

Fontex[®]

MR EF

Lilly

Dispergerbar tablett 20 mg

(Vita, odragerade, ovala tabletter med skåra, märkta 4400. Storlek 8,0 x 13,0 mm)

Antidepressivum, selektiv serotoninåterupptagshämmare

Aktiv substans:

Fluoxetin

ATC-kod:

N06AB03

Läkemedel från Lilly omfattas av Läkemedelsförsäkringen.

Miljöpåverkan

Fluoxetin

Miljörisk: Användning av fluoxetin har bedömts medföra låg risk för miljöpåverkan.

Nedbrytning: Fluoxetin är potentiellt persistent.

Bioackumulering: Fluoxetin har låg potential att bioackumuleras.

Detaljerad miljöinformation

Environmental Risk Classification

Predicted Environmental Concentration (PEC)

$PEC (\mu\text{g/l}) = (A \times 1000000000 \times (100 - R)) \div (365 \times P \times V \times D \times 100)$

$= 0,00000137 \times A \times (100 - 0)$

$= 0,00000137 \times 607,0332539 \times 100$

$= 0.08316 \mu\text{g/l}$

Where:

A = 607,0332539 kg (total amount sold in Sweden in 2023 as fluoxetine, data from IQVIA). This number is not adjusted for metabolism.

API form	Sales in 2023 kg
fluoxetine hydrochloride	678,47686811

fluoxetine	607,0332539*
------------	--------------

*calculated by multiplying the kg of fluoxetine hydrochloride sold by the molecular weight ratio of fluoxetine free base:fluoxetine hydrochloride salt (309.33:345.79)

R = Assumed 0% removal rate in a sewage treatment plant

P = $10 \cdot 10^6$ population of Sweden

V = 200 L of wastewater per capita per day (default from ECHA, 2012)

D = 10 dilution of wastewater by surface water flow (default from ECHA, 2012)

Measured Environmental Concentration (MEC)

Barclay et al (2012) have reported concentrations of fluoxetine and its major metabolite, norfluoxetine, in raw and treated waste water in Uppsala, Sweden that ranged from 0.001 to 0.016 µg/l. The Swedish Environmental Research Institute has conducted a national screening program for 101 pharmaceuticals in urban wastewater treatment plants and their receiving waters (Fick et al 2011, 2015). In 2010, samples of wastewater treatment plant influent and effluent (total of 24 samples) were analyzed and 21 samples had measurable levels of fluoxetine ranging from 0.005 to 0.240 µg/l, with a mean concentration of 0.041 µg/l and a median concentration of 0.017 µg/l. In 2014, samples of wastewater treatment plant influent and effluent (total of 15 samples) were analyzed and 7 had measurable levels of fluoxetine ranging from 0.0051 to 0.024 µg/l, with a mean concentration of 0.012 µg/l and a median concentration of 0.010 µg/l. In the river that received effluent from the Uppsala WWTP, fluoxetine was detected in both 2010 and 2014 at 0.032 and 0.0065 µg/l near the effluent outfall but only in 2010 150 m downstream (0.006 µg/l). Sampling points further downstream and in other surface waters did not have measurable levels (limit of quantitation was 0.005 µg/l). The surface water data reported by Fick et al (2011, 2015) are in agreement with previous reports of detection of fluoxetine in surface water (Kolpin et al 2002; Conley et al 2008; Lajeunesse et al 2008; Metcalfe et al 2003; Gros et al 2009) in that the detections are infrequent and the levels are less than 0.05 µg/l. Therefore, the calculated PEC of 0.08316 µg/l is greater than actual measured concentrations detected in surface water. Nonetheless, the PEC of 0.08316 µg/l will be used to assess the environmental risk.

Predicted No Effect Concentration (PNEC)

Ecotoxicological Studies

For the required ecotoxicity studies for FASS classification, a guideline-driven study conducted under Good Laboratory Practices (GLP) is listed, if available. If not available, literature data is cited as there are several published studies describing the ecotoxicity of fluoxetine. Chronic studies from the literature were restricted to those that tested fish and daphnids, reported apical endpoints of survival, growth and/or reproduction, and could be judged to be of acceptable quality and design (e.g. sufficient description of methods, use of appropriate statistics, exposure solution renewal or analytical confirmation of exposure, concentration spacing that supported NOEC determination).

Algae

Study J01292 with Pseudokirchneriella subcapitata (FDA TAD 4.01; GLP)

This study was 14 days and biomass endpoints are based on the 14 day data. Growth rate was determined over the period during which exponential growth occurred (the first 4 days).

EC50 (growth rate) = 30.5 µg/l

NOEC (growth rate) = 7.4 µg/l

While the NOEC for decrease in biomass after 14 days (1.2 µg/l) is lower, the NOEC for the growth rate is the preferred endpoint for classification (ECHA Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7b 2016).

Crustacean (Daphnids)

Acute toxicity

Study C03992 with *Daphnia magna* (FDA TAD 4.08; GLP)

EC50 48 h (immobilization) = 940 µg/l

Chronic toxicity

Oakes et al 2010 with *Daphnia magna* (21-day exposure; survival, growth, reproduction; standard test design, OECD 211)

NOEC = 60 µg/l

Stanley et al 2007 with *Daphnia magna* (21-day exposure; survival, growth, reproduction; standard test design, OECD 211)

NOEC = 174 µg/l

Henry et al 2004 with *Ceriodaphnia dubia* (7-day exposure; survival, reproduction; standard test design, EPA Method 1002)

NOEC = 89 µg/l

Brooks et al 2003 with *Ceriodaphnia dubia* (7-day exposure; survival, reproduction; standard test design, EPA Method 1002)

NOEC = 56 µg/l

Péry et al 2008 with *Daphnia magna* (2-generation test; survival, reproduction, length of offspring)

NOEC = 8.9 µg/l (nonstandard test design)

Fish

Acute Toxicity

Study F02892 with rainbow trout, *Oncorhynchus mykiss* (OECD 203; GLP)

LC50 96 h (mortality) = 1400 µg/l

Chronic toxicity

Henry and Black 2008 with mosquitofish, *Gambusia affinis* (91-day exposure with larvae; survival, sex ratio, weight and length, development; nonstandard test design)

NOEC = 5 µg/l (highest concentration tested)

Henry and Black 2008 with mosquitofish, *Gambusia affinis* (100-day exposure starting at age 59 days; survival, growth; nonstandard test design)

NOEC = 7 µg/l (next highest concentration was 71 µg/l)

Weinberger and Klaper 2014 with fathead minnow, *Pimephales promelas* (4-week exposure to adults; reproduction and behavior; nonstandard test design)

NOEC (based on reproduction) = 10 µg/l

Foran et al 2004 with medaka, *Oryzias latipes* (4 week exposure to adults; reproduction; nonstandard test design)

NOEC = 5 µg/l (highest concentration tested)

Lister et al 2009 with zebrafish, *Danio rerio* (7-day exposure to adults; reproduction; nonstandard test design)

NOEC = 3.2 µg/l

Stanley et al 2007 with fathead minnows, *Pimephales promelas* (7-day exposure to 6-day old larvae; survival, weight; US EPA Method 1000.0)

NOEC = 9 µg/l

Calculation of PNEC

$PNEC = 3,2 \mu\text{g/l} \div 10 = 0,32 \mu\text{g/l}$

PNEC is the lowest NOEC from a chronic study divided by an assessment factor of 10. 10 was chosen as the assessment factor because apical endpoints from chronic exposures to algae, daphnids and fish are available. The lowest NOEC with standard study designs is 7,4 µg/l for growth rate in algae. While no standard chronic studies are available in fish, there are several nonstandard studies in the literature which demonstrate that fish exposed to fluoxetine are susceptible to specific effects on growth and reproduction at high enough concentrations. Therefore, nonstandard studies from the literature that assessed effects on apical endpoints were considered. The lowest NOEC of 3.2 µg/l was by Lister et al (2009) in which adult

zebrafish were exposed to three concentrations of fluoxetine for 7 days and evaluated for egg production (see Appendix for brief study design). In this study, a control was included, water quality was evaluated, and test solutions were renewed daily.

Environmental risk classification (PEC/PNEC Ratio)

$$\text{PEC/PNEC} = 0.08316 \div 0,32 = 0,26$$

The PEC/PNEC ratio of less than 1 but greater than 0,1 justifies the phrase "Use of fluoxetine has been considered to result in low environmental risk."

Degradation

Biotic Degradation

Ready degradability:

Fluoxetine was not readily biodegradable in an OECD 301B test (N00793).

Inherent degradability:

Very little evidence of transformation of fluoxetine was observed when radiolabeled compound was incubated for 28 days with a microbial inoculum prepared from soil and secondary effluent from a wastewater treatment plant. (Study 40227, FDA 3.11).

Simulation studies:

Kwon and Armbrust (2006) evaluated the fate of fluoxetine in two natural water sediment systems using a study design similar to the OECD 308 (but only 30 days rather than 100). Due to partitioning to sediment, fluoxetine disappeared quickly from the water layer with DT50 values of 4,6 and 6,6 days. The distribution of fluoxetine in the two water sediment systems was used to calculate the DT50 values of fluoxetine from the total system (assuming first order disappearance kinetics). Fluoxetine disappeared from the total system with DT50 values of 32 days (lake sediment) and 114 days (creek sediment) which is likely due, in part, to unextractable binding to the sediment. Fluoxetine slowly disappears from water sediment systems.

Abiotic Degradation

Hydrolysis:

Fluoxetine is hydrolytically stable (Study 40229, FDA 3.09).

Photolysis:

Based on a lack of absorbance maxima between 800 and 290 nm, fluoxetine is expected to be photolytically stable (Study 40233, FDA 3.05).

Removal during sewage treatment

The log K_{oc} values for sludge, sediment and soil range from 2,73 to 5,18 (summarized by Oakes et al 2010). In a batch sorption study with a suspended sewage solids level of 3000 mg/l, more than 90% of fluoxetine was removed from the water column due to binding to sludge (Yamamoto et al 2005). Comparison of influent and effluent concentrations of fluoxetine in a Swedish waste water treatment facility confirmed a removal rate of greater than 90% (Zorita et al 2009). However, Barclay et al (2012) and Fick et al (2011) report that at five other waste water treatment plants in Sweden, there is little difference between the concentrations of fluoxetine in the influent and the effluent. Therefore, for this classification, minimal to no removal during sewage treatment is assumed.

Justification of the degradation phrase:

While fluoxetine disappears from a water sediment system with a calculated half-life of less than 120 days, the study design was for 30 days rather than the 100-day duration of the OECD 308. Therefore, the degradation phrase "Fluoxetine is potentially persistent" is based on the absence of a standard water sediment degradation study and the lack of ready and inherent biodegradability.

Bioaccumulation

Partitioning coefficient:

The log of the octanol water partition coefficients at pH values of 5, 7, and 9 were 0,73 to 1,06, 1,77 to 1,79, and 2,62 to 2,64, respectively (Study 40230, FDA 3.02).

Bioconcentration factor (BCF):

Measured concentrations of fluoxetine in fish confirm a low bioaccumulation of fluoxetine with reported BCF values of 84 L/kg (14 days exposure in rainbow trout, Zhou et al 2008), 74 and 80 L/kg (7 days in Japanese medaka, Paterson and Metcalfe 2008), and 13, 37, 330 L/kg (at approximate pH values of 7, 8, and 9, respectively, after 30 days in Japanese medaka. (Nakamura et al 2008). See Appendix for brief study designs of these nonstandard studies.

Justification of chosen bioaccumulation phrase:

The BCF is less than 500, justifying the use of the phrase “Fluoxetine has low potential for bioaccumulation.”

Excretion (metabolism)

Fluoxetine is well absorbed and then metabolized in humans (Lemberger et al 1985; Bergstrom et al 1988; Altamura et al 1994). Up to about 33 percent of the administered dose is excreted as fluoxetine or the active metabolite norfluoxetine or conjugates that might be transformed back to these active chemicals in a sewage treatment facility. For this classification, no reduction by metabolism was assumed for fluoxetine entering the environment.

PBT/vPvB ASSESSMENT

Because the measured BCF values are less than 2000 L/kg, fluoxetine does not meet the REACH criteria for bioaccumulative (ECHA, 2012). Therefore, fluoxetine is not classified as PBT or vPvB.

References

- Altamura A, Moro A, and Percudani M. 1994. Clinical pharmacokinetics of fluoxetine. *Clin Pharmacokinet* 26(3): 201-214.
- Barclay VKH, Tyrefors NL, Johansson IM, Pettersson CE. 2012. Trace analysis of fluoxetine and its metabolite norfluoxetine. Part II: Enantioselective quantification and studies of matrix effects in raw and treated wastewater by solid phase extraction and liquid chromatography-tandem mass spectrometry. *J Chromatog A* 1227:105-114.
- Bergstrom R, Lemberger L, Farid N, Wolen R. 1988. Clinical pharmacology and pharmacokinetics of fluoxetine: A Review. *Br J Psychiatry*. 153 (suppl. 3) 47-50.
- Brooks BW, Turner PK, Stanley JK, Weston JJ, Glidewell EA, Foran CM, Slattery M, La Point TW, Huggett DB. 2003. Waterborne and sediment toxicity of fluoxetine to select organisms. *Chemosphere* 52:135-142.
- Conley JM, Symes SJ, Schorr MS, Richards SM. 2008. Spatial and temporal analysis of pharmaceutical concentrations in the upper Tennessee River basin. *Chemosphere* 73:1178-1187.
- ECHA, European Chemicals Agency. 2012 Guidance on information requirements and chemical safety assessment. Chapter R.11: PBT Assessment and Chapter R.16: Environmental Exposure Estimation.
- Fick J, Lindberg RH, Kaj L, Brorström-Lundén E. 2011. Results from the Swedish National Screening Programme 2010. Subreport 3. Pharmaceuticals. Swedish Environmental Research Institute. IVL Report B2014. 56 pp.
- Fick J, Linberg RH, Fång J, Magnér J, Kaj L, Brorström-Lundén E. 2015. Screening 2014 Analysis of pharmaceuticals and hormones in samples from WWTPs and receiving waters. 2015. IVL Swedish Environmental Research Institute. Number C135. 51 pp.
- Foran CM, Weston J, Slattery M, Brooks BW, Huggett DB. 2004. Reproductive assessment of Japanese medaka (*Oryzias latipes*) following a four-week fluoxetine (SSRI) exposure. *Arch Environ Contam Toxicol* 46:511-517.
- Gros M, Petrović M, Barceló D. 2009. Tracing pharmaceutical residues of different therapeutic classes in environmental waters by using liquid chromatography/quadrupole-linear ion trap mass spectrometry and automated library searching. *Anal Chem* 81:898-912.

Henry TB, Black MC. 2008. Acute and chronic toxicity of fluoxetine (selective serotonin reuptake inhibitor) in western mosquitofish. *Arch Environ Contam Toxicol* 54:325-330.

Henry TB, Kwon J-W, Armbrust KL, Black MC. 2004. Acute and chronic toxicity of five selective serotonin reuptake inhibitors in *Ceriodaphnia dubia*. *Environ Toxicol Chem* 23:2229-2233.

Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. stream, 1999-2000: A national reconnaissance. *Environ Sci Technol* 36:1202-1211

Kwon J-W, Armbrust KL. 2006. Laboratory persistence and fate of fluoxetine in aquatic environments. *Environ Toxicol Chem* 25:2561-2568.

Lajeunesse A, Gagnon C, Sauve S. 2008. Determination of basic antidepressants and their N-desmethyl metabolites in raw sewage and wastewater using solid-phase extraction and liquid chromatography-tandem mass spectrometry. *Anal Chem* 80 (14): 5325-5333

Lemberger L, Bergstrom R, Wolen R, Farid N, Enas G, Aronoff G. 1985. Fluoxetine: Clinical pharmacology and physiologic disposition. *J Clin Psychiatry* 46:14-19.

Lister A, Regan C, Van Zwol J, Van Der Kraak G. 2009. Inhibition of egg production in zebrafish by fluoxetine and municipal effluents: A mechanistic evaluation. *Aq Toxicol* 95:320-329.

Metcalfe CD, Miao X-S, Koenig BG, Struger J. 2003. Distribution of acidic and neutral drugs in surface waters near sewage treatment plants in the lower great lakes, Canada. *Env Toxicol Chem* 22:2881-2889

Nakamura Y, Yamamoto H, Sekizawa J, Kondo T, Hirai N, Tatarazako N. 2008. The effects of pH on fluoxetine in Japanese medaka (*Oryzias latipes*): Acute toxicity in fish larvae and bioaccumulation in juvenile fish. *Chemosphere* 70:865-873.

Oakes KD, Coors A, Escher BI, Fenner K, Garric J, Gust M, Knacker T, Küster A, Kussatz C, Metcalfe CD, Monteiro S, Moon TW, Mennigen JA, Parrott J, Péry ARR, Ramil M, Roennefarht I, Tarazona JV, Sánchez-Argüello P, Ternes TA, Trudeau VL, Boucard T, Van Der Kraak GJ, Servos M. 2010. Environmental risk assessment for the serotonin re-uptake inhibitor fluoxetine: Case study using the European risk assessment framework. *Integrated Environ Assess Manag* 6:524-539.

Paterson G, Metcalfe CD. 2008. Uptake and depuration of the anti-depressant fluoxetine by the Japanese medaka (*Oryzias latipes*). *Chemosphere* 74:125-130.

Péry ARR, Gust M, Vollat B, Mons R, Ramil M, Fink G, Ternes T, Garric J. 2008. Fluoxetine effects assessment on the life cycle of aquatic invertebrates. *Chemosphere* 73:300-304.

Prozac Safety Data Sheet. Revision 09/04/2015. <http://ehs.lilly.com/msds/Prozac.pdf>

Stanley JK, Ramirez AJ, Chambliss CK, Brooks BW. 2007. Enantiospecific sublethal effects of the antidepressant fluoxetine to a model aquatic vertebrate and invertebrate. *Chemosphere* 69:9-16.

Study 40223. 1993. Determination of the ultraviolet-visible absorbance spectrum of fluoxetine hydrochloride. FDA TAD 3.05. Eli Lilly and Company.

Study 40227. 1993. Aerobic biodegradation of ¹⁴C-fluoxetine hydrochloride in water. FDA TAD 3.11. Eli Lilly and Company.

Study 40229. 1993. Hydrolysis of ¹⁴C-fluoxetine hydrochloride as a function of pH. FDA TAD 3.09. Eli Lilly and Company.

Study 40230. 1993. Determination of the octanol/water partition coefficient of fluoxetine hydrochloride. FDA TAD 3.02. Eli Lilly and Company.

Study C03992. 1993. The 48-hour acute toxicity of fluoxetine hydrochloride (LY110140) to *Daphnia magna* in a static test system. FDA TAD 4.08. Eli Lilly and Company.

Study F02892. 1993. The acute toxicity of fluoxetine hydrochloride (LY110140) to rainbow trout (*Oncorhynchus mykiss*) in a static test system. FDA TAD 4.11. Eli Lilly and Company.

Study N00793. 1993. A study to determine the aerobic biodegradation of fluoxetine hydrochloride (LY110140) in water using a closed bottle test. OECD 301. Eli Lilly and Company.

Study J01292. 1993. The 14-day acute toxicity of fluoxetine hydrochloride (LY110140) to the freshwater green alga (*Selenastrum capricornutum*) in a static test system. FDA TAD 4.01. Eli Lilly and Company.

Weinberger J, Klaper R. 2014. Environmental concentrations of the selective serotonin reuptake inhibitor fluoxetine impact specific behaviors involved in reproduction, feeding and predator avoidance in the fish *Pimephales promelas* (fathead minnow). *Aquatic Toxicol* 151:77-83.

Yamamoto H, Hayashi A, Nakamura Y, Sekizawa J. 2005. Fate and partitioning of selected pharmaceuticals in the aquatic environment. *Environ Sci* 12:347-358.

Zhou SN, Oakes KD, Servos MR, Pawliszyn J. 2008. Application of solid-phase microextraction for in vivo laboratory and field sampling of pharmaceuticals in fish. *Environ Sci Technol* 42:6073-6079.

Zorita S, Martensson L, Mathiasson L. 2009. Occurrence and removal of pharmaceuticals in a municipal sewage treatment system in the south of Sweden. *Sci. Total Environ.* 407:2760-2770

Appendix: Summary of design of nonstandard studies

Title:	<i>Inhibition of egg production in zebrafish by fluoxetine and municipal effluents: A mechanistic evaluation</i>	
Authors:	<i>A Lister, C Regan, J Van Zwol, G Van Der Kraak</i>	
Journal:	<i>Aquatic Toxicology</i>	
Citation:	<i>95(2009) 320-329</i>	
Materials & Methods		
Test Substance	Name:	Fluoxetine and ethinylestradiol
Test Organism	Species:	<i>Danio rerio</i> (zebrafish)
	Age at initiation:	Sexually mature adults
Exposure Design	Dilution Water:	Well water
	Exposure System	Static renewal, 100% renewal daily
	Duration:	7 days
	Nominal test concentrations:	0.32, 3.2, 32 µg/l fluoxetine 10 ng/l ethinylestradiol (EE2) Solvent control (DMSO)
	Replication:	3 replicates for each treatment 8 females:6 males per replicate
	Feeding:	Twice daily with salmon fry pellets and occasional supplements of blood worms
	Exposure aquaria:	4-liter beaker; 3.5 L volume
	Conditions:	28°C with 12H:12H light:dark cycle
	Solution renewal:	100% daily
Endpoints	Apical endpoints:	Fecundity, weight
Results		
Water Quality	Dissolved oxygen:	96.28 ± 0.54 percent saturated
	pH:	8.62 ± 0.19
	Total ammonia	0.25 ± 0.08 mg/l
Body Weight	No significant differences	
Fecundity	Average clutch size (# eggs/female) was significantly reduced at 32 µg fluoxetine/L compared to control.	
		Approximate eggs per female per day (estimated from graph)

	<i>Control</i>	~70 ^A
	<i>10 ng/l EE2</i>	~55 ^{AB}
	<i>0.32 µg/l fluoxetine</i>	~50 ^{AB}
	<i>3.2 µg/l fluoxetine</i>	~45 ^{AB}
	<i>32 µg/l fluoxetine</i>	~15 ^B

Title:	<i>Application of solid-phase microextraction for in vivo laboratory and field sampling of pharmaceuticals in fish</i>
Authors:	<i>SN Zhou, KD Oakes, MR Servos, J Pawliszyn</i>
Journal:	<i>Environmental Science and Technology</i>
Citation:	<i>42 (2008) 6073-6079</i>

Materials & Methods		
Test Substance	Name:	Fluoxetine
Test Organism	Species:	<i>Oncorhynchus mykiss</i> (rainbow trout)
	Size at initiation:	15.6 ± 0.3 cm; 28.7 ± 1.4 g (immature)
Exposure Design	Exposure System:	Static renewal, 50% renewal daily
	Duration of exposure:	7 and 14 days
	Nominal test concentrations:	3.2, 32, 320 µg fluoxetine/L Solvent control (ethanol in reference water)
	Replication:	N = 3 at each timepoint
	Exposure aquaria:	34-liter aquaria
	Temperature:	12.3-12.7°C
Analytical Methods	Total concentration in muscle:	Muscle tissue sampled on Day 7 and on Day 14 of exposure; analysis by liquid extraction followed by LC/MS/MS of extract
Calculations	<i>Bioaccumulation factor (BAF) calculated from the slope of the line generated by the total concentration in muscle plotted against the nominal concentration in water.</i>	

Results		
Water Quality	Dissolved oxygen:	10.09 ± 0.03 mg/l
	pH:	8.17 ± 0.06
	Unionized ammonia:	23.5 ± 1.5 µg/l
Bioaccumulation factors (L/kg)	7 days	14 days
	62	84

Title:	<i>Uptake and depuration of the anti-depressant fluoxetine by the Japanese medaka (<i>Oryzias latipes</i>)</i>
Authors:	<i>G Paterson, CD Metcalfe</i>
Journal:	<i>Chemosphere</i>
Citation:	<i>74 (2008) 125-130</i>

Materials & Methods		
Test Substance	Name:	Fluoxetine HCl

Test Organism	Species:	<i>Oryzias latipes</i> (medaka)
	Size at initiation:	0.36 ± 0.02 g (adult)
Exposure Design	Dilution Water:	Dechlorinated tap water
	Exposure System:	Static renewal, 100% renewal daily
	Duration:	7 days uptake (exposure), 21 depuration (in clean water)
	Nominal test concentrations:	0.64 µg fluoxetine/L Solvent control (acetone)
	Replication:	4 to 7 fish were collected on Days 0.2 (5 hr), 3, 7 of uptake and on days 7, 14, 21 of depuration
	Feeding:	Twice daily with live artemia
	Chambers:	10-liter aquaria
	Conditions:	23 to 27°C with 16H:8H light:dark cycle
Analytical Methods	Water:	Extraction by SPE; extracts analyzed by LC-MS/MS
	Whole fish:	Extracted by ASE; extracts analyzed by LC-MS/MS
Results		
Water Quality	Alkalinity:	60 to 75 mg/l
	pH:	7.2 to 7.5
	Hardness:	75 to 90 mg/l
	Fluoxetine Concentration:	0.55 ± 0.14 µg/l
Bioconcentration Factor (L/kg)		Ratio of concentration in fish on Day 7 to water concentration
		74
		Ratio of uptake and depuration rate constants
		80

Title:	<i>The effects of pH on fluoxetine in Japanese medaka (Oryzias latipes): Acute toxicity in fish larvae and bioaccumulation in juvenile fish</i>	
Authors:	Y Nakamura, H Yamamoto, J Sekizawa, T Kondo, N Hirai, N Tatarazako	
Journal:	Chemosphere	
Citation:	70 (2008) 865-873	
Materials & Methods		
Test Substance	Name:	Fluoxetine HCl
Test Organism	Species:	<i>Oryzias latipes</i> (medaka)
	Age at initiation:	2-month old
Exposure Design	Dilution Waters:	Three dechlorinated buffers: pH 7, 8, 9
	Exposure System:	Flow-through
	Duration:	30 days uptake (exposure)
	Nominal test concentrations:	10 µg fluoxetine/L in each buffer system Control for each buffer system

	Replication:	8 fish exposed to each buffer system with fluoxetine 4 to 7 fish were collected on Days 0.2, 3, 7 of uptake and on days 7, 14, 21 of depuration			
	Chambers:	2.3-liter glass tanks			
	Conditions:	23 to 27°C with 16H:8H light:dark cycle			
Analytical Sampling	Water:	Every 10 days			
	Fish (whole and liver):	Termination			
Analytical Methods	Water:	HPLC-UV/vis/FLD			
	Whole fish:	Two fish and two livers extracted together with fluvoxamine as an internal standard; extracts were analyzed by GC-MS			
Results					
Bioconcentration Factor		pH	7.2	8.1	8.9
		Fluoxetine in water µg/l	13.8	15.0	14.5
		BCF (body+liver) L/kg	13	37	330