014243

[DPX-JW062]

Special Mechanistic study

EPA Reviewer: Marion Copley, DVM
Registration Action Branch 1 (7509C)
EPA Secondary Reviewer: P.V. Shah, PhD
Registration Action Branch 1 (7509C)

Marion Copley, Date 1/19/00
P.V. Shah, Date 1/19/00

DATA EVALUATION RECORD

STUDY TYPE: Special Mechanistic Study, Distribution of Erythrocytes - Rat; OPPTS # NA

DP BARCODE: D259948
P.C. CODE: 067710

SUBMISSION CODE: S569303
TOX. CHEM. NO.: none

TEST MATERIAL (PURITY): DPX-JW062-119 (50% DPX-KN128, 50% IN-KN127) (94.5% a.i.)

SYNONYMS: (R,S)-methyl 7-chloro-2,5-dihydro-2-[[(methoxycarbonyl)[4-(trifluoromethoxy) phenyl] amino] carbonyl] indeno[1,2-*e*][1,3,4]oxadiazine-4a(3H)-carboxylate

CITATION: Anderson, J.J.. (1999) ^{14}DPX-JW062 (A Racemic Mixture of DPX-KN128 and IN-KN127): Distribution of Erythrocytes of Rats. E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Elkton Road, P.O. Box 50, Newark, DL 19714. Laboratory Project ID: DuPont-1952. February 15, 1999. MRID 44879801. Unpublished.

SPONSOR: Dupont Agricultural Products, E.I. du Pont de Nemours and Company, Wilmington, DE.

EXECUTIVE SUMMARY: MRID 44879801.(Summary taken from the study report page 12) Following oral (via gavage) dosing of rats with [trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062], a significant portion of the dosed radioactivity was associated with the erythrocytes. Washed erythrocytes from blood of male rats obtained 72 hr following single dosing with [trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062] at a nominal dose of 130 mg/kg, contained on average 29.6 μg/g [trifluoromethoxyphenyl (U)-^{14}C]DPX-JW062 equivalents.

Most of the radioactivity was distributed approximately evenly between the intracellular fluid and hemoglobin fractions; only traces of radioactivity were associated with the toluene and stroma fractions. The major identified radioactive species in erythrocytes was the trifluoromethoxyaniline, [^{14}C]IN-P0036. During characterization of erythrocyte-associated radioactivity, recovery of total radioactivity was 53.3 %. Lost radioactivity is believed due to loss of a volatile component, possibly [^{14}C]IN-P0036 itself. Data are consistent with HLR 283-96 (MRID 4447153) which showed that IN-P0036 was excreted in the urine, along with other polar

metabolites.

This special mechanistic study, distribution of erythrocytes in the rat with DPX-JW062 is classified **acceptable/nonguideline** because it is a special study and therefore does not satisfy any specific guideline requirement.

COMPLIANCE: Signed and dated Quality Assurance, Good Laboratory Practice, Data Confidentiality, and Flagging statements were included.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-JW062
   Description: white powder
   Lot/Batch No.: Haskell Sample No.: 20822 (DPX-JW062-33, 98.6% pure) and 21764 (DPX-JW062-119, 98.5% pure)
   CAS No.:144171-61-9 (for the active ingredient)

   Radiolabelled - [trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062 - 99.0 % radiochemical purity
   Description: not provided
   Lot/Batch No.: DuPont HOTC File No.: 429
   CAS No.: not given
   Specific activity: 52.8 μCi/mg

   [^{14}C]IN-P0036 - [^{14}C]4trifuloromethoxyaniline HCl - Analytical Standard - 90.1 % radiochemical purity
   Description: not provided
   Lot/Batch No.: DuPont HOTC File No.: 424
   CAS No.: not given
   Specific activity: 113.110 μCi/mg

   Structures: (see attached figures - taken from page 9 of MRID 44879801)

2. Vehicle and/or positive control

   Polyethelyne Glycol (molecular weight: 400) (PEG400). No positive control was used in this study.

3. Test animals

Species: rat
Strain: CRL:CD® (SD)IGS BR
Age and weight at study initiation: approximately 8 weeks; 232-255 g
Source: Charles River Laboratories, Inc., Raleigh, NC
Housing: Animals were housed individually in cages (unspecified type)
Diet: PMI Nutritional International, Inc. Certified Rodent LabDiet®5002 and tap water were available *ad libitum*
Environmental conditions:
Temperature: 23 °C ± 1 °C
Humidity: 50% ± 10%
Air changes: not stated
Photoperiod: 12 hr light/dark
Acclimation period: quarantined for minimum of 6 days before release.

4. Preparation of dosing solutions

For DPX-JW062, dose solutions were prepared by dissolving an appropriate amount of the test material in PEG-400. No degradation products were observed following more than two-months storage at -20 °C.

B. STUDY DESIGN AND METHODS

Animals (6 males) were placed in glass metabolism cages one day prior to dosing. They were fasted and then dosed (gavage) with 130 mg/kg trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062 (except 1 rat which received 111 mg/kg due to a shortage of test material). Rats were sacrificed 72 hours[1] post dosing with CO_2. Whole blood was collected (in heparinized vials, weighed, stored on wet ice). Erythrocytes were collected by centrifuging whole blood at 4 C for 15 min (2000 x g with a Sorvall RT6000 Refrigerated centrifuge). The erythrocytes were washed with distilled water. Prior to further analysis, the erythrocyte layer—including trapped plasma—was analyzed using a Packard Tri-Carb 2500 TR Liquid Scintillation Analyzer. The erythrocyte fraction was further analyzed for subcellular distribution of radioactivity and characterization of radioactivity associated with the erythrocytes. Fractionation Procedure of erythrocytes: Aliquots of washed erythrocytes were lysed by adding distilled water, vortex mixing and incubating for 2 hours at room temperature. Then the erythrocytes were further washed with toluene and centrifuged to separate intracellular content from the stromal fraction. The intracellular fraction was further separated into hemoglobin and intracellular fluid. The radioactivity incorporated into these fractions was quantitated using a Liquid Scintillation Analyzer.

[1]Based on MRID 44477153 time course data for plasma and erythrocytes. It was the time occurring between tC_{max} and $tC_{max/2}$.

C. RESULTS

1. **In Life** - There were no deaths.

2. **Fractionation of Erythrocyte Associated Radioactivity** - Most of the radioactivity was located in the intracellular fluid and hemoglobin fractions (see table 2).

Table 2 Location of Erythrocyte Associated Radioactivity

Fraction	% Total Radioactive Residue (TRR)
Toluene	0.2
Stroma	2.9
Intracellular fluid	44.1
Hemoglobin	52.8
Total	100

3. **Erythrocyte Associated Radioactivity**

a. Pepsin digestion followed by metabolite profiling - See table 3 for the resultant radioactivity profile.

Table 3 After Pepsin Digestion and SPE Purification

Sample	%TRR
Combined Acetonitrile Wash	19.0
Pellet Acetonitrile Extract	14.7
NaOH solubilized pellet	17.7
pH 7 load eluate	1.9
Total Recovery	53.3

HPLC System 2 analysis of the combined acetonitrile wash resulted in a single radioactive species which co-eluted with [^{14}C]IN-P0036] (column recovery was 93.9%).

HPLC analysis of the pellet acetonitrile extract resulted in [^{14}C]IN-P0036] as the primary radioactive component (column recovery was 54.1% - low recovery could be due to irreversible loss of material on the column, or through loss of volatile material from collected fractions).

D. CONCLUSIONS

Most of the radioactivity was distributed approximately evenly between the intracellular fluid and hemoglobin fractions; only traces of radioactivity were associated with the toluene and stroma fractions. The major identified radioactive species in erythrocytes was the trifluoromethoxyaniline, [^{14}C]IN-P0036. During characterization of erythrocyte-associated radioactivity, recovery of total radioactivity was 53.3 %. Lost radioactivity is believed due to loss of a volatile component, possibly [^{14}C]IN-P0036 itself. Data are consistent with HLR 283-96 (MRID 4447153) which showed that IN-P0036 was excreted in the urine, along with other polar metabolites.