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Toxicity of pharmaceuticals to earthworms

Nowadays, most studies were conducted to assess effects of heavy metals to earthworms. However, there are limited data about toxicity of medical drugs to earthworms. The following study was conducted to assess toxicity of pharmaceuticals to them. The toxicity experiment was performed according to Organisation for Economic Co-operation and Development (OECD) guideline toxicity test. Three pharmaceuticals (Acetaminophen, Ibuprofen and Naproxen) were used to test their toxicity to *Eisenia fetida* earthworms in 0.1 mg/kg, 1.0 mg/kg, 10 mg/kg and 100 mg/kg concentrations. It was found that earthworms are sensitive to pharmaceutical pollution. Acetaminophen caused fatality in high concentration and other pharmaceuticals deteriorated the development of worms.

Key words: pharmaceuticals, *Eisenia fetida*, Acetaminophen, Naproxen, Ibuprofen, earthworms, soil characteristics.

In the past people believed that only heavy metals, toxic/carcinogenic pesticides and industrial intermediates can cause chemical pollution. However, recent years studies showed that also pharmaceuticals pollute environment [1]. Pharmaceuticals enter to the environment in many ways. They excrete as the metabolites and people emit and dispose them. Also, medical drugs can be found in the landfills [2]. Furthermore, some pharmaceuticals are not biodegradable. Therefore, they can be met even in effluent sewage treatment [3].

Nowadays, there were two well-known examples, when pharmaceuticals had adverse effects on the environment. First of all, ethinyl estradiol (EE2), which lead to feminization of male fish and diclofenac, which contribute to poisoning of tens of millions of vultures in Asian countries. It was found that EE2 had adverse impact on aquatic organisms and especially it reduced the reproduction development of fish. EE2 had big impacts on fish even in small concentration [4]. In 2004 Reuters News Services mentioned that British male fish had become female one and it had been caused by exposure of contraceptive pill, which contain hormones produced by females [5].

Another one was non-steroidal anti-inflammatory drug, which was found as high toxic for birds. Indeed this chemical lead to mortality in vultures in Pakistan and India [6, 7].

Cleuvers conducted toxicity research on several mixed pharmaceuticals: diclofenac, ibuprofen, naproxen and acetylsalicylic acid. The experiments were performed using acute *Daphnia* and algal test. The result of the study showed that individually these pharmaceuticals had little effects, however, mixed combination of them was toxic [8].

Earthworms are commonly used for identification the pollution level of heavy metals in soil. They play a significant role in the soil system due to their effects on soil structure and function. Earthworms are able to increase soil fertility because they form organic matter layer in topsoil [9]. Paoletti suggested that earthworms are useful for monitoring contamination of agricultural land and urban areas. Earthworms are very sensitive to fungicides. It was detected that worms species such as *Lumbricus terrestris* and *Allolobophora* will be killed if the soil (1,8 kg ha⁻¹) is contaminated with benomyl. High concentration (998, 85 mg/L) of copper sulfates cause mortality of *Eisenia fetida* [10].

Nowadays, the study of pharmaceuticals as chemical pollutants is very limited. There were some research on effects of pharmaceuticals on aquatic organisms, but very little work was done on toxicity of med-

ical drugs on soil organisms. Therefore, the aim of this research was assessing the toxicity of pharmaceuticals to the earthworms. The data from this study can be used to identify the need for further research on bioaccessibility of pharmaceuticals to wildlife. As earthworms play an important role in the food chain of wildlife, pharmaceuticals can accumulate chemicals that may then be passed on to other living organisms.

Methods

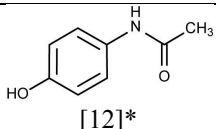
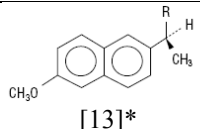
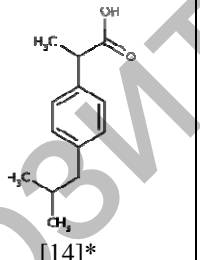
The species used in the toxicity studies was *Eisenia fetida* (Savigny). The earthworms were collected by hand digging from a field near the University of York, York, UK. All worms that were used for the experiments were adults. Their average weight varied from 0.3 to 2.7 g. After collection, the earthworms were kept in soil in an incubator for 5 days before the beginning of the experiments. This allowed for their acclimatization. The temperature of incubator was +15 °C. At the end of the experiments, the contaminated soil was incinerated and worms were released back to the field.

The experiment soil was collected from the same place as the worms. The pretreatment of the soil was conducted before the experiment. All soils were sieved and the pore size of the sieve was 22 mm.

The toxicity of acetaminophen, naproxen and ibuprofen was assessed by using the Organisation for Economic Co-operation and Development (OECD) guideline toxicity test [11]. The following pharmaceuticals were used for assessing their toxicity to worms (Table 1).

Table 1

The characteristics of pharmaceuticals

Name	Chemical structure	Molecular formula	Log Kow	pKa	S, mg/L	Purity, %	Supplier
Acetaminophen	 [12]*	C ₈ H ₉ NO ₂ [12]*	0.46	9.38	12.9	99.0	Sigma Aldrich
Naproxen	 [13]*	C ₁₄ H ₁₄ O ₃ [13]*	3.2	4.2	15.9	≥98	Sigma Aldrich
Ibuprofen	 [14]*	C ₁₃ H ₁₈ O ₂ [15]*	3.97	4.91	21	≥98	Sigma Aldrich

The first part of the experiment was to define appropriate soil condition for worms. Therefore, the suitability of the study soil to maintain earthworm under three different concentrations of water (40 ml, 55 ml, 60 ml) content with two different rates (5 g and 10 g) of feeding was evaluated. This stage of experiment lasted 10 days.

The next part of study was assessing the toxicity of acetaminophen to earthworms. Acetaminophen solutions were made in 3 concentrations: 5 g/L, 0.5 g/L, 0.05 g/L and 0.005 g/L. The solution of this pharmaceutical was added to worms in soil. This assessment took 21 days. Everyday, worms in soil were weighted. The condition of worms was checked in Day 14 and 21.

Then, toxicity study of Naproxen and Ibuprofen to earthworms was conducted. The initial stock solutions of Naproxen and Ibuprofen were prepared in methanol. The following test concentrations were prepared from stock solutions: 0.1 g/L, 1.0 g/L, 10 g/L and 100 g/L. This part of study also lasted 21 days. In Day 14 and 21 earthworms were collected and weighted individually and their morphological condition was assessed.

Results

Define appropriate condition of soil for earthworms

At the beginning of experiment the average weight of the earthworms was $0.62\text{g} \pm 0.39$. The average weight of worms at Day 10 was $0.66\text{g} \pm 0.41$. The high moisture content had the lowest growth followed by the low moisture content and the middle one had highest (Fig. 1). In addition, worms with feeding 5 g oats showed high development, whereas development of worms with 10 g oats feeding showed low development.

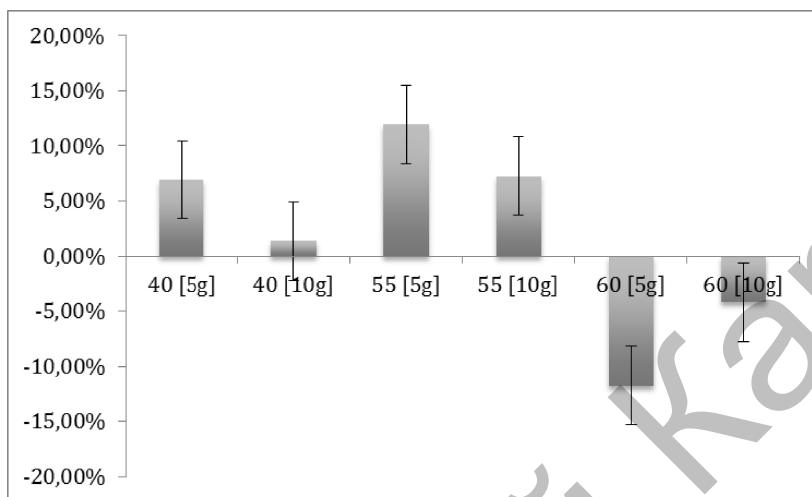


Figure 1. The growth of earthworms during 10 days

Toxicity study of Acetaminophen to earthworms

At Day 1, the mean weight of worms was $1.16\text{g} \pm 0.14$. During the experiment earthworms demonstrated $2.8\% \pm 0.12$ of growth. During the test, there was seen mortality of one worm in Day 5 in Control sample. The next dead worm was found in Day 19 in the sample of concentration 100 mg/kg. Then, other 2 worms died at the end of experiment (Table 2).

Figure 2 shows the average percentage of growth worms in different concentration during 3 weeks. The highest concentration of chemical had the lowest growth during 21 days, but it also had the highest development during 2 weeks. The highest growth of worms during the test was seen in 0.1 mg/kg of concentration.

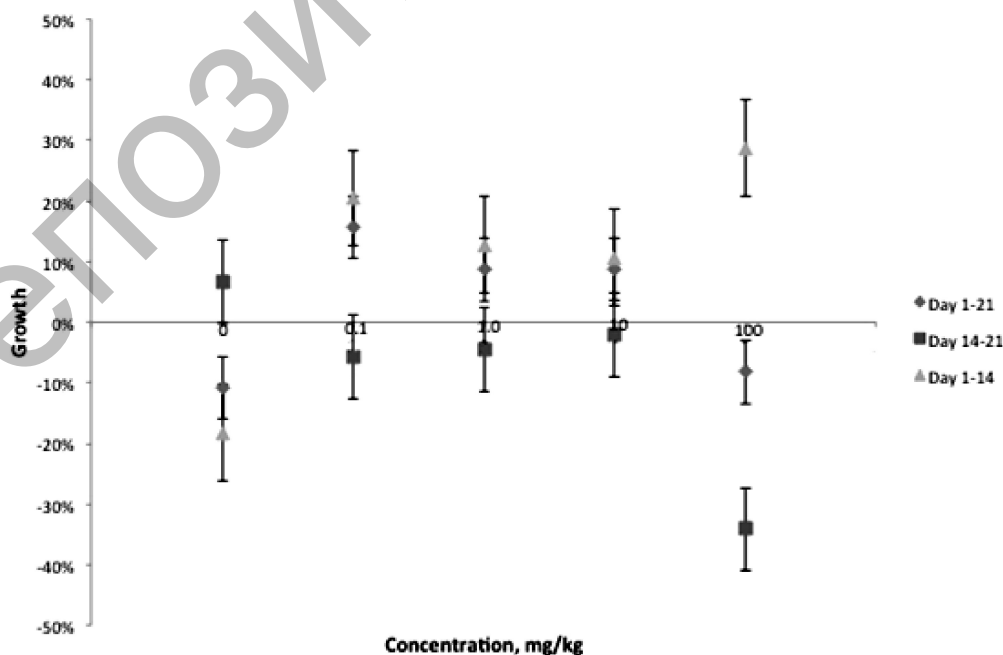


Figure 2. The percentage of development of worms during 3 weeks in toxicity test of acetaminophen

Table 2

Results of acetaminophen toxicity test to worms

Concentration, mg/kg	Fatality after 14 days	Average weight in Day 14, g	Fatality after 21 days	Average weight in Day 21, g
0	1	1.04	0	1.11
0.1	0	1.56	0	1.47
1.0	0	1.33	0	1.27
10	0	1.40	0	1.37
100	0	1.29	3	0.85

Moreover, the condition of worms in concentration 100 mg/kg was quite inferior. It was found that in Day 14, worms from 100 mg/kg concentration samples were not active while worms from control samples were very active. Furthermore, the body of worms from control samples became longer in comparison with other worms in Day 21.

Toxicity study of Naproxen to earthworms

The mean weight of worms in Naproxen toxicity experiment at Day 1 was $1.01\text{g} \pm 0.33$. After 3 weeks, their average weight was $1.41\text{g} \pm 0.52$. Naproxen did not show toxicity to the earthworms, as there were not seen any fatality during the experiment.

The highest concentration of naproxen showed low percentage of growth in comparison with the other concentrations (Fig. 3). It can be noted that worms with low concentration of pharmaceutical developed gradually.

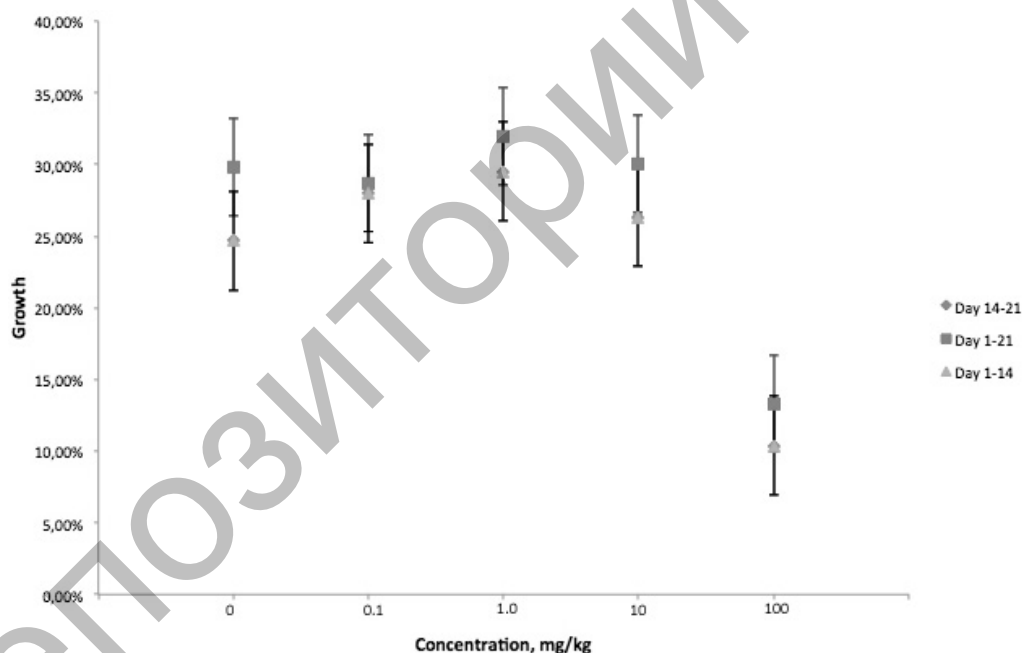


Figure 3. The average percentage of development of earthworms in Naproxen toxicity test in 3 weeks

Toxicity study of Ibuprofen to earthworms

Figure 4 illustrates the rate of growth of worms in 3 weeks in toxicity study of Ibuprofen. In this case, the highest concentration of chemical had the highest rate of growth followed by the low concentration and control samples had lowest.

Ibuprofen exposure to worms did not have a considerable effect to the earthworms. At the end of the experiment, no mortality occurred. Overall, there was $29.47\% \pm 0.06$ growth of worms during 21 days.

Table 3 provides data on comparison of average worm growth in toxicity test of 3 different chemicals. Acetaminophen showed the most toxic effect while Ibuprofen was the less toxic to the earthworms.

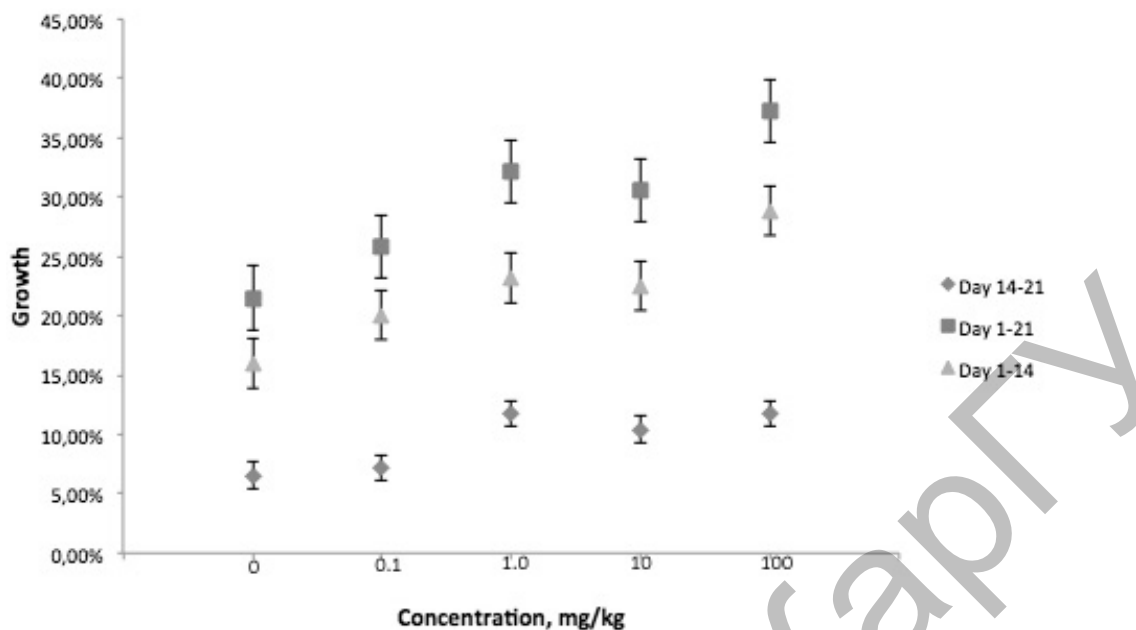


Figure 4. The percentage of worm growth in different concentration of Ibuprofen during 3 weeks

Table 3

The average growth of worms in different concentration in different pharmaceutical toxicity test

Concentration	Acetaminophen, %	Naproxen, %	Ibuprofen, %
0 mg/kg	-10.81	29.81	21.50
0.1 mg/kg	15.65	28.71	25.84
1.0 mg/kg	8.66	31.94	32.17
10 mg/kg	8.76	30.00	30.60
100 mg/kg	-8.24	13.33	37.25
Average	2.80	26.76	29.47
St. deviation	0.12	0.08	0.06

Discussion

It is significant to maintain moisture in earthworms as 75 % of their body consist of water [16]. According to the results, it can be concluded that the most suitable conditions for the toxicity test was the addition of 55 ml of water with 5 g of feeding oats. Lower amounts of water resulted in the soil drying out while higher amounts probably resulted in anaerobic conditions meaning that the earthworms were starved of oxygen and their body started to lose weight [17].

During the experiment, only acetaminophen showed toxic effect to worms. The high concentration of this chemical led to the mortality of earthworms. In addition, there also was death in the control sample at the beginning of the experiment. The mortality of the control worm may be due to sickness of worm before the experiment. Lukkari et al conducted toxicity study of heavy metals to worms. Their results in control samples did not show reproduction in the earthworms. They suggested that it was due to insufficient trace elements in control samples of soil. Thus, it is possible that worms in control containers did not develop well because of limited trace elements in soil [18].

Earthworms showed tolerance to naproxen and ibuprofen toxicity. In earlier work conducted by Spurgeon and Hopkin it was shown that adult *Eisenia fetida* worms were not much sensitive to contaminated soil with heavy metals in comparison to juvenile worms [19]. Thus, previously adult worms showed less sensitive to pollutants than juvenile worms and the present study used adult worms for toxicity tests. It is likely that worms were tolerant to drugs because they were adults, not juvenile worms. Furthermore, on Laskowski and Hopkin work indicated that worms could develop quite and well and be reproductive if the concentration of Zn below 1000 µg. It can be suggested, that 0.1–100 mg/kg of concentrations of ibuprofen and naproxen

were not enough toxic to worms and may be the higher concentration of these pharmaceuticals can lead to toxicity of worms [20].

Previous Spurgeon and Hopkin study found that chemicals are toxic in artificial soil than in the soil from the field. There was a significant mortality of *Eisenia fetida* worms in artificial soil that was contaminated with different concentration (various from 0 to 1000 µg/g) of cadmium, copper, lead and zinc. However, the same concentrations exposure by these metals in field soils did not lead to considerable mortality. Regarding to their study, it can be concluded that toxicity of chemicals in the field soils is not high than in artificial soils. Earthworms can be sensitive to chemicals in artificial soil due to bioavailability of chemicals [19]. The present study used field soil and it is likely that pharmaceuticals were not bioavailable in test soil and that is why drugs did not show toxicity effect to worms.

Conclusions and recommendations

Currently, pharmaceuticals are used very widely. Their impacts to the soil and earthworms are still poorly understood. Mostly, pharmaceuticals are not biodegradable in soils. Thus, contamination of soil by drugs can lead to exposure of pharmaceuticals to earthworms. There a little known about pharmaceuticals and earthworms relationship and it still need further research. However, it can be concluded mostly pharmaceuticals play a negative role to earthworm development.

Further research of the present study is very significant. It is known, that earthworms are prey of birds and small mammals. It means, they have an important role in a food chain and can uptake pharmaceuticals in their tissues. As a result, some concentration of pharmaceuticals can be transferred to terrestrial vertebrate species from worms. In that case, it is still limited data of consequences of bioaccessibility of pharmaceuticals to wildlife.

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Жауын құрттарына дәрілік заттардың улылығы

Мақала жауын құрттарына дәрілік заттардың улылығын бағалауға арналған. Қазіргі кезде ауыр металдардың жауын құрттарына әсері көп зерттелген. Соған қарамастан, дәрілік заттардың жауын құрттарына улы әсері аз анықталған. Осы улылықты анықтау улылық тәжірибелерін жүргізу нормативтерін дамыту және экономикалық бірлестік ұйымына сәйкес жүргізілген. Үш фармацевтикалық препарат (ацетаминофен, ибупрофен және напроксен) 0,1 мг/кг; 1,0; 10 және 100 мг/кг концентрациясында *Eisenia fetida* жауын құрттарына улылығын анықтау үшін пайдаланылған. Жауын құрттары фармацевтикалық ластануға сезімталдығы байқалған. Ацетаминофен жоғары концентрацияда өлімге әкеледі, ал қалған фармацевтикалық препараттар құрттардың дамуын нашарлатады.

А.Боксалл, Б.Н.Аубакирова, М.Р.Хантурин, Р.Р.Бейсенова

Токсичность лекарственных препаратов для дождевых червей

В статье отмечено, что в настоящее время есть много исследований по оценке воздействия тяжелых металлов на дождевых червей, однако сведений о токсичности лекарственных препаратов имеется недостаточно. Было проведено исследование с целью оценки токсичности лекарственных средств в соответствии с нормативами проведения опытов токсичности Организации экономического сотрудничества и развития (ОЭСР). Три фармацевтических препарата (ацетаминофен, ибупрофен и напроксен) были использованы для определения токсичности в экспериментах на дождевых червях *Eisenia fetida* в концентрациях 0,1 мг/кг; 1,0; 10 и 100 мг/кг. Было установлено, что дождевые черви чувствительны к фармацевтическому загрязнению. Ацетаминофен вызвал смертность в высокой концентрации, другие фармацевтические препараты ухудшали развитие червей.