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Radiolytic Removal of Selected Pesticides From Waters and Waste Using Ionizing Radiation

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The aim of this work was to investigate radiolytic decomposition of selected widely used compounds from different classes of pesticides, including imidazole fungicides (carbendazim) and organophosphorous pesticides (chlorfenvinphos). Using HPLC with UV/Vis and fluorometric detection, ion-chromatography and LC/MS methods, the effectiveness of decomposition was examined in different irradiation conditions, together with the identification of the products of decomposition. Monitoring of toxicity changes as a result of irradiation was carried out using Microtox bioluminescence test and Daphtoxkit test.

Keywords advanced oxidation process; carbendazim; chlorfenvinphos; HPLC; ionizing radiation; LC/MS; MCPA; pesticides decomposition; toxicity

INTRODUCTION

Intensive growth of various branches of industry and intensive use of chemicals in agriculture results in increasing flux of anthropogenic toxic pollutants to the natural environment. A significant group of these pollutants are pesticides, because of the wide use in contemporary agriculture, their toxicity, and slow degradation in the natural environment in many cases. Pesticide residues are detected both in the environment (waters, soil), and also in vegetables and fruits, as well as in processed food, hence a constant need for the improvement and simplification of analytical methods for the determination of pesticides in various samples is obvious, as well as the development of technologies for their effective removal from industrial wastes and environmental media.

Fungicides are chemicals used for the destruction of unwanted fungi in various applications, which have been employed on a large scale since the 1960s, and since then their use constantly increases. Species of the most common use for this purpose are thiabendazole, fuberidazole, benomyl, methyl thiophante, and carbendazim (MBC-methyl-2-benzimidazole carbamate) (1). Carbendazim is also a product of hydrolysis of benomyl and methyl thiophante. Carbendazim is used for the protection of crops, fruits, and vegetables against fungal diseases, and also for the protection of harvested products during their storage and transportation. Its toxicity is well documented (2), hence its residues are considered as environmental pollutants. Methods of decomposition of carbendazim, described so far in literature, are based on photolysis by UV irradiation in various chemical conditions (3–6). The main subject of those works was the determination of yield of carbendazim photolysis at different pH of irradiated solutions and various concentrations of dissolved oxygen.

The organophosphorus pesticides belong to most toxic environmental pollutants. Their toxicity is based on inhibiting of the activity of enzymes that regulates a functioning of the nervous system of animals and humans—mostly acetylcholinesterase (AChEs) (7). Decrease of AChE activity results in an increase of the amount of acetylcholine in synaptic junctions, and then in excitement, paralysis of muscles and the central breathing system. Pesticide chlorfenvinphos (CFV) examined in this work is insecticide and acaricide, commonly used for protection against insecticides in agriculture, and also against ticks and flies on horses, and goats as well as fleas on dogs. It is weakly

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soluble in water (120 mg L^{-1}), and its typical soil half-lives range from roughly 14 to 161 days (8,9). The World Health Organization qualifies chlorfenvinphos to toxicity class Ib (1). Chlorfenvinphos is most commonly determined using gas chromatography with a flame-ionization detector or flame-photometric sensitive to nitrogen containing compounds (10,11).

The aim of this study was to develop a high-performance liquid-chromatography (HPLC) method for simultaneous determination of each pesticide together with products of its radiolytic decomposition. Also, identification of products of radiolytic decomposition was carried out for both pesticides, together with an examination of different factors affecting the efficiency of their radiolytic degradation, including dose rate, initial concentration, and the pH of irradiated solutions.

EXPERIMENTAL PART

Materials

All chemicals used were of highest purity grade available. Carbendazim, 2-hydroxybenzimidazole, 2-aminobenzimidazole, 1,2-phenylenediamine, 2-methyl-2-propanol, and ammonium acetate were purchased from Sigma Aldrich, while aniline and benzimidazole were purchased from Fluka. Chlorfenvinphos (CFV) was purchased from the Institute of Organic Industry (Warsaw, Poland), 2,2′,4′-trichloroaceton from Fluka, 2,4-dichlorophenol (2,4-DCP), 2,4-dichlorobenzoic acid (2,4-DCBA), 2,4-dichoroacetophenon from Sigma-Aldrich, and diethyl phosphate from Chem Service (West Chester, PA, USA). All stock solutions were kept at 4°C in the dark. HPLC grade acetonitrile (ACN) from J.T. Bakers was used for the preparation of the HPLC eluent. It was filtered through a 0.2-μm filter before use.

Apparatus

Irradiation Source

For γ-irradiation a ^{60}Co source Issledovatel from Russia was used with a dose rate of 1.2 kGy/h. Irradiation was carried out in 100 mL conical flasks fully filled with solution (without gas above surface solution), and in 10 mL custom made flasks allowing saturation of irradiation solutions with the required gas.

HPLC Analysis

The chromatographic determinations of pesticides and products of their radiolytic decomposition were performed by reserved-phase HPLC with Shimadzu chromatograph equipped with UV/Vis diode array detector and fluorescent detector RF-10Axl, a Luna ODS2 (250 × 4.6 mm, 5 μm) analytical column and a guard column from Phenomenex (Torrance, CA, USA), with a flow rate 1 ml/min in gradient elution. The sample injection volume was 100 μL for a synthetic solution of carbendazim and 20 μL for waste analysis. The optimization of the eluent composition is discussed below. Ion-chromatographic measurements were carried out using a Dionex chromatograph model 20000i/p with a conductivity detector. Separations were carried out using an anion-exchange column model AS9 HC (Dionex) with pre-column AG9 HC, and electrochemical suppressor model ASRS-1.

Procedures

Irradiaton

The solutions of the examined pesticides were prepared for irradiation in deionized water and the pH was adjusted to the required values with sulfuric acid or potassium hydroxide solutions. A small amount of added sulfate ion does not affect the radiolytic decomposition of pesticides, due to low reactivity of SO_4^{2-} toward primary radicals formed during radiolysis of water ($OH^{\cdot}$, H, e_{aq}^{-}).

Microtox Test

A Microtox M500 Toxicity Analyser from Azur Environment (Wokingham, England) was used. Residual amounts of H_2O_2 (formed during radiolysis of water) and ozone were removed by a small addition of solid sodium thiosulfate. Measurements of toxicity were performed within 24 h after irradiation. The toxicity was expressed in toxicity units ($TU = 100/EC_{50}$), where EC_{50} is a concentration which causes 50% reduction of the bioluminescence after 15 min incubation.

Daphtoxkit Test

For these measurements the Daphtoxkit F™ was employed which is 24–48 h acute toxicity test, based on cladoceran crustacean *Daphnia magna* in the form of ''dormant eggs''. The test is carried out by the use of the ''neonates'' which are hatched in about 3 days from the eggs. The bioassays are conducted in disposable multi-well test plates and counting alive, immobile, and dead species under the microscope. Toxicity was determined before and after irradiation of the synthetic solution of carbendazim.

RESULTS AND DISCUSSION

Optimization of HPLC Determination

For monitoring of the effectiveness of radiolytic decomposition of carbendazim and chlorfevinphos, an investigation on the development of the HPLC method enabling simultaneous determination of selected pesticides and products of their degradation was carried out. In case of carbendazim these expected products of decomposition include benzimidazole, 2-aminobenzimidazole, aniline, o-phenylenodiamine, and 2-hydroxybenzimidazole, whereas for chlorfenvinphos: 2,4-dichlorobenzoic acid,

2,4-dichlorophenol, 2,2′,4′-trichloroacetophenon, and 2,4-dichloroacetophenon.

In case of carbendazim it was found that the pH of the eluent significantly affects the retention time of the determined compounds and shape of peaks in chromatograms. Reported earlier, the RP-HPLC method for the determination of carbendazim (12) does not permit simultaneous determination of carbendazim and its decomposition products. This can be achieved with a two-solution gradient elution employing 10 mM ammonium acetate aqueous solution containing 5% acetonitrile and pure acetonitrile with a gradient from 5 to 25% in 30 min. The pH 8.0 and wavelength 277 nm were selected as optimum for separation in further measurements.

For chlorfenvinphos the optimized factors included the mode of elution, conditions of UV detection, composition of the eluent, and the volume of the injected sample. As the optimum conditions a gradient elution has been considered, obtained by mixing of acetonitrile (ACN) with 5% of ACN in aqueous $2\,g\,L^{-1}$ solution of citric acid (in time up to 7 min concentration of ACN increased from 60 to 75%, and then up to 1 min it reached 100%). The injected sample volume was 50 μL, and detection was carried out at 250 nm. In these conditions the detection limits (for $S/N = 3$) were $62\,\mu g\,L^{-1}$ for CFV, $9.3\,\mu g\,L^{-1}$ for 2,4-DCBA and $15\,\mu g\,L^{-1}$ for 2,4-DCP.

Irradiation of Chlorfenvinphos

The aim of this work was to examine the effect of γ-irradiation on decomposition of chlorfenvinphos which may indicate a possibility of the use of such treatment for purification of waters and waste. The aerated, aqueous $50\,mg\,L^{-1}$ solution of CFV was irradiated in ^{60}Co source with doses from 0.1 to 1.0 kGy. The chromatogram obtained after irradiation with 100 Gy dose is shown in Fig. 1, and the assignment of the observed signals is given in Table 1.

As is shown in Fig. 2a, the decomposition of chlorfenvinphos with yield 90% occurs at 100 kGy dose. The effect of the applied dose magnitude was also examined in terms

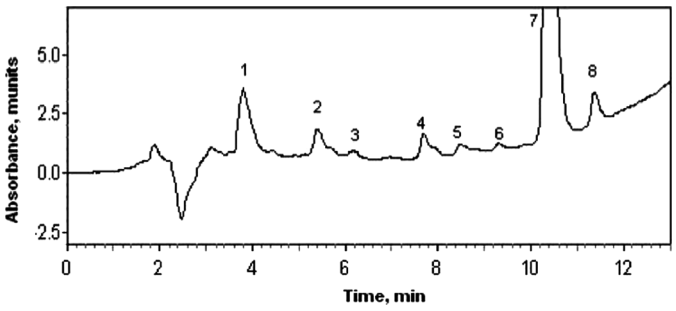


FIG. 1. HPLC chromatogram of aqueous $50\,mg\,L^{-1}$ solution of chlorfenvinphos irradiated with 100 Gy dose (see peak assignment in Table 1).

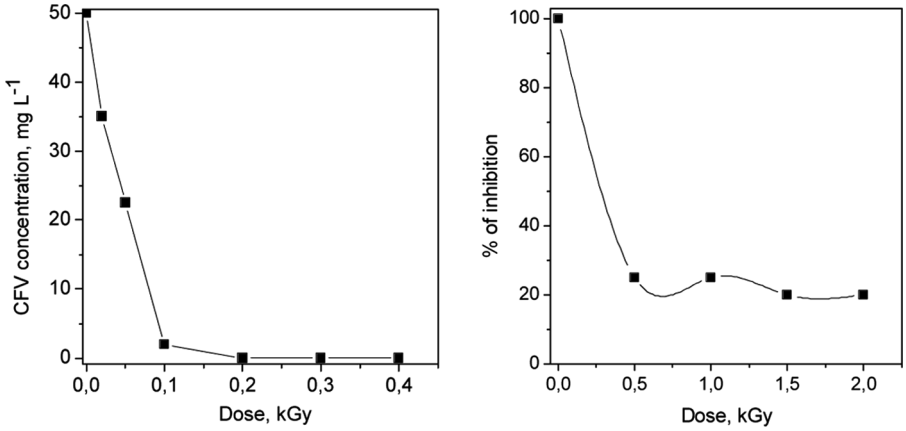
TABLE 1
Products of decomposition of chlorfenvinphos in $50\,mg\,L^{-1}$ solution by γ-irradiation with an applied dose 100 Gy (recorded chromatogram is shown in Fig. 1)

Peak No.	Retention time, min	Compound
1	3.8	Unidentified
2	5.4	2,4-dichlorobenzoic acid
3	6.2	2,4-dichlorophenol
4	7.7	Unidentified
5	8.5	2,2′,4′-trichloroacetophenon
6	9.3	2,4-dichloroacetophenon
7	10.4	Chlorfenvinphos
8	11.4	Unidentified

of decrease of activity of AChE mutant B394 obtained from the Department of Biochemistry at the University of Toulouse, France (Fig. 2b). Solution $50\,mg\,L^{-1}$ of CFV inhibits AChE 100%, while after irradiation with 500 Gy dose, where complete decomposition of CFV takes place, still some residual inhibition of 26% is observed. This indicates that certain products of CFV exhibit inhibitive properties towards AChEs.

Ionized products of radiolytic decomposition of CFV were monitored using high performance ion-chromatography with conductivity detection. Their concentration in irradiated solutions changes significantly with an applied dose of radiation (Fig. 3). The concentration of chloride about $3\,mg\,L^{-1}$, observed in the dose range from 0.3 to 0.6 kGy, corresponds to 20% of chlorine present in decomposed CFV. This means that at 0.6 kGy still about 80% of chlorine remains in organic products, which are not decomposed in this range of radiation doses.

In the same measurements it was shown that only trace amounts of orthophosphate can be detected in irradiated solutions (Fig. 3). This indicates that most phosphorus remains in organic form after decomposition of CFV in this range of radiation doses. Based on earlier publications (13,14), one can expect that as a result of radiolytic decomposition of CFV, diethyl phosphate (DEP) can be formed. In order to confirm the formation of DEP in irradiated solutions, HPLC measurements were also carried out with tandem MS detection at the Laboratory of Structural Research of the Department of Chemistry, University of Warsaw, using an HPLC Shimadzu LC 20 chromatograph with Applied Biosystems 3200 Q-Trap mass spectrometer. Measurements were carried out with atmospheric pressure ionization with positive polarity. It was found that at doses above 200 Gy, about 80% of CFV is decomposed to DEP, and the amount of DEP decreases with further increase of the radiation dose.



(A) (B)

FIG. 2. A) Effect of dose magnitude on yield of radiolytic decomposition of chlorfenvinphos in 50 mg L^{-1} solution. B) Inhibition of acetylcholinesterase mutant B394 by chlorfenvinphos solution 93 mg L^{-1} irradiated with various doses.

Irradiation of Carbendazim

In studies of carbendazim the effect of initial concentration was examined, and it was found that the radiation dose needed for 90% degradation of these compounds increases almost linearly with the initial concentration of fungicide in the examined concentration range 20–100 μmol L^{-1} (Fig. 4a). The reported measurements were carried out in fungicide solutions prepared in distilled water at pH 7. In γ-irradiated aerated solutions of carbendazim 100 μmol L^{-1}, in pH range from 3 to 10, practically a complete decomposition has been observed at small doses up to 0.6 kGy. In alkaline solutions it occurs slightly faster, and this can be attributed to simultaneously occurring hydrolysis of carbendazim (Fig. 4b). It is contrary to observations for photochemical decomposition, which was strongly affected by the pH of the initial solution of carbendazim (4).

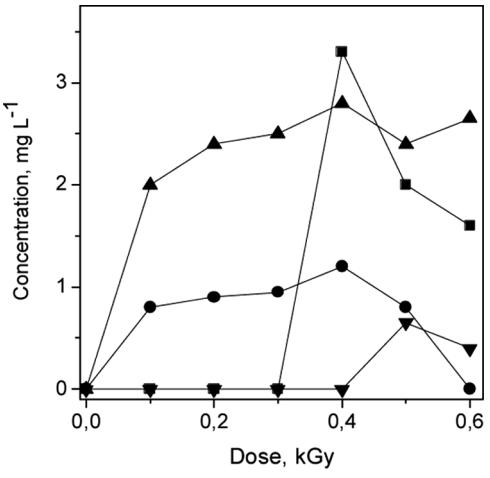


FIG. 3. Effect of dose magnitude on formation of ionic product as result of irradiation of 50 mg L^{-1} chlorfenvinphos solution: (■) acetate, (●) formate, (▲) chloride, (▼) orthophosphate.

The identification of products formed in radiolytic decomposition of carbendazim in various conditions is important for the elucidation of the mechanism of occurring processes. In photochemical processes as the main product of decomposition of carbendazim, 2-aminobenzimidazole has been identified (3), while when the process was carried out in the presence of hydrogen peroxide, the hydroxyl derivatives of carbendazim were postulated (15). In our experiments carried out in differrent conditions, with HPLC measurements using mass spectrometry detection, different products of decomposition were found. When oxidation with hydroxyl radicals produces from water radiolysis predominate, as the products of radiolysis hydroxybenzimidazole and two isomers of hydroxycarbendazim were identified, while in reducing conditions as the main product benzimidazole was found. The same results of comparison of kinetic modeling with experimental data for the effect of initial concentration and irradiation of carbendazim solutions in different conditions are shown in Fig. 5. The observed correlation is especially satisfactory for irradiation in different conditions with different doses.

Toxicity Changes

The important additional measurements to chromatographic ones, where the effectiveness of radiolytic decomposition of a particular pollutant is carried out, there are measurements of toxicity changes vs. the applied radiation dose. For this purpose a routine bacterial test Microtox was employed. CFV does not exhibit toxicity against bacteria *Vibrio fisheri* used in Microtox, which is opposite to some transient products of its decomposition such as

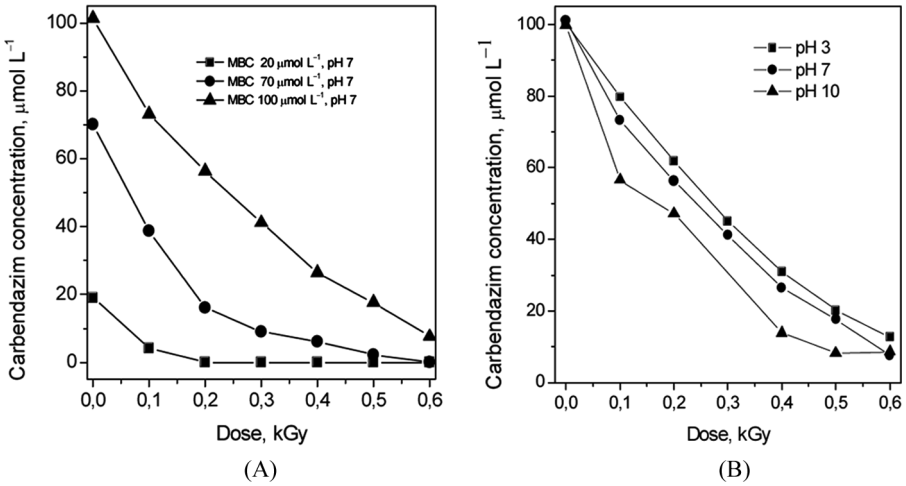


FIG. 4. A) The effect of initial concentration of carbendazim in γ-irradiated aqueous solutions on yield of decomposition at different irradiation doses. B) The effect of pH of irradiated solutions of carbendazim on yield of decomposition at initial concentration 100 μM.

2,4-DCP or 2,4-DCBA (Table 2). Toxicity of 2,4-dichloro- and 2,2′,4′-trichloroacetophenon was not determined as yet, and it was not found in available literature. The toxicity of 2,4-DCP and 2,4-DCBA seems to be a reason of certain increase of toxicity at small doses, and its decrease with an increase of the applied dose (Fig. 6). This leads to the conclusion that in order to decompose CFV at examined range of concentrations and to obtain non-toxic products, the applied radiation dose should exceed 1 kGy.

Also, for the decomposition of carbendazim toxicity monitoring was conducted. In this case it was observed that the Micrtotox test does not give any response. Most probably it is due to the fact that carbendazim and the expected products of its radiolytic degradation are not toxic for bacteria *Vibrio fisheri*. The chemical structure of the carbendazim molecule, containing in its structure the benzimidazole ring, indicates that this relationship should be toxic for higher organisms (17,18). As it was mentioned earlier, carbendazim is recognized as a substance which is very toxic for water organisms ($EC50 < 1\,mg\,L^{-1}$). Thereby, measurements of changes of the toxicity of carbendazim solution during γ-irradiation, were performed with the use of the Daphtoxkit test. The most toxic is carbendazim, but also 1,2-fenylenodiamine, especially after 48 hours. 2-aminobenzimidazole and 2-hydroxybenzimidazole

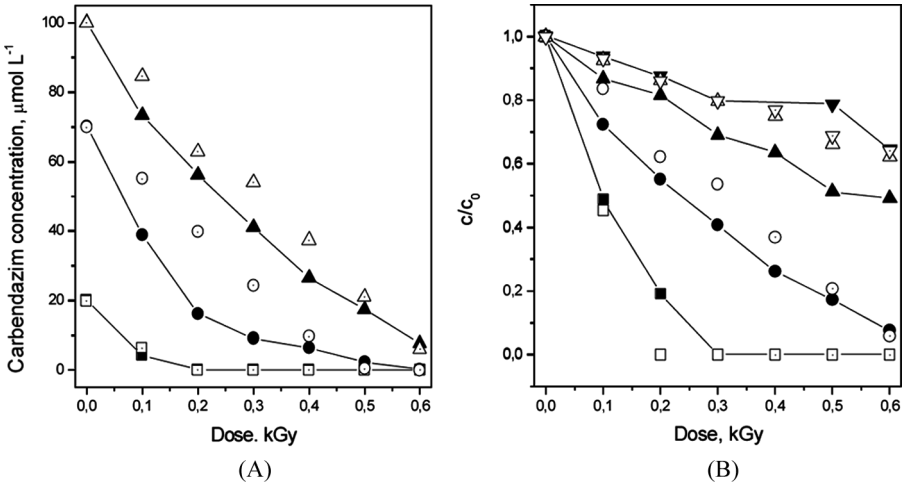


FIG. 5. Comparison of experimental results with kinetic modeling for radiolytic decomposition in different conditions: A – effect of initial concentration, B – irradiation of aqueous 100 μM solutions of carbendazim in different conditions: solution at pH 7.0 saturated with N_2O, (●), saturated solution at pH 7(■), argon saturated solution with added 106 μM *tert*-butanol at pH 1.5, (▲) and Ar saturated solution at pH 7.0 with added 106 μM *tert*-butanol (▼). Full points connected with lines – experimental results, open points – calculated data.

TABLE 2
Toxicity of chlorfenvinphos and expected products of its radiolytic decomposition determined by bioluminescence test Microtox

Compound	EC_{50} 15 min, mg L^{-1}	Reference
CFV	Not toxic	This work
2-DCBA	<5.0	(16)
2,4-DCP	1.2–6.1	(16)

cause in turn the stimulation of the movement of daphnia in concentrations above 10 below sea level (Table 3)

After irradiation with dose 0.1 kGy the toxicity increased 4 times, and with further increase of irradiation dose the toxicity of the irradiated solution decreases (Fig. 7). At dose 0.6 kGy, where the practical efficiency of carbendazim decomposition is practically 100%, the TU value is about 17 units smaller from the toxicity of the solution before irradiation. It can be expected that with the use of a larger dose a complete reduction of the toxicity occurs.

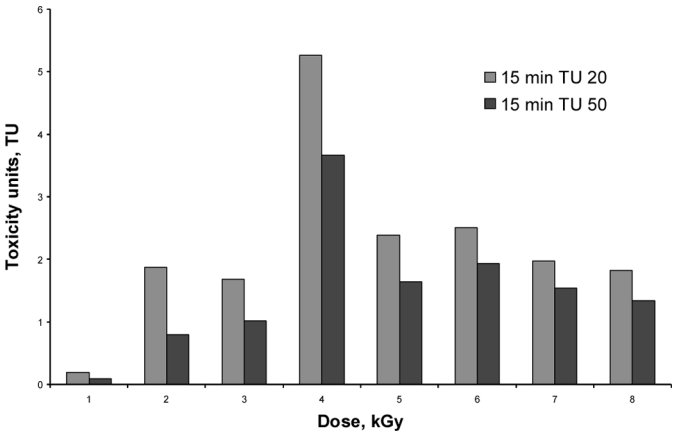


FIG. 6. Changes of toxicity of irradiated 50 mg L^{-1} solution of chlorfenvinphos with applied radiation dose measured with Microtox test.

TABLE 3
Toxicity of carbendazim and expected products of its radiolytic decomposition determined by Daphtoxkit test

Examined compounds	EC50 24 h, mg L^{-1}	EC50 48 h, mg L^{-1}
Carbendazim	0.45	0.34
Aniline	>100	>100
Benzimidazole	>100	70
1,2-fenylenodiamine	15.4	2.9
2-aminobenzimidazole	>20	15.4
2-hydroksybenzimidazole	>20	>20

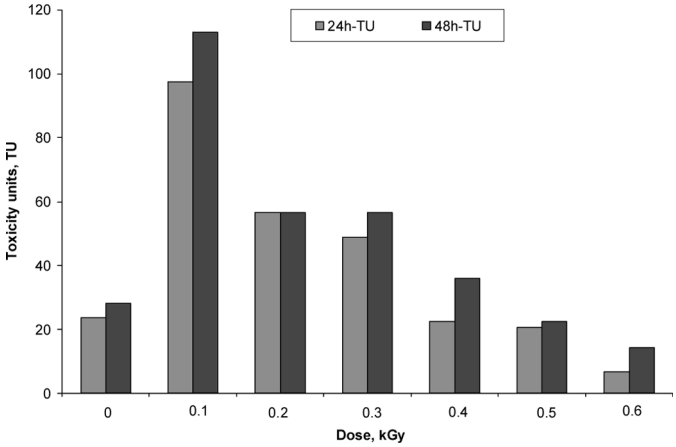


FIG. 7. Changes of toxicity of irradiated 100 μmol L^{-1} pH 7 solution of carbendazim with applied radiation dose measured with Daphtoxkit test.

CONCLUSIONS

It was found that the pH of the solution of carbendazim before γ irradiation did not cause essential changes in the efficiency of its decomposition. Preliminary investigations showed also that the dose needed for the decomposition of 50 mg L^{-1} chlorfenvinphos with a 90% yield occurs at 100 Gy dose. For complete decomposition of carbendazim at 20 μmol L^{-1} level, a radiation dose 200 Gy is needed. A good correlation of the experimental results with kinetic calculations for changes of the efficiency of the radiolytic decomposition of carbendazim was obtained. It was also shown that toxicological examination of solutions before and after radiation were an essential supplement of the chemical analysis.

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