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Research Article

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Influences of organic pollutants in water on antioxidant enzyme in zebra fish

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ABSTRACT

Zebra fish was used as testing animal to be stimulated by the organic solvents (phonel and bisphonel A).The superoxide dismutase(SOD) and catalase (CAT) were used as biomarker to study the influence of organic contaminants in water (phenol and bisphonel A) on the autioxidant enzyme activities of zebra fish. Research results indicated that the activities of SOD and CAT increased with time under the low concentration of phonel, and the trend became stronger along increased concentrations. The activities of SOD increased at the initial period and then decreased with time and concentration increasing in water containing bisphonel A. However, for the activities of CAT, with time and its concentration increasing, the variation trend became weaker.

Key words: Zebra fish, Phenol, Bisphonel A (PBA), Superoxide Dismutase(SOD), Catalase(CAT)

INTRODUCTION

Water environment is the place where various contaminants concentrate, and it is also an important place where pollution hazard occurs. In the absorption, migration and transformation process of pollutants, different stages will induce the biomarkers react accordingly [1]. Biomarker was widely used in the ecological toxicology research and ecological pollution risk evaluation of water environment. So, the use of biomarker as the early warning indicator in toxic effect is becoming the priority research areas [2-3]. The superoxide dismutase (SOD) and catalase (CAT) are the most common biomarkers used as the environmental toxicity indicator.

Bisphenol A and phenol are the important raw materials in chemical industry, medicine and pesticide. They have great potential harmful impact on the growth and reproduction of the aquatic organism [4]. It is simple to cause the pollution of water and soil quality. There will be a small mount of residual in the drinking water, its great harmful to our health if we drink or eat the polluted water or polluted fish [5-8]. Zebra fish is a common experimental animal used as indoor standard toxicology, is also recommended by ISO to test the toxicity of water in river. The report about the the bio-toxicology effect on bisphenol A and phenol towards zebra fish research is rare.

In this paper, we have studied the influence of bisphenol A and phenol at different concentration and time on the activity of SOD and CAT in the zebra fish muscle tissue. It will provide a scientific basis on the research of toxicological and biochemical indicators by accumulating data for the research of aquatic toxicology.

EXPERIMENTAL SECTION

2.1 Experiment fish

The fresh zebra fish (*Danio rerio*) with body length about 30.25±3.12 mm was purchased from the Nanchang Qianhuaban flower market. 25 zebra fishes were added into pollutant water with no feeding in the experimental period. In the process, the photoperiod was 14 or 10 hour, the temperature of the water was 27±1 ^{o}C, the time of continuous aeration was 24 hours. The activities of the zebra fish in the various pollutants were recorded.

2.2 Experimental method

2.2.1 Toxicity test

The medial lethal concentration of zebra fish in phonel and bisphonel A was 0.160 mg/L and 6.300 mg/L respectively according to our early experiments. So the $1/5LC_{50}$, $1/10LC_{50}$, $1/20LC_{50}$ of phonel and bisphonel A concentration were 0.008 mg/L, 0.016 mg/L ,0.032 mg/L ,0.315 mg/L, 0.630 mg/L and 1.260 mg/L respectively.

2.2.2 The determination of superoxide dismutase (SOD) activity

Add some buffer solution and millliQ water into a tube, after suffered for 20minutes at 25 ℃, adding the 25 ℃ warmed pretreated pyrogallol and mixing them uniformity. Then pour them into the quartz color dish, and detect the light absorption value every 30 s at the wave number 325 nm, the A0 is the data within 4 minutes. Before adding the warmed pretreated pyrogallol, adding some superoxide dismutase protein extracting solution and MillliQ water for adjusting, to detect the light absorption value every 30 s at the wavenumber 325 nm, the data within 4 minutes is the A_{SOD}. It was showed in Table 1.

Table 1 Protocol of velocity determination of pyorgallol autoxidation

reagent	standard	A_0	A_{SOD}
0.1 mol/L Tris-HCl (mL)	1.5	1.5	1.5
millliQ water	1.5	1.4	1.3
Protein extraction solution(mL)	-	-	0.1
pyrogallol (mL)	-	0.1	0.1
total volum(mL)	3.0	3.0	3.0

2.2.3 The determination of catalase (CAT) activity

Add the buffer solution (2.0 mL), protein extraction solution (0.1 mL), 8% H_2SO_4 (0.75 mL) and H_2O_2 (0.15 mL) into the contrast tube respectively. Add the buffer solution (2.0mL), protein extraction solution (0.1 mL) and H_2O_2 (0.75 mL) into the sample tubes, after 4 minutes adding the 8% H_2SO_4 (0.75 mL) to terminate reaction. Then use the distilled water as zero reference to measure the sample tubes and contrast tube's absorbance at the wave number 240 nm.

2.3 Statistics and data processing

The formula of the SOD activity is list in Equation (1) below.

$$\text{Inhibition ratio} = \frac{v_1}{v_2}\left(\Delta A_0 - \Delta A_{SOD}\right)/\Delta A_0 \times 100\% \quad (1)$$

where v_1 refers to the volume of the adding sample (mL), v_2 refers to the total volume (mL), ΔA_0 refers to the oxidation rate of pyrogallol, A_{SOD} refers to the inhibition oxidation rate of pyrogallol.

The formula of the CAT activity is list in Equation (2) below.

$$E(\text{mg } H_2O_2 / \text{min}\cdot\text{g}) = \frac{a\left(A_k - A_s\right)+b}{W_t} \times \frac{10}{m} \quad (2)$$

where A_k refers to the contrast tube absorbance, A_S refers to the samples absorbance, W_t refers to the reaction time, m refers to the mass of sample solution, a,b refer to the constant of the equation of linear regression.

RESULTS AND DISCUSSION

3.1 1/20 LC_{50} phenol and bisphenol A exposure

The sample mass and the protein concentration of the sample in $1/20LC_{50}$ phenol and Bisphenol A exposure were shown in Table 2.

Table 2 Concentration and mass of the sample in 1/20 LC_{50} phenol and bisphenol A exposure

Sample		0.008 mg/L phenol		0.315 mg/L bisphenol A	
		Sample Quality（g）	Sample concentration（mg/mL）	Sample Quality（g）	Sample concentration（mg/mL）
1d	1	0.254	1.090	0.206	1.039
	2	0.350	1.021	0.321	1.035
	3	0.475	1.084	0.412	1.045
7d	1	0.327	1.034	0.373	1.037
	2	0.303	1.068	0.311	1.028
	3	0.290	1.026	0.437	1.002
14d	1	0.348	1.053	0.219	1.015
	2	0.394	1.041	0.374	1.085
	3	0.356	1.014	0.284	1.015

The SOD activity was measured according to the absorbance of sample in 1/20 LC_{50} phenol and bisphenol A exposure at 325nm and the results were shown in Table 3.

Table 3 Absorbance of sample at 325 nm in 1/20 LC_{50} phenol and bisphenol A exposure

Sample		phenol				bisphenol A			
		30s	240s	ΔA	ΔA0-ΔASOD	30s	240s	ΔA	ΔA0-ΔASOD
1d		0.700	2.350	1.650		0.700	2.350	1.650	
	1	0.723	2.227	1.504	0.146	0.661	2.205	1.544	0.106
	2	0.732	2.231	1.499	0.151	0.637	2.181	1.544	0.106
	3	0.725	2.199	1.474	0.176	0.706	2.241	1.535	0.115
7d		0.984	2.936	1.952		0.984	2.936	1.952	
	1	0.954	2.763	1.809	0.143	0.718	2.567	1.849	0.103
	2	0.886	2.704	1.818	0.134	0.744	2.586	1.842	0.110
	3	0.684	2.510	1.826	0.126	0.684	2.521	1.837	0.115
14d		0.832	2.594	1.762		0.832	2.594	1.762	
	1	0.673	2.323	1.650	0.112	0.653	2.307	1.654	0.108
	2	0.791	2.422	1.631	0.131	0.731	2.376	1.645	0.117
	3	0.853	2.486	1.633	0.129	0.774	2.432	1.658	0.104

The measurement results were substituted into the formula (1) and SOD inhibition rates in 1/20 LC_{50} phenol and bisphenol A exposure were shown in Table 4.

Table 4 Inhibition rates of SOD in 1/20 LC_{50} phenol and Bisphenol A exposure

Sample	0.008 mg/L phenol	0.315 mg/L bisphenol A
1d	0.090 ± 0.008	0.064 ± 0.005
7d	0.066 ± 0.004	0.055 ± 0.004,
14d	0.062 ± 0.006	0.057 ± 0.006

As shown in Table 4, the SOD activity of zebra fish at 0.008 mg/L phenol was stimulated to enhance with time. As for adopt to the stimulation of pollutants to zebra fish, SOD activity in the body gradually restored. Mainly SOD was strongly suppressed by the contaminants in the initial stimulation, but over time, the increased in free radicals, SOD played the role of decomposition and its activity gradually increased. Combined with the knowledge of hormesis, we can speculate that in such lower concentrations stimulation of organic compounds, SOD activity might promote. The SOD activity of zebra fish at 0.315 mg/L bisphenol A was stimulated to enhance with time and formed β-type curve. The zebra fish superoxide dismutase enzyme stimulated by pollutants, enhance the activity first, gradually adapted to the stimulate pollutants over time, the activity will drop. The main reason was that zebra fish body exposed to the stimulate pollutants produced more reactive free radicals in the initial stimulation, SOD degradation result in the increased activity, so late as to reduce the free radicals , SOD activity reduced.

The CAT activity was measured according to the absorbance of sample at 240 nm in 1/20 LC_{50} phenol and bisphenol A exposure and the results were shown in Table 5.

Table 5 Absorbance of sample at 240 nm in 1/20 LC_{50} phenol and bisphenol A exposure

Sample		phenol			bisphenol A		
		Ak	As	Ak-As	Ak	As	Ak-As
1d	1	1.046	1.017	0.029	1.063	1.037	0.026
	2	1.054	1.019	0.035	1.067	1.032	0.035
	3	1.060	1.028	0.032	1.088	1.057	0.031
7d	1	1.088	1.029	0.059	1.047	1.033	0.014
	2	1.041	0.977	0.064	1.075	1.061	0.014
	3	1.050	0.987	0.063	1.049	1.032	0.017
14d	1	1.091	1.012	0.079	1.094	1.062	0.032
	2	1.089	1.007	0.082	1.090	1.054	0.028
	3	1.069	0.983	0.084	1.071	1.048	0.023

The measurement results were substituted into the formula (2) and CAT activity in 1/20 LC_{50} phenol and bisphenol A exposure were shown in Table 6.

Table 6 Activity of CAT in 1/20 LC_{50} phenol and Bisphenol A exposure

Sample	0.008 mg/L phenol	0.315 mg/L bisphenol A
1 d	0.410±0.036mgH_20_2/min·g	0.323±0.028mgH_20_2/min·g
7 d	0.649±0.058mgH_20_2/min·g	0.454±0.027mgH_20_2/min·g
14 d	0.706±0.043mgH_20_2/min·g	0.497±0.036mgH_20_2/min·g

As shown in Table 6, the CAT activity of zebra fish at0.008 mg/L Phenol was stimulated to enhance with time. The main reason was that H_2O_2 generated by products also increased, with the enhancement of the activity of CAT, CAT will gradually begin to break down it into harmless products, so CAT activity enhanced gradually. Combined with the knowledge of hormesis, we can speculate that in such lower concentrations stimulation of organic compounds, CAT activity might promote. CAT activity in vivo zebra fish under stimulation of 0.315 mg/L bisphenol A enhanced with the growth over time, the main reason was identical as 1/20 LC_{50} of bisphenol A .

3.2 1/10 LC_{50} phenol and bisphenol A exposure

The sample mass and the protein concentration of the sample in 1/10LC_{50} phenol and Bisphenol A exposure were shown in Table 7.

Table 7 Concentration and mass of the sample in 1/10 LC_{50} phenol and bisphenol A exposure

Sample		0.016 mg/L phenol		0.63 mg/L bisphenol A	
		Sample Quality（g）	Sample concentration（mg/mL）	Sample Quality（g）	Sample concentration（mg/mL）
1 d	1	0.463	1.083	0.368	1.047
	2	0.339	1.091	0.256	1.066
	3	0.585	1.066	0.398	1.065
7 d	1	0.536	1.046	0.439	1.027
	2	0.308	1.008	0.356	1.019
	3	0.298	1.064	0.313	1.051
14 d	1	0.251	1.088	0.201	1.026
	2	0.236	1.037	0.299	1.087
	3	0.173	1.032	0.234	1.004

The SOD activity was measured according to the absorbance of sample in 1/10 LC_{50} phenol and bisphenol A exposure at 325nm and the results were shown in Table 8.

Table 8 Absorbance of sample at 325 nm in 1/10 LC_{50} phenol and bisphenol A exposure

Sample		phenol				bisphenol A			
		30s	240s	ΔA	$\Delta A_0-\Delta A_{SOD}$	30s	240s	ΔA	$\Delta A_0-\Delta A_{SOD}$
1 d		0.700	2.350	1.650		0.700	2.350	1.650	
	1	0.797	2.288	1.491	0.159	0.591	2.176	1.585	0.065
	2	0.731	2.211	1.480	0.170	0.623	2.197	1.574	0.076
	3	0.764	2.243	1.479	0.171	0.642	2.221	1.579	0.071
7 d		0.984	2.936	1.952		0.984	2.936	1.952	
	1	0.803	2.631	1.828	0.124	0.777	2.595	1.818	0.134
	2	0.758	2.595	1.837	0.115	0.970	2.772	1.802	0.150
	3	0.831	2.676	1.845	0.107	0.707	2.523	1.816	0.136
14 d		0.832	2.594	1.762		0.832	2.594	1.762	
	1	0.823	2.478	1.653	0.109	0.804	2.501	1.669	0.093
	2	0.807	2.466	1.659	0.103	0.781	2.436	1.655	0.107
	3	0.796	2.432	1.636	0.126	0.742	2.401	1.659	0.103

The measurement results were substituted into the formula (1) and SOD inhibition rates in 1/10 LC_{50} phenol and bisphenol A exposure were shown in Table 9.

Table 9 Inhibition rates of SOD in 1/10 LC_{50} phenol and bisphenol A exposure

Sample	0.016 mg/L phenol	0.630 mg/L bisphenol A
1d	0.094±0.005	0.041±0.003
7d	0.057±0.005	0.070±0.006
14d	0.049±0.004	0.057±0.006

Table 9 were showed that the activity of zebra fish SOD at 0.016 mg/L of phenol was stimulated to grow with time and enhance. Activity trend was the same as the trend under the stimulus at 0.008 mg/L of phenol, but a higher rate. The main reason was identical as 1/20 LC_{50} of phenol. The SOD activity of zebra fish in the stimulation of 0.63 mg/L bisphenol A increased with time and formed a U-shaped curve, which was because zebra fish SOD was stimulated by contamination, firstly activity decreased with time, then zebra fish gradually adapted to the stimulation of contaminants, the enzyme activity would rise. The main reason was the growth of bisphenol A in the early exposure experiments, zebra fish SOD enzyme was inhibited, which resulted to an increase in the activity of free radicals in their body, to the late, SOD activity began to degrade these free radicals, so it performed an increased in activity .

The CAT activity was measured according to the absorbance of sample at 240 nm in 1/10 LC_{50} phenol and bisphenol A exposure and the results were shown in Table 10.

Table10 Absorbance of sample at 240 nm in 1/10 LC_{50} phenol and bisphenol A exposure

Sample		phenol			bisphenol A		
		A_k	A_s	A_k-A_s	A_k	A_s	A_k-A_s
1d	1	1.062	1.039	0.023	1.059	1.041	0.018
	2	1.061	1.039	0.022	1.089	1.070	0.019
	3	1.066	1.037	0.029	1.066	1.048	0.018
7d	1	1.046	0.990	0.056	1.067	1.041	0.026
	2	1.046	0.994	0.052	1.066	1.044	0.022
	3	1.049	1.003	0.046	1.096	1.064	0.032
14d	1	1.082	1.021	0.061	1.076	1.044	0.032
	2	1.095	1.027	0.068	1.099	1.063	0.036
	3	1.060	0.989	0.071	1.078	1.049	0.029

The measurement results were substituted into the formula (2) and CAT activity in 1/10 LC_{50} phenol and bisphenol A exposure were shown in Table 11.

Table11 Activity of CAT in 1/10 LC_{50} phenol and bisphenol A exposure

Sample	0.016 mg/L phenol	0.630 mg/L bisphenol A
1d	0.350±0.023mgH_2O_2/min·g	0.308±0.027 mgH_2O_2/min·g
7d	0.569±0.0431mgH_2O_2/min·g	0.381±0.031mgH_2O_2/min·g
14d	0.614±0.045mgH_2O_2/min·g	0.401±0.037mgH_2O_2/min·g

Table 11 were showed that the CAT activity of zebra fish at 0.016 mg/L of phenol was stimulated to grow with time

and enhance. Activity trend was the same as the trend under the stimulus at 0.008 mg/L of phenol, but a higher rate. The main reason was identical as 1/20 LC_{50} of phenol. The CAT activity of zebra fish at 0.63 mg/L bisphenol A was stimulated to grow with time and enhance. Activity trend was the same as the trend under the stimulus at 0.008 mg/L of phenol. The main reason was identical as 1/20 LC_{50} of bisphenol A.

3.3 1/5 LC_{50} phenol and bisphenol A exposure

The sample mass and the protein concentration of the sample in 1/5 LC_{50} phenol and bisphenol A exposure were shown in Table 12.

Table12 Concentration and mass of the sample in 1/5 LC_{50} phenol and bisphenol A exposure

Sample		0.032 mg/L phenol		1.260 mg/L bisphenol A	
		Sample Quality（g）	Sample concentration(mg/mIample Quality		Sample concentration(mg/mL
1d	1	0.301	1.044	0.326	1.000
	2	0.343	1.011	0.302	1.032
	3	0.184	1.004	0.342	1.064
7d	1	0.300	1.026	0.480	1.081
	2	0.326	1.010	0.413	1.034
	3	0.294	1.071	0.321	1.021
14d	1	0.366	1.005	0.200	1.002
	2	0.295	1.087	0.189	1.045
	3	0.249	1.061	0.204	1.033

The SOD activity was measured according to the absorbance of sample in 1/5 LC_{50} phenol and bisphenol A exposure at 325 nm and the results were shown in Table 13.

Table 13 Absorbance of sample at 325 nm in 1/5 LC_{50} phenol and bisphenol A exposure

Sample		phenol				bisphenol A			
		30s	240s	ΔA	ΔA_0-ΔA_{SOD}	30s	240s	ΔA	ΔA_0-ΔA_{SOD}
1d		0.700	2.350	1.650		0.700	2.350	1.650	
	1	0.770	2.212	1.442	0.208	0.683	2.205	1.522	0.128
	2	0.780	2.256	1.476	0.174	0.751	2.269	1.518	0.132
	3	0.818	2.263	1.445	0.205	0.738	2.266	1.528	0.122
7d		0.984	2.936	1.952		0.984	2.936	1.952	
	1	0.837	2.703	1.866	0.086	0.682	2.501	1.819	0.133
	2	0.690	2.543	1.853	0.099	0.608	2.432	1.824	0.128
	3	0.746	2.598	1.852	0.100	0.687	2.52	1.833	0.119
14d		0.832	2.594	1.762		0.832	2.594	1.762	
	1	0.797	2.462	1.665	0.097	0.882	2.546	1.664	0.098
	2	0.814	2.489	1.675	0.087	0.931	2.581	1.650	0.112
	3	0.803	2.469	1.666	0.096	0.787	2.442	1.655	0.107

The measurement results were substituted into the formula (1) and SOD inhibition rates in 1/5 LC_{50} phenol and bisphenol A exposure were shown in Table14.

Table 14 Inhibition rates of SOD in 1/5 LC_{50} phenol and bisphenol A exposure

Sample	0.032 mg/L phenol	1.260 mg/L bisphenol A
1d	0.116±0.01	0.075±0.008
7d	0.045±0.003	0.062±0.006
14d	0.042±0.004	0.047±0.003

Table 14 was showed that the SOD activity of zebra fish in the stimulation of 0.032 mg/L phenol increased with time and formed a U-shaped curve. Enzyme activity trend was the same as the trend under the same stimulus of 0.016 mg/L, 0.008 mg/L of phenol, but a higher rate and an early greater rate of change. The main factors of early period in SOD activity might be hormesis theory, the latter was mainly produced a certain amount of free radicals in vivo activity, which is harmful substances, SOD played an important role in its degradatio. SOD activity in vivo zebra fish at 1.26 mg/L of Bisphenol A was stimulated to grow with time and enhance. As for zebra fish adapted to stimulate of pollutation, superoxide dismutase activity gradually restored. The main reason was the same as the condition of 1/20 LC_{50} bisphenol A.

The CAT activity was measured according to the absorbance of sample at 240 nm in 1/5 LC_{50} phenol and bisphenol A exposure and the results were shown in Table 15.

Table15 Absorbance of sample at 240 nm in 1/5 LC_{50} phenol and bisphenol A exposure

Sample		phenol			bisphenol A		
		A_k	A_s	A_k-A_s	A_k	A_s	A_k-A_s
1d	1	1.056	1.039	0.017	1.047	1.029	0.018
	2	1.061	1.039	0.022	1.082	1.056	0.026
	3	1.070	1.053	0.017	1.083	1.056	0.027
7d	1	1.036	1.005	0.031	1.058	1.007	0.051
	2	1.031	0.995	0.036	1.062	1.009	0.053
	3	1.042	1.000	0.042	1.065	1.024	0.041
14d	1	1.088	1.050	0.038	1.081	1.024	0.057
	2	1.041	0.997	0.044	1.076	1.013	0.063
	3	1.079	1.032	0.047	1.083	1.019	0.064

The measurement results were substituted into the formula (2) and CAT activity in 1/5 LC_{50} phenol and bisphenol A exposure were shown in Table 16.

Table16 Activity of CAT in 1/5 LC_{50} phenol and bisphenol A exposure

Sample	0.032 mg/L phenol	1.260 mg/L bisphenol A
1d	0.323±0.028mgH_20_2/min·g	0.357±0.039mgH_20_2/min·g
7d	0.454±0.027mgH_2O_2/min·g	0.542±0.044mgH_2O_2/min·g
14d	0.497±0.036 mgH_2O_2/min·g	0.576±0.051 mgH_2O_2/min·g

Table 16 was showed that the CAT activity of zebra fish at t 0.032 mg/L of phenol was stimulated to grow with time and enhance. Activity trend was the same as the trend under the stimulus at 0.008 mg/L and 0.016 mg/L of phenol, but both were lower than the prior rate. Due to the high SOD activity, resulted in a large amount of H_2O_2, which affected the latter CAT activity causing a reduction of change rate. The CAT activity of zebra fish at 1.26 mg/L of bisphenol A was stimulated to grow with time and enhance. Activity trend was the same as the trend under the stimulus at 0.008 mg/L of phenol, the main reason was identical as 1/20 LC_{50} of bisphenol A.

CONCLUSION

Exposure experiment on zebra fish had been done by using 1/20, 1/10, 1/5 organic solvents (phenol and PBA) of median lethal concentration in this paper. It separately measured SOD and CAT activity of 1 d, 7 d and 14 d with the changed trend of concentration and time in zebra fish body. In the water which contains phenol, the activity of SOD and CAT enhanced with the increasing of time and concentration. The trend of change was also increasing. Our analysis in the water containing phenol showed that the active freedom group in zebra fish will be degraded by SOD and prompt the activity of SOD enhancing with increasing of time. Similarly, H_2O_2 which was SOD byproduct of degradation will be degraded into harmless substances by CAT. So the activity of CAT showed the trend of increasing with the time. Phenol played a role in promoting the activity of SOD and CAT under the concentration of experiment.In the water containing PBA, the trend of SOD activity was raised at first, and then decreased with the increasing of concentration and time. The activity of CAT enhances with the increasing of time and concentration and the changed trend was decreasing. Our analysis in the water containing PBA showed that SOD is subject to a certain inhibition in the short term. However, as time went on, the trend raised at first, then decreased because that SOD began to degrade active freedom groups. When SOD was suppressed in the early and the content of H_2O_2 in appropriate range the activity of CAT is higher; as time went on, the degradation of the CAT was the strongest and activity was the highest with content of H_2O_2 increasing. But as the H_2O_2 content increasing, organism ability of zebra fish were affected and the growth trend of CAT activity was weakened

In conclusion, SOD and CAT which own certain dose relationship can be used as biological indicator of organic pollution in the water that was exposure of organic pollutants.

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