*National Toxicology Program*
*U.S. Department of Health and Human Services*


## Center For The Evaluation Of Risks To Human Reproduction

# NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Fluoxetine

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## Preface

The National Toxicology Program (NTP) established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in 1998. The CERHR is a publicly accessible resource for information about adverse reproductive and/or developmental health effects associated with exposure to environmental and/or occupational chemicals. The CERHR is located at the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health and Dr. Michael Shelby is the director.[1]

The CERHR broadly solicits nominations of chemicals for evaluation from the public and private sectors. The CERHR follows a formal process for review and evaluation of nominated chemicals that includes multiple opportunities for public comment. Chemicals are selected for evaluation based upon several factors including the following:

- potential for human exposure from use and occurrence in the environment
- extent of public concern
- production volume
- extent of data from reproductive and developmental toxicity studies

The CERHR convenes a scientific expert panel that meets in a public forum to review, discuss, and evaluate the scientific literature on the selected chemical. Public comment is invited prior to and during the meeting. The expert panel produces a report on the chemical's reproductive and developmental toxicities and provides its opinion of the degree to which exposure to the chemical is hazardous to humans. The panel also identifies areas of uncertainty and where additional data are needed. The CERHR expert panels use explicit guidelines to evaluate the scientific literature and prepare the expert panel reports. Expert panel reports are made public and comments are solicited.

Next, the CERHR prepares the NTP-CERHR monograph. The NTP-CERHR monograph includes the NTP brief on the chemical evaluated, the expert panel report, and public comments on that report. The goal of the NTP brief is to provide the public, as well as government health, regulatory, and research agencies, with the NTP's interpretation of the potential for the chemical to adversely affect human reproductive health or children's health. The NTP-CERHR monograph is made publicly available electronically on the CERHR web site and in hard copy or CD-ROM from the CERHR.

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[1]Information about the CERHR is available on the web at <*http://cerhr.niehs.nih.gov*> or by contacting the director:

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Information about the NTP is available on the web at <*http://ntp-server.niehs.nih.gov*> or by contacting the NTP Liaison and Scientific Review Office at the NIEHS:

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## Introduction

In 1999, the CERHR Core Committee, an advisory committee composed of representatives from NTP member agencies, recommended fluoxetine for expert panel review. Fluoxetine (Prozac®, Sarafem™, CAS RN 54910-89-3), an antidepressant, is a widely prescribed drug in the United States. The CERHR selected fluoxetine hydrochloride for evaluation because of:

(1) numerous reproductive and developmental studies in laboratory animals and humans
(2) human exposure information
(3) changing prescription patterns

Fluoxetine hydrochloride, under the name Sarafem™, is now being prescribed to treat premenstrual dysphoric disorder, potentially increasing the number of exposures for women of childbearing age. The U.S. Food and Drug Administration (FDA) recently approved fluoxetine for use in 7-17 year-olds thereby increasing exposures of children. As part of the evaluation of fluoxetine, the CERHR convened a panel of scientific experts (Appendix I) to review, discuss, and evaluate the scientific evidence on the potential reproductive and developmental toxicities of the chemical. There was a public meeting of the CERHR Fluoxetine Expert Panel on March 3-5, 2004 in Alexandria, VA.

This fluoxetine monograph includes the NTP brief on fluoxetine, a list of the expert panel members (Appendix I), the expert panel report on fluoxetine (Appendix II), and all public comments received on the expert panel report on fluoxetine (Appendix III). The NTP-CERHR monograph is intended to serve as a single, collective source of information on the potential for fluoxetine to adversely affect human reproduction or development. Those interested in reading this monograph may include individuals, members of public interest groups, and staff of health and regulatory agencies.

The NTP brief included within this monograph presents the NTP's interpretation of the potential for fluoxetine exposure to cause adverse reproductive or developmental effects in people. The brief is intended to provide clear, balanced, and scientifically sound information. It is based on the contents of the expert panel report on fluoxetine, public comments on that report, and additional scientific information that became available following the expert panel meeting.

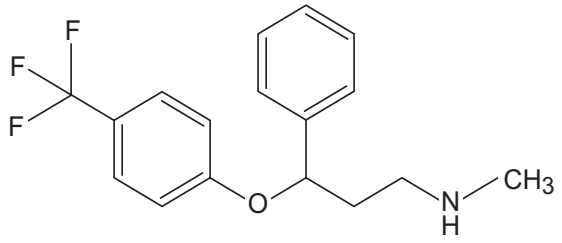
# NTP Brief on Fluoxetine

## What is Fluoxetine?

Fluoxetine is a pharmaceutical drug prescribed for a variety of psychiatric disorders, particularly depression. Fluoxetine is used in the treatment of depression, premenstrual dysphoric disorder (severe premenstrual syndrome), obsessive-compulsive disorder, panic disorder, and bulimia nervosa. It is also approved by the FDA to treat depression and obsessive-compulsive disorder in children 7-17 years old. On October 15, 2004 the FDA issued a press release (FDA, 2004) noting that manufacturers of antidepressant medications, including fluoxetine, were being directed to add a **"black box" warning** to labels on these medications. In addition, a Patient Medication Guide for patients receiving these medications will be developed. These precautions are being taken because FDA determined that there is an increased risk of suicidal thoughts and behavior in children and adolescents taking antidepressant medications.

Fluoxetine is classified as a selective serotonin reuptake inhibitor (SSRI). Its presumed mode of action involves the specific inhibition of uptake of the neurotransmitter serotonin at nerve terminals (synapses).

***Figure 1.***
*Chemical structure of Fluoxetine*




Fluoxetine hydrochloride, the medicinal form of this drug, is marketed under the names Prozac® and Sarafem™ by Eli Lilly and Company, Indianapolis, IN. The FDA has approved production of unbranded (generic) fluoxetine hydrochloride by at least 20 companies. Fluoxetine and its demethylated metabolite, norfluoxetine, are both therapeutically active. Fluoxetine hydrochloride is marketed in 10 mg, 20 mg, and 40 mg tablets, as an oral solution of 20 mg/5 ml, and in a 90 mg capsule for single weekly dosage.

The annual production volume for fluoxetine is not available. However, according to the FDA, in 2002, there were 1.2 billion tablets or teaspoons of fluoxetine sold to US pharmacies and approximately 26.7 million prescriptions were dispensed for fluoxetine. Of these prescriptions, 1.2 million were dispensed to pediatric and adolescent patients (1-18 years old) and 8.4 million were dispensed to women of childbearing age (19-44 years old).

## How Are People Exposed to Fluoxetine?

People are exposed to fluoxetine through medication. Little information is available on the occurrence of fluoxetine in the environment but water contamination appears to be very low. No information was located on occupational exposures associated with manufacture, packaging, or distribution. Recommended doses of fluoxetine are 10 to 80 mg/day or a single, weekly dosage of 90 mg for adults and 10 to 60 mg/day for children. Differences in recommended doses are based on the disorder being treated and on the patient's response to treatment.

Fluoxetine crosses the placenta and is found in breast milk. Thus, taking fluoxetine during pregnancy or lactation exposes the unborn child or infant to this drug. Fluoxetine and its metabolites have been detected in umbilical cord blood at birth. Fluoxetine has also been detected in blood and milk of breastfeeding women and in the blood of their infants. Blood levels in infants are related to maternal fluoxetine dose and maternal serum concentrations of fluoxetine and norfluoxetine.

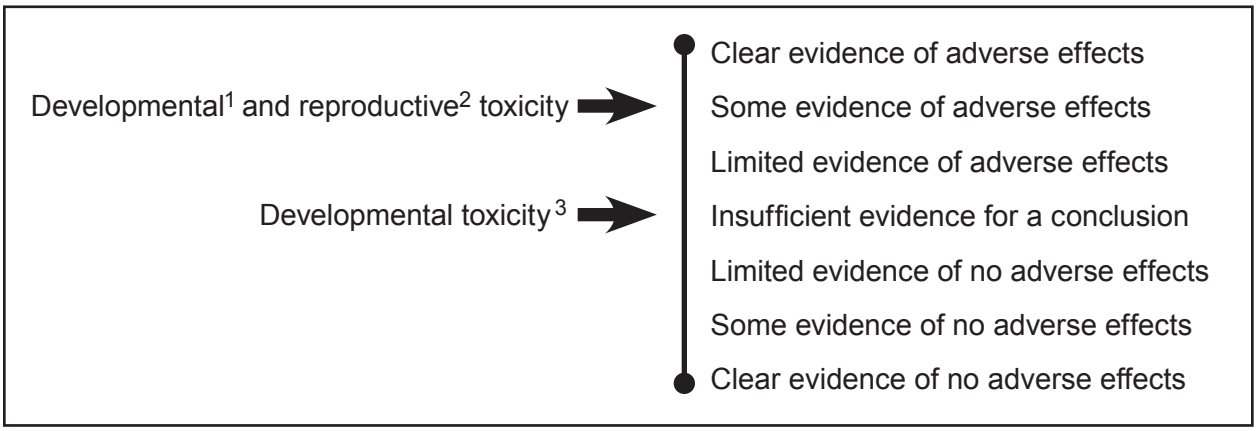
**Can Fluoxetine Affect Human Development or Reproduction?***

*Probably.* Studies reviewed by the expert panel show that oral exposure of pregnant women to therapeutic doses of fluoxetine may result in developmental toxicity in the infant as evidenced by shortened gestation, reduced growth in infants in the first 6 months of life, and an increased incidence of poor neonatal adaptation, *e.g.*, jitteriness, poor muscle tone, weak or absent cry (Figure 2a).

Fluoxetine therapy can cause impaired sexual function in both men and women, specifically a delay in or inability to achieve orgasm. Studies reviewed by the expert panel also reported an alteration in menstrual cycle length in some women. In experimental animals, altered estrous behavior, altered sexual receptivity, and reduced sexual motivation were observed (Figure 2b). These results support observations from human studies.

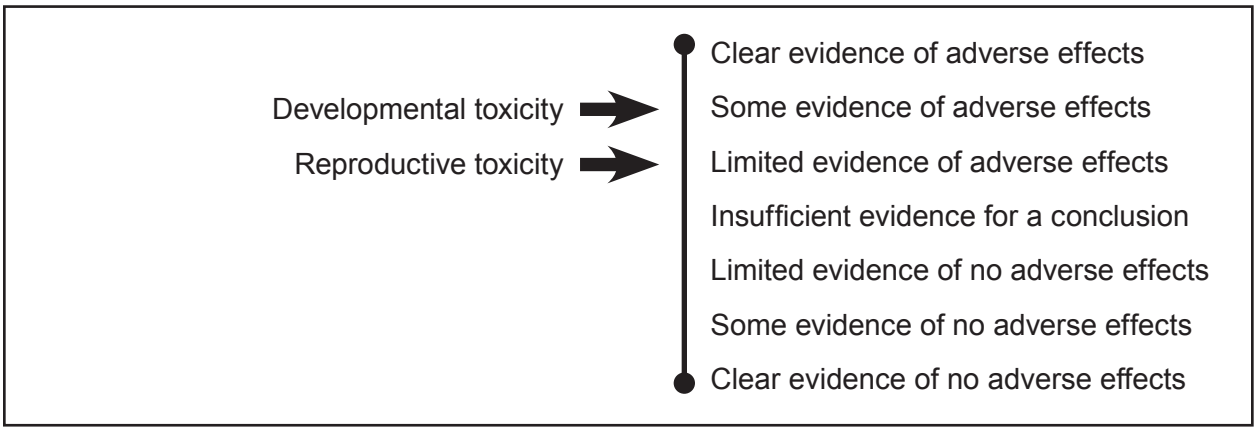
* Answers to this and subsequent questions may be: *Yes, Probably, Possibly, Probably Not, No or Unknown*

***Figure 2a.*** *The weight of evidence that fluoxetine causes adverse developmental or reproductive effects in humans*




[1] for fetus/infant of pregnant/breastfeeding women
[2] adverse effects limited to orgasmic dysfunction
[3] for children on fluoxetine therapy

***Figure 2b.*** *The weight of evidence that fluoxetine causes adverse developmental or reproductive effects in laboratory animals*



**Supporting Evidence**

The expert panel report (Appendix II) provides details and citations regarding studies on the possible reproductive and developmental toxicity of fluoxetine. The expert panel concluded that fluoxetine produces developmental toxicity in humans as characterized by an increased rate of poor neonatal adaptation (*e.g.* jitteriness, rapid breathing, low blood sugar, low body temperature, poor muscle tone, weak or absent cry, and inability to maintain blood oxygen levels while nursing) at maternal therapeutic doses (20-80 mg/day). These effects were more common when exposure occurred late in gestation. The expert panel noted that although these effects are transient and reversible, long-term follow-up studies to detect possible residual effects have not been conducted. However, in a study published after the expert panel report was released, Ansorge et al. (2004) treated young mice with 10 mg/kg bw/day fluoxetine from age 4 days to 21 days. Treatment was stopped at that time and, when the animals reached 12 weeks of age, the investigators began conducting tests to determine if the early exposure to fluoxetine affected emotional behavior in the adult animals. Based on effects observed in tests for exploratory behavior, anxiety-related and depression-related behaviors, and shock avoidance behavior, the authors concluded that exposure of young mice to fluoxetine over a period of brain development corresponding to the third trimester of pregnancy to 8 years of age in humans resulted in abnormal behavior in adult mice. The authors further concluded that these affects in mice may result from a fluoxetine-induced disruption of the serotonin transporter function during early development of the central nervous system and, in humans, "…may entail unexpected risks for affective function later in life."

The panel also concluded that exposure to fluoxetine during pregnancy can result in shortened gestation and reduced birth weight at term. Furthermore, exposure during pregnancy and/or through breastfeeding can result in reduced postnatal growth for infants less than 6 months of age. However, due to a lack of data, the panel could not evaluate the long-term implications of these findings. These results were, in part, supported by developmental studies in experimental animals. Rats exposed late in gestation to fluoxetine at 12 mg/kg bw/day (milligrams per kilogram body weight per day) had decreased birth weights and decreased postnatal survival. However, in these animal studies, neither the duration of pregnancy nor the ability to maintain a pregnancy was affected.

The expert panel noted that it is often difficult to separate drug-induced adverse effects from effects resulting from the disease process itself. However, a recent study (Andersson et al., 2004) compared pregnancy outcomes between women with depressive disorders and/or anxiety disorders and women without such disorders. The authors concluded that there were no differences in pregnancy outcomes between these groups. Another study (Suri et al., 2004) prospectively followed women over the course of pregnancy to assess the impact of depression and/or antidepressant treatment on pregnancy outcome. These investigators found no significant impact of depression or of fluoxetine therapy during pregnancy on pregnancy outcome.

The expert panel concluded there were insufficient data from studies in humans to evaluate the incidence of major malformations in newborns. However, there was sufficient evidence from developmental studies in rats (doses up to 12.5 mg/kg bw/day) and rabbits (doses up to 15 mg/kg bw/day) to conclude that oral administration of fluoxetine during pregnancy does not result in an increase in the incidence of malformations.

The panel noted that the assessment of human sexual dysfunction associated with fluoxetine therapy is complicated because sexual dysfunction is common in the general population and

can be associated with depression. However, in human studies, effects of fluoxetine therapy on sexual function, i.e., ability to achieve orgasm, have been observed at doses of 20 mg/day or higher. Data from experimental animal studies support observations in humans. Treatment of female rats with 10 mg/kg bw/day by subcutaneous or intraperitoneal injection results in altered estrous behavior and sexual receptivity, but had no effect on estrous cycle length. Treatment of male rats with ≥0.75 mg/kg bw/day by intraperitoneal injection results in reduced ejaculatory function and 10 mg/kg bw/day results in reduced sexual motivation.

## Should Exposures to Fluoxetine Cause Concern?

### *Adults*

*Probably Not.* The only clear reproductive effect of fluoxetine observed in exposed adults is an effect in some people on reproductive function, specifically a delay in or inability to achieve orgasm. This effect appears to be reversible upon cessation of fluoxetine therapy.

### *Pregnant Women*

*Probably.* Fluoxetine therapy is associated with shortened gestation and poor adaptation of newborns. Data are not sufficient to determine if long-term neurobehavioral effects or effects on growth and development result from in utero exposures.

### *Children*

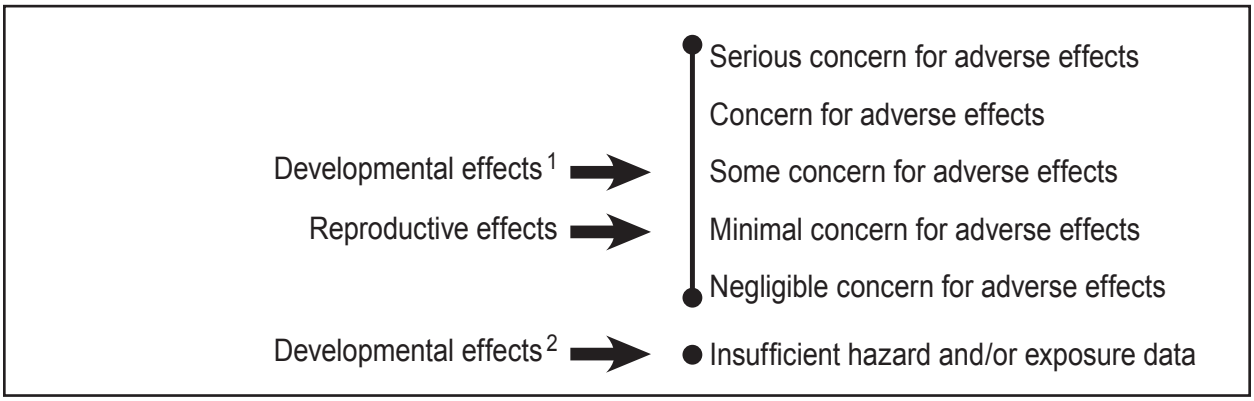
*Unknown.* Data are not sufficient to evaluate possible developmental effects in children exposed to fluoxetine through breast milk or therapy (Figure 3).

**The NTP concurs with the CERHR Fluoxetine Expert Panel that there is some concern for developmental effects, specifically shortened gestation and poor neonatal adaptation at therapeutic doses (20-80 mg/day).**

This conclusion is based on evidence from human studies that fluoxetine produces an increased rate of poor neonatal adaptation and that fluoxetine exposure during pregnancy can result in a shortened gestation and reduced birth weight at term. As noted by the expert panel, any risks associated with fluoxetine treatment must be weighed against the risks of untreated disease, particularly major depression. The health care provider and patient are best qualified to assess such risks.

**The NTP concurs with the CERHR Fluoxetine**

***Figure 3.*** *NTP conclusions regarding the possibilities that human development or reproduction might be adversely affected by exposure to fluoxetine*




[1] for the fetus and infant

[2] for pregnancy loss or for children exposed through breast milk or fluoxetine therapy

**Expert Panel that there is minimal concern for adverse reproductive effects in fluoxetine-exposed adults.**

This conclusion is based on evidence from human studies that therapeutic doses of fluoxetine may, in both men and women, result in reversible, impaired sexual function, specifically a delay in or an inability to achieve orgasm.

**The NTP concurs with the CERHR Fluoxetine Expert Panel that there are insufficient data to draw conclusions on how breast milk or therapeutic exposures to fluoxetine might affect development.**

The report that, in mice, early fluoxetine exposure can affect adult behavior (Ansorge et al., 2004) suggests that additional data are needed to confirm and extend these findings and determine if such effects might possibly occur in humans.

**The NTP concurs with the CERHR Fluoxetine Expert Panel that there are insufficient data to draw conclusions on an association between fluoxetine therapy in pregnant women and pregnancy loss.**

**These conclusions are based on the information available at the time this brief was prepared. As new information on toxicity and exposure accumulate, it may form the basis for either lowering or raising the levels of concern expressed in the conclusions.**

## References

Andersson L, Sundström-Poromaa I, Wulff M, Åström M, Bixo M. Neonatal outcome following maternal antenatal depression and anxiety: A population-based study. American Journal of Epidemiology 159:872-881 (2004).

Ansorge M, Zhou M, Lira A, Hen R, Gingrich J. Early-Life Blockade of the 5-HT Transporter Alters Emotional Behavior in Adult Mice. Science 306:879-881 (2004).

FDA. Available at *<http://www.fda.gov/cder/drug/antidepressants/SSRIPHA200410.htm>* (cited November 16, 2004)

Suri R, Altshuler L, Hendrick V, Rasgon N, Lee E and Mintz J. The impact of depression and fluoxetine treatment on obstetrical outcome. Archives of Women's Mental Health 7:193-200 (2004).

# Appendix I. NTP-CERHR Fluoxetine Expert Panel

A 12-member panel of scientists covering disciplines such as toxicology, epidemiology, and medicine was recommended by the CERHR Core Committee and approved by the Associate Director of the National Toxicology Program. Prior to the expert panel meeting, the panelists critically reviewed articles from the scientific literature, as well as a variety of other relevant documents. Based on this material, they identified key studies and issues for discussion. At a public meeting held March 3-5, 2004, the expert panel discussed these studies, the adequacy of available data, and identified data needed to improve future assessments. The expert panel reached conclusions on whether exposures to fluoxetine might result in adverse effects on human reproduction or development. Panel conclusions were based on the scientific evidence available at the time of the public meeting. The NTP-CERHR released the final expert panel report for public comment on April 19, 2004, and the deadline for public comments was June 17, 2004 (*Federal Register Vol. 69 No. 83, 23517-23518, April 2004*). The expert panel report on fluoxetine is provided in Appendix II and the public comments received on the report are in Appendix III. Input from the public and interested groups throughout the panel's deliberations was invaluable in helping to assure completeness and accuracy of the reports. The Expert Panel Report on Fluoxetine is also available on the CERHR website <*http://cerhr.niehs.nih.gov*>.

## Appendix I. NTP-CERHR Fluoxetine Expert Panel

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# Center For The Evaluation Of Risks To Human Reproduction

Appendix II

# NTP-CERHR EXPERT PANEL REPORT ON THE REPRODUCTIVE AND DEVELOPMENTAL TOXICITY OF FLUOXETINE

*April 2004* *NTP-CERHR-Fluoxetine-04*

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# ABBREVIATIONS

| | |
|---|---|
| $^{3}H$ | tritium labeled |
| 5-HT | 5-hydroxytryptamine (serotonin) |
| 5-$HT_{1A}$, 5-$HT_{2}$, 5-$HT_{2A/2C}$ | serotonin receptors |
| 5-HIAA | 5-hydroxyindoleacetic acid (serotonin metabolite) |
| 5HTTLPR | serotonin transporter gene-linked polymorphic region |
| 8-OH-DPAT | (±)-8-hydroxy-2-dipropylaminotetraline |
| ACTH | adrenocorticotropic hormone |
| AERS | Adverse Events Reporting System |
| ANCOVA | analysis of covariance |
| ANOVA | analysis of variance |
| AUC | area under the concentration versus time curve |
| BDI | Beck Depression Inventory |
| BMDL | benchmark dose 95th percentile lower confidence limit |
| bw | body weight |
| C | Celsius |
| $^{14}C$ | carbon-14 |
| $C_0$ | pre-dose level |
| cm | centimeter(s) |
| $C_{max}$ | maximum concentration |
| CAS RN | Chemical Abstracts Service Registry Number |
| CERHR | Center for the Evaluation of Risks to Human Reproduction |
| CES-D | Center for Epidemiologic Studies Depression |
| CI | confidence interval |
| CNS | central nervous system |
| CSF | cerebrospinal fluid |
| CYP | cytochrome P450 |
| dL | deciliter(s) |
| DMSO | dimethyl sulfoxide |
| DNA | deoxyribonucleic acid |
| DOI | (±)-4-iodo,2,5-dimethoxyphenylisopropylamine |
| EEG | electroencephalogram |
| Eq | equivalent |
| F1 | first filial generation |
| FDA | Food and Drug Administration |
| g | gram(s) |
| GABA | -amino-butyric acid |
| GC | gas chromatography |
| GD | gestation day |
| GLP | Good Laboratory Practice |
| GnRH | gonadotropin-releasing hormone |
| h | hour(s) |
| hCG | human chorionic gonadotropin |
| HCl | hydrochloride |

HPLC .....high performance liquid chromatography
HSDB.....Hazardous Substances Data Bank
$IC_{50}$.....concentration that results in 50% inhibition
IMI .....imipramine
KCl.....potassium chloride
kg.....kilogram(s)
$K_i$.....inhibition constant
i.p. .....intraperitoneal
iv .....intravenous
L .....liter(s)
LH .....luteinizing hormone
LOAEL.....lowest observed adverse effect level
M.....molar
m2 .....meter(s) squared
MDD .....Major Depressive Disorder
min .....minute(s)
mL.....milliliter(s)
mg .....milligram(s)
mM.....millimolar
MRS .....magnetic resonance spectroscopy
MS.....mass spectrometry
msec .....millisecond(s)
n or no. .....number
NICU.....neonatal intensive care unit
NIEHS.....National Institute of Environmental Health Sciences
ng.....nanogram
nM.....nanomolar
nmol .....nanomole(s)
NOAEL .....no observed adverse effect level
NS .....nonsignificant
NTP.....National Toxicology Program
OCD .....Obsessive-Compulsive Disorder
OPDRA.....Office of Postmarketing Drug Risk Assessment
OR:.....odds ratio
oz.....ounce(s)
pg.....picograms
PMDD.....Premenstrual Dysphoric Disorder
PND.....postnatal day
$pCO_2$ .....partial pressure carbon dioxide
$pO_2$.....partial pressure oxygen
sc .....subcutaneous
SD .....standard deviation
SE.....standard error
sec .....second(s)
sem.....standard error of the mean

SRI .....serotonin reuptake inhibitor
SSRI .....selective serotonin reuptake inhibitor
TCA.....tricyclic antidepressant
$T_{max}$ .....time to maximum levels
U.....unit
UV.....ultraviolet
WPPSI-R.....Wechsler Preschool and Primary Scale of Intelligence™–Revised
µg.....microgram(s)
µM.....micromolar
µmol .....micromole(s)
U.S.....United States

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# PREFACE

The National Toxicology Program (NTP) and the National Institute of Environmental Health Sciences (NIEHS) established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in June 1998. The purpose of the Center is to provide timely, unbiased, scientifically sound evaluations of human and experimental evidence for adverse effects on reproduction, to include development, caused by agents to which humans may be exposed.

Fluoxetine, an antidepressant that is widely-prescribed in the United States, was selected for evaluation by the CERHR based on (1) sufficient reproductive and developmental studies, (2) human exposure information, (3) changing prescription patterns, and (4) public concern about potential reproductive and/or developmental hazards associated with exposure. Fluoxetine hydrochloride, under the name Sarafem™, is prescribed to treat premenstrual dysphoric disorder (PMDD), potentially increasing the number of exposures for women of childbearing age. In addition, the Food and Drug Administration recently approved Prozac® for use in 7-17 year-olds thereby increasing exposures of children.

This evaluation results from the effort of a twelve-member panel of government and non-government scientists that culminated in a public expert panel meeting held March 3-5, 2004. This report has been reviewed by CERHR staff scientists, and by members of the Fluoxetine Expert Panel. Copies have been provided to the CERHR Core Committee, which is made up of representatives of NTP-participating agencies. This report is a product of the Expert Panel and is intended to (1) interpret the strength of scientific evidence that fluoxetine is a reproductive or developmental toxicant based on data from in vitro, animal, or human studies, (2) assess the extent of human exposures to include the general public, occupational groups, and other sub-populations, (3) provide objective and scientifically thorough assessments of the scientific evidence that adverse reproductive/developmental health effects may be associated with such exposures, and (4) identify knowledge gaps to help establish research and testing priorities to reduce uncertainties and increase confidence in future assessments of risk.

This Expert Panel Report will be a central part of the subsequent NTP-CERHR Monograph on Fluoxetine. The monograph will include the NTP-CERHR Brief, the expert panel report, and all public comments on the expert panel report. The NTP-CERHR Monograph will be made publicly available and transmitted to appropriate health and regulatory agencies.

The NTP-CERHR is headquartered at NIEHS, Research Triangle Park, NC and is staffed and administered by scientists and support personnel at NIEHS and at Sciences International, Inc., Alexandria, Virginia.

Reports can be obtained from the website <*http://cerhr.niehs.nih.gov/*> or from:

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**Note to Reader:**

This report is prepared according to the Guidelines for CERHR Panel Members established by NTP/NIEHS. The guidelines are available from the CERHR web site <*http://cerhr.niehs.nih.gov/*>. The format for Expert Panel Reports includes synopses of studies reviewed, followed by an evaluation of the Strengths/Weaknesses and Utility (Adequacy) of the study for a CERHR evaluation. Statements and conclusions made under Strengths/Weaknesses and Utility evaluations are those of the Expert Panel and are prepared according to the NTP/NIEHS guidelines. In addition, the Panel often makes comments or notes limitations in the synopses of the study. **Bold, square brackets** are used to enclose such statements. As discussed in the guidelines, square brackets are used to enclose key items of information not provided in a publication, limitations noted in the study, conclusions that differ from authors, and conversions or analyses of data conducted by the panel.

# 1.0 CHEMISTRY, USAGE, AND EXPOSURE

As noted in the CERHR Expert Panel Guidelines, the Exposure section is initially based on secondary review sources. Primary study reports are addressed by the Expert Panel if they contain information that is highly relevant to a CERHR evaluation of developmental or reproductive toxicity or if the studies were released subsequent to the reviews. For primary study reports that the Expert Panel reviewed in detail, statements are included about the strengths, weaknesses, and adequacy of the studies for the CERHR review process.

As described below (Section 1.2.2.), fluoxetine is a serotonin reuptake inhibitor (SRI) that is prescribed for a variety of psychiatric disorders, particularly depression. The Expert Panel acknowledges that in most instances, it is not possible to differentiate drug-induced adverse effects from those induced by the disease process itself. At the same time, studies on the effects of major depression on pregnancy and child developmental outcomes typically have not taken medication exposure into account. Recognizing that this problem impacts many of the conclusions drawn from this evaluation, the Panel felt it important to emphasize this problem as a preamble to this report. Further, the Expert Panel also recognizes that any risks associated with fluoxetine treatment must be weighed against the very real risks associated with leaving untreated the more severe forms of the disease. Such a risk-benefit analysis is best performed by the patient and responsible health care provider and should benefit from the evaluation and conclusions offered by this report.



## *1.1 Chemistry*

### *1.1.1 Nomenclature*

Fluoxetine (CAS RN 54910-89-3) is N-methyl-gamma-(4-(trifluoromethyl)phenoxy)-, (+-)-benzenepropanamine. Other names identified in ChemID (*1*) are:

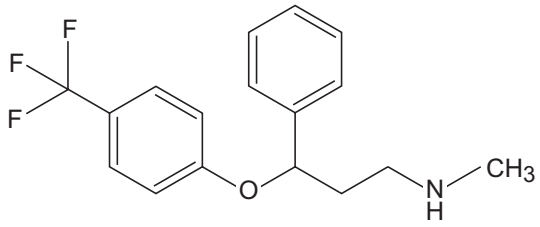
(+) or (-)-N-methyl-3-phenyl-3-((alpha,alpha,alpha-trifluoro-p-tolyl)oxy)propylamine
(+) or (-)-N-methyl-gamma-(4-(trifluoromethyl)phenoxy)benzenepropanamine
(+-)-N-methyl-3-phenyl-3-((alpha,alpha,alpha-trifluoro-p-tolyl)oxy)propylamine
(+-)-N-methyl-gamma-(4-(trifluoromethyl)phenoxy)benzenepropanamine
N-methyl-gamma-(4-(trifluoromethyl)phenoxy)-, (+-)-benzenepropamine
N-methyl-3-(p-trifluoromethylphenoxy)-3-phenylpropylamine
dl-3-(p-Trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine

Fluoxetine hydrochloride (CAS RN 59333-67-4) is marketed under the names Prozac® and Sarafem™ by Eli Lilly and Company, Indianapolis IN. The two trade names represent identical chemical formulations. In early literature, fluoxetine hydrochloride (HCl) was referred to as Lilly 110140 (*2*). In this report, fluoxetine and fluoxetine HCl are used according to the designation of study report authors. The Expert Panel recognizes that the administered medicinal form is fluoxetine HCl, and the active compound at the tissue level is fluoxetine.

### *1.1.2 Formula and Molecular Weight*

The chemical formula for fluoxetine is $C_{17}H_{18}F_3NO$. The molecular mass is 309.33. The structure is shown in Figure 1a. Fluoxetine HCl has a molecular mass of 345.79.

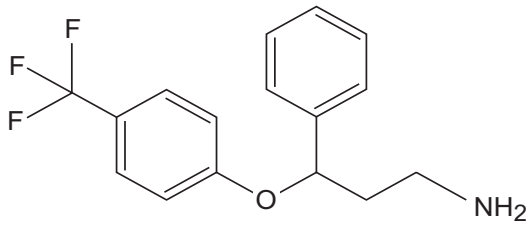
***Figure 1a:*** *Chemical Structure of Fluoxetine*




Fluoxetine concentrations are expressed in the literature as nM or ng/mL. For conversion, 1 nM = 0.31 ng/mL and 1 ng/mL = 3.23 nM. **[In this report, when study authors use ng/mL, concentrations have been left as stated; when given as nM, concentrations have been given as stated by the authors and have been converted by the Expert Panel to ng/mL].**

Fluoxetine is metabolized to norfluoxetine (Figure 1b), which also is an active SRI. The chemical formula for norfluoxetine is $C_{16}H_{16}F_3NO$ *(1)*. For conversion 1 ng/mL norfluoxetine = 3.34 nM and 1 nM norfluoxetine = 0.299 ng/mL.

***Figure 1b:*** *Chemical Structure of Norfluoxetine*






### *1.1.3 Chemical and Physical Properties*

Fluoxetine is a 50/50 racemic mixture of R- and S-enantiomers. Fluoxetine HCl is a white to off-white crystalline solid with a melting point of 158.4 – 158.9°C *(3)* and a solubility of 14 mg/mL in water *(4)*. S-Fluoxetine is dextrorotatory (+1.60) in methanol, but is levorotatory (-10.85) in water *(5)*.

The fluoxetine metabolite norfluoxetine is also a racemic mixture of R- and S-enantiomers *(4)*. The S-enantiomer is more potent than the R-enantiomer, as discussed in Section 2.1. No other information is available on the chemical and physical properties of norfluoxetine.

### *1.1.4 Technical Products and Impurities*

According to the product label for the Prozac® brand of fluoxetine HCl, the medication comes in 10 mg tablets and "pulvules," (capsules) and 20 and 40 mg pulvules. Prozac® is also available as a liquid containing 20 mg per 5 mL *(4)*. Each pulvule contains fluoxetine HCl equivalent to 10 mg (32.3 µmol), 20 mg (64.7 µmol), or 40 mg (129.3 µmol) of fluoxetine. The pulvules also contain starch, gelatin, silicone, titanium dioxide, iron oxide, and other inactive ingredients. The 10 and 20 mg pulvules also contain FD&C Blue No. 1, and the 40 mg pulvule also contains FD&C Blue No. 1 and FD&C Yellow No. 6. Each tablet contains fluoxetine HCl equivalent to 10 mg (32.3 µmol) of fluoxetine. The tablets also contain microcrystalline cellulose, magnesium stearate, crospovidone, hydroxypropyl

methylcellulose, titanium dioxide, polyethylene glycol, and yellow iron oxide. In addition to the above ingredients, the 10 mg tablet contains FD&C Blue No. 1 aluminum lake and polysorbate 80. The oral solution contains fluoxetine HCl equivalent to 20 mg (64.7 µmol) per 5 mL of fluoxetine. It also contains alcohol 0.23%, benzoic acid, flavoring agent, glycerin, purified water, and sucrose. Prozac® Weekly capsules, a delayed-release formulation, contain enteric-coated pellets of fluoxetine HCl equivalent to 90 mg (291 µmol) of fluoxetine. The capsules also contain D&C Yellow No. 10, FD&C Blue No. 2, gelatin, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose acetate succinate, sodium lauryl sulfate, sucrose, sugar spheres, talc, titanium dioxide, triethyl citrate, and other inactive ingredients.

Each Sarafem™ pulvule contains fluoxetine HCl equivalent to 10 mg (32.3 µmol) or 20 mg (64.7 µmol) of fluoxetine *(6)*. The pulvules also contain dimethicone, FD&C Blue No. 1, FD&C Red No. 3, FD&C Yellow No. 6, gelatin, sodium lauryl sulfate, starch, and titanium dioxide.

## *1.2 Use and Human Exposure*

### *1.2.1 Production*

S-Fluoxetine is synthesized from S-(-)-3-chloro-1-phenylpropanol by sequential reaction with sodium iodide, methylamine, sodium hydride, and 4-fluorobenzotrifluoride *(5)*. Besides Eli Lilly and Company, the manufacturer of branded Prozac® and Sarafem™, the FDA *(7)* also lists companies that have been approved to produce unbranded (generic) fluoxetine including Ranbaxy Laboratories, Ltd., Carlsbad Technology, Inc., Dr. Reddy's Laboratories Ltd., Sidmark Laboratories Inc., Eon Labs Manufacturing Inc., Mallinckrodt Inc., Alphapharm Pty Ltd., Ganes Chemicals for Siegfried Ltd., Apothecon Inc., TEVA Pharmaceuticals USA, IVAX Pharmaceuticals Inc., Zenith Goldline Pharmaceuticals Inc., Mylan Pharmaceuticals, Geneva Pharmaceuticals Inc., Barr Laboratories Inc., ESI Lederle, Alpharma, Hi-Tech Pharmaceutical Co. Inc., Marlon Grove Pharmaceuticals USA, and Novex Pharma. Some of these companies have been marketing fluoxetine overseas even while the U.S. patent precluded them from marketing the medication in this country. Eli Lilly and Company's initial patent application for fluoxetine was filed in 1974, and its most recent patent was issued in December, 1986. This last patent was declared invalid by the Court of Appeals for the Federal Circuit in August, 2000 *(8)*.

Production volume figures are not available. According to Eli Lilly and Company *(9)*, Prozac® and Sarafem™ together accounted for $2.57 billion in worldwide sales in the year 2000, or 24% of the company's sales in that year. The 2001 Eli Lilly and Company annual report states that 2001 U.S. sales of fluoxetine products (Prozac®, Prozac® Weekly, and Sarafem™) had decreased by 26% to $1.66 billion in the U.S., representing 14% of the company's annual sales. The decrease was attributed to the appearance of generic fluoxetine, implying that overall fluoxetine use was not believed to have decreased. A 1994 article in *Psychology Today* was quoted by Baum and Misri *(10)* as estimating that 1 million prescriptions per month were written for Prozac®.

According to the FDA *(11)*, 1.2 billion tablets (or teaspoons) of fluoxetine were sold to U.S. pharmacies in 2002. Fluoxetine was the most commonly prescribed SRI in 1998 and dropped to the third most commonly prescribed SRI during the past 3 years. Currently, fluoxetine represents 20.5% of all SRI prescriptions in the U.S. In 2002, about 26.7 million prescriptions were dispensed for fluoxetine,

with 1.2 million dispensed to pediatric and adolescent patients (1–18 years old) and 8.4 million dispensed to women of child bearing age (19–44 years old). The 20 mg strength is most commonly prescribed and accounts for about 70% of all dispensed prescriptions. The number of patients for whom these prescriptions were written is not known. The three physician specialties that most commonly prescribe fluoxetine include family practice, psychiatry, and internal medicine.

### *1.2.2 Use*

Fluoxetine is a serotonin reuptake inhibitor (SRI), indicated by the FDA for the treatment of major depressive disorder (MDD), obsessive-compulsive disorder (OCD), bulimia nervosa, panic disorder, and premenstrual dysphoric disorder (PMDD) *(4, 9*). Though indicated for treatment of major depression, fluoxetine is often prescribed for ill-defined dysthymia, frequently by non-psychiatric practitioners who may be reluctant to prescribe other classes of antidepressants *(10*). Fluoxetine was reported to be effective for the treatment of all degrees of depression, ranging from mild to severe *(12*). Some studies found that fluoxetine was as effective as tricyclic antidepressants (TCA) in treatment of severe depression *(2, 12*).

The FDA recently approved fluoxetine to treat MDD and OCD in children and adolescents (7–17 years old) *(7*). Eli Lilly and Company *(4*) indicates that although the efficacy of fluoxetine has been demonstrated for OCD and MDD, its safety and effectiveness in children younger than 7 years with OCD and younger than 8 years with MDD have not been established. Side effects that may be associated with fluoxetine treatment in children are reported in Section 3.1.3. The Prozac® product label mentions decrements in height and weight noted in children in one clinical trial (discussed in Section 3.1.3) and states, "The safety of fluoxetine treatment for pediatric patients has not been systematically assessed for chronic treatment longer than several months in duration. In particular, there are no studies that directly evaluate the longer-term effects of fluoxetine on the growth, development, and maturation of children and adolescent patients. Therefore, height and weight should be monitored periodically in pediatric patients receiving fluoxetine."

Fluoxetine is marketed under the name Sarafem™ solely for the treatment of PMDD *(6*). Effectiveness of Sarafem™ was not evaluated in combination with oral contraceptives *(6*).

### *1.2.3 Human Exposure*

#### ***1.2.3.1 Dosing***

According to the product label for Prozac® *(4*), the initial fluoxetine dose for MDD in adults is 20 mg each morning, with a dose increase "after several weeks" if needed, up to a maximum of 80 mg per day. For weekly therapy in adults, the dose is 90 mg once per week with Prozac® Weekly™ capsules. Dosing in children with MDD is initiated with 10–20 mg per day *(4*). After 1 week at 10 mg per day, the dose can be increased to 20 mg per day. However, due to higher plasma levels in lower-weight children, the recommended starting and target is 10 mg per day; a dose of 20 mg per day may be considered after several weeks if symptoms have not sufficiently improved.

The dosing recommendations for OCD, bulimia nervosa, and panic disorder are similar, except that the maximum dose is indicated as 60 mg per day for adults. The label notes that 80 mg per day has been used to treat OCD in adults, but that doses higher than 60 mg per day have not been systemati-

cally studied in the other conditions. For children with OCD, a starting dose of 10 mg per day is recommended *(4)*. Gradual dose increases over a period of weeks can be considered, with maximum doses not to exceed 60 mg per day in higher-weight children and adolescents and 20–30 mg per day in lower-weight children. No pediatric dose recommendation is made for the other disorders (for which the medication is not approved). The 90 mg once weekly dose is not discussed in the product label for any indication other than depression.

The product label for Sarafem™ recommends a dose of 20–60 mg per day and indicates that the maximum dose is 80 mg per day *(6)*. The label states that the dose may either be given on each day of the menstrual cycle or from 14 days prior to estimated start of menstruation through the first full day of menses during each cycle.

Off-label use of fluoxetine has included the treatment of anxiety disorders other than panic disorder, anorexia nervosa, and obesity (reviewed by Stokes and Holtz *(12)*). Based on the experience of some members, the Panel notes that fluoxetine has also been used in the treatment of OCD-spectrum disorders (e.g., paraphilias, compulsive sexual behavior, trichotillomania, kleptomania, and pathological gambling).

The duration of therapy for a first episode of depression is typically 6–9 months after remission of symptoms (reviewed by Stokes and Holtz *(12)*). Recurrence of symptoms is common, and lifetime therapy may be recommended for patients with recurrent disease. In OCD and luteal phase dysphoric disorder, symptom recurrence after discontinuation of medication is common, and prolonged therapy is often recommended.

Based on the statement that fluoxetine is excreted in human milk, nursing while on fluoxetine is not recommended by Eli Lilly and Company *(4)*.

Mood disorders are common in women of child-bearing years and it has been estimated that 15.6% of women meet criteria for major depression (by self-administered Center for Epidemiologic Studies Depression Scale) during the third trimester of pregnancy *(13)*. Medication kinetics may be influenced by physiologic changes of pregnancy, which require changes in dosing to maintain therapeutic benefit. These changes include an increased volume of distribution for drugs distributing in plasma or in total body water, decreased protein binding due to the dilutional effect of increased plasma volume, decreased gastric motility (delaying gastric emptying and permitting prolonged contact with gastric acid), increased hepatic enzyme production, and alterations in the activity of gut wall enzymes such as steroid-inducible CYP3A4 (modified from Hostetter et al. *(14)*).

Hostetter et al. *(14)* evaluated dosing requirements of 34 pregnant women treated during pregnancy with SRIs (9 on fluoxetine, 12 on paroxetine, and 13 on sertraline). Fourteen women were on medication from the prenatal period, another 14 discontinued the medication on learning of their pregnancies and restarted medication due to disease relapse, and 6 experienced new onset of depression during pregnancy. Women underwent monthly evaluation (Clinical Global Impression [GDI]) by a psychiatrist and completed a monthly Beck Depression Inventory (BDI). Medication doses were adjusted **[after an unspecified interval]** to achieve euthymia, defined as a GDI = 1 and a BDI < 9.

Of the 34 women, 22 required a dose increase during pregnancy. Of the 14 women who began pregnancy while taking an antidepressant medication and stayed on therapy, 8 (57%) required a dose increase. Among the 14 women who became pregnant while taking medication but stopped the medication when they learned of their pregnancies (at unspecified gestational ages), the mean gestational week at restarting therapy was 13.9±5.6 **[the errors from this report are presumably SD]**. The mean gestational age at initiation of therapy in the 6 women who were first treated during pregnancy was 18.8±7.0 weeks. The gestational age when the first increase in dose occurred was 24.4±9.5, 28.4±6.6, and 28.0±7.4 weeks, respectively, among the women who continued medication during pregnancy, the women who restarted medication during pregnancy, and the women initiating medication during pregnancy. The mean dose of fluoxetine at delivery was reported to be 32.0±19.2 mg/day and 25.0±10.0 mg/day in women who did and did not require a dose increase during pregnancy, respectively. The authors concluded that late second or early third trimester dose increase during pregnancy is commonly necessary, although they admit that a worsening of depression due to pregnancy cannot be excluded as the reason for the increased dose requirement**. [The Panel noted that the initial BDI is given as 12.3±11.9 (probably mean±SD). The BDI may be viewed as a rank, and the distribution of ranks may not be optimally expressed using a mean. Based on the large standard deviation, the distribution appears to have been quite skewed. The Panel notes that the BDI is scored such that the nondepressed range is from 0 to 8 on the self-administered interview and a score of 9–15 is considered "mild depression." For study purposes, women were dosed so that their BDI would be lower than 9. It may be that some of the women should have been treated with higher doses of fluoxetine from the start, but it may not have seemed necessary for those with only mild depression. No information was provided on how many in this group had scores higher than 9. The Panel concluded that the dose increase was probably due to alterations attributable to pregnancy. The need for this dose increase, however, might well have been missed had the women not come under increased scrutiny by being assessed each month by virtue of their being in the study.]**



#### *1.2.3.2 Intrauterine Exposure*

A limited number of studies measured blood fluoxetine and norfluoxetine levels in infants exposed to fluoxetine *in utero*. Norfluoxetine, the major metabolite of fluoxetine, is also an active SRI. Spencer *(15)* reported cord blood levels of 26 ng/mL fluoxetine and 54 ng/mL norfluoxetine following the birth of a prenatally exposed infant; at 96 hours of age, fluoxetine levels were below the detection limit (<25 ng/mL) and norfluoxetine was measured at 55 ng/mL in the infant. Mhanna et al. *(16)* reported serum levels of 129 ng/mL fluoxetine and 227 ng/mL norfluoxetine in one 2-day-old infant exposed to fluoxetine *in utero*. Mohan and Moore *(17)* reported a blood fluoxetine and norfluoxetine level of 92 ng/mL and 34 ng/mL, respectively, in a 96-hour-old infant exposed to fluoxetine *in utero*. Laine et al. *(18)* reported mean umbilical vein fluoxetine+norfluoxetine at 278 nM **[86.2 ng/mL]** (range 209–366 nM **[64.8–113.5 ng/mL]**). At 2 days and 2 weeks of age, mean fluoxetine+norfluoxetine (range) values were 319 nM **[~99 ng/mL, using the same molecular mass for fluoxetine and norfluoxetine]** (range 151–573 nM **[~47–178 ng/mL]**), and 153 nM **[~47 ng/mL]** (range 58–345 nM **[~18–107 ng/mL]**). **[Whether these infants also were exposed to fluoxetine and norfluoxetine in milk is not stated].** Heikkinen et al. *(19)* reported mean umbilical cord plasma concentrations (± SD) of fluoxetine and norfluoxetine of 112±75 and 209±79 nM **[34.7±23.2 and 64.8±24.5 ng/mL]**, respectively after maternal therapy with 20–40 mg/day fluoxetine (n=8). When corrected for a standard dose of 20 mg/day, mean fluoxetine+norfluoxetine was estimated as 278±85 nM **[~86±26**

**ng/mL]** in umbilical cord plasma at delivery.

*Strengths/Weaknesses:* These studies used adequate methods and can be considered reliable estimates of fluoxetine/norfluoxetine exposure at term. The use of combined fluoxetine + norfluoxetine concentrations is acceptable given the pharmacologic activity of both compounds. The derivation of ng/mL concentrations from combined molar concentrations of the two compounds introduces an error due to the different molecular mass of norfluoxetine and fluoxetine; however, the small size of this difference in molecular mass makes the resultant approximation reasonable. These data are limited by their applicability only to pregnancy exposures at or near term.

*Utility (Adequacy) for CERHR Evaluation Process:* These data can be used to estimate exposure in human fetuses at or near term.

#### *1.2.3.3 Exposure in Milk*

Fluoxetine and norfluoxetine levels in breast milk and/or blood of nursing mothers or their infants were reported in several studies *(20-27*). The most comprehensive studies were conducted by Hendrick et al. *(22*), Kristensen et al. *(28*), Taddio et al. *(26*), Heikkinen et al. *(19*), Yoshida et al. *(25*), and Suri et al. *(29*).

Hendrick et al. *(22*) examined 19 nursing mothers (24 – 40 years old) and 20 infants (5 – 34 weeks old; 1 set of twins). Mothers were taking 10 – 60 mg/day fluoxetine for a minimum of 6 weeks. Serum samples were obtained from 18 mothers and 20 infants. Nine of the mothers collected milk samples every 3 – 5 hours over a 24-hour period. Samples were analyzed by HPLC separation followed by UV detection. Data were analyzed by parametric statistics (e.g., Pearson *r*, *t*-test) and confirmed by nonparametric tests (Spearman *r*, robust *t*, or Wilcoxon rank-sum). Results for blood and milk levels of drug and metabolite are listed in Table 1 according to dose levels. Milk-to-plasma ratios are listed in Table 2. Drug and metabolite levels in milk paralleled each other with 2- to 3-fold variations over 24 hours with a peak level occurring about 8 hours after dosing. Fluoxetine was detected in 6 of 20 infant serum samples (30%) and norfluoxetine was detected in 17 of 20 infant serum samples (85%). As noted in Table 3, norfluoxetine levels in infant serum correlated highly with fluoxetine and norfluoxetine levels in maternal serum and milk and with maternal dose. Maternal doses ≥ 30 mg/day were more likely to result in detectable levels of fluoxetine and norfluoxetine levels in infant serum than doses ≤ 20 mg/day ($P = 0.02$) and resulted in higher levels of norfluoxetine in infant serum (67.3 vs. 8.9 ng/mL, $P = 0.05$). Concentrations of fluoxetine and norfluoxetine were likely to be very low in infants whose mothers had total serum drug and metabolite levels <150 ng/mL. Infant ages and weights did not correlate with drug or metabolite serum levels.

***Table 1. Levels of Fluoxetine and Norfluoxetine in Nursing Mothers and Their Infants***

| *Maternal Dose (mg/day)* | *Fluoxetine Levels (ng/mL)* | | | *Norfluoxetine Levels (ng/mL)* | | | *Reference* |
|---|---|---|---|---|---|---|---|
| | *Maternal Plasma or Serum* | *Milk* | *Infant Plasma or Serum* | *Maternal Plasma or Serum* | *Milk* | *Infant Plasma or Serum* | |
| 10 | 21–39 [n=2] | 31/<2[b] [n=1] | <1 [n=2] | 43 [n=2] | 16/<2[b] [n=1] | <1–4 [n=2] | Hendrick et al. *(22)* |
| 15 | 47 [n=1] | NE | <1 [n=1] | 90 [n=1] | NE | 3 [n=1] | |
| 20 | 28–242 [n=5] | 81–156/ 30–40[b] [n=2] | <1–84 [n=5] | 47–236 [n=5] | 124–131/ 39–50[b] [n=2] | <1–28 [n=5] | |
| 20 | 71–142 [n=3] | 29–87/ 37–103[a] [n=3] | <5–<20 [n=2] | 67–152 [n=3] | 7–44/ 11–74[a] [n=3] | <5–<20 [n=2] | Yoshida et al. *(25)* |
| | 124–135 [n=1] | 67/17[a] [n=1] | NE | 141–149 [n=1] | 52/13[a] [n=1] | NE | Burch and Wells *(21)* |
| | NE | 69 [n=1] | 340 [n=1] | NE | 90 [n=1] | 208 [n=1] | Lester et al. *(24)* |
| | NE | 38–68 [n=1] | 61 [n=1] | NE | 28–68 [n=1] | 57–58 [n=1] | Brent and Wisner *(20)* |
| 20–40 (values = mean±SD) | 2 d: 48±33 [n=11] | 2 d: NE | 2 d: 37±32 [n=11] | 2 d: 82±26 [n=11] | 2 d: NE | 2 d: 64±21 [n=11] | Heikkinen *(19)* |
| | 4 d: 57±38 [n=11] | 4 d: 49±36 [n=11] | 4 d: 22±16 [n=11] | 4 d: 84±26 [n=11] | 4 d: 43 ±34 [n=11] | 4 d: 51±15 [n=11] | |
| | 2 w: 105±51 [n=9] | 2 w: 57±35 [n=9] | 2 w: 7±10 [n=2] | 2 w: 110±33 [n=9] | 2 w: 26±18 [n=9] | 2 w: 42±26 [n=10] | |
| | 2 m: 120±59 [n=8] | 2 m: 60±27 [n=8] | 2 m: < 3 [n=8] | 2 m: 93±48 [n=8] | 2 m: 28±10 [n=8] | 2 m: 6±4 [n=8] | |
| 30 | 220 [n=1] | 163/99[b] [n=1] | <1 [n=1] | 224 [n=1] | 196/131[b] [n=1] | 88 [n=1] | Hendrick et al. *(22)* |
| 40 | 22–506 [n=10] | 97–235/ 14–162[b] [n=4] | <1–18 [n=10] | 88–674 [n=10] | 96–222/ 35–169[b] [n=4] | 12–265 [n=10] | |
| | 250 [n=1] | 61/132[a] [n=1] | NE | 177 [n=1] | 11/17[a] [n=1] | NE | Yoshida et al. *(25)* |
| | 453 [n=1] | 114[c] [n=1] | <40 [n=1] | 422 [n=1] | 124[c] [n=1] | 86–142 [n=1] | Hale et al. *(27)* |
| 60 | NE | 193/64[b] [n=1] | <1 [n=1] | NE | 177 / 69[b] [n=1] | 27 [n=1] | Hendrick et al. *(22)* |

*Table 1 (continued)*

| *Maternal Dose (mg/kg bw/ day)* | *Fluoxetine Levels (ng/mL)* | | | *Norfluoxetine Levels (ng/mL)* | | | *Reference* |
|---|---|---|---|---|---|---|---|
| | *Maternal Plasma or Serum* | *Milk* | *Infant Plasma or Serum* | *Maternal Plasma or Serum* | *Milk* | *Infant Plasma or Serum* | |
| 0.17–0.24 | NE | 23.1–35.9 [n=2] | <1 [n=1] | NE | 41.6–71.0 [n=2] | <1 [n=1] | Taddio et al. *(26)* |
| 0.24 | 38–49 [n=2] | 26–53 [n=2] | <10–104 [n=2] | 59–106 [n=2] | 50–52 [n=2] | <10–100 [n=2] | Kristensen et al. *(28)* |
| 0.27–0.35 | NE | 35.2–93.2 [n=5] | NE | NE | 31.0–95.7 [n=5] | NE | Taddio et al. *(26)* |
| 0.28–0.36 | 77–151 [n=4] | 29–135 [n=4] | 25 [n=1] | 106–180 [n=4] | 25–106 [n=4] | 17 [n=1] | Kristensen et al. *(28)* |
| 0.46 | NE | 143.6 [n=1] | NE | NE | 107.3 [n=1] | NE | Taddio et al. *(26)* |
| 0.46 | 91 [n=1] | 32 [n=1] | NE | 135 [n=1] | 33 [n=1] | NE | Kristensen et al. *(28)* |
| 0.56–0.66 | 182–335 [n=5] | 136–202 [n=5] | <10–30 [n=4] | 165–393 [n=5] | 88–274 [n=5] | <10–164 [n=4] | |
| 0.65 | NE | 122.9 [n=1] | NE | NE | 169.4 [n=1] | NE | Taddio et al. *(26)* |
| 0.85 | NE | 189.1 [n=1] | NE | NE | 143.2 [n=1] | NE | |
| 0.90–0.94 | 356–412 [n=2] | 344–384 [n=2] | <10–252 [n=2] | 339–397 [n=2] | 296–321 [n=2] | 185–187 [n=2] | Kristensen et al. *(28)* |

n = number of subjects studied; NE = not examined; d = days; w = weeks; m = months

[a] Level measured in foremilk/hindmilk

[b] Peak/trough level

[c] 10 days earlier

***Table 2. Breast Milk-to-Plasma Ratios for Fluoxetine and Norfluoxetine***

| *Number of Mothers Sampled* | *Fluoxetine Milk-to-Plasma Ratio (Range and Mean)* | *Norfluoxetine Milk-to-Plasma Ratio (Range and Mean)* | *Reference* |
|---|---|---|---|
| 8 | Peak levels:<br>0.34 – 6.09<br>**[Mean: 1.6]**<br>Trough levels:<br>0.05 – 2.91<br>**[Mean: 0.80]** | Peak levels:<br>0.33 – 2.08<br>**[Mean: 0.84]**<br>Trough levels:<br>0.1 – 0.79<br>**[Mean: 0.43]** | Hendrick et al.<br>*(22)* |
| 14 | 0.24 – 1.13<br>(Mean: 0.68)<br>(95% CI: 0.52 – 0.84) | 0.22 – 1.00<br>(Mean: 0.56)<br>(95% CI: 0.35 – 0.77) | Kristensen et al.<br>*(28)* |
| 4 | **[0.37 – 1.5][a]**<br>**[Mean: 0.65]** | **[0.085 – 1.1][a]**<br>**[Mean: 0.35]** | Yoshida et al.<br>*(25)* |
| 1 | **[0.29]** | **[0.21]** | Isenberg<br>*(23)* |
| 1 | **[0.14][a]** | **[0.092][a]** | Burch and Wells<br>*(21)* |
| 3 | 0.52 – 1.51<br>(0.88 ± 0.44)[b] | 0.60 – 1.15<br>(0.82 ± 0.3)[b] | Taddio et al.<br>*(26)* |

**[ ]** = Calculated by CERHR
[a] Values are only summarized for hindmilk
[b] Mean ± SD

***Table 3. Maternal Infant Drug Correlations Observed by Hendrick et al. (22)***

| *Parameter* | *Correlation Coefficient, r* | *Degrees of Freedom* | *P* |
|---|---|---|---|
| Infant serum norfluoxetine × Maternal serum fluoxetine | 0.73 | 17 | 0.0004 |
| Infant serum norfluoxetine × Maternal serum norfluoxetine | 0.74 | 17 | 0.0003 |
| Infant serum norfluoxetine × Peak milk fluoxetine | 0.77 | 7 | 0.01 |
| Infant serum norfluoxetine × Peak milk norfluoxetine | 0.64 | 7 | 0.06 |
| Maternal serum norfluoxetine × Peak milk fluoxetine | 0.80 | 6 | 0.02 |
| Maternal serum norfluoxetine × Peak milk norfluoxetine | 0.72 | 6 | 0.04 |
| Infant serum norfluoxetine × Maternal fluoxetine dose | 0.70 | 18 | 0.0006 |

*Strengths/Weaknesses:* This study featured a large sample size in comparison to other evaluated studies, careful ascertainment of maternal and infant serum concentrations and breast milk, and assessment of relationships to infant age, weight, and maternal dose. Fetal exposure status was noted and because most infants exposed during lactation had also been exposed *in utero*, these findings relate to this type of exposure scenario. The sample was not large enough to test multiple relationships. Some specific findings may be spurious. For example, only 3 infants were nursed by mothers on a fluoxetine dose of less than 20 mg. The "safe" doses noted may not be generalizeable, because the therapeutic dose may be higher, and the majority of mothers used doses of 40 mg/day or more. There was significant variability among subjects and, along with the small sample size, the variability may have permitted the results to be overly influenced by a few outlying cases. The convenience sample may have been biased. The inclusion of only Caucasians in the sample reduces generalizeability. With regard to infant outcomes, maternal perceptions of infants may have been affected by the mothers' depressed state, educational level, or socioeconomic status, none of which are described in this study, as well as by maternal denial or a maternal desire to minimize negative observations.

*Utility (Adequacy) for CERHR Evaluation Process:* This study permits the estimation of exposure of nursing infants to fluoxetine in milk, keeping the limitations discussed in mind.

Kristensen et al. *(28)* studied 14 nursing mothers (ages 23–44 years) and their infants (ages 0.1–15 months). Mothers were taking 20–80 mg/day fluoxetine (doses equal to 0.24–0.94 mg/kg bw/day) for 13–750 days. Ten of the subjects were tested in a limited sampling protocol that involved collecting a blood sample at 1.1–23.5 hours following dosing and a milk sample prior to and following feeding. Intensive sampling was conducted on the remaining 4 subjects by collecting blood and milk samples at 0, 1, 2, 3, 4, 6, 8, 12, and 24 hours post-dosing and calculating the 24-hour AUC. Blood samples were taken from a total of nine infants. Samples were analyzed by HPLC with UV detection. Data were analyzed by Students *t*-test for paired or independent data groups. Results according to dose levels are listed in Table 1. Fluoxetine and norfluoxetine were detected in five of nine and seven of nine infants, respectively. Norfluoxetine levels were generally highest in infants ≤1.5-months old. However, the authors noted that all of those infants were exposed to fluoxetine *in utero* and this exposure could have contributed to postnatal blood levels. Levels of drug and metabolite were higher in post- than in pre-feeding milk samples. The authors stated that this result was expected due to the increase in lipid content of milk during feeding. Study authors estimated percent infant doses compared to maternal doses according to concentrations detected in milk and obtained an average milk intake of 0.151 L/kg bw/day. The mean total dose of fluoxetine+norfluoxetine was 6.8% of the weight-adjusted maternal dose, but 5 infant doses were in the range of 8.6–12%.

*Strengths/Weaknesses:* Strengths include the intensive sampling in one arm of the study, multiple methods used to calculate infant dose, and statistical analysis using confidence intervals (CI). Weaknesses include the small sample size, great variability in maternal age, and lack of information on other maternal characteristics. It was not known if infants were pre-term and whether gestational ages were corrected in calculating age. Drug abusers appear to have been included. No exclusion criteria were indicated. Referral biases were possible. There was large variability in duration of therapy, infant age, and whether or not an infant was exposed *in utero*.

*Utility (Adequacy) for CERHR Evaluation Process:* This study permits an estimation of infant exposure to fluoxetine through milk.

Taddio et al. *(26*) examined 10 nursing women (24–38 years old) taking 0.17–0.85 mg/kg bw/day fluoxetine for at least 2 weeks. Infants were 20–747 days old during this study. Mothers collected and submitted 3–6 milk samples per dosing period (i.e., 2, 5, 8, 12, and 24 hours following dosing) for analysis of fluoxetine and norfluoxetine levels by GC/MS with an electron capture detector. Levels of fluoxetine and norfluoxetine in milk ranged from 17.4 to 293 ng/mL and 23.4 to 379.1 ng/mL, respectively. Mean levels of fluoxetine and norfluoxetine at various dose levels are reported in Table 1. In 8 women, fluoxetine levels in milk peaked within 6 hours of dosing, but in 2 women, maximum concentrations occurred more than 12 hours after dosing. Levels of fluoxetine and norfluoxetine paralleled each other and gradually declined toward the end of the dosing period. Concentrations in breast milk were linearly correlated with maternal dose, and hence estimated infant dose ($r^2$=0.89, $P$<0.001 for maternal dose vs. estimated infant dose). Milk and maternal plasma samples were simultaneously collected from three women on four occasions. Milk-to-plasma ratios were reported at 0.52–1.51 (mean±SD = 0.88±0.44) for fluoxetine and 0.60–1.15 (mean±SD = 0.82±0.3) for norfluoxetine. **[Individual levels in plasma and milk were not reported.]** Infant doses were estimated by multiplying the AUC concentration in milk by the volume of milk ingested per day (1,000 mL). Mean infant doses of fluoxetine and norfluoxetine were estimated at 0.077 and 0.084 mg/day, respectively. The total equivalent fluoxetine dose (0.165 mg/day) was calculated by combining the fluoxetine and norfluoxetine estimates. A dose of 0.165 mg/day is equivalent to 0.041 mg/kg bw/day [41 **µg/kg bw/day]** in a 4-kg newborn infant, and was estimated to be about 10.8% of the maternal dose on a weight-adjusted basis.

Fluoxetine and norfluoxetine levels were measured in the plasma of one infant and in randomly collected urine samples from five infants. The mean duration of infant drug exposure was 64.8 days. Fluoxetine and norfluoxetine levels in the plasma of one infant and the urine of a second infant were below the detection limit (1 ng/mL). In 4 infants, urine levels of fluoxetine ranged from 1.7 to 17.4 ng/mL. Norfluoxetine concentrations exceeded the detection limit in urine from 2 infants and were reported at 10.5 and 13.3 ng/mL.

*Strengths/Weaknesses:* Strengths of this study included the use of multiple milk samples. Weaknesses include the very small sample size and variability in age (2 years). The sample may have been biased because mothers were self-selected by having called a counseling program. There was no information on selection, attrition, or refusals and no control for maternal dose. The range of exposure was broad. There were no controls for any other factors. Relying on maternal report for infant observations entails problems similar to those noted in the Kristensen study *(28*).

*Utility (Adequacy) for CERHR Evaluation Process:* This study permits an estimation of infant exposure to fluoxetine through milk.

Yoshida et al. *(25*) studied 4 women taking fluoxetine for a mean duration of 21 weeks while breastfeeding. Dose levels were 20 mg/day in 3 women and 40 mg/kg/day in the fourth. One or two samples of breast milk and maternal and infant blood and urine were collected in the morning, approximately 12–15 hours after the last dose. Both foremilk and hindmilk samples were collected and

analyzed separately. Samples were analyzed for fluoxetine and norfluoxetine levels by GC/MS with an electron capture detector. Fluoxetine and norfluoxetine concentrations in plasma and milk are reported in Table 1. Concentrations of fluoxetine and norfluoxetine were higher in hindmilk samples, which had higher mean fat levels (11.3%) than did foremilk samples (5.5%). However, there was no significant correlation between fat levels and drug concentrations in milk. With the exception of one sample, levels of fluoxetine and norfluoxetine were higher in maternal plasma than in milk (see Table 1). In mothers taking 20 mg/day, levels of fluoxetine and norfluoxetine in urine were 235 – 426 and 131 – 597 ng/mL, respectively, indicating active excretion. Urinary levels of fluoxetine and norfluoxetine were 349 and 73 ng/mL, respectively, in the mother taking 40 mg/day. Infant urine concentrations of both fluoxetine and norfluoxetine were below the quantification limit of 2 ng/mL. Based on concentrations reported in hindmilk, the study authors estimated that infants receive fluoxetine-equivalent doses that are 3 – 10% of the mothers' doses on a weight-adjusted basis.

*Strengths/Weaknesses:* There were multiple measures of maternal plasma, urine, and foremilk and hindmilk taken at consistent time intervals across the sample. The infants were all full-term and underwent standardized assessments. This study was, however, a small case series with no information on recruitment. The infants were only followed up to 13 months, which is not predictive of later outcome. Maternal behaviors may have been responsible for outcome rather than fluoxetine dosage. There was no control group and no information on potentially important maternal characteristics such as depression and IQ. There was no standardized assessment of maternal depression.

*Utility (Adequacy) for CERHR Evaluation Process:* This study can be used to estimate infant fluoxetine exposure through milk. Infant outcome information may not be reliable (see Section 3.1.2).

Heikkinen et al. *(19*) measured maternal and infant plasma concentrations of fluoxetine and norfluoxetine at delivery, and 2 days, 4 days, 2 weeks, and 2 months after birth. Eleven nursing mother-infant pairs contributed data. Milk concentrations were evaluated at 4 days, 2 weeks, and 2 months after birth. Maternal plasma, infant plasma, and milk samples were obtained just prior to the mother's daily dose of fluoxetine and prior to a feeding, which were characterized as trough levels. Results of this study are listed in Table 1. Infant serum levels of both fluoxetine and norfluoxetine appeared to decline with age, despite continuing exposure to these compounds in milk.

*Strengths/Weaknesses:* Strengths include the prospective nature of the study and the evaluation period that spanned pregnancy through lactation. There was a limited range of drug dosage and controlling for gestational age, parity, and delivery mode. Weaknesses include the small sample size and multiple drug exposures.

*Utility (Adequacy) for CERHR Evaluation Process:* This study is adequate for estimation of infant fluoxetine and norfluoxetine exposure through milk.

Suri et al. *(29*) measured fluoxetine and norfluoxetine in milk and serum from ten mother-infant pairs in a study sponsored by Eli Lilly and Company. Milk measurements were made by HPLC and UV detection after liquid/liquid and solid phase extraction. Serum measurements were performed using an isocratic HPLC separation. Infant dose was estimated based on milk concentration **[which was not given in the paper]** and milk volume consumed, and ranged from 0.041 to 0.16 mg/day for fluoxetine

and 0.037 to 0.14 mg/day for norfluoxetine. The children weighed 3.4–5.7 kg; on a weight-adjusted basis **[calculations by CERHR]**, estimated fluoxetine intake was 8–35 µg/kg bw/day and estimated norfluoxetine intake was 6–41 µg/kg bw/day, or about 2–3 orders of magnitude lower than the usual adult dose on a body weight basis.

*Strengths/Weaknesses:* The technical methods appear to be appropriate. The use of children at different ages gives a wide range of intake estimates, which may be less useful in evaluating potential exposures in children at a particular time of concern (e.g., infancy). The lack of information on milk concentration of fluoxetine and norfluoxetine is a weakness of this paper.

*Utility (Adequacy) for CERHR Evaluation Process:* This paper is adequate for estimation of a range of exposure levels for nursing infants.

One mother/infant pair each was examined in the remaining studies of fluoxetine intake during breastfeeding and those values are reported in Table 1 *(20, 21, 23, 24, 27*). Burch and Wells *(21)* estimated the infant dose at 15–20 µg/kg bw/day norfluoxetine+fluoxetine by assuming that the milk contained 120 ng/mL fluoxetine+norfluoxetine and that the infant consumed 150 mL of milk per kg bw per day.

A case report of an infant with possible fluoxetine toxicity (somnolence) was reported by Hale et al. *(27*). Measurements of fluoxetine and norfluoxetine in maternal serum were 453 and 422 ng/mL, respectively (1,309 and 1,219 nM, respectively in the paper). **[Study authors may be using the molecular mass of fluoxetine HCl for their calculations. Using the molecular masses of fluoxetine and norfluoxetine, the concentrations are 1461 and 1287 nmol, respectively.]** Infant serum fluoxetine was below the limits of detection (<40 ng/mL) **[<129 nM]** and infant serum norfluoxetine was 142 ng/mL **[458 nM calculated by CERHR; the value in the paper appears to be incorrect].** Milk concentrations measured 10 days earlier were 114 ng/mL and 124 ng/mL for fluoxetine and norfluoxetine, respectively (329 and 358 nM, respectively) **[368 and 378 nM by CERHR calculations]**. Milk-to-plasma ratios were not calculated or included in Table 2 due to the difference in the timing of milk and plasma collections.

There is a report of one infant whose plasma fluoxetine levels exceeded those typically observed in mothers *(24*). One infant had plasma levels of drug and metabolite that were near the lower range of maternal values *(20*), while values were below the detection limit in three other infants *(25*). Milk-to-plasma ratios in most cases are reported to be lower than one (Table 2). In the report by Hendrick *(22*), however, there was one individual with a high milk-to-plasma ratio (>2) for fluoxetine and norfluoxetine and another individual with a milk-to-plasma ratio of 6.09 (for peak values), suggesting variation in biotransformation, protein binding, or distribution among women. This latter woman, in fact, had a milk-to-plasma ratio of 0.85 for norfluoxetine, suggesting a decreased capacity for biotransformation of the parent compound. Symptoms observed in infants breastfed by mothers taking fluoxetine are reported in Section 3.1.2.

These smaller case studies can direct attention to extreme ranges of exposure or to unusual and unique moderating factors; for example, the Lester paper *(24*) suggests the possibility that infant exposure may be far greater than that indicated in maternal dosage and may exceed normative ranges.

#### *1.2.3.4 Environmental and occupational exposure*

Fluoxetine has been reported in U.S. surface waters, presumably derived from urine and feces of people on therapy. A maximum surface water concentration of 0.012 µg/L has been estimated, with wastewater treatment plant effluent concentrations up to 0.540 µg/L (reviewed by Brooks et al. *(30)*). A second study reported that levels of fluoxetine were below the detection limit (25.5 ng/L) in water samples obtained from Louisiana (i.e., two surface water bodies, sewage plant effluent, and drinking water treatment plant) and Ontario, Canada (i.e., one surface water body, a drinking water treatment plant, and a pilot plant) *(31)*. Brooks et al. *(30)* noted that environmental levels of norfluoxetine have not been reported. An abstract reported that SRIs were detected at unspecified concentrations in tissues of bluegill fish collected from an effluent dominated stream in north Texas *(32)*. There is no known information on biodegradability of fluoxetine or norfluoxetine. No information was identified on occupational exposure to fluoxetine in the pharmaceutical industry.

*Strengths/Weaknesses:* Because norfluoxetine is the primary metabolite produced and excreted, and because norfluoxetine has biologic/pharmacologic properties similar to those of fluoxetine, the environmental levels of norfluoxetine are of much greater importance than the levels reported for fluoxetine (it is difficult to imagine how large amounts of fluoxetine would end up in wastewater other than from a manufacturing facility). Given other reports of pharmacologically active materials or metabolites being found in wastewater and hypotheses proposed for the effects of these chemicals on environmental organisms, the presence of fluoxetine/norfluoxetine in wastewater/groundwater/sediment should be investigated.

*Utility (Adequacy) for CERHR Evaluation Process:* These data predict negligible exposure from environmental contamination; however, the lack of information on norfluoxetine concentrations makes this interpretation unreliable.

### *1.3 Utility of Exposure Data*

The data set for fluoxetine consists of studies measuring fluoxetine and/or norfluoxetine levels in umbilical cord blood, blood of newborn infants, maternal blood, breast milk, and/or blood of breast-feeding infants. The database was sufficient for estimating ranges of fetal exposures in late pregnancy and infant exposure during breast feeding. A very limited amount of information was available regarding fluoxetine, but not norfluoxetine levels, in surface water. Though exposures are expected to be negligible, data were not sufficient to evaluate environmental contamination.

### *1.4 Summary of Human Exposure*

Fluoxetine is a medication marketed for the treatment of MDD, OCD, bulimia nervosa, panic disorder, and PMDD in adults and MDD and OCD in children 7–17 years old. It is believed that virtually all human fluoxetine exposure is through medication; environmental fluoxetine exposure appears to be trivial *(30)*. No information was identified on occupational exposure. Recommended fluoxetine doses are 10–80 mg/day or 90 mg/week in adults and 10–60 mg/day in children. Differences in recommended dose are based on the disorder being treated and on the patient's response to treatment. The 20 mg strength is most widely prescribed and accounts for about 70% of all dispensed prescriptions *(11)*. In 2002, about 26.7 million prescriptions were dispensed for fluoxetine, with 1.2 million dispensed to pediatric and adolescent patients (1–18 years old) and 8.4 million dispensed to women of child-bearing age (19–44 years old) *(11)*. The number of people for whom these prescriptions were written is not known.

It has been estimated that 15.6% of pregnant women meet criteria for depression *(13)*; it is not known what proportion of these women are treated with fluoxetine. Physiologic changes of pregnancy may require that fluoxetine dosing be increased to maintain clinical effectiveness *(14)*. The exposure of fetuses from use of fluoxetine by pregnant women has been estimated using umbilical cord blood concentrations of the medication shortly after birth; these concentrations have ranged from 26 to 112 ng/mL *(15-17, 19)*. Fluoxetine is metabolized to norfluoxetine, which is also pharmacologically active. Norfluoxetine levels in cord blood have been measured at 54–209 ng/mL *(15, 19)*. Fetal/neonatal exposure has also been estimated using combined fluoxetine+norfluoxetine cord blood concentrations. Values for the combined parent and active metabolite range from about 65 to 114 ng/mL *(18)*.

Fluoxetine concentrations have been measured in blood and milk of lactating women and in the blood of their infants *(19, 22, 25, 26, 28, 29)*. The ranges of milk concentrations for fluoxetine and norfluoxetine, respectively, are <2–384 ng/mL and <2–321 ng/mL (Table 1). Infant blood fluoxetine and norfluoxetine concentrations range from undetectable to 340 ng/mL and 265 ng/mL, respectively (Table 1). Maternal blood concentrations have been measured at 21–506 ng/mL and 43–674, respectively, for fluoxetine and norfluoxetine (Table 1). Fluoxetine appears to be concentrated in the more lipid-rich hindmilk than in foremilk *(25)*. Milk-to-plasma ratios range from 0.05 to 6.09 for fluoxetine and 0.085 to 2.08 for norfluoxetine; most ratios are lower than 1 (Table 2). The large variations in milk and plasma values may be due to outlying values from women with unusual pharmacokinetic variations in the handling of fluoxetine *(22)*. Infant exposure, as estimated by norfluoxetine serum concentration, is strongly related to maternal fluoxetine dose and maternal serum concentrations of fluoxetine and norfluoxetine (Table 3 *(22)*).

Data are also available on exposure levels when fluoxetine is used for pediatric indications. In 8–12 year old children (n=52) medicated with 20 mg/day for at least 4 weeks, the steady-state concentrations of fluoxetine and norfluoxetine in blood were 145±76 and 167±60 ng/mL respectively (see Table 6, pg 39). Similarly in 13–17 year old children (n=42), the levels were 79±49 and 113±41 ng/mL (see Table 6, pg 39 ).

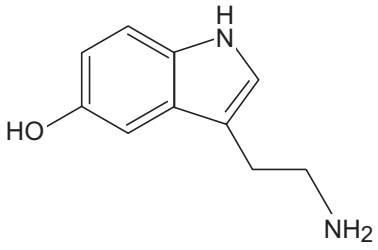
## 2.0 GENERAL TOXICOLOGY AND BIOLOGIC EFFECTS

### *2.1 Pharmacodynamics*

Fluoxetine, a racemic mixture of R- and S-enantiomers, was the first marketed member of a group of compounds known initially as selective serotonin reuptake inhibitors (SSRI). These agents now are more commonly called serotonin reuptake inhibitors (SRI), to avoid the implication that their activity is confined to serotonergic systems. Other SRIs marketed in the U.S. include sertraline, paroxetine, fluvoxamine, and citalopram. These agents are marketed for several indications, but their best known activity is in the treatment of depression.

The pharmacologic action of fluoxetine and other SRIs has been reviewed (Grimsley and Jann *(33)*; Wong et al. *(2)*; Stokes & Holtz *(12)*). Serotonin is 5-hydroxytryptamine (Figure 2), a regulatory neurotransmitter that also has physiologic functions in platelets, the gastrointestinal tract, and elsewhere in the body. In the brain, serotonin-containing neurons have their cell bodies primarily in the midline of the brainstem, but the axonal projections of these neurons are widespread throughout the brain. Serotonergic neurons play a role in regulation of mood, sleep, sexual activity, motor activity, neuroendocrine function, and cognition.

***Figure 2. Serotonin***




The following evidence obtained from various studies suggests that serotonin plays a role in depression and led to the development of fluoxetine:

- Reduced serotonin and 5-hydroxyindoleacetic acid (5-HIAA) levels in brain tissue or cerebrospinal fluid of suicide victims *(2, 12)*
- Antidepressive effects following treatment with tryptophan or 5-hydroxytryptophan, alone or in combination with monoamine oxidase inhibitors (MAOI) *(2)*
- Tendency of depressed patients to have defective serotonin transport and 5-$HT_2$ receptor activity in platelets *(12)*

In serotonergic neurons, serotonin is synthesized through the hydroxylation of tryptophan to 5-hydroxytryptophan which is then decarboxylated *(2)*. The newly produced serotonin is stored in vesicles until it is released into the synaptic cleft following nerve impulse. When released, serotonin may activate one of several postsynaptic serotonin receptor subtypes (e.g., 5-$HT_{1A,B,D,E,\text{ or }F}$, 5-$HT_{2A,C}$, 5-$HT_3$, 5-$HT_4$, 5-$HT_5$, 5-$HT_6$, and 5-$HT_7$). The action of serotonin is terminated when it binds to the presynaptic transporter for reuptake into the presynaptic nerve terminal and conversion to 5-HIAA by monoamine oxidase. The serotonin transporter is blocked by fluoxetine and other SRIs, leading to a 1.5- to 4-fold increase in serotonin in the synaptic cleft *(2)*. SRIs also block the serotonin transporter in blood platelets. There is a serotonin transporter in the placenta (reviewed by Nguyen et

al. *(34)*); however, fluoxetine interaction with this receptor has not been studied.

Evidence suggests that inhibition of serotonin uptake following fluoxetine dosing occurs within minutes in animals and presumably minutes-to-hours in humans *(2, 12)*. However, it takes several weeks for antidepressant effects to occur. The delay may be related to changes in the autoregulatory serotonin receptor on the presynaptic neuron. Initial fluoxetine dosing may increase serotonin levels in the raphe nuclei, leading to overactivity of somatodendritic and/or terminal serotonin autoreceptors and attenuated serotonin neuronal firing *(2, 12, 35)*. However, it is postulated that repeated fluoxetine dosing results in a compensatory down-regulation of serotonin receptors to restore the normal rate of neuronal firing, thus leading to an augmentation of serotonin release and neurotransmission. This down-regulation process takes time (up to 14 days in experimental preparations), and may account for the delay in antidepressant action that is typically seen with SRIs. One must also consider emerging evidence on the role of fluoxetine in facilitating hippocampal neurogenesis as a putative mechanism underlying its efficacy *(36-38)*. The stimulation and completion of neurogenesis in association with fluoxetine treatment temporally corresponds with the timing of symptom reduction in animal models of depression and anxiety. Human studies of individuals with major depressive disorder have reported reduced hippocampal volume of unknown etiology *(39)*. The ability of fluoxetine to stimulate neurogenesis is an important mechanism to consider not only with respect to the mediation of drug efficacy but also to its possible developmental toxicity.



Fluoxetine and its major metabolite, norfluoxetine, have high affinity for the serotonin transporter and selectively bind to the transporter according to a saturable process requiring sodium *(2)*. In contrast, fluoxetine has low affinity for norepinephrine uptake sites and neurotransmitter receptors such as $\alpha_1$-adrenergic, $\alpha_2$-adrenergic, β-adrenergic, dopaminergic, muscarinic, histaminergic, $H_1$, opiate, GABA, and benzodiazepine. Fluoxetine also has relatively low affinity for most serotonin receptors including 5-$HT_{1A,B,D}$ , 5-$HT_{2A}$, and 5-$HT_3$. However, the affinity of the R-enantiomer for the $5HT_{2C}$ receptor is approximately 20 times greater than that of the S-enantiomer, resulting in an overall affinity for the $5HT_{2C}$ receptor that is approximately 1–2 orders magnitudes higher than affinities for the other receptors. Although a possible interaction with the $5HT_{2C}$ receptor was observed, Wong et al. *(2)* stated that, "…blockade of 5-HT uptake most likely accounts for the pharmacological activity of fluoxetine."

Fluoxetine and other marketed SRIs were selected for development based on their inhibition of the transport protein for serotonin and lack of effect on the norepinephrine reuptake transporter. Norepinephrine is another neurotransmitter important in mood, sleep, and other central nervous system (CNS) activities. Fluoxetine and norfluoxetine each have an inhibition constant ($K_i$) of 17 nM for the serotonin transporter and more than 2,000 nM for the norepinephrine transporter. Fluoxetine administered at 2 mg/kg to rhesus monkeys at about 10 months of age produced decreases in cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid, but little or no effect on CSF norepinephrine or its metabolite *(40)*. **[The Panel based this interpretation on figures in the article; the paper does not present analysis of the norepinephrine data except for a visual display of the mean and standard error.]** The selectivity for the serotonin transporter increased the theoretical appeal of the SRIs, but in spite of the low activity for the norepinephrine transporter, these agents decrease the activity of dopamine hydroxylase, the rate-limiting step in the synthesis of norepinephrine.

Effects of fluoxetine administration in rodents include a decrease in food consumption, aggression, and

dominance behaviors. Experimental animal models of depression, such as learned helplessness, respond to fluoxetine administration. In learned helplessness, experimental animals are subjected to inescapable stress to the point that they stop trying to escape when given the opportunity. Fluoxetine administration fosters escape behavior in this situation. Short-term administration of fluoxetine increases anxiety in rodents while long-term administration is anxiolytic (reviewed by Wong et al. *(2)*).

Kelly et al. *(41)* evaluated 13 depressed patients after 6 weeks of treatment with fluoxetine. Subjects had Hamilton Depression scores of 20 or higher prior to therapy. The fluoxetine dose could be raised over the 6 weeks to as high as 60 mg/day. Responders were counted either as subjects with Hamilton Depression scores $\leq$ 6 with at least a 75% decrease in score compared to pre-treatment or as subjects with a Clinical Global Index score of 1 or 2. By either method of diagnosing response, there was no relation of response to either serum fluoxetine or serum norfluoxetine or to the squares of serum fluoxetine or norfluoxetine. The dose taken at week 6 was also unrelated to response, although this dose was related to serum fluoxetine and norfluoxetine levels. However, as noted by study authors, only 13 patients were included in this pilot study and it is therefore possible that a type II error could have occurred.

The S-enantiomer of norfluoxetine is about 20 times more potent a SRI than is the R-enantiomer (reviewed by Jannuzzi et al. *(42)*). In spite of this selectivity of the S-enantiomer, Jannuzzi et al. *(42)* did not find a relationship between total active fluoxetine (R-fluoxetine + S-fluoxetine + S-norfluoxetine) concentration in plasma and response to antidepressant therapy.



## *2.2 Pharmacokinetics*

### *2.2.1 Absorption*

According to the product label for Prozac®, a single oral 40 mg dose produces peak plasma fluoxetine concentrations in humans of 15 – 55 ng/mL after 6 – 8 hours *(4)*. In an FDA review, mean (± SD) peak plasma levels ($C_{max}$) following dosing of humans with 20 mg fluoxetine in the form of tablets or caplets were reported at 8.88 ± 3.42 and 8.99 ± 2.95 ng/mL, respectively *(43)*. **[Data on individual subjects and ranges detected in all subjects were redacted from the report.]** The pulvule, tablet, oral solution, and weekly capsule dosage forms are bioequivalent, although the weekly form contains enteric-coated pellets that resist dissolution below a pH of 5.5 *(4)*. The enteric coating delays the onset of absorption of fluoxetine 1 – 2 hours relative to the immediate release formulations. Food is reported not to affect the systemic bioavailability of fluoxetine, although it may delay its absorption by 1 – 2 hours *(4, 44)*.

*Strengths/Weaknesses:* The major limitation of the product label and FDA review *(43)* is the lack of actual data to substantiate the information provided. The data contained herein were accepted at face value.

*Utility (Adequacy) for CERHR Evaluation Process:* The information contained in the product label *(4)* is useful if taken at face value. Conclusions based on these data will be tentative unless corroborating data are available.

Harvey and Preskorn *(45)* reported pharmacokinetic parameters in 14 young adults (aged 20 – 39 years) and in 16 elderly subjects (aged 65 – 78 years). The maximum plasma concentration ($C_{max}$) of fluoxetine after an initial 20 mg dose was 10.6 ± 4.0 ng/mL (mean ± SD). After 6 weeks of daily

therapy with this dose, $C_{max}$ was 83.9 ± 22.2 ng/mL, and after 6 additional weeks on 40 mg/day fluoxetine, $C_{max}$ was 276 ± 56 ng/mL. While the fluoxetine $AUC_{0-24}$, $C_0$, and $C_{max}$, were not different between the young and elderly subjects, the half-life for fluoxetine and norfluoxetine were 25 and 33% longer, respectively, in the elderly subjects when compared to the younger subjects.

*Strengths/Weaknesses:* The paper by Harvey and Preskorn *(45)* provides useful information regarding the pharmacokinetic parameters found following an initial 20 mg dose in a population of healthy young and elderly patients, thereby indirectly providing information on absorption of the drug. The paper used analytic techniques with very good interassay coefficients of variation, providing confidence in the blood profiles provided. The AUC and half-life values were determined by the linear trapezoidal method and linear regression of the terminal portion of the curve, respectively. The blood levels of fluoxetine and norfluoxetine after 6 weeks of dosing with 20 mg/day and 40 mg/day also provide an indication of the blood levels that can be achieved in these 2 populations with these commonly prescribed dosing regimens.

*Utility (Adequacy) CERHR Evaluation Process:* This paper can be used to estimate internal exposure levels in nonpregnant adults on fluoxetine therapy. At oral fluoxetine doses ranging from 20 to 80 mg, $C_{max}$ values were reported to be proportional to dose *(44)*.

### 2.2.2 Distribution

#### *2.2.2.1 Nonpregnant individuals*

***Humans***

Fluoxetine is about 94.5% protein-bound in human plasma, mostly to albumin and $\alpha_1$-glycoprotein *(4)*. Volume of distribution in humans has been reported as 20 – 42 L/kg (reviewed by Altamura et al. *(44)*). According to the product label for Prozac®, human plasma concentrations after 30 days of dosing at 40 mg/day are 91 – 302 ng/mL for fluoxetine and 72 – 258 ng/mL for norfluoxetine, the N-demethylated metabolite *(4)*.

With once-weekly dosing using the enteric-coated preparation, peak concentrations are in the range of the average concentration for 20 mg once-daily dosing, according to the product label. Average trough concentrations are 76% lower for fluoxetine and 47% lower for norfluoxetine than the concentrations maintained by 20 mg once-daily dosing. Average steady-state concentrations of either once-daily or once-weekly dosing are in relative proportion to the total dose administered. Average steady-state fluoxetine concentrations are approximately 50% lower following the once-weekly regimen compared with the once-daily regimen *(4)*.

*Strengths/Weaknesses:* The values provided in the product label for Prozac® *(4)* are useful in providing an expected range of concentrations following repeated drug exposure. The large variability reported for these values reveal a 3 – 4 fold difference in steady-state plasma levels in patients receiving this drug. The original data supporting these statements were not provided. In addition, the levels should not be assumed to represent fluoxetine preparations other than Prozac®, because the bioavailability may differ among product formulations.

*Utility (Adequacy) CERHR Evaluation Process:* These data can be tentatively used in the evaluation process. The Panel notes that Harvey and Preskorn *(45*) report plasma levels in a range similar to those described in the product label.

In the study by Harvey and Preskorn *(45*), $AUC_{0-24}$ values in adults after a single 20 mg fluoxetine dose, after 6 weeks of fluoxetine 20 mg/day, and after an additional 6 weeks of fluoxetine 40 mg/day were 134 ± 83, 1,723 ± 475, and 5,730 ± 1,320 ng•h/mL, respectively. The time required for younger patients to reach steady-state at dosages of 40 mg/day was estimated at 8.5 weeks, due to the long half-life for the drug. A 2-fold increase in dosage (from 20 to 40 mg/day) resulted in a 3.2-fold increase in plasma concentration.

*Strengths/Weaknesses:* The analytic methodology in the Harvey and Preskorn *(45*) paper resulted in excellent interassay coefficients of variation and enantiomer nonspecific quantification of both fluoxetine and norfluoxetine levels. There is a good description of the test subjects and their disposition within the study time course.

*Utility (Adequacy) CERHR Evaluation Process:* This paper can be used to estimate internal fluoxetine exposure in nonpregnant adults on therapy.

Unpublished studies by Eli Lilly and Company on the use of fluoxetine in children and adolescents (ages 6 – 17) were summarized in a Clinical Pharmacology and Biopharmaceutics Review *(46*). Three studies were summarized with respect to pharmacokinetic parameters. In the first study, children 8 – 17 years old were given fluoxetine 10 mg/day for 1 week, then increased to 20 mg/day. After 8 weeks at this dose, some nonresponders were increased to 40 mg/day. An increase to 60 mg/day was possible for subjects not responding to 40 mg/day. Blood for pharmacokinetic studies was collected after at least 4 weeks on the 20 mg/day dose. The study sampled 52 children (8 – 12 years old) and 42 adolescents (13 – 17 years old). The steady-state concentration of fluoxetine at all ages was 116.6 ± 73.7 ng/mL (mean ± SD). For children and adolescents, the steady-state concentrations were 144.8 ± 76.4 and 78.8 ± 49.4 ng/mL, respectively (mean ± SD) **[*P* < 0.0001 children vs. adolescents by *t*-test performed by CERHR]**. Norfluoxetine concentrations in the whole sample, children, and adolescents, were 144.1 ± 58.9, 167.2 ± 59.6, and 113.1 ± 41.4 ng/mL, respectively (mean ± SD) **[*P* < 0.0001 children vs. adolescents by *t*-test performed by CERHR]**. Differences by sex of the subject were not apparent. The differences in fluoxetine and norfluoxetine concentrations between children and adolescents were attributed to differences in body weight. Other studies of steady-state blood levels produced similar results. **[The range of concentrations has been redacted from the FDA document. Given the large coefficients of variation, the variability among children and adolescents may have been large.]** The FDA review included an estimate of oral clearance at 11.8 L/kg, and a volume of distribution of 1,480 L. Variability of these values was said to be 85.7 and 44.2%, respectively. Weight and age accounted for significant portions of the variability; gender did not. A model incorporating weight and age still left unexplained 50% of the variability in oral clearance. When plasma fluoxetine concentrations were normalized by weight, pediatric and adult concentrations were considered equivalent. The report includes graphs, presumably to show this equivalence; however, the graphs have been redacted from the report.

*Strengths/Weaknesses:* The data summarized in the Clinical Pharmacology and Biopharmaceutics

Review *(46)* provide useful information concerning blood levels found in pre-adolescent and adolescent populations. The major limitation for acceptance of these data is the lack of detail regarding analytical methodology, range of blood values observed in the two populations, and other information redacted from the Review. Although authors of the Review conclude that the differences observed in blood levels of fluoxetine and norfluoxetine between pre-adolescent, adolescent, and adult patients are due to differences in body weight among these populations, there is considerable variability in blood levels resulting from the same oral dose within each of these populations and the reason for these differences is largely unexplained. Up to 50% of the variance observed could not be explained by body weight alone.

*Utility (Adequacy) CERHR Evaluation Process:* The data presented can be tentatively used to estimate internal fluoxetine dose in children and adolescents on therapy. Confidence in these data would be increased if corroboration were available from sources that included the underlying data.

Bolo et al. *(47)* used magnetic resonance spectroscopy (MRS) to estimate brain concentrations of fluoxetine+norfluoxetine in three men and one woman being treated for depression. The subjects were 42–50 years of age. One subject each was on 10 and 20 mg/day and 2 subjects were on 40 mg/day of the medication. Plasma fluoxetine+norfluoxetine was measured within an hour of the MRS study. Brain concentrations of fluoxetine+norfluoxetine ranged from 5 to 17 μM (about 1.6–5.3 μg/mL), while plasma concentrations ranged from 0.3 to 2.6 μM (about 0.09–0.81 μg/mL). The mean brain-to-plasma ratio (± SD) was 10±6. In 2 subjects who stopped therapy, brain half-life was 349 and 416 hours (14 and 17 days) and plasma half-life was 284 and 528 hours (12 and 22 days). The authors reported no association between brain concentration of fluoxetine+norfluoxetine and fluoxetine dose, duration of therapy, or cumulative dose of fluoxetine.

*Strengths/Weaknesses:* The paper by Bolo et al. *(47)* is useful in providing concentrations of the active forms of the drug in the target organ (brain) and in describing the relationship between brain and plasma concentrations. The number of patients was too small to allow conclusions to be drawn regarding predicted blood levels following exposure to 10, 20, or 40 mg/day, but the 4 patients with pair-wise comparisons of brain and plasma levels did allow approximation of the ratio between these two tissues. Drug concentration at the level of the receptor was not addressed. The lack of association between brain concentration and dose, dose duration, or cumulative dose makes any correlation between an adverse event involving the brain and administered dose problematic.

*Utility (Adequacy) CERHR Evaluation Process:* This study can be used to estimate brain concentrations of fluoxetine in nonpregnant adults on therapy.

***Experimental Animals***

According to Altamura et al. *(44)*, in experimental animals, fluoxetine is widely distributed in body tissues with the highest concentrations in lung and liver. The steady-state volume of distribution in rats after intravenous (i.v.) fluoxetine is about 16–20 L/kg, depending on the administered dose *(48)*. In rats given fluoxetine by oral gavage, $C_{max}$ for the parent compound normalized for a 5 mg/kg bw dose was 0.1, 0.2, and 0.2 nmol/mL (32, 64, and 64 ng/mL) after single oral gavage doses of 5, 10, and 20 mg/kg bw, respectively. **[It is not clear how values were normalized. Inspection of the graphic representation of the actual data suggests $C_{max}$ values of 0.1, 0.2, and 0.4 nmol/mL (32, 64, and 128 ng/mL), respectively after 5, 10, and 20 mg/kg.]** The normalized AUCs after these 3 doses were 2.0, 3.0, and

## 2.4 Genetic toxicology

According to the Prozac® product label, fluoxetine and norfluoxetine were negative in genotoxicity tests including a bacterial mutation assay, a DNA repair assay in cultured rat hepatocytes, a mouse lymphoma assay, and a sister chromatid exchange assay in Chinese hamster bone marrow cells *(4)*.

No published studies on fluoxetine genotoxicity testing were located.

**[The lack of study reports makes it impossible to judge and interpret these studies.]**

## 2.5 Carcinogenicity

### 2.5.1 Humans

Lawlor et al. *(67)* summarized available information on trials and epidemiological studies examining associations between antidepressant use and breast cancer. The only information presented specifically for fluoxetine was obtained from an unpublished report of 31 primary efficacy trials conducted in the U.S. Results of the trials were pooled and the trials included 4,397 individuals in the fluoxetine group and 2,918 individuals in the placebo group. Breast cancer was not a primary measurement but was assessed through an adverse-event reporting system. One case of breast cancer was reported in the treatment group and one case was reported in the placebo group. Lawlor et al. *(67)* noted several limitations of the study. The data were pooled by simple addition without considering factors such as age, socioeconomic class, and primary diagnosis. In addition, the follow-up time period of 5 – 60 weeks was not sufficient for detecting an association with breast cancer.

Kelly et al. *(68)* evaluated 5,814 women with primary breast cancer diagnosed in the preceding year, 5,095 women with primary cancers of other sites, and 5,814 women who were hospitalized for a non-cancer condition. Women were identified through a hospital-based case-control surveillance system using selected hospitals in Boston, New York, Baltimore, and Philadelphia. Subjects were interviewed during their hospitalizations by trained nurses and information on medication use was solicited. The medications of interest in this study were grouped by class (SRIs, TCAs, other antidepressants, phenothiazines, and antihistamines) and use was defined as regular if it occurred 4 days/week for at least 4 weeks. Logistic regression was performed to evaluate effects independent of age, region, race, religion, year of interview, age at menarche, age at first birth, body mass index, history of benign breast disease, menopausal status, history of breast cancer in mother or sister, current alcohol consumption, and number of lifetime hospitalizations. There were 28, 15, and 19 regular SRI users among breast cancer cases, cancer controls, and non-cancer controls, respectively. Relative risk (95% CI) for regular SRI use in cancer controls and non-cancer controls, respectively, were 1.6 (0.8, 3.2) and 1.5 (0.8, 2.8). When controls were combined and fluoxetine was examined separately, 23 of 5,814 breast cancer cases used fluoxetine regularly compared to 27 of 10,909 controls (multivariate relative risk 1.5 [95% CI: 0.8, 2.7]). For regular users of SRIs (taken together) and controls (combined), relative risk by duration of use was of borderline statistical significance for 1 – 2 years of use: relative risk 2.0 (95%CI 1.0, 4.3) based on 16 cases and 15 controls with 1 – 2 years of regular use. Durations of $<1$ and $\geq 3$ years were associated with relative risk (95% CI) of 1.2 (0.4, 3.5) and 1.3 (0.5, 3.7), and did not suggest a gradation of effect by length of use.

In their review of the Kelly study *(68)*, Lawlor et al. *(67)* noted that a causal breast cancer associa-

4.5 nmol/mL·h (620, 930, and 1,395 ng/mL·h). These values were obtained by the trapezoidal method using only the 48-hour study period. **[Actual values were estimated by CERHR from the graph in the paper using GraphPad Prism software as 2.1, 5.4, and 14.9 nmol/mL·h (620, 1,674, and 4,619 ng/mL·h.)]** Normalized norfluoxetine $C_{max}$ after these 3 doses was 0.4, 0.4, and 0.3 nmol/mL (120, 120, and 90 ng/mL), respectively. **[Actual norfluoxetine $C_{max}$ values were estimated from the graph as 0.4, 0.8, and 1.2 nmol/mL (120, 239, 359 ng/mL).]** The ratio of AUC for norfluoxetine-to-fluoxetine was 5.3, 4.1, and 3.0 at these three doses, respectively. The fluoxetine half-life after oral fluoxetine was 7–13 hours, and the norfluoxetine half-life after oral fluoxetine was 14–16 hours *(48)*.

*Strengths/Weaknesses:* This study used adequate methods to sample rat blood after i.v. and oral fluoxetine. Interpretation of the results is substantially impaired by the unexplained normalization process and the need to estimate the actual data from a graph. The interpretation of the AUC data is impaired by the use of the 48-hour sampling frame. Visual inspection of the graphs in the paper suggests that for the highest administered doses (20 mg/kg), plasma fluoxetine had not returned to baseline by the end of the sampling frame. In addition, norfluoxetine concentrations appeared not to have returned to baseline. The time-concentration curves for fluoxetine and norfluoxetine appeared not to be parallel after administration of fluoxetine, and a comparison of the AUC values for the limited sampling frames may not be informative with regard to chronic therapy.

*Utility (Adequacy) CERHR Evaluation Process:* The information contained within the paper by Caccia et al. *(48)* is important in allowing a comparison of external dose (gavage) to blood levels of fluoxetine and norfluoxetine in rats after a single dose. This information is helpful in the interpretation of the experimental animal toxicity studies and using these results to predict outcomes in humans.

Fluoxetine given intraperitoneally (i.p.) to rats at 2.5–20 mg/kg produces concentrations in plasma and whole brain that were related linearly to dose *(49)*. Norfluoxetine concentration in plasma and brain varied exponentially with dose, suggesting saturable metabolism. Platelet serotonin and brain 5-hydroxyindoleacetic acid decreased with increasing fluoxetine dose; however, brain serotonin did not decrease after administration of fluoxetine. Platelet serotonin and brain serotonin decreased 46 and 13%, respectively, after i.p. administration of 10 mg/kg bw norfluoxetine *(49)*.

*Strengths/Weaknesses:* This study used an i.p. route of administration, decreasing its interpretability for human therapeutic exposures, which are by mouth.

*Utility (Adequacy) CERHR Evaluation Process:* This study *(49)* is of limited value for this exercise due to the route of administration used. Blood levels were approximately equal with both routes at the 5 mg/kg dose level, while the levels following a 10 mg/kg i.p. injection were approximately twice the blood levels found following oral administration. The demonstration of saturable fluoxetine metabolism is useful for the evaluation process.

#### *2.2.2.2 Pregnancy*

***Humans***

Heikkinen et al. *(19)* measured fluoxetine and norfluoxetine in the plasma of 11 fluoxetine-treated women at 36–37 weeks gestation. Mean (± SD) fluoxetine and norfluoxetine concentrations prior to

the daily dose (trough levels) were 152 ± 107 nM (47 ± 33 ng/mL) and 364 ± 73 nM (109 ± 22 ng/mL), respectively. These women were on chronic doses of 20 – 40 mg/day fluoxetine. When a correction was made to correspond to a standard 20 mg/day dose, combined fluoxetine + norfluoxetine plasma concentration was estimated at 480 ± 115 nM (~144 ± 34 ng/mL). The authors noted that plasma fluoxetine concentrations in the pregnant women were considerably lower than concentrations typically seen in nonpregnant individuals on therapy. They also noted that plasma concentrations increased by 2 weeks postpartum (see Table 1) and postulated that plasma fluoxetine concentrations during pregnancy might be decreased by increased hepatic blood flow, increased volume of distribution, and decreased protein binding of fluoxetine. The mean ratio (± SD) of norfluoxetine-to-fluoxetine concentration during pregnancy (3.3 ± 1.4) was higher than at 2 months postpartum (1.4 ± 0.8, $P < 0.0072$), suggesting increased fluoxetine demethylation during pregnancy. At delivery, cord blood plasma concentrations of fluoxetine and norfluoxetine were 65% and 72% of concentrations in maternal plasma sampled at delivery. The milk-to-maternal plasma ratios ranged from 0.3 to 2.2 for fluoxetine and from 0.1 to 1.7 for norfluoxetine. Exposure of breastfed infants (as determined by plasma levels) decreased from 14 days postnatally to 2 months. Fluoxetine and norfluoxetine (combined and standardized to a 20 mg maternal dose level) in infant plasma ranged from a mean of 278 nmol/L at delivery to a mean of 155 nmol/L 2 weeks after delivery. These same units for concentrations in breast milk ranged from a mean of 244 to 296 nM from 4 days to 2 months after delivery. It is readily apparent that significant transfer of fluoxetine and norfluoxetine occurs in humans across the placenta and into the breast milk. The availability of the drugs from ingestion of breast milk is not understood as infant plasma levels were decreased 7 – 10-fold at 2 months even though the concentration in milk remained elevated.



*Strengths/Weaknesses:* This study by Heikkinen et al. *(19)* compared the pharmacokinetics of fluoxetine in 11 treated patients and 10 well-matched controls, which is a robust number of subjects for a kinetics study. The study included multiple measures of maternal, infant, and milk concentrations of both fluoxetine and the active metabolite norfluoxetine. Samples were included at the end of pregnancy as well as early after delivery: up to 2 months thereafter. These well-coordinated measures allow for a thorough analysis of the comparative kinetics of fluoxetine during pregnancy and in early development in the human. One weakness, acknowledged by the authors, is that the half-life estimations were often made with only two data points, which is not sufficient. Hence the elimination kinetic data can only be considered as rough estimates. A greater weakness is that the dose was quite variable among the patients. It is described that the patients received 20 – 40 mg fluoxetine, but no indication of the duration of the various dose levels is given. Also, some of the patients began taking fluoxetine at various weeks of gestation, while others apparently had been taking fluoxetine from the beginning of pregnancy, although this information was not directly given. Hence, the duration of therapy and thus the total dose could have been quite varied among the patients, which is important because the authors compare their results to data on nonpregnant women in another study. It is difficult to accept their conclusions regarding this comparison because the doses and durations of therapy may have differed largely between the pregnant and nonpregnant subjects in the two studies. Finally, it is difficult to understand how the authors obtained the norfluoxetine-to-fluoxetine ratios that they report during pregnancy (3.3) and at 2 months (1.4) from the data given in the tables.

*Utility (Adequacy) for CERHR Evaluation Process:* This study by Heikkinen et al. *(19)* is useful for the evaluation process because it compares the pharmacokinetic parameters during pregnancy to those after pregnancy in the same subjects, thus allowing for a direct comparison. It also useful for

understanding placental transfer of the drug and metabolite and it shows a direct comparison of the kinetics in the mother and simultaneously in the breastfed infant. The results of the Heikkinen et al. *(19*) study allowed the Expert Panel to conclude that blood levels of fluoxetine and norfluoxetine may be lower during pregnancy than those following similar dosing regimens in the nonpregnant state.

***Experimental Animals***

Pohland et al. *(50*) examined placental transfer and fetal distribution of fluoxetine in Wistar (Hsd:(WI) BR) rats using dissection and whole-body autoradiographic techniques. Unlabeled (99.3% purity) and $^{14}$C-labeled (98.3% radiochemical purity) fluoxetine HCl in water were administered to rats by gavage at a dose of 12.5 mg/kg. The authors stated that 12.5 mg/kg was the highest dose that resulted in negative results in an unpublished teratogenicity study. **[The Panel notes that 12.5 mg/kg was the highest dose used in the rat teratogenicity study by Byrd and Markham *(51*), reviewed in Section 3.2.1.1.]** In the dissection study, rats were treated on gestation day (GD) 12 (during organogenesis) and GD 18 (postorganogenesis). Five rats/time point/GD were sacrificed and examined at 1, 4, 8, and 24 hours post-dosing. Maternal blood, brain, kidney, liver, and lung were collected. Placentas, amniotic fluid, and embryos/fetuses were collected and pooled. Samples were analyzed by liquid scintillation spectrometry and levels of fluoxetine and norfluoxetine were measured by GC with electron capture detection. On GD 12 and 18, radiocarbon levels peaked at 4–8 hours post-exposure and declined slightly at 24 hours post-exposure in embryos, fetuses, placentas, amniotic fluid, and most maternal tissues. The exceptions were maternal plasma and liver, which had peak radiocarbon concentrations at 24 hours and 1 hour following exposure, respectively. The highest concentration of radiocarbon was found in maternal lung (mean peak values of ~147–157 µg-eq/g). Moderate levels of radiocarbon were detected in placenta and maternal brain and kidney (mean peak values of ~18–34 µg-eq/g in each organ); liver also contained moderate levels of radiocarbon (61–71 µg-eq/g at 1 hour post-exposure). Low levels of radiocarbon (expressed as peak values) were found in embryonic tissues (3.60 µg-eq/g), fetal tissues (5.54 µg-eq/g), amniotic fluid (0.04–0.1 µg-eq/g), and maternal plasma (1–2 µg-eq/g). Radiocarbon levels were higher in GD 18 fetuses than in GD 12 embryos at 4, 8, and 24 hours after dosing. Combined fluoxetine and norfluoxetine represented 63–80, 79–91, and 12–29% of total radiocarbon levels in embryonic/fetal tissues, placental tissues, and maternal plasma, respectively. Levels of fluoxetine in maternal and embryo/fetal tissues were higher at 1 and 4 hours post-dosing, while norfluoxetine levels were higher at the 24-hour time point.

In the whole-body autoradiography study, Pohland et al. *(50*) gavage dosed a rat with 12.5 mg/kg $^{14}$C-labeled fluoxetine on GD 18 and sacrificed it at 4 hours following exposure, the time shown to result in near maximum fetal concentrations in the dissection study. The animal was sectioned and exposed to film, which was analyzed visually or by taking optical density readings. The autoradiogram revealed that maternal lung, liver, brain, kidney, spleen, adrenal gland, gastrointestinal contents, Harderian gland, and salivary gland contained the highest concentrations of radiocarbon. Moderate concentrations of radiocarbon were observed in maternal myocardium, bone marrow, placenta, and mammary tissue. Moderate levels of radiocarbon passed through the placenta and were distributed throughout the fetus. The highest concentrations of radiocarbon in the fetus were seen in the brain and thymus; lower levels were observed in fetal liver and eyes. Uterine luminal fluid surrounding individual fetal-placental units also contained significant levels of radiocarbon. A quantitative analysis of radioactivity in maternal and fetal brain and thymus revealed that the level in fetal tissues was about half the level measured in maternal tissues.

*Strengths/Weaknesses:* The Pohland et al. *(50)* study offers a thorough analysis of the maternal and fetal distribution of fluoxetine and norfluoxetine (and total radioactive label) after dosing rats with radiolabeled fluoxetine. Studies were conducted on 2 different days of gestation using a high dose, roughly 10 times the therapeutic dose. In addition, multiple tissues were examined at 4 time points over a 24-hour period following dosing, which allows for an excellent analysis of kinetic changes. A weakness of the study lies in the difficulty in resolving conflicts in some of the data. For example, the fetal concentration of fluoxetine is higher on day GD 18 than on day GD 12, yet the relationship of placental concentrations on the 2 days are reversed. Also, the overall fetal concentration is very low compared to maternal tissues in the dissection study, but the concentration of fluoxetine in fetal tissues like the brain is as much as 50% of that in the maternal tissue in the autoradiographic analysis. Neither of these points is noted or discussed in the article.

*Utility (Adequacy) for CERHR Evaluation Process:* The Pohland et al. *(50)* study is useful in confirming that significant amounts of fluoxetine and norfluoxetine can cross the placenta into the fetus. The data demonstrated placental transfer of radiolabel to the embryo (GD 12) and fetus (GD 18) following oral dosing of the rat dam with 12.5 mg/kg of $C^{14}$-labeled fluoxetine. Several important pieces of information presented in this paper include that 63 – 80% of the radiolabel in the embryo/fetus was in the form of fluoxetine/norfluoxetine, that the time course for the radiolabeled species within the embryo/fetus follows a roughly similar time course as the maternal plasma, and that the thymus and brain contain the largest amount of radiolabel within the fetus. The presence of the majority of the radiolabel as fluoxetine/norfluoxetine within the rat fetus suggests that rat and human embryo/fetuses are exposed to similar chemicals (parent and/or metabolite), eliminating some uncertainty regarding metabolic differences between species. The time course of the fluoxetine/norfluoxetine within the embryo/fetus suggests that following a single dose, exposure during the first few hours is primarily to fluoxetine with norfluoxetine becoming the dominant exposure by 24 hours. Finally, knowledge that the radiolabel has the highest concentration in the brain and thymus provides a signal of where first to look for potential effects in the fetus.



Kim et al. *(52)* examined stereoselective pharmacokinetics of fluoxetine and norfluoxetine in pregnant Dorset Suffolk sheep and their fetuses. Five pregnant sheep were implanted with catheters between GD 117 and 126. Between GD 124 and 137 (gestation length = 145 days), fluoxetine chloride **[purity not specified]** was administered via the maternal femoral vein or via the fetal tarsal vein. All sheep were treated with fluoxetine via maternal and fetal exposure on different days in randomized order. The maternal dose was 50 mg and the fetal dose was 10 mg. Blood was collected from the fetal and maternal vein at 21 time points from 5 minutes prior to treatment to 72 hours post treatment. Blood removed from fetuses was replaced with blood from the mother or another ewe. Amniotic and fetal tracheal fluid samples were obtained from 5 minutes to 1 hour following treatment and at the time of blood collection beyond that time point. Maternal urine was collected every hour during the first 4 hours and with each blood sample 6 hours after dosing. Fluoxetine, norfluoxetine, and their glucuronide and sulfate conjugates were measured in samples by GC/MS. Statistical analyses included paired and unpaired t tests and two-way analysis of variance for repeated measures with *post hoc* test if necessary.

Following maternal administration of fluoxetine, maternal AUC for the S isomer of fluoxetine was significantly higher and clearance and volume of distribution were significantly lower compared to the R isomer. Half-lives of elimination were similar for the R and S isomer. Norfluoxetine did not

demonstrate stereoselective toxicokinetic differences in ewes. Fluoxetine and norfluoxetine rapidly crossed the placenta. Consistent with maternal findings, the AUC for the S isomer of fluoxetine was significantly greater compared to the R isomer in fetuses. Fetal half-lives of elimination for both the R and S isomers were significantly greater than maternal values. Although fetal elimination half-lives for R and S isomers of norfluoxetine did not differ significantly from maternal values, a stereoselective difference was noted by an S/R ratio significantly less than unity. Levels of fluoxetine and norfluoxetine in amniotic fluid and fetal tracheal fluid were slightly lower than fetal plasma levels, but there were no significant differences in levels of R and S isomers. Fluoxetine, norfluoxetine, and their glucuronides were detected in maternal urine. Together, parent drug and metabolites represented 3.4% of the administered dose. Urinary levels of fluoxetine and norfluoxetine did not plateau 72 hours following dosing, but the experiment was ended at that point due to ethical concerns about catheterizing the sheep for longer time periods.

Norfluoxetine or glucuronides of fluoxetine and norfluoxetine were not detected in fetal plasma following administration of fluoxetine to the fetus. Fetal levels of the S isomer were significantly higher than the R isomer and clearance for the S isomer was significantly lower. It was determined that placental clearance represented (mean ± SD) 89.4 ± 36.9% and 94.0 ± 37.3% of total fetal clearance for the R and S fluoxetine isomers, respectively. Fetal non-placental clearance values did not differ significantly from zero. In order to obtain more information about fetal versus maternal metabolic capability, two ewes were killed on GD 135 and 139 to obtain hepatic microsomes from ewes and fetuses. Incubation of the microsomal preparations with fluoxetine HCl resulted in norfluoxetine formation with maternal microsomes but not fetal microsomes.

*In vitro* and *ex vivo* protein binding of fluoxetine and norfluoxetine was also compared. A large portion of fluoxetine and norfluoxetine (~95%) was bound to plasma proteins. Stereoselective differences in binding were apparent in that S/R ratios for maternal and fetal fluoxetine values were significantly below unity. In both *ex vivo* and *in vivo* studies, the percentage of unbound fluoxetine was higher in fetuses compared to ewes.

In this study fetal blood gas and acid base status was also determined. Transient changes in fetal blood oxygenation, pH, and lactate levels were observed, but the effects will not be discussed here since they were stated to be similar to effects noted in an earlier study *(53*), which is summarized in detail in Section 3.2.1.4.

The study authors concluded that disposition of fluoxetine is stereoselective, most likely due to the differential plasma protein binding of the R and S isomers, and that sheep fetuses do not produce detectable level of norfluoxetine or glucuronides of fluoxetine or norfluoxetine.

*Strengths/Weaknesses:* Strengths of this study include extensive detail of experimental procedures and reporting of results. Data were generated from numerous samples collected over a 72-hour period. An *in vitro* method was used to verify *in vivo* observations of fetal metabolism. A weakness of the study is that the i.v. route of administration is not relevant to human exposures.

*Utility (Adequacy) for the CERHR Evaluative Process:* This study has utility in demonstrating maternal to fetal transfer of fluoxetine and metabolites, stereoselective differences in disposition, and lack

of fluoxetine metabolism by the fetus in a mammalian model.

### 2.2.3. Metabolism

Fluoxetine is N-demethylated to norfluoxetine by cytochrome P450 (CYP) enzymes (reviewed by Caccia *(54)*; see also Section 2.6.1). *In vitro* preparations of human microsomal enzymes (baculovirus-expressed) show a number of these enzymes to be active in the N-demethylation process. For R-, S-, and racemic fluoxetine, CYP2D6 produced the greatest clearance values (calculated from a pharmacokinetic model), followed in order by CYP2C9, CYP3A4, and CYP2C19 for R-fluoxetine and by CYP3A4, CYP2C9, and CYP2C19 for S-fluoxetine *(55)*. When the *in vitro* values were corrected to account for the prevalence of the CYP isoforms in human liver, CYP2C9, CYP3A4, and CYP2D6 were estimated to account for 43, 32, and 20% of the clearance of fluoxetine *in vivo*. Both fluoxetine and norfluoxetine are glucuronidated in the liver *(44)*. Another metabolite in humans is hippuric acid, a glycine conjugate of benzoic acid *(44)*. Further metabolic fates have not been well-characterized in humans.

In 13 adults 20–39 years old, norfluoxetine pharmacokinetic parameters were evaluated after administration of fluoxetine by mouth *(45)*. After 6 weeks of administration of fluoxetine 20 mg/day, norfluoxetine $C_{max}$ and $AUC_{0\text{-}24}$ were 165 ± 38 ng/mL and 3,635 ± 829 ng/mL•h, respectively (mean ± SD). After an additional 6 weeks of fluoxetine at 40 mg/day, norfluoxetine $C_{max}$ and $AUC_{0\text{-}24}$ were 306 ± 71 ng/mL and 7177 ± 1542 ng/mL•h, respectively (mean ± SD).

*Strengths/Weaknesses:* This study presents information in humans on chronic therapy, providing a better estimate of internal dose with respect to the usual therapeutic use of this medication.

*Utility (Adequacy) for CERHR Evaluation Process:* The Expert Panel found the metabolism of fluoxetine to norfluoxetine to be well characterized. The further metabolism of norfluoxetine is poorly understood, other than the conjugation pathways described above. Because both fluoxetine and norfluoxetine are pharmacologically active, the saturation of the demethylation pathway is of minor consequence for the primary mode of action (serotonin reuptake inhibition). Fluoxetine and norfluoxetine appear to inhibit several different CYP isoforms and can thereby affect metabolism, clearance, and blood levels of other medications the patient may be receiving. Fluoxetine and norfluoxetine can also inhibit the CYP isoforms that are responsible for fluoxetine/norfluoxetine metabolism (autoinhibition or "suicide" inhibition). Which CYP isoform is responsible for fluoxetine and norfluoxetine metabolism can depend on fluoxetine dose, with one CYP isoform being responsible for metabolism at low concentrations and another CYP isoform becoming dominant as concentrations within the body increase with repeated dosing. It is informative that the time required for patients to reach steady-state is on the order of 3 months and the time required for the patients to be considered "drug-free" is the same. The information on inhibition of CYP enzymes may relate primarily to interaction with other medications, not on the clinical effects of fluoxetine because the demethylated form is also pharmacologically active.

### 2.2.4. Elimination

In humans, about 80% of fluoxetine is excreted in the urine and 15% in stool. Urine excretory products consist of 11% fluoxetine, 7% fluoxetine glucuronide, 7% norfluoxetine, 8% norfluoxetine glucuronide, and 20% hippuric acid *(44)*. The plasma half-life of fluoxetine is 1–4 days and the half-life of norfluoxetine is 7–10 days. Renal impairment does not influence these half-lives, but hepatic failure increases the half-lives.

According to the product label for Prozac®, following chronic administration, the elimination half-lives for fluoxetine and norfluoxetine are increased to 4 – 6 and 4 – 16 days, respectively *(4)*. Accumulation of fluoxetine is expected to occur with chronic dosing, and active compound is described in the product label as present for "weeks" after termination of therapy.

In the study by Harvey and Preskorn *(45)*, after 12 weeks of fluoxetine therapy (6 weeks at 20 mg/day followed by 6 weeks at 40 mg/day), fluoxetine half-life was 3.9 ± 1.5 days and norfluoxetine half-life was 15.0 ± 6.5 days (mean ± SD), consistent with the product label.

*Strengths/Weaknesses:* The strengths of the Harvey and Preskorn study are discussed above. This study is considered reliable.

*Utility (Adequacy) CERHR Evaluation Process:* The Expert Panel found the very long half-lives in humans to be important. Exposure to fluoxetine or norfluoxetine during gestation in a woman on chronic therapy would be expected to occur unless the woman discontinued fluoxetine therapy 2 – 3 months (5 – 6 half-lives) before becoming pregnant.

## *2.3 General Toxicity*

### *2.3.1 Humans*

#### *2.3.1.1 Side effects of medication therapy*

Fluoxetine became widely used as an antidepressant soon after its introduction because of the impression that it produced fewer, milder side effects than did the TCA and MAOI antidepressants that previously were the mainstays of medication therapy for depression. The most common side effects are listed in Table 4 *(5)*. This table does not list sexual side effects, which are discussed in Section 4.1.4. Other reviews *(56)* report dermatologic side effects to be among the most common fluoxetine adverse effects, occurring in 13% of subjects in one study. These side effects include rash, urticaria, and a serum-sickness like illness (serum sickness is characterized by urticaria, edema, fever, lymphadenopathy, joint pain, and albuminuria, typically due to immune complexes arising from foreign protein administration).

***Table 4. Side Effects of Fluoxetine Therapy (Excluding Sexual Side Effects) (5)***

| *Side effect* | *Incidence (%)* |
|---|---|
| Nausea | 21 |
| Anxiety, Insomnia* | 15 |
| Diarrhea | 12 |
| Anorexia | 9 |
| Dyspepsia | 6 |
| Rash | 4 |
| Pruritus | 2 |

*Sufficient to result in stopping the medication

Effects of SRI therapy on weight are variable. Fluoxetine is more likely to produce appetite suppression and weight loss than to produce weight gain (reviewed by Goldstein and Goodnick *(56)*), leading

to off-label use of this medication in obesity treatments.

Case reports of abnormal bleeding during fluoxetine therapy have appeared (reviewed in Alderman et al. *(57)*), suggesting decreased platelet aggregation in response to serotonin reuptake inhibition. Seven patients receiving fluoxetine 20 mg/day were evaluated for platelet aggregation in response to adenosine diphosphate, arachidonic acid, collagen, epinephrine, or ristocetin without evidence of altered platelet function at 2 or 4 weeks of therapy *(57)*. These authors also published a case report of a 43 kg man who developed deficient platelet aggregation in response to the same stimulators while on fluoxetine 20 mg/day. The aggregation abnormality resolved on discontinuation of the fluoxetine therapy *(58)*. The authors postulated that the low body weight of this man may have led to unusually high fluoxetine or norfluoxetine concentrations; however, these concentrations were not measured.

Psychiatric side effects of fluoxetine therapy include nervousness, irritability, aggression, insomnia, lethargy, apathy, and akathisia (inability to stand or sit still) (reviewed by Goldstein and Goodnick *(56)*). The appearance of case reports of suicides on fluoxetine led to concern that suicidality might be increased by this medication, but controlled studies have shown suicidal thoughts and behaviors on fluoxetine to occur either less often or with the same frequency as on placebo or on TCAs (reviewed by Stokes and Holtz *(12)*). Mania has been reported on fluoxetine, but occurs with a low incidence (about 1%) and less often than with TCAs (reviewed by Goldstein and Goodnick *(56)*).



#### *2.3.1.2 Serotonin syndrome*

A syndrome attributed to excessive serotonergic neurotransmission results from an interaction of medications stimulating this system. This so-called serotonin syndrome can include confusion, hypomania, agitation, diarrhea, shivering, fever, diaphoresis, blood pressure effects, nausea, vomiting, myoclonus, hyperreflexia, incoordination, and tremor (reviewed by Goldstein and Goodnick *(56)*). The serotonin syndrome has been particularly severe in patients treated with SRIs and MAOIs, but has also been seen with SRIs combined with TCAs.

#### *2.3.1.3 Discontinuation symptoms*

An SRI discontinuation syndrome has been described consisting variably of dizziness, vertigo, ataxia, nausea, vomiting, lethargy, myalgia, chills, paresthesias, sleep disturbance, agitation, anxiety, and irritability (reviewed by Goldstein and Goodnick *(56)*; Haddad *(59)*). Symptoms may occur within the first 10 days after discontinuing therapy and persist for 3 weeks and are more common in people who have been on therapy for more than 2 months. Discontinuation symptoms are more common with shorter acting SRIs than with fluoxetine, for which the long elimination half-life and active metabolite result in a gradual taper off effect, but these symptoms have occasionally been described with fluoxetine.

#### *2.3.1.4 Overdosage*

The potential to commit suicide by overdosing on fluoxetine appears low. Stokes and Holtz *(12)* reviewed five deaths associated with fluoxetine overdosage. In three instances, other medications were coadministered, preventing assessment of the contribution of the fluoxetine to the death. In one case, fluoxetine was taken with ethanol. Blood ethanol concentration was 48 mM, and concentrations of fluoxetine and norfluoxetine were each 800 ng/mL. Only in the fifth case was fluoxetine overdose alone associated with death; this patient is estimated to have taken 1,200–2,000 mg of fluoxetine.

Goeringer et al. *(60*) examined 60 fatalities in which fluoxetine was measured in postmortem blood samples. The highest concentration of fluoxetine and norfluoxetine identified were 6.66 and 20.27 mg/L **[6,660 and 20,270 ng/mL].** This decedent also had measurable levels of trazodone, another antidepressant. The death was ruled as due to atherosclerotic cardiovascular disease, although the authors indicate that this cause was most likely incorrect. The only case they presented that was certified as a suicide due to fluoxetine overdose had fluoxetine and norfluoxetine blood concentrations of 3.67 and 0.38 mg/L **[3670 and 380 ng/mL]**, respectively.

Among 67 adults reporting overdose of fluoxetine alone to a poison control center, 30 had no symptoms after doses as high as 1,200 mg. In those adults with symptoms, 15 (22%) complained of tachycardia, 14 (21%) complained of drowsiness, 8 (12%) complained of nausea or vomiting, and 5 (7%) complained of tremor. Of 20 children with reported overdose, 18 were asymptomatic. A 2-year-old child who had taken 10 mg fluoxetine had hyperactivity and another 2-year-old who had taken an unknown amount became drowsy *(61*). A separate case report of a 4-year-old child who may have taken 7,000 mg fluoxetine found fluoxetine and norfluoxetine serum concentrations of 3080 and 423 ng/mL, respectively. The child demonstrated a brief period of unresponsiveness, sinus tachycardia, agitation, and dyskinesia, but was generally well and recovered completely *(62*).

**[The usefulness of the information provided from overdose cases for this exercise is limited. One important point would be that a pregnant woman could very well consume an overdose of fluoxetine and appear to recover completely. The effect of these high doses on the developing embryo would be unknown as the dose levels used in the animal studies are generally limited by overt maternal toxicity.]**

#### *2.3.1.5 Drug interactions*

In addition to being metabolized by CYP2D6, fluoxetine and norfluoxetine are also inhibitors of CYP2D6 *(63-66*). Fluoxetine inhibition of CYP2D6 can explain drug-drug interactions with TCAs, other SRIs, and some antipsychotic agents (e.g., haloperidol, thioridazine, perphenazine, clozapine, and risperidone). Other medications for which metabolism might be inhibited by fluoxetine and/or norfluoxetine include codeine (metabolic bioactivation to morphine), beta-blockers, and Type 1C antiarrhythmic agents (e.g., encainide, flecainide, and propafenone). Fluoxetine and norfluoxetine also are inhibitors of CYP2C enzymes, which metabolize diazepam, warfarin, tolbutamide, and phenytoin, and of CYP3A4, which metabolizes benzodiazepines, carbamazepine, cyclosporine, terfenadine, quinidine, erythromycin, and lidocaine and as such, can also contribute to drug-drug interactions through these mechanisms.

### *2.3.2. Experimental Animals*

According to the Prozac® product label, the median lethal oral dose is 452 mg/kg/day in rats and 248 mg/kg in mice *(4*). Acute high oral doses produce irritability and convulsions in "several species." In dogs, the lowest plasma concentration at which seizures occurred was twice the maximum plasma concentration seen in humans on chronic therapy with fluoxetine 80 mg/day.

**[The lack of study reports makes it impossible to judge to and interpret these studies.]**

tion with SRIs but not TCAs is inconsistent with animal studies and proposed biologic mechanisms that suggest an increased risk by both classes of drugs (see summary for Brandes et al. *(69)* study in Section 2.5.2). They noted that the putative association with SRIs was based on a very low number of cases and could have resulted by chance.

**[The studies presented in this section are limited and thus not useful for the CERHR evaluation process.]**

### *2.5.2 Experimental Animals*

Studies in experimental animals have examined fluoxetine's effects on tumor promotion and carcinogenicity.

Tutton and Barkla *(70)* examined the effects of fluoxetine treatment on cell proliferation and tumor growth. Fluoxetine treatment (10 mg/kg, i.p.) of Sprague Dawley rats (n = 6/group) with dimethylhydrazine-induced colonic tumors resulted in suppressed tumor cell division. In addition, fluoxetine treatment (10 or 20 mg/kg bw/day, i.p.) of immuno-deprived mice bearing xenografts (10 – 13 xenografts/group) of human adenocarcinoma colonic tumor cell lines resulted in slowed growth in 2 of the 3 cell lines **[time of fluoxetine treatment was not specified and data were not clearly presented in figures]**. The SRI citalopram was also tested and found to have effects similar to those of fluoxetine.

Abdul et al. *(71)* examined the effects of fluoxetine on three human prostatic carcinoma cells lines (PC-3, DU-145, and LNCaP). *In vitro* fluoxetine HCl treatment resulted in a dose-related inhibition of cell proliferation in all three cell lines, with a cytostatic effect noted at 10 μM. Higher concentrations were cytotoxic. Fluoxetine was also effective in blocking uptake of a radiolabeled serotonin analog in all three cell lines. Similar effects on growth and serotonin uptake inhibition were noted with two other antidepressants tested (zimelidine and 6-nitroquipazine), with fluoxetine reported to be the most potent drug. In an *in vivo* study, 6 athymic nude mice bearing SCPC-3 xenografts were subcutaneously (sc) injected with 40 μg/day fluoxetine for 6 weeks. Fluoxetine treatment significantly inhibited xenograft growth compared to control animals.

Brandes et al. *(69)* conducted a series of studies to determine if clinically relevant doses of fluoxetine (equivalent to ~20 – 80 mg/day in humans) or the TCA amitriptyline promote tumor growth or development in rodents. The studies were conducted due to both drugs' structural similarity to the anti-estrogen binding site histamine receptor ligand N,N-diethyl-2-[(phenylmethyl)phenoxy]ethan amine HCL, which stimulates tumor growth in *in vivo* studies. Fluoxetine treatment (40 mg/m$^2$) accelerated the formation of palpable tumors by about 30% in C3H mice (n = 10/group) injected with C-3 fibrosarcoma cells, with tumors first appearing at 3 versus 6 days following fibrosarcoma cell injection in the fluoxetine- and saline-treated animals, respectively. An *in vitro* study demonstrated that accelerated tumor formation was correlated with a fluoxetine-induced increase in DNA incorporation of $^3$H-thymidine in C-3 cells. Fluoxetine treatment (12 or 20 mg/m$^2$) in C57Bl mice (n = 10/group) s.c. injected with B16f10 melanoma cells resulted in larger tumors compared to saline-treated controls at day 17. No difference in survival between the fluoxetine and saline groups was noted with intravenous (i.v.) injection of melanoma cells. Fluoxetine treatment (11.5 or 28.5 mg/m$^2$) reduced latency of mammary tumor formation by 30 – 40% in Sprague-Dawley rats (n = 7 – 8/group) treated with dimethylbenzanthracene; 15 weeks following DMBA treatment there were 5 tumors in 4 of 7

rats in the saline group, 12 tumors in 7 of 7 rats in the 11.5 mg/m$^2$ fluoxetine group, and 13 tumors in 8 of 8 rats in the 28.5 mg/m$^2$ fluoxetine group. **[For both mouse and rat studies, there were some discrepancies between fluoxetine doses presented in the methods section vs. figures in the results section.]** Similar promotion effects were noted with amitriptyline.

A 2 year carcinogenicity study in C57BL/6 × C3H $F_1$ mice and Fischer rats was conducted by Bendele et al. *(72)* according to Good Laboratory Practice (GLP). Sixty rats/sex/group received 0, 0.5, 2.0, or 10 mg/kg bw/day fluoxetine HCl and 60 mice/sex/group received 0, 1.0, 5.0, or 10 mg/kg bw/day fluoxetine HCl through diet. The only detailed data presented were histopathologic findings of neoplasia, because the purpose of the report was to communicate carcinogenicity findings. Increased mortality related to CNS pharmacologic effects was observed in mice but greater than 50% survival was achieved in all groups of animals. Decreased body weight gain related to reduced food intake was observed in rats in the 10 mg/kg bw/day group. The only significant histopathologic finding related to treatment in rats was reported to be multifocal pulmonary histiocytosis related to phospholipid accumulation in males and females primarily from the 10 mg/kg bw/day group. In mice, the only treatment-related histologic effects were reported as minimal-to-moderate hepatic fatty changes in females from the 5 and 10 mg/kg bw/day groups and increased incidence and prominence of hepatocellular cytomegaly in males exposed to ≥5 mg/kg bw/day and females exposed to 10 mg/kg bw/day. **[Non-neoplastic histology data were not presented for rats or mice.]** No increased incidence of neoplasms was noted in either rats or mice. A significant dose-related decrease was observed for incidences of pituitary adenomas in male and female rats, mammary adenomas, and fibroadenomas in female rats, hepatocellular carcinomas in male mice, and pituitary adenomas in female mice. The antineoplastic findings were not replicated in a second study conducted with 60 mice per group.

The Panel notes the ongoing debate regarding the relevancy of the tumor promotion study by Brandes et al. *(69)* and the carcinogenicity study by Bendele et al. *(72)*. Based on findings of tumor promotion following fluoxetine and amitriptyline treatment, Brandes et al. *(69)* stated that epidemiologic studies should be conducted to determine the effects of antidepressants in cancer development and that tumor promotion should be studied in addition to carcinogenicity in drug screening procedures. Bendele et al. *(72)* stated that the lifetime rodent test allows for the evaluation of carcinogenic initiation as well as promotion of spontaneously occurring neoplasms and remains the most appropriate model to assess a chemical's effects in humans.

**[Reconciliation of opposing viewpoints about tumor promotion is beyond the scope of this exercise. One important point to investigate for this exercise would be the expression of the gene for the anti-estrogen binding site histamine receptor during development. If the gene is expressed during development or postnatal maturation, adverse effects could potentially occur in some organs as a result of fluoxetine exposure.]**

## *2.6 Potentially Sensitive Subpopulations*

### *2.6.1. Pharmacogenetics of Fluoxetine Metabolism*

Fluoxetine and norfluoxetine undergo oxidation followed by conjugation. The steps involved in oxidation and conjugation of these compounds and possible differences among populations in the responsible enzymes have not been well-characterized. Rather, attention has been drawn to variations

within the population in CYP enzymes that catalyze N-demethylation of fluoxetine to norfluoxetine. These enzymes may also play a role in further oxidation steps. The most important of these enzymes appears to be CYP2D6, previously known as debrisoquine hydroxylase or sparteine hydroxylase, discussed below. CYP2C19 also has been reported to be important in N-demethylation of fluoxetine to norfluoxetine *(73*). Individuals with inactivating mutations for CPY2C19 were found to have higher fluoxetine and lower norfluoxetine concentrations than individuals with the wild type enzyme. Inasmuch as fluoxetine and norfluoxetine are both pharmacologically active, it is not clear whether CYP2C19 polymorphisms have implications for fluoxetine toxicity.

The gene for CYP2D6 is located on the long arm of human chromosome 22. Polymorphisms for CYP2D6 are associated with at least 12 variants that alter enzyme activity (reviewed by DeVane *(74*) Gaedigk et al. *(75*) and Bertilsson et al. *(76*)). People with the usual CYP2D6 activity are called extensive metabolizers and people with lower levels of activity are called poor metabolizers. Poor metabolizer phenotypes occur in 5 – 8% of whites and 2 – 10% of blacks and Asians. There is considerable variation within racial groups; for example, there is a higher incidence in African Americans (8.5%) than in Zimbabweans (1.8%) of one of the inactive CYP2D6 alleles and up to 29% of Ethiopians carry duplicated or multiduplicated CYP2D6 alleles. The consequences of poor metabolizer status on fluoxetine and norfluoxetine kinetic parameters are shown in Table 5 (from Hamelin et al. *(77*)). Gene duplication in CPY2D6 may also be associated with increased enzyme activity, perhaps accounting for failure of fluoxetine to be effective at the usual doses in some patients.

***Table 5. Kinetic Parameters for Fluoxetine and Norfluoxetine After a Single 20 mg Fluoxetine Dose in Extensive and Poor Metabolizers of Debrisoquine (taken as a measure of CYP2D6 activity). [Hamelin et al. (77)]***

| *Parameter* | *Fluoxetine* | | *Norfluoxetine* | |
|---|---|---|---|---|
| | *Extensive Metabolizer (n=9)* | *Poor Metabolizer (n=10)* | *Extensive Metabolizer (n=9)* | *Poor Metabolizer (n=10)* |
| $C_{max}$ (µg/L) | 14 ± 3[a] | 22 ± 5* | 11 ± 3 | 5 ± 1* |
| $t_{max}$ (h) | 6 ± 2 | 7 ± 1 | 44 ± 32 | 79 ± 39* |
| $AUC_{0\rightarrow\infty}$ (µg/L·h) | 481 ± 245 | 1,871 ± 328* | 1,579 ± 396 | 736 ± 148* |
| Elimination rate constant ($h^{-1}$) | 0.03 ± 0.01 | 0.009 ± 0.002* | – | – |
| Half-life (h) | 24 ± 7 | 76 ± 14* | – | – |
| Drug excreted in urine (µg) | 225 ± 89 | 719 ± 208* | 1,047 ± 292 | 524 ± 173* |
| Renal clearance (L/h) | 0.7 ± 0.4 | 0.5 ± 0.2 | – | – |
| Clearance of fluoxetine to norfluoxetine (L/h) | – | – | 4.3 ± 1.9 | 0.4 ± 0.1* |

*$p < 0.05$ compared to extensive metabolizer
[a] Data are means ± SD

Based on Table 5, poor metabolizer status would be expected to confer increased risk of dose-related fluoxetine toxicity but decreased risk of norfluoxetine dose-related toxicity; however, norfluoxetine levels may not be decreased in poor metabolizers on chronic fluoxetine therapy due to compensatory

alternative mechanisms of fluoxetine demethylation. There is a case report of a fluoxetine-exposed 9-year-old boy with an inactive CYP2D6 genotype who died with symptoms suggesting fluoxetine intoxication *(78*). **[Although the child's poor metabolizer status may have contributed to his death, he was also on an unusually high dose of fluoxetine (100 mg/day) and was taking other medications (clonidine, methylphenidate, and promethazine).]** The child had very high postmortem blood concentrations of both fluoxetine and norfluoxetine (each 21,000 ng/mL, about 1,000 times the usual concentration found in the blood of adults on therapy), demonstrating that norfluoxetine could be produced even in the absence of functioning CYP2D6. Indeed, *in vitro* studies using human microsome preparations did not show complete inhibition of fluoxetine N-demethylation when quinidine, a CYP2D6 inhibitor, was added to the incubation *(55*).

**[Given all of the confounding variables in the clinical case presented by Sallee *(78*), it is not at all clear whether the ability of the child to metabolize fluoxetine or norfluoxetine had any bearing on the outcome. The child was receiving 100 mg fluoxetine/day (4 mg/kg bw/day) for approximately 10 months prior to his death. This dose would translate to a 280 mg daily dose for a 70 kg adult. The authors of the case report considered the blood measures from samples collected at autopsy questionable because the measured values may represent drug that fluxed from tissue back into the blood prior to sample collection. The fluoxetine and norfluoxetine levels were approximately equal (21,000 ng/mL). While it is clear from overdose cases that fluoxetine and norfluoxetine levels can reach extremely high levels with minimal-to-no clinical consequence, the exposure of this child was clearly in excess of other reported cases in children.]**

Polymorphisms have been described in the serotonin transporter. These polymorphisms have thus far been characterized as influencing the response of depression to SRI treatment rather than influencing toxicity potential *(79*). However one recent preliminary study in 36 Caucasian adult subjects taking up to 60 mg fluoxetine suggests that a short allele in the serotonin transporter gene-linked polymorphic region (5HTTLPR) may be associated with increased adverse effects from fluoxetine treatment *(80*). In the 9 subjects homozygous for the short 5HTTLPR allele, 78% experienced onset or worsening of insomnia and 67% developed agitation. In the 27 non-homozygous subjects, 22% experienced development or worsening of insomnia and 7% became agitated. Study design limitations noted by study authors included small sample size, no structured assessment of adverse effects, and an inability to distinguish agitation from akathisia. The study authors noted that these preliminary findings need to be confirmed in larger studies.

**[According to the Panel, if the basis for defining a sensitive subpopulation is determined by the pharmacologic activity of fluoxetine, then the difference between the "slow" and "extensive" metabolism populations is expected to make little difference in sensitivity, because the primary metabolite (norfluoxetine) is also active for inhibition of serotonin uptake. If the sensitive population is defined by a toxicity characteristic that is separate from the pharmacologic activity, then there may well be a difference between fluoxetine and norfluoxetine and the "slow" vs. "extensive" metabolism argument could make a difference. However, the Panel found no evidence of increased sensitivity due to a toxicity characteristic in the studies they reviewed. The Panel found no studies describing toxicity differences between fluoxetine and norfluoxetine, although there was one paper describing different interactions of norfluoxetine and fluoxetine with a specific receptor (see Section 2.1). Given the extensive metabolism of fluoxetine to norfluoxetine (even in the "slow"**

**group for metabolism), toxicity studies in effect examine a combination of these two chemicals. Overall, while the difference between "poor" and "extensive" metabolizers may account for a differing ratio of these two chemicals in the blood, it appears to have little consequence as far as the pharmacologic action or adverse clinical outcome.]**

### *2.6.2 Sex*

Women have a higher incidence of depression than do men, and there is evidence of differences between men and women in pharmacokinetic parameters for some antidepressants (reviewed by Frackiewicz et al. *(81)*). Differences in fluoxetine toxicity by sex have not been characterized.

### *2.6.3 Children*

Antidepressant medications, including SRIs, are used in children. Use of these agents has produced concern based on the fact that neurotransmitter systems are developing in children (reviewed by Vitiello and Jensen *(82)*). Theoretical concerns about SRI therapy in children were reviewed by Murphy et al. *(83)*. These authors believe that children may be particularly vulnerable to activation, hypomania, and irritability as side effects of SRIs; however, the reports on which they base their concern were anecdotal and possibly a reflection of the use in children of the usual adult dose of fluoxetine rather than a reduced dose. Possible adverse developmental effects of fluoxetine in children are discussed in Section 3.1.3.

**[The Panel concluded that in terms of the pediatric population, the pharmacokinetic evidence suggesting this group to be a sensitive subpopulation is easily understood based on weight differences. The data available to determine sensitivity based on a pharmacodynamic difference are not available.]**

## *2.7 Summary of General Toxicology and Biologic Effects*

### *2.7.1 Pharmacodynamics*

Fluoxetine, a racemic mixture of R- and S-enantiomers, is a compound known as a serotonin reuptake inhibitor. Serotonin is 5-hydroxytryptamine, a neurotransmitter that plays a role in regulation of mood, sleep, sexual activity, motor activity, neuroendocrine function, cognition, and depression *(2, 12, 33)*. Cell bodies of serotonergic neurons are found primarily in the midline of the brainstem, but axonal projections are widespread throughout the brain. Serotonergic neurons synthesize and release serotonin into the synaptic cleft upon nerve impulse. Upon release, serotonin may activate one of several postsynaptic serotonin receptor subtypes. The action of serotonin is terminated when it binds to the presynaptic transporter for reuptake into the presynaptic nerve terminal and conversion to 5-hydroxyindoleacetic acid (5-HIAA) by monoamine oxidase. The serotonin transporter is blocked by fluoxetine, norfluoxetine, as well as other SRIs, leading to a 1.5- to 4-fold increase of serotonin in the synaptic cleft *(2)*. SRIs also block the serotonin transporter in blood platelets.

Although inhibition of serotonin uptake occurs within minutes-to-hours following treatment with fluoxetine, antidepressant effects occur several weeks later. It is postulated that initial increases in serotonin levels in raphe nuclei lead to overactivity of serotonin autoreceptors and attenuate serotonin neuronal firing *(2, 12, 35)*. Repeated dosing with fluoxetine is postulated to lead to a compensatory down-regulation of serotonin receptors and restored neuronal firing that results in an augmentation

of serotonin release and neurotransmission within 14 days. Fluoxetine is also known to induce hippocampal neurogenesis according to a timetable coincident with symptom reduction in animal models of depression and anxiety *(38)*.

Fluoxetine and its major metabolite, norfluoxetine, have high affinity for the serotonin transporter and selectively bind to the transporter according to a saturable process requiring sodium *(2)*. In contrast, fluoxetine has low affinity for norepinephrine uptake sites and neurotransmitter receptors such as $\alpha_1$-adrenergic, $\alpha_2$-adrenergic, β-adrenergic, dopaminergic, muscarinic, histaminergic, $H_1$, opiate, GABA, and benzodiazepine receptors. Fluoxetine also has relatively low affinity for most serotonin receptors including 5-$HT_{1A,B,D}$ , 5-$HT_{2A}$, and 5-$HT_3$. Although SRIs have low affinity for the norepinephrine transporter, they reduce activity of dopamine hydrolase, which is involved norepinephrine synthesis *(33)*.

### *2.7.2 Pharmacokinetics and Metabolism*

#### ***2.7.2.1 General Pharmacokinetics and Metabolism***

Fluoxetine is absorbed following oral intake in humans and maximum blood levels are reported to be proportional to dose following intake of 20 – 80 mg *(44)*. Single oral doses of 20 and 40 mg fluoxetine were reported to result in peak plasma fluoxetine levels of ~9 – 11 ng/mL *(43, 45)* and 15 – 55 ng/mL *(4)*, respectively. Time to reach maximum plasma levels was reported at 6 – 8 hours for a 40 mg dose. Maximum fluoxetine plasma levels were reported to be similar in young and elderly individuals *(45)*. Bioequivalent forms of fluoxetine are available as pulvules, tablets, oral solution, and weekly capsules, although the coating on the weekly capsule delays onset of absorption by 1 – 2 hours *(4)*. Food does not affect systemic bioavailability but may delay absorption by 1 – 2 hours *(4, 44)*.

The volume of distribution for fluoxetine in humans was reported at 20 – 42 L/kg *(44)*. In humans, fluoxetine is 94.5% bound, mostly to albumin and $\alpha_1$-glycoprotein *(4)*. The mean brain-to-plasma ratio (± SD) of fluoxetine + norfluoxetine was estimated at 10 ± 6 in 4 subjects taking 10 – 40 mg/day fluoxetine *(47)*. One study estimated that the time to reach steady-state concentrations of fluoxetine is 8.5 weeks in non-elderly subjects due to the long half-life of the drug *(45)*; the half-life in elderly subjects was reported to be 25% longer. $AUC_{0-24}$ values after an additional 6 weeks of fluoxetine 40 mg/day were 134 ± 83, 1,723 ± 475, and 5,730 ± 1,320 ng•h/mL, respectively *(45)*. A 6-week administration of fluoxetine 20 mg/day to adults aged 20-39 years resulted in a norfluoxetine $AUC_{0-244}$ value of 3,635 ± 829 ng•h/mL, and after an additional 6 weeks of fluoxetine at 40 mg/day, norfluoxetine $AUC_{0-24}$ was 7,177 ± 1,542 ng•h/mL *(45)*.

Plasma levels of fluoxetine and norfluoxetine were reported for adults and children following repeated dosing; those values are summarized in Table 6. The Expert Panel noted that original data, analytical methodology, and ranges of values were not available for information referenced from the Prozac® product label *(4)* and obtained from the FDA Clinical Pharmacology and Biopharmaceutics Review *(46)* discussing effects in children. As noted from Table 6, blood levels of fluoxetine and norfluoxetine differ between pre-adolescents, adolescents, and adults. An FDA review *(46)* concluded that the age-related differences in blood levels are due to body weight, but the CERHR Expert Panel noted that there is considerable variation among individuals within the same age group and receiving the same dose. Up to 50% of variance could not be explained by body weight alone and the reason for

the variance is unknown. Average steady-state fluoxetine concentration for the once-weekly regimen is reported to be 50% lower compared to the daily regimen *(4)*.

***Table 6. Plasma Levels of Fluoxetine or Norfluoxetine in Humans Following Repeat Dosing***

| ***Subjects*** | ***Dose and Treatment Duration*** | ***Plasma Fluoxetine Level (ng/mL)*** | ***Plasma Norfluoxetine Level (ng/mL)*** | ***Reference*** |
|---|---|---|---|---|
| Children (ages 8–12) | 20 mg/day for ≥4 weeks | 144.8±76.4 | 167.2±59.6 | FDA *(46)* |
| Adolescents (13–17) | 20 mg/day for ≥4 weeks | 78.8±49.4 | 113±41.4 | |
| Adults (ages 20–39) | 20 mg/day for 6 weeks | 83.9±22.2 | 165±38 | Harvey and Preskorn *(45)* |
| | 20 mg for 6 weeks, then 40 mg for 6 weeks | 276±56 | 306±71 | |
| Adults | 40 mg for 30 days | 91–302 | 72–258 | Lilly *(4)* |

In experimental animals, fluoxetine is widely distributed with highest concentrations in lung and liver *(44)*. Steady-state volume of distribution in rats administered fluoxetine i.v. is about 16–20 L/kg *(48)*. In rats gavage-dosed with 5, 10, or 20 mg/kg fluoxetine, maximum plasma levels of fluoxetine were estimated at 32, 64, and 128 ng/mL, respectively, and maximum plasma levels of norfluoxetine were estimated at 120, 239, and 359 ng/mL, respectively *(48)*. However, the Expert Panel noted that the estimates were somewhat uncertain due to unexplained normalization procedures used in the study. Half-lives for fluoxetine and norfluoxetine were estimated at 7–13 hours and 14–16 hours, respectively.

In humans, fluoxetine is N-demethylated to norfluoxetine by CYP enzymes *(44)*. CYP enzymes involved in metabolism of fluoxetine in humans include CYP2D6, CYP2C9, CYP3A4, and CYP2C19, *(55)*. Both fluoxetine and norfluoxetine are glucuronidated in the liver *(44)*. Another metabolite in humans is hippuric acid, a glycine conjugate of benzoic acid *(44)*. Further metabolic fate has not been well-characterized in humans.

A study in rats demonstrated that plasma and brain levels of norfluoxetine varied exponentially between doses of 2.5 and 20 mg/kg administered by i.p. injection, thus demonstrating saturable metabolism *(49)*.

In humans, about 80% of an administered fluoxetine dose is excreted in the urine and 15% in stool. Urine excretory products consist of 11% fluoxetine, 7% fluoxetine glucuronide, 7% norfluoxetine, 8% norfluoxetine glucuronide, and 20% hippuric acid *(44)*. Half-lives for fluoxetine and norfluoxetine were reported at 1–6 days and 4–16 days, respectively *(4, 44, 45)*. Hepatic failure but not renal impairment is expected to increase the half-life of fluoxetine *(44)*. Accumulation of fluoxetine

is expected to occur with chronic dosing, and active compound is described in the product label as present for "weeks" after termination of therapy. The Panel noted that a woman on chronic therapy would have to discontinue fluoxetine therapy 2–3 months (5–6 half-lives) before becoming pregnant in order to avoid exposure to drug or metabolite during pregnancy.

***2.7.2.2 Pharmacokinetics in pregnant humans or experimental animals***

A limited amount of information is available on the distribution of fluoxetine in pregnant humans and rats. In pregnant women (36–37 weeks gestation) taking 20–40 mg/day fluoxetine, trough plasma levels of fluoxetine and norfluoxetine were measured at 47±33 ng/mL and 109±22 ng/mL, respectively *(19*). Study authors noted that plasma fluoxetine levels in pregnant women were considerably lower than typical levels observed in individuals who are not pregnant. However, the Expert Panel noted that a direct comparison to other studies is complicated by the variability of doses and duration of treatment in pregnant women. Two weeks into the postpartum period, blood levels of fluoxetine were increased (105±51 ng/mL). It was postulated that decreased fluoxetine-plasma level during pregnancy could be due to increased liver blood flow and volume of distribution, and decreased protein binding. Measurement of fluoxetine and norfluoxetine levels in cord blood and breast milk demonstrated that the drug and metabolite are transferred across the placenta and into breast milk. Numerous other studies have demonstrated the presence of fluoxetine in cord blood or newborn infants *(15-18*) and in milk *(22, 25-28*). Detailed discussions of fluoxetine and norfluoxetine levels in infants and milk are included in Sections 1.2.3.2 and 1.2.3.3.



Placental transfer of fluoxetine and norfluoxetine was demonstrated in rats on GD 12 (during organogenesis) and GD 18 (post-organogenesis) following dosing of dams with 12.5 mg/kg radiolabeled fluoxetine *(50*). The study demonstrated that 63–80% of the radiolabel in embryo or fetus was in the form of fluoxetine/norfluoxetine, that the time course of radiolabel in the fetus is similar to that in maternal plasma, and that fetal thymus and brain contained the highest amount of radiolabel. Detection of most radiolabel as fluoxetine/norfluoxetine in the fetus suggests that humans and rat fetuses are exposed to similar chemical moieties and eliminates some of the uncertainty regarding metabolic differences between species.

Placental transfer of the enantiomers of fluoxetine and norfluoxetine have been investigated in sheep *(52*). The R- and S-enantiomers of fluoxetine and norfluoxetine were administered to the maternal or fetal venous circulation between GD 124 and 137 (gestation length = 145 days). The AUC of the S isomer of fluoxetine was significantly higher and the volume of distribution and clearance significantly lower in both the maternal and fetal compartments when compared to the R isomer of fluoxetine. This difference was probably related to differences in binding to plasma proteins between the R- and S-enantiomers of fluoxetine demonstrated in this experiment. Elimination half-lives in the maternal and fetal compartments were similar with the R and S isomers of fluoxetine. Norfluoxetine did not demonstrate stereoselective differences in kinetics. Placental transfer was rapid for both enantiomers of fluoxetine and norfluoxetine and was the primary means (90%) of elimination of the drugs from the fetal circulation. Based on both *in vivo* and *in vitro* experiments, it was apparent that the fetal compartment in sheep was unable to metabolize fluoxetine to norfluoxetine, and in *in vivo* experiments, no conjugation of either fluoxetine or norfluoxetine was observed.

### *2.7.3 General Toxicology and Biologic Effects*

#### ***2.7.3.1 Human Data***

Non-reproductive side effects associated with fluoxetine use by adults are summarized in this section. Side effects in children are summarized in Section 3.1.3 and reproductive side effects are summarized in Section 4.1.

Fluoxetine side effects are perceived to be milder than those of TCAs and MAOIs. The most common side effects in order of higher to lower prevalence include nausea, anxiety/insomnia, diarrhea, anorexia, dyspepsia, rash, and pruritus *(5*). Fluoxetine can produce variable effects on body weight, but appetite suppression and weight loss are most commonly reported *(56*). Several case reports of abnormal bleeding in patients treated with fluoxetine suggested that fluoxetine may decreased platelet aggregation in response to serotonin reuptake inhibition *(57*). In one case report, a man taking 20 mg/day fluoxetine experienced abnormal platelet aggregation that resolved following discontinuation of therapy *(58*), but there was no evidence of altered platelet function in one study of 7 patients taking 20 mg/day fluoxetine for 2 or 4 weeks *(57*).

Psychiatric side effects reported with fluoxetine use include nervousness, irritability, aggression, insomnia, lethargy, apathy, and akathisia (inability to stand or sit still) *(56*). A low incidence of mania (about 1%) has also been reported with fluoxetine use *(56*). There are several case reports of suicides committed by patients on fluoxetine, but according to a review by Stokes and Holtz *(12*), controlled studies demonstrated suicidal thoughts and behaviors in fluoxetine treated patients to occur less often or with the same frequency as patients taking placebo or TCAs.

A serotonin syndrome attributed to excessive serotonergic neurotransmission results from interaction of medications stimulating this system (e.g., co-treatment with SRIs and MAOIs or TCAs) *(56*). Symptoms of the syndrome include confusion, hypomania, agitation, diarrhea, shivering, fever, diaphoresis, blood pressure effects, nausea, vomiting, myoclonus, hyperreflexia, incoordination, and tremor *(56*).

Discontinuation symptoms that can occur within the first 10 days of ending therapy and persist for up to 3 weeks have been reported for fluoxetine but occur more often with SRIs with shorter half-lives. Symptoms can variably include dizziness, vertigo, ataxia, nausea, vomiting, lethargy, myalgia, chills, paresthesias, sleep disturbance, agitation, anxiety, and/or irritability *(56, 59*).

Fluoxetine and norfluoxetine are inhibitors of CYP2D6 and fluoxetine is an inhibitor of CYP2C and CYP3A4 *(63-66*). These enzymes are involved in the metabolism of other drugs as described in Section 2.3.1.5. The Panel noted that fluoxetine can affect the metabolism and clearance of other drugs, thus impacting the desired drug action or resulting in adverse reactions.

Possible symptoms that can occur following fluoxetine overdose in adults and children, include tachycardia, drowsiness, nausea or vomiting, hyperactivity, unresponsiveness, agitation, dyskinesia and/or tremor *(61, 62*). Dosing information is incomplete, but the limited amount available indicates that response to fluoxetine varies, with no symptoms occurring in some patients ingesting up to 1,200 mg fluoxetine *(61*). Symptoms of unresponsiveness, sinus tachycardia, agitation, and dyskinesia were reported in a 4-year-old child who later made a full recovery; fluoxetine intake was estimated at 7,000

mg and resulted in blood levels of 3,080 ng/mL fluoxetine and 423 ng/mL norfluoxetine *(62)*. In one case presented as a suicide due to fluoxetine overdose, the blood level of fluoxetine and norfluoxetine was measured at 3,670 and 380 ng/mL, respectively *(60)*. The Expert Panel noted that there is no known information on embryo or fetal effects following a fluoxetine overdose by the mother during pregnancy.

#### *2.7.3.2 Experimental animal data*

According to the Prozac® product label, the median lethal oral dose in rats and mice, respectively, is 452 and 248 mg/kg *(4)*. Irritability and convulsions were reported in "several species" administered high acute oral doses and seizures were reported in dogs with plasma concentrations twice those of humans on chronic therapy with 80 mg/day fluoxetine.

### *2.7.4 Genetic Toxicity*

The product label for Prozac® indicates that fluoxetine and norfluoxetine tested negative in genotoxicity tests including a bacterial mutation assay, a DNA repair assay in cultured rat hepatocytes, a mouse lymphoma assay, and a sister chromatid exchange assay in Chinese hamster bone marrow cells *(4)*.

### *2.7.5 Carcinogenicity*

The Expert Panel found that studies on carcinogenicity in humans exposed to fluoxetine were limited and were not optimal for use in assessing this endpoint. Two-year dietary GLP carcinogenicity studies were conducted in C57BL/6 mice dosed with 1.0 – 10 mg/kg bw/day and Fischer rats dosed with 0.5 – 10 mg/kg bw/day fluoxetine and the findings of histopathologic neoplasia were reported in a published study *(72)*. Effects reported in rats receiving 10 mg/kg bw/day included decreased body weight gain related to reduced food intake and multifocal pulmonary histiocytosis related to phospholipid accumulation. In mice, increased mortality related to pharmacologic CNS effects occurred at an unspecified dose and hepatic fatty changes and cytomegaly were noted with exposure to ≥5 mg/kg bw/day. No increased incidence of neoplasms was noted in mice or rats dosed with up to 10 mg/kg bw/day.

Mixed results were obtained in fluoxetine tumor promotion studies *(69-71)*. There has been ongoing debate regarding the relevancy of tumor promotion studies and the need for such studies in drug screening procedures. However, resolution of the issue is beyond the scope of the CERHR review on developmental and reproductive toxicity.

### *2.7.6 Potentially sensitive subpopulations*

No information is available on processes of fluoxetine and norfluoxetine oxidation and conjugation and how variable activity of metabolic enzymes involved in such reactions could affect susceptibility to fluoxetine. The majority of publications focus on variations in CYP enzymes involved in the conversion of fluoxetine to norfluoxetine, most notably CYP2D6 and CYP2C19. Individuals with inactivating mutations of *CYP2C19* have been identified *(73)*. Polymorphisms for *CYP2D6* are associated with at least 12 variants that alter enzyme activity *(75)*. Poor metabolizer phenotypes are estimated to occur in 5 – 8% of whites and 2 – 10% of blacks and Asians with considerable variations within racial groups. *CYP2D6* gene duplication can also result in increased enzyme activity.

The Panel noted that polymorphisms in CYP enzymes might affect the ratios of fluoxetine and norfluoxetine in blood. However, the Panel noted that since the drug and metabolite both inhibit serotonin uptake, there may not be differences in sensitivity based on pharmacologic action. If fluoxetine and norfluoxetine exhibited toxic characteristics separate from pharmacological activity, then differences in sensitivity among "slow" and "extensive" metabolizers might be expected. However, the Panel found no evidence of increased sensitivity related to a toxic characteristic and is not aware of studies that describe toxicity differences between fluoxetine and norfluoxetine. The Panel concluded that polymorphisms in CYP enzymes likely have little consequence in terms of desired pharmacologic action or adverse clinical outcome associated with fluoxetine.

Polymorphisms identified in the serotonin transporter may influence response to SRI treatment *(79)*. In addition, a small, preliminary study suggested that individuals who are homozygous for a short 5HTTLPR allele may be more prone to insomnia and agitation following fluoxetine treatment, but results need to be verified in a larger study *(80)*.

Concerns have been raised about use of SRIs and other antidepressants in children because their neurotransmitter systems are developing *(82)*. Based on a series of anecdotal reports, Murphy et al. *(83)* concluded that children may be particularly vulnerable to activation, hypomania, and irritability during SRI therapy. The Panel noted that pharmacokinetic data suggest that children may be more vulnerable based on lower body weights, but there are no data to determine sensitivity based on pharmacodynamic differences. Potential developmental effects in children exposed to fluoxetine are discussed in Section 3.1.3.

# 3.0 DEVELOPMENTAL TOXICITY DATA

## *3.1 Human Data*

### *3.1.1. Exposure During Prenatal Development*

#### *3.1.1.1. Case reports*

The first case of neonatal toxicity attributed to maternal fluoxetine use was published in 1993 *(15)*. A male infant weighing 3580 g was born at 38 weeks gestation to a 17-year-old mother who took fluoxetine 20 mg/day throughout most of the pregnancy. The infant was initially hypoglycemic (capillary blood sugar 33 mg/dL) and was given oral dextrose. Capillary blood sugar values were normal over the next 4 hours. Symptoms developed at 4 hours of age and were characterized by acrocyanosis, jitteriness, and tachypnea with a respiratory rate of 70. At 8 hours of age, the child had temperature instability and became increasingly jittery with stuffy nose and poor suck. There was opisthotonic positioning of the head with roving eye movements and increased tone. Evaluation for sepsis and illicit drug exposure was negative. Symptoms peaked at 36 hours of age and began to decrease at 83 hours of age, with complete resolution by 96 hours of age. Cord blood fluoxetine was 26 ng/mL (adult therapeutic range 40–250 ng/mL) and norfluoxetine was 54 ng/mL (adult range on therapy 30–325 ng/mL). At 96 hours of age, the infant's fluoxetine level was undetectable (< 25 ng/mL) and norfluoxetine was 55 ng/mL.

In 1995, Venditelli et al. *(84)* presented a case of lipomeningocele, confirmed at in a 1-month-old child with first trimester exposure to fluoxetine. The mother was also taking alprazolam, vitamins $B_1$ and $B_6$, and heptaminol, but doses were not specified.

In 1997, Mhanna et al. *(16)* reported a 3020-g infant delivered at term to a woman who was treated with 60 mg fluoxetine per day. The infant was jittery and hypertonic with grunting, flaring, and retractions. There were petechiae on the face and trunk, a cephalohematoma, a subdural hematoma, and a nondisplaced clavicular fracture. Sepsis work-up and toxicology evaluations were negative. Serum fluoxetine and norfluoxetine on the second day of life were 129 and 227 ng/mL, respectively, levels that are within the usual adult range. The jitteriness was improved at 2 weeks and examination at 5 months was normal. **[The authors attribute the petechiae and subdural hematoma to possible bleeding abnormalities associated with fluoxetine. The Expert Panel notes that these findings plus the clavicular fracture could be evidence of traumatic delivery.]**

In 2000, Mohan and Moore *(17)* described a 3270-g male delivered to a woman who took 40 mg fluoxetine per day. The infant was delivered at 35 weeks gestation with tachypnea and respiratory distress at 4 hours of age. The tachypnea resolved, but between 24 and 36 hours of age, jitteriness, agitation, and seizure-like activity (with negative electroencephalography) occurred. There was an erythematous rash on the cheeks with petechiae on the abdomen, chest, and extremities. There was opisthotonic positioning of the head and increased tone. Jitteriness and tremulousness decreased by 144 hours of age with resolution over the next 48 hours. A neurodevelopmental examination at 4 months of age was normal. Fluoxetine and norfluoxetine levels at 96 hours of age were 92 and 34 ng/mL, respectively, within the expected adult range. The mother had reported fluttering movements *in utero*, leading the authors to postulate that abnormal motor activity had begun prior to delivery.

**[Although the infant was described as pre-term (35 weeks gestation) by study authors, the infant weighed approximately 7 pounds and 3 ounces.]**

In 2001, Nordeng et al. *(85)* reported five infants with symptoms attributed by the authors to withdrawal from SRI therapy. One infant had been exposed antenatally to 20 mg/day fluoxetine, 3 were exposed antenatally to 10–40 mg/day paroxetine, and 1 was exposed antenatally to 30 mg citalopram. None of the mothers appeared to have undergone toxicologic screening. The child exposed to fluoxetine was born at 27 weeks gestation and weighed 860 g. On the second day of life, he became irritable and agitated and was started on phenobarbital for presumed seizures. The electroencephalogram and cerebral ultrasound were normal. The phenobarbital was discontinued after 1 week with no further evidence of abnormal motor activity.

Abebe-Campino et al. *(86)* described a neonate with premature atrial and ventricular contractions born to a woman who discontinued fluoxetine therapy (≤30 mg/day from week 28 of pregnancy) 5 days prior to delivery. An irregular fetal heart rhythm was also appreciated *in utero* upon admission for labor. The arrhythmia, still present at discharge, had resolved by 1 month of age.

*Strengths/Weaknesses:* While most case studies evaluated infants for sepsis and sometimes CNS involvement, not all included toxicologic evaluations. Clinical approaches for ruling out other drug exposures, infection, CNS injury, etc. are important to determine if causes other than fluoxetine exposure contributed to observed symptoms. It seems that the neurodevelopmental effects were minor and short-term because most infants were reported to be normal at follow-up during infancy. The single case of arrhythmia is unconvincing regarding causation, as this arrhythmia is a very common finding in neonates. Similarly, the single case of tumor, though uncommon, is hardly an indication of a direct relationship. Corroboration would be required.

*Utility (Adequacy) for CERHR Evaluation Process:* The case reports by themselves are not adequate for the evaluation process.

An FDA OPDRA Postmarketing Safety Review of neonatal withdrawal associated with antidepressants used the Adverse Event Reporting System (AERS) to identify four cases of neonatal withdrawal to fluoxetine, three of which had been reported in the literature *(87)*. The report lists two children each with hypotonia and irritability and one child each with shivering, trembling, seizure, hypertonia, extremity spasms, grimacing, hyperreflexia, agitation, hyperactivity, excitability, shallow respirations, sleep apnea, trouble feeding, malaise, and EEG agitation **[sic]**.

In response to a request from CERHR, the FDA submitted a summary of postmarketing surveillance data on adverse reproductive or developmental effects reported for fluoxetine *(88)*. Data were obtained from AERS, an electronic database containing postmarketing reports of adverse drug reactions submitted by health care professionals, consumers, or pharmaceutical manufacturers. Two electronic searches were conducted of the AERS database: one for all reports of patients less than 2 years old and a second using the search key "COMPLICATIONS OF MATERNAL EXPOSURE TO THERAPEUTIC DRUGS." Reports from both searches, containing U.S. and foreign data, were combined and efforts were made to remove duplicate reports. The time period covered by the search was from December 29, 1987 (the date of marketing approval) through May 28, 2003. However,

the analysis included only reports from 1997 forward because reports generated prior to that time period were not available electronically. A total of 383 unique reports were identified in the search. When available, the FDA summarized information on event history, duration of exposure, dose, concomitant medications, behaviors and illnesses, and age of mother. However, in many cases such information was not provided in reports submitted to the FDA. Table 7 contains a summary of cases that the FDA placed in the most appropriate category, although it was noted that some cases could be placed in more than one category.

The FDA *(88*) noted several limitations that confound interpretation of the AERS data. The quality of data submitted is highly variable and pertinent information is often lacking from reports. Because reporting of adverse effects is voluntary, adverse drug reports are most likely under-reported. In addition, it is not possible to relate exposures to any denominator of total pregnancy exposure. Many of the cases involve exposure to other drugs, making it difficult to assess the effects of fluoxetine by itself. In addition there may be confounding by the maternal condition for which the drug was prescribed (e.g., depression). Despite these limitations, the FDA observed some patterns in the fluoxetine data. The most frequent congenital abnormalities (cardiac, neural tube, limb, and genito-urinary defects) are commonly reported in the general population. The most frequently reported chromosomal abnormality was trisomy 21, which also occurs commonly among the U.S. population. The 35 cases of prematurity were difficult to interpret because the rate of prematurity in the U.S. is close to 12%. Also common were reports of "neonatal drug withdrawal reactions," respiratory distress in non-premature infants, and other neonatal adverse events. Possible neonatal withdrawal reactions were previously addressed in an FDA OPDRA review *(87*). Also submitted were a number of reports describing colic or jitteriness in breastfed infants.

The FDA *(88*) concluded that results of the AERS survey of fluoxetine exposures are not inconsistent with findings and concerns previously reported in the literature, especially cases of neonatal withdrawal, premature birth, and neonatal complications *(18, 89*). However, no firm conclusions can be made due to limitations associated with the postmarketing data.

*Strengths/Weaknesses:* The AERS reports give information on adverse events that may be associated with fluoxetine use, but do not account for events associated with the underlying disease, other medications, lifestyle factors, or chance. The lack of a denominator limits the interpretation of this information.

*Utility (Adequacy) for CERHR Evaluation Process:* The AERS reports by themselves are not adequate for the evaluation process.

***Table 7. Summary of AERS Postmarketing Reports of Fluoxetine Exposures During Pregnancy, via Breastfeeding, or by Direct Ingestion in Children Younger than 2 Years [FDA (88)]***

| *Category* | *Number of Cases* |
|---|---|
| Congenital anomalies (total) | 102 |
| Limb | 11 |
| Genito-urinary | 11 |
| Respiratory | 1 |
| Eye | 4 |
| Ear | 2 |
| Cardiac | 20 |
| Neural tube | 14 |
| Orofacial/craniofacial | 9 |
| Hernia | 3 |
| Gastrointestinal | 3 |
| Dermatologic | 5 |
| Miscellaneous musculoskeletal | 2 |
| CNS | 3 |
| Multiple anomalies | 14 |
| Stillbirths/Spontaneous abortions | 35 |
| Chromosomal abnormalities | 16 |
| Dermatologic | 3 |
| Jaundice | 5 |
| Gastrointestinal disorders | 19 |
| Colic | 11 |
| Withdrawal syndromes and other CNS effects | 53 |
| Respiratory distress at birth | 32 |
| Failure to thrive | 3 |
| Sudden infant death syndrome | 4 |
| Developmental delay | 16 |
| Hypoglycemia | 7 |
| Hemolytic anemia | 1 |
| Events from prescribed/accidental ingestion by young children | 8 |
| Intracranial hemorrhage | 2 |
| Prematurity (with or without complications) | 35 |
| Cardiac rhythm abnormalities | 3 |
| Abnormal labor | 28 |
| **TOTAL** | **383** |

#### *3.1.1.2 Reports with denominators*

The Pastuszak et al. 1993 study *(90)* is described by authors as a prospective cohort study involving women who called any of four teratology information services about exposure to fluoxetine (n=128), tricyclics (n=74), or "non-teratogens" (n=128)." Non-teratogens were defined in the paper as exposures not associated with birth defects in large studies and examples given are acetaminophen, dental x-rays, and penicillin. **[The actual exposures in this control group were not given in the paper.]** Due to the small number of TCA cases available for age-matching, two separate comparisons were conducted. The first comparison examined 128 cases in the fluoxetine group and 128 controls in the non-teratogen group. In the second comparison, 74 fluoxetine cases were compared to 74 TCA controls and 74 non-teratogen controls. The TCA control group had only 74 women who could be age-matched ± 2 years to fluoxetine-exposed women. No other matching criteria were mentioned. The fluoxetine-exposed women had called the teratology information service in Toronto (n=45), Philadelphia (n=44), Camden (n=21), and Salt Lake City (n=18). Fluoxetine exposure occurred during the first trimester in 128 women, first and second trimester in 2, and throughout pregnancy in 6. All the control women were derived from the files of the Toronto group, which coordinated the study. **[The paper does not say when the control cases were collected; it is assumed that they were collected prior to the fluoxetine cases, but it is possible that some or all were collected concurrently. The gestational age at accession is not given, nor is it clear that women in all groups were comparable in gestational age at enrollment.]**

Information collected about the mother, father, and exposures was said to have been obtained in a "similar" manner in the four centers, although different forms were used in each center. Follow-up information on pregnancy outcome was collected 8 – 12 months after the expected birth of the child. Telephone interviews with parents appeared to be the primary method of collecting information, but some information was apparently also collected by mail. It is written, "At follow-up information was corroborated by written documentation from the child's physician." **[The way the study is written, it is not clear if written documentation was obtained only for Toronto cases or for all cases. It is also not stated that written documentation was obtained for presumed normal infants as well as for reportedly abnormal infants. Other studies from this group have apparently included written documentation only for children reported 'abnormal' by their parents.]**

Results are shown in Table 8. There were no differences among groups in pregnancy outcome except for a stated increase in spontaneous abortion when the fluoxetine-exposed pregnancies were compared to non-teratogen-exposed pregnancies (the table indicates a statistical difference, but the text says there was a nonsignificant "trend." **[The rate in the control group appears low, suggesting incomplete ascertainment or differences in gestational age at enrollment; neither possibility can be evaluated with the data reported in the paper. The stated difference between these groups could not be confirmed when CERHR repeated the Fisher test.]** Information on spontaneous abortion is described in more detail in Section 4.1.1. The table in the paper indicated a significant difference in the rate of vaginal delivery between the fluoxetine and non-teratogen groups; however, the text does not identify such a difference **[and calculation using the Fisher test by CERHR confirms the lack of significant difference].**

*Table 8. Birth Outcomes after Human Pregnancy Exposure from Pastuszak et al. (90)*

| *Outcome* | *Fluoxetine/Non-Teratogen Comparison (n = 128/group)* | | *Fluoxetine/TCA/Non-Teratogen Comparison (n = 74/group)* | | |
|---|---|---|---|---|---|
| | *Fluoxetine* | *Non-Teratogen* | *Fluoxetine* | *TCA* | *Non-Teratogen* |
| Live birth | 98 **[76.6%]** | 110 **[85.9%]** | 58 **[78.4%]** | 60 **[81.1%]** | 67 **[90.5%]** |
| Elective abortion | 11 **[8.6%]** | 8 **[6.3%]** | 6 **[8.1%]** | 5 **[6.8%]** | 2 **[2.7%]** |
| Spontaneous abortion | 19 **[14.8%]** | 10 **[7.8%]*** | 10 **[13.5%]** | 9 **[12.2%]** | 5 **[6.8%]** |
| Major congenital anomaly | 2/98 **[2.0%]** | 2/110 **[1.8%]** | 2/58 **[3.4%]** | 0/60 **[0%]** | 2/67 **[3.0%]** |
| Gestational age, mean weeks ± SD | 39.4 ± 1.7 | 39.4 ± 1.8 | 39.4 ± 1.6 | 39.1 ± 2.3 | 39.6 ± 1.9 |
| Birth weight, mean g ± SD | 3,459.7 ± 660.2 | 3,421 ± 563 | 3,421.9 ± 664.1 | 3,515.9 ± 672.3 | 3,408.6 ± 602.2 |
| ≥ 4000 g | 15/84 **[17.9%]** | 9/81 **[11.1%]** | | | |

**[Percentages calculated by CERHR; they do not add to 100% due to rounding]**
*$P = 0.03$ according to authors **[$P = 0.11$ calculated by CERHR using Fisher test]**

The anomalies in the fluoxetine group included one child with jejunal obstruction and one child with ventricular septal defect. In the non-teratogen controls, there was one child with pulmonary atresia and one child with ventricular septal defect. Neonatal complications were listed (Table 9) but not analyzed. The authors stated that when examined individually, none of the complications were significantly more common in the antidepressant-exposed groups. **[Combining complications shows a significant difference ($P = 0.034$) using $3 \times 2$ chi-square, performed by CERHR, assuming that the denominator is 74 as in the other comparisons of the three exposure groups.]**

*Table 9. Human Neonatal Complications in Pastuszak et al. (90)*

| *Fluoxetine* | *TCAs* | *Non-Teratogens* |
|---|---|---|
| Jaundice (2) | Metatarsus adductus | Jaundice |
| Shoulder dystocia and apnea | Congenital dislocation of the hip | Clipped tongue |
| Patent ductus arteriosus and cyanosis | Slight hypotonia | |
| Sepsis and seizures | β-hemolytic streptococcus | |
| Hemangioma (2) | Apnea | |
| Lacrimal stenosis | Hydrocele | |
| Aspiration pneumonia | Respiratory distress syndrome | |
| Club feet | Meconium aspiration and sepsis | |
| Congenital dislocation of the hip | Metatarsus varus | |

*Strengths/Weaknesses:* It is questionable whether the study by Pastuszak et al. *(90)* is actually a prospective study**.** It appears that women retrospectively reported exposures and related information when they called the teratology information services. Follow-up information about the infant was

collected mostly by phone 8 – 18 months after the expected date of delivery. Therefore, the data are not captured in a truly prospective fashion. In addition, the study had no unexposed group for comparison. Also, it appears that the percentage of missing data varies by exposure status. For example, the Expert Panel estimated that 13% of subjects in the fluoxetine group have missing data compared to 25% of non-teratogen controls. If the Expert Panel estimates are correct, information bias according to exposure status is a key concern of this study. There is additional concern about the likelihood of recall bias, especially for the relatively minor neonatal problems several months later. Mothers who were concerned about fluoxetine exposure may be more likely to report problems than those who were reassured about the "non-teratogen" exposure.

*Utility (Adequacy) for CERHR Evaluation Process:* The Pastuszak et al. *(90*) study has low utility due to methodological limitations.

Brunel et al. *(91*) reported the outcome of antidepressant-exposed pregnancies for which there was contact with the Lyon, France poison control center. Between 1986 and 1991, there were 17 fluoxetine-exposed pregnancies, of which 16 were exposed during the first trimester. Outcome information was obtained from the initial informant using a questionnaire **[presumably a postal questionnaire, but no details were given]**. Outcome information was available for 11 of the first-trimester fluoxetine-exposed pregnancies. Four were voluntarily aborted and the remaining seven resulted in reportedly normal children. In the overall sample of 114 pregnancies with first trimester exposure to antidepressants, there were 24 voluntary abortions, 11 spontaneous abortions, 1 stillbirth, 4 malformed children, and 5 children with neonatal problems. **[Based on these numbers, the Expert Panel estimated rates of 12% loss of clinically recognized pregnancy, 1% stillbirth, and 5% birth defects; these pregnancy outcomes are within expected ranges, although the suitability of using estimates of general population incidences as a comparator has not been established.]**

*Strengths/Weaknesses:* This report involves a very small sample, and is equivalent to a case series. There is no control for pregnancy outcome in depressed women on other medications or on no medication.

*Utility (Adequacy) for CERHR Evaluation Process:* This report is not useful in the Evaluation Process due to the small size of the sample and the lack of control information.

Rosa *(92*) published results in abstract from his Michigan Medicaid data analysis. Two fluoxetine-exposed infants with malformation diagnoses were identified where four would have been expected. **[The abstract does not detail the study methods; however, it is known from other sources that Dr. Rosa used Medicaid prescriptions to identify exposures and used diagnoses connected to Medicaid services to identify abnormal children. This is a record-linkage study in which the Michigan Medicaid claims database was linked to pediatric files with birth defects invoices for completed pregnancies. The accuracy of the linkages (hence, under-ascertainment) is dependent upon the algorithm used for the linkage and the receipt of services in either of the registries.]** This abstract is mentioned for completeness but will not be further considered.

McElhatton et al. *(93*) reported a case series of women calling any of 12 European teratology information services with concerns about exposure to 1 or more antidepressants. The countries represented were France, Italy, Israel, Germany, the Netherlands, Spain, Switzerland, and the United Kingdom.

Information was obtained from callers at the time of the initial contact with regard to other exposures and demographic parameters. **[The gestational age at contact is not stated.]** One month after the expected date of delivery, a postal questionnaire was sent to the woman's obstetric provider. Telephone follow-up was said to have been used on occasion. The postal questionnaire solicited information on pregnancy outcome and on other exposures or complications since the woman's initial contact with the service. Follow-up was not possible for 16–20% of subjects due to loss of contact or change in health-care provider. **[It is implied but not stated that loss to follow-up was similar across centers. More importantly, it is not known if attrition varied by exposure status of mothers or infant outcomes.]** Follow-up was available for 689 pregnancies. Most of the pregnancies included exposure to TCAs.

There were 21 women exposed to fluoxetine alone and 96 women exposed to a TCA plus fluoxetine. Among the 21 pregnancies exposed to fluoxetine alone, there was one child with a ventricular septal defect. Of the 96 women exposed to a TCA plus fluoxetine, there were 15 voluntary abortions, 13 spontaneous abortions, 1 late fetal death, 6 normal pre-term babies, 3 babies with a neonatal disorder, 2 liveborn babies, and 2 liveborn babies with anomalies. **[The difference between the terms "malformations" and "anomalies" is not clear in the paper; it is possible that "anomaly" is used to mean minor malformation.]** There were two children exposed to fluoxetine plus another non-tricyclic antidepressant with anomalies consisting of pilonidal sinus and an angioma. **[The denominator for fluoxetine+non-tricyclic antidepressants is not given.]** The neonatal disorders associated with fluoxetine exposure (either alone or in combination with other medications) included a child with asphyxia and bradycardia with periventricular bleeding, a preterm infant with withdrawal and pneumonia, and a child with gastroesophageal reflux associated with bradycardia. **[This large case series is strongly oriented toward TCAs. It is difficult to identify details regarding fluoxetine exposures.]**

*Strengths/Weaknesses:* The study by McElhatton et al. *(93)* has limitations that are inherent to a case series including highly self-selective sample, small sample size of fluoxetine-exposed women, no unexposed women, multiple comparisons, and potential for selection and information bias.

*Utility (Adequacy) for CERHR Evaluation Process:* This report may be useful as supplemental information but is not useful by itself for the Evaluation Process.

Chambers et al. *(89)* reported pregnancy outcomes among 228 women (from approximately 500 callers) who called the California Teratogen Information Service with concerns about fluoxetine exposure. **[A preliminary version of this report with a smaller number of subjects was published in abstract *(94)*.]** Women were selected based on willingness to participate and access to a telephone. During the index period, 1989–1995, the authors reported about 1,500 fluoxetine calls, of which about 1/3 were from pregnant women exposed to the drug during the first trimester. A comparison group consisted of 254 women who called with concerns about early pregnancy exposure to acetaminophen, dental x-ray, or ethanol < 1 oz (absolute alcohol) per week prior to pregnancy recognition. This comparison group was selected based on proximity of the call-in time to the call made by the fluoxetine-exposed women. Information on demographic characteristics and other exposures was obtained by questionnaire and telephone interview. A diary was used to document additional exposures occurring during pregnancy. Outcome information was obtained from the mother, the pediatrician, and examination by a single dysmorphologist (Kenneth L. Jones). In the children examined by the dysmorphologist, minor anomalies were assessed using a 132-item checklist. These anomalies were defined as includ-

ing characteristics observed in less than 4% of the general population.

Women were divided into early-exposed and late-exposed groups based on whether they took fluoxetine prior to 25 weeks gestation. Of the 100 women exposed to fluoxetine only prior to 25 weeks, 93 were exposed to the medication and discontinued it in the first trimester (i.e., only 7 continued the medication into the second trimester). Of the 73 women in the late-exposed group, 60 took fluoxetine throughout pregnancy (i.e., they were also exposed early). Sixty-six women in the late-exposed group (90.4%) took fluoxetine within 2 days of delivery. Indications for fluoxetine are shown in Table 10.

***Table 10. Indications for Fluoxetine in Chambers et al. (89)***

| | |
|---|---|
| Depression | 133 women (76.9%) |
| Anxiety | 14 women (8.1%) |
| Panic disorder | 11 women (6.4%) |
| Bipolar disorder | 10 women (5.8%) |
| OCD | 7 women (4.0%) |

Approximately 30% of the women in the fluoxetine groups were taking other psychotherapeutic medications: a benzodiazepine (clonazepam or alprazolam) in 17.5%, trazodone in 5.2%, and a TCA in 5.2%. Alcohol use above 1 oz absolute alcohol per week was reported in 5% of the early-exposed group, 1.5% of the late-exposed group, and none of the control group. Less than 1% of all women reported use of recreational drugs. More fluoxetine-exposed women continued to smoke after they knew they were pregnant (exposed-early group, 10.0%; exposed-late group, 17.8 %) than in the control group (3.8%).

Abnormalities are shown in Table 11. The authors compared prevalence of major malformations in liveborn babies with first trimester exposure to fluoxetine and controls and found no difference. Inclusion of the aborted fetuses with the liveborn babies in the fluoxetine group would elevate the incidence of congenital anomalies to 6.7%; however, there still would not have been a significant difference from the control group **[Fisher Exact test, performed by CERHR]**. Information was not provided on congenital anomalies diagnosed in children with only late pregnancy exposure to fluoxetine. There appear to have been only 13 such women.

An increase in the proportion of infants with multiple minor anomalies is also shown in Table 11. **[It is not clear how the pair-wise statistical comparisons were made within rows; chi-square performed by CERHR shows an overall *P* value of 0.0027 for the distribution.]**

*Table 11. Abnormalities in the Chambers Study (89)**

| *Variable* | *First trimester fluoxetine exposure* | *Control infants* | *P*** |
|---|---|---|---|
| ***Major anomalies*** | ***n=164*** | ***n=226*** | |
| VATER association | 0 | 1 | |
| Ventricular septal defect | 1 | 1 | |
| Ventricular septal defect, bilateral cryptorchidism | 1 | 0 | |
| Atrial septal defect | 1 | 0 | |
| Nasal dermal sinus | 1 | 0 | |
| Coccygeal dermal sinus | 1 | 0 | |
| Hypospadias | 1 | 2 | |
| Bilateral inguinal hernia | 0 | 2 | |
| Cleft palate | | 1 | |
| Sagittal synostosis | 1 | 0 | |
| Bilateral hip dysplasia | 2 | 0 | |
| Unilateral hip dysplasia | | 1 | |
| Total | 9 (5.5%) | 9 (4.4%) | 0.63 |
| ***Minor abnormalities*** | ***n=97*** | ***n=153*** | |
| 0 or 1 | 56 (57.7%) | 119 (77.8%) | 0.002 |
| 2 | 26 (26.8%) | 24 (15.7%) | 0.04 |
| 3 or more | 15 (15.5%) | 10 (6.5%) | 0.03 |

*Excludes one voluntary abortion of an infant with Down syndrome and one spontaneous abortion of an infant with hypoplastic femur-unusual facies syndrome in the group exposed to fluoxetine

**Chi-square or Fisher test.

Table 12 shows other outcomes in liveborns with fluoxetine exposure during pregnancy compared to controls. Late but not early pregnancy exposure was associated with an increased incidence of prematurity, a decrease in birth weight and length in full-term infants, and poorer neonatal condition characterized by admission to the special care nursery and adaptation problems. Using pregnancies with early fluoxetine exposure as a reference group, late pregnancy exposure to fluoxetine was associated with a relative risk for prematurity of 4.8 (95% CI: 1.1, 20.8; adjusted for multiparity; previous spontaneous abortion; preeclampsia, eclampsia, and hypertension; smoking status; maternal age; socioeconomic status; race; average dose of fluoxetine; gestational diabetes; use of other psychotherapeutic drugs; alcohol use; and evidence of maternal or neonatal infection near delivery).

A discussion of spontaneous abortion is included in Section 4.1.1.

*Table 12. Non-malformation Outcomes in the Chambers Study (89)**

| | *Fluoxetine Exposure Period* | | *Control Group* | *P*** |
|---|---|---|---|---|
| | *Early* | *Late* | | |
| ***Liveborn:*** | ***n=98*** | ***n=70*** | ***n=220*** | |
| Liveborn <37 weeks gestation | 4 (4.1%) | 10 (14.3%) | 13 (5.9%) | 0.03 |
| 37–42 weeks gestation | 91 (92.8%) | 59 (84.3%) | 203 (92.3%) | |
| >42 weeks gestation | 3 (3.1) | 1 (1.4) | 4 (1.8%) | |
| ***Clinical condition:*** | ***n=101*** | ***n=73*** | ***n=226*** | |
| Admission to special care nursery | 12 (11.9%) | 23 (31.5%) | 20 (8.8%) | <0.001 |
| Poor neonatal adaptation | 9 (8.9%) | 23 (31.5%) | - | <0.001 |
| ***Full term neonates:*** | ***n=95*** | ***n=61*** | ***n=209*** | |
| Weight, g (mean±SD) | 3,589±500 | 3,392±485 | 3,556±50 | 0.04 |
| Length, cm (mean±SD) | 51.5±2.5 | 50.4±2.7 | 51.5±2.5 | 0.01 |
| Head circumference, cm (mean±SD) | 34.8±1.5 | 34.3±1.6 | 34.5±1.5 | 0.19 |
| Birth weight <10$^{th}$ percentile | 3 (3.2%) | 7 (11.5%) | 7 (3.3%) | 0.02 |
| Microcephaly (<3$^{rd}$ percentile) | 2 (2.2%) | 2 (3.3%) | 2 (1%) | 0.41 |

*Gestation age and body measurements exclude twins and second pregnancies of women whose first pregnancies were previously included

**Analysis of variance, Fisher, or chi-square, as appropriate.



The relative risk for admission to a special care nursery was 2.6 (95% CI: 1.1, 6.9; adjusted for prematurity; preeclampsia, eclampsia, and hypertension; smoking status; maternal age; socioeconomic status; race; average dose of fluoxetine; gestational diabetes; mode of delivery; alcohol use; evidence of maternal or neonatal infection near delivery; and therapy with other psychotherapeutic drugs near delivery). The relative risk of poor neonatal adaptation was 8.7 (95% CI: 2.9, 26.6; adjusted for prematurity; use of pre-term labor medications; preeclampsia, eclampsia, and hypertension; smoking status; maternal age; socioeconomic status; race; average dose of fluoxetine; gestational diabetes; alcohol use; evidence of maternal or neonatal infection near delivery; and therapy with other psychotherapeutic drugs near delivery).

The authors recognized that admission to special care nurseries and evaluations of neonatal adaptation may have been biased by knowledge of fluoxetine use by the mother; however, they noted that infants were delivered in 109 different hospitals, with no hospital contributing disproportionately to these adverse outcomes. Particular concern about the increase in infants with multiple minor abnormalities was based on the belief that multiple minor abnormalities may be a harbinger of a potential for an increase in major abnormalities. The authors cite two prior studies (both from other investigators) that identified three or more minor abnormalities in 0.5 and 3.7% of children in the general population. The major anomaly rates in children with 3 or more malformations were cited as 90 and 20% in these 2 prior studies, respectively.

Additional investigation into late vs. early pregnancy exposure to SRIs was performed by the Slone

Epidemiology Center Birth Defects Study and has been published as an abstract *(95*). There was a relative risk of 2.5 (95% CI: 1.0, 6.3) for pre-term delivery in women using SRIs during the third trimester compared with women not exposed to SRIs. The risk estimate associated with first or second trimester SRI use was said to be similar to that of unexposed pregnancies. **[The Expert Panel considers this abstract to generally confirm the results of Chambers et al. *(89*), recognizing that the abstract does not provide adequate detail for a full evaluation and that the abstract concerns SRIs as a group as opposed to fluoxetine as an individual agent.]**

*Strengths/Weaknesses:* Chambers et al. *(89*) is a relatively good study with well-defined procedures and outcome measures and more effective and thorough ascertainment of outcome than earlier studies. It is important to note that many of the highly significant findings are based on case-case comparisons among fluoxetine users. Limitations include small numbers of subjects and models that have many covariates. A potential concern about the comparison of women with late pregnancy exposure to fluoxetine and women with early pregnancy exposure is that women with late pregnancy exposure had more severe depression and that the depression may have mediated the adverse neonatal outcomes rather than the medication exposure. The conflating of medication effects and effects of the underlying illness is an issue for the study of any therapeutic intervention, and it may be more useful to accept that studies of medication effects are usually studies of medication use in a clinical context of an illness being treated rather than in isolation. In addition, the assumption that the adverse effects in the late-exposed group in Chambers et al. *(89*) had more severe depression, and that the depression caused the adverse events represents only one possible scenario. It is possible that the early-exposed group contained more women with inadequately treated depression. If depression were the mediator of the adverse neonatal effects, the study of Chambers et al. *(89*) would have underestimated the medication effects if there were more women with undiagnosed or untreated depression in the control group than in the late-exposed group.

*Utility (Adequacy) for CERHR Evaluation Process:* This report is adequate for use in the Evaluation Process; however, the speculation regarding the significance of multiple minor anomalies should be interpreted with caution. Additional studies would be helpful for confirming or refuting the hypothesis that fluoxetine exposure during pregnancy increases the incidence of minor anomalies. The association of fluoxetine exposure with prematurity is potentially important, and the apparent confirmation of this finding in the Slone sample is useful supplementary information.

Goldstein et al. *(96*) reported outcomes of some of the cases of fluoxetine exposure during pregnancy reported to Eli Lilly and Company, manufacturer of Prozac®. Preliminary data that contributed to this study appeared previously in abstracts *(97, 98*) and a letter *(99*).

Pregnancy exposures about which Eli Lilly and Company were notified included reporting of the pregnant woman's age, fluoxetine dose, indication for use, time of exposure, concomitant medications, expected delivery date, and prior pregnancy history. Patient names were not collected. At an unspecified time after the delivery date, the reporter was contacted. **[The reporter could have been the pregnant woman herself or her healthcare provider.]** Information solicited from the reporter included pregnancy outcome, abnormalities, and complications. **[How the information differs by these terms is not indicated.]** The authors write, "The absence of a patient name increased the proportion lost to follow-up because the reporter often could not remember the specific case."

Women were included only if there was fluoxetine exposure in the first trimester. Elective terminations for malformations were included as adverse outcomes, but elective terminations for non-medical reasons were not included. For women with multiple fluoxetine-exposed pregnancies, only the first pregnancy was included. The abnormalities reported were taken as reported; no confirmation was attempted. A comparison was made to three historic controls, namely three papers on incidence of malformations published in 1954, 1958, and 1964, respectively.

There were 2,072 pregnancies in the exposure registry for which exposure was identified prior to awareness of pregnancy outcome **[gestational age at enrollment not provided]**. At the time the report was written, 314 were still *in utero*, 155 had been electively aborted, and 39 were excluded because exposure could not be confirmed to have occurred in the first trimester. Of the remaining 1,564 pregnancies, 768 **[49%]** were lost to follow-up and 796 had evaluable outcomes. Of evaluable pregnancies, 37 were from clinical trials and 759 **[95%]** from spontaneous reports. The authors distinguished malformations according to whether they were diagnosed near birth or subsequently (called postneonatal). Combining the malformations, there were 33 affected children among the 796 evaluable pregnancies **[4.1%]**. The perinatal malformations were reported by system as follows (number of affected children in parentheses): cardiovascular (1), chromosomal (5), craniofacial (2), gastrointestinal (6), neural tube defect (3), and miscellaneous (4). The postneonatal **[written postperinatal in the table]** abnormalities were reported as hypotonia/movement disorder (1), pyloric stenosis (3), strabismus (1), tracheomalacia (3), and umbilical hernia (1). Neonatal disorders in children without structural malformations included hyperbilirubinemia (12), irritability (2), colic (2), respiratory distress (1), low birth weight (2), infection (2), somnolence (2), hypoglycemia (1), tachypnea (2), hydramnios **[the Panel notes that this diagnosis is a maternal disorder, not a neonatal diagnosis]** (1), seizures (2), pneumothorax (1), gastroesophageal reflux (1), apnea/bradycardia (1), tremors (1), and hypotonia (1).

There were an additional 426 pregnancy exposures reported to Eli Lilly and Company after the outcome was known. Eighty-nine **[21%]** of the pregnancies resulted in a child with anomalies. The diverse nature of the anomalies did not suggest a pattern. **[The number of children with each diagnosis is reported in Table 3 in the original paper.]**

*Strengths/Weaknesses:* The compilation of available postmarketing reports is of interest, but the excessive loss to follow-up is an important weakness. In addition, the sample is self-selected and the use of the historical control is unacceptable. The ascertainment of outcome was not acceptable in that it relied on reporters who were likely to have different levels of interest, training, and experience in the diagnosis of abnormalities.

**Utility (Adequacy) for CERHR Evaluation Process:** Although this report might be used to support better quality studies, it is insufficiently reliable in and of itself, due to the loss to follow-up, the lack of an appropriate reference group, and the unknown quality of the outcome data.

A previous report by Goldstein *(100)* evaluated the outcome of pregnancies exposed during the third trimester without regard to whether there were also exposures in other trimesters. **[It is not stated, but some overlap is likely with regard to patients ascertained in the 1997 study of first trimester exposures; a woman exposed throughout pregnancy would have been eligible to have been included**

**in both reports. Loss to follow-up was not discussed in the current study.]** The methods appear to have been identical to the 1997 report with respect to enrollment of exposures and determination of outcome. Results were displayed by dose of fluoxetine and by trimester of exposure. Only three pregnancies included only third trimester exposures; 89 **[79%]** were exposed during all 3 trimesters. There was no apparent pattern of abnormal infant condition by fluoxetine dose (which was unreported for 20 infants **[18%]**). Outcomes are summarized in Table 13 without regard to maternal dose. None of the classes of abnormal infant condition appeared greater than expected based on general population surveys.

***Table 13. Selected Abnormalities Described with Third Trimester Exposure to Fluoxetine[a] [Adapted from Goldstein (100)]***

| *Abnormality[b]* | *Number (%)* |
|---|---|
| Irritability/jitteriness | 5 (4.5) |
| Hyperbilirubinemia/jaundice | 3 (2.7) |
| Gastrointestinal obstruction/stenosis | 2 (1.8) |
| Prematurity | 6 (5.4) |
| Somnolence | 4 (3.6) |

[a] All doses, any combination of other trimester exposures

[b] Some children had more than one abnormality.

**[Descriptions in original paper interpreted by Expert Panel]**

*Strengths/Weaknesses:* This report appears equivalent to the previous study and has the same weaknesses including unstated but possibly large loss to follow-up and reliance for outcome data on reporters of questionable reliability.

*Utility (Adequacy) for CERHR Evaluation Process:* Although this report might be used to support better quality studies, it is insufficiently reliable by itself for use in the Evaluation Process.

Wilton et al. *(101)* contacted general practitioners concerning pregnancy outcome of women who received a prescription for fluoxetine as part of a prescription-monitoring program. There were 52 exposed pregnancies resulting in 27 births. Two pregnancies were ectopic, 6 aborted spontaneously, 6 were electively aborted, and outcomes were unknown for 11 pregnancies. There were two children with congenital abnormalities, one of whom had spina bifida with hydrocephalus and one of whom had congenital hypothyroidism. A third child had single palmar creases, which the authors considered a minor malformation. **[The authors do not formally compare the proportion of pregnancies with complications for any medication groups, but it is possible to do so given the data they present. Using the Fisher Exact test, 6 spontaneous abortions in 52 pregnancies exposed to fluoxetine (12%) and 88 spontaneous abortions in 779 pregnancies exposed to other medications (11%) are not different ($P$=1), and 2 congenital malformations in 52 fluoxetine-exposed pregnancies (3.8%) and 10 congenital malformations in 497 pregnancies exposed to other medications (2.0%) are not significantly different ($P$=0.3).]**

*Strengths/Weaknesses:* Prescription monitoring programs are a fairly effective means of identifying exposure and are less likely to result in biased ascertainment than self-reporting or calling a Teratol-

ogy Information Service. Outcome ascertainment is less rigorous. The small number of fluoxetine-exposed pregnancies limits the ability to identify an increase in adverse outcomes.

*Utility (Adequacy) for CERHR Evaluation Process:* This study has limited utility in the evaluation of fluoxetine developmental effects due to the small sample.

Ericson et al. *(102*) used the Swedish Medical Birth Registry to identify women reporting antidepressant use on their first prenatal visit and to evaluate pregnancy outcome. The Swedish Registry of Congenital Malformations was used to supplement the outcome data. During the observation period **[not stated but may have been 1995–1997 based on information elsewhere in the paper]**, 281,728 infants appeared in the Medical Birth Registry, including 969 whose mothers reported antidepressant use. Of the antidepressant-exposed infants, 546 were exposed to SRIs and 438 were exposed to non-SRI antidepressants (15 were exposed to both). Among the SRIs, citalopram was most commonly used (364 monotherapy, 11 combination therapy), and among the non-SRIs, clomipramine was most common (333 monotherapy, 12 combination therapy). Fluoxetine monotherapy was identified in 15 pregnancies and fluoxetine combination therapy in 1 pregnancy. Antidepressant use was associated with premature delivery (crude OR: 1.67, 95% CI: 1.32, 2.11; adjusted OR: 1.43, 95% CI: 1.14, 1.80), but there was no difference by class of antidepressant, suggesting an association with the disease rather than the treatment. **[The Expert Panel believes that an alternative explanation for these findings is that a similar mode of action among these medications may be affecting the rate of prematurity.]** Low birth weight and congenital malformations were not significantly associated with treatment.

*Strengths/Weaknesses:* A population-based study in a stable population in a well organized healthcare system should result in near-complete ascertainment. There were few fluoxetine cases, but the data from a closely-related drug are still useful. Of note is the low rate of use of antidepressants compared to the U.S.

*Utility (Adequacy) for CERHR Evaluation Process:* Extrapolation from data on a related drug must be done with caution, but because of the above-mentioned strengths, the report adds useful information to the existing body of knowledge.

Cohen et al. *(103*) reviewed obstetric and neonatal records of 64 mother-infant pairs with fluoxetine exposure during pregnancy. The mothers came to the attention of the investigators when they requested information about medication exposure during pregnancy or by presenting with untreated depression during pregnancy. The investigators compared neonatal outcomes among pregnancies exposed early (first or second trimester) and late (including the third trimester) during pregnancy. The outcomes of interest involved neonatal adaptation and exposure throughout pregnancy appears to have been classified as late exposure. There were 11 mother-infant pairs in the early exposure group and 53 pairs in the late exposure group. Twenty-five women used concurrent psychotropic medications, particularly benzodiazepines. One of the 11 early-exposed babies had neonatal complications and was admitted to the special care nursery compared to 16 (30.2%) of the 53 babies with late exposure (*P* not significant). The authors believed the effect identified in their study to be real inasmuch as the proportions of children affected were similar to the proportions in Chambers et al. *(89*). They attributed the lack of statistical significance to the small sample. The study was supported by Eli Lilly and Company.

*Strengths/Weaknesses:* The small sample size is an important limitation to the utility of this report.

*Utility (Adequacy) for CERHR Evaluation Process:* Together with other studies, these outcome data are useful in the Evaluation Process.

Simon et al. *(104*) used pharmacy records from the Group Health Cooperative in Washington state to identify women believed to be exposed to antidepressant medications during pregnancy (i.e., 1 or more prescriptions filled within the 270-day interval before delivery). Medical records were reviewed to identify pregnancy outcomes. TCAs were considered as a group and SRIs were considered as a group and included fluoxetine, fluvoxamine, sertraline, and paroxetine. Of the 185 infants prenatally exposed to SRIs, 129 were exposed to fluoxetine. The TCA group consisted of 209 subjects. Unexposed pregnancies were selected for comparison (n=185 for SRIs and n=209 for TCA comparisons). Exposure to SRIs was associated with a decrease in gestational age of 0.9 weeks (95% CI: 0.5, 1.3 weeks, after adjusting for maternal tobacco use, other substance use, race, and number of prior births) and an increase in pre-term birth (gestational age≤36 weeks, OR: 4.38, 95% CI: 1.57, 12.22). In contrast to previous studies, this association was identified for both early and late (third trimester) exposure. Decrements in mean birth weight (-172 grams; 95% CI: -46,-299) disappeared after controlling for gestational age. No differences were significant when infants exposed to TCAs were compared with matched unexposed infants. **[The Expert Panel noted that the percent of preterm birth (<37 weeks) was comparable in the SRI- and TCA-exposed groups (10.3 and 10.0%, respectively]**. Apgar score was depressed at 5 minutes with an OR: for Apgar ≤ 7 of 2.78 (95% CI: 1.17, 6.26). There was no difference between the SRI group and the reference group in congenital anomalies, seizure disorder, motor delay, speech delay, or other motor abnormality. Minor anomalies and postnatal problems with adaptation or neonatal intensive care unit (NICU) admissions were not examined.

*Strengths/Weaknesses:* The population-based design is a strength of the Simon et al. *(104*) study, which linked hospital discharge records for live births between 1986 and 1998 with pharmacy records. The study demonstrated decrements in mean gestational age associated with SRIs. The study was relatively well designed within the limitations of a linkage study. It had the power to detect only large risks for birth defects. In Table 1 of the study, Apgar scores were analyzed and reported as continuous variables, which is incorrect; however, a more appropriate nonparametric analysis was also performed. The absence of information on the full distribution of gestational age in each of the groups precludes a more meaningful interpretation of these data.

*Utility (Adequacy) for CERHR Evaluation Process:* These data are useful for the Evaluation Process, and support the possibility that SRI exposure during pregnancy is associated with a reduction in gestational age at birth. However, the study is not adequate for the evaluation of seizures, motor or speech delays, and other motor abnormalities.

Laine et al. *(18*) reported a prospective study of 20 pregnancies exposed to antidepressants (10 fluoxetine, 10 citalopram) and 20 control pregnancies (except for thyroxine in one woman, no medications). **[It is not clear how controls were selected.]** Neonates underwent a structured evaluation for serotonergic symptoms including myoclonus, restlessness, tremor, shivering, hyperreflexia, incoordination, and rigidity. The evaluation was performed by a pediatrician who was supposed to have been blind to exposure status but "this blinding was not completely sustained in this clinical setting…"

Antidepressant-exposed pregnancies included 15 women with first trimester exposure (9 citalopram, 6 fluoxetine). Duration of antidepressant exposure was 7 –41 weeks. **[It is implied, though not stated, that all exposures involved the immediate pre-delivery period.]** Duration of pregnancy was similar in both groups (274 vs. 279 days [median] in SRI vs. control groups, $P=0.06$). Apgar at 15 minutes was said to be significantly lower in the SRI group. **[The Panel notes that the Apgar scores, which are the sums of ranks, were expressed as means and apparently analyzed with a *t*-test, which is not an appropriate analytic approach.]** Serotonergic symptoms were said to be significantly greater in the SRI group on days 1–4 of life. **[Symptoms scores were created by adding ranks for different domains. The sum of ranks was then considered as the individual's rank for use in a Wilcoxon signed rank test. The difference between SRI and control groups was quite large, and there were more children with symptoms and more days with symptoms in the SRI group than the control group.]** The difference in serotonergic symptom scores between groups were no longer seen at 2 weeks or 2 months of age. **[Possible lactational exposure was not mentioned.]** When the SRIs were considered separately, citalopram exposure was not associated with an increase in serotonergic symptom score at 1–4 days of age, but fluoxetine was associated with serotonergic symptoms **[data not shown in the paper]**.

Biochemical testing of these children showed an inverse correlation between serotonergic symptom score and 5-hydroxyindolacetic acid cord blood concentration in SRI-exposed but not control children. There were decreases in SRI-exposed children in cord blood serotonin (69%), 5-hydroxyindolacetic acid (18%), homovalinic acid (23%), and norepinephrine (46%). Fluoxetine, but not citalopram, was associated with a 49% decrease in cord blood prolactin concentration.

*Strengths/Weaknesses:* The prospective design and directed assessment done specifically to asses symptoms, rather than relying on chart review, are strengths; however, it is difficult to imagine how the investigators assessed nausea in newborns. The analyses of Apgar scores was inappropriate and unreliable. The symptom score analysis is suspect, as noted above, but the differences are so large that they would almost surely persist were the data analyzed more optimally. Incomplete blinding is a concern, but again probably not a decisive factor.

*Utility (Adequacy) for CERHR Evaluation Process:* This study adds to the limited body of knowledge about postnatal symptoms. The study is acceptable for use in the Evaluation Process, despite the above issues, and supports the association of near-term fluoxetine therapy and subsequent neonatal side effects.

Heikkinen et al. *(19*) followed 11 women treated for depression or panic disorder with fluoxetine at daily doses of 20–40 mg during pregnancy and compared outcomes with a control group of 10 women not on psychotropic medication matched for age, gravidity, parity, gestational weeks, and mode of delivery. **[One control subject dropped out. It is not indicated how or when the control group was recruited. Because there was matching for gestational age and mode of delivery, recruitment after delivery may have occurred.]** Of the 11 fluoxetine-exposed pregnancies, 5 women began the medication "later in pregnancy," by which the authors meant 22, 27, 31, 32, and 35 weeks gestation. **[It is presumed that the other six women were on medication throughout pregnancy, but no statement is made on this issue.]** Six were taking fluoxetine before becoming pregnant and continued to take the drug throughout pregnancy and lactation. One of the fluoxetine-exposed pregnancies was

characterized by "mild polyhydramnios" at 37 weeks, but the infant was said to be normal and healthy. Outcomes were said to be comparable in the fluoxetine and exposed groups. **[The outcome table includes gestational age and route of delivery; however, inasmuch as these parameters factors were matched, they cannot be considered relevant outcome measures. The authors indicate that there was a difference in 15-minute Apgar, but the table does not show a difference (in addition, the Panel noted that Apgar score was apparently compared inappropriately using means).]** There were no congenital malformations. Of specific interest is the comparability of birth weight and weight at 12 months between the groups (birth weight, fluoxetine exposed vs. control = 3,380±390 g vs. 3,510±550 g; weight at 12 months, fluoxetine vs. control 9,760±1,120 g vs 9,830±980 g). Developmental outcome at 1 year, assessed by a neurologic exam and Gesell developmental scales, was "normal" in all infants.

*Strengths/Weaknesses:* Heikkinen et al. *(19)* refer to their study as a prospective clinical trial. A limitation of the study is that there is no randomization. In addition it was unclear how controls were selected. A strength lies in the availability of developmental assessment at 1 year of age. However, the outcome is only given as normal or abnormal, which could obscure modest developmental impairment. "Normal" is not defined. It is not clear how the women were recruited into the study and there may have been selection bias. Controls appear to have been retrospectively selected. The matching was suboptimal (e.g., mean maternal age differed by 5 years [$P=0.08$]). Once again, Apgar scores were inappropriately treated as continuous variables. Consequently, the analysis is suspect, and the table does not match the text. In addition, the small sample size provides limited power to detect differences.

*Utility (Adequacy) for CERHR Evaluation Process:* This paper adds some useful information to our knowledge of long-term growth and development. Due to the limitations noted above, the conclusions are tentative.

Hendrick et al. *(105)* presented outcome information on 138 non-smoking women who took antidepressant medication during pregnancy, 73 of whom used fluoxetine. Women had apparently consulted one of the authors for psychiatric care during pregnancy. Follow-up information was obtained from obstetric and pediatric medical records. Fourteen (19%) of the pregnancies exposed to fluoxetine had "birth complications," compared to 14 (22%) of the remaining 65 women who used other antidepressants. Among the fluoxetine-exposed pregnancies, there were 5 pre-term births (6.8%), 3 low-birthweight babies at term (each 2.4 kg), 2 babies characterized as "floppy," and 1 case each of nuchal cord with need for assisted ventilation, meconium aspiration with hyperbilirubinemia, heavy meconium, and fractured clavicle with hyperbilirubinemia. The authors note that the low birth-weight term babies were born to women on 40 or 80 mg/day fluoxetine, perhaps implying that higher maternal doses might be more problematic for fetal growth. Of the 14 pregnancies with complications, 1 each occurred in a woman taking 5 and 10 mg/day fluoxetine, 2 occurred in women taking 20 mg/day fluoxetine, 8 occurred in women taking 40 mg/day fluoxetine, and 2 (both low birth weight) occurred in women taking 80 mg/day fluoxetine. It is not known whether this distribution of doses differed from that of the women without complications, but the authors report that medication dose did not correlate with either birth weight or gestational age for the entire sample. No fluoxetine-exposed infant was reported to have a congenital malformation.

*Strengths/Weaknesses:* The study by Hendrick et al. *(105)* is similar to a case series, although the

authors do estimate "incidence." A limitation is the lack of a comparison group. Exposed women were enrolled at any stage during pregnancy providing they had no other teratogenic exposures. Birth outcomes were abstracted from medical records. There were a substantial number of subjects with fluoxetine exposure. Outcome data were obtained from existing records, not collected prospectively.

*Utility (Adequacy) for CERHR Evaluation Process:* This report adds some useful reassuring information, but the lack of controls and reliance solely on medical records limits usefulness.

#### *3.1.1.3 Pregnancy outcome meta-analysis*

Addis and Koren *(106*) published a meta-analysis of epidemiology studies on first trimester use of fluoxetine with major malformation as the outcome of interest. Searches were conducted in standard bibliographic databases up to August or November 1996 (depending on the database). Criteria included studies in which first trimester exposure was ascertained before pregnancy outcome was known. Cohort studies were included whether or not they had a control group. A random-effects model was used to combine data for the different studies. For studies with a control group, a Mantel-Haenszel summary odds ratio was calculated. After excluding reports that were not original or that represented abstracts or letters followed by full reports, four studies remained for evaluation *(89-91, 93*). Two of these *(91, 93*) reported groups of exposed women without a reference group. The 4 studies represented the experience of 367 pregnancies. **[The Brunel report contributed only seven subjects.]** Ten infants (2.6% **[the Panel calculates it as 2.7%]**) had congenital anomalies. There were four infants with ventricular septal defect, two infants with hypospadias, and one infant each with atrial septal defect, jejunal obstruction, nasal-dermal sinus, and coccygeal-dermal sinus. The overall malformation rate was judged not different from the expected 1–3% population incidence of congenital malformation. The summary OR: using the 2 controlled studies was 1.33 (95% CI: 0.49–3.58). This report did not address minor malformations or neurodevelopmental outcomes. In addition, three abnormalities reported by Chambers et al. *(89*) were not included, one sagittal synostosis and two hip dysplasias. Chambers et al. also indicated that they excluded an electively-aborted fetus with trisomy 21 and a spontaneously aborted fetus with femoral hypoplasia-unusual facies syndrome. These two fetuses were not included in the Addis and Koren meta-analysis.

*Strengths/Weaknesses:* This report is useful in identifying available studies; however, the authors' analysis sheds no new light on the subject.

*Utility (Adequacy) for CERHR Evaluation Process:* This paper is of limited usefulness in the Evaluation Process due to the limitations of some of the underlying studies, which have already been identified.

#### *3.1.1.4 Neurobehavioral evaluation in children with prenatal exposure*

Nulman et al. 1997 *(107*) presented a study done by Motherisk, a teratology information service in Canada. **[A preliminary communication was published in abstract *(108*).]** This report presents the results of neurobehavioral testing of children born to women who called Motherisk with concerns about an exposure during the first trimester of pregnancy to either TCAs or fluoxetine, and compared these children to children born to women who had called about an "innocuous exposure." **[Examples given are acetaminophen, penicillin, and dental x-ray, but actual exposures are not indicated.]** At the time of intake **[the gestational age of which is unspecified]** women were asked about alcohol

use, smoking, lifestyle **[not otherwise specified]**, medical and nutritional status, and sexually transmitted diseases. Genetic and obstetric history and concomitant medications were recorded.

Assessment was initially made 6–9 months after delivery and consisted of an interview with the mother. Duration of antidepressant treatment was recorded as well as illnesses and complications that occurred during the pregnancy. Mothers were asked about the type of delivery, "the perinatal period" **[information not otherwise specified]**, and the times at which the child reached developmental milestones. A written report was obtained from the child's physician **[the content or form of this report was not indicated]**.

At an unspecified time after birth, children and mothers underwent testing by a psychometrician who did not know the mother's exposure status. **[It is not stated, but there appear to have been differences in age at testing among the children.]** The tests used were as follows:

Children:
- Bayley Scales of Infant Development (16–30 months of age)
- McCarthy Scales of Children's Abilities (children older than 30 months)
- Carey Temperament Scales (children up to 24 months old)
- Achenbach Behavior Checklist (children older than 24 months)
- Reynell Developmental Language Scales (all children)

Mothers:
- Wechsler Adult Intelligence Scale—Revised
- Hollingshead Four Factor Index (for socioeconomic status)
- Global Assessment Scale (for level of depression and function from the birth of the infant)
- Center for Epidemiologic Studies Depressed Mood Scale
- Index of Parental Attitudes

Outcomes were compared by one-way analysis of variance with Tukey's multiple range test.

There were 80 evaluated pregnancies in the TCA group (of an original 129 women who had been counseled by Motherisk since 1985: 24 were lost to follow-up, 8 declined participation, 3 were exposed to agents with known adverse developmental effects, 12 had spontaneous abortions, and 2 had elective abortions). There were 55 fluoxetine-exposed pregnancies for evaluation (of an original 88 women who were counseled about fluoxetine: 6 were lost to follow-up, 8 declined participation, 12 had spontaneous abortions, and 7 had therapeutic abortions). The control group consisted of 84 pregnancies. **[No information was given on spontaneous abortion in the control group, precluding evaluation of possible increases associated with treatment.]**

Of the women with TCA exposure, 40 used the medication in the first trimester, 36 throughout pregnancy, 2 during the first and second trimesters, and 2 during the first and third trimesters. Nine different medications were included in the TCA group. Of women with fluoxetine exposure, 37 used the medication during the first trimester and 18 throughout pregnancy.

Gravidity, parity, and previous elective abortion were said to differ among groups. **[However, it does**

**not appear that these comparisons were appropriately analyzed by ANOVA; distributions were either highly skewed or were not continuous.]** There were no differences among mothers in severity of depression or Index of Parental Attitudes. A trend toward decreased gestational age at delivery associated with antidepressant exposure was not significant ($P < 0.1$).

Child outcomes were the same among the groups with regard to gestational age, birth weight, percentile height and weight at testing, and percentile head circumference at testing. There were no differences in test scores (Table 14). **[There is no information on how many children underwent each test, the age at testing, or the comparability of ages among groups at testing. The only comment in this regard is that children were tested between the ages of 16 and 86 months. The numbers at the top of each column are from the authors' table, but cannot represent the number of children tested with each instrument inasmuch as the instruments were applied at different ages.]**

***Table 14. Neurobehavioral Test Results from Nulman et al. (107)***

| *Test* | *TCA (n=80)* | *Fluoxetine (n=55)* | *Control (n=84)* | *Adjusted difference (95% CI)* | |
|---|---|---|---|---|---|
| | | | | *TCA vs. control* | *Fluoxetine vs. control* |
| Bayley Mental Development Index | 118±17[a] | 117±17 | 115±14 | 2.4 (-4.5, 9.4) | 2.1 (-5.0, 9.2) |
| McCarthy General Cognitive Index | 117±10 | 114±16 | 114±13 | 2.7 (-2.3, 7.6) | 4.7 (-4.0, 13.4) |
| Reynell Verbal Comprehension Scale | 1.3±0.8 | 1.2±1.2 | 1.1±0.9 | 0.3 (-0.1, 0.5) | 0.3 (-0.1, 0.6) |
| Reynell Expressive Language Scale | 0.3±0.9 | -0.2±1.0 | 0.1±1.0 | 0 (-0.3, 0.3) | -0.1 (-0.4, 0.3) |

[a] Error is SD

**[Figures are reproduced from author table in spite of inappropriate use of mean±SD for some data representations.]**

*Strengths/Weaknesses:* Strengths include utilization of a comparison group of infants exposed to TCAs, and quantification of alcohol and cigarette use as well as lifestyle factors. In addition, multiple outcome domains were studied, examiners were blinded, standardized assessments of depression were used, and maternal behavioral factors were included. There were controls for maternal IQ and social class and information on recruitment and attrition was provided. Weaknesses include the convenience sample, consisting of women who might be at greater risk because they consulted an information service. In addition, only 60% of those identified were studied, outcomes were assessed at multiple ages with great variability in age range (from 1 to 7 years), no direct drug assays or measures were used, and interviews were the only source for history. The numbers of children studied at each age and subjected to each test were not given. The sample size was too small to control for confounding factors and to look into specific trimester exposures; in particular, cigarette and alcohol use are confounded with fluoxetine use. Only global tests of outcome were used, which may have been inadequate to identify specific teratogenic effects. Despite treatment, groups were marginally different on depression measures, which do not appear to have been controlled. Medication doses do

not appear to have been considered. Those confounders that were controlled in the new analysis were not identified.

*Utility (Adequacy) for CERHR Evaluation Process:* This report is of minimal utility in the Evaluation Process. Although it is generally supportive of a lack of effect of pregnancy exposure to fluoxetine on subsequent neurobehavioral testing, the methodologic difficulties could have permitted an important medication effect to escape detection.

Nulman et al. *(109*) published an additional study from Motherisk on children who had been exposed throughout pregnancy rather than only during the first trimester. Eighteen of the fluoxetine-exposed and 36 of the TCA-exposed mother-child pairs had been included in the first study *(107*). Pregnancies exposed to more than one antidepressant were excluded. The mothers and children were tested in a manner similar to the 1997 study. Children underwent neurobehavioral testing between the ages of 15 and 71 months. Exact ages at testing were not given, but children exposed to fluoxetine were on average younger than the control children (28.0 ± 10.9 months versus 41.6 ± 19.4 months, mean ± SD, $P < 0.003$). Children in the fluoxetine group were said to weigh less than children in the TCA group; this difference was based on ANOVA applied to mean percentiles (46.9 ± 31.1 vs. 63.5 ± 28.4 percentile). There were no differences among groups in maternal age, IQ, or socioeconomic status. According to the authors, "women in both antidepressant groups tended to consume more ethanol and to smoke more cigarettes during the index pregnancy" than women in the comparison group; data were not shown. Women in the fluoxetine group took a greater number of anxiolytics during pregnancy than women in the other group, and had higher scores on the Center for Epidemiologic Studies Depression (CES-D) questionnaire than women in the other groups. Women in the TCA group had higher scores than the control women on the CES-D. Women in the fluoxetine group had more episodes of depression between delivery and assessment than women in the TCA group. The Global Assessment of Functioning scores were higher in the control women than in the other two groups of women. Scores of the children on the cognitive tests are shown in Table 15 and demonstrated little difference by exposure group. In contrast to the previous study, the number of children subjected to each test was given in this paper. In addition, there were said to be no differences between the three groups across the nine temperament scales or three behavioral scales of the Child Behavior Checklist; data were not shown. A multiple regression analysis was used to consider maternal IQ, socioeconomic status, ethanol and cigarette use, depression severity, depression duration, treatment duration, number of depressive episodes after delivery, and medications used for depression. Medication treatment was not significantly associated with any of the cognitive test outcomes. Duration of maternal depression was negatively associated with the McCarthy Global Cognitive Index, and number of depressive episodes in the mother since delivery was negatively associated with language scores.

*Table 15. Neurobehavioral Results from Nulman et al (109)*

| Test | TCA | | Fluoxetine | | Control | |
|---|---|---|---|---|---|---|
| | *n* | *mean ± SD (95% CI)* | *n* | *mean ± SD (95% CI)* | *n* | *mean ± SD (95% CI)* |
| Bayley Scales of Infant Development | | | | | | |
| Mental | 28 | 110.9 ± 18.0 (104.0, 118.0) | 33 | 104.4 ± 15.5 (98.9, 109.9) | 18 | 104.1 ± 13.7 (97.3, 110.9) |
| Psychomotor | 28 | 100.1 ± 12.5 (95.3, 105.0) | 33 | 97.7 ± 11.0 (93.8, 101.6) | 18 | 98.3 ± 9.7 (94.0, 103.2) |
| Global Cognitive Index from McCarthy Scales of Children's Abilities | 18 | 117.8 ± 10.4 (112.6, 122.9) | 6 | 108.7 ± 19.9 (87.8, 129.5) | 16 | 118.4 ± 9.1 (113.6, 123.3) |
| Reynell Verbal Comprehension Scale | 45 | 1.1 ± 0.9 (0.8, 1.4) | 38 | 0.2 ± 1.3 (−0.2, 0.7) | 34 | 0.4 ± 1.0 (0.0, 0.7) |
| Reynell Expressive Language Scales | 45 | 0.2 ± 1.0 (−0.1, 0.4) | 37 | −0.3 ± 1.1 (− 0.7, 0.1) | 34 | −0.1 ± 1.2 (−0.5, 0.3) |

*Strengths/Weaknesses:* This study shares many of the strengths and weaknesses of the previous study. The inclusion of the number of children subjected to each test is an improvement; however, the tests remain relatively insensitive. The multiple regression is helpful in controlling the effects of potential confounders.

*Utility/Adequacy in CERHR Evaluation Process:* This report is of limited utility in the Evaluation Process. Although it is generally supportive of a lack of effect of pregnancy exposure to fluoxetine on subsequent neurobehavioral testing, the methodologic difficulties could have permitted an important medication effect to escape detection.

Heikkinen et al. *(19*) followed 11 women treated for depression or panic disorder with fluoxetine at 20–40 mg/day during pregnancy and compared outcomes with a control group of 10 women not on psychotropic medication matched for age, gravidity, parity, gestational weeks, and mode of delivery. **[One control subject dropped out. It is not indicated how or when the control group was recruited. Because there was matching for gestational age and mode of delivery, recruitment after delivery may have occurred. Alternatively, the author statement that matching was on gestational weeks may have referred to gestational age at first prenatal visit.]** Apgar scores were not significantly different between the groups. **[Apgar scores were presented as means ± SD in a table, raising the concern that they were analyzed using a parametric statistical test.]** Infants were followed to 12 months of age. Neurologic development was assessed by "modified Gesell developmental schedules including gross and fine motor functions, tonus, speech development, sensory screening, and social behavior." Outcome was classified as normal or abnormal. All 21 children were said to be normal at 12 months of age.

*Strengths/Weaknesses:* It is a strength that this study was prospective, spanning pregnancy and lactation with a limited range of exposure and with controlling for age, gestational age, parity, and delivery mode. Weaknesses include the inadequate and very small sample size, multiple drug exposures, and use of Gesell schedules, which are outdated and too global to detect subtle neurocognitive effects. In

addition, outcome classification was dichotomized rather than evaluated as continuous and therefore was insensitive. Children were followed only to 12 months of age, which is inadequate. There was inadequate information on important maternal factors such as level of depression, IQ, socioeconomic status, and behavioral style, as well as on other drug usage.

*Utility (Adequacy) for CERHR Evaluation Process:* This study is of minimal utility in the Evaluation Process due to methodologic limitations.

Oberlander et al. *(110*) studied acute pain response in infants exposed to psychotropic agents during prenatal development to determine if potential changes in neurodevelopment could be evident as altered pain responses. Facial responses and cardiac autonomic reactivity were recorded in healthy, full-term 2-day-old infants during a heel lance test for phenylketonuria. Infants in the medicated group were exposed during the last two trimesters of gestation. Twenty-two of the infants were exposed to an SRI (fluoxetine: n = 7, paroxetine: n = 11, sertraline: n = 4). Sixteen of the infants were exposed to both clonazepam (a GABA agonist) and an SRI (fluoxetine: n = 2, paroxetine: n = 14). Infants had detectable levels of drugs in their plasma during testing and the mean fluoxetine level was measured at 40.7 ng/mL. The control group consisted of 23 infants who were not exposed to medications *in utero*. Data were analyzed by ANOVA, *post hoc* comparisons, and/or analysis of covariance. Factors considered in the analyses included duration of exposure, breast feeding, age at time of test, use of maternal analgesia, and maternal antidepressant dose at time of delivery. During heel lance, infants exposed to SRIs or to SRIs and clonazepam had less facial activity and infants exposed to SRIs had a slower heart rate compared to control infants. Parasympathetic cardiac modulation was determined through measures of heart rate variability and the transfer relationship between heart rate and respiration. While the control infants were found to have a sustained sympathetic cardiac response, infants exposed to SRIs and SRIs plus clonazepam had greater maintenance of parasympathetic cardiac modulation. The authors concluded that attenuated pain response and increased parasympathetic cardiac modulation in exposed infants could have been due to direct pharmacologic actions of the drugs, which were still present in the infants, or altered brain development as a result of *in utero* drug exposure. The authors also noted their study was unable to distinguish between effects caused by drug exposure vs. stress because it did not include a depressed group of mothers who were not treated with antidepressants.

*Strengths/Weaknesses:* This study is the best and most elegant of the studies on the effects of prenatal exposure reviewed. It was hypothesis-driven and included appropriate narrow band tests that can be used to detect specific, subtle neurobehavioral assessments considered to be at risk from this exposure. Endpoints are selected that fit a model of underlying mechanisms that may be perturbed by exposure to fluoxetine during pregnancy. Exposure is based on detectable levels of drugs in the infants' plasma during testing rather than only on maternal report. Appropriate data analyses and covariates were used. This sophisticated study was designed to examine whether fluoxetine has an impact on facial responses and cardiac autonomic reactivity. Attenuated pain responses and increased parasympathetic cardiac modulation were found in exposed infants. The authors acknowledge that inclusion of a depressed group of mothers who were not treated with antidepressants would have strengthened the interpretation of the findings that these effects were due to drug exposure rather than stress experienced by the mothers treated with antidepressants. This study was conducted with full-term infants, so the impact on potentially more vulnerable groups, such as pre-term infants, is not known. Mothers were recruited during pregnancy as part of a larger study of psychotropic medication

use pre- and postpartum. However, data are based on a subgroup of infants who were recruited by a research nurse following delivery. Although the levels of maternal drug treatment during pregnancy were known for the mothers, the analyses were based on group membership rather than on maternal levels during pregnancy, presumably because median doses of SRIs and benzodiazepines and length of exposure did not differ between groups. However, looking at the data in Table 2 of the study, there do appear to be potential differences in the drug exposure of the two treatment groups. The authors acknowledge that they cannot rule out the contribution of benzodiazepines co-administered to 41% of the depressed women in the study. Nor can they determine whether the blunted facial expressions and increased parasympathetic cardiac modulation to the heel stick at 2 days are due to prenatal alterations in the fetal brain or the continued presence of the drugs received via placental transfer still in the infants 2 days postpartum.

*Utility (Adequacy) for CERHR Evaluation Process:* This study is adequate for an evaluation of potential neonatal neurobehavioral effects of maternal fluoxetine use prior to delivery.

Chambers et al. *(89*) was reviewed in Section 3.1.1.2. with respect to congenital abnormalities, but presents some findings regarding poor neonatal adaptation. This study involved 228 women who called the California Teratology Information Service with concerns about fluoxetine exposure. A comparison group of 254 women who called with concerns about early pregnancy exposure to drugs or treatments considered benign and whose alcohol exposure was below levels found to be detrimental (< 1.0 oz absolute alcohol or the equivalent of < 2 standard drinks/week) were matched to the fluoxetine group based on proximity of the call in time to the call made by the fluoxetine-exposed women. The study found that infants exposed throughout pregnancy as contrasted to those exposed only during the first trimester or controls were more likely to show an increased incidence of prematurity, a decrease in birth weight and length, to be admitted to a special care nursery, and to have an increased risk of poor neonatal adaptation, defined as reported jitteriness, tachypnea, hypoglycemia, hypothermia, poor tone, respiratory distress, weak or absent cry, or desaturation on feeding. These items were obtained from newborn nursery records and classified by two independent investigators.

*Strengths/Weaknesses:* The use of the proximity of the phone call measure is not explained and seems less optimal than matching on the basis of gestational weeks of pregnancy. Nonetheless, this prospective study was carefully conducted. There is some ambiguity regarding the statistical analysis, given that it appears that group was entered into the multiple linear regression analyses and that dose of fluoxetine was also entered as a potential confounder or additional risk factor. This approach would seem to lead to an underestimate of the actual relative risk of poor neonatal adaptation and admission to a special nursery, since some of the variance attributable to group would be reduced by its correlation with dose.

*Utility (Adequacy) for CERHR Evaluation Process:* This report is adequate for an evaluation of potential neonatal effects of antenatal fluoxetine exposure and its findings are consistent with abnormalities of neonatal adaptation associated with exposure to fluoxetine in late pregnancy.

In a report published only in abstract, Mattson et al. *(111*) performed a "comprehensive neuropsychological evaluation" on 66 children aged 4–6 years born to women who took fluoxetine during pregnancy. Comparisons were made with 30 children born to women with pregnancy exposures "not

deemed to be teratogenic." **[This report originated from San Diego and shares some authors with Chambers et al. *(89)*, so it is assumed to include women who called the California Teratology Information Service. It is not known whether any of the children reported in Chambers et al. 1996 are included in this abstract.]** Comparisons were made in verbal and nonverbal subtests of the Wechsler Preschool and Primary Scale of Intelligence™–Revised (WPPSI-R), verbal learning/memory, academic skill, language, short-term memory/attention, motor, and parent-rated behavior, and the abstract authors reports no significant difference between children with and without prenatal fluoxetine exposure**.**

*Strengths/Weaknesses:* This abstract describes a study that used a comprehensive battery of tests to assess children recruited retrospectively at 4–6 years from the California Teratology Information Service. No data are available in the abstract regarding the dose or timing of fluoxetine exposure, whether other drugs were taken in addition to fluoxetine, or the socioenvironmental or medical background of the subjects and controls. It is not clear whether this study is a follow-up of some of the children in the Chambers et al. 1996 study. In that case Mattson et al. may have access to data collected prospectively in the Chambers study, but such quantitative data are not mentioned or used in the study described in the abstract. As presented in the abstract, no significant group effects were found. The lack of effects could be due to a retrospective maternal report.

*Utility (Adequacy) for CERHR Evaluation Process:* This abstract is not adequate for use in the Evaluation Process based on the lack of available detail. Review of the full report, when available, would be expected to provide more detailed information.

Zeskind and Stephens *(112*) evaluated SRI-exposed and unexposed term infants at 14–39 hours of age. SRI exposure was determined based on medical record review. Of 17 SRI-exposed women who consented to participate and who were not taking other psychotropic medications, 1 woman used fluoxetine (30 mg/day) up to the time of delivery, and another woman used fluoxetine (dose not stated) during part of her pregnancy but was on another SRI at the time of delivery. The non-fluoxetine SRIs taken by women in this study included sertraline, paroxetine, and citalopram. Buproprion was also used by one of the women, in combination with SRIs. A control sample of 17 women were matched on maternal cigarette use (5 women per group), maternal age (± 2 years), and Medicaid status (3 women per group). Infants were evaluated using the Brazelton Neurobehavioral Assessment Scale, sleep organization, number of startles and tremulousness (rated on a 3-point scale), motor activity (using motion detectors on the wrists and ankles), and heart rate variability (an assessment of autonomic function). There were no differences between the groups of mothers in amount of ethanol used during pregnancy. Marijuana was used by four women in the SRI group, but their infants' results did not differ from the results in infants without maternal marijuana use and the authors did not use marijuana exposure as a covariate in their analysis. Infants born to women on SRIs displayed fewer different behavioral states, fewer state changes, a higher score for tremulousness, and fewer bouts of active sleep with an increase in the length of active sleep. Unadjusted data suggested an increase in motor activity and a decrease in heart rate variability associated with SRI exposure; when the data were adjusted for gestational age at delivery, the *P* values increased to 0.08 and 0.07 for these two outcome parameters. The authors concluded that SRI-exposed healthy term infants show "increased tremulousness, less flexible and dampened state regulation, greater amounts of uninterrupted REM sleep, greater numbers of startles or sudden arousals, more generalized motor activity, and greater

autonomic dysregulation than comparable infants in the term nursery."

*Strengths/Weaknesses:* The adequacy of maternal depression treatment was not assessed and could have had an influence on neonatal behavior. Intrapartum events, such as analgesic/anesthetic use and mode of delivery, were not considered. Exposure to illicit drugs was assessed only by record review and by infant urine drug screen "when the infant or the mother seemed to be at risk for drug use/exposure." Breastfeeding status was not addressed and may have influenced outcome, either through additional medication exposure or through effects of nursing on behavior. Therefore, a number of unmeasured factors could have influenced neonatal behavior and the other endpoints evaluated in this study. The arbitrary scores appears to have been analyzed by t-tests, rather than by a method more appropriate for ranked data. Multiple comparisons were made without a correction in the analysis. Only 17 (71%) of 24 eligible mothers participated. Finally, the use of fluoxetine by only two mothers, only one of whom was using it at term, decreases the applicability of this study to a consideration of fluoxetine effects. A strength of this study was the performance of the neonatal evaluations by a single evaluator who was blind to the exposure status of the infants.

*Utility (Adequacy) for CERHR Evaluation Process:* By itself, this study cannot be used to evaluate the possible developmental effects of fluoxetine, both because of the small number of fluoxetine-exposed infants and because of the methodologic considerations discussed above. The Panel notes, however, that the results of the study, taken at face value, are generally supportive of other studies identifying alterations in neonatal behavior following maternal use of fluoxetine.



### *3.1.2. Exposure During Breast Feeding*

A limited number of studies reported symptoms in infants breastfed by mothers taking fluoxetine. Many of the studies measured fluoxetine and norfluoxetine levels in milk and those values are reported in Section 1.2.3.3. Hale et al. *(27)* reported an infant, born to a women taking 40 mg fluoxetine per day, who was brought to medical attention on day 11 of life with somnolence, grunting, hypotonia, and a rectal temperature of 102°F. The mother reported that the child had begun to look ill at 3 days of age. Serum fluoxetine in the baby was below limits of detection (<40 ng/mL), but the serum norfluoxetine concentration was 142 ng/mL, which is at the upper end of the range reported for breastfed infants (see Section 1.2.3.3). The child had a blood leukocyte count of 22,500 with 11% bands, 65 segs, 15 lymphs, 1 atypical lymph, and 8 monocytes, a platelet count of 488,000, and a hematocrit of 49.1%. The authors reported negative bacterial and viral cultures of blood, cerebrospinal fluid, and urine. The toxicology screen was negative. Breastfeeding was discontinued and symptoms resolved over 3 weeks.

In a study involving 20 infants exposed to fluoxetine in milk, mothers reported no symptoms such as gastrointestinal effects, lethargy, changes in sleep pattern, or easy bruising *(22)*. No symptoms were reported in a study of 10 mothers and 11 infants *(26)* and in a case report of 1 infant *(21)*. A study with 14 infants reported colic in 1 infant, colic and hyperactivity in 1 infant, and "withdrawal symptoms" (i.e., uncontrolled crying, irritability, and poor feeding) in 2 infants, 1 of whom may have also been exposed to methadone *in utero (28)*. Increased irritability was observed by the father but not the mother or pediatrician of one infant *(23)*. Colic, increased crying, reduced sleep, increased vomiting, and watery stools were observed in one infant *(24)*; the symptoms were relieved when the infant was fed formula and symptoms resumed when fed breast milk. It was noted that the mother's breast milk was not tested for antigens that can cause colic. Seizure-like activity at 3 weeks and 4 months of age and cyanosis at

5½ months of age were reported in one breastfed infant whose mother was taking carbamezepine and buspirone in addition to fluoxetine *(20)*. Results of neurological evaluations, electroencephalographs, and brain magnetic resonance imaging tests were normal. At 1 year of age, no further episodes were reported and the infant was developing normally. **[The studies described in the first two paragraphs in this section were not designed to determine if there is an association between fluoxetine exposure and the reported symptoms. The studies to consider are those that attempt to define a denominator (study population) and employ an analytical design with a comparison group.]**

Yoshida et al. *(25)* examined mental and psychomotor performance using the Bayley Scale of Infant Development in four infants breastfed by mothers taking fluoxetine. The infants were 1–18 weeks old when mothers began taking fluoxetine and duration of breastfeeding during fluoxetine treatment was 12–52 weeks. Three infants were assessed up until 12–13 months of age and the fourth was lost to follow-up at 5 months of age. All of the infants were observed to have normal development and there were no abnormal neurological symptoms noted.

*Strengths/Weaknesses:* Although the Yoshida et al. *(25)* study appears to have a case series design, the authors collected maternal plasma, urine, and blood samples and infant urine samples. The convenience sample is subject to selection bias so external validity is limited. The length of follow-up was inadequate and the results were not predictive due to the young age at testing. The variability in age further compromises the sample size at any given developmental stage.

*Utility (Adequacy) for CERHR Evaluation Process:* This report is not adequate for use in the Evaluation Process.

Chambers et al. *(113)* conducted a retrospective cohort study to examine weight gain and possible symptoms in infants breastfed by women taking fluoxetine. Subjects for this study were selected from a prospective cohort of women enrolled in a CTIS study of fluoxetine exposure during pregnancy *(89)*. To be included in the study, the women had to have taken fluoxetine during pregnancy between 1989 and 1997, given birth to a full-term infant with no major malformations, breastfed exclusively for at least 2 weeks while taking fluoxetine, and not taken other psychotherapeutic medications or agents such as alcohol that can affect infant growth. Groups consisted of 26 women who took fluoxetine while pregnant and breastfeeding and 38 women (control group) who took fluoxetine while pregnant but not while breastfeeding. Percentages of women taking fluoxetine during the third trimester were 100 and 10.5% for the exposed and control groups, respectively. Fluoxetine doses during breastfeeding were 20–40 mg/day, with 21 of the 26 mothers exposed to the lower dose. Confounding factors that were considered included maternal age, parity, gestational age at birth, ethnicity, and socioeconomic status. The CES-D questionnaire to assess the severity of depression was completed by 77% of women in the fluoxetine group and 58% of women in the control group during mid-pregnancy but not while breastfeeding. Characteristics between the 2 subject groups were similar, except that women using fluoxetine while breastfeeding had a greater frequency of fluoxetine use during the third trimester of pregnancy (100 vs. 10.5%, $P<0.01$) and had infants with lower birth weight (3,479.5 vs. 3,711.7 g, $P=0.04$) and greater frequency of admission to special care nurseries (19.2 vs. 2.6%, $P=0.04$). Pediatric records of postnatal weight gain up to 6 months of age were reviewed to determine the effects of fluoxetine on growth. A linear regression analysis of infant weight gain demonstrated a significantly lower growth curve in infants nursed by mothers taking fluoxetine; a 392 g (95% CI:

-5 g, -780 g) deficit in body weight gain between 2 weeks and 6 months of age was noted for infants in the fluoxetine group. A repeated-measures analysis of covariance conducted in infants for which at least 2 weight measurements were available (n=19 and 11 in the fluoxetine and control groups, respectively), revealed that weight gain in the fluoxetine group was ~1.2 SD below the control group ($P=0.005$). Mothers were interviewed about symptoms in their infants and no unusual symptoms were reported. The study authors concluded that "…although there was no excess of infants in the fluoxetine group with postnatal weight measurements  2 standard deviations below the mean, these data indicate that breastfeeding while taking fluoxetine is associated with reduced growth that may be of clinical importance in situations in which infant weight gain is already of concern."

*Strengths/Weaknesses:* The retrospective study by Chambers et al. *(113*) is relatively good. A strength of the study is that pediatric records were obtained to assess growth during infancy. Other strengths included exclusion of preterm infants as well as infants with major malformations and infants exposed to other medications or agents affecting infant growth. Multiple confounding factors were considered and linear regression was used as a statistical technique. Infant gender was considered in the study. The use of a control group of breastfed infants without exposure to medication was a strength and growth curve analysis is a sensitive and sophisticated statistical technique. Although there were no concurrent measures of maternal depressive symptoms during breast feeding, the Panel finds it reasonable to assume that women in the unmedicated group could be experiencing some level of depression. Therefore, unlike most other studies lacking an unmedicated depressed comparison group, this study provides evidence of infant deficits specifically related to fluoxetine and not the underlying depressive disorder. Weaknesses include the retrospective cohort study design, and possible selection bias in using a sample enrolled on the basis of contact with a teratology information service. In addition, there were no direct measures of fluoxetine in breast milk or maternal blood; therefore, the reliability of the self-report data could not be evaluated. The reliance on maternal report for infant behavioral outcomes is a weakness.

*Utility (Adequacy) for CERHR Evaluation Process:* This report is adequate for an evaluation of possible effects of lactational exposure to fluoxetine and supports the conclusion that fluoxetine exposure in infants is associated with a decrease in growth velocity. However, because all of the postnatally exposed infants versus 10.5% of control infants were exposed to fluoxetine in the third trimester, it is not possible to rule out growth deficits resulting from prenatal exposure or residual levels of fluoxetine/norfluoxetine from third trimester exposure.

The American Academy of Pediatrics *(114, 115*) classified fluoxetine as a drug "whose effect on nursing infants is unknown but a concern." **[The Panel agrees with the American Academy of Pediatrics classification of fluoxetine.]**

### *3.1.3. Exposure During Childhood*

The FDA Medical Review *(116*) contains a summary of effectiveness and safety studies submitted by Eli Lilly and Company in support of the application for the pediatric indication. Adverse effects identified in this summary include manic reaction, hyperkinesia, rash, personality disorder, agitation, constipation, headache, nervousness, somnolence, suicide attempt, depression, endometrial hyperplasia, hostility, euphoria, and migraine. Manic reaction was reported in four subjects, hyperkinesia, rash, and personality disorder in two subjects, and the remaining effects in one subject (overlap

among symptoms was not discussed). There were 228 subjects on fluoxetine in this report. As a part of the report, the reviewing medical officer expressed concern regarding two possible adverse effects, impaired growth and prolonged QTc interval. The decrement in growth was described in terms of height increase in the fluoxetine and control subjects of 1.0 and 2.0 cm, respectively, and weight increases in the fluoxetine and control subjects of 1.2 and 2.3 kg, respectively in the 19-week study. Variances were not given but a *P* value for both is given as 0.008. Analysis by Z-score (normed for age and gender) or percentile yielded similar apparent decrements in growth.

The prolongation of the QTc interval was identified in an early study and reportedly not confirmed in later studies. The medical officer reported that the sponsor attributed the early report to random variation. The medical officer, however, wrote, "I am not persuaded that the finding from the initial reading is an artifact of variability attributable to sinus arrhythmia. There would have to be some reason why this factor would affect the QT intervals of fluoxetine and placebo patients differently. The finding of an increase with fluoxetine was especially robust with the Fridericia correction (P-value = 0.009); such p-values are by definition unlikely to be produced by random variability…Thus I feel that the most likely explanation for QTc interval prolongation…is that this is a true drug effect, and not an artifact of random variability. In part I suspect this is a true finding because the r-isomer of fluoxetine is known to prolong the QT interval in adults." The underlying data table showed in this initial analysis a mean ± SD QTc interval of 387.25 ± 15.98 msec with a mean ± SD change from baseline of 7.38 ± 19.2 msec **[the method of statistical comparison was not given]**. The Clinical Pharmacology and Biopharmaceutics Review *(46)* contains a box-plot analysis of the change in QTc intervals in the pediatric studies, and concluded that "these changes are not major."

Side effects of fluoxetine therapy appear similar in children to those in adults, consisting most commonly of headache, asthenia, nausea, diarrhea, insomnia, nervousness, anxiety, and somnolence *(4)*. Studies in children also demonstrated thirst, hyperkinesia, agitation, personality disorders, epistaxis, urinary frequency, and menorrhagia as treatment-emergent side effects in children and adolescents *(4)*. Many of the side effects reported in individuals using fluoxetine were also reported by subjects on placebo in these trials.

Particular concern has been expressed by some authors regarding the activating side effects of fluoxetine and the impression that children are particularly sensitive to excessive arousal or irritability. DeVane and Sallee *(117)* reviewed case reports and retrospective reports that identified behavioral problems (e.g., irritability, excessive energy) and manic symptoms associated with fluoxetine therapy. Go et al. *(118)* present three cases of manic behavior in children or adolescents associated with SRI therapy for OCD and report three others. They indicate that in an open-label trial, 5 of 15 children and adolescents treated with fluoxetine for OCD developed manic behaviors. Riddle et al. *(119)* reported that 4 of 10 children treated for OCD displayed agitation/activation as a fluoxetine side effect.

Other studies do not support activation as the most prominent side effect of fluoxetine therapy in children. Birmaher et al. *(120)* treated 37 children with fluoxetine and 37 with placebo and found activating side effects (excitement, giddiness, or disinhibition) in 7 children on fluoxetine and 4 on placebo (*P* NS). Only gastrointestinal side effects (abdominal pain and nausea, 46 vs. 22%, fluoxetine vs. placebo) and neurologic side effects (drowsiness or headache, 44 vs. 14%, fluoxetine vs. placebo) were more common in the fluoxetine than the placebo group; however, 5 children in the fluoxetine

group dropped out of the study because of behavioral disinhibition versus none in the control group. Scahill et al. *(121*) treated 12 children with fluoxetine and 12 with placebo in a study of Tourette's disorder. Motor restlessness was the only side effect more common in the fluoxetine group, occurring in 7 (58.3%) of these children compared to 2 (16.6%) of children on placebo. Fairbanks et al. *(122*) treated 16 children for anxiety in an open-label study of fluoxetine and found drowsiness to be the most common side effect, occurring in 5 children (31%). Sleep problems were reported in 3 children (19%). In a multicenter study of depression treatment sponsored by Eli Lilly and Company, Emslie et al. *(123*) treated 109 children with fluoxetine and 110 with placebo. Headache was reported to be the only side effect more commonly encountered in fluoxetine-treated children than in placebo-treated children **[the number of affected children was not given].** Another study sponsored by Eli Lilly and Company *(124*) did not include any difference in side effects among 71 children given fluoxetine and 32 children given placebo. Hyperkinesis was noted in 9 fluoxetine-exposed children (12.7%) and 1 placebo-exposed child (3.1%, $P = 0.167$ Fisher exact test).

Birmaher et al. *(125*) reviewed the charts of 21 children who began fluoxetine at ages of 11 – 17 years for treatment of anxiety disorders. Most of the children improved and none worsened, suggesting that activation/agitation were not problems in this sample.

Armitage et al. *(126*) did sleep studies on 6 children before and during fluoxetine therapy for depression (2 boys and 4 girls, average age 12.0 ± 1.9 years **[presumably SD]).** The percent time in Stage 2 sleep was reported to be decreased from 49.1 ± 6.5 to 45.2 ± 7.4% (mean ± SD; $P < 0.1$). Stage 1 sleep was significantly increased ($P < 0.05$). The Expert Panel found the clinical significance of this finding to be questionable, although the authors emphasize subjective assessment by the subjects, suggesting the sleep experience to be of lesser quality. The Panel also found there to be a multiple comparison issue in the analysis of these results. The number of arousals prior to fluoxetine therapy was 23.2 ± 7.8 and after therapy was 36.7 ± 13.3 (mean ± SD). **[The authors indicate this difference as significant at $P < 0.02$; however, performance of *t*-test by CERHR showed $P = 0.06$. The authors describe the use of ANOVA, but it is not clear how ANOVA was used with two sets of sleep data (before and during therapy).]** The most prominent finding in this study was an increase in myoclonic leg movements from 12.2 ± 5.3 to 63.3 ± 36.5 (mean ± SD; $P < 0.02$). The authors commented that fluoxetine disturbance of sleep in children is similar to adults **[although they present no data on adults]**. The Expert Panel found this study to suffer from a small number of subjects and the relative subjectivity of EEG interpretation. The interpreters do not appear to have been blinded, nor is it clear whether the same observer read the pre- and post-treatment studies. Interobserver variability may be an issue.

There has been concern that fluoxetine therapy in children may impair growth. This concern was prompted by a case report of a 13½-year-old boy with diabetes mellitus and OCD who experienced severe growth failure on 60 mg/day fluoxetine *(127*). When the fluoxetine was discontinued, the boy's growth resumed. Weintrob et al. *(128*) reported 4 children aged 11.3 – 13.7 years with growth attenuation and decreased growth hormone secretion in response to provocative testing. One of the children was on fluoxetine and three were on fluvoxamine, another SRI. A possible mediator of growth impairment is suppression of growth hormone. Noradrenergic $\alpha_2$ receptors in the arcuate nucleus are involved in growth hormone secretion (mediated by growth hormone releasing hormone). Desipramine, a TCA that blocks noradrenergic reuptake, stimulates growth hormone release. This desipramine-mediated growth hormone release was shown to be abolished in 12 depressed adults treated with fluoxetine

*(129)*. **[The Expert Panel questions the accuracy of the assay in this report. It is strange that all six subjects had exactly no (0) change from baseline growth hormone values. Every assay is expected to have some inherent variability in the results and some random scatter of values would have been expected here.]** Conclusions about the effects of serotonin modification of growth hormone are tentative, however, due to differences in growth hormone effects associated with type of serotonin receptor, species, and age (Pinilla et al. *(130)* and references therein). In addition, depression and panic disorder in humans are associated with suppression of growth hormone response to provocative testing *(131-133)*. In a review on SRIs and neuroendocrine function, Raap and Van de Kar *(134)* presented data suggesting that fluoxetine does not decrease basal growth hormone secretion but decreases growth hormone secretion in response to the 5-$HT_{1A}$ receptor agonist ipsapirone.

A review published in 2000 *(135)* represented the "Proceedings of a consensus meeting held in Geneva, Switzerland, October 5–6, 1998." This article emphasized the importance of depression in children and adolescents, summarizing studies indicating that suicide is one of the leading causes of death in children 8–18 years old and citing a 1982 report that more than 12,000 American children under the age of 15 are admitted to hospitals for suicidal behavior. This report states that 10–20% of adolescent patients will experience adverse events during SRI treatment, including gastrointestinal disturbance, headache, dizziness, insomnia, and weight gain. Based on possible lack of effectiveness of TCAs and potentially fatal consequences of TCAs in overdose, the article concludes that SRIs "should be considered the first-line treatment option in children and adolescents." No information was given concerning membership in the consensus group or the methods by which the deliberations were conducted. The consensus meeting was sponsored by Eli Lilly and Company, which was the employer of one of the authors of the report. **[The Expert Panel did not consider this review to be a reliable source of information for the Evaluation Process, although the Panel acknowledged that major depression is an important problem in children and that medication therapy may be indicated.]**

On October 27, 2003, the FDA issued a Public Health Advisory on reports of suicidality (both suicidal ideation and suicide attempts) in pediatric patients being treated with antidepressant medications for MDD *(136)*. According to the advisory, "preliminary data suggest an excess of such reports for patients assigned to several of these antidepressant drugs compared to those assigned to placebo. FDA has completed a preliminary review of such reports for eight antidepressant drugs (citalopram, fluoxetine, fluvoxamine, mirtazapine, nefazodone, paroxetine, sertraline, and venlafaxine) studied under the pediatric exclusivity provision, and has determined that additional data and analysis, and also a public discussion of available data, are needed." In their latest Public Health Advisory, the FDA stated that the contribution of antidepressants to suicidal thinking and behavior is not yet clear, and cautioned clinicians, patients, families, and caregivers to closely monitor children or adults receiving fluoxetine or other antidepressants for worsening of depression or suicidal thoughts, especially during initiation of therapy and following dose adjustments *(137)*. Manufacturers were asked to update their labels with stronger cautions and warnings about the need for monitoring of symptoms.

**[The Expert Panel finds the literature on consequences of childhood exposures to be markedly deficient. Most studies have very small sample sizes, with inadequate follow-up ranging from 6 to 13 weeks. In some studies, the number of children who completed follow-up is too few for comparison of symptoms; therefore, conclusions that there are no differences from control treatments are unwarranted. These studies are also limited by high dropout rates and multiple diagnoses.]**

## 3.2. Experimental Animal Data

### 3.2.1. Prenatal Developmental Studies

#### 3.2.1.1. Standard Segment II studies

Byrd and Markham *(51)* examined developmental toxicity in 25 Fischer 344 (F344/NHsd) rats/group gavage dosed with fluoxetine HCl (96.0% purity) in distilled water at 0, 2, 5, or 12.5 mg/kg bw/day on GD 6–15 (plug=GD 0). Dose selection was based on the results of an unpublished preliminary study that demonstrated increased maternal death and reduced fetal viability at doses ≥20 mg/kg bw/day. Results of the preliminary study are published in an FDA review by Tabacova *(138)*. **[It was not stated if concentrations of fluoxetine were verified in dosing solutions.]** Evaluations of maternal toxicity included body weight gain, food intake, and clinical signs. On GD 20, dams were sacrificed, euthanized, and necropsied. Corpora lutea were counted and implantation sites were examined. Fetuses were weighed and examined for external anomalies. A third of the fetuses were fixed in Bouin's solution for an examination of the viscera. The remaining fetuses were examined for skeletal effects. The litter was considered the unit of evaluation in statistical analyses that included analysis of covariance, Student's *t*-test, and Dunnett's *t*-test. Significant maternal effects and results for the major fetal parameters evaluated are listed in Table 16.

***Table 16. Prenatal Toxicity Study of Fluoxetine in Rats [Byrd and Markham (51)]***

| *Effects* | *Fluoxetine doses (mg/kg bw/day)* | | | |
|---|---|---|---|---|
| | ***0*** | ***2*** | ***5*** | ***12.5*** |
| Maternal weight change on GD 7–14 (g) | ~12 | ~15 | ~12 | ~2** |
| Maternal weight change on GD 14–20 (g) | ~36 | ~42 | ~45* | ~45* |
| Total maternal weight change (g) | ~57 | ~63 | ~62 | ~48* |
| Maternal food intake on GD 7–14 (g/day) | ~13 | ~12 | ~11** | ~7** |
| Number of live litters | 25 | 19 | 21 | 17 |
| Number of live fetuses [d] | 7.9±0.6 | 8.8±0.6 | 8.8±0.5 | 7.1±0.9 |
| % Postimplantation loss [d] | 7.4±1.9 | 8.0±2.5 | 7.6±1.7 | 12.6±5.8 |
| Fetal weight (g)[d] | 3.20±0.11 | 3.20±0.06 | 3.10±0.03 | 3.29±0.10 |
| % Fetuses with variations [a,d] | 0 | 0 | 0 | 0 |
| % Fetuses with deviations [b,d] | 0.3±0.3 | 0.5±0.5 | 1.3±0.9 | 0.6±0.6 |
| % Fetuses with malformations [c,d] | 1.2±0.6 | 0.5±0.5 | 0.5±0.5 | 0 |
| NOAELs | | | Maternal | Fetal |

25 dams/dose group were used initially
*$P \leq 0.05$; **$P \leq 0.01$
[a]Transitory or permanent but innocuous anomalies that occur in ≥5% of historical control fetuses
[b]Transitory or permanent but innocuous anomalies that occur in <5% of historical control fetuses
[c]Anomalies that are disfiguring or incompatible with survival, growth, development, fertility, or longevity
[d]Mean±SE

Maternal weight gain was reduced in the 12.5 mg/kg bw/day group on GD 7–14, as was total weight gain throughout pregnancy. However, weight gain was significantly increased in the 5 and 12.5 mg/kg

bw/day groups on GD 14–20, the time period including the last 2 days of treatment and 5 days following termination of treatment. Food intake was significantly lower in dams of the 5 and 12.5 mg/kg bw/day groups on GD 7–14 (~15 and 50% lower than controls, respectively). Totals of 17–25 litters/group were evaluated and no significant effects were noted for fetal viability, weight, or morphology. The authors identified maternal and fetal NOAELs of 5 and 12.5 mg/kg bw/day, respectively. **[The Expert Panel agrees with the author selection of NOAELs, noting that the slight increase in weight gain and reduction in food intake in the 5 mg/kg bw/day animals were not toxicologically significant. The Expert Panel agrees with the author assessment that the results are likely due to the known pharmacologic effects of fluoxetine on feeding behavior. The FDA Pharmacologist Review of NDA 18-936, dated March 14, 1984 *(139)*, also contains a summary of this study, but was not judged by the Panel to be more useful than the published paper by Byrd and Markham.]**

*Strengths/Weaknesses:* This study in rats used suitable controls, adequate numbers of dams/group, appropriate measures of maternal and developmental toxicity, multiple dose levels, an appropriate route of administration, and appropriate methods of analysis. The weakness of the study is the fairly low fertility rate across groups, which would not have been treatment-related with this study design.

*Utility (Adequacy) for CERHR Evaluation Process:* The Byrd and Markham study is very useful for the Evaluation Process and indicates maternal and fetal NOAELs in rats of 5 and 12.5 mg/kg bw/day, respectively. A benchmark dose for developmental toxicity using the developmental data cannot be calculated because there was no significant developmental toxicity at any of the doses tested.

Byrd and Markham *(51)* examined developmental toxicity in 15 Dutch Belted rabbits/group gavage dosed with fluoxetine HCl (96.0% purity) in distilled water at 0, 2.5, 7.5, or 15 mg/kg bw/day on GD 6–18. Dose selection was based on the results of an unpublished preliminary study that demonstrated abortion and reduced maternal body weight gain and food intake at ≥7.5 mg/kg bw/day. Results of the preliminary study are provided in an FDA review by Tabacova *(138)*. **[It was not stated if concentrations of fluoxetine were verified in dosing solutions.]** Evaluations of maternal toxicity included body weight gain, food intake, and clinical signs. Does were sacrificed and necropsied on GD 28. Corpora lutea were counted and implantation sites were examined. Fetuses were weighed and examined for external, visceral, and skeletal anomalies. Viscera were evaluated using a fresh tissue technique. The litter was considered the unit of evaluation in statistical analyses that included analysis of covariance, Student's *t*-test, and Dunnett's *t*-test. Significant maternal effects and results for the primary fetal parameters evaluated are listed in Table 17. Following a period of anorexia and weight loss, two does from the 15 mg/kg bw/day dose group died on GD 14 and 27; the postmortem evaluation revealed acute pneumonia. Three other does from the 15 mg/kg bw/day group aborted between GD 26 and 27. Noting that other investigators reported abortions in rabbits following reduced food intake, Byrd and Markham *(51)* opined that the abortions were not directly induced by fluoxetine, but were likely an indirect result of fluoxetine's established pharmacologic activity (i.e., reduced maternal food consumption). Significant maternal weight loss occurred in all treated groups on GD 6–12, and the effect remained significant in the 15 mg/kg bw/day group on GD 12–18. Rebound increases in body weight gain occurred following cessation of fluoxetine treatment. However, the 15 mg/kg bw/day group maintained a significant net loss in body weight across the duration of pregnancy. Food intake was significantly reduced in all dose groups on GD 6–12 and in the 7.5 and 15 mg/kg bw/day dose groups on GD 12–18. Fetuses from 7–15 litters/group were evaluated and no effects were noted for

fetal viability, weight, or morphology. Although statistical significance was not obtained, Tabacova *(138)*, considered an increase in postimplantation lethality (8.5 vs. 16%), decrease in live fetuses per litter (7.0 vs. 6.5), and increase in variations (an extra 13th rib and wavy ribs per fetus [total of 5 vs. 9], litter analysis not presented) at the 15 mg/kg bw/day group compared to control group to be treatment related. **[The Expert Panel disagrees with Tabacova's interpretation because historical control ranges were not discussed, the fetal endpoints do not show a dose-response when the two lower treatment groups are included, and maternal toxicity at the high dose resulted in only seven litters/eight pregnancies for evaluation.]** The authors identified a developmental NOAEL of 15 mg/kg bw/day and noted that a maternal NOAEL was not identified. Tabacova *(138)* identified a maternal NOAEL of 7.5 mg/kg bw/day **[the decrease in feed consumption during the dosing interval at all exposure levels was not considered]** and a developmental toxicity NOAEL of 7.5 mg/kg bw/day. **[The Expert Panel agrees with the NOAELs identified by Byrd and Markham *(51)*. The FDA Pharmacologist Review of NDA 18-936, dated March 14, 1984 *(139)*, also contains a summary of this study, but was not judged by the Panel to be more useful than the published paper by Byrd and Markham.]**

***Table 17. Prenatal Toxicity Study of Fluoxetine in Rabbits [Byrd and Markham (51)]***

| *Effects* | *Fluoxetine doses (mg/kg bw/day)* | | | |
|---|---|---|---|---|
| | ***0*** | ***2.5*** | ***7.5*** | ***15*** |
| Number of maternal deaths (of 15 does/group) | 0 | 0 | 0 | 2 |
| Number of abortions | 0 | 0 | 0 | 3 |
| Maternal weight change on GD 6–12 (g) | ~20 | ~-50** | ~-120** | ~-260** |
| Maternal weight change on GD 12–18 (g) | ~30 | ~-10 | ~-30 | ~-120** |
| Maternal weight change on GD 18–27 (g) | ~60 | ~50 | ~180* | ~210* |
| Total maternal weight change (g) | ~90 | ~-10 | ~40 | ~-110* |
| Maternal food intake on GD 6–12 (g/day) | ~125 | ~100* | ~60** | ~25** |
| Maternal food intake on GD 12–18 (g/day) | ~110 | ~75 | ~65* | ~10** |
| No. live litters | 15 | 14 | 13 | 7 |
| No. live fetuses [d] | 7.0±0.7 | 6.7±0.6 | 7.7±0.4 | 6.5±0.6 |
| % Postimplantation loss [d] | 8.5±3.8 | 8.2±4.6 | 7.1±2.6 | 16±7.6 |
| Fetal weight (g) [d] | 33.05 ±1.58 | 32.27±1.45 | 31.73±0.82 | 30.09±2.81 |
| % Fetuses with variations [a,d] | 3.8±1.7 | 11.9±4.0 | 14.3±4.2 | 15.5±8.4 |
| % Fetuses with deviations [b,d] | 0 | 0 | 0.8±0.8 | 2.4±2.4 |
| % Fetuses with malformations [c,d] | 0 | 0 | 0 | 0 |
| NOAELs | | | | Fetal |

*$P \leq 0.05$; **$P \leq 0.01$

[a]Transitory or permanent but innocuous anomalies that occur in ≥5% of historical control fetuses

[b]Transitory or permanent but innocuous anomalies that occur in <5% of historical control fetuses

[c]Anomalies that are disfiguring or incompatible with survival, growth, development, fertility, or longevity.

[d]Mean±SE

*Strengths/Weaknesses:* Strengths of the Byrd and Markham *(51)* studies include use of adequate numbers of rabbits, presentation of fetal anomalies as individual fetal incidence and number of litters affected, and performance of pilot studies to establish maximum tolerated doses as limited by the pharmacologic effects of fluoxetine (i.e., decrease in food consumption/anorexia). Confidence in the results of this study is reduced by the inadequate numbers of litters per group, especially at the highest dose (n = 7 litters), which results in decreased statistical power.

*Utility (Adequacy) for CERHR Evaluation Process:* The Byrd and Markham *(51)* study is adequate for the evaluation of prenatal developmental toxicity (e.g., malformations, live litter size, and fetal weights) in rats and rabbits. The developmental NOAEL is 15 mg/kg bw/day, and the maternal NOAEL is < 2.5 mg/kg bw/day. A benchmark dose calculation is not possible for the developmental data due to the lack of a significant treatment effect.

#### *3.2.1.2 Late pregnancy exposure*

da-Silva et al. *(140)* examined the effects of late pregnancy fluoxetine exposure on postnatal development of rat pups. Wistar rats (10–12/group) were administered 0, 8, or 16 mg/kg bw/day fluoxetine **[purity not specified]** in water by gavage on GD 15–20. Doses were selected to be slightly lower and higher than the developmental NOAEL of 12.5 mg/kg bw/day reported by Byrd and Markham *(51)*. Food and water intake and weight gain were evaluated in dams during treatment. Maternal data were evaluated for statistical significance using the Kruskal-Wallis test followed by the Mann-Whitney *U* test. Dams were allowed to litter and at birth, litters were culled to six pups. Postnatal growth and survival were evaluated in pups up until weaning on PND 25. On PND 60, 1 male and 1 female pup from each litter were i.p. injected with 6 mg/kg 5-methoxy-N,N-dimethyltryptamine, a $5HT_1$ receptor agonist, and assessed for behavioral responses. Litters were the unit of evaluation in statistical analyses that included two-way or one-way ANOVA, Student's *t*-test, the Kruskal-Wallis test, or the Mann-Whitney *U* test. Food intake and weight gain were reduced in dams of the 16 mg/kg bw/day group, but it was not clear if statistical significance was achieved. Gestation duration was shortened by about half a day in both the 8 and 16 mg/kg bw/day groups and the effect was said to be significant when the 8 and 16 mg/kg bw/day groups were combined and compared to the control group. **[The range of delivery days (GD 21–22) was identical in the control and both fluoxetine groups. It is unlikely that a mean of 0.4–0.5 days difference is biologically relevant within this standard range, especially because only 10–12 animals/group were included and delivery status was determined only twice daily.]** There were no effects on the number of live pups at birth or stillborn pups. Pup body weights at birth were lower in males and females of the 8 and 16 mg/kg/day groups and statistical significance was achieved for male pups in both dose groups. **[There did not appear to be a dose-related response since pup body weights were approximately equal in both dose groups and group mean litter size, known to be inversely related to pup birth weights, for both fluoxetine groups was slightly larger than the control value.]** There were no effects on pup weights at weaning or on pup survival. No effects on behavior were noted following treatment with 5-methoxy-N,N-dimethyltryptamine and authors considered this finding to be preliminary evidence that the serotonergic system was unaffected. However, they noted that additional studies on serotonin brain levels and turnover are needed before definitive conclusions can be made. Venlafaxine, a non-selective reuptake inhibitor, was also tested. Venlafaxine had no effect on gestation length, although the range of delivery days (GD 21–24) included at least 1 unusually long gestation length in the high-dose group. Venalfaxine's effects on the remaining parameters were similar to those observed

in both the control and fluoxetine-treated groups, but its effects on the remaining parameters were similar to those observed with fluoxetine treatment.

*Strengths/Weaknesses:* The study by da-Silva et al. *(140)* used appropriate sample sizes, dose levels, and statistics. The route of administration was appropriate. Although the purity of fluoxetine was not reported, it was obtained directly from the manufacturer and was most likely of sufficient quality. The effect on gestation length is questionable. The effect on male pup body weights at birth is questionable due to the lack of a dose-response relationship.

*Utility (Adequacy) for CERHR Evaluation Process:* The study by da-Silva et al. *(140)* is adequate for use in the CERHR evaluation process and supports a developmental NOAEL of 16 mg/kg bw/day for late pregnancy exposure to fluoxetine.

#### *3.2.1.3. Serotonergic, dopaminergic, and neurotoxicity endpoints*

As noted in Section 2.1, changes in serotonin transporters and receptors may be associated with depression. A number of studies examined changes in these serotonergic structure endpoints in animals exposed during prenatal or postnatal development. These studies are summarized below. Due to the radiochemical instability of $^{3}$H-fluoxetine, these studies commonly use ligands such as $^{3}$H-imipramine ($^{3}$H-IMI), which has affinity for serotonin transporters and receptor sites, and $^{3}$H-paroxetine and $^{3}$H-citalopram, which have affinity for serotonin transporters *(2)*.

In a series of studies with similar design, $^{3}$H-IMI binding sites *(141)* and phosphoinositide hydrolysis and 5-$HT_2$ receptors *(142)* in the cerebral cortex were examined in rats exposed to fluoxetine during the prenatal period. In these studies Wistar rats **[number treated not specified]** were given 0 or 2.5 mg/kg bw/day fluoxetine **[purity not specified]** in drinking water from GD 6 until parturition. The dose of 2.5 mg/kg bw/day was selected because preliminary studies demonstrated toxicity to fetuses at higher doses *(141)*. Offspring were killed at either PND 25 or 90 for an examination of the parameters listed in Table 18. One study examined 5 – 10 pups per endpoint *(142)* and it appears that similar numbers were examined in the second study *(141)*. **[Neither report specified how many treated litters were represented in the testing assessments, or whether the litter or individuals were used as the statistical unit.]** Statistical analysis included one-way ANOVA followed by Student's *t*-test. Fluoxetine treatment had no effect on dam body weights or litter size, which averaged ten pups *(141)*. Results for serotonergic endpoints are outlined in Table 18. As noted in Table 18, prenatal exposure to fluoxetine reduced density of $^{3}$H-IMI binding sites and inositol phosphate accumulation occurred at 25 days of age but not 90 days of age in rats with prenatal fluoxetine exposure. To examine the differential sensitivity of the brain following prenatal vs. adult exposure, the same parameters examined in prenatally exposed rats were evaluated in adult rats **[age, number treated, and sexes not specified]** that were given 2.5 mg/kg bw/day fluoxetine through drinking water for 15 days and killed 72 hours following withdrawal of treatment. No effects were observed following adult rat exposure. In addition, no short-term effects on phosphoinositide hydrolysis and 5-$HT_2$ receptors characteristics were noted following exposure in 25-day-old rats receiving 2.5 mg/kg fluoxetine by i.p. injection and examined 1 hour later *(142)*. Numerous other antidepressants were tested but those results will not be discussed here.

***Table 18. Effects of Prenatal Fluoxetine Exposure on Cortical Serotonergic Endpoints in Rats on PND 25 and 90***

| *Endpoint* | *Significant effects observed in offspring prenatally exposed to fluoxetine* | | *Reference* |
|---|---|---|---|
| | ***PND 25*** | ***PND 90*** | |
| Density of $^3$H-IMI binding sites in dorsal cortex | ↓30% | No effect | Montero et al. *(141)* |
| $^3$H-IMI dissociation constant in dorsal cortex | No effect | No effect | Montero et al. *(141)* |
| Serotonin-induced $^3$H- inositol phosphate accumulation in cerebral cortex | ↓[a] | No effect | Romero et al. *(142)* |
| 5-$HT_2$ receptor density and dissociation constant in cerebral cortex[b] | No effect | No effect | Romero et al. *(142)* |

$^3$H-IMI=$^3$H-imipramine
↓= Statistically significant decrease compared to rats that were not exposed to fluoxetine *in utero*
[a] Quantitative comparison with control values is not possible due to the manner of data presentation
[b] Determined by $^3$H-ketanserin binding

*Strengths/Weaknesses:* The main weaknesses of the studies by Montero et al. *(141)* and Romero et al. *(142)* are the lack of information on number of litters and animals exposed and lack of information about the methods of selecting pups for assessment at each age. If indeed multiple pups from only one or two litters/assessment/age were used for evaluations, then the modest percent change noted in density of binding sites may be more likely related to litter-to-litter or animal-to-animal variation, or slight differences in litter developmental events, than to prenatal exposures. However, many of the endpoints evaluated were not significantly affected. The findings raise the issue of the biologic relevance of such changes, because there were no "functional" endpoints monitored to correlate with the apparent neurochemical alterations. Only a single dose level of fluoxetine was used in these studies, which precludes a dose-response evaluation.

*Utility (Adequacy) for CERHR Evaluation Process:* The lack of information on pup assignments mentioned above reduces the utility of the studies by Montero et al. *(141)* and Romero et al. *(142)*. These reports do suggest that a serotonin-related endpoint may be altered immediately following weaning when there has been prenatal exposure to fluoxetine.

A series of studies examined the effects of prenatal fluoxetine exposure on serotonergic systems in rats *(143-145)*. In these studies, Sprague-Dawley rats were s.c. injected with 0 (0.9% saline) or 10 mg/kg bw/day fluoxetine HCl **[purity not reported]** on GD 13–20. The authors noted that GD 13–20 is a time when serotonergic neurons are rapidly dividing, differentiating, and establishing axonal projections in target regions. **[Rationale for dose selection was not discussed.]** At birth, litters were culled to nine pups (five males and four females) and the pups were fostered to untreated dams. Various structural and functional endpoints (see Table 19) were examined in prenatally exposed rats on PND 25 (prepubescence) and PND 70 (adulthood). In 1 study, male and female rats were examined on PND 25 but only male rats were examined on PND 70 *(143)*. Only male rats were examined in the other two studies *(144, 145)*. The authors chose not to examine female offspring on PND 70 in order

to avoid effects associated with varying hormonal responses during different stages of the estrous cycle. For each analysis, three to ten offspring/group, obtained from different litters within the same treatment group, were examined. Statistical analyses included one- or two-way ANOVA, Student's *t*-test, and/or the Newman-Keuls' test.

***Table 19. Effects of Prenatal Fluoxetine Exposure on Serotonergic Endpoints in Forebrain and Midbrain of Rats on PND 25 and 70***

| ***Endpoint*** | ***Significant effects observed in offspring prenatally exposed to fluoxetine*** | | ***Reference*** |
|---|---|---|---|
| | ***PND 25*** | ***PND 70*** | |
| Hypothalamic 5-$HT_{2A/2C}$ receptor density [a] | No effect | ↓ 35% | Cabrera and Battaglia *(143)* |
| Cortical 5-$HT_{2A/2C}$ receptor density [a] | No effect | No effect | Cabrera and Battaglia *(143)* |
| Hypothalamic and cortical 5-$HT_{2A/2C}$ receptor affinity for DOI | No effect | No effect | Cabrera and Battaglia *(143)* |
| Density of hypothalamic serotonin uptake sites (a measure of serotonin innervation) [b] | No effect | No effect | Cabrera and Battaglia *(143)* |
| Density of serotonin uptake sites in frontal cortex, hypothalamus, hippocampus, striatum, or midbrain [b] | No effect | No effect | Cabrera-Vera et al. *(144)* |
| Density of serotonin transporters in hippocampal subregions of the telencephalon [c] | 47% ↑ in CA2 and 38% ↑ in CA3 area of Ammon's horn | No effect | Cabrera-Vera and Battaglia *(145)* |
| Density of serotonin transporters in amygdala subregions of the telencephalon [c] | 32% ↑ in baso-lateral nucleus and 44%↑ in medial nucleus | No effect | Cabrera-Vera and Battaglia *(145)* |
| Density of serotonin transporters in cortex, septum, and basal ganglia subregions of telencephalon [c] | No effect | No effect | Cabrera-Vera and Battaglia *(145)* |
| Density of serotonin transporters in hypothalamic subregions of the diencephalon [c] | 21% ↓ in dorso-medial nucleus and 21% ↑ in lateral hypothalamus | No effect | Cabrera-Vera and Battaglia *(145)* |
| Density of serotonin transporters in the tegmentum subregions of the mesencephalon [c] | 19% ↓ in substantia nigra | No effect | Cabrera-Vera and Battaglia *(145)* |
| Density of serotonin transporters in the raphe nuclei subregions of the mesencephalon [c] | No effect | No effect | Cabrera-Vera and Battaglia *(145)* |

*Table 19 (continued)*

| *Endpoint* | *Significant effects observed in offspring prenatally exposed to fluoxetine* | | *Reference* |
|---|---|---|---|
| | *PND 25* | *PND 70* | |
| 5-$HT_{2A/2C}$-mediated neuroendocrine responses to a DOI agonist challenge dose (measure of receptor function determined by blood levels of adrenocorticotropin, corticosterone, and renin) | No effect | 58% ↓ in blood adrenocorticotropin | Cabrera and Battaglia *(143)* |
| Basal serotonin levels in frontal cortex, hypothalamus, hippocampus, striatum, or midbrain | 28% ↓ in frontal cortex | 28% ↓ in midbrain | Cabrera-Vera et al. *(144)* |
| Basal 5-HIAA levels in frontal cortex, hypothalamus, hippocampus, striatum, or midbrain | No effect | No effect | Cabrera-Vera et al. *(144)* |
| Basal serotonin turnover (ratio of 5-HIAA/serotonin) in frontal cortex, hypothalamus, hippocampus, striatum, or midbrain | No effect | No effect | Cabrera-Vera et al. *(144)* |
| Basal dopamine levels in hypothalamus, striatum, and midbrain | No effect | No effect | Cabrera-Vera et al. *(144)* |
| Basal norepinephrine levels in frontal cortex, hypothalamus, hippocampus, striatum, or midbrain | No effect | No effect | Cabrera-Vera et al. *(144)* |
| Serotonin levels in frontal cortex, hypothalamus, hippocampus, following injection with p-chloroamphetamine | No effect | ↓ in midbrain (~50% in controls vs. 20% in treated) | Cabrera-Vera et al. *(144)* |

DOI = (±)-4-iodo,2,5-dimethoxyphenylisopropylamine
↓= Statistically significant decrease compared to rats that were not exposed to fluoxetine *in utero*
↑= Statistically significant increase compared to rats that were not exposed to fluoxetine *in utero*
[a]Determined with $^{125}$I-DOI
[b]Determined with $^{3}$H-paroxetine
[c]Determined with $^{3}$H-citalopram and autoradiography

In the study by Cabrera and Battaglia *(143)*, gestational fluoxetine treatment had no effect on maternal weight gain or litter sizes. Body weights of both male and female offspring of the fluoxetine group were significantly reduced by about 8% on PND 0. No effects on offspring body weight were noted on PND 28, but male body weights were significantly lower than controls (~14%) on PND 70. Results of forebrain and midbrain serotonergic endpoints are outlined in Table 19. As noted in Table 19, prenatal fluoxetine exposure resulted in age- and region-specific effects on select serotonergic endpoints in rats. While this study reported no effects on brain indices examined at 25 days of age, reduced hypothalamic 5-$HT_{2A/2C}$ receptor density and reduced neuroendocrine responses to an agonist for these receptors were seen in males examined at 70 days of age. The authors noted that alterations in serotonergic pathways have been implicated in human psychiatric disorders. However the study authors noted that

more research is required to determine the implications of these study results for humans.

Cabrera-Vera et al. *(144*) used the same prenatal exposure regimen to examine biochemical effects on the functional integrity of forebrain and midbrain serotonergic neurons at prepubescent (PND 26) and adult ages (PND 70) in rats. As shown in Table 19, age- and region-specific effects were seen with significant effects at both ages. Prepubescent male rats prenatally exposed to fluoxetine showed reduced 5HT content in the frontal cortex, while at adult ages, effects were seen in midbrain 5HT content at basal level and following p-chloroamphetamine injection.

In the third study by these investigators *(145*), serotonin transporter densities in forebrain and midbrain areas were evaluated in prepubescent and adult male rats following the same prenatal exposure regimen. As shown in Table 19, age-dependent and site-specific alterations in the density of 5HT transporters were found. Multiple alterations were seen in several forebrain areas (i.e., hypothalamus, hippocampus, amygdala, and substantia nigra) in 25-day-old male rats, but none were found in adults. These effects would likely manifest as altered serotonergic neurotransmission in the limbic areas at day 25. The authors pointed out that the failure to find effects on transporter density at the later age does not rule out the possible presence of functional alterations.

*Strengths/Weaknesses:* A strength of these studies *(143-145*) is the inclusion of a single functional endpoint that is modulated by central serotonergic pathways (a 58% decrease in blood adrenocorticotropin). In addition, procedures for assessing offspring endpoints were appropriately described and conducted. A strong rationale was presented for selection of the prenatal exposure period. A weakness of the studies is that only a single dose level of fluoxetine was used, thus precluding a dose-response evaluation. Exclusion of female offspring assessment at PND 70 in 1 study and total exclusion from 2 of the studies is problematic from a risk assessment perspective; estrous cycles stages can easily be standarized using vaginal cytology observations.

*Utility (Adequacy) for CERHR Evaluation Process:* These studies *(143-145*) provide suggestive evidence for alterations in serotonin mediated/modulated function after developmental exposure. However, the relatively modest degree of change in parameters (usually < 50%), lack of dose-response data, and clear patterns of effects suggesting that certain CNS areas/pathways may be affected by exposure decrease the utility of these studies in a quantitative estimate of risk.

Del Rio et al. *(146*) also reported reduced density of $^{3}$H-IMI binding sites but no effect on dissociation constant in the cortices of 25-day-old rats the mothers of which were exposed to 3 mg/kg bw/day fluoxetine in drinking water during the last 15 days of gestation. **[The reduced density of these binding sites implicates alterations in serotonin uptake mechanisms. No details of experimental procedures were provided in the publication.]**

Effects of prenatal fluoxetine exposure on dopamine-related endpoints were examined in a study to determine mechanisms of cocaine-induced behavioral alterations in rats *(147*). Eight pregnant Sprague-Dawley rats received a peroral dose of fluoxetine 12.5 mg/kg in saline on GD 8 – 20 **[specific route was not specified but assumed to be by gavage; fluoxetine purity not specified]**. A control group of 12 dams received 0.9% saline by s.c. injection. **[Cocaine, the primary compound of interest in this study, was administered by the s.c. route. There was not a separate control group treated**

**by the oral route.]** Weight gain and food intake were monitored in dams. At birth, pups were sexed and weighed, culled to five males and five females per litter when possible, and fostered to untreated dams. Developmental landmarks including tooth eruption and eye opening were recorded. Litters were considered the unit of analysis, and statistical analyses included Student's *t*-test or ANOVA with *post hoc* analysis by Duncan's multiple range test. Compared to saline controls, fluoxetine had no effect on weight gain or food intake in dams **[data not shown]**. Fluoxetine treatment had no effect on pup weights on PND 1 or 20. There was also no effect on litter size, sex ratio, number stillborn, and developmental landmarks **[data not shown for any of these endpoints]**. On PND 19, 1 male and 1 female from each litter were randomly selected for a quinpirole challenge test to assess catecholamine function. Pup behavior stereotypy and locomotion were observed following s.c. injections of either 0.03 or 0.09 mg/kg quinpirole-HCl (a dopamine $D_2$ receptor agonist). On PND 20, 4 male pups per litter that were not treated with quinpirole were killed. Striatal tissues from those pups were dissected and pooled from each litter for an examination assessment of dopamine $D_1$ and $D_2$ receptors. Fluoxetine treatment had no effect on behavior in the quinpirole challenge test or on dopamine receptor binding. In contrast, prenatal cocaine treatment increased quinpirole-induced behavioral stereotypy and motor activity. Cocaine treatment had no effect on dopamine receptor binding ($K_d$ or $B_{max}$). No effects on the quinpirole challenge test or dopamine-receptor binding were noted for the other drugs tested, including desipramine, GBR 12909, and lidocaine.

*Strengths/Weaknesses:* A strength of this study by Stewart et al. *(147*) is that procedures for assessing offspring endpoints were appropriately described and conducted. In addition, the study included standard background maternal and offspring measurements with which to compare any treatment-related changes in dopaminergic endpoints of primary interest. A weakness of this study is that only a single dose level of fluoxetine was used, thus precluding a dose-response evaluation. In addition, background data were not presented. In contrast to other fluoxetine studies, decreased dam weights and food consumption, decreased offspring birth weights, and/or postnatal survival decreases were not observed. The lack of adverse maternal effects at the dose used in this study (12.5 mg/kg bw/day) is unusual and raises questions about lack of findings in other endpoints monitored.

*Utility (Adequacy) for CERHR Evaluation Process:* The study by Stewart et al. *(147*) is only useful for suggesting that fluoxetine exposure produces no strong interactive effects on early neurochemical and behavioral endpoints of dopaminergic function.

Vorhees et al. *(148*) evaluated neurotoxicity in offspring of Sprague-Dawley CD (VAF, Charles River) rats gavage dosed with 1, 5, or 12 mg/kg bw/day fluoxetine HCl **[purity not reported]** in water on GD 7–20. In order to obtain at least 25 litters/treatment group with ≥12 pups/litter, 25–47 dams were treated in each group using continuous breeding procedures. Dose selection was based on doses used in a prenatal developmental toxicity study *(51*), an *in vitro* study *(149*), and an embryo/fetal distribution study *(50*). Two control groups with 24–28 dams/group were gavaged with water on GD 7–20. One control group received food and water *ad libitum*, while the other control group was pair-fed and pair-watered to achieve the same food and water intake rates as the 12 mg/kg bw/day fluoxetine group. Maternal body weights and food and water intake were monitored. Dams were allowed to litter and at birth, pups were examined, sexed, weighed, and culled to six males and six females/litter. Offspring weight gain and survival were monitored up to PND 77. Neurobehavior testing was conducted in offspring during 3 stages of development: preweaning (PND 16), juvenile (PND 45), and adult (PND

75). During each stage, two male-female pairs/litter/group were tested on a certain cluster of measures. Two pairs/litter/group were examined for locomotor activity, acoustic startle response, and startle response at 1 hour or 0.5 hour following i.p. administration of a pharmacological challenge dose of 10 mg/kg fluoxetine or 1 mg/kg apomorphine, respectively. On PND 45, learning and memory were also evaluated in two pairs/litter by spontaneous alternation, passive avoidance, and the Cincinnati water maze. On PND 18, 71, and 79, 2 males and females from each of 6 litters/treatment group were sacrificed and their brain weights were measured. **[This behavioral testing battery is consistent with typically used comprehensive batteries, with the exception of the inclusion of a fluoxetine challenge during auditory startle testing.]** Statistical analyses included Fisher's test for uncorrelated proportions to evaluate offspring mortality and ANOVA procedures using litter means for other endpoints.

Body weight loss occurred in dams from the 12 mg/kg bw/day and pair-fed control groups during treatment and weights remained significantly lower than the *ad libitum* control group throughout gestation. Reproductive and developmental effects are listed in Table 20.

***Table 20. Reproductive and Developmental Effects in Rats [Vorhees et al. (148)]***

| *Effects* | *Doses (mg/kg bw/day)* | | | | |
|---|---|---|---|---|---|
| | *0: ALC[a]* | *0: PFC[b]* | *1* | *5* | *12* |
| No. of litters with <10 liveborn/no. of sperm positive dams (%) | 1/28 (3.6) | 0/24 (0) | 1/29 (3.4) | 0/25 (0) | 7/47 (15) |
| No. of litters with sex ratio >8:4 / no. of sperm positive dams (%) | 1/28 (3.6) | 0/24 (0) | 2/29 (6.9) | 0/25 (0) | 3/47 (6.4) |
| No. of litters with all offspring dead by PND 7 | 0 | 0 | 0 | 0 | 1 |
| No. of litters (%) reaching weaning | 25/28 (92.6) | 24/24 (100) | 25/29 (89.3) | 25/25 (100) | 36/47 (76.6)* |
| Gestation length (days)[c] | 21.4±0.1 | 21.9±0.1** | 21.4±0.1 | 21.6±0.1 | 21.6±0.1 |
| Male pup birth weight/litter (g)[c] | 6.3±0.1 | 6.5±0.1 | 6.3±0.1 | 6.3±0.1 | 6.0±0.1** |
| Female pup birth weight/litter (g)[c] | 6.0±0.1 | 6.2±0.1 | 6.0±0.1 | 5.8±0.1 | 5.6±0.1*** |
| Number of dead pups/number of pups born on PND 0 (%) | 10/430 (2.3) | 2/389 (0.5)[#] | 8/445 (1.7) | 9/405 (2.2) | 95/737 (12.9)[##] |
| No. of pups dead/no. of retained on PND 1–7 (%) | 9/297 (3.0) | 2/294 (0.7)[#] | 2/300 (0.7)[#] | 10/298 (3.4) | 47/440 (10.7)[##] |

[a] ALC = *Ad libitum* controls
[b] PFC = Pair-fed controls
[c] [It appears that data was presented as mean±sem, but this was not stated for all parameters]
* $P<0.05$ compared to PFC group, but not ALC group
** $P<0.05$ compared to any other group
*** $P<0.05$ compared to either control group
[#] $P<0.05$ compared to ALC
[##] $P<0.01$ compared to ALC

Litter sizes were reduced in the 12 mg/kg bw/day group and more dams had to be enrolled in that treatment group in order to obtain a sufficient number of pups for evaluation. Compared to all other groups,

gestation length was significantly prolonged in the pair-fed control group. Pup birth weights were lower in the 12 mg/kg bw/day group with statistical significance achieved for male pups compared to all other groups and females compared to either control group. Using the decrease in birth weight for female offspring (which appeared more sensitive than males), the BMD10 (benchmark dose corresponding to a 10% effect level) was 17 mg/kg bw/day and the BMDL (benchmark dose corresponding to the lower bound of the 95% confidence limit at a 10% effect level) was 15 mg/kg bw/day. The numbers of pups dying on PND 0 and between PND 1 and 7 was significantly higher in the 12 mg/kg bw/day group compared to the *ad libitum* control group. There were no effects on offspring survival or growth at later time periods. Though the number of litters reaching weaning in the 12 mg/kg bw/day group was significantly reduced compared to pair-fed controls, significance was not achieved compared to the *ad libitum* group. No significant effects were noted on tests for locomotor activity, spontaneous alternation, passive avoidance, or water maze performance. Though some significant interaction effects were observed for auditory startle response and challenge testing, the study authors noted that there were no patterns of treatment-related changes. Regional brain weights were not affected by treatment. The study authors concluded that fluoxetine is not a developmental neurotoxicant in rats.

*Strengths/Weaknesses:* Although behavioral effects were not seen in the Vorhees et al. *(148*) study, possible bias in the 12 mg/kg bw/day offspring may have resulted from reduced litter size and increased postnatal death in this group. Further, the ability of the neurobehavioral battery to examine the functional integrity of forebrain and midbrain serotonergic systems is unclear. The fluoxetine challenge used in the context of auditory startle testing would likely reflect functioning in certain hindbrain systems, but may not have served to test the functional integrity of the forebrain and midbrain systems, which are implicated as vulnerable to prenatal exposure in the studies discussed at the beginning of this section (Section 3.2.1.3).

*Utility (Adequacy) for CERHR Evaluation Process:* Although the study by Vorhees et al. *(148*) does not show developmental neurotoxicity, this study is adequate for an evaluation of developmental toxicity of fluoxetine and identifies a treatment-related effect on birth weight at the 12 mg/kg bw/day dose. Although a decrease in maternal food intake may have been responsible for some of the decreased pup weight, the decrease in pup weight was significant in comparison to a pair-fed control. Using pup weight, a benchmark dose can be calculated. The BMDL was 15 or 17 mg/kg bw/day, depending on the use of a polynomial or a linear model, respectively.

A study available only as an abstract examined neurobehavioral alterations in 38–41 offspring of rats orally exposed to 0 (n=41 offspring) or 2.5 (n=38 offspring) mg/kg bw/day fluoxetine during the entire gestation period *(150*). Offspring in the fluoxetine group experienced retarded body growth during the first 2 weeks of the postnatal period, altered performance on emotional or motivational responsiveness to some environmental challenge tests (e.g., righting reflex, grip strength, homing test, and auditory startle), motor hyperactivity, and learning and memory deficits as measured by passive avoidance tests. **[The abstract does not provide study details on numbers of litters/group or on litter/group representation in the rats that were tested.]**

A second study available only as an abstract reported enduring "cognitive and behavioral effects" in 10 adult female offspring of representing each of 10 litters born to Long-Evans rats gavage dosed with 10 mg/kg bw/day fluoxetine from 14 days prior to mating through either GD 10 or PND 14

*(151*). **[The abstract describes the visual discrimination tasks used, but does not present results. The suggestion of effects is made only by the title of the abstract.]**

**[In summary, rodent studies conducted to evaluate the effects of prenatal exposure to fluoxetine have examined midbrain and forebrain serotonin content and function at adult ages, the density of serotonin transporters in forebrain and midbrain areas at prepubescent and adult ages, and the developmental and neurobehavioral sequelae from birth through adulthood. While prenatal exposure has been shown to cause age-dependent and site-specific effects in certain midbrain and forebrain areas that are likely to impact serotonergic function in these areas, robust effects on postnatal behavioral measures have not yet been seen or adequately explored. Studies designed to determine the functional consequences of the prenatally-induced alterations in serotonergic systems would be more informative.]**

#### *3.2.1.4. Other developmental endpoints*

Stanford and Patton *(152*) examined hematoma frequency in rats exposed to fluoxetine *in utero*. Sprague-Dawley rats were gavaged with water (n = 18) or 5.62 mg/kg bw/day fluoxetine HCl [**purity not specified]** in water (n = 25) from GD 7 until parturition. The dose was said to be approximately five times the human dose on a mg/kg basis. At birth, pups were weighed, assessed for viability, and examined for hematomas by an individual who was not blinded to the treatment condition. There was minimal discussion of statistical procedures in this report. At birth, body weights of dams in the treatment group were significantly lower **[4.5%]** than the control group. Pup birth weights did not differ significantly between the control and treatment group**.** There were no significant differences in numbers of live and stillborn pups. Hematoma frequency was significantly higher in pups born to treated vs. control dams, with an incidence of 29.3 and 1.8% in exposed and control pups, respectively. Hematoma frequency was also significantly higher in the treated group when analyzed on a per litter basis **[data not presented]**. Hematomas were similar in appearance in control and treated animals; they were absorbed within 3 – 5 days with no additional evidence of vascular effects. Study authors postulated that rat offspring exposed to fluoxetine *in utero* are highly sensitive to bruising due to serotonergic effects on vascular activity.

*Strengths/Weaknesses:* Strengths of the Stanford and Patton *(152*) study are that it assessed a peripheral endpoint related to serotonin function and that dosing was continued to term. Weaknesses of the study include the use of only a single dose level of fluoxetine, precluding a dose-response evaluation; the lack of blinding of the examiner; the lack of historical control data; and the lack of appropriate statistical methods. Of particular concern is the extremely high background rate of stillborn rat pups in both the control (17.1%) and treated (23.7%) groups, suggesting poor animal husbandry practices throughout the study.

*Utility (Adequacy) for CERHR Evaluation Process:* Based on its methodologic weaknesses, this study is not adequate for consideration in the CERHR Evaluation Process.

Singh et al. *(153*) studied aggression in rats exposed to fluoxetine *in utero*. Pregnant rats **[strain and number not specified]** were i.p. injected with 10 mg/kg bw/day fluoxetine **[purity not specified]** on GD 13 – 21. A control group was injected with the 0.9% saline vehicle. The rats were allowed to litter and within 16 hours after delivery, litters were culled to 8 pups. Pups were nursed by foster dams for

3 weeks. At 8 weeks of age, pups were paired by sex and weight and tested for foot shock-induced aggressive behavior. Seven pairs of rats in the control group and ten pairs of rats in the fluoxetine group were evaluated. **[The numbers of litters represented and the numbers of each sex tested were not specified.]** Aggression was measured by latency to fighting and the number of fighting bouts occurring within 120 seconds. Statistical analysis included the two-tailed Mann-Whitney *U*-test. Fluoxetine treatment had no effect on latency to fighting (63.60 ± 25.27 seconds in fluoxetine group vs. 68.57 ± 10.08 seconds in the control group). However, fluoxetine-treated rats had a significantly greater number of fighting bouts (67.30 ± 10.37 in fluoxetine group vs. 41.70 ± 9.55 in controls). Similar effects were also observed with other drugs tested, including diazepam, phenobarbital, and haloperidol. The authors concluded that prenatal fluoxetine exposure enhanced aggression, as measured by number of fighting bouts.

*Strengths/Weaknesses:* The study by Singh et al. *(153*) used a measure of elicited social interaction to assess effects of prenatal fluoxetine exposure in the offspring. A weakness of the study is that only a single dose level of fluoxetine was used, thus precluding a dose-response evaluation. The lack of information on strain, sex, and number of litters represented are major weaknesses of this study. In addition, i.p. injection during late gestation is problematic.

*Utility (Adequacy) for CERHR Evaluation Process:* Lack of procedural details makes it difficult to interpret the utility of the study by Singh et al. *(153*). Also, the findings of similar increased aggression following late gestational treatment with other CNS drugs with very different pharmacologic actions suggests that these unspecified procedures (litter/sex, etc) may contribute to significant differences observed, or that within this design, the control group may have been less aggressive than normally expected. This study did not provide the critical procedural details to allow utility of the resulting data for risk assessment purposes.

Morrison et al. *(154*) conducted a study to determine if *in utero* fluoxetine exposure produces behavioral changes in sheep fetuses. Twenty-one pregnant Dorset/Suffolk sheep were implanted with catheters and fetal electrodes to permit monitoring of fetal physiologic functions between GD 118 and 132. Three days following surgery, 11 sheep were given 70 mg fluoxetine **[purity not specified]** in water by bolus i.v. infusion and were then continuously infused at a rate of 0.036 mg/minute (98.5 µg/kg bw/day) for 8 days. Dosing was based on volume of distribution and clearance data previously collected in that laboratory. The same exposure protocol was conducted in ten control sheep infused with water. Blood was collected daily for an analysis of blood gases. Fetal eye movements, breathing movements, and electrocortical activity were monitored continuously. Fetal activity data were analyzed by three-way and two-way repeated-measures ANOVA, followed by *post hoc* Fisher's *t*-tests. Data for gestational age and birth weight were analyzed by unpaired *t*-tests. Plasma levels of fluoxetine were measured by GC/MS and reported at 46.9 – 173.3 ng/mL and 106.1 ng/mL on infusion days 1 and 8, respectively, in maternal sheep. The fetal fluoxetine-plasma level was reported at 58.9 ng/mL on infusion day 8. There were no significant changes in maternal blood gas values. On the first infusion day, fluoxetine treatment resulted in significant reductions in $pO_2$, pH, and oxygen saturation and significantly increased $pCO_2$ compared to pre-infusion levels. The reductions in $pO_2$ and oxygen saturation in the fluoxetine group were seen throughout the treatment period, with statistical significance obtained on some individual infusion days (days 2, 6, and 7 for $pO_2$; days 2, 4, 6, and 7 for oxygen saturation). Qualitatively similar changes in $pO_2$ and oxygen saturation appeared to occur in the control fetuses, but the apparent

changes were of smaller magnitude and did not reach statistical significance. Compared to pre-infusion values, the fluoxetine group experienced significant reductions in daily incidence of fetal breathing movements (40% pre-infusion vs. 29% post-infusion), eye movements (50% preinfusion vs. 39% postinfusion), and low voltage electrocortical activity (54% preinfusion vs. 45% postinfusion) during the first infusion day. The daily incidence of low voltage electrocortical activity was also lower in the fluoxetine group compared to the control group throughout the treatment period. Reduced incidence of eye movements and low voltage electrocortical activity persisted throughout the infusion period in the fluoxetine group. Fetal breathing movements continued to decline in both treated and control fetuses with no inter-group differences noted. Incidence of high voltage electrocortical activity was reported to increase from 39% during pre-infusion to 68% post-infusion **[data not shown and it is not clear how this value compared to control animals]**. Fluoxetine treatment resulted in no significant differences in gestational age at birth or fetal weight compared to control treatment. The study authors concluded that maternal intake of fluoxetine results in altered fetal behavioral states.

*Strengths/Weaknesses:* This study used appropriate methods and adequate sample size and controls. The statistical analysis was appropriate. Weaknesses include the i.v. route of administration and the use of a single dose level group, which precludes a dose-response evaluation.

*Utility (Adequacy) for CERHR Evaluation Process:* This study is adequate for consideration; however, the restriction of the findings to fetal physiologic parameters with a lack of effect on gestational age and fetal weight raises the question of the relevance of the findings to human risk evaluation.



Morrison et al. *(53*) conducted a study to assess the effects of fluoxetine exposure during late pregnancy. Twenty-nine pregnant Dorset/Suffolk sheep were implanted with catheters and fetal electrodes between GD 118 and 122. Following 1 pre-infusion day, 14 sheep were given 70 mg fluoxetine in water by bolus i.v. infusion and were then continuously infused with 2.77 mg/mL fluoxetine **[purity not specified]** at a rate of 0.036 mL/minute (fluoxetine 100 µg/minute) for 8 days. The same protocol was conducted in 15 control sheep infused with water. Blood was collected daily from ewes and fetuses for an analysis of blood gases with an IL 1306 pH/blood gas analyzer; fluoxetine and norfluoxetine levels were analyzed by GC/MS. Blood gases, cardiovascular effects, and uterine artery blood flow were analyzed by ANOVA followed by *post hoc* Fisher's *t*-tests. Data for gestational age and birth weight were analyzed by unpaired *t*-tests. Prenatal and postnatal offspring growth was also monitored. The peak fluoxetine-plasma level on infusion day 1 was measured at 173.3 ng/mL in ewes and 26.8 ng/mL in fetuses. During the first 6 hours following treatment, norfluoxetine-plasma levels increased from 4.5 to 25.3 ng/mL in ewes and from 0 to 8 ng/mL in fetuses. During the 8-day infusion period, fluoxetine plasma levels peaked at 166.5 ng/mL in ewes and 58.9 ng/mL in fetuses; plasma norfluoxetine levels peaked at ~200 ng/mL in ewes and ~70 ng/mL in fetuses. There were no significant changes in maternal blood gas values in the fluoxetine-treated group compared to control animals. Fluoxetine treatment resulted in acute, transient effects that were noted within 15 minutes following exposure. Those effects included decreases in uterine artery blood flow, fetal $pO_2$, oxygen saturation, and pH, and increases in fetal $pCO_2$ and heart rate. In most cases the effects were consistently significant compared to control values only within the first 1 – 2 hours following treatment initiation. A significant increase in lactate levels during the 6 hours following treatment, compared to pre-infusion levels, was only seen in fluoxetine-treated fetuses. No significant changes in uterine artery blood flow, blood gas values, or cardiovascular measurements in the fluoxetine group compared to the control group were

noted after the first day of treatment. There were ten live births in the control group and nine live births in the fluoxetine group. Fluoxetine treatment had no effect on gestational age at birth, birthweight, percent live births, or head or abdominal circumference. Compared to controls, postnatal weight gain was significantly lower in treated animals on PND 2, but significantly higher on PND 5. The study authors concluded that fluoxetine treatment during pregnancy has transient effects on fetal status that could be of consequence following repetitive occurrences of these effects.

*Strengths/Weaknesses:* Confidence in these data is high due to appropriate analytic and statistical methods, adequate sample size, and appropriate controls. The administration of fluoxetine by i.v. bolus is problematic and the single dose level weakens the findings by precluding the evaluation of a dose-response relationship.

*Utility (Adequacy) for CERHR Evaluation Process:* This study is adequate for assessment, but relevance to human risk is decreased by the route of exposure and the transitory nature of the effects (effects occurring within 1 hour on the first day of treatment). In spite of the acute transient effects on uterine blood flow and blood gas measurements in the fetuses, there were no effects on gestational age at birth, birthweight, percentage of live births, or head or abdominal circumference, suggesting no significant toxicologic effect. The temporary decrease in postnatal weight gain on PND 2 could have been due to the pharmacologic effects of fluoxetine in blood or in milk.

### *3.2.2 Postnatal Developmental Studies*

Bastos et al. *(155*) examined the immediate effects of chronic fluoxetine treatment on the development and lesion-induced plasticity of retinotectal axon projections in Lister Hooded rats. Two exposure periods were used. In one group, rat pups were injected i.p. with 0 (0.9% saline) or 7.5 mg/kg bw/day fluoxetine on PND 1 – 10. In the second group, the rats were i.p. injected with 0 or 10 mg/kg bw/day fluoxetine **[purity not specified]** on PND 14 – 28. On PND 21, a lesion was induced in the left retina of some of the rats in the second group. To allow for tracing of the retinotectal pathway, the right eye was injected with horseradish peroxidase on PND 9 or 27 in the 2 groups, respectively. On the day following the tracer injection, the animals in each group were killed for removal and sectioning of the brain. **[A total of 57 animals were examined but the numbers treated and examined within most treatment groups were not specified.]** In vehicle-treated rats receiving either the early or later treatments, uncrossed retinotectal pathways were arranged in discrete clusters of terminal labeling in the rostral portion of the tectum. Rearrangements in these pathways were observed in 33% (4 of 12) of rats treated with fluoxetine from days 14 to 28 and an unspecified percentage of rats treated from days 1 to 10. The changes were characterized by decreased density of terminal rostral tectum labeling and abnormal spreading of retinal terminal fields along the rostra-caudal axis. These results suggest that fluoxetine treatment induced an active reorganization of the retinotectal axons. Fluoxetine treatment was also found to increase plasticity of retinotectal axon projections following the induction of retinal lesions. Following lesion induction in vehicle-treated rats, there was a small reorganization of intact uncrossed projections with only a few terminals invading the denervated tectal surface. In 53% (8 of 15) of rats in the PND 14 – 28 fluoxetine-treated group, amplified reorganization characterized by the obvious spreading of uncrossed retinal axons into denervated areas was noted following lesion induction. The study authors interpreted the data as suggesting that fluoxetine treatment induces axonal rearrangements and amplifies neural plasticity in the CNS of young rats.

*Strengths/Weaknesses:* Because Bastos et al. *(155*) do not clarify the number of animals in each group and assay, it is difficult to determine the reliability of the findings. The study authors' use of the terms "neonatal" and "juvenile" to describe the 2 treatment periods, PND 1–10 and 14-28, respectively, may not be fully accurate for the specific windows of treatment.

*Utility (Adequacy) for CERHR Evaluation Process:* The study by Bastos et al. *(155*) is of low utility for use in a CERHR review for two reasons. First, the fluoxetine-associated changes in retinotectal axon development in juvenile rats is not clearly a model for human risk. Had fluoxetine been shown to alter neuronal architecture in a manner leading to cognitive impairment, the applicability to human risk would have been clearer. Second, the i.p. injection of what may have been a high dose of fluoxetine decreases the relevance to oral exposure in humans. It is also noted that the early postnatal period in this study corresponds to the fetal period in humans, making the dose route and size potentially less relevant to humans.

Wegerer et al. *(35*) studied the effects of fluoxetine treatment on serotonergic and noradrenergic system development in rats administered fluoxetine during prepubertal and pubertal stages. Male Wistar rats were administered 0 or 5 mg/kg bw/day fluoxetine **[purity not specified]** in drinking water for 2 weeks starting at 25 or 50 days of age. The dose was said to be 10 times higher than the human dose; however, it has been shown to be the minimum dose that produces serotonin reuptake inhibition in adult rats. Weight gain was monitored daily. Six rats per treatment group were sacrificed at various time periods. The day 25–39 treated group of rats was killed at either PND day 50 (n=6 animals) or PND 90 (n=6), either 10 days or 8.5 weeks, respectively, following discontinuation of dosing. The day 50–64 treatment group was killed at 90 days of age. Brains were removed and homogenized for an examination of $^{3}$H-paroxetine and $^{3}$H-nisoxetine binding to serotonin and noradrenaline transporters, respectively. Statistical significance of data from the control vs. treated group was determined by ANOVA followed by two-tailed *post-hoc t*-test. Fluoxetine and norfluoxetine levels in blood were analyzed by HPLC and UV detection. Plasma levels of fluoxetine and norfluoxetine were similar in both age groups of rats and ranged from 27 to 29.9 ng/mL and 242.4 to 271.8 ng/mL, respectively. There was no effect on body weight gain and no obvious behavioral changes. The earlier fluoxetine treatment that started at 25 days of age resulted in a significantly (~20%) increased density of $^{3}$H-paroxetine binding sites in the frontal cortex when measured at 50 and 90 days of age. This persistent effect was not seen in 90-day-old rats that received fluoxetine treatment starting at 50 days of age. In neither of the two ages evaluated were effects seen on the density of $^{3}$H-paroxetine binding sites in other brain regions examined, including the parietal cortex, occipital cortex, hypothalamus, and midbrain. Further, fluoxetine treatment had no effect on dissociation constants for $^{3}$H-paroxetine or $^{3}$H-nisoxetine or density of $^{3}$H-nisoxetine binding sites. The study authors postulated that the 2-week fluoxetine treatment beginning at day 25 may have caused serotonin-induced production and release of astrocytic growth factor. The study authors indicated that the biologic significance of these effects in rodents is not known, and this lack does not permit extrapolation to humans. However, they cautioned that this study suggests that fluoxetine exposure during the development of the serotonergic system is capable of inducing persistent changes in the brain's structural architecture that are not produced following treatment of the mature brain.

*Strengths/Weaknesses:* In the study by Wegerer et al. *(35*), it appears that six rats/condition were used. While this number is low, it appears acceptable for publication standards within this area of work. The route of administration (drinking water) may not be relevant to human medication exposure, and the

use of a single dose level precludes a dose-response evaluation.

*Utility (Adequacy) for CERHR Evaluation Process:* The study by Wegerer et al. *(35*) is adequate for use in the CERHR evaluation. Although the increase in serotonergic but not adrenergic projections in the frontal cortex in 25-day-old rats is intriguing, the significance of this alteration in rats for human risk is difficult to predict.

Norrholm and Ouimet *(156*) examined hippocampal dendritic spine density in juvenile Sprague-Dawley rats receiving acute or chronic fluoxetine treatment. In the acute study, rats were i.p. injected with a single dose of 5 mg/kg fluoxetine HCl **[purity not specified]** on PND 21. One control group was i.p. injected with 0.9% saline and a second control group was not handled. The rats were sacrificed 24 hours later, at PND 22. In the chronic study, rats were treated in the same manner as rats in the acute study, but dosing was continued (i.p. injection of 5 mg/kg bw/day) for 3 weeks. Half of the animals were killed 24 hours after the last injection (PND 42), while the remaining animals were killed 21 days following the last injection (PND 62). Brain samples were prepared for a determination of dendritic spine density in the CA1 region of the hippocampus and the dentate gyrus. Each treatment group contained three or four rats. Data were analyzed by two-tailed Student's *t*-test. The only significant effects observed in the acutely fluoxetine-treated rats were a 25.9% increase in total number of secondary dendrites and an 18.9% increase in summed dendritic length, which were significant when compared to a pooled group of saline and non-handled controls. Chronic fluoxetine treatment inhibited the age-related increase in CA1 dendritic spine density that was observed in saline and nonhandled controls between PND 22 and 62. CA1 dendritic spine density in fluoxetine-treated animals was significantly lower than in saline and non-handled control groups 24 hours after the chronic treatment ended (17.1 and 25.5% lower than saline and non-handled controls, respectively) and after the 3-week recovery period following chronic treatment (20.0 and 23.6% lower than saline and non-handled controls, respectively). Dendritic spine length in the CA1 was not affected by chronic fluoxetine treatment. No effects occurred in the dentate gyrus following acute or chronic treatment with fluoxetine. **[These region-specific effects on spine density immediately after acute treatment, and 3 weeks following chronic treatment suggest that the development of dendritic spines was arrested during a period in which rapid growth would normally occur.]** The study authors suggest that these results may reflect interference with either the formation or retention of new spines, which typically occurs during the second postnatal month in the rat hippocampus. Additional drugs were also examined and results are reported in the study but will not be reviewed here.

*Strengths/Weaknesses:* The study by Norrholm and Ouimet *(156*) uses a small sample size, as is common with these types of studies. The i.p. route of administration and the use of only a single dose level of fluoxetine are important weaknesses in the study.

*Utility (Adequacy) for CERHR Evaluation Process:* The study by Norrholm and Ouimet *(156*) is of low utility in a CERHR review. It is difficult to predict whether the fluoxetine-associated effects on formation or retention of dendritic spines can be extrapolated to humans.

Mendes-da Silva et al. *(157*) examined the effects of neonatal fluoxetine exposure on forced-swim behavior in Wistar rats. The forced-swim procedure has been widely used as a rodent model of learned helplessness or depression. Beginning 1 day following birth (PND 1) and continuing to PND 21, 26

rats/group **[gender not specified]** received saline or 10 mg/kg bw/day fluoxetine **[purity not specified]** by s.c. injection. Body weight gain was monitored and data were analyzed by Students *t*-test. Body weight gain was significantly reduced from PND 9 to 21 in the fluoxetine group; however, by PND 60, body weights were equivalent in the 2 treatment groups. At 60 days of age, the rats were subjected to a forced-swim test. For the test, rats were placed in a tank of water from which they could not escape and were forced to swim for 15 minutes. One day later, the rats were returned to the tank for 5 minutes and latency to the first escape attempt and duration of behavioral immobility were measured. Swim-test data were evaluated by the Mann-Whitney two-tailed test. The study authors stated that fluoxetine-treated rats displayed reduced depressive behavior, as evidenced by an increased latency to escape and decreased behavioral immobility; however, these statements are not consistent with the tabular data. [**Based on tables in the study it appears that the opposite is true. Latency to escape attempt was smaller in fluoxetine-treated (97.5 seconds) vs. control rats (154.5 seconds) and behavioral immobility was increased in the fluoxetine (24.5 seconds) vs. control group (9 seconds).]**

*Strengths/Weaknesses:* The study by Mendes-da Silva et al. *(157*) contains ambiguous results. Additional weaknesses are the s.c. route of administration and the use of only a single dose level of fluoxetine.

*Utility (Adequacy) for CERHR Evaluation Process:* The study by Mendes-da Silva et al. *(157*) has no utility for a CERHR review due to the ambiguity in the presentation of the results.



Dow-Edwards *(158*) treated pre-weaning Sprague-Dawley rats with 25 mg/kg fluoxetine **[purity unspecified]** s.c. on days 11, 13, 15, 17, and 19 (the morning that pups were discovered with dams was day 1). Pups (5 males and 5 females/litter) were reared by their dams and weaned on day 21. All pups within a litter received the same treatment and it is suggested that 15 litters were exposed to each of 3 treatment regimens (cocaine, fluoxetine, or vehicle). On day 75 and 76, animals were tested for auditory-startle reactivity and habituation. Behavioral data were examined with data collapsed across members of the same litter and same sex. The study was performed primarily to determine whether cocaine's effects on development of the nervous system are consistent with effects upon 5HT reuptake inhibition. Thus, a fluoxetine group was used to directly explore the effects of serotonin reuptake inhibition independent of cocaine's other possible mechanisms. At the time of behavioral testing, body weights did not differ between the fluoxetine-treated animals and the controls. Upon initial auditory-startle measurement, the fluoxetine-treated males but not females showed increased startle amplitudes in the latter trial blocks of the session ($P = 0.062$). This finding was interpreted as increased sensitization, a phenomenon that has been seen following lesion of raphe nuclei as well as in response to increased background noise or stimulus intensity. On the second day of testing, the fluoxetine-treated males were also more reactive to the startle stimulus. The authors interpreted these findings as consistent with a subtle reduction in function of neurons in the raphe complex, pathways known to have an inhibitory influence on startle responding. Similar findings have been reported in a tactile-startle paradigm following acute fluoxetine administration to adult rats (Geyer and Tapson, 1988; cited in Dow-Edwards *(158*)).

*Strengths/Weaknesses:* The study by Dow-Edwards *(158*) was well done. Although all members of a litter received the same treatment, data were reduced across the male vs. female members of each litter. Thus, it is suggested that an effective number of 15 animals per group may have been used. The group

number is large compared to other studies presented in this section of the report. Weaknesses of this study are the use of the s.c. route of administration and the use of a single dose level of fluoxetine. *Utility (Adequacy) for CERHR Evaluation Process:* The study by Dow-Edwards *(158*) is adequate for use in a CERHR review, but the conclusions are of low utility given the *P* value of 0.062.

**[In conclusion, most studies in this section appear to use solid measurement techniques. However, measurements were generally made on small numbers of subjects, which introduces statistical power concerns. In addition, the i.p. or s.c. routes of exposure and the single-dose level designs are important limitations in an evaluation of human risk.]**

### *3.2.3. Mechanistic and In Vitro Studies*

Serotonin transporter mRNA has been identified in neural crest-derived structures and sensory pathways of the rat embryo, supporting a role for serotonin in embryo development *(159*). Immunohistochemical studies using cultured mid-gestation mouse embryos show localization of serotonin to mesenchyme adjacent to epithelia of the craniofacial region *(160*). In this study, serotonin immunoreactivity was abolished in the cultured embryos when fluoxetine $10^{-5}$ M **[3100 ng/mL]** was added to the culture 1 hour prior to a 3-hour incubation with serotonin precursors.

Shuey et al. *(149*) conducted an *in vitro* developmental toxicity assay with fluoxetine in order to determine if defects induced in mouse embryos by the antidepressant sertraline may have been due to serotonin uptake inhibition. At least 12 ICR mouse embryos (GD 9) were incubated for 48 hours in a medium containing 1 or 10 µM **[310 or 3,100 ng/mL]** fluoxetine. **[It does not appear that there was a concurrent control group, although controls were used in the sertraline experiment.]** Following the incubation period, malformations were examined and statistical significance was determined by chi-square analysis. No malformations were reported in the 1 µM **[310 ng/mL]** group. A significant increase in the percentage of embryos with both nasal prominence deficiency and lack of forebrain expansion (54%) was observed in the 10 µM **[3,100 ng/mL]** group. Embryos with first visceral arch deficiency (38%) were also significantly increased in the 10 µM group. The defects were similar to those caused by sertraline exposure.

Shuey et al. *(149*) noted that results of the *in vitro* study were inconsistent with *in vivo* studies that demonstrated no fluoxetine-induced malformations, despite the fact that fluoxetine and its metabolite norfluoxetine were shown to cross the placenta in rats *(50*). Shuey et al. speculated that these compounds may not have been present in the conceptus at a concentration sufficient to produce malformations. Byrd and Markham *(51*) disagreed with this theory. They estimated that the 10 µM concentration used by Shuey et al. converted to about 3.5 µg-eq/mL, which is lower than concentraions of fluoxetine and norfluoxetine measured in rat embryos (3.60–5.45 µg-eq/g) following oral dosing with 12.5 mg/kg *(50*), a dose that does not cause malformations in *in vivo* rat studies *(51*). Byrd and Markham speculated that inconsistencies between *in vitro* mouse studies and *in vivo* rat studies may be due to varying inter-species sensitivity. **[The Expert Panel notes that the myriad differences between *in vitro* vs. *in vivo* systems could account for the apparent inconsistencies.]**

*Strengths/Weaknesses:* Weaknesses in the study by Shuey et al. *(149*) include the lack of a concurrent control, no clear specification of the "normal" range of these types or other types of findings in control embryos in this test system, and the small number of embryos evaluated. Conclusions of direct

relevance or expectation of an identical type of effect in the *in vivo* teratology studies or in humans are inappropriate.

*Utility (Adequacy) for CERHR Evaluation Process:* The authors' *(149*) use of two different SRIs in the same *in vitro* model suggests that the pharmacologic action of SRIs, rather than the drugs *per se*, may be responsible for the findings observed in this test system. This view is consistent with the conclusion in this and other studies of a role for serotonin in normal embryo development. However, Shuey et al. used unusual logic in stating that the *in vivo* studies were inadequate to characterize risk since craniofacial findings did not occur in rat and rabbit studies *(51*). The many differences between *in vitro* and *in vivo* test systems are just as likely to account for the apparent inconsistencies. This study has no direct utility in a risk assessment process.

Yavarone et al. *(161*) cultured GD 9–12 mouse embryos in the presence of serotonin and showed immunostaining in the heart. On day 9, the heart tube was uniformly stained but by day 10, staining was confined to the outflow tract and atrioventricular canal. Co-incubation with fluoxetine 10 μM **[3,100 ng/mL]** greatly reduced immunostaining. Cardiac cell proliferation, measured by $^{3}$H-thymidine labeling was decreased in mesenchymal cells in the outflow tract and atrioventricular canal with GD 10 exposure to fluoxetine. The authors concluded that serotonin uptake from maternal-embryo circulation (as opposed to synthesis) is involved in the development of the endocardial cushions.

*Strengths/Weaknesses:* The study by Yavarone et al. *(161*) used fluoxetine as a tool to help distinguish the role of serotonin in heart development. The study provided limited speculation regarding SRIs and *in vivo* malformations. Weaknesses of the study included no indication of embryo numbers used for each assessment or whether the source of embryos for each assessment was from a single or multiple litters.

*Utility (Adequacy) for CERHR Evaluation Process:* The study by Yavarone et al. *(161*) has no utility for a risk assessment process.

Moiseiwitsch et al. *(162*) explanted mandibles from GD 13 mouse embryos and cultured them for 8 days with 0.01–100 μM serotonin, a known stimulator of tooth-germ development in this system. Co-culture with fluoxetine 1 μM (310 ng/mL) prevented the decrease in S-100β, a calcium-binding protein, caused by serotonin, but had no effect on expression of cartilage proteoglycan core protein or of tenascin, an extracellular matrix molecule. **[These authors cite a previous study *(163*) showing that fluoxetine inhibits tooth bud development in this preparation, but they did not report fluoxetine effects on tooth bud development in the 1998 study.]**

*Strengths/Weaknesses:* The study by Moiseiwitsch et al. *(162*) used fluoxetine as a tool to help distinguish the role of serotonin in tooth development. It provided limited speculation regarding SRIs and *in vivo* malformations. Weaknesses of the study included no indication of embryo numbers used for each assessment or whether the source of embryos for each assessment was from a single or multiple litters.

*Utility (Adequacy) for CERHR Evaluation Process:* The study by Moiseiwitsch et al. *(162*) has no utility for a risk assessment process.

### *3.3 Utility of Developmental Toxicity Data*

The data set for human developmental toxicity consists of studies examining pregnancy outcome, malformations, postnatal adaptation, and childhood neurobehavioral development following prenatal exposures, clinical signs and growth as a result of late pregnancy and breast milk exposures, and growth during childhood exposure. The most complete study for evaluating prenatal exposures *(89*), along with supporting data from more limited studies, provided sufficient data for evaluating the effects of prenatal exposures on malformations, premature births or shortened gestation, and neonatal adaptation. There were insufficient data to determine if prenatal fluoxetine exposure affects childhood neurobehavioral development. A study by Chambers et al. *(113*) was adequate for evaluating growth in infants exposed to fluoxetine prenatally or through breast milk. The data were not sufficient for an evaluation of the effects of childhood fluoxetine exposures on growth, cardiac function, or suicidality.

Animal data included a study examining prenatal toxicity in rats and rabbits exposed by gavage and were sufficient for evaluating prenatal endpoints such as malformations and mortality *(51*). Data were sufficient to address postnatal growth and survival in rat pups born to dams gavage dosed with fluoxetine during pregnancy *(148*). Although limited examinations of neurological function in rats exposed during pre- or postnatal development suggested no major effects, the database did not include an examination of multiple endpoints of neuroanatomy and function.

### *3.4 Summary of Developmental Toxicity Data*

#### *3.4.1 Human Data*

##### ***3.4.1.1 In utero exposures***

Case reports and case series reported adverse effects in infants exposed to fluoxetine *in utero* but the Expert Panel noted that such studies are not adequate for evaluating developmental effects of fluoxetine. The Expert Panel focused their evaluation of prenatal fluoxetine toxicity on studies with denominators, i.e., the sampling frame that was used for selecting study subjects could be identified.

The most complete study for evaluating toxicity in infants following prenatal exposure to fluoxetine was conducted by Chambers et al. *(89*). The study evaluated pregnancy outcomes in 228 women taking fluoxetine and a comparison group of 254 women exposed to either acetaminophen, dental x-ray, or < 1 oz. alcohol/week prior to learning of pregnancy. The fluoxetine group was divided into early or late exposures (before or after 25 weeks gestation, respectively). No difference in major malformations was found between liveborn infants exposed to fluoxetine in early gestation and controls. However, the proportion of infants with multiple minor anomalies was increased. Late pregnancy exposures were associated with increased incidence of prematurity, reduced birth weight and length at full term, and poorer neonatal condition characterized by admission to special care nursery and adaptation problems (e.g., jitteriness, tachypnea, hypoglycemia, hypothermia, poor tone, respiratory distress, weak or absent cry, or desaturation on feeding). Relative risks for late exposure were calculated by comparing late and early exposures and adjusting for numerous confounding factors. Relative risks were 4.8 (95% CI: 1.1 – 20.8) for prematurity, 2.6 (95% CI: 1.1 – 6.9) for admission to a special care nursery, and 8.7 (95% CI: 2.9 – 26.6) for poor neonatal adaptation. Though conclusions about major malformations are limited by small sample size, the Expert Panel found this study to

have well-defined procedures and outcome measures and a thorough assessment of outcome compared to other studies. However, the Panel urged caution in interpreting the long-term implications of multiple minor anomalies. Also, the possible confounding effects of maternal depression need to be considered.

Two other large studies involving medical record reviews conducted by Simon et al. *(104)* and Ericson et al. *(102)* were felt to be well-designed and contribute to the utility of available data, although the Expert Panel noted that the medical record reviews in these studies do not provide outcome measures as strong as those in Chambers et al. *(89)*.

Simon et al. *(104)* performed a record linkage study among members of a population based prepaid health plan to identify pregnancies for whom 1 or more prescriptions were filled for antidepressants within a 270 day interval before delivery. Significant reductions in gestational age at delivery (-0.9; 95% CI: -0.5,-1.3) were observed for women with SRI exposure (129/185 women using fluoxetine) during pregnancy in comparison to matched unexposed women after adjusting for maternal tobacco use, other substance use, race, and number of prior births. Risk of preterm birth dichotomized as <36 weeks gestation conferred a significantly increased risk for SRI exposure (OR:: = 4.38; 95% CI: 1.57, 12.22). Mean birth weights were significantly lower among SRI exposed infants in comparison to unexposed (-172 grams; 95% CI: -46, -299, controlling for the above potential confounders), but no effects were noted on birth weight when corrected for gestational age. None of these differences was significant when infants exposed to TCAs were compared with matched unexposed infants. The authors concluded that the observed effects are specific to SRI exposure rather than underlying maternal depression. The Expert Panel noted that the percent of preterm birth (<37 weeks) was comparable in the SRI- and TCA-exposed groups (10.3 and 10.0%, respectively). The absence of information on the full distribution of gestational age in each of the groups precludes a more meaningful interpretation of these data.



In a review of the Swedish Medical Birth Registry, Ericson et al. *(102)* found that use of antidepressants (fluoxetine [n=15], other SRIs [n=531], and non-SRI antidepressants [n=438]) was associated with premature delivery (adjusted OR: 1.43, 95%; CI 1.14, 1.80), but no associations were noted by class of antidepressants; therefore, the authors suggested that the disease rather than the treatment was associated with prematurity. The Expert Panel believes that an alternative explanation for these findings is that a similar mode of action among these medications may be affecting the rate of prematurity.

The additional studies of prenatal fluoxetine toxicity were found to be limited by small numbers of subjects and/or study design. Though these studies alone were of limited utility, the Panel found some of the studies useful when evaluated together as a group, especially with better quality studies. No increase in major congenital malformations following *in utero* exposure was identified in any of the other studies reviewed by the Panel *(19, 90, 96, 101, 104)*, although the Panel found the methods of most of these studies to be inadequate (i.e., limited sample size and statistical power for hypothesis testing) to detect a potentially important risk for congenital anomalies.

In a review of medical records, Cohen et al. *(103)* found that neonatal complications requiring admission to a special care nursery occurred in 1 of 11 (9%) pregnancies with first or second trimester exposure to fluoxetine vs. 16 of 53 (30%) pregnancies with third trimester exposure. Laine et al. *(18)* evaluated sero-

tonergic symptoms (e.g., myoclonus, restlessness, tremor, shivering, hyperreflexia, incoordination, and rigidity) in infants from ten fluoxetine- and ten citalopram-exposed pregnancies in which exposures were implied to have occurred during the period just prior to delivery. Compared to a control group (n=20), there was no difference in pregnancy duration, but serotonergic symptoms were greater in the SRI group during the first 4 days of life; there was no difference at 2 weeks of age. The study by Oberlander et al. *(110*) found that 2-day-old infants exposed to SRIs (fluoxetine: n=7, paroxetine: n=11, sertraline: n=4) *in utero* responded to pain with less facial activity, a slower heart rate, and greater maintenance of cardiac modulation compared to control infants. It is not known if effects were due to prenatal brain alterations or continued presence of fluoxetine in infant blood. Findings from another study on a broader range of prenatal SRI exposures were consistent with these neonatal findings *(112*).

In contrast to the Chambers et al. study, Goldstein et al. *(100*) reported a lower incidence of neonatal irritability or jitteriness (4.5%) compared to other investigators and stated there were no apparent patterns of abnormal infant conditions in cases of fluoxetine exposure during the third trimester. The Goldstein study was not considered to be reliable due to methodologic limitations such as unstated but possibly large loss to follow-up and reliance for outcome data on reporters of questionable reliability. Other neurotoxicity studies, limited by study design and found to be of little utility, suggested that prenatal fluoxetine exposure had no effect on neurodevelopment in children up 12 months *(19*) and 71 months of age *(107, 109*).

Although no differences were found by Simon et al. *(104*) in seizure disorder, motor delay, speech delay, or other motor abnormalities when comparing children with pregnancy exposure to fluoxetine and an unexposed reference group, the data collected to demonstrate this lack of developmental deficits were considered inadequate by the Expert Panel. No difference was found in birth weight or growth during the first 12 months for infants born to 11 women treated with 20–40 mg fluoxetine during pregnancy as compared to 10 control women *(19*), but the small size of this study provided limited power to detect differences.



#### *3.4.1.2 Breast milk exposures*

Symptoms in infants breastfed by mothers taking fluoxetine were reported in case studies as outlined in Section 3.1.2. While some studies reported symptoms similar to those reported with prenatal exposure (e.g., hyperactivity, crying, irritability, reduced sleep, and poor feeding), no symptoms were reported in other breastfed infants exposed to fluoxetine. There were no controlled studies designed to evaluate symptoms in infants exposed to fluoxetine through breast milk.

In a well designed retrospective-cohort study conducted by Chambers et al. *(113*), it was found that infants nursed by mothers taking fluoxetine (n=26) had a 392 g deficit in body weight gain (95% CI: 5–780 g), with weight gain at ~1.2 SD below control group (n=38) values from 2 weeks to 6 months of age. However, all of the postnatally exposed infants had been prenatally exposed to fluoxetine in the third trimester, as contrasted to only 10.5% of the controls. Thus, the growth deficits found in this study may have been due to prenatal exposure or the effects attributed to postnatal exposure may have been partly due to residual levels of fluoxetine/norfluoxetine from third trimester exposure. A major strength of this study is that unlike most other fluoxetine studies which lack an unmedicated depressed comparison group, this study provides the only evidence of infant deficits specifically related to fluoxetine and not the underlying depressive disorder.

#### *3.4.1.3 Childhood exposures*

Side effects in children taking fluoxetine are similar to those of adults and include manic reaction, hyperkinesia, rash, personality disorder, agitation, constipation, diarrhea, headache, nervousness, somnolence, insomnia, suicide attempt, depression, endometrial hyperplasia, hostility, euphoria, and migraine *(4, 116*). Additional side effects reported for children include thirst, hyperkinesia, epistaxis, urinary frequency, and menorrhagia *(4*). Some reviews expressed concern that children may be particularly sensitive to excessive arousal and irritability *(117-119*). However, other studies did not find activation to be the most common side effect, as more commonly observed effects included gastrointestinal effects, drowsiness, and headache *(120, 122, 123*).

In a medical review, the FDA *(116*) expressed concern about prolonged QTc interval and growth decrements in children taking fluoxetine. Although prolonged QTc interval was not replicated in later studies, the significance was found to be robust using Fridericia correction, which is unlikely to result in statistical significance for random variability. In addition the R-isomer of fluoxetine prolonged QTc intervals in adults. Therefore, the FDA Medical Officer believed prolonged QTc interval to be a true drug effect in children.

FDA concerns about growth decrements in children were based upon a 19-week study that reported height and weight increases of 1.0 cm and 1.2 kg in children treated with fluoxetine vs. 2.0 cm and 2.3 kg in control children ($P=0.008$) *(116*). The original study examining growth in children was not available to CERHR. Impaired growth was reported in abstracts but there are no known published studies examining this endpoint.

In October, 2003, the FDA issued a Public Health Advisory regarding their review of suicidality in children taking fluoxetine or seven other antidepressant drugs *(136*). It was concluded that preliminary data suggest an excess of reports of suicidal ideation and suicide attempts and that additional data, analysis, and public discussion of available data on this issue are needed. In their latest Public Health Advisory, the FDA stated that the contribution of antidepressants to suicidal thinking and behavior is not yet clear, and cautioned clinicians, patients, families, and caregivers to closely monitor children or adults receiving fluoxetine or other antidepressants for worsening of depression or suicidal thoughts, especially during initiation of therapy and following dose adjustments *(137*). Manufacturers were asked to update their labels with stronger cautions and warnings about the need for monitoring of symptoms.

The Expert Panel finds the literature on childhood exposures to be markedly deficient due to small sample sizes, inadequate follow-up ranging from 6 to 13 weeks, high attrition, and multiple diagnoses. Therefore, it is not possible to reach a conclusion regarding possible differences between fluoxetine and control treatments in the context of underlying methodologic limitations.

### *3.4.2 Experimental Animal Data*

The main studies reviewed for an evaluation of developmental toxicity in animals are summarized in Table 21.

*Table 21. Summary of Key Fluoxetine Animal Developmental Toxicity Studies*

| *Doses (mg/kg bw/day)* | *Exposure regimen* | *Species/Strain* | *Dose: Effect* | *Reference* |
|---|---|---|---|---|
| 2.5<br>7.5<br>15 | GD 6–18, gavage | Dutch Belted rabbits | **Dams:**<br>**2.5–15:** Weight loss, ↓ food intake<br>**Fetuses:**<br>**NOAEL = 15** | Byrd and Markham *(51)* |
| 2<br>5<br>12.5 | GD 6–15, gavage | Fischer 344 rats | **Dams:**<br>**NOAEL = 5**<br>**12.5:** ↓weight gain and food intake<br>**Fetuses:**<br>**NOAEL = 12.5** | Byrd and Markham *(51)* |
| 8<br>16 | GD 15–20 gavage | Wistar rat | **Dams:**<br>**16:** ↓weight gain and food intake[a]<br>**Pups:**<br>**NOAEL = 16**<br>No effects on pup mortality, pup weight at weaning, or behavior | da-Silva et al. *(140)* |
| 12.5 | GD 8–20 peroral | Sprague-Dawley rat | **Pups:**<br>**NOAEL = 12.5**<br>No effect on pup birth weight, weight gain, mortality, or behavior | Stewart et al. *(147)* |
| 1<br>5<br>12 | GD 7–20 gavage | Sprague-Dawley rat | **Dams:**<br>**NOAEL = 5**<br>**LOAEL = 12:** weight loss<br>**Pups:**<br>**NOAEL = 5**<br>**LOAEL = 12:** ↑death on PND 0 and PND 1–7, ↓ birthweight<br>No effects on behavior | Vorhees et al. *(148)* |
| 5.62 | GD 7–parturition, gavage | Sprague-Dawley rat | **Dams:** ↓ body weight<br>**Pups:** ↑hematomas | Stanford and Patton *(152)* |

[a]Statistical significance not known
↑= statistically significant increase; ↓= statistically significant decrease

#### *3.4.2.1 Rabbits*

A study conducted by Byrd and Markham *(51)* demonstrated no effects on fetal morphology, viability, or body weight in rabbit fetuses following treatment of does with up to 15 mg/kg bw/day fluoxetine by gavage on GD 6–18. Maternal toxicity was evident by reduced food intake and weight loss occurring at all doses ≥2.5 mg/kg bw/day. In addition, treatment with 15 mg/kg bw/day resulted in death in two does and abortion in three does. The fetal NOAEL in rabbits was identified as 15 mg/kg bw/day. No maternal NOAEL in rabbits was identified due to effects occurring at all dose levels.

#### *3.4.2.2 Rats*

A study conducted by Byrd and Markham *(51)* demonstrated no effects on fetal morphology, viability,

or body weight in rat fetuses following treatment of dams with up to 12.5 mg/kg bw/day fluoxetine by gavage on GD 6–15. Maternal toxicity was evident by reduced food intake and decreased weight gain at the high dose, 12.5 mg/kg bw/day. Maternal and fetal NOAELs in rats were identified as 5 and 12.5 mg/kg bw/day, respectively.

A number of studies examined the effects of fluoxetine on biochemical and structural aspects of the serotonergic system, with the most thoroughly reported studies conducted by Cabrera and colleagues *(143-145*). In the studies, pregnant rats were injected s.c. daily with fluoxetine during GD 13–20 and male offspring were examined on PND 25 and 70. As noted in Table 19, fluoxetine treatment resulted in age-specific and region-specific changes in serotonergic parameters such as density of 5HT receptors and serotonin transporters and serotonin content in forebrain areas. The Panel noted that the studies suggested altered serotonin-mediated function following prenatal fluoxetine exposure, but the utility of these studies is questionable due to the modest degrees of change (<50%) for most endpoints and the lack of a clear pattern of effects.

Studies examining postnatal neurobehavioral function in rats exposed to fluoxetine *in utero* found no effects on locomotor activity, acoustic startle response, learning, or memory in preweanling, juvenile, or adult offspring challenged with fluoxetine or apomorphine *(148*); behavior in adult offspring following injection with a $5HT_1$ receptor agonist *(140*); or behavior stereotypy and locomotion in 19-day-old offspring following injection with a dopamine $D_2$ receptor agonist *(147*). Details regarding prenatal dose levels and exposure duration are included in Table 21. The Panel notes that these behavioral studies demonstrate no major effects on neurobehavioral endpoints; however, the studies examined only a small subset of the multiple endpoints of neuroanatomy and function.

Decreased birth weight and increased pup death on PND 0 and PND 1–7 were reported following gavage dosing of dams with 12 mg/kg bw/day fluoxetine on GD 7–20 *(148*). Other studies with smaller group sizes and shorter exposure periods found no or only questionable effects on birth weight and no increase in prenatal mortality following gavage dosing of dams with up to 12.5–16 mg/kg bw/day *(140, 147*). None of the studies with prenatal exposure reported postnatal decrements in weight gain. A transient increase in hematoma frequency at birth was reported in the offspring of rats gavage dosed with 5.62 mg/kg bw/day from GD 7 until parturition *(152*).

A number of studies in rats examined the effects of postnatal exposure to fluoxetine. Increased reactivity to a startle stimulus, interpreted by authors as a subtle reduction in neuronal function in the raphe complex, was noted in male but not female rats (75–76 days of age) s.c. injected with 25 mg/kg fluoxetine every other day from PND 11 to 19 *(158*). Other studies examining postnatal fluoxetine effects in rats used small sample sizes, which could limit statistical power, but applied solid measurement techniques. In those studies, treatment of immature rats (≤28 days old) with 5–10 mg/kg bw/day for 10–15 days resulted in effects such as reorganization of retinotectal pathways and increased plasticity of retinotectal axon projections *(155*), increased density of serotonin but not noradrenaline transporters *(35*), and inhibition of CA1 dendritic spine density increases during periods of normal growth *(156*).

***3.4.2.3 Sheep***

Two studies by Morrison et al. *(53, 154*) monitored the effects of fluoxetine in fetuses of sheep administered a bolus i.v. injection of 70 mg fluoxetine and then infused with up to 98.5 µg/kg bw/day

for 8 days, beginning around GD 121 and 135. Transient effects were noted for uterine artery blood flow, fetal $pO_2$, oxygen saturation, pH, $pCO_2$, and heart rate following administration of the bolus dose. Fetal breathing movements were transiently reduced following the bolus fluoxetine dose, while reductions in eye movements and low voltage electrocortical activity persisted throughout the infusion period. Fluoxetine treatment had no significant effects on gestational age, birth weight, percent live births, or head or abdominal circumference. Postnatal weight gain was lower on PND 2 but higher on PND 5 in lambs from the fluoxetine group.

**The Expert Panel concluded there is sufficient evidence in humans to determine that prenatal exposure to fluoxetine results in poor neonatal adaptation (e.g., jitteriness, tachypnea, hypoglycemia, hypothermia, poor tone, respiratory distress, weak or absent cry, diminished pain reactivity, or desaturation on feeding) at typical therapeutic exposures (20–80 mg/day orally) during the third trimester of pregnancy. Whether this effect represents developmental toxicity or a direct pharmacologic effect cannot be determined based on the existing data. Data are insufficient to determine whether prenatal fluoxetine exposure affects rates of major malformations or postnatal neurologic development.** Therapeutic fluoxetine exposure during early pregnancy may result in an increased incidence of minor anomalies. Shortening of gestation and reduced birthweight are also suspected, although the evidence is not sufficient to exclude the underlying disorder, depression, as a cause or contributor to these effects. The evidence is suggestive that exposure to fluoxetine through breast milk can result in reduced infant growth; however, these effects may be related to prenatal exposure. Reduced growth in children (age not specified) with 19-week exposure to fluoxetine is also suspected, but the Panel could not evaluate the sufficiency of the original data without access to these data. **Data are not sufficient to evaluate other developmental effects following childhood exposures to fluoxetine**.

The Panel concluded there is sufficient evidence in rats to demonstrate that treatment of dams with 12 mg/kg bw/day fluoxetine by the oral route on GD 7–20 results in developmental toxicity in the form of decreased birth weight and impaired pup survival *(148)*. **The Panel notes that there was a decrease in maternal weight gain at this dose, but that the decrease in birth weight was significant in comparison to a pair-fed control. Using the decrease in birth weight in female offspring, which appeared more sensitive than males, the $BMD_{10}$ (benchmark dose corresponding to a 10% effect level) was 17 mg/kg/day and the BMDL (benchmark dose corresponding to the lower bound of the 95% confidence limit at a 10% effect level) was 11 mg/kg bw/day.** The Panel concluded there is sufficient evidence in rats and rabbits to demonstrate that gavage administration during embryogenesis with dose levels of up to 15 mg/kg in rabbits or 12.5 mg/kg in rats does not result in developmental toxicity in the form of abnormal morphology or reduced fetal viability. The rat and rabbit data are assumed relevant to consideration of human risk. The Panel concluded that data in sheep were insufficient to evaluate the possible developmental toxicity of fluoxetine.

# 4.0 REPRODUCTIVE TOXICITY DATA

## *4.1 Human Data*

### *4.1.1 Female reproductive function*

Two cases of anovulatory women who became ovulatory on fluoxetine were presented by Strain *(164*). No information was provided on the cause of the anovulation in either case, but neither woman had responded to clomiphene, suggesting a hypothalamic cause. In both instances, improvement in depression was reported to have occurred prior to correction of the ovulation problem. Two women with hypogonadotropic hypogonadism associated with Prader-Willis syndrome developed menstrual-like episodes of genital bleeding on fluoxetine *(165*). The authors postulated a hypothalamic effect of the fluoxetine therapy. Hormonal evaluations were not reported. The Expert Panel notes that in the patients with hypogonadotropic hypogonadism associated with Prader-Willis syndrome, apparent menstruation occurred after 7 – 9 months of fluoxetine exposure while the effects noted in the report by Strain *(164*) began almost immediately after starting fluoxetine treatment. It was not determined if the women with Prader-Willis syndrome were actually ovulating.

A clinical trial found 2 of 16 women to complain of shortening menstrual cycles *(166*). This report led the investigators of a fluoxetine efficacy study to conduct a *post hoc* analysis of menstrual cycle data to identify possible effects of fluoxetine on menstrual cycle length *(167*). The original study was a multicenter study of fluoxetine vs. placebo for premenstrual mood changes, sponsored by Eli Lilly and Company in Canada. Women were excluded if they had 2 cycles of fewer than 24 days or more than 35 days in the previous 6 months or were on oral contraceptives. A single-blind two-cycle placebo phase was used to exclude placebo responders, following which subjects were randomized to 6 months therapy with fluoxetine at either 20 or 60 mg/day. A daily calendar was used to monitor cycle length. Cycle-length change was defined as ≥1 SD from the mean change between baseline cycles 1 and 2, which turned out to be 4 days. Subjects with baseline-cycle variation >2 SD (8 days) were excluded from analysis. Evaluation was made based on the first fluoxetine-exposed cycle.

One of 61 women on placebo experienced a change in her cycle (shortening), compared to 7 of 70 and 11 of 62 on fluoxetine, 20 and 60 mg/day, respectively ($P = 0.011$ by chi-square). The 7 women with altered cycles in the fluoxetine 20 mg/day group included 4 with shortening and 3 with lengthening cycles. The 11 with altered cycles in the fluoxetine 60 mg/day group consisted of 6 with shortening and 5 with lengthening cycles. The authors speculated that fluoxetine-mediated increases in serotonin in the brain could inhibit hypothalamic gonadotropin-releasing hormone (GnRH), delaying ovulation, and that fluoxetine inhibition of CYP3A4 would decrease the metabolism of estrogen, thereby advancing ovulation. These two effects could be offsetting, or one or the other could predominate, which would result in variability in response among women.

*Strengths/Weaknesses:* The results presented by Steiner et al. *(167*) represent a *post hoc* analysis of data collected from a study investigating the efficacy of fluoxetine in the treatment of PMDD. The data necessary to determine changes in cell cycle length had already been collected as part of the original study (to determine which symptoms were premenstrual and which were not) and were simply analyzed for this additional endpoint. **[The Expert Panel notes that women with dramatic changes in cycle time (either shortening or lengthening) were excluded from the sample because this change was**

**considered a deviation in the original study. Therefore, these data represent those subjects with a slight-to-moderate response, rather than all of the subjects, specifically excluding those with more dramatic responses. Although fluoxetine, at either at 20 or 60 mg/day, was associated with an increased incidence of changes in cycle length in the test subjects, there was no uniformity to the response and the changes noted would not necessarily be considered adverse.]**

*Utility (Adequacy) for CERHR Evaluation Process:* This study is adequate for an evaluation of potential reproductive effects of fluoxetine in women.

The relation between fluoxetine use and spontaneous abortion has not been fully investigated in such a manner to ensure that all pregnancies have been captured in relation to use of this medication. Chambers et al. *(89*) did not observe a significant relation between fluoxetine use in early pregnancy and spontaneous abortion in an unadjusted analysis of birth outcome. However, other significant differences were reported in relation to fluoxetine use impacting the interpretation of findings , (i.e., smaller percentage of live born infants and a higher percentage of therapeutic abortions among users). Moreover, loss to follow-up was higher among users than non-users. These differences have tremendous implications for the interpretation of pregnancy outcome results with regard to competing risks and other potential biases impacting the conclusions. In addition, an unstated number of women contributed more than one pregnancy to the study sample, and statistical models that can address the known clustering in pregnancy outcomes were not used by the investigators. In a second study, Pastuszak et al. *(90*) reported a significant difference in the percentage of spontaneous abortions reported by women using fluoxetine (14.8%) in comparison to the non-teratogen group (7.8%) ($P = 0.03$). **[The Expert Panel calculated a *P* value of 0.11 using Fisher's exact test.]**

*Strengths/Weaknesses:* Both studies address only clinically recognized pregnancy losses (approximately one-third of all postimplantation losses) and neither paper clearly states the methodology for ascertaining spontaneous abortion (though much seems to be retrospective reporting after the expected date of delivery). This approach may underascertain pregnancy loss.

*Utility (Adequacy) for CERHR Evaluation Process:* The Expert Panel determined that these data on pregnancy loss were not adequate for the evaluation process.

### *4.1.2 Galactorrhea*

There are two case reports describing teenagers on fluoxetine who developed hyperprolactinemia and galactorrhea *(168, 169*). One was also on pimozide. A Netherlands network of centers for the collection of spontaneous adverse event reports presented 15 cases of galactorrhea associated with SRIs, 4 of which involved fluoxetine *(170*). A comparison of the reports in their database suggested that galactorrhea was reported more often than expected in association with antidepressants compared to other adverse effects, and that serotonin-active agents were more involved with galactorrhea reports than were other kinds of antidepressants. An increase in serum prolactin, characterized as an increase in amplitude of diurnal prolactin peaks, was shown in menopausal women treated with fluoxetine, supporting a role of serotonergic drugs in galactorrhea *(171*).

*Strengths/Weaknesses:* The study by Urban and Veldhuis *(171*) provides support but not definitive evidence for fluoxetine as a cause of galactorrhea in women of child-bearing age via an increase

in prolactin levels. The subjects were postmenopausal women selected based on lack of exposure to exogenous estrogens and demonstrated to have appropriately low estrogen-blood levels. Prolactin levels were described as normal. Subjects served as their own controls. Fluoxetine (60 mg/day) increased the amount of prolactin found in the blood during the pulsatile release characteristic of this hormone. The study authors described the subjects as being at "steady-state" for fluoxetine blood levels due to a half-life of "1 – 3 days" after 6 days of treatment. However, the data presented in the pharmacokinetics section of this report show that patients receiving 60 mg/day of fluoxetine require up to 3 months to be at steady-state, especially when considering the blood levels of the active primary metabolite norfluoxetine. How the control of prolactin release would respond in cycling females after 3 months of fluoxetine treatment is unknown. The authors acknowledged that the results from this study cannot be directly extrapolated to ovulating women with normal cycles but suggested that fertile women may be more sensitive to these effects than postmenopausal women. How fertile women with a steady-state blood level of fluoxetine would respond in terms of prolactin release has not been investigated, although the case reports of galactorrhea suggests indirectly that some do respond to fluoxetine-induced prolactin release.

*Utility (Adequacy) for CERHR Evaluation Process:* This study is adequate for the evaluation of reproductive effects of fluoxetine in women. It shows increased prolactin in menopausal women treated with this medication. The relevance of this finding for women of child-bearing age has not been demonstrated, but case reports of galactorrhea on fluoxetine therapy are suggestive.



### *4.1.3 Male reproductive effects*

A case study reported bilateral asymmetric gynecomastia with no hormonal disorder in a 21-year-old man, occurring approximately 4 months after he had taken 20 mg/day fluoxetine for 1 month *(172)*. **[The Expert Panel notes that case studies by themselves are not adequate for the evaluation process.]**

Nine healthy men were treated with fluoxetine 60 mg/day in 3 divided doses of 20 mg. There were no changes in serum luteinizing hormone (LH) *(173)*.

*Strengths/Weaknesses:* The study by Urban and Veldhuis *(173)* did not show a change in serum LH values in men following fluoxetine administration. Fluoxetine (60 mg/day in 3 equal doses) exposure began 6 days prior to the study and continued throughout the 30-hour sampling period. The authors assumed the subjects were at steady-state because the half-life of fluoxetine was reported to be 1 – 3 days. It is apparent from the pharmacokinetics section of this report that the time to reach a steady-state with 60 mg/day is up to 3 months and the blood levels of norfluoxetine should be considered as well because the primary metabolite is also pharmacologically active.

*Utility (Adequacy) for CERHR Evaluation Process:* This study is of limited value in assessing male reproductive effects because steady-state was unlikely to have been reached at the time of sampling.

To assess the effects of fluoxetine on vas muscle contractility, Medina et al. *(174)* obtained ring segments of vasa deferentia from 32 men undergoing vasectomy for elective sterilization. Muscle preparations were placed in a bath of modified Krebs-Henseleit solution and contractile response to electrical stimulation or to potassium chloride was evaluated in the presence or absence of nifedipine, a calcium channel blocker. Fluoxetine $10^{-5}$ M (3,100 ng/mL) produced a 40% reduction in contrac-

tion response to electrical stimulation and to potassium chloride. In the presence of nifedipine, there was about a 40% decrease in response to electrical stimulation and to potassium chloride, with no further decrease in response in the presence of fluoxetine $10^{-5}$ M. The addition of norepinephrine to the bath resulted in contractions that were partially inhibited by $10^{-5}$ M fluoxetine but not by $10^{-6}$ or $10^{-7}$ M (310 or 31 ng/mL) fluoxetine. The authors concluded that fluoxetine has a "moderate inhibitory effect on $Ca^{2+}$ entry," and that fluoxetine would have a low risk of inhibiting vas function unless toxic concentrations were reached.

*Strengths/Weaknesses:* The paper by Medina et al. *(174*) describes effects on ring segments of vasa deferentia exposed *in vitro* to several different concentrations of fluoxetine. A weakness of the study is that the levels of fluoxetine required to cause an effect were so high that these results have little application except under conditions of an acute overdose, in which case the contractility of the vasa deferentia is of little or no concern. The authors are correct in their interpretation that fluoxetine has a very low risk of directly inhibiting the function of the vasa deferentia under normal exposure and use conditions.

*Utility (Adequacy) for CERHR Evaluation Process:* This paper is not useful in an evaluation of possible reproductive toxicity of fluoxetine due to the high exposure levels and the use of an *in vitro* model.

Seo et al. *(175*) duplicated the study of Medina et al. *(174*) to compare SRIs with one another and with clomipramine with regard to inhibition of vasal contraction to norepinephrine. Vasa deferentia were obtained from 15 healthy men undergoing sterilization vasectomy and from 2 men with bladder cancer who were undergoing radical cystectomy. Contractions of vasal strips were produced using $10^{-4}$ M norepinephrine or 70 mM KCl in HEPES-buffered physiologic saline (pH 7.4). Vasal contractions were not inhibited by $10^{-5}$ M (3,100 ng/mL) fluoxetine and were nearly completely inhibited by $10^{-4}$ M (31,000 ng/mL) fluoxetine. The mean inhibitory concentration of fluoxetine was $2.4 \times 10^{-5}$ M (7,400 ng/mL).

*Strengths/Weaknesses:* The paper by Seo et al. *(175*) duplicates the findings of Medina et al. *(174*). Comments provided for the Medina paper apply here.

*Utility (Adequacy) for CERHR Evaluation Process:* This paper is not useful in an evaluation of possible reproductive toxicity of fluoxetine due to the high exposure levels and the use of an *in vitro* model.

### *4.1.4 Sexual dysfunction*

Terms used for sexual abnormalities include the following:

- *Abnormal sexual function*: a general term that can refer to any of the sexual dysfunctions.
- *Decreased sexual response*: an imprecise term that can refer to female arousal disorder, male erectile disorder, female orgasmic disorder, or male orgasmic disorder.
- *Arousal problem*: either female arousal disorder, male erectile disorder, or both.
- *Female orgasmic disorder*: persistent delay in or absence of orgasm following normal sexual excitement. In the text, the following terms are used: *anorgasmia, orgasm problem, and delayed orgasm*.

- *Female sexual arousal disorder*: persistent inability to attain or maintain an adequate lubrication-swelling response during sexual activity. In the text, *arousal problem* refers to this syndrome.
- *Hypoactive sexual desire disorder*: persistent deficient or absent sexual fantasies and desire for sexual activity. In the text, *decreased libido* and *desire problems* are used to indicate this syndrome.
- *Male erectile disorder*: persistent inability to obtain or maintain an adequate erection. In the text, *erectile problem* is used to indicate this disorder.
- *Male orgasmic disorder*: persistent delay or absence of orgasm during sexual activity. The following terms are used to indicate this disorder: *ejaculatory problem, delayed ejaculation, retarded ejaculation, anejaculation, and ejaculatory incompetence*.

Abnormal sexual function is not unusual in the general population and is common in association with depression and with antidepressant medication (Angst *(176*); also reviewed by Baldwin *(177*)). **[When first released, fluoxetine and other SRIs were not expected to have significant sexual side effects. After they were in general usage, case reports of sexual side effects began accumulating.]** The first report of sexual side effects of fluoxetine was a letter describing one woman and one man on therapy with anorgasmia *(178*). In the product label for Prozac®, decreased libido was reported by 4% of 2444 people randomized to fluoxetine for the treatment of depression, OCD, or bulimia, compared to none of 1,331 people randomized to placebo. A published comparison of fluoxetine and bupropion reported impotence in 4.2%, anorgasmia in 1.7%, and decreased libido in 1.7% of depressed subjects treated with fluoxetine *(179*). There is evidence, however, that the incidence of sexual dysfunction associated with fluoxetine is considerably higher than 4%. Early case series suggested anorgasmia or other orgasmic difficulties in 8–16% of patients *(180-182*). A comparison of fluoxetine and paroxetine in the treatment of depression-identified sexual dysfunction in 7% of subjects on fluoxetine in one study *(183*) and abnormal ejaculation or impotence in 20% of men in another study *(184*). Clinical trials of fluoxetine for PMDD or luteal phase dysphoric disorder report sexual dysfunction in 8.5% *(185*) and 17% *(186*) of subjects.

The incidence of sexual dysfunction with fluoxetine is higher if patients are directly queried about symptoms than if they are expected to volunteer sexual complaints. It is commonly assumed that many individuals are reluctant for various reasons to tell their physicians about drug-induced sexual side effects. In an office-based private practice, 54 (34%) of 160 fluoxetine-treated patients reported the onset of sexual dysfunction that had not been present prior to treatment *(187*). Of the 54 patients, 16 reported decreased libido, 21 reported decreased sexual response, and 17 reported both decreased libido and decreased sexual response. Sexual response improved with a decrease in fluoxetine dose and normalized 1–3 weeks after discontinuation of fluoxetine. **[The number of patients who discontinued the drug is not stated.]** In another report, 7 (37%) of 19 patients given fluoxetine 20 mg/day for depression experienced sexual problems *(188*). Patients had been carefully questioned about symptoms prior to therapy and monthly thereafter. Four women had decreased libido, one man had erectile problems, and two men had orgasm or ejaculation problems. When the dose was changed to 20 mg every other day, the sexual problems resolved in 5 of the 7 affected patients. The problems resolved in the remaining two patients when the dose was decreased to 20 mg once per week. A retrospective chart review of 30 men on fluoxetine identified sexual complaints in 12 (40%) *(189*). Interview of a convenience sample of 60 outpatients (22 men and 38 women) on SRIs showed an

incidence of sexual dysfunction in 43%, identical to the incidence of sexual dysfunction (6 of 14) in the patients on fluoxetine in this report *(190*). Patterson *(191*) in a letter indicates that 45 (75%) of 60 men on fluoxetine in his practice reported retarded ejaculation or ejaculatory incompetence, and that the symptoms improved in all 30 men who reduced the fluoxetine dose.

A lower incidence of sexual dysfunction was reported in a retrospective chart review of 596 outpatients on SRIs, half of whom were on fluoxetine *(192*). Patients were said to have been asked, "Have you had any problems with the medication such as upset stomach, jitteriness, or sexual difficulty?" Overall, 16.3% of the sample reported sexual dysfunction, including 23.4% of men and 13.5% of women ($P<0.01$ by chi-square). Orgasm problems were the most common, occurring in 59.3% of the patients on fluoxetine who complained of sexual problems. Desire problems occurred in 30.5% and arousal problems in 10.2% of patients on fluoxetine who complained of sexual problems. **[These percentages add to 100%, suggesting either that patients had only one complaint or that they were recorded only under a single complaint type.]** There was no difference among SRIs in the incidence of sexual complaints.

*Strengths/Weaknesses:* The Expert Panel notes that the incidence of sexual dysfunction in the general population is considerable. Sexual dysfunction is commonly associated with depression, yet when this population is retrospectively investigated for sexual dysfunction in trials with fluoxetine, there are no pre-treatment reports of sexual dysfunction in depressed individuals. While many of these studies claim to study only "new onset" sexual dysfunction, there is little-to-no evidence that these patients were interviewed specifically to address these endpoints prior to treatment with fluoxetine. After the patients had used fluoxetine for a period of time, intensive interviews were done to investigate possible sexual dysfunction. This design flaw as well as others (e.g., lack of use of placebo, interviewers not blind to treatment) may introduce a bias in these reports that is difficult to resolve.

*Utility (Adequacy) for CERHR Evaluation Process:* These reports are useful as supplemental information to the results of better controlled studies.

Hsu and Shen *(189*) found 21 (30%) of 69 women to have sexual side effects including 5 women with only loss of libido, 3 women with only orgasm problems, 12 women with both libido and orgasm problems, and 1 woman with nonspecific complaints *(193*).

*Strengths/Weaknesses:* This study by Hsu and Shen *(189*) is one of two retrospective chart reviews done by this group. Retrospective chart reviews are generally regarded as hypothesis-generating rather than hypothesis-testing. Since retrospective studies using selection methodology are inappropriate for determining a prevalence rate for sexual side effects in a treatment population, this represents a weakness in this study.

*Utility (Adequacy) for CERHR Evaluation Process:* The study by Hsu and Shen *(189*) has limited utility for the CERHR evaluation process.

Zajecka et al. *(194*) used the Rush Sexual Inventory to evaluate 42 outpatients before and after SRI therapy (21 patients used fluoxetine). Treatment-emergent sexual dysfunction was identified in 60% of men and 57% of women after 8 weeks of therapy **[breakdown by specific SRI is not given]**.

Orgasm problems were the most common sexual difficulties. Some improvements in sexual function were also noted as described by the following statement made by authors in their results section: "Males treated with fluoxetine showed a statistically significant increase in desire and frequency to initiate sexual activity and an increased overall degree of sexual satisfaction, and females treated with fluoxetine showed a statistically significant increase in the frequency of pleasurable sexual thoughts and an increase in the desire to initiate sexual activity at the end of 8 weeks of treatment compared to baseline measures." **[These statements are based on analysis of visual analog scales. The percent change from baseline is shown graphically and *P* values are indicated, but there is no indication of what statistical method was used for the comparison.]**

*Strengths/Weaknesses:* The paper by Zajecka et al. *(194*) addresses an important point when considering sexual dysfunction in patients receiving fluoxetine treatment. While a significant portion of the treated population noted sexual dysfunction after fluoxetine treatment, another portion of the treated population reported a significant increase in positive outcomes of primary sexual dysfunction (that may have been present prior to treatment). Since the authors collected baseline data and the study was conducted in a prospective manner, both an increase and decrease in sexual dysfunction due to fluoxetine treatment could be determined. The lack of information regarding the incidence of treatment-emergent sexual dysfunction by specific SRI treatment and the lack of detail of statistical methodology detract from this paper. In addition, this study used the Rush Sexual Inventory, an instrument that has not been validated and has been used only minimally outside of the hospital where it originated. The study did not use a double blind condition, and a placebo was not employed.

*Utility (Adequacy) for CERHR Evaluation Process:* The study by Zajecka et al. *(194*) can be used in an evaluation of fluoxetine reproductive effects, although there are limitations based on design considerations (use of an unvalidated instrument, lack of a control).

In a prospective study of sexual side effects among 31 patients starting SRI therapy, including 8 on fluoxetine, Labbate et al. *(195, 196*) (in what appears to be a duplicate publication of the same or similar data) identified delayed orgasm and a decrease in orgasm quality in men and women. **[Monthly visual analog scales were evaluated using ANOVA with *post hoc t*-testing, an approach that is not optimal for visual analog scale analysis. The effects were large, however, and would probably be confirmed by more appropriate analytic methods.]** In the first month on therapy, 6 of 18 women reported anorgasmia; this proportion had decreased to 2 of 17 by the third month of therapy.

*Strengths/Weaknesses:* The papers by Labbate et al. *(195, 196*) describe a group of patients suffering from anxiety disorders (versus depression in the majority of the other studies) that were evaluated prospectively for sexual dysfunction. The fluoxetine patients comprise only 8 of the 31 patients and the results are presented for SRIs as a whole and are not separated by individual drug. Therefore, it is not possible to separate the effects due to fluoxetine from those due to other SRIs. This open-label study had a small sample size, used an unvalidated instrument, and had no placebo. These major weaknesses in the reporting of the study results limit the usefulness of these papers.

*Utility (Adequacy) for CERHR Evaluation Process:* The papers by Labbate et al. *(195, 196*) can be used in an evaluation of fluoxetine reproductive effects, although there are limitations based on design considerations (use of an unvalidated instrument, lack of a control).

A large, multicenter study sponsored by Eli Lilly and Company assessed sexual side effects as part of an efficacy study of fluoxetine 20 mg/day and 90 mg/week as continuation therapy *(197)*. Subjects were initially treated with fluoxetine 20 mg/day in an open-label fashion for 13 weeks, following which responders were randomized to 25 additional weeks of fluoxetine 20 mg/day, fluoxetine 90 mg/week, or placebo. Subjects self-rated in response to four questions, using a five-point rating scale for each question. **[The methods section indicated that nonparametric analytic methods were used and changes in depression ratings were considered as covariates, but the tables present means and SD.]** The proportions of subjects self-rating at each level of impairment are shown in Table 22 and Table 23. The authors concluded no difference in the proportions at each rating scale. **[However, statistical analysis by CERHR shows a significant shift toward less impaired ratings in several of the domains, as marked in the tables.]**

***Table 22. Number and Percent (%) of Women at Each Level of Impairment [Adapted from Michelson et al. (197)]***

| *Sexual function* | *Level of impairment* | | | | |
|---|---|---|---|---|---|
| | *None* | *Minimal* | *Mild* | *Moderate* | *Severe* |
| ***Sexual interest/desire (n=330)*** | | | | | |
| Before therapy | 86 (26.1) | 32 (9.7) | 52 (15.8) | 74 (22.4) | 86 (26.1) |
| After 13 weeks of therapy | 164 (49.7) | 67 (20.3) | 45 (13.6) | 30 (9.1) | 24 (7.1) |
| ***Lubrication (n=325)*** [a] | | | | | |
| Before therapy | 186 (57.2) | 45 (13.8) | 35 (10.8) | 35 (10.8) | 24 (7.4) |
| After 13 weeks of therapy | 239 (73.5) | 37 (11.4) | 24 (7.4) | 14 (4.3) | 11 (3.4) |
| ***Orgasm (n=317)*** [a] | | | | | |
| Before therapy | 120 (37.9) | 42 (13.2) | 44 (13.9) | 53 (16.7) | 58 (18.3) |
| After 13 weeks of therapy | 176 (55.5) | 51 (16.1) | 30 (9.5) | 31 (9.8) | 29 (9.1) |
| ***Overall sexual function (n=320)*** | | | | | |
| Before therapy | 104 (32.5) | 32 (10.0) | 52 (16.3) | 72 (22.5) | 60 (18.8) |
| After 13 weeks of therapy | 170 (53.1) | 65 (20.3) | 40 (12.5) | 19 (5.9) | 26 (8.1) |

[a] Difference by ANOVA performed by CERHR; authors state no difference among any groups

*Table 23. Number and Percent (%) of Men at Each Level of Impairment [Adapted from Michelson et al. (197)]*

| *Sexual function* | *Level of impairment* | | | | |
|---|---|---|---|---|---|
| | *None* | *Minimal* | *Mild* | *Moderate* | *Severe* |
| ***Sexual interest/desire (n=155)*** | | | | | |
| Before therapy | 51 (32.9) | 21 (13.5) | 34 (21.9) | 32 (20.6) | 17 (11.0) |
| After 13 weeks of therapy | 83 (53.5) | 30 (19.4) | 15 (9.7) | 16 (10.3) | 11 (7.1) |
| ***Erection (n=155)*** [a] | | | | | |
| Before therapy | 82 (52.9) | 17 (11.0) | 25 (16.1) | 17 (11.0) | 14 (9.0) |
| After 13 weeks of therapy | 97 (62.6) | 22 (14.2) | 10 (6.5) | 18 (11.6) | 8 (5.2) |
| ***Orgasm (n=154)*** [a] | | | | | |
| Before therapy | 79 (51.3) | 17 (11.0) | 27 (17.5) | 15 (9.7) | 16 (10.4) |
| ***After 13 weeks of therapy*** | ***90 (58.4)*** | ***21 (13.6)*** | ***14 (9.1)*** | ***16 (10.4)*** | ***13 (8.4)*** |
| ***Overall sexual function (n=155)*** [a] | | | | | |
| Before therapy | 59 (38.1) | 28 (18.1) | 30 (19.4) | 20 (12.9) | 18 (11.6) |
| After 13 weeks of therapy | 84 (54.2) | 29 (18.7) | 14 (9.0) | 17 (11.0) | 11 (7.1) |

[a] Difference by ANOVA performed by CERHR; authors state no difference among any groups



The proportions of patients who were improved, unchanged, or worsened are shown in Figure 3. During this initial 13-week treatment phase, the likelihood of experiencing a decrement in sexual function was associated with age greater than 50 years but not with sex. **[A possible association between sexual function and change in depression rating was not reported for the initial 13-week phase.]**

The changes in sexual function during the continuation phase are shown in Figure 4. Only subjects who responded to the antidepressant effects of fluoxetine 20 mg/day during the initial 13-week phase were randomized to different treatments in the continuation phase. During this phase, improvement in sexual function was associated with improvements in depression, as estimated by the Hamilton Rating Scale for Depression. There were no differences among treatment groups in improvement or worsening of any self-rated sexual function score. **[The figure was derived from a table that mixes men and women and does not indicate the number of persons providing data.]**

*Figure 3. Percent Reporting Overall Sexual Function or Interest, Orgasm, and Lubrication/ Erection Improvement or Worsening during the First 13 Weeks of Fluoxetine Therapy in Michelson et al. (197)*

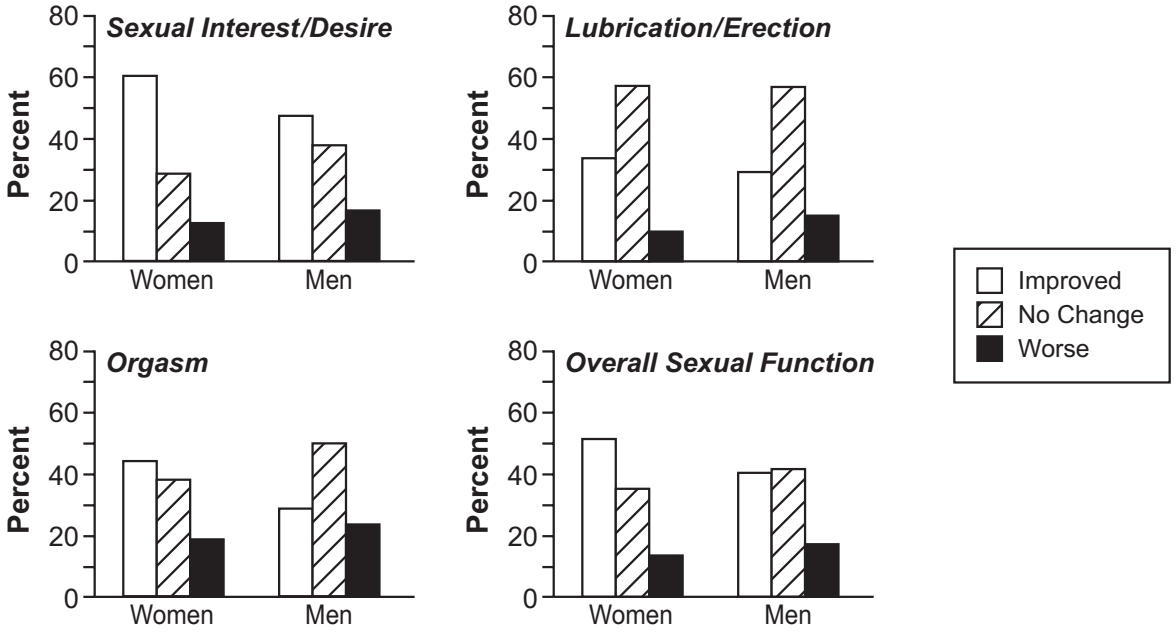
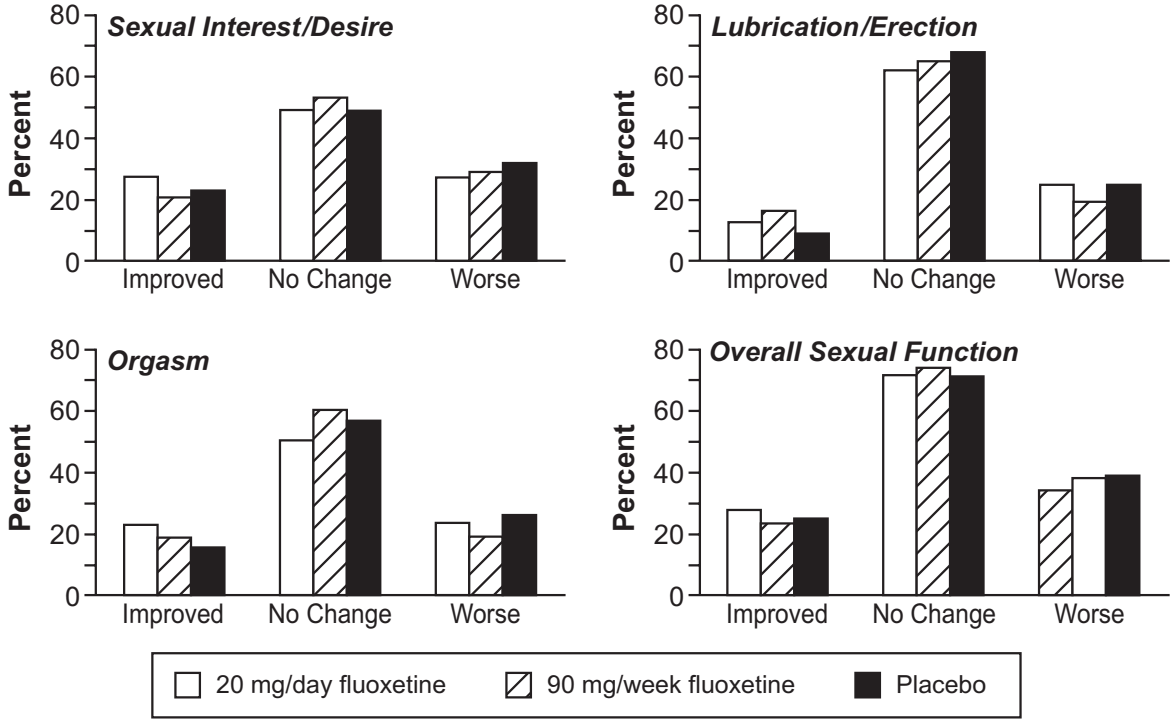



*Figure 4. Change in Overall Sexual Function, Orgasm, Lubrication/Erection, and Sexual Interest/Desire during the 26-week Continuation Treatment [Adapted from Michelson et al. (197)]*



*Strengths/Weaknesses:* The paper by Michelson, et al. *(197*) describes a prospective assessment of sexual dysfunction prior to fluoxetine treatment, at the end of the open-label treatment, and after a double-blind placebo-controlled continuation period. The rating system used to rank various aspects of sexual dysfunction had not been validated *per se*, but had been used previously and had compared well with other, more standard measurement instruments. Another limitation was that the primary purpose of the study was to test efficacy with assessment of sexual dysfunction as an add-on feature. No attempt was made to exclude patients with underlying sexual dysfunction from enrolling in this study. Clinical signs of depression without other signs of psychiatric disease were the only criteria for enrollment. The study demonstrates the problems associated with trying to separate changes in sexual function occurring concurrently with depression that is responding to treatment. Improved achievement of orgasm was recognized as separate from changes in depression. This effect has been noted in other studies, many of which lacked a prospective design and a double-blind placebo-controlled arm. The strongest correlation noted by the authors was a coincident worsening of sexual dysfunction and features of depression in patients starting to fail treatment. Failure of treatment that occurred during the continuation phase could have been due to treatment with placebo, reduced dose level of fluoxetine (to 90 mg/week), or failure of the 20 mg/day dosing regimen.

*Utility (Adequacy) for CERHR Evaluation Process:* This study by Michelson, et al. *(197*) has limited utility in evaluating the effect of fluoxetine on sexual function.



A multicenter, randomized prospective comparison of fluoxetine, sustained-release bupropion, and placebo, sponsored by Glaxo Wellcome (manufacturer of bupropion [Wellbutrin®]) was reported by Coleman et al. *(198*). Fifteen centers were involved in recruiting about 150 subjects for each treatment arm (bupropion 150, fluoxetine 154, placebo 152). Patients were depressed to a similar degree as evaluated by the Hamilton Rating Scale for Depression. All of the patients enrolled in this study were required to have "normal sexual functioning," defined as absence of sexual arousal disorder or orgasm dysfunction. However, patients were allowed to have a "sexual desire disorder" (deficiency of sexual fantasy and desire for sexual activity) as long as they were having sexual activity at least once every 2 weeks. The authors noted that decreased sexual desire is common in depression. The study design consisted of a 1-week screening phase followed by an 8-week treatment phase. At baseline, sexual-desire disorder was present in 24 and 23% of subjects in the bupropion and fluoxetine groups, respectively, compared to 14% of the placebo group. About 2/3 of subjects in each arm completed the 8-week protocol (bupropion 94, fluoxetine 97, placebo 102). All 3 treatments were effective in a proportion of patients: a reduction of at least 50% in the Hamilton, which had been defined as response, occurred in 56, 57, and 50% of subjects in the bupropion, fluoxetine, and placebo groups, respectively (*P* NS). Remission was defined as a decrease in the Hamilton to a rating of lower than 8 and was achieved in 47, 40, and 32% of subjects in the bupropion, fluoxetine, and placebo groups, respectively. The remission rate was significantly different between bupropion and placebo but not between bupropion and fluoxetine or fluoxetine and placebo.

Sexual dysfunction was evaluated by trained interviewers who met with subjects each week to assess whether they met predetermined criteria for a sexual-function disorder. About 1/3 of subjects in the fluoxetine group met criteria for orgasm dysfunction by the end of the 8-week trial, compared to about 10% of subjects on either bupropion or placebo ($P < 0.001$). The significant increase in orgasm dysfunction on fluoxetine compared to the other two arms persisted when the analysis was restricted

to subjects whose depression remitted. **[The authors identified an association between high-dose fluoxetine and orgasm disorder that was not seen for bupropion at high doses; however, ANOVA performed by CERHR did not show a statistically significant relationship.]**

Although patients with sexual-arousal disorder and orgasm disorder were excluded from enrolling in the study, sexual-desire disorder was present in 24 and 23% of subjects in the bupropion and fluoxetine groups at baseline, respectively, compared to 14% of the placebo group. The study attempted to evaluate only "substance induced arousal disorder and orgasm dysfunction" because these were endpoints of "sexual functioning" rather than "sexual desire disorder," although data were collected for all three endpoints. Over the 8-week course of the study, the prevalence of desire disorder did not change in the fluoxetine and placebo groups while the prevalence in the bupropion group decreased to a level similar to placebo.

At baseline, the percentage satisfied with their sexual function was 84, 84, and 83% in the bupropion, fluoxetine, and placebo groups, respectively. Of these subjects, about 7, 23, and 3% became dissatisfied with their sexual function in the bupropion, fluoxetine, and placebo groups, respectively **[estimated from a figure in the paper]**.

*Strengths/Weaknesses:* The paper by Coleman et al. *(198*) is the only double-blind, placebo-controlled, multicenter study using direct inquiry about sexual side effects. The paper provides useful information regarding the onset of sexual dysfunction in patients receiving fluoxetine. The selection criteria for this study was different than that used for Michelson, et al. *(197*), in that the patients had to have clinical signs of depression without sexual arousal disorder or orgasm disorder. Sexual desire disorder was allowed. These selection criteria eliminated a group of patients present in the Michelson et al. study and therefore this report could not replicate the Michelson study findings of an improvement in sexual functioning and depression symptoms with fluoxetine treatment. The Coleman et al. study presents information that patients with no underlying sexual arousal disorder or orgasm disorder receiving fluoxetine experienced orgasm dysfunction on the order of 30–35%, as compared to approximately 10% in the placebo group. The percentage of patients with sexual desire disorder did not change in either the fluoxetine-exposed group or the group receiving a placebo. The percentage of patients with sexual arousal disorder increased over the 8-week treatment period in both the fluoxetine and placebo groups, although the difference between these two groups was statistically significant at three of the nine weekly time points. However, at the end of the 8-week treatment period, there was no statistically significant difference between the fluoxetine-treated and placebo groups. The percentage of patients satisfied with their sexual functioning at baseline (prior to drug or placebo exposure) was increased in the fluoxetine group when compared to placebo over the course of the 8-week study. This finding is not surprising as this curve roughly follows the curve for orgasm dysfunction, although the maximum level is only 20–25% of patients. These data provide additional evidence that fluoxetine exposure can cause an increased incidence of orgasm dysfunction in patients receiving the drug. The data supporting an effect on sexual desire or arousal are less robust. The removal of patients with an underlying problem with sexual arousal prior to study start eliminated the possibility of fluoxetine improving this disorder and therefore affecting the overall rate within patients suffering from depression.

*Utility (Adequacy) for CERHR Evaluation Process:* The paper by Coleman et al. *(198*) is a valuable study that is useful for the CERHR process.

Clayton et al. *(199*) conducted a multicenter, double blind study to compare the effects of the antidepressant reboxetine to fluoxetine and placebo. Adult outpatients (ages 18–65 years) with MDD and a Hamilton Rating Scale for Depression score >22 randomly received placebo, 20–40 mg/day fluoxetine, or 8–10 mg/day reboxetine for up to 8 weeks. Each group contained 150 subjects with 51–60 males and 90–99 females. Demographics such as age, baseline sexual function, and depression rating were similar among the three groups. None of the subjects was taking other drugs. Sexual dysfunction was assessed at baseline and weeks 4 and 8 using the Rush Sexual Inventory. Two-way ANOVA was used to examine continuous data and the Cochran-Mantel-Haenszel test was used to assess categorical data. Data were presented for week 8. Due to premature withdrawal, the final number of male/female subjects in each group were 47/73 for placebo, 45/85 for fluoxetine, and 50/78 for reboxetine. Compared to the placebo group, fluoxetine significantly reduced the subjects' ability to become sexually excited. A significant decrease in overall sexual satisfaction with fluoxetine treatment was also noted when subjects were evaluated together as a group, but not separately by sex. The number of women who were unable to achieve orgasm was significantly increased following 8 weeks of fluoxetine treatment **[data not shown]**. In subjects who symptomatically responded to fluoxetine, ability to become sexually excited and overall sexual satisfaction were significantly reduced compared to placebo. Compared to the reboxetine group, subjects on fluoxetine had less difficulty with achieving erections, obtaining full erections, and had less pain during sex and ejaculation.

*Strengths/Weaknesses:* The strengths of this study include its prospective, randomized, double blinded design and use of a placebo group. Baseline information on sexual function was collected and demographic variables such as age and severity of depression were kept constant between groups. A weakness of the study is the use of the Rush Sexual Inventory, which has not been validated.

*Utility (Adequacy) for CERHR Evaluation Process:* This is a valuable study which is useful for the CERHR evaluation of fluoxetine.

In a study not sponsored by any pharmaceutical company, Modell et al. *(200*) showed no difference by frequency distribution of questionnaire responses between SRIs including fluoxetine and bupropion with respect to libido, arousal, duration of time from arousal to orgasm, intensity of orgasm, and duration of orgasm. **[Study authors reported a decrement in ratings for sexual function on SRIs compared to bupropion; however, their scoring system appears to have been analyzed incorrectly by application of positive and negative integer values to ranks with subsequent use of *t*-tests and ANCOVA.]**

*Strengths/Weaknesses:* In the study by Modell et al. *(200*), an unvalidated questionnaire was given to patients who had received an antidepressant in their clinic. The response rate was approximately 33%. There is no way to know if the sample responding were representative of the general sample treated. In addition, the method of statistical analysis used in this paper precludes useful interpretation of the data. The purported decrement in ratings for sexual function on SRIs compared to bupropion was based on inappropriate analysis by application of positive and negative integer values to ranks with subsequent use of *t*-tests and ANCOVA.

*Utility (Adequacy) for CERHR Evaluation Process:* The study by Modell et al. *(200*) is not useful for the CERHR process.

A Spanish study of 1,022 outpatients on different antidepressant medications used a structured questionnaire to estimate the incidence of sexual dysfunction (Montejo et al. *(201*); this information appears in a preliminary version as Montejo-Gonzalez et al. *(202*)). There were 279 patients on fluoxetine (166 women and 113 men), 57.7% of whom reported sexual dysfunction. Decreased libido and delayed orgasm/ejaculation were the most common problems, occurring in 50.2 and 49.5% of fluoxetine-exposed individuals, respectively. Anorgasmia/anejaculation and erectile function/ decreased vaginal lubrication occurred in 39.1 and 21.8% of patients on fluoxetine, respectively. **[Percentages add to more than 100; each subject may have had more than one complaint.]**

*Strengths/Weaknesses:* The major strengths of the Montejo et al. *(201*) study are that it approximated general practice without altering physician prescribing patterns and that it evaluated treatment-emergent sexual dysfunction in a prospective fashion. The paper provides information on sexual dysfunction in a large group of patients receiving one of ten different psychotropic agents. The authors used a questionnaire (informally validated) to assess changes in sexual function in patients with "normal sexual function" who had started one of the ten medications. The patients had to recall the state of their sexual functioning prior to receiving the medication (no information is presented as to how long a period of time elapsed between starting the medication and symptom recall). The pre-treatment sexual function status was then compared to the current sexual function status while receiving the medication. There is no mention of an entry bias (patients with sexual dysfunction prior to treatment who lied in order to be included in the study) nor is it apparent that the authors looked for this phenomenon. The method of statistical analysis did not appear to control for multiple comparisons, nor was there a comparison with patients who did not receive any medications.

*Utility (Adequacy) for CERHR Evaluation Process:* The study by Montejo et al. *(201*) can be used as long as its weaknesses are noted.

A multicenter, cross-sectional study on the prevalence of sexual dysfunction in people taking antidepressant medication was sponsored by Glaxo Wellcome, Inc *(203*). Subjects were recruited from 1,101 primary care clinics in the U.S. and consisted of sexually active adults taking a single antidepressant medication for depression. Patients completed a 14-item, gender-specific questionnaire consisting of questions in 5 domains (sexual pleasure, desire/frequency, desire/interest, arousal, orgasm) plus a global score. Sexual dysfunction was defined based on threshold scores determined in a previous study using the same instrument. Questionnaires were administered, reviewed, and scored by the primary care physician, who discussed the results with the patient. Additional information on possible contributors to sexual dysfunction was obtained by interview with the primary care physician. In addition to the overall population, a "target population" was identified consisting of patients without other possible causes of sexual dysfunction, such as use of other medications that might cause sexual dysfunction, or the presence of other illnesses. Patients in the target population were on their respective antidepressant medications for at least 3 months, which, according to the authors, would reduce the likelihood that sexual dysfunction was due to depression or to previous antidepressant medication. Of the 6,297 patients in the total population, 1,531 (24.3%) were taking fluoxetine. Of the 798 patients in the target population, 245 (30.7%) were taking fluoxetine. There were seven other antidepressant medications represented in the sample, two of which were counted separately as their immediate release and delayed release preparations. About 35% **[estimated from a graph]** of the patients on fluoxetine were considered to have sexual dysfunction based on reaching

the threshold score **[presumably for global sexual dysfunction]**. The percent of patients with sexual dysfunction on other medications ranged from about 20 to 40% **[estimated from a graph]** and the overall percent with sexual dysfunction appeared identical to that for fluoxetine. The prevalence of sexual dysfunction in the target group was about 25% **[estimated from a graph]**, which was similar to that for the group overall. The range for sexual dysfunction associated with antidepressant medication in the target group ranged from about 5 to 30% **[estimated from a graph].** Logistic regression was used to assess the influence of a number of demographic and health factors in the overall clinical population on the likelihood of sexual dysfunction. The OR: for fluoxetine (taking sustained release bupropion as the reference) was 2.23 (95% CI: 1.75, 2.87). Four other antidepressant medications had elevated ORs with 95% CIs that excluded unity. The highest of these ORs was 2.89. Other statistically significant contributors to sexual dysfunction in the overall clinical population from the regression included ages 50 – 59, not currently married or widowed, college graduation, employment less than full-time, retirement, tobacco use 6 – 20 times/day, previous sexual side effects on another antidepressant, co-morbid illness, concomitant medication, history of little or no sexual enjoyment, and sexual enjoyment being rated as somewhat or not important. Using a high vs. a low dose of antidepressant medication overall was associated with an increased OR: for sexual dysfunction, but for fluoxetine, there was no association with low ($\leq$20 mg/day) vs. high dose ($\geq$30 mg/day).

*Strengths/Weaknesses:* The paper from Clayton et al. *(203*) provides another source of information regarding the effect of antidepressant medication on sexual functioning. The strengths of this study include a large number of patients evaluated across several different treatment modalities, employment of a well validated instrument, approximation of normal physician practices, use of a questionnaire that had been used previously to elicit information from patients regarding sexual functioning, and an attempt to evaluate the primary care physicians who were collecting the data at the treatment site. A possible weakness is that the "threshold" scores used to define sexual dysfunction came from untreated control patients from another study investigating sexual function in depressed patients and were not further defined in the paper. The authors "prospectively" attempted to identify a target population that was expected to be free of sexual dysfunction based on parameters historically associated with sexual dysfunction. The authors attempted to correct for pre-existing sexual dysfunction by excluding patients with known organic causes of sexual dysfunction and thus formed a subgroup assumed to consist of treatment-emergent sexual dysfunction only. While this design was "prospective" from the standpoint of the analyzing data, it was not a true prospective study design in that the patients were not selected into this group prior to receiving antidepressant medication. How sexual dysfunction was determined is not explained fully in the paper, but is cited only as a reference by the lead author. In addition, the scores from the individual parts (arousal, orgasm) of the exam were not reported, only the global score. It is not possible, therefore, to compare with other studies in which these parameters were reported individually. The data presented from patients treated with fluoxetine in the overall clinical population did not differ from any other treatment groups and were within the range for the entire treated population.

*Utility (Adequacy) for CERHR Evaluation Process:* The study by Clayton et al. *(203*) is useful for the CERHR evaluation process and demonstrates disturbance of sexual function during fluoxetine therapy for depression.

Consistent with an effect in inhibiting orgasm, fluoxetine has been reported anecdotally and in

controlled reports to be useful in the treatment of premature ejaculation *(204-210*). One mechanism by which fluoxetine may affect premature ejaculation is through a decrease in penile somatosensory threshold *(211*). There are several case reports of successful treatment of paraphilias with fluoxetine *(212-215*).

There have also been reports of improved erectile function *(216, 217*) and prolonged erection *(218, 219*) associated with fluoxetine therapy in men. There have also been reports of spontaneous sexual experiences experienced by patients on fluoxetine *(220-222*). These spontaneous experiences included sexual arousal without penile erection in a man, arousal with or without sexual fantasies in several women, and one case of clitoral engorgement and orgasm associated with yawning.

**[Reports of improved sexual function reinforce the point that the individual response to fluoxetine is highly variable and cannot be predicted. At least in certain cases, the effects observed are opposite to what would be expected based upon the larger studies. The effects observed appear to be highly dependent on dose, with a doubling or halving of the dose rate either inducing or relieving the associated symptoms.]**

## *4.2 Experimental Animal Data*

### *4.2.1 Female Reproduction*

#### *4.2.1.1 In vivo*

Unpublished reproductive toxicology studies from Lilly Research Laboratories were abstracted by Tabacova *(138*) of the FDA National Center for Toxicological Research. **[The original reports were requested from Eli Lilly and Company but were not received. The information presented here is from the Tabacova summary. The data are presented in tabular form in the Tabacova paper with designations of statistical differences and of "statistically non-significant change." The changes are frequently described in percent change from control, without an indication of variance, precluding formal trend testing. Only the changes marked in the tables as statistically significant are indicated here—in no instances do the NOAELs appear to have been based on "statistically non-significant" determinations. The FDA Pharmacologist Review of NDA 18-936, dated March 14, 1984 *(139*), also contains a summary of this study, but was not judged by the Panel to be more useful than the Tabacova review.]** A fertility study in the female Wistar rat used fluoxetine doses of 0, 2, 5, and 12.5 mg/kg bw/day by oral gavage (n = 30 females/dose group) for 2 weeks prior to mating, plus gestation and lactation. Approximately 10 dams per group were sacrificed on GD 20 for an evaluation of prenatal developmental toxicity and about 20 dams per group were allowed to deliver and nurse their litters for postnatal developmental toxicity evaluations. Weight gain was decreased at the top dose during the 2-week pre-mating period. There were no adverse effects of fluoxetine on the proportion of mated females that were pregnant. There were statistically significant decreases in birth weight per litter, pup weight gain on PND 7, and pup survival on PND 7 at the top dose. The Tabacova review noted several effects that were not statistically significant but were considered **[by unstated criteria]** to be dose related. Those effects included decreased numbers of corpora lutea and implants at the two highest doses; decreased litter size and live fetuses at the high dose; and increased embryolethality at the high dose. The summary data in the Tabacova tables do not give variances for continuous data or litter proportions for categorical data, precluding the calculation of a

benchmark dose. The adult reproductive NOAEL was 12.5 during pregnancy. **[The decrease in pre-mating weight was not considered in determining the NOAEL for reproductive toxicity but was considered in determining the NOAEL for adult general toxicity.]** The developmental NOAEL was 5 mg/kg bw/day.

*Strengths/Weaknesses:* This study appears to have been a standard reproductive study; however, the lack of access to the description of the methods and the data in the original report precludes an evaluation of strengths and weaknesses.

*Utility (Adequacy) for CERHR Evaluation Process:* The available level of detail is insufficient for use of this study in the Evaluation Process.

Matuszczyk et al. *(223*) studied the effects of subchronic fluoxetine treatment on sexual behavior in female rats in two sets of experiments reported in one publication. Both experiments were conducted in 74-day-old female rats. In the first experiment, estrous cyclicity was examined through vaginal smears. Rats were observed for signs of behavioral receptivity (e.g., lordosis, hop/darting, and ear wiggling) when placed near a male, but not allowed to copulate. Observations were made daily starting 1 week before treatment. Fifteen rats/group were then injected **[specific route not specified]** with 10 mg/kg bw/day fluoxetine **[purity not specified]** in saline or saline alone for 3 weeks. Daily observations of estrous cyclicity and behavior continued during the injection period. The proportion of rats displaying behavioral estrus at least once per week was recorded and analyzed by the chi-square test. Results are listed in Table 24.



***Table 24. Percentage of Female Rats Displaying Estrous Behavior at Least Once per Week [Matuszczyk et al. (223)]***

| *Fluoxetine dose (mg/kg bw/day)* | *% Females with estrous behavior on following days of fluoxetine treatment* | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Pre-test* | *7* | *14* | *21* | *28[a]* | *35[a]* | *42[a]* |
| 0 | 100 | 100 | 100 | 95 | 100 | 100 | 100 |
| 10 | 100 | 95 | 70* | 30** | 50** | 80 | 100 |

[a] Post-treatment
*$P<0.05$, **$P<0.01$ compared to controls
Values estimated by CERHR from a graph

A reduction in the percentage of animals displaying behavioral estrus was noted during the first week of treatment and reached statistical significance during the second and third weeks of treatment. The effect remained statistically significant for a week following treatment, but animals were fully recovered within 3 weeks after treatment ended. Vaginal cyclicity remained normal in both fluoxetine-treated (n = 8) and saline-treated (n = 7) animals (data not shown).

In the second experiment by Matuszczyk et al. *(223*), 29 rats were ovariectomized and allowed to recover for a 1-week period. The rats were then primed with estradiol benzoate and progesterone injections. One behavioral test was conducted and rats were then injected with 10 mg/kg bw/day fluoxetine in saline or saline alone **[number treated in each group not specified; route not specified]** for 42 days. Sexual behavior and motivation were tested on treatment days 7, 14, 21, 28, 35, and 42.

For the last test conducted on day 42, the estradiol benzoate level was doubled. In the sexual behavior tests, females were allowed to mate with males for a total of ten mounts and receptive behaviors were evaluated, as described above. Sexual motivation was determined by the amount of time the female rat spent near a male rat vs. a female rat in estrus. Data were analyzed by Mann-Whitney *U*-test. Results and levels of statistical significance are listed in Table 25. As noted in Table 25, females displayed less hop/dart and ear wiggling behavior between days 21 and 42 and less lordosis behavior between days 21 and 35. The only time at which fluoxetine-treated animals spent significantly less time with males compared to control animals was day 7.

***Table 25. Sexual Behavior in Female Rats [Matuszczyk et al. (223)]***

| *Parameter* | *Dose (mg/kg bw/day)* | *Days following fluoxetine treatment* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *Pre-test* | *7* | *14* | *21* | *28* | *35* | *42* |
| Number of hop/dart and ear wiggling responses | 0 | 7 | 28 | 23 | 25 | 26 | 29 | 28 |
| | 10 | 6 | 23 | 19 | 20* | 18 | 20* | 19* |
| Median lordosis quotient | 0 | 100 | 100 | 100 | 100 | 99 | 100 | 100 |
| | 10 | 100 | 95 | 99 | 93* | 85* | 85*** | 100 |
| % Time with male | 0 | 24 | 30 | 19 | 17 | 26 | 23 | 25 |
| | 10 | 28 | 15* | 20 | 19 | 25 | 20 | 27 |

*$P<0.05$, ***$P<0.001$ compared to controls
Values estimated by CERHR from graphs
Lordosis quotient = (number of lordosis divided by number of mounts) × 100

Matuszczyk et al. *(223*), concluded that subchronic fluoxetine treatment impairs estrous behavior in normally cycling rats, decreases receptive behavior in ovariectomized rats primed with estradiol benzoate and progesterone, but only marginally affects female sexual motivation.

*Strengths/Weaknesses:* The lack of identification of the injection route for these studies is an important weakness. There is no mention of whether females were selected based on proven cycling, a standard criterion for this kind of study that should have been used. The use of a single dose level of fluoxetine precludes evaluation of a dose-response relationship.

*Utility (Adequacy) for CERHR Evaluation Process:* This study provides minimal utility for the CERHR Evaluation Process based on the inappropriate route of administration and the lack of information on proven cycling of the females.

Frye and Rhodes *(224*) examined the effects of acute fluoxetine **[purity not specified]** and zaprinast treatment on sexual behavior in female hamsters. Sixteen sexually inexperienced hamsters in the peak of estrus (~60-days-old) were subjected to a series of studies involving treatment with vehicle, fluoxetine, or zaprinast, a phosphodiesterase-5 inhibitor. All hamsters received each treatment in randomized, counterbalanced order, no more than once per week. Following the administration of each treatment, the females were placed in the proximity of a male hamster and female sexual behavior was assessed by measuring lateral displacement, pelvic adjustments made in response to

sexual stimuli. Data were analyzed by ANOVA and least-square means *post hoc* tests. Dosing with fluoxetine in saline at 10 mg/kg bw i.p. at 60 minutes prior to contact with a male hamster, significantly reduced lateral displacement compared to the vehicle saline group. Intraperitoneal administration of 3 mg/kg bw zaprinast 40 minutes following fluoxetine treatment attenuated the fluoxetine response and lateral displacement was equivalent to control levels.

*Strengths/Weaknesses:* A strength of this study is the randomized crossover design that exposed animals to all treatment conditions. Measurement of lateral displacement provided a quantitative assessment of sexual behavior. Use of hamsters allows for a comparison of effects to typical rodent species used in laboratory studies. Weaknesses of this study include the acute, single-dose level exposure and use of the i.p. route, neither of which are relevant to human exposures. In addition, the use of a single dose level precludes a dose-response assessment.

*Utility (Adequacy) for CERHR Evaluation Process:* This study has limited usefulness since the treatment conditions are not relevant to human exposures.

Sullivan et al. *(225*) examined the effects of fluoxetine on estrous cycle lengths of fasted mice in a study designed to examine the role of the serotonergic system as a mediator of leptin effects on the reproductive system. In the study, C57B16-J mice (8–10 weeks-old) with normal estrous cycles received one of several treatments (n=5–7 per group) during diestrus, prior to or during a 48-hour fast. Body weights were measured and estrous cycles were observed daily until the mice resumed estrous cycling. Statistical analyses included ANOVA followed by *post hoc* pair-wise analysis with Tukey-Kramer, Student-Newman-Keuls, and Fisher-protected least significant difference tests when indicated. Mice s.c. injected with 32 mg/kg fluoxetine **[purity not specified]** at the start of fasting had a cycle length (4.7±0.6 days) equivalent to those of mice that were i.p. injected with 0.1 mg/kg leptin every 12 hours (4.6±0.7 days) and those of mice that were not fasted and injected with saline (4.5±0.2 days). In contrast, the cycles of mice that were fasted and received twice daily i.p. saline injections were significantly longer (10.2±0.5 days) due to a prolonged diestrus stage. Mice treated with fluoxetine or leptin resumed estrous cycles at body weights below pre-fast levels, while mice in the other groups did not resume cycling until returning to or surpassing pre-fast body weights. Co-administration of fluoxetine and leptin with 1 mg/kg and 2 mg/kg of the 5HT 1/2/7 receptor antagonist metergoline, respectively, blocked the protective effects on estrous cycle length. Percent body weight loss during fasting and body weight gain and food intake 24 hours after feed resumption were equivalent in all fasted animals. Fluoxetine was also administered to leptin-deficient or leptin receptor-deficient mice and found to have no effect on body weight or initiation of estrous cycles or fertility in the normally infertile animals. According to the study authors, the results of this study are consistent with the hypothesis that leptin signals are conveyed to gonadotropin-releasing hormones by serotonergic neurons.

*Strengths/Weaknesses:* The dose of fluoxetine used in this study was excessively high and the route is not relevant to human exposure. The 48-hour fast in this species is equivalent to starvation. The authors do not indicate whether the female mice were proven cyclers.

*Utility (Adequacy) for CERHR Evaluation Process:* This study is not adequate for the CERHR Evaluation Process.

Van de Kar et al. *(226)* conducted a study to determine if long-term fluoxetine treatment alters estrous cycles or sensitivity of hypothalamic 5-$HT_{1A}$ or 5-$HT_{2A}$ receptor systems in cycling female Sprague-Dawley rats. Rats (n=20–34/group; 60 days old) were i.p. injected with saline or 10 mg/kg bw/day fluoxetine HCl **[purity not specified]** for 3 consecutive estrous cycles starting on metestrus and ending 1 day prior to metestrus. Vaginal smears were conducted prior to and during treatment with fluoxetine to monitor estrous cycles and plasma estradiol levels were measured during metestrus. One day after the last fluoxetine injection, rats were administered saline, 8-OH-DPAT (a 5-$HT_{1A}$ agonist), or DOI (a 5-$HT_{2A}$ agonist), and sacrificed 15–30 minutes later. Blood was collected for an analysis of plasma oxytocin, ACTH, and corticosterone, as peripheral indicators of hypothalamic 5-$HT_{1A}$ or 5-$HT_{2A}$ sensitivity. Blood prolactin and renin levels were also measured. Estradiol data were analyzed by Student *t*-test and all other hormonal data by two-way ANOVA. The Newman Keuls' multiple-range test was used to compare group means. The fluoxetine-treated rats lost weight and their body weight was significantly lower than control values by day 3 of the study. Fluoxetine treatment had no effect on estrous cycle length or plasma estradiol levels (n=7–8 rats/group examined). An increase in plasma ACTH, oxytocin, and corticosterone levels that occurs following injection with 8-OH-DPAT in saline-treated rats was completely blocked by fluoxetine pre-treatment. 8-OH-DPAT had no effect on plasma prolactin or renin levels in saline-treated rats but significantly increased prolactin levels in the fluoxetine group. Fluoxetine had no effect on DOI-induced increases in plasma ACTH, corticosterone, oxytocin, or renin. DOI treatment significantly increased plasma prolactin levels in the fluoxetine but not saline group. The study authors concluded that fluoxetine treatment of rats for three cycles desensitizes hypothalamic postsynaptic 5-$HT_{1A}$ signaling without affecting estrous cycling.

*Strengths/Weaknesses:* This study used appropriate methods, controls, numbers of animals, and statistical analyses; however, females were not selected for cyclicity, and the i.p. dose is not relevant to human exposure. The reduction in female body weight is consistent with other reports using this dose. The use of a single dose level precluded an evaluation of the dose-response relationship. Although females were housed together to synchronize cycles, the introduction of males in the vivarium would have been more effective for synchronization.

*Utility (Adequacy) for CERHR Evaluation Process:* Due to the single high i.p. dose level and the lack of proven cyclicity of the females selected for study, this report is not adequate for the Evaluation Process.

Fıçıcıoglu et al. *(227)* postulated that fluoxetine-induced hyperprolactinemia could produce a rat model of adenomyosis. They treated 7–8 week-old female Wistar rats (190–250 g), some of which had been ovariectomized, with fluoxetine 0.5 mg/rat (about 2.5–2.6 mg/kg) or an unspecified placebo by daily oral gavage for 14 weeks. The fluoxetine was obtained by opening 20 mg capsules that had been manufactured for human use. **[No information is given on what portion of the contents of the capsule consisted of fluoxetine.]** Fifty rats were divided into four groups: Ovariectomized+fluoxetine, ovariectomized+placebo, intact+fluoxetine, and intact+placebo. **[It does not appear that intact animals were sham operated.]** One data table indicates 12 rats per group **[two animals are not accounted for]**. Serum prolactin was measured in blood obtained from conscious rats by cardiac puncture. A commercial immunometry kit was used with an intra-assay variation of 3.5–4.7% and an interassay variation of 6.8–8%. In the animals receiving fluoxetine (ovariectomized or intact), prolactin-serum levels were elevated compared to rats receiving placebo. Mean serum prolactin

concentrations (±SD) for fluoxetine-treated animals were 74.88±2.30 and 73.58±2.07 ng/mL in intact and ovariectomized rats, respectively. Mean serum prolactin concentrations in placebo-treated rats were 11.54±3.20 and 10.99±2.06 ng/mL in intact and ovariectomized rats, respectively. Animals were decapitated **[apparently without anesthesia]** and uteri were examined for evidence of adenomyosis. The uteri of fluoxetine-treated rats were said to be "2–2.5 times the size of those of their controls" but no data were provided on uterine measurements or weights. In the intact rats receiving fluoxetine, "all but one" demonstrated adenomyosis. No adenomyosis was apparently seen in any other uteri. The authors concluded that fluoxetine-associated hyperprolactinemia can produce adenomyosis in the presence of functioning ovaries.

*Strengths/Weaknesses:* The lack of data on uterine weight is an important shortcoming of this study. The effect of fluoxetine on prolactin in humans is already known and this study adds little if anything to our understanding. The use of gavage dosing is a strength; however, the likely presence of an unspecified amount of inert material in the capsule makes it impossible to know what dose of fluoxetine was actually administered.

*Utility (Adequacy) for CERHR Evaluation Process:* This study is not adequate for the CERHR Evaluation Process.

Pecins-Thompson and Bethea *(228*) examined fluoxetine effects on hormone levels of spayed Rhesus macaques in a study designed to determine mechanisms of progesterone-induced prolactin secretion. Five spayed female monkeys (5–6.0 kg) **[age not specified]** were used in the study. During the first week of the study, the monkeys were infused with saline and received Silastic implants containing estrogen. An s.c. injection of 20 mg progesterone was administered during the second week of the study. During this time period, blood samples were collected twice daily on 1 day prior to and 3 days following the progesterone injection. Three days later the animals received 5 mg/day **[0.8–1 mg/kg bw/day]** fluoxetine **[purity not specified]** i.v. for 4 weeks. A second s.c. injection of 20 mg progesterone was administered on the second day into the fourth week of fluoxetine infusion. Blood samples were again collected during this time period twice daily for 1 day prior to and 3 days following the progesterone injection. Plasma levels of estrogen, progesterone, and prolactin were compared prior to and following fluoxetine treatment, with each monkey serving as its own control. Data were analyzed by two-way ANOVA, *post hoc* comparisons with the Tukey-Kramer multiple comparisons test, and/or the Student-Newman-Keuls multiple-comparisons test.

Plasma progesterone levels were below detection limits prior to the progesterone injection. Following progesterone injection, plasma progesterone levels were measured at 73.0±12.1 and 50.2±10.9 ng/mL in the saline- and fluoxetine-infused animals, respectively. Four days later, the plasma progesterone levels were measured at ~5 ng/mL. There were no significant differences in plasma progesterone levels prior to or following fluoxetine treatment. Plasma estrogen levels were equivalent in saline- and fluoxetine-treated animals and mean levels were reported at 206±3.4 pg/mL and 177±3.2 pg/mL in each group, respectively. The estrogen values were reported to be within physiological range by study authors. Prior to the progesterone injection, prolactin levels were similar in saline- and fluoxetine-treated animals. A progressive increase in prolactin levels occurred in both groups following the progesterone injection. Prolactin levels were higher in the fluoxetine vs. the saline group and those values reached statistical significance 2 days following progesterone injection (~15 ng/mL vs. ~30

ng/mL prolactin in saline vs. fluoxetine groups, respectively).

To block nuclear, but not membrane, progestin receptors, treatment with RU 486 was also tested. RU 486 blocked the progesterone-induced increase in prolactin. According to the study authors, this study suggests that progesterone induces prolactin secretion through a genomic mechanism and that serotonin plays a role in neural regulation of progesterone-induced prolactin secretion.

*Strengths/Weaknesses:* This study provides additional information on the mechanism of prolactin release by fluoxetine. The most important weakness is the use of i.v. dosing, which is not relevant to human exposure.

*Utility (Adequacy) for CERHR Evaluation Process:* This study is marginally adequate for the Evaluation Process. Although it provides information on the release of prolactin, which may constitute a clinically important adverse effect of fluoxetine therapy, the i.v. route calls into question the relevance of this mechanistic study for human risk assessment.

#### *4.2.1.2 In vitro*

Vedernikov et al. *(229*) examined the effects of fluoxetine on spontaneous and serotonin-induced contractility in Sprague-Dawley rat uterine rings *in vitro*. Uterine rings were prepared from 6 rats sacrificed on GD 14 (mid-gestation) and 6 rats sacrificed on GD 22 (term gestation). The rings were incubated in Krebs' buffer to which fluoxetine was added in 1.0-log unit increments ($10^{-9}$ through $10^{-5}$ M **[0.31 – 3,100 ng/mL]**) every 10 minutes. Fifteen minutes after the last fluoxetine dose, the dose-response to serotonin ($10^{-10}$ through $10^{-5}$ M) was measured. Organ chambers were then washed and tissue viability was confirmed with potassium chloride. **[A time solvent control was used but treatment of that sample was not described in detail.]** Data were analyzed in terms of integral activity at each dose, serotonin concentration resulting in 50% maximal effect, the –log50% of maximal effect, and AUC-response curves. Statistical significance was determined by one-way ANOVA and Tukey multiple comparison tests. Fluoxetine had no effect on spontaneous contractile activity in mid- or term-gestation uterine samples. Fluoxetine attenuated the serotonin-induced concentration-dependent increase in activity. In both mid- and term-gestation samples, fluoxetine treatment significantly shifted the serotonin concentration-response curve to the right and reduced the AUC. Similar effects were observed with the other drugs tested, which included imipramine and nortriptyline. The authors stated that reported increases in premature delivery in women treated with fluoxetine cannot be explained by direct myometrial action by fluoxetine; however, this study cannot rule out CNS effects on uterine contractility.

*Strengths/Weaknesses:* The conclusion of the authors that premature delivery cannot be explained by a direct action of fluoxetine on the myometrium is limited by the *in vitro* study design.

*Utility (Adequacy) for CERHR Evaluation Process:* This study is adequate as supplemental information to *in vivo* studies.

Rudolf et al. *(230*) conducted a study that focused on determining the role of oxytocin on uterine serotonin uptake in albino mice. *In vitro* uptake of serotonin by mouse uterine horns was found to be sodium-dependent, saturable, and inhibited by fluoxetine**,** imipramine, and 6-nitroquipazine. The $IC_{50}$

for fluoxetine was reported at 0.09 nM **[28 pg/mL][data not shown]**. Myometrial uptake was found to be localized in uterine mast cell cells. Serotonin uptake into uterine mast cells was inhibited by oxytocin in uteri obtained from mice in estrus but not from mice that were ovariectomized and treated with progesterone. Inhibition was reversed by addition of the oxytocin antagonist, $OVT_{16}$. *In vitro* uterine contractility was measured in the presence of serotonin and serotonin plus 6-nitroquipazine, a serotonin uptake inhibitor. Addition of 6-nitroquipazine moved the concentration-response curve to the left and increased the magnitude of contractions by an order of magnitude. This study is difficult to interpret in the context of assessing fluoxetine safety. **[The Expert Panel noted this study for completeness but did not find the study results helpful in the consideration of possible fluoxetine reproductive effects.]**

### *4.2.2 Male Reproduction*

#### ***4.2.2.1 In vivo***

A number of studies examined fluoxetine-induced effects on male rat reproductive performance following acute *(231, 232*) or repeated *(233-235*) dosing. With the exception of an oral dosing study *(232*), and a s.c. dosing study *(235*), all dosing was conducted through the i.p. route.

Yells et al. *(231*) studied the effects of fluoxetine on sexual behavior in 90 – 120-day-old male Sprague-Dawley rats in 2 experiments. Rats were screened for sexual behavior before inclusion in either experiment. In the first experiment, 16 male rats were i.p. injected with saline or 5, 10, or 20 mg/kg fluoxetine HCl **[purity not specified]** in saline. A counter-balanced design was utilized in which all males received each dose, with a minimum of 9 days between treatments. Forty-five minutes after receiving the injection, mating with a receptive female was observed until males became sexually exhausted (i.e., 30 minutes without mounting or intromission). Parameters evaluated included percent ejaculating, mean number of ejaculations, and mean latency to exhaustion. Data for intromission frequency, ejaculation latency, copulatory efficiency, and post-ejaculatory interval were presented separately for the first and last ejaculatory series. **[Definitions for these terms were not provided.]** Statistical significance of data was evaluated by Cochran's Q statistic, ANOVA, and/or Scheffe's test for multiple comparisons. Results obtained for 10 – 16 animals in each group are summarized in Table 26. As noted in Table 26, reductions in the mean number of ejaculations occurred with doses of ≥10 mg/kg bw/day and the percentage ejaculating was reduced at 20 mg/kg bw/day. During the first ejaculatory series, an increase in the post-ejaculatory interval at ≥10 mg/kg bw/day was the only effect that obtained statistical significance. Effects of fluoxetine treatment were more pronounced during the last ejaculatory series, as all doses caused significant increases in intromission frequency, ejaculation latency, and post-ejaculatory interval, and a significant decrease in copulatory efficiency.

**Table 26. Sexual Performance Parameters in Male Rats following Acute Fluoxetine Treatment [Yells et al. *(231)*]**

| *Parameter* | *Ejaculation Series* | *Dose (mg/kg bw/day)* | | | |
|---|---|---|---|---|---|
| | | *0* | *5* | *10* | *20* |
| % Ejaculating | N/A | 100 | 100 | 100 | 62.5* |
| Mean number of ejaculations[a] | N/A | 5.7 | 5.8 | 4.6** | 4.3** |
| Mean latency to exhaustion (min)[a] | N/A | 107.2 | 119.4 | 106.9 | 100.9 |
| Intromission frequency (sec)[b] | First<br>Last | 5<br>5 | 5.5<br>6.5* | 5.5<br>8* | 6<br>9.75* |
| Ejaculation latency (sec)[b] | First<br>Last | 350<br>300 | 300<br>500* | 275<br>600* | 400<br>1,100* |
| Copulatory efficiency[b] | First<br>Last | 80<br>80 | 70<br>40* | 75<br>55* | 60<br>30* |
| Post-ejaculatory interval (sec)[b] | First<br>Last | 350<br>700 | 400<br>800*** | 500**<br>875*** | 575**<br>1,000*** |

* $P<0.001$, ** $P<0.005$, *** $P<0.01$
N/A Non-applicable
[a]Includes only animals that ejaculated
[b]Values estimated by CERHR from a bar graph

In the second experiment by Yells et al. *(231)*, a lesion in the nucleus paragigantocellularis was produced in one group of sexually experienced male Sprague-Dawley rats and a sham operation was conducted in a second group. Rats were screened for sexual behavior 2 weeks following surgery and on the following week received i.p. injections of either saline or 20 mg/kg fluoxetine. There were four groups of eight rats: a sham surgery group receiving saline; a lesion group receiving saline; a sham surgery group receiving fluoxetine; and a lesion group receiving fluoxetine. Forty-five minutes after treatment, rats were allowed to mate until sexual exhaustion, as described above for the first experiment. Brains were examined at the end of the experiment to verify lesion placement. Data were analyzed by a chi-square test and ANOVA. Results are presented in Table 27. Eight animals were evaluated in all treatment groups, with the exception that only four were examined in the sham fluoxetine group for values reported in the first and last ejaculatory series. As noted in Table 27, fluoxetine inhibited sexual function, while lesion induction facilitated function. A reduction in percent rats achieving ejaculation in the sham-operated fluoxetine group was statistically significant. For the first ejaculatory series, the authors reported statistical significance for lesion effects on ejaculation latency, intromission frequency, post-ejaculatory interval, and copulatory efficiency, and drug effects on intromission frequency and ejaculation latency. For the final ejaculatory series, statistical significance was reported for lesion effects on ejaculation latency, intromission frequency, post-ejaculatory interval, and copulatory efficiency and drug effects on ejaculation latency, intromission frequency, post-ejaculatory interval, and copulatory efficiency. The authors noted some inconsistencies in statistical significance obtained in the first and second experiments but indicated that changes occurred in the same direction. Study authors concluded that inhibitory effects on sexual function by fluoxetine may be due in part to interactions with neurons in the nucleus paragigantocellularis.

*Table 27. Sexual Performance in Male Rats following Lesions to the Nucleus Paragigantocellularis or Sham Surgery and Acute Fluoxetine Treatment [Yells et al. (231)]*

| Parameter | Ejaculation Series | Surgery Status/ Treatment | | | |
|---|---|---|---|---|---|
| | | Sham/ Saline | Sham/ 20 mg/kg Fluoxetine | Lesion/ Saline | Lesion/ 20 mg/kg Fluoxetine |
| % Ejaculating | N/A | 100 | 50*** | 100 | 100 |
| Mean number of ejaculations [a] | N/A | 5.00 | 2.83 | 8.33 | 5.83 |
| Mean latency to exhaustion (min) [a] | N/A | 146.5 | 114.0 | 205.7 | 186.6 |
| Intromission frequency (sec) [b] | First<br>Last | 7<br>6.5 | 11<br>10.5 [c] | 5.5<br>3.5 | 7<br>5 |
| Ejaculation latency (sec) [b] | First<br>Last | 400<br>600 | 600<br>900 [c] | 300<br>200 | 450<br>300 |
| Copulatory efficiency [b] | First<br>Last | 75<br>55 | 55<br>25 [d] | 80<br>65 | 70<br>50 |
| Post-ejaculatory interval (sec) [b] | First<br>Last | 400<br>700 | 400<br>875 [c] | 350<br>425 | 300<br>500 |

***$P < 0.01$

N/A Non-applicable

[a] Includes only animals that ejaculated

[b] Values estimated by CERHR from a bar graph

[c] Four animals were examined for these parameters, while eight were examined in all other groups

[d] See text for discussion of statistical significance

*Strengths/Weaknesses:* A strength of these two experiments is the replication in the second study of some of the findings in the first study. The report also provides reasonable data on site(s) of action. The high fluoxetine dose and the i.p. route of administration are weaknesses of the experiments. The use of a single dose of fluoxetine does not permit dose–response modeling.

*Utility (Adequacy) for CERHR Evaluation Process:* This report is marginally useful in the evaluation of adverse effects of fluoxetine on reproduction, specifically sexual function, due to the irrelevant route of exposure.

Mos et al. *(232*) examined the effects of acute fluoxetine exposure on sexual behavior in male Wistar rats in a series of three experiments. In each of the experiments, fluoxetine **[purity not specified]** was orally administered in a tragacanth vehicle **[assumed but not stated to be by oral gavage]** at doses of 0, 3, 10, or 30 mg/kg. Dose selection was based on previous observation and was designed to avoid sedation. A Greek-Latin square design was used in which doses were separated by 1-week intervals. Parameters evaluated included mount/intermission latency, mount frequency, intromission frequency, mount and intromission frequency, ejaculation latency, post-ejaculatory interval, copulatory efficiency, and activity. Data were analyzed by a proportional hazard model with likelihood-ratio test, followed by pair-wise comparison against vehicle, with the Cochran-Mentel-Haenszel method followed by signed rank-sum test, or by Kruskal-Wallis ANOVA followed by the Mann-Whitney *U*-test.

In the first experiment by Mos et al. *(232)*, male rats (200–225 g) were pretested and matched for sexual performance. Selected rats were divided into groups of 12 and administered either drug or vehicle. One hour following treatment, sexual behavior with a receptive female was observed for 25 minutes or until the first post-ejaculatory action. Results for parameters in which statistical significance was obtained at one or more doses are listed in Table 28. Inhibitory effects induced by fluoxetine treatment included modest increases in mount/intromission latency at 3 and 30 mg/kg bw/day and post-ejaculatory interval at 30 mg/kg bw/day. An unexpected enhancement of sexual performance was suggested by significantly reduced ejaculation latency and mount/intromission frequency and increased copulatory efficacy at the 3 mg/kg bw/day dose.

***Table 28. Sexual Performance of Male Rats following Acute Fluoxetine Treatment [Mos et al. (232)]***

| *Parameter* | *Dose (mg/kg bw/day)* | | | |
|---|---|---|---|---|
| | *0* | *3* | *10* | *30* |
| Mount/intromission latency in sec | 3.7 (0.7)[a] | 4.8 (1.3)* | 3.9 (0.9) | 6.0 (1.8)* |
| Number of mounts/intromission frequency | 17.5 (2.7) | 10.0 (1.7)* | 13.5 (3.1) | 18.5 (4.1) |
| Ejaculation latency in sec | 375 (49) | 198 (47)* | 216 (51) | 513 (115) |
| Post-ejaculatory interval in sec | 278 (23) | 240 (32) | 270 (21) | 316 (21)* |
| Copulatory efficacy | 0.64 (0.04) | 0.81 (0.06)* | 0.66 (0.07) | 0.55 (0.09) |

[a]Results presented as median (standard error of median)
*$P < 0.05$ compared to vehicle controls

In the second experiment by Mos et al. *(232)*, the effects of fluoxetine on sexual performance were tested in sexually naive male rats (200–225 g) to determine if they were more sensitive than sexually experienced rats. The naive rats (12/group) were subjected to the same protocol described above for the first experiment. No statistically significant effects were observed at doses up to 30 mg/kg. Although the number of mounts appeared to be reduced by fluoxetine treatment, the results were not statistically significant.

In a third experiment, Mos et al. *(232)* studied the effects of fluoxetine treatment in rats that were allowed to mate until sexual exhaustion. Sexually active males (375–400 g) were selected for this study based on their performance in preliminary tests. The selected rats were randomly assigned to groups dosed with drug or vehicle. Ten rats/group were treated, but due to the loss of a block of data, nine rats/group were evaluated. Sexual performance was tested 30 minutes following treatment and continued for 4 hours or until the rats became sexually exhausted (i.e., no activity for 30 minutes). No statistically significant effects or dose-related trends in male sexual performance were noted at doses up to 30 mg/kg. The study authors noted that neither the enhancement nor the inhibitory effects seen in the first experiment were replicated in this experiment. Noting the small magnitude of effect in the first experiment, the authors did not find the lack of replication surprising.

Three additional SRIs, paroxetine, sertraline, and fluvoxamine, were tested in the study by Mos et al. *(232)*, and the study authors concluded that although paroxetine and sertraline had slightly stronger effects than fluoxetine or fluvoxamine, none of the SRIs administered at non-sedating doses produced major

inhibitory effects on male rat sexual behaviors. The authors also concluded that male rat sexual behavior is not an appropriate model for studying mechanisms of SRI sexual inhibition in human males.

*Strengths/Weaknesses:* Fluoxetine was administered at only a single time, which does not model the chronic dose schedule of human exposure. Evaluation of animal response also was restricted to a single time point. The lack of an effect under these conditions is not informative. The use of males pre-selected for normal sexual function is a strength.

*Utility (Adequacy) for CERHR Evaluation Process:* This report is not adequate for an evaluation of fluoxetine reproductive effects with chronic dosing over time, which is the typical human exposure scenario. This study is adequate for evaluating effects of single doses, but such an evaluation would not be expected to be informative.

Taylor et al. *(233*) examined the effects of chronic fluoxetine treatment on the reproductive system of adult male Long-Evans rats (150–200 days old). Sexually naive rats (9 per group) were i.p. injected with 0 or 0.75 mg/kg bw/day fluoxetine **[purity not specified]** in 0.9% saline for 4 weeks. Tests were conducted to assess sexual behavior, circulating hormones, and sex organ weight. Data were analyzed by ANOVA and/or Tukey's Honestly Significant Difference tests. Three types of behaviors were assessed in the treated rats: sexual performance, sexual motivation, and aggression. To evaluate sexual performance, latencies to first intromission and frequencies of intromissions and ejaculations were observed during 45-minutes contact with a receptive female. Sexual motivation was assessed by observing time spent near a female in estrus vs. a non-estrous female. Inter-male aggressiveness was evaluated by observing behavior with an untreated male. Behavior testing was conducted 60 minutes after dosing on 3 occasions on separate days of each week, during weeks 2–4 of treatment. Results of behavior testing collapsed over all 3 weeks tested are presented in Table 29.

***Table 29. Results of Behavior Testing in Male Rats Administered Fluoxetine [Taylor et al. (233)]***

| *Parameter* | *Dose (mg/kg bw/day)* | |
|---|---|---|
| | *0* | *0.75* |
| Sexual performance: | | |
| Latency to first intromission[a] | 1.2 ± 0.2[b] | 12.2 ± 2.8* |
| Intromission frequency[a] | 34.8 ± 2.2 | 11.0 ± 1.8* |
| Ejaculation frequency[a] | 3.2 ± 0.9 | 0.9 ± 0.2* |
| Sexual Motivation: | | |
| Proximity to estrous female (min) | 13.0 ± 0.3 | 12.5 ± 0.5 |
| Number of urinary marks by estrous female | 113 ± 17 | 98 ± 14 |
| Aggression | | |
| Total aggressive responses | 25.9 ± 0.7 | 19.5 ± 1.2* |

*$P < 0.05$.
[a]Units not specified.
[b]Results presented as mean ± sem

Fluoxetine treatment inhibited sexual performance as noted by increased latency to intromission and decreased intromission and ejaculation frequency. However, fluoxetine had no effect on sexual motivation. Fluoxetine treatment also reduced aggression. Behaviors were measured in the receptive

female and male rats and it was noted that those animals responded differently to control vs. treated rats. Female rats were less solicitous and male rats were less aggressive to the fluoxetine-treated animals than control animals.

Rats were sacrificed 24 hours following the last treatment. Blood was collected for measurement of serum testosterone and corticosteroid in six rats per treatment group. Concentrations of dopamine, serotonin, and their metabolites were measured by HPLC in olfactory tubercles, a primary projection area for the mesolimbic system. Results for hormones and neurotransmitter levels are outlined in Table 30.

***Table 30. Hormone and Neurotransmitter Levels in Rats following Fluoxetine Exposure [Taylor et al. (233)]***

| *Parameter* | *Dose (mg/kg bw/day)* | |
|---|---|---|
| | *0* | *0.75* |
| Serum testosterone (ng/mL serum) | 172 ± 0.1 [a] | 155 ± 0.1 |
| Serum corticosteroid (ng/mL serum) | 44 ± 6 | 84 ± 9 |
| Dopamine (DA) | 100% ± 3 | 167% ± 15* |
| 3,4-dihydroxyphenylacetic acid (DOPAC) | 100% ± 2 | 110% ± 6* |
| Homovanillic acid (HVA) | 100% ± 3 | 113% ± 7* |
| 5-HT | 100% ± 3 | 149% ± 10* |
| 5-HIAA | 100% ± 6 | 80% ± 13* |
| DA/DOPAC | 1.78 ± 0.1 | 1.41 ± 0.1* |
| DA/HVA | 5.26 ± 0.2 | 4.20 ± 0.2* |
| 5-HT/5-HIAA | 1.67 ± 0.1 | 1.13 ± 0.2* |

*$P < 0.05$

[a] Results presented as mean ± sem

As noted in Table 30, fluoxetine treatment did not affect serum testosterone levels, but did cause significant changes in serum corticosteroid levels. Fluoxetine treatment also affected neurotransmitter and metabolite levels and neurotransmitter turnover in olfactory tubercles. Sex organs were collected and weighed at sacrifice. Fluoxetine treatment significantly reduced relative (to body weight) pituitary weight but had no effect on relative weights of adrenals, epididymides, testes, penis, seminal vesicles, bulbospongiosus muscles, and ventral prostate. The authors stated that gross histopathologic assessments were conducted in peripheral structures removed at necropsy and there were no indications of pathologic changes. **[Histopathology procedures were not discussed and the data were not presented.]**

The TCA trimipramine was also tested by Taylor et al. *(233)* and effects were found to be similar to but of greater magnitude than those of fluoxetine. Taylor et al. *(233)* concluded that fluoxetine suppresses copulatory and aggressive responses in rats without affecting sexual motivation, circulating testosterone levels, or peripheral structures of the reproductive system.
*Strengths/Weaknesses:* The dose of fluoxetine is more appropriate than in many of the previous studies, although the i.p. route is a weakness.

*Utility (Adequacy) for CERHR Evaluation Process:* This study is adequate for an evaluation of male reproductive effects of fluoxetine, with caution about interpreting results from the inappropriate route of exposure.

Cantor et al. *(234*) studied the effects of acute and chronic fluoxetine exposure on sexual performance in male rats. Groups of 8–10 sexually experienced male Long-Evans rats (300–500 g) were i.p. injected with fluoxetine HCl **[purity not specified]** in water at doses of 0, 1, 5, or 10 mg/kg bw/day. Sexual performance was tested every 4 days, in a total of 11 trials. **[Therefore, the treatment period was assumed to be 44 days.]** On trial days, rats were injected 60 minutes prior to testing. Tests were conducted by placing a male in a bi-level cage and then placing a receptive female on the other level. Anticipatory sexual excitement was measured by the number of times the male changed levels. The rats were allowed to copulate for 30 minutes. Measures of copulatory performance included latencies to mount, intromission, and ejaculation; numbers of mounts without intromission, mounts with intromission, and ejaculations; post-ejaculatory interval; and intromission ratio. To evaluate acute effects, the mean of three baseline trials was subtracted from results of the first trial. Statistical analyses for acute effects included a one-way multivariate ANOVA with Wilks' lambda criterion, univariate ANOVA, and stepdown analysis. In the analysis of chronic treatment, results from the first, middle, and final three trials were respectively averaged. Statistical analyses included ANOVA and *post hoc* comparisons using protected one-tailed *t*-tests. During the study, four fluoxetine-treated rats died. Body weight gain was significantly reduced in the 5 and 10 mg/kg bw/day groups. Acute exposure to 10 mg/kg bw/day fluoxetine resulted in significantly increased latency-to-level change compared to vehicle controls (41.4 vs. 16.7 seconds, $P<0.02$, in treated vs. controls, respectively) and post-ejaculatory interval (441 vs. 318 seconds in treated vs. controls, $P<0.003$, respectively). **[Levels of significance appeared to vary between text and table or were unclearly stated in the table.]** During chronic treatment, level-change frequency and ejaculation frequency were the only dose-related effects observed, as presented in Table 31. [**These are the only chronic data presented in the study.]** In the 5 mg/kg bw/day group, a significant reduction in level change frequency occurred only during the early stage of treatment. Significant reductions in level-change frequencies at all time periods and in ejaculation frequency during the mid-to-late periods were noted in the 10 mg/kg bw/day group. Fluoxetine treatment had no effect on copulatory efficiency **[data not shown in study report]**.

In the next phase of the study, Cantor et al. *(234*) conducted four additional trials to examine the effects of oxytocin treatment on fluoxetine-induced sexual dysfunction. Following the last of the fluoxetine trials, the fluoxetine treatment groups were i.p. injected with 0.0002 mg/kg oxytocin 1 hour prior to the first and fourth trials and saline 1 hour before the second and third trials. The control group continued to receive only saline vehicle. Daily fluoxetine or saline injections were continued throughout this phase of the study. Results from the two oxytocin and non-oxytocin trials were respectively collapsed and compared to final trials with only fluoxetine treatment. Data were analyzed by one-way ANOVA. Two additional fluoxetine-treated rats died during this phase of the study. Oxytocin treatment had no effect on level-change frequency, but significantly increased the number of ejaculations in the 5 and 10 mg/kg bw/day fluoxetine groups compared to late treatment with fluoxetine alone. The study authors concluded that "The reversal by oxytocin of the fluoxetine-induced deficit in ejaculations is consistent with the hypothesis that serotonin suppresses ejaculatory mechanisms by interrupting the action of oxytocin, which normally accompanies sexual behavior."

***Table 31. Sexual Performance of Male Rats Chronically Treated with Fluoxetine [Cantor et al. (234)]***

| Parameter/time period | Dose (mg/kg bw/day) | | | |
|---|---|---|---|---|
| | *0* **(n=9)** | *1* **(n=8)** | *5* **(n=10)** | *10* **(n=8)** |
| Number of level changes | | | | |
| Baseline | 10.5 | 9.5 | 8.5 | 12 |
| Early | 12.5 | 9.5 | 7* | 5** |
| Mid | 12 | 11 | 10 | 5.5** |
| Late | 13 | 11 | 10 | 7** |
| Number of ejaculations | | | | |
| Baseline | 3.25 | 2.8 | 3.1 | 3.3 |
| Early | 3.1 | 3 | 2.8 | 2.6 |
| Mid | 3 | 3.1 | 2.8 | 2.1** |
| Late | 2.6 | 2.9 | 2 | 1.6* |

*$P<0.05$, **$P<0.01$ from controls
Values estimated from a line graph by CERHR
Six animals died and were removed from consideration prior to analysis; the dose group assignments of the dead animals were not given

*Strengths/Weaknesses:* The death of six animals (dose groups unspecified) and the body weight decreases at the two highest doses raise the question of the appropriateness of the strength of the doses and the i.p. route. The reversal of sexual effects with oxytocin is interesting, but the relevance of this study is questionable given the toxicity experienced by fluoxetine-treated animals.

*Utility (Adequacy) for CERHR Evaluation Process:* This study is of use in providing mechanistic clues to the sexual effects of fluoxetine, but the relevance for predicting risks to human reproduction is questionable.

Matuszcyk et al. *(235*) examined the effects of subchronic fluoxetine treatment on male rat sexual performance. Two sets of experiments were conducted in which sexually experienced male Wistar rats (74 days old) were exposed daily to fluoxetine HCl **[purity not specified]** in saline by s.c. injection. Sexual motivation tests were conducted by determining the times male rats spent in the proximity of an estrous female rat vs. a male rat. Sexual behavior tests measured number of mounts with and without penile intromission, ejaculation latencies, and post-ejaculatory interval. Sexual behavior tests were ended when no intromission occurred within 15 minutes of female presentation, when no ejaculation occurred within 30 minutes of the first intromission, after the first intromission following ejaculation, or when no further intromission occurred within 15 minutes of ejaculation. Statistical analyses included Mann-Whitney *U*-test for between-group comparisons and the Wilcoxon test for within-group comparisons of behavioral effects. Body weight data were analyzed by *t*-test.

In the first experiment by Matuszcyk et al. *(235*), 23 rats/group were s.c. injected with saline or 10 mg/kg bw/day fluoxetine for 28 days. Sexual motivation was tested prior to treatment and at 3 hours following treatment on days 7, 14, 21, and 28. Fluoxetine treatment progressively reduced the time spent near the estrous female and on days 21 and 28, the differences were significantly lower than controls (12 vs. 27% and 8 versus 30% of time on days 21 and 28, respectively).

In the second experiment by Matuszcyk et al. *(235)*, 20 rats/group were s.c. injected with saline or 10 mg/kg bw/day fluoxetine for 14 days. Sexual motivation was tested prior to treatment and on day 14. Copulatory behavior was evaluated prior to treatment and on days 3, 6, 9, and 13. Testing was conducted 3 hours after the rats were dosed. Results of sexual behavior and motivation testing are listed in Table 32.

***Table 32. Sexual Performance and Motivation in Male Rats Treated with Fluoxetine [Matuszcyk et al. (235)]***

| ***Parameter*** [a] | ***Treatment Group*** | ***Treatment day*** | | | | |
|---|---|---|---|---|---|---|
| | | ***Pre-treatment*** | ***3*** | ***6*** | ***9*** | ***13 or 14*** [b] |
| Ejaculation latency (min) | Control<br>Fluoxetine | 11<br>8 | 6<br>7 | 6.5<br>9 | 7<br>11* | 6<br>17*** |
| Number of mounts | Control<br>Fluoxetine | 7<br>10 | 6.5<br>7 | 7<br>8 | 6.5<br>8 | 6<br>13** |
| Number of intromissions | Control<br>Fluoxetine | 13<br>11 | 10<br>13 | 12<br>17* | 12<br>15.5* | 12<br>15 |
| % Time near estrous female | Control<br>Fluoxetine | 50<br>42 | NE | NE | NE | 40<br>35* |

*$P<0.05$, **$P<0.02$, ***$P<0.01$ compared to vehicle controls
[a] Behavior parameters examined on day 13 and motivation parameters on day 14
[b] Values estimated by CERHR from graphs
NE = not examined

Fluoxetine treatment progressively increased ejaculation latency and the number of mounts compared to controls beginning on days 9 and 13, respectively. Rats treated with fluoxetine also spent significantly less time near an estrous female than control rats. No other sexual parameters were consistently affected. Body weight gain was significantly lower in the fluoxetine-treated rats. Matuscyzk et al. *(235)* concluded that fluoxetine treatment affected both sexual motivation and copulatory behavior.

*Strengths/Weaknesses:* The use of high doses and the s.c. route makes this study of questionable significance for the assessment of risk to human reproduction. The decrease in sexual motivation appears to be in contrast to other studies, which did not show an effect on motivation.

*Utility (Adequacy) for CERHR Evaluation Process:* This study is adequate for use in evaluating the effect of fluoxetine on reproductive function in rats. The use of these data for human risk assessment must be tempered by the s.c. route of administration, which is not used in human treatment.

Hsieh et al. *(236)* examined the effectiveness of fluoxetine and other serotonergic agents in treating premature ejaculation by measuring seminal vesicle pressure in response to electrical nerve stimulation in 12–14-week-old Male Wistar rats. At 10-minute intervals, fluoxetine **[purity not specified]** was administered to anesthetized rats **[number treated not specified]** through 7 i.v. injections for a

cumulative dose of 0.1 mg/kg. Ten minutes following each injection, the lesser splanchnic nerve of the vas deferens was electrically stimulated and intraluminal pressure was measured. Drug responses were compared to an initial baseline response to electrical stimulation. Blood pressure was monitored throughout the procedure and found to be unaffected by drug treatment. Data were analyzed by Student *t*-test. Fluoxetine reduced pressure responses with a mean ± sem maximum inhibition value of 84.1 ± 8.9% at 0.1 mg/kg and an $IC_{50}$ value of 0.00166 mg/kg. Pressure responses were also reduced by some of the other serotonergic agents (serotonin and clomipramine, but not imipramine or indatraline) and by prazosin (an $\alpha_1$-adrenergic antagonist). The authors concluded that fluoxetine was the most effective inhibitory agent and possibly the most valuable for treatment of ejaculatory disorders.

*Strengths/Weaknesses:* This study used an appropriate design to answer a well-focused question concerning the effects of fluoxetine on ejaculatory function. The i.v. route of administration detracts from the utility of the study; however, the small divided dose regimen makes this atypical route less problematic.

*Utility (Adequacy) for CERHR Evaluation Process:* This report is useful as a mechanistic study in evaluating potential adverse effects of fluoxetine on male reproduction.

Acute i.p. treatment of male rats with fluoxetine 5 mg/kg results in increased serum cortisol and progesterone *(237*). Daily administration of the same dose for 21 days prevented the response of both hormones to the acute challenge. **[The Expert Panel did not find this report useful in their evaluation of reproductive effects of fluoxetine.]**

#### *4.2.2.2 In vitro*

Busch et al. *(238*) evaluated the effects of fluoxetine on norepinephrine-, serotonin-, and calcium-induced contractions in rat vas deferens *in vitro*. Vas deferens were obtained from Wistar rats (250 – 350 g) **[number of rats not stated]** and the epididymal portion was incubated in a bath with Krebs-Hensseleit buffer. Pre-treatment concentration-response curves were obtained for norepinephrine, serotonin, and calcium and these curves served as controls. The samples were then washed, equilibrated in buffer, and incubated with $10^{-6} – 10^{-4}$ M fluoxetine (dissolved in a DMSO vehicle) for 30 minutes. Responses of the fluoxetine-treated samples to norepinephrine, serotonin, and calcium were calculated as a percentage of control maximum response (six experiments conducted for each compound). Results were analyzed by two-way ANOVA followed by the Student-Newman-Keuls multiple comparison test. $10^{-5}$ M **[3,100 ng/mL]** Fluoxetine had no effect on serotonin-induced contraction. The effects of fluoxetine on norepinephrine-induced contraction depended upon the dose. No effect was observed with $10^{-6}$ fluoxetine **[310 ng/mL]**, while significant inhibition was noted with $10^{-4}$ **[31,000 ng/mL]** M (data not shown). A dual effect was noted with $10^{-5}$ M fluoxetine, with a significant increase in vas deferens response at low doses of norepinephrine, but inhibition of maximal contraction response at higher norepinephrine doses.

Busch et al. *(238*) conducted a series of experiments to determine the mechanism for fluoxetine potentiation of contraction at low norepinephrine doses. The effects of fluoxetine were compared to desipramine and cocaine, inhibitors of norepinephrine neuronal uptake. At low norepinephrine doses, the increment in vas deferens response observed with $10^{-5}$ M fluoxetine occurred similarly with exposure to $10^{-6}$ M desipramine or cocaine. Synergism between $10^{-7}$ M desipramine and $10^{-6}$ M

fluoxetine was examined and it was found that the combination of drugs produced a greater increase in vas deferens response to norepinephrine than occurred with either drug alone. Because uptake of norepinephrine occurs by a $Na^+$ and $Cl^-$ transporter, the effects of fluoxetine, desipramine, and cocaine were studied in a low $Na^+$ and $Cl^-$ buffer. Both fluoxetine and desipramine failed to increase low-dose norepinephrine-induced vas deference contraction in the presence of low $Na^+$ and $Cl^-$. These results suggest that fluoxetine could interact with the norepinephrine transporter. Binding of $^3$H-prazosin, an $\alpha_1$-adrenergic receptor antagonist, to vas deferens membranes was examined (4 experiments with a pool of 15 animals/group) and no effect was found on either receptor density or affinity. Results of the binding study further suggest that fluoxetine effects on vas deference do not occur through a postsynaptic mechanism.

Additional experiments were conducted by Busch et al. *(238*) to determine if the fluoxetine-induced decrease in vas deferens contraction at high norepinephrine doses is due to inhibition of calcium entry through voltage-operated calcium channels. Fluoxetine effects on norepinephrine-induced contraction in a high-calcium medium and on calcium-induced contractions in KCl-depolarized vas deferens were studied. High concentrations of calcium partially reduced the fluoxetine inhibition of norepinephrine-induced contraction. $10^{-5}$ M fluoxetine was found to inhibit calcium-induced vas deferens contraction.

According to Busch et al. *(238*), this study suggests that fluoxetine increased responses to low doses of norepinephrine though inhibition of neuronal norepinephrine uptake and inhibited responses to high norepinephrine concentrations by antagonizing calcium transport through voltage-dependent channels.

*Strengths/Weaknesses:* These experiments were straightforward, but the Expert Panel could not tell if there was synergism; that is, if responses were greater than additive. The Panel notes that the question of synergism is not relevant to the evaluation of possible reproductive effects of fluoxetine, although these data may increase the understanding of mechanisms of adverse reproductive effects in males.

*Utility (Adequacy) for CERHR Evaluation Process:* This report has utility as a mechanistic study in the consideration of possible male reproductive toxicity of fluoxetine but is not of utility in estimating human risk.

Busch et al. *(239*) next conducted a study to determine the influence of testosterone and fluoxetine on *in vitro* contractile responses in rat vas deferens. Male Wistar rats were either left intact, castrated 21 days before the study, or castrated then treated with testosterone starting at 15 days following castration and continued for a total of 7 days **[number of rats in each group was not stated]**. The vasa deferentia were removed from six rats/group/treatment and *in vitro* contractile responses were studied and analyzed as described above for the Bush et al. *(238*) study. Pre-treatment concentration-response curves obtained with norepinephrine and calcium were compared to curves obtained with those two compounds in the presence of $10^{-5}$ M fluoxetine **[3100 ng/mL]** (in a DMSO vehicle).

Busch et al. *(239*) found that vas deferens weights were reduced in the castrated rats that did not receive testosterone replacement and these vasa, in contrast to vasa deferentia from intact or testosterone-treated castrated rats, were found to be spontaneously active. Norepinephrine or calcium-induced

contractile responses were significantly smaller in vasa deferentia from castrated rats compared to intact and testosterone-treated castrated rats.

Consistent with results from the Busch et al. *(238*) study, fluoxetine treatment of the vas deferentia from intact rats resulted in increased response at low norepinephrine doses but inhibition of maximum response. Vasa deferentia from the castrated rats that did not receive testosterone replacement were the only ones that did not exhibit a fluoxetine-induced enhancement of contraction at low doses of norepinephrine and the inhibition of maximal response was greater than that observed in intact rats. Addition of prazosin, a non-selective $\alpha_1$-adrenergic receptor antagonist, inhibited contractions in vasa deferentia in intact, castrated, and testosterone-treated rats. According to the study authors, this finding confirms that the $\alpha_1$ adrenergic receptor is involved in contractile responses from all three groups of rats. $^3$H-prazosin binding density and affinity were reduced in vasa deferentia from castrated rats compared to intact rats but fluoxetine treatment had no effect on receptor binding or affinity in any of the three groups. Treatment with cocaine shifted the norepinephrine response curve to the left in all three groups, leading authors to suggest that the neuronal norepinephrine uptake mechanisms remains intact in castrated rats. Addition of a nitric oxide (smooth muscle relaxant) synthase inhibitor had no effect on norepinephrine-induced contraction either in the presence or absence of fluoxetine in any of the three groups. Fluoxetine treatment inhibited calcium-induced contraction of vasa deferentia in all three groups of rats but the effect was more pronounced in the castrated vs. intact or testosterone-treated rats. According to the study authors, this finding suggests that castration can lead to altered response of vas deferens to calcium in rats.

Busch et al. *(239*) concluded that "…vas deferens contractile response is testosterone dependent and that this behaviour [sic] modifies the effects of drugs such as fluoxetine that have dual effect on contractility."

*Strengths/Weaknesses:* This study appears to have been a competently performed mechanism study.

*Utility (Adequacy) for CERHR Evaluation Process:* This study may be of value as a mechanism study but has no utility in the evaluation of possible human reproductive risk of fluoxetine exposure.

#### *4.2.2.3 Testicular weight*

The FDA Pharmacologist Review of NDA 18-936, dated March 14, 1984 *(139*), contains descriptions of toxicology studies in experimental animals in which testicular weight was reported to be altered. A 3-month oral toxicity study in B6C3F1 mice administered fluoxetine in the diet at 0, 0.001, 0.0045, or 0.02%, giving mean daily doses estimated at 0, 1.6, 6.9, and 31 mg/kg. Six of 20 males in the top dose died, compared to 1 of 20 in each of the other groups **[*P*=0.0245, chi-square by CERHR]**. Weight in the males was said to have decreased 8% in the high dose group; the underlying data and significance testing were not provided. Absolute and relative testis weight were described as decreased in the high dose group. No data were given, but relative testis weight was said to be 12% below the control at the end of the dosing period and 26% below the control in a subset of 5 animals after a 1-month recovery period. Testicular histopathology **[fixation and staining methods unstated]** showed "hypospermatogenesis, usually bilateral" in 6 of 15 high dose animals, compared to 0/15, 1/15, and 0/14 control, low, and mid-dose animals, respectively **[*P*= 0.0013, chi-square by CERHR]** and 0/5, 0/5, 0/5, and 4/5 animals after recovery in the control, low, mid, and high-dose

groups **[*P*=0.3671, chi-square by CERHR]**.

In a 1-year oral toxicity study in beagle dogs reported in the same FDA review *(139)*, fluoxetine was given to 5 males/dose group at 1, 4.5, or 20 mg/kg/day for six months followed by a decrease at the high dose to 10 mg/kg/day for the balance of the year. There were four males in the control group. Two males at the high dose were removed from treatment once or twice for 1–17 days due to severe side effects. None of the males died, but of females in the same study, three died at the high dose. Most of the high dose animals lost weight initially, but weight subsequently recovered **[no data shown].** Plasma fluoxetine was measured, but not reported in this summary. Absolute and relative testis weights at the high dose were described as decreased 26 and 33% below the control, respectively **[data and statistical analysis not shown]**. Gross pathology showed "unilateral retained, small testes" in 0/4, 1/3, 1/3, and 1/3 dogs in the control, low-, mid-, and high- dose groups, respectively. Histopathology was described as showing abnormalities in 1 of 3 dogs in each of the fluoxetine groups.

**[The Expert Panel notes these studies from the FDA Pharmacologist Review *(139)*. It is not possible to tell from this report whether the testicular effects in the mouse study were secondary to excessive toxicity in the males, or whether the putative decrease in testis weight in the dog study represented testicular toxicity or represented a chance finding given the small number of dogs in the study. The lack of experimental detail and the absence of data render this report inadequate for use in the CERHR process.]**



### *4.2.3 Fertility/Reproductive Function*

Results of an unpublished two-generation study in rats were reported in an abstract by Hoyt et al. *(240)* and a review by Tabacova *(138)* of the FDA National Center for Toxicological Research **[Eli Lilly and Company declined CERHR's request for a copy of this report. Eli Lilly and Company did provide a copy of the Hoyt et al. poster of this study. The information presented here is from the Tabacova summary and Hoyt abstract. The data are presented in tabular form in the Tabacova paper with designations of statistical differences and of "statistically non-significant change." The changes are frequently described in percent change from control, without an indication of variance, precluding formal trend testing. Only the changes marked in the tables as statistically significant are indicated here—in no instances do the NOAELs appear to have been based on "statistically non-significant" determinations. The FDA Pharmacologist Review of NDA 18-936, dated March 14, 1984 *(139)*, also contains a summary of this study, but was not judged by the Panel to be more useful than the Tabacova review.]** A two-generation feeding study in Wistar rats summarized by Tabacova *(138)* used dietary fluoxetine concentrations of 0, 0.002, 0.005, and 0.0125%, resulting in estimated fluoxetine intakes in males of 0, 1.5, 3.9, and 9.7 mg/kg bw/day, and in females of 0, 1.3, 3.1, and 7.4 mg/kg bw/day. Exposure in males started 10 weeks before mating and continued throughout breeding. Exposure of females began 3 weeks prior to mating and continued through pregnancy and lactation. There were 40 animals of each sex in each dose group in the parental generation. Male offspring were exposed to the test diet from weaning and females were started 6 weeks later. On GD 20, half the females were sacrificed for an assessment of fetal morphology, viability, and weight. The rest of the females delivered and nursed their offspring until weaning. There were decreases in body weight, weight gain, and food intake in parental animals at the high dose. Tabacova considered there to have been a dose-dependent decrease in fertility that was observed in the two highest doses with 9% and 11% reductions in fertility compared to con-

trols, respectively, although statistical significance was not shown. There was a 15–17% incidence of preimplantation loss at all fluoxetine doses, representing a 100–134% increase above the control rate. There were no increases in visceral or skeletal malformations in the offspring and neurobehavioral testing measuring sensory or motor function was not affected by treatment. Pup weight was decreased at the high dose on PND 7 and at the two highest doses on PND 58. Reduced pup survival was noted at the two highest doses on PND 1 and at the high dose through PND 21. **[No statistical significance is indicated in one table of the Tabacova report, while a second table states that reduced postnatal survival was statistically significant in the high-dose group during the first week of life.]** Fertility and reproductive performance of the $F_1$ offspring were not adversely affected. The adult NOAELs for general toxicity were considered to be 3.1 and 3.9 mg/kg bw/day in females and males, respectively. The NOAELs for reproductive toxicity were 1.3 and 1.5 mg/kg bw/day in females and males, respectively. The NOAEL for developmental toxicity was 1.3 mg/kg bw/day. It was noted that the reliability of the study might be somewhat limited by imprecise determination of feed intake, by non-comparability of initial body weight among male dose groups, and by imprecise timing of pregnancy onset, with some dams delivering their litters prior to scheduled cesarean section on GD 20. The summary data in the Tabacova tables do not give variances for continuous data or litter proportions for categorical data, precluding the calculation of a benchmark dose.

*Strengths/Weaknesses:* This study appears to have been a GLP study that used suitable controls, adequate numbers of rats per dose group, appropriate endpoints of reproductive performance, multiple dose levels, and an oral route of administration. Given the known pharmacologic effects of fluoxetine in reducing food intake, administration of the test article in the diet is a weakness of the study design. The highest dose achieved in the females was only 7.4 mg/kg bw/day.

*Utility (Adequacy) for CERHR Evaluation Process:* This report is of limited utility because the original data are not available for inspection. The availability of a poster from a meeting presentation is of some help, and supports the decrease in $F_1$ pup survivability that is reported to have occurred in the high-dose group. The possible technical limitations reported in the Tabacova paper decrease confidence in the study results, although again, inspection of the original report would be useful in determining how important these technical issues may have been.

### *4.3 Utility of Reproductive Toxicity Data*

The majority of reproductive studies conducted in humans examined sexual function and the data set was found sufficient for assessing sexual function, specifically orgasm, in both men and women. Although case studies suggested increased prolactin secretion in post-menopausal women, there was a lack of studies examining the effect in premenopausal women. The data set was not sufficient for an examination of other possible effects on the human reproductive system such as potential disruptions in menstrual cycles or ovulation.

Because an unpublished report describing a two-generation study in rats was not made available to the Expert Panel, the animal data were insufficient for an assessment of male and female fertility. Studies in rats suggested decrements in male and female sexual performance but no effect on estrous cycles. A study in monkeys demonstrated that fluoxetine increases progesterone-induced prolactin secretion. Although the studies in rats and monkeys provide qualitative support for findings observed in humans, the studies did not provide dose-response information and were conducted by exposing

animals through non-relevant exposure routes.

## 4.4 Summary of Reproductive Toxicity Data

### 4.4.1 Human Data

Possible reproductive effects of fluoxetine exposure in women include menstrual cycle changes and galactorrhea. Reports of anovulatory women who began menstruating when taking fluoxetine are available only as case studies (see Section 4.1.1); therefore, the effects could not be evaluated by the Panel. A single-blind randomized-placebo study demonstrated menstrual cycle length changes in 1 of 61 women on placebo, 7 of 70 women on 20 mg/day fluoxetine, and 11 of 62 women on 60 mg/day fluoxetine *(167)*. In each fluoxetine group, menstrual changes consisted of lengthened cycles in approximately half of the women and shortened cycles in the other half. Hypotheses provided by authors for the variability in response were delayed ovulation resulting from serotonin inhibition of hypothalamic GnRH or advanced ovulation resulting from reduced estrogen metabolism due to fluoxetine inhibition of CYP34A. Cases of galactorrhea were reported in women taking fluoxetine and a study demonstrated that fluoxetine increased prolactin levels in post-menopausal women *(171)*.

One study demonstrated that 60 mg/day fluoxetine does not result in a change in LH levels in men, but the Panel noted that the subjects had not reached steady-state concentrations of fluoxetine *(173)*. *In vitro* studies conducted with human vasa deferentia demonstrated that fluoxetine is unlikely to inhibit function of vasa deferentia under normal conditions *(174, 175)*.

The majority of fluoxetine reproductive studies in humans focused on sexual dysfunction. The assessment of sexual dysfunction in patients taking fluoxetine is complicated by the fact that sexual dysfunction is not uncommon in the general population and is commonly associated with depression *(176)*. In controlled studies, rates of sexual dysfunction were reported at 33–60% in adults taking fluoxetine *(194, 198, 201, 203)*. Male and female orgasmic disorders are the most commonly reported sexual disorders in patients on SRIs including fluoxetine and the rates range from 12 to >50% among individuals with sexual dysfunction *(195, 196, 198, 201)*. Other symptoms reported with fluoxetine use included delayed or no ejaculation, impaired erectile function, and reduced vaginal lubrication *(201)*. Two studies have reported that fluoxetine treatment resulted in enhanced sexual function (e.g., improved desire, lubrication, orgasm, and/or erection) *(194, 197)*. One study suggested that improved sexual function was related to improvement of depression with fluoxetine treatment *(197)*. The Panel noted that effects of fluoxetine on sexual function are variable and cannot be predicted for individuals.

### 4.4.2 Experimental Animal Data

Fertility and reproduction in female rats and in two generations of male and female rats were assessed in two unpublished reports but data were available to the Panel only as an abstract and poster presentation *(240)* and as a summary in an FDA report *(138)*. Because the original data were not available, the Panel was not able to draw conclusions about these studies.

Various aspects of female reproductive toxicity were evaluated in animal studies. Injection of rats with 10 mg/kg bw/day fluoxetine for about 2–3 weeks had no effect on estrous cycles *(223, 226)*. Estrous behavior and receptive activities (e.g., lordosis, hop/darting, ear wiggling) were impaired in rats following injection with 10 mg/kg bw/day fluoxetine s.c. or i.p. for 3–6 weeks *(223)*. Prolactin

levels were increased in rats gavaged with 2.5 – 2.6 mg/kg bw/day fluoxetine for 14 weeks *(227)* and in monkeys receiving 5 mg/day [~1 mg/kg bw/day] fluoxetine by i.v. for 4 weeks *(228)*. In monkeys, blockade of nuclear progestin receptors by RU 486 and the resulting inhibition of progesterone-induced increase in prolactin implied that serotonin is involved in the neural regulation of progesterone-induced prolactin release, thus suggesting a possible mechanism of prolactin release by fluoxetine. An *in vitro* study with rat uterine rings demonstrated that fluoxetine has no direct effect on uterine myometrium *(229)*.

A number of studies measured sexual performance in male rats repeatedly dosed with fluoxetine, primarily by the i.p. route. Although the route is not relevant to human exposures, the studies are well conducted and provide some insight on sexual performance in rats treated mostly with high doses. Fluoxetine doses of 0.75 or 10 mg/kg bw/day adversely affected ejaculation (e.g., reduced ejaculation frequency or increased latency to ejaculation) and doses of 10 mg/kg bw/day reduced sexual motivation (e.g., time spent near estrous female) *(233-235)*. Oxytocin improved ejaculatory function in one study, suggesting that serotonin may inhibit ejaculation by interfering with oxytocin action in rats *(234)*. Seminal vesicle pressure in response to electrical nerve stimulation was reduced in rats receiving a cumulative dose of 0.1 mg/kg bw fluoxetine i.v. *(236)*. *In vitro* studies with rat vasa deferentia demonstrated that fluoxetine affected norepinephrine- or calcium-induced contraction only at very high doses ($10^{-5}$ M=3,100 ng/mL) *(238, 239)*.

Treatment of male rats with 0.75 mg/kg bw/day fluoxetine by i.p. injection resulted in a reduction in relative (to body weight) pituitary weight but had no effect on relative weights of adrenals, epididymides, testes, penis, seminal vesicles, bulbospongiosus muscles, and ventral prostate; testosterone levels were also unaffected *(233)*.

**The Expert Panel concluded there is sufficient evidence in humans that fluoxetine produces reproductive toxicity in men and women manifested as impairment of sexual function, specifically orgasm.** This impairment of sexual function may result from the same serotonergic mode of action as the pharmacologic effects of the medication. Effects on individual sexual function are unpredictable. Depression is associated with impaired sexual function, and successful treatment of depression may be associated with improvements in sexual function. Fluoxetine effects on sexual function in humans have been observed even at 20 mg/day. The Expert Panel notes that sexual dysfunction associated with fluoxetine is reversible, but does not consider reversibility to nullify the determination of reproductive toxicity. Fluoxetine also may increase prolactin secretion in menopausal women and, based on case reports, may also do so in women of child-bearing age.

**The data in female rats are sufficient to qualitatively demonstrate that fluoxetine treatment with 10 mg/kg bw/day by s.c. or i.p. injection results in altered estrous behavior and sexual receptivity, but has no effect on estrous cycle length. The data in male rats are sufficient to qualitatively demonstrate that i.p. or s.c. injection with ≥ 0.75 mg/kg bw/day results in reduced ejaculatory function and i.p. injection with 10 mg/kg bw/day results in reduced sexual motivation. However, most of the data in male and female rats were not sufficient to evaluate dose–response relationships and were generated using a route irrelevant to human exposures. Data in female monkeys are sufficient to demonstrate that i.v. infusion with ~1 mg/kg bw/day fluoxetine results in a progesterone-induced increase in prolactin, but no dose-response in formation is available and the route of administration is not relevant to human exposures.**

# 5.0 SUMMARIES, CONCLUSIONS AND CRITICAL DATA NEEDS

## *5.1 Summary of Reproductive and Developmental Toxicity*

### *5.1.1 Developmental Toxicity*

The Expert Panel concluded that there was sufficient evidence to permit evaluation of developmental toxicity in humans. Birth weight, prematurity or shortened gestation, neonatal adaptation, and early infant growth (<6 months) could be evaluated, but there were insufficient data to examine incidence of major malformations, long-term neurobehavioral development, and growth in children (6–24 months). There were insufficient data to discriminate effects of prenatal vs. postnatal fluoxetine exposure on postnatal growth. The Panel could not evaluate the impact of childhood therapeutic exposures to fluoxetine on development. Although the effects of the underlying disorder as an explanation of the observed effects cannot be excluded, a few studies provided evidence comparing medicated and unmedicated pregnant women with depression or other underlying disorders.

The Expert Panel concluded that third trimester exposure to therapeutic doses of fluoxetine (20–80 mg/day orally) is associated with an increased incidence of poor neonatal adaptation (e.g., jitteriness, tachypnea, hypoglycemia, hypothermia, poor tone, respiratory distress, weak or absent cry, diminished pain reactivity, or desaturation with feeding), as well as increased admissions to special care nurseries. Shortening of gestation and reduced birth weight at term were also suspected by the Panel. Exposure to fluoxetine through breast milk may result in reduced postnatal growth during early infancy. However, the possibility that this diminished growth may be related to prenatal rather than postnatal exposure could not be excluded. The long-term implications of these findings cannot be evaluated without further longitudinal data.

### *5.1.2 Reproductive Toxicity*

Human reproduction comprises a series of highly interrelated and timed processes, requiring investigators to examine a spectrum of endpoints including sexual function, menstruation, semen quality, ovulation, conception, and postimplantation pregnancy loss. Comparable endpoints are available for experimental animal research.

The weight of evidence in humans supports a relation between fluoxetine exposure and orgasmic dysfunction in both men and women and altered menstrual cycle length in women. The Panel considers orgasmic dysfunction (delay and inability to achieve orgasm) in both men and women as evidence of reproductive toxicity. Implications of orgasmic dysfunction for conception probability are unknown. It is important to note that the effect on orgasmic dysfunction is reversible and may be related to the pharmacological mode of action. The Expert Panel believes the reversibility of the effect does not obviate the finding of reproductive toxicity. Other evidence of reproductive toxicity is the reported alteration in menstrual cycle length in some women.

Experimental animal evidence is largely lacking with regard to reproductive endpoints. Further, the data are limited by use of single dose levels in most studies and irrelevant routes of exposure. Duration of pregnancy or the ability of females to maintain pregnancy has not been shown to be affected by fluoxetine exposure in developmental toxicity studies.

## 5.2 Summary of Human Exposure Data

Fluoxetine belongs to a class of therapeutic agents referred to as serotonin reuptake inhibitors. It has undergone evaluation by the FDA and has been approved for the treatment of major depressive disorder, obsessive compulsive disorder, bulimia nervosa, panic disorder, and premenstrual dysphoric disorder in adults and major depressive disorder and obsessive compulsive disorder in children 7–17 years old. Off-label use in younger children is known to occur. Virtually all human fluoxetine exposure is through medication, while environmental fluoxetine exposure appears to be trivial. Recommended fluoxetine doses are 10–80 mg/day or 90 mg/week in adults and 10–60 mg/day in children. In 2002, about 26.7 million prescriptions were dispensed for fluoxetine, with 1.2 million dispensed to pediatric and adolescent patients (1–18 years old) and 8.4 million dispensed to women of child-bearing age (19–44 years old) *(11)*.

Usage of this medication includes maternal exposure during pregnancy, related intrauterine and lactational exposure, as well as direct pediatric exposure. The database was sufficient for estimating ranges of fetal exposures in late pregnancy and maternal and infant exposure during breast feeding. Fluoxetine is metabolized to norfluoxetine, which is also pharmacologically active. In pregnant women (36–37 weeks gestation) taking 20–40 mg/day fluoxetine, trough plasma levels of fluoxetine and norfluoxetine were measured at 47±33 ng/mL and 109±22 ng/mL, respectively *(19)*. During the postpartum period, maternal blood levels of fluoxetine and of norfluoxetine are quite variable and dose-dependent (21–506 and 43–674 ng/mL, respectively). Intrauterine fetal exposure, using umbilical cord blood concentrations of fluoxetine shortly after birth, have ranged from 26 to 112 ng/mL *(15-17, 19)*. Norfluoxetine levels in cord blood have been measured at 54–209 ng/mL *(15, 19)*.

In lactating women *(19, 22, 25, 26, 28, 29)*, the ranges of milk concentrations for fluoxetine and norfluoxetine, respectively, are <2–384 ng/mL and <2–321 ng/mL. In nursing infants, blood fluoxetine and norfluoxetine concentrations range from undetectable to 340 ng/mL and 265 ng/mL, respectively. Milk-to-plasma ratios range from 0.05 to 6.09 for fluoxetine and 0.085 to 2.08 for norfluoxetine; most ratios are lower than 1. Infant exposure is better estimated by infant norfluoxetine serum concentration, which is strongly related to maternal fluoxetine dose and maternal serum concentrations of fluoxetine and norfluoxetine *(22)*. In 8–12 year old children (n=52) medicated with 20 mg/day for at least 4 weeks, the steady-state concentrations of fluoxetine and norfluoxetine in blood were 145±76 and 167±60 ng/ mL respectively. Similarly in 13–17 year old children (n=42), the levels were 79±49 and 113±41.

## 5.3 Overall Conclusions

### 5.3.1. Developmental Toxicity

Sufficient evidence exists for the Panel to conclude that fluoxetine exhibits developmental toxicity as characterized by an increased rate of poor neonatal adaptation (e.g., jitteriness, tachypnea, hypoglycemia, hypothermia, poor tone, respiratory distress, weak or absent cry, diminished pain reactivity, or desaturation with feeding) at typical maternal therapeutic doses (20–80 mg/day orally). These effects appear to result more readily from *in utero* exposure late in gestation. The observed toxicity may be reversible, although long-term follow-up studies have not been conducted to look for residual effects. The evidence suggests that developmental toxicity can also occur in the form of shortened gestational duration and reduced birth weight at term *(89, 104)*.

Results in humans were supported by animal data. In particular, Vorhees et al. *(148*) observed developmental toxicity in the form of decreased birth weight and impaired pup survival in rats exposed late in gestation to fluoxetine at 12 mg/kg bw/day.

### *5.3.2 Reproductive Toxicity*

The Expert Panel concluded that there is sufficient evidence in humans that fluoxetine can produce reproductive toxicity in men and women as manifested by reversible, impaired sexual function, specifically orgasm.

Although reproductive toxicity data in animals were obtained using study designs incorporating irrelevant routes of exposure and mostly single doses, they were sufficient to demonstrate qualitatively that fluoxetine treatment can result in altered estrous behavior, altered sexual receptivity, and reduced sexual motivation. As such, these studies are supportive of the human observations.

The mechanism(s) by which fluoxetine can cause reproductive and developmental toxicity is unknown. However, the Panel suspects both the adverse and desired pharmacological actions of this and other SRIs are mediated by their serotonergic activity. As such, the Expert Panel acknowledges that in many instances, it is not possible to differentiate drug-induced adverse effects from those induced by the disease process itself or the pharmacological action of the drug. Further, the Expert Panel also recognizes that any risks associated with fluoxetine treatment must be weighed against the known risks associated with untreated disease, particularly major depression. Such a risk-benefit analysis is best performed by the patient and responsible health care provider and should benefit from the evaluation and conclusions offered by this report.

The Panel concluded there are insufficient data to draw conclusions regarding concern for drug-induced toxicity in infants exposed to fluoxetine through breast milk or children on fluoxetine therapy. There also are insufficient data on possible drug associations with maternal and/or embryonic/fetal toxicity leading to pregnancy loss. The Panel concluded there is some concern for fluoxetine-associated shortened gestational duration and poor neonatal adaptation at exposure levels encountered in therapy (20 – 80 mg/day), particularly since the follow-up data in the latter are not available to determine whether or not long-term neurobehavioral end-points might be affected. Finally, the Panel expresses minimal concern for fluoxetine-induced reproductive toxicity (orgasmic dysfunction) at exposure levels encountered in therapy based on the reversible nature of these effects and the difficulties in distinguishing between these endpoints and the pharmacological action of this drug.

## *5.4. Critical Data Needs*

Critical data needs are defined as research or studies that would provide information to substantially reduce uncertainty and increase confidence in assessment of human reproductive and developmental risks. The fluoxetine Expert Panel found that studies in humans were generally limited in statistical power by small sample sizes and were not designed or reported in a manner that would allow a clear distinction between the effects of the underlying disease and the effects of the medication. Data were generally not available to permit a comparison of the pregnancy outcome effects of medication with the effects of nonmedication therapies, e.g., cognitive behavioral or interpersonal therapies, in pregnant women. Further, information was generally lacking on criteria for diagnosis of depression

and on severity of disease. In addition, confounding factors such as smoking, alcohol consumption, use of other medications including dietary supplements, age, prior reproductive history, and comorbid illnesses often were not adequately reported or controlled. Future studies should take these factors into consideration, because such a design would permit longitudinal ascertainment of exposure data and other relevant covariates. Additional and better comparisons of fluoxetine effects with effects of other SRIs are needed.

Specific critical data needs identified by the Expert Panel were:

**Developmental Toxicology**

*Human Studies:*

- Data from prospective cohort studies of women planning pregnancies to capture all hCG-detected pregnancies and determine effects of fluoxetine on critical windows of human development including at or shortly after conception
- Additional data on the possible effects of fluoxetine on gestational length, prematurity, fetal growth, and neonatal adaptation
- Data from longitudinal prospective studies on whether prenatal fluoxetine exposure affects postnatal growth, neuroanatomy, and neurobehavioral development
- Data from studies on neonatal growth and neurobehavioral function in neonates exposed to fluoxetine through breast milk
- Data from longitudinal prospective studies on neuropsychological functioning using standardized and sensitive measurements in children taking the medication

*Experimental Animal Studies:*

- Data from rodent studies that comply with current testing guidelines
- Data from developmental neurobehavioral studies, including brain histology
- Data examining prenatal exposure effects on hippocampal development

**Reproductive Toxicology**

*Human Studies:*

- Data on the effects of fluoxetine on male and female fertility
- Data on spontaneous abortion that can address separation of the effects of medication from effects of the underlying disorder
- Additional data from sexual function studies based on underlying disease (indication for therapy)

*Animal Studies:*

- Data on the effects on semen quality, ovulation, conception, and pregnancy loss

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*National Toxicology Program*
*U.S. Department of Health and Human Services*


# Center For The Evaluation Of Risks To Human Reproduction

# PUBLIC COMMENTS ON THE EXPERT PANEL REPORT ON FLUOXETINE

Appendix III

# Eli Lilly and Company Comments on the NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Fluoxetine

The report of the NTP-CERHR Expert Panel on the Reproductive and Developmental Toxicity of Fluoxetine was recently issued for public comment. Early on in their evaluation, the Expert Panel concluded that the potential for environmental exposure to fluoxetine was trivial. The NTP-CERHR Expert Panel went on to evaluate the safety of fluoxetine for patients.

Comments by Eli Lilly and Company on the specific findings in the report of the NTP-CERHR Expert Panel can be summarized as follows:

1. There is not sufficient evidence to conclude that fluoxetine causes developmental toxicity manifested as poor neonatal adaptation, increased incidence of minor anomalies, reduced gestation or birth weight, or reduced size of breastfed infants.

2. Beneficial or unwanted side effects of SSRIs on sexual function appear to be reversible and any risks should be evaluated by patients and their physicians. Since effects on sexual function from the use of SSRIs appear to be confounded by the disease state and to be based on reversible pharmacology, fluoxetine should not be classified as a reproductive toxin in humans.

3. Untreated depression can result in substantial medical risks. Fluoxetine is approved in the United States for the treatment of major depressive disorder. Fluoxetine is only available through prescription, so the benefits and potential risks of use should be evaluated on a case-by-case basis by patients and their physicians.

4. Communications about the safety of patients using pharmaceuticals should remain the responsibility of the US FDA.

## 1. Developmental Toxicity of Fluoxetine

According to the Expert Panel (Section 3.4 Summary),

> *"…there is sufficient evidence in humans to determine that prenatal exposure to fluoxetine results in poor neonatal adaptation (e.g., jitteriness, tachypnea, hypoglycemia, hypothermia, poor tone, respiratory distress, weak or absent cry, diminished pain reactivity, or desaturation on feeding) at typical therapeutic exposures (20–80 mg/day orally) during the third trimester of pregnancy.";*

*"Therapeutic fluoxetine exposure during early pregnancy may result in an increased incidence of minor anomalies.";*

*"Shortening of gestation and reduced birthweight are also suspected, although the evidence is not sufficient to exclude the underlying disorder, depression, as a cause or contributor to these effects.";*

and

*"The evidence is suggestive that exposure to fluoxetine through breast milk can result in reduced infant growth; however, these effects may be related to prenatal exposure."*

Eli Lilly and Company does not believe that there is sufficient evidence to support any of the conclusions by the NTP-CERHR Expert Panel regarding developmental toxicity.

### ***Poor Neonatal Adaptation***

There are published reports about increases in a variety of clinical observations in newborns from mothers treated with SSRIs during the third trimester of pregnancy. The observations resolve within days after birth. Their cause cannot be effectively separated from the underlying disease condition and other confounding factors. Some of the clinical observations are consistent with observations that might be anticipated from pharmacological activity, or withdrawal from exposure to an SSRI. Many of these observations have also been reported in babies from mothers suffering from untreated depression. Given the minor and transient nature of the observations and their similarity to some of the observations that might be expected from pharmacological activity, Eli Lilly and Company does not believe that there is sufficient evidence to classify fluoxetine as a developmental toxin.

The Expert Panel appears to have primarily based their conclusion on the work of Chambers et al. (1996). The Expert Panel noted some weaknesses in this report, including a small number of subjects and models that have many covariates (due to confounding factors such as smoking, alcohol consumption, use of other psychotherapeutic medications, and differences in maternal age). Other epidemiologists consider the study conclusions to be limited precisely because of these confounding factors (Llewellyn et al., 1997).

The description of "poor neonatal adaptation" itself is not a clinically defined category for a distinct pattern of responses that are routinely associated with newborns from women treated with fluoxetine. Instead it is a catch-all descriptor for individual or multiple observations that, when combined, form a statistically significant grouping. All the observations used to define this catch-all grouping are not clearly related to the pharmacology of fluoxetine. Chambers et al. (1996) did not list the frequency of findings for each type of observation, only the frequency for the combined grouping. It is not

clear if the statistical significance of this grouping would change if the observations were grouped differently.

Weaknesses of the study by Chambers et. al (1996) have been summarized by Wisner et al. (1999) and Robert (1996). Probably the most significant weakness of this study was that the control group does not allow separation of fluoxetine effects from those of the underlying maternal depression. The control group for the study by Chambers et al. (1996) consisted of normal pregnant women who called the California Teratogen Information Service and Clinical Research Program with questions about drugs and procedures not considered to be teratogenic. This control group had a lower incidence of confounding factors than the fluoxetine treatment groups. There was no assessment of the incidence of untreated depression in the control group. For the treatment groups, fluoxetine was used either early in pregnancy (exposed-early treatment group) or throughout pregnancy for most of the rest of the women (exposed-late treatment group). Comparison between the exposed-early group and the exposed-late group led Chambers et al. (1996) to conclude that exposure to fluoxetine in late pregnancy increases the risk of poor neonatal adaptation. This finding might also be explained by the fact that severe depression could have required treatment throughout pregnancy in the exposed-late group (Robert, 1996). The exposed-early group may have had a mild form of depression, allowing treatment with fluoxetine to be stopped. From this study, there is no way to determine if the results represented the effects of the underlying severity of the disease and maternal condition or long-term exposure to fluoxetine. Chambers et al. (1996) acknowledged that the "extent to which these findings may be due to the underlying maternal condition is unknown."

The risks of untreated depression can include poor nutrition, disrupted sleep patterns, difficulty following medical and prenatal care recommendations, suicide, worsening of co-morbid medical illness, and increased exposure to tobacco, alcohol, or drugs (Llewellyn et al., 1997). Maternal depressive symptoms can also lead to prematurity and low birth weight (Orr and Miller, 1995; Steer et al. 1992). Pre-term birth can be associated with postnatal complications. Clearly, without treatment for depression, pregnant women and their infants can face significant health risks. Even with antidepressant treatment, the severity of depression and maternal condition can complicate the interpretation of results from studies like those of Chambers et al. (1996).

### ***Increased Incidence of Minor Anomalies***

The expert panel states "Therapeutic fluoxetine exposure during early pregnancy may result in increased incidence of minor anomalies." Eli Lilly and Company does not believe that there is sufficient evidence for this statement.

The expert panel based their conclusion regarding minor anomalies on the work of Chambers et al. (1996). As previously noted, there are weaknesses in the design of the work by Chambers et al. (1996) that preclude any definitive conclusions regarding the potential for fluoxetine to cause minor anomalies in infants. These weaknesses include a

small number of subjects, multiple confounders due to factors such as smoking, alcohol consumption, use of other psychotheropeutic medications and differences in maternal age, and the inability to separate fluoxetine effects from those of the underlying maternal depression.

It is critically important to note that the statistical evaluation of minor anomalies utilized a univariate categorical analysis in which confounding risk factors were not controlled. The increase in minor anomalies reported by Chambers et al. (1996) was only statistically significant when all of the confounding risk factors were left uncontrolled. Acknowledging this significant design flaw and potential bias in outcome, Chambers et al. (1996) re-evaluated the minor anomaly data excluding infants from mothers that were also exposed to benzodiazepines. When infants exposed to benzodiazepines were excluded from analysis, Chambers et al. (1996) reported that the incidence of minor anomalies were not significantly different between the fluoxetine exposed and control groups. The change in study outcome when controlling for only one confounding risk factor highlights the importance of proper study design and controls. Given the lack of significant differences in minor anomalies when infants exposed to benzodiazepines were removed from the population and the lack of control for the other potential confounding risk factors, no definitive conclusions regarding increased minor anomalies can be drawn from the Chambers et al. (1996) work.

Multiple publications have addressed the characteristics, criteria and evidence that are needed to conclude that an environmental agent or a drug produces structural anomalies in humans (Brent 1978, Brent 1995 and Shepard 1998). Of primary importance in these criteria are A) proven exposure, B) consistent findings in two or more high quality epidemiology studies, including the control of confounding factors, C) careful delineation of the clinical cases identifying a specific defect or syndrome of effects, and D) similar positive findings in an animal model demonstrating a dose-response. The available data on fluoxetine does not fit the above criteria and does not support drawing the conclusion that therapeutic fluoxetine exposure during early pregnancy may result in an increased incidence of minor anomalies. This position is based on the following.

A. There was no quantitative proof of exposures in the study by Chambers et al. (1996). Analytical analysis of fluoxetine in maternal blood was not conducted in the Chambers et al. (1996) study. Fluoxetine exposure was assumed based on patient and physician response or records, however, quantitative exposure-response relationships could not be established.

B. There are no consistent findings in two or more high quality epidemiology studies demonstrating a significant effect on minor anomalies in humans. Only one human study deemed acceptable by the expert panel reported an increase in minor anomalies following fluoxetine exposure (Chambers et al. 1996). This study, however, cannot be considered of high quality relative to evaluating minor anomalies due to failure to control for multiple confounding factors. As previously described, when just one of the confounding risk factors (exposure to

benzodiazepines) was removed from the analysis, the authors reported that there was no significant difference in minor anomalies between the fluoxetine and control groups. Furthermore, the selection of infants for evaluation was apparently not conducted randomly or systematically with specific selection criteria. The lack of scientifically rigorous and acceptable selection criteria introduces additional bias and further compromises the validity of the study conclusions regarding minor anomalies.

C. There is no specific defect or syndrome of effects following fluoxetine exposure. Most, if not all agents known to cause malformations in humans are associated with a specific syndrome of effects or a specific defect (Shepard 1998). Chambers et al. (1996) concluded that no pattern of anomalies was recognized. Only when the data was grouped such that 132 different types of minor anomalies were considered, did the authors find statistical significance between the fluoxetine treated group and the control group. This difference was not present when controlling for one of the many risk confounders.

D. Similar findings of increased minor anomalies in animal studies have not been demonstrated. Two scientifically rigorous studies evaluating anomalies in rats and rabbits were conducted by Byrd and Markham (1994). Neither of these studies demonstrated an increase in fetal anomalies following exposure. Both of these studies were reviewed by the expert panel and the FDA and were found to be of high quality and sufficient to evaluate the effects of fluoxetine on embryo and fetal development in an animal model. Importantly, the expert panel also concluded that "The rat and rabbit data are assumed relevant to consideration of human risk."

***Shortening of Gestation and Reduced Birth Weights***

The expert panel suspected that exposure to fluoxetine could lead to shortening of gestation and reduced birth weights. Eli Lilly and Company does not believe that there is sufficient evidence to support this conclusion.

One of the two references quoted to support this assertion (Simon et al., 2002) was a study of SSRI and tricyclic antidepressants. The statistical evaluations were by class of compounds. The authors noted that any statistical significance disappeared when the effects of individual pharmaceuticals were evaluated. Minor, but statistically significant differences by class appeared to be as influenced by changes in the mean values for the randomly selected controls as they were by changes due to exposure to a class of chemicals. For example, the mean estimated gestational age for infants with mothers treated with tricyclic antidepressants was 38.8 ± 1.9 weeks. The mean estimated gestational age for infants with mothers treated with SSRI antidepressants was 38.5 ±1.8 weeks. The result for the tricyclic antidepressant was not different from the mean gestational age (39.1±1.7 weeks) of a randomly selected set of infants from a matched set of untreated mothers. Another random selection of infants to match the SSRI treatment

resulted in a gestational age of 39.4 ±1.5 weeks for infants from untreated mothers. The increase in the control gestational age and reduction in variability contributed at least as much as the slight decline in gestational age of the SSRI treatment group to allow the authors to claim a statistically significant difference for the SSRI treatment. This control bias is also present in the odds ratio evaluation for the percent of infants with gestational ages less than or equal to 36 weeks. Birth weight differences found in this study disappeared when normalized for gestational age. While the authors of this paper caution about the over interpretation of marginally significant results given the large number of comparisons, they go on to draw large conclusions from minor differences found for data sets that could have a control bias. They also suggest that the outcome of their research could be confounded by the underlying medical condition. Symptoms of maternal depression can themselves lead to prematurity and low birth weight (Orr and Miller, 1995; Steer et al. 1992). Based on this information, the study by Simon et al. (2002) should not be the basis for the NTP-CERHR Expert Panel to suspect shortened gestation or reduced birth weights for infants from mothers treated with fluoxetine.

The Expert Panel also referenced Chambers et al. (1996) for their suspicion about birth weight. The average birth weights listed by Chambers et al. (1996) for controls and treatments are within the normal range for infants at birth. There was a higher proportion of infants with gestational ages less than 37 weeks for women treated throughout their pregnancy for depression (10/70) than for women treated only in the first and second trimester (4/98) or for untreated controls (13/220). Chambers et al. (1996) did not determine whether this was due to treatment with fluoxetine or to the severity of the symptoms of the underlying depression. As already described, the symptoms of depression can lead to prematurity and low birth weight.

Since neither of the studies quoted by the NTP-CERHR panel evaluated the effect of depression as a variable on gestational age or birth weight, the panel has no real basis in humans to suspect that treatment of women with fluoxetine results in either of these effects in infants.



### ***Reduced Size of Breastfed Infants***

The NTP-CERHR Expert Panel indicated that there is suggestive evidence about the size of breastfed infants. This appears to be primarily based on the presence of fluoxetine in breast milk and the results from a different study by Chambers et al. (1999). Chambers et al. (1999) indicated "that infants who are breastfed by mothers who take fluoxetine track a growth curve significantly below that of infants breastfed without medication." Fluoxetine levels were not actually measured in breast milk by Chambers et al. (1999), but infant exposure was assumed to have occurred from breastfeeding. They did note, however, that the possibility of direct effects of fluoxetine on weight gain in nursing infants is not supported by the dose they could have received. Less than 10 percent of a maternal dose of fluoxetine is transferred to a nursing infant (Taddio et al., 1996). Chambers et al. (1999) also acknowledged "women with an underlying condition requiring a psychotherapeutic medication may breastfeed less often and engage in other

behaviors that influence postnatal weight gain in their infants." So the results of this study do not support the NTP-CERHR Expert Panel conclusions about the direct effects of fluoxetine on the size of breastfed infants. Chambers et al. (1999) even wrote that "there is no evidence from these data to indicate that mothers who breastfeed their infants while taking fluoxetine should be concerned about side effects attributable to the medication."

## 2. Reproductive Toxicity of Fluoxetine

According to the Expert Panel (Section 4.4 Summary),

> *…there is sufficient evidence in humans that fluoxetine produces reproductive toxicity in men and women manifested as impairment of sexual function, specifically orgasm.*

The Expert Panel also noted that effects on individual sexual performance are unpredictable. The Expert Panel acknowledged that, in many instances, it is not possible to differentiate drug-induced adverse effects from those induced by the disease process itself or the pharmacological action of the drug. Depression is associated with impaired sexual function, and successful treatment of depression may be associated with improvements in sexual function. Some evidence does suggest that SSRIs can cause untoward sexual experiences. At least one report indicates that improvement in sexual function (reversal of sexual dysfunction) occurs when the dose of an SSRI is diminished or the drug is withdrawn (Montejo-Gonzalez et. al, 1997). Reliable estimates of the incidence and severity of these experiences involving sexual desire, performance, and satisfaction are difficult to obtain. While it is difficult to know the precise risk of sexual dysfunction associated with the use of SSRIs, the product information for fluoxetine recommends that physicians should routinely inquire about such possible side effects with their patients. Important to note is that sexual function is not necessarily equivalent to reproductive performance. Since effects on sexual function from the use of SSRIs can be confounded by the disease state and appear to be based on reversible pharmacology, fluoxetine should not be classified as a reproductive toxin in humans.

## 3. Risk/Benefit Considerations in the Use of Fluoxetine

***Subpopulation of patients: Children***

The U.S. Food and Drug Administration recently approved Prozac® (fluoxetine hydrochloride) for the treatment of major depressive disorder and obsessive-compulsive disorder in the pediatric population. The clinical data reviewed by the FDA indicate that Prozac 20 mg has a comparable profile of safety and efficacy in both adults and children. Eli Lilly and Company submitted four pediatric studies: two clinical studies for depression, one for obsessive-compulsive disorder, and one pharmacokinetic study. The FDA based its decision on placebo-controlled clinical trials.

Lilly is working closely with the FDA on the design of a Phase IV post-marketing study to further evaluate whether there is any long-term effect on either weight or height of children who take Prozac. The FDA stated in its announcement that "the clinical significance of height and weight differences on long-term growth is unknown." (www.fda.gov) The Phase IV studies should help provide additional information in this area. Nonetheless, the FDA has found the product to be both safe and effective as seen in its approval.

On December 10, 2003 the MHRA (Medicines and Healthcare Products Regulatory Agency) in Europe announced, "On the basis of a review of the safety and efficacy of the SSRI class in the treatment of pediatric major depressive disorder undertaken by the Expert Working Group of the Committee on Safety of Medicines (CSM), the CSM has advised that the balance of risks and benefits for the treatment of major depressive disorder in under 18s is judged to be unfavorable for sertraline, citalopram and escitalopram and unassessable for fluvoxamine. Only fluoxetine (Prozac) has been shown in clinical trials to have a favorable balance of risks and benefits for the treatment of MDD in the under 18s" (www.mhra.gov.uk). The MHRA has asked Eli Lilly and Company to submit documents for the pediatric approval of Prozac in Europe.

***General Patient Population***

The stated purpose of the NTP-CERHR is to address the "widespread concern among health professionals, environmental scientists, and the public that environmental exposures may be contributing to human reproductive and developmental disorders". The chemicals previously reviewed by the NTP-CERHR reach the general population through environmental exposure. Some of these chemicals have the potential for a health risk, with no potential for a health benefit. The results of exposure of individuals to most environmental chemicals are only rarely monitored by a health care professional.

Unlike the chemicals that can reach entire populations by environmental exposure, fluoxetine is available only through prescription. Individuals are monitored by physicians qualified to evaluate the potential benefits and risks of fluoxetine to their patients. All of the approved uses of fluoxetine have been evaluated by the U.S. Food and Drug Administration.

Product information that describes potential benefits and risks of fluoxetine has been made available to physicians and the public. For example, the presence of fluoxetine in breast milk is noted in the product information and nursing is not recommended. Eli Lilly and Company also recently agreed to class labeling to include the published associations of poor neonatal adaptation in babies from mothers with depression who were treated with SSRIs. It is noted in the product information that fluoxetine should only be used during pregnancy, labor and delivery if the benefit justifies the potential risk. Determining the potential for certain developmental or reproductive hazards from the use of fluoxetine is complicated by the same hazards that result from maternal

depression. Improvement in depression from the use of fluoxetine might reduce the risk of these hazards. This risk/benefit consideration is best made on a case-by-case basis by physicians and their patients.

## 4. Communications about pharmaceutical safety in patients

The role adopted by the Expert Panel to review literature for a registered pharmaceutical product overlaps the statutory responsibility of the United States Food and Drug Administration (US FDA). The US FDA collects published information and proprietary data available for its safety and efficacy reviews and requires detailed explanations of the risks and benefits from the use of a pharmaceutical to be packaged with the product. While the report of the Expert Panel has no direct regulatory implications for the use of fluoxetine, generalized media reports already written about the findings of the "government report" have the potential to alarm people being treated for depression into discontinuing their treatment before they talk to their physicians. Patients could even decide to unnecessarily terminate healthy pregnancies.

The US FDA has the sole regulatory responsibility in the US government for evaluating the safety and efficacy of pharmaceuticals, and for determining appropriate communications to the public about them. Terminologies used in the NTP-CERHR report were developed for population exposures to environmental chemicals. The guidelines for the NTP-CERHR Expert Panel actually have no narrative criteria for observed effects that warrant categorizing a pharmaceutical as having reproductive or developmental toxicity. Nor do the guidelines provide direction for evaluating therapeutic drugs that may improve reproductive performance. The panel members had to partially develop and apply a new definition of reproductive toxicity in the report for fluoxetine, indicating that reversible pharmacology qualifies as toxicity to patients treated for depression, apparently even if reproductive function is improved. Even though the NTP-CERHR Expert Panel provides a thorough review of published information, interpreting the information for pharmaceuticals and communicating potential risks to patients should remain the responsibilities of the US FDA.

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June 16, 2004

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RE: NTP-CERHR Report on Fluoxetine

By regular mail and electronic mail (shelby@niehs.nih.gov )

Dear Sirs:

The following comments are in follow-up to my testimony before the NTP-CERHR Expert Panel given on March 3, 2004. I am attaching a curriculum vitae which highlights my work in the area of reproductive psychiatry over the last 18 years. I am Director of the Perinatal and Reproductive Psychiatry Clinical Research Program at Massachusetts General Hospital and Associate Professor of Psychiatry at Harvard Medical School. I have sub-specialty training and longstanding clinical activity in the area of treatment of mood and anxiety disorders. This has been complemented by over fifteen years of dedicated work as a clinical researcher in the area of mood disorders in women and, specifically, the treatment of depression during pregnancy and the puerperium. As noted during my comments in Washington, I have also served as a consultant to Eli Lilly since the original launch of fluoxetine and have carefully followed and examined the accumulating data regarding the reproductive safety of the compound since it came to market in the United States.

The Program I direct at Massachusetts General Hospital is designed to help patients make decisions about the potential use of psychiatric medications, including antidepressants, during pregnancy. Our group consults on approximately 1000 women per year to review the relative risks of continuing pharmacotherapy during pregnancy versus the risks of depressive relapse and the impact of such relapse on maternal and fetal well-being. Our web-driven Perinatal Information Resource Center (see http://www.womensmentalhealth.org) receives approximately 17,000 visits per month, with the majority of queries focusing on the use of psychiatric medications during pregnancy.

NTP Comments
Lee S. Cohen, MD

**NTP-CERHR Expert Panel Report: Points of Concern**

Introduction:

The conclusion of the NTP Expert Panel that fluoxetine is a "reproductive toxin," has the potential to dramatically affect the treatment of major depression in women of reproductive age. Major depression is a highly prevalent illness and clusters in women during the childbearing years. There is already stigma associated with depression and its treatment; treatment of depression during pregnancy is a particular concern for patients and lay literature and scientific reports are frequently conflicting or inconclusive. Much of the data referenced and reviewed by the Expert Panel, and on which conclusions have been made regarding the reproductive safety of fluoxetine, have clear flaws acknowledged by the original investigators in many cases.

Risk for Congenital Malformations:

While the Expert Panel did not conclude that fetal exposure to fluoxetine was associated with an increased risk for major congenital malformations, they did suggest that prenatal exposure *was* associated with increased risk for minor congenital anomalies. This is ironic since, while there was an increase in two and three minor anomalies in the exposed versus unexposed children, there was no apparent pattern of minor anomalies noted. Given the absence of a consistent pattern with respect to minor anomalies, the authors clearly acknowledge the inability to make a causal link between fetal exposure to fluoxetine and increased risk for minor anomalies. It is also noteworthy, that the dysmorphologic evaluation with respect to minor anomalies was conducted on only half the original sample, hence introducing a bias which is not acknowledged by the NTP Panel in the Expert report (see Cohen and Rosenbaum, 1994, NEJM for comment).

Poor "Neonatal Adaptation" Associated with Prenatal Exposure to Fluoxetine

The Expert Panel report also addresses the risk for "perinatal toxicity," which typically includes symptoms of jitteriness and autonomic reactivity in the newborn. Reports have accumulated over the last decade suggesting that prenatal exposure, particularly during the third trimester, to several selective serotonergic reputable blockers (SSRIs), including fluoxetine, may be associated with an increased risk of transient symptoms as noted above. Most reports have not associated such exposure with adverse longer-term sequelae. Fluoxetine is the only SSRI for which we have long-term neurobehavioral data, including follow-up of exposed children through ages 4-7. No differences in long-term neurobehavioral outcome between exposed and unexposed children were noted.

Another concern regarding conclusions made by the Expert Panel lies with the failure to acknowledge an important potential confound in the literature reviewed by the committee, namely, the extent to which untreated maternal mood may have adverse effect on fetal and neonatal outcome. Recent literature (see Orr and Miller, 2002) supports earlier preliminary concerns regarding the adverse effects of untreated maternal mood on fetal and neonatal well-being, with data suggesting higher rates of obstetrical and neonatal complications in offspring of mothers who suffer from depression during pregnancy. In our own work, we note that the threshold for patients' willingness to use antidepressant during pregnancy is frequently high. So greater duration of treatment during pregnancy, as noted in some of the studies reviewed by the

NTP Expert Panel, really implies that the study population suffers from more severe depression compared for example, to a group exposed only "early in pregnancy" ( see Chambers, 1996). It is my opinion that the failure to address the confound of untreated depression during pregnancy, and its potential effect on the observed outcome, constitutes a serious omission.
Our group has spent nearly twenty years counseling women about the relative risks of antidepressant use during pregnancy, including fluoxetine. Decisions are best made on a case-by-case basis since, when presented with the same information, patients make very different decisions as a function of wishes and individual perception of risk and benefit. These decisions are best made collaboratively between patient and physician around a specific clinical situation.

Fluoxetine is used to treat a serious illness; it is not a potential environmental toxin, such as those reviewed by other NTP panels. The compound in question is a therapeutic agent where safety is actually reviewed in an ongoing fashion by a separate governmental agency. The report does not indicate that decisions about whether to use fluoxetine during pregnancy are clinical choices made by patients in the context of some risk-benefit analysis made collaboratively between the patient, her family, and the physician.

My colleagues and I have described high rates of relapse in women with a history of recurrent major depression who discontinue antidepressants in pregnancy. Depression during pregnancy is associated with compromised fetal and neonatal outcomes, risks that are not reflected in the report. Discontinuation of antidepressant medication near the end of pregnancy also appears to increase the risk for postpartum depression.

The Expert Panel notes in the report that any risks of fluoxetine need to be weighed against the risks of untreated disease. But this brief statement, embedded in a lengthy document that describes fluoxetine as "a reproductive toxin," is inadequate. I am particularly concerned as to how this report will impact the decisions made by my patients, and patients of my colleagues, who use these therapeutic agents to treat a disease like major depression with its attendant morbidity and potential mortality.

Sincerely,

Lee S. Cohen, M.D.

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RE: NTP-CERHR Report on Fluoxetine

Gentlemen:

I am responding to the most recent NTP-CERHR report on fluoxetine dated April 2004. I had previously reviewed the document published that was dated November 2003. I am specifically addressing the issues with regard to some aspects of developmental toxicity.

Background:

Received BA in 1948, M.D. with honor in 1953 and a Ph.D. in embryology and radiation biology in 1944 from the University of Rochester. First postdoctoral fellow of the March of Dimes in the area of birth defects 1953-54, Pediatric Residency at the Massachusetts General Hospital. I am a Board Certified pediatrician who was chairman of a large pediatric department for 30 yeas at the Jefferson Medical College with pediatric programs at the Thomas Jefferson University Hospital, the Methodist Hospital, Christiana Hospital and the duPont Hospital for Children. We have had a large neonatal program with intensive care nurseries in two of the hospitals and as many as 20,000 deliveries per year, all told, during some of our peak years. My own area of interest and research is in teratology, developmental toxicology and genetics. I had a NIH training program for many years and our trainees are heads of developmental biology programs at universities and in industry. I was funded continuously during my research career by the NIH and the DOE and have been consulted and continue to be consulted by the FDA, CDC, NIH, DOE, industry and the Attorney General's office of the United States. I have published 400 papers, six (6) books and numerous abstracts in my areas of expertise. I am a member of the IOM of the NAS, the NCRP, HESI, HPS, and a number of other research societies. One of the societies of which I am a charter member is the Teratology Society, having been elected president in 1966-67, as well as editor of the Journal, Teratology for three five-year terms. Although I no longer am chairman of the Pediatrics department, I still have a laboratory, teach medical students and residents and have students in our laboratory. I am presently a member of the Dental Institute's Dental Amalgam Committee and have just been appointed to a new committee of the NAS dealing with the

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The Nemours Foundation
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Thomas Jefferson University

Appendix III

environmental toxicology. Other consultations include solving environmental toxicology issues concerning new drugs and chemicals that are quite similar to the issues raised in the fluoxetine report. My most recent publication is a supplement to the April issue of "Pediatrics," which is a series of 30 chapters dealing with environmental toxicology issues. I was the senior editor and Michael Weitzman was co-editor. The preface and four (4) chapters were written by the both of us and two chapters were authored solely by myself (Brent 2004). My present position is Distinguished Professor of Pediatrics, Pathology and Radiology.

Consultation with Lilly:

Before being consulted by Eli Lilly, I had an interest in this topic because my oldest son is Chairman of Child Psychiatry at the University of Pittsburgh and his field is childhood depression and suicide. He is involved in extensive research in treating depressed children and is involved with the evaluation of the impact of the controversial papers recently published dealing with the risks of SSRI's. Therefore, it is important that future publications contain conclusions that are supported by definitive data and not presumptions or hypotheses. Depressed adults and children need all the assistanee we can give them.

My general reaction to the report with regard to developmental toxicity is that the expert panel is required to follow past guidelines set forth by NTP CERHR with regard to drawing conclusions about developmental toxicity and are requested to respond with a yes or no answer (i.e. is the substance developmentally toxic or not). Having to follow this guideline places the expert panel in an untenable position of having to draw a firm conclusion when firm data is not available. It is like having to label and individual as a dangerous criminal because he has committed a crime, regardless of what the crime is (i.e. serious felony vs. minor traffic violation). It would be much better if the committee would describe what they consider to be the developmental findings that are of concern and the quality of the data on which the conclusion is based. Labeling an agent as a developmental toxicant on the basis of minimal or controversial data is not appropriate.

Congenital Malformations:

The animal studies and the epidemiological studies indicate that therapeutic doses of fluoxetine in the human and in animals at pharmacokinetically equivalent doses do not result in an increase in major birth defects. Although the expert panel is in agreement with the lack of data demonstrating an effect on major malformations, they state that fluoxetine exposure during early gestations may result in an increased incidence of minor anomalies. With regard to the issue of minor anomalies the expert committee focused on the paper by Chambers et al. published in the New England Journal of Medicine in October 1996. In that paper, the author's conclusions do not include any inference that the minor anomalies were an issue in their study. They reported a

decrease in the incidence of one minor anomaly in the exposed population compared to the controls, and an increase in two or three minor anomalies in the exposed population compared to the controls. All three of these comparisons were statistically significant. The authors felt that they could not conclude that this was a causal association because the spectrum of minor anomalies had no consistent pattern in the exposed group as compared to the controls. This is a very important principle in teratology that is utilized in evaluating major malformation syndromes as well as clusters of minor anomalies. Minor anomalies can be used in teratology studies in many ways. For instance repeated clusters of minor anomalies may be associated with an increase in major anomalies at a higher exposure. Minor anomalies may occur at lower exposures as sentinels of a teratogenic exposure. But if you do not have major malformations either clinically or in animal studies, then the occurrence of minor anomalies cannot be interpreted to be a definitive sign of teratogenicity. If a child was exposed to thalidomide but exhibited no major malformations but did have a band around the little finger and a small sacral dimple, the experienced dysmorphologist would know that these minor malformations were not the result of the thalidomide exposure, because those are not minor malformations that have been identified in the thalidomide syndrome.

The other issue with regard to the minor anomalies is that Dr. Kenneth Jones, who is a well-known authority on dysmorphology and an expert in examining patients with dysmorphology effects, did not examine all of the patients in the Chambers study. Approximately 50% of the patients were examined by him so that the incidence and statistics for major malformations were determined from the whole group of exposed population and the minor anomalies were only determined from a portion of the exposed population. The most important aspect of the Chambers et al. paper is that they themselves did not believe that the minor anomalies issue was a causal association. I called Dr. Chambers and spoke with her in detail about the minor anomalies and she was surprised that the expert committee would draw any conclusions abut the data on minor anomalies, since the authors themselves did not infer that there was a causal association based on their data. It might be worthwhile for the Committee to discuss the issue of minor anomalies with her directly. She is very approachable and very forthright.

<u>Neonatal Adaptation:</u>

Another issue that the expert committee dealt with was neonatal adaptation. As the committee pointed out, these were all transient signs that were present in the newborn nursery and at higher incidence in the fluoxetine-exposed mothers than in the controls. I would point out to the committee that this is an extremely difficult study to perform because there was no randomization of the patients being sent to the intensive care nursery versus those going to the regular nursery. Patients in the intensive care nursery or intermediate nurseries are more carefully monitored compared to the normal nursery. The intensive care nurseries have more

attendants and the babies are evaluated more closely. The physical and behavioral signs are recorded in more detail in these nurseries as compared to the regular newborn nurseries. It is more likely that a mother who has been depressed and on fluoxetine is going to have her baby sent to an intensive care nursery or intermediate care nursery for observation compared to the patients who would go to a normal nursery who are not depressed and on fluoxetine. As a result of the differences in patient care, you do not have a comparable control group for the exposed patients with regard to the intensity of observing these infants. Many normal newborn babies have these transient neonatal adaptive findings in the normal nurseries that are not recorded. An important point that the panel determined was that these adaptive findings were not permanent and that when the babies were examined weeks or months later all these newborns adaptive findings were gone.

Method of Evaluation:

In developmental biology, five important criteria are considered in the process of evaluating and concluding whether or not an agent causes developmental toxicity in humans. These five areas include: 1) Consistent findings in two or more high quality epidemiology studies, 2) Ecological or secular trend analysis, 3) Animal studies that include teratology and developmental biology studies, 4) Pharmacokinetic or toxicokinetic studies in the animal models that include the equivalent human exposures, 5) Biological principles, method of action studies, receptor studies, biological plausibility and the application of teratology and developmental biology principles.

Using the above methodology we can arrive at the following conclusions:

1) The human epidemiology studies and animal studies indicate that fluoxetine does not cause congenital malformations. In the area of minor anomalies, we do not have consistency of multiple epidemiology studies that describe a cluster of minor anomalies that is associated with fluoxetine exposure. The Chambers study is the only one that deals with minor anomalies and the authors conclude that their study was not a causal association.

2) Secular trend or ecological analysis cannot be utilized because fluoxetine is utilized in only a small percentage of pregnant women.

3) and 4) The animal studies and animal toxicology studies clearly indicate that fluoxetine is not a teratogen and that you do not get any developmental effects until you raise the exposures high enough to produce maternal toxicity. This also is an important finding that can be used in evaluating the clinical aspects of fluoxetine exposure in pregnant women.

5) The last area that is considered by developmental biologists in evaluating the clinical significance of drug and chemical exposures in pregnancy is biological plausibility. Since there is an absence of animal studies and human studies to indicate that fluoxetine produces major malformation, biologic plausibility is not utilized to evaluate the occurrence of major malformations, since it is mainly used to support the presence of a teratogenic or developmental effect. On the other hand, biological plausibility does not support the concept that fluoxetine would be causally related to the etiology of minor malformations, since we do not have teratogens that only produce minor malformations at low doses, but no severe major malformations at higher exposures.

Fetal Growth Retardation:

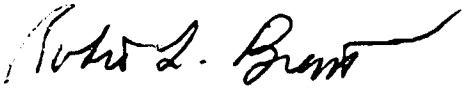
The expert panel concluded that they were concerned about the possibility that fluoxetine may have been responsible for growth retardation in one population of newborns exposed in utero (Chambers et al. 1999). Growth retardation is an important, sensitive developmental effect. Although it was reported that the fluoxetine-exposed fetuses were smaller than controls (3419 gms vs. 3711 gms), both groups were above the national average at birth. It is difficult to draw any conclusions about fluoxetine's effect on growth when the average weight of the fluoxetine-exposed newborns was above the national average in this study.

Conclusion:

The expert panel had three concerns, which in their opinion, was sufficient to label fluoxetine as a developmental toxicant. Such a labeling may seem appropriate to the panel as a precaution, but does the available data warrant such a label. As I indicated in my discussion, an essential characteristic of a causal or statistically associated effect is consistency of the finding in epidemiological studies, which is presently not available. The minor malformation data, the growth retardation data and the transient neurological data have yet to be confirmed as definitive positive findings. This group of drugs is important for the treatment of very serious and debilitating diseases and the committee has a responsibility to act on the basic definitive results of developmental toxicity studies that are not available at this time. It is appropriate to describe the "positive" findings and the quality of the data on which these findings are based. Reversible effects (neonatal adaptation) and inconsequential results (statistical growth retardation in groups of normal sized babies) need to be carefully evaluated before determining that they are causal effects or significantly detrimental.

If you have any questions about these comments, please feel free to contact me.

Sincerely,



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June 17, 2004

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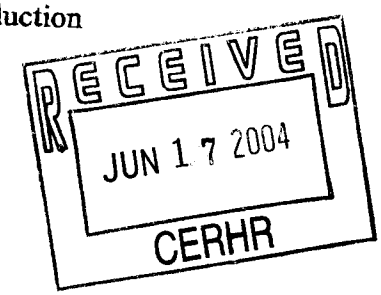


**3 pages via electronic mail and fax: 919-316-4511**

Dear Dr. Shelby:

The following comments are submitted on behalf of the 800,000 members and supporters of People for the Ethical Treatment of Animals (PETA) in response to the National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) report on the reproductive and developmental toxicity of fluoxetine, which was made available on April 19, 2004. Public comments on the report were solicited in the *Federal Register* on April 29, 2004 (vol. 69, no. 83, pp. 23517-23518).

Fluoxetine is better known as Prozac®, since it is marketed under this and several other trade names. Based on the response received on June 16 to our Freedom of Information Act request, fluoxetine was nominated for further study by a single, anonymous individual and "no reason for nomination [was] provided." This is completely contrary to the procedure for nominations outlined on the NTP's website at http://cerhr.niehs.nih.gov/nominate/index.html.

**The Recommended Animal Tests Are Unjustifiable and Unnecessary**

The report concluded that additional mammalian studies are necessary in the following areas (p. 138):

(i) Rodent toxicity (using studies that comply with the current testing guidelines)
(ii) Developmental behavioral neurotoxicity, including brain histology
(iii) Effects of prenatal exposure on hippocampal development
(iv) Effects on semen quality
(v) Effects on ovulation, conception, and abortion

We cannot estimate how many animals will suffer and die in the course of these studies, but the number will undoubtedly be large. Furthermore, some of the studies for areas (ii) and (iii) will almost certainly be carried out on primates, since the behavioral effects of neurotoxicity and the emergence of the hippocampus from the archipallium are more pronounced in primates than in other mammals. In addition, neurotoxicity studies are often particularly cruel, as animals are often subjected to stressful and abusive practices, such as electric shock, food/water deprivation, and the deliberate infliction of pain or anxiety (OECD, 2000), in crude attempts to measure motor, sensory, cognitive, and other functional parameters, many of which bear little resemblance to neurological assessment methods used clinically in humans (Anger, 1990). Many common neurotoxicity tests rely heavily on measures of the animals' behavior, rather than other,

more objective physiological measures, which has raised concerns about the potential for extreme variability in test results and the subjectivity of their interpretation (Claudio et al, 2000; Tilson, 1995; Gerber and O'Shaughnessy, 1986). In fact, one EPA scientist has acknowledged that "the outcome of a study can depend on the inherent variability of a test measure" (Tilson, 2000).

**Human Data Are Available and More Appropriate**

Further testing on animals for fluoxetine is not only unnecessary, it is immoral, since human data are so readily available and will be much more applicable to the endpoints that the NTP wishes to study. The U.S. development program for this drug involved the full set of animal experiments required by the Food and Drug Administration (FDA), and equivalent development programs were followed in all major industrialized countries. All required clinical (i.e., human) studies were also carried out. Since its U.S. launch 17 years ago in 1987, fluoxetine has been administered worldwide to tens of millions of patients from almost every demographic group, and it has become the most widely prescribed antidepressant.

The diversity and sheer number of people who take this drug have provided Eli Lilly and other interested parties with an ideal opportunity to carry out large-scale Phase-IV (post-marketing) studies on its side effects. If all necessary data on the human effects of fluoxetine have not been obtained from such studies, this would represent a case of astonishing negligence.

The report states that additional data are needed in several specific areas. The effects on semen quality and ovulation should be investigated by studies on adult males and adult, non-pregnant females, who compose the overwhelming majority of the patients to whom fluoxetine is administered (i.e., an enormous number of patients). Investigation of the effects on conception, abortion, and developmental neurotoxicity would require studies on children and/or pregnant women. However, despite the concerns that have been raised, fluoxetine has been, and continues to be, widely administered to these groups, and there is, therefore, no reason that the necessary information could not be obtained by means of data analyses from human studies.

With regard to developmental toxicity, the report acknowledges that the principal developmental toxicity data deficiency results from the failure to maintain long-term follow-up to the studies that have been carried out (p. 136). It is also arguable that sufficient developmental toxicity data are already available to justify the decision that fluoxetine should not be administered to pregnant women or women who have the potential to become pregnant, as it is known to result in a deterioration of neonatal adaptation (p. 136). Finally, the statement that rodent data are needed from studies that comply with the current guidelines is not explained; nowhere else in the report is the failure of previous studies to meet the guidelines mentioned. It is important to stress that animal data are widely known to be of limited use for predicting side effects in humans. It is therefore incomprehensible that the NTP-CERHR believes that more experiments on thousands of animals will provide data relevant to humans that could not be obtained by statistical analysis of the real effects on millions of humans.

**Conclusion**

Under separate cover, we are sending you a letter regarding our concerns with the NTP-CERHR's system of test-substance nominations and solicitation of public comments. The NTP needs to explain its policy of accepting anonymous nominations, which violates the interests of open and transparent government procedures, as well as the fact that this particular nomination was pursued with "no reason for nomination provided." Both are contrary to your own instructions for nominating chemicals.

Further, the NTP-CERHR needs to assess and critique recommendations for additional animal tests and give proper consideration to possible overlap between its programs and those of other sections of the NTP and other governmental bodies. In this particular case, it is unclear what role the FDA should play in requesting additional data on Prozac.

To conclude, we strenuously object to the recommendation that large numbers of animals, whether they be rats or primates, be subjected to these experiments. We urge the NTP to state very clearly in its final report that additional animal experimentation on Prozac is not a priority and should not be pursued. While we understand that the NTP has frequently not addressed the recommended data needs in its final monographs, its failure to do so in this case would be highly irresponsible.

Sincerely,

J. Sandler

Jessica Sandler, MHS
Federal Agency Liaison

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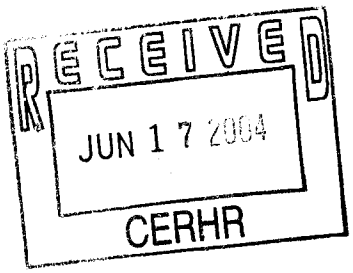


June 17, 2004

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***Via electronic mail to shelby@niehs.nih.gov and fax to 919-316-4511***

Dear Dr. Shelby:

I am writing in response to the National Toxicology Program's notice on the Expert Panel Report regarding questions on the reproductive and developmental toxicity of fluoxetine (Prozac). The action taken (e.g., convening the expert panel) thus far is puzzling and certainly is in conflict with the approval for use of this drug in younger populations and in premenstrual dysphoric disorder by the very agency—the Food and Drug Administration—whose mission it is to determine drug safety. This smacks of redundancy and waste of federal resources. But what is most outrageous is the lack of proper research perspective on the part of the panel.

The panel report acknowledges that tens of thousands of persons over the entire age span have been treated with fluoxetine for years, yet does not understand that it is the human outcomes data that should be the focus of any further inquiry—NOT basic dosing experiments on nonhuman animals.

As an epidemiologist, I can vouch for the vast superiority of data on reproductive outcomes and development collected from humans under realistic dosages and in the diverse milieu of everyday life. The latter includes variation in body weight and fat distribution, other drugs, smoking, sleep duration, family history, health care access, comorbidity, and other exposures. These are extremely important modifiers of the outcomes in question and can never be accounted for in laboratory experiments on nonhuman species living in situations far from human conditions. The basic laboratory toxicology data so collected on nonhuman species can never be generalized, even remotely, to answering the questions being asked regarding the safety of fluoxetine in younger ages and women of child bearing years. We are all too aware of the errors that have been made in the past in extrapolating safety from narrow high dose experiments on nonhuman animals to pregnant women, for example.

Most importantly, we are indeed fortunate to have the truly relevant data (or the means to easily obtain it) at hand in this particular case. In addition to already collected data and ongoing studies on fluoxetine, long term follow-up on thousands of persons who participated in previous trials can be carried out quickly. Furthermore, nested case-control and historical cohort studies could be performed on the numerous ongoing population-based cohorts, many of which include random samples of children being followed, with data being used to address many epidemiological questions. Indeed, at present I am aware of several ongoing clinical trials of

fluoxetine in children and adolescents, and there are abundant data sets on women that include pregnancies over the course of observation.

I am appalled that, if the panel's recommendations are accepted, the millions of NIH taxpayer dollars that have gone into collecting priceless, relevant human data that could truly address the questions about fluoxetine would go to waste, and virtually useless (dangerously so) data will be collected in painful experiments on nonhuman animals (most likely rats, dogs and primates). Something in the scientific and regulatory process has gone badly awry here, with one government agency (NTP) apparently duplicating the mission of another federal agency (that of the FDA).

If allowed to proceed, we will all suffer the consequences of bad data and wasted research dollars, and many animals will suffer being subjected to useless, painful, and deadly experimentation.

Sincerely,

Terry Young, MS, PhD
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