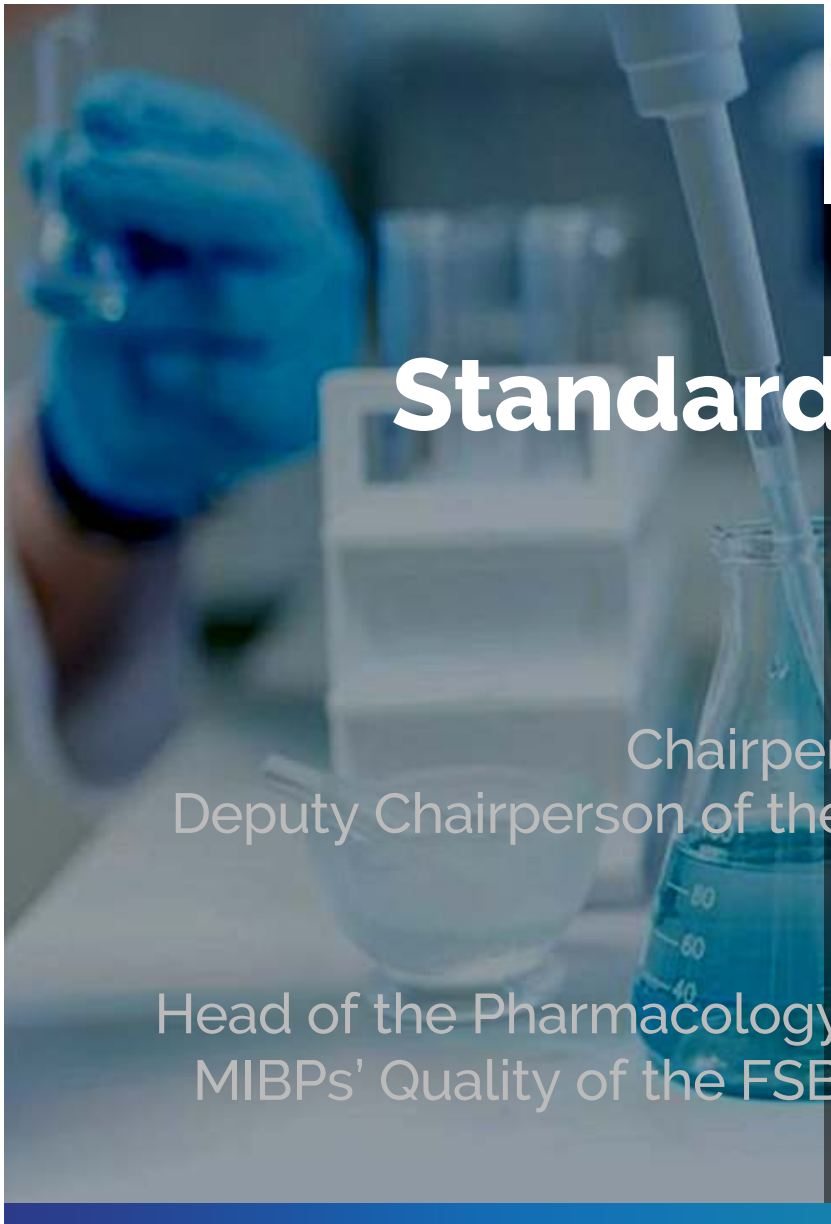

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Abnormal Toxicity. Standardisation and Quality Control of Medicinal Products in the Russian Federation

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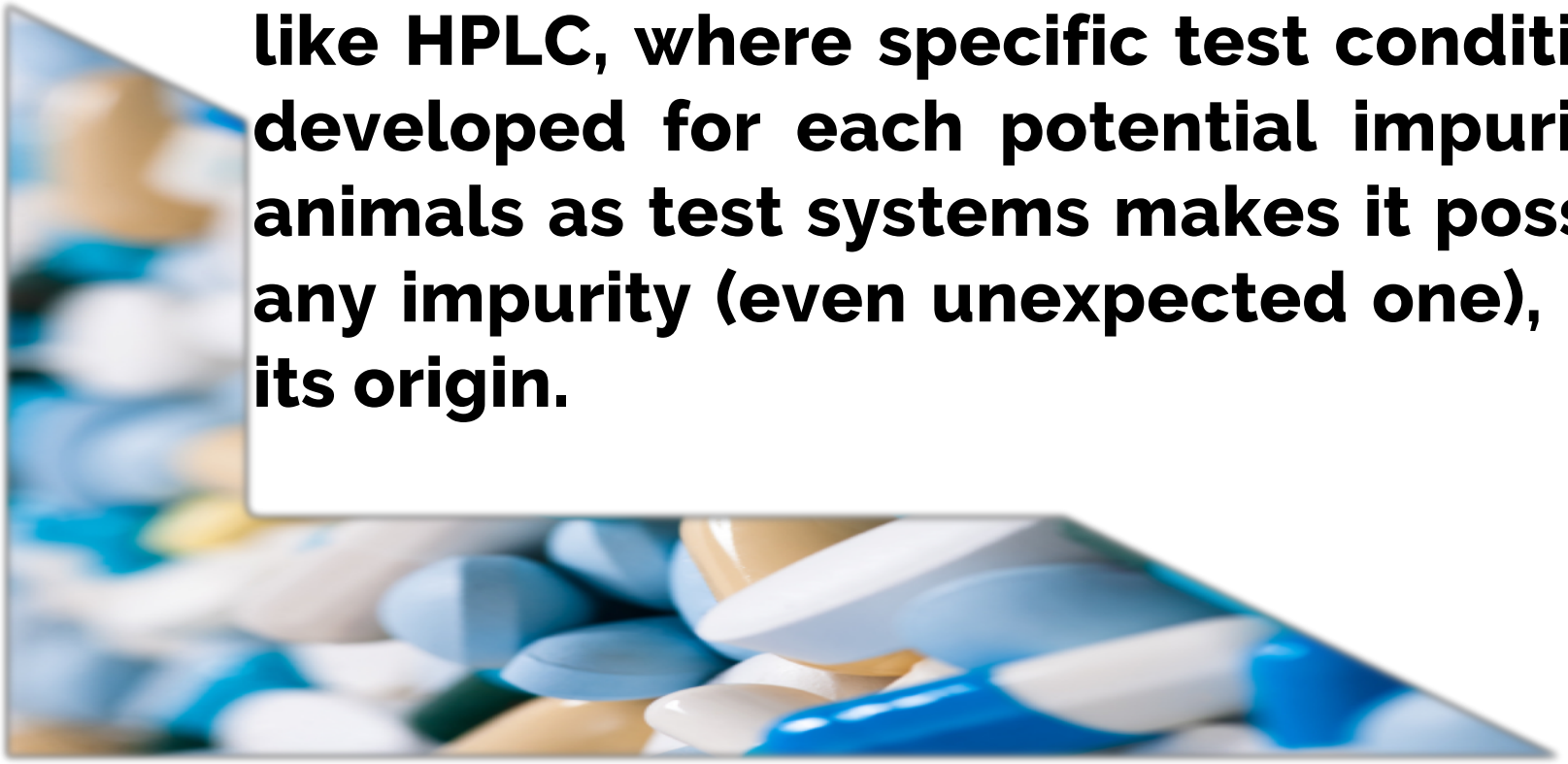
The Abnormal toxicity test is a multipurpose method of detecting unwanted toxic impurities. Unlike highly specific physico-chemical methods, like HPLC, where specific test conditions should be developed for each potential impurity, the use of animals as test systems makes it possible to detect any impurity (even unexpected one), irrespective of its origin.



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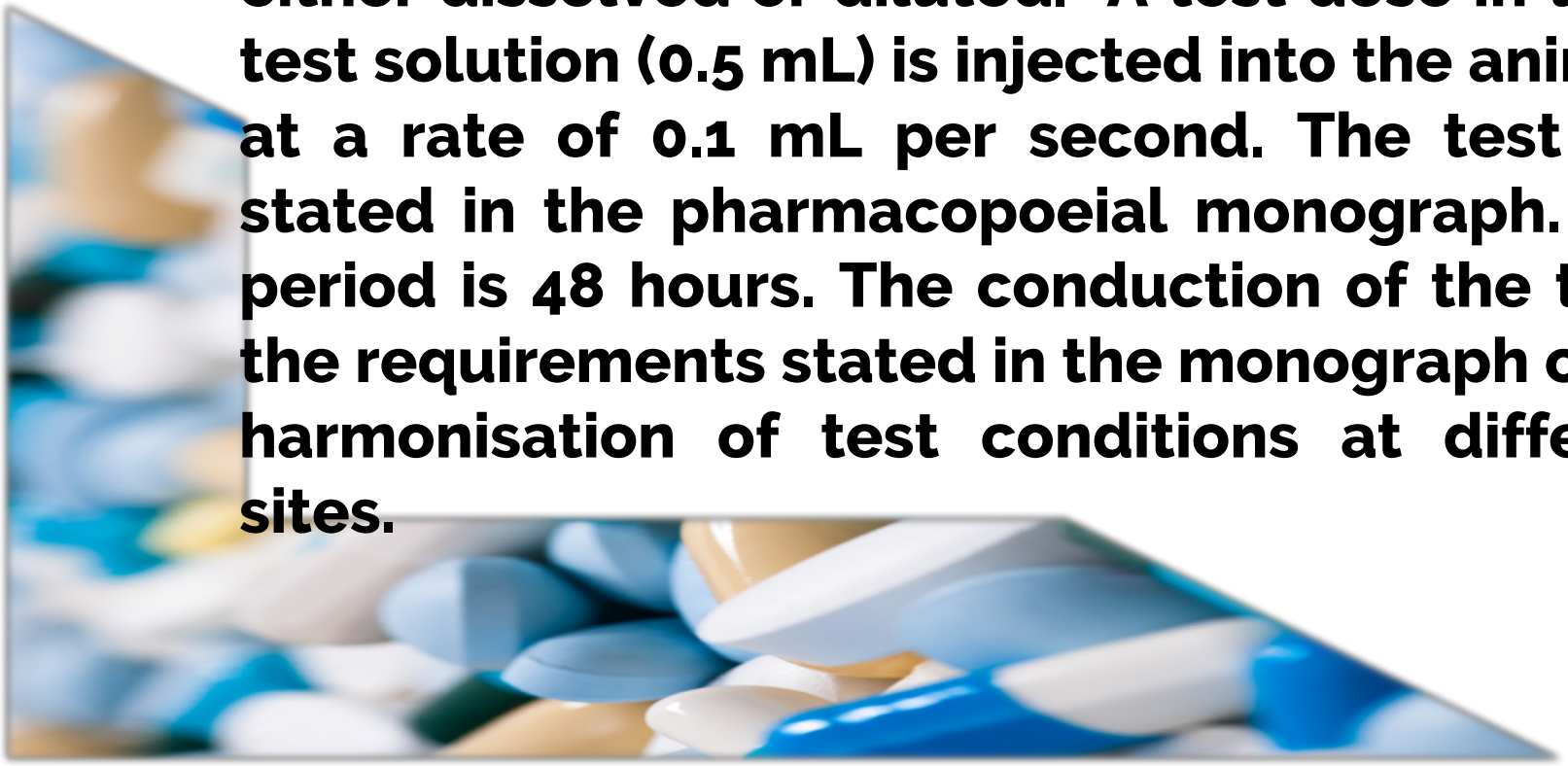
The test is carried out on 5 healthy white mice of both genders, with a body weight of 19 to 21 g, that haven't been used previously in experiments. The tested medicine is either dissolved or diluted. A test dose in the portion of the test solution (0.5 mL) is injected into the animal's caudal vein at a rate of 0.1 mL per second. The test dose should be stated in the pharmacopoeial monograph. The observation period is 48 hours. The conduction of the test according to the requirements stated in the monograph contributes to the harmonisation of test conditions at different production sites.



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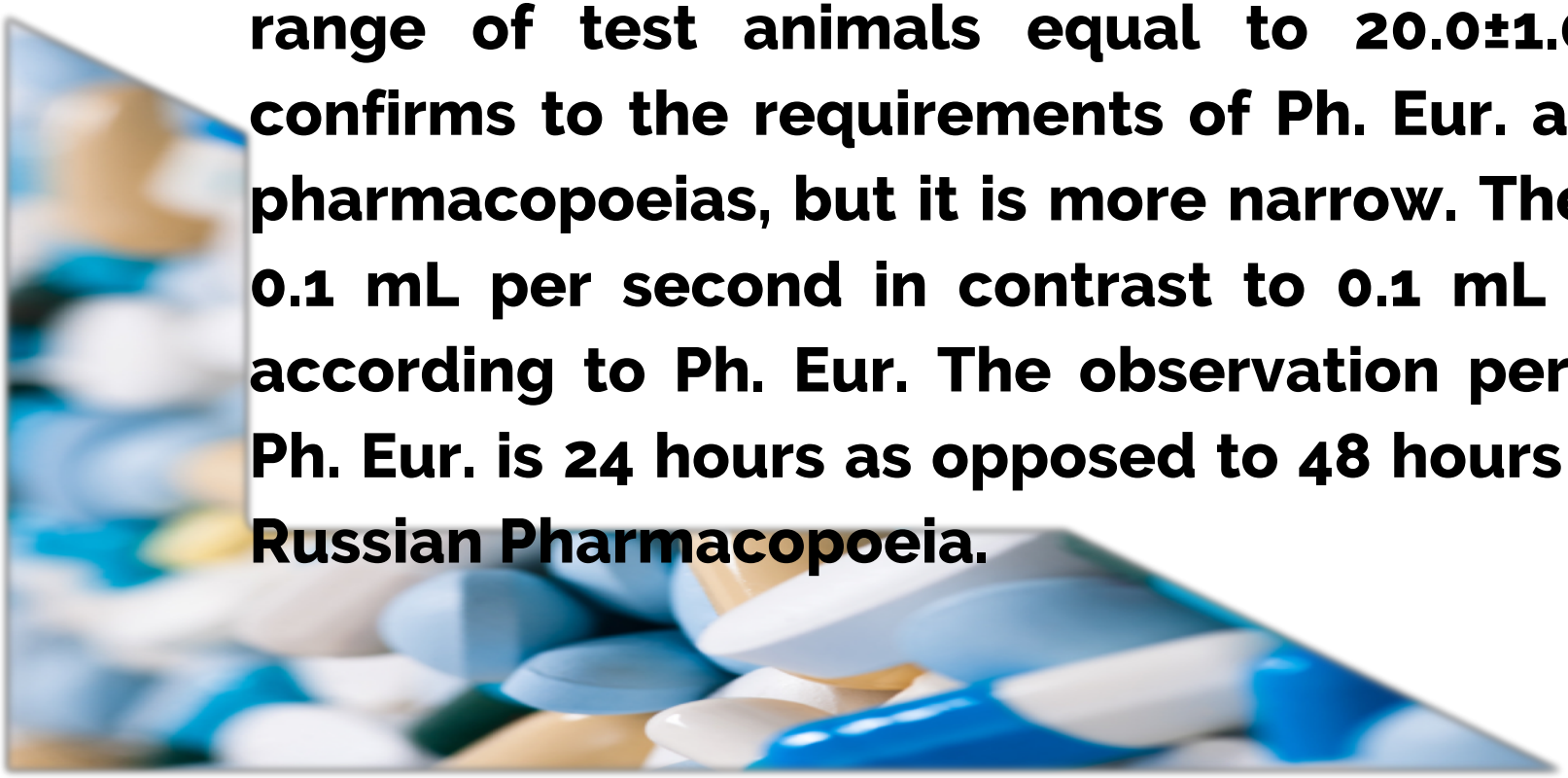
In order to obtain more accurate test results the "Abnormal toxicity" general monograph of the Russian Pharmacopoeia, XIV edition, stipulates the body weight range of test animals equal to 20.0±1.0 g. This range confirms to the requirements of Ph. Eur. and other leading pharmacopoeias, but it is more narrow. The injection rate is 0.1 mL per second in contrast to 0.1 mL per 3-6 seconds according to Ph. Eur. The observation period according to Ph. Eur. is 24 hours as opposed to 48 hours according to the Russian Pharmacopoeia.



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The test dose is a critical parameter in the evaluation of the Abnormal Toxicity Test. The optimal dose lies within the range between the maximum tolerated dose (MTD) and the lethal dose LD_{10}, where the MTD is the maximum dose of a medicinal product that does not cause the death of an animal after injection but may lead to intoxication, and the LD_{10} is the dose, injection of which leads to the death of 10 % of animals.

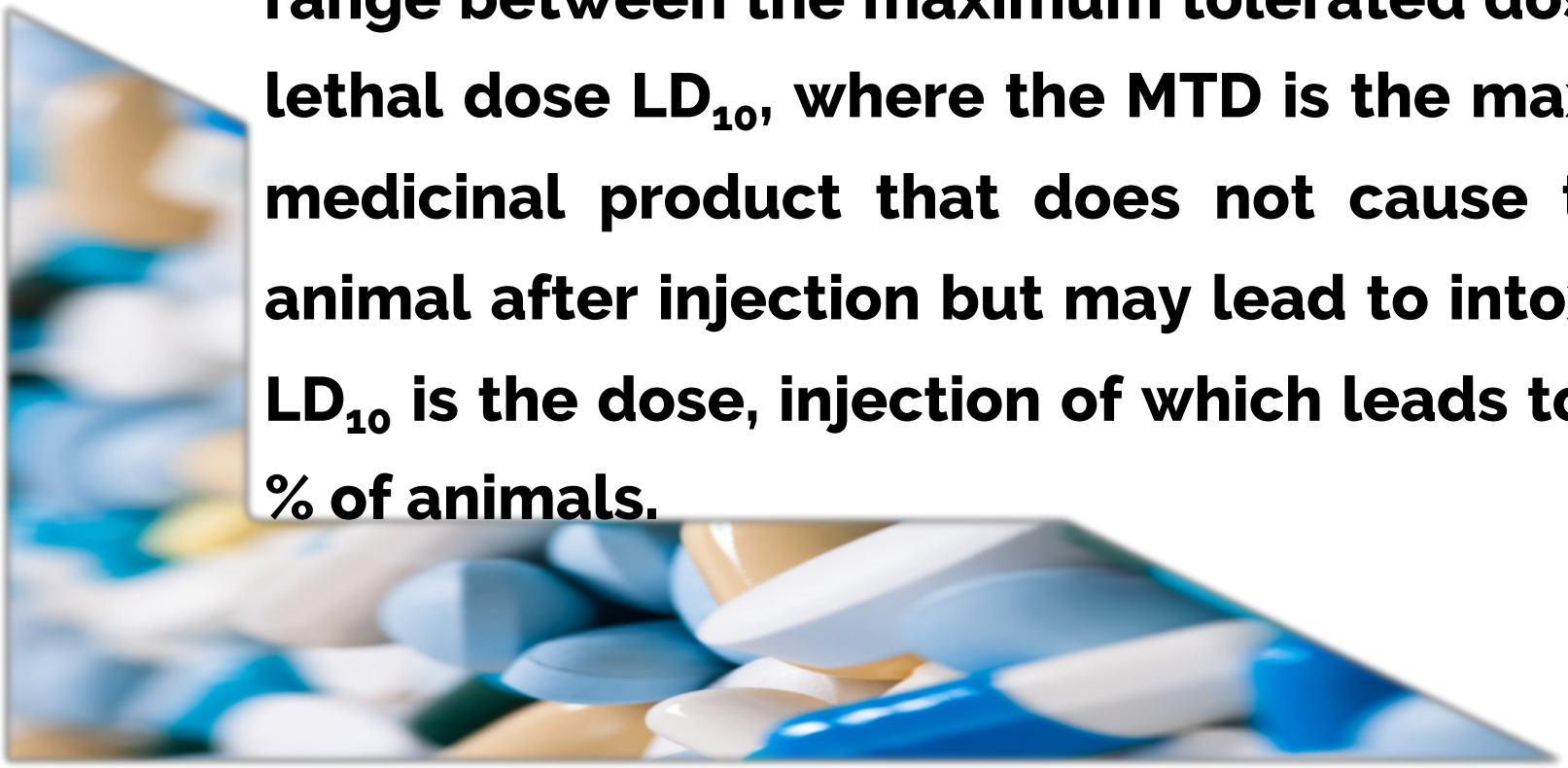

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Table 1
Comparative analysis of experimental conditions of the Abnormal Toxicity Test in the leading pharmacopoeias

Conditions and requirements	Pharmacopoeias					
	USP 41		Ph. Eur. IX ed.	International Pharm., 4 ed.,2018	Russian Pharm., XIV ed.	Pharmacopoeia of EEU, draft monograph
	Safety Test	Acute Toxicity	Abnormal Toxicity	Undue toxicity	Abnormal Toxicity	
Animal species	Mice		Mice	Mice	Mice	Mice
Number of animals (1 injection)	5	5	5	5	5	5
Weight (g)	17-23	Monographs	17-22	18-22	19-21	19-21
Administration	intravenous	intravenous	intravenous	intravenous	intravenous	intravenous
Injection volume	0.5 mL	Monographs	0.5 mL	0.5 mL	0.5 mL	0.5 mL
Injection rate/duration	100 µL/s 5 s	100 µL/s 5 s	0.1 mL/3-6 s 15-30 s	0.1 mL/s 5 s	0.1 mL/s 5 s	0.1 mL/s 5 s
Observation period	48 hours	48 hours	24 hours	48 hours	48 hours	48 hours
Defect (death)	2 mice	2 mice	2 mice	2 mice	2 mice	2 mice

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The testing of ceftriaxone substance, produced by five independent manufacturers, demonstrates the critical role of the injection rate in the Abnormal Toxicity Test (see Table 2). Intravenous administration of samples 1 and 2 at 30 mg per mouse at a rate of 0.1 mL/s did not lead to intoxication or death of any animal during the whole observation period.

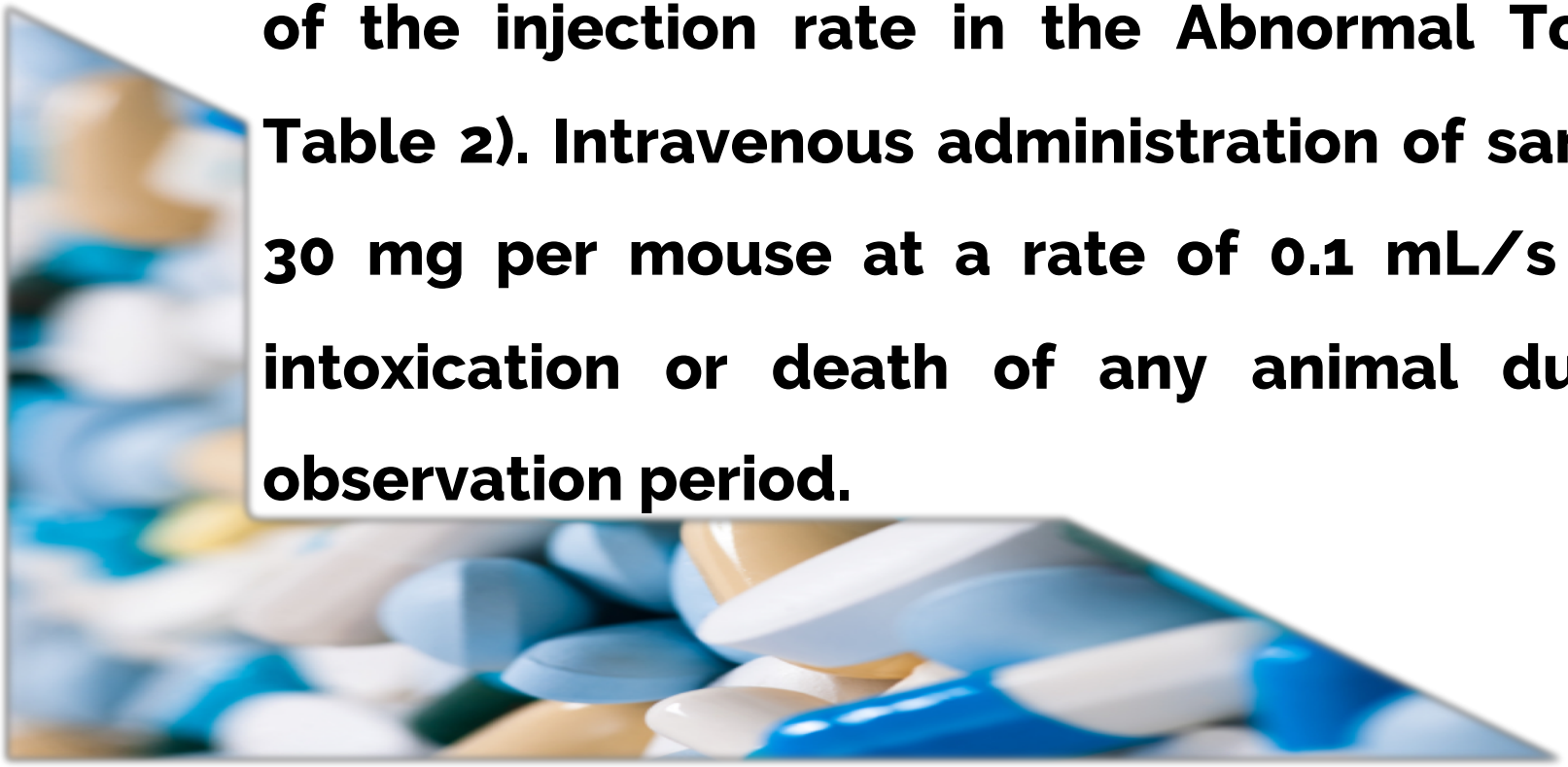

Table 2

Results of the Abnormal Toxicity Test of Ceftriaxone samples at 30 mg per mouse at different injection rates

Manufacturer	1	2	3	4	5
Injection rate/ (duration)	Death				
0.1 mL/s (5 s)	0/5	0/5	3/10	1/10	2/10
0.1 mL/3 s (15 s)	0/5	0/5	0/5	0/5	0/5
0.1 mL/6 s (30 s)	0/5	0/5	0/5	0/5	0/5

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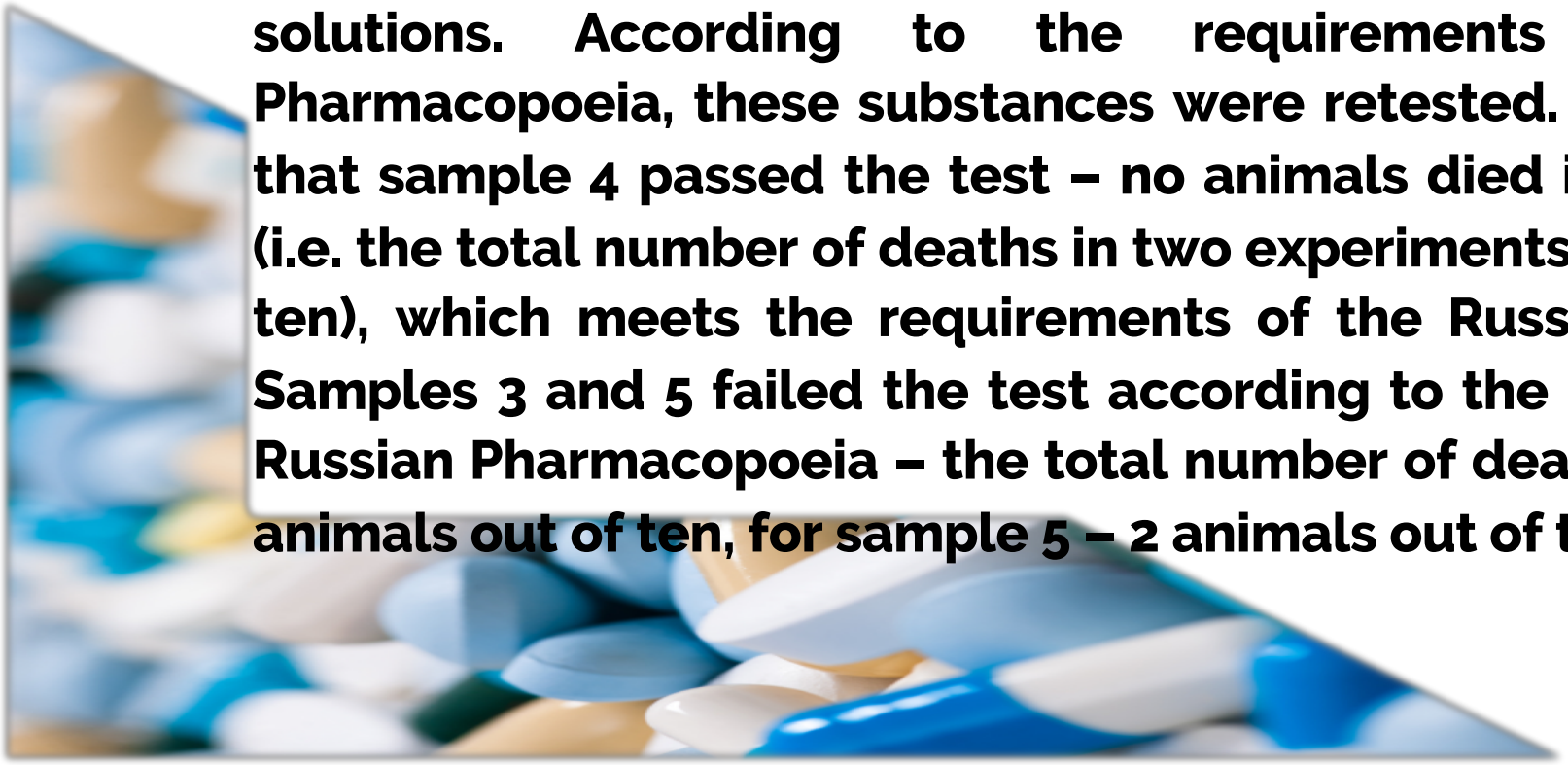
However, administration of the same dose of samples 3, 4 and 5 at the same injection rate caused the symptoms of intoxication, such as respiratory disturbance and dystaxia, tonic-clonic muscle activity. One animal in each group died 5 minutes after the injection of test solutions. According to the requirements of the Russian Pharmacopoeia, these substances were retested. The re-test showed that sample 4 passed the test – no animals died in the second group (i.e. the total number of deaths in two experiments is one animal out of ten), which meets the requirements of the Russian Pharmacopoeia. Samples 3 and 5 failed the test according to the requirements of the Russian Pharmacopoeia – the total number of deaths for sample 3 is 3 animals out of ten, for sample 5 – 2 animals out of ten.



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Acceptable tolerance without any intoxication symptoms or deaths was observed for all samples at 30 mg per mouse injected at a rate of 0.1 mL per 3 s or 6 s.

The increase of the test-dose to LD_{50} (50 mg per mouse) administered intravenously led to the aggravation of intoxication symptoms and the increase in the number of deaths. None of the samples passed the test.

The results of injection of substance samples at a test-dose of 50 mg per mouse at a rate of 0.1 mL per 3 s are comparable with the response to the test-dose of 30 mg per mouse injected at a rate of 0.1 mL per 1 s. In both cases only three substances pass the test (see Table 3). Therefore, when the testing is conducted according to the Ph. Eur., the increase of injection duration should lead to the increase of the test-dose – to 50 mg per mouse.

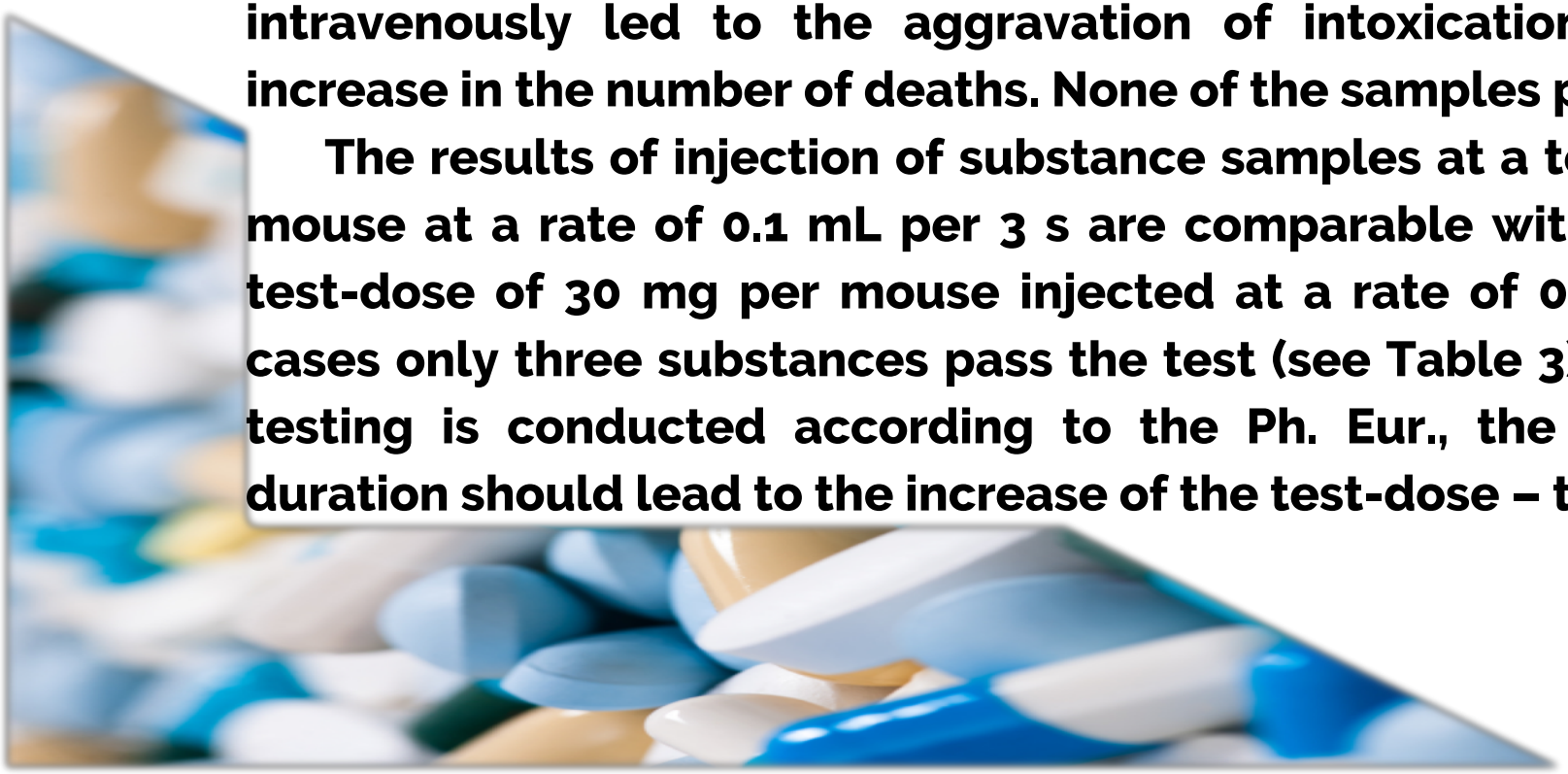

Table 3

Results of Abnormal Toxicity Test of substances produced by foreign manufacturers

Manufacturer	1	2	3	4	5
	Dose of 50 mg per mouse				
Injection rate	Number of deaths				
0.1 mL/s	2/10	3/10	7/10	5/10	9/10
0.1 mL/3 s	0/5	0/5	2/10	0/5	5/10
0.1 mL/6 s	0/5	0/5	0/5	0/5	1/10

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The acute toxicity test of Cefepim antibiotic demonstrates the effect of the injection rate on the toxicity value. Table 4 shows that, depending on the rate of injection of test samples, the intravenous administration of Cefepim at the dose range from 20 to 55 mg per mouse leads to toxic reactions of different intensity followed by the death of animals. After the injection of a dose of 35 mg per mouse during 5 s 83 % of animals in the experiment die. After the injection of the same dose during 30 s all the mice in the experiment survive.

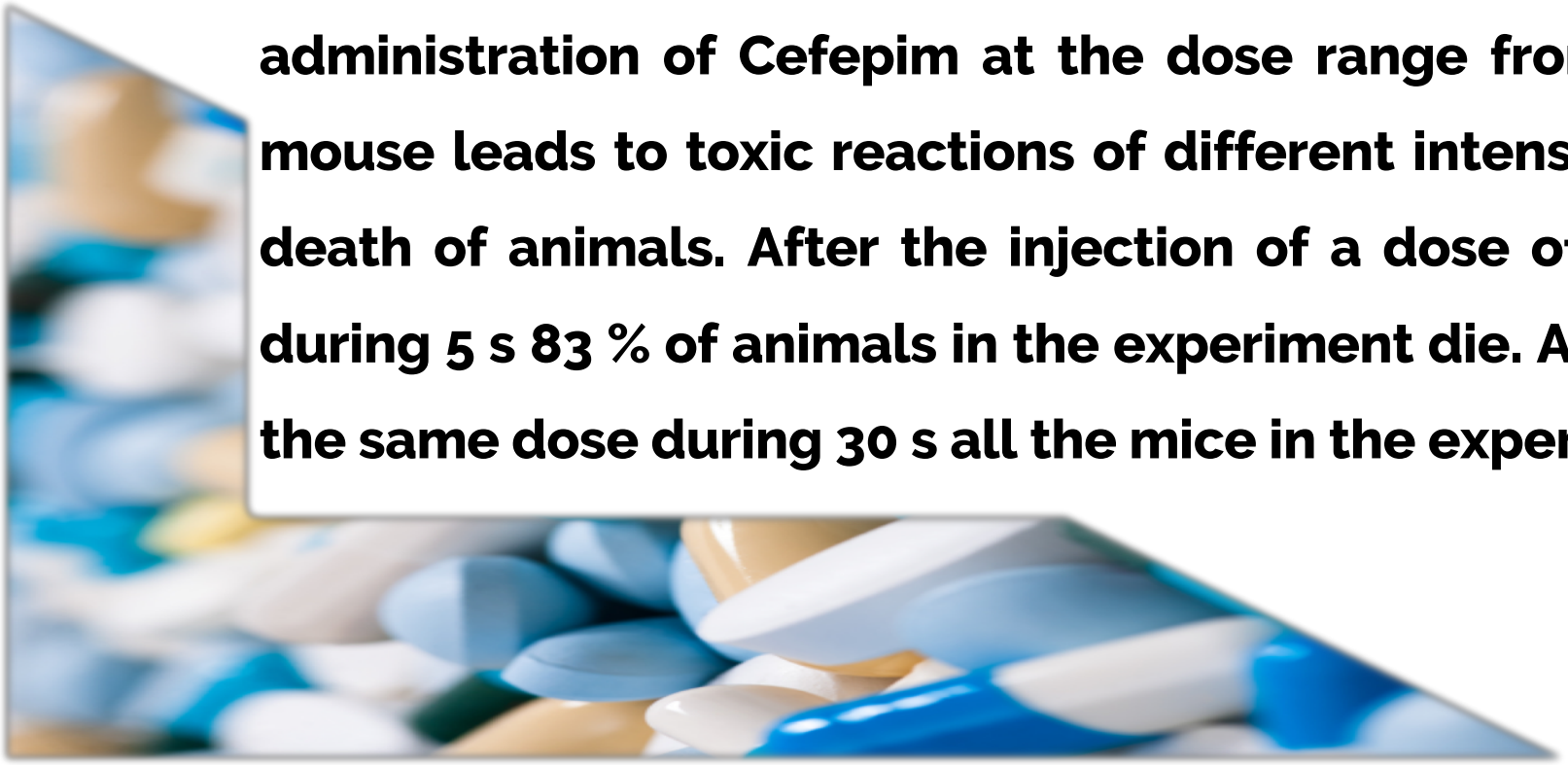

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Table 4

Acute toxicity values of Cefepim depending on the injection rate of test samples

Experiment No.	*Death rate* of white mice after intravenous injection of sample doses (mg per mouse):*								Calculated values *(mg per mouse)*
	20	*25*	*30*	*35*	*40*	*45*	*50*	*55*	
Injection rate of test sample 0.5 mL per 5 s									
1	*0/5*	*0/5*	*2/5*	*4/5*	*5/5*	*5/5*	*-*	*-*	LD_{10}= 27,5 LD_{16} = 28,9 LD_{84} = 39,3 LD_{50} = 34,2 (31,9÷36,4)
2	*0/5*	*0/5*	*1/5*	*2/5*	*4/5*	*5/5*	*-*	*-*	
3	*0/5*	*0/5*	*1/5*	*2/5*	*4/5*	*5/5*	*-*	*-*	
Total	*0/15*	*0/15*	*4/15*	*8/15*	*13/15*	*15/15*	*-*	*-*	
Injection rate of test sample 0.5 mL per 30 s									
1	*-*	*-*	*0/5*	*0/5*	*2/5*	*3/5*	*4/5*	*5/5*	LD_{10}= 36,5 LD_{16} = 37,9 LD_{84} = 47,9 LD_{50} = 42,9 (40,3÷45,5)
2	*-*	*-*	*0/5*	*0/5*	*2/5*	*3/5*	*5/5*	*5/5*	
3	*-*	*-*	*0/5*	*0/5*	*1/5*	*4/5*	*5/5*	*5/5*	
Total	*-*	*-*	*0/15*	*0/15*	*5/15*	*10/15*	*14/15*	*15/15*	

** - number of deaths/ number of mice used in the experiment*

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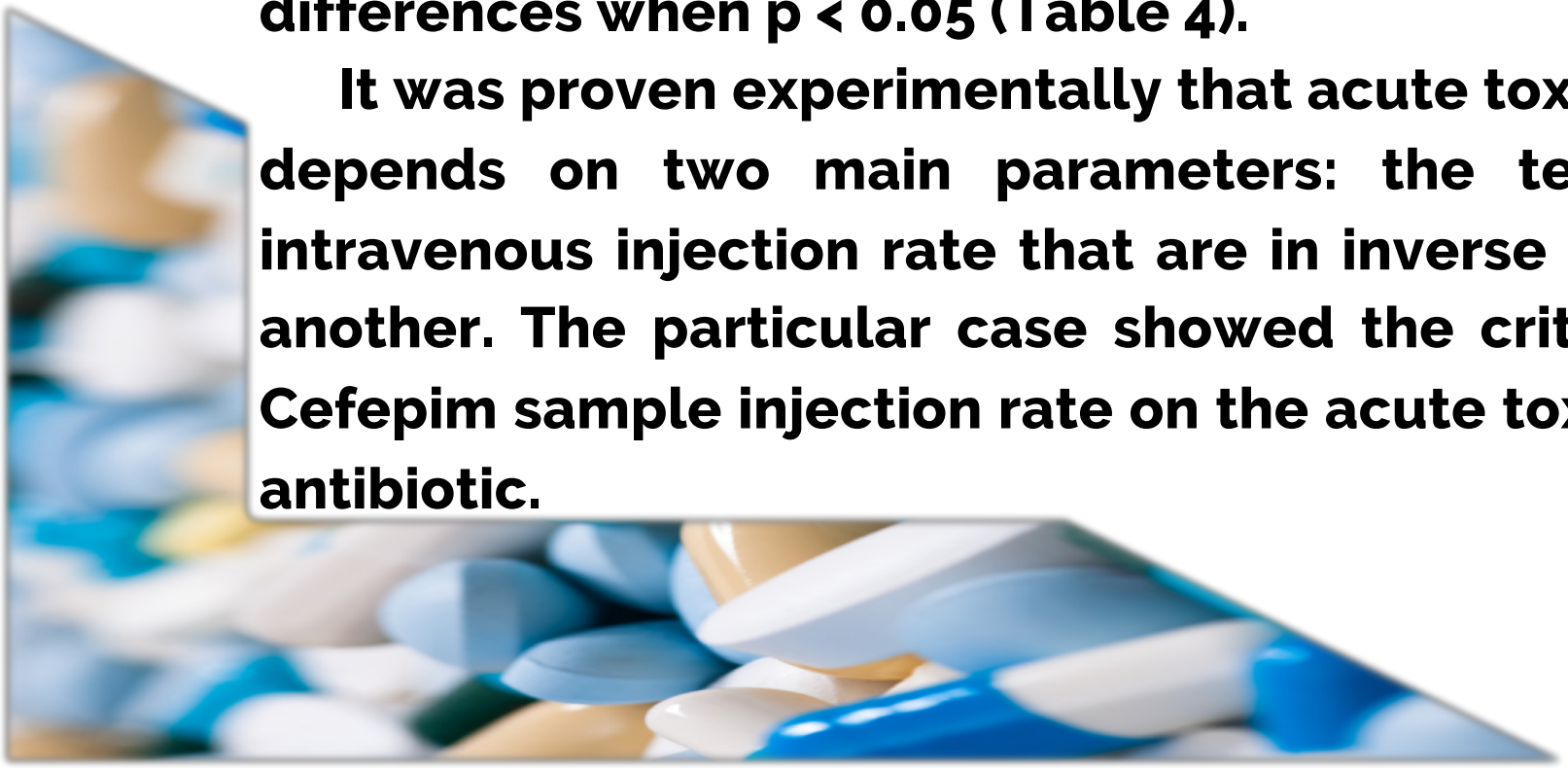
Comparative statistic analysis of acute toxicity values of Cefepim calculated according to the results of experiments with different sample injection rates demonstrated reliability of differences when $p < 0.05$ (Table 4).

It was proven experimentally that acute toxicity of antibiotics depends on two main parameters: the test-dose and the intravenous injection rate that are in inverse proportion to one another. The particular case showed the critical effect of the Cefepim sample injection rate on the acute toxicity value of this antibiotic.



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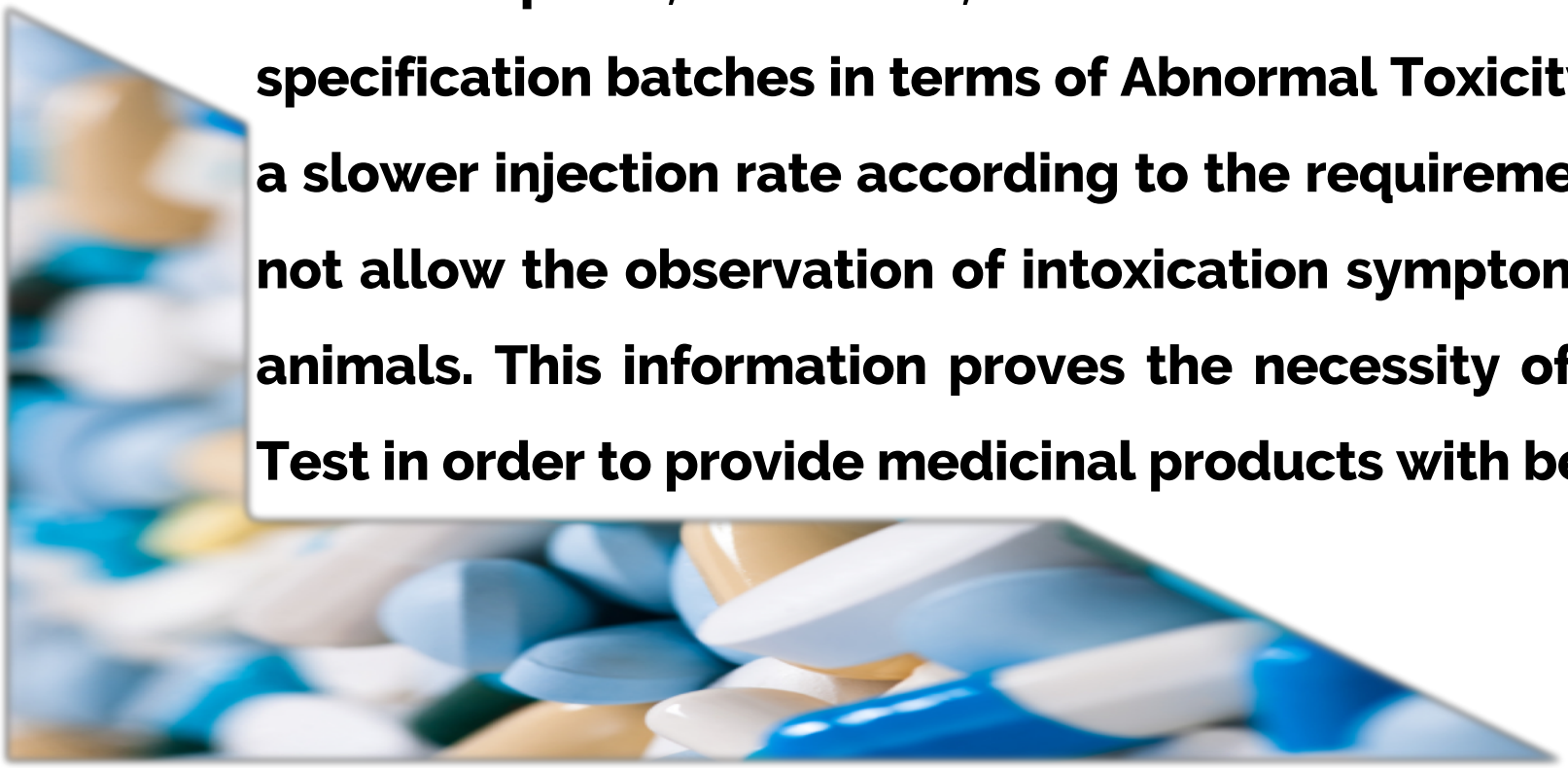
Thus, the given experimental data demonstrate that the correct test conditions (injection rate and test-dose) according to the Russian Pharmacopoeia, XIV ed., allow the identification of out-of-specification batches in terms of Abnormal Toxicity. At the same time, a slower injection rate according to the requirements of Ph. Eur. does not allow the observation of intoxication symptoms and the death of animals. This information proves the necessity of Abnormal Toxicity Test in order to provide medicinal products with better safety.



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