In Item 1, Section 7, new clinical safety data are provided from three prospective placebo-controlled studies of acetaminophen administered at maximum-labeled doses over two-to-five days in chronic alcohol abusers under controlled conditions. Subjects began acetaminophen dosing within 24 to 48 hours after cessation of drinking. This period has been proposed as the time within which the risk of acetaminophen hepatotoxicity is highest based primarily on metabolism studies in animal models with ethanol and toxic doses of acetaminophen.

5.5 Overview of Acetaminophen Metabolism and Toxicology

Acetaminophen undergoes mixed-competitive and sequential biotransformation as outlined in Scheme 1. Only about 2% to 5% of a dose is excreted unchanged in urine. Acetaminophen is mainly conjugated with glucuronic acid by UGT enzymes, specifically the isoforms UGT1A6 and UGT1A9 [8,9]. It is also a substrate for two sulfotransferases, SULT1A1 and SULT1A3 [10,11]. Sulfation of acetaminophen is partly governed by the availability of inorganic sulfate, which is rate limiting in the formation of the cofactor of sulfation, 3'-phosphoadenosine-5'phosphosulfate (PAPS). The other rate-limiting reaction is sulfotransferase activity. A small fraction of an acetaminophen dose is oxidized by cytochrome P4502A6 (CYP2A6) to form stable nontoxic catechols eventually found in the urine as sulfate and glucuronide conjugates [12].

Scheme 1. Acetaminophen Metabolism†

Enzyme	Cofactor	Amount Formed	Metabolite
UGT1A6 UGT1A9	glycuronic acid	45 to 60%	Glucuronide
SULT1A1 SULT1A3	sulfate PAPS	25 to 35%	Sulfate
CYP2E1	glutathione	5 to 10%	Thiols
CYP2A6		3 to 6%	Catechols

† The balance of an acetaminophen dose is excreted as acetaminophen in the urine.

About 5% to 10% of an acetaminophen dose is oxidized by cytochrome P4502E1 (CYP2E1) to produce N-acetyl-p-benzoquinoneimine (NAPQI) [13], a highly reactive, short-lived electrophile, which is conjugated with glutathione. This conjugate is subsequently cleaved to result in chemically stable, nontoxic thiol metabolites: the cysteine, mercapturate, methylthio, and methanesulfinyl adducts of acetaminophen. The contribution of CYP isoenzymes, other than CYP2E1, to NAPQI formation is negligible *in vivo* in humans and is clinically insignificant [14]. This fact is crucial to navigating the differences among animal studies, *in vitro* studies in human liver microsomes, and expressed human CYP isoforms that do not frequently correlate well with *in vivo* observations, even though they are often used as a means to suggest forms responsible for *in vivo* human drug metabolism.

The toxicity profile of acetaminophen in acute overdoses, in which excessive amounts are consumed within a short time, has been well characterized [15]. Clinical evidence demonstrates that glucuronidation of acetaminophen is not saturated, even among those individuals who have taken considerable overdoses. However, if life-threatening injury is observed, drug metabolism including glucuronidation is substantially slowed, but not saturated [16,17] in the Michelis-Menten manner. The reported urinary excretion of the glucuronide metabolite ranges from 45% to 60% of therapeutic doses and from 40% to 75% of overdoses above 137 mg/kg (approximately 10g) [18,19]. In contrast, sulfate conjugation is capacity-limited at higher doses as evidenced by a decrease in the fractional urinary excretion of sulfate metabolites [20]. The mechanism seems to be depletion of activated sulfate for conjugation and, perhaps, actual saturation of the enzyme at very high doses.

Because of the limited capacity of sulfation at higher doses, the fraction of an acetaminophen dose metabolized by glucuronidation and CYP2E1 oxidation to NAPQI increases. As hepatic glutathione is consumed by NAPQI, it is continuously replenished at an estimated rate of 20 to 30 μmol/min in humans, corresponding to approximately 14 g/d [1,21,22]. The rate of consumption must substantially exceed this rate of production for an appreciable period to cause enough depletion of glutathione to allow necrosis to occur from free NAPQI. The exact mechanisms by which NAPQI causes hepatocellular death are not completely understood, but seem to include oxidative stress, loss of function of critical macromolecules due to NAPQI adduct formation, blebbing of the cellular and mitochondrial membranes, and loss of calcium homeostasis. The resulting hepatocellular necrosis is observed centrilobularly. N-acetylcysteine replenishes glutathione and protects against necrotic cell death if administered early enough in the sequence of events [15,23].

5.6 Hierarchy of Evidence-Based Scientific and Clinical Data

Diminished hepatic glutathione concentrations and/or enhanced CYP2E1 activity have been hypothesized to increase the risk of acetaminophen hepatotoxicity at therapeutic doses. For example, these metabolic alterations have been said to increase risk in adults who drink moderate-to-excessive quantities of alcohol. Lower glutathione concentrations have also been said to increase risk in adults with liver disease, human immunodeficiency virus (HIV) infection, malnutrition, and when fasting. Alterations in other acetaminophen metabolic pathways have been hypothesized to increase the risk of hepatotoxicity, such as the impaired ability to conjugate xenobiotics via glucuronidation in individuals with Gilbert's Syndrome. These speculations are often based on a superficial construction of theory that has not been upheld in direct experimentation nor refined as new evidence becomes available that more fully and reliably adds to our understanding of acetaminophen metabolism.

A renowned early investigator in the area of glutathione research, Bernard Lauterburg, recently critiqued the experimental and clinical data pertaining to those populations supposed to have enhanced risk of acetaminophen-induced injury due to diminished abundance of hepatocellular glutathione [1]. He argues that true depletion of glutathione, defined as an intracellular concentration below 10% of normal, has not been documented in humans aside from states of intoxication by some drugs, including excessive overdoses of acetaminophen. Lauterburg further concludes that, "except for anecdotal reports, there is no convincing evidence that other populations [those other than cases of actual drug overdose] in which low glutathione has been observed—such as patients with human immunodeficiency virus (HIV) infection or chronic hepatitis C, malnourished patients, and patients with cirrhosis—are at higher risk of experiencing adverse events from acetaminophen."

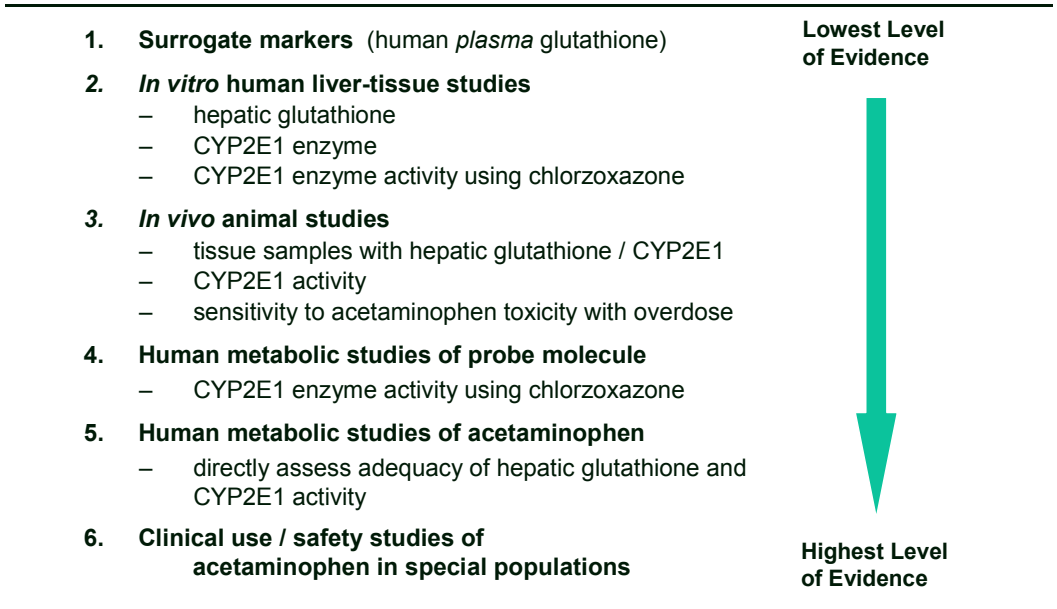
Further supporting Dr. Lauterburg's assertion that acetaminophen use is not inherently more risky in these populations is the high incidence of these conditions in the adult American population and the ubiquitous use of acetaminophen estimated at 20% of the adult US population (approximately 45 million adults) each week, according to 2005 Slone Survey data [24]. Considering the widespread awareness among health care professionals of the possibility of liver injury being caused by acetaminophen, there is the absence of anything substantial in these populations beyond isolated case reports.

Extrapolation of metabolic theories to increased risks of hepatotoxicity is largely based upon the assumption that any alteration in acetaminophen metabolism is clinically meaningful. In fact, the weight of experimental and clinical evidence described in this section runs counter to such supposition and does not suggest that populations with mildly altered acetaminophen metabolism are at risk of hepatotoxicity from therapeutic doses. Alterations that have been linked to disease states are indeed minor. However, data pertaining to increased risk following considerable acetaminophen overdose in certain populations is less clear.

In a 2004 review [2] of misconceptions associated with acetaminophen metabolism and toxicity at therapeutic doses, Rumack highlights the seemingly discrepant data from *in vitro*, animal, and *in vivo* human studies that make extrapolation to the clinical setting problematic. He suggests the following aspects of the literature should be considered when evaluating safety issues: animal to human data validity and species differences; *in vitro* versus *in vivo* data; class I versus class II or III literature; and case selection bias including confounding variables.

A hierarchy of evidence-based experimental and clinical data that has been used to suggest diminished clinical safety at therapeutic doses is outlined in Scheme 2. Suppositions on risk from altered glutathione abundance and CYP2E1 protein or activity have been generated primarily from studies in which relatively minor differences are concluded to have clinical significance. These data will be reviewed in this section.

Scheme 2. Hierarchy of evidence-based science and clinical data



5.7 Effect of Liver Disease on Glutathione and CYP2E1 Levels/Activity

5.7.1 Organization of Long-Standing and New Study Data

When generating hypotheses on potential effects of liver disease on acetaminophen metabolism, results from different CYP2E1 and hepatic glutathione studies should be evaluated simultaneously rather than in isolation [2]. Because CYP2E1 and hepatic glutathione contribute to the formation and detoxification, respectively, of NAPQI from acetaminophen, the effect of liver disease on both are relevant to the characterization of liver safety. This dual consideration was recently addressed in a review on the therapeutic use of acetaminophen in patients with liver disease [25].

The etiology and severity of liver disease can influence levels of plasma and hepatic glutathione, and of CYP2E1 protein abundance and activity. Investigators often group patients differently, which makes organizing data for assessment and interpretation challenging. Other factors may influence the measured values, including underlying genetics, diet, multiple medical conditions, nutritional status, and length of abstinence from alcohol before liver biopsy, to name a few. Details of such factors are often omitted from published reports.

To facilitate review of key findings, the long-standing and new study data in liver disease are grouped loosely into four categories: viral hepatitis, alcoholic liver disease, nonalcoholic fatty liver disease (NAFLD), and cirrhosis (various etiologies). Within these categories, the effect of liver disease on glutathione and CYP2E1 concentrations is described in the context of the hierarchy of evidence-based experimental and clinical data. Some data fit into more than one category, and are addressed, as appropriate, in each section.

5.7.2 Viral Hepatitis

Plasma glutathione concentrations are considered a surrogate marker for hepatic glutathione concentrations, as the two have been correlated [26]. This finding is consistent with the liver being the major source of circulating glutathione. The analytical measurement of plasma glutathione is not trivial in that erythrocyte glutathione concentration is approximately 100 times that of plasma. Thus, small amounts of hemolysis in a blood sample can substantially contaminate the measurement. On the other hand, glutathione in a sample can readily oxidize to form glutathione disulfide and mixed disulfides if not controlled.

Plasma glutathione concentrations were reported to be less than 10% of normal [27] in a study of adults with hepatitis C, a level that many would expect to put hepatocyte mitochondria in a life-threatening state of oxidative stress. In contrast, several other research groups could not confirm this observation in their studies of adults with hepatitis C, where plasma concentrations were normal [28], slightly higher [29], or only moderately lower than those of healthy adults [26]. The severe depletion reported in the first study cited, the number of studies that report very different observations, and the challenge of analyzing glutathione in plasma suggest that this finding is unreliable.

The approximately normal plasma concentrations found in adults with hepatitis C are consistent with hepatic concentrations determined from liver tissue biopsies that were also in the normal range, despite the presence of mild-to-moderate inflammation due to viral hepatitis [30]. In another study [31], hepatic glutathione concentrations measured in adults with chronic viral or alcoholic hepatitis were higher than those in biopsy specimens from normal livers (2.27 ± 0.42 versus 1.05 ± 0.11 μmol/g liver).

Chlorzoxazone is used as a specific *in vivo* probe for CYP2E1 activity because the CYP2E1 enzyme oxidizes about 75% of a dose to 6-hydroxychlorzoxazone [32]. Chlorzoxazone clearance from the body and the ratio of 6-hydroxy metabolite to chlorzoxazone (an putative surrogate for chlorzoxazone clearance) were estimated in 14 adults with chronic hepatitis C as a measure of CYP2E1 activity [33]. None of the enrolled subjects consumed alcohol regularly, and Ludwig's score was used to grade liver biopsy specimens for severity of lobular and portal inflammation, and the degree of fibrosis. These adults exhibited relatively mild hepatic impairment with low scores for inflammation and fibrosis, and their CYP2E1 activity ($CL/F = 21.5 \pm 10.1$ L/h) was similar to that reported for healthy adults without liver disease. This finding is consistent with plasma and hepatic glutathione concentrations being within normal ranges in adults with chronic hepatitis C infection.

Taken together, these experimental and clinical studies show that adults with chronic hepatitis C infection have plasma and hepatic glutathione concentrations within normal ranges. In addition, CYP2E1 activity measured clinically with the chlorzoxazone probe is very similar to healthy adults. However, the production of glutathione in chronic hepatitis C patients who have progressed to cirrhosis is about 50% slower compared with healthy adults [22]. Yet, in cirrhosis, *in vivo* metabolism studies show that CYP2E1 activity is similarly decreased, as discussed further in Section 5.7.5. Because CYP2E1 and hepatic glutathione contribute to the formation and detoxification of NAPQI from acetaminophen,

the effect of liver disease on both are equally important to consider. In cirrhosis, both glutathione and CYP2E1 activity are decreased.

5.7.3 Alcoholic Liver Disease

Alcoholic liver disease includes cases of alcoholic steatosis (fatty liver), hepatitis, fibrosis, and cirrhosis. Plasma glutathione concentrations of chronic alcohol abusers with histological fatty liver (2.7 ± 1.2 nmol/mL) and biopsy-proven cirrhosis (2.1 ± 1.3 nmol/mL) were statistically lower than control subjects (8.5 ± 2.6 nmol/mL) [34]. Reported values of hepatic glutathione concentrations are limited and may depend on the severity of disease and the time at which alcohol drinking had ceased before biopsy. Hepatic glutathione concentrations were higher than normal controls in patients with chronic hepatitis (6/12 alcoholic) by 116% in one study [31], and with chronic hepatitis by 42% in another study [30]. By contrast, hepatic glutathione concentrations in a group of adults having alcohol-induced disease were 44% lower than nonalcoholic control subjects [35]. In more severe alcoholic liver disease, hepatic glutathione was 62% lower in a group comprised mainly of adults (5/8) who had progressed to cirrhosis from alcoholic hepatitis [36].

Measurements of hepatic glutathione have been compared among subgroups of chronic alcohol abusers by the degree of histological alterations (steatosis versus hepatitis-fibrosis-cirrhosis) and by the presence or absence of biochemical test abnormalities [37]. Although mean values were lower than that in normal livers, no differences were detected among these subgroups, confirming that hepatic glutathione is a poor index of mild versus advanced alcohol-induced liver disease [37].

These studies show that adults with alcoholic liver disease generally have lower-than-normal concentrations of plasma and hepatic glutathione, although some studies have found higher hepatic concentrations. Nevertheless, CYP2E1 mRNA levels were reduced nearly 70% along with a decrease in hepatic glutathione concentrations in a study of six chronic alcohol abusers with biopsy-proven alcoholic liver disease (stable hepatitis with either fibrosis or cirrhosis) that simultaneously considered CYP2E1 expression [38]. The investigators suggest that reasons for lower hepatic glutathione concentrations are likely multifactorial in chronic alcohol abusers, including malnutrition, decreased precursor availability, and decreased capacity to synthesize glutathione, and that malnutrition may also cause reduced CYP2E1 expression. In fact, the CYP2E1 protein content of 21 control and 50 cirrhotic liver specimens were shown to be negatively correlated with nutritional status using multivariate analysis ($p < 0.02$) [39]. The regression equation in Table 5.1

shows the relationship of decreasing CYP2E1 protein with poor nutrition. Again, these latter findings underscore the need to consider several studies on the effect of liver disease on both hepatic glutathione and CYP2E1 concentrations and activity.

Table 5.1 Influence of Nutritional Status on CYP2E1 Protein from Control and Diseased Livers

CYP2E1 Protein (%) = 119.98 – 0.63 × age (y)	– 13.75 (if normal nutrition) – 27.50 (if impaired nutrition) – 41.25 (if cachectic) – 8.82 (if hepatocellular liver disease) – 41.25 (if cholestatic liver disease)

The metabolic ratio of chlorzoxazone was used as a probe for CYP2E1 activity in a metabolism study of adults with alcoholic liver disease [40]. Alcohol-abstaining adults were sorted into three groups with increasing degrees of liver damage: alcoholic fatty liver (n=17), hepatitis (n=11), and cirrhosis (n=20). When not induced by alcohol, no differences in CYP2E1 activity were detected between healthy subjects and the adults with alcoholic fatty liver or hepatitis. By contrast, CYP2E1 activity was decreased by more than 50% in the group with alcoholic cirrhosis having moderate-to-severe impairment. The major finding of this study was the decreasing CYP2E1 activity in alcohol-abstaining adults with progressively severe manifestations of alcoholic liver disease ($p = 0.0008$). All 48 adults with alcoholic liver disease were included in the latter analysis, and the metabolic chlorzoxazone ratio ranged from 0.50 ± 0.28 in 14 healthy controls to 0.19 ± 0.10 in 20 the alcoholic cirrhotic subjects.

Collectively, results from the experimental and chlorzoxazone-probe clinical studies show that both hepatic glutathione concentrations and CYP2E1 concentrations/activity decrease in alcohol-abstaining adults with increasing severity of alcoholic liver disease. Therefore, the amount of toxic intermediate, NAPQI, formed from therapeutic doses of acetaminophen would be expected to be within normal ranges, or even less for alcoholic-abstaining adults who have progressed to more severe cirrhosis. Clinical evidence supporting this hypothesis is provided from a metabolism study of acetaminophen and disulfiram in five adults with compensated, biopsy-proven, alcoholic cirrhosis [41]. The investigators found that the formation clearance of thiols, representing detoxified NAPQI, was comparable to that in five control subjects (47 ± 35 and 44 ± 21 mL/min, respectively). Although the study size is small, it refutes the supposition that risk is greater in patients with alcoholic cirrhosis.

Additional clinical evidence is provided in Item 1, Section 6 from other acetaminophen metabolism studies of adults with advanced liver disease showing no change in thiols. In these latter studies, investigators generally combine data for alcoholic liver disease and cirrhosis of various etiologies.

5.7.4 Nonalcoholic Fatty Acid Liver Disease (NAFLD)

NAFLD has a histological spectrum ranging from generally benign fatty liver to bland steatosis with inflammation to damage (ie, nonalcoholic steatohepatitis, or NASH, with or without cirrhosis). Only limited information on plasma and hepatic glutathione specifically attributed to NAFLD is available, because most data are grouped in studies with liver diseases of different etiologies. Hepatic glutathione associated with steatosis of unknown etiology was modestly lower than normal by 22% in one study [30], but modestly higher by 53% in another study [31]. Other information on hepatic glutathione is available from a study of hepatocytes from fatty mouse livers in which the investigators found mitochondrial adaptations to obesity-related chronic oxidant stress [42]. Compared with mitochondria from normal livers, the fatty-liver mitochondria had 25% more glutathione ($p < 0.005$).

To date, there is only one published study by Weltman et al. on CYP2E1 in adults with NASH [43]. Protein concentrations of CYP2E1 in liver biopsy specimens from 31 subjects with NASH were higher than those for 10 control subjects. However, the estimated concentrations were only semiquantitative in that the intensity of immunostaining was assigned a categorical score from 0 (no specific staining) to +3 (intense staining).

The same research group had earlier suggested a connection between CYP2E1 and NASH based on experimental data from a rat nutritional model of hepatic steatosis [44]. They fed rats a diet devoid of methionine-choline for four weeks resulting in severe macrovesicular steatosis and inflammation. Microsomal CYP2E1 protein and hepatic CYP2E1 messenger RNA levels were increased in the rat livers. Additionally, wild-type mice fed the methionine-choline-deficient diet produces hepatic steatosis and inflammation [45]. In this animal model, CYP2E1 was also induced, but after 10 weeks of feeding.

In contrast, another mouse model of NASH produced with a different diet has shown opposite findings to the methionine-choline-deficient diet where CYP2E1 levels are actually decreased [46]. About half the obese mice fed a high-fat diet for nine weeks developed steatohepatitis with increased alanine aminotransferase, neutrophilic infiltration, and fibrosis. The obese mice had a 63% reduction in CYP2E1 concentrations and decreased

activity, as assessed by the generation of 6-hydroxychlorzoxazone ($p < 0.05$). In addition, CYP4A was increased 2.5 fold, which further supports a recent report [45] that induction of the isozymes, CYP4A10 and CYP4A14, plays a pivotal role in lipid peroxidation in experimental NASH in *Cyp2e1*-knockout mice.

In the study [45] of *Cyp2e1*-knockout mice, the methionine-choline-deficient diet was used to produce experimental NASH, and surprisingly, mice lacking CYP2E1 displayed comparable or worse liver injury than controls on the same diet. The investigators also found that there was significant up-regulation of both CYP4A10 and CYP4A14. One possibility for CYP2E1 induction in mice fed the methionine-choline-deficient diet as opposed to decreases in CYP2E1 concentrations and activity in obese mice fed the high-fat diets, is that the former diet imposes a more potent oxidant stress on the liver, which questions the validity of extrapolating this model and its findings to NASH in humans.

More recently, another *in vivo* model showed that high-dose acetaminophen hepatotoxicity was attenuated in mice with dietary steatotic liver [47]. The mice were fed either normal chow or a western high-fat/high carbohydrate diet for four months, producing severe hepatic steatosis. Compared with the controls, they had about 90% lower ALT activity, less centrilobular injury, and a 42% decrease in CYP2E1 mRNA concentrations after a hepatotoxic overdose of acetaminophen.

Chlorzoxazone has been used as a probe for CYP2E1 activity in nondiabetic adults with biopsy-proven NASH [48] and in morbidly obese adults with NAFLD [49]. Hepatic activity of CYP2E1, as reflected by the oral clearance of chlorzoxazone, was a modest 24% higher ($p< 0.03$) in the NASH group [48]. Importantly, the amount of chlorzoxazone transformed to metabolite was similar to the control group comprised of adults who had been matched for body mass index. In the morbidly obese subjects with NALFD, oral chlorzoxazone clearance was about 3-fold higher compared with controls ($p < 0.001$) [49]. However, the latter study did not account for the strong direct relationship of chlorzoxazone clearance with body weight, a correlation found even among young healthy volunteers without fatty liver [32]. Nevertheless, these results suggest CYP2E1 induction in morbidly obese adults with NAFLD.

There are no reported studies of acetaminophen metabolism in humans with biopsy-proven NASH or NALFD. However, the effect of obesity on acetaminophen clearance and metabolism has been evaluated previously in two studies. Acetaminophen clearance increases with body weight, a correlation found even in young healthy adults, and the

increase in obese adults was consistent with this trend [50]. So, when adjusted for body weight, both clearance and distribution volume did not differ between obese and control groups. This finding is consistent with hepatic clearance being a function of lean body mass. A subsequent clinical study was conducted to determine the effect of obesity on drug glucuronidation using acetaminophen and two other drugs that are mainly conjugated with glucuronic acid [51]. Clearances of the three drugs were highly correlated, indicating that the glucuronide conjugating capacity increases in proportion to body weight.

Further support for increased glucuronidation in obesity is provided by a study in genetically obese rats where increasing hepatic UDP glucuronyltransferase (UGT) activity was found with acetaminophen as the probe substrate [52]. Although the formation clearance of acetaminophen glucuronide is unchanged when expressed per gram of liver weight, UGT activity is markedly increased compared with matched lean rats when expressed as total hepatic activity.

In summary, animal studies show an increase of mitochondrial glutathione in fatty rat livers due to obesity-related oxidative stress. Use of the methionine-choline-deficient diet in rodents, which has questionable validity as a model for NASH, shows induction of CYP2E1 activity upon development of steatohepatitis, but use of high-fat diets in obese mice leads to reduction of CYP2E1 mRNA, protein, and activity. The limited studies in humans show modest increases or decreases of hepatic glutathione content in adults with NALFD, and one *in vitro* study of liver biopsy specimens from adults with NASH suggests an increase in CYP2E1 content. Chlorzoxazone metabolism studies show a modest 24% increase in CYP2E1 activity in NASH, and a larger 3-fold increase in NAFLD, although the latter study did not account for the general effect of body weight on clearance.

Clinical data from acetaminophen metabolism studies show that clearance increases with body weight and that this increase is due to higher conjugating capacity of the liver at heavier weights [50,51]. Taking the findings of the previous chlorzoxazone and acetaminophen studies together, the balance between activation of acetaminophen to NAPQI via CYP2E1 and direct conjugation with glucuronic acid and sulfate would not be expected to change in NASH or NALFD.

5.7.5 Cirrhosis of Different Etiologies

Cirrhosis is an end-stage liver disease that is characterized by injured liver cells, fibrosis within the liver, and the formation of regenerative nodules. Cirrhosis can progress from either hepatocellular (viral, alcoholic, nonalcoholic steatosis) or cholestatic (primary biliary, genetic) liver diseases. The rate of glutathione production is slower in patients who have progressed to cirrhosis [21,22], which results in lower plasma and hepatic glutathione concentrations [34,36,37,38].

An *in vitro* comparison of CYP2E1 protein concentrations in livers of adults with end-stage, cirrhosis and normal livers found no differences [53], whereas a decrease in CYP2E1 protein concentrations of 59% ($p < 0.05$) was found in another study of cirrhotic liver samples compared with normal livers [54]. The activity of CYP2E1 was also measured *in vitro* simultaneously with CYP2E1 protein concentrations in microsomal fractions from 42 adults with liver disease undergoing diagnostic biopsies [55]. Specimens were sorted into groups based on severity of liver disease, not etiology. Using the metabolism of chlorzoxazone as a probe, CYP2E1 activities were highly correlated with protein content ($r=0.75$, $p < 0.001$), and the activities were found to vary from 8- to 20-fold across specimens. No differences in CYP2E1 concentrations or activities were detected between samples from normal livers or those with mild liver disease (n=24) and the samples with severe liver disease (n=18).

The apparently discrepant *in vitro* findings may be explained, in part, by a subsequent study in which both mRNA and protein content of CYP2E1 were measured in 20 normal biopsy specimens, 32 specimens from adults with hepatocellular cirrhosis, and 18 specimens from adults with cholestatic cirrhosis [56]. CYP2E1 mRNA was diminished in all hepatocellular and cholestatic cirrhotic livers, whereas reductions in median CYP2E1 protein concentrations were 19% and 51% for hepatocellular and cholestatic cirrhosis, respectively. The investigators and others have suggested that these *in vitro* data infer a differential effect of cirrhosis on CYP2E1 mRNA and protein concentrations depending on disease etiology [56].

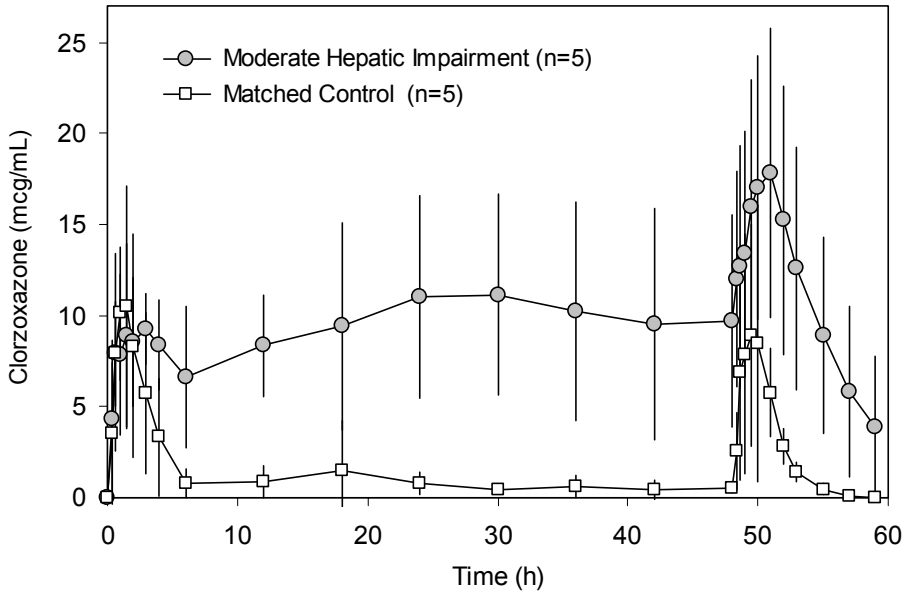
The first clinical evidence of a decline in CYP2E1 activity with cirrhosis was reported in adults with moderate-to-severe liver disease using chlorzoxazone as a probe [57]. Twenty adults with different etiologies and severity of hepatocellular liver disease and 20 matched-control healthy adults completed the study. Liver disease severity was categorized by the Child-Pugh score, and all adults with Child's class B (moderate) or C (severe) had biopsy-

proven cirrhosis. None had cholestatic disease, and two adults had alcoholic liver disease, but no recent history of alcohol consumption. Compared with control subjects, no statistical difference in the metabolite-to-chlorzoxazone ratio at four hours was detected for adults with mild liver impairment and a Child-Pugh score of 5 to 6, which is consistent with the findings reported previously for adults having hepatitis C infection and mild impairment [33]. In the cohort with moderate-to-severe impairment and a Child-Pugh score from 7 to 11, CYP2E1 activity was markedly reduced compared with control subjects. Median (range) ratios were 0.21 (0.02-0.75) versus 0.83 (0.40-1.39), respectively, which were significant at $p < 0.05$ [57].

McNeil has sponsored a multiple-dose metabolism study of chlorzoxazone in adults with mild-to-moderate liver disease [58]. Although the data have not been published, they were submitted to FDA in an annual report to the new drug application for Parafon Forte™, a chlorzoxazone product. Fifteen adults with liver disease of different etiologies and severity, and 15 matched-control healthy adults completed the study. Liver disease severity was categorized by the Child-Pugh score, and all, except two, adults had biopsy-proven disease with or without cirrhosis. One subject had cholestatic liver disease (primary biliary cirrhosis), and the remaining adults had hepatocellular diseases, primarily hepatitis C infection. No subject had documented alcoholic liver disease.

Compared with 10 matched-control subjects, no statistical difference was detected in the clearance of chlorzoxazone estimated after the last dose (at steady state) for the 10 adults with mild liver impairment and Child-Pugh scores of 5. This result also is consistent with the findings reported previously for adults having hepatitis C infection and mild impairment [33,57]. In the cohort with moderate liver impairment and a Child-Pugh score from 6 to 8, CYP2E1 activity was markedly reduced compared with control subjects. The difference in mean chlorzoxazone clearances (1.33 ± 0.44 versus 3.63 ± 1.52 mL/kg/min, respectively) was significant at $p < 0.004$ [58]. Figure 5.1 illustrates the higher plasma concentrations of chlorzoxazone in the moderately liver-impaired subjects.

Figure 5.1 Effect of Moderate Hepatic-Impairment on Multiple-Dose Chlorzoxazone Pharmacokinetics [58]



These two clinical metabolism studies [57,58] show a decrease in CYP2E1 activity in adults with hepatocellular liver disease that had progressed to cirrhosis and moderate-to-severe impairment (Child-Pugh Scores from 7 to 11). Moreover, a decrease in CYP2E1 activity was shown in adults with alcoholic liver disease that had progressed to cirrhosis [40]. This contrasts with previous suggestions [56,59] that CYP2E1 activity may only be reduced in severe cases of cirrhosis of cholestatic origin, and that activity would be relatively unchanged in cirrhosis of hepatocellular origin. This discrepancy is likely due to the uncertainty associated with extrapolating *in vitro* data to *in vivo* metabolism and clearance, because the latter depend on other *in vivo* parameters, such as hepatic mass, hepatic blood flow, and drug-transport limiting systems of hepatocytes.

5.7.6 Summary of Experimental and Clinical Metabolism Data

There is a complete lack of evidence to support the supposition that adults with liver disease may be at risk when using acetaminophen products at therapeutic doses due to differences in hepatic glutathione concentrations or CYP2E1 activity. Suppositions of increased risk must not consider changes in glutathione or CYP2E1 in isolation [2], but rather they must consider the spectrum of processes involved in acetaminophen metabolism. Often, suppositions fail to recognize that both glutathione concentrations and

CYP2E1 activity can change in the same direction at the same time in a disease state. Toward this end, the experimental and clinical data on glutathione and CYP2E1 reviewed in this section are summarized together in Table 5.2 by the four categories of liver disease. The key points are

- Adults with chronic hepatitis C infection have hepatic glutathione concentrations within normal ranges. In addition, *in vivo* CYP2E1 activity measured clinically with the chlorzoxazone probe is within normal ranges [33]. Production of plasma glutathione concentrations in chronic hepatitis C patients who have progressed to cirrhosis is slower compared with control subjects [22]. However, metabolism studies show that *in vivo* CYP2E1 activity is similarly decreased in cirrhosis [57,58]. The balance does not appear to shift; thus, acetaminophen is no less safe in hepatitis C, even when it has progressed to cirrhosis.

- Adults with alcoholic liver disease who continuously consume alcohol have lower plasma and hepatic glutathione concentrations. Although alcohol induces *in vivo* CYP2E1 activity, it also acts as competitive inhibitor of CYP2E1, thereby effectively diminishing the oxidation of acetaminophen to NAPQI while present even in the face of increased CYP2E1 abundance [69]. Adults with alcoholic liver disease who abstain from consuming alcohol have both lower hepatic glutathione and *in vivo* CYP2E1 activity with increasing severity of disease [40,57].

- Adults with nonalcoholic fatty liver disease (NALFD) have modest increases or decreases in hepatic glutathione concentrations. Recent studies of high-fat diets in obese mice show a reduction of CYP2E1 mRNA, protein, and activity with development of fatty liver and steatohepatitis [46,47]. The clearance of chlorzoxazone, which is 75% oxidized by CYP2E1, shows moderate increases in CYP2E1 activity in adults with NASH and higher increases in NAFLD, although that latter was not adjusted for body weight. Importantly, acetaminophen metabolism studies also show that clearance increases with body weight, but animal and clinical data indicate that this increase is due to higher glucuronide conjugating capacity of the liver at heavier weights [50,51].

- Adults with liver cirrhosis of various etiologies may have a slower rate of plasma glutathione production [21,22]; however, CYP2E1 expression and *in vivo* activity also declines as chronic liver disease (both hepatocellular and cholestatic) progresses to moderate and severe cirrhosis [38,40,57,58]. Again, the balance between activation and detoxification is sustained by simultaneous compensating changes.

Table 5.2 Summary of Experimental and Clinical Metabolism Data on Glutathione and CYP2E1 in Liver Diseases

Disease Category	Cofactor/ Enzyme	Model	Effect	Protein/mRNA or Activity	References
Viral Infections	GSH	Human plasma	↔ Hepatitis	Protein	28,29
		Human liver	↔,↑ Hepatitis	Protein	30,31
	CYP2E1	Clinical CZX probe	↔ Hepatitis	Activity	33
Alcoholic Liver Disease[a]	GSH	Human plasma	↓ Steatosis	Protein	22
		Human liver	↑ Hepatitis	Protein	30,31
		Human liver	↓ Hepatitis	Protein	35,37
	CYP2E1	Clinical CZX probe	↔,↓ Mix Case	Activity	40
	GSH & CYP2E1	Clinical APAP metabolism	↔ Compensated	----	41
NAFLD / NASH	GSH	Human liver	↓ Steatosis	Protein	30
		Human liver	↑ Steatosis	Protein	31
		Rat mitochondria	↑ Steatosis	Protein	42
	CYP2E1	Human liver	↑ Steatosis	Protein	43
		Rodent MCD diet	↑ Steatosis	Protein /mRNA	44,45
		Mouse high-fat diet	↓ Steatosis	Protein/Activity	46,47
		Clinical CZX probe	↑ Steatosis	Activity	48,49
	GSH & CYP2E1	Clinical APAP metabolism	↔ Obese	----	50,51
Cirrhosis of Different Etiology[b]	GSH	Human plasma	↓ Mix Case	Protein	26,27,22
		Human liver	↔,↓ Mix Case	Protein	30,36,37,38
	CYP2E1	Human liver	↓ Alcoholic	mRNA	38
		Human liver	↓ Mix Case	Protein/mRNA	39,56
		Human liver	↓,↔ Mix Case	Protein	54,53
		Human liver	↔ Mix Case	Protein/Activity	55
		Clinical CZX probe	↓ Mix Case	Activity	40,57,58

a: Includes data mainly from alcohol-abstaining adults, but some studies are unclear of the time between cessation of alcohol and when data were collected, the latter of which may affect reported means and their interpretation.

b: Includes cases of cirrhosis resulting from both hepatocellular (viral, alcoholic, nonalcoholic steatosis) and cholestatic (primary biliary and other genetic) diseases that are either reported separately or mixed.

Key: APAP – acetaminophen; CYP2E1 – cytochrome P450 2E1; CZX – chlorzoxazone; GSH – glutathione; MCD – methionine-choline-deficient diet; NAFLD – nonalcoholic fatty liver disease; NASH – nonalcoholic steatohepatitis; ↑ increase, ↓ decrease, ↔ no change.

5.8 Effect of Liver Disease on Glucuronide and Sulfate Conjugation

Direct glucuronide and sulfate conjugation accounts for about 85% of the metabolism of acetaminophen. The isoform UGT1A6 mainly catalyzes the glucuronidation of acetaminophen with some contribution by UGT1A9 [8,9]. Pharmacokinetic studies in adults with cirrhosis have shown that metabolism of drugs by glucuronidation is often unchanged in cirrhosis. Hypotheses regarding the mechanism of observed preservation of glucuronidation in liver disease are that latent UGT enzymes may be activated with liver injury and/or extrahepatic glucuronidation may be induced. However, an *in vitro* study [60] of UGT expression in human liver samples revealed an increase of UGT in the remaining viable hepatocytes following a range of liver injuries. Test samples included normal livers, and livers from adults with nonalcoholic steatosis, a spectrum of chronic alcohol abuse, primary biliary cirrhosis, and hemochromatosis. These experimental data suggest that up-regulation of UGT enzymes in viable hepatocytes is the only known molecular basis for the preservation of glucuronidation in liver disease. The study also demonstrated that UGT enzymes had a predominantly centrizonal distribution (zone 3 accentuation), which had not been previously characterized. Additional evidence for up-regulation is provided by measurements of UGT mRNA in human liver biopsy samples mainly from adults with hepatitis C and B infections graded by inflammation or fibrosis [61]. Although not statistically significant, mRNA concentrations of UGT1A6 and UGT1A9 isoforms trended higher with moderate fibrosis and no change with more severe fibrosis.

In a review of experimental and clinical data published over 15 years ago [62], Hoyumpa and Schenker suggest that glucuronidation generally may be preserved in mild-to-moderate liver disease, but may be impaired in severe liver disease. Unfortunately, the authors misstate findings on acetaminophen metabolism from a study cited on page 788 of their review. Specifically, they write that investigators “noted decreased formation of glucuronide in patients with abnormal prothrombin time ratio and low serum albumin (severe disease) but not in those in whom both tests were normal (mild disease)” [62]. Instead, acetaminophen half-life was associated with the clinical laboratory tests. The study clearly shows no difference in the urinary excretion data of all acetaminophen metabolites, including the glucuronide, among normal subjects and those with mild and severe liver disease [63].

In a subsequent *in vitro* metabolism study [64] using hepatic microsomes derived from healthy and severe cirrhotic livers, changes in the glucuronidation of probe drugs were found to depend on UGT isoforms responsible for conjugation of the probe. No difference was detected in the metabolic clearance of umbelliferone, a specific probe for UGT1A6,

between the healthy and cirrhotic liver samples. Acetaminophen is mainly conjugated by UGT1A6.

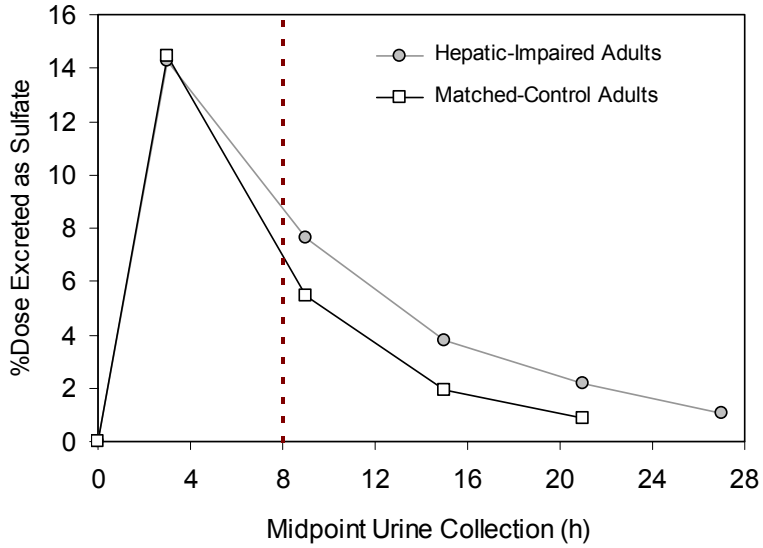
There is limited information on the effect of liver disease on sulfation. Sulfation of acetaminophen partly reflects availability of inorganic sulfate, subsequent availability of its high-energy form, 3'-phosphoadenosine-5'phosphosulfate (PAPS), and sulfotransferase activity (both SULT1A1 and SULT1A3) [10,11]. In an *in vitro* study of liver biopsy samples from healthy adults and adults with chronic hepatitis or cirrhosis, activities of six conjugation enzymes were determined using test substrates [65]. The mean activity of sulfotransferases was statistically lower in the diseased samples, but no difference was detected in the activity of glucuronyltransferases. Neither the specificities of test substrates for different metabolic pathways nor isoforms of the enzymes were reported, severely limiting the use of these experimental findings to *in vivo* acetaminophen metabolism.

In a rat-liver perfusion study, the effect of cirrhosis on p-nitrophenol metabolism was assessed after one pass through the liver [66]. p-Nitrophenol undergoes sulfation by SULT1A1 and glucuronidation. The results show that the sulfate formation rate was slower in cirrhotic rat livers compared with control livers, and the glucuronide formation rate was unchanged. Again, lack of isoform specificity of this test substrate makes extrapolation of these findings to *in vivo* acetaminophen metabolism unreliable.

A comparison of acetaminophen sulfate conjugation by Davies et al found no differences in the median fractional urine recovery of sulfate metabolite between 21 adults with various liver diseases and 28 healthy adults [67]. However, a statistically significant difference in median sulfate, but not glucuronide, conjugate recovery was reported between 28 adults with primary biliary cirrhosis (PBC) and the healthy adults (4.5%, range 0.1% to 21.3%, versus 9.6%, range 0.6% to 31.4%). Yet, these findings are unreliable because urine was collected for only eight hours after the acetaminophen dose, which is an insufficient interval to collect metabolites fully. For example, the reported median fractions of acetaminophen dose recovered as sulfate and glucuronide conjugates in the healthy adults were only 9.6% and 13.5% in this study, which are markedly lower than expected, strongly suggesting incomplete recovery [67].

In studies of acetaminophen disposition, urine is collected for 24 hours. To further illustrate this point, sulfate excretion data from an acetaminophen metabolism study [4] sponsored by McNeil is shown in Figure 5.2. Sulfate excretion patterns between moderately hepatic-impaired and matched-control subjects differ, and a line drawn at eight hours after the dose shows that conclusions may change, depending on whether results for an 8- versus 24-hour collection are considered.

Figure 5.2 Fraction of Acetaminophen Sulfate Excreted in Urine After a Single 1000 mg Dose Over Time, Which Shows Incomplete Excretion at 8 Hours [4]



In another more relevant study, the biotransformation of acetaminophen was compared among 27 adults with primary biliary cirrhosis (PBC), 15 adults with other chronic liver diseases (noncirrhotic n = 9 and cirrhotic n = 6), and 10 healthy females [68]. Urine was collected for 24 hours after a 500-mg intravenous dose ensuring adequate collection of the metabolites. In addition, hepatic cytosolic bile acid sulfotransferase activity was measured using ^{35}S-PAPS. Table 5.3 shows mean percents of sulfate conjugate excreted that are similar among groups, even with late-stage PBC. Additionally, there were no differences detected in glucuronide excretion, the percent of total acetaminophen dose excreted, or sulfotransferase activity between any groups.

Table 5.3 Percent of Acetaminophen Dose Excreted as the Sulfate Conjugate

PBC Stage 1	PBC Stage 2/3	PBC Stage 4	Other Liver Diseases	Healthy Controls
n = 7	n = 7	n = 13	n = 15	n = 10
37.3 ± 21%	28.5 ± 4.7%	29.4 ± 11.3%	24.4 ± 9.6%	30.0 ± 7.3%

Taken together, the human experimental and clinical data show that glucuronidation is preserved in liver disease with evidence that UGT may be up-regulated in the remaining

viable cells. Yet, without clear specificity of the metabolic pathway(s) of test substrates, the validity of extrapolating experimental *in vitro* and animal findings to *in vivo* acetaminophen metabolism is questionable. The most reliable scientific evidence on the effect of liver disease on both glucuronidation and sulfation is provided by metabolism studies of acetaminophen in adults with liver disease of various etiology and severity.

The recent McNeil-sponsored study [4] and five other published acetaminophen metabolism studies unequivocally show that the amounts of acetaminophen glucuronide and sulfate formed and excreted in urine are similar between healthy adults and those with liver disease after single and multiple doses of the drug. The latter studies are discussed in detail in Item 1, Section 6.

5.9 Metabolic Interactions of Alcohol and Acetaminophen

At the September 19, 2002 meeting of the Nonprescription Drug Advisory Committee, participants were asked to consider if alcohol users might be more susceptible to hepatotoxicity with acetaminophen. The experimental and clinical metabolism data are reviewed below in this section, and clinical safety data from new prospective, clinical studies of acetaminophen in alcohol users and abusers are provided in Item 1, Section 7.

5.9.1 Key Points from Experimental and Metabolism Data

The key points from McNeil's review of experimental and clinical metabolism data on alcohol and acetaminophen follow.

- There are no published data suggesting that alcohol lowers concentrations of plasma or hepatic glutathione below normal in moderate or occasional binge drinkers of alcoholic beverages. Using chlorzoxazone as a probe in metabolism studies of moderate drinkers, having both controlled and uncontrolled alcohol consumption, CYP2E1 activity was only modestly induced about 30 to 35% on average.
- With chronic excessive alcohol consumption, plasma or hepatic glutathione concentrations in alcohol abusers are often below normal ranges. Compared with nondrinking control subjects, metabolism studies using chlorzoxazone as a probe show about a twofold higher CYP2E1 activity in recently alcohol-abstaining chronic alcohol abusers.
- Alcohol both induces and competitively inhibits CYP2E1, resulting in simultaneous induction and inhibition when it is present. When alcohol and acetaminophen are consumed together by humans or experimental animals, alcohol competitively inhibits CYP2E1 and less of the toxic intermediate, NAPQI, is produced while alcohol is present in the blood.
- Available evidence from chlorzoxazone metabolism data suggests that there is about a one-to-two day window after alcohol is removed from the blood when CYP2E1 activity might be increased. However, in a study of *healthy* adults examining the effect of alcohol induction on acetaminophen metabolism immediately after alcohol was cleared, the fraction of the acetaminophen dose converted to NAPQI, and indirectly measured as detoxified urinary cysteine and mercapturic

conjugates, was modestly increased by 21.6% ($p < 0.03$). While supporting the operational mechanism, the increase from 0.075 to 0.092 in the fraction of dose excreted as total thiols is clinically insignificant, causing no additional risk of acetaminophen hepatotoxicity at therapeutic doses in adults who are moderate or occasional binge drinkers of alcoholic beverages.

- Several clinical metabolism studies were conducted comparing the biotransformation of acetaminophen between nondrinking control subjects and chronic alcohol abusers after cessation of alcohol drinking. Overall, the fact that there are no or only fairly small changes in thiols produced within the first few days after cessation of alcohol consumption shows that the effect of alcohol on acetaminophen oxidation by CYP2E1 induction is moderate at best. These data provide metabolic evidence that chronic alcohol abusers do not have an increased risk of acetaminophen-induced hepatotoxicity at therapeutic doses of acetaminophen

5.9.2 Effect of Ethanol on Glutathione and CYP2E1

There are no published data suggesting that ethanol lowers steady-state concentrations of plasma or hepatic glutathione below normal in moderate or occasional binge drinkers of alcoholic beverages. To the contrary, plasma and hepatic glutathione concentrations are often lower than normal in chronic alcohol abusers [36] due in part to decreased precursor availability, decreased capacity to synthesize glutathione, and malnutrition. However, expression of CYP2E1 is also reduced with malnutrition in chronic alcohol abusers [39].

Ethanol both induces and competitively inhibits CYP2E1, resulting in simultaneous induction and inhibition when ethanol is present. Higher catalytic activity is only observed once ethanol is eliminated from the body, thus the activation of acetaminophen to the toxic intermediate is generally limited by ethanol [69]. Moreover, induction of CYP2E1 activity measured in animal models and humans differs mechanistically by species, and by the duration, amount, and manner of ethanol exposure. For example in rats, CYP2E1 is induced through stabilization of the enzyme at peak ethanol concentrations between 1.0 to 2.5 mg/mL [70,71]. However, at ethanol concentrations $\geq$ 2.5 mg/mL, the synthesis of CYP2E1 protein increases through messenger ribonucleic acid (mRNA) and/or translation efficiency. In humans, both CYP2E1 protein and mRNA levels were found to be higher in liver biopsy samples from recently drinking alcoholic abusers compared with nondrinking control subjects [72,73].

Three clinical studies showed that chronic alcohol exposure induces CYP2E1 activity as measured by the metabolism of chlorzoxazone [74,75,76]. Induction begins relatively soon after ingestion of alcohol in both moderate drinkers and chronic alcohol abusers, with CYP2E1 activity continuing to rise over time. After one week of 40 grams alcohol (about three drinks) daily, CYP2E1 activity in moderate drinkers increased by 31% ($p < 0.05$) with additional increases up to four weeks [74]. Another study in moderate drinkers with uncontrolled alcohol consumption found a 35% ($p < 0.05$) higher CYP2E1 activity compared with the nondrinking control subjects [75]. These latter moderate drinkers had a history of drinking an average of two-to-three drinks per day over the last five years, and had consumed alcohol within 48 hours of the study.

A study in five severe chronic alcohol abusers showed a twofold higher mean ratio of plasma metabolite-to-chlorzoxazone concentrations measured at two hours after the dose of chlorzoxazone ($p < 0.01$). This test was conducted within 24 hours of the last drink and also compared with that measured again 3, 8, and 15 days later [74]. The subjects were all smokers who consumed an estimated 282 ± 41 g alcohol per day over the last year, and had been drinking excessively for 23.4 ± 2.8 years.

Following cessation of chronic alcohol exposure, clinical studies of chlorzoxazone metabolism have demonstrated that CYP2E1 induction is transient, lasting at most a few days [77]. The half-life time for the waning of CYP2E1 induction during a period of alcohol abstinence is estimated at 2.5 days with CYP2E1 activity reverting to normal within three to eight days [74,77,78].

Studies in humans using both acetaminophen and other probe CYP2E1 substrates have shown that combined inducer-inhibitors such as ethanol diminish CYP2E1 activity while present in blood, and that the effect on induction on CYP2E1 activity is seen only after the inducer-inhibitor is eliminated from blood. This, available evidence actually shows that ethanol and CYP2E1 effectors acting by similar mechanisms most likely protect against acetaminophen-induced hepatotoxicity in humans when present in blood. *There is neither human or animal scientific evidence to the contrary.*

In summary, there are no published data suggesting that alcohol lowers steady-state concentrations of plasma or hepatic glutathione below normal in moderate or occasional binge drinkers of alcoholic beverages. In clinical metabolism studies of moderate drinkers,

having both controlled and uncontrolled alcohol consumption, CYP2E1 activity was modestly induced about 30 to 35% on average using chlorzoxazone as a probe.

With chronic excessive alcohol consumption, plasma or hepatic glutathione concentrations in alcohol abusers are often below normal ranges. Animal studies show that CYP2E1 protein concentrations are higher due to either stabilization and/or increased synthesis, depending on ethanol exposure. Compared with nondrinking control subjects, human liver biopsy samples show about a fourfold higher CYP2E1 protein concentration, whereas *in vivo* clinical studies using chlorzoxazone as a probe show only about a twofold higher CYP2E1 activity in recently alcohol-abstaining chronic alcohol abusers. The latter differences illustrate the difficulty of extrapolating *in vitro* experimental research to *in vivo* clinical effects.

5.9.3 Alcohol and Acetaminophen Metabolism

In a 1997 review of ethanol metabolism, cirrhosis, and alcoholism and referring to the mainly experimental research available at the time, Charles Lieber writes, “Obviously the clinically most relevant results are those obtained with alcohol in humans” [79]. During the next ten years, subsequent experimental and human research has challenged the relevance of some earlier experimental findings and clinical supposition regarding the role of ethanol and CYP2E1 in alcoholic liver disease. For example, in a study of ethanol-induced liver damage, no differences in steatosis, inflammation, and necrosis were found between wild-type and *CYP2E1*-knock-out mice [80]. Instead, activation of Kupffer cells and tumor necrosis factor-α were found to have essential roles in early ethanol liver injury in mice [81,82].

Likewise, the clinically most relevant data with regard to the safety of acetaminophen in moderate drinkers and chronic alcohol abusers are those obtained prospectively with alcohol and acetaminophen in humans. Several clinical metabolism and safety studies do not support the supposition [79] that these populations have an increase risk of acetaminophen hepatotoxicity at therapeutic doses. Results from the clinical metabolism studies, delineating *in vivo* interactions of alcohol and acetaminophen, are discussed in the remaining parts of this section; whereas the recently completed clinical safety studies of alcohol and maximum-labeled daily doses of acetaminophen in moderate and chronic alcohol abusers are discussed in Item 1, Section 7.

5.9.3.1 Adults Who Consume Alcohol Moderately

5.9.3.1.1 Concomitant Usage of Alcohol and Acetaminophen

When alcohol and acetaminophen are consumed together by humans or experimental animals, alcohol competitively inhibits CYP2E1 and less of the toxic intermediate, NAPQI, is produced while alcohol is present in the blood. Animal studies have demonstrated that alcohol, when administered acutely and concomitantly with acetaminophen, can decrease NAPQI production and protect against hepatotoxicity [83,84]. A decrease in thiol metabolites formed via NAPQI has also been reported in studies of concomitant usage of alcohol and acetaminophen in healthy adults.

In one crossover study of nonalcoholic adults having a history of low alcohol consumption (0.5 to 1.5 ounces of ethanol 3 to 4 times per week), metabolites of acetaminophen in the urine were measured after 1 g acetaminophen was ingested without and with alcohol one hour before the dose [85]. With prior alcohol ingestion, a transient elevation in acetaminophen excretion and suppression of acetaminophen mercapturate excretion, a product of CYP2E1 oxidative metabolism, was observed for up to 12 hours. A second crossover study reported similar findings in a group comprised of moderate and heavy drinkers [86]. The acetaminophen dose was taken about one hour after an initial 0.6 g/kg ethanol dose, followed by 0.1 and 0.16 g/kg hourly doses for eight hours in the moderate and heavy drinkers, respectively. The amount of unchanged acetaminophen excreted in urine was about doubled ($p < 0.01$) and thiols (conjugates of NAPQI) decreased by 67% to 70% ($p < 0.01$) with ethanol present.

5.9.3.1.2 One-to-Two Day Window After Cessation of Alcohol Drinking

In occasional, moderate, or binge drinkers of alcohol, experimental and *in vivo* CYP2E1 metabolism studies suggest that the amount of thiol metabolites formed from acetaminophen may increase immediately after alcohol is cleared from the body and is no longer available for competitive inhibition with acetaminophen. Available evidence suggests that there is about a one-to-two day window after alcohol is removed from the blood when CYP2E1 activity might be increased.

A pharmacokinetic study by Thummel *et al* [87] in healthy adults, designed to identify the biphasic effects on CYP2E1 activity and acetaminophen metabolism, confirmed that the amount of thiol metabolites produced by CYP2E1 depends on the relative times of acetaminophen and ethanol ingestion. This crossover study simulated an evening of binge

drinking. Ethanol (approximating one 750-mL bottle of wine, six 12-ounce cans of beer, or 9 ounces of 80-proof liquor) was infused intravenously over a six-hour interval (6 PM to 12 AM) followed by eight hours of abstinence before dosing with 500 mg acetaminophen. The fraction of the acetaminophen dose converted to NAPQI, and indirectly measured as detoxified urinary cysteine and mercapturic conjugates, was modestly increased by 21.6% ($p < 0.03$). While supporting the operational mechanism, the increase from 0.075 to 0.092 in the fraction of dose excreted as total thiols is clinically insignificant, causing no additional risk of acetaminophen hepatotoxicity at therapeutic doses.

Another key conclusion from the Thummel study [87] was that short-term ethanol exposure in healthy adults had no effect on body clearance of the acetaminophen dose with and without alcohol: 25.3 ± 9.72 versus 25.3 ± 9.74 L/h. The latter result is expected with selective modification of a quantitatively minor route (CYP2E1) of elimination.

5.9.3.2 Adults Who Chronically Abuse Alcohol

5.9.3.2.1 Active Excessive Alcohol Drinking

The acute effect of alcohol consumed concurrently (1.72 g/kg over eight hours) on the metabolism of acetaminophen was evaluated in a small group of heavy drinkers [88]. About a 50% reduction in mean urinary recovery of thiol metabolites (cysteine and mercapturic acid conjugates) was reported. The investigators conclude on the basis of these findings that acute intoxication with ethanol would be expected to reduce the risk of liver damage following acetaminophen overdose.

5.9.3.2.2 One-to-Two Day Window After Cessation of Alcohol Drinking

The metabolism of a 2-g acetaminophen dose with concurrent measurements of plasma glutathione concentrations was assessed in chronic alcoholics who had been drinking heavily (estimated average consumption of 180 g ethanol/day) within two days after entering an alcohol treatment program [36]. None had clinical evidence of alcoholic liver disease, and they were dosed with acetaminophen on the second day of hospitalization.

Most importantly, the data in this study [36] show that the rates of glutathione consumption and repletion with acetaminophen metabolism were similar between chronic alcoholic and control subjects even though baseline plasma glutathione concentrations were lower for the chronic alcoholic subjects (4.66 ± 1.87 versus 8.37 ± 2.65 μM; $p < 0.05$). Minimum plasma glutathione concentrations were reached at three hours after the 2-g dose for both groups.

In addition, the amounts of glutathione consumed were nearly identical, as the approximate decreases from baseline were 2.26 and 2.11 μM for the chronic alcoholic and control subjects, respectively. Lastly, the biotransformation of acetaminophen was comparable between groups, as the recovery, relative proportions, and absolute amounts of the metabolites, including the thiols (cysteine and mercapturate), did not differ. These data in the alcohol-abstaining chronic alcoholics provide evidence that hepatic glutathione concentrations, reflected by surrogate plasma concentrations, were adequate to metabolize a supratherapeutic acetaminophen dose despite lower baseline values.

Several clinical metabolism studies were conducted comparing the biotransformation of acetaminophen between nondrinking control subjects and chronic alcohol abusers after cessation of alcohol drinking. Results are summarized in Table 5.4 in which four studies [36,89,90,91] show no difference and two studies [92,93] show a modest increase in the amount of thiol metabolites produced by chronic alcohol abusers. The timing of the acetaminophen dose with regard to when alcohol was ceased may account for some differences in these results, but overall, the fact that there are no or only fairly small changes in thiols produced shows that the effect of alcohol on acetaminophen oxidation by CYP2E1 induction is moderate. These data provide metabolic evidence that chronic alcohol abusers do not have an increased risk of acetaminophen-induced hepatotoxicity at therapeutic doses of acetaminophen.

Table 5.4 Comparison of Urinary Thiol Metabolites in Chronic Alcoholic and Nondrinking Control Subjects from Metabolism Studies of Acetaminophen

Study	Populations	Dose	Urinary Thiol Metabolites	Timing of Dose Relative to Alcohol Cessation
Critchley 1982 [89]	16 heavy drinkers 12 healthy controls	20 mg/kg	No difference	Not reported
Villeneuve 1983 [92]	9 chronic alcoholics 6 healthy controls	12 mg/kg	Increase	Dosed 24 to 48 hours after ceasing alcohol ingestion
Lauterberg 1988 [21]	5 chronic alcoholics 3 healthy controls	2 g	No difference	Drinking heavily up to 2 days before the dose
Skinner 1990 [91]	5 chronic alcoholics	1. 5 g	No difference	Tested once within 48 h and again at least 10 days abstinence
Critchley 1990 [86]	20 chronic alcoholics 26 healthy controls	1.5 g	No difference	Not reported
Chern 1993 [93]	11 chronic alcoholics 9 healthy controls	36 mg/kg	Increase	Dosed within 7 days of last alcohol intake

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Section 6 Table of Contents

6. NEW AND PUBLISHED ACETAMINOPHEN METABOLISM STUDIES SHOW NO GREATER RISK ASSOCIATED WITH LIVER DISEASE

In Part A, Preexisting Liver Disease as a Risk Factor for Acetaminophen Hepatotoxicity, of Section IV in the Proposed Rule [71 FR 77328], FDA has *"reconsidered its previous position on this issue and now believes that current evidence supports a warning"*. FDA has proposed the labeling text, "ask a doctor before use if you have liver disease", be added to acetaminophen OTC products. FDA based this labeling proposal on its following tentative conclusions from Part A [71 FR 77329]:

- a single prospective clinical study [1] found by FDA in the literature that evaluated the susceptibility of the diseased liver to acetaminophen toxicity was not definitive
- analyses of an acetaminophen overdose database and a review of the AERS case reports suggest that people with a history of liver disease may have increased susceptibility to acetaminophen-induced hepatotoxicity
- depletion of hepatic GSH has been found in both alcoholic and nonalcoholic liver diseases, suggesting that the diseased liver may have less capacity to inactivate the toxic metabolite of acetaminophen
- expression of hepatic P450–2E1, a major enzyme for metabolic activation of acetaminophen, tends to be increased in stable chronic liver diseases particularly in nonalcoholic fatty liver disease

6.1 McNeil's Position

McNeil disagrees with FDA's tentative conclusions based on their collection of data cited in the Proposed Rule. McNeil believes that stable chronic liver disease, in the presence or absence of alcohol, does not pose a risk for developing acetaminophen hepatotoxicity at therapeutic doses and that proposed labeling text, "ask a doctor before use if you have liver disease" is not warranted. McNeil bases its position on (1) an extensive analysis of the literature published up to April 2007 on clinical metabolism studies of acetaminophen and liver disease, and (2) new metabolism data from a recently completed study in hepatic-impaired adults sponsored by McNeil [2].

In our analysis of the literature, new and more sophisticated evidence of the role of critical cytochrome P450 isoforms (CYP2E1), hepatocellular glutathione homeostasis, and glucuronyltransferase systems show that the suppositions regarding risk in individuals with

liver disease are unfounded. These experimental and scientific data, detailed previously in Item 1 of Section 5, do not support the hypothesis of a risk of acetaminophen hepatotoxicity in individuals with liver disease at recommended doses. As such, these data and metabolism data from several clinical studies do not support the idea that individuals with liver diseases need to take a lower dose of acetaminophen.

6.2 Key Points from the Scientific and Clinical Data

- New and published metabolism data do not show either *"decreased deactivation and/or increased metabolic activation of acetaminophen in the diseased liver".* The amount of thiol metabolites (a marker of NAPQI[†] formation) is similar in individuals with and without liver disease, demonstrating no decrease in glutathione conjugation (decreased deactivation) or increase in metabolism by CYP2E1 (increased metabolic activation) in individuals with liver disease.
    - The amounts of thiols produced are similar after single doses or after repeat dosing of acetaminophen at 4 g/d in individuals with and without liver disease.
    - The metabolic formation clearance of thiol metabolites is similar in the diseased liver after a single dose, and it does not change after repeat dosing of acetaminophen 4 g/d for four days.

- New metabolism data from a McNeil-sponsored study show that acetaminophen metabolism increases with the administration of the maximum-labeled daily dose of acetaminophen for four days in individuals with diseased and healthy livers. Consecutive daily dosing of acetaminophen induces glucuronidation (a nontoxic metabolic pathway), resulting in increased total body clearance of acetaminophen and increased production of the glucuronide metabolite.
- New and published pharmacokinetic data show that individuals with liver disease have a modest increase in the elimination half-life of acetaminophen (about 1 to 2 h on average) that does not lead to progressive plasma accumulation of acetaminophen after repeat dosing or to a change in the amount metabolized via CYP2E1 compared with healthy individuals. While total body clearance is about 15 to 60% lower on average, which reflects slower acetaminophen removal, this is not relevant to the biotransformation to metabolites, which is essentially the same as that in healthy adults.

[†] NAPQI (N-acetyl-*p*-benzoquinoneimine) is a highly reactive intermediate, which is conjugated with glutathione to produce the inert, nontoxic thiol metabolites: cysteine, mercapturate, methylthioacetaminophen, and methanesulfinylacetaminophen.

- Safety data from five prospective clinical studies of subjects with liver disease of varying severity and etiology show no evidence of acetaminophen hepatotoxicity at therapeutic doses.
- Hepatologists and gastroenterologists often prefer acetaminophen use by patients with liver disease over nonsteroidal anti-inflammatory drugs (NSAIDs). Indeed, acetaminophen is commonly used to treat flu-like symptoms associated with interferon treatment in hepatitis C patients.

6.3 Overview of Published Acetaminophen Pharmacokinetic and/or Metabolism Studies in Liver Disease

Acetaminophen toxicity is understood to be dose dependent. Hepatotoxicity is not imparted directly by acetaminophen plasma concentrations, but rather by the activated metabolite, NAPQI, if not adequately detoxified by glutathione after excessive overdoses. The question of whether the diseased liver can adequately detoxify therapeutic doses of acetaminophen can be addressed by measuring the amounts, fractions of dose, and formation clearances of metabolites produced. Toward this end, McNeil sponsored a new metabolism study in hepatic-impaired adults that includes both single- and multiple-dose acetaminophen pharmacokinetics [2]. Only biotransformation data for repeat administration at the maximum dose of 4 g/d have been lacking. A description of the study design and methods is provided as Attachment 1 to this section, whereas key findings from this study are integrated herein.

Acetaminophen disposition has been studied extensively in about 60 children and 362 adults with a wide variety of liver diseases, including various types of cirrhosis, hepatitis, nodular transformation, congenital hepatic fibrosis, and α_1-antitrypsin deficiency. Table 6.1 lists the pharmacokinetic and/or metabolism studies published from 1961 through 2006; additional details on each study are provided in a summary table as Attachment 2 to this section. There are 22 single-dose pharmacokinetic studies: five studies in hepatic-impaired children of which two measured urine metabolites; 16 studies in hepatic-impaired adults of which nine measured urine metabolites, and one study in children and adults that measured metabolites. Additionally, there are two repeat-dose pharmacokinetic studies in hepatic-impaired adults, but neither measured metabolites.

Table 6.1 Listing of Published Acetaminophen Pharmacokinetics and/or Metabolism Studies

Study [Reference]	Populations	Size	Age[a] (y)	Acetaminophen Dose	Urine Metabolites
Al-Obaidy 1996 [3]	• Mild liver disease • Moderate liver disease • Severe liver disease	4 4 5	7 to 11 3 to 8 0.6 to 3.1	SD; 10 mg/kg	yes; 36 h
Andreasen 1979 [4]	• Cirrhosis • Healthy	11 12	32 to 69 41 to 69	SD: 1000 mg	no
Andreasen[b] 1979 [4]	• Cirrhosis	4	32 to 69	MD; 1000 mg Q8H x 5 days	no
Arnman 1978 [5]	• Cirrhosis • Liver cancer • Healthy	21 4 15	22 to 81 48 to 82 18 to 86	SD; 15 mg/kg	no
Benson[c] 1983 [1]	• Cirrhosis	6	42 to 66	MD; 1000 mg QID x 5 days	no
Benson 1983 [1]	• Cirrhosis • Alcoholic liver disease • Chronic active hepatitis • Chronic persistent hepatitis • Primary biliary cirrhosis	5 2 7 3 3	NR	MD; 1000 mg QID x 13 days	no
Brazier 1993 [6]	• Cirrhosis; compensated • Cirrhosis; decompensated • Healthy	7 14 6	NR	SD: 1000 mg	no
Careddu 1961 [7]	• Hepatitis; acute phase • Healthy	15 10	Children (NR)	SD; 10mg/kg IV	no
Cormack 2006 [8]	• Chronic liver diseases	17	3 to 15	SD; 40 mg/kg rectal	no
Davies 1995 [9]]	• Primary biliary cirrhosis • Other liver diseases • Healthy	28 21 27	Median 55 Median 43 Median 33	SD: 500 mg	yes; 8 h
El-Azib 1999 [10]	• Cirrhosis;schistosomal infection • Healthy	8 8	9 to 65	SD: 1000 mg	yes; 8 h
Fevery 1969 [11]	• Parenchymatous disease • Obstructive jaundice • Gilbert's syndrome • Healthy	26 9 3 14	NR	SD; 10mg/kg	yes; variable

a: Age reported as range unless otherwise indicated.
b: Cohort was assessed with multiple-dose regimen; c: Pilot study
Abbreviations: MD – multiple-dose; NR- not reported; SD – single-dose; Q8H – every eight hours; and QID – four times daily

Table 6.1 Listing of Published Acetaminophen Pharmacokinetics and/or Metabolism Studies

Study [Reference]	Populations	Size	Age[a] (y)	Acetaminophen Dose	Urine Metabolites
Forrest 1979 [12]	• Mild liver disease • Severe liver disease • Healthy	8 7 8	29 to 67 27 to 60 21 to 34	SD: 1500 mg	yes, 24 h
Froomes 1999 [13]	• Severe liver cirrhosis • Healthy	10 5	39 to 64 26 to 47	SD: 1000 mg	no
Hayes 1989 [14]	• Alcoholic cirrhosis • Chronic active hepatitis cirrhosis • Cryptogenic cirrhosis • Portal Fibrosis	8 2 1 1	33 to 70	SD: 1000 mg	no
Jorup-Ronstrom 1986 [15]	• Viral hepatitis; acute and recovery phases • Healthy	10 10	18 to 57 19 to 50	SD: 1000 mg	no
Leung 1989 [16]	• Hepatitis B cirrhosis • Alcoholic cirrhosis • Hepatocellular carcinoma	29 13 8	NR	SD: 1500 mg	yes; 24 h
Misra 1984 [17]	• Cirrhosis • Healthy	5 5	0.5 to 5	SD: 30 mg/kg	no
Poulsen 1991 [18]	• Alcoholic cirrhosis; compensated • Healthy	5 5	34 to 63 35 to 79	SD: 500 mg	yes; 24 h
Shamszad 1975 [19]	• Cirrhosis • Alcoholic active hepatitis • Alcoholics with normal liver function • Alcoholics given alcohol with acetaminophen	5 7 5 5	NR	SD: 975 mg	no
Turner 1990 [20]	• Primary biliary cirrhosis • Other chronic liver diseases • Healthy	27 15 10	NR	SD; 500 mg IV	yes; 24 h
Vendemiale 1989 [21]	• Cirrhosis	6	55 to 65	SD; 1000mg	yes; NR
Vest 1961 [22]	• Hepatitis; acute and recovery phases	6	5 to 15	SD; 10 or 20 mg/kg	yes; 24 h
Villeneuve 1983 [23]	• Chronic alcoholism • Alcoholic cirrhosis • Healthy	9 11 6	mean 42 mean 56 mean 31	SD; 12 mg/kg	yes; 24 h
Zapater 2004 [24]	• Mild-moderate cirrhosis • Severe cirrhosis • Healthy	9 5 7	64 ± 7 55 ± 10 NR	SD: 1000 mg	yes; 24 h

a: Age reported as range unless otherwise indicated.
Abbreviations: IV – intravenous; MD – multiple-dose; NR- not reported; SD – single-dose; Q8H – every eight hours; and QID – four times daily

Studies that describe the pharmacokinetics of acetaminophen from plasma data alone are of little use. Metabolite data are needed to elucidate the effect of liver disease, if any, on the biotransformation of acetaminophen. Pharmacokinetic parameters, such as elimination half-life (t½) and total body clearance (CL/F), depict time-dependent changes in plasma concentrations of the pharmacologically active analgesic (acetaminophen itself) and give vanishingly small information on the formation or disposition of the toxic intermediate, NAPQI. Nevertheless, to avoid seeming to disregard published data of acetaminophen disposition in individuals with liver disease, this response also includes studies that gathered acetaminophen plasma data, but neglected to collect the crucial metabolite data.

6.4 Integrated Urinary Metabolite Results Across Studies

6.4.1 Single-Dose Acetaminophen Biotransformation by Healthy and Diseased Livers is Similar

The data of utmost importance to gauge the metabolism of acetaminophen in liver disease are the fractional amounts of metabolite excreted in urine. Detailed biotransformation data of acetaminophen from single doses are available from McNeil Study 11-005 [2] and four published studies [12,16,23,24]. Data on the sulfate conjugation of acetaminophen are available from a published abstract [20]. In each of these studies, urine was collected over 24 to 36 hours after a single dose to ensure maximal recovery of unchanged acetaminophen and all its metabolites.

Available information describing the hepatic-impaired populations for three published studies is summarized in Table 6.2. No additional information describing the subjects beyond cirrhosis due to hepatitis B and alcoholism is available from the fourth published study [16]. Demographic information and screening clinical laboratory values associated with hepatic function are summarized in Table 6.3 for the subject populations in McNeil Study 11-005. Additional characteristics of the hepatic-impaired subjects are provided in Table 6.4. These subjects had diagnoses of hepatocellular cirrhosis secondary to hepatitis C and/or previous alcohol abuse, and they had a Child-Pugh score of 7 to 9, indicating moderate hepatic impairment. Ten subjects had chronic hepatitis C, and eight had been chronic alcohol abusers.

Table 6.2 Description of the Hepatic-Impaired Populations in Published Studies

Study	
Zapater 2004 [24]	Fourteen subjects with cirrhosis were enrolled: nine were grouped as having mild-to-moderate liver dysfunction (Child A and B; Child-Pugh score 6.8 ± 1.5) and five as having severe liver dysfunction (Child C; Child-Pugh score 10.4 ± 0.5) ($p < 0.05$). Nine subjects had esophageal varices; eight had ascites, of which four had both conditions.
Villeneuve 1983 [23]	Eleven subjects were enrolled with biopsy-proven alcoholic cirrhosis. All were hospitalized at the time of the study: seven for ascites and four for ruptured esophageal varices. None had consumed alcohol for at least 30 days before the study.
Forrest 1979 [12]	Fifteen subjects with chronic liver disease were enrolled: eight were grouped as having mild liver disease in whom albumin and/or prothrombin ratio were normal, and seven were grouped as having severe liver disease in whom both were abnormal. The following diagnoses comprise the group with mild liver disease: alcoholic cirrhosis (3), cryptogenic cirrhosis (1), chronic active hepatitis (1), primary biliary cirrhosis (2), and nodular transformation (1). The following diagnoses comprise the group with severe liver disease: alcoholic cirrhosis (5), cryptogenic cirrhosis (1), and chronic active hepatitis (1).

Table 6.3 Description of the Study Population in McNeil Study 11-005 [2]

	Hepatic-Impaired Subjects (n = 12)	Matched-Control Subjects (n = 13)
Demographics		
Age (y)	49.9 ± 4.8	50.5 ± 5.2
Gender (M / F)	4 / 8	5 / 8
Smoking Status (S / NS)	5 / 7	5 / 8
Race (A / B / W)	1 / 3 / 8	1 / 3 / 9
Body Mass Index (kg/m^2)	28.4 (22.1 to 36.8)	28.7 (22.5 to 36.1)
Lean Body Mass (kg)	51.2 ± 4.3	54.5 ± 12.6
Screening Laboratories		
Alanine Aminotransferase (U/L)	62.9 ± 33.2	24.5 ± 14.2
Aspartate Aminotransferase (U/L)	79.7 ± 50.5	22.2 ± 7.21
Gamma-Glutamyl Transferase (U/L)	159.7 ± 165.2	23.2 ± 15.2
Total Bilirubin (mg/dL)	1.53 ± 0.98	0.67 ± 0.29
Alkaline Phosphatase (U/L)	119.9 ± 44.4	71.8 ± 22.1
Albumin (g/dL)	3.78 ± 0.57	4.50 ± 0.40
Platelets (thous/UL)	149 ± 127	305 ± 67
Prothrombin Time (sec)	10.35 ± 0.93	9.51 ± 0.59

Abbreviations: M – male, F – female, S – smoker, NS – nonsmoker, A – Asian, B – Black, and W – white.

Table 6.4 Characteristics of Moderately Hepatic-Impaired Subjects in McNeil Study 11-005 [2]

Subject Number	Gender	Age (y)	Smoking Status	Alcohol History	Cirrhosis Etiology	Time and Details of Diagnosis	Childs-Pugh Score
1	M	62	NS; Quit	Abuse (27 y)	Alcohol	Cirrhosis (1994); hepatocelluar dyfunction	8
2	F	49	NS	No Use	Hepatitis C	Ongoing hepatitis infection (1995); moderate hepatosplenomegaly	7
3	F	54	S	Abuse (28 y)	Hepatitis C / Alcohol	Cirrhosis (1998); ongoing hepatitis infection (1993); moderate hepatomegaly	7
4	M	49	S	Abuse (34 y)	Hepatitis C / Alcohol	Cirrhosis (2004); ongoing hepatitis infection (2000)	9
5	M	51	NS; Quit	Occasional Use	Hepatitis C	Cirrhosis (2004)	9
6	F	45	S	Abuse (NR)	Alcohol	Cirrhosis (1996)	8
7	F	47	S	No Use	Hepatitis C	Cirrhosis (2002)	7
8	F	50	NS	Abuse (NR)	Alcohol	Cirrhosis (2001)	7
9	F	51	NS; Quit	Abuse (NR)	Hepatitis C	Cirrhosis (2002); splenomegaly; portal hypertension	7
10	F	45	NS; Quit	Occasional Use	Hepatitis C	Cirrhosis (2002); hepatitis infection (1987)	7
11	M	45	NS	Abuse (NR)	Hepatitis C / Alcohol	Cirrhosis (NR); severe splenomegaly	8
12	F	51	S	Abuse (10 y)	Hepatitis C / Alcohol	Cirrhosis (2000); hepatitis infection (2000)	7

Abbreviations: S- smoker, NS – nonsmoker, and NR – time not recorded.

In the abstracted metabolism study [20], acetaminophen biotransformation was compared among 27 adults with primary biliary cirrhosis (PBC), 15 adults with other chronic liver diseases (noncirrhotic n = 9 and cirrhotic n = 6), and 10 healthy females. Urine was collected for 24 hours after a 500-mg intravenous dose of acetaminophen. Additionally, hepatic cytosolic bile acid sulfotransferase activity was measured using ^{35}S-PAPS, a cofactor for sulfotransferase enzymes. Table 6.5 lists the mean (± sd) percents of sulfate conjugate excreted, which are similar among groups, even the group with late-stage PBC. No differences were reported in glucuronide excretion, percent of total acetaminophen dose excreted, or sulfotransferase activity between any groups. These results show that acetaminophen conjugation to sulfate and glucuronide metabolites was not altered by liver disease in general, and by PBC in particular.

Table 6.5 Percent of Acetaminophen Dose Excreted as the Sulfate Conjugate

PBC Stage 1	PBC Stage 2/3	PBC Stage 4	Other Liver Diseases	Healthy Controls
n = 7	n = 7	n = 13	n = 15	n = 10
37.3 ± 21%	28.5 ± 4.7%	29.4 ± 11.3%	24.4 ± 9.6%	30.0 ± 7.3%

The detailed acetaminophen biotransformation data from the other five studies are listed in Table 6.6. Overall, mean (± sd) percents of unchanged acetaminophen and the glucuronide, sulfate, and oxidative thiol metabolites (sum of cysteine and mercapturate) were similar between healthy control and hepatic-impaired groups across studies. Within studies [12,23,24], no statistical differences were detected in means for any metabolite between the healthy control and hepatic-impaired groups. In McNeil Study 11-005 [2], a one-way analysis of variance detected no statistical differences in mean percents of unchanged acetaminophen and all metabolites.

Regarding the biotransformation of acetaminophen, the investigators of each study conclude:

- Forrest *et al*: “Of particular significance is the excretion of normal amounts of the cysteine and mercapturic acid conjugates.” “With increasing hepatotoxic doses of paracetamol [acetaminophen] the cysteine and mercapturic acid conjugates are excreted in proportionately larger amounts and the present findings provide no evidence to suggest that a therapeutic dose of paracetamol is more likely to cause liver damage in patients with chronic liver disease than in healthy subjects [12].”

Table 6.6 Urine Excretion Pattern of Acetaminophen and Metabolites From a Single Dose, Reported as Mean (±sd) Percent[a]

Study	Sample Size	Acetaminophen	Glucuronide	Sulfate	Thiols[b]	Total Recovered
McNeil 2007 [2][c]						
Control Subjects	11[d]	2.4 ± 0.8	49.0 ± 7.43	22.4 ± 7.0	5.5 ± 2.1	82.4 ± 14.1
Mild/Mod Liver Disease	12	2.9 ± 2.0	40.2 ± 13.3	27.4 ± 12.0	6.8 ± 2.5	79.0 ± 7.6
Zapater 2004 [24]						
Control Subjects	7	1.2 ± 0.6	59.9 ± 4.2	27.9 ± 4.4	9.7 ± 2.2	NR
Mild/Mod Liver Disease	9	1.6 ± 1.2	57.1 ± 7.6	30.4 ± 7.1	9.0 ± 2.8	NR
Severe Liver Disease	5	1.0 ± 0.5	53.5 ± 7.4	35.4 ± 7.9	9.2 ± 4.4	NR
Leung 1989 [16]						
Hepatitis B Cirrhosis	29	5 ± 2	58 ± 9	28 ± 7	9[e]	NR
Alcoholic Cirrhosis	13	3 ± 1	62 ± 9	27 ± 10	8[e]	NR
Villeneuve 1983 [23]						
Control Subjects	6	4.8 ± 0.7	57.3 ± 2.9	33.3 ± 3.3	4.6[e]	88.6 ± 3.4
Cirrhosis	11	5.0 ± 0.5	51.7 ± 3.3	37.1 ± 2.6	6.0[e]	73.9 ± 4.8
Forrest 1979 [12]						
Control Subjects	8	3.7 ± 0.2	54 ± 1.4	33 ± 1.2	8.6[e]	92 ± 0.6
Mild Liver Disease	8	2.7 ± 0.3	59 ± 2.3	29 ± 1.9	8.7[e]	81 ± 3.1
Severe Liver Disease	7	4.6 ± 0.8	50 ± 3.7	35 ± 3.1	8.4[e]	84 ± 5.5

a: Data are reported as percent of acetaminophen dose recovered [2,12,24] or as percent of total acetaminophen and metabolites recovered [16,23].

b: Catechols were also measured in McNeil Study 11-005: 3.1 ± 2.6 and 2.4 ± 0.9 for control and hepatic-impaired subjects, respectively.

c: Thiols are the sum of cysteine and mercapturate metabolites.

d: Urine data for two matched-control subjects were not available due to an error at the clinical site. The sample collection vials were inadvertently switched between them, resulting in mixed pools.

e: Mean data for cysteine and mercapturate metabolites were reported separately in the publication, but are added here.

Abbreviations: sd – standard deviation, NR – not reported

- Leung *et al*: "In both the groups of patients without malignancy [n = 29 hepatitis B cirrhosis; n = 13 alcoholic cirrhosis], the relative proportions of unchanged paracetamol and its 4 conjugates were similar to the proportions found in healthy Caucasians which are 6%, 54%, 31%, 4% & 5%, respectively [16]."
- Villeneuve *et al*: "In abstinent subjects with alcoholic cirrhosis, the amount of cysteine and N-acetylcysteine in the urine was comparable to that of the control subjects. Their capacity for producing these reactive metabolites is therefore similar to that of normal subjects, but it is difficult to determine if the cirrhotic liver is more sensitive to the toxic effects of the same amount of reactive metabolite [NAPQI] [23]."
- Zapater *et al*: "These two metabolites [cysteine and mercapturate] reflect the conversion of the drug to the reactive hepatotoxic intermediate which undergoes conjugation with reduced glutathione and then our data support previous findings that a therapeutic dose of acetaminophen is not more likely to cause liver damage in patients with chronic liver disease than in healthy subjects [24]."

6.4.2 Repeat-Dose Acetaminophen Biotransformation by Healthy and Diseased Livers is Similar

As shown in the previous section, acetaminophen biotransformation by the diseased liver is clearly similar to that by healthy livers after a single dose, a finding replicated across six studies comprised of 135 individuals with liver diseases of different etiology and severity. The next question is whether the metabolite pattern is similar after repeat dosing of acetaminophen at the maximum-labeled daily 4-g dose. To date, there are no published, repeat-dose, acetaminophen metabolism studies in individuals with liver disease. McNeil Study 11-005 [2] is the first study in hepatic-impaired adults that evaluates acetaminophen biotransformation after repeat dosing of 1000 mg every six hours for four days, totaling 17 g. The duration of four days of dosing was chosen based on (i) estimates of half-lives for the metabolites in the hepatic-impaired subjects to project the time to reach steady-state pharmacokinetics, and (ii) the total number of days that the hepatic-impaired subjects would be housed in the clinic during the study. The latter included two days before dosing, three days for the single-dose evaluation, and six days for the multiple-dose evaluation.

Time-dependent changes in acetaminophen biotransformation from the first dose to steady-state doses are shown in Table 6.7. For the matched-control subjects, the amount of glucuronide metabolite increased whereas the amount of sulfate metabolite decreased with

Table 6.7 Comparison By Dose of Mean (± sd) Percent Acetaminophen or Metabolite Excreted for Hepatic-Impaired and Matched-Control Subjects

	Hepatic-Impaired (n=12)			Matched-Control (n=11)		
	First Dose	**Last Dose**	**p-value[a]**	**First Dose**	**Last Dose**	**p-value[a]**
Acetaminophen	2.90 ± 0.1.99[b]	4.72 ± 1.66[b]	0.001	2.40 ± 1.78	2.53 ± 1.06	ns
Glucuronide	40.2 ± 13.3	52.1 ± 16.4	0.0001	49.0 ± 7.42	59.9 ± 11.7	0.008
Sulfate	27.4 ± 12.0	19.8 ± 4.94	0.016	22.4 ± 6.98	17.9 ± 4.16	0.01
Total Catechols[c]	2.44 ± 0.90[d]	2.91 ± 0.77[d]	0.038	3.09 ± 2.61	2.62 ± 0.72	ns
Total Thiols[e]	6.84 ± 2.47	5.77 ± 1.73	ns	5.51 ± 2.08	5.74 ± 1.86	ns

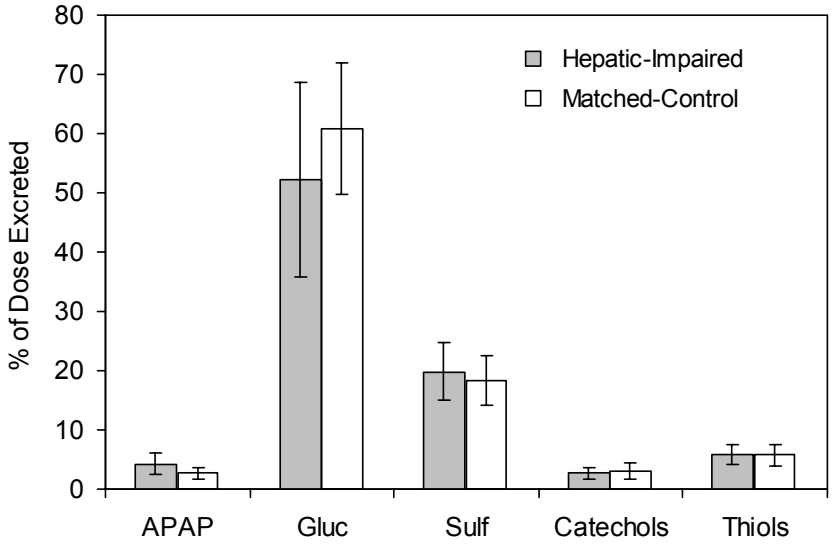
a: p-value from matched-pair t-test
b: n=10 subjects
c: The sum of methoxyacetaminophen (MO)-glucuronide and methoxyacetaminophen (MO)-sulfate
d: n=11 subjects
e: The sum of cysteine and mercapturate
ns – not significant

repeat dosing. These differences are statistically significant, and consistent with changes reported in a disposition study of young healthy adults with repeat acetaminophen dosing of 1000 mg, 1500 mg, and 2000 mg every six hours [25,26]. The latter study reported increases in glucuronide formation that more than offset decreases in sulfate conjugation over time. Although the data suggest cofactor depletion with possible saturation of the sulfate pathway, increases in glucuronide produced with repeat dosing were more pronounced, indicating induction of glucuronosyltransferase, presumably UGT1A6 [25]. Enhanced glucuronidation with repeat dosing of acetaminophen is discussed in more detail in Section 6.5.3.

Similarly for the hepatic-impaired subjects, the amount of glucuronide metabolite increased and the amount of sulfate metabolite decreased with repeat dosing, and these differences are statistically significant. The amount of acetaminophen excreted unchanged in the urine was statistically higher after repeat dosing, which is consistent with more acetaminophen being cleared directly by renal elimination. An important finding is that there were no changes in the amount of thiol metabolites formed in either group with repeat dosing, as they are nearly identical between groups after several grams of acetaminophen exposure. Enhanced glucuronidation and increased renal excretion of unchanged acetaminophen in the hepatic-impaired group show biotransformation that supports an expectation of diminished risk of hepatotoxicity with repeat therapeutic dosing at the maximum daily dose (4 g/d). This is in direct opposition to the naïve expectation before the discovery that acetaminophen induces its own glucuronidation with repeat dosing.

Figure 6.1 shows the urinary excretion pattern of acetaminophen and its metabolites excreted in urine as the percent of acetaminophen dose at steady state. Comparison of means between study groups using a one-way analysis of variance detected no statistical differences in the metabolites, indicating that the biotransformation of acetaminophen by the diseased liver is not different than the healthy liver. The percent of unchanged acetaminophen excreted is greater in the hepatic-impaired group, 4.26 ± 1.88% (n=12) versus 2.64 ± 1.09 (n=13) at $p = 0.014$, as this difference reflects higher renal elimination in the hepatic-impaired adults with repeat acetaminophen dosing.

Figure 6.1 Comparison of the Urinary Excretion Pattern of Acetaminophen and Its Metabolites By Study Group



6.5 Integrated Pharmacokinetic Results Across Studies

6.5.1 Lack of Progressive Acetaminophen Plasma Accumulation with Repeat Dosing

As noted previously, studies that describe the pharmacokinetics of acetaminophen from plasma data alone are of little use in elucidating the effect of liver disease, if any, on the biotransformation of acetaminophen. Pharmacokinetic parameters, such as elimination half-life (t½) and total body clearance (CL/F), depict time-dependent changes in plasma concentrations of the pharmacologically active analgesic (acetaminophen itself) and give vanishingly small information on the formation or disposition of the toxic intermediate, NAPQI.

Drugs having linear pharmacokinetics will reach steady-state plasma concentrations or be eliminated from the body after a time equal to four-to-five times the half-life. Thus, acetaminophen concentrations will reach steady state in healthy adults after 10 to 15 hours of multiple or repeat dosing because of its short half-life, which ranges from two to three hours. Moreover, the short half-life and dosing interval (τ) of four-to-six hours results in minimal accumulation of acetaminophen. Drug accumulation (Ac) after multiple doses can be estimated using the following standard equation:

$$Ac = \frac{1}{1 - 2^{-\varepsilon}}, \quad \text{where } \varepsilon = \tau / t½.$$

Pharmacokinetic data available from the acetaminophen studies in adults and children with liver disease of different severity and etiology show modest increases in the elimination half-life, about one-to-two hours on average. Means listed in Table 6.8 are consistent across the studies for the hepatic-impaired and control groups.

Table 6.8 Acetaminophen Half-Life[a] (t½; hour) After a Single Dose

Study	Liver Disease Groups	Control Group
McNeil [2]	3.4 ± 1.1; n=12	2.8 ± 1.4; n=12
Zapater [24]	3.7 ± 1.3; n=9 and 4.0 ± 0.6; n=5	2.0 ± 0.4; n=7
Andreasen [4]	3.7 ± 0.8; n=11	2.1 ± 0.6; n=12
Jorup-Ronstrom [15]	3.2 ± 0.4; n=10	2.1 ± 0.2; n=10
Villeneuve [23]	3.1 ± 0.2; n=11	2.0 ± 0.2; n=6
Arnman [5]	4.8 ± 2.4; n=21	2.9 ± 1.5; n=14
Forrest [12]	2.2 ± 0.5; n=8 and 4.3 ± 1.2; n=7	2.4 ± 0.2; n=8

a: Data reported as mean ± standard deviation; sample size per group.

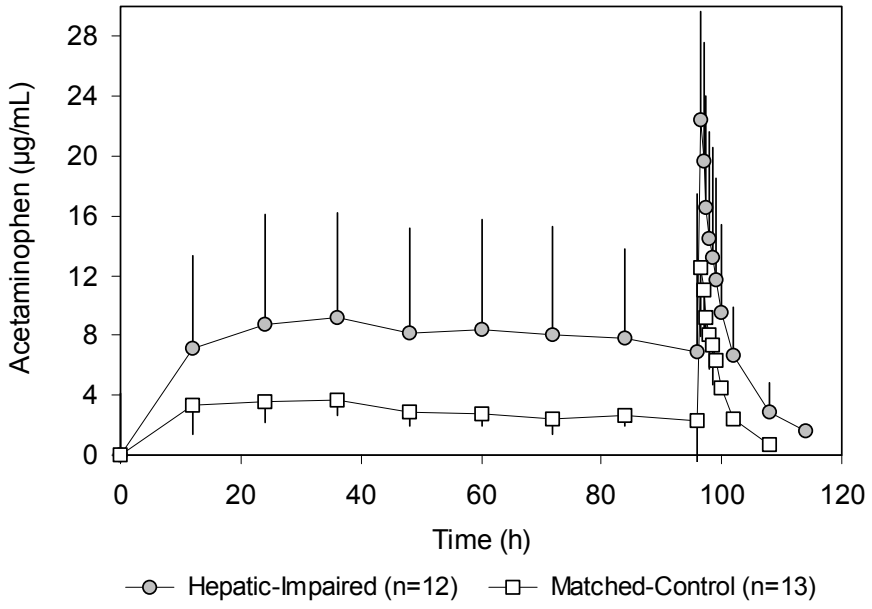
To place the magnitude of change in acetaminophen's short half-life in perspective relative to other drugs having longer half-lives, Table 6.9 contains examples of half-life changes due to liver disease. Based on the previous equations, the projected change in the time to reach steady-state plasma concentrations and drug accumulation are also shown compared with healthy adults. A two-fold increase in a short half-life drug (2 to 4 h), leads to a modest 50% increase in plasma accumulation, whereas a two-fold increase an intermediate half-life drug (12 to 29 hours) leads to a three-fold increase in drug accumulation. Marked accumulation up to 16-fold can occur with a two-fold increase in a long half-life drug (6.2 to 10.0 days).

The one-to-two hour increase in acetaminophen half-life on average in hepatic-impaired adults leads to modestly higher steady-state acetaminophen concentrations that do not progressively increase with repeated dosing. As illustrated in Figure 6.2, steady-state concentrations are reached in about one day in hepatic-impaired adults with no further accumulation, whereas they are typically reached in a half-day in healthy adults. Most importantly, the acetaminophen molecule itself imparts analgesic and antipyretic activities, and it does not cause adverse effects directly as other drugs generally do. These modestly elevated concentrations of acetaminophen are not of concern.

Table 6.9 Comparison of Drugs Having Changes in Half-life and Accumulation With Liver Disease

Drug	Study Population	τ (hour)	t½ (hour)	Time,ss (day)	R
Acetaminophen 1000mg	Healthy	6	2	0.4	1.1
	Liver Disease	6	4	0.8	1.5
S-Ibuprofen 100mg	Healthy	6	1.8	0.4	1.1
	Liver Disease	6	3.4	0.7	1.4
Chlorzoxazone 500 mg	Healthy	6	1.2	0.3	1.0
	Liver Disease	6	3.7	0.8	1.5
Fleroxacin 200mg	Healthy	24	12	2.6	1.3
	Liver Disease	24	29	6.0	2.9
Cyclobenzaprine 5mg	Healthy	8	23	4.8	4.3
	Liver Disease	8	46	9.6	8.2
Toremifene 60mg	Healthy	24	6.2 (d)	31	9.5
	Liver Disease	24	10.9 (d)	54	16

Figure 6.2 Multiple-Dose Acetaminophen Pharmacokinetic Profile from McNeil Study 11-005 [2]



In contrast to acetaminophen, elevated concentrations of other analgesic agents can exert adverse effects directly in people with liver disease. For example, nonsteroidal anti-inflammatory drugs (NSAIDS), including aspirin, ibuprofen, and naproxen exert an antiplatelet effect by blocking the formation of thromboxane A_2 via inhibition of platelet cyclooxygenase. Aspirin is unique in that it does so irreversibly. Adults with chronic liver disease who take NSAIDs have an increased risk of experiencing a bleeding event, not only because of the coagulopathy caused by disease itself, but also because of the increase in potential sites for hemorrhage (eg, esophageal varices, ulcers, and hemorrhoids) that result from portal hypertension [27].

6.5.2 Understanding Changes in Acetaminophen Clearance in Liver Disease

6.5.2.1 Basic Principles

To appreciate new and published findings on the effect of liver disease on the total body clearance of acetaminophen, a few general concepts are highlighted. Total clearance, CL (mL/min), is a measure of the efficiency with which a drug is removed from the body, and it is estimated from pharmacokinetic data as the dose divided by area under the plasma concentration – time curve: CL/F = Dose / AUC. Because the fraction (F) of the dose that is actually absorbed is not known, total clearance is reported as CL/F.

Total clearance is comprised of the sum of hepatic, renal, and other organ clearances:

$$CL/F = CL_H + CL_R + CL_O.$$

For acetaminophen, hepatic clearance, CL_H, predominates, as it contributes about 96% to total body clearance. Hepatic clearance is comprised of the sum of formation clearances fCL of the metabolites:

$$CL_H = fCL_{GLUC} + fCL_{SULF} + fCL_{CATECHOLS} + fCL_{THIOLS}$$

where the formation clearance, fCL, of a metabolite is estimated by multiplying the fraction, fe, of the dose excreted as metabolite in urine with total clearance: $fCL = fe \times CL/F$.

Some published acetaminophen pharmacokinetic studies in adults with liver disease and other conditions report changes in total acetaminophen clearance without urinary metabolite data, and then hypothesize potential changes in the different metabolites, including the formation of thiols, without any scientific evidence. This practice often leads to

highly speculative and inaccurate conclusions, especially with regard to extrapolating the study's results and its implication on the safety of acetaminophen at therapeutic doses.

6.5.2.2 Effect of Liver Disease on the Clearance of Drugs, Including Acetaminophen

Cirrhosis is a consequence of chronic liver disease characterized by replacement of liver tissue by fibrotic scar tissue and regenerative nodules, leading to progressive loss of liver function. Cirrhosis is most commonly caused by alcoholism and hepatitis C, and was the 12th leading cause of death in the United States in 2004 (26,549 deaths, - 5% versus 2003) [28]. Ascites is the most common complication of cirrhosis and is associated with a poor quality of life, increased risk of infections, and a poor long-term outcome. The pathological hallmark of cirrhosis is the development of scar tissue that replaces normal parenchyma, blocking the portal flow of blood through the organ and disturbing normal function.

Cirrhosis may cause loss of hepatic enzyme activity, altered hepatic blood flow, and reduced drug binding in the blood. These factors influence the distribution and clearance of drugs and their free concentrations in plasma. Drugs can be classified by their hepatic extraction ratio. If the hepatic extraction ratio is high then elimination is mainly dependent on blood flow, whereas, if the hepatic extraction ratio is low then elimination is sensitive to the liver's intrinsic ability to metabolize the drug.

Acetaminophen has a low hepatic extraction ratio and its protein binding is low, reported at 24% [29]. Drugs in this classification are generally unaffected by changes in hepatic blood flow and plasma protein binding, thus findings from disposition studies in liver disease show that hepatic elimination may be affected by loss of cell mass, reduction in enzyme mass per cell, or restricted access to hepatocytes (ie, sinusoidal capillarization) [30]. Anatomical and functional studies suggest that cirrhotic livers may in fact contain relatively normal hepatocytes, but less of them.

The total clearance of acetaminophen is slower in adults with liver disease. Estimates by study group are listed in Table 6.10, and the difference in means for the liver disease and control groups is about 50% across studies. Despite the slower clearance of acetaminophen, the relative amounts of each metabolite formed, including the thiols via NAPQI, are similar to those in healthy adults [2,12,16,23,24].

Table 6.10 Acetaminophen Total Clearance[a] (CL/F; mL/min) of a Single Dose

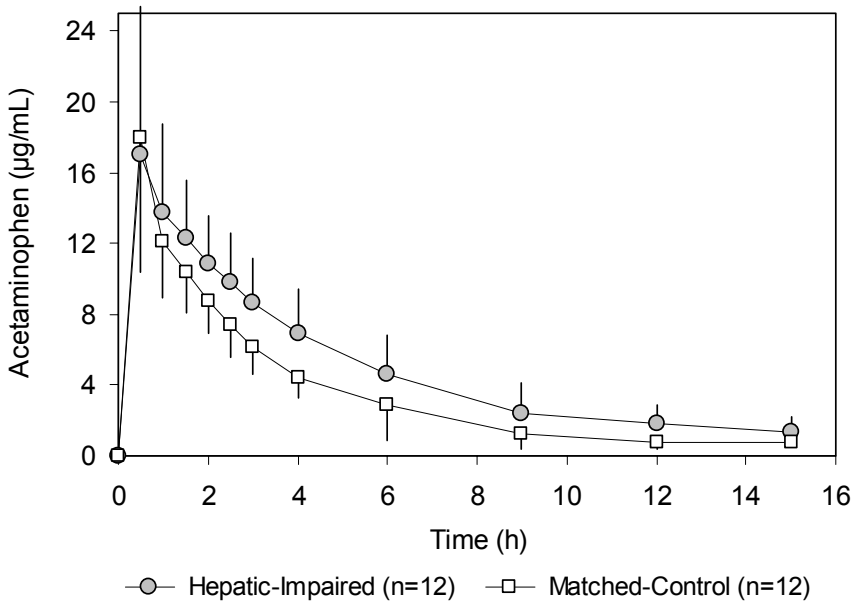
Study	Liver Disease Groups	Control Group
McNeil [2]	248 ± 108; n=12	335 ± 93; n=12
Zapater [24]	182 ± 96; n=9 and 139 ± 60; n=5	368 ± 62; n=7
Andreasen [4]	162 ± 51; n=11	355 ± 68; n=12
Jorup-Ronstrom [15]	224 ± 37; n=10	284 ± 37; n=10
Villeneuve [23]	231 ± 11; n=11	470 ± 46; n=6
Froomes [13]	181 ± 50; n=9	364 ± 184; n=5
Poulsen [18][b]	230 ± 41; n=5	268 ± 77; n=5
Hayes [14]	203 ± 353; n=6 and 289 ± 158; n=6	

a: Data reported as mean ± standard deviation; sample size per group.
b: Clearance (CL; mL/min) estimated with an intravenous dose.

Moderate-to-severe hepatic impairment due to cirrhosis mainly decreases the velocity of formation of each acetaminophen metabolite, resulting in modestly higher acetaminophen plasma concentrations compared with healthy adults, but the overall biotransformation remains unchanged. Caffeine, like acetaminophen, is classified as having a low hepatic extraction ratio and low protein binding. Moreover, total clearance of caffeine is slower in cirrhosis, but the biotransformation is similar to that in healthy subjects [31,32]. These findings support the proposition that hepatic elimination of acetaminophen and caffeine by cirrhotic livers may be affected by loss of cell mass (relatively normal hepatocytes, but less of them) and/or restricted access to hepatocytes [33].

In a health professional guide [34] on managing drug therapy in patients with severe liver disease, maintenance doses may or may not need adjustment for drugs with low-to-intermediate hepatic extraction. However, adjustments in both initial and maintenance doses of drugs with high hepatic extraction are highly recommended because of clinically relevant increases in plasma concentrations. In the case of acetaminophen, a low extraction drug, dose adjustment is not necessary in liver disease because the slower clearance results in modest increases in acetaminophen plasma concentrations after single (Figure 6.3) or steady-state (Figure 6.2) doses. For drugs that cause adverse effects directly, increases in plasma concentrations may be of concern. In contrast, the acetaminophen molecule itself imparts favorable analgesic and antipyretic activities, whereas the reactive metabolite, NAPQI, causes hepatotoxicity at excessive overdoses.

Figure 6.3 Single-Dose Acetaminophen Pharmacokinetic Profile (McNeil Study 11-005) [2]



6.5.3 Acetaminophen Induces Glucuronidation in Healthy and Diseased Livers With Repeat Dosing

Recently, acetaminophen was discovered to increase its own total clearance through enhanced glucuronidation when dosed repeatedly with 1000, 1500, and 2000 mg, totaling 4, 6, and 8 grams per day in healthy young adults [25,26]. Increases in total clearance from the first to final doses ranged from about 20% to 30% for all dose levels studied, and these changes were each statistically significant at $p < 0.0001$. In addition, plasma concentrations of the glucuronide metabolite were higher after repeat dosing than would predicted from single-dose pharmacokinetic data.

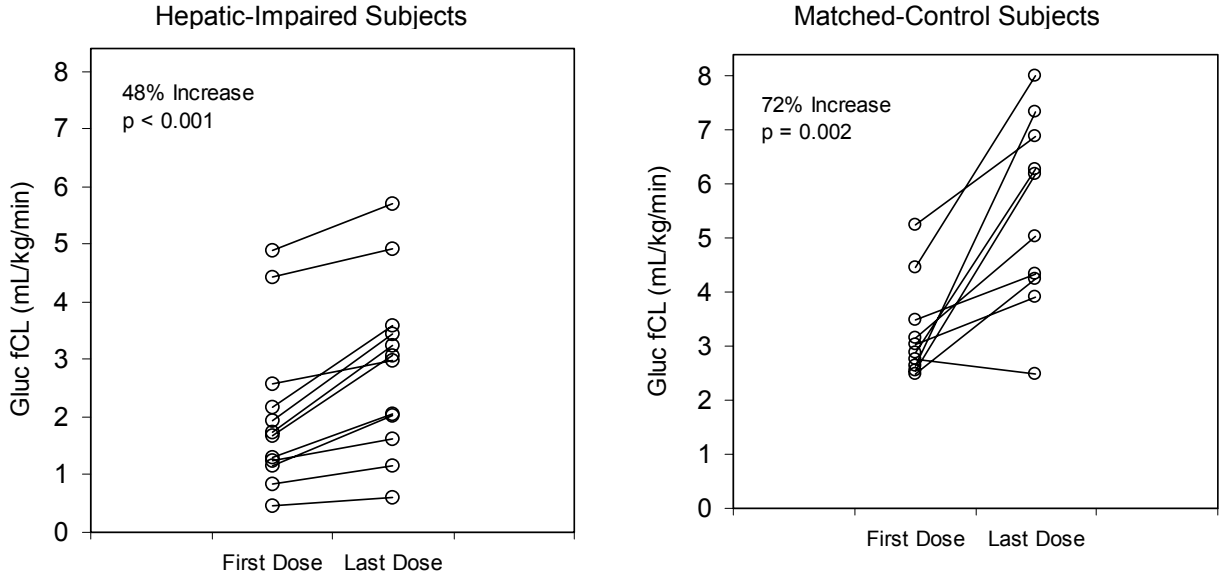
A comprehensive review of the historic literature revealed supportive evidence for increased acetaminophen glucuronidation with repeat dosing [35,36,37], although it was not recognized at the time or its potential implications in the safety and tolerability of multiple days of continuous acetaminophen use. In one study, the amount of glucuronide metabolite excreted in urine by nine healthy subjects increased from 47.7 ± 9.3% after a single 650-mg dose to 55.6 ± 6.8% ($p < 0.05$) after a regimen of 650 mg acetaminophen every six hours [35]. A similar increase in urinary glucuronide metabolite was reported in

another group of healthy subjects dosed with 650 mg acetaminophen every six hours [36]. In an early study of adults with Gilbert's syndrome, metabolites were measured in two subjects after an intravenous 10-mg/kg dose of acetaminophen was administered before and again after 20 oral doses of 1.5 g acetaminophen [37]. The amount of the glucuronide excreted increased from 37% to 59% and 45% to 62% of the acetaminophen dose in each subject, respectively.

In McNeil Study 11-005 [2], increases in total clearance were found with repeat dosing of 1000 mg acetaminophen every six hours. Total clearance from the first to final doses increased from 4.80 ± 1.89 to 5.48 ± 2.28 mL/kg/min ($p = 0.037$) in the hepatic-impaired group, and from 6.46 ± 1.65 to 8.23 ± 2.40 mL/kg/min ($p = 0.0001$) in the matched-control group. This change was accompanied by an increase in the fraction of acetaminophen dose excreted as the glucuronide metabolite, despite an expected decrease in the sulfate metabolite, from the first to final doses. Specifically, the fractional amount of glucuronide produced increased from 40.2 ± 13.3% to 52.1 ± 16.4% in the hepatic-impaired subjects ($p < 0.001$) and from 49.0 ± 7.4% to 60.8 ± 11.1% in the matched-control subjects ($p = 0.008$). Moreover, no differences were detected in these increases in glucuronide between groups using one-way analysis of variance ($p = 0.808$), indicating similar responses to repeat dosing of 1 g every six hours in both the diseased and healthy liver.

Time-dependent increases in acetaminophen clearance due to induction of UDP-glucuronyltransferases, despite hepatic impairment, are analogous with increases found in caffeine clearance due to induction of hepatic enzymes by smoking in both cirrhotic and healthy subjects [31,32]. These findings suggest that cirrhotic livers may contain relatively normal hepatocytes, but perhaps less of them, able to metabolize acetaminophen and caffeine similar to healthy livers, except somewhat slower. Figure 6.4 shows the increase over time in the formation clearance of the glucuronide metabolite for each subject.

Figure 6.4 Increase in Formation Clearance of the Glucuronide Metabolite After Repeat Acetaminophen Dosing in Both the Diseased and Healthy Liver.



An *in vitro* study in hepatocytes has also demonstrated that acetaminophen can increase glucuronidation [38]. This study was designed to explore potential mechanisms of enhanced glucuronidation by testing the glucuronidation of p-nitrophenol, a substrate of UGT1A6, after pretreatment with various agents that consume hepatic glutathione, including acetaminophen. Note that acetaminophen is glucuronidated by UGT1A6. The investigators show that a decrease in the ratio of reduced-to-oxidized glutathione by these agents shifts glycogen metabolism in the direction of glycogenolysis and accumulates UDP-glucose, oversupplying it for glucuronidation. They believe that the availability of UDP-glucose is the major determinant in glutathione depletion-stimulated glucuronidation reported previously *in vivo* [39].

The decrease in the reduced-to-oxidized glutathione ratio, an important indicator of oxidative activity in hepatocytes, appears to initiate useful compensatory pathways: shifting of glycogen metabolism toward UDP-glucose formation that may stimulate both ascorbate synthesis and glucuronidation. The investigators suggest that glucuronidation prevents further oxidation of aglycones, protecting cells from the generation of toxic electrophilic compounds and from the consequent progressive lowering of the cellular reduced

glutathione pool. Thus, these mechanisms described in their research study may serve as a useful protective pathway in the liver.

With repeat acetaminophen dosing in individuals with and without liver disease, acetaminophen may induce UDP-glucuronyltransferase activity directly and/or indirectly by continuous consumption of hepatic glutathione. As reviewed by Lauterburg [40], replenishment of glutathione begins immediately after taking a therapeutic dose of acetaminophen, stimulated by decreasing concentrations of reduced hepatic glutathione during detoxification of the reactive metabolite, NAPQI. He estimates that hepatic glutathione concentrations may decrease by less than 10% after a 1000-mg dose, and that they rapidly recover because of increased synthesis. With repeat dosing of acetaminophen every six hours, the associated fluctuations in glutathione concentrations may also stimulate glucuronidation by shifting glycogen metabolism toward UDP-glucose formation, which was shown *in vitro* to stimulate both ascorbate synthesis and glucuronidation [38]. However, prospective clinical metabolism studies are needed to confirm such a hypothesis for the observed increases in glucuronidation.

In summary, clinical metabolism studies of acetaminophen show an increase in glucuronidation with repeat dosing from 650 mg up to 2000 mg per dose, in young and older healthy adults, and in adults with liver disease and possibly Gilbert's syndrome. These metabolism data have important implications for our understanding of the well-established safety profile of repeat acetaminophen dosing at the maximum-labeled daily dose in the general population, including individuals with liver disease.

6.5.4 No Change in the Formation Clearance of Thiol Metabolites

As discussed in Section 6.4, the amount of thiol metabolites, reflective of the amount of NAPQI produced via CYP2E1, after single and repeat doses of acetaminophen was similar between healthy adults and adults with liver disease of different severity and etiology [2,12,16,23,24]. Additionally, there were no changes over time in the amounts of thiols produced or the formation clearances between the first and final, steady-state doses in the hepatic-impaired adults in McNeil Study 11-005 [2]. Using the matched-pair t-test, no differences were detected in mean percent of thiols excreted (6.84 ± 2.45 and 5.77 ± 1.72 %) and in mean formation clearance of thiols (0.326 ± 0.139 and 0.324 ± 0.169 mL/kg/min) for the first and final doses, respectively. These data are noteworthy in that they clearly show that the diseased liver can readily metabolize therapeutic doses of

acetaminophen up to the maximum-labeled daily dose, demonstrating no changes in deactivation (glutathione conjugation) or activation (metabolism by CYP2E1).

6.6 Acetaminophen Use in Adults and Children with Acute and Chronic Viral Hepatitis

6.6.1 Use of Acetaminophen in Clinical Practice

The most common chronic blood-borne infection in the United States is caused by hepatitis C virus, an RNA virus transmitted by direct percutaneous exposure to blood, with an estimated overall prevalence of 3.9 million people [41]. Disease progression varies among subpopulations, but up to 85% of hepatitis C virus infections become chronic (3.3 million), and 20% of cases progress to cirrhosis (660,000). In addition, an estimated 240,000 children are infected with hepatitis C virus; of these, 68,000 to 100,000 are chronically infected.

Clinical practitioners commonly recommend acetaminophen for the management of side effects during therapy for hepatitis C viral infection [42]. Standard therapies for hepatitis C infection are interferon or pegylated-interferon with ribavirin. An important obstacle to successful treatment is intolerance of interferon. Adverse effects of interferon-based combination therapy can be grouped broadly into influenza-like symptoms, neuropsychiatric symptoms, and hematological abnormalities. In large treatment trials, adverse events prompted therapy discontinuation in 10 to 14% of patients and dose reductions in 32 to 42% [43]. Because of this challenge, management of these adverse events is included *a priori* in clinical trial designs by the permitting acetaminophen use as needed [44]. For the general population, supportive care can minimize side effects to these therapies, thereby improving treatment tolerance and overall health-related quality of life for infected patients. So, prophylactic acetaminophen is often used to manage the flu-like symptoms associated with treatment.

6.6.2 Clinical Studies of Acetaminophen in Individuals with Viral Hepatitis

A prospective, double-blind placebo-controlled clinical study was designed to evaluate the effect of repeated acetaminophen administration on ALT level and viral load (HCV RNA) in adults with chronic hepatitis C virus [45]. Patients received either 3 g/d acetaminophen (n=17) or placebo (n=17) for seven days. No differences between treatment groups were noted with respect to ALT levels or viral load that were monitored before and at the end of

treatment, and when measured again three days later. The clinical investigators conclude that acetaminophen can be used as an analgesic or antipyretic drug in adults with chronic hepatitis C.

Three clinical pharmacology studies compared the pharmacokinetics of acetaminophen during the acute and recovery phases of infectious viral hepatitis: two studies in children and one in adults [7,15,22]. Elimination of acetaminophen from an intravenous 10-mg/kg dose was estimated in children experiencing an acute phase of infectious hepatitis [7]. The mean half-life during the acute phase was only slightly elevated compared with that in healthy children (2.97 ± 0.37 h versus 2.23 ± 0.30 h; $p < 0.001$). Approximately one week later, elimination half-lives were estimated in four children during the recovery phase when serum bilirubin was markedly below initial values. The half-lives were also lower (mean 2.34 ± 0.29 h) and comparable with values in healthy children. As noted previously, studies that describe the pharmacokinetics of acetaminophen from plasma data alone are of little use in elucidating the effect of liver disease, if any, on the biotransformation of acetaminophen. Half-life changes depict time-dependent changes in plasma concentrations of acetaminophen itself and give little information on the formation or disposition of NAPQI.

In another study, the acetaminophen half-life after an intravenous dose in 10 children, ages 5 to 15 y, with viral hepatitis was longer during the acute infectious phase compared with the recovery phase, and a lesser amount of glucuronide was excreted in the urine [22]. The amount of sulfate metabolite was similar in the acute and recovery phases for the 10-mg/kg dose, but higher for the 20-mg/kg dose. These results show that even though the amount of glucuronide formed decreases during the acute phase of hepatitis in children, the amount of sulfate formed increases at the higher acetaminophen dose. Sulfation is the predominant conjugation pathway for acetaminophen in infants and young children [46], which differs from adults for whom glucuronidation is the major metabolite.

Acetaminophen pharmacokinetics was assessed during the acute and recovery phases of the disease in adults with acute viral hepatitis (A, B, non-A/non-B) [15]. Ten adults received 1000 mg of acetaminophen during the acute phase and again approximately one month after recovery. The mean half-life of acetaminophen was modestly longer in the acute phase compared with the recovery phase (3.2 ± 0.44 versus 2.3 ± 0.13 h; $p < 0.05$), and when compared with control subjects (2.1 ± 0.15 h; $p < 0.05$). The clinical investigators conclude that acetaminophen can be given to adults with hepatitis at conventional doses

because maximum plasma acetaminophen concentrations were unaffected by hepatitis and that clearance did not differ significantly between control subjects and adults with hepatitis in either the acute or recovery phases.

Overall, modest increases in acetaminophen half-life (about one hour on average) are reported during the acute phase of viral hepatitis in three clinical pharmacology studies. As discussed in Section 6.5.1, such changes do not lead to accumulation of acetaminophen plasma concentrations nor increase in peak concentrations. The slower elimination rate appears consistent with altered transport from hepatocytes proposed by Fevery et al [11]. They suggest that since free bilirubin is often present in the serum in increasing amounts during the initial stages of acute viral hepatitis, acetaminophen may then compete with higher bilirubin concentrations for uptake and transport mechanisms within the hepatocytes. Alternatively, higher concentrations of both free bilirubin and acetaminophen may reflect similar diminished uptake without competition.

6.6.3 Key Points of Acetaminophen Use in Viral Hepatitis

- Clinical practitioners commonly recommend acetaminophen for the management of flu-like symptoms associated with interferon-combination therapy for hepatitis C viral infection, the most common type of viral hepatitis in the United States.
- Only a modest increase in acetaminophen half-life is found during the acute phase of viral hepatitis, and it returns to lower values during the recovery phase.

6.7 Children with Chronic Liver Disease

The metabolism of a single, 10-mg/kg dose of acetaminophen was evaluated in 13 children, ages seven months to 12 years, with chronic liver disease of varying severity [3]. Diagnosis of liver disease included biliary atresia, Alagille's syndrome, α1-antitrypsin deficiency, chronic active hepatitis, congenital hepatic fibrosis, and TPN cholestasis. The investigators compared the biotransformation of acetaminophen with published data reported in healthy children of similar ages. They noted that the relative amount of the glucuronide conjugate was higher but the sulfate conjugate was no different from that in healthy children. Although there are no reports of the amounts of cysteine and mercapturate excreted in healthy children, the investigators note that their data do not suggest any change in magnitude sufficient to cause concern when compared with either healthy adults or adults with liver disease. No significant differences were observed among children with mild,

moderate, or severe liver disease in the half-life, total exposure, oral clearance, or distribution of acetaminophen.

In a small study, the elimination of acetaminophen after a 30 mg/kg oral dose was compared in five children with cirrhosis with five healthy children (ages six months to 5 years). The mean half-lives were 1.78 ± 0.69 h and 1.90 ± 0.32 h, respectively [17]. The investigators conclude that glucuronidation and other conjugation pathways are probably not altered in children with cirrhosis because the elimination rate of acetaminophen was not impaired.

6.8 Prospective Clinical Safety Studies in Adults With Liver Disease

The therapeutic use of acetaminophen in patients with liver disease has been reviewed recently [47]. In this section, the safety and tolerability of therapeutic doses of acetaminophen from five prospective clinical studies in 77 adults with liver disease of varying severity and etiology are reviewed. Repeated acetaminophen dosing at 4 g/d was studied for four, five, and 13 days in adults with chronic liver disease [1,2]. Repeated doses of 4 g/d for five days were also assessed in a placebo-controlled study of chronic alcohol abusers who were reactive for the hepatitis C virus (HCV) antibody [48]. In addition, two clinical studies assessed 3 g/d in adults with cirrhosis for five days [4] and in adults with chronic hepatitis C infection for seven days [45]. The cumulative doses ranged from 15 to 56 g acetaminophen across studies. The key clinical findings are summarized in Table 6.11, and overall they show that multiple therapeutic doses of acetaminophen over several days were well tolerated. There were no increases in liver function tests including ALT, INR, bilirubin, no changes in viral load in adults with hepatitis, and no hepatic-related clinical adverse events.

Table 6.11 Summary of Clinical Outcomes for Therapeutic Acetaminophen Use in Adults with Liver Disease

	Benson [a] 1983 [1]	Benson 1983 [1]	McNeil 2007 [2]	Andreasen 1979 [4]	Green 2005 [48]	Dargère 2000 [45]
Population Evaluated						
Alcoholic Cirrhosis	–	2	2	4	–	–
Alcoholic / Hep C	–	–	6	–	18	–
Hep C Cirrhosis	–	3	4	–	–	–
Hepatitis C	–	7	–	–	–	17
Other [b]	6	8	–	–	–	–
Dosing Regimen	4g x 5d	4g x 14d	4g x 4d [c]	3g x 5d	4g x 5d	3g x 7d
Cumulative Exposure	20 g	56 g	17 g	15 g	20 g	21 g
Number Exposed	6	20	12	4	18	17
Clinical Outcomes	+	+	+	+	+	+
Change in ALT	↔	↔	↔	↔	↔	↔
Change in INR	NR	NR	NR	NR	↔	NR
Change in Other Labs[d]	↔	↔	↔	↔	NR	NR
Hepatic AEs[e]	None	None	None	None	None	None

a: Pilot study
b: Other includes Laennec's cirrhosis, cirrhosis (type unspecified), and primary biliary cirrhosis
c: One additional dose given on the morning of the fifth day.
d: Clinical laboratory tests associated with liver function.
e: Other clinically significant adverse hepatic events beyond changes in INR and laboratory tests.
Key: NR – not reported; NA – not applicable

6.8.1 Additional Data and Information From Benson's Published Study of 4 g/d Acetaminophen in Liver Disease

In 1983, Benson published the first prospective, placebo-controlled study of acetaminophen at the maximum-labeled dose in adults with liver disease [1]. FDA critiqued this study in Docket No. 77N-094, "Literature review to assess whether acetaminophen can be used safely by people with liver disease", issued on March 3, 2003, which is reference 26 of the proposed rule. Additional data and information from the Benson study addressing some questions raised by FDA in the review is highlighted in herein with further details provided in Attachment 3 of this section. Key findings from the pilot and full studies are summarized below.

Initially, an open-label pilot study in six adults with chronic liver disease was conducted to determine if acetaminophen accumulates with continued daily doses of four grams for five days [1]. The hepatic-impaired adults were evaluated clinically, which included laboratory tests for direct bilirubin, alkaline phosphatase, AST, ALT, GGTP, creatinine, prothrombin time, partial prothromboplastin time, and protein electrophoresis. No changes in clinical status or in laboratory tests were observed. In addition, no progressive increases in acetaminophen plasma concentration were measured over the five-day study period, indicating that acetaminophen does not continue to accumulate as the mean half-life was only modestly prolonged to 3.4 ± 2.5 h, and plasma concentrations ranged between 4.5 to 26.7 µg/mL across subjects.

A larger double-blind, placebo-controlled, crossover study was designed to assess whether adults with stable chronic liver disease could safely use four grams of acetaminophen daily for two weeks without developing adverse reactions or have deterioration in liver-related laboratory tests [1]. Twenty adults, who completed the study per protocol, were randomized to either placebo or acetaminophen initially after a baseline period of seven days, and then crossed over to the other treatment. Diagnoses of liver disease were confirmed by liver biopsy in all adults, and they included alcoholic liver disease, Laennec's cirrhosis, cirrhosis (type unspecified), postnecrotic cirrhosis, chronic active hepatitis, chronic persistent hepatitis, and primary biliary cirrhosis.

Pregnant women and subjects with histories of drug reactions or with known allergies to acetaminophen were excluded from the study. Subjects were instructed to continue their normal intake of medication and/or alcohol intake without change in schedule during the 35-day study period. No additional concomitant medication was to be taken during that period. If additional drugs were to be taken during the study period, that subject would be excluded from the analysis.

The study began with an initial one-week baseline period of observation to assess the stability of the disease process. Twenty-eight subjects were enrolled to complete 20 subjects, and they were randomly assigned to one of two treatment sequences. Group 1 received 4-g/d acetaminophen for 13 days then crossed over to placebo in identical capsules for another 13 days, whereas Group 2 began with placebo then crossed over to 4 g/d acetaminophen. Detailed subject evaluations, including the laboratory tests were performed initially and at 7, 14, 21, and 35 days. Subjects were instructed to take two

capsules at 8:00 AM, 12:00 noon, 4:00 PM, and 8:00 PM each day during the 7-day baseline period (placebo) and the two 13-day treatment periods (1 g acetaminophen or placebo).

In its review, FDA suggests that potential carryover effects likely existed in this crossover study thus affecting the reported results. Additional information from the protocol and statistical report shows that the data analysis included the appropriate statistical models to test for carryover effects. The safety endpoints of this study were the clinical laboratory test values measured after 13 days of acetaminophen or placebo treatment. Because the endpoints were compared sufficiently late in each crossover treatment (in this case at the 13-day mark), the potential for carryover effects, if any, had been minimized. In fact, no statistically significant findings for crossover effects were detected in these laboratory values.

FDA noted that the subjects were not stratified by underlying liver disease. Instead, they were randomly assigned to one of the two treatment sequences. Stratification is generally not necessary with a crossover design because each subject serves as his or her control. Nevertheless, the similarity of the two groups with respect to baseline clinical laboratory values had been assessed by comparing means of raw and log-transformed data measured on Day 7 (end of baseline period) for sequence Groups 1 and 2 using a student's t test. Additionally, the Wilcoxon rank-sum test was used to compare medians between groups. No statistical differences in mean or median baseline values between Groups 1 and 2 were detected for any of the 19 variables considered.

The clinical assessment showed that acetaminophen was well tolerated with continuous use of maximum-labeled daily doses of 4 g/d with no change in the clinical status of any subject. Serum aminotransferase activities remained unchanged during the 13 days of acetaminophen 4 g/d, as shown by the ratio of alanine aminotransferase (ALT) to its upper limit of reference range in Figure 6.5 and Figure 6.6

Although one adult had increases in liver function laboratory tests while taking acetaminophen, he tolerated subsequent challenges with four grams of acetaminophen for periods of 10 and 14 days without such increases. Therefore, the clinical investigators did not attribute the original changes in laboratory tests during the study to acetaminophen, but rather as the result of an exacerbation of his chronic liver disease. No other abnormalities indicative of adverse reaction to acetaminophen were observed, so the investigators concluded that therapeutic acetaminophen doses of 4 g/d may be used in adults with chronic stable liver disease.

Figure 6.5 Measurements of ALT per ULRR for Group 1 of 10 Hepatic-Impaired Subjects. Adapted from Data on File in Attachment 2.

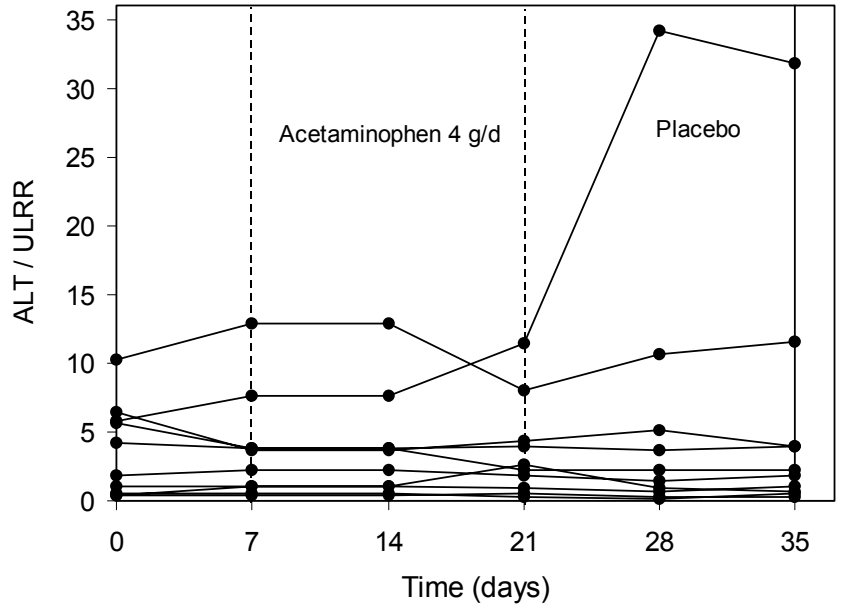
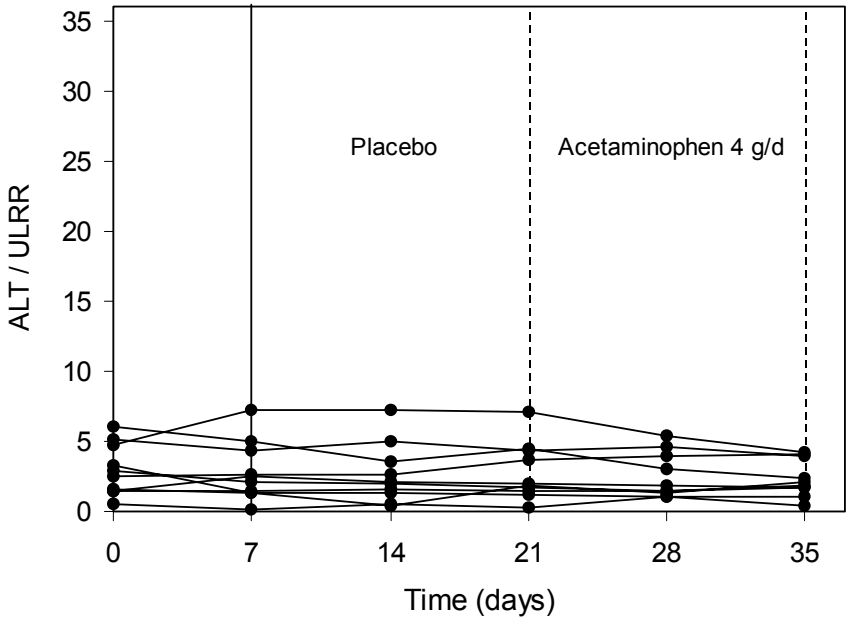


Figure 6.6 Measurements of ALT per ULRR for Group 2 of 10 Hepatic-Impaired subjects. Adapted from Data on File in Attachment 2.



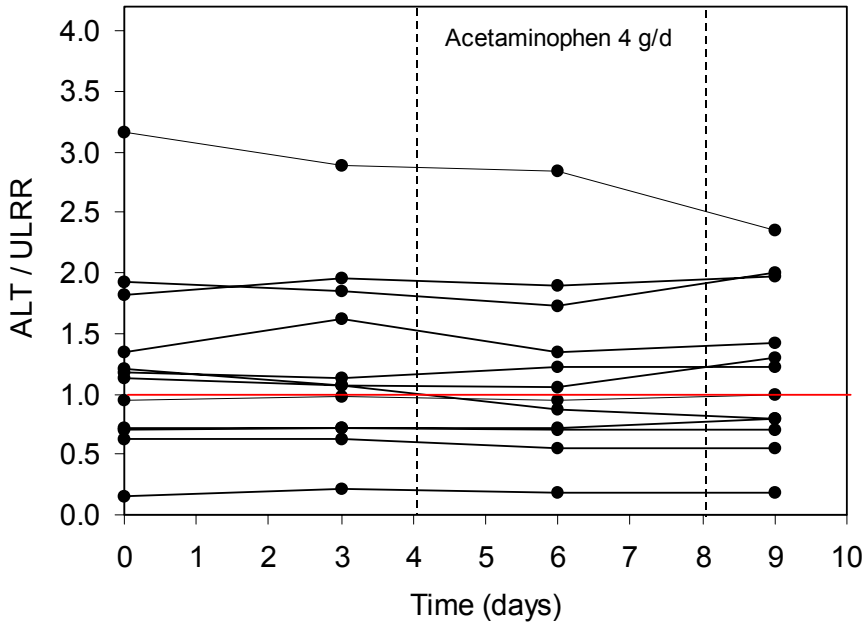
6.8.2 Clinical Safety Data From McNeil-Sponsored Study 11-005

The new McNeil-sponsored study [2] shows that acetaminophen at 4 g/d for four days was clinically well tolerated, as no increases in serum aminotransferases above baseline were observed during and 24 hours after the multiple-dose regimen in the subjects with chronic hepatitis C and/or alcoholic liver cirrhosis. Results for the ratio of ALT to ULRR are shown for the hepatic-impaired subjects in Figure 6.7. Aminotransferase activities for some of the control subjects without hepatic impairment had elevations above baseline during the multiple-dose regimen. Two control subjects were discontinued from the study due to mild elevations in aminotransferases (ALT of 87 and 117 U/L) on the third day of repeat dosing.

Transient mild elevations in aminotransferases have been observed and reported in some individuals without liver disease while taking repeat maximum-labeled daily doses of acetaminophen (3.9 and 4 g/d). In a retrospective analysis of seven clinical studies in over 1000 acetaminophen-treated adults with osteoarthritis [49], aminotransferase elevations (ALT and/or AST) greater than the ULRR were observed in subjects taking acetaminophen at a higher incidence, about 17%, than in subjects taking comparator medications or placebo. These ALT elevations were generally less than 1.5 times the ULRR. The analysis also showed that these transient ALT elevations usually resolve or decrease with continued acetaminophen treatment, are unaccompanied by signs or symptoms of hepatic injury, and appear to be clinically insignificant.

No serious adverse events occurred during McNeil Study 11-00 [2]. Five hepatic-impaired subjects reported eight mild or moderate adverse events: weight gain, skin tightness, urinary tract infection, venipuncture site pain, venipuncture site swelling, haematuria, and ocular hyperanemia. The investigator deemed these events definitely not related to study drug. Eight control subjects reported 14 mild adverse events. Six of these adverse events were deemed definitely not related to study drug: anemia, herpes simplex, hemorrhoidal pain, dizziness (2), and neck pain. The remaining eight adverse events were elevations in aminotransferases that were deemed possible (1), probably (6), and definitely (1) related to study drug.

Figure 6.7 Measurements of ALT per ULRR for the Hepatic-Impaired Subjects in McNeil Study 11-005 [2]



6.8.3 Summary of Results From the Remaining Prospective Clinical Studies

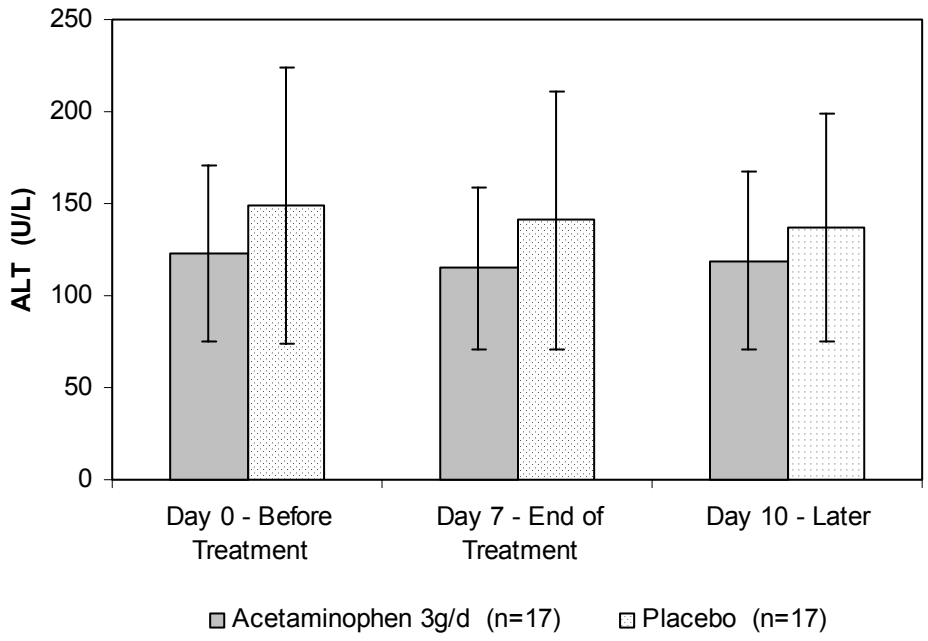
In a randomized, double-blind, placebo-controlled study of active alcoholic adults, the tolerability of acetaminophen 4 grams/day for five days, dosed after the cessation of drinking in a detoxification center, was assessed by monitoring hepatic laboratory values and adverse events [48]. Exclusion criteria included a baseline serum acetaminophen > 20 µg/ml, AST or ALT > 200 IU/L, or INR > 1.5. Of the 100 patients who completed the study (acetaminophen, n=49; and placebo, n=51), 18 (37%) patients taking acetaminophen and 23 (45%) taking placebo were reactive for hepatitis C virus antibody. No significant differences were detected in ALT, AST, and INR measurements between treatment subgroups. The investigators conclude that maximal therapeutic dosing of acetaminophen did not affect hepatic function as measured by ALT, AST, or INR in the alcoholic patients, even in the presence of elevated baseline ALT or in those patients with hepatitis.

In a repeat-dose, matched-control study [4], acetaminophen pharmacokinetics of repeated dosing of 3 g/d for five days were assessed in adults with cirrhosis. The mean trough acetaminophen concentrations were 9.2 µg/ml in four adults with cirrhosis and 3.2 µg/ml in nine control subjects. Progressive accumulation of acetaminophen, signs of clinical

hepatotoxicity, or significant changes in tests of liver function (ALT, ALP, PT, and bilirubin) were not observed during the study.

The effect of repeated acetaminophen administration on ALT level and viral load (HCV RNA) in adults with chronic hepatitis C virus was evaluated in a double-blind, placebo-controlled clinical study [45]. Patients received either 3 g/d acetaminophen (n=17) or placebo (n=17) for seven days. No differences within and between groups were noted with respect to ALT levels or viral load that were monitored before and at the end of treatment, and when measured again three days later. The results for ALT are displayed in Figure 6.8. The clinical investigators conclude that acetaminophen could be used as an analgesic and antipyretic drug in adults with chronic hepatitis C.

Figure 6.8 Comparison of ALT Measurements in Patients With Chronic Hepatic C by Treatment. Adapted from Dargère [45].



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SECTION 6 ATTACHMENTS

Attachment 1: Study Design and Methods for McNeil Study 11-005

Single- and Multiple-Dose Pharmacokinetics of Acetaminophen and Its Metabolites in Hepatic-Impaired Subjects at the Maximum Daily Dose

Protocol 11-005

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Study Design

McNeil Study 11-005 has a multiple-dose, open-label, parallel-group design. Twelve moderately hepatic-impaired adults and 13 healthy matched-control adults completed the study and were included in the pharmacokinetic analysis. Inclusion criteria for subjects with moderate hepatic impairment included a Child-Pugh score of 7 to 9, and a known medical history of liver disease with or without a known history of alcohol abuse. In addition, they were required to have previous confirmation of liver cirrhosis or chronic hepatitis transforming to cirrhosis by liver biopsy, macroscopic evaluation by laparoscopy or computerized tomography scan, or ultrasonography associated with an unambiguous medical history. The control subjects were matched by gender, race, and smoking status, and they were within ± 5 years of age and within ± 10% of body mass index of the hepatic-impaired subjects. They were considered healthy based on medical history, physical examination, clinical laboratory profiles, and an electrocardiogram within the range of clinical acceptability.

The main exclusion criteria for subjects with moderate hepatic impairment included aminotransferase activities more than five times upper limit of reference range; fluctuating or rapidly deteriorating hepatic function; ascites greater than moderate; moderate or severe (grade 3 or 4) encephalopathy; and Class III or IV heart failure as defined by the New York Heart Association functional classification system. In addition, subjects would be excluded if they, within the two months before screening, had a history of significant drug or alcohol abuse or any indication of regular use of more than two units per day of alcoholic beverages (one unit equals: ½ pint of beer, or one 4 oz glass of wine, or 1½ measures of spirit).

Subjects checked into the clinical center two days before dosing (Day –2) and remained housed at all times during the study. On Day 1 of the study, two 500-mg acetaminophen caplets were administered. Single-dose pharmacokinetics of parent and metabolites were estimated from serial blood and urine samples collected over 36 hours. After a three-day washout period, the multiple-dose regimen began. On Day 4, two 500-mg acetaminophen caplets were administered every six hours (8 AM, 2 PM, 8 PM, and 2 AM) for 17 doses. Blood samples were collected before the 8 AM and 8 PM doses on Days 4, 5, 6, and 7, and serially for 30 hours after the last 8 AM dose on Day 8. Urine samples were collected for 24 hours beginning on Day 7. Subjects fasted before the morning doses on Days 1 and 8, and the remaining doses were administered at least two hours after a meal or snack.

During each day of serial blood sample collection, smoking and chewing of tobacco were prohibited from the time of morning wake-up until two hours after dosing. The average daily consumption of cigarettes and all other tobacco products was recorded on each subject's case report form. Consumption of alcohol was restricted from 72 hours before the start of dosing and while housed at the clinical center.

Aminotransferase activities (ALT and AST) were measured at screening and on Days –1, 3, 6, and 9, and subjects were monitored for adverse events. A complete panel of clinical laboratory tests were obtained at screening, and repeated on the morning of Day 3 (before the first of the multiple doses) and on the morning of Day 9 (reviewed before discharge on Day 10). At the conclusion of the study, subjects were given a complete physical exam.

Methods

Assay of Biological Samples

Plasma samples were quantified for acetaminophen, glucuronide, sulfate, cysteine, mercapturate, and methoxyacetaminophen using a validated high-pressure liquid chromatographic (HPLC) method. Aliquots of urine samples were assayed directly for acetaminophen, glucuronide, sulfate, cysteine, mercapturate, and methoxyacetaminophen, and twice after digestion with glucuronidase and sulfatase using another validated HPLC method.

The sum of methoxyacetaminophen concentrations after digestion with enzymes accounts for the total amount of catechols excreted per acetaminophen dose. The sum of free concentrations and those after digestion with enzymes for cysteine and mercapturate

accounts for the total amount of thiols. The latter metabolites are produced via cytochrome P4502E1 that forms the highly reactive intermediate, NAPQI, which is detoxified by glutathione.

For the quantification of acetaminophen and metabolites in plasma, 200-μL samples of plasma were diluted with water, glucuronidase, or sulfatase, as applicable. After incubation, the samples were extracted with acetonitrile: methanol (65:35), evaporated to dryness, reconstituted in 0.2% hydrochloric acid and centrifuged. For the quantification of acetaminophen and metabolites in urine, 50-μL samples of urine were diluted with water, glucuronidase, or sulfatase, as applicable. After incubation, the samples were extracted with acetonitrile, evaporated to dryness, and reconstituted with mobile phase. All final extracts from both plasma and urine samples were analyzed using HPLC with ultraviolet absorbance detection.

Pharmacokinetic Analysis

Pharmacokinetic parameters for acetaminophen and its metabolites were estimated using noncompartmental methods and a validated software program, WinNonLin Enterprise Version 4.1 (Pharsight Corp., Mountainview CA).

From the single dose on Day 1, the following pharmacokinetic parameters for acetaminophen and metabolites were estimated using plasma concentrations: area under the curve (AUC_{INF}); maximum plasma concentration ($C_{MAX,1}$); time to maximum concentration ($T_{MAX,1}$); total body clearance ($CL/F_{,1}$) for acetaminophen only; volume of distribution ($Vd/F_{,1}$) for acetaminophen only; apparent terminal rate constant and half-life (λ_Z, t½)

From the last multiple dose on Day 8, the following steady-state pharmacokinetic parameters for acetaminophen and metabolites were estimated using plasma concentrations: area under the curve ($AUC\tau$); maximum plasma concentration ($C_{MAX,SS}$); time to maximum concentration ($T_{MAX,SS}$); total body clearance ($CL/F_{,SS}$) for acetaminophen only; volume of distribution ($Vd/F_{,SS}$) for acetaminophen only; apparent terminal rate constant and half-life (λ_Z, t½); accumulation ratio (R); and minimum plasma concentration ($C_{MIN,SS}$).

From the single dose on Day 1, the following pharmacokinetic parameters for acetaminophen and metabolites (as appropriate) were estimated using urine concentrations: fraction of acetaminophen dose excreted in urine (fe); percent of acetaminophen dose excreted in urine (%D); metabolite formation clearance ($fCL_{,1}$); and renal clearance ($CL_{R,1}$).

From the multiple doses on Day 7, the following steady-state pharmacokinetic parameters for acetaminophen and metabolites (as appropriate) were estimated using urine concentrations: fraction of total acetaminophen dose excreted in urine (fe_{24}); percent of total acetaminophen dose excreted in urine (%D); metabolite formation clearance ($fCL_{,SS}$); and renal clearance ($CL_{R,SS}$).

The current definition of obesity according to the World Health Organization is a body mass index (BMI) = 30 kg m^2, and about 50% of the subjects enrolled in McNeil Study 11-005 had BMI $\geq$ 30 kg m^2. Therefore, lean body mass (LBM) was used in the pharmacokinetic analysis rather than body weight, because the former size descriptor has been shown to best describe the clearance of drugs in obese individuals [1]. The clearance and volume of distribution parameters for acetaminophen and metabolites are reported as estimated or after adjusting by LBM (kg).

Statistical Tests

To assess the effect of moderate liver disease on acetaminophen pharmacokinetics and metabolism, differences in parameters between the hepatic-impaired and matched-control subjects were analyzed with a one-way analysis of variance (ANOVA). Time-dependent differences between the initial single dose on Day 1 and the final steady-state dose on Day 8 for the acetaminophen and metabolite formation clearances, and the fractions of metabolites excreted were assessed within each group (hepatic-impaired and matched-control). A matched-pair t-test was used to test whether the difference in these parameters on Day 8 minus Day 1 are greater than zero.

Using the matched-pair t-test, ALT, AST, alkaline phosphatase, gamma-glutamyl transferase, prothrombin time, and total bilirubin were compared within group (hepatic-impaired and control) to test whether the difference between Day 9 (after treatment) and Day 3 (before treatment) was significantly different from zero.

Results

Data from this study are integrated into Item 1, Section 6 of this document.

Reference List

1. Green B, Dufful SB. What is the best size descriptor to use for pharmacokinetic studies in the obese? Brit J Clin Pharmacol 2004; 58:119-133.

Attachment 2: Summary Table of Acetaminophen Studies in Liver Disease

Summary of Published Acetaminophen Pharmacokinetic/Metabolism Studies in Liver Disease (Years 1961 to 2006)

Citation	Study Design	APAP Dose Duration	Study Populations, Results, Conclusions
Al-Obaidy SS et al: *Eur J Clin Pharmacol* 1996;50:69-76	OL SD	10 mg/kg APAP	**Population:** Mild liver disease, children ages 7 to 11 y, n = 4 Moderate liver disease, children ages 3 to 8 y, n = 4 Severe liver disease, children ages 0.6 to 3 1 y, n = 5 **Pharmacokinetics:** Acetaminophen half-lives for children with mild, moderate, and severe liver disease were 2.0, 2.5, and 3.0 h, respectively. Formation of glucuronide metabolite appears higher that that reported in normal children, but formation of sulfate was not affected. Amounts of cysteine and mercapturate conjugates were comparable to those reported in healthy adults or adults with liver disease. **Conclusion**: The authors conclude this study provides reassuring additional data that, at least for single doses, there is no cause for concern in the use of acetaminophen in children with chronic liver disease.
Andreasen PB and Hutters L: *Acta Med Scand*, 1979, Suppl 624:99-105	OL SD MD	1000 mg APAP Single dose Multiple dose Q8h x 5 days	**Population:** Normal subjects, ages 41 to 69 y, n = 12 Cirrhotic subjects, ages 32 to 69 y, n = 11 **Pharmacokinetics:** Statistically significant differences were detected in half-life (2.1 vs 3.7 h) and clearance (337 vs 162 mL/min) of acetaminophen between normal and cirrhosis patients, respectively. Half-lives for each group were the same after single and multiple doses, and steady-state concentrations were statistically higher in cirrhotics than in normals. No clinical or biochemical signs of hepatotoxicity were observed during the study.
Arnman R and Olsson R: *Acta Hepato-Gastroenterol* 1978;25:283-286	OL SD	15 mg/kg APAP	**Population:** Control subjects, ages 18 to 86 y, n = 15 Cirrhotic subjects, ages 22 to 81 y, n =21 Liver Cancer subjects, ages 48 to 82 y, n = 4 **Pharmacokinetics:** Terminal half-life of APAP was 50% longer in cirrhotic subjects than in control subjects. Cirrhotic AUCs were also higher, but C_{MAX} and T_{MAX} were the same for all subjects. Most importantly, no significant differences in the 24-hour urinary excretion of acetaminophen between the liver cirrhosis patients and controls.

Abbreviations: APAP – acetaminophen; DB – double blind, OL – open-label; PL – placebo control; MD – multiple dose; SD – single dose.

Citation	Study Design	APAP Dose Duration	Study Populations, Results, Conclusions
Brazier JL et al *Alcohol Clin Exp Res* 1993;17:170-173	OL	1000 mg APAP Single dose	**Population:** Control subjects, n = 6 Cirrhotic subjects (compensated), n = 7 Cirrhotic subjects, (decompensated), n = 14 **Pharmacokinetics:** Acetaminophen terminal half-life similar between control and compensated cirrhotic subjects (2.7±0.5 h and 3.0±0.8 h) but lower in decompensated cirrhotic subjects(5.6±2.8 h).
Benson G: *Clin Pharmacol Therap* 1983;33:95-101p	DB PL XO	1000 mg APAP Multiple dose Q4h x 5 days Q4h x 13 days	**Population:** Cirrhotic subjects, ages 42 to 66 y, n = 6 (pilot) 20 subjects with cirrhosis (5), alcoholic liver disease (2), chronic active hepatitis (7); chronic persistent hepatitis (3), primary biliary cirrhosis (3) **Pharmacokinetics:** Pilot Study (n=6) Terminal half-life of acetaminophen was 3.4 h. No acetaminophen accumulation with repeated dosing of 4 gram/day. **Conclusion**: Clinical safety data support the use at therapeutic doses at maximum recommended dose 4g/d in adults with of stable chronic liver disease.
Careddu P et al: *Minerva Pediatr* 1961; 13:1619	OL SD	10mg/kg APAP IV	**Population:** Children with infectious hepatitis – acute phase, n = 15 Healthy children, n = 10 **Pharmacokinetics:** Terminal acetaminophen half-lives statistically higher in children with hepatitis (2.96 *vs* 2.24 h) after a 10 mg/kg intravenous dose.
Cormack CRH et al. *Ped Anesthesia* 2006;16:417-423	OL SD	40 mg/kg rectal	**Population:** Children with mild liver diseases ages 5 to 15 y, n = 17 **Pharmacokinetics:** Estimates of clearance 0.73 L/kg/h and time-concentration profiles for the children with liver diseases were similar to that reported in the healthy pediatric population. **Conclusion**: A single dose of rectal acetaminophen (40 mg/kg) is a satisfactory analgesic alternative.
Davies M, et al. *J Hepatol* 1995;22:551-560.	OL SD	500 mg	**Population:** Primary Biliary Cirrhosis, median age 55 y, n = 28 Other Liver Diseases, median age 43 y, n = 21 Healthy adults, median age 33 y, n = 27 **Pharmacokinetics:** Urine was collected for 8 hours, and the sulfate and sulfate:glucuronide ratio were reduced in primary biliary cirrhosis ($p<0.05$), but not in other liver diseases. **Conclusion**: Collection of the metabolites was incomplete, which makes the authors conclusion of impairment of sulfation in primary biliary cirrhosis questionable.

Abbreviations: APAP – acetaminophen; DB – double blind, OL – open-label; PL – placebo control; MD – multiple dose; SD – single dose.

Citation	Study Design	APAP Dose Duration	Study Populations, Results, Conclusions
El-Azib G, et al: *Inter J Clin Pharmacol Ther* 1999;7:299-303	OL SD	1000 mg APAP	**Population:** Control subjects, n = 8 Cirrhosis due to schistosomal infection, ages 9 to 65 y, n = 8 **Pharmacokinetics:** Half-life was about 1.5 to 1.7 fold higher in patients with cirrhosis due to schistosomal infection. Plasma glucuronide was higher, but the sulfate conjugate was comparable to control subjects. Urine collection was incomplete, only about 25% for the first eight hours, so data are inconclusive. **Conclusion:** Schistosomal infection is known to cause marked histopathological changes in the liver and to have an inhibitory effect on various drug-metabolizing enzymes. The increased acetaminophen half-life is consistent with cirrhosis from other causes, but the metabolic profile was not fully characterized to determine whether differences exist among the comparator groups.
Fevery J, de Groote J. *Acta Hepato-Splenologica* 1969;16:11-18	OL SD	10 mg/kg APAP	**Population:** Control subjects, n = 14 Hepatic-impaired subjects, n = 38 Parenchymatous disease (26), obstructive jaundice (9), Gilberts disease (3) **Pharmacokinetics:** Urinary excretion of unconjugated acetaminophen was elevated in hepatic-impaired subjects. Subjects with hyperbilirubinemia above 50 mg/L had statistically higher serum concentrations of acetaminophen measured at 3 and 6 hours after the dose. **Conclusion:** The authors conclude that jaundice is associated with lower metabolism of acetaminophen either by a decreased conjugation rate, or by competition in the uptake by the hepatocyte, or in the transport to the blood of the already conjugated form.
Forrest JAH et al: *Eur J Clin Pharmacol* 1979;15:427-431	OL SD	1500 mg APAP	**Population:** Control subjects, ages 21 to 34 y, n = 8 Mild Liver Disease subjects, ages 29 to 67 y, n = 8 Severe Liver Disease subjects, ages 27 to 60 y, n = 7 **Pharmacokinetics:** Acetaminophen half-life was similar in control subjects (2.4 h) and patients with mild liver disease (2.2 h), but was prolonged in those with severe liver disease (4.3 h, $p<0.001$). Importantly, no differences were detected in 24-h urinary excretion of acetaminophen and its glucuronide, sulfate, cysteine, and mercapturic acid conjugates in the three groups. Thus, the biotransformation, including glutathione conjugation, is not impaired in liver disease. **Conclusion:** No evidence of increased risk of hepatotoxicity in patients with chronic liver disease when given a 1500 mg dose of acetaminophen. The biotransformation of acetaminophen, including the thiol conjugates, is not altered in liver disease.

Abbreviations: APAP – acetaminophen; DB – double blind, OL – open-label; PL – placebo control; MD – multiple dose; SD – single dose.

Citation	Study Design	APAP Dose Duration	Study Populations, Results, Conclusions
Froomes PR, et al: *Gastroenterol* 1999;118:915-920	OL SD	1000 mg APAP dosed with and without oxygen	**Population:** Control subjects, ages 26 to 47 y, n = 5 Severe Liver Cirrhosis, ages 39 to 64 y, n = 10 **Pharmacokinetics:** Half-life was 4.5 ± 1 h in patients with sever liver disease awaiting transplant compared with 2.3 ± 0.8 h in control subjects, and clearance was about 50% lower. Oxygen supplementation did not have an effect on APAP pharmacokinetics. No metabolites were measured in this cohort. **Conclusion:** Capillarization in chronic liver disease results in a diffusional barrier to the exchange of solutes between the sinusoidal lumen and hepatocytes. It may contribute to lower drug elimination in cirrhosis by affecting uptake of drugs, bilirubin, or cofactors required for metabolism.
Hayes PC, et al: *Amer J Gastroenterology* 1989; 84:723-726	PL DB	1000 mg APAP ± 160 mg propranolol Single dose QD x 6 mo QD x 12 mo	**Population:** Acetaminophen + placebo subjects, ages 38 to 68 y, n = 6 Acetaminophen + propranolol subjects, ages 33 to 70 y, n = 6 (All subjects had chronic liver disease with cirrhosis) **Pharmacokinetics:** Propranolol did not affect the clearance of total or unconjugated acetaminophen either acutely or at 6 and 12 months. **Conclusion:** Chronic propranolol administration for up to 12 months does not interfere with hepatic metabolism of acetaminophen in patients with chronic liver disease. Propranolol is used to reduce portal pressure, prevent bleeding from esophageal varices, and improve salt and water homeostasis in these patients.
Jorup-Ronstrom C, et al: *Clin Pharmacokin* 1986;11:250-256.	PL OL SD	1000 mg APAP 1000 mg aspirin	**Population:** Control subjects, ages 19 to 50 y, n = 10 Viral Hepatitis subjects, ages 18 to 57 y, n = 10 (Acute versus recovery phases) **Pharmacokinetics:** Relative to control subjects, maximum acetaminophen plasma concentrations were similar to those for subjects in acute and recovery phases of viral hepatitis. No statistical difference in acetaminophen clearance was detected for control vs hepatitis subjects in the recovery phase, but clearance was statistically lower when hepatitis subjects were in the acute vs recovery phase. Half-life was statistically higher in acute hepatitis vs control subjects (3.2 and 2.1 h), but not in recovery hepatitis (2.3 h). **Conclusion:** The clinical investigators conclude that no differences in acetaminophen pharmacokinetics between control subjects and those with hepatitis in the recovery phase, and that normal doses can be used when patients are in this phase and the acute phase, except for severe cases.

Abbreviations: APAP – acetaminophen; DB – double blind, OL – open-label; PL – placebo control; MD – multiple dose; SD – single dose.

Citation	Study Design	APAP Dose Duration	Study Populations, Results, Conclusions
Misra PK et al. *Indian Ped* 1984;21:127-131	OL SD	30 mg/kg	**Population:** Children with cirrhosis (n = 5), ages 0.5 to 5 y Healthy children (n=5), ages 0.5 to 5 y **Pharmacokinetics:** Elimination half-life is 1.9 ± 0.32 h in healthy control children and 1.78 ± 0.69 h in children with cirrhosis. **Conclusion:** The rate of elimination of acetaminophen is not impaired in children with cirrhosis, suggesting that the glucuronidation and other conjugation pathways are probably not altered during the liver disease.
Poulsen HE et al: *Xenobiotica* 1991;21:243-249	OL SD	500 mg APAP IV Before and after 5 days 500 mg disulfiram	**Population:** Compensated alcoholic cirrhotic subjects, ages 34 to 63 y, n = 5 Control subjects, ages 35 to 79 y, n = 5 **Pharmacokinetics:** Acetaminophen plasma clearance decreased after disulfiram for all subjects, but formation clearances to the glucuronide, sulfate, and thiol metabolites did not change. Slight decrease in clearance in cirrhotic subjects compared with controls. **Conclusion:** The authors conclude that the results reveal no major effects of liver cirrhosis on acetaminophen clearance. This is consistent with data in which cirrhotic patients were not considered more susceptible to acetaminophen overdose than others without such a disease.
Shamszad M et al: *Gastroenterol* 1975; 69:865	OL SD	975 mg APAP	**Population:** Cirrhotic subjects, n = 5 Alcoholic active hepatitis subjects, n = 7 Alcoholic subjects with normal liver function, n = 5 Alcoholic subjects, given alcohol with acetaminophen, n = 5 **Pharmacokinetics:** Statistical differences were detected in half-life for cirrhotic (3.5 h) and alcoholic hepatitis patients (4.5 h) compared with alcoholics without liver disease (2.2 h) and alcoholics given 80 g alcohol simultaneously (2.8 h). **Conclusion:** Prolonged half-life observed in adults with biopsy proven cirrhosis and active alcoholic hepatitis. Alcohol taken with acetaminophen does not change its plasma elimination.

Abbreviations: APAP – acetaminophen; DB – double blind, OL – open-label; PL – placebo control; MD – multiple dose; SD – single dose.

Citation	Study Design	APAP Dose Duration	Study Populations, Results, Conclusions
Turner IB, et al. *Hepatol* 1990;12:896 (Abstract)	OL SD	500 mg IV	**Population:** Primary Biliary Cirrhosis, n = 27 Other Liver Diseases, n = 15 Healthy adults,n = 10 **Pharmacokinetics:** Urine was collected for 24 hours. No differences in the sulfate or glucuronide conjugate excreted, percent of total acetaminophen dose excreted, or sulfotransferase activity between any groups. **Conclusion**: These results show that acetaminophen conjugation to sulfate and glucuronide metabolites was not altered by liver disease in general, and by primary biliary cirrhosis in particular.
Vendemiale G, et al. J *Hepatol* 1989; 9(S1),S240 (Abstract)	OL SD	1000 mg	**Population:** Child B cirrhotics; ages 55 to 65y; (n = 6) **Pharmacokinetics:** Plasma AUC for sulfate and glucuronide increased after one week of s-adenosylmethione (SAMEe) administration. The percents of sulfate and mercapturate excreted in urine increased and decreased, respectively, ($p < 0.001$) versus baseline. **Conclusion:** The authors conclude that SAMe may promote acetaminophen inactivation, exerting a protective effect in patients with cirrhosis.
Vest MF, Fritz E. *J Clin Path* 1961; 14:482-487.	OL SD	10 or 20 mg/kg IV APAP	**Population:** Children with infectious hepatitis – acute and recovery phases (n = 6) **Pharmacokinetics:** Terminal acetaminophen half-lives longer during acute infectious phase after an intravenous dose. Amount of glucuronide excreted in the urine was higher during the recovery phase, but sulfate was similar for the 10 mg/kg dose and higher for the 20 mg/kg dose. Thiols formed via oxidation were not measured. **Conclusion:** Results show a decrease in the formation clearance of the glucuronide metabolite during the acute phase of hepatitis in children, but sulfate higher at higher dose.

Abbreviations: APAP – acetaminophen; DB – double blind, OL – open-label; PL – placebo control; MD – multiple dose; SD – single dose.

Citation	Study Design	APAP Dose Duration	Study Populations, Results, Conclusions
Villeneuve JP, et al: *Gastroenterol Clin Biol* 1983; 7(11), 898-902	OL SD	12 mg/kg APAP	**Population:** Control subjects, mean age 31 y, n = 6 Chronic alcoholic abusers, mean age 42 y, n = 9 Alcoholic cirrhotic subjects, mean age 56 y, n = 11 **Pharmacokinetics:** Half-life and clearance of acetaminophen are similar in chronic alcoholic and normal subjects, whereas half-life was prolonged and clearance was reduced by about 50% in alcoholic cirrhotics. Percents of cysteine and N-acetylcysteine in excreted in urine were similar between cirrhotic and normal subjects, but they were statistically higher in chronic alcoholic abusers who stopped consumption before dosing. **Conclusion:** Results suggest an increase in production of thiol metabolites within 24 to 48 hours of stopping alcohol consumption by the chronic alcoholic subjects, consistent with CYP2E1 induction. In abstinent subjects with alcoholic cirrhosis, production of the thiols metabolites was comparable to control subjects.
Zapater P, et al: *Aliment Pharmacol Ther* 2004; 20:29-36	OL SD	12 mg/kg APAP	**Population:** Control subjects, n = 7 Mild-moderate cirrhotic subjects, mean age 64 y, n = 9 Severe cirrhotic subjects, mean age 55 y, n = 5 **Pharmacokinetics:** Half-life was prolonged (3.8 $\pm$ 1.1h vs 2.0 $\pm$ 0.4h) and clearance was reduced by about 50% in cirrhotics vs controls. However, plasma and urinary excretion of metabolites, including the thiols, did not differ between groups despite an increase in acetaminophen plasma concentrations. Recovered metabolites were not different according to severity of liver dysfunction, ascites, or presence of esophageal varices. **Conclusion:** The authors conclude that their data support previous findings that a therapeutic dose of acetaminophen is not more likely to cause liver damage in patients with chronic liver disease than in healthy subjects.

Abbreviations: APAP – acetaminophen; DB – double blind, OL – open-label; PL – placebo control; MD – multiple dose; SD – single dose.

Attachment 3. Information on Benson Acetaminophen Clinical Study in Liver Disease

INTRODUCTION

Acetaminophen use by individuals with liver disease was assessed by Dr. Gordon Benson, "Acetaminophen in Chronic Liver Disease", and published in Clinical Pharmacology and Therapeutics in 1983 [1]. This article covers two studies designed to evaluate the safety of acetaminophen at the maximum daily dose (4 g/d) in subjects with chronic liver disease. The first was a pilot study with six subjects. The second was a larger double-blind study in 20 subjects.

The completed OTC Drug Clinical Review for FDA Docket Number 77N-094 (reference 26 from FDA's Proposed Rule) discusses the two studies within this publication. These data were identified in the agency's literature search for clinical studies primarily dealing with the use of acetaminophen in subjects with chronic liver disease. FDA provides comments in their review of the safety data available from the publication.

McNeil has located additional information from copies of the original study protocol, a statistical report, and case report forms for the larger comprehensive study (N=20). This document provides supplementary data and information to address some FDA comments on subject stratification and disposition, potential for crossover effect, and statistical analysis.

STUDY OBJECTIVE

The objective of the study stated in the protocol [2] was "To establish the safety of acetaminophen given at a dose of 4 grams per day for 13 days to subjects having pre-existing liver disease. The study will be a double-blind crossover with an initial one week baseline period of observation to ascertain stability of the patient population."

METHODOLOGY

Study Design

The study had a double-blind, placebo-control, two-period crossover design. Per protocol, 20 subjects were to complete the study, and they were evaluated clinically and with laboratory studies, including those for levels of total and direct bilirubin, alkaline phosphotase, 5-nucleotidease, serum glutamic oxalacetic transaminase (SGOT), serum glutamic pryruvic transaminase (SGPT), gamma glutamyl transpeptidase (GGTP), bile

acids (fasting), creatinine, prothrombin time, partial thromboplastin time, and protein electrophoresis.

Pregnant women and subjects with histories of drug reactions or with known allergies to acetaminophen were excluded from the study. Subjects were instructed to continue their normal intake of medication and/or alcohol intake without change in schedule during the 35-day study period. No additional concomitant medication was to be taken during that period. If additional drugs were to be taken during the study period, that subject would be excluded from the analysis.

Detailed subject evaluations, including the laboratory tests were performed initially and at 7, 14, 21, and 35 days. Subjects were instructed to take two capsules at 8:00 AM, 12:00 noon, 4:00 PM, and 8:00 PM each day during the 7-day baseline period (placebo) and the two 13-day periods (1 g acetaminophen or placebo).

The study began with an initial one-week baseline period of observation to assess the stability of the disease process. The diagnosis of the liver disease in all subjects had already been confirmed by liver biopsy. All subjects had a planned one-week wash out before receiving blinded treatment. Twenty-eight subjects were enrolled to complete 20 subjects, and they were randomly assigned to one of two treatment sequences. Group 1 received 4-g/d acetaminophen for 13 days then crossed over to placebo in identical capsules for another 13 days, whereas Group 2 began with placebo then crossed over to 4-g/d acetaminophen.

Discussion of Study Design

The study had a double-blind, two-sequence, two-period crossover design. In its review, FDA suggests that potential carryover effects likely existed in this crossover study. Additional information from the protocol and statistical report shows that the data analysis included the appropriate statistical method to test for potential carryover effects. Moreover, the crossover design was considered advantageous over a parallel design because variability between subjects is minimized as each subject, in effect, serves as his or her own control. This was an important consideration in recruiting subjects from a special population of hepatic-impaired adults who, by the nature and severity of liver disease, present with a broad range of clinical chemistries. The safety endpoints of this study are the clinical laboratory test values measured after each 13 days of acetaminophen or placebo treatment. Because the endpoints were compared sufficiently late in each

crossover treatment (in this case at the 13-day mark), the potential for carryover effects, if any, had been minimized. In fact, no statistically significant findings for crossover effects were detected in the parameters evaluated.

Subjects were not stratified by underlying liver disease; instead, they were randomly assigned to one of the two treatment sequences. Stratification is generally not necessary with a crossover design because, as stated previously, each subject is his or her control. The similarity of the two groups with respect to baseline clinical laboratory values had been assessed by comparing means of raw and log-transformed data measured on Day 7 (end of baseline period) for sequence Groups 1 and 2 using a student's t test. Additionally, the Wilcoxon rank-sum test was used to compare medians between groups. No statistical differences in mean or median baseline values between Groups 1 and 2 were detected for any of the 19 variables considered. The statistical results for the comparison of baseline mean values on raw data are shown in Table 1. While it is not necessary to stratify within a crossover design, it is noteworthy that differences were not observed between the sequence groups.

Table 1 . Comparison of Mean Baseline Values (Day 7) Between Groups 1 and 2

Variable	t-test, p value	Variable	t-test, p value
Weight	0.609	Serum Bile Acids	0.569
Temperature	0.423	Serum Creatinine	0.596
Total Bilirubin	0.813	Prothrombin Time	0.576
Direct Bilirubin	0.644	Partial Prothrombin Time	0.282
Alkaline Phosphatase	0.370	Albumin	0.716
5-Nucleotidase	0.294	Alpha 1	0.277
SGOT	0.637	Alpha 2	0.569
SGPT	0.530	Beta	0.589
GGT	0.694	Gamma	0.159
		Total	0.299

Details on Subject Disposition

In its review of the study, FDA was not clear on the disposition of subjects, so further details on subject disposition are provided in this section. Twenty-eight subjects were enrolled in the study to complete 20 subjects for the data analysis per protocol. Five subjects withdrew

consent due to personal reasons during the baseline period and were not included in the analysis, and they are listed in Table 2.

Table 2 Subjects that Withdrew Consent Before or During the Placebo Baseline Period

Subject Number	Day of Withdrawal	Diagnosis	Reason for Withdrawal of Consent
3877	0	Alcoholic Cirrhosis	Anxiety
3886	1	Chronic Persistent Hepatitis	Obtained job
3887	7	Laeneecs Cirrhosis	Hospitalized for other reason
3888	7	Unknown	Death in family and left city
3894	6	Cryptogenic Cirrhosis	Language problem

Of the remaining three subjects who were not included in the data analysis, two were discontinued from the study due to adverse events and one was a protocol violation. Subject 3879 developed a skin rash suggestive of a hypersensitivity reaction after taking acetaminophen for 10 days, and Subject 3884 developed nausea and malaise during the placebo period followed by deterioration during the acetaminophen period. Subject 3883 was excluded because of an obvious change in alcohol intake, which was a protocol violation. These three subjects are listed in Table 3.

Table 3 Subjects not Included in Statistical Analysis

Subject Number	Diagnosis	Reason for Early Discontinuation
3879	Laeneecs Alcoholic Cirrhosis	Discontinued drug (acetaminophen) on day 10 due to skin rash.
3883	Alcoholic Cirrhosis	Discontinued due to protocol violation (increase in alcohol consumption).
3884	Chronic Active Hepatitis	Discontinued drug (acetaminophen) on day 12 due to nausea, malaise and abdominal discomfort that began during Baseline Period (placebo).

Subject 3884 was evaluated and subsequently given acetaminophen in a single 1-g dose and in doses of 4 g daily for periods of 10 and 14 days at a time where his disease appeared stable (three months later). During this period, the subject experienced no adverse symptoms and there were no changes in his laboratory findings [1]. The

investigator concluded that the resulting adverse events during the study had been the result of an exacerbation of his chronic liver disease.

Table 4 and Table 5 summarize demographic information for the subjects who were included in the analysis and randomized to Group 1 and 2, respectively.

Table 4. Demographic Information for Subjects in Group 1

Patient	Age (Yrs)	Sex	Diagnosis	Duration (yrs)
3869	56	M	Post necrotic cirrhosis; portocaval shunt	2
3870	59	F	Laennec's cirrhosis	1
3872	55	F	Primary biliary cirrhosis	4
3874	32	F	Primary biliary cirrhosis	8
3878	57	M	Laennec's cirrhosis	5
3881	45	F	Alcoholic liver disease (fatty liver)	1.17
3891	21	F	Chronic liver hepatitis; post necrotic cirrhosis	5
3893	23	M	Chronic persistent hepatitis	1.5
3895	52	M	Cirrhosis; portocaval shunt	2
3896	25	M	Chronic active hepatitis	3

Table 5. Demographic Information for Subjects in Group 2

Patient	Age (Yrs)	Sex	Diagnosis	Duration (yrs)
3871	60	M	Cirrhosis	5
3873	41	M	Chronic active hepatitis	3.5
3875	24	M	Chronic active hepatitis	2
3876	45	M	Laennec's cirrhosis	1
3880	58	F	Alcoholic Liver Disease	5
3882	38	M	Chronic active hepatitis	2.5
3885	25	F	Chronic persistent hepatitis	6
3889	32	F	Chronic persistent hepatitis	0.75
3890	22	F	Chronic active hepatitis; post necrotic cirrhosis	5
3892	21	F	Chronic active hepatitis; post necrotic cirrhosis	7

Statistical Analysis

1.1.1 Clinical Endpoints

The study assessed differences in 17 clinical laboratory test variables as well as weight and oral temperature as it related to both one-week on treatment and two weeks on treatment. The laboratory test variables included both total and direct bilirubin, alkaline phosphotase, 5-nucleotidease, serum glutamic oxalacetic transaminase (SGOT), serum glutamic pryruvic transaminase (SGPT), gamma glutamyl transpeptidase (GGTP), bile acids (fasting), creatinine, prothrombin time, partial thromboplastin time, and protein electrophoresis.

To assess clinical status of the subjects during the study, eight variables related to history of disease and seven variables relating to physical examination were considered. These fifteen variables included: skin rash, fever, nausea, vomiting, abdominal pain, jaundice, dark urine, itching, jaundice, spider angiomas, palamar erythmea, hepatomegaly, splenomeagaly, ascites and edema.

1.1.2 Additional Information on Statistical Tests

An analysis of variance for a two-period crossover design was performed for each of the 17 laboratory tests as well as weight and temperature. The model included subject, drug, period, and visit as factors. The analysis was performed on raw, log-transformed, and ranked data measured after one week on treatment (that is, days 14 vs 28) and measured after two weeks on treatment (that is, days 21 vs 35). The results of the three ANOVA's for are presented in Table 6 after one week on treatment and in Table 7 after two weeks on treatment.

Review of statistical results shows no carryover effects. ANOVA yielded essentially the same results for raw, log-transformed, and ranked data. There are no significant differences between acetaminophen and placebo after one week on treatment for all variables except alkaline phosphatase, 5-nucleotidase and gamma glutamyl transpeptidase. There were no significant differences between acetaminophen and placebo after two weeks on treatment except for albumin.

Table 6 Comparisons Between Treatments After One Week on Treatment (Days 14 and Day 28)

	ANOVA on Raw Data			ANOVA on Log Transformed Data			ANOVA on Ranked Data		
	1st Per. Treatment	Carry Over	Both Per. Treatment	1st Per. Treatment	Carry Over	Both Per. Treatment	1st Per. Treatment	Carry Over	Both Per. Treatment
Weight	0.630	0.668	0.200	0.630	0.676	0.161	0.528	0.630	0.280
Temperature	0.173	0.051	0.603	0.164	0.052	0.603	0.248	0.052	0.854
Total Bilirubin	0.545	0.383	0.438	0.561	0.366	0.296	0.854	0.528	0.482
Direct Bilirubin	0.392	0.395	0.573	0.438	0.445	0.668	0.684	0.630	0.352
Alkaline Phosphatase	0.398	0.338	0.027	0.493	0.369	0.041	0.684	0.528	0.012
5-Nucleotidase	0.290	0.262	0.075	0.355	0.273	0.037	0.684	0.630	0.024
SGOT	1.000	0.476	0.226	0.603	0.755	0.545	0.912	0.970	0.436
SGPT	0.723	0.374	0.267	0.826	0.864	0.809	0.970	0.970	0.280
GGT	0.616	0.511	0.065	0.713	0.864	0.070	0.630	0.854	0.036
Serum Bile Acids	0.668	0.616	0.844	0.921	0.921	1.000	0.854	0.970	0.740
Serum Creatinine	0.723	0.324	0.129	0.794	0.369	0.163	0.970	0.578	0.166
Prothrombin Time	0.516	0.472	1.000	0.520	0.476	1.000	0.630	0.528	0.912
Partial Prothrombin Time	0.312	0.260	0.161	0.310	0.222	0.219	0.436	0.166	0.106
Albumin	0.502	0.386	1.000	0.530	0.417	1.000	0.740	0.630	0.436
Alpha 1	0.506	0.703	0.502	0.511	0.723	0.493	0.740	0.912	0.630
Alpha 2	0.273	0.196	0.516	0.275	0.200	0.535	0.436	0.394	0.854
Beta	0.610	0.660	0.864	0.567	0.603	0.921	0.684	0.630	0.970
Gamma	0.210	0.273	0.420	0.158	0.204	0.147	0.314	0.218	0.218
Total	0.560	0.826	0.069	0.623	0.889	0.085	0.394	0.854	0.076

Table 7 Comparisons Between Treatments After Two Week on Treatment (Days 21 and 35)

	P Values Two Week on Treatment (Days 21 and 35)								
	ANOVA on Raw Data			ANOVA on Log Transformed Data			ANOVA on Ranked Data		
	1st Per. Treatment	Carry Over	Both Per. Treatment	1st Per. Treatment	Carry Over	Both Per. Treatment	1st Per. Treatment	Carry Over	Both Per. Treatment
Weight	0.616	0.685	0.187	0.623	0.685	0.247	0.528	0.630	0.314
Temperature	0.668	0.921	0.516	0.668	0.921	0.520	0.630	0.912	0.740
Total Bilirubin	0.411	0.472	0.864	0.456	0.460	0.921	0.578	0.528	0.740
Direct Bilirubin	0.404	0.452	0.768	0.484	0.511	1.000	0.912	0.740	0.970
Alkaline Phosphatase	0.345	0.304	0.108	0.380	0.312	0.177	0.528	0.394	0.124
5-Nucleotidase	0.244	0.257	0.109	0.191	0.226	0.179	0.482	0.528	0.124
SGOT	0.497	0.264	0.243	0.889	1.000	0.456	0.740	0.684	0.394
SGPT	0.545	0.331	0.219	1.000	0.864	0.660	0.854	0.854	0.352
GGT	0.383	0.452	0.213	1.000	1.000	0.637	0.740	0.912	0.124
Serum Bile Acids	0.864	0.921	0.794	0.864	0.744	0.282	0.970	0.970	0.740
Serum Creatinine	0.328	0.380	0.545	0.350	0.438	0.476	0.528	0.684	0.436
Prothrombin Time	0.088	0.207	0.238	0.085	0.206	0.255	0.064	0.166	0.218
Partial Prothrombin Time	0.259	0.310	0.445	0.229	0.294	0.452	0.280	0.314	0.578
Albumin	0.168	0.324	0.288	0.177	0.350	0.264	0.436	0.436	0.028
Alpha 1	0.585	1.000	0.502	0.561	1.000	0.489	0.740	0.796	0.578
Alpha 2	0.099	0.125	0.217	0.097	0.126	0.196	0.218	0.314	0.166
Beta	0.257	0.414	0.273	0.254	0.389	0.312	0.248	0.394	0.528
Gamma	0.374	0.340	1.000	0.292	0.260	1.000	0.578	0.280	0.796
Total	0.676	1.000	0.135	0.603	1.000	0.097	0.796	0.912	0.106

SUMMARY

As stated in Dr. Benson's original paper and confirmed by the original statistical report for this study, twenty subjects completed per protocol and were included in the statistical analysis. This document provides additional information regarding the demographic characteristics and disposition of the subject population.

Subjects were randomly assigned to one of the two treatment sequences, effectively serving as their own control in this two-period crossover study. Because of this design, there was no need for stratification by underlying liver disease. Also, statistical tests showed that the mean laboratory values for the two different sequence groups were comparable at the end of the baseline period on Day 7. Importantly, additional results from the statistical analyses show that were no carryover effects after one or two weeks of treatment for any variable in this study

REFERENCE LIST

1. Benson GD. Acetaminophen in chronic liver disease. Clin Pharmacol Ther. 1983;33:95-101
2. Benson GD. Investigational Protocol. Phase II study comparing the safety of acetaminophen with placebo in subjects with liver disease. July 1976. (*On File*)

Section 7 Table of Contents

7 PROVEN SAFETY OF THERAPEUTIC DOSES OF ACETAMINOPHEN IN PEOPLE WHO CONSUME ALCOHOL

In this section, McNeil (1) reviews the data that FDA cites within the proposed rule as it relates to an association between acetaminophen, alcohol, and hepatotoxicity from retrospective case series and databases (2) addresses specific criticisms FDA has raised about studies in Section VII, Part B [71 FR 77336]; and (3) provides additional new clinical safety data from prospective studies that support the safety of the maximum-labeled daily dose of acetaminophen in people who consume alcohol.

7.1 FDA Comments from the Proposed Rule

On October 23, 1998 a final regulation was published in the Federal Register requiring that any OTC drug product labeled for adult use and containing acetaminophen, aspirin, carbaspirin calcium, choline salicylate, ibuprofen, ketoprofen, magnesium salicylate, naproxen sodium, or sodium salicylate have an alcohol warning statement in its labeling. For products containing acetaminophen this warning stated: "If you consume three or more alcoholic drinks every day, ask your doctor whether you should take acetaminophen or other pain relievers/fever reducers. Acetaminophen may cause liver damage."

At the September 19 and 20, 2002 Nonprescription Drugs Advisory Committee (NDAC) meeting, participants were asked to consider if alcohol users might be more susceptible to hepatotoxicity with acetaminophen and if the maximum allowable daily dose of acetaminophen should be used by individuals consuming three or more drinks per day. In its December 26, 2006, proposed rule in the Federal Register, FDA summarized the following points from that meeting:

- "NDAC was concerned about the lack of available data on which to base such advice, noting that there is a lack of information about how to determine the amount of alcohol, that may be harmful to any individual. NDAC noted that reducing the risk of drug adverse events is the goal, but believed that more data are essential for them to make specific recommendations." [71 FR 77324]
- "NDAC stated that, intuitively, a lower dose would decrease potential toxicity, but noted that there is a lack of information to support such labeling." [71 FR 77324]
- "At the September 19, 2002, NDAC meeting, FDA described cases of hepatotoxicity involving the use of prescription combination

(narcotic/acetaminophen) products. Many of these cases involved people with a history of alcohol abuse." [71 FR 77336]

- "NDAC was unable to recommend a reduced maximum daily acetaminophen dose for alcohol abusers, because of lack of specific data." [71 FR 77336]

Additional FDA comments in the proposed rule with respect to alcohol are as follows.

FDA is asking for additional comments and data on specific dosage for safe and effective use of acetaminophen in people who consume alcohol. "Both comment and data on specific dosage for safe and effective use of acetaminophen in people who consume alcohol. " [71 FR 77346]

FDA's analysis of 282 cases from AERS noted, "For those cases with acetaminophen dose information, the mean dose associated with toxicity was lower for alcohol users compared to nonalcohol users." [71 FR 77321]

"Risk factors, such as alcohol use or pre-existing liver disease, were identified and may have increased the risk for hepatotoxicity." [71 FR 77321]

"Risk factors—multiple data sources identify alcohol and underlying liver disease as risk factors that may increase the potential for hepatotoxicity" [71 FR 77322]

"Based on the data presented by Dr. Lee on liver failure, the experience in the University of Pennsylvania Hospital series, and data from the AERS database, FDA believes that alcohol users are a significant percentage of persons who develop severe liver injury." [71 FR 77336]

"Acetaminophen products already have an alcohol warning to alert consumers of the risk for developing hepatotoxicity. It is important to determine whether the labeling should include a lower daily dose for chronic alcohol users." "At this time, FDA is seeking both comments and data to support a specific dosage for acetaminophen as safe and effective in people who consume alcohol." [71 FR 77336]

In the proposed rule, FDA is proposing to remove the current alcohol warning and incorporate the following alcohol warning in the new liver warning for acetaminophen:

"Severe liver damage may occur if you take three or more alcoholic drinks every day while using this product." [71 FR 77333]

7.2 McNeil's Position

An alcohol warning on acetaminophen-containing products as it relates to therapeutic doses of acetaminophen is not warranted. New clinical safety data from multiple prospective, double-blind, randomized, placebo controlled trials demonstrate that alcoholics can safely take the maximum labeled daily dose of acetaminophen (4 g/day).

FDA's proposed labeling that "severe liver damage may occur if you take three or more alcoholic drinks every day while using this product" is inappropriate because it suggests that there is a risk when taking the maximum labeled daily dose of acetaminophen. FDA's proposed labeling does not have a sound scientific basis, is inconsistent with known and theorized acetaminophen metabolism in the presence of alcohol, and is not supported by new clinical safety data.

Some data suggest that alcohol use may be a risk factor for taking more than the recommended dose (overdose) and the development of hepatotoxicity, although even those data are conflicting. Therefore, if FDA deems that a new or reformatted alcohol warning is necessary, this warning should reflect the most current scientific data and apply only to taking more than the recommended dose (overdose) not therapeutic doses ($\leq$ 4 g/day).

Also, because alcohol use is not a credible risk factor for the development of hepatotoxicity, hepatic failure or hepatic dysfunction at the maximum labeled daily dose of acetaminophen, 4 g/day, data do not support reducing the current maximum labeled daily dose of acetaminophen for people who consume any amount of alcohol.

7.3 Key Points from McNeil's Response Supported by Clinical Data

- New clinical safety data from multiple prospective, double-blind, randomized, placebo controlled trials demonstrate that alcoholics can safely take the maximum labeled daily dose of acetaminophen (4 g/day).
- In light of new prospective data, which was not available at the time of FDA's 2002 NDAC meeting and which FDA has not taken into consideration in its justification for its proposed labeling, it is no longer appropriate for FDA to use historical