CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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ENVIRONMENTAL ASSESSMENT

Environmental Assessment
Finding of No Significant Impact

NDA 22-516

Cymbalta (duloxetine HCl)

Food and Drug Administration
Center for Drug Evaluation and Research
October 30, 2009

FINDING OF NO SIGNIFICANT IMPACT

NDA 22-516

Cymbalta (duloxetine HCl)

The National Environmental Policy Act of 1969 (NEPA) requires Federal agencies to assess the environmental impact of their actions. The Food and Drug Administration (FDA) is required under NEPA to consider the environmental impact of approving certain drug product applications as an integral part of its regulatory process.

NDA 22-516 requests approval of Cymbalta (duloxetine HCl) for the treatment of chronic pain, as in osteoarthritis and chronic lower back pain. In support of its application, Eli Lilly and Company prepared an environmental assessment (attached) in accordance with 21 CFR Part 25 which evaluates the potential environmental impact from the use and disposal of this product.

The Food and Drug Administration, Center for Drug Evaluation and Research, has carefully considered the potential environmental impact due to approval of this application and has concluded that this action is not expected to have a significant effect on the human environment. Therefore, an environmental impact statement will not be prepared.

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Attachment: 10/30/09, Environmental Assessment (confidential appendices removed)

Environmental Assessment for the Use of Duloxetine Hydrochloride in the Management of Chronic Pain

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May 2009

Table of Contents

Page

Table of Contents

Environmental Assessment for the Use of Duloxetine Hydrochloride in the Management of Chronic Pain

Description of the Proposed Action

Requested Approval

Eli Lilly and Company has filed a supplement (22-516) to the NDA for duloxetine hydrochloride pursuant to the Federal Food, Drug, and Cosmetic Act. An updated Environmental Assessment has been submitted to support the supplement to the NDA for duloxetine hydrochloride pursuant to 21 CFR part 25.

An environmental assessment of duloxetine hydrochloride was submitted with the original NDA 21-427. The current proposed supplement to the NDA is not categorically excluded from assessment of environmental impact as dictated in the Federal Register (July 29, 1997, 21 CFR 25.31) because of the estimated concentration at the point of entry into the aquatic environment and because the new indication could result in increased use of duloxetine hydrochloride in the United States.

The use of duloxetine hydrochloride will result in one major pathway to the environment: sewage treatment facilities receiving influent from the general public. Wastes generated from production facilities are regulated by federal, state, and local environmental protection agencies and are not considered in this environmental assessment.

Need for Action

Duloxetine hydrochloride, a naphthyl ether amine, inhibits the uptake of serotonin and norepinephrine. Duloxetine is currently approved for use in the United States for major depressive disorder, generalized anxiety disorder, fibromyalgia, and diabetic peripheral neuropathic pain. In the current application, duloxetine is being proposed for the management of chronic pain through additional studies in osteoarthritis and chronic low back pain.

Locations of Use

The location of the use of duloxetine hydrochloride will be primarily in the patient's home and workplace. There is no reason to expect use to be concentrated in a particular geographic region.

Disposal Sites

Empty or partially empty packages containing duloxetine hydrochloride will typically be disposed of by a community's solid waste management system, which may include landfills, incineration, and recycling, although minimal quantities of unused drug could be disposed of in the sewer system.

Identification of the Chemical Substance

The identification of the chemical substance has not changed from that described in the original Environmental Assessment filed with NDA 21-427.

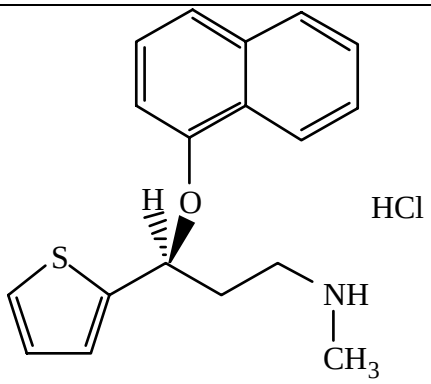
Nomenclature

Established Name (USAN)

(+)-N-methyl-γ-(1-naphthalenyloxy)-2-thiophenepropanamine hydrochloride

Brand/Proprietary Name/Tradename

Cymbalta

Chemical Abstracts Service Number:	136434-34-9
Molecular Formula:	$C_{18}H_{19}NOS \cdot HCl$
Molecular Weight:	333.88
Structural Formula:	

Environmental Issues

Environmental Fate of Released Substances

The environmental fate of the released substance was described in the original Environmental Assessment filed with NDA 21-427. Since that assessment, an additional fate study has been conducted in a water-sediment system to support registration requirements in the European Union and was described in an NDA supplement application in 2007. While the total use of duloxetine is expected to increase with the new indication, it will still be below the maximum total amount estimated in the original Environmental Assessment. Thus, the expected environmental concentrations associated with all current and proposed indications will not be higher than those predicted in the original Environmental Assessment.

Report summaries for all supporting environmental chemistry and fate studies are located in Appendix B. Full reports are available upon request.

Physical and Chemical Characterization

The water solubility of duloxetine hydrochloride was determined to be 21.6, 2.74, and 0.331 g/L at pH 4, 7, and 9, respectively (Study 1982.6114). The dissociation constant (pK_a) of duloxetine hydrochloride was determined to be 9.34 (Study 1982.6115). At pH 4, 7, and 9, the log of the n-octanol/water partition coefficient (log D_{ow}) of duloxetine (both ionized and nonionized species) was determined to be 0.781, 1.54, and 3.35, respectively (Study 1982.6127). The K_d was measured in sewage sludge and ranged between 1166 to 1731 (Study 1982.6123). The K_d can be normalized for the amount of organic carbon in the sludge to calculate a K_{oc} of 2893 to 4296 for duloxetine hydrochloride.

Characteristic	Duloxetine Hydrochloride		
pK_a	9.34		
K_{oc}	2893 to 4296		
K_d	1166 to 1731		
	pH 4	pH 7	pH 9
Solubility g/L	21.6	2.74	0.331
Log D_{ow}	0.781	1.54	3.35

The log D_{ow} at pH 9 is less than 3.5 indicating that the probability for bioaccumulation is low. However, it is greater than 3 suggesting that sorption to biosolids in the sewage treatment plant will occur. Indeed, the K_d confirms that duloxetine hydrochloride will adsorb to biosolids in wastewater treatment plants.

Vapor pressure of duloxetine hydrochloride was not determined because thermogravimetric analysis of duloxetine hydrochloride showed no weight loss below 160°C. Above this temperature, decomposition and melting occurs. Thus, in the environment, release to the atmosphere is not expected.

Environmental Depletion Mechanisms

Duloxetine is extensively metabolized by humans; less than 10% of the administered dose is excreted as parent compound (Confidential Appendix D).

Duloxetine hydrochloride hydrolyzes slowly at temperatures lower than or equal to 30°C, with a half-life ranging from approximately 1.5 to 3.5 months at 30°C (Study 1982.6120).

Based on its ultraviolet-visible absorption spectrum, the theoretical phototransformation of duloxetine hydrochloride is estimated to be 100% within 1 month (Study 1982.6130). The algae toxicity study also suggests that duloxetine will be susceptible to photolysis.

In the algae study, a control (no algae) sample spiked with 0.029 mg/L duloxetine had no detectable duloxetine after 3 days of incubation in continuous light (Study 1982.6118). While duloxetine binds to glass to some degree (there is a 30% decrease in aqueous concentration in a glass test tube over 7 days, Study 1982.6112), the majority of the rapid disappearance is most likely due to photolysis.

Duloxetine hydrochloride was not significantly biodegraded when incubated with activated sewage sludge for 8 days (Study 1982.6123). However, the presence of a small non-duloxetine radioactive peak indicates that there is potential for transformation of duloxetine. In the same study, duloxetine did adsorb to sludge biosolids (see discussion of K_d above).

The transformation of duloxetine in a water-sediment system has also been investigated (Study 807566). Radiolabeled duloxetine was incubated in two different water-sediment systems, one of which contained sediment characterized as sand while the other sediment was a silt loam. When incubated in water-sediment systems in aerobic conditions for 100 days, radiolabeled duloxetine disappeared from the overlying water with a half-life of 3 days and was extensively degraded. At the end of the 100 days, only 37% to 58% of the dosed radioactivity was recovered as duloxetine from the sand and silt loam water-sediment systems, respectively. Up to 45 separate degradation products were observed over the course of the study. Ultimate degradation of duloxetine also occurred: 5% to 11% of the radioactivity was recovered as CO_2.

Thus, the depletion mechanisms of duloxetine hydrochloride from the aqueous environment are sorption, biodegradation, and photolysis.

It is not expected that duloxetine will persist in the environment. Its extensive metabolism in humans and sediments suggests that duloxetine will be subjected to biodegradation. In addition, duloxetine will photolyze and slowly hydrolyze in the aqueous environment.

Environmental Concentrations

Expected Introduction Concentration (EIC) in Water

Even with the increase in use expected with the supplemental indication, the maximum amount used annually in the United States is still predicted to be less than 100,000 kg of duloxetine (free base) as estimated in the original environmental assessment. From this forecast, the expected introduction concentration (EIC) of duloxetine at the point of entry into the aquatic environment is calculated as follows:

$$\mathrm{EIC}_{\mathrm{aquatic}}\ (\mathrm{ppb}) = \frac{100{,}000\ \mathrm{kg} \times 1{,}000{,}000{,}000\ \mu\mathrm{g/kg}}{1.3 \times 10^{11}\ \mathrm{L/day} \times 365\ \mathrm{days}} = 2.1\ \mu\mathrm{g/L}$$

where 1.3x10^{11} L/day is the volume of water entering publicly owned treatment works in the United States (Environmental Protection Agency, *2000 Needs Survey, Report to Congress*). This calculation assumes that all duloxetine used enters the publicly owned

treatment works system, drug product usage occurs throughout the United States in proportion to population and the amount of wastewater generated, and there is no human metabolism or microbial degradation.

This $EIC_{aquatic}$ can be adjusted for sorption to biosolids. The measured K_d for sorption to biosolids at 2.5 g/L was 1166. K_d is defined as:

$$K_d = \frac{\left(\frac{\text{Duloxetine}_{\text{biosolids}}}{\text{Mass}_{\text{biosolids}}}\right)}{\left(\frac{\text{Duloxetine}_{\text{water}}}{\text{Mass}_{\text{water}}}\right)}$$

where $Duloxetine_{water}$ and $Duloxetine_{biosolids}$ are the amounts of duloxetine in water and biosolids, respectively. If the total amount of duloxetine in the water and the sludge is $Duloxetine_{total}$, then the above equation can be rearranged to give:

$$\text{Duloxetine}_{\text{water}} = \frac{\text{Duloxetine}_{\text{total}} \times \text{Mass}_{\text{water}}}{\text{Mass}_{\text{biosolids}} \times \left(K_d + \frac{\text{Mass}_{\text{water}}}{\text{Mass}_{\text{biosolids}}}\right)}$$

A typical water treatment facility has a biosolids concentration in the aerator basin of 3 to 6 g/L (Metcalf and Eddy 1991). Using the more conservative value, in 1 liter of water, $Duloxetine_{total}$ is 2.1 µg, $Mass_{water}$ is 1000 g (or 1000 mL), and $Mass_{biosolids}$ is 3 g. Solving for $Duloxetine_{water}$, the expected introduction concentration (EIC) in the aqueous phase adjusted for sorption to solids is:

$$EIC_{aquatic} = 0.5\mu g / L$$

Schultz and Furlong (2008) have published a report on the detection of duloxetine in a wastewater treatment effluent in St. Paul, Minnesota as well as in a wastewater effluent-dominated stream, Pecan Creek in Texas. The concentrations reported in both the wastewater effluent in Minnesota and Pecan Creek in Texas were approximately 0.002 µg/L, 250 times lower than the expected introduction concentration adjusted for sorption calculated above.

Expected Environmental Concentration (EEC) in Water

The Expected Environmental Concentration, EEC, can be calculated for the aquatic environment after consideration of dilution of treatment facility effluent by receiving waters. Based on dilution factors for treatment facilities available from the EPA, a dilution factor of 10 is appropriate. This concentration is considered for long-term exposure scenarios.

$$EEC_{aquatic\,(afterdilution)} = 0.05\mu g / L$$

Expected Introduction Concentration (EIC) in Biosolids and Soil

It is also possible to predict the amount of duloxetine bound to the biosolids in a wastewater facility. The total duloxetine in 1 liter is 2.1 µg so in this case, the amount that must be sorbed to 3 g of biosolids is 1.6 µg. Thus:

$$EIC_{biosolids} = 530 \mu g / kg$$

The residence time for sludge in wastewater facilities is 5 to 10 days. Study 1982.6123 is a biodegradation study with duloxetine hydrochloride in which it was observed that after 8 days in contact with sludge, at least one degradation product of duloxetine was detected. The lag time to detection of a degradation product may indicate that microorganisms must become adapted in order to use duloxetine as a food source. In a wastewater treatment plant, it is assumed that the duloxetine concentration will be constant and thus the microorganisms will be continually exposed to duloxetine. This could result in greater biodegradation than observed in the laboratory study. Therefore, it is not unreasonable to assume that, in a wastewater facility, some degradation of duloxetine will occur.

Biosolids from treatment facilities are often applied to land, e.g. as fertilizer for crops. The rate of application is limited by the quantity of pollutants in the biosolids and by the nitrogen concentration. The total amount of nitrogen in biosolids ranges from 3% to 8% on a dry weight basis (Sullivan 1998). The total nitrogen includes ammonium nitrogen and organic nitrogen. Ammonium nitrogen is immediately available for crop use, but is also susceptible to loss through volatilization upon application. The organic nitrogen is available following mineralization by soil microbes. For this assessment, it is assumed that all of the nitrogen is essentially available to the crops. Therefore, the least amount of nitrogen in biosolids would be 3% on a dry weight basis. Corn silage utilizes a maximum rate of nitrogen at 480 pounds/acre (539 kg/ha, Hammond et al. 1994). Using this application rate of nitrogen, a maximum rate of application of biosolids to agricultural land can be calculated.

$$\frac{539\text{kg}_{\text{Nitrogen}}}{\text{ha}} \times \frac{100\text{kg}_{\text{biosolids}}}{3\text{kg}_{\text{Nitrogen}}} = 17{,}967\text{kg}_{\text{biosolids}} / \text{ha} = 18\text{metrictons}_{\text{biosolids}} / \text{ha}$$

An incorporation depth of 15 cm into the top layer is typical in land application of biosolids (EPA 1993). Assuming that the mass of soil is 1500 kg/m^3, the concentration of duloxetine in the soil after application of biosolids with 530 µg duloxetine/kg concentration is estimated to be:

$$\frac{18{,}000 kg_{biosolids} \times 530 \mu g_{duloxetine} / kg_{biosolids}}{10{,}000 m^2 \times 0.15 m \times 1500 kg_{soil} / m^3} = 4.2 \mu g_{duloxetine} / kg_{soil}$$

Expected Introduction Concentration (EIC) in Sediment

In the water-sediment transformation study (Study 807566), after 100 days approximately 88% of the radioactivity was associated with the sediments of both the sand and silt loam water-sediment systems. At this time point, the concentrations of duloxetine residues in the sediment layers were 2.6 and 5.9 mg/kg and the concentrations in the overlying waters were 81 and 18 µg/L for the sand and silt loams systems, respectively. Thus, the ratio of the concentration of duloxetine residues in sediment to water ranged from 31 (in the sand system) to 330 (silt loam). If the highest concentration of duloxetine residue diluted in surface water is expected to be 0.05 µg/L, then the highest concentration of duloxetine residue in sediment would be no more than 16.5 µg/kg.

Summary of Environmental Fate

Duloxetine will enter the environment through its use by the general population. While human metabolism of duloxetine is extensive, estimations of concentrations of duloxetine in the environment were calculated based on total elimination as the parent compound (that is, a total residue approach). The Expected Introduction Concentration of total residues of duloxetine in a sewage treatment facility could be as high as 2.1 µg/L. The primary depletion mechanism of duloxetine from the aqueous environment is sorption to biosolids at water treatment facilities. Consideration of this depletion mechanism is used to calculate an adjusted Expected Introductory Concentration ($EIC_{aquatic}$) of 0.5 µg/L. The concentration in biosolids could be as high as 530 µg/kg. If biosolids are applied to land, then duloxetine may enter the terrestrial environment at a concentration in the soil ($EIC_{terrestrial}$) of 4.2 µg/kg. Duloxetine is not expected to volatilize and therefore will not enter the atmospheric environment. Duloxetine is not expected to persist in the environment due to its potential for degradation and photolysis.

Environmental Effects of Released Substances

The environmental effects of duloxetine in aquatic organisms were investigated in a battery of toxicity studies conducted according to OECD guidelines. In 2006, an updated Environmental Assessment was submitted with a supplement to NDA 21-427 which included an additional toxicity study conducted with earthworms. Since then, additional chronic toxicity studies have been conducted with fish and sediment invertebrates to satisfy registration requirements for the European Union and these results were described in an NDA supplement application in 2007. The results of all the toxicity studies are discussed below. Report summaries for all supporting environmental toxicity studies are located in Appendix B. Full reports for the environmental toxicity studies are available upon request.

Microbial Inhibition (Tier One)

The effect of duloxetine on sewage microorganisms was tested by incubating activated sludge with duloxetine for 3 hours (Study 1982.6126). The endpoint measured was respiration rate. The no-observed-effect concentration (NOEC) was 2 mg/L and the EC_{50} was determined to be 36.5 mg/L (expressed, as for all of the following data, as duloxetine free base).

Fish Acute Toxicity (Tier Two)

The acute toxicity of duloxetine to rainbow trout was determined in juvenile fish following exposure to the compound for 96 hours (Study 1982.6125). The endpoint measured was mortality. The NOEC was 0.45 mg/L and the 96-hour LC_{50} was estimated to be 1.3 mg/L.

Invertebrate Acute Toxicity (Tier Two)

The acute toxicity of duloxetine to *Daphnia magna* was determined following exposure to the compound for 48 hours (Study 1982.6116). The endpoint measured was immobilization. The NOEC was determined to be 1.1 mg/L and the 48-hour EC_{50} was estimated to be 2.4 mg/L.

Algal Toxicity (Tier Two)

The acute toxicity of duloxetine to green algae was determined using the species *Psuedokirchneriella subcapitata* (Study 1982.6118). The algal cells were exposed for 72 hours and the endpoints measured were inhibition of biomass and average growth rate. Biomass, the area under the growth curve, was the most sensitive endpoint for duloxetine, with a 72-hour EC_{50} of 0.064 mg/L and a NOEC of 0.011 mg/L.

Earthworm Toxicity (Tier Two)

The acute toxicity of duloxetine to *Eisenia fetida* was determined following exposure to the compound incorporated in an artificial soil for 14 days (Study 1982.6133). The endpoints measured were mortality and growth (weight change). The NOEC was determined to be $\geq$1000 mg/kg (the highest concentration tested) and the 14-day LC_{50} was >1000 mg/kg.

Chronic Toxicity (Tier Three)

Daphnia magna

The chronic toxicity of duloxetine to *Daphnia magna* was determined in a full life-cycle test with endpoints of size, survival, and reproduction (Study 1982.6129). Along with body length, the most sensitive endpoint in the study was reproduction. The EC_{50} and NOEC values of 0.28 mg/L and 0.011 mg/L, respectively, were determined for reproduction.

Fish

Two chronic early life-stage toxicity tests with duloxetine have been conducted in fathead minnows (*Pimephales promelas*).

In the first, organisms were exposed to average duloxetine concentrations ranging from 0.005 to 0.45 mg/L during embryo development and for 28 days post-hatch (Study 1982.6268). Survival and hatching success were not affected. Reduction in length was the most sensitive endpoint with minimal (6% to 14% compared to control), but statistically significant, differences from control at all levels. Weight was also reduced compared to control, but only at 0.05 mg/L and higher. Statistically, the NOEC in this study was lower than the lowest treatment concentration tested, 0.005 mg/L. Because the biological significance of a 6% or 7% reduction in length is not clear, it is possible that the NOEC could be as high as 0.012 mg/L in this study.

In the second early life-stage study with fathead minnows, a lower range of concentrations was tested: 0.00018 to 0.037 mg/L. Survival and hatching success were not affected by treatment. There were significant reductions in both length and dry weight at the highest treatment concentration (0.037 mg/L). There were no statistically significant reductions in length or weight at or below the NOEC of 0.012 mg/L.

When considered together, the results from both early life-stage studies indicate that the NOEC for biologically significant effects is 0.012 mg/L.

Sediment Invertebrates

The chronic toxicity of ^{14}C duloxetine to sediment-dwelling midges was determined in a 28-day exposure of *Chironomus riparius* (Study 1982.6278). No significant effects on emergence or development rate were observed at the highest concentration tested in sediment, 92 mg total ^{14}C duloxetine residues/kg. HPLC/RAM profiling demonstrated that more than 90% of the radioactivity in the sediment was duloxetine.

Risk Assessment

To assess the environmental risk of duloxetine in the environment, the median effect concentration or no-observed-effect concentration was compared to the Maximum Expected Environmental Concentration, or MEEC. To protect all species, the quotient of the two numbers (the Assessment Factor) must be above 1000 for Tier One screening, above 100 for Tier Two, and above 10 for Tier Three screening as suggested by guidance from the FDA.

Effects Concentrations Compared to Expected Environmental Concentrations

Aquatic Environment					
Acute Studies					
Species	NOEC (µg/L free base)	LC50 or EC50 (µg/L free base)	MEEC (µg/L free base)	LC50 or EC50 ÷ MEEC	Required Assessment Factor
Sewage microorganisms (3 hours)	2000	36,500	0.5	73,000	≥1000
Rainbow trout (96 hours)	450	1300	0.5	2600	≥100
Daphnia magna (48 hours)	1100	2400	0.5	4800	≥100
Pseudokirchneriella subcapitata (72 hours)	N/A	64	0.5	128	≥100
Chronic Studies					
Pseudokirchneriella subcapitata (72 hours)	11	N/A	0.05*	220	≥10
Daphnia magna (21 days)	11	280	0.05*	220	≥10
Pimephales promelas (4 embryo days + 28 post-hatch days)	12	N/A	0.05*	240	≥ 10
Chironomus riparius (28 days)	≥ 92,000 µg/kg	N/A	16.5 µg/kg	≥ 5575	≥ 10
Terrestrial Environment					
Eisenia fetida (14 days)	≥ 1,000,000	>1,000,000	4.2	>238,095	≥ 100

*Note: for chronic exposure, a dilution factor of 10 was utilized.

The calculated assessment factors in all cases are greater than the required factors and in no case were sub-lethal effects observed at concentrations equal to the MEEC. These results indicate that duloxetine release to sewage treatment plants and subsequently to the environment does not pose an environmental risk.

Other Issues

Risk of Pharmacological Effects in Fish

Duloxetine has two mechanisms of action: inhibition of serotonin reuptake transporters and inhibition of norepinephrine reuptake transporters. Evidence for the existence of both serotonin and norepinephrine transporters in fish has been reported (Wang et al. 2006; Roubert et al. 2001). In order to assess the risk of a pharmacological effect in fish, the steady-state plasma concentrations of duloxetine in humans were compared to the calculated concentration of duloxetine that might be found in fish plasma, using the predicted environmental concentration in surface water. At the lowest starting therapeutic dose in humans of 20 mg twice per day, the steady state plasma concentration is 31 µg/L (see Confidential Appendix D). Fish plasma concentration can be estimated from the predicted environmental concentration using a model described by Huggett et al. (2005) based on the plasma:water relationship published by Fitzsimmons et al. (2001). The log D_{ow} value of duloxetine at pH 7 is 1.54. Using this value, the blood:water partitioning coefficient ($P_{b:w}$) is 1.75 using the relationship log $P_{b:w}$ = 0.73 x log P - 0.88, and this coefficient is then multiplied by the predicted environmental concentration (Fitzsimmons et al. 2001). Using the maximum surface water concentration of duloxetine residues (0.05 µg/L), the estimated plasma concentration in fish is 0.09 µg/L. This level is 344 times lower than the steady state duloxetine concentration in humans. So even if serotonin and norepinephrine transporters in fish had the same sensitivity to duloxetine as those in humans, pharmacological effects in fish would not be expected because of the very low exposure levels.

Potential Effects on Humans

If a human were to drink 2 liters of surface water at the maximum EEC of 0.05 µg/L duloxetine, the dose would be 0.1 µg. This dose would be at least 400,000 times less than the lowest daily therapeutic dose of duloxetine (40 mg). Thus, it is not expected that humans will be adversely affected by environmental concentrations of duloxetine.

Summary

Duloxetine and related metabolites enter the environment through wastewater facilities. In wastewater facilities, duloxetine is expected to partition to the solids resulting in a reduction of the aqueous concentration. The expected duloxetine environmental concentration in water (a maximum of 0.05 µg/L in surface water) is not expected to affect aquatic organisms based on the toxicity of duloxetine to fish, invertebrates, and algae. Additionally, comparison of predicted plasma concentrations in fish with the therapeutic plasma levels in humans indicates that pharmacological effects are not

expected in environmental species. Duloxetine is not expected to persist in the aquatic environment because it is subject to degradation, hydrolysis, and photolysis. The maximum concentration of duloxetine expected in soil resulting from agricultural land application of biosolids is not expected to affect terrestrial organisms based on the lack of toxicity of duloxetine to earthworms. The amount of duloxetine that humans could be exposed to by drinking surface water with the maximum expected environmental concentration of duloxetine would be substantially less than the therapeutic dose range. In summary, no adverse environmental effects have been identified from the use of duloxetine in the treatment of human populations.

Mitigation Measures

As no adverse environmental effects have been identified in this environmental assessment from any use of duloxetine, no mitigation measures are needed. This action has no known effects on endangered or threatened species or historic properties.

Alternatives to the Proposed Action

As no adverse environmental effects have been identified from any use of duloxetine, there is no need for alternatives to the proposed action.

List of Preparers

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See Appendix C for curriculum vitae

References

[EPA] Environmental Protection Agency. 1993. Rules and regulations: standards for the use or disposal of sewage sludge. Fed Regist, February 58(32):9285.

[EPA] Environmental Protection Agency. 1996. Draft - Ecological effects test guidelines: OPPTS 850.1300 Daphnid chronic toxicity test, EPA 712-C-96-120. Washington: US EPA.

Fitzsimmons PN, Fernandez JD, Hoffman AD, Butterworth BC, Nichols JW. 2001. Branchial elimination of superhydrophobic organic compounds by rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicol 55:23-34.

Hammond C, Segars B, Gould C. 1994. Land application of livestock and poultry manure. In: University of Georgia College of Agricultural & Environmental Sciences Cooperative Extension Service. Circular 826. Athens: University of Georgia. p 1-9.

Huggett DB, Benson WH, Chipman K, Cook JC, Gray LE, Kinter LB, Meyerhoff RD, Trudeau VL. 2005. Role of mammalian data in determining pharmaceutical responses in aquatic species. In: Williams RT, editor. Human Pharmaceuticals Assessing the Impacts on Aquatic Ecosystems. Pensacola (FL): Society of Environmental Toxicology

and Chemistry. p 149-181.

Metcalf & Eddy Inc, Tchobanoglous G, Burton FL. 1991. In: Clark BJ, Morriss JM. Wastewater engineering: treatment, disposal, and reuse. 3rd ed. Boston: Irwin/McGraw-Hill 1334 p.

[OECD] Organisation for Economic Cooperation and Development. 1981. Dissociation constants in water, No. 112. In: OECD guideline for testing chemicals.

[OECD] Organisation for Economic Cooperation and Development. 1981. Hydrolysis as a function of pH, No. 111. In: OECD guideline for testing of chemicals.

[OECD] Organisation for Economic Cooperation and Development. 1981. Inherent biodegradability: modified SCAS test, No. 302A. In: OECD guideline for testing of chemicals.

[OECD] Organisation for Economic Cooperation and Development. 1984. Activated sludge, respiration inhibition test, No. 209. In: OECD guidelines for testing of chemicals. Paris: OECD.

[OECD] Organisation for Economic Cooperation and Development. 1984. Alga, growth inhibition test, No. 201. In: OECD guidelines for testing of chemicals. Paris: OECD.

[OECD] Organisation for Economic Cooperation and Development. 1984. *Daphnia* sp., acute immobilisation test and reproduction test, No. 202. In: OECD guidelines for testing of chemicals. Paris: OECD.

[OECD] Organisation for Economic Cooperation and Development. 1984. Earthworm, acute toxicity tests, No. 207. In: OECD guideline for the testing of chemicals.

[OECD] Organisation for Economic Cooperation and Development. 1984. Fish acute toxicity test, No. 203. In: OECD guidelines for testing of chemicals. Paris: OECD.

[OECD] Organisation for Economic Cooperation and Development. 1992. Fish, early-life stage toxicity test, No. 210. In: OECD guideline for the testing of chemicals.

[OECD] Organisation for Economic Cooperation and Development. 1995. Partition coefficient (n-octanol/water): shake flask method, No. 107. In: OECD guidelines for testing of chemicals. Paris: OECD.

[OECD] Organisation for Economic Cooperation and Development. 1995. Water solubility, No. 105. In: OECD guideline for the testing of chemicals.

[OECD] Organisation for Economic Cooperation and Development. 1998. *Daphnia magna* reproduction test, No. 211. In: OECD guidelines for testing of chemicals.

[OECD] Organisation for Economic Cooperation and Development. 2000. Phototransformation of chemicals in water - direct and indirect photolysis. In: Draft OECD guideline for testing of chemicals. 1-59.

[OECD] Organisation for Economic Cooperation and Development. 2002. Aerobic and anaerobic transformation in aquatic sediment systems, No. 308. In: OECD guideline for the testing of chemicals.

[OECD] Organisation for Economic Cooperation and Development. 2004. Sediment-water chironomid toxicity test using spiked sediment, No. 218. In: OECD guidelines

for the testing of chemicals.

Roubert C, Sagne C, Kapsimali M, Vernier P, Bourrat F, Giros B. 2001. A Na^+/Cl^--dependent transporter for catecholamines, identified as a norepinephrine transporter, is expressed in the brain of the teleost fish medaka (*Oryzias latipes*). Mol Pharmacol 60(3):462-473.

Schultz M, Furlong E. 2008. Trace analysis of antidepressant pharmaceuticals and their select degradates in aquatic matrixes by LC/ESI/MS/MS. Anal Chem 80:1756-1762.

Sullivan D. 1998. Fertilizing with biosolids. PNW 508. Corvallis: Oregon State University, Extension Service. p 1-12.

Wang Y, Takai R, Yoshioka H, Shirabe K. 2006. Characterization and expression of serotonin transporter genes in zebrafish. Tohoku J Exp Med 208(3):267-274.

Nonconfidential Appendices

Appendix A: Duloxetine Data Summary Table

DULOXETINE DATA SUMMARY TABLE	
PHYSICAL/CHEMICAL CHARACTERIZATION	
Water Solubility	At pH 4 21.6 g/L At pH 7 2.74 g/L At pH 9 0.331 g/L
Dissociation Constant	$pK_a = 9.34$
Log Octanol/Water Partition Coefficient	Log D_{ow} At pH 4 0.781 At pH 7 1.54 At pH 9 3.35
Vapor Pressure or Henry's Law Constant	Not determined, assumed to be nonvolatile. Thermogravimetric analysis indicates decomposition and melting do not occur until 160°C.
Sorption/Desorption: (K_{oc}) (K_d)	2893 to 4296 1166 to 1731
DEPLETION MECHANISMS	
Hydrolysis	Half life at temperatures equal to or lower than 30°C is 1.5 to 3.5 months
Aerobic Biodegradation in Sewage Sludge	No significant degradation in 8 days. Small radioactive degradation product indicates eventual degradation.
Degradation in Water-Sediment Systems	After 100 days under aerobic conditions, only 40% to 60% of the applied radioactivity could be recovered as duloxetine. DT_{50} from water was 3 days. 5% to 11% evolved as $^{14}CO_2$. Up to minor 45 degradation products were identified.
Photolysis	Theoretical phototransformation is 100% loss within 1 month in pH 4, 7, and 9 aqueous buffers.
Metabolism	Human metabolism is extensive, <10% excreted as parent compound.

ENVIRONMENTAL EFFECTS	
Microbial Inhibition	Sewage Sludge Microorganisms EC50 36.5 mg/L NOEC 2 mg/L
Acute Toxicity	*Daphnia magna* (48 hour) EC50: 2.4 mg/L NOEC: 1.1 mg/L *Oncorhynchus mykiss* (96 hour) EC50: 1.3 mg/L NOEC: 0.45 mg/L *Pseudokirchneriella subcapitata* (72 hour) $EC_{biomass}50$: 0.064 mg/L $NOEC_{biomass}$: 0.011 mg/L $EC_{growthrate}50$: 0.20 mg/L $NOEC_{growthrate}$: 0.029 mg/L *Eisenia fetida* (14 days) LC50: >1000 mg/kg NOEC: ≥1000 mg/kg
Chronic Toxicity	Full Life-Cycle Toxicity Test with *Daphnia magna* (21 days) LOEC: 0.037 mg/L NOEC: 0.011 mg/L $EC_{survival}50$: 0.45 mg/L $EC_{reproduction}50$: 0.28 mg/L Early Life Stage Toxicity Tests with *Pimephales promelas* (4 day egg exposure and 28-day larvae exposure) overall results from 2 studies LOEC: 0.037 mg/L NOEC: 0.012 mg/L Larvae development and adult emergence study *Chironomus riparius* (28 days) LOEC: >92 mg/kg NOEC: ≥92 mg/kg

Appendix B: Report Summaries

Report Summary - Study: 1982.6112

Report Title: Duloxetine Hydrochloride - Validation of the Analytical Method for the Determination of Duloxetine in Aqueous Solutions

Study date: June 2001

Methods:

An HPLC/uv method for duloxetine was validated in freshwater. Freshwater was fortified with three concentrations of duloxetine hydrochloride and evaluated using the HPLC/uv method. Accuracy, precision, specificity, and linearity were evaluated. The adsorption of duloxetine hydrochloride to glass and plastic was evaluated by measuring aqueous concentrations at Day 0 and after 1 and 7 days of incubation in the dark at 20ºC.

Results:

The mean recovery of duloxetine hydrochloride (accuracy) was 102% with a standard deviation of 2.14% and a precision (RSD) of 2.09%. Control samples had no peaks which might interfere with the quantitation of duloxetine (specificity). The correlation coefficient of the standard curve was 0.9997 with a y-intercept of 0.0803.

The recoveries for the adsorption to glass and plastic on Day 0 ranged from 91.4% to 101%. The mean recoveries on Day 1 were 96.7% (glass) and 83.8% (plastic). The mean recoveries on Day 7 were 70.5% (glass) and 44.2% (plastic)

Report Summary - Study: 1982.6114

Report Title: Duloxetine Hydrochloride - Determination of the Water Solubility of a Test Substance Following OECD Guideline 105

Study date: June 2001

Methods:

The aqueous solubility of duloxetine hydrochloride was determined in pH 4, 7, and 9 aqueous buffers. Duloxetine hydochloride was added to 250 mL round bottomed flasks containing 100 mL of the buffer solutions. Test samples were agitated on a shaker table in a 30°C environmental chamber for equilibration periods of 24, 48, or 72 hours. After the equilibration period, the flasks were moved to an environmental chamber at 20°C for 24 hours with continued shaking. Duplicate samples were taken from the flasks and centrifuged at 25,848 g for 20 minutes. The supernatants were analyzed for duloxetine by HPLC.

Results:

The length of the equilibration time at 30°C did not affect the water solubility. Solubility decreased with increasing pH.

	Mean water solubility of duloxetine hydrochloride at 20°C (g/L)
pH 4	21.6
pH 7	2.74
pH 9	0.331

Report Summary - Study: 1982.6115

Report Title: Duloxetine Hydrochloride - Determination of the Dissociation Constant for a Test Substance Following OECD Guideline 112

Study date: June 2001

Methods:

The dissociation constant of duloxetine was determined at 20°C by a titration method using a Brinkman Titrino Workcell Version 4.0, Metrohm titrator. Two concentrations of duloxetine hydrochloride were prepared in CO_2-free water: 2.98 mM and 0.596 mM. The 2.98 mM solution was titrated with 0.150 mL aliquots of 0.1 M hydrochloric acid. The 0.596 mM solution was titrated with 0.020 mL aliquots of 0.1 M sodium hydroxide. The software program recorded the cumulative milliliters added and the resulting pH after each addition.

Results:

The dissociation constant (pK_a) was determined from the titration curve with 0.1 M sodium hydroxide. Titration with 0.1 M hydrochloric acid did not result in a titration curve. The mean pK_a for duloxetine hydrochloride was determined to be 9.34 at 20°C.

Report Summary - Study: 1982.6127

Report Title: Duloxetine Hydrochloride - Determining the Partitioning Coefficient (n-Octanol/Water) of a Test Substance by the Flask-Shaking Method Following OECD Guideline 107

Study date: June 2001

Methods:

The octanol/water partition coefficient (D_{ow}) of duloxetine hydrochloride was determined at pH 4, 7, and 9. A stock concentration of 201 mg/L duloxetine hydrochloride was prepared in buffer-saturated n-octanol. Solutions were prepared in duplicate for each pH using the volume ratios of 1:16, 1:8, and 1:4 of n-octanol-saturated buffer to duloxetine n-octanol stock. The mixtures were placed in centrifuge tubes with Teflon®-lined caps and rotated for 5 minutes at 20°C, centrifuged, and re-equilibrated. Each phase was then analyzed by HPLC.

Results:

The partition coefficients were dependent on pH but independent of concentration.

	Mean D_{ow} (range)	Log D_{ow}
pH 4	6.05 (5.76 to 6.39)	0.781
pH 7	34.7 (33.2 to 36.3)	1.54
pH 9	2250 (2110 to 2320)	3.35

Report Summary - Study: 1982.6123

Report Title: Duloxetine Hydrochloride - Determination of the Inherent Biodegradability and Adsorption of a Test Substance by the SCAS Test, Modified from OECD Guideline 302A

Study date: June 2001

Methods:

[^{14}C]Duloxetine hydrochloride was used to determine the kinetics of adsorption to sewage sludge and the aerobic biodegradability of duloxetine in activated sludge.

For adsorption determination, duplicate 500 mL flasks containing 200 mL 0.01 M $CaCl_2$ and 2500, 1250, 625, or 313 mg/L sludge solids were incubated with 1.01 mg/L [^{14}C]duloxetine hydrochloride. The flasks were stirred in an environmental chamber at 22 ± 3°C for 4 hours. At time points 0, 1, 2, and 4 hours, 30 mL homogenous samples were taken from each flask. Samples were split with one portion being extracted and analyzed for parent material by HPLC/RAM and LSC and the other portion centrifuged to isolate the supernatant for assay of parent material. The organic carbon content of the sludge was also determined.

For assessment of biodegradation potential, duplicate 500 mL flasks containing 250 mL of sewage sludge with 2500 mg/L solids were incubated with 1.00 mg/L of [^{14}C]duloxetine hydrochloride. The flasks were stirred in an environmental chamber at 22 ± 3°C. The flasks were stoppered and connected to a volatiles trapping system. Samples (20 mL) were taken from the flasks at 0, 8, 24, 72, 96, 120, 144, and 192 hours. The volatiles traps were sampled at 96 and 192 hours. Sludge samples were analyzed by HPLC/RAM following extraction of the whole sample. Volatile trap samples were assayed by LSC.

Results:

Adsorption of duloxetine hydrochloride to solids reached a plateau by 2 hours incubation with the sewage sludge. The adsorption coefficients ($K_{d(sludge)}$) at 4 hours were calculated to be 1166, 1269, 1197, and 1731 for 2500, 1250, 625, and 313 mg solids/L, respectively. The adsorption coefficients expressed as a function of the organic carbon content ($K_{oc(sludge)}$) of the activated sludge were calculated to be 2893, 3150, 2970, and 4296.

During the biodegradation study, duloxetine concentrations dropped from 91.3% at 0 hour to 62.1% by 8 hours. There was no further decline in duloxetine concentration over the remaining 8 days. Therefore, this initial decline is most likely attributable to extraction inefficiency as duloxetine becomes more tightly bound to the sludge solids. After 8 days, a small degradation peak was observed accounting for approximately 1.5% of the total radioactivity. The presence of this degradation product indicates the eventual biodegradability of duloxetine.

Report Summary - Study: 807566

Report Title: The Aerobic Transformation of [^{14}C]-Duloxetine Hydrochloride in Aquatic Sediment Systems

Study date: July 2007

Methods:

The rate of aerobic transformation of [^{14}C]-duloxetine was studied according to OECD Guideline 308 at a temperature of 20 ± 2ºC for 100 days in two aerobic sediments, one characterized as a sand and the other as a silt loam. A series of incubation vessels were established for each sediment containing approximately 50 to 65 g (dry weight) sediment with 167 to 297 mL of overlying water. On Day 0, the overlying water was dosed with 1 mg/L [^{14}C]-duloxetine. Vessels were connected to traps for the collection of CO_2 and volatile organic compounds. On Days 0, 7, 14, 30, 60, and 100, duplicate vessels from each water-sediment system were sacrificed. At sacrifice, overlying water was assayed for total radioactivity by LSC and profiled by HPLC with radiometric detection to identify parent and degradation products. The sediment was extracted with a series of solvents. The extracts were pooled, assayed for total radioactivity, and profiled by HPLC with radiometric detection. Finally, the remaining sediment (post-extraction) was combusted to evaluate bound residue. Satellite vessels for each sediment:water system were not dosed with duloxetine. These vessels were incubated along with the treated vessels and used to monitor sediment characteristics and to evaluate the ability of the sediments to metabolize radiolabelled glucose over the course of the study.

Results:

Water/sediment viability was assessed at Days 0, 30, 60, and 100. The evolved $^{14}CO_2$ after incubation with radiolabelled glucose indicated that the water sediment systems maintained microbial viability throughout the period of incubation.

Following application of ^{14}C-duloxetine, mass balance ranged from 94% to 100% of the applied radioactivity for the sediments over the course of the study.

Duloxetine rapidly partitioned to sediment from the overlying water, the DT50 from water was approximately 3 days. At the end of the study, only 2% to 8% of the applied radioactivity was located in the overlying water, 56% to 66% of the applied radioactivity was extractable from the sediment, and 21% to 32% of the applied radioactivity could not be extracted from the sediment.

There was evidence of primary and ultimate degradation. At the end of the study, 5% to 10% of the applied radioactivity was recovered as $^{14}CO_2$ and only 37% to 58% was identified as the parent duloxetine. There were 19 to 45 degradation products observed in the HPLC profiles of sediment extracts and overlying water over the course of the study. The majority of these products were transient and accounted for less than 1% to 2% of the applied radioactivity. At their maximum occurrence, four of the products were observed to be 5% to 21% of the applied radioactivity, but these levels had decreased to either less than 5% or less than detection by Day 100.

This study demonstrated that duloxetine disappears rapidly from overlying water and is degraded extensively under aerobic conditions in water sediment systems.

Report Summary - Study: 1982.6120

Report Title: Duloxetine Hydrochloride - Determination of the Abiotic Degradation of the Test Substance by Hydrolysis at Three Different pH Values Following OECD Guideline 111

Study date: June 2001

Methods:

Preliminary Test:

A hydrolysis study with duloxetine was conducted in three aqueous buffers, pH 4, 7, and 9. Duloxetine hydrochloride was added to the buffers for a final concentration of 10 mg/L (expressed as duloxetine free base). Aliquots of each solution were incubated in 50 mL volumetric flasks in a 50°C water bath for 5 days. All flasks were wrapped in foil. Analysis for duloxetine concentration was performed on Days 0 and 5.

Definitive Test:

A hydrolysis study with duloxetine was conducted in the same three aqueous buffers above. Two 200 mL aliquots of each solution containing 10 mg/L duloxetine were incubated in volumetric flasks for 28 days in a 40°C water bath. A third 200 mL aliquot was incubated for 35 days at 30°C. All flasks were wrapped in foil. At Days 0, 3, 7, 10, 12, 14, 17, 20, and 28, samples were removed from the 40°C incubation for analysis. Samples were taken from the 30°C incubation at Days 0, 3, 7, 10, 12, 17, 28, and 35.

Results:

Preliminary Test:

The percent duloxetine remaining after 5 days at pH 4, 7, and 9 was 56.4%, 75.9%, and 60.7%, respectively.

Definitive Test:

The following first order hydrolysis rate characteristics for duloxetine were calculated.

pH	°C	Initial [Duloxetine] on Day 0 (mg/L)	[Duloxetine] at end of test (mg/L)	Hydrolysis Rate Constant (Day^{-1})	Half Life (Days)
4	30	10.4	6.07	0.0165	41.88
7	30	10.0	8.05	0.0069	100.62
9	30	10.0	7.43	0.0096	72.48
4	40	9.98	2.92	0.0440	15.73
7	40	10.2	5.67	0.0219	31.69
9	40	9.84	4.21	0.0306	22.64

Report Summary - Study: 1982.6130

Report Title: Duloxetine Hydrochloride - Determination of the Ultraviolet-Visible Absorption Spectrum in Aqueous Solution Following OECD Proposed Guideline for Phototransformation of Chemicals in Water

Study date: June 2001

Methods:

Solutions of 0.0015 M duloxetine hydrochloride were prepared in pH 4 and pH 7 buffers and in unbuffered pure reagent water. A solution of 0.0003 M duloxetine hydrochloride was prepared in pH 9 buffer. The absorption spectra of the test solutions were measured using a Hewlett-Packard Model 8453 UV-Vis spectrophotometer. Absorbance peaks recorded in the wavelength range for natural sunlight (i.e. 295 to 800 nm) were used to calculate the propensity for phototransformation of duloxetine.

Results:

Absorbance peaks were observed in the range of 295 to 325 nm. The molar absorption coefficient was determined for each peak and using these values it was calculated that within 30 days, 100% of duloxetine would be phototransformed at pH 4, 7, and 9 and in pure reagent water.

Report Summary - Study: 1982.6126

Report Title: Duloxetine Hydrochloride - Activated Sludge Respiration Inhibition Following OECD Guideline 209

Study date: June 2001

Methods:

Duloxetine hydrochloride was incubated with synthetic sewage feed and activated sludge (1.5 g/L solids concentration) in a volume of 500 mL in 1000 mL beakers. There were five treatment levels consisting of one replicate each. Four treatment levels of 3,5-dichlorophenol were incubated as above as a reference control for the study. There were two controls consisting of synthetic sewage feed and activated sludge only and an abiotic control with synthetic sewage feed only. The nominal concentrations of duloxetine (expressed as free base) were 2, 6, 18, 54, and 162 mg/L. The stock solution (500 mg/L) used to make the test concentrations was analyzed by HPLC and determined to be 498 mg/L duloxetine (free base). The nominal concentrations of 3,5-dichlorophenol were 3.0, 10, 30, and 100 mg/L. After 3 hours and 25 minutes of incubation during which the test systems were aerated, homogenous samples from each replicate and control were collected. The pH was measured and the dissolved oxygen was monitored over 10 minutes in a Strathkelvin Instruments oxygen system while the samples were continuously stirred in a water bath. From these measurements, the oxygen consumption rate was calculated for each treatment level and control.

Results:

The temperature of the test solutions was maintained between 18.5 and 21.9°C during the incubation and the water bath used during the oxygen measurements was maintained at approximately 21°C. The pH in all treatments was between 7.27 and 7.59. The respiration rates for the control vessels were 29.3 and 31.1 mg O_2/L/h. The abiotic control respiration rate was -1.8 mg O_2/L/h. The respiration rates for the reference compound were 26.9, 15.4, 3.5, and 0.6 mg O_2/L/h for 3.0, 10, 30, and 100 mg/L, respectively. The EC50 of 3,5-dichlorophenol was calculated to be 11.1 mg/L which is within the acceptable limits (5.0 to 30 mg/L) as specified in the OECD 209 Guideline. Respiration rates for the treatment levels were 30.7, 21.8, 26.0, 7.7, and -0.8 mg O_2/L/h for 2, 6, 18, 54, and 162 mg/L duloxetine, respectively. The no-observed-effect concentration for duloxetine was 2 mg/L and the EC50 was calculated to be 36.5 mg/L.

Report Summary - Study: 1982.6125

Report Title: Duloxetine Hydrochloride - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Static-Renewal Conditions

Study date: June 2001

Methods:

The acute toxicity of duloxetine to rainbow trout was assessed according to OECD Guideline 203. Juvenile trout (mean weight 0.75 g, mean length 42 mm) were exposed to mean measured concentrations of duloxetine of 0 (control), 0.45, 0.89, 1.9, 3.8, 8.6, and 17 mg/L (here and below expressed as duloxetine free base) for 96 hours. A total of 10 fish were exposed to each treatment level in a volume of 15 L. At 48 hours, the fish were transferred to fresh exposure solutions. Daily mortality and behavioral changes were recorded.

Results:

Temperature in the test system was maintained between 13 and 14°C. The pH and dissolved oxygen ranged from 6.7 to 7.4 and 6.2 to 10.2 mg/L, respectively. At 96 hours, the cumulative mortality at concentrations ≥1.9 mg/L was 100%. There was no mortality in lower treatment levels or the control. Lethargic swimming behavior was observed in the 0.89 mg/L. The 96 hour LC50 was determined to be 1.3 mg/L duloxetine with 95% confidence intervals of 0.89 to 1.9. The 96 hour no-observed-effect concentration was 0.45 mg/L duloxetine.

Report Summary - Study: 1982.6116

Report Title: Duloxetine Hydrochloride - Acute Toxicity to Daphnids (*Daphnia magna*) Under Static Conditions

Study date: June 2001

Methods:

The acute toxicity of duloxetine to daphnids was assessed according to OECD Guideline 202. Daphnids (≤24 hours old) were exposed to mean measured concentrations of duloxetine of 0 (control), 0.10, 0.52, 1.1, 2.1, 4.2, and 8.5 mg/L (expressed as duloxetine free base) for 48 hours. Four replicates were included at each treatment level. Each replicate contained 5 animals in 200 mL of test solution. The test solutions were prepared with fortified well water (initially pH 8.0, conductance 550 µmhos/cm, total hardness as $CaCO_3$ 180 mg/L, and total alkalinity as $CaCO_3$ 120 mg/L). At 24 and 48 hours, water quality measurements were made and the number of immobilized daphnids in each replicate was recorded.

Results:

During the testing period, the temperature ranged from 19 to 21°C, the pH from 7.9 to 8.2, and the dissolved oxygen from 8.6 to 10.3. No immobilization or other adverse effects (e.g. lethargy) were observed in treatment levels ≤1.1 mg/L duloxetine and the control. Immobilization occurred in 35%, 100%, and 100% of daphnids exposed to 2.1, 4.2, and 8.5 mg/L duloxetine, respectively. The surviving daphnids in the 2.1 mg/L group were observed to be lethargic. The 48-hour EC50 and 95% confidence limits were calculated to be 2.4 mg/L and 1.1 to 4.2 mg/L duloxetine, respectively. The no-observed-effect concentration was 1.1 mg/L duloxetine.

Report Summary - Study: 1982.6118

Report Title: Duloxetine Hydrochloride - Acute Toxicity to the Freshwater Green Alga *Pseudokirchneriella subcapitata*, Following OECD Guideline #201

Study date: June 2001

Methods:

A static toxicity test was conducted to evaluate the effects of duloxetine hydrochloride on the green alga, *Pseudokirchneriella subcapitata*. There were six treatment levels containing duloxetine hydrochloride and three replicates at each treatment. The initial measured concentrations in the treatments were 0.0053, 0.011, 0.029, 0.070, 0.20, and 0.47 mg/L duloxetine (concentrations and all results below are expressed as the free base). There were six replicates for the control. To each replicate, approximately one million algal cells were added to 100 mL of appropriately treated Algal Assay Procedure medium in sterile 250 mL flasks to give an initial cell concentration of 10,000 cells/mL. The cells were cultured under continuous illumination at 400 to 490 footcandles and continuous shaking for 72 hours. The pH and conductivity during the test ranged from 7.4 to 8.2 and 80 to 90, respectively. The temperature was 24°C. At 24, 48, and 72 hours, a sample was removed from each flask and the cells were counted using a hemocytometer. These measurements were used to calculate the growth rate and biomass for each replicate.

Results:

After 72 hours, the concentration of duloxetine in all treatments was less than 10% of the nominal concentration and below the limit of quantification of the chemical assay. An additional replicate in the 0.029 mg/L treatment in which no cells were added also contained less than 10% of the initial concentration after 72 hours. Thus the disappearance of duloxetine was probably due in large part to photolysis. There is no established method to maintain constant exposure concentrations in algal toxicity studies if test material declines over the study. After 72 hours, the control growth rate was 1.61 $days^{-1}$ (standard deviation = 0.020) and for treatment concentrations ≥0.070 mg/L the rate was significantly reduced (≤1.51 $days^{-1}$). Thus, the no-observed-effect concentration (NOEC) for growth rate was 0.029 mg/L. The median effective duloxetine concentration on reduction of growth rate (EC50) was 0.20 mg/L with 95% confidence limits of 0.088 to 0.31 mg/L. After 72 hours, the biomass (the area under the growth curve) of the control cells was 10,500 cells·days/mL. At 0.47 mg/L, duloxetine biomass was significantly reduced. Based on these results, the NOEC for biomass would be 0.20 mg/L. However, duloxetine concentrations ≥0.029 mg/L duloxetine caused >10% reduction of biomass. Thus, the NOEC for biomass was considered to be 0.011 mg/L rather than 0.20 mg/L. The EC50 at 72 hours was calculated to be 0.064 mg/L with 95% confidence limits of 0.019 to 0.23 mg/L. Biomass was the most sensitive endpoint and, therefore, the most conservative EC50 and NOEC for this study were initial duloxetine concentrations of 0.064 and 0.011 mg/L, respectively.

Report Summary - Study: 1982.6133

Report Title: Duloxetine Hydrochloride - Acute Toxicity to Earthworms (*Eisenia fetida*) following OECD Guideline #207

Study date: February 2002

Methods:

The acute toxicity of duloxetine to earthworms was assessed according to OECD Guideline 207. Adult earthworms (300-600 mg) were exposed to 63, 130, 250, 500, and 1000 mg/kg duloxetine (as free base) in artificial soil for 14 days. Four replicates of 10 earthworms each were exposed to each concentration and a blank control in 750 g (wet weight) of amended artificial soil. Mortality and observations of surviving earthworms were recorded on Days 7 and 14. On Day 14, the surviving earthworms were collectively weighed on a per replicate basis after being rinsed with deionized water and blotted dry.

Results:

Temperature, pH, and moisture content in the test system ranged from 19 to 21°C, 5.8 to 6.5, and 21% to 39%, respectively. There was 100% survival in all treatment levels and controls. The 14-day NOEC was 1000 mg/kg and the LC50 was >1000 mg/kg. After 14 days, the mean change in body weight of earthworms exposed to 63, 130, 250, 500, and 1000 mg/kg duloxetine was -16.0%, -16.0%, -16.3%, 17.2%, and 26.8%, respectively. The mean weight change in the control group was -14.2%.

Report Summary - Study: 1982.6129

Report Title: Duloxetine Hydrochloride - Full Life-Cycle Toxicity Test with Water Fleas, *Daphnia magna* Under Flow-Through Conditions, Following FIFRA Guideline 72-4, OECD Guideline #211, and OPPTS Draft Guideline 850.1300.

Study date: June 2001

Methods:

Daphnia magna, ≤24 hours old, were exposed to duloxetine hydrochloride for 21 days in a flow-through exposure system. There were six treatment levels and a control with four replicate vessels in each treatment. Each replicate vessel held 10 daphnids in a volume of 1.4 L. Test solutions were delivered to the vessels at a rate of six vessel volumes per 24-hour period to provide a 90% solution replacement rate of approximately 9 hours. The mean measured concentrations in the treatments were 0 (control), 0.011, 0.037, 0.080, 0.14, 0.26, and 0.50 mg/L duloxetine (expressed here and below as the free base) prepared in fortified well water. Conditions during the exposure were 19 to 22°C and a light:dark cycle of 16:8 hours at 30 to 70 footcandles. The number of immobilized adult daphnids and observations of abnormal behavior were recorded daily. Assessments of offspring released were determined beginning on Day 7 and three times per week through Day 21.

Results:

Water quality parameters monitored during the test included pH (7.9 to 8.2), conductivity (500 µmhos/cm), total hardness (180 mg/L as $CaCO_3$), and total alkalinity (110 to 120 mg/L as $CaCO_3$). After 21 days, mean percent survival in the treatments was 95%, 100%, 93%, 93%, 100%, 100%, and 38% in the control, 0.011, 0.037, 0.080, 0.14, 0.26, and 0.50 mg/L duloxetine, respectively. The EC50 for survival was calculated to be 0.45 mg/L. After 21 days, the mean body length of daphnids exposed to ≥0.037 mg/L duloxetine was significantly reduced from the control average of 5.1 mm. The mean dry weight of daphnids exposed to ≥0.14 mg/L was significantly reduced compared to the control average of 1.1 mg. After 21 days, the mean cumulative number of offspring released per female daphnid in the treatments was 161, 166, 140, 131, 113, and 72 for control, 0.011, 0.037, 0.080, 0.014, and 0.26 mg/L duloxetine, respectively. The reproduction for the 0.50 treatment was not analyzed in the statistics because of the significant survival effect. Offspring numbers in treatment levels ≥0.037 were significantly different from the control. The no-observed-effect concentration and the EC50 for reproduction were calculated to be 0.011 and 0.28 mg/L duloxetine, respectively.

Report Summary - Study: 1982.6268

Report Title: Duloxetine HCl - Early Life-Stage Toxicity Test with Fathead Minnow, (*Pimephales promelas*), Following OECD Guideline #210

Study date: July 2007

Methods:

The chronic toxicity of duloxetine to fathead minnows was assessed according to OECD Guideline 210. Fathead minnow embryos (<24 hours old) were exposed to mean measured concentrations of duloxetine of 0 (control), 0.005, 0.012, 0.05, 0.14, and 0.45 mg duloxetine/L through hatch until 28 days post-hatch. The test was initiated with two replicates of 60 eggs per treatment level. At hatch, the two replicates were impartially reduced to 20 larvae per replicate.

Results:

There were no significant effects on hatching success (80% in control), appearance of live larvae at hatch (100% normal in control), or survival of fry 28 days after hatch (95% in control). At test termination, total length of larvae exposed to the control averaged 27.4 mm. The mean total length of larvae exposed to the 0.0050, 0.012, 0.050, 0.14, and 0.45 mg duloxetine/L treatment levels was 25.6, 25.8, 24.5, 23.7, and 25.3 mm, respectively. Statistical analysis (Williams' Test) demonstrated a significant difference in larval length among larvae exposed to all treatment levels tested compared to the control (27.4 mm). The percent decreases from control for total length were 7%, 6%, 11%, 14%, and 8% for 0.005, 0.012, 0.05, 0.14, and 0.45 mg duloxetine/L, respectively. Dry weight of larvae in the control averaged 0.0416 g. Mean dry weights of larvae exposed to the 0.0050, 0.012, 0.050, 0.14, and 0.45 mg duloxetine/L treatment levels averaged 0.0346, 0.0362, 0.0311, 0.0285, and 0.0342 g, respectively. Statistical analysis (Williams' Test) demonstrated a significant difference in dry weight among larvae exposed to the 0.050, 0.14, and 0.45 mg duloxetine/L treatment levels compared to the control (0.0416 g). The percent decreases from control in dry weight were 17%, 13%, 25%, 31%, and 18% for 0.005, 0.012, 0.05, 0.14, and 0.45 mg duloxetine/L, respectively.

Strong concentration-response relationships were not found in the study. No significant effects on weight were found at the lowest two treatment concentrations of 0.005 and 0.012 mg duloxetine/L. At these two treatment levels, minimal, but statistically significant reductions (7% and 6%) in length were found. Statistically, the lowest-observed-effect concentration (LOEC) and the no-observed-effect concentration (NOEC) were at or below the lowest concentration tested, 0.005 mg/L. Because the biological significance of a 6% or 7% reduction in length is not clear, it is possible that the NOEC could be as high as 0.012 mg/L.

Report Summary - Study: 1982.6273

Report Title: Duloxetine HCl - Early Life-Stage Toxicity Test with Fathead Minnow, (*Pimephales promelas*), Following OECD Guideline #210

Study date: July 2007

Methods:

The chronic toxicity of duloxetine to fathead minnows was assessed according to OECD Guideline 210. Fathead minnow embryos (<24 hrs old) were exposed to mean measured concentrations of duloxetine of 0 (control), 0.00015, 0.00045, 0.0016, 0.004, 0.012, and 0.037 mg duloxetine/L through hatch until 28 days post-hatch. The test was initiated with two replicates of 60 eggs per treatment level. At hatch, the two replicates were impartially reduced to 20 larvae per replicate.

Results:

There were no significant effects on embryo hatching success (83% in control), appearance of live larvae at hatch (100% normal in control), or survival of fry 28 days after hatch (95% in control). At test termination, total length of larvae exposed to the control averaged 30.4 mm. The mean total length of larvae exposed to the 0.00015, 0.00045, 0.0016, 0.004, 0.012, and 0.037 mg duloxetine/L treatment levels was 30.0, 29.3, 29.8, 29.4, 28.9, and 27.7 mm, respectively. Statistical analysis (Williams' Test) demonstrated a significant difference in larval length compared to the control at the 0.037 mg duloxetine/L treatment level only. The percent decreases from control for total length were 1%, 4%, 2%, 3%, 5%, and 9% for 0.00015, 0.00045, 0.0016, 0.004, 0.012, and 0.037 mg duloxetine/L, respectively. Dry weight of larvae in the control averaged 0.0554 g. Mean dry weights of larvae exposed to the 0.00015, 0.00045, 0.0016, 0.004, 0.012, and 0.037 mg duloxetine/L treatment levels averaged 0.0549, 0.0508, 0.0529, 0.0543, 0.0514, and 0.0457 g, respectively. Statistical analysis (Williams' Test) demonstrated a significant difference in dry weight compared to control at the 0.037 mg duloxetine/L treatment level only. The percent decreases from control in dry weight were 1%, 8%, 5%, 2%, 7%, and 18% for 0.00015, 0.00045, 0.0016, 0.004, 0.012, and 0.037 mg duloxetine/L, respectively.

In this early life stage study with fathead minnows, growth was the most sensitive endpoint. There was a concentration-response relationship for both weight and length. The LOEC and NOEC values were 0.037 mg/L and 0.012 mg/L, respectively.

Report Summary - Study: 1982.6278

Report Title: [^{14}C]-Duloxetine - Full Life-Cycle Toxicity Test with Sediment-Dwelling Midges (*Chironomus riparius*) Under Static Conditions, Following OECD Guideline 218

Study date: July 2007

Methods:

The chronic toxicity of duloxetine to midges was assessed according to OECD Guideline 218. Test vessels consisted of approximately 75 mL artificial sediment with 300 mL of overlying water. The sediment was dosed with radiolabelled duloxetine at nominal concentrations from 3.1 to 100 mg/kg. A blank control and a solvent control were included. Larval midges (2 to 3 days post-hatch) were introduced to the test vessels (8 replicates per treatment, 20 midges per replicate) to initiate the exposure. Four vessels per treatment were used for biological observations. The remaining vessels were used for analytical measurements only. Vessels were observed daily for emergence of adult midges for 28 days.

Results:

The mean total radioactive residue measured in the sediments on Days 0, 7, and 28 was 2.8, 6.0, 12, 27, 42, and 92 mg/kg (expressed as duloxetine). HPLC/RAM analysis confirmed that >90% of the radioactive residue in sediments was duloxetine throughout the study. The total ^{14}C radioactive residue concentration in the pore water at the highest treatment level ranged from 16 to 49 mg/L. The total ^{14}C radioactive residue concentration in the overlying water ranged from 0.82 to 2.2 mg/L.

There were no alterations in the ratio of male and female midges which emerged over the treatment range. There were no treatment-related effects on emergence of midges up to the highest level tested compared to pooled control (78%). There were no treatment-related effects on development rate of midges compared to the pooled control (0.0545 days^{-1}). There was a minimal decrease at 27 mg/kg which was considered neither biologically significant nor treatment related. The LOEC was determined to be >92 mg total ^{14}C duloxetine residue/kg and the NOEC was 92 mg total ^{14}C duloxetine residue/kg.

Appendix C: Curriculum Vitae of Preparers

Alison Nimrod Perkins

Lilly Research Laboratories, Indianapolis, IN

Ph.D.	Pharmacology/Toxicology, University of Mississippi	1996
B.S.	Chemistry, Tulane University	1988

Previous Experience: Research Scientist, University of Mississippi in the National Center for Natural Products Research (1997 to 1999). Supervised technical staff responsible for screening extracts and pure compounds from natural products for various biological activities and developed new assays. Senior Toxicologist, Lilly Research Laboratories (2000 to 2005). Managed toxicology issues and studies for drug discovery and development. Author on several publications and abstracts in the natural products arena as well as environmental toxicology. Guest lecturer for undergraduate and graduate level courses in pharmacology and toxicology.

Current Responsibility: Senior Research Scientist, Health, Safety and Environmental. Prepares environmental risk assessments for animal and pharmaceutical products for submission to the FDA and Europe. Prepares guidelines for production facilities for containment of active products. Designs and monitors GLP environmental chemistry, fate, and toxicity studies.

Professional Activities:

Editorial Board: Environmental Toxicology and Chemistry
Member: Society of Environmental Toxicology and Chemistry
Reviewer: ETC, Journal of Natural Products, Journal of Biomolecular Screening

Roger D. Meyerhoff

Lilly Research Laboratories, Indianapolis, IN

Ph.D. Fisheries/Pharmacology & Toxicology, Oregon St. Univ. 1980
M.S. Fisheries/Limnology & Water Pollution, Oregon St. Univ. 1976
B.S. Fisheries and Wildlife Biology, Univ. Calif. at Davis 1974

Previous Experience: Senior Toxicologist up to Research Advisor and Head of Environmental Science and Hazard Communications (1980 to 2004). Conducted acute and chronic environmental toxicology studies with over 20 aquatic and terrestrial species and coordinated aquatic and terrestrial field studies. Author of a number of abstracts, papers, and chapters on the results of these studies and lecturer on environmental risk assessment to undergraduates and graduate students at several universities. Has prepared risk assessments for pesticides, animal products, and pharmaceutical products to support submissions to the EPA, FDA, Europe, Australia, and Japan since 1982. As Head of Environmental Science and Hazard Communications, was responsible for personnel and operations supporting environmental safety at production facilities, registration of new products (conduct of inhalation, aquatic, wildlife, microbial, and environmental chemistry studies), and workplace safety (material safety data sheets, caution statements, and risk assessments for human exposure).

Current Responsibility: Senior Research Advisor for Health, Safety and Environmental in Lilly Research Laboratories. Responsible for human and environmental risk assessments to support product registrations, workplace safety, product safety, and environmental safety at production facilities.

Professional Activities:
Chairman (1993-1995), SETAC Foundation for Environmental Education
President (1991-1992), Society of Environmental Toxicology & Chemistry (SETAC)
Board of Directors (1987-1993), SETAC
Member (1991-Present), PhRMA Environmental Working Group
Member (1987-Present), An. Health Inst. Sci. Com., Env. Working Group
Member (1985-1987), National Agricultural Chemical Association
Subcommittee on Environmental Toxicology and Chemistry

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/s/

EMILY A MCVEY
10/30/2009

JON E CLARK
11/02/2009

MOHEB M NASR
11/02/2009

Food and Drug Administration
Center for Drug Evaluation and Research
Office of Pharmaceutical Science/Immediate Office

Memorandum

Date: October 30, 2009

From: Emily A. McVey, Ph.D.
OPS/IO/PARS

To: Don Henry,
ONDQA

Through: Jon Clark, M.S.
OPS/IO/PARS

Subject: **NDA 22-516:** Cymbalta (duloxine HCl)
Sponsor: Eli Lilly and Company
Review of Environmental Assessment

A. Background

Eli Lilly and Company requests approval of Cymbalta (duloxetine HCl) for the treatment of chronic pain, as in osteoarthritis and chronic low back pain (NDA 22-516). An Environmental Assessment (EA) has been submitted pursuant to 21 CFR part 25.

B. Discussion

Executive Summary

This Environmental Assessment, dated May 2009, supports the new drug supplemental application for Cymbalta (duloxetine HCl) for the treatment of chronic pain, as in osteoarthritis and chronic low back pain. The EA was prepared in accordance with 21 CFR Part 25 by Eli Lilly and Company.

The sponsor estimates an EIC of 2.1µg/L in water, based on an estimate of less than 100,000 kg of duloxetine used in the United States, despite the added indication addressed in this supplemental NDA for treatment of chronic pain, with no sorption, metabolism or degradation taken into account. Since the EIC is greater than 1 ppb, the environmental assessment was submitted and reviewed. The submitted information was as recommended in

the CDER/CBER Guidance for Industry: Environmental Assessment of Human Drug and Biologics Applications (July 1998).

Duloxetine and related metabolites enter the environment through wastewater facilities, where duloxetine is expected to partition to the solids, resulting in a lower aqueous concentration. The adjusted expected EIC in water (a maximum of 0.5 µg/L in surface water) is not expected to affect aquatic organisms, based on the toxicity tests performed herein on fish, invertebrates and algae. In addition, comparison of predicted plasma concentrations in fish with therapeutic plasma levels in humans indicates that pharmacological effects are not expected in aquatic organisms. Duloxetine is not expected to persist in the aquatic environment because it is subject to degradation, hydrolysis and photolysis. The maximum concentration of duloxetine expected in soil from application of biosolids is not expected to harm terrestrial organisms based on the earthworm toxicity assay performed. No adverse environmental effects have been identified in this environmental assessment that would warrant further investigation or mitigation.

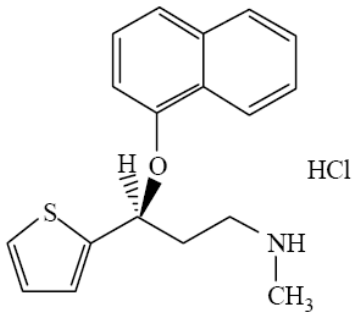
A FONSI is recommended.

C. Environmental Assessment Review

1. **Date:** May, 2009
2. **Applicant:** Eli Lilly and Company
3. **Address:** Lilly Corporate Center, Indianapolis, IN 46285
4. **Proposed Action:** Eli Lilly and Company is filing a supplemental NDA pursuant to section 505(b)(1) of the Federal Food, Drug and Cosmetic Act for Cymbalta (duloxetine HCl) for the short term treatment of chronic pain, as in osteoarthritis and chronic low back pain.

5. **Identification of Chemicals**

(i) Established Name: (+)-N-methyl-©-(1-naphthalenyloxy)-2-thiophenepropanamine hydrochloride
(ii) Brand/Proprietary Name/Tradename: Cymbalta
(iii) Chemical Abstract Registration Number: 136434-34-9
(iv) Molecular Formula: $C_{18}H_{19}NOS \cdot HCl$
(v) Molecular Weight: 333.88
(vi) Chemical Structure:



6. Environmental Characterization

Physical/Chemical Values

Water solubility
21.6, 2.74, and 0.331 g/L at pH 4, 7, and 9, respectively

Dissociation constants (pKa)
9.34

Octanol/Water Partition Coefficient
log K_{ow} = 0.781, 1.54, and 3.35 at pH 4, 7, and 9, respectively

Vapor Pressure
Not determined, assumed to be nonvolatile. Thermogravimetric analysis indicates decomposition and melting do not occur until 160°C.

Sorption/Desorption
K_{oc} = 2893 to 4296
K_d = 1166 to 1731

Environmental Depletion Mechanisms

Duloxetine is almost completely metabolized after administration; less than 10% of the administered dose is excreted as parent compound. It hydrolyzes slowly at $\leq$ 30°C, with a ½ life ranging from 1.5 to 3.5 months (at 30°C).

The theoretical phototransformation of duloxetine HCl is estimated to be 100% within 1 month, based on its UV absorption spectrum. Also, in the algal toxicity study, a control algae spiked with 0.029 mg/L duloxetine had no detectable duloxetine after 3 days of incubation with continuous light. While duloxitine binds to glass at low levels (30% decrease in aqueous concentration in a glass test tube over 7 days), the majority of this disappearance is likely due to photolysis. Duxloxetine HCl was not biodegraded when incubated with sewage sludge for 8 days, but the presence of a small non-duloxetine radioactive peak indicated that there was potential for transformation of duloxetine. Duloxetine did absorb to sludge biosolids.

The transformation of duloxetine in a water-sediment system suggested a ½ life of 3 days with extensive degradation in aerobic conditions. 37% to 58% of radioactivity was recovered as duloxetine from sand and silt loam water-sediment systems, respectively, and up to 45 separate degradation products were observed (including 5 to 11% ultimate degradation to CO_2.

Duloxetine should not persist in the environment. Extensive metabolism in humans, and under aerobic conditions in sediments, suggest vigorous biodegradation, as well as photolysis and hydrolysis in the aqueous environment.

Environmental Fate and Effects

Duloxetine will enter the environment through general use throughout the population. The expected introduction concentration (EIC) of total residues of duloxetine in a sewage treatment facility could be as high as 2.1 µg/L. The primary depletion mechanism will be sorption to biosolids at water treatment facilities, giving an adjusted $EIC_{aquatic}$ of 0.5 µg/L. The concentration in biosolids could be as high as 530 µg/L. If these biosolids were then land-applied, duloxetine might enter the terrestrial environment at a soil concentration ($EIC_{terrestrial}$) of 4.2 µg/kg. It is not expected to volatilize.

The following ecotoxicity effects studies were conducted in accordance with OECD guidelines:

ENVIRONMENTAL EFFECTS	
Microbial Inhibition	Sewage Sludge Microorganisms EC50 36.5 mg/L NOEC 2 mg/L
Acute Toxicity	*Daphnia magna* (48 hour) EC50: 2.4 mg/L NOEC: 1.1 mg/L *Oncorhynchus mykiss* (96 hour) EC50: 1.3 mg/L NOEC: 0.45 mg/L *Pseudokirchneriella subcapitata* (72 hour) $EC_{biomass}50$: 0.064 mg/L $NOEC_{biomass}$: 0.011 mg/L $EC_{growthrate}50$: 0.20 mg/L $NOEC_{growthrate}$: 0.029 mg/L *Eisenia fetida* (14 days) LC50: >1000 mg/kg NOEC: ≥1000 mg/kg
Chronic Toxicity	Full Life-Cycle Toxicity Test with *Daphnia magna* (21 days) LOEC: 0.037 mg/L NOEC: 0.011 mg/L $EC_{survival}50$: 0.45 mg/L $EC_{reproduction}50$: 0.28 mg/L Early Life Stage Toxicity Tests with *Pimephales promelas* (4 day egg exposure and 28-day larvae exposure) overall results from 2 studies LOEC: 0.037 mg/L NOEC: 0.012 mg/L Larvae development and adult emergence study *Chironomus riparius* (28 days) LOEC: >92 mg/kg NOEC: ≥92 mg/kg

To assess the environmental risk of duloxetine in the environment, the median effect concentration or no-observed-effect-concentration (MEC or NOEC) was compared to the Maximum Expected Environmental Concentration (MEEC). The quotient of the two numbers (or Assessment Factor) must be above 1000 for Tier One screening, above 100 for Tier Two, and above 10 for Tier Three (see Guidance document for details). The following table summarizes the results of this comparison for each species.

Effects Concentrations Compared to Expected Environmental Concentrations

Aquatic Environment					
Acute Studies					
Species	NOEC (µg/L free base)	LC50 or EC50 (µg/L free base)	MEEC (µg/L free base)	LC50 or EC50 ÷ MEEC	Required Assessment Factor
Sewage microorganisms (3 hours)	2000	36,500	0.5	73,000	≥1000
Rainbow trout (96 hours)	450	1300	0.5	2600	≥100
Daphnia magna (48 hours)	1100	2400	0.5	4800	≥100
Pseudokirchneriella subcapitata (72 hours)	N/A	64	0.5	128	≥100
Chronic Studies					
Pseudokirchneriella subcapitata (72 hours)	11	N/A	0.05*	220	≥10
Daphnia magna (21 days)	11	280	0.05*	220	≥10
Pimephales promelas (4 embryo days + 28 post-hatch days)	12	N/A	0.05*	240	≥ 10
Chironomus riparius (28 days)	≥ 92,000 µg/kg	N/A	16.5 µg/kg	≥ 5575	≥ 10
Terrestrial Environment					
Eisenia fetida (14 days)	≥ 1,000,000	>1,000,000	4.2	>238,095	≥ 100

*Note: for chronic exposure, a dilution factor of 10 was utilized.

Since the ratio of the toxicity values to the Maximum Expected Environmental Concentration is greater than the relevant assessment factor, no additional studies are

required and adverse environmental effects are not anticipated as a consequence of the use of Duloxetine

Cumulative Environmental Fate and Effects

Since duloxetine HCl is still under patent with Eli Lilly as Cymbalta, the estimates contained above include cumulative effects for this compound.

7. Mitigation Measures and Alternatives

Since no adverse environmental impacts are expected, no mitigation or alternatives were provided.

D. Literature Reviewed

[1-6]

1. Bercu JP, Parke NJ, Fiori JM, Meyerhoff RD. 2008. Human health risk assessments for three neuropharmaceutical compounds in surface waters. *Regulatory Toxicology and Pharmacology* 50:420-427.

2. 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.

3. 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.

4. Nalecz-Jawecki G. 2007. Evaluation of the in vitro biotransformation of fluoxetine with HPLC, mass spectrometry and ecotoxicological tests. *Chemosphere* 70:29-35.

5. Pery 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.

6. Schultzt MM, Furlong ET. 2008. Trace analysis of antidepressant pharmaceuticals and their select degradates in aquatic matrixes by LC/ESI/MS/MS. *Analytical Chemistry* 80:1756-1762.

Findings: Evidence for the existence of serotonin and norepinephrine transporters in fish has been reported, so the sponsor compared the steady-state plasma concentrations in humans to the calculated concentration that might be found in fish plasma, based on the predicted environmental concentration in surface water. They estimate the predicted plasma concentration in fish to be 0.09 µg/L, 344 times lower than the steady state concentration in humans. They conclude that even if the serotonin receptors in fish had the same sensitivity to

duloxetine as those in humans, pharmacological effects would not be expected due to the very low exposure levels.

E. Comments and Conclusions

Based on an evaluation of the information provided in this EA and previous EAs, in FDA guidance, and on the scientific validity of the "no effects" conclusions of the EA, no significant adverse environmental impacts are expected from the use of duloxetine HCl for chronic pain.

A Finding of No Significant Impact (FONSI) is recommended.

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/s/

EMILY A MCVEY
10/30/2009

JON E CLARK
11/02/2009