

THE INVESTIGATIONS OF QUALITY OF VEGETABLE OIL DELIVERED AS "HUMANITARIAN AID"

**M.B. Rajković, Ksenija Pićurić-Jovanović, Jovanka Laličić,
Divna Kovačević, Gordana Pantelić and Irena Petrović ***

Abstract: The result of examination of oil of unknown origin from the crisis territory as follows: the examined oil has qualities permissible for human nutrition. The examination of contained quantity of contaminants and heavy metals showed that ingredients were not higher than values prescribed by the Statute, except for lead. It is on the upper limit. Contents of radionuclides range within the limits of average values. The quantity is not dangerous for human food.

However, the present methods and the existing apparatus are not developed enough, so there is ignorance of the total structure of the sample. There is a possibility of substances being present in very low quantities, first of all, cancerogenic substances or substances which can provoke somatic or genetic changes.

Paracelsius said: "Everything is toxic, but it depends of dose". That "dose" is the main cause of consequences (therapeutic, toxic or lethal) which begin as a result of carrying contaminated substances into human organism.

Key words: oil quality, corn oil, cotton oil, heavy metals, Tocopherol, Tocotrienols.

*Dr Miloš B. Rajković, Associate Professor, Dr Ksenija Pićurić-Jovanović, Associate Professor, Jovanka Jovičić, Assistant, Institute of Food Technology and Biochemistry, Divna Kovačević, M.Sc., Senior Research Associate, Institute of Plant Protection and Food Products, Faculty of Agriculture, University of Belgrade, 11081 Belgrade-Zemun, Nemanjina 6, FR Yugoslavia

Gordana Pantelić, M.Sc., Section Head Department of Radiology and Irena Petrović, M.Sc., Researcher, Institute of Occupational and Radiological Health "Dr Dragomir Karajović", 11000 Belgrade, FR Yugoslavia

Introduction

Edible vegetable oils are triglycerides of fatty acids that may also contain small quantities of other substances which are natural oil contents: free and bound stearins, phosphatides and vitamins, giving flavour, aroma and colour to the oils (Regulations, 1999; Rekalic i Vitorovic, 1975; Đurđić, 1996).

Edible vegetable oils include foods obtained from fruits (seeds) of oleaceous plants or plant parts that contain oil, and are produced according to an established technological procedure:

- *cold pressed raw edible vegetable oil*, produced without heating, by pressing with previous cleaning (removing of the impurities). peeling and automatic chopping up. Cold pressed oil may be purified only by washing with water, precipitating, filtration and centrifugation.

- *virgin edible oil*, produced by pressing, with previous cleaning, peeling and automatic chopping up. To separate the oil, it is permitted to use heating for pressing. Virgin edible vegetable oil may be purified only by water washing, precipitation, filtration and centrifugation.

- *edible refined oil*, produced by cleaning, peeling and automatic chopping up, pressing, i.e. extraction with an organic solvent and refining (Regulations, 1999).

During the last decade of the XX century, there appeared, within the territory of former SFR Yugoslavia, during the war actions, various foods intended for human nutrition, but of suspicious quality or with time limit expired long ago (e.g. flour from 1939, etc.). As a great number of people remained without anything, due to migrations and loss of all their property, this aid was mostly welcomed and received with gratitude. After consuming of thus delivered food, there appeared later on certain medical problems, so the good quality of the foods and the good will of the donors became justifiably suspicious.

During military actions in the territory of Kosovo and Metohia in March–April 1999, a whole hanger full of food products intended for soldiers of the so-called Liberation Army of Kosovo was seized. Our attention was caught by a big can of edible oil without any specification or date of production, but only with imprinted inscription: *USA Refined Vegetable Oil. Not to be Sold or Exchanged Usaid "4 liters - 3.7 kg"*, with big letters USA, which positively proves the origin of the oil (Fig. 1).

Suspecting the quality of the oil as well as the way the storehouse had been comparatively easily seized, we posed the question: Was the quality of the oil good enough for human nutrition or it had been left on purpose – thrown away, because of bad quality?

For these reasons, a sample of the oil was examined in detail, in order to establish its physico-chemical properties and main quality parameters, and to determine characteristic parameters to reveal its origin and good quality. In addition, there were performed heavy metal content and radionuclide presence determinations in order to achieve reliable conclusions on the possibility of using this oil in human nutrition.



Fig. 1. – The investigated canned oil

Materials and Methods

For the examination of the oil sample, standard methods for determination of oil quality and its characterization were applied (Yugoslav Standard 43, 1962a i 1962b).

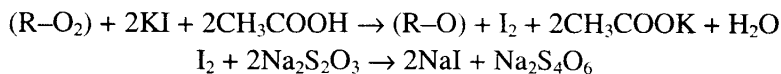
Moisture determination: water content in oil was determined by distillation with xylol, because the actual content of water is being determined, and this cannot be achieved by drying procedure at 105°C, because at this temperature other substances evaporate that have the boiling points close to that of water. Oil aliquot taken for the analysis depends on moisture percent being determined. Usually, enough is taken to get 2–5 cm³ of water.

Acidic or neutralization number is the measure of fat hydrolyzation degree. It is defined as the number of mg KOH necessary for neutralization of free fatty acids in 1 g of fats or for neutralization of 1 g of fatty acids. Fat acidity is often expressed also as wt. % or free fatty acids (FFA), and in calculation it is assumed that all fatty acids have mole mass of oleic acid. The relation between acid number and % FFA (approximated as oleic acid) may be expressed in the following way:

$$\text{One unit of acidic number} = 0.503 \% \text{ SMK}$$

Procedure: Oil is dissolved in a convenient solvent (96 wt. % ethyl alcohol in diethyl eter), and subsequently titrated with KOH. The quantity of utilized alkali is the measure of the oil or fat acidity.

Peroxide number (PB) represents oxygen mass (in μg) bound in the form of peroxide (active oxygen, in 1 g oil). The method for peroxide number determination is based on determination of iodine extracted by peroxide action on iodides in low pH surrounding.



Simultaneously, a "blank" is analysed in order to check the purity of reagents. Calculation:

$$\text{PB} = (\text{A}-\text{B}) \cdot 5/\text{O} = (\text{milimol O}_2/\text{kg})$$

A – $\text{Na}_2\text{S}_2\text{O}_3$ utilized for the main assay

B – $\text{Na}_2\text{S}_2\text{O}_3$ utilized for the "blank" assay

O – weighed sample aliquot (in g)

Relative oil density represents the ratio of mass of the given oil aliquot volume at $t^\circ\text{C}$ under atmospheric conditions, against the mass of the same volume of water at 20°C in the atmosphere. Relative oil density may be determined by: aerometer, hydrostatic balance and pycnometer.

Refraction index represents the ratio between the sine of the angle of the incoming ray and the sine of the angle of refracted light ray, with the wavelength of 589.3 nm (mean value D of sodium band) when it passes from air into the oil at a given temperature. For expressing the results of refraction index, the temperature of 40°C (n_D^{40}) has been adopted. Determination of refraction index was performed by Abbe's refractometer.

Saponification number signifies KOH mass (in mg) which is necessary for a complete saponification of free and bound fatty acids in 1 g oil. Oil saponification is performed with alcohol solution of KOH of known concentration, and the excess of free alkalies is retitrated by HCl solution of known concentration. Difference between HCl solution volume (in cm^3) utilized for the titration of the main and "blank" assays shows how many cm^3 or KOH solution has been utilized for oil saponification:

$$\text{SB} = (\text{A}-\text{B}) \cdot 28.1/\text{Ok}$$

where:

A – HCl utilized for "blank" assay;

B – HCl utilized for main assay;

Ok – weighed sample aliquot (in g)

$$1 \text{ cm}^3 0.5 \text{ mol/dm}^3 \text{ HCl is equivalent } 28.1 \text{ mg KOH}$$

Iodine number is expressed by the mass of iodine (in g) added to unsaturated fatty acids in 100 g oil, and it describes the degree of unsaturation. The

determination was performed by Hanus's method and it is based on the fact that unsaturated fatty acids, both free or bound in glycerides, add to their double bond a molecule of halogenes.

$$\text{Iodine number} = (A-B) \cdot 0.0127 \cdot 100$$

where:

A – $\text{Na}_2\text{S}_2\text{O}_3$ known concentration utilized for the "blank" assay (in cm^3);

B – $\text{Na}_2\text{S}_2\text{O}_3$ known concentration utilized for the "blank" assay (in cm^3);

Ok – weighed sample aliquot

1 cm^3 0.1 mol/dm^3 solution of iodine is equivalent 0.0127 g iodine

Heavy metal determination. For the determination of heavy metal content in food products, there is a range of methods of instrumental chemical analysis: colorimetry, atomic absorption spectrophotometry (AAS), method of inductively coupled plasma (ISP), polarography, direct potentiometry (ionometry), etc. Atomic absorption spectrophotometry is an analytical method based on light absorption by the atoms of the chemical element the content of which is being determined. Essentially, the method is spraying of a solution of the analysed element into flame and exposing it to the light with the wavelength best absorbed by the given element. In passing through the flame, the light intensity decreases, which is due to the presence of the atoms of the analysed element. This decrease of light intensity depends on concentration of the analysed element in the solution. AAS method is widely applied in practice, because of its high sensitivity (many elements have been determined in concentrations of 10^{-6} g and lower) and precision.

Analytical procedure: Ten grams (10 cm^3) of oil were weighed for determination of lead, copper and iron, 2 cm^3 of 25% solution of $\text{Mg}(\text{NO}_3)_2$ were added and the mixture was warmed on a hot plate and after that burned in an oven at 450°C for 12 h. Into the white powder, 10 cm^3 of 2% nitrous acid were added, it was filtered and transferred into 10 cm^3 measuring vessel. For arsenic determination, 1 g of the oil was weighed, 20 cm^3 of conc. HNO_3 were added into it and the mixture warmed on a hot plate almost to dryness, after that 10 cm^3 of H_2O_2 were added and the mixture warmed to dryness. 10 cm^3 of 1% HCl were added, the mixture filtered and 1% HCl added up to 25 cm^3 (Regulations, 1999; Official Gazette of SFRJ, br. 53, 1991; Regulations, 1992).

Measurement performed by an AA Spectra – 200 Varian, Varian Australia Pty, Ltd, springvale Rd, Malgrave, Victoria, Australia (Atomic Absorption News Letter, 1976).

Gamma spectrometric analyses were performed by homogenization of oil sample, its subsequent drying at 105°C and keeping airtight (30 days) in order to achieve radioactive equilibrium. Gamma spectrometric measurements were performed by three pure germanium detectors manufactured by EG&G "ORTEC",

with the efficiency of 25–30%, and energy resolution of 1.75–1.95 keV. The measurement time for one sample was 60,000 to 100,000 sec, and the basic radiation was measured after 250,000 sec.

Measurement of the total beta activity was performed by α - β anticoincident proportional gass counter (“COUNTMASTER”) with the basic radiation 1 imp/min. Planchet radius was 2.3 cm. Efficiency of the counter was 24 % (Antić et al., 2000; Pantelić et al., 1999; Pantelić and Petrović, 2000; Pantelić, 1999).

Results and Discussion

Although fats and oils are triglycerides, i.e. esters of saturated and unsaturated fatty acids and trihydroxile alcohol glycerol, they are divided into animal and plant triglycerides that by its consistency may be solid or liquid. Beside glycerine esters in oils and fats, there are free and bound pigments, phosphatides and vitamins that give them flavour, aroma and colour. In animal fats, there is cholesterine in unsaponified part, and in plant fats there is phytosterine.

Oils differ from one another by their physical and chemical properties, their differences originating from containing different fatty acids which give chemical character to the triglyceride, because they comprise the reactive part of the molecule.

In natural tryglicerides, about 60 fatty acids have been detected, which may be divided into the following groups:

1. *Saturated fatty acids*, with general formula $C_nH_{2n}O_2$, for example $C_{18}H_{36}O_2$ – *stearinic acid*

2. *Unsaturated fatty acids* with:

a) one double (covalent) bond, with general formula $C_nH_{2n-2}O_2$

for example $C_{18}H_{34}O_2$ – *oleic acid*

b) two double (covalent) bonds, with general formula $C_nH_{2n-4}O_2$

for example $C_{18}H_{32}O_2$ – *linoleic acid*

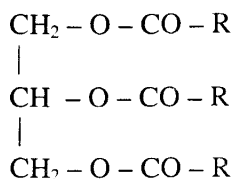
c) three double (covalent) bonds, with general formula $C_nH_{2n-6}O_2$

for example $C_{18}H_{30}O_2$ – *linolenic acid* and etc.

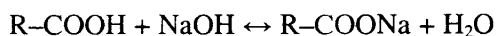
3. *Oxi fatty acids*

4. *Dicarbon acids, cyclic and keto-acids*

If structural formula of a triglyceride is observed, it may be seen that the whole molecule may be divided in two parts: glycerine moiety (C_3H_5) and three fatty acid moieties ($3RCOO$):



Fatty acids have general formula R-COOH. At room temperature, saturated fatty acids are solid, and unsaturated ones with lower mole masses, are liquid. They are insoluble in water (except for low molecular ones), but they are soluble in: alcohol, ether, benzene and other organic solvents. Each fatty acid molecule can bind one molecule of sodium and potassium hydroxide and thus to be transformed into salt (*soap*).



In addition, fatty acids are weak acids, and their solutions do not change the colour of methyl-orange and Congo-red. Their titrations are performed in the presence of phenolphthaleine, litmus and alkali blue.

Organoleptic investigation of the can content was performed at its opening and it was established that the oil had an aroma inherent to the raw material, without foreign aromas and that it was not rancid. The colour of the oil was light yellow, which speaks in favour of its being cottonseed oil, because an oil with the same qualities, as for instance maize oil, would be dark yellow, and sesame oil - green (also too expensive to be processed).

The results of the investigations of the oil of unknown origin by physical and chemical methods are represented in the Table 1.

T a b . 1. - Physico-chemical characteristics of investigated oils

Parameter of quality	Obtained Values	Maximal Allowed Concentrations
Content of water and other evaporable substances (in wt. %)	0.03	0.20
Content of soap (in wt. %)	Ni in traces	0.0050
Acid number (mg KOH/g)	0.09	0.60
Peroxide number (mmol/kg)	2.4	2.5
Relative oil density ($x^\circ\text{C}/\text{water } 20^\circ\text{C}$)	0.923	-
Refraction index (ND 40°C)	1.467	-
Saponification number (mg KOH/g oil)	198	-
Iodine number (Hanus)	100.95	-

Heavy metals include the metals with densities higher than 5 g/cm^3 , and they are characterized by various chemical properties and biological influence. Some heavy metals (Fe, Cu, Zn) have an important physiological role in human organism, while metals like lead, mercury, nickel and cadmium are very toxic

substances. The third group includes metals: silver, gold, molybdenum, chromium and cobalt which are less toxic compared to the second group (Official gazette of FR Yugoslavia, 1992; Jakovljević and Blagojević, 1998).

The results of heavy metal content determination in the investigated oil are represented in the Table 2.

T a b . 2. – Heavy metal content in the investigated oil (mg/kg) determined by method of AAS

Heavy metal	Pb	Cu	Fe	As
Content	0.1	0.05	0.1	< 0.05
Refined oil*	0.1	0.1	1.5	0.1
Unrefined oil*	0.4	0.4	5.0	0.4
Hydrogenic oil*	0.1	0.5	1.5	0.1

*Allowed values for heavy metal content in various oil types according to Regulations

The results of gammaspectrometric analyses of radionuclides in the investigated oil (in Bq/dm³) are represented in the Table 3.

T a b . 3. – Presence of radionuclides in the investigated oil

Radionuclide	⁴⁰ K	¹³⁴ Cs	¹³⁷ Cs	²³² Th	²²⁶ Ra	²³⁸ U	²³⁵ U
Radioactivity (Bq/dm ³)	< 3.2	< 0.1	< 0.2	< 0.8	< 3.2	< 11.7	< 0.3

On the basis of the obtained results represented in Table 1, and in accordance with regulations (Official gazette of FR Yugoslavia, 1999), it may be concluded that the oil originates either from maize seedlings or from cottonseed, which comparative characteristics presented in Table 4. (Swern, 1972).

T a b . 4. – Comparative characteristics of examined oil, the oil originates either from maize seedlings or from cottonseed

	Investigated oil	Oil originates from maize seedlings	Oil originates from cottonseed
Relative oil density (x°C/water 20°C)	0.923	0.917–0.925	0.916–0.918
Refraction index (ND 40°C)	1.467	1.465–1.468	1.468–1.472
Saponification number (mg KOH/g oil)	198	187–195	189–198
Iodine number (Hanuš)	100.95	107–135	99–113

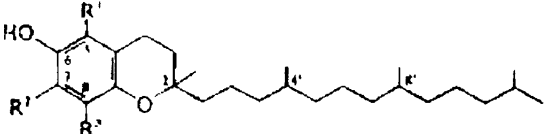
Investigations of heavy metal content in the oil showed that, except lead content that almost reaches the limit of the allowed values, contents of remaining heavy metals are far below those allowed by regulations.

The results of measurements of natural radionuclide activity in the investigated oil showed that they are within the limits of natural radioactivity levels. The findings of artificial radiation in the oil sample obtained by gammaspectrometric measurements are the result of Chernobyl accident (¹³⁷Cs)

(Pantelić et al., 1996) and they vary in the analysed samples between 0.12 Bq/kg (minimal activity) and 0.50 Bq/kg (maximal activity) - i.e. 0.43 Bq/kg (mean activity). Activity of long-living radionuclides of artificial origin is low, with the result that effective doses of the radiation of these nuclides introduced by ingestion is significantly below the recommended annual limit for the received dose per person, which amounts to 1 mSv/year (ICRP, 1990).

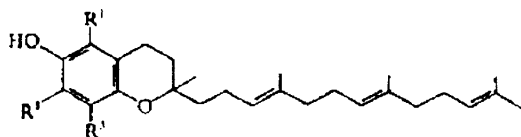
Either different substances having vitamin E activity occur in nature. They belong to two families with the generic names *tocols* and *tocotrienols*. The members of each family are designated with α , β , γ or δ , depending on the number and position of the methyl groups attached to a chromane ring. The side chain is saturated in the tocols and unsaturated in the tocotrienols. Commonly, the tocols are also called *tocopherols*. Structures are shown in Table 7. (Shuler, 1990).

T a b . 7. – Structure and properties of vitamin E and related compounds

Compound	Formula Molecular weight	Structura
Tocols		
		
Tocol	$C_{26}H_{44}O_2$ 388.64	$R^1: H$ $R^2: H$ $R^3: H$
8-Methyltolcol (δ -Tocopherol)	$C_{27}H_{46}O_2$ 402.67	$R^1: H$ $R^2: H$ $R^3: CH_3$
5.8-Dimethyltolcol (β -Tocopherol)	$C_{28}H_{48}O_2$ 416.69	$R^1: CH_3$ $R^2: H$ $R^3: CH_3$
7.8 Dimethyltolcol (γ -Tocopherol)	$C_{28}H_{48}O_2$ 416.69	$R^1: H$ $R^2: CH_3$ $R^3: CH_3$
5.7.8-Trimethyltolcol (α -Tocopherol)	$C_{29}H_{50}O_2$ 430.72	$R^1: CH_3$ $R^2: CH_3$ $R^3: CH_3$

The structures of the tocopherols indicate three centres of asymmetry at C-2, C-4' and C-8' respectively. The tocotrienols possess one centre of asymmetry at C-2 in addition to the sites of geometrical isomerism at C-3' and at C-7'. Thus, of each tocopherol and tocotrienol four stereoisomers exist.

Tocotrienoli



8-Methyltocotrienol (δ -Tocotrienol)	$C_{27}H_{40}O_2$ 396.62	$R^1: H$ $R^2: H$ $R^3: CH_3$
5,8-Dimethyltocotrienol (β -Tocotrienol)	$C_{28}H_{42}O_2$ 410.65	$R^1: CH_3$ $R^2: H$ $R^3: CH_3$
7,8-Dimethyltocotrienol (γ -Tocotrienol)	$C_{28}H_{42}O_2$ 410.65	$R^1: H$ $R^2: CH_3$ $R^3: CH_3$
5,7,8-Trimethyltocotrienol (α -Tocotrienol)	$C_{29}H_{44}O_2$ 424.67	$R^1: CH_3$ $R^2: CH_3$ $R^3: CH_3$

Tocopherols have antioxidant activity and are important in stopping the destructive activity of free oxygen radicals and peroxides that occur in metabolism. Especially the structures of cell membranes, or more precisely, polyunsaturated acids which are integrative part of membrane lipids, are susceptible to these oxidative activities.

Tocopherol and tocotrienol contents in the oil from maize seedlings and in cottonseed oil are presented in Table 5, and the content of desmethyl sterine in total sterol (in wt. %) in Table 6.

Table 5. – Contents of tocoferols and tocotrienols (Руководств, 1969)

	Oil originates from maize seedlings	Oil originates from sesam
α -tocoferol	23–573	40–500
β -tocoferol	ND–356	ND
γ -tocoferol	268–2468	270–410
δ -tocoferol	23–75	ND
α -tocotrienol	ND	ND
γ -tocotrienol	ND	ND
δ -tocotrienol	ND	ND
Total	331–3716	81–160

Note: Oil originates from maize seedlings contents and ND–52 mg/kg β -tocotrienol.

T a b . 6. – Portion of desmethylsterin in total sterol, in wt. %

	Oil originates from maize seedlings	Oil originates from cottonseed
Holesterol	0.2–0.6	–
Brasikasterol	ND–0.2	–
Kampesterol	18.6–24.1	8–9
Stigmasterol	4.3–7.7	–
β -sitosterol	54.8–66.6	91–92
δ -5-avenasterol	4.2–8.2	–
δ -7-avenasterol	1.0–4.2	–
δ -7-avenasterol	0.7–2.7	–
Other	ND–2.4	–
Total sterols (mg/kg of oil)	7950–22150	2600–5700

All the obtained results indicate that the oil is probably cottonseed oil of standard, good quality, without traces of contaminators. It must be stressed that the applied methods are routine ones, included in standard analyses. There remains the problem of the method sensitivity, i.e. whether potentially present contaminators or cancerogenic substances are at the detection level or not. Technological lagging surely takes its toll, making the application of instrumental and modern equipment for sample investigation a necessity, in order to detect and eliminate completely even the traces of contaminators.

Conclusion

Investigation of the oil of unknown origin from a critical area showed that it was oil of good quality, suitable for human nutrition. Analyses of contaminator and heavy metal contents showed that the traces were not above those allowed by regulations, except for lead that reached the upper limit value.

Radionuclide content was well within the limits of average values and did not represent danger for people consuming this oil.

However, it is necessary to be cautious, because current methods and existing equipment are not sufficiently improved, and substances present in very low concentrations remain undetected – first of all, these include cancerogenic agents or substances causing somatic or genetic disorders.

Paracelsius said: "Everything is toxic, but it is depend of dose". That "dose" is the main cause of consequences (therapeutic, toxic or lethal) which begin as a result of carrying contaminated substances into human organism.

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ISPITIVANJE KVALITETA ULJA DOPREMLJENOG KAO "HUMANITARNA POMOĆ"

**M.B. Rajković, Ksenija Pićurić-Jovanović, Jovanka Laličić,
Divna Kovačević, Gordana Pantelić i Irena Petrović ***

Re z i m e

Ispitivanje ulja nepoznatog porekla sa kriznih područja pokazalo je da se radi o ulju kvaliteta dozvoljenog za ljudsku ishranu. Ispitivanja sadržaja kontaminatora i teških metala pokazalo je da primese nisu više od dopuštenih i zakonom determinisanih vrednosti, osim olova koje se nalazi na samoj gornjoj granici.

Sadržaj radionuklida nalazi se u granicama prosečnih vrednosti i ne predstavlja opasnost za ljude pri konzumiranju ulja.

Ono što upućuje na oprez je da sadašnje metode i postojeća aparatura nisu dovoljno razvijene pa ostaje nepoznanica o supstancama koje se nalaze u veoma niskim koncentracijama – u prvom redu kancerogenih agenasa ili supstanci koje izazivaju somatske ili genetske promene.

Ali, kao što je rekao još Paracelzijus "sve je otrovno samo zavisi od doze", ta "doza" je glavni uzrok posledica (terapeutskim, toksičnim ili letalnim) koje nastaju kao rezultat konzumiranja kontaminiranih supstanci.

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* Dr Miloš B.Rajković, vanredni profesor, dr Ksenija Pićurić-Jovanović, vanredni profesor, Jovanka Laličić, asistent-pripravnik, Institut za prehrambenu tehnologiju i biohemiju, mr Divna Kovačević, viši stručni saradnik, Institut za zaštitu bilja i prehrambenih proizvoda, Poljoprivredni fakultet, 11081 Beograd-Zemun, Nemanjina 6, SR Jugoslavija

Mr Gordana Pantelić, načelnik Odeljenja za radiologiju i mr Irena Petrović, istraživač-saradnik, KCS Institut za medicinu rada i radiološku zaštitu "Dr Dragomir Karajović", 11000 Beograd, SR Jugoslavija