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MARIANO-da-SILVA, Samuel; de OLIVEIRA, Silvio Luiz; Oliveira LEITE, César Augusto; Silva do PRADO, Renata; Pimenta de FARIA, Francys; Nunes OLIVEIRA, Rangel Camillo; de Siqueira MARIANO-da-SILVA, Fabiana Maria

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Ação tóxica do cádmio sobre a levedura Saccharomyces cerevisiae PE-2: influência do ph e conteúdo de dextrose e extrato de levedura no meio

Samuel MARIANO-da-SILVA[1]*, Silvio Luiz de OLIVEIRA[1], César Augusto Oliveira LEITE[2], Renata Silva do PRADO[2], Francys Pimenta de FARIA[3], Rangel Camillo Nunes OLIVEIRA[2], Fabiana Maria de Siqueira MARIANO-da-SILVA[4]

Abstract

The aim of this study was to evaluate the effects of pH, dextrose and yeast extract on the cadmium toxicity on *Saccharomyces cerevisiae* PE-2. In the first assay, the YED mediums with different pH (2, 3, 4, 5, 6, 7, and 8) containing 0.0 and 0.05 mmol Cd L^{-1} were inoculated with yeast suspension and incubated at 30 °C for 18 hours. During the anaerobic growth, the biomass concentration was determined. The yeast trehalose content, cell viability, and the growth rate were assessed at the beginning and at the end of the growth stages. In the second assay the YED mediums were diluted to the total, ½, and ¼ content of dextrose and yeast and 0.0 and 0.05 mmol Cd L^{-1} were added. The pH of the mediums was adjusted to 5. The culture mediums were inoculated and incubated at 30 °C for 18 hours. The yeast growth was not affected by cadmium at high pH, but at low pH the yeast becomes more sensitive to the toxic effect. The yeast susceptibility to cadmium was enhanced by the decrease of yeast extract strength and the increase of dextrose strength.
Keywords: *yeast; cadmium; dextrose; yeast extract; pH.*

Resumo

O objetivo deste estudo foi avaliar a influência de alguns fatores químicos na sensibilidade da levedura *Saccharomyces cerevisiae* PE-2 ao cádmio. Em um primeiro ensaio, os meios YED com diferentes valores de pH (2, 3, 4, 5, 6, 7, e 8) e acrescidos de 0,0 e 0,05 mmol Cd L^{-1} foram inoculados e incubados a 30 °C por 18 horas. Durante o crescimento anaeróbio foi determinada a concentração celular. No início e ao final do estágio de crescimento determinaram-se os teores de trealose, a viabilidade celular e a taxa de brotamento da levedura. Em um segundo ensaio, os meios foram diluídos a ½ e ¼ para dextrose e extrato de levedura e adicionados de 0,0 e 0,05 mmol Cd L^{-1}. O pH dos meios foi ajustado para 5. Após a inoculação, os frascos foram incubados a 30 °C por 18 horas. O crescimento não foi afetado pelo cádmio, porém em níveis mais baixos os sintomas de toxidade apareceram. O decréscimo da concentração de extrato de levedura e o incremento na concentração de dextrose aumentaram a susceptibilidade da levedura ao cádmio.
Palavras-chave: *levedura; cádmio; dextrose; extrato de levedura; pH.*

1 Introduction

The pollution of the environment with toxic heavy metals has been increasing throughout the world with industrial progress. It is important to establish an efficient and low-cost method for the removal of metal pollutants. The ability of microorganisms to remove heavy metals from aqueous solution has long been of scientific interest. Recently, such microbial ability has attracted considerable attention to remove heavy metals at low concentration from aqueous wastes because the methods suitable for removing high concentrations of metals are ineffective or costly when applied to diluted metals. There are many reports on algae, bacteria, fungi, or higher plants that can help remove and/or accumulate large amounts of heavy metals from or to their external environment (ALLBERTINI, 1999; ALBERTINI; CARMO; PRADO-FILHO, 2007; GADD; MOWLL, 1983; GRAFL; SCHWANTES, 1983; MARIANO-DA-SILVA, 1998; MARIANO-DA-SILVA; BASSO, 2004; MARIANO-DA-SILVA; PRADO-FILHO, 1998; MARIANO-DA-SILVA; PRADO-FILHO, 1999; MARIANO-DA-SILVA, 2001; RÖSICK; MANGIR; LOCHMANN, 1986; SILVA, 1995; and VOLESKY, 1990). A variety of mechanisms are known for metal uptake, e.g. adsorption to cell wall, diffusion into the cells, and metabolism-dependent ion transport. These processes depend on many factors such as metal species and growth conditions of the organisms.

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[1] *Departamento de Ciências Biológicas, Universidade Federal de Goiás, UFG/CAJ CP 03, CEP 75800-970, Jataí – GO, Brasil, E-mails: smarianos@uol.com.br; s3oliveirac@yahoo.com.br*
[2] *Universidade Federal de Goiás – UFG/CAJ, CP 03, CEP 75800-970, Jataí – GO, Brasil*
[3] *Programa de Pós-Graduação em Agronomia – UFG/CAJ, CP 03, CEP 75800-970, Jataí – GO, Brasil, E-mail: francysbiopimenta@hotmail.com*
[4] *Departamento de Tecnologia de Alimentos, UEG/CJ, Av. 31 de maio s/n, setor Epaminondas, CEP 75800-000, Jataí – GO, Brasil, E-mail: fmdsmds@uol.com.br*
**A quem a correspondência deve ser enviada*

Only the past decade has the potential of metal biosorption by microbial biomass materials been well established. The types of microbial biomass of interest can quite pragmatically be those that can be easily obtained in larger quantities.

One of the most ubiquitous biomass types of yeasts utilized on a large scale by man for centuries is Strains of *Saccharomyces cerevisiae*, which when propagated aerobically is known as "baker's yeast". Other strains of the same species when used anaerobically to producing ethanol have been labeled "distiller's or brewer's yeast". However, *S. cerevisiae* is not only the key microorganism in the brewing or fermentation process, but it is also an easily manipulated eukaryotic cell. It serves as an excellent model to study many important problems in eukaryotic biology (BROCK et al., 1994), is easy to handle, grows rapidly, and provides a large number of homogeneous individuals.

Moreover, yeasts have shown to take up have metals ions (GÖKSUNGUR; UREN; GÜVENÇ, 2005; GRAFL; SCHWANTES, 1983; MARIANO-da-SILVA; PRADO-FILHO, 1998; MARIANO-DA-SILVA; PRADO-FILHO, 1999; MARIANO-DA-SILVA, 2001; PARK; LEE; JUNG, 2003; RÖSICK; MANGIR; LOCHMANN, 1986; VASUDEVAN; PADMAVATHY; DHINGRA, 2002), and have a potential as effective biosorbents. Hence, cell growth (MARIANO-da-SILVA; BASSO, 2004), cell viability (MARIANO-da-SILVA, 1998; MARIANO-da-SILVA, 2001; MARIANO-da-SILVA et al., 2007), electron transport (BITTON et al., 1984), and mitochondrial respiration (HAUBENSTRICKER et al., 1990) of *Sacharomyces cerevisiae* have all been selected as parameters for pollution assessment.

However, metal/*Saccharomyces* interaction is influenced by a number of environmental and experimental factors, e.g. conditions of pH and temperature and the presence of additional ions in solutions. Those factors influence considerably the composition of all yeast cells and, consequently, the binding abilities of cells for metal ions (AVERY; TOBIN, 1993; NORRIS; KELLY, 1977; BRADY; DUNCAN, 1994; MARIANO-da-SILVA et al., 2007).

Since previous studies indicated that the susceptibility of microorganisms to environmental toxicants can be influenced by pH, organic matter, and nutrient strength (BAGY; EL-SHAAROUNY; EL-SHANAWANY, 1991; DOSTALEK; PATZAKA; MATEIKAB, 2004; ENGL; KUNZ,1995; GÖKSUNGUR; UREN; GÜVENÇ, 2005; HSU; LEE; CHANG, 1992; KUJAN; VOTRUBA; KAMENIK, 1995; MARIANO-DA-SILVA, 2001; MARIANO-DA-SILVA et al., 2007; MARIANO-DA-SILVA; BASSO, 2004; MARQUES, et al., 1999), we attempted to examine the effect of pH, yeast extract and dextrose contents of the cadmium toxicity on *S. cerevisiae* PE-2 in order to optimize a bioassay with this organism.

2 Materials and methods

2.1 Preparation of glasswere

All reusable glasswere (glass, quartz, polyethylene, teflon, etc.) was rinsed with detergent and ultra pure water, and it was then soaked for four hours in a mixture of nitric acid, hydrocloric acid, and water (1 + 2 + 9) followed by rinsing with ultra pure water and heat drying (McDANIEL, 1992).

2.2 Preparation of solutions

To limit metal contamination, all aqueous solutions were prepared with ultra-pure water (millipore Milli-Q purification system).

2.3 Yeast

Saccharomyces cerevisiae PE-2, caracterized by the karyotyping profile (BASSO et al., 1993), was kindly provided by the Yeast Collection of the Biological Science Department (ESALQ/USP).

2.4 Yeast pre-growth

Yeast was reactivated in YEPD medium (Yeast Extract Peptone Dextrose) by a pure-culture (lyophilizated) and was pre-grown anaerobically at 30 °C, in a sterilized (autoclaving at 1 ATM, 120 °C for 20 minutes) molasses medium with 6% ART (total reducing sugars), supplemented with KH_2PO_4 (8.36 mmol L^{-1}), $(NH_4)_2SO_4$ (5 mmol L^{-1}), urea (38.75 mmol L^{-1}), $MgSO_4.H_2O$ (3.57 mmol L^{-1}), $ZnSO_4.7H_2O$ (0.10 mmol L^{-1}), $MnSO_4.H_2O$ (0.12 mmol L^{-1}) and linolenic acid (0.11 mmol L^{-1}). Cells from the late-exponential growth phase were harvested by centrifugation (800 G, 20 minutes) and resuspended in distilled deionized water to a final concentration of 1g (fresh wt) 100 mL^{-1} (MARIANO-DA-SILVA, 2001).

2.5 First growth assay

Fermentation was carried out with (autoclaving at 1 ATM, 120 °C for 20 minutes) 75 mL of YED sterilized (yeast extract 1% and dextrose 2%) medium in 125 mL Erlenmeyer flasks sealed with aluminum foil with different cadmium concentrations (0.0 and 0.05 mmol L^{-1}) and different pH (2, 3, 4, 5, 6, 7 e 8). The pH of the suspensions was adjusted using 0.1 mol L^{-1} H_2SO_4 or 0.1 mol NaOH. The flasks were inoculated in aseptic conditions with 1 mL of 1% (wet basis) yeast suspension and incubated at 30 °C and 70 RPM for 18 hours in an orbital shaker. At specific times during fermentation (0, 2, 4, 6, 8, 10, 12, 14, 16 and 18 hours), 1 mL portions of cell suspension were withdrawn and transferred to test-tubes with 9 mL of deionized water. The biomass concentration was determined by turbidity measurements at 570 nm (Bausch and Lomb) using a standard-line previously performed.

2.6 Second growth assay

To compare the effects of cadmium on *S. cerevisiae* under different dextrose and yeast extract contents, the same procedure was used. Medium was prepared by dilution (the total, 1/2, and 1/4 content of dextrose and yeast) and with the addition of 0,0 and 0,05 mmol Cd L^{-1}. The pH of the media was adjusted to 5 by using 0.1 mmol L^{-1} H_2SO_4. The flasks were incubated at 30 °C and 70 RPM for 18 hours in an orbital shaker. The experiments were made in triplication.

2.7 Cell counting

After growth (18 hours), 0.5 mL of each cell suspension was sampled, diluted, colored using eritrosine, and directly counted

with a microscopic for yeast viability and growth rate evaluation according to AMORIM et al. (1989).

2.8 Yeast trehalose

Trehalose was extracted from 60 mg of washed cells (fresh wt) with 2 mL of 0.5 mol L^{-1} trichloroacetic acid in ice bath for 20 minutes (the suspension was frequently shaken), centrifuged (Trevelyan, 1956a; Trevelyan, 1956b), and 0.2 mL of each supernatant was subjected to anthrone reaction according to BRIN (1966).

2.9 Alcohol measurement

The alcohol was determined using yeast free wine distillation followed by alcoholic determination in digital densimeter (ZAGO et al., 1989).

2.10 Statistical analysis

Variance analysis (F-test) was used to analyze the variables, following by a completely casual experimental delineation, in factorial scheme with 3 replicates per treatment. The averages comparisons were made by multiple comparison Tukey tests in a factorial delineation (BISHOP, 1966; SNEDECOR; COCHRAN, 1967).

3 Results and discussion

Increased pH can result in precipitation of metal hydroxides or oxides reducing the free metal ions concentration. The formation of $CdOH^+$ begins at pH 7.5 and that of $Cd(OH)_2$ at pH 9. Thus, at pH 8, most of the Cd would be in the form of $Cd^{2+}$with little $CdOH^+$ and no $Cd(OH)_2$. Whereas at pH 9, the Cd^{2+} and $CdOH^+$ ionic species would predominate, with little $Cd(OH)_2$ being present (PARK; LEE; JUNG, 2003). At pH below 7, cadmium exists predominately as the free divalent ion. A pH between 4 and 7 is widely accepted as optimal for metal interaction with almost all types of biomasses (PARK; LEE; JUNG, 2003; GÖKSUNGUR; UREN; GÜVENÇ, 2005; MARQUES et al., 1999). Hydrolysis reactions occur with nearly all the cadmium cations, and because of this the interaction is facilitated (BAES; MESMER, 1976).

Similar results with *S. cerevisiae* PE-2 (Figures 1 and 2) were obtained. The growth of *S. cerevisiae* PE-2 was not significantly affected by cadmium at pH 7 and 8. At low pH, the yeast becomes more sensitive to the toxic effect (Table 1), and pH between 3 and 6 was considered optimal for metal/yeast interaction. The little differences observed in the literature (GÖKSUNGUR; UREN; GÜVENÇ, 2005; MARQUES et al., 1999; PARK; LEE; JUNG, 2003; VASUDEVAN; PADMAVATHY; DHINGRA, 2002) were probably due to the growth medium. The pH at which there are substantial quantities of Cd^{+2}, $CdOH^+$, or $Cd(OH)_2$ in the complex system of a microbial growth medium has not been clearly established. In this situation, the chemical behavior of a given metal can be a complicated function of pH and medium matter.

HSU et al., (1992) concluded that the dilution of YE medium (3 g yeast extract, 3 mg malt extract, 5 mg peptone, and 10 g glucose in 1 liter of distilled water) to ½ and ¼ with distilled water did not significantly alter the effect of cadmium on *S. cerevisiae*. In contrast, our results showed the high correlation of the growth medium dilution and the cadmium effects (Table 2). The *S. cerevisiae* PE-2 growth was significantly affected by 0.05 mmol L^{-1} of cadmium at 1 YE, and at ½ and ¼ YE the growth was totally inhibited.

It was observed that the susceptibility of *S. cerevisiae* PE-2 to cadmium was dramatically enhanced by the decrease of yeast extract strength. The cadmium ion, as previously related by RAMAMOORTY (1975); KUSHNER (1975), has a strong affinity for organic materials such as yeast extract. Thus, there are two possible explanations for the toxicity decrease of cadmium when increasing yeast extract strength: the organic matter reacts

Table 1. Trehalose content, viability and building rate in *S. cerevisiae* PE-2 cultured in YED mediums with cadmium (0.0 and 0.05 mmol L^{-1}) and with different pH (2, 3, 4, 5, 6, 7, and 8).

Treatment	Trehalose (g $100g^{-1}$)	Viability (%)	Building (%)
Initial	5.85[a]	95.91[a]	25.61[a]
pH 2.0 – 0.0 mmol L^{-1} Cd	0.00[e]	7.35[d]	0.00[c]
pH 3.0 – 0.0 mmol L^{-1} Cd	3.09[c]	63.88[c]	24.93[a]
pH 4.0 – 0.0 mmol L^{-1} Cd	4.87[b]	98.53[a]	23.64[a]
pH 5.0 – 0.0 mmol L^{-1} Cd	4.84[b]	99.50[a]	20.03[ab]
pH 6.0 – 0.0 mmol L^{-1} Cd	4.77[b]	99.13[a]	24.57[a]
pH 7.0 – 0.0 mmol L^{-1} Cd	4.84[b]	99.27[a]	23.78[a]
pH 8.0 – 0.0 mmol L^{-1} Cd	4.87[b]	99.54[a]	23.63[ab]
pH 2.0 – 0.05 mmol L^{-1} Cd	0.00[e]	0.00[e]	0.00[c]
pH 3.0 – 0.05 mmol L^{-1} Cd	0.10[e]	0.00[e]	0.00[c]
pH 4.0 – 0.05 mmol L^{-1} Cd	0.99[d]	62.26[c]	15.01[b]
pH 5.0 – 0.05 mmol L^{-1} Cd	1.45[d]	85.49[b]	23.10[ab]
pH 6.0 – 0.05 mmol L^{-1} Cd	3.19[c]	90.30[b]	27.55[a]
pH 7.0 – 0.05 mmol L^{-1} Cd	4.87[b]	98.14[a]	27.39[a]
pH 8.0 – 0.05 mmol L^{-1} Cd	4.97[b]	98.72[a]	25.10[a]
Coefficient of Variation (%)	5.986	2.056	12.09

The averages followed by the same letters do not differ among themselves according to the Tukey test to 1% of confidence.

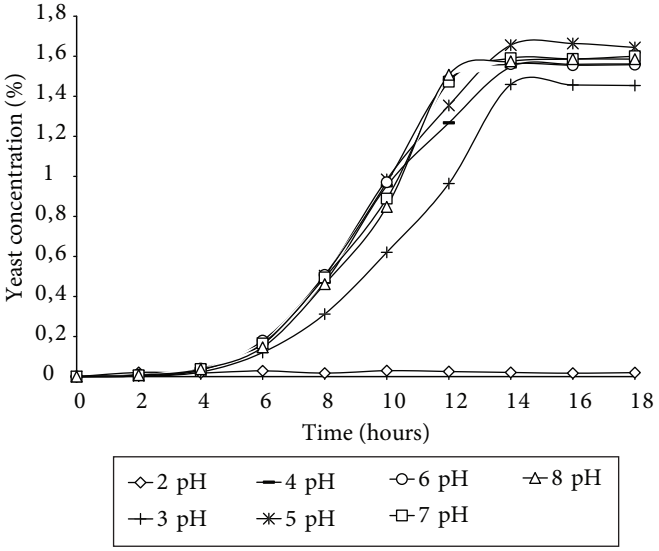

Figure 1. Effects of pH on the growth of *S. cerevisiae* PE-2 in medium without cadmium.

Table 2. Yeast biomass, viability, growth rate, and trehalose content in *S. cerevisiae* cultured in YED mediums with cadmium (0.0 and 0.05 mmol L^{-1}) and with different content of yeast extract (1YE = full; ½YE = half; ¼YE = quarter) and dextrose (1D = full; ½D = half; ¼D = quarter).

Treatment	Yeast	Viability	Building	Trehalose
	(g 100 mL^{-1})	(%)	(%)	(g 100 g^{-1})
initial	–	99.78[a]	25.66[a]	5.23[a]
1YE + 1D – 0.0 mmol L^{-1} Cd	1.6750[a]	99.51[a]	26.13[a]	4.51[a]
1YE + ½D – 0.0 mmol L^{-1} Cd	1.6409[a]	99.60[a]	22.10[a]	6.22[a]
1YE + ¼D – 0.0 mmol L^{-1} Cd	1.5768[a]	99.63[a]	11.54[b]	6.28[a]
½YE + 1D – 0.0 mmol L^{-1} Cd	1.5077[a]	98.69[a]	19.51[a]	1.60[b]
½YE + ½D – 0.0 mmol L^{-1} Cd	1.4534[a]	98.63[a]	10.97[b]	5.22[a]
½YE + ¼D – 0.0 mmol L^{-1} Cd	1.4972[a]	99.00[a]	15.91[ab]	6.07[a]
¼YE + 1D – 0.0 mmol L^{-1} Cd	1.5728[a]	84.02[b]	19.93[a]	0.19[c]
¼YE + ½D – 0.0 mmol L^{-1} Cd	0.6872[b]	92.22[a]	11.73[b]	0.03[c]
¼YE + ¼D – 0.0 mmol L^{-1} Cd	0.5243[b]	96.70[a]	19.33[a]	0.01[c]
1YE + 1D – 0.05 mmol L^{-1} Cd	0.7722[b]	56.12[b]	12.09[b]	0.91[b]
1YE + ½D – 0.05 mmol L^{-1} Cd	0.7662[b]	45.89[b]	26.12[a]	1.16[b]
1YE + ¼D – 0.05 mmol L^{-1} Cd	0.8085[b]	44.12[b]	28.22[a]	2.54[a]
½YE + 1D – 0.05 mmol L^{-1} Cd	0.0062[c]	0.00[c]	0.00[c]	0.02[c]
½YE + ½D – 0.05 mmol L^{-1} Cd	0.0074[c]	0.00[c]	0.00[c]	0.01[c]
½YE + ¼D – 0.05 mmol L^{-1} Cd	0.0065[c]	0.00[c]	0.00[c]	0.03[c]
¼YE + 1D – 0.05 mmol L^{-1} Cd	0.0097[c]	0.00[c]	0.00[c]	0.03[c]
¼YE + ½D – 0.05 mmol L^{-1} Cd	0.0073[c]	0.00[c]	0.00[c]	0.03[c]
¼YE + ¼D – 0.05 mmol L^{-1} Cd	0.0066[c]	0.00[c]	0.00[c]	0.01[c]
Coefficient of Variation (%)	9.98	9.05	19.90	12.31

The averages followed by the same letters do not differ among themselves according to the Tukey test to 1% of confidence.

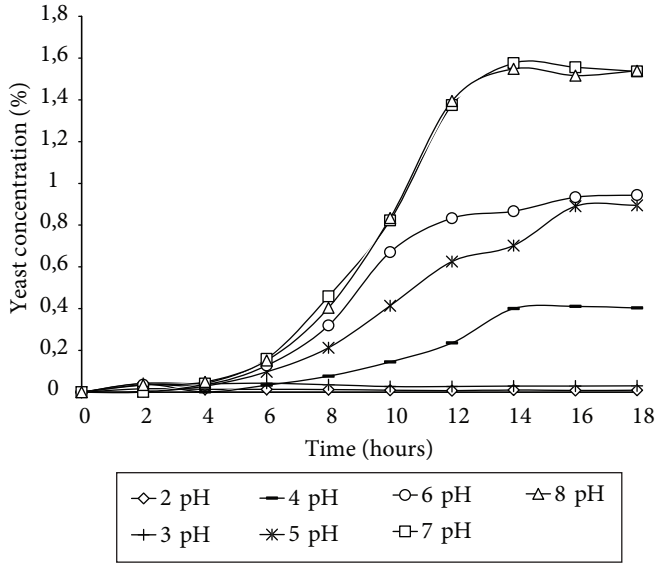

Figure 2. Effects of pH on the growth of *S. cerevisiae* PE-2 in medium with 0.05 mmol L^{-1} of cadmium.

with cadmium ions to form compounds that are less toxic than the ions themselves, and/or the ions adsorbed on the surface of particles are rendered less toxic (BAGY et al., 1991).

The decrease of dextrose strength lowered the susceptibility of S. cerevisiae PE-2 to cadmium. This is probably due to the decrease of metabolic activity. It was found that the cadmium transport into the yeast cell (when the cadmium is more toxic) depends on energy therefore it is glucose dependent (GADD; MOWLL, 1983; RÖSICK, 1986). Thus, in low dextrose the cadmium was less toxic (Table 2).

Yeast viability decreased in parallel with trehalose content; apparently in response to cadmium toxicity. Therefore, trehalose concentrations may be an important indicator of cadmium stress on yeast.

4 Conclusions

The growth of *S. cerevisiae* PE-2 was not significantly affected by cadmium at pH 7 and 8, but at low pH the yeast becomes more sensitive to the toxic effect. The susceptibility of *S. cerevisiae* PE-2 to cadmium was enhanced by the decrease of yeast extract strength, and the decrease of dextrose strength lowered the susceptibility of *S. cerevisiae* PE-2 to cadmium.

The toxicity of cadmium to *Saccharomyces cerevisiae* PE-2 is apparently dependent on the chemical characteristics of the growth medium. The toxicity of a toxicant may be reduced by some of the specific properties of a growth medium whereas for others, with different chemical characteristics, the toxicity of an equivalent dose of some toxicant may be increased. Thus, to achieve success in toxicity studies, the influence of the chemical factors on toxicant toxicity should be considered.

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