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APPLICATION NUMBER:

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NON-CLINICAL REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	NDA 209471
Supporting document/s:	SDN 37, 46, and 47 (Electronic Document Room Sequence number 36, 45, and 46)
Applicant's letter and CDER Stamp date:	July 27, 2021 (SDN 37), November 29, 2021 (SDN 46), and December 7, 2021 (SDN 47)
Product:	Combogesic® (Acetaminophen 325 mg and Ibuprofen 97.5 mg) tablet
Indication:	For the short-term management of mild to moderate acute pain
Applicant:	AFT Pharmaceuticals Limited
Review Division:	Division of Anesthesiology, Addiction Medicine, and Pain Medicine (DAAP)
DPT-N Reviewer:	Carlic K. Huynh, PhD
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Template Version: September 1, 2010

Disclaimer

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1 Executive Summary

1.1 Introduction

The Applicant, AFT Pharmaceuticals Limited, has developed Combogesic® (acetaminophen 325 mg/ibuprofen 97.5 mg) tablets for the short-term management of mild to moderate acute pain using the oral route of administration. This is a 505(b)(2) application referencing Ultracet® (NDA 21123) and Motrin® (NDA 17463) for the Agency's previous determination of safety for acetaminophen and ibuprofen, respectively. This is the third cycle review. All nonclinical deficiencies were adequately resolved at the conclusion of the second review cycle and no new nonclinical deficiencies were identified. However, a Complete Response was issued for the proposed product after the second cycle review due to facility inspections issues.

1.2 Brief Discussion of Nonclinical Findings

No new toxicology studies were submitted to support this NDA resubmission (see previous nonclinical reviews). The Applicant submitted a new formulation, elemental impurities evaluation, and new drug substance and drug product specifications that were reviewed by the Pharmacology Toxicology review team. The new formulation does not contain any novel excipients. The drug substance specifications for acetaminophen and ibuprofen, drug product specifications for Combogesic®, and elemental impurities evaluations were reviewed and deemed acceptable.

1.3 Recommendations

1.3.1 Approvability

From the nonclinical Pharmacology Toxicology perspective, NDA 209471 may be approved with the following recommended changes in labeling (see 1.3.3 below).

1.3.2 Additional Nonclinical Recommendations

None at this time.

1.3.3 Labeling

The following table contains the draft labeling proposed by the Applicant with the changes proposed by this Reviewer and the rationale for the proposed changes. Several proposed labeling changes are from a literature review previously conducted for acetaminophen (Appendix 1) and ibuprofen (Appendix 2). The reader is referred to the final action letter for the final drug product labeling.

Applicant's proposed labeling	***Reviewer's proposed changes***	***Rationale for changes***

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

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Background and Prior Regulatory History (Nonclinical):
The Applicant, AFT Pharmaceuticals Limited, has developed Combogesic® (acetaminophen 325 mg/ibuprofen 97.5 mg) tablets for the short-term management of mild to moderate acute pain using the oral route of administration. As such, the proposed product is considered an acute use product. Combogesic®, which is developed specifically for the U.S. market, is a lower strength formulation of Maxigesic® (acetaminophen 500 mg/ibuprofen 150 mg) that maintains the same ratio of acetaminophen and ibuprofen. Maxigesic® is marketed in New Zealand, Australia, United Arab Emirates (UAE), Singapore, Malaysia, and more than 15 European countries. This is a 505(b)(2) application referencing Ultracet® (NDA 21123) and Motrin® (NDA 17463) for the Agency's previous determination of safety for acetaminophen and ibuprofen, respectively.

There were no nonclinical deficiencies raised at the conclusion of the second cycle review. In this submission, the Applicant submitted a new formulation, as well as new drug substance and drug product specifications that requires review from the Pharmacology Toxicology review team.

The maximum daily dose MDD is based on 12 tablets, which totals 3900 mg of acetaminophen (325 mg/tablet x 12 tablets = 3900 mg) and 1170 mg of ibuprofen (97.5 mg/tablet x 12 tablets = 1170 mg).

Formulation:
The updated formulation (commercial batches) is illustrated in the following table:

Combogesic® Excipient Quantities at the MDD of 3.9 g/day APAP			
Excipient	**Quantity per tablet (mg)**	**Quantity at MDD of 3.9 g/day APAP (12 tablets)**	**Comments**
Sodium lauryl sulfate	(b) (4)		Adequately qualified via FDA-approved oral products
Microcrystalline cellulose			
Povidone-30 (Povidone K-30)			
Croscarmellose sodium			
Lactose monohydrate			
Magnesium stearate			
(b) (4)			(b) (4) does not appear to be in the FDA Inactive Ingredient Database. However, each individual ingredient is adequately qualified via FDA-approved oral products at the worse-case of 156 mg/day maximum daily intake.
Hypromellose Titanium dioxide Polydextrose Talc Maltodextrin	NS	NS	
(b) (4)	(b) (4)	(b) (4)	(b) (4)
	--	--	
	NS	NS	

(b) (4)			(b) (4)

NS- not specified

There are no novel excipients in the formulation, as each excipient at the MDD of 12 tablets/day are within levels in FDA approved drug products. As shown in the table above, the formulation is acceptable.

Drug Substance:
The following table illustrates the acetaminophen drug substance manufacturer specifications from (b) (4), which was formally (b) (4) (from the Applicant's submission):

Impurity	Structure	Specification	Comment
(b) (4)			

[redacted]

As shown in the table above, the specifications in the acetaminophen drug substance manufacturer specifications are acceptable with the exception of [redacted]. Moreover, APAP drug substance from [redacted]) has been in numerous FDA approved oral formulations.

[redacted] The current drug substance specification for [redacted] is NMT [redacted]% (per Ph Eur), however, [redacted] contains a structural alert for mutagenicity. A search of the published literature did identify several studies that suggested that the compound has been tested in the Ames assay (*Salmonella* mutagenesis only) and found to be negative [redacted]

[redacted] tested the ability of [redacted] to form mutations in several *Salmonella* strains (TA100, TA1535, TA1537, and TA98) with and without S9 metabolic activation. The doses used in [redacted]. were up to 1000 mcg for non-toxic compounds. [redacted]. is a review paper and does not contain the raw data as seen in final study reports. Although the standard 5000 mcg/plate and *E. coli* WP2 strain were not used, Bruce Ames was one of the authors listed as contributing to the study and more than likely, the methods used are appropriate for a valid study.

[redacted] tested the ability of [redacted] to form mutations in several *Salmonella* strains (TA1535, TA1537, TA98, and TA100) with and without S9 metabolic activation. The doses used in [redacted]. were up to 10 mg/plate, which is greater than the 5 mg/plate. The appropriate positive controls were used for each strain. However, the *E. coli* WP2 strain was not used and this paper does not contain the raw data as seen in final study reports. Nonetheless, the method developed by Bruce Ames was used. Thus, the methods used are more than likely appropriate for a valid study.

Moreover, [redacted] is very similar to the profile for the well-known impurity [redacted]. The Agency has requested that [redacted] be reduced to as low as technically feasible in the drug substance. In previous review cycles, [redacted] has been reduced to NMT [redacted]%, which was considered as low as technically feasible (the reader is referred to the quality review for further details). Thus, the acetaminophen drug substance specifications are acceptable.

The following table illustrates ibuprofen drug substance manufacturer specifications from [redacted] (from the Applicant's submission):

Impurity	Structure	Specifications	Acceptable?
(b) (4)			

As shown in the table above, the ibuprofen drug substance manufacturer specifications from (b) (4) are acceptable as they meet ICH Q3A(R2) qualification thresholds.

Drug Product:
The drug product specifications for Combogesic® tablets (release and shelf-life) are illustrated in the following tables (from the Applicant's submission):

Degradant	Specification (Release and Stability)	Comments
(b) (4)		

As shown in the table above, the DP specification are acceptable.

Elemental Impurities:
The elemental impurities assessment was performed and the results are illustrated in the following table (from the Applicant's submission):

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Table 14 Evaluation of the elemental impurities in reformulated Combogesic by option 1

(b) (4)

As shown in the table above, the elemental impurities in the elemental impurities assessment meet ICH Q3D limits (oral PDE) at the maximum daily intake of 12 tablets/day and therefore are deemed acceptable.

Conclusions and Recommendations:
No new toxicology studies were submitted to support this NDA submission. There are no safety issues regarding the formulation and the elemental impurities assessment. The drug substance specifications for acetaminophen and ibuprofen as well as the drug product specifications are acceptable. From the nonclinical Pharmacology Toxicology perspective, there are adequate data to support an approval recommendation.

References:

(b) (4)

Appendix 1: Nonclinical Recommendations for Acetaminophen Rx Labeling

The following labeling recommendations for acetaminophen (MDD of 3.9 grams/day via 12 tablets/day) are based upon a review of the literature completed by R. Daniel Mellon, PhD in 2010 and updated by Carlic Huynh, PhD in 2016.

Recommended Labeling	Rationale/Comment
8 USE IN SPECIFIC POPULATIONS **8.1 Pregnancy** **Nonclinical Risk Summary Statement (Human data to be provided by Maternal Health Review Team)** Animal reproduction studies have not been conducted with IV acetaminophen. Reproductive and developmental studies in rats and mice from the published literature identified adverse events at clinically relevant oral doses of acetaminophen. Treatment of pregnant rats with doses of acetaminophen approximately equal to the maximum human daily dose (MHDD) showed evidence of fetotoxicity and increases in bone variations in the fetuses. In another study, necrosis was observed in the liver and kidney of both pregnant rats and fetuses at doses approximately equal to the MHDD. In mice and rats treated with acetaminophen at doses within the clinical dosing range, cumulative adverse effects on reproductive capacity were reported. In mice, a reduction in number of litters of the parental mating pair was observed as well as retarded growth, abnormal sperm in their offspring, and reduced birth weight in the next generation. In rats, female fertility was decreased following in utero exposure to acetaminophen [see *DATA*].	See labeling for human risk summary statement The proposed risk summary statement is based on the data in the animal data section below.
The estimated background risk of major birth defects and miscarriage for the indicated population is unknown. All	PLLR Boilerplate to date.

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Recommended Labeling	Rationale/Comment
pregnancies have a background risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.	
Animal Data Studies in pregnant rats that received oral acetaminophen during organogenesis at doses up to 0.85 times the maximum human daily dose (MHDD = 3.9 grams/day, based on a body surface area comparison) showed evidence of fetotoxicity (reduced fetal weight and length) and a dose-related increase in bone variations (reduced ossification and rudimentary rib changes). Offspring had no evidence of external, visceral, or skeletal malformations.	The statement regarding bone effects in the rat model represents collective review of the data reported in the studies from Burdan (2000, 2001, and 2003).
When pregnant rats received oral acetaminophen throughout gestation at doses of 1.2-times the MHDD (based on a body surface area comparison), areas of necrosis occurred in both the liver and kidney of pregnant rats and fetuses. These effects did not occur in animals that received oral acetaminophen at doses 0.3-times the MHDD, based on a body surface area comparison.	These rat data regarding liver and kidney toxicity are from Neto et al (2004).
In a continuous breeding study, pregnant mice received 0.25, 0.5, or 1.0% acetaminophen via the diet (357, 715, or 1430 mg/kg/day). These doses are approximately 0.43, 0.87, and 1.7 times the MHDD, respectively, based on a body surface area comparison. A dose-related reduction in body weights of fourth and fifth litter offspring of the treated mating pair occurred during lactation and post-weaning at all doses. Animals in the high dose group had a reduced number of litters per mating pair, male offspring with an increased percentage of abnormal	The mouse data are from the NTP reproductive toxicology study as summarized by Reel et al. (1992).

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Recommended Labeling	Rationale/Comment
sperm, and reduced birth weights in the next generation pups.	
8.3 Females and Males of Reproductive Potential Published animal studies report that oral acetaminophen treatment of male animals at doses that are 1.2 times the MHDD and greater (based on a body surface area comparison) result in decreased testicular weights, reduced spermatogenesis, reduced fertility, and reduced implantation sites in females given the same doses. Additional published animal studies indicate that acetaminophen exposure in utero adversely impacts reproductive capacity of both male and female offspring at clinically relevant exposures [see *Nonclinical Toxicology (13.1)*].	Section 8.3 was added as several published studies report effects of acetaminophen on reproductive potential. The multigenerational study was conducted by the NTP (Lamb, 1997; Program, 1984; and Reel et al., 1992). Fertility studies were summarized in the literature (Boyd and Hogan, 1968; Jacqueson et al., 1984; Lamb, 1997; Yano and Dolder, 2002; and Holm et al., 2016).
13 NONCLINICAL TOXICOLOGY	
13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility <u>Carcinogenesis</u> Long-term studies in mice and rats have been completed by the National Toxicology Program to evaluate the carcinogenic potential of acetaminophen. In 2-year feeding studies, F344/N rats and B6C3F1 mice were fed a diet containing acetaminophen up to 6000 ppm. Female rats demonstrated equivocal evidence of carcinogenic activity based on increased incidences of mononuclear cell leukemia at 0.8 times the maximum human daily dose (MHDD) of 3.9 grams/day, based on a body surface area comparison. In contrast, there was no evidence of carcinogenic activity in male rats (0.7 times) or mice (1.2-1.4 times the MHDD, based on a body surface area comparison).	Based on current CDER ECAC standards, the mononuclear cell leukemia findings were deemed significant. However, the ECAC concluded that the finding is considered to have limited relevance to humans. We elected to leave the “equivocal” statement in the labeling, as that is what the NTP concluded at the time and is how the results were described in the study report.

Recommended Labeling	Rationale/Comment
Mutagenesis Acetaminophen was not mutagenic in the bacterial reverse mutation assay (Ames test). In contrast, acetaminophen tested positive in the in vitro mouse lymphoma assay and the in vitro chromosomal aberration assay using human lymphocytes. In the published literature, acetaminophen has been reported to be clastogenic when administered a dose of 1500 mg/kg/day to the rat model (3.6-times the MHDD, based on a body surface area comparison). In contrast, no clastogenicity was noted at a dose of 750 mg/kg/day (1.8-times the MHDD, based on a body surface area comparison), suggesting a threshold effect.	Findings from the Ames and in vitro chromosomal aberrations assays are from the studies conducted by the NTP. Although there are several studies in the literature that suggest the same conclusion, the NTP studies are the most definitive studies and result in the same basic message. The NTP has not conducted an in vivo assay for chromosomal damage. There are, however, numerous reports, as summarized in several articles (Rannug et al., 1995, Bergman et al., 1996) that demonstrate positive findings for clastogenicity in both animals and humans. The summary article (Bergman et al. 1996) suggests that there should be a threshold for these effects and that they likely occur at hepatotoxic doses. Definitive concurrence with such a conclusion would require careful evaluation of the underlying data that are not available to the Agency. The proposed labeling reflects the results for the pivotal in vivo study described in the Bergman review. Since the data are consistent with the carcinogenicity study results, and the study was apparently completed for the German regulatory authorities, the reported results will be included in product labeling.
Impairment of fertility In studies conducted by the National Toxicology Program, fertility assessments with acetaminophen have been completed in Swiss CD-1 mice via a continuous breeding study. There were no effects on fertility parameters in mice consuming up to 1.7 times the MHDD of acetaminophen, based on a body surface area comparison. Although there was no effect on sperm motility or sperm density in the epididymis, there was a significant	To date, the results of the fertility endpoints from Reel et al. (1992) serve as the primary data on fertility.

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Recommended Labeling	Rationale/Comment
increase in the percentage of abnormal sperm in mice consuming 1.7 times the MHDD (based on a body surface area comparison) and there was a reduction in the number of mating pairs producing a fifth litter at this dose, suggesting the potential for cumulative toxicity with chronic administration of acetaminophen near the upper limit of daily dosing. Published studies in rodents report that oral acetaminophen treatment of male animals at doses that are 1.2 times the MHDD and greater (based on a body surface area comparison) result in decreased testicular weights, reduced spermatogenesis, reduced fertility, and reduced implantation sites in females given the same doses. These effects appear to increase with the duration of treatment.	The statement regarding testicular findings is derived from studies in rats by Boyd and Hogan (1968) and Jacqueson et al (1984) which reported decreased testicular weights and spermatogenesis following dosing of acetaminophen at 1.2 times the MHDD for longer than 30-days. There are data in the rat reporting effects on sexual behavior, sperm parameters, early implantation, and fertility at doses that are also only 1.2 fold the maximum human dose (Ratnasooriya and Jayakody, 2000). Ultrastructural changes in the testes have been reported after a single dose of oral APAP (1.6 times the MHDD) to the male rat by Yano et al. (2002).
In a published mouse study, oral administration of 50 mg/kg acetaminophen to pregnant mice from Gestation Day 7 to delivery (0.06 times the MHDD) reduced the number of primordial follicles in female offspring and reduced the percentage of full term pregnancies and number of pups born to these females exposed to acetaminophen in utero.	Data Source (Holm et al., 2016).
In a published study, pregnant rats oral administration of 350 mg/kg acetaminophen (0.85 times the MHDD) from Gestation Day 13 to 21 (dams), reduced the number of germ cells in the fetal ovary and decreased ovary weight and reduced number of pups per litter in F1 females as well as reduced ovary weights in F2 females.	Data Source (Dean et al., 2016).

REVIEW OF SUPPORTING DATA:

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 United States National Toxicology Program (NTP) has conducted in vitro mutagenicity and clastogenicity studies on APAP (National Toxicology Program, 1993). The results of the NTP studies indicate that acetaminophen tests negative as a mutagen; however, it tests positive as a clastogen in vitro (induced sister chromatid exchanges and chromosomal aberrations in CHO cells). These data are available to the public, employed current protocols and were conducted in accordance with Good Laboratory Practices (GLPs). These findings also should be included in product labeling. Although there are numerous published studies that support the conclusion that APAP is clastogenic in vitro (Ibrulj et al., 2007), the results of the NTP studies provide adequate data to support such a statement 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. The NTP has not 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 publicly available. 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 are not 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 obtained by the review team and determined to be an abstract of a presentation. As this reference did not provide actual data, it normally would 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.

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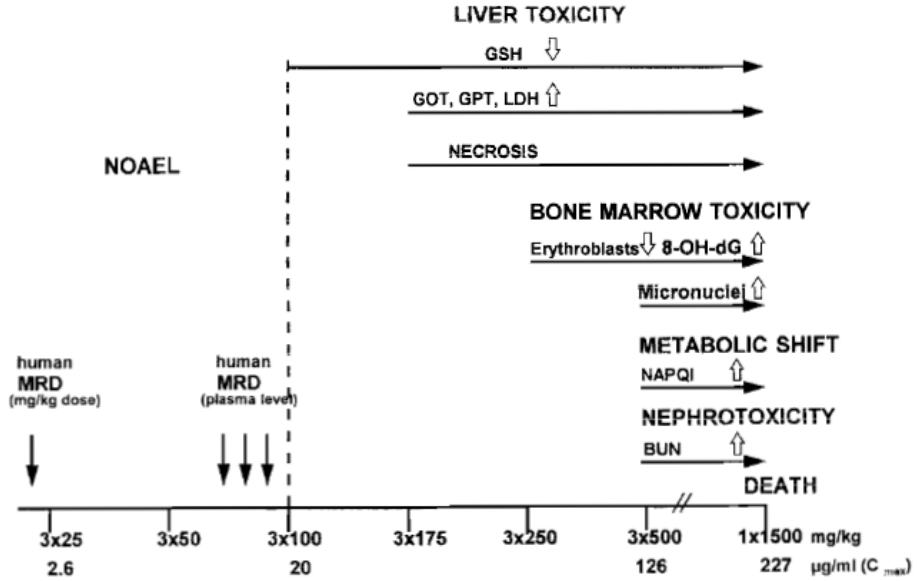

Fig. 1. Paracetamol: Oral gavage toxicity and micronucleus studies in rats. Onset of various toxicity related effects in relation to dosages used and peak plasma levels determined. Arrows illustrate increases or decreases in the relevant parameters determined. The human maximum recommended dosage (MRD) 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 would be reviewed by the Agency. However, the proprietary data could not be obtained; therefore, these results cannot be independently verified. However, as this finding is key to the conclusion that a NOEL 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.

Oshida and colleagues examined the in vivo effects of APAP in the comet assay (Oshida et al., 2008). These investigators treated mice with 12, 60, or 300 mg/kg APAP, IP and examined the liver, kidney, and bone marrow for evidence of DNA damage via the comet assay and examined cytotoxicity via hematology and clinical chemistry parameters. The high dose of APAP produced a positive response in the liver only, suggesting a threshold for genotoxicity, however, the effect also correlated with cytotoxicity. Therefore, the positive comet assay results may be due to cytotoxicity rather than genotoxicity. Based on a body surface area basis, the NOEL of 60 mg/kg

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(180 mg/m^2) provides an exposure margin of 0.07. The high dose of 300 mg/kg (900 mg/m^2) provides an exposure margin of 0.36, suggesting that the hepatotoxicity or genotoxicity occurs at clinically relevant exposures. However, given the uncertainty if the finding is due to cytotoxicity or actual genotoxicity in this study, this study is not recommended to be included in the product labeling.

Carcinogenicity of APAP. Studies conducted by the NTP to evaluate the carcinogenic potential of acetaminophen can be used to support oral acetaminophen drug product prescription labeling. The study reports are available publicly. 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:

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Acetaminophen

Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Feed concentration			
0, 600, 3,000, or 6,000 ppm acetaminophen	0, 600, 3,000, or 6,000 ppm acetaminophen	0, 600, 3,000, or 6,000 ppm acetaminophen	0, 600, 3,000, or 6,000 ppm acetaminophen
Body weights			
Dosed groups similar to controls	Dosed groups similar to controls	Dosed groups lower than controls	Dosed groups lower than controls
2-Year survival rates			
27/50; 28/50; 23/50; 24/50	30/50; 34/50; 34/50; 28/50	32/50; 40/50; 31/50; 31/50	27/50; 32/50; 25/50; 38/50
Nonneoplastic effects			
Kidney: nephropathy severity grades (2.30, 2.56, 2.64, 2.78); renal tubule hyperplasia (1/50, 5/50, 3/50, 5/50) Parathyroid gland: hyperplasia (0/42, 4/45, 6/46, 8/45)	Kidney: nephropathy severity grades (1.44, 1.58, 1.64, 1.72)	Thyroid gland: follicular cell hyperplasia (0/49, 6/49, 12/50, 15/50)	Thyroid gland: follicular cell hyperplasia (2/48, 8/50, 11/50, 25/50)
Neoplastic effects			
None	None	None	None
Uncertain findings			
None	Mononuclear cell leukemia: 9/50; 17/50; 15/50; 24/50	None	None
Level of evidence of carcinogenic activity			
No evidence	Equivocal evidence	No evidence	No evidence
Genetic toxicology			
Salmonella typhimurium (gene mutation)	Negative in strains TA100, TA1535, TA1537, and TA98 with and without S9		
Sister chromatid exchanges (Chinese hamster ovary cells *in vitro*)	Positive with and without S9		
Chromosomal aberrations (Chinese hamster ovary cells *in vitro*)	Positive with and without S9		

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Mean daily doses of APAP consumed were calculated based on mean food consumption over the course of the above studies 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	1.3
Female Mice	114	603	1187	3561	1.5

*Exposure margin based on body surface area comparison to the maximum adult human dose of 3900 mg/day (2405 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 (ECAC) on February 2, 2010. Based upon current CDER criteria, the mononuclear cell leukemia noted in the female rats were significant rather than equivocal; however, the ECAC 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.”

Effects on Fertility. A review of the literature identified three publications that provide some data relevant to fertility studies (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.

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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 cytosol glutathione transferase activity and of lipid peroxides. The authors note that the treatment did not result in decreased testicular glutathione levels; therefore, the toxicity cannot 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 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 NTP 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 cannot be obtained via this study.

Reference ID: 4913649

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.

TABLE 5
Sperm Analysis for F_1 Male Mice at Necropsy following Continuous Exposure to Acetaminophen (Task 4)[a]

	% Acetaminophen in the diet	
Parameter[b]	0	1.0
Percentage motile sperm	51 ± 5	55 ± 3
Sperm density (number of sperm × 10^3/mg cauda epididymis)	925 ± 56	1038 ± 62
Percentage abnormal sperm[c]	7.3 ± 0.8	16.4 ± 2.6*

[a] These F_1 male mice are the same ones used in the mating trial (Table 3).
[b] Values are means ± SEM. Number of males: 19 for the control group and 20 for the 1.0% group.
[c] Tailless sperm not included in determination of percentage abnormal sperm.
* Significantly different ($p < 0.01$) from the control group.

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).

A rat study on male fertility was conducted by Kristensen et al. (2011) where Wistar rat dams were dosed 150, 250, and 350 mg/kg/day of APAP (0.36x, 0.61x, and 0.85x the maximum daily human dose of 4000 mg based on a body surface area comparison) from Gestational Day (GD) 13 to 21 via oral gavage. Following dosing, the anogenital distance (AGD), which is the distance from the anus to the genitalia (mm), was measured and an AGDi was calculated by dividing the AGD by the cube root of the body weight in kg for each rat. Rat studies using testosterone and an anti-androgen have indicated that androgen deficiency during a critical male programming window from GD 15.5 to 17.5 leads to cryptorchidism, hypospadias, compromised fertility, and reduction in the AGD (Welsh et al., 2008). On GD 21, Caesarean sections were performed on each dam. The following figure illustrates the changes in the AGDi of the male pups following maternal exposure to APAP on GD 13 to 21 (from Kristensen et al., 2011):

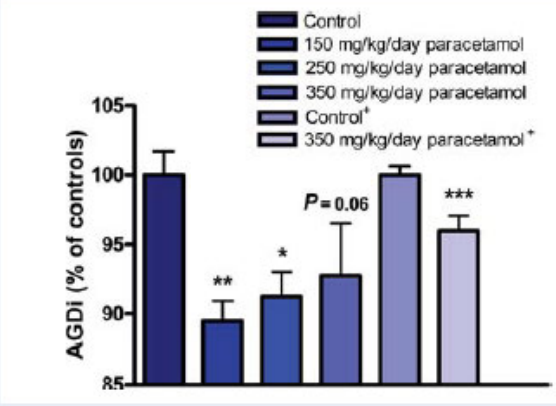

Figure 3 Intrauterine exposure from GD13 to 21 to paracetamol in rats led to a reduction in the testosterone-dependent AGD of male offspring. Results from initial experiment performed with four to five litters per dose group and the verifying experiment (+) performed with six litters per dose group. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$. *P*-values are from ANOVA followed by Dunnett's *post hoc* test with litter as the statistical unit and included as a random factor in a mixed model. There is no significant difference between treated groups. Data represent the mean ± SEM.

As shown in the figure above, a significant decrease in the AGDi was observed in the male pups following maternal exposure to APAP.

Additionally, testosterone production was analyzed on fetal testes at the time of fetal testosterone production initiation at GD 14.5 in an ex vivo culture system where the testes were dissected out of male rats from GD 14.5 and cultured in the presence of APAP for 3 days duration. As shown in the figure below, testosterone levels were significantly decreased for up to 3 days whereas prostaglandin levels were significantly decreased for 1 day (Kristensen et al., 2011).

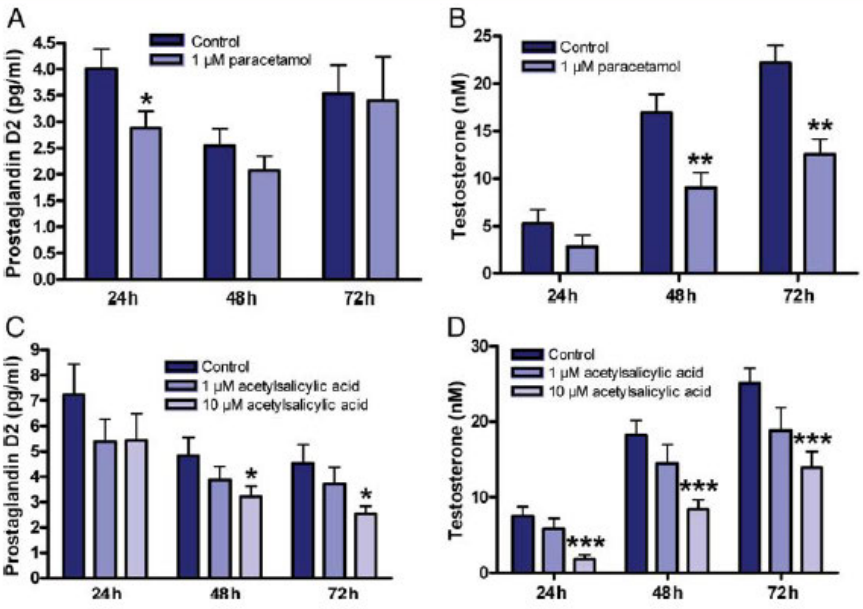

Figure 4 Mild analgesic compounds paracetamol and acetylsalicylic acid reduced PGD2 and testosterone production in *ex vivo* cultured GD14.5 rat testes. (**A**) Paracetamol ($n = 10$) and (**C**) acetylsalicylic acid ($n = 8$) reduced PGD2 secretion during *ex vivo* culture. (**B**) Paracetamol ($n = 10$) and (**D**) acetylsalicylic acid ($n = 13$) reduced testosterone secretion from the fetal rat testes during 3 days of *ex vivo* culture. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$. *P*-values are from a two-sided unpaired Student's *t*-test. Data represent the mean ± SEM.

Testosterone levels were not measured under in vivo conditions but assayed using an ex vivo culture system. Nonetheless the results are consistent with an impact of APAP on testosterone production early in development of male fetuses. These data are also consistent with the current labeling which states that APAP can reduce testicular weights, spermatogenesis, and fertility in rodents at clinically relevant exposures. However, importantly, these data suggest a smaller exposure margin for adverse effects, such as decreased testosterone production during development. Overall, the Kristensen et al., 2011 study does provide additional information suggesting an impact on testosterone production early in development at lower exposures than the currently listed 1.2 times the MHDD margins.

The effects of APAP and aniline on male fertility were studied in mice by Holm et al. (Holm et al., 2015). In this study, mated female C57BL/6J mice were divided into five treatment groups that either received water control, acetaminophen, or aniline. Mice in the acetaminophen groups, either received 50 or 150 mg/kg/day of APAP (0.06x and 0.18x the maximum daily human dose of 4000 mg based on a body surface area comparison). Mice in the aniline groups either received 31 or 93 mg/kg/day of aniline (which is converted into APAP). The doses of APAP or aniline were dissolved in 0.5 mL of water and administered from Gestational Day 7 to delivery. This study demonstrated that aniline is converted to acetaminophen by the liver. The following figure illustrates the conversion of aniline to APAP with reported APAP levels in the urine collected in mice at 4 and 24 hours after oral gavage administration of aniline or acetaminophen (see the following figure from Holm et al., 2015).

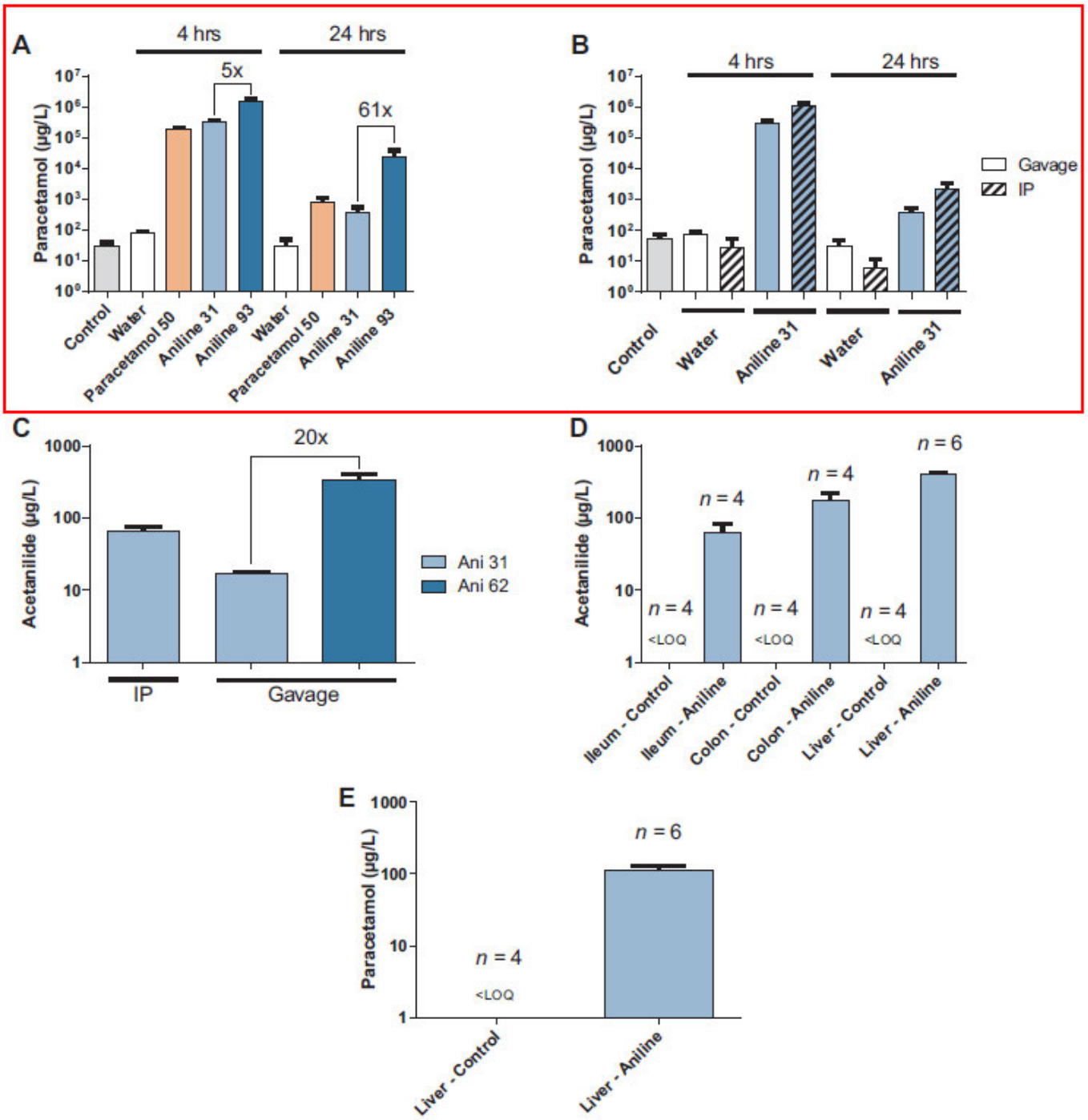

FIG. 3. Aniline is metabolized and excreted in urine as the analgesic paracetamol. A, Concentration of paracetamol in the urine collected from untreated male mice (control) and subsequently after 4 and 24 h postgavaging with water, aniline (31 and 62 mg/kg) or paracetamol (50 mg/kg). B, Concentration of paracetamol in the urine after 4 and 24 h after IP and gavage with aniline. C, Concentration of intermediate metabolite acetanilide in the urine 4 h after intraperitoneal injection (IP) and gavage. D, Concentration of intermediate metabolite acetanilide after *ex vivo* exposure of the luminal side of ileum and colon with 10 µM aniline for 3 h, and incubation of the perfused liver for 2 h with 10 µM aniline. E, Concentration of paracetamol in liver after perfusion and 2 h incubation with 10 µM aniline. No paracetamol was detected in ileum or colon samples after 3 h *ex vivo* exposure and no acetanilide or paracetamol were detected in the perfusion medium prior to experiments. Concentrations are depicted as mean ± SEM on a logarithmic y-axis ($n = 5$).

To measure any anti-androgenic effects, the anogenital distance (AGD) was measured and the AGD index (AGDi) was calculated by dividing the AGD by the cube root of the

body weight. Anogenital distance is the length between the anus and genital tubercle and increases during development. Males have a larger anogenital distance than females and this is believed to be due to testosterone production. Therefore, reduced anogenital distance in males is believed to be a surrogate for reduced testosterone production and has been associated with reduced fertility. The AGDi was significantly reduced in males from all APAP groups compared to control for up to 10 weeks post-birth (see figure below, from Holm et al., 2015). This study demonstrated that administration of acetaminophen or aniline impaired male reproductive development.

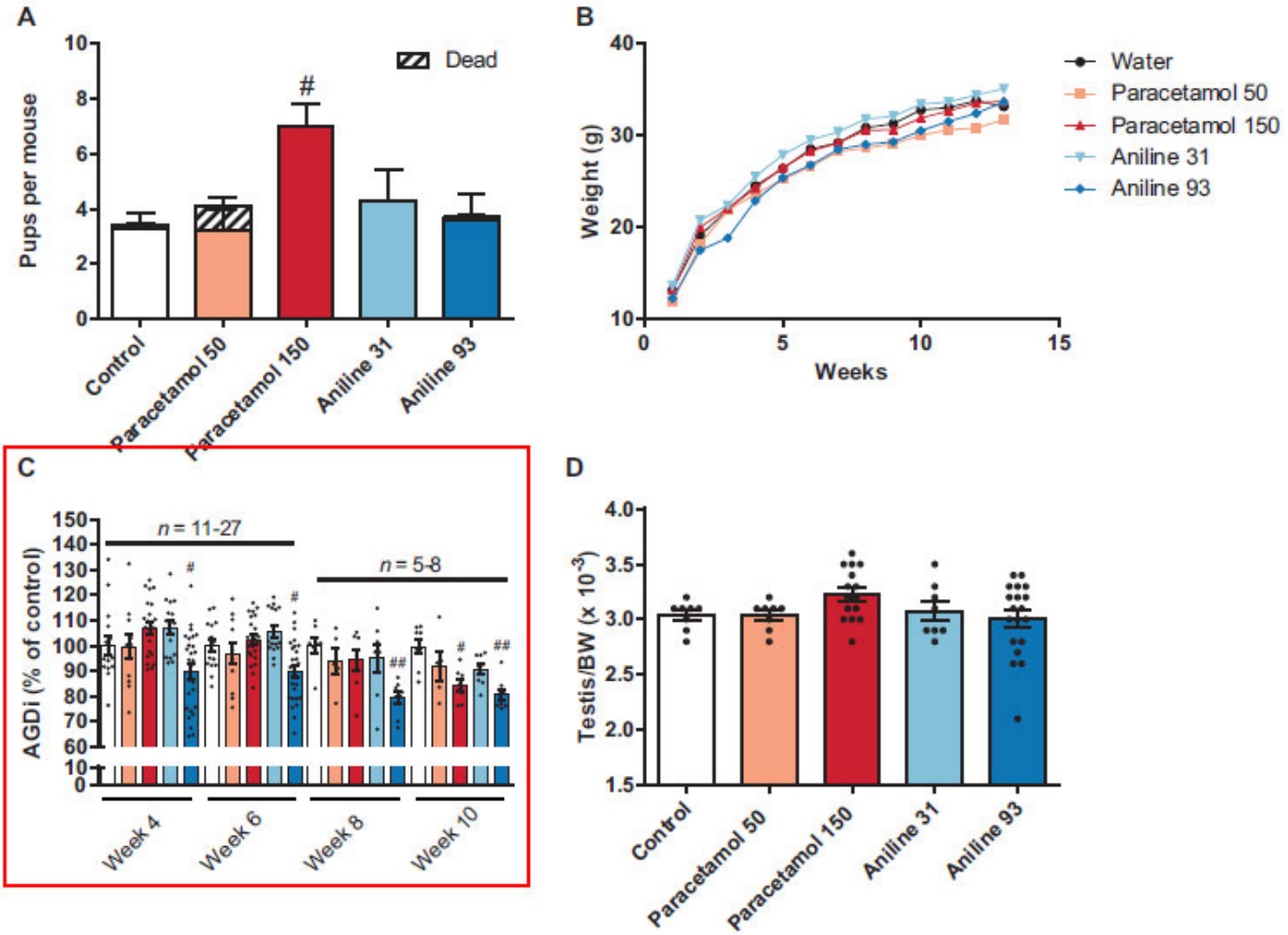

FIG. 4. Intrauterine exposure to aniline (31 and 93 mg/kg/day) or paracetamol (50 and 150 mg/kg/day) from GD7-20 disturbed aspects of the male reproductive development. A, Average number of pups per dam that where born and died within the 2 first postnatal weeks. B, Weight development in male pups in each dose group (n as in C). C, AGDi normalized to controls in male in relation to postnatal age. D, Weight of testes relative to whole body weight from mice killed at 6–7 weeks of age. Statistical tests performed were 2-way ANOVA followed by Bonferroni post hoc test with each pup being the statistical unit from 8 to 10 litter per dose group. #P ≤ .05; ##P ≤ .01. Results are depicted as mean ± SEM.

In a different study by the same group, the effects of APAP and aniline on female fertility were evaluated in mice by Holm et al. (2016). In this study, C57BL/6J mice were divided into five treatment groups that either received water control, acetaminophen, or aniline (Holm et al., 2016). Mice in the acetaminophen groups, either received 50 or 150 mg/kg/day of APAP (0.06x and 0.18x the maximum daily human dose of 4000 mg

based on a body surface area comparison). Mice in the aniline groups either received 31 or 93 mg/kg/day of aniline (which is converted into APAP as reported previously). The doses of APAP or aniline were dissolved in 0.5 mL of water. Females and males were initially caged and dosed together. Following determination of pregnancy, the males were separated from the females but were kept as sentinels for toxicity. To determine the effects of intrauterine exposure (Days 7 to 13.5 post-coitum) to APAP on fetal gonads, a separate set of C57BL/6J dams from the 50 mg/kg/day APAP oral gavage dose group was sacrificed on Day 13.5 post-coitum and the fetal gonads were dissected out and analyzed. To test female reproductive capacity after intrauterine exposure to APAP, eight female offspring from the control group were randomly selected and caged pairwise with eight female offspring from the 50 mg/kg/day APAP dose group. These female pairs were then caged and mated with a male C57BL/6J mouse that was not previously exposed to APAP. Thereafter, the dams were separated and pregnancies, time of birth, weight of pups, number of live and dead born pups, and lactating dams (with suckling pups) were recorded until 14 days after the last birth. Mating was performed twice at 6 months and 10 months of age. In another set of experiments, fetal gonad/mesonephros complexes from inbred C56BL/6 mice and outbred CD 1 mice were dissected out at Day 12.5 post-coitum and cultured for 3 days with and without APAP in an ex vivo culture system. The ex vivo culture system has not been validated for regulatory use and therefore the results of this assay will not be included in the label.

The AGDi was reduced in female off-spring in APAP or aniline treatment groups compared to control (see figures below, from Holm et al., 2016).

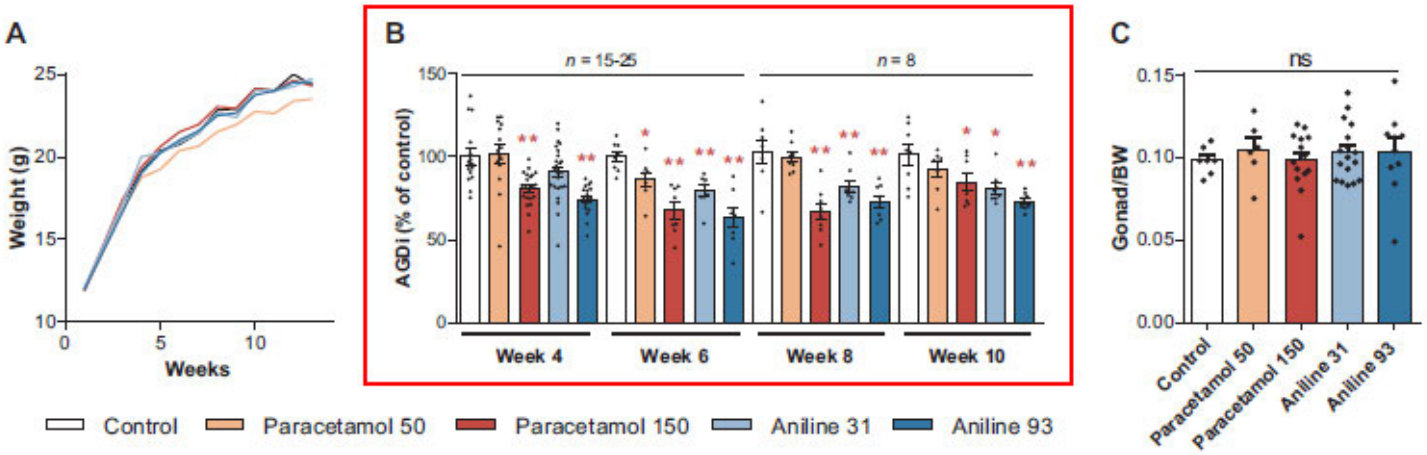

FIG. 1. Intrauterine exposure to aniline (31 and 93 mg/kg/day) or paracetamol (50 and 150 mg/kg/day) from 7 dpc to delivery reduced anogenital distance (AGD) in female offspring. A, Average weight of the female offspring from postnatal week 1 to 13. B, AGD index (AGDi) of the female offspring relative to postnatal growth and control group. C, Weight of gonads relative to whole body weight from mice at 6–7 weeks of age (n = 6–17). B and C, Results are depicted as mean ± SEM. Statistics were performed using one-way ANOVA followed by Holm-Sidak multiple comparison test. *$P \leq .05$ and **$P \leq .01$.

The follicle population was depleted after 7 weeks post-birth of female pups born to dams exposed to APAP (50 and 150 mg/kg/day) as shown in the figure below (from Holm et al., 2016).

Reference ID: 4913649

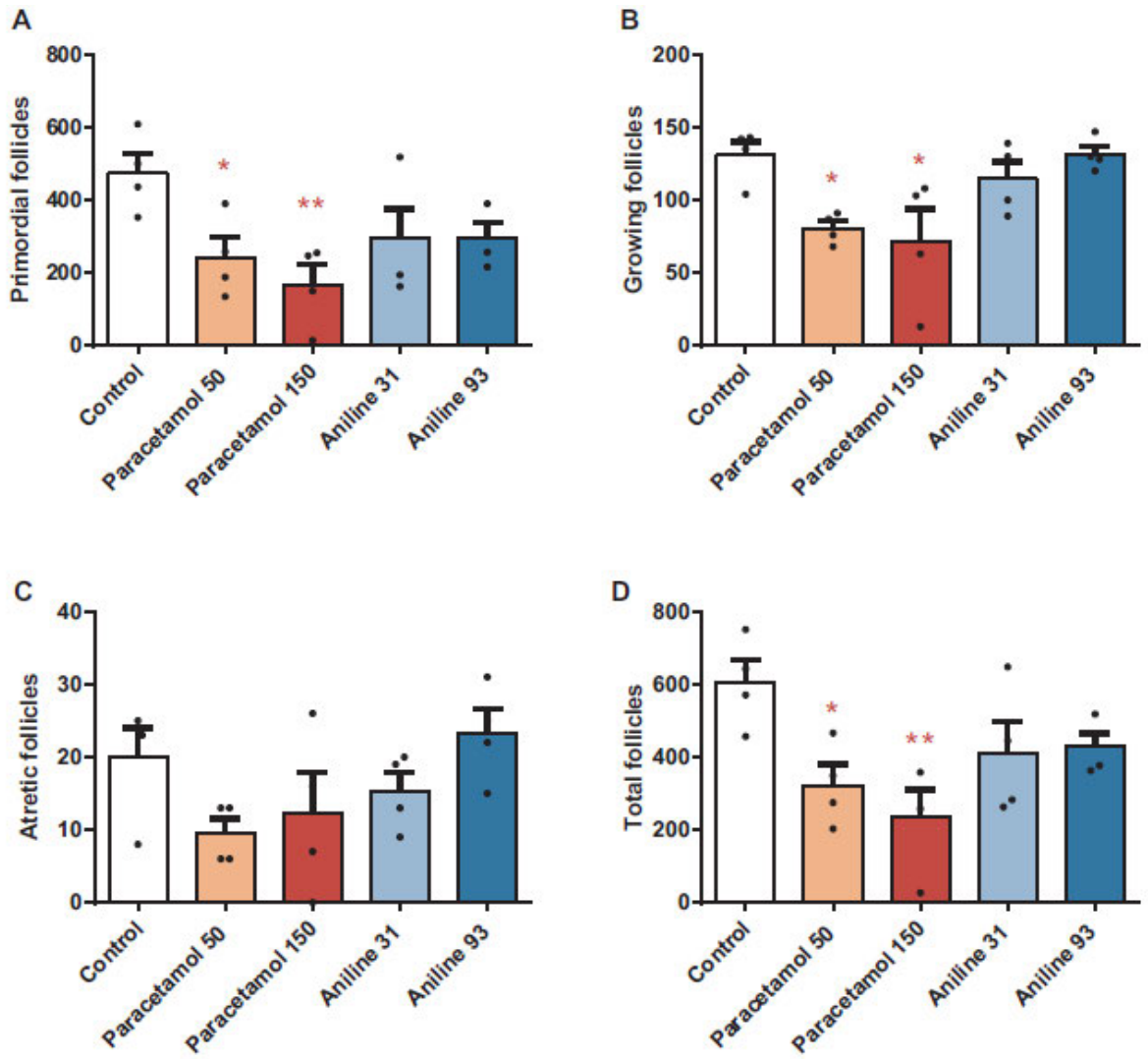

FIG. 3. Quantification of follicle populations illustrates depleted reserves after intrauterine exposure to paracetamol (50 and 150 mg/kg/day) or aniline (31 and 93 mg/kg/day). Data represent the mean number of (A) primordial (quiescent) germ cells, (B) growing follicles, (C) atretic follicles, and (D) total follicles per ovary ± SEM. The follicles were counted from four randomly picked ovaries from the five different groups after labeling of oocytes with Ybx2, examining every fifth sections. Follicle counts were analyzed using one-way ANOVA followed by Holm-Sidak multiple comparison test. *$P \leq .05$ and **$P \leq .01$.

Primordial follicles, growing follicles, atretic follicles, and total follicles were all significantly reduced in a dose-dependent manner in pups born to dams treated with APAP (see figure above).

Fertility of female mice exposed to APAP in utero was significantly reduced in terms of % of full term pregnancies as well as pups per dam at both the 6 month and 10-month time point in female rats (dams) dosed with 50 mg/kg/day of APAP (150 mg/m^2; see table below, from Holm et al., 2016).

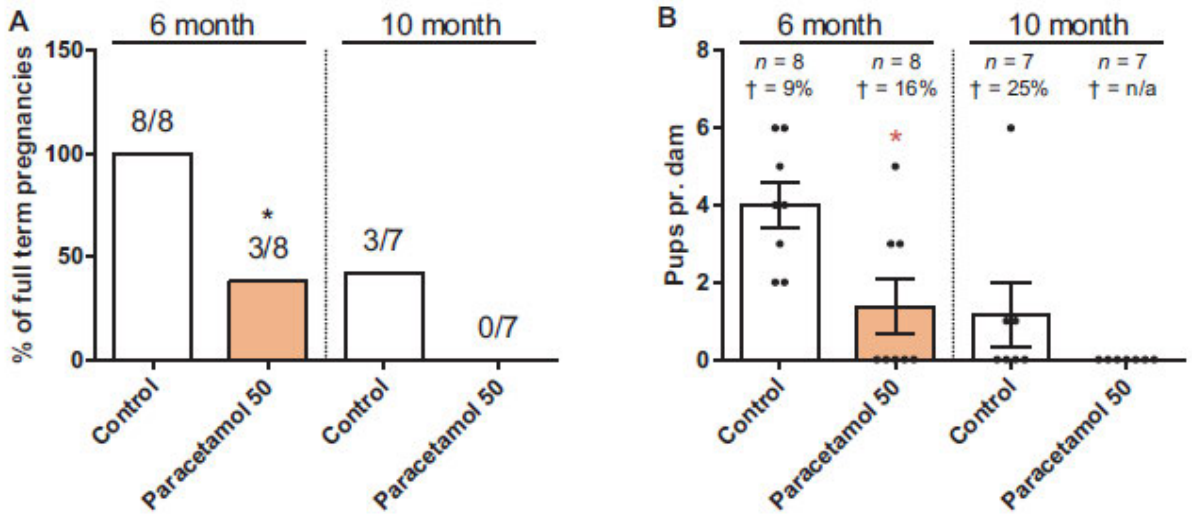

FIG. 4. Intrauterine exposure to paracetamol reduced fertility in mice. A, Percentage of full-term pregnancies with the fraction pregnant/total number of dams stated above the bars. B, Number of pups per dam mated with a male for 14 days at 6 and 10 months of age. † indicates percentage of pups found dead after birth. Results are depicted as mean ± SEM. Full-term pregnancies data were analyzed as a table of contingency using Fischer exact test, and number of pups per dam with two-way ANOVA followed by Holm-Sidak multiple comparison test. *$P \leq .05$.

There was significantly reduced expression of *Mvh*, a marker of fetal germ cells, in female pups born to dams exposed to APAP on Day 13.5 post-coitum.

It is noted that Holm et al., 2016 only dosed the dams using 2 doses of APAP (50 and 150 mg/kg/day) and a NOAEL for follicle depletion in the female pups or other measured endpoints was not identified. Although follicle depletion as well as the AGD and AGDi are not typical endpoints for fertility, the study does examine % of full term pregnancy and pups per dam, both of which were significantly reduced with APAP treatment. A reduction in the percentage of full term pregnancies and number of pups was reported from dosed dams exposed to APAP from Day 7 post-coitum to delivery at a dose that was 0.06x the maximum daily human dose of 4000 mg based on a body surface area comparison. These data suggest an adverse impact on female fertility following in utero exposure. These findings extend previous mouse male fertility findings, and the results of this study are appropriate to include in the label. In support of the findings from this paper (Holm et al., 2016), Dean et al. (2016) also investigated female fertility as a toxicological endpoint in a pre- and post-natal development study (see below).

Effects on Embryo-Fetal Development. Information in the published domain were reviewed to determine whether any results can be used to inform the Pregnancy section of acetaminophen product labeling (Lambert and Thorgeirsson, 1976, Lubawy and Garrett, 1977, Reel et al., 1992, Burdan, 2000, Laub et al., 2000, Neto et al., 2004). As a single entity oral prescription drug label for acetaminophen has not previously been approved by the Agency, it appears as though a Pregnancy Category for oral 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. 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 oral APAP on pregnancy.

The published nonclinical literature 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).

Lubawy 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 cannot 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).

Reference ID: 4913649

TABLE 2
Effect of Continuous Exposure to Acetaminophen on Postnatal Body Weights of F_1 Mice from the Final Litter of P Pairs (Task 4)

	% Acetaminophen in the diet			
Age (days)	0	0.25	0.5	1.0
	Body weight (g)			
Birth (0)[a]				
Male	1.64 ± 0.03 (37)	1.68 ± 0.04 (17)	1.69 ± 0.04 (16)	1.68 ± 0.07 (16)
Female	1.59 ± 0.03 (36)	1.61 ± 0.03 (17)	1.63 ± 0.04 (17)	1.61 ± 0.06 (17)
Weaning (28)[b]				
Male	17.25 ± 0.49 (42)	15.95 ± 0.54 (40)	14.38 ± 0.56 (45)*	11.37 ± 0.61 (24)*
Female	15.46 ± 0.43 (34)	13.88 ± 0.50 (32)*	13.62 ± 0.45 (33)*	11.08 ± 0.55 (33)*
Mating trial (74 ± 10.0)[b]				
Male	35.39 ± 0.74 (19)	33.38 ± 0.44 (20)*	32.49 ± 0.51 (20)*	28.92 ± 0.43 (20)*
Female	29.04 ± 0.70 (19)	26.04 ± 0.56 (20)*	26.28 ± 0.54 (20)*	24.23 ± 0.34 (20)*

[a] Values are mean pup weights per litter ± SEM (number of litters).
[b] Values are mean mouse weights per group ± SEM (number of mice).
* Significantly different ($p < 0.05$) from the control group.

Laub et al. administered APAP to female mice only during the first few days of gestation; therefore this study cannot 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).

Table 1. Occurence of skeletal variations (%) in foetuses of rats treated with paracetamol and in the control groups

	Control groups			Paracetamol-treated groups		
	T	K	CON	P1	P2	P3
Number of examined alizarin staining specimens	118	176	294	66	75	70
Nasal, reduced ossification	—	—	—	—	1 (1.33)	—
Frontal, reduced ossification	—	—	—	1 (1.51)	1 (1.33)	—
Parietal, reduced ossification	10 (8.47)	9 (5.11)	19 (6.46)	2 (3.03)	8 (10.66)	11 (15.71)
Interparietal, reduced ossification	12 (10.17)	10 (5.68)	22 (7.48)	7 (10.60)	11 (14.66)	13 (18.57)
Supraoccipital, missing	—	—	—	—	1 (1.33)	—
Supraoccipital, reduced ossification	5 (4.24)	5 (2.84)	10 (3.40)	2 (3.03)	3 (4.00)	4 (5.71)
Hyoid, missing	—	—	—	—	2 (2.66)	1 (1.43)
Hyoid, reduced ossification	1 (0.85)	1 (0.57)	2 (0.68)	—	—	2 (2.86)
13th rib, unilateral short	1 (0.85)	1 (0.57)	2 (0.68)	—	—	—
13th rib, wavy	1 (0.85)	6 (3.40)	7 (2.38)	2 (3.03)	1 (1.33)	—
Supernumerary rib, bud unilateral (L1)	—	1 (0.57)	1 (0.34)	1 (1.51)	—	3 (4.29)
Supernumerary rib, short unilateral (L1)	—	1 (0.57)	1 (0.34)	—	—	1 (1.43)
Supernumerary rib, short bilateral (L1)	—	—	—	—	—	1 (1.43)
Metacarpal, unilateral missing of one bone	2 (1.70)	1 (0.57)	3 (1.02)	—	—	—
Metacarpal, bilateral missing of one bone	2 (1.70)	2 (1.14)	4 (1.36)	1 (1.51)	2 (2.66)	7 (10.00)
Metacarpal, unilateral missing of two bones	—	—	—	—	—	1 (1.43)
Metatarsal, unilateral missing of two bones	4 (3.40)	6 (3.40)	10 (3.40)	3 (4.54)	3 (4.00)	8 (11.43)

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).

Table 1. Tested parameters (Mean ± Standard Deviation/litter) in the common control (CON) and acetaminophen-treated (P) groups

	CON	P1	P2	P3
Fetal weight (g)	3.80 ±0.42	3.83 ± 0.24	3.67 ± 0.39	3.46 ± 0.27**
Fetal length (mm)	38.55 ± 1.09	38.10 ± 0.91	37.62 ± 1.50	37.53 ± 0.99*
Tail length (mm)	11.85 ± 0.52	11.82 ± 0.42	11,55 ± 0.43	11.60 ± 0.29
Placental weight (g)	0.59 ± 0.06	0.57 ± 0.04	0.62 ± 0.05	0.61 ± 0.04
Number of luteum corpuscle	15.13 ± 0.43	14.62 ± 1.59	15.87 ± 2.47	15.14 ± 2.96
Number of fetuses	14.31 ± 2.25	13.87 ± 2.23	14.37 ± 4.13	13.57 ± 3.50
Number of resorptions	0,55 ± 0.68	0.50 ± 1.06	1.12 ± 1.12	1.14 ± 1.77
Preimplantation mortality	2.08 ± 4.37	1.85 ± 3.27	3.16 ± 6.14	3.70 ± 6.95
Postimplantation mortality	3.40 ± 4.19	3.62 ± 7.61	9.13 ± 10.58	7.30 ± 10.29
Number of ecchymosis	0.20 ± 0.41	0.12 ± 0.35	0.50 ± 0.53	0.28 ± 0.48

* Differ significantly from the common control value, ** Differ significantly from group P1.

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). 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).

Reference ID: 4913649

Effects on Pre- and Postnatal Development. Pre- and postnatal developmental data have been reported by Reel et al.; however, this study did not include comparable endpoints as a dedicated pre- and postnatal development study. Liver and kidney findings were reported by Neto et al. as described above (Neto et al., 2004). As the effects reported in Neto et al. represent adverse effects on the fetus, they should be included in the product labeling, unless superseded by adequate human data.

Blecharz-Klin et al. studied the levels of neurotransmitters in various regions of the nervous system of 2-month old male rats born to mothers that were exposed to APAP in the drinking water at doses of 5 and 15 mg/kg/day (0.012x and 0.036x the maximum daily human dose of 4000 mg based on a body surface area comparison) during pregnancy and lactation (Blecharz-Klin et al., 2015b, a, Blecharz-Klin et al., 2016). Immediately following birth, these male rat pups stayed with their mother for 28 days until weaning and then were exposed to APAP in the drinking water for an additional 28 days. The dosing duration of this study represents the dose duration of a typical pre- and postnatal development study. The following tables illustrate the changes in the level of monoamines and their metabolites and changes in amino acids levels in the medulla oblongata, cerebellum, and spinal cord:

Medulla Oblongata (Blecharz-Klin et al., 2015a):

Table 1
Effect of prenatal and early postnatal paracetamol administration on the level of monoamines and their metabolites (mean ± SE) in the medulla oblongata of rat pups.

Monoamine and metabolite levels in the medulla oblongata ng/g wet tissue (mean ± SE)			
	Con (n = 10)	P5 (n = 10)	P15 (n = 10)
NA	1523.51 ± 72.40	**1183.62 ± 40.97*****	**884.74 ± 68.43**$^{***,\#\#\#}$
MHPG	647.85 ± 61.38	591.33 ± 38.33	**749.43 ± 54.60**■
MHPG/NA	0.43 ± 0.04	0.51 ± 0.03	**0.89 ± 0.08**$^{***,\#\#\#}$
DA	90.80 ± 4.21	79.52 ± 6.42	**67.31 ± 2.58****
DOPAC	351.92 ± 19.02	335.40 ± 29.72	353.10 ± 19.71
DOPAC/DA	4.60 ± 0.52	4.91 ± 0.63	5.36 ± 0.45
HVA	666.91 ± 45.67	**516.82 ± 56.76***	**437.03 ± 21.31*****
HVA/DA	8.56 ± 0.97	7.63 ± 1.14	6.61 ± 0.49
5-HT	432.12 ± 49.90	393.90 ± 17.57	**701.69 ± 80.34**$^{***,\#\#\#}$
5-HIAA	183.76 ± 16.24	178.87 ± 11.52	182.67 ± 9.38
5-HIAA/5-HT	0.48 ± 0.07	0.45 ± 0.02	**0.31 ± 0.05**●

Bold font indicate a significant difference versus controls.
* P5, P15 *vs* Control, $p < 0.05$ (Newman–Keuls).
** P15 *vs* Control $p < 0.01$ (Newman–Keuls).
*** P5, P15 *vs* Control, $p < 0.005$ (Newman–Keuls).
$^{\#\#\#}$ P5 *vs* P15, $p < 0.005$ (Newman–Keuls).
● P15 *vs* Control, $p < 0.05$ (Fisher's Exact Test).
■ P5 *vs* P15 $p < 0.05$ (Fisher's Exact Test).

Table 2
Effect of prenatal and early postnatal paracetamol administration on the level of amino acids (mean ± SE) in the medulla oblongata of the rat pups.

Amino acid levels in the medulla oblongata ng/mg wet tissue (mean ± SE)			
	Con (n = 10)	P5 (n = 10)	P15 (n = 10)
Glutamic acid	1008.45 ± 25.45	967.60 ± 18.42	953.94 ± 17.60
Taurine	222.52 ± 6.05	**207.77 ± 4.57***	210.13 ± 3.72
Alanine	209.52 ± 5.96	**193.32 ± 3.06•**	193.23 ± 5.15
γ-Aminobutyric acid	287.92 ± 10.77	286.33 ± 6.67	285.84 ± 7.29
Aspartic acid	438.36 ± 14.34	415.69 ± 8.03	431.59 ± 21.87
Histidine	7.19 ± 0.30	6.98 ± 0.13	6.78 ± 0.30

Bold font indicate a significant difference versus controls.
* P5 *vs* Control $p < 0.05$ (Newman–Keuls).
• P5 *vs* Control $p < 0.05$ (Fisher's Exact Test).

At 5 mg/kg/day, the levels of noradrenaline and homovanillic acid were significantly decreased compared to control. At 15 mg/kg/day, the levels of noradrenaline (NA), dopamine (DA), homovanillic acid (HVA), and 5-hydroxyindoloacetic acid/5-hydroxytryptamine (5-HIAA/5-HT) ratio were significantly decreased compared to control. Moreover, the levels of 3-methoxy-4-hydroxyphenylglycol (MHPG), the MHPG/NA ratio, and 5-hydroxytryptamine (5-HT) were significantly increased compared to control. The decreases in noradrenaline and homovanillic acid were dose-dependent. At 5 mg/kg/day, the levels of taurine and alanine were significantly decreased compared to control. There were no changes at 15 mg/kg/day dose group making the changes in taurine and alanine not dose-dependent.

Cerebellum (Blecharz-Klin et al., 2016):

Table 1
Effect of the prenatal and postnatal paracetamol administration on the level of monoamines and their metabolites (mean ± SE) in the cerebellum of rat pups.

Monoamine and metabolite levels in the cerebellum ng/g wet tissue (mean ± SE)				
	Con	P5	P15	F value
NA	400.47 ± 39.50	463.69 ± 19.50	451.48 ± 12.57	$F_{(2,27)} = 1.61$
MHPG	**48.47 ± 6.37**	46.47 ± 2.91	**62.54±4.11***	$F_{(2,27)} = 3.49$
MHPG/NA	0.12 ± 0.01	**0.10±0.01**	**0.14±0.01•**	$F_{(2,27)} = 4.14$
DA	26.42 ± 2.84	27.30 ± 1.18	30.89 ± 1.67	$F_{(2,27)} = 1.38$
DOPAC	6.06 ± 0.81	8.15 ± 1.29	9.86 ± 1.13	$F_{(2,27)} = 3.02$
DOPAC/DA	0.35 ± 0.15	0.30 ± 0.04	0.33 ± 0.04	$F_{(2,27)} = 0.10$
HVA	11.87 ± 2.36	16.32 ± 1.77	17.18 ± 2.21	$F_{(2,27)} = 1.80$
HVA/DA	0.51 ± 0.10	0.60 ± 0.06	0.56 ± 0.07	$F_{(2,27)} = 0.32$
5-HT	30.03 ± 5.40	47.90 ± 6.05	34.61 ± 4.18	$F_{(2,27)} = 3.11$
5-HIAA	**29.09±3.84**	**49.68±7.28***	**28.37±4.40•**	$F_{(2,27)} = 5.05$
5-HIAA/5-HT	1.20 ± 0.19	1.02 ± 0.08	0.83 ± 0.10	$F_{(2,27)} = 1.89$

* P5, P15 *vs.* Con, $p < 0.05$ (Newman-Keuls).
• P5 *vs.* P15, $p < 0.05$ (Newman-Keuls).

Table 2
Effect of the prenatal and postnatal paracetamol administration on the level of amino acids (mean ± SE) in cerebellum of the rat pups.

Amino acid levels in the cerebellum ng/mg wet tissue (mean ± SE)				
	Con	P5	P15	F value
Glutamic acid	1728.10 ± 80.28	1773.71 ± 37.72	1891.43 ± 39.62	$F_{(2,27)} = 2.26$
Taurine	765.94 ± 25.60	762.04 ± 12.85	804.93 ± 10.94	$F_{(2,27)} = 1.83$
Alanine	315.55 ± 13.55	**299.65 ± 11.86**	**344.29 ± 7.82•**	$F_{(2,27)} = 3.99$
γ-Aminobutyric acid	302.95 ± 14.50	322.55 ± 8.12	330.10 ± 9.68	$F_{(2,27)} = 1.60$
Aspartic acid	278.61 ± 18.76	294.50 ± 10.23	326.10 ± 11.20	$F_{(2,27)} = 3.02$
Histidine	10.89 ± 0.81	12.11 ± 0.31	11.58 ± 0.54	$F_{(2,27)} = 1.07$

• P5 *vs.* P15, $p < 0.05$ (Newman-Keuls).

At 15 mg/kg/day, there was an increase in the levels of 3-methoxy-4-hydroxyphenylglycol (MHPG). All other changes were not dose-dependent and were not different compared to control.

Spinal Cord (Blecharz-Klin et al., 2015b):

Monoamine and metabolite levels in the spinal cord ng/g tissue (mean ± SE)

	Con	P5	P15
NA	304.148 ± 21.92	283.105 ± 25.78	268.627 ± 11.47
MHPG	21.714 ± 1.68	15.914 ± 1.79•	16.755 ± 1.45*
MHPG/NA	0.073 ± 0.01	0.060 ± 0.01	0.063 ± 0.01
DA	42.488 ± 3.21	30.239 ± 3.08*	31.635 ± 1.49**
DOPAC	5.494 ± 1.51	5.699 ± 1.36	3.870 ± 0.50
DOPAC/DA	0.140 ± 0.05	0.240 ± 0.08	0.123 ± 0.02
HVA	30.898 ± 4.00	18.545 ± 3.02*	25.721 ± 3.06
HVA/DA	0.726 ± 0.07	0.706 ± 0.15	0.812 ± 0.08
3-MT	8.449 ± 1.97	5.099 ± 1.47	3.196 ± 1.04•
3-MT/DA	0.197 ± 0.04	0.195 ± 0.06	0.099 ± 0.03
5-HT	196.771 ± 24.88	208.496 ± 35.44	236.661 ± 11.74
5-HIAA	87.666 ± 9.16	85.905 ± 14.43	107.494 ± 5.59
5-HIAA/5-HT	0.456 ± 0.02	0.415 ± 0.04	0.454 ± 0.01

* P5, P15 vs control, $p < 0.05$ (Newman–Keuls).
** P15 vs control, $p < 0.01$ (Newman–Keuls).
• P5, P15 vs control, $p < 0.05$ (Fisher's Exact Test).

Amino acid levels in the spinal cord ng/mg tissue (mean ± SE)

	Con	P5	P15
Glutamic acid	1280.81 ± 84.92	1399.32 ± 181.53	1700.87 ± 118.00•
Taurine	282.25 ± 14.48	251.10 ± 32.28	315.54 ± 28.29
Alanine	433.60 ± 36.94	417.54 ± 46.10	509.54 ± 41.09
γ-Aminobutyric acid	364.44 ± 23.49	366.34 ± 40.17	402.57 ± 32.93
Aspartic acid	397.24 ± 43.68	512.28 ± 54.97	686.84 ± 52.68***,#
Histidine	16.65 ± 1.45	15.17 ± 2.04	18.96 ± 1.08

*** P15 vs control, $p < 0.005$ (Newman–Keuls).
# P5 vs P15, $p < 0.05$ (Newman–Keuls).
• P15 vs control, $p < 0.05$ (Fisher's Exact Test).

There were significant dose-dependent decreases in the levels of 3-methoxy-4-hydroxyphenylglycol (MHPG) and 3-methoxytyramine (3-MT) in the 5 and 15 mg/kg/day dose groups compared to control. The levels of dopamine (DA) were significantly decreased in both the 5 and 15 mg/kg/day dose groups; however, the decreases were not dose-dependent. At 15 mg/kg/day, there were significant increases in the levels of glutamic acid and aspartic acid.

These studies did not utilize a typical pre- and postnatal development study design as the typical reproductive and developmental endpoints were not evaluated. Moreover, the F_1 pups did not undergo behavioral or functional assessments to determine the biological significance of these biochemical changes in the medulla oblongata, cerebellum, and spinal cord. In fact, the researchers do not clearly understand the significance of these changes in the cerebellum, medulla oblongata, and spinal cord; however, they acknowledge that these changes may play a role in developmental and neurological disorders such as ADHD. Thus, the significance of these changes in neurotransmitter levels is unknown and therefore do not warrant inclusion in the label at this time.

Dean et al. (2016) studied exposure to APAP during pregnancy in the F_0 dams and its effects on germ cell development and reproductive function in the F_1 and F_2 generation using Wistar rats (Dean et al., 2016). Wistar rats were dosed with 350 mg/kg/day APAP (0.85x the maximum daily dose of 4000 mg based on a body surface area comparison) via oral gavage as a suspension in corn oil. Timed-matings were established by the presence of a vaginal plug and defined as embryonic day 0.5 (e0.5). Pregnant dams were dosed with 350 mg/kg/day APAP on e13.5 (embryonic day or gestation day) to e21.5 to encompass dosing during the masculinization programming window of e15.5 to e18.5. Control and APAP dams were sacrificed on e15.5, e16.5, e17.5, e18.5, e21.5, or allowed to give birth. The resulting offspring were then sacrificed on either postnatal (PND) 25, which represents early puberty, or PND 90, which represents adulthood. Fetuses sacrificed on e21.5 were weighed and the gonads were dissected out. Post-natal pups were weighed, sacrificed, and their gonads were dissected out. Female adult rats (F_1 generation) that had been exposed in utero to APAP were each placed with an untreated control male for 4 days to allow mating and the number of pups per litter was counted on the day of birth. Male adult rats (F_1 generation) that had been exposed in utero to APAP, each of which was paired with an untreated control female for 4 days and the number of pups per litter was counted on the day of birth. The resulting F_2 offspring were studied at PND 25 (puberty) or PND 90 (adulthood) with the focus on the female F_2 offspring as the male F_2 offspring did not exhibit any obvious reproductive phenotypic change. Fetal gonads from pups who were exposed to APAP in utero were observed with reduced expression of *Ep2* (PGE_2 receptor) mRNA and PGE_2 levels but the expression of *Cox2* (cyclooxygenase) mRNA was not reduced (see below, from Dean et al., 2016).

Reference ID: 4913649

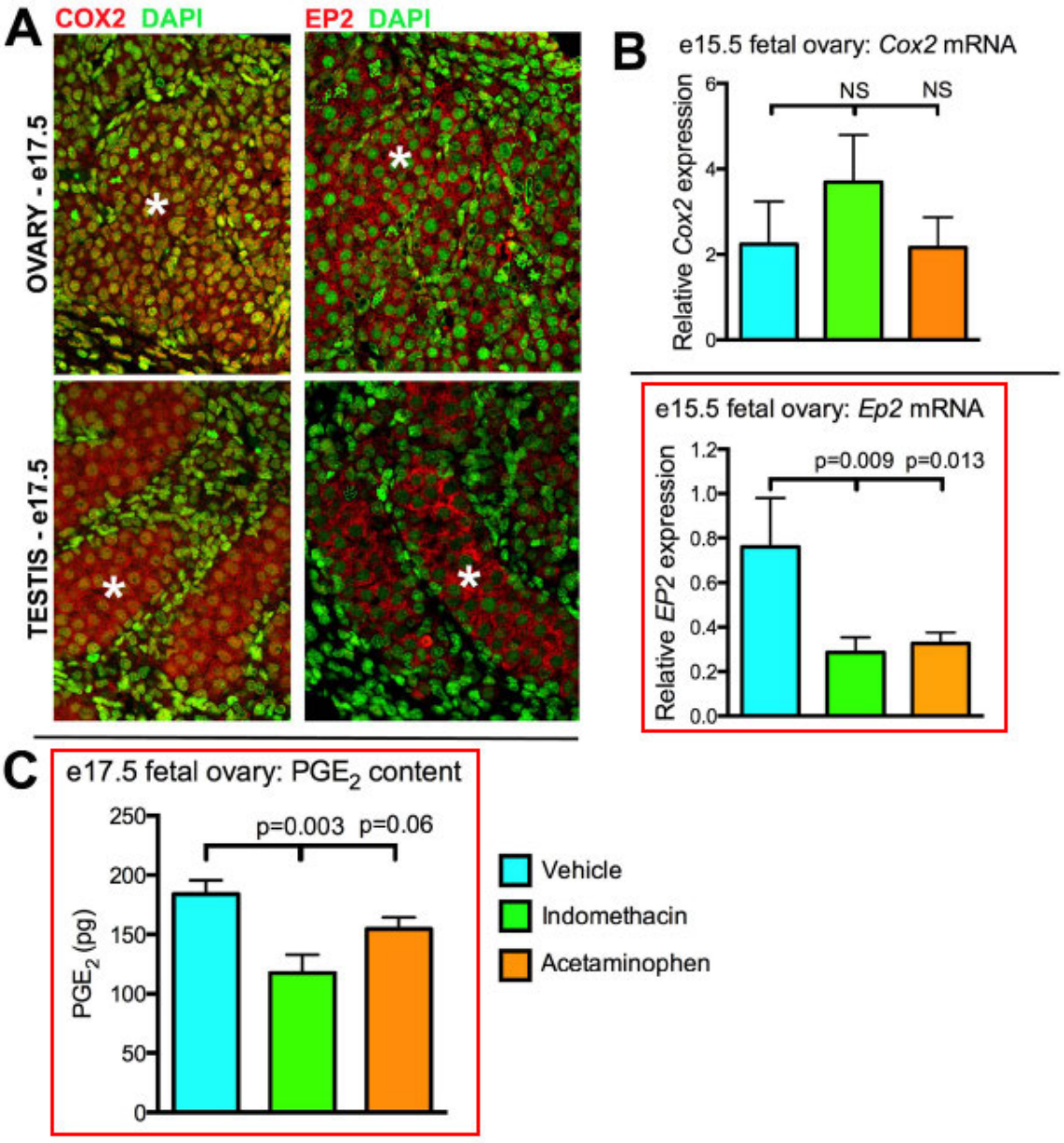

Figure 1. The fetal rat gonads as a source and target for prostaglandins (PGs). (A) Immunoexpression of cyclo-oxygenase-2 (COX2) and PGE_2 receptors (EP2) in germ cells (asterisks) of the fetal (e17.5) rat ovary and testis. (B) The effect of exposure to indomethacin or acetaminophen on *Cox2* and *Ep2* mRNA expression at e15.5 in the F1 fetal ovary (Values are Means ± SEM for n = 6–9). (C) Effect of analgesic exposure on F1 fetal rat ovarian PGE_2 content 3h after a single administration of vehicle or analgesic on e17.5 (Means ± SEM for n = 5).

The researchers argue that germ cells express COX2 and prostaglandin receptors such as EP2 and therefore, are potential targets for prostaglandins (PG), which demonstrates that COX2 and PG receptors might play a common and conserved role in fetal germ cell development. APAP has been shown to alter prostaglandin pathways and is commonly used during pregnancy. Intra-uterine exposure to APAP reduced the number of germ cells and weight of the ovary in adult females (see below, from Dean et al., 2016). Adult female fertility was reduced slightly as well.

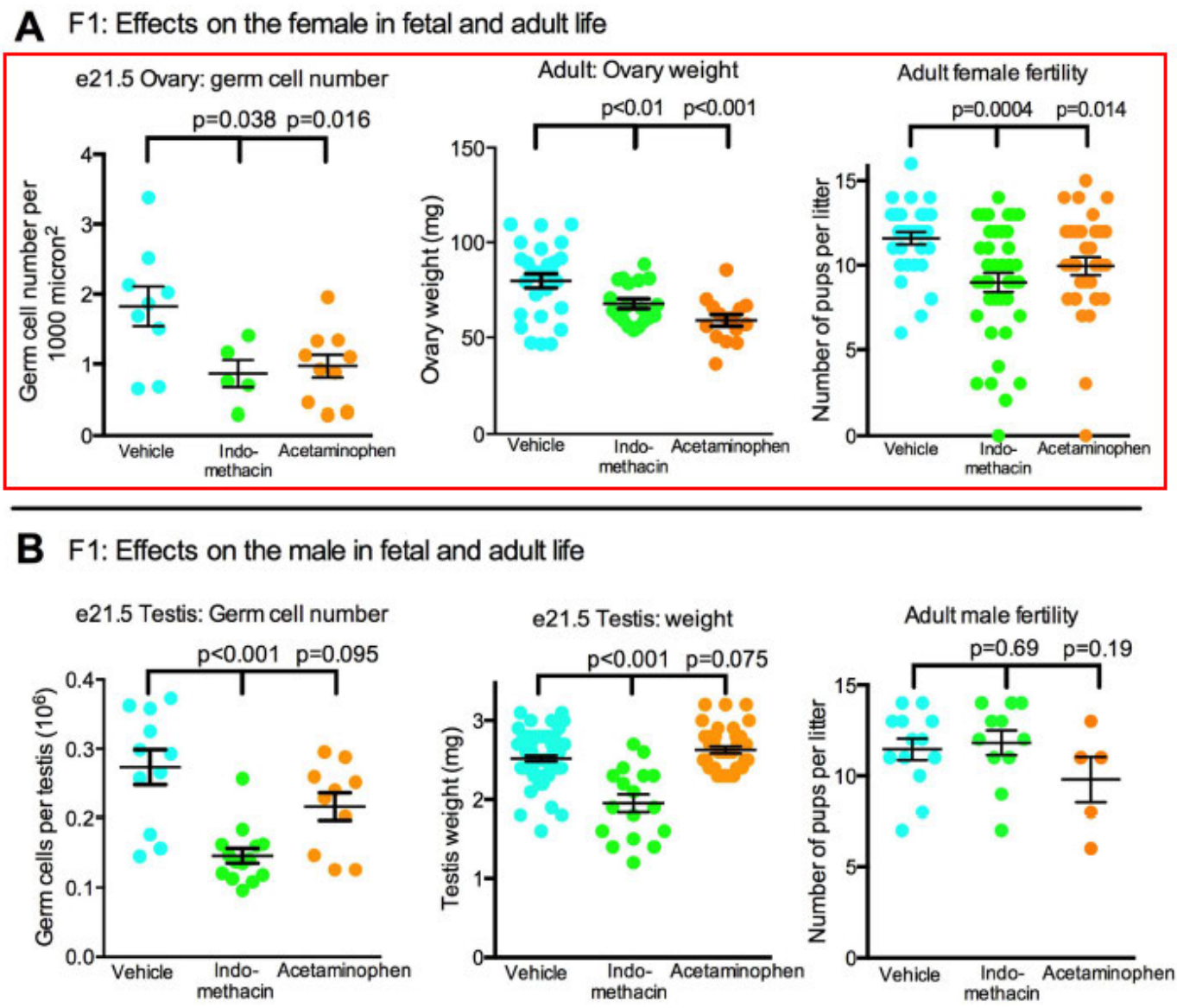

Figure 2. Effect of fetal exposure to analgesics on (top) ovarian development and fertility in F1 female rats, and (bottom) corresponding changes in F1 males. (A) Germ cell number at e21.5 (n = 5–10 animals per group), adult ovarian weight (n = 15–27 animals per group) and fertility after mating with normal untreated stud males (n = 30–36 animals per group). (B) Germ cell number at e21.5 in the testis (n = 10–14 animals per group), together with testis weight at the same age (n = 17–64 animals per group) and fertility after mating with normal untreated adult females (n = 5–13 animals per group). Black horizontal bars show Means ± SEM. Note that controls used for indomethacin and acetaminophen studies were pooled for analysis as they did not differ significantly for any of the measured parameters. In each group, animals were from 4–11 different litters.

In the female ovary, the developmental pathway for germ cells is to switch off pluripotency factors and enter meiosis. The researchers monitored meiosis in F_1 female fetuses by measuring expression of DMRT1, which is germ cell specific after e15.5 in rats, and as such, germ cell loss of DMRT1 expression is an index of completion of meiotic entry. The markers of meiotic entry are *Dmrt1* and *Stra8*. The pluripotency marker *Lin28* was used, expression on which is lost in ovarian germ cells in rats and

humans prior to meiotic entry. There was increased expression of *Dmrt1* and *Stra8* mRNA on e18.5 compared to no statistical differences on e17.5. Expression of *Lin28* mRNA was increased on e18.5 compared to no statistical differences on e17.5. Male fetal germ cells do no enter meiosis but undergo a differentiation step when pluripotency markers (such as OCT4) are switched off at approximately e15.5 to e19.5 in rats (see below, from Dean et al., 2016). The expression of OCT4 was monitored in the male gonads from pups born to dams exposed to APAP. The expression of OCT4 was significantly reduced on e15.5, e16.5, and e17.5 compared to age-matched controls (see below, from Dean et al., 2016).

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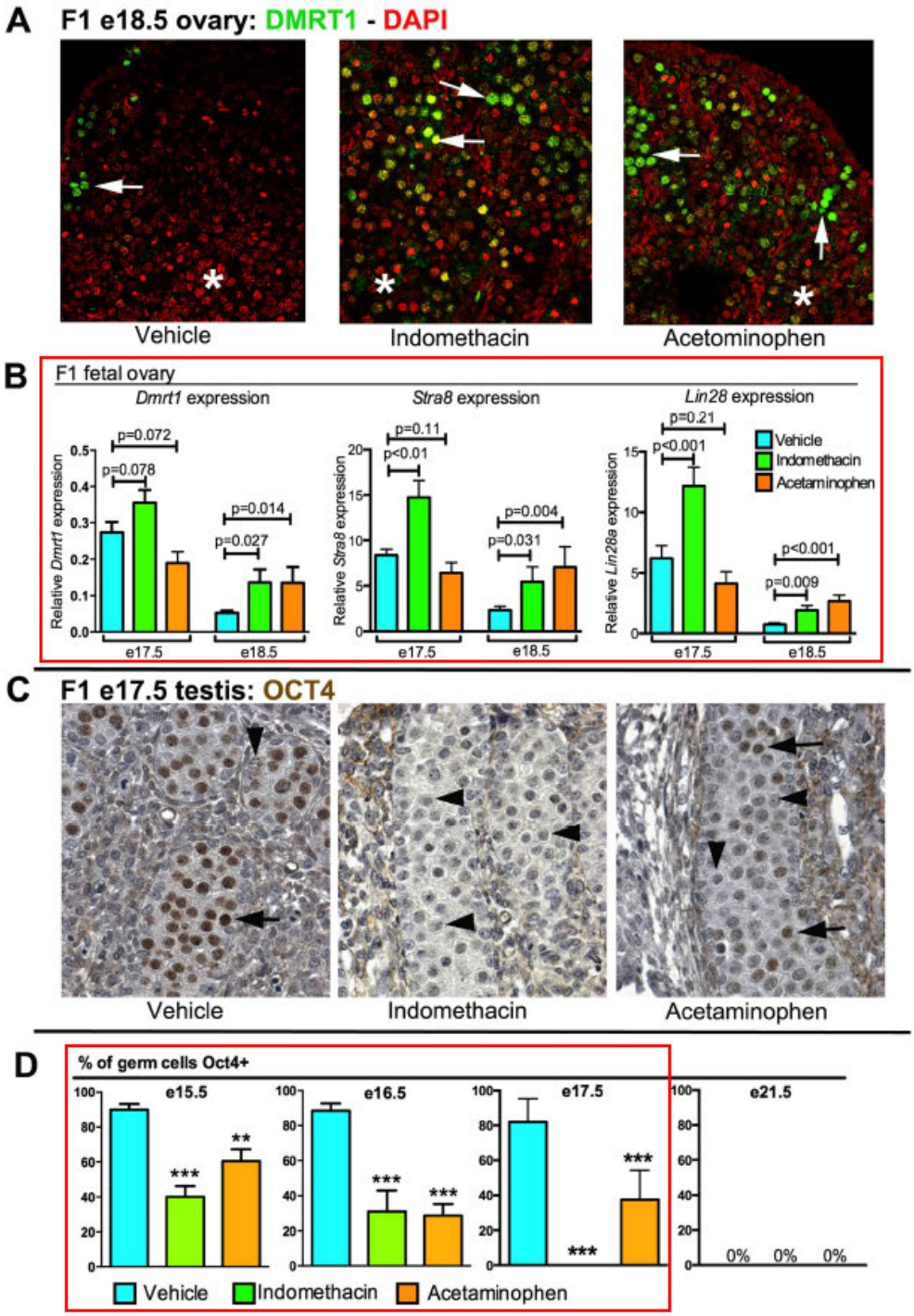

Figure 3. Effect of fetal analgesic exposure on the tempo of fetal germ cell development in the F1 fetal ovary (A,B) and testis (C,D). (A) Representative immunoexpression results for DMRT1 (green) in e18.5 ovaries from fetuses exposed to vehicle or to analgesic, highlighting regional differences in the proportion of oocytes still expressing DMRT1 (green) and those in which DMRT1 has been switched off (asterisks). (B) Evidence for delayed oocyte development in analgesic-exposed fetuses based on the temporal change in expression of *Dmrt1*, *Stra8* and *Lin28* mRNA expression at e17.5 and e18.5 (Means ± SEM for 9–18 animals per group from 3–5 different litters). (C) Germ cell-specific nuclear expression of OCT4 (brown; black arrows) in the fetal testis was reduced substantially by exposure to analgesic with all (indomethacin) or most (acetaminophen) germ cells prematurely losing expression of OCT4 by e17.5, unlike in controls. (D) The proportion of germ cells expressing OCT4 was quantified at e15.5, e16.5, e17.5 and e21.5 for 5–6 animals per group at each age from a minimum of 5 litters (Means ± SEM). $^{**}p < 0.01$, $^{***}p < 0.001$, in comparison with respective control group.

Analgesic exposure caused advanced differentiation of fetal germ cell development in males and an opposite effect in females. The F_2 female offspring whose F_1 parent was exposed to APAP in utero showed a significant reduction in ovary weight at PND25 as well as in adulthood. Ovarian follicle numbers in F_2 female offspring whose F_1 parent was exposed to APAP in utero were also investigated at PND25. There was a significant reduction in primordial follicles and total follicles (see below, from Dean et al., 2016).

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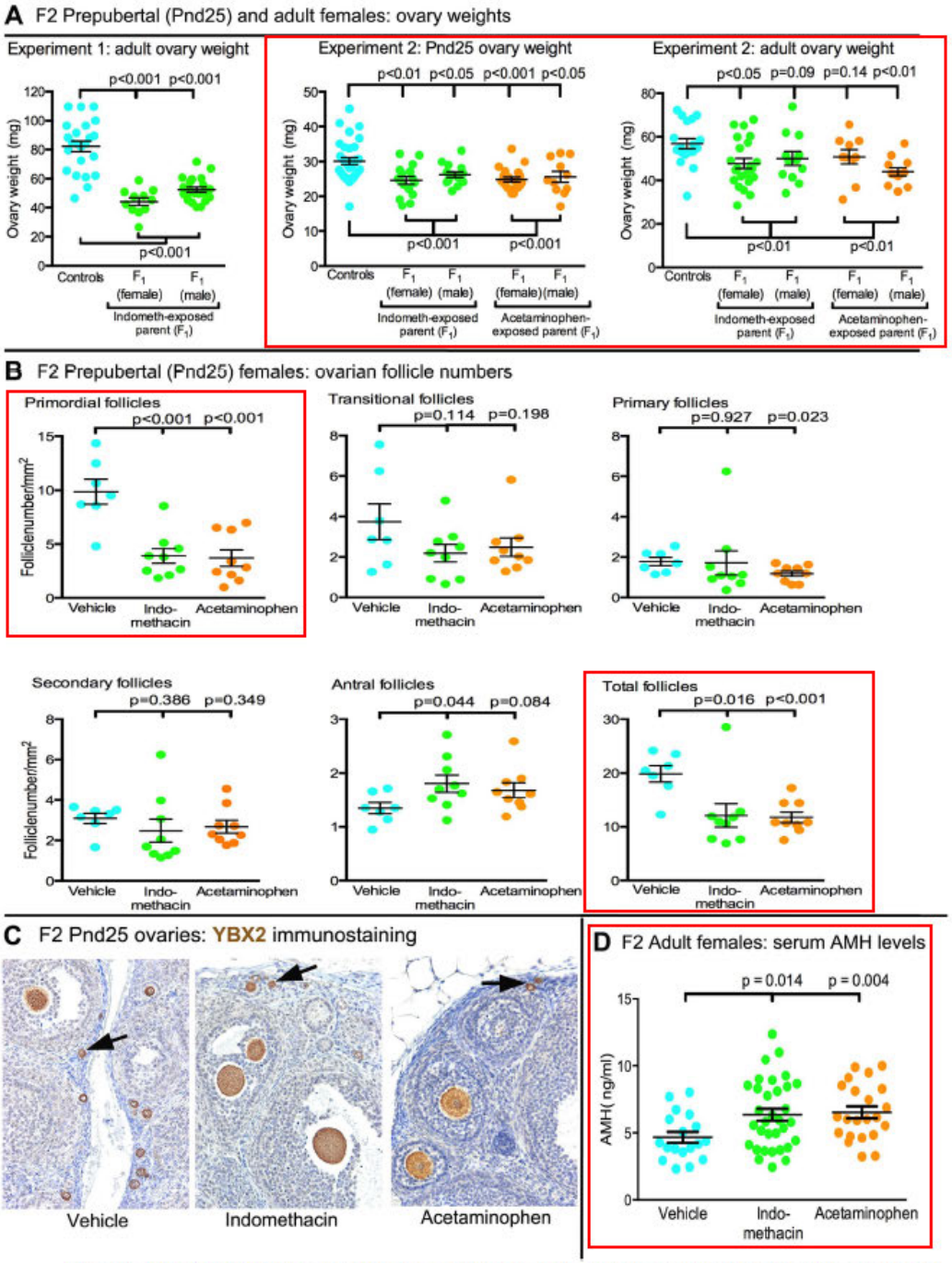

Figure 5. Effect of fetal exposure of the F1 generation to analgesics on ovarian size and function in second generation (F2) females. A Ovarian weights at postnatal day 25 (Pnd25) or adulthood were reduced overall in F2 females of analgesic-exposed parents, an effect that was generally evident irrespective of which F1 parent had been exposed to analgesic in utero. B Follicle classification and counts in the ovaries of representative prepubertal females from experiment 2 (panel A) based on immunostaining of oocytes for YBX2 (panel C). Follicle counts revealed a highly significant decrease in primordial follicle number (arrowed in panel C) in Pnd25 females derived from an F1 parent exposed in utero to analgesic and there was a trend towards increased numbers of antral follicles in the same ovaries (data derived from equal numbers of paternal and maternal 'treatment exposed' parents). D Serum AMH levels in adult F2 females (N=18–33 per group). Black horizontal bars show Means ± SEM. Mated F1 animals derived from 7 separate litters whilst data for F2 animals derived from 5-6 litters.

There was also an increase in serum AMH levels in adult F_2 females whose F_1 parent was exposed to APAP in utero. AMH is anti-Müllerian hormone and is produced in large preantral/small antral follicles. The increased serum levels in AMH are thought to explain the slight increase in antral follicles at PND25.

The results from the study by Dean et al., 2016 describe data on developmental fate of germs cells in the ovary and testis as well as prostaglandin mRNA expression and follicle population in the ovary, which are endpoints that are not the typical endpoints of GLP reproductive and development toxicology studies. Although these atypical endpoints were used, the study did use typical endpoints such as ovary weight and number of pups per litter. The study did demonstrate decreases in the ovary weight and the number of pups per litter in the F_1 females born to dams that were exposed to APAP. The decrease in ovary weight persisted to the F_2 females. Interestingly, the fetal weights of the testis in F_1 males were not different compared to control and male fertility in adults was slightly decreased but not statistically significant. The male findings may be due to lower doses and the shorter dosing period compared to the continuous breeding study in the existing label. The results from the Dean et al., 2016 study as well as the Holm et al., 2016 study that describe the impact of acetaminophen on female fertility are relevant and warrant inclusion in the label.

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Appendix 2: Nonclinical Recommendations for Labeling for Ibuprofen

This memo documents the labeling recommendations for ibuprofen, which are based upon the Agency's review of the literature. Several studies from the scientific literature were reviewed to inform Section 8, pregnancy (teratogenic and nonteratogenic effects) and Section 13, animal studies including mutagenesis, carcinogenesis, and impairment of fertility. The following labeling recommendations are based on a literature review and the is 1170 mg ibuprofen (97.5 mg/tablet x 12 tablets).**Recommended Changes**	**Rationale/Comment/Source**
8 Use in Specific Populations	
8.1 Pregnancy	
Risk Summary	
In published animal reproduction studies, there were no clear developmental effects at doses up to 2.7-times the maximum recommended human dose (MRHD) in the rabbit and 1.5-times in the MRHD rat when dosed throughout gestation. In contrast, an increase in membranous ventricular septal defects was reported in rats treated on Gestation Days 9 & 10 with 2.2-times the MRHD.	Risk summary based on Adams et. al (1969) and Cappon et. al (2003)
Based on animal data, prostaglandins have been shown to have an important role in endometrial vascular permeability, blastocyst implantation and decidualization. In animal studies, administration of prostaglandin synthesis inhibitors such as ibuprofen, resulted in increased pre- and post-implantation loss.	NSAID Class Labeling Nonclinical Risk Summary Statement
Data *Animal data*	
In a published study, female rabbits given 7.5, 20, or 60 180 mg/kg ibuprofen (0.3, 1.0, or 2.7-times the maximum human daily dose of 1170 mg of ibuprofen based on a body surface area comparison) from Gestation Days 1 to 29, no clear treatment-related adverse developmental effects were noted This dose was associated with significant maternal toxicity (stomach ulcers, gastric lesions) In the same publication, female rats were administered 7.5, 20, 60, 180 mg/kg ibuprofen (0.06, 0.2, 0.5, 1.5-times the maximum daily dose) did not result in clear adverse developmental effects. Maternal toxicity (gastrointestinal lesions) was noted at 20 mg/kg and above.	Adams et. al (1969)

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In a published study, rats were orally dosed with 300 mg/kg ibuprofen (2.5 fold the maximum human daily dose of 1170 mg based on a body surface area comparison) during Gestation Days 9 and 10 (critical time points for heart development in rats). Ibuprofen treatment resulted in an increase in the incidence of membranous ventricular septal defects. This dose was associated with significant maternal toxicity including gastrointestinal toxicity. One incidence each of a membranous ventricular septal defect and gastroschisis was noted fetuses from rabbits treated with 500 mg/kg (8-times the maximum human daily dose) from Gestation Day 9 to 11.	Cappon et. al (2003)
13 Nonclinical Toxicology **13.1 Carcinogenesis, Mutagenesis, and Impairment of Fertility** <u>Carcinogenesis</u> Adequate long-term animal studies have not been conducted to evaluate the carcinogenic potential of ibuprofen.	Data from Adams et. al (1970) is not adequate by current standards to include in labeling. Carcinogenicity data were not included in the original Motrin® label.
<u>Mutagenesis</u> In published studies, ibuprofen was not mutagenic in the in vitro bacterial reverse mutation assay (Ames assay).	From Oldham et. al (1986) and Philipose et. al (1997) Although Philipose reports positive findings in the SCE assay in the mice, several studies examining SCE rates in human lymphocytes do not report any significant differences before and after two-week treatment duration of ibuprofen (800 to 1200 mg/day) or other NSAIDs. The rat SCE data need not be included in labeling.
<u>Impairment of Fertility</u> In a published study, dietary administration of ibuprofen to male and female rats 8-weeks prior to and during mating at dose levels of 20 mg/kg (0.2-times the MRHD based on a body surface area comparison) did not impact male or female fertility or litter size.	Adams et. al (1969)
In other studies, adult mice were administered ibuprofen at a dose of 5.6 mg/kg/day IP (0.02-times the MRHD based on a body surface area comparison) for 35 or 60 days in males and 35 days in females. There was no effect on sperm motility or viability in males but decreased ovulation was reported in females.	From Stutz et. al (2000) and Martini et. al (2008)

REVIEW OF SUPPORTING DATA:

Genetic Toxicity Studies:

Reference ID: 4913649

Publication: Mutagenicity Testing of Selected Analgesics in Ames *Salmonella* Strains (Oldham et. al, 1986)

Methods:

Strains:	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA 1538
Concentrations in definitive study:	1, 10, 100, 500, 750, and 1000 mcg/plate
Basis of concentration selection:	The 5000 mcg/plate dose resulted in toxicity in all strains.
Negative control:	DMSO
Positive control:	Without S9 activation: Dexon (200 mcg, TA98 and TA1538); 2-aminoantracene (50 mcg, TA1537); sodium azide (0.5 mcg, TA100 and TA1535); With S9 activation: 2-aminoanthracene (2 mcg; all strains)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Ibuprofen in vehicle (DMSO), vehicle alone (negative control), or positive control was pre-incubated with the bacteria with and without S9 mix in the top agar. The solution was mixed and overlaid onto a minimal bottom agar. After the overlay solidified, the plates were inverted and incubated at 37˚C for 46 to 50 hrs. Afterwards, the plates were scored. All concentrations and controls (both positive and negative) in this toxicity-mutation assay were plated in duplicate. There was no confirmatory test performed.

Additonal Methods/Validity:

Ibuprofen was obtained from [redacted] (b) (4) with no purity information given. The study was conducted at [redacted] (b) (4). Up to 1000 mcg/plate of ibuprofen was tested as higher doses caused toxicity in the strains tested. The strains tested were *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 (Kamber et al., 2009 and McCann et al., 1975). TA100, and TA1535 were used to detect base pair substitutions; however, these were C:G and not A:T base pair substitutions. TA98, TA1537, and TA1538 were used to detect frameshift mutations (Kamber et al., 2009 and McCann et al., 1975). The positive controls used in the study were appropriate as they caused reliable positive results. This study is appropriate to test frameshift mutations as well as C:G base pair substitutions; however, this study did not test A:T base pair substitutions.

Results:

Reference ID: 4913649

The following tables illustrate the results with strains TA98, TA100, TA1535, TA1537, and TA1538 with and without S9 metabolic activation treated with ibuprofen (adapted from the publication):

Table 1. Mutagenicity testing of acetaminophen, aspirin, phenacetin and ibuprofen in the Ames test with no metabolic activation

	Mean revertant counts[a]				
Treatment[b]	TA98	TA100	TA1535	TA1537	TA1538
Negative control					
50.0 μl DMSO	25 (6)	146 (8)	11 (2)	9 (3)	17 (2)
IB (μg)					
1.0	30 (2)	112 (13)	13 (6)	10 (1)	16 (1)
10.0	22 (7)	140 (10)	8 (0)	6 (0)	17 (11)
100.0	25 (1)	137 (7)	10 (5)	8 (5)	19 (3)
500.0	11 (3)	34[c]	4 (3)	2 (1)	12 (6)
750.0	0	40 (4)	3 (1)	0	2[c]
1000.0	0	0	0	0	0
Positive indicators					
50.0 μg 9-AA·HCl	—	—	—	236 (25)[d]	—
0.5 μg Na Azide	—	405 (3)[d]	272 (10)[d]	—	—
200.0 μg Dexon	1882 (86)[d]	—	—	—	537 (21)[d]

[a] Negative control values represent the mean colony counts on six plates. All other treatment values are the mean from two plates. Standard deviations are in parentheses.
[b] DMSO, dimethylsulfoxide; APAP, acetaminophen; ASA, aspirin; PA, phenacetin; IB, ibuprofen; 9-AA·HCL = 9-aminoacridine·HCl.
[c] No revertant colonies present on one plate due to toxicity.
[d] Represents at least a doubling of negative control values.

Table 2. Mutagenicity testing of acetaminophen, aspirin, phenacetin and ibuprofen in the Ames test with activation by Aroclor 1254-induced rat liver S-9

	Mean revertant counts[a]				
Treatment[b]	TA98	TA100	TA1535	TA1537	TA1538
Negative control					
50.0 μl DMSO	46 (8)	129 (6)	16 (3)	11 (3)	26 (3)
IB (μg)					
1.0	41 (11)	140 (9)	18 (1)	8 (0)	29 (4)
10.0	41 (18)	133 (11)	17 (4)	10 (6)	28 (8)
100.0	38 (0)	141 (24)	15 (0)	7 (2)	26 (7)
500.0	37 (1)	107 (18)	10 (3)	5 (6)	23 (3)
750.0	35 (5)	82 (3)	0	5 (1)	0
1000.0	21 (6)	71 (12)	0	0	0
Positive indicator					
2.0 μg 2-AA	848 (8)[d]	1088 (15)[d]	241 (27)[d]	97 (3)[d]	790 (38)[d]

[a] Negative control values represent the mean colony counts on six plates. All other treatment values are the mean from two plates. Standard deviations are in parentheses.
[b] DMSO, dimethylsulfoxide; APAP, acetaminophen; ASA, aspirin; PA, phenacetin; IB, ibuprofen; 2-AA, 2-anthramine.
[c] No revertant colonies present on one plate due to toxicity.
[d] Represents at least a doubling of negative control values.

As shown in the tables above, there were no dose-dependent changes in the number of revertants in any of the ibuprofen treatment groups with and without S9 metabolic activation.

Thus, ibuprofen was not mutagenic under the conditions of this study.

Publication Title: Comparative mutagenic and genotoxic effects of three propionic acid derivatives ibuprofen, ketoprofen and naproxen (Philipose et. al, 1997)

Methods:

Strains:	*Salmonella typhimurium* strains TA97a, TA100, and TA102
Concentrations in definitive study:	1, 10, 100, 1000, and 5000 mcg/plate
Basis of concentration selection:	The 5000 mcg/plate dose resulted in toxicity in all strains tested.
Negative control:	DMSO
Positive control:	Without S9 activation: 4-nitro-*o*-phenylenediamine (20 mcg; all strains); With S9 activation: 2-aminofluorene (10 mcg; all strains)
Formulation/Vehicle:	DMSO

Reference ID: 4913649

Incubation & sampling time: Ibuprofen in vehicle (DMSO), vehicle alone (negative control), or positive control was pre-incubated with the bacteria with and without S9 mix in the top agar. The solution was mixed and overlaid onto a minimal bottom agar. After the overlay solidified, the plates were inverted and incubated at 37°C for 48 hrs. Afterwards, the plates were scored. All concentrations and controls (both positive and negative) in this toxicity-mutation assay were plated in duplicate. There was no confirmatory test performed.

Additional Methods/Validity:

Ibuprofen was obtained from the (b) (4). The study was conducted in (b) (4) The doses of ibuprofen tested were up to 1000 mcg/plate as the 5000 mcg/plate dose caused toxicity in the strains tested. The strains tested were *Salmonella typhimurium* strains TA97a, TA100, and TA102. TA97a tested for frameshift mutations, TA100 tested for C:G base pair substitutions, and TA102 tested for A:T base pair substitutions (Kamber et al., 2009). The positive controls used in the study were appropriate as they caused reliable positive results. Taken together, these methods represent a valid study.

Results:

The following tables illustrate the results with strains TA97a, TA100, and TA102, with and without S9 metabolic activation (adapted from the publication):

Table 1
Number of revertants induced by ibuprofen, ketoprofen and naproxen in the Salmonella plate incorporation test using TA97a with or without S9

Chemicals (μg/plate)	Number of revertants/plate			
	− S9 [a]		+ S9 [a]	
	Mean ± SD of 1st Expt.	Mean ± SD of 2nd Expt.	Mean ± SD of 1st Expt.	Mean ± SD of 2nd Expt.
Control (DMSO)	115.00 ± 1.41	117.00 ± 12.72	109.50 ± 16.26	113.00 ± 1.41
Ibuprofen				
1	142.50 ± 9.19 [c]	133.50 ± 6.36	134.00 ± 1.41	158.50 ± 14.84 [c]
10	133.00 ± 7.07 [b]	139.00 ± 12.72	136.50 ± 4.95	145.50 ± 10.60 [c]
100	119.00 ± 12.72	127.50 ± 4.95	131.00 ± 11.31	135.00 ± 1.41 [c]
1 000	116.50 ± 4.95	108.00 ± 9.89	126.50 ± 3.53	110.00 ± 26.87
5 000	Toxic	Toxic	Toxic	Toxic
Positive control				
NPD (20 μg/plate)	1191.00 ± 19.79	1119.00 ± 43.84		
2-AF (10 μg/plate)			957.00 ± 45.25	1056.00 ± 50.91

− S9, without metabolic activation; + S9, with metabolic activation.
[a] Mean ± SD of two plates. Results of each concentration were compared with the solvent control by Dunnett's *t*-test.
[b] $p < 0.05$.
[c] $p < 0.01$.

Reference ID: 4913649

Table 2
Number of revertants induced by iboprofen, ketoprofen and naproxen in the Salmonella plate incorporation test using TA100 with or without S9

Chemicals (μg/plate)	Number of revertants/plate			
	− S9 [a]		+ S9 [a]	
	Mean ± SD of 1st Expt	Mean ± SD of 2nd Expt.	Mean ± SD of 1st Expt.	Mean ± SD of 2nd Expt.
Control (DMSO)	133.00 ± 8.48	146.00 ± 14.14	151.00 ± 15.55	143.00 ± 16.97
Ibuprofen				
1	172.00 ± 2.83 [c]	166.50 ± 17.68	175.50 ± 28.99	161.00 ± 1.41
10	179.00 ± 1.41 [c]	177.00 ± 19.79	173.00 ± 16.97	177.00 ± 14.14
100	158.00 ± 9.90	166.50 ± 19.09	165.50 ± 13.00	157.00 ± 4.24
1 000	140.50 ± 9.19	121.50 ± 13.43	153.50 ± 2.12	137.50 ± 3.53
5 000	Toxic	Toxic	Toxic	Toxic
Positive control				
Sodium azide (1.5 μg/plate)	1292.50 ± 17.68	1167.50 ± 7.78		
2-AF (10 μg/plate)			1094.50 ± 86.97	1140.00 ± 97.58

− S9, without metabolic activation; + S9, with metabolic activation.
[a] Mean ± SD of two plates. Results of each concentration were compared with the solvent control by Dunnett's *t*-test.
[b] $p < 0.05$.
[c] $p < 0.01$.

Table 3
Number of revertants induced by ibuprofen, ketoprofen and naproxen in the Salmonella plate incorporation test using TA102 with or without S9

Chemicals (μg/plate)	Number of revertants/plate			
	− S9 [a]		+ S9 [a]	
	Mean ± SD of 1st Expt.	Mean ± SD of 2nd Expt.	Mean ± SD of 1st Expt.	Mean ± SD of 2nd Expt.
Control (DMSO)	327.50 ± 31.82	308.00 ± 31.11	355.00 ± 12.73	301.50 ± 19.09
Ibuprofen				
1	409.00 ± 29.70	371.00 ± 29.69	292.50 ± 67.17	319.00 ± 29.69
10	338.00 ± 28.28	324.00 ± 21.21	283.00 ± 21.21	336.50 ± 6.36
100	295.00 ± 12.72	345.00 ± 8.48	315.50 ± 23.33	285.50 ± 26.16
1 000	Toxic	Toxic	Toxic	Toxic
Positive control				
MMS (1 μl/plate)	3001.00 ± 172.53	3032.50 ± 168.99		

− S9, without metabolic activation; + S9, with metabolic activation.
[a] Mean ± SD of two plates. Results of each concentration were compared with the solvent control by Dunnett's *t*-test.
[b] $p < 0.05$.

As shown in the table above, there were no dose-dependent changes in the number of revertants in the ibuprofen treatment groups with and without S9 metabolic activation.

Thus, ibuprofen was not mutagenic under the conditions of this study.

In Vivo Sister Chromatid Exchange Assay

Methods: Paraffin-coated BrdU tablets were implanted in mice prior to treatment with ibuprofen (25, 50, or 100 mg/kg via IP injection). Control mice received an injection of vehicle (75 mcL DMSO). Mitomycin C (1.5 mg/kg) was used as a positive control. Colchicine (4 mg/kg, IP) was injected 22 hours after the BrdU tablet and two hours later bone marrow was expelled using 0.075 M KCl. Cells were lysed with hypotonic treatment, fixed and chromosomes were differentiated and stained for evaluation. In a separate group of animals, ibuprofen was dosed at 270 mg/kg via oral gavage. Cyclophosphamide (10 mg/kg) served as the positive control.

In vivo sister chromatid exchanges induced by ibuprofen, ketoprofen and naproxen in mice after i.p. administration

Treatment	SCE/cell of 5 animals	SCE/cell (mean ± SD) [a]	Value of trend statistics	Replicative indices (mean ± SD) [a]
Solvent control (75 µl of DMSO)	5.1, 4.3, 4.4, 4.3, 4.4	4.50 ± 0.34		1.85 ± 0.06
IB (mg/kg)				
25	4.7, 5.6, 4.5, 5.3, 5.1	5.04 ± 0.44		1.88 ± 0.09
50	5.7, 5.0, 5.7, 6.1, 6.0	5.70 ± 0.43 [b]	6.133 [b]	1.87 ± 0.07
100	6.1, 5.6, 6.2, 6.0, 6.1	6.00 ± 0.23 [b]		1.75 ± 0.06
KP (mg/kg)				
25	5.0, 4.6, 5.4, 5.9, 7.1	5.60 ± 0.97		1.85 ± 0.04
50	5.7, 5.8, 6.5, 4.7, 5.6	5.66 ± 0.64 [b]	8.250 [b]	1.78 ± 0.08
100	7.5, 6.1, 5.5, 6.1, 6.6	6.36 ± 0.75 [b]		1.76 ± 0.07
NP (mg/kg)				
25	5.2, 4.8, 5.8, 5.1, 4.5	5.08 ± 0.49		1.82 ± 0.08
50	4.9, 5.1, 5.3, 5.0, 6.0	5.26 ± 0.44	5.190 [b]	1.80 ± 0.06
100	5.6, 5.3, 6.1, 7.0, 6.1	6.02 ± 0.65 [b]		1.90 ± 0.08
Positive control				
Mitomycin-C (1.5 mg/kg)	18.6, 19.8, 23.0, 25.5, 21.3	21.64 ± 2.72		1.89 ± 0.10

[a] Mean ± SD of 5 animals (30 cells per animal). Results of each dose were compared with the control using Dunnett's test.
[b] $p < 0.01$.

In vivo sister chromatid exchanges induced by ibuprofen, ketoprofen and naproxen in mice after oral administration

Treatment	SCE/cell of 5 animals	SCE/cell (mean ± SD) [a]	Replicative indices (mean ± SD) [a]
Solvent control (Gum acacia)	4.4, 4.0, 5.6, 5.4, 4.0	4.68 ± 0.77	1.87 ± 0.08
Ibuprofen (270 mg/kg)	5.9, 5.6, 5.9, 7.7, 5.7	6.16 ± 0.87 [b]	1.79 ± 0.07
Ketoprofen (270 mg/kg)	5.8, 5.6, 4.7, 4.8, 4.8	5.14 ± 0.52	1.82 ± 0.04
Naproxen (270 mg/kg)	5.0, 5.7, 7.1, 6.8, 8.5	6.62 ± 1.35 [b]	1.88 ± 0.06
Positive control			
Cyclophosphamide (10 mg/kg)	23.2, 18.5, 18.3, 20.5, 18.8	19.86 ± 2.06	1.89 ± 0.08

[a] Mean ± SD of 5 animals (30 cells per animal). Results of each dose were compared with the control using Dunnett's test.
[b] $p < 0.05$.

The results suggest that ibuprofen demonstrated clastogenic activity in vivo.

Publication Title: Sister Chromatid Exchange in Patients Treated with Nonsteroidal Anti-Inflammatory Drugs (Sardas et al., 1991)

Reference ID: 4913649

Patients with degenerative rheumatic diseases and invertebral disc disorders who were receiving NSAIDs: diclofenac (Voltaren, 200 mg/day); ibuprofen (Brufen, 1200 mg/day); or indomethacin (Indocid, 75 mg/day) were evaluated to determine whether NSAID treatment for two weeks resulted in genotoxic effects on peripheral lymphocytes. Peripheral heparinized blood samples were collected before NSAID treatment and after a 2-week treatment period with one NSAID. Chromosomal preparations were stained by fluorescence plus the Giemsa technique. An average of 30 metaphase plates with 46 intact chromosomes and well differentiated SCE were scored (SCE/cell). No significant differences in SCE frequency in 40 patients were observed when treated patients with NSAIDs were compared to results obtained from untreated patients as illustrated in the following table (from the publication):

Table I. Mean sister chromatid exchange (SCE)/cell in human lymphocytes before and after therapy with nonsteroidal anti-inflammatory drugs (not significant at $p > 0.05$ by paired t-test)

Drug	No. of subjects	Mean SCE/cell (± SD)	
		before	after
Diclofenac	15	7.60 ± 1.59	7.67 ± 1.64
Ibuprofen	12	5.98 ± 1.70	6.02 ± 2.05
Indomethacin	13	6.60 ± 1.07	6.90 ± 1.60

Publication Title: Investigations of the influence of nonsteroidal antirheumatic drugs on the rates of sister-chromatid exchange (Kullich and Klein, 1986)

Sister-chromatid exchange (SCE) rates before and after treatment with several NSAIDs including diclofenac (100 mg/day), fluriprofen (200 mg/day), ibuprofen (1200 mg/day), indomethacin (75 mg/day), isoxicam (200 mg/day), ketoprofen (150 mg/day), piroxicam (20 mg/day), pirprofen (800 mg/day), and tiaprofenic acid (600 mg/day) were evaluated in human lymphocytes in vivo. Herparinised blood samples were collected from healthy, non-smoking volunteers prior to commencing therapy and after a 2-week treatment period. Samples were stained with 2.5% Giemsa solution with 20 metaphases that contained complete, well-spread chromosome sets were counted. SCE rates of patients treated with an NSAID and controls were not significantly different and were within normal variation and in the normal range as illustrated in the following table (from the publication):

Mean values = $\bar{X}$; standard deviation = s; number of subjects = n.

Substance	n	SCE/cell				Significance
		Untreated		Treated		
		$\bar{x}$	s	$\bar{x}$	s	
Diclofenac	7	5.57 ± 1.21		6.16 ± 0.78		n.s.
Flurbiprofen	7	5.40 ± 1.57		4.84 ± 1.26		n.s.
Ibuprofen	7	5.17 ± 0.61		4.77 ± 0.24		n.s.
Indometacin	6	5.30 ± 1.04		5.67 ± 1.46		n.s.
Isoxicam	11	4.40 ± 0.56		4.55 ± 0.57		n.s.
Ketoprofen	7	5.49 ± 1.33		5.94 ± 2.16		n.s.
Piroxicam	6	3.72 ± 0.59		4.37 ± 1.10		n.s.
Pirprofen	7	5.04 ± 0.30		5.54 ± 0.78		n.s.
Tiaprofenic acid	6	4.60 ± 0.99		4.42 ± 0.61		n.s.
Controls	34	5.7 ± 1.8				
		without		with		
Mitomycin C	in vitro	4.2 ± 1.7		30.0 ± 4.3		

Publication Title: Do non-steroidal anti-inflammatory drugs induce sister chromatid exchanges in T lymphocytes? (Ozkul et al, 1996)

A total of 48 patients were treated with NSAID drugs (ibuprofen - 800 mg/day; ketoprofen - 150 mg/day, naproxen – 1000 mg/day, indomethacin - 75 mg/day; diclofenac - 100 mg/day, acetylsalicylic acid) for two weeks. Sister chromatid exchanges in cultured lymphocytes from patients before and after NSAID treatment were compared. There were no differences in average SCE rates between patients before and after any NSAID treatment as illustrated in the following table (from the publication):

	Mean SCEs/cell (± SD)	
Drug	**Before treatment**	**After treatment**
Ibuprofen	4.34 ± 1.57	4.50 ± 1.80
Indomethacin	5.12 ± 1.04	5.47 ± 1.57
Naproxen	5.01 ± 2.01	5.10 ± 1.32
Acetylsalicylic acid	4.75 ± 1.08	4.82 ± 0.98
Ketoprofen	4.37 ± 1.62	4.48 ± 1.22
Diclofenac	5.32 ± 1.52	5.63 ± 1.48

Differences between the means before and after treatment were not significant = ($P > 0.05$) according to the paired t-test.

Reviewer's Comments on the overall genotoxicity of ibuprofen:

The Ames assays with ibuprofen from both Oldham et al., 1986 and Philipose et al., 1997 represent an overall weight of evidence approach to test the mutagenicity of ibuprofen. Taken together, ibuprofen is not mutagenic (frameshift mutations as well as C:G and A:T base pair substitutions). The results from these papers are appropriate to be included in the label.

Reference ID: 4913649

Philipose also conducted in vivo studies in mice to examine the clastogenic activity of ibuprofen. These authors report a “weakly clastogenic” effect in an in vivo sister-chromatid exchange assay in mice. However, several publications have examined whether ibuprofen (800-1200 mg/day) treatment for 2-weeks altered SCE rates in human lymphocytes and reported no significant differences before and after ibuprofen treatment. It was also noted that NSAID as a class after a two week treatment duration did not alter SCE rates before and after treatment. Collectively, the in vivo results reported in mice have not been observed in humans and therefore it is recommended the clastogenic effects observed in mice be omitted from the label.

Carcinogenicity:

Publication Title: Some Aspects of the Pharmacology, Metabolism, and Toxicology of Ibuprofen (Adams et. al, 1970)

Mouse Carcinogenicity study

Methods:

Doses:	300 mg/kg for 43 weeks, reduced to 100 mg/kg from Week 43 to 80 due to GI toxicity.
Frequency of dosing:	Daily
Dose volume:	No reported
Route of administration:	Not reported, presumably oral
Formulation/Vehicle:	Not reported
Species/Strain:	Mouse
Number/Sex/Group:	50/sex
Satellite groups:	None
Study design:	Not reported
Deviation from study protocol:	Unknown

Results:
The authors provided the following summary tables.

Reference ID: 4913649

MICE ALIVE AFTER 43 AND 80 WEEKS ON IBUPROFEN

	Ibuprofen Dosage		
	300 mg./kg./day 0 to 43 weeks		100 mg./kg./day 43 to 80 weeks
	Initial number	After 43 weeks	After 80 weeks
Males:			
Control	50	48	15
Dosed	50	32	6
Females:			
Control	50	42	12
Dosed	50	41	24

TUMOUR INCIDENCE IN MICE ON IBUPROFEN FOR LONGER THAN 43 WEEKS

	Males		Females	
	Control	Dosed	Control	Dosed
Mice examined/Mice with tumours	43/35	29/21	40/34	40/26
Types of tumour:				
Hepatomas	12	5	5	5
Liver haemangiomas	1	1	1	1
Lymphomas	13	14	22	19
Breast adenocarcinomas	0	0	9	1
Others (benign)	16	9	12	15

Rat Carcinogenicity study

Methods:

Doses:	180 mg/kg for 56 weeks, reduced to 60 mg/kg from Week 56 to 104 due to GI toxicity.
Frequency of dosing:	Daily
Dose volume:	No reported
Route of administration:	Not reported, presumably oral
Formulation/Vehicle:	Not reported
Species/Strain:	Rat
Number/Sex/Group:	30/sex
Satellite groups:	None
Study design:	Not reported
Deviation from study protocol:	Unknown

Reference ID: 4913649

Results:
The authors provided the following summary tables.

TABLE VI

RATS ALIVE AFTER 56 AND 104 WEEKS ON IBUPROFEN

	Ibuprofen Dosage		
	180 mg./kg./day 0 to 56 weeks		60 mg./kg./day 56 to 104 weeks
	Initial number	After 56 weeks	After 104 weeks
Males:			
Control	30	30	16
Dosed	30	22	10
Females:			
Control	30	30	23
Dosed	30	21	11

TUMOUR INCIDENCE IN RATS ON IBUPROFEN FOR LONGER THAN 56 WEEKS

	Males		Females	
	Control	Dosed	Control	Dosed
Rats examined/Rats with tumours	30/13	22/4	30/16	21/12
Types of tumour:				
Hepatomas	1	0	0	2
Liver haemangiomas	0	1	0	0
Lymphomas	5	1	2	3
Others (malignant)	3	1	5	1
Others (benign)	8	2	20	11

The Authors report no evidence of tumors in ibuprofen-treated animals. However, the studies are limited by modern standards as there is only one dose tested and there were limited number of animals alive by the end of the study. Therefore, we do not recommend including these data in the product labeling.

Reproductive and Developmental Toxicology:

Publication Title: Absorption, Distribution and Toxicity of Ibuprofen (Adams et. al, 1969)

Rat embryofetal study

Methods:

Doses:	0, 7.5, 20, 60, and 180 mg/kg/day of ibuprofen
Frequency of dosing:	Once daily from the 1st to the 20th day of pregnancy
Dose volume:	Presumably 10 mL/kg as this was the control volume
Route of administration:	Oral intubation
Formulation/Vehicle:	Control females were given 10 mL/kg of water daily
Species/Strain:	Rats/Albino rats
Number/Sex/Group:	No information given as to the number of maternal dams
Satellite groups:	Additional group to receive 7.5 and 20 mg/kg/day of ibuprofen throughout pregnancy to birth
Study design:	No information given
Deviation from study protocol:	N/A

Additional Methods:

Ibuprofen was presumably obtained from (b) (4). with no information on its purity. The study was conducted in the (b) (4). Primiparous females and proven males were caged together and the presence of spermatozoa in the vaginal smear is considered the first day of pregnancy. Thereafter, dosing with ibuprofen took place until the 20th day of pregnancy. Uterine contents were examined on Day 21 of pregnancy and the fetuses were examined for external, visceral, and skeletal abnormalities. The number of live, dead, and resorbed fetuses, and the number of corpora lutea were recorded. All live fetuses were weighed and examined for external and visceral abnormalities. The brain, eyes, gonads, kidneys, liver, and lungs for some were examined histologically. Skeletal examination of the fetuses included staining by Dawson's method.

Additional groups of rats received 7.5 or 20 mg/kg/day of ibuprofen throughout pregnancy until birth and the young were examined 3 weeks after delivery in order to show certain types of developmental abnormalities at weaning which may not be visible before birth. All pups in these groups were examined in detail for gross abnormalities.

Results:

Females receiving 20, 60, and 180 mg/kg/day of ibuprofen on Days 1 to 20 of pregnancy were observed with gastrointestinal lesions with a dose-dependent increase in severity. There were no such lesions in the control and 7.5 mg/kg/day dose groups. There was a diminished growth rate in the 180 mg/kg/day dose group only. There was a decrease in the number of litters and % alive fetuses in the 180 mg/kg/day dose group as illustrated in the following table (from the publication):

Reference ID: 4913649

TABLE 5

EMBRYOTOXIC ACTIVITY OF IBUPROFEN GIVEN ORALLY TO RATS FROM DAY 1 TO 20 OF PREGNANCY

Dosage (mg/kg/day)	Number of litters	Number of fetuses		Live litter size (mean ± SE)	Fetal body weight (g, mean ± SE)	Implantation index (%)[b]
		Alive (%)	Dead (%)[a]			
180	4	35 (89.7)	4 (10.3)	8.8 ± 0.6	2.73 ± 0.19	93
60	15	121 (90.3)	13 (9.7)	8.1 ± 0.6	2.99 ± 0.03	92
20	13	115 (89.9)	13 (10.1)	8.8 ± 0.5	2.94 ± 0.08	90
7.5	11	95 (92.2)	8 (7.8)	8.6 ± 0.7	2.81 ± 0.11	94
Control	11	91 (92.9)	7 (7.1)	8.3 ± 0.6	2.94 ± 0.28	89

[a] Dead fully formed fetuses *in utero* and resorbed fetuses fused with the placenta.
[b] Ratio of implants to corpora lutea.

The dams that received ibuprofen throughout pregnancy until birth had uneventful pregnancies and delivered their pups without difficulty as illustrated in the following table (from the publication):

TABLE 6

POSTNATAL OBSERVATIONS ON YOUNG OF RATS RECEIVING IBUPROFEN ORALLY FROM DAY 1 OF PREGNANCY UNTIL PARTURITION

Dosage (mg/kg/day)	Number of litters	Number of young		Live litter size (mean ± SE)	Viability index (%)[a]	Weaning weight (g, mean ± SE)
		Alive	Stillborn			
20	10	89	1	8.9 ± 0.6	89.2	32.1 ± 0.9
7.5	6	51	2	8.5 ± 0.9	97.9	37.7 ± 1.6
Control	15	143	2	9.5 ± 0.4	96.8	32.5 ± 0.9

[a] The number of young weaned expressed as the percentage of the number born alive less those killed at birth.

As shown in the table above, a similar number of live young per litter was obtained from ibuprofen-treated and control rats.

The following table illustrates that there were no malformed fetuses from any of the ibuprofen-treated dams in both sets of treatments (those dams receiving ibuprofen from Days 1 to 20 of pregnancy and those receiving ibuprofen throughout pregnancy until birth), from the publication:

TABLE 7

NUMBER OF MALFORMED FETUSES AND YOUNG OBTAINED FROM RATS RECEIVING IBUPROFEN ORALLY DURING PREGNANCY

Type of malformation	Treatment from day 1 to 20 of pregnancy (mg/kg/day)					Treatment from day 1 of pregnancy to parturition (mg/kg/day)		
	180	60	20	7.5	Control	20	7.5	Control
External and visceral								
Hydrocephalus	—	—	—	1	—	—	—	—
Anophthalmia	—	—	—	—	—	1	—	—
Microphthalmia	—	—	—	—	—	1	1	1
Subcutaneous edema	—	1[a]	—	—	—	—	—	—
Diaphragmatic hernia	—	—	—	—	—	—	—	2
Dextrorotation of viscera	—	1	—	—	—	—	—	—
Unilateral hydronephrosis	—	—	—	—	1	—	—	—
Blood cyst on liver	—	—	—	—	—	—	—	1
Testis underdeveloped	—	—	—	—	—	—	—	1
Skeletal								
13th rib shortened	—	3	2	—	1	4	1	3
13th rib absent	—	—	—	—	—	1	1	1
Rib articulation misplaced	—	—	—	—	—	—	—	1
Swelling on ribs	—	—	—	—	—	1	1	—
Fused ribs and fused sternebrae	—	—	—	—	—	—	1	—
Fused ribs and misaligned vertebral bodies	—	1	—	—	—	—	—	—
Misplaced ribs, fused sternebrae and accessory sternebrae	—	—	—	—	—	1	—	—
Sternebrae underdeveloped	—	2	1	—	5	—	—	1
Accessory ossified center in sternum	—	—	—	—	—	1	5	22
Number of litters	4	15	13	11	11	10	6	15
Number of fetuses or young examined	35	121	115	95[b]	91	90	53	145
Number of litters with malformed fetuses or young	0	7	2	1	4	6	5	10

[a] Skeletal malformations included wavy ribs and bilateral curvature of radius and ulna.
[b] Skeletons of this group were not examined.

Reviewer's Comments:

The study appeared to use the methods seen in standard embryofetal developmental toxicity studies. However, maternal observations appear to be limited to body weight and examination of the gastrointestinal tract. A maternal NOAEL cannot be determined in this study although the local GI effects are expected evidence of maternal toxicity for an NSAID. Use of COX inhibitors (such as ibuprofen) could increase postimplantation loss, induce skeletal, midline, diaphragmatic, heart defects, and fetal growth retardation as well as complicate pregnancy if taken near delivery-time due to pulmonary hypertension, secondary to the constriction of ductus arteriosis (Cappon et al., 2003; Cook et al., 2003; and Ostensen and Skomsvoll, 2004). As such, decreased fetal weight and reduced number of litters and the % alive number of young in dams treated with ibuprofen were observed in this study. The rat dose of 180 mg/kg/day that resulted in reduced number of litter, % alive number of fetuses, and fetal weight represents a human dose of 1751 mg in an average human weighing 60 kg, based on a body surface area comparison. This rat dose represents an exposure margin of 0.55 based on the maximum daily dose of 3200 mg for ibuprofen.

Publication Title: Absorption, Distribution and Toxicity of Ibuprofen (Adams et al., 1969)

Reference ID: 4913649

NOTE to reader: Dr. Stewart Adams led the research team in Boots Pure Drug Company (Nottingham England) that discovered ibuprofen. The studies described in his 1969 and 1970 papers may or may not have served as the basis of the original Motrin® drug product NDA submission to the FDA (NDA 17463, Approved September 19, 1974). The prescription labeling for Motrin® states "Reproductive studies conducted in rats and rabbits have not demonstrated evidence of developmental abnormalities."

Rabbit embryofetal study

Methods:

Doses:	0, 7.5, 20, and 60 mg/kg/day of ibuprofen
Frequency of dosing:	Once daily from the 1st to the 29th day of pregnancy
Dose volume:	1 mL/kg as this was the dose volume in the control
Route of administration:	Oral
Formulation/Vehicle:	Control females were given 1 mL/kg daily of water
Species/Strain:	Rabbit/New Zealand White
Number/Sex/Group:	Information not given
Satellite groups:	None
Study design:	Information not given
Deviation from study protocol:	N/A

Additional Methods:

Ibuprofen was presumably obtained from (b) (4) with no information on its purity. The study was conducted in the (b) (4) Virgin females weighing 2.5 to 5 kg were paired with proven males and the day of mating was termed Day 0 of pregnancy. The following day was the start of dosing with 0, 7.5, 20, or 60 mg/kg/day of ibuprofen daily until Day 29 of pregnancy. All females were sacrificed on Day 30 and their uterine contents were examined. The number of live, dead, and resorbed fetuses, and the number of corpora lutea were recorded. All live fetuses were weighed and examined for external and visceral abnormalities. The brain, eyes, gonads, kidneys, liver, and lungs for some were examined histologically. Skeletal examination of the fetuses included staining with Alizarin Red S by Crary's method.

Results:

Females given 60 mg/kg/day on Days 1 to 29 of pregnancy grew less than control and were observed with stomach ulcers. These females were also observed with pneumonia and a mild degree of focal hepatitis that is thought to be a secondary infection to the gastric lesions. Two females from the 60 mg/kg/day dose group gave birth prematurely to normal pups on Days 26 and 28 of pregnancy. Females given 20 mg/kg/day were similar although less affected. Females given 7.5 mg/kg/day grew normally but some had gastric ulcers or erosions. Minimal gastric damage was observed in 2/23 controls. The number of litters and the % alive number of fetuses decreased in a dose-dependent manner as shown in the following table (from the publication):

TABLE 3

EMBRYOTOXIC ACTIVITY OF IBUPROFEN GIVEN ORALLY TO RABBITS FROM DAY 1 TO 29 OF PREGNANCY

Dosage (mg/kg/day)	Number of litters	Number of fetuses		Live litter size (mean ± SE)	Fetal body weight (g, mean ± SE)	Implantation index (%)[b]
		Alive (%)	Dead (%)[a]			
60	17	113 (82.5)	24 (17.5)	6.7 ± 0.7	49.6 ± 2.2	76.5
20	19	151 (83.0)	31 (17.0)	8.0 ± 0.4	47.6 ± 1.5	94.8
7.5	22	178 (84.0)	34 (16.0)	8.1 ± 0.6	48.2 ± 1.8	88.3
Control	23	209 (87.8)	29 (12.2)	9.1 ± 0.5	49.6 ± 1.0	92.3

[a] Dead fully formed fetuses *in utero* and resorbed fetuses fused with the placenta.
[b] Ratio of implants to corpora lutea.

There were no consistent pattern of malformations and the small number of cases showed no tendency relatable to dose except for the 4 cases of congenital malformations in the 60 mg/kg/day dose group (see the table below, from the publication):

Reference ID: 4913649

TABLE 4

NUMBER OF MALFORMED FETUSES OBTAINED FROM RABBITS RECEIVING IBUPROFEN ORALLY FROM DAY 1 TO 29 OF PREGNANCY

Type of malformation	Dosage (mg/kg/day)			
	60	20	7.5	Control
External and visceral				
Cyclopia and associated malformations	4[a]			
Cranioschisis		1		
Exophthalmos		3		
Microphthalmia		1	1	
Forelimb flexure				2
Incomplete skin-covering around umbilicus, duplication of falciform ligament, and small lobe of lung absent				1[b]
Diverticulum of ileum				1
Gall bladder absent		1	1	
Accessory gall bladder			1	
Bilobed gall bladder		1		
Swelling on bile duct			1	
Small lobe of lung absent	3		2	
Skeletal				
Lumbar scoliosis				1
1st rib shortened			1	
Swelling on ribs	1			
Xiphoid underdeveloped				1
Sternebra misshapen			1	2
Sternebrae joined by ossified strand		2		
5th sternebra underdeveloped	5	2	3	10
Number of litters	17	19	22	23
Number of fetuses examined	113	151	178	209
Number of litters with malformed fetuses	6	6	7	11

[a] All 4 fetuses in the same litter.
[b] Skeletal malformations included unilateral rib fusion and fusion of the 4th and 5th sternebrae.

Reviewer's Comments:

Reference ID: 4913649

The study appeared to use the methods seen in standard embryofetal developmental toxicity studies although dosing was over the entire gestational period. However, maternal observations appear to be limited to body weight, clinical observations, and examination of the gastrointestinal tract. A maternal NOAEL cannot be determined in this study based on dose-dependent gastric lesions at all doses; however the low dose could be considered a LOAEL. Use of COX inhibitors (such as ibuprofen) could increase postimplantation loss, induce skeletal, midline, diaphragmatic, heart defects, and fetal growth retardation as well as complicate pregnancy if taken near delivery-time due to pulmonary hypertension, secondary to the constriction of ductus arteriosis (Cappon et al., 2003; Cook et al., 2003; and Ostensen and Skomsvoll, 2004). Reduced number of litter and % alive number of fetuses from does treated with ibuprofen were observed in this study. The 60 mg/kg/day dose in rabbits that resulted in 4 cases of cyclopia related malformations. This dose represents a human dose of 1,167.6 mg in an average human weighing 60 kg, based on a body surface area comparison. This rat dose represents an exposure margin of 0.36 based on the maximum daily of 3200 mg for ibuprofen.

The authors state that “Apart from 4 young in 1 litter with multiple malformations characteristic of cyclopia (Ballantyne, 1904), there was no consistent pattern of malformations, and the small number of cases showed no tendency related to dose.”

Publication Title: Absorption, Distribution and Toxicity of Ibuprofen (Adams et al., 1969)

Reproductive study

Methods:
Ten male and 20 female rats of proven fertility were given a powdered diet containing 0.035% of ibuprofen (equivalent to a daily dose of 20 mg/kg) along with a control group on a plain diet for a 60 day pre-mating period. After 60 days, all rats that received ibuprofen were given a plain diet and the females were kept (14 days, 2 to a cage) with 1 male of the same dietary group (mating period). Records were kept of females becoming pregnant and of litter sizes at birth.

Results:
Female rats given a diet containing 0.035% of ibuprofen showed a small loss in weight while similarly treated male rats gained weight. Two control females were sacrificed humanely, one having a mammary abscess and the other having an umbilical hernia. The number of females that became pregnant after the 14-day mating period is 15/20 in the ibuprofen group versus 16/18 in the control. All the males in the 2 groups mated successfully apart from 1 male receiving ibuprofen, which may account for 2 of the 5 ibuprofen-treated females to become pregnant.

Reference ID: 4913649

Shortly before giving birth, 2 ibuprofen-treated females had vaginal hemorrhage. Of these, 1 died with 10 fully formed fetuses in the uterus and extensive hemorrhage surrounding the placenta, gastric ulcers, and anemia. The other female was sacrificed humanely with lung congestion, a large blood clot in one uterine horn, and no dead or resorbed fetuses. The remaining ibuprofen-treated females had uneventful pregnancies and give birth to litters with an average of 7.9 live young, compared to 7.3 live young in the control.

Reviewer's Comments:

This study is appropriate for examining fertility especially in males as 60 days should be sufficient to examine epididymal transit and possibly spermatogenesis. However, sperm parameters were not taken (sperm motility, sperm count, appearance, etc.) and female fertility parameters, such as fertility index, were not determined. In other words, the only fertility measure was successful mating for both the males and females in this study. The rat dose of 20 mg/kg is equivalent to a human dose of 194.6 mg to an average human weighing 65 kg, based on a body surface area comparison. The rat dose of 20 mg/kg yields an exposure margin of 0.061 based on the maximum daily of 3200 mg for ibuprofen.

Publication Title: Developmental Toxicity Evaluation of Ibuprofen and Tolmentin Administered in Triple Daily Doses to Wistar CRL:(WI)WUBR Rats (Burdan 2004)

Rat embryofetal study

Methods:

Doses:	0, 25.5, 255, and 600 mg/kg of ibuprofen (these are total daily dose as the rats were dosed 3 times daily)
Frequency of dosing:	Three times daily (8 hours apart) from the 8th to the 21th day of pregnancy
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Distilled water
Species/Strain:	Rats/Wistar CRL:(WI)WUBR (albino rats)
Number/Sex/Group:	Group sizes were not provided
Satellite groups:	None
Study design:	N/A
Deviation from study protocol:	N/A

Additional Methods:

Reference ID: 4913649

Ibuprofen was presumably obtained from [redacted] (b) (4) and is >99% pure. The study was conducted by [redacted] (b) (4). The vehicle appears to be distilled water. The dose volume is 10 mL/kg. The rats were dosed three times daily (8 hours apart) from Day 8 to Day 21 of gestation via oral gavage. Mortality checks were performed daily before treatment and 3 times daily during treatment. Body weight gains are monitored daily from Day 8 to the end of pregnancy.

On Gestation Day 21, the dams were sacrificed and the blood, uterus, and abdominal organs were collected. For each blood sample, plasma levels of ALT, AST, urea, and total protein were determined. Liver, kidney, and spleen weights were recorded. Organs of the gastrointestinal tract were prepared and stained for histopathological examination.

Ovarian corpora lutea were counted. The uteri were examined for the presence and position of resorption sites, dead or live fetuses, and the number of implantation sites. Fetuses were removed, sexed, and examined macroscopically for external malformations. The weight of the fetuses and placentas, the fetal crown-rump length, and tail length were measured. The pre- and postimplantation loss was calculated using the following formula (from the publication):

$$\text{Preimplantation loss}\,(\%) = ((\text{no. corpora lutea} - \text{no. implantations})/\text{no. corpora lutea}) \times 100$$

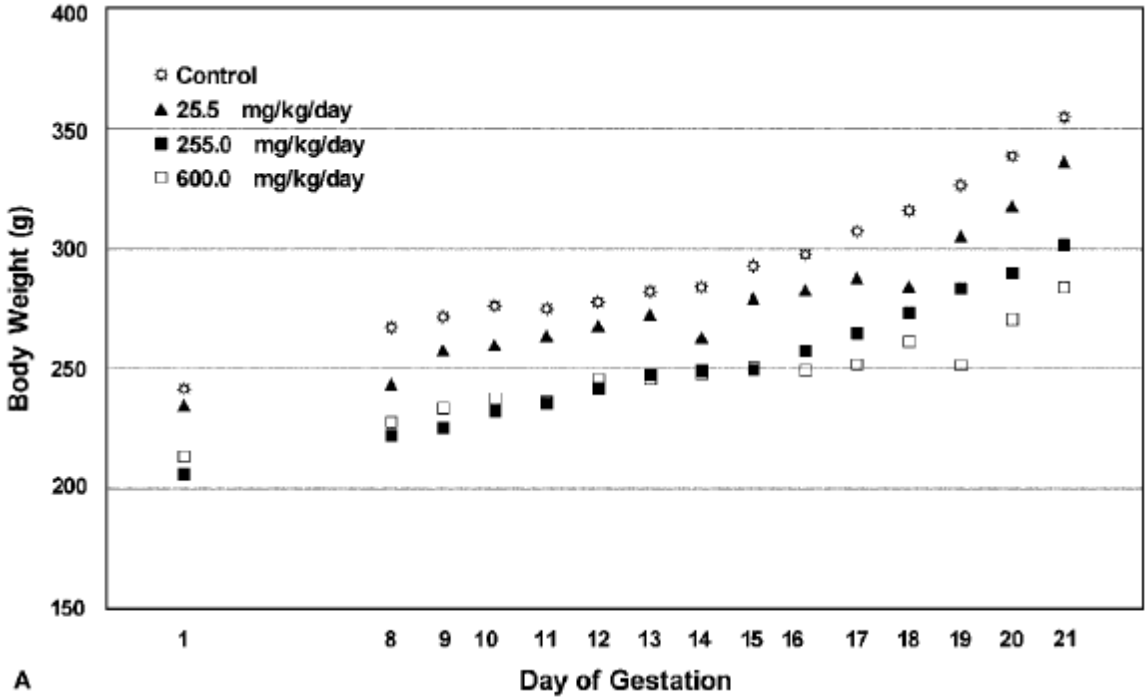
$$\text{Postimplantation loss}(\%) = ((\text{no. implantations} - \text{no. live fetuses})/\text{no. implantations}) \times 100$$

One in 10 fetuses from each experimental group were randomly selected for ultrastructural and genetic examination. Two-thirds randomly selected fetuses from each litter were stained with alcian blue and alizarin red-S to study skeletal malformations. Internal organs were grossly examined to evaluate possible pathological changes. The remaining fetuses were selected to study soft tissue abnormalities.

Results:

There appears to be a dose-dependent decrease in the body weights of the dams treated with ibuprofen as shown in the following figure (from the publication):

Reference ID: 4913649



In the blood, there was a dose-dependent increase in AST, ALT, and urea as well as a dose-dependent decrease in total protein (TP) in dams treated with ibuprofen; however, only the changes at the 600 mg/kg/day dose group was significantly different from control (from the publication):

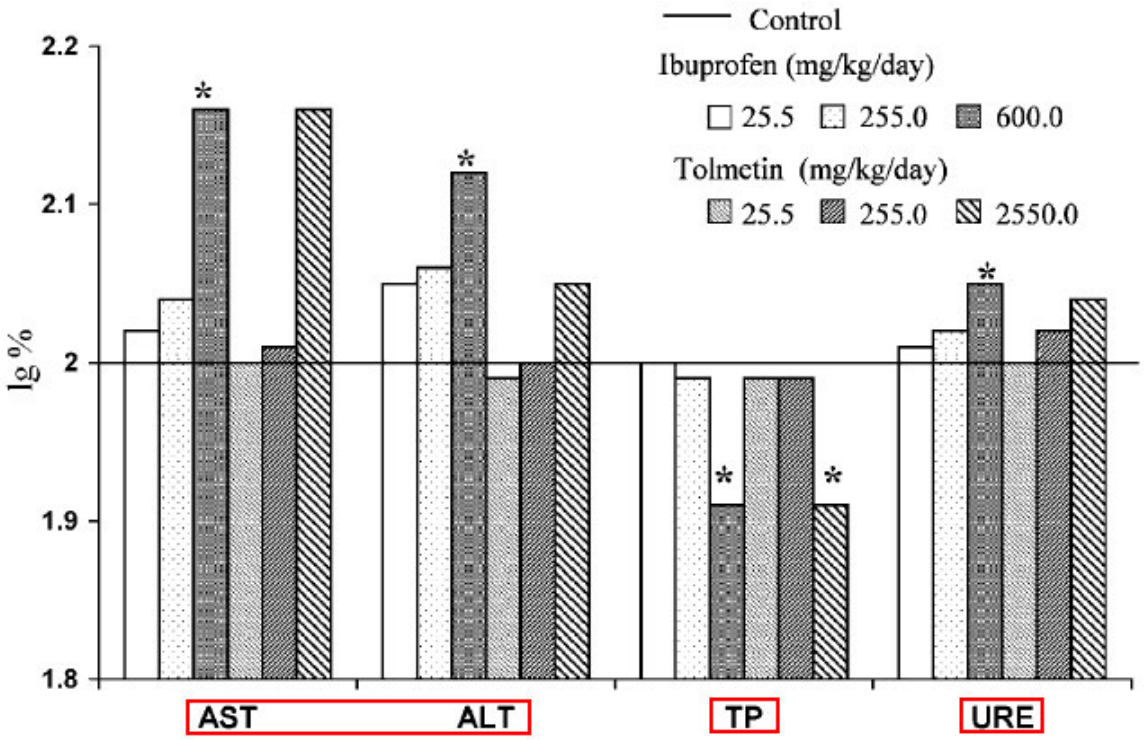


Fig. 2. Relative activity of alanine (ALT) and aspartate aminotransferase (AST), level of total protein (TP) and urea (URE) in dams treated with ibuprofen and tolmetin. Data presented as a lg% of the control value. *$p<0.05$.

There is a reduction in the fetal weight, fetal length, and the % live fetuses/litter with skeletal variations in the 600 mg/kg/day dose group as shown in the following table (from the publication):

Table 1
Maternal Reproductive, Litter and Fetal Alteration Observations in Control, Ibuprofen-, and Tolmetin-Exposed Groups

		Ibuprofen (mg/kg/day)			Tolmetin (mg/kg/day)		
	Control	25.5	255.0	600.0	25.5	255.0	2550.0
Initially selected dams (n)	20	20	20	20	20	20	20
Found dead dams (n)	0	0	4	14	0	3	16
Pregnant dams sectioned on GD 21	20	20	16	6	20	17	4
Corpora lutea	14.20±2.37	13.90±1.86	13.93±1.73	14.16±1.47	13.60±2.16	13.59±2.43	13.25±0.95
Number of fetuses	12.50±2.52	12.60±2.25	12.18±1.72	11.83±3.92	12.35±2.23	12.53±2.67	9.50±4.04
Males	5.95±2.37	6.70±2.07	6.18±1.27	6.33±1.63	6.15±1.98	6.06±2.33	5.50±1.91
Females	6.55±1.09	5.90±1.68	6.00±1.50	5.50±2.94	6.20±1.67	6.49±0.94	4.00±2.16
Number of early resorptions (n/litter)	8 (7)	3 (2)	7 (5)	5 (4)	5 (5)	4 (3)	1 (1)
Number of late resorptions (n/litter)	0	0	0	0	0	0	0
Number of dead fetuses (n/litter)	0	0	0	4 (1)	0	0	10 (2)
Implantation	12.90±2.71	12.75±2.09	12.62±1.50	13.33±1.96	12.60±2.16	12.76±2.33	12.25±0.50
Preimplantation loss (%)	9.51±7.15	8.43±6.07	9.13±5.97	6.21±6.14	7.52±1.17	6.07±3.10	7.28±5.83
Postimplantation loss (%)	2.84±4.20	1.33±4.24	3.59±6.20	13.41±23.06	2.05±3.68	2.35±5.62	22.91±31.45
Fetal weight (g)	3.68±0.19	3.65±0.12	3.54±0.29	3.24±0.26[a]	3.69±0.19	3.52±0.26	3.29±0.27[a]
Fetal length (mm)	37.54±1.76	37.38±1.12	36.42±2.07	35.74±1.82[a]	37.17±2.35	36.67±2.15	32.95±1.77[a]
Placenta, weight (g)	0.54±0.05	0.53±0.04	0.54±0.05	0.51±0.02	0.55±0.06	0.55±0.06	0.49±0.04
% live fetuses/litter with external malformation	0.31±1.40	0.87±2.68	0.00	0.00	0.00	0.59±2.43	6.25±12.50
% live fetuses/litter with visceral malformation	1.25±5.59	0.00	3.65±10.08	0.00	0.00	0.00	12.50±25.00
% live fetuses/litter with skeletal malformation	0.62±2.79	0.00	0.78±3.12	3.51±5.46	0.00	0.74±3.03	11.11±15.71
% live fetuses/litter with external variations	5.73±6.18	5.28±6.32	9.41±3.09[a]	21.12±26.97	6.51±4.57	5.37±5.13	28.85±16.30[a]
% live fetuses/litter with visceral variations	10.42±25.20	4.17±13.11	13.54±28.03	50.00±77.46	13.16±28.64	0.00	0.00
% live fetuses/litter with skeletal variations	20.97±15.07	17.58±16.09	23.31±13.07	95.00±8.36[a]	18.11±14.49	27.99±14.61	100.00[a]

[a]Significantly different ($p \leq 0.05$) from control value.

Morphologic abnormalities in the fetuses from dams that were treated with ibuprofen include increased incidence of asymmetric palate rugae (external variation), number of fetuses with skeletal variations, frontal bone (reduced ossification), parietal bone (reduced ossification), interparietal bone (reduced ossification), supraoccipital bone (reduced ossification), sternebra (unossified), sternebra (reduced ossification), sternebra (other variations), and reduced ossification of the whole skeleton. Additionally, there was decreased incidence of number of fetuses with external variations and metatarsal bodies (reduced ossification, skeletal variation). These changes are shown in the following table notes (from the publication):

Table 2
Morphologic Abnormalities in Rat Fetuses in Control, Ibuprofen, and Tolmetin-Exposed Groups[a]

		Ibuprofen (mg/kg/day)			Tolmetin (mg/kg/day)		
	Control	25.5	255.0	600.0	25.5	255.0	2,550.0
Total fetuses examined[b]	250 (20)	252 (20)	195 (16)	71 (6)	247 (20)	213 (17)	38 (4)
Total double-stained specimens	175 (20)	175 (20)	125 (15)	30 (4)	169 (20)	126 (15)	20 (3)
Total Alizarin-stained specimens	—	—	8 (1)	20 (2)	—	18 (2)	6 (1)
Total examined by Wilson's method	52 (20)	54 (20)	43 (16)	12 (6)	55 (20)	49 (17)	7 (4)
External malformation[b]							
No. of fetuses with malformation	1 (1)	2 (2)	—	—	—	1 (1)	1 (1)
Short tail	1 (1)	—	—	—	—	1 (1)	—
Cleft palate	—	2 (2)	—	—	—	—	—
Myelomeningocele	—	—	—	—	—	1 (1)	—
Umbilical hernia	—	—	—	—	—	—	1 (1)
Visceral malformation[c]							
No. of fetuses with malformation	1 (1)	—	2 (2)	—	—	—	1 (1)
Interventricular septal defect	1 (1)	—	2 (2)	—	—	—	1 (1)
Skeletal malformation							
No. of fetuses with malformation	1 (1)	—	1 (1)	2 (2)	—	1 (1)	2 (2)
Coccygeal vertebra, missing	1 (1)	—	—	—	—	1 (1)	1 (1)
Sacro-coccygeal vertebra, missing	—	—	—	—	—	1 (1)	—
Transversal foramen, cleft	—	—	—	1 (1)	—	—	—
Frontal bone, misshapen	—	—	1 (1)	—	—	1 (1)	—
Fused rib	—	—	—	1 (1)	—	—	—
Additional sternebrae	—	—	1 (1)	—	—	—	1 (1)
Cleaved sternebra	—	—	—	—	—	—	—
External Variations[b]							
No. of fetuses with variations	14 (11)	13 (10)	18 (16)	10[d] (6)	16 (15)	11 (10)	9[d] (4)
Hematoma	7 (5)	—	8 (7)	5 (3)	10 (6)	4 (4)	2 (1)
Anasarca	—	—	2 (1)	—	—	—	1 (1)
Asymmetric palate rugae	7 (6)	13 (10)	12 (14)	8[d] (6)	10 (9)	7 (6)	6[d] (4)
Visceral Variations[c]							
No. of fetuses with variations	4 (4)	2 (2)	3 (3)	4 (3)	6 (5)	—	—
Malpositioned kidney[c]	4 (4)	2 (2)	1 (1)	4 (2)	4 (4)	—	—
Enlarged lateral ventricle	—	—	2 (2)	1 (1)	2 (1)	—	—
Skeletal variations							
No. of fetuses with variations	35 (17)	31 (14)	30 (16)	47[d] (6)	29 (16)	39 (16)	26[d] (4)
Nasal bone, reduced ossification[e]	—	—	2 (2)	3 (2)	—	—	1 (1)
Frontal bone, reduced ossification	8 (5)	9 (6)	3 (3)	21[d] (6)	11 (7)	9 (6)	18[d] (4)
Parietal bone, reduced ossification[e]	3 (3)	2 (2)	—	7[d] (4)	—	3 (2)	9[d] (4)
Interparietal bone, reduced ossification	7 (4)	7 (4)	12 (8)	12[d] (6)	5 (4)	5 (4)	8[d] (4)
Supraoccipital bone, reduced ossification	5 (3)	3 (3)	8 (8)	9[d] (5)	4 (4)	—	7[d] (4)
Hyoid bone, unossified	—	—	—	—	—	2 (2)	—
Hyoid bone, reduced ossification	2 (2)	—	7 (3)	5 (4)	4 (3)	—	5 (3)
Hyoid bone, additional ossification center	—	1 (1)	—	—	—	2 (2)	—
Wavy ribs[e]	—	—	—	1 (1)	—	1 (1)	—
13th rib, wavy[e]	2 (2)	4 (2)	—	3 (3)	—	4 (4)	7 (4)
13th rib, short[e]	2 (2)	1 (1)	3 (2)	4 (2)	2 (1)	—	2 (2)
Supernumerary cervical ribs[e]	—	—	—	—	1 (1)	—	1 (1)
Supernumerary lumbar ribs[e]	3 (2)	1 (1)	3 (3)	—	—	2 (2)	3 (1)
Sternebra, unossified	5 (3)	3 (2)	—	14[d] (4)	3 (2)	3 (3)	11[d] (4)
Sternebra, reduced ossification	9 (6)	7 (7)	14 (6)	19[d] (6)	9 (7)	8 (6)	14[d] (4)
Sternebra, other variations[f]	7 (5)	6 (6)	12 (7)	23[d] (6)	9 (5)	3 (2)	21[d] (4)
Asymmetric vertebral body	5 (3)	10 (6)	2 (2)	4 (3)	4 (2)	5 (3)	7[d] (3)
Unossification of sacro-coccygeal vertebrae	—	—	—	—	—	1 (1)	—
Metacarpal bodies, reduced ossification[e]	6 (2)	2 (2)	9 (3)	4 (2)	—	7 (6)	11[d] (4)
Metatarsal bodies, reduced ossification[e]	9 (6)	4 (4)	9 (6)	8[d] (5)	6 (5)	5 (5)	6[d] (3)
Reduced ossification of the whole skeleton	—	—	—	5 (4)	—	—	5 (3)
Increase of ossification in primary ossification centers	—	—	—	—	9 (1)	—	—

[a]A single fetus may be represented more than once in the listing of individual defects. The number of litters is in parentheses.
[b]All fetuses were examined for external abnormalities, including cleft palate.
[c]Head and neck structures were evaluated in the Bouin's solution stained fetuses by Wilson's method. The abdominal and thorax structure were examined in the Bouin's solution stained fetuses and in most of the eviscerated alteration fetuses.
[d]Significantly different ($p \leq 0.05$) from control value.
[e]Uni- or bilateral.
[f]Bifurcated at the distal end (VI), dumbbell-shaped (III and V).

Reviewer's Comments:

This study appears to use methods employed in standard embryofetal developmental toxicity studies. However, the dosing in this study is from Day 8 to Day 21 of gestation, which may miss critical developmental landmarks that occur at earlier time points (Gestation Days 6 and 7). Maternal observations include body weight and plasma levels of liver enzymes, total protein, and urea. No maternal NOAEL can be obtained in this study. Use of COX inhibitors (such as ibuprofen) could increase postimplantation loss, induce skeletal, midline, diaphragmatic, heart defects, and fetal growth retardation as well as complicate pregnancy if taken near delivery-time due to pulmonary hypertension, secondary to the constriction of ductus arteriosis (Cappon et al., 2003; Cook et al., 2003; and Ostensen and Skomsvoll, 2004). As such, decreased fetal weight and reduced number of litter and % alive number of fetuses as well as the various external and skeletal variations (reduced ossification) in dams treated with ibuprofen were observed in this study. The rat dose of 600 mg/kg/day that resulted in these observations represents a human dose of 5,838 mg in an average human weighing 60 kg, based on a body surface area comparison. The rat dose of 600 mg/kg yields an exposure margin of 1.82 based on the maximum daily dose of 3200 mg for ibuprofen. The NOAEL for developmental effects would appear to be 25.5 mg/kg (0.08-times the maximum recommended human daily dose of 3200 mg).

Publication Title: Relationship Between Cyclooxygenase 1 and 2 Selective Inhibitors and Fetal Development When Administered to Rats and Rabbits During the Sensitive Periods for Heart Development and Midline Closure (Cappon et al., 2003)

Methods:

Ibuprofen was obtained from (b) (4) but no information was given regarding the purity of ibuprofen. One hundred forty timed-pregnant female Sprague-Dawley rats were obtained from (b) (4), were 9 to 12 weeks old, and were on 1, 2, or 4 days of gestation at arrival. Dams were randomly assigned to seven groups of 20 rats each (1 control group and an ibuprofen treatment group). The ibuprofen dose was 300 mg/kg/day via oral gavage at a dose volume of 10 mL/kg on Gestation Days 9 and 10. The control rats received 0.5% methylcellulose using the same route and dosing regimen. Rats were sacrificed on Day 21.

One hundred fifty timed-pregnant female New Zealand White rabbits were obtained from (b) (4), were 5 to 6.5 months of age, weighed 2.83 to 4.25 kg, and were on Gestation Day 8 upon arrival. Does were randomly assigned to six groups of 20 rabbits each (1 control group and an ibuprofen treatment group). The ibuprofen dose was 500 mg/kg/day via oral gavage at a dose volume of 2 mL/kg on Gestation Days 9, 10, and 11. The control rabbits received 0.5% methylcellulose using the same route and dosing regimen. Rabbits were sacrificed on Day 29.

Reference ID: 4913649

During the study, all animals were observed twice daily for morbidity and mortality. Body weights and food consumption were determined on the day of arrival and daily beginning on Gestation Day 8. Following sacrifice, the abdominal, thoracic, and pelvic viscera were examined grossly. The uteri and ovaries were removed and weighed. The number of corpora lutea as well as the number, type, and location of implantation sites were recorded. Viable fetuses were removed and weighed. For each viable fetus, a detailed external examination was performed including examination of the eyes, palate, and external orifices. The viable fetuses were sacrificed and examined for internal abnormalities. The hearts were examined for anomalies. The brain and ventricles were examined for dilatations or abnormalities.

Results:

Rat Data: The following table illustrates that there were greater numbers of dams dead, gravid at Cesarean section, and more unscheduled deaths in dams treated with 300 mg/kg/day ibuprofen on Gestation Days 9 and 10 (see following table, adapted from the publication):

Table 1
Rat Pregnancy Rate, Mortality, and Necropsy Findings From Dams Treated With NSAIDs on GDs 9 and 10

					GI toxicity at necropsy	
Dose group	Dose level[a]	Number dosed	Number died[b]	Number gravid at cesarean section	Unscheduled death	Scheduled examination
Control	0	20	0	20	0	No findings
Ibuprofen	300	20	1	19	1	No findings

[a]Dose levels are expressed as milligrams of active moiety of test article per kilogram of body weight.
[b]Number died includes animals found dead and killed moribund.
GD, gestational day; GI, gastrointestinal; NSAID, nonsteroidal anti-inflammatory drug.

The body weight of dams treated with 300 mg/mg/day ibuprofen on Gestation Days 9 and 10 were significantly lower than control on all time points where body weights were recorded following dosing as shown in the following table (from the publication):

Table 2
Rat Body Weights (g) During Gestation of Dams Treated with NSAIDs on GDs 9 and 10

GD	Control	CJ-19,209	Meloxicam	Diclofenac	Diflunisal	Ibuprofen	Ketorolac
9	282 ± 11	280 ± 13	279 ± 14	284 ± 7	287 ± 14	276 ± 18	282 ± 14
10	288 ± 12	284 ± 12	278 ± 18[c]	274 ± 11[c]	272 ± 13[c]	258 ± 14[c]	275 ± 17[c]
11	294 ± 13	292 ± 10	277 ± 21[c]	248 ± 13[c]	262 ± 12[c]	245 ± 12[c]	267 ± 20[c]
12	299 ± 14	300 ± 11	280 ± 28[c]	252 ± 15[c]	256 ± 17[c]	243 ± 19[c]	277 ± 27[c]
15	317 ± 17	315 ± 10	309 ± 13	261 ± 21[c]	282 ± 23[c]	277 ± 23[c]	303 ± 27[b]
18	352 ± 30	353 ± 12	348 ± 16	315 ± 35[c]	321 ± 34[c]	319 ± 39[c]	345 ± 24
21	402 ± 23	398 ± 13	404 ± 17	362 ± 37[c]	358 ± 53[c]	368 ± 46[c]	394 ± 28
Corrected BW gain[a]	19.9 ± 13.6	19.8 ± 13.2	18.5 ± 15.9	−13.6 ± 25.3[c]	−6.1 ± 36.8[c]	−5.4 ± 26.4[c]	11.1 ± 17.7

Data presented as mean ± standard deviation.
[a]Corrected BW gain equals the body weight gain from GD 9 to 21 minus the gravid uterine weight.
[b]$p \leq 0.05$.
[c]$p \leq 0.01$.
BW, body weight; GD, gestational day; NSAID, nonsteroidal anti-inflammatory drug.

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The fetal weight from dams treated with 300 mg/kg/day ibuprofen on Gestation Days 9 and 10 were significantly lower than control as shown in the following table (adapted from the publication):

Table 3
Rat Cesarean Section Observations and Fetal Weights of Dams Treated with Nonsteroidal Anti-Inflammatory Drugs on Gestational Days 9 and 10

Dose group (n[a])	Corpora lutea	Viable fetuses	Pre-implantation loss (%)	Post-implantation loss (%)	Fetal weight (g)	Placental weight (g)
Control (20)	14.2±1.4	13.6±1.2	4.0±5.8	3.7±4.9	5.72±0.4	0.53±0.07
Ibuprofen (19)	14.9±2.2	14.0±2.7	5.9±14.5	6.2±9.5	5.29±0.64[b]	0.55±0.07

Data presented as mean± standard deviation.
[a]Number of pregnant females examined at cesarean section.
[b]$p \leq 0.05$.
[c]$p \leq 0.01$.

The fetuses from dams treated with 300 mg/kg/day ibuprofen on Gestation Days 9 and 10 were observed with membranous ventricular septal defects in the heart at a higher incidence than control as well as increased incidence by 1 for blood vessels (subclavian retroesophageal), eyes (microphthalmia), and kidney (renal papilla absent) as shown in the following table (from the publication):

Table 4
Rat Fetal Evaluations From Dams Treated With Non-steroidal Anti-Inflammatory Drugs on Gestational Days 9 and 10

	Dose group[a]						
	Control 262 (20)	CJ-19,209 192 (15)	Meloxicam 217 (16)	Diclofenac 177 (14)	Diflunisal 185 (14)	Ibuprofen 252 (19)	Ketorolac 247 (19)
External findings							
Polydactyly	—	—	—	2(1)[b]	—	—	—
Syndactyly	—	—	—	1(1)[b]	—	—	—
Hindlimb hypoflexion	—	—	—	1(1)[c]	—	—	—
Acaudate	—	—	—	1(1)[c]	—	—	—
Anus imperforate	—	—	—	1(1)[c]	—	—	—
Visceral findings							
Heart							
VSD, membranous	1(1)	1(1)	—	1(1)	8(7)	12(8)	3(3)
Blood vessels							
Subclavian retroesophageal	—	—	—	—	—	1(1)	—
Eyes							
Microphthalmia	—	—	—	—	—	1(1)	—
Kidneys							
Renal papilla absent	—	—	—	—	—	1(1)	—

[a]Fetuses (litters)
[b,c]Multiple findings for one fetus.
VSD, ventricular septal defect.

Rabbit Data: The following table illustrates that there were greater numbers of does dead, gravid at Cesarean section, and more unscheduled deaths in does treated with 500 mg/kg/day ibuprofen on Gestation Days 9 to 11 (see following table, adapted from the publication):

Reference ID: 4913649

Table 5
Rabbit Pregnancy Rate, Mortality, and Necropsy Findings from Does Treated with Nonsteroidal Anti-Inflammatory Drugs on Gestational Days 9, 10, and 11

Dose group	Dose level[a]	Number dosed	Number died[b]	Number gravid at cesarean section	GI toxicity at necropsy: Unscheduled death	GI toxicity at necropsy: Scheduled examination
Control	0	20	0	18	0	No findings
Ibuprofen	500	20	1	19	1	No findings

[a]Dose levels are expressed as milligrams of active moiety of test article per kilogram of body weight.
[b]Includes animals found dead and killed moribund.
[c]Two does aborted and were killed.
GI, gastrointestinal.

The body weight of does given 500 mg/kg/day ibuprofen on Gestation Days 9 to 11 were significantly lower at all time points that body weights were recorded following dosing as illustrated in the following table (from the publication):

Table 6
Body Weights of Does Treated with Nonsteroidal Anti-Inflammatory Drugs on Gestational Days 9, 10, and 11

GD	Control	CJ-19,209	Meloxicam	Diclofenac	Diflunisal	Ibuprofen	Ketorolac
9	3.51±0.20	3.43±0.20	3.33±0.23	3.54±0.30	3.46±0.20	3.42±0.21	3.56±0.19
10	3.52±0.22	3.46±0.20	3.36±0.25	3.59±0.31	3.45±0.21	3.27±0.23[c]	3.59±0.20
11	3.55±0.19	3.46±0.21	3.37±0.25	3.56±0.32	3.39±0.21[b]	3.20±0.29[c]	3.59±0.21
12	3.55±0.18	3.45±0.22	3.38±0.26	3.48±0.33	3.31±0.21[c]	3.13±0.22[c]	3.56±0.21
13	3.61±0.19	3.45±0.25[b]	3.38±0.27	3.41±0.30[b]	3.27±0.18[c]	3.11±0.22[c]	3.56±0.21
14	3.65±0.19	3.46±0.27[b]	3.39±0.27	3.39±0.29[c]	3.29±0.20[c]	3.18±0.24[c]	3.61±0.22
19	3.75±0.20	3.57±0.24[b]	3.50±0.27	3.54±0.31[b]	3.48±0.15[c]	3.45±0.21[c]	3.72±0.20
24	3.87±0.20	3.70±0.22[b]	3.62±0.27	3.67±0.27[b]	3.63±0.18[c]	3.55±0.22[c]	3.85±0.18
29	3.98±0.22	3.80±0.22[b]	3.71±0.28	3.79±0.28[b]	3.74±0.18[c]	3.70±0.23[c]	3.95±0.20
Corrected BW gain[a]	−0.06±0.14	−0.11±0.11	−0.10±0.13	−0.19±0.12[c]	−0.15±0.10[b]	−0.19±0.11[c]	−0.14±0.11

Data presented as mean±standard deviation. Meloxicam body weight was compared statistically to its concurrent control group; body weight data for the meloxicam concurrent control group are not shown (see Materials and Methods for more details).
[a]Corrected body weight gain equals the BW gain from GD 9 to 21 minus the gravid uterine weight.
[b]$p \leq 0.05$.
[c]$p \leq 0.01$.
BW, body weight; GD, gestational day.

The fetal weight from does treated with 500 mg/kg/day ibuprofen on Gestation Days 9 to 11 were significantly lower than control as shown in the following table (adapted from the publication):

Table 7
Rabbit Cesarean Section Observations and Fetal Weights from Does Treated with Nonsteroidal Anti-Inflammatory Drugs on Gestational Days 9, 10, and 11

Dose group (n[a])	Corpora lutea	Viable fetuses	Pre-implantation loss (%)	Post-implantation loss (%)	Fetal weight (g)	Placental weight (g)
Control (18)	10.5±2.6	8.2±2.3	15.0±23.7	5.7±8.9	45.2±4.1	5.8±0.5
Ibuprofen (19)	9.6±2.0	8.3±1.9	9.4±12.6	3.9±9.6	41.0±4.4[c]	5.6±0.8

Data presented as mean± standard deviation.
[a]Number of pregnant females examined at cesarean section.
[b]$p \leq 0.05$.
[c]$p \leq 0.01$.

There was greater incidence of gastroschisis (external findings) and membranous ventricular septal defect in the heart in the fetuses of does treated with 500 mg/kg/day ibuprofen on Gestation Days 9 to 11 as illustrated in the following table (from the publication):

Table 8
Rabbit Fetal Evaluations from Does Treated with Nonsteroidal Anti-Inflammatory Drugs on Gestational Days 9, 10, and 11

	Dose group[a]						
	Control 147 (18)	CJ-19,209 157 (20)	Meloxicam 132 (16)	Diclofenac 124 (16)	Diflunisal 109 (14)	Ibuprofen 158 (19)	Ketorolac 156 (18)
External findings							
Petechia	—	—	—	—	—	—	1(1)
Gastroschisis	—	—	—	—	—	1(1)	—
Forepaw hyperflexion	1(1)	—	—	—	—	—	—
Omphalocele	—	—	—	—	1(1)	—	—
Visceral findings							
Heart							
VSD, membranous	—	—	—	—	2(2)[d]	1(1)	—
VSD, muscular					1(1)[b]		
Blood vessels							
Enlarged aortic arch	—	—	—	—	1(1)[b]	—	—
Subclavian retroesophageal	—	—	—	—	1(1)	—	—
Absent innominate artery	—	—	—	—	1(1)	—	—
Accessory vessels	—	—	—	—	6(4)	—	1(1)
Brain							
Lateral ventricles dilated	1(1)	—	—	—	—	—	2(2)
Diaphragm							
Diaphragmatic hernia	—	—	—	—	1(1)	—	—
Eyes							
Microphthalmia	—	—	—	—	6(4)[c,d,e]	—	—
Hemorrhage	—	—	—	—	2(2)[c,e]	—	—
Hemorrhagic ring	—	1(1)	—	—	—	—	—

[a]Fetuses (litters).
[b–e]Multiple findings for one fetus.
VSD, ventricular septal defect.

Reviewer's Comments:

The methods in this study appear to be a modification of standard embryofetal developmental toxicity studies. In this study, only Gestation Days 9 and 10 in rats and Days 9-11 in rabbits were dosed to study the effect of ibuprofen on heart development. Maternal observations were limited to body weight evaluations. A maternal NOAEL cannot be determined in this study. Fetal observations appear to be adequate with the exception that there is detail examination of the heart structures. Use of COX inhibitors (such as ibuprofen) could increase postimplantation loss, induce skeletal, midline, diaphragmatic, heart defects, and fetal growth retardation as well as complicate pregnancy if taken near delivery-time due to pulmonary hypertension, secondary to the constriction of ductus arteriosis (Cappon et al., 2003; Cook et al., 2003; and Ostensen and Skomsvoll, 2004). As such, decreased fetal weight in dams and does treated with ibuprofen were observed in this study. The rat dose that resulted in ventricular septal defects in rat pups (300 mg/kg/day) is equivalent to a human dose of 2919 mg in an average human weight 60 kg, based on a body surface area comparison. The rat dose of 300 mg/kg/day yields an exposure margin of 0.912 based on the maximum daily dose of 3200 mg for ibuprofen. The 500 mg/kg/day rabbit dose used in this study is equivalent to a human dose of 9730 mg in an average human weighing 60 kg, based on a body surface area comparison. The rabbit dose of 500 mg/kg/day yields an exposure margin of 3.04 based on the maximum daily dose of 3200 mg for ibuprofen.

Publication Title: Congenital Ventricular Septal Defects and Prenatal Exposure to Cyclooxygenase Inhibitors (Burdan et. al, 2006)

Methods:
The authors conducted a retrospective analysis of teratology studies conducted in their own laboratory using the Wistar rat strain between 1997 and 2004. They compared these findings with reported findings of developmental toxicology studies with selective and nonselective COX-2 inhibitors. In all studies, rats were administered study medication on Gestation Day 7 to 16. Pregnancies were terminated on GD 21 by Caesarian section and fetuses were examined macroscopically, weighed, and fetal crown-rump length were checked. Body mass index and pre- and post-implantation mortality rates were determined. One third of the fetuses were dissected in situ or stained with Bouin solution and internally examined. The remaining fetuses were eviscerated and prepared for skeletal examinations.

Results:
Although not statistically significant, the authors report a greater increase in the incidence of ventricular septal defects in offspring exposed to aspirin and ibuprofen compared to control. They extrapolate predicted incidence rates as follows: aspirin (46.26/10000 fetuses) and ibuprofen (106.95/10000 fetuses) compared to control animals (5.38-19.72/10000 fetuses). According to the authors, the predicted incidence is higher than the control data predictions in studies reported by Pfizer (9.59/10000), Middle Atlantic Reproduction and Teratology Association (19.72/10000), WIL Research Laboratories (4.56/10000), and Cappon et al. (5.38/10000).

Reference ID: 4913649

Publication Title: Functional Activity of Mouse Sperm was not Affected by Low Doses of Aspirin-like Drugs (Stutz et. al, 2000)

Methods:

There was no information regarding where ibuprofen was obtained or its purity. The study was conducted in (b) (4) Albino Swiss mice were divided into different dose groups. Group 1 was female mice housed with males without treatment for 4 days. Pregnant females were selected and treated from Day 5 to Day 18 of pregnancy. The males from this dam were them studied after reaching sexual maturity (approximately 70 days after birth). Group 2 was adult males (70 days old) treated with ibuprofen (5.6 mg/kg/day, IP) for 35 days (time to cover epididymal transit). Group 3 was adult males (70 days old) treated with ibuprofen (5.6 mg/kg/day, IP) for 60 days (time to cover spermatogenesis and epididymal transport).

Results:

The following figure illustrates the sperm parameters (motile, swollen, and viable) in the different ibuprofen treatment groups (from the publication):

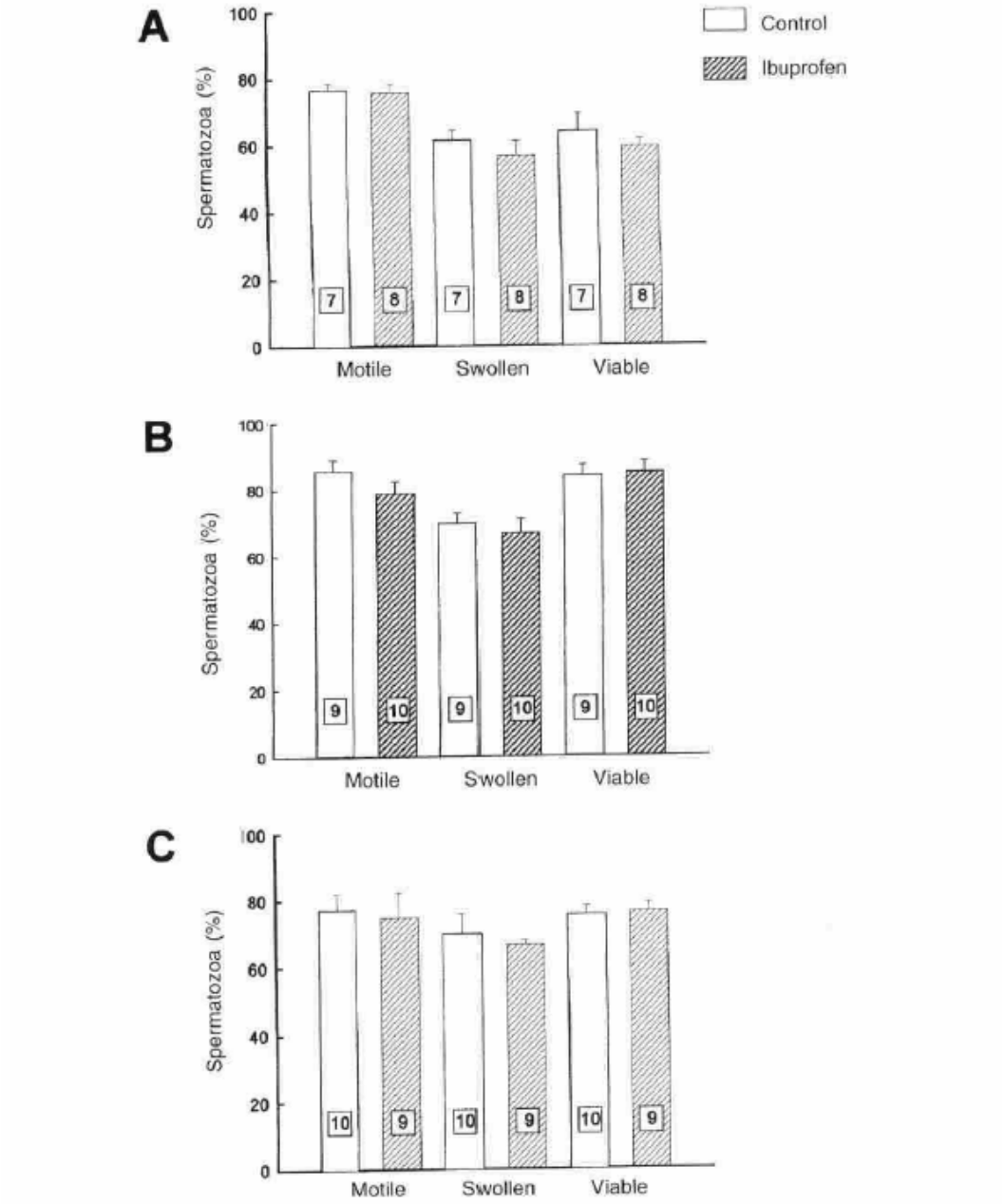


Figure 2. Effect of ibuprofen (ip, 5.6 mg/kg day^{-1}) administered to female mice from the 5th to 18th day after 4 days housed with males. Pregnancy: (A) male cohort assayed at 70 days from birth; adult male mice during (B) 35 days; or (C) 60 days on the percentage of motile, swollen, and viable spermatozoa. Control animals were injected with the vehicle (propylene glycol) in the same volume and period. Results are expressed as means ± standard errors. Numbers in boxes indicate number of animals.

As shown in the figure above, ibuprofen did not affect the sperm parameters in any of the treatment groups.

The following figure illustrates the plasma levels of testosterone after treatment with ibuprofen (from the publication):

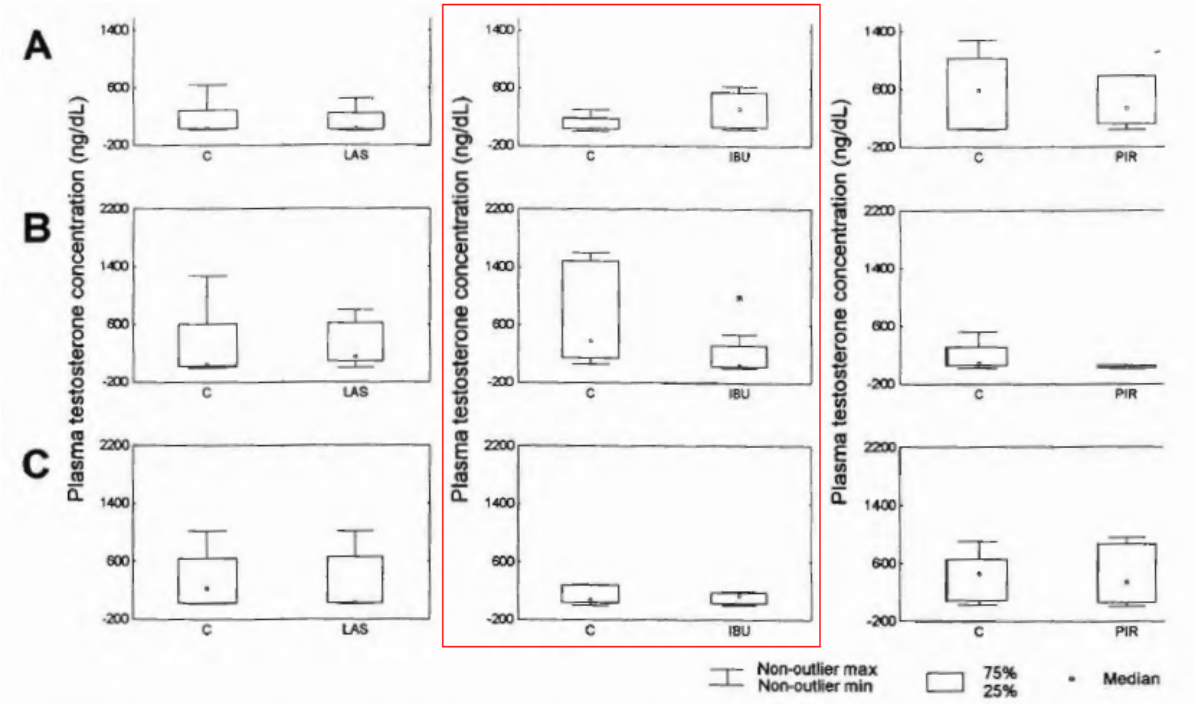


Figure 4. Effect of lysine acetyl salicylate (LAS) (im, 14.3 mg/kg day^{-1}), ibuprofen (IBU) (ip, 5.6 mg/kg day^{-1}), or piroxicam (PIR) (ip, 0.28 mg/kg day^{-1}) administered to female mice from the 5th to 18th day after 4 days housed with males. Pregnancy: male cohort assayed at (A) 70 days from birth and at adulthood during (B) 35 or (C) 60 days on the plasma testosterone levels. Control animals were injected with the vehicle (distilled water, propylene glycol, and dimethilsulfoxide, respectively) in the same volume and period. $n = 8$ in all the treatments. *$p < .05$ vs control.

As shown in the figure above, plasma testosterone levels were significantly diminished after treatment with 5.6 mg/kg/day of ibuprofen for 35 days in adult male mice. There was no change in the testosterone levels in adult male mice treated with 5.6 mg/kg/day of ibuprofen for 60 days.

Reviewer's Comments:

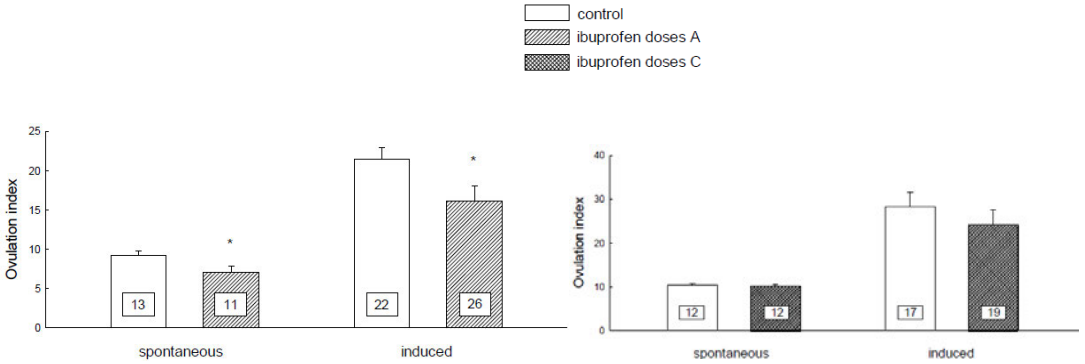
Ibuprofen was administered via the IP route, which may result in similar pharmacokinetics as oral administration because uptake of ibuprofen is through absorption in the gut, possibly small intestine. However, IP administration does not address exposure in the esophagus and stomach as ibuprofen passes these tissues. The study has some notable features mainly exposure of ibuprofen to cover epididymal transit and spermatogenesis in mice and the effect of ibuprofen in male pups via maternal exposure. The mouse dose of 5.6 mg/kg/day represents a human dose of 27.2 mg in an average human weighing 60 kg, based on a body surface area comparison. The mouse dose of 5.6 mg/kg/day yields an exposure margin of 0.0085 based on the maximum daily dose of 3200 mg for ibuprofen.

Publication Title: Chronic Administration of Nonsteroid-Antiinflammatory Drugs (NSAIDs): Effects upon Mouse reproductive Functions (Martini et. al, 2008)

Methods:

Male or female Albino Swiss mice (70 days old) were injected daily for 35 or 60 days, respectively, with either IP ibuprofen (dissolved in propylene glycol in NaCl solution) at doses of 5.6, 11.2 or 16.8 mg/kg/day or IP piroxicam (dissolved in DMSO in NaCl solution) at doses of 0.28, 0.56 or 0.84/kg/day. Female fertility parameters evaluated were spontaneous and induced ovulation, oocyte maturity, and spermatozoa migration through the female genital tract. Male fertility parameters examined were epididymal spermatozoa concentration, motility, viability, resistance to hypoosmotic shock, acrosomal status, and membrane maturity. Additional parameters that were evaluated included in vitro and in vivo fertilization, reproductive hormones plasma levels, and cyclooxygenase inhibition in reproductive tissues. Ibuprofen was obtained from (b) (4) . with no mention of purity.

Results:
The following tables and figures summarize the effects of ibuprofen on female reproductive function (from the publication):



Ovulation indices (# of oocytes/females) of adult female mice injected for 35 days with ibuprofen. Dose A is 5.6/mg/kg/day and Dose B is 16.8 mg/kg/day.

As seen in the above figure, ibuprofen at a dose of 5.6 mg/kg/day but not 16.8 mg/kg/day produced a reduction in spontaneous and induced ovulation rates. Oocyte maturation, FSH levels on oestrus day, and sperm parameters in the uterus and oviduct were not affected by ibuprofen administration.

Variables	IBUPROFEN				PIROXICAM			
	doses A		doses C		doses A		doses C	
	Control	NSAIDs	Control	NSAIDs	Control	NSAIDs	Control	NSAIDs
% of in vitro fertilized oocytes	81, 9% (199)	82.6% (218)	90.7% (237)	89.0% (137)	85.4% (226)	81.7% (274)	80.3% (132)	83.5% (91)
In vivo % of pregnant mice	76.9% (13)	91.7% (12)	80.0% (5)	60.0% (10)	80.0% (10)	70.0% (10)	70.0% (10)	65.0% (10)
Number of fetuses	8.4 ± 0.4 (10)	9.5 ± 0.7 (11)	9.2 ± 0.5 (4)	8.5 ± 0.2 (6)	9.6 ± 0.7 (8)	8.1 ± 1.2 (7)	9.2 ± 1.1 (6)	9.0 ± 1.0 (5)

Ibuprofen (doses A: 0.56 mg/100g/day or doses C: 1.68 mg/100g/day; i.p.) or piroxicam (doses A: 0.028 mg/100g/day or doses C: 0.084 mg/100g/day; i.p.) were injected daily to adult female mice for 35 days. Control animals were injected with the solvent (propylene glycol or dimethylsulfoxide respectively) in the same volume and period. In vitro fertilization indices, are expressed as percentages of fertilized oocytes that were obtained by ampullar puncture after induced superovulation. In vivo fertilization indices are expressed as percentages of pregnant mice after housing for 4 days (one oestral cycle) with untreated males; 12 days after, females were sacrificed and the number of fetuses was determined. Results are expressed as percentages or Mean ± SEM. In parentheses: number of oocytes or female mice evaluated.

SPERM VARIABLE	IBUPROFEN				PIROXICAM			
	CONTROL UTERUS	CONTROL OVIDUCT	UTERUS	OVIDUCT	CONTROL UTERUS	CONTROL OVIDUCT	UTERUS	OVIDUCT
Progressive (%)	45.7 ± 6.1 [a] (12)	20.0 ± 11.5 [a] (3)	46.2 ± 5.7 [b] (9)	7.0 ± 7.0 [b] (2)	43.6 ± 6.5 (8)	25.0 ± 14.4 (3)	22.9 ± 8.3 (9)	---
Non-progressive (%)	10.5 ± 2.9 (12)	13.3 ± 6.7 (3)	12.8 ± 2.7 (9)	13.5 ± 13.5 (2)	12.9 ± 2.1 (8)	11.0 ± 11.0 (3)	15.8 ± 4.8 (9)	—
Non-motile (%)	43.7 ± 5.1 [c] (12)	66.7 ± 17.6 [c] (3)	41.1 ± 5.7 [d] (9)	79.5 ± 6.5 [d] (2)	43.6 ± 5.1 (8)	64.0 ± 7.4 (3)	61.2 ± 8.9 (9)	—
Viable (%)	47.7 ± 6.3 [e] (12)	24.3 ± 5.0 [e] (10)	49.6 ± 6.6 [f] (10)	27.1 ± 6.9 [f] (7)	56.9 ± 6.2 [g] (8)	33.1 ± 5.6 [g] (7)	40.0 ± 7.0 [h] (9)	18.4 ± 3.4 [h] (5)
Acrosome reacted (%)	51.4 ± 10.2 (11)	70.3 ± 13.0 (6)	54.1 ± 12.2 (9)	74.1 ± 7.5 (8)	33.3 ± 2.7 [i] (7)	55.5 ± 4.7 [i] (8)	32.4 ± 6.0 [j] (7)	60.3 ± 8.3 [j] (5)

Ibuprofen (doses A: 0.56 mg/100g/day; i.p.) or piroxicam (doses A: 0.028 mg/100g/day; i.p.) were administered to adult female mice during 35 days. Control animals were injected with the solvent (propylene glycol or dimethylsulfoxide respectively) in the same volume and period. The spermatozoa were evaluated 110 min after the female accepted the untreated male for mating. Values indicate Mean ± SEM. In parentheses: number of animals evaluated. In each row, identical letters indicate significant differences ($p < 0.05$).

The following summary table illustrates the effects of ibuprofen on epididymal spermatozoa (from the publication):

SPERM VARIABLE	IBUPROFEN		PIROXICAM	
	CONTROL	NSAIDs	CONTROL	NSAIDs
Body weight (g)	27.6 ± 0.5 (24)	27.5 ± 0.5 (19)	26.7 ± 1.1 (10)	26.5 ± 0.5 (13)
Concentration (x 10^6/ml)	11.6 ± 0.9 (37)	11.1 ± 1.0 (30)	15.3 ± 1.9 (20)	14.6 ± 2.1 (18)
Motile (progressive + non-progressive) (%)	63.7 ± 2.2 (30)	62.8 ± 2.8 (29)	74.2 ± 2.0 (17)	67.2 ± 3.1 (18)
Viable (%)	71.3 ± 2.1 (16)	71.7 ± 2.2 (16)	73.2 ± 1.3 (17)	75.6 ± 2.4 (18)
Swollen (HOST) (%)	60.3 ± 3.1 (17)	57.5 ± 3.6 (15)	69.9 ± 1.4 (17)	69.4 ± 2.2 (17)
Acrosome intact (%)	78.9 ± 1.1 (8)	77.4 ± 3.5 (8)	88.4 ± 1.4 (9)	88.9 ± 1.2 (10)
Bending and/or with cytoplasmatic drop (%)	17.0 ± 1.9 (16)	17.4 ± 2.0 (19)	14.4 ± 1.7 (24)	17.4 ± 2.3 (22)

Ibuprofen (doses B: 1.12 mg/100g/day; i.p.) or piroxicam (doses B: 0.056 mg/100g/day; i.p.) were administered to adult male mice for 60 days. Control animals were injected with the solvent (propylene glycol or dimethylsulfoxide respectively) in the same volume and period. The spermatozoa were obtained from the caudal epididymis. Values indicate Mean ± SEM. In parentheses: number of animals. HOST= hypoosmotic swelling test.

No effects on epididymal spermatozoa was observed after adult mice were treated for 60 days with IP ibuprofen at a dose of 11.2 mg/kg/day

In vitro fertilization index of spermatozoa obtained from males was significantly lower than vehicle controls (from the publication). No effect on proportion of pregnant females mated with treated males or litter sizes were reported when comparing ibuprofen-treated males versus control males.

Variables	IBUPROFEN				PIROXICAM			
	doses B		doses C		doses B		doses C	
	Control	NSAIDs	Control	NSAIDs	Control	NSAIDs	Control	NSAIDs
% of in vitro fertilized oocytes	73.5%[a] (196)	59.1%[a] (171)	66.1% (121)	62.4% (117)	80.9% (89)	73.8% (126)	83.0% (94)	85.5% (124)
% of pregnant mice	13.0% (23)	43.7% (16)	21.4% (14)	37.5% (16)	33.3% (18)	21.4% (14)	14.3% (14)	16.7% (12)
litter size	7.0 ± 1.5 (3)	7.9 ± 1.0 (7)	8.7 ± 0.3 (3)	7.8 ± 1.2 (6)	6.8 ± 1.6 (6)	3.3 ± 0.9 (3)	7.5 ± 2.5 (2)	8.5 ± 0.5 (2)

Ibuprofen (doses B: 1.12 mg/100g/day or doses C: 1.68 mg/100g/day; i.p.) or piroxicam (doses B: 0.056 mg/100g/day or doses C: 0.084 mg/100g/day; i.p.) were injected to adult male mice for 60 days. Control animals were injected with the solvent (propylene glycol or dimethylsulfoxide respectively) in the same volume and period. In vitro fertilization indices, are expressed as percentages of fertilized oocytes that were obtained by ampullar puncture after induced superovulation. In vivo fertilization indices are expressed as percentages of untreated pregnant mice after housing for 4 days (one estral cycle) with treated males; 12 days after, females were sacrificed and the number of fetuses was determined. Results are expressed as percentages or Mean ± SEM. In parentheses: number of oocytes or female mice evaluated. **a**= $p < 0.05$.

It was also reported that plasma concentrations of FSH and testosterone in males were not altered by ibuprofen treatment.

Reviewer's Comments:

This study was an extension of the Stutz et al. (2000) paper, which previously examined lower doses of ibuprofen on predominantly male fertility parameters. In this study by Martini et al., (2008), female fertility parameters in addition to male parameters were evaluated. In addition, higher doses of ibuprofen were examined in this study. In males, similar results were obtained as the last study by Stutz et al. in which sperm parameters and resulting litter sizes were not altered by ibuprofen treatment; however, there were no differences in testosterone plasma concentrations in the ibuprofen-treated males. In females, ibuprofen treatment resulted in changes in ovulation indices at the low dose but not high dose with no changes in sperm parameters when analyzed from the uterus or oviduct. The authors described this dose independent effect on ovulation has been reported previously and may be due to the different prostaglandins that are inhibited and due to the synthesis of prostaglandins recovering at different rates. It was also discussed that unchanged in vivo fertilization rates appears to be contradictory with lower ovulation indices. However the authors reconcile this contradiction by stating that the study was designed to avoid implantation inhibition and/or abortions, ibuprofen injections were interrupted the day before male and female were mated.

Publication Title: Effect of Ibuprofen and Naproxen on Implantation and Pregnancy in Rat (Gupta et. al, 1984)

Methods:

Ibuprofen was obtained from (b) (4) with no purity information. The study was conducted in (b) (4) Female albino rats of established fertility were left overnight with male rats of proven fertility. The next morning, mating was confirmed by clumps of spermatozoa in the vaginal smear, which is considered Day 1 of pregnancy. Ibuprofen was administered orally on Days 3, 4, and 5 of pregnancy in two dose groups, 20 mg/100 g bw and 30 mg/100 g bw, which is 200 mg/kg and 300 mg/kg. The number of implantation sites and number of viable fetuses were counted on Day 10 and Day 19 of pregnancy, respectively

Results:

The following table illustrates the number of implantation sites and the number of viable fetuses following ibuprofen administration (from the publication):

Table 2—Effect of Oral Administration of Ibuprofen on Pregnancy in Rat

[Values are mean ± SE of 9 animals]

Treatment	No. of animals with implantation	No. of animals with resorption sites	No. of implantation sites (Day 10)	No. of fetuses (Day 19)
Control (5% $NaHCO_3$)	9	0	8.77 ± 0.52	8.00 ± 0.53
Ibuprofen (20 mg/100 g)	6	1	3.71 ± 1.46**	3.28 ± 1.28*
Ibuprofen (30 mg/100 g)	3	3	3.44 ± 1.14***	0.11 ± 0.11***

P values: * < 0.02; ** < 0.05; *** < 0.001

Reference ID: 4913649

As shown in the table above, there is a dose-dependent decrease in the number of implantation sites and number of fetuses.

Reviewer's Comments:
The dosing of ibuprofen was not sufficient to study male and female fertility and any possible effects on sperm characteristics such as motility. The doses used in the study is equivalent to the human doses of 1946 and 2919 mg in an average human weighing 60 kg, based on a body surface area comparison. This study was useful to study implantation sites and the number of fetuses. The rat doses used in this study yield exposure margins of 0.6 and 0.9 based on the maximum daily dose of 3200 mg for ibuprofen. These data confirm the general statements made in the NSAID class labeling; therefore, it does not appear to be necessary to include the specific details in the product labeling.

Integrated Summary and Safety Evaluation

Section 8.1: Pregnancy

Embryo-Fetal Development

The 1994 Motrin® labeling only states that (b) (4) The current Motrin® labeling states "Reproductive studies conducted in rats and rabbits have not demonstrated evidence of developmental abnormalities." This statement provides no data regarding the doses of the drugs given to the animals or the periods of development covered by the studies. It cannot be used to create a PLLR appropriate label without review of the original study reports, which are proprietary data and cannot be referenced to support a 505(b)(2) application without a letter of authorization granting the Agency permission to review the actual data in the Motrin® NDA. As such, our review must rely on the existing published information to inform the labeling.

The Adams' data summaries from the 1969 and 1970 publications are presumably the based on the studies originally completed by Boots Pharmaceuticals, the group that discovered ibuprofen. Based on the data available to us for this submission, we cannot state if the Adams' studies are the same studies that were submitted to the FDA in support of the Motrin® NDA.

Reference ID: 4913649

The rabbit teratology study described by Adams reports 4 pups in one high dose litter with evidence of cyclopia-related malformations. The authors dismissed this finding because it only occurred in one litter and there was no obvious dose-dependency. However, this is a rather unusual finding which unfortunately occurred in the high dose group (60 mg/kg). Therefore it is hard to dismiss the finding as irrelevant due to lack of dose dependency. Cappon et. al 2003 do report results of a rabbit study testing a higher dose of ibuprofen (200 mg/kg); however, ibuprofen was only administered on Gestation Day 9 to 11 in this study.

Cyclopia is a malformation resulting in failure of the embryonic forebrain to divide into two hemispheres. It is a rare form of holoprosencephaly characterized by the failure of the embryonic proencephalon to properly divide the orbits into two eye cavities. This results in not only the presence of a single eye in the center of the face but also one optic nerve and optic lobe in the brain (Cohen and Shiota, 2002). “Cyclopia” was not reported in the historical control database for New Zealand White Rabbits published by Charles River (2008-2010). The closest malformation reported was “close set eye socket” which occurred at a very low incidence (close-set eye socket was reported in one fetus in one litter in 5859 fetuses and 696 litters examined). That being said, cyclopia was reported in a single control animal in a study of the teratogenicity of diflunisal, published around the same time period (Clark et.al, 1984). These authors also suggest that diflunisal teratogenicity is characterized by axial skeletal defects, possibly resulting from anemia as a result of significant gastrointestinal lesions.

There are several known causes of cyclopia in humans, including genetic mutations. The toxin cyclopamine derived from the plant *Veratrum californicum* has also been reported to cause cyclopia in sheep (Keeler, 1970). Keeler also reports that administration of cyclopamine between Gestation Days 6 and 9 results in cyclopia in rabbits. That toxin did not produce cyclopia when it was dosed from Gestation Day 9 to 12 or 12 to 15, suggesting that the insult must occur early in gestation. Other causes of holoprosencephaly have been proposed, including maternal diabetes, ethyl alcohol, and retinoic acid (Cohen and Shiota, 2002).

Reference ID: 4913649

The observation that all 4 fetuses reported with cyclopia-like malformations occur in the same litter suggests that the cause was likely something unique to that doe, possibly a genetic defect or unique toxicity, rather than a direct effect of the ibuprofen drug treatment. Individual animal data are not available to know details of that particular doe; however, the authors do note that the 60 mg/kg dose produced stomach ulcers and a few animals also had “pneumonia and a mild degree of focal hepatitis that was probably due to infection secondary to the gastric lesions.” This significant maternal toxicity, that likely includes anemia from the stomach ulcers, confounds the study result interpretation. Given the finding of cyclopia restricted to a single litter, and the lack of a signal for cyclopia in other NSAIDs, it seems unlikely that the finding is related to ibuprofen. Likewise, even if the finding was related to ibuprofen, the severity of the maternal toxicity present in this dose suggests limited potential for clinical relevance. As such, we do not recommend that this finding be included in the product label. In the absence of other clear adverse effects, the Adams study results appear reasonable to include in the product labeling in order to provide greater detail, including exposure margins.

The findings reported by Cappon et. al (2003) of an increased incidence of membranous ventricular septal defects (VSD) in rats treated with ibuprofen during the critical periods of heart formation appear to present a finding that was not previously identified in the original embryo-fetal development studies conducted to support Motrin® and/or by Boots Pharmaceuticals, likely because Cappon et al were able to obtain higher doses by dosing over a shorter dosing interval. Of the NSAID drugs tested by these authors, ibuprofen did appear to increase the incidence of membranous ventricular septal defects more than the other drugs. Burdan et. al (2004) reported one incidence of membranous VSD in control animals and two in fetuses from rats treated with 255 mg/kg ibuprofen (none in the high dose group). In a more comprehensive evaluation of the multiple studies, Burdan et. al report that there appears to be an increase in membranous VSD in rats treated with aspirin and ibuprofen compared to background incidence (Burdan et. al, 2006). This effect is thought to be associated with drugs that block COX-1 more than drugs that are selective COX-2 inhibitors, possibly since the ventricular septum development occurs between GD8 and 16 and COX-2 expression does not appear to occur in rats until GD16 and is specifically located in the skin, heart, cartilage, and kidney fetuses at this time; whereas COX-1 is expressed throughout the embryonic and fetal periods. Although it is possible that maternal toxicity contributed to this finding, we believe it is prudent to include these data in the product labeling to enhance the ability to detect such an association in humans, should one exist.

Reference ID: 4913649

Burdan and colleagues also report data for high doses of ibuprofen (≥225 mg/kg) administered from Day 8 to 21 of gestation resulted in reduced fetal weight and length and reduced ossification in many bones and increased postimplantation losses. The significance of these findings is complicated by the fact that there was significant moribundity and even 20% mortality was also noted at these doses. In surviving dams, profound stomach changes were noted as well as intestinal lesions in the illium. When these lesions resulted in severe gastrointestinal lesions, including perforations, hepatic lesions were also noted. Although these doses are below the human equivalent doses based on a body surface area comparison, the clinical significance of these adverse effects is questionable given the greater sensitivity of the animal models to the gastrointestinal effects of NSAIDs, which is particularly true for ibuprofen. Given the extent of the maternal toxicity and the unclear clinical relevance, these findings need not be included in product labeling.

Pre- and Postnatal Development

There is no reference to a pre- and postnatal development study in the original Motrin® labeling. Adams (1969) does suggest that Boots Pharmaceuticals did conduct a study in rats that could be considered similar to a pre- and postnatal development study. Adams dosed pregnant female rats throughout gestation until parturition. The young were examined 3 weeks after delivery. According to the paper, “Litters were reduced to 9 at birth (the normal practice in our breeding colony), and any underweight or neglected young were killed during the suckling period. All were examined in detail for gross abnormalities. From the litter records, the viability index at weaning was estimated.” The authors report that there was no impact on litter size, viability index, or weaning weight. However, as it is not clear if underweight animals were omitted or not, the results of this study cannot be included in the product labeling as an adequate pre- and postnatal development study.

A search of the published literature failed to identify any other published information that could be used to characterize the effect of ibuprofen on pre- and postnatal development. Given the impact of ibuprofen on the ductus arteriosis, a standard study design could not be easily completed with any expectation of useful data.

Juvenile Animal Data

We have not identified any juvenile animal studies in the published literature.

Section 13.1: Carcinogenesis, Mutagenesis, and Impairment of Fertility

Section 13 should be updated to reflect the existing knowledge, if available.

Reference ID: 4913649

Carcinogenesis. The original Motrin® labeling does not contain any carcinogenicity data. There is a published reference article describing studies conducted by Boots Pharmaceuticals (Adams et al., 1970); however, these studies do not appear to have been included in the Motrin® labeling and do not appear to be adequate by current standards. No additional published studies were identified in the published literature. The section should state that ***adequate*** long-term animal studies to evaluate the carcinogenic potential of ibuprofen have not been completed.

Mutagenesis. The original Motrin® labeling does not include any genetic toxicology data. The two published Ames assays from Oldham et al., 1986 and Philipose et al., 1997 demonstrated that ibuprofen is not mutagenic in what could collectively be called a standard set of bacterial strains by modern standards. These data should be included in the product labeling. Although Philipose did include data from an in vivo sister chromatid exchange assay, this assay has fallen out of favor (Dearfield et. al, 2011) and is no longer even listed in ICH S2(R1). Further, several publications were identified that suggest that ibuprofen is not clastogenic in humans; therefore the rat clastogenicity data are not necessary for product labeling. As per the regulations, human data cannot be included in Section 13; therefore, the negative human data will not be added to labeling either.

Impairment of Fertility. The original Motrin® labeling did not contain any specific data or information regarding the effects of ibuprofen on fertility and/or early embryonic development. As a result our review will only rely on the existing published information to inform the label. Adams et. al (1969) summarizes findings from a fertility study in which 10 male and 20 female rats were dosed with ibuprofen in the diet at a dose level of approximately 20 mg/kg or normal feed for 8-weeks prior to and presumably during the 2-week mating period.

Reference ID: 4913649

The rat fertility study described by Adams reported that 15 of 20 (75%) females receiving ibuprofen were pregnant and 16 of 18 (89%) control females were pregnant after the 14-day mating period. Although female fertility appears reduced, one male was considered infertile. After taking into consideration that 2 of the 5 failed pregnancies were considered to be the result of a single incidence of male infertility, the female fertility index can be adjusted to 15 of 18 (83%) females becoming pregnant, which does not appear to be different than 16 of 18 (89%) becoming pregnant in controls. In another study by Stutz et al. (2000), adult males were dosed with ibuprofen for 35 days or 60 days to determine if ibuprofen affected epididymal transit or spermatogenesis and epididymal transit, respectively. There were no effects on any sperm parameters examined, which included motility, response to hypoosmotic shock, and viability, after ibuprofen treatment. In a follow-up study by Martini et al. (2008), male and female fertility parameters were examined. Similar results were obtained in males supportive of the previous data collected. In females, the only notable effects from ibuprofen IP administration for 35 days were a reduction in ovulation indices, both induced and spontaneous. Taken together, these data suggest that female ovulation is affected by ibuprofen treatment whereas male fertility, resulting litter sizes and sperm characteristics are not significantly impacted by ibuprofen administration. These results should be included in the updated product labeling.

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Reference ID: 4913649

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/s/

CARLIC K HUYNH
01/03/2022 01:09:00 PM

NEWTON H WOO
01/03/2022 05:33:27 PM

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	NDA 209471
Supporting document/s:	SDN 24 (Electronic Document Room Sequence number 23)
Applicant's letter date:	May 7, 2020 (SDN 24)
CDER Stamp Date:	May 7, 2020 (SDN 24)
Product:	Combogesic® (Acetaminophen 325 mg and Ibuprofen 97.5 mg) tablet
Indication:	For the short-term management of mild to moderate acute pain
Applicant:	AFT Pharmaceuticals Limited
Clinical Review Division:	Division of Anesthesiology, Addiction Medicine, and Pain Medicine (DAAP)
Reviewer:	Carlic K. Huynh, PhD
Team Leader:	Newton H. Woo, PhD
Supervisor:	R. Daniel Mellon, PhD
Clinical Division Director:	Rigoberto A. Roca, MD
Project Manager:	Sandy Truong

Template Version: September 1, 2010

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Executive Summary:
The NDA was originally submitted on March 1, 2017 and was issued a Complete Response (see Complete Response Letter dated December 22, 2017). This is a second cycle NDA review. In this NDA resubmission, the Applicant has tightened the DS specification for (b) (4) to NMT (b) (4)% to be as low as technically feasible as per discussions with the CMC review team. Therefore, there are no safety concerns with the DS specification for acetaminophen. The Applicant has performed an elemental impurities assessment. The levels of the elemental impurities meet the limits of ICH Q3D. There are no safety concerns with the elemental impurities assessment. Thus, all nonclinical concerns have been addressed and are considered adequately resolved. From a Pharmacology Toxicology perspective, the proposed product may be approved.

Background and Prior Regulatory History (Nonclinical):
The Applicant, AFT Pharmaceuticals Limited, has developed Combogesic® (acetaminophen 325 mg/ibuprofen 97.5 mg) tablets for the short-term management of mild to moderate acute pain using the oral route of administration. Combogesic® is a lower strength formulation of Maxigesic® (acetaminophen 500 mg/ibuprofen 150 mg) that maintains the same ratio of acetaminophen and ibuprofen and was developed specifically for the U.S. market. Maxigesic® is marketed in New Zealand, Australia, UAE, Singapore, Malaysia, and more than 15 European countries. This is a 505(b)(2) application referencing Ultracet® (NDA 21123) and Motrin® (NDA 17463) for the Agency's previous determination of safety for acetaminophen and ibuprofen, respectively.

The first cycle review identified several nonclinical deficiencies and recommended a Complete Response. The following nonclinical deficiencies were communicated to the Applicant (see Complete Response Letter from December 22, 2017):

3. You have not provided adequate justification for the proposed drug substance specification for (b) (4) Because (b) (4) has been reported to produce chromosomal aberrations in human lymphocytes, reduce the drug substance specification for (b) (4) to as low as technically feasible.

4. Your application does not address the potential presence of elemental impurities in your drug product in accordance with ICH guidance document: *Q3D Elemental Impurities*, available at https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM371025.pdf.

   To address this deficiency, analyze the final drug product for elemental impurities as per the above guidance taking into consideration the maximum daily dose of 12 tablets per day. Provide safety justification for any elemental impurity that exceeds the ICH permissible daily exposures for the oral route of administration.

Applicant's Response to Item 3:
The Applicant has tightened the acetaminophen drug substance (DS) specification for (b) (4) as seen in the following table (adapted from the Applicant's submission):

Impurity	Structure	Specification	Comment
(b) (4)		NMT (b) (4) %	• Tightened to NMT (b) (4) % • As low as technically feasible per discussion with CMC • Acceptable

The reader is referred to the quality review for further details.

There are no safety concerns with the DS specification for acetaminophen and this deficiency is considered adequately resolved.

Applicant's Response to Item 4:
The Applicant has submitted an elemental impurities assessment as per ICH Q3D that has taken into consideration the maximum daily dose of 12 tablets/day of the proposed drug product. The potential sources of the elemental impurities were determined to be from the APIs and excipients in the drug product formulation. The following table illustrates actual and/or predicted levels of potential impurities taking into consideration the data supplied by the API and excipient manufacturers (adapted from the Applicant's submission):

Elemental Impurity	Total Maximum Daily Intake (mcg)	Control Threshold, (b) (4) of PDE (mcg/day)	ICH Q3D Oral PDE (mcg/day)	Adequate?
(b) (4)				Yes, adequate as per ICH Q3D and additional controls are not required.

As shown in the table above, the levels of the elemental impurities at the maximum daily dose of 12 tablets/day are below the (b) (4) PDE and PDE limits specified in ICH Q3D. As such, there are no safety concerns with the elemental impurities assessment and this deficiency is considered adequately resolved.

Conclusions and Recommendations:
To address the nonclinical deficiencies in the complete response letter, the Applicant tightened the DS specification for (b) (4) to NMT (b) (4) %. This specification appears to be as low as technically feasible as per discussions with the CMC review team. In addition, the Applicant submitted an elemental impurities assessment. The levels of the elementals in the elemental impurities assessment meet the limits of ICH Q3D. Taken together, there are no safety concerns with the (b) (4) specification and with the elemental impurities assessment.

Thus, all nonclinical concerns have been addressed and are considered adequately resolved. From a Pharmacology Toxicology perspective, the proposed product may be approved.

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/s/

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10/14/2020 12:58:07 PM

NEWTON H WOO
10/14/2020 01:09:38 PM

RICHARD D MELLON
10/16/2020 02:22:18 PM
I concur.

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	209471
Supporting document/s:	SDN 1, 10, 13, and 16 (Electronic Document Room Sequence Number 0, 9, 12, and 15)
Applicant's letter date:	March 1, 2017 (SDN 1), June 1, 2017 (SDN 10), July 24, 2017 (SDN 13), and September 10, 2017 (SDN 16)
CDER stamp date:	March 1, 2017 (SDN 1), June 1, 2017 (SDN 10), July 24, 2017 (SDN 13), and September 11, 2017 (SDN 16)
Product:	Combogesic® (Acetaminophen 325 mg and Ibuprofen 97.5 mg) tablet
Indication:	For the short-term management of mild to moderate acute pain
Applicant:	AFT Pharmaceuticals Limited
Review Division:	Division of Anesthesia, Analgesia, and Addiction Products (DAAAP)
Reviewer:	Carlic K. Huynh, PhD
Team Leader:	Newton H. Woo, PhD
Supervisor:	R. Daniel Mellon, PhD
Division Director:	Sharon Hertz, MD
Project Manager:	Allison Meyer

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1 Executive Summary

1.1 Introduction

The Applicant, AFT Pharmaceuticals Limited, has developed Combogesic® (acetaminophen 325 mg/ibuprofen 97.5 mg) tablets for the short-term management of mild to moderate acute pain using the oral route of administration. Combogesic® is a lower strength formulation of Maxigesic® (acetaminophen 500 mg/ibuprofen 150 mg) that maintains the same ratio of acetaminophen and ibuprofen and was developed specifically for the U.S. market. Maxigesic® is marketed in New Zealand, Australia, UAE, Singapore, Malaysia, and more than 15 European countries. This is a 505(b)(2) application referencing Ultracet® (NDA 21123) and Motrin® (NDA 17463) for the Agency's previous determination of safety for acetaminophen and ibuprofen, respectively.

1.2 Brief Discussion of Nonclinical Findings

No new toxicology studies were required to support this NDA submission due to the lack of concerns regarding pharmacokinetic and pharmacodynamic interactions and the extensive clinical and nonclinical safety data accumulated for acetaminophen and ibuprofen individually and as a combination. The existing nonclinical data are sufficient to support the safety of the proposed indication and dosing regimen. There are no safety issues regarding the formulation, the drug substance specification for ibuprofen, and the drug product specifications. The acetaminophen drug substance specification for (b) (4) (NMT (b) (4) %) must be tightened to as low as technically feasible and is included in our comments to the Applicant. The Applicant did not address the potential for elemental impurities to be present in the final drug product.

Due to several publications that suggest enhanced gastrointestinal (GI) and/or renal toxicity in rats co-administered ibuprofen and acetaminophen, the Applicant conducted two non-standard toxicology studies: an acute oral GLP toxicity study of acetaminophen, ibuprofen, and phenylephrine HCl alone and in two combinations in rats (Study 1247-12131) and a 7-day oral GLP gastrointestinal and renal toxicity study of ibuprofen and acetaminophen in female rats (Study 1247-12072). Although these studies have several limitations, co-administration of acetaminophen and ibuprofen did not increase GI or renal toxicity and did not identify any new safety concerns with the combination.

1.3 Recommendations

1.3.1 Approvability

From the nonclinical Pharmacology Toxicology perspective, there are inadequate data to support an approval recommendation. Therefore, a complete response is recommended.

The following deficiencies, which overlap with CMC deficiencies, must be addressed in the second review cycle.

1. You have not provided adequate justification for the proposed drug substance specification for (b) (4) Because (b) (4) has been reported to product chromosomal aberrations in human lymphocytes, reduce the drug substance specification for (b) (4) to as low as technically feasible.

2. Your application does not address the potential presence of elemental impurities in your drug product in accordance with ICH guidance document: *Q3D Elemental Impurities*, available at https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM371025.pdf. To address this deficiency, analyze the final drug product for elemental impurities as per the above guidance taking into consideration the maximum daily dose of 12 pills per day. Provide safety justification for any elemental impurity that exceeds the ICH permissible daily exposures for the oral route of administration.

1.3.2 Additional Nonclinical Recommendations

None at this time. The need for a juvenile animal study to support studies in pediatric patients below the age of 2 will be addressed if this application is approved and when an appropriate pediatric formulation is proposed.

1.3.3 Labeling

In the original submission, the Applicant did not propose labeling in PLLR format and no Section 13 was proposed. Updated labeling based in literature review was requested in an information request. The following recommended changes are to the Applicant's proposed labeling from 7/24/2017, which was not annotated by the Applicant nor adjusted for the daily exposures via this drug product. These have not been discussed with the review team or the Applicant as of the date of this review. The reader is ultimately referred to the approval letter for final labeling recommendations.

Note: exposure ratios are based on the current proposed maximum daily dose of 12 tablets per day (3900 mg acetaminophen and 1170 mg ibuprofen per day). Annotation of the literature references that support this labeling has been provided by the review team.

Applicant's proposed labeling	***Reviewer's proposed changes***	***Rationale for changes***
USE IN SPECIFIC POPULATIONS	**USE IN SPECIFIC POPULATIONS**	

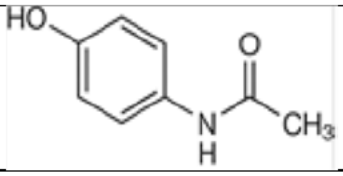
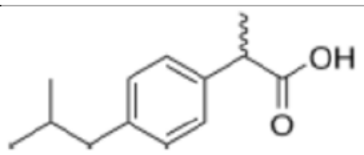
Reference ID: 4190546
8 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

(b) (4)

2 Drug Information

2.1 Drug

	Acetaminophen	**Ibuprofen**
CAS Registry Number:	103-90-2	15687-27-1
Generic Name	Acetaminophen, paracetamol	Ibuprofen
Code Name	APAP	
Chemical Names	N-(4-hydroxyphenyl)acetamide p-Hydroxyacetanilide p-Acetamidophenol N-Acetyl-p-aminophenol	α-Methyl-4-(2-methylpropyl)-benzene acetic acid p-Isobutyl hydratropic acid 2-(4-Isobutylphenyl) propionic acid
Molecular Formula	$C_8H_9NO_2$	$C_{13}H_{18}O_2$
Molecular Weight	151.16 g/mol	206.28 g/mol

Structure or Biochemical Description		
Pharmacologic Class	There is no FDA-established pharmacologic class for acetaminophen due to the lack of a clear understanding of the mechanism of action of acetaminophen.	Nonsteroidal Anti-inflammatory Drug (FDA Established Pharmacologic Class)

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND#	Drug Name	Division	Status	Indication	Sponsor
107435	Maxigesic® (Acetaminophen and Ibuprofen)	DAAAP	Active (May 8, 2010)	For the relief of acute pain and pain associated with osteoarthritis	AFT Pharmaceuticals Ltd.

NDA#	Drug Name	Div	Strength (route)	Marketing Status	AP Date	Indication	Company
21123	Ultracet® (Acetaminophen and Tramadol HCl)	DAAAP	325 mg Acetaminophen and 37.5 mg Tramadol (oral)	Prescription	August 15, 2001	Management of moderate to moderately severe acute and chronic pain	Janssen Pharmaceuticals Inc.
17463	Motrin® (Ibuprofen)	DAAAP	300, 400, 600, and 800 mg (oral)	Withdrawn (not for safety or efficacy reasons)	January 5, 2015 (withdrawn effective date)	For relief of the signs and symptoms of rheumatoid arthritis and osteoarthritis, for relief of mild to moderate pain, and for the treatment of primary dysmenorrhea	McNeil Consumer Healthcare Div McNeil-PPC Inc

MF#	Subject of MF	Holder	Submit Date	Reviewer's Comment
		(b) (4)	February 7, 2002 (active)	Deemed acceptable in various solid oral formulations (see quality review dated August 29, 2016).
			July 3, 1987 (active)	Deemed acceptable for solid oral formulations (see quality reviews dated December 15, 2015 and April 6, 2016).

2.3 Drug Formulation

Combogesic® is composed of 325 mg of acetaminophen and 97.5 mg of ibuprofen. The following table illustrates the composition of Combogesic® tablets (from the Applicant's submission):

Table 1: Composition of Combogesic Tablets

3.2.P.1.2 Composition

Component	Quantity per tablet (mg)	Quality Standard	Function
(b) (4)			
Acetaminophen**	325.00	USP/Ph. Eur.	Drug substance
Ibuprofen***	97.50	USP/Ph. Eur.	Drug substance
(b) (4)	(b) (4)	USP/Ph. Eur.	(b) (4)
Microcrystalline cellulose#		USP/Ph. Eur.	
(b) (4)		USP/Ph. Eur.	
Croscarmellose sodium		USP/Ph. Eur.	
(b) (4)			
		USP/Ph. Eur.	
		In-house	
		USP/Ph. Eur.	
		USP/Ph. Eur.	
Talc		USP/Ph. Eur.	
Magnesium stearate		USP/Ph. Eur.	
(b) (4)			
(b) (4)		In-house *Ph. Eur./USP/JP* *Ph. Eur./NF/JP* *Ph. Eur./USP/FCC/JP* *Ph. Eur/NF* *Ph. Eur./USP/JP/JSFA*	
HPMC (b) (4) *Hypromellose* (b) (4) *Lactose Monohydrate* *Titanium Dioxide* (b) (4) (b) (4) (b) (4)			
(b) (4)		USP/Ph. Eur.	
(b) (4)		In-house	
Total weight of coated tablet			

The following table illustrates the total weight of the excipients in the proposed formulation (data from the Applicant's submission):

Table 2: Combogesic® Excipients at the MDD of 4 g/day APAP

Combogesic® Excipient Quantities at the MDD of 4 g/day APAP			
Excipient	**Quantity per tablet (mg)**	**Quantity at MDD of 4 g/day APAP (12 tablets)**	**Comments**
(b) (4)	(b) (4)		Adequately qualified via FDA-approved oral products
Microcrystalline cellulose			
(b) (4)			
Croscarmellose sodium			
Talc			
Magnesium stearate			
(b) (4)			In an FDA-approved oral product to treat a chronic condition, the
HPMC (b) (4) Hypromellose			

(b) (4) Lactose monohydrate Titanium dioxide (b) (4) (b) (4)	(b) (4)		total daily dose of this excipient is (b) (4) mg. However, each individual ingredient is adequately qualified via FDA-approved oral products.

2.4 Comments on Novel Excipients

There are no novel excipients in the formulation.

2.5 Comments on Impurities/Degradants of Concern

Acetaminophen Drug Substance Specifications:
The drug substance acetaminophen is supplied by (b) (4). (DMF (b) (4)) and the finished product manufacturer is (b) (4).
The following table illustrates the acetaminophen drug substance "finished product manufacturer" specifications (data from the Applicant's submission):

Table 3: Acetaminophen Drug Substance Specifications

Impurity	Structure	Specification	Comment
(b) (4)			

(b) (4)

(b) (4) ((b) (4) The current drug substance specification for (b) (4) is NMT (b) (4)% and would normally be acceptable as per ICH Q3A(R2). However, (b) (4) contains a structural alert for mutagenicity. A search of the published literature did identify several studies that suggested that the compound has been tested in the Ames assay (*Salmonella* mutagenesis only) and found to be negative (b) (4)

(b) (4) tested the ability of (b) (4) ((b) (4) to form mutations in several *Salmonella* strains (TA100, TA1535, TA1537, and TA98) with and without S9 metabolic activation. The doses used in (b) (4). were up to 1000 mcg for non-toxic compounds. (b) (4). is a review paper and does not contain the raw data as seen in final study reports. Although the standard 5000 mcg/plate and *E. coli* WP2 strain were not used, Bruce Ames was one of the authors listed as contributing to the study and more than likely, the methods used are appropriate for a valid study.

(b) (4) tested the ability of (b) (4) ((b) (4) to form mutations in several *Salmonella* strains (TA1535, TA1537, TA98, and TA100) with and without S9 metabolic activation. The doses used in (b) (4). were up to 10 mg/plate, which is greater than the 5 mg/plate. The appropriate positive controls were used for each strain. However, the *E. coli* WP2 strain was not used and this paper does not contain the raw data as seen in final study reports. Nonetheless, the method developed by Bruce Ames was used. Thus, the methods used are more than likely appropriate for a valid study.

Moreover, [redacted] is very similar to the profile for the well-known impurity [redacted] The Agency has requested that [redacted] be reduced to as low as technically feasible in the drug substance. To date, [redacted] has not been detected in stability batches (BDL < [redacted]%). These stability data show that the specification for [redacted] can be tightened further. Thus, the Applicant will be tasked to tighten their DS specification for [redacted] in the acetaminophen drug substance. As this NDA will be given a Complete Response during this cycle, this outstanding item was not addressed via an IR at this time. It will be listed as a deficiency in this cycle.

Ibuprofen Drug Substance Specifications:
The drug substance ibuprofen is supplied by [redacted]) and the finished product manufacturer is [redacted]. The following table illustrates the ibuprofen drug substance "finished product manufacturer" specifications (data from the Applicant's submission):

Table 4: Ibuprofen Drug Substance Specifications

Impurity	Specification	Comment
[redacted]		

(b) (4)

As shown in the table about, the ibuprofen drug substance specifications as well as the levels of the residual solvents in the ibuprofen drug substance are acceptable.

Drug Product Specifications:
The following table illustrates the drug product specifications at release and upon stability for (data from the Applicant's submission):

Table 5: Drug Product Specifications

Degradant	Specification	Batch				Comments
		BAD1201 Feb 2012	BAD1202 Feb 2012	BAD1203 Mar 2012	BAD5401 Nov 2014	
(b) (4)						

As shown in the table above, the drug product specifications are acceptable. In the batch analysis, stability data was provided for up to 36 months under normal conditions (25°C ± 2° / 60 ± 5% RH) and up to 6 months under accelerated conditions (40 °C ± 2° / 75 ± 5% RH).

Elemental Impurities:
There are no data in the application regarding the testing for elemental impurities. This must be addressed in accordance with ICH Q3D. This will be listed as a deficiency.

Container Closure System:
(b) (4) The container closure materials are compliant with the guidelines for packaging of food and pharmaceutical products. There are no safety concerns with the container closure system.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed drug product is intended for adult patients and not for pediatric patients under 18 years of age. The adult dose is 3 tablets (975 mg of acetaminophen and 292.5 mg of ibuprofen) up to four times a day (every 6 hours) as needed for pain relief, to a maximum of 12 tablets per day (3900 mg of acetaminophen and 1170 mg of ibuprofen).

2.7 Regulatory Background

The drug development program for the proposed drug product was conducted under IND 107435 under the name Maxigesic®. The original IND was submitted on April 8, 2010 and was allowed to proceed on May 8, 2010 (see nonclinical review dated May 10, 2010). At the time of the nonclinical review, the Division communicated with the Applicant that no additional nonclinical studies were required. There was a preNDA meeting with the Applicant on July 9, 2017 (see meeting minutes dated August 5, 2015). There is Agency concurrence with the Applicant's iPSP (see letter dated February 6, 2017).

3 Studies Submitted

3.1 Studies Reviewed

The following table illustrates the studies that were reviewed:

Study Number	Study Title
1247-12072	Seven-Day Oral GLP Gastrointestinal and Renal Toxicity Study of Ibuprofen and Paracetamol in Female Rats

1247-12131	Acute Oral GLP Toxicity Study of Paracetamol, Ibuprofen, and Phenylephrine HCl Alone and In Two Combinations in Rats

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

There were no previous reviews referenced.

4 Pharmacology

4.1 Primary Pharmacology

There were no primary pharmacology studies with the combination of APAP and ibuprofen or either constituent alone submitted in this NDA.

4.2 Secondary Pharmacology

There were no secondary pharmacology studies with the combination of APAP and ibuprofen or either constituent alone submitted in this NDA.

4.3 Safety Pharmacology

There were no safety pharmacology studies with the combination of APAP and ibuprofen or either constituent alone submitted in this NDA.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

There were no PK/ADME studies with the combination of APAP and ibuprofen or either constituent alone submitted in this NDA.

5.2 Toxicokinetics

There were no toxicokinetic studies with the combination of APAP and ibuprofen or either constituent alone submitted in this NDA.

Reference ID: 4190546

6 General Toxicology

6.1 Single-Dose Toxicity

During development, the Applicant noted that there were several published reports that suggested the potential for additive toxicity with the combination of ibuprofen and acetaminophen (Derle & Gujar, 2001; Bhattacharya, et al., 1991; Kalra, et al., 2009; Kumar, et al., 2010). The Applicant concluded that the older two articles were not very useful and the two newer articles provided conflicting results. Therefore, they completed a study of their own to assess the potential for drug interactions on the target organs of toxicity.

The Applicant submitted an acute oral GLP toxicity study that evaluated APAP, Ibuprofen, and phenylephrine HCl alone and in two combinations in rats (1247-12131) and a seven-day oral GLP gastrointestinal and renal toxicity study of ibuprofen and APAP in female rats (1247-12072). There were no required toxicology studies to support the safety of the proposed product.

Study title: Acute Oral GLP Toxicity Study of Paracetamol, Ibuprofen, and Phenylephrine HCl Alone and In Two Combinations in Rats

Study no.:	1247-12131
Study report location:	\\cdsesub1\evsprod\nda209471\0000\m4\42-stud-rep\423-tox\4231-single-dose-tox\1247-12131\1247-12131-pre-clinical-study-report.pdf
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 13, 2012
GLP compliance:	Yes. Signature provided on August 13, 2012
QA statement:	Yes. Signature provided on August 13, 2012
Drug, lot #, and % purity:	Ibuprofen, Lot 2AI8020, 100% pure; Paracetamol (Acetaminophen) , Lot 2AE0414, 100.3% pure; Phenylephrine HCl, Lot ZK0044, 99.7% pure

Key Study Findings

This study was performed in 2 phases:

- In Phase A, the MTD study, rats were administered single doses of either 1000 mg/kg of acetaminophen, 500 mg/kg of ibuprofen, or 11 or 500 mg/kg of phenylephrine via oral gavage and then were observed for 6 days prior to sacrifice.
  - Staining of the nares was the only clinical observation in the 1000 mg/kg acetaminophen group. Dehydration was observed in the 500 mg/kg ibuprofen group. Dehydration, decreased activity, piloerection,

and tachypnea were observed in the 500 mg/kg phenylephrine group as well as all female rats were found dead the morning of the day following dosing and thus, the phenylephrine dose was reduced to 11 mg/kg. In the 11 mg/kg phenylephrine group, scab around the mouth was the only clinical observation.
    - There were body weight decreases of 2, 5, and 7% in the 500 mg/kg phenylephrine (24-hours post-dose period in the males), 1000 mg/kg acetaminophen (6-day post-dose period in the females), and 500 mg/kg ibuprofen (6-day post-dose period in the females).
    - The only macroscopic findings were in the 3 females found dead in the 500 mg/kg phenylephrine group including foamy content in the trachea in 1 female, and dark or discolored lungs and bilateral dark red kidney medullas in all 3 females, where are considered phenylephrine-related.
    - From these results, 1000 mg/kg of acetaminophen, 300 mg/kg of ibuprofen, and 10 mg/kg of phenylephrine were selected as the doses studied Phase B.
- In Phase B, the acute toxicity study, rats were administered single doses of either 1000 mg/kg of acetaminophen, 300 mg/kg of ibuprofen, 10 mg/kg of phenylephrine, 1000 and 300 mg/kg of acetaminophen and ibuprofen, or 1000, 300 and 10 mg/kg of acetaminophen, ibuprofen, and phenylephrine via oral gavage and then were observed for 7 days prior to sacrifice.
    - All rats survived to the scheduled necropsy.
    - There were no treatment-related changes in body weight.
    - The clinical observations of decreased activity, stained on head, and dehydration appear to be additive (combined acetaminophen and ibuprofen vs these compounds alone).
    - Macroscopic changes include discoloration of the stomach in the combination acetaminophen and ibuprofen group in 1/5 females. There were no further treatment-related macroscopic changes.
    - No NOAEL was determined as histopathology was not examined and only 1 dose was studied.

Methods

Doses:	See table below
Frequency of dosing:	One single oral dose in both phases
Route of administration:	Oral gavage for both phases
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% carboxymethylcellulose (CMC) and 0.1% Tween 80 in water
Species/Strain:	Rat/Sprague Dawley
Number/Sex/Group:	3/sex/group Phase A and 5/sex/group Phase B, see table below
Age:	6-8 weeks
Weight:	225-275 g
Satellite groups:	None
Unique study design:	Did not include histopathology although gross pathology was performed and there was no vehicle control group
Deviation from study protocol:	None

The following table illustrates the 2 phases of this study: The maximum tolerated dose (MTD) study and the acute toxicity study (from the Applicant's submission):

Table 6: Study Design of Phase A and Phase B

Phase A – MTD Study

Group (DIA)	Test Article	Dosage mg/kg	Conc. mg/mL	# of Animals		Animal No.	
				M	F	M	F
1	Paracetamol	1000	100	3	3	1-3	28-30
4	Ibuprofen	500	50	3	3	4-6	31-33
7	Phenyl-ephrine	500	50	3	3	7-9	34, 36, 55
8	Phenyl-ephrine	11	1.1	3	3	16-18	43-45

Phase B – Acute Toxicity Study

Group (DIA)	Test Article(s)	Dosage mg/kg	Conc. mg/mL	# of Animals		Animal No.	
				M	F	M	F
10	Para-cetamol	1000	100	5	5	1-5	26-30
11	Ibuprofen	300	30	5	5	6-10	31-35
12	Phenyl-ephrine	10	1.0	5	5	11-15	36-40
13	Para-cetamol	1000	200	5	5	16-20	41-45
	Ibuprofen	303	60.6				
14	Para-cetamol	1000	303	5	5	21-25	46-50
	Ibuprofen	300	90.9				
	Phenyl-ephrine	10	3.0				

The doses used in Phase A were selected on the basis of studies done in the published scientific literature. For acetaminophen, 1000 mg/kg is considered the limit dose as per ICH M3(R2). Moreover, rats tolerated single oral doses of up to 900 mg/kg acetaminophen (Prescott, 2001). For ibuprofen, 500 mg/kg was selected due to the determination of the LD_{50} of 1225 mg/kg in the Summary Basis of Approval for NDAs 21393 and 21394 (Advil® PM Liqui-Gels and Advil® PM Caplets, respectively). For phenylephrine, 500 mg/kg was selected due to rats tolerating dosing in feed at up to 216 mg/kg phenylephrine for 14 days without any toxicity (Bucher et al., 1988). The subsequent dose of 11 mg/kg phenylephrine was given based on the resulting lethality of the 500 mg/kg phenylephrine dose. In Phase A, the test articles were administered as a single dose via oral gavage and then the rats were observed for 6 days prior to sacrifice.

In Phase B, 1000 mg/kg acetaminophen was selected because 1000 mg/kg acetaminophen was well tolerated in Phase A. The ibuprofen dose of 300 or 303 mg/kg was selected to match the composition of Maxigesic® and Maxigesic PE® tablets based on an acetaminophen dose of 1000 mg/kg. The phenylephrine dose of 10 mg/kg was selected to match the composition of Maxigesic PE® tablets based on an acetaminophen dose of 1000 mg/kg. It is noted that the doses of ibuprofen and phenylephrine used in Phase B were below the doses of these drugs used in Phase A. In Phase B, the test articles were administered as a single dose via oral gavage and then the rats were observed for 7 days prior to sacrifice.

Observations

Mortality

In both phases, all rats were observed cage-side twice daily for signs of ill health, morbidity, mortality, injury, viability, and availability of food and water.

Clinical Signs
In both phases beginning on Day 1 after the first dose cycle and continuing through the day of scheduled sacrifice, examinations for general signs of toxicity, including fecal and urine quality, were conducted for all rats twice daily. Each rat was examined in its home cage as well as more detailed examination of the head, neck, limbs, trunk, tail, body orifices, and genitalia. Each rat was observed for changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions, lacrimation, piloerection, pupil size, unusual respiratory pattern, and the cage for abnormal feces, urine, or other excretions or secretions.

Body Weights
In both phases beginning on the day of dosing prior to the first dose, body weights were recorded daily.

Food Consumption
Food consumption was not measured and recorded in this study.

Ophthalmoscopy
Ophthalmoscopy was not performed in this study.

ECG
ECG recordings were not performed in this study.

Hematology
Hematology was not performed in this study.

Clinical Chemistry
Clinical chemistry was not performed in this study.

Urinalysis
Urinalysis was not performed in this study.

Gross Pathology
In both phases, postmortem examinations were performed on all rats dying spontaneously or euthanized at the scheduled necropsies. At necropsy, all rats were examined visually for external abnormalities including palpable masses. The abdominal, thoracic, and cranial cavities and their contents were examined for abnormalities and the organs examined.

Organ Weights
Organ weights were not recorded in this study.

Histopathology
Histopathology was not performed in this study.

Reference ID: 4190546

Adequate Battery
N/A

Peer Review
N/A

Histological Findings
N/A

Special Evaluation
There were no special evaluations performed in this study.

Toxicokinetics
Toxicokinetics were not performed in this study.

Dosing Solution Analysis
Dosing solution analysis was performed for Phase B only. Duplicate top, middle, and bottom samples of up to 5 mL each were collected from each dosing formulation at the time of preparation. The following table illustrates the dosing formulations (from the Applicant's submission):

	Concentration (mg/mL)		
Group (DIA)	Ibuprofen	Paracetamol	Phenylephrine HCl
1	---	100	---
4	50	---	---
7	---	---	50
8	---	---	1.1
10	---	100	---
11	30	---	---
12	---	---	1.0
13	60.6	200	---
14	90.9	303	3.0

Results for Phase A

Mortality
All three females in the 500 mg/kg phenylephrine dose group were found dead the morning of the following dosing day. This dose clearly exceeds the maximum tolerated dose for phenylephrine. There were no further deaths in the Phase A rats from any of the other dose groups.

Clinical Observations
The following clinical observations were noted in each group:

Reference ID: 4190546

Acetaminophen at 1000 mg/kg:
Staining of the nares was observed in 2 males and 1 female.

Ibuprofen at 500 mg/kg:
Staining of the nares and dehydration were observed in both sexes. Females were also observed with generalized pallor and 1 female was observed with decreased activity.

Phenylephrine at 500 mg/kg:
There were numerous clinical observations indicative of generalized toxicity that were observed in both sexes, including cool to touch, staining and/or wetness of body surfaces, dehydration, decreased activity, unkempt appearance, piloerection, tachypnea, and/or increased lacrimation. All 3 females in this dose group were found dead.

Phenylephrine at 11 mg/kg:
Scab round the mouth was observed in a single male.

Body Weight
The Sponsor noted a 2% decrease in mean body weight over the first 24 hours post-dose in the male 500 mg/kg phenylephrine dose group, a 5% decrease in the mean body weight over the 6-day post-dose period in the female 1000 mg/kg acetaminophen dose group, and a 7% decrease in the mean body weight over the 6-day post-dose period in the female 500 mg/kg ibuprofen dose group. There were no further treatment-related changes to body weight or body weight gain in the remaining dose groups.

Macroscopic Pathology
The following table illustrates the macroscopic changes in the Phase A rats (from the Applicant's submission):

Table 7: Macroscopic Findings in Males and Females (Phase A)

Phase A

Males

Observation	Group 1	Group 4	Group 7	Group 8
Not Remarkable	3 / 3	3 / 3	3 / 3	3 / 3
Scheduled Sacrifice	3 / 3	3 / 3	3 / 3	3 / 3

Females

Observation	Group 1	Group 4	Group 7	Group 8
Eye: opacity	--	--	1 / 3	--
Kidneys: discolored	--	--	3 / 3	--
Lungs: discolored	--	--	2 / 3	--
Lungs: dark	--	--	1 / 3	--
Trachea: foamy contents	--	--	1 / 3	--
Not Remarkable	3 / 3	3 / 3	--	3 / 3
Found Dead	--	--	3 / 3	--
Scheduled Sacrifice	3 / 3	3 / 3	--	3 / 3

Group 1 – acetaminophen; Group 4 – ibuprofen; Group 7 & 8 - phenylephrine

The only macroscopic changes noted were in the 3 females found dead in the 500 mg/kg phenylephrine dose group. Foamy contents in the trachea was noted in 1 of these females. Dark or discolored lungs and bilateral dark red kidney medullas were observed in all 3 females. An opacity in the right eye was observed in 1 female and was considered incidental. The lung, trachea, and kidney findings were considered related to 500 mg/kg phenylephrine administration. There were no further treatment-related macroscopic changes in any of the other dose groups.

Due to the above changes in body weight, clinical observations, and macroscopic changes, 1000 mg/kg acetaminophen, 300 mg/kg ibuprofen, and 10 mg/kg phenylephrine were selected as doses in Phase B of the study.

Results for Phase B

Toxicities in the acetaminophen only, ibuprofen only, and the acetaminophen + ibuprofen groups are noted in this review to describe the additive effects of acetaminophen and ibuprofen as the proposed Combogesic® is a combination of acetaminophen and ibuprofen.

Mortality

All rats in Phase B survived to the scheduled sacrifice.

Clinical Observations

The following table illustrates the clinical signs that are potentially related to the test articles (from the Applicant's submission):

Table 8: Clinical Signs (Phase B)

Incidence of Clinical Signs Potentially Related to Test Article

Paracetamol (mg/kg) =	1000	---	---	1000	1000
Ibuprofen (mg/kg) =	---	300	---	303	300
Phenylephrine (mg/kg) =	---	---	10	---	10
Decreased activity	7/10	0/10	0/10	9/10	8/10
Stained on head (around eyes, nose, and/or mouth)	2/10	3/10	1/10	7/10	7/10
Appears dehydrated	1/10	1/10	0/10	2/10	1/10
Stained body (including urogenital area)	0/10	1/10	0/10	0/10	2/10

As shown in the table above, decreased activity was observed in both the acetaminophen only (7/10) and the acetaminophen + ibuprofen (9/10) groups with none in the ibuprofen only group. Decreased activity may be an effect of acetaminophen only. Stained on head was observed in the acetaminophen only (2/10) and ibuprofen only (3/10) and in the acetaminophen + ibuprofen (7/10) groups with an additive effect. Appears dehydrated was observed in the acetaminophen only (1/10) and ibuprofen only (1/10) and in the acetaminophen + ibuprofen (2/10) groups with an additive effect. There is no additive effect with stained body as it only occurs in the ibuprofen only (1/10) group.

Body Weight

The following table illustrates the changes in body weight (from the Applicant's submission):

Table 9: Body Weight (Phase B)

Summary of Mean Body Weight Data

Paracetamol (mg/kg) =	1000	---	---	1000	1000
Ibuprofen (mg/kg) =	---	300	---	303	300
Phenylephrine (mg/kg) =	---	---	10	---	10
Day –1	263.6	263.2	266.3	264.3	271.6
Day 2	255.1	265.1	267.2	257.6	263.7
Weight change Days –1 to 2	*–3%*	*+1%*	*±0%*	*–3%*	*–3%*
Day 7	265.9	264.8	272.4	260.1	269.6
Weight change Days –1 to 7	*+1%*	*+1%*	*+2%*	*–2%*	*–1%*

As shown in the table above, there does not appear to be any additive effects of acetaminophen in combination with ibuprofen on the mean body weight and the body weight gain. In fact, the changes in mean body weight and body weight gain appear transient in all dose groups.

Macroscopic Pathology
The following table illustrates the macroscopic changes (from the Applicant's submission):

Table 10: Macroscopic Findings in Males and Females (Phase B)

Phase B

Males

Observation	Group 10	Group 11	Group 12	Group 13	Group 14
Liver: supernumerary lobe	--	--	1 / 5	--	--
Not Remarkable	5 / 5	5 / 5	4 / 5	5 / 5	5 / 5
Scheduled Sacrifice	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5

Females

Observation	Group 10	Group 11	Group 12	Group 13	Group 14
Stomach: discolored	--	--	--	1 / 5	--
Not Remarkable	5 / 5	5 / 5	5 / 5	4 / 5	5 / 5
Scheduled Sacrifice	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5

Group 10 – acetaminophen; Group 11 – ibuprofen; Group 12 – phenylephrine; Group 13 – acetaminophen/ibuprofen; Group 14 – all three

As shown in the table above, macroscopic observations include supernumerary lobe of the liver in the phenylephrine group and discolored stomach in the female acetaminophen + ibuprofen group. Supernumerary lobe of the liver was observed in the phenylephrine group only and is not related to acetaminophen, ibuprofen, and acetaminophen + ibuprofen and as such, can be dismissed. Discolored stomach in the female acetaminophen + ibuprofen group is an expected effect of acetaminophen, ibuprofen alone and in combination.

Dosing Solution Analysis
The sample preparations for acetaminophen and phenylephrine ranged from 90-101% and 99-105%, respectively, demonstrating accurate preparations and homogeneity.

For ibuprofen, the preparation ranged from 73-85% (mean 80%) for the 30 mg/mL preparation. For the 60.6 mg/mL preparation, the preparation ranged from 84-93% (mean 88%) and for the 90.0 mg/mL preparation, the preparation ranged from 87-93% (mean 89%). Based on these results, Group 11 rats received a 240 mg/kg dose instead of the intended 300 mg/kg dose of ibuprofen. The concentration of ibuprofen in Group 13 rats (acetaminophen with ibuprofen) was considered acceptable at 88% of nominal and for homogeneity.

6.2 Repeat-Dose Toxicity

Study title: Seven-Day Oral GLP Gastrointestinal and Renal Toxicity Study of Ibuprofen and Paracetamol in Female Rats

Study no.:	1247-12072
Study report location:	\\cdsesub1\evsprod\nda209471\0000\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\1247-12072\1247-12072-pre-clinical-study-report.pdf
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 4, 2012
GLP compliance:	Yes. Signature provided on August 13, 2012
QA statement:	Yes. Signature provided on August 13, 2012
Drug, lot #, and % purity:	Ibuprofen, Lot 2AI8020, 100% pure; Paracetamol (Acetaminophen), Lot 2AE0414, 100.3% pure

Key Study Findings

Rats were administered 80 mg/kg of acetaminophen and 24 mg/kg of ibuprofen, alone or in combination, once daily via oral gavage for 7 days. A vehicle control group is included.

- All rats survived to the scheduled sacrifice.
- There were no treatment-related changes in clinical signs, body weights, food consumption, urinalysis, and gross pathology.
- There were increases in monocytes, lymphocytes, large unstained cells, basophils, reticulocytes, and white blood cells in the acetaminophen, ibuprofen, and combination groups that were within normal ranges.
- There was an 8.54% increase in the AST levels in the ibuprofen dose group with no additive effects in the combination acetaminophen and ibuprofen group.
- There were no treatment-related histopathological changes in the stomach and gut-associated lymphoid tissue (duodenum). There were focal areas of inflammation with and without fibrosis as well as hyaline casts and hyaline droplets in the kidney; however, these kidney findings were in all dose groups with no additive effects.
- No NOAEL was determined as only one dose of acetaminophen and ibuprofen, alone and in combination, was used in the study.

Methods

Doses:	24 mg/kg/day ibuprofen and 80 mg/kg/day acetaminophen once daily for 7 days
Frequency of dosing:	Once daily for seven days
Route of administration:	Oral gavage
Dose volume:	10 mL/kg for Groups 1-3, and 5 mL/kg ibuprofen with 5 mg/kg paracetamol (total of 10 mL/kg) for Group 4
Formulation/Vehicle:	0.5% carboxymethylcellulose (CMC) and 0.1% Tween 80 in water
Species/Strain:	Rat/Sprague Dawley
Number/Sex/Group:	10 female rats/group (females only)
Age:	6-8 weeks
Weight:	225-275 g
Satellite groups:	There were no toxicokinetics group
Unique study design:	This renal toxicology study used female rats only because they are less prone than males to develop chronic progressive nephropathy. Organ weights were taken and recorded. The study does not evaluate an adequate histopathology battery as only the stomach, duodenum, and kidneys were examined microscopically. Toxicokinetics were not performed.
Deviation from study protocol:	None

The following table illustrates the study design (from the Applicant's submission):

Table 11: Study Design

Group	Test Material(s)	Dosage (mg/kg/day) Ibuprofen	Dosage (mg/kg/day) Paracetamol	Animal No.
DHW1	0.5% CMC/ 0.1% Tween 80 in water (vehicle)	0	0	1-10
DHW2	Ibuprofen	24	0	11-20
DHW3	Paracetamol	0	80	21-30
DHW4	Ibuprofen + Paracetamol	24	80	31-40

Observations and Results

Mortality

All rats were observed cage-side twice daily for signs of ill health, morbidity, mortality, injury, viability, and availability of food and water. All rats survived to the scheduled sacrifice.

Clinical Signs

Reference ID: 4190546

Beginning on Day 1 after the first dose and continuing through the day of scheduled sacrifice, examinations for general signs of toxicity including fecal and urine quality were conducted twice daily. Each rat was examined in its home cage and subsequently hands-on to include an examination of the head, neck, limbs, trunk, tail, body orifices, and genitalia. Changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions, lacrimation, piloerection, pupil size, unusual respiratory pattern were noted as well as in the cage for abnormal feces, urine, or other excretions or secretions. There were no treatment-related changes in clinical signs.

Body Weights
Body weights were recorded for all rats prior to randomization to dose groups and daily beginning on the day prior to first dose through to the schedule sacrifice. There were no treatment-related changes in mean body weight or body weight gain in any of the dose groups.

Food Consumption
Food consumption was recorded for all rats weekly beginning prior to the first dose through to the scheduled sacrifice. There were no treatment-related changes in food consumption.

Ophthalmoscopy
Ophthalmoscopy was not performed in this study.

ECG
ECG recordings were not performed in this study.

Hematology
Rats were fasted overnight prior to blood sampling for clinical chemistry analysis prior to the scheduled sacrifice. The following hematology parameters were measured (from the Applicant's submission):

White blood cell count	Mean cell volume
Absolute differential leukocyte count	Mean cell hemoglobin
Red blood cell count	Mean cell hemoglobin concentration
Hemoglobin	Platelet count
Hematocrit	Reticulocyte count

The following table illustrates the changes in hematology (data from the Applicant's submission):

Table 12: Hematology Changes

Potential Drug-Related Differences in Hematology Parameters				
	Vehicle	**Ibuprofen**	**Acetaminophen**	**Combination**
Ibuprofen (mg/kg/day)	**0**	**24**	**0**	**24**
Acetaminophen (mg/kg/day)	**0**	**0**	**80**	**80**

Monocytes Mean count (per mcL) % Control	140 ---	210 +50%	190 +36%	290 +107%
Lymphocytes Mean (per mcL) % Control	6,380 ---	7,140 +12%	7,700 +21%	8,250 +29%
Large Unstained cells Mean (per mcL) % Control	60 ---	70 +17%	90 +50%	100 +67%
Basophils Mean (per mcL) % Control	40 ---	40 ±0%	60 +50%	70 +75%
Reticulocytes Mean (% of RBCs) % Control	2.17 ---	2.01 -7%	2.42 +12%	2.61 +20%
White Blood Cells (WBC) Mean (per mcL) % Control	7,310 ---	8,200 +12%	8,850 +21%	9,600 +31%

As shown in the table above, the changes in monocytes, lymphocytes, large unstained cells, basophils, and reticulocytes are additive in nature. Monocytes, lymphocytes and basophils are all white blood cells or leukocytes. Historical control of leukocytes in female Sprague Dawley rats of this age is 4000 to 14,000 per mcL (Derelanko 2008). As such, the monocytes, lymphocytes, and basophils collectively fall within the historical control range. This is in agreement with the Applicant's Final Study Report. Reticulocytes and large unstained cells are increased in an additive manner and are both within the range of normal variation for female rats per the Applicant's Final Study Report. In addition, the white blood cell count (WBC) was increased by 31.3% in the acetaminophen + ibuprofen dose group (9,600 per mcL). This is within the historical control of leukocytes (white blood cells) in female Sprague Dawley rats of this age is 4000 to 14,000 per mcL (Derelanko 2008). The increase in WBC is dismissed in the Applicant's Final Study Report. There were no further treatment-related changes in hematology.

Clinical Chemistry

The following clinical chemistry parameters were measured (from the Applicant's submission):

Sodium	Total cholesterol
Potassium	Triglycerides
Chloride	Total protein
Alkaline phosphatase	Albumin
Alanine aminotransferase	Globulin (calculated)
Aspartate aminotransferase	Albumin/globulin ratio (calculated)
Glucose	Calcium
Blood urea nitrogen	Inorganic phosphorus
Creatinine	Total bilirubin

There was a decrease of 8.54% in the aspartate aminotransferase (AST) levels in the ibuprofen only dose group. There were no additive effects of the AST level between the acetaminophen and ibuprofen alone and in combination dose groups. There were no further treatment-related changes in clinical chemistry.

Urinalysis
Rats were administered not more than 20 mL/kg of water by oral gavage and then placed in metabolism cages for an overnight fast prior to urine collection. Urine was collected from the first 5 rats from each dose group prior to necropsy. The following urinalysis parameters were measured (from the Applicant's submission):

Specific gravity	Urobilinogen
pH	Bilirubin
Nitrite	Occult blood
Protein	Color and appearance
Glucose	Microscopy of sediment (if protein equals or exceeds 100 mg/dL or occult blood equals or exceeds 50 ery/µL)
Ketones	

There were no treatment-related changes in urinalysis.

Gross Pathology
At necropsy, the rats were examined visually for external abnormalities including palpable masses. The abdominal cavity was opened and the stomach, duodenum, and kidneys were removed. These organs were examined for any abnormalities.

There was 1/10 female from the control group that was observed with a focus in the glandular mucosa of the stomach. As this observation in the stomach was only observed in the control, it is deemed not treatment-related. There were no further treatment-related changes in gross pathology including the stomach, duodenum, or kidneys in the remaining rats.

Organ Weights
Organ weights were not recorded in this study.

Histopathology
Adequate Battery
Only the stomach, duodenum, and kidneys were examined microscopically. This is not an adequate battery for a general toxicology study.

Peer Review
Yes. A Pathology Report was included in the Final Study report for this study.

Histological Findings
The following table illustrates the histopathological findings (from the Applicant's submission):

Reference ID: 4190546

Table 13: Histopathology Changes in the Stomach, Duodenum, and Kidney

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
Necropsy Status: TERMINAL SACRIFICE GROUP (K0)

Sex		Females			
Dose Group		1	2	3	4
No. Animals per Dose Group		10	10	10	10
INTEST-SM, DUODENUM	No.Examined	10	10	10	10
	NAD	7	10	9	9
- Increased GALT		3	-	1	1
KIDNEY	No.Examined	10	10	10	10
	NAD	9	5	7	5
- Fibrosis; Capsular		-	-	-	1
- Hyaline Casts		1	3	-	1
- Hyaline Droplets		1	-	-	-
- Inflammation; Interstitial with Fibrosis		-	2	3	1
- Inflammation; Interstitial with Mucinous Material		-	-	-	1
- Mineralization		-	1	-	1
STOMACH	No.Examined	10	10	10	10
	NAD	10	10	10	10

NAD = Nothing abnormal discovered
Group 1, DHW1, females: Ibuprofen (0 mg/kg/day)/Paracetamol (0 mg/kg/day)
Group 2, DHW2, females: Ibuprofen (24 mg/kg/day)/Paracetamol (0 mg/kg/day)
Group 3, DHW3, females: Ibuprofen (0 mg/kg/day)/Paracetamol (80 mg/kg/day)
Group 4, DHW4, females: Ibuprofen (24 mg/kg/day)/Paracetamol (80 mg/kg/day)

There were no histopathological findings in the stomach that were considered treatment-related. There were minimally increased gut-associated lymphoid tissue (GALT) in the duodenum in all treatment groups including the control that was not considered treatment-related. There were several histopathological findings in the kidney including focal areas of inflammation with fibrosis that were observed in all 3 treatment groups with similar incidence and no additive effects. According to the Pathology Report, these areas of inflammation with fibrosis (lesions) were generally chronic in nature, were most probably pre-existed prior to dosing, were distributed randomly throughout the cortex of the kidney, and each of which was morphologically distinct. In the Pathology Report, it was noted that while the affected rats all had features of chronic inflammation and fibrosis, the assortment of inflammatory cells and the nature of the surrounding tissue reaction were not consistent with a common inciting cause.

There were no further treatment-related changes in histopathology.

Special Evaluation
There were no special evaluations in this study.

Toxicokinetics
Toxicokinetics were not determined in this study.

Dosing Solution Analysis
Duplicate top, middle, and bottom samples of 1.0 mL each were collected from each dosing formulation at the time of preparation. The following table illustrates the formulation preparations for ibuprofen and acetaminophen (from the Applicant's submission):

	Concentration (mg/mL)	
Group	Ibuprofen	Paracetamol
DHW2	2.4	0
DHW3	0	8.0
DHW4	4.8	16.0

Sample results for the acetaminophen formulation preparations ranged from 92-98% confirming accurate preparations and homogeneity.

Sample results for the 2.4 mg/mL preparation of ibuprofen ranged from 76-92%, mean of 85%, which was done to match the composition of Maxigesic®. The 2.4 mg/mL preparation of ibuprofen was administered from Group 2 (24 mg/kg/day ibuprofen). Sample results for the 4.8 mg/mL preparation of ibuprofen ranged from 85-87%, mean 86%, which was done to match the composition of Maxigesic®. The 4.8 mg/mL preparation of ibuprofen was administered to Group 4 (24 mg/kg/day ibuprofen with 80 mg/kg/day acetaminophen). As the assay values for ibuprofen were within 15% of the nominal value, the ibuprofen preparations were considered adequately homogenous.

7 Genetic Toxicology

There were no genetic toxicology studies with the combination of APAP and ibuprofen or either components that were submitted in this NDA.

8 Carcinogenicity

There were no carcinogenicity studies with the combination of APAP and ibuprofen or either components that were submitted in this NDA.

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9 Reproductive and Developmental Toxicology

There were no reproductive and developmental toxicology studies with the combination of APAP and ibuprofen or either components that were submitted in this NDA.

10 Special Toxicology Studies

There were no special toxicology studies with the combination of APAP and ibuprofen or either components that were submitted in this NDA.

Juvenile Animal Studies and Status of PSP:
As per the agreed PSP, the Applicant will be obtaining PK data in pediatric patients between 2 and 12 years of age. No juvenile animal studies were deemed necessary to support that age range. Efficacy and Safety will be studied in children under 2 years of age. For this younger age group, an oral suspension formulation will be developed. The final formulation is not yet determined and we will have to examine the safety of the proposed excipients at that time. During review of the iPSP, the Division indicated that NSAIDs can have adverse effects on the liver, kidney, and lung and indicated that the Applicant would have to address these potential target organs of toxicity in children under the age of 2 as these target organs are not fully formed until the age of two. The Applicant did not specifically address this in the current submission. The agreed PSP does state that a juvenile animal study to specifically address the safety of acetaminophen and ibuprofen on the development of the liver, kidney, and lungs will be completed if required by the Agency to support clinical studies in children under 2 years of age. At this point, we expect a juvenile animal study will be required to support clinical studies under 2 years of age. The Agency has agreed to deferral of the clinical study in children under 2 until data in older patients is obtained.

11 Integrated Summary and Safety Evaluation

Although no new toxicology studies were required to support this NDA submission, the Applicant submitted an acute oral GLP toxicity study of acetaminophen, ibuprofen, and phenylephrine HCl alone and in two combinations in rats (Study 1247-12131) and a 7-day oral GLP gastrointestinal and renal toxicity study of ibuprofen and acetaminophen in female rats (Study 1247-12072). These studies were completed to assess the potential for ibuprofen and acetaminophen to produce additive toxicity as reported in the published literature.

In the acute oral GLP toxicity study of acetaminophen, ibuprofen, and phenylephrine HCl alone and in two combinations in rats (Study 1247-12131), the study was performed in 2 phases (Phase A and Phase B). In Phase A, the MTD study, rats were administered single doses of either 1000 mg/kg of acetaminophen, 500 mg/kg of ibuprofen, or 11 or 500 mg/kg of phenylephrine via oral gavage and then were observed for 6 days prior to sacrifice. In Phase A, staining of the nares was the only clinical observation in the 1000 mg/kg acetaminophen group. Dehydration was observed in the 500 mg/kg ibuprofen group. Dehydration, decreased activity, piloerection, and

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tachypnea were observed in the 500 mg/kg phenylephrine group as well as all female rats were found dead the morning of the day following dosing and thus, the phenylephrine dose was reduced to 11 mg/kg. In the 11 mg/kg phenylephrine group, scab around the mouth was the only clinical observation. In Phase A, there were body weight decreases of 2, 5, and 7% in the 500 mg/kg phenylephrine (24-hours post-dose period in the males), 1000 mg/kg acetaminophen (6-day post-dose period in the females), and 500 mg/kg ibuprofen (6-day post-dose period in the females). In Phase A, the only macroscopic findings were in the 3 females found dead in the 500 mg/kg phenylephrine group including foamy content in the trachea in 1 female, and dark or discolored lungs and bilateral dark red kidney medullas in all 3 females, where are considered phenylephrine-related. From the results in Phase A, 1000 mg/kg of acetaminophen, 300 mg/kg of ibuprofen, and 10 mg/kg of phenylephrine were selected as the doses studied Phase B. In Phase B, the acute toxicity study, rats were administered single doses of either 1000 mg/kg of acetaminophen, 300 mg/kg of ibuprofen, 10 mg/kg of phenylephrine, 1000 and 300 mg/kg of acetaminophen and ibuprofen, or 1000, 300 and 10 mg/kg of acetaminophen, ibuprofen, and phenylephrine via oral gavage and then were observed for 7 days prior to sacrifice. In Phase B, all rats survived to the scheduled necropsy. In Phase B, there were no treatment-related changes in body weight. In Phase B, the clinical observations of decreased activity, stained on head, and dehydration appear to be additive (combined acetaminophen and ibuprofen vs these compounds alone). In Phase B, macroscopic changes include discoloration of the stomach in the combination acetaminophen and ibuprofen group in 1/5 females, which is an expected effect of acetaminophen. There were no further treatment-related macroscopic changes. No NOAEL was determined as only 1 dose was studied.

In the 7-day oral GLP gastrointestinal and renal toxicity study of ibuprofen and acetaminophen in female rats (Study 1247-12072), female rats were administered 80 mg/kg of acetaminophen and 24 mg/kg of ibuprofen, alone or in combination, once daily via oral gavage for 7 days. A vehicle control group is included. All rats survived to the scheduled sacrifice. There were no treatment-related changes in clinical signs, body weights, food consumption, urinalysis, and gross pathology. There were increases in monocytes, lymphocytes, large unstained cells, basophils, reticulocytes, and white blood cells in the acetaminophen, ibuprofen, and combination groups that were within normal ranges. There was an 8.54% increase in the AST levels in the ibuprofen dose group with no additive effects in the combination acetaminophen and ibuprofen group. There were no treatment-related histopathological changes in the stomach and gut-associated lymphoid tissue (duodenum). There were focal areas of inflammation with and without fibrosis as well as hyaline casts and hyaline droplets in the kidney; however, these kidney findings were in all dose groups with no clear additive effects. No NOAEL was determined as only one dose of acetaminophen and ibuprofen, alone and in combination, was used in the study. Nonetheless the study does not suggest a significant drug interaction in terms of expected toxicities of these two drugs. Further, as the GI tract and kidney are known target organs of toxicity for these two drugs, these endpoints and NSAID-induced GI toxicity in animals is more pronounced than in humans, these endpoints were monitored in the clinical studies.

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There were no safety issues regarding the formulation, the drug substance specifications for ibuprofen, and the drug product specifications. However, there are data suggesting that (b) (4) is clastogenic and the Division has requested that this drug substance impurity be reduced to as low as reasonably possible. Further, there was no assessment for elemental impurities in the drug product. This must be addressed in order to approve this application.

APPEARS THIS WAY ON ORIGINAL

12 Appendix/Attachments

References

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(b) (4)

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Appendix 1: Nonclinical Recommendations for Acetaminophen Rx Labeling

The following labeling recommendations for acetaminophen (MDD of 4 grams per day) are based upon a review of the literature completed by R. Daniel Mellon, PhD in 2010 and updated by Carlic Huynh, PhD in 2016.

Recommended Labeling	Rationale/Comment
8 USE IN SPECIFIC POPULATIONS **8.1 Pregnancy** **Nonclinical Risk Summary Statement (Human data to be provided by Maternal Health Review Team)** Animal reproduction studies have not been conducted with IV acetaminophen. Reproductive and developmental studies in rats and mice from the published literature identified adverse events at clinically relevant oral doses of acetaminophen. Treatment of pregnant rats with doses of acetaminophen approximately equal to the maximum human daily dose (MHDD) showed evidence of fetotoxicity and increases in bone variations in the fetuses. In another study, necrosis was observed in the liver and kidney of both pregnant rats and fetuses at doses approximately equal to the MHDD. In mice and rats treated with acetaminophen at doses within the clinical dosing range, cumulative adverse effects on reproductive capacity were reported. In mice, a reduction in number of litters of the parental mating pair was observed as well as retarded growth, abnormal sperm in their offspring, and reduced birth weight in the next generation. In rats, female fertility was decreased following in utero exposure to acetaminophen [see *DATA*].	See labeling for human risk summary statement The proposed risk summary statement is based on the data in the animal data section below.
The estimated background risk of major birth defects and miscarriage for the indicated population is unknown. All	PLLR Boilerplate to date.

Recommended Labeling	Rationale/Comment
pregnancies have a background risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.	
Animal Data Studies in pregnant rats that received oral acetaminophen during organogenesis at doses up to 0.85 times the maximum human daily dose (MHDD = 4 grams/day, based on a body surface area comparison) showed evidence of fetotoxicity (reduced fetal weight and length) and a dose-related increase in bone variations (reduced ossification and rudimentary rib changes). Offspring had no evidence of external, visceral, or skeletal malformations.	The statement regarding bone effects in the rat model represents collective review of the data reported in the studies from Burdan (2000, 2001, and 2003).
When pregnant rats received oral acetaminophen throughout gestation at doses of 1.2-times the MHDD (based on a body surface area comparison), areas of necrosis occurred in both the liver and kidney of pregnant rats and fetuses. These effects did not occur in animals that received oral acetaminophen at doses 0.3-times the MHDD, based on a body surface area comparison.	These rat data regarding liver and kidney toxicity are from Neto et al (2004).
In a continuous breeding study, pregnant mice received 0.25, 0.5, or 1.0% acetaminophen via the diet (357, 715, or 1430 mg/kg/day). These doses are approximately 0.43, 0.87, and 1.7 times the MHDD, respectively, based on a body surface area comparison. A dose-related reduction in body weights of fourth and fifth litter offspring of the treated mating pair occurred during lactation and post-weaning at all doses. Animals in the high dose group had a reduced number of litters per mating pair, male offspring with an increased percentage of abnormal	The mouse data are from the NTP reproductive toxicology study as summarized by Reel et al. (1992).

Recommended Labeling	Rationale/Comment
sperm, and reduced birth weights in the next generation pups.	
8.3 Females and Males of Reproductive Potential Published animal studies report that oral acetaminophen treatment of male animals at doses that are 1.2 times the MHDD and greater (based on a body surface area comparison) result in decreased testicular weights, reduced spermatogenesis, reduced fertility, and reduced implantation sites in females given the same doses. Additional published animal studies indicate that acetaminophen exposure in utero adversely impacts reproductive capacity of both male and female offspring at clinically relevant exposures [see *Nonclinical Toxicology (13.1)*].	Section 8.3 was added as several published studies report effects of acetaminophen on reproductive potential. The multigenerational study was conducted by the NTP (Lamb, 1997; Program, 1984; and Reel et al., 1992). Fertility studies were summarized in the literature (Boyd and Hogan, 1968; Jacqueson et al., 1984; Lamb, 1997; Yano and Dolder, 2002; and Holm et al., 2016).
13 NONCLINICAL TOXICOLOGY	
13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility <u>Carcinogenesis</u> Long-term studies in mice and rats have been completed by the National Toxicology Program to evaluate the carcinogenic potential of acetaminophen. In 2-year feeding studies, F344/N rats and B6C3F1 mice were fed a diet containing acetaminophen up to 6000 ppm. Female rats demonstrated equivocal evidence of carcinogenic activity based on increased incidences of mononuclear cell leukemia at 0.8 times the maximum human daily dose (MHDD) of 4 grams/day, based on a body surface area comparison. In contrast, there was no evidence of carcinogenic activity in male rats (0.7 times) or mice (1.2-1.4 times the MHDD, based on a body surface area comparison).	Based on current CDER ECAC standards, the mononuclear cell leukemia findings were deemed significant. However, the ECAC concluded that the finding is considered to have limited relevance to humans. We elected to leave the “equivocal” statement in the labeling, as that is what the NTP concluded at the time and is how the results were described in the study report.

Recommended Labeling	Rationale/Comment
Mutagenesis Acetaminophen was not mutagenic in the bacterial reverse mutation assay (Ames test). In contrast, acetaminophen tested positive in the in vitro mouse lymphoma assay and the in vitro chromosomal aberration assay using human lymphocytes. In the published literature, acetaminophen has been reported to be clastogenic when administered a dose of 1500 mg/kg/day to the rat model (3.6-times the MHDD, based on a body surface area comparison). In contrast, no clastogenicity was noted at a dose of 750 mg/kg/day (1.8-times the MHDD, based on a body surface area comparison), suggesting a threshold effect.	Findings from the Ames and in vitro chromosomal aberrations assays are from the studies conducted by the NTP. Although there are several studies in the literature that suggest the same conclusion, the NTP studies are the most definitive studies and result in the same basic message. The NTP has not conducted an in vivo assay for chromosomal damage. There are, however, numerous reports, as summarized in several articles (Rannug et al., 1995, Bergman et al., 1996) that demonstrate positive findings for clastogenicity in both animals and humans. The summary article (Bergman et al. 1996) suggests that there should be a threshold for these effects and that they likely occur at hepatotoxic doses. Definitive concurrence with such a conclusion would require careful evaluation of the underlying data that are not available to the Agency. The proposed labeling reflects the results for the pivotal in vivo study described in the Bergman review. Since the data are consistent with the carcinogenicity study results, and the study was apparently completed for the German regulatory authorities, the reported results will be included in product labeling.
Impairment of fertility In studies conducted by the National Toxicology Program, fertility assessments with acetaminophen have been completed in Swiss CD-1 mice via a continuous breeding study. There were no effects on fertility parameters in mice consuming up to 1.7 times the MHDD of acetaminophen, based on a body surface area comparison. Although there was no effect on sperm motility or sperm density in the epididymis, there was a significant	To date, the results of the fertility endpoints from Reel et al. (1992) serve as the primary data on fertility.

Recommended Labeling	Rationale/Comment
increase in the percentage of abnormal sperm in mice consuming 1.7 times the MHDD (based on a body surface area comparison) and there was a reduction in the number of mating pairs producing a fifth litter at this dose, suggesting the potential for cumulative toxicity with chronic administration of acetaminophen near the upper limit of daily dosing. Published studies in rodents report that oral acetaminophen treatment of male animals at doses that are 1.2 times the MHDD and greater (based on a body surface area comparison) result in decreased testicular weights, reduced spermatogenesis, reduced fertility, and reduced implantation sites in females given the same doses. These effects appear to increase with the duration of treatment.	The statement regarding testicular findings is derived from studies in rats by Boyd and Hogan (1968) and Jacqueson et al (1984) which reported decreased testicular weights and spermatogenesis following dosing of acetaminophen at 1.2 times the MHDD for longer than 30-days. There are data in the rat reporting effects on sexual behavior, sperm parameters, early implantation, and fertility at doses that are also only 1.2 fold the maximum human dose (Ratnasooriya and Jayakody, 2000). Ultrastructural changes in the testes have been reported after a single dose of oral APAP (1.6 times the MHDD) to the male rat by Yano et al. (2002).
In a published mouse study, oral administration of 50 mg/kg acetaminophen to pregnant mice from Gestation Day 7 to delivery (0.06 times the MHDD) reduced the number of primordial follicles in female offspring and reduced the percentage of full term pregnancies and number of pups born to these females exposed to acetaminophen in utero.	Data Source (Holm et al., 2016).
In a published study, pregnant rats oral administration of 350 mg/kg acetaminophen (0.85 times the MHDD) from Gestation Day 13 to 21 (dams), reduced the number of germ cells in the fetal ovary and decreased ovary weight and reduced number of pups per litter in F1 females as well as reduced ovary weights in F2 females.	Data Source (Dean et al., 2016).

REVIEW OF SUPPORTING DATA:

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 United States National Toxicology Program (NTP) has conducted in vitro mutagenicity and clastogenicity studies on APAP (National Toxicology Program, 1993). The results of the NTP studies indicate that acetaminophen tests negative as a mutagen; however, it tests positive as a clastogen in vitro (induced sister chromatid exchanges and chromosomal aberrations in CHO cells). These data are available to the public, employed current protocols and were conducted in accordance with Good Laboratory Practices (GLPs). These findings also should be included in product labeling. Although there are numerous published studies that support the conclusion that APAP is clastogenic in vitro (Ibrulj et al., 2007), the results of the NTP studies provide adequate data to support such a statement 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. The NTP has not 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 publicly available. 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 are not 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 obtained by the review team and determined to be an abstract of a presentation. As this reference did not provide actual data, it normally would 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

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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.

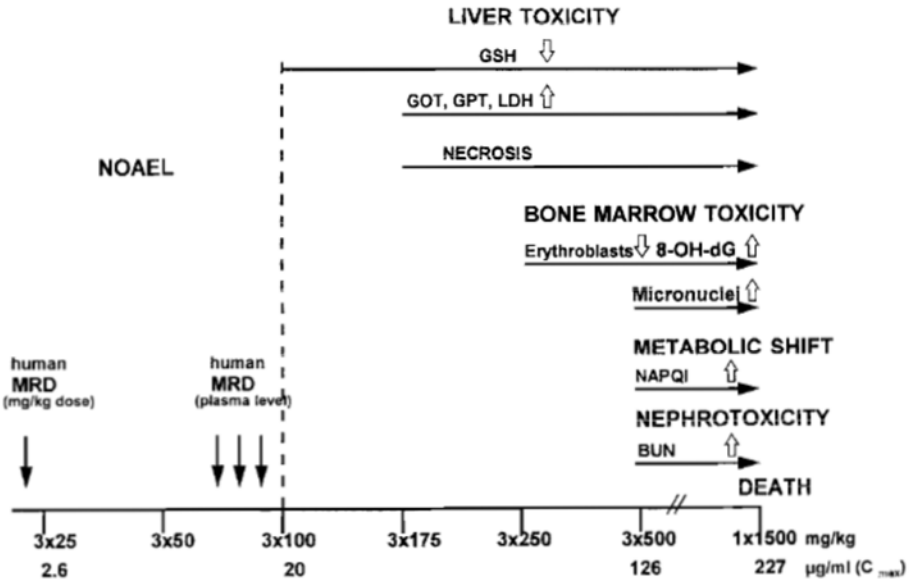

Fig. 1. Paracetamol: Oral gavage toxicity and micronucleus studies in rats. Onset of various toxicity related effects in relation to dosages used and peak plasma levels determined. Arrows illustrate increases or decreases in the relevant parameters determined. The human maximum recommended dosage (MRD) 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 would be reviewed by the Agency. However, the proprietary data could not be obtained; therefore, these results cannot be independently verified. However, as this finding is key to the conclusion that a NOEL 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.

Oshida and colleagues examined the in vivo effects of APAP in the comet assay (Oshida et al., 2008). These investigators treated mice with 12, 60, or 300 mg/kg APAP, IP and examined the liver, kidney, and bone marrow for evidence of DNA damage via the comet assay and examined cytotoxicity via hematology and clinical chemistry parameters. The high dose of APAP produced a positive response in the liver only, suggesting a threshold for genotoxicity, however, the effect also correlated

with cytotoxicity. Therefore, the positive comet assay results may be due to cytotoxicity rather than genotoxicity. Based on a body surface area basis, the NOEL of 60 mg/kg (180 mg/m^2) provides an exposure margin of 0.07. The high dose of 300 mg/kg (900 mg/m^2) provides an exposure margin of 0.36, suggesting that the hepatotoxicity or genotoxicity occurs at clinically relevant exposures. However, given the uncertainty if the finding is due to cytotoxicity or actual genotoxicity in this study, this study is not recommended to be included in the product labeling.

Carcinogenicity of APAP. Studies conducted by the NTP to evaluate the carcinogenic potential of acetaminophen can be used to support oral acetaminophen drug product prescription labeling. The study reports are available publicly. 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:

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Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Acetaminophen

Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Feed concentration			
0, 600, 3,000, or 6,000 ppm acetaminophen	0, 600, 3,000, or 6,000 ppm acetaminophen	0, 600, 3,000, or 6,000 ppm acetaminophen	0, 600, 3,000, or 6,000 ppm acetaminophen
Body weights			
Dosed groups similar to controls	Dosed groups similar to controls	Dosed groups lower than controls	Dosed groups lower than controls
2-Year survival rates			
27/50; 28/50; 23/50; 24/50	30/50; 34/50; 34/50; 28/50	32/50; 40/50; 31/50; 31/50	27/50; 32/50; 25/50; 38/50
Nonneoplastic effects			
Kidney: nephropathy severity grades (2.30, 2.56, 2.64, 2.78); renal tubule hyperplasia (1/50, 5/50, 3/50, 5/50) Parathyroid gland: hyperplasia (0/42, 4/45, 6/46, 8/45)	Kidney: nephropathy severity grades (1.44, 1.58, 1.64, 1.72)	Thyroid gland: follicular cell hyperplasia (0/49, 6/49, 12/50, 15/50)	Thyroid gland: follicular cell hyperplasia (2/48, 8/50, 11/50, 25/50)
Neoplastic effects			
None	None	None	None
Uncertain findings			
None	Mononuclear cell leukemia: 9/50; 17/50; 15/50; 24/50	None	None
Level of evidence of carcinogenic activity			
No evidence	Equivocal evidence	No evidence	No evidence
Genetic toxicology			
Salmonella typhimurium (gene mutation)	Negative in strains TA100, TA1535, TA1537, and TA98 with and without S9		
Sister chromatid exchanges (Chinese hamster ovary cells *in vitro*)	Positive with and without S9		
Chromosomal aberrations (Chinese hamster ovary cells *in vitro*)	Positive with and without S9		

Mean daily doses of APAP consumed were calculated based on mean food consumption over the course of the above studies 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 (ECAC) on February 2, 2010. Based upon current CDER criteria, the mononuclear cell leukemia noted in the female rats were significant rather than equivocal; however, the ECAC 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."

Effects on Fertility. A review of the literature identified three publications that provide some data relevant to fertility studies (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 cytosol glutathione transferase activity and of lipid peroxides. The authors note that the treatment did not result in decreased testicular glutathione levels; therefore, the toxicity cannot 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 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 NTP 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 cannot 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.

TABLE 5
Sperm Analysis for F_1 Male Mice at Necropsy following Continuous Exposure to Acetaminophen (Task 4)[a]

	% Acetaminophen in the diet	
Parameter[b]	0	1.0
Percentage motile sperm	51 ± 5	55 ± 3
Sperm density (number of sperm × 10^3/mg cauda epididymis)	925 ± 56	1038 ± 62
Percentage abnormal sperm[c]	7.3 ± 0.8	16.4 ± 2.6*

[a] These F_1 male mice are the same ones used in the mating trial (Table 3).
[b] Values are means ± SEM. Number of males: 19 for the control group and 20 for the 1.0% group.
[c] Tailless sperm not included in determination of percentage abnormal sperm.
* Significantly different ($p < 0.01$) from the control group.

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).

A rat study on male fertility was conducted by Kristensen et al. (2011) where Wistar rat dams were dosed 150, 250, and 350 mg/kg/day of APAP (0.36x, 0.61x, and 0.85x the maximum daily human dose of 4000 mg based on a body surface area comparison) from Gestational Day (GD) 13 to 21 via oral gavage. Following dosing, the anogenital distance (AGD), which is the distance from the anus to the genitalia (mm), was measured and an AGDi was calculated by dividing the AGD by the cube root of the body weight in kg for each rat. Rat studies using testosterone and an anti-androgen have indicated that androgen deficiency during a critical male programming window from GD 15.5 to 17.5 leads to cryptorchidism, hypospadias, compromised fertility, and reduction in the AGD (Welsh et al., 2008). On GD 21, Caesarean sections were performed on each dam. The following figure illustrates the changes in the AGDi of the male pups following maternal exposure to APAP on GD 13 to 21 (from Kristensen et al., 2011):

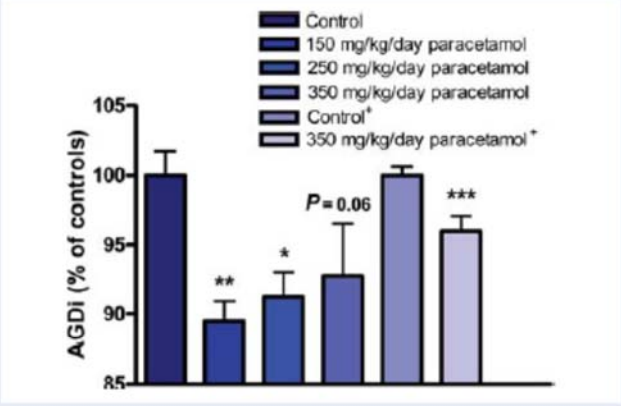

Figure 3 Intrauterine exposure from GD13 to 21 to paracetamol in rats led to a reduction in the testosterone-dependent AGD of male offspring. Results from initial experiment performed with four to five litters per dose group and the verifying experiment (+) performed with six litters per dose group. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$. *P*-values are from ANOVA followed by Dunnett's *post hoc* test with litter as the statistical unit and included as a random factor in a mixed model. There is no significant difference between treated groups. Data represent the mean ± SEM.

As shown in the figure above, a significant decrease in the AGDi was observed in the male pups following maternal exposure to APAP.

Additionally, testosterone production was analyzed on fetal testes at the time of fetal testosterone production initiation at GD 14.5 in an ex vivo culture system where the testes were dissected out of male rats from GD 14.5 and cultured in the presence of APAP for 3 days duration. As shown in the figure below, testosterone levels were significantly decreased for up to 3 days whereas prostaglandin levels were significantly decreased for 1 day (Kristensen et al., 2011).

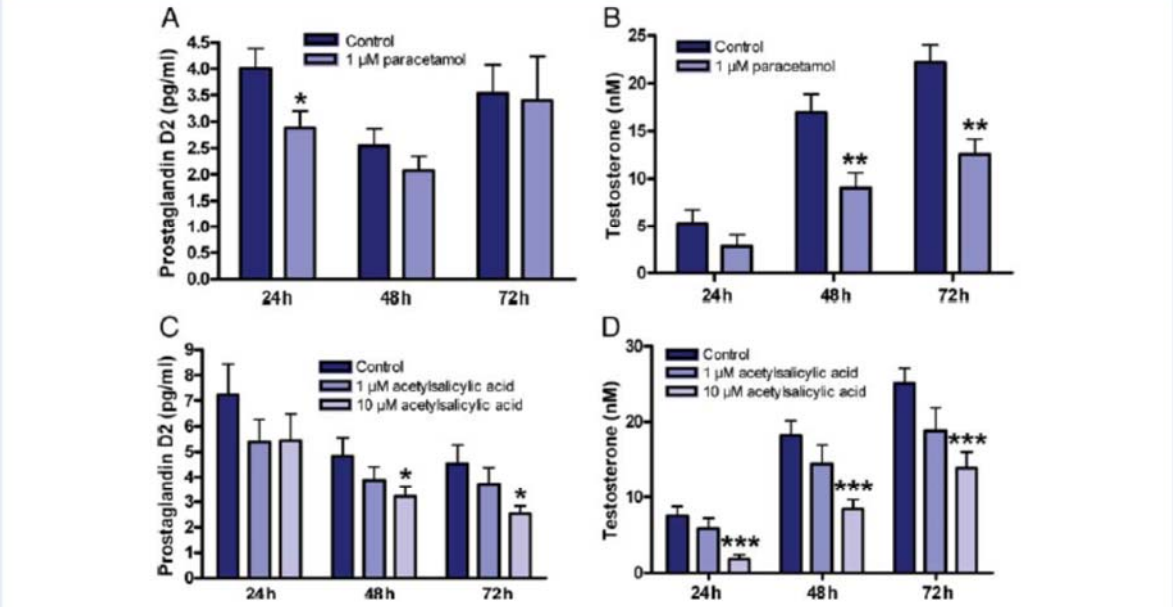

Figure 4 Mild analgesic compounds paracetamol and acetylsalicylic acid reduced PGD2 and testosterone production in *ex vivo* cultured GD14.5 rat testes. (**A**) Paracetamol ($n = 10$) and (**C**) acetylsalicylic acid ($n = 8$) reduced PGD2 secretion during *ex vivo* culture. (**B**) Paracetamol ($n = 10$) and (**D**) acetylsalicylic acid ($n = 13$) reduced testosterone secretion from the fetal rat testes during 3 days of *ex vivo* culture. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$. *P*-values are from a two-sided unpaired Student's *t*-test. Data represent the mean ± SEM.

Testosterone levels were not measured under in vivo conditions but assayed using an ex vivo culture system. Nonetheless the results are consistent with an impact of APAP on testosterone production early in development of male fetuses. These data are also consistent with the current labeling which states that APAP can reduce testicular weights, spermatogenesis, and fertility in rodents at clinically relevant exposures. However, importantly, these data suggest a smaller exposure margin for adverse effects, such as decreased testosterone production during development. Overall, the Kristensen et al., 2011 study does provide additional information suggesting an impact on testosterone production early in development at lower exposures than the currently listed 1.2 times the MHDD margins.

The effects of APAP and aniline on male fertility were studied in mice by Holm et al. (Holm et al., 2015). In this study, mated female C57BL/6J mice were divided into five treatment groups that either received water control, acetaminophen, or aniline. Mice in the acetaminophen groups, either received 50 or 150 mg/kg/day of APAP (0.06x and 0.18x the maximum daily human dose of 4000 mg based on a body surface area comparison). Mice in the aniline groups either received 31 or 93 mg/kg/day of aniline (which is converted into APAP). The doses of APAP or aniline were dissolved in 0.5 mL of water and administered from Gestational Day 7 to delivery. This study demonstrated that aniline is converted to acetaminophen by the liver. The following figure illustrates the conversion of aniline to APAP with reported APAP levels in the urine collected in mice at 4 and 24 hours after oral gavage administration of aniline or acetaminophen (see the following figure from Holm et al., 2015).

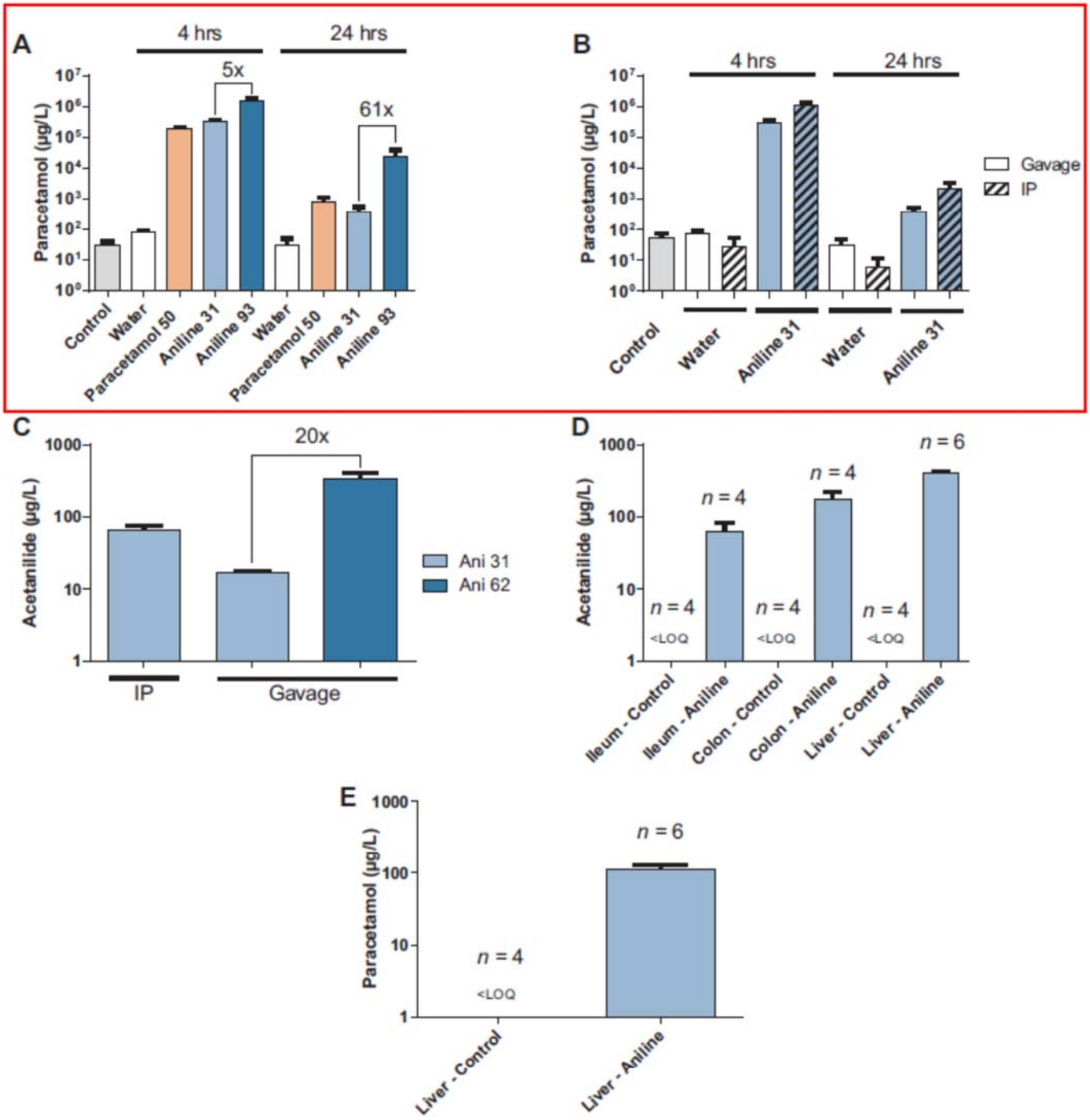

FIG. 3. Aniline is metabolized and excreted in urine as the analgesic paracetamol. A, Concentration of paracetamol in the urine collected from untreated male mice (control) and subsequently after 4 and 24 h postgavaging with water, aniline (31 and 62 mg/kg) or paracetamol (50 mg/kg). B, Concentration of paracetamol in the urine after 4 and 24 h after IP and gavage with aniline. C, Concentration of intermediate metabolite acetanilide in the urine 4 h after intraperitoneal injection (IP) and gavage. D, Concentration of intermediate metabolite acetanilide after *ex vivo* exposure of the luminal side of ileum and colon with 10 µM aniline for 3 h, and incubation of the perfused liver for 2 h with 10 µM aniline. E, Concentration of paracetamol in liver after perfusion and 2 h incubation with 10 µM aniline. No paracetamol was detected in ileum or colon samples after 3 h *ex vivo* exposure and no acetanilide or paracetamol were detected in the perfusion medium prior to experiments. Concentrations are depicted as mean ± SEM on a logarithmic y-axis ($n = 5$).

To measure any anti-androgenic effects, the anogenital distance (AGD) was measured and the AGD index (AGDi) was calculated by dividing the AGD by the cube root of the

body weight. Anogenital distance is the length between the anus and genital tubercle and increases during development. Males have a larger anogenital distance than females and this is believed to be due to testosterone production. Therefore, reduced anogenital distance in males is believed to be a surrogate for reduced testosterone production and has been associated with reduced fertility. The AGDi was significantly reduced in males from all APAP groups compared to control for up to 10 weeks post-birth (see figure below, from Holm et al., 2015). This study demonstrated that administration of acetaminophen or aniline impaired male reproductive development.

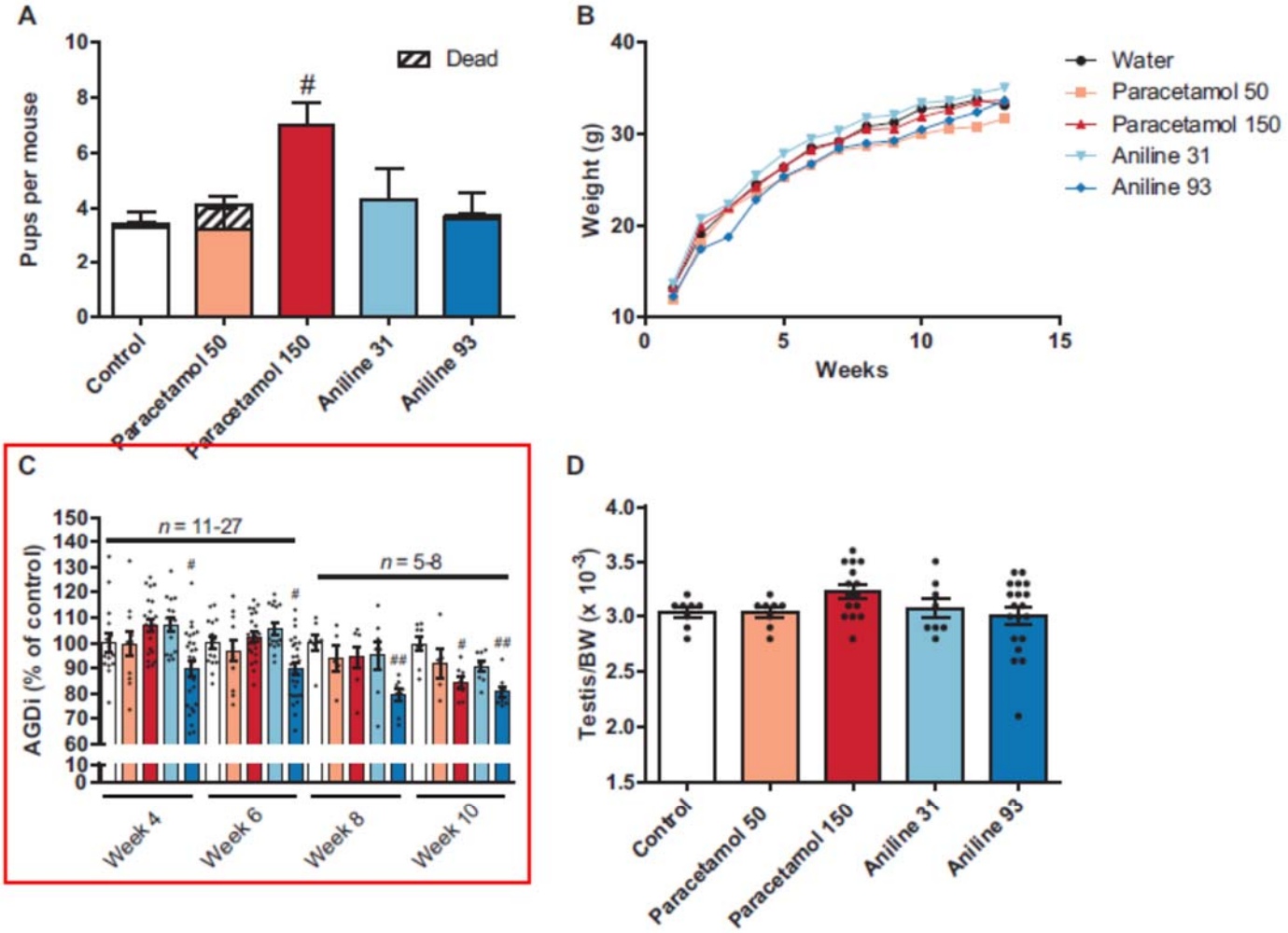

FIG. 4. Intrauterine exposure to aniline (31 and 93 mg/kg/day) or paracetamol (50 and 150 mg/kg/day) from GD7-20 disturbed aspects of the male reproductive development. A, Average number of pups per dam that where born and died within the 2 first postnatal weeks. B, Weight development in male pups in each dose group (n as in C). C, AGDi normalized to controls in male in relation to postnatal age. D, Weight of testes relative to whole body weight from mice killed at 6–7 weeks of age. Statistical tests performed were 2-way ANOVA followed by Bonferroni post hoc test with each pup being the statistical unit from 8 to 10 litter per dose group. #P ≤ .05; ##P ≤ .01. Results are depicted as mean ± SEM.

In a different study by the same group, the effects of APAP and aniline on female fertility were evaluated in mice by Holm et al. (2016). In this study, C57BL/6J mice were divided into five treatment groups that either received water control, acetaminophen, or aniline (Holm et al., 2016). Mice in the acetaminophen groups, either received 50 or 150 mg/kg/day of APAP (0.06x and 0.18x the maximum daily human dose of 4000 mg

based on a body surface area comparison). Mice in the aniline groups either received 31 or 93 mg/kg/day of aniline (which is converted into APAP as reported previously). The doses of APAP or aniline were dissolved in 0.5 mL of water. Females and males were initially caged and dosed together. Following determination of pregnancy, the males were separated from the females but were kept as sentinels for toxicity. To determine the effects of intrauterine exposure (Days 7 to 13.5 post-coitum) to APAP on fetal gonads, a separate set of C57BL/6J dams from the 50 mg/kg/day APAP oral gavage dose group was sacrificed on Day 13.5 post-coitum and the fetal gonads were dissected out and analyzed. To test female reproductive capacity after intrauterine exposure to APAP, eight female offspring from the control group were randomly selected and caged pairwise with eight female offspring from the 50 mg/kg/day APAP dose group. These female pairs were then caged and mated with a male C57BL/6J mouse that was not previously exposed to APAP. Thereafter, the dams were separated and pregnancies, time of birth, weight of pups, number of live and dead born pups, and lactating dams (with suckling pups) were recorded until 14 days after the last birth. Mating was performed twice at 6 months and 10 months of age. In another set of experiments, fetal gonad/mesonephros complexes from inbred C56BL/6 mice and outbred CD 1 mice were dissected out at Day 12.5 post-coitum and cultured for 3 days with and without APAP in an ex vivo culture system. The ex vivo culture system has not been validated for regulatory use and therefore the results of this assay will not be included in the label.

The AGDi was reduced in female off-spring in APAP or aniline treatment groups compared to control (see figures below, from Holm et al., 2016).

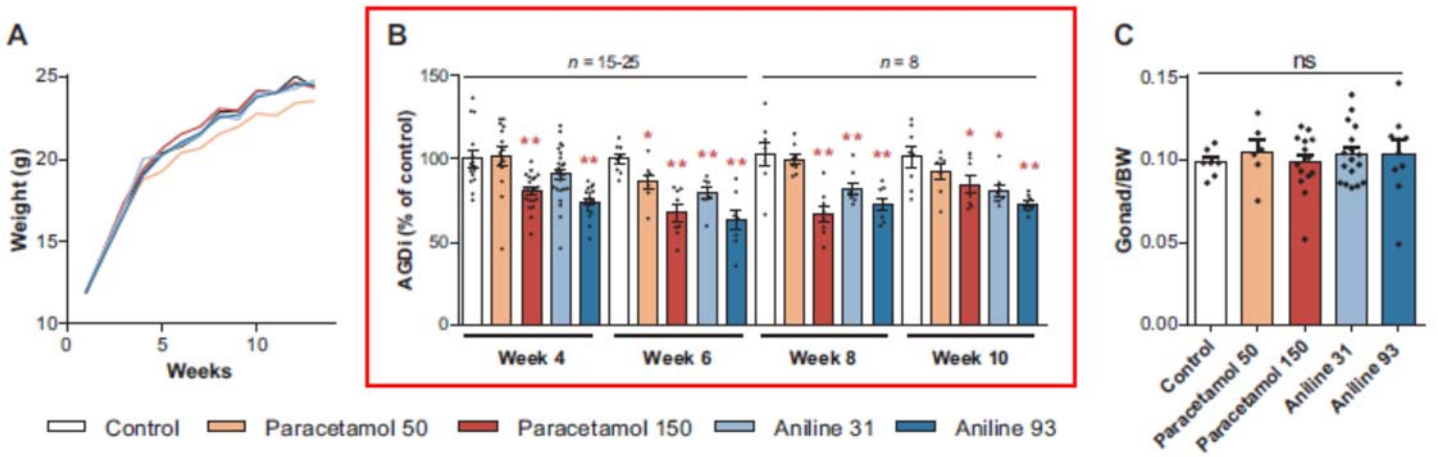

FIG. 1. Intrauterine exposure to aniline (31 and 93 mg/kg/day) or paracetamol (50 and 150 mg/kg/day) from 7 dpc to delivery reduced anogenital distance (AGD) in female offspring. A, Average weight of the female offspring from postnatal week 1 to 13. B, AGD index (AGDi) of the female offspring relative to postnatal growth and control group. C, Weight of gonads relative to whole body weight from mice at 6–7 weeks of age (n = 6–17). B and C, Results are depicted as mean ± SEM. Statistics were performed using one-way ANOVA followed by Holm-Sidak multiple comparison test. *$P \leq .05$ and **$P \leq .01$.

The follicle population was depleted after 7 weeks post-birth of female pups born to dams exposed to APAP (50 and 150 mg/kg/day) as shown in the figure below (from Holm et al., 2016).

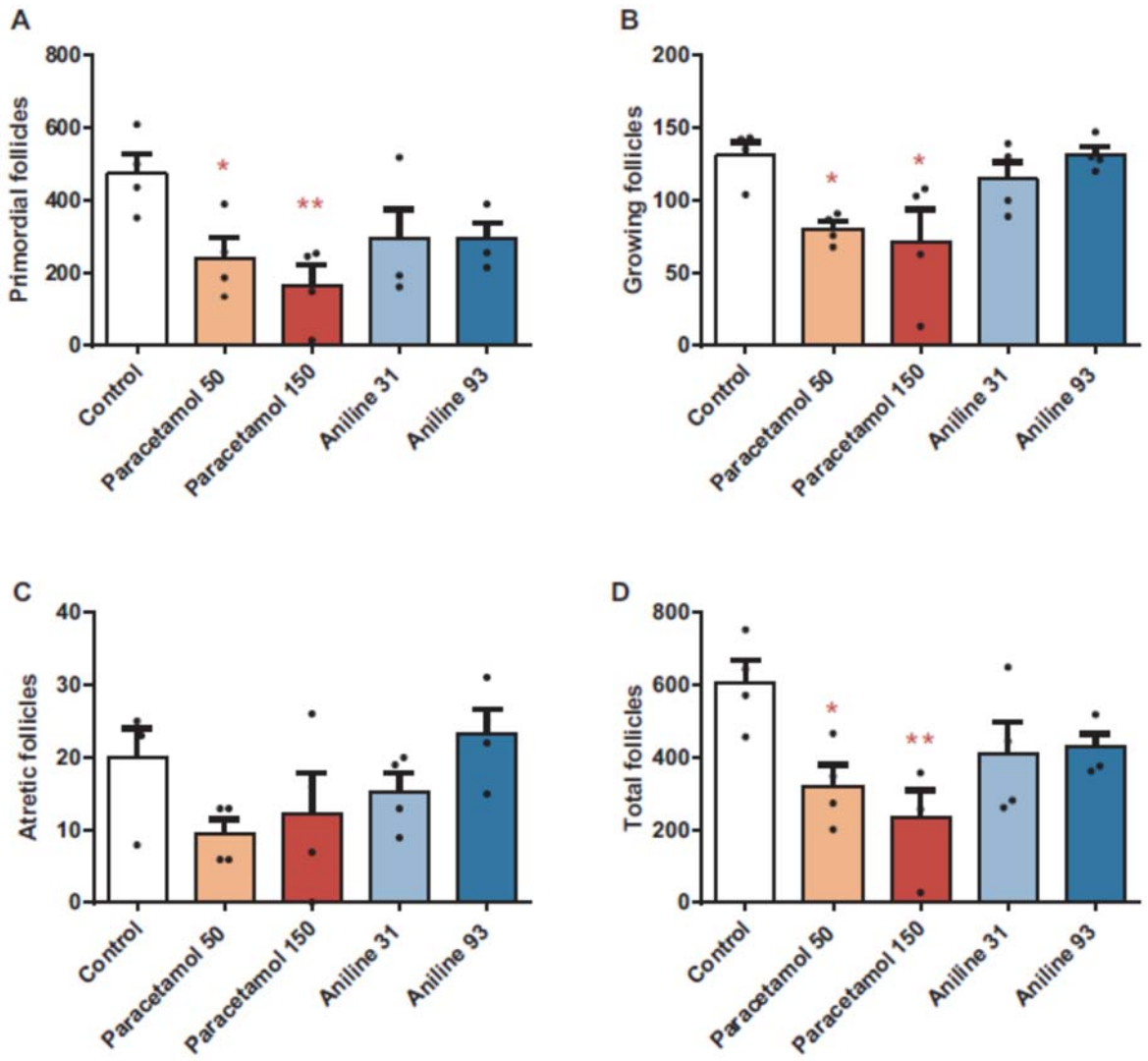

FIG. 3. Quantification of follicle populations illustrates depleted reserves after intrauterine exposure to paracetamol (50 and 150 mg/kg/day) or aniline (31 and 93 mg/kg/day). Data represent the mean number of (A) primordial (quiescent) germ cells, (B) growing follicles, (C) atretic follicles, and (D) total follicles per ovary ± SEM. The follicles were counted from four randomly picked ovaries from the five different groups after labeling of oocytes with Ybx2, examining every fifth sections. Follicle counts were analyzed using one-way ANOVA followed by Holm-Sidak multiple comparison test. *$P \leq .05$ and **$P \leq .01$.

Primordial follicles, growing follicles, atretic follicles, and total follicles were all significantly reduced in a dose-dependent manner in pups born to dams treated with APAP (see figure above).

Fertility of female mice exposed to APAP in utero was significantly reduced in terms of % of full term pregnancies as well as pups per dam at both the 6 month and 10-month time point in female rats (dams) dosed with 50 mg/kg/day of APAP (150 mg/m^2; see table below, from Holm et al., 2016).

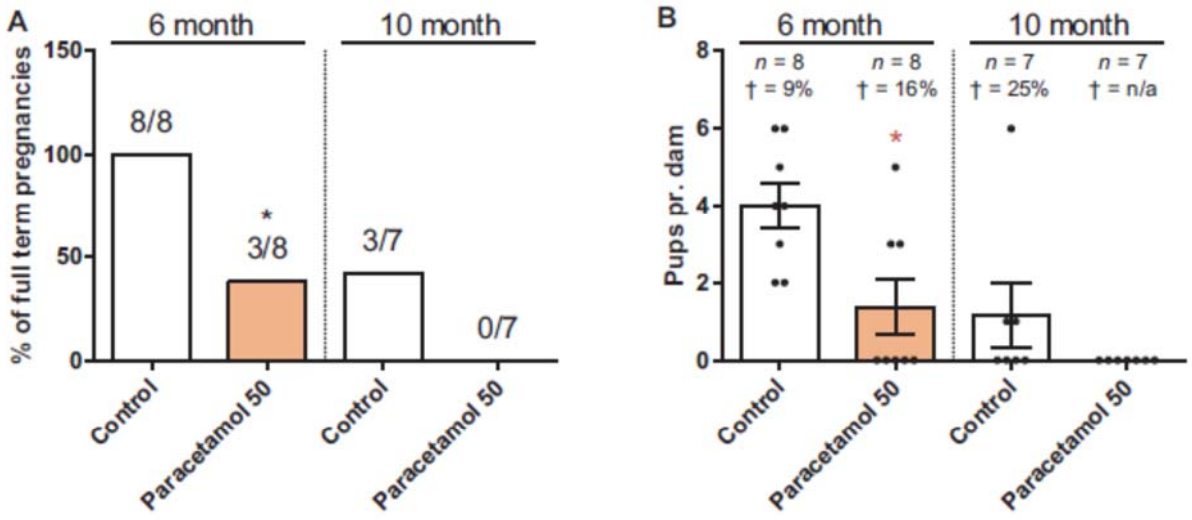

FIG. 4. Intrauterine exposure to paracetamol reduced fertility in mice. A, Percentage of full-term pregnancies with the fraction pregnant/total number of dams stated above the bars. B, Number of pups per dam mated with a male for 14 days at 6 and 10 months of age. † indicates percentage of pups found dead after birth. Results are depicted as mean ± SEM. Full-term pregnancies data were analyzed as a table of contingency using Fischer exact test, and number of pups per dam with two-way ANOVA followed by Holm-Sidak multiple comparison test. *$P \leq .05$.

There was significantly reduced expression of *Mvh*, a marker of fetal germ cells, in female pups born to dams exposed to APAP on Day 13.5 post-coitum.

It is noted that Holm et al., 2016 only dosed the dams using 2 doses of APAP (50 and 150 mg/kg/day) and a NOAEL for follicle depletion in the female pups or other measured endpoints was not identified. Although follicle depletion as well as the AGD and AGDi are not typical endpoints for fertility, the study does examine % of full term pregnancy and pups per dam, both of which were significantly reduced with APAP treatment. A reduction in the percentage of full term pregnancies and number of pups was reported from dosed dams exposed to APAP from Day 7 post-coitum to delivery at a dose that was 0.06x the maximum daily human dose of 4000 mg based on a body surface area comparison. These data suggest an adverse impact on female fertility following in utero exposure. These findings extend previous mouse male fertility findings, and the results of this study are appropriate to include in the label. In support of the findings from this paper (Holm et al., 2016), Dean et al. (2016) also investigated female fertility as a toxicological endpoint in a pre- and post-natal development study (see below).

Effects on Embryo-Fetal Development. Information in the published domain were reviewed to determine whether any results can be used to inform the Pregnancy section of acetaminophen product labeling (Lambert and Thorgeirsson, 1976, Lubawy and Garrett, 1977, Reel et al., 1992, Burdan, 2000, Laub et al., 2000, Neto et al., 2004). As a single entity oral prescription drug label for acetaminophen has not previously been approved by the Agency, it appears as though a Pregnancy Category for oral 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. 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 oral APAP on pregnancy.

The published nonclinical literature 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).

Lubawy 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 cannot 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).

Reference ID: 4190546

TABLE 2
Effect of Continuous Exposure to Acetaminophen on Postnatal Body Weights of F_1 Mice from the Final Litter of P Pairs (Task 4)

	% Acetaminophen in the diet			
Age (days)	0	0.25	0.5	1.0
	Body weight (g)			
Birth (0)[a]				
Male	1.64 ± 0.03 (37)	1.68 ± 0.04 (17)	1.69 ± 0.04 (16)	1.68 ± 0.07 (16)
Female	1.59 ± 0.03 (36)	1.61 ± 0.03 (17)	1.63 ± 0.04 (17)	1.61 ± 0.06 (17)
Weaning (28)[b]				
Male	17.25 ± 0.49 (42)	15.95 ± 0.54 (40)	14.38 ± 0.56 (45)*	11.37 ± 0.61 (24)*
Female	15.46 ± 0.43 (34)	13.88 ± 0.50 (32)*	13.62 ± 0.45 (33)*	11.08 ± 0.55 (33)*
Mating trial (74 ± 10.0)[b]				
Male	35.39 ± 0.74 (19)	33.38 ± 0.44 (20)*	32.49 ± 0.51 (20)*	28.92 ± 0.43 (20)*
Female	29.04 ± 0.70 (19)	26.04 ± 0.56 (20)*	26.28 ± 0.54 (20)*	24.23 ± 0.34 (20)*

[a] Values are mean pup weights per litter ± SEM (number of litters).
[b] Values are mean mouse weights per group ± SEM (number of mice).
* Significantly different ($p < 0.05$) from the control group.

Laub et al. administered APAP to female mice only during the first few days of gestation; therefore this study cannot 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).

Table 1. Occurence of skeletal variations (%) in foetuses of rats treated with paracetamol and in the control groups

	Control groups			Paracetamol-treated groups		
	T	K	CON	P1	P2	P3
Number of examined alizarin staining specimens	118	176	294	66	75	70
Nasal, reduced ossification	—	—	—	—	1 (1.33)	—
Frontal, reduced ossification	—	—	—	1 (1.51)	1 (1.33)	—
Parietal, reduced ossification	10 (8.47)	9 (5.11)	19 (6.46)	2 (3.03)	8 (10.66)	11 (15.71)
Interparietal, reduced ossification	12 (10.17)	10 (5.68)	22 (7.48)	7 (10.60)	11 (14.66)	13 (18.57)
Supraoccipital, missing	—	—	—	—	1 (1.33)	—
Supraoccipital, reduced ossification	5 (4.24)	5 (2.84)	10 (3.40)	2 (3.03)	3 (4.00)	4 (5.71)
Hyoid, missing	—	—	—	—	2 (2.66)	1 (1.43)
Hyoid, reduced ossification	1 (0.85)	1 (0.57)	2 (0.68)	—	—	2 (2.86)
13th rib, unilateral short	1 (0.85)	1 (0.57)	2 (0.68)	—	—	—
13th rib, wavy	1 (0.85)	6 (3.40)	7 (2.38)	2 (3.03)	1 (1.33)	—
Supernumerary rib, bud unilateral (L1)	—	1 (0.57)	1 (0.34)	1 (1.51)	—	3 (4.29)
Supernumerary rib, short unilateral (L1)	—	1 (0.57)	1 (0.34)	—	—	1 (1.43)
Supernumerary rib, short bilateral (L1)	—	—	—	—	—	1 (1.43)
Metacarpal, unilateral missing of one bone	2 (1.70)	1 (0.57)	3 (1.02)	—	—	—
Metacarpal, bilateral missing of one bone	2 (1.70)	2 (1.14)	4 (1.36)	1 (1.51)	2 (2.66)	7 (10.00)
Metacarpal, unilateral missing of two bones	—	—	—	—	—	1 (1.43)
Metatarsal, unilateral missing of two bones	4 (3.40)	6 (3.40)	10 (3.40)	3 (4.54)	3 (4.00)	8 (11.43)

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).

Table 1. Tested parameters (Mean ± Standard Deviation/litter) in the common control (CON) and acetaminophen-treated (P) groups

	CON	P1	P2	P3
Fetal weight (g)	3.80 ±0.42	3.83 ± 0.24	3.67 ± 0.39	3.46 ± 0.27**
Fetal length (mm)	38.55 ± 1.09	38.10 ± 0.91	37.62 ± 1.50	37.53 ± 0.99*
Tail length (mm)	11.85 ± 0.52	11.82 ± 0.42	11,55 ± 0.43	11.60 ± 0.29
Placental weight (g)	0.59 ± 0.06	0.57 ± 0.04	0.62 ± 0.05	0.61 ± 0.04
Number of luteum corpuscle	15.13 ± 0.43	14.62 ± 1.59	15.87 ± 2.47	15.14 ± 2.96
Number of fetuses	14.31 ± 2.25	13.87 ± 2.23	14.37 ± 4.13	13.57 ± 3.50
Number of resorptions	0,55 ± 0.68	0.50 ± 1.06	1.12 ± 1.12	1.14 ± 1.77
Preimplantation mortality	2.08 ± 4.37	1.85 ± 3.27	3.16 ± 6.14	3.70 ± 6.95
Postimplantation mortality	3.40 ± 4.19	3.62 ± 7.61	9.13 ± 10.58	7.30 ± 10.29
Number of ecchymosis	0.20 ± 0.41	0.12 ± 0.35	0.50 ± 0.53	0.28 ± 0.48

* Differ significantly from the common control value, ** Differ significantly from group P1.

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). 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).

Effects on Pre- and Postnatal Development. Pre- and postnatal developmental data have been reported by Reel et al.; however, this study did not include comparable endpoints as a dedicated pre- and postnatal development study. Liver and kidney findings were reported by Neto et al. as described above (Neto et al., 2004). As the effects reported in Neto et al. represent adverse effects on the fetus, they should be included in the product labeling, unless superseded by adequate human data.

Blecharz-Klin et al. studied the levels of neurotransmitters in various regions of the nervous system of 2-month old male rats born to mothers that were exposed to APAP in the drinking water at doses of 5 and 15 mg/kg/day (0.012x and 0.036x the maximum daily human dose of 4000 mg based on a body surface area comparison) during pregnancy and lactation (Blecharz-Klin et al., 2015b, a, Blecharz-Klin et al., 2016). Immediately following birth, these male rat pups stayed with their mother for 28 days until weaning and then were exposed to APAP in the drinking water for an additional 28 days. The dosing duration of this study represents the dose duration of a typical pre- and postnatal development study. The following tables illustrate the changes in the level of monoamines and their metabolites and changes in amino acids levels in the medulla oblongata, cerebellum, and spinal cord:

Medulla Oblongata (Blecharz-Klin et al., 2015a):

Table 1
Effect of prenatal and early postnatal paracetamol administration on the level of monoamines and their metabolites (mean ± SE) in the medulla oblongata of rat pups.

Monoamine and metabolite levels in the medulla oblongata ng/g wet tissue (mean ± SE)			
	Con (n = 10)	P5 (n = 10)	P15 (n = 10)
NA	1523.51 ± 72.40	**1183.62 ± 40.97*****	**884.74 ± 68.43**$^{***,\#\#\#}$
MHPG	647.85 ± 61.38	591.33 ± 38.33	**749.43 ± 54.60**■
MHPG/NA	0.43 ± 0.04	0.51 ± 0.03	**0.89 ± 0.08**$^{***,\#\#\#}$
DA	90.80 ± 4.21	79.52 ± 6.42	**67.31 ± 2.58****
DOPAC	351.92 ± 19.02	335.40 ± 29.72	353.10 ± 19.71
DOPAC/DA	4.60 ± 0.52	4.91 ± 0.63	5.36 ± 0.45
HVA	666.91 ± 45.67	**516.82 ± 56.76***	**437.03 ± 21.31*****
HVA/DA	8.56 ± 0.97	7.63 ± 1.14	6.61 ± 0.49
5-HT	432.12 ± 49.90	393.90 ± 17.57	**701.69 ± 80.34**$^{***,\#\#\#}$
5-HIAA	183.76 ± 16.24	178.87 ± 11.52	182.67 ± 9.38
5-HIAA/5-HT	0.48 ± 0.07	0.45 ± 0.02	**0.31 ± 0.05**●

Bold font indicate a significant difference versus controls.
* P5, P15 *vs* Control, $p < 0.05$ (Newman–Keuls).
** P15 *vs* Control $p < 0.01$ (Newman–Keuls).
*** P5, P15 *vs* Control, $p < 0.005$ (Newman–Keuls).
$^{\#\#\#}$ P5 *vs* P15, $p < 0.005$ (Newman–Keuls).
● P15 *vs* Control, $p < 0.05$ (Fisher's Exact Test).
■ P5 *vs* P15 $p < 0.05$ (Fisher's Exact Test).

Table 2
Effect of prenatal and early postnatal paracetamol administration on the level of amino acids (mean ± SE) in the medulla oblongata of the rat pups.

Amino acid levels in the medulla oblongata ng/mg wet tissue (mean ± SE)			
	Con (n = 10)	P5 (n = 10)	P15 (n = 10)
Glutamic acid	1008.45 ± 25.45	967.60 ± 18.42	953.94 ± 17.60
Taurine	222.52 ± 6.05	**207.77 ± 4.57***	210.13 ± 3.72
Alanine	209.52 ± 5.96	**193.32 ± 3.06•**	193.23 ± 5.15
γ-Aminobutyric acid	287.92 ± 10.77	286.33 ± 6.67	285.84 ± 7.29
Aspartic acid	438.36 ± 14.34	415.69 ± 8.03	431.59 ± 21.87
Histidine	7.19 ± 0.30	6.98 ± 0.13	6.78 ± 0.30

Bold font indicate a significant difference versus controls.
* P5 *vs* Control $p < 0.05$ (Newman–Keuls).
• P5 *vs* Control $p < 0.05$ (Fisher's Exact Test).

At 5 mg/kg/day, the levels of noradrenaline and homovanillic acid were significantly decreased compared to control. At 15 mg/kg/day, the levels of noradrenaline (NA), dopamine (DA), homovanillic acid (HVA), and 5-hydroxyindoloacetic acid/5-hydroxytryptamine (5-HIAA/5-HT) ratio were significantly decreased compared to control. Moreover, the levels of 3-methoxy-4-hydroxyphenylglycol (MHPG), the MHPG/NA ratio, and 5-hydroxytryptamine (5-HT) were significantly increased compared to control. The decreases in noradrenaline and homovanillic acid were dose-dependent. At 5 mg/kg/day, the levels of taurine and alanine were significantly decreased compared to control. There were no changes at 15 mg/kg/day dose group making the changes in taurine and alanine not dose-dependent.

Cerebellum (Blecharz-Klin et al., 2016):

Table 1
Effect of the prenatal and postnatal paracetamol administration on the level of monoamines and their metabolites (mean ± SE) in the cerebellum of rat pups.

Monoamine and metabolite levels in the cerebellum ng/g wet tissue (mean ± SE)				
	Con	P5	P15	F value
NA	400.47 ± 39.50	463.69 ± 19.50	451.48 ± 12.57	$F_{(2,27)} = 1.61$
MHPG	**48.47 ± 6.37**	46.47 ± 2.91	**62.54±4.11***	$F_{(2,27)} = 3.49$
MHPG/NA	0.12 ± 0.01	**0.10±0.01**	**0.14±0.01•**	$F_{(2,27)} = 4.14$
DA	26.42 ± 2.84	27.30 ± 1.18	30.89 ± 1.67	$F_{(2,27)} = 1.38$
DOPAC	6.06 ± 0.81	8.15 ± 1.29	9.86 ± 1.13	$F_{(2,27)} = 3.02$
DOPAC/DA	0.35 ± 0.15	0.30 ± 0.04	0.33 ± 0.04	$F_{(2,27)} = 0.10$
HVA	11.87 ± 2.36	16.32 ± 1.77	17.18 ± 2.21	$F_{(2,27)} = 1.80$
HVA/DA	0.51 ± 0.10	0.60 ± 0.06	0.56 ± 0.07	$F_{(2,27)} = 0.32$
5-HT	30.03 ± 5.40	47.90 ± 6.05	34.61 ± 4.18	$F_{(2,27)} = 3.11$
5-HIAA	**29.09±3.84**	**49.68±7.28***	**28.37±4.40•**	$F_{(2,27)} = 5.05$
5-HIAA/5-HT	1.20 ± 0.19	1.02 ± 0.08	0.83 ± 0.10	$F_{(2,27)} = 1.89$

* P5, P15 *vs.* Con, $p < 0.05$ (Newman-Keuls).
• P5 *vs.* P15, $p < 0.05$ (Newman-Keuls).

Table 2
Effect of the prenatal and postnatal paracetamol administration on the level of amino acids (mean ± SE) in cerebellum of the rat pups.

Amino acid levels in the cerebellum ng/mg wet tissue (mean ± SE)				
	Con	P5	P15	F value
Glutamic acid	1728.10 ± 80.28	1773.71 ± 37.72	1891.43 ± 39.62	$F_{(2,27)} = 2.26$
Taurine	765.94 ± 25.60	762.04 ± 12.85	804.93 ± 10.94	$F_{(2,27)} = 1.83$
Alanine	315.55 ± 13.55	**299.65 ± 11.86**	**344.29 ± 7.82•**	$F_{(2,27)} = 3.99$
γ-Aminobutyric acid	302.95 ± 14.50	322.55 ± 8.12	330.10 ± 9.68	$F_{(2,27)} = 1.60$
Aspartic acid	278.61 ± 18.76	294.50 ± 10.23	326.10 ± 11.20	$F_{(2,27)} = 3.02$
Histidine	10.89 ± 0.81	12.11 ± 0.31	11.58 ± 0.54	$F_{(2,27)} = 1.07$

• P5 *vs.* P15, $p < 0.05$ (Newman-Keuls).

At 15 mg/kg/day, there was an increase in the levels of 3-methoxy-4-hydroxyphenylglycol (MHPG). All other changes were not dose-dependent and were not different compared to control.

Spinal Cord (Blecharz-Klin et al., 2015b):

Monoamine and metabolite levels in the spinal cord ng/g tissue (mean ± SE)

	Con	P5	P15
NA	304.148 ± 21.92	283.105 ± 25.78	268.627 ± 11.47
MHPG	21.714 ± 1.68	15.914 ± 1.79•	16.755 ± 1.45*
MHPG/NA	0.073 ± 0.01	0.060 ± 0.01	0.063 ± 0.01
DA	42.488 ± 3.21	30.239 ± 3.08*	31.635 ± 1.49**
DOPAC	5.494 ± 1.51	5.699 ± 1.36	3.870 ± 0.50
DOPAC/DA	0.140 ± 0.05	0.240 ± 0.08	0.123 ± 0.02
HVA	30.898 ± 4.00	18.545 ± 3.02*	25.721 ± 3.06
HVA/DA	0.726 ± 0.07	0.706 ± 0.15	0.812 ± 0.08
3-MT	8.449 ± 1.97	5.099 ± 1.47	3.196 ± 1.04•
3-MT/DA	0.197 ± 0.04	0.195 ± 0.06	0.099 ± 0.03
5-HT	196.771 ± 24.88	208.496 ± 35.44	236.661 ± 11.74
5-HIAA	87.666 ± 9.16	85.905 ± 14.43	107.494 ± 5.59
5-HIAA/5-HT	0.456 ± 0.02	0.415 ± 0.04	0.454 ± 0.01

* P5, P15 vs control, $p < 0.05$ (Newman–Keuls).
** P15 vs control, $p < 0.01$ (Newman–Keuls).
• P5, P15 vs control, $p < 0.05$ (Fisher's Exact Test).

Amino acid levels in the spinal cord ng/mg tissue (mean ± SE)

	Con	P5	P15
Glutamic acid	1280.81 ± 84.92	1399.32 ± 181.53	1700.87 ± 118.00•
Taurine	282.25 ± 14.48	251.10 ± 32.28	315.54 ± 28.29
Alanine	433.60 ± 36.94	417.54 ± 46.10	509.54 ± 41.09
γ-Aminobutyric acid	364.44 ± 23.49	366.34 ± 40.17	402.57 ± 32.93
Aspartic acid	397.24 ± 43.68	512.28 ± 54.97	686.84 ± 52.68***,#
Histidine	16.65 ± 1.45	15.17 ± 2.04	18.96 ± 1.08

*** P15 vs control, $p < 0.005$ (Newman–Keuls).
# P5 vs P15, $p < 0.05$ (Newman–Keuls).
• P15 vs control, $p < 0.05$ (Fisher's Exact Test).

There were significant dose-dependent decreases in the levels of 3-methoxy-4-hydroxyphenylglycol (MHPG) and 3-methoxytyramine (3-MT) in the 5 and 15 mg/kg/day dose groups compared to control. The levels of dopamine (DA) were significantly decreased in both the 5 and 15 mg/kg/day dose groups; however, the decreases were not dose-dependent. At 15 mg/kg/day, there were significant increases in the levels of glutamic acid and aspartic acid.

These studies did not utilize a typical pre- and postnatal development study design as the typical reproductive and developmental endpoints were not evaluated. Moreover, the F_1 pups did not undergo behavioral or functional assessments to determine the biological significance of these biochemical changes in the medulla oblongata, cerebellum, and spinal cord. In fact, the researchers do not clearly understand the significance of these changes in the cerebellum, medulla oblongata, and spinal cord; however, they acknowledge that these changes may play a role in developmental and neurological disorders such as ADHD. Thus, the significance of these changes in neurotransmitter levels is unknown and therefore do not warrant inclusion in the label at this time.

Dean et al. (2016) studied exposure to APAP during pregnancy in the F_0 dams and its effects on germ cell development and reproductive function in the F_1 and F_2 generation using Wistar rats (Dean et al., 2016). Wistar rats were dosed with 350 mg/kg/day APAP (0.85x the maximum daily dose of 4000 mg based on a body surface area comparison) via oral gavage as a suspension in corn oil. Timed-matings were established by the presence of a vaginal plug and defined as embryonic day 0.5 (e0.5). Pregnant dams were dosed with 350 mg/kg/day APAP on e13.5 (embryonic day or gestation day) to e21.5 to encompass dosing during the masculinization programming window of e15.5 to e18.5. Control and APAP dams were sacrificed on e15.5, e16.5, e17.5, e18.5, e21.5, or allowed to give birth. The resulting offspring were then sacrificed on either postnatal (PND) 25, which represents early puberty, or PND 90, which represents adulthood. Fetuses sacrificed on e21.5 were weighed and the gonads were dissected out. Post-natal pups were weighed, sacrificed, and their gonads were dissected out. Female adult rats (F_1 generation) that had been exposed in utero to APAP were each placed with an untreated control male for 4 days to allow mating and the number of pups per litter was counted on the day of birth. Male adult rats (F_1 generation) that had been exposed in utero to APAP, each of which was paired with an untreated control female for 4 days and the number of pups per litter was counted on the day of birth. The resulting F_2 offspring were studied at PND 25 (puberty) or PND 90 (adulthood) with the focus on the female F_2 offspring as the male F_2 offspring did not exhibit any obvious reproductive phenotypic change. Fetal gonads from pups who were exposed to APAP in utero were observed with reduced expression of *Ep2* (PGE_2 receptor) mRNA and PGE_2 levels but the expression of *Cox2* (cyclooxygenase) mRNA was not reduced (see below, from Dean et al., 2016).

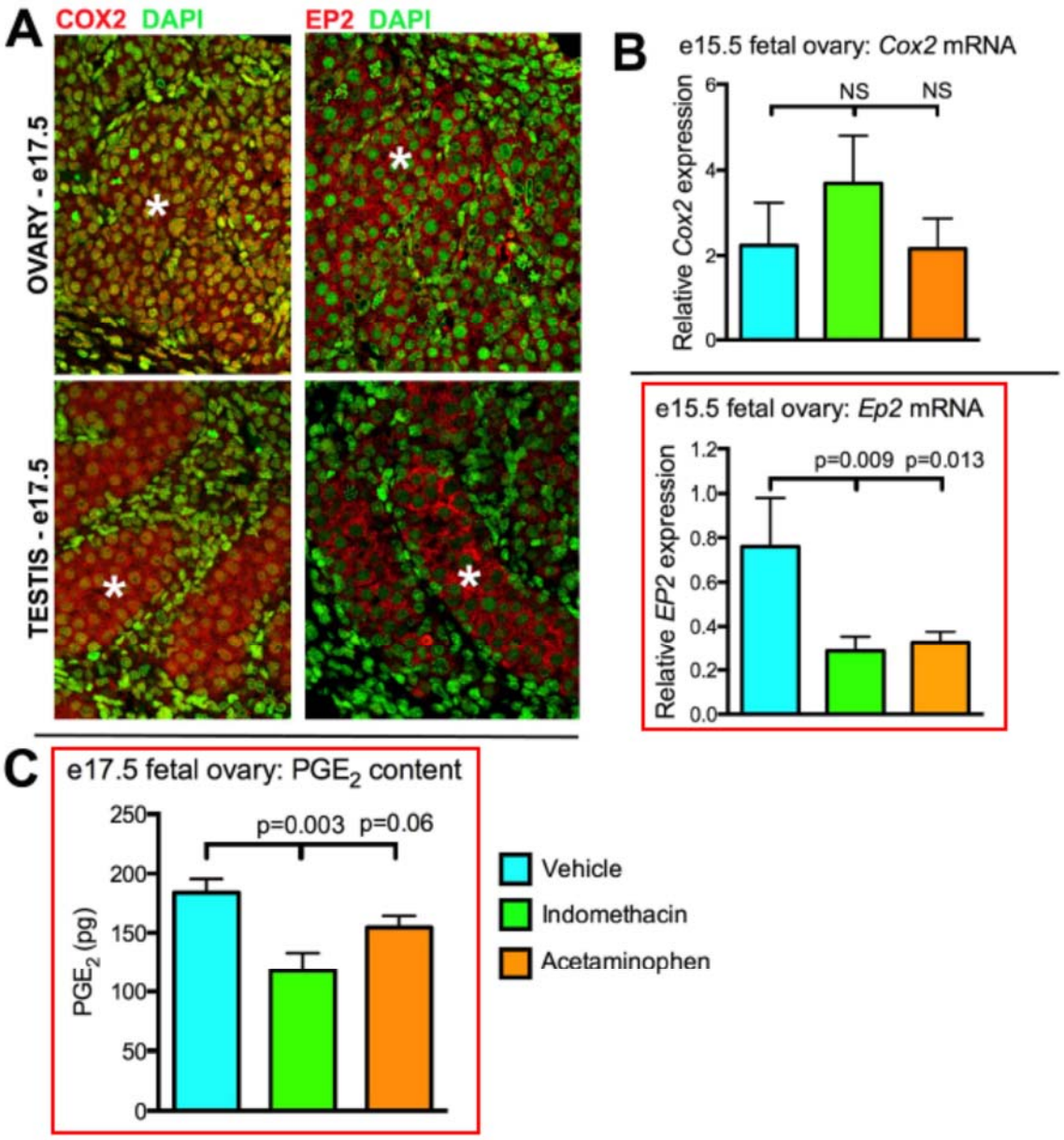

Figure 1. The fetal rat gonads as a source and target for prostaglandins (PGs). (A) Immunoexpression of cyclo-oxygenase-2 (COX2) and PGE_2 receptors (EP2) in germ cells (asterisks) of the fetal (e17.5) rat ovary and testis. (B) The effect of exposure to indomethacin or acetaminophen on *Cox2* and *Ep2* mRNA expression at e15.5 in the F1 fetal ovary (Values are Means ± SEM for n = 6–9). (C) Effect of analgesic exposure on F1 fetal rat ovarian PGE_2 content 3h after a single administration of vehicle or analgesic on e17.5 (Means ± SEM for n = 5).

The researchers argue that germ cells express COX2 and prostaglandin receptors such as EP2 and therefore, are potential targets for prostaglandins (PG), which demonstrates that COX2 and PG receptors might play a common and conserved role in fetal germ cell development. APAP has been shown to alter prostaglandin pathways and is commonly used during pregnancy. Intra-uterine exposure to APAP reduced the number of germ cells and weight of the ovary in adult females (see below, from Dean et al., 2016). Adult female fertility was reduced slightly as well.

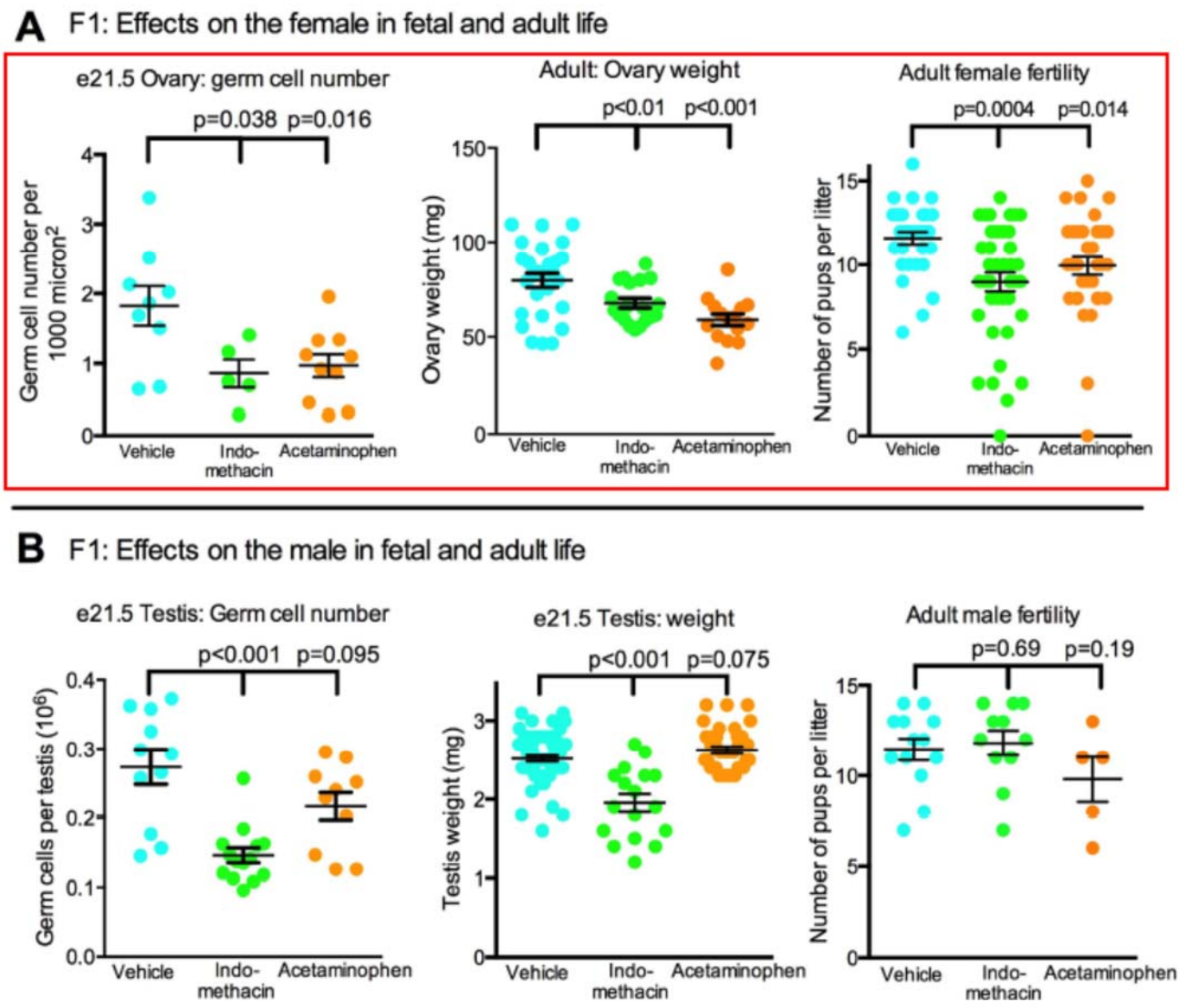

Figure 2. Effect of fetal exposure to analgesics on (top) ovarian development and fertility in F1 female rats, and (bottom) corresponding changes in F1 males. (A) Germ cell number at e21.5 (n = 5–10 animals per group), adult ovarian weight (n = 15–27 animals per group) and fertility after mating with normal untreated stud males (n = 30–36 animals per group). (B) Germ cell number at e21.5 in the testis (n = 10–14 animals per group), together with testis weight at the same age (n = 17–64 animals per group) and fertility after mating with normal untreated adult females (n = 5–13 animals per group). Black horizontal bars show Means ± SEM. Note that controls used for indomethacin and acetaminophen studies were pooled for analysis as they did not differ significantly for any of the measured parameters. In each group, animals were from 4–11 different litters.

In the female ovary, the developmental pathway for germ cells is to switch off pluripotency factors and enter meiosis. The researchers monitored meiosis in F_1 female fetuses by measuring expression of DMRT1, which is germ cell specific after e15.5 in rats, and as such, germ cell loss of DMRT1 expression is an index of completion of meiotic entry. The markers of meiotic entry are *Dmrt1* and *Stra8*. The pluripotency marker *Lin28* was used, expression on which is lost in ovarian germ cells in rats and

humans prior to meiotic entry. There was increased expression of *Dmrt1* and *Stra8* mRNA on e18.5 compared to no statistical differences on e17.5. Expression of *Lin28* mRNA was increased on e18.5 compared to no statistical differences on e17.5. Male fetal germ cells do no enter meiosis but undergo a differentiation step when pluripotency markers (such as OCT4) are switched off at approximately e15.5 to e19.5 in rats (see below, from Dean et al., 2016). The expression of OCT4 was monitored in the male gonads from pups born to dams exposed to APAP. The expression of OCT4 was significantly reduced on e15.5, e16.5, and e17.5 compared to age-matched controls (see below, from Dean et al., 2016).

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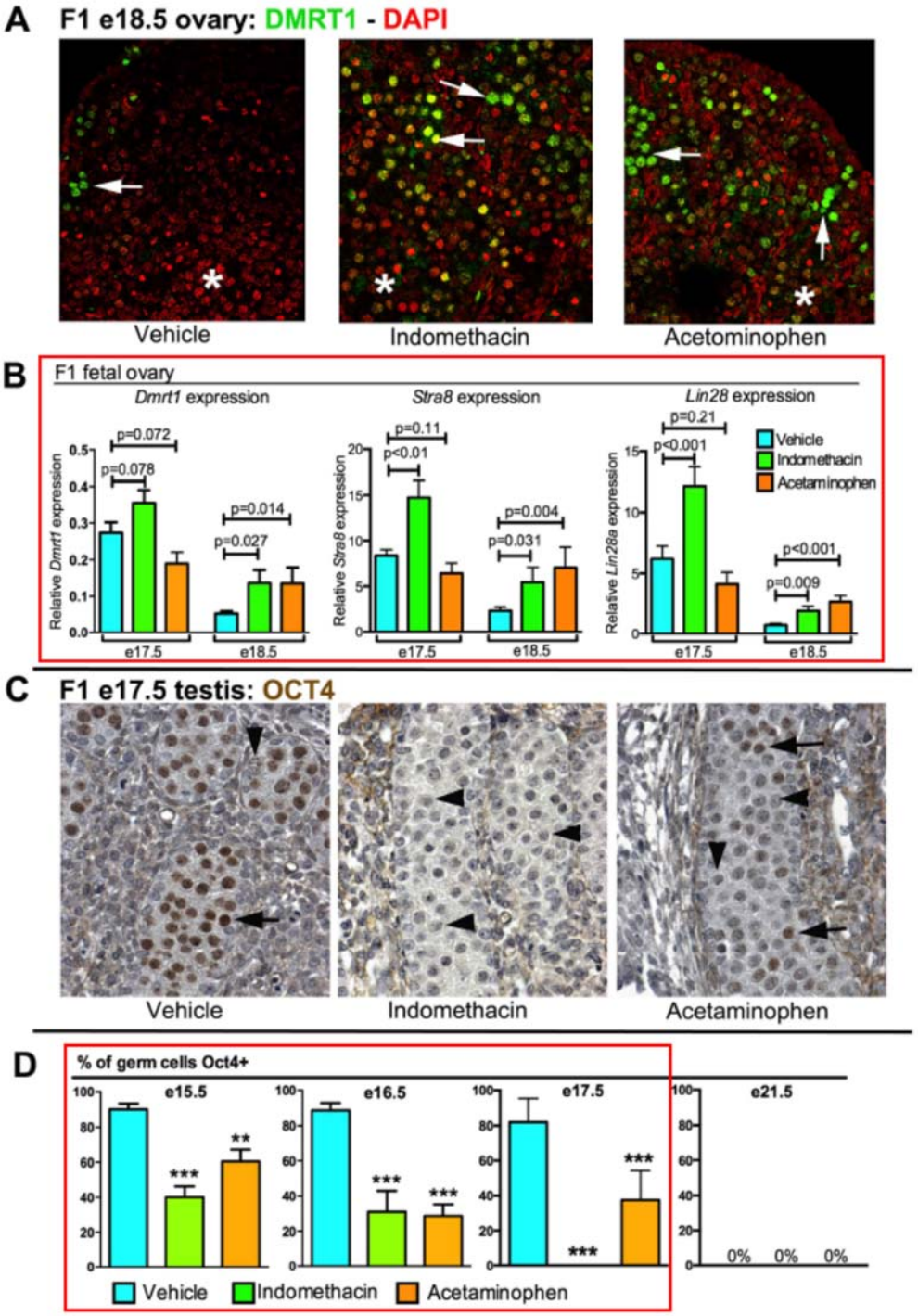

Figure 3. Effect of fetal analgesic exposure on the tempo of fetal germ cell development in the F1 fetal ovary (A,B) and testis (C,D). (A) Representative immunoexpression results for DMRT1 (green) in e18.5 ovaries from fetuses exposed to vehicle or to analgesic, highlighting regional differences in the proportion of oocytes still expressing DMRT1 (green) and those in which DMRT1 has been switched off (asterisks). (B) Evidence for delayed oocyte development in analgesic-exposed fetuses based on the temporal change in expression of *Dmrt1*, *Stra8* and *Lin28* mRNA expression at e17.5 and e18.5 (Means ± SEM for 9–18 animals per group from 3–5 different litters). (C) Germ cell-specific nuclear expression of OCT4 (brown; black arrows) in the fetal testis was reduced substantially by exposure to analgesic with all (indomethacin) or most (acetaminophen) germ cells prematurely losing expression of OCT4 by e17.5, unlike in controls. (D) The proportion of germ cells expressing OCT4 was quantified at e15.5, e16.5, e17.5 and e21.5 for 5–6 animals per group at each age from a minimum of 5 litters (Means ± SEM). $^{**}p < 0.01$, $^{***}p < 0.001$, in comparison with respective control group.

Analgesic exposure caused advanced differentiation of fetal germ cell development in males and an opposite effect in females. The F_2 female offspring whose F_1 parent was exposed to APAP in utero showed a significant reduction in ovary weight at PND25 as well as in adulthood. Ovarian follicle numbers in F_2 female offspring whose F_1 parent was exposed to APAP in utero were also investigated at PND25. There was a significant reduction in primordial follicles and total follicles (see below, from Dean et al., 2016).

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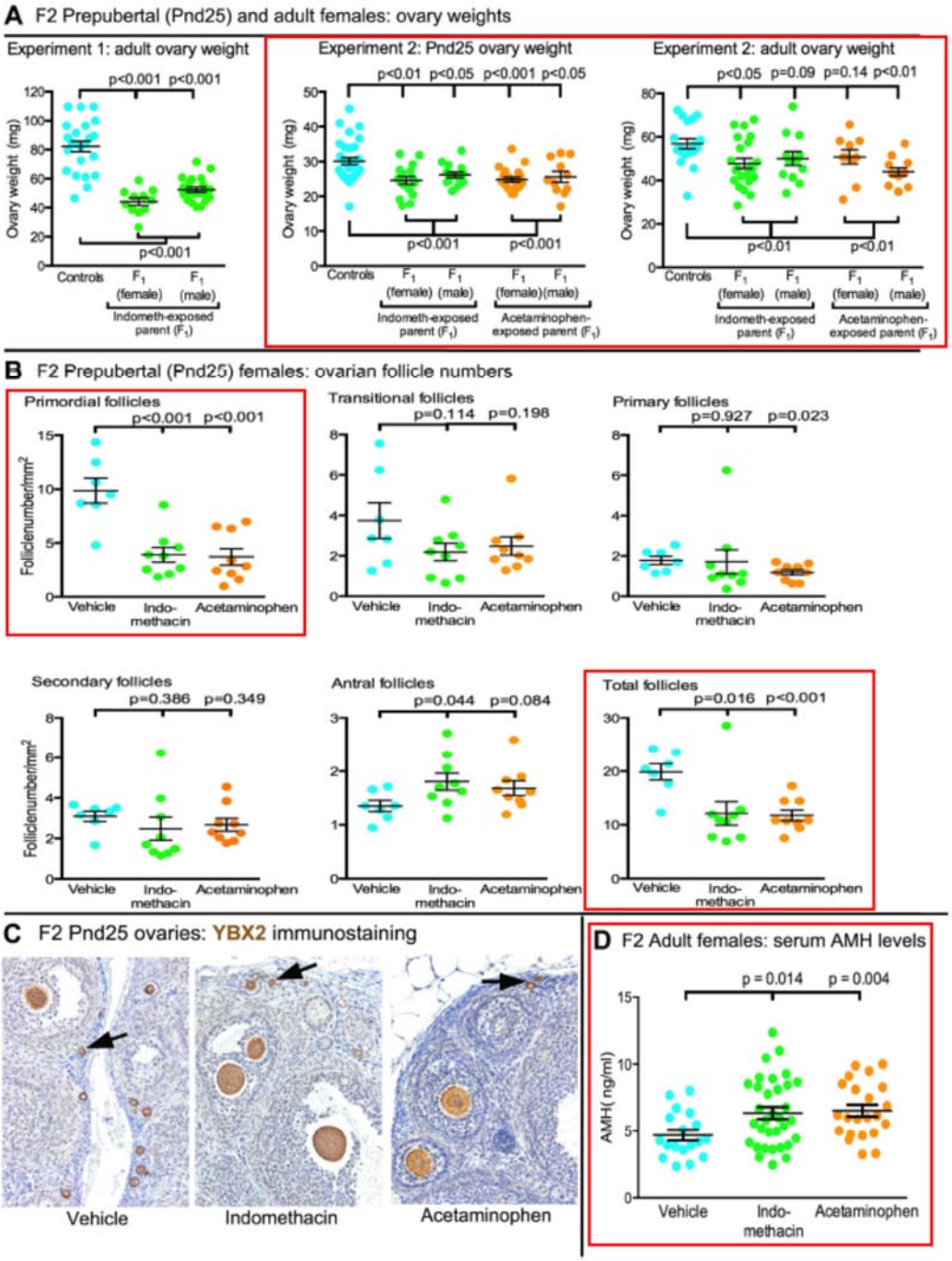

Figure 5. Effect of fetal exposure of the F1 generation to analgesics on ovarian size and function in second generation (F2) females. A Ovarian weights at postnatal day 25 (Pnd25) or adulthood were reduced overall in F2 females of analgesic-exposed parents, an effect that was generally evident irrespective of which F1 parent had been exposed to analgesic in utero. B Follicle classification and counts in the ovaries of representative prepubertal females from experiment 2 (panel A) based on immunostaining of oocytes for YBX2 (panel C). Follicle counts revealed a highly significant decrease in primordial follicle number (arrowed in panel C) in Pnd25 females derived from an F1 parent exposed in utero to analgesic and there was a trend towards increased numbers of antral follicles in the same ovaries (data derived from equal numbers of paternal and maternal 'treatment exposed' parents). D Serum AMH levels in adult F2 females (N=18–33 per group). Black horizontal bars show Means ± SEM. Mated F1 animals derived from 7 separate litters whilst data for F2 animals derived from 5-6 litters.

There was also an increase in serum AMH levels in adult F_2 females whose F_1 parent was exposed to APAP in utero. AMH is anti-Müllerian hormone and is produced in large preantral/small antral follicles. The increased serum levels in AMH are thought to explain the slight increase in antral follicles at PND25.

The results from the study by Dean et al., 2016 describe data on developmental fate of germs cells in the ovary and testis as well as prostaglandin mRNA expression and follicle population in the ovary, which are endpoints that are not the typical endpoints of GLP reproductive and development toxicology studies. Although these atypical endpoints were used, the study did use typical endpoints such as ovary weight and number of pups per litter. The study did demonstrate decreases in the ovary weight and the number of pups per litter in the F_1 females born to dams that were exposed to APAP. The decrease in ovary weight persisted to the F_2 females. Interestingly, the fetal weights of the testis in F_1 males were not different compared to control and male fertility in adults was slightly decreased but not statistically significant. The male findings may be due to lower doses and the shorter dosing period compared to the continuous breeding study in the existing label. The results from the Dean et al., 2016 study as well as the Holm et al., 2016 study that describe the impact of acetaminophen on female fertility are relevant and warrant inclusion in the label.

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Appendix 2: Nonclinical Recommendations for Labeling for Ibuprofen

This memo documents the labeling recommendations for ibuprofen, which are based upon the Agency's review of the literature. Several studies from the scientific literature were reviewed to inform Section 8, pregnancy (teratogenic and nonteratogenic effects) and Section 13, animal studies including mutagenesis, carcinogenesis, and impairment of fertility. For this appendix, the MDD was considered to be 800 mg ibuprofen 4 times a day (3200 mg/day).

Genetic Toxicity Studies:

Publication: Mutagenicity Testing of Selected Analgesics in Ames *Salmonella* Strains (Oldham et. al, 1986)

Methods:

Strains:	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA 1538
Concentrations in definitive study:	1, 10, 100, 500, 750, and 1000 mcg/plate
Basis of concentration selection:	The 5000 mcg/plate dose resulted in toxicity in all strains.
Negative control:	DMSO
Positive control:	Without S9 activation: Dexon (200 mcg, TA98 and TA1538); 2-aminoantracene (50 mcg, TA1537); sodium azide (0.5 mcg, TA100 and TA1535); With S9 activation: 2-aminoanthracene (2 mcg; all strains)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Ibuprofen in vehicle (DMSO), vehicle alone (negative control), or positive control was pre-incubated with the bacteria with and without S9 mix in the top agar. The solution was mixed and overlaid onto a minimal bottom agar. After the overlay solidified, the plates were inverted and incubated at 37˚C for 46 to 50 hrs. Afterwards, the plates were scored. All concentrations and controls (both positive and negative) in this toxicity-mutation assay were plated in duplicate. There was no confirmatory test performed.

Additonal Methods/Validity:

Ibuprofen was obtained from (b) (4) with no purity information given. The study was conducted at (b) (4) Up to 1000 mcg/plate of ibuprofen was tested as higher doses caused toxicity in the strains tested. The strains tested were *Salmonella typhimurium* strains TA98, TA100, TA1535,

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TA1537, and TA1538 (Kamber et al., 2009 and McCann et al., 1975). TA100, and TA1535 were used to detect base pair substitutions; however, these were C:G and not A:T base pair substitutions. TA98, TA1537, and TA1538 were used to detect frameshift mutations (Kamber et al., 2009 and McCann et al., 1975). The positive controls used in the study were appropriate as they caused reliable positive results. This study is appropriate to test frameshift mutations as well as C:G base pair substitutions; however, this study did not test A:T base pair substitutions.

Results:
The following tables illustrate the results with strains TA98, TA100, TA1535, TA1537, and TA1538 with and without S9 metabolic activation treated with ibuprofen (adapted

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from the publication):

Table 1. Mutagenicity testing of acetaminophen, aspirin, phenacetin and ibuprofen in the Ames test with no metabolic activation

	Mean revertant counts[a]				
Treatment[b]	TA98	TA100	TA1535	TA1537	TA1538
Negative control					
50.0 μl DMSO	25 (6)	146 (8)	11 (2)	9 (3)	17 (2)
IB (μg)					
1.0	30 (2)	112 (13)	13 (6)	10 (1)	16 (1)
10.0	22 (7)	140 (10)	8 (0)	6 (0)	17 (11)
100.0	25 (1)	137 (7)	10 (5)	8 (5)	19 (3)
500.0	11 (3)	34[c]	4 (3)	2 (1)	12 (6)
750.0	0	40 (4)	3 (1)	0	2[c]
1000.0	0	0	0	0	0
Positive indicators					
50.0 μg 9-AA·HCl	—	—	—	236 (25)[d]	—
0.5 μg Na Azide	—	405 (3)[d]	272 (10)[d]	—	—
200.0 μg Dexon	1882 (86)[d]	—	—	—	537 (21)[d]

[a] Negative control values represent the mean colony counts on six plates. All other treatment values are the mean from two plates. Standard deviations are in parentheses.
[b] DMSO, dimethylsulfoxide; APAP, acetaminophen; ASA, aspirin; PA, phenacetin; IB, ibuprofen; 9-AA·HCL = 9-aminoacridine·HCl.
[c] No revertant colonies present on one plate due to toxicity.
[d] Represents at least a doubling of negative control values.

Table 2. Mutagenicity testing of acetaminophen, aspirin, phenacetin and ibuprofen in the Ames test with activation by Aroclor 1254-induced rat liver S-9

	Mean revertant counts[a]				
Treatment[b]	TA98	TA100	TA1535	TA1537	TA1538
Negative control					
50.0 μl DMSO	46 (8)	129 (6)	16 (3)	11 (3)	26 (3)
IB (μg)					
1.0	41 (11)	140 (9)	18 (1)	8 (0)	29 (4)
10.0	41 (18)	133 (11)	17 (4)	10 (6)	28 (8)
100.0	38 (0)	141 (24)	15 (0)	7 (2)	26 (7)
500.0	37 (1)	107 (18)	10 (3)	5 (6)	23 (3)
750.0	35 (5)	82 (3)	0	5 (1)	0
1000.0	21 (6)	71 (12)	0	0	0
Positive indicator					
2.0 μg 2-AA	848 (8)[d]	1088 (15)[d]	241 (27)[d]	97 (3)[d]	790 (38)[d]

[a] Negative control values represent the mean colony counts on six plates. All other treatment values are the mean from two plates. Standard deviations are in parentheses.
[b] DMSO, dimethylsulfoxide; APAP, acetaminophen; ASA, aspirin; PA, phenacetin; IB, ibuprofen; 2-AA, 2-anthramine.
[c] No revertant colonies present on one plate due to toxicity.
[d] Represents at least a doubling of negative control values.

As shown in the tables above, there were no dose-dependent changes in the number of revertants in any of the ibuprofen treatment groups with and without S9 metabolic

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activation.

Thus, ibuprofen was not mutagenic under the conditions of this study.

Publication Title: Comparative mutagenic and genotoxic effects of three propionic acid derivatives ibuprofen, ketoprofen and naproxen (Philipose et. al, 1997)

Methods:

Strains:	*Salmonella typhimurium* strains TA97a, TA100, and TA102
Concentrations in definitive study:	1, 10, 100, 1000, and 5000 mcg/plate
Basis of concentration selection:	The 5000 mcg/plate dose resulted in toxicity in all strains tested.
Negative control:	DMSO
Positive control:	Without S9 activation: 4-nitro-*o*-phenylenediamine (20 mcg; all strains); With S9 activation: 2-aminofluorene (10 mcg; all strains)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Ibuprofen in vehicle (DMSO), vehicle alone (negative control), or positive control was pre-incubated with the bacteria with and without S9 mix in the top agar. The solution was mixed and overlaid onto a minimal bottom agar. After the overlay solidified, the plates were inverted and incubated at 37˚C for 48 hrs. Afterwards, the plates were scored. All concentrations and controls (both positive and negative) in this toxicity-mutation assay were plated in duplicate. There was no confirmatory test performed.

Additional Methods/Validity:

Ibuprofen was obtained from the (b) (4) The study was conducted in (b) (4) The doses of ibuprofen tested were up to 1000 mcg/plate as the 5000 mcg/plate dose caused toxicity in the strains tested. The strains tested were *Salmonella typhimurium* strains TA97a, TA100, and TA102. TA97a tested for frameshift mutations, TA100 tested for C:G base pair substitutions, and TA102 tested for A:T base pair substitutions (Kamber et al., 2009). The positive controls used in the study were appropriate as they caused reliable positive results.

Taken together, these methods represent a valid study.

Results:
The following tables illustrate the results with strains TA97a, TA100, and TA102, with

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and without S9 metabolic activation (adapted from the publication):

Table 1
Number of revertants induced by ibuprofen, ketoprofen and naproxen in the Salmonella plate incorporation test using TA97a with or without S9

Chemicals (μg/plate)	Number of revertants/plate			
	− S9 [a]		+ S9 [a]	
	Mean ± SD of 1st Expt.	Mean ± SD of 2nd Expt.	Mean ± SD of 1st Expt.	Mean ± SD of 2nd Expt.
Control (DMSO)	115.00 ± 1.41	117.00 ± 12.72	109.50 ± 16.26	113.00 ± 1.41
Ibuprofen				
1	142.50 ± 9.19 [c]	133.50 ± 6.36	134.00 ± 1.41	158.50 ± 14.84 [c]
10	133.00 ± 7.07 [b]	139.00 ± 12.72	136.50 ± 4.95	145.50 ± 10.60 [c]
100	119.00 ± 12.72	127.50 ± 4.95	131.00 ± 11.31	135.00 ± 1.41 [c]
1 000	116.50 ± 4.95	108.00 ± 9.89	126.50 ± 3.53	110.00 ± 26.87
5 000	Toxic	Toxic	Toxic	Toxic
Positive control				
NPD (20 μg/plate)	1191.00 ± 19.79	1119.00 ± 43.84		
2-AF (10 μg/plate)			957.00 ± 45.25	1056.00 ± 50.91

− S9, without metabolic activation; + S9, with metabolic activation.
[a] Mean ± SD of two plates. Results of each concentration were compared with the solvent control by Dunnett's *t*-test.
[b] $p < 0.05$.
[c] $p < 0.01$.

Table 2
Number of revertants induced by iboprofen, ketoprofen and naproxen in the Salmonella plate incorporation test using TA100 with or without S9

Chemicals (μg/plate)	Number of revertants/plate			
	− S9 [a]		+ S9 [a]	
	Mean ± SD of 1st Expt	Mean ± SD of 2nd Expt.	Mean ± SD of 1st Expt.	Mean ± SD of 2nd Expt.
Control (DMSO)	133.00 ± 8.48	146.00 ± 14.14	151.00 ± 15.55	143.00 ± 16.97
Ibuprofen				
1	172.00 ± 2.83 [c]	166.50 ± 17.68	175.50 ± 28.99	161.00 ± 1.41
10	179.00 ± 1.41 [c]	177.00 ± 19.79	173.00 ± 16.97	177.00 ± 14.14
100	158.00 ± 9.90	166.50 ± 19.09	165.50 ± 13.00	157.00 ± 4.24
1 000	140.50 ± 9.19	121.50 ± 13.43	153.50 ± 2.12	137.50 ± 3.53
5 000	Toxic	Toxic	Toxic	Toxic
Positive control				
Sodium azide (1.5 μg/plate)	1292.50 ± 17.68	1167.50 ± 7.78		
2-AF (10 μg/plate)			1094.50 ± 86.97	1140.00 ± 97.58

− S9, without metabolic activation; + S9, with metabolic activation.
[a] Mean ± SD of two plates. Results of each concentration were compared with the solvent control by Dunnett's *t*-test.
[b] $p < 0.05$.
[c] $p < 0.01$.

Table 3
Number of revertants induced by ibuprofen, ketoprofen and naproxen in the Salmonella plate incorporation test using TA102 with or without S9

Chemicals	Number of revertants/plate			
(μg/plate)	− S9 [a]		+ S9 [a]	
	Mean ± SD of 1st Expt.	Mean ± SD of 2nd Expt.	Mean ± SD of 1st Expt.	Mean ± SD of 2nd Expt.
Control (DMSO)	327.50 ± 31.82	308.00 ± 31.11	355.00 ± 12.73	301.50 ± 19.09
Ibuprofen				
1	409.00 ± 29.70	371.00 ± 29.69	292.50 ± 67.17	319.00 ± 29.69
10	338.00 ± 28.28	324.00 ± 21.21	283.00 ± 21.21	336.50 ± 6.36
100	295.00 ± 12.72	345.00 ± 8.48	315.50 ± 23.33	285.50 ± 26.16
1 000	Toxic	Toxic	Toxic	Toxic
Positive control				
MMS (1 μl/plate)	3001.00 ± 172.53	3032.50 ± 168.99		

− S9, without metabolic activation; + S9, with metabolic activation.
[a] Mean ± SD of two plates. Results of each concentration were compared with the solvent control by Dunnett's *t*-test.
[b] $p < 0.05$.

As shown in the table above, there were no dose-dependent changes in the number of revertants in the ibuprofen treatment groups with and without S9 metabolic activation.

Thus, ibuprofen was not mutagenic under the conditions of this study.

In Vivo Sister Chromatid Exchange Assay

Methods: Paraffin-coated BrdU tablets were implanted in mice prior to treatment with ibuprofen (25, 50, or 100 mg/kg via IP injection). Control mice received an injection of vehicle (75 mcL DMSO). Mitomycin C (1.5 mg/kg) was used as a positive control. Colchicine (4 mg/kg, IP) was injected 22 hours after the BrdU tablet and two hours later bone marrow was expelled using 0.075 M KCl. Cells were lysed with hypotonic treatment, fixed and chromosomes were differentiated and stained for evaluation. In a separate group of animals, ibuprofen was dosed at 270 mg/kg via oral gavage.

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Cyclophosphamide (10 mg/kg) served as the positive control.

In vivo sister chromatid exchanges induced by ibuprofen, ketoprofen and naproxen in mice after i.p. administration

Treatment	SCE/cell of 5 animals	SCE/cell (mean ± SD) [a]	Value of trend statistics	Replicative indices (mean ± SD) [a]
Solvent control (75 µl of DMSO)	5.1, 4.3, 4.4, 4.3, 4.4	4.50 ± 0.34		1.85 ± 0.06
IB (mg/kg)				
25	4.7, 5.6, 4.5, 5.3, 5.1	5.04 ± 0.44		1.88 ± 0.09
50	5.7, 5.0, 5.7, 6.1, 6.0	5.70 ± 0.43 [b]	6.133 [b]	1.87 ± 0.07
100	6.1, 5.6, 6.2, 6.0, 6.1	6.00 ± 0.23 [b]		1.75 ± 0.06
KP (mg/kg)				
25	5.0, 4.6, 5.4, 5.9, 7.1	5.60 ± 0.97		1.85 ± 0.04
50	5.7, 5.8, 6.5, 4.7, 5.6	5.66 ± 0.64 [b]	8.250 [b]	1.78 ± 0.08
100	7.5, 6.1, 5.5, 6.1, 6.6	6.36 ± 0.75 [b]		1.76 ± 0.07
NP (mg/kg)				
25	5.2, 4.8, 5.8, 5.1, 4.5	5.08 ± 0.49		1.82 ± 0.08
50	4.9, 5.1, 5.3, 5.0, 6.0	5.26 ± 0.44	5.190 [b]	1.80 ± 0.06
100	5.6, 5.3, 6.1, 7.0, 6.1	6.02 ± 0.65 [b]		1.90 ± 0.08
Positive control				
Mitomycin-C (1.5 mg/kg)	18.6, 19.8, 23.0, 25.5, 21.3	21.64 ± 2.72		1.89 ± 0.10

[a] Mean ± SD of 5 animals (30 cells per animal). Results of each dose were compared with the control using Dunnett's test.
[b] $p < 0.01$.

In vivo sister chromatid exchanges induced by ibuprofen, ketoprofen and naproxen in mice after oral administration

Treatment	SCE/cell of 5 animals	SCE/cell (mean ± SD) [a]	Replicative indices (mean ± SD) [a]
Solvent control (Gum acacia)	4.4, 4.0, 5.6, 5.4, 4.0	4.68 ± 0.77	1.87 ± 0.08
Ibuprofen (270 mg/kg)	5.9, 5.6, 5.9, 7.7, 5.7	6.16 ± 0.87 [b]	1.79 ± 0.07
Ketoprofen (270 mg/kg)	5.8, 5.6, 4.7, 4.8, 4.8	5.14 ± 0.52	1.82 ± 0.04
Naproxen (270 mg/kg)	5.0, 5.7, 7.1, 6.8, 8.5	6.62 ± 1.35 [b]	1.88 ± 0.06
Positive control			
Cyclophosphamide (10 mg/kg)	23.2, 18.5, 18.3, 20.5, 18.8	19.86 ± 2.06	1.89 ± 0.08

[a] Mean ± SD of 5 animals (30 cells per animal). Results of each dose were compared with the control using Dunnett's test.
[b] $p < 0.05$.

The results suggest that ibuprofen demonstrated clastogenic activity in vivo.

Publication Title: Sister Chromatid Exchange in Patients Treated with Nonsteroidal Anti-Inflammatory Drugs (Sardas et al., 1991)

Patients with degenerative rheumatic diseases and invertebral disc disorders who were receiving NSAIDs: diclofenac (Voltaren, 200 mg/day); ibuprofen (Brufen, 1200 mg/day); or indomethacin (Indocid, 75 mg/day) were evaluated to determine whether NSAID treatment for two weeks resulted in genotoxic effects on peripheral lymphocytes. Peripheral heparinized blood samples were collected before NSAID treatment and after a 2-week treatment period with one NSAID. Chromosomal preparations were stained by fluorescence plus the Giemsa technique. An average of 30 metaphase plates with 46 intact chromosomes and well differentiated SCE were scored (SCE/cell). No significant differences in SCE frequency in 40 patients were observed when treated patients with NSAIDs were compared to results obtained from untreated patients as

illustrated in the following table (from the publication):

Table I. Mean sister chromatid exchange (SCE)/cell in human lymphocytes before and after therapy with nonsteroidal anti-inflammatory drugs (not significant at $p > 0.05$ by paired t-test)

Drug	No. of subjects	Mean SCE/cell (± SD)	
		before	after
Diclofenac	15	7.60 ± 1.59	7.67 ± 1.64
Ibuprofen	12	5.98 ± 1.70	6.02 ± 2.05
Indomethacin	13	6.60 ± 1.07	6.90 ± 1.60

Publication Title: Investigations of the influence of nonsteroidal antirheumatic drugs on the rates of sister-chromatid exchange (Kullich and Klein, 1986)

Sister-chromatid exchange (SCE) rates before and after treatment with several NSAIDs including diclofenac (100 mg/day), fluriprofen (200 mg/day), ibuprofen (1200 mg/day), indomethacin (75 mg/day), isoxicam (200 mg/day), ketoprofen (150 mg/day), piroxicam (20 mg/day), pirprofen (800 mg/day), and tiaprofenic acid (600 mg/day) were evaluated in human lymphocytes in vivo. Herparinised blood samples were collected from healthy, non-smoking volunteers prior to commencing therapy and after a 2-week treatment period. Samples were stained with 2.5% Giemsa solution with 20 metaphases that contained complete, well-spread chromosome sets were counted. SCE rates of patients treated with an NSAID and controls were not significantly different and were within normal variation and in the normal range as illustrated in the following

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table (from the publication):

Mean values = $\bar{X}$; standard deviation = s; number of subjects = n.

Substance	n	SCE/cell		Significance
		Untreated	Treated	
		$\bar{x}$ s	$\bar{x}$ s	
Diclofenac	7	5.57 ± 1.21	6.16 ± 0.78	n.s.
Flurbiprofen	7	5.40 ± 1.57	4.84 ± 1.26	n.s.
Ibuprofen	7	5.17 ± 0.61	4.77 ± 0.24	n.s.
Indometacin	6	5.30 ± 1.04	5.67 ± 1.46	n.s.
Isoxicam	11	4.40 ± 0.56	4.55 ± 0.57	n.s.
Ketoprofen	7	5.49 ± 1.33	5.94 ± 2.16	n.s.
Piroxicam	6	3.72 ± 0.59	4.37 ± 1.10	n.s.
Pirprofen	7	5.04 ± 0.30	5.54 ± 0.78	n.s.
Tiaprofenic acid	6	4.60 ± 0.99	4.42 ± 0.61	n.s.
Controls	34	5.7 ± 1.8		
		without	with	
Mitomycin C	in vitro	4.2 ± 1.7	30.0 ± 4.3	

Publication Title: Do non-steroidal anti-inflammatory drugs induce sister chromatid exchanges in T lymphocytes? (Ozkul et al, 1996)

A total of 48 patients were treated with NSAID drugs (ibuprofen - 800 mg/day; ketoprofen - 150 mg/day, naproxen – 1000 mg/day, indomethacin - 75 mg/day; diclofenac - 100 mg/day, acetylsalicylic acid) for two weeks. Sister chromatid exchanges in cultured lymphocytes from patients before and after NSAID treatment were compared. There were no differences in average SCE rates between patients before and after any NSAID treatment as illustrated in the following table (from the publication):

	Mean SCEs/cell (± SD)	
Drug	**Before treatment**	**After treatment**
Ibuprofen	4.34 ± 1.57	4.50 ± 1.80
Indomethacin	5.12 ± 1.04	5.47 ± 1.57
Naproxen	5.01 ± 2.01	5.10 ± 1.32
Acetylsalicylic acid	4.75 ± 1.08	4.82 ± 0.98
Ketoprofen	4.37 ± 1.62	4.48 ± 1.22
Diclofenac	5.32 ± 1.52	5.63 ± 1.48

Differences between the means before and after treatment were not significant = ($P > 0.05$) according to the paired t-test.

Reviewer's Comments on the overall genotoxicity of ibuprofen:

The Ames assays with ibuprofen from both Oldham et al., 1986 and Philipose et al., 1997 represent an overall weight of evidence approach to test the mutagenicity of ibuprofen. Taken together, ibuprofen is not mutagenic (frameshift mutations as well as C:G and A:T base pair substitutions). The results from these papers are appropriate to

be included in the label.

Philipose also conducted in vivo studies in mice to examine the clastogenic activity of ibuprofen. These authors report a “weakly clastogenic” effect in an in vivo sister-chromatid exchange assay in mice. However, several publications have examined whether ibuprofen (800-1200 mg/day) treatment for 2-weeks altered SCE rates in human lymphocytes and reported no significant differences before and after ibuprofen treatment. It was also noted that NSAID as a class after a two week treatment duration did not alter SCE rates before and after treatment. Collectively, the in vivo results reported in mice have not been observed in humans and therefore it is recommended the clastogenic effects observed in mice be omitted from the label.

Carcinogenicity:

Publication Title: Some Aspects of the Pharmacology, Metabolism, and

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Toxicology of Ibuprofen (Adams et. al, 1970)

Mouse Carcinogenicity study

Methods:

Doses:	300 mg/kg for 43 weeks, reduced to 100 mg/kg from Week 43 to 80 due to GI toxicity.
Frequency of dosing:	Daily
Dose volume:	No reported
Route of administration:	Not reported, presumably oral
Formulation/Vehicle:	Not reported
Species/Strain:	Mouse
Number/Sex/Group:	50/sex
Satellite groups:	None
Study design:	Not reported
Deviation from study protocol:	Unknown

Results:

The authors provided the following summary tables.

MICE ALIVE AFTER 43 AND 80 WEEKS ON IBUPROFEN

	Ibuprofen Dosage		
	300 mg./kg./day 0 to 43 weeks		100 mg./kg./day 43 to 80 weeks
	Initial number	After 43 weeks	After 80 weeks
Males:			
Control	50	48	15
Dosed	50	32	6
Females:			
Control	50	42	12
Dosed	50	41	24

TUMOUR INCIDENCE IN MICE ON IBUPROFEN FOR LONGER THAN 43 WEEKS

	Males		Females	
	Control	Dosed	Control	Dosed
Mice examined/Mice with tumours	43/35	29/21	40/34	40/26
Types of tumour:				
Hepatomas	12	5	5	5
Liver haemangiomas	1	1	1	1
Lymphomas	13	14	22	19
Breast adenocarcinomas	0	0	9	1
Others (benign)	16	9	12	15

Rat Carcinogenicity study

Methods:

Doses:	180 mg/kg for 56 weeks, reduced to 60 mg/kg from Week 56 to 104 due to GI toxicity.
Frequency of dosing:	Daily
Dose volume:	No reported
Route of administration:	Not reported, presumably oral
Formulation/Vehicle:	Not reported
Species/Strain:	Rat
Number/Sex/Group:	30/sex
Satellite groups:	None
Study design:	Not reported
Deviation from study protocol:	Unknown

Results:

The authors provided the following summary tables.

TABLE VI

RATS ALIVE AFTER 56 AND 104 WEEKS ON IBUPROFEN

	Ibuprofen Dosage		
	180 mg./kg./day 0 to 56 weeks		60 mg./kg./day 56 to 104 weeks
	Initial number	After 56 weeks	After 104 weeks
Males:			
Control	30	30	16
Dosed	30	22	10
Females:			
Control	30	30	23
Dosed	30	21	11

TUMOUR INCIDENCE IN RATS ON IBUPROFEN FOR LONGER THAN 56 WEEKS

	Males		Females	
	Control	Dosed	Control	Dosed
Rats examined/Rats with tumours	30/13	22/4	30/16	21/12
Types of tumour:				
Hepatomas	1	0	0	2
Liver haemangiomas	0	1	0	0
Lymphomas	5	1	2	3
Others (malignant)	3	1	5	1
Others (benign)	8	2	20	11

The Authors report no evidence of tumors in ibuprofen-treated animals. However, the studies are limited by modern standards as there is only one dose tested and there were limited number of animals alive by the end of the study. Therefore, we do not

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recommend including these data in the product labeling.

Reproductive and Developmental Toxicology:

Publication Title: Absorption, Distribution and Toxicity of Ibuprofen (Adams et. al, 1969)

Rat embryofetal study

Methods:

Doses:	0, 7.5, 20, 60, and 180 mg/kg/day of ibuprofen
Frequency of dosing:	Once daily from the 1st to the 20th day of pregnancy
Dose volume:	Presumably 10 mL/kg as this was the control volume
Route of administration:	Oral intubation
Formulation/Vehicle:	Control females were given 10 mL/kg of water daily
Species/Strain:	Rats/Albino rats
Number/Sex/Group:	No information given as to the number of maternal dams
Satellite groups:	Additional group to receive 7.5 and 20 mg/kg/day of ibuprofen throughout pregnancy to birth
Study design:	No information given
Deviation from study protocol:	N/A

Additional Methods:
Ibuprofen was presumably obtained from (b) (4). with no information on its purity. The study was conducted in the (b) (4) Primiparous females and proven males were caged together and the presence of spermatozoa in the vaginal smear is considered the first day of pregnancy. Thereafter, dosing with ibuprofen took place until the 20th day of pregnancy. Uterine contents were examined on Day 21 of pregnancy and the fetuses were examined for external, visceral, and skeletal abnormalities. The number of live, dead, and resorbed fetuses, and the number of corpora lutea were recorded. All live fetuses were weighed and examined for external and visceral abnormalities. The brain, eyes, gonads, kidneys, liver, and lungs for some were examined histologically. Skeletal examination of the fetuses included staining by Dawson's method.

Additional groups of rats received 7.5 or 20 mg/kg/day of ibuprofen throughout pregnancy until birth and the young were examined 3 weeks after delivery in order to show certain types of developmental abnormalities at weaning which may not be visible

before birth. All pups in these groups were examined in detail for gross abnormalities.

Results:
Females receiving 20, 60, and 180 mg/kg/day of ibuprofen on Days 1 to 20 of pregnancy were observed with gastrointestinal lesions with a dose-dependent increase in severity. There were no such lesions in the control and 7.5 mg/kg/day dose groups. There was a diminished growth rate in the 180 mg/kg/day dose group only. There was a decrease in the number of litters and % alive fetuses in the 180 mg/kg/day dose group as illustrated in the following table (from the publication):

TABLE 5

EMBRYOTOXIC ACTIVITY OF IBUPROFEN GIVEN ORALLY TO RATS FROM DAY 1 TO 20 OF PREGNANCY

Dosage (mg/kg/day)	Number of litters	Number of fetuses		Live litter size (mean ± SE)	Fetal body weight (g, mean ± SE)	Implantation index (%)[b]
		Alive (%)	Dead (%)[a]			
180	4	35 (89.7)	4 (10.3)	8.8 ± 0.6	2.73 ± 0.19	93
60	15	121 (90.3)	13 (9.7)	8.1 ± 0.6	2.99 ± 0.03	92
20	13	115 (89.9)	13 (10.1)	8.8 ± 0.5	2.94 ± 0.08	90
7.5	11	95 (92.2)	8 (7.8)	8.6 ± 0.7	2.81 ± 0.11	94
Control	11	91 (92.9)	7 (7.1)	8.3 ± 0.6	2.94 ± 0.28	89

[a] Dead fully formed fetuses *in utero* and resorbed fetuses fused with the placenta.
[b] Ratio of implants to corpora lutea.

The dams that received ibuprofen throughout pregnancy until birth had uneventful pregnancies and delivered their pups without difficulty as illustrated in the following table (from the publication):

TABLE 6

POSTNATAL OBSERVATIONS ON YOUNG OF RATS RECEIVING IBUPROFEN ORALLY FROM DAY 1 OF PREGNANCY UNTIL PARTURITION

Dosage (mg/kg/day)	Number of litters	Number of young		Live litter size (mean ± SE)	Viability index (%)[a]	Weaning weight (g, mean ± SE)
		Alive	Stillborn			
20	10	89	1	8.9 ± 0.6	89.2	32.1 ± 0.9
7.5	6	51	2	8.5 ± 0.9	97.9	37.7 ± 1.6
Control	15	143	2	9.5 ± 0.4	96.8	32.5 ± 0.9

[a] The number of young weaned expressed as the percentage of the number born alive less those killed at birth.

As shown in the table above, a similar number of live young per litter was obtained from ibuprofen-treated and control rats.

The following table illustrates that there were no malformed fetuses from any of the ibuprofen-treated dams in both sets of treatments (those dams receiving ibuprofen from

Days 1 to 20 of pregnancy and those receiving ibuprofen throughout pregnancy until birth), from the publication:

TABLE 7

NUMBER OF MALFORMED FETUSES AND YOUNG OBTAINED FROM RATS RECEIVING IBUPROFEN ORALLY DURING PREGNANCY

Type of malformation	Treatment from day 1 to 20 of pregnancy (mg/kg/day)					Treatment from day 1 of pregnancy to parturition (mg/kg/day)		
	180	60	20	7.5	Control	20	7.5	Control
External and visceral								
Hydrocephalus	—	—	—	1	—	—	—	—
Anophthalmia	—	—	—	—	—	1	—	—
Microphthalmia	—	—	—	—	—	1	1	1
Subcutaneous edema	—	1[a]	—	—	—	—	—	—
Diaphragmatic hernia	—	—	—	—	—	—	—	2
Dextrorotation of viscera	—	1	—	—	—	—	—	—
Unilateral hydronephrosis	—	—	—	—	1	—	—	—
Blood cyst on liver	—	—	—	—	—	—	—	1
Testis underdeveloped	—	—	—	—	—	—	—	1
Skeletal								
13th rib shortened	—	3	2	—	1	4	1	3
13th rib absent	—	—	—	—	—	1	1	1
Rib articulation misplaced	—	—	—	—	—	—	—	1
Swelling on ribs	—	—	—	—	—	1	1	—
Fused ribs and fused sternebrae	—	—	—	—	—	—	1	—
Fused ribs and misaligned vertebral bodies	—	1	—	—	—	—	—	—
Misplaced ribs, fused sternebrae and accessory sternebrae	—	—	—	—	—	1	—	—
Sternebrae underdeveloped	—	2	1	—	5	—	—	1
Accessory ossified center in sternum	—	—	—	—	—	1	5	22
Number of litters	4	15	13	11	11	10	6	15
Number of fetuses or young examined	35	121	115	95[b]	91	90	53	145
Number of litters with malformed fetuses or young	0	7	2	1	4	6	5	10

[a] Skeletal malformations included wavy ribs and bilateral curvature of radius and ulna.
[b] Skeletons of this group were not examined.

Reviewer's Comments:

The study appeared to use the methods seen in standard embryofetal developmental toxicity studies. However, maternal observations appear to be limited to body weight and examination of the gastrointestinal tract. A maternal NOAEL cannot be determined in this study although the local GI effects are expected evidence of maternal toxicity for an NSAID. Use of COX inhibitors (such as ibuprofen) could increase postimplantation loss, induce skeletal, midline, diaphragmatic, heart defects, and fetal growth retardation as well as complicate pregnancy if taken near delivery-time due to pulmonary hypertension, secondary to the constriction of ductus arteriosis (Cappon et al., 2003; Cook et al., 2003; and Ostensen and Skomsvoll, 2004). As such, decreased fetal weight and reduced number of litters and the % alive number of young in dams treated with ibuprofen were observed in this study. The rat dose of 180 mg/kg/day that resulted in reduced number of litter, % alive number of fetuses, and fetal weight represents a human dose of 1751 mg in an average human weighing 60 kg, based on a body surface area comparison. This rat dose represents an exposure margin of 0.55 based on the

maximum daily dose of 3200 mg for ibuprofen.

Publication Title: Absorption, Distribution and Toxicity of Ibuprofen (Adams et al., 1969)

NOTE to reader: Dr. Stewart Adams led the research team in Boots Pure Drug Company (Nottingham England) that discovered ibuprofen. The studies described in his 1969 and 1970 papers may or may not have served as the basis of the original Motrin® drug product NDA submission to the FDA (NDA 17463, Approved September 19, 1974). The prescription labeling for Motrin® states "Reproductive studies conducted in rats and rabbits have not demonstrated evidence of developmental abnormalities."

Rabbit embryofetal study

Methods:

Doses:	0, 7.5, 20, and 60 mg/kg/day of ibuprofen
Frequency of dosing:	Once daily from the 1st to the 29th day of pregnancy
Dose volume:	1 mL/kg as this was the dose volume in the control
Route of administration:	Oral
Formulation/Vehicle:	Control females were given 1 mL/kg daily of water
Species/Strain:	Rabbit/New Zealand White
Number/Sex/Group:	Information not given
Satellite groups:	None
Study design:	Information not given
Deviation from study protocol:	N/A

Additional Methods:

Ibuprofen was presumably obtained from (b) (4) with no information on its purity. The study was conducted in (b) (4) (b) (4) Virgin females weighing 2.5 to 5 kg were paired with proven males and the day of mating was termed Day 0 of pregnancy. The following day was the start of dosing with 0, 7.5, 20, or 60 mg/kg/day of ibuprofen daily until Day 29 of pregnancy. All females were sacrificed on Day 30 and their uterine contents were examined. The number of live, dead, and resorbed fetuses, and the number of corpora lutea were recorded. All live fetuses were weighed and examined for external and visceral abnormalities. The brain, eyes, gonads, kidneys, liver, and lungs for some were examined histologically. Skeletal examination of the fetuses included staining with Alizarin Red S by Crary's method.

Results:

Females given 60 mg/kg/day on Days 1 to 29 of pregnancy grew less than control and were observed with stomach ulcers. These females were also observed with pneumonia and a mild degree of focal hepatitis that is thought to be a secondary infection to the gastric lesions. Two females from the 60 mg/kg/day dose group gave birth prematurely to normal pups on Days 26 and 28 of pregnancy. Females given 20 mg/kg/day were similar although less affected. Females given 7.5 mg/kg/day grew normally but some had gastric ulcers or erosions. Minimal gastric damage was observed in 2/23 controls. The number of litters and the % alive number of fetuses decreased in a dose-dependent manner as shown in the following table (from the publication):

TABLE 3

EMBRYOTOXIC ACTIVITY OF IBUPROFEN GIVEN ORALLY TO RABBITS FROM DAY 1 TO 29 OF PREGNANCY

Dosage (mg/kg/day)	Number of litters	Number of fetuses		Live litter size (mean ± SE)	Fetal body weight (g, mean ± SE)	Implantation index (%)[b]
		Alive (%)	Dead (%)[a]			
60	17	113 (82.5)	24 (17.5)	6.7 ± 0.7	49.6 ± 2.2	76.5
20	19	151 (83.0)	31 (17.0)	8.0 ± 0.4	47.6 ± 1.5	94.8
7.5	22	178 (84.0)	34 (16.0)	8.1 ± 0.6	48.2 ± 1.8	88.3
Control	23	209 (87.8)	29 (12.2)	9.1 ± 0.5	49.6 ± 1.0	92.3

[a] Dead fully formed fetuses *in utero* and resorbed fetuses fused with the placenta.
[b] Ratio of implants to corpora lutea.

There were no consistent pattern of malformations and the small number of cases showed no tendency relatable to dose except for the 4 cases of congenital malformations in the 60 mg/kg/day dose group (see the table below, from the

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publication):

TABLE 4

NUMBER OF MALFORMED FETUSES OBTAINED FROM RABBITS RECEIVING IBUPROFEN ORALLY FROM DAY 1 TO 29 OF PREGNANCY

Type of malformation	Dosage (mg/kg/day)			
	60	20	7.5	Control
External and visceral				
Cyclopia and associated malformations	4[a]			
Cranioschisis		1		
Exophthalmos		3		
Microphthalmia		1	1	
Forelimb flexure				2
Incomplete skin-covering around umbilicus, duplication of falciform ligament, and small lobe of lung absent				1[b]
Diverticulum of ileum				1
Gall bladder absent		1	1	
Accessory gall bladder			1	
Bilobed gall bladder		1		
Swelling on bile duct			1	
Small lobe of lung absent	3		2	
Skeletal				
Lumbar scoliosis				1
1st rib shortened			1	
Swelling on ribs	1			
Xiphoid underdeveloped				1
Sternebra misshapen			1	2
Sternebrae joined by ossified strand		2		
5th sternebra underdeveloped	5	2	3	10
Number of litters	17	19	22	23
Number of fetuses examined	113	151	178	209
Number of litters with malformed fetuses	6	6	7	11

[a] All 4 fetuses in the same litter.
[b] Skeletal malformations included unilateral rib fusion and fusion of the 4th and 5th sternebrae.

Reviewer's Comments:

The study appeared to use the methods seen in standard embryofetal developmental toxicity studies although dosing was over the entire gestational period. However, maternal observations appear to be limited to body weight, clinical observations, and examination of the gastrointestinal tract. A maternal NOAEL cannot be determined in this study based on dose-dependent gastric lesions at all doses; however the low dose could be considered a LOAEL. Use of COX inhibitors (such as ibuprofen) could increase postimplantation loss, induce skeletal, midline, diaphragmatic, heart defects, and fetal growth retardation as well as complicate pregnancy if taken near delivery-time due to pulmonary hypertension, secondary to the constriction of ductus arteriosis (Cappon et al., 2003; Cook et al., 2003; and Ostensen and Skomsvoll, 2004). Reduced

number of litter and % alive number of fetuses from does treated with ibuprofen were observed in this study. The 60 mg/kg/day dose in rabbits that resulted in 4 cases of cyclopia related malformations. This dose represents a human dose of 1,167.6 mg in an average human weighing 60 kg, based on a body surface area comparison. This rat dose represents an exposure margin of 0.36 based on the maximum daily of 3200 mg for ibuprofen.

The authors state that "Apart from 4 young in 1 litter with multiple malformations characteristic of cyclopia (Ballantyne, 1904), there was no consistent pattern of malformations, and the small number of cases showed no tendency related to dose."

Publication Title: Absorption, Distribution and Toxicity of Ibuprofen (Adams et al., 1969)

Reproductive study

Methods:
Ten male and 20 female rats of proven fertility were given a powdered diet containing 0.035% of ibuprofen (equivalent to a daily dose of 20 mg/kg) along with a control group on a plain diet for a 60 day pre-mating period. After 60 days, all rats that received ibuprofen were given a plain diet and the females were kept (14 days, 2 to a cage) with 1 male of the same dietary group (mating period). Records were kept of females becoming pregnant and of litter sizes at birth.

Results:
Female rats given a diet containing 0.035% of ibuprofen showed a small loss in weight while similarly treated male rats gained weight. Two control females were sacrificed humanely, one having a mammary abscess and the other having an umbilical hernia. The number of females that became pregnant after the 14-day mating period is 15/20 in the ibuprofen group versus 16/18 in the control. All the males in the 2 groups mated successfully apart from 1 male receiving ibuprofen, which may account for 2 of the 5 ibuprofen-treated females to become pregnant.

Shortly before giving birth, 2 ibuprofen-treated females had vaginal hemorrhage. Of these, 1 died with 10 fully formed fetuses in the uterus and extensive hemorrhage surrounding the placenta, gastric ulcers, and anemia. The other female was sacrificed humanely with lung congestion, a large blood clot in one uterine horn, and no dead or resorbed fetuses. The remaining ibuprofen-treated females had uneventful pregnancies and give birth to litters with an average of 7.9 live young, compared to 7.3 live young in the control.

Reviewer's Comments:
This study is appropriate for examining fertility especially in males as 60 days should be sufficient to examine epididymal transit and possibly spermatogenesis. However, sperm parameters were not taken (sperm motility, sperm count, appearance, etc.) and

female fertility parameters, such as fertility index, were not determined. In other words, the only fertility measure was successful mating for both the males and females in this study. The rat dose of 20 mg/kg is equivalent to a human dose of 194.6 mg to an average human weighing 65 kg, based on a body surface area comparison. The rat dose of 20 mg/kg yields an exposure margin of 0.061 based on the maximum daily of 3200 mg for ibuprofen.

Publication Title: Developmental Toxicity Evaluation of Ibuprofen and Tolmentin Administered in Triple Daily Doses to Wistar CRL:(WI)WUBR Rats (Burdan 2004)

Rat embryofetal study

Methods:

Doses:	0, 25.5, 255, and 600 mg/kg of ibuprofen (these are total daily dose as the rats were dosed 3 times daily)
Frequency of dosing:	Three times daily (8 hours apart) from the 8th to the 21th day of pregnancy
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Distilled water
Species/Strain:	Rats/Wistar CRL:(WI)WUBR (albino rats)
Number/Sex/Group:	Group sizes were not provided
Satellite groups:	None
Study design:	N/A
Deviation from study protocol:	N/A

Additional Methods:

Ibuprofen was presumably obtained from (b) (4) and is >99% pure. The study was conducted by (b) (4) (b) (4) The vehicle appears to be distilled water. The dose volume is 10 mL/kg. The rats were dosed three times daily (8 hours apart) from Day 8 to Day 21 of gestation via oral gavage. Mortality checks were performed daily before treatment and 3 times daily during treatment. Body weight gains are monitored daily from Day 8 to the end of pregnancy.

On Gestation Day 21, the dams were sacrificed and the blood, uterus, and abdominal organs were collected. For each blood sample, plasma levels of ALT, AST, urea, and total protein were determined. Liver, kidney, and spleen weights were recorded. Organs of the gastrointestinal tract were prepared and stained for histopathological examination.

Ovarian corpora lutea were counted. The uteri were examined for the presence and position of resorption sites, dead or live fetuses, and the number of implantation sites.

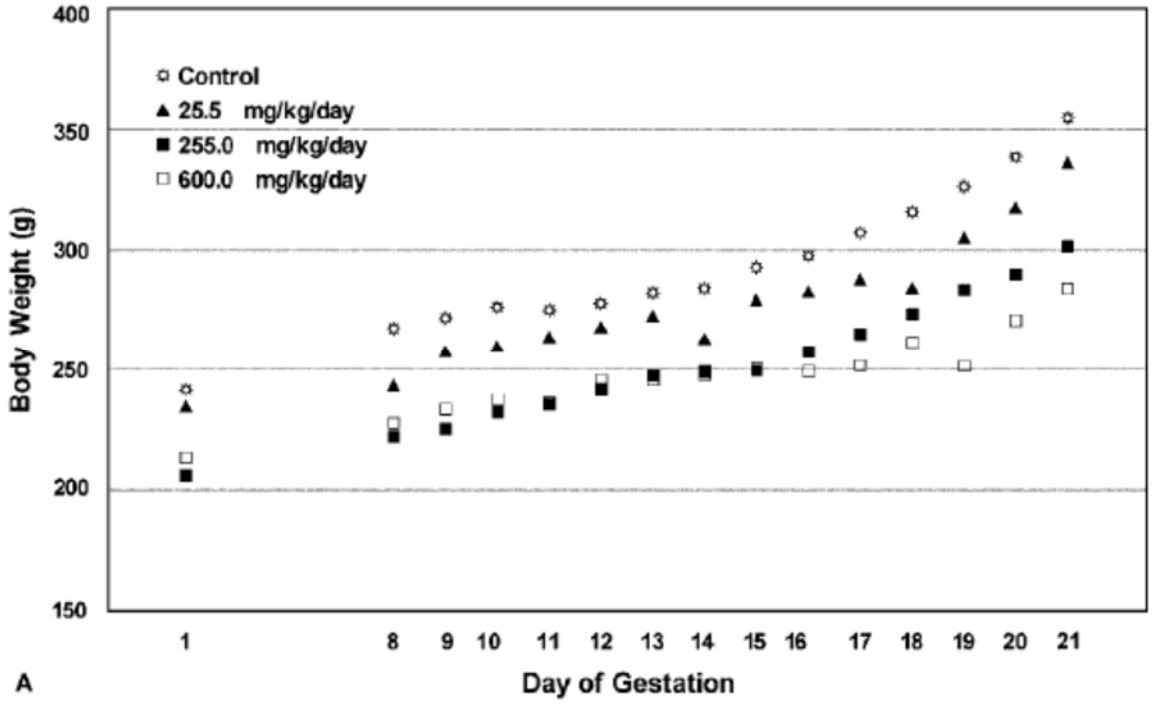
Fetuses were removed, sexed, and examined macroscopically for external malformations. The weight of the fetuses and placentas, the fetal crown-rump length, and tail length were measured. The pre- and postimplantation loss was calculated using the following formula (from the publication):

$$\text{Preimplantation loss}\,(\%) = ((\text{no. corpora lutea} - \text{no. implantations})/\text{no. corpora lutea}) \times 100$$

$$\text{Postimplantation loss}(\%) = ((\text{no. implantations} - \text{no. live fetuses})/\text{no. implantations}) \times 100$$

One in 10 fetuses from each experimental group were randomly selected for ultrastructural and genetic examination. Two-thirds randomly selected fetuses from each litter were stained with alcian blue and alizarin red-S to study skeletal malformations. Internal organs were grossly examined to evaluate possible pathological changes. The remaining fetuses were selected to study soft tissue abnormalities.

Results:
There appears to be a dose-dependent decrease in the body weights of the dams treated with ibuprofen as shown in the following figure (from the publication):



In the blood, there was a dose-dependent increase in AST, ALT, and urea as well as a dose-dependent decrease in total protein (TP) in dams treated with ibuprofen; however, only the changes at the 600 mg/kg/day dose group was significantly different from

control (from the publication):

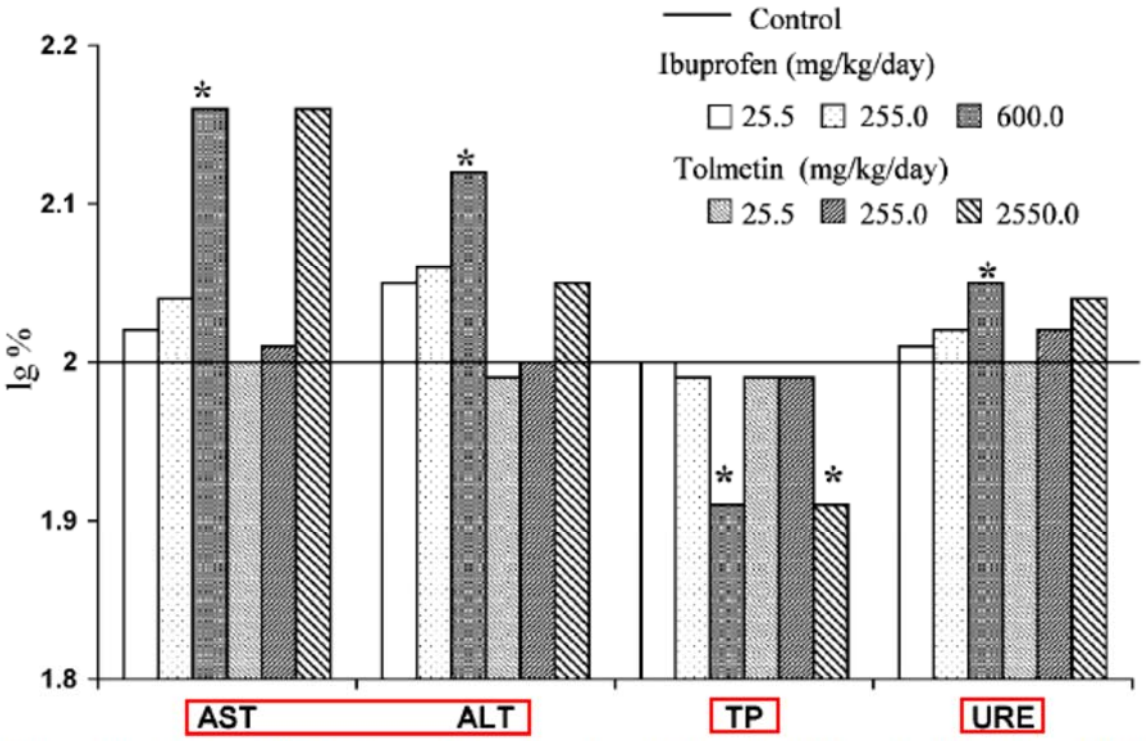


Fig. 2. Relative activity of alanine (ALT) and aspartate aminotransferase (AST), level of total protein (TP) and urea (URE) in dams treated with ibuprofen and tolmetin. Data presented as a lg% of the control value. *$p<0.05$.

There is a reduction in the fetal weight, fetal length, and the % live fetuses/litter with skeletal variations in the 600 mg/kg/day dose group as shown in the following table

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(from the publication):

Table 1
Maternal Reproductive, Litter and Fetal Alteration Observations in Control, Ibuprofen-, and Tolmetin-Exposed Groups

		Ibuprofen (mg/kg/day)			Tolmetin (mg/kg/day)		
	Control	25.5	255.0	600.0	25.5	255.0	2550.0
Initially selected dams (n)	20	20	20	20	20	20	20
Found dead dams (n)	0	0	4	14	0	3	16
Pregnant dams sectioned on GD 21	20	20	16	6	20	17	4
Corpora lutea	14.20±2.37	13.90±1.86	13.93±1.73	14.16±1.47	13.60±2.16	13.59±2.43	13.25±0.95
Number of fetuses	12.50±2.52	12.60±2.25	12.18±1.72	11.83±3.92	12.35±2.23	12.53±2.67	9.50±4.04
Males	5.95±2.37	6.70±2.07	6.18±1.27	6.33±1.63	6.15±1.98	6.06±2.33	5.50±1.91
Females	6.55±1.09	5.90±1.68	6.00±1.50	5.50±2.94	6.20±1.67	6.49±0.94	4.00±2.16
Number of early resorptions (n/litter)	8 (7)	3 (2)	7 (5)	5 (4)	5 (5)	4 (3)	1 (1)
Number of late resorptions (n/litter)	0	0	0	0	0	0	0
Number of dead fetuses (n/litter)	0	0	0	4 (1)	0	0	10 (2)
Implantation	12.90±2.71	12.75±2.09	12.62±1.50	13.33±1.96	12.60±2.16	12.76±2.33	12.25±0.50
Preimplantation loss (%)	9.51±7.15	8.43±6.07	9.13±5.97	6.21±6.14	7.52±1.17	6.07±3.10	7.28±5.83
Postimplantation loss (%)	2.84±4.20	1.33±4.24	3.59±6.20	13.41±23.06	2.05±3.68	2.35±5.62	22.91±31.45
Fetal weight (g)	3.68±0.19	3.65±0.12	3.54±0.29	3.24±0.26[a]	3.69±0.19	3.52±0.26	3.29±0.27[a]
Fetal length (mm)	37.54±1.76	37.38±1.12	36.42±2.07	35.74±1.82[a]	37.17±2.35	36.67±2.15	32.95±1.77[a]
Placenta, weight (g)	0.54±0.05	0.53±0.04	0.54±0.05	0.51±0.02	0.55±0.06	0.55±0.06	0.49±0.04
% live fetuses/litter with external malformation	0.31±1.40	0.87±2.68	0.00	0.00	0.00	0.59±2.43	6.25±12.50
% live fetuses/litter with visceral malformation	1.25±5.59	0.00	3.65±10.08	0.00	0.00	0.00	12.50±25.00
% live fetuses/litter with skeletal malformation	0.62±2.79	0.00	0.78±3.12	3.51±5.46	0.00	0.74±3.03	11.11±15.71
% live fetuses/litter with external variations	5.73±6.18	5.28±6.32	9.41±3.09[a]	21.12±26.97	6.51±4.57	5.37±5.13	28.85±16.30[a]
% live fetuses/litter with visceral variations	10.42±25.20	4.17±13.11	13.54±28.03	50.00±77.46	13.16±28.64	0.00	0.00
% live fetuses/litter with skeletal variations	20.97±15.07	17.58±16.09	23.31±13.07	95.00±8.36[a]	18.11±14.49	27.99±14.61	100.00[a]

[a]Significantly different ($p \leq 0.05$) from control value.

Morphologic abnormalities in the fetuses from dams that were treated with ibuprofen include increased incidence of asymmetric palate rugae (external variation), number of fetuses with skeletal variations, frontal bone (reduced ossification), parietal bone (reduced ossification), interparietal bone (reduced ossification), supraoccipital bone (reduced ossification), sternebra (unossified), sternebra (reduced ossification), sternebra (other variations), and reduced ossification of the whole skeleton. Additionally, there was decreased incidence of number of fetuses with external variations and metatarsal bodies (reduced ossification, skeletal variation). These

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changes are shown in the following table notes (from the publication):

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Table 2
Morphologic Abnormalities in Rat Fetuses in Control, Ibuprofen, and Tolmetin-Exposed Groups[a]

		Ibuprofen (mg/kg/day)			Tolmetin (mg/kg/day)		
	Control	25.5	255.0	600.0	25.5	255.0	2,550.0
Total fetuses examined[b]	250 (20)	252 (20)	195 (16)	71 (6)	247 (20)	213 (17)	38 (4)
Total double-stained specimens	175 (20)	175 (20)	125 (15)	30 (4)	169 (20)	126 (15)	20 (3)
Total Alizarin-stained specimens	—	—	8 (1)	20 (2)	—	18 (2)	6 (1)
Total examined by Wilson's method	52 (20)	54 (20)	43 (16)	12 (6)	55 (20)	49 (17)	7 (4)
External malformation[b]							
No. of fetuses with malformation	1 (1)	2 (2)	—	—	—	1 (1)	1 (1)
Short tail	1 (1)	—	—	—	—	1 (1)	—
Cleft palate	—	2 (2)	—	—	—	—	—
Myelomeningocele	—	—	—	—	—	1 (1)	—
Umbilical hernia	—	—	—	—	—	—	1 (1)
Visceral malformation[c]							
No. of fetuses with malformation	1 (1)	—	2 (2)	—	—	—	1 (1)
Interventricular septal defect	1 (1)	—	2 (2)	—	—	—	1 (1)
Skeletal malformation							
No. of fetuses with malformation	1 (1)	—	1 (1)	2 (2)	—	1 (1)	2 (2)
Coccygeal vertebra, missing	1 (1)	—	—	—	—	1 (1)	1 (1)
Sacro-coccygeal vertebra, missing	—	—	—	—	—	1 (1)	—
Transversal foramen, cleft	—	—	—	1 (1)	—	—	—
Frontal bone, misshapen	—	—	1 (1)	—	—	1 (1)	—
Fused rib	—	—	—	1 (1)	—	—	—
Additional sternebrae	—	—	1 (1)	—	—	—	1 (1)
Cleaved sternebra	—	—	—	—	—	—	—
External Variations[b]							
No. of fetuses with variations	14 (11)	13 (10)	18 (16)	10[d] (6)	16 (15)	11 (10)	9[d] (4)
Hematoma	7 (5)	—	8 (7)	5 (3)	10 (6)	4 (4)	2 (1)
Anasarca	—	—	2 (1)	—	—	—	1 (1)
Asymmetric palate rugae	7 (6)	13 (10)	12 (14)	8[d] (6)	10 (9)	7 (6)	6[d] (4)
Visceral Variations[c]							
No. of fetuses with variations	4 (4)	2 (2)	3 (3)	4 (3)	6 (5)	—	—
Malpositioned kidney[c]	4 (4)	2 (2)	1 (1)	4 (2)	4 (4)	—	—
Enlarged lateral ventricle	—	—	2 (2)	1 (1)	2 (1)	—	—
Skeletal variations							
No. of fetuses with variations	35 (17)	31 (14)	30 (16)	47[d] (6)	29 (16)	39 (16)	26[d] (4)
Nasal bone, reduced ossification[e]	—	—	2 (2)	3 (2)	—	—	1 (1)
Frontal bone, reduced ossification	8 (5)	9 (6)	3 (3)	21[d] (6)	11 (7)	9 (6)	18[d] (4)
Parietal bone, reduced ossification[e]	3 (3)	2 (2)	—	7[d] (4)	—	3 (2)	9[d] (4)
Interparietal bone, reduced ossification	7 (4)	7 (4)	12 (8)	12[d] (6)	5 (4)	5 (4)	8[d] (4)
Supraoccipital bone, reduced ossification	5 (3)	3 (3)	8 (8)	9[d] (5)	4 (4)	—	7[d] (4)
Hyoid bone, unossified	—	—	—	—	—	2 (2)	—
Hyoid bone, reduced ossification	2 (2)	—	7 (3)	5 (4)	4 (3)	—	5 (3)
Hyoid bone, additional ossification center	—	1 (1)	—	—	—	2 (2)	—
Wavy ribs[e]	—	—	—	1 (1)	—	1 (1)	—
13th rib, wavy[e]	2 (2)	4 (2)	—	3 (3)	—	4 (4)	7 (4)
13th rib, short[e]	2 (2)	1 (1)	3 (2)	4 (2)	2 (1)	—	2 (2)
Supernumerary cervical ribs[e]	—	—	—	—	1 (1)	—	1 (1)
Supernumerary lumbar ribs[e]	3 (2)	1 (1)	3 (3)	—	—	2 (2)	3 (1)
Sternebra, unossified	5 (3)	3 (2)	—	14[d] (4)	3 (2)	3 (3)	11[d] (4)
Sternebra, reduced ossification	9 (6)	7 (7)	14 (6)	19[d] (6)	9 (7)	8 (6)	14[d] (4)
Sternebra, other variations[f]	7 (5)	6 (6)	12 (7)	23[d] (6)	9 (5)	3 (2)	21[d] (4)
Asymmetric vertebral body	5 (3)	10 (6)	2 (2)	4 (3)	4 (2)	5 (3)	7[d] (3)
Unossification of sacro-coccygeal vertebrae	—	—	—	—	—	1 (1)	—
Metacarpal bodies, reduced ossification[e]	6 (2)	2 (2)	9 (3)	4 (2)	—	7 (6)	11[d] (4)
Metatarsal bodies, reduced ossification[e]	9 (6)	4 (4)	9 (6)	8[d] (5)	6 (5)	5 (5)	6[d] (3)
Reduced ossification of the whole skeleton	—	—	—	5 (4)	—	—	5 (3)
Increase of ossification in primary ossification centers	—	—	—	—	9 (1)	—	—

[a]A single fetus may be represented more than once in the listing of individual defects. The number of litters is in parentheses.
[b]All fetuses were examined for external abnormalities, including cleft palate.
[c]Head and neck structures were evaluated in the Bouin's solution stained fetuses by Wilson's method. The abdominal and thorax structure were examined in the Bouin's solution stained fetuses and in most of the eviscerated alteration fetuses.
[d]Significantly different ($p \leq 0.05$) from control value.
[e]Uni- or bilateral.
[f]Bifurcated at the distal end (VI), dumbbell-shaped (III and V).

Reviewer's Comments:
This study appears to use methods employed in standard embryofetal developmental toxicity studies. However, the dosing in this study is from Day 8 to Day 21 of gestation, which may miss critical developmental landmarks that occur at earlier time points (Gestation Days 6 and 7). Maternal observations include body weight and plasma levels of liver enzymes, total protein, and urea. No maternal NOAEL can be obtained in this study. Use of COX inhibitors (such as ibuprofen) could increase postimplantation loss, induce skeletal, midline, diaphragmatic, heart defects, and fetal growth retardation as well as complicate pregnancy if taken near delivery-time due to pulmonary hypertension, secondary to the constriction of ductus arteriosis (Cappon et al., 2003; Cook et al., 2003; and Ostensen and Skomsvoll, 2004). As such, decreased fetal weight and reduced number of litter and % alive number of fetuses as well as the various external and skeletal variations (reduced ossification) in dams treated with ibuprofen were observed in this study. The rat dose of 600 mg/kg/day that resulted in these observations represents a human dose of 5,838 mg in an average human weighing 60 kg, based on a body surface area comparison. The rat dose of 600 mg/kg yields an exposure margin of 1.82 based on the maximum daily dose of 3200 mg for ibuprofen. The NOAEL for developmental effects would appear to be 25.5 mg/kg (0.08-times the maximum recommended human daily dose of 3200 mg).

Publication Title: Relationship Between Cyclooxygenase 1 and 2 Selective Inhibitors and Fetal Development When Administered to Rats and Rabbits During the Sensitive Periods for Heart Development and Midline Closure (Cappon et al., 2003)

Methods:
Ibuprofen was obtained from (b) (4) but no information was given regarding the purity of ibuprofen. One hundred forty timed-pregnant female Sprague-Dawley rats were obtained from (b) (4), were 9 to 12 weeks old, and were on 1, 2, or 4 days of gestation at arrival. Dams were randomly assigned to seven groups of 20 rats each (1 control group and an ibuprofen treatment group). The ibuprofen dose was 300 mg/kg/day via oral gavage at a dose volume of 10 mL/kg on Gestation Days 9 and 10. The control rats received 0.5% methylcellulose using the same route and dosing regimen. Rats were sacrificed on Day 21.

One hundred fifty timed-pregnant female New Zealand White rabbits were obtained from (b) (4), were 5 to 6.5 months of age, weighed 2.83 to 4.25 kg, and were on Gestation Day 8 upon arrival. Does were randomly assigned to six groups of 20 rabbits each (1 control group and an ibuprofen treatment group). The ibuprofen dose was 500 mg/kg/day via oral gavage at a dose volume of 2 mL/kg on Gestation Days 9, 10, and 11. The control rabbits received 0.5% methylcellulose using the same route and dosing regimen. Rabbits were sacrificed on Day 29.

During the study, all animals were observed twice daily for morbidity and mortality.

Body weights and food consumption were determined on the day of arrival and daily beginning on Gestation Day 8. Following sacrifice, the abdominal, thoracic, and pelvic viscera were examined grossly. The uteri and ovaries were removed and weighed. The number of corpora lutea as well as the number, type, and location of implantation sites were recorded. Viable fetuses were removed and weighed. For each viable fetus, a detailed external examination was performed including examination of the eyes, palate, and external orifices. The viable fetuses were sacrificed and examined for internal abnormalities. The hearts were examined for anomalies. The brain and ventricles were examined for dilatations or abnormalities.

Results:

Rat Data: The following table illustrates that there were greater numbers of dams dead, gravid at Cesarean section, and more unscheduled deaths in dams treated with 300 mg/kg/day ibuprofen on Gestation Days 9 and 10 (see following table, adapted from the publication):

Table 1
Rat Pregnancy Rate, Mortality, and Necropsy Findings From Dams Treated With NSAIDs on GDs 9 and 10

					GI toxicity at necropsy	
Dose group	Dose level[a]	Number dosed	Number died[b]	Number gravid at cesarean section	Unscheduled death	Scheduled examination
Control	0	20	0	20	0	No findings
Ibuprofen	300	20	1	19	1	No findings

[a]Dose levels are expressed as milligrams of active moiety of test article per kilogram of body weight.
[b]Number died includes animals found dead and killed moribund.
GD, gestational day; GI, gastrointestinal; NSAID, nonsteroidal anti-inflammatory drug.

The body weight of dams treated with 300 mg/mg/day ibuprofen on Gestation Days 9 and 10 were significantly lower than control on all time points where body weights were recorded following dosing as shown in the following table (from the publication):

Table 2
Rat Body Weights (g) During Gestation of Dams Treated with NSAIDs on GDs 9 and 10

GD	Control	CJ-19,209	Meloxicam	Diclofenac	Diflunisal	Ibuprofen	Ketorolac
9	282 ± 11	280 ± 13	279 ± 14	284 ± 7	287 ± 14	276 ± 18	282 ± 14
10	288 ± 12	284 ± 12	278 ± 18[c]	274 ± 11[c]	272 ± 13[c]	258 ± 14[c]	275 ± 17[c]
11	294 ± 13	292 ± 10	277 ± 21[c]	248 ± 13[c]	262 ± 12[c]	245 ± 12[c]	267 ± 20[c]
12	299 ± 14	300 ± 11	280 ± 28[c]	252 ± 15[c]	256 ± 17[c]	243 ± 19[c]	277 ± 27[c]
15	317 ± 17	315 ± 10	309 ± 13	261 ± 21[c]	282 ± 23[c]	277 ± 23[c]	303 ± 27[b]
18	352 ± 30	353 ± 12	348 ± 16	315 ± 35[c]	321 ± 34[c]	319 ± 39[c]	345 ± 24
21	402 ± 23	398 ± 13	404 ± 17	362 ± 37[c]	358 ± 53[c]	368 ± 46[c]	394 ± 28
Corrected BW gain[a]	19.9 ± 13.6	19.8 ± 13.2	18.5 ± 15.9	−13.6 ± 25.3[c]	−6.1 ± 36.8[c]	−5.4 ± 26.4[c]	11.1 ± 17.7

Data presented as mean ± standard deviation.
[a]Corrected BW gain equals the body weight gain from GD 9 to 21 minus the gravid uterine weight.
[b]$p \leq 0.05$.
[c]$p \leq 0.01$.
BW, body weight; GD, gestational day; NSAID, nonsteroidal anti-inflammatory drug.

The fetal weight from dams treated with 300 mg/kg/day ibuprofen on Gestation Days 9 and 10 were significantly lower than control as shown in the following table (adapted

from the publication):

Table 3
Rat Cesarean Section Observations and Fetal Weights of Dams Treated with Nonsteroidal Anti-Inflammatory Drugs on Gestational Days 9 and 10

Dose group (n[a])	Corpora lutea	Viable fetuses	Pre-implantation loss (%)	Post-implantation loss (%)	Fetal weight (g)	Placental weight (g)
Control (20)	14.2±1.4	13.6±1.2	4.0±5.8	3.7±4.9	5.72±0.4	0.53±0.07
Ibuprofen (19)	14.9±2.2	14.0±2.7	5.9±14.5	6.2±9.5	5.29±0.64[b]	0.55±0.07

Data presented as mean± standard deviation.
[a]Number of pregnant females examined at cesarean section.
[b]$p \leq 0.05$.
[c]$p \leq 0.01$.

The fetuses from dams treated with 300 mg/kg/day ibuprofen on Gestation Days 9 and 10 were observed with membranous ventricular septal defects in the heart at a higher incidence than control as well as increased incidence by 1 for blood vessels (subclavian retroesophageal), eyes (microphthalmia), and kidney (renal papilla absent) as shown in the following table (from the publication):

Table 4
Rat Fetal Evaluations From Dams Treated With Non-steroidal Anti-Inflammatory Drugs on Gestational Days 9 and 10

	Dose group[a]						
	Control 262 (20)	CJ-19,209 192 (15)	Meloxicam 217 (16)	Diclofenac 177 (14)	Diflunisal 185 (14)	Ibuprofen 252 (19)	Ketorolac 247 (19)
External findings							
Polydactyly	—	—	—	2(1)[b]	—	—	—
Syndactyly	—	—	—	1(1)[b]	—	—	—
Hindlimb hypoflexion	—	—	—	1(1)[c]	—	—	—
Acaudate	—	—	—	1(1)[c]	—	—	—
Anus imperforate	—	—	—	1(1)[c]	—	—	—
Visceral findings							
Heart							
VSD, membranous	1(1)	1(1)	—	1(1)	8(7)	12(8)	3(3)
Blood vessels							
Subclavian retroesophageal	—	—	—	—	—	1(1)	—
Eyes							
Microphthalmia	—	—	—	—	—	1(1)	—
Kidneys							
Renal papilla absent	—	—	—	—	—	1(1)	—

[a]Fetuses (litters)
[b,c]Multiple findings for one fetus.
VSD, ventricular septal defect.

Rabbit Data: The following table illustrates that there were greater numbers of does dead, gravid at Cesarean section, and more unscheduled deaths in does treated with 500 mg/kg/day ibuprofen on Gestation Days 9 to 11 (see following table, adapted from

the publication):

Table 5
Rabbit Pregnancy Rate, Mortality, and Necropsy Findings from Does Treated with Nonsteroidal Anti-Inflammatory Drugs on Gestational Days 9, 10, and 11

Dose group	Dose level[a]	Number dosed	Number died[b]	Number gravid at cesarean section	GI toxicity at necropsy: Unscheduled death	GI toxicity at necropsy: Scheduled examination
Control	0	20	0	18	0	No findings
Ibuprofen	500	20	1	19	1	No findings

[a]Dose levels are expressed as milligrams of active moiety of test article per kilogram of body weight.
[b]Includes animals found dead and killed moribund.
[c]Two does aborted and were killed.
GI, gastrointestinal.

The body weight of does given 500 mg/kg/day ibuprofen on Gestation Days 9 to 11 were significantly lower at all time points that body weights were recorded following dosing as illustrated in the following table (from the publication):

Table 6
Body Weights of Does Treated with Nonsteroidal Anti-Inflammatory Drugs on Gestational Days 9, 10, and 11

GD	Control	CJ-19,209	Meloxicam	Diclofenac	Diflunisal	Ibuprofen	Ketorolac
9	3.51±0.20	3.43±0.20	3.33±0.23	3.54±0.30	3.46±0.20	3.42±0.21	3.56±0.19
10	3.52±0.22	3.46±0.20	3.36±0.25	3.59±0.31	3.45±0.21	3.27±0.23[c]	3.59±0.20
11	3.55±0.19	3.46±0.21	3.37±0.25	3.56±0.32	3.39±0.21[b]	3.20±0.29[c]	3.59±0.21
12	3.55±0.18	3.45±0.22	3.38±0.26	3.48±0.33	3.31±0.21[c]	3.13±0.22[c]	3.56±0.21
13	3.61±0.19	3.45±0.25[b]	3.38±0.27	3.41±0.30[b]	3.27±0.18[c]	3.11±0.22[c]	3.56±0.21
14	3.65±0.19	3.46±0.27[b]	3.39±0.27	3.39±0.29[c]	3.29±0.20[c]	3.18±0.24[c]	3.61±0.22
19	3.75±0.20	3.57±0.24[b]	3.50±0.27	3.54±0.31[b]	3.48±0.15[c]	3.45±0.21[c]	3.72±0.20
24	3.87±0.20	3.70±0.22[b]	3.62±0.27	3.67±0.27[b]	3.63±0.18[c]	3.55±0.22[c]	3.85±0.18
29	3.98±0.22	3.80±0.22[b]	3.71±0.28	3.79±0.28[b]	3.74±0.18[c]	3.70±0.23[c]	3.95±0.20
Corrected BW gain[a]	−0.06±0.14	−0.11±0.11	−0.10±0.13	−0.19±0.12[c]	−0.15±0.10[b]	−0.19±0.11[c]	−0.14±0.11

Data presented as mean±standard deviation. Meloxicam body weight was compared statistically to its concurrent control group; body weight data for the meloxicam concurrent control group are not shown (see Materials and Methods for more details).
[a]Corrected body weight gain equals the BW gain from GD 9 to 21 minus the gravid uterine weight.
[b]$p \leq 0.05$.
[c]$p \leq 0.01$.
BW, body weight; GD, gestational day.

The fetal weight from does treated with 500 mg/kg/day ibuprofen on Gestation Days 9 to 11 were significantly lower than control as shown in the following table (adapted from

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the publication):

Table 7
Rabbit Cesarean Section Observations and Fetal Weights from Does Treated with Nonsteroidal Anti-Inflammatory Drugs on Gestational Days 9, 10, and 11

Dose group (n[a])	Corpora lutea	Viable fetuses	Pre-implantation loss (%)	Post-implantation loss (%)	Fetal weight (g)	Placental weight (g)
Control (18)	10.5±2.6	8.2±2.3	15.0±23.7	5.7±8.9	45.2±4.1	5.8±0.5
Ibuprofen (19)	9.6±2.0	8.3±1.9	9.4±12.6	3.9±9.6	41.0±4.4[c]	5.6±0.8

Data presented as mean± standard deviation.
[a]Number of pregnant females examined at cesarean section.
[b]$p \leq 0.05$.
[c]$p \leq 0.01$.

There was greater incidence of gastroschisis (external findings) and membranous ventricular septal defect in the heart in the fetuses of does treated with 500 mg/kg/day ibuprofen on Gestation Days 9 to 11 as illustrated in the following table (from the

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publication):

Table 8
Rabbit Fetal Evaluations from Does Treated with Nonsteroidal Anti-Inflammatory Drugs on Gestational Days 9, 10, and 11

	Dose group[a]						
	Control 147 (18)	CJ-19,209 157 (20)	Meloxicam 132 (16)	Diclofenac 124 (16)	Diflunisal 109 (14)	Ibuprofen 158 (19)	Ketorolac 156 (18)
External findings							
Petechia	—	—	—	—	—	—	1(1)
Gastroschisis	—	—	—	—	—	1(1)	—
Forepaw hyperflexion	1(1)	—	—	—	—	—	—
Omphalocele	—	—	—	—	1(1)	—	—
Visceral findings							
Heart							
VSD, membranous	—	—	—	—	2(2)[d]	1(1)	—
VSD, muscular					1(1)[b]		
Blood vessels							
Enlarged aortic arch	—	—	—	—	1(1)[b]	—	—
Subclavian retroesophageal	—	—	—	—	1(1)	—	—
Absent innominate artery	—	—	—	—	1(1)	—	—
Accessory vessels	—	—	—	—	6(4)	—	1(1)
Brain							
Lateral ventricles dilated	1(1)	—	—	—	—	—	2(2)
Diaphragm							
Diaphragmatic hernia	—	—	—	—	1(1)	—	—
Eyes							
Microphthalmia	—	—	—	—	6(4)[c,d,e]	—	—
Hemorrhage	—	—	—	—	2(2)[c,e]	—	—
Hemorrhagic ring	—	1(1)	—	—	—	—	—

[a]Fetuses (litters).
[b–e]Multiple findings for one fetus.
VSD, ventricular septal defect.

Reviewer's Comments:

The methods in this study appear to be a modification of standard embryofetal developmental toxicity studies. In this study, only Gestation Days 9 and 10 in rats and Days 9-11 in rabbits were dosed to study the effect of ibuprofen on heart development. Maternal observations were limited to body weight evaluations. A maternal NOAEL cannot be determined in this study. Fetal observations appear to be adequate with the exception that there is detail examination of the heart structures. Use of COX inhibitors (such as ibuprofen) could increase postimplantation loss, induce skeletal, midline, diaphragmatic, heart defects, and fetal growth retardation as well as complicate pregnancy if taken near delivery-time due to pulmonary hypertension, secondary to the constriction of ductus arteriosis (Cappon et al., 2003; Cook et al., 2003; and Ostensen and Skomsvoll, 2004). As such, decreased fetal weight in dams and does treated with ibuprofen were observed in this study. The rat dose that resulted in ventricular septal defects in rat pups (300 mg/kg/day) is equivalent to a human dose of 2919 mg in an average human weight 60 kg, based on a body surface area comparison. The rat dose of 300 mg/kg/day yields an exposure margin of 0.912 based on the maximum daily dose of 3200 mg for ibuprofen. The 500 mg/kg/day rabbit dose used in this study is

equivalent to a human dose of 9730 mg in an average human weighing 60 kg, based on a body surface area comparison. The rabbit dose of 500 mg/kg/day yields an exposure margin of 3.04 based on the maximum daily dose of 3200 mg for ibuprofen.

Publication Title: Congenital Ventricular Septal Defects and Prenatal Exposure to Cyclooxygenase Inhibitors (Burdan et. al, 2006)

Methods:
The authors conducted a retrospective analysis of teratology studies conducted in their own laboratory using the Wistar rat strain between 1997 and 2004. They compared these findings with reported findings of developmental toxicology studies with selective and nonselective COX-2 inhibitors. In all studies, rats were administered study medication on Gestation Day 7 to 16. Pregnancies were terminated on GD 21 by Caesarian section and fetuses were examined macroscopically, weighed, and fetal crown-rump length were checked. Body mass index and pre- and post-implantation mortality rates were determined. One third of the fetuses were dissected in situ or stained with Bouin solution and internally examined. The remaining fetuses were eviscerated and prepared for skeletal examinations.

Results:
Although not statistically significant, the authors report a greater increase in the incidence of ventricular septal defects in offspring exposed to aspirin and ibuprofen compared to control. They extrapolate predicted incidence rates as follows: aspirin (46.26/10000 fetuses) and ibuprofen (106.95/10000 fetuses) compared to control animals (5.38-19.72/10000 fetuses). According to the authors, the predicted incidence is higher than the control data predictions in studies reported by Pfizer (9.59/10000), Middle Atlantic Reproduction and Teratology Association (19.72/10000), WIL Research Laboratories (4.56/10000), and Cappon et al. (5.38/10000).

Publication Title: Functional Activity of Mouse Sperm was not Affected by Low Doses of Aspirin-like Drugs (Stutz et. al, 2000)

Methods:
There was no information regarding where ibuprofen was obtained or its purity. The study was conducted in (b) (4) Albino Swiss mice were divided into different dose groups. Group 1 was female mice housed with males without treatment for 4 days. Pregnant females were selected and treated from Day 5 to Day 18 of pregnancy. The males from this dam were them studied after reaching sexual maturity (approximately 70 days after birth). Group 2 was adult males (70 days old) treated with ibuprofen (5.6 mg/kg/day, IP) for 35 days (time to cover epididymal transit). Group 3 was adult males (70 days old) treated with ibuprofen (5.6 mg/kg/day, IP) for 60 days (time to cover

spermatogenesis and epididymal transport).

Results:
The following figure illustrates the sperm parameters (motile, swollen, and viable) in the

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different ibuprofen treatment groups (from the publication):

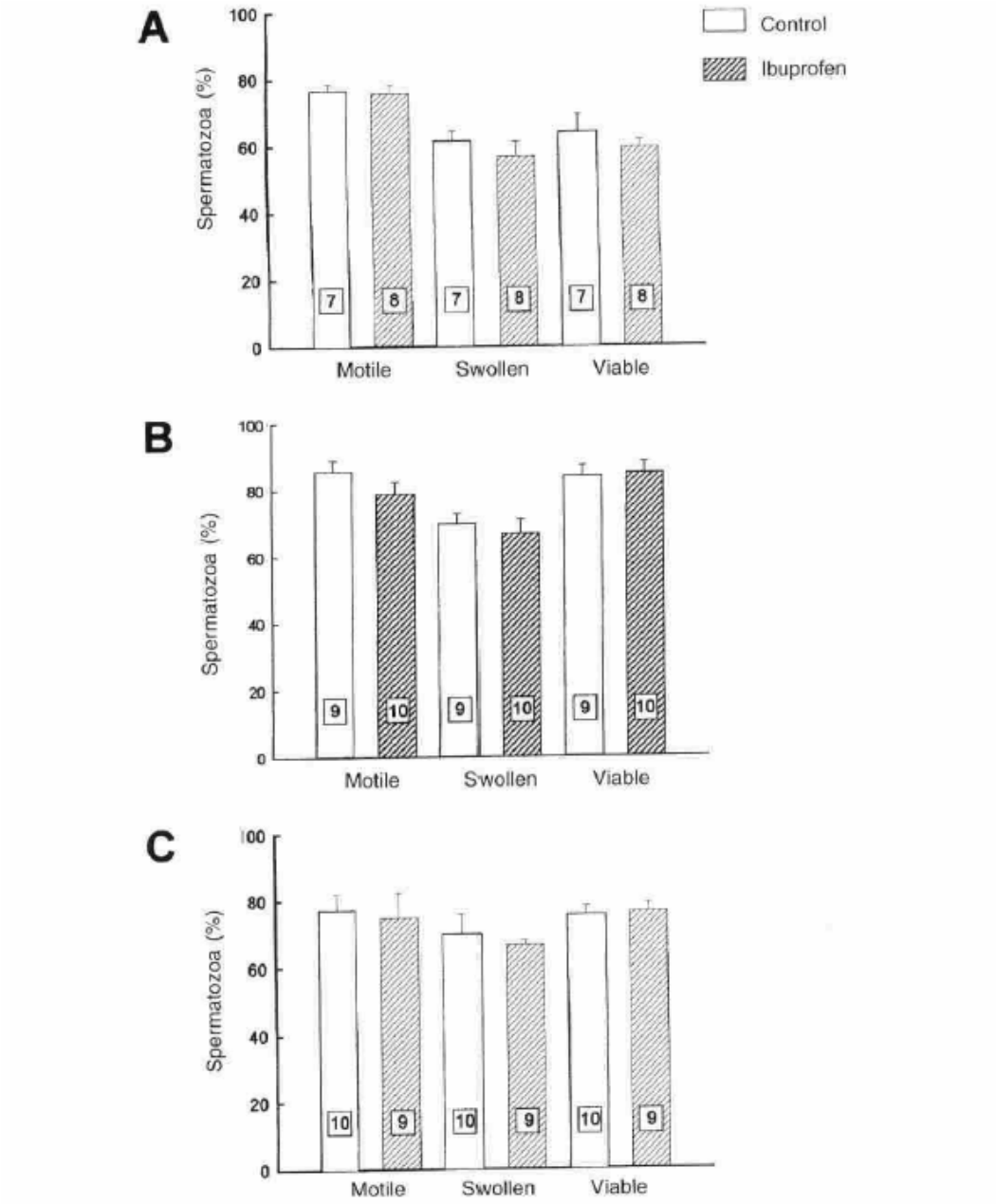


Figure 2. Effect of ibuprofen (ip, 5.6 mg/kg day^{-1}) administered to female mice from the 5th to 18th day after 4 days housed with males. Pregnancy: (A) male cohort assayed at 70 days from birth; adult male mice during (B) 35 days; or (C) 60 days on the percentage of motile, swollen, and viable spermatozoa. Control animals were injected with the vehicle (propylene glycol) in the same volume and period. Results are expressed as means ± standard errors. Numbers in boxes indicate number of animals.

As shown in the figure above, ibuprofen did not affect the sperm parameters in any of the treatment groups.

The following figure illustrates the plasma levels of testosterone after treatment with

ibuprofen (from the publication):

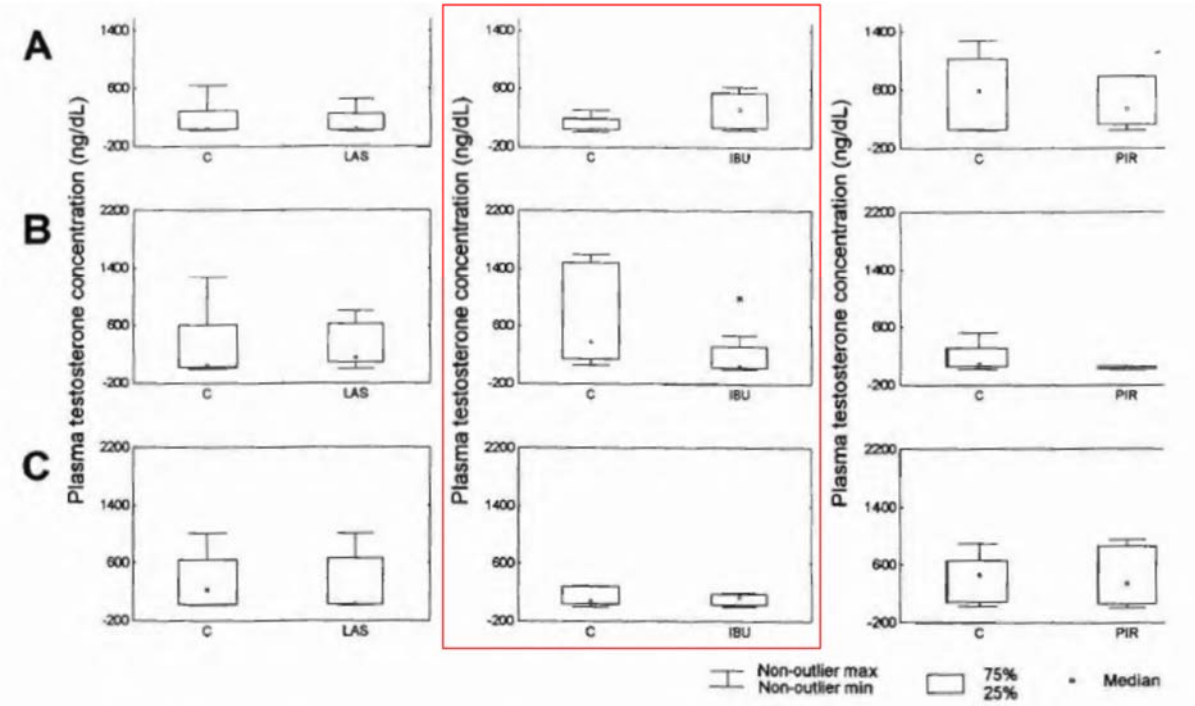


Figure 4. Effect of lysine acetyl salicylate (LAS) (im, 14.3 mg/kg day^{-1}), ibuprofen (IBU) (ip, 5.6 mg/kg day^{-1}), or piroxicam (PIR) (ip, 0.28 mg/kg day^{-1}) administered to female mice from the 5th to 18th day after 4 days housed with males. Pregnancy: male cohort assayed at (A) 70 days from birth and at adulthood during (B) 35 or (C) 60 days on the plasma testosterone levels. Control animals were injected with the vehicle (distilled water, propylene glycol, and dimethilsulfoxide, respectively) in the same volume and period. $n = 8$ in all the treatments. *$p < .05$ vs control.

As shown in the figure above, plasma testosterone levels were significantly diminished after treatment with 5.6 mg/kg/day of ibuprofen for 35 days in adult male mice. There was no change in the testosterone levels in adult male mice treated with 5.6 mg/kg/day of ibuprofen for 60 days.

Reviewer's Comments:

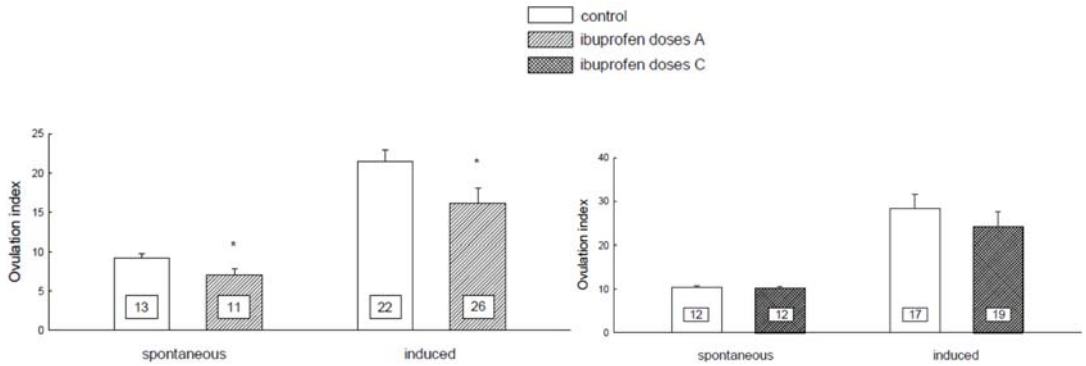
Ibuprofen was administered via the IP route, which may result in similar pharmacokinetics as oral administration because uptake of ibuprofen is through absorption in the gut, possibly small intestine. However, IP administration does not address exposure in the esophagus and stomach as ibuprofen passes these tissues. The study has some notable features mainly exposure of ibuprofen to cover epididymal transit and spermatogenesis in mice and the effect of ibuprofen in male pups via maternal exposure. The mouse dose of 5.6 mg/kg/day represents a human dose of 27.2 mg in an average human weighing 60 kg, based on a body surface area comparison. The mouse dose of 5.6 mg/kg/day yields an exposure margin of 0.0085 based on the maximum daily dose of 3200 mg for ibuprofen.

Publication Title: Chronic Administration of Nonsteroid-Antiinflammatory Drugs

(NSAIDs): Effects upon Mouse reproductive Functions (Martini et. al, 2008)

Methods:
Male or female Albino Swiss mice (70 days old) were injected daily for 35 or 60 days, respectively, with either IP ibuprofen (dissolved in propylene glycol in NaCl solution) at doses of 5.6, 11.2 or 16.8 mg/kg/day or IP piroxicam (dissolved in DMSO in NaCl solution) at doses of 0.28, 0.56 or 0.84/kg/day. Female fertility parameters evaluated were spontaneous and induced ovulation, oocyte maturity, and spermatozoa migration through the female genital tract. Male fertility parameters examined were epididymal spermatozoa concentration, motility, viability, resistance to hypoosmotic shock, acrosomal status, and membrane maturity. Additional parameters that were evaluated included in vitro and in vivo fertilization, reproductive hormones plasma levels, and cyclooxygenase inhibition in reproductive tissues. Ibuprofen was obtained from (b) (4) . with no mention of purity.

Results:
The following tables and figures summarize the effects of ibuprofen on female reproductive function (from the publication):



Ovulation indices (# of oocytes/females) of adult female mice injected for 35 days with ibuprofen. Dose A is 5.6/mg/kg/day and Dose B is 16.8 mg/kg/day.

As seen in the above figure, ibuprofen at a dose of 5.6 mg/kg/day but not 16.8 mg/kg/day produced a reduction in spontaneous and induced ovulation rates. Oocyte maturation, FSH levels on oestrus day, and sperm parameters in the uterus and oviduct

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were not affected by ibuprofen administration.

Variables	IBUPROFEN				PIROXICAM			
	doses A		doses C		doses A		doses C	
	Control	NSAIDs	Control	NSAIDs	Control	NSAIDs	Control	NSAIDs
% of in vitro fertilized oocytes	81, 9% (199)	82.6% (218)	90.7% (237)	89.0% (137)	85.4% (226)	81.7% (274)	80.3% (132)	83.5% (91)
In vivo % of pregnant mice	76.9% (13)	91.7% (12)	80.0% (5)	60.0% (10)	80.0% (10)	70.0% (10)	70.0% (10)	65.0% (10)
Number of fetuses	8.4 ± 0.4 (10)	9.5 ± 0.7 (11)	9.2 ± 0.5 (4)	8.5 ± 0.2 (6)	9.6 ± 0.7 (8)	8.1 ± 1.2 (7)	9.2 ± 1.1 (6)	9.0 ± 1.0 (5)

Ibuprofen (doses A: 0.56 mg/100g/day or doses C: 1.68 mg/100g/day; i.p.) or piroxicam (doses A: 0.028 mg/100g/day or doses C: 0.084 mg/100g/day; i.p.) were injected daily to adult female mice for 35 days. Control animals were injected with the solvent (propylene glycol or dimethylsulfoxide respectively) in the same volume and period. In vitro fertilization indices, are expressed as percentages of fertilized oocytes that were obtained by ampullar puncture after induced superovulation. In vivo fertilization indices are expressed as percentages of pregnant mice after housing for 4 days (one oestral cycle) with untreated males; 12 days after, females were sacrificed and the number of fetuses was determined. Results are expressed as percentages or Mean ± SEM. In parentheses: number of oocytes or female mice evaluated.

SPERM VARIABLE	IBUPROFEN				PIROXICAM			
	CONTROL UTERUS	CONTROL OVIDUCT	UTERUS	OVIDUCT	CONTROL UTERUS	CONTROL OVIDUCT	UTERUS	OVIDUCT
Progressive (%)	45.7 ± 6.1 [a] (12)	20.0 ± 11.5 [a] (3)	46.2 ± 5.7 [b] (9)	7.0 ± 7.0 [b] (2)	43.6 ± 6.5 (8)	25.0 ± 14.4 (3)	22.9 ± 8.3 (9)	---
Non-progressive (%)	10.5 ± 2.9 (12)	13.3 ± 6.7 (3)	12.8 ± 2.7 (9)	13.5 ± 13.5 (2)	12.9 ± 2.1 (8)	11.0 ± 11.0 (3)	15.8 ± 4.8 (9)	—
Non-motile (%)	43.7 ± 5.1 [c] (12)	66.7 ± 17.6 [c] (3)	41.1 ± 5.7 [d] (9)	79.5 ± 6.5 [d] (2)	43.6 ± 5.1 (8)	64.0 ± 7.4 (3)	61.2 ± 8.9 (9)	—
Viable (%)	47.7 ± 6.3 [e] (12)	24.3 ± 5.0 [e] (10)	49.6 ± 6.6 [f] (10)	27.1 ± 6.9 [f] (7)	56.9 ± 6.2 [g] (8)	33.1 ± 5.6 [g] (7)	40.0 ± 7.0 [h] (9)	18.4 ± 3.4 [h] (5)
Acrosome reacted (%)	51.4 ± 10.2 (11)	70.3 ± 13.0 (6)	54.1 ± 12.2 (9)	74.1 ± 7.5 (8)	33.3 ± 2.7 [i] (7)	55.5 ± 4.7 [i] (8)	32.4 ± 6.0 [j] (7)	60.3 ± 8.3 [j] (5)

Ibuprofen (doses A: 0.56 mg/100g/day; i.p.) or piroxicam (doses A: 0.028 mg/100g/day; i.p.) were administered to adult female mice during 35 days. Control animals were injected with the solvent (propylene glycol or dimethylsulfoxide respectively) in the same volume and period. The spermatozoa were evaluated 110 min after the female accepted the untreated male for mating. Values indicate Mean ± SEM. In parentheses: number of animals evaluated. In each row, identical letters indicate significant differences ($p < 0.05$).

The following summary table illustrates the effects of ibuprofen on epididymal

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spermatozoa (from the publication):

SPERM VARIABLE	IBUPROFEN		PIROXICAM	
	CONTROL	NSAIDs	CONTROL	NSAIDs
Body weight (g)	27.6 ± 0.5 (24)	27.5 ± 0.5 (19)	26.7 ± 1.1 (10)	26.5 ± 0.5 (13)
Concentration (x 10^6/ml)	11.6 ± 0.9 (37)	11.1 ± 1.0 (30)	15.3 ± 1.9 (20)	14.6 ± 2.1 (18)
Motile (progressive + non-progressive) (%)	63.7 ± 2.2 (30)	62.8 ± 2.8 (29)	74.2 ± 2.0 (17)	67.2 ± 3.1 (18)
Viable (%)	71.3 ± 2.1 (16)	71.7 ± 2.2 (16)	73.2 ± 1.3 (17)	75.6 ± 2.4 (18)
Swollen (HOST) (%)	60.3 ± 3.1 (17)	57.5 ± 3.6 (15)	69.9 ± 1.4 (17)	69.4 ± 2.2 (17)
Acrosome intact (%)	78.9 ± 1.1 (8)	77.4 ± 3.5 (8)	88.4 ± 1.4 (9)	88.9 ± 1.2 (10)
Bending and/or with cytoplasmatic drop (%)	17.0 ± 1.9 (16)	17.4 ± 2.0 (19)	14.4 ± 1.7 (24)	17.4 ± 2.3 (22)

Ibuprofen (doses B: 1.12 mg/100g/day; i.p.) or piroxicam (doses B: 0.056 mg/100g/day; i.p.) were administered to adult male mice for 60 days. Control animals were injected with the solvent (propylene glycol or dimethylsulfoxide respectively) in the same volume and period. The spermatozoa were obtained from the caudal epididymis. Values indicate Mean ± SEM. In parentheses: number of animals. HOST= hypoosmotic swelling test.

No effects on epididymal spermatozoa was observed after adult mice were treated for 60 days with IP ibuprofen at a dose of 11.2 mg/kg/day

In vitro fertilization index of spermatozoa obtained from males was significantly lower than vehicle controls (from the publication). No effect on proportion of pregnant females mated with treated males or litter sizes were reported when comparing ibuprofen-treated males versus control males.

Variables	IBUPROFEN				PIROXICAM			
	doses B		doses C		doses B		doses C	
	Control	NSAIDs	Control	NSAIDs	Control	NSAIDs	Control	NSAIDs
% of in vitro fertilized oocytes	73.5%[a] (196)	59.1%[a] (171)	66.1% (121)	62.4% (117)	80.9% (89)	73.8% (126)	83.0% (94)	85.5% (124)
% of pregnant mice	13.0% (23)	43.7% (16)	21.4% (14)	37.5% (16)	33.3% (18)	21.4% (14)	14.3% (14)	16.7% (12)
litter size	7.0 ± 1.5 (3)	7.9 ± 1.0 (7)	8.7 ± 0.3 (3)	7.8 ± 1.2 (6)	6.8 ± 1.6 (6)	3.3 ± 0.9 (3)	7.5 ± 2.5 (2)	8.5 ± 0.5 (2)

Ibuprofen (doses B: 1.12 mg/100g/day or doses C: 1.68 mg/100g/day; i.p.) or piroxicam (doses B: 0.056 mg/100g/day or doses C: 0.084 mg/100g/day; i.p.) were injected to adult male mice for 60 days. Control animals were injected with the solvent (propylene glycol or dimethylsulfoxide respectively) in the same volume and period. In vitro fertilization indices, are expressed as percentages of fertilized oocytes that were obtained by ampullar puncture after induced superovulation. In vivo fertilization indices are expressed as percentages of untreated pregnant mice after housing for 4 days (one estral cycle) with treated males; 12 days after, females were sacrificed and the number of fetuses was determined. Results are expressed as percentages or Mean ± SEM. In parentheses: number of oocytes or female mice evaluated. a= $p < 0.05$.

It was also reported that plasma concentrations of FSH and testosterone in males were not altered by ibuprofen treatment.

Reviewer's Comments:

This study was an extension of the Stutz et al. (2000) paper, which previously examined

lower doses of ibuprofen on predominantly male fertility parameters. In this study by Martini et al., (2008), female fertility parameters in addition to male parameters were evaluated. In addition, higher doses of ibuprofen were examined in this study. In males, similar results were obtained as the last study by Stutz et al. in which sperm parameters and resulting litter sizes were not altered by ibuprofen treatment; however, there were no differences in testosterone plasma concentrations in the ibuprofen-treated males. In females, ibuprofen treatment resulted in changes in ovulation indices at the low dose but not high dose with no changes in sperm parameters when analyzed from the uterus or oviduct. The authors described this dose independent effect on ovulation has been reported previously and may be due to the different prostaglandins that are inhibited and due to the synthesis of prostaglandins recovering at different rates. It was also discussed that unchanged in vivo fertilization rates appears to be contradictory with lower ovulation indices. However the authors reconcile this contradiction by stating that the study was designed to avoid implantation inhibition and/or abortions, ibuprofen injections were interrupted the day before male and female were mated.

Publication Title: Effect of Ibuprofen and Naproxen on Implantation and Pregnancy in Rat (Gupta et. al, 1984)

Methods:

Ibuprofen was obtained from (b) (4) with no purity information. The study was conducted in (b) (4) Female albino rats of established fertility were left overnight with male rats of proven fertility. The next morning, mating was confirmed by clumps of spermatozoa in the vaginal smear, which is considered Day 1 of pregnancy. Ibuprofen was administered orally on Days 3, 4, and 5 of pregnancy in two dose groups, 20 mg/100 g bw and 30 mg/100 g bw, which is 200 mg/kg and 300 mg/kg. The number of implantation sites and number of viable fetuses were counted on Day 10 and Day 19 of pregnancy, respectively

Results:

The following table illustrates the number of implantation sites and the number of viable

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fetuses following ibuprofen administration (from the publication):

Table 2—Effect of Oral Administration of Ibuprofen on Pregnancy in Rat

[Values are mean ± SE of 9 animals]

Treatment	No. of animals with implantation	No. of animals with resorption sites	No. of implantation sites (Day 10)	No. of fetuses (Day 19)
Control (5% $NaHCO_3$)	9	0	8.77 ± 0.52	8.00 ± 0.53
Ibuprofen (20 mg/100 g)	6	1	3.71 ± 1.46**	3.28 ± 1.28*
Ibuprofen (30 mg/100 g)	3	3	3.44 ± 1.14***	0.11 ± 0.11***

P values: * < 0.02; ** < 0.05; *** < 0.001

As shown in the table above, there is a dose-dependent decrease in the number of implantation sites and number of fetuses.

Reviewer's Comments:

The dosing of ibuprofen was not sufficient to study male and female fertility and any possible effects on sperm characteristics such as motility. The doses used in the study is equivalent to the human doses of 1946 and 2919 mg in an average human weighing 60 kg, based on a body surface area comparison. This study was useful to study implantation sites and the number of fetuses. The rat doses used in this study yield exposure margins of 0.6 and 0.9 based on the maximum daily dose of 3200 mg for ibuprofen. These data confirm the general statements made in the NSAID class labeling; therefore, it does not appear to be necessary to include the specific details in the product labeling.

Integrated Summary and Safety Evaluation

Section 8.1: Pregnancy

Embryo-Fetal Development

The 1994 Motrin® labeling only states that (b) (4) The current Motrin® labeling states "Reproductive studies conducted in rats and rabbits have not demonstrated evidence of developmental abnormalities." This statement provides no data regarding the doses of the drugs given to the animals or the periods of development covered by the studies. It cannot be used to create a PLLR appropriate label without review of the original study reports, which are proprietary data and cannot be referenced to support a 505(b)(2) application without a letter of authorization granting the Agency permission to review the actual data in the Motrin® NDA. As such, our review must rely on the existing published

information to inform the labeling.

The Adams' data summaries from the 1969 and 1970 publications are presumably the based on the studies originally completed by Boots Pharmaceuticals, the group that discovered ibuprofen. Based on the data available to us for this submission, we cannot state if the Adams' studies are the same studies that were submitted to the FDA in support of the Motrin® NDA.

The rabbit teratology study described by Adams reports 4 pups in one high dose litter with evidence of cyclopia-related malformations. The authors dismissed this finding because it only occurred in one litter and there was no obvious dose-dependency. However, this is a rather unusual finding which unfortunately occurred in the high dose group (60 mg/kg). Therefore it is hard to dismiss the finding as irrelevant due to lack of dose dependency. Cappon et. al 2003 do report results of a rabbit study testing a higher dose of ibuprofen (200 mg/kg); however, ibuprofen was only administered on Gestation Day 9 to 11 in this study.

Cyclopia is a malformation resulting in failure of the embryonic forebrain to divide into two hemispheres. It is a rare form of holoprosencephaly characterized by the failure of the embryonic proencephalon to properly divide the orbits into two eye cavities. This results in not only the presence of a single eye in the center of the face but also one optic nerve and optic lobe in the brain (Cohen and Shiota, 2002). "Cyclopia" was not reported in the historical control database for New Zealand White Rabbits published by Charles River (2008-2010). The closest malformation reported was "close set eye socket" which occurred at a very low incidence (close-set eye socket was reported in one fetus in one litter in 5859 fetuses and 696 litters examined). That being said, cyclopia was reported in a single control animal in a study of the teratogenicity of diflunisal, published around the same time period (Clark et.al, 1984). These authors also suggest that diflunisal teratogenicity is characterized by axial skeletal defects, possibly resulting from anemia as a result of significant gastrointestinal lesions.

There are several known causes of cyclopia in humans, including genetic mutations. The toxin cyclopamine derived from the plant *Veratrum californicum* has also been reported to cause cyclopia in sheep (Keeler, 1970). Keeler also reports that administration of cyclopamine between Gestation Days 6 and 9 results in cyclopia in rabbits. That toxin did not produce cyclopia when it was dosed from Gestation Day 9 to 12 or 12 to 15, suggesting that the insult must occur early in gestation. Other causes of holoprosencephaly have been proposed, including maternal diabetes, ethyl alcohol, and retinoic acid (Cohen and Shiota, 2002).

The observation that all 4 fetuses reported with cyclopia-like malformations occur in the same litter suggests that the cause was likely something unique to that doe, possibly a genetic defect or unique toxicity, rather than a direct effect of the ibuprofen drug treatment. Individual animal data are not available to know details of that particular doe; however, the authors do note that the 60 mg/kg dose produced stomach ulcers and a few animals also had "pneumonia and a mild degree of focal hepatitis that was probably

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due to infection secondary to the gastric lesions." This significant maternal toxicity, that likely includes anemia from the stomach ulcers, confounds the study result interpretation. Given the finding of cyclopia restricted to a single litter, and the lack of a signal for cyclopia in other NSAIDs, it seems unlikely that the finding is related to ibuprofen. Likewise, even if the finding was related to ibuprofen, the severity of the maternal toxicity present in this dose suggests limited potential for clinical relevance. As such, we do not recommend that this finding be included in the product label. In the absence of other clear adverse effects, the Adams study results appear reasonable to include in the product labeling in order to provide greater detail, including exposure margins.

The findings reported by Cappon et. al (2003) of an increased incidence of membranous ventricular septal defects (VSD) in rats treated with ibuprofen during the critical periods of heart formation appear to present a finding that was not previously identified in the original embryo-fetal development studies conducted to support Motrin® and/or by Boots Pharmaceuticals, likely because Cappon et al were able to obtain higher doses by dosing over a shorter dosing interval. Of the NSAID drugs tested by these authors, ibuprofen did appear to increase the incidence of membranous ventricular septal defects more than the other drugs. Burdan et. al (2004) reported one incidence of membranous VSD in control animals and two in fetuses from rats treated with 255 mg/kg ibuprofen (none in the high dose group). In a more comprehensive evaluation of the multiple studies, Burdan et. al report that there appears to be an increase in membranous VSD in rats treated with aspirin and ibuprofen compared to background incidence (Burdan et. al, 2006). This effect is thought to be associated with drugs that block COX-1 more than drugs that are selective COX-2 inhibitors, possibly since the ventricular septum development occurs between GD8 and 16 and COX-2 expression does not appear to occur in rats until GD16 and is specifically located in the skin, heart, cartilage, and kidney fetuses at this time; whereas COX-1 is expressed throughout the embryonic and fetal periods. Although it is possible that maternal toxicity contributed to this finding, we believe it is prudent to include these data in the product labeling to enhance the ability to detect such an association in humans, should one exist.

Burdan and colleagues also report data for high doses of ibuprofen (≥225 mg/kg) administered from Day 8 to 21 of gestation resulted in reduced fetal weight and length and reduced ossification in many bones and increased postimplantation losses. The significance of these findings is complicated by the fact that there was significant moribundity and even 20% mortality was also noted at these doses. In surviving dams, profound stomach changes were noted as well as intestinal lesions in the illium. When these lesions resulted in severe gastrointestinal lesions, including perforations, hepatic lesions were also noted. Although these doses are below the human equivalent doses based on a body surface area comparison, the clinical significance of these adverse effects is questionable given the greater sensitivity of the animal models to the gastrointestinal effects of NSAIDs, which is particularly true for ibuprofen. Given the extent of the maternal toxicity and the unclear clinical relevance, these findings need not

be included in product labeling.

Pre- and Postnatal Development

There is no reference to a pre- and postnatal development study in the original Motrin® labeling. Adams (1969) does suggest that Boots Pharmaceuticals did conduct a study in rats that could be considered similar to a pre- and postnatal development study. Adams dosed pregnant female rats throughout gestation until parturition. The young were examined 3 weeks after delivery. According to the paper, "Litters were reduced to 9 at birth (the normal practice in our breeding colony), and any underweight or neglected young were killed during the suckling period. All were examined in detail for gross abnormalities. From the litter records, the viability index at weaning was estimated." The authors report that there was no impact on litter size, viability index, or weaning weight. However, as it is not clear if underweight animals were omitted or not, the results of this study cannot be included in the product labeling as an adequate pre- and postnatal development study.

A search of the published literature failed to identify any other published information that could be used to characterize the effect of ibuprofen on pre- and postnatal development. Given the impact of ibuprofen on the ductus arteriosis, a standard study design could not be easily completed with any expectation of useful data.

Juvenile Animal Data

We have not identified any juvenile animal studies in the published literature.

Section 13.1: Carcinogenesis, Mutagenesis, and Impairment of Fertility

Section 13 should be updated to reflect the existing knowledge, if available.

Carcinogenesis. The original Motrin® labeling does not contain any carcinogenicity data. There is a published reference article describing studies conducted by Boots Pharmaceuticals (Adams et al., 1970); however, these studies do not appear to have been included in the Motrin® labeling and do not appear to be adequate by current standards. No additional published studies were identified in the published literature. The section should state that ***adequate*** long-term animal studies to evaluate the carcinogenic potential of ibuprofen have not been completed.

Mutagenesis. The original Motrin® labeling does not include any genetic toxicology data. The two published Ames assays from Oldham et al., 1986 and Philipose et al., 1997 demonstrated that ibuprofen is not mutagenic in what could collectively be called a standard set of bacterial strains by modern standards. These data should be included in the product labeling. Although Philipose did include data from an in vivo sister chromatid exchange assay, this assay has fallen out of favor (Dearfield et. al, 2011) and is no longer even listed in ICH S2(R1). Further, several publications were identified that

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suggest that ibuprofen is not clastogenic in humans; therefore the rat clastogenicity data are not necessary for product labeling. As per the regulations, human data cannot be included in Section 13; therefore, the negative human data will not be added to labeling either.

Impairment of Fertility. The original Motrin® labeling did not contain any specific data or information regarding the effects of ibuprofen on fertility and/or early embryonic development. As a result our review will only rely on the existing published information to inform the label. Adams et. al (1969) summarizes findings from a fertility study in which 10 male and 20 female rats were dosed with ibuprofen in the diet at a dose level of approximately 20 mg/kg or normal feed for 8-weeks prior to and presumably during the 2-week mating period.

The rat fertility study described by Adams reported that 15 of 20 (75%) females receiving ibuprofen were pregnant and 16 of 18 (89%) control females were pregnant after the 14-day mating period. Although female fertility appears reduced, one male was considered infertile. After taking into consideration that 2 of the 5 failed pregnancies were considered to be the result of a single incidence of male infertility, the female fertility index can be adjusted to 15 of 18 (83%) females becoming pregnant, which does not appear to be different than 16 of 18 (89%) becoming pregnant in controls. In another study by Stutz et al. (2000), adult males were dosed with ibuprofen for 35 days or 60 days to determine if ibuprofen affected epididymal transit or spermatogenesis and epididymal transit, respectively. There were no effects on any sperm parameters examined, which included motility, response to hypoosmotic shock, and viability, after ibuprofen treatment. In a follow-up study by Martini et al. (2008), male and female fertility parameters were examined. Similar results were obtained in males supportive of the previous data collected. In females, the only notable effects from ibuprofen IP administration for 35 days were a reduction in ovulation indices, both induced and spontaneous. Taken together, these data suggest that female ovulation is affected by ibuprofen treatment whereas male fertility, resulting litter sizes and sperm characteristics are not significantly impacted by ibuprofen administration. These results should be included in the updated product labeling.

Conclusion and Recommendation:

The following labeling recommendations are based on the literature review above:

Recommended Changes	Rationale/Comment/Source
8 Use in Specific Populations	
8.1 Pregnancy	

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Risk Summary	
In published animal reproduction studies, there were no clear developmental effects at doses up to 0.4-times the maximum recommended human dose (MRHD) in the rabbit and 0.5-times in the MRHD rat when dosed throughout gestation. In contrast, an increase in membranous ventricular septal defects was reported in rats treated on Gestation Days 9 & 10 with 0.8-times the MRHD.	Risk summary based on Adams et. al (1969) and Cappon et. al (2003)
Based on animal data, prostaglandins have been shown to have an important role in endometrial vascular permeability, blastocyst implantation and decidualization. In animal studies, administration of prostaglandin synthesis inhibitors such as ibuprofen, resulted in increased pre- and post-implantation loss.	NSAID Class Labeling Nonclinical Risk Summary Statement
Data *Animal data*	
In a published study, female rabbits given 7.5, 20, or 60 180 mg/kg ibuprofen (0.04, 0.12, or 0.36-times the maximum human daily dose of 3200 mg of ibuprofen based on a body surface area comparison) from Gestation Days 1 to 29, no clear treatment-related adverse developmental effects were noted This dose was associated with significant maternal toxicity (stomach ulcers, gastric lesions In the same publication, female rats were administered 7.5, 20, 60, 180 mg/kg ibuprofen (0.02, 0.06, 0.18, 0.54-times the maximum daily dose) did not result in clear adverse developmental effects. Maternal toxicity (gastrointestinal lesions) was noted at 20 mg/kg and above.	Adams et. al (1969)

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In a published study, rats were orally dosed with 300 mg/kg ibuprofen (0.9 fold the maximum human daily dose of 3200 mg based on a body surface area comparison) during Gestation Days 9 and 10 (critical time points for heart development in rats). Ibuprofen treatment resulted in an increase in the incidence of membranous ventricular septal defects. This dose was associated with significant maternal toxicity including gastrointestinal toxicity. One incidence each of a membranous ventricular septal defect and gastroschisis was noted fetuses from rabbits treated with 500 mg/kg (3-times the maximum human daily dose) from Gestation Day 9 to 11.	Cappon et. al (2003)
13 Nonclinical Toxicology **13.1 Carcinogenesis, Mutagenesis, and Impairment of Fertility** Carcinogenesis Adequate long-term animal studies have not been conducted to evaluate the carcinogenic potential of ibuprofen.	Data from Adams et. al (1970) is not adequate by current standards to include in labeling. Carcinogenicity data were not included in the original Motrin® label.
Mutagenesis In published studies, ibuprofen was not mutagenic in the in vitro bacterial reverse mutation assay (Ames assay).	From Oldham et. al (1986) and Philipose et. al (1997) Although Philipose reports positive findings in the SCE assay in the mice, several studies examining SCE rates in human lymphocytes do not report any significant differences before and after two-week treatment duration of ibuprofen (800 to 1200 mg/day) or other NSAIDs. The rat SCE data need not be included in labeling.
Impairment of Fertility In a published study, dietary administration of ibuprofen to male and female rats 8-weeks prior to and during mating at dose levels of 20 mg/kg (0.06-times the MRHD based on a body surface area comparison) did not impact male or female fertility or litter size.	Adams et. al (1969)
In other studies, adult mice were administered ibuprofen at a dose of 5.6 mg/kg/day IP (0.0085-times the MRHD based on a body surface area comparison) for 35 or 60 days in males and 35 days in females. There was no effect on sperm motility or viability in males but decreased ovulation was reported in females.	From Stutz et. al (2000) and Martini et. al (2008)

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/s/

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12/05/2017

NEWTON H WOO
12/05/2017

RICHARD D MELLON
12/05/2017
I concur.