide. The detection of radionuclide ^{137}Cs only in samples of wild boar meat hunted in the counties of Argeș, Covasna, Harghita, and Sibiu - all of which are mountainous counties - indicates that in the ecosystem of deciduous forests in the mountainous areas of these counties, ^{137}Cs still exists in concentrations high enough so that, through ingestion by wild boars, this radionuclide reaches their muscle tissue at a detectable level through high-resolution gamma spectrometry with an HPGe detector. No values exceeding the maximum permissible level of 600 Bq/kg for ^{137}Cs were recorded (according to current regulations for radiation protection and animal health). However, identifying values of 290 Bq/kg in a 24-month-old wild boar and 246 Bq/kg in a 36-month-old wild boar - values that are close to half of the maximum permitted level - still represent a cause for concern. Due to the bioaccumulation effect, the meat from wild boars hunted in these 4 counties could become a risk factor for food safety and human health.

Keywords: wild boar meat, radionuclide ^{137}Cs, gamma spectrometry, food safety, human health

Determinarea prezenței radionuclizilor ^{134}Cs și ^{137}Cs prin teste de laborator reprezintă una dintre formele de evaluare a contaminării radioactive a apei, produselor alimentare de origine animală şi nonanimală şi a hranei pentru animale. Efectuarea de teste prin spectrometrie gama de înaltă rezoluţie cu detector de HPGe (germaniu hiperpur) pe 189 probe de carne de mistreţi vânaţi pe teritoriul României şi identificarea prezenței radionuclidului ^{137}Cs în 24 probe, cu valori ale activității specifice cuprinse între 2,18 Bq/kg și 333 Bq/kg (cea mai mare valoare înregistrată în cadrul studiului), a relevat o prevalență de 12,69% pentru acest radionuclid. Detectarea radionuclidului ^{137}Cs numai în probele de carne de mistreți vânați de pe raza județelor Argeș, Covasna, Harghita și Sibiu - toate fiind județe cu topografie montană - arată că în ecosistemul pădurilor de foioase din zonele montane ale acestor județe există în continuare ^{137}Cs în concentrații suficient de mari astfel că, prin ingerare de către mistreți, acest radionuclid ajunge în țesutul muscular al acestora la un nivel detectabil prin spectrometrie gama de înaltă rezoluţie cu detector de HPGe. Nu au fost înregistrate valori care să depășească nivelul maxim permis de 600 Bq/kg pentru ^{137}Cs (conform reglementărilor în vigoare pentru radioprotecție și sănătatea animală). Dar identificarea valorilor de 290 Bq/kg la un mistreț de 24 de luni și de 246 Bq/kg la un mistreț de 36 de luni - valori care se situează aproape de jumătatea nivelului maxim permis - reprezintă totuși un semnal de alarmă. Din cauza efectului de bioacumulare, carnea de la mistreții vânați în cele 4 județe s-ar putea transforma într-un factor de risc pentru siguranța alimentară și pentru sănătatea umană.

Cuvinte cheie: carne de mistreț, radionuclid ^{137}Cs, spectrometrie gama, siguranță alimentară, sănătate umană

All forms of life on our planet are continuously exposed to ionising radiation originating from both natural sources (cosmic, terrestrial, or solar radiation) and artificial or anthropogenic sources, generated by radionuclides resulting from human activities (nuclear weapons testing, nuclear power plants and other nuclear energy production facilities, medical imaging, scientific research, and industrial use of radiation) (1, 14). Radionuclides are atoms of certain chemical elements whose nuclei are unstable and emit radiation when they decay in a process called radioactivity. Once released into the environment, radionuclides

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contaminate radioactively, depending on their nature, the amount emitted, and the environmental conditions. When present in significant quantities, they have a major impact on human health and the environment through the phenomenon of bioaccumulation. Radionuclides can be assimilated and accumulated by organisms in the food chain, especially those taken up by plants and subsequently consumed by animals. This can lead to increased concentrations of radionuclides in organisms at higher trophic levels, which can increase the risk of radiation exposure for these organisms and for those who consume the respective products (4, 7). Forests are considered nearly closed ecosystems, so pollutants or contaminants return to the soil when flora and fauna die, and perennial plants and animals with longer lifespans - existing in that ecosystem - can constantly uptake them, leading to a cumulative effect of contaminants in organisms. Wildlife animals are considered bioindicators of environmental pollution, including radionuclide contamination. After the Chernobyl accident, wild boars with elevated levels of radioactive contamination were detected throughout Europe (2,3).

Studies conducted in forests in Germany and Austria (3) have shown wild boars with specific activity of radionuclide ^{137}Cs exceeding the maximum permitted level of 600 Bq/kg, especially in forests in northern Austria and Bavaria[3], even after 30 years since the Chernobyl nuclear accident (April 26, 1986). Since few studies are available at the national level regarding the level of radioactive contamination of game meat and at the same time the risk to food safety, this study aimed to measure the activity of radionuclides ^{137}Cs and ^{134}Cs in wild boar meat hunted in several counties of the country and additionally, to assess the magnitude of the values obtained relative to the maximum permitted level, the number of cases, and their correlations with the age categories of the tested wild boars.

MATERIALS AND METHODS

The study tests were conducted between January 19, 2023, and February 9, 2024, during which 189 wild boars were investigated. Their ages ranged from 12 to 136 months, and they were hunted in hunting grounds across 11 counties of the country. Among these counties, 5 were border counties: Dolj, Giurgiu, Olt, Satu-Mare, and Tulcea, while the other 6 were counties from various inland areas of the country: Argeș, Covasna, Harghita, Hunedoara, Ialomița, Sibiu.

The analysed samples consisted of 400-500 grammes of striated muscle tissue from each hunted wild boar. Tissue samples were taken from either the front or hind limb region, depending on accessibility, to avoid areas with consecutive blood infiltrations due to shooting. The working technique required muscle tissue to be as well cleaned as possible from fatty tissue, connective tissue, or blood infiltrations.

Laboratory tests were performed at the Institute of Hygiene and Veterinary Public Health (I.I.S.P.V.), following a specific procedure developed by the National Reference Laboratory for the Control of Radioactive Contamination to determine the specific activity of radionuclides (^{137}Cs, ^{134}Cs, and others) using high-resolution gamma spectrometry with an HPGe (hyper-pure germanium) detector, adapted for food and feed analysis in the laboratory. This method is referenced to SR EN ISO 10703:2016[8] through Specific Procedure No. 030. The equipment used for determining the specific activity of radionuclides ^{137}Cs and ^{134}Cs is a Canberra Packard Central Europe GmbH (Austria) gamma spectrometer (Fig.1), which includes: a digital spectrum analyser - DSA.1000, with 8192 energy channels, a coaxial hyper-pure germanium detector with a crystal diameter of 57 mm, a cryostat (capable of maintaining the detector at the temperature of liquid nitrogen), a lead shielding system for the detector (Pb castle). Additionally, the equipment is equipped with a computer and software package for quantitative analysis and control gamma spectrometry type GENIE 2000 with calibration software S574/Lab.SOCS/ISOCS, which, with the help of a mathematical model, allows for the identification and quantification of all gamma-emitting radionuclides present in a sample.

Fig. 1. Canberra Packard Equipment

The main steps of the test according to Specific Procedure No. 030 "Determination of the specific activity of radionuclides (^{137}Cs, ^{134}Cs, and others) by high-resolution gamma spectrometry with HPGe detector, in foods and feeds" are as follows:

1.Sample processing and preparation for measurement: removal of fatty tissue, connective tissue, aponeuroses, membranes, mincing by chopping with an electric robot, determination of the weight of the measurement box by weighing, homogenisation, and distribution in the measurement box, ensuring that it occupies the entire volume up to a level similar to that of the closed standard sources used for calibration and verification, weighing the box to accurately determine the mass contained by subtracting the weight of the measurement box.

2.Equipment verification and calibration by:

a)establishing the correspondence relationship between spectrum channels and their energy level, and

b)determining the counting efficiency of the detector as a function of energy.

3.Determination of the specific activity of radionuclides ^{137}Cs and ^{134}Cs: the sample box is introduced using a special clamp into the measurement chamber (Lead castle), positioned directly on the detector win-

dow. The acquisition time of the gamma spectrum is established through the software, the identification data of the sample and the mass of the sample (in kg), including the weighing error on the balance, are entered, and the spectrum acquisition process is initiated. Through the analysis and interpretation of the acquired spectrum, the equipment software calculates and establishes the minimum detectable activity (MDA) and the specific activity of the two radionuclides (^{137}Cs and ^{134}Cs) expressed in Bq/kg (Becquerel).

RESULTS AND DISCUSSION

The results obtained during the readings were interpreted taking into account the minimum detectable activity (MDA), a parameter defined as the smallest activity of a radioactive source that can be detected with a confidence level of 95% by dedicated equipment for detecting the specific activity of radionuclides. MDA is a variable calculated by the equipment software and is influenced by: the sensitivity of the detector, the natural background radiation, the measurement time and geometry, and the characteristics of the sample (the composition, density, and dimensions of the analysed sample can influence how radiation is emitted and detected, which can affect the MDA).

The lower the MDA the more sensitive the equipment is, and it can detect lower concentrations of radionuclides in a sample. Knowing the value of MDA, calculated by the software following a laboratory test to determine the activity of radionuclides is crucial in interpreting the obtained results. For this reason, all veterinary health laboratories involved in official control present, along with the obtained result, the values of MDA for the respective test in their analysis reports.

Following the investigations, it was found that in 24 out of the 189 tested samples, the presence of ^{137}Cs was identified, and its specific activity was measured. This represents a prevalence of 12.69% for this radionuclide in this study.

In none of the samples was a specific activity of over 600 Bq/kg of ^{137}Cs measured, which is the maximum level allowed by national and European regulations:Commission Implementing Regulation (EU) 1158 /2020 (10) and Regulation (Euratom) No. 52/15.01. 2016 of the EU Council (8).

The measured specific activity ranged between 2.18 Bq/kg and 333 Bq/kg (the highest value recorded in the study). Table 1 provides detailed values obtained for each of the 24 samples where the presence of the radionuclide ^{137}Cs was recorded.

Although the equipment software allows for it, the presence of the radionuclide ^{134}Cs was not identified, which is understandable because the half-life for ^{134}Cs is 2.065 years compared to ^{137}Cs, which has a half-life of 30.17 years.

The non-identification of the radionuclide ^{134}Cs in the wild boar meat hunted in various counties of Romania is consistent with the results of studies conducted on wild boars in Italy, Germany, and Austria. The authors of the study observed the presence of only ^{137}Cs.

Table 1

Values obtained for ^{137}Cs and 134Cs with the corresponding MDA for the samples where specific activity for ^{137}Cs was identified

CTY	No.	Crotal number	Origin	Sex	Age/ Months	Value Cs-137	Value Cs-134	Date of analysis
AG	1	Fara nr crotaliu	FV - 1 - AG	F	24	65,1; (MDA=3,32)	<3,05; (AMD=3,05)	07-02-2024
CV	1	20031RO0829458	FV - 1 - CV	F	24	7,71; (MDA=2,61)	<3,17; (AMD=3,17)	28-11-2023
CV	2	21003RO0777843	FV - 2 - CV	M	36	4,58; (MDA=1,97)	<3,15; (AMD=3,15)	15-12-2023
HR	1	20173	FV- 08-H	M	20	15,7; (MDA=2,94)	<3,30; (AMD=3,30)	08-05-2023
HR	2	20174	FV- 08-H	M	20	24,5; (MDA=3,18)	<3,17; (AMD=3,17)	09-05-2023
HR	3	20189	FV- 01-H	M	60	35,7; (MDA=3,01)	<2,63; (AMD=2,63)	09-05-2023
HR	4	20496	FV- 01-H	M	24	57,4; (MDA=2,64)	<3,00; (AMD=3,00)	09-05-2023
HR	5	20545	FV- 05-H	M	12	5,84; (MDA=2,34)	<2,96; (AMD=2,96)	30-05-2023
HR	6	20549	FV- 01-H	M	24	7,20; (MDA=2,13)	<2,96; (AMD=2,96)	30-05-2023
HR	7	20629	FV- 05-H	M	136	20,1; (MDA=2,72)	<3,13; (AMD=3,13)	30-05-2023
HR	8	21360	FV- 04-H	M	36	3,36; (MDA=2,29)	3,55; (AMD=3,55)	05-02-2024
HR	9	21433	FV- 07-H	M	36	7,00; (MDA=2,33)	<2,78; (AMD=2,78)	05-02-2024
HR	10	21460	FV- 03-H	M	72	90,4; (MDA=3,98)	<2,96; (AMD=2,96)	05-02-2024
HR	11	20002	FV- 06-H	M	36	**246; (MDA=3,56)**	<3,70; (AMD=3,70)	07-02-2024
HR	12	20004-1	FV- 03-H	M	8	4,55; (MDA=2,50)	<3,09; (AMD=3,09)	08-02-2024
HR	13	20019	FV- 06-H	M	36	40,4; (MDA=2,52)	<3,40; (AMD=3,40)	08-02-2024
HR	14	20046	FV- 04-H	M	84	6,45; (MDA=2,08)	<3,21; (AMD=3,21)	08-02-2024
HR	15	20067	FV- 02-H	F	60	**333; (MDA=4,28)**	<3,97; (AMD=3,97)	08-02-2024
HR	16	20068	FV- 02-H	M	24	**290; (MDA=4,26)**	<3,77; (AMD=3,77)	09-02-2024
HR	17	20084	FV- 06-H	F	24	47,3; (MDA=3,22)	<3,45; (AMD=3,45)	09-02-2024
SB	1	ROSBFC19004	FV - 1 - SB	M	8	2,5; (MDA=1,53)	<3,15; (AMD=3,15)	13-04-2023
SB	2	ROSBFC19011	FV - 1 - SB	F	11	3,58; (MDA=2,12)	<3,26; (AMD=3,26)	19-04-2023
SB	3	ROSBFV23VURPA	FV - 2 - SB	M	72	2,18; (MDA=2,16)	<3,05; (AMD=3,05)	24-04-2023
SB	4	ROSBFV2010036	FV - 3 - SB	F	54	5,67; (MDA=2,67)	<3,26; (AMD=3,26)	25-04-2023

To assess how statistically significant the difference is between the mean values for the activity of radionuclide ^{137}Cs in the 24 samples and the reference value of 600 Bq/kg, the value of "t" was calculated using the t-Test for independent samples, according to the formula:

$$t_{calc} = \frac{\bar{x} - \mu}{\sigma_{\bar{x}}}$$

where:

- $\bar{X}$=the mean value obtained from the experiments
- μ = the reference value we are comparing to (600 Bq/kg);
- σ_x = the standard error of the sample mean

σ_x was calculated according to the formula:

$$\sigma_{\bar{x}} = \frac{s}{\sqrt{n}}$$

where: S = standard deviation of the sample
n = sample size

S (standard deviation of the sample) and $\bar{X}$ (mean of the values obtained from the experiments) were calculated using Excel, resulting in the following values:

- $\bar{X}$ = 55.26X^-=55.26 Bq/kg (the mean of the 24 values obtained from the experiments)
- S = 94.33S=94.33 Bq/kg (the standard deviation of the 24 values obtained from the experiments)
- n =24N=24 (the number of samples where values for the activity of radionuclide ^{137}Cs were identified)

The value of the critical t, against which the calculated t was reported to evaluate if there is a statistically significant difference, was obtained from the specific values table [14] for the t-distribution based on the significance level α (α = 1 - P) and the degrees of freedom determined by the formula:

n-1 (df = n – 1 = 24 – 1 = 23).

α = 0.05 for a probability P of 95%.

$$\frac{\bar{X} - \mu}{S/\sqrt{n}} \quad \frac{55,26Bq/kg - 600Bq/kg}{94,33/\sqrt{24}} \quad \frac{55,26Bq/kg - 600Bq/kg}{94,33/4,90} \quad \frac{-544,74Bq/kg}{19,26} \quad t_{calculat} = -28,29 \; Bq/kg$$

The selected value from the table for the critical t was -2.06866, according to the correlation between degrees of freedom and α/2, following the formula:

t_{critic} = t (df;0.05/2) = -2.06866

The calculated t-value (-28.29) is greater than the critical t-value (-2.06866), which means there is a statistically significant difference between the mean values for the activity of radionuclide ^{137}Cs in the 24 samples and the reference value of 600 Bq/kg.

Analysing the data obtained from the tests, it was observed that, at the national level, the specific activity for ^{137}Cs is not a widespread phenomenon, but it cannot be neglected either, as it was identified in 4 out of the 11 counties included in the study, representing 36.3% of the total tested counties. This indicates an uneven distribution of radionuclides in the environment across different geographical areas within Romania (Fig. 2).

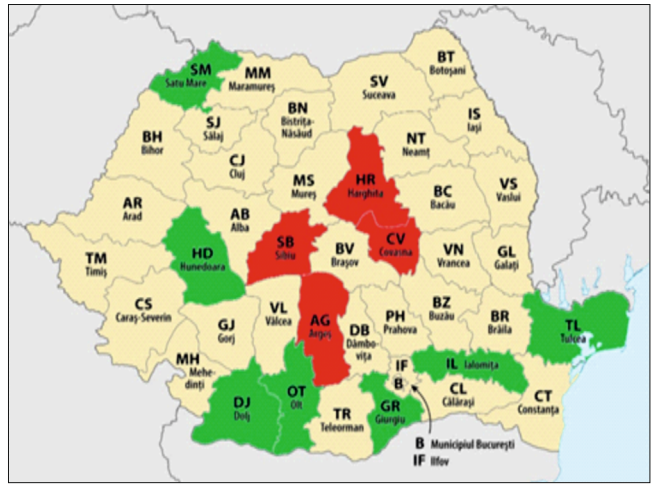

Fig. 2. Map of the counties included in the study and the geographical distribution of cases where samples with specific activity for radionuclide ^{137}Cs were identified

Evaluating the data regarding the age of the wild boars from which the meat samples with the presence of radionuclide ^{137}Cs were identified, a wide range of ages was observed, between 8 and 136 months. For a more detailed analysis, the wild boars were divided into 5 age categories: age group 1 for wild boars aged 0-12 months,age group 2 for wild boars aged 13-36 months, age group 3 for wild boars aged 37-72 months, age group 4 for wild boars aged 73-120 months, and age group 5 for wild boars older than 120 months.

The age was provided by the personnel from the hunting reserves, along with other data (sex, hunting reserve, identification number) necessary for the traceability of the analysed samples.

The assessment of the age of wild boars was based on morphological aspects: the size of the specimen, hair colour, constitutional type, skull shape, and especially on the basis of dental changes: the shape, growth, and degree of wear of incisors, canines, and the blunting of premolars and molars.

The results obtained regarding the age of the wild boars from which the meat samples with the presence of radionuclide ^{137}Cs were identified are presented in Table 2, which shows that even in the meat of young wild boars (8-12 months), specific activity for radionuclide ^{137}Cs was identified, but the predominant group is group 2 (13-36 months).

Correlating the data from Table 2 with the age data and the values obtained for radionuclide ^{137}Cs from Table 1, it can be observed that for 2 wild boars from group 2 (13-36 months), values of 246 Bq/kg and 290 Bq/kg were recorded, and for one wild boar from group 3, aged 60 months, values of 333 Bq/kg were found. All three wild boars were from Harghita County, and 2 of them were from the same hunting reserve (HR-02-H).

The values of 333 Bq/kg, 290 Bq/kg, and 246 Bq/kg - being at or near half the maximum permissible level (600 Bq/kg) - can be an alarming indicator considering the action of radionuclides when they enter the human or animal body and their effect on the health of

Table 2

Number of wild boars with the presence of radionuclide ^{137}Cs and age categories

Age category	Group 1 (0-12 months)	Group 2 (13-36 months)	Group 3 (37-72 months)	Group 4 (73-120 months)	Group 5 (>120 months)
The number of wild boars where the presence of the radionuclide ^{137}Cs was identified.	4 (11,33% of 24 total)	13 (54,17% of 24 total)	5 (20,08% of 24 total)	1 (4,17% of 24 total)	1 (4,17% of 24 total)

wildlife and consumers (humans).

When radionuclides enter the body, they emit radiation, which is a form of energy. The greater the quantity of radionuclides, the greater the energy released. This energy can be extremely harmful as it can cause changes at the cellular level. The radiation emitted by radionuclides has the ability to penetrate the cells of the body and can interact with internal structures, including the DNA in the nucleus, with these interactions altering the cell and potentially causing genetic damage (1,7).

For the health of wildlife (wild boars in the case of the study): ^{137}Cesium accumulates in muscles and soft tissues and is rapidly metabolised, allowing for quick elimination from the body. Within 50-150 days, the ingested dose is reduced by half. However, it should not be overlooked that low levels of radiation, including those to which we are normally exposed, can cause cellular changes by damaging DNA, potentially leading to genetic mutations or various forms of tumour conditions (7). For the safety of consumers of game meat (food safety), it should not be overlooked that if the quantities ingested exceed the rate of metabolism and elimination of ^{137}Cesium from the body (bioaccumulation), values exceeding the maximum permissible level for the specific activity of ^{137}Cs can be reached. This was identified in February 2018, when a wild boar from Suceava was found with values of over 600 Bq/kg.

CONCLUSIONS

High-resolution gamma spectrometry with an HP Ge (hyperpure germanium) detector is an analytical technique capable of detecting the activity of radionuclide ^{137}Cesium in wild boar meat based on the spectrum of gamma radiation emitted by it. The presence and measurement of the specific activity of radionuclide ^{137}Cs in 24 samples of wild boar meat, hunted in Romania, out of a total of 189 samples tested through high-resolution gamma spectrometry, revealed a prevalence of 12.69% for this radionuclide in this study. The identification of Cesium-137 in the meat of wild boars hunted in 4 out of the total of 11 counties included in the study indicates heterogeneous geographic distribution for this radionuclide. In all 4 counties, the predominance of mountainous terrain suggests that the soil in the mountainous area acts as a reservoir for radionuclides that have been released into the environment in the past, both through natural events and as a result of anthropogenic activities such as nuclear tests or accidents. In these regions, radionuclides have accumulated in the soil, and the wildlife, particularly the wild boar population, is exposed to these radionuclides through feeding and interaction with the surrounding environment. Although no samples with values of specific activity for radionuclide ^{137}Cs exceeding the maximum permissible level (600 Bq/kg) were found (8,10), the values of 246 Bq/kg, 290 Bq/kg, 330 Bq/kg - identified in this study - can be considered concerning because even low levels of radiation, to which living organisms are naturally exposed, can affect cells (7) by damaging DNA and inducing or developing tumor conditions in wildlife, including wild boars. The values of 290 Bq/kg recorded in the meat from a 24-month-old wild boar and the values of 246 Bq/kg recorded in the meat from a 36-month-old wild boar can be considered alarming and worrisome because, through the effect of bioaccumulation, wild boars with such values, in the following years, if they survive, may reach values exceeding 600 Bq/kg, posing a risk factor for food safety and human health (consumers). No presence of radionuclide ^{134}Cs was identified in the study, similar to other studies conducted previously in Italy, Germany, and Austria (2,3), and the authors of those studies did not identify this radionuclide either. Prospective studies to evaluate the presence of radionuclide ^{137}Cs in wild boar meat from the other counties of the country are necessary to have comprehensive data from all counties, which are very useful for developing programs and strategies aimed at wildlife health and human health, but can also be very useful in the event of nuclear accidents or incidents.

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