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DOI: 10.1515/cdem-2018-0007 CHEM DIDACT ECOL METROL. 2018;23(1-2):113-126

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DETERMINATION OF TOXICOLOGICAL PARAMETERS OF SELECTED BIOACTIVE ORGANIC CHEMICALS USING THE OSTRACODTOXKIT F^{TM}

WYZNACZANIE PARAMETRÓW TOKSYKOLOGICZNYCH WYBRANYCH ORGANICZNYCH SUBSTANCJI BIOAKTYWNYCH Z ZASTOSOWANIEM TESTU OSTRACODTOXKIT F^{TM}

Abstract: Assessment of the impact of pharmaceutical residues on living organisms is very complex subject. Apart from taking into account the toxicity of individual compounds also their presence in mixtures should be taken into account. In this work, attempts were made to assess the ecotoxicity of biologically active substances (with 50 % effective concentration (EC_{50}) values growing from fluoxetine (EC_{50} = 4.431 nM) >> gemfibrozil ≈ 17α-ethinylestradiol ≈ ketorolac > indomethacin > theophylline ≈ progesterone > naproxen ≈ trypsin > 2-(2,4,5-trichlorophenoxy)propionic acid > chloramphenicol > acetylsalicylic acid > ibuprofen > ketoprofen > 19-norethindrone to bezafibrate as the least toxic drug among studied ones) to the ISO standardized Ostracodtoxkit F^{TM} bioassay. The Ostracodtoxkit F^{TM} was proven to be very sensitive tool with respect to responding to presence of pharmaceuticals. Results of studies justify the statement that more research is needed in field of assessment of chronic exposure to pharmaceuticals and other newly emerging pollutants especially when they are present in complex mixture.

Keywords: bioassays, pharmaceuticals, toxicity, *Heterocypris incongruens*, Ostracodtoxkit F^{TM}

Introduction

Among all inorganic and organic pollutants present in the environment, pharmaceuticals are becoming a serious problem due to their increasing amount and bioaccessibility. In recent years many studies have shown that pharmacological substances are present in environment at the measurable level [1-8]. It is estimated that in the European Union (EU) countries about 3000 substances are used as medicine including antibiotics, analgesics and anti-inflammatory drugs, beta-blockers, contraceptives, lipid regulators, neuroactive compounds and many others. In addition to medical purposes many biologically and endocrine active substances are also used in veterinary medicine [9, 10].

For certain and most frequently applied drugs the continuous monitoring and statistical documentation is preformed (statistics may vary depending on the list of drugs and the law

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in force in a given country), and so for the class of non-steroidal anti-inflammatory drugs (NSAID) including acetylsalicylic acid, paracetamol, ibuprofen and diclofenac (consumption of these drugs in Germany reached, respectively, 836.3, 622.0, 345.0, 35.1 Mg), naproxen (e.g. 35.07 Mg per year in England in 2000) and the antiepileptic carbamazepine (e.g. 88 Mg per year in Germany 2001) [9]. As a result of considerable consumption (some of these drugs are commercially available without a prescription - so called over the counter drugs) they may exist in the environment at high levels. The presence and the stability of drugs in the environment is related to theirs pharmacokinetics (metabolism, half-life, urinary and fecal excretion *etc*.) and environmental conditions [11, 12].

Literature data concerning concentration levels and properties of selected drugs present in the environment are summarized in Table 1. The data indicate that residues of pharmaceuticals can be found in all water compartments: the wastewater (both in influents and effluents), surface waters and even in drinking water. Even most modern sewage treatment plants are unable to cope with the accumulated quantities of pharmaceuticals in waters.

Despite the constantly increasing number and amount of drugs reaching the environment their fate and way of affecting the organisms inhabiting ecosystems is still not fully understood - mainly due to synergic, antagonistic or additive character of interactions occurring between the chemicals [13, 14]. There are gaps in knowledge on toxicological studies from the clinical trials of drugs and ecotoxicological studies (after they reach the environment). The aim of this study presented was to determine the 50 % effective concentration (EC_{50}) and 50 % lethal concentration values (LC_{50}) of selected substances to aid performing environmental risk assessment with respect to pharmaceuticals residues toxicity to higher organisms and environmentally stated concentration levels.

Materials and methods

Chemicals, reagents and instruments

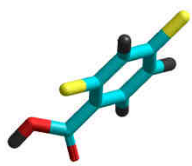
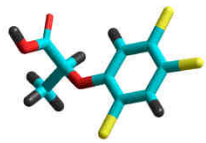
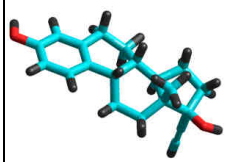
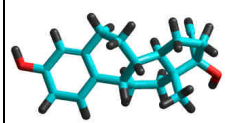
Compounds of analytical purity grade: 2,4-dichlorobenzoic acid, 2-(2,4,5-trichlorophenoxy)propionic acid, 17α-ethinylestradiol, 19-norethindrone, acetylsalicylic acid, bezafibrate, caffeine, chloramphenicol, fluoxetine hydrochloride, gemfibrozil, indomethacin, ibuprofen, ketoprofen, ketorolac tris salt, naproxen, progesterone, trypsin and theophylline were purchased from Sigma-Aldrich (Germany). For assessing EC_{50} and LC_{50} parameters of easily soluble compounds (trypsin, theophylline, HEPES, fluoxetine hydrochloride, 2,4-dichlorobenzoic acid, acetylsalicylic acid, ketorolac tris salt, chloramphenicol) the standard solutions were prepared and studied. Insoluble compounds were weighed in proper amounts and added to the wells in the test plates in mass ratios (also mixed in sediment volume) and for this reason results for these analytes toxicity studies are given in mmol/kg. Sediments in test plates were spiked with proper nominal amounts of chemicals on the 0^{th} day of performing the test, equilibration time was 4 hours.

The Ostracodtoxkit F^{TM} chemicals and utensils (vials with algal food for chronic toxicity tests and matrix dissolving medium, Spiruline, 6-well test plates, certified reference sediment, certified dormant eggs of *Heterocypris incongruens*) were purchased from MicroBioTests Inc. (Belgium). The instruments and equipment used during the study were:

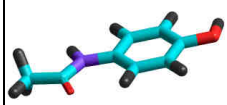
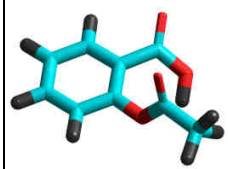
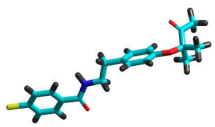
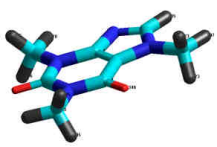
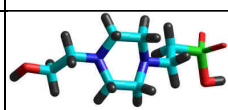
a) electronic pipettes (Rainin, Eppendorf, Germany),
b) analytical balance from Radwag (Poland),
c) CP411 Metron pH-meter,
d) binocular from Ceti NV (Belgium).

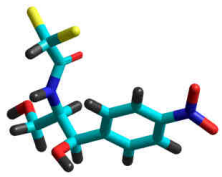
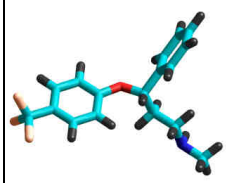
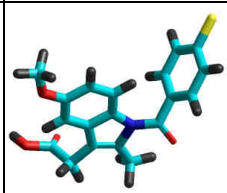
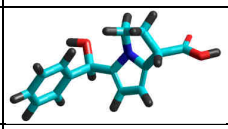
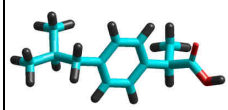
The basic characteristics of the standard sediment was as follows: OC < 1 %, Mg^{2+} 1.12 mg/kg, and Ca^{2+} 54.36 mg/kg. Distilled water was used instead of standard EPA medium because the standard solutions were prepared in such water; thus, all tests were run at approximately the same ion strength and osmotic pressure.

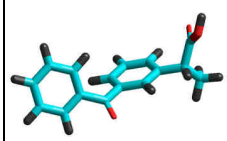
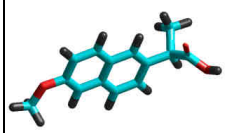
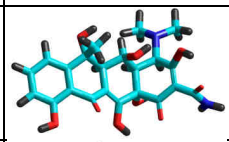
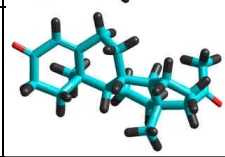
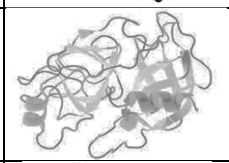
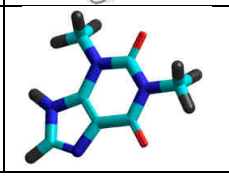
Table 1
Information on pharmaceuticals/biologically active compounds studied, their properties, functions and environmental concentration levels determined

Analyte / CAS no.	**Structure[2]**	**logP/ M_m [Da]**	**Effect on living organisms**	**Environmental problem**		
				Concentration stated in the environment	**Sample matrix and localization**	**Ref.**
2.4-dichlorobenzoic acid / 50-84-0		2.82/ 191.01	synthetic growth regulator with properties similar to the auxin (inhibits the growth and development of plants)	80-620 ng/g	Soil samples, Central Bohemia, Czech Republic	[4]
				100-3500 ng/dm^3	Waste water samples (influents) Weisbaden, Germany	[1]
2-(2.4.5-trichloro-phenoxy) propionic acid / 93-72-1		3.8/ 269.51	herbicide and plant growth regulator	< MDL = 10 ng/dm^3	STPs Taiwan	[15]
17α-ethinylestradiol / 57-63-6		3.67/ 296.40	a semisynthetic alkylated estradiol often used as the estrogenic component in oral contraceptives	< LOD		
19-norethindrone / 68-22-4		2.97/ 298.42	a synthetic progestational hormone with actions similar to those of progesterone	26-224 ng/dm^3	Bleached kraft mill effluent, domestic wastewater effluents, Canada	[16]

[2] Structure optimized for lowest energy, with HyperChem 8.0 software, blue - carbon atoms, black - hydrogen atoms, red - oxygen atoms, yellow - chlorine atoms, green - sulphur atoms

Analyte / CAS no.	Structure[2]	logP/ M_m [Da]	Effect on living organisms	Environmental problem		
				Concentration stated in the environment	Sample matrix and localization	Ref.
acetaminophen / 103-90-2		0.5/ 151.16	non-steroidal anti-inflammatory drug	< LOQ-37458 ng/dm^3	Wastewater samples (input and output of Alcalá de Henares), Madrid, Spain	[7]
				< 10-1400 ng/dm^3	Wastewater samples, USA	[2]
				1.8-73 ng/dm^3	Surface, drinking, and waste waters samples (effluent), Jeolla province, Jeju Island, (Youngsan River), South Korean	[5]
acetylsalicylic acid / 50-78-2		1.19/ 180.16	analgesic. antipyretic and anti-inflammatory	Municipal waste water influent 6290±3390 ng/dm^3 Effluent 18±11 ng/dm^3 Hospital waste water Influent - effluent 2670-78 ng/dm^3 River water 54±48 ng/dm^3	Waste, hospital waste, river water samples, five rivers in Busan Region, Korea	[16]
				41-50 ng/dm^3	Waste, river water samples (Llobregat River basin, Barcelona), Spain	[17]
bezafibrate / 41859-67-0		3.8/ 361.82	an antilipemic agent	200-700 ng/dm^3	Wastewater (effluents), Whidbey, Peterborough, Ontario, Canada	[18]
				< LOD-4600 ng/dm^3	Waste, river water samples, Frankfurt, Germany	[19]
caffeine / 58-08-2		–0.1/ 194.19	stimulant	< 8.5-152 ng/dm^3	Coastal ocean and water bodies adjacent samples, Oregon Coast, USA	[20]
				< LOD-10 ng/dm^3	Surface and groundwater samples, Hanelai Bay, Kauai	[21]
HEPES / 7356-45-9		–4.07*/ 238.30	a dipolar ionic buffer used in cellular biotests	*Data not available*	*Data not available*	*Data not available*

Analyte / CAS no.	Structure[2]	logP/ M_m [Da]	Effect on living organisms	Environmental problem		
				Concentration stated in the environment	Sample matrix and localization	Ref.
chloramphenicol / 56-75-7		1.14/ 323.13	antibiotic	< LOD -2.43 ng/dm^3	Wastewater samples (influent and effluent) Guangzhou, China	[22]
				27.1-75 ng/dm^3	Surface waters samples (influents and effluents), Han River, China	[23]
fluoxetine hydrochloride / 54910-89-3		4.05/ 309.33	used to treat depression and obsessive-compulsive disorders	< LOQ-1827 ng/dm^3	Wastewater samples (input and output of Alcalá de Henares), Madrid, Spain	[7]
				< 43.2 ng/dm^3	Stream water samples (Boulder Creek, Colorado, Fourmile Creek, Iowa), USA	[24]
gemfibrozil / 25812-30-0		4.77*/ 250.33	lipid regulator	20-60 ng/dm^3	Wastewater, Whitby, Peterborough, Canada	[18]
				< 17055 ng/dm^3	Wastewater samples (input and output of Alcalá de Henares), Madrid, Spain	[7]
indomethacin / 53-86-1		4.3/ 357.79	non-steroidal anti-inflammatory agent (NSAID)	> 20 ng/dm^3	Waste water samples, Peterborough, Canada	[18]
				< LOD-240 ng/dm^3	Raw water samples, Bosnia and Herzegovina, Croatia, Serbia	[25]
ketorolac tris salt / 74103-07-4		1.9*/ 255.27	analgesic	< LOQ-2793 ng/dm^3	Wastewater samples (input and output of Alcalá de Henares), Madrid, Spain	[7]
ibuprofen / 15687-27-1		3.8/ 206.28	nonsteroidal anti-inflammatory agent with analgesic properties used in the therapy of rheumatism and arthritis	< 12-34 ng/dm^3	Surface water (Lao-Jie and Zen-Wen River) samples, Taiwan	[26]
				influent 1966 ±662 ng/dm^3 effluent 40 ±32 ng/dm^3	Waste water (effluents, influents) samples, Sapporo, Japan	[27]

Analyte / CAS no.	Structure[2]	logP/ M_m [Da]	Effect on living organisms	Environmental problem		
				Concentration stated in the environment	Sample matrix and localization	Ref.
ketoprofen / 22071-15-4		3.1/ 254.28	an ibuprofen-type anti-inflammatory analgesic and antipyretic	< 351 ng/dm^3	Surface/waste water samples, Vancouver Island, British Columbia, Canada	[28]
				0-180 ng/dm^3	Waste water effluents samples, (Aa Uster and Aabach Moenchaltorf rivers), lake Greifensee, Switzerland	[25]
naproxen / 22204-53-1		3.3/230.26	an anti-inflammatory agent with analgesic and antipyretic properties	< 5228 ng/dm^3	Wastewater samples (input and output of Alcalá de Henares), Madrid, Spain	[7]
				< 0.5-1338 ng/dm^3	Waste water samples, USA	[2]
oxytetracycline / 79-57-2		–0.09/ 460.43	one of the family of tetracycline antibiotics	influent-effluent 712±95 - 235±55 ng/dm^3	Waste water (Xiao River), China	[29]
progesterone / 57-83-0		3.9/ 314.46	progestational steroid	66±36 ng/dm^3	Wastewater samples (influents and effluents), Beijing, China	[3]
trypsin / 9002-07-7		- /23800	digestive enzyme produced by the bovine pancreas	*Data not available*	*Data not available*	*Data not available*
theophylline / 58-55-9		–0.02/ 180.16	used to treat asthma and chronic obstructive bronchopulmonary disease	Average 242 ng/g	Sludge samples, Japan	[30]

*XLogP3-AA. ND - not detected, LOD - limit of detection, LOQ - limit of quantification, MDL - method detection limit

Methodology of Ostracodtoxkit F^{TM}

As the spectrum of microbiotests available to assess the quality of soils or sediments is much narrower than that for water, the possibility of using *H. incongruens* as a direct contact test to determine the toxicity of soils and sediments contaminated with pharmaceuticals and their residues is very valuable. The applicability of Ostracodtoxkit F^{TM} is being proven by an increasing number of studies and manuscripts undertaking the

problem of toxicity assessment of different environmental compartment with given bioassay. The *Heterocypris incongruens* are small organisms living in the bottom area of seas and fresh-water bodies. Due to their high sensitivity to the presence of organic contaminants as well as heavy metals they show high usefulness in the monitoring of the degree of contamination of waters and bottom sediments.

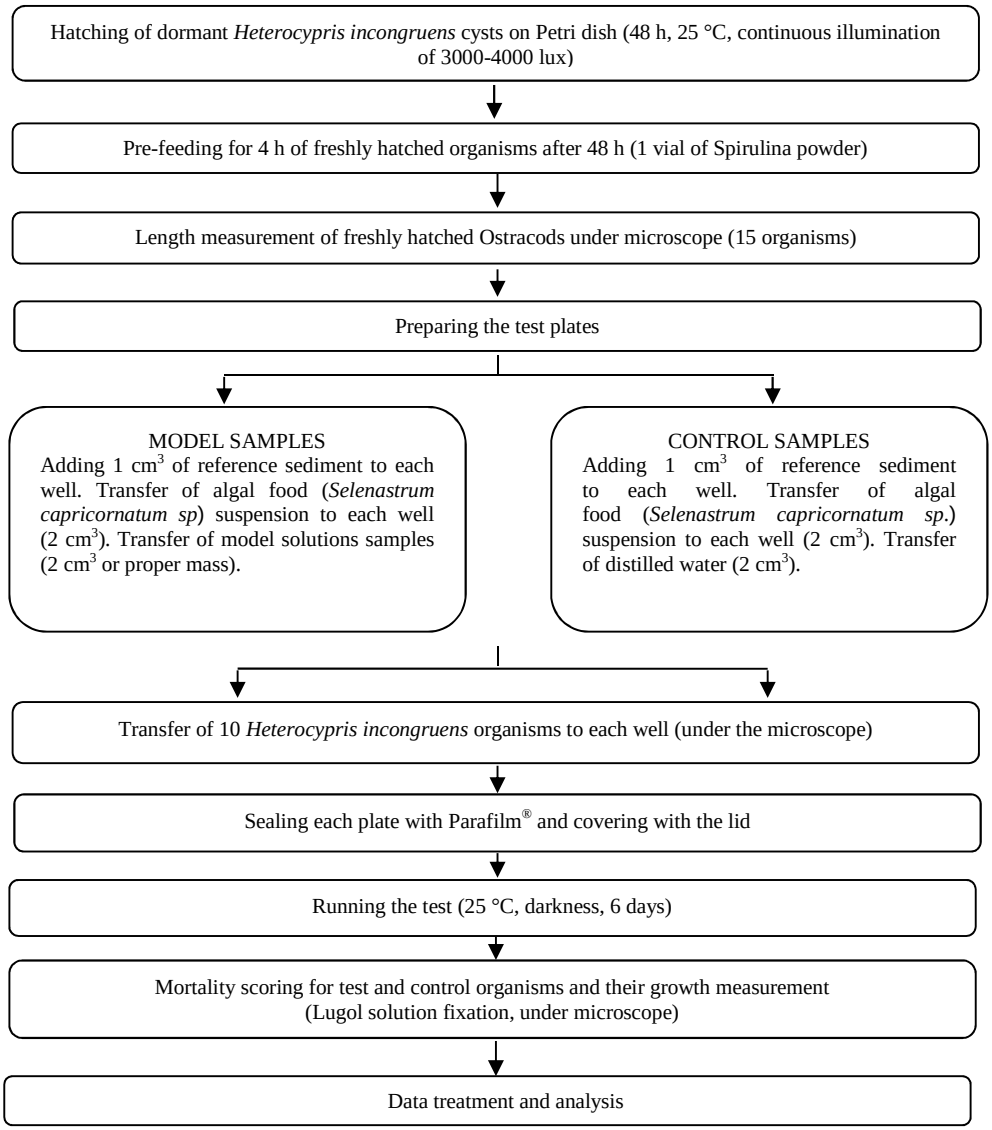


Fig. 1. Schematic presentation of *Heterocypris incongruens* sub-chronic toxicity determination procedure

The extended duration of the test (6 days) makes it possible to assess the disadvantageous biological effects related to long-term exposure of the organism to

xenobiotics and assess the exposure occurring at various developmental stages, from the larval stage to a mature specimen. The toxicity level is determined on the basis of observing two effects, including inhibition of the rate of growth and determination of the mortality of organisms as a result of contact with harmful agents. Figure 1 presents a diagram of the analytical procedure used to estimate the level of chronic toxicity.

The toxicity parameters EC_{50} and LC_{50} were evaluated applying Ostracodtoxkit F^{TM} bioassay that was purchased from Microbiotest Ltd. (Belgium). The tests were run according to a slightly modified version of the original procedure that had to be adapted to the conditions of the toxicity determinations described in [31].

The experiments were carried out in triplicates for all studies with solutions of varying concentrations of a drugs in standard sediments. The test was considered to be performed correctly if the mortality of the reference sediment organisms was < 20 % and if the mean length increment was > 400 μm. This requirement was fulfilled in all the experiments. The experimental data were analyzed by regression analysis following logarithmic transformations and EC_{50}/LC_{50} values were expressed as toxicant concentrations. After range finding tests proper experiments were carried out to produce linear dose-response curves for particular chemicals.

Results and discussion

In Table 2 values of EC_{50} and LC_{50} determined in the present study for soluble substances while in Table 3 for insoluble chemicals are given together with their comparison to literature data for other bioindicating organisms. As already stated in case of water insoluble substances the final EC_{50} and LC_{50} data are expressed in relation to sediment content. It is significant in case of using given organism in environmental risk assessment of poorly soluble drugs residues. It was shown that the most toxic (under conditions of study) after 6 days of experiment was highly soluble fluoxetine (with EC_{50} and LC_{50} reaching as low levels as 4.331 and 5.259 nM, respectively), and poorly soluble: gemfibrozil (29.038 and 33.795 ng/kg for EC_{50} and LC_{50}, respectively), 17α-ethinylestradiol (36.754 and 92.477 ng/kg for EC_{50} and LC_{50}, respectively), ketorolac (35.227 and 52.330 nM for EC_{50} and LC_{50}, respectively) and indomethacin (59.035 and 88.374 ng/kg for EC_{50} and LC_{50}, respectively). Interestingly, difference of potential small difference of concentrations divides possibility of inhibiting the growth of *Heterocypris incongruens* from lethal values - in cases of fluoxetine and gemfibrozil these differences are very narrow what underlines high environmental risk of these substances when present in the environment. The Ostracodtoxkit F^{TM} was noted to be at least 4 times more sensitive (e.g. in case of 17α-ethinylestradiol) than in case of other bioassays like those using *Daphnia sp.*, *Hyalella azteca*, or even *Dunaliella tertiolecta* algae - which are generally recognized as sensitive organisms. It may be result of *H. incongruens*'s habituation - feeding in the borderland between sediments/soils and close-to-bottom water what makes it prone to both soluble and insoluble pollutants reaching its body with gastrointestinal tract. As can be seen presence of water insoluble chemicals (e.g. bezafibrate, gemfibrozil, indomethacin or naproxen) would almost not be notified with other crustaceans. Anti-inflammatory drugs appear to have low impact on *Heterocypris incongruens* (under conditions of performing given research).

Summarizing, among most potent substances tested in the current research one can mention (in the order of increasing EC_{50} to the bioassay organism): fluoxetine >>

gemfibrozil ≈ 17α-ethinylestradiol≈ketorolac > indomethacin > theophylline ≈ progesterone > naproxen ≈ trypsin > 2-(2,4,5-trichlorophenoxy)propionic acid > chloramphenicol > acetylsalicylic acid > ibuprofen > ketoprofen > 19-norethindrone > bezafibrate.

Table 2

Results of studies on determination of EC_{50}/LC_{50} and other toxicological data for selected chemicals toward bioassay organisms and their direct comparison to values for other organisms for soluble analytes

Substance	***Heterocypris incongruens* (Ostracodtoxkit F^{TM})**		**Referenced ecotoxicity data**	
	EC_{50} [nM]	**LC_{50} [nM]**	**Bioassay organisms**	
acetylsalicylic acid	167.5489	#	*Daphnia magna* (*crustacean*)	EC_{50} = 8.15±0.91 mmol/dm^3 [32] EC_{50} (48 h) = 88.1 mg/dm^3 [33]
			Daphnia pulex (*crustacean*) (Daphtoxkit F^{FM})	EC_{50} = 2.0±0.05 mmol/dm^3 [32]
			Desmodesmus subspicatus (algae)	EC_{50} = 106.7 mg/dm^3 [33]
caffeine	47.771	250.505	*Daphnia magna* (*crustacean*)	EC_{50} = 3.521±0.394 mmol/dm^3 [32] EC_{50} (24 h) = 683.752 mg/dm^3 EC_{50} (24 h) = 161.18 mg/dm^3 [34]
chloramphenicol	217.466	278.538	*Daphnia magna* (*crustacean*)	EC_{50} = 1.681±0.082 mmol/dm^3 [32] EC_{50} = 542.86 mg/dm^3 EC_{50} = 1095.42 mg/dm^3 [34]
fluoxetine hydrochloride	4.331	5.259	*Daphnia magna* (*crustacean*)	NOEC (28 d) = 33 µg//dm^3 LOEC (28 d) = 100 µg/dm^3 [33]
			Hyalella azteca (amphipod)	NOEC (21 d) = 8.9 µg/dm^3 LOEC (21d) = 31 µg/ dm^3 LOEC (10 d) = 5.6 mg/kg [33]
			Potamopyrgus antipodarum (freshwater snail)	NOEC = 13 µg/dm^3 LOEC = 69 µg/dm^3 [33]
			Dunaliella tertiolecta (algae)	EC_{50} (96 h) = 169.81 µg/dm^3 [33]
			Pimephales promelas (fish)	LC_{50} (48 h) = 705 µg/dm^3 [33]
HEPES	> 28.596	#	-	-
ketorolac	35.227	52.330	*Daphnia magna* (*crustacean*)	EC_{50} = 155.123 mg/ dm^3 LC_{50} = 473.836 mg/ dm^3 [34]
theophylline	37.834	> 37.834	*Daphnia magna* (*crustacean*)	EC_{50} = 0.861±0.04 mmol/dm^3 [32]
			Daphnia pulex (crustacean) (Daphtoxkit F^{TM})	EC_{50} = 1.82±0.48 mmol/dm^3 [32]
trypsin	> 0.380	> 0.380	-	-
2.4-dichlorobenzoic acid	#	#	*Daphnia magna* (*crustacean*)	EC_{50} = 1.124±0.084 mmol/dm^3 [32]
			Daphnia pulex (*crustacean*) (Daphtoxkit F^{FM})	EC_{50} = 1.47±0.18 mmol/dm^3 [32]

Substance	***Heterocypris incongruens* (Ostracodtoxkit F™)**		**Referenced ecotoxicity data**	
	EC_{50} **[nM]**	LC_{50} **[nM]**	**Bioassay organisms**	
acetaminophen	#	#	*Streptocephalus proboscideus* (*crustacean*) (Streptoxkit F)	EC_{50} (24 h) = 9.2 mg/dm^3 [35]
			Daphnia magna (*crustacean*)	EC_{50} (24 h) = 55.5 mg/dm^3 [35] EC_{50} (48 h) = 30.1 mg/dm^3 EC_{50} (96 h) = 26.6 mg/dm^3 [33]
			Vibrio fischeri (bacteria)	EC_{50} (15 min) = 567.5 mg/dm^3 EC_{50} (30 min) = 650 mg/dm^3 [33]
			Oryzias latipes (fish)	LC_{50} (48 h) > 160 mg/dm^3 LC_{50} (96 h) > 160 mg/dm^3 [33]
			Scenedesmus subspicatus (algae)	EC_{50} (72 h) = 134 mg/dm^3 [33]
			Tetrahymena pyriformis (ciliates)	EC_{50} (48 h) = 112 mg/dm^3 [33]
			Barilius rerio (fish)	LC_{50} (48 h) = 378 mg/dm^3 [33]

- impossible to determine (> 1 mM), "-" - data not available, d - days, h - hours

Table 3

Results of studies on determination of EC_{50}/LC_{50} and other toxicological data for selected chemicals toward bioassay organisms and their direct comparison to values for other organisms for insoluble analytes

Substance	***Heterocypris incongruens* (Ostracodtoxkit F™)**		**Referenced ecotoxicity data**	
	EC_{50} **[ng/kg]**	LC_{50} **[ng/kg]**	**Bioassay organisms**	
17α-ethinylestradiol	36.754	92.477	*Pimephales promelas* (fish)	LOEC (21 d) = 1 ng/dm^3 [33]
			Danio rerio (fish)	LOEC = 2 ng/dm^3 [33]
19-norethindrone	690.544	779.175	-	-
2-(2.4.5-trichlorophenoxy) propionic acid	129.904	289.184	-	-
bezafibrate	1055.429	1806.205	*Hydra attenuate* (cnidarian)	LC_{50} (96 h) = 70.71 mg/dm^3 EC_{50} (96 h) = 25.85 mg/dm^3 LOEC (96 h) = 1 mg/dm^3 NOEC (96 h) = 0.1 mg/dm^3 [33]
			Brachionus calyciflorus (rotifer)	LC_{50} (24 h) = 60.91 mg/dm^3 EC_{50} (48 h) = 0.44 mg/dm^3 LOEC (48 h) = 0.3125 mg/dm^3 NOEC (48 h) = 0.156 mg/dm^3 [33]
			Thamnocephalus platyurus (*crustacean*)	LC_{50} (24 h) = 39.69 mg/dm^3 [33]
			Daphnia magna (*crustacean*)	EC_{50} (24 h) = 100.08 mg/dm^3 [33]
			Ceriodaphnia dubia (*crustacean*)	EC_{50} (48 h) = 75.79 mg/dm^3 EC_{50} (7 d) = 0.13 mg/dm^3 LOEC (7 d) = 0.047 mg/dm^3 NOEC (7 d) = 0.023 mg/dm^3 [33]

Substance	*Heterocypris incongruens* (Ostracodtoxkit F™)		Referenced ecotoxicity data	
	EC_{50} [ng/kg]	LC_{50} [ng/kg]	Bioassay organisms	
gemfibrozil	29.038	33.795	*Hydra attenuate* (cnidarian)	LC_{50} (96 h) = 1.18 mg/dm^3 EC_{50} (96 h) = 22.36 mg/dm^3 LOEC (96 h) = 1 mg/dm^3 NOEC (96 h) = 0.1 mg/dm^3 [33]
			Vibrio fischeri (bacteria)	EC_{50} (30 min) = 85.74 mg/dm^3 EC_{50} (24 h) = 64.6 mg/dm^3 EC_{50} (48 h) = 45.1 mg/dm^3 [33]
			Chlorella vulgaris (algae)	EC_{50} (24 h) = 195 mg/dm^3 EC_{50} (48 h) = 161 mg/dm^3 EC_{50} (72 h) = 150 mg/dm^3 [33]
			Daphnia magna (*crustacean*)	EC_{50} (24 h) = 57.1 mg/dm^3 EC_{50} (48 h) = 42.6 mg/dm^3 EC_{50} (72 h) = 30 mg/dm^3 [33]
			Brachionus calyciflorus (rotifer)	LC_{50} (24 h) = 77.30 mg/dm^3 EC_{50} (48 h) = 0.44 mg/dm^3 LOEC (48 h) = 0.312 mg/dm^3 NOEC (48 h) = 0.156 mg/dm^3 [33]
			Thamnocephalus platyurus (*crustacean*)	LC_{50} (24 h) = 161.05 mg/dm^3 [33]
			Ceriodaphnia dubia (*crustacean*)	EC_{50} (7 h) = 0.53 mg/dm^3 LOEC (7 h) = 0.156 mg/dm^3 NOEC (7 h) = 0.078 mg/dm^3 [33]
ibuprofen	293.536	404.515	*Vibrio fischeri* (bacteria) ToxAlert	EC_{50} = 12.1 mg/dm^3 [35]
			Vibrio fischeri (bacteria) Microtox	EC_{50} = 19.1 mg/dm^3 [35]
			Lepomis macrochirus (fish)	LC_{50} (96 h) = 173 mg/dm^3 [35] NOEC (96 h) = 10 mg/dm^3 [35]
			Daphnia magna (*crustacean*)	LC_{50} (48 h) = 9.06 mg/dm^3 [35] NOEC (48 h) = 3.37 mg/dm^3 [35] EC_{50} (48 h) = 108 mg/dm^3 [33]
			Skeletonema costatum (algae)	EC_{50} (96 h) = 7.1 mg/dm^3 [35]
			Desmodesmus subspicatus (algae)	EC_{50} = 315 mg/dm^3 [33]
			Lemna minor (duckweed)	EC_{50} (7 d) = 22 mg/dm^3 [33]
			Hydra attenuate (cnidarian)	LC_{50} (96 h) = 22.36 mg/dm^3 [33]
			Pareas carinatus (mollusk)	LC_{50} (72 h) = 17.1 mg/dm^3 [33]
indomethacin	59.035	88.374	*Thamnocephalus platyurus* (crustacean)	LC_{50} (24 h) = 16.14 mg/dm^3 [33]
			Oryzias latipes (fish)	LC_{50} (96 h) = 81.92 mg/dm^3 [33]
ketoprofen	476.012	643.583	*Vibrio fischeri* (bacteria) ToxAlert 100	EC_{50} = 15.6 mg/dm^3 [35]
			Microtox *Vibrio fischeri* (bacteria)	EC_{50} = 19.3 mg/dm^3 [35]

Substance	*Heterocypris incongruens* (Ostracodtoxkit F™)		Referenced ecotoxicity data	
	EC_{50} [ng/kg]	LC_{50} [ng/kg]	Bioassay organisms	
naproxen	112.137	307.627	*Vibrio fischeri* (bacteria) ToxAlert 100	EC_{50} = 21.2 mg/dm^3 [35]
			Vibrio fischeri (bacteria) Microtox	EC_{50} = 35 mg/dm^3 [35]
			Daphnia magna *(crustacean)*	EC_{50} (48 h) = 174 mg/dm^3 [33]
			Desmodesmus subspicatus (algae)	EC_{50} (48 h) > 320 mg/dm^3 [33]
			Ceriodaphnia dubia (crustaceans)	EC_{50} (24 h) = 66.37 mg/dm^3 [33]
			Lemna minor (duckweed)	EC_{50} (7 h) = 24.2 mg/dm^3 [33]
			Brachionus calyciflorus (rotifer)	LC_{50} (24 h) = 62.48 mg/dm^3 [33]
oxytetracycline	145.035	378.473	*Daphnia magna (crustacean)*	LC_{10}, LOEC (48 h) = 100 mg/dm^3 - the highest concentration [35]
			Selenastrum capricornutum (algae)	EC_{50} (72 h) = 4.18 mg/dm^3 [35]
			Folsomia fimetaria (springtail)	EC_{50}, NOEC (21 d) > 5000 mg/kg dry weight [35]
			Enchytraeus crypticus (white pot-worm)	EC_{50}, LC_{50}, NOEC (21 d) > 2000 mg/kg dry weight [35]
			Aporrectodea caliginosa (earthworm)	EC_{50}. NOEC (21 d) > 3000 mg/kg dry weight [35]
			Lemna gibba (duckweed)	EC_{50} (7 d) = 1.01 mg/dm^3 LOEC (7 h) = 1 mg/dm^3 [35]
			Vibrio fischeri (bacteria)	EC_{50} (30 min) = 64.5 mg/dm^3 [33]
progesterone	92.137	129.558	-	-

- impossible to determine under test conditions (>1mM), "-" - data not available, d - days, h - hours

Conclusions

Even brief literature search enables statement that there is a huge gap in the exotoxicological datasets reflecting the chronic sensitivity of bioassaying organisms to organic pollutants of pharmaceutical character. By many it can be considered as drawback of using chronic assays in ecotoxicological studies. Fortunately, studies in this field are being more and more often performed and new data is available for researchers. It helps elaborating modelling data/formulae and reference values in case of preparing standard models trying to reflect anthropogenic impact on the surrounding environment.

The promising fact on applicability of ISO standardized Ostracodtoxkit F™ is that it was proven to be more sensitive in comparison to other reviewed organisms while the problem of bioactive chemicals present in the environment keeps and will keep growing due to insufficient removal processes. For ecotoxicological researchers these values are not surprising due to both longer time of exposure of *H. incongruens* (what can be translated in more rational way to reflect toxicological threat posed to mammals etc.) and more active uptake of pollutants present in feed and sediments due to presence of gills and gastrointestinal tract. The next steps of utilizing Ostracodtoxkit F™ should be directed to

determining interactions occurring between chemicals under real environmental conditions in case of their co-presence and varying physicochemical parameters, the problem should be addressed in the nearest future for selected biotests (including Ostracodtoxkit F^{TM}) as the problem of pollution with pharmaceuticals residues will be of growing concern [14].

Acknowledgments

The work has been co-financed by the Polish Ministry of Science and Higher Education grant no. IP2011 028071. We formally state that all studies involving humans or experimental animals were conducted in accordance with national and institutional guidelines for the protection of human subjects and animal welfare.

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WYZNACZANIE PARAMETRÓW TOKSYKOLOGICZNYCH WYBRANYCH ORGANICZNYCH SUBSTANCJI BIOAKTYWNYCH Z ZASTOSOWANIEM TESTU OSTRACODTOXKIT F^{TM}

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Abstrakt: Ocena wpływu pozostałości farmaceutyków na organizmy żywe jest bardzo złożonym zagadnieniem. Oprócz brania pod uwagę toksyczności tylko pojedynczych związków również współobecność wielu zanieczyszczeń musi być rozważana. W niniejszej pracy podjęto próbę wyznaczenia stopnia ekotoksyczności biologicznie aktywnych substancji (o wartościach stężeń efektywnych (EC_{50}) rosnących w szeregu fluoksetyna (EC_{50} = 4.431 nM) >> gemfibrozyl ≈ 17α-etynyloestradiol≈ketorolak > indometacyna > teofilina ≈ progesteron > naproksen ≈ trypsyna > kwas 2-(2,4,5-trichlorofenoksy)propionowy > chloramfenikol > kwas acetylosalicylowy > ibuprofen > ketoprofen > 19-noretyndron ≈ bezafibrat jako najmniej toksyczny lek pośród badanych) wobec testu Ostracodtoxkit F^{TM}, standaryzowanego normą ISO. Test Ostracodtoxkit F^{TM} okazał się bardzo czułym narzędziem pod względem odpowiedzi na obecność farmaceutyków. Wyniki badań uzasadniają stwierdzenie, że więcej badań jest koniecznych do przeprowadzenia w obszarze oceny ekspozycji chronicznej na farmaceutyki i inne nowo pojawiające się zanieczyszczenia, zwłaszcza w przypadku ich obecności w złożonych mieszaninach.

Słowa kluczowe: biotesty, farmaceutyki, toksyczność, *Heterocypris incongruens*, Ostracodtoxkit F^{TM}