CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

022450Orig1s000

PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

SUPERVISOR'S SECONDARY REVIEW
PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-450**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**13-May-2009**
PRODUCT:	**BRANDNAME (acetaminophen) Injection**
INTENDED CLINICAL POPULATION:	**Adults, Adolescents and Children 2 to 12 years old as indicated for acute pain and fever**
SPONSOR:	**Cadence Pharmaceuticals, Inc.**
REVIEW DIVISION:	**Division of Anesthesia, Analgesia, and Rheumatology Products (HFD-170)**
PHARM/TOX REVIEWER:	**Carlic K. Huynh, Ph.D.**
PHARM/TOX SUPERVISOR:	**R. Daniel Mellon, Ph.D.**
DIVISION DIRECTOR:	**Bob A. Rappaport, M.D.**
PROJECT MANAGER:	**Sharon M. Turner Rinehardt** **Ramani V. Sista**
PDUFA DEADLINE:	**13-Nov-2009, extended to 13-Feb-2009**
Date review submitted to DARRTs:	**10-Feb-2010**

Executive Summary

I. Recommendations

A. Recommendation on approvability

From a nonclinical pharmacology toxicology perspective, NDA 22-450 may be approved pending agreement on the drug product labeling including a Pregnancy Category C.

B. Recommendation for nonclinical studies

No further studies are required at this time.

C. Recommendations on labeling

The labeling recommendations for acetaminophen below are based upon the review team's independent literature searches in conjunction with the citations and summary articles submitted by the Applicant. The Applicant submitted revised labeling in their January 13, 2010 submission, which was also taken into consideration. As the recommendations below are prior to discussion with the full review team and the Applicant, the reader is referred to the action letter for final labeling.

5 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

(b) (4)

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Dr. Carlic Huynh completed the primary review of the nonclinical pharmacology and toxicology sections of this NDA and has recommended that the application may be approved at this time. I concur with this recommendation.

The Applicant has relied upon the Agency's previous finding of safety and efficacy for Tylenol CR Oral Tablets (an over-the counter product), literature references, and several bridging studies they have conducted to support this NDA.

The Applicant conducted a pivotal 28-day repeat-dose toxicology study of the intravenous formulation in the rat model. The study demonstrated a NOAEL that provides an acceptable exposure margin for the maximum daily dose of APAP. The levels of drug product degradants in the formulation tested were not provided;

(b) (4)

Regulatory history: IND 58,362 was originally submitted to the Division of Anti-Inflammatory, Analgesic, and Ophthalmic Drug Products (HFD-550) by Bristol-Myers Squibb Company (BMS) on May 24, 1999 (FDA receipt date). Dr. Hamid Amouzadeh completed the review of the nonclinical pharmacology toxicology data submitted at that time to support the safety of the proposed Phase 3 clinical trial. The pivotal nonclinical studies in the original IND included the 28-day repeat-dose toxicology study in the rat model (Study number 980100T) and a local tolerance study in the rabbit. The proposed study was allowed to proceed based in part on the previously clinical experience with the drug product.

The Division (HFD-550) met with BMS on April 30, 2003 via a Type B guidance meeting with objectives that included obtaining concurrence on the preclinical safety data necessary for registration. The official meeting minutes contain the following nonclinical question and response provided by Drs. Josie Yang and Hamid Amouzadeh:

PRECLINICAL
Acetaminophen has been extensively studied in animals and the results of preclinical studies support the safety for human use. No data gaps were identified that would suggest the need for additional preclinical studies at this time. To support NDA registration requirements, proprietary preclinical data for Acetaminophen Injection and references to available public domain data for acetaminophen will be provided in the NDA. Does the Agency concur that no additional preclinical studies are required to support the NDA approval of Acetaminophen Injection? If the Agency does not agree, BMS requests guidance on this matter.

FDA Response:
Please provide updated information on the reproductive and genetic toxicity and carcinogenicity. If information is not available, non-clinical studies may be needed.

The IND was transferred from BMS to Cadence Pharmaceuticals effective March 30, 2006. An End-of-Phase 2 meeting was held with Cadence Pharmaceuticals on August 14, 2006; however, the nonclinical development program was not discussed at this time. The Sponsor did not request a preNDA meeting prior to submission of this NDA.

B. Pharmacologic activity

Acetaminophen (paracetamol, N-acetyl-p-aminophenol) is an analgesic and antipyretic agent with little anti-inflammatory activity. However, the exact pharmacological mechanism(s) of action mediating these effects have not been clearly delineated.

C. Nonclinical safety issues relevant to clinical use

There are two main nonclinical issues that must be addressed by the Applicant: providing adequate drug product labeling and providing adequate justification for the proposed drug product stability specifications, specifically the levels of p-aminophenol, which are significantly greater in liquid formulations than solid oral dosage forms. These two issues will be discussed in detail below.

Drug Product Labeling

To my knowledge, a drug product label for acetaminophen as a stand alone prescription drug product has not previously been approved by the FDA. The Applicant is relying upon information in the published literature as the basis for the proposed product labeling.

Genetic Toxicology of APAP. There are numerous studies in the literature that report positive genotoxicity findings for acetaminophen. As summarized in one review article of the genotoxic effects of acetaminophen (Rannug, et al., 1995), "[a]n overall evaluation of the data indicates that genotoxic effects of paracetamol contribute to the total burden of genetic damage observed in humans." Given the widespread use of acetaminophen, careful

consideration of these findings must be made in order to provide as accurate information as possible for product labeling. The original submission proposed the following language:

(b) (4)

In their January 13, 2010 submission, they proposed the following language:

(b) (4)

The Sponsor submitted four new genetic toxicology studies on APAP in this application using bulk acetaminophen drug substance. The results of the Ames bacterial reverse mutation assay and an in vivo unscheduled DNA synthesis assay in isolated livers of male rats were both negative. In contrast, the results from the in vitro chromosomal aberrations assay and mouse lymphoma assay were positive. The results from the chromosomal aberrations assay indicate that acetaminophen is clastogenic in vitro. The results of the mouse lymphoma assay, which can measure both mutagenicity and clastogenicity, indicate that acetaminophen is clastogenic and suggest the potential for mutagenicity. As these studies were conducted employing current protocols and were conducted in accordance with GLPs, the findings are appropriate for product labeling. However, the unscheduled DNA synthesis assay is not part of the ICH standard genotoxicity battery and has very low sensitivity therefore I do not recommend including the negative findings from that study in the product labeling.

In addition to the studies completed by the Applicant, the United States National Toxicology Program (NTP) has also conducted in vitro mutagenicity and clastogenicity studies on APAP with comparable results (National Toxicology Program, 1993). The results of the two NTP studies indicate that acetaminophen tests negative as a mutagen; however, it tests positive as a clastogen in vitro. These data confirm the Applicant's findings, are available to the public, employed current protocols and were conducted in accordance with Good Laboratory Practices (GLPs). Therefore, these findings also should be included in the product labeling.

As per current standards, positive in vitro clastogenicity results must be further assessed via an adequate in vivo study which can also provide information with respect to a potential No Observed Effect Level (NOEL) for clastogenicity. Neither the Applicant nor the NTP have conducted an in vivo assay for clastogenicity, such as the micronucleus assay. Although there are many references to in vivo clastogenicity studies in the literature (for reviews see (Rannug, et al., 1995; Bergman, et al., 1996)), the cited studies that would most closely resemble current study protocols are not publically available, and the applicant provided only the review articles in their submission. However, as results from in vivo studies provide data

regarding a potential threshold for clastogenicity, results from adequate in vivo studies should also be included in the product labeling.

Shortly after the Rannug article was published, three European Regulators (Medical Products Agency in Sweden, Federal Institute for Drugs and Medical Devices in Germany and Medicines Control Authority in Norway) published a second review on the subject of the genotoxicity and carcinogenicity of acetaminophen (Bergman, et al., 1996). This summary review cites several studies that were apparently conducted at the request of the German regulatory authorities to more definitively characterize the in vivo clastogenic potential of acetaminophen. These original studies were not submitted with the NDA application nor are they apparently available as they are owned by Ciba-Geigy and Hazelton Labs and/or the German regulatory authorities. However, according to Bergman, they were published by Baumeister in 1995. This citation was not provided by the Applicant; however it was obtained by the review team and determined to be an abstract of a presentation. As this reference did not provide actual data, it can not be used to inform product labeling. Bergman et al. conclude that there is "convincing evidence that genotoxic effects of paracetamol appear only at dosages inducing pronounced liver and bone marrow toxicity and that the threshold level for genotoxicity is not reached at therapeutic dosages." The Bergman paper conclusion that the genotoxic effects of the acetaminophen only occur at doses that exceed the hepatotoxic doses in the rat model is illustrated in the diagram below, reproduced from that article.

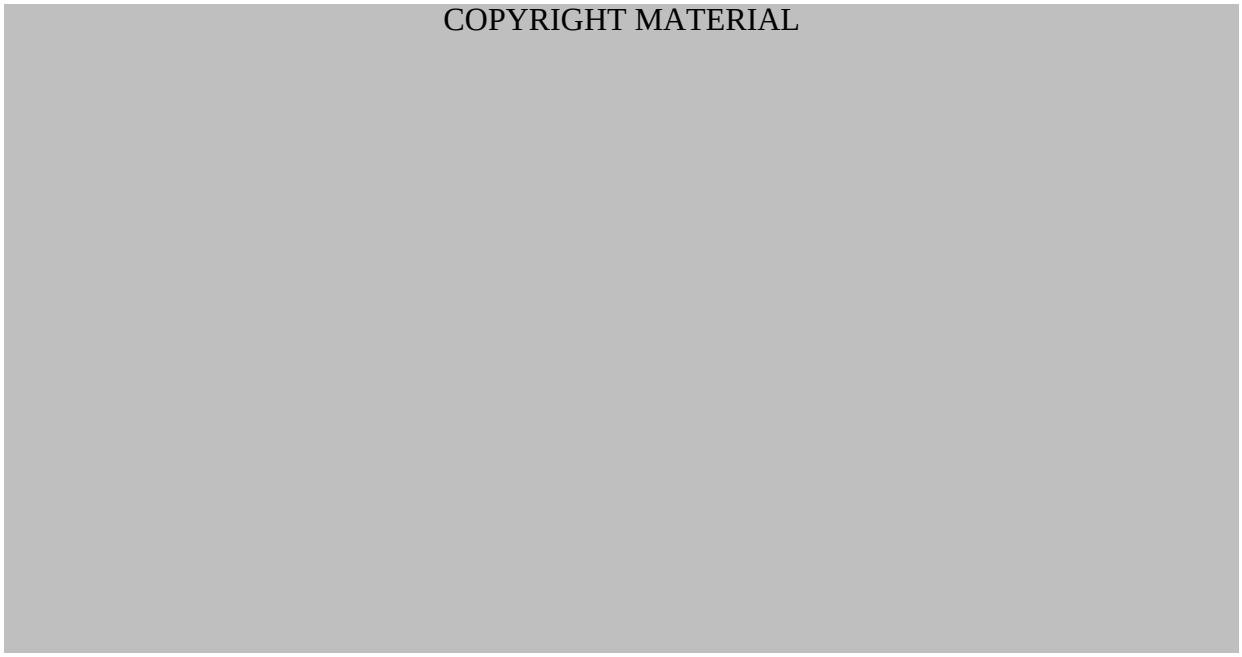

Fig. 1. Paracetamol: Oral gavage toxicity and micronucleus studies in rats. Onset of various toxicity related effects in relation to dosages used and peak plasma levels determined. Arrows illustrate increases or decreases in the relevant parameters determined. The human maximum recommended dosage (MRD) exposure is marked on a mg/kg basis as well as on the basis of peak plasma levels (for abbreviations see abbreviation list).

Collectively the existing genotoxicity data support the conclusion that acetaminophen is clastogenic, the effect is dose-dependent, and a NOEL can be obtained that provides an

apparent safety margin based on body surface area comparisons. Ideally, a safety margin would be based on exposure data, and therefore, if at all possible, the pivotal study reports referenced by Bergman should be submitted to the Agency. The Sponsor confirmed that the proprietary data could not be obtained; therefore, these results could not be independently verified. However, as this finding is key to the conclusion that a NOEL level for clastogenicity exists, and the study was conducted by the German regulatory authorities, the finding should be reported in the labeling. Due to the lack of toxicokinetic data, the exposure comparison must be made based on a body surface area comparison. According to the Bergman summary, 3 x 250 mg/kg (4500 mg/m^2) dose of acetaminophen (4 hour intervals) did not result in an increase in micronuclei formation. In contrast, either 3 x 500 or 1 x 1500 mg/kg dose (9000 mg/m^2) resulted in an increase in micronuclei formation. The NOEL dose for clastogenicity as defined in the studies reported in Bergman et al. (4500 mg/m^2) is 1.8 times the maximum daily dose of APAP (4000 mg/60 kg = 2467 mg/m^2) based on a body surface area comparison.

Carcinogenicity of APAP. The original NDA submission proposed the following language for the carcinogenesis portion of the product labeling:

(b) (4)

The January 13, 2010 revised draft labeling contains the following statement regarding the nonclinical data derived from the NTP studies:

(b) (4)

The wording is based on studies conducted by the National Toxicology Program (NTP) to evaluate the carcinogenic potential of acetaminophen. The study reports are available publically. The NTP study reports do not contain toxicokinetic data; therefore, the exposure margins for the product labeling will have to be based on body surface area comparisons for the label. The summary of the study results are reproduced from the NTP report in the table below:

Mean daily doses of APAP consumed were calculated based on mean food consumption over the course of the above studies in order to determine the exposure margin that should be included in the product labeling, as summarized in the table below:

Mean Dose (mg/kg)/Day APAP consumption (NTP Studies)

Group	600 ppm	3000 ppm	6000 ppm	6000 ppm (mg/m^2)	Exposure Margin* (HD = 6000 ppm)
Male Rats	30	149	295	1770	0.7
Female Rats	33	163	318	1908	0.8
Male Mice	91	448	1010	3030	1.2
Female Mice	114	603	1187	3561	1.4

*Exposure margin based on body surface area comparison to the maximum adult human dose of 4000 mg/day (2466.6 mg/m^2 for an average 60 kg person) for the high dose (HD) group.

Based on the results of this study, the NTP panel concluded that there was no evidence of carcinogenic activity in male rats. The NTP concluded that there was equivocal evidence of carcinogenic activity of acetaminophen in female rats based on an increase in the incidence of mononuclear cell leukemia that reached statistical significance in the high dose group. There was no evidence of carcinogenic activity of acetaminophen in male or female mice.

The results of this study were discussed by the Executive Carcinogenicity Assessment Committee on February 2, 2010. Based upon current CDER criteria, the mononuclear cell leukemias noted in the female rats were significant rather than equivocal; however, the Committee specifically noted that the NTP F344 rat strain is known to have a high background incidence of certain tumors, including mononuclear cell leukemia (Haseman, et al., 1998; Caldwell, 1999; Ishmael and Dugard, 2006). In fact, the NTP has discontinued use of the F344/N rat strain and began using a commercial source of the F344 rat (King-Herbert and Thayer, 2006). In terms of the finding regarding the increased incidence of mononuclear cell leukemia, the ECAC minutes note that "The committee recommended that the labeling of the product describe the results of the studies but note that this is of limited relevance."

The revised annotated labeling submitted by the Applicant on January 13, 2010 also included the following clinical statement:

(b) (4)

The annotated labeling cites four publications (Kreiger, et al., 1993; Mellemgaard, et al., 1994; Fortuny, et al., 2006; Viswanathan, et al., 2008). These references specifically look at the incidence of renal, bladder and endometrial cancers and therefore on their surface do not appear to support the proposed language. The reader is referred to the Clinical team leader review (Dr. Ellen Fields) for evaluation of these study reports and recommendations regarding this aspect of the proposed labeling.

Effects on Fertility. The Applicant did not conduct fertility studies for this application. In lieu of studies, they submitted a literature review on the subject. In the original NDA submission, the Applicant has proposed to include the following language in the product labeling:

(b) (4)

The proposed statement does not include information on the doses that produce the described effects nor how the exposure compares to the human exposure obtained with this product. In the original application, the Applicant references three publications from the literature to support this statement (Boyd and Hogan, 1968; Jacqueson, et al., 1984; Lamb, 1997). Boyd and Hogan administered acetaminophen via oral gavage to Wistar rats at doses of 500 mg/kg to 4000 mg/kg for 100 days. The publication notes that changes in testicular weight were not

noted for treatment durations of less than one month. In animals that survived the 100 day treatment, decreased testicular weights were noted even at the lowest dose tested (500 mg/kg corresponds to 3000 mg/m^2) which is only 1.2 times the maximum human dose of 4000 mg/day on a body surface area comparison. The decrease in weight of the testes was attributed to "almost complete atrophy of spermatogenic tissue." A NOAEL for testicular changes following longer than 1 month treatment was not obtained.

Studies conducted by Jacqueson and colleagues report that a 70-day treatment of male rats with 500 mg/kg dose of APAP resulted in a similar decrease in testicular weight, an increase in testicular cytotosol glutathione transferase activity and of lipid peroxides. The authors note that the treatment did not result in decreased testicular glutathione levels; therefore, the toxicity can not be readily attributed to a mechanism similar to APAP-induced hepatotoxicity (Jacqueson, et al., 1984).

Although changes in testicular weight following 30-day treatment with 500 mg/kg APAP to rats was not noted by Boyd and Hogan (1968) and Jacqueson et al. (1984), a literature search conducted by the review team notes that this dose has also been reported to result in impairment of libido, sexual vigor/performance, fertility index, implantation index and number of implantation sites in the rat (Ratnasooriya and Jayakody, 2000). The reference was not submitted by the Applicant but was able to be obtained and reviewed during the review cycle by the review team. The authors administered either 500 or 1000 mg/kg APAP to male rats via oral gavage for 30 consecutive days and then examined their sexual behavior and fertility via interactions with untreated females. The 500 mg/kg dose (3000 mg/m^2) reduced sexual behavior parameters, reduced vaginal sperm counts, impaired sperm motility and reduced fertility (pregnancy rate, implantation index and fertility index). This dose is 1.2 times the human maximum daily dose based on a body surface area comparison. Time course studies using 1000 mg/kg APAP demonstrated a reduction in ejaculated sperm number as measured by vaginal sperm counts following treatment for 17 days; whereas no effects were noted on day 3 or day 7. Based on these results, a NOEL levels of adverse effects on male sexual behavior and fertility was not established.

Yano et al. reported that a single dose of APAP (650 mg/kg = 3990 mg/m^2) administered orally to the male rat produced ultrastructural changes in the testes when measured 5, 10 and 15 days following treatment (Yano and Dolder, 2002). These changes included deformed seminiferous tubules, dilated blood vessels, edema of interstitial tissue, advanced spermatids with unusual amounts of residual cytoplasm, and well developed endoplasmic reticulum and Golgi complexes. A NOEL was not reported for these effects, which were noted with a dose that is 1.6 times the maximum human daily dose on a body surface area basis.

The National Toxicology Program conducted a continuous breeding study in Swiss CD-1 mice which were given APAP at 0.0, 0.25, 0.5 and 1.0% in feed (National Toxicology Program, 1984; Reel, et al., 1992; Lamb, 1997). These doses resulted in exposures estimated from food consumption of 357, 715, and 1430 mg/kg/day (Reel, et al., 1992). Although designed as a continuous breeding study, this study reports that continuous exposure of mice to up to 1.0% APAP indirectly (in utero and lactational exposure) and directly from Day 28 (weaning) to Day 74 ± 10 had no significant effect on mating or fertility. Although there was

no significant difference in sperm motility or sperm density in the cauda epididymis between 0 and 1.0% APAP groups, there was a significant increase in the percentage of abnormal sperm from the cauda epididymis relative to controls (see table below reproduced from the publication). Of note, based on the Lamb et al. summary, only the high dose group and control group appear to have been evaluated for sperm parameters; therefore, a NOEL level for sperm effects can not be obtained via this study. The high dose tested, 1430 mg/kg (4290 mg/m^2) is 1.7 times the maximum daily dose of APAP (4000 mg/60 kg = 2467 mg/m^2) based on a body surface area comparison.

Cumulative exposure to APAP appeared to reduced fecundity of mating pairs, since 6 of 19 high-dose pairs failed to produce a fifth litter and of the 13 mating pairs that did produce a litter, there was a reduction in the number of live pups born (Reel, et al., 1992).

In the revised product labeling received from the Applicant on January 13, 2010, the Applicant elected to remove all references to testicular atrophy. There was no justification or rationale provided. They provided the following revised language and reference the data from the 1984 NTP study (National Toxicology Program, 1984), which was also summarized by Reel et al. (1992).

(b) (4)

Effects on Embryo-Fetal Development. No new nonclinical embryo-fetal developmental studies were submitted with this NDA. The Applicant is relying upon information in the published domain to inform the Pregnancy section of the product labeling (Lambert and Thorgeirsson, 1976; Lubawy and Garrett, 1977; Reel, et al., 1992; Laub, et al., 2000; Burdan, 2000; Neto, et al., 2004). As a single entity prescription drug label for acetaminophen has not

previously been approved by the Agency, it appears as though a Pregnancy Category for acetaminophen has never been officially designated. Given the long history of clinical use of oral APAP during pregnancy, the nonclinical review team recognizes that there may be adequate and well-controlled clinical studies with oral acetaminophen to justify a Pregnancy Category B for oral drug products. However, this must be confirmed via review of the large amount of published clinical literature. As the Applicant did not submit this information with the original NDA, this information was requested and as it constituted a major amendment to the application the submissions contributed to the need to extend the regulatory clock. The reader is referred to the Maternal Health Team Consult Response for evaluation of the existing human data and product labeling recommendations regarding the clinical effects of APAP on pregnancy.

The original NDA application proposed the following nonclinical information be included in the animal toxicology section of the label.

(b) (4)

In the revised product labeling received from the Applicant on January 13, 2010, the Applicant proposed the following language to support the proposed Pregnancy Category:

(b) (4)

They reference the publication by Lubawy and Garrett (1997) as the basis for the first paragraph of the above statement and the Reel et al. (1992) report as the basis for the second paragraph above.

The published literature submitted by the Applicant and/or identified by the review team is summarized below:

Lambert and Thorgeirsson report no teratogenic effect of acetaminophen in B6 and AK strains of mice treated from gestation day 6 through 13 with APAP does of 100 and 250 mg/kg via IP injection. Based on a body surface area comparison, the dose of 250 mg/kg in a mouse (750 mg/m^2) is only 0.3-times the maximum recommended human dose of 4000 mg/60 kg person

(2466.6 mg/m^2). However, as the parameters examined were not described in the publication, it is not possible to confirm the adequacy of the study (Lambert and Thorgeirsson, 1976).

Lubway and Garrett report that there were no adverse effects of 125 mg/kg or 250 mg/kg APAP when administered to pregnant rats from gestation day 8 through 19. However, this study does not appear to examine visceral or skeletal malformations and therefore can not be considered an adequate embryo-fetal development study (Lubawy and Garrett, 1977). Based on a body surface area comparison, the dose of 250 mg/kg in a rat (1500 mg/m^2) is only 0.6-times the maximum recommended human dose of 4000 mg/60 kg person (2466.6 mg/m^2).

As referenced above in the fertility discussion, Reel et al. report the results of NTP's continuous breeding study in mice; however, these studies do not appear to have been designed to specifically monitor for visceral or skeletal malformations (Reel, et al., 1992). The authors, however, report a decreased number of live pups in the fifth litter. Assessment of the F1 mice from the fourth and fifth litter indicated that pup weights at birth were not affected by APAP treatment; however, body weights of the F1 mice during the lactational and postweaning periods were depressed in a dose-related manner, as noted in the table below (reproduced from the Reel et al. publication).

Laub et al. administered APAP to female mice only during the first few days of gestation; therefore this study can not be deemed an embryo-fetal development study since dosing did not cover the period of organogenesis. This study did suggest that APAP doses of 800 or 1430 mg/kg did not affect the development of preimplantation embryos (Laub, et al., 2000). Based on a body surface area comparison, the dose of 1430 mg/kg in a mouse (4290 mg/m^2) is 1.7-times the maximum recommended human dose of 4000 mg/60 kg person (2466.6 mg/m^2).

Burdan treated pregnant Wistar rats via oral gavage with 3.5, 35, or 350 mg/kg APAP from gestation day 8 to 14 and examined macroscopically for external malformation and for skeletal malformations (Burdan, 2000). The author concludes that the APAP treatment did

not lead to statistically significant differences in bone anomalies; however, there were some dose-related increases in the incidence of reduced ossification that exceeded control levels. Historical control data was not discussed in this publication. The highest dose tested (350 mg/kg = 2100 mg/m^2) is only 0.85-times the maximum recommended human dose of 4000 mg/60 kg person (2466.6 mg/m^2) based on body surface area. Although visceral malformations were not evaluated in this study, the treatment duration was consistent with a standard embryo-fetal development study. The skeletal variations reported in this publication are reproduced in the table below (emphasis added by reviewer).

Although not reviewed by the Applicant, Burdan published a second article that contributes to the understanding of the potential embryo-fetal effects of APAP. In the subsequent study (Burdan, et al., 2001), Burdan reported decreased weight and length of gestation day 21 fetuses removed from the dams treated with 350 mg/kg APAP compared to those removed from the controls or the low dose, respectively (see table below, reproduced from the publication).

Burdan has also examined the potential external, visceral and skeletal effects of the combination of APAP and caffeine (doses of APAP are the same as in the two previous studies). The author reports no evidence of malformations in any group (Burdan, 2003). Collectively, the work of Burdan and colleagues suggests that treatment of the rat during organogenesis results in evidence of fetotoxicity (reduced fetal weight and length), statistically insignificant increases in altered bone morphology, but no evidence of external, visceral, or skeletal malformations. None of the studies that examined APAP alone demonstrated evidence of maternal toxicity; however, the top dose represents only 0.85-times the maximum recommended human daily dose on a body surface area basis.

Neto et al. treated pregnant rats via oral gavage with 0, 125, 500, or 1500 mg/kg APAP from gestation day 1 to term pregnancy and examined the effects on maternal and fetal liver and kidney via light and electron microscopy. As this study did not examine either visceral or skeletal tissues, it is not an adequate embryo-fetal development study. The two higher doses tested produced necrotic areas in both the liver and kidney in both maternal and fetal tissues (Neto, et al., 2004). Based on a body surface area comparison, the dose of 500 mg/kg in a rat (3000 mg/m^2) is only 1.2-times the maximum recommended human dose of 4000 mg/60 kg person (2466.6 mg/m^2). The authors report a NOEL for APAP-induced microscopic liver and kidney changes of 125 mg/kg. Based on a body surface area comparison, the dose of 125 mg/kg in a rat (750 mg/m^2) is only 0.3-times the maximum recommended human dose of 4000 mg/60 kg person (2466.6 mg/m^2). This reference appears to be the basis of the sponsor's original proposed labeling. However, the study supports the conclusion that in the rat model, APAP treatment will produce both maternal and fetal liver and kidney histopathology at a dose between 125 and 500 mg/kg (between 0.3 and 1.2-times the maximum recommended human dose based on body surface area). This does not represent a "high dose" and indicates that the rat model is unlikely to provide an adequate safety margin

via either oral or parenteral routes to justify a Pregnancy Category B based on nonclinical data. In the absence of adequate clinical data with an IV APAP formulation, these adverse findings would dictate a Pregnancy Category C.

Effects of Pre- and Postnatal Development. Pre- and postnatal developmental studies were not submitted with this NDA. The Applicant is relying upon information in the published domain to inform the product labeling, primarily the data reported by Reel et al.; however, this study did not include comparable endpoints as a dedicted pre- and postnatal development study. As noted above, the original NDA submission, the Applicant proposed the following information be included in section 13.2 of the label:

(b) (4)

The original submission did not clearly identify the source for this statement; however, the liver and kidney findings were reported by Neto et al. as described above (Neto, et al., 2004). In the revised product labeling received from the Applicant on January 13, 2010, the above statement was removed with no justification or rationale. However, as the effects reported in Neto et al. represent adverse effects on the fetus, they should be included in the product labeling and support a Pregnancy Category C, unless superseded by adequate human data.

Drug Substance Impurities

Acetaminophen (APAP) drug substance is known to contain two impurities that have structural alerts for mutagenicity, p-aminophenol (PAP; aka 4-aminophenol or 4-AP) and p-chloroacetanilide. These two impurities have been restricted in the drug substance as per the USP to NMT 0.005% and 0.001%, respectively, since ~1970. According to the USP APAP monograph contact (Dr. Clyde Anthony), the USP changed the drug substance specification to be consistent with the British Pharmacopeia (personal communication). To date, the review team has not been able to determine definitively what specifically was driving these extremely low USP specifications. The referenced DMF for the drug substance has been previously found adequate by the Agency for numerous acetaminophen drug products and therefore, although the specifications for these drug substances would exceed the proposed NMT (b) (4) mcg/day as recommended in the December 2008 Draft FDA Guidance for Industry titled "Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches," based on the long history of use, this will not be deemed a deficiency in the DMF for the drug substance. In the submission dated September 14, 2009 (SDN 16) the Applicant confirmed that the specification for p-chloroacetanilide is present to show compliance with the requirements in the USP monograph. However, based on the synthetic scheme, Mallinckrodt confirmed that this impurity can not be present in their drug substance. Of note, although p-chloroacetanilide levels do not increase in the drug product; PAP is also a drug product degradant and will be discussed below.

Drug Product Impurities

The Applicant originally proposed a specification of NMT (b) (4) for PAP in the drug product. This is based primarily on their stability data which indicates that levels of this drug product degradant reached up to (b) (4) in some batches over the submitted 36 months stability data. For a maximum daily dose of 4 grams of acetaminophen per day, a specification of NMT (b) (4) will result in a maximum exposure to 4 mg of PAP (4000 mcg/day). It is not possible to reduce this impurity to NMT (b) (4) mcg/day in the formulation due to the inherent instability of acetaminophen in liquid formulations. Therefore, the safety qualification for the proposed specification must be based on data. Typically, safety qualification requires a minimal genetic toxicology screen and a repeat dose toxicology study in a single species of a duration adequate to support the proposed indication (between 14 and 90 days duration, as per ICHQ3B(R2), unless otherwise justified. Since the Applicant did not request a pre-NDA meeting, this requirement for adequate safety qualification of this drug product degradant was communicated to the Applicant in the 74-day filing letter.

In their submission dated August 12, 2009 (received by FDA August 13, 2009), the Applicant provided a toxicological risk assessment for PAP. This risk assessment did not include new toxicology studies; rather, the assessment was based on a literature review that included evidence that PAP is a metabolite of APAP and summarized existing toxicology data for PAP from the published literature. Each will be discussed below.

NOTE: In their submission dated September 10, 2009 (SDN 15), the Applicant proposed to revise the drug product degradant specification for PAP to NMT (b) (4) (to be discussed below). In terms of general toxicity (non-genotoxic impurities) ICH Q3B(R2) the threshold for safety qualification for a drug with a maximum daily dose (b) (4) However, given the genotoxic potential of the impurity and the long history of very low drug substance specifications listed in the USP with unclear origin, standard qualification thresholds are not applicable. Adequate safety justification in terms of both genotoxic potential and general toxicity must be provided.

Is PAP a significant metabolite of APAP?

As per ICHQ3B(R2), "Degradation products that are also significant metabolites present in animal and/or human studies are generally considered qualified." The guidance document does not define the term "significant." According to the submission received August 13, 2009, the Applicant proposes that PAP is a recognized metabolite of APAP. The submission cites 4 different references to support their statement (Newton, et al., 1983; Elder, 1988; Scientific Committee on Consumer Products (SCCP), 2005; Hogestatt, et al., 2005). The data from these citations are summarized in the table below:

Reference	Information in reference	Adequacy to support claim
(Elder, 1988)	This article is from Clairol (hair dye manufacturer) and actually cites two other references to support the statement "Analgesics, such as acetaminophen (paracetamol, N-acetyl-p-aminophenol) and phenacetin (4-ethoxyphenyl-N-acetamide) are partially metabolized to PAP.[11-12]" Reference 11 is: (Gemborys and Mudge, 1981)	Reference alone does not support claim that PAP is a significant metabolite of APAP. Secondary reference by Gemborys was obtained by the Agency and summarized below. Secondary reference by Shibasaki

	Reference 12 is: (Shibasaki, et al., 1971)	was requested and submitted by the Applicant as part of a major amendment and is summarized below.
(Scientific Committee on Consumer Products (SCCP), 2005)	This is a review of the safety of PAP in hair dyes conducted by a European Commission. The report does not contain original data; however, it does refer to studies that were available to the Commission at that time.	The pivotal references cited by this review were requested and their submission, in part, contributed to the major amendment.
(Newton, et al., 1983)	This reference actually reports differential nephrotoxicity of PAP in Sprague-Dawley (SD) vs. Fischer rats. SD rats are relatively resistant to PAP-induced nephrotoxicity, whereas Fischer rats are more sensitive. The study reports that renal cortical necrosis was not observed after a single dose of 50 mg/kg (300 mg/m^2), but was observed at doses of 100, 200, and 400 mg/kg PAP (600, 1200, and 2400 mg/m^2).	This report does not support the conclusion that PAP is a significant metabolite of APAP. However, it does refer to a different article that was obtained by the review team and is reviewed below (Newton, et al., 1982).
(Hogestatt, et al., 2005)	The study presents data that suggests that APAP can be deacetylated to PAP, which can then be converted by the body to AM404 via the enzyme Fatty Acid Amide Hydrolase (FAAH).	This study suggests that PAP can be formed from APAP, and that the formation of PAP in the brain can contribute to the therapeutic effects of APAP. This finding has not been repeated by other laboratories; however, it contributes to the conclusion that PAP is a metabolite of APAP. The study does not provide adequate information to establish a safe level of PAP in this product.
Studies obtained and reviewed by the Agency		
(Gemborys and Mudge, 1981)	The study examined evidence for trace metabolites of APAP in the hamster, which has been reported to generate a higher fraction of reactive intermediates of APAP metabolism than the mouse or rat. APAP doses of 50, 200, 300, 425, and 600 mg/kg IP were examined, which resulted in PAP levels in the urine (free and conjugated) of 0.1, 0.7, 0.9, 1.2 and 2.9%, respectively.	Strengths of the study: The study contains data suggesting that PAP may be a minor metabolite of APAP in the hamster. Weaknesses of the study: We do not know how much PAP was present in the APAP material administered. We do not have hamster toxicology studies to know if this level of impurity has been qualified for safety in this species.
(Shibasaki, et al., 1971)	The publication describes results of ADME studies in the rabbit following intravenous administration of APAP. APAP was administered intravenously via the rabbit ear vein at a dose of 300 mg and levels of free APAP and conjugated APAP metabolites were evaluated in blood and urine. The results support the	The report supports the conclusion that APAP is largely metabolized to the glucuronide and sulfate conjugates. The paper does not contain data to support the Applicant's conclusion that APAP

	conclusion that APAP is largely excreted in a conjugated form.	can be metabolized to 4-AP and therefore is significant metabolite.
(Newton, et al., 1982)	The publication reports data that administration of APAP (0, 250, 500, 750, and 900 mg/kg, SC) to male F344 rats (the strain most sensitive to PAP induced nephrotoxicity) results in urinary levels of PAP. PAP excreted in the urine over 24 hours increased with dose up to the 750 mg/kg dose but then dropped off at 900 mg/kg. This drop off was attributed to reduced urinary output, likely related to nephrotoxicity. On a pure mg/kg basis, following a dose of 500 mg/kg APAP, approximately 2% of the administered dose was detected in the urine as PAP. Similar results were noted in the isolated perfused kidney model. Kidney function was assessed in studies with PAP administered to the F344 rat. PAP (50 mg/kg, SC) did not elevate blood urea nitrogen (BUN) levels when measured at 24 or 48 hours post injection. In contrast, the higher doses of PAP significantly elevated BUN and resulted in histopathological data consistent with nephrotoxicity. This article also references another citation (Carpenter and Mudge, 1981) that notes that in a mouse isolated kidney preparation, APAP can be deacetylated to form PAP. This citation also references two Smith and Griffiths papers that appear to also characterize the potential deacetylation of APAP via intestinal microflora (Smith and Griffiths, 1974; Smith and Griffiths, 1976).	Strengths of the study: The results of the study support the conclusion that PAP is a minor metabolite of APAP in the F344 rat in vivo and in an isolated perfused kidney. On a mg/kg basis, the study suggests that approximately 2% of SC administered APAP is excreted in the urine of F344 rats as PAP over a 24 hour period. Weaknesses of the study: We do not know how much PAP was present in the APAP material administered or in the solutions employed in the isolated perfused kidney study.
(Carpenter and Mudge, 1981)	This citation provides data to suggest that the mouse kidney is capable of deacetylating APAP to form the more nephrotoxic PAP molecule. This citation also provides data to suggest that PAP can be reacetylated to from APAP. However the citation also states that PAP in solution can oxidize to PBQI, which is equally cytotoxic as NAPQI, the reactive intermediate of APAP metabolism that is believed to be responsible for the hepatotoxicity of APAP, particularly with depleted glutathione.	
(Smith and Griffiths, 1974)	This study reports that rat intestinal microflora is able to deacetylate APAP to form PAP in vitro.	The study does not provide a quantitative evaluation of the levels formed in order to determine if PAP could be deemed a significant metabolite.
(Smith and Griffiths, 1976)	The study examined the metabolism of radiolabeled [acetyl-^{14}C]paracetamol in the rat and reported that 6% of the administered radiolabel could be detected as $^{14}CO_2$, providing indirect evidence of	The study provides qualitative support for the conclusion that PAP is likely a metabolite of APAP. It suggests indirectly that up to 6% of

	deacetylation.	APAP is deacetylated to form PAP.

Collectively, although it is not definitively clear why the British Pharmacopeia restricted the levels of PAP in APAP drug substance to NMT 0.005%, this limited review of the historical literature on this subject suggests that the action may have been largely driven by the concern for nephrotoxic effects of PAP (Green, et al., 1969). This concern was probably enhanced by the earlier reports of nephrotoxicity associated with phenacetin metabolite p-phenitidine, particularly since phenacetin is also metabolized to acetaminophen. The studies noted in the table above suggest that APAP can be deacetylated to form PAP in the body or in vitro. Although the levels of PAP in the solutions administered were never reported, and may have been formed in the solution via degradation; the studies are consistent with the conclusion that PAP is a minor metabolite of APAP.

There is no definition of what a significant metabolite is, as described in ICHQ3B(R2). The Agency has proposed that a major metabolite is one that can be found at 10% or greater in the circulation. There is no evidence that PAP is a major metabolite in man or animals. It is not clear from the literature references reviewed to date that PAP would be considered a significant metabolite; therefore, the conclusion that it is a minor metabolite does not, in and of itself, provide adequate safety qualification for the proposed levels.

As part of the major amendment, the Applicant submitted a clinical pharmacology study that was designed to measure plasma levels of 4-AP in human subjects who took oral APAP in the form of Extra Strength Tylenol. This study was reviewed in detail by Dr. Ping Ji (Clinical Pharmacology Reviewer). Dr. Ji concludes that although very low levels of 4-AP were measured following administration of 4 grams of APAP per day, the levels could only account for 0.05% of the administered APAP. Therefore, the data provided by the Applicant indicates that based on the measurable levels of 4-AP obtain, 4-AP could be considered a very minor metabolite at best. Further Dr. Ji notes that the exposures to 4-AP via the existing approved oral tablet/capsule formulations are far below those that would occur via this IV drug product at the proposed specification.

Of note, the Applicant was asked to measure the levels of 4-AP in the Extra Strength Tylenol capsules they tested and reported that the levels were below the LOQ or MNT (b) (4) for 5 of 6 samples and NMT (b) (4) for one of the six samples. At the highest level detected, (b) (4) of 4 grams per day would result in a maximum total daily oral dose of 24 mcg/day. Therefore, the total daily exposure via the liquid formulation with a spec of NMT (b) (4) (b) (4) mcg/day) is ~133-times greater than one would get via the maximum dose of Extra Strength Tylenol.

Did the Sponsor provide adequate toxicology data to qualify the proposed level of PAP?

Adequate qualification of an impurity typically entails a minimal genetic toxicology screen (in vitro gene mutation assay and an in vitro assay for chromosomal damage) as well as a repeat-dose toxicology study of adequate duration to support the proposed clinical indication. For the acute post-operative indication for this IV product, a study 28 days duration should be

provided. The applicant did not conduct a toxicology study for PAP. Further, although the applicant did conduct 2 and 4 week toxicology studies with APAP, the levels of PAP in the product administered are not known. Therefore, it is not possible to conclude that the study provided adequate qualification of the impurity. Review of the protocol for the 2-week study employed in that study indicated that the APAP solutions were manufactured on March 27, 1998 (expiry on May 31, 1999). The experimental phase of the main study was initiated on September 14, 1998; therefore, the materials were likely in solution for approximately 6-7 months. The certificate of analysis indicates that the specification for PAP should be NMT 0.5 mg/100 mL. The results of the analysis report an absence of PAP; therefore it is not possible to conclude that the animal study submitted provided adequate coverage for the impurity.

In their submission dated 10-Sept-2009, the Sponsor revised their drug product specification for PAP to NMT (b) (4). For a maximum daily dose of 4 grams of APAP, this would result in an exposure of a maximum of 3.2 mg/day of PAP. According to the applicant, this specification is in line with the specification set for the marketed product in France (NMT (b) (4)). The specification of NMT (b) (4) is necessary as the HPLC method employed by Baxter, which reports the results, reports only two digits after the decimal place.

In lieu of conducting definitive studies, the Sponsor submitted a literature review of the general toxicology of PAP. This review was submitted 7/28/2009, before the last three months of the review cycle and therefore can not be deemed a major amendment. However, the submission largely relied upon summary articles rather than the original publications and therefore is deemed inadequate to serve as justification for the safety of the proposed specification. The original publications or study reports were requested and those that were able to be obtained by the Applicant were submitted for review.

Further, the studies summarized in the Applicant's submission employed the oral route of administration. These studies may not reflect the same C_{max} values as via the IV route of administration of the proposed product and therefore it is not clear if they provide an adequate assessment of the potential IV toxicity via this product. The secondary citations in the SCCP document that could be obtained were reviewed in order to determine the exposure margins based on body surface area; however, as the studies do not employ the IV route of administration and did not employ multiple doses of the product, a 28-day toxicology study with PAP would provide the most definitive information on the safety of the proposed degradant levels.

In order to definitively establish a NOAEL level for the impurity PAP in an APAP formulation, an intravenous general toxicology study of at least 28-days duration should be conducted. Given the comparable toxicity profile of PAP and APAP, a study of APAP with spiked levels of PAP must be conducted to define an adequate safety margin.

A NOAEL level for IV PAP can not be extrapolated from the submitted literature. However, the most definitive characterization of the general toxicity of oral PAP was recently reported (Harada, et al., 2008). These authors represent the Japanese National Institute of Health Sciences, and report that the NOAEL for general toxicity in a 28-day repeat-dose toxicology

study was 20 mg/kg/day (120 mg/m^2) which is 60-times the maximum human daily dose of PAP via 4 grams/day APAP if the specification was set to NMT (b) (4) of 4000 mg/day APAP = 3.2 mg/60 kg adult = 2 mg/m^2). The 28-day repeat dose toxicology study was completed to determine doses for reproductive and developmental toxicology study (discussed below) and was likely in accordance with OECD test guidelines and GLP compliance. In the reproductive toxicology study, a dose of 500 mg/kg resulted in mortality with evidence of tubular necrosis of the kidney. The reported 60x safety margin in the rat 28-day study would be adequate to support the safety of an oral formulation. Due to the difference in route of administration, in addition to the normal 10x uncertain factor for species extrapolation, even if an additional 3x uncertainty factor was added based on different route of administration, the data suggests an adequate safety margin for general toxicity.

Does the submitted clinical studies that employed this product provide adequate safety qualification for the proposed specification of PAP?

As a drug substance degradant that continues to form during stability, freshly prepared solutions contain far less PAP than those stored on stability. The sponsor did not provide data regarding the levels of PAP in the drug solutions tested in the clinical studies.

Due to the inherent reactivity of the PAP (irreversible binding to renal macromolecules) PAP has not been readily detected in the urine of humans and therefore is not discussed as a human metabolite. Unfortunately, this also precludes the ability to quantitatively determine the amount of PAP formed in the body.

Is PAP metabolically converted to APAP in the body? In the Applicant's submission dated August 12, 2009 (received by FDA August 13, 2009), the Applicant proposes that PAP can be metabolically converted to APAP in several species, including humans. The reference provided is the 2005 summary opinion from SCCP. The supporting citations were requested so that the conclusion could be independently reviewed. In a submission dated October 20, 2009 (received by FDA October 21, 2009), the Applicant provided additional references to support this statement. Key citations are reviewed below:

Reference	Information in reference	Adequacy to support claim
(Newton, et al., 1983)	Fischer and Sprague-Dawley rats were treated with PAP (200 and 400 mg/kg, SC) and urinary metabolites over 24 hours were collected. The results suggest that metabolites of PAP included APAP, APAP-conjugates, and PAP-conjugates. The urinary metabolites formed are reproduced in the table below, reproduced from the article.	Although not all of the administered dose of PAP was recovered, the results suggest that a significant portion of the PAP was converted to APAP.

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(Song and Chen, 2001)	Male mice were injected intraperitoneally with PAP (100-700 mg/kg) and plasma levels of APAP and its metabolites were measured by HPLC after PAP administration. Hepatocellular damage was assessed via serum GPT and SDH levels 12 hours later. The metabolic conversion of PAP to APAP occurred via N-acetylation. Cites Klos et al. 1992 paper that reports that about 70% of the PAP administered to the rat can be detected in bile as APAP and APAP metabolites (Klos, et al., 1992). Song and Chen investigated if similar conversion occurred in the mouse. Levels of APAP and conjugates following IP injection of PAP in the rat and mouse are presented in the figure below, reproduced from the report: COPYRIGHT MATERIAL **FIGURE 6.** Plasma concentrations of APAP, APAP-glucuronide, and APAP-sulfate after PAP administration (400 mg/kg, i.p.) in the mouse and rat. (A) APAP, (B) APAP-glucuronide, and (C) APAP-sulfate. Each point represents the mean ± SD (n = 3–4).	The mouse has been reported to be the most sensitive species for PAP-induced hepatotoxicity. The rat has been reported to be resistant to PAP-induced hepatotoxicity. Although data were not provided that allow an estimate of the amount of PAP converted to APAP in the mouse, the report does provide reasonable evidence that a percentage of PAP is metabolically converted to APAP in both rats and mice.

Although these citations support the Sponsor's claim that PAP can be metabolically converted to APAP in the mouse and rat models, they do not provide data to specifically demonstrate the safety of the proposed specification of 4-AP.

Is PAP Genotoxic? The Applicant's submission dated August 12, 2009 (received by FDA August 13, 2009) cites that there are numerous articles describing the potential genotoxicity of PAP. Collectively, these citations suggest that the PAP tests negative as a mutagen; however, it tests positive as a clastogen both in vitro and in vivo. These studies were recently reviewed in the context of the genotoxicity of aniline and its metabolites (Bomhard and Herbold, 2005). The summary tables below were reproduced from that reference (for full references cited in the tables, the reader is referred to the Bomhard manuscript):

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The in vivo clastogenic effects are presumed to have a threshold for toxicity. The references that administered the PAP via IP injection from the above table were requested in order to attempt to define a NOEL for clastogenicity via a parenteral route of administration.

Wild et al. reported that intraperitoneal doses of p-aminophenol increased the incidence of micronucleated polychromatic erythrocytes at all doses tested (Wild, et al., 1980). A NOEL for clatogenicity was not obtained via this study. The results are summarized in the table below:

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Sicardi et al. (1987) administered 112 or 225 mg/kg p-aminophenol to Swiss mice via intraperitoneal injection and report an increase in the incidence of micronucleated polychromatic erythrocytes at both doses compared to controls animals (Sicardi, et al., 1987). A NOEL for clastogenicity was not obtained. The results are summarized in the table below:

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Cliet et al. administered 53, 107, and 214 mg/kg 4-AP to CD-1 mice via an intraperitoneal injection and evaluated the number of micronucleated hepatocytes per 1000 cells 24 hours later (Cliet, et al., 1989). The results are summarized in the table below:

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The data above suggest a NOEL for clastogenicity via intraperitoneal injection of 53 mg/kg in the mouse. Based on body surface area comparisons, the mouse dose of 53 mg/kg/day (=159 mg/m^2) would represent 79.5-times the maximum human daily dose of PAP via 4 grams/day APAP if the specification was set to NMT (b) (4) of 4000 mg/day APAP = 3.2 mg/60 kg adult = 2 mg/m^2).

Review of the Sicardi reference from 1991, indicates that a dose of 5 mg/kg, IP did not result in an increase in micronucleated polychromatic erythrocyte frequency in bone marrow cells; however, doses of 50, 100 and 200 mg/kg were positive for micronuclei formation suggestion (Sicardi, et al., 1991). The NOEL level for clastogenicity could be established at 5 mg/kg. The results of the study are reproduced in the table below.

Compound	Number of mice	Dose (mg/kg)	Micronucleated erythrocytes/1000 cells	P
Vehicle	10	0	2.44 ± 0.26	
4-AP	10	5	2.33 ± 0.38	n.s.
	10	50	6.40 ± 0.36	*
	10	100	21.40 ± 1.08	*
	10	200	7.70 ± 0.54	*
n.s. = not significant, * = p< 0.05				

Based on body surface area comparisons, the mouse dose of 5 mg/kg/day (=15 mg/m^2) would represent 7.5-times the maximum human daily dose of PAP via 4 grams/day APAP if the

specification was set to NMT [redacted] (b) (4) of 4000 mg/day APAP = 3.2 mg/60 kg adult = 2 mg/m^2). Interestingly, the Sicardi study from 1991 contradicts the NOEL reported by Cliet et al. (1989).

The above published data suggest that although parenteral 4-AP is clastogenic, a threshold for clastogenicity exists. Based on the most conservative NOEL for clastogenicity, there appears to be a safety margin of approximately 7.5-times the maximum human daily dose. A definitive determination of the safety margin for the IV formulation would require a GLP in vivo clastogenicity study conducted via the IV route of administration, as the NOEL may differ via this route pending target tissue examined or an adequate carcinogenicity study given the positive findings to date.

Is PAP Carcinogenic? The Applicant's submission dated August 12, 2009 (received by FDA August 13, 2009) referenced an oral carcinogenicity study of PAP conducted in the Sprague-Dawley rat model (Bomhard and Herbold, 2005). The applicant states that the study was conducted according to OECD guideline and was GLP complaint; however, the reference cited by the applicant is actually a review article of the genotoxicity of aniline and its metabolites and not an actual carcinogenicity study of PAP. Review of the SCCP position paper on PAP suggest that an oral carcinogenicity study was conducted by the Centre Internationale de Toxicologie – Evreux, France (Scientific Committee on Consumer Products (SCCP), 2005). The actual study report was not originally submitted for independent review in the original NDA. However, the SCCP report indicates that the study was GLP compliant and conducted according to OECD guideline 451. Sprague-Dawley rats were administered 0, 2, 5, 12 and 30 mg/kg/day PAP via oral gavage for 101 weeks. The SCCP position paper noted "a marginal increase in the number of malignant lymphoma in males given 30 mg/kg/d (3 cases of heterog. mal. lymphoma compared to 1 in the control group and 1 in the low dose group)." The SCCP concluded that "the test substance showed neither a carcinogenic potential nor an effect on the incidence of spontaneous occurring tumours at any dose level."

Upon request by the Agency, the Sponsor was able to obtain the carcinogenicity study report. According to the report, statistical analysis employed a trend test according to the method of Peto. Doses administered were 0, 2, 5, 12 and 30 mg/kg with the high dose tested representing the predicted MTD based on a 13 week range-finding toxicology study. The incidences of malignant lymphoma findings are reproduced in the table below.

	Males					Females				
	0	**2**	**5**	**12**	**30**	**0**	**2**	**5**	**12**	**30**
Lymphoblastic Malignant Lymphoma	0/50	0/30	1/32	2/38	0/50					
Lymphocytic Malignant Lymphoma	0/50	0/30	1/32	1/38	0/50	1/50	0/37	0/35	1/34	1/50
Heterogeneous Malignant Lymphoma	1/50	1/30	0/32	0/38	**3/50**	1/50	1/37	0/35	0/34	0/50

Based on the summary by SCCP, assuming the worst case scenario that the marginal increase in lymphomas was real, the NOEL for heterogeneous malignant lymphomas could be estimated to be the 12 mg/kg/day dose. No toxicokinetic data were reported to determine exposures; however, based on body surface area comparisons, the rat dose of 12 mg/kg/day (= 72 mg/m^2) would represent 36-times the maximum human daily dose of PAP via 4 grams/day APAP if the specification was set to NMT [redacted] (b) (4) of 4000 mg/day APAP = 3.2 mg/60 kg adult = 2 mg/m^2).

The results of the carcinogenicity study support the conclusion that the positive genotoxic effects reported in the literature with PAP do not translate into carcinogenicity in the rat at exposures that would result from use of this product.

The No Adverse Effect Level for general toxicity in this carcinogenicity study was 5 mg/kg (30 mg/m^2) for males and 12 mg/kg (72 mg/m^2) for females. These doses represent 15- and 36-times the maximum human daily dose of PAP via 4 grams/day APAP if the specification was set to NMT [redacted] (b) (4) of 4000 mg/day APAP = 3.2 mg/60 kg adult = 2 mg/m^2).

Is PAP Teratogenic? The Applicant's submission dated August 12, 2009 (received by FDA August 13, 2009) provided the following references regarding the embryo-fetal developmental effects of PAP (Elder, 1988; Burnett, et al., 1989; Scientific Committee on Consumer Products (SCCP), 2005).

As noted previously, the Elder and SCCP review papers reference original studies that were obtained/requested for review in order to independently evaluate the potential teratogenicity of PAP and are summarized below.

Rutkowski and Ferm report that intranperitoneal and intravenous administration of ≥100 mg/kg PAP to pregnant Syrian golden hamsters resulted in evidence of teratogenicity without compromised maternal health (Rutkowski and Ferm, 1982). In contrast, these authors report that oral administration of up to 200 mg/kg PAP did not result in evidence of teratogenicity (see table below reproduced from the Rutkowski and Ferm publication).

TABLE 1

EFFECT OF DOSE AND ROUTE OF ADMINISTRATION OF THE AMINOPHENOLS IN THE LKV STRAIN OF THE SYRIAN GOLDEN HAMSTER

The teratogenic effects reported following IP or IV PAP administration are summarized in the table below (reproduced from the publication).

TABLE 2

EFFECT OF THE AMINOPHENOLS IN THE LKV STRAIN OF THE SYRIAN GOLDEN HAMSTER

The lowest IV dose tested, 100 mg/kg (500 mg/m^2), is 250-times the maximum human daily dose of PAP via 4 grams/day APAP if the specification was set to NMT [redacted] (b) (4) of 4000 mg/day APAP = 3.2 mg/60 kg adult = 2 mg/m^2). However, as noted in the table above, a NOEL for IV administration was not obtained in this study so a safety margin can not be established for IV administration based on this study.

Burnett et al. administered 0.07, 0.2, or 0.7% PAP to male and female Sprague-Dawley rats via the diet as part of a 6-month toxicology study that included assessments of reproductive toxicology as well. For the teratology portion of the study, female animals were treated via the diet for 13 weeks prior to mating with an untreated male. Following confirmation of insemination, females were returned to the diet containing APAP until gestation day 20 (time of sacrifice for teratology assessment). Compared to control animals, maternal body weights were reduced compared to control in both the mid dose and high dose groups at the time of mating. During the gestational period, maternal weight gains were statistically significantly reduced in the high dose group compared to controls between gestation days 0 and 20, indicative of maternal toxicity. Examination of the fetuses from these animals demonstrated a dose-related increase in post-implantation losses. There was a significant increase in the rudimentary ribs and in the mid dose and high dose groups and in unossified sternebrae in the high dose group (Burnett, et al., 1989). Based on the information in the publication, the low dose of 0.07% would be the NOAEL for teratogenicity. The dose of PAP consumed by the low dose group in this study was not reported, however, there was no clear evidence of decreased food consumption and the high dose group was estimated by the authors to have consumed approximately 520 mg/kg body weight per day. Therefore, the low dose could be estimated at 52 mg/kg (312 mg/m^2) which would be approximately 156-times the maximum human daily dose of PAP via 4 grams/day APAP if the specification was set to NMT [redacted] (b) (4) of 4000 mg/day APAP = 3.2 mg/60 kg adult = 2 mg/m^2).

Due to the lack of GLP studies on the reproductive and developmental effects of PAP, the Japanese National Institute of Health Sciences completed a study reported to be in accordance with OECD test guidelines and GLP compliance. The results were recently reported in the published literature. Harada et al. (2008) administered 4-aminophenol (0, 20, 100, or 500 mg/kg/day) via oral gavage to male and female rats (Harada, et al., 2008). Males were treated for a total of 49 days, beginning 14 days before mating. Females were dosed for a total of 40-60 days, starting 14 days before mating, throughout gestation to Day 3 of lactation. Standard endpoints, with the exception of skeletal malformation examinations, were evaluated. Adverse effects on developmental endpoints were noted at the high dose, including terminated estrus cycles, prolonged gestation periods, decreased delivery index, lower pup weights, increased stillborns and decreased viability of pups. The authors report a NOAEL for reproductive and developmental toxicity as 100 mg/kg/day (600 mg/m^2). Although the route of administration in this study was oral rather than intravenous, the oral NOAEL of 100 mg/kg/day is 300-fold higher than the maximum human daily dose of PAP via 4 grams/day APAP if the specification was set to NMT [redacted] (b) (4) of 4000 mg/day APAP = 3.2 mg/60 kg adult = 2 mg/m^2).

To date, the reviewed material suggests that although PAP is teratogenic following high oral doses, two studies demonstrated a NOAEL for teratogenicity and suggest an adequate safety margin for the oral route of administration. The results in the hamster model comparing different routes of administration indicate that the teratogenic potential of PAP following IP or SC dosing is greater that the teratogenic potential following oral administration. Similar results would be expected via the IV route of administration. A NOAEL for teratogenicity of PAP via the IV route of administration is not known and a safety margin can not be calculated. Therefore, it is not possible to definitively conclude that the maximum exposure to PAP via this product at the proposed specification will not result in teratogenicity. To definitively determine that IV PAP is not teratogenic at these levels, an embryo-fetal development study employing the IV route of administration should be completed. However, given the estimated oral safety margins for teratogenicity of 156x and 300x, even if an additional 3x uncertainty factor is applied to the standard 10x safety margin due to the different route of administration, there appears to be little concern regarding the teratogenic potential of the PAP impurity at the specified level. However, the difference in teratogenic sensitivity for the different routes of administration for PAP raises further concern that the existing human oral pregnancy data may not be directly extrapolatable to the safety of the IV drug product. As adequate IV data are not available in either animal models or human experience for the IV APAP formulation, a Pregnancy Category C should be assigned to this product.

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Ref Type: Online Source

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List of Nonclinical Submissions to NDA:

SDN in DARRTs	Submission Date & FDA Received Date	eCTD Sequence Number (Life Cycle Sequence in Global Submit Review)	Content
1	5/13/2009 & 5/13/2009	0000	• Original NDA
12	8/12/2009 & 8/13/2009	0010	• Toxicological risk assessment for 4-AP • Additional literature references • Revised draft labeling and identification of key references supporting labeling.
16	9/14/2009 & 9/15/2009	0015	• CMC info related to specification for p-chloroacetanilide in DS • Reduced specification for 4-AP from NMT (b) (4)
21	10/21/2009 & 10/21/2009	0020	• Annotated Key References for the toxicological risk assessment of 4-AP
25	11/4/2009 & 11/4/2009	0024	• Clinical PK study orally administered Extra Strength Tylenol Capsules
27	11/9/2009 & 11/10/2009	0028	• Additional literature references, including full NTP reproductive and toxicology study report from NTIS
30	12/4/2009 & 12/4/2009	0029	• Oral rat carcinogenicity study of 4-AP
34	12/14/2009 & 12/15/2009	0033	• Analysis of levels of 4-AP present in the orally administered Extra Strength Tylenol Capsules • Sponsor's justification for why the levels of 4-AP administered do not impact the clinical study results • Sponsor's justification for why levels of 4-AP noted support their conclusion that 4-AP is a significant metabolite.
35	1/13/2010 & 1/14/2010	0034	• Revised annotated labeling

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22450	ORIG-1	CADENCE PHARMACEUTICALS INC	Ofirmev (acetaminophen for injection)

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/s/

RICHARD D MELLON
02/10/2010

Pharmacology Toxicology Review Addendum
Executive Summary

I. Recommendations

A. Recommendation on approvability
From the nonclinical pharmacology perspective, NDA 22-450 is recommended for approval.

B. Recommendation for nonclinical studies
At this time, there are no nonclinical studies needed that will impact approvability.

C. Recommendation on labeling
The table below contains the draft labeling submitted by the sponsor, the proposed changes and the rationale for the proposed changes for the nonclinical toxicology section only. Please see the secondary review for final labeling recommendations.

(b) (4)

(b) (4)

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

From the previous pharmacology toxicology review of this NDA, acetaminophen is not a mutagen (as demonstrated by negative results in the bacterial Ames test) but is a clastogen (as demonstrated by positive results in the chromosomal aberration assay in cultured human peripheral blood lymphocytes).

From the previous pharmacology toxicology review of this NDA, the results of the NTP carcinogenicity studies showed that there was no evidence of carcinogenic activity of acetaminophen in male F344/N (Fischer) rats, there was some evidence of carcinogenic activity in female rats based on an increased incidence of mononuclear cell leukemia (although this finding is believed to be of limited human relevance), and there was no evidence of carcinogenic activity in male and female B6C3F1 mice.

From the previous pharmacology toxicology review of this NDA, testicular atrophy and reduction in testis weight was noted in fertility studies of acetaminophen performed on rats at all doses tested. There were no fetal morphological abnormalities but dose-dependent microscopic lesions of the maternal liver and kidney in rats as well as retarded growth and abnormal sperm in F_1 mice and reduced birth weight of F_2 pups in embryo and teratogenicity studies done in rodents.

The Sponsor has submitted a continuous feed study (NTP-1984) of up to 1.0% APAP in CD-1 mice to demonstrate decreased fertility in the F_0 generation and decreased pup weights in the F_1 and F_2 generations. There was also an increased amount of abnormal sperm in the F_1 males. However, the fertility and reproductive study on APAP was conducted using orally administered APAP. Although the fertility and reproductive study on APAP did not examine the skeletal malformations, the pups were examined grossly for any malformations.

B. Pharmacologic activity

Acetaminophen is a non-opioid analgesic and non-salicylate antipyretic compound. A definitive mechanism of action for acetaminophen has not been elucidated. The use of IV acetaminophen in the proposed indication of the treatment of acute pain and fever in adult and pediatric patients is supported through the various mechanisms of action of acetaminophen, including a central site of action, prostaglandin inhibition, and cannabinergic and serotonergic effects. Namely, transformation of acetaminophen in the CNS to form AM404 can then stimulate vanilloid receptors (TRPV1) to increase the activity of endogenous cannabinoids and to exert anti-nociceptive and hypothermic effects.

C. Nonclinical safety issues relevant to clinical use

The Sponsor submitted a study reproductive and developmental toxicology conducted by the National Toxicology Program (NTP-1984). The study was a continuous feed study of up to 1.0% APAP in CD-1 mice to demonstrate decreased fertility in the F_0 generation and decreased pup weights in the F_1 and F_2 generations. There was also an increased amount of abnormal sperm in the F_1 males. Moreover, exposure to APAP leads to testicular changes in various rat studies from the published scientific literature (Boyd-1968, Ratnasooriya-2000, and Yano and Dolder-2002).

There is a drug product degradant, 4-aminophenol (4-AP). In the proposed drug product specifications, 4-AP is NMT (b) (4). The Sponsor has submitted references and studies to elucidate the metabolism, reproductive toxicity, genetic toxicity, and carcinogenicity of 4-AP. 4-AP is not mutagenic but is clastogenic. From a clinical PK study of orally administered 1-4 g APAP, 4-AP is not a major metabolite. Up to 30 mg/kg/day 4-AP did not increase the incidence of neoplastic lesions in a 2-year rat carcinogenicity assay. The reproductive toxicology studies from the literature demonstrate that 4-AP is associated with dose-related increase in fetal malformations, a high incidence of fetal resorption, decrease in maternal body weight, decreased postpartum pup weight, tail abnormalities, and/or hind limb paralysis, as well as perinatal loss. These reproductive toxicology studies submitted by the Sponsor employed the oral route of administration.

FDA Center for Drug Evaluation and Research
Division of Anesthesia, Analgesia, and Rheumatology Products
10903 New Hampshire Avenue, Silver Spring, MD 20993

ADDENDUM
MEMO TO FILE

IND number:	22-450
Sponsor:	Cadence Pharmaceuticals, Inc. 12481 High Bluff Drive, Suite 200 San Diego, CA 92130
Submission Type:	Original NDA
Supporting Doc Number:	21, 25, 29, 30, 34, and 35
Submission Date/Receipt:	October 21, 2009 / October 21, 2009 (SDN 21) November 4, 2009 / November 4, 2009 (SDN 25) December 1, 2009 / December 2, 2009 (SDN 29) December 4, 2009 / December 4, 2009 (SDN 30) December 14, 2009 / December 15, 2009 (SDN 34) January 13, 2010 / January 14, 2010 (SDN 35)
Drug Substance:	Acetaminophen
Reviewer name:	Carlic K. Huynh, Ph.D.
Supervisor name:	R. Daniel Mellon, Ph.D.
Division name:	Division of Anesthesia, Analgesia, and Rheumatology Products
Review completion date:	January 20, 2010

Recommendation: From a nonclinical pharmacology/toxicology perspective, NDA 22-450 is recommended for approval.

Background/Prior Regulatory History:
On November 10, 2009, the Sponsor was granted a 3-month extension to the review cycle due to the submission of a major amendment. Additional supporting documents have been submitted by the Sponsor or have been obtained by the nonclinical pharmacology toxicology review team since the nonclinical review of this NDA was sent to the Sponsor on November 3, 2009 and are evaluated in this addendum.

Reproductive toxicology of APAP:
Regarding the reproductive toxicology of APAP, there is a final report of a reproduction and fertility study preformed on CD-1 mice when administered APAP in feed from the NTP (1984). NOTE: Acetaminophen (APAP) is abbreviated as ACET in this study.

CD-1, (ICR)BR outbred albino mice were fed a diet consisting of 0.25, 0.5, and 1.0% APAP in the continuous breeding phase of the study. It is noted that 0.25, 0.5, and 1.0% APAP correspond to 2.5, 5.0, and 10.0 mg/g APAP, respectively. It is noted that Reel-1992 had summarized this NTP study and determined the mice ingested 357, 715, and 1430 mg/kg APAP, respectively. The continuous breeding phase consists of a 7-day premating exposure, a 98-day cohabitation period, and a 21-day segregation period. The continuous breeding phase lasts for a total of 18 weeks.

Continuous exposure of CD-1 mice to 0.25, 0.5, and 1.0% APAP had no effect on the proportion of pairs able to produce at least one litter.

Table 1 shows the reproductive performance of the fertile pairs during treatment with dietary APAP.

Table 1: Reproductive Performance of Fertile Pairs During Continuous Breeding

	Treatment Group (% ACET)			
Reproductive Parameter[a,b]	0.0	0.25	0.5	1.0
Litters Per Pair	4.83 ± 0.11 (40)	4.94 ± 0.13 (18)	4.84 ± 0.09 (19)	4.68 ± 0.11 (19)[c]
Live Pups per Litter				
Male	5.87 ± 0.22 (40)	6.29 ± 0.35 (18)	5.64 ± 0.42 (19)	5.20 ± 0.23 (19)[c]
Female	5.66 ± 0.20 (40)	5.71 ± 0.31 (18)	5.35 ± 0.33 (19)	5.32 ± 0.25 (19)[d]
Combined	11.53 ± 0.33 (40)	12.00 ± 0.57 (18)	10.99 ± 0.65 (19)	10.52 ± 0.38 (19)[d]
Proportion of Pups Born Alive	0.98 ± 0.01 (40)	0.99 ± 0.00 (18)	0.99 ± 0.00 (19)	0.98 ± 0.01 (19)
Sex of Pups Born Alive (Males/Total)	0.51 ± 0.01 (40)	0.53 ± 0.02 (18)	0.51 ± 0.02 (19)	0.50 ± 0.02 (19)
Live Pup Weight (g)				
Male	1.64 ± 0.01 (40)	1.66 ± 0.02 (18)	1.71 ± 0.04 (19)	1.62 ± 0.03 (19)[e]
Female	1.57 ± 0.01 (40)	1.60 ± 0.02 (18)	1.65 ± 0.03 (19)	1.57 ± 0.02 (19)
Combined	1.61 ± 0.01 (40)	1.63 ± 0.02 (18)	1.68 ± 0.04 (19)	1.60 ± 0.02 (19)
Adjusted Live Pup Weight (g)[f]				
Male	1.65 ± 0.02 (40)	1.68 ± 0.02 (18)	1.70 ± 0.02 (19)[g]	1.60 ± 0.02 (19)[d,h]
Female	1.58 ± 0.01 (40)	1.62 ± 0.02 (18)	1.64 ± 0.02 (19)[i]	1.55 ± 0.02 (19)[d,h]
Combined	1.61 ± 0.01 (40)	1.65 ± 0.02 (18)	1.67 ± 0.02 (19)[g]	1.58 ± 0.02 (19)[j]

[a]Mean ± S.E. for all litters delivered per fertile pair.
[b]Number of fertile pairs providing data indicated in parenthesis.
[c]Significantly different ($p<0.05$) from the 0.0 and 0.25% APAP groups.
[d]Significantly different ($p<0.05$) from the 0.25% APAP group.
[e]Significantly different ($p<0.05$) from the 0.5% APAP group.
[f]Mean adjusted for total number of live and dead pups per litter by analysis of covariance.
[g]Significantly different ($p<0.05$) from the 0.0% APAP group.
[h]Significantly different ($p<0.01$) from the 0.5% APAP group.
[i]Significantly different ($p<0.01$) from the 0.0% APAP group.
[j]Significantly different ($p<0.01$) from the 0.25 and 0.5% APAP groups.

Exposure to 1.0% APAP in the diet caused a significant reduction ($p<0.05$) in the number of litters per pair relative to the 0.0 and 0.25% APAP groups. Moreover, mice fed dietary levels of 1.0% APAP had a decreased number of live male pups per litter ($p<0.05$) as compared to mice receiving 0.0 or 0.25% APAP in their diet. Dietary APAP had no effect on the number of live female pups per litter. There was no effect of these dietary levels of APAP on the proportion of pups born alive, the sex of pups born alive, and the

absolute pup weight. There was no dose-dependent effect of these dietary levels of APAP on the adjusted live pup weight.

Since APAP had no effect on fertility and only relatively minor effects on reproductive performance in F_0 breeding pairs, the reproductive effects of APAP in the F_1 generation was evaluated. The litters from the continuous breeding phase (0, 0.25, 0.5, and 1.0% APAP dose groups) were weaned at 28 days of age and 9 or 10 litters were randomly selected for rearing. The litter groups were maintained at the same dietary levels as their parents (F_0 generation). At 74 ± 10 days of age, one to three males and females from each of these litters were randomly selected for breeding within each dose group and were cohabitated for 7 days. The pairs were then separated and the females were allowed to deliver their litters (F_2 generation). Exposure to dietary levels of APAP continued during cohabitation and pregnancy. At the conclusion of this phase of the study, gross necropsy and histopathology was performed.

The body weights of the F_1 mice at birth (day 0), at weaning (28 days of age), and at mating (74 ± 10 days of age) were taken and shown in Table 2.

Table 2: Body Weight of F_1 Generation at Birth (Day 0), Weaning (Day 28), and at the Outset of the Mating Trial (Day 74 ± 10): Effect of Continuous Exposure to APAP

	Body Weight (g), $\bar{X}$ ± S.E.							
	% ACET in Diet							
Age (Days)	0.0		0.25		0.5		1.0	
	♂	♀	♂	♀	♂	♀	♂	♀
Birth (Day 0)[a]	1.64 ± 0.03 (37)	1.59 ± 0.03 (36)	1.68 ± 0.04 (17)	1.61 ± 0.03 (17)	1.69 ± 0.04 (16)	1.63 ± 0.04 (17)	1.68 ± 0.07 (16)	1.61 ± 0.06 (17)
Weaning (Day 28)[b]	17.25 ± 0.49 (42)	15.46 ± 0.43 (34)	15.95 ± 0.54 (40)	13.88 ± 0.50[c] (32)	14.38 ± 0.56[d] (45)	13.62 ± 0.45[d] (33)	11.37 ± 0.61[d] (24)	11.08 ± 0.55[d] (33)
Mating Trial (Day 74 ± 10)[e]	35.39 ± 0.74 (19)	29.04 ± 0.70 (19)	33.38 ± 0.44[c] (20)	26.04 ± 0.56[d] (20)	32.49 ± 0.51[d] (20)	26.28 ± 0.54[d] (20)	28.92 ± 0.43[d] (20)	24.23 ± 0.34[d] (20)

[a]Mean male or female pup weight per litter; number of litters evaluated indicated in parenthesis below mean Bale and female pup weight per litter.
[b]Mean for individual pup weights; number of pups weighed indicated in parenthesis below the mean pup weight.
[c]Significantly different from the 0% APAP group ($p<0.05$).
[d]Significantly different from the 0% APAP group ($p<0.01$).
[e]Mean for individual mice; number of mice weighed indicated in parenthesis below the mean mouse weight.

Table 2 shows that there was a significant dose-dependent decrease in the body weights of F_1 pups at weaning and at mating ($p<0.05$ or $p<0.01$), indicating APAP toxicity during lactational and postweaning periods. In contrast, continuous exposure of F_1 mice to APAP in utero and from birth to 74 ± 10 days of age had no statistically significant effects on the proportion of detected matings, fertile pairs, pups born alive or sex of pups born alive (males/total) relative to the control F_1 mice.

Table 3 shows the reproductive performance of fertile pairs of F_1 mice after continuous exposure to APAP.

Table 3: Reproductive Performance of Fertile Pairs of F_1 Mice After a Mating Trial to Determine the Effect of Continuous Exposure to APAP

Reproductive Parameter[a,b]	Treatment Group (% ACET) 0.0	0.25	0.5	1.0
Live Pups per Litter				
Male	5.06 ± 0.42 (16)	5.53 ± 0.35 (19)	6.40 ± 0.41 (20)[c]	5.11 ± 0.50 (18)
Female	6.56 ± 0.63 (16)	4.74 ± 0.33 (19)[d]	5.55 ± 0.40 (20)	5.11 ± 0.47 (18)
Combined	11.63 ± 0.60 (16)	10.26 ± 0.34 (19)	11.95 ± 0.48 (20)[e]	10.22 ± 0.57 (18)
Proportion of Pups Born Alive	0.99 ± 0.01 (16)	0.97 ± 0.02 (19)	1.00 ± 0.00 (20)[f]	0.99 ± 0.01 (18)
Sex of Pups Born Alive (Males/Total)	0.44 ± 0.03 (16)	0.54 ± 0.03 (19)	0.53 ± 0.03 (20)	0.50 ± 0.04 (18)
Live Pup Weight (g)				
Male	1.57 ± 0.04 (16)	1.54 ± 0.03 (19)	1.58 ± 0.03 (20)	1.42 ± 0.03 (18)[g]
Female	1.50 ± 0.03 (16)	1.49 ± 0.03 (19)	1.50 ± 0.02 (20)	1.36 ± 0.02 (18)[g]
Combined	1.53 ± 0.03 (16)	1.52 ± 0.03 (19)	1.54 ± 0.02 (20)	1.39 ± 0.02 (18)[g]
Adjusted Live Pup Weight (g)[h]				
Male	1.58 ± 0.03 (16)	1.53 ± 0.03 (19)	1.59 ± 0.03 (20)	1.41 ± 0.03 (18)[g]
Female	1.51 ± 0.03 (16)	1.48 ± 0.02 (19)	1.52 ± 0.02 (20)	1.34 ± 0.03 (18)[g]
Combined	1.54 ± 0.03 (16)	1.50 ± 0.02 (19)	1.56 ± 0.02 (20)	1.37 ± 0.03 (18)[g]

[a]Mean ± S.E. for the one litter delivered per fertile pair.
[b]Number of fertile pairs providing data indicated in parenthesis.
[c]Significantly different ($p<0.05$) from the 0.0 and 1.0% APAP groups.
[d]Significantly different ($p<0.05$) from the 0.0% APAP group.
[e]Significantly different ($p<0.01$) from the 0.25 and 1.0% APAP groups.
[f]Significantly different ($p<0.05$) from the 0.25% APAP group.
[g]Significantly different ($p<0.01$) from the 0.0, 0.25 and 0.5% APAP groups.
[h]Mean adjusted for total number of live and dead pups per litter by analysis of covariance.

As shown in Table 3, there was a significant decrease ($p<0.01$) in the absolute and relative live pup weight (F_2 generation) in the 1.0% APAP treatment group versus that in the 0.0, 0.25, and 0.5% APAP treatment groups.

Mice from the 0.0 and 1.0% APAP-exposed F_1 mice were weighed and necropsied at the conclusion of this phase of the study.

Sperm assessment was performed in the high dose male mice and are shown in Table 4.

Table 4: Sperm Analysis for F_1 Mice: Effect of Continuous Exposure to APAP

Parameter[a,b]	Treatment Group (% ACET) 0.0	1.0
% Motile Sperm	51.42 ± 5.33 (19)	55.45 ± 2.90 (20)
Sperm Concentration (No. sperm x 10^3/mg caudal tissue)	924.82 ± 55.81 (19)	1037.65 ± 61.75 (20)
% Abnormal Sperm[c]	7.28 ± 0.84 (19)	16.38 ± 2.62 (20)[d]
% Tailless Sperm[e]	20.88 ± 1.37 (19)	24.65 ± 1.82 (20)

[a]Mean ± S.E.
[b]Number of observations indicated in parenthesis.
[c]Tailless sperm were not included in the determination of abnormal sperm.
[d]Significantly different ($p<0.01$) from the 0.0% APAP group.

[e]Tail-less sperm were found to be an artifact of the method used to prepare sperm suspensions. Maceration of the cauda epididymis appeared to cause the loss of tails from many of the spermatozoa.

As shown in Table 4, sperm assessment indicated no significant difference in the % motile sperm or sperm concentration in the cauda epididymis between male mice given 0.0 and 1.0% APAP in the diet. In contrast, the % abnormal sperm in the cauda was significantly elevated ($p<0.01$) in the males exposed to 1.0% APAP in the diet compared to the male control group.

Body and organ weights of the F_1 males following dietary treatment of 0.0 and 1.0% APAP are presented in Table 5.

Table 5: Male Body Weight and Organ Weights of F_1 Rats at Necropsy

	Treatment Group (% ACET)	
Variable[a,b]	0.0	1.0
Body Weight (g)	33.08 ± 0.58 (19)	29.61 ± 0.40 (19)[c,d]
Liver (g)	1.92 ± 0.05 (19)	1.73 ± 0.03 (20)[d]
Brain (g)	0.48 ± 0.01 (19)	0.44 ± 0.00 (20)[d]
Pituitary (mg)	2.2 ± 0.1 (19)	1.9 ± 0.1 (19)[e,f]
Left Testis/Epididymis (mg)	169 ± 4.4 (19)	157 ± 4.7 (20)
Right Testis (mg)	123 ± 3.4 (19)	114 ± 4.1 (20)
Right Epididymis (mg)	47 ± 1.1 (19)	42 ± 1.3 (20)[d]
Prostate (mg)	32 ± 3.2 (19)	25 ± 2.2 (20)
Seminal Vesicles (mg)	346 ± 18.8 (19)	318 ± 8.9 (20)

[a]Mean ± S.E.
[b]Number of observations indicated in parenthesis.
[c]One male body weight was inadvertently not taken in the 1.0% APAP group.
[d]Significantly different ($p<0.01$) from the 0.0% APAP group.
[e]Significantly different ($p<0.05$) from the 0.0% APAP group.
[f]One pituitary gland lost in the 1.0% APAP group due to improper dissection.

As shown in Table 5, body weight ($p<0.01$) and liver ($p<0.01$), brain ($p<0.01$), pituitary ($p<0.05$) and right epididymal ($p<0.01$) weights were significantly decreased in the F_1 male mice fed 1.0% APAP in the diet relative to F_1 male mice given the control diet.

Body and organ weights of the F_1 females following dietary treatment of 0.0 and 1.0% APAP are presented in Table 6.

Table 6: Female Body Weight and Organ Weights of F_1 Rats at Necropsy

Variable[a,b]	Treatment Group (% ACET) 0.0	1.0
Body Weight (g)	30.94 ± 0.74 (17)[c]	28.38 ± 0.47 (19)[d,e]
Liver (g)	1.86 ± 0.06 (19)	1.84 ± 0.05 (20)
Brain (g)	0.47 ± 0.01 (19)	0.45 ± 0.00 (20)[f]
Pituitary (mg)	2.9 ± 0.1 (19)	2.3 ± 0.1 (20)[e]
Ovary/Oviduct (mg)	38 ± 1.5 (19)	36 ± 1.3 (20)
Uterus (mg)	328 ± 23.9 (19)	334 ± 17.6 (20)

[a]Mean ± S.E.
[b]Number of observations indicated in parenthesis.
[c]Two female body weights inadvertently not taken.
[d]One female body weight inadvertently not taken.
[e]Significantly different ($p<0.01$) from the 0.0% APAP group.
[f]Significantly different ($p<0.05$) from the 0.0% APAP group.

As shown in Table 6, body weight ($p<0.01$) as well as brain ($p<0.05$) and pituitary ($p<0.01$) weight also were significantly decreased in F_1 female mice given 1.0% APAP in their diet versus F_1 female mice that received the control diet.

The reproductive organs of both F_1 male and female rats underwent gross morphologic and histopathologic examination. There were no relevant treatment-related gross morphology or histopathologic findings in the testis, epididymis, prostate and seminal vesicles in the F_1 male mice, as well as in the ovary, oviduct, uterus or vagina in the F_1 female mice. Moreover, histological evaluation of the cell types in the vaginal mucosa revealed that there were no relevant treatment-related effects on the estrous cycle.

Several conclusions can be made from the results of this reproductive toxicity study. Exposure of the F_0 generation of mice to 1.0% APAP in the diet over an 18-week period resulted in a significant reduction ($p<0.05$) in the number of litters per pair and the number of live male pups per litter versus the control group. In addition, body weights of the F_1 mice at 28 days of age and at 74 ± 10 days of age were significantly depressed ($p<0.05$ or $p<0.01$) in a dose-related manner, indicating APAP toxicity during the lactational and postweaning periods. Live pup weights were significantly decreased ($p<0.01$) for offspring (F_2 generation) produced by F_1 breeding pairs exposed continuously to 1.0% APAP in the diet. The % abnormal sperm also were significantly elevated ($p<0.01$) in the F_1 males. Body weights for the F_1 parents were significantly depressed ($p<0.01$) at necropsy, which is indicative of the toxicity of dietary 1.0% APAP in the diet. These conclusions are in concurrence of the study authors.

A clear NOAEL cannot be determined from this study. Although the amount of APAP was identified in the feed for each treatment group, it is not clear how much APAP was ingested by the mice in each of the treatment groups. Moreover, the fertility and reproductive studies on the F_1 generation only employed the control and 1.0% APAP dose. Therefore, a clear NOAEL cannot be established from these fertility and reproductive toxicity studies.

Testicular Findings with APAP:
The reproduction and fertility study from the National Toxicology Program (NTP)-1984 reported a significant elevation in % abnormal sperm in the F_1 males. Additionally, several papers in the published literature that were either submitted by the Sponsor or obtained by the Division highlight testicular changes in animals treated with APAP.

The study by Boyd-1968 examined the effect of APAP when administered to Wistar albino rats for 100 days. The end point measurement was testicular weight. Twenty treated rats for each dose of APAP (0.5, 0.7, 1.1, 1.4, 2.5, 3.0, 3.5 and 4.0 g/kg/day) and 8 water-fed control rats were used. Testicular atrophy occurred at all doses even at the lowest dose of 0.5 g/kg/day APAP tested (the testicular weight decreased by around 40%). The doses used in Boyd (1968) represent an exposure margin of 1.2, 1.7, 2.7, 3.4, 6.1, 7.3, 8.5, and 9.7, respectively based on body surface area comparison, for the average human weighing 60 kg given the maximum daily dose of 4000 mg APAP. Because the testicular atrophy finding was present in all doses of APAP tested, a NOAEL for testicular atrophy cannot be obtained.

Ratnasooriya-2000 investigated reproductive competence of male albino rats given large doses of acetaminophen. For the fertility study, male albino rats (12 rats/group) were given either 1000 mg/kg acetaminophen or vehicle at 12 hours daily for 30 consecutive days. Other studies used 500 and 1000 mg/kg acetaminophen (6 rats/group). In the mating studies, pre-coital sexual behavior of rats treated with the higher dose of acetaminophen was essentially similar to those of control. The vaginal sperm count of rats mated with acetaminophen treated rats was significantly reduced (by 60%). Moreover, the motility of the ejaculated spermatozoa of the treated rats was impaired but was neither agglutinated nor decapitated nor grossly abnormal. The fertility of the acetaminophen treated rats was also significantly reduced, measured in terms of quantal pregnancy (by 50%), number of uterine implants (by 62%), implantation index (by 62%) or fertility index (by 50%). Moreover, 5 out of 12 (42%) rats were totally infertile. The acetaminophen treatment also caused a significant increased (by 173%) in the pre-implantation losses. However, post-implantation loss was unchanged. Following cessation of treatment, the affected fertility parameters were completely or partially reversed. Chronic treatment of 1000 mg/kg of acetaminophen induced a significant increase (by 60%) in SGOT activity but had no significant effect on SGPT activity, suggesting a damaging effect on the liver. The high dose of acetaminophen (1000 mg/kg) caused significant reduction in the relative weights of both testes (by 33%) and corpus epididymides (by 25%). Furthermore, the lengths of the testes of treated rats were unaltered, but their interstitial fluid volume was significantly and moderately reduced (by 32%). In the seminiferous tubules of acetaminophen treated rats, there was marked apoptosis of both spermatocytes and early spermatids (71.23 ± 3.9%). This reviewer concurs with the findings and conclusions of the researchers.

The study by Yano and Dolder 2002 investigated the effects of acetaminophen on rat testicles. Male Wistar rats (6 rats/group) were treated with a single dose of 4.4 mmol/kg acetaminophen or control. After 5, 10, and 50 days, the rat testicles were fixed and

examined under microscopy. The results obtained after acetaminophen administration revealed various changes in the seminiferous tissue. Light microscope analysis showed that the animals treated with acetaminophen had some deformed seminiferous tubules, with a separation of the basal cells. Blood vessels were dilated or even ruptured and edema of the interstitial tissue was observed. Many typical tubules in the various stages of sperm development were found but 10 days after treatment, advanced spermatids had unusual amounts of residual cytoplasm remaining in these cells. Under electron microscopy, the loss of contact between spermatic cells was observed near areas of fragmentation of the Sertoli cells. Nearer to the lumen, the spermatic cells had exceptionally well-developed endoplasmic reticulum and Golgi complexes. The nuclei (containing the chromatin) of the spermatids was poorly condensed. The loss of contact between basal cells is apparently due to Sertoli cell fragmentation, seen after 5 to 10 days. Defects of these cells that provide support and nutrition for the spermatic cells result in loss of spermatic cells and may lead to the destruction of this tissue and infertility. The frequent observation of poorly condensed chromatin in late spermatids could be the result of the covalent binding of some acetaminophen metabolites to DNA, a finding that has contributed to the classification of this drug as genotoxic. Blood vessels are strongly affected by acetaminophen. In all of the testicles investigated, the blood vessels appeared dilated and even ruptured. Recuperation of the effected blood vessels was not observed in the 15-day period after drug administration. The inefficiency of dilated blood vessels led to edema and a degree of anoxia is to be expected when blood circulation is diminished. This probably results in oxidative defects in the spermatic epithelium contributing to the alterations of this tissue. This reviewer concurs with the findings and conclusions of the researchers. It is noted that the dose of 4.4 mmol/kg (665 mg/kg = 3990 mg/m^2) in rats is very close to the dose therapeutically used for man (maximum of 4 g APAP to 60 kg human = 2466.7 mg/m^2). The safety margin of the rat dose of 4.4 mmol/kg is 1.62 based on a body surface comparison in an average human weighing 60 kg.

Carcinogenicity of APAP:
The carcinogenic potential of APAP was evaluated by the National Toxicology Program (NTP-1993). In the first study, groups of 50 male and 50 female B6C3F1 mice, eight to nine weeks old, were given APAP at concentrations of 0, 600, 3,000, or 6,000 mg/kg (ppm) in food for up to 104 weeks. The results show that there was no evidence of carcinogenic activity in male and female mice. Second, groups of 50 male and 50 female Fischer 344/N rats, seven to eight weeks old, were given APAP in the diet at concentrations of 0, 600, 3,000, or 6,000 mg per kg (ppm) in food for up to 104 weeks. The results show that there was no treatment-related increase in tumor incidence was found in male rats and there was equivocal evidence of carcinogenic activity in female rats based on increased incidences of mononuclear cell leukemia.

The NTP-1993 carcinogenicity study appears to follow GLP guidelines. An adequate number of rats and mice have been used. In this study, groups of 50 rats and mice of each sex were administered 0, 600, 3000, or 6000 mg/kg (ppm) APAP through the feed for up to 104 weeks. At 15 months, 10 rats and mice per sex from each dose group were randomly selected by interim evaluation. The appropriate protocols were used and

included histological examinations of various tissues. Statistical analyses included pair comparisons and identification of dose-related trends. The results of the NTP carcinogenicity studies showed that there was no evidence of carcinogenic activity of APAP in male F344/N rats, there was equivocal evidence of carcinogenic activity in female rats based on increased incidences of mononuclear cell leukemia, and there was no evidence of carcinogenic activity in male and female B6C3F1 mice. Regarding the observation of mononuclear cell leukemia in the female F344/N rats, 9/50, 17/50, 15/50, and 24/50 rats were affected by this observation after treatment with 0, 600, 3000, and 6000 mg/kg APAP, respectively (see Table 7).

Table 7

Incidence of Mononuclear Cell Leukemia in Female Rats in the 2-Year Feed Study of Acetaminophen[a]

	0 ppm	600 ppm	3,000 ppm	6,000 ppm
Overall rates[b]	9/50 (18%)	17/50 (34%)	15/50 (30%)	24/50 (48%)
Adjusted rates[c]	26.4%	42.8%	35.1%	56.3%
Terminal rates[d]	6/30 (20%)	12/34 (35%)	8/34 (24%)	10/28 (36%)
First incidence (days)	575	581	485	554
Life table tests[e]	P=0.003	P=0.116	P=0.188	P=0.003
Logistic regression test[e]	P=0.003	P=0.070	P=0.120	P=0.001

[a] 2-year historical incidence for untreated controls at study laboratory (mean): 66/399 (16.5%); 2-year historical incidence for untreated control groups in NTP studies (mean ± SD): 425/2,043 (20.8% ± 8.1%)
[b] Number of neoplasm-bearing animals/number of animals examined at site
[c] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal.

As shown in Table 7 above, only the high dose group was statistically significant compared to control ($P<0.05$). Although each rat and mouse was given 0, 600, 3000, or 6000 mg/kg APAP through the feed, the actual dose of APAP each animal received was different. For example, the male rats given 600, 3000, and 6000 mg/kg APAP actually received 22, 109, and 222 mg/kg APAP, respectively. The female rats given 600, 3000, and 6000 mg/kg APAP actually received 24, 118, and 240 mg/kg APAP, respectively. The male mice given 600, 3000, and 6000 mg/kg APAP actually received 79, 411, and 880 mg/kg APAP, respectively. The female mice given 600, 3000, and 6000 mg/kg APAP actually received 98, 534, and 987 mg/kg APAP, respectively. The NOAEL for the male rats is 268 mg/kg which corresponds to a safety margin of 0.54 based on a body surface area comparison, for the average human weighing 60 kg given the maximum daily dose of 4000 mg APAP. The NOAEL for the female rats is 118 mg/kg based on the incidence of mononuclear cell leukemia which corresponds to a safety margin of 0.29 based on a body surface area comparison, for the average human weighing 60 kg given the maximum daily dose of 4000 mg APAP. The NOAEL for the male mice is 880 mg/kg which corresponds to a safety margin of 1.07 based on a body surface area comparison, for the average human weighing 60 kg given the maximum daily dose of 4000 mg APAP. The NOAEL for the female mice is 987 mg/kg which corresponds to a

safety margin of 1.20 based on a body surface area comparison, for the average human weighing 60 kg given the maximum daily dose of 4000 mg APAP.

Mononuclear cell leukemia occurred in the female Fischer rats in the NTP-1993 carcinogenicity study. According to Caldwell-1999 and Ishmael and Dugard-2006, mononuclear cell leukemia occurs at a high and variable background incidence in Fischer rats but it has a very low background rate in other rat strains such as Sprague-Dawley, Osborne-Mendel, and Wistar rats. In fact, mononuclear cell leukemia is the most common life-threatening neoplasm in the Fischer rat (Caldwell-1999) and its background rate has increased over time (Ishmael and Dugard-2006).

Ishmael and Dugard (2006) addressed whether mononuclear cell leukemia in the Fischer rats has any relevance in man. According to Ishmael and Dugard-2006, the neoplastic mononuclear cells in mononuclear cell leukemia in the Fischer rat are consider to be derived from large granular lymphocytes (LGLs) and that most, if not all, Fischer rat LGL leukemia tumor cells are considered to be of the true NK type. In general, LGL leukemia in man is uncommon and that the great majority of cases are of T cell CD3+ cell type. In fact, only a small portion of LGL leukemia in man (15%) is of the NK CD3- cell type. The disease in the Fischer rat appears to bear a greater similarity to the much rarer NK CD3- cell type in man. Moreover, the Fischer rat appears to be predisposed to NK-LGL leukemia whereas there is no such predisposition to NK-LGL in man.

As such, mononuclear cell leukemia is believed to be species specific to the Fischer rat and is of limited relevance to man. This assessment is made with eCAC concurrence.

Metabolism of APAP to 4-AP:
Regarding the metabolism of APAP to 4-AP, the clinical pharmacology review team has reviewed a clinical PK study (b) (4) submitted by the Sponsor. The clinical review team has reviewed the study and portions of the review are incorporated herein. (b) (4) In the study, six subjects received oral acetaminophen 1000 mg (2 x 500 mg caplets) under fasting condition. Twelve subjects assigned to the repeated dose group received three additional 1000 mg doses (2 x 500 mg caplets) of oral acetaminophen (APAP) at T4, T8, and T12 hours (for a total daily dose of 4000 mg). The drug taken was Tylenol® Extra Strength. Criteria for evaluation included measurement of plasma concentrations of APAP and 4-aminophenol (4-AP) at various time points. After single dose administration of oral acetaminophen 1000 mg, the mean Cmax and $AUC_{0\text{-}12\ hr}$ of 4-aminophenol was approximately 0.027% and 0.025% of that of acetaminophen, respectively. After multiple dose administration of oral acetaminophen 1000 mg, the mean Cmax and AUC of 4-aminophenol after the fourth dose was approximately 0.031% and 0.025% of that of acetaminophen, respectively. A mean AUC of APAP and 4-AP was determined after oral administration of 1 g of APAP every 4 hours. The ratio of $AUC_{0\text{-}4\ hr}$, $AUC_{0\text{-}12\ hr}$, and $AUC_{12\text{-}16\ hr}$ of 4-AP to APAP was 0.028, 0.025, and 0.025%, respectively. The clinical pharmacology review team has thus determined that 4-AP is not a major metabolite of APAP, when given as a 1 g APAP oral

dose. Additionally, the clinical pharmacology review team has noted that "[s]tability studies demonstrated that during storage, (b) (4) APAP occurs in solution to produce increasing amounts of the impurity, 4-AP, with time. The amount of 4-AP is (b) (4) at release and (b) (4) at room temperature. The limiting factor for shelf life is the allowed amount of 4-AP at expiry (b) (4) If assuming the systemic clearance of 4-aminophenol and acetaminophen is same, the AUC ratio is (b) (4) at this maximum specification level".

The mean amount of 4-AP in the Tylenol® Extra Strength Caplets used in this clinical study is below LOQ (0.197 µg/mL) which corresponds to (b) (4) relative to acetaminophen with one sample having an 4-AP content greater than LOQ (0.291 µg/mL) which corresponds (b) (4) According to the DP specifications for IV APAP, there are NMT (b) (4) 4-AP in the drug product formulation of this NDA. Moreover, in an analysis of the clinical batches of APAP IV formulation, there is (b) (4) 4-AP in the clinical batches. Thus, there is a several hundred fold more 4-AP in the IV APAP drug formulation of this NDA than in the Tylenol® Extra Strength Caplets.

It is noted that a study employing the IV route of administration of APAP has not been conducted. Moreover, a comparison of the exposure data between oral and IV APAP doses was not performed in this PK clinical study.

Reproductive Toxicity of 4-AP:

A. Embryo-fetal Development Studies:

Previously, the Syrian Golden Hamster study described in SCCP-2005 was cited. The data from the study can be found in the reference Rutowski-1982. Briefly, pregnant female Syrian Golden Hamsters were administered 4-AP on day 8 of gestation via three different routes (single IP doses of 100, 150, or 200 mg/kg, IV doses of 100, 150, 200, or 250 mg/kg, or oral gavage doses of 100 or 200 mg/kg). Control animals received saline. There was a "dose-related increase in a variety of malformations coupled with a high incidence of fetal resorption occurred over the tested dose range by both the IP and IV routes of administration with no evidence of maternal toxicity" (SCCP-2005). A NOAEL cannot be determined as malformations and fetal resorptions occurred at all doses tested. It is not clear what the number of animals used in each dose group were however, as discussed in Rutkowski-1982, the total number of fetuses from the 0, 100, 150, 200, and 250 mg/kg dose groups was 88, 145, 70, 28, and 3 fetuses, respectively. The study employed examination of external gross malformations. In additional, three fetuses from every litter demonstrating at least one malformation were examined by dissection for soft tissue anomalies of the abdomen. It is clear that the skeletal malformations were not examined in the study and that only gross external examination for malformations was performed. Although the ideal study would include an examination of skeletal malformations, Rutkowksi-1982 did evaluate external gross malformations and thus is considered relevant because the findings are significant.

B. Peri/Postnatal Study:

Previously, a peri/postnatal study was described in SCCP-2005. The peri/postnatal study was described in the reference by Kavlock-1990. Briefly, pregnant female Sprague-Dawley rats were administered 4 doses of 4-AP (100, 333, 667, and 1000 mg/kg 4-AP) by oral gavage on day 11 of gestation. Following dosing of the mothers, there were monitoring for any overt signs of toxicity (tremors, convulsions, vocalizations, labored breathing, salivation, lacrimation, etc). The mothers were allowed to deliver the offspring and were sacrificed at weening. The fetuses were examined for external malformations only. The results show "[m]aternal body weight was significantly reduced at doses of 667 and 1,000 mg/kg, associated with decreased postpartum pup weight, tail abnormalities and/or hind limb paralysis at 667 and 1,000 mg/kg and perinatal loss at 1,000 mg/kg" (SCCP-2005). The appropriate maternal observations were made however, the pups were only observed for external malformations. The NOAEL was 333 mg/kg 4-AP can be established based on maternal body weight and pup weight, tail abnormalities and/or hind limb paralysis, and perinatal loss. For an average human weighing 60 kg, the rat NOAEL represents a safety margin (b) (4) based on a body surface comparison[1]. Although the ideal study would include an examination of skeletal malformation, Kavlock-1990 did evaluate external gross malformations and thus is considered relevant because the findings are significant.

The reproductive and developmental toxicity of 4-AP (PAP) is also studied by Harada-2008. The group of authors in Harada-2008 is from the Division of Risk Assessment of the National Institute of Health Sciences in Tokyo, Japan, which is the regulatory authority in Japan. Sprague Dawley rats (12/sex/group) were given 4-AP via gavage at 0, 20, 100, or 500 mg/kg/day. Males were dosed for 49 days, beginning at Day 14 before mating and the females were dosed for 40-60 days, from Day 14 before mating to Day 3 of lactation (females were dosed throughout the mating and gestation periods). The doses for the reproductive and developmental toxicity study were determined previously by a 28-day toxicity study conducted by the Japan Existing Chemical Data Base (JECDB-1995).

In the Harada-2008 study, 4 males and 2 females died at 500 mg/kg/day, and the remaining surviving males and females showed brown urine at 100 mg/kg/day and above. Body-weight gain was lower in males and females at 500 mg/kg/day throughout the study but was significantly lower for days 1-15 in males and days 1-8 and days 0-7 of pregnancy in females. Food consumption was decreased significantly in males at 500 mg/kg/day (for days 1-11) and in females at 100 and 500 mg/kg/day (for days 1-8 and 1-11, respectively). There was a significant decrease in the absolute and relative weights of the testes (by -32.0 and -23.4%, respectively) and epididymides (by -27.6 and -17.4%, respectively) at 500 mg/kg/day. Histopathological examinations of the surviving males revealed decreased spermatocyte and spermatid levels in the testis, debris of germ cell in

[1] The maximum daily human exposure to the IV APAP product of this NDA is 4000 mg/kg. According to the DP specifications for IV APAP, 4-AP is present at NMT (b) (4) The NOAEL for a peri/postnatal study of 4-AP in rats is 333 mg/kg/day. For rats, 333 mg/kg/day x 6 = 1998 mg/m^2. For an average human weighing 60 kg, the safety margin is (b) (4) based on a body surface area comparison.

the epididymis lumen, basophilic tubules in the kidney, and deposits of hemosiderin in the red pulp and extramedullary hematopoiesis in the spleen in males at 500 mg/kg/day. The gestation period was significantly increased (by 4.95%), the delivery index was significantly decreased (by -22.6%), the viability index at day 4 of lactation was significantly decreased (by -75.1%), the body weight of pups on postnatal day (PND) 0 was significantly lowered (male pups by -29.0%, female pups by -30.8%) with a non-significant decrease on PND 4, and the number of stillborns was significantly increased (by 1,186%) in females at 500 mg/kg/day. Moreover, the percentage of females showing abnormal estrous cycles was significantly increased from 0% in the controls to 45.5% in the 500 mg/kg/day 4-AP dose group.

Several conclusions can be made from the Harada-2008 study. There were no adverse effects on reproduction or development that were detected at 20 and 100 mg/kg/day and as such, the NOEL for reproductive and developmental toxicity is 100 mg/kg/day 4-AP. The NOAEL for reproductive and developmental toxicity as well as for general toxicity is 500 mg/kg/day 4-AP. The NOEL of 100 mg/kg/day represents a safety margin of 304 for an average human weighing 60 kg. The NOAEL of 500 mg/kg/day represents a safety margin of 1523 for an average human weighing 60 kg. According to Harada-2008, these findings indicate that 4-AP is general and reproductive/developmental toxic, but is unlikely to be teratogenic, in rats. This reviewer concurs with this assessment.

Genetic toxicity of 4-AP:
DeFlora-1984 conducted a study of the bacterial reverse mutation test (Ames assay) on 4-AP using various tester strains of *S. typhimurium* (TA1535, TA100, TA1538, TA98, and TA1537). The results were negative and 4-AP is not mutagenic in these tester strains. The Ames assay was not conducted on any *E. coli* tester strain or *S. typhimurium* TA102 and therefore only tests for potential GC base pair reversions. It is not clear what doses of 4-AP were used however, it dose not appear that the standard protocol for the Ames assay was employed. Thus, the study in DeFlora-1984 may be considered relevant although this report does not address the potential impact of 4-AP on AT base pair primary reversions.

Zeiger-1988 conducted a study of the bacterial reverse mutation test (Ames assay) on 4-AP using various tester strains of *S. typhimurium* (TA97, TA98, TA100, TA1535, and TA1537). No tester stains of *E. coli* or *S. typhimurium* TA102 were used. The results were negative and 4-AP was not mutagenic in these tester strains. It is not clear the doses of 4-AP used but it appears that a maximum dose of 10 mg/plate was used. Thus, the study in Zeiger-1988 is considered relevant although it does not employ the current standard testing strains (missing the AT base pair reversion).

An in vivo test for chromosome aberrations and a mouse micronucleus test could not be obtained by the Division and were not submitted by the Sponsor.

Carcinogenicity of 4-AP:

The carcinogenicity of 4-AP was conducted (b) (4) in the study entitled "Potential Carcinogenic Effects by Oral Route (Gavage) in Rats" and is reviewed below.

Study title: Potential Carcinogenic Effects by Oral Route (Gavage) in Rats

Key study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model:
NOTE: Due to the late date of submission during the review cycle, the study results were not discussed with the eCAC. This carcinogenicity study used a common laboratory animal, Sprague-Dawley rats, to test the carcinogenicity of 4-AP.

Evaluation of tumor findings:
The animals were observed for palpable masses. After six months of treatment, all animals were palpated every two weeks and the date and any changes in palpable masses were recorded.

Table 8 is the number of palpable masses that were confirmed as tumors at microscopic examination for the control and each dose group.

Table 8: Number of Tumors Following Treatment with 4-AP for 101 weeks

Dose-levels (mg/kg/day)	0	2	5	12	30
Males					
. Number of animals in study	50	50	50	50	50
. Number of palpable masses	8	16	11	7	9
. Number of animals bearing palpable masses	6	12	11	7	8
Mean per animal	1.3	1.3	1.0	1.0	1.1
Week of appearance of the first mass	27	65	22	71	31
Females					
. Number of animals in study	50	50	50	50	50
. Number of palpable masses	89	84	76	72	71
. Number of animals bearing palpable masses	39	36	40	39	37
Mean per animal	2.3	2.3	1.9	1.8	1.9
Week of appearance of the first mass	45	35	33	43	33

As shown in Table 8 above, the number of palpable masses in males for control and 2, 5, 12, and 30 mg/kg/day 4-AP were 8, 16, 11, 7, and 9, respectively. In females, the number of palpable masses for control and 2, 5, 12, and 30 mg/kg/day 4-AP were 89, 84, 76, 72, and 71, respectively. In males, the mean number of palpable masses per animal

for control and 2, 5, 12, and 30 mg/kg/day 4-AP were 1.3, 1.3, 1.0, 1.0, and 1.1, respectively. In females, the mean number of palpable masses per animal for control and 2, 5, 12, and 30 mg/kg/day 4-AP were 2.3, 2.3, 1.9, 1.8, and 1.9, respectively.

Tumor findings from specific organs will be discussed in the histopathology section below.

Study no.:	11902 TCR (95/2/023)
Volume #, and page #:	10 volumes, 3277 pages total
Conducting laboratory and location:	Centre International de Toxicologie (C.I.T.) Miserey 27005 Evreux France
Date of study initiation:	September 8, 1994

GLP compliance: Yes, signature provided on January 9, 1998.
QA report: yes (X) no ( ), signature provided on January 9, 1998.

Drug, lot #, and % purity:	para-aminophenol; lot # 2070155; 99.9% purity
CAC concurrence:	There was no CAC involvement.

NOTE: Para-aminophenol is the same compound as 4-aminophenol (4-AP).

Methods

Doses:
The final doses used in this carcinogenicity study were 2, 5, 12, and 30 mg/kg.

Basis of dose selection (MTD, MFD, AUC etc.):
The doses were based on a previous study, a 13-week toxicity study in rats orally administered 30 and 100 mg/kg/day daily dose of 4-AP, that produced dose-dependent minimal to marked tubular nephrosis associated with slight to marked tubular basophila. The results (kidney histopathological findings) show that 30 mg/kg/day was considered the maximum tolerated dose. The previous 13-week toxicity study was not submitted and was not reviewed by this reviewer.

Species/strain:
Rat/Sprague-Dawley Crl CD®.

Number/sex/group (main study):
50/sex/group.

Route, formulation, volume:
4-AP was delivered by oral gavage. 4-AP was formulated in vehicle, 0.5% (w/w) aqueous carboxymethylcellulose in water for injection. The dose volume was 5 mL/kg/day.

Frequency of dosing:

Daily.

Satellite groups used for toxicokinetics or special groups:
No toxicokinetics were performed in this study.

Age:
The animals were approximately 6 weeks old at the time of study initiation.

Animal housing:
The animals were housed in cages that contained 2 rats of the same sex and group. The standard conditions of temperature, humidity, ventilation, and light were employed. The animals had free access to food and water.

Restriction paradigm for dietary restriction studies:
No dietary restrictions were needed as the test compound (4-AP) was administered via oral gavage and not via feed.

Drug stability/homogeneity:
The following is from the homogeneity section of the materials and methods as supplied (b) (4):

> A preparation was made at the lowest concentration (i.e. 0.4 mg/ml) to be used in the study under the conditions representative of those of the study.
>
> Samples were taken in duplicate from the suspension at three different levels (top, middle, bottom) and analysed for homogeneity.

The following is from the stability section of the materials and methods as supplied (b) (4):

> The suspension (0.4 mg/ml concentration level) prepared for homogeneity analysis was stored either at room temperature and sampled after 0 and 5 hours storage or at +4 °C and sampled after 2, 4 and 7 days storage. Samples were taken in duplicate for each sampling time and diluted with a degassed solvent containing a stabilizer (ascorbic acid at 0.01%).
>
> The samples taken on days 2 and 4 were stored frozen at -20 °C pending analysis at the last sampling occasion (day 7) when all samples were assayed.

Dual controls employed:
A vehicle control group is used as a negative control for both males and females.

Interim sacrifices:
There was no interim sacrifice as necropsy was performed at one time interval upon completion of the study.

Deviations from original study protocol:
There were various amendments to the protocol, most notably the duration of the study. The study was originally scheduled for 104 weeks. The animals were to be killed before week 104 if mortality reaches or passes 80% in either the control or any of the treatment

groups for both males and females combined. Indeed, the 30 mg/kg/day females had a survival rate of 20% or a mortality rate of 80% at week 101 and the control males had a survival rate of 26% or a mortality rate of 74% at week 101.

Observation times

Mortality:

All animals were checked twice a day for morbidity and mortality. The survival rates for rats at 78 weeks and 101 weeks are in Table 9.

Table 9: Survival Rate

Males					
Dose-levels (mg/kg/day)	0	2	5	12	30
Onset of mortality (in week)	30	6	4	17	9
Survival rate in week 78 (%)	70	84	80	62	70
Survival rate in week 101 (%)	26	44	38	28	28
- found dead	21	23	22	26	23
- killed prematurely	16	5	9	10	13
Females					
Dose-levels (mg/kg/day)	0	2	5	12	30
Onset of mortality (in week)	54	36	46	13	20
Survival rate in week 78 (%)	82	72	86	72	68
Survival rate in week 101 (%)	32	30	30	34	20
- found dead	12	17	13	18	18
- killed prematurely	22	18	22	15	22

As shown in Table 9 above, there was no dose-dependent relationship in the onset of mortality, survival rate in week 78, and survival rate in week 101. In males and females, there was a shortened onset of mortality where treatment of 2, 5, 12, and 30 mg/kg/day 4-AP were 6, 4, 17, and 9 weeks, respectively in males and 36, 46, 13, and 20 weeks, respectively in females compared to 30 weeks in the male control and 54 weeks in the female control. The survival rate in week 78 was 70, 84, 80, 62, and 70% in males treated with 0, 2, 5, 12, and 30 mg/kg/day 4-AP, respectively and 82, 72, 86, 72, and 68% in females treated with 0, 2, 5, 12, and 30 mg/kg/day 4-AP, respectively. The survival rate in week 101 was 26, 44, 38, 28 and 28% in males treated with 0, 2, 5, 12, and 30 mg/kg/day 4-AP, respectively and 32, 30, 30, 34, and 20% in females treated with 0, 2, 5, 12, and 30 mg/kg/day 4-AP, respectively. In fact, the survival rates of the male rats in all 4-AP treatment groups were better than in the control group however, the high dose females had a lower survival rate compared to control (20 vs. 32% survival rate, respectively).

Clinical signs:

All animals were observed for clinical signs daily. Blood samples were taken at 52 and 78 weeks as well as at the end of the study for hematology. The following hematology parameters were measured:

Parameter	Apparatus/Method	Units
Erythrocytes (RBC)	Bayer Diagnostics H1 (1) Haematology Analyzer/laser	T/l
Haemoglobin (HB)	Bayer Diagnostics H1 Haematology Analyzer/Drabkin	g/dl
Mean Cell Volume (MCV)	Bayer Diagnostics H1 Haematology Analyzer/laser	fl
Packed Cell Volume (PCV)	Bayer Diagnostics H1 Haematology Analyzer/calculated	l/l
Mean Cell Haemoglobin Concentration (MCHC)	Bayer Diagnostics H1 Haematology Analyzer/calculated/ laser	g/dl
Mean Cell Haemoglobin (MCH)	Bayer Diagnostics H1 Haematology Analyzer/calculated	pg
Thrombocytes (PLAT)	Bayer Diagnostics H1 Haematology Analyzer/laser	G/l
Leucocytes (WBC)	Bayer Diagnostics H1 Haematology Analyzer/ peroxidase cytochemistry/laser morphometry	G/l
Differential White Cell Count with cell morphology	Bayer Diagnostics H1 Haematology Analyzer/ peroxidase cytochemistry/laser morphometry (a)	
. neutrophils (N)		% and G/l
. eosinophils (E)		% and G/l
. basophils (B)		% and G/l
. lymphocytes (L)		% and G/l
. monocytes (M)		% and G/l

(a) Blood smears were prepared for all sampled animals. If the samples were not accepted by the H1 Analyser, a microscopic evaluation was performed after May Grünwald Giemsa staining.

(1) Bayer Diagnostics (92807 Puteaux, France)

There were no relevant treatment-related changes in clinical signs except for the observation of orange colored urine in the high dose groups in both males and females. Orange colored urine was observed in 96% of males from the 30 mg/kg/day 4-AP group compared to 0% from the control group and in 2, 30, and 94% of females from the 5, 12, and 30 mg/kg/day 4-AP groups, respectively compared to 0% from the control group. There were no relevant treatment-related changes in hematology. It is noted that liver enzymes or kidney function was not measured.

Body weights:
Body weights of each animal were recorded before treatment, on the first day of treatment, weekly during the first 13 weeks of the study, and every four weeks until the end of the study. In both males and females, there were no relevant treatment-related changes in body weight compared to control as well as no dose-dependent changes in body weight over the course of the study. Moreover, all body weight changes were within ±10% compared to control throughout the course of the study for both sexes.

Food consumption:
Food consumption was recorded over a seven-day period during the first 13 weeks of the study and every four weeks until the end of the study. There were no relevant treatment-related changes in food consumption.

Histopathology: Peer review: yes ( ), no (X)
It is not clear whether a peer review of the histopathology was conducted. The following tissues were preserved and microscopically examined at necropsy:

adrenals	lymph nodes (mandibular, mesenteric and regional to masses if visible)	skin
aorta	mammary glands	spinal cord (cervical, thoracic and lumbar)
brain including medulla/ pons, cerebellar and cerebral cortex	oesophagus	spleen
caecum	optic nerve	sternum with bone marrow
colon	ovaries	stomach with forestomach
duodenum	pancreas	testes and epididymides
eyes with Harderian glands	pituitary gland	thymus
femoral bone with articulation	prostate	thyroids with parathyroids
heart	rectum	tongue
ileum	salivary glands (sublingual and submaxillary)	trachea
jejunum	sciatic nerve	urinary bladder
kidneys	seminal vesicles	uterus (horns and cervix)
liver	skeletal muscle	vagina
lungs with bronchi		

Additionally, the following organs were weighed at necropsy:

adrenals	liver	prostate
brain	lungs	spleen
heart	ovaries	testes
kidneys	pituitary gland	thyroid with parathyroids

There were a number of gross morphological changes observed in the animals. Enlarged liver was observed in 9/50, 5/50, 7/50, 7/50, and 12/50 male animals treated with 0, 2, 5, 12, and 30 mg/kg/day 4-AP, respectively. Paleness of the liver was observed in 3/50, 4/50, 4/50, 4/50, and 8/50 male animals treated with 0, 2, 5, 12, and 30 mg/kg/day 4-AP, respectively. Reduction in the size of the seminal vesicles was observed in 11/50, 5/50, 8/50, 12/50, and 16/50 males treated with 0, 2, 5, 12, and 30 mg/kg/day 4-AP, respectively. Soft testes were observed in 18/50, 15/50, 11/50, 18/50, and 22/50 males treated with 0, 2, 5, 12, and 30 mg/kg/day 4-AP, respectively. Reduction in size of the

epididymides was observed in 5/50, 8/50, 4/50, 12/50, and 10/50 males treated with 0, 2, 5, 12, and 30 mg/kg/day 4-AP, respectively. Enlarged liver was observed in 6/50, 11/50, 6/50, 4/50, and 11/50 female animals treated with 0, 2, 5, 12, and 30 mg/kg/day 4-AP, respectively. Paleness of the kidney was observed in 3/50, 0/50, 3/50, 4/50, and 6/50 females treated with 0, 2, 5, 12, and 30 mg/kg/day 4-AP, respectively. There were no other relevant treatment-related changes in the gross examination of organs.

There were a number of non-neoplastic microscopic findings (see Table 10).

Table 10: Summary of Non-neoplasic Microscopic Findings After 4-AP Treatment

	4-AP Treatment (mg/kg/day)				
Males	0	2	5	12	30
Spleen					
plasmocytosis	1/50 (2.0%)	1/35 (2.9%)	0/33	2/39 (5.1%)	7/50 (14%)
Lung					
chronic interstitial pneumonia	6/50	18/50	18/50	5/50	11/50
Testes					
degeneration of the seminiferous tubules	13/50 (26%)	15/38 (39%)	10/34 (29%)	18/43 (42%)	17/50 (34%)
Females					
Liver					
altered cell foci, acidophilic	21/50	26/50	33/50	30/50	25/50
coagulative hepatocellular necrosis	2/50	2/50	8/50	9/50	6/50

As shown in Table 10 above, plasmocytosis of the spleen was observed in 1/50, 1/35, 0/33, 2/39, and 7/50 male animals treated with 0, 2, 5, 12, and 30 mg/kg/day 4-AP, respectively. Chronic interstitial pneumonia of the lung was observed in 6/50, 18/50, 18/50, 5/50, and 11/50 males treated with 0, 2, 5, 12, and 30 mg/kg/day 4-AP, respectively. Degeneration of the seminiferous tubules of the testes was observed in 13/50, 15/38, 10/34, 18/43, and 17/50 males treated with 0, 2, 5, 12, and 30 mg/kg/day 4-AP, respectively. Altered cell foci, acidophilic, in the liver was observed in 21/50, 26/50, 33/50, 30/50, and 25/50 female animals treated with 0, 2, 5, 12, and 30 mg/kg/day 4-AP, respectively. Coagulative hepatocellular necrosis (areas, focal, and multifocal) of the liver was observed in 2/50, 2/50, 8/50, 9/50, and 6/50 females treated with 0, 2, 5, 12, and 30 mg/day/day 4-AP, respectively.

There were no relevant treatment-related or dose-dependent neoplastic lesions. The statistical analysis performed on the incidence of neoplastic lesions revealed there was no statistical significance ($p>0.05$) between the 4-AP treatment groups and control.

There was a significant decrease in the mean % body weight of the prostate gland and testes in the male rats treated with 30 mg/kg/day 4-AP. In the prostate, the mean % change relative to body weight was 0.24% and 0.16% in the control and 30 mg/kg/day 4-AP treatment groups, respectively. In the testes, the mean % change relative to body

weight was 0.52% and 0.42% in the control and 30 mg/kg/day 4-AP treatment groups, respectively. There were no other relevant treatment-related changes in organ weights.

Toxicokinetics:
Toxicokinetics was not studied.

Conclusions:
Treatment of rats of 2, 5, 12, and 30 mg/kg/day 4-AP did not increase the incidence of neoplastic lesions over control. Therefore, a NOEL for carcinogenicity of 30 mg/kg/day is established. A NOAEL for adverse events of 12 mg/kg/day in males can be established based on mortality, clinical signs, organ weights, and histopathological findings of the spleen and testes. A NOAEL for adverse events of 5 mg/kg/day in females can be established based on histopathological findings of coagulative hepatocellular necrosis of the liver. However, these doses are based a MTD in rats and that exposure to these levels allowed for the rats to survive throughout a 2-year carcinogenicity study and thus may not be best suited for a general toxicity study.

Repeat-dose Toxicity of 4-AP:
A NOAEL for the general toxicity of 4-AP was best studied in JECBD-1995. Sprague Dawley rats (4/sex/group) were administered 0, 4, 20, 100, or 500 mg/kg/day 4-AP orally for 28 days with a 14-day recovery period in this repeat dose toxicity study. Following treatment and recovery, all animals were measured for body weight changes, food and water consumption, hematology, blood chemistry, and urinalysis. In addition, pathology (macro- and microscopically) was performed. This study appears to be GLP and the parameters in a typical repeat-dose toxicity study were measured.

Figure 1 represents the body weight changes in the animals. In the 500 mg/kg/day males, there was a significant decrease in body weights at day 7. The body weights in the 500 mg/kg/day males during the remainder of the study were decreased albeit not significantly. There were no other relevant treatment related changes in body weight.

Figure 1: Body Weight Changes Following Oral Administration of 4-AP

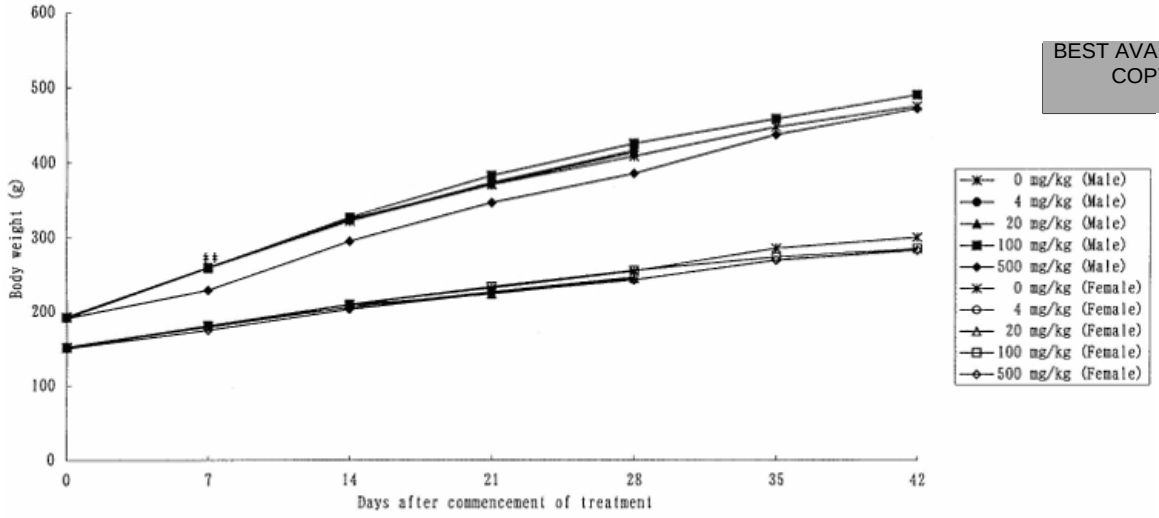

Fig. 1 Body weight changes of rats treated orally with 4-aminophenol in 28-day repeat dose toxicity test
Significantly different from 0 mg/kg group.**: $p<0.01$.

There were no relevant treatment related changes in hematology in both males and females. There were no relevant treatment related changes in blood chemistry in both males and females.

Brown colored urine was observed in 0, 0, 0, 2/6, and 5/6 males and 0, 0, 0, 6/6 and 6/6 females treated with 0, 4, 20, 100, and 500 mg/kg/day 4-AP. Brown colored urine was not observed in the recovery animals. There were no other relevant treatment related changes in urinalysis.

The relative organ weights of the liver and kidneys were significantly elevated (by 17.6 and 22.9%, respectively) in the males treated with 500 mg/kg/day 4-AP. The changes in the relative organ weight of the kidneys were reversible but remained elevated in the liver (by 17.0%) in the recovery males. Additionally, there was a significant elevation in the absolute and relative organ weight of the thymus (by 19.1 and 19.0%, respectively) in recovery males treated with 500 mg/kg/day 4-AP. There was a significant increase in the absolute organ weight of the liver (by 26.9%) and in the relative organ weights of the liver, kidneys, and spleen (by 29.9, 13.7, and 16.7%, respectively) in females treated with 500 mg/kg/day 4-AP. In addition, there was a significant increase in the absolute organ weight of the kidneys in the females treated with 100 and 500 mg/kg/day 4-AP (by 13.4 and 11.7%, respectively). The changes in the absolute organ weight of the liver and the relative organ weigh of the spleen were reversible in the recovery females. There was a significant decrease in the absolute organ weight of the brain in the recovery females treated with 100 and 500 mg/kg/day 4-AP (by -4.5 and -4.0%, respectively). There was a significant increase in the relative organ weight of the thymus (by 26.1%) in the recovery females treated with 100 mg/kg/day 4-AP. There was a significant increase in the relative organ weights of the liver and kidneys (by 15.3 and 13.8%, respectively) in the recovery females treated with 500 mg/kg/day 4-AP.

There were changes in the gross examination of the animals. White streak of the corticomedullary junction of the kidney was observed in both sexes (3/5 males and 6/6 females) compared to no control animals in the animals treated with 500 mg/kg/day 4-AP. White streak of the corticomedullary junction of the kidney was not seen in the recovery animals. In addition, dark reddish spleen was observed in 6/6 females in the 500 mg/kg/day 4-AP group compared to no control animals. Dark reddish spleen was still seen in 6/6 recovery females treated with 500 mg/kg/day 4-AP.

Basophilic proximal tubule of the kidney was observed in 4/5 males from the 500 mg/kg/day 4-AP group compared to no control males and persisted in 3/6 recovery males from the 500 mg/kg/day 4-AP group compared to no control males. In the spleen, there was an increase in extramedullary hematopoiesis and an increase in hemosiderin pigment in 5/6 females and 5/6 females from the 500 mg/kg/day 4-AP group compared to no controls females. The changes in the spleen did not reverse as 2/6 females and 6/6 females from the 500 mg/kg/day 4-AP group still had an increase in extramedullary hematopoiesis and an increase in hemosiderin pigment, respectively compared to no control females. Basophilic proximal tubule in the kidney was observed in 4/6 and 6/6 females treated with 100 and 500 mg/kg/day 4-AP compared to no control females. The observation of basophilic proximal tubule in the kidney was reversible in the recovery females.

According to JECDB-1995, the NOEL is 20 mg/kg/day for both sexes. This reviewer concurs with this assessment of the NOEL. The NOAEL is 100 mg/kg/day for both males and females. The safety margin for the NOEL is 60.9 for an average human weighing 60 kg based on a body surface comparison. The safety margin for the NOAEL is 305 for an average human weighing 60 kg based on a body surface comparison.

Overall Conclusions and Recommendations:
From the nonclinical pharmacology/toxicology perspective, NDA 22-450 is recommended for approval.

Several conclusions can be made from the results of the fertility and reproductive toxicity study. Exposure of the F_0 generation of mice to 1.0% APAP in the diet over an 18-week period resulted in a significant reduction in the number of litters per pair and the number of live male pups per litter versus the control group. Body weights of the F_1 mice at 28 days of age and at 74 ± 10 days of age were significantly depressed in a dose-related manner. Live pup weights were significantly decreased for offspring (F_2 generation) produced by F_1 breeding pairs exposed continuously to 1.0% APAP in the diet. The % abnormal sperm also were significantly elevated in the F_1 males. However, a clear NOAEL cannot be determined from this study. Although the amount of APAP was identified in the feed for each treatment group, it is not clear how much APAP was ingested by the mice in each of the treatment groups.

The carcinogenicity of APAP was studied in Fischer rats and B6C3F1 mice. The rats and mice were given 0, 600, 3000, or 6000 mg/kg APAP through the feed for up to 104 weeks. There was no evidence of carcinogenic activity of APAP in the mice and male

rats. There was evidence of carcinogenic activity of APAP in the female rats based on an increased incidence of mononuculear cell leukemia. However, following discussions with the Executive Carcinogenicity Assessment Committee (ECAC), the mononuclear cell leukemia is believed to be species specific and of limited relevance in humans, as discussed in the literature (Caldwell-1999 and Ishmael and Dugard-2006).

From a clinical PK study of orally administered APAP, a mean AUC of APAP and 4-AP was determined after oral administration of 1 g of APAP every 4 hours. The ratio of 4-AP to APAP was (b) (4) Based on these data, 4-AP is not considered a major human metabolite. However, a study employing the IV route of administration of APAP containing the proposed levels of 4-AP has not been conducted. It is important to note that the amount of 4-AP used in the clinical PK study of orally administered APAP was (b) (4) relative to acetaminophen. According to the DP specifications for IV APAP, there are NMT (b) (4) 4-AP in the drug product formulation of this NDA. Thus, there is a several hundred fold more 4-AP in the IV APAP drug formulation of this NDA than in the Tylenol® Extra Strength Caplets.

Reproductive toxicology studies of 4-AP from Rutkowski-1982 and Kavlock-1990 do not address skeletal malformations however, the reported findings are relevant to inform labeling. Rutkowski-1982 demonstrated a dose-related increase in malformations coupled with a high incidence of fetal resorption that occurred over all 4-AP doses tested by the IV route of administration with no evidence of maternal toxicity. A NOAEL cannot be determined as malformations and fetal resorptions occurred at all doses tested. Kavlock-1990 demonstrated that maternal body weight was significantly reduced at doses of 667 and 1,000 mg/kg 4-AP, associated with decreased postpartum pup weight, tail abnormalities and/or hind limb paralysis at 667 and 1,000 mg/kg 4-AP and perinatal loss at 1,000 mg/kg 4-AP. The appropriate maternal observations were made however, the pups were only observed for external malformations. The NOAEL was 333 mg/kg 4-AP can be established based on maternal body weight and pup weight, tail abnormalities and/or hind limb paralysis, and perinatal loss. For an average human weighing 60 kg, the rat NOAEL represents a safety margin (b) (4) based on a body surface comparison.

4-AP appears to be mutagenic through studies done in the published literature. Information regarding the potential for in vivo clastogenicity of 4-AP cannot be addressed because the Division was not able to obtain or the Sponsor was not able to provide the in vivo chromosome aberrations test and in vivo mouse micronucleus test.

Treatment of rats of 2, 5, 12, and 30 mg/kg/day 4-AP in a 2-year carcinogenicity assay did not increase the incidence of neoplastic lesions over control. Therefore, a NOEL for carcinogenicity of 30 mg/kg/day can be established. This represents a safety margin of 91.4 for 4-AP based on a body surface comparison[2].

[2] The maximum daily human exposure to the IV APAP product of this NDA is 4000 mg/kg. According to the DP specifications for IV APAP, 4-AP is present at NMT (b) (4) The NOEL for carcinogenicity of 4-AP from the 2-year rat carcinogenicity study is 30 mg/kg/day. For rats, 30 mg/kg/day x 6 = 180 mg/m^2. For an average human weighing 60 kg, the safety margin is 180/1.97 = 91.4 based on a body surface area comparison.

Treatment of rats of 0, 4, 20, 100, and 500 mg/kg/day 4-AP in a 28-day repeat dose toxicity study produced a NOEL of 20 mg/kg/day and a NOAEL of 100 mg/kg/day. The safety margin for the NOEL is 60.9 for an average human weighing 60 kg based on a body surface comparison. The safety margin for the NOAEL is 305 for an average human weighing 60 kg based on a body surface comparison.

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Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22450	ORIG-1	CADENCE PHARMACEUTICALS INC	Ofirmev (acetaminophen for injection)

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/s/

CARLIC K HUYNH
02/10/2010

RICHARD D MELLON
02/10/2010

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-450**
SERIAL NUMBER:	**N 000 (original submission)**
DATE RECEIVED BY CENTER:	**May 13, 2009**
PRODUCT:	**BRANDNAME (Acetaminophen injection)**
INTENDED CLINICAL POPULATION:	**Treatment of acute pain and fever in adult and pediatric patients**
SPONSOR:	**Cadence Pharmaceuticals, Inc.** **12481 High Bluff Drive, Suite 200** **San Diego, CA 92130**
DOCUMENTS REVIEWED:	**Electronic, Vols. 1-4**
REVIEW DIVISION:	**Division of Anesthesia, Analgesia, and Rheumatology Products (HFD-170)**
PHARM/TOX REVIEWER:	**Carlic K. Huynh, Ph.D.**
PHARM/TOX SUPERVISOR:	**R. Daniel Mellon, Ph.D.**
DIVISION DIRECTOR:	**Bob A. Rappaport, M.D.**
PROJECT MANAGER:	**Sharon Turner-Rinehardt**
Date of review submission to DARRTs:	**November 3, 2009**

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

This NDA is deemed a complete response from a nonclinical pharmacology/toxicology perspective. There are several deficiencies that are outlined below.

B. Recommendation for nonclinical studies

Fertility, embryo toxicity, and teratogenicity studies were cited from the published literature (Boyd-1968, Reel-1992, Neto-2004 and Lubawy-1977). Although the majority of these literature references regarding the fertility and embryo and teratogenicity studies of acetaminophen were not done following GLP guidelines, it remains clear that there were animal studies conducted to assess the reproductive toxicology of acetaminophen and that the adverse findings from these studies are noted. The Sponsor must submit exposure data from these studies and how these doses relate to the clinical dose of this NDA in order for accurate information to be put in the label.

There are a number of deficiencies in the justification of 4-AP that are delineated here. Regarding the reproductive toxicity of 4-AP, the following references are needed:

> Rutowski, J.V. and Ferm, V.H. (1982) Comparison of the Teratogenic Effects of the Isomeric Forms of Aminophenol in the Syrian Golden Hamster. Toxicol and Applied Pharmacol 63, pp. 264-269
>
> Kavlock, R. J. Structure-Activity Relationships in the Developmental Toxicity of Substituted Phenols: *in vivo* Effects. Teratology 41, 43-59 (1990)

Regarding the genetic toxicity of 4-AP, the following references are needed:

> DeFlora, S., Zanacchi, P., Camoirano, A., Bennicelli, C., and Badolati, G.S. (1984a). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test. *Mutat. Res.* **133**:161–198
>
> Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1988). *Salmonella* mutagenicity tests: IV. Results from the testing of 300 chemicals. *Environ. Molec. Mutagen.* **11**(Suppl. 12):1–158
>
> Microtest, York, UK. Study To Evaluate the Potential of *p*-Aminophenol To Induce Unscheduled DNA Synthesis in Rat Liver Using an *in vivo/in vitro* Procedure. Report N°: ILUREBRP.029 (9/1989)

Microtest, York, UK. Study To Evaluate the Chromosome Damaging Potential of *p*-Aminophenol By Its Effects on Cultured Human Lymphocytes Using an *in vitro* Cytogenetics Assay. Report N°: 1HLRREBRP.029 (1/1990)

Centre International de Toxicologie – Evreux, France. p-Aminophenol – Micronucleus Test by the Oral Route in Mice. Report No. 7757 MAS (CIAUL 91053) (5/1992)

Regarding the carcinogenicity of 4-AP, the Sponsor referenced Bomhard-2005 as having the 2-year rat carcinogenicity bioassay. No such study is in the Bomhard-2005 reference. The 2-year rat carcinogenicity bioassay must be submitted for an evaluation of the carcinogenicity of 4-AP.

If the Sponsor considers other references as more pivotal studies to address the above, they must submit those references as well.

C. Recommendations on labeling

The table below contains the draft labeling submitted by the sponsor, the proposed changes and the rationale for the proposed changes.

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(b) (4)

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Acetaminophen is not a mutagen (as demonstrated by negative results in the bacterial Ames test) but is a clastogen (as demonstrated by positive results in the chromosomal aberration assay in cultured human peripheral blood lymphocytes).

The results of the NTP carcinogenicity studies showed that there was no evidence of carcinogenic activity of acetaminophen in male F344/N rats, there was equivocal evidence of carcinogenic activity in female rats based on increased incidences of mononuclear cell leukemia, and there was no evidence of carcinogenic activity in male and female B6C3F1 mice

Testicular atrophy and reduction in testis weight was noted in fertility studies of acetaminophen performed on rats at all doses tested. There were no fetal morphological abnormalities but dose-dependent microscopic lesions of the maternal liver and kidney in rats as well as retarded growth and abnormal sperm in F_1 mice and reduced birth weight of F_2 pups in embryo and teratogenicity studies done in rodents.

B. Pharmacologic activity

Acetaminophen is a non-opioid analgesic and non-salicylate antipyretic compound. A definitive mechanism of action for acetaminophen has not been elucidated. The use of IV acetaminophen in the proposed indication of the treatment of acute pain and fever in adult and pediatric patients is supported through the various mechanisms of action of acetaminophen, including a central site of action, prostaglandin inhibition, and cannabinergic and serotonergic effects. Namely, transformation of acetaminophen in the CNS to form AM404 can then stimulate vanilloid receptors (TRPV1) to increase the activity of endogenous cannabinoids and to exert anti-nociceptive and hypothermic effects.

C. Nonclinical safety issues relevant to clinical use

Although the majority of these literature references regarding the fertility and embryo and teratogenicity studies of acetaminophen were not done following GLP guidelines, it remains clear that there were animal studies conducted to assess the reproductive toxicology of acetaminophen and that the adverse findings from these studies are noted. The Sponsor must submit exposure data from these

studies and how these doses relate to the clinical dose of this NDA in order for accurate information to be put in the label.

The presence of the drug product degradant, 4-aminophenol (4-AP) remains a concern. In the drug product specifications, 4-AP is NMT (b) (4). The Sponsor makes several conclusions regarding the toxicological risk assessment of 4-AP. However, most of the references that the Sponsor submits in support of these conclusions were from review papers that cited studies from the published literature. The review papers that were submitted, SCCP-2005 and Bomhard-2005, do not contain data but is rather a summary of studies done. As such, the data from the studies regarding the metabolism, reproductive toxicity, genetic toxicity, and carcinogenicity of 4-AP were not fully reviewed. Therefore, there is insufficient evidence to make any conclusions with regard to the metabolism, reproductive toxicity, genetic toxicity, and carcinogenicity of 4-AP. A list of studies need to address these deficiencies are listed above.

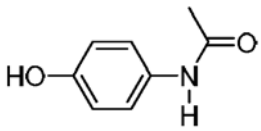
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number:	22-450
Review number:	1
Sequence number/date/type of submission:	N 000/May 13, 2009/ 505(b)(2) NDA
Information to sponsor:	Yes () No (X)
Sponsor and/or agent:	Cadence Pharmaceuticals, Inc. 12481 High Bluff Drive, Suite 200 San Diego, CA 92130
Manufacturer for drug substance:	Mallinckrodt Inc. (Covidien) 8801 Capital Blvd. Raleigh, NC 27616
Reviewer name:	Carlic K. Huynh, Ph.D.
Supervisor name:	R. Daniel Mellon, Ph.D.
Division name:	Division of Anesthesia, Analgesia, and Rheumatology Products
HFD #:	170
Review completion date:	October 20, 2009

Drug:

Trade name:	BRANDNAME
Generic name:	acetaminophen; paracetamol
Code name:	
Chemical name:	N-acetyl-p-aminophenol; 4'-hydroxyacetanilide; p-hydroxyacetanilide; p-acetamidophenol; p-acetaminophenol; p-acetylaminophenol
CAS registry number:	103-90-2
Molecular formula/molecular weight:	$C_8H_9NO_2$ / 151.16 g/mol
Structure:	



Relevant INDs/NDAs/DMFs:

NDA#	Drug Name	Div	Strength (route)	Marketing Status	AP Date	Indication	Company

19-872	Tylenol	ONP/ DNCE	650 mg (oral tablet, extended release)	Approved Over-the-Counter	8-June-1994	• temporary relieves minor aches and pains due to: ▪ muscle aches ▪ backache, ▪ headache ▪ toothache ▪ the common cold ▪ menstrual cramps ▪ minor pains of arthritis • temporarily reduces fever	McNeil Consumer Health

IND#	Drug	Status	Division	Indication	Stamp Date	Sponsor
58,362	acetaminophen	Active	OND/ DAARP	Treatment of postoperative pain following lower abdominal surgery	24-May-1999	Cadence Pharmaceuticals

DMF#	Subject of DMF	Holder	Submit Date	Reviewer's Comment
5326	acetaminophen USP manufactured in Raleigh, North Carolina Plant	Mallinckrodt, Inc.	5-April-1984	Active, referenced by numerous OGD products

Drug class: Non-opioid analgesic and non-salicylate antipyretic

Intended clinical population: Treatment of acute pain and fever in adult and pediatric patients

Clinical formulation:
BRANDNAME is supplied in a ready-to-use 100 mL, glass vial containing 1000 mg of acetaminophen (10 mg/mL). The Sponsor has provided the following table to describe the composition of the drug product:

Table 1. Unit Composition of Acetaminophen, Injection for Intravenous Use

Component	Unit Formula	Function	Quality Standard
Acetaminophen	1,000 mg	Active substance	USP, current edition
Mannitol	3,850 mg	(b) (4)	USP, current edition
Cysteine hydrochloride, monohydrate	25.00 mg		USP, current edition
Dibasic sodium phosphate, anhydrous	10.40 mg		USP, current edition
Sodium hydroxide[1]	(b) (4)		NF, current edition
Hydrochloric acid[2]			NF, current edition
(b) (4)			

As shown in the table above, the drug product contains 1000 mg of acetaminophen per vial. It is important to note that the maximum daily exposure to acetaminophen is 4000 mg/day as per the proposed labeling.

The Sponsor has also provided the following table of excipient concentrations and characteristics:

Table 2. Excipient Concentration and Characteristics

Excipient	Concentration	Characteristic
Mannitol	3.85%	(b) (4)
Cysteine hydrochloride, monohydrate	0.025%	
Dibasic sodium phosphate, anhydrous[1]	0.01%	
Sodium hydroxide	(b) (4)	
Hydrochloric acid		
(b) (4)		

[1] The drug product formulation during development was manufactured (b) (4) (b) (4) the proposed commercial formulation uses dibasic sodium phosphate, anhydrous, as discussed in Section 3.2.P.2.2.1.2.3.

All of the excipients are listed in the IIG. The following table shows the maximum potency (as listed in the IIG) for each excipient:

Table 3. Maximum Potency of the Excipients

Excipient	Amount in Formulation	Daily Exposure (4 doses/day)	Maximum Potency (IIG)
Mannitol	3.85% (3850 mg/vial)	3.85% (15,400 mg/day)	(b) (4)
Cysteine hydrochloride, monohydrate	0.025% (25 mg/vial)	0.025% (100 mg/day)	
Sodium phosphate, dibasic anhydrous	0.01%	0.01%	
Sodium hydroxide	(b) (4)		
Hydrochloric acid	(b) (4)		

NOTE: Currently, the maximum daily dose of acetaminophen is 4 g/day or 4 doses/day of this drug product. Thus all of the excipients at maximum daily exposure levels are below the maximum potency according to the IIG. According to the Drug Product Reference File (DPRF), the potency of mannitol in approved products may be as high as (b) (4) the potency of cysteine hydrochloride in approved products may be as high as (b) (4) and the potency of sodium phosphate, dibasic anhydrous in approved products may be as high as (b) (4).

Impurities:
The following table is the drug substance specifications as per USP:

Table 4. Impurities in Acetaminophen (USP Monograph)

Impurity	Limit
Water	NMT 0.5%
Chloride	NMT 0.014%
Sulfate	NMT 0.02%
Sulfide	Negative Test
Heavy Metals	Less than 0.001%
Free p-aminophenol (4-aminophenol)	NMT 0.005%
p-chloroacetanilide	NMT 0.001%
Readily carbonizable substances	NMT Standard
Organic volatile impurities	Meets requirements

NMT: Not more than

Two impurities, namely 4-aminophenol and p-chloroacetanilide, contain structural alerts for genotoxicity. The impurity 4-aminophenol (4-AP) is also called p-aminophenol (PAP). These impurities have been known to be in acetaminophen drug substance for

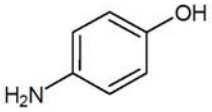
quite some time. Their levels have been restricted as indicated as per USP specifications that were established back in 1970 (Clyde Anthony, USP, personal communication to Dan Mellon, CDER). For 1000 mg of acetaminophen, the presence of 4-aminophenol and p-chloroacetanilide are 0.005% and 0.001%, respectively. For 4 doses or 4000 mg of acetaminophen, the presence of 4-aminophenol and p-chloroacetanilide are (b) (4) respectively. According to ICH Q3A(R2) guidelines, the identification and qualification thresholds for an impurity in a drug substance with a maximum daily dose of > 2 g are (b) (4) Thus 4-aminophenol and p-chloroacetanilide meet ICH Q3A(R2) thresholds for non genotoxic impurities.

The drug substance impurity p-chloroacetanilide has been reduced to a reasonably low technically feasible level of NMT 0.001% and thus the proposed specification, which complies with all other acetaminophen drug substances as per the USP, is acceptable.

The drug substance impurity 4-aminophenol has also been reduced to a reasonably low technically feasible level of NMT 0.005%, and thus the proposed specification, which complies with all other acetaminophen drug substances as per USP, is acceptable.

The following tables are the degradants found in the drug product and the drug product specifications:

Table 5. Impurities Found in Acetaminophen, Injection for Intravenous Use

RRT	Name	Structure	Amounts Found in Drug Product Lots
0.4	4-aminophenol		(b) (4)
			(b) (4)

[1] 4-aminophenol values (b) (4) are typical values observed at drug product release (see Section 3.2.P.5.4). Stability studies show (b) (4) at 36 months when stored at 30 °C. Stability results are provided in Section 3.2.P.8.3 and discussed in Section 3.2.P.8.1.

Table 6. Specification for Acetaminophen, Injection for Intravenous Use

(b) (4)

The drug substance impurity p-chloroacetanilide was not found in the drug product as a degradant. The impurity 4-aminophenol was detected (b) (4) in the drug product stability testing (Table 5). However, 4-aminophenol levels were (b) (4) in stability testing at 36 months when stored at 30˚C (Table 5). In the drug product specification, 4-aminophenol levels were present (b) (4) (Table 6). The impurity 4-aminophenol contains a structural alert for mutagenicity (amino group) and must be reduced to NMT (b) (4). Since the maximum daily dose to acetaminophen is 4 g/day or 4 doses/day of this drug product, the daily exposure to 4-aminophenol is 0.40% or 4.0 mg/day (4,000 mcg/day). Therefore, 4-aminopenol fails to meet the NMT (b) (4) limit. Although 4-aminophenol fails to meet the NMT (b) (4) limit, the Sponsor has submitted a toxicological risk assessment of 4-aminophenol to justify the safety of 4-aminophenol in their product and an evaluation of the Sponsor's assessment is in Appendix 1 and the conclusions are summarized below.

According to ICH Q3B(R2), the identification and qualification thresholds of an impurity for drug products with a maximum daily dose of > 2 g is (b) (4) respectively. Individual unknown impurities are NMT (b) (4) in the drug product specifications. For 4 doses or 4000 mg of acetaminophen, individual unknown impurities is NMT (b) (4) and thus fails to meet ICH Q3B(R2) thresholds.

The Sponsor has submitted a toxicological risk assessment of 4-aminophenol. According to the Sponsor, several conclusions can be made regarding the toxicological risk assessment of 4-AP. 4-AP appears to be a minor metabolite in experimental animals with 0.16% in blood and 0.66%. 4-AP appears to cause similar toxic events as acetaminophen. The various embryo-fetal development and peri/postnatal studies done with 4-AP demonstrate that there was a dose dependent increase in the number of fetal malformations. The results of the bacterial and *in vivo* rodent genotoxicity studies with 4-AP appear to show 4-AP is not mutagenic (as demonstrated by negative results from the Ames test) but may be clastogenic (as demonstrated by equivocal results in the chromosomal aberrations assay). From a 2-year rat carcinogenicity study, 4-AP is not carcinogenic. However, most of the references that the Sponsor submits in support of these conclusions were from review papers that cited studies from the published literature. The review papers that were submitted, SCCP-2005 and Bomhard-2005, do not contain data but is rather a summary of studies done. As such, the data from the studies regarding the metabolism, reproductive toxicity, genetic toxicity, and carcinogenicity of 4-AP were not fully analyzed by this reviewer. Therefore, there is insufficient evidence to make any conclusions with regard to the metabolism, reproductive toxicity, genetic toxicity, and carcinogenicity of 4-AP.

Extractables and Leachables:
Extractables testing (b) (4) is found in DMF 4681 (to Baxter Healthcare Corporation) and is summarized here. The DMF holder conducted extractables testing using standard procedures described in most Pharmacopoeia (Ph. Eur., USP, JP, etc.). The extractables found during testing were common (b) (4).

Route of administration: Injection for intravenous administration

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Data reliance: Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 22-450 are owned by Cadence Pharmaceuticals, Inc. or are data for which Cadence Pharmaceuticals, Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 22-450 that Cadence Pharmaceuticals, Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved

application that Cadence Pharmaceuticals, Inc. does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 22-450.

As per the date of this review, this NDA is submitted via the 505(b)(2) pathway with the referenced drug being Tylenol® (NDA 19-872). The application also relies extensively upon published literature for product labeling, since Tylenol, as a nonprescription product, does not have prescription product labeling.

Studies reviewed within this submission:

Study Title	Document #	Submission (Date)
Pharmacology and Pharmacokinetics		
Toxicity		
2-Week Tolerance Study of PERFALGAN® Administered 4 Times a Day as 15-Minute Intravenous Infusion in Rats Followed by a 2-Week Treatment-Free Period	20455 TSR	May 13, 2009
PERFALAGAN® 28-Day Repeat Dose Infusion Toxicity Study in the Rat followed by a 28 Day Reversibility Period	980100T	May 13, 2009
Other Toxicity		
Study to Evaluate the Chromosome Damaging Potential of Paracetamol by its Effects on Cultured Human Lymphocytes Using an *in vitro* Cytogenetics Assay	UBF 1/HLC	May 13, 2009
Study to Determine the Ability of Paracetamol to Induce Mutations at the Thymidine Kinase (*tk*) locus in Mouse Lymphoma L5178Y Cells Using a Fluctuation Assay	UBF 1/TK	May 13, 2009
Study to Evaluate the Potential of Paracetamol to Induce Unscheduled DNA Synthesis in Rat Liver Using an *in vivo*/*in vitro* Procedure	UBF 1/ILU	May 13, 2009
Skin Sensitization Test in Guinea-Pigs	19379 TSG	May 13, 2009
Local Tolerance Study in the Rabbit	980101T	May 13, 2009
Evaluation of the Dependence Potential of Propacetamol, Paracetamol and Diethylglycine after Intravenous Administration on the Naloxone-precipitated Withdrawal Test, in Mice	BG206P134	May 13, 2009

Studies <u>not</u> reviewed within this submission:

(b) (4)

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Acetaminophen is a non-opioid analgesic and non-salicylate antipyretic compound. A definitive mechanism of action for acetaminophen has not been elucidated. The Sponsor has conducted a literature review of the mechanism of acetaminophen, which is presented below.

2.6.2.2 Primary pharmacodynamics

Mechanism of action:
Although the exact mechanism of action of acetaminophen is not clearly defined, there are several potential mechanisms of action including central effects upon prostaglandin synthesis, the cannabinoid receptor system, and/or the serotonergic system.

The various mechanisms of action of acetaminophen are summarized in the Sponsor's non-clinical overview:

> **Central Site of Action**
> Many authors have speculated over the years that acetaminophen acts centrally rather than peripherally. Recent experiments showing pretreatment with oral acetaminophen capable of blocking the hyperalgesia stimulated by intrathecal delivery of substance P supports this hypothesis (Crawley-2008). In addition, the effect of acetaminophen on prostaglandin synthesis in the cerebrospinal fluid of mice (Ayoub-2004) and cats (Feldberg-1973), and the correlated antipyretic response argues for a central site of action.
>
> Acetaminophen may have its most potent effects on the central cyclooxygenase (COX) activity because peroxide tone and arachidonic acid levels are likely to be lower than at peripheral sites of substantial inflammation, such as in rheumatoid arthritis.
>
> **Prostaglandin Inhibition**
> Unlike NSAIDs, acetaminophen does not appear to have the capacity to inhibit peripheral COX activity and it is generally considered to be a weak inhibitor of the synthesis of prostaglandins centrally. The literature implicating acetaminophen as an inhibitor of COX-1 or COX-2 is contradictory. Results of experiments with broken cell systems suggest weak inhibition of COX-1 and to a lesser extent COX-2, whereas results from intact cells are more variable, and depend upon the cell type and experimental conditions (Swierkosz-2002). COX inhibition appears to be affected by the local arachidonic acid and peroxide concentrations (tone). At sites of inflammation in the periphery, peroxide tone and arachidonic acid levels will be higher, likely inhibiting the effects of acetaminophen on COX-1 and COX-2. In the central nervous system (CNS), peroxide tone is low, and inhibition of PG synthesis by acetaminophen may be more important.
>
> The COX enzymes are more accurately termed prostaglandin H2 synthases (PGHS), because they catalyze the oxygenation of arachidonic acid to yield PGH2 in two steps at distinct active sites within the same enzyme, the COX site and the peroxidase (POX) catalytic site (reviewed by Aronoff-2006). Acetaminophen appears to inhibit PGH2 activity through its capacity to serve as a reducing co-substrate for the POX active site, and it has also been suggested that intracellular hydroperoxide levels (the "peroxide tone" of the cell) might best explain the ability of the drug to block prostanoid synthesis in a

cell-specific manner. Peroxide tone is low in the CNS and high in cells at peripheral sites of inflammation.

However, to date, no clear correlation has emerged between the inhibition of a specific cyclooxygenase and acetaminophen's mechanism of action, yet whatever its mechanism, acetaminophen does not cause the negative gastrointestinal (GI), renal, or bleeding (platelet) side effects associated with the COX-1 inhibition of NSAIDs.

Cannabinergic Effects
Another possible mechanism involves cannabinoid (CB) receptor, CB1 antagonism. Högestätt and colleagues recently published an elegant series of experiments that proposes an explanation for inhibition seen on the analgesic effect of acetaminophen with specific CB1 antagonists (Högestätt-2005). They discovered an inactive metabolite of acetaminophen in the rat that after reacting with arachidonic acid forms N-arachidonoylphenolamine (AM404). AM404 acts as a potent transient receptor potential vanilloid type 1 (TRPV1) agonist.

The results of their studies suggest that acetaminophen goes through a two-step transformation, with oxidative deacylation in the liver to form *p*-aminophenol, followed by fatty acid amide hydrolase (FAAH) catalyzed conjugation in the CNS to form AM404. This active metabolite then stimulates vanilloid receptors (TRPV1) to increase the activity of endogenous cannabinoids and to exert anti-nociceptive and hypothermic effects. Inhibitor studies suggest that the effects are mediated by CB1 receptors. A possible interaction between cannabinoid and PG effects is suggested by the fact that AM404 inhibits PGE2 production by isolated PGHS-1 and PGHS-2 (COX-1 and COX-2) (Högestätt-2005).

Serotonerigic Effects
Serotonin or 5-hydroxytryptamine (5-HT) receptors are broadly located on nerve and smooth muscle cells. Three receptor subtypes are involved in serotonin modulation of mood, body temperature, and sleep (5-HT1A), appetite and metabolism (5-HT2), and nausea/emesis (5-HT3).

Early research implicated serotonin pathways in the anti-nociceptive effects of acetaminophen. These studies included the finding of decreased responses to acetaminophen in rats following injury to the serotonergic bulbospinal pathways (Tjølsen-1991), inhibition of the antinociceptive activity of acetaminophen when serotonin was depleted in the CNS (Pini-1996), and an acetaminophen stimulated increase in the release of serotonin from rat brain slices at clinically relevant concentrations (100 μM or 15 mg/kg) (Chen-2003).

The specificity toward serotonin receptors antagonized by acetaminophen has been widely investigated, and suggests that acetaminophen may exert analgesic effects through the 5-HT2 receptor (Ruggieri-2008). Other investigators have implicated the 5-HT1A receptor (Bonnefont-2003), the 5-HT3 receptor (Pelissier-1996), and the 5-HT4 receptors (Arranz-1998).

Serotonergic neurons are part of a pathway that is anatomically coextensive and interactive with the opioid-mediated pain modulatory circuit. It is interesting that

antagonist blockage of the μ- and κ-opioid receptors reverses the increase in serotonin levels and serotonergic turnover induced by acetaminophen (Ruggieri-2008).

In conclusion, central serotonin-related pathways and centers appear to be involved in the antinociceptive properties of acetaminophen, at least in animal models. However, the neural effects are complex, and may also involve cannabinoid and opioidergic pathways. In Cadence-sponsored clinical studies, the use of 5-HT3 antagonist anti-emetic agents did not compromise the analgesic efficacy of acetaminophen.

Other Potential Mechanisms
Other experimental evidence suggests the involvement of opioid receptors, such as the μ1 and κ-receptors (Ruggieri-2008), modulation of the analgesic effect by N-methyl-D-aspartate (NMDA), an excitatory amino acid receptor complex (Björkman-1995), and an inhibitory action on spinal nitric oxide mechanisms (Björkman-1995, Björkman-1994, Piletta-1991).

Drug activity related to proposed indication:
The use of IV acetaminophen in the proposed indication of the treatment of acute pain and fever in adult and pediatric patients is supported through the various mechanisms of action of acetaminophen, including a central site of action, prostaglandin inhibition, and cannabinergic and serotonergic effects. In addition, recent studies suggest that transformation of acetaminophen in the CNS to form AM404 can then stimulate vanilloid receptors (TRPV1) to increase the activity of endogenous cannabinoids and to exert anti-nociceptive and hypothermic effects.

2.6.2.3 Secondary pharmacodynamics
According to the Sponsor, "[l]iterature review shows that acetaminophen exerts no discernible adverse secondary effects on the major peripheral organ systems at therapeutic doses" and may exert beneficial effects on various organ systems.

The Sponsor presents the beneficial gastric and cardiac effects of acetaminophen:

... Gastric Effects
Acetaminophen protected against mucosal damage caused by a variety of gastro-toxic agents. At doses of 15-250 mg/kg, acetaminophen protected male and female rats against erosive gastric injury produced by aspirin in a dose-dependent manner without influencing the rapid decrease in histamine-stimulated gastric acid output that follows exposure to aspirin (Seegers-1978, Seegers-1979). Acetaminophen also antagonized gastric injury caused by naproxen, indomethacin, aspirin, and ethanol in rats (Ivey-1978, Trautmann-1991) and the acute gastric mucosal injury produced by ischemia and reperfusion (Nakamoto-1997). These findings suggest that acetaminophen should be safe to use in patients with active gastric disease.

... Cardiac Effects
Investigators explored the effects of acetaminophen in the post-ischemic, reperfused myocardium in dogs. Infarct size was significantly reduced by acetaminophen at 375 mg IV given before the onset of ischemia and 375 mg IV given 90 minutes after reperfusion (equating to 30 mg/kg/day total) (Merrill-2004). Other researchers examined acetaminophen effects in both large (ovine) and small (rabbit) collateral-deficient animal models to study the reperfused myocardium. Acetaminophen had no adverse effect on

any cardiac parameter examined post-infarction and post-reperfusion (Leshnower-2006). In canine myocardium, acetaminophen at 15 mg/kg IV protected against hydrogen peroxide-induced oxidative injury (Jaques-Robinson-2008).

In light of the increased risks associated with NSAID use before and after acute myocardial infarction (MI) or decreased survival after an acute MI with the use of NSAIDs and COX-2 inhibitors, the demonstration of safe use of acetaminophen on the cardiovascular system in animal MI models suggests that it should be safe to use in patients with heart disease...

2.6.2.4 Safety pharmacology

According to the Sponsors, "acetaminophen is not considered to be toxic to the cardiovascular, respiratory, GI, endocrine, immune, or central nervous systems" at doses below the hepatotoxic threshold. In fact, "[a]t doses below the hepatotoxic threshold, there is little or no evidence of secondary adverse pharmacology in animal modes or humans". The following table is representative of the studies in various organ systems:

Table 7. Animal Model Systems Tested with No Adverse Effects from Acetaminophen

Organ System Evaluated	Species	Tested with no effect (Doses below hepatotoxic threshold)	Reference
Cardiovascular	Rat	Systemic vascular bed	Nossaman-2007
Respiratory	Rat	Pulmonary vascular bed	Nossaman-2007
Immune	Human peripheral monocytes	Phagocytic activity *in vitro*	Klein-1982
	Human polymorphonuclear leucocytes	Degranulation and migration *in vitro*	Kankaanranta-1994
Renal	Rats	Ethanol diuresis	Morato-1992
		Excretion of water, creatinine, electrolytes or prostaglandins	Muth-Selbach-1999
Gastrointestinal	Rabbits	Gastric acid secretion	Levine-1991
	Rats	Synthesis of PGE_2 by gastric mucosal and parietal cells	Poon-1988
	Dogs	Gastric mucosal blood flow and acid secretion	Bennet-1977
Nervous	Rats	Action potential of isolated phrenic nerve	Brodin-1987
		Glutathione reserves in brain	Bien-1992

The following is a summary of the animal and *in vitro* studies done on the safety pharmacology of acetaminophen from the Sponsor:

Acetaminophen effects observed on the GI system in animal studies were determined to be of little significance (Bennett-1977, Nakamoto-1997).

There are no significant CNS toxic effects of acetaminophen administered to animals below the threshold hepatotoxic dose.

The effect of acetaminophen on responses to vasoactive agents was investigated in a rat model of pulmonary and systemic vascular bed functions (Nossaman-2007). The

administration of acetaminophen, in doses of 10, 25, and 50 mg/kg IV, did not alter responses to injections of arachidonic acid or norepinephrine indicating a lack of effects of acetaminophen in the cardiovascular system.

At levels toxic to the liver, acetaminophen depressed the respiratory rate in rats (Sewell-1984), but in fetal lambs high doses had an opposite effect, reducing the plasma concentrations of PGE2 and increasing breathing moments (Walker-1998).

Acetaminophen had no effects on *in vitro* assays of human immune cell functions, such as phagocytic activity of peripheral monocytes (Klein-1982), degranulation and migration of human polymorphonuclear leucocytes (Kankaanranta-1994), adherence (Farber-1992), or stimulation of cytokine production (Griswold-1993).

Abuse liability:
There was no information with regards to the abuse liability of acetaminophen submitted from the Sponsor. Acetaminophen is not a scheduled drug.

2.6.2.5 Pharmacodynamic drug interactions
See Pharmacokinetic drug interactions below.

2.6.3 PHARMACOLOGY TABULATED SUMMARY
[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

None.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary
The pharmacokinetics/toxicokinetics of acetaminophen have been well studied in the published literature. The Sponsor summarizes the PK studies in various animal species after IV administration of acetaminophen (see Table 8).

Table 8. Pharmacokinetic Parameters in Animals after IV Acetaminophen

Species	Dose (mg/kg)	Clearance (mL/min/kg)	Volume of Distribution (L/kg)	Terminal Half-life (min)	Reference
Rat	15 30 150 300	44.0 42.3 9.3 6.4	1.02 0.88 0.83 1.13	16.4 14.8 61.7 122.0	Galinsky-1981
Dog	150	4.04	0.6	107	St Omer-1984
Sheep	15	15.3	0.90	58.2	Wang-1990
Horse	10	4.84	0.83	118.0	Engelking-1987

As shown in the table above, the volume of distribution is approximately 1 L/kg across species; however, the clearance and terminal half-life varies across species. The Sponsor

notes that "[i]n the rat, clearance has been shown to decrease at high doses as metabolism by sulfation saturates and half-life increases".

For comparison, the terminal elimination half life in human is 2 to 3 hours in children, adolescents, and adults.

2.6.4.2 Methods of Analysis

[see under individual study reviews]

2.6.4.3 Absorption

The Sponsor has provided a literature review of the absorption of acetaminophen. Oral acetaminophen is absorbed from the GI tract primarily by non-ionic diffusion (Bagnall-1979). The Sponsor notes that "[t]he best method of estimating oral absorption of acetaminophen is to quantify its excretion as unchanged drug and its various metabolites in urine." Accordingly, "nearly 100% of an oral dose is absorbed in rats (Miller-1974), mice (Corcoran-1984), rabbits (Nakamura-1987), cats, and dogs (Savides-1984, Podder-1988)" and that "[t]here is a good correlation between the extent of absorption of acetaminophen in animals and humans (Chiou-1998, Goicoechea-1998)".

Moreover, the Sponsor believes that "[g]iven the near complete absorption from the GI tract across multiple species, it is unlikely that the route of administration will alter the fundamental pharmacological or toxicological properties of acetaminophen". The Sponsor notes that "[i]n humans, PO acetaminophen is 93% bioavailable, indicating nearly complete oral absorption after accounting for first-pass hepatic metabolism. The first dose area under the concentration versus time curve (AUC) for oral acetaminophen is similar to that for the same dose given intravenously, indicating similar overall systemic exposure".

2.6.4.4 Distribution

The volume of distribution for acetaminophen is approximately 1 L/kg (see Table 8). The Sponsor notes that "[i]n rats, the plasma and brain concentrations of acetaminophen were similar to each other and to concentrations in other tissues (Green-1984, Fischer-1981)" and that "[t]he binding of acetaminophen to plasma proteins ranged from only from 10% to 25% (Clissold-1986)". Moreover, "[i]n rats, acetaminophen will bind to red blood cells in the circulation; this can reduce the rate of entry into liver cells and affect the rate of sulfate conjugation (Pang-1995)" however, "for humans, there is no appreciable binding of acetaminophen to red blood cells (Gazzard-1973)".

2.6.4.5 Metabolism

The metabolism of acetaminophen occurs predominantly in the liver in all species. According to the Sponsor, "[a] correlation has been found between species sensitivity to the hepatotoxicity of acetaminophen and the balance between two key metabolic pathways: (1) conjugation to glucuronide and sulfate esters, which form non-toxic metabolites that are excreted in the urine, and (2) CYP450 mixed function oxidase activation to form a reactive intermediary, NAPQI, which rapidly reacts with glutathione to form nontoxic glutathione, cysteine and mercaptate conjugates. At sufficiently high

doses of acetaminophen, glutathione is depleted and the reactive metabolite binds covalently to cell macromolecules, causing cellular and tissue damage". Moreover, the Sponsor believes "[t]he relative contribution of the toxic and nontoxic pathways across animal species can be inferred from the amount of metabolite from each found in the urine after a dose of acetaminophen. The major elimination pathway for many species, including humans, is glucuronidation. Sulfation is the dominant form of metabolism in cats and rats. The mouse and hamster exhibit extensive metabolic activation as evidenced by high levels of cysteine and mercapturate conjugates (>15% for both)".

The following figure illustrates the metabolism of acetaminophen (reproduced from Bertolini-2006):

NOTE: Paracetamol = acetaminophen

As shown in the figure above, acetaminophen is metabolized by various mechanisms. Glucuronic acid conjugation is the predominant mechanism of acetaminophen metabolism in adults. Forty to 67% of acetaminophen is metabolized via glucuronic acid conjugation. Sulphuric acid conjugation, which is predominant in fetal and early life, accounts for 20-46% of acetaminophen metabolism. Five to 15% of acetaminophen is converted to the toxic metabolite, N-acetyl-p-benzoquinoneimine (NAPQI), via P450 enzymes. Under conditions of normal levels of glutathione and therapeutic levels of acetaminophen, NAPQI binds to glutathione to form non-toxic conjugates. However, under conditions of depletion of glutathione or overdosage of acetaminophen, NAPQI can lead to cellular toxicity. Three percent of acetaminophen is metabolized via cysteine

conjugation, leading to safe urinary excretion. 1-2% of acetaminophen undergoes biliary excretion. One to 4% of acetaminophen is excreted unchanged in urine. Acetaminophen can also be deacetylated to p-aminophenol in the liver, which is then conjugated with arachidonic acid via enzymatic reaction by fatty acid amide hydrolase (FAAH) to form N-arachidonoyl-phenolamine (AM404) in the CNS. The function of AM404 is discussed above in the mechanism of action section. It is not clear what percentage of acetaminophen is deacetylated to p-aminophenol in the liver, what percentage of p-aminophenol is in the circulation, or what percentage of p-aminophenol is converted to AM404 in the CNS.

However, according to Bertolini, "after intraperitoneal injection of 300 mg/kg of acetaminophen, the AM404 levels in the brain were 10.3 ± 1.9 pmol/g" (2006). This represents at least 0.00000343% of acetaminophen is converted to AM404.

It is important to note that p-aminophenol (PAP), which is also called 4-aminophenol (4-AP), is an impurity in the DS and a degradant in the DP. Based on the information above, there are no clear data to suggest that p-aminophenol is a major metabolite of acetaminophen.

The Sponsor provides further details regarding the metabolism of acetaminophen in the nonclinical overview:

> **… Sulfate Conjugation of Acetaminophen**
> Phenolsulfotransferase acts upon acetaminophen in the liver to produce sulfate conjugates in a high-affinity, low-capacity, saturable elimination system (Klaassen-1997). The rate of sulfation is limited by the availability of inorganic sulfate (Seo-1999). Sulfation predominates in the human fetus and newborn, with a shift to the adult pattern (predominance of glucuronidation) during infancy …
>
> **…Glucuronide Conjugation of Acetaminophen**
> UDP-glucuronosyl-transferase (UGT) acts upon acetaminophen to produce glucuronide conjugates in a low affinity, high capacity system that is inducible in many species. Glucuronide conjugates are the major urinary metabolite of acetaminophen in humans (Prescott-1980) and many animal species (Wong-1981, Smolarek-1990, Altomare-1984).
>
> Glucuronide conjugation is not saturable in isolated perfused rat liver or with isolated dog hepatocytes, even at high acetaminophen concentrations (Grafström-1979, Bolcsfoldi-1981).
>
> *In vivo,* no evidence of saturation of glucuronidation is found in mice as the dose increases from 50 to 400 mg/kg (Miners-1984).
>
> When human volunteers were given acetaminophen at a high dose of 8 g/day, drug clearance increased over a few days, and a hepatoprotective upregulation of glucuronidation occurred (Gelotte-2007).
>
> **… Oxidation of Acetaminophen**
> It is accepted that the majority of acetaminophen toxicity may be attributed to the formation by P450 CYP(s) of a reactive intermediate, NAPQI, via a direct two electron

transfer mixed function oxidase reaction (reviewed in Prescott-2001, Chapter 6). When hepatic glutathione stores are adequate, NAPQI rapidly reacts with reduced glutathione to form nontoxic glutathione cysteine, and mercaptate conjugates. At sufficiently high doses of acetaminophen, glutathione is depleted and the reactive metabolite binds covalently to cell macromolecules, causing cellular and tissue damage.

… Liver Cytochromes P450

The sensitivity to hepatic toxicity of acetaminophen in animal models can be increased by treatments that enhance the activity of any of several P450 isoenzymes, and can be decreased by P450 inhibitors. The identity of the most important isoenzymes for the formation of NAPQI may vary by species and by physiological or pharmacological conditions, so extrapolation of animal data to humans must be done with caution. CYP2E1 appears to play a major role in the microsomal bioactivation of acetaminophen under most conditions and in most species, including humans. …

When glutathione is depleted, NAPQI reacts covalently with proteins to cause cellular damage. Covalent reaction with liver proteins by NAPQI is not random and occurs selectively in relation to specific target proteins. However, the identity of the protein targets responsible for hepatocellular damage remains to be determined. Nonetheless, this damage eventually leads to apoptosis and necrosis (Cohen-1997). …

Administration of toxic amounts of acetaminophen by any route can deplete the available hepatic glutathione. Starvation or inadequate dietary intake of precursors can reduce hepatic stores of glutathione.

2.6.4.6 Excretion

The Sponsor has described the excretion of acetaminophen as follows:

> The main route of excretion of acetaminophen and its metabolites is renal. It undergoes glomerular filtration followed by extensive tubular reabsorption. Excretion is independent of urinary pH but appears to be weakly correlated with urine flow rate. Recovery of drug dose in the urine approaches 100% when all major and minor metabolites are quantified.

Moreover, "[a]cetaminophen is excreted in breast milk". The Sponsor describes the excretion of acetaminophen in breast milk in humans and animals as follows:

> Intravenous acetaminophen given to lactating rabbits resulted in acetaminophen concentrations in milk that paralleled concentrations in serum with a milk to serum acetaminophen concentration ratio of 0.58 (McNamara-1991). However, acetaminophen is excreted in human breast milk at levels typically between 0.1 – 2% of the orally administered dose, and peak levels occur at about 2 hours and are not detected at 12 hours (Berlin-1980, Bitzén-1981, Notarianni-1987).

2.6.4.7 Pharmacokinetic drug interactions

The following table is a representation of compounds that modulate levels of acetaminophen-glutathione conjugates (Prescott-2001):

Table 9: Drugs that Alter Levels of Acetaminophen-Glutathione Conjugates

Source: Prescott 2001, Chapter 16, pp 519-526.

The Sponsor further notes the effects of alcohol, paclitaxel, troglitazone, retinol, and doxapram on acetaminophen metabolism and toxicity.

> Effects of alcohol on acetaminophen metabolism and toxicity are complex, and include P450 induction, competitive inhibition, and reduction of glutathione stores. Chronic ethanol feeding in rats increased hepatic CYP2E1 activity, and selectively depleted mitochondrial reduced glutathione (GSH) (Zhao-2002). However, these effects disappeared when ethanol was withdrawn.
>
> A review of the recent scientific literature shows only limited examination of other drugs that may exert an effect on the PK behavior of acetaminophen through regulation of P450 enzymes. These include the anticancer drug, paclitaxel (Kostrubsky-1997) and a drug to improve glycemic control in type 2 diabetes, troglitazone (Li-2002), both of which affect CYP3A3/4 systems.
>
> Another drug, retinol, appears to exert an influence on acetaminophen-induced hepatotoxicity by reducing glucuronidation and causing a build-up of acetaminophen in the liver, leading to increased production of the toxic metabolite and hepatic injury (Bray-2001). In one more case, a drug to treat respiratory insufficiency, doxapram, was shown to potentiate acetaminophen-induced hepatotoxicity in both mouse primary cultured hepatocytes and in mice (Kanno-2000). However, no mechanistic explanation was proposed.

2.6.4.8 Other Pharmacokinetic Studies

The Sponsor summarized repeat dose tolerance studies published in the literature and is presented below:

> Rats and mice may develop tolerance to the toxic effects of high doses of acetaminophen. Rats appear to adapt by developing a protective enhanced glutathione repletion, through up-regulation of glucose-6-phosphate dehydrogenase and glutathione reductase activities (O'Brien-2000). Tolerance to the cytotoxicity of acetaminophen was also observed in cultured mouse fibroblasts maintained in exponential growth following sequential exposures to acetaminophen at 9-270 mg/L over a period of three weeks (Clothier-1997). Protective pretreatment with acetaminophen in mice resulted in the centrilobular localization of protein adducts, selective down-regulation of CYPP450, CYP2E1, and CYP1A2, increased hepatic glutathione, and increased hepatocellular proliferation (Shayiq-1999).

2.6.4.9 Discussion and Conclusions

See overall conclusions and recommendations below.

2.6.4.10 Tables and figures to include comparative TK summary

None.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

None.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

Hepatotoxicity

According to the Sponsor, "the most studied toxic effect of high doses of acetaminophen is hepatotoxicity".

As shown in figure 1 above, acetaminophen can be converted to the toxic metabolite, N-acetyl-p-benzoquinoneimine (NAPQI), via P450 enzymes. "At sub-toxic doses, this molecule is rapidly conjugated with glutathione and eliminated in urine. However, at toxic doses of acetaminophen, glutathione is eventually depleted, and NAPQI reacts with critical liver proteins. The covalent binding of NAPQI was demonstrated in studies with precision-cut rat liver and kidney slices (Brittebo-1997). Such covalent binding is an essential component of acetaminophen hepatotoxicity (Nelson-1995)".

The Sponsor has detailed the typical microscopic findings of hepatotoxicity in the following passage:

> Histological studies of acetaminophen-induced hepatic necrosis in rats after single oral doses of 2,500 and 3,500 mg/kg have detailed the typical microscopic findings of hepatotoxicity (Dixon-1971). In animals dying within 24 hours, there was loss of basophilic granules in the cytoplasm of centrilobular hepatocytes, with fine hydropic vacuolation and mild to moderate sinusoidal congestion. When death occurred between 24-48 hours, there was marked hepatic congestion with dilatation of the central vein and disruption of the surrounding sinusoids that were packed with red blood cells. Necrosis of centrilobular cells was advanced with nuclear disintegration, pyknosis, karyorrhexis, and cytoplasmic eosinophilia. The structure of the liver was largely preserved but in some areas eosinophilic anuclear cells coalesced to form amorphous masses. The extent of necrosis varied from small foci surrounding the central vein to confluent areas involving the centrilobular and midzonal regions. In more severely affected animals, hydropic vacuolation spread into periportal hepatocytes and bridging necrosis extended to the remaining areas so that only the portal tracts were preserved amid an amorphous mass of necrotic hepatocytes and red blood cells. These findings have been confirmed in mice, hamsters, cats, sheep, rabbits, dogs, and pigs (Savides-1984, Baumik-1995, Vagena-1998, Xue 1999).

Other Observed Toxicities

The Sponsor has also summarized other toxicities observed in animals given acetaminophen at doses above the hepatotoxic threshold.

… Methemoglobulinemia
In animals receiving high doses of acetaminophen, methemoglobinemia, a form of hemoglobin that binds a water molecule instead of oxygen, is sometimes observed. While acetaminophen has been shown to cause methemoglobinemia in a number of animal models (rats, cats, dogs, and guinea pigs), it has not been shown to do so in humans even in the presence of oral acetaminophen overdose (Jones-1997).

… Platelet Effects
Acetaminophen does not affect platelet numbers or function in humans, probably due to their high intracellular peroxide tone. *In vitro*, exposure to very high levels of acetaminophen (150 mg/L) can inhibit aggregation of human platelets in response to arachidonic acid or collagen, but the clinical significance is uncertain (Shorr-1985). Similar results were obtained with equine platelets *in vitro* (Heath-1994). These findings contrast with NSAIDs, which are known to inhibit the synthesis of thromboxane, interfere with platelet function, and cause clinical bleeding events (Knijff-Dutmer-2002).

… Renal Effects
The kidney contains relevant CYP450 isoforms, and some local metabolic activation of acetaminophen to NAPQI may occur. In animal models, renal effects usually occur with doses of acetaminophen that exceed the hepatotoxic threshold (Zenser-1978, Martin-1994).

… Gastrointestinal Effects
Gastrointestinal mucosal inflammation, hemorrhage and edema have been observed in rats and dogs following single high toxic doses of oral or lavage acetaminophen (Boyd-1966, Piperno-1978), but at nontoxic doses acetaminophen effects observed on the GI system in animal studies were determined to be minor and of little significance (Bennett-1977, Nakamoto-1997).

… Central Nervous System Effects
There are no significant CNS toxic effects of acetaminophen administered to animals below the threshold hepatotoxic dose. Central nervous system effects that occurred at higher or lethal doses include depression, listlessness, hypo-reactivity, lethargy, weakness, loss of righting reflex, tail extension, ataxia, over-reaction to noise, tremor, somnolence, stupor, coma, and convulsions described in high dose acute toxicity studies in mice, rats, cats, dogs, and pigs (Boyd-1966, Mitchell-1973, Finco-1975, Savides-1984, Francavilla-1989).

… Cardiovascular and Respiratory Effects
In the isolated rat heart, acetaminophen decreased the output of PG in response to arachidonic acid and reduced the duration of coronary vasodilatation (Shaffer-1981). It took toxic doses to depress the respiratory rate in rats (Sewell-1984), but in fetal lambs high doses had an opposite effect, reducing the plasma concentrations of PGE2 and increasing breathing moments (Walker-1998).

Genetic toxicology:
Acetaminophen is not mutagenic (as demonstrated by negative results in the Ames test) but has tested positive as a clastogen (as demonstrated by positive results in the chromosomal aberrations assay).

Carcinogenicity:
The results of the NTP 2-year carcinogenicity studies in rodents showed that there was no evidence of carcinogenic activity of acetaminophen in male F344/N rats, there was equivocal evidence of carcinogenic activity in female rats based on increased incidences of mononuclear cell leukemia, and there was no evidence of carcinogenic activity in male and female B6C3F1 mice (NTP-1993).

Reproductive toxicology:
Testicular atrophy and reduction in testis weight was noted in fertility studies of acetaminophen performed on rats. There were no fetal morphological abnormalities but dose-dependent microscopic lesions of the maternal liver and kidney in rats as well as retarded growth and abnormal sperm in F_1 mice and reduced birth weight of F_2 pups in embryo and teratogenicity studies done in rodents.

Special toxicology:
Acetaminophen did not induce a withdrawal opiate-like syndrome in a withdrawal test in mice.

2.6.6.2 Single-dose toxicity
The Sponsor did not submit any single-dose toxicity studies.

According to the Sponsor, "[t]here is a very large amount of literature on the nonclinical toxicology of acetaminophen administered orally (usually in feed or by lavage), but solubility and formulation challenges have limited the number of studies using the IV or IP routes". The Sponsor has provided a table for the LD_{50} of acetaminophen in a number of common experimental species (see Table 10).

Table 10. Single Dose LD_{50} of Acetaminophen in Various Species

Species	Route	LD_{50} (mg/kg)	Reference
Rat	PO	>4000	Boyd-1968
	IP	1600	Mancini-1980
Mouse	PO	900-1200	Guash-1990
	IP	800	Mancini-1980
Guinea pig	PO	3500	Boxill-1958
Rabbit	PO	3000	Berkovitch-1999
Dog	PO	200	Savides-1984
Cat	PO	120	Villar-1998

IP = intraperitoneal; LD_{50} = lethal dose of a compound for 50% of animals exposed; PO = oral

2.6.6.3 Repeat-dose toxicity
The Sponsor submitted two older repeat-dose toxicity studies with this NDA. These two older repeat-dose toxicity studies involve treatment with PERFALGAN® (1000 mg/100 mL of paracetamol or acetaminophen). NOTE: PERFALGAN is the name of an injectable acetaminophen drug product owned by Bristol Myers Squibb and approved overseas. It has not been approved in the United States. Further, the current NDA

contains the original study reports, since this drug product was purchased from BMS by Cadence. There are no patent certification issues associated with this reference that would preclude approval of the product as a 505(b)(2) application.

Study title: 2-Week Tolerance Study of PERFALGAN® Administered 4 Times a Day as 15-Minute Intravenous Infusion in Rats Followed by a 2-Week Treatment-Free Period.

Key study findings:

- Sprague-Dawley rats were administered 0, 80, and 200 mg/kg/day of acetaminophen by IV infusion as a 15-minute infusion for 2 weeks.
- Induration was observed in 2 males and females from the low dose group compared to 3 males and 2 females in the control group.
- Greenish contents at infusion sites 2 and 3 were observed in 1 female from the low dose and 2 females from the high dose treatment groups. This observation was reversed in infusion site 2 recovery animals but was not reversed in high dose recovery females in infusion site 3. Moreover, greenish contents at infusion site 3 were observed in 1 male from the low dose recovery group.
- The Sponsor did not explain the presence and significance of greenish contents at the infusion site. A NOAEL cannot be established for this finding and this study cannot be used in support of this NDA.
- The 28-day repeat-dose toxicity is the more relevant study in support of this NDA.

Study no.: 20455 TSR (laboratory study number)
Volume #, and page #: This is an electronic submission (1 volume)
Conducting laboratory and location: (b) (4)
Date of study initiation: August 22, 2000 (males); August 31, 2000 (females)
GLP compliance: Yes
QA report: yes (X) no ( ) Signatures were provided on December 7, 2000.
Drug, lot #, and % purity:
Paracetamol (acetaminophen, (b) (4)); FD 00007/05925; complies with specifications (no information on purity).

Methods

Doses: 0, 80, and 200 mg/kg/day PERFALGAN®
Species/strain: Rats/Sprague-Dawley
Number/sex/group or time point (main study): 10/sex/group
Route, formulation, volume, and infusion rate: Continuous intravenous infusion, four times a day at 6-hour intervals. Formulated with 0.9% NaCl solution. Volume was 20 mL/kg/day). Infusion rat was 0.2 mL/h. This formulation

appears to be identical to the formulation of this NDA (although the specification is in French with a partial translation to English).

Satellite groups used for toxicokinetics or recovery: 5/sex/group for recovery
Age: 12 weeks old
Weight: 370 g (range of 329 to 426 g) for males;
228 g (range of 202 to 257 g) for females
Sampling times: See observations and times below.
Unique study design or methodology (if any): This is a standard repeat-dose toxicity study.

NOTE: PERFALGAN® is an intravenous formulation of acetaminophen and will be referred to as IV acetaminophen. PRO-DAFALGAN® is a prodrug formulation of acetaminophen and does not represent the acetaminophen formulation used in this NDA. As such, the results obtained using PRO-DAFALGAN® will not be considered.

Results

Mortality:
All animals were checked for morbidity and mortality twice daily.

There were no deaths in animals from the 200 mg/kg/day IV acetaminophen group. One female from the 80 mg/kg/day IV acetaminophen group was found dead and another female from the same group was sacrificed early on days 18 and 25, respectively.

The pathology report for the female found dead on day 18 concluded that the cause of death was from the moderate septic embolus observed in the microscopic examination of the heart. According to the pathology report, the finding is not treatment related and is believed to be a result of the permanent venous cannulation used to deliver IV acetaminophen.

The pathology report for the female sacrificed early on day 25 noted poor clinical condition such as piloerection, round back and emaciation, pallor of the eyes and extremities, cold to touch, hypokinesia, and enophthalmos, macroscopic changes such as a reduction in size of the spleen and thymus, and histopathology changes such as atrophy of the bone marrow. The histopathology report noted that the death in this female was not clearly identified.

Clinical signs:
All animals were observed at least once a day for clinical signs.

The incidence of induration along the catheter or at infusion site is illustrated in the following table:

Table 11. Incidence of Induration

Dose-level (mg/kg/day)	0		0		160		80		200	
	(NaCl)		(PLACEBO)		(PRO-DAFALGAN®)		(PERFALGAN®)			
Sex	M	F	M	F	M	F	M	F	M	F
Number at start	15	15	15	15	15	15	15	15	15	15
Induration	3	2	0	0	5	3	2	2	0	0
Number of animals without clinical signs	12	12	14	12	10	9	13	11	11	9

As shown in the Table 11 above, induration was observed in 2 males and females from the 80 mg/kg/day IV acetaminophen group, as well as in 3 males and 2 females from the vehicle placebo control group. Induration was not observed in any of the animals from the placebo control group. Due to the lack of a dose-dependency, induration is not considered a test substance related change.

There were no other relevant treatment related changes in clinical signs.

Body weights:
The body weights of each animal was recorded once prior to grouping, on the day before the beginning of the treatment period, and on days -1, 4, 8, 15, 22, and 29. There were no relevant treatment related changes in body weight in both the treatment and recovery groups.

Food consumption:
Food consumption was measured once weekly. There were no relevant treatment related changes in food consumption.

Ophthalmoscopy:
Ophthalmological examinations were performed on all animals before the beginning of the treatment period and on weeks 2 and 4. There were no relevant treatment related changes seen in ophthalmological examinations.

EKG:
An EKG was not performed.

Hematology:
All animals were deprived of food prior to blood sampling. Hematology was determined during weeks 3 (end of treatment period) and 4 (end of recovery period). The hematology parameters that were determined included erythrocytes, hemoglobin, mean cell volume, packed cell volume, mean cell hemoglobin concentration, mean cell hemoglobin, thrombocytes, leucocytes, differential white cell count with cell morphology (including neutrophils, eosinophils, basophils, lymphocytes, and monocytes), reticulocytes, prothrombin time, activated partial thromboplastin time, and fibrinogen.

There were no relevant treatment related changes seen in hematology.

Clinical chemistry:
All animals were deprived of food prior to blood sampling. Clinical chemistry was determined during weeks 3 (end of treatment period) and 4 (end of recovery period). The clinical chemistry parameters included sodium, potassium, chloride, calcium, inorganic phosphorous, magnesium, glucose, urea, creatinine, total bilirubin, direct bilirubin, total proteins, albumin, albumin/globulin ratio, cholesterol, triglycerides, alkaline phosphate, aspartate aminotransferase, alanine aminotransferase, creatine kinase, and gamma-glutamyl transferase.

There was a slight increase in the sodium ion and chloride ion levels in the 200 mg/kg/day IV acetaminophen groups but these levels recovered in the recovery animals. There were no other relevant treatment related changes in clinical chemistry.

Urinalysis:
All animals were deprived of food and were placed in individual metabolism cages for an overnight period of at least 14 hours during urine collection. Urinalysis was performed during week 3 (end of the treatment period). The Sponsor notes that there were no relevant changes in urinary parameters and thus no further urinalysis was carried out to week 4 (end of recovery period). The urinalysis parameters included volume, pH, specific gravity, proteins, glucose, ketones, bilirubin, nitrates, blood, urobilinogen, cytology of sediment (including leucocytes, erythrocytes, cylinders, magnesium ammonium phosphate crystals, calcium phosphate crystals, calcium oxalate crystals, and cells), appearance, and color.

There were no relevant treatment related changes in urinalysis.

Gross pathology:
Macroscopic *post-mortem* examinations were performed on all animals including examination of the external surfaces, all orifices, the cranial cavity, the external surfaces of the brain and spinal cord, the thoracic, abdominal, and pelvic cavities with their associated organs and tissues, and the neck with its associated organs and tissues.

There were no relevant treatment related changes in gross pathology.

Organ weights (specify organs weighed if not in histopath table):
See histopathology inventory table below. There were no relevant treatment related changes in organ weights.

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( ) Signature provided on December 7, 2000 (b) (4) (no other signatures were on pathology report).

Treatment and control animals had 3 infusion sites. In infusion site 1, thickened adjacent tissue was observed in 1/10 control females and in 1/10 males each from the 80 and 200 mg/kg/day IV acetaminophen groups. The observation of the thickened adjacent tissue

was reversed in the recovery animals with only 1/5 males from the 200 mg/kg/day recovery group affected. In infusion site 2, greenish contents and thickened adjacent tissue were observed. Greenish contents were observed in no control animals and in 1/11 females from the 80 mg/kg/day IV acetaminophen group and in 2/10 females from the 200 mg/kg/day IV acetaminophen. Thickened adjacent tissue was observed in 1/10 males from the control group and in 3/10 males and 3/10 females from the 80 mg/kg/day IV acetaminophen group and in 2/10 males and 3/10 females from the 200 mg/kg/day IV acetaminophen group. The observations of the greenish contents and thickened adjacent tissue were completely reversed in the recovery animals. In infusion site 3, dilatation, greenish contents, and thickened adjacent tissue were observed. Dilatation was observed in no control animals and in 1/10 males and 1/11 females from the 80 mg/kg/day IV acetaminophen group and in 1/10 male and 1/10 females from the 200 mg/kg/day IV acetaminophen group. Greenish contents were observed in no control animals and in 1/11 females and 2/10 females from the 80 and 200 mg/kg/day IV acetaminophen groups, respectively. Thickened adjacent tissue was observed in no control animals and in 1/10 males and 3/10 females from the 200 mg/kg/day IV acetaminophen group. The observations of dilatation and thickened adjacent tissue were completely reversed in the recovery animals however, greenish contents were observed in 1/5 males from the 80 mg/kg/day IV acetaminophen recovery group and in 1/5 females from the 200 mg/kg/day IV acetaminophen recovery group.

The study pathologist noted the greenish contents at the infusion site but did not offer any interpretation. It is noted that in this 2-week study, the supplier of the IV formulation of acetaminophen is (b) (4). In the 28-day study reviewed below, the supplier of the IV formulation of acetaminophen is (b) (4) and greenish contents in the infusion site were not observed. A comparison of the ingredients in the IV formulation of acetaminophen from (b) (4) and the current NDA would be helpful. The significance of greenish contents at the infusion site is unknown and the Sponsor did not explain the presence and significance of greenish contents in the pathology report. Since the Sponsor did not explain the presence of greenish contents, a NOAEL cannot be defined for this observation and this 2-week toxicity study cannot be used to support this NDA.

There were no other relevant treatment related changes in histopathology.

Toxicokinetics:
Toxicokinetic studies were not performed.

Histopathology inventory (optional)

Study	20455 TSR
Species	Sprague-Dawley Rats
Adrenals	X, *
Aorta	
Bone Marrow smear	
Bone (femur)	

Brain	X, *
Cecum	X
Cervix	
Colon	X
Duodenum	X
Epididymis	X
Esophagus	X
Eye	X
Fallopian tube	
Gall bladder	
Gross lesions	X
Harderian gland	X
Heart	X, *
Ileum	X
Injection site	X
Jejunum	X
Kidneys	X, *
Lachrymal gland	
Larynx	
Liver	X, *
Lungs	X
Lymph nodes, cervical	
Lymph nodes mandibular	X
Lymph nodes, mesenteric	X
Mammary Gland	X
Nasal cavity	
Optic nerves	
Ovaries	X, *
Pancreas	X
Parathyroid	X, *
Peripheral nerve	
Pharynx	
Pituitary	X
Prostate	X
Rectum	X
Salivary gland	X
Sciatic nerve	
Seminal vesicles	
Skeletal muscle	
Skin	
Spinal cord	
Spleen	X, *
Sternum	
Stomach	X
Testes	X, *
Thymus	X, *
Thyroid	X, *
Tongue	
Trachea	X

Urinary bladder	X
Uterus	X
Vagina	
Zymbal gland	

X, histopathology performed
*, organ weight obtained

It is noted that the aorta, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, sternum with bone marrow, tongue, and vagina were preserved. However, these tissues were neither examined histologically nor weighed. In additional, the femoral bone with articulation was preserved and examined histologically.

Thus, the 28-day repeat-dose toxicity study is the more relevant study in support of this NDA.

Study title: PERFALAGAN® 28-Day Repeat Dose Infusion Toxicity Study in the Rat followed by a 28-Day Reversibility Period.

Key study findings:

- Sprague-Dawley rats were administered 0, 80, 200, and 400 mg/kg/day acetaminophen by IV infusion at 6-hour intervals for 28 days.
- There were no histological liver changes or changes in liver enzymes or bilirubin in the treatment groups.
- NOAEL was 400 mg/kg/day.
- The maximum dose of acetaminophen in humans is 4000 mg. For an average human weighing 60 kg, the exposure margin is 0.97 based on body surface area.
- The safety margin of a single dose administration of 1000 mg of acetaminophen in humans based on the human AUC_{0-6} value and the rat NOAEL AUC_{0-6} value is 3.10.

Study no.: 980100T
Volume #, and page #: This is an electronic submission (1 volume)
Conducting laboratory and location: (b) (4)
Date of study initiation: September 14, 1998
GLP compliance: Yes
QA report: yes (X) no ( ) Signatures were provided on May 12, 1999.
Drug, lot #, and % purity: PERFALGAN® (acetaminophen, (b) (4)); lot # PMC 0397/B-8083A; complies with specifications (99.6% purity)

Methods

Doses: 0, 80, 200, and 400 mg/kg/day PERFALGAN®
Species/strain: Rat/SPF (Specific Pathogen Free) Sprague-Dawley
Number/sex/group or time point (main study): 12/sex/group

Route, formulation, volume, and infusion rate: Continuous intravenous administration, four times a day at 6 hour intervals. Formulated with isotonic saline solution. The volume varied (10, 2, 5, and 10 mL/kg/administration for 0, 80, 200 and 400 mg/kg/day PERFALGAN®, respectively). The infusion rate was 0.2 mL/h, lasting 15 minutes (0.4 mL/h of isotonic saline solution in between treatments). The formulation contains disodium edetate, which is not in the formulation of this NDA.

Satellite groups used for toxicokinetics or recovery: 10/sex/group (control and 400 mg/kg/day PERFALGAN®) for recovery

Age: Over 9 weeks
Weight: About 250 g
Sampling times: See observations and times below.
Unique study design or methodology (if any): This is a standard repeat-dose toxicity study.

NOTE: PERFALGAN® is an intravenous formulation of acetaminophen and will be referred to as IV acetaminophen.

Results

Mortality:
All animals were checked at least twice daily for mortality or signs of morbidity.
In the treatment group, the following table details the deaths of each animal:

Table 12. Lethality During the Treatment Period

GROUP	ANIMAL No.	SEX	DAY	REASON
CONTROL	982148	Male	D28	Pyelonephritis/Pulmonary septic thrombi
	982424	Male	D16	◆
PERFALGAN® 400 mg/kg/day	982176	Male	D7	◆
	982210	Female	D9	Internal bleeding
	982213	Female	D16	Cerebellar medulloblastoma
	982484	Female	D22	Pyelonephritis/Septic thrombus at the catheter site/ Embolic gastroenteritis/Encephalitis/Osteomyelitis
	982490	Female	D6	◆
	982497	Female	D7	◆

As shown in the table above, 2 males from the control group and 1 male and 5 females from the 400 mg/kg/day IV acetaminophen group died. One male from the control group died of pyelonephritis/pulmonary septic thrombi. The 3 females from the 400 mg/kg/day IV acetaminophen group died of internal bleeding cerebellar medulloblastoma, or pyelonephritis/septic thrombus at the catheter site/embolic gastroenteritis/encephalitis/

osteomyelitis. All other animals died of unknown causes although it is noted that mild changes associated with the catheter, hepatic lobar embolic damage, and some individual hepatocytic necrosis were observed in those animals . A total of 8 animals died.

There were no deaths in the recovery group animals.

There were a total of seven animals that died from the toxicokinetic study. Three males and 3 females from the 400 mg/kg/day IV acetaminophen group and 1 female from the 200 mg/kg/day IV acetaminophen group died during the course of the study. There were no gross lesions noted of these animals.

Clinical signs:
All animals were examined clinically at least once a day. Animals for all groups had wounds and scabs under the forelegs and back due to the jacket maintaining the infusion system. There were no other relevant treatment related changes in clinical signs.

Body weights:
All animals were weighed on the day of randomization, days 7, 14, 21 and 28, and on the day of necropsy. There were no relevant treatment related changes in body weights.

Food consumption:
Food consumption for all animals was measured weekly. There were no relevant treatment related changes in food consumption.

Ophthalmoscopy:
Ophthalmologic examination was performed on the day following the last treatment and on the day of necropsy. There were no relevant treatment related changes in ophthalmoscopy.

EKG:
An EKG was not performed.

Hematology:
Hematologic parameters were determined on the day following the last treatment and on the day of necropsy. Hematologic parameters included white blood cell count, differential white cell count, red blood cell counts, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet counts, reticulocytes, prothrombin time, and activated partial thromboplastin time.

Mean corpuscular volume in males increased 6.5 and 6.1% after treatment with 200 and 400 mg/kg/day IV acetaminophen, respectively. Mean corpuscular volume in females increased 3.9% after treatment with 400 mg/kg/day IV acetaminophen. However, these changes in mean corpuscular volume were reversible. Mean corpuscular hemoglobin in males increased 6.0 and 4.0% after treatment with 200 and 400 mg/kg/day IV acetaminophen, respectively. Mean corpuscular hemoglobin in females increased 4.3%

after treatment with 400 mg/kg/day IV acetaminophen. However, these changes in mean corpuscular hemoglobin were reversible. Lymphocyte counts in males decreased 18.8% after treatment with 400 mg/kg/day IV acetaminophen and remained decreased 25.3% after the recovery period. Hemoglobin in females increased 13.4% after treatment with 400 mg/kg/day IV acetaminophen and remained increased 4.0% after the recovery period. Hematocrit in females increased 12.0% after treatment with 400 mg/kg/day IV acetaminophen and remained increased 4.7% after the recovery period.

There were no other relevant treatment related changes in hematology.

Clinical chemistry:
Clinical chemistry was determined on the day following the last treatment and on the day of necropsy. Clinical chemistry parameters included alanine aminotransferase, apartate aminotransferase, total bilirubin, direct bilirubin, calcium, chloride, total cholesterol, free cholesterol, creatinine, gamma GT, glucose, alkaline phosphatase, magnesium, creatinine phosphokinase, phosphorus, potassium, sodium, triglycerides, urea, total protein electrophoresis, and αGST.

Gamma globulin in males decreased 25.5% after treatment with 400 mg/kg/day IV acetaminophen and remained decreased 16.3% after the recovery period. Gamma globulin in females decreased 25.9% after treatment with 400 mg/kg/day IV acetaminophen. However, these changes in gamma globulin in females were reversible. Alpha-1 globulin in females increased 24.4% after treatment with 400 mg/kg/day IV acetaminophen. However, these changes in alpha-1 globulin were reversible. Urea in females decreased 10.2% after treatment with 400 mg/kg/day IV acetaminophen and remained increased 10.4% after the recovery period. Potassium in females increased 4.9% after treatment with 400 mg/kg/day IV acetaminophen and remained increased 10.3% after the recovery period.

There were no other relevant treatment related changes in clinical chemistry including no changes in liver enzymes and no changes in creatinine levels, an indicator of renal damage.

Urinalysis:
All animals were placed in individual metabolism cage for 16 hours and food and water were withheld during urine collection. Urinalysis parameters included protein, glucose, ketones, urobilinogen, bilirubin, blood, pH, volume, color, and specific gravity. There were no relevant treatment related changes in urinalysis including no changes in bilirubin, an indicator of liver injury.

Gross pathology:
All animals at necropsy were subjected to examinations of the external surface, all orifices, the cranial cavity, the external surface of the brain and samples of the spinal cord, the thoracic and abdominal cavities and organs, and the carcass.

There was induration of the vein in most animals due to the method of infusion used during the study. There were no other relevant treatment related changes in gross pathology.

Organ weights (specify organs weighed if not in histopath table):
The absolute organ weight of the pituitary in females decreased 19.3% and the weight of the pituitary as a % body weight in females decreased 19.4% after treatment with 400 mg/kg/day IV acetaminophen. However, there was no change in the weight of the pituitary as a % brain weight in females and the changes in the absolute organ weight of the pituitary and the weight of the pituitary as a % body weight in females were reversible.

There were no other relevant treatment related changes in the organ weights.

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( ) Pathology report signed (b) (4) on May 10, 1999 (no other signatures were on the pathology report).

There were no relevant treatment related changes in histopathology including histological liver findings.

It is noted that the greenish contents at the infusion site was not observed in this 28-day study as they were in the 2-week study.

Toxicokinetics:
Toxicokinetic studies were performed on 3 males and 3 females per time and day of sampling and per dose level. At such sampling periods, the blood samples were collected for drug analysis. Blood samples were collected after the first administration on Day 1 and after the last administration on Day 28 at the end of the infusion (T0), and 1, 3, and 6 hours after each infusion.

The following table from the Sponsor shows the C_{max} and AUC values on Days 1 and 28 at each dose:

Table 13. Pharmacokinetic Parameters for IV Acetaminophen in Rats

		C_{max} (µg/mL)			AUC_{0-6} (µg·h/mL)		
Dose (mg/kg/day)	**Sex**	**Day 1**	**Day 28**	**Ratio**	**Day 1**	**Day 28**	**Ratio**
80	M	16.8	17.9	1.06	12.1	12.7	1.05
	F	21.3	20.0	0.94	23.4	19.9	0.85
200	M	50.9	44.6	0.88	49.1	36.5	0.74
	F	49.6	47.8	0.96	56.2	48.1	0.86
400	M	105	92.9	0.89	133	113	0.85
	F	102	98.0	0.96	143	114	0.80

Definitions: AUC = Area under the concentration versus time curve from time 0 to 6 hours postdose;
C_{max} = Maximum plasma concentration

As shown in the table above, C_{max} values range from 16.8 to 17.9 and 21.3 to 20.0 µg/mL for 80 mg/kg/day acetaminophen give to males and females, respectively. C_{max} values range from 50.9 to 44.6 and 49.6 to 47.8 µg/mL for 200 mg/kg/day acetaminophen give to males and females, respectively. C_{max} values range from 105 to 92.9 and 102 to 98.0 µg/mL for 400 mg/kg/day acetaminophen give to males and females, respectively. The Day 28 to Day 1 ratio for C_{max} ranges from 0.88 to 1.06. The AUC values range from 12.1 to 12.7 and 23.4 to 19.9 µg·h/mL for 80 mg/kg/day acetaminophen give to males and females, respectively. The AUC values range from 49.1 to 36.5 and 56.2 to 48.1 µg/mL for 200 mg/kg/day acetaminophen give to males and females, respectively. The AUC values range from 133 to 113 and 143 to 114 µg/mL for 400 mg/kg/day acetaminophen give to males and females, respectively. The Day 28 to Day 1 ratio for AUC ranges from 0.74 to 1.05, indicating that there was no accumulation of acetaminophen after repeated dosing over a 28 day period for each of the 3 dose levels.

For comparison, the AUC_{0-6} in humans is 42.9 µg·h/mL for 1000 mg of IV acetaminophen (Study CPI-APA-103). Based on the AUC_{0-6} and using the NOAEL of 400 mg/kg/day in the rats and the AUC_{0-6} in humans, the safety margin for an average human weighing 60 kg is 3.10 (if the human was given a single dose of 1000 mg/kg acetaminophen).

Histopathology inventory (optional)

Study	980100T
Species	SPF Sprague-Dawley Rats
Adrenals	X, *
Aorta	X
Bone Marrow smear	X
Bone (femur)	
Brain	X, *
Cecum	X
Cervix	
Colon	X
Duodenum	X
Epididymis	X
Esophagus	X
Eye	X
Fallopian tube	
Gall bladder	
Gross lesions	
Harderian gland	X
Heart	X, *
Ileum	X
Injection site	X
Jejunum	X
Kidneys	X, *
Lachrymal gland	
Larynx	

Liver	X, *
Lungs	X, *
Lymph nodes, cervical	
Lymph nodes mandibular	
Lymph nodes, mesenteric	X
Mammary Gland	X
Nasal cavity	
Optic nerves	
Ovaries	X, *
Pancreas	X
Parathyroid	X
Peripheral nerve	
Pharynx	
Pituitary	X, *
Prostate	X, *
Rectum	X
Salivary gland	X, *
Sciatic nerve	X
Seminal vesicles	X
Skeletal muscle	X
Skin	X
Spinal cord	X
Spleen	X, *
Sternum	X
Stomach	X
Testes	X, *
Thymus	X, *
Thyroid	X, *
Tongue	X
Trachea	X
Urinary bladder	X
Uterus	X, *
Vagina	X
Zymbal gland	

X, histopathology performed
*, organ weight obtained

In addition, the femur, tibia, and the femoro-tibial joint were collected and examined histologically.

It is noted that the Sponsor concludes there may be a limited utility of this study as there were methodological issues with the study and the IV acetaminophen formulation used in this study contains disodium edetate, which is not in the formulation of this NDA. However, control animals were subjected to the same vehicle (containing disodium edetate) as the test animals exposed to IV acetaminophen. Therefore, the disodium edetate should not have an impact on the results of this study. Further, it should be noted that this study was deemed acceptable to support the clinical program when submitted with the original IND.

2.6.6.4 Genetic toxicology

The Sponsor presents the following table to highlight the genetic toxicology testing of acetaminophen from the published literature:

Table 14: Genotoxicity Testing of Acetaminophen

Assay Type	Species	Result[1]		Reference
		(+)	(-)	
In Vitro				
Mutagenicity (Ames test)[2] ± S-9 activation	*S-typhimurium*		X	Oldham-1986, Burke-1994
Chromosome aberrations	V79 Chinese hamster cells	X		Muller-1991
DNA synthesis inhibition	Human, HL-60 cells	X		Wiger-1997
Induction of oubain resistance	Cultured mouse embryo cells		X	Patierno-1989
DNA strand breaks	Cultured human skin fibroblasts	X	X	Nordenskjöld-1983 Wilmer-1981
DNA repair	Rat hepatocytes		X	Milam-1985
Sex-linked recessive lethality	*Drosphilia*		X	King-1979
Intrachromosomal recombination	Yeast		X	Brennan-1997
In Vivo				
Chromosomal aberrations sister chromatid exchange	Mice bone marrow cells	X		Giri-1992
			X	Anantha Reddy-1985
Micronuclei formation	Mice		X	Muller-1995
	Rat	X		King-1979
DNA single strand breaks	Rat liver and kidney		X	Hongslo-1994

[1] (+) indicates potentially genotoxic effects were observed, (-) indicates genotoxic effects were not observed.
[2] *S-typhimurium* Strains TA 97, 98 100, 1535, 1537, 1538 were tested.

As shown in the table above, acetaminophen is negative in the Ames test (Oldham-1986 and Burke-1994), positive in a chromosomal aberration test (Muller-1991), and negative in the mouse micronuclei formation test (Muller-1995). However, there are mixed results with acetaminophen in assays that investigate DNA strand breaks in cultured human skin fibroblasts (Nordenskjold-1983 and Wilmer 1981) having negative and positive results, respectively, and rat micronuclei formation, the results of which was positive (King-1979).

The National Toxicology Program (1993) has also conducted genotoxicity testing on acetaminophen. The Ames test of acetaminophen is deemed valid because the investigators have used the appropriate strains of *Salmonella typhimurium* (TA100, TA1535, TA1537, and TA98). The investigators found that acetaminophen did not induce mutations in these strains. These results correspond to the negative Ames test results of Oldham-1986 and Burke-1994. In contrast, acetaminophen induced sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells. The genetic toxicity study done on Chinese hamster ovary cells was deemed valid because the appropriate number of cells in metaphase were scored (up to 200 first-division metaphase cells and 25 or 50 second-division metaphase cells were scored). It is noted that the

National Toxicology Program has included the protocols for both the Ames test and the Chinese hamster ovary cell study (1993; see Appendix E).

The Sponsor has also conducted and submitted a number of genetic toxicology studies, which are reviewed below.

Study title: Study to Evaluate the Chromosome Damaging Potential of Paracetamol by its Effects on Cultured Human Lymphocytes Using an *in vitro* Cytogenetics Assay

Key findings:

- This chromosomal aberration study was deemed valid.
- Blood cultures (lymphocytes) were obtained from a single male donor and were exposed to 26.40, 37.71, 53.87, 76.96, 109.9, 157.1, 224.4, 320.5, 457.9, 654.2, 934.5, and 1335 µg/mL acetaminophen for 20 hrs (+S-9), 3 + 17 hrs (+ and –S-9), 44 hrs (–S-9), and 3 + 41 hrs (+ S-9).
- Acetaminophen induced chromosome aberrations in cultured human peripheral blood lymphocytes.

Study no.: UBF 1/HLC

Volume #, and page #: This is an electronic submission, 1 volume
Conducting laboratory and location: (b) (4)

Date of study initiation: July 29, 1991
GLP compliance: Yes
QA reports: yes (X) no ( ) Signature was provided on May 28, 1992
Drug, lot #, and % purity:
Paracetamol (acetaminophen, (b) (4)); Lot # 0.2712; no percentage given for purity (contains (b) (4) of 4-aminophenol)

Methods

Strains/species/cell line:
Blood cultures were obtained from a single male donor. The mammalian liver post-mitochondrial fraction (S-9) used for metabolic activation was prepared from male Sprague-Dawley rats.

Doses used in definitive study:
Doses of 26.40, 37.71, 53.87, 76.96, 109.9, 157.1, 224.4, 320.5, 457.9, 654.2, 934.5, and 1335 µg/mL acetaminophen were used.

Basis of dose selection:

Preliminary solubility testing showed acetaminophen was soluble in 2% (w/v) tribasic sodium citrate at a maximum concentration of 12 mg/mL. Test chemical solutions of acetaminophen were prepared by dissolving acetaminophen in 2% (w/v) tribasic sodium citrate to give 8.9 mg/mL. Sonication was required to achieve dissolution. Test chemical solutions were used with 2.25 hours of dissolution.

According to the Sponsor's protocol for the selection of doses for cytogenetic analysis, "[t]he top dose for analysis was to be one at which a 50-80% reduction in MI occurred or 10 mM whichever was the lower. Initial analysis was to be from 20+0 hour treatments - S-9 and 3+17 hour treatments + S-9… . Slides from the selected top dose and the next 2 lower doses, plus controls (A and B) in each case, were to be analyzed".

Negative controls:
The negative control was the test chemical solvent, 2% (w/v) tribasic sodium citrate.

Positive controls:
The positive controls were 4-nitroquinoline-1-oxide (in the absence of metabolic activation) and cyclophosphamide (in the presence of metabolic activation). Both were dissolved in sterile anhydrous dimethyl sulphoxide immediately prior to use.

Incubation and sampling times:
The Sponsor provides the following tables to illustrate the incubation and sampling times:

Treatment	S-9	Number of cultures/hours			
		3+17	20+0	44+0	3+41
Untreated control	-	2	2	2	-
(all doses)	+	2	-	-	2
Negative control	-	4	4	4	-
	+	4	-	-	4
Test chemical	-	2	2	2	-
(all doses)	+	2	-	-	2
Positive controls	-	-	2	-	-
(all doses)	+	2	-	-	-

Treatment §	S-9	Duration of treatment (hours)	Harvest time after start of treatment (hours)
20 + 0 hours	-	20	20†
44 + 0 hours	-	44	44
3 + 17 hours	-	3	20
3 + 17 hours	+	3	20†
3 + 41 hours	+	3	44

§ hours treatment + hours recovery
† includes positive control treatment

As shown in the tables above, all doses of the untreated and positive controls as well as the negative control were carried out with or without S-9 activation. The human lymphocytes isolated from blood cultures were treatment for 3, 20 or 44 hours. The lymphocytes treated for 3 hours were subjected to 17 or 41 hours recovery. Only experiments with the lymphocytes exposed to 3 hours of treatment with 17 hours recovery were carried out with and without S-9 activation. The experiments with the lymphocytes treated for 20 and 44 hours without recovery were carried out without S-9 activation and the experiments with the lymphocytes treated for 3 hours and 41 hours recovery were carried out with S-9 activation.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):
The study was valid for a number of reasons (reproduced verbatim from the submission):

1. no evidence of significant heterogeneity between replicate cultures was obtained in the binomial dispersion test;
2. the proportion of cells with structural aberrations (excluding gaps) in negative control cultures fell within the normal range;
3. at least 160 cells out of an intended 200 were analyzed at each treatment level; and
4. the positive control chemicals, 4-nitroquinoline-1-oxide and cyclophosphamide, induced statistically significant increases in the number of cells with structural aberrations.

The study appears valid because of the following reasons:

1. The appropriate controls were used. 4-nitroquinoline-1-oxide was used as a positive control in the absence of metabolic activation and cyclophosphamide was used as a positive control in the presence of metabolic activation.

2. The appropriate exposure conditions were used. In this study, 3 hours treatment with 17 hours recovery time both in the absence and presence of metabolic activation and 20 hours treatment with no recovery time in the absence of metabolic activation.
3. The appropriate number of cells was scored. In this study, 160 out of an intended 200 cells were analyzed.
4. The appropriate number of cells in metaphase was used. In this study, 100 metaphases from each culture were used where possible.
5. The dose selection based upon the mitotic index (MI) was acceptable. In this study, 50-80% reduction in MI at the top dose.

Study outcome:
For 20 hours of treatment and no recovery time without S-9 activation, the following table illustrates the structural aberrations observed:

Treatment	Solvent - S-9			157.1 µg/ml - S-9			224.4 µg/ml - S-9			320.5 µg/ml - S-9			5 µg/ml NQO - S-9		
Culture	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
Cells scored	98	100	198	100	100	200	100	73	173	94	90	184	25	25	50
Gaps	0	0	0	2	0	2	7	2	9	4	3	7	2	10	12
Chr. del.	0	0	0	10	10	20	0	2	2	4	1	5	0	4	4
Chr. exch.	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Ctd. del.	0	0	0	2	5	7	3	1	4	6	2	8	13	7	20
Ctd. exch.	0	0	0	0	0	0	0	0	0	0	0	0	7	6	13
Other	0	0	0	0	0	0	0	0	0	1	0	1	2	3	5
Total inc. gaps	0	0	0	14	15	29	10	6	16	15	6	21	24	30	54
Total excl. gaps	0	0	0	12	15	27	3	4	7	11	3	14	22	20	42

As shown in the table above, the number of structural aberrations was 27, 7, and 14 structural aberrations in cultured human lymphocytes treated with 157.1, 224.4, and 320.5 µg/mL acetaminophen, respectively (for 20 + 0 hours without S-9 activation), compared to 42 structural aberrations in the positive control and no structural aberrations in the negative control. The number of structural aberrations excludes gaps. The increase in the number of structural aberrations is not dose-dependent.

For 3 hours of treatment and 17 hours recovery time with S-9 activation, the following table illustrates the structural aberrations observed:

Treatment	Solvent + S-9			654.1 µg/ml + S-9			934.5 µg/ml + S-9			1335 µg/ml + S-9			25 µg/ml CPA + S-9		
Culture	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
Cells scored	100	100	200	100	100	200	100	100	200	100	100	200	25	25	50
Gaps	4	1	5	0	3	3	2	1	3	0	1	1	7	6	13
Chr. del.	0	0	0	0	0	0	0	1	1	2	0	2	0	5	5
Chr. exch.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ctd. del.	0	0	0	1	2	3	1	1	2	1	0	1	12	6	18
Ctd. exch.	0	0	0	0	1	1	0	0	0	0	1	1	2	0	2
Other	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total inc. gaps	4	1	5	1	6	7	3	3	6	3	2	5	21	17	38
Total excl. gaps	0	0	0	1	3	4	1	2	3	3	1	4	14	11	25

As shown in the table above, the number of structural aberrations was 4, 3, and 4 structural aberrations in cultured human lymphocytes treated with 654.1, 934.5, and 1335 µg/mL acetaminophen, respectively (for 3 + 17 hours with S-9 activation), compared to 25 structural aberrations in the positive control and no structural aberrations in the negative control. The number of structural aberrations excludes gaps.

For 3 hours of treatment and 17 hours recovery time without S-9 activation, the following table illustrates the structural aberrations observed:

Treatment	Solvent - S-9			1335 µg/ml - S-9		
Culture	A	B	A+B	A	B	A+B
Cells scored	100	100	200	100	100	200
Gaps	3	1	4	1	5	6
Chr. del.	0	0	0	4	3	7
Chr. exch.	0	0	0	0	0	0
Ctd. del.	1	1	2	2	3	5
Ctd. exch.	0	0	0	0	0	0
Other	0	0	0	0	0	0
Total inc. gaps	4	2	6	7	11	18
Total excl. gaps	1	1	2	6	6	12

As shown in the table above, the number of structural aberrations was 12 structural aberrations in cultured human lymphocytes treated with 1335 µg/mL acetaminophen (for 3 + 17 hours without S-9 activation), compared to 2 structural aberrations in the negative control. The number of structural aberrations excludes gaps.

For 44 hours of treatment and no recovery time without S-9 activation and 3 hours treatment and 41 hours recovery time with S-9 activation, the following table illustrates the structural aberrations observed:

	44 + 0 hours, –S-9						3 + 41 hours, +S-9					
Treatment	Solvent - S-9			76.96 µg/ml - S-9			Solvent + S-9			1335 µg/ml + S-9		
Culture	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
Cells scored	85	100	185	71	89	160	100	100	200	100	100	200
Gaps	5	6	11	7	10	17	0	1	1	1	4	5
Chr. del.	0	0	0	4	9	13	0	1	1	0	1	1
Chr. exch.	0	0	0	0	0	0	0	0	0	0	0	0
Ctd. del.	1	1	2	5	6	11	0	0	0	0	0	0
Ctd. exch.	0	0	0	0	0	0	0	0	0	0	0	0
Other	0	0	0	0	0	0	0	0	0	0	0	0
Total inc. gaps	6	7	13	16	25	41	0	2	2	1	5	6
Total excl. gaps	1	1	2	9	15	24	0	1	1	0	1	1

As shown in the table above, the number of structural aberrations was 24 structural aberrations in cultured human lymphocytes treated with 79.96 µg/mL acetaminophen (for 44 + 0 hours without S-9 activation), compared to 2 structural aberrations in the negative control. The number of structural aberrations excludes gaps. The number of structural aberrations was 1 structural aberrations in cultured human lymphocytes treated with 1335 µg/mL acetaminophen (for 3 + 41 hours with S-9 activation), compared to 1 structural aberrations in the negative control. The number of structural aberrations excludes gaps.

There were no changes to the frequency of cells with numerical aberrations under all treatment conditions.

Acetaminophen induced chromosome aberrations in cultured human peripheral blood lymphocytes; however, this effect was not clearly concentration dependent.

Study title: Study to Determine the Ability of Paracetamol to Induce Mutations at the Thymidine Kinase (*tk*) locus in Mouse Lymphoma L5178Y Cells Using a Fluctuation Assay

Key findings:

- This Mouse Lymphoma study was deemed valid.
- L5178Y mouse lymphoma cells were treated with 500 to 5000 µg/mL acetaminophen, in the absence and presence of S-9 activation.
- The top dose of 5000 µg/mL acetaminophen was based on the solubility limit of acetaminophen in the culture medium.
- At 500 to 5000 µg/mL, in the absence and presence of S-9 activation, acetaminophen induced mutation at the *tk* locus of L5178Y mouse lymphoma cells.

Study no.: UBF 1/TK

Volume #, and page #: This is an electronic submission, 1 volume
Conducting laboratory and location: (b) (4)

Date of study initiation: July 29, 1991
GLP compliance: Yes
QA reports: yes (X) no ( ) Signature was provided on March 31, 1992
Drug, lot #, and % purity: Paracetamol (acetaminophen, (b) (4)); Lot # 0.2712; no percentage was given for purity (contains (b) (4) of 4-aminophenol)

Methods

Strains/species/cell line:
L51787 TK $^{+/-}$ mouse lymphoma cells was used.

Doses used in definitive study:
Doses of 500, 1000, 2000, 4000, and 5000 µg/mL of acetaminophen were used.

Basis of dose selection:
According to the Sponsor's protocol, "[m]aximum exposure (5000 µg/ml) could be achieved in the assay by dissolving [acetaminophen] in tissue culture medium".

Negative controls:
The negative control was the tissue culture medium containing 2% (w/v) sodium citrate tribasic.

Positive controls:
The positive controls were 4-nitroquinoline-1-oxide (NQO) in the absence of S-9 activation and benzo(a)pyrene (BP) in the presence of S-9 metabolic activation.

Incubation and sampling times:

At least 10^7 cells in the culture medium were exposed to the negative control, various concentrations of acetaminophen, or positive controls in the presence or absence of S-9 activation for 3 hours.

Following treatment, the cultures were allowed a period of 2 days to develop and express any possible TK^- mutations. Based on observations of the growth of the cultures during the expression period, the Sponsor provided the following table to illustrate how the cultures were selected to be plated for viability and 5-trifluorothymidine (TFT) resistance:

Experiment 1 (µg/ml)			Experiment 2 (µg/ml)		
- S-9	+ S-9		- S-9	+ S-9	
0	0		0	0	
500	500		500	500	
1000	1000		1000	1000	
2000	2000		2000	2000	
4000	4000		4000	4000	
5000	5000		5000	5000	
0.05 NQO	2	BP	0.05 NQO	2	BP
0.1 NQO	3	BP	0.1 NQO	3	BP

Note from the table above, the study was done in duplicate (experiment 1 and 2) each was preformed in the presence and absence of S-9 activation. Each experiment consisted of 2 plates for survival or viability and 4 plates for TFT resistance.

Following the expression period, 10^4 cells/mL were plated and then diluted to 8 cells/mL to assess cell viability. Cells are plated in 96-well microtiter plates and incubated until scorable (9-14 days).

Following the expression period, 10^4 cells/mL (diluted to 2 x 10^3 cells/well) were plated and incubated in 96-well microtiter plates to assess TFT resistance. Cells were incubated in a final concentration of 3 µg/mL of TFT until scorable (11-14 days).

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):
The study was valid for the following reasons:

1. The appropriate doses and dosing criteria were used. The highest dose (5000 µg/mL) was based on the solubility limit of acetaminophen in the culture medium.
2. The appropriate positive controls were used. Benzo(a)pyrene (BP) is an appropriate positive control in the presence of S-9 metabolic activation. 4-

nitroquinoline-1-oxide (NQO) is an appropriate positive control in the absence of S-9 activation in the cultured human lymphocytes. 4-nitroquinoline-1-oxide (NQO) is appropriately used in this assay because the positive control generated an increased mutant frequency of several fold higher than the negative control.

3. The criteria for positive results were appropriate. All treatment groups generated mutant frequencies that were several fold higher than the negative control.
4. The appropriate measure of survival was used. For the microwell technique, relative survival (reported as %RS) was used. The Sponsors have taken into account the differential growth that can occur during treatment in the wells.
5. The mutant frequency (MF) was determined appropriately. In this study,

   $$MF = [PE\ (mutant)/PE\ (viable)] \times 10^6,$$

   where PE (mutant) is the plating efficiency of the mutant cells and PE (viable) is the plating efficiency of the viable cells.

Study outcome:
The following table illustrates the relative survival (%RS), mutant frequency (MF) for acetaminophen and the positive control and the test for linear trend of the MF for experiment 1 in the absence of S-9 activation:

Table 15. Relative Survival, Mutant Frequency, and Linear Trend for Experiment 1 (-S-9)

Treatment µg/ml	%RS	MF(1)
0	100.0	157.42
500	83.7	319.59
1000	55.0	436.20
2000	44.7	621.96
4000	38.3	296.54
5000	35.3	285.54

Test For Linear Trend	
Slope	2.24E-08
Variance	5.53E-17
b^2/Sb	9.067**

Positive Control (NQO)

Treatment µg/ml	%RS	MF(1)
0.05	89.9	908.81
0.1	71.2	1E+03

As shown in the table above, there was a dose-dependent decrease in the %RS in L51787 TK $^{+/-}$ mouse lymphoma cells where there was a 35.3 %RS in cells treated with 5000 µg/mL acetaminophen. There was a dose-dependent increase in MF for up to 2000 µg/mL acetaminophen. At 4000 and 5000 µg/mL acetaminophen, the MF leveled off. However there was a positive linear trend of an increase in the MF with acetaminophen treatment, as demonstrated by $b^2/Sb = 9.097$.

The following table illustrates the relative survival (%RS), mutant frequency (MF) for acetaminophen and the positive control and the test for linear trend of the MF for experiment 1 in the presence of S-9:

Table 16. Relative Survival, Mutant Frequency, and Linear Trend for Experiment 1 (+S-9)

Treatment µg/ml	%RS	MF(1)
0	100.0	182.51
500	70.6	295.55
1000	45.2	471.65
2000	31.9	525.97
4000	26.6	305.64
5000	26.6	274.87

Test For Linear Trend	
Slope	1.56E-08
Variance	6.15E-17
b^2/Sb	3.979*

Positive Control (BP)

Treatment µg/ml	%RS	MF(1)	LMF
2	52.1	997.45	-6.910
3	26.9	2E+03	-6.200

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As shown in the table above, there was a dose-dependent decrease in the %RS in L51787 TK $^{+/-}$ mouse lymphoma cells where there was a 26.6 %RS in cells treated with 5000 µg/mL acetaminophen. There was a dose-dependent increase in MF for up to 2000 µg/mL acetaminophen. At 4000 and 5000 µg/mL acetaminophen, the MF leveled off. However there was a positive linear trend of an increase in the MF with acetaminophen treatment, as demonstrated by $b^2/Sb = 3.979$.

The following table illustrates the relative survival (%RS), mutant frequency (MF) for acetaminophen and the positive control and the test for linear trend of the MF for experiment 2 in the absence of S-9:

Table 17. Relative Survival, Mutant Frequency, and Linear Trend for Experiment 2 (-S-9)

Treatment µg/ml	%RS	MF(1)
0	100.0	183.61
500	75.7	344.78
1000	52.8	447.75
2000	42.3	364.16
4000	41.4	295.87
5000	30.5	275.38

Test For Linear Trend	
Slope	1.35E-08
Variance	5.89E-17
b^2/Sb	3.101*

Positive Control (NQO)

Treatment µg/ml	%RS	MF(1)	LMF
0.05	52.8	2E+03	-6.460
0.1	35.2	1E+03	-6.524

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As shown in the table above, there was a dose-dependent decrease in the %RS in L51787 TK $^{+/-}$ mouse lymphoma cells where there was a 30.5 %RS in cells treated with 5000 µg/mL acetaminophen. There was a dose-dependent increase in MF, which peaks at 1000 µg/mL acetaminophen, followed by a dose-dependent decrease in MF for 2000 to 5000 µg/mL acetaminophen. However there was a positive linear trend of an increase in the MF with acetaminophen treatment, as demonstrated by $b^2/Sb = 3.101$.

The following table illustrates the relative survival (%RS), mutant frequency (MF) for acetaminophen and the positive control and the test for linear trend of the MF for experiment 2 in the presence of S-9:

Table 18. Relative Survival, Mutant Frequency, and Linear Trend for Experiment 2 (+S-9)

Treatment µg/ml	%RS	MF(1)
0	100.0	133.35
500	84.9	180.39
1000	51.4	214.54
2000	39.4	289.04
4000	34.0	159.86
5000	28.3	200.17

Test For Linear Trend	
Slope	7.01E-09
Variance	4.25E-17
b^2/Sb	1.154

Positive Control (BP)

Treatment µg/ml	%RS	MF(1)	LMF
2	83.1	764.63	-7.176
3	57.7	1E+03	-6.847

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As shown in the table above, there was a dose-dependent decrease in the %RS in L51787 TK $^{+/-}$ mouse lymphoma cells where there was a 28.3 %RS in cells treated with 5000 µg/mL acetaminophen. There was a dose-dependent increase in MF, which peaks at 2000 µg/mL acetaminophen. At 4000 and 5000 µg/mL acetaminophen, the MF was 159.86 and 200.17, respectively. However there was a positive linear trend of an increase in the MF with acetaminophen treatment, as demonstrated by $b^2/Sb = 1.154$.

The following table illustrates the small and large colony mutant frequencies for negative and positive controls and doses of acetaminophen showing a significant increase in mutant frequency:

Experiment	Concentration µg/ml	S-9	Mutant frequency* Small colony	Large colony	Proportion small colony mutants
1	0	-	87	63	0.58
	500		195	120	0.62
	1000		256	127	0.67
	2000		428	177	0.71
	4000		186	89	0.68
	5000		188	104	0.64
	NQO 0.05		506	262	0.66
	0.10		835	273	0.75
	0	+	84	86	0.49
	500		168	110	0.60
	1000		325	128	0.72
	2000		307	164	0.65
	4000		186	103	0.64
	BP 2		488	352	0.58
	3		1221	519	0.70
2	0	-	94	78	0.55
	500		215	112	0.66
	1000		272	137	0.67
	2000		251	102	0.71
	4000		185	107	0.63
	NQO 0.05		770	489	0.61
	0.10		884	342	0.72
	0	+	60	67	0.47
	1000		132	78	0.63
	2000		156	113	0.60
	BP 2		379	236	0.62
	3		689	245	0.74

* per 10^6 viable cells

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As shown in the table able, both small and large colony mutants were formed following treatment with acetaminophen, with a higher portion of small colony mutants. Small colony mutants are indicative of chromosomal damage whereas large colony mutants are indicative of DNA damage. From the results of this study, it appears acetaminophen is more likely to generate chromosomal, rather than DNA, damage.

At 500 to 5000 µg/mL, in the absence and presence of S-9, acetaminophen induced mutation at the *tk* locus of L5178Y mouse lymphoma cells.

Study title: Study to Evaluate the Potential of Paracetamol to Induce Unscheduled DNA Synthesis in Rat Liver Using an *in vivo*/*in vitro* Procedure

Key findings:

- This Unscheduled DNA Synthesis study was deemed valid.

- Hepatic cultures from male Wistar rats were treated with 79.06, 250, and 350 mg/kg acetaminophen in a valid assay for 12-14 hours in one experiment and for 2-4 hours in the second experiment.
- The top dose of 350 mg/kg was based on the solubility limit of acetaminophen in water.
- Treatment of acetaminophen did not induce unscheduled DNA synthesis in hepatocyte cultures prepared from male Wistar rats.

Study no.: UBF 1/ILU

Volume #, and page #: This is an electronic submission, 1 volume
Conducting laboratory and location: (b) (4)

Date of study initiation: February 24, 1992
GLP compliance: Yes
QA reports: yes (X) no ( ) Signature was provided on December 15, 1992
Drug, lot #, and % purity:
Paracetamol (acetaminophen, (b) (4)); Lot # 0.2712; no percentage given for purity (contains (b) (4) of 4-aminophenol)

Methods

Strains/species/cell line:
Male Wistar rats. 6 male rats were used per treatment group.

Doses used in definitive study:
Doses of 79.06, 250.0, and 350.0 mg/kg of acetaminophen were used.

Basis of dose selection:
From the Sponsor's description of the methods, "[a] top dose of 350 mg/kg was chosen based on the solubility limit of [acetaminophen] in water. A second dose of 250 mg/kg was also selected as being equimolar for [acetaminophen] with the top dose of Propacetamol chlorhydrate used in [previous studies]. A third dose of 79.06 mg/kg, a half-log dilution of 250 mg/kg, was chosen as an appropriate lower dose level for the UDS [Unscheduled DNA Synthesis] study".

Negative controls:
The negative control was water, the vehicle for acetaminophen.

Positive controls:
The positive controls were 2-acetamidofluorene (2-AAF) and dimethylnitrosamine (DMN). 2-AAF was suspended in corn oil at 7.5 mg/mL and was used as the positive

control for the 12-14 hours experiment. DMN was dissolved in distilled water at 1.0 mg/mL and was used as the positive control for the 2-4 hours experiment.

Incubation and sampling times:
Animals were dosed daily. The animals were sacrificed at 12-14 hours after dosing (experiment 1) or 2-4 hours after dosing (experiment 2).

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):
The study was valid for a number of reasons (reproduced verbatim from the submission):

1. the mean net grain count for vehicle-treated animals was less than or equal to 0 (0.0 and -3.9 for Experiments 1 and 2, respectively);
2. The positive control chemicals 2-AAP and DMN induced increases in mean net grain count of 5 or more (7.8 and 12.6, respectively), and 50% or more of cells (73.2% and 89.6%, respectively) had net grain counts of 5 or more (This result showed that the test system was sensitive to 2 known DNA damaging agents requiring metabolism for their action and that the experiment was valid); and
3. The data in TABLE 8 shows that the percentage of cells in S-phase for one treatment group exceeded 1% of the population (0.2% to 3.0% for Experiment 1 and 0.5% to 0.8% for Experiment 2) (Solvent controls gave values of 2.6% and 0.5% for Experiment 1 and 2 respectively. Similar values were found for solvent control treatments in 2 related studies and in view of the limited database available and upon which the 1% limit was based, there is insufficient grounds to reject the data and the experiment is considered valid).

Furthermore, the study was valid for the following reasons:

1. The appropriate positive controls were used. 2-AAF was used as the positive control for the 12-14 hours experiment. DMN was used as the positive control for the 2-4 hours experiment.
2. The appropriate dose range of acetaminophen was used where the top dose of 350 used in the assay was the solubility limit of acetaminophen in water.
3. The appropriate number of cells per animal was used. In this assay, 100 cells/animal were scored.
4. The net nuclear grain (NNG) was appropriately calculated by subtracting the cytoplasmic grains (CG) from the nuclear grains (NG).

Study outcome:
The following table shows the net nuclear grain counts at both 12-14 and 2-4 hours sacrifice time.

Table 19. Group Mean Net Grain Count Values

A.

12-14 hours sacrifice time

Dose (mg/kg)		Net nuclear grain count (NG)		Net grain count of cells in repair		Percent of cells in repair (NG ≥ 5)	
		mean	SD	mean	SD	mean	SD
0	WATER	0.0	0.6	9.1	5.8	0.8	1.3
79.06		0.5	0.6	9.2	4.4	1.8	1.1
250		0.3	0.5	5.3	0.2	1.4	0.9
350		0.5	0.5	6.4	1.2	2.0	1.6
75	2-AAF	7.8	1.1	9.4	0.8	73.2	7.0

B.

2-4 hours sacrifice time

Dose (mg/kg)		Net nuclear grain count (NG)		Net grain count of cells in repair		Percent of cells in repair (NG ≥ 5)	
		mean	SD	mean	SD	mean	SD
0	WATER	-3.9	0.9	6.8	0.4	0.8	1.3
79.06		-3.0	1.0	8.0	0.0	1.2	2.7
250		-4.1	0.7	5.5	0.7	0.4	0.5
350		-3.8	1.3	0	-	-	-
10	DMN	12.6	3.2	13.9	3.0	89.6	6.3

As shown in the table above, treatment of IV acetaminophen at 79.06, 250, and 350 mg/kg did not induce unscheduled DNA synthesis in hepatocyte cultures prepared from male Wistar rats. For example, treatment with 79.06, 250, and 350 mg/kg acetaminophen yielded a net nuclear grain count of 0.5, 0.3, and 0.5, respectively compared to 0.0 for water and 7.8 for 2-AAF at the 12-14 hours sacrifice time. Moreover, treatment with 79.06, 250, and 350 mg/kg acetaminophen yielded a net nuclear grain count of -3.0, -4.1, and -3.8, respectively compared to -3.9 for water and 12.6 for DMN at the 2-4 hours sacrifice time.

Treatment of acetaminophen did not induce unscheduled DNA synthesis in hepatocyte cultures prepared from male Wistar rats.

2.6.6.5 Carcinogenicity

The Sponsor did not submit any carcinogenicity studies in this NDA. The Sponsor did submit a literature review of the carcinogenicity of acetaminophen:

> The carcinogenicity of acetaminophen has been extensively reviewed (*eg* WHO-1999). The authors concluded that there was not evidence that acetaminophen is carcinogenic, based on research in experimental animals, including many studies of long-term exposure. There is no evidence to suggest that a change in the route of administration from oral/rectal to IV would alter its carcinogenic potential. It is unlikely that short-term exposure to IV acetaminophen, such as that envisioned in this NDA, would cause a carcinogenic risk.
>
> A thorough and often cited animal study on carcinogenicity of acetaminophen was performed by the National Toxicology Program (NTP-1993). First, groups of 50 male and 50 female B6C3F1mice, eight to nine weeks old, were given acetaminophen at concentrations of 0, 600, 3,000, or 6,000 mg per kg (ppm) of food for up to 104 weeks. There was no difference in survival between control and exposed mice, but a dose-related decrease in body-weight gain was recorded in animals of each sex. Under the conditions of this study, there was no evidence of carcinogenic activity. Second, groups of 50 male and 50 female Fischer 344/N rats, seven to eight weeks old, were given acetaminophen in the diet at concentrations of 0, 600, 3,000, or 6,000 mg per kg (ppm) of food for up to 104 weeks. There was no difference in survival between control and exposed groups and no effect of treatment on body-weight gain. No treatment-related increase in tumor incidence was found in male rats.
>
> Acetaminophen does not interact with known carcinogens or modifying factors to promote renal or intestinal tumors. In a model of urinary bladder carcinogenesis, acetaminophen did not significantly increase the incidences of tumors of the renal tubules, renal pelvis, ureter or urinary bladder when compared with the initiated control group (Shibata-1995). In a model of intestinal carcinogenesis, less than 50% of the animals receiving 3-2-dimethyl-4-aminobiphenyl (DMAB) survived, the deaths being due mainly to intestinal obstruction from tumor growth. No intestinal tumors occurred in the controls or those receiving acetaminophen alone.
>
> Acetaminophen does not interact with other factors that induce liver damage to cause tumors. In a short-term model of fatty liver and liver cirrhosis, acetaminophen did not significantly alter the number or size of liver foci when compared with the relevant control values (Maruyama-1990). In a long-term model of fatty liver and liver cirrhosis, acetaminophen did not alter the number or size of liver foci when compared with the relevant control groups (Maruyama-1990).
>
> Some earlier literature suggested an association between acetaminophen usage and human renal tumors, but this was not confirmed in more recent, well-controlled studies. For example, in a population-based case-control study conducted in Ontario, Canada, involving all histologically confirmed cases of renal-cell carcinoma newly diagnosed in 1986 or 1987 among residents aged 25–69 years, the odds ratio for past acetaminophen use (adjusted for age, smoking habits, and body mass index) was 0.9 (95% confidence interval [CI], 0.4–1.8) for men and 0.6 (95% CI, 0.4–1.6) for women (Kreiger-1993). In another population-based study, all histologically confirmed cases of renal-cell carcinoma

> diagnosed between 1989 and 1991 in Danish inhabitants aged 20–79 years at the time of diagnosis were evaluated. Among the study subjects with the highest life-long intake of acetaminophen (> 1 kg), the odds ratios were 0.9 (95% CI, 0.2–4.0) for men and 0.5 (95% CI, 0.1–1.8) for women (Mellemgaard-1994).

The WHO-1999 reference is a review paper that summarizes studies that have been done to evaluate the carcinogenicity of acetaminophen. No data submitted in the WHO-1999 paper and was not analyzed by this reviewer. Thus, the WHO-1999 reference is not considered relevant.

The NTP (1993) carcinogenicity study appears to follow GLP guidelines. An adequate number of rats and mice have been used. In this study, groups of 60 rats and mice of each sex were administered 0, 600, 3000, or 6000 ppm acetaminophen through the feed for up to 103 weeks. At 15 months, 10 rats and mice per sex from each dose group were randomly selected by interim evaluation. The appropriate protocols were used and included histological examinations of various tissues. The results of the NTP carcinogenicity studies showed that there was no evidence of carcinogenic activity of acetaminophen in male F344/N rats, there was equivocal evidence of carcinogenic activity in female rats based on increased incidences of mononuclear cell leukemia, and there was no evidence of carcinogenic activity in male and female B6C3F1 mice (NTP-1993). Regarding the observation of mononuclear cell leukemia in the female F344/N rats, 9/50, 17/50, 15/50, and 24/50 rats were affected by this observation after treatment with 0, 600, 3000, and 6000 ppm acetaminophen, respectively (NTP-1993). Although each rat and mouse was given 0, 600, 3000, or 6000 ppm acetaminophen through the feed, the actual dose of acetaminophen each animal received was different. For example, the male rats given 600, 3000, and 6000 ppm acetaminophen actually received 22, 109, and 222 mg/kg acetaminophen, respectively. The female rats given 600, 3000, and 6000 ppm acetaminophen actually received 24, 118, and 240 mg/kg acetaminophen, respectively. The male mice given 600, 3000, and 6000 ppm acetaminophen actually received 79, 411, and 880 mg/kg acetaminophen, respectively. The female mice given 600, 3000, and 6000 ppm acetaminophen actually received 98, 534, and 987 mg/kg acetaminophen, respectively. The NOAEL for the male rats is 268 mg/kg which corresponds to a safety margin of 0.54 based on body surface area of rats, for the average human weighing 60 kg given the maximum daily dose of 4000 mg acetaminophen. The NOAEL for the female rats is 118 mg/kg based on the incidence of mononuclear cell leukemia which corresponds to a safety margin of 0.29 based on body surface area of rats, for the average human weighing 60 kg given the maximum daily dose of 4000 mg acetaminophen. The NOAEL for the male mice is 880 mg/kg which corresponds to a safety margin of 1.07 based on the body surface area of mice. The NOAEL for the female mice is 987 mg/kg which corresponds to a safety margin of 1.20 based on the body surface area of mice. According to the NTP (1993), "[e]quivocal evidence of carcinogenic activity describes studies that are interpreted as showing a marginal increase of neoplasms that may be chemically related" and "[n]o evidence of carcinogenic activity describes studies that are interpreted as showing no chemically related increases in malignant or benign neoplasms".

2.6.6.6 Reproductive and developmental toxicology

No new reproductive and developmental studies of acetaminophen were submitted with this NDA. The Sponsor did present a review of the published literature regarding the reproductive and developmental toxicology of acetaminophen.

Fertility and Early Embryonic Development

Regarding fertility studies, the Sponsor cited the following studies:

> Testicular atrophy was noted in rats given toxic oral doses of acetaminophen over a period of 100 days (Boyd-1968) and testis weight was reduced following daily administration of toxic doses of acetaminophen to rats for 70 days (Jacqueson-1984). The addition of up to 1% of acetaminophen in the diet of Swiss CD-1 mice did not affect fertility (Lamb-1997).

The studies by Boyd-1968 and Jacqueson-1984 were not done according to GLP guidelines but did demonstrate testicular changes specifically testicular atrophy in the Boyd study and reduction in testis weight in the Jacqueson study.

The study by Boyd-1968 examined the effect of acetaminophen when administered to Wistar albino rats for 100 days. The end point measurement was testicular weight. Twenty treated rats for each dose (0.5, 0.7, 1.1, 1.4, 2.5, 3.0, 3.5 and 4.0 g/kg/day) and 8 water-fed control rats were used. Testicular atrophy occurred at doses as low as 0.5 g/kg/day (the testicular weight decreased by around 40%). The doses used in Boyd (1968) represent an exposure margin of 1.2, 1.7, 2.7, 3.4, 6.1, 7.3, 8.5, and 9.7, respectively based on body surface area, for the average human weighing 60 kg given the maximum daily dose of 4000 mg acetaminophen. However, it should be noted that most of these doses exceeded the estimated LD_{50} for this species (estimated at 0.77 g/kg/day for 100 days) and therefore represent significantly toxic doses. Furthermore, all the doses tested resulted in testicular atrophy, where treatment with the low dose, 0.5 g/kg/day, resulted in a 40% decrease in testicular weight. Because the testicular atrophy finding was present in all doses of acetaminophen tested, a NOAEL for testicular atrophy cannot be obtained.

In the Jacqueson study (1984), male Sprague-Dawley rats were administered 500 mg/kg/day acetaminophen via gastric intubation for 70 days. Treatment with 500 mg/kg/day acetaminophen resulted in a significant decrease in testes weight, a significant increase in testicular GST activity during spermatogenesis but testicular content is not affected. Although a decrease of testes weight was shown, Jacqueson only used one dose. Neither a NOAEL nor a dose-dependency of the effect can be determined from this study. Thus, the Jacqueson study is not considered ideal for informing product labeling. Moreover, the dose employed also appears to exceed the estimated LD_{50} in the rat for this duration of treatment.

The Lamb study (1997) was done for the National Toxicology Program to evaluate the effects of acetaminophen in reproduction and fertility in CD-1 mice. The full study report is not available for this study as this reference is an abstract of the fully study. CD-1 mice were administered the equivalent of 370, 770, and 1400 mg/kg/day

acetaminophen in the diet in F_0 to F_2 generations. F_1 pup body weights were reduced for both sexes by 6 to 18%. Following the birth of the F_2 pups, the high dose male F_1 adults were 10% less and the females were 8% less compared to control. Sperm abnormalities in the F_1 adults increased from 7% in the control to 16% in the high dose group. The Lamb-1997 reference was an abstract of the reproduction and fertility study done on acetaminophen. Moreover, it is not clear whether the Lamb study (1997) is GLP compliant; however, the study was conducted according to well-established protocols employed by the NTP. The Lamb study (1997) also refers to Reel-1992, which was GLP compliant. Although there were no data included in the reference, the Lamb-1997 is considered relevant because the study was conducted according to well-established protocols employed by the NTP.

Embryofetal Development

Regarding embryo and teratogenicity studies, the Sponsor cited the following studies:

> In the rat, acetaminophen at 250 mg/kg/day during organogenesis did not affect fetal length or weight or the incidence of resorptions, and did not cause malformations or fetotoxicity (Lubawy-1977). When acetaminophen was administered to pregnant rats by gavage at 150, 500, or 1,500 mg/kg/day from the first day of pregnancy up to term, there were no morphological abnormalities, but dose-dependent microscopic lesions of the maternal liver and kidney were observed (Neto-2004).
>
> In the mouse, although pre-implantation embryos were sensitive to acetaminophen *in vitro*, no adverse effects on embryo development to term were observed after treatment of female mice with 1,430 mg/kg/day acetaminophen from day -8 to day 3 of gestation (Laub-2000). Continuous exposure of Swiss CD-1 mice to 1% acetaminophen in the diet (equivalent to 1,430 mg/kg/day) led to cumulative effects on reproduction with retarded growth and abnormal sperm in the F1 mice, and to reduced birth weight of F2 pups, although there were no signs of embryo- or teratogenicity at lower doses (Reel-1992). No teratogenic effects of acetaminophen were observed at doses of 100 and 250 mg/kg/day given to C57BL/6 mice between days 6 and 13 of gestation (Lambert-1976).

In the Lubawy study (1977), groups of 21 female Sprague-Dawley sperm-positive rats were administered 0, 125, and 250 mg/kg acetaminophen or aspirin by gavage from day 8 to day 19 of gestation (organogenesis). The rats were sacrificed on day 20. The study employed an appropriate number of animals and used the proper endpoints of an embryofetal development or teratogenicity study (such as fetal length, weight, incidence of resorptions, number of implant sites, and placental weight). However, it is not clear if the typical endpoints of a teratogenicity study were evaluated, such as visceral and skeletal malformations of the embryos and fetuses. Even though the study only used 2 doses, the study did demonstrate that acetaminophen had no affect on fetal length, weight or incidence of resorptions and did not cause malformations (insofar as fetal length is concerned) or fetotoxicty in the rats in the doses tested. The NOAEL for the embryotoxic effects can be determined to be 250 mg/kg, which represents a safety margin of 0.61 based on body surface area, for the average human weighing 60 kg given the maximum daily dose of 4000 mg acetaminophen. The Lubawy study (1977) is of limited utility as

far as teratogenicity is concerned because it is not clear if the visceral and skeletal malformations of embryos and fetuses we evaluated.

In the Neto study (2004), 40 pregnant female Wistar rats were divided into four groups and treated with 0, 125, 500, or 1500 mg/kg acetaminophen from the 1st to the 20th day of gestation. The rats were sacrificed on day 20. The endpoint of the study was to examine the liver and kidney (gross and histopathologic), both maternal and fetal. It is noted that none of the endpoints of the study assessed the skeletal structures of the fetuses, a typical measure for teratogenicity in GLP reproductive toxicity studies. There were no changes to the fetal liver and kidney in all treatment groups. There were dose-dependent microscopic lesions of the maternal liver and kidney. There were necrotic areas in the maternal liver and degeneration and necrotic foci in the maternal kidney. These observations occurred more frequently and severely in the 1500 mg/kg group compared to the 500 mg/kg group. There were no such observations in the low dose group. A NOAEL of 125 mg/kg can be established for the maternal liver and kidney findings, which represents a safety margin of 0.36 based on body surface area, for the average human weighing 60 kg given the maximum daily dose of 4000 mg acetaminophen. However, the Neto study (2004) is of limited utility because the typical endpoint of a teratogenicity study, namely an evaluation of the fetal skeletal structure, was not assessed.

In the Laub study (2000), female pubertal NSA mice were mated and the pregnant mice were treated with various concentrations of acetaminophen. Two-cell stage embryos were treated with 0, 375, 750, and 1500 μM acetaminophen. Female mice were treated with 800 or 1430 mg/kg acetaminophen from day -8 to day 1 or 3 of gestation. GSH levels were taken from adult livers and the embryos. Due to the time of dosing, the Laub study is not fully considered because acetaminophen was not dosed throughout gestation and the embryos and fetuses were not fully developed.

The Reel-1992 study was conducted under GLP guidelines. Male and female Swiss CD-1 mice are treated for 1 week, then paired for 14 weeks and continuously treated with the dose equivalent of 0, 357, 715, and 1430 mg/kg acetaminophen. The resultant pups were also examined for signs of toxicity (e.g. birth weight). However, an evaluation of the fetal skeletal structures was not assessed, a typical endpoint in teratogenicity studies. The results show that mice given 1% acetaminophen (1,430 mg/kg) continuously led to retarded growth and abnormal sperm in F_1 mice and reduced birth weight of F_2 pups, but there were no signs of embryotoxicity at lower doses of 0.25 and 0.5% (357 and 715 mg/kg). A NOAEL of 715 mg/kg can be established for embryotoxicity, which represents a safety margin of 0.95 based on body surface area, for the average human weighing 60 kg given the maximum daily dose of 4000 mg acetaminophen. The Reel-1992 study is of limited utility because the typical endpoint of teratogenicity studies, an evaluation of the fetal skeletal structure, was not assessed. Therefore, Reel-1992 did not properly assess teratogenicity.

In the Lambert study (1976), the teratogenicity of acetaminophen was studied in pregnant C57BL/6N mice given 100-250 mg/kg acetaminophen intraperitoneally during days 6-13

of gestation. The Lambert study (1976) also investigated glutathione levels in the developing mouse. It is not clear how the teratogenicity of acetaminophen was evaluated as the investigators cite Monie-1965 in their methods (Monie IW, Kho KG, and Morgan J. 1965. Supplement to Teratology Workshop Manual, p. 163. University of Chicago Press, Berkeley, California). According to the Sponsors, the results show there were no teratogenic effects observed in the mice treated with 100-250 mg/kg acetaminophen. There were no significant differences in the number of fetal resorptions, in fetal weight, or in fetal size compared to control mice. The Lambert study does not account for all of gestation in mice and the number of mice in each treatment group as well as how the teratogenicity of acetaminophen is evaluated remains unclear. For these reasons, the Lambert study was not considered adequate for labeling purposes.

Regarding the embryo and teratogenicity studies of acetaminophen, Lubawy (1977), Neto (2004), and Reel (1992) best demonstrated the embryotoxic effects of acetaminophen but not the teratogenicity of acetaminophen.

Fertility studies done by Boyd-1968 demonstrated testicular atrophy and reduction in testis weight in rats treated with acetaminophen. The embryo and teratogenicity studies done by Reel-1992 demonstrated retarded growth and abnormal sperm in F_1 mice and reduced birth weight of F_2 pups in rats treated with acetaminophen. Moveover, embryo and teratogenicity studies done by Neto-2004 demonstrated no fetal morphological abnormalities but dose-dependent microscopic lesions of the maternal liver and kidney in rats treated with acetaminophen. However, the embryo and teratogenicity studies done by Lubawy-1977 demonstrated that acetaminophen had no affect on fetal length, weight or incidence of resorptions and did not cause malformations or fetotoxicty in rats. Although the majority of these literature references regarding the fertility and embryofetal development studies of acetaminophen were not done following GLP guidelines, it remains clear that there were animal studies conducted to assess the reproductive toxicology of acetaminophen and that the adverse findings from these studies are noted. The Sponsor must submit exposure data from these studies and how these doses relate to the clinical dose of this NDA in order for accurate information to be put in the label.

In a Micromedex search, (b) (4) revealed several clinical case studies on acetaminophen use during pregnancy and fetal outcomes. According to this database, acetaminophen use during pregnancy does not appear to increase the risk of adverse outcomes. A Maternal Health Team consult was requested to address this issue; however, as the Applicant did not submit a detailed review of the existing clinical literature, lack of adequate information to inform the pregnancy category based on the clinical data will be listed as a deficiency (See Maternal Health Review during second cycle submission).

As it was noted in the excretion section of the toxicokinetics section, acetaminophen is excreted in human milk in small amounts (Bitzén-1981; Berlin-1980).

2.6.6.7 Local tolerance

Study title: Skin Sensitization Test in Guinea-Pigs

Key study findings:

- Control and acetaminophen did not induce delayed contact hypersensitivity in guinea-pigs when administered either via the intradermal or cutaneous routes of administration.

Study no.: 19379 TSG
Volume #, and page #: This is an electronic submission, 1 volume
Conducting laboratory and location: (b) (4)

Date of study initiation: November 25, 1999
GLP compliance: Yes
QA reports: yes (X) no ( ) Signatures were provided on April 5, 2000.
Drug, lot #, and % purity: Paracetamol (acetaminophen, (b) (4)); Lot # FD99037/D5916; 99.99% purity

Formulation/vehicle:
The formulation used contains the following:

DENOMINATION	UNIT FORMULA	PERCENT FORMULA
PARACETAMOL	1000.00	1.00
MANNITOL	3850.00	3.85
L-CYSTEINE HYDROCHLORIDE	(b) (4)	
DIHYDRATE DISODIUM PHOSPHATE		
DISODIUM DIHYDRATE ETHYLENE DIAMINE TETRAACETIC		
SODIUM HYDROXYDE	s	
WATER FOR INJECTABLE PREPARATION	s	
	100.00 ml	100.00 %

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The vehicle used is 0.9% NaCl.
NOTE: paracetamol = acetaminophen.

Methods

Doses:
Doses of 75% (w/w) and undiluted preparations of acetaminophen were used in the main study.

Study design:
Male and female Harley Crl: (HA) BR guinea-pigs were treated with different concentrations of acetaminophen administered during a preliminary test and the main study. The main study consists of an induction phase followed by a challenge phase. The Sponsor provided a description of the study as follows:

2.3 TREATMENT

2.3.1 Preliminary test

For the test and control substances, a preliminary test was conducted in order to determine the concentrations to be tested in the main study.

By intradermal route (tested concentrations: 75% and 50% (w/w)):
- 24 hours before treatment, the dorsal region of the animals was clipped,
- intradermal administrations of the dosage form preparations (0.1 ml) were performed in the interscapular region,
- cutaneous reactions were evaluated approximately 24, 48 hours and 6 days after the injections.

By cutaneous route (tested concentrations: 100% and 50% (w/w)):
- 24 hours before treatment, both flank regions of the animals were clipped,
- the filter paper of a chamber (Finn Chamber®) was fully-loaded with the dosage form preparations. The chamber was then applied to the clipped area of the skin (one concentration per flank). The chamber was held in place by means of an occlusive dressing for 24 hours,
- cutaneous reactions were evaluated approximately 24 and 48 hours after removal of the dressings.

Criteria for selection of concentrations

The following criteria were used:
- the concentrations should be well-tolerated systemically and locally,
- intradermal injections should cause moderate irritant effects (no necrosis or ulceration of the skin),
- cutaneous application for the induction should cause at most weak or moderate skin reactions or be the maximal practicable concentration,
- cutaneous application for the challenge phase should be the highest concentration which does not cause irritant effects.

2.3.2 Main study

2.3.2.1 Preparation of the animals

For all animals, the application sites were:
- clipped on days -1 and 7 (interscapular region 4 cm x 2 cm),
- clipped and shaved on day 21 (each flank 2 cm x 2 cm).

2.3.2.2 Study design

The study design was as follows:

Groups	Number of animals	Induction		Challenge application (day 22)	
		Intradermal injection (day 1)	Cutaneous application (day 8)	right flank	left flank
1	10	vehicle	vehicle	PARACETAMOL INJECTABLE	Control substance
2	20	Control substance	Control substance	Control substance	vehicle
3	20	PARACETAMOL INJECTABLE	PARACETAMOL INJECTABLE	PARACETAMOL INJECTABLE	vehicle

2.3.2.3 Induction phase by intradermal and cutaneous routes

2.3.2.3.1 Intradermal route

On day 1, six injections were made deep into the dermis of a 4 cm x 2 cm clipped interscapular area, using a needle (diameter: 0.50 x 16 mm) mounted on a 1 ml plastic syringe (0.01 ml graduations).

Three injections of 0.1 ml were made into each side of this interscapular region (i.e. three pairs of sites), as follows:

Injection	Site	Treated groups	Control group
1	Anterior	FCA at 50% (v/v) in 0.9% NaCl	FCA at 50% (v/v) in 0.9% NaCl
2	Middle	test substance (group 3) or control substance (group 2) at 75% (w/w) in 0.9% NaCl	0.9% NaCl
3	Posterior*	test substance (group 3) or control substance (group 2) at 75% (w/w) in a mixture FCA /0.9% NaCl 50/50 (v/v)	vehicle at 50% (w/w) in a mixture FCA /0.9% NaCl 50/50 (v/v)

FCA: Freund's complete adjuvant

* : The test substance was first dissolved in the aqueous phase prior to mixing with FCA. The final concentration of the test substance was equal to that used in injection 2.

The anterior and middle pairs of injections were performed close to each other and nearest the head, while the posterior pair was performed towards the caudal part of the test area.

2.3.2.3.2 Cutaneous route

On day 7, the interscapular area was clipped.
The animals of all groups were treated with 0.5 ml of sodium lauryl sulfate at the concentration of 10% (w/w) in vaseline, in order to induce local irritation.

On day 8, a pad of filter paper (approximately 8 cm^2) was fully-loaded with the undiluted test substance and was then applied to the interscapular region of the animals of the treated group 3. The pad was held in place for 48 hours by means of an adhesive hypoallergenic dressing and an adhesive anallergenic waterproof plaster.

The animals of the control group 1 received an application of the vehicle alone and the animals of the treated group 2 received an application of the undiluted control substance under the same experimental conditions.

2.3.2.4 Challenge phase

On day 22, the filter paper of a chamber (Finn Chamber®) was fully-loaded with the undiluted test substance and was then applied to a clipped area of the skin of the posterior right flank of the animals of the treated group 3. The left flank received an application of the vehicle alone.
The chambers were held in contact with the skin for 24 hours by means of an adhesive anallergenic waterproof plaster.

The animals of the treated group 2 received an application of the undiluted control substance to the right flank and of the vehicle to the left flank under the same experimental conditions.
The animals of the control group 1 received an application of the undiluted test substance to the right flank and of the undiluted control substance to the left flank under the same experimental conditions.

Results:
In the preliminary study, the results for the administration of acetaminophen via the intradermal route are presented in the following tables:

Control substance

Animal number	Concentration of the test substance % (w/w)	Scoring after treatment 24 hours	48 hours	6 days
male 301	75 + FCA	I	I	I
	75	LI	LI	LI
	50 + FCA	I	I	I
	50	LI	LI	LI
female 302	75 + FCA	I	I	I
	75	LI	LI	LI
	50 + FCA	I	I	I
	50	LI	LI	LI

Test substance

Animal number	Concentration of the test substance % (w/w)	Scoring after treatment 24 hours	48 hours	6 days
male 303	75 + FCA	I	I	I
	75	LI	LI	LI
	50 + FCA	I	I	I
	50	LI	LI	LI
female 304	75 + FCA	I	I	A
	75	LI	LI	LI
	50 + FCA	I	I	I
	50	LI	LI	LI

FCA : mixture Freund's Complete Adjuvant/0.9% NaCl 50/50 (v/v)
I : irritation
LI : slight irritation
A : crusts

As shown in the tables above, irritation or slight irritation was observed in all treatment animals with the exception of 1 female in the 75 + FCA group where crusts were observed. Based on the observations from the tables above, the Sponsor believes "the concentration should be well-tolerated systemically and locally, intradermal injections should cause moderate irritant effect but no necrosis or ulceration of the skin" and thus, the "concentrations of test and control substances chosen for the main study were 75% (w/w)".

In the preliminary study, the results of the administration of acetaminophen via the cutaneous route are presented in the following tables:

Control substance

Animal number	Concentration of the test substance %		Scoring after removal of the dressing 24 hours	48 hours
male 301	100	RF	0	0
	50 (w/w)	LF	0	0
female 302	100	RF	0	0
	50 (w/w)	LF	0	0

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Test substance

Animal number	Concentration of the test substance %		Scoring after removal of the dressing 24 hours	48 hours
male 303	100	RF	0	0
	50 (w/w)	LF	0	0
female 304	100	RF	0	0
	50 (w/w)	LF	0	0

RF: right flank
LF: left flank

As shown in the tables above, there were no skin reactions observed in any of the treatment animals. Based on the observations from the tables above, the Sponsor believe "the concentrations should be well-tolerated systemically and locally, cutaneous application for the induction should cause at most weak or moderate skin reactions or be the maximal practicable concentration, cutaneous application for the challenge phase should be the highest concentration which does not cause irritant effect" and thus, the "concentrations of test and control substances chosen for the topical application of the induction phase (day 8) and for the challenge application (day 22) were 100%".

In the challenge phase of the main study, there were no observed skin reactions in any of the treated animals with the exception of dry skin observed in 1/20 animals from group 3 (undiluted test substance).

The control and acetaminophen groups did not induce delayed contact hypersensitivity in guinea-pigs at 24 hours and 48 hours after injection via the intradermal and cutaneous routes.

Study title: Local Tolerance Study in the Rabbit

Key study findings:

- There were no relevant treatment related changes at the injection site when acetaminophen was administered via the intravenous and perivenous routes.
- Slight increases in perivascular hemorrhage and perivascular acute inflammation were observed in animals administered acetaminophen via the intra-arterial route compared to placebo.

Study no.: 980101T
Volume #, and page #: This is an electronic submission, 1 volume
Conducting laboratory and location: (b) (4)

(b) (4)

Date of study initiation: April 27, 1998
GLP compliance: Yes
QA reports: yes (X) no ( ) Signature was provided on May 12, 1999.
Drug, lot #, and % purity: PERFALGAN® (acetaminophen, (b) (4)); Lot # 8083A; purity: no percentage given but no detectable 4-aminophenol (≤ 0.5 mg/100 mL spec)

Formulation/vehicle:
The formulation of PERFALGAN® contains acetaminophen, mannitol, phosphates, disodium edetate, and cysteine. The vehicle control contains mannitol, phosphates, disodium edetate, and cysteine.
NOTE: PERFALGAN® is IV acetaminophen and will be referred to as acetaminophen. PRO-DAFALGAN® is a prodrug formulation of acetaminophen and does not represent the acetaminophen formulation used in this NDA. As such, the results obtained using PRO-DAFALGAN® will not be considered.

Methods

Doses:
20 mg/kg of acetaminophen was used.

Study design:
Female New Zealand white Albino rabbits were treated with 20 mg/kg of acetaminophen at a flow of 0.13 mL/kg/min via intravenous infusion for 15 minutes, 4 times a day at 6 hour intervals. There were 4 rabbits per treatment group. The groups were IV infusion of acetaminophen (group 1), vehicle control (group 2), and intra-arterial infusion of acetaminophen (group 3).

Results:
The following table illustrates the clinical observations of the injection sites when acetaminophen was administered via the intravenous route:

Table 20. Clinical Observation of the Injection Sites Intravenous Route

	Oedema	Erythema	Haematoma	Dry blood	Scabbing
PERFALGAN®	Site : 4/5	Site : 4/5	Site : 5/5	Site : 2/5	Site : 1/5
placebo of PERFALGAN®.	Site : 5/5	Site : 4/5	Site : 2/5	Site : 2/5 Upstream : 1/5	Site : 1/5

As shown in the table above, hematoma was observed in 2/5 animals in the placebo groups and in 5/5 animals from the acetaminophen group. The observations of edema, erythema, dry blood, and scabbing affected the same number of animals in both the placebo and acetaminophen groups.

The following table illustrates the clinical observations of the injection sites when acetaminophen was administered via the perivenous route:

Table 21. Clinical Observation of the Injection Sites Perivenous Route

	Haematoma
PERFALGAN®	Site : 5/5
placebo of PERFALGAN®.	Site : 4/5

As shown in the table above, there was a slight increase in number of animals affected with hematoma in the acetaminophen group (5/5 animals) compared to the placebo group (4/5 animals).

The following table illustrated the clinical observations of the injection sites when acetaminophen was administered via the intra-arterial route:

Table 22. Clinical Observation of the Injection Sites Intra-Arterial Route

	Oedema	Erythema	Haematoma
PERFALGAN®	Site : 0/5	Site : 2/5	Site : 5/5 Upstream : 2/5
placebo of PERFALGAN®.	Site : 0/5	Site : 2/5	Site : 5/5 Upstream : 1/5

As shown in the table above, there was a slight increase in the number of animals affected with hematoma upstream of the injection site in the acetaminophen group (2/5 animals) compared to the placebo group (1/5 animals). The observations of edema, erythema, and hematoma at the injection site affected the same number of animals in both the placebo and acetaminophen groups.

There were no relevant treatment related changes at the injection site when acetaminophen was administered via the intravenous and perivenous routes.

Perivascular hemorrhage and perivascular acute inflammation were observed in animals administered acetaminophen via the intra-arterial route. Perivascular hemorrhage was observed in 4/5 animals from the acetaminophen groups compared to 2/5 animals from the placebo group. Perivascular acute inflammation was observed in 3/5 animals from the acetaminophen group compared to 1/5 animals from the placebo group.

2.6.6.8 Special toxicology studies

Study title: Evaluation of the Dependence Potential of Propacetamol, Paracetamol and Diethylglycine after Intravenous Administration on the Naloxone-precipitated Withdrawal Test, in Mice

Key study findings:

- Acetaminophen did not induce a withdrawal opiate-like syndrome.

Study no.: BG206P134
Volume #, and page #: This is an electronic submission, 1 volume
Conducting laboratory and location: (b) (4)

Date of study initiation: not known
GLP compliance: Yes
QA reports: yes ( ) no (X) Signatures were provided regarding the report is a true and accurate presentation of the original data on November 28, 1997.
Drug, lot #, and % purity: paracetamol (acetaminophen, (b) (4)); batch 44873; no information for the % purity
Formulation/vehicle: Acetaminophen is in saline for injection

NOTE: paracetamol = acetaminophen

Methods:

Doses:
10, 30, and 100 mg/kg (intravenous administration) of acetaminophen.

Study design:
Male CDR-1(ICR)BR (b) (4)) weighing 17 to 26 g were used. Based on the methodology of Saelens *et al.* (1971) and Malis *et al.* (1975) the mice received, 3 times a day for 2 days *(t.i.d)* and twice a day *(b.i.d.)* on the third day an intravenous administration of the test compounds (e.g. acetaminophen) or vehicles. Thirty minutes after the last injection, the animals received an intraperitoneal administration of naloxone l00 mg/kg (10 ml/kg body weight). Then each mouse was immediately placed in separate plexiglas cylinder. The number of jumps (all 4 paws off the bottom surface) executed by each mouse during 10 minutes following the injection of naloxone was recorded. Nine to 10 mice were included in the treated groups and 10 in the control groups. Results are presented as the mean of jumps (m = SEM) and as the percentage of jumping mice for each group.

Results:
The number of jumps after acetaminophen (paracetamol) administration is illustrated in the table below:

Table 23. Effect of Acetaminophen (Paracetamol)-induced Physical Dependence in Mice Jumping Test

Dose levels mg/kg i.v.	pH	Osmolality mOsm/l	Number of jumps m ± SEM	% of jumping mice

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Paracetamol					
	saline	5.87	287	1.70 ± 1.59	20
	10	5.80	297	3.10 ± 2.99	20
	30	5.88	307	0.30 ± 0.30	10
	100	5.79	351	1.10 ± 0.99	20
Morphine	30	5.65	333	99.00 ± 22.45	80

As seen in Table 23, acetaminophen (paracetamol) did not induce any jumps compared to the saline control. Morphine induced dramatically more jumps than the saline control and acetaminophen (paracetamol) treatment.

Morphine was used as positive control and confirmed the findings of Marshall and Grahame-Smith (1971) that demonstrated a close relationship between the number of jumps and the degree of physical dependence. According to the study report, acetaminophen (paracetamol) "did not induce a withdrawal opiate-like syndrome since no jumping behavior was observed". This reviewer concurs with the Sponsor's conclusions because the number of jumps was not dose-dependent and the number of jumps following acetaminophen administration was dramatically lower than the number of jumps following morphine administration.

2.6.6.9 Discussion and Conclusions

See overall conclusions and recommendations below.

2.6.6.10 Tables and Figures

None.

2.6.7 TOXICOLOGY TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

None.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

There are no issues with regard to toxicity of acetaminophen as well as no issues with regard to extractables and leachables, and the drug substance specifications (which are present in the USP monograph).

Acetaminophen is not a mutagen (as demonstrated by negative results in the bacterial Ames test) but is a clastogen (as demonstrated by positive results in the chromosomal aberration assay in cultured human peripheral blood lymphocytes).

The results of the NTP carcinogenicity studies showed that there was no evidence of carcinogenic activity of acetaminophen in male F344/N rats, there was equivocal

evidence of carcinogenic activity in female rats based on increased incidences of mononuclear cell leukemia, and there was no evidence of carcinogenic activity in male and female B6C3F1 mice.

Testicular atrophy and reduction in testis weight was noted in fertility studies of acetaminophen performed on rats at doses that were close to the LD_{50} dose for the duration of the study. There were no fetal morphological abnormalities but dose-dependent microscopic lesions of the maternal liver and kidney in rats as well as retarded growth and abnormal sperm in F_1 mice and reduced birth weight of F_2 pups in embryo and teratogenicity studies done in rodents. Although the majority of these literature references regarding the fertility and embryo and teratogenicity studies of acetaminophen were not done following GLP guidelines, it remains clear that there were animal studies conducted to assess the reproductive toxicology of acetaminophen and that the adverse findings from these studies are noted. The Sponsor must submit exposure data from these studies and how these doses relate to the clinical dose of this NDA in order for accurate information to be put in the label.

There is a drug product degradant in the drug product specification, namely 4-AP, which the proposed DP specification is NMT (b) (4). The Sponsor makes several conclusions regarding the toxicological risk assessment of 4-AP. 4-AP appears to be a minor metabolite in experimental animals with 0.16% in blood and 0.66% in liver tissue. 4-AP appears to cause similar toxic events as acetaminophen. The various embryo-fetal development and pre- and postnatal studies done with 4-AP demonstrate that there was a dose dependent increase in the number of fetal malformations. The results of the bacterial and *in vivo* rodent genotoxicity studies with 4-AP appear to show 4-AP is not mutagenic (as demonstrated by negative results from the Ames test) but may be clastogenic (as demonstrated by equivocal results in the chromosomal aberrations assay). From a 2-year rat carcinogenicity study, 4-AP is not carcinogenic. However, most of the references that the Sponsor submits in support of these conclusions were from review papers that cited studies from the published literature. The review papers that were submitted, SCCP-2005 and Bomhard-2005, do not contain data but is rather a summary of studies done. As such, the data from the studies regarding the metabolism, reproductive toxicity, genetic toxicity, and carcinogenicity of 4-AP were not fully reviewed. Therefore, there is insufficient evidence to make any conclusions with regard to the metabolism, reproductive toxicity, genetic toxicity, and carcinogenicity of 4-AP.

Unresolved toxicology issues (if any):
There is insufficient information to write the fertility and embryo-fetal development sections of the acetaminophen label. There is also insufficient information to characterize the impurity, 4-AP. The recommendations to address these deficiencies are addressed below in the "recommendations" section.

Recommendations:
This NDA is deemed a complete response from the nonclinical pharmacology/toxicology perspective due to deficiencies that are delineated below.

In regards to the fertility and embryo-fetal development studies of acetaminophen cited in the published literature, the Sponsor must submit exposure data from these studies and how these doses relate to the clinical dose of this NDA in order for accurate information to be put in the label.

The presence of the drug product degradant, 4-aminophenol (4-AP) remains a concern. In the drug product specifications, 4-AP is NMT (b) (4). The Sponsor makes several conclusions regarding the toxicological risk assessment of 4-AP. However, most of the references that the Sponsor submits in support of these conclusions were from review papers that cited studies from the published literature as well as proprietary studies they do not own. The review papers that were submitted, SCCP-2005 and Bomhard-2005, do not contain data but is rather a summary of studies done. As such, the data from the studies regarding the metabolism, reproductive toxicity, genetic toxicity, and carcinogenicity of 4-AP were not available for full review. Therefore, there is insufficient evidence to make any conclusions with regard to the metabolism, reproductive toxicity, genetic toxicity, and carcinogenicity of 4-AP. A list of studies that is needed to address these deficiencies is listed below:

Centre International de Toxicologie – Evreux, France. p-Aminophenol – Micronucleus Test by the Oral Route in Mice. Report No. 7757 MAS (CIAUL 91053) (5/1992)

DeFlora, S., Zanacchi, P., Camoirano, A., Bennicelli, C., and Badolati, G.S. (1984a). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test. *Mutat. Res.* 133:161–198

Kavlock, R. J. Structure-Activity Relationships in the Developmental Toxicity of Substituted Phenols: *in vivo* Effects. Teratology 41, 43-59 (1990)

Microtest, York, UK. Study To Evaluate the Potential of *p*-Aminophenol To Induce Unscheduled DNA Synthesis in Rat Liver Using an *in vivo/in vitro* Procedure. Report N°: ILUREBRP.029 (9/1989)

Microtest, York, UK. Study To Evaluate the Chromosome Damaging Potential of *p*-Aminophenol By Its Effects on Cultured Human Lymphocytes Using an *in vitro* Cytogenetics Assay. Report N°: 1HLRREBRP.029 (1/1990)

Rutowski, J.V. and Ferm, V.H. (1982) Comparison of the Teratogenic Effects of the Isomeric Forms of Aminophenol in the Syrian Golden Hamster. Toxicol and Applied Pharmacol 63, pp. 264-269

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1988). *Salmonella* mutagenicity tests: IV. Results from the testing of 300 chemicals. *Environ. Molec. Mutagen.* 11(Suppl. 12):1–158

Suggested labeling:
There are proposed changes to the label with regards to genetic toxicity, carcinogenicity, and reproductive toxicity of acetaminophen. These changes are outlined in the executive summary.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

Appendix 1: Review of the Toxicological Risk Assessment of 4-Aminophenol (4-AP)

Introduction

According to the Sponsor, "[t]he proposed drug product specifications for acetaminophen 10 mg/mL injection for intravenous use include a limit of (b) (4) for 4-AP". As such, "4-aminophenol (4-AP) is the main degradation product detected in the intravenous (IV) formulation of acetaminophen". In addition to 4-AP as a degradation product, [it] "is also a recognized metabolite of acetaminophen, formed by hydrolysis in the liver and kidney following the oral administration of acetaminophen".

Metabolism

Regarding the metabolism of 4-AP, the Sponsor submitted the following discussion:

> 4-AP metabolism is remarkably similar to that of acetaminophen. Oral administration of 4-AP to rabbits resulted in recovery of 4-AP, 4-AP glucuronide, and 4-AP sulfate in the urine indicating that 4-AP is orally bioavailable, and is conjugated in a similar fashion to acetaminophen (SCCP-2005). Also, oxidation of 4-AP to a reactive metabolite, p-benzoquinoneimine (BQI) has been associated with the toxic manifestations of 4-AP …, with depletion of GSH apparently being a necessary step for manifestation of toxicity.
>
> 4-AP is also a recognized metabolite of acetaminophen, formed by hydrolysis in the liver and kidney following the administration of acetaminophen (Elder-1988, SCCP-2005, Newton-1983). In recent studies of Högestätt and colleagues (Högestätt-2005), in rats given 300 mg/kg acetaminophen, both 4-AP and acetaminophen were detected in the liver, blood, brain, and spinal cord, with the highest concentrations found in blood and liver. In the blood, 4-AP was detected at a level of 30 nmol/g and acetaminophen at 3.3 µmol/g. Concentrations in the blood were 8 nmol/g for 4-AP and 3.5 µmol/g for acetaminophen. A calculation of the amounts of 4-AP relative to acetaminophen yields 0.16% in blood and 0.66% in liver tissue. This physiological conversion thus results in more 4-AP exposure than would be provided by the (b) (4) limit proposed for the drug product.
>
> 4-AP has also been shown to be acetylated to acetaminophen in multiple species, including humans (SCCP-2005). In fact, no 4-AP was detected in the circulation after subcutaneous (SC) administration of 12.5 mg/kg 4-AP to rats, but acetaminophen (28 to 45%) and its glucuronide (0-18%) and sulfate (47 to 70%) metabolites were detected. After oral administration of 400 mg/kg 4-AP to rabbits, 45% of the analytes were related to acetaminophen (25% acetaminophen, 16% acetaminophenylglucuronide, and 4% acetaminophenylsulphate). Administration of 500 mg of 4-AP to humans (by an unspecified route) resulted in plasma acetaminophen levels that were 5 to 16-fold that of 4-AP (SCCP-2005). Thus, metabolic conversion of 4-AP to acetaminophen appears to be quite substantial in all evaluated species.

Sponsor has submitted a number of references from the published literature to determine the metabolism of 4-AP.

The Newton-1982 reference studied the deacetylation of acetaminophen to 4-AP in rats. The investigators measured the concentration of 4-AP in urine as a measure of the

conversion of acetaminophen to 4-AP. The concentration of 4-AP in the plasma or liver was not determined. Therefore, the Newton-1982 reference is of limited utility.

The Högestätt-2005 reference is used to characterize the mechanism by which acetaminophen is converted to AM404 (see figure 1). The acetaminophen was administered intraperitoneally. The percentage of acetaminophen that is deacetylated to p-aminophenol in the liver was determined in Högestätt-2005. The percentage of p-aminophenol that is in the circulation, and the percentage of p-aminophenol that is converted to AM404 in the CNS were not determined in Högestätt-2005. However, according to the Sponsor's assessment of Högestätt-2005, the amount of 4-AP relative to acetaminophen is 0.16% in blood and 0.66% in liver tissue and it appears that 4-AP is not a major metabolite of acetaminophen. Thus, Högestätt-2005 may be of limited utility because the study employed intraperitoneal administration, rather than intravenous administration, of acetaminophen.

Regarding the metabolic conversion of 4-AP to acetaminophen, the metabolic studies of 4-AP that were reviewed in the SCCP-2005 and Elder-1988 references were summaries of the findings and did not contain data. As such, the data from metabolic studies investigating the conversion of 4-AP to acetaminophen were not submitted and not analyzed by this reviewer. It is not clear whether any of the studies cited in these review papers were GLP compliant. Moreover, the studies that were conducted employed other methods of administration that were not intravenous. For example, various routes of administration, namely subcutaneous and oral administration, were used in the SCCP-2005 reference and intraperitoneal administration was used in the Högestätt-2005 reference. Therefore, a more definitive study on the pharmacokinetics of the conversion between 4-AP to acetaminophen as well as the conversion between acetaminophen to 4-AP needs to be conducted using the same route of administration as in this NDA.

Single Dose Toxicity

A number of studies have been conducted to evaluate the acute hepatotoxicity and nephrotoxicity of 4-AP. The Sponsor has conducted a literature search of the acute hepatotoxicity of 4-AP.

> A study of the hepatotoxic potential of 4-AP was conducted in male C57BL/6 mice and SD rats (Elder-1988). Single intraperitoneal (IP) 4-AP doses in the range of 100 to 700 mg/kg were administered. Effects on liver function were evaluated by quantifying serum levels of alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH). Livers were also excised for histopathology evaluation. Significant changes in liver function were observed 12 hours after dosing in mice in the dose range of 400 to 700 mg/kg, but not in rats up to the lethal dose of 400 mg/kg. This indicates a limited hepatotoxic potential for 4-AP.
>
> A study was conducted to evaluate the role of N-acetylation of 4-AP to acetaminophen in 4-AP-induced hepatotoxicity in mice (Song-2001). Male C57BL/6 mice were given various single IP doses of 4-AP and euthanized 12 hours later for assessment of serum ALT and SDH levels and determination of the extent of non-protein sulfhydryl (NPSH) and GSH depletion. Plasma levels of acetaminophen and its metabolites were also measured. 4-AP administration depleted NPSH at doses ≥ 400 mg/kg. Buthionine

sulfoximine, an inhibitor of GSH synthesis, potentiated 4-AP-induced hepatotoxicity. Ascorbate, a reducing agent, did not affect 4-AP-induced hepatotoxicity or NPSH depletion. After 4-AP treatment, acetaminophen and its sulfates (glucuronide and GSH conjugates) were detected in the plasma. These results suggest that GSH depletion, as well as N-acetylation of 4-AP to form acetaminophen, and its subsequent metabolism to its oxidative metabolite, may play a role in 4-AP-induced hepatotoxicity at high IP doses in mice.

The Sponsor has conducted a literature search of the acute nephrotoxicity of 4-AP.

Male Fischer 344 (F344) rats received single SC injections of 0, 250, 500, 750, or 900 mg/kg acetaminophen or 0, 25, 50, 100, or 200 mg/kg 4-AP, with sacrifice and blood and urine sampling 24 hours after dosing (Elder-1988, SCCP-2005, Newton-1982). 4-AP did not affect liver function at any tested dose. No effects of 4-AP on the kidney were noted at 25 mg/kg, but accumulation of p-aminohippurate (PAH) was reduced (indicating damage to the straight section of proximal tubules) at doses ≥ 50 mg/kg and serum BUN was elevated at doses ≥ 100 mg/kg. Acetaminophen affected liver function at all tested doses. Kidney function was only affected at acetaminophen doses of 750 (BUN increased 3.3-fold) and 900 mg/kg (BUN increased 5.8-fold). 4-AP was recovered in the urine of acetaminophen treated rats, providing evidence that 4-AP is a metabolite of acetaminophen in rats. This was confirmed when the addition of acetaminophen to the perfusion medium resulted in excretion of 4-AP from isolated perfused kidneys.

4-AP-induced kidney toxicity was further evaluated and strain differences in toxicity were investigated in F344 and SD rats (Elder-1988, SCCP-2005, Newton-1983). Groups of 4 male F344 and SD rats were dosed SC with 0, 50 100, 200, or 400 mg/kg 4-AP. Urine was collected for evaluation of urinary metabolites of 4-AP, and the rats were sacrificed 24 hours post-dose. While no kidney damage was identified microscopically at the 50 mg/kg dose with either rat strain, both strains exhibited dose related proximal tubular necrosis (occurring primarily in the straight segments at lower doses, but extending to the convoluted segments of proximal tubules and possibly distal tubules at higher doses), protein precipitates, casts in proximal and distal tubules, as well as collecting ducts, and numerous protein droplets in the tubular epithelial cells. The extent of damage was greater at comparable doses in F344 rats vs. SD rats. Serum BUN and accumulation of PAH in kidney slices was not affected in either strain at the 50 mg/kg dose. PAH accumulation was reduced in F344 kidney slices at ≥ 100 mg/kg as compared to reduction of accumulation only at 400 mg/kg in SD rats, and serum BUN was elevated at ≥ 200 mg/kg in F334 rats compared to increases at only 400 mg/kg in SD rats. There was evidence of covalent binding of radiolabeled 4-AP to kidney microsomes in both strains. The difference in renal effects of acetaminophen in these two strains of rats may be related to a difference in the rate of conversion to 4-AP.

Additional studies, summarized in the assessments of the safety of 4-AP as a cosmetic ingredient (Elder-1988, SCCP-2005), have been conducted to elucidate the role of 4-AP metabolism in the induction of nephrotoxicity and cellular changes possibly associated with nephrotoxicity. Oxidation of 4-AP apparently plays a role in the induction of nephrotoxicity. The antioxidant ascorbic acid reduced glutathione depletion and cell death in rabbit renal tubular epithelial cells incubated with 4-AP and in rats dosed simultaneously with ascorbic acid and 4-AP (SCCP-2005). In studies conducted with rat renal slices, depletion of reduced GSH occurred prior to 4-AP-induced toxicity becoming manifest and oxidized GSH increased, suggesting induction of oxidative stress (Harmon-

2005). Co-incubation with ascorbic acid maintained GSH levels and prevented 4-AP induced toxicity. Based on these findings, it was hypothesized that ascorbic acid protected renal tissue by preventing redox cycling of 4-AP to BQI with concomitant release of oxygen free radicals. Marked GSH depletion was considered critical to development of 4-AP toxicity.

Repeat Dose Toxicity

The Sponsor submits the following table to illustrate the repeat dose toxicology studies conducted in rats with oral administration of 4-AP:

Table 24. Repeat Dose Toxicology Studies Conducted in Rats with Oral 4-AP

Strain	Dosing Duration	Doses (mg/kg/day)	Compound-Related Findings	Reference
Wistar	13 days	55	None **NOAEL**[1]	SCCP-2005
Wistar	12 weeks	20	None **NOAEL**	SCCP-2005
Sprague-Dawley	9 months	43.5	None **NOAEL**	SCCP-2005, Miller-1948
Sprague-Dawley	13 weeks	0 (Control)	Hyaline droplets in renal epithelial cells of some M/F	SCCP-2005, Burnett-1989
		47	No effects on body wt, food consump, clinical signs, hematology, clin chem, organ wts; increased incidence & severity of hyaline droplets in renal epithelial cells M/F **TDL**[2]	
		133	No effects on body wt, food consump, clinical signs, hematology, clin chem., organ wts; increased incidence & severity of hyaline droplets in renal epithelial cells M/F	
		467	Body wt & food consump ↓ M/F; RBC indices ↓ F but not clinically significant; relative liver & kidney wt ↑ M/F considered an adaptive change due to increased xenobiotic metabolism; increased incidence & severity of hyaline droplets in renal epithelial cells M/F with epithelial degeneration in more severe cases	
Sprague-Dawley	6 months	0 (Control)	Hyaline droplets in renal epithelial cells of some M/F	SCCP-2005, Burnett-1989
		47	No effects on body wt, food consump, clinical signs, hematology, clin chem, organ wts; histopathology not evaluated	
		133	No effects on body wt, food consump, clinical signs, hematology, clin chem., organ wts; histopathology not evaluated	
		467	Body wt ↓ M/F, recovery not complete 11 wks post dosing; no effects on food consump, clinical signs, hematology (including, clin chem; relative liver & kidney wt ↑ M/F with recovery 11 wks post dosing; increased incidence and severity of hyaline droplets in renal epithelial cells M/F, also present in recovery M	

Definitions: NOAEL = No observed adverse effect level; TDL = Toxic dose level

As shown in the table above, there were no relevant treatment-related findings reported in a 13-day study in Wistar rats, in a 12-week study in Wistar rats, and in a 9-month study in Sprague-Dawley rats. A 6-month study in Sprague-Dawley rats with an interim sacrifice at 13 weeks tested the effects of 47, 133, and 467 mg/kg/day 4-AP. The control animals at both the 13-week and 6-month sacrifice showed hyaline droplets in renal epithelial cells in some males and females.

At the 13-week interim sacrifice, there were no effects of body weight, food consumption, clinical signs, hematology, clinical chemistry, and organ weights; however, there was an increased incidence and severity of the hyaline droplets in renal epithelial cells in males and females in both the 47 and 133 mg/kg/day 4-AP groups. In the 467

mg/kg/day 4-AP group, there was a decrease in the body weight and food consumption in males and females, a non-clinically significant decrease in the red blood cell indices in females, a relative increase in liver and kidney weights in males and females that is considered to be an adaptive change due to increased xenobiotic metabolism, and an increased incidence and severity of hyaline droplets in renal epithelial cells in males and females with epithelial degeneration in more severe cases.

At the 6-month sacrifice, there were no effects of body weight, food consumption, clinical signs, hematology, clinical chemistry, and organ weights in both the 47 and 133 mg/kg/day 4-AP groups. It is noted that histopathology was not evaluated in these two treatment groups. In the 467 mg/kg/day 4-AP group, there was a decrease in body weights in males and females that was not completely recovered 11 weeks post dosing, no change in food consumption, clinical signs, hematology, and clinical chemistry, an increase in relative liver and kidney weight in males and females with recovery 11 weeks post dosing, and an increased incidence and severity of hyaline droplets in renal epithelial cells in males and females that was also present in recovery males. From these results, a NOAEL of 467 mg/kg/day in the Sprague-Dawley rats can be established.

The 13-day study in Wistar rats, 12-week study in Wistar rats, 9-month study in Sprague-Dawley rats, and the 6-month study in Sprague-Dawley rats with an interim sacrifice at 13 weeks were in the reference from the SCCP-2005 opinion paper. However, the data was not submitted in the SCCP-2005 opinion paper. As such, the data was not analyzed by this reviewer.

The 13-day study in Wistar rats, 12-week study in Wistar rats, 9-month study in Sprague-Dawley rats investigated the effects of only one dose of 4-AP. Therefore, these studies are of limited utility because no dose-response information can be obtained from these studies.

The 6-month study in Sprague-Dawley rats with an interim sacrifice at 13 weeks investigated the effects of three doses of 4-AP, 47, 133, and 467 mg/kg/day 4-AP. The appropriate parameters of a GLP repeat-dose toxicity study were investigated such as gross examinations, clinical chemistry, hematology, and histopathologic examinations, etc. However, histopathologic examinations were not performed in the 47 and 133 mg/kg/day 4-AP dose groups. Despite having the proper methodology, the SCCP-2005 opinion paper is a summary of data obtained in the studies. As such, the data was not analyzed by this reviewer and the data will be needed in order to give a full assessment of the repeat-dose toxicity of 4-AP. Thus, the repeat-dose toxicity of 4-AP cannot be evaluated.

The Burnett study (1989) investigated the effects of three doses (0.07, 0.2, and 0.7% in feed) of 4-AP in Sprague-Dawley rats for up to 6 months. The appropriate parameters for a repeat-dose toxicity study were evaluated such as blood samples for clinical chemistry and gross and histopathologic examination of organs and tissues. An appropriate number of animals for each dose group was used (groups of up to 40 males and 45 females were used). However, there was no explanation of the actual doses ingested by the rats in the

Burnett study (1989) and how they relate to the doses used in the SCCP-2005 opinion paper. Thus, the Burnett study (1989) is of limited utility.

Reproductive Toxicity

A. Embryo-fetal Development Studies

The Sponsor cites several studies from the published literature that investigated the embryo-fetal development studies on 4-AP.

> Embryo-fetal development was also assessed as part of the previously described chronic toxicology study conducted with oral administration of 4-AP to SD rats (Elder-1988, SCCP-2005, Burnett-1989). After 13 weeks of feeding diet containing 0, 0.07, 0.2, or 0.7% 4-AP, 25 females/group were housed individually, mated to single untreated males and then maintained on control diet throughout the mating period. Once pregnancy was confirmed, females were returned to their original test diets with the same dose of 4-AP throughout the gestation period. Females were euthanized on Day 20 of gestation. The uterus and ovaries were exposed, the numbers of live and dead fetuses and early and late resorptions were recorded, and the total number of corpora lutea on both ovaries was determined. Live fetuses were examined for external gross malformations. Half the live fetuses were then processed for evaluation of soft-tissue anomalies and half for examination of skeletal defects.
>
> Decreased body weight was noted in the mid and high dose females relative to the control group both prior to mating and throughout gestation. There were statistically significant increases in post-implantation loss, reduction in pup weight and delayed ossification in the high dose group, which were considered secondary to maternal toxicity. While there was a statistically significant increase in the incidence of rudimentary 14th ribs in the high dose group, the incidence of litters with this variation was comparable to the historical control incidence for the performing laboratory. Malformations were restricted to a single fetus with an absent tail (also seen in the control group) and 5 fetuses from one litter with bent limb bones in the high dose group (observed previously in control groups at the performing laboratory). In the absence of other malformations, these findings were considered more likely to have resulted from maternal stress or toxicity rather than being indicative of a teratogenic response.
>
> In another embryo-fetal development study conducted in SD rats, 4-AP was administered by oral gavage at doses of 0, 25, 85, or 250 mg/kg/day to groups of 23 or 28 pregnant females from Day 6 to 15 of gestation (Elder-1988, SCCP-2005). No effects were noted at the 25 mg/kg/day dose in this study and maternal toxicity (reduced body weight gain) occurred with no evidence of embryotoxicity or teratogenicity at the 85 mg/kg/day dose. In the 250 mg/kg/day group, reduced maternal body weight gain was accompanied by a low incidence of fetal external (0.8%), visceral (3.9%) and skeletal (4.7%) malformations, indicating teratogenicity at this maternally toxic dose.
>
> In a further embryo-fetal development study, pregnant female Syrian Golden Hamsters were administered 4-AP on day 8 of gestation via three different routes (SCCP-2005, Bomhard-2005). Separate groups of hamsters were administered single IP doses of 100, 150, or 200 mg/kg, IV doses of 100, 150, 200, or 250 mg/kg, or oral gavage doses of 100 or 200 mg/kg. Control animals received saline. A dose-related increase in a variety of malformations coupled with a high incidence of fetal resorption occurred over the tested dose range by both the IP and IV routes of administration with no evidence of maternal toxicity. The finding that BQI, an oxidative metabolite of 4-AP, produced effects similar

> to 4-AP suggests that the teratogenicity of 4-AP may be related to BQI formation and its capacity to readily bind macromolecules when GSH is depleted.

In the first embryo-fetal development study conducted by Burnett-1989, 25 females/group were dose 0, 0.07, 0.2, or 0.7% 4-AP in the diet throughout gestation. Females were euthanized on day 20. However, the actual doses of 4-AP that the rats ingested were not given. The appropriate measures were taken such as the numbers of live and dead fetuses, early and late resorptions, the total number of corpora lutea on both ovaries, and fetal body weight. Live fetuses were examined for external gross malformations such as soft-tissue anomalies and skeletal defects. No NOAEL can be obtained from this study because the doses of 4-AP ingested by the rats were not give. Therefore, this study is of limited utility and is not considered relevant.

The another embryo-fetal development study conducted by Elder-1988, 23 or 28 SD rats were administered 0, 25, 85, or 250 mg/kg/day 4-AP via oral gavage from day 6 to day 15 of gestation. The appropriate measures were taken such as maternal and fetal weight, as well as fetal external, visceral, and skeletal malformations. Therefore, this study is considered relevant. Although there was reduced body weight gain in the 85 mg/kg/day 4-AP group, there was no evidence of embryotoxicity or teratogenicity and thus the NOAEL for embryotoxicity or teratogenicity is 85 mg/kg/day.

In the third study described in SCCP-2005, pregnant female Syrian Golden Hamsters were administered 4-AP on day 8 of gestation via three different routes (single IP doses of 100, 150, or 200 mg/kg, IV doses of 100, 150, 200, or 250 mg/kg, or oral gavage doses of 100 or 200 mg/kg). Control animals received saline. The appropriate measure were taken such as fetal resorptions, fetal malformations. Although the number of hamsters in a group was not stated, this was the only study to include IV administration of 4-AP. It is noted that the data was not submitted in the SCCP-2005 opinion paper. As such, the data was not analyzed by this reviewer. Despite the appropriate methodology, this study is not considered relevant because no data was presented in the SCCP-2005 opinion paper. In fact, the data was presented in the Rutowski-1982 reference.

B. Peri/Postnatal Study

The Sponsor draws upon one study discussed by the Scientific Committee on Consumer Products (SCCP) of the European Commission.

> A single oral gavage dose of 0, 100, 333, 667, or 1,000 mg/kg 4-AP was administered to pregnant female SD rats on Day 11 of gestation (SCCP-2005). Maternal body weight was significantly reduced at doses of 667 and 1,000 mg/kg, associated with decreased postpartum pup weight, tail abnormalities and/or hind limb paralysis at 667 and 1,000 mg/kg and perinatal loss at 1,000 mg/kg. Thus, oral administration of 4-AP to SD rats resulted in peri/postnatal toxicity at high, maternally toxic doses.

The peri/postnatal study was in the reference from the SCCP-2005 opinion paper. However, the data was not submitted in the SCCP-2005 opinion paper. As such, the data was not analyzed by this reviewer. The peri/postnatal study presented in the SCCP-2005 opinion paper was done using 4 doses of 4-AP (100, 333, 667, and 1000 mg/kg 4-AP), administered by oral gavage on day 11 of gestation. The appropriate end points were

measured such as maternal body weight, pup body weight, gross examination of the pup, etc. Despite investigation of the appropriate end points, this study is not considered relevant because the data was not presented in the SCCP-2005 opinion paper. In fact, the data is found in the cited reference Kavlock-1990.

In conclusion, there is insufficient evidence from the embryo-fetal development and peri/postnatal studies done on 4-AP to adequately address the reproductive toxicity of 4-AP. Moreover, the Sponsor has submitted review papers that only summarizes the studies done to address the reproductive toxicity of 4-AP. For example, the SCCP-2005 opinion paper cites Rutowski-1982 for an embryo-fetal development study of 4-AP and Kavlock-1990 for a peri-postnatal study of 4-AP.

Genotoxicity

A. Bacterial Mutagenicity

The Sponsor cites several references from the published literature to address the bacterial mutagenicity of 4-AP. The various studies done on 4-AP evaluated the ability of 4-AP to induce mutations in various strains of *S. typhimurium* and *E. coli*.

> The capacity of 4-AP to induce reverse mutations has been evaluated in the various *S. typhimurium* tester strains (TA97, 98, 100, 102, 1535, 1538) in over 20 separate tests (Bomhard-2005). Most of these studies were performed using standard methods in the absence or presence of liver homogenates from rats as a metabolic activation system (S9). The vast majority of the tests showed no reverse mutations, and only a single positive result (strain TA1537, –S9) was reported. This positive finding was marginal (< 3-fold increase) and not replicated in 9 other studies utilizing the TA1535 tester strain with 4-AP doses as high as 10 mM/plate. Thus, 4-AP is not mutagenic toward the standard tester strains utilized in the Ames *S. typhimurium* reverse mutation assay.
>
> Several bacterial mutagenicity tests have also been conducted with 4-AP utilizing various strains of *E. coli* with the majority of tests showing no mutagenic potential (Bomhard-2005). Positive findings were noted with WP2 Mutoxitest strains IC203 and IC188 but not with IC204, 206 or 208 (Martinez-2000). While results were marginal in strain IC188 (also known as *E. coli* WP2*uvrA*/pKM101), containing the OxyR gene that confers resistance to oxidative stress in *E. coli and S. typhimurium* (Storz-1987), a clearly positive finding was noted with the OxyR deficient strain IC203 in the absence of S9 at the highest tested concentration of 1,000 µg/plate. This suggests that 4-AP elicited a mutagenic response in this *E. coli* tester strain through the induction of oxidative stress. 4-AP also elicited a marginal mutagenic response (a maximum of 3-fold increase at 1,300 µg/plate) in another study which utilized the *E. coli* tester strain WP2*uvrA*/pKM101 (Yoshida-1998). This strain has higher sensitivity to certain oxidative mutagens compared to other bacterial tester strains and mutagenic activity was suppressed with addition of dimethylsulfoxide or catalase, again suggesting involvement of active oxygen species in the bacterial mutagenic process induced by 4-AP.

The Ames test using various *S. typhimurium* and *E. coli* tester strains was cited in the Bomhard-2005 review paper. The Bomhard-2005 reference is a review paper and summarizes data from a number of laboratories that conducted Ames testing of 4-AP. In fact, there are numerous labs that have conducted Ames testing of 4-AP including, but not limited to, DeFlora-1984a and Zeiger-1988 for testing in *S. typhimurium*. As such,

the data was not presented in Bomhard-2005 and was not analyzed by this reviewer. Therefore, the Bomhard-2005 reference is not considered relevant.

The bacterial mutagenicity tests with 4-AP conducted in Martinez-2000, Storz-1987, and Yoshida-1998 did not appear to be GLP compliant. Up to 1300 µg/plate 4-AP were used in these studies. The Martinez-2000 study used *E. coli* WP2 Mutoxitest strains IC203, IC188, IC204, IC206 and IC208. The Storz-1987 study used *E. coli* WP2*uvrA*/pKM101. The Yoshida-1998 study also used WP2*uvrA*/pKM101. Therefore, these studies are not considered relevant.

B. *In Vivo* Rodent Genotoxicity

The Sponsor cited several references in the published literature of various *in vivo* studies done in rodents to address the genotoxicity of 4-AP. These studies include the *in vivo/in vitro* rat liver unscheduled DNA synthesis (UDS) assay, chromosomal aberration assay in the bone marrow of Wistar rats, and a bone marrow micronucleus test.

> A GLP compliant *in vivo/in vitro* rat liver unscheduled DNA synthesis (UDS) assay was conducted with single oral administration of 0, 285, or 1,425 mg/kg 4-AP to groups of male Wistar rats (SCCP-2005). The highest tested dose was 80% of the dose that is lethal to 50% of test animals (LD50) and considered the maximum tolerated dose based on clinical signs and toxic reactions in the treated rats. Positive control compounds (AAF and DMN) were also tested to confirm the sensitivity of the assay procedure. Hepatocytes were isolated from groups of 3 rats per time point 4 and 12 hours post-dose and UDS was evaluated by autoradiography. No increase in mean net nuclear grain counts or increase in percentage of cells in repair occurred at either time point with either of the tested doses of 4-AP. Thus, oral dosing of 4-AP to rats did not cause liver DNA damage that triggered excision repair under the conditions employed in this assay.
>
> The ability of 4-AP to induce chromosomal aberrations in the bone marrow of Wistar rats was evaluated in a GLP compliant study (SCCP-2005). Groups of 5 male and female rats were given single oral doses of 0, 200, 400, or 800 mg/kg 4-AP. The 800 mg/kg dose was considered the MTD based on a preliminary dose range-finding study. Groups of rats were euthanized 24 hours post-dose for all dose groups with a group of rats at the highest tested dose also being euthanized 48 hours post-dose. Mitotic index was determined based on 1,000 cells/animal and at least 100 metaphases/animal were evaluated for chromosome aberrations. A statistically significant increase in cells with chromosome aberrations occurred only in the high dose group 24 hours post-dose, with lethality also noted in this group. However, while there was a statistically significant increase relative to the concurrent control group, the frequency of aberrant cells in this control group was lower than usual and the frequency seen in the high dose group was similar to that seen in the historical control data. Thus, the results of this study were equivocal.…
>
> A repeat dose bone marrow micronucleus test was conducted under GLP conditions and in accordance with OECD guidelines with daily oral administration of 0, 12, or 30 mg/kg 4-AP to groups of 10 SD rats/sex for 13 weeks (SCCP-2005). Slides were prepared from bone marrow taken from each rat, a total of 1,000 erythrocytes were examined. The ratio of PCEs to NCEs was calculated as a measure of toxicity to the bone marrow, and the frequency of micronucleated cells in the PCE population was determined. The ratio of PCEs to NCEs was not affected by 4-AP treatment, and there was no statistically or biologically significant increase in micronucleated bone marrow PCE frequency in 4-AP

> treated rats relative to concurrent control values in this study. Thus, 4-AP is not considered to elicit clastogenic or aneugenic effects in the bone marrow of rats orally administered 4-AP daily at doses as high as 30 mg/kg/day for 13 weeks.

The unscheduled DNA synthesis assay, the chromosomal aberrations assay, and the micronucleus test were in the reference from the SCCP-2005 opinion paper. However, the data was not submitted in the SCCP opinion paper. As such, the data was not analyzed by this reviewer.

The UDS assay presented in SCCP-2005 appears consistent with GLPs. Five (5) male rats/group were used in the study. The maximum dose of 1425 mg/kg was chosen on the basis of clinical signs and toxic reactions. Two doses were given, 285 and 1425 mg/kg 4-AP with 2 sampling times of 4 and 12 hrs. The appropriate positive controls were used (AAF and DMN). Despite the appropriate methodology used in the UDS assay of 4-AP, the SCCP-2005 opinion paper did not present any data from this UDS assay. In fact, the data is found in the reference by Microtest, Report number ILUREBRP (9/1989).

The chromosomal aberrations assay presented in SCCP-2005 is GLP compliant and follows OECD 473 guidelines. Human lymphocytes were obtained from a healthy donor and duplicate cultures were used. However, the positive controls were not given. The appropriate protocol was used (3 hrs treatment with 17 hrs recovery with and without S-9 activation and 20 hrs treatment with no recovery without S-9 activation). Despite the appropriate methodology used in the chromosomal aberrations assay of 4-AP, the SCCP-2005 opinion paper did not present any data from this chromosomal aberrations assay. In fact, the data is found in the reference by Microtest, Report number 1HLRREBRP (1/1990).

The micronucleus test presented in SCCP-2005 appears consistent with GLPs. Five (5) male and 5 female Swiss mice were present in each group. The maximum dose of 500 mg/kg was chosen on the basis of clinical signs and toxic reactions. Three dose levels of 4-AP were used (170, 250, and 500 mg/kg) with either a 24 or 48 hrs sacrifice time. Although the negative and positive controls were not listed, they are stated to be OECD compliant. A total of 1000 erythrocytes from each animal were scored. Despite the appropriate methodology used in the micronucleus test of 4-AP, the SCCP-2005 opinion paper did not present any data from this micronucleus test. In fact, the data is found in the reference by the Centre International de Toxicologie, Report number 7757 MAS (5/1992).

There was no data submitted in the SCCP-2005 opinion paper. As such, a full evaluation of the Ames test using various *S. typhimurium* and *E. coli* tester strains, unscheduled DNA synthesis assay, the chromosomal aberrations assay, and the micronucleus test done on 4-AP cannot be performed. Thus, there is insufficient information on which to determine the genetic toxicity of 4-AP.

Carcinogenicity

There was a 2-year rat carcinogenicity study done on 4-AP in the published literature.

> 4-AP was assessed for carcinogenic activity in SD rats in a GLP compliant study conducted in accordance with OECD guidelines (Bomhard-2005). Groups of 50 rats/sex were orally administered daily doses of 0, 2, 5, 12, or 30 mg/kg 4-AP. The high dose was selected for this study based on the occurrence of minimal to marked tubular nephrosis with daily doses of 30 and 100 mg/kg 4-AP in a previously conducted 13-week study in SD rats, with 30 mg/kg/day being considered the MTD. The duration of dosing was initially intended to extend to 104 weeks, but due to reduced survival in the control groups (26% male and 32% female survivors) after 101 weeks of dosing, the study was terminated and surviving animals were euthanized. No major differences were noted in 4-AP treated rats vs. controls during the course of dosing with respect to body weight changes or lethality, except for reduced survival in the high dose females relative to control females (20% compared to 32% at Week 101). Orange colored urine was noted from Week 10 onward in almost all rats in the high dose group. The incidence of benign and malignant tumors in the 4-AP treated rats vs. controls was similar, except for a marginal increased incidence of malignant lymphoma in the high dose males (3 in the high dose group vs. 1 in the control and 1 in the low dose group). It was concluded that 4-AP showed neither carcinogenic potential nor any effect on the incidence of spontaneously occurring tumors at any dose level in this study.

The carcinogenicity study cited in Bomhard-2005 was referenced in a review paper. As such, the data was not presented in Bomhard-2005 and was not analyzed by this reviewer. The Bomhard-2005 reference discussed the genetic toxicity of various aniline compounds including 4-AP. In fact, the carcinogenicity of 4-AP is not discussed at all in the Bomhard-2005 review. As such, it is not certain what reference the carcinogenicity study of 4-AP came from. Thus, there is insufficient information to evaluate on the carcinogenicity of 4-AP.

Conclusion

The Sponsor makes several conclusions regarding the toxicological risk assessment of 4-AP. 4-AP appears to be a minor metabolite in experimental animals with 0.16% in blood and 0.66% in liver tissue. 4-AP appears to cause similar toxic events as acetaminophen. The various embryo-fetal development and peri/postnatal studies done with 4-AP demonstrate that there was a dose dependent increase in the number of fetal malformations. The results of the bacterial and *in vivo* rodent genotoxicity studies with 4-AP appear to show 4-AP is not mutagenic (as demonstrated by negative results from the Ames test) but may be clastogenic (as demonstrated by equivocal results in the chromosomal aberrations assay). From a 2-year rat carcinogenicity study, 4-AP is not carcinogenic. However, most of the references that the Sponsor submits in support of these conclusions were from review papers that cited studies from the published literature. The review papers that were submitted, SCCP-2005 and Bomhard-2005, do not contain data but is rather a summary of studies done. As such, the data from the studies regarding the metabolism, reproductive toxicity, genetic toxicity, and carcinogenicity of 4-AP were not fully reviewed. Therefore, there is insufficient evidence to make any conclusions with regard to the metabolism, reproductive toxicity, genetic toxicity, and carcinogenicity of 4-AP.

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Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22450	ORIG-1	CADENCE PHARMACEUTICALS INC	ACETAMINOPHEN FOR INJECTION FOR IV USE

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

CARLIC K HUYNH
11/03/2009

RICHARD D MELLON
11/03/2009
See secondary pharmacology/toxicology review for further discussion of deficiencies identified during this review cycle.

PHARMACOLOGY/TOXICOLOGY NDA FILEABILITY CHECKLIST

NDA/BLA Number: 22-450 **Applicant: Cadence Pharmaceuticals, Inc.** **Stamp Date: May 13, 2009**

Drug Name: Acetaminophen injection **NDA/BLA Type: 505(b)(2)** **DAARP/OND/CDER/FDA**

On **initial** overview of the NDA application for Refuse to File (RTF): **This NDA may be filed**

	Parameters	Yes	No	Comment
1	On its face, is the pharmacology section of the NDA/BLA organized (in accord with 21 CFR 314 and current guidelines for format and content) in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section of the NDA/BLA indexed and paginated in a manner allowing substantive review to begin?	X		
3	On its face, is the pharmacology/toxicology section of the NDA/BLA legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505(b1) and (b2) including referenced literature) completed and submitted in this NDA (carcinogenicity*, mutagenicity*, teratogenicity*, effects on fertility*, juvenile studies, acute and repeat dose adult animal studies*, maximum tolerated dose determination, dermal irritancy, ocular irritancy, photo co-carcinogenicity, animal pharmacokinetic studies, safety pharmacology, etc)?			Not applicable. The Sponsor did not conduct any new nonclinical studies. The submitted 505(b)(2) New Drug Application (NDA) included referenced nonclinical studies. The Sponsor relies upon the literature for carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, maximum tolerated dose determination, dermal irritancy, ocular irritancy, photo co-carcinogenicity, animal pharmacokinetic studies, safety pharmacology, etc. However, the Sponsor has previously submitted a pharmacology study to test the analgesic activity of acetaminophen after IV administration in mice, two repeat dose toxicity studies of IV administered acetaminophen in rats, genotoxicity studies, a skin sensitization test in guinea pigs, an IV infusion study in rabbits, and a

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				dependence study after IV administration.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies been conducted with the appropriate formulation?	X		The formulation of this NDA is a solution for injection and is not the same as the referenced products (Ultracet® and Tylenol®), which are oral tablet formulations. This NDA contains two repeat-dose toxicity studies in rats administered acetaminophen intravenously.
6	Is (are) the excipient(s) appropriately qualified (including interaction between the excipients if applicable)?	X		This NDA contains excipients that are found in the FDA IIG. The amount of excipients in this formulation is below the maximum potency.
7	On its face, does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted a rationale to justify the alternative route?	X		This NDA contains toxicity studies of acetaminophen in rats administered intravenously.
8	Has the sponsor submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?		X	NOTE: The pharmacology study was not performed in accordance with GLP. The repeat-dose toxicity studies in rats were performed in accordance with GLP. The genotoxicity studies were conducted according to GLPs in the UK. Local tolerance studies were performed in accordance with GLP. Dependence studies were performed in accordance with GLP.
9	Has the sponsor submitted all special studies/ data requested by the Division during pre-submission discussions with the sponsor?			Not applicable. There was no pre-NDA meeting with the Sponsor. The Sponsor did not conduct any new nonclinical studies. The submitted 505(b)(2) New Drug Application (NDA) included referenced nonclinical studies.
10	Are the proposed labeling sections relative to pharmacology, reproductive toxicology, and carcinogenicity appropriate (including human dose multiples expressed in either mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?	X		

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11	Has the sponsor submitted any toxicity data to address impurities, new excipients, leachables, etc. issues.		X	Justification provided in module 3 based on manufacturing capabilities for proposed (b) (4) expiry. Acceptable as per ICHQ3B(R2) for non-structural alert, but not acceptable based on FDA guidance on genotoxic impurities. NOTE: The Sponsor did not submit extractables/leachables testing data (b) (4) or justification for the safety of the proposed 4-aminophenol via IV route.
12	Has the sponsor addressed any abuse potential issues in the submission?	X		The Sponsor submitted an "evaluation of dependence after IV of the naloxone-precipitated withdrawal test in mice".
13	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable. This is a 505(b)(2) New Drug Application (NDA) submitted to support a Rx.
14	From a pharmacology/ toxicology perspective, is the NDA/BLA fileable? If ``no`` please state below why it is not.		X	PROPOSED FILING ISSUES: DP impurity safety information has not been provided to support the proposed stability specification. Extractables/leachables testing (b) (4) (CMC review team indicated that information is in DMF 4681, therefore, this is a review issue).

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Note: Although the DAARP nonclinical review team recommended that the NDA not be filed until adequate justification for the safety of proposed levels of 4-AP in the drug product is provided, Drs. Paul Brown and David Jacobson-Kram, Associate Directors of Pharmacology and Toxicology for the ODE2 and OND, respectively, indicated that this should not be considered a filing issue. Therefore, the NDA may be filed.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Comments to Sponsor:
Please provide the following information:

- Your NDA submission does not contain a toxicological risk assessment for the safety of the drug product degradant, 4-aminophenol, which contains a structural alert for genotoxicity and carcinogenicity. As per the published FDA Draft Guidance to Industry, levels of this impurity should be reduced to NMT (b) (4) unless otherwise justified based on a toxicological risk assessment. In the absence of adequate justification, adequate safety qualification for any potential genotoxic impurities must include:

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- Minimal genetic toxicology screen (two in vitro genetic toxicology studies (point mutation assay and chromosomal aberration assay) with the isolated impurity, tested up to the limit dose for the assay.

- Should either of these genetic toxicology studies yield positive or equivocal results, the impurity specification must be set at NMT (b) (4) or otherwise justified. Justification may require an assessment for carcinogenic potential in either a standard 2-year rodent bioassay or an appropriate transgenic mouse model.

- Submit a comprehensive toxicological risk assessment for 4-aminophenol which specifically address the potential for this compound to contribute to the risk of genetic toxicity, carcinogenicity, reproduction and developmental toxicity and general toxicity (specifically hepatic and renal toxicity). This assessment must include data for the exposures of the animal in your toxicology studies to this impurity via the batches tested and how these levels compare to NOAELs for toxicity.

- Submit the toxicological risk assessment for the safety of the drug product degradant, 4-aminophenol by August 13, 2009.

Reviewing Pharmacologist: _____
Date

Team Leader: _____
Date

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/s/

Carlic K Huynh
7/9/2009 02:36:34 PM
PHARMACOLOGIST

R. Daniel Mellon
7/9/2009 02:39:33 PM
PHARMACOLOGIST
I concur.