

**ATTACHMENT B.3**  
**INHIBITION STUDY: RESULTS**

## Results

Incubations were conducted three times for this study. In the initial incubations, (1) phenacetin was added in the incubations for the evaluation of CYP2A6 and incubated for 10 minutes instead of 30 minutes; (2) coumarin was added in the incubations for the evaluation of CYP1A2 and incubated for 30 minutes instead of 10 minutes; and (3) no substrate was included in the incubations containing 30  $\mu\text{M}$  metaxalone for the evaluation of CYP2B6. In the second inhibition, CYP3A4 activities in incubations containing ketoconazole failed to meet the criterion set in the protocol. Data generated from the failed or erroneous incubations are filed with the study documentation and not reported here.

### Positive Control

CYP3A4 activity in pooled human liver microsomes was characterized by the formation of 6 $\beta$ -hydroxytestosterone from testosterone. The mean specific activity of CYP3A4 in the positive control samples treated with 1  $\mu\text{M}$  ketoconazole was <13.5% and <11.8% (Table 1) of the mean specific activity in the corresponding vehicle control (VC) in the initial and third incubations, respectively, which meets the criterion stated in the protocol.

### CYP1A2

CYP1A2 activity in pooled human liver microsomes was characterized by the formation of acetaminophen from phenacetin. The mean CYP1A2 activities in samples treated with metaxalone at the concentrations of 0.3, 1, 3, 30, and 100  $\mu\text{M}$  were 97.2, 92.3, 90.3, 89.8, and 82.0% of the mean CYP1A2 activity in the VC, respectively (Table 2). Metaxalone inhibited CYP1A2 activity in a dose-dependent pattern. The IC<sub>50</sub> value is greater than 100  $\mu\text{M}$  since the inhibition was only 18% at the concentration of 100  $\mu\text{M}$ . No chromatographic interference from metaxalone was detected with the assay method (data not shown).

### CYP2A6

CYP2A6 activity in pooled human liver microsomes was characterized by the formation of 7-hydroxycoumarin from coumarin. The mean CYP2A6 activities in samples treated with metaxalone at the concentrations of 0.3, 1, 3, 30, and 100  $\mu\text{M}$  were 96.9, 95.7, 102, 107, and 94.8% of the mean CYP2A6 activity in the VC, respectively (Table 3). The decrease in CYP2A6 activity observed in the presence of 0.3, 1, and 100  $\mu\text{M}$  metaxalone was not statistically significant ( $p > 0.05$ , unpaired two-tailed t test). Therefore, metaxalone did not inhibit CYP2A6 activity in pooled human liver microsomes under the conditions tested. No chromatographic interference from metaxalone was detected with the assay method (data not shown).

### CYP2B6

CYP2B6 activity in pooled human liver microsomes was characterized by the formation of nirvanol from *S*-mephenytoin. The mean CYP2B6 activities in samples treated with metaxalone at the concentrations of 0.3, 1, 3, 30, and 100  $\mu\text{M}$  were 90.2, 99.5, 90.6, 96.6, and 83.7% of the mean CYP2B6 activity in the VC, respectively (Table 4). The decrease of CYP2B6 activity following treatment with metaxalone at the concentrations of 1 and 30  $\mu\text{M}$  was not statistically significant ( $p > 0.05$ ; unpaired two-tailed *t* test). The maximal inhibition observed at 100  $\mu\text{M}$  of metaxalone was 16.3%. No chromatographic interference from metaxalone was detected with the assay method (data not shown).

### CYP2C8

CYP2C8 activity in pooled human liver microsomes was characterized by the formation of 6-hydroxypaclitaxol from paclitaxol. The mean CYP2C8 activities in samples treated with metaxalone at the concentrations of 0.3, 1, 3, 30, and 100  $\mu\text{M}$  were 92.7, 103, 109, 101, and 97.8% of the mean CYP2C8 activity in the VC, respectively (Table 5). The apparent decrease of CYP2C8 activity following treatment with metaxalone at the concentrations of 0.3 and 100  $\mu\text{M}$  was not statistically significant ( $p > 0.05$ ; unpaired two-tailed *t* test). Therefore, metaxalone did not inhibit CYP2C8 activity in pooled human liver microsomes under the conditions tested. No chromatographic interference from metaxalone was detected with the assay method (data not shown).

### CYP2C9

CYP2C9 activity in pooled human liver microsomes was characterized by the formation of 4'-methylhydroxytolbutamide from tolbutamide. The mean CYP2C9 activities in samples treated with metaxalone at the concentrations of 0.3, 1, 3, 30, and 100  $\mu\text{M}$  were 81.4, 81.6, 81.2, 91.0, and 87.4% of the mean CYP2C9 activity in the VC, respectively (Table 6). The apparent decrease of CYP2C9 activity following treatment with metaxalone was not statistically significant ( $p > 0.05$ ; unpaired two-tailed *t* test). Therefore, metaxalone did not inhibit CYP2C9 activity in pooled human liver microsomes under the conditions tested. No chromatographic interference from metaxalone was detected with the assay method (data not shown).

### CYP2C19

CYP2C19 activity in pooled human liver microsomes was characterized by the formation of 4'-hydroxymephenytoin from *S*-mephenytoin. The mean CYP2C19 activities in samples treated with metaxalone at the concentrations of 0.3, 1, 3, 30, and 100  $\mu\text{M}$  were 84.6, 67.0, 67.7, 63.8, and 64.9% of the mean CYP2C19 activity in the VC, respectively (Table 7). Therefore, metaxalone inhibited CYP2C19 activity in pooled human liver microsomes under the conditions tested. The inhibition ranged from 15.4% to 36.2%. The  $\text{IC}_{50}$  value is greater than 100  $\mu\text{M}$ . No chromatographic interference from metaxalone was detected with the assay method (data not shown).

### **CYP2D6**

CYP2D6 activity in pooled human liver microsomes was characterized by the formation of dextrorphan from dextromethorphan. The mean CYP2D6 activities in samples treated with metaxalone at the concentrations of 0.3, 1, 3, 30, and 100  $\mu$ M were 81.3, 84.2, 89.3, 85.1, and 85.9% of the mean CYP2D6 activity in the VC, respectively (Table 8). Therefore, metaxalone inhibited CYP2D6 activity in pooled human liver microsomes under the conditions tested. The inhibition ranged from 10.7% to 18.7% and was not dose-dependent. No chromatographic interference from metaxalone was detected with the assay method (data not shown).

### **CYP2E1**

CYP2E1 activity in pooled human liver microsomes was characterized by the formation of 6-hydroxychlorzoxazone from chlorzoxazone. The mean CYP2E1 activities in samples treated with metaxalone at the concentrations of 0.3, 1, 3, 30, and 100  $\mu$ M were 83.3, 84.0, 88.6, 86.1, and 87.2% of the mean CYP2E1 activity in the VC, respectively (Table 9). Therefore, metaxalone inhibited CYP2E1 activity in pooled human liver microsomes under the conditions tested. The inhibition ranged from 11.4% to 16.7% and was not dose-dependent. No chromatographic interference from metaxalone was detected with the assay method (data not shown).

### **CYP3A4**

CYP3A4 activity in pooled human liver microsomes was characterized by the formation of 6 $\beta$ -hydroxytestosterone from testosterone. The mean CYP3A4 activities in samples treated with metaxalone at the concentrations of 0.3, 1, 3, 30, and 100  $\mu$ M were 87.1, 88.1, 90.0, 85.2, and 80.3% of the mean CYP3A4 activity in the VC, respectively (Table 10). Therefore, metaxalone inhibited CYP3A4 activity in pooled human liver microsomes under the conditions tested. The inhibition ranged from 10.0% to 19.7%. No chromatographic interference from metaxalone was detected with the assay method (data not shown).

## **Conclusions**

Metaxalone inhibited activities of CYP1A2, CYP2B6, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in human liver microsomes. The inhibition ranged from 12.8% (CYP2E1) to 35.1% (CYP2C19) at the concentration of 100  $\mu$ M.

Metaxalone did not inhibit activities of CYP2A6, CYP2C8, and CYP2C9 in human liver microsomes.