FOLLOW-UP MEASURE
FINAL JOINT ASSESSMENT REPORT
MUTUAL RECOGNITION

Name of the medicinal product in the EU:	Prozac oral liquid 20mg/5ml Prozac 20mg capsule Prozac 20 mg dispersible tablets
Name(s) of the active substances :	Fluoxetine hydrochloride
Pharmacotherapeutic classification :	N06A B03
Pharmaceutical form and strength :	Oral liquid 20mg/5ml Oral capsule 20mg Dispersible tablets 20 mg
Reference No. for the Mutual Recognition Procedure :	UK/H/0636/01,03 FR/H/242/01
Concerned Member States :	UK/H/0636/03: AT, DE, EL, ES, FR, HU, IE, IT, NL, PT, SE UK/H/0636/01: AT, BE, CZ, DE, EL, ES, FR, IE, IT, LU, MT, NL, PT, SI. FR/H/242/01: BE, DE, DK, EL, ES, IS, IT, LU, NL, NO, SE.
Reference Member State :	UK for Oral liquid 20mg/5ml and Oral capsule 20mg FR for Dispersible tablets 20 mg
Marketing Authorisation holder's name and address :	Eli Lilly and company Limited Priestley Road Basingstoke Hampshire RG24 9NL United Kingdom
Name, telephone and telefax number of the contact point in the Member State :	**FR** [redacted] **UK** [redacted]
Names of assessors :	**UK** [redacted] **FR** [redacted]
Date of Preliminary Assessment Report (Day 40) :	09/07/2008
Comments due from CMSs (Day 55)	23/07/2008
Date of Final Assessment Report	14/10/2008

Contents Page

TABLES

FINAL EXECUTIVE SUMMARY

Product Name: Prozac (UK/H/636/01, 03/II/02, FR/H/242/01)

Follow up measure: To assess the findings of juvenile toxicity studies conducted to investigate the effect of Fluoxetine (Prozac) on neurohormonal sexual maturation and testicular pathogenesis in rodents.

1. GENERAL RECOMMENDATIONS

There are a number of outstanding issues arising from the assessment of the testicular pathology and neurohormonal sexual maturation study in juvenile rats with Fluoxetine hydrochloride that will need to be addressed by the MAH. These are discussed in section 5 and 6 of this report.

The MAH is requested to submit the responses to the list of questions within 3 months of the date of circulation of this AR.

2. INTRODUCTION

On 1 June 2006, a positive opinion was adopted by the CHMP for the use of fluoxetine in the EU for the treatment of major depressive episodes unresponsive to psychological therapy after 4–6 sessions in children aged 8 to 17 years.

Some pre-clinical issues remained unresolved at the time of the opinion. A study published by Beck (2004) (study WIL-353039) investigating the effect of fluoxetine hydrochloride on juvenile CD rats identified several toxicological effects on developmental parameters: delays in sexual maturation, testicular degeneration, and potential effects on emotional behaviour. As part of the CHMP conditions of approval of the paediatric indication, the Marketing Authorisation Holder (MAH) agreed a risk management plan which included a commitment to conduct additional preclinical studies to investigate the mechanism of toxicity of these effects and to determine their relevance to the intended paediatric patient population. They also committed to co-operate with clinical investigators in developing a NIMH sponsored study in children. The MAH submitted draft protocols for the following studies for assessment on 30th June 2006 and the concerned member states agreed the final protocol for the studies on October 2006:

- **Investigate the effect of fluoxetine hydrochloride on neurohormonal sexual maturation in the juvenile rat.**
- **Characterise testicular pathogenesis in the juvenile rat.**
- **Characterise the effect of fluoxetine hydrochloride on specified emotional behaviours in the juvenile rat.**

The neurohormonal and testicular toxicity studies have been completed and the MAH has submitted the final study reports for assessment. The study on emotional behaviours is still in progress. It should be noted that the MAH also submitted a report of their preliminary findings of the testicular toxicity study in March 2007. This preliminary report revealed severe cutaneous effects that had not been observed in the Beck 2004 study, and is discussed further in this report.

Two preliminary assessment reports have been written on D40 of the procedure by the two reference member states (UK and FR). Comments from Sweden were received on D55. The following report is a Joint Assessment Report between UK and FR that integrates the comments from Sweden.

3. STUDY 901144 "A TESTICULAR PATHOGENESIS CHARACTERIZATION OF LY110140 ADMINISTERED BY ORAL GAVAGE IN YOUNG RATS FOLLOWED BY A RECOVERY PERIOD". Charles River Laboratories. November 2007.

The purpose of this study was to characterize the development and potential reversibility of testicular toxicity through neurohormonal and histopathologic evaluations in male juvenile CD rats when LY110140 (fluoxetine hydrochloride) was orally administered from Day 21 *post partum* (pp) through to Days 55, 70 or 91 pp.

Study Design

Table 1. Study 901144: Design

Animals were randomized into the groups as follows:

Group Number Identification	Dose Level[a] mg/kg/day	Animal Numbers - Dosing Schedule: Males Day 21 to 55* *post partum, inclusive*	Males Day 21 to 70* *post partum, inclusive*	Males Day 21 to 91* *post partum, inclusive*
1/ Vehicle Control	0	20	20	20
2/ Low LY110140	10	20	20	20
3/ High LY110140	30	20	20	20

a Dose volume = 5 mL/kg

* 9 to 10 rats/group/time point were sacrificed at the end of each respective treatment period. The remaining 10 rats/time point/group were observed until Day 181 *post partum.*

Group Number Identification	Dose Level[a] mg/kg/day	Toxicokinetic Subgroup - Dosing Schedule: Males Day 21 to 55* *post partum,* inclusive	Males Day 21 to 91* *post partum*, inclusive
1/ Vehicle Control	0	3	3
2/ Low LY110140	10	16	16
3/ High LY110140	30	16	16

a Dose volume = 5 mL/kg

* Males were bled on Day 55 and 91 *post partum* for toxicokinetic evaluation. Three male pups from each group were bled via the jugular vein a maximum of 3 times for the treated groups and at 1 timepoint for the control group.

Treated animals received LY110140 in vehicle (deionized water) at daily doses of 10 and 30 mg/kg/day. Controls were given vehicle orally by gavage. At approximately 2 hours after the last dose on Days 55, 70 or 91 pp, and on Day 181 (recovery phase) rats were euthanized by decapitation and blood was collected for hormone analysis. The reproductive tracts of these animals were examined (organ weights and gross and histopathology).

Results

Treatment-related deaths were observed in the main and recovery phase animals in the 30 mg/kg/day dose group.

Among main and recovery phase males there was a mortality rate of 2/60, 0/60 and 4/60 for the control, 10 and 30 mg/kg/day groups, respectively. Two of the four males at 30 mg/kg/day were euthanized in poor condition on Day 86 or 89 pp and the other 2 males were found dead on Day 36 or 43 pp. In the toxicokinetic phase 0, 1 and 3 males at 0, 10 and 30 mg/kg/day, respectively died during or following a bleed at either the Day 55 pp or Day 91 pp and two more toxicokinetic males at 30 mg/kg/day were found dead on Day 41 pp. The poor condition of the males in the 30 mg/kg/day may have been a contributory factor in the deaths in this group. Interpretation of mortality in the satellite group of toxicokinetic animals is confounded by increased stress associated with different handling conditions (i.e., repeated blood collections) of these animals.

Table 2. Study 901144: clinical signs

Summary of Clinical Signs in Males from Main and Recovery Subsets for Days 21 to 55 pp, Days 21 to 70 pp and Days 21 to 91 pp Considered to be Treatment Related

Clinical Signs	Males - Main (Recovery) Group 3 - 30 mg/kg/day		
	Day 21 to 55 pp	Day 21 to 70 pp	Day 21 to 91 pp
Thin	0/10 (0/10)	0/10 (1/10)	4/10 (6/10)
Fur ungroomed	0/10 (2/10)	0/10 (0/10)	5/10 (6/10)
Hypersensitive	0/10 (0/10)	1/10 (2/10)	4/10 (6/10)

Hypersensitivity, thinness and ungroomed fur were typically noted at the last week of dosing and/or recovery in the 30 mg/kg/day males from the Day 21 to 91 pp subset. One previously unreported finding of this study was an increased incidence and severity of skin lesions (redness, swelling, scabbing) on the forepaws, forelimb, digit and/or tail of animals in the 30/kg/day group. In the more severely affected animals, additional veterinary observations included severed and or/necrotic digits. In 3 of the 10 animals from the Day 21-91 pp group, some of the cutaneous findings were considered to be a result of self-mutilation due to the presence of lesions along the tail and necrotic digits and animals observed licking/chewing affected forepaws. The reported self-mutilation was observed on day 80 pp but had improved somewhat prior to the scheduled euthanasia or by Day 92 pp. The MAH stated that there were a number of references to various animal models of self-injury which usually occurs secondarily to a primary insult or event such as the display of stereotypy and can be observed under circumstances of overall poor health, severe environmental restriction and severe weight reduction in rodents. The relationship of rodent data to the complexities of the human behavioural disorder or even primate behaviour, in general, is not straightforward. SSRIs such as fluoxetine have, in fact, shown some efficacy in treatment of self-injurious behaviour in dogs, primates, and humans. Other than severe weight reduction and overall poor health, changes in pain or tactile sensation have not been associated with fluoxetine administration. The MAH concluded that self-mutilation observed in the present study could be associated with the severe reduction in body weights and overall poor health of the high-dose animals.

Assessor's comments

The poor condition of the animals noted at the last week of dosing and/or recovery for the Day 21 to 91 pp subset, indicated that the maximum tolerated dose had been achieved following more than 49 days of exposure to 30 mg/kg/day fluoxetine.

The self-mutilation observed at the top dose group was not reported in the earlier study by Beck (2004), or in the neurohormonal study by Adamo-Trigiani (2007); however, comparison of the systemic exposures at the 30mg/kg dose achieved in each study showed that the highest exposures were attained in the testicular pathology study:
AUC of 14.16 μg•hr/mL at PND 91 in the Beck, 2004 study;
AUC of 22.4 μg•hr/mL at PND 90 in the testicular pathology study;
AUC of 12.9 μg•hr/mL at PND 61 in the neurohormonal study
The MAH has concluded that the self-mutilation is caused by low body weight and overall poor health. This is considered to be unlikely. Self-mutilation is known to be an effect of supra-pharmacological doses of compounds affecting the central nervous system. The self-mutilation is more likely to be an exaggerated pharmacological response to the high systemic exposures and probably high body burden due to bioaccumulation, as evidenced by the non-linear pharmacokinetic profile. The other reported effects (e.g. increased mortality, severe decreases in body weight and body weight gain and other overt signs of toxicity [i.e. hypersensitivity, thinness, and ungroomed fur]) at this dose were also probably a reflection of this exaggerated pharmacological effect.

Table 3. Study 901144: body weight

Summary of Body Weight Gains: Percent Change in Body Weight Gains for Males Treated with LY110140 Compared to Controls

Subset	Percent Change for Overall Body Weight Gain in Males Group 3 - 30 mg/kg/day		
	Day 21 to 52 pp (Day 56-180pp)	Day 21 to 66 pp (Day 70 to 180 pp)	Day 21 to 87 pp (Day 91-180 pp)
Animals Dosed Day 21 to 55 pp	-23.2% (17.4%)	-	-
Animals Dosed Day 21 to 70 pp	-	-16.0% (35.8%)	-
Animals Dosed Day 21 to 91 pp	-	-	-35.2% (116.6%)

For males at 30 mg/kg/day, lower body weight gains were most pronounced between Days 21 and 23 pp and continued to a lesser extent throughout the treatment period, resulting in lower body weights. By Day 91 pp, some individual animals in the high dose group experienced weight decreases of up to 45% relative to the control mean value. Body weights were comparable to controls by recovery Day 180 pp.

Assessor's comments
A decrease in body weight gain was observed in the 30 mg/kg/day treated group. This was reversible following arrest of the treatment and is consistent with the known pharmacological effect of fluoxetine on food consumption (Halford JC et al. Serotoninergic drugs: effects on appetite expression and use for the treatment of obesity. *Drugs* **67**(1), 27-55.)

With regard to the **hormone level determinations** the MAH reported some deviations from the study protocol[*], but these are not considered to have affected the study outcome. Treatment with 10 or 30 mg/kg had no effect on circulating levels of **LH, FSH and testosterone** examined in rats on **days 55, 70 and 91 pp** or at the end of the recovery period. There was a significant decrease in LH levels in 181 day old males that had previously been given 30 mg/kg until day 70 pp. However, the MAH stated that the lower level of LH was not associated with changes in other hormones measured; nor was it observed at any other time point evaluated, and the MAH regarded the change as a likely result of the pulsatile nature of basal hormone secretion due to the overlapping of LH levels in this group with the levels in control animals. Overall it was concluded that Fluoxetine hydrochloride had no effect on circulating levels of LH, FSH and testosterone. A treatment related, statistically significant decrease in serum **inhibin B** levels was observed in 91 day old rats. However the MAH concluded that the changes in serum inhibin B levels were of no biological significance because the changes were not accompanied by an effect on FSH (the levels of which are regulated by inhibin B) as shown by the group and individual animal data. The inhibin B levels returned to control values by the end of the recovery period.

Organ weights typically reflected the marked differences in body weight at termination (Day 55 or 70 or 91 pp), except for lower testes weights at recovery (Day 181 pp) for the 30 mg/kg/day subsets dosed from Days 21 to 91 pp.

[*] The light in the housing area of the Day 21-55 rats was not switched off on Day 54 which might have compromised the hormone assay results because of the effect of light cycle interruptions, the animals were swapped with those in the Day 21-90 pp group. This enabled a sufficient number of animals for the analysis of hormone status after 55 days and allowed the "affected" animals re-allocated to the 21-90 pp group sufficient time for effects of the light cycle to pass before study termination. It is agreed that the data from the affected groups is unlikely to have been compromised

Table 4. Study 901144: histopathology

Summary of Histopathology Changes in Males from Main and Recovery Subsets for Days 21 to 55 pp, Days 21 to 70 pp and Days 21 to 91 pp

Histopathology	Males - Main (Recovery) Group 3 - 30 mg/kg/day		
	Day 21 to 55 pp	Day 21 to 70 pp	Day 21 to 91 pp
Testis, right: degeneration of the seminferous epithelium	0/10 (0/10)	3/9 (0/10)	7/9 (5/9)
Testis, left: degeneration of the seminferous epithelium	0/10 (0/10)	3/9 (2/10)	7/9 (8/9)
Epididymides: epithelial vacuolation	9/9 (2/10)	9/9 (1/10)	9/9 (2/9)
Epididymides: degeneration/sloughing with necrosis).	0/10 (0/10)	3/9 (0/10)	7/9 (0/9)

There were no treatment-related effects on organ weights or histopathologic changes at 10 mg/kg/day.

Macroscopic examination revealed treatment-related pathology characterised as mass or nodule in the epididymides, small epididymides, prostate, seminal vesicles and testes, soft testes and dark area, scab and ulceration of the skin in some of the animals treated with 30mg/kg up to day 91 PP. At the end of the recovery period (i.e. day 181), treatment-related changes caused by administration from Day 21 to Day 70, or 91 pp were seen infrequently and included raised area, mass, and enlargement of the epididymides and small, soft and pale area in the testes. Therefore, whilst lessened, the adverse effects were still evident. Microscopic examination of the testes, epididymides, prostate and seminal vesicles from the 30mg/kg/day group also revealed lesions, the severity (in terms of frequency and extent of damage) of which was associated with the duration of exposure to Fluoxetine (i.e., lesions more severe in animals treated up to Day 91 pp compared to those observed in animals treated for a shorter duration) as seen in the previous study by Beck 2004, and which were irreversible.

The seminiferous epithelium in the **testes** of animals treated up to day 70 and day 90 pp showed degeneration. These animals also had epithelial vacuolation and degeneration/sloughing with necrosis in the **epididymis**. Affected tubules in day 70 rats had loss of germ and/or Sertoli cells. Adverse effects in Sertoli cells (vacuolation) were previously reported in the Beck (2004) study. Lesions were still present in the epididymides and/or testes at the end of the recovery period (Day 181 pp) in animals that had been treated for up to days 70 or 91, whilst the animals treated up to day 55 only had lesions in the epididymis. The testicular changes recorded in the recovery rats treated up to Day 91 pp correlated with decreases in the testicular absolute and relative (to body and brain) weights. There were some signs of improvement at the end of the recovery period (i.e. reduction in the number of affected tubules of the seminiferous epithelium) but the morphology of the remaining affected tubules showed extensive loss of the germinal epithelium and was indicative of an irreversible injury. These effects therefore showed partial irreversibility with the most severely affected tubules progressing to irreversible injury.

Assessor's comments

The same range and type of histopathological findings previously reported in the Beck 2004 study (WIL-353039) was observed in the present study, indicating clear reproducibility of the effects of fluoxetine hydrochloride on male reproductive organs. As the present study included treatment periods of increasing duration (e.g. day 21 to 55 pp ; day 21 to 71 pp and day 21 to 91 pp, instead of day 21 to day 91 pp in the previous study), it could be concluded that lesions tended to be more pronounced with

increased duration of treatment. Changes were still observed in the epididymides and testes, along with lower testes weights, at the end of the recovery period following treatment with 30 mg/kg/day for 91 days. Data indicated irreversibility of the effects at the end of the recovery period spanning from Day 90 to 126, corresponding to approximately 2 to 3 spermatogenesis cycle. It could be concluded that spermatogenesis was ineffective to reverse the effects.

Although there was a statistically significant decrease in the LH levels at the end of the recovery period of the 30 mg/kg animals, statistical changes were not reported during the treatment period, when denegation of the male gonads would have been in progress so there was no evidence to support its involvement in the aetiology of this effect. Furthermore no statistically significant effect was reported with the other hormones investigated with the exception of inhibin B. As fluoxetine had no effect on plasma levels of hormones regulated by GnRH (e.g. FSH and LH) this indicates that a suppression of GnRH release due to the excessive stimulation of serotonin release is not the mechanism by which fluoxetine induced testicular toxicity and delays in sexual maturation. However the data does not rule out the possibility of a local pharmacodynamic action for fluoxetine, especially because it has been shown that serotonin exerts local effects on male sexual organs in the rat (Jimenez-Trejo F *et al*. Serotonin concentration, synthesis, cell origin, and targets in the rat caput epididymis during sexual maturation and variation associated with adult mating status: morphological and biochemical studies. *Journal of Andrology*. **28** (1), 136-149, 2007) or in the hamster (Frungieri MB *et al* Serotonin in golden hamster testes: testicular levels, immunolocalization and role during sexual development and photoperiodic regression-recrudescence transition. *Reproductive Neuroendocrinology*. **69**, 299-308, 1999).

Although the statistically significant change in inhibin B levels did not appear to have any physiological significance because of the absence of corresponding changes in FSH plasma levels, it is likely that this finding is a marker of the testicular pathology and an indicator of a localised mechanism of action.

The Sertoli cells responsible for the synthesis of inhibin B provide nutrients to the developing spermatids (and other surrounding cells); therefore, loss/damage of this cell population, as evidenced in the Beck study and replicated in the present study, would naturally be reflected in the levels of inhibin B relative to control, and would have consequential effects on the survival of the cell populations being supported by this vital cell line. This would be a possible explanation for the widespread necrosis and apoptosis reported. Treatment related changes in plasma inhibin B levels were also reported in the neurohormonal study (as discussed below) so this effect is reproducible. The testicular effects only occurred at systemic exposures that were associated with exaggerated pharmacological effects, and it is likely that this was also a factor in the aetiology of the testicular pathology.

The systemic exposure to fluoxetine and norfluoxetine at 10mg/kg/day was not associated with any adverse effects in the testes in the three studies conducted to date; therefore, it can be regarded as the NOAEL for these findings

Overall the findings of the study do not support a GnRH mediated affect, therefore the potential for fluoxetine to be inducing toxicity through a localised mechanism of action in the male reproductive organs needs to be considered, and the changes in inhibin B plasma levels might be an indicator of a local effect.

The MAH will be requested to investigate possible mechanisms leading to the epithelial vacuolation and necrosis in the male reproductive tract taking these observations into account during their investigations.

The mechanism of toxicity remains unknown.

Toxicokinetics

Table 5. Study 901144: Systemic exposure (AUC0-t) to LY110140 and its metabolite (norfluoxetine) and peak plasma concentration (Cmax):

	Parameter	Administered Fluoxetine Dose			
		10 mg/kg/day		30 mg/kg/day	
		Fluoxetine	Norfluoxetine	Fluoxetine	Norfluoxetine
Day 55 pp	C_{max} (ng/mL)	260	599	829	2820
	AUC_{0-t} (µg•hr/mL)	2.42	10.6	13.6	51.1
Day 91 pp	C_{max} (ng/mL)	395	513	988	3940
	AUC_{0-t} (µg•hr/mL)	2.96	10.1	22.4	85.8

Abbreviations: AUC_{0-t} = the area under the plasma concentration-time curve from 0 hours to time (t) where t is the time of the last quantifiable time point, C_{max} = maximum observed plasma concentration.

The exposure (Cmax and AUC0-t) to fluoxetine and norfluoxetine increased with increasing fluoxetine dose. The Cmax values for fluoxetine increased proportionally to the administered fluoxetine dose on Day 55 and 91 pp. However, AUC0-t values for fluoxetine as well as exposure values for norfluoxetine (Cmax and AUC0-t) increased disproportionately with increasing fluoxetine dose.

Assessor's comments

Compared to the first study performed in the juvenile rat (study WIL-353039, Beck, 2004), same levels of exposure were achieved, except at day 91 post partum (corresponding to 70 days of treatment) where higher exposures were achieved in WIL-353039 study for animals treated with 10 mg/kg fluoxetine) (please refer to annex 2). In a similar manner, exposure to norfluoxetine is higher than fluoxetine. Accumulation following time is observed, mostly for norfluoxetine.

According to previous assessments, approximate children exposure was 3.0 µg•hr/mL for fluoxetine and 3.6 µg•hr/mL for norfluoxetine following single oral administration of 20 mg fluoxetine (children aged 8 to 15 years).

4. STUDY 901143 "A NEUROHORMONAL STUDY OF LY110140 ADMINISTERED BY ORAL GAVAGE IN YOUNG RATS". Charles River Laboratories. Date of the report: September 2007.

The purpose of this study was to evaluate the potential effects of LY110140 on the hypothalamic-pituitary-gonadal (HPG) axis when administered daily by oral gavage to weanling and juvenile rats during the peripubertal period for up to 41 days from Day 21 pp up to Day 61 pp, inclusive.

Study design

Table 6. Study 901143: Design

Group No. Identification	Dose Level[a] mg/kg/day	Number of Males - Dosing Schedule			
		Day 21 to 28 *pp*	Day 21 to 40 *pp*	Day 21 to 50 *pp*	Day 21 to 61 *pp*
1/ Vehicle Control	0	10	10	10	10
2/ Low LY110140	10	10	10	10	10
3/ High LY110140	30	10	10	10	10

a Dose Volume = 5 mL/kg
pp = post partum

Group No. Identification	Dose Level[a] (mg/kg/day)	Number of Females - Dosing Schedule					
		Day 21 to 28 *pp*	Day 21 to 30 *pp*	Day 21 to 33 *pp*	Day 21 to 35 *pp*	Day 21 to 44 *pp*	Day 21 to 50 *pp*
1/ Vehicle Control	0	10	10	10	10	10	10
2/ Low LY110140	10	10	10	10	10	10	10
3/ High LY110140	30	10	10	10	10	10	10

a Dose Volume = 5 mL/kg
pp = post partum

Group No. Identification	Dose Level[a] (mg/kg/day)	Dosing Schedule **Toxicokinetic Subgroup**			
		Males*		Females*	
		Day 21 to 28[b]	Day 21 to 61[b]	Day 21 to 28[b]	Day 21 to 50[b]
1/ Vehicle Control	0	3	3	3	3
2/ Low LY110140	10	24	24	24	24
3/ High LY110140	30	24	24	24	24

a Dose Volume = 5 mL/kg
* Males were bled on Days 28 and 61 *post partum* and females were bled on Day 28 and 50 for toxicokinetic evaluation. Three pairs of pups/sex from each group were bled via the abdominal aorta at predose, 0.5, 1, 2, 4, 6, 10 and 24 hours post dose for theLY110140 treated groups and at 2 hours for the control group.
b *pp = post partum*

Males were evaluated for preputial separation and females were evaluated for vaginal opening only on their respective day of termination for each group. Treated animals received LY110140 in vehicle (deionized water) at daily doses of 10 and 30 mg/kg/day. Controls (10 rats/sex/subset) were given vehicle orally by gavage. At approximately 2 hours after the last dose, rats were euthanized by decapitation and blood was collected for hormone analysis.

Results

a) Males

There were no treatment-related effects on survival, clinical signs, body weight and body weight gain in the 10mg/kg group. However treatment with 30mg/kg day was associated with clear signs of systemic toxicity the severity of which worsened with increasing duration of exposure. **Lower body weight** and **body weight gain** compared to controls (statistical significance unknown) were observed from Day 23 pp onwards and persisted throughout the rest of the dosing period in all subsets. Animals treated for a minimum of 40 days and above experienced an increased incidence of salivation, fur staining, and wet fur of the muzzle and/or lower jaw. Survival was not affected in this study. Self-mutilation reported in the testicular pathology study was not observed in this study. Nevertheless these findings are indicative of an exceedance of the maximum tolerated dose and an exaggerated pharmacological response,

Table 7. Study 901143: body weights

Summary of Body Weight Gains: Percent Change in Body Weight Gains for Males Treated with LY110140 Compared to Controls

Subset	Males Group 3 - 30 mg/kg/day			
	Overall Body Weight Gain - Day 21 to 27 pp	Overall Body Weight Gain - Day 21 to 39 pp	Overall Body Weight Gain - Day 21 to 49 pp	Overall Body Weight Gain - Day 21 to 60 pp
Animals Dosed Day 21 to 28 pp	-38%	-	-	-
Animals Dosed Day 21 to 40 pp	-	-14%	-	-
Animals Dosed Day 21 to 50 pp	-	-	-16%	-
Animals Dosed Day 21 to 61 pp	-	-		-17%

A delay in **sexual maturation** was not observed at the doses tested throughout the duration of the study (table 5) which is in contrast to the delayed sexual maturation previously reported at 10 mg/kg/day in rats.

Table 8. Study 901143: Male physical development

Summary of Male Physical Development

Group No. Identification	Day Post Partum No. of Pups with Preputial Separation/Subset			
	28 (Day 21 to 28 pp)	40 (Day 21 to 40 pp)	50 (Day 21 to 50 pp)	61 (Day 21 to 61 pp)
Group 1 - Vehicle Control	0/10	0/10	8/10	10/10
Group 2 - 10 mg/kg/day	0/10	0/10	10/10	10/10
Group 3 - 30 mg/kg/day	0/10	0/10	6/10	10/10

Treatment with 10 and 30mg/kg fluoxetine hydrochloride up to Days 28, 40, 50 and 61 post partum (i.e. the period covering pre-pubescence to sexual maturity) had **no effect on circulating levels of follicle stimulating hormone, luteinising hormone, prolactin and testosterone**.

There was a statistically significant change in the levels of circulating **inhibin B** but the changes were not temporal- or dose-related. There was a significant **decrease** in inhibin B in animals treated with 10mg/kg for 28 days, but no effect in animals treated up to days 40 and 50 pp. Conversely treatment with 30mg/kg was associated with an **increase** in circulating inhibin B levels. The MAH notes that these alterations in inhibin B plasma levels did not appear to be physiologically significant. Inhibin B plays a physiological role in the feedback control of FSH secretion (it is secreted by Sertoli cells in response to follicle stimulating hormone (FSH) and then in turn exerts an inhibitory effect on FSH

production) yet alterations in the plasma concentrations of inhibin B were not accompanied with a corresponding change in circulating FSH levels as shown by the group and individual animal data (no trend showing males with high inhibin B levels having low FSH plasma levels).

Table 9. Study 901143: circulating inhibin B levels from male rats

Male: Inhibin B					
	Statistic	Animal Age at Collection			
		28	40	50	61
Control	Mean	185.48	116.36	105.07	107.76
	SEM	16.848	10.169	9.581	6.900
	N	10	10	10	10
10 mg/kg LY110140	Mean	121.94	145.04	84.07	114.12
	SEM	12.723	25.421	11.498	8.447
	N	10	10	9	10
	p value	0.0287†	0.6810†	0.2070	0.5948†
30 mg/kg LY110140	Mean	209.79	134.55	101.15	145.52
	SEM	27.036	18.658	5.985	13.846
	N	9	10	10	10
	p value	0.9888†	0.9049†	0.9334	0.0365†
† Dunnett's t-test conducted on Rank Average transformed data.					

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Table 10. Comparison of the systemic exposures achieved in the **three juvenile toxicity studies** conducted to date in juvenile male CD rats (pharmacokinetic data = peak plasma concentrations [C_{max}] and area under the concentration curve [AUC]).

Analyte	**Parameter**	**Juvenile toxicity study** (Beck 2004)	**Neurohormonal study** (Adamo-Trigiani, 2007)		**Testicular pathogenesis study** (Adamo-Trigiani, 2007)	
		PND 90	**PND 28**	**PND 61**	**PND 55**	**PND 91**
Fluoxetine						
10mg/kg/day	AUC $_{0-\tau}$ (µg•hr/mL)	6.71	2.54	2.09	2.42	2.96
	C_{max} (ng/mL)	1290	253	256	260	395
30mg/kg/day	AUC $_{0-\tau}$ (µg•hr/mL)	14.16	14.9	12.9	13.6	22.4
	C_{max} (ng/mL)	1223	855	734	829	988
Norfluoxetine						
10mg/kg/day	AUC $_{0-\tau}$ (µg•hr/mL)	28.27	9.36	10.5	10.6	10.1
	C_{max} (ng/mL)	3617	476	556	599	513
30mg/kg/day	AUC $_{0-\tau}$ (µg•hr/mL)	64.23	39.5	50.5	51.1	85.8
	C_{max} (ng/mL)	3792	2010	2360	2820	3940

Assessor's comments

Concerns were raised regarding the lack of a NOAEL (and thus lack of a safety margin) for delayed sexual maturation in males treated with 10mg/kg/day fluoxetine hydrochloride (AUC of 6.713 µg•hr/mL in the Beck 2004 study). However, a delay in male sexual maturation was not observed in the neurohormonal study at the same dose of 10mg/kg/day, and even at the higher dose of 30mg/kg/day. The systemic exposure following treatment with 10mg/kg/day up to day 61pp was 56% lower in the neurohormonal study compared to the exposures recorded in the Beck (2004) study following treatment up to day 91 pp (2.09 µg•hr/mL versus 6.7 µg•hr/mL), which might explain the absence of the delayed sexual maturation at this dose level.

As discussed earlier, there was a statistically significant change in inhibin B levels. This could be regarded as a marker for effects in Sertoli cells which are responsible for the synthesis of this hormone. Pathology was not assessed in this study so effects in this cell population are unknown. As

delayed sexual maturation was not observed in this study, although there did appear to be a slight lag in the onset of preputial separation at day 50 pp in animals treated with 30 mg/kg, an association between the inhibin B levels and delayed sexual maturation in the Beck study cannot be established. As there were no effects on the levels of the other hormones investigated it has to be tentatively considered that fluoxetine had no physiologically relevant effect on endocrine function in male rats at all doses tested; therefore there is no evidence to support the theory that the aetiology of the previously observed delayed male sexual maturation involved a suppression of hypothalamic GnRH secretion.

b) Females

There was a *statistically significant **delay in sexual maturation at 30 mg/kg.** Onset of vaginal opening was observed from day 28 pp in the controls and was completed by day 35pp, but was only evident in the 30mg/kg group from Day 44 pp. Female rats in the 10 mg/kg/day group appeared to lag slightly behind the control group in reaching sexual maturation however this was not a statistically significant finding. (*only statistically significant in the Day 21 to 33 pp and Day 21 to 35 pp subsets).

Table 11. Study 901143: Female sexual development

Summary of Female Physical Development

Group No. Identification	Day Post Partum No. of Pups with Vaginal Opening/Subset					
	28 (Day 21 to 28 pp)	30 (Day 21 to 30 pp)	33 (Day 21 to 33 pp)	35 (Day 21 to 35 pp)	44 (Day 21 to 44 pp)	50 (Day 21 to 50 pp)
Group 1 - Vehicle Control	1/10	4/10	9/10	10/10	8/10	10/10
Group 2 - 10 mg/kg/day	0/10	2/10	5/10	6/10	10/10	10/10
Group 3 - 30 mg/kg/day	0/10	0/10	0/10*	0/10*	7/10	8/10

* Significantly different ($p \leq 0.05$) from controls

A reduction in **body weight and body weight gain** (statistical significance unknown) was observed in the 30 mg/kg/day group.

Table 12. Study 901143: Summary of female bodyweight gains compared to controls

Summary of Body Weight Gains: Percent Change in Body Weight Gains for Females Treated with LY110140 Compared to Controls

Subset	Females Group 3 - 30 mg/kg/day					
	Overall Body Weight Gain - Day 21 to 27 pp	Overall Body Weight Gain - Day 21 to 29 pp	Overall Body Weight Gain - Day 21 to 32 pp	Overall Body Weight Gain - Day 21 to 34 pp	Overall Body Weight Gain - Day 21 to 43 pp	Overall Body Weight Gain - Day 21 to 49 pp
Animals Dosed Day 21 to 28 pp	-49%	-	-	-	-	-
Animals Dosed Day 21 to 30 pp	-	-33%	-	-	-	-
Animals Dosed Day 21 to 33 pp	-	-	-27%	-	-	-
Animals Dosed Day 21 to 35 pp	-	-	-	-24%	-	-
Animals Dosed Day 21 to 44 pp	-	-	-	-	-6%	-
Animals Dosed Day 21 to 50 pp	-	-	-	-	-	-18%

Statistically significant changes in **FSH** levels were seen in the 10 mg/kg dose group compared to control from day 22. In the 30mg/kg group statistically significant changes in **LH** and **FSH** levels were evident on day 35. The MAH did not consider these changes to be physiologically significant as

the levels in the treated rats overlapped the levels in control animals which reflected the pulsatile nature of basal hormone secretions.

Table 13. Study 901143: FSH levels in female rats

Female: FSH							
	Statistic	Animal Age at Collection					
		28	30	33	35	44	50
Control	Mean	2.94	2.98	3.00	2.60	4.08	3.00
	SEM	0.401	0.660	0.259	0.225	0.599	0.419
	N	9	10	10	10	10	10
10 mg/kg LY110140	Mean	5.22	3.29	4.97	5.36	3.40	2.81
	SEM	0.423	0.351	0.929	1.272	0.538	0.390
	N	10	10	10	10	10	10
	p value	0.0034	0.1507†	0.3057†	0.0567†	0.6805†	0.8865†
30 mg/kg LY110140	Mean	4.16	3.10	2.80	4.23	3.18	3.40
	SEM	0.530	0.330	0.258	0.499	0.622	0.318
	N	10	10	10	10	10	10
	p value	0.1282	0.3073†	0.7888†	0.0158†	0.2853†	0.5465†

† Dunnett's t-test conducted on Rank Average transformed data.

Progesterone and **estradiol** levels reflected the maturational state of the animals. The levels of the two hormones were comparatively lower in the 30mg/kg group compared to control and the animals treated at the lower dose of 10mg/kg and this was attributed to the sexually immature state of the animals at the top dose and was evidence of the delayed development of the ovarian structure responsible for the synthesis and release of these hormones.

There were no treatment related effects on serum **prolactin**. Furthermore, basal levels of prolactin were seen in the control and treated groups indicating that the rats were not in a state of stress at the time of blood sample collection. **Inhibin A** levels in the 10 mg/kg fluoxetine hydrochloride dose group were statistically different from control levels at Day 50 pp. The MAH stated that a closer examination of individual animal data showed that the difference in inhibin A levels between the control and 10 mg/kg/day dose group was likely to be related to the distribution of animals to the different phases of the oestrous cycle with more rats in the 10 mg/kg fluoxetine hydrochloride dose group in the follicular phase than controls, and **not** related to treatment with fluoxetine hydrochloride.

By Day 44 pp, the majority of female rats in the 30 mg/kg group were sexually mature. No differences in serum levels of LH, FSH, PRL, estradiol and progesterone were noted between control and fluoxetine treated rats at 44 or 50 days pp, reflecting the comparable maturational state of all the treatment groups. Inhibin A levels in the 10 mg/kg dose group were statistically different from control levels at Day 50 pp. The MAH stated that a closer examination of individual hormone data showed evidence that the difference in inhibin A between the control and 10 mg/kg dose group, was most likely to be related to the distribution of animals to the different phases of the oestrous cycle with more rats in the 10 mg/kg dose group in the follicular phase than controls and not related to treatment with fluoxetine. Overall it was concluded that fluoxetine had no physiologically relevant effect on the HPG axis.

Table 14. Comparison of the systemic exposures achieved in the **two juvenile toxicity studies** conducted to date in juvenile female CD rats (pharmacokinetic data = peak plasma concentrations [C_{max}] and area under the concentration curve [AUC]).

Analyte	**Parameter**	**Juvenile toxicity study** (Beck 2004)	**Neurohormonal study** (Adamo-Trigiani, 2007)	
		PND 90	**PND 28**	**PND 50**
Fluoxetine				
10mg/kg/day	AUC $_{0-\tau}$ (µg•hr/mL)	5.13	3.26	3.06
	C_{max} (ng/mL)	568	328	362
30mg/kg/day	AUC $_{0-\tau}$ (µg•hr/mL)	25.36	16.7	16.8
	C_{max} (ng/mL)	1348	893	935
Norfluoxetine				
10mg/kg/day	AUC $_{0-\tau}$ (µg•hr/mL)	25.08	11.0	13.3
	C_{max} (ng/mL)	1476	621	645
30mg/kg/day	AUC $_{0-\tau}$ (µg•hr/mL)	72.71	44.2	44.7
	C_{max} (ng/mL)	4223	2030	2050

Toxicokinetics

Table 15. Study 901143: toxicokinetics

Values for the peak plasma concentration (C_{max}) and the area under the concentration curve (AUC_{0-t}) are shown in the following table.

	Parameter	Administered Fluoxetine Dose			
		10 mg/kg/day		30 mg/kg/day	
		Fluoxetine	Norfluoxetine	Fluoxetine	Norfluoxetine
Female					
Day 28 pp					
	AUC_{0-t} (µg•hr/mL)	3.26	11.0	16.7	44.2
	C_{max} (ng/mL)	328	621	893	2030
Day 50 pp					
	AUC_{0-t} (µg•hr/mL)	3.06	13.3	16.8	44.7
	C_{max} (ng/mL)	362	645	935	2050
Male					
Day 28 pp					
	AUC_{0-t} (µg•hr/mL)	2.54	9.36	14.9	39.5
	C_{max} (ng/mL)	253	476	855	2010
Day 61 pp					
	AUC_{0-t} (µg•hr/mL)	2.09	10.5	12.9	50.5
	C_{max} (ng/mL)	256	556	734	2360

Abbreviations: AUC_{0-t} = the area under the plasma concentration-time curve from 0 hours to time (t) where t is the time of the last quantifiable time point, C_{max} = maximum observed plasma concentration.

Assessor's comments

A delay in sexual maturation in female rats was observed at 30 mg/kg, and there was evidence of this effect at 10mg/kg, although not statistically significant. The Beck (2004) study also showed treatment-related immaturity of the female reproductive tract at 30mg/kg/day, delayed onset of vaginal patency, decreased numbers of corpora lutea, lower organ weights; and indications of delayed sexual maturity at 10mg/kg (the treated group had higher mean body weights at the onset of vaginal patency compared

to controls although there was no statistically significant effect on age of onset). In both cases, these effects were accompanied by a notable reduction in bodyweight and bodyweight gain (although the statistical significance is unknown). Comparison of the male and female body weight analysis shows that the reductions in bodyweight gain was more severe in the females than the males, also the systemic exposure of fluoxetine in female rats at 10 and 30 mg/kg after 29 days exposure was higher than that measured in the male rats (see tables 11 and 15). These differences might partly account for the delayed sexual maturation observed in females but absent in the males in the present study.

With regard to the statistically significant increase in inhibin A levels at 10 mg/kg at Day 50 pp, the MAH stated that a closer examination of individual hormone data showed evidence that the difference in inhibin A between the control and 10 mg/kg dose group, was most likely related to the distribution of animals to the different phases of the oestrous cycle with more rats in the 10 mg/kg dose group in the follicular phase than controls and not related to treatment with fluoxetine. **However the MAH did not address the possibility that fluoxetine might have been responsible for the distribution of the animals to the different phases through potential effects on the cycle. The MAH will be requested to discuss this finding further**.

The MAH's explanation for the statistically significant changes in estradiol and progesterone is plausible. However, the data on plasma levels of FSH and LH over time does not provide adequate evidence to rule out an effect of fluoxetine on hypothalamic GnRH secretion because it only shows the concentrations at set time points, not the full profile of hormone release. Therefore if fluoxetine did cause a dose-related effect on the height of the peak or at the trough of each pulse, then it would not necessarily be reflected in the table presented. **The MAH will need to provide a profile of FSH and LH release over the duration of treatment for each dose level to determine whether there was a dose-related trend.**

5. ASSESSOR'S OVERALL CONCLUSION

Two studies were performed in the juvenile rat in order to investigate delays in sexual maturation and testicular toxicity induced by exposure to fluoxetine hydrochloride in a previous study in juvenile rats carried out by Beck 2004 (study WIL-353039, Beck, September 2004), and to determine if these effects were due to suppression of GnRH release mediated by the pharmacological action of fluoxetine on serotonin release. To investigate the mechanism for delays in male and female sexual maturation and testicular pathology an analysis of the effect of treatment over the period of pre-pubescence through to sexual maturation on (a) plasma levels of gonadotrophin hormones (e.g. FSH, LH), (b) onset of sexual maturation, (c) histopathology (d) reversibility was conducted. In addition to confirming the previously observed results, the data derived from these two new studies provided the information on the male and female reproductive discussed below.

Female reproductive system

There was no evidence to clearly correlate the delay in sexual maturation observed in the 30mg/kg/day females with suppression of hypothalamic GnRH secretion/action. There was a statistically significant change in mean FSH and LH plasma levels relative to controls in the 30mg/kg group however the MAH stated that this was due to the pulsatile nature of hormone secretion (also observed in the controls) and therefore not of any physiological significance. However the MAH will need to provide a profile of FSH and LH release over the duration of treatment for each dose level to confirm that the change in hormone levels was not treatment related because the data presented on plasma levels of FSH and LH over time does not provide adequate evidence to rule out an effect of fluoxetine on hypothalamic GnRH secretion as it only shows the concentrations at set time points, not the full profile of hormone release. Therefore if fluoxetine did cause a dose-related effect on the height of the peak or at the trough of each pulse, then it would not necessarily be reflected in the table presented.

The only finding that appeared to correlate with the delayed onset of vaginal patency was body weight because delays in sexual maturation observed in males and females in the neurohormonal study and the study conducted be Beck only occurred when body weight gain was significantly affected; delays in sexual maturation were not reported in animals whose body weight gain was not affected. Therefore the mechanism for the delayed sexual maturation appeared to be related to the effects of fluoxetine on body weight gain. The information reported in the Beck 2004 study shows that the reduction in body weight gain was due to a reduction in food consumption. To confirm if reduced body weight gain is related to delayed sexual maturation, the MAH will be requested to provide a table correlating food consumption, bodyweight data and onset of sexual maturation on an individual animal basis.

Overall, the evidence presented does not indicate that the effects of fluoxetine on sexual maturation were mediated by suppression of hypothalamic GnRH secretion/action, although this needs to be confirmed by the MAH.

Male reproductive system

Testicular toxicity

Overall, the testicular pathology study confirmed fluoxetine hydrochloride to be a testicular toxin; however, it did not generate information that would clarify the mechanism of toxicity leading to the degenerative effects in the testes, epididymides, seminal vesicles and prostate. Beyond the awaited loss of weight linked to the pharmacological effect of fluoxetine, it can be considered that the MTD was achieved at the highest dose level after more than 49 days of exposure. Results indicated reproducibility of the findings previously observed on testes and epididymis (testes showed degeneration of the seminiferous epithelium, and the epididymides had epithelial vacuolation and degeneration/sloughing with necrosis) irreversible following 2 to 3 spermatogenesis cycles. It was shown in the previously submitted study that such effects led to functional alteration of the reproductive performances. As the present study included different treatment period (day 21 to 55 pp ; day 21 to 71 pp and day 21 to 91 pp, instead of day 21 to day 91 pp in the previous study), it could be concluded that lesions tended to be more pronounced with increased duration of treatment
The significant reductions in relative testicular weights, body weight and body weight gain might have played a role in the pathogenesis of the male reproductive tract but it is unlikely that this alone would lead to such a specific finding (irreversible testicular degeneration and necrosis, epididymal epithelial vacuolation). Analysis of the toxicokinetic data in the 30mg/kg/day animals treated up to Day 91 pp (from the testicular pathogenesis study) revealed a supraproportional increase in the serum levels of fluoxetine and its active metabolite norfluoxetine indicating that were was some bioaccumulation. Fluoxetine has a long elimination half-life and it is probable that the elimination pathway was overwhelmed by the high chronic exposures, hence the higher than expected systemic exposures in these animals. This higher body burden was associated with reversible and irreversible degenerative effects in the male reproductive tract and self-mutilation and is indicative of exaggerated pharmacology.

Fluoxetine did not appear to affect the GnRH mediated hormone release because there were no differences in the circulating levels of FSH, LH and testosterone between the treated animals and controls during the treatment period when signs of the testicular effects first appeared. The only hormone affected by treatment was inhibin B; treatment related changes in inhibin B were also observed in the neurohormonal study. The MAH did not consider this to be a physiologically relevant finding because the inhibin B levels did not lead to changes in serum FSH levels. The role of inhibin B has been investigated extensively and its physiological role is currently understood to be in the feedback control of FSH secretion. It is thought to be secreted by Sertoli cells in response to follicle stimulating hormone (FSH) and in turn to exert an inhibitory effect on FSH production. Therefore the effects on inhibin B were not considered to be of any physiological significance. However these changes might in fact be indicative of an effect on the Sertoli cells, and thus a potential marker of toxicity. The testicular pathology study and the Beck (2004) study both reported loss or damage of Sertoli cells, and considering the essential role played by the Sertoli cells in providing support to the

developing spermatids/spermatozoa, it is possible that effects on this cell line might be the trigger for, or at least play a part in, the pathogenesis in the male reproductive tract.

Local pharmacological effects of fluoxetine have been shown previously: Serotonin exerts local effects on male sexual organs in the rat (Jimenez-Trejo F *et al*). Serotonin concentration, synthesis, cell origin, and targets in the rat caput epididymis during sexual maturation and variation associated with adult mating status: morphological and biochemical studies. *Journal of Andrology*. **28** (1), 136-149, 2007) or in the hamster (Frungieri MB *et al* Serotonin in golden hamster testes: testicular levels, immunolocalization and role during sexual development and photoperiodic regression-recrudescence transition. *Reproductive Neuroendocrinology*. **69**, 299-308, 1999).

Overall, the evidence provided by the MAH (e.g. FSH and LH plasma levels) from the two new studies does not indicate that the toxicity of fluoxetine is mediated by an effect on GnRH activity (but does not completely rule this out either). Therefore, the possibility of a mechanism of toxicity occurring locally in the male reproductive tract should be further investigated. The fact that changes in inhibin B have occurred in two independant studies is evidence that it might be an important marker of toxicity, and its role (if any) in the pathogenesis of the testicular findings should be further investigated. The mechanism of toxicity of the degenerative testicular effects remains unknown which is a concern because it is an irreversible effect.

Delays in male sexual maturation

The delays in sexual maturation observed in the Beck study at 10mg/kg/day were not observed at the same dose (and same study conditions) in the neurohormonal study. Differences in the systemic exposures achieved at this dose might be the reason for this (Beck study = **AUC** of **6.71** μg•hr/mL and $\mathbf{C_{max}}$ of **1290** ng/mL at PND 90; Adamo-Trigiani neurohormonal study = **AUC** of **2.09** μg•hr/mL and $\mathbf{C_{max}}$ of **259** ng/mL); however, delays in sexual maturation were also not observed at the top dose where significantly higher systemic exposures were achieved. It is unclear why there should be such a disparity in the response to treatment between the Beck study and the recently conducted study by Adamo-Trigiani (2007).

The aim of the neurohormonal study was to establish if the delay in sexual maturation in male rats, for which there is no safety margin, was mediated by suppression of GnRH release; however, as a delay in sexual maturation did not occur at all doses tested in the recent study, it is not possible to determine whether an association exists. Fluoxetine hydrochloride did not modify FSH and LH serum levels in the males treated in this study. Delayed sexual maturation was observed at the top dose in the female rats but the effect did not correlate with the changes in plasma levels of FSH and LH during the treatment period (although the MAH needs to provide a hormone release profile to confirm this). It should be noted that the reduction in body weight and body weight gain throughout the treatment period at the top dose observed in the females was also reported in the males yet a delay in sexual maturation was not observed in males, however differences in the systemic exposure of fluoxetine achieved, and in the severity of the reduction in body weight gain between the two sexes might be partly responsible for the delayed sexual maturation in females and its absence in males. Nevertheless, the critical factors that led to the delayed sexual maturation in males (Beck, 2004) remain unclear.

Overall the neurohormonal study did not provide evidence of an association between delayed male sexual maturation and inhibition of GnRH release.

With regard to the patient population the previously calculated safety margin for delayed sexual maturation was between 0.8 to 8.8 for pre-adolescents at steady state, at the NOAEL of 10 mg/kg/day.

6. RECOMMENDATIONS

FURTHER RESEARCH

- In order to address potential specific effect of fluoxetine to the rat, mechanistic studies in the juvenile rat could be performed. For example, carrying out a study in the juvenile rat expose to norfluoxetine could provide data indicating whether toxicity is mediated by fluoxetine or norfluoxetine. As levels of norfluoxetine are higher in the rat than in the human, slightly higher safety margins could be achieved if norfluoxetine is demonstrated to be the active component of the testicular toxicity (*i.e.* safety margin of 3.6 to 16.3 based on norfluoxetine levels at steady state for preadolescents as previously reported, compared to 0.8-8.8 based on fluoxetine levels) (see annex 1). Performing a juvenile toxicity study in a second species could also allow addressing species specificity.

- Taken into account that SSRI, also sharing a common pharmacological activity, possess some specificity, such as a long plasmatic half life for fluoxetine, potential toxicological differences between fluoxetine compared to other SSRI should be address. Data derived from available juvenile toxicity studies performed in the rat with other SSRI should be reviewed.

SPC AMENDMENTS

The current SPC text reflects the findings in the Beck study. The text in section 5.3 of the SPC is as follows:

Current section 5.3 of Prozac SPC:

'There is no evidence of carcinogenicity or mutagenicity from in vitro or animal studies.

In a juvenile toxicology study in CD rats, administration of 30mg/kg/day of fluoxetine hydrochloride on postnatal days 21 to 90 resulted in irreversible testicular degeneration and necrosis, epididymal epithelial vacuolation, immaturity and inactivity of the female reproductive tract and decreased fertility. Delays in sexual maturation occurred in males (10 and 30mg/kg/day) and females (30mg/kg/day). The significance of these findings in humans is unknown. Rats administered 30mg/kg also had decreased femur lengths compared with controls and skeletal muscle degeneration, necrosis and regeneration. At 10mg/kg/day, plasma levels achieved in animals were approximately 0.8 to 8.8-fold (fluoxetine) and 3.6 to 23.2-fold (norfluoxetine) those usually observed in paediatric patients. At 3mg/kg/day, plasma levels achieved in animals were approximately 0.04 to 0.5-fold (fluoxetine) and 0.3 to 2.1-fold (norfluoxetine) those usually achieved in paediatric patients.

A study in juvenile mice has indicated that inhibition of the serotonin transporter prevents the accrual of bone formation. This finding would appear to be supported by clinical findings. The reversibility of this effect has not been established.

Another study in juvenile mice (treated on postnatal days 4 to 21) has demonstrated that inhibition of the serotonin transporter had long lasting effects on the behaviour of the mice. There is no information on whether the effect was reversible. The clinical relevance of this finding has not been established'

Assessor's comments.
The reported self-injury appears to be an exaggerated pharmacological response and is not considered to be relevant to clinical exposures therefore this finding does not need to be added to the SPC.

Once data confirming an absence of a treatment related effect on FSH and LH levels has been provided, the MAH will be requested to submit a type II variation to update section 5.3 of the Prozac SPCs with the study findings in order to inform prescribers that the testicular pathology and delayed sexual maturation are not SSRI class effects mediated through an action on the hypothalamus.

7. LIST OF QUESTIONS TO BE ADDRESSED BY THE MAH

Female reproductive toxicity

1. With regard to the statistically significant increase in inhibin A levels at 10 mg/kg at Day 50 pp, the MAH stated that a closer examination of individual hormone data showed evidence that the difference in inhibin A between the control and 10 mg/kg dose group, was most likely related to the distribution of animals to the different phases of the oestrous cycle with more rats in the 10 mg/kg dose group in the follicular phase than controls and not related to treatment with fluoxetine. However the MAH must discuss the possibility that fluoxetine might have been responsible for the distribution of the animals to the different phases of the oestrous cycle through potential effects on the cycle.

2. The MAH must provide a profile of FSH and LH release over the duration of treatment for each dose level to determine whether there was a dose related trend.

3. The MAH must provide a table correlating food consumption, bodyweight data and onset of sexual maturation on an individual animal basis for the male and female rats.

Male reproductive toxicity

4. The MAH must investigate the possibility of a mechanism of toxicity occurring locally in the male reproductive tract. The fact that statistically significant changes in inhibin B have occurred in two independant studies is evidence that it might be an important marker of toxicity, and its role (if any) in the pathogenesis of the testicular findings should be further investigated.

5. The applicant should discuss the potential species specificity of the effects observed in the juvenile rat, in relation to the effects observed on inhibin B and to exposure to fluoxetine versus Norfluoxetine. The relevance of performing a study in a second species should be addressed.

6. The applicant should discuss the potential specificity of the effects observed in the juvenile rat with fluoxetine compared to other SSRI, based on available data.

Annex 1: Study WIL-353039, Beck, And September 2004: Safety margins, as calculated by the applicant.

Margins of safety (MOS) for testes and skeletal muscle changes and femur length decreases (excerpted from Table 2): The juvenile rat NOAEL for these parameters is 10 mg/kg/day.

	Fluoxetine MOS		Norfluoxetine MOS	
	Single dose EM[a]	SteadyState EM[a]	Single dose EM[a]	Steady State EM[a]
Preadolescents	0.8 (0.4-5.0)	1.4 (0.8-8.8)	1.1 (0.7-3.0)	5.7 (3.6-16.3)
Adolescents	1.6 (0.7-4.7)	2.9 (1.2-8.2)	1.8 (1.3-4.3)	9.9 (6.8-23.2)

a. Single dose exposure multiple (EM) = single dose rat AUC/steady state clinical AUC; Steady state EM multiple = steady state rat AUC/steady state clinical AUC

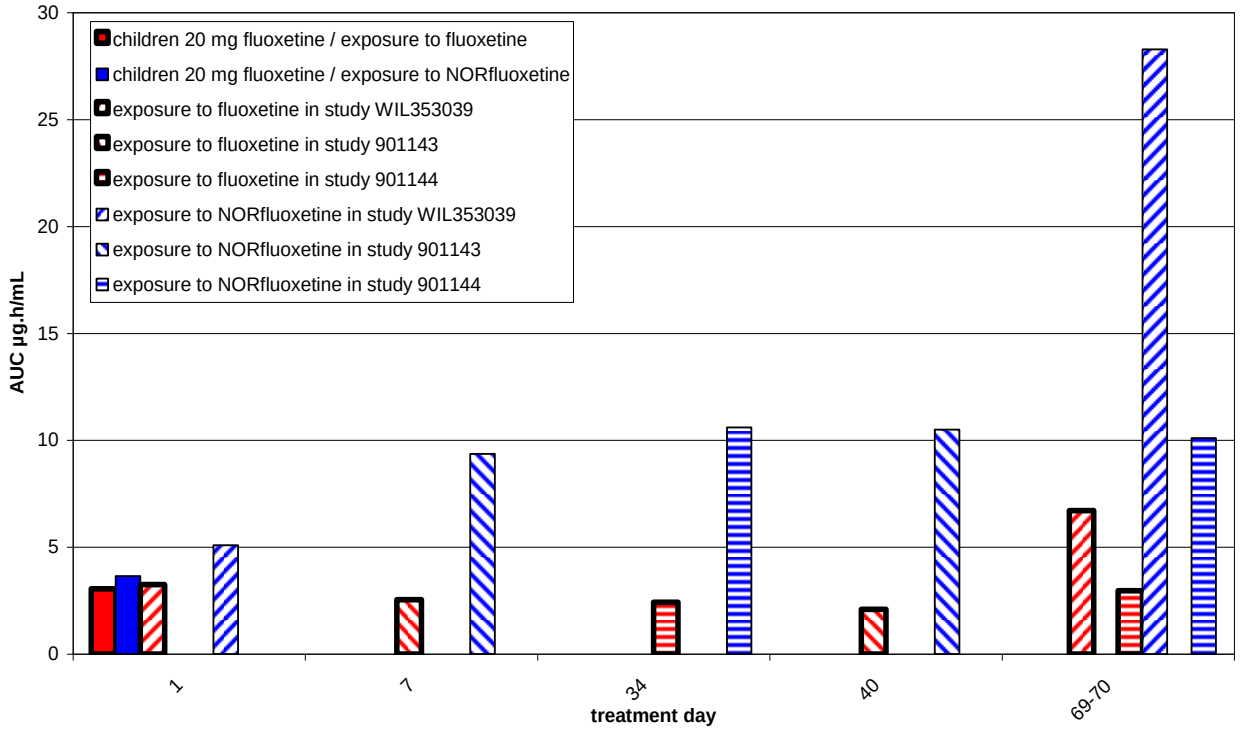
Margins of safety (MOS) for delays in sexual maturation and decreased body weight gain (excerpted from Table 2): The juvenile rat NOAEL for these parameters is 3 mg/kg/day.

	Fluoxetine MOS		Norfluoxetine MOS	
	Single dose EM[a]	SteadyState EM[a]	Single dose EM[a]	Steady State EM[a]
Preadolescents	0.1 (0.1-0.7)	0.1 (.04-0.5)	0.3 (0.2-0.8)	0.5 (0.3-1.5)
Adolescents	0.2 (0.1-0.6)	0.2 (0.1-0.5)	0.5 (0.3-1.2)	0.9 (0.6-2.1)

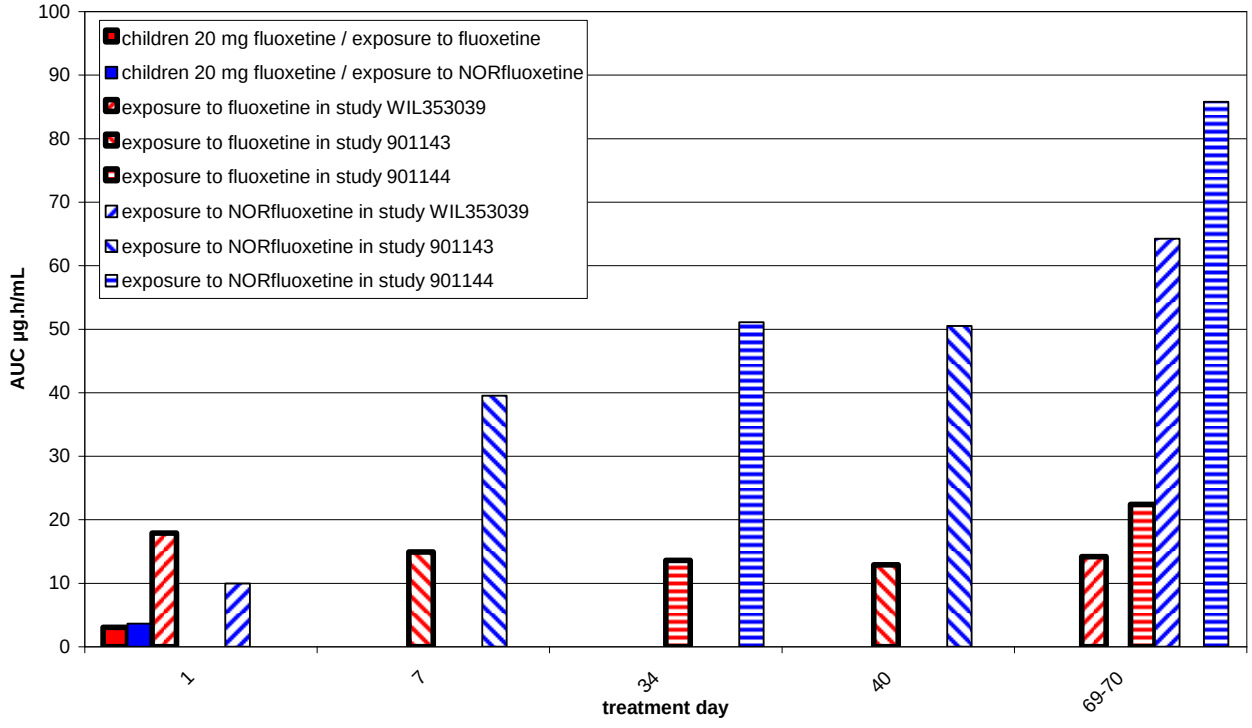
a. Single dose exposure multiple (EM) = single dose rat AUC/steady state clinical AUC; Steady state EM multiple = steady state rat AUC/steady state clinical AUC

Annex 2: Exposure to fluoxetine and NORfluoxetine according to the time

group 10 mg/kg fluoxetine orally administered in juvenile rats: exposure to fluoxetine and NORfluoxetine among studies and comparison with children exposure



group 30 mg/kg fluoxetine orally administered in juvenile rats: exposure to fluoxetine and NORfluoxetine among studies and comparison with children exposure



Annex 3. Testicular Pathogenesis study

Hormone Data Tables

LH: Treatment Phase				
		Animal Age at Collection		
	Statistic	55	70	91
Control	Mean	0.62	0.24	0.18
	SEM	0.229	0.040	0.056
	N	10	10	9
10 mg/kg LY110140	Mean	0.37	0.35	1.47
	SEM	0.092	0.200	1.238
	N	10	10	10
	p value	0.6271†	0.2550†	0.5977†
30 mg/kg LY110140	Mean	0.27	0.17	0.11
	SEM	0.064	0.022	0.009
	N	9	9	9
	p value	0.1907†	0.5014†	0.2574†

† Dunnett's t-test conducted on Rank Average transformed data.

LH: Recovery Phase				
		Animal Age at Dosing Termination		
	Statistic	55	70	91
Control	Mean	0.38	0.53	0.17
	SEM	0.070	0.296	0.030
	N	8	10	10
10 mg/kg LY110140	Mean	0.36	3.15	0.21
	SEM	0.103	2.984	0.056
	N	10	10	10
	p value	0.6235†	0.9944†	1.0000†
30 mg/kg LY110140	Mean	0.33	0.13	3.73
	SEM	0.062	0.023	3.286
	N	10	10	9
	p value	0.8577†	0.0258†	0.1748†

† Dunnett's t-test conducted on Rank Average transformed data.

FSH: Treatment Phase				
		Animal Age at Collection		
	Statistic	55	70	91
Control	Mean	9.44	6.56	6.20
	SEM	0.819	0.251	0.384
	N	10	10	9
10 mg/kg LY110140	Mean	8.17	7.35	9.22
	SEM	0.348	0.511	1.639
	N	10	10	10
	p value	0.4788†	0.3682	0.1461†
30 mg/kg LY110140	Mean	9.48	7.46	7.11
	SEM	0.469	0.559	0.649
	N	9	9	9
	p value	0.5871†	0.2977	0.5306†

† Dunnett's t-test conducted on Rank Average transformed data.

FSH: Recovery Phase				
		Animal Age at Dosing Termination		
	Statistic	55	70	91
Control	Mean	5.99	6.48	5.01
	SEM	0.327	0.676	0.435
	N	8	10	10
10 mg/kg LY110140	Mean	6.19	8.12	4.72
	SEM	0.322	3.131	0.218
	N	10	10	10
	p value	0.8726	0.3641†	0.8398†
30 mg/kg LY110140	Mean	6.61	8.80	10.79
	SEM	0.331	4.590	4.957
	N	10	10	9
	p value	0.3262	0.0690†	0.6569†

† Dunnett's t-test conducted on Rank Average transformed data.

Testosterone: Treatment Phase				
		Animal Age at Collection		
	Statistic	55	70	91
Control	Mean	3.16	1.36	1.02
	SEM	0.873	0.230	0.197
	N	10	10	9
10 mg/kg LY110140	Mean	2.04	1.45	0.95
	SEM	0.521	0.350	0.152
	N	10	10	10
	p value	0.2507†	0.9311†	0.9982†
30 mg/kg LY110140	Mean	1.35	1.25	0.51
	SEM	0.199	0.332	0.189
	N	9	8	9
	p value	0.1272†	0.7881†	0.0508†

† Dunnett's t-test conducted on Rank Average transformed data.

Testosterone: Recovery Phase				
		Animal Age at Dosing Termination		
	Statistic	55	70	91
Control	Mean	0.89	1.09	0.92
	SEM	0.146	0.226	0.238
	N	8	10	10
10 mg/kg LY110140	Mean	0.81	1.01	1.10
	SEM	0.124	0.111	0.140
	N	10	10	10
	p value	0.8665	0.8543†	0.6964
30 mg/kg LY110140	Mean	1.09	0.97	1.15
	SEM	0.122	0.158	0.169
	N	10	10	9
	p value	0.4735	0.9999†	0.5893

† Dunnett's t-test conducted on Rank Average transformed data.

Inhibin B: Treatment Phase				
		Animal Age at Collection		
	Statistic	55	70	91
Control	Mean	44.19	40.15	50.48
	SEM	8.319	3.369	4.464
	N	10	10	9
10 mg/kg LY110140	Mean	54.62	38.70	40.31
	SEM	6.355	5.230	3.387
	N	10	10	10
	p value	0.4990	0.9649	0.1248
30 mg/kg LY110140	Mean	61.54	39.40	35.64
	SEM	7.144	5.424	3.684
	N	9	9	9
	p value	0.1908	0.9910	0.0237

† Dunnett's t-test conducted on Rank Average transformed data.

Inhibin B: Recovery Phase				
		Animal Age at Dosing Termination		
	Statistic	55	70	91
Control	Mean	38.10	39.30	40.15
	SEM	4.459	4.344	5.896
	N	8	10	10
10 mg/kg LY110140	Mean	42.14	38.82	41.08
	SEM	5.317	4.730	5.191
	N	10	10	10
	p value	0.8004	0.9956	0.9917
30 mg/kg LY110140	Mean	41.71	43.94	41.44
	SEM	5.129	4.145	7.697
	N	10	10	9
	p value	0.8361	0.6805	0.9851

† Dunnett's t-test conducted on Rank Average transformed data.

Annex 4. Neurohormonal study

Male: Testosterone

	Statistic	Animal Age at Collection			
		28	40	50	61
Control	Mean	0.17	0.83	1.68	1.62
	SEM	0.050	0.274	0.454	0.250
	N	10	10	10	10
10 mg/kg LY110140	Mean	0.13	0.63	1.37	1.96
	SEM	0.192	0.342	0.349	0.331
	N	10	10	9	10
	p value	0.8332†	0.3609†	0.9518†	0.5615
30 mg/kg LY110140	Mean	0.1	0.23	0.93	1.09
	SEM	0	0.051	0.229	0.201
	N	9	10	10	10
	p value	0.1835†	0.0526†	0.5164†	0.2900

† Dunnett's t-test conducted on Rank Average transformed data.

Male: Inhibin B

	Statistic	Animal Age at Collection			
		28	40	50	61
Control	Mean	185.48	116.36	105.07	107.76
	SEM	16.848	10.169	9.581	6.900
	N	10	10	10	10
10 mg/kg LY110140	Mean	121.94	145.04	84.07	114.12
	SEM	12.723	25.421	11.498	8.447
	N	10	10	9	10
	p value	0.0287†	0.6810†	0.2070	0.5948†
30 mg/kg LY110140	Mean	209.79	134.55	101.15	145.52
	SEM	27.036	18.658	5.985	13.846
	N	9	10	10	10
	p value	0.9888†	0.9049†	0.9334	0.0365†

† Dunnett's t-test conducted on Rank Average transformed data.

Female: LH

	Statistic	Animal Age at Collection					
		28	30	33	35	44	50
Control	Mean	0.542	0.39	0.33	0.28	0.31	0.17
	SEM	0.140	0.071	0.071	0.094	0.072	0.030
	N	9	10	10	10	10	10
10 mg/kg LY110140	Mean	1.32	0.51	0.34	0.55	0.39	0.14
	SEM	0.375	0.168	0.051	0.160	0.122	0.019
	N	10	10	10	10	10	10
	p value	0.4653†	0.9886†	0.8989†	0.1543†	0.9718†	0.9940†
30 mg/kg LY110140	Mean	0.81	0.59	1.06	0.76	0.40	0.24
	SEM	0.452	0.131	0.519	0.177	0.086	0.062
	N	10	10	10	10	10	10
	p value	0.3408†	0.6681†	0.7141†	0.0166†	0.7870†	0.7528†

† Dunnett's t-test conducted on Rank Average transformed data.

Female: FSH							
	Statistic	Animal Age at Collection					
		28	30	33	35	44	50
Control	Mean	2.94	2.98	3.00	2.60	4.08	3.00
	SEM	0.401	0.660	0.259	0.225	0.599	0.419
	N	9	10	10	10	10	10
10 mg/kg LY110140	Mean	5.22	3.29	4.97	5.36	3.40	2.81
	SEM	0.423	0.351	0.929	1.272	0.538	0.390
	N	10	10	10	10	10	10
	p value	0.0034	0.1507†	0.3057†	0.0567†	0.6805†	0.8865†
30 mg/kg LY110140	Mean	4.16	3.10	2.80	4.23	3.18	3.40
	SEM	0.530	0.330	0.258	0.499	0.622	0.318
	N	10	10	10	10	10	10
	p value	0.1282	0.3073†	0.7888†	0.0158†	0.2853†	0.5465†

† Dunnett's t-test conducted on Rank Average transformed data.

Female: Prolactin							
	Statistic	Animal Age at Collection					
		28	30	33	35	44	50
Control	Mean	5.40	2.48	2.49	2.40	7.729	3.68
	SEM	1.925	0.732	0.751	1.099	4.304	1.131
	N	9	10	10	10	10	10
10 mg/kg LY110140	Mean	4.44	2.70	5.38	1.91	3.69	4.74
	SEM	1.798	0.891	3.120	0.370	1.227	0.887
	N	10	10	10	10	10	10
	p value	0.7364†	0.9505†	0.6888†	0.7745†	0.9958†	0.4900†
30 mg/kg LY110140	Mean	1.91	1.37	2.41	1.25	4.15	8.11
	SEM	0.369	0.122	0.406	0	1.514	2.264
	N	10	10	10	10	10	10
	p value	0.1430†	0.2571†	0.5533†	0.3907†	0.9958†	0.2371†

† Dunnett's t-test conducted on Rank Average transformed data.

Female: Estradiol							
	Statistic	Animal Age at Collection					
		28	30	33	35	44	50
Control	Mean	17.94	10.08	14.58	10.36	12.04	14.593
	SEM	4.99	1.989	2.301	1.708	2.457	3.125
	N	9	10	10	10	10	10
10 mg/kg LY110140	Mean	9.03	7.23	10.91	10.45	10.91	18.27
	SEM	0.616	0.552	2.748	2.183	0.760	2.986
	N	10	10	10	10	10	10
	p value	0.0477†	0.3267†	0.2000†	0.9888†	0.4216†	0.3998†
30 mg/kg LY110140	Mean	9.28	8.63	7.43	8.68	11.418	12.41
	SEM	0.365	0.884	0.485	1.261	2.643	1.776
	N	10	10	10	10	10	10
	p value	0.0537†	0.9445†	0.0609†	0.7139†	0.9623†	0.9547†

† Dunnett's t-test conducted on Rank Average transformed data.

Female: Progesterone							
	Statistic	Animal Age at Collection					
		28	30	33	35	44	50
Control	Mean	1.44	4.84	5.86	8.75	8.56	9.67
	SEM	0.285	1.846	1.378	2.847	1.346	0.907
	N	9	10	10	10	10	10
10 mg/kg LY110140	Mean	0.73	1.01	4.47	5.53	16.04	14.16
	SEM	0.080	0.200	1.301	1.742	2.769	3.560
	N	10	10	10	10	10	10
	p value	0.0403†	0.0071†	0.4674†	0.4323†	0.0710†	0.9883†
30 mg/kg LY110140	Mean	3.04	2.08	1.59	1.98	8.11	8.14
	SEM	0.648	0.472	0.391	0.745	2.098	2.273
	N	10	10	10	10	10	10
	p value	0.2026†	0.4429†	0.0033†	0.0065†	0.8822†	0.4400†

† Dunnett's t-test conducted on Rank Average transformed data.

Female: Inhibin A							
	Statistic	Animal Age at Collection					
		28	30	33	35	44	50
Control	Mean	361.33	356.72	297.74	247.59	207.27	188.08
	SEM	29.823	60.519	50.130	67.132	38.756	38.570
	N	9	10	10	10	10	10
10 mg/kg LY110140	Mean	383.68	328.54	255.90	160.04	231.24	330.91
	SEM	26.945	34.694	41.618	26.029	44.935	30.321
	N	10	10	10	10	10	10
	p value	0.8276	0.8598	0.7921†	0.6530†	0.8868	0.0115
30 mg/kg LY110140	Mean	338.12	286.23	212.20	267.47	205.27	273.08
	SEM	34.454	28.398	20.967	36.630	40.791	32.397
	N	10	10	10	10	10	10
	p value	0.8157	0.4217	0.5617†	0.2099†	0.9992	0.1539

Hormone Data Tables

Male: LH					
	Statistic	Animal Age at Collection			
		28	40	50	61
Control	Mean	0.29	0.38	0.21	0.21
	SEM	0.103	0.070	0.022	0.054
	N	10	10	10	10
10 mg/kg LY110140	Mean	0.18	0.28	1.39	0.16
	SEM	0.050	0.066	1.093	0.028
	N	10	10	9	10
	p value	0.9270†	0.2324†	0.8092†	0.4999†
30 mg/kg LY110140	Mean	0.41	1.35	0.197	0.27
	SEM	0.232	1.060	0.051	0.086
	N	10	10	10	10
	p value	0.6648†	0.5616†	0.3039†	0.9822†

† Dunnett's t-test conducted on Rank Average transformed data.

Male: FSH					
	Statistic	Animal Age at Collection			
		28	40	50	61
Control	Mean	8.28	8.14	6.10	4.81
	SEM	0.558	0.464	0.741	0.242
	N	10	10	10	10
10 mg/kg LY110140	Mean	9.275	6.19	5.60	5.91
	SEM	0.772	0.865	0.412	0.634
	N	10	10	9	10
	p value	0.5419	0.2110†	0.8070	0.5596†
30 mg/kg LY110140	Mean	9.56	7.69	7.23	5.12
	SEM	0.850	1.074	0.659	0.829
	N	10	10	10	10
	p value	0.3785	0.8430†	0.3506	0.8945†

† Dunnett's t-test conducted on Rank Average transformed data.

Male: Prolactin					
	Statistic	Animal Age at Collection			
		28	40	50	61
Control	Mean	1.25	1.57	1.67	2.82
	SEM	0	0.187	0.228	0.704
	N	10	10	10	10
10 mg/kg LY110140	Mean	1.32	1.91	2.54	2.21
	SEM	0.059	0.346	1.042	0.338
	N	10	10	9	10
	p value	0.1364†	0.9083†	0.9659†	0.9233†
30 mg/kg LY110140	Mean	1.25	2.15	1.50	2.04
	SEM	0	0.493	0.171	0.359
	N	10	10	10	10
	p value	1.0000†	0.8614†	0.8119†	0.7835†

† Dunnett's t-test conducted on Rank Average transformed data.