# Interaction of radionuclide $^{131}$I and cadmium chloride in an alternative bioassay with *Artemia franciscana* evaluated by a digital record

Ladislav Kovářík[1], Petr Dvořák[1,2], Katarína Beňová[4], Jana Doležalová[2,3], Martin Tomko[4], Michaela Špalková[4]

[1]University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Veterinary Hygiene and Ecology, Department of Biology and Wildlife Diseases, Brno, Czech Republic
[2]University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Veterinary Hygiene and Ecology, Centre for Ionizing Radiation Application, Brno, Czech Republic
[3]University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Veterinary Hygiene and Ecology, Department of Animal Origin Food and Gastronomic Sciences, Brno, Czech Republic
[4]University of Veterinary Medicine and Pharmacy in Košice, Institute of Biology, Zoology and Radiobiology, Košice, Slovak Republic

*Received April 3, 2020*
*Accepted December 21, 2020*

**Abstract**

The interaction of radionuclide $^{131}$I and cadmium chloride was investigated by an alternative bioassay using the crustaceans *Artemia franciscana*. Fifty individuals were placed in each Petri dish. Due to radiation protection, evaluation of the experiment was performed using digital recordings taken by a camera. In the group containing a cadmium solution with an added radionuclide with a volumetric activity of 32 MBq·l$^{-1}$, the lethality was significantly lower than in the group containing only a cadmium solution of 0.250 mmol·l$^{-1}$. In the cadmium solution group and higher volumetric activity of radionuclide $^{131}$I (370 MBq·l$^{-1}$), the lethality was significantly higher than in the control group, which demonstrated a synergistic effect. It was found that lethality was lower in the group containing only radionuclide $^{131}$I with a volumetric activity of 138 MBq·l$^{-1}$ than in the control group. This result supports the theory of radiation hormesis.

*Radioactive iodine, internal contamination, external irradiation, digital recording, FIJI*

The environment has been burdened by the impact of the adverse effects of an ever-increasing global production. Undesirable products with varying degrees of toxicity enter the soil, surface water and air in each sector of production. This also applies to ionizing radiation, which is part of the natural background, but has also been increasingly used in healthcare, industry and other areas of human activities.

The impact of these factors on living organisms needs to be investigated in order to prevent damage resulting from their possible undesirable combined effects.

The level of cosmic radiation and the terrestrial part of the natural background in the early period of life on this planet were about three times higher than they are today (Karam and Leslie 1999). Recently, new sources of ionizing radiation have appeared as a result of human activities, particularly the use of ionizing radiation in medical diagnostics and therapy. It also includes the release of radioactive substances in nuclear power plant accidents, nuclear weapon testing, and the use of atomic bombs in Japan at the end of World War II. In the first phase of nuclear accidents following the release of radionuclides into the atmosphere, the main food contaminants are iodine radioisotopes (Pöschl and Nollet 2006).

The effects of ionizing radiation on living organisms have been studied for a long time. They can be divided according to the type of emitted particles, the dose size (absorbed energy) and the exposure time. Constant low doses of natural background or episodically occurring higher doses during medical examinations are common.

**Address for correspondence:**
Jana Doležalová
Palackého tř. 1946/1
612 42 Brno, Czech Republic

Phone: +420 541 562 623
E-mail: dolezalovaj@vfu.cz
http://actavet.vfu.cz/

The effect of higher doses of ionizing radiation (i.e. doses higher than 500 mSv) has been thoroughly studied in vertebrates and described by several authors (e.g. Sesztáková et al. 1996; Falis et al. 2004; Beňová et al. 2006; Beňová et al. 2007), though it is less studied in invertebrates, e.g. *Artemia salina* (Dvořák and Beňová 2002).

The effect of intermediate doses and doses comparable to the natural background, i.e. up to about 250 mSv (in vertebrates), is relatively difficult to prove statistically. This area of the impact of ionizing radiation on organisms is at the stage of intense investigation (Špalková et al. 2015). The course of dose-risk dependence has been described by several hypotheses. The most common are the linear threshold-free model (LNT), the threshold model, the linear quadratic model and the hormesis model (Luckey 1991; Luckey 1994; Sanders 2010). The combination of internal contamination and external irradiation with $^{131}I$ was used in the experiment that best describes a model of the real situation after nuclear accidents.

Cadmium and other heavy metals are among the most important environmental pollutants. They are usually present at very low concentrations but those concentrations are gradually increasing locally due to industrial and agricultural production. Cadmium is released as a by-product in ore mining and processing in industry, and in power engineering when fossil fuels are burned. It enters the soil as part of fertilizers such as phosphates. When dissolved in water, along with other substances, it may get through the root system into plants that feed livestock and other animals and thereby enter the food chain and represent an important source of contamination for humans as well (Jinadasa et al. 1997).

Cadmium and its compounds have been classified as human carcinogens. It may cause various oncological diseases, such as lung, kidney and prostate cancer. The harmfulness of cadmium results from its very slow excretion by the organism. The reported half-life is between 10 and 30 years (Ercal et al. 2001). For this reason, its use is regulated and controlled by the applicable laws of the Czech Republic and the EU, specifically by the EP and Council Directive (2002/95/EC 2003). The effect of cadmium and other heavy metals on animals has long been studied (Lyn 2003; Falis et al. 2014).

In line with the 3R concept (Russell and Burch 1959), i.e. Replacement, Reduction, Refinement, an alternative second-generation bioassay was chosen for this experiment as a substitute for higher organisms. This is also subject to the EP and Council Directive on the protection of animals used for scientific purposes (2010/63/EC 2010). Freshly hatched nauplionic stages of *Artemia franciscana* were used as a model organism (Dvořák et al. 2005).

The aim of this paper was to investigate the effects of cadmium chloride at a concentration lower than $LC_{50}$ in 216 h (Dvořák et al. 2005) on the nauplionic stages of *A. franciscana* in interaction with various volumetric activities of radionuclide $^{131}I$. In this study, the counting of *A. franciscana* individuals was performed by digital record evaluation in order to avoid irradiation when reading the results.

## Materials and Methods

In the prolonged *A. franciscana* toxicity bioassay, a 3% pure glucose solution was used as an energy source. Sea water (4.7% salinity) was prepared from chemicals of p.a. purity (Dvořák et al. 2005).

This experiment was prolonged to 456 h over and above the standard time of 264 h (Dvořák et al. 2005).

Clear square polystyrene Petri dishes (120 × 120 mm) were used in this experiment. Each dish contained 45 ml of sea water with 3% glucose. Fifty randomly selected freshly hatched *A. franciscana* nauplium stages were placed in each dish. The dishes were shielded against each other by a 110-mm layer of copper plates of a layer thickness higher than the level of solution in the dish. This prevented the irradiation of nearby dishes (including the control).

The $CdCl_2$ 2.5 $H_2O$ (Ing. Petr Švec - Penta s.r.o., Prague, Czech Republic) stock solution was prepared at a 10-fold concentration to be added to the dishes at one tenth of the total dish volume (5 ml). The concentration of this solution used in the Petri dishes was 0.25 $mmol \cdot l^{-1}$ $Cd^{2+}$.

The initial volumetric activity of the radioisotope was 37 MBq·ml$^{-1}$ on the reference date. The ampoule of the clear colourless solution contained 2 mg NaI in 1 ml; the pH was declared in a range of 8 to 10. The manufacturer was the Institute of Isotopes Co. Ltd., Budapest, Hungary.

NaI solution was diluted to achieve approximately the required volumetric activities in the individual dishes: 30, 125, and 375 MBq·l$^{-1}$.

The exact volumetric activity of $^{131}$I in the dishes was determined by gamma spectrometry after the end of the experiment, converting the volumetric activity to the start time of the experiment. Determination of $^{131}$I activity was performed by gamma spectrometry systems (Canberra, USA) using HPGe GC4018 germanium detectors (40% efficiency) with a resolution of 1.8 keV, verified by the Czech Metrology Institute. These activities are listed in Table 1.

Table 1. Experimental design.

| Dish | Group | $Cd^{2+}$ concentration (mmol·l$^{-1}$) | $^{131}$I initial activity (MBq·l$^{-1}$) |
|---|---|---|---|
| 1 | C | - | - |
| 2 | I138 | - | 138 |
| 3 | Cd | 0.250 | - |
| 4 | I32Cd | 0.250 | 32 |
| 5 | I132Cd | 0.250 | 132 |
| 6 | I370Cd | 0.250 | 370 |

The difference in activity between plates #2 and #5 is consistent with the magnitude of standard uncertainty in the gamma spectrometry assay. In terms of the biological activity of $^{131}$I in the experiment, the volume activities (132 and 138 MBq·l$^{-1}$) were considered identical.

The room temperature was measured at the time of reading and ranged from 19.2 to 20.7 °C, with a mean of 20.2 °C.

The bioassay evaluation criterion was lethality, assessed as the absolute loss of movement. Plate counts were performed every 24 h after the beginning of the experiment (0 h). To improve the quality of the photographic image, the test was carried out in bowls without lids. Evaporated water was regularly replenished to its initial volume.

The experiment was recorded by taking digital photos with a Nikon D7200 camera with a Nikon 18-140 mm lens (Nikon Corporation, Minato City, Tokyo, Japan). Photographs taken at the maximum possible resolution of 6000 × 4000 pixels were used. A series of 12 photographs of Petri dishes with individuals was taken when reading. A delay of 2 s was set between each burst. Free software developed specially for processing visual data – ImageJ, specifically the distribution named FIJI, was used for evaluation.

The evaluation was based on a comparison of two consecutive images in each series. Changes in the position of moving (active) *A. franciscana* individuals in the Petri dish were observed. The Analyze Particles function implemented in the FIJI software used was applied to determine the number of changes. Before evaluating the photographs, the parameters of the functions used were set and tested appropriately in the scripts according to the given resolution and their exposure level.

The procedure for counting individuals in the photograph was as follows. The number of individuals with changed positions was determined in the file "Artemia-n.jpg". The following file "Artemia-(n + 1).jpg" was for reference only. When both files were opened, colour photos were converted to monochrome with 256 levels and inverted. Subsequently, the function "Calculator Plus" was subtracted, after which only the changes were visible. With the "setThreshold" function, a decision level was set to separate the data from an undesired digital noise. Then the number of particles was determined by the "Analyze Particles..." function, and the result was saved. The evaluation was followed by a check and possible correction of the parameters used for the calculation.

The results in plates 4, 5, and 6 represented the dependence of lethality on $^{131}$I. Differences were tested by one-way analysis of variance.

## Results

The results of the experiment were divided into two graphs due to the validation of lethality of the control group being exceeded (20%). Data were interpolated with grade 4–6 polynomials. Correlation indices for each group were as follows: IC = 0.996, II138 = 0.983, ICd = 0.996, II32Cd = 0.995, II132Cd = 0.989, II370Cd = 0.997.

Figure 1 shows the observed dependence of lethality on exposure time and the activity of added radionuclide $^{131}$I from the start of the experiment to 264 h when the lethality in the control group did not exceed 20%.

Lethality (individuals) was monitored up to 456 h due to continuous irradiation and activity decrease, although the lethality of the control group had already risen above the recommended value of 20% due to poor nutrition. Figure 2 shows the lethality dependence at this extended experiment time from 264 h up to 456 h.

The lethality of control group C increased only slightly to 12% in 240 h (Fig. 1). From 240 h it increased approximately linearly to 72% at 456 h (Fig. 2).

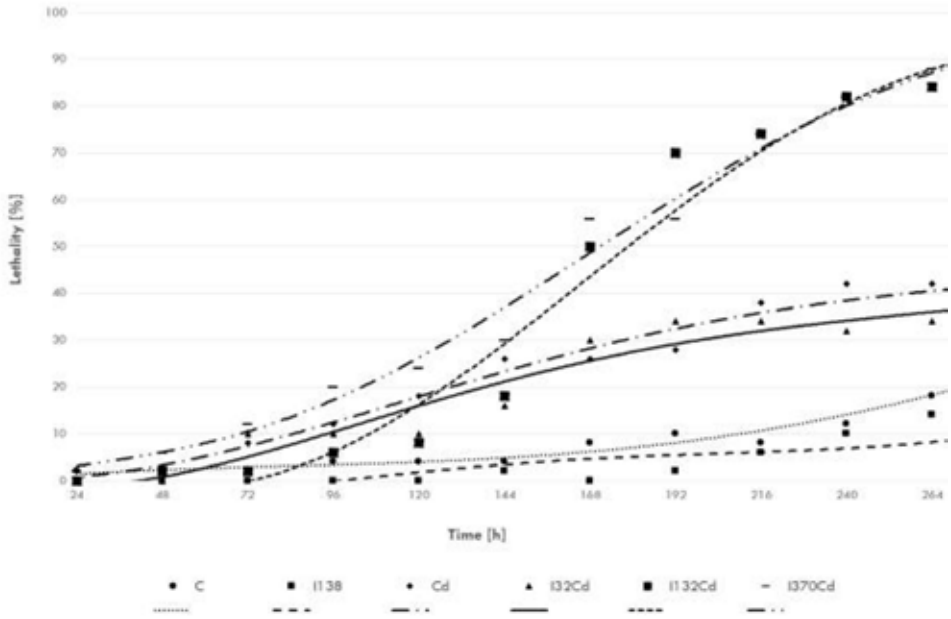


Fig. 1. Lethality (%) as a function of time and volumetric activity of 131I 0.250 mmol·l-1 Cd2+ up to 264 h.

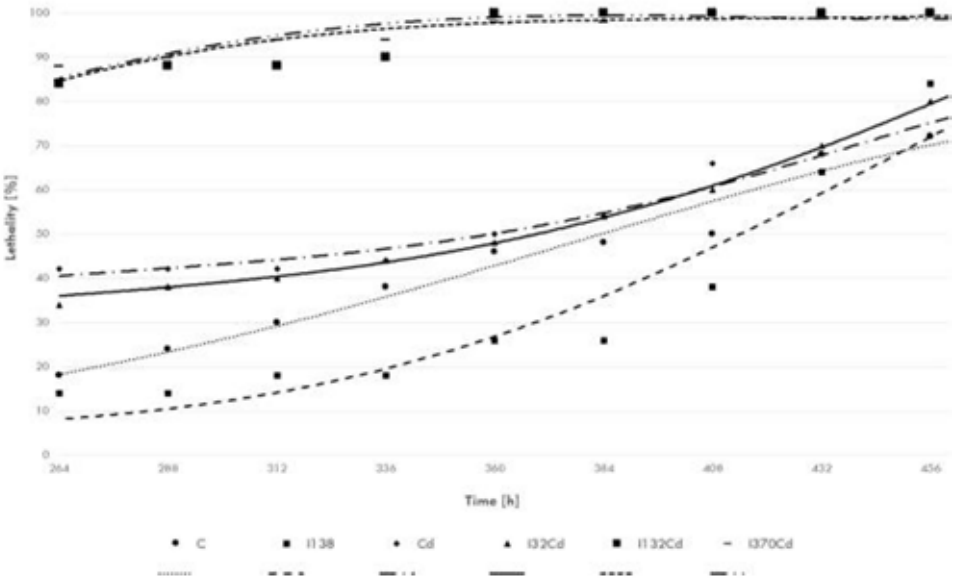


Fig. 2. Lethality (%) as a function of time and volumetric activity of 131I 0.250 mmol·l-1 Cd2+ from 264 h to 456 h.

The shape of the lethality/exposure curves varied significantly between groups. The difference was mainly caused by the higher volumetric activity of $^{131}$I in combination with cadmium.

The lethality of the I138 group (self-acting radioiodide) was increased only very slightly up to 168 h. There was an increase to 14% between 192 h and 264 h (Fig. 1). The curve differs from that of the control group between 264 h and 384 h (Fig. 2). The addition of radionuclide $^{131}$I of a volumetric activity of 138 MBq·l$^{-1}$ to the test environment therefore led to a decrease in lethality compared to the control group throughout the experiment, with the exception of 456 h.

As can be seen in both graphs, the lethality of the I32Cd group was very similar to that of the Cd group, although it contained additional $^{131}$I with a low volumetric activity of 32 MBq·l$^{-1}$. Between 144 h and 264 h, the lethality in both groups was significantly higher compared to control C. Comparing the two groups (Figs 1 and 2), the lethality of I32Cd up to 408 h was non-significantly decreased compared to the Cd group.

In the I132Cd group, the lethality at the start of the experiment was insignificantly reduced compared to the lethality in the control group. Up to 144 h, it was insignificantly reduced compared to the lethality of the Cd group. Up to 120 h, it was also insignificantly reduced compared to the lethality of the I32Cd group. At 168 h there was a significant increase in lethality relative to all groups. After 360 h, the lethality reached 100%.

In the I370Cd group the lethality was significantly increased from 96 h and its growth was faster than in the I132Cd group. Lethality reached 100% at 408 h. Although the volumetric activity of $^{131}$I in this group was almost three times that of the I132Cd group, the course of lethality of both groups from 216 h was practically identical. The difference between activities was, therefore, only seen in the early stages of the experiment.

However, when comparing the curves within the three groups of lethality (4, 5, and 6), ANOVA demonstrated significant ($P = 2.95 \cdot 10^{-9}$) lethality depence upon activity concentration $^{131}$I.

## Discussion

In this study, the counting of *A. franciscana* individuals was performed using a digital recorder which allows evaluation of experiments with a higher overall activity of all dishes. The method of visual reading was not appropriate due to the risk of significant exposure of the observer to ionizing radiation, e.g. when handling the dishes. Photos were taken instead of video because the resolution of the pictures taken is more than 10 × higher. For instance, images taken by this device had a resolution of 24 megapixels, while the resolution of an HD video record may be approximately 2 megapixels.

Use of this high-resolution imaging also makes it possible to perform this bioassay employing 50 nauplia in one Petri dish. This provided a homogeneous environment for all individuals, in contrast to alternative bioassays employing this number as the total number of individuals.

It is also necessary to consider whether the subjects were mechanically initiated during the recording when evaluating photographs. Non-moving subjects were considered dead. The lethality at a given time might, therefore, be slightly higher than the lethality at another time. This effect can be reduced by repeating the records at short intervals and averaging the values obtained.

The specificity of the experiment using the $^{131}$I radionuclide was that the observed lethality of *A. franciscana* was also dependent on its continuously decreasing activity. The radionuclide used has a relatively short half-life of only 8.025 days (192.6 h). On the other hand, this isotope is one of the most abundant in environmental contamination immediately after nuclear accidents and nuclear weapon explosions. The subjects were exposed

to ionizing radiation continuously from the beginning till the end of this experiment. In a different experiment, irradiation (e.g. $^{60}Co$) occurred at a single dose (Beňová et al. 2007) or continuously at the same dose rate (Ahlers et al. 1992). However, the total effect of ionizing radiation was a combination of internal contamination and external irradiation with radionuclide $^{131}I$.

Therefore, when assessing the lethality values obtained during the experiment, it ought to be considered that the decrease in the activity of $^{131}I$ (a half-life of 192.6 h) is half, and after 385.2 h equals one quarter of the baseline at the start of the experiment.

The experiment was intentionally prolonged due to this decrease in volumetric activity and its effect on lethality, for which reason the experiment continued even though the lethality of the control group was higher than the recommended value from 288 h. It would be optimal for validation for the lethality of the control group not to exceed 20% of the prolonged 240 h test with *A. franciscana* (Dvořák et al. 2005). However, the duration of exposure to the test substance (especially ionizing radiation) is chosen to exhibit a maximum difference in values between the experimental and control groups, thereby allowing a less common test extension to 456 h. It was found, thanks to this extension, that the lethality of group I138, containing only the radionuclide, was reduced significantly from 336 h to 384 h compared to the control group. Low doses of ionizing radiation may, therefore, result in lower lethality of the subjects, in this case the I138 group, compared to the control group.

The results of this work support the theory of radiation hormesis (Hrnčíř 1999; Sanders 2010). As suggested in many other biological and medical studies (Clewell et al. 2019), it appears that the natural mechanisms controlling living organisms are much more complex and defy the generally accepted concept of a simple linear cause-effect. Our results also support the notion that low doses of ionizing radiation might not be unambiguously negative (Luckey 2008).

Higher doses of ionizing radiation in combination with the toxic effects of cadmium, the I370Cd and I132Cd groups, show a synergistic negative effect. Cadmium toxicity has been described by several authors, such as Ochi and Ohsawa (1985). Bencko et al. (1995) reported the relationship between a higher Cd content in soil and the mortality of the population in the monitored area. Statistics have shown that there is a small (but demonstrable) risk of an increased incidence of ovarian cancer. High concentrations of cadmium in the liver and kidneys have been linked to the ability of these organs to produce more melatonin. Lethal doses of cadmium may inhibit mitochondrial phosphorylation in the liver (Kottferová and Koréneková 1998). The mechanism of action is believed to be based on the blocking of the (SH-) groups contained in proteins. This effect is similar to ionizing radiation. Cadmium binds to metallothionein in tissues. It is bonded via sulphhydryl groups. It is reported that they can reach about 10% of the molecular weight of metallothionein (Frazier and Rayner 1982; Kottferová et al. 2002). Its toxicity becomes evident when metallothionein synthesis is low. The cadmium chloride concentration we used was low for *A. franciscana.*

Several studies have also demonstrated the radioprotective effect of cadmium, both at the cellular level and throughout the whole organism (Privezencev et al. 1996). In our experiment, however, the radioprotective effect of cadmium was not demonstrated. The low volume activity of $^{131}I$ (32 $MBq \cdot l^{-1}$) had no significant effect on the lethal cadmium chloride curve, although it was found to be somewhat lower almost throughout the experiment.

From the point of view of our experiment, the volume activity of $^{131}I$ 32 $MBq \cdot l^{-1}$ appeared to be non-significant; independent activity of 138 $MBq \cdot l^{-1}$ represented the radiation hormone; and activities of 132 and 370 $MBq \cdot l^{-1}$ already represented interactions that significantly increased cadmium chloride toxicity.

In comparison with the limiting activities for drinking water, which are 5 to 6 orders of magnitude lower for mammals than those used in this experiment, it should be noted that

radiosensitivity of *A. franciscana* is also about 2 orders of magnitude lower ($LC_{50}$ for 96 h 600 Gy) than in mammals ($LD_{50}$ for 30 day 4–6 Gy) (Dvořák and Beňová 2002).

The group containing only the radionuclide showed lower lethality than the control group, which is in conflict with the linear threshold-free model, thereby supporting the theory of the hormesis effect. The observed cadmium concentrations and higher $^{131}I$ radioisotope activity showed a synergistic effect. Digital recording allows the evaluation of experimental results when working with high activities of open sources of ionizing radiation without a risk of exposing the experimenter to ionizing radiation.

The results of this work model a real situation after a nuclear accident, when the interaction of radioiodine and cadmium salts contaminating the environment occurs.

**References**


2002/95/EC, Directive 2003: Restriction of the use of certain hazardous substances in electrical and electronic equipment. In: Official Journal of the European Union, pp. 19-23

2010/63/EC, Directive 2010: Enhancing the protection of animals used for scientific purposes. Environmental Law and Management, pp. 33-79

Ahlers I, Ahlersová E, Toropila M, Ďatelinka I 1992: Thymus lipids in continuously irradiated rats. Physiol Res **41**: 417-421

Bencko V, Cikrt M, Lener J 1995: Toxic metals in human living and working environment (in Czech). Praha: Grada, 282 p.

Beňová K, Cigánková V, Falis M, Šmajda B 2006: Clinical symptoms and histological changes in *Poecilia reticulata* following gamma-rays irradiation. Acta Vet Brno **75**: 557-560

Beňová K, Dvořák P, Falis M, Sklenář Z 2007: Interaction of low doses of ionizing radiation, potassium dichromate and cadmium chloride in *Artemia franciscana* biotest. Acta Vet Brno **76**: 35-40

Clewell RA, Thompson CM, Clewell HJ 2019: Dose-dependence of chemical carcinogenicity: Biological mechanisms for thresholds and implications for risk assessment. Chem Biol Interact **301**: 112-127

Dvořák P, Šucman E, Beňová K 2005: The development of a ten day biotest using *Artemia salina* nauplii. Biologia **60**: 593-597

Dvořák P, Beňová K 2002: The investigation of interactions of low doses of ionizing radiation and risk factors by means of *Artemia salina* biotest. Folia Vet **46**: 195-197

Ercal N, Gurer-Orhan H, Aykin-Burns N 2001: Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. Curr Top Med Chem **1**: 529-539

Falis M, Beňová K, Toropila M, Sesztáková E, Legáth J 2004: Changes in the activity of selected adaptive enzymes in chicken liver after single gamma irradiation. Bull Vet Inst Pulawy **48**: 503-506

Falis M, Špalková M, Legáth J 2014: Effects of heavy metals and pesticides on survival of *Artemia franciscana*. Acta Vet Brno **83**: 95-99

Frazier L, Rayner K 1982: Making and correcting errors during sentence comprehension: Eye movements in the analysis of structurally ambiguous sentences. Cogn Psychol **14**: 178-210

Hrnčíř E 1999: Theory of hormesis and controversies on its application to the problem of ionizing radiation. Hygiena **44**: 156-162

ImageJ. Available at: https://imagej.net/Fiji, last modified December 2007

Jinadasa KBPN, Milham PJ, Hawkins CA, Cornish PS, Williams PA, Kaldor CJ, Conroy JP 1997: Survey of cadmium levels in vegetables and soils of greater Sydney, Australia. J Environ Qual **26**: 924-933

Karam PA, Leslie SA 1999: Calculations of background beta-gamma radiation dose through geologic time. Health Phys **77**: 662-667

Kottferová J, Koréneková B, Skalická M 2002: Zinc levels in poultry meat. Trace Elem Electroly **19**: 91-93

Kottferová J, Koréneková B 1998: Distribution of Cd and Pb in the tissues and organs of free-living animals in the Territory of Slovakia. Bull Environ Contam Toxicol **60**: 171-176

Luckey TD 1991: Radiation Hormesis. Boca Raton, CrC Press, 306 p.

Luckey TD 1994: Radiation hormesis in cancer mortality. Chin Med J **107**: 627-630

Luckey TD 2008: Atomic bomb health benefits. Dose-Response **6**: 369-382

Lyn PND 2003: Toxic metals and antioxidants: part II. The role of antioxidants in arsenic and cadmium toxicity. Altern Med Rev **8**: 106-128

Ochi T, Ohsawa M 1985: Participation of active oxygen species in the induction of chromosomal aberrations by cadmium chloride in cultured Chinese hamster cells. Mutat Res **143**: 137-142

Pöschl M, Nollet LML 2006: Radionuclide concentrations in food and the environment. CRC Press, New York, 459 p.

Privezencev KV, Sirota NP, Gaziev AI 1996: Vlijanie sočetannogo bozdejstvia Cd i γ-radiacii na povreždenie i reparaciu DNK b limfoidnych tkanjach myšej. Radiacionnaja biologija, radioekologija **36**: 234-239

Russell WMS, Burch R L 1959: The Principles of Humane Experimental Technique. London, UK: Methuen, 238 p.

Sanders CL 2010: Radiation Hormesis and the Linear-No-Threshold Assumption. Berlin, Heidelberg: Springer Berlin Heidelberg, 217 p.
Sesztáková E, Toropila M, Beňová K 1996: Post-irradiation changes in the peripheral blood of chickens. Folia Vet **40**: 87-90
Špalková M, Beňová K, Dvořák P, Falis M, Renčko A 2015: Study of interaction of low doses of ionizing radiation with selected pesticides on *Artemia franciscana*. Acta Vet Brno **84**: 37-42