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The pilot study on Ibuprofen uptake by carrot

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Abstract : *The increasing consumption of pharmaceuticals is reflected in contamination of surface water and soil which are then source of possible contamination of crops. Our study uncovers possibility of Ibuprofen (IBU) uptake by carrot when water and soil contaminated by Ibuprofen were used for its cultivation. The basic monitoring of IBU presence in Moravian-Silesian surface water was performed by bag method before growth tests. The maximal detected IBU amount was 21 ng on gram of sorbent per 24 hours. The growth tests started by germination test which uncovers 6,6% reduction in germination in case of slow delivery of IBU and total inhibition of germination in case of fast delivery of IBU. At the end of five-month growth test, carrots were regularly harvested and methanol extracts of plant parts were prepared and analyzed. The detected IBU uptake did not exceed 2 ng/g of green parts and 1.1 ng/g of carrot roots. The study showed that the IBU uptake by carrot is possible. The potential risks to human health associated with consumption of carrots watered by contaminated water or cultivated in contaminated soil are small. Unfortunately, potential risks are real mainly for small animals and it is necessary to solve this problem.*

Keywords **-** *carrot, consumer risk, contamination, Ibuprofen uptake, surface water for watering*

I. Introduction

At present, the pharmaceuticals are produced industrially in huge amounts and therefore they are in many cases cheap. Industrial production thus allows their unnecessary overuse. The amount of non-metabolized pharmaceuticals excreted in urine then grows with their increasing consumption. The higher content in urine together with liquidation of the expired pharmaceuticals by flushing into the toilet have an impact on the increasing pharmaceutical content in wastewater [1, 2]. The pharmaceuticals from wastewater get primarily into the surface water [3-5] due to imperfections in wastewater treatment plants [3, 6]. The second way how the pharmaceuticals can get into the surface water is by liquidation of expired pharmaceuticals with municipal waste. The pharmaceuticals are subsequently rinsing from municipal waste on dump into soil and water.

The various kinds of pharmaceuticals in waters around the world were detected – for example antibiotics [7, 8], antidepressants [9, 10], group of sedatives, hypnotics and opiates [11, 12], estrogens [13, 14] or non-steroid anti-inflammatory drugs [5, 15, 16]. The contamination of the surface water is the basis for drinking and ground water [17], soil [18] or crops [19, 20] contamination. The contamination of water and crops also represents a danger for aquatic organisms, animals and humans [21-25].

The contamination of agricultural crops (for example carrot, spinach, cabbage or wheat) is caused mainly by watering of crops with the contaminated surface water. The watering with contaminated water represents a danger for example for gardeners which can use (by our knowledge) up to 300 L of water from natural sources for watering daily. The supply for example of Ibuprofen into the soil in the garden and into the crops than can be up to 3 μg per m^2 and per a day. The contamination of crops can also be caused by their cultivation in the contaminated soil or by fertilization with contaminated sludge from wastewater treatment plants. The possibility of crops contamination by pharmaceuticals was confirmed for example by works published by Cortés [19], Sabourin [20], Shenker [26], Winker [27] or Wu [28]. They used different types of pharmaceuticals and they test the pharmaceutical uptake into the different kinds of crops (for example carrot, cucumbers, tomatoes or lettuce) by different ways such as watering or fertilization. However, they performed experiments under conditions which were very distant to conditions in the real environment.

The main goal of our study is the evaluation of potential uptake of anti-inflammatory pharmaceutical Ibuprofen (IBU) by *Daucus carota* when contaminated soil or water with high concentration of IBU (much higher than was detected in surface water and in soil) were used for their cultivation. The IBU was chosen with regard to the fact that IBU is one of the most popular painkiller in the Czech Republic. Moreover, IBU removing effectiveness in wastewater treatment plants does not exceed 80% [3, 6]. The higher IBU concentrations were used for ensuring of conclusiveness of performed tests. The use of soil for cultivation instead of more often exploited perlite [29, 30] was caused by the attempt to get closer to properties of the real environment.

II. Experimental

2.1 Materials and chemicals

The Ibalgin 400 tablets (Zentiva, k.s., Dolní Měcholupy, Czech Republic) were used as a cheapest source of Ibuprofen (IBU). The tablets which IBU content (according to producer) 400 mg/tablet were used in the intact form and in the form of powder which was prepared by crushing of the tablets in a mortar. Aqueous solution used to check of IBU concentration in tablets and for watering of one group of carrots in this experiment was also prepared from tablets Ibalgin 400.

The carrot (*Daucus carota*) of the Chantenay type (NOHEL – GARDEN, Dobříš, Czech Republic) with shorter root was used as test crop. The carrot was grown in the universal substrate (Agro CS, Říkov, Czech Republic) which usage was caused by the attempt to simulate properties of the real environment. The universal substrate contained 150-400 mg/L of nitrogen, 80-250 mg/L of phosphorus pentoxide and 250-600 mg/L of potassium oxide according to the producer.

Deionized water and IBU solution with concentration 100 mg/L were used for watering of carrots. The IBU solution was prepared by dissolution of tablets Ibalgin 400 in 1 L of deionized water every 14 days with regard to the IBU stability in solution [31]. Deionized water was prepared in Aqual 25 (AQUAL s.r.o., Brno, Czech Republic).

The acetonitrile (ACN), methanol (MeOH) and water (W) (MS purity, Merck KGaA, Darmstadt, Germany) was used for the preparation of a mobile phase for UHPLC/MS. Methanol was also used as an extraction agent for IBU extraction from plant parts after growth test.

Bags used in monitoring of the IBU content in Moravian-Silesian surface waters were prepared from white non-woven textile Startex 17 (NOHEL GARDEN a.s., Dobříš, Czech Republic). They were filled by dried crushed leaves of *Reynoutria Bohemica* harvested from the banks around Odra river (Fig. 1 point OD).

2.2 Content of Ibuprofen in tablets

The determination of IBU content in the Ibalgin 400 tablets was performed before the beginning of growth tests. Six tablets from three different packages of the pharmaceutical Ibalgin 400 (from each package two tablets) were taken for determination of IBU content. Each tablet was dissolved in 1 L of deionized water. Created solution was analyzed after 24 hour standing in the fridge by proposed UHPLC/MS method five times. The obtained average concentration was used in the next steps.

2.3 Ibuprofen in environment

The IBU content in Moravian – Silesian surface waters was determined before growth tests. The sampling was performed by bag method [32] during five months (May – September 2013) at five sampling locations (Fig. 1) – two rivers (Ostravice and Odra) and three lakes (Kališok, Antošovice and Hlučín).

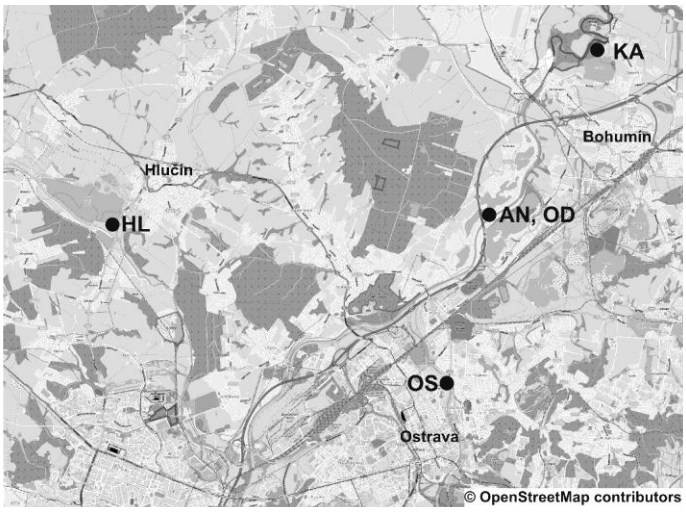

Fig. 1. Sampling locations in Moravian – Silesian Region, Czech Republic (OS – Ostravice river, OD – Odra river, AN – lake Antošovice, KA – lake Kališok, HL – lake Hlučín). The cartography in the map tiles and documentation are licensed under the Creative Commons Attribution-ShareAlike 2.0 license (CC BY-SA). The maps are available under the Open Database License on address - http://www.openstreetmap.org/#map=12/49.8718/18.2208 [33].

The determination of IBU content was performed in a three steps – adsorption on bag, desorption from bag and detection. The sampler with bags was thrown up into the surface water in the selected areas and IBU was adsorbed on sorbent in bag at the first day. The exact locations of the samplers with bags were chosen so as sampler not to interfere and the risk of their disappearance was reduced. The bags were removed from surface water at the second day after 24 hours exposition. Each removed bag was immediately inserted into Erlenmayer flask with 50 ml of extraction agent - mixture 1:1 (V:V) ACN:W. The extracts were filtered through a syringe filter (PTFE, 0.2 μm, Whatman Inc., New Jersey, USA) into vials and analyzes by UHPLC/MS (supplier Bruker s.r.o., Brno, Czech Republic) after 24 hours of extraction.

2.4 Growth experiment

The growth experiment was carried out in 12 separate flowerpots which were divided into 4 groups (one group = 3 flowerpots) labeled as A, B, C and D. The group A was a control group watered only by deionized water, in group B substrate was mixed with five crushed tablets of Ibalgin 400, in group C the substrate was contaminated by 5 intact tablets of Ibalgin 400. The final IBU amount supplied into groups B and C was approximately 2 g into 1 kg of substrate. The group D was watered by IBU solution with concentration 100 mg/L. The final amount of IBU supplied into group D was approximately 1 g into 1 kg of substrate.

Each flowerpot was filled with approximately 1 kg of universal substrate (in group B and C the substrate was enriched by IBU) and 10 seeds of the carrot were planted in it. The groups A, B and C were regularly watered by deionized water and group D was regularly watered by IBU solution with concentration 100 mg/L. The final volume of used deionized water (IBU solution) was approximately 10 L during 5 months of growth experiment. The flowerpots were placed into the laboratory at the temperature between 20 and 21°C, the humidity was between 27 and 34%. Experiment was performed under normal light conditions with 30% irradiance reduction. The used arrangement was chosen with regard to efforts to conduct an experiment under conditions approximating as closely as possible to the real environment. The experiment was terminated after 5 months, the stalks with leaves and the roots of carrots were gradually harvested and the methanol extracts of both parts were prepared. The extracts were filtered and analyzed by UHPLC/MS method.

2.5 Detection methods

The one gram of sample (stalks with leaves or roots) was weighed into Erlenmayer flask and it was filled up with 50 mL of extraction agent – 100% methanol. The extraction solvent was chosen on the basis of its similar polarity as Ibuprofen. The samples have been shaken for 24 hours at ambient pressure and approximately 20°C. The extract was filtered through the syringe filter (PTFE, 0.2 μm, Whatman Inc., New Jersey, USA) and analyzed.

Determination of IBU concentrations was performed by UHPLC/MS system Ultimate 3000/micrOTOF-QII (supplier Bruker s.r.o., Brno, Czech Republic) which was equipped with a column Kinetex 5μ C18 100A (Phenomenex, Torrance, USA). The chromatographic separation was carried out under following isocratic conditions: mobile phase – 70:20:10 (V:V:V) acetonitrile:methanol:water, flow of mobile phase – 0.2 mL/min, temperature of column – 20°C. Detection was performed by MS with ESI in negative ionization mode. Pressure of nebulizer gas was 2 bar, flow of drying gas was 10 L/min and its temperature was 200°C, respectively. Detection mass was 205.1 Da. The analytical method was developed and tested for three volumes of injection – 1 μL (for concentrations in mg/L), 10 μL (for concentrations in μg/L) and 100 μL (for concentrations in ng/L). The validation parameters of detection method are shown in the Table 1.

Table 1 Validation parameters of UHPLC/MS method

Parameter	Value
Limit of detection	0.1 ng/L
Limit of quatitation	0.5 ng/L
Determination coeficients of linearity	0.990 – 0.994
Repeatibility (maximal deviation)	6.36%
Reproducibility (maximal deviation)	9.46%
Precision (maximal deviation)	2.85%
Accuracy (maximal deviation)	1.37%

2.6 Human and animal rights

People, animals and genetically modified plants have not been used during this research. Human and animal rights have not been violated during the research.

III. Results and Discusion

3.1 Content of Ibuprofen in tablets

The tablets used to solve of health problems are not the reference material and the precise concentration of active compound is unknown. Therefore, the content of Ibuprofen (IBU) in Ibalgin 400 tablets was examined before growth tests. According to producer, these tablets should include 400 mg of IBU per a tablet. The average value 379 ± 2 mg per tablet obtained by analysis of tablets showed a lower content of IBU in tablets in comparison with data provided by the producer. We think that this deviation was caused by imperfect transfer of IBU from tablets to the analyzed solution and by imperfections of detection method (Table 1). The average content of IBU (379 mg per tablet) obtained from these tests was used in the next steps

3.2 Ibuprofen in environment

The monitoring of IBU content in surface waters was performed by bag method **[**32**]** between May and September 2013 at five locations of Moravian – Silesian region – two rivers (Ostravice and Odra) and three lakes (Antošovice, Hlučín, Kališok)(Fig. 1).

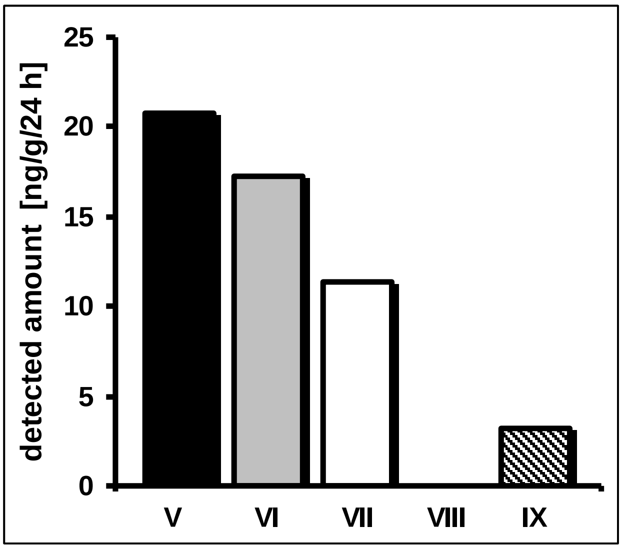

Fig. 2. Detected Ibuprofen amount in Ostravice river after 24 hours bag exposition (V – May, VI – June, VII – July, VIII – August, IX – September).

The presence of Ibuprofen in surface water has been confirmed for example by Ascar [15], Paiga [4] or Bu [7] whose detected IBU in the surface water in concentrations from tens to hundreds ng/L. We found quantifiable IBU amounts only in the Ostravice river (Fig. 1 point OS). The results (Fig. 2) obtained for Ostravice river show very interesting trend. We assume that this is caused by water flow rate in Ostravice river when the IBU content (see Fig. 2 colums V – VIII) decreased with a decrease of the amount of water in the Ostravice river due to no raining. Detected IBU content subsequently increased with the coming of rain which increased water flow.

The detected IBU amount in Ostravice river represents a danger for gardeners around Odra river which used water from this river for watering. The average gardener used (by our knowledge) up to 300 L of water from natural sources for watering daily. Thus IBU supply into soil and crops can be up to 6.3 μg per m^2 and per a day, 176.4 μg per m^2 and per a month or 882 μg per m^2 and per five months. Therefore, we also used higher amounts of IBU in our experiment.

The concentration of IBU in the remaining sampling points was below the limit of quantification or IBU has not been detected.

3.3 Germination of seeds

To our knowledge, this is the first study where the influence of Ibuprofen (IBU) on germination of carrot seeds were examined (Table 2). The tests were performed using three forms of IBU source: crushed tablets added into the soil (group B), intact tablets added into the soil (group C) and IBU solution used for watering (group D). Two forms of soil contamination (intact and crushed tablets) were chosen in order to determine influence of slow releasing of IBU– intact tablet and influence of fast releasing of IBU – crushed tablet. Moreover, intact tablets simulate one of the ways how people get rid of unused expired pharmaceuticals **-** liquidation in municipal waste. The results showed significant influence on germination only in group B where no germination was observed. The total inhibition of carrot growth was probably caused by mixing of the soil with crushed tablets which contained roughly 2 g of IBU. This phenomenon has not been previously described in available literature. We supposed that this total inhibition was caused by fast supply of IBU from crushed tablets to the seeds which were unable to germinate under the influence of IBU.

Table 2 Influence of administration form of Ibuprofen on germination of seeds of carrot (average value from 3 repeats)

Group	Number of germinated seeds	Germination percentage [%]
A	25	83.33
B	0	0
C	23	76.77
D	23	76.77

A– control watered with deionized water, B – crushed tablets, C **-** intact tablets, D –watering with solution of Ibuprofen

The other two groups (C and D) showed 6,6% lower germination in comparison with control group A (Table 2). This phenomenon has not been described in the available literature, too. We assume that this phenomenon was caused by fact that the supply of Ibuprofen was in the groups C (slow release from tablet) and D (gradual supply by plant watering) slower than in the case of group B.

3.4 Content of Ibuprofen in tablets

The main goal of our study was to determine the potential uptake of Ibuprofen (IBU) by carrot when contaminated soil and water were used for its cultivation (Table 3). The growth test was terminated after five months of growth and carrots were harvested. The carrots were regularly suffused by deionized water (groups A, B and C) and by IBU solution (group D) during cultivation. The overall amount of liquid used for watering was approximately 10 L. The supplied quantities of IBU were 1g (group D) and 2g (group C) into 1 kg of universal substrate. The extraction of harvested plant parts were performed by simple extraction procedure with 50 mL of 100% methanol. It has been tested more solvents such as acetonitrile. Methanol has emerged as the best extraction solvent due to its similar polarity as Ibuprofen. Then extracts were analyzed by UHPLC/MS after filtration through above-mentioned syringe filter.

Table 3 Content of Ibuprofen in stalks with leaves and in carrots after five months growth (n=3)

Group	Designation of flowerpot	Ibuprofen content in stalks with leaves [ng/g]	Ibuprofen content in roots [ng/g]
A	A1	n.d.	n.d.
	A2	n.d.	n.d.
	A3	n.d.	n.d.
C	C1	<LOQ	1.03
	C2	<LOQ	0.98
	C3	<LOQ	0.88
D	D1	1.01	n.d.
	D2	1.77	n.d.
	D3	1.83	n.d.

A– control group watered with deionized water, C **-** intact tablets, D – watered with IBU solution, n.d. – not detected

The highest IBU uptake (1.01 – 1.88 ng/g) was achieved in green parts of plants from group D which were watered by IBU solution. The IBU uptake was observed also in green parts were in group C (into soil were inserted intact tablets), but detected amounts were under the quantification limit (LOQ) of detection method. The IBU uptake by roots of carrots was observed only in carrots grown in contaminated soil (group C) and it did not exceed 1.03 ng/g. The above-mentioned results showed one interesting phenomenon. If watering with IBU solution was used, this pharmaceutical got primarily into the green parts of plant (stalk with leaves). In contrast, when soil with tablets was used, IBU uptake was mainly roots of carrots. This phenomenon might be related to the processes of acquirement of nutrients and moisture by plants. Further research would be carry out for explanation of this phenomenon.

The similar IBU uptake tests were presented for example by Cortés [19], Sabourin [20] or Winker [27] who used detection methods with similar detection limits (around 1.5 ng/L) as our detection method. The our uptake test were more successful than tests presented by above-mentioned authors who not detected IBU in their tests crops or IBU uptake was below the limit of quantification of their detection method. The difference could be caused mainly by concentrations of IBU in sources. The sources containing IBU in ng were used in works of the above-mentioned authors. In contrast, our study used sources containing IBU in milligrams. The reason for these milligram concentrations was ensuring a conclusiveness of performed tests. We assume that the IBU uptake would be significantly lower if natural sources (surface water, soil) would be used for the cultivation.

The possibility of food contamination which was confirmed for example by works of by Cortés [19], Sabourin [20], Shenker [26] or Wu [28] represents danger for human or animals – for example Ottonello [21], Rahman [22] or Brozinski [23]. Our results showed that IBU uptake by carrot is possible but does not exceed units of nanograms although carrot was exposed to high concentrations of this pharmaceutical. The contamination of the surface water by IBU does not exceed quantity of 1 μg/L [15]. We confirmed this fact in Ostravice River by tests with the bag method by which IBU has been detected in amount 21 ng/g/24 hours. It corresponds to 0.52 μg of IBU in one 25L large pot of water used to watering of garden. The IBU uptake by carrots would be much smaller when sources (water, soil, fertilizers) with content of IBU in hundreds of nanograms (which were detected in nature) would be used. Thus, the potential risk for human consumers is very low because humans eat only one part of carrot – root and it is mainly heat treated. However, the consumers are not only humans but also animals [28]. The potential risks especially for small herbivorous animals such as rabbit is not negligible because animals consume both parts (stalks with leaves and roots) uncooked. Moreover, the supplied amount of pharmaceutical/mass of consumer ratio is much higher for animals than for human.

IV. Conclusion

The aim of our study was to evaluate the possibility of Ibuprofen (IBU) uptake by carrot associated with its cultivation using water and soil contaminated by IBU. The germination tests performed on start of research uncovers 6,6% reduction of germination in case of slow delivery of IBU and total inhibition of germination in case of fast delivery of IBU higher amounts. The final results showed that very small uptake of non-steroid pharmaceutical Ibuprofen took place during growth using soil or water contaminated with high concentrations of IBU. Thus, there is very low risk for human consumer, because Ibuprofen is present in much smaller amounts in nature (in real soil or in real surface water), but there is considerable risk mainly for small animals (for example rabbits) which should be reduced. The solution would be removement of contaminants from water, soil and fertilizers which are used during the cultivation. The removement could be obtained for example by sorption.

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