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Registration Action Branch 1

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Rat
OPPTS 870.7485 [§85-1]

DP BARCODE: D245135
P.C. CODE: 067710

SUBMISSION CODE: S539237
TOX. CHEM. NO.:

TEST MATERIAL (PURITY): Indeno[1,2-*e*][1,3,4]oxadiazine-4a(3*H*)-carboxylic acid, 7-chloro-2,5-dihydro-2-[[methoxycarbonyl)[4-(trifluoromethoxy)phenyl]amino]carbonyl],methyl ester, (R,S)- (purity 96.0%)

SYNONYMS: (R,S)-7-Chloro-3-[methoxycarbonyl-(4-trifluoromethoxy-phenyl)-carbamoyl]-2,5-dihydro-indeno[1,2-*e*][1,3,4]oxadiazine-4*a*(3*H*)-carboxylic acid methyl ester; DPX-MP062; DPX-JW062-33; DPX-JW062-119; DPX-JW062EL; (R,S)-methyl7-chloro-2,5-dihydro-2-[[(methoxycarbonyl)[4-(trifluoromethoxy)phenyl]amino]carbonyl]indeno[1,2-*e*][1,3,4]oxadiazine-4*a*(3*H*)-carboxylate

DPX-MP062 is a mixture of ~75% DPX-KN128 (S-isomer) and ~25% DPX-KN127(R-isomer)
DPX-JW062 is a racemic mixture of DPX-KN128 (S-isomer) and DPX-KN127(R-isomer)

CITATION: Himmelstein, M. (1997) ^{14}C-DPX-MP062 (a 3:1 mixture of DPX-KN128 and IN-KN127): Metabolism in the Rat. Haskell Laboratory Report No. HL-1997-00439. E.I. duPont de Nemours and Co. Newark, DE. MRID 44477152.

Himmelstein, M. (1997) ^{14}C-DPX-JW062 (a racemic mixture of DPX-KN128 and IN-KN127): Metabolism in the Rat. Haskell Laboratory Report No. 283-96. E.I. DuPont de Nemours and Co. Newark, DE. MRID 44477153.

SPONSOR: E.I. DuPont de Nemours and Co. Wilmington, DE.

EXECUTIVE SUMMARY: In a metabolism and disposition study, groups of Crl:CD®(SD)BR rats (3-8/sex/dose depending on the experiment) were given DPX-MP062 (96% purity) or DPX-JW062 (98.6-99.6% purity) in PEG-400 by gavage (Haskell Laboratory Sample Numbers provided but no batch numbers provided). Dosing regimens for DPX-JW062 included a single low dose (5 mg/kg) and a single high dose (150 mg/kg) in male and female rats, and a 14-day repeated low dose (5 mg/kg/day) in female rats only. For the single-dose experiments two different radiolabels were used; [indanone-1-^{14}C]DPX-JW062 and [trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062. The 14-day repeated dose experiments used only [trifluoromethoxyphenyl(U)-^{14}C]DPX-

JW062. Biliary excretion experiments were conducted in male and female rats given either a single low dose (5 mg/kg) or a single high dose (150 mg/kg) of [indanone-1-^{14}C]DPX-JW062, or a single low dose of [trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062. For DPX-MP062, only a single low-dose (5 mg/kg) experiment was conducted to determine pharmacokinetics, tissue residues, and metabolite profiles. Intravenous administration studies were not conducted due to the limited solubility (<20 ppb) of the test material. Expired air was not collected because results of pilot studies indicated that expired air contained no CO_2 or volatile metabolites.

There were no deaths that could be attributed to the test material. Total recovery of administered radioactivity in the experimental groups ranged from 90-98%. Both DPX-JW062 and DPX-MP062 were rapidly absorbed and eliminated following oral administration. The absorption of DPX-JW062 was dose dependent and appeared be saturated at the high dose. Both DPX-JW062 and DPX-MP062 were widely distributed following oral administration. The distribution pattern did not vary with dosing regimen and overall tissue burden was limited to only 3.4-12.9% of the administered dose. Fat tissue contained the greatest level of radioactivity (1.76-8.76% of the administered dose) and, for both compounds, was greater in female rats. Clearance from this tissue was notably extended in females following administration of a single oral dose. For all doses and regimens, the remaining tissues contained <1% of the administered radioactivity at 168 hours after dosing. Most of the absorbed dose of either DPX-JW062 or DPX-MP062 was excreted via the urine within 96 hours regardless of dosing regimen. Most (85-86%) of fecal elimination occurred within 48 hours. Due to saturated absorption at the high dose, urinary and biliary excretion were correspondingly lower. Radioactivity associated with the feces included unabsorbed material as well as biliary excretion of absorbed radioactivity (50-61% of total radioactivity in the feces). Gender-specific variability was observed for the fecal and urinary elimination of DPX-MP062 but not for DPX-JW062.

Studies assessing whole blood, plasma and blood cell kinetics provided values for elimination half-time ($t_{1/2}$), area-under-the-curve (AUC), mean time to maximum concentration (tC_{max}), half-peak time ($tC_{max/2}$), maximum concentration (C_{max}) and half maximum concentration ($C_{max/2}$). For DPX-MP062 and low dose [indanone-1-^{14}C]DPX-JW062, plasma tC_{max} and $tC_{max/2}$ were similar for males and females but for the high doses and the [trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062 these indices were greater for female rats. Plasma $t_{1/2}$ values were notably shorter for male rats than for female rats at both doses and for both radiolabels. Pharmacokinetic findings were consistent with a dose-dependent absorption. The plasma $t_{1/2}$ values for the trifluoro-methoxyphenyl(U) were quite variable and inexplicably long for female rats.

Metabolite studies revealed that both DPX-MP062 and DPX-JW062 were extensively metabolized and that the metabolites were eliminated in the urine, feces, and bile. With the exception of parent compound (DPX-JW062, which accounted for 19.2% of a single low dose in the feces of female rats), none of the metabolites from any source represented more than 12.3% of the administered dose. The metabolite profile for DPX-JW062 was dose dependent and varied quantitatively between males and females. Differences in metabolite profiles were also observed for the different label positions. For DPX-JW062, none of the biliary metabolite retention times matched those of fecal metabolites, thereby indicating that all of the biliary metabolites undergo

further biotransformation in the gut. Biliary excretion studies were not performed for DPX-MP062. A metabolic pathway was proposed for both DPX-MP062 and DPX-JW062.

This metabolism study in the rat is classified as acceptable and does satisfy the guideline requirement for a metabolism study (85-1) in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided in both study reports.

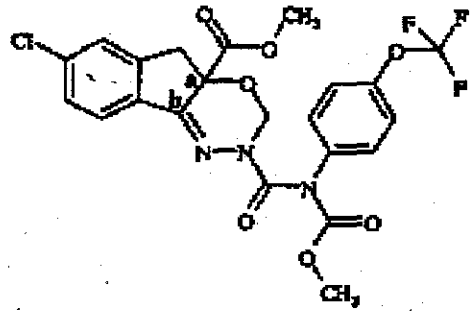
I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound

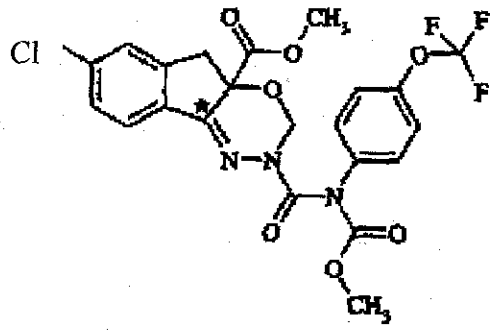
Purity: 96% (^{14}C-DPX-MP062); 98.6-99.6% (^{14}C-DPX-JW062 depending on sample)
Lot No.: Haskell Sample No. 22071 (^{14}C-DPX-MP062); Haskell Sample Nos. 20406, 20822, 20822-2, 21764, 22122 (^{14}C-DPX-JW062), no batch or lot numbers were provided
Description: white solid (^{14}C-DPX-JW062); white solid (^{14}C-DPX-MP062)
Contaminants: none noted
Stability: stated to be stable (data not provided)
CAS No.: 144171-61-9
Structure:

DPX-MP062



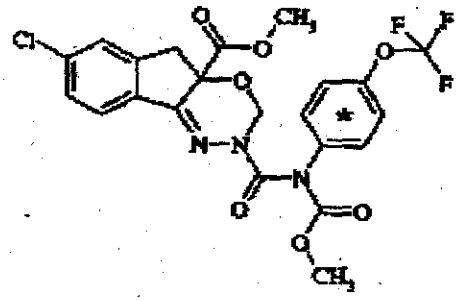
[a] Denotes position of chiral center
[b] Denotes position of ^{14}C Label

[indanone-1-^{14}C]DPX-JW062



* Denotes position of ^{14}C Label

[trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062



* Denotes position of ^{14}C Label

2. Vehicle

PEG-400 was used as the vehicle for both DPX-MP062 and DPX-JW062.

3. Test animals

Species: rat
Strain: Crl:CD®(SD)BR
Age and weight at study initiation:
males: 7-12 weeks; 179-267 g (all experiments)
females: 7-12 weeks; 166-242 g (all experiments)
Source: Charles River Laboratories, Inc., Raleigh, NC. (all experiments)
Housing: Housed individually in either glass metabolism cages or stainless steel cages
Diet: Purina® Certified Rodent Chow® #5002 (Ralston Purina Co.) or Rodent Pellet Certified Formula A/1 Chow (P.J. Noyes Co., Inc.) *ad libitum*
Water: tap water provided *ad libitum* (water analyzed for total bacteria count and presence of coliforms, lead, and other contaminants

Environmental conditions:
Acclimation period: Rats were held in quarantine for at least 5 days
Temperature: 23±2°C (metabolism chamber)
Humidity: relative humidity 50±10%
Air changes: not provided
Photoperiod: 12-hr light/12-hr dark

4. Preparation of dosing solutions

For DPX-MP062, dose solutions were prepared by dissolving an appropriate amount of the test material in PEG-400 and sonicating at 40°C for 1-4 hours. For DPX-JW062, the test material was initially dissolved in acetonitrile (later removed by nitrogen convection) prior to dissolution in PEG-400. The test material was again dissolved in PEG-400 and sonicated as described for DPX-MP062. The radioactivity concentration of the dose solutions was verified by liquid scintillation counting (LSC). The overall average dpm value for three samples of three diluted aliquots was used to calculate the radioactivity concentration (μCi/g). Chemical stability, concentration, and radioactivity were verified before and after administration to the test animals. HPLC analysis showed that the dosing solutions maintained chemical concentration, radiochemical purity and radioactive concentration throughout the dosing period and for an extended period (1-2 months). No degradation products were observed following more than two-months storage at -20°C.

B. STUDY DESIGN AND METHODS

1. Group arrangements

Animals of similar age and body weight were randomly assigned to the definitive experimental groups described in Table 1.

Pilot Studies: Prior to conducting the definitive experiments described in Table 1, pilot studies were conducted to evaluate dosing vehicles, determine the stability of ^{14}C-DPX-JW062, provide urine and fecal samples for extraction, develop analytical procedures, determine major urinary and fecal metabolites, and provide information on the routes and rates of excretion. Data from the pilot studies were not presented in the studies reviewed (MRID 44477152 and -53) in this Data Evaluation Report.

TABLE 1. Dosing groups for metabolism and disposition studies with DPX-MP062 and DPX-JW062.

Test Group	Dose (mg/kg)[a]	Number/sex	Remarks[b]
Exp. 1	5[c] 150[c] 5[d] 150[d]	5M/4F 3M/3F 3M/3F 4M/3F	time-course in blood; single oral administration of [indanone-1-^{14}C]DPX-JW062 or trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062; jugular vein cannulated (one day recovery period)
Exp. 2	5[c] 150[c] 5[d] 150[d]	5M/5F 5M/5F 5M/5F 5M/5F	excretion kinetics, tissue residue; single oral administration of [indanone-1-^{14}C]DPX-JW062 or trifluoromethoxy phenyl(U)-^{14}C]DPX-JW062
Exp. 3	5[c] 150[c] 5[d] 150[d]	8M/8F 8M/8F 8M/8F 8M/8F	tissue residue, tC_{max}, $tC_{max/2}$ for single oral administration; 4 rats of each sex sacrificed at various time periods (see section B.2)
Exp. 4	5[c] 150[c] 5[d]	3M/4F 3M/3F 4M/4F	biliary excretion; single oral dose; DPX-JW062
Exp. 5	control	3M/3F	PEG-400 vehicle only
Exp. 7	5 (3-6μCi) daily	24F	14-day multiple oral dose to determine bioaccumulation and elimination of [trifluoromethoxy(U)-^{14}C]DPX-JW062; 3 rats each were sacrificed 24 hours after the 4th, 8th, 11th, and 14th dose. Four more groups (n=3) sacrificed at 3, 7, 14, and 21 days after last dose
Exp. 8	5 (25μCi)	5M/5F	plasma, RBC, excretion kinetics, tissue residue, and metabolite profile; single oral dose of [indanone-1-^{14}C]DPX-MP062; 3 males and 3 females were cannulated in the jugular vein for time-course study in blood; maintained for 168 hrs post-dosing

[a] For Exp. 1-5,each rat received at leats 15 μCi; [b] Intravenous administration not performed due to insolubility of test material (<20 ppb); [c] [indanone-1-^{14}C]; [d] [trifluoromethoxyphenyl(U)-^{14}C]
Note: no experiment #6 was described in the study reports

2. Dosing and sample collection

Dosing – Animals were fasted 24 hours prior to dosing and, depending on the experiment, placed individually into glass metabolism units or stainless steel cages. The test article was administered by gavage.

Sample collection – Biologic samples from the various experimental groups were collected as described below. At terminal sacrifice, the rats were killed by CO_2 asphyxiation. Blood was immediately obtained by cardiac puncture in heparinized syringes. Collection of other tissue samples followed.

Expired air– Pilot studies (summary of results presented in MRID 44477153) indicated that rats dosed with DPX-JW062 (a racemic mixture of the S- and R-isomers) eliminated <1% of the administered radioactivity in exhaled air and, therefore, expired air was not collected.

Blood – Exp. 1: Blood was collected from the jugular vein cannulae at predose, and at 0.5, 1, 2, 4, 8, 12, and 24 hours, and every 24 hours to 168 hours after administration of a single oral dose.
Exp. 2: Blood was collected at terminal sacrifice (168 hours).
Exp. 3: Blood was collected from four male and four female rats of each dose group (see Table 1) at times corresponding to the specific tC_{max} as determined in Exp. 2. Blood was also collected from an additional four rats of each sex at the time corresponding to one-half the C_{max} ($tC_{max/2}$).
Exp. 4: Not collected
Exp. 5: Blood was collected 24 hours following a single gavage dose of the vehicle, PEG-400.
Exp. 7: Whole blood was collected at terminal sacrifice.
Exp. 8: Blood (~0.2 mL) was collected from the jugular cannula at predose, 0.5, 1, 2, 4, 6, 8, 12, and every 24 hours to 168 hours. In instances where the jugular cannula became nonfunctional, blood was collected from a tail vein.

All blood samples (whole blood, plasma, and red blood cells) were refrigerated until analysis.

Urine – Exp. 1: Not collected
Exp. 2: Urine was collected over dry ice pre-dose, at 8 hours, and every 24 hours until terminal sacrifice at 168 hours.
Exp. 3: Urine was collected (no specifics provided) but not analyzed.
Exp. 4: Urine was collected over dry ice during the 48-hour experimental period.
Exp. 5: Urine was collected over dry ice for 24 hours after a single gavage dose of the vehicle, PEG-400.

Exp. 7: Urine from one group of rats (three females) was collected throughout the 14-day treatment period and for 7 days thereafter.
For Exp. 8 (single 5 mg/kg oral dose), urine samples were collected over dry ice at pre-dose, at 8 hours and ever 24 hours thereafter up to 168 hours.

Feces – Exp. 1: Not collected
Exp. 2: Feces were collected over dry ice at pre-dose and at 8 hours and every 24 hours until terminal sacrifice at 168 hours.
Exp. 3: Feces were collected (no specifics provided) but not analyzed.
Exp. 4: Feces were collected over dry ice during the 48-hour experimental period.
Exp. 5: Urine was collected over dry ice for 24 hours after a single gavage dose of the vehicle, PEG-400.
Exp. 7: Feces from one group of rats (three females) were collected throughout the 14-day treatment period and for 7 days thereafter.
For Exp. 8 (single 5 mg/kg oral dose), fecal samples were collected over dry ice at pre-dose, at 8 hours and ever 24 hours thereafter up to 168 hours.

Bile- Bile was collected only from rats in Exp. 4. Bile samples were collected prior to dosing and at the following intervals: 0-8 hours, 8-24 hours, and 24-48 hours. The bile cannulae were connected to swivel adapters to allow for collection from unanesthetized rats over the 48-hour period. The bile samples accumulated into a dry ice-cooled vial that was exterior to the metabolism chamber.

Cage wash – Exp. 1: Not collected.
Exp. 2: Cage wash (and feed residue) was collected presumably at 168 hours (time intervals not specified)
Exp. 3: Not collected.
Exp. 4: Not collected.
Exp. 5: Not collected.
Exp. 7: Cage wash and feed residue from one group of rats (three females) were collected throughout the 14-day treatment period and for 7 days thereafter.
For Exp. 8 (single 5 mg/kg oral dose), cage wash (successive dilute detergent, water, acetone washes)and feed residue were collected at the time of termination of the test animals at 168 hours.

Tissues – Where mandated by experimental protocol, the following tissues were collected: whole blood, plasma, red blood cells, liver, lung, heart, kidney, spleen, adrenals, muscle, bone (marrow and mineral), brain, fat, testes or ovaries, uterus, gastrointestinal tract and contents, and carcass.
Exp. 2: The aforementioned tissues were collected immediately after terminal sacrifice at 168 hours.
Exp. 3: Tissues (as noted for Exp. 2) were collected from four male and four female rats of each dose group (see Table 1) at times corresponding to the

specific tC_{max} as determined in Exp. 2. Tissues were also collected from an additional four rats of each sex at the time corresponding to one-half the C_{max} ($tC_{max/2}$).
Exp. 4: Tissues were not collected.
Exp. 5: At 24 hours after a single gavage dose of PEG-400, tissues were collected as noted for Exp. 2.
Exp. 7: Whole blood, plasma, red blood cells, brain, fat, and liver were collected at necropsy.
For Exp. 8 (single 5 mg/kg oral dose), liver, testes, adrenals, kidney, heart, lung, ovaries, skin, spleen, brain, fat, uterus, bone (marrow and mineral), gastrointestinal tract (and contents), and carcass were collected immediately after sacrifice at 168 hours and stored at -20°C.

3. Sample preparation/analysis

Expired air – Not performed.

Blood - Blood samples were placed on ice immediately after collection. Plasma and red blood cells were separated by centrifugation. Plasma was stored at -20°C; red cells and whole blood were stored at 4°C. ^{14}C content in plasma and red blood cells was analyzed by LSC in duplicate (Exp. 1 and prior to animal termination) or triplicate (Exp. 2, 3, 8 and at terminal sacrifice). Whole blood and red blood cells (Exp. 1, 2, 3, and 7) were combusted prior to LSC analysis. Blood was not collected in Exp. 4.

Urine – Urine from each collection interval in Exp. 2, 4, 7, and 8 was analyzed by LSC in triplicate. Urine samples pooled across collection intervals were used for quantitation of metabolites. Pooled samples were counted to confirm total radioactivity in each pool and filtered prior to HPLC analysis. Unpooled samples from Exp. 2 were used by the sponsors laboratory for metabolite characterization.

Feces – Feces from each collection interval in Exp. 2, 7 (rats terminated 7 days post 14-day repeated dose), and 8 were homogenized, combusted, and analyzed by LSC in triplicate. Pooled fecal samples from each sex were used for quantitation of metabolites. Two to four-gram aliquots of each pooled sample were extracted three times with 100% acetonitrile. The samples were centrifuged and the supernatant extract counted by LSC. The residual pellet was combusted and counted by LSC. The extracts were concentrated by centrifugal vacuum desiccation, reconstituted in 0.5-1.0 mL acetonitrile, and filtered prior to HPLC analysis. According to the study author, the acetonitrile extraction provided a 71-119% extraction efficiency.

For Exp. 2, analyses on samples from both males and females of all subgroups (5 mg/kg and 150 mg/kg of either the indanone or trifluoromethoxyphenyl label

position) were performed to determine the metabolite profile. Additionally, samples from an Exp. 7 subgroup (14-day repeated dose [5 mg/kg/day] with 7-day post-dose sacrifice) were analyzed to determine if and how the metabolite profile changed throughout the repeated dosing regimen. The samples (Days 0-1 and Days 13-14) were prepared as previously explained and analyzed by HPLC.

Bile – Triplicate aliquots of individual bile samples from Exp. 4 were counted by LSC to determine percent of dose secreted in the bile. The remaining bile samples were pooled (two collection periods pooled to obtain highest radioactivity content) and used for identification and quantitation of metabolites.

Bile samples from three male rats given a single 5 mg/kg dose of [indanone-1-^{14}C]DPX-JW062 were treated with glucuronidase or sulfatase enzymes to determine the presence of glucuronide or sulfate conjugate metabolites. The samples were incubated for 4 or 24 hours in a shaker bath at 37°C. Both a frozen and incubated control (no enzymes) were also prepared. Following centrifugation, the samples were analyzed by HPLC.

Tissues – Following homogenization and combustion, tissue and carcass samples for Exp. 2, 3, 7, and 8 were analyzed in duplicate or triplicate by LSC. Fat samples were extracted at the sponsor's lab (DuPont Experimental Station) and resulting extracts assayed for radioactivity. Extracts were then used for identification and quantification of metabolites.

Cage wash – Cage wash and feed residue for Exp. 2, 7, and 8 were analyzed for radioactivity in duplicate or triplicate by LSC. Feed residue was homogenized and combusted prior to LSC but cage wash (dilute detergent, water, and acetone successive rinses)was counted directly.

Analytical techniques

Scintillation counting – Samples were counted in triplicate using a Packard 2500 TR Liquid Scintillation Counter and various scintillation cocktails depending on the sample. Non-liquid samples (whole blood, red blood cells, feed residue, tissues, and feces) were combusted and the liberated $^{14}CO_2$ trapped in Carbosorb and mixed with Permafluor cocktail. A scintillation fluid blank was run concurrently with the samples. Most samples were counted for 10 minutes or until 200,000 counts accumulated (low activity samples were counted for 50 minutes or until 1600 counts accumulated).

High Performance Liquid Chromatography (HPLC): Two HPLC systems were utilized for analysis of metabolites and parent compound. Both System 1 and 2 utilized a Hewlett-Packard Series 1050 HPLC with a Quaternary pump, autosampler, and column heater. The columns were operated at 40°C. Both systems used UV detectors set at 230 and/or 280 nm, and in-line radioactivity

detectors (System one used either a 220 μL calcium fluoride solid cell or a 500 μL liquid flow cell; System 2 used only the 220μL calcium fluoride solid cell). Structural identification of DPX-MP062 metabolites was performed by direct comparison to HPLC radiochromatograms from rats dosed with DPX-JW062 while structural identification of DPX-JW062 metabolites was made by co-chromatography with known standards (structures and specifications provided in study reports). Several HPLC methods with different columns, mobile phases, and varying flow gradients were used to analyze metabolites in feces, urine, and tissues (fat tissue was analyzed by the sponsor, E.I. du Pont de Nemours & Co).

Mass Spectrometry (MS): MS was used to identify metabolites of DPX-JW062.

4. Pharmacokinetic determinations

Plasma and red blood cell kinetics (C_{max}, tC_{max}, $C_{max/2}$, $t_{Cmax/2}$, half-life [$t_{1/2}$], and AUC) were determined from rats with cannulated jugular veins administered a single 5 mg/kg oral dose of [indanone-1-^{14}C]DPX-MP062 (Exp. 8) or [indanone-1-^{14}C]DPX-JW062 (Exp. 1). For this experiment, blood was collected at 0.5, 1, 2, 4, 6, 8, 12, and 24 hours and daily for up to 168 hours after dosing.

5. Metabolite identification

Metabolites in the bile, urine, and feces that represented >5% of the administered dose were characterized if possible. Several metabolites representing <5% of the dose were also structurally identified. Additionally, fat tissue and urine samples were submitted to the project sponsor (E.I. du Pont de Nemours and Co.) for metabolite characterization. Initial characterization of urinary metabolites was conducted by the contractor laboratory (Haskell Laboratories).

6. Statistics

Statistical analyses included mean and standard deviations for ^{14}C residue in tissues, fluids, and excreta. Coefficient of variation was used to assess scintillation count results. Linear regression was used to construct standard curves of dose concentration and to determine values for kinetic parameters.

II. RESULTS

A. PILOT STUDIES

Results of the pilot studies indicated that the test material could be successfully administered in PEG-400 and that the dosing solution was stable throughout the

duration of the study. Neither [indanone-1-^{14}C]DPX nor [trifluoromethoxyphenyl(U)-^{14}C]DPX were exhaled as CO_2 or volatile metabolites. Recovery of orally administered radioactivity was >90% over the 168-hr study duration. Raw data for these studies were not presented in the reviewed study reports (MRID 44477152 and MRID 4477153) but the results of subsequent study experiments affirmed the findings reported for the pilot studies.

B. DISTRIBUTION/EXCRETION STUDIES

1. Mass balance

The total recovery for each test article (DPX-MP062 and DPX-JW062) was greater than 90%, thereby indicating acceptable accounting of administered radioactivity. Mass balance values were similar regardless of the dosing regimen and ranged from 90-98%. Data for the indanone-1-^{14}C label (IND-DPX-JW062) are shown in Table 2 and data for the trifluoromethoxyphenyl(U)-^{14}C label (TMP-DPX-JW062)are shown in Table 3.

TABLE 2. Recovery (% of dose) of radioactivity in rats following a single oral low dose (5 mg/kg) of IND-DPX-MP062 or IND-DPX-JW062, and single oral high dose (150 mg/kg) of IND-DPX-JW062

Label	Sex	Urine	Feces	Tissues	Total[a]
IND-MP062	M	35±5	47±4	4.4±0.8	90±3
	F	45±6	33±4	12.9±4.8	95±2
IND-JW062 (low dose)	M	41±6	44±2	3.4±1.0	95±2
	F	37±9	44±9	7.8±1.6	94±2
IND-JW062 (high dose)	M	14±5	78±8	1.4±0.6	98±2
	F	13±4	75±7 (83±7.7)[b]	3.1±1.9	97±4

[a] Includes cage wash and feed residue (4.14% for males and 3.08% for females); [b] calculated by reviewer based upon data from Table 3, p. 57, MRID 44477153

Data taken from Table 2, p. 39 of MRID 44477152, and Table 2, p. 56 of MRID 44477153. Values are mean ± S.D.

TABLE 3. Recovery (% of dose) of radioactivity in rats following single oral low dose (5 mg/kg), 14-day repeated doses of TMP-DPX-JW062 (5 mg/kg), or single high dose (150 mg/kg) of TMP-DPX-JW062

Label	Sex	Urine	Feces	Tissues	Total
TMP-JW062 (single low dose)	M	55±7	27±3	10.3±2.2	97±3
	F	47±14	30±11	16.6±5.0	98±2
TMP-JW062 (14-day repeated dose)	M	-	-	-	-
	F	56±8	25±2	8.4±4.2	96±3
TMP-JW062 (single high dose)	M	20±7	65±2	3.6±1.7	93±5
	F	14±2	71±6	4.4±1.6	95±2

Data taken from Table 2, p. 56 of MRID 44477153. Values are mean ± S.D.

2. Absorption

Based upon pharmacokinetic, tissue burden, and excretion data, it appears that the test materials (both DPX-MP062 and DPX-JW062) were rapidly absorbed following oral administration. For DPX-MP062 (Exp. 8) peak plasma concentrations were attained 6-8 hours following a single 5 mg/kg oral dose. At 0.5 hours, mean plasma concentrations were already 27% and 36% of peak levels for males and females, respectively thereby indicating rapid absorption. Based upon urinary excretion, absorption following a single high dose of DPX-JW062 appeared to exhibit some degree of saturation (only 20 and 14% was excreted by high-dose males and females, respectively, compared to 55% and 47% for low-dose males and females). Fecal elimination (percent of dose) was greater for high-dose rats than for low-dose rats, also implying greater elimination of unabsorbed test material. Biliary contribution to the total fecal radioactivity was substantially lower (19% and 6% for males and females, respectively)in high-dose rats than in low-dose rats (61% and 50% for males and females, respectively) also implying decreased absorption. The decreased biliary excretion also indicated that the greater fecal radioactivity observed in the high-dose group represented radioactivity unabsorbed from the gastrointestinal tract.

Based upon urinary and biliary excretion data, and retention of radioactivity in the tissues/carcass, overall absorption of [indanone-1-^{14}C]DPX-JW062 was approximately 69-81% in the low dose group and 8-14% in the high-dose group. Somewhat higher absorption was observed for males rats in both of these groups but statistical significance was not indicated. Overall absorption for [trifluoro-methoxyphenyl(U)-^{14}C]DPX-JW062 was 77-79% following a single low dose. Overall absorption for other treatment regimens could not be determined reliably without biliary excretion data.

3. Tissue distribution

Both DPX-MP062 and DPX-JW062 were widely distributed following oral administration (single dose) but deposition in tissues (at 168 hours post dosing) was limited to only 3.4 - 12.9% of the total dose. With the exception of fat tissue and skin, individual tissue burdens following a single oral dose represented <1% of the administered dose.

For rats receiving a single oral dose (5 mg/kg) of [indanone-1-^{14}C]DPX-MP062 or [indanone-1-^{14}C]DPX-JW062 (Exp. 8, MRID 44477152), overall tissue burden represented only 4.4±0.8% (DPX-MP062) and 3.4±1.0% (DPX-JW062) in males, and 12.9±4.8% (DPX-MP062) and 7.8±1.6% (DPX-JW062) in females of the total administered radioactivity(0.3-0.7% less if digestive tract contents excluded). For DPX-MP062, females exhibited an overall tissue burden that was 2.9-fold greater than that for males based upon recovered radioactivity. For DPX-JW062, overall tissue burden in female rats was 2.3-

fold greater than for males. Most of the radioactivity in both sexes and for both test articles was found in fat tissue (1.76-8.76%) with female rats exhibiting somewhat greater amounts than males (3.3-fold for DPX-MP062 and 2.7-fold for DPX-JW062). Fat burden for DPX-MP062 and DPX-JW062 derived radioactivity was similar in males but that for DPX-MP062 was 1.9-fold greater in females. The variations in fat burden appeared to account for the differences in overall tissue burden between DPX MP-062 and DPX-JW062. Generally, overall tissue burden based upon ^{14}C retention was slightly greater in rats dosed with DPX-MP062 than those dosed with DPX-JW062. No parent compound was detected in the fat tissue. At all time points examined, the red blood cells of rats dosed with the trifluoromethoxyphenyl label consistently contained much greater levels of radioactivity than did plasma. This relationship was not observed for rats given the indanone label. For rats dosed with the triflouromethxyphenyl label, red blood cells consistently contained greater amounts of radioactivity at all time points than was observed for the indanone label.

For the 14-day repeated dose experiment, tissue distribution was similar to that observed for the single-dose groups. At 8-14 days of treatment, plasma concentration appeared to be approaching a steady state with an elimination $t_{1/2}$ of 7.4, 12.0, and 12.4 days for plasma, whole blood and red blood cells, respectively. Fat tissue contained the greatest percent of the administered dose (12.55% at Day 9). Whole blood exhibited radioactivity levels as high as 6.32% of the administered dose at Day 5 but the levels declined to 1.57% by Day 35. With the exception of fat tissue and whole blood, concentrations in other tissues (expressed as percent of administered dose) never exceeded 1% at any time during the 14-day dose period or during the 21-day post-dose period.

4. Excretion

Excretion data for rats given a single 5 mg/kg dose of [indanone-1-^{14}C]DPX-MP062 or [indanone-1-^{14}C]DPX-JW062 (Exp. 8, MRID 44477152) are shown in Table 2. Both urine and feces represented major routes of excretion (35-45% and 33-47%, respectively). No substantial quantitative differences in excretion profiles were observed between DPX-MP062 and DPX-JW062 at the low doses.

Time course for urinary and fecal excretion of a single oral dose of [indanone-1-^{14}C]DPX-MP062(5 mg/kg) are shown in Table 4. Most urinary excretion (25.72 and 34.31% of administered dose in males and females, respectively) occurred within 24 hours of dosing. This represented 74% (males)and 76% (females) of the total urinary excretion. Within 96 hours, 94% (males) and 88% (females) of the total radioactivity in the feces was eliminated.

TABLE 4. Urinary and fecal excretion (% of dose)of [indanone-1-^{14}C]DPX-MP062 in rats following a single oral low dose (5 mg/kg)[a].

Time Interval (hr)	Urine		Feces	
	Males	Females	Males	Females
0-0	0.001±0.001	0.000±0.000	0.000±0.000	0.000±0.000
0-8	8.777±1.248	11.118±3.697	1.239±1.040	0.143±0.118
8-24	16.946±4.906	23.200±2.681	18.915±4.280	8.267±2.530
24-48	4.705±1.162	5.683±1.260	18.182±3.670	15.153±4.251
48-72	1.504±0.245	2.181±0.135	3.605±0.891	3.641±1.216
72-96	1.158±0.443	1.215±0.252	1.753±0.335	2.229±0.373
96-120	0.639±0.145	0.838±0.179	1.183±0.480	1.505±0.547
120-144	0.498±0.225	0.601±0.124	1.073±0.421	1.264±0.640
144-168	0.413±0.181	0.463±0.050	0.685±0.172	1.142±0.410
Total	34.64	45.29	46.63	33.34

[a]Values are mean ± S.D
Data taken from Tables 3 and 4, pp. 40-41, MRID 44477152

Most (69-90%) of the radioactivity in the feces of rats given a single 5 mg/kg dose of DPX-MP062 (Exp. 8) was associated with metabolites. Parent compound accounted for 1.4% (males) and 1.8% (females) of the administered dose. The remainder of the radioactivity (2.5 - 7.5% of fecal radioactivity) remained in the aqueous phase pellet and was not characterized.

Time-course data for excretion of radioactivity by rats given a single low dose of DPX-JW062 are shown in Table 5. Most fecal elimination occurred with 48 hours. Urinary excretion peaked between 8 and 24 hours. The 48 hour time period accounted for approximately 85-86% of urinary excretion in both male and female rats given a single low dose of [indanone-1-^{14}C]DPX-JW062.

Table 5. Urinary and fecal excretion (% of dose)of [indanone-1-^{14}C]DPX-JW062 in rats following a single oral low dose (5 mg/kg)[a].

Time Interval (hr)	Urine		Feces	
	Males	Females	Males	Females
0-0	0.000±0.000	0.001±0.000	0.000±0.000	0.000±0.000
0-8	13.383±3.853	12.545±4.256	1.202±1.149	1.091±0.697
8-24	16.322±4.009	13.843±3.005	19.639±5.771	13.104±10.464
24-48	5.129±1.145	5.330±1.691	16.606±5.268	19.559±6.915
48-72	2.000±0.582	2.054±0.463	3.579±1.014	4.875±2.608
72-96	1.461±0.504	1.351±0.491	1.443±0.496	1.789±0.518
96-120	1.032±0.419	0.808±0.309	1,053±8.245	1.216±0.344
120-144	0.775±0.480	0.672±0.176	0.841±10.326	1.224±0.663
144-168	0.619±0.477	0.680±0.292	0.724±0.384	1.427±0.937
Total	40.72	37.28	45.09	44.29

[a]Values are mean ± S.D
Data taken from Tables 3 and 4, pp. 57 and 59, MRID 44477153

Time-course data for rats given a single high-dose (150 mg/kg) of DPX-JW062 (Table 6) varied compared to the low-dose groups (Table 5). It is evident that fecal elimination represented a greater percent of the administered radioactivity in the high-dose rats than was observed for the low-dose group over the 168-hour observation period. Additionally, for the low-dose rats, 76-83% of the radioactivity associated with the feces was eliminated within 48 hours, whereas for the high-dose group only 43-47% of fecal radioactivity was eliminated within the same time frame. No gender differences were observed.

Table 6. Urinary and fecal excretion (% of dose)of [indanone-1-^{14}C]DPX-JW062 in rats following a single oral high dose (150 mg/kg)[a].

Time Interval (hr)	Urine		Feces	
	Males	Females	Males	Females
0-0	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000
0-8	3.355±0.925	2.714±0.676	0.087±0.119	0.352±0.522
8-24	3.727±1.366	3.358±1.295	5.294±4.019	7.433±8.536
24-48	2.889±0.665	3.009±1.425	28.001±27.372	31.342±22.407
48-72	1.697±0.865	1.552±0.773	20.062±19.534	28.063±17.938
72-96	0.899±0.605	0.817±0.495	10.732±7.948	11.151±8.447
96-120	0.719±0.464	0.497±0.290	7.202±8.827	3.346±3.100
120-144	0.454±0.357	0.327±0.126	5.895±10.180	0.972±0.669
144-168	0.264±0.105	0.260±0.110	0.877±1.124	0.546±0.343
Total	14.00	12.53	78.15	83.20

[a]Values are mean ± S.D.
Data taken from Tables 3 and 4, pp. 57 and 59, MRID 44477153

Biliary excretion data are shown in Table 7. Most biliary excretion occurred from 8-48 hours and was substantially reduced in the high-dose group. At similar doses (5 mg/kg), little difference was observed in the biliary excretion of [indanone-1-^{14}C]DPX-JW062 and [trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062. There were no statistically significant differences in biliary excretion between males and females or between the two dose groups. Results of the bile duct cannulation studies showed that in the low-dose rats, biliary excretion accounted for 50% (females) and 61% (males) of the radioactivity associated with the feces over the 48-hr collection period. In the high-dose group, however, biliary excretion accounted for 19% of the radioactivity in the feces of males but only 6% in females over the 48-hr collection period. The gender difference as well as the decreased overall biliary excretion in this group relative to others were notably different.

TABLE 7. Biliary excretion (% of dose) of DPX-JW062 in rats following a single oral dose (5 or 150 mg/kg)[a]		
Collection Interval(hrs)	**% of Dose**	**Cumulative % of Dose**
5 mg/kg [indanone-1-^{14}C]DPX-JW062		
Males		
0-0	0.000±0.000	0.000±0.000
0-8	5.077±4.028	5.077±4.028
8-24	14.667±3.920	19.745±6.484
24-48	2.814±0.717	22.559±6.167
Females		
0-0	0.000±0.000	0.000±0.000
0-8	1.225±1.772	1.225±1.772
8-24	12.629±4.566	13.854±6.102
24-48	3.277±1.918	17.130±5.949
150 mg/kg [indanone-1-^{14}C]DPX-JW062		
Males		
0-0	0.000±0.000	0.000±0.000
0-8	3.554±0.566	3.554±0.566
8-24	1.932±0.667	5.486±0.125
24-48	0.911±0.166	6.397±0.240
Females		
0-0	0.000±0.000	0.000±0.000
0-8	0.467±0.500	0.468±0.499
8-24	0.556±0.362	1.023±0.850
24-48	0.808±0.264	1.831±0.663
5 mg/kg [trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062		
Males		
0-0	0.000±0.000	0.000±0.000
0-8	8.442±6.522	8.442±6.522
8-24	7.878±3.035	16.320±6.366
24-48	2.668±1.119	18.987±6.663
Females		
0-0	0.000±0.000	0.000±0.000
0-8	6.171±3.142	6.171±3.142
8-24	7.645±1.125	13.816±4.097
24-48	3.475±0.461	17.291±4.159

[a] Values mean ± S.D.
Data taken from Table 7, p. 78, MRID 4477153.

C. PHARMACOKINETICS STUDIES

1. Plasma and Red Blood Cell Kinetics

Data from Exp. 1 and 8 indicated that the blood kinetics for DPX-MP062 and DPX-JW062 were not substantially different from one another for low dose single exposure (Table 8). A comparison of the kinetics of the [indanone-1-^{14}C]DPX-JW062 and the [trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062, however, revealed notable differences as shown in Table 9. Additionally, differences between low dose (5 mg/kg) and high dose (150 mg/kg) kinetics were shown for DPX-JW062 (Table 9).

For DPX-MP062, peak time (tC_{max})and half peak times ($tC_{max/2}$) in plasma were similar for males and females (Table 8). Elimination half-time ($t_{1/2}$)in plasma was greater for females than for males; this relationship was reversed for red blood cell $t_{1/2}$. For both males and females, red blood cell $t_{1/2}$ values were greater than plasma $t_{1/2}$ values indicating longer retention of radioactivity in the red blood cells than in the plasma; this was more pronounced in male rats. The mean values for plasma AUC in females of both dose groups for both DPX-MP062 and DPX-JW062 were higher than that for males. However, individual animal data indicated considerable variability in this parameter that could account for such differences.

Kinetic parameters for DPX-JW062 exhibited gender-related differences as did DPX-MP062 (Table 8).

Table 8. Pharmacokinetic parameters in red blood cells and plasma for DPX-MP062 and DPX-JW062 following a single oral dose (5 mg/kg)[a]

Parameter	Plasma		Red blood cells	
	Male	Female	Male	Female
DPX-MP062				
$t_{1/2}$ (hr)	39±1	49±10	91±16	74±4
AUC (μg/g·hr)	83±9	117±11	80±11	91±22
tC_{max} (hr)	8.0±3.5	7.3±1.2	8.7±3.1	6.0±3.5
$tC_{max/2}$ (hr)	25±4	27±12	35±1	33±8
C_{max} (μg/g)	2.3±0.6	2.9±1.1	1.1±0.1	1.4±0.5
$C_{max/2}$ (μg/g)	1.2±0.3	1.4±0.6	0.6±0.1	0.7±0.3
DPX-JW062				
$t_{1/2}$ (hr)	35±1	52±10	97±25	68±7
AUC (μg/g·hr)	80±27	135±17	82±17	94±13
tC_{max} (hr)	6.8±3.9	5.3±3.4	6.8±2.7	3.3±3.2
$tC_{max/2}$ (hr)	24±4	24±3	38±11	32±9
C_{max} (μg/g)	2.4±0.8	3.0±0.3	1.1±0.4	1.5±0.3
$C_{max/2}$ (μg/g)	1.2±0.4	1.5±0.2	0.6±0.2	0.7±0.1

[a] Values mean ± S.D.

Data taken from Table 1, p. 38, MRID 44477152

Table 9. Pharmacokinetic parameters in red blood cells and plasma of rats following a single oral dose of DPX-JW062 (5 mg/kg or 150 mg/kg)[a]

Parameter	Plasma		Red blood cells	
	Male	Female	Male	Female
[indanone-1-^{14}C]DPX-JW062 (5 mg/kg)				
$t_{1/2}$ (hr)	35±1	52±10	97±25	68±7
AUC (μg/g·hr)	80±27	135±17	82±17	94±13
tC_{max} (hr)	6.8±3.9	5.3±3.4	6.8±2.7	3.3±3.2
$tC_{max/2}$ (hr)	24±4	24±3	38±11	32±9
C_{max} (μg/g)	2.4±0.8	3.0±0.3	1.1±0.4	1.5±0.3
$C_{max/2}$ (μg/g)	1.2±0.4	1.5±0.2	0.6±0.2	0.7±0.1
[indanone-1-^{14}C]DPX-JW062 (150 mg/kg)				
$t_{1/2}$ (hr)	45±2	59±5	84±26	75±6
AUC (μg/g·hr)	536±295	785±111	437±133	545±53
tC_{max} (hr)	8±4	19±9	4.0±3.5	9±13
$tC_{max/2}$ (hr)	39±8	64±13	57±6	88±13
C_{max} (μg/g)	9.2±3.5	9.3±1.3	4.7±1.4	4.7±0.9
$C_{max/2}$ (μg/g)	4.6±1.7	4.6±0.7	2.4±0.7	2.4±0.4
[trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062 (5 mg/kg)				
$t_{1/2}$ (hr)	92±43	114±20	112±6	119±23
AUC (μg/g·hr)	28±10	55±18	1519±337	2627±327
tC_{max} (hr)	8±0	8±0	24±0	24±0
$tC_{max/2}$ (hr)	16±3	19±4	140±3	150±26
C_{max} (μg/g)	0.6±0.1	0.8±0.2	8.4±1.4	13.9±3.6
$C_{max/2}$ (μg/g)	0.3±0.1	0.4±0.1	4.2±0.7	7.0±1.8
[trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062 (150 mg/kg)				
$t_{1/2}$ (hr)	96±49	188±49	95±17	138±28
AUC (μg/g·hr)	241±102	597±343	7656±1620	16648±2296
tC_{max} (hr)	3.3±1.5	27±39	40±14	72±24
$tC_{max/2}$ (hr)	22±2	118±136	168±5	225±17
C_{max} (μg/g)	2.7±0.4	2.6±0.3	39±10	63±17
$C_{max/2}$ (μg/g)	1.4±0.2	1.3±0.1	19±5	31±8

[a] Values mean ± S.D.

Data taken from Table 1, p. 55, MRID 44477153

2. Tissue elimination kinetics: 14-day repeated dose

Results of the 14-day repeated dose (5 mg/kg [trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062) revealed a biphasic elimination of radioactivity from the blood and tissue. For blood plasma and tissues (fat, liver, and brain), there appeared to be a notable decline in radioactivity from the time of the last dose (Day 14) to 6 or 7 days later (Days 20-21). For the remaining period (Days 21-35), the decline in radioactivity was much slower. For whole blood, the concentration appeared to remain at steady state for 3 days after the last dose whereupon a second and more rapid elimination occurred and was maintained through Day 35 (experiment termination). For red blood cells, there was a notable decline from the last dose (Day 14) to Day 16. From Day 16 to Day 21, elimination changed little, whereupon steady decline occurred until Day 35. Based upon 6, 24, and 168-hour data for both labels of DPX-JW062 and 168-hour data for DPX-MP062, radioactivity in fat tissue was cleared more slowly in females than in males for both the low and high doses.

D. METABOLITE CHARACTERIZATION STUDIES

1. Urine

Urine samples pooled from individual rats from both [indanone-1-^{14}C]DPX-JW062 (Exp. 2) and [indanone-1-^{14}C]DPX-MP062 (Exp. 8) treated rats each contained 13 metabolites (Tables 10 and 11). For DPX-MP062, only three metabolites exceeded 5% of the administered dose and only two metabolites exceeded 5% of the administered dose for DPX-JW062. Urine from female rats treated with DPX-MP062 contained somewhat greater amounts (although not designated as statistically different) of all metabolites except the unidentified region 1 product. For DPX-JW062, gender-related quantitative differences were unremarkable. For rats give a single low dose of DPX-JW062 with the trifluoromethoxyphenyl label, fewer (10) metabolites were detected but only two represented >4% of the administered dose. These two metabolites were MG195 (13.7-17.0% of the administered dose) and MC218 (21.1-23.5% of the administered dose). High dose treatment with the trifluoromethoxyphenyl label resulted in a qualitatively similar metabolite profile in the urine but with notably lower quantities due to less absorption.

Table 10. Metabolites (% of dose) in urine of rats given a single oral dose of [indanone-1-^{14}C]DPX-MP062 (5 mg/kg)

Region[a]	Males	Females	Identification
1	1.0±0.4	0.7±0.4	not identified
2	4.5±0.6	4.6±0.5	not identified
3	1.8±0.2	2.0±0.4	ML440-conjugate
4	6.7±1.3	10.2±0.7	MU716 (and MX828-sulfate, minor component)
5	2.7±0.8	3.4±0.6	MA576-conjugate
6	2.2±0.5	2.6±0.6	not identified
7	3.1±0.6	4.2±1.2	HO-JU874
8	3.2±0.7	4.8±2.1	MA576
9	5.6±0.7	6.9±1.3	MX829-sulfate
10	0.9±0.2	2.0±0.8	JU874
11	0.5±0.1	0.7±0.2	not identified
12	2.2±0.4	3.5±1.3	MX829
13	0.4±0.1	0.3±0.1	not identified
Total	34.80	45.90	

[a] Region based upon HPLC retention time.
Data taken from Table 11a, p. 51, MRID 44477152
Values mean ± S.D.

Table 11. Metabolites (% of dose) in urine of rats given a single oral dose of [indanone-1-^{14}C]DPX-JW062 (5 mg/kg)

Region[a]	Males	Females	Identification
1	1.8±0.9	0.4±0.1	not identified
2	6.1±0.8	4.5±0.8	not identified
3	1.4±0.6	1.3±0.3	ML440-conjugate
4	12.3±2.6	11.9±3.0	MU716 (and MX828-sulfate, minor component)
5	2.1±0.7	2.5±0.5	MA576-conjugate
6	2.5±0.3	2.0±0.7	not identified
7	3.1±1.0	3.1±1.1	HO-JU874
8	2.5±0.5	2.7±0.9	MA576
9	4.1±0.9	3.3±0.8	MX829-sulfate
10	0.9±0.3	1.7±0.9	JU874
11	1.3±0.2	1.3±0.4	not identified
12	2.2±0.7	1.4±0.3	MX829
13	0.5±0.3	0.3±0.1	not identified
Total	40.8	36.4	

[a] Region based upon HPLC retention time.
Data taken from Table 5b, p. 52, MRID 44477152.

2. Feces

Fecal samples from male and female rats in Exp. 2 (DPX-JW062) and Exp. 8 (DPX-MP062) were individually pooled, extracted with acetonitrile, and analyzed by HPLC. Acetonitrile extraction removed 69-90% of the radioactivity from the fecal samples. Ten metabolites were detected in fecal samples from rats given DPX-MP062 (Table 12) and nine metabolites detected for rats given DPX-JW062 (Table 13). For DPX-MP062, four of the ten metabolites were identified and for DPX-JW062, four of nine metabolites were identified. Quantitation revealed that only three DPX-MP062 metabolites and four DPX-JW062 equaled or exceeded 5% of the administered radioactivity. Quantitative gender-related differences were apparent for four to five of the metabolites from each test article. Parent compound represented 6-20% of the administered DPX-JW062 dose in low-dose groups and 68-79% of the administered dose in the high-dose groups. For the low dose groups, greater amounts of parent compound were detected in the feces of female rats than male rats. For rats given a single low dose DPX-JW062 with the trifluoromethoxyphenyl label, 5-HO-JW062, DPX-JW062 and IN-JT333 were also detected and at similar quantities as observed for the indanone label. Unidentified metabolites with similar HPLC retention times as those observed for the indanone label were detected and represented <2% of the administered dose.

For DPX-MP062, parent compound represented 1.4-1.8% of the administered dose for males and females, respectively.

Table 12. Metabolites (% of dose) in feces of rats given a single oral dose of [indanone-1-^{14}C]DPX-MP062 (5 mg/kg)

Region[a]	Males	Females	Identification
1	1.6±0.3	2.3±0.8	not identified
2	12.1±2.2	6.7±2.1	not identified
3	2.0±0.3	0.9±0.2	not identified
4	8.5±1.0	2.2±0.6	HO-MP062[b]
5	8.1±1.2	5.2±2.5	HO-MP062[c]
6	1.7±0.3	2.4±0.8	not identified
7	0.4±0.1	0.4±0.2	not identified
8	0.4±0.1	0.6±0.3	not identified
9	1.4±0.5	1.8±0.3	DPX-JW062
10	0.4±0.1	1.8±1.2	IN-JT333
Total[d]	36.6	24.3	

[a] Region based upon HPLC retention time; [b] Haskell sample no. 22105; [c] Haskell sample no. 22106; [d] Total radioactivity in feces (Table 2) includes unextractable residue of water soluble pellet and, therefore, is greater than that represented by metabolites.
Data taken from Table 9a, p. 48, MRID 44477152.
Values mean ± S.D.

Table 13. Metabolites (% of dose) in feces of rats given a single oral dose of [indanone-1-^{14}C]DPX-JW062 (5 mg/kg)

Region[a]	Males	Females	Identification
1	2.0±1.3	1.7±1.1	not identified
2	10.7±8.8	5.9±3.4	not identified
3	1.1±0.6	0.4±0.2	not identified
4	5.0±3.0	1.2±0.6	5-HO-JW062[b]
5	7.2±3.5	3.9±1.4	5-HO-JW062[c]
6	2.3±1.2	2.0±0.3	not identified
7	0.7±0.6	0.5±0.2	not identified
8	5.7±5.2	19.2±14.7	DPX-JW062
9	0.8±0.6	2.2±1.8	IN-JT333
Total[d]	35.5	37.0	

[a] Region based upon HPLC retention time; [b] Haskell sample no. 22105; [c] Haskell sample no. 22106; [d] Total radioactivity in feces (Table 2) includes unextractable residue of water-soluble pellet and, therefore, is greater than that represented by metabolites. Data taken from Table 9b, p. 49, MRID 44477152. Values mean ± S.D.

3. Tissues

In Exp.1, fat contained the greatest amount of radioactivity (3-9% of the administered dose). Because only minimal radiolabel was detectable in other tissues (generally <1%), only fat tissue was analyzed for metabolites. Following extraction of the radioactivity-containing fraction with acetonitrile (96% efficiency) and analysis by HPLC, it was found that 91-92% of the radioactivity in fat tissue was associated with the metabolite, IN-JT333. Based on the total radioactivity in the fat tissue (3-9%) and the IN-JT333 contribution of 91-92% to this tissue burden, IN-JT333 accounted for 2.8-8.3% of the administered radioactivity. Parent compound was not detected in fat tissue of rats given the single low dose of [indanone-1-^{14}C]DPX-JW062 and represented only 0.2 to 4.2% of the radioactivity detected in fat tissues of high-dose rats. For rats receiving [trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062, parent compound represented <0.1 to 1.6% of the total fat residue following a single low dose, and 0.6 to 1.0% in rats receiving multiple 5 mg/kg/day doses.

An analysis of the IN-JT333 in fat tissue of individual animals indicated greater amounts of the IN-KN125 enantiomer (biologically more active) than the IN-KN124 enantiomer (biologically less active). The IN-KN125:IN-KN124 ratio ranged from 1.3 to 2.8 for a single low dose of [indanone-1-^{14}C]-DPX-JW062, 1.4 to 1.8 for a single high dose of [indanone-1-^{14}C]-DPX-JW062, 1.3 to 3.5 for a repeated low dose of [trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062, 1.1 to 1.8 for a single low dose of [trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062, and 1.1 for a single high dose of [trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062.

4. Bile

Fifteen metabolites were detected in bile collected from rats in Exp. 4 (5 or 150 mg/kg indanone-1-^{14}C]DPX-JW062, or 5 mg/kg [trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062). However, no one metabolite represented more than 4.5% of the administered dose. Treatment of the bile samples with glucuronidase/sulfatase did not change the chromatographic profile indicating that none of the biliary metabolites were glucuronide or sulfate conjugates. Parent compound in the bile represented <1% of the administered dose. None of the biliary metabolite retention times matched those of metabolites detected in the feces.

5. Proposed metabolic pathway

Metabolic pathways for DPX-MP062 and DPX-JW062 were proposed (Figure 1 & 2; Appendix). The pathway proposed for DPX-MP062 is based largely on the findings for DPX-JW062. The metabolism of DPX-JW062 is extensive and includes three initial processes as described by the study author: 1) hydrolysis of the carboxymethyl group from the amino nitrogen of the trifluoromethoxyphenyl moiety to form IN-JT333, 2)hydroxylation of the benzylic position to form 5-HO-JW062, and 3) formation of IN-KG433 (determined by in vitro experiments hepatic microsomal preparations). Fecal excretion of 5-HO-JW062 represented a large portion of the administered dose of DPX-JW062. The formation of several conjugates from JT333 and from 5-HO-JW062 was validated by known intermediates.

III. DISCUSSION

A. DISCUSSION

In a metabolism and disposition study, groups of Crl:CD®(SD)BR rats (5/sex/dose group)were given DPX-MP062 (96% purity) or DPX-JW062 (98.6-99.6% purity) in PEG-400 by gavage. Dosing regimens for DPX-JW062 included single low dose (5 mg/kg) and single high dose (150 mg/kg) in male and female rats, and a 14-day repeated low dose (5 mg/kg/day) in female rats only (24 rats). For the single-dose experiments two different radiolabels were used; [indanone-1-^{14}C]DPX-JW062 and [trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062. The 14-day repeated dose experiments used only [trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062. Additionally, a biliary excretion experiment was conducted in which male and female rats (3-4/sex/dose) received either a single low dose (5 mg/kg) or a single high dose (150 mg/kg) of [indanone-1-^{14}C]DPX-JW062, or a single low dose of [trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062. For DPX-MP062, only a single low-dose (5 mg/kg) experiment was conducted (5 male and 5 females) to determine kinetic parameters, absorption, excretion, tissue burdens, and metabolite profiles in male and female rats. Intravenous administration studies were not conducted due to the limited solubility (<20 ppb) of the test material. Expired air was not collected

because results of pilot studies indicated that expired air contained no CO_2 or volatile metabolites.

There were no deaths that could be attributed to the test material. Total recovery of administered radioactivity in the various experimental groups ranged from 90-98% indicating an acceptable mass balance for all of the experiments.

Both DPX-JW062 and DPX-MP062 were rapidly absorbed in rats following oral administration. The absorption of DPX-JW062 was dose dependent and appeared saturated at the high dose. Based upon urinary and biliary excretion, and retention of radioactivity in the carcass, total absorption was 69-81% for the low dose, and 8-14% for the high dose. Both DPX-JW062 and DPX-MP062 were widely distributed following oral administration but overall tissue burden was limited to only 3.4-12.9% of the administered dose. Tissue burden was correspondingly less in high dose animals due to the decreased absorption. Fat tissue contained the greatest level of radioactivity (1.76-8.76% of the administered dose). Remaining tissue generally contained <1% of the administered radioactivity. For both DPX-JW062 and DPX-MP062, radioactivity in fat tissue was greater in female rats (2.3-fold for DPX-JW062 and 2.9-fold for DPX-MP062) and clearance from this tissue was notably extended in females following administration of a single oral dose. Tissue distribution was similar following the 14-day repeated dosing. Most of the absorbed dose of either DPX-JW062 (37-41% for low dose [indanone-1-^{14}C]DPX-JW062 and 47-55% for low dose [trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062) or DPX-MP062 ([indanone-1-^{14}C] DPX-MP062)was excreted via the urine. Urinary excretion was similar (56% of administered dose) in rats receiving the 14-day repeated low dose treatment. Because of the saturated absorption at high dose, urinary excretion was substantially lower (13-14% for [indanone-1-^{14}C]DPX-JW062 and 14-20% for [trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062). A high-dose group for DPX-MP062 was not included in the study protocol. Radioactivity associated with the feces included unabsorbed material as well as biliary excretion of absorbed radioactivity. Results of experiments with rats fitted with bile duct cannulae and given a single low dose of DPX-JW062showed that at the low dose, 50% (females) and 61% (males) of the fecal radioactivity was associated with biliary excretion. At the high dose, however, biliary excretion accounted for only 19% (males) and 6% (females) of the radioactivity eliminated in the feces suggesting substantially reduced absorption. A concomitant reduction in urinary elimination affirmed a reduction in absorption at the high dose, probably the function of a saturated absorption process.

Time-course data on the elimination of absorbed radioactivity via the urine and feces showed that following a single low dose of DPX-MP062, 95-96% of the urinary excretion occurred within 96 hours and 71-83% of fecal elimination occurred within 48 hours. Based upon percent of administered DPX-MP062 dose, female rats exhibited greater total urinary elimination and lower fecal elimination than did male rats. For DPX-JW062, neither the low dose nor high dose treatment

groups exhibited any biologically significant sex-related differences in urinary or fecal elimination. Approximately 85-86% of a low dose of DPX-JW062 was eliminated in the urine within 48 hours by females and males, respectively. Elimination within 48 hours accounted for 83% (males) and 76% (females) of the total fecal elimination following a single low dose of DPX-JW062. For rats in the high dose treatment groups, the time-course for urinary elimination was similar to that of the low-dose group. Approximately 70% of the urinary excretion occurred within 48 hours for both males and females. Fecal elimination was somewhat prolonged compared to the low dose group. At 48 hours, only 43% (males) and 47% (females) of the radioactivity associated with the feces was eliminated. This increased to 82% and 94% for males and females, respectively, by 96 hours and was likely a function of both gastrointestinal motility and the increased levels of test material in the gut due to decreased absorption at this dose.

Studies were performed to assess kinetics in plasma and blood cells following single oral doses of 5 mg/kg and 150 mg/kg of DPX-MP062 and DPX-JW062 (both the indanone and trifluoromethoxyphenyl labels). These studies produced values for elimination half-time ($t_{1/2}$), area-under-the-curve (AUC), mean time to maximum concentration (tC_{max}), half-peak time ($tC_{max/2}$), maximum concentration (C_{max}) and half maximum concentration ($C_{max/2}$). For DPX-MP062 and low dose [indanone-1-^{14}C]DPX-JW062, plasma tC_{max} and $tC_{max/2}$ were similar for males and females but for the high doses of [indanone-1-^{14}C]DPX-JW062 and [trifluoromethoxyphenyl (U)-^{14}C]DPX-JW062, these indices were greater for female rats. The plasma and whole blood $t_{1/2}$ were shorter for male rats than for female rats. The latter is consistent with the somewhat greater retention of radioactivity in fat tissue by female rats. The $t_{1/2}$ was longer in female rats for both radiolabels and both mixtures, again reflecting the greater accumulation of radioactivity in fat tissue. However, dose did not affect the $t_{1/2}$ which is consistent with the observed saturation of absorption. The plasma $t_{1/2}$ was greater for the trifluoromethoxyphenyl(U) label than for the indanone label. The variability associated with the plasma $t_{1/2}$ for the trifluoromethoxyphenyl(U) was also inexplicably great and was reasonably explained by the study author as being due to a macromolecular binding of a trifluoromethoxyphenyl-derived metabolite. Elimination from tissues appeared to parallel that of plasma with the exception of fat tissue in which retention was prolonged especially in female rats.

Comparison of AUC values for rats in the high- and low-dose groups reflect saturated absorption as shown by only a 6.7-fold and 8.6-fold increase, respectively, for the high dose groups ([indanone-1-^{14}C]DPX-JW062 and [trifluoromethoxyphenyl (U)-^{14}C]DPX-JW062) relative to their respective low dose groups. If absorption were not saturated, these increases would be expected to more closely approximate the 30-fold difference between the doses (i.e., 5 mg/kg to 150 mg/kg). The contention of saturated absorption is affirmed when one considers the corresponding biliary excretion and urinary elimination data (both reduced in the high dose groups), fecal elimination (increased in the high dose due to unabsorbed material as

determined by the reduced biliary excretion), and a substantial increase in the percent of dose found in gastrointestinal contents in high-dose animals. Red blood cell AUC values for DPX-JW062 with the trifluoromethoxyphenyl label were much higher than those for DPX-MP062 or indanone DPX-JW062. The study author stated that this might be explained by extensive macromolecular binding of a trfluoromethoxyphenyl-derived metabolite. Although data were not available to confirm this hypothesis, it is considered by the reviewer to be a reasonable contention.

Metabolite studies revealed that both DPX-MP062 and DPX-JW062 are extensively metabolized and that metabolites are eliminated in the urine, feces, and bile. With the exception of parent compound (DPX-JW062, which accounted for 19.2% of a single low dose in the feces of female rats), none of the metabolites from any source represented more than 12.3% of the administered dose. The metabolite profile for DPX-JW062 was dose dependent and varied quantitatively between males and females. Additionally, differences in metabolite profiles were observed for the different label positions (i.e., indanone and trifluoromethoxyphenyl DPX-JW062). For DPX-JW062, none of the biliary metabolite retention times matched those of fecal metabolites. Therefore, it may be assumed that all of the biliary metabolites undergo further biotransformation in the gut. Gender-related quantitative differences were also observed for fecal and urinary metabolites of DPX-MP062 (fecal metabolite profile more so). Biliary excretion studies were not performed for DPX-MP062. A metabolic pathway was proposed for both DPX-MP062 and DPX-JW062. The proposed pathway included data obtained from *in vitro* experiments with hepatic microsomal preparations in addition to data obtained from the animal studies.

In analysis of data from the 14-day repeated high-dose study with [indanone-1-^{14}C]DPX-JW062, the concentrations of the enantiomers, IN-KN125 and IN-KN124, were higher (6.5-fold and 8.6-fold, respectively, based upon a comparison of μg equiv./g fat tissue) in the fat tissue of females compared to that of males. Although consistent with greater μg equiv./g values for the IN-JT333 metabolite (which is comprised of the enantiomers) observed for females than for males, this finding demonstrates a greater propensity for accumulation of the more active enantiomer (IN-KN125) by female rats than by male rats. Stereoselective metabolism and disposition was hypothesized by the study authors; a reasonable explanation. Determining the toxicologic ramifications of this gender-specific metabolism/ disposition was not an objective the studies described in MRID 44477152 and MRID 44477153.

B. STUDY DEFICIENCIES

These studies (MRID 4477152 and 4477153) appeared to be properly conducted and exhibited no major procedural flaws. Although routine descriptive statistics

were reportedly performed, no indication of significance levels were provided for tabled data thereby resulting in uncertainties in interpreting some results (i.e., total absorption betweeen males and females) Graphic representation of data did, however, contain error bars.

Some minor discrepancies in calculations were found, an example of which pertains to fecal elimination of radioactivity for high-dose female rats (IN-JW062) being somewhat higher than reported (83% vs. 78%; data for female rats presented in Table 3, p. 57 [Group 2F] do not sum to 78%. Such discrepancies did not compromise the validity of the study or the conclusions drawn from the results.

Repeated dose experiments were not performed for DPX-MP062. The available studies indicated that both DPX-MP062 and DPX-JW062 accumulate in fat (more so in female rats) and that most of this burden is a metabolite (IN-JT333) comprised primarily of the active enantiomer (IN-KN125). The data from the reviewed studies suggest that, for a given dose, DPX-MP062 is absorbed to a slightly greater extent and that the fat burden, at least in female rats, is somewhat greater than following a similar dose of DPX-JW062. As such, it would appear that determining the extent of IN-KN125 accumulation in fat tissue of female rats following a repeated dose regimen may provide useful information on the enriched formulation (DPX-MP062). Repeated dose experiments with DPX-JW062 (5 mg/kg/day) indicated that IN-JT333 content in fat tissue was measurable (13.7 μg equiv./g tissue) at 21 days after the last dose of the 14-day repeated dose experiment. This leaves unanswered the question regarding the extent of this residue following repeat dosing with DPX-MP062 which exhibits greater absorption and somewhat greater deposition into fat tissue.

It is also curious that for metabolism characterization studies in which rats were dosed with DPX-MP062, the JW062 formulation is indicated as a metabolite (e.g. Table 9a,p. 48 of MRID 44477152. If this is not a typographical error, it needs explanation.

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY.
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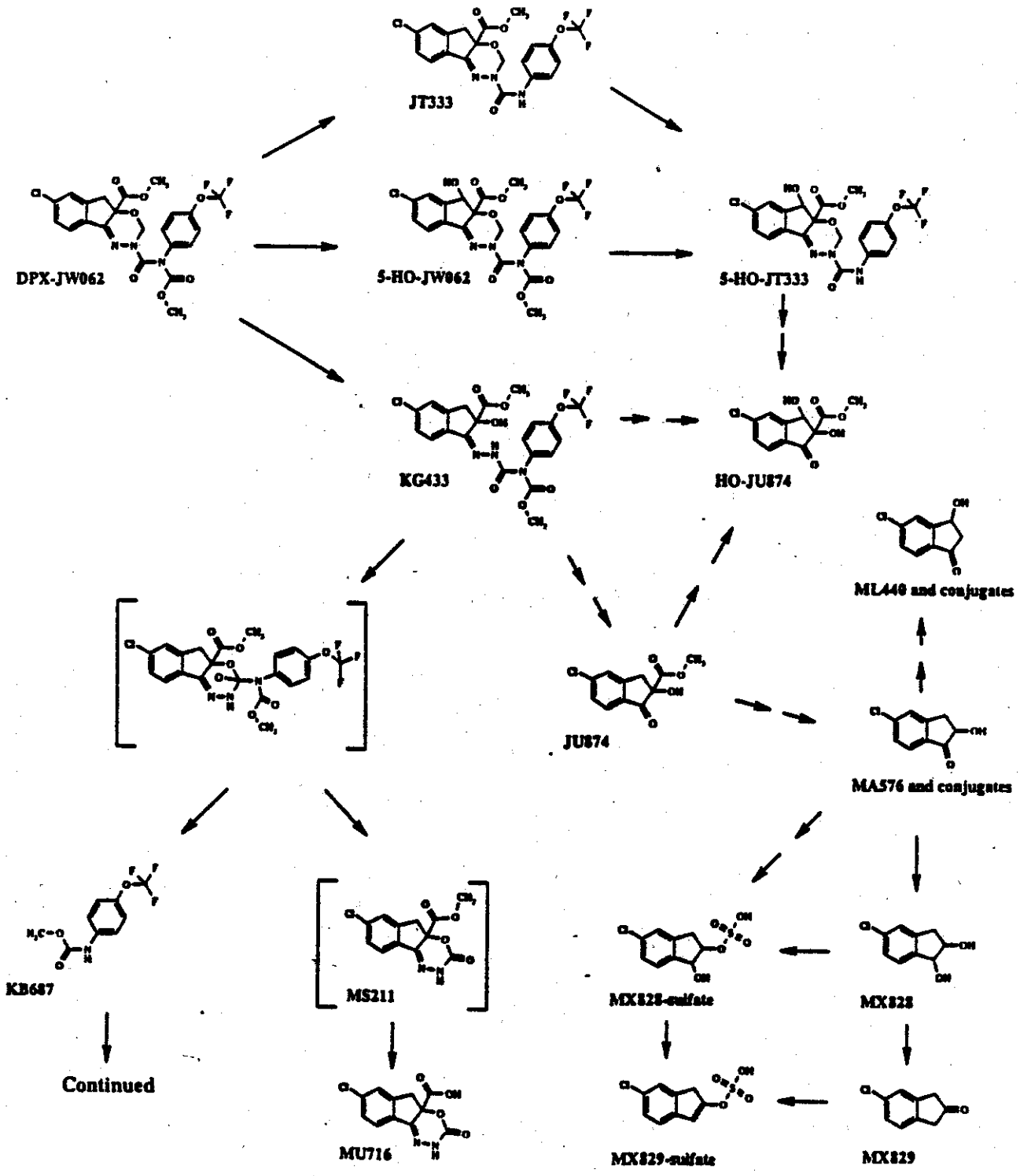
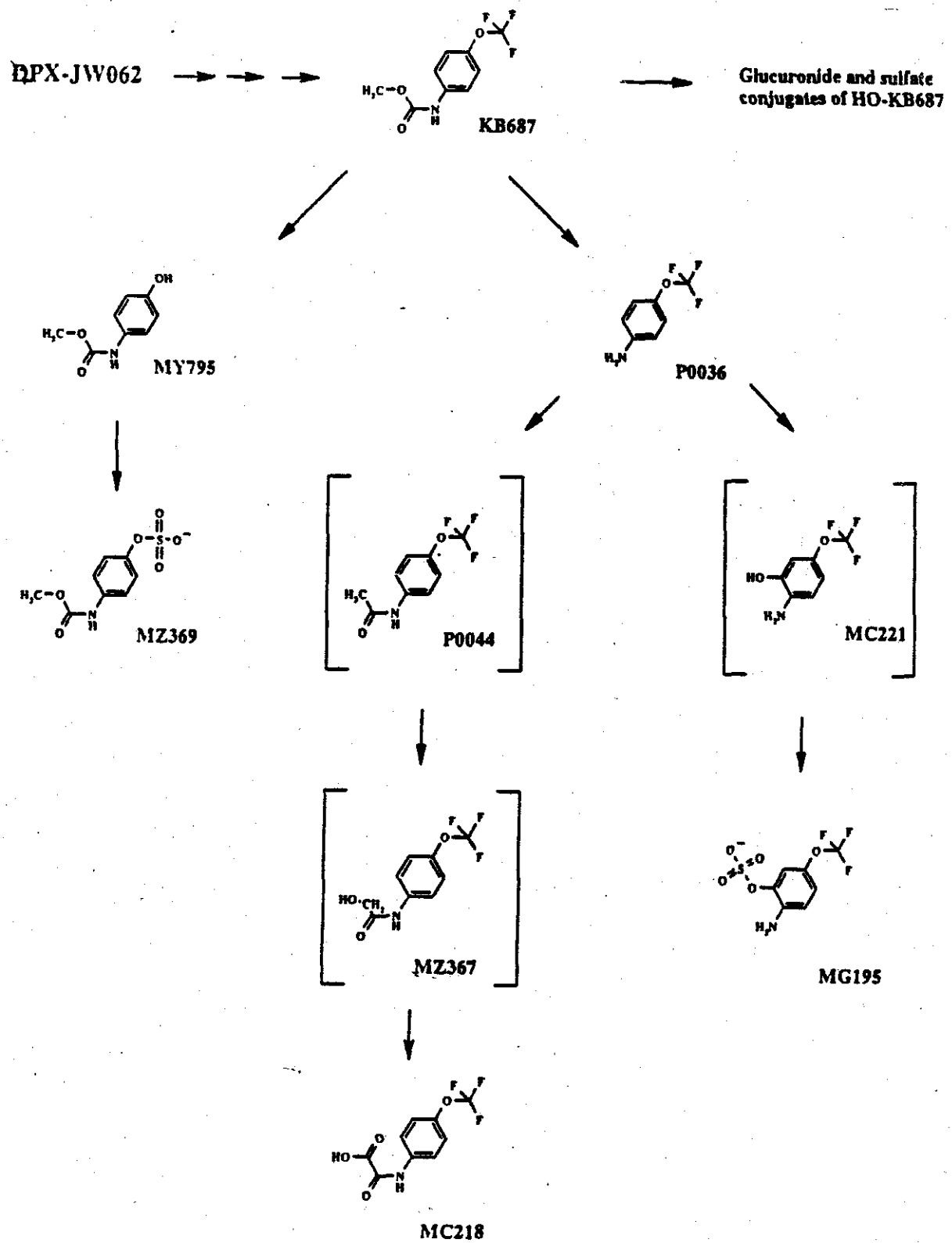
FIGURE 1. Proposed Metabolic Pathway for DPX-JW062 and DPX-MP062 in the Rat



Taken from MRID 44477153, p. 136.

FIGURE 1 (cont.). Proposed Metabolic Pathway for DPX-JW062 and DPX-MP062 in the Rat.



Taken from MRID 44477153, p. 137.

DPX-JW062 (50% KN128, 50% KN127): Mutagenicity

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: DPX-JWO62-106
   Description: Off-white solid
   Lot/batch number: DPX-JWO62-106
   Purity: 95.03% ai
   CAS Number: 144171-61-9
   Receipt date: Not provided
   Stability: "appeared to be stable under the conditions of the study."
   Solvent used: Dimethyl sulfoxide (DMSO)
   Other provided information: The storage conditions of the test material were not reported. Dosing solutions were prepared immediately prior to use. Achieved concentrations were not verified analytically.

2. Control Materials:

   Negative: None

   Solvent/final concentration: DMSO / 0.1 mL/plate

   Positive:

Nonactivation:	
Sodium azide	2 µg/plate TA1535, TA100
2-Nitrofluorene	25 µg/plate TA98
ICR-191 Acridine	2 µg/plate TA97a
Methyl methanesulfonate	1000 µg/plate WP2 uvrA
Activation:	
2-Aminoanthracene	2 µg/plate TA1535, TA98
	1 µg/plate TA97a, TA100
	25 µg/plate WP2 uvrA

3. Activation: S9 derived from male Sprague Dawley

x Aroclor 1254	x induced	x rat	x liver
_ phenobarbital	_ noninduced	_ mouse	_ lung
_ none	_ hamster	_ other	
_ other	_ other		

The rat liver S9 homogenate (Lot Nos. 0539 and 0571) were purchased from Molecular Toxicology. The S9 mix was prepared as follows:

Component:	Concentration
$MgCl_2$	8 mM
KCL	33 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
Phosphate buffer	100 mM, pH 7.4
S9	1.6 mg S9 protein/mL S9 mix

4. Test Organism Used: S. typhimurium strains
__ TA97 _x_ TA98 _x_ TA100 __ TA102 __ TA104
_x_ TA1535 __ TA1537 __ TA1538
list any others: Salmonella typhimurium TA97a and E. coli WP2 uvrA (pKM101)

Test organisms were properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes

5. Test Compound Concentrations Used:

Mutation assay: Two independent trials were performed with seven doses (10, 50, 100, 500, 1000, 2500 and 5000 µg/plate +/- S9) in both trials. Triplicate plates per dose, per strain, per condition were prepared.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay: _x_ Standard plate test
__ Pre-incubation (___) minutes
__ "Prival" modification
__ Spot test
__ Other (describe)

2. Mutation Assay: Two independent trials were performed. For both trials, 0.1 mL inoculations of the appropriate tester strain at a cell density of 10^9 bacteria and 0.1-mL volumes of the selected test material concentration or solvent control and 0.5 mL of phosphate buffered saline were added to tubes containing 2 mL of top agar (supplemented with L-histidine and biotin for the S. typhimurium strains or tryptophan for the E. coli strain). The contents of each tube were poured onto plates of Davis minimal agar. For the S9-activated test, 0.5 mL of the S9 mix replaced the phosphate buffered saline. Positive controls were included in the mutation assays. Triplicate plates were used per strain, per dose, per condition. Following incubation

at 37°C for ≈48 hours, the background lawn of growth was examined and revertant colonies were counted; means and standard deviations were calculated.

3. Evaluation Criteria:

(a) Assay validity: The assay was considered acceptable if (1) the appropriate genetic markers were verified for each tester strain, (2) the number of spontaneous revertants for each tester strain was within specified limits, (3) the density of the tester strain cultures was $\geq 1.0 \times 10^9$ cells/mL, and (4) the nonactivated and S9-activated positive controls induced a positive response.

(b) Positive response: The test material was considered positive if it caused a dose-related ≥2-fold increase in the mean number of revertants per plate of at least one strain.

C. REPORTED RESULTS:

Mutation Assays : Representative results from the two independently performed mutation assays are presented in Tables 1 and 2. As shown, compound precipitation was observed at the two highest levels (2500 and 5000 µg/plate) both with and without S9 activation in both trials. Compound insolubility was also occasionally reported at 1000 µg/plate. DPX-JWO62-106 was neither cytotoxic nor mutagenic in any trial. By contrast, the positive control compounds induced marked increases in the number of revertant colonies of the corresponding tester strain in all trials.

Based on these findings, the study author concluded that DPX-JWO62-106 was not mutagenic in this bacterial mutation assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study author's interpretation of the data was correct. The test material was assayed to insoluble levels (≥2500 µg/plate +/-S9) but failed to induce a cytotoxic or mutagenic response in a well-controlled study. In addition, the sensitivity of the test system to detect mutagenesis was adequately demonstrated; all tester strains responded in the expected manner to the appropriate positive controls. We conclude, therefore, that DPX-JWO62-106 was adequately tested and found to be negative in this bacterial assay system.

E. STUDY DEFICIENCIES: NONE.

Table 1: Representative Results of Trial 1 of the Salmonella typhimurium/ Escherichia coli Microsome Mutation Assay with DPX-JWO62-106

Substance	Dose per Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a] TA1535	TA97a	TA98	TA100	WP2uvrA
Solvent Control							
Dimethylsulfoxide	0.1 mL	-	16	127	22	154	186
	0.1 mL	+	16	158	24	185	170
Positive Controls							
Sodium azide	2 µg	-	406	--	--	591	--
ICR-191acridine	2 µg	-	--	1749	--	--	--
2-Nitrofluorene	25 µg	-	--	--	1564	--	--
Methyl methane sulfonate	1000 µg	-	--	--	--	--	1534
2-Aminoanthracene	25 µg	+	--	--	--	--	1505
	2 µg	+	317	--	1371	--	--
	1 µg	+	--	856	--	943	--
Test Material							
DPX-JWO62-106	2500 µg[b]	-	12	166	17	157	181
	2500 µg[b]	+	10	185	21	177	143

[a] Average counts from triplicate plates.

[b] Lowest insoluble dose; compound precipitation was also occasionally seen at 1000 µg/plate. Results for the highest dose (5000 µg/plate+/ -S9) or lower doses (10, 50, 100, 500 or 1000 µg/plate+/ -S9) did not suggest a mutagenic effect.

Note: Data were extracted from the Study Report, Tables 1-5, pp. 14-18.

Table 2: Representative Results of Trial 2 of the Salmonella typhimurium/ Escherichia coli Microsome Mutation Assay with DPX-JWO62-106

Substance	Dose per Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a] TA1535	TA97a	TA98	TA100	WP2uvrA
Solvent Control							
Dimethylsulfoxide	0.1 mL	-	13	103	18	108	167
	0.1 mL	+	13	132	18	158	167
Positive Controls							
Sodium azide	2 µg	-	237	--	--	730	--
ICR-191acridine	2 µg	-	--	1755	--	--	--
2-Nitrofluorene	25 µg	-	--	--	1467	--	--
Methyl methane sulfonate	1000 µg	-	--	--	--	--	1554
2-Aminoanthracene	25 µg	+	--	--	--	--	1717
	2 µg	+	311	--	1556	--	--
	1 µg	+	--	779	--	800	--
Test Material							
DPX-JWO62-106	2500 µg[b]	-	6	118	14	135	159
	2500 µg[b]	+	7	151	20	153	159

[a] Average counts from triplicate plates.

[b] Lowest insoluble dose; compound precipitation was also occasionally seen at 1000 µg/plate. Results for the highest dose (5000 µg/plate+/ -S9) or lower doses (10, 50, 100, 500 or 1000 µg/plate+/ -S9) did not suggest a mutagenic effect.

Note: Data were extracted from the Study Report, Tables 6-10, pp. 19-23.