Original Article

Assessment of 2,4-difluoroaniline Aquatic Toxicity Using A Zebrafish (*Danio rerio*) Model

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Abstract

Bio-monitoring, in the control strategies for pollution, has several advantages over other tests in aquatic organisms. Bio-effects may link the bioavailability of the compounds of interest with their concentration at target organs and intrinsic toxicity. Potential harmful effects of 2,4-difluoroaniline (DFA), on a 96-hour acute static test, on adult zebrafish (*Danio rerio*) were followed. According to OECD 203: Fish Acute Toxicity Test, LC50 was determined using 70 adult zebrafish AB strain and 14 zebrafish as control group, to evaluate the effects and action at different concentrations of DFA. Obtained data were analyzed using Minitab Statistical Software as Goodness-of-Fit correlation tests. Logarithmic value obtained for LC50 was of 2.30311, which corresponded to LC50 of 200.96 mg/L. Results from the performed work reinforce the idea of the zebrafish adults as simple and easy reproducible model organisms in ecotoxicology. Although there are more comprehensive tests available, this particular one is both rapid and relatively cheap. The results obtained for LC50, in our opinion, are useful and applicable to a large number of substances, especially in field conditions when overall effectiveness is more important than meticulousness of the method.

Keywords: acute static test, adult Danio rerio model, aquatic toxicity, 2,4-difluoroaniline

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Introduction

In order for ecotoxicogenomics to fulfil their immense potential, collaborative efforts are necessary through parallel use of diverse model microorganisms (e.g. *Saccharomyces cerevisiae*), aquatic (e.g. *Oryzias latipes*, *Poecilia reticulata, Danio rerio, Daphnia magna, Lemna minor* and *Xenopus tropicalis*), terrestrial (e.g. *Arabidopsis thailiana, Caenorhabdites elegans* and *Eisenia foetida*) and other (e.g. macroinvertebrates, snails, tadpoles, etc.) (Abdul Rida et al., 1997; Hood et al., 2000; Hawkins et al., 2003; Fraker and Smith, 2004; Snape et al., 2004; Sanchez-Hernandez, 2006; Poynton et al., 2007; Coogan and La Point, 2008; Karathia et al., 2011).

Ecotoxicogenomic tools may also provide better mechanistic understanding of the aquatic ecotoxicology. In this context, advantages of the use of acute toxicity test are represented by a wide range of applicability for chemical substances that can be reproduced easily in laboratory conditions. The purpose of the acute test is to identify the dose which produces 50% mortality of the test organisms, being an important tool for toxicological research (Pennie et al., 2000; Snape et al., 2004).

Currently zebrafish (*Danio rerio*) serve as models for a very wide variety of research. Compared with other species of animals used in experiments, researchers have shown that zebrafish are accepted by the scientific community as having a great applicability in many fields of human and veterinary medicine. For example, in 2008 in the European Union 1,087,155 fish were used in experiments and 440.852 in fundamental biological research (Lawrence, 2007; Reed and Jennings, 2011).

Researches on *Danio rerio* have evolved into several areas: biology, oncology, toxicology, reproduction, teratology, genetics, neurobiology, environmental sciences, stem cell research and regenerative medicine (Khudoley, 1984; Lele and Krone, 1996; Amatruda et al., 2002; Johansen et al., 2006; Sullivan and Kim, 2008; Peterson and Freeman, 2009; Mione and Trede, 2010; Goldshmith et al., 2012).

Due to effortless stock maintenance in captivity and their great advantages, e.g. reduced size, rapid and good reproduction with short life cycle, clear sexual dimorphism, great number of non-adesive transparent eggs per spawning, rapid embrio-development, etc., zebrafish have prevailed among other known test organisms and become a valious tool (Hill et al., 2005; Albertson and Kocher, 2006; Major and Poss, 2007; Spence et al., 2008; De Oliveira, 2009; Reed and Jennings, 2011). For example, as biological models, zebrafish have the advantage of complete genome sequence. Zebrafish larvae, for example, are able to rapidly regenerate fins, skin, heart, larval stages, etc.; these stages are used in several researches (Lele and Krone, 1996; Lawrence, 2007; Johansen et al., 2008; De Oliveira, 2009).

Among the widespread substances, potentially toxic to the environment, aniline is used in various fields of applications (e.g. organic syntheses, tire industry, varnishes, paints, explosives, plastics, antioxidants, antiseptics and disinfectants, etc.) and considered as a major source of industrial pollution. 2,4-difluoroaniline (DFA) is produced as a fine chemical for use in pharmaceutical industries. Moreover, disinfection of raw water for the production of drinking water is an important issue and, in this respect, disinfection products, including 2,4-difluoroaniline (DFA), may exert toxic effects being studied in human and animal models (Boogaard et al., 1994). Repeated and prolonged exposure to 2,4-difluoroaniline can be dangerous for mammals. Potential harmful health effects of 2,4-difluoroaniline on living organisms are on eye (ocular irritation, conjunctivitis, corneal burns), skin (sensibility, allergic reaction, dermatitis), digestive (nausea, vomit, diarrhoea, dizziness, red-brown blood), and respiratory (irritations, pulmonary oedema, CNS depression, asphyxia, methemoglobinemia) (The *NIST WebBook*). In mammals, the metabolic activation of DFA starts with N-oxidation to corresponding hydroxylamine which may be further oxidized in an autocatalytic co oxidation process with haemoglobin (Hb) yielding the nitrosoarene and methemoglobin (met-Hb) (Eadsforth et al., 1984). Being an aniline compound, long-term adverse effects in the aquatic environment can be expected. DFA may also act as harmful aquatic substance (OPPT Chemical Fact Sheets).

In this respect, our goal was to assess the potential harmful and/or toxic effects of DFA on aquatic organisms by acute static test, using an adult zebrafish (*Danio rerio*) model and the monitoring of behavioural disorders arising, dependent on the used substance concentration and in correlation with temperature, pH and dissolved oxygen.

Materials and Methods

The study was performed in compliance with good laboratory practice in accordance with the European Convention principles for the protection of vertebrate animals used in experimental and other scientific purposes, adopted in 1986, in Strasbourg (Council of Europe, 1986); the 2010/63/EU Directive of the European Parliament and of the European Council on the protection of animals used for scientific purposes, adopted on 22 September 2010 (European Council, 2010) in accordance with Romanian law for animal experimentation (Romanian Government, 2014); and with the approval of the Scientific Ethics Committee of the Faculty of Veterinary Medicine Timisoara.

Test Substance: Test substance used in our study was 2,4-difluoroaniline (C6H5F2N) (synonims: 1-amino 2,4-difluorobenzene or 2,4-difluorobenzenamine), a disinfectant with molecular weight of 129.11g/mol and relative density of 1.268 g/mL at 25°C. The solubility of the test substance in water was of 1-5g/100 ml at 20.55°C, constituting a stable solution, strongly incompatible with oxidizing agents, acids, acid chloride, anhydrous acid and becoming susceptible to air exposure in time. 2,4-difluoroaniline (DFA) 99% (D101400_ALDRICH) purchased from Sigma Aldrich Hungary (Budapest, Hungary) was oily liquid, ranging in colour from colourless to dark red (OPPT Chemical Fact Sheets; The *NIST WebBook*). The test substance was

mixed up and successfully homogenized with the water using an S-250D model digital sonifier (Branson Ultrasonics, Danbury, USA), for two minutes at 40°C. The chemical concentration was obtained accurately and then adjusted for the needed concentrations in the fish tanks.

Zebrafish: According to the OECD 203, 70 adult zebrafish AB strain and 14 adult zebrafish as control group were used to evaluate the effects of the test substance and its action at different concentrations on the living organisms (OPPT Chemical Fact Sheets). The chosen zebrafish were aged between 6 and 24 months (between 2.5-3 cm long and between 0.2-0.3 g per individual). The fish were maintained in three litre plastic test aquariums ZebTEC rack model (Tecniplast, Italy), with Active Blue technology static system to maintain the test water within the allowed limits. Room temperature was 23°C and water temperature in the aquariums was 26°C. To record the room temperature, the space was fitted with a thermostat and an air conditioning system to maintain optimum parameters and avoid overheating. The parameters of temperature, pH and oxygen were recorded using a Hach HQ40D meter (Hach Lange GmbH, Germany). At the start of the test, the parameters registered were: temperature (26°C), conductivity (515 mS), and pH of the water in the system (7.5). Seven fish were introduced in each study aquarium, which meant a total weight of 2.1 g./aquarium. Prior to the test the fish were fed twice a day with SDS (Small granular feed) (Dietex International, UK) but during the 96 hours of the testing period, the fish were not fed at all. Photoperiod cycle was maintained for 16 h light/8 h dark daily, with an oxygen saturation of 80%. In the cases where the dissolved oxygen fell below 60%, water filtration was carried out and the aeration rate was settled at 10 liters/minute. Behaviour of the fish in each replicate was inspected daily.

Test Methodology: Prior to the introduction of water into the aquariums, from the total three liters of water, an equal amount representing the test solution which was added at the start of experiment was removed. Then, the fish were placed rapidly in the test aquariums (in a period of just 30 minutes after the dilutions) and their behaviour was followed and recorded at 0, 3, 6, 24, 48, 72 and 96 hours after the experiment started. A fish was considered dead if no gill movements were noticed and if there was no reaction when the caudal peduncle was touched. LC50 was determined using the OECD guidelines which describes the Fish Acute Toxicity Test (Test No. 203). The samples were done in duplicate (groups A and B). An amount of 1.2302 ml, from the previously homogenised test substance, was diluted with 100 ml of water. Then, as previously developed, "range finding test", five concentrations were chosen for testing purposes as follows: 100 mg/L as maximum, 75 mg/L, 50 mg/L, 25 mg/L as intermediate, and 10 mg/L as minimum concentrations. The concentration which killed all the fish in the test was 100 mg/L and the concentration where no fish died was 10 mg/L, therefore the maximum concentration of the solution to a fish did not exceeded 1 g/L (Table 1).

Table 1 Proportions used for dilution in testing

Testing solution's concentration (mg/L)	Concentration's logarithm	Substance quantity used (ml)	Water quantity used (ml)
100	1.00000	19.23	2980.77
75	1.39794	14.42	2985.58
50	1.69897	9.61	2990.39
25	1.87506	4.80	2995.20
10	2.00000	1.92	2998.08

Table 2 Evolution of mortalities, temperature, pH and dissolved oxygen during the entire test period

Dilution / determination time interval (h)		No. of death fish				pH				Temperature (°C)				O_2 (%)			
		24	**48**	**72**	**96**	**24**	**48**	**72**	**96**	**24**	**48**	**72**	**96**	**24**	**48**	**72**	**96**
Lot																	
Control	**1**	0	0	0	0	24	48	72	96	24	48	72	96	24	48	72	96
	2	0	0	0	0	7.45	7.07	7.77	7.54	23.7	24.3	24.5	28.2	91.3	61.9	95.5	91.1
10 mg/L	**A**	0	0	0	0	7.52	7.21	7.62	7.37	23.5	24.2	24.3	27.9	90.3	57.5	92.5	72.4
	B	0	0	0	0	7.47	7.17	7.70	7.28	23.5	24.3	24.2	27.9	83.1	60.1	97.2	65.5
25 mg/L	**A**	0	0	0	0	7.52	7.16	7.72	7.26	23.5	24.4	24.3	27.9	91.2	69.3	99.7	67.6
	B	0	0	0	0	7.53	7.17	7.61	7.36	23.8	24.4	24.5	27.8	81.1	68.8	98.4	74.2
50 mg/L	**A**	3	2	0	0	7.46	7.24	7.78	7.39	23.9	24.4	24.4	27.8	83.5	61.2	95.8	77.9
	B	3	4	-	-	7.39	7.13	7.82	7.26	24.1	24.5	24.4	28.0	80.4	69.9	97.2	63.7
75 mg/L	**A**	6	1	-	-	7.50	7.12	-	-	24.2	24.5	-	-	84.1	76.3	-	-
	B	7	-	-	-	7.55	7.08	-	-	24.2	24.6	-	-	90.5	79.8	-	-
100 mg/L	**A**	7	-	-	-	7.57	7.07	-	-	24.2	24.6	-	-	92.9	78.2	-	-
	B	6	1	-	-	7.47	7.11	-	-	23.7	24.4	-	-	89.5	69.2	-	-

Statistical Analysis: The obtained data was analyzed using the Minitab Statistical Software (Minitab Ltd. Coventry, UK) as Goodness-of-Fit correlation tests (GOF). This is a statistical model describing how a set of observations are fitting, measuring the discrepancy between the observed values and the values expected under the model studied. Differences were considered to be significant when $p < 0.05$.

Results

In Table 2 the mortality trends for A and B groups are shown, at 24 and 48 hours after testing. It should be noted that at 24 hours after the start of the test at concentrations of 75 mg/L and 100 mg/L in the case of group B, all fish died. The chemical parameter analysis revealed that the pH remained at values above 7.39 on the first day of testing, decreased to 7.07 after 48 hours and then increased again until the end of the test period (Table 2).

The thermal curve remained in the comfort zone of the zebrafish until the last day of testing, when a slight increase of the water temperature was observed. Dissolved oxygen in the water was within normal limits on the first day, but after 48 hours the concentration decreased considerably that it was necessary to introduce aeration filters, in the case of aquariums where the oxygen levels dropped below 60%.

In our observations all fish exposed to DFA excerted behavioural changes associated with a visible anxiety. The main behavioural and critical points observed after 24 hours were intense stress manifested by the fish trying to escape (especially at the concentration of 100 mg/L) and general refusal to swim and move away from the aquarium base. The most obvious behavioural disorders were shaking of the body (at the concentration of 75 mg/L) as well as swimming sideways, and motion incoordination (at the concentration of 100 mg/L). By processing the Minitab Software statistical program as Goodness-of-Fit correlation tests, it was suggested that the value distribution was in concordance with the obtained data ($p = 0.029$). Logarithmic value obtained for LC50 was 2.30311, which corresponded to a LC50 of 200.96 mg/L.

Under testing it was observed that the survival rate gradually progressed in direct correlation with the concentration's logarithm reported to the deaths recorded from the beginning of the test. The survival rates according to the number of dead fish, the cumulative failure reported to the log concentration and the probability reported to the log concentration are presented in Figures 1, 2 and 3, respectively.

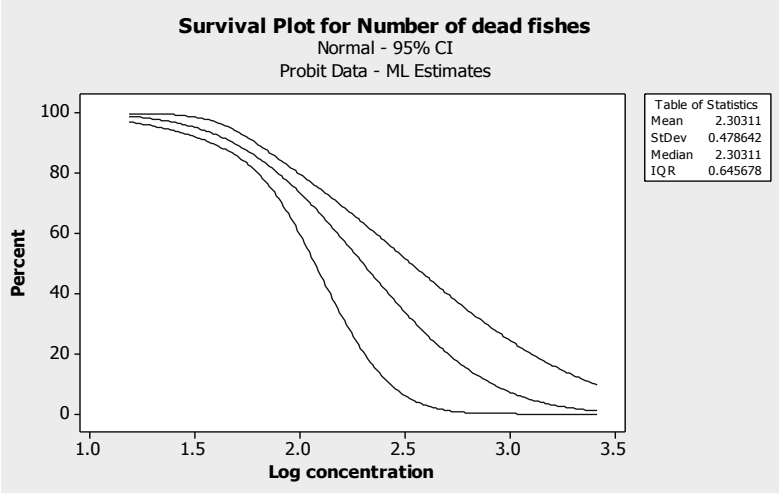

Figure 1 Survival rates according to the number of dead fish

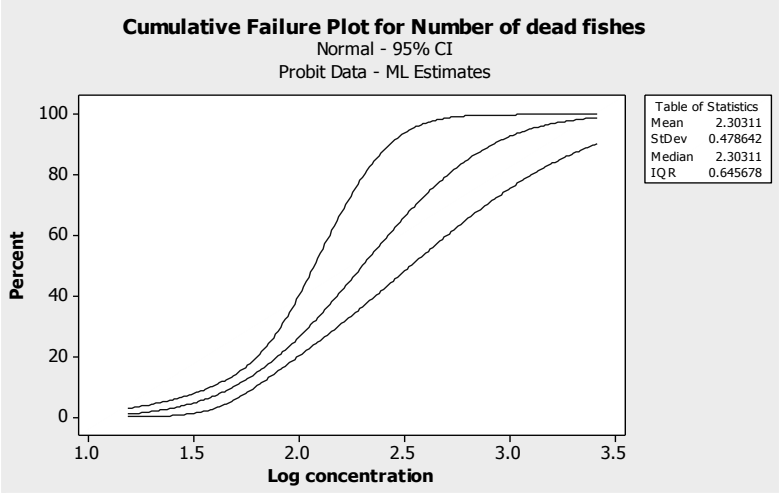

Figure 2 Cumulative failure reported to the log concentration

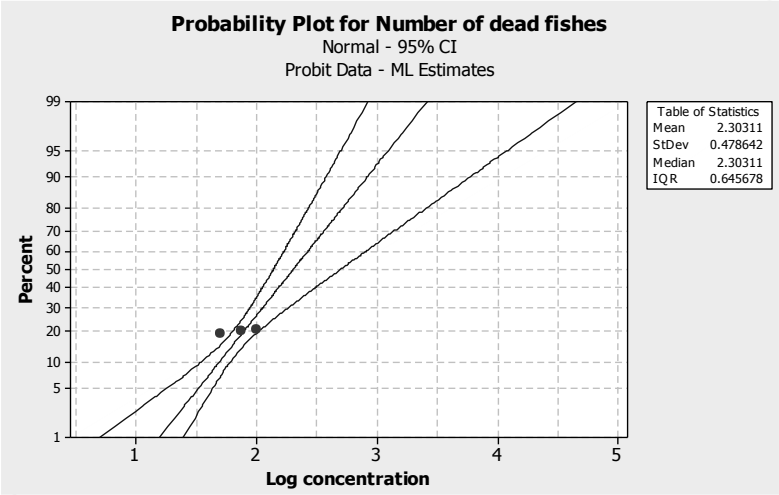

Figure 3 Probability reported to the log concentration

Discussion

Until now, numerous guidelines have been developed for the ecotoxicological biomonitoring based to the importance of fish in aquatic pollution. Among these, the *Danio rerio* models on adult fish and embryos are one the most frequently studied, compared and finally used as screening assays (Nagel, 2002; Scholz et al., 2008; Lammer et al., 2009). Numerous papers focusing on this topic underlie the opportunity of new laboratory techniques and protocols for the standardization of zebrafish as model for the ecotoxicological evaluation (Langheinrich, 2003; Spitsbergen and Kent, 2003; Van der Oost et al., 2003; Braunbeck et al., 2005; Zon and Peterson, 2005).

At international level, the Organization of Economic Co-operation and Development (OECD) and International Organization for Standardization (ISO) have proposed protocols for ecotoxicity assessment with zebrafish. Initially, the protocols were established for acute toxicity assessment in adult fish and early-life stage (OECD 203 and OECD 210), short-term effects on early-life stage (OECD 212) and juvenile growth (OECD 215). At present, modifications in old guidelines have been discussed and new draft guidelines that include more sophisticated endpoints for ecotoxicity assessment have been proposed. For example, the new guidelines proposed give more emphasis on specific mode of action of compounds (e.g. endocrine disruptors) and focus on full life cycle studies. However, the limitations of bio-monitoring, such as confounding factors that are not related to pollution, should be carefully considered when interpreting data (Scholz and Mayer, 2008; Zounková et al., 2011; OECD, Work Related to Endocrine Disrupters, 2012).

Rácz et al. (2012) evaluated the toxic effects of 4-ethylbenzaldehyde (EBA) and 2,4-difluoroaniline (DFA) on a similar model on adult zebrafish like ours. The reserchers observed the effect of DFA on the same main behavioural and critical points exerced by zebrafish under contact with this substance. Bencsik et al. (2013) tested 2,4-difluoroaniline in a 120-hour test on a zebrafish embryo model and obtained a LC50 value of 171.6 mg/L, comparable to our results on adult fish, for the same substance.

Analyzing the LC50 value obtained, we consider it to be a certain value for DFA in the acute 96-hour static test in zebrafish model. It is to mention that water pH, temperature and oxygen levels can significantly influence the final values, justifying small differences between the final values and another. In our case the registered values for 96 hours ranged between: 7.07 and 7.82 for pH, 23.5 and 28.8^{o}C for temperature, and 57.5 and 99.7% for disolved oxygen, at a water conductivity of 515 mS.

In conclusion, results from current work reinforce the idea of using the adult zebrafish as simple and easy reproducible model organisms in water ecotoxicology. Although there are more comprehensive tests available, this particular one is both rapid and relatively cheap. The results obtained for LC50, in our opinion, are useful, especially in field conditions when overall effectiveness is more important than meticulousness of the method. In accord with previous studies, we suggest zebrafish as the ideal and attractive choice for initial drug toxicity screening and other biomedicine tests.

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References

Abdul-Rida AM, Bouché, MB 1997. Earthworm toxicology: from acute to chronic tests. Soil Biol Biochem 29: 699-703.

Albertson RC, Kocher TD 2006. Genetic and developmental basis of cichlid trophic diversity. Heredity 97: 211-221.

Amatruda JF, Shepard JL, Stern HM, Zon LI 2002. Zebrafish as a cancer model system. Canc Cell 1: 229–231.

Bencsik D, Kanizsai B, Bakos K, Kovacs R, Gazsi G, Csepeli A, Racs G, Szende B, Urbanyi B, Csenki Z 2013. Hagyományos víztisztítási technológiák során visszamaradó melléktermék vegyületek vizsgálata halembrió- modellen (in Hungarian) Halaszati Tudomanyos Tanacskazas (HTT) XXXVII: 38.

Boogaard PJ, Fokkema GN, Beulink GD, Bouskill J, van Sittert NJ 1994. Molecular dosimetry of 2,4-difluoroaniline in humans and rats by determination of hemoglobin adducts Environ Health Perspect 102: 27–29.

Braunbeck T, Boettcher M, Hollert H, Kosmehl T, Lammer E, Leist E, Rudolf M, Seitz N 2005. Towards an alternative for the acute fish LC(50) test in chemical assessment: the fish embryo toxicity test goes multi-species - an update. ALTEX 22: 87-102. Coogan MA, La Point, TW 2008. Snail bioaccumulation of triclocarban, triclosan, and methyltriclosan in a North Texas, USA, stream affected by wastewater treatment plant runoff. Environ Toxicol Chem 27: 1788–1793.

Council of Europe 1986. ECPVAEOSP: European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. Strasbourg, France: Council of Europe. [Online]. Available: http://conventions.coe.int/treaty/en/treaties/html/123.htm. Accesed 10 Dec 2013.

De Oliveira R 2009. Zebrafish early life-stages and adults as a tool for ecotoxicology assessment. Disertation, Biology Department, University of Aveiro, Portugal. [Online]. Available: https://ria.ua.pt/bitstream/10773/8838/1/6237.pdf. Accesed 10 Dec , 2013.

Eadsforth CV, Logan CJ, Morrison BJ, Warburton PA 1984. 2,4-difluoroaniline and 4-fluoroaniline exposure: monitoring by methaemoglobin and urine analyses. Int Arch Occup Environ Health 54: 223-232.

European Council (2010). Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes. Brussels, Belgium: European Council. [Online]. Available: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF. Accesed 10 Dec , 2013.

Fraker SL, Smith GR 2004. Direct and interactive effects of ecologically relevant concentrations of organic wastewater contaminants on *Rana pipiens* tadpoles. Environ Toxicol 19: 250–256.

Goldshmith Y, Sztal TE, Jusuf P, Hall TE, Nguyen-Chi M, Currie PD 2012. Fgf- Dependent glial cell bridges facilitate spinal cord regeneration in zebrafish. The J Neurosci 32: 7477-7492.

Hawkins WE, Walker WW, Fournie JW, Manning CS, Krol RM 2003. Use of the Japanese medaka (*Oryzias latipes*) and guppy (*Poecilia reticulata*) in carcinogenesis testing under national toxicology program protocols. Toxicol Pathol 31: 88-91.

Hill AJ, Teraoka H, Heideman W, Peterson RE 2005. Zebrafish as a model vertebrate for investigating chemical toxicity. Toxicol Sci 86: 6–19. (doi: 10.1093/toxsci/kfi110).

Hood TE, Calabrese EJ, Zuckermann BM 2000. Detection of an estrogen receptor in two nematode species and inhibition of binding and development by environmental chemicals. Ecotoxicol Environ Saf 47: 74–81.

Johansen R, Needham JR, Colquhoun DJ, Poppe TT, Smith AJ 2006. Guidelines for health and welfare monitoring of fish used in research. Lab Animal 40: 323–340.

Karathia H, Vilaprinyo E, Sorribas A, Alves R 2011. *Saccharomyces cerevisiae* as a model organism: a comparative study. PLoS One 6: e16015.

Khudoley VV 1984. Use of aquarium fish, *Danio rerio* and *Poecilia reticulata*, as test species for evaluation of nitrosamine carcinogenicity. Natl Canc Inst Monogr 65: 65–70.

Lammer E, Carr GJ, Wendler K, Rawlings JM, Belanger SE, Braunbeck T 2009. Is the fish embryo toxicity test (FET) with the zebrafish (*Danio rerio*) a potential alternative for the fish acute toxicity test? Comp Biochem Physiol C Toxicol Pharmacol 149: 196-209.

Langheinrich U 2003. Zebrafish: a new model on the pharmaceutical catwalk. BioEssays 25: 904-912.

Lawrence C 2007. The husbandry of zebrafish (*Danio rerio*): a review. Aquaculture 269: 1-20.

Lele Z, Krone PH 1996. The zebrafish as a model system in developmental, toxicological and transgenic research. Biotechnol Adv 14: 57-72

Major RJ, Poss KD 2007. Zebrafish heart regeneration as a model for cardiac tissue repair. Drug Discov Today Dis Models 4: 219–225.

Mione MC, Trede NS 2010. The zebrafish as a model for cancer. Dis Model Mech 3: 517–523.

Nagel R 2002. DarT: the embryo test with the zebrafish *Danio rerio* - a general model in ecotoxicology and toxicology. ALTEX 19: 38-48.

OECD, Work Related to Endocrine Disrupters (2012) [Online]. Available: http://www.oecd.org/env/ehs /testing/50067203.pdf. Accesed 20 Feb. 2013.

OPPT Chemical Fact Sheets. Aniline Fact Sheet: Support Document (CAS No. 62-53-3). [Online]. Available: http://www.epa.gov/chemfact/anali-sd.pdf - Accesed 20 Feb. 2013.

Pennie WD, Tugwood JD, Oliver GJ, Kimber I 2000. The principles and practice of toxicogenomics: applications and opportunities. Toxicol Sci 54: 277–283.

Peterson SM, Freeman JL 2009. Cancer cytogenetics in the zebrafish. Zebrafish 6: 355-360.

Poynton HC, Varshavsky JR, Chang B, Cavigiolio G, Chan S, Holman PS, Loguinov AV, Bauer DJ, Komachi K, Theil EC, Perkins EJ, Hughes O, Vulpe CD 2007. *Daphnia magna* ecotoxicogenomics provides mechanistic insights into metal toxicity. Environ Sci Technol 41:1044-1050.

Rácz G, Csenki Z, Kovács R, Hegyi A, Baska F, Sujbert L, Zsákovics I, Kis R, Gustafson R, Urbányi B, Szende B 2012. Subacute toxicity assessment of

water disinfection byproducts on zebrafish. Pathol Oncol Res 18: 579-584.

Reed B, Jennings M 2011. Guidance on the housing and care of zebrafish *Danio rerio*, Research Animals Department, Science Group, RSPCA, West Sussex, UK.

Romanian Government (2014). Legea nr. 43, din 11 aprilie 2014, privind protecţia animalelor utilizate în scopuri ştiinţifice (in Romanian). [Online]. Available: http://www.cdep.ro/pls/legis/legis_pck.htp_act?ida=123790. Accesed, 07 May 2014.

Sanchez-Hernandez JC 2006. Earthworm biomarkers in ecological risk assessment. Rev Environ Contam Toxicol 188: 85–126.

Scholz S, Fischer S, Gundel U, Kuster E, Luckenbach T, Voelker D 2008. The zebrafish embryo model in environmental risk assessment - applications beyond acute toxicity testing. Environ Sci Pollut Res 15: 394–404.

Scholz S, Mayer I 2008. Molecular biomarkers of endocrine disruption in small model fish. Mol Cell Endocrinol 293: 57-70.

Snape JR, Maund SJ, Pickford DB, Hutchinson TH 2004. Ecotoxicogenomics: the challenge of integrating genomics into aquatic and terrestrial ecotoxicology. Aquat Toxicol 67: 143-54.

Spence R, Gerlach G, Lawrence C, Smith C 2008. The behaviour and ecology of the zebrafish, *Danio rerio*. Biol Rev Camb Philos Soc 83: 13-34.

Spitsbergen JM, Kent ML 2003. The state of the art of the zebrafish model for toxicology and toxicologic pathology research - advantages and current limitations. Toxicol Pathol 31: 62-87.

Sullivan C, Kim CH 2008. Zebrafish as a model for infectious disease and immune function. Fish Shellfish Immunol 25: 341-350.

Test No. 203. Fish, acute toxicity test. OECD, July 17, 1992. [Online]. Available: http://www.oecd-ilibrary.org/environment/test-no-203-fish-acute-toxicity-test_9789264069961-en. Accesed 20 Feb., 2013.

Test No. 210. Fish, Early-Life Stage Toxicity Test, OECD, 17 July 1992, pages: 18, [Online]. Available: http://www.oecd-ilibrary.org/environment/test-no-210-fish-early-life-stage-toxicity-test_9789264070103-en. Accesed 20 Feb., 2013.

Test No. 212. Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages. OECD, 21 Sep 1998, pages: 20, [Online]. Available: http://www.oecd-ilibrary.org/environment/test-no-212-fish-short-term-toxicity-test-on-embryo-and-sac-fry-stages_9789264070141-en.- Accesed 20 Feb., 2013.

Test No. 215. Fish, Juvenile Growth Test, OECD, 21 Jan 2000, pages: 16, [Online]. Available: http://www.oecd-ilibrary.org/environment/test-no-215-fish-juvenile-growth-test_9789264070202-en. Accesed 20 Feb., 2013.

The NIST WebBook - NIST Standard Reference Data, 2,4-difluoroaniline. [Online]. Available: http://webbook.nist.gov/cgi/cbook.cgi?ID=C367259&Mask=20. Accesed 20 Feb. 2013.

Van der Oost R, Beyer J, Vermeulen NPE 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharmacol 13: 57-149.

Zon LI, Peterson RT 2005. In vivo drug discovery in the zebrafish. Nat Rev Drug Discov 4: 35-44.

Zounková R, Klimešová Z, Nepejchalová L, Hilscherová K, Bláha L 2011. Complex evaluation of ecotoxicity and genotoxicity of antimicrobials oxytetracycline and flumequine used in aquaculture. Environ Toxicol Chem 30: 1184-1189.

บทคัดย่อ

การประเมินความเป็นพิษในน้ำของ 2,4-difluoroaniline โดยใช้โมเดลปลาม้าลาย (*Danio rerio*)

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การตรวจติดตามทางชีวภาพซึ่งเป็นกลยุทธ์ที่ใช้ควบคุมมลพิษนั้น มีประโยชน์หลายประการซึ่งเหนือการทดสอบอื่นในสิ่งมีชีวิตที่อาศัยในน้ำ ผลกระทบทางชีวภาพอาจเชื่อมโยงกับความคงอยู่ทางชีวภาพของสารประกอบที่สนใจ ที่มีความเข้มข้นในอวัยวะเป้าหมายและความเป็นพิษโดยธรรมชาติ มีการศึกษาติดตามผลที่อันตรายของ 2,4-difluoroaniline (DFA) โดยการทดสอบแบบ acute static test ที่ 96 ชั่วโมงต่อปลาม้าลาย (*Danio rerio*) ที่เจริญเต็มที่ จาก OECD 203: การทดสอบความเป็นพิษอย่างเฉียบพลันในปลา ได้มีการวัด LC50 โดยใช้ปลาม้าลายสายพันธุ์ AB ที่เจริญเต็มที่จำนวน 70 ตัว และจำนวน 14 ตัวเป็นกลุ่มควบคุม เพื่อประเมินผลกระทบและการแสดงออกที่ความเข้มข้นของ DFA ที่แตกต่างกัน ข้อมูลที่ได้ได้ถูกนำมาทดสอบ Goodness-of-Fit correlation โดยใช้ซอฟท์แวร์ทางสถิติ Minitab ค่า logarithmic ที่ได้จาก LC50 มีค่า 2.30311 ซึ่งสอดคล้องกับ LC50 200.96 mg/L ผลจากงานที่ได้ทำเน้นถึงแนวคิดว่าปลาม้าลายที่เจริญเต็มที่สามารถนำมาใช้เป็นต้นแบบของสิ่งมีชีวิต ในด้านนิเวศน์พิษวิทยาที่ไม่ซับซ้อนและทำซ้ำได้ ถึงแม้ว่ามีการทดสอบที่ละเอียดกว่านี้ การทดสอบชนิดนี้มีทั้งความไวและมีราคาค่อนข้างย่อมเยา จากความเห็นของผู้วิจัย ผลการทดลองที่ได้รับจาก LC50 นั้นมีประโยชน์และเหมาะกับสารประกอบที่มีจำนวนมาก โดยเฉพาะในสภาพท้องที่เมื่อประสิทธิภาพโดยรวมมีความสำคัญมากกว่าความละเอียดของวิธีการ

คำสำคัญ: acute static test, ต้นแบบ *Danio rerio* ที่เจริญเต็มที่, ความเป็นพิษในน้ำ, 2,4-difluoroaniline

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