Acetaminophen

All rats receiving acetaminophen survived the one-day dosing period. Acetaminophen was generally well tolerated with no adverse clinical signs observed in any of the control or treated groups. Significant treatment-related decreases in group mean body weights were observed at the second interim and final necropsies. Clinical chemistry summaries for six relevant analytes indicative of hepatic effects are summarized in Table 1.

There were no treatment-related gross findings in rats given acetaminophen and no direct treatment-related changes in liver weights. At the second interim necropsy (18 hours post dose), an increase in the mean relative liver weight of treated rats was statistically significant (about 1.08X control mean) but this slight difference was attributed to decreased terminal body weight. Mean absolute liver weights were not affected by treatment at any time point.

The principal treatment-related histopathologic finding was centrilobular necrosis evident in 5 of 5 rats at 18 hours post dose. In addition to coagulative necrosis, centrilobular necrosis also included mixed inflammatory cell infiltration, congestion, hemorrhage, and/or single cell necrosis. Slightly increased numbers of hepatocytes with mitotic figures were evident in controls, as compared to treated rats. The relevance of this finding is unknown since occasional hepatocyte mitoses are not uncommon in laboratory rodents. Many control livers also had slight focal or multifocal infiltration of mononuclear cells.
Mononuclear cell infiltration of the liver is a common spontaneous background change in laboratory rodents (8).

In summary, treatment with acetaminophen, which causes hepatocellular death at high acute doses, resulted in significant ALT elevations 18 hours post-dose, but not at 8 hours or 72 hours post-dose. Several hematology endpoints also increased, and body weight decrements were noted. The principal treatment-related histopathologic finding was centrilobular necrosis evident in 5 of 5 rats at 18 hours post dose. The experimental design was successful; acute administration of acetaminophen resulted in progressive hepatocellular necrosis, an effect that subsided after a 54 hour recovery period.

ANIT
All rats receiving ANIT survived the 12, 48, or 120 hour post-dosing period. ANIT was generally well tolerated at all dose levels. Group mean absolute and/or relative liver weights were significantly increased in treated rats compared to controls at the second interim and final necropsies. Group mean body weights were significantly decreased in treated rats compared to control animals at the first interim and final necropsies. Numerous serum markers and hematology parameters were significantly altered by drug treatment, especially at 48 hours post-dose. Summaries for six relevant analytes are tabulated in Table 1.

There were no treatment-related gross findings in rats given ANIT. Group mean absolute and/or relative liver weights were significantly increased in treated rats compared to
controls at the second interim and final necropsies. The increased weights were greatest at the second interim necropsy (about 1.1 to 1.2X control mean at 48 hours post dose); at the final necropsy (120 hours post dose) absolute weight was similar to control mean, but relative liver weight remained increased due to decreased terminal body weights.

Twelve hours post dose, one treated rat had slight multifocal single cell hepatocyte necrosis and another treated rat had pericholangial inflammation (two portal areas within the sections contained scattered inflammatory cells and were edematous). At 48 hours post dose, 4 of 5 treated rats had single cell hepatocyte necrosis, and all five treated rats had pericholangial inflammation. At this time point, the inflammatory change was qualitatively similar to that seen at the earlier time point (acute inflammation), but the severity was increased (increased inflammatory cells with edema, and greater number of pericholangial areas affected). At 120 hours post dose, single cell hepatocyte necrosis was absent but pericholangial inflammation persisted (5 of 5 affected). At this time point, pericholangial inflammation appeared more subacute (mixed inflammatory cells with more mononuclear cells) but the inflammation had diminished in severity (less edematous, fewer areas affected).

ANIT selectively damages bile ducts. In this study, numerous serum markers indicative of liver damage were significantly elevated at the midpoint (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, 5’ nucleotidase, total bilirubin, bile acids), with recovery noted for most as expected. Group mean absolute and/or relative liver weights were significantly increased in treated rats compared to controls at the
second interim and final necropsies. In the fulminant dose group, 4 of 5 treated rats had single cell hepatocyte necrosis, and all five treated rats had pericholangial inflammation. These results confirmed the adequacy of the experimental design for modeling cholestatic injury.

**Phenobarbital**

All rats receiving phenobarbital survived the one to three-day dosing period. Phenobarbital was generally well tolerated with no adverse clinical signs observed in any of the control or treated groups other than decreased activity. No significant treatment-related changes in group mean body weights were observed at either interim necropsy when comparisons were made to concurrent control animals; however, at the 3rd and final necropsy, a significant decrease in mean body weight was noted in the treated animals. Although total was protein decreased 10% on day 2 and cholesterol was increased 50% on day 5, when compared to concurrent vehicle controls, no statistically significant changes in the group means were noted for the six clinical chemistry markers tabulated in Table 1.

There were no treatment-related gross findings in rats given phenobarbital. Group mean absolute and/or relative liver weights were significantly increased in treated rats compared to controls at all time points. The increased weights were greatest at the second interim necropsy, but were approaching baseline at the final necropsy. Two days post dose, liver weights were increased about 1.1X control mean, whereas at five days,
the increase was about 1.5X control mean. At the final necropsy (8 days post dose), absolute liver weights were about the same as control, but relative liver weight was still increased about 1.06X control mean. Increased liver weights were attributable to centrilobular hypertrophy, which was evident in 5 of 5 treated rats two days post dose. Increased numbers of hepatocytes with either apoptotic bodies or mitotic figures accompanied centrilobular hypertrophy in 3 of 5 treated rats at the same time point. These histopathologic findings were not evident in livers obtained at either two or twelve days.

Treatment with Phenobarbital, a potent cytochrome P450 inducer, resulted in significant increases in absolute and relative liver weights, particularly in the intermediate necropsy group. These results indicated that the experimental design was successful in producing hepatomegaly.

Wy-14,643

All rats receiving Wy-14, 643 survived the one-day or 3-day dosing period. Wy-14, 643 was generally well tolerated with no adverse clinical signs observed in any of the control or treated groups. No significant treatment-related changes in group mean body weights were observed at interim or final necropsies when comparisons were made to concurrent control animals. Statistically significant changes in the group means were noted for several clinical chemistry markers when compared to concurrent vehicle controls. These are summarized in Table 1.
There were no treatment-related gross findings in rats given Wy-14, 643. Group mean absolute and/or relative liver weights were increased in treated rats compared to controls at all time points, but not all increases were statistically significant. The increased weights were greatest at the second interim necropsy, but were approaching baseline at the final necropsy. Two days post dose, liver weights were increased about 1.1-1.2X control mean, whereas four days post dose, the increase was about 1.4X control mean. At the final necropsy (fourteen days post dose), absolute liver weights were about the same as control, but relative liver weight was still increased about 1.1X control mean.

Two days post dose, the principal histopathologic change was an increased number of hepatocytes with mitotic figures (4 of 5). Four days post dose, histopathologic changes were limited to centrilobular hypertrophy and karyomegaly in 5 of 5 treated rats. Increased liver weights were attributed to these changes. Hypertrophy and karyomegaly persisted at the fourteen-day necropsy; however, there was a reduction in severity of hypertrophy and in the incidence of karyomegaly. A few rats in both control and treated groups had slight multifocal infiltration of mononuclear cells in the liver. The incidence was essentially similar between control and treated groups. Mononuclear cell infiltration of the liver is a common spontaneous background change in laboratory rodents.

Treatment with Wy-14, 643, which causes hepatic cell proliferation and peroxisome proliferation in rodents, resulted in significant increases in absolute and relative liver weight, particularly in the intermediate necropsy group. Depressed hematology indicators suggested hematotoxicity after 4 days and serum clinical chemistry markers
suggested a complex effect on liver function with depression of one hepatic enzyme noted at all three time points. These results indicated that the experimental design adequately modeled hepatomegaly.