auto-injector. The resulting flare responses at 5 minutes post injection were measured using an

Analysis of variance was performed with effects due to volunteer, period, alosetron dose, site of 5-HT administration, 5-HT dose and the interaction of 5-HT dose and alosetron dose.

At every 5-HT dose level there were statistically significant decreases in flare size with each alosetron dose level, and the decrease in flare size was larger with increasing alosetron dose (See Table 30).

Table 30 Suppression of 5-HT Induced Flare Response by IV Alosetron

5-HT Dose (μM)	Placebo	Alosetron Dose					
		0.1 mg		1 mg		4 mg	
		Alosetron Plasma Concentrations (ng/ml) Geometric Mean					
		10 min	35 min	10 min	35 min	10 min	35 min
		2.5	0.8	28.1	8.6	136.1	42.0
	Flare Response (cm^2)	Flare Response (cm^2) % Decrease Compared to Placebo (95% CI)					
10	3.07	1.33 57% (38, 70)		0.94 69% (56, 79)		0.86 72% (60, 80)	
40	4.25	1.99 53% (32, 68)		1.10 74% (62, 82)		0.88 79% (70, 86)	
160	6.66	2.58 61% (44, 73)		1.50 77% (68, 84)		0.90 87% (81, 91)	
640	12.25	4.76 61% (38, 76)		3.36 73% (56, 83)		2.53 79% (67, 87)	
2,560	17.60	12.01 32% (16, 44)		12.13 31% (16, 44)		9.63 45% (33, 55)	
10,240	27.10	18.93 30% (20, 39)		17.39 36% (26, 44)		14.93 45% (37, 52)	

The decrease in flare size was significantly smaller at the 5-HT 2,560 and 10,240 μM doses and likely reflects greater competitive binding of 5-HT at these higher doses. This should be surmountable with higher alosetron doses.

The sponsor was not able to demonstrate a relationship between flare size and alosetron plasma concentration due to the variability of both parameters.

The variability in flare size is understandable, as local innervation near the site of injection, and consequently flare response, would be expected to vary between sites of injection, between periods, and between individuals.

The reason for the high variability in alosetron concentrations is less well understood but has been seen consistently across studies.

(2) Flare Response - PO Alosetron (Study GHP:90:27)

Study GHP:90:27 was a randomized, double-blind, placebo controlled, four period cross over study in 12 healthy male volunteers. Each subject received three does levels of alosetron (0.05 mg, 0.25 mg, 1 mg) in 100 ml of grapefruit juice and placebo (grapefruit juice). Alosetron was administered after an overnight fast and 2 hours after completing a standardized breakfast. Intradermal injections of 5-HT 160 µM were made in duplicate into sites on the back using an auto-injector pre-dose and at 1, 2, 4, 8, and 12 hours post-dose. The resulting flare responses at 5 minutes post injection were measured using an Venous blood was sampled for determination of alosetron concentrations predose and at 10 minutes after each intradermal injection.

The extent and duration of suppression of the flare response increased with dose (See APPENDIX 3), and the duration of suppression lasted for several hours longer than alosetron is detectable. In fact, suppression lasted until estimated mean concentrations were in the range of 0.1 - 0.15 ng/ml (0.3 - 0.45 nM/L). These concentrations are similar to aloseton's Ki (0.4 nM/L). (The Ki is the concentration required for half of the receptors to be occupied. It should be noted that both the plasma concentrations and Ki are reported as total drug. Although free Ki's are more appropriate they were not reported.)

It should be noted that alosetron was administered with grapefruit juice in the flare response study. Consequently, alosetron concentrations might have been higher than they would be otherwise, although they appear similar to concentrations seen in other studies.

This reviewer finds that the duration of suppression tends to support dosing every 10 hours. This is based upon a mean suppression of the flare response of 25% or greater (mean duration 8.9 hours) in men (See APPENDIX 3).

The sponsor claims that dosing qd or bid is supported; however, the criteria for this assessment is unclear.

It's currently unknown if blockade of the $5HT_3$ receptor is required for efficacy, the extent of blockade required, or the duration of blockage. If $5HT_3$ receptor blockade is required for effect, it's reasonable to assume that blockade has to be present for a sufficient length of time and that long periods without blockade will result in a lack of efficacy. The data presented suggest that women will have a longer duration of $5HT_3$ blockade than men. In addition, since the drug was dosed bid, the dosing intervals were approximately 10 & 14 hours. Drug administration was also with food, which decreases absorption and would result in a shorter duration of blockade. Consequently, men may have had an insufficient sufficient duration of blockade for clinical efficacy.

The sponsor claimed that there was a lack of a PK/PD relationship due to the high variability in both flare response and plasma concentrations. In fact, the subject with the highest concentrations had one of the smallest responses at each dose level. Thus, either there is either a large inherent variability in the technique, differences in pharmacodynamics between subjects, differences in metabolite exposure, or a combination of factors.

In fact, the most potent metabolite studied, 6-OH alosetron has a Ki of approximately 0.2 nM/L. Although, it was not detected, this is 1/6 the level of detection (0.4 ng/ml / 1.15 nM/L) and 1/29th the lower limit of quantitation (2 ng/ml / 5.77 nM/L). Other metabolites could also be contributing to effect since most of the circulating metabolites have not been identified and the receptor affinities have not be determined for all of the metabolites.

(3) PD - Colonic Motility (Study SB331007)

Study SB331007 was a randomized, double-blind, placebo controlled, two-way cross over study of the pharmacokinetic-pharmacodynamic relationship of alosetron. Subjects included 6 male and 7 female subjects with IBS excluding those with constipation predominant (*'according to the Rome criteria'*) and 12 healthy control subjects matched for age and sex (6 male and 6 female). Alosetron 4 mg po was dosed bid with breakfast and dinner for 7 days.

The pharmacodynamic effect of alosetron was assessed by conducted from 12 midnight falling between day 6 and day 7, to 12 midnight falling between day 7 and day 8.

Venous blood samples for determination of alosetron concentrations were obtained at 90 minutes after the evening dose on day 6 and prior to breakfast on day 8.

Subjects on bulking agents used them throughout study and women were allowed to remain on oral contraceptives. Either of which might effect the results.

Alosetron concentration variability was very high with the limited sampling. In fact, a sequence effect was observed in alosetron 90 minute post-dose concentrations (See Table 31).

Table 31 Alosetron 90 min Concentrations

Subject Group	Treatment Sequence	n	Geometric LS Mean	95% CI
Patients	PBO - Alosetron	5	9.27	2.61, 32.90
	Alosetron - PBO	8	20.37	7.48, 55.48
Volunteers	PBO - Alosetron	7	28.94	9.92, 84.45
	Alosetron - PBO	8	4.93	1.81, 13.41

LS - Least Squares

The high variability in plasma concentrations and limited sampling, associated with the high variability in the pharmacodynamic endpoints (contraction frequency, motility index, and High Amplitude Propulsive Contractions) and the small numbers of subjects, prevent any pharmacokinetic-pharmacodynamic correlation. In fact, the high variability in the pharmacodynamic endpoints results in a lack of any statistically significant effects due to treatment, as assessed by most parameters.

In general, mean motility indices tend to be higher while on alosetron with an increase in colonic activity after dinner, in both treatment groups. This has prompted the sponsor to suggest that alosetron may 'normalize' intestinal motility in IBS patients with diarrhea predominance. This reviewer finds little support for this mechanistic hypothesis for alosetron. Secondary analyses of High Amplitude Propulsive Contractions (HAPC) failed to find any gender differences.

2. 5-HT_3 Receptor Affinities for Alosetron and Metabolites

Receptor affinities are reported in this section (See Table 32). It should be noted that affinities are reported for total drug concentration and not free drug. Also we do not know the protein binding for any metabolite. Consequently, we can't determine the relevance to in vivo concentrations. Finally, the sponsor did not examine glucuronide conjugates claiming that glucuronides are not active, although we know that morphine glucuronide is highly active and crosses into the CNS. Although, the sponsor might have additional structure activity relationships from similar compounds that would shed light on this issue. Nor did the sponsor examine the affinity of N-desmethyl-alosetron and its' metabolites claiming that it is not

found in humans, in spite of the fact that it was found in high amounts in Japanese subjects. See APPENDIX 2 Alosetron Metabolic Pathways for alosetron and metabolite structures.

Table 32 5-HT$_3$ Receptor Affinities for Alosetron and Metabolites

Code Number	Descriptive Name	pKI	Ki (nM)	Potency Relative to Alosetron (according to sponsor)	Comments
GR68755	Alosetron	9.45±0.06	0.4	1	
GR96105X	6-OH-Alosetron	9.55±0.12	0.2	1	May be twice as potent (or more)
GR163860A	7-OH-Alosetron	7.43±0.03	3.72	1:11	
GR169307X	Hydroxylated Methyl Imidazole	8.39±0.02	41.0	1:105	
GR168355X	Monocarbonyl	6.95±0.08	112.2	1:316	
GR153732X	Dicarbonyl	<6	>1000.0	~1:1000	*'Significant Metabolite in Man'*
GR87620	N-Desmethyl-Alosetron	Not Reported	—	—	Claimed that it Not Detected in Man

In no case was the Hill slope significantly different from 1, probably indicating a single binding site on the 5-HT$_3$ receptor.

3. Metabolite Plasma Concentrations

Approximately 66% of the total circulating radioactivity has not been assigned to any specific compounds (See Table 33).

Table 33 Reported Circulating Concentrations of Alosetron and Metabolites

Analyte	Concentration[a] (ng/ml)	Percent	Potency Relative to Alosetron
Alosetron	5	11.4	1
6-OH Alosetron	< 2	< 4.5	2
7-OH Alosetron	< 2	< 4.5	1:11
N-Desmethyl-Alosetron	1.5	3.4	?
Hydroxymethyl-Alosetron	5	12	1:105
Total *(Metabolites)*	~15 *(~10)*	34 (22.7)	—
Unaccounted for	~29	66	—
Total Radioactivity (Alosetron Base Equivalent)	44	100	—

a - alosetron base equivalent

See Table 36 Summary of Alosetron Metabolite Information for a summary of concentrations, and potencies.

a) N-Desmethyl-Alosetron (GR87620)

N-Desmethyl-Alosetron (GR87620) was reported in the receptor affinity study as not detected in man. This reviewer presumes that this is based upon the mass balance data. However, it was reported as a

circulating metabolite in three Japanese studies with peak concentrations usually ⅓ to ½ alosetron peak concentrations as reported in the following two studies.

Study AS-01

N-Desmethyl-Alosetron concentrations achieved after dosing of 1, 2, or 4 mg tablets were reported from the phase I single rising dose crossover study in Japanese males, study AS-01. The highest mean concentrations achieved with each dose are shown in Table 34.

Table 34 Peak Mean Concentrations of N-Desmethyl-Alosetron after Single Doses in Japanese Males

Dose (mg)	Highest Mean Concentration (ng/ml)	Time of Highest Mean Concentration (Hours)
1	1.54 ± 0.47	2.0
2	3.42 ± 0.48	1.0
4	5.67 ± 0.83	2.0

Study AS-02

In study AS-02, alosetron was administered 1 mg bid for 7 days. Mean Cmax for alosetron was 3.36 ng/ml. Samples were taken for N-desmethyl-alosetron 2 hours after the dose on day 1, at 2 and 4 hours on day 5, and at 6 time points on day 7. Tmax was always at 2 hours due to the limited sampling.

Mean peak concentrations are shown in Table 35 and are approximately 1/3 of the mean alosetron peak concentration. However, due to the limited sampling peak concentrations may be higher. In fact, the highest measured concentration of N-desmethyl alosetron was 2.52 ng/ml, whereas the peak alosetron concentration in the same subject was 2.49 ng/ml, i.e. approximately a 1:1 ratio. The mean peak concentrations over time do not indicate any accumulation of N-desmethyl-alosetron.

Table 35 N-Desmethyl-Alosetron Mean Peak Concentrations

	Day 1	Day 5	Day 7
Cmax (ng/ml) Mean +/- sd	0.99 ± 0.19	1.71 ± 0.54	1.09 ± 0.21

b) Hydroxymethyl-Alosetron (GR169307)

Hydroxymethyl-alosetron (GR169307) was reported with a maximum peak concentration of 4.9 ng/ml in the Japanese food effect study, study AS-03. Alosetron was administered as 1 mg po in this study and approximately equimolar concentrations of alosetron and hydroxymethyl-alosetron were achieved. Since, hydroxymethyl-alosetron is approximately 1:105 as potent as alosetron it is not likely to contribute to activity mediated via 5-HT_3 blockade.

c) 6-OH and 7-OH-Alosetron

Neither 6-hydroxy alosetron (GR96105) nor 7-hydroxy-alosetron (GR163860) were detected using an assay with a lower limit of detection of < 2 ng/ml in the Japanese food effect study, AS-03.

Although these metabolites were not detected in plasma in this study, the lower limit of detection is sufficiently high that significant activity could still be attributable these metabolites in spite of their not being detected. In fact, the assay for 6-OH-alosetron has a level of detection of 0.4 ng/ml (1.15 nM/L) and a lower limit of quantitation of 2 ng/ml (5.77 nM/L). These concentrations are 6 and 29 fold greater than the Ki (0.2 nM/L). A similar situation could exist with 7-OH-alosetron since it's 1/10 as potent as alosetron.

This is supported by the oral alosetron 5-HT flare response study. In this study suppression of flare response lasted for several hours longer than alosetron is detectable. In fact, suppression lasted until estimated alosetron concentrations were in the range of 0.15 ng/ml.

Finally, the relatively high percent eliminated in the feces in combination with delayed fecal elimination suggests enterohepatic recycling, prolonged low level exposure, and possible accumulation of one or both of these two metabolites with multiple dosing (See APPENDIX 2 Alosetron Metabolic Pathways).

d) bis-Oxidized Alosetron (Dicarbonyl)

According to the sponsor, the bis-oxidized Alosetron metabolite (GR153732) that is formed through oxidation of imidazole ring may have a structure that is consistent with either a dicarbonyl (C=O) or an epoxide. Plasma concentrations of this compound were not mentioned, although it is reported to account for 9-14% of the dose. The dicarbonyl has a potency of approximately 1:1000 of alosetron and is unlikely to contribute to activity mediated via $5HT_3$ receptor blockade. The NIH shift in the position of the methyl group on the imidazole ring that occurs with dicarbonyl formation, indicates that the formation of the dicarbonyl proceeds through an epoxide intermediate. There is no mention anywhere if any of the minor metabolites is a diol. A diol would indicate inactivation of a relatively stable epoxide by epoxide hydrolase, but lack of a diol does not preclude metabolism to an epoxide intermediate.

Epoxides can be toxic to metabolizing organs and terratogenic, especially if administered with other compounds that are metabolized to epoxides, such as phenytoin or carbamazepine, or if administered in combination with an epoxide hydrolase inhibitor such as valproic acid. However, epoxide exposure with phenytoin and carbamazepine are quite high compared to what would be possible with alosetron. Consequently, any epoxide formation with alosetron via this pathway, even if induced, would be unlikely to have clinical consequences. However, this does not preclude expoxide exposure subsequent to N-desmethylation (See APPENDIX 2 Alosetron Metabolic Pathways).

To illustrate, for phenytoin a typical single 300 mg dose of sodium phenytoin is 1.09 milliMoles, and approximately 60-90% of the dose proceeds through an epoxide intermediate. For carbamazepine a typical single 200 mg dose is 0.85 milliMoles, and 25-90% of the dose is metabolized to an epoxide, and free epoxide concentrations in plasma are on the order of 10,000 nM/L. In comparison, a 1 mg dose of alosetron is 3.4 microMoles and even if 50% or more proceeds through an expoxide the exposure is approximately 1/500 that seen with phenytoin or carbamazepine (See APPENDIX 1).

Table 36 Summary of Alosetron Metabolite Information

Report	Code	Description	% in urine (Most data after 4 mg dose)	Plasma Concentrations	Activity	
					pKi	Potency Relative to Alosetron
Alterations to Indole Ring						
WBP/91/002 JJD/94/003	GR87620	Desmethyl from indole ring	not observed in US Studies 6-9% of dose in Japanese Food effect Study	<2 ng/ml at all times - US Japanese - 2.74 ng/ml		
WD1998004 02/00 JJD/94/003	GR96105	6-OH-alosetron (indole hydroxylation)	Both 6 and 7 OH 18% Not Resolved 6-OH is 15% 7-OH is 3%	Not detected (<2 ng/ml) in Japanese food effect study	9.55±0.12	1
		Alosetron-6-glucuronide	6-O-GLUC (14%?) (5 fold 7-O-Gluc in urine)			
	GR163860	7-OH-alosetron (indole hydroxylation)	Both 6 and 7 OH 18% Not Resolved 6-OH is 15% 7-OH is 3%	Not detected (<2 ng/ml) in Japanese food effect study	7.43±0.03	1:11
		Alosetron-7-glucuronide	7-O-Gluc (~4%?) (1/5 6-O-Gluc in urine)			
Alterations to Imidazole Ring						
	GR169307	Hydroxymethyl-alosetron Hydroxylated methyl on imidazole	3%	highest peak 4.9 ng/ml in Japanese food effect study (Double alosetron peak concentration)	8.39±0.02	1:105
	GR168355	monocarbonyl (C=O) [oxidation (C=O) of imidazole]	4%	Concentrations not mentioned	6.95±0.08	1:316
WBP/91/105	GR153732	bis-oxidized may be either dicarbonyl (C=O) or an epoxide [oxidation of imidazole]	9-14% of dose	Concentrations not mentioned	<6	Weak binding - Inactive
	GR168557	dicarbonyl (C=O) with opened imidazole ring	<1%	Concentrations not mentioned		
	GR62202	N-dealkyl of imidazole ring. Synthetic Intermediate **Mutagenic**		<lod (0.5 ng/ml) "Not observed in man"		

J. Drug Interactions

1. *In Vitro* Inhibition by Alosetron

The results of *in vitro* inhibition by alosetron for some P450 isozymes are conflicting and thus the results are confusing. One would expect to see an increase in inhibition with both increasing alosetron concentration and with increasing probe substrate concentration, with the most extensive inhibition at near saturating concentrations of both the probe substrate and alosetron. From Table 37 inhibition is most likely to occur with 1A2 and 2E1, and possibly with 3A4.

The potential of alosetron to inhibit a variety of P450 isozymes was studied at 2 alosetron concentrations, 0.2 and 2.0 μM. These molar concentrations are equivalent to alosetron concentrations of 165.4 and 1654 ng/ml respectively. The 0.2 μM (165.4 ng/ml) concentration was selected because it is in the range of the peak concentration (up to ~180 ng/ml) seen with the largest dose used in the alosetron dose ranging studies (16 mg). The 2.0 μM is simply 10 fold higher. Substrates were studied at concentrations equivalent to their Km's and also sometimes at saturating concentrations (See Table 37).

Table 37 P450 Inhibitory Potential of Alosetron

Isoform	Probe & Metabolite	Km (μM)	Saturating Conc. (μM)	Alosetron Concentration (μM)	% Inhibition (probe concentration equivalent to Km)	% Inhibition (probe concentration is saturating)
1A2	Exthoxyresorufin O-deethyl	0.4	2.5	0.2	0	24.8
				2.0	60	55.8
2C9	Tolbutamide 4-hydroxy	250	—	0.2	8.8	not studied
				2.0	11.7	not studied
2C19	(S)-mephenytoin 4-hydroxy	100	—	0.2	0	not studied
				2.0	0	not studied
2D6	Bufuralol 1-hydroxy	10	—	0.2	6.1	not studied
				2.0	10.1	not studied
2E1	Chlorozoxazone 6-hydroxy	50	500	0.2	17	10.3
				2.0	51	10.4
3A4	Midazolam 1-hydroxy	10	60	0.2	18.6	0
				2.0	12.9	0

The inhibition of the various isozymes by alosetron in the low micromolar concentration range indicates that alosetron may be a very potent inhibitor of P450. For example, ketoconazole has a Ki for 3A4 in the range of 100 - 700 nM/L of free drug, whereas mean peak concentrations are 68 nM/L (See APPENDIX 1).

Like ketoconazole alosetron is an imidazole with type II binding to heme, and it's likely that it's the imidazole moiety that results in inhibition. Although, alosetron is substituted at the 4 and 5 positions of the imidazole ring, typically such substitutions result in less potent inhibitors than 1 substituted imidizoles such as ketoconazole. Due to this binding to heme one would expect any inhibition to be noncompetitive in nature, with changes in Vmax observed.

Peak concentrations of alosetron 1 mg po are generally less than 10 ng/ml, which would be equivalent to a concentration of 30.2 nM/L and free concentrations 6 nM/L. Although alosetron concentrations ranged up to 75 nM/L with a 1 mg dose (15 nM/L free drug). Consequently, concentrations achieved with clinical doses are so low that significant inhibition of P450's would likely only occur under limited circumstances.

Due to the much higher localized concentrations in the GI lumen compared to plasma, *in vivo* inhibition could occur with drugs that have extensive first pass due to metabolism by the gastrointestinal lumen.

This is significant with drugs with high intrinsic clearance by CYPIIIA4 and due to localization of CYPIIIA4 on the tips of the intestinal microvilli. *In vivo* inhibition of P450 isozymes would thus be mainly of clinical concern with narrow therapeutic index drugs in two situations, in overdoses and with drugs with high gastrointestinal first pass.

For overdoses, narrow therapeutic index drugs would be of the most concern. For CYPIA2 this would include compounds such as theophylline and other methylxanthines. Whereas for 3A4, this would include the usual compounds that have clinically significant interactions with ketoconazole, such as cisapride, midazolam, cyclosporine, etc..

2. *In Vivo* Inhibition by Alosetron

a) Metabolic Probes

Study S3BB1011

In study S3BB1011, the 29½ day time invariance study, the effect of alosetron on the elimination of various probe substrates was examined. Probe cocktail was administered on day -8, and 1 hour after the last dose of alosetron, when peak alosetron concentrations would be expected. Two subjects had peak concentrations of approximately 25 ng/ml on day 1 and approximately 15 ng/ml on day 29. A few other subjects had peak concentrations of around 15. Most others had peaks in 3 - 10 ng/ml range and a few had peak concentrations around 1 ng/ml.

Table 38, shows clinically significant inhibition of P450 IA2 with alosetron doses of 1 mg bid. This is somewhat inconsistent with the *in vivo* results with theophylline and may be a spurious finding, or may reflect inhibition by a metabolite(s), or a cumulative inhibitory effect with multiple dosing.

Table 38 Summary of Change in Probe-Drug Cocktail Ratios between Day -8 and Day 30

Probe Compound	Metabolite	Sample	Enzyme Probed	n	Mean Ratio (Day -8 : Day 30) 90% CI	p-Value	Conclusion
Caffeine	1,7-Dimethly-xanthine	8 hour plasma	IA2	20	1.48 (1.24, 1.77)	0.001	Inhibition
Chorzoxazone	6-hydroxy-chlorzoxazone	4 hour plasma	2E1	21	0.87 (0.72, 1.07)	0.257	No Change
Dapsone	Dapsone Hydroxylamine	0 - 8 hour urine	3A	24	0.97 (0.90, 1.05)	0.524	No Change
Mephenytoin	4-hydroxy-mephenytoin	0 - 8 hour urine	2C19	29	0.97 (0.79, 1.18)	0.775	No Change
Dapsone	Monoacetyl-dapsone		NAT2	19	1.43 (1.23, 1.65)	<0.001	Inhibition

Dapsone is a substrate for both CYP3A and NAT2. NAT2 is polymorphic and tends to metabolize complex arylamines and hydrazines such as sulfamethazine, procainamide, and hydralazine. The inhibition of NAT2 is not surprising, since alosetron is an imidazole and inhibition of NAT might be expected. The sponsor has indicated that since NAT proceeds via a ping-pong mechanism inhibition does not occur. This is clearly incorrect and probably a misunderstanding of the enzyme kinetics. Ping-pong mechanisms and NAT specifically, definitely can exhibit enzyme inhibition although the initial velocity rate plots in the presence of varying concentrations of inhibitors are atypical compared to what is seen with P450's.

NAT2 acetylation status may be clinically important with a number of xenobiotics. For example, phenelzine toxicity, isoniazid hepatotoxicity, hydrazine induced bladder cancer, and procainamide induced systemic lupus erythymatosis. In fact, the highest approved dose of hydralazine is limited because of toxicity at doses greater than 400 mg in slow acetylators. The distribution of slow and fast acetylators varies with ethnicity and is thus a factor to consider.

An isozyme that may be similar to NAT2 is found in high concentrations in the intestinal lumen and inhibition of this NAT could occur in the GI lumen.

Consideration should also be given to whether alosetron may inhibit NAT1. NAT1 metabolizes simple amines, and inhibition of small simple arylamines such as para-amino-salicylic acid in the GI tract, could possibly result in salicylate toxicity.

b) Theophylline (IA2)

Based upon this study, this reviewer concludes that there is no clinically significant inhibition of CYPIA2.

Study S3BA1004 administered alosetron 1 mg or placebo po bid x 15½ days, with theophylline 200 mg administered for the last 7½ days to healthy female volunteers. Pharmacokinetic metrics for theophylline and metabolites were determined after the last dose.

According to the sponsor, *'The results of this study showed no effect of alosetron on theophylline metabolism, measured in terms of either plasma theophylline concentrations or urinary recovery of theophylline and three metabolites (3-methyl-xanthine, 1-methyl-urate, and 1,3-dimethyl-urate). This result demonstrated the need for caution in interpreting earlier evidence that alosetron might diminish CYP1A2 activity. In addition to these results, a small increase in steady-state fluctuation was observed which, in the absence of increased peak concentrations, is unlikely to affect the potential for theophylline toxicity. Thus, this effect is not likely to be clinically significant.'*

Table 39 Effect of Alosetron on Theophylline and Theophylline Metabolite Kinetics (CYPIA2)

Parameter (Units)[a]	Placebo (n = 10)	1 mg Alosetron (n = 10)	LS Mean Ratio
Theophylline:			
C_{max} (μg/mL)	8.57 (7.20-10.22)	9.30 (7.81-11.09)	1.09
t_{max}[b] (h)	6.0 (1.0-8.4)	3.0 (0.5-8.0)	-1.50
AUC_{12} (mcg•h/mL)	91 (76-109)	93 (78-111)	1.02
$t_{½}$ (h)	8.2 (6.9-9.7)	8.8 (7.4-10.4)	1.07
Cmin (mcg/ml)	6.67 (5.43 - 8.20)	6.16 (5.10 - 7.57)	0.92
Fluctuation Index	0.21 (0.17 - 0.26))	0.32 (0.26 - 0.40)	1.54
Theophylline Ae12 (mg)	30.4mg (23.2-39.8)	35.5mg (27.1-46.5)	1.17
Metabolites:			
3-methyl xanthine Ae12 (mg)	25.8mg (20.3-32.8)	24.6mg (19.4-31.3)	0.96
1-methyl urate Ae12 (mg)	37.7mg (30.0-47.3)	40.6mg (32.4-50.9)	1.08
1,3-dimethyl urate Ae12 (mg)	97.5mg (87.9-108.2)	99.1mg (89.3-110.0)	1.02

a Values are Geometric Least Squares Means with (95% CI) except where noted
b median (range)

The *in vivo* results with theophylline are inconsistent with the *in vivo* probe study. However, since both were multiple dose studies (2 weeks and 4 weeks), this reviewer is inclined to believe the theophylline interaction study. This reviewer believes that any inhibition due to some sort of cumulative effect would probably be apparent to some extent at two weeks, yet there was no indication of a time dependence for inhibition in this study.

The 'inhibition' with the probe study could be due to caffeine intake by subjects, prior to the probe cocktail administration on day -8. Although there was some detection of caffeine and paraxanthine in predose urine samples, examination of the data indicate that the amounts present were minimal. Examination of the caffeine and paraxanthine data does not provide a clear answer to this apparent inhibition. Graphical analysis might be useful but was not employed.

c) Min Ovral

According to the sponsor, *'Study S3BA1002 was conducted to examine the potential effect of alosetron on a representative oral contraceptive containing ethinyl estradiol and levonorgestrel, owing to the likelihood of concomitant use. The results showed small decreases in steady-state plasma concentrations of ethinyl estradiol and levonorgestrel (9% and 13%, respectively) in the presence of alosetron. These changes were statistically significant, but are not likely to be clinically significant.'*

Table 40 Pharmacokinetic Metrics for Levonorgestrel and Ethinyl Estradiol in Presence and Absence of Alosetron

	Levonorgestrel			Ethinyl Estradiol		
	MIN-OVRAL QD x 21 days	MIN-OVRAL QD + 1mg alosetron BID x 21 days	Ratios	MIN-OVRAL QD x 21 days	MIN-OVRAL QD + 1mg alosetron BID x 21 days	Ratios
AUC** (ng•h/mL)	102.3 (84.9-123.1)	89.1 (74.0-107.3)	0.87* (0.78, 0.97)	1041 (897-1208)	948 (817-1101)	0.91* (0.85, 0.98)
C_{max} (ng/mL)	7.84 (6.64-9.26)	7.29 (6.18-8.61)	0.93 (0.85, 1.02)	104.8 (89.5-122.9)	102.4 (87.4-120.0)	0.98 (0.90, 1.06)
t_{max} (h)	1.27 (1.25-1.51)	1.51 (1.25-2.00)	0.49† (p = 0.136)‡	1.50 (1.25-1.51)	1.50 (1.26-1.75)	0.00† (p = 0.183)‡
$t_{½}$ (h)	32.8 (27.1-39.6)	32.5 (26.8-39.3)	0.99 (0.84, 1.16)	15.5 (13.5-17.6)	15.1 (13.2-17.3)	0.98 (0.89, 1.08)

All values of Cmax, tmax, AUC, $t_{½}$, are shown as geometric LS mean (95% confidence interval) except as noted:
* $p \leq 0.05$
** AUC24
† median difference in tmax
‡ p value for Wilcoxon signed rank test

d) Haloperidol

According to the sponsor, *'Study S3B-201 was conducted to examine the potential for interaction with haloperidol in support of a potential therapeutic indication for schizophrenia that was abandoned. The results showed no effect of alosetron on the pharmacokinetics of haloperidol at maintenance doses in schizophrenic patients.'*

Study S3B-201 was a randomized, double-blind placebo controlled crossover study in 13 schizophrenic subjects (11 male and 2 female) 26 to 62 years of age (See Table 41).

Table 41 Haloperidol Pharmacokinetic Metrics in the Presence and Absence of Alosetron

(n= 10)	Haloperidol 5-20mg QD + Alosetron 1 mg QD x 2 wks	Haloperidol 5-20mg QD + Placebo QD x 2 wks	ANOVA
C_{max} (ng/mL)	10.13 ± 5.72	8.69 ± 5.58	NS
t_{max} (hr)	2.50 ± 0.94	5.10 ± 3.81	NS
AUC_{24} (ng•h/mL)	123.6 ± 64.7	104.3 ± 70.0	NS
$t_{½}$ (hr)	15.03 ± 4.52	17.88 ± 12.91	NS

NS - not significant at 0.05 level

There was no differences in reduced haloperidol AUC's between treatment groups, or in reduced haloperidol to haloperidol AUC ratios.

e) Cisapride (CYPIIIA4)

Study S3BA1001 was a randomized, double-blind, placebo controlled, crossover trial comparing the pharmacokinetics and cardiac pharmacodynamics of cisapride in the presence and absence of alosetron 1 mg bid. The subjects included 12 healthy male and female volunteers (6/6 m/f) 19 to 44 years of age.

According to the sponsor, *'Study S3BA1001 was carried out, at the agency's request, to examine the potential effect of alosetron on cisapride metabolism. The results showed no effect of alosetron on cisapride plasma concentrations or the ratio of 6-β-hydroxycortisol to cortisol, an in vivo measure of the activity of CYP3A4, the primary enzyme responsible for metabolism of cisapride. In addition, no effect was observed on QTc interval duration, a measure of cardiotoxicity from elevated cisapride concentrations caused by metabolic inhibition of CYP3A4.'*